Strategies to Address Low Drug Solubility in Discovery and Development

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ABBREVIATIONS: ABT-229; 8,9-anhydro-4-deoxy-39-N-desmethyl-39-N-ethylerythromycin 3B,6-b-hemiacetate; AMG 517, N-(4-[6-[4-(trifluoromethyl)phenyl]-pyrimidin-4-yl]oxy)-benzothiazol-2-yl)-acetamide; AUC, area under the curve; BCRP, breast cancer–resistant protein; BCS, Biopharmaceutical Classification System; CD, cyclodextrin; CMC, critical micelle concentration; CNT, classical nucleation theory; CRA13, naphthalen-1-yl(4-(pentyloxy)naphthalen-1-yl)methanone; DG, diglycercide; DMA, dimethylacetamide; DMSO, dimethyl sulfoxide; DSC, differential scanning calorimetry; FA, fatty acid; FABP, fatty acid-binding protein; FaSSGF, fasted-state simulated gastric fluid; FaSSIF, fasted-state simulated intestinal fluid; FATP, fatty acid transport protein; FeSSIF, fed-state simulated intestinal fluid; FTIR, Fourier transform infrared spectroscopy; GI, gastrointestinal; HDL, high-density lipoprotein; HPC, hydroxypropyl cellulose; HLB, hydrophilic-lipophilic balance; HPMC, hydroxypropyl methylcellulose; HPH, high-pressure homogenization; HPMCAS, hydroxypropyl methylcellulose acetate succinate; K-832, 2-benzyl-5-(4-chlorophenyl)-6-[4-(methylthio)phenyl]-2-yl
carbamoyl]-5-methyl-[1,3]dioxan-5-yl]-morpholin-4-yl-methanone; L-883555, N-cyclopropyl-1-[3-[6-(1-hydroxy-1-methyl)-1-oxidoxyridin-3-yl]phenyl]-1,4-dihydro-[1,8]naphthyridin-4-one 3-carboxamide; LBF, lipid-based formulations; LC, long chain; LCQ789, 5-(4-chlorophenyl)-1-phenyl-6-(4-(pyrazin-2-yl)phenyl)-1H-pyrazo[3,4-d]pyrimidin-4(5H)-one; LDL, low-density lipoprotein; LFCS, Lipid Formulation Classification System; LFC, lysophosphatidylcholine; MC, medium chain; mdr or MDR, multi-drug resistant; MG, monoglyceride; MRP, multiresistance protein; NMR, nuclear magnetic resonance; NSC-639829, N-(4-[6-(4-bromo-2-pyrimidinyl)-3-methylphenyl]-(dimethyaminomethyl))benzopyran-3-yl-acetic acid; OZ209, cis-adamantane-2-spiro-3’-8’-(aminomethyl)-1’2’4’-tioxaspiro[4,5]decane mesylate; PEG, polyethylene glycol; PG-300995, 2-(2-thiophenyl)-4-azabenzimidazole; P-gp, P-glycoprotein; PL, phospholipid; PPI, polymer precipitation inhibitor; PV, polyvinylpyrrollidone; RR200765, I-2-[4-(4-fluoro-phenyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-5-methyl-(1H)dioxan-5-yl)morpholin-4-yl-methanone; SCF, super critical fluids; SEDDS, self-emulsifying drug-delivery systems; SD, solid dispersion; SGP, simulated gastric fluid; SLN, solid lipid nanoparticle; TC, triglyceride; TPGS, β-α-tosylpheryl polyethylene glycol succinate; TRl, triglyceride-rich lipoprotein; UWL, unstirred water layer; XRPD, X-ray powder diffraction.
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I. Introduction

High hydrophobicity and intrinsically low water solubility are increasingly common characteristics of hits, leads, development candidates, and ultimately marketed drugs. Many hypotheses have been put forward as to why these trends have emerged, and the true explanation is clearly multifaceted. The application of combinatorial chemistries to generate large chemical libraries and the common application of high-throughput screening modalities, often in nonaqueous media (or mixed-solvent media), have probably played a role. The desire for increased potency, coupled with the realization that receptor binding is mediated, at least in part, by hydrophobic interactions, further magnifies the likelihood that drug candidates will have limited aqueous solubility. Finally, the quest to explore unprecedented drug targets, some of which are associated with intracellular signaling pathways, lipid processing architecture, or highly lipophilic endogenous ligands, only amplifies the requirement for highly lipophilic, poorly water-soluble drug candidates to access and interact with the target.

These drives ultimately bias the identification of poorly water-soluble “hits” during early drug screens. Poor water solubility is a significant risk factor in low oral absorption because drug molecules must, in most cases, be in solution to be absorbed, and oral bioavailability is usually a required characteristic in a target product profile of an orally administered medicine. As such, medicinal chemistry strategies during lead optimization typically seek to modify physicochemical properties (including solubility) such that drug leads have more developable characteristics. Many decision gates, or idealized character panels, are used to identify and reject drug candidates with inappropriate developability properties and, subsequently, to synthetically modify structures to improve physicochemical characteristics. Perhaps the best known of these is Chris Lipinski’s “rule of 5” (Lipinski et al., 1997), but there are many others.
In all cases, however, at least moderate water solubility is usually a focus.

Nonetheless, even with contemporary medicinal chemistry programs and increasingly sophisticated lead optimization strategies, it is apparent that for some targets, reducing lipophilicity and increasing water solubility will result in an unacceptable reduction in potency. In spite of attempts to circumvent solubility problems, approximately 40% of currently marketed drugs (Fig. 1) and up to 75% of compounds currently under development have been suggested to be poorly water-soluble (Di et al., 2009, 2011). Furthermore, the problems of low water solubility do not seem to be diminishing and may well be increasing (Takagi et al., 2006). Low water solubility therefore continues to be a challenge to successful drug development.

This review seeks to provide an overview of the strategies that may be taken to address the problems of low solubility during drug discovery and development and includes a comprehensive review of formulation approaches to support the clinical development of oral and parenteral drug products for poorly water-soluble drugs. In addition, even when lead optimization is successful in increasing aqueous solubility, early preclinical studies are still required with less than optimal leads to provide the data sets to allow informed progression or rejection; therefore, we also address the strategies that might be used when dealing with the complexities of low solubility during the discovery phase. In the former case, at least for oral drug products, the market typically dictates the need for traditional solid dosage forms (e.g., capsules, tablets). In the latter, where studies are usually conducted in small animals (rodents), liquid formulations are often used to allow oral dosing, and therefore a slightly different approach must be taken.

This review is structured to provide an initial and relatively brief introduction to the determinants of low water solubility to provide the theoretical basis for the approaches that might be taken to address solubility challenges. We subsequently present summary sections that outline potential strategies for addressing solubility issues, first in vitro and second in vivo, with the latter section addressing both parenteral and oral administration. Subsequently, we provide a comprehensive review of the technologies that can be used to promote solubility or dissolution. These latter sections are necessarily dense and are intentionally separated from the higher-level strategy summaries provided in the introduction. The technology overviews provide a reference source for the summaries that precede them. A schematic representation of each of these formulation approaches is provided in Fig. 2 and is intended to highlight the variety of proven strategies available to those working with poorly water-soluble drugs.

A. What Is Low Drug Solubility?

Although low water solubility of drug candidates presents varied and significant challenges throughout drug discovery and development, the greatest concern is generally the risk of reduced and variable absorption after oral administration. The value at which limited solubility begins to impact absorption is difficult to state definitively since it is dependent on a number of other system variables, including drug permeation, dose, and the environment present within the gastrointestinal (GI) tract. To understand these variables, and therefore, the factors that impact the required solubility for a drug candidate, it is instructive first to appreciate the drivers of flux across an absorptive membrane since these in turn will determine whether the drug dose can be absorbed over the timescale available.

Assuming appropriate chemical and metabolic stability and an absence of transporter or carrier-mediated processes, flux \( F \) across an absorptive membrane is the product of the concentration gradient and the permeability across the membrane and is described as follows:

\[
F = \frac{D \cdot K \cdot A}{h} (C_m - C_0)
\]

where \( C_m \) is the drug concentration immediately adjacent to the membrane, \( C_0 \) is the drug concentration on the abluminal side of the membrane, \( K \) is the partition coefficient between the aqueous solution overlaying the membrane and the membrane itself, \( h \) is the width of the diffusion layer, \( D \) is the diffusion coefficient of the drug in the membrane, and \( A \) is the surface area. Assuming that the drug concentration on the abluminal side of the absorptive membrane is low relative to the concentration at the absorptive site (i.e., \( C_m \gg C_0 \)), eq. 1 can be simplified to the following:

---

**Fig. 1.** A comparison of the distribution of solubilities for the top 200 oral drug products in the United States (US), Great Britain (GB), Spain, and Japan and from the World Health Organization (WHO) Essential Drug List. Very soluble drugs: over 1000 mg/ml; freely soluble drugs: 100–1000 mg/ml; soluble drugs: 33–100 mg/ml; sparingly soluble drugs: 10–33 mg/ml; slightly soluble drugs: 1–10 mg/ml; very slightly soluble drugs: 0.1–1 mg/ml; practically insoluble drugs: <0.1 mg/ml. Adapted from Takagi et al. (2006).
The values for $D$, $K$, and $h$ are typically fixed for a particular system and are used to define the permeability coefficient ($P$) where

$$P = \frac{D \times K}{h}$$

In the absence of supersaturation, the maximum concentration that can be attained at the surface of an absorptive membrane is equivalent to the equilibrium solubility ($C_s$) of the drug, and therefore the maximum flux (per unit area) ($F'$) is the product of solubility and permeability:

$$F' = P \times C_s$$

Appreciation of this relationship illustrates that knowledge of the solubility alone is insufficient to anticipate whether solubility will limit flux (or absorption) since flux is also a function of permeability. To some extent, therefore, low solubility can be offset by high permeability; similarly, if permeability is low, the requirements for solubility to generate appropriate flux increase.

A well recognized approach applied in early drug discovery for estimating the required solubility and permeability needed to achieve good oral absorption is the concept of a maximum absorbable dose (MAD), originally derived by Johnson and Swindell (1996) and further applied by Curatolo (1998) and Lipinski (2000):

$$MAD = C_s \times k_{abs} \times SIWV \times SITT$$

where $C_s$ is the solubility (mg/ml) at pH 6.5 (representing the pH of the small intestine); $k_{abs}$ is the rate constant (h$^{-1}$) for intestinal absorption (which is related to the permeability); SIWV is the small intestinal water volume (in milliliters), which is typically assumed to be $\sim 250$ ml (the volume of fluid assumed to be present in the fasted GI tract after a glass of water has been drunk when taking medication orally); and SITT is the small intestinal transit time (min) of $\sim 270$ min (4.5 h). Rearranging this relationship provides an expression for the necessary or target solubility for a given dose and $k_{abs}$ (or permeability) and provides an initial indication as to whether solubility is likely to limit oral absorption. This concept is shown graphically in Fig. 3, the data from which are taken from a seminal review that shows the theoretical required solubility to provide good oral absorption for drugs with projected doses ranging from 0.1 to 10 mg/kg and permeabilities ranging from low to high. At one end of the spectrum, highly potent drugs for which the dose is low and the membrane permeability is high have relatively low solubility requirements to achieve good oral absorption. At the other extreme, low-potency drugs for which the dose is high and the permeability is low need...
considerably higher solubility for good oral absorption (by several orders of magnitude in this example). As a broad initial estimation, and assuming moderate potency (1.0 mg/kg) and moderate permeability \( (k_{\text{abs}} = 1.0 \text{ h}^{-1}) \), this approach indicates that where aqueous solubilities are \(<50 \mu\text{g/ml}, problems associated with low water solubility might be anticipated. It is clear, however, that the required solubility to support drug absorption must be evaluated in light of both the potency (or dose) and the permeability characteristics. It is also clear that at stages during the development pathway, in particular during preclinical toxicity testing, exposure at doses considerably in excess of the predicted clinical dose will be required, magnifying the need for solubility support.

A further application of the solubility-permeability relationship to oral drug absorption is the Biopharmaceutics Classification System (BCS) (Fig. 4), originally developed by Amidon et al. (1995), with subsequent variations by others (Wu and Benet, 2005; Butler and Dressman, 2010; Chen et al., 2011). The principles of the BCS are well described elsewhere (Amidon et al., 1995; Yu et al., 2002; Dahan et al., 2009), but in brief, the BCS allows classification of drug molecules as a function of their solubility and permeability properties. Originally proposed to provide a scientific basis for bio waivers based on a correlation of in vitro drug dissolution and in vivo drug absorption, this classification system has found much broader applicability across many areas of drug discovery and development. According to the BCS, class I molecules are those having both high solubility and high permeability (and therefore likely few problems with oral absorption); class II compounds are those that have low solubility and high permeability (where solubility is the primary limitation to absorption); class III compounds have high solubility but low permeability (where absorption is limited by membrane permeation and not solubility); and class IV compounds are those in which both poor solubility and poor permeability limit drug absorption. The focus of the current review is therefore BCS class II compounds, which often exhibit solubility-limited absorption. Class IV compounds are also relevant, although they have additional problems associated with low permeability.

Drug dose is also an important factor in the BCS because highly soluble drugs are defined as those in which the highest dose will dissolve in 250 ml over the pH range of the GI tract (i.e., pH 1–6.8). Several examples of dose, solubility, and volume requirements (taken from Amidon et al., 1995) are shown in Table 1. As with the MAD, these calculations are not designed to be definitive but rather illustrate that low-dose compounds, such as digoxin, can have good absorption and bioavailability, even when GI solubility is low, whereas the absorption of high-dose compounds, such as griseofulvin, is more often low, variable, and highly formulation dependent as a result of their solubility limitations.

A complication of the BCS definition of high solubility is that the highest strength dose must be soluble in 250 ml of water at all pH values that might be encountered in the GI tract. Therefore, drugs may be classified as class II even though they have good solubility at one end of this pH range. For example, many weak acids have low solubility at pH 1 and are strictly classified as BCS class II compounds but are quite soluble at intestinal pH (pH 6–7) and in many cases do not exhibit solubility-limited absorption. As such, a BCS class II designation does not always dictate that solubility will be a limitation to absorption; rather, compounds in this BCS class are more likely to be solubility limited than are those in class I. Indeed,

**TABLE 1**  
Examples of dose, solubility, and volume requirements for a range of poorly water-soluble drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>Solubility (mg/ml)</th>
<th>Volume Required for Complete Solubilization (ml)</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piroxicam</td>
<td>20</td>
<td>0.007</td>
<td>2857</td>
<td>Low, variable</td>
</tr>
<tr>
<td>Digoxin</td>
<td>0.5</td>
<td>0.024</td>
<td>21</td>
<td>Good</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>500</td>
<td>0.015</td>
<td>33,333</td>
<td>Low, variable</td>
</tr>
<tr>
<td>Chlorthiazide</td>
<td>500</td>
<td>0.78</td>
<td>636</td>
<td>Reasonable</td>
</tr>
</tbody>
</table>

From Amidon et al. (1995).
a more practical description of BCS class II may be drugs that do not have high solubility rather than those that have low solubility since the classification system specifically identifies compounds that fall into class I and all those with solubilities below this fall inherently into class II (or class IV if permeability is also low) (Fig. 4).

The preceding discussion serves to illustrate further that low solubility is a somewhat arbitrary concept when assessing the likelihood that solubility will limit drug absorption and that additional knowledge of the likely dose and membrane permeability is inevitably required to put a solubility value into an appropriate context.

B. Determinants of Aqueous Solubility

A detailed description of the thermodynamic determinants of drug solubility in aqueous media is beyond the scope of the current discussion; for more information, the interested reader is directed to the following references: Grant and Higuchi (1990), Yalkowsky (1999), and Murdande et al. (2010a). An overview of the broad physicochemical drivers of drug solubility is warranted, however, since these drivers underpin the approaches that can be taken to enhance solubility. Simplistically, the potential for a drug (solute) to pass into solution in an aqueous fluid (solvent) is dictated by three separate events, as shown in Fig. 5. First, solute molecules must be abstracted from the solid state, a process that involves breaking solute-solute bonds. The strength of solute-solute attractive forces in different solids varies significantly and is typically higher for electrolytes than for nonelectrolytes (since ionic attractive forces in the solid state are stronger than nonionic forces), for crystalline solids compared with amorphous materials, and for planar nonelectrolytes (which pack more effectively in the solid state) compared with nonplanar molecules. The melting point provides a reasonable indication of the strength of intermolecular solute-solute interactions in the solid material. Second, a void must be created in the solvent that is sufficient to accommodate the abstracted solute molecule. Since intermolecular forces in the liquid state are much lower than those in the solid state, the energy required to create a void in the solvent is low and is usually ignored when assessing energy changes during dissolution. Finally, the solute molecule is inserted into the solvent void. For molecules with some affinity for a polar solvent such as water, this process is energetically favorable and therefore drives drug solubility. This last concept is the rationale behind the often quoted maxim “like dissolves like.” This is largely true, but it captures only half the story since it ignores the impact of changes to solid-state properties on solubility. Since the energy transitions associated with changes to solvent structure are small, it is apparent that there are two primary determinants of drug solubility: 1) the energy required to overcome the strength of intermolecular forces in the solute solid state and 2) the energy generated on the interaction of solute and solvent molecules in solution (solvation).

For an analogous structural series, solubility is therefore generally lower for molecules that have higher melting points (stronger attractive forces) and lower affinity for water (poor solvation).

1. Ideal Versus Nonideal Solubility. Solubility theory defines an idealized condition or an ideal solution, where the intermolecular forces between solute and solvent are equivalent to those between solute and solute and those between solvent and solvent. Under these circumstances, the mixing of solute and solvent molecules in the liquid state results in no net energy
change. Where this is the case, solubility is dependent on the strength of the solute crystal lattice and may be defined by

$$\log X = -\frac{\Delta H_f}{2.303R} \left( \frac{T_m - T}{T_m T} \right)$$

(6)

where X is the ideal mole fraction solubility of solute, \(\Delta H_f\) is the enthalpy of fusion of solute, \(T_m\) is solute melting point, T is the absolute temperature, and R is the gas constant.

In reality, ideal solutions are highly unusual, and solution conditions close to ideality exist pharmaceutically only in solutions of highly lipophilic drugs in nonaqueous (lipidic) solutions. In contrast, in aqueous solution, the differences between solute-solute interactions and solute-solvent interactions are highly significant, leading to nonideal solution behavior and much lower solubility than would otherwise be predicted. In this case, the difference between ideal and nonideal behavior is captured by a correction factor termed the activity coefficient (\(\gamma\)), where

$$\log X = -\frac{\Delta H_f}{2.303R} \left( \frac{T_m - T}{T_m T} \right) - \log \gamma$$

(7)

In turn, the activity coefficient is a function of the molar volume of the solvent (\(V_s\)), the volume fraction of the solute (\(\phi_1\)), and the difference in the solubility parameters for the solvent (\(\delta_1\)) and solute (\(\delta_2\)):

$$\log \gamma = \frac{V_s \phi_1^2}{2.303RT} (\delta_1 - \delta_2)^2$$

(8)

The solubility parameters provide a measure of the cohesive forces in either solute or solvent, and from eq. 8, it is evident that smaller differences between the solubility parameters of the solvent and solute give rise to lower activity coefficients and, therefore, an increase in solubility toward ideality (i.e., \(\delta_1 = \delta_2\)) (Rubino, 2002).

The solubility equation (eq. 7) captures the determinants of solubility as dictated by changes to the solid-state properties of a drug (and usually manifest by changes to melting point) and the activity coefficient (reflecting differences between the solubility parameters), and it reiterates the importance of solute-solute interactions in the solid state and the need to minimize differences between solute and solvent properties to maximize solvation (i.e., like dissolves like). These principles underpin all the approaches to solubility enhancement that are subsequently described in this review, as each of these approaches either change solid-state properties or change the nature of the interaction between drug (solute) and solvent molecules.

C. Hydrophobic or Lipophilic Drug Candidates?

An understanding of the primary drivers of drug solubility allows an important distinction to be made between poorly water-soluble drugs that are limited by solid-state properties (e.g., the strength of the crystal lattice), those that are limited by solvation (i.e., solute-solvent interactions in solution), and those that are limited by both. In practice, most compounds fall into two categories since almost all poorly water-soluble compounds have limited affinity for water (i.e., they are hydrophobic) and are therefore solvation-hydration limited. Where compounds are hydrophobic and also show strong intermolecular forces in the solid state, they are typically poorly soluble in both aqueous and nonaqueous solvents. In contrast, hydrophobic molecules, in which solubility is not limited by solid-state properties, often show varying degrees of solubility in nonaqueous solvents such as lipids (since the molecular properties that reduce hydration in aqueous media often promote solvation in nonaqueous media). The latter compounds are therefore both hydrophobic and lipophilic, with the former simply being hydrophobic.

The difference between these two types of molecules can be illustrated using the analogies of "brick dust" for hydrophobic molecules with poor solubility in all solvents and "grease balls" for compounds that are hydrophobic and lipophilic and show reasonable solubility in lipids (Stella and Nti-Addae, 2007). This distinction is important since the formulation options available for either hydrophobic or lipophilic compounds differ considerably. Simplistically, the increased lipid solubility of lipophilic drug candidates allows access to liquid surfactants and lipid-based delivery technologies that can be filled into soft gelatin capsules (or sealed hard gelatin capsules), whereas the lack of solubility for hydrophobic molecules in almost all vehicles precludes formulation in anything other than modified solid dosage forms.

D. Solubility of Electrolytes, Weak Electrolytes, and Nonelectrolytes

The solvation properties of drug molecules, and therefore a significant part of the driving force for drug solubility in aqueous media, are highly dependent on the extent of ionization. The presence of a charged functional group provides an opportunity for favorable ion-dipole interactions with polar solvents such as water, which directly enhance hydration and water solubility. Strong electrolytes (such as NaCl) that completely dissociate in water are generally highly water soluble. This is not always the case, however, as solubility remains a composite function of the energy required to break the crystal lattice versus the energy liberated on hydration of the ions formed. In some extreme cases, for example, an inorganic salt such as AgCl, the crystal lattice energy is sufficiently high that aqueous solubility remains low. As a strong electrolyte, therefore, dissociation of AgCl molecules in solution is complete, but as a poorly water-soluble strong electrolyte, solubility is limited.

In reality, most drugs are organic materials that are either nonelectrolytes (which do not dissociate to form
ionic species in water) or weak electrolytes that dissociate only partially in water such that both un-ionized solute and the dissociated ions are present in solution.

The solubility of weak electrolytes is highly dependent on the degree of ionization (dissociation) since the affinity of the ionized species for water is markedly higher than that of the un-ionized species. The degree of dissociation is in turn dependent on the pKₐ of the weak electrolyte and the pH of the solution into which it is dissolving. Simplistically, at pH values above the pKₐ of a weak acid and below the pKₐ of a weak base, solubility increases significantly as a result of ionization. For weak acids and bases with a single ionizable group, solubility increases by a factor of 10 for every pH unit away from the pKₐ (although this trend does not continue indefinitely and solubility is ultimately limited by the solubility product of the salts that are formed in situ with available counterion; see Section IV). Nonetheless, optimization of solution pH is an effective means by which solubility can be enhanced and is commonly used to enhance the solubility properties of solution formulations for weak electrolytes. For solid dosage forms, the principles of pH-dependent solubility may be manipulated by the isolation of a drug (or drug candidate) as an appropriate salt form. The use of pH and salt selection to enhance solubility and the dissolution rate is described in more detail in Section IV. For nonelectrolytes, solubility behavior is not complicated by the effects of ionization and remains a function of hydration and the strength of the crystal lattice.

E. Solubility and Dissolution Rate

The solubility of a drug in aqueous solution is a fundamentally important property that affects not only the potential for drug absorption after oral administration and the ability to administer the drug parenterally but also the ease of manipulation and testing in the laboratory and during manufacture. However, drug solubility is an equilibrium measure and the rate at which solid drug or drug in a formulation passes into solution (i.e., the dissolution rate) is also critically important when the time available for dissolution is limited. This rate is particularly relevant after oral administration since intestinal transit time is finite and the rate of drug dissolution must significantly exceed the rate of transit for absorption to be maximized. For example, the absorption of a drug with reasonable solubility may still be poor if the rate of dissolution is low since the solubility limit may never be reached during the intestinal transit time. Similarly, even where the rate of dissolution is relatively rapid, if the equilibrium solubility is low, the quantity of drug available in solution for absorption is unlikely to support rates of flux across the intestine that are sufficient to absorb the entire drug dose in the time available. For different drugs, and under different circumstances, either solubility or dissolution rate (or both) may be the limiting feature.

The process of drug dissolution from the solid state is summarized in Fig. 6. An unstirred water layer is present on the surface of every dissolving solid and provides the barrier to drug equilibration with the well-stirred bulk solution. The dissolution rate is therefore defined by the rate at which drug diffuses across the unstirred water layer, and the equations that describe drug dissolution (i.e., the Noyes Whitney Equation, eq. 9) are analogous to simple diffusion equations. The rate of transfer across the unstirred layer is a function of the concentration gradient across the unstirred layer, the width of the diffusion layer (h), the surface area of contact of the solid with the dissolution fluid (A), and the diffusion rate of the drug in water (D). The concentration gradient in turn is a function of the maximum drug concentration at the surface of the dissolving solid (drug solubility Cₛ) and the concentration in the well-stirred bulk (C) (Noyes and Whitney, 1897):

\[
\frac{dc_s}{dt} = \frac{D \cdot A}{h} (C_s - C) \tag{9}
\]

If we assume sink conditions where the concentration of drug in bulk solution (C) is low relative to the concentration on the surface of the dissolving solid (Cₛ), then this relationship collapses to

\[
\frac{dc_s}{dt} \approx \frac{D \cdot A}{h} C_s \tag{10}
\]
For most small molecules, the diffusion rate constant in water (D) is relatively high and manipulation of drug structure does not typically affect D to the extent that it has a significant impact on dissolution rate. Similarly, whereas the width of the diffusion layer can be altered by agitation or stirring in vitro, this aspect cannot be easily manipulated in vivo. The major determinants of in vivo drug dissolution rate are therefore solubility and surface area. Since solubility is a function of the strength of the crystal lattice and the affinity of the solute (drug) for the aqueous environment, three major strategies can be defined to increase the solubility or dissolution rate (realizing that the dependence of dissolution on solubility dictates that increases in solubility inherently increase the dissolution rate):

- Reducing intermolecular forces in the solid state (increases solubility and dissolution rate)
- Increasing the strength of solute-solvent interactions in solution (increases solubility and dissolution rate)
- Increasing the surface area available for dissolution (increases dissolution rate, potential to moderately increase solubility at very small particle sizes (<1 μm)

F. Summary

Low aqueous solubility and reduced dissolution rates are a common property of many new drug candidates, and these properties create a number of challenges during drug discovery and development. An understanding of the determinants of solubility and dissolution provides a framework from which approaches to enhance solubilization may be developed. In subsequent sections of this review, we first address the complexities of working with poorly water-soluble drugs in vitro and subsequently summarize the approaches that can be taken to assist in the development of both parenteral and oral formulations. The main body of the review follows and provides a detailed account of the technological approaches that can be taken to support the development of effective formulations for poorly water-soluble drugs. Comment is made as to the many different approaches that might be taken during lead optimization and preclinical development and also those strategies that are also appropriate for extension into clinical development and ultimately to the market. To constrain the scope of this review, synthetic medicinal chemistry approaches to solubility manipulation are not addressed and the discussion is limited to approaches that do not result in the generation of a fundamentally new chemical entity. For more information on approaches to solubility manipulation via structural modification, the interested reader is directed to the following reviews: Fleisher et al. (1996), Stella and Nti-Addae (2007), Di et al. (2009), Keseru and Makara (2009), and Ishikawa and Hashimoto (2011).

II. In Vitro Complexities of Working with Poorly Water-Soluble Drugs

Despite the drive to identify “drug-like” leads with acceptable physicochemical properties, lead series are often plagued by poor aqueous solubility. The basis for this trend was discussed earlier in this introduction, but regardless of the source, working with inherently poorly water-soluble compounds creates a number of challenges throughout drug discovery, beginning with primary activity screens and progressing through to secondary in vitro assays and in vivo assessment.

In many in vitro assays, there is little scope for improving solubility given the poor tolerability of many in vitro biologic test systems for solubilizing components. In this instance, the focus must be on understanding the consequences of simple solution preparation and manipulation (e.g., dilution), appreciating the potentially confounding effects of compound precipitation on assay results, and grasping the impact of commonly used buffer components in dictating “free” compound concentrations in solution. The sections that follow highlight some of the common problems associated with the in vitro testing of poorly water-soluble compounds and, where available, approaches that can be taken to overcome, or at least minimize, these issues.

A. Drug Precipitation, Adsorption, Binding, and Complexation in In Vitro Assays

Beyond the realms of chemical synthesis, most in vitro evaluations of compound performance are conducted using aqueous-based biologic buffers and related media. The range of in vitro testing protocols is, of course, broad but might include binding or displacement assays, enzyme inhibition studies, activity screening in cell culture, traditional organ-bath pharmacology, assessment of uptake and transport in cell culture systems, or excised tissues and metabolic stability studies using microsomes or hepatocytes. In all cases, an accurate knowledge of the concentration of drug in solution is required as it is critical to the determination of the experimental endpoint. For example, most in vitro assays of drug activity are based on a concentration-response relationship, with the endpoint being some measure of the inhibitory or effective concentration (e.g., IC_{50} or EC_{50}). In other assays, changes to drug concentrations in solution during the assay are used as an indicator of cellular uptake or transport, metabolism, or binding, all of which rely on a known concentration of compound in solution.

In most drug discovery settings, moderate- to high-throughput assay formats (i.e., 96-, 384-, or 1536-well plates) are used, and compounds are introduced into aqueous biologic media via dilution of concentrated
stock solutions prepared in water miscible organic solvents, the most common being dimethyl sulfoxide (DMSO). DMSO is an excellent solvent for many poorly soluble compounds, including those with diverse chemical structures, and allows for the preparation of highly concentrated stock solutions for subsequent dilution for most compounds. However, compound precipitation after dilution of concentrated cosolvent solutions is common (see Section VI) and can lead to variable responses depending on how the dilution is conducted and the composition of the final assay media. This in turn can lead to a high degree of variability and inconsistent results between assays where these variables may be different. Different biologic assays also vary in their tolerance to the final DMSO (or other cosolvent) concentrations, making it difficult to standardize experimental conditions and dilution procedures across different assay formats.

For many in vitro assays (with the exception of high-throughput assays specifically designed to assess solubility), it may be difficult, if not impossible, to detect the presence of finely precipitated material on dilution into aqueous media, and measuring the final concentration of drug may be impractical. Working with compounds with inherently low aqueous solubilities generally limits the available range of drug concentrations that can be used to define concentration-dependent processes. Under these circumstances, complete binding or inhibition profiles may be difficult to define since the solubility limit is reached before saturation of binding sites or approach to maximal inhibition.

Furthermore, the physicochemical properties that predispose compounds to low aqueous solubility can also lead to an association of drug molecule with hydrophobic environments and surfaces. In fact, this phenomenon is often a driving force for biologic potency since partitioning into and across cell membranes and interaction with cellular and molecular targets are thermodynamically favored compared with residence within an aqueous solution. However, these characteristics also lead to an inherent propensity for nonspecific adsorption to surfaces such as tubes, pipette tips, filters, syringes, multiwell plates and cellular supports. Under these circumstances, a decrease in drug concentration in solution may be reflective of nonspecific adsorption rather than binding, uptake, or transport, artificially reducing the concentration of drug in solution and leading to an erroneous endpoint determination if concentrations are assumed on the basis of only the dilution factor. Clearly, there are advantages to obtaining measured concentrations to provide confidence in the experimental results when practical and also to provide an assurance of mass balance, which is a critical control measurement in any mass transport experiment.

The adsorption of drug to filter membranes requires special mention since separation of free drug from bound, complexed, or precipitated drug is a common procedure during the conduct of many in vitro assays. Assessment of the potential for adsorption during filtration is an important validation step but is particularly critical for poorly water-soluble drugs. Where significant adsorption is evident, and unavoidable, then it may be necessary to avoid the process of filtration. Equilibrium dialysis may provide an alternative under such circumstances, but the potential for adsorption to the dialysis membrane is also high. A final approach is to use ultracentrifugation to separate larger drug complexes from free drug in solution, but these measurements are time consuming, require specialized equipment and a significant density difference between species, and ultimately still carry the risk of drug adsorption to centrifuge tubes or plates.

Several different approaches can be taken to overcome issues of adsorption. The first is the choice of tube, filter, culture flask, multiwell plate, filter or pipette tip, as many manufacturers supply materials with modified surface properties to reduce nonspecific adsorption. It is important to appreciate the consequences of using different surfaces as those specifically designed to, for example, promote cell adhesion by making them more hydrophilic, will also increase the likelihood of nonspecific drug adsorption. In contrast, more hydrophilic surfaces will generally reduce adsorption by reducing the thermodynamic favorability of drug leaving the largely aqueous solution environment. Another approach involves pre-exposing potential adsorption sites to drug solution with a view to saturating adsorption before conduct of the experiment. Finally, alteration of the solution properties can reduce adsorption by increasing drug affinity for the bulk solution and reducing the effective partition coefficient between solution and drug adsorption sites. Most commonly, this is achieved via the inclusion of small quantities of a cosolvent (depending on the sensitivity of the particular assay) or by manipulating solution pH to increase solute-solvent interactions in solution. The use of pH and cosolvents to enhance solubility is described in more detail in Sections IV and VI, respectively.

Adsorption may also be reduced via the addition of a complexation or solubilization agent such as a surfactant (see Section VII), cyclodextrin (see Section VIII), or protein. Although these approaches are often highly effective, they introduce a further complexity, namely, changes to the chemical potential or thermodynamic activity of free (unbound) drug in solution as discussed in the following section.

B. Changes to Thermodynamic Activity Resulting from Complexation, Binding, or Solubilization

The effective concentration of a species in solution is most accurately defined by its thermodynamic activity (a), which is related to the concentration (C) and the activity coefficient (γ):
A detailed evaluation of thermodynamic activity and chemical potential is beyond the scope of this review, but in simple terms, the effective concentration or thermodynamic activity can be considered as the concentration of drug in solution that is unconstrained by interaction with any other molecular species and is therefore available to exert its effect, regardless of whether the effect is to bind to a receptor or to diffuse across a membrane. In concentrated solutions, for example, the close molecular proximity of drug molecules to each other may promote solute-solute interactions (i.e., enhance intramolecular association), thereby reducing thermodynamic activity. Under these circumstances, the activity coefficient is less than unity, and the effective concentration or activity is less than the measured concentration. In typical dilute solutions, the degree of dilution is expected to reduce solute-solute intermolecular interactions in solution such that each molecule effectively acts independently, and under these circumstances, the activity coefficient is unity and activity is equal to concentration. This allows the definition of equilibrium constants, for example, in dilute solution using drug concentration instead of the thermodynamically correct use of activities.

The relevance of this discussion to the in vitro testing of poorly water-soluble drugs is that many strategies that promote drug solubility in aqueous solution and assay media can change the thermodynamic activity of the drug in solution. This is most readily illustrated by considering the impact of the addition of a surfactant to an aqueous solution of drug. Surfactants are amphiphilic molecules, and in aqueous solutions, they can exist as either monomers or as micellar structures. As surfactant concentrations are increased above the critical micelle concentration (CMC), the concentration of monomeric surfactant remains constant while the concentration of surfactant present as micelles in solution increases. Surfactant micelles typically enhance drug solubility by providing a hydrophobic environment in the micellar core to solubilize poorly water-soluble drugs as they have a greater affinity for this hydrophobic environment than for the surrounding aqueous environment (see Section VII). However, under this circumstance, the thermodynamic activity of the drug is much lower than the total concentration as the activity coefficient of the drug is lower than unity. In the case of drug solubilized in a surfactant micelle, drug can be considered as existing in equilibrium between solubilized drug within the micelle and free drug in an intermicellar phase or bulk solution phase. Here, solubilized drug is highly constrained by the surrounding micellar structure, and the effective concentration (i.e., the thermodynamic activity) is more accurately represented by the free (nonmicellar) concentration of drug.

In addition to the use of surfactants, other common circumstances where the thermodynamic activity of a compound might be reduced include the introduction of a complexation agent, such as a cyclodextrin, or the addition of plasma or serum proteins to cell-based assay media. For the latter situation, the so-called serum shift phenomenon is widely recognized where in vitro activity changes in response to changing concentrations of serum present in the media. For a more comprehensive review of the effect of protein binding on the pharmacological activity of drugs and the complexities of interpreting static (in vitro) versus dynamic (in vivo) situations, the reader is referred to the excellent review by Smith et al. (2010). In each of these cases, the total concentration of drug is significantly higher than the effective or free concentration, and the situation is further confounded by the difficulty in accurately quantifying free drug concentrations. In contrast, solubility enhancement through pH manipulation or cosolvency will typically have limited impact on thermodynamic activity of drug in solution.

III. In Vivo Assessment of Poorly Water-Soluble Compounds

In vivo assessment of new drug candidates begins with early in vivo efficacy studies in animal models and continues through pharmacokinetic and dose-limiting toxicology studies in various preclinical species and ultimately into human clinical trials. Preclinical pharmacokinetic studies are heavily used to support human dose and pharmacokinetic predictions and, along with results from toxicology studies, are used to select a safe starting dose in humans. For in vivo evaluations in animals, there are multiple approaches that can be used to facilitate i.v. administration and to improve exposure after oral dosing by the use of enabling formulations. However, there is always a risk that early incorporation of an enabling formulation approach may shift the focus away from structural optimization. Even in circumstances where lead optimization strategies are successful in identifying drug candidates with improved solubility properties, it is likely that at some stage during drug discovery there will be the need to assess less soluble early leads for intrinsic activity and proof of concept efficacy to justify compound or series progression.

A complication of poor aqueous solubility in the in vivo assessment of compounds throughout drug discovery and development is that compound supply is frequently limited (particularly in early discovery), and material that is available most likely will not have the final, or optimal, solid-state properties. As compounds progress through discovery and into development, the solid-state properties will almost certainly change; crystal forms will become better defined, particle size reduction may be introduced, and salt forms will likely
become available for compounds with ionizable functional groups. The timing at which these changes occur during development will impact early preclinical data, and this needs to be appreciated and factored into study design and data interpretation.

A. Parenteral Administration

1. Complexities with Parenteral Administration of Poorly Water-Soluble Drugs. In comparison with the large number of drugs intended for oral administration, parenteral formulations constitute a more limited proportion of marketed products. However, the generation of useful parenteral formulations remains a key component of drug discovery and drug development for almost all drug molecules (regardless of the intended route of clinical administration) since data obtained after i.v. administration is necessary to generate fundamental pharmacokinetic parameters (volume of distribution, clearance, half-life, absolute bioavailability) to support compound optimization and progression, and such data are also useful to support early stage pharmacodynamic assessment of drugs intended for acute administration.

Low aqueous solubility significantly complicates the development of i.v. formulations since, in almost all cases, simple solution formulations are required. The following section describes several different approaches that can be used as i.v. solution formulations, depending on the physicochemical properties of the drug and the intended use (e.g., animal or human). Depending on the formulation strategy adopted, increases in solubility of several orders of magnitude are possible. The risks associated with i.v. administration of poorly water-soluble drugs include, first, the potential for drug precipitation on administration and, second, the potential for formulation components to cause pain, phlebitis, inflammation, hemolysis, or unacceptable toxicology. Precipitation is more likely for formulations where pH adjustment or cosolvents form the basis for drug solubilization since both are likely to be altered significantly following injection (Gad et al., 2006; Li and Zhao, 2007). A summary of parenteral formulation approaches is given in Table 2, and these are briefly described as follows.

pH adjustment (see Section IV) is a powerful mechanism by which the solubility of weak acids and weak bases may be enhanced, but the approach is limited by the pKₐ of the compound and the acceptable pH range of the formulation. In this regard, acidic solutions are generally more readily tolerated than are basic solutions, and working pH ranges of 2–9 (Li and Zhao, 2007) or 4–9 (Lee et al., 2003) have been suggested. The pH of parenteral solutions can be manipulated via the addition of small quantities of strong acids or bases, such as HCl or NaOH but is more usefully achieved via the use of a buffer, generally with as low a buffer concentration as possible while still maintaining the desired pH. Common buffer systems used in parenteral formulations include phosphate and bicarbonate at neutral or slightly basic pH and citrate, lactate, tartrate, acetate, and maleate for more acidic solutions (Flynn, 1980; Kipp, 2007).

Cosolvents (see Section VI) are also commonly used to promote solubility in parenteral formulations, and ethanol, propylene glycol (PG), low-molecular-weight
Potential hierarchy of formulation approaches for the development of parenteral formulations for poorly water-soluble drugs

Previously published suggestions for maximum allowable quantities provided in parentheses. Further details are provided in individual sections. Note that common examples are provided, and this is not intended to represent an exhaustive list.

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple isotonic solutions</td>
<td>Normal saline</td>
</tr>
<tr>
<td>pH-adjusted solutions</td>
<td>pH 6–9 for weak acids; common buffers include phosphate, bicarbonate</td>
</tr>
<tr>
<td>pH 2–6 for weak bases; common buffers include citrate, acetate, lactate</td>
<td></td>
</tr>
<tr>
<td>Cosolvents</td>
<td>Examples:</td>
</tr>
<tr>
<td></td>
<td>Propylene glycol (30–60%) (50% × 0.5 ml/kg)</td>
</tr>
<tr>
<td></td>
<td>PEG 400 (40–100%) (50% × 1 ml/kg)</td>
</tr>
<tr>
<td></td>
<td>Dimethylacetamide (10–30%)</td>
</tr>
<tr>
<td></td>
<td>N-Methylpyrrolidone (10–20%)</td>
</tr>
<tr>
<td></td>
<td>DMSO (10–20%) (100% × 0.1 ml/kg)</td>
</tr>
<tr>
<td></td>
<td>Ethanol (10%) (20% × 0.5 ml/kg)</td>
</tr>
<tr>
<td></td>
<td>Common cosolvent combinations:</td>
</tr>
<tr>
<td></td>
<td>Propylene glycol:ethanol:water 40:10:50</td>
</tr>
<tr>
<td></td>
<td>PEG 400:ethanol:water 40:10:50</td>
</tr>
<tr>
<td></td>
<td>Propylene glycol:ethanol:dimethylacetamide:water 20:10:10:60</td>
</tr>
<tr>
<td>Cosolvents + pH adjustment</td>
<td>As above plus buffered aqueous component</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Examples:</td>
</tr>
<tr>
<td></td>
<td>Cremophor EL (5–10%) (10% × 0.5 ml/kg, single dose only, potential for anaphylaxis in dogs)</td>
</tr>
<tr>
<td></td>
<td>Tween 80 (5–10%) (2% × 0.25 ml/kg, potential for anaphylaxis in dogs)</td>
</tr>
<tr>
<td></td>
<td>Pluronic F68 (20–50%) (15% × 0.5 ml/kg)</td>
</tr>
<tr>
<td></td>
<td>Common surfactant combinations:</td>
</tr>
<tr>
<td></td>
<td>Hydroxypropyl-β-cyclodextrin (20–40%)</td>
</tr>
<tr>
<td></td>
<td>Sulfobutylether-β-cyclodextrin (Captisol) (20–40%) (20% × 5 ml/kg)</td>
</tr>
<tr>
<td>Complexation agents</td>
<td>As above plus buffered aqueous component</td>
</tr>
<tr>
<td>Surfactants or complexation agents</td>
<td>Lipid emulsions</td>
</tr>
<tr>
<td>+ pH adjustment</td>
<td>Phospholipid stabilized soybean oil emulsion (10–20% lipid) (Intralipid)</td>
</tr>
<tr>
<td>Nanomilled drug crystals</td>
<td>Nanocrystals</td>
</tr>
<tr>
<td>Advanced colloids</td>
<td>Liposomes, polymeric or lipid nanoparticles</td>
</tr>
</tbody>
</table>

a Concentration range based on rat or mouse and single administration at 10 ml/kg (Li and Zhao, 2007).
b Concentration range and maximum dose volume for acute and subchronic doses in rodents (≤7 days) (Neervannan, 2006).
c Descriptions of toxicological outcomes after specific dose regimens in several species for many common excipients also available in Gad et al. (2006).

d Polysorbates and poloxamers (Pluronics, including Pluronic F68; BASF Corporation). Whereas surfactants provide for relatively robust solubilization, a complexity is the growing realization that many surfactants can affect transport processes, such as cellular efflux, and may also result in significant adverse effects, including hypersensitivity reactions (Gelderblom et al., 2001; ten Tije et al., 2003). Cremophor EL, in particular, seems to promote an immune reaction in some species (most notably dogs) when administered parenterally. Surfactants bring additional complexities to data interpretation and, where possible, might usefully be avoided in favor of pH changes, cosolvents, or the use of cyclodextrins.

Complexation agents (see Section VIII), such as cyclodextrins, provide a mechanism for enhancing solubility for many drugs and have been increasingly used as modified cyclodextrins, such as hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutylether-β-cyclodextrin (SBE-β-CD), have become more cost effective. HP-β-CD and SBE-β-CD have higher aqueous solubilities than do unmodified cyclodextrins (therefore allowing the support of higher concentrations of complexed drug) and, importantly, are becoming well characterized in terms of their safety profile. In rare situations where the binding constant between drug and cyclodextrin is particularly high (e.g., ~10^6 M^-1), there is the potential for alteration of the pharmacokinetic distribution and elimination processes (Charman et al., 2006), but most drugs exhibit significantly lower binding constants (<10^4 M^-1) and are efficiently “released” from the
complex via dilution and displacement by endogenous species (Stella and Rajewski, 1997; Stella et al., 1999; Stella and He, 2008). HP-β-CD and SBE-β-CD are commonly used in parenteral formulations in concentration ranges of up to 20 to 40% w/v in both humans and animals, and combining complexation with pH adjustment can, in some cases, provide additional solubility enhancement.

Intravenous emulsion formulations (see Section XI) can be used for lipophilic, poorly water-soluble drugs that have high solubility in lipids. Typically, lipid emulsion formulations contain between 10 and 20% lipid, which limits drug load for compounds that do not have high lipid solubility. Intravenous emulsion formulations have been used for many years as nutritional supplements (e.g., Intralipid; Baxter Healthcare, Deerfield, IL) and are usually formulated using soybean oil as the dispersed phase and phospholipids as the emulsifier. Emulsions can be formulated de novo by dissolving drug into the oil phase of the emulsion before emulsification; however, this requires the use of specialized high-pressure homogenization equipment to provide emulsions with submicron particle sizes. Alternatively, drug may be incorporated into preformed commercial emulsions by dissolving the drug in a small volume of cosolvent with subsequent slow incorporation using sonication (El-Sayed and Repta, 1983).

More complex formulation approaches, such as the use of nanosuspensions (see Section IX), liposomes, or microemulsions, have all been used to facilitate parenteral administration but can substantially affect drug disposition. These approaches are better avoided when the intent of the formulation is to define fundamental pharmacokinetic parameters of a drug. In many cases, combination approaches to solubilization in parenteral formulations are beneficial. The overarching premise for this approach is that the benefits of solubilization are often additive, whereas the effects on toxicity are typically a function of reaching a critical level for a single component. Maintaining concentration of one solubilizer below that threshold, but combining multiple approaches, therefore, may have benefit. In some cases, however, combination approaches add complexity without generating benefit or, indeed, are detrimental. Combination approaches are summarized in Table 3 and are further described in detail in each individual section of this review.

### TABLE 3

<table>
<thead>
<tr>
<th>pH Adjustment</th>
<th>Cosolvents</th>
<th>Surfactants</th>
<th>Cyclodextrins</th>
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</tbody>
</table>

- **pH adjustment**: ++, additive effect on drug solubilization; ++++, no clear effect on drug solubilization; +++- possible decrease in drug solubilization.

B. Oral Administration

In contrast to parenteral formulations, the approaches that are commonly used to support the oral delivery of poorly water-soluble drugs vary significantly depending on the purpose of the in vivo study, the species in which studies are conducted, the quantity of material available, and the level of exposure required. Distinction between the approaches taken at different stages of the discovery-development continuum is difficult, and different organizations will have different approaches depending on in-house experience and expertise. Nonetheless, the formulation strategies that are commonly adopted during discovery and development are summarized as follows.

1. Formulations to Support Drug Discovery. In proof-of-concept pharmacology studies, the focus is generally placed on generating sufficient exposure to allow early indication of the efficacy of a particular pharmacophore or compound series. At this stage, only small quantities of compound are usually available, and control of crystallinity is limited, with early batches often including a significant proportion of amorphous material. Before a salt screening program, ionizable compounds may also be available only as the free acid or base or potentially as a non-optimal salt form. Under these circumstances, simple solution formulations are preferred since they circumvent the complexities of dissolution and, therefore, obviate any variability in exposure resulting from differences in solid-state characteristics. Solution formulations facilitate ease and reproducibility of administration via oral gavage, enable ready conduct of single and multiple dose studies, and support dosing strategies such as cassette dosing. The approaches taken to develop oral solution formulations are similar to those used for parenteral formulations, however, the acceptable limits on formulation components are typically wider. A useful order of potential formulation approaches to achieve simple oral solution formulations is provided in Table 4. In early stage proof-of-concept studies, relatively aggressive absorption enabling formulations may be used to overcome solubility and dissolution rate limitations, depending on the frequency and length of dosing; however, caution should be used to ensure that the formulation components do not confound the efficacy readout, and vehicle controls are essential.

Oral delivery during lead optimization is typically focused on prioritizing lead compounds from within a series with more favorable absorption properties and on identifying the key structural and physicochemical factors that contribute to poor oral bioavailability, in an effort to guide subsequent structural modification. Understanding the basis for poor oral absorption is fundamental to designing a strategy to overcome this limitation. The approaches used for oral formulations of poorly water-soluble compounds in lead optimization
TABLE 4
Possible hierarchy of formulation approaches for the development of oral solution formulations

Previously published suggestions for maximum allowable quantities of formulation excipients provided in parentheses. Exemplar formulations—for example, cosolvent combinations, surfactant formulations, and lipid-based formulations—are provided, realizing that they represent a limited “snapshot” of possible formulations, concentration ranges, etc. (Further details are provided in the sections of this review that describe each technology.)

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple Solutions ± pH</td>
<td>As Table 2</td>
</tr>
<tr>
<td>Cosolvents ± pH</td>
<td>Propylene glycol (30–60%) (80% × 2 ml/kg)&lt;br&gt;PEG 400 (40–100%)&lt;sup&gt;b&lt;/sup&gt; (100% × 2 ml/kg)&lt;sup&gt;b&lt;/sup&gt;&lt;br&gt;DMA (10–30%)&lt;sup&gt;b&lt;/sup&gt;&lt;br&gt;N-Methylpyrrolidone (10–20%)&lt;sup&gt;b&lt;/sup&gt;&lt;br&gt;DMSO (10–20%)&lt;sup&gt;b&lt;/sup&gt;&lt;br&gt;Ethanol (10%)&lt;sup&gt;b&lt;/sup&gt; (50% × 0.5 ml/kg)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Surfactants</td>
<td>As Table 2, but also solid and semisolid surfactants including: n-α-Tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS 1000) (20–50%)&lt;sup&gt;c&lt;/sup&gt;&lt;br&gt;Lauroyl macrogol-32 glycerides (Gelucire 44/14) (20–50%)&lt;sup&gt;c&lt;/sup&gt;&lt;br&gt;Caprylocaproyl macrogol-8-glycerides (Labrasol) [40–60%]&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Complexation agents</td>
<td>As Table 2</td>
</tr>
<tr>
<td>Cosolvent/surfactant combinations</td>
<td>PEG-based cosolvent/surfactant combinations, e.g., 55% PEG: 25% propylene glycol: 25% Cremophor EL</td>
</tr>
<tr>
<td>Simple lipid formulations</td>
<td>Lipid solution formulations, e.g., 100% soybean oil, corn oil&lt;br&gt;Self-emulsifying products, e.g., Labrasol, Labrafil, or Gelucire up to 50% in water</td>
</tr>
<tr>
<td></td>
<td>30% long-chain triglyceride (e.g., soybean oil): 30% long-chain diglycerides/monoglycerides (e.g., Maisine 35-1): 30% Cremophor EL: 10% ethanol, usually dispersed in water, or,&lt;br&gt;30% medium-chain triglyceride (e.g., Captex): 30% medium-chain diglycerides/monoglycerides (e.g., Capmul): 30% Cremophor EL: 10% ethanol, usually dispersed in water</td>
</tr>
</tbody>
</table>

<sup>a</sup> Concentration range based on rat or mouse and single administration at 10 ml/kg (Li and Zhao, 2007).
<sup>b</sup> Concentration range and maximum dose volume for acute and subchronic doses in rodents (≤7 days) (Neervannan, 2006).
<sup>c</sup> Descriptions of toxicological outcomes after specific dose regimens in several species for many common excipients are also available in Gad et al. (2006).
dissolution rate. The availability of larger quantities of material also allows more complex particle size reduction strategies to be explored including micronized formulations and nanosuspensions (see Section IX).

2. Use of Enabling Formulations to Promote Oral Absorption. In situations where attempts to increase polarity and water solubility lead to significant decreases in potency, and where solubility or dissolution has been identified as the rate-limiting process for absorption, a strategic decision may be made to progress a compound series via the use of enabling formulations to improve bioavailability and exposure. This approach may be taken in an effort to obtain rapid proof-of-concept data in humans against a new target or simply to enable early pharmacokinetic and safety data to be obtained for a novel compound series.

The preclinical assessment of more specialized oral formulations will depend heavily on the type of formulation being assessed. Generally, rodents require liquid formulations that can be easily administered via a gavage tube. Recent developments in the availability of minicapsules and tablets allows dosing of tablets or encapsulated materials to rodents; however, the requirement for specialized equipment to prepare these dosage forms and the low liquid volumes in the rodent GI tract (which may limit the rate of capsule/tablet disintegration) make this approach less common.

Alternate crystal forms such as cocrystals, polymorphs, or amorphous drug may be explored, although the inherent instability of metastable crystal and amorphous forms dictates the need for close monitoring of crystallization properties, and formulation approaches to stabilize against solid-state transitions may be necessary, depending on the required stability period. Cocrystals provide some advantage in this respect as their crystal lattice energy is significantly higher than amorphous drug, allowing the generation of formulations with improved stability. Stabilization of polymorphs or amorphous drug may be achieved by the use of (usually polymeric) solid dispersion formulations, where reduced molecular mobility, often achieved via intermolecular interactions between carrier and drug, reduces the risk of changes to the amorphous-crystal structure during storage. Solid dispersions require relatively complex equipment to provide clinical formulations, such as spray drying or hot melt extrusion; however, trial batches can be formulated using simple benchtop equipment such as a rotary evaporator or freeze-driers.

As drug candidates progress through development, preclinical investigations in nonrodent species will be conducted, usually in dogs or nonhuman primates. The physical form of materials used in these studies will vary, and simple solutions or suspensions will often still be used. Larger species generally provide the first opportunity to assess the performance of prototype tablet and capsule formulations that might ultimately progress to the clinic. For ease of preparation on a relatively small scale, hand filling of dry powder blends or granules into hard gelatin capsules or liquid filling into preformed soft gelatin capsules can be used. Sealed hard-gelatin capsules provide an alternative for liquid fills and may be sealed by hand or, more effectively using specialized capsule-sealing equipment, realizing that such equipment may not be readily available at this stage. For poorly water-soluble drugs, the shift to encapsulated and solid dosage forms dictates renewed concentration on solid-state formulation strategies such as salts, cocrystals, and particle size reduction alongside more complex formulation approaches, including solid dispersions and lipid-based formulations.

3. Preclinical Toxicology Formulations. Preclinical toxicology evaluation is a particular challenge for poorly water-soluble drugs. The need to escalate doses to the point where in vivo exposure is sufficient to demonstrate limiting toxicity generally requires absorbed doses (in milligram per kilogram terms) 30 to 100 times greater than might be envisaged with a clinically relevant dose and formulation. Where water solubility is low, this is often impossible with solution or suspension formulations, since beyond a certain point, drug exposure remains constant regardless of increases in dose, dictating the need for more complex formulation approaches; however, this requirement is tempered by potential concerns regarding the toxicology of the large quantities of excipient often required to afford the higher exposure. Relatively little information is publicly available that describes the quantities of different combinations of excipients that do not lead to toxic endpoints over a range of dosing periods. One notable exception is an excellent review by Gad et al. (2006), which provides a summary of the effects of a range of excipients in toxicity testing protocols of different lengths. Neervannan (2006) also provide some data regarding the maximum quantities of common excipients that might be safely used in acute and subchronic dosing protocols for up to 7 days. However, these communications provide data on a limited number of surfactants, lipids, and polymers and do not address the potential toxicity of the combinations of components often used.

Toxicity concerns for many of the excipients used in more complex formulations are attenuated by the realization that many polymers and surfactants have high molecular weights and are relatively polar, or amphiphilic. As such, the extent of absorption of these excipients after oral administration is low. Many surfactants are also digestible (Cuiné et al., 2008). Under these circumstances, the risks of systemic toxicity are low, and the oral LD₅₀ of most hydrophilic polymers and nonionic surfactants are high (Rowe et al., 2009). The major concerns therefore revolve around the possibility of GI side effects, including loose stools and emesis (Reppas et al., 1991; Andersen, 1994; Schulze et al., 2005; Ruble et al., 2006). By incorporation of
vehicle controls, many of these effects can be accounted for during data analysis. Confounding data interpretation issues arise, however, where GI symptoms manifest as a consequence of the candidate drug’s desired biologic activity or toxicity profile.

Cosolvents, in particular ethanol, typically exhibit more significant toxicity profiles and, hence, cosolvent combinations are commonly used to maintain the concentration of one single component at a relatively low level. Finally, several surfactants have also been shown to inhibit efflux transporters (Batrakova et al., 1999; Yamagata et al., 2007a) and GI cytochrome P450 enzymes (Wandel et al., 2003; Bravo González et al., 2004; Christiansen et al., 2011) and may therefore affect drug bioavailability after oral administration. Evidence of systemic effects, however, is limited except after parenteral administration. After parenteral administration, surfactants such as Tween 80 and Cremophor EL may stimulate hypersensitivity reactions (Weiss et al., 1990; Gelderblom et al., 2001; ten Tije et al., 2003), especially in dogs (Lorenz et al., 1982).

4. Development of Clinical Formulations for Poorly Water-Soluble Drugs. The strategies used when developing clinical formulations typically fall into two categories. The first strategy is the development of simple formulations that maximize the speed to first-in-human studies but provide no functional link to the likely formulations used in later stage clinical trials and that might be intended for commercialization. Second is the development of mature formulations, with the expectation of progressing essentially similar formulations through clinical development to commercialization. Where possible, the former approach uses nonformulated drug to simplify dosage form development, and, where solubility permits, simple direct filling of drug in capsules or drug-in-bottle approaches can be used. The relatively recent application of direct capsule-filling instruments such as Xcelodose (Capsugel, Greenwood, SC) that facilitate accurate filling of doses ranging from 100 μg to 100 mg have greatly simplified this process.

For many poorly water-soluble compounds, however, low solubility limits the possibility of adopting an accelerated route to first-in-human studies, and in these instances, more sophisticated formulations are required to ensure exposure. Formulations with the potential to progress through clinical trials, thereby circumventing the need for repeated bioequivalence bridging studies, are typically evaluated. The escalation of formulation complexity in this case (Table 6) commonly follows a pathway similar to that previously described for preclinical formulations, although in this case, the potential to progress through to full scale tableting or encapsulation methods and the use of Good Manufacturing Practice processing equipment becomes more important. Where simple solid-state or particle size approaches to enhanced solubility such as salt formation, cocrystals, or nanocrystals fail to provide sufficient exposure, the major routes of progression are the use of lipid-based formulations or solid dispersions. The choice of approach is dictated largely by the physicochemical properties of the drug candidate, where lipid-based formulations lend themselves to poorly water-soluble, lipophilic drugs (i.e., those with sufficient solubility in lipids, surfactants, and cosolvents to allow the target dose to be dissolved in the capsule fill material), whereas solid dispersions are commonly explored for drug candidates with insufficient lipophilicity to allow the use of lipid-based formulations.

In conclusion, the general strategies that may be taken to identify and overcome the challenges of low water solubility in the drug discovery and development environment are summarized in the proceeding discussion. The tools that have been suggested as approaches to overcome the limitations of poor solubility are described in detail in following sections.

### IV. Buffers and Salt Formation

Adjusting solution pH, usually via the use of a buffer, provides an effective means of increasing the proportion of a weakly acidic or basic drug that is present in the ionized form. In turn, increasing ionization increases polarity and therefore increases drug solubility in polar (aqueous) solutions. Salt formation results in a similar endpoint, and in many ways salts of weak acids and bases are the solid-state equivalent of pH adjustment. Salts are formed via an ionic interaction between weakly acidic or basic drugs and an oppositely charged basic or acidic counterion. When salts are subsequently allowed to dissolve and dissociate in water, the acidic or basic counterion from which they were derived causes a shift in the pH of the solution to provide an analogous endpoint to that obtained by pH adjustment. Isolation of drugs as a particular salt form also changes the nature of the crystal lattice, resulting in differences in dissolution rate from alterations in solid-state properties compared with the free acid or free base (Huang and Tong, 2004; Corrigan, 2007).
Diclofenac provides a good example of the potential advantages of salt formation. In the un-ionized form, diclofenac has a low aqueous solubility (<0.02 mg/ml) (Fini et al., 1999), whereas the sodium or potassium salt forms of diclofenac are >400 times more water soluble (Fini et al., 1996). Similar trends are also evident with weak bases (which make up the larger proportion of ionizable drug candidates); for example, the aqueous solubility of enalapril maleate is 25 mg/ml (Kasim et al., 2004), whereas that of the free base is ~0.21 mg/ml. In unbuffered solutions, salts therefore provide an opportunity for significant increases in dissolution and solubility. In contrast, in buffered solutions that favor the un-ionized free acid or free base, the aqueous solubility advantages of pharmaceutical salts are attenuated, although significant increases in dissolution rate are still evident since the pH in the unstirred water layer reflects the pH of the dissolving salt rather than the bulk (buffered) pH (Serajuddin and Jarowski, 1985b; Li et al., 2005b; Serajuddin, 2007).

Exploiting the enhanced solubility and dissolution rate of the ionized form of a drug, either through pH adjustment or salt formation, is therefore a common approach for oral and parenteral routes of drug delivery and should be regarded as a primary strategy for addressing low aqueous solubility (Berge et al., 1977; Stahl and Wermuth, 2002; Tao et al., 2009). Although this approach is applicable only to ionizable compounds, weak acids and weak bases account for up to two-thirds of marketed drugs and drugs in development (Stahl and Wermuth, 2002); consequently, the strategies described here are relevant across a wide range of drug candidates.

In this section, the fundamental principles of ionization potential and the solubility of weak electrolytes and their respective salts are described. Subsequently, the formulation considerations when using buffered systems and salts for parenteral delivery and oral delivery are addressed before finally providing an overview of common salt-screening strategies.

A. Solution Behavior of Weak Electrolytes and Their Salts

1. Ionic Equilibria. On addition to water, strong electrolytes such as hydrochloric acid and sodium hydroxide dissociate almost completely. In contrast, weak electrolytes, such as the weakly acidic and weakly basic molecules that constitute most ionizable drugs, dissociate only partially at most pH values. For a weak acid or weak base, the total concentration in solution at a given pH is therefore the sum of the concentrations of the ionized and un-ionized species. The degree of dissociation into the ionized and un-ionized form is described by the dissociation constant ($K_a$) for the equilibrium reactions shown in eq. 12 for a monoprotic weak acid (HA) (left equation) and weak base (BH$^+$) (right equation) in water, with the associated expressions for $K_a$ shown in eq. 13.

$$HA + H_2O \rightleftharpoons H_3O^+ + A^- \quad \text{BH}^+ + H_2O \rightleftharpoons H_3O^+ + B \quad \text{eq. 12}$$

$$K_a = \frac{[A^-][H_3O^+]}{[HA]} \quad K_b = \frac{[B][H_3O^+]}{[BH^+]} \quad \text{eq. 13}$$

As the dissociation constant may vary by several orders of magnitude, the $pK_a$ or negative logarithm of $K_a$ ($pK_a = -log K_a$) is more typically used to compare acids and bases (Table 7). Decreasing $pK_a$ denotes stronger acids (i.e., species that more readily donate a proton), whereas increasing $pK_a$ reflects stronger bases (i.e., species that more readily accept a proton).

The Henderson-Hasselbalch equation (eq. 14) further describes the pH dependency of the ionization properties of weak acids (left equation) or bases (right equation) and relates the degree of ionization to the pH of a solution and the $pK_a$ of the drug.

$$\text{pH} = pK_a + \log \left( \frac{[A^-]}{[HA]} \right) \quad \text{pH} = pK_a + \log \left( \frac{[B]}{[BH^+]} \right) \quad \text{eq. 14}$$

From eq. 14, it is apparent that under conditions where the pH of a solution is the same as the $pK_a$ of a dissolved weak acid or base, the concentrations of ionized and un-ionized species are equivalent. A change in pH by one log unit away from the $pK_a$ subsequently results in a 10-fold change in the degree of ionization.

2. pH Solubility Relationships for Weak Electrolytes and Salts of Weak Electrolytes. The intrinsic solubility of a nonelectrolyte is governed by the relative strengths of the intermolecular forces in the solute (i.e., its solid-state properties) and the affinity of the solute for the solvent (i.e., the strength of solute-solvent interactions) (see Section 1.B). This is also the case for a weak electrolyte but is complicated by differences in ionization of the solute with changes in pH that, in turn, alter polarity and the affinity of the solute for the solvent. Thus, a weak electrolyte will initially dissolve to an extent governed by the intrinsic solubility of the

<table>
<thead>
<tr>
<th>Acidic Groups</th>
<th>$pK_a$ Value</th>
<th>Basic Groups</th>
<th>$pK_b$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphonic acids</td>
<td>&lt;1</td>
<td>Aliphatic amines</td>
<td>8–11</td>
</tr>
<tr>
<td>Phosphonates</td>
<td>2</td>
<td>Saturated nitrogen heterocycles</td>
<td>9–11</td>
</tr>
<tr>
<td>Carboxylic acids</td>
<td>2.5–5</td>
<td>Pyridines</td>
<td>4–6</td>
</tr>
<tr>
<td>Imides</td>
<td>8–11</td>
<td>Anilines</td>
<td>3–5</td>
</tr>
<tr>
<td>Thiods</td>
<td>7–10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>8–10</td>
<td>Weak base</td>
<td>4.5–9.5</td>
</tr>
<tr>
<td>Phens</td>
<td>8–10</td>
<td>Very weak base</td>
<td>0.0–4.5</td>
</tr>
<tr>
<td>Weak acid</td>
<td>4.5–9.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very weak acids</td>
<td>9.5–14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
un-ionized form. Depending on the pH, a proportion of the dissolved species subsequently ionizes in accordance with the Henderson-Hasselbalch equation (eq. 14), leading to further dissolution of solid drug to maintain a constant concentration (i.e., the saturated solubility) of the un-ionized form. This will continue within an unbuffered solution until the saturated solubility of both un-ionized and ionized species is reached. As the ionized species is able to form favorable ion-dipole interactions with polar solvents, it is more soluble than the un-ionized species, and therefore, drugs are more soluble in polar solvents at pH values that favor the ionized form. The total solubility (S) of a weak acid (HA) or weak base (B) at a given pH is therefore the sum of the solubility of the ionized and un-ionized forms (Yalkowsky, 1999).

\[
S = [\text{A}^-] + [\text{HA}] \quad S = [\text{B}] + [\text{BH}^+] \quad (15)
\]

Combinations of eq. 14 and eq. 15 generate the following solubility equations for weak acids (left, eq. 16) and weak bases (right, eq. 16) where total drug solubility at a particular pH is a function of the solubility of the un-ionized form (S₀) and the pKₐ of the weak acid or base.

\[
S = S₀ \left[1 + 10^{(p\text{H} - pKₐ)}\right] \quad S = S₀ \left[1 + 10^{(pKₐ - p\text{H})}\right] \quad (16)
\]

From eq. 16, it is evident that for a monoprotic weak acid or base, changes in pH of 1 unit away from the pKₐ lead to 10-fold (1 log unit) differences in solubility. For diprotic species, similar relationships may be developed (eq. 17) where total solubility is dependent on the pKₐ of two ionization centers (Yalkowsky, 1999). Accordingly, diprotic species are expected to show a 100-fold (2-log unit) change in solubility with a change in pH of one unit (Garren and Pyter, 1990; Venkatesh et al., 1996). The top equation is for a weak diprotic acid, and the bottom equation is for a weak diprotic base:

\[
S = S₀ \left[1 + 10^{(p\text{H} - pK_{a1})} + 10^{(2p\text{H} - pK_{a1} - pK_{a2})}\right]
S = S₀ \left[1 + 10^{(pK_{a1} - p\text{H})} + 10^{(pK_{a1} - pK_{a2} - 2p\text{H})}\right] \quad (17)
\]

The solubility equations for weak electrolytes shown in the preceding should be viewed with two significant caveats. First, eq. 16 and eq. 17 suggest that drug solubility changes indefinitely with changing pH. Ultimately, however, the achievable solubility is regulated in most cases by the presence of counterions that may be intentionally present (in the case of a salt) or unintentionally present (in the case of ions present in blood, GI fluids, buffers, and so forth). In this case, the total solubility is regulated by the limit of solubility of the ionized weak acid or base in the presence of the corresponding basic or acidic counterion, ultimately resulting in precipitation of the solid salt of the acid-base pairs. The solubility of the salt is therefore the limiting condition to the aqueous solubility of drug in the ionized form when a counterion is present. Where multiple counterions are present, the limiting salt solubility typically reflects the salt with the lowest solubility, or the salt formed with the counterion in highest concentration if common ion effects are significant. Salt solubility, including aspects of common ion effects, is described in more detail in Section IV.4. Second, classic ionization texts usually ignore the possibility of ion-pair formation or self-association. Where ion-pair formation or self-association leads to the formation of supramolecular complexes in solution (including multimers, micelles, and liquid crystalline species), apparent solubility values may be much greater than anticipated (Serajuddin et al., 1987; King et al., 1989; Kriwet and Muller-Goyman, 1993; Fini et al., 1995).

Exemplar pH solubility profiles for a weak acid and a weak base, and the salts formed by association with a corresponding basic or acidic counterion, are presented in Fig. 7. The total drug solubility (S in eq. 16) of a weak base is low at high pH where the un-ionized form (B) predominates (region 1 of the pH solubility profile for a weak base). Where the solubility of the ionized drug species (BH⁺) is significantly in excess of that of the un-ionized form, B (which is typical), decreasing pH results in an increase in the proportion of BH⁺ and an increase in total solubility. The increase in solubility becomes particularly significant from pH values of approximately 1 pH unit above the pKₐ (i.e., where the degree of dissociation increases beyond ~10%) to pH values below the pKₐ, where, as described previously, solubility increases 10-fold for every unit pH decrease (for a weak base). At a critical point (and critical pH, denoted pHmax), the equilibrium solubility of the ionized species (as the salt) is reached, and no further increases in solubility are seen with decreasing pH. This limiting solubility is set by the solubility product of the salt (Ksp):

\[
(\text{BH}^+ \text{X}^-)_{\text{solid}} K_{\text{sp}} = [\text{BH}^+] + [\text{X}^-] \quad (18)
\]

When the concentration of solid phase (i.e., salt) is in excess and, therefore, approximately constant:

\[
K_{\text{sp}} = [\text{BH}^+] [\text{X}^-] \quad (19)
\]

In region 2 of Fig. 7 (i.e., at the solubility limit), excess solid phase is present as the salt, and the saturated species in solution is largely the ionized form. At pH values <pHmax, the total solubility of a weak base is given by [BH⁺] plus a small (and decreasing) quantity of B (free base) (Kramer and Flynn, 1972; Serajuddin, 2007). The total solubility in region 2 of Fig. 7 is given by eq. 20. for weak acids (top equation) and weak bases (bottom equation).
Scheme 1 summarizes the solid-solution equilibria for a weak base (B), where the counterion is provided by a strong acid (HX) to form a salt (BH\(^+\)X\(^-\)\)solid. This scheme brings together the relationships between dissociation (described by \(K_a\) and the dominant factor in region 1 of Fig. 7) and the equilibrium solubility of the salt (described by \(K_{sp}\) and the dominant factor in region 2 of Fig. 7). In some cases, additional equilibria, including the potential for hydrate formation or self-association, may be present but have been omitted here for the sake of clarity.

\[
S = [A^-] \left[ 1 + \frac{[H_3O^+]}{K_a} \right] = [A^-] \left[ 1 + 10^{(pK_a - \text{pH})} \right] \\
S = [BH^+] \left[ 1 + \frac{[K_a]}{H_3O^+} \right] = [BH^+] \left[ 1 + 10^{(\text{pH} - pK_a)} \right]
\]  

(20)

Scheme 1. Phase solubility scheme for salt formation between a weak base (B) with a strong acid (X). Adapted from Pudipeddi et al. (2002).

In a solution of a monovalent salt, dissociation leads to equal concentrations of [BH\(^+\)] and [X\(^-\)]. From eq. 19, the maximum solubility of [BH\(^+\)] (i.e., the limiting value for salt solubility) is equal to the square root of the solubility product (\(\sqrt{K_{sp}}\)). \(K_{sp}\) is a constant for a particular salt and defines the maximum concentration of drug ions and corresponding counterions that a solvent can maintain in solution (Bhattachar et al., 2006). Essentially, the higher the \(K_{sp}\) value, the greater the solubility of the salt. \(K_{sp}\) is therefore a critical parameter in understanding the solubility of salts or the solubility of ionized drugs where salt formation in situ is likely. Unfortunately, techniques that accurately predict \(K_{sp}\) do not exist, largely as a result of the unpredictability of the nature of the crystal lattice. Screening programs that are able to rationally and rapidly assess the limit of solubility of different salt forms are therefore critical to support effective development programs, and are described in more detail in Section IV.E.2.

3. Factors Affecting Salt Formation at pH\(_{max}\).

The pH\(_{max}\) defines the limiting pH above which (for a weak acid) and below which (for a weak base) the solid phase constitutes the salt rather than the free acid or free base. To facilitate salt formation, the bases or acids that are used to provide the counterion must be sufficiently strong to shift solution pH past pH\(_{max}\) (into region 2 of Fig. 7). In general, salts of weak bases will form more readily with more strongly acidic counterions such as hydrochloride compared with more weakly acidic counterions such as citrate. As a general rule, salt formation will occur only between a weak acid or weak base and counterion if the difference in p\(K_a\) for the two is greater than 2 (Pudipeddi et al., 2002; Stahl and Wermuth, 2002; Black et al., 2007). It is also evident that conditions that cause a shift in pH\(_{max}\) will change the ease of salt formulation. Thus, conditions that result in an increase in pH\(_{max}\) for a weak base and a decrease in pH\(_{max}\) for a weak acid will assist in salt formation by reducing the pH shift that must be generated by the counterion to move into region 2 of
Fig. 7. For example, decreasing the pH_max for a weak acid will allow salt formation with a more weakly basic counterion, thereby increasing the possible choices of counterion.

At the pH_max value, the salt and the free acid or free base are present in the solid state, and this situation is unique to this transition point in the pH-solubility profile. Under these circumstances, eq. 16 and eq. 20 are both applicable. Setting eq. 16 equal to eq. 20 and solving for pH (i.e., pH_max) results in eq. 21 and a relationship that provides an indication of the dependence of pH_max on pK_a, the intrinsic solubility of the un-ionized free acid or free base (S_0) and K_sp (Bogardus and Blackwood, 1979; Serajuddin, 2007). Note that the equation on the left applies for weak acids while the equation on the right applies for weak bases:

\[
\text{pH}_\text{max} = \text{pK}_a + \log \frac{K_{\text{sp}}}{S_0}, \quad \text{pH}_\text{max} = \text{pK}_a + \log \frac{S_0}{\sqrt{K_{\text{sp}}}} \tag{21}
\]

From eq. 21, it is clear that the value for pH_max is dependent on the properties of the drug namely, intrinsic solubility (S_0), relative acidity/basicity (pK_a) and the solubility of the salt (K_sp). The effect of changing these three terms on pH_max is illustrated for a typical weak base in Fig. 8. Increasing the pK_a (i.e., increasing basicity) (Fig. 8A), increasing S_0 (i.e., increasing intrinsic solubility) (Fig. 8B), and decreasing K_sp (i.e., forming salts with lower solubilities), all assist salt formation by increasing pH_max (realizing that lower solubility salts may not be preferred in many cases).

4. Determinants of Salt Solubility. The maximum solubility of a salt is the boundary condition for in vivo solubility and dissolution and, therefore, the parameter against which alternate salts are screened (at least for their impact on solubility). The accurate determination of salt solubility, however, is not a trivial task due to the potential for interconversion between the salt form and the free base or free acid (see Section IV. D.1.c), and the impact of a number of system variables. These are described below.

a. pH_max. To ensure that solubility data reflect the equilibrium solubility of the salt, measurements must be taken at pH values that ensure that the solid phase in equilibrium with solution is the salt and not the free acid or base. As such, solubility measurement should be taken at pH values above (for weak acids) or below (weak bases) the corresponding pH_max. If this condition is not met, the solution is not saturated with respect to drug in the ionized form, leading to an underestimation of the saturated salt solubility. It is advisable during solubility measurements to determine both the pH of the solution and to characterize the nature of the solid phase (e.g., using XRPD). The presence of salt alone in the analyzed solid provides evidence of location within region 2 of the pH-solubility phase diagram (Fig. 7) and, therefore, that the data obtained reflect the true solubility of the salt. Conversely, the presence of free acid or free base suggests that insufficient salt has been added to reach the pH_max and that the system has equilibrated in region 1 (Alsenz and Kansy, 2007; Hasa et al., 2011). Under these circumstances, the addition of larger quantities of salt is required to provide further counterion, which in turn results in a pH change (Pudipeddi et al., 2002). Typically, a relatively high excess of salt is required to ensure that the pH approaches pH_max. Determination of the solubility of a salt in a buffered solution will reveal only the solubility of the un-ionized plus ionized form of the drug at that particular pH, and where the pH is above pH_max (for a weak base) or below pH_max (for a weak acid), the solubility of the drug is dependent only on pH and the solubility of the ionized form (eq. 16) and is independent of the counterion. This provides no indication of K_sp, and therefore, no indication of the solubility limit for the salt. The reader is directed to a recent article by Murdande et al. (2011a) for a more detailed description of the factors to be considered when measuring the equilibrium solubility of crystalline solids (and applicable here to salts).

b. Choice of Counterion to Maximize Salt Solubility. Table 8 provides a list of common counterions that have been used to isolate salts of weakly acidic and weakly basic drugs for the development of oral and parenteral drug products. An indication of the frequency of use is also provided (Paulekuhn et al., 2007). As K_sp is counterion specific, the solubilities of salts

![Fig. 8](image-url). The effect of (A) pK_a, (B) intrinsic drug solubility (S_0) and (c) salt solubility (K_sp) on the position of the pH_max of a weak base. Salt formation of a weak base becomes more attainable (an increase in pH_max) with increasing pK_a of the base, an increase in intrinsic solubility, or a decrease in salt solubility. Adapted from Pudipeddi et al. (2002).
formed with a variety of different counterions can vary significantly. For example, solubility differences of up to 100-fold have been described for a series of amine salts of diclofenac (O'Connor and Corrigan, 2001), and differences in solubility of up to four orders of magnitude have been described for salts of ibuprofen (David et al., 2012). Similarly, for the weak base enalapril, salts formed with a range of aliphatic or aromatic carboxylic acids exhibit solubilities ranging from 22 mg/ml up to 350 mg/ml (Kumar et al., 2008a).

A better understanding of the factors that affect salt solubility (and therefore counterion selection) may be gained by examination of the determinants of the molar free energy of solution ($\Delta G_{\text{soln}}$) (Chowhan, 1978):

$$\Delta G_{\text{soln}} = \Delta G_{\text{solvation}} - \Delta G_{\text{lattice}}$$  \hspace{1cm} \text{(22)}

$$\Delta G_{\text{solvation}} = \Delta G_{\text{cation}} - \Delta G_{\text{anion}}$$  \hspace{1cm} \text{(23)}

Solubility is a function of the free energy gain achieved by solvation of the anion and cation (eq. 23) offset by the free energy of the crystal lattice (eq. 22). Salt formation has the capacity to alter both the free energy of the crystal lattice ($\Delta G_{\text{lattice}}$) (by changing the nature of packing and the strength of intermolecular interactions in the solid state) and the degree of solvation ($\Delta G_{\text{solvation}}$). Solvation is typically enhanced by salt formation since dissociation into ions facilitates the formation of ion-dipole interactions with water molecules that are more energetically favorable than, for example, hydrogen bond interactions between water and un-ionized drug. In contrast, the crystal lattice energy is typically increased by salt formation via the generation of stronger intramolecular ionic interactions, and this is usually evident in an increase in melting point (Corrigan, 2007; David et al., 2012). From eq. 22, the increase in ($\Delta G_{\text{lattice}}$) is expected to be detrimental to solubility. However, the higher solvation energies resulting from ionization typically offset the energy cost of breaking ionic interactions in the crystal lattice, leading to a net increase in solubility for salts compared with the free acid or base.

The importance of solvation of both anion and cation to the overall solvation energy dictates that hydrophilic counterions, by virtue of higher solvation energies, might be expected to form more soluble salts than hydrophobic counterions (Menegon et al., 2011; David et al., 2012), and indeed this is often the case. However, the choice of counterion affects both solvation and lattice energy, and as such, definitive structure-
solubility relationships for differing counterions do not exist (Stahl, 2003; Corrigan, 2007; Serajuddin, 2007). Thus, monovalent and divalent alkali metals (K\(^+\), Na\(^+\), Mg\(^{2+}\), Ca\(^{2+}\)) are widely used counterions for salt formation for weakly acidic drugs (Table 8), and increasing valency and decreasing counterion ionic radius might be expected to increase hydrophilicity and facilitate solvation. However, salts of monovalent and larger cations (of equivalent valency) are typically the most soluble (Chowhan, 1978; Anderson and Conradi, 1985; Forbes et al., 1995). For example, Fig. 9 depicts the solubility of metal salts of 7-methylsulfinyl-2-xanthonecarboxylic as a function of pH (Chowhan, 1978) and illustrates that maximum salt solubility is evident for larger monovalent counterions [i.e., K\(^+\) (most soluble) > Na\(^+\), > Ca\(^{2+}\) > Mg\(^{2+}\)]. These trends suggest that counterion effects on lattice energy (where larger, less highly charged species reduce intermolecular attractive forces and typically result in lower crystal lattice energies) are more critical determinants of the solubility of a salt than corresponding effects on solvation (where higher-charge densities are expected to increase solvation). Hydrophilic counterions may therefore show higher solvation energies but result in lower solubility (compared with a less hydrophilic counterion) by virtue of higher lattice energies. For example, the lactate and mesylate salts of the experimental antimalarial drug [\(-\)-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol] are more soluble than salts formed with the freely soluble hydrochloride counterion (Agharkar et al., 1976), and saccharin salts of lamotrigine are more soluble than those formed with the more hydrophilic succinic acid and fumaric acid counterions (Galcera and Molins, 2009). Kumar et al. (2008a) have also shown that malate salts of enalapril are more than 10 times more soluble than maleate, besylate, and tosylate salts, despite each of the counterions showing very high aqueous solubilities. Finally, the solubilities of 22 salts formed between various \(p\)-substituted benzoic acid derivatives and benzylamine have been shown to vary significantly, despite broadly similar energies of solvation (Parshad et al., 2002). Across different salt series, lattice energy effects on solubility therefore appear to dominate over solvation effects in many cases.

Consistent with the potential importance of differences in lattice energy on salt solubility, several authors have described correlations between the melting point of pharmaceutical salts (as a measure of lattice energy) and saturated aqueous solubility (Anderson and Conradi, 1985; Gould, 1986; Thomas and Rubino, 1996; Ledwidge and Corrigan, 1998; O’Connor and Corrigan, 2001; Guerrieri et al., 2010). Figure 10, for example, shows the relationship between melting point and solubility for a range of flurbiprofen salts formed with different organic and inorganic amine cations and provides good evidence of a relationship between decreasing melting point and increasing solubility (Anderson and Conradi, 1985). The properties of the counterion that affect packing within the salt lattice, and therefore the lattice energy, include symmetry and size, the ability to form hydrogen-bonds with the drug (in addition to ionic interactions), and the capacity to delocalize charge (Chowhan, 1978; Gould, 1986; Parshad et al., 2004). For example, salts of the weakly basic drug candidate (UK-47880) formed with planar and aromatic acids were found to be highly crystalline and to have higher melting temperatures compared with an oily, low melting salt formed with two aliphatic and more flexible carboxylic acids (citric acid and dodecyl benzene sulfonic acid) (Gould, 1986). Malate and maleate salts of ephedrine also show higher melting...
temperatures compared with fumarate and tartrate salts (Black et al., 2007). This has been suggested to reflect the small size of the maleate counterion and the symmetrical nature of the maleate counterion, both factors that favor hydrogen bonding within the salt lattice such that the lattice energy is increased (Gould, 1986). Similarly, diclofenac salts formed with the freely soluble tris(hydroxymethyl)aminomethane (TRIS) counterion were found to be less soluble than expected, a situation later ascribed to the ability of TRIS to hydrogen bond within the salt lattice (Fini et al., 1996; O’Connor and Corrigan, 2001). In this example, the TRIS salt had the highest melting temperature and lowest solubility of a range of diclofenac salts formed with organic amine counterions and therefore provides another example of the potential for more polar counterions to result in lower salt solubilities via stronger packing within the crystal lattice (Corrigan, 2007). In contrast, increasing the alkyl chain length of amine counterions led to a decrease in the solubility of salts of several weak acids (ibuprofen, gemfibrozil, etodolac), although increasing the alkyl chain length on the counterion lowered the salt melting temperature (David et al., 2012). The overall effect of counterion polarity on solubility therefore reflects the net effect of changes to both lattice energy (where more polar counterions typically increase lattice energy, decreasing solubility) and hydration (where increasing counterion polarity typically increases hydration and increases solubility).

Interestingly, mesylate (methanesulphonate) salts appear to be finding increasing favor for the delivery of weak bases both orally and parenterally (Paulekuhn et al., 2007; Elder et al., 2010; Branham et al., 2012). The wider use of the mesylate counterion in salt selection strategies for solubility enhancement may be in part attributed to its high-charge density but also to its retention of a degree of hydrophobicity, which appears to affect molecular packing in the crystal lattice. As a result, salts formed with the mesylate counterion are likely to show high solvation energies compared with salts formed with other organic counterions (because of the high-charge density) but lower lattice energies compared with salts formed with inorganic counterions (which also show a high charge density). For example, mesylate salts of α-(2-piperidyl)-3,6-bis (trifluoromethyl)-9-phenanthrenemethanol (Agharkar et al., 1976), RPR200765 (Bastin et al., 2000), and haloperidol (Li et al., 2005b) all showed lower melting temperatures compared with equivalent hydrochloride salt forms. The mesylate ion also has a very low pKₐ (the lowest of all common organic acidic counterions) (Table 8), in turn facilitating salt formation with weak and very weak bases (Stahl, 2003). Mesylate salts are also less susceptible to the common ion effect in vivo, and this is described in more detail in Section IV.D.1.d.

Finally, the choice of counterion requires appreciation of the impact of the counterion on a range of pharmaceutical factors, including solubility, but also on other properties, such as flow, compression, and stability. Currently, the ability to predict differences in the solid-state properties of salts isolated with differing counterions is limited by a lack of understanding of the factors that control packing arrangements during crystallization and the potential for ionized drug molecules to self-associate in solution. This in turn limits accurate de novo prediction of counterion effects on solubility. Consequently, it is more typical to select the most appropriate salt via a salt-screening process. Salt screens are described in more detail in Section IV.E.2.

c. The Effect of Common Ions on Salt Solubility.
The solubility of a salt is highly sensitive to the presence of common ions. This effect can be rationalized according to the theoretical descriptions of salt solubility as described in Section IV.A.3, in particular eq. 19 and Scheme 1, where it is apparent that excess counterion will result in suppression of salt solubility to maintain \( K_{sp} \) (Berge et al., 1977; Streng et al., 1984; Serajuddin, 2007). The presence of common ions has frequently been shown to reduce salt solubility in vitro (Miyazaki et al., 1980; Streng et al., 1984; Fini et al., 1995; Wang et al., 2002; Bergstrom et al., 2004; Li et al., 2005a; Larsen et al., 2007), and in some cases common ions can suppress the solubility of a salt below that of the free base/acid (Li et al., 2005a). The common ion effect also has potential implications for parenteral formulations where ions present in various additives (e.g., buffers, salts) are common to those in the salt. Common ion effects are more critical for drugs of low solubility where small changes to common ion concentrations can lead to relatively large compensatory changes to drug concentrations (Serajuddin, 2007).

Effects in vivo are also evident where salts come into contact with high concentrations of common ions. The most familiar example is that of changes to the solubility and dissolution rate of hydrochloride salts in the regions of the GI tract that contain endogenous chloride ions, such as the stomach (from acid secretion) and the intestine (due to the presence of sodium chloride) (Miyazaki et al., 1981; McConnell et al., 2008), but this effect is also evident for sodium salts of weak acids where concentrations of endogenous sodium ion are high. The use of counterions other than hydrochloride or sodium (i.e., counterions not found in high concentration in vivo) might be expected to reduce the possibility of common ion effects. However, interconversion of salt forms during dissolution is possible. For example, Li et al. (2005a) have described a situation where mesylate and phosphate salts of haloperidol that were exposed to high concentrations of free chloride subsequently converted to the hydrochloride salt, which showed a slower rate of dissolution resulting from the presence of the common ions.

In vivo salt conversion (most commonly to the hydrochloride or sodium salt) therefore runs the risk
of a decrease in solubility due to a change in salt form, and may also increase susceptibility to common ion effects. These possibilities dictate that the $K_{sp}$ for chloride and sodium salts is typically measured, regardless of the salt form that is ultimately developed, to provide an indication of the potential ramifications of in vivo conversion.

Interestingly, although salts formed with hydrochloride and sodium ions are perhaps the most susceptible to common ion effects, they are also by far the most common counterions for weak bases and acids, respectively (see Table 8). This situation may be attributed to the high acidity/basicity of these counterions and, therefore, the potential to form salts with even weakly ionizable drug compounds (Bastin et al., 2000; Pudipeddi et al., 2002) and the realization that hydrochloride and sodium salts are often the default option during drug crystallization and purification. In the absence of obvious problems with factors such as solubility, hygroscopicity, stability, and crystallinity, these salt forms are often progressed, and as such, their high prevalence does not necessarily provide any indication of advantage with respect to solubility.

d. Effect of Organic Solvents on Salt Solubility. As a culmination of the synthetic steps required to obtain a new chemical entity, organic solvents or cosolvent-water mixtures are commonly used to facilitate isolation of a crystalline solid. Isolation as a crystalline solid assists in purification, and the choice of the initial salt form often reflects ease of purification rather than intrinsic downstream pharmaceutical or biopharmaceutical properties. Nonetheless, almost all salt forms are commonly isolated from organic solvents, mixtures of organic solvents or cosolvent/water mixtures (Serajuddin and Pudipeddi, 2002; Kumar et al., 2007). The organic solvents (water miscible and immiscible) that may be used for developing drug products are restricted by safety and toxicology issues; a comprehensive review of the use of nonaqueous solvents for oral drug products was recently published (Pole, 2008).

In addition to changing bulk solubility properties (and promoting crystallization), the presence of cosolvent can significantly affect the process of salt formation. For example, in less polar environments (e.g., in the presence of a cosolvent), the ionized form of the drug and counterion is less favored. As such, weak acids and bases become weaker, increasing $pK_a$ in the case of weakly acidic drugs and acidic counterions or decreasing $pK_a$ in the case of weakly basic drugs and basic counterions. Under these conditions, the use of solvents or cosolvents disfavors salt formation by reducing the ability of the acidic or basic counterion to shift the pH to pH$_{max}$ (Tarsa et al., 2010). In contrast, where drug and counterion $pK_a$ remain largely unchanged, solvents and cosolvents promote salt formation via increases in the solubility of the un-ionized form (Fig. 8C) (Kramer and Flynn, 1972). Ideal solvents and cosolvents for salt formation have good solvent properties for un-ionized drug, but they have limited effects on acidity/basicity (Rubino and Thomas, 1990). Despite the frequency of use of cosolvents during purification and isolation of salt forms, Serajuddin and Pudipeddi (2002) caution that cosolvent use may result in the isolation of salts that would not ordinarily form in water. Such salts therefore are at high risk of conversion to the free acid/base on contact with aqueous fluids and moisture (Serajuddin and Pudipeddi, 2002).

B. pH adjustment Strategies for Addressing Low Drug Solubility

Solution formulations can be readily adjusted to a target pH such that a drug candidate is ionized to a degree that affords solvation of the target drug dose. This approach applies equally to solutions of salts or the corresponding free acid or free base since the solubility is the same at an equivalent pH and counterion concentration (assuming the solubility limit of the salt has not been reached).


The most commonly used pharmaceutical buffers comprise weak acids (HA) and the conjugate base ($A^-$). Since physiological pH covers mainly neutral and acidic pH, buffers of weak bases (B) and their conjugate acids (BH$^+$) are less frequently used. To exemplify, the acid-conjugate base pair of acetate buffer is illustrated in Scheme 2.

In this system, an increase in hydrogen ion concentration is buffered by the conjugate base (CH$_3$COO$^-$), and the addition of hydroxide ion is similarly neutralized by the acid (CH$_3$COOH). Buffer capacity is widely used to measure the ability of a buffer to resist a change in pH and is a measure of the quantity of acid/base required to change the pH of a buffered solution by 1 pH unit. Increasing buffer concentrations leads to increases in buffer capacity, and at a constant buffer concentration, maximum buffering capacity is achieved when the solution pH is close to the $pK_a$ of the buffer.

Details of a wide selection of buffers have been previously published (Flynn, 1980; Merck-Index, 2006). Buffer systems most commonly applied to parenteral pharmaceutical formulations and their effective buffer pH range are listed in Table 9 (Lee et al., 2003; Li and Zhao, 2007; Nema and Brendel, 2011). The most effective buffer range is usually limited to one pH unit either side of the $pK_a$, although buffer combinations may be used to extend the buffer range.

$$\text{CH}_3\text{COOH} + \text{H}_2\text{O} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{H}_3\text{O}^+$$

\[ K_a = 1.8 \times 10^{-5} \quad (pK_a = 4.76) \]

Scheme 2. Dissociation of a commonly used (acetate) buffer.
During parenteral administration, pain on administration is likely when using solutions buffered to high or low pH. Formulations are therefore typically limited to a working pH range of 4–9, although a wider pH range (2–9) may be used acutely in a preclinical setting (Li and Zhao, 2007). Extremes of pH may also lead to chemical instability, and in these cases, drug stability should be monitored in the formulation for the intended period of use (Pasini and Indelicato, 1992; Islam and Narurkar, 1993; Carstensen, 1995; Zhu et al., 2002; Chen et al., 2006). Buffer concentrations normally exceed 0.05 M and are generally less than 0.5 M (Wang and Kowal, 1980; Sinko, 2006). Increasing buffer capacity does not increase drug solubility but can prove more effective in maintaining drug in solution on dilution or with the addition of a formulation additive. High buffer concentrations, however, increase the risk of pain on injection and in high volume may alter physiological levels of biologically important buffer components such as lactate (Nema and Brendel, 2011). The buffer will also contribute to the overall ionic strength of the formulation, and high buffer concentrations can promote salting out of drug from solution (McDevit and Long, 1952; Raghavan and Brendel, 2011).

A pH adjustment is a simple and powerful mechanism for solubility adjustment and, as such, is typically the first approach for solution formulations of ionizable drugs. For example, naproxen is a weak acid with a $pK_a$ of 4.57 and intrinsic solubility (of the free acid) of 16 $\mu$g/ml (Fini et al., 1995). As a comparatively “strong” weak acid, the low solubility of naproxen can be increased dramatically in a pH range acceptable for parenteral administration. Indeed, solution concentrations of $\sim$45 mg/ml might be expected in pH 8 phosphate buffer and $\sim$450 mg/ml in pH 9 sodium bicarbonate buffer. Under these circumstances, pH adjustment could be used to deliver a wide range of doses (Lee et al., 2003). In some cases, and in particular for weak(er) acids of higher $pK_a$ and weak(er) bases with lower $pK_a$ values, pH adjustment alone may be insufficient to allow the administration of an appropriate dose. For example, attempts to formulate a drug candidate that was a weaker acid than naproxen (e.g., a compound with $pK_a = 7$), but of equivalent intrinsic solubility, would provide a maximum concentration of only $\sim$1.6 mg/ml in bicarbonate buffer (pH 9). In this case, pH adjustment alone is unlikely to offer a viable solubilization strategy, and a combination of strategies would need to be explored, such as the addition of a cosolvent, surfactant, or cyclodextrin. The advantages (and complications) associated with these combination approaches are discussed in the following section, and the benefits of cosolvents, surfactants; cyclodextrins as individual solubility enhancers are described in more detail in Sections VI, VII, and VIII, respectively.

2. Impact of Cosolubilizers and Electrolytes on pH-Mediated Solubilization.

a. pH Adjustment and Cosolvents. Cosolvents—including propylene glycol, ethanol, PEG, and DMSO—increase the solubility of nonpolar molecules in polar solvents (such as water) by reducing solvent polarity (see Sections VI and V.L.D.1). The presence of a cosolvent may therefore provide additional solubilization for solution formulations where pH manipulation is insufficient. However, cosolvents also alter the strength of the buffers that are included in a pH-cosolvent combination formulation, potentially altering their application (Narazaki et al., 2007b). For example, acidic buffers are less acidic in the presence of cosolvents since the less polar conditions favor the un-ionized form of the buffering species, resulting in an increase in the apparent $pK_a$ (Rubino, 1987; Yalkowsky, 1999). Similarly, decreasing concentrations of the ionized species in basic buffers leads to a decrease in $pK_a$ and a reduction in basicity. As acids donate protons, they increase the number of free ions in solution; therefore, the $pK_a$ of acidic species is more sensitive to changes in solvent polarity compared with bases, which have no effect on the concentration of ions in the system (Rubino, 1987; Yalkowsky, 1999; Narazaki et al., 2007b). The effect of cosolvents on the $pK_a$ of both acids and bases is therefore predictably more enhanced with decreasing polarity of the cosolvent (i.e., reduced dielectric

### TABLE 9
Buffers widely used in parenteral formulations, including details of their buffering range and concentration used in some example marketed formulations

<table>
<thead>
<tr>
<th>Buffering Agent</th>
<th>$pK_a(s)$</th>
<th>Most Effective pH Range</th>
<th>Example Formulations, Buffer Concentration (M); Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maleic acid</td>
<td>1.9, 6.2</td>
<td>2–3</td>
<td>Librium; 0.14 M i.v.</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>2.9, 4.2</td>
<td>2.5–4</td>
<td>Priscoline; 0.04 M i.v.</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>3.8</td>
<td>3–4.5</td>
<td>Ciproxin; $-0.012$ M i.v. infusion</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3.1, 4.8, 6.4</td>
<td>3–7</td>
<td>Amikin; $-0.13$ M i.v. or i.m.</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>4.75</td>
<td>4–6</td>
<td>Novantrone; $-0.08$ M i.v. infusion</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>6.3, 10.3</td>
<td>4–9</td>
<td>Yutopor; $-0.07$ M i.v. infusion</td>
</tr>
<tr>
<td>Sodium phosphate</td>
<td>2.5, 7.2, 12.4</td>
<td>6–8</td>
<td>Ivanz; $0.03–0.3$ M i.v. or i.m.</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3.1, 4.8, 6.4</td>
<td>3–7</td>
<td>Coumadin; $-0.04$ M i.v.</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>4.5</td>
<td>4.5</td>
<td>Zantac; $-0.02$ M i.v. or i.m.</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>6.3, 10.3</td>
<td>4–9</td>
<td>Norcuron; $-0.012$ M i.v.</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3.1, 4.8, 6.4</td>
<td>3–7</td>
<td>Librium; 0.14 M i.v.</td>
</tr>
<tr>
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<td>4.5</td>
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<td>Ciproxin; $-0.012$ M i.v. infusion</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>4.5</td>
<td>4.5</td>
<td>Amikin; $-0.13$ M i.v. or i.m.</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3.1, 4.8, 6.4</td>
<td>3–7</td>
<td>Novantrone; $-0.08$ M i.v. infusion</td>
</tr>
<tr>
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<td>Yutopor; $-0.07$ M i.v. infusion</td>
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</tr>
<tr>
<td>Sodium phosphate</td>
<td>2.5, 7.2, 12.4</td>
<td>6–8</td>
<td>Coumadin; $-0.04$ M i.v.</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3.1, 4.8, 6.4</td>
<td>3–7</td>
<td>Zantac; $-0.02$ M i.v. or i.m.</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>4.5</td>
<td>4.5</td>
<td>Norcuron; $-0.012$ M i.v.</td>
</tr>
</tbody>
</table>

$^a$ Selected LD$_{50}$ values (i.v. administration): Acetic acid: 525 mg/kg (mouse), sodium acetate: 1195 mg/kg (mouse), citric acid: 330 mg/kg (rabbit), sodium (mono) citrate: 379 mg/kg (rabbit), trisodium citrate: 143 mg/g (rat).

$^b$ Summarized from Li and Zhao (2007), Lee et al. (2003) and Nema and Brendel (2011).
capacity). The potential outcome of this effect is that the addition of a cosolvent to a buffer system may alter its capacity to maintain pH by causing a shift in $pK_a$ of the buffering species. The effect is further complicated by the ability of cosolvents to shift the $pK_a$ of weakly acidic and weakly basic drugs such that there is a reduced fraction of ionized drug at a given pH (Kramer and Flynn, 1972; Narazaki et al., 2007b).

Importantly, however, the net effect of using a cosolvent with pH manipulation is usually an increase in total drug solubility. This reflects the fact that changes in dissociation are usually offset by cosolvent-mediated enhancements in the solubility of the un-ionized form. Cosolvents may in some cases also increase the solubility of the ionized species. Table 10 describes the effect of the addition of a range of cosolvents on the solubility of a weak base across a wide pH range (1–7).

In all cases, cosolvent addition increases solubility, and the maximum solubility is evident at the lowest pH (reflecting the largest ionized fraction) (Jain et al., 2001). However, the relative gain that can be attributed to the cosolvent progressively decreases with decreasing pH. This reflects the decreased solubility advantage provided by cosolvents for increasingly ionized species.

Since the addition of a cosolvent to a pH-buffered solution leads to a net increase in drug solubility, this approach has been widely implemented in marketed parenteral formulations, including chlordiazepoxide [Librium; Hoffmann-LaRoche, Inc., Nutley, NJ (propylene glycol 20%, pH 3)], dihydroergotamine [DHE-45; Novartis AG, Basel, Switzerland (15% glycerin, 6.1% ethanol, pH 3.6)], fenoldopam [Corlopam; Ben Venue Laboratories, Inc., Bedford, OH (propylene glycol 50%, pH 3)], oxytetracycline [Terramycin; Pfizer Labs, NY (propylene glycol 65–75%, pH 3)], pentobarbital [Nembutal; Lundbeck, Inc., Deerfield, IL (propylene glycol 40%, ethanol 10%, pH 9.5)], and phenytoin [Dilantin; Park-Davis, NY (propylene glycol 40%, ethanol 10%, pH 10–12.3)] (Strickley, 2004).

One concern with the use of pH-cosolvent combinations is the potential for an increased risk of drug precipitation on dilution resulting from a reduction in buffer capacity. This is well exemplified by a relatively recent study of the solubility properties of the weak acid phenytoin in a formulation containing 50 mM carbonate buffer (pH ~9.8) with and without 15% ethanol. After dilution with phosphate buffer (pH 7.4), formulations containing ethanol showed a greater shift (decrease) in pH as a result of the increased $pK_a$ of the buffer in the presence of the cosolvent, in turn leading to increased drug precipitation in comparison with ethanol-free formulations (Narazaki et al., 2007b).

b. pH Adjustment and Strong Electrolytes. Strong electrolytes, such as sodium chloride, are often added to parenteral formulations to ensure isotonicity. In contrast to the effect of cosolvents, the addition of polar species such as sodium chloride increases the polarity of the solvent, causing a reduction in the solubility of the un-ionized form and an increase in the solubility of the ionized form (effectively increasing acid/base strength) (Windheuser and Higuchi, 1963; Flynn, 1980; Yalkowsky, 1999). Electrolytes therefore decrease the $pK_a$ of acidic buffers and increase the $pK_a$ of basic buffers, with once again the effect being more pronounced for acids. There is also the potential for electrolytes to modify solubility via the common ion effect or the formation of salts of lower solubility.

c. pH Adjustment and Surfactants. Surfactants usually enhance drug solubility via solubilization in the hydrophobic micelle core (see Section VIII). Consistent with the effect of cosolvents on buffered solutions, the beneficial effects of surfactants are therefore progressively lost with increasing drug ionization. This is also evident in Table 10, where decreasing pH (and therefore increasing ionization for a weak base) leads to a decrease in the advantage provided by micellar solubilization in a 20% Tween 80 solution (Jain et al., 2001). Nonetheless, the net effect is again an increase in total drug solubility in the presence of both surfactant and reduced pH since both approaches are beneficial to solubilization. An additional ramification of the combination of pH and surfactant approaches is the realization that a pH change can affect surfactant properties. Thus, changes to pH can significantly alter the properties of ionic surfactants (including solubility, critical micelle concentration, and solubilization capacity). However, ionic surfactants are in general more toxic than nonionic surfactants (Strickley, 2004) and are therefore rarely used in parenteral formulations. In contrast, nonionic surfactants are more commonly used and have self-association properties that are more independent of pH. The potential for pH to affect the solubilization of drugs in micellar formulations is described in more detail in Section VII.D.2.

d. pH Adjustment and Cyclodextrins. Many non-polar drugs are readily solubilized by cyclodextrins via the formation of an inclusion complex within the hydrophobic cyclodextrin core (see Sections VIII and VII.C). Given the hydrophobic nature of this interaction,

<table>
<thead>
<tr>
<th>Table 10</th>
<th>The effect of cosolvent and surfactant addition on the solubility of a weak base at differing pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7</td>
<td>pH 2</td>
</tr>
<tr>
<td>Buffer alone</td>
<td>0.00003</td>
</tr>
<tr>
<td>20% Ethanol</td>
<td>0.003 (100)</td>
</tr>
<tr>
<td>20% DMSO</td>
<td>0.002 (66.7)</td>
</tr>
<tr>
<td>20% PEG 400</td>
<td>0.006 (200)</td>
</tr>
<tr>
<td>40% Propylene glycol</td>
<td>0.017 (567)</td>
</tr>
<tr>
<td>20% Tween 80</td>
<td>1.8 (60 000)</td>
</tr>
</tbody>
</table>

Data taken from Jain et al. (2001).

Values in parentheses represent the ratio of solubility in the presence of cosolvent/surfactant to solubility in buffer alone.
ionized drugs typically have lower cyclodextrin binding constants ($K_i$) than do the un-ionized species ($K_u$) (Cirri et al., 2006). A pH adjustment to achieve conditions where the ionized species is more dominant therefore reduces the efficiency of cyclodextrin complexation, and the $K_u/K_i$ ratio has been used to describe the loss of binding affinity resulting from increasing drug ionization (Okimoto et al., 1996). However, in a fashion similar to that described for surfactants or cosolvents, formulations using pH manipulation in combination with cyclodextrins typically retain improved solubilization capacities compared with pH manipulation or cyclodextrin addition alone (Li et al., 1998a, 1999c; Ni et al., 2002; Ran et al., 2005; He et al., 2006). This reflects a greater gain in solubility via pH shift compared with the decrease in efficiency of cyclodextrin complexation. The gain in solubility with a change in pH is a combination of the higher solubility of the ionized form of the drug and the potential complexation of ionized drug. Even though the binding constant for the ionized drug species is usually lower than that of the un-ionized species, the total quantity of complexed ionized drug is typically greater than that of the un-ionized form because the concentration of ionized drug in solution is much higher. Interestingly, Kim et al. have shown that the mesylate salt of ziprasidone dissociates incompletely in solution (providing additional evidence of the possibility of stable ion pairs in solution) and also that it is the ion pair that preferentially interacts with the cyclodextrin rather than the ionized drug (Kim et al., 1998). The nature of the counterion used during salt formation may therefore play a role in drug-cyclodextrin binding (Kim et al., 1998; Mura et al., 2001b).

Where the cyclodextrin itself is ionized, the potential for enhanced complexation through ion pairing may provide additional benefits. This has been observed with the weak bases prazosin, papaverine, and miconazole with anionic sulfobutylether-$\beta$-cyclodextrin where the binding constant for the ionized form of these drugs ($K_i$) was similar to that of the un-ionized forms ($K_u$) (Okimoto et al., 1996; Zia et al., 2001).

3. Effects of Dilution on Drug Solubilization by pH Adjustment. The effect of a pH change and dilution on the solubilization capacity of an acid-adjusted (i.e., not buffered) solution of a weak base is illustrated graphically in Fig. 11. In the absence of buffer, dilution is expected to increase pH and, therefore, decrease the maximum solubility of the drug. The drop in solubility is linear when plotted against $[H^+]$ (but exponential when plotted against pH). Where dilution curves are above the solubility curve (between points X and Y), the solution is supersaturated and at risk of precipitation.

For a buffered solution, dilution is expected to reduce buffer capacity such that pH may increase and drug solubility may decrease in a manner that already described. Figure 12 shows the effect of diluting formulations of phenytoin (a weak acid) buffered to pH ~10. The x-axis shows dilution in terms of the formulation fraction (ff). The ff is defined by eq. 24, where $V_f$ is the volume of the formulation and $V_{DM}$ is the volume of the dilution medium, in this case, pH 7.4 Sorensen’s phosphate buffer (SPB) (Narazaki et al., 2007a):

$$ff = \frac{V_f}{V_f + V_{DM}}$$

Figure 12A is analogous to Fig. 11 and compares the susceptibility to precipitation on dilution of two formulations containing either low (0.5 mg/ml) or high (1.0 mg/ml) drug concentrations. As expected, the onset of precipitation occurs at a lower dilution factor for the more concentrated formulation. Figure 12, B and C shows the importance of buffer type and buffer concentration on the potential for drug precipitation on dilution. In this example, carbonate buffer is more effective in preventing precipitation compared with phosphate buffer as a result of the superior buffer capacity of carbonate buffer over the investigated pH range (Narazaki et al., 2007a). Therefore, there is a need to consider not only the ideal pH range in which a buffer can operate but also the buffer capacity within this range. In this case, phosphate buffer lacks sufficient buffer capacity between the pH of the dilution medium (pH 7.4) and the initial pH of the formulation (pH 10), and consequently the shift in pH in phosphate buffer after dilution is greater than that in carbonate and leads to drug precipitation. The precipitation risk on dilution of buffered solutions may also be predicted; however, predictive models are likely to portray the worst-case scenario and do not take into consideration the possibility of periods of supersaturation before precipitation (Narazaki et al., 2007a).
Methods for Assessing Precipitation Potential for Buffered Parenteral Formulations. The importance of drug precipitation on injection has led to the development of a number of models to assess precipitation potential. Drug precipitation can be visually assessed in vivo using various animal models, including the rabbit ear, rat tail vein, dog femoral vein, or monkey jugular vein, with phlebitis at or near the injection site providing indirect evidence of drug precipitation (Yalkowsky et al., 1998; Johnson et al., 2003). However, these in vivo methods are slow (sometimes symptoms associated with precipitation may take 24 h to materialize) and largely qualitative unless efforts are made to quantitate precipitation by monitoring, for example, an increase in temperature at the site of administration (Ward and Yalkowsky, 1993; Johnson et al., 2003). Alternatively, in vitro precipitation models may be used (Yalkowsky et al., 1988, 1998; Li et al., 1998b; Dai, 2010). Briefly, simple serial dilutions of the formulation are generated with a variety of solutions, including water, saline, dextrose 5%, human plasma, tris buffer, or Sorensen's phosphate buffer (SPB) (Reed and Yalkowsky, 1985; Yalkowsky et al., 1998) and evidence of drug precipitation detected visually or spectrophotometrically following filtration (or another appropriate separation means) of the solutions. Similarly, the dropwise addition method may be used where small aliquots of formulation are slowly introduced (approximately every 30 s) into a static or continuously stirred dilution medium (El-Sayed and Repta, 1983; Li et al., 1998b). The latter method has the advantage of revealing the minimum volume ratio of formulation-to-dilution medium that promotes precipitation. Dynamic precipitation methods are designed to mimic better the effects in vivo via the addition of formulation to a circulating medium (normally pumped through plastic tubing using a peristaltic pump) (Yalkowsky et al., 1983, 1998; Johnson et al., 2003). A major advantage of this approach is that the effect of injection rate on the likelihood of drug precipitation can be assessed (Li et al., 1998b), and although dynamic tests are more complex than static in vitro tests, they have been shown to better correlate with in vivo bioavailability data (Cox et al., 1991; Dai, 2010).

The duration of an in vitro precipitation test is important since drug precipitation may not be immediate and may occur at varying rates and extents, depending on the formulation and the nature of the dilution medium. Li and Zhao (2007) proposed that 3-5 min is a sufficient to assess the likelihood of drug precipitation during static serial dilution tests as an administered formulation is likely to have been significantly diluted in this time after circulation with the blood. Also, in many cases, the administered drug will bind to components of human plasma (e.g., albumin and lipoproteins); therefore, in vitro precipitation methods that use simple solutions and buffers may overestimate the extent of drug precipitation (Cox et al., 1991). The use of more complex dilution media (i.e., containing plasma proteins) may serve to alleviate these differences, although conscious omission of these materials provides a more conservative estimate of precipitation potential.

Buffer Systems in Nonparenteral Formulations. Solution formulations intended for—for example, oral, ocular, or nasal administration—also commonly use buffers. Similar to parenteral formulations, buffers may be used in these systems to alter pH such that...
conditions favor ionization of the drug (and therefore increased solubility), and tighter control of pH might also be used to ensure adequate drug chemical stability or permeability (Chung et al., 1970; Mitra and Mikkelsen, 1982; Washington et al., 2000). In ocular systems, pH buffering can also be used to reduce irritation associated with the use of solutions containing acidic molecules (Suhonen et al., 1995; Vaddi et al., 2007). Buffers may also enhance drug absorption across the cornea by increasing the apparent lipophilicity of the drug via the formation of a drug-buffer ion pair (Higashiyama et al., 2004).

The basic principles of pH buffering are largely consistent across all routes of administration, although the intended route of drug absorption will dictate buffer type and concentration. The acceptable pH range in ophthalmic solutions is narrow (e.g., between pH 6 and 8) so that the pH of the product is consistent with the pH of lacrimal fluid for maximum comfort (Carney and Hill, 1976). Buffer concentrations in ophthalmic systems are likely to be low to avoid irritancy. Lacrimal fluid has very limited buffer capacity but still can influence the pH of a topically applied solution if the applied volumes are low (Longwell et al., 1976).

Buffered solutions may also be used after oral administration; however, the intrinsic buffering properties of GI contents in either fasted or fed conditions is likely to be higher than the formulation; therefore, reversion to GI pH is likely in most cases.

Although manipulation of ionization (and therefore solubility) may be achieved using buffers or direct pH modification in preclinical studies, the more common approach in the clinic is the use of salts to allow the administration of solid dosage forms. These approaches are discussed below.

C. The Use of Salts to Address Low Aqueous Solubility in Parenteral Formulations

Since salts are often the preferred pharmaceutical form of a drug (because of ease of isolation, speed of dissolution, solid-state characteristics, improved processability, and chemical stability), parenteral formulations are often prepared using buffered solutions of salts, even though similar endpoints may be achieved using the free acid or free base. Simple solutions of the salt (in the absence of additional buffer components) are also possible, although in this case the limited buffer capacity of the salt usually leads to a solution that is sensitive to pH shifts and, therefore, precipitation.

In the absence of an additional buffer, solutions of a salt formed using a weak acid and strong base will be basic and those from a weak base and strong acid will be acidic. The pH at the point at which a solution is saturated with respect to the salt is the $pH_{\text{max}}$ value, and this varies depending on the nature of the counterion and the solubility of the formed salt. In undersaturated solutions, the pH value after the addition of different salts of the same drug also varies depending on the acid/base strength of the counterion.

In situations where complete dissociation of a salt in solution results in a pH value that is outside the acceptable range for parenteral administration, a buffer can be added to adjust the pH. However, this approach may lead to drug precipitation if the use of a buffer to lower the pH reduces solubility (by decreasing drug ionization) below the target drug concentration.

An additional factor that warrants consideration is the effect of common ions in the buffer since this may lead to precipitation of the drug salt. For example, the use of buffers containing sodium to promote the solubility of sodium salts of weak acids may exceed the solubility product of the salt resulting in drug precipitation. Under these circumstances, a potassium-based buffer may be more appropriate.

D. The Use of Salt Forms to Address Low Aqueous Solubility in Oral Formulations

Pharmaceutical salts are widely used in oral solution and suspension formulations. Most commonly, salts are used to enhance solubility, either directly to facilitate the formulation of a solution or indirectly to enhance the in vivo dissolution of a suspension, tablet, or capsule fill material or to aid in the in vitro reconstitution of, for example, antibiotic solutions for pediatric treatments. Alternatively, low solubility salts have been used to taste-mask some antibacterials (Jones et al., 1969; Menegon et al., 2011).

The isolation of differing salt forms may also be used to improve other pharmaceutical properties such as stability, flow, compaction, and so forth. The effect of salt formulation and counterion choice on nonsolubility-related phenomena are discussed in detail elsewhere (Giron and Grant, 2002; Stahl, 2003; Huang and Tong, 2004).

1. The Use of Pharmaceutical Salts to Enhance Dissolution. The potential for salts to dissociate in water to form ionized species leads to increases in solubility compared with the un-ionized free acid or free base. The solubility difference between a salt and the free base or free acid may be large (>100-fold), especially where the drug is highly hydrophobic. From the Noyes-Whitney equation (eq. 9), the greater solubility of salts compared with the free acid or base also suggests the potential for increases in dissolution rate, and in general, this is the case. For example, sodium and potassium salts of ampicillin show superior dissolution rates compared with the free acid trihydrate (Hitzenberger and Jaschek, 1974), and the dissolution rates of hydrochloride and mesylate salts of haloperidol are significantly higher than the free base under pH conditions reflective of the small intestine (Li et al., 2005b). Different salt forms also show differences in dissolution rate. Monovalent salts of p-aminosalicylic acid, for example, generate faster intrinsic rates of
dissolution compared with the equivalent divalent salts, and the dissolution rates of the mesylate salt of haloperidol is higher than that of the hydrochloride salt (Forbes et al., 1995; Li et al., 2005a). The differences in dissolution rate generally reflect differences in the solubility of the salts, and in some cases, it has been possible to correlate salt solubility with the intrinsic dissolution rate (Nicklasson et al., 1981; Forbes et al., 1995; O’Connor and Corrigan, 2001).

The potential for salts to enhance solubility and dissolution rate has led to widespread application in oral formulations for poorly water-soluble drugs (Stahl and Wermuth, 2002), and several examples of improved oral bioavailability and/or rate of oral absorption (i.e., speed of onset) have been described (Nelson, 1957; Morozowich et al., 1962; Nelson et al., 1962; Lin et al., 1972; Jaschek, 1974; Meanwell et al., 1992; Kwei et al., 1995; Patt et al., 1999; Desjardins et al., 2002; Gwak et al., 2005; Guzmán et al., 2007; Han and Choi, 2007; Hitzenberger and Kesisoglou and Wu, 2008; Chiang et al., 2009; Menon et al., 2009). It is apparent, however, that the degree of ionization (and therefore solubility) of an orally administered weak acid or weak base is likely to be dictated in large part by the pH of the GI fluids rather than the intrinsic pH attained by the dissolution and dissociation of the salt of a weak acid or base in water. For example, where GI pH is above the pH$_{\text{max}}$(for a weak base) or below pH$_{\text{max}}$(for a weak acid), solubility (and therefore potentially dissolution rate) is expected to be dictated by the effect of pH on ionization of the free acid or base and will therefore be largely independent of salt form.

In contrast, the dissolution rates of pharmaceutical salts are often significantly higher than equilibrium solubility measurements at GI pH would suggest. Salts therefore commonly provide benefits in in vivo dissolution and absorption, even when equilibrium solubilities at intestinal pH suggest little advantage over free acid or free base. A classic example is provided by doxycycline, which, as a hydrochloride salt, shows significantly higher dissolution rates than the free base at pH 4 and pH 7 despite similar solubility for both forms of the drug under these pH conditions (Bogardus and Blackwood, 1979; Serajuddin, 2007). This effect can be ascribed to the self-buffering potential of salts during dissolution as described below.

\textit{a. Effect of Self-Buffering on Salt Dissolution.}

The dissolution rate of a dissolving solid is limited by drug diffusion across an unstirred layer of water that is present on the surface of every dissolving solid. The rate of dissolution (dW/dt) is a function of the solubility of the drug in the unstirred layer (C$_{\text{u}}$), the diffusion coefficient of the drug in the unstirred layer (D), the width of the unstirred layer (h) and the drug concentration in the bulk solvent (C) (eq. 9).

The dissolution rate is therefore expected to reflect the saturated drug solubility (C$_{\text{s}}$). However, the critical point of difference when assessing the dissolution of a salt, rather than a nonionizable drug or a free acid or free base, is the potential for dissolution of the salt to alter the pH in the unstirred layer, which in turn affects the solubility of the drug (C$_{\text{s}}$) in this layer. Since by definition, this water layer is unstirred, significant differences in bulk pH and microenvironment pH at the surface of a dissolving salt can be generated and maintained. It is this pH difference that explains the often higher drug solubility in the unstirred layer (when compared with solubility at bulk pH) and benefits to dissolution rate. In essence, the salt has the potential to buffer the pH of the immediate microenvironment into which it dissolves, hence the use of the term \textit{self-buffering} (Serajuddin and Jarowski, 1985b).

The self-buffering capacity of salts is well exemplified by comparison of the dissolution profiles of two different haloperidol salts with that of the free base in Fig. 13 (Li et al., 2005b). Haloperidol is a poorly soluble weak base (pK$_{\text{a}}$ ~-8), and the free base shows a very slow rate of dissolution at pH values above ~2 (Fig. 13A). In contrast, both the hydrochloride (Fig. 13B) and mesylate (Fig. 13C) salts show evidence of reasonable dissolution at pH 5 and pH 7. The dissolution rate of the mesylate salt is some 6-fold higher than that of the hydrochloride, a difference consistent with the relative differences in the solubilities (K$_{\text{sp}}$) of the two salts. The rationale for the greater dissolution rate of the two salts at higher bulk pH values has been explored by estimating the likely pH in the unstirred water layer. This was achieved by measuring the pH of a concentrated slurry of haloperidol or the haloperidol salts in unbuffered dissolution media (Serajuddin and Jarowski, 1985a,b). This analysis revealed that dissolution of the free base increased the pH in the unstirred water layer relative to bulk pH, effectively suppressing solubility and dissolution rate (Fig. 13D). In contrast, dissolution of the salts resulted in equivalent pH conditions in the bulk solution and the unstirred water layer up to ~pH 3 for the mesylate salt and pH 5 for the hydrochloride salt, beyond which the pH remained consistent (i.e., was effectively buffered) in the unstirred water layer, even with increases in bulk pH up to 8 (Fig. 13D). Substitution of saturated drug solubilities at the effective pH in the diffusion layer, rather than solubility at bulk pH, subsequently allowed good correlation of drug solubility with dissolution rate (Serajuddin and Jarowski, 1985a,b; Li et al., 2005b). The self-buffering capabilities of salts therefore provide the potential for faster rates of dissolution across a much larger pH range than is possible with either the free acid or free base and facilitates, for example, enhanced dissolution of an acidic drug in the low pH environment in the stomach (Mooney et al., 1981; Serajuddin and Jarowski, 1985a; McNamara and Amidon, 1986, 1988; Southard et al., 1992).
the hydrochloride and mesylate salt forms at pH ~1 were all low (Fig. 13). This slow rate of dissolution of the hydrochloride salt form in the acidic media can be explained by the common ion effect, whereas the slow rate of dissolution of the free base and mesylate salt may reflect an in situ conversion to the slowly dissolving hydrochloride salt. The effect of in situ salt interconversions and common ions on dissolution of salts is described in more detail in Sections IV.D.1.c and IV.D.1.d, respectively.

b. Effect of pH Changes on Drug Supersaturation and Precipitation. Although the self-buffering effect of salts is likely to encourage faster dissolution rates, diffusion of the ionized species across the unstirred water layer ultimately leads to entry into the bulk fluids of the GI tract (or dissolution medium in vitro) where mixing precludes the maintenance of the buffered pH in the unstirred layer. Under these conditions, contact with the bulk solution results in supersaturation relative to the solubility of the drug at bulk pH and the potential for drug precipitation. Where the pH of the dissolution fluid encourages conversion of dissolved salt to the free acid or the free base, the bulk solution is referred to as a “reactive” medium (Higuchi et al., 1964). Significant pH change is also evident as, for example, drug in solution passes from the gastric to intestinal environment.

The implications of pH change on drug precipitation in the GI tract have been extensively discussed (Perng et al., 2003; Kostewicz et al., 2004; Gu et al., 2005; Dai et al., 2007; Guzmán et al., 2007; Carlert et al., 2010). Many other factors beyond pH may also play a role in the propensity for salts to precipitate in vivo, including in situ conversion to less soluble salts (see Section IV.D.1.c), the common ion effect (see Section IV.D.1.d), and differences in the solubilization capacity of the GI tract owing to the presence of bile salt-phospholipid mixed micelles (Kaukonen et al., 2004a; Carlert et al., 2010; Bevernage et al., 2011). Figure 14 provides a simple summary of the potential implications of pH

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**Fig. 13.** Effect of dissolution media pH on the dissolution rate of (A) haloperidol free base and (B) hydrochloride and (C) mesylate salt forms, and (D) the pH at the surface of a solid as a function of bulk pH of the dissolution media. Dissolution values are expressed as means (n = 6). Dashed lines are used for pH 7.01 as only data during the first 30 min of dissolution was provided. Adapted from Li et al. (2005b).
change on the dissolution (and potential precipitation) of pharmaceutical salts. Issues pertaining to pH gradients in a single GI compartment (e.g., the self-buffering effects of salts) and during GI transit (e.g., when moving from the stomach to the intestine) are described. First, for the salt of a weak acid in the stomach (Fig. 14A), dissolution is driven initially by self-buffering (to more basic pH) in the diffusion layer. Ultimately, however, conversion of the ionized drug to the free acid is favored in the bulk acidic pH in the stomach, leading to solution conditions that are supersaturated and, therefore, prone to precipitation of the free acid. Where precipitation of the free acid is evident, this may manifest either as a finely divided powder dispersed in the stomach fluids or as a poorly soluble layer of free acid on the surface of the dissolving salt particles (Higuchi et al., 1965b; Serajuddin and Jarowski, 1985b; Serajuddin et al., 1986). However, this effect is expected to be limited as the drug empties from the stomach into the higher pH conditions of the intestine that favor ionization (and dissolution) of either the free acid or the salt.

In the case of the dissolution of a weak base in the acidic gastric environment (Fig. 14B), gastric pH is expected to favor ionization, resulting in rapid initial dissolution. On transfer into the intestine, however, the increase in pH favors conversion to the free base, resulting in supersaturation. Several eventualities are possible. First, where intestinal absorption is rapid, the drug may be absorbed before precipitation. Alternatively, precipitation of the free base may occur either as the crystalline or amorphous form. If dissolution was incomplete in the stomach and the precipitated free base forms a poorly soluble layer on the surface of dissolving salt particles, dissolution in the intestinal environment will slow significantly. Where precipitation results in phase separation of either crystalline or amorphous drug in the GI fluids, the likelihood of redisolution and absorption is dictated by the dissolution rate of the particles formed. Fortunately, precipitation from supersaturated solutions often leads to the generation of particles with small particle sizes or amorphous content, both conditions that favor redisolution, even from the free base.

Additives may also be incorporated into the dosage form in an attempt to stabilize supersaturation. For example, various water-soluble polymers have been used to stabilize the supersaturated solutions formed by self-buffered dissolution of salts of weak acids in the stomach (Guzmán et al., 2004, 2007; Brouwers et al., 2009) and on gastric emptying of solutions of weak bases (e.g., itraconazole) (van Speybroeck et al., 2011). Alternatively, enteric materials may be used to prevent the release and precipitation of salts of a weak acid in the stomach or weak bases in the intestine (Miller et al., 2008; DiNunzio et al., 2010a). Salts selected to provide more controlled (i.e., slower) rates of dissolution may also limit the quantity of drug in the supersaturated state so that the driving force for precipitation is reduced.

c. Potential for Salt Conversion to Un-Ionized Drug/ Hydrates/Other Salt Forms In Situ. As described in the preceding section, several scenarios may be envisaged as a dissolved salt is transported along the changing pH conditions of the GI tract, with the potential for salt conversion to the free acid or free base in situ. The fundamental origins for such conversions are illustrated in Fig. 15, which shows the position of pH_max in relation to the physiologic pH of the GI tract for weak acids of differing acidity (top panels) and for weak bases of different basicity (bottom panels). In general, for salts of weak acids, the risk of conversion to the free acid, and therefore the risk of precipitation, is increased where pH < pH_max. Similarly, for salts of weak bases, conversion to the free base and the likelihood of precipitation increase where pH > pH_max (Serajuddin, 2007). Accordingly, salts of very weak acids (i.e., higher pK_a values) and very weak bases (i.e., lower pK_a values) show higher risks of conversion to the free acid/base since they have higher pH_max values (for acids) or lower pH_max values (for bases) compared with salts of stronger acids/bases (see Fig. 15). However, the rate of conversion of a salt to the un-ionized species in response to a change in pH remains unpredictable (Agoram et al., 2010).

Depending on the availability of counterions in solution and the K_sp of the resulting salt complex, drugs administered as one salt form may also precipitate out of solution as a different salt, resulting in marked changes in properties and behavior (Li et al., 2005a; Serajuddin, 2007). Such salt interconversions are likely where counterions are present in reasonable concentration and have the potential to form a salt with a lower K_sp. Salt interconversions may be further exacerbated by the common ion effect. For example, conversion of salts of weak bases (and in particular very weak bases) to the hydrochloride salt in the stomach may render an orally administered salt form sensitive to a chloride common ion effect (even though the originally administered salt was not a hydrochloride). In situ conversions of this type have been reported during in vitro dissolution of mesylate and phosphate salts of haloperidol in the presence of chloride ions, although in this case enhancing the dissolution rate of the original salt forms (by increasing available drug surface area) was able to reduce in situ conversion (Li et al., 2005a).

Issues surrounding hydrate formation are most commonly observed on storage, where conversion of the anhydrous salt to the hydrate typically results in a reduction in solubility of the drug product. In some
A Salt of a weakly acidic drug

- **Incomplete dissolution**
  - Dissolution of a salt of a weak acid in the acidic stomach is promoted by the self-buffering effect of salts. Drug in solution in the stomach may enter the intestine and be absorbed, or it may convert to the un-ionized free acid in the stomach, typically resulting in supersaturation and increasing the risk of precipitation.
  - Precipitated free acid may exist as fine particles (either amorphous or crystalline) or may form a layer of poorly soluble free acid around the salt particle, inhibiting further gastric dissolution. On gastric emptying, dissolution of precipitated free acid is subsequently promoted by the higher pH in the intestine. Incomplete dissolution of the salt of a weak acid in the stomach leads to gastric emptying of undissolved salt and the expectation of improved dissolution in the higher pH environment of the small intestine.

B Salt of a weakly basic drug

- **Incomplete dissolution**
  - Dissolution of the salt of a weak base in the stomach is favored by the low pH environment. Drug in solution subsequently enters the intestine, where the increase in pH commonly leads to transient supersaturation as the degree of ionization (and therefore drug solubility) is decreased. Depending on the rate of absorption, supersaturated drug may be absorbed or precipitate (in either the amorphous or crystalline form). Precipitated free base may form discrete particles or coat incompletely dissolved salt particles, inhibiting further dissolution. Redissolution is possible, however, as drug is absorbed and sink conditions return. Incomplete dissolution of the salt of a weak base in the stomach results in gastric emptying of the undissolved salt, the dissolution of which is slow in the small intestine but assisted by the self-buffering effect of the salt.

Salt = light shaded purple, free acid/base = dark shaded purple.
cases, conversion to the hydrate may also occur during the early stages of dissolution, in turn reducing the dissolution rate. Finally, where crystallization from supersaturated solutions occurs (in response, e.g., to self-buffering of a salt, gastric emptying of a weak base, or rapid dissolution of the anhydrous form), recrystallization as the less soluble hydrate is also possible. The hydrate and anhydrous forms of a particular salt may show different propensities for conversion to the free acid or free base, resulting in seemingly unusual differences in dissolution. For example, the \( \text{pH}_{\text{max}} \) of siramesine hydrochloride is 4.8 for the anhydrate and 5.1 for the monohydrate (the higher \( \text{pH}_{\text{max}} \) for the hydrate form likely reflecting the lower intrinsic solubility of the drug; see Fig. 8). Dissolution of both the anhydrous and monohydrate forms at pH 6.4 (i.e., above the \( \text{pH}_{\text{max}} \)) therefore predicates the formation of a supersaturated solution and the likelihood of precipitation of the free base. In this case, recrystallization of the free base occurred more readily from solutions of the anhydrous salt compared with the hydrate, with the surprising outcome of a slower rate of dissolution of the anhydrate (Zimmermann et al., 2009). Differences in the rates of crystallization of the free base were attributed to crystals of the anhydrate acting as more effective nucleation substrates compared with the hydrate. In contrast, under more acidic conditions (i.e., \( \text{pH}_{\text{max}} \)), where conversion to the free acid is evident under gastric conditions and potentially in the small intestine (depending on \( \text{pH}_{\text{max}} \)). For even weaker acids (\( \text{p}K_a \sim 7 \)) conversion under most physiologic conditions is likely. Note the difference between \( \text{pH}_{\text{max}} \) and \( \text{p}K_a \) has been arbitrarily shown here as \( \sim 2.5 \) pH units to allow simple illustration but will vary for differing salts.

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d. Effect of Common Ions on Dissolution in the Gastrointestinal Tract. As described in the previous section, the presence of common ions reduces salt solubility. In light of the relationship between solubility and dissolution rate, common ions may therefore also suppress dissolution rate. For example, the dissolution of tetracycline hydrochloride in 0.1 M
hydrochloric acid is slower than that of the free base, and as a result, the oral bioavailability of tetracycline hydrochloride is reduced compared with that of the free base (Miyazaki et al., 1975, 1981). The addition of sodium chloride to a dissolution medium can similarly reduce the dissolution rate of sodium salts (Mooney et al., 1981; Serajuddin et al., 1987). Although different counterions may be sought to circumvent the effect of common ions, it is apparent that the selection of an alternative counterion does not necessarily preclude a common ion effect since there is potential for in situ conversion provided sufficient concentrations of the alternate counterion are present (Li et al., 2005a).

2. Physical Properties of Pharmaceutical Salts. Salts can exist in many chemical forms, including 1) highly ordered crystalline phases, 2) crystalline solvates and hydrates (“pseudopolymorphs”), 3) liquid crystals, and 4) irregular amorphous states. The propensity for a salt to exist in the crystalline or noncrystalline amorphous form is dependent on the properties of the drug molecule, the counterion used, and also the solvent composition used to isolate the salt (Tong and Zografi, 1999; Giron and Grant, 2002; Black et al., 2007; Towler et al., 2008; Brittain, 2010). Crystalline salts are generally favored for development over noncrystalline forms owing to their superior stability and higher melting temperatures. Salts formed using strongly acidic or basic counterions typically show a greater inclination to higher crystallinity (Hirsch et al., 1978; Bastin et al., 2000; Remenar et al., 2003a; Black et al., 2007; Gross et al., 2007). Amorphous salts of various drugs have been isolated (Tong and Zografi, 1999, 2001; Towler et al., 2008; Hasa et al., 2011); however, these are rarely pursued unless a formulation strategy that subsequently stabilizes the amorphous form (such as a solid dispersion; see Section X) is envisaged (Towler et al., 2008; Agoram et al., 2010).

Salts have also been widely explored as a means to improve the chemical stability of weak acids (Walton et al., 1970; Cotton et al., 1994) and weak bases (Bartolini et al., 1981; Walkling et al., 1983; Gould, 1986; Lin, 1999; Ki et al., 2008). The stability benefits accrued by isolation as a salt can be reversed, however, if the salt shows increased evidence of hygroscopicity since moisture absorption can lead to reduced chemical stability and poorer flow and compression properties of the solid drug (Gordon and Chowhan, 1987, 1990; Adam et al., 2000). Salts absorb moisture by forming hydrogen bonds with water in a mechanism analogous to interactions with water molecules in solution. Salts that tend to show greater hygroscopicity include those containing more hydrophilic counterions, ostensibly as a result of the higher polarity of the crystal surface (Berger et al., 1977; Visalakshi et al., 2005; Gross et al., 2007; Guerrieri et al., 2010). More hydrophilic counterions include hydrochloride, dihydrochloride, and sulfate for weak bases and sodium and potassium for weak acids. Metal alkali salts tend to be more hygroscopic than organic amine salts; however, metal salts are often more soluble (Schwartz and Buckwalter, 1962; Hirsch et al., 1978). Increases in solubility and dissolution rate through salt formation must therefore be considered in light of the stability properties of the new solid. For hygroscopic materials, interaction with water vapor on storage may also result in hydrate formation, with the attendant risks of a reduction in solubility and dissolution rate (see Section IV.D.1.c). The transformation of a salt to a hydrate or other physical form on storage or hydration is often detrimental to performance (i.e., aqueous solubility and dissolution rate) and highly dependent on choice of counterion. However, such transformations are hard to predict a priori, and as a consequence, the optimal salt form is normally chosen after a salt screen (Gould, 1986; Tong and Whitesell, 1998; Bastin et al., 2000).

Pharmaceutical solids with low melting temperatures are typically “soft and plastic,” whereas those with higher melting temperature show “hard and brittle” characteristics (Corrigan, 2007). Low melting, soft, and plastic materials typically process less readily into free-flowing powders with good behavior on deformation (during compression), and this is often detrimental to the characteristics of the final dosage form, such as disintegration, friability, and content uniformity. Salt formation usually leads to increases in melting temperature compared with the free acid or free base and therefore has the potential to enhance processability. However, differences in packing efficiency, and therefore differences in melting point (Gould, 1986; O’Connor and Corrigan, 2001), also alter solubility, and as a result, there is some temptation to select the salt with the lowest melting temperature as this salt is likely to show the highest solubility (see Section IV.A.4). Indeed, salt solubility commonly decreases by a factor of 10 for every increase of 100°C in the melting temperature (Corrigan, 2007). A compromise is usually required between lower melting points to encourage solubility and higher melting points to permit adequate processing. Salts with melting points below 100°C are rarely progressed (Stahl and Wermuth, 2002).

3. Potential Toxicity of Pharmaceutical Counterions. The potential for toxicity has limited the exploration of many novel counterions; however, the wide range in current use is usually sufficient for most applications. Of the counterions that have been previously used, lithium salts are now generally avoided because of their known pharmacological activities (Berge et al., 1977), and bromide ions are also infrequently used owing to their toxic effects and potential to accumulate in the kidney (Torosian et al., 1973). Calcium salts affect renal function (Schou, 1958; Berge et al., 1977), and maleic acid salts cause renal tubular lesions in the dog (Everett et al., 1993), although both are still widely
used (Paulekuhn et al., 2007). Nitrates are also known to cause GI irritation leading to nausea and vomiting (Berge et al., 1977). More recently, safety concerns have been voiced surrounding the use of sulfonic acid counterions (mesylates, besylates, and tosylates). These counterions are strongly acidic (pKₐ values between −1.34 and 0.7; see Table 8) and consequently are widely used to form salts with weak bases (Engel et al., 2000; Gross et al., 2007; Kumar et al., 2007; Elder et al., 2010). The antiviral product Viracept, for example, contains nelfinavir as a mesylate salt; however, its use was suspended by the European Medicines Agency in 2007 over concerns that it contained high levels of the sulfonate ester, ethyl methanesulfonate (EMS) (Elder et al., 2010), which is genotoxic and possibly carcinogenic (Snodin, 2006). More recent evidence, however, suggests that the high levels of EMS in Viracept resulted from contamination of the starting material rather than being a side reaction during salt formulation (EMEA, 2007). The use of equimolar concentrations of drug and counterion can eliminate the potential for EMS to form during salt manufacture (Teasdale et al., 2009; Elder et al., 2010), and mesylates remain popular as potential counterions.

In an attempt to simplify counterion choice based on toxicity, counterions for oral or parenteral delivery have been categorized into three classes in accordance with their GRAS (“Generally Recognized as Safe”) status and Accepted Daily Intake values. The classification is summarized in Table 11, and more details on regulatory guidance for novel salt-formers and new salt forms for a previously approved drug are available in Verbeeck et al. (2006) and Stahl and Wermuth (2002).

### E. Feasibility of Salt Formation and Salt Selection Strategies

Early identification of the final chemical form for a potential drug product is of significant benefit to rapid development. For poorly water-soluble drugs in particular, finalizing a potential salt form early in the discovery program is expected to aid improved drug exposure in vivo and facilitate more confident progression through pharmacokinetic and toxicokinetic studies. However, selecting the optimal salt is not straightforward, as many of the properties that dictate the potential in vivo performance of a salt (e.g., Kₐp, the potential for in situ conversions to a less soluble salt, the formation of hydrates, self-association properties, and so forth) cannot be predicted using the fundamental solubility principles of the ionized form (i.e., via the Henderson-Hasselbalch equation, eq. 14). The large number of potential counterions (Table 8) also precludes the synthesis and detailed evaluation of all potential salt forms. A more efficient approach, therefore, is to develop experimental methodologies that rapidly screen potential salt forms that meet predetermined criterion. Shanker et al. (1994) described one of the first screening protocols to search for the most promising salt forms for new chemical entities, and several companies subsequently adopted similar screening approaches, with each company setting its own selection criterion. The successes of salt screening methodologies have spawned an era of automation and the creation of specialty companies dedicated to salt and polymorph screening.

The development of small-scale characterization techniques that require low (milligram) quantities of raw material (Shanker et al., 1994; Tong and Zografi, 1999; Bastin et al., 2000; Morissette et al., 2004) and the availability of high-throughput screening methods for multiple physical forms now enable physicochemical profiling of salt forms to be a routine process that is undertaken earlier in drug development (Kern, 2001; Kerns and Di, 2003). One such example suggests that as little as 100 mg of drug material may be sufficient to complete pharmaceutical salt profiling (Balbach and Korn, 2004).

#### 1. Salt Formation Feasibility.

A simple but effective indicator of the potential for salt formation is the presence of a 2-unit difference in pKₐ between the drug and the oppositely charged counterion (Tong and Whitesell, 1998; Pudipeddi et al., 2002; Black et al., 2007). This stems from the realization that the wider the difference in acidity and basicity between the drug

<table>
<thead>
<tr>
<th>Class</th>
<th>Criteria</th>
<th>Acids</th>
<th>Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salt formers that can be used without restriction because they contain physiologically ubiquitous ions and/or ions that occur as intermediate metabolites in biochemical pathways. Frequently used in the past and present</td>
<td>Acetic, citric, fumaric, maleic, hydrochloric, sulphuric, succinic</td>
<td>t-Arginine, calcium, lysine, magnesium, sodium, potassium</td>
</tr>
<tr>
<td>2</td>
<td>Salt formers that while not naturally occurring have through a number of applications shown low toxicity and good tolerability</td>
<td>Beaylate, mesylate, napsylate nicotinate, tosylate,</td>
<td>Diethylamine, tromethamine,</td>
</tr>
<tr>
<td>3</td>
<td>Salt formers that are occasionally used, mainly for the purposes of achieving ion-pair formation. Sometimes suitable to solve particular problems</td>
<td>Nitric, formic, hydrobromide</td>
<td>Piperazine, ethylenediamine</td>
</tr>
</tbody>
</table>

Data taken from Stahl and Wermuth (2002).

The different classes are designed to categorize salt counterions according GRAS (“generally recognized as safe”) status and Accepted Daily Intake values.
and the counterion, the greater the probability of proton transfer during the acid-base reaction. Consequently, stronger counterions are often required for salt formation using weakly acidic/basic drugs. For example, phenytoin is a very weak acid (pKₐ = -8-9), and hence sodium (a strongly basic counterion) salts are used in nearly all commercial products (exceptions include pediatric formulations and oral suspensions). Notably, however, even the sodium salt is prone to conversion to the free acid during dissolution. Itraconazole is a very weak diprotic base (pKₐ,1 = 3.7, pKₐ,2 = 2), and documented exploration of the potential to form salts is therefore limited. In a small salt-screening study, however, itraconazole reportedly formed several crystalline “hits” with a variety of different acid counterions, including phosphoric acid, sulfonic acid, methanesulfonic acid, formic acid, and succinic acid. In this study, however, the pKₐ values of the fumarate (pKₐ = 3.03) and succinate (pKₐ = 4.2) counterions resulted in differential pKₐ values that did not meet the “rule of 2,” and the crystalline materials recovered may have been itraconazole salts, but they may also have been hydrates or cocrystals (Tarsa et al., 2010).

When attempting to develop salts of very weak bases, the potential for reversion to the free base (disproportionation) in the solid state must be addressed. Disproportionation can occur when basic excipients increase the microenvironmental pH in the dosage form above the pHₘₐₓ of the salt, resulting in the loss of a proton (Guerrieri and Taylor, 2009). Factors that increase the risk of disproportionation include weak basicity (low pKₐ values), high salt-base solubility ratios, and low pHₘₐₓ (Guerrieri and Taylor, 2009). Disproportionation can lead to decreased rates of dissolution (Robhrs et al., 1999), decreased potency (Zannou et al., 2007), and changes to tablet hardness and disintegration (Williams et al., 2004).

Additional complexity also surrounds the formation of salts of diprotic drug molecules. For a dibasic compound, for example, avitriptan (pKₐ,1 = 8.0, pKₐ,2 = 3.6), decreasing pH results in the protonation of the most basic moiety and subsequent formation of the monosalt on reaching the first (highest) pHₘₐₓ. Further decreases in pH protonate the second (less basic) moiety and eventually the formation of the disalts at pH values below the second pHₘₐₓ (pHₘₐₓ,2). To form the disalts, the counterion must therefore be sufficiently acidic to protonate both basic groups. In the case of avitriptan, the hydrochloride anion was sufficiently acidic to form the disalts while mesylate, acetate, lactate, tartrate, and succinate were all unable to lower the pH below pH 5 and therefore formed only monosalts (Serajuddin and Pudipeddi, 2002). Similarly, dibasic quetiapine (pKₐ,1 = 6.83, pKₐ,2 = 3.56) formed disalts with hydrochloride, whereas, with fumaric acid, only a monosalt was formed (Volgyi et al., 2010).

2. Salt-Screening Strategies. Because of the complexities associated with salt formation and the limited capacity to predict salt properties, salt selection remains largely empirical, and salt-screening techniques remain the mainstay of salt selection protocols. The major advances in this area have been the development of faster high-throughput processes that require less compound. Compared with traditional salt synthesis, a high-throughput method may require only milligrams (1–10 mg) of free acid/base per test, and with integrated automated or semiautomated processes, this can significantly reduce the number of steps required to isolate the salt form and increase the accuracy with which this is performed. Strategies for the preparation of pharmaceutical salts have been recently reviewed (Morissette et al., 2004; Kumar et al., 2007).

High-throughput salt screens provide an opportunity to assess rapidly a number of different counterions and to examine several experimental conditions that may affect salt formulation (e.g., solvent type and composition). For example, Ware and Lu (2004) describe a method to evaluate 104 potential salts of a candidate weak base using 13 potential counterions and 8 different solvents. In the first stage, 100 μL of a solution containing 5 mg of drug (in this case, trazadone free base) was robotically dispensed into individual wells of a 96-well plate, followed by 200 μL solvent and 15 μL of acidic counterion (in an equimolar ratio). The 96-well plates were incubated at ambient temperature for 12 h, during which time the solvent evaporated and salts (if formed) precipitated or crystallized. Samples of solid material from each well were subsequently analyzed using polarized-light microscopy for crystallinity. In stage 2, 25-mg batches of 34 potential “hits” from the first stage were characterized by XRPD and DSC before evaluation of hygroscopicity and melting point. Finally, 1-g batches of four salts were prepared, and the particle size, surface area, and solubility were determined (Ware and Lu, 2004).

High-throughput salt-screening methods similar to those described by Ware and Lu allow a large number of drug-counterion combinations to be randomly screened for potential salt hits. However, smaller, more directed screens are possible if the counterions tested are chosen only from those that are likely to provide a shift in pH sufficient to shift the pH above (for weak acids) or below (for weak bases) the corresponding pHₘₐₓ (Serajuddin and Pudipeddi, 2002; Serajuddin, 2007). This approach has the potential to eliminate several potential counterions by virtue of an inappropriate pKₐ.

It is also apparent that general screening methods and selection criteria might not be appropriate for drugs where solubility is a critical limitation to oral bioavailability since salts that provide significant solubility advantage might be discarded early in a general screen because of “failing” progression criteria for other properties such as hygroscopicity. For these compounds, the selection of hygroscopic salts may be acceptable where solubility properties are sufficiently advantageous.
and where hygroscopicity can be addressed through the design of suitable packaging materials or via a more tailored choice of excipients to minimize water uptake on storage (Kumar et al., 2008b). Solubility-focused screens have therefore been proposed (Huang and Tong, 2004) where an in situ measurement of salt solubility is chosen as the primary screening outcome. Solubility-screening methods have been described by Shankar et al. (1994) and later by Tong and Whitesell (1998), where the primary objective of the screen was to select a salt based on solubility improvements sufficient to support development. The counterions investigated (hydrochloride, mesylate, phosphate, citrate, succinate, or tartrate) were chosen based on $pK_a$ values that were more than two units below the $pK_a$ of the drug to ensure a pH shift sufficient to lower pH below the $pH_{max}$ and to provide greater assurance that any isolated solid was a salt. In each case, a counterion concentration of 0.1 M was sufficient to ensure that the counterion was in excess (relative to the quantity of drug) and, accordingly, that the quantity of dissolved drug was dependent on the solubility of the formed salt.

Several other strategies for salt selection have been described (Gould, 1986; Morris et al., 1994; Bastin et al., 2000) and reviewed (Corrigan, 2007; Serajuddin, 2007; Kumar et al., 2008b), and the interested reader is directed to these references for more detail.

F. Summary

Many new chemical entities contain functional groups that are ionizable under physiologic conditions. In general, the ionized form has greater aqueous solubility than the un-ionized species; therefore, methods that promote ionization typically enhance aqueous solubility. This can be achieved in solution dosage forms by modification of solution pH (commonly via the use of buffers) and in the case of solid dosage forms via the isolation of salts.

For parenteral formulations, an increase in drug solubility via simple pH adjustment can be sufficient for complete solvation of the target drug dose, and as a rule of thumb, differences in pH of 1 unit away from the $pK_a$ of the drug result in 10-fold enhancements in solubility. In the presence of buffers or other counterions, however, the upper solubility limit is determined by the solubility product of the salt that is formed in situ. Buffer choice and appreciation of the potential counterions that might be present in, for example, plasma, are therefore important determinants of practical solubility limits. Where pH adjustment alone is insufficient to allow appropriate solubilization, pH adjustment may be used in combination with other strategies, including cyclodextrins, cosolvents, and nonionic surfactants.

Isolation of a salt and the subsequent dissolution of the salt in vivo provide many of the advantages of pH adjustment but also allow development of a solid dosage form, with the attendant advantages in solid-state stability. Under conditions where bulk solution pH is buffered, the solubility advantages of the pH shift promoted by salt formation may be reduced; however, the dissolution rate often remains high as a result of a “self-buffering effect” where the microenvironment pH in the unstirred water layer surrounding a dissolving salt form promotes drug ionization and dissolution. Subsequent diffusion of dissolved drug into the bulk pH may result in precipitation, but where the rate of absorption is faster than the rate of precipitation, or where supersaturation is stabilized, significant increases in absorption remain possible. Several examples highlighting the ability of salts to improve oral bioavailability are evident in the literature, and the abundance of salt forms in marketed products is tribute to the potential advantages that may accrue as a result of isolation as the salt form.

However, prediction of the solubility benefits of different salt forms remains difficult, and salt-screening techniques provide the mainstay industrial approach for salt selection. Salts may also suffer from reductions in apparent solubility because of the presence of ions in the dissolution media (in vitro or in vivo) that are common with the counterion used for salt formation (the “common ion effect”) or as a result of in situ conversion to salts with lower solubility to the free acid or free base or to a hydrate (Table 12). The presence of ionizable groups also does not guarantee the formation of a soluble and stable salt, and drug and counterion should be matched to provide differences in $pK_a$ of at least 2 units to ensure proton transfer.

Assessment of the feasibility of salt isolation and the choice of counterion are therefore critical determinants of the success of pharmaceutical salts. However, for ionizable compounds, the relative simplicity of pharmaceutical salts, the potential benefits in dissolution rate and solubility, and the presence of a significant industrial experience base dictate that the isolation of

| TABLE 12
| Summary of factors affecting the formation, solubility and dissolution rate of salts |
| Key factors that affect salt formation | $pK_a$ difference between drug and available counterion, Drug solubility, Salt solubility, Organic solvents |
| Key factors that affect salt solubility | Combined energies of solvation of drug and counterion, Lattice energies, Common ions |
| Key factors that affect salt dissolution rate | Salt solubility, Self-buffering capacity, Environmental pH of the surrounding fluids, Common ions |
| In situ conversion from salt to free acid/free base | Hydrate formation during storage or during dissolution |
| In situ interconversion between salt forms | |
V. Optimization of Crystal Habit: Polymorphism and Cocrystal Formation

Crystal engineering is traditionally defined as the deliberate design and control of molecular packing within a crystal structure with the intention of generating a solid that shows a particular and desirable characteristic (Desiraju, 2001, 2010). Since the solid-state properties of a drug will influence a range of different drug properties, including solubility, the level of interest in crystal engineering strategies has increased dramatically over recent years (Desiraju, 2001; Datta and Grant, 2004; Blagden et al., 2007).

In the broadest sense, crystal engineering may encompass any manipulation that results in altered crystal packing, including both traditional approaches, such as the isolation of different salts (Peterson et al., 2010), solvates (Blagden et al., 2007), or polymorphs, and more recent approaches, such as cocrystal formation (Aakeroy and Salmon, 2005). In each of these approaches, a change to solid-state properties is likely to affect solubility and may be advantageous in overcoming solubility limitations that stem from the strength of the crystal lattice. In the case of salt formation, however, changes to solubility reflect both changes to the crystal lattice and coincident changes to pH and ionization (and where changes to pH and ionization provide the primary beneficial effects). Salt formation is therefore addressed in a separate section (see Section IV).

Here the discussion of crystal engineering strategies is limited to polymorphism and cocrystal formation (i.e., approaches that alter the crystal habit). A somewhat broader use of the term crystal engineering has also emerged (Biradha et al., 2011) to describe strategies that include not only deliberate manipulation of the crystal lattice but also manipulation of crystallization conditions to isolate drug crystals of a particular size and shape (Nokhodchi et al., 2003; Blagden et al., 2007). The latter strategy, however, is arguably better described as particle (rather than crystal) engineering since it is the physical properties of the particle that are changed rather than the crystal form (see Section IX). Indeed, the same polymorphic form can form particles with widely differing characteristics (Nokhodchi et al., 2003; Iacocca et al., 2010).

The potential for a drug to form one or more crystalline solids that differ only by the molecular arrangement of drug molecules in the crystal lattice is referred to as polymorphism, and different crystalline forms of the same compound are described as polymorphs. Materials lacking crystalline character are said to be amorphous. Since they differ in crystal habit, polymorphs or the amorphous form exhibit different free energies, which in turn give rise to unique physicochemical properties. In the context of the current review, different crystalline polymorphs (or the amorphous form) have different solubilities and, therefore, have the potential to impact oral bioavailability for drugs where low aqueous solubility is a significant limitation to absorption (Aguiar and Zelmer, 1969; Law et al., 2004).

Cocrystals constitute a molecular complex between a drug and cocrystal former and also result in changes to the crystal lattice. In some respects, cocrystals are analogous to pharmaceutical salts in that they constitute a complex between drug and an additional species (counterion, coformer). Unlike the salt complex formed between a weak acid or weak base and the respective counterion, in the case of a complex between a drug and a cocrystal former, proton exchange does not occur (Aakeroy et al., 2007). Unlike salts, cocrystallization strategies are therefore not limited to ionizable compounds.

Isolation of a compound as a high-energy crystalline polymorph or in the amorphous form can have a profound impact on apparent drug solubility. Indeed, on dissolution, transient differences in the amorphous-to-crystalline drug solubility ratio may cover several orders of magnitude, leading to potentially dramatic increases in dissolution rate. These solutions are, however, thermodynamically unstable, and over time, drug in solution will recrystallize, reverting to more thermodynamically stable (and generally less soluble) forms (Hancock and Zografi, 1997; Murdande et al., 2011a). These may include, for example, more stable crystalline polymorphs or hydrates (Pudipeddi and Serajuddin, 2005; Agoram et al., 2010; Murdande et al., 2011a), but in both cases, solubility is usually reduced. This transformation can occur very rapidly on dissolution, complicating solubility measurements for the high energy form, and can also occur during storage in the solid state, particularly on exposure to moisture or heat (Yu, 2001; Pudipeddi and Serajuddin, 2005; Janssens and Van den Mooter, 2009). A well known example of the complexities of polymorphic transitions is that of ritonavir (Norvir; Abbott Laboratories, North Chicago, IL), which was initially launched by Abbott Laboratories in 1992 as a capsule formulation containing dissolved drug at a loading that was believed to be below the solubility of the thermodynamically stable crystal form (to avoid the potential for drug crystallization during storage). However, a previously unidentified polymorph of enhanced stability, but correspondingly lower solubility, was subsequently identified during a change to process chemistry. The lower solubility of the more stable form drew into question the long-term stability of the original formulation, prompting product recall and reformulation prior to rerelease (Chemburkar et al., 2000; Bauer et al., 2001; Huang and Tong, 2004).
Deliberate isolation of high-energy crystal forms for the purpose of improved solubility is therefore possible only when coupled with formulation strategies that impart a level of stabilization to the crystal form sufficient to cover the required shelf-life. In this regard, recent increases in the understanding of crystal transitions and the widespread availability of thermal analytical technologies have allowed a more rational approach to developing formulations that are stable over timescales relevant to the typical shelf-life of a drug product (Hancock and Zografi, 1997; Weuts et al., 2011), and coformulation with (usually polymeric) materials that are capable of stabilizing drug in a high-energy form over long periods is becoming routine (Taylor and Zografi, 1997; Friesen et al., 2008; Curatolo et al., 2009). The most common of these approaches is the formulation of polymorphic or amorphous drug in polymeric solid dispersions, and this formulation technology is described in more detail in Section X.

In the current section, we introduce the fundamental concepts of crystallinity and polymorphism in pharmaceutical solids and explore the relationship between the solid-state properties of a drug and solubility. The latter provides the rationale for the isolation of high-energy polymorphs and also serves as background to subsequent sections that discuss formulation approaches that may be used to stabilize high-energy solids (usually the amorphous form) such as solid dispersions. The concept of crystal engineering via nontraditional means is subsequently introduced and, in particular, the design and isolation of cocrystals. Cocrystals provide the potential opportunity to match the benefits of high-energy polymorphs or amorphous drug, such as improved solubility and dissolution characteristics, with the desired stability traits of a stable crystal form (Blagden et al., 2007; Desiraju, 2010).

A. Polymorphs

1. Crystal Packing, Polymorphism, and Phase Transformations. In an ideal crystal, identical structural components, or unit cells, are repeated regularly and indefinitely in three dimensions in the absence of imperfections or defects (Vippagunta et al., 2001). In pharmaceutical crystals, the unit cell comprises drug molecules in an orientation that is constant throughout the crystal lattice. The nature and strength of the intermolecular interactions that form between drug molecules within the unit cell and between adjacent cells are determined by the chemical properties of the drug molecule and their orientation within the unit cell.

Molecular interactions within a crystal lattice are either attractive or repulsive and include relatively weak van der Waals forces, π-π stacking, and electrostatic interactions (0.5–2 kJ per mol), stronger hydrogen bond interactions (5–30 kJ per mol), and much stronger (∼150 kJ per mol) ionic interactions. The summation of the energies from all intermolecular interactions determines the total packing energy of a crystal or the crystal lattice energy. Since stronger intermolecular interactions minimize the molecular energy (and mobility) of the molecules within the crystal, the higher the lattice energy, the greater the stability (and lower the solubility) of the crystal (Datta and Grant, 2004; Lohani and Grant, 2006). The most stable crystal form therefore allows the largest number of molecular interactions with adjacent molecules. Accordingly, a crystal lattice in which the orientation of the drug molecule does not permit the maximum number of interactions with adjacent molecules is unstable, or metastable, and over time or after exposure to heat or moisture, molecules within the crystal lattice will reorientate (relax) to adopt a more thermodynamically stable conformation. It is important to recognize the potential confusion that can occur between reference to unstable polymorphs as “high-energy” solids (where the reference to high energy refers to thermodynamic instability) and the realization that the crystal lattice energy of these systems is, in fact, low compared with the thermodynamically stable crystal form.

Polymorphism is the term most frequently used to describe the ability of an organic molecule to exist in more than one crystalline form (i.e., polymorph) (see Fig. 16), and it has been estimated that approximately 80% of pharmaceutical compounds show more than one polymorph (Grunenberg et al., 1996; Datta and Grant, 2004; Lohani and Grant, 2006). In addition to “true” polymorphs, where different crystal packing arrangements result entirely from differences in packing

![Fig. 16. Schematic diagram illustrating common solid-state arrangements of drug molecules in the crystalline state and the amorphous form. Drug polymorphs vary only in the molecular organization of individual drug molecules in the crystalline state. Solvates and hydrates (often referred to as pseudopolymorphs) are molecular complexes of drug and molecules of water (in the case of hydrates) or other solvent molecules (in the case of solvates) that form intermolecular interactions with the drug and become incorporated within the crystalline lattice. Cocrystals are also molecular complexes, consisting of drug and a cocrystal former (coformer), which interact primarily via hydrogen bonds. Popular definitions of cocrystals in the pharmaceutical literature state that the coformer must be solid at room temperature, distinguishing cocrystals from solvates and clathrates. Salts are in many ways analogous to cocrystals but differ by virtue of an electrostatic interaction that forms between a charged drug molecule and an oppositively charged counterion.](image-url)
efficiency of drug alone, alternate crystal structures may also form following a disturbance to the crystal packing arrangement induced by the presence of an additional molecule. For example, where molecules favor hydrogen bond formation with water or other polar solvents, the solvent molecules may be incorporated into the host lattice during crystallization, forming stoichiometric or nonstoichiometric hydrates (where the solvent is water), or solvates (for other solvents). The presence of solvent molecules within the crystal lattice alters the pattern of drug-drug interactions and, in doing so, gives rise to altered crystal arrangements. However, solvates and hydrates are not polymorphs in the true sense and, thus, are often referred to as pseudopolymorphs. Similarly, the amorphous state should not, in theory, be described as a different polymorphic form as this state lacks long-range order (Datta and Grant, 2004) and can demonstrate only a certain degree of local order, for example, symmetrical carboxylic dimerization in the case of indomethacin molecules (Taylor and Zografi, 1997; Rodriguez-Spong et al., 2004). Pragmatically, however, the US Food and Drug Administration (FDA) and the International Conference on Harmonization (ICH) classify hydrates, solvates, and amorphous forms all as polymorphs (Ku, 2010).

2. Effect of Polymorphism on Drug Solubility, Dissolution Rate, and Oral Absorption. Recent analysis of the effect of polymorphism on the solubility and dissolution rate of 55 compounds suggested that the differences in apparent solubility between a high-energy crystalline polymorph and the most stable crystal form are, in most cases, relatively moderate. In this analysis, the solubility ratio between high-energy crystal forms and the most stable crystalline polymorphs was between 2 and 3 for most of the compounds and less than 5 for all but one compound (Pudipeddi and Serajuddin, 2005). In contrast, the amorphous:crystalline solubility ratio is typically much higher (Hancock and Parks, 2000; Murdande et al., 2010b). Solubility comparisons have also been made between hydrates and anhydrous crystalline forms across a collection of 17 compounds, and only three compounds showed a greater than 3-fold difference in solubility between the hydrate and the anhydrous form (Pudipeddi and Serajuddin, 2005). Although there are occasional exceptions, hydrates are generally less water-soluble compared with anhydrous forms of the same drug (Huang and Tong, 2004), a situation reflecting the reduction in available sites for drug interaction with water in the hydrate.

On the basis of solubility alone, there would appear to be only moderate potential benefit (2- to 5-fold increase in solubility) for poorly water-soluble drugs in using different crystalline polymorphic forms or by switching between an anhydrous and hydrated form (Singhal and Curatolo, 2004). However, the use of high-energy crystalline polymorphs can lead to significant increases in oral bioavailability for drugs including chloramphenicol palmitate (Aguiar et al., 1967), sulfameter (Khalafallah et al., 1974), phenylbutazone (Pandit et al., 1984), amobarbital (Kato and Kohketsu, 1981), and more recently celecoxib (Lu et al., 2006) and rifaximin (Viscomi et al., 2008) (Fig. 17, A and B). The seemingly contradictory nature of in vitro solubility assessments for high-energy polymorphs and in vivo observations may result from the inherent difficulties in assessing the true solubility advantage of a thermodynamically unstable solid in vitro. Thus, only one true equilibrium solubility value is evident for a drug (the solubility of the lowest energy, thermodynamically stable crystal form). In practice, equilibration to form the thermodynamically stable crystal form may be rapid or may occur over an extended time. It may be possible, therefore, to obtain a solubility value for the thermodynamically unstable solid, provided solubility measurements are made before transformation to the

Fig. 17. (A) Kinetic solubility of selected rifaximin polymorphs in water; (B) mean concentration-time profiles of rifaximin polymorphs after oral administration of 100 mg/kg to four dogs. Adapted from Viscomi et al. (2008).
more thermodynamically stable species. In many cases, however, recrystallization to the lower-energy (more stable) polymorph is rapid, leading to underestimation of the apparent solubility of the high-energy polymorph. Nonetheless, the rate of dissolution of a high-energy polymorph can be many times faster than that of the equivalent lower-energy material, and drug concentrations in aqueous solution that are orders of magnitude higher than the solubility of the thermodynamically stable crystal may be obtained.

This transient increase in apparent solubility may be described as the kinetic solubility of the high-energy polymorph, that is, the solubility that is maintained for a finite period (and that reflects the solid-state properties of the high-energy polymorph rather than the equilibrium solubility of the stable crystal) but is effectively transient. Where the rate of recrystallization of the stable crystal is slow or deliberately delayed, high initial drug concentrations may be maintained in the GI tract for sufficient periods to drive significant increases in drug absorption. When assessing the in vitro solubility and dissolution behavior of different polymorphic or amorphous forms, data obtained over shorter (non-equilibrium) timescales may therefore provide a more accurate indication of in vivo dissolution (Singhal and Curatolo, 2004; Blagden et al., 2007). In Fig. 17A, for example, different rifaximin crystalline polymorphs show significant differences in kinetic solubility, and these are reflected in differences in in vivo oral absorption (Fig. 17B). In contrast, estimates closer to the equilibrium solubility of the various polymorphs (e.g., the data in Fig. 17A after ~5 h) suggest much more moderate differences in drug solubility and values that correlate less effectively with the in vivo data.

The isolation and formulation of high-energy polymorphs (or amorphous drug), therefore, have the potential to enhance significantly the bioavailability of poorly water-soluble drug molecules when compared with the most stable crystal form. It is also evident that this is complicated by thermodynamic instability when compared with the most stable crystal form, both in terms of the stability of the polymorph in the dosage form and the generation of a supersaturated solution on dissolution. In practice, the key to harnessing the utility of high-energy polymorphs lies in stabilizing the polymorph in the formulation and in stabilizing the supersaturated solution that forms in vivo. As such, high-energy polymorphs or amorphous drug are rarely administered unformulated or as a simple suspension [with one notable exception being Zinnat tablets (GlaxoSmithKline), which contains amorphous cefuroxime axetil] and instead are most commonly coformulated with stabilizing agents such as polymers to form, for example, solid dispersion formulations. Broader description of the application of polymorphs, or amorphous drug, to the enhancement of drug dissolution and solubility is therefore also found in the following sections of this review that address the specific formulation approaches that might be used to stabilize amorphous drug, in particular, the use of solid dispersion technologies (see Section X).

Nonetheless, amorphous drug has the potential to be used in preclinical settings to afford increased drug exposure for efficacy and safety assessment and where long-term stability of the formulated drug candidate is not essential (Nagapudi and Jona, 2008; Byrn et al., 2010; Palucki et al., 2010; Zheng et al., 2011). Indeed, it is possible that the materials emerging from early chemistry programs (i.e., before formal polymorph and crystallization screens) may contain at least some amorphous material, and this may provide assistance in early exposure studies. The corollary to this suggestion, however, is that the advent of stricter control of polymorphic form in subsequent drug batches may lead to a decrease in oral bioavailability for poorly watersoluble drugs as the amorphous content is reduced. Early identification of possible polymorphs and confirmation of the stable crystal form is therefore important, with the latter providing a stable comparative baseline for subsequent bioavailability-enhancing approaches.

Beyond effects on solubility, dissolution rate, and stability, different polymorphs also exhibit differences in a range of pharmaceutical properties (e.g., hygroscopicity, flow, compaction, and compatibility), all of which may be a critical in determining whether a drug candidate becomes a marketed product. These properties, although important, lie outside the scope of the current review. Further information concerning the physical properties of drug polymorphs and pseudopolymorphs can be found in relevant reviews (Morris et al., 2001; Zhang et al., 2004).

B. Cocrystals

Cocrystals provide an alternative crystal engineering strategy for improved drug solubility (Vishweshwar et al., 2006; Henck and Byrn, 2007; Childs and Zaworotko, 2009; Friščić and Jones, 2010). Cocrystal formation places greater emphasis on prospective molecular design of the crystal form, and has the added advantage over the isolation of high-energy crystal forms of superior thermodynamic stability in the solid-state (Fleischman et al., 2003; Good and Rodriguez-Hornedo, 2009).

1. Cocrystals as a Mechanism of Enhanced Drug Solubility and Dissolution. Pharmaceutical cocrystals are defined as mixed crystals consisting of two or more molecular species (that alone are solid at ambient conditions) held together by noncovalent and nonionic forces (Vishweshwar et al., 2006; Arora and Zaworotko, 2009). Although there is considerable debate over the definition of a cocrystal (Vishweshwar et al., 2006; Blagden et al., 2007; Schultheiss and Newman, 2009), here we exclude crystal solvates, hydrates, and clathrates as a component of each of these complexes
will exist in either the liquid or gaseous state at ambient temperature (Morissette et al., 2004; Aakeroy and Schultheiss, 2007).

Cocrystals are distinguished from salts by the presence of neutral molecular species within the cocrystal lattice rather than a molecular complex containing drug and counterion in a charged form (Fig. 16) (Aakeroy et al., 2007; Shan and Zaworotko, 2008; Frisčić and Jones, 2010; Stevens et al., 2010). However, analogous to salts, cocrystals may provide dramatic increases in dissolution rate compared with simple drug crystals, and this effect can be ascribed to an improvement in the drug solubility (Banerjee et al., 2005; Good and Rodriguez-Hornedo, 2009).

The molecular components of cocrystal complexes are the target molecule (traditionally the drug molecule) and the cocrystal former(s) (also known as the cocrystallizing agent or coformer). Cocrystals are formed by intermolecular interactions, or synthons, that form between the drug and a potential cocrystal former, which in turn leads to the creation of supramolecular assemblies. Synthons common to pharmaceutical cocrystals are based largely on hydrogen bonding motifs and are categorized either as heterosynthons (e.g., carboxylic acid-amide, pyridine-carboxylic acid, and imidazole-carboxylic acid dimers) or as homosynthons (e.g., carboxylic acid-carboxylic acid and amide-amide dimers) (Fig. 18). Cocrystal formation is favored when noncovalent intermolecular interactions between complementary functional groups on the drug and the cocrystal former are more energetically favorable than intramolecular interactions between two drug molecules (Chow et al., 2008). Conversely, where drug-drug rather than drug-coformer interactions dominate, recrystallization of two distinct solid phases (i.e., drug and coformer) is evident rather than cocrystallization to form a single solid phase (Aakeroy and Šalmon, 2005).

Similar to interactions between a drug and its counterion in a sparingly soluble salt, cocrystal solubility can be defined by the equilibrium constant that describes cocrystal dissociation. Thus, for a 1:1 cocrystal of drug A and a coformer B in equilibrium with A and B in solution, the equilibrium relationship (eq. 25) and equilibrium constant or solubility product \( K_{sp} \) in eq. 26 can be described. Note that in this case, the activity coefficients for A and B in solution are assumed to be unity and the activity of the solid crystal invariant—both assumptions that hold true for sparingly soluble systems (Good and Rodriguez-Hornedo, 2009):

\[
A_\alpha B_\beta \rightleftharpoons A_\alpha + B_\beta
\]  

(eq. 25)

\[
K_{sp} = [A]^\alpha[B]^\beta
\]  

(eq. 26)

The solubility product \( K_{sp} \) is unique for a cocrystal in a particular solvent and describes the dissociation of the molecular complex in solution. \( K_{sp} \) is dependent on the strength of the solid-state interactions between the drug and cocrystal former relative to their interaction with the solvent (Good and Rodriguez-Hornedo, 2009). From eq. 26, it follows that for a 1:1 drug-coformer complex (i.e., \( \alpha = \beta = 1 \)), the solubility of the cocrystal is equal to the square root of \( K_{sp} \). It is also apparent that the solubility of the cocrystal is dependent on the concentration of coformer in solution, such that increases in coformer concentration reduce the solubility of the cocrystal in a similar fashion to the concept of the common ion effect for sparingly soluble electrolytes.

Depending on the choice of coformer, the solubility of the cocrystal may be higher or lower than the equilibrium solubility of drug alone. Cocrystals may be designed to address a number of pharmaceutical issues, but here we focus on the use of cocrystals to promote solubility and dissolution rate, and under these circumstances, cofomers are chosen such that the solubility of the cocrystal is in excess of that of the drug alone. In these instances, drug concentrations in solution resulting from dissolution of the cocrystal will be in excess of the solubility of the thermodynamically stable drug crystal (Nehm et al., 2006; Good and Rodriguez-Hornedo, 2009; Schultheiss and Newman, 2009). The system is therefore metastable, and the solution is supersaturated with respect to drug solubility in the absence of coformer. This is a similar situation to that encountered on dissolution of a high-energy polymorph, where the initial solubility is high, but over longer timescales, reversion to the solubility of the stable crystal form will occur. In practice, however, significant increases in dissolution and apparent solubility are possible, depending on the rate of recrystallization from the supersaturated solution (see Section V.B.5).

In contrast to most high-energy polymorphs, the intermolecular interactions between drug and coformer in the solid state (which are a necessary part of cocrystal formation) stabilize the cocrystal lattice such that
issues surrounding solid-state stability are circumvented (or at least reduced). Simplistically, cocrystals potentially provide solubility benefits that are analogous to those provided by high-energy crystalline polymorphs or amorphous drug but with solid-state stability advantages similar to that of salts.

2. Solubility Assessment of Cocrystals. As with high-energy polymorphs, equilibrium solubility testing of cocrystals is complex since the kinetic solubility of the cocrystal (reflecting $K_{sp}$) is usually greater than that of the equilibrium solubility of the stable drug crystal. Dissolution of a cocrystal, therefore, results in drug concentrations that are typically greater than the solubility of the stable crystal, and drug precipitation is thermodynamically favored until such time as the intrinsic solubility limit of the most thermodynamically stable solid species (i.e., the most stable drug polymorph) is reached. Moreover, as cocrystals give rise to increases in transient solubility, which in turn may lead to significant increases in dissolution rate and absorption, measurement of the equilibrium solubility is likely to underestimate the potential in vivo advantages of cocrystals. Therefore, simple methods to assess cocrystal solubility and to distinguish cocrystal solubility from the equilibrium solubility of the system (which intrinsically moves toward the solubility of the thermodynamically stable crystal form of pure drug) are required.

A simple measure of cocrystal solubility may be gained via assessment of the kinetic solubility of the complex using traditional phase solubility methods. In this case, the assumption must be made that limited recrystallization occurs from the metastable solution that results on dissolution (Schultheiss and Newman, 2009). Alternatively, Good and Rodriguez-Hornedo (2009) recently described a method to determine more accurately the solubility of a cocrystal based on an understanding of cocrystal-phase solubility relationships (Good and Rodriguez-Hornedo, 2009). This approach is predicated on the realization that increasing the concentration of coformer in solution decreases the solubility of the cocrystal (eq. 26) in a situation analogous to the common ion effect with sparingly soluble salts. For cocrystals, where the cocrystal solubility is higher than that of the parent drug, adding coformer to the system reduces cocrystal solubility, eventually leading to a point where the solubility of the drug and that of the cocrystal are equal. This is an invariant point on the phase solubility diagram, and measurement of drug and coformer solution concentrations in equilibrium with both solid drug and solid cocrystal at this transition point allows for calculation of $K_{sp}$. From $K_{sp}$, the intrinsic solubility of the cocrystal can be calculated. A further advantage of this method is the identification of regions in the phase solubility diagram where the cocrystal is the thermodynamically stable species—knowledge that greatly assists in the isolation of pure cocrystal (see Section V.B.4).

The effect of increasing the coformer concentration on the solubility of a cocrystal is illustrated in the phase solubility diagram in Fig. 19, where the solid red line depicts decreasing cocrystal solubility with increasing coformer concentration and the horizontal dashed line represents the solubility of the drug crystal in the absence of coformer. The solubility of the coformer is assumed to be significantly in excess of that of drug and cocrystal and is therefore not shown. Four regions are evident in the phase diagram. Region 1 is where excess solid phase comprises crystalline drug, since the concentrations of drug and coformer in solution are greater than the drug crystal solubility limit but below the solubility of the cocrystal; region 2 is where concentrations in solution are in excess of both drug and cocrystal and excess solid phase consists of the species with the highest solubility (i.e., the cocrystal); region 3 is where the solution is undersaturated with respect to both drug and cocrystal (and therefore no solid phase is evident); and region 4 is where cocrystal alone is the thermodynamically stable solid phase as concentrations are lower than the solubility of drug but greater than that of the cocrystal. At the transition point (labeled X), the two solid phases (drug and cocrystal) coexist in equilibrium with a solution containing dissolved drug and coformer (Good and Rodriguez-Hornedo, 2009). The transition point provides the coformer concentration at which the solubility of the drug is equal to the cocrystal solubility and, consequently, allows calculation of $K_{sp}$ and ultimately the solubility of the cocrystal (Good and Rodriguez-Hornedo, 2009).

Experimentally, the transition point concentrations of drug and coformer can be determined using the reaction crystallization method (RCM), in which drug crystals are added to a near-saturated solution of coformer.
As drug is dissolved in the coformer solution, the concentration of drug in solution passes the phase solubility limit of the cocrystal (where the green arrow crosses the red line, marked Y in Fig. 19), resulting in precipitation of cocrystal. This leads to a reduction in coformer (and drug) concentrations in solution shifting the position on the phase diagram to the left (since coformer precipitates) and down (since drug also precipitates with the cocrystal). However, in the presence of excess drug, the decrease in drug concentration is only transient as more drug immediately dissolves and stimulates further cocrystal precipitation. The process, therefore, continues, moving the composition in solution to the left of the phase diagram as drug concentrations in solution are maintained by drug dissolution but where coformer concentrations are reduced as a result of precipitation of the cocrystal (i.e., following the green arrow). Ultimately, the concentration of coformer in solution is reduced such that it is no longer in excess and therefore no longer limiting to cocrystal solubility. This condition is denoted by the transition point X, which reflects the solubility limit of both drug and cocrystal in solution. Alternatively, the same transition point X can be determined by adding excess cocrystal to a saturated solution of drug (i.e., the blue arrow in Fig. 19). In this approach, dissolution of cocrystal results in increases in drug concentrations greater than the saturated solubility, prompting drug crystallization from the supersaturated solution. This process again continues until the solubility of the cocrystal is reached, at which point additional increases in drug concentration in solution are not possible on addition of excess cocrystal, and the transition point is reached (i.e., point X).

At the transition point, the existence of a mixed solid phase (crystallized drug and cocrystal) can be verified by x-ray powder diffraction (XRPD) and drug-coformer concentrations in solution measured by HPLC. From these concentrations, $K_{sp}$ is calculated using eq. 26; subsequently, the equilibrium solubility of the cocrystal is calculated from $K_{sp}$. For the dissolution of a 1:1 cocrystal complex, this occurs when drug and coformer concentrations in solution are equal and the equilibrium solubility of the cocrystal is equal to the square root of $K_{sp}$.

Interestingly, solubility values predicted by $K_{sp}$, are often lower than those obtained experimentally (Rodriguez-Hornedo et al., 2006). This can be attributed to the formation of drug-coformer complexes in solution, which in the case of poorly water-soluble drugs (which are usually less soluble than the coformer) leads to increased cocrystal solubility (Nehm et al., 2006). Where $K_{11}$ is the binding constant used to describe the solution complex (AB) between drug (A) and its coformer (B) in a 1:1 ratio:

$$K_{11} = \frac{[AB]}{[A][B]} = \frac{[AB]}{K_{sp}}$$

(27)

The concentration of complex in solution is given by:

$$[AB] = K_{11}K_{sp}$$

(28)

and the total concentration of A in solution ($A_T$) is a function of the solution concentration resulting from the solubility of the cocrystal (determined by $K_{sp}$) plus the additional quantity, reflecting the presence of the association complex AB (determined by $K_{11}$). For a 1:1 cocrystal complex in the solid state and in solution (Nehm et al., 2006):

$$[A]_T = \frac{K_{sp}}{[B]_T} + K_{11}K_{sp}$$

(29)

From eq. 29, a graph of $[A]_T$ against the inverse of $[B]_T$ (total coformer in solution) reveals $K_{sp}$ from the slope and $K_{11}K_{sp}$ from the intercept.

3. Solubility Advantages of Cocrystals. Depending on the choice of coformer, the solubility ratio of cocrystal to drug crystal may vary considerably, from less than 1 to values in excess of 100. Therefore, the potential solubility enhancement provided by cocrystals will likely exceed that of most crystalline polymorphs, and solubility enhancement similar to or above that of amorphous drug is possible (Remenar et al., 2007; Good and Rodriguez-Hornedo, 2009). Cocrystal solubility ratios are also proportional to the solubility of the coformer (Fig. 20), and data obtained for a series of cocrystals of

![Fig. 20. The relationship between cocrystal solubility and the solubility of the coformer. Drugs used were caffeine, theophylline, and carbamazepine. Open symbols are solubilities determined in water, closed symbols in organic solvents. Adapted from Good and Rodriguez-Hornedo (2009).](image)
carbamazepine, theophylline, and caffeine suggest that coformer solubility values must be at least 10-fold higher than drug solubility to generate cocrystals with improved solubility greater than that of crystalline drug (Good and Rodriguez-Hornedo, 2009).

Given that cocrystal solubility is dependent on coformer solubility, the use of highly soluble coformers is common. For ionizable coformers, this results in significant differences in cocrystal solubility with changes in pH even for cocrystals with nonionizable drugs. For example, Fig. 21 illustrates theoretical pH solubility profiles for a cocrystal containing a nonionizable drug and either a weakly acidic (Fig. 21A) or a zwitterionic coformer (Fig. 21B). The corresponding effects for an ionizable drug in the presence of both coformers are also illustrated (Fig. 21, C and D) (Bethune et al., 2009). Note that ionizable drugs may form cocrystals (rather than salts) with ionizable coformers where the difference in pKₐ between drug and conformer is insufficient to allow salt formation.

The solubility advantages provided by isolation of a cocrystal have been shown to correlate broadly with differences in melting point of the cocrystal and the drug for a series of carbamazepine, theophylline, and caffeine cocrystals (Good and Rodriguez-Hornedo, 2009). This trend was also replicated for 10 different cocrystals of AMG 517 (Stanton and Bak, 2008), providing evidence of the role of decreased crystal lattice energy in the solubility improvement. However, this relationship was not apparent in later investigations of cocrystals of AMG 517 using a more diverse range of coformers (Stanton et al., 2009), and closer scrutiny of the data sets for carbamazepine and theophylline revealed that the decrease in melting point of the cocrystal was not sufficient to explain the magnitude of the increase in solubility (Good and Rodriguez-Hornedo, 2009). These data are consistent with the view that enhanced solute-solvent interactions (such as the formation of drug-coformer complexes in solution) and the use of coformers with increasing aqueous solubility may be

Fig. 21. Theoretical pH solubility profiles of various ionizable and nonionizable compounds and their cocrystals. (A) carbamazepine-succinic acid, (B) carbamazepine-4-aminobenzoic acid hydrate, (C) itraconazole-L-tartaric acid, and (D) gabapentin-3-hydroxybenzoic acid. The plots show that even for nonionizable drugs [i.e., (A) and (B)], pH can affect the solubility of the cocrystal via effects on the solubility of the coformer. Adapted from Bethune et al. (2009).
more significant determinants of increased cocrystal solubility than changes to solid-state properties (Good and Rodriguez-Hornedo, 2009; Schultheiss and Newman, 2009).

Because the dissolution of a cocrystal with higher solubility than drug alone leads to the creation of a solution that is supersaturated with respect to drug solubility, the biopharmaceutical advantages of cocrystals are based on increases in kinetic or apparent solubility. The stability of this supersaturated solution is therefore of paramount importance. The generation (and stabilization) of supersaturated drug solutions is an important aspect of the performance of many contemporary formulation approaches, including solid dispersions and lipid-based formulations, and is addressed elsewhere in this review (see Sections X and XI, respectively). Regardless of the manner in which supersaturation is generated (often described as the “spring” required to generate supersaturation), a means of reducing the rate of recrystallization of the stable crystal form (i.e., a “parachute”) is important. In the case of cocrystals, this may be achieved by judicious choice of cocrystal designs that do not necessarily promote the highest kinetic solubility (since this also generates the greatest driving force for recrystallization) but rather provide the slowest rate of transformation to the less-soluble form. Alternatively, formulation additives may be used in an attempt to stabilize the supersaturated solution; to this end, surfactants and polymers have recently been explored as a means of stabilizing supersaturated cocrystal solutions (Remenar et al., 2007; Huang and Rodriguez-Hornedo, 2010; Good et al., 2011).

4. Design and Preparation of Cocrystals. Traditionally, pharmaceutical cocrystals have been formed via empirical attempts to cocrystallize a drug of interest with a wide range of pharmaceutically approved or acceptable cocrystallizing agents or coformers. The advent of in silico crystal engineering methods now provides an opportunity for more rational cocrystal design. Central to this design step is the identification of intermolecular interactions, or synthons, that form between the drug and a potential cocrystal former. These interactions ultimately lead to the formation of supramolecular assemblies and a crystal structure when extended into three-dimensional space. Synthon selection during cocrystal design has been aided significantly by large number of cocrystal structures listed in the Cambridge Structural Database (Allen, 2002; Shan and Zaworotko, 2008).

Although the rational design of the supramolecular synthon provides a starting point for cocrystal assembly, it is not always possible to translate this into reality (Aakeroy and Salmon, 2005). Indeed, factors other than the theoretical molecular compatibility between a drug and a cocrystal former may dominate, including the intrinsic unpredictability of crystallization and the difficulties associated with distinguishing between the interactional hierarchies that commonly exist when the drug contains multiple functional groups with the capacity of forming multiple supramolecular synthons with a coformer (Haynes et al., 2004; Aakeroy and Salmon, 2005). Therefore, both computational and empirical studies are required to support cocrystal design (Shan and Zaworotko, 2008; Arora and Zaworotko, 2009), and it is common to screen for potential cocrystal hits using a range of coformers. These may be chosen preferentially from coformers sharing the same functional groups (Remenar et al., 2005b; Childs et al., 2004; Childs and Hardecastle, 2007; Stanton et al., 2011) but might also include, for example, a mixture of acids and amides to explore whether assemblies of homosynthons or heterosynthons are preferred (Fleischman et al., 2003; Cheney et al., 2010).

Cocrystals can be prepared from solutions containing stoichiometric amounts of drug and cocrystal former followed by removal of the solvent through evaporation or by cooling the mixture to induce precipitation of the solid phase (Etter and Reutzel, 1991; Fleischman et al., 2003; Walsh et al., 2003; Childs et al., 2004). Other common preparation methods include ball mill cogrinding, antisolvent addition, melt cooling, sublimation, ultrasonication, slurry conversion, and supercritical technologies (Morissette et al., 2004; Arora and Zaworotko, 2009; Padrela et al., 2009). More recently, the solvent-drop method has been increasingly used, in which small amounts of solvent are added during cogrinding of the drug and coformer (Trask et al., 2004). Solvent methods are limited by virtue of the often wide differences in solubility of the drug and coformer (Morissette et al., 2004; Qiao et al., 2011), but they offer the advantage of more rapid rates of crystallization and the absence of physical and thermal stresses. In general, however, these techniques do not assure isolation of the cocrystal and may result in crystallization of the single crystal phases of drug or coformer alone. Phase diagrams are beneficial in this regard as they reveal the solvent:drug:coformer ratios at which the most stable phase is the cocrystal (Chiarella et al., 2007; Ainouz et al., 2009). This is particularly important when the drug and the coformer show wide differences in solubility in the solvent (Blagden et al., 2007; Chiarella et al., 2007). An understanding of the phase solubility behavior of drug, coformer, and solvent underpins the reaction crystallization method (RCM) of cocrystal formation where excess drug is added to saturated or near-saturated solutions of coformer. The RCM is described in more detail in Section V.B.2 and in Fig. 19. The methods used to prepare cocrystals can strongly influence the possibility of success, and a large number of potential coformers can be investigated. Consequently, several studies have presented high-throughput screening approaches to cocrystal synthesis.
and physicochemical characterization (Remenar et al., 2003a; Morissette et al., 2004; Seefeldt et al., 2007; Zhang et al., 2007; Childs et al., 2008; Lu et al., 2008; Kojima et al., 2010).

As with all solid-state screening methods, the isolated solid phase must be characterized, and common spectroscopic techniques used to verify the presence of a cocrystal include XRPD, NIR, FTIR, and Raman Spectroscopy. The presence of a single melting endotherm by DSC has been widely used to verify the presence of a cocrystal. Single-crystal X-ray diffractometry is also increasingly being used to determine structural details of the cocrystal (McNamara et al., 2006; Remenar et al., 2007), and NMR and HPLC are being used to verify the stoichiometric ratio of the complex (Bak et al., 2008). An additional characterization step is required where drug and coformer have the potential to undergo an acid-base reaction to form a salt rather than a cocrystal. By definition, components of cocrystals are neutral. However, many drug compounds contain potential sites of ionization that may cooperate with an oppositely charged group on the coformer. To identify whether a salt or cocrystal results, the position of the proton of interest must be characterized, most commonly via single crystal X-ray diffractometry, solid-state NMR, or X-ray photoelectron spectroscopy (Aakeroy et al., 2007; Stevens et al., 2010; Remenar et al., 2007), and each cocrystal showed superior rates of dissolution in comparison with AMG 517 and increased bioavailability after oral administration to rats. In these studies, cocrystals were dosed in suspensions supplemented with 1% PVP to reduce the rate of recrystallization of drug (rather than cocrystal) since this process was shown, in vitro, to be rapid (<1 h for most of the investigated cocrystals) (Stanton et al., 2010).

### TABLE 13

<table>
<thead>
<tr>
<th>Drug</th>
<th>Coformer(s)</th>
<th>Effect of Cocrystallization on Drug Dissolution and/or In Vivo Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exemestane (Shiraki et al., 2008)</td>
<td>Maleic acid</td>
<td>A faster intrinsic dissolution rate compared with drug alone, although enhancement in dissolution rate was limited by a rapid rate of transformation of the cocrystal to the drug in solution.</td>
</tr>
<tr>
<td>Megestrol acetate (Shiraki et al., 2008)</td>
<td>Saccharin</td>
<td>A 3.8-fold increase in dissolution rate generating supersaturation but with rapid (within 20 min) transformation and recrystallization of drug alone. After 4 h, concentrations of dissolved drug were 2-fold greater than the equilibrium solubility of the drug alone.</td>
</tr>
<tr>
<td>Indomethacin (Jung et al., 2010)</td>
<td>Saccharin</td>
<td>A 1.7- and 3.6-fold increase in intrinsic dissolution at pH 1.2 and 7.4, respectively. Approximately a 2-fold increase in AUC in beagle dogs and a &gt;6-fold increase in Cmax.</td>
</tr>
<tr>
<td>Meloxicam (Cheney et al., 2011)</td>
<td>Aspirin</td>
<td>A &gt;40-fold increase in kinetic solubility. A &gt; 4-fold increase in AUC in rats. The PABA cocrystal showed faster (&gt;1.4-fold) intrinsic dissolution rate compared with drug alone. The dissolution rate of an arginine salt was faster than the urea cocrystals but was more susceptible to hydrate formation.</td>
</tr>
<tr>
<td>Nitrofurantoin (Cherukuvada et al., 2011)</td>
<td>p-Aminobenzoic acid (PABA) Urea</td>
<td>Theobromine</td>
</tr>
</tbody>
</table>

Tmax, time after administration that a drug is at its maximal concentration.
Cocrystal formulations have been described for the poorly water-soluble sodium channel blocker, CFPC [2-[4-(4-chloro-2-fluorophenoxy)-phenyl]pyrimidine-4-carboxamide]. CFPC has a low pK_a, which precludes facile salt formation with most acidic counterions, and a low glass transition temperature (T_g = 43°C), rendering it highly sensitive to recrystallization from amorphous formulations (McNamara et al., 2006). Cocrystal approaches to solubility enhancement were therefore attempted, and a glutaric acid containing cocrystal was selected for in vitro and in vivo analysis. In comparison with the free base, the glutaric acid cocrystal showed an 18-fold increase in intrinsic dissolution rate (Fig. 22A). Consistent with the increase in in vitro dissolution rate, a 2.5- to 3.3-fold increase in bioavailability was also observed in fasted beagle dogs compared with the drug crystal when the cocrystal was dosed at 5 or 50 mg/kg (McNamara et al., 2006) (Fig. 22B).

Cocrystal strategies have also been used to improve the in vivo pharmacokinetic profile (increased exposure, faster onset of action) of L-883555 (a novel phosphodiesterase-IV inhibitor complexed with l-tartaric acid) (Variankaval et al., 2006), indomethacin (with saccharin) (Jung et al., 2010), and meloxicam (with aspirin) (Cheney et al., 2011). In the last example, an unconventional approach to cocrystal design was used where meloxicam-aspirin cocrystals were successfully formed after only consultation of the Cambridge Structural Database and a priori knowledge of a pharmacological interaction between these two compounds (coadministration of commercial meloxicam (Mobic; Boehringer Ingelheim, Ridgefield, CT) and aspirin had previously led to an unexplained increase in AUC] (Cheney et al., 2011).

C. Summary

Polymorphism in pharmaceutical solids is common. Decreases in intermolecular attractive forces in high-energy polymorphs and the amorphous form (compared with the stable crystal form) dictate that these materials exhibit higher apparent solubilities and have the potential to improve dissolution rate, solubilization, and absorption for poorly water-soluble drugs. However, the use of high-energy polymorphs (or more commonly the amorphous form) is complicated by thermodynamic instability in the solid state. Additional approaches must therefore be taken to stabilize formulations to provide acceptable shelf-life. This is commonly achieved by combination with excipients such as polymers to form solid dispersion formulations (these are described in more detail in Section X).

Crystal-engineering strategies provide an additional approach for the manipulation of crystal habit and an additional opportunity to reduce the strength of the crystal lattice and to promote solubility and absorption for poorly water-soluble drugs. Cocrystals constitute a molecular complex between a drug and a water-soluble cocrystal former and are in some respects structurally similar to salts. In contrast to salts, however, the complex between drug and cocrystal former does not involve proton exchange; therefore, cocrystals can be formed by nonionizable drug molecules.

VI. Cosolvents

A. Rationale for the Use of Cosolvents for the Solubilization of Poorly Water-Soluble Drugs

Cosolvents are water-miscible organic solvents and are widely used to increase the solubility of poorly water-soluble substances (Yalkowsky, 1999; Rubino, 2002). Cosolvents are less polar than water and, therefore, enhance aqueous drug solubility by lowering
the polarity of the bulk solvent to a level that more closely reflects the polarity of the nonpolar solute (e.g., drug).

Cosolvent formulations are used routinely for the parenteral delivery of drugs during preclinical assessment and are widely used in marketed parenteral products, including diazepam (Valium; Hoffmann-La Roche, Inc., Nutley, NJ), digoxin (Lanoxin; GlaxoSmithKline, Research Triangle Park, NC), melphalan hydrochloride (Alkeran; GlaxoSmithKline), and lorazepam (Ativan; Wyeth-Ayerst, Radnor, PA). Their popularity can be attributed to the relative ease of use in formulation design and the potential to increase dramatically the level of solubilized drug.

Drug candidates most well suited to cosolvency include those that lack ionicizable functionalities (and therefore are not suitable for pH adjustment strategies) and those with moderate log P (between 1 and 3) that show relatively low affinity for solubilization by surfactants or lipids (Kipp, 2007). In addition, cosolvents are commonly used in combination with other solubilization strategies. For example, the use of a cosolvent with a surfactant can allow high concentrations of solubilized drug to be obtained (via discrete mechanisms) at lower concentrations of each excipient. Surfactants are also widely used in conjunction with cosolvents to reduce the risk of drug precipitation as cosolvent-based formulations are diluted in vitro or in situ.

Cosolvents are also widely used in formulations designed for oral delivery. In this case, however, cosolvents are commonly a minor component of the formulation, and other excipients provide the major solubilization enhancement. Lipid-based formulations are typical of systems of this type and may comprise lipids, surfactants, and cosolvents. Under these circumstances, cosolvents may be included to reduce viscosity, to facilitate dispersion, and to enhance drug solubility in the formulation. These formulations are described in more detail in Section XI.

The focus of the present section is on the utility of cosolvents in enhancing drug solubility for parenteral delivery where the most commonly used cosolvents include ethanol, propylene glycol, and low-molecular-weight polyethylene glycol (PEG). Here we describe the fundamental concepts that underpin the solubilization potential of cosolvents, the potential of the log-solubility model to predict drug solubility in cosolvent systems, the key formulation/nonformulation factors that can affect solubilization, and, finally, the potential pharmacological effects of cosolvents.

### B. Commonly Used Organic Cosolvents in Parenteral Drug Delivery

Cosolvents are used in 10 to 15% of FDA-approved parenteral products (Nema et al., 1997), and the most widely used cosolvents are propylene glycol, ethanol, glycerol, and PEG 300/400. Examples of the cosolvent concentrations currently used in marketed formulations are shown in Table 14, where it is apparent that the concentrations of cosolvent used are often high and many formulations contain >50% (v/v) cosolvent (e.g., Prograf (Astellas Pharma US, Inc, Deerfield, IL), melphalan (Alkeran; GlaxoSmithKline, Research Triangle Park, NC), and lorazepam (Ativan; Wyeth-Ayerst, Radnor, PA). In this case, formulations for i.v. administration are diluted before use to minimize irritation at the injection site (see Section VI.F). However, dilution of the formulation will significantly reduce the solubilization capacity of the cosolvent, thereby introducing the risk of drug precipitation. Alongside the possibility of incompatibility or toxicity, the potential for drug precipitation either on dilution before administration or on injection is a key criterion.

<table>
<thead>
<tr>
<th>Cosolvent</th>
<th>Recommended Range for Preclinical Use (w/v)</th>
<th>Frequency of Use in Commercial Formulations</th>
<th>Concentrations Used in Some Commercial Formulations</th>
<th>Final Cosolvent Concentration Administered (%w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>30–60%</td>
<td>17</td>
<td>70% in Multitest CMI</td>
<td>Not diluted before s.c. use.</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>50%</td>
<td>32</td>
<td>80% in Ativan</td>
<td>≤40% for i.v. bolus dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40% in Dilantin</td>
<td>Direct i.v. injection with a maximum rate of administration of 1 ml/min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40% in Valium</td>
<td>Direct i.v. injection with a maximum rate of administration of 1 ml/min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40% in Lanoxin</td>
<td>≤25% before i.v. infusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>68% in Luminal</td>
<td>≤68% before i.v. bolus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60% in Alkeran</td>
<td>≤1% before i.v. infusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100% in alprostadil injection USP</td>
<td>≤4% for i.v. infusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>49% in Taxol</td>
<td>≤10% before i.v. infusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>80% in Prograf</td>
<td>≤0.32% before i.v. infusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42% in Vumon</td>
<td>≤0.84% before i.v. infusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50% in methacarbamol</td>
<td>Direct i.v. injection with a maximum rate of administration of 3 ml/min.</td>
</tr>
<tr>
<td>Polyethylene glycol 300</td>
<td>10%</td>
<td>34</td>
<td>67% in Busulfex</td>
<td>≤5.6% before i.v. infusion</td>
</tr>
<tr>
<td>Polyethylene glycol 400</td>
<td>40–100%</td>
<td>4</td>
<td>33% in Busulfex</td>
<td>≤2.75% before i.v. infusion</td>
</tr>
<tr>
<td>DMPS</td>
<td>10–20%</td>
<td>2</td>
<td>4% before i.v. infusion</td>
<td>≤2.5% before i.v. infusion</td>
</tr>
<tr>
<td>DMA</td>
<td>10–30%</td>
<td></td>
<td>5.6% before i.v. infusion</td>
<td></td>
</tr>
</tbody>
</table>

* % w/v, on the basis of single dosing 10 ml/kg to a mouse or rat. Li and Zhao (2007). See text for additional details.

* Values obtained from Nema and Brendel (2011), Strickley (2004), and Bouquet and Wagner (2012).
in the design of parenteral cosolvent formulations. As lower formulation volumes are typically used during i.m. administration, higher cosolvent concentrations (i.e., lower dilutions) may be used if the formulation is intended for this route (Gad et al., 2006). Table 14 provides some guidance on the recommended concentrations of cosolvents for preclinical use (Li and Zhao, 2007). Although formulations typically do not contain ethanol at greater than 10% v/v, because of potential central nervous system and respiratory depressive effects, there are many exceptions. For example, ethanol concentrations between 15% and 20% v/v have been administered intravenously to canines (Chang et al., 2007; Gu et al., 2009); in rats, ethanol concentrations up 80% have been used (Hanafy et al., 2007). Recommended concentrations of propylene glycol and PEG 400 that may be administered intravenously are usually much higher than ethanol (Table 14). Propylene glycol has been administered intravenously to beagles at concentrations between 40 and 60% (Joshi et al., 2004; Merritt et al., 2007), and formulations containing up to 100% propylene glycol (PG) have been administered to rats (Gershkovich and Hoffman, 2007; Usach and Peris, 2011). A recent study reported incidences of reversible renal toxicity and increased water consumption/dry mouth in dogs that were intravenously dosed with up to 8.75 g/kg PEG 400 per day over 30 consecutive days, confirming that this cosolvent is generally well tolerated (Li et al., 2011a).

Dimethyl sulfoxide (DMSO) is not used in commercial parenteral formulations because of the potential for membrane penetration and cell lysis and other potential hemodynamic disturbances (e.g., clotting, changes to arterial blood pressure) (Mottu et al., 2000). Nonetheless, DMSO is widely used in preclinical formulations (usually up to 20% v/v) (Vennerstrom et al., 2004; Liddle et al., 2008; Caliph et al., 2009; Kapetanovic et al., 2010; Usach and Peris, 2011), and a DMSO containing busulfan intravenous formulation (containing up to 2% v/v DMSO, equivalent to ≤0.5 ml) has been tested in canine models without showing any signs of adverse effects (Ehniger et al., 1995) and in human subjects receiving a single i.v. dose who were also free from pain at the injection site (Schuler et al., 1998). Cosolvent combinations are widely used in preclinical i.v. formulations. Ethanol has been frequently used in combination with propylene glycol (Kamath et al., 2005; Zhang et al., 2006; Otaegui et al., 2009) or PEG 400 (Stella et al., 1995; Chan et al., 1998; Choo et al., 2011); DMSO has been used with propylene glycol combinations (Chen et al., 2007; Hu et al., 2009; Usach and Peris, 2011).

Examples of oral products that contain significant amounts of cosolvent include liquid-filled bexarotene capsules (Targetetin; Eisai Inc., Woodcliff Lake, NJ), which use PEG 400 as the cosolvent; etoposide capsules (VePesid; Bristol-Myers Squibb, Stamford, CT), which also contains PEG 400 but with glycerin, citric acid, and a small amount of water; amprenavir capsules and oral solution (Agenerase; GlaxoSmithKline), which contain PEG 400, propylene glycol, TPGS, sodium chloride, sodium citrate, and citric acid.

C. Solubilization by Cosolvents

1. Cosolvent Effects on Solubility Parameters. Cosolvents increase the aqueous solubility of poorly water-soluble drugs by disrupting the intermolecular hydrogen bonding networks that are present in aqueous systems. This leads to a decrease in the polarity of the solvent and the creation of an environment with physicochemical properties that are more similar to that of the solute (i.e., drug). Simplistically, this results in an increase in drug solubility according to the general principle of “like dissolves like.”

More accurately, the effect of cosolvents on drug solubility may be assessed using the solubility parameter approach. The role of solubility parameters in ideal and nonideal solubility was discussed in Section IB.1 of this review. In brief, differences between the solubility parameters of solute (drug) and solvent provide an indication of the differences in intermolecular forces in the solute (i.e., solute-solute interactions) and solvent (i.e., solvent-solvent interactions) and in turn provide an indication of how close the properties of the solution are to an ideal solution (eq. 8).

In an ideal solution, intermolecular forces between solute-solute and solvent-solvent molecules are equal, and under these conditions, solubility is essentially maximal (and solvent independent). However, the large differences between intermolecular bonds in water and most poorly water-soluble solutes dictate that almost all aqueous solutions are nonideal. Deviations from ideality result in a decrease in solubility, and the magnitude of the deviation from ideality is captured by the activity coefficient (γ) (eq. 7), which in turn is a function of the difference in the solubility parameters of solute and solvent (eq. 8).

The solubility parameter (δ) may be determined experimentally by measuring the energy required to vaporize (E_v) a solute or solvent of molar volume V_m where

δ = \sqrt{\frac{\Delta E_v}{V_m}} \tag{30}

More commonly, however, solubility parameters are estimated using the group contribution approach, as the total cohesive forces of a molecule reflect the cohesive properties of the individual nonpolar and polar fragments (Fedors, 1974). The contributions to total cohesive force (δ_v) of dispersive forces (δ_d), the polar permanent dipole (δ_p), and hydrogen bonding forces (δ_h) are provided by the Hansen partial solubility parameters (eq. 31):


Experimentally calculated solubility parameters for most cosolvents are readily available in the literature, and values for some of the most commonly used cosolvents are shown in Table 15. As a general rule, the solubility parameter decreases with decreasing cosolvent polarity as the capacity to self-associate via hydrogen bonding is reduced. In contrast, water has the highest solubility parameter. The solubility of nonpolar solutes (the solubility parameters for which are low relative to that of water) is therefore higher in cosolvents or in mixed cosolvent-water solutions with lower solubility parameters.

The dielectric constant (\(\varepsilon\)), defined by the ability of a material to concentrate electric lines of flux, also provides a measure of cosolvent polarity. In the context of cosolvent systems, dielectric properties provide a measure of the ability of the solution to allow electrolyte dissociation into respective ions. Water, for example, has a high dielectric constant, readily allows ionic dissociation, and therefore is a good solvent for polar and ionizable compounds. In contrast, the high dielectric constant and polarity of water result in lower solubility for nonpolar molecules. Dioxane, by comparison, is miscible with water but has a very low dielectric constant (\(\varepsilon = 2.25\), Table 15). Blending dioxane with water in differing ratios, therefore, generates a series of standard solutions with known dielectric constant. Evaluation of the solubility of a solute in these dioxane-water mixtures allows the dielectric constant required for maximum solubility to be identified [the dielectric requirement (DR)]. Since maximum solubility will occur at the same (or within a narrow range) DR value in other cosolvent systems, the DR value can be used to calculate the proportion of cosolvent(s) required to achieve maximum solubility in a defined solvent-cosolvent pair (Paruta et al., 1964). Importantly, however, the solubility value at this maximum will vary depending on the nature of the cosolvent pairs used; hence, the DR is able to provide only the ideal ratio for combinations of specific solvents (see the following section below).

Other commonly used descriptors of cosolvent polarity include interfacial tension and oil-water partition coefficient. Values of these parameters for commonly used cosolvents are also presented in Table 15.

2. Predicting Solubility in Cosolvent Systems: The Log-Linear Solubility Model. A number of models have been described that aim to predict drug solubility in cosolvent combinations, and for further information, the interested reader is directed to a recent review by Jouyban (Jouyban 2008). Of these, the log-linear solubility model, proposed by Yalkowsky, is perhaps the most widely used (Li et al., 1999b,d; Yalkowsky, 1999; Millard et al., 2002; Kawakami et al., 2004; Kawakami et al., 2006; Tomsic et al., 2006; Dong et al., 2008). The log-linear model suggests that the molar solubility of a solute in a mixed solvent system is a function of drug solubilities in the individual components (Yalkowsky, 1999; Millard et al., 2002). Equation 32 describes the relationship between the solubility of a solute in a binary water-cosolvent system (\(S_{\text{mix}}\)), where \(S_w\) is the molar solubility of the solute in water, \(f_c\) is the volume fraction of the cosolvent, and \(\sigma\) is the solubilization power of the cosolvent. Equation 33 may be further used to estimate drug solubility in a system containing multiple cosolvents, although it assumes that the different cosolvents exhibit no specific nonideal interactions (i.e., that the solubilization powers of the different solvents are simply additive) (Millard et al., 2002):

\[
\log S_{\text{mix}} = \log S_w + \sigma f_c \tag{32}
\]

\[
\log S_{\text{mix}} = \log S_w + \sum \sigma f_c \tag{33}
\]

Knowledge of the intrinsic solubility of the solute in water and the solubilization power of the cosolvent (\(\sigma\)) can therefore be used to predict drug solubility at a particular cosolvent concentration. The value for \(\sigma\) may also be calculated using eq. 34, where \(s\) and \(t\) are constants for a particular solvent (but are solute independent) and have been determined for a variety of solvents.

<table>
<thead>
<tr>
<th>Cosolvent</th>
<th>Solubility Parameter(^a) ((\delta))</th>
<th>Dielectric Constant(^b) ((\varepsilon))</th>
<th>Interfacial Tension(^c) ((\gamma))</th>
<th>Log K(_{ow})(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>23.4</td>
<td>81.0</td>
<td>72.0</td>
<td>−4.00</td>
</tr>
<tr>
<td>Glycerol</td>
<td>16.5</td>
<td>42.5</td>
<td>64.9</td>
<td>−2.60</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>14.6</td>
<td>37.7</td>
<td>48.8</td>
<td>−1.93</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>12.6</td>
<td>37.7</td>
<td>37.1</td>
<td>−1.40</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.7</td>
<td>24.3</td>
<td>22.2</td>
<td>−0.31</td>
</tr>
<tr>
<td>DMA</td>
<td>12.0</td>
<td>46.7</td>
<td>38.0</td>
<td>−1.09</td>
</tr>
<tr>
<td>PEG 400</td>
<td>11.3</td>
<td>13.6</td>
<td>46.0(^a), 11.7(^a)</td>
<td>−1.30</td>
</tr>
<tr>
<td>DMSO</td>
<td>10.8</td>
<td>37.8</td>
<td>35.7</td>
<td>−0.66</td>
</tr>
<tr>
<td>Dioxane</td>
<td>9.9</td>
<td>2.2</td>
<td>33.6</td>
<td>−0.42</td>
</tr>
</tbody>
</table>

\(K_{ow}\), the oil-water partition coefficient.

\(^a\) Values obtained from Yalkowsky (1999).

\(^b\) Values obtained from Rubino (2002).

\(^c\) Values obtained from Fauconnet (1999).

\(^d\) Values obtained from Robins (2002).
of commonly used cosolvents using experimental solubility data for different polar and nonpolar compounds (Millard et al., 2002). $K_{ow}$ is the oil-water partition coefficient for the solute:

$$\sigma = \text{slog} K_{ow} + t$$  \hspace{1cm} (34)

An example of the applicability of the log solubility model is provided in Fig. 23, which plots the solubility of a series of alkyl $p$-aminobenzoates as a function of propylene glycol concentration in water (Yalkowsky et al., 1972). For each solute, excellent agreement is evident between the experimentally determined solubility (symbols) and predicted solubility (solid lines) according to eq. 32. It is also apparent that the effect of cosolvent concentration (i.e., solubilizing power) becomes increasingly more pronounced with increasing alkyl chain length (and therefore hydrophobicity) of the solute, as suggested by eq. 34. Indeed, near-linear relationships have been identified between the solubilization power ($\sigma$) of many commonly used cosolvents (ethanol, propylene glycol, PEG 400, glycerol) and the hydrophobicity of the solute (as described by log $K_{ow}$) (Millard et al., 2002).

Since $s$ and $t$ are constants for a particular cosolvent, eq. 34 also predicts that the rank order of solubilization power for a range of cosolvents should be consistent across different solutes. However, this is not always the case, and the literature provides many examples where rank-order solubility for different solvent systems is highly compound specific (Table 16). For example, solubilization of carbendazim at pH 7 (where the drug is predominantly un-ionized) follows the rank order of solubility (as described by log $K_{ow}$) (Millard et al., 2002).

A limitation to the use of the log-linear solubility model is the lack of applicability for semipolar solutes (i.e., those solutes that are more polar than cosolvent but less polar than water). These compounds show a solubility maximum at cosolvent mixtures between 0 and 100% cosolvent. This is in contrast to the more common scenario of polar or nonpolar solutes (where the log-linear model appears to have broader applicability), where solubility is greatest in 100% water or 100% cosolvent, respectively (Morris et al., 1988; Yalkowsky, 1999; Millard et al., 2002; Rubino, 2002). Experimentally determined log solubility versus cosolvent fraction plots also often show deviation from the model at high cosolvent concentrations, where measured solubility is higher or lower than the solubility predicted by the linear log model. Negative deviations (i.e., solubility lower than predicted) are more prevalent at low cosolvent concentrations (e.g., $<50\%$) and may be due to the ability of water to retain its ordered structure and the formation of some water-cosolvent intermolecular hydrogen bonds, in turn decreasing the potential number of solvent-solute interactions.

![Fig. 23. Log-linear solubility relationship for a series of alkyl $p$-aminobenzoates in propylene glycol-water mixtures. A decreasing slope reflects decreasing log $P$ values for the various solutes. Alkyl chain lengths on the drug were ethyl ($C_2$), butyl ($C_4$), hexyl ($C_6$), octyl ($C_8$), dodecyl ($C_{12}$). Adapted from Yalkowsky et al. (1972).](image)

### Table 16

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rank Order in Solubilization Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antalarmin (Sanghvi et al., 2009)</td>
<td>Ethanol &gt; PG &gt; PEG 400</td>
</tr>
<tr>
<td>Phenytoin (Kawakami et al., 2004; 2006)</td>
<td>DMA &gt; ethanol = PEG 400 &gt; DMSO</td>
</tr>
<tr>
<td>NSC-638289, pH 7 (un-ionized) (Jain et al., 2001)</td>
<td>Ethanol &gt; DMSO &gt; PEG 400 &gt; PG</td>
</tr>
<tr>
<td>NSC-638289, pH 2 (Jain et al., 2001)</td>
<td>Ethanol &gt; PEG 400 &gt; PG &gt; DMSO</td>
</tr>
<tr>
<td>NSC-638289, pH 1 (ionized) (Jain et al., 2009)</td>
<td>PG &gt; ethanol &gt; PEG 400 &gt; DMSO</td>
</tr>
<tr>
<td>Carbendazim (Ni et al., 2002)</td>
<td>PEG 400 &gt; ethanol &gt; PG &gt; glycerol</td>
</tr>
<tr>
<td>N-Epoxymethyl-1,8-naphthalimide (Dong et al., 2008)</td>
<td>Ethanol &gt; PEG 400 &gt; PG &gt; glycerol</td>
</tr>
<tr>
<td>PG-300995 (Ran et al., 2005)</td>
<td>Ethanol &gt; PEG 400 &gt; PG &gt; glycerol</td>
</tr>
</tbody>
</table>

PG, propylene glycol.

$^a$ Solubilization was determined over a 0–20% w/v cosolvent range, with the exception of studies by Jain et al. (2001) and Dong et al. (2008), where maximum cosolvent concentrations were 40–40% and 50%, respectively.
(Kimura et al., 1975; Rubino and Obeng, 1991). At higher cosolvent concentrations (e.g., >50%), there is a significant breakdown of ordered water structure. More water molecules are therefore available to participate in solute-solvent interactions, potentially leading to positive deviations (i.e., solubility higher than predicted) in log solubility plots (Rubino and Yalkowsky, 1987; Rubino and Obeng, 1991). Alternatively, these positive deviations from predicted solubility have been attributed to changes to the solid-state drug properties, in particular, the formation of more soluble solvates (Flynn et al., 1979; Nicklasson and Brodin, 1984).

D. Impact of Solubilizers and Electrolytes on Cosolvent-Mediated Drug Solubilization

1. Cosolvents and pH Adjustment. Buffers are widely used to improve the solubilization of ionizable compounds (see Section IV). In the presence of a cosolvent, the total solubility of an ionizable solute is a function of the concentration of both the un-ionized and ionized species and the solubilization power of the cosolvent toward the un-ionized \( \sigma_u \) and ionized forms \( \sigma_i \). Since cosolvent solubilization power is dependent on the hydrophobicity of the solute (eq. 34), the level of cosolvency decreases with increasing drug ionization, potentially reducing the solubilization advantage of the cosolvent.

This effect is illustrated in Fig. 24, where increasing concentrations of a variety of cosolvents (ethanol, propylene glycol, DMSO, and PEG 400) increase the solubility of NSC-639829 (a poorly soluble weak base), but the effect (power) of each cosolvent diminishes with decreasing pH (as drug ionization increases) (Jain et al., 2001). However, the reduction in solubilization capacity with increased drug ionization is not sufficient to cause a decrease in the total drug solubility, and in all cases, the beneficial effect of increasing ionization on solubility outweighs the reduction in solubilization power of the cosolvent. Thus, a solubility advantage of a combination strategy of cosolvent plus pH manipulation is evident, although the beneficial effects of pH adjustment are reduced at high cosolvent fractions. As such, cosolvent-pH buffering combination strategies are widely used in the development of preclinical parenteral formulations and for commercial formulations, for example, chloridrazepoxide (Librium; propylene glycol 20%, pH 3), D.H.E. 45 (15% glycine, 6.1% ethanol, pH 3.6), fenoldopam (Corlopam; propylene glycol 50%, pH 3), oxytetracycline (Terramycin; propylene glycol 65–75%, pH 3), pentobarbital (Nembutal; Lundbeck Inc., Deerfield, IL (propylene glycol 40%, ethanol 10%, pH 9.5]), and phenytoin (Dilantin; propylene glycol 40%, ethanol 10%, pH 10–12.3) (also see Section IV.B.2.a).

2. Cosolvents and Surfactants. Micellar solubilization is a common mechanism by which the solubility of poorly water-soluble drugs may be enhanced (see Section VII), and cosolvent-surfactant combination strategies have been widely explored. Kawakami and coworkers, for example, showed that combining DMA or DMSO with a nonionic surfactant, Gelucire 44/14 (Gattefossé Pharma, Lyon, France) led to a moderate increase in the solubility of both phenytoin and indomethacin and that the solubility of phenytoin increased with the addition of Tween 80 or sodium dodecyl sulfate (SDS) to a variety of cosolvent formulations (ethanol, PEG 400, glycerol or DMA) (Kawakami et al., 2004, 2006). In all cases, however, the additive effect on total solubility was small. The lack of a more significant increase in solubility is first due to the ability of the cosolvent to increase the aqueous solubility of the surfactant, thereby increasing the CMC and decreasing micelle volume, both factors that reduce the solubilization potential of the surfactant (see Section VIII). Second, cosolvent is expected to partition into the micelle, leading to an increase in polarity of the micelle core (rendering it less capable of solubilizing hydrophobic drugs) and reducing the effective extramicellar concentration of cosolvent.

However, despite the limited advantage of surfactant-cosolvent combinations on total solubilization, surfactants have the additional (and highly significant) benefit of reducing the potential risk of drug precipitation on dilution of cosolvent formulations (Li and Zhao, 2007; Jain et al., 2010; Tonsberg et al., 2011). This protective effect reflects the exponential decrease in the solubilizing power of cosolvents with increasing dilution, whereas dilution of surfactant micelles above the CMC leads to a more linear solubility-dilution profile. Surfactant-cosolvent combinations are therefore widely used, even though the effects on total solubility are only moderate (see also Section VII.D.1).

3. Cosolvents and Cyclodextrins. Cyclodextrins, including hydroxpropyl-\( \beta \)-cyclodextrin and sulfobutylether-\( \beta \)-cyclodextrin, have been used in the development of parenteral formulations since they can increase the apparent solubility of a number of poorly water-soluble drugs by forming drug-cyclodextrin inclusion complexes (see Section VIII).

In general, the combination of cyclodextrins with cosolvents is uncommon, as several factors may contribute to a lack of a positive effect on solubility or even a decrease in total drug solubility on the addition of a cyclodextrin to a cosolvent formulation. First, the cyclodextrin may complex with the cosolvent itself. Analogous to the effect of surface cyclodextrin, this leads to a decrease in effective cosolvent concentration (He et al., 2003), and simultaneous competition between drug and cosolvent for the cyclodextrin hydrophobic cavity will reduce the complexation efficiency of the cyclodextrin toward the drug (van Stam et al., 1996; Miyake et al., 1999b; Evans et al., 2000; Partyka et al., 2001). The complexation efficiency may also decrease
in the presence of a cosolvent by virtue of the drug showing more affinity for the bulk medium (which is less polar in the presence of a cosolvent) and, in turn, a decreased tendency toward the formation of the drug-cyclodextrin inclusion complex. In contrast, factors that may contribute to increased drug solubility in cosolvent-cyclodextrin solutions include 1) higher concentrations of free drug in the presence of a cosolvent, providing an increased driver toward complexation; and 2) the potential for the formation of highly soluble ternary complexes of drug-cosolvent-cyclodextrin, particularly when the cosolvent and drug molecules are small, as this may facilitate simultaneous drug and cosolvent complexation by the cyclodextrin (Reer and Muller, 1993; Li et al., 1999d; Yalkowsky, 1999; Nandi et al., 2003). The latter effect is exemplified by studies showing that the solubility of fluasterone in aqueous ethanol solutions is higher in the presence of hydroxypropyl-β-cyclodextrin and that as little as 0.2% cosolvent (which is insufficient to solubilize a significant amount of drug via cosolvency alone) increases drug solubility in the presence of the cyclodextrin (Li et al., 1999d; He et al., 2003). Consistent with these data, the currently marketed Sporanox IV solution (itraconazole; Janssen Pharmaceuticals, Titusville, NJ) contains 40% hydroxypropyl-β-cyclodextrin and 2.5% propylene glycol (Strickley, 2004) (see also Section VIII.D.2).

4. Cosolvents and Strong Electrolytes. Strong electrolytes such as sodium chloride increase solution polarity, and this acts to oppose the decrease in polarity stimulated by addition of cosolvent. Consequently, the addition of salts to cosolvent systems is generally avoided as it will decrease drug solubility (Yalkowsky, 1999).

Fig. 24. Solubilization of NSC-639829 by ethanol, propylene glycol, DMSO, and PEG 400 at pH 1, 2, and 7. DMSO, dimethyl sulfoxide; PEG, polyethylene glycol. Adapted from Jain et al. (2001).
E. Effects of Dilution on Drug Solubilization by Cosolvents

Dilution of cosolvent solutions in aqueous media, including biologic fluids such as blood, leads to a linear decrease in drug concentration with increasing dilution. In contrast, the solubilizing capacity of cosolvent formulations drops exponentially on dilution (green line, Fig. 25). In many cases, particularly where cosolvent systems contain drug dissolved at concentrations approaching the maximum solubility in the system (e.g., the red line in Fig. 25), dilution has the potential to generate conditions where drug concentrations are supersaturated (i.e., the required concentration on dilution is greater than the drug solubility in the system, X to Y in Fig. 25), and under these circumstances, drug precipitation is likely. Drug precipitation after parenteral administration may cause mechanical or chemical irritation at the injection site, leading to phlebitis (Falchuk et al., 1985; Avis et al., 1986), and potentially more serious systemic effects such as pulmonary embolism (Taniguchi et al., 1998). Solid precipitates are also likely to cause pain during the administration of i.m. injections.

Approaches to minimize the potential risk of precipitation are therefore critical. This may be achieved by 1) reducing the drug load in the formulation (i.e., moving toward the blue line in Fig. 25 rather than the red line); 2) reducing the rate of administration (injection); 3) injecting into larger blood vessels (allowing additional time and a greater supply of plasma proteins, lipoproteins, and red blood cells to which drugs may bind); and 4) the use of combination formulations such as cosolvent-surfactant and cosolvent-pH systems that are less susceptible to solubility decreases on dilution.

These approaches, however, are not always effective in eliminating the risk of drug precipitation on dilution of cosolvent formulations. Indeed, in an analysis of 21 marketed formulations, Johnson et al. (2003) showed that the effect of injection rate on drug precipitation was drug/formulation dependent and that slowing the injection rate did not always reduce precipitation. For example, slowing the rate of infusion reduced precipitation from Valium, Cordarone, nafcillin, and phytonadione formulations, but the same approach led to increased precipitation from Dilantin and dobutamine formulations. Similarly, using a dynamic precipitation model, Ward and Yalkowsky (1993) showed that it was possible to reduce amiodarone precipitation by increasing (rather than reducing) the rate of drug injection into the circulating medium. The authors suggested that the use of a high infusion rate reduced the efficiency of mixing (i.e., the dilution rate) in blood, thereby reducing the risk of precipitation (Ward and Yalkowsky, 1993). In vitro methods for assessing the likelihood of drug precipitation on dilution of solubilizing formulations are described in more detail in Section IV.B.3.a.

F. Potential Pharmacological Properties of Cosolvents

1. In Vitro Assessment of Cosolvent Toxicity. Pain on administration of cosolvent formulations is common and has been largely attributed to hemolysis of red blood cells (Strickley, 2004; Amin and Dannenfelser, 2006). It is therefore routine to screen potential cosolvent formulations for their potential toxicity to red blood cells in vitro. In the method of Reed and Yalkowsky (1985), 0.1 ml of formulation is vortex-mixed with various volumes of blood and incubated at 25°C for 2 min before mixing with 5 ml of saline to quench the potential hemolytic properties of the cosolvent. The level of hemolysis is detected via an increase in optical density of the mixture (i.e., a change in color resulting from the release of hemoglobin after cell lysis). In analogous dynamic tests, the formulation is continuously infused into blood pumped at a given rate before the mixture passes into a saline solution to quench the hemolysis potential. A formulation is considered compatible if it causes hemolysis in less than 10% of cells, whereas formulations resulting in 25% hemolysis during a static test and 2% hemolysis during the dynamic test are classified as hemolytic (Amin and Dannenfelser, 2006).

Data obtained using hemolysis models are highly dependent on experimental variables. For example, levels of in vitro hemolysis are highly dependent on the cell-cosolvent contact time and static methods typically result in higher levels of cell lysis compared with results obtained from dynamic tests and in vivo studies (Krzyszeniak et al., 1997). As such, very short (1 s) contact times and formulation-to-blood ratios of 0.1 have been suggested to capture most adequately the potential toxic effect of a formulation. Traditionally, human blood is used to assess the lytic properties of parenteral
formulations, although rabbit blood has generated consistent cell lysis (Amin and Dannenfelser, 2006).

2. Lytic Effects of Commonly Used Cosolvents. The potential for commonly used cosolvents to cause hemolysis has been widely reported (Mottu et al., 2000). In a static cell lysis model, the concentrations (% v/v) of cosolvent required to induce lysis in 50% of blood cells varied in the following order: dimethyl isosorbide (least toxic = 39.5%) < DMA (37.0%) < PEG 400 (30.0%) < ethanol (21.2%) < propylene glycol (5.7%) < DMSO (5.1%) (Reed and Yalkowsky, 1985). The same rank order of hemolytic potential was reported in a later study using similar methods (Fu et al., 1987). Dynamic models have also suggested that propylene glycol may be more toxic than ethanol (Krzyzaniak et al., 1997). Extending these suggestions into in vivo studies in rats (Fu et al., 1987) and dogs (Fort et al., 1984), where animals were intravenously dosed various cosolvent formulations on a daily basis over a 2-week period, ethanolic (10%) formulations containing propylene glycol (40 and 50%) again showed more irritation and hemolysis (detected via increased hemoglobin content in the urine) compared with equivalent ethanolic formulations containing up to 40% PEG 400.

The toxic effects of propylene glycol toward red blood cells have been attributed to the hypotonicity of propylene glycol solutions, although there is evidence that this may be attenuated both in vitro and in vivo when used in combination with ethanol (Reed and Yalkowsky, 1985; Amin and Dannenfelser, 2006) or PEG 400 (Fu et al., 1987; Amin and Dannenfelser, 2006) and in saline or isotonic phosphate (compared with water) (Reed and Yalkowsky, 1986; Fu et al., 1987; Amin and Dannenfelser, 2006). Indeed, it should be noted that propylene glycol and ethanol are by far the most widely used cosolvents in parenteral formulations. Ativan injection (containing lorazepam), for example, contains 80% propylene glycol and is diluted only 1:1 with saline, dextrose 5%, or lactated Ringer’s solution before administration or following mixing with blood) and the cell-based toxicity of cosolvent, in particular, after i.v. administration.

VII. Surfactants

A. Rationale for the Use of Surfactants to Enhance the Solubilization of Poorly Water-Soluble Drugs

Surfactants are commonly used to solubilize poorly water-soluble drugs via drug incorporation into surfactant micelles, and they may also support enhanced delivery via improvements to wetting and in the stabilization of suspension and nanosuspension formulations (Attwood and Florence, 1983; Yalkowsky, 1999; Malmsten, 2002). Micellar solution–based formulations are simple and effective and as such have been widely used, largely after i.v. administration. Some examples of marketed parenteral products include paclitaxel (Taxol; Bristol-Myers Squibb), cyclosporine (Sandimmune; Novartis AG, Basel Switzerland), amiodarone hydrochloride (Cordarone Intravenous; Pfizer), and calcitriol (Calcijex; Abbott Laboratories).

Several surfactant-based parenteral formulations have been developed as nonaqueous pre concentrates (docetaxel; Sanofi-Aventis, Bridgewater, NJ), Prograf, Taxol) and therefore require significant dilution before use. Sandimmune, for example, comprises drug dissolved in a combination of surfactant and cosolvent (65% Cremophor EL, 35% ethanol) that is diluted at least 50- to 100-fold with normal saline or 5% dextrose before i.v. administration. The requirement for dilution before administration, and the inevitable dilution that occurs postinjection, leads to the possibility of drug precipitation; to avoid this, a number of combination surfactant-containing formulation approaches have been explored (Li et al., 1999c; Li and Zhao, 2003; Jain et al., 2004; Strickley, 2004; Li and Zhao, 2007). These include surfactant-cosolvent combinations and surfactants combined with pH-adjustment strategies. These approaches reflect the realization that a single solubilization strategy is often insufficient to solubilize the entire dose of drug. Combination approaches allow the use of lower concentrations of a single component, a strategy that has found favor since high surfactant concentrations have the potential to cause local or systemic adverse reactions after parenteral administration, including pain at the injection site or more serious hypersensitivity reactions (Gelderblom et al., 2001; ten Tije et al., 2003; Strickley, 2004).

G. Summary

Cosolvents are widely used to solubilize poorly watersoluble drugs after oral and parenteral administration. Cosolvents may be used for both neutral and ionizable compounds and have been used successfully in combination with other solubilization strategies, including surfactants, cyclodextrins, pH manipulation, and lipids. The factors that should be considered when using cosolvent formulations include the likely loss of solubilization power on dilution (both before administration or following mixing with blood) and the cell-based toxicity of cosolvent, in particular, after i.v. administration.

VII. Surfactants

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Unlike most drug solubilization technologies, where in vivo pharmacological effects of the formulation components are limited, in the case of surfactants increasing evidence suggests the potential for inhibitory effects on both metabolic and drug transport or anti-transport processes (Martin-Facklam et al., 2002b; Singla et al., 2002; Bogman et al., 2003). In particular, inhibition of cellular efflux processes might be expected to enhance drug absorption for compounds where intestinal efflux transporters such as P-glycoprotein (P-gp) limit oral bioavailability or provide enhanced cellular access to, for example, multidrug-resistant tumor populations where resistance is mediated by overexpression of P-gp. Surfactant inhibition of P-gp activity at the blood-brain barrier has also been suggested to promote drug access to the central nervous system (Kabanov et al., 2003).

In products for oral administration, surfactants are rarely used as the only solubilizing agent, either in liquid formulations or in solubilizing formulations destined for filling into soft or hard gelatin capsules. For these applications, surfactants are typically used in combination approaches that include surfactant, cosolvent, and lipid. Such formulations are usually described under the generic heading of “lipid-based formulations”, regardless of the quantity of traditional “lipid” incorporated. Notable examples of formulations of this type include ampranavir (Agenerase; GlaxoSmithKline), cyclosporine (Neoral; Novartis Pharmaceuticals), and ritonavir (Norvir SEC; Abbott Laboratories). These systems are described in more detail in Section XI of this review. Surfactants are also commonly used to stabilize nanosuspension formulations and are therefore an important constituent of formulations described in Section IX. Finally, surfactants may be used to improve wetting of hydrophobic materials in simple solid dosage forms and to stabilize traditional suspension-based formulations. The interested reader is directed to the following reviews for further details (Nielloud and Marti-Mestres, 2000; Tadros, 2005).

The emphasis of the current section is on the use of surfactants in parenteral formulations. In comparison with oral delivery (where most surfactants are not

**TABLE 17**

Cosolvent regimens “well tolerated” in various animals

Summarized in this table are the data obtained in a number of in vivo studies performed by four separate organizations between 1991 and 2006 and summarized in Gad et al. (2006). In each study, a vehicle control group was evaluated, with the results summarized in this table.

<table>
<thead>
<tr>
<th>Cosolvent</th>
<th>Species</th>
<th>Duration of Study</th>
<th>Route of Administration</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>Dog</td>
<td>1 mo</td>
<td>i.v.</td>
<td>1.25 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 mo</td>
<td>i.v.</td>
<td>200 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 mo</td>
<td>i.p.</td>
<td>5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 mo</td>
<td>Oral</td>
<td>5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Guinea pig</td>
<td>1 mo</td>
<td>i.v.</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>1 mo</td>
<td>Oral</td>
<td>5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>1 mo</td>
<td>s.c.</td>
<td>1 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Primate</td>
<td>“Efficacity”</td>
<td>Oral</td>
<td>3 ml/kg/day</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Dog</td>
<td>1 mo</td>
<td>Oral</td>
<td>5 ml/kg (7.5% solution)</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>3 mo</td>
<td>Oral</td>
<td>5 ml/kg (5.0% solution)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 wk</td>
<td>Oral</td>
<td>10 ml/kg (10% solution)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>6 mo</td>
<td>Oral</td>
<td>2.5 g/day</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>1 mo</td>
<td>i.p.</td>
<td>2.5 ml/day (5% solution)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Acute</td>
<td>i.p.</td>
<td>5 ml/kg (5% solution)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>13 wk</td>
<td>Cutaneous</td>
<td>100 μl/animal/day (70% solution)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Rat</td>
<td>1 mo</td>
<td>Oral</td>
<td>1000 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>—</td>
<td>s.c.</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Guinea pig</td>
<td>1 mo</td>
<td>Oral</td>
<td>500 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>1 mo</td>
<td>i.v.</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Acute</td>
<td>s.c.</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>1 mo</td>
<td>i.p.</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>—</td>
<td>i.v.</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>PEG 300</td>
<td>Mouse</td>
<td>1 mo</td>
<td>Oral</td>
<td>10 ml/kg/day</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>1 mo</td>
<td>Oral</td>
<td>500 mg/kg</td>
</tr>
<tr>
<td>PEG 400</td>
<td>Mouse</td>
<td>1 mo</td>
<td>Oral</td>
<td>1000 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>1 mo</td>
<td>Oral</td>
<td>10 ml/kg/day</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>1 mo</td>
<td>i.p.</td>
<td>500 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 mo</td>
<td>Oral</td>
<td>5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 mo</td>
<td>i.v.</td>
<td>0.5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 mo</td>
<td>Cutaneous</td>
<td>5 ml/kg (35% solution)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>104 wk</td>
<td>Cutaneous</td>
<td>2.5 ml/kg</td>
</tr>
<tr>
<td>PG</td>
<td>Minipig</td>
<td>2 wk</td>
<td>Cutaneous</td>
<td>2.5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 mo</td>
<td>Oral</td>
<td>2.5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 mo</td>
<td>s.c.</td>
<td>2.5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Minipig</td>
<td>26 wk</td>
<td>Cutaneous</td>
<td>2.5 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>1 mo</td>
<td>Oral</td>
<td>10 ml/kg (50% solution)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>1 mo</td>
<td>i.p.</td>
<td>2.5 ml/kg (40% solution)</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>1 mo</td>
<td>Oral</td>
<td>2.5 ml/kg</td>
</tr>
</tbody>
</table>

ADME, absorption, disposition, metabolism, and elimination; PG, propylene glycol; mo, month; wk, week.
absorbed), this introduces additional safety concerns since injected formulations are directly introduced into the systemic circulation and rapidly interact with vascular tissue after administration. Here we describe the surfactants that are commonly used in parenteral formulations, introduce the theory underpinning drug solubilization by micellar systems, address the formulation and nonformulation factors that may affect solubilization, and finally, and discuss the potential pharmacological effects of surfactants.

B. Commonly Used Nonionic Surfactants in Drug Delivery

The surface activity of surfactants is derived from the presence of both hydrophilic and lipophilic environments in the molecular architecture. Depending on the charge on the hydrophilic segment (often referred to as the “head group,” as opposed to the lipophilic “tail”), surfactants may be categorized as either ionic (anionic or cationic) or nonionic. Nonionic surfactants are more common in drug delivery owing to their better safety, superior capacity to solubilize nonpolar solutes at lower concentrations, and good compatibility with other pharmaceutically relevant excipients (Elworthy and Treon, 1966; Davis et al., 1970; Nielloud and Marti-Mestres, 2000). Table 18 lists a range of nonionic surfactants that are frequently used in pharmaceutical formulations. Nonionic surfactants are generally esters of saturated, unsaturated fatty acids or glycerides, monoesters of fatty alcohols, or block copolymers (polyethers) of polyethylene oxide and polypropylene oxide. They may be grouped according to the nature of the hydrophilic head group, which includes sorbitan (used in Spans) and its ethoxylated derivatives (used in Tweens) or polyoxyethylene chains of varying length (present in surfactants such as Cremophor, Gelucire, Brij, Labrasol, and Labrafil). Surfactants can be manufactured via ethoxylation (e.g., reaction of sorbitan esters with ethylene oxide in the case of Tween surfactants) or esterification (e.g., esterification of medium-chain-length fatty acids in the case of Labrasol). Surfactants obtained by ethoxylation may show higher purity, although molecular comparison of surfactants manufactured via different processes is complex (Schick, 1966).

The poloxamer block copolymers (Pluronics, Lutroils, Symperonics) comprise a hydrophobic poly (propylene oxide) central segment flanked on either side by more hydrophilic ethylene oxide blocks (Kabanov et al., 2002) and are a class of nonionic polymeric surfactants that have attained significant recent interest for drug solubilization and targeted drug release (Adams et al., 2003; Chiappetta and Sosnik, 2007).

### Table 18

<table>
<thead>
<tr>
<th>General Class</th>
<th>Primary Hydrophilic Component</th>
<th>Primary Hydrophobic Component</th>
<th>Cloud Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span</td>
<td>Sorbitan</td>
<td>Monolaurate (Span 20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monopalmitate (Span 40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monostearate (Span 60)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sesquioleate (Span 83)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trioleate (Span 85)</td>
<td></td>
</tr>
<tr>
<td>Tween</td>
<td>Polyethoxylated sorbitan</td>
<td>Monolaurate (Tween 20)</td>
<td>76°C</td>
</tr>
<tr>
<td></td>
<td>(polysorbate)</td>
<td>Monopalmitate (Tween 40)</td>
<td>73°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monostearate (Tween 60)</td>
<td>80°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monoleoleate (Tween 80)</td>
<td>65°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trioleate (Tween 85)</td>
<td>73°C</td>
</tr>
<tr>
<td>Cremophor</td>
<td>Polyoxyethylene</td>
<td>Hydrogenated castor oil</td>
<td>93°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Cremophor RH40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Castor oil (Cremophor EL)</td>
<td>72°C</td>
</tr>
<tr>
<td>Polyoxyglycerides</td>
<td>Polyoxyethylene</td>
<td>Caprylic and capric glycerides</td>
<td>64°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Labrasol)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oleyl or linoleyl glycerides</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Labrafil M1944CS, M2125CS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stearoyl or lauroyl glycerides</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Gelucire 50/13, 44/14)</td>
<td></td>
</tr>
<tr>
<td>Tocols</td>
<td>Polyethylene glycol</td>
<td>n-α-Tocopherol succinate</td>
<td>~40°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lauryl alcohol (Brij 35)</td>
<td>&gt;100°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cetyl alcohol (Brij 58)</td>
<td>&gt;100°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stearyl alcohol (Brij 78)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oleyl alcohol (Brij 98)</td>
<td></td>
</tr>
<tr>
<td>Poloxamers</td>
<td>Ethylene oxide units</td>
<td>Poloxamer 188 (Pluronic F68)</td>
<td>&gt;100°C</td>
</tr>
<tr>
<td></td>
<td>Propylene oxide units</td>
<td>Poloxamer 184 (Pluronic L64)</td>
<td>58°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poloxamer 331 (Pluronic L101)</td>
<td>15°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poloxamer 335 (Pluronic P105)</td>
<td>91°C</td>
</tr>
</tbody>
</table>

LS, low aqueous solubility limits accurate determination of cloud point.

* Ethoxylated castor oil derivatives contain a composite mix of monoesters, diesters and triesters of polyethoxylated fatty acids (primarily ricinoleic acid) and hydrophilic, nonfatty acid esterified materials, including glycerol, polyoxyethylene ether, and free polyethylene glycol.

* Also known as macroglglycerides and are composed of a well defined mixture of monoglycerides, diglycerides, and triglycerides, monoesters and diesters of PEG, and some free PEG.
Surfactants are also commonly classified by their hydrophilic-lipophilic balance (frequently abbreviated to HLB), which categorizes surfactants on the basis of the molecular weight (MW) ratio of their hydrophilic and lipophilic components, expressed as a percentage divided by five (Griffin, 1949). For traditional nonionic surfactants, HLB values range from 0 (most lipophilic) to 20 (most hydrophilic); for poloxamers, the HLB value is determined by the ratio of the lengths of ethylene oxide and propylene oxide blocks. Apparent HLB values for Pluronics and other block copolymers can exceed 20 (Kozlov et al., 2000).

The highest concentrations of several nonionic surfactants that have been used in commercial parenteral formulations are presented in Table 19 and provide an indication of the maximum quantities that might be tolerated in human subjects. Higher concentrations may be possible during preclinical testing, especially over short periods, although the potential pharmacological effects of surfactant must be considered when developing formulations for toxicology testing. Concentrations of administered surfactant are often higher if the formulation is to be given via an i.m. injection, although lower injection volumes must be used (Nema and Brendel, 2011).

C. Solubilization by Surfactants

Effective micellar solubilization of poorly water-soluble drugs is dependent on several factors, including surfactant properties, drug properties, and a variety of external factors such as the presence of other solubilizing agents, electrolytes, and dilution. An understanding of the factors that determine solubilization efficiency is critical to the rational design of micellar formulations and allows early identification of circumstances where alternative solubilization strategies might be more appropriate (Li et al., 1999a,c).

1. Micelle Formation and Drug Solubilization.

The amphiphilic nature of surfactants promotes self-association in aqueous environments and facilitates the formation of micelles above a critical concentration, frequently referred to as the critical micelle concentration (CMC). Surfactant micelles have the capacity to enhance the effective aqueous solubility of poorly water-soluble drugs, largely via solubilization in the hydrophobic micelle core but also via interaction with head groups and orientation/incorporation into the polar/nonpolar water-micelle interface. The location of solubilization is largely dependent on drug hydrophilicity and charge (Mukerjee, 1979; Yalkowsky, 1999; Rangel-Yagui et al., 2005). Highly lipophilic drugs are expected to be primarily solubilized by lipophilic surfactant “tails” (e.g., alkyl chains) orientated to the micelle core, whereas less lipophilic compounds are predominantly solubilized at the polyoxyethylene-rich core/mantle interface (Elworthy and Patel, 1982; Fahelelbom et al., 1993; Malcolmson et al., 1998).

The CMC reflects the saturated solubility of surfactant monomers in the solvent (e.g., water) at a particular temperature (Becher, 1967). Micelle formation is driven thermodynamically by the liberation of structured water that is involved in the hydration of the hydrophobic alkyl chain in monomeric solution, back into bulk solution. Micelle formation also results in removal of hydrophobic regions of the surfactant molecule from an aqueous environment (when surfactant monomers are present in free solution) and incorporation into the largely hydrophobic micellar core. This results in a gain in entropy through increased mobility of the alkyl chain in the micelle core and reduction in water structure.

The higher aqueous solubility of ionic surfactants and their potential to repel adjacent surfactant molecules via electrostatic repulsion at the micellar

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### Table 19

Commonly used surfactants: published recommendations for concentrations in preclinical testing and maximum concentrations observed in clinical parenteral products

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Recommended Range for Preclinical Use</th>
<th>Frequency of Use in Commercial Formulations</th>
<th>Concentrations Used in Some Commercial Preconcentrates</th>
<th>Final Surfactant Concentration Administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cremophor EL</td>
<td>5–10%</td>
<td>3</td>
<td>65% (Sandimmune)</td>
<td>≤5% after a 20- to 100-fold dilution, i.v. infusion.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51% (Taxol)</td>
<td>Average volume per single dose = 3.5 ml²</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50% (Vumon)</td>
<td>≤10% after 5- to 20-fold dilution, i.v. infusion.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50% (Valstar)</td>
<td>Average volume per single dose = 25.8 ml²</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7%</td>
<td>≤5% after a 10- to 100-fold dilution, IV infusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20% (Prograf)</td>
<td>18% after a ~4-fold dilution, intravesical instillation</td>
</tr>
<tr>
<td>Cremophor RH 40</td>
<td>5–10%</td>
<td>1</td>
<td>100% (Taxotere)</td>
<td>≤1% after a 250- to 1000-fold dilution, i.v. infusion</td>
</tr>
<tr>
<td>Cremophor RH 60</td>
<td>5–10%</td>
<td>22</td>
<td>100% (Panitot)</td>
<td>≤2% after a ~50- to 150-fold dilution, i.v. infusion</td>
</tr>
<tr>
<td>Tween 20</td>
<td>20–50%</td>
<td>72</td>
<td>100% (Panitot)</td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>5–10%</td>
<td>72</td>
<td>100% (Panitot)</td>
<td></td>
</tr>
<tr>
<td>Solutol HS-15</td>
<td>20–50%</td>
<td>50% (Panitot)</td>
<td>100% (Panitot)</td>
<td></td>
</tr>
</tbody>
</table>

² % w/v on the basis of single 10 ml/kg excipient dose to a mouse or rat, from Li and Zhao (2007).
³ From Nema and Brendel (2011).
⁴ From Strickley (2004).
⁵ Significant evidence of local/systemic adverse reactions.
⁶ It was not possible to identify the product.
⁷ Licensed for use in Mexico.
interface results in a higher CMC in comparison with nonionic surfactants (with similar hydrophobic segments) (Malmsten, 2002). At equal total surfactant concentration, therefore, a lower concentration of an ionic surfactant will be present as micelles.

Several physicochemical properties of surfactant solutions change dramatically at the CMC, and, therefore, CMC measurement is readily achieved via a change in surface tension, light scattering, molecular conductivity, osmotic pressure, self-diffusion, and magnetic resonance with increasing surfactant concentration. The physical properties, including CMC, for a range of ionic and nonionic surfactants are well described in detail elsewhere (Elworthy et al., 1968; Yalkowsky, 1999); cloud-point data (providing a measure of the aqueous solubility of a surfactant) for a number of commonly used nonionic surfactants are included in Table 18. Surfactants typically form spherical micelles in water, as this conformation maximizes the surface area of interaction of the polar head groups with the external aqueous environment and minimizes the aqueous interaction of the nonpolar tail (Tanford, 1974, 1980). For a long-chain nonionic surfactant, the number of surfactant monomers within a spherical micelle may be as many as 150 (Arnarson and Elworthy, 1982), although further growth of a spherical micelle is usually limited by steric constraints (Attwood and Florence, 1983). Certain amphiphiles adopt nonspherical micellar conformations, typically rod-shaped micelles, and these may further aggregate to form ordered hexagonal, cubic, or lamellar liquid crystal phases (Attwood and Florence, 1983; Laughlin, 1994; Lawrence, 1994). Dispersed, self-assembled liquid crystals comprising amphiphilic oils (e.g., phospholipid, monoolein) alone or in combination with other surfactants have been examined as solubilizers, most commonly liposomes (dispersed lamellar phase), but also cubosomes and hexasomes (Lawrence, 1994; Boyd et al., 2009). Liposomes, cubosomes, and hexasomes may be used to overcome low drug solubility in i.v. drug formulations; however, their complex structure commonly alters the pharmacokinetics and pharmacodynamics of encapsulated drug molecules. As such, they have found greater application in the development of targeted drug delivery systems with controlled drug release profiles. Here we focus on simple micellar systems for drug solubilization; the interested reader is directed to alternate texts describing the use of liquid crystalline systems for drug delivery (Lawrence, 1994; Shah et al., 2001; Smart et al., 2008; Boyd et al., 2009).

2. Quantification of Micellar Solubilization. For poorly water-soluble drugs, increasing surfactant concentrations above the CMC results in linear increases in drug solubility (Fig. 26). Where the CMC is low, this also provides for a solubilization profile that is largely linear on dilution, a situation in contrast to cosolvent formulations where dilution leads to a nonlinear decrease in drug solubility and, typically, drug precipitation. The molar solubilization capacity \( \kappa \) is frequently used to quantify surfactant solubilization and is defined as the mole ratio of micellar solubilized drug \( (S_M) \) to the concentration of micellar surfactant \( (C_{mic}) \) (Yalkowsky, 1999):

\[
\kappa = \frac{(S_{tot} - S_0)}{(C_{surf} - CMC)} = \frac{S_M}{C_{mic}}
\]

where \( S_0 \) is the intrinsic solubility of the drug, \( S_{tot} \) is the total concentration of drug in solution (solubilized plus free), and \( C_{surf} \) is the total surfactant concentration. The determination of \( \kappa \) provides a rapid, effective means of comparing the solubilization efficiency of various surfactants for a particular drug. It does not, however, account for potential differences in the intrinsic drug solubility. The micelle-water partition coefficient \( (K_M) \) is therefore a more appropriate index for comparing total solubilization for different drugs:

\[
K_M = \frac{(S_{tot} - S_0)}{S_0} = \frac{S_M}{S_0}
\]

The aforementioned solubilization descriptors have been used widely to quantify and compare surfactant solubilization by various surfactants and to explore the impact of drug physicochemical properties on solubilization (Yalkowsky, 1999; Alvarez-Nunez and Yalkowsky, 2000; Croy and Kwon, 2004; He et al., 2006; Kadam et al., 2009; Kadam et al., 2011). For example, using Tween 80 as a model surfactant, Alvarez and co-workers showed that increasing the octanol-water
partition coefficient (log P) of a solubilized drug resulted in a corresponding increase in $K_M$ (Alvarez-Nunez and Yalkowsky, 2000) (Fig. 27) and led to the suggestion that micellar solubilization is likely to be of most potential benefit for drugs with log $P$ values $> 3$. The exceptions to this suggestion include drugs with high potency that require relatively low levels of solubilization and drugs where interaction at the micellar interface is the prevalent mechanism of solubilization.

3. **Effect of Surfactant Type and Structure on Drug Solubilization.** Increasing the length of the hydrophobic region of an ionic or nonionic surfactant leads to a decrease in the water solubility of surfactant monomers, in turn leading to a decrease in the CMC (Hall and Pethica, 1967; Attwood and Florence, 1983; Chandler, 2005). Micelle formation from surfactants with larger hydrophobic chains also typically results in an increase in the volume of the micelle core (El-Eini et al., 1976; Lindman et al., 1980; Ong and Manoukian, 1988; Hurter et al., 1993; Wanka et al., 1994; Chen et al., 1998; Tsui et al., 2010). Together, these trends predict an improvement in micellar drug solubilization with surfactants of increasing alkyl chain length. The effect of surfactant alkyl chain length has been observed in many solubilization studies using Tween, Brij, and Pluronic surfactants (Ismail et al., 1970; Samaha and Cadalla, 1987; Ong and Manoukian, 1988; Kozlov et al., 2000; Ahmed, 2001; Tommasini et al., 2004; Kovacs et al., 2009).

Other reports suggest that the impact of surfactant chain length on drug solubilization is significant only when the drug is highly nonpolar and has a high affinity for the micelle core (Alvarez-Nunez and Yalkowsky, 2000). For example, indomethacin solubilization is similar in Tween 20 or Tween 80 micelles, whereas solubilization of the more lipophilic vitamin A palmitate is higher in micelles constructed with the longer-chain Tween 80 surfactant (Yalkowsky, 1999). The effect of surfactant chain length also diminishes above a chain length of 14–16 carbons atoms (Elworthy and Patel, 1982; Ong and Manoukian, 1988; Yalkowsky, 1999) (Fig. 28). In general, the lower CMC values of nonionic versus ionic surfactants promote more effective drug solubilization, although this is not always the case, and examples of higher drug solubilization with ionic surfactants have been documented (Krishna and Flanagan, 1989; Bhat et al., 2006; Bhat et al., 2008; Sanghvi et al., 2009). In these circumstances, it is likely that solubilization within the micelle-water interface or direct interaction with ionic head groups dominates interaction patterns rather than solubilization within the micelle core (Malcolmson et al., 1998). Where interfacial interactions dominate, the molecular weight of the polyoxylethylene component of the surfactant may prove to be the most significant factor in determining solubilization properties.

4. **Effect of Temperature on Drug Solubilization.** Micellar formulations may be subjected to elevated temperatures on storage or during autoclaving to provide for sterility following manufacture (Schott and Han, 1975; Na et al., 1999). Conversely, low temperatures may be encountered where refrigeration is required to provide appropriate drug stability. An understanding of the potential impact of temperature on micellar solubilization is therefore important during formulation design.

At a fixed surfactant concentration, increasing temperature results in a reduction in the degree of
hydration of the polyoxyethylene chains of nonionic surfactant monomers (Schott and Han, 1975; Na et al., 1999). Ultimately, this leads to phase separation of the surfactant as the solubility limit is broached. This is the cloud point, that is, the point at which an increase in temperature results in the generation of a cloudy solution (usually defined as an increase in solution turbidity of 50%). As a result, the CMC for nonionic surfactants (the opposite is true for ionic surfactants) decreases on heating (Wanka et al., 1994; Tadros, 2005), which increases the potential for drug solubilization. However, temperatures in excess of the cloud point are likely to lead to formulation instability. The effect of changes to temperature on drug solubilization in micellar systems is complex since temperature also impacts on the intrinsic aqueous solubility of the drug. In most cases, a drug will show greater affinity for the bulk solution with increasing temperature and, in turn, a lower driving force for partitioning into the micellar phase. Although this suggests a possible decrease in micellar solubilization, the net effect under conditions of increasing temperature is usually an increase in overall solubility in nonionic surfactant solutions (Humphreys and Rhodes, 1968; Barry and Eleini, 1976; Yalkowsky, 1999). At lower temperatures, drug solubility in solution is reduced and the CMC of nonionic surfactants is expected to increase (as surfactant solubility increases). Decreasing temperature therefore increases the risk of drug precipitation.

D. Impact of Cosolubilizers and Electrolytes on Surfactant-Mediated Drug Solubilization

1. Surfactants and Cosolvents. Cosolvency aims to reduce the polarity of an aqueous medium to improve the solvation of a hydrophobic drug and is described in more detail in Section VI. Cosolvents may also be used in combination with surfactants. In this case, cosolvents impact not only the solution properties of the drug but also the efficiency of micellar solubilization. Thus, by reducing the polarity of the solution, cosolvents enhance the solvation of nonionic surfactants, giving rise to increases in CMC and decreases in micelle size. Consistent with this suggestion, a wide range of pharmaceutical cosolvents (e.g., ethanol, propylene glycol, polyethylene glycol, DMA, and glycerol) have been shown to increase the CMC of aqueous solutions of polyoxyethylene surfactants (Eicke, 1980; Yalkowsky, 1999; Kawakami et al., 2004; D’Errico et al., 2005) and the block copolymer surfactants (Na et al., 1999; Ivanova et al., 2001; Kawakami et al., 2006; Tomsic et al., 2006; Sarkar et al., 2010). For example, the addition of 10% ethanol to a micellar solution of Tween 80 led to an ~4-fold increase in CMC, and the same concentration of PEG and DMA led to even greater (~10-fold and ~18-fold, respectively) increases. The larger effect of PEG and DMA was suggested to reflect their greater hydrophobicity and, therefore, their increased ability to alter the polarity of the solvent compared with ethanol (Kawakami et al., 2006). Interestingly, alcohols of more than four carbon atoms have the opposite effect, causing a decrease in the CMC of nonionic surfactants (Armstrong et al., 1996; Bharatiya et al., 2007).

To consider the net effect of cosolvents on drug solubilization in a micellar formulation requires the appreciation of a number of factors. As described already, cosolvents usually lead to an increase in surfactant CMC, but they also increase drug solubility in the bulk phase, causing drug partitioning into the micelle to decrease. This may be further complicated if the cosolvent itself is solubilized by the surfactant as the micelle core will become more polar and, therefore, less attractive to drug solubilization (Zana, 1995; Kawakami et al., 2006). The combined use of cosolvents with surfactants may therefore lead to a decrease in micellar drug solubilization, particularly when the drug shows high affinity for the surfactant core (Kawakami et al., 2004; Dong et al., 2008). In contrast to the potentially detrimental effect of cosolvents on micellar solubilization, cosolvency increases drug solubility in bulk solution. As such, the net impact on drug solubilization is difficult to predict a priori and reflects the relative efficiencies of cosolvency versus micellar solubilization on drug solubility.

Surfactants are also commonly added to cosolvent systems in an attempt to reduce susceptibility to precipitation on dilution (see Section VI.D.2) (Tonsberg et al., 2011) and are found in a number of commercial parenteral formulations (Ni et al., 2002; Strickley, 2004), including Taxol and Sandimmune. Both formulations are nonaqueous solutions of Cremophor EL and dehydrated alcohol, and both require dilution with significant quantities of aqueous phase before i.v. administration. The presence of cosolvent aids in dispersion and dilution of the surfactant solution.

2. Surfactants and pH Adjustment. The properties of micelles formed by nonionic surfactants are largely independent of pH. However, changing pH can alter the micellar solubilization of ionizable drugs.

For ionizable compounds, the total quantity of dissolved drug is the sum of the concentrations of ionized [Di] and un-ionized [Du] drug in free solution and the concentrations of ionized [DiM] and un-ionized drug [DuM] in the micellar phase. The micelle-water partition coefficients for ionized drug (Ki) are predictably lower than the corresponding values for the un-ionized form (Ku) as a result of differences in solubility in the aqueous solvent (Jain et al., 1999, 2001; Li and Zhao, 2003; He et al., 2006). Under conditions of differing pH, the concentration of un-ionized drug in solution (Du) remains unchanged, and assuming no change to the properties of the nonionic surfactant micelle with pH, then the affinity of the un-ionized form for the micelle phase is also
unchanged. $K_a$ and $D_o$ are therefore pH independent (Li et al., 1999b,c).

In contrast, changing pH will alter the concentration of ionized drug in free solution [$D_i$] and, depending on the magnitude of $K_a$ (which describes the affinity of the charged drug for surfactant micelles), may increase the quantity of micellar ionized drug, [$D_iM$]. Two extremes might be envisaged. First, where the affinity of ionized drug for micelles is low, the slope of drug solubility vs. surfactant concentration plots at varying pH will be unaffected (and reflect $K_M$, eq. 36) (Yalkowsky, 1999) but will be shifted upward, reflecting the higher solubility of ionized drug in free solution (Ran et al., 2005). Second, where ionized drug has significant affinity for micelles, the slope of the drug solubility versus surfactant concentration plots will change with a change in pH, reflecting contributions from both micellar ionized and un-ionized drug.

The latter scenario is exemplified in Fig. 29, where the increase in solubility of flavopiridol, a poorly soluble weak base ($pK_a = 5.68$), is shown as a function of pH and Tween 20 concentrations (Li et al., 1999b). In this example, the micellar association constant for un-ionized flavopiridol ($K_a$) is $333.3 \text{ M}^{-1}$ and for ionized drug is $185.4 \text{ M}^{-1}$. Under conditions of decreasing pH, an increase in [$D_i$] is therefore apparent; in addition, micellar solubilization of the increasing quantity of the ionized form is also enhanced, and as a consequence, the increase in drug solubility with increasing surfactant concentration is greater than that observed when only un-ionized drug is available in solution (i.e., at higher pH) (Li et al., 1999b). Similar trends in solubility with changing pH and surfactant concentration have been observed by others (Ikeda et al., 1977; Jain et al., 2001, 2004; Ran et al., 2005; Sheng et al., 2006; Kovacs et al., 2009). The potentially beneficial effect of the combined use of pH adjustment and the presence of a nonionic surfactant has been achieved in several commercial micellar formulations, including Cordarone (10% Tween 80, pH 4.1) and Librium (4% Tween 80, pH 3) (see also Section IV.B.2.c).

The effect of pH change on micellar drug solubilization is more complex for ionic surfactants since pH change alters the ionization state of both the drug and the head group of the surfactant. For an anionic surfactant, an increase in pH is expected to lead to greater ionization of the head group and an increase in the CMC through the combined effect of increased solubility and electrostatic repulsion between adjacent surfactant head groups at the micellar interface. Under these circumstances, a decrease in drug solubilization is expected for nonionizable or anionic drugs (where electrostatic repulsion from micellar head groups may reduce solubility further). In contrast, the potential to form ion pairs may increase drug solubilization for a weak base. Indeed, the rank order of surfactant solubilization efficiency for a weak base (under conditions where both drug and anionic/cationic surfactant are ionized) has been suggested to be anionic surfactant $>$ nonionic surfactant $>$ cationic surfactant (Bhat et al., 2008). The opposite trend is expected for a weakly acidic compound.

3. Surfactant Mixtures. A combination of surfactants may be used to promote drug solubilization where the required concentration for a single surfactant is high and likely to give rise to adverse effects. The CMC of a binary mixture of two surfactants can be predicted using eq. 37, where $x_1$ and $x_2$ are the relative mole fractions of the different surfactants in micellar form (Tadros, 2005):

$$\text{CMC} = x_1\text{CMC}_1 + x_2\text{CMC}_2 \quad (37)$$

In accordance with eq. 37, the CMC of a surfactant mixture will be an average of the CMC of the single components. However, this relationship applies only to systems where the two surfactants are structurally related (i.e., of the same class with similar polar head groups but different alkyl chain lengths).

In practice, it is more common to investigate systems containing two or more structurally unrelated surfactants since their combined use more commonly leads to the formation of mixed micelles with drug solubilization properties that are greater than the simple additive effect of the two surfactants. A classic example of this type of synergy is the formation of bile salt-phospholipid mixed micelles, which show substantial increases in solubilization capacity when used in combination. Evidence of mixed micelles in solutions containing both ionic and nonionic surfactants has also been widely reported (Treiner, 1994; Bhat et al., 2008), and the combination of hydrophilic and hydrophobic Pluronics has been explored to obtain micelles of improved size, stability, and solubilization capacity (Danson et al., 2004; Oh et al., 2004; Wei et al., 2009;
Yang et al., 2009). Surfactant combinations are widely used as components in lipid-based formulations and are described in more detail in Section XI.

4. Surfactants and Cyclodextrins. Cyclodextrins—including hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin—increase the apparent drug solubility of a number of poorly water-soluble drugs by forming drug-cyclodextrin inclusion complexes (see Section VIII). Cyclodextrins can also complex with both nonionic (Veiga and Ahsan, 1998; Buschmann et al., 2000; Eli et al., 2000) and ionic surfactants (Junquera et al., 1993; Park and Song, 1989). As a result, the drug-cyclodextrin binding constant (a descriptor of drug solubilization by cyclodextrins) is typically lower in the presence of surfactant as a result of direct competition for available cyclodextrin. Furthermore, the complexed surfactant fraction is unable to participate in micelle formation, leading to a reduction in micellar solubilization of poorly water-soluble drugs (Junquera et al., 1993). Drug solubilization in a solution containing both a surfactant and cyclodextrin is therefore unfavorable, and their combined use commonly provides no additional benefit to total drug solubilization. Indeed, in some cases, surfactant-cyclodextrin combinations lead to a decrease in the concentration of solubilized drug (Veiga and Ahsan, 1998; Yang et al., 2004; Rao et al., 2006) (see also Section VIII.D.3).

5. Surfactants and Strong and Weak Electrolytes. Weak electrolytes (such as ionic buffers) and strong electrolytes (e.g., sodium chloride that may be included in a diluent) increase the polarity of aqueous solutions and, therefore, lead to the opposite effect on surfactant properties compared with cosolvents (i.e., electrolytes decrease surfactant solubility and CMC, increase micelle size, and typically increase surfactant solubilization capacity) (Attwood and Florence, 1983; Yalkowsky, 1999; Tadros, 2005). Equation 38 provides a means of predicting the CMC of a surfactant in the presence of a strong electrolyte:

\[
\text{LogCMC} = \text{LogCMC}_{\text{no salt}} - k_s m_s \tag{38}
\]

where \(k_s\) and \(m_s\) are the salt constant (describing salting-out potential; see forthcoming text) and the salt molality, respectively (Ray and Nemethy, 1971; Carale et al., 1994). The capacity of a salt to lower the CMC correlates with its Hofmeister-like properties (Hofmeister, 1888), where salts showing the greatest ability to restructure water (multivalent ions and those with small ionic radii and high charge densities) exhibit the greater capacity to “salt-out” a surfactant from solution.

The presence of charged ions in solution can also shield electrostatic repulsive forces between charged ionic surfactants; hence, the effect of salts on the CMC is comparatively greater for ionic surfactants compared with nonionic surfactants (Carale et al., 1994). The impact of electrolyte addition on drug solubilization in ionic and nonionic micellar solutions has been explored using a range of sulfonylurea-type compounds and glitazones (Seedher and Kanojia, 2009). These studies report a greater than 10-fold increase in the solubilization capacity of mixed micelles formed between anionic (i.e., sodium lauryl sulfate) and nonionic (Tween 80) surfactants toward glyburide, pioglitazone, and rosiglitazone on the addition of 0.15 M sodium chloride (Seedher and Kanojia, 2009). Strong electrolytes have also been shown to depress the CMC of Pluronic block copolymers (Bahadur et al., 1992; Jain et al., 1999, 2000; Desai et al., 2001).

E. Effects of Dilution on Drug Solubilization by Surfactants

Parenteral and oral micellar formulations are diluted in situ after administration, and formulations for i.v. use are frequently diluted before administration. Understanding the potential for loss of solubilization capacity on dilution is therefore an important aspect of dose form design for micellar formulations. As illustrated in Fig. 30, the dilution of a micellar formulation will typically only cause drug precipitation when the concentrations of surfactant approach the CMC (marked in Fig. 30) and, in particular, when the micellar formulation contains a level of drug (or drug dose) close to the maximum solubilization capacity of the formulation (i.e., the red curve, Fig. 30). The potential for precipitation is reduced when the CMC is low and is therefore less prevalent for nonionic surfactants compared with ionic surfactants. Indeed, micellar formulations are much less susceptible to drug precipitation on dilution than, for example, cosolvent systems, since solubility increases linearly with increasing surfactant concentration above the CMC and therefore decreases linearly with linear sample

![Fig. 30. The effect of dilution of a surfactant solution containing a high or a low drug concentration. Points on the dilution curves at which theoretical drug concentrations exceed maximum solubility (labeled X and Y) indicate areas of precipitation risk. S_0 is the intrinsic drug solubility in the absence of surfactant.](image-url)
dilution. In contrast, the exponential increase in solubilization capacity observed on addition of cosolvent results in nonlinear decreases in solubilization capacity on dilution and greater potential for precipitation.

Surfactants are commonly added to both cosolvent and buffered formulations to reduce the risk of precipitation, regardless of an effect on intrinsic solubility (Nassar et al., 2004; Dong et al., 2008; Sanghvi et al., 2009; Tonsberg et al., 2011). For example, low HLB block copolymers have shown recent utility in reducing drug precipitation on dilution of micellar formulations and pH-adjusted formulations of amiodarone (Elhasi et al., 2007) and an unnamed poorly water-soluble drug (Dai et al., 2008b). The mechanisms of precipitation inhibition may simply reflect the additional solubilizing power gained from the presence of surfactant, even at high dilution, since the CMCs of the block copolymers are low compared with traditional nonionic surfactants (the CMC of Pluronic F127 is $2.8 \times 10^{-6}$ M, whereas the CMC of vitamin E TPGS 1000 succinate is $1.3 \times 10^{-3}$ M (Kataoka et al., 2001; Kabanov et al., 2002; Adams et al., 2003). Alternatively, the block copolymers may inhibit drug crystallization directly (Dai et al., 2008a) in a manner analogous to the inhibitory effects of nonionic high-molecular-weight polymers on drug crystallization from supersaturated solutions (Warren et al., 2010).

### F. Potential Pharmacological Properties of Nonionic Surfactants

A relatively narrow range of nonionic surfactants has been used in parenteral drug delivery, a situation perpetuated by concerns surrounding toxicity and pharmacological activity and the limited number of surfactants with preexisting regulatory approval. The adverse effects reportedly resulting from surfactant administration are described in the following section, noting that these effects may reflect surfactant composition directly or the presence of lower quantities of reaction products from the surfactant synthesis in the final product.

#### 1. Toxicity

Table 20 lists the LD$_{50}$ values for a variety of nonionic surfactants commonly used for parenteral drug delivery (Rowe et al., 2009). The mechanisms by which surfactants mediate toxicity are not well described; however, injury to the local vasculature, blood cells, and other tissues may be mediated through the capacity to adsorb to cells and to

1) disrupt the integrity of the cell membrane (Shalel et al., 2002) or 2) at higher concentrations (above the CMC) to solubilize lipid membranes (Jones, 1999). The potential for surfactant (or indeed any excipient) to promote toxicity associated with blood incompatibility is commonly screened in vitro by monitoring hemolysis in human or animal red blood cells after incubation with excipient (e.g., surfactant) or a complete formulation (Krzyzaniak and Yalkowsky, 1998). The typical experimental design used was described in Section V.I.F.2, and studies of this type have revealed that increasing the chain length of nonionic surfactants appears to promote surfactant toxicity against red blood cells (Ernst and Arditti, 1980), although this toxicity increase is not maintained above a chain length of approximately 14 carbons (Schott, 1973; Ferguson and Potten, 1976). The nature of the hydrophobic portion of a surfactant may also impact on cell penetration, and in this regard, triglycerides are bulkier than monoalkyl materials and, therefore, may reduce surfactant cell interaction. Nonionic surfactants of equivalent/similar hydrophobic character have also been shown to exhibit wide differences in lytic effects, implicating a role for the hydrophilic component in dictating toxicity differences (Zaslavsky et al., 1978; Tragner and Csordas, 1987). For example, nonionic surfactants comprising hydrophilic segments made up of polyoxyethylene (e.g., Brij and Solutol HS-15) exhibit significantly less cellular toxicity compared with a range of sugar-based surfactants (Shalel et al., 2002; Soderlind et al., 2003), and the toxicity toward an epithelial cell line of a series of commonly used surfactants with similar C18 chain-length lipophilic regions and only small differences in surface activity (CMC values were similar) varied more than 100-fold in the following rank order: Simulsol 4000 (least toxic) < Cremophor EL < Tween 80 < Tween 20 < Solutol HS-15 < TPGS 1500 (Maupas et al., 2011). It was further proposed that this rank order reflected variation in the size of the hydrophilic group, with bulkier polyoxyethylene chains providing a greater steric

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Median Lethal Dose (LD$_{50}$) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brij 35</td>
<td>Mouse: 0.16 g/kg i.p. Mouse: 1 g/kg i.v. Rat: 0.027 g/kg i.v. Rat: 8.6 g/kg p.o.</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Mouse: 1.42 g/kg i.v. Mouse: 37 g/kg p.o.</td>
</tr>
<tr>
<td>Tween 40</td>
<td>Mouse: 4.5 g/kg i.v.</td>
</tr>
<tr>
<td>Tween 60</td>
<td>Mouse: 7.6 g/kg i.p.</td>
</tr>
<tr>
<td>Tween 80</td>
<td>Rat: 8.8 g/kg i.v. Mouse: 25 g/kg p.o.</td>
</tr>
<tr>
<td>Labrasol</td>
<td>Rat: &gt;22 ml/kg day p.o.</td>
</tr>
<tr>
<td>Labrafil</td>
<td>Dog: 0.64 g/kg i.v.</td>
</tr>
<tr>
<td>Cremophor EL</td>
<td>Mouse: 6.5 g/kg i.v. Rat: &gt;6.4 g/kg p.o.</td>
</tr>
<tr>
<td>Cremophor RH40</td>
<td>Mouse: 12.5 g/kg i.p.</td>
</tr>
<tr>
<td>Cremophor RH60</td>
<td>Mouse: &gt;12.5 g/kg i.p.</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>Mouse: &gt;16 g/kg p.o.</td>
</tr>
<tr>
<td></td>
<td>Mouse: 1 g/kg i.v.</td>
</tr>
<tr>
<td></td>
<td>Rat: 7.5 g/kg i.v.</td>
</tr>
<tr>
<td></td>
<td>Rat: 9.4 g/kg p.o.</td>
</tr>
</tbody>
</table>

Data taken from Rowe et al. (2009).
Several nonionic surfactants also can cause more widespread systemic effects after parenteral use, and the risks associated with the systemic administration of Cremophor and Tween surfactants have been well documented (Gelderblom et al., 2001; ten Tije et al., 2003). Indeed, the Taxol formulation (paclitaxel; Bristol-Myers Squibb) has been the subject of considerable discussion owing to its high Cremophor EL content (see Table 19). Used primarily for the treatment of breast cancer, Taxol is a nonaqueous solution that contains 30 mg of paclitaxel dissolved in 5 ml of a mixture of 51% Cremophor EL and 49% dehydrated alcohol. It is diluted at least 20-fold with saline or 5% dextrose before administration by i.v. infusion (Bristol-Myers Squibb Company, 2007). Despite dilution and slow rates of infusion, however, acute hypersensitivity reactions are commonly observed and manifest as dyspnea, flushing, urticaria, hypotension, and angioedema (Weiss et al., 1990; Rowinsky et al., 1991; Gelderblom et al., 2001; Singla et al., 2002). Paclitaxel (i.e., aside of any vehicle effect) may also cause hypersensitivity reactions (Essayan et al., 1996); however, an increasing body of evidence suggests that the hypersensitivity reactions associated with Taxol are, at least in part, related to the Cremophor component of the formulation. Data to support this contention includes evidence of 1) histamine release after administration of ethoxylated oleic acid (a minor component of Cremophor EL) to dogs (Lorenz et al., 1982), 2) complement pathway activation at Cremophor EL plasma concentrations above 2 μL/ml and a reduction in the incidence of hypersensitivity at lower infusion rates that minimize Cremophor plasma concentrations (van Zuylen et al., 2000), and 3) hypersensitivity reactions after the i.v. administration of other Cremophor-containing formulations, such as Sandimmune (Volcheck and Van Dellen, 1998). In the latter study, the same patients showed no evidence of allergic reaction on receiving oral doses of the same formulations, providing additional support to the suggestion that Cremophor is either digested or not absorbed (or both) after oral administration and, therefore, that the toxicity concerns that arise after parenteral administration are minimized (Volcheck and Van Dellen, 1998; Gelderblom et al., 2001). Sensitivity to parenteral Cremophor now dictates that patients receiving Taxol be premedicated with antihistamines and corticosteroids to reduce histamine-related adverse reactions (Weiss et al., 1990; Pazdur et al., 1993), although minor reactions (facial flushing and rash) remain common (Gelderblom et al., 2001). Significant interspecies differences toward hypersensitivity reactions to Cremophor EL are also apparent. Dogs are highly sensitive to drugs and excipients that cause histamine release and are therefore more prone to the hypersensitivity reactions associated with Cremophor compared with rodent animal models (Lorenz et al., 1982; Park et al., 2009). The i.v. use of Cremophor-containing formulations has also been associated with peripheral neuropathy (Wiernik et al., 1987; van Zuylen et al., 2001).

The oral LD50 for Cremophor EL and RH40 in rats is 6.4 and 16 g/kg, respectively (Table 20) (Rowe et al., 2009). Rats dosed with 100 mg/kg Cremophor EL for 1 month showed no evidence of adverse reactions (Gad et al., 2006), and in human subjects dosed with 5 g of Cremophor EL, only one in eight reported symptoms that were attributed to the surfactant (in this case, mild GI disturbance was observed) (Martin-Facklam et al., 2002b). Indeed, evidence of the tolerability of oral Cremophor can be drawn from the clinical use of Norvir SEC (ritonavir) oral capsules for the treatment of HIV where daily doses of Cremophor EL of ~1.2 g are common (Strickley and Oliyai, 2007).

Docetaxel (Taxotere) (Table 19) is formulated as a nonaqueous solution in 100% Tween 80; like Taxol, it is routinely diluted before i.v. administration (Strickley, 2004). Although Taxotere has been associated with some incidences of acute hypersensitivity (Schrijvers et al., 1993) and peripheral neuropathy (Hilkens et al., 1997a,b), the frequency of adverse effects is lower than that of the Cremophor-containing formulations, suggesting a tolerability benefit for Tween 80 (Gelderblom et al., 2001; ten Tije et al., 2003).

The potential toxicity of some surfactants has limited wider application except at low concentrations. To this end, a significant effort has been directed toward reengineering older micellar formulations for parenteral use, and a number of Cremophor-free paclitaxel formulations have been developed to circumvent the issues surrounding the use of Taxol (van Zuylen et al., 2001; Singla et al., 2002). One notable example is Genexol-PM (Samyang Corporation, Seoul Korea), where paclitaxel is solubilized by micelles of a polyactic acid-polyethylene oxide block copolymer (Kim et al., 2004b). Compared with Taxol, the maximum tolerable dose of Genexol in mice is three times higher (Kim et al., 2001). The formulation was also able to solubilize the drug in a micellar form at up to 10 mg/ml, which is ~1.7-fold higher than the original Taxol formulation (Kwon and Forrest, 2006; Zhang and Feng, 2006). Abraxane (Celgene, Summit, NJ) is another Cremophor-free formulation for paclitaxel where the drug is bound to albumin particles (Green et al., 2006). Abraxane is currently approved by the FDA for the treatment of breast cancer. A recent advance in the use of surfactants for parenteral solubilization is the move to toward purified surfactants. For example, ultrapurified polysorbate 80 (HX2) is now available from the NOF Corporation (White Plains, NY) for oral and parenteral use. HX2 is derived from vegetable-based components and is designed to offer a reduction in hypersensitivity
and better stability compared with traditional polysorbate surfactants (Tween).

Solutol HS-15 (BASF Corporation) has also been investigated for use in oral and parenteral drug delivery (Ku and Velagaleti, 2010). Solutol consists of ethoxylated (15 moles) glycerides of 12-hydroxystearic acid and is much like the Cremophor-class of surfactants. Similar levels of histamine release have been observed after administration of Solutol and Cremophor to various animals (Ku and Velagaleti, 2010). Solutol has approval for use in injectable formulations in Mexico, Argentina, and Canada (Strickley, 2004), but in Europe and the US, it is still regarded as a novel excipient (Ku and Velagaleti, 2010).

2. Pharmacokinetic Effects. Changes in the pharmacokinetics of several compounds—including paclitaxel, cyclosporine, doxorubicin, and etoposide—have all been linked with the use of Cremophor in parenteral formulations (Ellis et al., 1996; Millward et al., 1998; van Zuylen et al., 2001; Bittner and Mountfield, 2002; Buggins et al., 2007), and Tween 80 has been shown to alter drug disposition after coadministration with doxorubicin, etoposide, and digoxin (Colombo et al., 1996, 1999; Zhang et al., 2003). The general consensus to emerge from these studies is that the presence of nonionic surfactants (at high concentration) may lead to an increase in systemic exposure of a coadministered drug compared with administration in, for example, a cosolvent formulation (van Zuylen et al., 2001; ten Tije et al., 2003). By way of illustration, Fig. 31 shows the pharmacokinetics of paclitaxel dosed to mice in formulations containing Cremophor EL, Tween 80, or DMA (a cosolvent). Administration of paclitaxel in the Cremophor EL formulation shows evidence of higher plasma concentrations in comparison with the other formulations, suggesting that changes to drug clearance are possible, although in this case, changes were specific to Cremophor EL rather than Tween 80 (Gelderblom et al., 2001). van Zuylen et al. (2001) have shown a similar increase in paclitaxel concentrations after coadministration with Cremophor EL and suggested that the mechanism of enhanced exposure reflected the low volume of distribution of Cremophor EL (~blood volume) and subsequent entrapment of paclitaxel in surfactant micelles in the plasma (van Zuylen et al., 2001). It is not clear why this effect was not observed with Tween 80 (Fig. 31), although Tween 80 is cleared more quickly than Cremophor (ten Tije et al., 2003) and Cremophor EL has been suggested to impact on excretory processes such as P-glycoprotein (P-gp) efflux (see Section XI). The existence of Cremophor micelles in the blood after administration of Taxol may also lead to altered pharmacokinetics for simultaneously administered drugs (ten Tije et al., 2003) and, therefore, the potential for drug interactions. Further descriptions of the effect of Cremophor EL on the pharmacokinetics of paclitaxel and other drugs are found in the following references: Gelderblom et al. (2001), ten Tije et al. (2003), and Buggins et al. (2007).

Beyond simple physicochemical mechanisms of solubilization (and therefore changes to pharmacokinetics presumably via differences in free concentration), it is increasingly apparent that surfactants (including Cremophor) can affect the activity of metabolic cytochrome P450 enzymes and excretory drug transporters, thereby potentially changing clearance and distribution processes directly (Bittner et al., 2003; Wandel et al., 2003; Bravo González et al., 2004; Christiansen et al., 2011). For example, several drugs, including many cytotoxic agents, are substrates for efflux transporters such as P-gp, breast cancer resistance protein (BCRP), and multidrug resistance proteins (MRP1 and MRP2) (Schinkel et al., 1996; Borst and Elferink, 2002; Leonard et al., 2003). Upregulation of these transporters is evident in many tumor types, leading to the phenomenon of multidrug resistance. P-gp expression at the luminal side of the blood-brain barrier (BBB) is also believed to limit drug access to the brain and, therefore, the cytotoxicity of some anticancer drugs that are P-gp substrates (van Asperen et al., 1996; Kemper et al., 2003). Cremophor EL has been reported to inhibit P-gp and may therefore reverse the implications of this MDR phenotype (Friche et al., 1990b; Schuurhuis et al., 1990; Woodcock et al., 1990). Similar P-gp inhibition has been shown in several cancer cell lines using Tween 80 (Friche et al., 1990b), Solutol HS-15 (Coon et al., 1991), TPGS (Dintaman and Silverman, 1999; Bogman et al., 2003), and Pluronic surfactants (Yang et al., 2007). Cremophor EL, Tween 20, Span 20, Brij 30, and

![Fig. 31. Effect of the formulation vehicle on paclitaxel concentration in female mice after an i.v. bolus administration of the drug at a concentration of 10 mg/kg. DMA, dimethylacetamide. Values are expressed as means ± SEM. Adapted from Sparreboom et al. (1999).](image-url)
Pluronic P85 have all been shown in vitro to inhibit the efflux properties of BCRP (Kabanov et al., 2003; Yamagata et al., 2007a, 2009), and recent in vitro studies have shown that Cremophor EL is able to inhibit MRP2 to a greater extent than probenecid (a small molecular inhibitor of MRP2). In contrast, in the same study, TPGS and Tween 80 showed only moderate effects on MRP2 inhibition (Hanke et al., 2010).

Coadministration of drugs that are substrates for efflux transporters, such as P-gp, BCRP, and MRP, with surfactants such as Cremophor therefore has the potential to inhibit P-gp activity and promote access to tumor cells, to the brain, or, in the case of brain tumors, to both. To this end, rapidly (≤6 h) infused doses of Taxol to human subjects have been shown to lead to plasma concentrations of Cremophor EL that are in excess of those seen to inhibit P-gp activity in vitro by more than 50% (Webster et al., 1993; Rischin et al., 1996). However, later in vivo studies have been unable to translate the in vitro inhibitory effects of Cremophor EL on P-gp activity into improved efficacy for Adriamycin (a P-gp substrate) in mice bearing MDR leukemia (Watanabe et al., 1996) or to increase paclitaxel uptake into the mouse brain (Kemper et al., 2003). The lack of correlation between in vitro and in vivo endpoints may reflect differences in in vivo and in vitro P-gp expression or difficulties in accurately reproducing the physical and biochemical barrier properties of the cell membrane in vitro.

In addition to potential effects on drug efflux from the brain and from cancer cells, surfactants have been widely studied for their effects on P-gp in the small intestine (where P-gp may limit drug absorption) (Rege et al., 2002; Bogman et al., 2003, 2005) and on cytochrome P450 enzymes in the small intestine that may contribute to the first-pass metabolism of orally administered drugs (Bravo González et al., 2004; Christiansen et al., 2011). Since surfactants in oral drug delivery are typically used in combination with other excipients such as lipids and cosolvents, their capacity to affect efflux and first-pass metabolic liabilities are discussed alongside effects on solubilization in the section of this review focused on lipid based formulations (see Section XI).

G. Summary

Above the critical micelle concentration, surfactants increase drug solubility via micellar solubilization. The lipophilic micelle core provides a nonpolar reservoir into which highly lipophilic compounds may partition, enhancing apparent aqueous solubility. In addition, poorly water-soluble drugs may also be solubilized by other regions of the micelle, in particular the polyoxyethylene-rich mantle. A large number of nonionic surfactants have been used in both oral and parenteral preparations, and surfactants may be used in conjunction with solubilization approaches, including cosolvency, pH adjustment, and lipid-based delivery systems. However, realizing the potential for surfactant-induced hypersensitivity after parenteral administration (e.g., Cremophor EL in commercial paclitaxel formulations) and the possibility of surfactant effects on free concentration and transporter/metabolic enzyme activity, care must be taken to interpret correctly the pharmacokinetic and pharmacodynamic data obtained in the presence of surfactants (especially after parenteral administration).

VIII. Cyclodextrins

A. Rationale for the Use of Cyclodextrins in the Solubilization of Poorly Water-Soluble Drugs

Cyclodextrins (CDs) are macrocyclic oligosaccharides consisting of a hydrophilic outer exterior and a hydrophobic inner cavity (Fig. 32). Drug molecules are able to form dynamic inclusion complexes within this cavity, and the higher solubility of the drug-CD complex relative to the solubility of drug alone increases apparent solubility, often over several orders of magnitude. As a result, there has been widespread interest in the use of CDs to address low solubility in both oral and parenteral drug products (Lofthson and Brewster, 1996; Rajewski and Stella, 1996; Stella and Rajewski, 1997; Uekama et al., 1998; Davis and Brewster, 2004; Brewster and Lofthson, 2007; Lofthson and Duchêne, 2007). Examples of marketed formulations that use CD complexation are listed in Table 21 and include oral solid dosage forms (e.g., Omebeta, Betafarm) and liquid dosage forms for both oral (Sporanox Oral Solution; Janssen Pharmaceuticals) and parenteral (e.g., Vfend, Pfizer) routes of administration.

Common CDs include those formed by six, seven, or eight saccharide monomers, and these are classified as α, β, or γ CD, respectively. The most widely used CDs in pharmaceutical preparations are based on β-CD (i.e., CDs containing seven sugar moieties) (Fig. 32). The broad utility of β-CD stems from the good alignment between β-CD cavity size and the aromatic scaffolds commonly encountered in poorly water-soluble drugs. Since the upper limit of the solubility of a drug-CD complex is that of the CD alone, the relatively poor water solubility of unmodified CDs limits their widespread utility. Modified CDs, such as hydroxypropyl-β-CD (HP-β-CD) and sulfobutylether-β-CD (SBE-β-CD) (Table 22), have therefore been introduced, the solubility of both being significantly higher than that of the unmodified material and typically in excess of 0.5 g/ml (see Table 22). The higher solubility of chemically modified CDs also reduces the nephrotoxicity previously associated with poorly water-soluble CDs (Brewster et al., 1989; Thompson, 1997; Stella and He, 2008). This improved toxicological profile of the modified CDs has enhanced interest in the use of CDs in parenteral
applications. CDs are generally not absorbed after oral administration; therefore, the potential for systemic toxicity is much lower than that after parenteral administration. Recent interest in the use of CDs as actives in of themselves (e.g., effects on cholesterol absorption and renal drug elimination) raises a number of potential complications with respect to excipient classification and the traditional assumption that excipients are “inactive.” This is increasingly evident across a number of excipient classes, including, for example, surfactants where activity in transporter and metabolic inhibition is increasingly described. However, the ramifications of this with respect to drug product registration are yet to unravel fully.

The utility of CDs in assisting the delivery of poorly water-soluble drugs is dictated by the affinity of the drug for the CD as indicated by the magnitude of the drug-CD binding constant. Increasing affinity increases the proportion of the total solubilized drug concentration that is present within the CD complex and reduces the quantity of CD required to dissolve a target drug mass. Increased drug affinity for the complex is therefore favored in most circumstances. High binding affinities, however, may lead to incomplete dissociation of the drug-CD complex after administration (and dilution) and limit the concentration of free drug available for absorption or activity. Very high binding affinities have also been associated with altered drug pharmacokinetics after i.v. administration of a drug-CD complex (Charman et al., 2006), although this appears to be the exception rather than the rule and only likely for drug molecules with CD binding constants in excess of $1 \times 10^5$ M$^{-1}$ (Stella and He, 2008).

The nature of the drug-CD complex dictates that solubilization with CDs is molecularly specific and driven by the relative “fit” of the drug molecule (or a portion of the molecule) within the CD cavity. CD approaches are therefore less generic than solubility enhancement strategies such as micellar solubilization. Nonetheless, CDs solubilize a wide variety of structurally distinct drug molecules, both neutral and ionized, and may be used with other solubilization strategies, such as pH adjustment.

Solid drug-CD complexes may also be isolated by, for example, freeze-drying drug-CD solutions. Parenteral drug-CD formulations that can be reconstituted before use and oral formulations containing solid CD-drug complexes are therefore possible. In such formulations, rates of in vitro dissolution of the drug-CD complex may be dramatically increased compared with uncomplexed drug due to the significant increases in apparent solubility of the drug-CD complex. The generation of an altered solid form of the drug also provides additional mechanisms of dissolution enhancement via changes to solid-state properties.

**TABLE 21**

Commercially available cyclodextrin-containing formulations

<table>
<thead>
<tr>
<th>Drug/Product</th>
<th>Cycloextrin (CD) Used</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporanox (itraconazole; Janssen Pharmaceuticals)</td>
<td>HP-$\beta$-CD</td>
<td>Oral solution/i.v. solution</td>
</tr>
<tr>
<td>MitoExtra, Mitoztrex (mitomycin; Novartis Pharmaceuticals)</td>
<td>HP-$\beta$-CD</td>
<td>i.v. infusion</td>
</tr>
<tr>
<td>Abilify (aripiprazole; Bristol-Myers Squibb and Otsuka Pharm)</td>
<td>SBE-$\beta$-CD</td>
<td>i.m. solution</td>
</tr>
<tr>
<td>Vfend (voriconazole; Pfizer)</td>
<td>SBE-$\beta$-CD</td>
<td>i.v. solution</td>
</tr>
<tr>
<td>Yaz (ethinylestradiol and drospirenone; Bayer)</td>
<td>$\beta$-CD</td>
<td>Tablet</td>
</tr>
<tr>
<td>Omebeza (omeprazole; Betafarm)</td>
<td>$\beta$-CD</td>
<td>Tablet</td>
</tr>
<tr>
<td>Caverject Dual (alprostadil; Pfizer)</td>
<td>$\alpha$-CD</td>
<td>i.v. solution</td>
</tr>
<tr>
<td>Nitropen (nitroglycerin; Nippon Kayaku)</td>
<td>$\beta$-CD</td>
<td>Sublingual tablet</td>
</tr>
</tbody>
</table>

HP, hydroxypropyl; SBE, sulfobutylether.
Indeed, CD formulations where drug is present in the amorphous form have been described, and under these circumstances, amorphous CD formulations have much in common with, and share the potential advantages of, solubilizing solid dispersion formulations (see Section X) and microporous adsorbent formulations (see Section XII.A). However, the disadvantages of formulations that contain drug in the noncrystalline form also apply to amorphous CD formulations; the risks of drug precipitation post dissolution and amorphous-crystalline solid-state transitions during storage are ever present.

In this section, the fundamental principles underpinning the use of CDs to increase drug solubility are reviewed and the drug and non-drug related factors that affect drug-CD complexation discussed. Examples of CD utility in oral and parenteral delivery are subsequently presented, including a brief discussion of the issues surrounding CD toxicity. Finally, a review of the mechanisms of drug release from the CD complex is presented and a brief assessment provided of the most common approaches to the manufacture CD formulations.

B. Drug-Cyclodextrin Inclusion Complexes

1. Binding Equilibria between a Drug and Cyclodextrin.

Complexation between a drug molecule (the “guest”) and CD molecule (the “host”) initially requires rearrangement or displacement of water molecules that are present within the CD cavity and that sovlate the drug molecule in solution. The complex is then formed via intermolecular (noncovalent) interactions between the guest (drug) and the CD, and water molecules subsequently reorganize around the newly formed drug-CD complex (Bekers et al., 1991).

The process of drug-CD complexation is typically characterized by a large and negative enthalpy (ΔH) (Ross and Rekharsky, 1996; Rekharsky and Inoue, 2000; Liu and Guo, 2002) that reflects the intermolecular interactions that favor complex formation. These interactions usually comprise van der Waals and hydrophobic interactions between the drug and the hydrophobic CD cavity. Changes in entropy (ΔS) are usually moderate and either negative or slightly positive Komiyama and Bender, 1978; Harrison and Eftink, 1982; Inoue et al., 1993). In contrast, the association of two nonpolar molecules in solution (or, for example, amphiphiles during micelle formation) is more commonly associated with an increase in entropy as structured water molecules are redistributed into free solution (Frank and Evans, 1945). The more moderate changes in entropy during drug-CD complexation have been suggested to reflect a loss of rotational freedom on complex formation that offsets the gain in entropy during water redistribution. The free energy of complexation is therefore driven by the negative enthalpy term rather than by an increase in entropy and provides an example of enthalpy-entropy compensation (Inoue et al., 1993; Rekharsky and Inoue, 2000; Omar et al., 2007; di Cagno et al., 2011). Ionic substituents located on the exterior segment of some CDs, for example, SBE-β-CD, may also interact with the drug via ion-dipole interactions or via an ion pair if the drug is oppositely charged. Drug interactions external to the cavity may also play a role in dictating the stoichiometry of drug-CD complexation (Okimoto et al., 1996).

Drug binding affinity to a CD is described by the equilibrium binding constant (K), also referred to as the stability constant. The binding constant provides a measure of the solubilizing potential of a particular CD toward the drug of interest. For a 1:1 complex of drug-CD, the binding constant K is defined by eq. 39, where [D] and [CD] are the concentration of the free drug and CD, respectively, and [D:CD] is the concentration of the formed complex:

$$D_f + CD_f \rightleftharpoons [D:CD]$$

$$K_{1:1} = \frac{[D:CD]}{[D_f][CD_f]} \quad (39)$$

From eq. 39, it is clear that the higher the binding constant (K), the greater the ratio of complexed drug to uncomplexed drug. Binding constants typically lie between 0 (no binding) and 1 × 10^6 M⁻¹ (high binding),

### TABLE 22

Examples of commonly used cyclodextrins to aid oral and parenteral delivery of poorly water-soluble drugs

<table>
<thead>
<tr>
<th>Cyclodextrin</th>
<th>Common Name</th>
<th>MS^a and Mw^b</th>
<th>Aqueous Solubility (mg/ml (20°C))</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CD (6 glucose units)</td>
<td>Alfadex</td>
<td>~972</td>
<td>14.5</td>
</tr>
<tr>
<td>β-CD (7 glucose units)</td>
<td>Betadex</td>
<td>~1135</td>
<td>1.85</td>
</tr>
<tr>
<td>γ-CD (8 glucose units)</td>
<td>Gammadex</td>
<td>~1297</td>
<td>23.2</td>
</tr>
<tr>
<td>2-Hydroxypropyl-α-CD (HP-α-CD)</td>
<td>Cavasol W6 HP (Wacker, Germany)</td>
<td>0.65/1199</td>
<td>&gt;500</td>
</tr>
<tr>
<td>2-Hydroxypropyl-β-CD (HP-β-CD)</td>
<td>Cavasol W7 HP (Wacker, Germany)</td>
<td>0.65/1400</td>
<td>&gt;600</td>
</tr>
<tr>
<td>Sulfobutylether-β-CD (SBE-β-CD)</td>
<td>Capitosol (CyDex Pharmaceuticals, USA)</td>
<td>0.9/2163</td>
<td>&gt;600</td>
</tr>
<tr>
<td>Randomly methylated-β-CD (RM-β-CD)</td>
<td>Cavasol W7 M (Wacker, Germany)</td>
<td>1.8/1312</td>
<td>&gt;600</td>
</tr>
<tr>
<td>Maltosyl-β-CD (Mal-β-CD)</td>
<td>Ensuiko Sugar Refining Co. (Japan)</td>
<td>0.14/1459</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

CD, cyclodextrin; HP, hydroxypropyl; SBE, sulfobutylether.

^a Average molar substitution.

^b Average molecular weight.

^c Also described as “hydroxypropyl betadex” and “betadex sulfobutyl ether sodium” by the United States Pharmacopeia and National Formulary.

^d CyDex Pharmaceuticals was recently acquired by Ligand Technology, La Jolla, CA.
although most drugs fall within the range of $1 \times 10^2$ and $1 \times 10^4 \text{M}^{-1}$ (Rajewski and Stella, 1996; Stella and Rajewski, 1997; Stella et al., 1999). Higher binding constants are desirable since lower levels of CD may be used in the formulation; however, there is a risk of incomplete dissociation of the drug-CD complex after administration if the binding constant is very high (i.e., in excess of $1 \times 10^5 - 1 \times 10^6 \text{M}^{-1}$). Incomplete dissociation may in turn affect drug pharmacokinetic and pharmacodynamic properties (Stella and He, 2008). This is described in more detail in Section VIII.E.

In saturated solutions (i.e., at equilibrium), $[D_d]$ in eq. 39 is the intrinsic drug solubility ($S_0$). The total concentration of dissolved drug in a CD solution ($S_T$) is therefore the sum of free drug concentration and drug complexed with the CD:

$$S_T = S_0 + [D:CD]$$

Merging eq. 39 and eq. 40 provides an expression that indicates the solubility gain that may be achieved at different CD concentrations:

$$S_T = S_0 + \frac{K_{1:1}S_0}{1 + K_{1:1}S_0} [CD_T]$$

where $[CD_T]$ is the total CD concentration added to the aqueous solution. CD concentrations in pharmaceutical preparations greater than 40% are unusual (Li and Zhao, 2007; Stella and He, 2008). Therefore, if the intrinsic drug solubility ($S_0$) is known, eq. 41 allows rapid assessment of the potential utility of CDs through substitution of the target drug concentration for $S_T$ and calculation of the required value for $K$ to achieve this target drug concentration. Where the value for $K$ exceeds values typical for pharmaceutical molecules (i.e., $> 1 \times 10^6 \text{M}^{-1}$), CD alone is unlikely to provide a suitable solubilization strategy. In these situations, an alternative strategy or a combined approach (e.g., CDs plus pH adjustment in the case of an ionizable drug) may be required. It should be noted that eq. 41 applies only for drugs that form 1:1 complexes. The formation of a higher-order (e.g., 2:1) drug-CD complex requires less CD to achieve the intended target concentration. Similarly, lower order complexes (e.g., 1:2 drug-CD complexes) suggest poor drug CD complexation and the need for larger quantities of CD to facilitate effective solubilization.

Where the binding constant of a drug of interest has already been determined or is available in the literature, the CD concentration required to dissolve the drug may also be calculated from eq. 41.

2. Measurements of Drug-Cyclodextrin Binding Constants. The binding constant of a drug-CD complex can be measured using several different approaches. In one of the most common approaches, the phase solubility approach, dissolved drug concentrations are determined in solutions of increasing CD concentration. An example phase-solubility profile is shown in Fig. 33A, with the gradient of the slope reflecting the solubilizing capacity of the CD. The binding constant for a 1:1 complex is calculated by solving eq. 42 using the linear phase solubility profile (gradient $m$, intercept $S_0$):

$$S_T = S_0 + \frac{K_{1:1}S_0}{1 + K_{1:1}S_0} [CD_T] = m[CD_T] + S_0$$

Rearranging to generate eq. 43:

$$m = \frac{K_{1:1}S_0}{1 + K_{1:1}S_0}$$

Eq. 43 can itself be arranged to calculate the binding constant, $K$:

$$K_{1:1} = \frac{m}{S_0 - m}$$

Other commonly used methods to determine drug-CD binding constants include NMR spectroscopy, UV spectroscopy, circular dichroism, potentiometry, microcalorimetry, freezing-point depression, HPLC/TLC, membrane permeation techniques, and capillary electrophoresis. Spectroscopic techniques (e.g., NMR) have proved useful in discerning drug-CD complex stoichiometry (Bettinetti et al., 1991; Piel et al., 1998; Miyake et al., 1999b; Ribeiro et al., 2005a; di Cagno et al., 2011). Provided the environmental factors likely to affect solubility (e.g., pH and temperature) are tightly controlled, different methods may generate highly consistent results (Kurkov et al., 2011). However, in some cases, higher-order complexes identified during phase solubility studies have not been able to be verified by NMR experiments or UV spectroscopy. These discrepancies may be attributed to the more dilute conditions used in the NMR/UV spectroscopy studies, in which there is a reduced likelihood of forming higher-order complexes in comparison with the higher concentration conditions in phase-solubility experiments (Loftsson et al., 2002; Magnusdottir et al., 2002; Loftsson et al., 2005).

Phase-solubility profiles are classified according to their shape (Higuchi and Connors, 1964), and vary depending on the solubility and stoichiometry of the complex (Fig. 33). Two main solubility profiles are evident and are described as either A-type or B-type. B-type profiles typically lead to limited increases in drug solubility and are more commonly (but not exclusively) associated with nonsubstituted CDs (Szejtli, 1994; Brewster and Loftsson, 2007). B$_S$-type profiles are reflective of situations where complexation initially results in an increase in drug solubility, but the maximum solubility of the complex in solution is soon reached (the plateau phase in Fig. 33B). Further increases in CD concentration result in precipitation of the complex.
decreasing solubilized drug concentrations (Szejtli, 1994; Brewster and Loftsson, 2007). As seen in Fig. 33B, B₁-type profiles are characteristic of situations where the complex has lower solubility than drug alone (Uekama et al., 1998; Brewster and Loftsson, 2007). The lack of solubility gain makes B-type CD complexes less attractive for most applications.

In contrast, significant increases in the quantity of drug dissolved (ST) with increasing CD concentration are evident in A-type phase solubility profiles. A-type profiles that increase linearly with increase in CD concentration are common within pharmaceutical sciences and are denoted A₁-type. Examples of poorly water-soluble drugs exhibiting A₁-type CD solubility profiles include nicardipine hydrochloride with β-CD (Fernandes et al., 2002), itraconazole with β-CD (Al-Marzouqi et al., 2006), indomethacin with β-CD (Jambhekar et al., 2004), cinnarizine with HP-β-CD and SBE-β-CD (Jarvinen et al., 1995), phenytoin with HP-β-CD and SBE-β-CD (Savolainen et al., 1998), danazol with HP-β-CD (Badawy et al., 1996), dexamethasone with HP-β-CD (Loftsson et al., 1994), and flavopiridol with HP-β-CD (Li et al., 1998a).

The slope of A₁-type profiles can provide an indication of the stoichiometry of complex formation (Fig. 34). Slopes of gradient unity indicate 1:1 drug-CD complexes. Slopes that are greater than unity suggest the formation of a higher-order complex with respect to the drug (i.e., more than one drug molecule per CD molecule) (Yalkowsky, 1999; Brewster and Loftsson, 2007; Loftsson and Brewster, 2010). Nonlinear increases in ST above a critical CD concentration are also possible and are classified as Aᵣ-type (i.e., a positive deviation) or Aᵣ-type profiles (i.e., a negative deviation) (Higuchi and Connors, 1964) (Fig. 33B). Aᵣ-type profiles are less common than A₁-type profiles but have been observed for several drugs, including cyclosporine A (Miyake et al., 1999a), itraconazole (Miyake et al., 1999b; Peeters et al., 2002; Brewster et al., 2008a), miconazole (Pedersen et al., 1993; Okimoto et al., 1996; Ribeiro et al., 2008), and tacrolimus (Arima et al., 2001b). Aᵣ-type profiles reveal greater increases in drug solubilization than expected from simple 1:1 complexes and therefore...
suggest the presence of higher-order complexes with respect to the CD (on reaching a certain CD concentration) (Fig. 34). The stoichiometry of higher-order complexes is inferred from the extent of the deviation in AN-type profiles (Brewster and Loftsson, 2007) and the goodness of fit to either quadratic (for 1:2 complexes, eq. 45) or cubic (for 1:3 complexes, eq. 46) functions (Peeters et al., 2002):

\[
S_T = S_0 + K_{11}S_0[CD] + K_{11}K_{12}S_0[CD]^2
\]

\[
S_T = S_0 + K_{11}S_0[CD] + K_{11}K_{12}S_0[CD]^2 + K_{11}K_{12}K_{13}S_0[CD]^3
\]

The accuracy of these models is limited by the assumption that the total CD concentration is equal to the free (uncomplexed) CD concentration. Errors occur where a significant proportion of the CD is in the complexed form (as is often the case) (Peeters et al., 2002; Brewster and Loftsson, 2007; Brewster et al., 2008a). Aside from higher-order molecular complexes with respect to the CD, AN-type profiles may also result from the formation of noninclusion complexes or via solubilization of drug by drug-CD aggregates. These potential situations are described in more detail in Section VIII.B.3.

Phase solubility profiles showing negative deviations, or AN-type profiles (Fig. 33B), describe situations where the solubilization efficiency of the CD decreases on reaching a critical concentration (Higuchi and Connors, 1964). This may occur as a result of 1) a decrease in solvent quality through increased solution viscosity at higher drug and CD concentrations, 2) self-association of free CD (thereby reducing the available concentration of free CD), or 3) where ionic interactions are evident, CD-mediated changes in the apparent pKₐ of the drug (Uekama et al., 1998; Brewster and Loftsson, 2007). AN-type profiles are comparatively rare in the pharmaceutical literature but have been observed for miconazole (Piel et al., 1998; Ribeiro et al., 2008), carvedilol (Miro et al., 2006), and gefitinib (Lee et al., 2009).

The complexation efficiency (CE) provides an alternative to the binding constant for quantification of CD solubilization capacity (Loftsson et al., 2007a). CE is calculated from the concentration ratio between complexed and uncomplexed CD:

\[
CE = \frac{[D\cdot CD]}{[CD]} = S_0 \cdot K_{11} = \frac{m}{1 - m}
\]

A CE value of 1 indicates that all added drug is complexed with CD (in a 1:1 ratio), whereas a CE of 0.1 indicates that 1 drug molecule of 11 has formed a complex (Loftsson et al., 2005). CE has been suggested to provide a more robust indication of the solubilization properties of different CDs since the intrinsic solubility (S₀) of poorly water-soluble drugs is low and may be overestimated by the intercept in a phase solubility curve (Loftsson et al., 2005, 2007a; Loftsson and Brewster, 2010).

3. Secondary Equilibria: Micelles and Cyclodextrin Aggregate Formation. All natural cyclodextrins self-associate in solution to form aggregates and micelles (Mele et al., 1998; Szente and Szefjli, 1999; Loftsson et al., 2002; Bonini et al., 2006). Chemically modified CDs (e.g., HP-β-CD and SBE-β-CD) may also acquire surfactant-like properties after incorporation of a hydrophobic/lipophilic drug molecule (Loftsson et al., 2002; Magnusdottir et al., 2002; Kurkov et al., 2011) or, in the case of CDs with hydrophobic modifications, in the absence of a guest (Auzely-Velty et al., 2000). In addition to forming typical guest-host molecular inclusion complexes, CDs also have the potential to form micellar aggregates and provide additional sites and mechanisms of drug solubilization.

For example, phase solubility profiles of the sodium salts of the nonsteroidal anti-inflammatory drugs ibuprofen and diflunisal with HP-β-CD have been reported to be AN type with slopes of greater than 1, indicative of the formation of higher-order (e.g., 2:1) drug-CD complexes (Magnusdottir et al., 2002; Kurkov et al., 2011). Space-filling docking analysis of the 1:1 complex, however, suggested that the CD cavity in HP-β-CD was too small to accommodate an additional drug molecule, and the authors concluded that HP-β-CD solubilization of ibuprofen and diflunisal was a combination of molecular complexation and drug solubilization via aggregates of the drug-CD complex (Magnusdottir et al., 2002; Kurkov et al., 2011). Similarly, the shape of phase solubility profiles for cholesterol–HP-β-CD and cholesterol–SBE-β-CD could not be explained by the formation of drug-CD inclusion complexes alone (Loftsson et al., 2002), prompting the suggestion of a role for drug solubilization via non-inclusion complexation in the overall solubility profile. Further evidence that cholesterol-HP-β-CD complexes may have intrinsic solubilization properties is apparent in studies showing an increase in cyclosporine A solubilization by HP-β-CD on the addition of cholesterol (Loftsson et al., 2002, 2005). In the latter case, the increase in cyclosporine A solubility occurred despite the expectation that the addition of cholesterol would result in a decrease in drug solubilization (as a result of competition for the CD cavity). A number of methods have also been proposed to allow detection of CD aggregates directly (rather than via indirect inference from phase solubility studies), and these methods have been the subject of a recent review (Messner et al., 2010). It remains unclear, however, whether drug-CD aggregates affect the in vivo performance of CD formulations since the aggregates are held together through weak interactions such as hydrogen bonding and van der Waals interactions, and deaggregation on
dilution in blood or in the GI fluids is likely (Messner et al., 2010, 2011b).

C. Drug Factors Affecting Complexation by Cyclodextrins

1. Size. As different CDs show differences in the size of the hydrophobic cavity and the degree and nature of chemical substitution, drug affinity toward a range of CD molecules may vary considerably. However, drug molecular size, relative to the size of the hydrophobic cavity, provides a strong indication of the likelihood of complexation and also the order of the complex (Loftsson et al., 1993; Loftsson and Brewster, 1996; Mura et al., 1999; Loftsson et al., 2002, 2005; Tønnesen et al., 2002). The size of the CD cavity varies depending on the number of glycopyranose monomers in the ring. Thus, α-CDs have the smallest cavity (∼4.7–5.3 Å in diameter) and γ-CDs the largest (∼7.5–8.3 Å in diameter). The size of the inclusion complex cavity for β-CD is ∼6.0 – 6.5 Å (Uekama, 2004).

The size of the hydrophobic cavity of the CD host determines the fit of the guest drug molecule (or molecular fragment) and, subsequently, the extent of the interaction of the guest with the host post-insertion. The dependence of the size of the CD cavity on the generation of ideal guest-host complexes is well exemplified by Cromwell et al. (1985), who examined the binding affinity of a carboxylate derived adamantane for α-CD, β-CD, and γ-CD. The carboxylate-derived adamantane was able to form complexes with both β-CD and γ-CD, but a better fit with β-CD led to an increased number of van der Waals interactions between the guest and host and, ultimately, a higher enthalpy of binding. This in turn favored complexation with the β-CD derivative, whereas the adamantane was too large to complex effectively with the smaller α-CD hydrophobic cavity (Cromwell et al., 1985).

In addition to the complexation of adamantanes (for which β-CD has particularly high affinity), the β-CD cavity is well suited to the insertion of aromatic benzene rings. Since this functional group is found widely in the molecular structure of many drugs β-CD, and its derivatives, are well suited for solubilization of a large number of pharmaceutical molecules (Marques et al., 1990; Nakai et al., 1990; Tong et al., 1991; Kearney et al., 1992; Takisawa et al., 1993). Importantly, the entire drug molecule does not need to enter the hydrophobic cavity for effective CD complexation since binding may be achieved in instances where only a small portion of the drug molecule is associated with the CD. This is evident in the CD complexation of high M_w compounds such as tacrolimus (Arima et al., 2001b) and itraconazole (Peeters et al., 2002), both of which showed good binding to β-CD but are too large to be entirely enclosed within the CD cavity.

As only a portion of the drug molecule is required to form a drug-CD complex, and since many molecules contain more than one aromatic group, larger drug molecules are well suited to forming higher-order complexes with respect to the CD (i.e., one drug molecule and multiple CD molecules). Itraconazole can form 1:2 complexes with HP-β-CD, and under neutral conditions, even 1:3 complexes are possible (Miyake et al., 1999b; Peeters et al., 2002).

One notable example of the use of CDs other than β-CD is the development of sugammadex (Bridion, formerly Organon, now Merck). Sugammadex is a modified γ-CD with a very high binding affinity for the anesthetic rocuronium [the sugammadex-rocuronium binding constant is in the order of 1 × 10^7 M⁻¹ which is at least 10-fold higher than any of the other drug-CD binding constants in the literature (Bom et al., 2002)]. The high binding affinity of sugammadex for rocuronium ensures that i.v. administration of sugammadex results in rapid sequestration of rocuronium into the CD complex and effective reversal of neuromuscular blockade without the need for the administration of anticholinesterases.

2. Polarity and Charge. As the inner cavity of a CD molecule is hydrophobic, increasing drug hydrophobicity typically increases binding affinity (Bergeron et al., 1978; Uekama et al., 1982, 1983; Jones and Parr, 1987; Marques et al., 1990; Liu and Guo, 2002). Solubility enhancement via CD solubilization is therefore more pronounced for drugs with increasing hydrophobicity. For example, the solubility of dithiolethione (log \( P = 1.58 \)) increases ∼9-fold on the addition of 10% HP-β-CD, whereas the solubility of the structurally related but more lipophilic 5-phenyldithiolethione (log \( P = 3.67 \)) and anetholetrithione (log \( P = 3.82 \)) increases ∼400-fold in the presence of HP-β-CD (Dollo et al., 1999). A similar trend was observed in the same study using SBE-β-CD. Albers and Muller (1992) also noted an increase in the effectiveness of HP-β-CD complexation for more hydrophobic derivatives of testosterone and estradiol. Structural changes that increase hydrophobicity, such as increasing alkyl chain length or the size and number of aromatic rings in the drug molecule, are likely to contribute to enhanced CD complexation, although structural modification of this type will also reduce intrinsic solubility (in water), thereby increasing the need for enhanced solubilization. The net effect of increasing drug hydrophobicity on total drug solubility is therefore difficult to predict.

For ionizable drugs, the degree of complexation of the un-ionized form is typically greater than the ionized (and more polar) equivalent (Tinwalla et al., 1993; Okimoto et al., 1996; Redenti et al., 2000). Binding constants can be measured as a function of pH to describe the relative binding affinities of the un-ionized (K_w) and ionized (K_i) forms (Okimoto et al., 1996). Comparison of the degree of CD complexation
with the un-ionized/ionized drug fraction (i.e., $K_u/K_i$) provides an indication of the loss of binding affinity through the introduction of charge on the drug molecule. Scheme 3 illustrates the effect of ionization on complexation between a neutral CD and an acidic or basic drug. Although both ionized and un-ionized forms of a drug may form an inclusion complex with the CD, it is more common that the more hydrophobic un-ionized form more readily complexes and thus, $K_u > K_i$. The scheme also highlights the potential for dissociation of drug in free solution ($K_a$) and as the complex ($K_{a}^{'}$).

From Scheme 3, total drug solubility under conditions of changing pH is the sum of four species (i.e., the total of free and complexed drug) in both the un-ionized form and the ionized form (Tinwalla et al., 1993; Okimoto et al., 1996):

For a weak acid:

$$S_T = [HA] + [HA:CD] + [A^-] + [A^-:CD]$$ (48)

For a weak base:

$$S_T = [B] + [B:CD] + [BH^+] + [BH^+:CD]$$ (49)

Although a pH change resulting in increased drug ionization may reduce CD binding affinity, the higher aqueous solubility of the ionized form is often sufficient to compensate for decreased CD association, and total solubilized drug concentrations increase (Li et al., 1998a, 1999c; Piel et al., 1998; Jain et al., 2001; Ni et al., 2002; Ran et al., 2005; He et al., 2006). This situation is analogous to the combined use of pH manipulation with cosolvents and surfactants, where increasing ionization reduces the impact of the cosolvent or micellar solubilization effect, but the net effect is to enhance total solubility.

An example of this synergy is provided in Fig. 35, where the solubility of the weak base flavopiridol increases with increasing acidity (i.e., with decrease in pH) and with increases in HP-$\beta$-CD concentration at both high and low pH, suggesting that both un-ionized and ionized drugs show some affinity for the CD (Li et al., 1998a). The marketed Sporanox formulations provide further evidence of the impact of combining pH with CD complexation. The i.v. formulation contains 10 mg/ml itraconazole complexed with 40% HP-$\beta$-CD, with the pH adjusted to pH 4.5, whereas the less stringent irritancy concerns associated with oral administration allow the oral formulation to be pH adjusted to pH 2.0 (Strickley, 2004).

The potential for CD ionization adds additional complexity to the understanding of pH effects on drug-CD complexation. SBE-$\beta$-CD, for example, is anionic and has been suggested to form ion pairs with the weakly basic drugs prazosin, papaverine, and miconazole in pH conditions where both drug and CD are (partially) ionized (Okimoto et al., 1996; Zia et al., 2001). In contrast, for weakly acidic drugs (e.g., naproxen, warfarin, and indomethacin), the situation was reversed, and binding affinity of the charged species was reduced. The authors suggested that this may be attributed to electrostatic repulsion between SBE-$\beta$-CD and the similar charged anionic drugs (Okimoto et al., 1996; Zia et al., 2001). Electrostatic repulsion may therefore limits complexation, although increasing the ionic strength of the solution may restore some degree of association via salting out and charge

\[ \text{Scheme 3.} \]

**Fig. 35.** Total experimental aqueous flavopiridol solubilities in hydroxypropyl-$\beta$-cyclodextrin (HP-$\beta$-CD) solutions at different pH values. Adapted from Li et al. (1998a).
shielding effects (Zia et al., 2001; Brewster et al., 2008a).

Finally, a change in solution pH may also influence the order of drug-CD complexes. Itraconazole is a weak base and complexes with HP-β-CD in a pH-dependent manner, as the three potential proton acceptor sites are, under conditions of increasing pH, progressively deprotonated and, therefore, increasingly available for interaction with CDs. Increases in pH from 1.5 to 2.5 to 4.0 leads to a progressive increase in the $K_{1:2}$ binding constant (i.e., an increase in affinity for the 1:2 drug-CD complex) (Miyake et al., 1999b; Peeters et al., 2002), and molecular mechanics simulations suggest the formation of a 1:3 drug-CD complex at neutral pH (Peeters et al., 2002).

The potential for electrostatic repulsion is generally thought to preclude charged CDs from forming higher-order complexes (with respect to CD) (Thompson, 1997; Loftsson et al., 2002); however, evidence of Δ-type increases in itraconazole solubility in the presence of SBE-β-CD (Brewster et al., 2008a) suggest that this may not always be the case. Whether Δ-type solubilization profiles using charged CDs are due to conventional complexation or solubilization via noninclusion complexes is currently not well understood and is likely to be further complicated in situations where the drug itself may be ionized (as ion-pair interactions between drug and CD are likely to contribute to drug solubilization).

3. Presence of Counterions. Several studies have reported an increase in CD solubilization for ionizable compounds in the presence of low-molecular-weight acidic/basic counterions, including tartaric and malic acid, bile acids, and various amino acids (Piel et al., 1997; Faucci et al., 2000; Mura et al., 2001b; Mura et al., 2003, 2005). The mechanism of solubilization enhancement is attributable to an ion-pair formation, where the CD and counterion interact via a “salt bridge” that stabilizes the drug-CD complex and promotes solubilization of ionized drug.

For example, naproxen solubility in HP-β-CD is higher in the presence of arginine (a basic counterion) (29.5 mg/ml) compared with HP-β-CD solutions of equivalent pH without arginine or in solutions containing arginine alone (11.3 mg/ml and 14.5 mg/ml, respectively) (Mura et al., 2003). Solubility advantages are also dependent on the nature of the counterion, and the solubility of an econazole-malic acid-α-CD ternary complex has been reported to be enhanced compared with equivalent ternary complexes formed in the presence of drug, CD, and lactate or citrate counterions (Mura et al., 2001b). Since the lactate and citrate salts of econazole were more soluble than the malate salt in the absence of CD, the authors concluded that steric effects and counterion size (and not salt solubility) were more important factors in determining the extent of solubility enhancement provided by the ternary complex (Mura et al., 2001b).

D. Impact of Cosolubilizers and Excipients on Cyclodextrin-Mediated Drug Solubilization

1. Cyclodextrins and Water-Soluble Polymers. Many hydrophilic polymers increase drug binding to CDs. The addition of HPMC or PVP increases vinpocetine binding to β-CD and SBE-β-CD (Fig. 36) (Ribeiro et al., 2003b, 2005a), and HP-β-CD binding constants for hydrocortisone, dexamethasone, and carbamazepine are all increased in the presence of HPMC (Loftsson et al., 1994; Smith et al., 2005; Kou et al., 2011). Similarly, the solubility of Δ9-tetrahydrocannabinol increases approximately 200-fold in the presence of 8% HP-β-CD and approximately 300-fold in solutions containing 8% HP-β-CD and 0.1% HPMC.

Fig. 36. Phase-solubility diagrams for vinpocetine at room temperature in the presence of (A) β-cycloDEXTRIN (β-CD) and (B) sulfolubulether-β-cycloDEXTRIN (SBE-β-CD) with or without the water-soluble polymers HPMC/PVP. Values are expressed as means. HPMC, hydroxypropyl methylcellulose; PVP, polyvinylpyrrolidone. Adapted from Ribeiro et al. (2003a).
(Jarho et al., 1998). Water-soluble polymers (at low concentrations), therefore, offer a potentially synergistic benefit for CD-mediated drug solubilization.

The mechanism by which water-soluble polymers enhance drug solubilization in the presence of CDs is not fully understood (polymers typically have little intrinsic effect on drug solubility), although it has been suggested that the polymers interact directly with the drug-CD complex to form a ternary drug-CD-polymer structures with enhanced solubilization capacity (Loftsson et al., 1994; Loftsson and Brewster, 1996; Messner et al., 2010). In support of this contention, solid-state analysis of vinpocetine-CD-polymer ternary complexes reveals evidence of hydrogen bond interactions between the polymer and the drug-CD complex (Ribeiro et al., 2003b). However, other interactions (such as van der Waals or ion-dipole interactions) cannot be discounted (Ribeiro et al., 2005a). Indeed, Valero et al. (2003) suggest that PVP is able to form ion-dipole interactions with naproxen sodium while the drug is enclosed within the hydrophobic cavity of HP-β-CD, and simultaneously to form hydrogen bonds with the CD. Interestingly, in the latter example, the addition of PVP did not enhance CD solubilization of naproxen, and Smith et al. (2005) report that PVP is unable to enhance the solubilization of carbamazepine by SBE-β-CD. Enhancement of CD solubilization via the addition of water-soluble polymers is therefore not universal.

Polymers may also stabilize drug-CD aggregates and reduce the potential for deaggregation on dilution or agitation (Loftsson et al., 2002; Messner et al., 2011b). Thus, 0.5% carboxymethyl cellulose (a negatively charged polymer) stabilizes hydrocortisone–HP-β-CD aggregates (Messner et al., 2011a), although in this case, the addition of 5% of a neutral (HPMC) or positively charged (hexadimethrine bromide) additive had little effect on aggregate stability (Messner et al., 2011a).

2. Cyclodextrins and Cosolvents. Cosolvents such as short-chain alcohols (e.g., ethanol), low-molecular-weight polyethylene glycols, and propylene glycol are often used to enhance drug solubility (see Section VI) and may also be used in combination with CDs. However, cosolvent molecules compete with drug molecules for the CD hydrophobic cavity and also promote the solubility of the noncomplexed drug in free solution (Loftsson et al., 1993). As such, drug-CD binding may be reduced (van Stam et al., 1996; Miyake et al., 1999b; Evans et al., 2000; Partyka et al., 2001). Nonetheless, combinations of CDs with cosolvents may lead to net increases in drug solubilization compared with cosolvent or CD alone (Reer and Muller, 1993; Yalkowsky, 1999; Li et al., 1999d; Nandi et al., 2003). Consistent with this suggestion, the marketed itraconazole formulations (Sporanox IV and Oral Solution) contain both CD and propylene glycol. Similarly, the solubility of fluasterone in a 20% HP-β-CD solution is 4.04 mM (He et al., 2003), and although similar increases in solubility are possible via the addition of cosolvent, this is evident only on the addition of large-volume fractions (>50% cosolvent). In contrast, appropriate combinations of 20% HP-β-CD with methanol, ethanol, and propanol are all capable of increasing solubility above 4.04 mM (He et al., 2003). Interestingly, at lower CD concentrations (i.e., ≤10% HP-β-CD), low concentrations of cosolvent (typically <25% cosolvent) decreased or had no impact on drug solubilization (He et al., 2003). The potential for cosolvent to lower drug solubilization in the presence of lower concentrations of CD likely reflects the competitive effects of the cosolvent for the hydrophobic cavity. He and coworkers (2003) noted that a higher-molecular-weight cosolvent (propanol) showed the highest capacity to lower drug solubility in the presence of CD and subsequently proposed that for the lower molecular weight cosolvents, CDs may simultaneously complex cosolvent and drug, thereby preventing the cavity competition effects evident with propanol (see also Section VI.D.3).

3. Cyclodextrins and Surfactants. Cyclodextrins form inclusion complexes with both nonionic (Veiga and Ahsan, 1998; Buschmann et al., 2000; Eli et al., 2000) and ionic surfactants (Park and Song, 1989; Junquera et al., 1993), leading to potential competition between drug and surfactant for CD binding sites. Furthermore, the complexed surfactant fraction is unable to participate in micelle formation, reducing micellar solubilization (Junquera et al., 1993). Combinations of CDs and surfactants therefore often lead to a decrease in the concentration of solubilized drug (Veiga and Ahsan, 1998; Tommasini et al., 2004; Yang et al., 2004; Rao et al., 2006) and are commonly avoided (see also Section VII.D.4).

E. In Vivo Utility of Drug-Cyclodextrin Complexes in Parenteral Formulations

Cyclodextrins have been widely explored for their potential to improve the aqueous solubility of poorly water-soluble drugs in parenteral formulations. CDs approved for use in marketed parenteral products include SBE-β-CD (Captisol; Ligand Technology, La Jolla, CA) and HP-β-CD (Table 21). Sporanox IV (Janssen Pharmaceuticals) contains 1% itraconazole, 40% HP-β-CD, and 2.5% propylene glycol with pH adjustment to pH 4.5. It is diluted 1:1 with saline before administration via i.v. infusion (Strickley, 2004). Vfend IV (Pfizer) is a freeze-dried mixture of voriconazole (0.667%) and SBE-β-CD (10.67%). Geodon (Pfizer) is also a freeze-dried product containing ziprasidone mesylate trihydrate (2%) and SBE-β-CD (29.4%). After reconstitution, Geodon is administered via i.m. injection.

1. Effect of Cyclodextrins on Drug Pharmacokinetics after Intravenous Administration. After i.v. administration, CD-based formulations are rapidly diluted in
plasma and interact with endogenous components in the blood that competitively displace drug molecules from the CD binding cavity. Dilution and displacement therefore promote drug dissociation from CDs, allowing distribution of the drug to sites of action (Rajewski and Stella, 1996; Stella and Rajewski, 1997; Stella et al., 1999; Stella and He, 2008).

For drugs with CD-binding constants of up to $2 \times 10^4$ M$^{-1}$, a 1 in 100 dilution factor has been suggested to facilitate drug dissociation from CDs (Thompson, 1997). Simply assuming a blood volume of 3.5 L, the administration of 5 ml of a CD formulation results in a 1:700 dilution. In practice, the volumes of distribution of most CDs are closer to the volume of extracellular water (~30% body weight) rather than blood volume; therefore, even greater dilution factors of up to 1:4200 are likely (Stella et al., 1999). Cholesterol (Uekama, 2004) and albumin (Frijlink et al., 1991; Lin et al., 2007a) compete for binding to the hydrophobic cavity of CDs and, together with the dilution effects, promote drug dissociation from the CD cavity under most circumstances. Consistent with these suggestions, many reports describe no difference in drug pharmacokinetics following administration as either a CD complex or in the uncomplexed form (Stella and He, 2008). Examples include isotretinoin (Lin et al., 2007a), melphalan (Kaltun et al., 2010), prednisolone (Arimori and Uekama, 1987), propofol (Egan et al., 2007a), melphalan (Koltun et al., 2010), prednisolone (Egan et al., 2007a), miconazole (Piel et al., 1999), and etomidate (McIntosh et al., 2004).

In contrast, where drug-CD binding affinity is very high ($>1 \times 10^5$ M$^{-1}$), CD complexation may perturb drug pharmacokinetics (Charman et al., 2006; McIntosh et al., 2010). For example, the volume of distribution and clearance of OZ209 (a novel antimalarial compound) is significantly decreased in rats when dosed as a complex of OZ209 and SBE-$\beta$-CD compared with an uncomplexed (pH-buffered solution) formulation (Charman et al., 2006) (Fig. 37). The binding constant of OZ209 with SBE-$\beta$-CD is notably high and between $1 \times 10^5 - 1 \times 10^6$ M$^{-1}$ (Perry et al., 2006). Stella and He (2008) have subsequently proposed a threshold binding constant of $1 \times 10^6$ M$^{-1}$, above which dilution on administration may be insufficient for complete dissociation of the complex.

A more detailed description of the effect of CDs on drug pharmacokinetics and distribution after parenteral administration has been provided by Buggins et al. (2007).

2. Effects of Dilution on Drug Solubilization by Cyclodextrins. Although dilution and displacement on administration provide a ready mechanism for drug liberation from CDs, dilution also increases the risk of drug precipitation under some circumstances. The effect of dilution of a solution containing a 1:1 drug-CD complex is shown graphically in Fig. 38A. Dilution leads to a linear reduction in the concentration of drug and CD and also results in a linear decrease in the solubilizing capacity of the system. In most cases, dilution of a 1:1 drug-CD complex is therefore unlikely to result in precipitation, regardless of the extent of dilution (Rajewski and Stella, 1996; Yalkowsky, 1999). This provides a significant advantage over other solubilization modalities such as the addition of cosolvent, where dilution leads to exponential decreases in solubilization capacity and significantly elevated risks of precipitation. In contrast, dilution of a 1:2 drug-CD complex leads to a nonlinear decrease in solubilization capacity with dilution, introducing an increased risk of drug precipitation after dilution to low CD concentrations (Rajewski and Stella, 1996) (Fig. 38B).

3. Potential Toxicological Properties of Parenterally Administered Cyclodextrins. The in vivo toxicology of parenterally administered CDs has been described in detail elsewhere (Rajewski and Stella, 1996; Irie and Uekama, 1997; Stella and He, 2008) and is therefore addressed only briefly here.

The major potential toxicities associated with CD administration stem from membrane disruption, cholesterol complexation, and precipitation of insoluble cholesterol-CD complexes in the kidney and commonly lead to nephrotoxicity (Irie and Uekama, 1997; Uekama, 2004; Stella and He, 2008). The hemolytic properties of a number of chemically modified CDs toward rabbit erythrocytes have been correlated with the ability to solubilize cholesterol. In these studies, HP-$\beta$-CD and SBE-$\beta$-CD were less hemolytic than the more lipophilic methylated CDs, and SBE-$\beta$-CD was less hemolytic than HP-$\beta$-CD (Irie and Uekama, 1997; Uekama, 2004). A 5% w/v SBE-$\beta$-CD formulation of etomidate also resulted in less (10-fold) hemolysis on
i.v. administration to dogs compared with a commercially available propylene glycol–based formulation (Amidate, Abbott Laboratories) (McIntosh et al., 2004). The potential for hemolysis after administration of HP-β-CD and SBE-β-CD, therefore, appears to be no greater than, and likely less than, many other solubilization strategies.

The renal toxicity of CDs is highly linked to CD solubility. Thus, i.v. administration of the relatively poorly water-soluble β-CD to rats at 0.9 g/kg dose leads to significant renal toxicity and some irritation at the site of administration (Frank et al., 1976; Irie and Uekama, 1997; Thompson, 1997). The renal toxicity of natural CDs follows the rank order β-CD (most toxic) > α-CD > γ-CD (Thompson, 1997), a trend consistent with solubility where γ-CD (most soluble) > α-CD > β-CD (Irie and Uekama, 1997). The low aqueous solubility and subsequent nephrotoxicity of β-CD preclude use in parenteral formulations. In contrast, modified CDs are more soluble in water, show lower renal toxicities, and are usually eliminated unchanged in the urine. The elimination half-lives of HP-β-CD and SBE-β-CD are less than 2 h in humans (~114 min and ~84 min for HP-β-CD and SBE-β-CD, respectively), with complete elimination within 6 to 12 h (Zhou et al., 1998; Stella and He, 2008). The rate of clearance of administered CDs may be lower under conditions of reduced glomerular filtration in patients suffering from renal insufficiency (Irie and Uekama, 1997), potentially leading to accumulation after repeated administration (Stella and He, 2008). More detailed information on maximum possible doses for commonly used CDs is available in reviews by Brewster and Loftsson (2007) and Stella and He (2008).

F. In Vivo Utility of Drug-Cyclodextrin Complexes in Oral (Nonparenteral) Formulations

Cyclodextrins have been widely explored as possible excipients in oral solution formulations, although currently, Sporanox Oral Solution is the only marketed product; the oral bioavailability of itraconazole from this formulation is estimated to be between 45 and 82% (Strickley, 2004). CDs may be used as components in solid oral dosage forms as rapid solvent removal from drug-CD solutions does not destroy the complex. The potential for CDs to improve oral bioavailability has been reviewed elsewhere, and the interested reader is directed to this reference for greater detail (Carrier et al., 2007).

1. Effect of Cyclodextrins on Oral Drug Bioavailability.

The ability of CD complexation to enhance the effective aqueous solubility of poorly water-soluble drugs results in increases in oral bioavailability in many cases. For example, formulations containing either SBE-β-CD or HP-β-CD increase the oral bioavailability of cinnarizine in fasted beagle dogs from 8.0 ± 4% after administration of a simple aqueous suspension formulation to 55 to 60% after administration of the CD formulation (Fig. 39) (Jarvinen et al., 1995). The oral bioavailability of resveratrol trimethyl ether (trans-3,5,4’-trimethoxystilbene) is also increased from <1% after administration of an oral suspension formulation (to rats), to 64.6 ± 8.0% after administration of a solution containing 0.3 M randomly methylated β-CD (RM-β-CD) (Lin and Ho, 2011). In the same study, dose escalation from 15 mg/kg to 60 mg/kg led to corresponding increases in exposure (Lin and Ho, 2011). Melarsoprol is a poorly water-soluble drug (aqueous solubility 6 μg/ml) used in the treatment of human African trypanosomiasis (HAT) (Gibaud et al., 2005). Because of its low water solubility, melarsoprol is administered i.v. as a cosolvent formulation in the commercially available product (Arsobal; Sanofi-Aventis). Complexation with RM-β-CD or HP-β-CD, however, increases melarsoprol solubility by more than 7000-fold (Gibaud et al., 2005), and oral administration of a reconstituted lyophilised drug-CD complex (RM-β-CD or HP-β-CD) over a 7-day period eliminated the
parasite from HAT-infected animals (Rodgers et al., 2011). Capsules containing lyophilized ziprasidone mesylate and SBE-β-CD complexes and HPMC acetate succinate (HPMCAS) dosed to fasted humans also showed increased oral bioavailability compared with commercial Geodon capsules (containing ziprasidone hydrochloride, lactose, pregelatinized starch and magnesium stearate) and a diminished food effect (Thombre et al., 2011). Paclitaxel-CD complexes (HP-β-CD or β-CD) encapsulated within poly(anhydride) nanoparticles also facilitate increased oral bioavailability for paclitaxel compared with the commercial Taxol formulation. The paclitaxel-CD nanoparticle formulations resulted in absolute bioavailability of 80% relative to i.v. administration of Taxol. In contrast, oral administration of the Taxol formulation did not provide plasma levels above the limit of quantification of the assay (Agueros et al., 2010). The authors suggested that the dramatic increase in oral bioavailability reflected increases in paclitaxel solubilization mediated by the CDs and bioadhesion resulting from the nanoparticle formulation.

2. Oral Administration of Physical Mixtures Versus Isolated Drug-Cyclodextrin Complexes. The simplest drug-CD formulations are obtained by blending drug and CD to form a physical powder mixture that can be blended with other excipients and compressed to form traditional solid dosage forms (see Section VIII.G.2). Drug-CD physical mixtures improve the rate of drug dissolution over that of drug alone, suggesting that CDs can enhance drug dissolution via either improved wetting (Guyot et al., 1995; Nagarsenker et al., 2000; Reddy et al., 2004; Shinde et al., 2008) or in situ formation of the drug-CD complex in the dissolution medium (Corrigan and Stanley, 1982; Guyot et al., 1995; Fukuda et al., 2008).

Alternatively, spray-drying or free-drying drug-CD solutions yield powders that can contain solid drug-CD complexes, and formulations containing such pre-formed CD complexes typically result in more significant gains in dissolution and oral bioavailability compared with physical mixtures (Fig. 40) (Corrigan and Stanley, 1982; Savolainen et al., 1998; Okimoto et al., 1999; Nagarsenker et al., 2000; Fernandes et al., 2002; Koester et al., 2003; Carrier et al., 2007; Fukuda et al., 2008). Techniques and approaches used to verify that drug-CD complexes are retained in the solid state are discussed in more detail in Section VIII.G.2.

3. Using Cyclodextrins to Stabilize Amorphous Drug. In the solid state, drug molecules complexed within the hydrophobic cavity of a CD molecule are constrained from interaction with other drug molecules and are typically unable to interact sufficiently to form a crystal lattice (Bettinetti et al., 1992; Mura et al., 1998; Szeflji, 1998; Kimura et al., 1999). This mechanism for stabilizing drug in the noncrystalline state is in some ways analogous to mesoporous systems, where crystal growth is limited by the narrow dimensions of the inner capillary network (see Section XII.A). Modified CDs are also commonly present in the amorphous form, and solid drug-CD inclusion complexes are therefore often isolated in the amorphous or microcrystalline form (Uekama et al., 1983; Veiga et al., 1996; Hirayama et al., 1997; Mura et al., 1998; Veiga et al., 2001; Sotthivirat et al., 2007). As such, CD complexation is an alternate approach to isolate drug in a noncrystalline amorphous form, and solid-state CD complexes may be regarded in a broad sense as a class of solid dispersion. The potential benefits of isolating a drug in the amorphous form to drug dissolution and bioavailability are described in more detail in Section X. In brief, however, the loss of crystalline character reduces the barrier to solubility presented by solute-solute molecular interactions in the crystal matrix and this change to solid-state properties of the drug leads to enhanced dissolution rates. In the case of a CD-based matrix, dissolution of the carrier also increases the solubilization capacity of the dissolution media, further enhancing the drivers of dissolution rate (Fig. 40).

As with all formulations containing amorphous (i.e., metastable) drug, rapid dissolution of the amorphous form may result in drug concentrations in the dissolution media that are supersaturated with respect to the solubility of the stable crystal. Supersaturation may be beneficial with respect to drug absorption but is also a driver of drug precipitation. The net benefit is therefore a balance between increased dissolution rate, enhanced supersaturation, and protection against precipitation. CDs, including HP-β-CD and SBE-β-CD, enhance the stability of supersaturated drug solutions (Vandecruys et al., 2007; Brewster et al., 2008a,b), suggesting that CDs are able to both promote the isolation
of the amorphous form and stabilize the supersaturated solutions that form on dissolution. Supersaturation stabilization likely reflects increases in apparent drug solubility in the presence of CD and a reduction in the degree of supersaturation relative to the solubility of the stable crystal form. Alternatively, and in a manner analogous to the use of polymers in solid dispersions or lipid-based formulations (Brewster et al., 2002; Chowdary and Srinivas, 2006; Cappello et al., 2007; Lee et al., 2009; Warren et al., 2010; Anby et al., 2012), CDs may stabilize supersaturation by slowing/inhibiting nucleation or crystal growth (Brewster et al., 2008a).

A number of studies have examined the effect of adding polymers to CD formulations. In most cases, however, the intent has been to exploit the capacity of polymers to enhance drug-CD complexation (see Section VIII.D.1) (Loftsson et al., 1994; Smith et al., 2005) or to generate controlled release CD-based dosage forms (Uekama et al., 1990; Rao et al., 2001; Koester et al., 2003, 2004; Ribeiro et al., 2005b) rather than to stabilize the supersaturated solutions that may result on dissolution (Brewster et al., 2002; Lee et al., 2009).

Consistent with other formulation approaches that contain amorphous solids, CD-based amorphous dispersions are thermodynamically unstable. Amorphous drug contained within CD solid complexes is therefore expected, over time, to revert to the more stable but less soluble crystalline form. Unfortunately, whether the drug-CD complex slows the process of recrystallization and whether this is dependent on the affinity of the drug-CD interaction is not well described, and compared with polymer-based solid dispersions, relatively few studies have investigated the stability of amorphous drug-CD formulations (Irie and Uekama, 1997; Hirayama et al., 2001; Uekama, 2002). However, given that the intermolecular interactions between drug and CD must be overcome for the drug to crystallize, it is conceivable that a correlation exists between the physical stability of the amorphous drug-CD complex and the strength of the interaction between drug and CD. This is comparable with the physical stability within amorphous solid dispersions, where evidence of intermolecular hydrogen bonds between drug and polymer is often considered beneficial to the long-term physical stability of the formulation.

4. Cyclodextrin Complexation as a Limitation to Oral Bioavailability. Enhanced drug absorption after oral administration of CD formulations is commonly ascribed to increases in the apparent solubility of the drug in the GI fluids (resulting from either complex formation, isolation of the amorphous form, or supersaturation stabilization). However, there are also several examples where CDs have increased drug solubility but have not improved oral bioavailability (Nambu et al., 1978; Oteroespinar et al., 1991; Miyake et al., 2000). Additional factors must therefore be considered when attempting to understand the performance of CD formulations in vivo (Jambhekar et al., 2004; Uekama, 2004; Carrier et al., 2007). In particular, drug association with a CD complex reduces thermodynamic activity compared with a simple solution formulation, and for drugs with high CD binding constants, this may be sufficient to reduce the rate or extent of drug absorption. In a similar fashion to drug dissociation from the complex after i.v. administration, drug dissociation in the GI tract is expected to be driven by dilution and competition for CD complexation by endogenous substances in the GI lumen, such

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**Fig. 40.** (A) Release profiles of ketoprofen in 900 ml of 0.1 N HCl and 0.1 N HCl containing either 25 mg of sulfobutylether-β-cyclodextrin (SBE-β-CD) or 25 mg of β-cyclodextrin (β-CD). (B) Effect of hot-melt extrusion processing with either SBE-β-CD or β-CD on ketoprofen release in 900 ml of 0.1 N HCl. Experiments carried out at 37 ± 0.5°C USP apparatus 2, 50 rpm. Values are expressed as means (n = 6) ± SD. Adapted from Fukuda et al. (2008).
as bile acids and cholesterol (Nakanishi et al., 1989; Rajewski and Stella, 1996; Miyake et al., 1999b).

In the case of the cinnarizine and HP-\(\beta\)-CD or SBE-\(\beta\)-CD complexes described previously, an increase in \(C_{\text{max}}\) and oral bioavailability and decrease in \(T_{\text{max}}\) compared with a suspension formulation suggest efficient and rapid dissociation of cinnarizine from the HP-\(\beta\)-CD or SBE-\(\beta\)-CD complex in vivo (Jarvinen et al., 1995). For cinnarizine, however, drug-CD binding constants are not high (<5000 M\(^{-1}\)) (Jarvinen et al., 1995), whereas poor dissociation and the potential for limitations to drug availability may be more likely for drugs with higher binding affinity (Tokumura et al., 1986; Bekers et al., 1991; Uekama et al., 1998; Stella et al., 1999; Carrié et al., 2007). Indeed, several studies have shown a decrease in in vitro membrane flux for drugs solubilized by CDs compared with solution formulations alone (Iervolino et al., 2000; Dias et al., 2003; Manca et al., 2005; Dahan et al., 2010), consistent with a decrease in thermodynamic activity and similar, for example, to the reduction in free concentration that results from micellar solubilization.

A relatively complex series of potentially conflicting processes is therefore evident during the dissolution of a CD-based formulation compared with a suspension formulation. The CD formulation is expected to lead to a significant improvement in the dissolution rate of the drug-CD complex, but subsequent dissociation of drug from the complex is required before absorption [as only noncomplexed (free) drug is absorbed]. The extent of dissociation reflects the magnitude of the drug-CD binding constant, and, ultimately, whether the strength of the association complex limits bioavailability will reflect a balance between the degree of solubility enhancement and the magnitude of the reduction in thermodynamic activity. The impact of complexation on bioavailability will also depend on drug permeability across the intestinal membrane, where increased permeability is expected to provide improved sink conditions and promote continuing dissociation of the drug-CD complex. Accordingly, CDs have, in general, a greater capacity to improve the oral absorption of drugs belonging to class II of the BCS (i.e., low-solubility, high-permeability drugs) (Loftsson et al., 2007b; Loftsson and Brewster, 2010). Nonetheless, improved oral absorption of BCS class IV drugs (i.e., those of low solubility and low permeability) is also possible, suggesting that bioavailability enhancement via increases in solubility are possible even for low permeability compounds (Loftsson and Brewster, 2010). Formulation design should ensure that the CD concentrations used are not higher than that required to provide adequate solubilization since surplus CD will decrease the free drug concentration thereby reducing flux (Dahan et al., 2010; Loftsson and Brewster, 2011).

5. Potential Toxicological Properties of Orally Administered Cyclodextrins. The oral toxicology of both natural and chemically modified CDs has been extensively reviewed elsewhere (Irie and Uekama, 1997; Thompson, 1997; Uekama et al., 1998; Stella and He, 2008), and in general, CDs are less toxic after oral administration compared with i.v. administration since oral bioavailability of CDs is low; natural \(\alpha\)-CDs and \(\beta\)-CDs are not substrates for salivary and pancreatic amylases and are also resistant to acid hydrolysis in the stomach. Conversely, \(\gamma\)-CD is rapidly digested into glucose and maltose by amylases in the GI tract (De Bie et al., 1998). All CD varieties may be subject to fermentation by microflora present in the large intestine (Flourie et al., 1993), although chemical substitution is thought to reduce the extent of breakdown (Irie and Uekama, 1997).

The high molecular weight (usually >1000 Da) and hydrophilicity of CDs limits membrane permeability; therefore, absorption after oral administration is low (Irie and Uekama, 1997; Stella and He, 2008). Indeed, less than 1% of an orally administered dose of HP-\(\beta\)-CD is absorbed in rats (Stevens, 1999), and similarly low (0.5–3.3%) absorption of HP-\(\beta\)-CD has been reported in humans (Thompson, 1997; Brewster and Loftsson, 2007). Much less oral safety data are available for SBE-\(\beta\)-CD; however, absorption is likely to be low (consistent with HP-\(\beta\)-CD), with most ingested CD excreted via the feces (Thompson, 1997). Predictably, more lipophilic CD derivatives, including randomly methylated (RM) and dimethylated (DM) \(\beta\)-CD derivatives, are absorbed to a greater extent, although oral bioavailabilities remain relatively low. For example, in rats, ~10% of DM-\(\beta\)-CD (Szatmari and Vargay, 1988; Thompson, 1997) and ~12% of RM-\(\beta\)-CD (Antlisperger and Schmid, 1996) are bioavailable after oral administration.

Cyclodextrins have been shown to complex with bile salts that are present in the intestinal lumen (Tan and Lindenbaum, 1991; Holm et al., 2011). Rats repeatedly dosed orally with 5 g/kg HP-\(\beta\)-CD developed pancreatic hypertrophy (and later, pancreatic neoplasia), and this was attributed to bile salt-CD complexation in the intestine and increased fecal excretion of bile salts, in turn promoting the release of cholecystokinin, a peptide with known mitogenic properties in rats (Gould and Scott, 2005). However, as cholecystokinin shows no mitogenic effects in humans and other species, the effects of HP-\(\beta\)-CD on the pancreas may be specific to the rat (Gould and Scott, 2005).

Similar to parenterally administered CDs, those that enter the systemic circulation via the GI tract are largely excreted through the kidneys. The combination of low absorption and the relatively low systemic toxicity of HP-\(\beta\)-CD and SBE-\(\beta\)-CD dictates that large quantities may be tolerated after oral administration, with doses of up to 8 g per day of HP-\(\beta\)-CD used in the clinic (Gould and Scott, 2005; Loftsson and Brewster, 2010). No marketed products for oral administration at
present contain SBE-β-CD, although doses via i.v. administration may be as high as 14 g per day (i.e., Vfend IV).

6. Effect of Cyclodextrins on Membrane Transport and Drug Metabolism. Several studies have suggested that CDs, in particular the modified CDs, may affect oral drug absorption via effects on intestinal membrane transporter functionality (e.g., via effects on the efflux transporter, P-gp) or via alterations in cytochrome P450–based first-pass metabolism (Arima et al., 2001a, 2004; Yunomae et al., 2003; Ishikawa et al., 2005; Fenyvesi et al., 2008). These effects are analogous to the potential effects of surfactants on drug efflux and intestinal metabolism (Friche et al., 1990b; Kemper et al., 2003; Buggins et al., 2007; Yang et al., 2007), however, the mechanisms by which CDs inhibit efflux or metabolism are less well described but presumably reflect complexation of membrane lipids, reorganization of membrane structures, or alterations to transporter or enzyme activities (Arima et al., 2001a, 2004; Ishikawa et al., 2005). Also consistent with surfactants, definitive data describing the effect of CDs on transporter or enzyme functionality are difficult to obtain in vivo for poorly water-soluble drugs since bioavailability is likely to be enhanced by both changes to dissolution and efflux/metabolism. Delineation of the two pathways is therefore difficult without recourse to knockout animals, the latter restricting studies to small rodent models.

G. Manufacture of Cyclodextrin Formulations

1. Parenteral Formulations. As discussed in Section VIII.D, CDs are widely used in conjunction with other formulation strategies during the development of parenteral formulations as the combined use of CDs with, for example, pH adjustment may lead to synergistic increases in drug solubilization. Consequently, CD formulations typically contain several different components, including polymers, buffers, cosolvents, and such. Parenteral formulations are usually prepared as simple aqueous solutions but may also be freeze-dried to form solid inclusion complexes (Brewster et al., 1991; Xiang and Anderson, 2002; Lockwood et al., 2003; Hong et al., 2011) that are reconstituted with water or saline immediately before administration (Kim et al., 2004a; Strickley, 2004). The quantity of CD required to dissolve drug to achieve a particular target concentration is determined by phase solubility analysis or, where published binding constant information is available, may be calculated using eq. 41 (see Section VIII.B.1).

2. Solid Drug-Cyclodextrin Complexes. Solid drug-CD complexes are generated most simply by trituration, during which a small amount of solvent (usually a mixture of organic solvent with water) is added to a mixture of drug and CD to form a slurry of paste-like consistency. The slurry is subsequently dried under a vacuum to remove the solvent (Arima et al., 2001b; Mura et al., 2001a; Patel and Vavia, 2006; Veiga et al., 1996). More commonly, however, solid complexes are isolated from freeze-dried or spray-dried solutions (Marques et al., 1990; Jarvinen et al., 1995; Veiga et al., 1996; Savolainen et al., 1998; Dollo et al., 1999; Jambhekar et al., 2004; Pralhad and Rajendrakumar, 2004; Villaverde et al., 2004) or after coprecipitation (Uekama et al., 1982; Badawy et al., 1999; Veiga et al., 1996). In the latter approach, solid drug-CD complexes are encouraged to precipitate from a supersaturated drug-Csolution. Where the aqueous solubility of the inclusion complex is low, water may be used as the solvent; however, small amounts of cosolvent are often added where the drug-CD solubility is high (as is often the case). The solvent is then removed by evaporation to stimulate precipitation of the complex (Kou et al., 2011).

Similar to the manufacture of nano and microparticulates (see Section IX) and solid dispersions (see Section X), supercritical fluid (SCF) techniques may be used in the manufacture of solid drug-Cs formulations. In these techniques, SCFs may be used as either solvent or antisolvent (Van Hees et al., 1999; Junco et al., 2002; Al-Marzouqi et al., 2006); however, use as an antisolvent is more common as the solubility of many drugs and CDs in SCFs is low, limiting the drug-CD concentrations that may be used (Tozuka et al., 2006).

Solid complexes should be analyzed to verify the physical form of the drug and to ascertain whether the integrity of the drug-CD inclusion complex has been maintained during processing (Pedersen, 1997; Thombre et al., 2012). Loss of drug crystalline character after precipitation/drying of drug-CD solutions and the maintenance of drug crystallinity in physical mixtures of drug and CD are often taken as evidence of the stability of drug-CD complexes during the manufacture of solid material. However, many of the common characterization techniques used to make these distinctions, including DSC and XRPD, lack the capacity to discriminate between complexed and uncomplexed amorphous drug. Spectroscopic methods such as Raman or FTIR provide a more robust means for characterizing the nature of drug-CD complexes in the solid state (Veiga et al., 1996; Van Hees et al., 1999; Fernandes et al., 2002; Jambhekar et al., 2004) since spectral shifts indicate the formation of drug-CD interactions, while the absence of spectral bands specific to the drug provide evidence that the drug is shielded from the spectroscopic signal and is therefore most likely located within the CD cavity (Jambhekar et al., 2004).

H. Summary

Cyclodextrins are cyclic oligosaccharides that are able to form host-guest inclusion complexes with poorly
water-soluble drugs. The affinity of drug molecules for solubilization in the hydrophobic core of the CD is defined by the CD binding constant, and, for most drugs, binding constants of $1 \times 10^2 - 1 \times 10^4 \text{ M}^{-1}$ are evident. Increases in the drug solubility of several orders of magnitude are possible, and solubilization efficiency may be enhanced by coformulation with polymers or cosolvents and via combination with pH manipulation strategies. Coformulation with surfactants is typically detrimental to solubilization. CDs have been used to facilitate the generation of oral and parenteral formulations and as simple solutions or as solid or reconstitutable solid dosage forms. Solid CD-drug complexes may also be used to isolate drug in the more soluble amorphous form, although detailed studies of the long-term physical stability of amorphous drug-CD complexes are limited. The toxicity of modified CDs appears to be similar or lower than that of common surfactants or cosolvents and is reduced after oral administration as a result of the limited absorption of the excipient.

**IX. Particle Size Reduction Strategies**

Particle size reduction leads to an increase in the surface area available for solvation and an increase in the rate of dissolution for solid drug products. Particle size reduction technologies are therefore routinely used to improve the oral bioavailability of poorly water-soluble drugs (Rabinow, 2004; Keck and Muller, 2006; Merisko-Liversidge and Liversidge, 2011) and when developing fine (nano) suspension formulations for parenteral delivery (Muller and Bohm, 1998; Shi et al., 2009).

Particle size reduction technologies may be characterized as either “top-down” processes, where larger particles are fragmented into smaller particles, or as “bottom-up” processes, where small particles are harvested after recrystallization of a drug from a supersaturated solution. Traditionally, small particles have been obtained via top-down, dry-impact processes that introduce considerable shear forces and reduce the particle size of a coarse drug powder using, for example, hammer mills, ball mills, and air-jet mills. This process, commonly referred to as micronization, results in the formation of particles in the micron size range with average diameters $<10 \mu\text{m}$ and particle sizes commonly between 2 and 5 $\mu\text{m}$ (Nykamp et al., 2002; Rasenack and Muller, 2002). Dry milling remains in wide use; however, a combination of recent technological developments and the wider utilization of polymeric and surfactant stabilizers have led to the ability to produce much smaller (nano) particulates with sizes in the 200–500-nm range. Bottom-up particle assembly processes have also become more widespread as crystallization methods have become more sophisticated, allowing the controlled crystallization or precipitation of nanoscale drug assemblies from highly supersaturated drug solutions.

The term *nanoparticle* is frequently used to describe a range of nanosized materials, most commonly polymeric, liposomal, or solid lipid nanoparticles, but it also correctly describes nanoparticulate drug crystals and suspensions (Wu et al., 2011). Drug nanoparticles have many advantages over simple micronized drug powders. Indeed, the increase in surface area to mass ratio for nanoparticulates is dramatic, sometimes covering several orders of magnitude (Fig. 41) (Junghanns and Muller, 2008; Merisko-Liversidge and Liversidge, 2008), and this has the potential to provide for substantial increases in dissolution rate, bearing in mind the dependence of dissolution rate on surface area (eq. 9).

Particles in the nanoscale may also be more soluble because of changes to particle curvature and the introduction of defects into the crystal lattice (Junghanns and Muller, 2008; Mauludin et al., 2009). As a result, drug nanocrystals may yield oral bioavailabilities that far exceed those obtained using conventional particles or micronized particles of poorly water-soluble drugs (Liversidge and Cundy, 1995; Jinno et al., 2006; Sun et al., 2011). The practical applicability of nanocrystals is evident in several marketed nanocrystal formulations (Table 23) (Chaubal, 2004; Rabinow, 2004; Merisko-Liversidge and Liversidge, 2011), and there is also increasing application of nanosuspensions in preclinical studies, where the ability to enhance dissolution at the same time as maximizing drug dose (since nanocrystals require little additional excipient) is often critical. Importantly, the development of top-down nanomilling technologies now allows the processing of drug batches relatively cheaply and in batch sizes suitable for application from early discovery phases (milligram) through to product marketing (kilogram) (Chen et al., 2006; Kesisoglou et al., 2007; Niwa et al., 2011b).

![Fig. 41. The change in surface area obtained when solid particles exist in the micron-size range (microparticle) and the nanometer-size range (nanoparticles). Both microparticles and nanoparticles can be prepared from top-down and bottom-up techniques. Adapted from Merisko-Liversidge and Liversidge (2008).](image)
The formation of nanocrystal formulations is not without complexity, and several challenges are evident. Nanoparticles are highly cohesive and must be stabilized to prevent aggregation. In addition, not all drug candidates have physicochemical properties suitable for effective particle size reduction, and nanoparticulate drugs may be susceptible to polymorphic transition on manufacture or storage (Van Eerdenbrugh et al., 2008; Wu et al., 2011). The current section of this review first covers the theoretical background that underpins the effect of particle size on dissolution rate, solubility and ultimately in vivo exposure for poorly water-soluble drugs. Second, the most widely used methods for nanoparticle production are introduced. Finally, examples of the potential utility of microparticulate and nanoparticulate formulations after oral and parenteral administration are discussed.

A. Particle Size Effects on Dissolution, Solubility, and In Vivo Performance

1. Effect of Particle Size on Dissolution Rate. The rate of drug dissolution from a solid dosage form surrounded by an unstirred diffusional layer is traditionally described by the Noyes-Whitney equation (eq. 9). Where particle size reduction yields an increase in surface area, eq. 9 (see Section I.E) suggests an equivalent increase in dissolution velocity and, therefore, an increase in bioavailability for drugs where exposure after oral administration is limited by dissolution rate. Consistent with this suggestion, micronization leads to an improvement in the oral bioavailability of many poorly water-soluble drugs, including cilostazol (Jinno et al., 2006), fenofibrate (Vogt et al., 2008), progesterone (Hargrove et al., 1989), proquipazon (Nimmerfall and Rosenthaler, 1980), and nitrofurantoin (Watari et al., 1983), all of which are believed to exhibit dissolution-rate limited absorption. However, the generation of micron-sized drug particles is often insufficient to overcome the challenges to absorption presented by compounds with very low aqueous solubility (<1 µg/ml) (Kondo et al., 1993; Muller et al., 2001; Keck and Muller, 2006; Gao et al., 2008), and alternative methods have been developed to reduce particle size further and to isolate particles with nanometer-scale dimensions.

The increase in surface area that may be achieved by the generation of nanoparticulate drug suspensions is significant (Fig. 41) and may lead to large improvements in dissolution rate compared with both unmodified drug powders and micronized drug. Indeed, in some cases, dissolution is sufficiently fast as to be practically instantaneous [e.g., >80% release within 2 min has been reported for nanocrystals of ibuprofen (Plakkot et al., 2011) and indomethacin (Liu et al., 2011; Niwa et al., 2011a)]. Figure 42 provides a recent illustration of the effect of particle size reduction on the dissolution of suspensions of itraconazole. Here, micronization (to 5.5 µm) leads to a 2- to 3-fold increase in dissolution rate, whereas further particle size reduction to produce nanoscale particulates (350–700 nm) elicits additional 3- to 5-fold increases in dissolution rate compared with the micronized product (and therefore an overall ~14-fold increase in dissolution compared with nonmicronized drug) (Sun et al., 2011).

Although increases in surface area are widely acknowledged as the primary driver of increased dissolution for microparticulates and nanoparticulates, it is also apparent that the improvements in dissolution velocity may exceed that predicted simply on the basis of available surface area. Under these circumstances, changes to drug solubility (C_s), to the thickness of the diffusional layer (h), and to particle shape may also

Table 23

<table>
<thead>
<tr>
<th>Drug</th>
<th>Brand Name</th>
<th>Technology</th>
<th>Route of Administration and Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamycin</td>
<td>Rapamune (Wyeth)</td>
<td>NanoCrystal</td>
<td>Oral, tablet</td>
</tr>
<tr>
<td>Aprepitant</td>
<td>Emend (Merk)</td>
<td>NanoCrystal</td>
<td>Oral, capsule</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>Tricor 145 (Abbott)</td>
<td>NanoCrystal</td>
<td>Oral, tablet</td>
</tr>
<tr>
<td>Megestrol</td>
<td>MegaceES (Par Pharmaceutical Companies)</td>
<td>NanoCrystal</td>
<td>Oral, liquid solution</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>Triglide (Sciele Pharma Inc)</td>
<td>NanoCrystal</td>
<td>Oral, tablet</td>
</tr>
<tr>
<td>Paliperidone palmitate</td>
<td>Invega Sustenna (Jansen Pharmaceuticals)</td>
<td>NanoCrystal</td>
<td>i.v., sustained release injection</td>
</tr>
<tr>
<td>Thymectacin</td>
<td>Theralux (Celmed)</td>
<td>NanoCrystal</td>
<td>Oral, tablet</td>
</tr>
</tbody>
</table>

* Currently in phase I/II clinical trials.

![Fig. 42. In vitro dissolution profiles of itraconazole from the suspensions of various particle sizes in 0.1 M HCl at 37°C. Values are expressed means (n = 3). Adapted from Sun et al. (2011)*
explain the increase in dissolution rate, and these factors are addressed in the sections below. (Anderberg et al., 1988; Bisrat and Nystrom, 1988, 1998; Elamin et al., 1994; Mosharraf and Nystrom, 1995).

2. Effect of Particle Size on Saturated Solubility. Traditional solubility theory suggests that decreases in particle size are expected to have little impact on equilibrium solubility since particle size changes typically affect neither solid-state properties nor the efficiency of solvation. However, the advent of technologies that support the isolation of particles with submicron particle sizes requires that this view be modified. Indeed, the Ostwald-Freundlich Equation (Freundlich, 1909) (eq. 50), suggests solubility increases on reducing particle size, with effects becoming significant below $1 \mu m$ ($r = 0.5 \mu m$):

$$\log \frac{C_s}{C_\infty} = \frac{2\sigma V_m}{3.03RTpr}$$

where $C_s$ is the saturated solubility, $C_\infty$ is the solubility of the solid consisting of large particles, $\sigma$ is the interfacial tension of the solid substance, $V_m$ is the molar volume of the particles, $R$ is the gas constant, $T$ is the absolute temperature, $\rho$ is density of the solid, and $r$ is the particle radius.

This phenomenon was initially described by Kelvin in a gas-liquid system, where increasing curvature of a liquid droplet (in response to decreasing radius) increased vapor pressure and the rate of transfer into a gas phase (described by Wu and Nancollas, 1998). The principle, however, applies equally to solid particles in a liquid medium where a reduction in particle size below $1 \mu m$ increases solvation pressure, subsequently giving rise to an increase in solubility (Junghanns and Muller, 2008).

In addition to Ostwald-Freundlich effects on curvature, high-energy impact milling and other top-down particle size reduction techniques may cause small defects in the crystal lattice (particularly on the particle surface). This is expected to increase solubility via a reduction in the limitations to solubility imparted by the strength of solute-solute interactions in the crystal lattice.

Although changes to kinetic solubility are evident for nanoparticulate drug suspensions via increased curvature (via the Ostwald-Freundlich effect) and the introduction of defects into the crystal lattice, the equilibrium solubility of the stable crystal remains unchanged. As such, rapid dissolution of drug nanoparticles leads to the generation of a transiently supersaturated solution (with respect to the solubility of the stable crystal form) and, therefore, the potential for recrystallization. The same situation occurs on dissolution of all nonequilibrium crystal structures such as high-energy polymorphs, solvates, cocrystals (see Section V), and, depending on pH, salts (see Section IV). In this case of nanoparticulates, however, the degree of supersaturation is usually low since particle size effects on saturated solubility are moderate ($<2$-fold owing to changes to curvature or lattice defects). As a result, the more prominent effects of nanosizing on drug absorption are typically driven by the much larger changes to dissolution rate.

Accurate measurement of the saturated solubility of a nanoparticulate is also complicated by the potential for very small particles to remain suspended in solution after conventional centrifugation (or even ultracentrifugation in some cases) and to pass through many of the membrane filters used to separate drug in solution from solid drug (Van Eerdenbrugh et al., 2010b). Bearing in mind the low intrinsic solubility of many of the drugs to which this technology is applied, only trace quantities of particle contamination are required to generate large errors in solubility assessment. Recent data to suggest the potential for solid nanocrystals to absorb in the UV/Vis light spectrum (Van Eerdenbrugh et al., 2011) further reinforce the suggestion that great care must be taken to exclude the possibility of particulate contamination during solubility and dissolution assessment.

To overcome some of the concerns regarding phase-separation techniques, light scattering and turbidity measurements have been explored as an alternate approach to solubility assessment (Lindfors et al., 2006; Van Eerdenbrugh et al., 2010b). In these methods, the first appearance of solid material (as an indication of the solubility limit) is determined from the intersection of two curves, one describing the small increases in scattering/turbidity (at 700 nm) that are evident in undersaturated solutions containing progressively higher amounts of dissolved drug and a second curve that describes the relationship between scattering/turbidity and particle load in a series of saturated solutions containing increasing amounts of undissolved drug (Van Eerdenbrugh et al., 2010b). In these studies, correction for scattering in the absence of particulate drug was required as all solutions/suspensions contained a small quantity of vitamin E TPGS (as a nanosuspension stabilizer) that resulted in scattering from surfactant micelles.

3. Effect of Particle Size and Shape on the Diffusional Layer Thickness. Particles smaller than $2 \mu m$ in diameter demonstrate faster rates of dissolution by virtue of a reduction in the thickness of the unstirred diffusional layer (Higuchi and Hiestand, 1963; Lu et al., 1993). The size of the diffusion layer is described by the Prandtl equation (eq. 51), where $h_{th}$ signifies the thickness of the hydrodynamic boundary layer (the diffusion layer in the Noyes-Whitney equation; eq. 9) around a particle of length (diameter) L over which a liquid flows at constant velocity $V$. $k$ is a constant (Mosharraf and Nystrom, 1995; Muller and Bohm, 1998):
The Prandtl equation predicts a decrease in $h_H$ with decreasing particle size, and from eq. 9, an increase in the dissolution rate. The velocity ($V$) of fluid flow over a particle in eq. 51 is dependent on particle shape, and notably slower rates of dissolution can occur where smaller particles are irregularly shaped and fluid flow is slower (Mosharraf and Nyström, 1995; Sugano, 2008).

### B. Common Methods to Reduce Particle Size

Traditionally, micronized drug product has been obtained using dry-impact milling, with the most popular equipment including hammer mills, ball mills, and more recently, air-jet mills (Hajratwala, 1982; Nykamp et al., 2002; Nakach et al., 2004). In these techniques, smaller particles are produced via the fracture of larger particles and the gradual attrition of the particle surface (Tavares, 2007). However, unless very long milling times are used, these techniques are unable to produce drug particles with average diameters in the nanoscale. Particles are also prone to phase transformations during dry milling, especially over extended processing times (Halebian and McCrone, 1969; Elamin et al., 1994; Mackin et al., 2002; Descamps et al., 2007), precluding application to thermally labile and low melting materials.

In contrast, wet-milling approaches, such as pearl milling, and high-shear liquid impaction methods, such as high-pressure homogenization, are highly effective in generating and stabilizing newly formed nanocrystals and are increasingly used to produce particles in the nanometer range (Van Eerdenbrugh et al., 2008; Merisko-Liversidge and Liversidge, 2011). These new methods have not yet been used to support the manufacture of a marketed product.

#### 1. “Top-Down” Particle Size Reduction

- **a. Pearl Milling.** Pearl milling (alternatively described as nanomilling or media milling) is perhaps the most established technique for the production of drug nanocrystals and has been described in detail in several recently published reviews (Rasenack and Muller, 2004; Peltonen and Hirvonen, 2010; Merisko-Liversidge and Liversidge, 2011).

  **In brief, pearl milling involves the continuous stirring and wet milling of an aqueous drug slurry with specialized— and extremely hard and durable— milling “pearls.” Nanocrystals smaller than 400 nm in diameter may be produced, and particle size reduction is achieved via a combination of high-energy shear and impaction forces between the milling pearls and the solid drug particles. The addition of a polymer or surfactant stabilizer(s) to the aqueous slurry is essential as this attenuates the increase in free energy associated with the creation of new particle surface and prevents nanocrystal aggregation. Stabilizers are usually added before milling initiation, however, recent studies suggest that the dynamic addition of stabilizers during milling may further facilitate the milling process (Bhakay et al., 2011).**

  The NanoCrystal technology platform (Elan Drug Technologies, Dublin, Ireland) is the most well-developed pearl-milling method for particle size reduction, although it is available only through a contractual agreement with Elan (recently merged with Alkermes, Dublin, Ireland). This technology utilizes a patented milling medium (PollyMill) consisting of small (<1 mm) highly cross-linked polystyrene beads (or pearls), the design of which facilitates the grinding process by maximizing the number of bead-drug particle contact points (Merisko-Liversidge and Liversidge, 2011). The NanoCrystal technology is scaleable from small-scale milligram batches (with application in early preclinical studies) to large-scale batches (500–1000 kg) that can be progressed to clinical use and marketing. Marketed products that use the NanoCrystal technology platform cover a range of applications and are listed in Table 23.

  Although the NanoCrystal technology has dominated pearl milling applications for some time, alternative pearl mill technologies have been developed de novo (e.g., Dyno-Mill; Willy A. Bachofen, Basel, Switzerland) (Jinno et al., 2006, 2008), and yet others have been developed in-house based on modifying existing milling equipment (Nekkanti et al., 2009; Juhnke et al., 2010; Niwa et al., 2011b; Plakkot et al., 2011). These new methods have not yet been used to support the manufacture of a marketed product.

  Early descriptions of pearl milling suggested that long milling times (several days) may be required to produce nanosized particles (Merisko-Liversidge et al., 1996; Muller et al., 2001; Hu et al., 2004a). However, it is apparent from the more recent literature that nanoscale particles may be achieved for some compounds after milling for much shorter periods of minutes or hours (Fig. 43) (Merisko-Liversidge and Liversidge, 2011). Longer milling times are likely required only when low milling speeds are used or during the milling of crystals that do not easily fragment (i.e., very hard or very soft crystals). One concern surrounding the use of pearl milling is the potential erosion of the milling media leading to contamination of the final product. The milling media must therefore be shown to withstand the milling process to avoid potential erosion and product contamination (Merisko-Liversidge et al., 2003). PolyMill, the milling medium used in the NanoCrystal platform, is said to be entirely resistant to the milling process (Merisko-Liversidge and Liversidge, 2011), although final formulations for parenteral delivery are routinely assessed for possible impurities.
Most drug powders are applicable to dry- and wet-milling techniques. However, those with low glass transition or melting temperatures (typically below 100°C) show a tendency to deform plastically under stress (rather than fracture) and are therefore at greater risk of polymorphic transitions (crystalline-crystalline or crystalline-amorphous) during the milling process.

b. High-Pressure Homogenization. High-pressure homogenization (HPH) is widely used to produce nano-sized particles and was recently reviewed (Keck and

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**Fig. 43.** Particle size reduction during milling. The particle size reduction of naproxen is shown in the processing chamber of the media mill. With increased milling time, the particle size distribution profile using laser light diffraction decreases dramatically (i.e., shifts to the left) and narrows. The particle size distribution curves are as follows: (A) the initial particle size of naproxen before processing, (B) 15 min postprocessing, (C) 30 min postprocessing, and (d) 60 min postprocessing in a media mill. The time required to process a poorly water-soluble compound is dependent on properties of the molecule, properties of the chosen stabilizer systems, and various processing parameters. For naproxen stabilized with povidone, a residence time of 60 min provides a nanosuspension with a mean particle size diameter ($D_{\text{mean}}$) of 200 nm. Adapted from Merisko-Liversidge and Liversidge (2011).
Muller, 2006; Muller et al., 2011a). Two different HPH techniques may be used to produce nanoparticles via a top-down approach, namely, microfluidization and piston-gap HPH; the latter is the most widely reported technique.

Piston-gap HPH, involves the passage of a stabilized aqueous drug suspension through a cylinder of approximately 3 cm in diameter before high-velocity streaming at high pressure into a narrow gap of around a 25-μm diameter. The dramatic increase in fluid pressure (up to 15,000–30,000 psi) causes water to boil at room temperature (in accordance with Bernoulli’s principle), and this leads to cavitation of fluid at the particle surface. These cavities subsequently collapse as the suspension leaves the narrow vessel (and as the pressure drops below the saturated vapor pressure of the liquid), and the resultant shockwaves cause particle disintegration into smaller nanocrystals (Muller and Bohm, 1998; Muller and Junghanns, 1998; Patravale et al., 2004; Keck and Muller, 2006).

Proprietary technologies that use HPH include the DissoCubes platform (SkyePharma AG, Muttenz, Switzerland) and NanoPure (PharmaSol, Berlin, Germany). The latter technology also permits the use of nonaqueous media, thereby allowing for the generation of water-free nanoparticle suspensions that may be filled into capsules. A number of piston-gap homogenizers have also been described (Patravale et al., 2004) and include the APV Micron Lab 40 (APV Deutschland GmbH, Lubeck, Germany), the EmulsiFlex-C3 and C5 (Avestin Inc., Ottawa, ON, Canada), and the Stansted 7400 (Stansted Fluid Power Ltd., Stansted, UK). Piston-gap HPH is readily scalable, and similar homogenizers are widely used to manufacture pharmaceutical and food-grade emulsions (e.g., milk) at commercial scale.

The particle size of the nanosuspensions formed by HPH typically ranges from 40 to 500 nm (Muller et al., 1999; Muller et al., 2001), although several process variables affect the size of the final product. First, owing to the high velocity of material transfer during HPH, the residence time within the piston gap is short. Therefore, material must be repeatedly cycled through the homogenizer (Moschwitzer et al., 2004; Sun et al., 2011) until the target particle size is attained or until no further change in size is evident with repeated cycling (Sahoo et al., 2011). Typically, 10 to 20 homogenization cycles are sufficient to achieve the minimum particle size (Xiong et al., 2008; Gao et al., 2010a; Pardeike and Muller, 2010), although larger cycle numbers may be required for drug crystals that fragment poorly (Moschwitzer et al., 2004; Gao et al., 2010b). Increased pressure may be used to reduce the number of homogenization cycles, however, this incurs greater cost and potential wear to the apparatus (Mishra et al., 2009). The use of more concentrated suspensions also provides a greater number of particle-particle interactions and, therefore, improves particle size reduction (Muller and Bohm, 1998). However, this benefit diminishes at loadings >10% w/w drug as a result of difficulties in stabilizing highly concentrated suspensions (Rabinow, 2004) and the increased energy required to process highly viscous particle slurries (Kraue and Muller, 2001).

Methods of HPH have been widely described for the development of parenteral and oral formulations containing poorly water-soluble drugs (Peters et al., 2000; Kraue and Muller, 2001; Kayser et al., 2003; Moschwitzer et al., 2004; Hecq et al., 2005, 2006; Wang et al., 2010b; Li et al., 2011c; Sahoo et al., 2011; Sun et al., 2011; Wang et al., 2011a) (see also Table 23). HPH has been suggested to be particularly suitable for parenteral delivery as there is no risk of contamination from milling media and the high-pressure environment is able to eliminate potential microbial contaminants (Krauel-Goellner, 2008).

Disadvantages of the HPH technique include the need for prolonged processing times when multiple homogenization cycles are required to reach the target particle size (Gao et al., 2010b; Lai et al., 2009; Muller and Junghanns, 1998) and higher minimum batch sizes compared with pearl milling by virtue of the higher void volume of most piston-gap homogenizers (Krauel-Goellner, 2008). The drug must also be pre-homogenized (at low pressure) or milled before HPH to prevent blockage of the aperture in the piston gap (Sahoo et al., 2011). Alternatively, drug crystals with small particle diameter may be harvested from supersaturated solutions (the basis for the bottom-up approaches to nanoparticle production are described in more detail in the section below) and further processed by HPH. This combination bottom-up/top-down approach is central to the NanoEdge technology (Baxter International Inc, Deerfield, IL), which generates thin and fragile needle-like crystals (by precipitation) that are highly susceptible to the subsequent homogenization process (Fig. 44) (Kipp et al., 2003; Rabinow, 2004; Rabinow et al., 2007).

2. “Bottom-up” Nanoparticle Assembly. Bottom-up strategies for microparticle and nanoparticle production are based on the controlled precipitation or crystallization of drug from a supersaturated solution (D’Addio and Prud’homme, 2011). The first step in this process is the generation of a supersaturated solution, followed by the formation of crystal nuclei, which subsequently act as a focal point for crystal growth. Crystal growth and phase separation of solid drug subsequently continue until drug concentrations in solution reach the saturated solubility.

Attainment of a high degree of supersaturation is critical to the success of nanoparticle formation as it encourages the formation of many small nuclei, the ongoing growth of which is restricted by rapid depletion of the supersaturated drug pool (through
extensive nucleation) (Hu et al., 2004a; Chan and Kwok, 2011; D’Addio and Prud’homme, 2011; de Waard et al., 2011). In contrast, at lower degrees of supersaturation, the generation of smaller numbers of crystal nuclei and slower depletion of the supersaturated pool allow crystal growth to continue for longer periods, in turn generating larger particles. Distinction between controlled precipitation/crystallization methods is dependent on differences in the way in which supersaturation is achieved (e.g., solvent shift or solvent evaporation) and the nature of the solvents used. A number of the most common approaches to the bottom-up production of microparticulate and nanoparticulate systems are described as follows.

a. Controlled Crystallization Via Solvent Shift. In solvent-shift methods, solvent containing drug at high concentration is mixed with an antisolvent (with limited solvency for the drug), causing the solubility of the drug in the mixed solvent to drop. This leads to supersaturation and ultimately drug crystallization. The selection of a solvent-antisolvent combination that generates a high degree of supersaturation is critical to the success of the solvent-shift method. The solvents should also be readily miscible as slow mixing will prevent the rapid establishment of a uniform degree of supersaturation in the solvent mixture. The latter is essential for the production of small particles with narrow particle size distribution.

The Hydrosol solvent-shift technique was first proposed by List and Sucker (1988) and involves the addition of a drug-containing organic solvent to a continuously stirring aqueous solution (Rasenack and Muller, 2002, 2004; Matteucci et al., 2007). It represents a relatively simple technique for producing nanoparticles, although wide particle size distributions are evident where mixing is nonuniform. Solvent carryover in the recovered product is possible but may be eliminated by subsequent spray-drying; however, this may also (unintentionally) yield amorphous drug particles (Rasenack and Muller, 2002).

Supercritical fluids (SCFs) may be used as an alternative to organic solvents in a number of pharmaceutical applications, including controlled crystallization. SCFs are typically gases at room temperature and pressure, but they exist at temperature and pressures above a critical point in a phase that is neither gaseous nor liquid but shares properties of both. SCFs therefore have the solvent properties of liquids but behave in many other respects like gases (Palakodaty and York, 1999; Pasquali et al., 2006). SCFs are of particular interest to particle synthesis methods as it is possible to fine-tune their solvent properties via small changes in pressure and temperature (Phillips and Stella, 1993). The gaseous properties of SCFs also afford rapid and efficient mixing (Chan and Kwok, 2011). Carbon dioxide (CO₂) is the most widely used SCF for pharmaceutical applications because of its moderate (and therefore practical) critical temperature (304.2 K, 31.1°C) and pressure (7.38 MPa; 1070 psi). Supercritical CO₂ is also nontoxic, nonflammable, inexpensive, and can be recycled during supercritical techniques (Bahrami and Ranjbarian, 2007).

SCFs have been used for a number of years as a means of microparticle isolation via the rapid expansion of supercritical solution (RESS) process (Phillips and Stella, 1993; Rasenack and Muller, 2004; Bahrami and Ranjbarian, 2007). In RESS, a drug-containing SCF is heated and passed through a nozzle into a precipitation chamber. Passage through the nozzle results in a drop in pressure, supersaturation, and ultimately drug crystallization (Phillips and Stella, 1993; Pasquali et al., 2006). Although RESS is
a viable approach for particle production, both at the laboratory scale and for large-scale manufacture (Subramaniam et al., 1997), particles are typically greater than 1 μm in size, largely because of unrestricted particle aggregation in the precipitation chamber (Moribe et al., 2005; Shinozaki et al., 2006; Chingunpitak et al., 2008; Su et al., 2009; Chen et al., 2010). Attempts to overcome this problem to generate nanosized material have included incorporating a cosolvent into the SCF (Thakur and Gupta, 2006) and submerging the output nozzle into an aqueous surfactant solution (Young et al., 2000; Turk et al., 2002). However, RESS remains limited by the often poor solvency of SCFs for poorly water-soluble drugs. Combining the SCF with a cosolvent may, in some cases, overcome this problem (Larson and King, 1986; Dobbs et al., 1987; Thakur and Gupta, 2006); however, it is now increasingly common to use SCFs as antisolvents in more traditional solvent shift methods of nanoparticle production (described as below).

Techniques that use SCFs as the antisolvent are broadly termed SCF antisolvent techniques (SAS). The various SAS approaches differ in the way in which the SCF interacts with the drug-containing solvent (Jung and Perrut, 2001). For example, in the gaseous antisolvent (GAS) method, the SCF is passed through the drug solution as a gas (Muhrer et al., 2006; Pasquali et al., 2006), whereas the aerosol solvent extraction system (ASES) or precipitation from compressed antisolvent (PCA) method requires the drug solvent solution to be sprayed as a fine mist into a chamber of compressed SCF. In PCA, the higher surface area of the aerosolized drug solution permits a faster rate of mass transfer into the antisolvent, leading to rapid attainment of higher levels of supersaturation than are possible with GAS techniques (Fusaro et al., 2005). Indeed, recognition of the importance of the speed of mass transfer (to generate high and uniform supersaturation) led York and coworkers to develop the solution enhanced dispersion by SCF (SEDS) method, where a two-channelled coaxial nozzle design permits a high-velocity stream of SCF (CO₂) to disperse and combine with a continuously flowing stream of drug-containing solvent. In the SEDS method, conditions within the mixing chamber are controlled such that the CO₂ remains in its supercritical state and the fast rate of mass transfer between the SCF and drug-containing solvent leads to rapid nucleation and subsequent particle formation (Hanna and York, 1998; Palakodaty and York, 1999; Pasquali et al., 2006).

Processing variables that influence the characteristics of the harvested particles (e.g., shape, size, and size distribution) include those that affect the rate of mass transfer of the saturated solution, such as the drug solubility in the solvent, the pressure and temperature within the precipitation chamber, and the rate at which the drug solution mixes with the antisolvent. Discussion of these factors is beyond the scope of the current article, however, details are available in the following references: Reverchon et al. (2002, 2007), Muhrer et al. (2006), and Sathigari et al. (2011).

b. Precipitation after Solvent Evaporation. Solvent evaporation provides an alternative means of generating a supersaturated drug solution during bottom-up particle generation methods. Rapid attainment of a high degree of supersaturation is again the main objective; therefore, a high surface area of the drug solution must be achieved to allow for rapid evaporation of the solvent. This has conventionally been achieved using spray-drying (Vehring, 2008) but until recently has been limited to the production of micron-sized particles. More recently, new-generation spray-dryers (e.g., the Buchi B90; BUCHI Labortechnik AG, Flawil, Switzerland) permit the production and collection of particles in the range of 300 to 5000 nm and require only milligram quantities of drug (Heng et al., 2011). A common drawback of spray-drying, however, is the potential for unintentional generation of amorphous drug particles rather than nanocrystals (Hu et al., 2004b; Rasenack and Muller, 2004), although for some applications, this may be advantageous.

Other common variants of the solvent evaporation method include evaporative precipitation into aqueous solution (EPAS) where a heated drug solution (normally an organic solvent of low boiling point to avoid thermal degradation of the drug) is sprayed as fine droplets into a heated aqueous solution containing various stabilizers (Hu et al., 2004a). The aqueous suspension obtained is then freeze-dried or spray-dried to generate solvent (water) free particles. EPAS has shown recent utility in generating amorphous nanosize particles of cyclosporine (Chen et al., 2002), quercetin (Gao et al., 2011b), and itraconazole (Chen et al., 2004a). A variety of other solvent evaporation techniques for producing nanoparticles have been developed and include electrospaying (Jaworek, 2007), cryogenic spray-freezing into liquid (SFL) (Hu et al., 2004a), and controlled crystallization during freeze-drying (CDFD) (de Waard et al., 2008), although these appear to have been less widely applied compared with the more established bottom-up techniques.

Interestingly, there have been few published comparisons of top-down versus bottom-up approaches to microparticle and nanoparticle production, although the greater commercial success that has been enjoyed by top-down methods may be attributable to more facile scale-up (de Waard et al., 2011). Nevertheless, owing to their simplicity, speed, and low cost, bottom-up techniques are highly appropriate for laboratory-scale and proof-of-concept experiments and, with wider use, may emerge as viable large-scale production techniques.
**C. Oral and Parenteral Delivery of Formulations Containing Nanosized Drug Particles**

**1. Oral Delivery.** Examples of drug nanoparticle-based formulations used in the oral delivery of poorly water-soluble drugs are provided in Table 24 and include the marketed products Rapamune (containing rapamycin) and Emend (containing aprepitant). Data comparing the oral bioavailability of aprepitant after administration to beagle dogs as a nanosuspension (120 nm) and a conventional microsuspension (5.5 µm) are reproduced in Fig. 45 (Wu et al., 2004). In this example, tablets containing micronized aprepitant had previously shown evidence of a positive food effect in human subjects (unpublished), and a food effect was also evident (3.2-fold increase in bioavailability) on dosing the micronized drug as a suspension to fed dogs. Administration of the NanoCrystal formulation, however, resulted in a 4.3-fold increase in bioavailability over the micronized drug in the fasted state and no difference in drug exposure after coadministration with food (Fig. 45). A lipid formulation showed similar performance (Wu et al., 2004), but low solubility in traditional lipidic excipients dictated that multiple capsules (>10) would have been required to achieve the projected human dose.

The microparticulate and nanoparticulate systems previously shown to enhance itraconazole dissolution in vitro in Fig. 42 were also subsequently examined in vivo after oral administration to rats, and the differences in dissolution were shown to translate to differences in bioavailability (Fig. 46). Thus micronization (to 5.5 µm) resulted in a 6-fold increase in oral bioavailability compared with a nonmicronized product, and further particle size reduction to yield nanoparticles with diameters of 350 or 700 nm (by high-pressure homogenization) elicited further (6.8-fold) increases in exposure compared with the micronized product (>40-fold compared with nonmicronized drug) (Sun et al., 2011). In this case, the additional benefits of a reduction in particle size from 700 to 350 nm were relatively small.

A significant advantage of drug nanosuspensions is the ability to incorporate readily into tablet and capsule formulations and to use the same technology to generate simple oral suspension formulations for preclinical pharmacokinetic, efficacy, and toxicity studies (Merisko-Liversidge et al., 1996; Langguth et al., 2005; Merisko-Liversidge and Liversidge, 2008). For example, formulation of a high permeability and low solubility (<1 µg/ml) developmental compound in an oral nanosuspension allowed the administration of higher doses than were possible using a nonaqueous lipid formulation (750 versus 350 mg/kg) and subsequently led to significantly increased drug exposure in rats compared with both nonmicronized material and a lipid-surfactant mixture (Kesisoglou et al., 2007) (Fig. 47). A recent example of the use of nanosuspensions to support dose escalation studies of an (unnamed) BCS class II model compound has also shown evidence of significantly higher drug exposure from a nanosuspension compared with micronized formulations and sufficient exposure to allow saturation of elimination at the highest dose (Sigfridsson et al., 2011b). Finally, oral administration of 100 mg/kg nanosuspension of the very poorly water-soluble antiprotozoal, atovaquone, to mice infected with Toxoplasma gondii, provided efficacy that exceeded that of a microsuspension and was comparable to that of a 10 mg/kg i.v. dose (Scholer et al., 2001).

In contrast, a small number of studies have reported a reduction in drug exposure after administration of oral nanosuspensions compared with conventional solubilized formations (Kesisoglou et al., 2007; Chiang et al., 2009), and in common with most delivery strategies designed to enhance oral exposure, apocryphal evidence suggests there are examples where nanosuspensions fail to provide significant increases in exposure, but they are not reported in the literature. Nonetheless, liquid nanosuspensions are effective in many situations, are relatively simple, and allow the administration of high doses, with low quantities of excipient, and therefore, should be considered particularly during preclinical studies where required doses are likely to be high. Furthermore, satisfactory results after the preclinical use of a nanosuspension provides a relative facile transition point for the progression and scale-up of the formulations into a solidified nanosuspension for clinical use.

**2. Nanosuspensions for Parenteral Delivery of Poorly Water-Soluble Drugs.** The risk of embolism after administration of particulate formulations has traditionally precluded the use of suspensions as i.v. formulations for poorly water-soluble drugs. Nanosuspensions, however, provide the potential for rapid in vivo dissolution and significantly reduced risks of embolism via the use of particles with diameters that are much smaller than that of most capillaries (Wong et al., 2008). As such, nanosuspensions are increasingly used during preclinical testing (Kipp, 2004; Rabinow, 2004; Kesisoglou et al., 2007; Rabinow et al., 2007; Sigfridsson et al., 2007), although progression into human clinical assessment has been slow. At this point, the only marketed example is a sustained release i.m. injection of paliperidone palmitate (Invega Sustenna or Xepilon;EU Janssen Pharmaceuticals); however, several i.v. nanoparticle formulations are currently under development, for example, thymectacin (Theralux, Celmed), which is currently in phase I/II clinical trials for the treatment non-Hodgkin lymphoma.

Interest in the use of parenteral nanosuspensions stems from their potential to deliver high drug loads in a rapidly dissolving form without the need for large quantities of excipients that may be irritant or toxic
<table>
<thead>
<tr>
<th>Top-Down Via Pearl-Milling</th>
<th>Median or Mean Particle Size and Stabilizer/Dispersants Used</th>
<th>Form Used during In Vitro/in vivo Testing</th>
<th>Dissolution/Bioavailability Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cilostazol (Jinno et al., 2006)</td>
<td>220 nm HPC and docusate sodium</td>
<td>Suspension</td>
<td>6.2- to 6.6-fold increase in exposure (AUC) in beagle dogs compared with jet-milled and hammer-milled drug Reduced food effect</td>
</tr>
<tr>
<td>Cilostazol (Jinno et al., 2008)</td>
<td>260 nm HPC and docusate sodium n-Mannitol</td>
<td>Wet-milled tablet</td>
<td>12.8-fold increase in bioavailability (5–64%) in fasted beagle dogs over a commercial tablet Diminished food effect</td>
</tr>
<tr>
<td>Danazol (Liversidge and Cundy, 1995)</td>
<td>169 nm PVP K-15</td>
<td>Suspension</td>
<td>15.1-fold increase in AUC in beagle dogs over a normal suspension &gt;95% drug dissolved within 10 min at pH 1.2 and 6.8 n-Mannitol aided redispersion of the dried nanoparticles</td>
</tr>
<tr>
<td>Phenytoin (Niwa et al., 2011b)</td>
<td>292 nm PVP K-30 and SLS n-Mannitol</td>
<td>Spray-dried suspension</td>
<td>Nanosuspensions containing either stabilizer showed rapid dissolution (pH 5) despite significant differences in particle size Nanosuspensions dosed to human subjects showed a diminished food effect compared with ziprasidone HCl (Geodon) (~1.3-fold and ~2-fold increase in AUC respectively). However, there was an increase in Cmax and decrease in Tmax with the nanosuspension, hence the nanoformulation and the branded product were not bioequivalent.</td>
</tr>
<tr>
<td>Undisclosed model compound (Sigfridsson et al., 2011b)</td>
<td>280 nm HPMC 1500 cPs and PVP K-30, SDS</td>
<td>Suspension</td>
<td>High-dose nanosuspensions showed higher exposure (~1.65- to 2.1-fold increase in AUC) in rats over a microsuspension formulation Both suspension and spray-dried suspensions showed &gt;85% release within 2 min in acidic media (pH 2)</td>
</tr>
<tr>
<td>Ibuprofen (Plakkot et al., 2011)</td>
<td>270 nm HPMC (6 cPs), PVP K-30 and SLS</td>
<td>Suspension</td>
<td>Nanosuspension with its stabilizer showed rapid dissolution (pH 5) despite significant differences in particle size.</td>
</tr>
<tr>
<td>Indomethacin (Liu et al., 2011)</td>
<td>394 nm/393 nm/986 nm Pluronic F68/Pluronic F127/Tween 80</td>
<td>Freeze-dried nanosuspension</td>
<td>Nanosuspensions containing either stabilizer showed rapid dissolution (pH 5) despite significant differences in particle size.</td>
</tr>
<tr>
<td>Ziprasidone (Thombre et al., 2011)</td>
<td>Particle size not described Pluronic F108/Tween 80 and Soybean lecithin</td>
<td>Suspension</td>
<td>Nanosuspensions dosed to human subjects showed a diminished food effect compared with ziprasidone HCl (Geodon) (~1.3-fold and ~2-fold increase in AUC respectively). However, there was an increase in Cmax and decrease in Tmax with the nanosuspension, hence the nanoformulation and the branded product were not bioequivalent.</td>
</tr>
<tr>
<td>Top-down via high pressure homogenization</td>
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<tr>
<td>Itraconazole (Sun et al., 2011)</td>
<td>316 nm Pluronic F127 and SLS</td>
<td>Freeze-dried nanosuspension</td>
<td>Nanosuspension showed a 51-fold/7.8-fold increase in AUC in rats compared with raw powder and microsuspension, respectively. See Fig. 42 and Fig. 46.</td>
</tr>
<tr>
<td>Deacety mycopeoxydiene (Wang et al., 2011a)</td>
<td>515 nm Lecithin, poloxamer 188, HPMC and PVP. n-Mannitol</td>
<td>Freeze-dried nanosuspension compressed into tablets</td>
<td>Near complete (&gt;80%) drug dissolution within 10 min across range of pH (1.2–7.0)</td>
</tr>
<tr>
<td>Diclofenac (Lai et al., 2009)</td>
<td>580 nm Pluronic F68</td>
<td>Freeze-dried nanosuspension</td>
<td>Improved dissolution rate over free drug, drug-stabilizer physical mixtures and coarse suspensions in water, and in simulated gastric and intestinal fluids</td>
</tr>
<tr>
<td>Rutin (Mauludin et al., 2009)</td>
<td>727 nm SDS</td>
<td>Freeze-dried nanosuspension compressed into tablets</td>
<td>80% release in water within 30 min Superior dissolution compared with a marketed formulation and microparticle formulation</td>
</tr>
<tr>
<td>Cefpodoxime proxetil (Gao et al., 2010b)</td>
<td>245 nm Poloxamer 188, HPMC E3-LV and glycerol n-Mannitol</td>
<td>Spray-dried nanosuspension</td>
<td>1.6-fold increase in AUC in rabbits compared with a marketed oral suspension 1.69-fold increase in Cmax</td>
</tr>
<tr>
<td>Spironolactone (Langguth et al., 2005)</td>
<td>400 nm Dioctyl sulfosuccinate</td>
<td>Suspension</td>
<td>3.3-fold increase in AUC of canrenone (spironolactone metabolite) over a microsuspension formulation However, higher exposures were obtained using a solid lipid nanoparticle formulation and combining suspensions with a surfactant.</td>
</tr>
<tr>
<td>Revaprazan hydrochloride (Li et al., 2011c)</td>
<td>562 nm Poloxamer 188</td>
<td>Freeze-dried nanosuspension</td>
<td>1.36- to 1.45-fold increase in AUC in rats compared with the coarse suspension and microsuspension Nanosuspension reduced Tmax from over 3 h (for the suspension/microsuspension) to under 1 h</td>
</tr>
<tr>
<td>Bottom-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole (Mou et al., 2011)</td>
<td>249 nm (via precipitation from solution). HPMC E50 n-Mannitol</td>
<td>Spray-dried suspension</td>
<td>1.8-fold increase in AUC in fasted rats over Sporanox pellets No change in AUC, Cmax or Tmax when administered in the fed state</td>
</tr>
</tbody>
</table>

(continued)
A further advantage is the potential to retain the chemical stability advantages associated with drug in the solid state. Physical stability concerns, however, are evident where the formulation is present as a predispersed suspension (via aggregation and particle size changes) and where there is potential for nanosuspensions to contain drug in anything other than the most stable crystal form (via polymorphic transitions).

Stability to particle aggregation and Ostwald ripening is enhanced by the addition of polymeric or surfactant-based stabilizer(s). Those that have been used in parenteral nanosuspensions include PVP, Pluronic F68, Tweens, and lecithin and are discussed in greater detail in the following references: Kesisoglou et al. (2007) and Wu et al. (2011). Tonicity modifiers (e.g., glycerol and sodium chloride) are also required in most i.v. formulations and have the potential to promote instability in a nanosuspension either by altering drug solubility (leading to Ostwald ripening) or by interacting with incorporated stabilizers (Jacobs et al., 2000). Where it is not possible to achieve adequate stability in liquid nanosuspensions, suspensions may be freeze-dried and then reconstituted with water or saline before use (Muller and Bohm, 1998). This step, however, introduces additional risks since removal of the suspending medium (on drying) may lead to the formation of particle aggregates. If particles are not readily dispersed during reconstitution of the dried suspension, the aggregates may cause irritation during injection and reduced drug efficacy following a slower rate of dissolution. Redispersion may be facilitated by the addition of a dispersing aid such as mannitol or sucrose before the drying process (Hecq et al., 2005; Van Eerdenbrugh et al., 2008).

The preclinical use of nanosuspensions has been reported to yield little, if any, irritation or pain at the site of administration (Xiong et al., 2008; Wang et al., 2004; Rabinow, 2004).
Further evidence for the favorable safety profile of nanosuspensions may be drawn from the development of several nanosuspension formulations for use in the clinic, although, as described already, these are yet to reach the market.

To avoid phlebitis and potentially more serious systemic side effects such as hypersensitivity and pulmonary embolism, nanosuspensions for i.v. use require particles with diameters of less than 300–400 nm (Jacobs et al., 2000; Muller et al., 2001; Li and Zhao, 2007). A risk of local irritation remains, however, if nanoparticles aggregate on contact with blood (Aramwit et al., 2006), and the i.v. compatibility of a nanosuspension should be assessed in a manner analogous to that commonly used for buffered/cosolvent/micellar formulations (see Sections IV, VI, and VII; and Yalkowsky et al., 1998). A number of studies have also linked the i.v. use of particulate formulations with hemodynamic effects, including a reduction in arterial blood pressure (Slack et al., 1981; Douglas et al., 1987; de Garavilla et al., 1998; Sigfridsson et al., 2011a). Reducing the rate of administration or use of histamine premedication may reduce the severity of these and other adverse effects associated with parenteral use of nanosuspensions (de Garavilla et al., 1996, 1998; Aramwit et al., 2006).

The reduced particle size of nanosuspension formulations provides the opportunity for parenteral formulations to dissolve sufficiently rapidly in situ that the pharmacokinetic profiles of a formulated drug are indistinguishable from administration in a cosolvent or cyclodextrin formulation. Indeed, many studies have observed similar pharmacokinetics after administration of a nanosuspension formulation compared with other parenteral formulations (Fig. 48) (Clement et al., 1992; Viernstein and Stumpf, 1992; Gassmann et al., 1994; Sigfridsson et al., 2007). However, there is also a significant body of evidence that has described differences in drug pharmacokinetics after i.v. administration of nanoparticle formulations. These are usually manifest as a marked reduction in $C_{\text{max}}$ and an increase in mean residence times after nanoparticle administration, presumably reflecting the incomplete initial dissolution and altered distribution and elimination profiles of particulate drug as opposed to drug in solution (White et al., 2003; Rabinow et al., 2007; Wang et al., 2010b; Zakir et al., 2011; Gao et al., 2011c). For example, the apparent plasma half-life of an itraconazole-cyclodextrin solution formulation (Sporanox) is approximately 5 h after i.v. administration to rats but increased to 15 h after administration of a crystalline nanosuspension (Fig. 49) (Rabinow et al., 2007). The nanosuspension was also associated with a reduction in $C_{\text{max}}$ (Fig. 49), although at an equivalent dose, it showed superior antifungal properties (Rabinow et al., 2007). Perhaps unsurprisingly, effects on pharmacokinetics are highly dependent on the particle size of the nanosuspension (Gao et al., 2008; Wang et al., 2010b), and amorphous (rather than crystalline) nanosuspensions appeared to show smaller reductions in $C_{\text{max}}$.

![Fig. 47. Comparison of nanosized active pharmaceutical ingredient to a nonaqueous vehicle suspension in support of toxicology formulation development for an insoluble compound (aqueous solubility <1 µg/ml). Utilization of nanosizing enabled an increase in maximum feasible dose and resulted in significantly higher exposure compared with a lipid-surfactant mixture (Merck; data on file). Adapted from Kesisoglou et al. (2007).](image1)

![Fig. 48. The mean plasma levels of AZ68 versus time after i.v. administration of AZ68 in a PEG 400:DMA:water (1:1:1) solution, as an amorphous nanosuspension, and as a crystalline nanosuspension to rats. The administered dose was 5 µmol/kg in all cases. Values are expressed as means (n = 3). PEG, polyethylene glycol; DMA, dimethylacetamide. Adapted from Sigfridsson et al. (2007).](image2)
compared with solution formulations. Together, these data suggest that it is the requirement for particle dissolution that leads to the decrease in C_{max} (Rabinow et al., 2007) after parenteral administration of nano-suspension formulations.

Nonetheless, by virtue of a reduction in C_{max}, nano-suspensions may also yield fewer drug-related toxic events for compounds where toxicity is correlated with maximum plasma concentrations and, therefore, provide the opportunity to use higher drug doses during dose escalation (Sharma et al., 2011) and clinical studies (Peters et al., 2000; Scholer et al., 2001; Margolis et al., 2007; Rabinow et al., 2007). In contrast, in some examples where nanoparticles have led to a reduced C_{max}, a reduction in clearance and a corresponding increase in total exposure are also evident. Under these circumstances, and where toxicity is more highly correlated with total exposure (rather than peak exposure i.e., C_{max}), nanosuspension administration may lead to greater toxicity. The propensity for altered pharmacokinetics after i.v. administration also likely precludes the use of nanoparticle formulations as an intravenous control during absolute bioavailability estimations since the requirement for identical systemic pharmacokinetic parameters (clearance, volume of distribution) after i.v. and oral administration might not be met.

Where the parenteral use of nanosuspensions is associated with prolonged drug exposures, there is also the potential for development of a controlled-release delivery platform (van’t Klooster et al., 2010), with the advantage of controlling the period of sustained release simply by modifying the particle size (Rabinow, 2004; Gao et al., 2008; Baert et al., 2009; Wang et al., 2010b). However, intact nanoparticles in the systemic circulation are likely to be eliminated rapidly after uptake into phagocytic cells of the mononuclear phagocytic system (MPS) (Ganta et al., 2009; Wang et al., 2011b), leading to accumulation in MPS organs, such as the liver and spleen (Muller et al., 2011a). The uptake of nanoparticles by the MPS may therefore limit rapid drug exposure to the site of action, although gradual drug release post-phagocytosis has the potential to provide sustained release back into the systemic circulation (Kipp, 2004; Patravale et al., 2004; Rabinow et al., 2007). The use of nanoparticulate systems also provides a means of directly targeting disease states within the MPS. The potential use of modified nano-suspension formulations to form the basis of targeted or site-specific delivery systems in a manner analogous to that of liposomes or nanoparticles is outside the scope of the current article but has been addressed elsewhere (Vonarbourg et al., 2006; Muller et al., 2011a).

D. Summary

Top-down (e.g., milling) or bottom-up (e.g., precipitation) techniques for decreasing particle size are effective and widely used techniques to increase the rate of dissolution of poorly water-soluble drugs. The mechanism for enhanced dissolution is largely via an increase in surface area with reduction in particle size. The advent of technologies that are able to generate nanosized (rather than micronized) drug particles has resulted in significant increases in surface area and dissolution rate and also the potential for additional advantage via increases in apparent drug solubility. Increases in solubility with particle size reduction may stem from the introduction of imperfections into the crystal structure during high-energy nanomilling or from changes in particle curvature, although the latter effect is evident only in particles with diameters smaller than 1 micron. Increases in solubility are relatively modest, however, and changes to dissolution rather than solubility remain the dominant advantage of nanocrystals.

The rapid dissolution rate of nanosized suspensions, the ability to generate particle sizes significantly smaller than blood capillary diameters, and the low irritancy of drug nanoparticles have also led to the use of nanocrystals by parenteral as well as oral routes of administration. Drug nanocrystals therefore provide a flexible approach to oral and parenteral administration and can be used both preclinically as a suspension and clinically after incorporation into more traditional tablet or capsule formulations. Particle size reduction, however, is not always effective at increasing exposure, and reaggregation phenomena may limit increases in dissolution rate. The need for relatively high shear to reduce particle sizes to submicron levels also runs the risk of inducing changes to crystal form and the introduction of amorphous character. Finally, nanosizing requires the use of specialized equipment and, until
recently, has been limited to application under license because of intellectual property considerations. Nonetheless, the use of a nanocrystalline drug as a means to enhance the delivery of poorly water-soluble drugs is an established technology, and examples of commercial oral and injectable products are evident.

X. Solid Dispersions

The prospect of using solid dispersions as a means of enhancing dissolution and oral bioavailability for poorly water-soluble drugs was first reported by Sekiguchi and Obi in 1961 (Sekiguchi and Obi, 1961) and has been widely explored over the subsequent 50 years (Hancock and Zografi, 1997; Serajuddin, 1999; Leuner and Dressman, 2000; Newman et al., 2012). Solid dispersions increase drug dissolution via several mechanisms, including a reduction in effective particle size, improved wetting, enhanced solubilization, and elimination of the impact of lattice energy via stabilization of the more soluble amorphous state. The mechanism of dissolution enhancement is largely dictated by the structure of the solid dispersion formed, but it is underpinned in many cases by the fundamental differences in solubility and dissolution behavior of a crystal and a glass of the same material (Grantscharova and Gutzow, 1986).

The term solid dispersion covers a range of different (but related) formulations, all containing drug dispersed within an inert carrier matrix (typically water-soluble sugars, polymers, or surface active emulsifiers). The principal difference between solid dispersion subtypes is the physical form of the drug and the carrier. Drug is either suspended in the carrier as phase-separated crystalline or amorphous particles, or it exists as a homogeneous molecular mixture of (amorphous) drug and carrier. The carrier can exist in the amorphous or crystalline form.

Given the often wide differences in crystalline and amorphous drug solubilities, different classes of solid dispersion often result in significant differences in dissolution rate. Solid dispersions that contain drug in the amorphous form usually show much faster dissolution rates than formulations containing crystalline drug, leading to significant improvements in drug absorption (Doherty and York, 1987; Law et al., 1992, 2004; Kennedy et al., 2008; Van Eerdenbrugh et al., 2009; Li et al., 2011b; Newman et al., 2012). Solid dispersions containing drug in the amorphous form are therefore often preferred. A notable example of a marketed amorphous solid dispersion is that of Sporanox capsules (Janssen Pharmaceuticals), an antifungal product containing the poorly water-soluble drug itraconazole molecularly dispersed in HPMC and coated onto inert sugar beads. Oral administration of the Sporanox solid dispersion formulation results in significantly enhanced oral bioavailability compared with the more slowly dissolving crystalline itraconazole (Mellaerts et al., 2008; Van Eerdenbrugh et al., 2009). Other marketed formulations that use solid dispersion formulations are listed in Table 25.

Despite their potential benefits, the number of commercially available solid dispersions is relatively low. This reflects the thermodynamic instability of drug in the amorphous form and the inevitable complexities of assuring drug stability, the inability to process many solid dispersion formulations using conventional manufacturing methods such as granulation, compression, and coating, and a lack of analytical characterization tools that can demonstrate, with high confidence, similarity among batches of materials sufficient to provide quality specifications for acceptance and rejection of the drug product. A classic example of the difficulty of progressing thermodynamically unstable formulations is provided by the antiviral ritonavir, a product that was initially marketed as a capsule formulation (Norvir; Abbott Laboratories) but was later withdrawn after the identification of a more stable (and less soluble) crystalline polymorph (Bauer et al., 2001).

Research in this area over the last 10 years, however, provides good evidence that the preparation of solid dispersion formulations with both improved dissolution and acceptable stability is possible. In particular, there is a growing understanding of the mechanisms by which high-molecular-weight polymers such as HPMC (and derivatives), PVP, and the methacrylates are able to attenuate the drivers of drug crystallization (Yoshioka et al., 1995; Van den Mooter et al., 1998; Matsumoto and Zografi, 1999) and, therefore, enhance physical stability. The mechanisms of amorphous stabilization include a reduction in molecular mobility by increasing local viscosity in the

<table>
<thead>
<tr>
<th>Commercial pharmaceutical products that use solid dispersion technology</th>
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<tbody>
<tr>
<td>Drug</td>
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<td>---------------------------------</td>
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<tr>
<td>Sporanox (itraconazole; Janssen Pharmaceuticals)</td>
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<tr>
<td>Intalence (etavirine; Tibotec)</td>
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<tr>
<td>Prograf (tacrolimus; Astellas Pharma US, Inc)</td>
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<tr>
<td>Nivadil (nilvadipine; Fujisawa Pharmaceutical Co., Ltd)</td>
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<tr>
<td>Certican or Zortress (everolimus; Novartis Pharmaceuticals)</td>
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<tr>
<td>Isotpin-SRE (verapamil; Abbott Laboratories)</td>
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<td>Zelboraf (vemurafenib; Roche)</td>
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<tr>
<td>Incivek (telaprevir; Vertex Pharmaceuticals)</td>
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<tr>
<td>Gris-PEG (griseofulvin; Pedini Pharmaceutical Inc)</td>
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<tr>
<td>Cesamet (nabulone; Valeant Pharmaceuticals)</td>
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<tr>
<td>Rezulin (trogilitazone; Parke-Davis)</td>
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<tr>
<td>Kaletra (lopinavir and ritonavir; Abbott Laboratories)</td>
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<td>Norvir tablets (ritonavir; Abbott Laboratories)</td>
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</table>

HPC, hydroxypropyl cellulose; HPMC, hydroxypropyl methylcellulose; HPMCAS, HPMC acetate succinate.

*The original Norvir capsule formulation was withdrawn from the market, but was subsequently relaunched as a PVP/VA melt-extended tablet (Norvir Film-coated Tablets) and as a lipid formulation (Norvir SRC).*

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solid dispersion matrix (Hancock and Zografi, 1997; Shamblin et al., 2000; Van den Mooter et al., 2001; Six et al., 2004), the generation of intermolecular interactions between polymer and drug that also restrict molecular movement (Taylor and Zografi, 1997; Khougaz and Clas, 2000; Vasanthavada et al., 2005), and the inhibition of crystal nucleation (Konno and Taylor, 2006; Lindfors et al., 2008; Marsac et al., 2008; Caron et al., 2010).

These processes all limit drug transformation from the desirable (from a solubility perspective) but thermodynamically unstable amorphous form to the less soluble but stable crystalline form. The effectiveness of crystallization inhibition is linked closely to the crystallization tendencies of the drug, the properties of the formulation (carrier), and the drug-to-carrier ratio since increasing drug load decreases the quantity of carrier available to stabilize the drug in the high-energy form. There is also the added risk that high drug loading will promote phase separation within the solid dispersion, in turn increasing the risk of instability (Marsac et al., 2009; Qian et al., 2010a). Nonformulation factors that affect stability include the method of solid dispersion manufacture (widely used techniques include hot-melt extrusion and spray- or freeze-drying) (Broman et al., 2001; DiNunzio et al., 2010b) and the presence of moisture and other plasticizers (Sethia and Squillante, 2004; Marsac et al., 2008; Rumondor et al., 2009b). Extensive, rapid advances in understanding of the fundamental physics of the glassy state (Johari, 2000; Angell et al., 2003; Ngai et al., 2010) have also contributed to a better understanding of the physical stability of amorphous systems, in particular, the importance of the kinetics of relaxation of high-energy solids (Lunkenheimer et al., 2000; Prevosto et al., 2009). Increased appreciation of the kinetic aspects of structural relaxation provides a platform to predict more accurately the molecular mobility of materials at temperatures below the glass transition temperature (Bras et al., 2008; Thayyil et al., 2008; Adrjanowicz et al., 2010b; Johari et al., 2011; Kaminski et al., 2011) and, therefore, to understand the determinants of the long-term physical stability of pharmaceutical formulations containing non-crystalline solids.

Finally, the fate of the drug after release from a solid dispersion is a key indicator of formulation performance, in particular, the propensity for drug recrystallization from the supersaturated solutions that are generated by dissolution of amorphous drug. In the absence of stabilization, recrystallization occurs, leading to a decrease in the quantity of drug in solution available for absorption. However, the inclusion of polymers within the formulation matrix has been shown to reduce crystallization tendency post-dissolution and to provide for significant increases in oral bioavailability in some cases (Tanno et al., 2004; Friesen et al., 2008; Brouwers et al., 2009).

Here we describe the various solid dispersion subtypes and the mechanisms of drug release that underpin solid dispersion utility. Common methods of manufacture are also briefly described. Finally, the manner in which polymers may be used to maintain the stability of the amorphous state in solid dispersions over prolonged storage periods is discussed.

A. Classification of Solid Dispersions

Solid dispersions can be classified according to the molecular arrangement of the drug within the carrier matrix and the properties of the carrier. In simple terms, drug may be 1) partially dissolved with excess drug suspended in the crystalline state, 2) partially dissolved with excess drug suspended in the amorphous state, or 3) completely dissolved (i.e., molecularly dispersed). The solid dispersion matrix may be either crystalline or amorphous. As such, there are six major types of solid dispersions (although mixtures of amorphous and crystalline particles are also possible). These are described in Table 26, and each arrangement is shown schematically in Fig. 50 (van Drooge et al., 2006). Solid dispersions containing amorphous drug particles or molecularly dispersed drug are usually described as amorphous solid dispersions and those based on an amorphous carrier as glasses. Where drug is present as a molecular dispersion, formulations are described as a solid solution (where the carrier is crystalline) or a glass solution (where the carrier is amorphous). Depending on the drug loading in the solid dispersion relative to drug solubility in the carrier, solid or glass solutions may be supersaturated (see Section X.G.5).

The different classes of solid dispersion are described in the following sections, and systems where the carrier is crystalline (i.e., types 1–3 in Table 26) are discussed first. The simplest of these systems comprise crystalline drug suspended in a crystalline carrier and are commonly referred to as eutectic mixtures. Eutectic mixtures are combinations of crystalline components (drug and a carrier) that are miscible in the molten liquid state but show little or no miscibility in the solid state (Sekiguchi and Obi, 1961; Chiou and Riegelman, 1970). A eutectic system has a single chemical composition that becomes a solid at a lower temperature than any other composition made with the same components. This unique composition is known as the eutectic composition and the temperature as the eutectic temperature. Below this eutectic temperature, a mixture of drug and carrier simultaneously crystallize to form an intimate mixture of fine crystalline drug particles within a crystalline carrier matrix. At all other drug-carrier compositions (i.e., noneutectics), one component preferentially solidifies during cooling until the remaining concentrations in the liquid phase reach the eutectic composition, at which point the remaining drug and carrier (in the liquid phase) simultaneously crystallize.
One of the first reports of the use of solid dispersions as a formulation approach for poorly water-soluble drugs described mixtures of sulfathiazole and urea and classified the formulations as eutectic mixtures (i.e., containing drug in the crystalline form) (Sekiguchi and Obi, 1961). Later work, however, suggests that sulfathiazole shows some degree of solid solubility in urea, hence the original sulfathiazole-urea systems might more accurately be defined as a solid solution (i.e., type 3 in Table 26). In retrospect, it is likely that many of the solid dispersions that were first described as eutectic mixtures were either solid solutions, non-eutectics, or monotectic systems, the latter comprising combinations of drug and carrier in proportions where only the melting temperature of the drug is depressed (i.e., where the melting temperature of the carrier is unchanged).

Within the class of solid dispersions described as solid solutions (i.e., type 3), there are two subtypes: 1) those in which the two materials are miscible at all compositions in both the liquid and solid state (continuous solid solutions) and 2) those in which complete drug-carrier miscibility is observed only within certain areas of the drug-carrier phase diagram (discontinuous solid solutions) (Leuner and Dressman, 2000). Continuous solid solutions are rare and are generally not reported in the pharmaceutical literature (Janssens and Van den Mooter, 2009). Discontinuous solid solutions are therefore the more relevant pharmaceutical system (Chiou and Riegelman, 1971). In regions of the phase diagram where drug and carrier are miscible (i.e., where solid solutions are formed), drug is mixed within the carrier at a molecular level, and drug in this form exhibits the maximum attainable surface area for hydration (right hand panel; Fig. 50). The rate of drug release from solid solutions is faster than that from equivalent multiphase systems containing suspended solid drug particles, regardless of whether the suspended material is amorphous (e.g., type 2) or crystalline (e.g., type 1).

Solid dispersions containing drug dispersed in an amorphous carrier (i.e., types 4–6) may be classified in a similar fashion to those where the carrier is crystalline, although in this case the solid dispersions are commonly referred to as “glasses” to reflect the non-crystalline character of the carrier. Thus, type 4 systems, or glass suspensions, are analogous to eutectics and contain crystalline drug suspended in an amorphous carrier; type 5 systems, or amorphous glass suspensions, are related but contain amorphous drug particles suspended in an amorphous drug carrier; type 6 solid dispersions or glass solutions are analogous to solid solutions and contain a molecular dispersion of drug in an amorphous carrier. The use of the term

### TABLE 26

<table>
<thead>
<tr>
<th>Type</th>
<th>Solid Dispersion</th>
<th>Drug Carrier</th>
<th>Total No. of Phases</th>
<th>Physical Drug Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eutectic mixture</td>
<td>Crystalline particles</td>
<td>2</td>
<td>Stable</td>
</tr>
<tr>
<td>2</td>
<td>Solid amorphous suspension</td>
<td>Amorphous particles</td>
<td>2</td>
<td>Unstable; crystallization risk</td>
</tr>
<tr>
<td>3</td>
<td>Solid solution&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Molecularly dispersed</td>
<td>1</td>
<td>Unstable (below crystalline solubility); crystallization risk</td>
</tr>
<tr>
<td>4</td>
<td>Glass suspension</td>
<td>Crystalline particles</td>
<td>2</td>
<td>Stable</td>
</tr>
<tr>
<td>5</td>
<td>Glass amorphous suspension</td>
<td>Amorphous particles</td>
<td>2</td>
<td>Unstable; crystallization risk</td>
</tr>
<tr>
<td>6</td>
<td>Glass solution</td>
<td>Molecularly dispersed</td>
<td>1</td>
<td>Unstable; risk of phase-separation and crystallization</td>
</tr>
</tbody>
</table>

<sup>a</sup> May include continuous/discontinuous and substitutional/interstitial; see Leuner and Dressman (2000).

Fig. 50. Schematic depicting the three different molecular arrangements of drug molecules within a solid dispersion. The panels illustrate the major arrangement of drug in each solid dispersion subtype (Table 26). In types 1–3, the carrier is crystalline; in types 4–6, the carrier is amorphous. Note that some molecularly dispersed drug will be present in each type (reflecting the solid solubility of the drug).
**glass** to describe amorphous pharmaceutical systems provides a facile point of differentiation between crystalline and noncrystalline carriers but is an oversimplification since glasses and amorphous solids, although both noncrystalline, are thermodynamically distinct. Gupta (1996) suggests a difference in amorphous and glassy materials based on the degree of short-range order such that short-range order in a glass is consistent with that in a melt (allowing the material to undergo a glass transition), whereas amorphous solids contain a higher level of short-range order precluding glass transition. In practice, however, both glasses and amorphous solids effectively circumvent the limitations to solubility provided by the crystal lattice, and a detailed discussion of differential thermodynamics is not warranted here. The interested reader is directed to the following references for more detail: Kauzmann (1948), Angell (1995a,b), and Gupta (1996).

**1. Nontraditional Solid Dispersions.** Several other formulation types have been described as solid dispersions, including drug-excipient complexes, polymer-stabilized nanoparticle formulations, and formulations containing amorphous drug where the amorphous form is stabilized via adsorption to a high-surface-area, solid carrier.

Solid drug-CD complexes may also contain amorphous drug, but in this case, drug molecules are usually confined to the hydrophobic core of the CD rather than being truly molecularly dispersed or suspended within an inert matrix (Davis and Brewster, 2004; Brewster and Loftsson, 2007; Stella and He, 2008). Drug-CD complexes that are further dispersed within a polymer matrix better reflect the composition of a solid dispersion, but they have the added complexity of the drug-CD interaction.

Polymer stabilized drug nanoparticles contain similar components to solid dispersions and may also be prepared using a similar approach, for example, solvent evaporation techniques (Chan and Kwok, 2011). A notable difference, however, is the lower drug-polymer ratio found in solid dispersion formulations (Rasenack and Muller, 2002) where the polymer is required to disperse the drug molecularly (thus rendering it noncrystalline) and to assist in solubilization and wetting. In contrast, in most nanoparticle formulations, polymers are included to stabilize the dispersion and to decrease agglomeration of small drug particles (Van Eerdenbrugh et al., 2008; Wu et al., 2011). Furthermore, the physical form of the drug in a nanoparticle formulation is usually crystalline, whereas in most recently developed solid dispersions, drug is present in the amorphous form. The fact that solid dispersions (where drug is embedded in a polymer matrix) may also be isolated in nanoparticle or microparticle form, especially after spray-drying (Won et al., 2005; Kennedy et al., 2008; Wu et al., 2009; Dontireddy and Crean, 2011), adds further complexity to the distinction between polymer stabilized nanoparticles and nanoparticulate solid dispersions.

Finally, there is increasing interest in the generation of amorphous drug formulations via drug adsorption to a high surface area carrier. These systems stabilize amorphous drug on the carrier surface and, where porous carriers are used, within the carrier pore structure (Van Speybroeck et al., 2010b). Adsorbed formulations such as these have occasionally been described as solid dispersions or surface solid dispersions (Kerc et al., 1998; Watanabe et al., 2001; Takeuchi et al., 2005; Wang et al., 2006). Differentiation between traditional solid dispersions and adsorbed amorphous systems can be made on the basis of the arrangement of drug; in the case of solid dispersions, drug is molecularly dispersed or suspended throughout an inert carrier and crystallization is slowed primarily via an increase in viscosity and a decrease in drug mobility. In adsorbed amorphous systems, drug crystallization is prevented through drug interaction with the surface of the carrier (with the resulting spatial separation preventing nucleation) and, in instances where the carrier is porous, the narrow dimensions of the fine capillary network (that reduce mobility and limit crystal growth) (Mellaerts et al., 2007). The use of high surface area adsorbents is described in more detail in Section XII.A.

**B. Mechanisms by which Solid Dispersions Enhance Dissolution Rate and Oral Bioavailability**

**1. Reduced Particle Size and Enhanced Drug Wetting and Solubilization.** A common mechanism by which all the solid dispersion subtypes in Table 26 enhance dissolution is via an effective reduction in drug particle size. Solid dispersions comprise drug (whether amorphous or crystalline) suspended or molecularly dispersed in a carrier. In most cases, suspended drug particles have smaller particle sizes than are usual in traditional solid dosage forms, and in the case of a molecular dispersion, the particle size of drug in the carrier is minimized, theoretically, to the size of a single molecule (Craig, 2002). The reduction in particle size provides for an increase in effective surface area for dissolution (in accordance with eq. 9). Secondary precipitation from the supersaturated solutions that may be formed by the dissolution of amorphous drug is also possible but commonly results in the formation of suspensions with relatively small particle size (Friesen et al., 2008; Alonzo et al., 2011; Kanaujia et al., 2011). Indeed, it is often difficult to distinguish between an increase in dissolution that stems from a reduction in the particle size of the aggregates present in the original solid dispersion and the size of particles that may be formed in situ via precipitation on dissolution. This is further complicated by the realization that some level of particle aggregation is also likely (Simonelli et al., 1976; Alonzo et al., 2011). Nonetheless, in most cases,
an increase in the dissolution rate resulting from a decrease in the size of dissolving drug particles is evident.

The use of highly water-soluble carrier materials also promotes rapid influx of hydration media into the solid dispersion matrix, resulting in an increase in wetting. To take advantage of this effect, 1st-generation solid dispersions used low-molecular-weight, highly water-soluble carriers such as urea (Goldberg et al., 1966a,c), aliphatic short-chain carboxylic acids such as citric and succinic acid (Goldberg et al., 1966b; Summers and Enever, 1976), and sugars such as sucrose, trehalose, sorbitol, and mannitol (Allen et al., 1978; Arias et al., 1995; Okonogi et al., 1997; Valizadeh et al., 2004). In one of the earliest studies, Sekiguchi and Obi (1961) described an increase in the rate of dissolution of sulfathiazole when the drug was fused with urea and then crash-cooled to form a solid dispersion (when compared with a physical mixture of drug and urea). The improvement in dissolution rate was attributed to the rapid dissolution of the eutectic and liberation of drug in the form of a microcrystalline dispersion and was ultimately shown to improve oral absorption in human subjects.

A growing awareness of the potential benefits associated with the use of polymeric or surfactant-based solid dispersions and the relatively high melting temperatures of many sugars (which complicates solid dispersion manufacture via melting/fusion techniques) (Leuner and Dressman, 2000) have resulted in a general decline in the use of 1st-generation carriers. Second-generation polymeric carriers—including PVP, PEG, polymethacrylates, and HPMC (and its derivatives) —and 3rd-generation carriers including self-emulsifying and surfactant-based excipients are now considerably more common (Serajuddin, 1999; Vasconcelos et al., 2007).

Polyethylene glycol (PEG) was the first widely explored 2nd-generation polymeric excipient and formed the basis for the first commercial solid dispersion product (Gris-PEG, Pedinol Pharmacal Inc., Farmingdale, NY). PEG has a low melting temperature (the melting point of PEG 20 000 is between 60 and 63°C) and can be readily fused with drug by quench-cooling a melt or via hot-melt extrusion (Craig, 1990; Leuner and Dressman, 2000). PEG-based solid dispersions enhance the dissolution of many low solubility compounds (Law et al., 1992, 2001; Saers and Craig, 1992; Save and Venkitachalam, 1992; Lloyd et al., 1997; Van den Mooter et al., 1998; Verheyen et al., 2002; Valizadeh et al., 2004; Baird and Taylor, 2011), however, benefits over and above simple physical mixtures of drug and polymer are not always apparent. For example, PEG 6000–based solid dispersions increase the dissolution rate of diazepam and temazepam compared with crystalline drug, however, the rate of dissolution is no better than that of equivalent PEG-drug physical mixtures (Verheyen et al., 2002). Similarly, physical mixtures of nifedipine with PEG 4000 and 6000 provide rates of dissolution equivalent to that of a solid dispersion, even though the fusion of drug and polymer during solid dispersion manufacture leads to nifedipine transformation to a metastable and more soluble physical form (Save and Venkitachalam, 1992). Zerrouk et al. (2001) also report that although the bioavailability of carbamazepine is lower after administration to rabbits in a PEG 6000 physical mix when compared with an equivalent solid dispersion, the differences in exposure were not statistically significant.

Nonetheless, and realizing that examples to the contrary are evident, in most cases, solid dispersion formulations outperform drug-polymer physical mixtures (McGinity et al., 1984; Dannenfelser et al., 1996; Law et al., 2001; Jun et al., 2005; Han et al., 2011; Kanaujia et al., 2011; Khan et al., 2011; Li et al., 2011b; Mues et al., 2011). Thus, no evidence of drug dissolution over a 30-min period was reported for a physical mixture of itraconazole-HPMC, whereas a hot-melt extruded solid dispersion containing drug in the amorphous form released ~80% drug within the same period (Verreck et al., 2003b). Amorphous ritonavir-PEG 8000 solid dispersions also show superior rates of dissolution compared with rates of equivalent physical mixtures (Fig. 54A) (Law et al., 2004). In both these cases, it is likely that the advantage of the solid dispersion stems from differences in solubility resulting from isolation of drug in the amorphous form. The advantages that accrue from the isolation of drug in the amorphous form are described in more detail in Section X.B.2.

Third-generation solid dispersions include surface-active excipients that promote emulsification of the formulation and solubilization of incorporated drug. Emulsifiers are widely used in solid dispersions either alone or in combination with polymers and can accelerate drug release through enhanced drug wetting and solubilization (Serajuddin et al., 1988, 1990). Emulsifiers also aid drug release by enhancing the ability of the formulation to disperse adequately on hydration (Serajuddin, 1999). Some of the first studies to explore solubilizing solid dispersions examined the utility of incorporation of polyethoxylated surfactants, including Labrasol, Tweens, and Gelucire, and showed improvements in dissolution rate and oral bioavailability over nonsolubilizing solid dispersions (Serajuddin et al., 1988, 1990; Veiga et al., 1993; Sheen et al., 1995; Khoo et al., 2000).

Solid emulsifiable glasses have also been prepared by drying emulsions (containing dissolved drug) in the presence of sugars, with or without the inclusion of surfactants. These systems are closely related to solid dispersions (and may also be prepared by similar techniques, for example, solvent evaporation); the difference is the substitution of a dispersed oil phase containing dissolved drug for the more typical
dispersion of crystalline or amorphous drug (Myers and Shively, 1993). Emulsifiable glasses self-emulsify on hydration to form a fine drug-in-oil dispersion (Myers and Shively, 1992; Porter et al., 1996) and provide for equivalent drug exposure for cyclosporine after oral administration to dogs compared with traditional liquid self-emulsifying formulations (Porter et al., 1996).

A number of surface-active agents have been incorporated into solid dispersions, including ionic (e.g., sodium lauryl sulfate) and nonionic surfactants (e.g., Pluronic block copolymers) (Chen et al., 2004b; Newa et al., 2007; Lakshman et al., 2008; Badens et al., 2009), and these excipients offer additional benefits to solid dispersion performance, including promotion of drug mixing/miscibility within the solid dispersion (i.e., promotion of solid solution formation) (Dannenfelser et al., 1996; Wulff et al., 1996; Shin and Kim, 2003; Linn et al., 2012). Since many of the surface active agents used in the formation of solid dispersions of this type are liquid or semisolid at room temperature, manufacture into traditional solid dosage forms (e.g., tablets) is difficult unless high-molecular-weight polymers are incorporated. For example, mixtures of Tween 80 and PEG (e.g., PEG 1000, 1450, 3350, and 8000) have been shown to form solid formulations up to 75% surfactant (Morris et al., 1992). Alternatively, direct filling of drug-carrier melts into hard gelatin capsules has also been attempted (Seraujdinn, 1999).

2. Administration of Drug in the Amorphous Form. Amorphous solids lack the ordered molecular lattice and defined molecular configuration and packing of crystalline solids of equivalent chemical composition. The strength of intermolecular solid-state interactions in an amorphous solid is therefore lower than in crystalline solids. From the solubility equations in Section I.B.1, a reduction in intermolecular forces in the solid state contributes to an increase in solubility. Indeed, such is the importance of solid-state interactions to solubility that the solubility gain attained by the use of drug in the metastable amorphous form may be up to several orders of magnitude. However, differences in experimentally determined amorphous and crystalline solubilities are in practice often much lower (Hancock and Parks, 2000; Murdande et al., 2011a). This has been suggested to reflect the complexities of measuring the “equilibrium” solubility of a thermodynamically unstable amorphous material, such that spontaneous crystallization results in underestimation of the initial (kinetic) solubility properties of the amorphous form (Hancock and Parks, 2000; Bhugra and Pikal, 2008; Murdande et al., 2010a,b, 2011a). Recent work also suggests that historical predictions of amorphous drug solubility (using the difference in heat capacity between crystalline and amorphous drug) may have been overestimated since amorphous solids are often hygroscopic and invariably absorb water. The resulting drug-water interactions decrease the free energy difference between amorphous and crystalline drug and reduce the solubility advantage compared with theoretical predictions (Murdande et al., 2010a,b).

Despite the potential for errors in the estimation of solubility, significant solubility advantages remain for noncrystalline drug, and these are manifest through increases in dissolution rate and, ultimately, oral bioavailability. This solubility advantage is the basis for much of the utility of amorphous solid dispersions.

3. Maintenance of Supersaturation after Drug Dissolution. Drug dissolution from dosage forms containing high-energy polymorphs or amorphous solids typically leads to the attainment of drug concentrations in solution that are supersaturated with respect to the saturated solubility of the stable crystal form. The attainment of supersaturation is beneficial in providing a greater concentration of dissolved drug and in generating drug solutions with increased thermodynamic activity. The induction of supersaturation in solution has been referred to as a concentration “spring” and may occur under many conditions, including dissolution of amorphous drug, but also the dispersion or digestion of lipid-based dosage forms, the dissolution of salts of weak acids or bases, and after the gastric emptying of basic drugs that are soluble in acidic gastric fluid, but less soluble in the intestine (Fig. 51) (Guzmán et al., 2004; Kostewicz et al., 2004; Brouwers et al., 2009; Warren et al., 2010; Porter et al., 2011; Shono et al., 2011; Anby et al., 2012).

Supersaturated solutions provide an advantage with respect to drug absorption but are unstable and at risk of precipitation. The rate of drug precipitation from supersaturated solutions can be markedly slowed by the presence of materials (including polymers) that inhibit crystal nucleation or slow crystal growth (Vandecruys et al., 2007; Brouwers et al., 2009; Warren et al., 2010). Consistent with the “spring” analogy for supersaturation induction, the reduction in the rate of drug precipitation from supersaturated solutions has been referred to as a “parachute” and the combined mechanism of supersaturation generation and precipitation inhibition as a “spring and parachute” (Guzmán et al., 2004, 2007) (Fig. 51).

The potential for the polymers that are used to fabricate solid dispersions also to inhibit drug precipitation from supersaturated solutions post dissolution is illustrated in Fig. 52A. In this example, three tacrolimus solid dispersions were manufactured using different carrier polymers. In each case, the formulations led to consistent (and rapid) initial rates of dissolution (Yamashita et al., 2003). However significant differences in the ability of the polymers to maintain supersaturation were subsequently evident. HPMC prevented recrystallization more effectively compared with PVP and PEG 6000, and HPMC-based solid dispersions were later shown to provide for a 10-fold
increase in C_{max} and AUC in dogs compared with administration of crystalline drug (Fig. 52B) (Yamashita et al., 2003). HPMC plays a similar role in enhancing the bioavailability of the poorly water-soluble weak base itraconazole after oral administration of the Sporanox formulation. In this case, drug dissolution under acidic conditions reflective of the gastric environment is unsurprisingly rapid, but it is widely believed that the robust bioavailability (∼55%) of itraconazole after administration of Sporanox is due, at least in part, to the ability of HPMC to prevent drug precipitation as dissolved drug leaves the acidic stomach and enters the less favorable solubilizing conditions (for a weak base) in the intestine (Jung et al., 1999; Six et al., 2005; Augustijns and Brewster, 2012).

Similar behavior with respect to the capacity for polymers to stabilize supersaturated drug has been attributed to HPMCAS-based solid dispersions (Friesen et al., 2008; Kennedy et al., 2008; Konno et al., 2008; Curatolo et al., 2009; Murdande et al., 2011a). HPMCAS is unusual compared with many of the most commonly used polymers in oral formulations in that it shows some degree of amphiphilicity, with hydrophobic domains available for interaction with poorly water-soluble drugs and hydrophilic domains available for interactions with the aqueous medium. These properties assist in the maintenance of supersaturation (Tanno et al., 2004). Figure 53 shows the ability of HPMCAS to maintain supersaturation for two experimental compounds at high (A) and low (B) drug loads (Curatolo et al., 2009).

HPMCAS also has a high glass transition temperature (∼120°C) and can therefore reduce mobility and protect against drug crystallization in the solid state [see Section X.F.3 and Section X.G]. In contrast, at least for weak bases, the bioavailability enhancements provided by solid dispersions of HPMCAS are unlikely to stem entirely from the maintenance of supersaturation since HPMCAS is largely insoluble in the acidic stomach contents, and on gastric emptying, drug precipitation may occur before complete
dissolution of the HPMCAS matrix (Van Speybroeck et al., 2010b).

a. The Impact of Dissolution Rate and Supersaturation Ratio on Precipitation Inhibition. The drivers of drug precipitation from supersaturated solutions include the degree of supersaturation (where increased supersaturation increases the potential for nucleation and subsequent precipitation or crystallization) (Turnbull and Fisher, 1949) and the rate of dissolution (where rapid attainment of highly supersaturated solution also increases the chances of nucleation and precipitation).

Formulation approaches that moderate the degree of supersaturation in solution by either reducing the supersaturation ratio or reducing the rate of dissolution therefore increase the stability of the supersaturated solution, reduce the likelihood of precipitation, and maximize the performance of the formulation (Augustijns and Brewster, 2012). Support for this contention is provided by the data described by Six et al. (2005), where slower rates of itraconazole release from HPMC solid dispersions led to better in vivo performance in human subjects compared with solid dispersions prepared using Eudragit E100 or mixtures of Eudragit E100 and PVPVA 64 that showed faster in vitro rates of dissolution. Solid dispersions of itraconazole prepared using enteric polymers (Eudragit L100-55 and Carbopol 947P) to reduce drug release in the stomach also showed enhanced performance compared with HPMC (Miller et al., 2008; DiNunzio et al., 2010a). Specifically, the enteric polymers reduced the quantity of the dose that was solubilized in the acidic gastric content and, therefore, reduced the solubilized bolus that was introduced into the higher pH environment of the intestine on gastric emptying. This in turn lowered the degree of supersaturation that was invoked by the increase in pH in the small intestine and the drop in itraconazole solubility (Miller et al., 2008; DiNunzio et al., 2010a). Subsequent bioavailability studies using an itraconazole-Eudragit L100-55 enteric solid dispersion and a conventional HPMC solid dispersion in rats revealed that the enteric matrix resulted in prolonged and improved exposure (although the results were highly variable) (Miller et al., 2008). Similarly, although the release of a sparingly soluble glycogen phosphorylase inhibitor was faster from solid dispersions formed with cellulose acetate phthalate (CAP) compared with HPMC, the highly supersaturated drug solutions that resulted from dissolution of the CAP formulation could not be maintained, and the slower releasing HPMC solid dispersion showed improved overall performance (Crew et al., 2007).

b. Mechanisms by which Polymers Maintain Supersaturation in Solution. Precipitation from a supersaturated solution follows two critical steps: the formation of particle nuclei and subsequent particle growth (Macie and Grant, 1986; Gebauer et al., 2008; Lindfors et al., 2008). However, differences in the nature of the matrix in which precipitation or crystallization occurs (i.e., in solution or within the polymer) result in important differences in assessment and stabilization.

One example of a means of reducing the potential for precipitation from solution is via an increase in drug solubility in solution as this will reduce the level of supersaturation and the thermodynamic drivers of drug precipitation. Supersaturation stabilization via an increase in solubilization is largely applicable to
surface-active carriers such as the surfactants and emulsifiers that are used in 3rd-generation solid dispersions. Polymeric carriers have also been suggested to enhance the stability of supersaturated solutions via an increase in drug solubilization (Usui et al., 1997), however, in most cases, (nonsurface active) polymers offer little, if any, direct enhancement of equilibrium solubility (Konno et al., 2008; Warren et al., 2010; Bevernage et al., 2011). Instead, polymers reduce drug precipitation via slowing the kinetics of drug precipitation or crystallization (Rodriguez-Hornedo and Murphy, 1999; Raghavan et al., 2001; Lindfors et al., 2008; Miller et al., 2008; Brouwers et al., 2009; Curatolo et al., 2009; Warren et al., 2010; Kanaujia et al., 2011).

Increased solution viscosity has also been proposed as a mechanism by which polymers inhibit precipitation in both the solid-state and in solution (Miller et al., 2008; Gao et al., 2009). However, precipitation inhibition in solution has been observed at polymer concentrations that are too low to have significant effects on solution viscosity (Usui et al., 1997; Warren et al., 2010), and polymers with similar viscosity enhancement properties can show marked differences in their ability to inhibit precipitation (Vandecruys et al., 2007; Warren et al., 2010; Bevernage et al., 2011). Taking into consideration the markedly higher molecular mobility of polymers in solution (compared with the solid state), it seems likely that polymer-mediated precipitation inhibition in solution is mediated more commonly by direct effects on nucleation or crystal growth rather than changes in viscosity. In this regard, polymers may slow nucleation by interacting with drug in solution and by adsorption to the interface of the emerging particle/crystal nucleus (Okimoto et al., 1997; Suzuki and Sunada, 1998; Yamashita et al., 2003; Gao et al., 2004; Yokoi et al., 2005; Brewster et al., 2008a). Polymer effects on nucleation and crystal growth in the solid state are described in more detail in Section X.F.

In situations where only particle growth is reduced, spontaneous nucleation leads to an initial decrease in dissolved drug concentration (due to nuclei formation) and a subsequent reduction in the rate of crystal growth (Konno et al., 2008). The ability of a polymer to adsorb to a developing drug nuclei also has the potential to reduce crystal growth and to disrupt the manner in which the crystal lattice is formed (Lindfors et al., 2008). This may promote drug precipitation as a high-energy crystalline polymorph or in the amorphous form (rather than as the thermodynamically stable crystal). In both cases, the precipitated material is expected to show more rapid redissolution compared with a precipitate containing the stable crystal. The physical form of drug as it phase-separates from supersaturated solution is therefore an area of growing interest (Friesen et al., 2008; Alonzo et al., 2011; Hsieh et al., 2012; Ozaki et al., 2012). Where the solid takes on the characteristics of a highly ordered molecular structure, phase separation of crystalline solid results. In contrast, where crystal structure is not maintained, drug is expected to precipitate in the amorphous form. Whether assembly of amorphous or crystalline particles occurs by two completely separate paths or whether an amorphous “preparticle” precedes the subsequent generation of a lower-energy drug crystal or higher energy amorphous precipitate is unclear (Gebauer et al., 2008; Warren et al., 2010). Friesen and coworkers, for example, speculate that most of the drug released from HPMCAS solid dispersions is stabilized in solution in the form of amorphous drug-polymer colloidal assemblies that are approximately 20 to 300 nm in diameter (Friesen et al., 2008). The presence of colloidal drug-polymer aggregates permits more sustained periods of supersaturation by decreasing the collision frequency of monomeric, solvated free drug molecules, which in turn decreases the kinetics of nucleation (Friesen et al., 2008; Gebauer and Coelfen, 2011; Murdande et al., 2011b). Recent studies have described the formation of similar nanoparticulate species in dissolution media after rapid drug release from felodipine-HPMC (Alonzo et al., 2011) and ketoconazole-PVP solid dispersions (Kanaujia et al., 2011).

The spontaneous formation of self-assembled molecular species in solution has been shown even for a single solute and has been attributed to conditions of sustained supersaturation (Ohgaki et al., 1992). These observations are consistent with a broadly applicable concept of the formation of prenucleation clusters that have “molecular” character in aqueous solution and create multiple equilibria among the various species present. This in turn decreases the collision frequencies of the solvated, unimolecular species and results in the creation of sustained, supersaturated solutions (Gebauer and Coelfen, 2011).

Although the formation of structured polymer-drug nanoassemblies in water appears to sustain the period of supersaturation, their presence complicates analysis since small aggregates are less likely to separate from free drug in solution using traditional centrifugal or filtration-based separation techniques and may also retain the spectroscopic properties of the parent (Alonzo et al., 2011; Van Eerdenbrugh et al., 2011). Whether drug is completely dissolved in solution or dispersed as nanosized aggregates is therefore difficult to define accurately (Murdande et al., 2011b). Furthermore, whether nanoaggregates reduce the thermodynamic activity of drug compared with a typical solution (and therefore reduce the driving force for absorption) is also unknown. In a situation analogous to the solubilization of drug in a microemulsion or micelle, the aggregate is expected to provide a solubilized reservoir that is in rapid equilibrium with drug in free solution (Friesen et al., 2008). However, there is also a risk that
nanoaggregates may grow and ultimately promote drug crystallization. Judicious selection of the polymer is expected to reduce this possibility.

Interestingly, although classic nucleation theory (CNT) is most commonly used to explain crystallization and provides a backdrop to the use of crystallization inhibitors in pharmaceutical formulations (see Section X.G.2), it is increasingly clear that CNT fails to capture the complexity of nucleation in many cases (Kashchiev and van Rosmalen, 2003) and that alternative mechanisms may be more appropriate. These include models where molecular aggregates are initially formed, followed by the generation of postcritical nuclei and subsequent nucleation of the crystal phase (Gebauer et al., 2008; Meldrum and Sear, 2008) and two-step mechanisms where the initial formation of metastable clusters that are several hundred nanometers in size precedes crystal nucleation within the clusters (Vekilov, 2010). The use of computational physics and molecular dynamics also provides insight into localized interactions between molecules, clusters, and the fluid phase and their relative contributions to the nucleation and crystallization of molecules in complex media (Nishino et al., 2011). Detailed discussion of these concepts is not warranted here; however, the importance of cluster formation in drug crystallization and the potential to disrupt this process via interaction with excipients, including polymers, is apparent, and a deeper understanding of the complexities of crystal nucleation will likely drive further improvements in formulation design.

At present, however, a priori prediction of the ability of an excipient to prevent drug crystallization remains difficult, and structurally related polymers often show significant differences in precipitation inhibition (Ziller and Rupprecht, 1988; Warren et al., 2010). As a result, the identification of polymeric carriers that increase dissolution rate and also stabilize supersaturation remains largely empirical and driven by high-throughput screening methods.

c. Screening for Potential Polymer Precipitation Inhibitors. Several recent papers have used solution-based screening methods to assess the potential for polymeric materials to limit drug precipitation from supersaturated solutions (Vandecruys et al., 2007; Curatolo et al., 2009; Janssens et al., 2010; Warren et al., 2010). The methods used have also been reviewed by Brouwers et al. (2009) and Warren et al. (2010). Although several approaches that assess polymeric precipitation inhibitors have been described, the most common approach is the solvent-shift (or cosolvent/solvent quench) method. In solvent-shift methods, supersaturation is stimulated via the introduction of a small volume of a concentrated solution of drug in a water-miscible solvent [such as DMSO, DMA, or dimethylformamide (DMF)] into a receptor medium containing dissolved polymer. Samples are subsequently removed, filtered, or centrifuged and dissolved drug concentrations determined by UV spectrophotometry (Vandecruys et al., 2007; Curatolo et al., 2009). Continuous monitoring of turbidity via nephelometry can also be used to track precipitation in real time (Warren et al., 2010). The receptor medium used is usually water or a buffered solution, although more complex screening methods may be performed using biorelevant media such as simulated gastric or intestinal fluids. Bevernage and coworkers recently compared the ability of polymeric excipients to prevent precipitation from supersaturated solutions using simple buffers, simulated intestinal fluids (fasted/fed), and aspirated human intestinal fluid. In this case, the capacity of the polymer to reduce precipitation was diminished when studies were run in aspirated intestinal fluid (Bevernage et al., 2011).

Of the carriers that are commonly used in the manufacture of solid dispersions, HPMC has been shown to inhibit precipitation for a number of different compounds and is arguably the least compound-specific precipitation inhibitor (Warren et al., 2010). Nonetheless, precipitation inhibition by HPMC remains variable. For example, in an examination of the ability of HPMC to inhibit drug precipitation from supersaturated solutions of a series of 24 compounds, precipitation inhibition was evident for only 12 compounds, whereas for 12 others, little evidence of stabilization was apparent (Vandecruys et al., 2007). Variability in the ability of polymers to inhibit precipitation has also been shown with polymers, including PVP and HPMCAS, where under some circumstances precipitation inhibition may be highly effective (some evidence for the benefits of HPMCAS are shown in Fig. 53), but under other conditions, effects may be limited (Warren et al., 2010).

Difficulties have also emerged in translating the data obtained from screening methods using polymer solutions to successful precipitation inhibition during the dissolution of a manufactured dosage form. For example, vitamin E TPGS 1000 (d-α-tocopheryl polyethylene glycol succinate) and PVPVA-64 effectively inhibit the precipitation of itraconazole from supersaturated solutions in vitro using solvent-shift methods (Janssens et al., 2008). In contrast, dissolution tests conducted using solid dispersion formulations containing the same components resulted in drug recrystallization from supersaturated solutions after ~1 h. The authors attributed the lack of inhibition of precipitation during the dissolution tests to the presence of seed crystals in the solid dispersion matrix. Contradictory results have also been reported for Eudragit 415F and PVP (Abu-Diak et al., 2011). In the latter case, PVP maintained the stability of supersaturated solutions of celecoxib formed via solvent shift more effectively than Eudragit 415F, whereas the reverse was true on examination of drug dissolution patterns from solid
dispersions containing the same polymers. The improved performance of Eudragit 4155F in the dissolution experiments was ascribed to superior solubilization and wetting properties (Abu-Diak et al., 2011).

The variability observed in the performance of polymeric precipitation inhibitors (PPI) currently precludes a priori selection of appropriate inhibitors without experimental testing and is compounded by a lack of understanding of the mechanisms by which PPIs act (Warren et al., 2010). This is further complicated by the realization that the most effective polymers must enhance dissolution and maintain supersaturation in solution and simultaneously maintain drug stability in the solid state in the formulation. Screening for polymer carriers on the basis of their ability to support supersaturation should therefore be used as only one indicator of an appropriate carrier (Yamashita et al., 2011).

C. Recent Examples of Bioavailability Enhancement Using Solid Dispersions

Solid dispersions containing drug in the amorphous form (i.e., types 2 and 3 and types 5 and 6, Fig. 50) commonly result in faster rates of drug dissolution compared with crystalline drug, drug-carrier physical mixtures, and solid dispersions containing drug in the crystalline form (i.e., types 1 and 4, Fig. 50). Most recent investigations of the utility of solid dispersion formulations have therefore focused on the development of formulations containing drug in the amorphous form (Newman et al., 2012). Law et al. (2004) have described PEG 8000–based solid dispersions containing amorphous ritonavir and provide evidence of significantly faster dissolution rates compared with simple physical mixtures (Fig. 54A) and marked increases in oral bioavailability in beagle dogs compared with crystalline drug (Fig. 54B). Increases in drug load, however, led to phase-separation of drug within the crystalline PEG carrier, giving rise to decreased dissolution rates and in vivo exposure (Law et al., 2004). Amorphous solid dispersions have also been explored as a potential formulation strategy for AMG 517, a poorly soluble vanilloid receptor-1 antagonist (Kennedy et al., 2008). Originally formulated as a drug suspension in 10% Pluronic F108 (OraPlus), AMG 517 showed good oral bioavailability during preclinical evaluation, although it was later revealed that this was due to AMG 517 forming a cocrystal with sorbic acid, the preservative in OraPlus (Bak et al., 2008) (see Section V.B.5). Unfortunately, the OraPlus formulation was unable to achieve sufficient exposure using higher AMG 517 doses intended for human clinical trials (Kennedy et al., 2008). Solid dispersions were therefore prepared by spray-drying solutions of drug and HPMC E5 (a low viscosity grade of HPMC) or HPMCAS, both approaches resulting in isolation of amorphous drug. Although improvements in dissolution were evident for both formulations, the HPMCAS formulation was selected for further testing on the basis of superior performance (28-fold faster intrinsic dissolution compared with crystalline drug and 2-fold higher than unformulated amorphous drug). Administration of the HPMCAS solid dispersion to cynomolgus monkeys resulted in a 1.4-fold increase in $C_{\text{max}}$ and a 1.6-fold increase in exposure compared with the OraPlus suspension (Kennedy et al., 2008).

A large volume of data describing the potential utility of HPMCAS solid dispersions has been described by Friesen and coworkers (Friesen et al., 2008). These studies summarized the use of HPMCAS to generate amorphous solid dispersions for a number of investigational Pfizer compounds. Preclinical exposure
data were obtained for more than 100 compounds using HPMCAS solid dispersions of amorphous drug, and the solid dispersions gave rise to 2- to 40-fold increases in bioavailability compared with crystalline drug. Twenty-one compounds were evaluated in clinical trials using the same technology, and in all cases, bioavailability was improved, with the worst performer showing a 2-fold increase in bioavailability compared with crystalline drug (Friesen et al., 2008). Another excipient experiencing notable interest is Soluplus (BASF Corp.), which is a polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer. Soluplus solid solutions of BCS class II drugs fenofibrate, danazol, and itraconazole have been prepared by hot-melt extrusion, with each system demonstrating superior in vivo performance in beagle dogs compared with the crystalline form of the drug (Linn et al., 2012).

The partial lipophilic character of this polymer explains its capacity to increase drug solubilization in aqueous media (Hardung et al., 2010). This solubilization capacity may minimize the risk of drug precipitation from supersaturated solutions (by decreasing the amount of supersaturated drug).

A powerful example of the potential clinical and commercial utility of amorphous solid dispersions is provided by Kaletra (Abbott Laboratories) (see Table 26). Kaletra is a combination of two HIV protease inhibitors, ritonavir (50 mg) and lopinavir (200 mg), in a polyvinylpyrrolidone/vinylacetate copolymer (PVPVA) solid dispersion prepared by hot-melt extrusion (see Section X.E.1) (Breitenbach, 2002; Gao, 2008). The solid-dispersion formulation was developed in preference to a liquid-filled gelatin capsule formulation on the basis of a diminished food effect in humans and an improved stability profile since the capsule formulation required refrigeration (Klein et al., 2007). Sporanox (Janssen Pharmaceuticals) provides a second commercial example of the utility of amorphous solid dispersions.

Although many studies have examined solid dispersion formulations preclinically in dogs and clinically in humans, it is also possible to evaluate system performance in smaller rodent models (Newman et al., 2012). In this case, administration is complicated by the difficulty of administering encapsulated or tableted dose forms in small animals. Hard gelatin minicapsules have been used (Hasegawa et al., 1985; Yoo et al., 2000; Newa et al., 2007; Van Speybroeck et al., 2010b); however, the low volume of the rodent stomach may limit the rate at which formulation is released from the dosage form. Solid dispersions may be administered as a suspension, but care must be taken to administer the dose as soon as possible after dispersion to minimize the potential for in situ crystallization or precipitation (Nagapudi and Jona, 2008). For small animals that are dosed by gavage, formulations are typically dispersed in the dosing syringe immediately before administration. Complications include increases in viscosity attributable to the dissolution of high-molecular-weight polymeric carriers and segregation of solid dispersion particles resulting in the generation of heterogeneous suspensions (Kawakami, 2009).

The ideal properties of a suspending vehicle are the subject of some debate. An aqueous suspending vehicle containing 1–2% HPMC or 1–5% poloxamer has been recommended (Nagapudi and Jona, 2008), however, others caution against the use of additives with solubilizing properties as this may encourage rapid dissolution of the drug and carrier, increasing the chance of drug recrystallization (Zheng et al., 2011). In an attempt to evaluate the impact of predispersion on utility, a solid dispersion of the experimental compound LCQ789 was recently administered to rats as an aqueous suspension in water, and the data were compared with administration of the solid dispersion as a powder-filled capsule in dogs (Zheng et al., 2011). In both cases, similar rank-order performance was evident. In this example, the solid dispersion led to superior exposure compared with a simple suspension or cocrystal formulation but lower exposure than that obtained from a microemulsion formulation. The solid dispersion, however, showed lower variability in dose-escalation trials and was therefore considered the most favorable approach for ongoing studies (Zheng et al., 2011).

D. Role of the Carrier and the Drug-Carrier Ratio in Dictating Drug Release Kinetics from Solid Dispersions

A molecular dispersion of drug within a solid dispersion is expected to provide the maximum surface area available for hydration and, therefore, faster dissolution rates compared with formulations where drug is present as a fine suspension of amorphous particles. Indeed, the dissolution rate of molecularly dispersed drug may be almost instantaneous. However, where drug dissolution is rapid, the dissolution rate of the carrier has the potential to limit drug release (Dubois and Ford, 1985; Craig, 2002; DiNunzio et al., 2010b). Under conditions of “carrier-controlled release,” drug dissolution is dependent on carrier hydrophilicity and viscosity.

Carrier-limited drug release from solid dispersions is typified by a positive correlation between the rate of release of drug and the rate of release or dissolution of the polymeric carrier (Corrigan, 1986; Craig, 1990; Esnaashari et al., 2005). This occurs more frequently when drug loading is low since this favors the formation of molecular dispersions (rather than amorphous suspensions). However, drug dissolution from solid dispersions containing suspended amorphous drug particles can also be controlled by the rate of polymer dissolution if dissolution of the amorphous particles is sufficiently rapid (Bansal et al., 2007; Khan et al., 2011).
The critical processes that occur during carrier-controlled drug release have been described in detail by Craig (2002). In situations where polymer dissolution is fast, drug is in contact with the polymer only transiently and is liberated into the bulk medium as a fine molecular dispersion. Increasing carrier viscosity leads to slower drug release, and an inverse relationship between drug release rate and carrier viscosity has been described for solid dispersions based on PEG (Dubois and Ford, 1985; Craig and Newton, 1992; Shah et al., 1995), PVP (Bansal et al., 2007), HPMC (Karavas et al., 2001, 2007; Tajarobi et al., 2011) and Eudragit (Miller et al., 2008; DiNunzio et al., 2010b; Abu-Diak et al., 2011) High-molecular-weight PVP and HPMC provide additional barriers to dissolution via the formation of viscous “gels” that restrict the rate of water ingress and egress from the matrix (Leuner and Dressman, 2000; Karavas et al., 2001, 2007). This is to some extent analogous to carrier-dependent release of drug from hydrophilic matrix tablets, albeit in the latter case, the rate of drug release is intended to occur much more slowly.

In addition to increases in dissolution, polymeric carriers require properties that enable the stabilization of the high-energy amorphous form. The ideal polymer properties for amorphous stabilization (high-molecular-weight, high-viscosity, nonhygroscopic) are largely contradictory to those that promote rapid release. Polymer selection is therefore a tradeoff between properties, including release rate and amorphous drug stabilization (Janssens and Van den Mooter, 2009).

Drug-release profiles also change with increasing drug-carrier ratio, and several studies have shown a reduction in drug dissolution rate from solid dispersion with increasing drug-carrier ratios (Corrigan, 1986; Veiga et al., 1993; Kapsi and Ayres, 2001; Verreek et al., 2003b; Law et al., 2004; Karavas et al., 2005; Janssens et al., 2008; Konno et al., 2008; Lakshman et al., 2008; Abu-Diak et al., 2011; Alonzo et al., 2011; Dontireddy and Crean, 2011; Ghosh et al., 2011; Han et al., 2011; Khan et al., 2011) with one example reproduced in Fig. 55 (Simonelli et al., 1976). The decrease in dissolution is usually attributed to a reduction in the quantity of drug in the molecularly dispersed form since increasing drug load reduces the intermolecular distance between drug molecules and the quantity of stabilizing polymer, both of which favor phase separation of amorphous/crystalline particulate drug in the carrier (Vasanthavada et al., 2005; Qian et al., 2010a).

At high drug-carrier ratios, the reduction in the relative quantity of polymer present may also lead to a decrease in drug wetting and the potential for incongruent release of drug and carrier, with the latter resulting in the formation of a carrier-depleted, drug-rich solid phase that is at increasing risk of phase separation or crystallization (Higuchi et al., 1965a; Craig, 2002). This phenomenon of “drug-controlled dissolution” has been used to explain the reduced release of sulfathiazole from PVP solid dispersions (Simonelli et al., 1976) and, more recently, the release of felodipine and indomethacin from PVP/HPMC solid dispersions (Alonzo et al., 2011). Under these conditions, the rate of drug release is dependent on the physical form of the drug in the drug-rich layer. Where drug is present as an amorphous suspension, the drug concentrations that are likely to be achieved during dissolution are expected to be supersaturated relative to the solubility of the crystal form but unlikely (thermodynamically) to exceed the saturated solubility of amorphous drug. In contrast, where drug is molecularly dispersed, rapid initial dissolution may lead to supersaturation relative to both crystal and amorphous drug solubilities. Consistent with this suggestion, felodipine-HPMC solid dispersions assembled at low drug-carrier ratios of 10:90 (that favor drug-polymer miscibility) show very fast initial rates of dissolution, and dissolved drug concentrations exceed the calculated solubility of amorphous drug (Alonzo et al., 2011). Similar observations have been made during dissolution testing of felodipine-PVP and indomethacin-PVP solid dispersions at the same 10:90 drug-carrier ratio. However, dynamic light scattering experiments have subsequently revealed that the concentration of felodipine “in solution” after release from the HPMC solid dispersions also include drug in the form of submicron-sized particles, which absorb in the same region of the UV spectrum as dissolved drug. The true concentration of drug in solution is therefore lower and more consistent with the amorphous drug solubility (Alonzo et al., 2011). Nonetheless, solid dispersions comprising drug molecularly dispersed throughout the carrier are likely to show maximal rates of drug release...
E. Common Methods to Manufacture Solid Dispersions

1. Melting/Fusion. One of the simplest methods of solid dispersion formation is via heating mixtures of drug and carrier above the melting or glass transition temperature, followed by solidification by rapid cooling. Adequate mixing to form a homogeneous molecular solution is critical to the physical stability and performance of solid dispersions formed via fusion techniques since this avoids formation of the drug-rich or polymer-rich microenvironments that promote phase separation and reduce dissolution rate. Rapid cooling is also essential as conditions where the cooling rate exceeds the rate of crystallization are required to facilitate isolation of drug in the more soluble amorphous form (see Section X.F.1). Where phase separation does occur, rapid cooling reduces the time available for particle growth and reduces the particle size of the (amorphous or crystalline) suspended material.

The first reported solid dispersions were formed by fusing sulfathiazole and urea above the eutectic temperature, prior to rapid cooling in an ice bath (Sekiguchi and Obi, 1961). Faster rates of cooling were later achieved by pouring the melt onto stainless steel plates to accelerate heat loss (Sekiguchi et al., 1964; Chiou and Riegelman, 1970). Ice bath and liquid nitrogen quenching remain widely used cooling techniques (Dordunoo et al., 1991; Saers et al., 1993; Hirasawa et al., 1999; Law et al., 2001). Melts may also be spray-cooled (spray-congealing). In this process, melts are sprayed into a cooled chamber that is continuously perfused with chilled air, allowing the formation of spherical drug/carrier particles that can be directly filled into a capsule or compressed into a solid tablet (Sancin et al., 1999; Fini et al., 2002). Drug release rates from solid dispersions typically increase with increasing cooling rate since this increases the likelihood of drug isolation in the noncrystalline form and minimizes the size of any suspended particles (McGinity et al., 1984; Save and Venkitachalam, 1992). In contrast, reports of slower dissolution rates with an increased cooling rate are also apparent (Saers et al., 1993; Doshi et al., 1997), illustrating the unpredictable nature of crystallization (Craig, 1990; Saers et al., 1993).

Limitations to hot-melt methods for preparing solid dispersions include the possibility of poor drug-carrier miscibility at elevated temperatures (Forster et al., 2001a) and the potential for thermal degradation of drug where it is necessary to hold the drug-carrier mixture at elevated temperatures for prolonged periods (Goldberg et al., 1965).

More recently, hot-melt extrusion (HME) has grown in popularity as it appears to address many of the limitations of simple fusion methods (Breitenbach, 2002; Crowley et al., 2007; Repka et al., 2007). In HME, a mixture of drug and carrier(s) is introduced via a hopper into an extruder that contains a rotating screw or auger inside a heated barrel. The extruder is rapidly heated to lower the viscosity of the drug-carrier blend. The rotating screw, in combination with heating, results in effective deaggregation of drug particles and incorporation into the molten carrier. The mixture is subsequently forced through a die to produce an extrudate of uniform shape (Crowley et al., 2007). The residence time of the blend in the extruder is short (~2–5 min), limiting thermal instability (Breitenbach, 2002). HME can be used in batch mode or adapted for continuous production (Breitenbach, 2002).

Proprietary HME methods include the Meltrex technique developed by SOLIQS (Abbott GmbH & Co. KG), which is used in the production of Kaletra and Isoptin-SRE tablets (both developed by Abbott Laboratories), and the KinetiSol dispersing (KSD) technology, which was recently developed at the University of Texas and now owned by DisperSol Technologies LLC (Austin, TX). KSD uses a series of rapidly rotating blades that generate a level of kinetic and thermal energy that is sufficient to promote rapid drug-carrier fusion without the need for an external heat source (DiNunzio et al., 2010a, b). The shorter time required to achieve an intimate mixture of drug with carrier compared with conventional HME may be of particular significance for drugs that are thermally labile. Indeed, HPMC-itraconazole solid dispersions have been prepared using KSD in <15 s, whereas conventional HME requires >5 min (DiNunzio et al., 2010b). In the same study, the KSD solid dispersions were also shown to be more stable than those formed by conventional HMEs, and the increase in physical stability was attributed to more homogeneous mixing and, therefore, a lower risk of phase separation. The ability to heat rapidly and melt drug-carrier blends using KSD has the additional advantage of reducing the need for plasticizers. Plasticizers are commonly added to facilitate melting during HME, but they may be detrimental to the long-term stability of solid dispersions because of their propensity to increase molecular mobility (Lakshman et al., 2008; DiNunzio et al., 2010a). Finally, HME and KSD also allow the formation of solid dispersion for high melting polymers such as HPMC and HPMCAS (Verreck et al., 2003b; Six et al., 2005; DiNunzio et al., 2010b; Hughley et al., 2010), which are most commonly manufactured using spray-drying or other solvent-removal methods owing to the high temperatures required to melt the polymer (Tg > 120°C) (Gilis et al., 1995; Jung et al., 1999; Han et al., 2011; Won et al., 2005).
surrounding traditional melting methods dictated the use of solvent evaporation techniques in the manufacture of solid dispersions, particularly when using high Tg carriers (Leuner and Dressman, 2000). The temperatures required to evaporate commonly used organic solvents are usually in the order of ~25–65°C (Masters, 1991) and lower than those required during melt/fusion methods. Solvent evaporation was therefore preferred for thermally labile drugs or for carriers with high Tg values, for example HPMC and PVP (Padden et al., 2011).

During the manufacture of solid dispersions via solvent evaporation, drug and carrier are dissolved in a common solvent before mixing and solvent evaporation under elevated temperatures or reduced pressure. This is typically achieved using spray-drying (Cal and Sollohub, 2010; Sollohub and Cal, 2010). Solvents include chloroform, dichloromethane, methanol, ethanol, acetone, and methylene chloride. Solvents may be used in combination and may also include small amounts of water to enhance drug or polymer solubility in the solvent mixture (Sinha et al., 2009; Padden et al., 2011).

The solvent must be completely removed after formation of the solid dispersion since residual solvents carry a toxicity liability and also plasticize the drug-polymer mixture. Plasticization enhances mobility and increases the likelihood of phase separation and crystallization. Solvent removal is usually achieved under vacuum at elevated temperature and may also require desiccation. For example, methanol removal from carbamazepine-PVP K30 solid dispersions has been reported to occur under vacuum in a rotary evaporator at 40°C over a 24-h period (Sethia and Squillante, 2004), ethanol from indomethacin-HPMC/ethylcellulose mixtures under vacuum at 35°C by rotary evaporation and then further drying for 12 h in a desiccator (Ohara et al., 2005), and chloroform from ofloxacin-PEG 4000/20000 dispersions under reduced pressure using a vacuum dryer at 40°C before further drying in a vacuum oven (at room temperature) for 24–48 h (Okonogi et al., 1997). More efficient drying is possible at elevated temperatures (Chiu and Riegelman., 1970), but increases in temperature also increase molecular mobility and, therefore, the likelihood of amorphous to crystalline transitions. Some solvents are easier to remove than others (Bitz and Doelker, 1996), however, solvent choice is largely driven by toxicity burden and the capacity to dissolve both drug and polymer rather than by the vaporization pressure.

More effective approaches to the removal of solvent include freeze-drying (Otsuka et al., 1993; Van Drooge et al., 2004), spray-drying (Jung et al., 1999; Van den Mooter et al., 2001; Weuts et al., 2005), and fluidized bed granulation (Ho et al., 1996). The Sporanox solid dispersion (itraconazole-HPMC; Janssen Pharmaceuticals), for example, is prepared by spray-drying from a dichloromethane-ethanol solution (Janssens and Van den Mooter, 2009). Etravirine-HPMC solid dispersions (Intelicence; Tibotec) are also prepared by spray-drying (Scholler-Gyure et al., 2009). More efficient solvent removal enhances product performance since it limits the time available for drug and polymer to migrate and phase separate. Thermal analysis of itraconazole-Eudragit E100 solid dispersions has shown that increasing the speed of solvent removal by spray-drying results in improved drug-polymer miscibility compared with equivalent cast-film solid dispersions that rely on solvent evaporation (Janssens et al., 2010). In this example, the use of spray-drying allowed higher drug loading without increasing the risk of phase separation.

In summary, solvent evaporation methods allow the use of lower temperatures than traditional melt/fusion techniques but are complicated by the identification of solvent systems with appropriate solvent capacity for both carrier (e.g., polymer) and drug. Where drug/carrier solubility is low and large batches are required, solvent volumes are high, resulting in significant environmental, cost, and procurement issues (Serajuddin, 1999). Solvent removal is also critical, and since the rate of solvent removal influences molecular miscibility and structure within the carrier, solid dispersion performance can vary significantly with small changes in manufacturing conditions.

3. Solvent Evaporation Using Supercritical Fluids. Supercritical fluids (SCFs) can be used to enhance the efficiency of solvent evaporation during the manufacture of solid dispersions. In a manner analogous to the advantages of spray-drying and freeze-drying, SCF methods facilitate rapid removal of solvent (Pasquali et al., 2006). SCF techniques also use lower volumes of organic solvents, minimize disposal costs, and reduce the potential for solvent carryover into the final product. SCFs that are commonly used in pharmaceutical processing and the different solvent and antisolvent methods that may be used with SCFs to isolate pharmaceutical products are discussed with reference to nanoparticle preparations in Section IX. The principles of the use of SCFs to promote solid dispersion manufacture are similar to those used during nanoparticle or microparticle isolation, the major difference being the addition of larger quantities of polymer, such that the polymer acts as a matrix rather than a stabilizer. In both cases, SCFs are now most commonly used as an antisolvent (SAS techniques) rather than the primary solvent because of the low solubility of many drugs in SCFs (Jun et al., 2005; Won et al., 2005; Majerik et al., 2007; Li et al., 2011b).

Supercritical solvent methods have been used to form solid dispersions for drugs, including carbamazepine (Moneghini et al., 2001), felodipine (Won et al., 2005), cefuroxime axetil (Jun et al., 2005), phenytoin (Muhrer et al., 2006), piroxicam (Wu et al., 2009), and ketoprofen (Manna et al., 2007). SCF-enabled solid
dispersions may provide advantages in both dissolution rate and processability. For example, carbamazepine-PEG 8000 solid dispersions prepared by gas antisolvent (GAS) precipitation result in improved flow properties and lower cohesion compared with those formed by conventional solvent evaporation (Sethia and Squillante, 2002). Piroxicam-PVP solid dispersions prepared by precipitation with compressed antisolvent (PCA) methods also result in small increases in dissolution rate compared with the equivalent spray-dried solid dispersion. In the latter example, piroxicam was present as an amorphous dispersion in both PCA and spray-dried solid dispersions, and the faster dissolution rate from the PCA product was ascribed to the presence of smaller suspended particles in the PCA-derived matrix (Wu et al., 2009). In contrast, solid dispersions prepared by antisolvent SCF techniques have in some cases been shown to exhibit slower dissolution rates compared with the equivalent spray- or freeze-dried product as a result of increased drug crystallinity in the manufactured product (Corrigan and Crean, 2002; Majerik et al., 2007; Badens et al., 2009).

4. Electrospinning and Microwave Irradiation. Electrospinning, or electrostatic spinning, can be used to generate very long nanofibers of uniform diameter and has the potential to assist in the manufacture of any polymer-based pharmaceutical product (Ignatious et al., 2010). In the manufacture of solid dispersions by electrospinning, a charged drug-polymer melt or solution is passed through a nozzle and subjected to a high voltage (5–30 kV direct current). At a critical point, the electrostatic repulsive forces that result from the applied charge overcome the surface tension in the fluid, and a fine stream of fluid (typically with a diameter that is orders of magnitude smaller than the nozzle diameter) is ejected from the fluid surface. The nanofibers that are produced have very small diameters (~100 nm) and very high surface area-to-volume ratios (Ignatious and Baldoni, 2001; Ignatious and Sun, 2004), resulting in rapid solidification via cooling or solvent evaporation. The efficiency of solvent evaporation also minimizes the potential for solvent residue. The dimensions of electrospun solid dispersions can be controlled via the magnitude of the applied voltage to provide customized rates of drug release (Fridrikh et al., 2003; Verreck et al., 2003a).

Finally, microwave irradiation has recently been used to promote solid dispersion formation. Microwaves rapidly achieve uniform heating in polymeric materials, and short periods of (<5 min) microwave irradiation have been used to manufacture solid dispersions of tibolone-PEG (Papadimitriou et al., 2008), nimesulide-Gelucre 50/13 and nimesulide-Poloxamer 188 (Moneghini et al., 2009), atorvastatin calcium-PEG 6000 (Maurya et al., 2010), and itraconazole-TPGS (Moneghini et al., 2010).

### F. Physical Stability of Amorphous Solid Dispersions

Physical stability is a critical issue during product development for solid dispersion formulations. For amorphous solid dispersions in particular, complications arise from the potential for amorphous drug to revert back to the more stable (but less soluble) crystalline form. Where drug is molecularly dispersed in the carrier [i.e., type 3 (crystalline carrier) or type 6 (amorphous carrier) systems; Table 26] transformation to an amorphous suspension (i.e., type 2/5) or crystalline suspension (i.e., type 1/4) is likely and must be controlled to provide appropriate product shelf life.

As the amorphous to crystalline drug conversion is usually associated with a decrease in drug dissolution and a decrease in oral bioavailability, efforts to better understand and minimize physical transformations are increasing. It is now widely recognized that an understanding of the thermodynamic drivers (i.e., the free energy difference between the amorphous and the more ordered crystalline phase) and the kinetic drivers (i.e., the level of mobility or freedom to move within the solid state) of crystallization is central to the design of solid dispersions that show adequate physical stability over pharmaceutically relevant periods. A complete understanding of the properties of pharmaceutical glasses, however, remains elusive. Indeed, in 2005, on the 125th anniversary of the journal *Science*, a section was devoted to 125 questions that point to gaps in our basic knowledge, and among these was the question, What is the nature of the glassy state? (Anonymous, 2005). The nature and properties of the glass state continue to be a topic of extensive research in physics and physical chemistry (Prevosto et al., 2008; Wondraczek and Mauro, 2009; Ngai et al., 2010; Angell, 2011; Berthier and Biroli, 2011; Johari, 2011a, b; Capaccioli et al., 2012), and it remains a challenge for pharmaceutical scientists to keep abreast of the advances made.

Detailed descriptions of transitions in the glass state and the mechanisms by which amorphous materials revert to the crystalline state are beyond the scope of this review, and the interested reader is directed toward Byrn et al. (1999), Hancock and Zografi (1997), Bhattacharya and Suryanarayanan (2009), and Johari and Shanker (2010) for detailed information on molecular mobility, relaxation, and crystallization. Nonetheless, a relatively brief discussion of the formation of pharmaceutical glasses and the mechanisms of (in)stability of amorphous material is required and is provided in the following as critical background to the design of (physically) stable pharmaceutical preparations.

1. Structural Changes at the Glass Transition Temperature. Amorphous solids are produced from the crystalline form using high-energy processing techniques such as melt extrusion, melt quenching, milling, grinding,
or compaction or by direct isolation from a supersaturated solution via precipitation, freeze-drying, or spray-drying (Hancock and Zografi, 1997). All amorphous solids lack the long-range order evident in crystalline solids, and in pharmaceutical applications, solid dispersions containing amorphous drug typically comprise drug in the “glass” state. Amorphous glasses are amorphous solids that convert to the liquid state on heating through a glass-liquid transition temperature and are formed during rapid cooling of the molten state. This is summarized in Fig. 56, which describes changes in molecular volume with changes in temperature. An understanding of the events that occur during the liquid-glass transition (on cooling) also provides a background to the issues surrounding the physical (in) stability of solid dispersions containing amorphous drug and the ability of excipients such as polymers to enhance physical stability.

The solid blue line in Fig. 56 traces the marked reduction in system volume that occurs as a melt is cooled below the melting temperature ($T_m$), leading to crystallization of the thermodynamically stable solid. In contrast, the solid green line traces the change in system volume under rapid cooling. Under these conditions, the rapid rate of cooling precludes molecular relaxation and crystallization below the melting temperature, and instead, the material enters a supercooled liquid phase. Further cooling of the metastable supercooled liquid leads to a critical point, the glass transition temperature ($T_g$), where, if the rate of cooling is faster than the rate of molecular relaxation, the material enters a glassy, amorphous state. The thermodynamic basis for the glass transition was proposed by Kauzmann (1948). In brief, Kauzmann (1948) suggests that a system that enters a supercooled liquid phase must at some point either enter into the glassy state or crystallize since these phase changes are necessary to avoid an “entropy crisis,” in which the extrapolated entropy of the supercooled liquid (i.e., the dashed green line) falls below that of the crystalline solid. This thermodynamic crisis (commonly referred to as Kauzmann’s paradox) is avoided through glass formation. The Kauzmann temperature ($T_K$) may be interpreted as the lowest possible $T_g$ for glass formation.

In the glassy state, entropy, enthalpy, and volume are higher than in the crystalline state, and amorphous solids are therefore thermodynamically unstable and liable to relax and recrystallize over extended periods. Over shorter times, molecular relaxation (and therefore crystallization) is inhibited by the dramatic reduction in molecular mobility below the $T_g$, resulting from the increase in the viscosity of the glass (Davies and Jones, 1953; Angell, 1995a). The increase in viscosity in turn limits molecular diffusion and reorientation into the position required to form a crystal lattice.

2. Glass-Forming Potential. All materials are theoretically capable of forming a glass in the manner described in the preceding section (Turnbull, 1969). In practice, however, many relax rapidly on cooling and crystallize rather than forming kinetically stable glasses (Turnbull, 1969; Yu, 2001). Drugs showing these characteristics are said to have low glass-forming potential and form glasses only under rapid rates of cooling (Kaushal and Bansal, 2008; Baird et al., 2010). The minimum rate of cooling at which a material vitrifies to form a glass is the critical cooling rate ($R_{crit}$), and this value may be used to compare differences in glass-forming ability across a drug series (Baird et al., 2010). Compounds with high $R_{crit}$ values are poor glass-formers, whereas low $R_{crit}$ values imply relatively facile glass formation.

An alternative descriptor for glass-forming potential and crystallization tendency is the fragility of a material (Angell, 1995a,b). Fragility provides an indication of the rate of structural relaxation and is evidenced by the viscosity changes on cooling toward $T_g$. Fragile materials show significant deviations from Arrhenius behavior on cooling, leading to rapid rates of relaxation and a collapse of liquid structure with decrease in temperature (Angell, 1995a). This propensity for rapid structural relaxation limits stabilization of an amorphous glass. In contrast, the viscosity of “strong” liquids declines in closer accordance with Arrhenius relaxation behavior and promotes glass formation (discussed in more detail in Section X.G.3).

The rapid structural relaxation of weak glassformers on cooling is also manifest in high heat-capacity changes ($\Delta C_p$) on passing through the $T_g$ (Angell and Tucker, 1980; Kerc and Srćic, 1995; Hancock and Zografi, 1997; Shamblin et al., 1999). The significant molecular reorganization that occurs in

![Fig. 56](image-url)
weak glass-formers as temperature changes dictates large heat capacity changes (i.e., high $\Delta C_p$) to dissipate energy. In contrast, limited molecular reorganization on cooling is evident in strong glass-formers, despite changing temperature, and as a result, systems exhibit much smaller $\Delta C_p$ values. $\Delta C_p$ therefore provides an additional indicator of glass-forming potential, with high $\Delta C_p$ values suggesting weak glass-forming potential.

3. Polymer Effects on the Glass Transition Temperature. The high molecular viscosity of macromolecular polymers, coupled with a marked reduction in system viscosity and molecular mobility on cooling, results in ready stabilization of the glass phase. The high viscosity of many polymers also results in high $T_g$ values, in excess of that of most drugs (Matsumoto and Zografi, 1999; Lakshman et al., 2008; Padden et al., 2011). For example, the $T_g$ of PVP K30 is 168°C, and the $T_g$ of HPMC is $\sim$170°C. The high $T_g$ of these polymers dictates that homogeneous dispersions of drug and polymer have $T_g$ values that are elevated relative to the $T_g$ of drug alone (assuming $T_g^{\text{drug}} < T_g^{\text{polymer}}$) and, therefore, that polymeric formulations have the potential to stabilize molecular transitions in the amorphous state. The ability of a high $T_g$ polymer to raise the apparent $T_g$ of a drug in a solid dispersion is shown graphically in Fig. 57, where gradual increases in PVP content in indomethacin-PVP binary mixtures leads to a progressive increase in the $T_g$ of the system (Yoshioka et al., 1995). For drugs with low glass-forming potential, fusion with a polymer provides a means of kinetic stabilization (and, therefore, isolation) of the noncrystalline amorphous glass that might not be possible in the absence of polymer because of rapid molecular relaxation and crystallization.

Even where it is possible to isolate amorphous drug in the absence of excipient (i.e., for drugs with higher glass-forming potential), polymer incorporation usually increases the physical stability of amorphous drug by increasing the $T_g$ of the system and reducing molecular mobility (Ford and Rubinstein, 1979; Yoshioka et al., 1995; Matsumoto and Zografi, 1999; Van den Mooter et al., 2001; Six et al., 2004; Karavas et al., 2005; Konno and Taylor, 2008). The use of high-molecular-weight polymers as vehicles for solid dispersion formulation, therefore, provides an opportunity to both increase the likelihood of glass formation and promote the stability of the amorphous system formed through increased $T_g$ (the capacity for polymers to stabilize amorphous drug via other mechanisms is further discussed in Section X.G).

Zografi and coworkers proposed that mobility within an amorphous system was reduced sufficiently to allow acceptable physical stability assuming storage at temperatures 50°C below the $T_g$ (Hancock et al., 1995; Hancock and Zografi, 1997). The $T_g\sim 50°C$ suggestion stemmed from a series of papers that examined the recrystallization of indomethacin alone and after the formation of a number of PVP solid dispersions. In the absence of polymer, indomethacin recrystallization occurred in less than 6 weeks at storage temperatures 20°C below the $T_g$. When the drug was molecularly dispersed in PVP solid dispersions, the difference between the $T_g$ of indomethacin in the formulations and the storage temperature increased to 40–50°C. Under these conditions, recrystallization of indomethacin was suppressed (Yoshioka et al., 1994, 1995).

Knowledge of the $T_g$ of a drug-polymer solid dispersion therefore provides insight into the likely physical stability of an amorphous solid dispersion, and solid dispersions of high $T_g$ are expected to show greater physical stability. $T_g$ may be determined experimentally using DSC or other calorimetric methods (Royall et al., 1998; Weuts et al., 2003; Baird and Taylor, 2012). Alternatively, the Gordon-Taylor equation (eq. 52) can be used to predict the $T_g$ of an ideally mixed solid solution from the sum of the weight fraction (W) and $T_g$ values of the individual components of the mixture (Gordon and Taylor, 1952):

$$T_g^{\text{mix}} = \frac{(w_1T_g^1 + Kw_2T_g^2)}{(w_1 + Kw_2)} \quad (52)$$

The parameter $K$ in the Gordon-Taylor equation is a constant and may be approximated from the densities ($\rho$) of the components in the amorphous state according to the Simha-Boyer rule (Simha and Boyer, 1962):

$$K = \frac{\rho_1}{\rho_2} \frac{T_g^1}{T_g^2} \quad (53)$$

In eq. 52 and eq. 53, subscripts 1 and 2 are used to represent individual components. Analogous equations may be derived for more complex mixtures (i.e., those containing more than two components) (Van den Mooter et al., 2001; Six et al., 2004; Karavas et al., 2005; Konno and Taylor, 2008).
Mootet el. al., 2001); alternatively, an approximation of total properties can be obtained by using the components with the highest and lowest $T_g$ values.

A comparison between measured and predicted $T_g$ (using the Gordon-Taylor equation) is shown in Fig. 57, and good agreement has also been reported elsewhere (Forster et al., 2001a; Konno and Taylor, 2006; van Drooge et al., 2006; Janssens and Van den Mooter, 2009; Weuts et al., 2011). Deviations between experimental $T_g$ values and those predicted from the Gordon-Taylor equation usually indicate nonideal mixing in the solid dispersion (Six et al., 2003a) resulting from poor drug-polymer miscibility or plasticization by residual water or other solvents (van Drooge et al., 2006; Patterson et al., 2008). Poor mixing also results in an increased propensity for phase separation on storage (see X.G.5). In contrast, predicted $T_g$ values that are consistent with experimental values are usually indicative of good mixing, although this is not always the case (Marsac et al., 2009; Baird and Taylor, 2012). Positive deviations from Gordon-Taylor behavior (where experimental $T_g$ values are greater than estimated values) usually indicate the presence of molecular interactions between drug and polymer (Shen and Torkelson, 1992; Khogaz and Clas, 2000).

4. Moisture Effects on Glass Transition Temperature. Although solid dispersions with increased $T_g$ provide for enhanced stability of incorporated amorphous drug, under some circumstances, solid dispersions with high $T_g$ values might still show evidence of crystallization. A common explanation for this phenomenon is the plasticizing effect of moisture adsorption during storage (Zhou et al., 2002; Miyazaki et al., 2004; Rumondor and Taylor, 2010). Water has a low $T_g$ (−137°C) and is a very effective plasticizer (Roos, 1997). As a rule of thumb, 1% adsorbed water increases molecular mobility by two orders of magnitude and reduces $T_g$ by $\sim 10^\circ$ C (Andronis and Zografi, 1998). The presence of water, therefore, facilitates drug relaxation to the more stable crystalline state. Vasanthavada et al. (2005) described a series of griseofulvin-PVP solid dispersions with very high $T_g$ values (>125°C) but that showed evidence of phase separation and drug recrystallization after storage at high relative humidity (40°C/69% relative humidity). Moisture-induced instability has also been reported for PVP solid dispersions containing felodipine and loperamide (Weuts et al., 2005; Konno and Taylor, 2006, 2008; Marsac et al., 2008). In this case, sensitivity to moisture was ascribed to the hygroscopic nature of PVP as similar solid dispersions containing the less hygroscopic polymers HPMC and HPMCAS showed reduced instability on exposure to moisture (Konno and Taylor, 2008). Tablet excipients, including fillers, may also be hygroscopic, and incorporation of such excipients into solid dispersion formulations should be approached with caution (Bruce et al., 2010). Absorbed water has the added complexity of the potential to hydrogen bond with hydrophilic polymers, thereby disrupting the drug-polymer interactions that may be critical to the physical stability of a solid dispersion (Konno and Taylor, 2008; Rumondor et al., 2009b; Rumondor and Taylor, 2010). Water absorption on storage can be minimized by appropriate storage conditions, packaging, or polymer choice where less hydrophilic, less hygroscopic polymers typically reduce water uptake.

G. Factors Affecting Drug Crystallization from Solid Dispersions

The use of high $T_g$ polymers to generate high $T_g$ solid dispersions is a common and often successful mechanism of stability enhancement. The $T_g$ of a solid dispersion, however, is not always predictive of physical stability. Thus, polymer addition may decrease drug crystallization within a solid dispersion but has little or no effect on $T_g$ (Yoshioka et al., 1995; Miyazaki et al., 2007; Konno et al., 2008) or, in some cases, may reduce crystallization and simultaneously decrease $T_g$ (Khogaz and Clas, 2000; Law et al., 2001; Janssens et al., 2010). A broader examination of the physical instability of amorphous drug in solid dispersions, beyond that dictated by $T_g$, is therefore warranted.

Most studies that have evaluated the stability of solid dispersions have focused on the properties of the formulation as a driver of drug stability. These include examinations of the effects of intermolecular interactions between drug and excipient, excipient-mediated inhibition of crystal nucleation, changes to molecular mobility, and differences in mixing and miscibility. These are addressed later herein. In addition, the intrinsic properties of the drug (rather than the formulation) also play a role in determining the potential for drug crystallization. Several authors have suggested that the propensity for drug crystallization from the unformulated amorphous form may provide a useful measure of the potential for crystallization from a formulated solid dispersion (Marsac et al., 2006a; Friesen et al., 2008; Janssens and Van den Mooter, 2009; Weuts et al., 2011). This is usually assessed via differences in the ease of glass formation (i.e., glass forming ability) and differences in the stability of the pure drug glass to crystallization (Aso et al., 2000; Six et al., 2001; Marsac et al., 2006a; Kaushal and Bansal, 2008; Baird et al., 2010; Van Eerdenbrugh et al., 2010a; Weuts et al., 2011).

As described in Section X.F.2, drugs with low glass-forming potential relax rapidly on cooling, and this is manifest in high $\Delta C_p$ at $T_g$. The predisposition of fragile liquids to structural relaxation facilitates crystallization, decreases the likelihood of glass formation, and reduces glass stability (Angell, 1995b; Mao et al., 2006b; Kaushal and Bansal, 2008; Baird et al., 2010). Where glass formation is encouraged by the addition of a high $T_g$ polymer, the intrinsic molecular
properties of drugs with low glass-forming potential may therefore reduce the physical stability of the amorphous material in formulated solid dispersions, in some instances despite favorable changes to other common indicators of stability, such as $T_g$ (Weuts et al., 2005; Miyazaki et al., 2007; Friesen et al., 2008).

The tendency for drugs to crystallize out of high-energy amorphous formulations is predicated by the excess volume, enthalpy, entropy, and free energy carried by amorphous glasses compared with the stable crystal state. To initiate crystallization, however, an activation energy barrier must be overcome that reflects the kinetic limitations to crystallization. This is the energy required for molecules to diffuse and appropriately align such that nucleation and the formation of a new solid phase are possible (James, 1985). The stability of amorphous drug is a function of both the inherent thermodynamic liability of the amorphous form and the kinetic barriers to crystallization provided by the viscosity of the glass and supplemented by the addition of excipients, such as polymers (Van den Mooter et al., 1998; Konno and Taylor, 2006; Bhugra and Pikal, 2008; Marsac et al., 2008).

Differences in the thermodynamic properties of drug in the amorphous versus the crystalline form are typically described in terms of configurational or conformational properties (i.e., differences in enthalpy, entropy, and free energy resulting from differences in structure) and are commonly calculated from the configurational heat capacity (i.e., the difference in $C_p$ between the amorphous and crystalline states).

A detailed description of the use of configurational thermodynamics to describe differences in amorphous drug stability is beyond the scope of the current discussion; however, two recent studies exemplify the potential role of thermodynamic liabilities and kinetic barriers in dictating appropriate stability for amorphous drug. Marsac et al. (2006a) examined the thermodynamic and kinetic drivers of drug stability to crystallization from felodipine and nifedipine amorphous glasses. Nifedipine crystallized more readily than felodipine, and this was attributed to a larger free-energy difference between the amorphous and the crystalline forms, resulting in a greater thermodynamic driver for crystallization. Differences in free energy were largely derived from differences in enthalpy. In contrast, no differences were evident in many determinants of the kinetic barriers to crystallization, including $T_g$ and mobility (although the activation energy for nifedipine crystallization was lower than that of felodipine). Zhou et al. (2002) similarly evaluated the crystallization properties of ritonavir, fenofibrate, acetaminophen, and ABT-229 (a motilin receptor agonist structurally related to erythromycin) and also showed little correlation between crystallization tendency and $T_g$. In this case, however, configurational entropy (and not enthalpy or free energy) and molecular mobility gave a better indication of crystallization tendency. Others have also suggested that increases in configurational entropy may be used to provide an indication of increased stability of amorphous materials (Zhou et al., 2008; Graeser et al., 2009; Baird and Taylor, 2012). In contrast, Johari and Shanker (2010) question the use of thermodynamic descriptors (such as heat capacity and melting enthalpy) to predict the kinetic stability of amorphous solids. This is described in more detail in Section X.G.3.

1. Drug-Polymer Intermolecular Association. Drug-polymer interactions, such as hydrogen bonding, decrease the ease of recrystallization by reducing drug mobility and by limiting the drug-drug molecular interactions that precede nucleation and crystallization (Khoudz and Clas, 2000; Weuts et al., 2003; Miyazaki et al., 2006; Bhugra and Pikal, 2008). Hydrogen bonds between indomethacin and PVP, for example, limit indomethacin crystallization by preventing the formation of drug-drug dimers through adjacent carboxylic acid groups—an interaction likely to be the initial step in indomethacin crystallization (Taylor and Zografi, 1997). Suppression of indomethacin crystallization in the presence of PVP has also been reported at polymer levels (5%) that are too low to have a significant impact on $T_g$, suggesting other mechanisms of stabilization (Yoshioka et al., 1995; Matsumoto and Zografi, 1999). PVP hydrogen bonds with many drug compounds in solid dispersions, and these provide the opportunity for increases in physical stability (Van den Mooter et al., 1998; Matsumoto and Zografi, 1999; Tantishayakul et al., 1999; Damian et al., 2002; Weuts et al., 2003; Jun et al., 2005; Shinde et al., 2008; Lima et al., 2011). PVP has two functional groups (=N- and C=O) with the capacity to accept hydrogen bonds, although steric hindrance around the nitrogen generally favors intermolecular hydrogen bonding via the carbonyl group (Van den Mooter et al., 1998). Drug-polymer hydrogen bonding has also been shown for several other drug-polymer combinations, for example, carbamazepine-PEG 4000/6000 (Doshi et al., 1997), benzidazole-PEG 6000 (Lima et al., 2011), cefturoxime-HPMC (Jun et al., 2005), and NVS981-HPMC phthalate (Ghosh et al., 2011). The absence of a drug-polymer intermolecular interaction within a solid dispersion, however, does not necessarily preclude the ability of a polymer to stabilize the amorphous form (Van den Mooter et al., 2001; Caron et al., 2010). The extent to which drug and polymer interact also determines the level of mixing and, therefore, miscibility. The importance of miscibility to stability in solid dispersions is described in more detail in Section X.G.5.

2. Inhibition of Crystal Nucleation. The potential for polymers to stabilize amorphous solid dispersions via direct inhibition of nucleation is well described (Konno and Taylor, 2006; Marsac et al., 2006b; Caron
et al., 2010). Thermodynamic drivers to nucleation are explained by classic homogeneous nucleation theory (CNT) (Turnbull and Fisher, 1949; James, 1985), and concepts from the CNT have been applied to solid dispersion systems to understand better the stability of amorphous drug in the presence of polymers.

The rate of nucleation (I) may be defined in terms of the free energy of activation for molecular diffusion across the nucleation interface to join the new lattice ($\Delta G_a$) and the free-energy change associated with the formation of a spherical nucleus of critical size ($\Delta G^*$) (Konno and Taylor, 2006; Marsac et al., 2006a):

$$I = A \cdot \exp\left(-\frac{(\Delta G_a + \Delta G^*)}{kT}\right)$$  \hspace{1cm} (54)

where A is a constant, $k$ is the Boltzmann constant, and $T$ is absolute temperature. The free-energy change ($\Delta G$) during the formation of a spherical nucleus is determined by the difference in the bulk free energy between the amorphous phase and the crystal phase ($\Delta G_v$) and the energy required to form a new solid surface ($\Delta G_a$). $\Delta G_v$ is volume dependent and favors nucleation as the free energy of the crystal is lower than that of the amorphous form (i.e., $\Delta G_v$ is negative). $\Delta G_a$ is a function of the surface area of the new phase and interfacial tension and disfavors nucleation as energy is required to form a new surface (i.e., $\Delta G_a$ is positive). As the size of the crystal nucleus increases, the magnitude of $\Delta G_v$ increases more rapidly than $\Delta G_a$ (since volume increases more rapidly than area with increasing diameter). $\Delta G$ therefore reaches a maxima in the $\Delta G$ versus crystal nucleus size plot, after which $\Delta G$ decreases without limit. This maxima is the free energy for formation of a nucleus of critical size ($\Delta G^*$), and it defines the minimum (critical) nucleus size for crystallization. Before reaching the critical nucleus size, small nuclei may still form but require additional free energy for growth. As such, their existence is only transient, and the nuclei rapidly decompose. However, above the critical nucleus size, crystal growth is thermodynamically favored.

$\Delta G_a$ is often incorporated into the pre-exponential term in eq. 54 (James, 1985; Bhugra and Pikal, 2008) but can also be expanded to enable discussion of the potential impact of polymers on nucleation rates in solid dispersions (Konno and Taylor, 2006; Marsac et al., 2006a). Konno and Taylor (2006) suggest that polymers increase $\Delta G_a$ (and, therefore, reduce nucleation rate in eq. 54) by three potential mechanisms: 1) by acting as a diluent and providing a physical impediment to nucleation; 2) by allowing for specific interactions between the drug and polymer, reducing drug diffusivity; and 3) by accumulating at the nucleation interface. Accumulation of a polymer-rich layer at the nucleation interface occurs when drug crystallization depletes drug concentrations in the vicinity of the crystal nucleus and drug-free polymer does not diffuse away from the interface. The local concentration of polymer therefore increases, reducing molecular mobility in the polymer-rich (high $T_g$) zone and reducing drug access to the crystal nucleus.

From eq. 54, nucleation is also decreased by increasing $\Delta G^*$ (i.e., the minimum free-energy change required to form a crystal nucleus) and, therefore, the thermodynamic barrier to crystal formation. As described previously, $\Delta G^*$ is dependent on $\Delta G_a$ and $\Delta G_v$. Increases in $\Delta G^*$ can arise from an increase in $\Delta G_a$ after polymer accumulation at the nucleation interface and a change in interfacial tension between the two phases, but such changes may also reflect changes to $\Delta G_v$, attributable to differences in free energy between crystalline and amorphous drug. The latter is possible since drug-polymer interactions can lead to an increase in the configurational entropy of the drug in the formulation compared with unformulated drug in the amorphous form (Konno and Taylor, 2006). Higher-configuration entropies may reduce the crystallization tendency of amorphous drug since increased entropy increases the number of possible configurations that the drug can hold, decreasing the probability that drug molecules are correctly orientated for crystallization to occur (Zhou et al., 2002; Marsac et al., 2006a; Baird and Taylor, 2011). However, relationships between the crystallization of amorphous drug and entropy are not always apparent (Konno and Taylor, 2006; Marsac et al., 2006a; Bhugra and Pikal, 2008).

3. Molecular Mobility, Strength, and Fragility. Molecular mobility and associated descriptors of mobility such as relaxation, fragility, and strength are commonly used to describe the physical stability of amorphous materials and solid dispersion formulations. A degree of molecular mobility is required to effect nucleation and crystallization; hence, decreases in mobility are expected to reduce crystallization rates (Hancock et al., 1995; Shamblin et al., 1999, 2000). Molecular mobility can be reduced by increasing viscosity, and the decrease in mobility inherent in an increase in $T_g$ has been implicated in the potential utility of many excipients to enhance the stability of solid dispersions (Van den Mooter et al., 1998, 2001; Verreck et al., 2003b; Sethia and Squillante, 2004; Six et al., 2004). Mobility is also reduced by intermolecular interactions between drug and excipient (polymer) (Taylor and Zografi, 1997; Matsumoto and Zografi, 1999; Khougaz and Clas, 2000; Gupta et al., 2004; Rawlinson et al., 2007).

During storage, the molecular structure of a thermodynamically unstable amorphous material relaxes and the degree of relaxation on storage may be used as an indicator of molecular mobility and stability. Relaxation and diffusion in glassy materials are complex and multifactorial and have been widely studied using
experimental and theoretical approaches, especially in the past decade (Capaccioli et al., 2008). Simplistically, relaxation may be subdivided into primary α-relaxation and secondary β-relaxation (Priestley et al., 2005). α-Relaxation involves rotational and translation re-orientation of molecules in a global sense (i.e., throughout a sample) and is most apparent at temperatures above and immediately below the glass transition temperature (i.e., where there is greater mobility). β-Relaxations are observed at lower temperatures and include localized rotation of molecules or molecular segments; β-relaxation processes include the well-described Johari-Goldstein (J-G) β-relaxations (Johari et al., 2005; Prevosto et al., 2009), which followed the work of Johari and Goldstein (1970), who identified secondary relaxation behaviors within rigid glass structures (i.e., below T_g). Both α- and β-relaxations appear to occur universally in glass-forming materials (Johari and Goldstein, 1970; Thayyil et al., 2008). Evidence that α-relaxation (as captured by methods describing relaxation and mobility changes on movement through T_g) cannot always be linked to changes in the physical state of amorphous solids (Johari et al., 2005; Vyazovkin and Dranca, 2005) and a realization that J-G β-relaxations are precursors of longer-range motion, including α-relaxation and the glass transition (Ngai, 1998; Johari et al., 2005; Prevosto et al., 2009), suggest that J-G β-mobility may better describe the molecular changes that lead to nucleation (Vyazovkin and Dranca, 2005; Bhugra and Pikal, 2008). Differences in J-G β-mobility below T_g may therefore provide an improved indication of crystallization potential in amorphous solids (Ngai et al., 2001; Tombari et al., 2008a,b). The contribution of α- and β-relaxation to stability in amorphous pharmaceuticals is well described elsewhere (Bhattacharya and Suryanarayanan, 2009).

Molecular mobility can be estimated via measurement of viscosity, dielectric relaxation time, or structural relaxation time, and a range of techniques—including rheometry, dielectric, spectroscopy, and solid-state NMR—have been used to quantify these properties (Andronis and Zografi, 1997; Yoshioka et al., 1999; Aso et al., 2000; Liu et al., 2002; Offerdahl et al., 2005). In pharmaceutical applications, most studies have used thermal methods, in particular DSC, to provide an indication of changes to molecular mobility. As described in the following section, this approach has limitations, but widespread application warrants a description of the methods used. In general, thermal analytical approaches to estimate molecular mobility within a glassy solid (below the T_g) are performed using annealing experiments or "enthalpy recovery experiments." These are described in detail elsewhere (Hancock et al., 1995; Mao et al., 2006a) but rely on molecular relaxation during storage below T_g (annealing), resulting in a loss of enthalpy. The loss of enthalpy (i.e., the relaxation enthalpy, ΔH_relax) is subsequently related to the degree of relaxation, which in turn is a function of molecular mobility: the greater the molecular mobility, the greater the degree of relaxation.

The enthalpy loss on relaxation (ΔH_relax) is estimated via measurement of the enthalpy recovered during sample reheating past the T_g (Hancock et al., 1995) and compared with ΔH_∞ (the maximum amount of recovered enthalpy at infinite time) to give the extent of relaxation (Φ) from eq. 55:

$$\Phi = 1 - \left( \frac{\Delta H_{relax}}{\Delta H_\infty} \right)$$  (55)

ΔH_∞ is calculated from the change in heat capacity (ΔC_p) of the drug between the glass transition temperature (T_g) and the annealing temperature (T):

$$\Delta H_\infty = \Delta C_p (T_g - T)$$  (56)

Where repeated measurements are obtained, the data describing relaxation (Φ) as a function of the annealing (storage) time (t) can be compared with models such as the Kohlrausch-Williams-Watts (KWW) equation that describe the approach to a fully relaxed state:

$$\Phi = \exp \left[ -\left( \frac{T}{\tau} \right)^\beta \right]$$  (57)

This provides an average relaxation time constant τ and a stretch parameter β that varies between 0 and 1 to indicate deviation from exponential relaxation behavior. Deviations are often seen since the level of structural relaxation during sample annealing may vary considerably within a sample and during the course of the annealing experiment. Relaxation is therefore nearly always nonlinear and nonexponential (Davies and Jones, 1953).

Structural relaxation is highly temperature dependent, and trends in relaxation as a function of temperature have been used to provide additional insight into the potential stability of amorphous materials. Several models have been used to describe the temperature dependence of relaxation time. The Vogel-Fulcher-Tammann (VFT) equation (eq. 58) is commonly used to describe relaxation above T_g, whereas the Adam-Gibbs-Vogel (AGV) equation (eq. 59) is used below the T_g:

$$\tau = \tau_0 \exp \left( \frac{D T_0}{T - T_0} \right)$$  (58)

$$\tau = \tau_0 \exp \left( \frac{D T_0}{T \left( 1 - \frac{T_0}{T_f} \right)} \right)$$  (59)

In eq. 58 and eq. 59, T_0 is the temperature at which the configurational entropy is zero (i.e., no further
relaxation is possible) and has the same physical meaning as the Kauzmann temperature (Fig. 56). \( \tau_0 \) is the relaxation time constant for an unrestricted material and is normally taken as being of the order of the lifetime of atomic vibrations (10\(^{-14}\) seconds) (Mao et al., 2006a), and \( T_f \) is the fictive temperature (the temperature at which the structure of a supercooled liquid is “frozen-in” and quantitatively similar to the \( T_g \)) (Badrinarayanan et al., 2007). \( T_f \) is determined by the point of intersection of lines extrapolated from above and below the \( T_g \) in thermal melting plots (similar to Fig. 56), and errors in \( T_f \) estimation may lead to significant differences in shelf-life estimations (Johari and Shanker, 2010). The strength parameter (D) provides an alternate measure of the fragility of pharmaceutical glasses. Most drugs are classified as either fragile (D < 10) or moderately fragile (D = 10–30) (Crowley and Zografi, 2001; Zhou et al., 2002; Mao et al., 2006a), and for these materials, additional formulation approaches are typically needed to facilitate glass formation and to ensure stability. Significant variations in crystallization tendency also exist even within the fragile or moderately fragile classifications (Baird et al., 2010). As described in the preceding section, annealing experiments are widely used to measure the level of molecular mobility below glass transition temperatures (Andronis and Zografi, 1998; Shamblin et al., 1999; Liu et al., 2002; Weuts et al., 2003; Mao et al., 2006a,b) and were used, for example, by Zografi and coworkers (Hancock and Zografi, 1997) to establish the \( T_g \)–50°C rule, described in Section X.F.3.

\[ T_{rg} = \frac{T_f}{T_g} \]  

(60)

Most drugs have \( T_{rg} \) values between 0.65 and 0.8 (Baird and Taylor, 2012) and the approximation that \( T_{rg} = 0.66 \) is widely used to predict glass transition temperatures from the melting temperature (the so-called “2/3 rule”). Poor glass-formers, however, show significantly higher melting temperatures relative to their glass transition temperatures and exhibit lower \( T_{rg} \) (typically \( T_{rg} < 0.66 \)) with decreasing \( T_f/T_m \) ratios indicative of materials with greater fragility and reduced glass-forming ability.

Friesen et al. (2008) investigated the ability of the reduced glass transition temperature to estimate the potential for polymers to enhance the stability of solid dispersion formulations. This study examined the properties of HPMCAS spray-dried solid dispersions for 139 experimental compounds. Across the data set, drugs with higher \( T_m/T_g \) ratios (lower \( T_{rg} \)) were more susceptible to crystallization (Fig. 58). The data also confirmed the importance of drug load in determining physical stability (Friesen et al., 2008), and at higher drug loads, the stabilizing effect of the polymer decreased. For poor glass-formers (i.e., those with higher \( T_m/T_g \) ratios in Fig. 58), adequate stability was achieved only at low drug loads (i.e., where the polymer-to-drug ratio was high). The importance of drug loading in solid dispersion stability is described in more detail in Section X.G.5.

Given the link between drug fragility and the long-term stability of formulated solid dispersions, attempts have subsequently been made to understand better the molecular properties that predetermine fragility. Using principal component analysis, poor glass-formers, that is, those showing high crystallization tendencies and lower physical stability (defined as “class I” molecules), were shown to have lower molecular weights and fewer rotational bonds than compounds showing higher glass forming potential (class III molecules) (Baird et al., 2010). This was suggested to reflect differences in configurational entropy, such that a reduction in the number of rotational bonds (a situation that is more likely for drugs with lower molecular weights) increased the probability of the drug existing in the correct orientation for crystallization (Baird et al., 2010). Class II compounds showed a similar level of glass-forming potential to class III but

![Fig. 58. \( T_m/T_g \) versus log P for low-solubility compounds successfully formulated as solid dispersions (all achieved supersaturation during in vitro dissolution tests). The boundaries of regions (i) to (iv) are arbitrary but are representative of general trends in decreasing physical stability with decreasing \( T_g \) of the drugs (moving vertically from region (i) up to region (iii)) and decreasing dissolution rate with increasing drug lipophilicity (moving horizontally from region (i), (ii) or (iii) into region (iv)). Because of the high \( T_g \) values, drugs in region (i) may be incorporated into solid dispersions at higher (>50% w/w) drug loadings; the lower stability of drug in the amorphous form in regions (ii) and (iii) requires lower drug loading for long-term solid dispersion stability (35–50% w/w and 10–35% w/w, respectively). Drug loading in region (iv) is low (10–35% w/w) and limited by a slow rate of dissolution of lipophilic drugs. \( T_g \), glass transition temperature; \( T_m \), melting temperature. Adapted from Friesen et al. (2008).](image-url)
greater crystallization tendencies—a property attributed to the ease of formation of crystal nuclei during cooling of drug melts. Class I compounds (i.e., those least likely to form amorphous material) included tolfenamic acid, griseofulvin, carbamazepine, and tolbutamide. Class II included acetaminophen, celecoxib, nifedipine, and cinnarizine; class III (i.e., those with a higher glass-forming potential and most stable in the amorphous form) included fenofibrate, felodipine, itraconazole, ritonavir, and indomethacin (Baird et al., 2010). Mahlin et al. (2011) used in silico techniques to predict glass-forming potential based on molecular structure, and consistent with the data described by Baird et al. (2010), suggest that larger molecules with larger numbers of rotational bonds and limited molecular symmetry show good glass-forming potential.

4. Limitations to the Use of Thermal Analysis to Estimate Molecular Mobility. Although estimations of molecular relaxation using changes in heat capacity remain common, in large part because of the wide availability of thermal analytical techniques, Johari and Shanker (2010) have highlighted a number of potential shortcomings of correlating measured or calculated thermodynamic quantities (such as heat capacity of a glass) with the molecular mobility of an amorphous pharmaceutical to estimate differences in stability. In these studies, relaxation dynamics in acetaminophen glasses were instead obtained using dielectric relaxation spectroscopy (Johari et al., 2005) and the data compared with previous studies using calorimetry (Zhou et al., 2002). In short, the data obtained using thermal analysis appeared to underestimate relaxation. The authors suggest that this finding reflected errors in estimation of configurational entropy from specific heat data since entropy originating from nonconfigurational sources may contribute significantly to heat-capacity changes on varying temperature but does not appear to have a role in structural relaxation (Johari et al., 2005). Dielectric relaxation spectroscopy may therefore represent a more accurate means of assessment of molecular mobility, although some limitations for pharmaceutical applications are apparent (Craig, 1992). Specifically, in their most preferred configuration, dielectric spectroscopy measurements are conducted using a sealed cell in which the sample is melted (Alie et al., 2004; Johari et al., 2005; Adrjanowicz et al., 2010a; Dantuluri et al., 2011). In contrast, pharmaceutical scientists are usually interested in monitoring stability over the projected pharmaceutical shelf-life and in samples that have been subjected to different humidity and temperature conditions. This requires the use of a “parallel plate” method (Adrjanowicz et al., 2010a; Grzybowska et al., 2010); however, this may introduce additional experimental error, including the entrapment of varying amounts of air and moisture (the dielectric constants of air and water are markedly different from that of the solid dispersion). The advantages and limitations of various instrumental techniques to measure molecular mobility are discussed further in Bhattacharya and Suryanarayanan (2009).

5. Drug-Polymer Miscibility and Relationship to Drug Loading. Solid dispersions containing drug molecularly dispersed throughout the polymer matrix (i.e., types 3 and 6 systems as defined in Table 26) are typically more resistant to drug crystallization. In contrast, systems containing higher local concentrations of phase separated amorphous drug (i.e., types 2 and 5) increase the likelihood of molecular alignment and nucleation and are more prone to crystallization. Physical instability in solid dispersions is therefore more common where there is heterogeneous mixing, where drug-polymer miscibility is poor and where drug loading exceeds the drug-polymer miscibility limit (van Drooge et al., 2006; Marsac et al., 2009; Paudel et al., 2010; Qian et al., 2010a). Understanding the relationship between drug loading and the drug-polymer miscibility limit (and therefore the molecular arrangement of drug in the formulation) is critical to the development of amorphous solid dispersions that maintain physical stability over prolonged periods.

The miscibility of amorphous drug in a polymer matrix is dependent only on adhesive and cohesive interactions between drug and polymer (whereas the solubility of crystalline drug is also dependent on the strength of the crystal lattice). For amorphous drug, where drug-polymer miscibility is thermodynamically favored, the free energy of mixing (ΔGmix) is negative (eq. 61):

$$
\Delta G_{mix} = \Delta H_{mix} - T \Delta S_{mix}
$$

The mixing of two components typically increases entropy (ΔSmix), favoring miscibility. Mixing enthalpy (ΔHmix) is therefore the more discriminating factor in determining miscibility, with negative or slightly positive values favoring spontaneous miscibility (Pajula et al., 2010). The value of ΔHmix is a function of the relative strength of cohesive interactions (drug-drug and polymer-polymer interactions) and adhesive interactions (drug-polymer interactions), where negative mixing enthalpy (and therefore miscibility) is favored when adhesive interactions dominate.

A single Tg value during a melting DSC cycle is usually considered satisfactory evidence of drug-polymer miscibility (assuming the Tg of the drug and polymer are different). In contrast, multiple Tg values suggest regions of heterogeneity resulting from incomplete drug-polymer mixing or limited drug-polymer miscibility (Six et al., 2003a,b; van Drooge et al., 2006). DSC is therefore routinely used to determine the miscibility of drug-polymer mixtures. However, drug-polymer miscibility at elevated temperatures (i.e., temperatures around the Tg) is not necessarily
predictive of miscibility at lower (storage) temperatures (Qi and Craig, 2010; Qian et al., 2010a,b; Baird and Taylor, 2012). Similarly, the lack of crystalline peaks in x-ray diffraction data (i.e., the presence of the characteristic amorphous halo) provides evidence of amorphous content but little additional information as to miscibility of the amorphous drug with the polymer (i.e., the degree of molecular dispersion).

The limitations of DSC and XRPD in assessing solid dispersion physical stability were recently highlighted by Qian et al. (2010b), who used hot-melt extrusion to prepare PVPVA solid dispersions of an unnamed experimental compound, using either a low (50 rpm) or high (225 rpm) extruder screw speed. On initial assessment, batches produced using either method had a single and equivalent \( T_g \) (88°C). XRPD assessments were also similar, and both methods of characterization suggested that variation in screw speed had little effect on the properties of the solid dispersion. In contrast, repeat testing after 2 months revealed that crystallization had occurred in the batch produced using the slower screw speed, whereas the batch prepared using the high screw speed was stable. Confocal Raman microscopy was subsequently used to chemically map samples of the two solid dispersions. Significant differences in homogeneity were obtained (Fig. 59), with the least stable solid dispersion (left) showing regions rich in amorphous drug (red areas in Fig. 59). These data provide a good example of the potential advantage of characterization methods beyond conventional DSC and XRPD, in particular, spatially specific spectroscopic methods, to assess the homogeneity of mixing in amorphous solid dispersions (Breitenbach et al., 1999; Qian et al., 2010b).

Higher-resolution imaging of drug distribution patterns in solid dispersions may be achieved using tapping-mode atomic force microscopy and has been used to assess a range of drug-polymer films and powders for areas of immiscibility (with resolution down to the nanometer range) (Weuts et al., 2011). Example images from a recent study are reproduced in Fig. 60 (Lauer et al., 2011). In this case, the drugs [a neurokinin 1 (NK1) receptor antagonist and an inhibitor of cholesterylester transfer protein (CETP)] show evidence of miscibility with PVP, PVPVA, and HPMCAS (i.e., topography consistent with that of the pure polymer), whereas discrete particles (~80 nm) are observed in Eudragit L100 films, suggesting phase-separation (Lauer et al., 2011). Friesen et al. (2008) also suggest that scanning electron microscopy can be used to detect crystallinity in solid dispersions at levels as low as 1%. Further details of the techniques that can be used to detect phase-separated drug within solid dispersions are provided in two recent reviews (Qi and Craig, 2010; Baird and Taylor, 2012).

To circumvent some aspects of the empirical nature of solid dispersion design and to inform the selection of carriers that are miscible with the drug of interest, initial estimates of drug-polymer miscibility may be achieved using the Flory-Huggins theory of mixing of regular solutions (Lloyd et al., 1997; Yao et al., 2005; Marsac et al., 2006b, 2009; Pajula et al., 2010; Sun et al., 2010; Baird and Taylor, 2011) or Hildebrand solubility parameters (Greenhalgh et al., 1999; Forster et al., 2001b; Paudel et al., 2010; Huang et al., 2011; Zhao et al., 2011).

![Fig. 59. Representative compositional Raman images of two experimental solid dispersions prepared by hot melt extrusion (HME 1 and HME 2, respectively). The red regions depict higher local concentration of drug in the formulation (an undisclosed compound). The size of each Raman map is 800 \( \mu \text{m} \times 800 \mu \text{m} \) and 1600 \( \times 40 \). Raman spectra were collected to construct each image. HME 1 = slow extruder screw speed (50 rpm); HME 2 = fast extruder screw speed (225 rpm). Reprinted from Qian F, Huang J, Zhu Q, Haddadin R, Gawel J, Garmise R, and Hussain M (2010b) Is a distinctive single \( T_g \) a reliable indicator for the homogeneity of amorphous solid dispersion? Int J Pharm 395:232–235, with permission from Elsevier.](image-url)
The Flory-Huggins equation for mixing of two polymers (eq. 62) (Flory, 1953) is shown below and is an extension of the Gibbs free-energy change on mixing (eq. 61):

$$\Delta G_{\text{mix}} = RT \left[ \left( \frac{\phi_A}{r_A} \right) \ln \phi_A + \left( \frac{\phi_B}{r_B} \right) \ln \phi_B + \chi \phi_A \phi_B \right] \quad (62)$$

In the Flory-Huggins equation, R and T are the gas constant and temperature, $\phi_A$ and $\phi_B$ are volume fractions of the two polymer components being mixed, $r_A$ and $r_B$ are the number of monomer segments in each polymer component, and $\chi$ is the interaction parameter between these two components. In the case of a solid dispersion, A and B refer to drug and polymer components of the mixture and for the drug, $r_A = 1$.

The first two terms on the right in eq. 62 represent the contribution of the combinatorial entropy of mixing of the two components, while the third term represents all noncombinatorial factors included in the enthalpy of mixing. Since mixing leads to an increase in entropy, the first two terms in the Flory-Huggins equation almost always favor mixing. The third term represents the change in local interactions and motions of the drug and polymer. In most cases, attractive energies between molecules of a single component are stronger than those between unlike pairs, causing the third term in eq. 62 to oppose mixing. This term is governed by the value of the Flory-Huggins interaction parameter ($\chi$). A large positive $\chi$ value causes the enthalpy component to dominate the equation and promotes immiscibility of the system. In contrast, a negative or marginally positive value for $\chi$ suggests good miscibility (Paudel et al., 2010).

The dependence of $\Delta G_{\text{mix}}$ on the quantity of drug in a solid dispersion is evident by plotting the solution of the Flory-Huggins equation (eq. 62) as a function of the volume fraction of drug ($\phi_A$). At fixed temperature, this plot has only one independent variable ($\phi_A$). However, the shape of the curve is temperature dependent as $\chi$ varies inversely with temperature. At low $\chi$ values (i.e., high temperature), all compositions are miscible, whereas at high $\chi$ values, immiscibility results. Most combinations of polymer and drug are not miscible over all temperature ranges. For these partially miscible systems, a plot of $\Delta G_{\text{mix}}$ versus $\phi_A$ shows a central maxima flanked by two free-energy minima. An example of such a plot is shown schematically in Fig. 61A. At a fixed temperature, miscibility is favored by volume fractions close to the free-energy minima. In
the central range of compositions, the maxima in the free energy profile leads to immiscibility. Since this immiscible portion of the curve exists between two miscible regions, it represents a miscibility gap. Once $\chi$ has been determined from the Flory-Huggins equation (eq. 62) and the dependence of the volume fraction of drug on $\Delta G_{\text{mix}}$ has been plotted (i.e., Fig. 61A), a spinodal curve can be calculated by taking the second derivative of eq. 62. The spinodal curve defines (and separates) the metastable and unstable regions within the two-phase (i.e., nonmiscible) portion of the phase diagram (shown in Fig. 61B) ( Favvas and Mitropoulos, 2008). Compositions within these two regions phase-separate via different mechanisms. Metastable compositions are located between the phase boundary and the spinodal curve and are relatively stable to small molecular fluctuations. Phase separation requires a significant local perturbation to the system and can be achieved only by the formation of a nucleus of critical size and subsequent particle growth (Favvas and Mitropoulos, 2008). In the case of drug-polymer solid dispersions, phase separation in this way is hindered by the low kinetic mobility of the dispersion (which limits diffusion of drug molecules within the polymer matrix) and the energy required to form a drug nucleus of critical size. The importance of local molecular mobility in dictating nucleation and growth is consistent with an important role for secondary or $\beta$-relaxation behavior in the physical stability of solid dispersions below $T_g$. In contrast, for volume-fraction compositions and temperatures located inside the spinodal line (the unstable region), the system is highly unstable to even small molecular fluctuations, and phase separation takes place spontaneously throughout the sample via spinodal decomposition (rather than local nucleation) and only diffusion limits phase separation. The global nature of spinodal decomposition suggests a closer link to primary or $\alpha$-relaxation behavior. Knowledge of whether the composition of a solid dispersion exists between the phase boundary and spinodal curve (metastable region) or inside the spinodal curve (unstable region) at the storage temperature provides an additional indication of physical stability (Fig. 61B). Although both systems are thermodynamically unstable, the risk of spontaneous phase separation via spinodal decomposition (in the case of systems that lie inside the spinodal curve) typically precludes the preparation of solid dispersions with acceptable physical stability over prolonged periods. In contrast, appropriate polymer choice may allow kinetic stabilization of metastable compositions and facilitate exploitation of formulations with higher drug load.

Solubility parameters (Hildebrand and Hansen) provide an alternative mechanism for estimation of the potential for drug-polymer miscibility, where drug-polymer combinations with the most similar solubility parameters are expected to provide the greatest likelihood of miscibility. Huang et al. (2011) examined the miscibility of polymeric solid dispersions of nifedipine and showed that predictions of miscibility using the Flory-Huggins parameter and Hildebrand solubility parameters both indicated greater nifedipine miscibility in Eudragit RL compared with ethylcellulose. These predictions were subsequently verified experimentally using FTIR and DSC. Similarly, Paudel et al. (2010) examined naproxen-PVP systems and showed that the Flory-Huggins interaction parameter was large and negative, and that this correlated well with high kinetic miscibility limits (> 50% w/w drug in PVP) for solid dispersions prepared by spray drying. In contrast, however, when the solubilities of crystalline and amorphous naproxen in PVP were estimated using solubility in a low-molecular-weight PVP analog (n-methylpyrrolidone), solubility was low (5–7% w/w) in both cases, suggesting that PVP solid dispersions prepared at drug loadings greater than ~7% w/w were highly supersaturated and, despite Flory-Huggins
predictions of high miscibility, at risk of phase separation. The limitations of the Flory-Huggins model in this example were ascribed to an inability to account for specific saturable interactions (e.g., drug-polymer H-bonds) and the fact that the interaction parameter was based on mixing data at high temperatures (i.e., near the melting temperature of the drug) where miscibility was favored (eq. 62) (Paudel et al., 2010).

Since attractive intermolecular interactions between drug and polymer provide a driver for enhanced miscibility, several spectroscopic techniques, including infrared, Raman, and NMR spectroscopy, have been used to probe the presence of drug-polymer interactions in solid dispersions (Taylor and Zografi, 1997; Van den Mooter et al., 1998; Tantishayakul et al., 1999; Khougaz and Clas, 2000; Konno and Taylor, 2006; Marsac et al., 2006a; Rumondor et al., 2009a). The absence of drug polymer interactions, however, does not always preclude miscibility, and Eudragit E100 and itraconazole, for example, are partially miscible but show no evidence of intermolecular bonding (Janssens et al., 2010).

Figure 62 provides a simplified interpretation of the relationships between drug loading relative to the crystalline solubility/amorphous miscibility limits in a solid dispersion and physical stability. Thermodynamically stable solid solutions are formed where drug load is lower than the crystalline drug solubility in the carrier (assuming homogeneous dispersion of drug within the carrier) (Vasanthavada et al., 2004; Qian et al., 2010a). However, this situation is rare since crystalline solubility is invariably low and below the concentrations required to provide an appropriate dose. For example, the crystalline solubility of itraconazole in Eudragit E100 is 0.012% w/w, whereas the amorphous solubility (i.e., miscibility) is approximately 7% (Janssens et al., 2010).

Higher drug loads (i.e., those greater than the crystalline solubility limit) result in solid solutions that are supersaturated with respect to drug crystal solubility. These systems are thermodynamically unstable, and over time, the drug is susceptible to crystallization. Where drug crystallization occurs, a two-phase solid
dispersion is produced comprising crystalline drug particles suspended in the carrier (i.e., reversion to type 1 or type 4 solid dispersions dependent on the physical form of the carrier). The potential for crystallization to occur via the initial formation of amorphous nuclei or particles (i.e., type 2 or type 5 systems) is also shown in Fig. 62, although this intermediate may be transient, and spontaneous crystallization of drug from a supersaturated solid solution is also possible (Rumondor and Taylor, 2010).

The greater the drug loading relative to the crystalline solubility, the higher the level of supersaturation and the higher the driving force for crystallization (since the thermodynamic barriers to crystallization (i.e., $\Delta G^*$) are reduced (eq. 54)). Increasing drug loading ultimately exceeds the amorphous drug solubility in the carrier (i.e., the drug-polymer miscibility limit). Above the miscibility limit, physical instability occurs via two separate mechanisms. Where the drug-polymer composition is located between the phase boundary and spinodal curve (see preceding discussion), solid dispersions are metastable and phase-separate via nucleation and particle growth. This generates areas of localized high concentration of amorphous drug in a manner analogous to that seen below the amorphous solubility limit. At higher drug loads, beyond the spinodal curve, solid dispersions are unstable and spontaneously phase-separate by spinodal decomposition rapidly to form domains rich in amorphous drug. The localized concentrations of polymer surrounding areas of phase separated amorphous drug are low, increasing the risk of drug crystallization (Six et al., 2004; Vasanthavada et al., 2004; Rumondor and Taylor, 2010).

Changes in physical form of a drug within solid dispersions with changing drug load may be monitored via effects on $T_g$ in situations where the drug plasticises the polymer (i.e., reduces polymer $T_g$) (Six et al., 2004; Van Drooge et al., 2004, 2006; Vasanthavada et al., 2004). For example, in diazepam-PVP solid dispersions (prepared by freeze-drying), increasing drug load leads to a progressive decrease in the $T_g$ of the drug-polymer mixtures, and above 35% w/w drug, two distinct $T_g$ emerge (i.e., drug and polymer glass transitions) as the drug-phase separates from the polymer. At higher drug loads, the absolute level of molecularly dispersed drug is largely unchanged, however, the level of drug arranged as amorphous clusters increases steadily. Solid dispersions greater than the diazepam-PVP miscibility limit (35%), therefore, show characteristics of both an amorphous suspension (type 5) and an amorphous solid solution (type 6) (van Drooge et al., 2006).

Unlike molecularly dispersed formulations, phase-separated drug present as suspended amorphous particles is no longer intimately mixed with the carrier. This more heterogeneous molecular rearrangement is more likely to crystallize compared with solid dispersions where drug is present in a molecularly dispersed form (i.e., type 3 or type 6 systems) that has increased kinetic stability as a result of intimate contact with the polymer (Six et al., 2004; Marsac et al., 2006b; Rumondor and Taylor, 2010). Solid dispersions with lower drug-polymer ratios and those with high drug-polymer miscibility therefore show better physical stability at ambient temperatures (Chan and Kazarian, 2004; Van Drooge et al., 2004; Weuts et al., 2005; Friesen et al., 2008; Qian et al., 2010a). These solid dispersions are also more physically stable at elevated temperature and in the presence of moisture (Van Drooge et al., 2004; Vasanthavada et al., 2004; Rumondor and Taylor, 2010). Water or other solvents (that may be carried over from manufacturing) reduce miscibility by promoting molecular interactions between solvent and drug or polymer and, therefore, reduce the number of possible drug-polymer intermolecular interactions. The plasticizing properties of solvents also encourage phase-separation in situations where drug loads exceed the miscibility limit through increased molecular mobility in the system. Rumondor and Taylor (2010) further propose that moisture affects the route of drug recrystallization, either by promoting phase-separation, and therefore crystallization from heterogeneous dispersions containing amorphous drug clusters, or by promoting spontaneous crystallization from homogeneous dispersions (see Fig. 62). The latter route of crystallization and crystal growth is likely to be slower given the higher surrounding concentrations of stabilizing polymer.

Finally, the processes depicted in Fig. 62 assume initial preparation of homogeneous solid dispersions. Where the method of manufacture does not provide for adequate mixing, drug and polymer phase-separation may occur below the miscibility limit. Under conditions of poor mixing, regions exist that are drug rich and polymer poor, and the (local) level of drug in these regions is likely to be in excess of the miscibility limit. This scenario leads to localized phase separation and drug crystallization, which may ultimately trigger more widespread (global) crystallization.

**H. Summary**

Solid dispersion formulations comprise drug physically dispersed within an inert and usually highly water-soluble carrier, often a high molecular weight polymer. Drug may exist in the carrier in multiple physical forms and may be dissolved, partially dissolved with excess drug suspended in the crystalline state, partially dissolved with excess drug in the amorphous state, or partially dissolved with excess drug present as a mixture of crystalline and amorphous states. The inert carrier matrix may also be present in either crystalline or amorphous forms. Academic researchers and industrial investigators have
used myriad terms to describe “solid dispersions” that comprise combinations of these physical states of solute and matrix. These include solid solutions, eutectic mixtures, solid amorphous dispersions, molecular dispersions, amorphous molecular dispersions, corecipients, and sugar glasses. All are used extensively in the pharmaceutical literature without the existence of a standard or widely accepted nomenclature. Here we simplify to account for six classes of solid dispersion where drug is present either molecularly mixed in solution or as a crystalline or amorphous dispersion and where the carrier is amorphous or crystalline (Table 26). Of these, systems containing amorphous drug provide for the most significant increases in solubility, dissolution, and oral bioavailability and are therefore most widely used. Indeed, the use of noncrystalline solids as a single or multicomponent system may be one of the most efficient means of achieving enhanced drug solubilization, especially for drugs with relatively high melting points.

Despite the great potential of solid dispersions, especially amorphous solid dispersions, for bioavailability enhancement, there are few successful examples of products on the market. This reflects a great many complexities with their use, in particular the intrinsic thermodynamic instability of drug in the noncrystalline form. High molecular-weight polymers have a proven capacity to kinetically stabilize drug in the high-energy amorphous form, however, many factors contribute to appropriate physical stability. These include the generation of homogeneous dispersions with a high degree of drug-carrier miscibility, identification of systems with high glass transition temperatures relative to storage temperatures, and the reduction of structural relaxation below the glass transition temperature. The stability of a solid dispersion is also dictated, at least in part, by the intrinsic crystallization tendencies of the drug—properties that are difficult to predict a priori.

The choice of excipients that are used to formulate solid dispersions remains empirical in many cases, and although experimentally determined indicators of utility, such as the long-held suggestion that storage at 50°C below Tg results in acceptable stability, have increased confidence in the progression of high-energy formulations, it is clear that these provide an incomplete understanding of behavior in the glass state. Indeed, crystallization has been shown at temperatures far below Tg, and it is increasingly apparent that crystallization behavior is not well correlated with global measures of structural relaxation and that inferring changes in mobility below Tg from studies conducted above Tg may not be ideal.

Instead, recent studies suggest that local molecular motions such as Johari-Goldstein relaxations act as a precursor to changes in global mobility and instability and might constitute a more accurate indicator of stability below Tg. A deep understanding of the drivers of recrystallization, however, remains elusive, and the use of thermal methods to estimate thermodynamic indicators of stability may incompletely capture the complexity of what is essentially a kinetic process. Ongoing studies in material sciences and physics to understand and predict better the behavior of amorphous and glassy solids should lead to better understanding of physical stability and ultimately in vitro/in vivo performance of solid dispersions. The continuing challenge for pharmaceutical scientists is to keep abreast with the advances in this area and to use these studies to build confidence and robustness into the development of drug products containing amorphous solids.

XI. Lipid-Based Formulations

The physicochemical properties that predispose poorly water-soluble drugs to low and variable oral bioavailability (i.e., low water solubility, high log D) are shared by many dietary lipids, fat-soluble vitamins, and sterols. Dietary lipids and lipophilic nutrients, however, are typically well absorbed, even after ingestion of lipid “doses,” which can be as high as 100 g per day, as part of an adult’s Western diet. The efficiency of lipid absorption reflects the evolution of highly specialized lipid-processing pathways that circumvent the problems of low water solubility and lead to efficient solubilization of dietary lipids in endogenous micellar species in the GI tract. In the simplest sense, coadministration of drugs with lipids in lipid-based formulations (LBF) seeks to harness the advantages of endogenous lipid processing pathways to support drug absorption.

It has been recognized for many years that lipids in food can assist the absorption of drugs with low water solubility. Perhaps the best known example, and certainly one of the earliest examples that benefit from coadministration with food, is griseofulvin (Crounse, 1961). Others—including danazol (Charman et al., 1993; Sunesen et al., 2005), halofantrine (Humberstone et al., 1996), atovaquone, and troglitazone (Nicolaides et al., 2001; Schmidt and Dalhoff, 2002) have subsequently been reported. Clinical reliance on coadministration with food is, however, fraught with difficulty because of the variability associated with differences in patterns of food ingestion (and food components) as a function of a variety of factors, including time, age, culture, and health status. In contrast, coadministration of poorly water-soluble drugs with formulated lipids provides a route to the advantages of lipid coadministration, without the variability that results from the use of food as a lipid source. The use of lipids in food as a mechanism of absorption enhancement is also impractical in many preclinical models where animals will not eat “on command.”
The potential for LBF to enhance the absorption of poorly soluble drugs has been recognized for more than 40 years. Groves and de Galindez (1976) described LBF with the potential to self-emulsify within the gastric environment in the 1970s, effectively paving the way for the development of the encapsulated, self-emulsifying formulations that are most commonly found in current use. Indeed, most contemporary LBF are not simply solutions or suspensions of drug in lipids but rather comprise combinations of glyceride lipids, lipophilic or hydrophilic surfactants, and cosolvents. These materials are usually filled into soft or hard gelatin capsules and may be liquid, semisolid, or solid at room temperature.

The utility of LBF and the possible advantages of self-emulsifying formulations are perhaps best exemplified by examination of the early formulations of the poorly water-soluble immunomodulator cyclosporine. The first of these products, Sandimmune, was marketed in 1981. Sandimmune comprised a solution of cyclosporine in olive oil, Labrafil M 2125 CS, and ethanol filled into soft gelatin capsules. Capsule rupture in the stomach resulted in the formation of a polydisperse oil-in-water emulsion. The oral bioavailability of cyclosporine from Sandimmune was low but was able to provide for effective exposure after oral administration and was marketed for many years. In 1994, Sandimmune was reformulated as Sandimmune Neoral, comprising corn oil glycerides, polyoxy 40 hydrogenated castor oil (Cremophor RH 40), propylene glycol, ethanol, and DL-α-tocopherol. Unlike the original Sandimmune formulation, Neoral was designed such that it emulsified spontaneously on contact with the GI fluids to form a microemulsion with a mean particle size smaller than 100 nm. The Neoral microemulsion formulation resulted in enhanced and more consistent exposure and a reduction in the variability associated with coadministered food (i.e., a reduced food effect) (Kovarik et al., 1994; Mueller et al., 1994a, b,c). The clinical and commercial success of Neoral stimulated a subsequent increase in interest in the use of LBF and ultimately led to the development of LBF for a range of drugs (Strickley, 2004, 2007). The success of Sandimmune Neoral also resulted in significant interest in LBF that spontaneously form emulsions with particle sizes in the nanometer size range on dispersion. Interestingly, although many of these subsequently were to provide for excellent exposure, a direct link to the particle size of the initial dispersion remains elusive. Indeed, rapid processing of the formulation in vivo (digestion, interaction with biliary components) likely leads to significant change in particle size. More recent data suggest that a better indicator of in vivo utility is the ability to maintain drug solubilization as the formulation is dispersed and digested in the GI tract, a property also displayed by the Neoral formulation.

Here we provide an overview of the mechanisms by which LBF promote the absorption of poorly water-soluble drugs, describe the range of potential formulations that fall under the broad umbrella of “LBF”, discuss the design of LBF and the methods of formulation assessment, and provide a number of recent studies that exemplify the potential utility of this approach.

A. Mechanisms of Bioavailability Enhancement by Lipid-Based Formulations

LBF are most commonly used to promote the oral bioavailability of poorly water-soluble drugs via enhancements in drug solubilization and dissolution rate. In addition, LBF can stimulate intestinal lymphatic drug transport (thereby providing increases in bioavailability via a reduction in first-pass metabolism), and emerging evidence suggests a potential role for components of LBF (primarily surfactants) in the inhibition of intestinal efflux and metabolism. With the exception of direct effects on transporter and enzyme functionality, the mechanisms by which LBF achieve these ends are intrinsically linked to their intercalation into, or stimulation of, endogenous lipid digestion and absorption pathways. These are described in the following section.

1. Stimulation of Intestinal Lipid Absorption and Transport Pathways. Several aspects of intestinal lipid absorption have been reviewed, in many cases with a focus on strategies to reduce the rising incidence of metabolic syndrome. The specific topics addressed include lipid digestion (Phan and Tso, 2001; Mu and Hoy, 2004; Lindquist and Hernell, 2010), nanostructures formed during lipid digestion (Boyd, 2012), membrane transport (Niot et al., 2009), intracellular trafficking of lipids (Mansbach and Siddiqi, 2010), lipoprotein assembly (Iqbal and Hussain, 2009; Mansbach and Siddiqi, 2010; Warnakula et al., 2011), and lymphatic transport (Dixon, 2010). The interested reader is directed to the listed references for more detail; however, the aspects most critical to drug absorption are summarized as follows.

Dietary lipid is largely composed of triacylglyceride or triglyceride (TG). Other dietary lipids include phospholipids (PL), sterols, glycolipids, and vitamins A, D, E, and K. Although TG is consumed in quantities of up to 100 g daily as part of a Western diet, it is digested and absorbed with remarkable (95%) efficiency. The process of digestion and absorption is initiated almost immediately on ingestion, as TG is partially hydrolyzed by lingual lipase, which is released from lingual serous glands on the tongue, and subsequently by gastric lipase in the stomach (Mu and Hoy, 2004). Approximately 10–30% of dietary TG is hydrolyzed by gastric and lingual lipase to form diglyceride (DG) and free fatty acid (FA) (Hamosh and Scow, 1973). The production of amphiphilic lipid
digestion products, such as DG and FA, and the mechanical mixing that occurs in the stomach (Nordskog et al., 2001) facilitates the formation of a coarse lipid emulsion (droplet size <50 μm) that increases the surface area of exposure for further digestion.

Entry of this coarse emulsion (chyme) into the duodenum via the pylorus subsequently triggers a series of events that lead to the chemical digestion and physical solubilization of dietary lipids and ultimately to highly efficient lipid absorption, mostly from the upper small intestine (Nordskog et al., 2001; Mu and Hoy, 2004). Thus, the presence of TG digestion products in the duodenum stimulates cholecystokinin release, gallbladder contraction, and bile and pancreatic enzyme secretion into the duodenum (Nordskog et al., 2001; Mu and Hoy, 2004). Undigested lipid in the lower small intestine can also activate an “ileal brake” that reduces small intestine motility and thus increases the time available for further digestion and absorption. In the duodenum, pancreatic lipase binds to the surface of chyme and continues the digestion of TG and DG to monoglyceride (MG) and free FA (Lowe, 1997). In the presence of bile salts, co-lipase is required to promote anchoring of lipase to the lipid droplet interface (Lowe, 1997). Other dietary lipids are also hydrolyzed in the duodenum. For example, phosphatidylcholine is digested by pancreatic phospholipase (PLA-2) to yield lysophosphatidylcholine (LPC) and free FA (Borgström et al., 1957; van den Bosch et al., 1965; Arnesjo and Grubb, 1971) and cholesterol ester, which accounts for 10–15% of dietary cholesterol (with the remainder present as free cholesterol) (Nordskog et al., 2001), is hydrolyzed by cholesterol esterase. Cholesterol esterase also has additional activity toward TG and PLs (Lombardo et al., 1980).

Solubilization of lipids occurs simultaneously with chemical digestion. Bile components—including bile salts, PL, and cholesterol—are released into the duodenum in the form of micelles after gallbladder contraction. Bile components facilitate further emulsification of chyme, leading to a reduction in lipid droplet size and an increase in the surface area available for lipase-mediated hydrolysis. Although the products of lipid digestion are somewhat more water-soluble than their glyceride precursors, they nonetheless have poor bulk water solubility and as such accumulate on the surface of digesting lipid droplets. As they are hydrated at the oil-water interface, these polar oils swell to form liquid crystal structures ranging from lamellar to cubic and hexagonal phase structures. The phase behavior is complex and a function of the types and quantities of lipids and lipid digestion products present and the concentration of bile salts, phospholipids, and other dietary/formulation components (Patton and Carey, 1979; Hernell et al., 1990; Staggers et al., 1990; Kossena et al., 2003; Fatouros et al., 2007a,b). In general, lipolytic products (FA, MG, DG) detach from the surface of TG droplets as vesicular structures (or other dispersed liquid crystalline structures) and are ultimately solubilized into mixed micellar structures comprising biliary derived PL, BS, and cholesterol. The formation of a mixed micellar phase during lipid digestion was first described by Hofmann and Borgström (Hofmann and Borgström, 1964). Subsequent studies have identified myriad liquid crystalline species that exist in equilibrium with intestinal mixed micelles at various stages during digestion of food or formulation-derived lipids (Patton and Carey, 1979; Patton et al., 1984; Rigler et al., 1986; Hernell et al., 1990; Staggers et al., 1990; Fatouros et al., 2007a,b; Warren et al., 2011). Progression through to mixed micelles, with small particle sizes (~3.5 nm) (Cohen and Carey, 1990) and high surface area-to-mass ratios is thought to facilitate effective diffusional transport to the absorptive membrane.

Integration of digested lipids into colloidal structures is required to maintain solubilization and at the same time to provide structures that are small enough to diffuse rapidly across the unstirred water layer (UWL) present on the surface of absorptive enterocytes (Wilson et al., 1971; Nordskog et al., 2001). The UWL is formed by water molecules trapped in a complex glycoprotein network consisting of mucus and glycolalyx and has a lower (acidic) pH compared with the bulk fluid in the intestine (Shiau et al., 1985; Orsenigo et al., 1990). Historically, FA absorption was believed to occur via passage across the UWL within mixed micelles and subsequent diffusion across the absorptive membrane after release from the mixed micelles in a process stimulated by the low pH microenvironment in the UWL (Shiau, 1981). In a seminal paper by Chow and Hollander, however, long-chain FA uptake across the absorptive membrane was shown to occur via both passive and carrier-mediated transport, depending on the FA concentration (Chow and Hollander, 1979). Subsequently, several lipid transporters have been identified (Niot et al., 2009) that interact specifically with free lipid (e.g., FA, cholesterol, PL) (Endemann et al., 1993; Febrario et al., 2001; Krieger, 2001; Thuahnai et al., 2001; Rajaraman et al., 2005). In some cases, endocytic uptake of larger lipid complexes has also been suggested (Pohl et al., 2002, 2004; Ring et al., 2002). However, unequivocal evidence of a role for endocytosis in colloidal lipid uptake in the intestine remains elusive.

Putative brush-border lipid membrane transporters include cluster of differentiation 36 (CD36), plasma membrane fatty-acid binding protein (FABPpm), fatty-acid transport protein 4 (FATP4), Niemann-Pick C1 Like 1 (NPC1L1), and Scavenger receptor class B member 1 (SR-B1). A cholesterol antitransporter or efflux protein ABCG5/8 is also present (Berk and Stump, 1999; Niot et al., 2009). The quantitative importance of these transporters to plasma membrane
uptake of lipids is controversial; however, there is strong evidence that each affects the metabolic fate of lipids inside enterocytes (Berk and Stump, 1999; Niot et al., 2009). Intracellular solubilization and transport of lipid digestion products also appears to be assisted by interaction with a number of families of lipid-binding proteins [e.g., fatty-acid binding proteins (FABP), acyl carrier proteins (ACP), sterol carrier proteins (SCP), cellular retinol binding proteins (CRBP)] (Agellon et al., 2002; Niot and Besnard, 2004; Niot et al., 2009). Interaction with intracellular lipid-binding proteins has been suggested to facilitate cytosolic transport and also to direct delivery to intracellular organelles, including the nucleus (to allow genetic moderation of lipid absorption via changes to the expression of enzymes and proteins involved in lipid absorption) and the endoplasmic reticulum, where re-esterification to TG and assembly into intestinal lipoproteins occurs (Niot et al., 2009; Mansbach and Siddiqi, 2010).

The absorption and intracellular trafficking of lipids are thus controlled by a highly specific network of transport proteins and metabolic enzymes. Simplistically, FA and MG with medium-length acyl chains, which are more polar than their long-chain equivalents, diffuse across the enterocyte, enter the underlying lamina propria, and access the systemic circulation via the intestinal vascular microvessels and portal vein (Mansbach et al., 1991). In contrast, long-chain FA and MG are in most cases trafficked to the endoplasmic reticulum (ER), where they are resynthesized to TG via either the 2-monoglyceride pathway (which predominates in the fed state) (Niot et al., 2009; Mansbach and Siddiqi, 2010) or glycerol-3-phosphate pathway (which predominates in the fasted state) (Johnston and Rao, 1965; Tso et al., 1995; Lehner and Kuksis, 1996). Synthesized TG then either enters a cytosolic pool (from where it may diffuse into underlying intestinal microvessels or be hydrolyzed to MG and FA and re-enter TG synthetic pathways) (Mansbach et al., 1991) or is transferred across the ER membrane by microsomal TG transfer protein (MTP) (Jamil et al., 1995; Hussain et al., 2003, 2008) and/or carnitine palmitoyltransferase (Washington et al., 2003). MTP also facilitates lipidation of apolipoprotein B48 (apoB48) with PL and cholesterol from the rough ER membrane to release a “primordial lipoprotein” into the rough ER lumen (Wu et al., 1996; Hussain et al., 2008). Fusion of the primordial lipoprotein with the TG droplet at the junction of the smooth ER and rough ER leads to formation of an immature lipoprotein or prechylomicron via a process termed core expansion (Black, 2007; Mansbach and Gorelick, 2007; Iqbal and Hussain, 2009; Mansbach and Siddiqi, 2010). The ultimate size of the lipoproteins formed is dictated by the size of the core lipid droplet, which in turn depends on the quantity and type of lipid consumed (Black, 2007; Mansbach and Gorelick, 2007; Iqbal and Hussain, 2009; Mansbach and Siddiqi, 2010). Very-low-density lipoproteins (VLDLs) are preferentially produced in the fasted state, whereas both VLDL and larger, less dense chylomicrons are formed after lipid ingestion.

Immature lipoproteins are subsequently trafficked to the Golgi within prechylomicron transport vesicles (PCTVs) (Kumar and Mansbach, 1999; Mansbach and Siddiqi, 2010), matured (Niot et al., 2009) and transferred to the basolateral membrane within vesicles containing multiple lipoproteins (Niot et al., 2009; Mansbach and Siddiqi, 2010). These vesicles fuse with the basolateral membrane, releasing lipoproteins into the intercellular space between enterocytes (Kindel et al., 2010; Kohan et al., 2010). After passing through the basement membrane, lipoproteins are taken up into lacteals and the lymphatic system rather than intestinal microvessels. The specific mechanism of access of lipoproteins into lacteals is the subject of some debate and is excellently reviewed by Dixon (Dixon, 2010). Nonetheless, numerous studies have shown chylomicron entry into lacteals either via paracellular transport through open intercellular gaps (Palay and Karlin, 1959; Casley-Smith, 1962; Papp et al., 1962; Ladman et al., 1963; Rubin, 1966; Tytgat et al., 1971) or via transcellular transport within vesicles (Palay and Karlin, 1959; Papp et al., 1962; Dobbins and Rollins, 1970; Dobbins, 1971; Dixon et al., 2009). The mesenteric lymph vessels that drain the small intestine, converge to form larger lymphatic vessels, and ultimately flow into the major lymphatic (the thoracic lymph duct), which drains directly into the systemic blood circulation via a union with the vasculature at the junction of the left internal jugular and left subclavian veins. This provides an access route for lipoproteins to the systemic circulation and also provides a potential alternate transport pathway for poorly water-soluble, lipophilic drugs to the systemic circulation. The latter provides a mechanism of drug transport from the small intestine that bypasses the liver and therefore has the potential to avoid hepatic first-pass metabolism.

In addition to the traditional mechanisms of lipid transport to the systemic circulation described above (i.e., diffusion directly into the mesenteric vasculature, or resynthesis, lipoprotein assembly, and uptake into mesenteric lacteals), an alternative pathway has been relatively recently described, where cholesterol and vitamin D are secreted from the enterocyte within apoB free/ApoAI containing lipoproteins that are essentially identical to high-density lipoprotein (HDL) (Iqbal et al., 2003; Iqbal and Hussain, 2005). The subsequent fate of HDL released from the intestine, however, is also controversial (Iqbal and Hussain, 2009), with some authors proposing direct uptake into the mesenteric lymphatics (Bearnot et al., 1982;
Forester et al., 1983; Magun et al., 1985), whereas others suggest that HDL in lymph is derived from plasma (Quintao et al., 1979; Sipahi et al., 1989; Oliveira et al., 1993; Brunham et al., 2006).

The absorption of poorly water-soluble dietary lipids and subsequent transport to the systemic circulation is therefore supported via a number of processes. These include the following:

- Stimulation of bile secretion and an increase in intestinal concentrations of biliary-derived solubilizers
- The combination of biliary secretions with lipid digestion products in the intestinal lumen to form colloidal species, including emulsion droplets, vesicles, and mixed micelles that have high solubilization capacities for poorly water-soluble molecules
- Promotion of mass transport across the unstirred water layer via transport within intestinal mixed micelles
- Absorption across the apical (absorptive) membrane of the enterocyte via passive and active transport mechanisms
- Transport across the enterocyte via interaction with cytosolic lipid-binding proteins
- Assembly into intestinal lipoproteins within the enterocyte, uptake into the intestinal lymph, and

Fig. 63. Schematic of the mechanisms by which poorly water-soluble drugs are absorbed from lipid-based formulations (LBF), including illustration of the mechanisms by which LBF promote the absorption or bioavailability of poorly water-soluble drugs. (1) Avoidance of the need for traditional drug dissolution by presenting the drug as a monomolecular dispersion (solution) in the LBF. (2) Formation of a dispersed emulsion within the stomach in which the drug remains solubilized after capsule rupture. (3) Stimulation of bile secretion, bile salt, and phospholipid micelle release into the intestinal lumen and thus an increase in intestinal concentrations of biliary-derived solubilizers. (4) Combination of biliary secretions with lipid digestion products in the small intestine lumen to form colloidal species—including emulsion droplets, vesicles, and mixed micelles—that have high solubilization capacities for poorly water-soluble drugs. Note that the release of pancreatic lipase and co-lipase from the gallbladder facilitates digestion of formulation lipids and thus the formation of more polar digestion products that assist in vesicle and mixed micelle formation. (5) Promotion of mass transport across the unstirred water layer (UWL) via transport within intestinal mixed micelles. (6) Enhanced drug permeability across the enterocyte apical membrane where formulation components may alter passive permeability or reduce efflux. (7) Transport across the enterocyte via interaction with cytosolic lipid-binding proteins. (8) Highly lipophilic drugs (typically log D > 5) and long-chain TG solubility > 50 mg/g) have the potential for association with developing lipoproteins in the enterocyte and uptake into the intestinal lymph rather than the mesenteric blood capillaries, and therefore transport to the systemic circulation via a route that avoids passage through the liver and first-pass hepatic metabolism. (9) Protection from enterocyte-based drug metabolism, either directly via excipient effects on enzyme activity or indirectly where lipoprotein association within the enterocyte occurs.
transport to the systemic circulation in a transport route that avoids the liver

The coadministration of lipophilic drugs with lipids, therefore, has the potential to recruit a number of endogenous lipid processing pathways to support drug absorption. These can be broadly grouped into mechanisms that support solubilization, those that facilitate absorption and permeability across the enterocyte, and those that stimulate intestinal lymphatic transport. These mechanisms are depicted in Fig. 63. In addition to lipids, LBF also often contain other excipients (e.g., surfactants and cosolvents) that may further assist in drug solubilization, absorption, or lymphatic transport. The mechanisms by which LBF support absorption are discussed below realizing that benefits accrue as a function of both stimulation of endogenous lipid processing pathways and the additional benefits that exogenous, formulation-derived materials provide.

2. Enhanced Drug Dissolution and Solubilization in the Intestinal Lumen. LBF typically comprise lipids or combinations of lipids, surfactants, and cosolvents that are chosen such that the drug dose can be completely dissolved in the combination of excipients. In most cases, this lipidic solution is filled into hard or soft gelatin capsules to allow convenient administration. On capsule rupture, LBF therefore present drug to the GI tract in solution, albeit in nonaqueous solution, providing an immediate benefit compared with traditional solid dose forms since it avoids the requirement for wetting and dissolution. Instead, LBF substitute dissolution for the requirement for continued solubilization as the formulation is processed in the GI tract and the need for transfer or partition into colloidal droplets that are sufficiently small to allow diffusion across the UWL and approach to the absorptive membrane. At the absorptive membrane, current understanding suggests that drug absorption occurs via the fraction of drug that is present in free solution and in equilibrium with the solubilized reservoir. Drug absorption depletes the free concentration in solution, and this is rapidly replenished by drug partition out of the solubilized reservoir to maintain the solubilization equilibrium. In essence, the traditional solid-liquid dissolution step that occurs with solid dose forms (and limited by the solid-state properties of the crystal lattice) is replaced by a rapid liquid-liquid partitioning step between the solubilized reservoir and drug in free solution. Continued solubilization is important, as drug precipitation recreates solid drug and results in reversion of the drug absorption process to one that reflects the situation on administration of a solid dose form or suspension. As a caveat to this general assumption, however, some benefits may be evident if precipitation leads to the generation of small drug particles or where phase separation from a LBF results in drug precipitation in the amorphous state (Sassene et al., 2010; Thomas et al., 2012), both situations that are expected to enhance dissolution rate.

Lipid suspension formulations have also been used as LBF (Bloedow and Hayton, 1976; Kaukonen et al., 2004a; Dahan and Hoffman, 2007; Jantratid et al., 2008; Larsoen et al., 2008a; Sachs-Barrable et al., 2008), especially where drug loads are sufficiently high to preclude the generation of a solution formulation. In this case, the benefits of LBF are limited to their ability to raise the solubilization capacity of the GI fluids and must rely on dissolution for the drug dose to be solubilized in situ. In this case, exposure profiles can be more variable than that achieved with lipid solution formulations. Processing and capsule filling complexities further confound the development of suspension formulations.

Solubilization of a lipophilic, poorly water-soluble drug in the GI content is therefore a function of the following:

- Avoidance of drug precipitation as LBF are initially dispersed (usually on capsule rupture in the stomach)
- Stimulation of secretion of endogenous solubilizing lipids (bile salt, phospholipid, cholesterol) in bile
- Supply of exogenous (formulation-derived) lipids, lipid digestion products, surfactants, and cosolvents
- Generation of mixed colloidal species from endogenous and exogenous solubilizers with the solubilization capacity to prevent drug precipitation during GI transit

These processes are depicted in Fig. 63.

The likelihood of drug precipitation during formulation dispersion is described in more detail in Sections XI.A.2 and XI.B.3. Factors affecting crystallization in oil-in-water emulsions have been reviewed in detail (McClements, 2012). Simplistically, drug precipitation is largely dictated by the hydrophilicity of the components present in the formulation. Thus, simple solutions of drug in lipids (the most lipophilic of LBF) carry little threat of drug precipitation as the formulation is dispersed (Porter and Charman, 2001a; Abdalla and Mader, 2009; Mohsin et al., 2009; Day et al., 2010). In contrast, incorporation of larger quantities of water-miscible surfactants and cosolvents increases the risk of precipitation as the solubilizing power of these components is lost on dilution. In general, less lipophilic drugs appear to more favorably partition out of digesting lipidic formulations and into the colloidal lipid phases formed by the incorporation of digestion products into endogenous micellar species (Kaukonen et al., 2004a; Day et al., 2010). In contrast, more lipophilic drugs accumulate in any undigested oil
that is present, limiting transfer into micellar components until all remaining lipid is digested (Sek et al., 2002; Kaukonen et al., 2004a; Day et al., 2010). As expected, drugs with higher affinities for bile salt and phospholipid mixed micelles equilibrate into the colloidal lipid phase more effectively (Christensen et al., 2004).

Solubilization is further influenced by the mass and type of lipid administered in the formulation, and, in general, administration of a higher lipid mass provides greater support for drug solubilization. This reflects the ability of larger quantities of lipid to maximize the release of bile salt (Kossena et al., 2007) and also the ability of larger quantities of lipid digestion products to more effectively swell endogenous bile salt micelles, thereby increasing solubilization capacity. Effective differentiation of the ability of different lipids to most effectively promote drug solubilization in the GI tract remains a goal of many research programs, including ours; however, it is apparent that the inclusion of, for example, glycerides based on medium-chain (MC) or long-chain (LC) FA leads to quite different colloidal behavior and, therefore, differences in drug solubilization. For example, Kossena et al. (2003, 2004) examined phase behavior and drug solubilization on dilution of MC and LC FA and monoglyceride (MG) (reflecting common digestion products) in simulated endogenous intestinal fluid (SEIF: 4 mM BS, 1 mM PL, and 0.25 mM cholesterol). At high concentrations of lipid, reflective of conditions likely to exist on the surface of a digesting oil droplet, liquid crystalline phases dominated with a lamellar phase evident in MC FA and MG systems and a more viscous cubic phase formed in the long-chain FA and MG systems. Further dilution resulted in the production of large vesicles and the coexistence of mixed micelles and unilamellar vesicles at concentrations likely to reflect the environment adjacent to the absorptive surface of the enterocyte. The MC systems were vesicle rich and had higher solubilization capacities at high FA and MG concentrations compared with the LC systems; however, the solubilization capacity of the MC system was rapidly lost on dilution. In contrast, the LC systems retained solubilization capacity through greater levels of dilution, coincident with the persistence of vesicular species at greater dilutions. Therefore, LC lipids appear to support more robustly drug solubilization at high dilution.

It is also apparent that the properties of formulation-derived lipids change significantly on digestion. Indeed, differential solubilization properties may become apparent only on digestion. For example, drug solubility in MC lipids is usually higher (on a milligram per gram basis) than in LC lipids, providing advantages in drug solubility in the formulation and therefore the potential for the administration of higher drug doses in the same mass of formulation. On digestion, however, ionized MC FAs, such as capric and caprylic acid, are significantly more polar and much less capable of swelling bile salt micelles (and therefore of increasing the solubilization capacity) than the equivalent glycerides. In contrast, the digestion products of LC glycerides, for example, oleic acid, are much more capable of retaining the capacity to support drug solubilization on digestion. The utility of LC lipids has been confirmed in several in vivo studies, where more effective support of drug absorption is evident compared with MC lipids (Palin and Wilson, 1984; Holmberg et al., 1990; Behrens et al., 1996; Christensen et al., 2004; Porter et al., 2004b; Han et al., 2009). This is not always the case, however, and others have described similar absorption from MC and LC systems (Grove et al., 2005, 2006) or benefits associated with the use of MC systems (Gallo-Torres et al., 1978; Myers and Stella, 1992; Dahan and Hoffman, 2006, 2007; Ahmed et al., 2012).

The inclusion of surfactants and cosolvents in LBF has a profound impact on drug solubilization, both on formulation dispersion and on digestion. As described, increased quantities of hydrophilic materials in LBF promote the formation of emulsions with smaller particle size (often in the nanometer size range) but also increase the risk of drug precipitation during dispersion owing to a loss of solubilization capacity Pouton, 2006; Cuiné et al., 2007; Mohsin et al., 2009). This is particularly true for cosolvents, the solubilization capacity of which decreases exponentially on dilution [see Section VI.E and Pouton, 2006; Pole, 2008]. In contrast, LBF containing less water-soluble surfactants are less able to generate emulsions with very small particle size but are more resistant to drug precipitation on dispersion.

One aspect of the performance of formulations containing surfactants after oral administration that is often overlooked is the potential for surfactant digestion in vivo. Many of the surfactants used in pharmaceutical preparations are FA esters of ethoxylated hydrophilic head groups (see Section VII) and as such have the potential for enzymatic cleavage of the ester bond. Surfactants such as polysorbate 80, Cremophor EL, and Cremophor RH 40 (Cuiné et al., 2008; Christiansen et al., 2010) (although Cremophor RH40 may be less effectively hydrolyzed than Cremophor EL (Cuiné et al., 2008), Labrasol, and Gelucire (Cuiné et al., 2008; Fernandez et al., 2008, 2009) are digestible, whereas TPGS and sucrose laurate appear to be stable in the presence of pancreatic enzymes (Christiansen et al., 2010). Whether digestion affects the properties of surfactants sufficiently to alter the solubilization capacity of the system, however, is less well understood. Digestion of surfactants leads to a loss of solvent capacity (Cuiné et al., 2008; Fernandez et al., 2008, 2009) during in vitro lipolysis, and this finding has also been correlated with reductions in absorption
in vivo in at least one study (Cuiné et al., 2008). The use of nondigestible surfactants has therefore been suggested to provide an avenue to the generation of more robust LBF (Cuiné et al., 2008). Surfactants can also inhibit the activity of pancreatic lipase, although it is uncertain how relevant this is to the in vivo processing of LBF where enzyme levels are high and probably in excess (MacGregor et al., 1997; Sek et al., 2006; Christiansen et al., 2010; Li and McClements, 2011; Wulff-Pérez et al., 2012).

The utility of LBF is therefore, at least in part, a function of the ability to maintain drug in a solubilized state during formulation dispersion, digestion, and interaction of exogenous lipids and surfactants with endogenous solubilizing species within the GI fluid. Interestingly, however, recent studies have shown that the generation of colloidal structures with high equilibrium solubilities may not be critical to absorption promotion. For example, where drug release from the LBF is slowed (e.g., via the formation of cubic liquid crystalline phases), absorption may be enhanced by the release of constant low concentrations of drug to the GI environment, thereby lowering the required solubilization capacity (Nguyen et al., 2010). LBF dispersion and digestion may also lead to a period when drug remains solubilized but is present at concentrations that are supersaturated compared with equilibrium drug solubility in the colloidal species present. Where supersaturation is retained for a period that is sufficient to support absorption, bioavailability may be enhanced despite only moderate changes to equilibrium solubility (Porter et al., 2011; Anby et al., 2010; Li et al., 2012; Thomas et al., 2012). The generation of supersaturation may also provide additional benefits with respect to absorption by increasing drug thermodynamic activity. Supersaturation as a potential driving force for absorption was reviewed (Brouwers et al., 2009; Augustijns and Brewster, 2012) and more specifically in the context of LBF by Warren et al. (2010) and Gao and Morozowich (2006).

The length of time for which drug must remain solubilized to promote drug absorption effectively is drug specific and reflects intestinal permeability. As such, solubilization may need to be maintained only briefly for highly permeable drugs but retained for longer periods for less permeable compounds where absorption is slower. The likely period of supersaturation depends partly on the mass ratio of drug solubilized in the supersaturated condition to the equilibrium solubility of the drug in the system (i.e., the supersaturation ratio) (Augustijns and Brewster, 2012). In general, the likelihood of crystal nucleation and precipitation is enhanced at higher supersaturation ratios (Gao and Morozowich, 2006; Warren et al., 2010; Anby et al., 2012; Augustijns and Brewster, 2012). The optimum supersaturation ratio is, however, a balance between enhanced solubilization and the potential for precipitation. It is currently not well defined across different formulation classes and drug types. Nonetheless, supersaturation is likely to occur when LBF lose solubilization capacity during GI processing [see Section XI.B.3] such as during dilution and dispersion of formulations with higher concentrations of surfactant or cosolvent (Mohsin et al., 2009; Do et al., 2011) or during digestion of glyceride-containing formulations (Kaukonen et al., 2004b; Anby et al., 2012; Williams et al., 2012b). Supersaturation might also be expected to occur more readily in MC LBF (which lose solubilization capacity more quickly during dispersion and digestion) compared with LC systems. As such, supersaturation ratios may be particularly high for highly lipophilic drugs after digestion of MC systems (Kaukonen et al., 2004b; Porter et al., 2004a; Williams et al., 2012b). The challenge in this case is the stabilization of supersaturation and prevention of precipitation.

Strategies to assist in the stabilization of supersaturation are therefore widely sought. In general, the same technologies as those used to stabilize supersaturation resulting from the dissolution of salts, high-energy crystal forms, or solid dispersions are typically used. Thus, the addition of polymeric precipitation inhibitors has been used to good effect with LBF, although the available database is relatively small (Gao and Morozowich, 2006; Brouwers et al., 2009; Warren et al., 2010; Anby et al., 2012; Li et al., 2012). Polymer-containing SEDDS systems have been referred to as “supersaturable” SEDDS (Erlich et al., 1999; Gao et al., 2004; Gao and Morozowich, 2006; Gao et al., 2009, 2011a). A range of polymers have been explored for their effect on supersaturation stabilization; however, to this point, cellulose-based polymers appear to be most effective (Warren et al., 2010). Polymers likely prevent precipitation by interfering with crystal nucleation or growth (Gao and Morozowich, 2006; Brouwers et al., 2009; Warren et al., 2010). Even in the event of precipitation, polymers may also retain some benefit by encouraging drug precipitation in the amorphous rather than the crystalline form, providing the potential for enhanced resolubilization (Gao et al., 2009).

3. Enhanced Intestinal Permeability and Inhibition of Intestinal Efflux and First-Pass Metabolism. The high lipophilicity and limited polarity of poorly water-soluble drugs dictate that most have intrinsically good passive membrane permeability (i.e., they behave as typical class II BCS compounds). However, this is not always the case, and several examples of low-solubility, low-permeability compounds are evident (BCS class IV). Poorly water-soluble, highly lipophilic drugs are also commonly substrates for intestinal efflux transporters such as P-glycoprotein (P-gp), multidrug resistance proteins (MRP) and breast cancer resistance protein (BCRP) and have an increased liability toward first-pass metabolism. The challenges provided by low
water solubility, therefore, often occur in tandem with issues of permeability, especially cellular efflux or metabolism. Under these circumstances, formulation components that assist solubility and at the same time enhance permeability or reduce first-pass metabolism are beneficial.

Many components present within LBF, including triglyceride digestion products (FA and MG), surfactants, cosolvents, and biliary components (bile salts, lysophospholipids, cholesterol) enhance passive paracellular and transcellular membrane diffusion (Aungst, 2000; Seelig and Gerebtzoff, 2006; Heerklotz, 2008; Goole et al., 2010). For example, MC FAs (sodium caprate) and glycerides (C8–C10) (Sekine et al., 1985; Unowsky et al., 1988; Yeh et al., 1994) are well known to enhance paracellular diffusion by opening tight junctions (Anderberg et al., 1992; Tomita et al., 1992; Lohikangas et al., 1994; Lindmark et al., 1998) and may also permeabilize the membrane and enhance transcellular permeation (Tomita et al., 1992). Longer-chain FAs and MGs similarly enhance passive permeability, although fewer studies have examined the effects of LCs compared with MC FAs (Lundin et al., 1997; Aungst, 2000). Surfactants and cosurfactants also enhance paracellular diffusion by opening tight junctions (Rama Prasad et al., 2003; Chang and Shojaei, 2004; Rama Prasad et al., 2004; Sha et al., 2005) or transcellular diffusion by permeabilizing or solubilizing the membrane (Rege et al., 2002; Prasad et al., 2003; Rama Prasad et al., 2003).

The potential for a surfactant to increase transcellular diffusion via an increase in membrane permeability is related to structure and physicochemical properties (Seelig and Gerebtzoff, 2006; reviewed in Heerklotz, 2008) and is determined by differences in both uptake into the membrane and perturbation of membrane structure. Uptake into the outer membrane leaflet is usually rapid; however, flip-flop to the inner membrane leaflet may occur slowly for some surfactants, in particular those with bulky, ionic, or highly hydrophilic headgroups. Membrane uptake is governed by the mole ratio partition coefficient, \( K_r \),

\[
K_r = \frac{n^b_r}{n^b_L \cdot C_{aq}}
\]  

(63)

where \( n^b_r \) and \( n^b_L \) are the mole numbers of surfactant and lipid in the membrane bilayer, respectively, and \( C_{aq} \) is aqueous surfactant concentration (Heerklotz, 2008). In general, \( K_r \) is proportional to the log P of the surfactant and approximates 1/CMC. Thus, more lipophilic surfactants have lower CMCs and partition more readily into membranes. However, deviations from this relationship are possible, for example, where the surfactant is charged and forms electrostatic interactions with the membrane or where the surfactant induces bilayer or monolayer curvature strain in the membrane.

Bilayer curvature strain occurs when a surfactant inserts quickly into the outer monolayer but does not translocate to the inner leaflet or flip-flops relatively slowly, resulting in bilayer asymmetry and strain. Flip-flop requires the polar head group of the surfactant to traverse the hydrophobic core of the membrane and to align with the polar headgroups of the inner rather than the outer membrane leaflet. As such, surfactants with large, charged, or very hydrophilic head groups are more likely to flip-flop slowly and to generate bilayer curvature strain. If the bilayer is unable to assume the spontaneous curvature required to accommodate asymmetry, strain occurs as a result of molecular compression. Where the surfactant head group is large and the hydrophobic surfactant tail is sufficiently small that it cannot completely fill the void between adjacent membrane lipids, monolayer curvature strain also leads to a disordering of the hydrophobic chains in the membrane and a thinner, more flexible, and more permeable membrane with reduced lateral packing density. Strong detergents are more able to enhance passive permeability and are considered those that partition readily into membranes (generally those with higher log P) and that have large head groups or small hydrophobic groups and therefore induce either monolayer or bilayer curvature strain. At higher surfactant concentrations, bilayer or monolayer curvature strain can result in mechanical failure of the membrane, membrane leakage, and increased passage of solutes across the membrane.

Surfactants also enhance passive drug diffusion via membrane solubilization. Membrane solubilization results when surfactants cause curvature strain to such an extent that lipids are expelled from the membrane in the form of lipid-saturated surfactant micelles. Solubilization is more likely to occur with strong detergents that induce curvature strain at relatively low concentrations and that are able to induce significant perturbations in the membrane without being squeezed out of the membrane by the induced curvature (typically surfactants that are anchored into the membrane by more than a single alkyl chain). Bile salts are particularly strong membrane solubilizers and are therefore very effective permeability enhancers in vitro (Scott Swenson and Curatolo, 1992; Aungst, 2000; Heerklotz, 2008). However, whether the concentrations of lipids or bile salts that are likely to be present in the GI tract during the digestion of LBF are sufficient to generate permeability enhancement in vivo is less clear. Assembly of bile salts and lipids into mixed micelles of bile salt, phospholipid, cholesterol, and exogenous lipids also significantly reduces the thermodynamic activity of the bile salts and reduces the likelihood of permeability enhancement.

Surfactants can therefore enhance passive diffusion across membranes via membrane permeabilization and solubilization. However, some degree of membrane
Summary of studies investigating the impact of lipid formulation components on drug efflux by major intestinal efflux transporters [P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance protein-2 (MRP-2)]

<table>
<thead>
<tr>
<th>Solubilizer</th>
<th>Transporter</th>
<th>Concentration</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brij 30</td>
<td>P-glycoprotein (Lo, 2003; Yamagata et al., 2007a)</td>
<td>20–200 μM</td>
<td>Inhibition of epirubicin or mitoxantrone transport in Caco-2 cells, P-gp expressing cells and everted gut segments</td>
</tr>
<tr>
<td>Brij 35</td>
<td>P-glycoprotein (Yu et al., 2011a)</td>
<td>30–120 μM</td>
<td>Inhibition of bis(12)-hupryridone efflux in Caco-2 cells</td>
</tr>
<tr>
<td>Cremophor EL</td>
<td>P-glycoprotein (Batrakova et al., 1998; Bogman et al., 2003; Cornaire et al., 2004; Hugger et al., 2002; Katneni et al., 2007; Martin-Facklam et al., 2002a; Mudra and Borchardt, 2010; Rege et al., 2002; Shono et al., 2004)</td>
<td>In vivo 1 mg/kg rats, 0–5000 mg in humans (i.e., 0, 100, 1000, or 5000 mg)</td>
<td>Altered pharmacokinetic profile of digoxin and celiprolol, and dose dependent increase in plasma Cmax and AUC of saquinavir, after oral administration to humans</td>
</tr>
<tr>
<td>MRP-2 (Bogman et al., 2003)</td>
<td></td>
<td>0.0002–2% (w/v)</td>
<td>No significant effect on methylfluorescein-glutathione conjugate transport by MDCK cells overexpressing MRP-2</td>
</tr>
<tr>
<td>Cremophor RH40</td>
<td>P-glycoprotein (Tayrouz et al., 2003; Yamagata et al., 2007a)</td>
<td>In vitro 50–100 μM</td>
<td>Inhibition of mitoxantrone transport in Caco-2 cells, P-gp overexpressing cells</td>
</tr>
<tr>
<td>BCRP (Yamagata et al., 2007a)</td>
<td></td>
<td>In vivo 600 mg, 3× daily</td>
<td>Altered pharmacokinetic profile of digoxin after oral administration to mice</td>
</tr>
<tr>
<td>CRL-1605 copolymer</td>
<td>P-glycoprotein (Banerjee et al., 2000; Jagannath et al., 1999)</td>
<td>132 mg/kg</td>
<td>Altered pharmacokinetic profile of tobramycin and amikacin following oral administration to mice</td>
</tr>
<tr>
<td>Gelucire 44/14</td>
<td>P-glycoprotein (Sachs-Barrable et al., 2007; Yamagata et al., 2007a)</td>
<td>0–0.02% (w/v), 1–15 μM</td>
<td>Inhibition of mitoxantrone or rhodamine transport in Caco-2 cells and P-gp overexpressing cells</td>
</tr>
<tr>
<td>BCRP (Yamagata et al., 2007a)</td>
<td></td>
<td>1–15 μM</td>
<td>No difference in mitoxantrone uptake in BCRP-expressing cells</td>
</tr>
<tr>
<td>Labrasol</td>
<td>P-glycoprotein (Cornaire et al., 2004; Hu et al., 2002; Lin et al., 2007b)</td>
<td>0.05–0.5% (w/v)</td>
<td>Inhibition of rhodamine or digoxin and celiprolol transport in vitro and in rat everted gut segments</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 mg/kg into rat colon</td>
<td>Altered pharmacokinetic profile of digoxin and celiprolol in rat bioavailability studies</td>
</tr>
<tr>
<td>Myrj 52</td>
<td>P-glycoprotein (Lo, 2003; Yamagata et al., 2007a)</td>
<td>20–200 μM in vitro, 200 μM in situ</td>
<td>Inhibition of epirubicin or mitoxantrone transport in vitro Caco-2 cells, P-gp expressing cells and in situ everted gut sacs</td>
</tr>
<tr>
<td>BCRP (Yamagata et al., 2007a)</td>
<td></td>
<td>100–200 μM</td>
<td>No effect on mitoxantrone transport in BCRP over-expressing cells</td>
</tr>
<tr>
<td>PEG 200, PEG 300, PEG 400, and PEG 20,000</td>
<td>P-glycoprotein (Hugger et al., 2002; Johnson et al., 2002; Shen et al., 2006, 2008)</td>
<td>0–20%</td>
<td>Inhibition of doxorubicin, taxol and rhodamine 123 transport in Caco-2 cells and MDR1-MDCK cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibition of prednisolone, methylprednisolone, quinidine and digoxin transport in situ or excised rat jejunum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No significant effect on methylfluorescein-glutathione conjugate transport in vitro MDCK cells overexpressing MRP-2</td>
</tr>
<tr>
<td>Pluronic F68</td>
<td>P-glycoprotein (Batrakova et al., 1998; Bogman et al., 2003; Yu et al., 2011a)</td>
<td>0–2%, 0–7050 μM</td>
<td>Inhibition of rhodamine 123 transport in Caco-2 cells but potency less than Pluronic F85 and L81</td>
</tr>
<tr>
<td>MRP-2 (Bogman et al., 2003)</td>
<td></td>
<td>0.0002–2% (w/v)</td>
<td>No significant effect on methylfluorescein-glutathione conjugate transport in vitro MDCK cells overexpressing MRP-2</td>
</tr>
<tr>
<td>Pluronic F108</td>
<td>P-glycoprotein (Miller et al., 1999)</td>
<td>100 μM</td>
<td>Inhibition of rhodamine 123 transport in KBv monolayers</td>
</tr>
<tr>
<td>MRP-2 (Miller et al., 1999)</td>
<td></td>
<td>100 μM</td>
<td>Inhibition of fluoroescin transport in pane-1 cell monolayers</td>
</tr>
<tr>
<td>Pluronic L81</td>
<td>P-glycoprotein (Batrakova et al., 1998; Miller et al., 1999)</td>
<td>40–100 μM</td>
<td>Inhibition of rhodamine 123 transport in Caco-2 cells and KBv monolayers</td>
</tr>
<tr>
<td>MRP-2 (Miller et al., 1999)</td>
<td></td>
<td>100 μM</td>
<td>Inhibition of fluoroescin transport in pane-1 cell monolayers</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Solubilizer</th>
<th>Transporter</th>
<th>Concentration</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pluronic P85</td>
<td>P-glycoprotein (Batrakova et al., 1998; Miller et al., 1999; Yamagata et al., 2007a)</td>
<td>0–220 μM</td>
<td>Inhibition of mitoxantrone transport in P-gp expressing cells Inhibition of digoxin transport in situ rat jejunum</td>
</tr>
<tr>
<td>MRP-2 (Miller et al., 1999)</td>
<td>100 μM</td>
<td></td>
<td>Inhibition of fluorescein transport in P-gp expressing cells</td>
</tr>
<tr>
<td>BCRP (Yamagata et al., 2007a,b)</td>
<td>In vitro 1–20 μM</td>
<td></td>
<td>Inhibition of mitoxantrone transport in BCRP-expressing cells In vivo 100–500 mg/kg Dose dependent increase in topotecan AUC in wild-type mice, maximum twofold increase and no effect on IV clearance. Less effective in increasing AUC after administration to BCRP (−/−) mice when compared with BCRP (+/+ ) mice (1.2-fold increase). Similar effects seen in everted gut sacs</td>
</tr>
<tr>
<td>Pluronic PE8100 and Pluronic PE6100</td>
<td>P-glycoprotein (Bogman et al., 2003)</td>
<td>0.0002–2% (w/v)</td>
<td>Inhibition of rhodamine 123 transport at in P388/MDR cells</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>P-glycoprotein (Bogman et al., 2005)</td>
<td>0.8% (w/v) solution</td>
<td>No effect on talinolol transport in Caco-2 cells or on absorption in vivo in humans</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>P-glycoprotein (Yamagata et al., 2007a)</td>
<td>1–15 μM</td>
<td>No significant effect on mitoxantrone transport in P-gp expressing cells</td>
</tr>
<tr>
<td>BCRP (Yamagata et al., 2007a)</td>
<td>1–15 μM</td>
<td></td>
<td>No significant effect on mitoxantrone transport in BCRP-expressing cells</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>P-glycoprotein (Bogman et al., 2003; Lo, 2003; Shono et al., 2004)</td>
<td>0.0002–2% (w/v)</td>
<td>No significant effect on rhodamine 123 transport</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20–200 μM</td>
<td>Inhibition of epirubicin transport in Caco-2 cells in a dose-dependent manner. Effect less pronounced than other excipients</td>
</tr>
<tr>
<td></td>
<td>MRP-2 (Bogman et al., 2003)</td>
<td>0.0002–2% (w/v)</td>
<td>No significant effect on methylfluorescein-glutathione conjugate transport by MRP-2 in MDCK cells overexpressing MRP-2</td>
</tr>
<tr>
<td>Softigen 767</td>
<td>P-glycoprotein (Cornaire et al., 2004)</td>
<td>In situ 0.05–0.5% (w/v)</td>
<td>Inhibition of digoxin and celiprolol transport in rat everted gut segments Significant increase in digoxin plasma AUC and modified plasma profile of celiprolol in vivo in rats</td>
</tr>
<tr>
<td>Solutol HS</td>
<td>P-glycoprotein (Bittner et al., 2002; Cornaire et al., 2004)</td>
<td>In situ 0.05–0.5% (w/v), In vivo 10% (w/v) solution</td>
<td>Inhibition of digoxin transport in rat everted gut segments 2-fold increase in colchicine bioavailability in rats</td>
</tr>
<tr>
<td>Span 20</td>
<td>P-glycoprotein (Yamagata et al., 2007a)</td>
<td>50–100 μM</td>
<td>Inhibition of mitoxantrone transport in P-gp expressing cells</td>
</tr>
<tr>
<td>BCRP (Yamagata et al., 2007a)</td>
<td>50–100 μM</td>
<td></td>
<td>Inhibition of mitoxantrone transport in BCRP-expressing cells</td>
</tr>
<tr>
<td>Span 80</td>
<td>P-glycoprotein (Lo, 2003)</td>
<td>20–200 μM</td>
<td>Inhibition of epirubicin transport in Caco-2 cells and in situ rat everted gut sacs</td>
</tr>
<tr>
<td>TPGS</td>
<td>P-glycoprotein (Bittner et al., 2002; Bogman et al., 2003, 2005; Brouwers et al., 2006; Chan et al., 2004; Chang and Shojaei, 2004; Chang et al., 1996; Dintaman and Silverman, 1999; Pan et al., 1996; Rege et al., 2002; Sokol et al., 1991; Varma and Panchagnula, 2005; Yamagata et al., 2007a)</td>
<td>In vitro 0–1.0 mM</td>
<td>Inhibition of Rhodamine 123, paclitaxel, mitoxantrone, and talinolol transport in Caco-2 cells and P388/MDR cells. Enhanced the cytotoxicity of P-gp substrates (doxorubicin, vinblastine, paclitaxel, and colchicines) in NIH-MDR1-GA85 cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0002–2% (w/v)</td>
<td>In vivo 50 mg/kg TPGS Increased ampprenavir, colchicine, cyclosporine, talinolol and paclitaxel bioavailability in rats or humans</td>
</tr>
<tr>
<td>MRP-2 (Bogman et al., 2003)</td>
<td>0.0002–2% (w/v)</td>
<td></td>
<td>No significant effect on methylfluorescein-glutathione conjugate transport in MDCK cells overexpressing MRP-2</td>
</tr>
<tr>
<td>BCRP (Yamagata et al., 2007a)</td>
<td>50–100 μM</td>
<td></td>
<td>No significant effect on mitoxantrone transport in BCRP-expressing cells</td>
</tr>
<tr>
<td>Tween 20</td>
<td>P-glycoprotein (Batrakova et al., 1998; Cornaire et al., 2004; Lo, 2003; Yamagata et al., 2007a)</td>
<td>20–250 μM</td>
<td>Inhibition of mitoxantrone, epirubicin and rhodamine 123 transport in P-gp expressing cells and Caco-2 cells</td>
</tr>
</tbody>
</table>

(continued)
solubilization (Aungst, 2000) appears to be required to enhance permeability, and there may be little difference in the concentrations required for permeability enhancement versus those that result in cytotoxicity (Seelig and Gerebtzoff, 2006).

More recently, an increasing number of studies have described the ability of LBF components to enhance drug permeability via inhibition of the major intestinal efflux transporters P-gp, BCRP, and MRP (see Table 27). Several recent reviews have also addressed excipient-based transporter inhibition (Seelig and Gerebtzoff, 2006; Constantinides and Wasan, 2007; Werle, 2008; Li-Blatter et al., 2009; Goole et al., 2010). The proposed mechanisms by which lipidic excipients influence efflux activity include direct interaction with the transporter (Fréchette et al., 1990a; Spoelstra et al., 1991; Zordan-Nudo et al., 1993; Orlowski et al., 1998), changes to membrane fluidity or the microenvironment.
of membrane lipid domains (leading to indirect effects on transporter activity via protein destabilization) (Woodcock et al., 1992; Loel and Sharom, 1993; Regev et al., 1999), or changes to efflux transporter expression (Risovic et al., 2003, 2004; Sachs-Barrable et al., 2007; Barta et al., 2008).

Seelig and coworkers suggest that surfactants that are able to anchor into the membrane most effectively (via the presence of a hydrophobic tail) and that also display P-gp binding motifs, for example, 1,2, dicaprylin, tricaprylin (Miglyol 808; Sasol, Hamburg, Germany), PEG oleate, Cremophor EL, Solutol HS-15, vitamin E, vitamin E acetate, vitamin E TPGS, Tween 80, octyl-β-glucoside, and Triton X-100, are most capable of enhancing drug permeability in vivo. In contrast, the same authors suggest that high-molecular-weight excipients, such as polyethylene glycols and polyoxyethylene-polyoxypropylene block copolymers (poloxamers), may be less likely to inhibit P-gp in vivo (despite in vitro evidence to the contrary) owing to difficulties in incorporation into the intestinal membrane, since the intestinal membrane has a greater lateral packing density than most in vitro cell lines (Seeleg and Gerebtzoff, 2006). They also suggest that further data are required to support whether membrane fluidization is relevant to the in vivo mechanism of P-gp inhibition for most surfactants. For example, Rege et al. (2002) concluded that membrane fluidization is an unlikely mechanism of permeability enhancement as Tween 80 and Cremophor EL fluidize lipid bilayers, whereas vitamin E TPGS rigidifies rather than fluidizes lipid bilayers, yet all inhibit multiple efflux transporters proficiently.

a. Correlation of In Vitro Effects of Lipid-Based Formulation Excipients on Permeability with Changes to In Vivo Exposure? Although several studies have shown an effect of lipidic excipients on permeability in vitro, reports of the effects on the permeability of poorly water-soluble drugs in vivo are more limited. In part, this reflects the difficulty in delineating permeability enhancement from solubilization since most of the amphiphiles that affect permeability also impact on solubilization. For poorly water-soluble drugs where permeability and solubility limit bioavailability, ascribing an increase in bioavailability to formulation effects on either permeability or solubility is therefore complex. Studies using probes where solubility is less likely to limit bioavailability (e.g., BCS class 3) provides for easier data interpretation and may allow effects of the same surfactant to be inferred in studies with drugs where solubility is a confounding issue.

Surfactant effects on permeability are likely to depend on high localized concentrations of surfactant, regardless of whether the change to permeability reflects inhibition of an efflux transporter or effects on passive transport. In vivo, however, this may be difficult to achieve since dilution of the formulation in the GI fluids, the large surface area of the GI tract, differences in dissolution rate of the drug and excipient, changes to intestinal pH and motility, and intersubject and intrasubject variability will conspire to limit excipient accumulation at the site of drug absorption. Increases in permeability may also be difficult to achieve in vivo as drug absorption and excipient effects on intestinal permeability are often site dependent. For example, drugs are commonly absorbed from the proximal small intestine, whereas some permeation enhancers are more effective at promoting passive diffusion in the distal intestine (Yamamoto et al., 1994; Yeh et al., 1994; Sekine et al., 1985; Aungst, 2000).

Finally, membrane properties and the expression and activity of efflux transporters differ between in vitro and in vivo models. For instance, the intestinal membrane is seemingly more resistant to enhancements in passive diffusion than in vitro models, possibly as a result of differences in the types of lipids in the membrane and the greater lateral packing density of the intestinal membrane compared with many cultured cell lines (Aungst, 2000; Seeleg and Gerebtzoff, 2006). The intestinal membrane is also covered in a thick layer of mucus that is absent in most cell lines, and many in vitro cell lines, particularly immortalized cell lines, overexpress a number of the common transport proteins. The intestinal membrane may therefore be less readily accessed or less readily penetrated by surfactants that might otherwise permeabilize/solubilize cell membranes or inhibit transporter efflux in vitro. Nonetheless, in vitro studies provide a useful tool to generate rank-order relationships for permeability enhancing effects (Quan et al., 1998; Aungst, 2000) and allow for greater insight into the mechanisms of permeation enhancement.

Despite the complexities of in vitro– in vivo correlation of permeability data, a number of in vivo studies have shown enhanced absorption of hydrophilic or macromolecular drugs after coadministration with lipidic excipients, including FA (Burcham et al., 1995; Constantinides et al., 1996), monoglycerides (Beskid et al., 1988; Constantinides et al., 1994; Hastewell et al., 1994; Lundin et al., 1997), and surfactants (Prasad et al., 2003; Rama Prasad et al., 2003), suggesting that lipidic excipients may enhance the absorption of poorly permeable (but water-soluble) molecules via increases in passive permeability.

In vivo studies in which lipidic excipients have been proposed to enhance oral absorption via inhibition of intestinal efflux include studies with both hydrophilic and poorly water-soluble, lipophilic drugs. These include talinolol (Bogman et al., 2005), saquinavir (Martin-Facklam et al., 2002a), amprenavir (Brouwers et al., 2006), digoxin (Tayrouz et al., 2003), cyclosporine A (Sokol et al., 1991; Chang et al., 1996; Pan et al., 1996), and paclitaxel (Varma and Panchagnula, 2005;
Constantinides and Wasan, 2007) (all of which are substrates for P-gp efflux) and topotecan (a substrate for BCRP) (Yamagata et al., 2007a). The latter (Yamagata et al., 2007a), provides an excellent example of a study design that has allowed differentiation of effects on permeability versus solubility for a poorly water-soluble drug. In this work, the authors showed increases in exposure after coadministration of topotecan with Tween 85 and Pluronic P85 to mice but went on to show no effects after i.v. administration (ruling out surfactant effects on systemic disposition) or after oral administration to BCRP knockout mice (ruling out effects on solubilization).

Coadministration of dietary lipids and LBF components may also affect presystemic metabolism. Examples of increases in drug exposure as a result of changes in presystemic metabolism stimulated by lipids (Patel and Brocks, 2009) or other solubilizing excipients (Buggins et al., 2007) have been reviewed recently. The potential for lipids to influence drug exposure via effects on metabolism is well illustrated by studies that show either increased (Gupta et al., 1990; Gupta and Benet, 1990) or decreased metabolism (Liedholm and Melander, 1986; Milton et al., 1989; Humberstone et al., 1996; Humberstone et al., 1998; Meng et al., 2001) after drug administration to humans (Liedholm and Melander, 1986; Milton et al., 1989; Gupta et al., 1990; Gupta and Benet, 1990; Meng et al., 2001) or beagle dogs (Humberstone et al., 1996, 1998) with a high-fat meal. Similar effects have been described in rats after administration with relatively large lipid doses (on a milligrams per kilogram basis). For example, coadministration with 2 ml/kg peanut oil containing 1% cholesterol decreased the metabolism of halofantrine and amiodarone (Brocks and Wasan, 2001) or beagle dogs (Humberstone et al., 1996, 1998; Liedholm and Melander, 1986; Milton et al., 1989; Meng et al., 2001) after drug administration to humans (Liedholm and Melander, 1986; Milton et al., 1989; Gupta et al., 1990; Gupta and Benet, 1990; Meng et al., 2001) or beagle dogs (Humberstone et al., 1996, 1998) with a high-fat meal. Similar effects have been described in rats after administration with relatively large lipid doses (on a milligrams per kilogram basis). For example, coadministration with 2 ml/kg peanut oil containing 1% cholesterol decreased the metabolism of halofantrine and amiodarone (Brocks and Wasan, 2001; Shayeganpour et al., 2005) and 50–200 μg/kg docosahexaenoic acid appeared to reduce the metabolism of cyclosporine, saquinavir, and midazolam by reducing intestinal but not hepatic metabolism (Hirunpanich et al., 2006, 2008; Hirunpanich and Sato, 2006). Metabolism of amiodarone was also reduced after incubation with intestinal segments isolated from rats preadministered 2 ml/kg peanut oil with 1% cholesterol compared with control rats (Shayeganpour et al., 2008). The metabolism of halofantrine by isolated intestinal segments was similarly reduced in the presence of lipids (Patel et al., 2010), and a trend toward lower systemic metabolite to parent ratios was evident after administration of anethol trithione to rats with lipid formulations (Yu et al., 2011b). These studies demonstrate that the glycerides and FA present in LBF can influence drug metabolism. However, further studies are required to confirm that this is the case after in vivo administration of the smaller quantities of lipid typically contained in LBF.

The mechanism by which lipids are able to perturb presystemic metabolism are drug and formulation dependent and include the potential for both direct effects on enzyme activity and indirect effects via changes to enzyme expression levels (Patel and Brocks, 2009). For example, in hepatic microsomes, FAs directly inhibit Cyp isoenzymes, and the efficiency of inhibition decreases in the following order: polyunsaturated FA > unsaturated FA > saturated FA (Hirunpanich et al., 2007). In contrast, the expression levels of Cyp isoenzymes are either increased or decreased on in vitro incubation with LC FA in rat hepatocytes (Li et al., 2006, 2007) and after long-term ingestion of certain dietary oils in rats (Yoo et al., 1991; Li et al., 1992; Brunner and Bai, 2000). Lipid coadministration can also influence systemic metabolism by altering systemic lipoprotein levels and therefore the nature of drug binding in the blood. Association with lipoproteins has been shown to both reduce and enhance drug uptake into metabolic cells and thus increase or decrease systemic metabolism (Wasan et al., 2008; Patel and Brocks, 2009). Decreases in free concentrations of halofantrine resulting from increased binding to plasma lipoproteins have also been suggested to reduce systemic clearance and volume of distribution (Humberstone et al., 1998). In contrast, increased lipoprotein binding has been reported to increase, decrease, or not change the area under the plasma concentration versus the time profile of cyclosporine, depending on the model of hyperlipidemia used (Wasan et al., 2008). It seems unlikely, however, that the small lipid doses contained in LBF will lead to changes in systemic lipoprotein concentrations that are sufficient to influence drug metabolism via an effect on binding.

In addition to lipids, excipients, including surfactants and cosolvents, also influence drug metabolism in vitro and in situ. Tween 20, Tween 80, vitamin E TPGS, Cremophor EL, Cremophor RH40, Brij 35, Solutol HS-15, and other nonionic surfactants inhibit Cyp3A4 and Cyp2C9 in a concentration-dependent manner in liver microsomes or isolated hepatocytes at concentrations below the CMC (Bravo González et al., 2004; Randall et al., 2011; Rao et al., 2010; Christiansen et al., 2011). PEG400 and Pluronic 85 inhibit verapamil metabolism in isolated rat intestinal segments, whereas in the same model, TPGS showed only marginal effects (Johnson et al., 2002). In an in situ rat perfusion model PEG-400, but not Cremophor EL, TPGS, or Tween 80 reduced verapamil metabolism (Mudra and Borchardt, 2010). Mountfield et al. (2000) further reported that a number of potential LBF components, including oils, cosolvents, and surfactants, were able to inhibit Cyp3A4 at relatively high concentrations, but they concluded that only the amphiphilic excipients (Tween 80 and oleic acid) were likely to be capable of significant enzyme inhibition at the concentrations one would expect to find in vivo. Fewer studies have attempted to examine the potential for
surfactants or cosolvents to inhibit Cyp-450-mediated metabolism in vivo. Ren et al. (2009) demonstrated inhibition of midazolam metabolism in vitro and some changes to in vivo metabolism of midazolam after administration with polysorbate 20, Cremophor EL, Myrj 52, and Pluronic F68. Inhibition of metabolism, in addition to effects on intestinal transporters, has also been suggested as the mechanism by which formulations containing TPGS and Cremophor EL enhance the absorption of cyclosporine (Chang et al., 1996) and saquinavir (Martin-Facklam et al., 2002a), respectively.

How surfactants and cosolvents inhibit metabolism and whether they are able to provide useful increases in exposure by inhibiting drug metabolism in vivo remain unclear. Proposed mechanisms of inhibition include indirect effects on metabolic enzyme activity via membrane fluidization or depletion of ATP (as has been described for P-glycoprotein mediated efflux (Christiansen et al., 2011) or direct effects on enzyme function or expression (Rao et al., 2010; Christiansen et al., 2011). A number of surfactants are also digestible in vivo (Fernandez et al., 2007, 2008; Cuiné et al., 2008; Christiansen et al., 2010). Digestion is likely to alter significantly the physicochemical properties of surfactants and, therefore, the potential for inhibition of metabolic or efflux processes. This point, however, has not been addressed directly. In many cases, surfactant digestion will also liberate FA and the liberated FA might also be expected to impact enzyme activity, although the quantity of FA produced via surfactant digestion will be limited. In addition to digestion, the potential for surfactant degradation in the acidic conditions in the stomach, dilution in the GI fluids, and poor absorption into enterocytes further complicate the likelihood of useful in vivo activity and in vitro-in vivo correlation.

The impact of excipients on efflux and metabolism is potentially linked by the proposed “metabolic alliance” between efflux transporters and metabolic enzymes in the GI tract (e.g., P-glycoprotein and Cyp3A4) (Benet and Cummins, 2001; Benet et al., 2004). In this model, efflux is believed to enhance the time available for enterocyte-based presystemic metabolism. As such, inhibition of efflux might be expected to have the additional benefit of providing protection from enterocyte-based metabolism by reducing the time available for metabolism.

4. Promotion of Lymphatic Drug Transport. In addition to potential effects on drug solubilization, permeability, and metabolism, for a small subset of highly lipophilic drugs, coadministration with lipids can affect the route of drug trafficking to the systemic circulation through the promotion of drug transport via the intestinal lymphatic system. Lymphatic drug transport has been described for many highly lipophilic drugs and has been the subject of several reviews (Porter and Charman, 2001b; O’Driscoll, 2002; Porter et al., 2007; Trevaskis et al., 2008; Yanez et al., 2011).

The process of lymphatic drug transport is summarized in Fig. 64. After oral administration, drug absorption typically involves uptake into and passage across enterocytes into the underlying lamina propria, where both blood and lymphatic capillaries are present. Blood drains from the mesenteric capillaries into the portal vein and subsequently flows via the liver to the systemic circulation. Blood flow from the intestine via the portal vein is some 500-fold faster than lymph flow through the mesenteric and thoracic lymph ducts; as such, the predominant drug absorption route for most drug molecules is via the blood.

As described in Section XI.A.1, ingestion of dietary fat promotes the assembly of triglyceride-rich lipoproteins (TRL), including chylomicrons and VLDL in the enterocyte, and the subsequent transport of these lipoproteins from the intestine to the systemic circulation occurs via the intestinal lymph (Kindel et al., 2010; Mansbach and Siddiqi, 2010). For some highly lipophilic drugs, association with these lipoprotein assembly and transport pathways leads to drug transport to the systemic circulation via the lymph rather than via the blood, and under some circumstances, notably after postprandial administration (where lipid absorption and
lipoprotein synthesis are high), lymphatic transport may be the dominant means of drug transport (Khoo et al., 2001; Trevaskis et al., 2008). Since lymph drains from the lymphatic capillaries into the mesenteric and thoracic lymph ducts before emptying directly into the systemic circulation, intestinal lymphatic drug transport avoids first-pass hepatic metabolism.

In addition to dietary lipids, formulation-derived lipids also stimulate TRL assembly and lymphatic drug transport. In general, long-chain and monounsaturated glycerides stimulate lipoprotein formation more effectively than medium-chain and saturated glycerides and more effectively promote lymphatic drug transport (Charman and Stella, 1986; Caliph et al., 2000; Khoo et al., 2003). TRL production and lymphatic drug transport are predictably increased by the administration of higher lipid loads (Charman and Stella, 1986; Khoo et al., 2003; White et al., 2009).

Historically, the physicochemical properties that have been ascribed to drugs that are amenable to significant lymphatic transport are a log $P > 5$ (or log $D$ for ionizable compounds) and solubility in long-chain triglycerides $>50$ mg/g (Charman and Stella, 1986; Porter and Charman, 2001b; O’Driscoll, 2002; Porter et al., 2007; Trevaskis et al., 2008). These properties reflect the necessity for avid drug partition into TRL to drive drug transport via the lymphatics. Most lymphatically transported drugs conform to these characteristics; however, more recent studies have also suggested that in some cases, drugs with lower lipid solubility but a specific affinity for lipoproteins beyond simple partitioning behavior (e.g., affinity for the lipoprotein interface) may also be significantly transported via the lymph (Gershkovich and Hoffman, 2005; Trevaskis et al., 2010a; Gershkovich et al., 2009). Nonetheless, all these compounds remain highly lipophilic as defined by log $P$.

For almost all lymphatically transported drugs, coadministration with lipids leads to increases in bioavailability and increases in lymphatic transport. This has led to the suggestion that stimulation of lymphatic drug transport is able to increase drug absorption. Definitive evidence to support this contention, however, is lacking since delineation of lipid effects on solubilization versus lymphatic transport is difficult. Nonetheless, promotion of lymphatic drug transport can increase the oral bioavailability of drugs that are subject to significant first-pass metabolism (Khoo et al., 2001; Shackleford et al., 2003; Trevaskis et al., 2009; White et al., 2009). For example, food has a substantial positive effect on the oral bioavailability of a lipophilic cannabinoid receptor agonist CRA13 in humans (Fig. 65A). To investigate the mechanism by which bioavailability is enhanced by food, the extent of absorption and bioavailability of CRA13 was compared in fasted beagles administered radiolabeled CRA13 and in lymph-cannulated greyhound dogs administered nonlabeled CRA13 after a meal (Trevaskis et al., 2009). Consistent with the data in humans, the absolute bioavailability of CRA13 was low in fasted dogs (8–20%). However, this was not due to poor absorption as
72–75% of radiolabeled CRA13 was recovered in the systemic circulation (Fig. 65B), suggesting significant first-pass metabolism. Bioavailability was increased to 47.5% in the dogs after postprandial administration (Fig. 65B), and most of the dose (43.7%) was transported to the systemic circulation via the lymphatic system (Fig. 65C). The total extent of absorption of CRA13 in the fed lymph-cannulated dogs was estimated at 63.2% (from addition of the extent of lymphatically transported drug and the systemic availability of drug in the blood, assuming 80% first-pass metabolism) (Fig. 65B). As CRA-13 was well absorbed in both fasted and fed dogs, the positive effect of food on the bioavailability of CRA13 appeared to reflect stimulation of lymphatic transport resulting in reduced first-pass metabolism.

For drugs with very high first-pass metabolism, lymphatic transport can be responsible for the delivery of most of the bioavailable drug to the systemic circulation, even when the overall extent of lymphatic transport is relatively low. For example, the oral bioavailability of testosterone and methyltestosterone is essentially zero owing to very high first-pass metabolism. The synthesis of lipophilic prodrugs of both compounds, however, increases the proportion of the dose that partitions into developing lymph lipoproteins and therefore facilitates lymphatic drug transport and the direct delivery of bioavailable drug to the systemic circulation. Although these prodrugs are relatively inefficient, the degree of exposure is sufficient to support an oral testosterone drug product (Andriol, Merck) (Shackleford et al., 2003; White et al., 2009). Recent evidence further suggests that lymphatic transport may provide some protection from enterocyte-based metabolism by sequestering drug and into developing lipoproteins (Trevaskis et al., 2006).

B. Design and Formulation of Lipid-Based Formulations

LBF comprise a diverse group of materials—including lipid solutions, emulsions, microemulsions, nanoemulsions, micellar solutions, liposomes, lipid nanoparticles, and emulsion preconcentrates (anhydrous formulations that spontaneously emulsify on contact with aqueous media and are commonly referred to as self-emulsifying drug delivery systems or SEDDS). LBF may be liquid, semisolid, or solid at room temperature and intended for oral or parenteral administration.

1. Lipid-Based Formulations for Parenteral Administration. A number of different types of formulations containing lipids—including lipid solutions, emulsions, microemulsions, micellar solutions, liposomes, and solid lipid nanoparticles (including variations such as nanostructured lipid carriers (NLCs) (see Section XII.B) and lipid-drug conjugate (LDC) nanoparticles)—have been used to facilitate effective i.v., s.c., i.m., or local (e.g., intra-articular) administration of poorly water-soluble drugs (Constantinides et al., 2008). In general, parenteral LBF have been used for two primary purposes. First, they have been used as a means to allow the delivery of lipophilic, poorly water-soluble drugs in a vehicle designed to be analogous to a cosolvent formulation or cyclodextrin solution (i.e., one that has little or no effect on systemic pharmacokinetic parameters). In contrast, many of the more complex parenteral LBF—such as liposomes, solid lipid nanoparticles, certain micellar solutions, and various liquid crystalline phases—have been used to intentionally influence pharmacokinetic and biodistribution profiles and to target drug delivery to specific anatomic or pathologic regions (e.g., tumors) (Shi and Li, 2005; Constantinides et al., 2008). For example, liposomal formulations of amphotericin (Janknegt et al., 1992) and doxorubicin (Lasic, 1996) have significantly improved patient therapy by modifying pharmacokinetics to improve activity and safety profiles. The formulation of lipid-based parenteral formulations as targeted drug delivery systems to optimize drug distribution patterns to sites of activity versus toxicity is beyond the scope of the current review. The interested reader is directed to the following excellent reviews for further information regarding liposomes: Lian and Ho (2001), Constantinides et al. (2008), and Puri et al. (2009); and the following reviews are recommended for solid lipid nanoparticles: Müller et al. (2000), Mehnert and Mader (2001), Wissing et al. (2004), Joshi and Muller (2009), Puri et al. (2009), and Bunjes (2010).

In contrast, parenteral lipid solutions or suspensions (Chien, 1981; Zuidema et al., 1994; Murdam and Florence, 2000; Larsen et al., 2009; Weng Larsen and Larsen, 2009) are simple to formulate and are commonly marketed for i.m., s.c. (predominantly for veterinary use), and intra-articular (Larsen et al., 2008b) administration of lipophilic drugs. For example, lipid solutions of lipophilic prodrugs have been used successfully in the treatment of schizophrenia and hormone replacement therapy for more than 30 years (Davis et al., 1994; Weng Larsen and Larsen, 2009; Leucht et al., 2011). The aim of these formulations is to sustain drug release by providing a “depot” at the site of administration. The release of lipophilic drugs from the lipid depot is slowed by partitioning behavior that favors drug association with the vehicle rather than the surrounding interstitial fluid. Validation of the reproducibility of drug release is therefore a critical design aspect to ensure against dose dumping (Larsen et al., 2009; Weng Larsen and Larsen, 2009). Long-acting parenteral lipid solutions typically consist of a lipophilic drug (or lipophilic prodrug derivative) dissolved in vegetable oil (e.g., sesame oil, soybean oil, safflower oil, Miglyols, castor oil, cottonseed oil) (Strickley, 2004). In some cases, a solubilizing agent
(e.g., benzyl benzoate) or antioxidant (e.g., tocopherol) may be added.

Conventional oil-in-water emulsions and nanoemulsions are also widely used for parenteral delivery of poorly water-soluble drugs during preclinical (Li and Zhao, 2007; Shah and Agnihotri, 2011) and clinical evaluation (Floyd, 1999; Driscoll, 2006b; Hippalgaonkar et al., 2010). Intravenous lipid emulsions have been used as a part of parenteral nutrition programs for more than 50 years: Intralipid (10% or 20% soybean oil, 1.2% egg phosphatides, and 2.5% glycerol) was introduced in 1961 and remains in use today (Floyd, 1999; Driscoll, 2006a; Hippalgaonkar et al., 2010). Lipid emulsions have subsequently been used to facilitate the delivery of lipophilic drugs via various parenteral administration routes, including i.v., s.c., i.m., and intra-articular administration. Indeed, several marketed products use lipid emulsions to facilitate i.v. administration—including devidipine, diazepam, propofol, etomidate, perfluorodecalin, perfluorotripropylamine, alprostadil, dexamethasone palmitate, flurbiprofen axetil, and vitamins A, D2, E, and K1 (Strickley, 2004; Hippalgaonkar et al., 2010). The composition of current products and considerations in the development, manufacture and approval (including safety issues) of lipid emulsions have been reviewed elsewhere (Floyd, 1999; Strickley, 2004; Cannon et al., 2008; Driscoll, 2006a; Date and Nagarsenker, 2008; Bunjes, 2010; Hippalgaonkar et al., 2010; Mirtallo et al., 2010) and are briefly addressed in the following section.

Lipid emulsions are most often used for drugs with very low water solubility, where more traditional parenteral formulation options (e.g., cosolvents, pH control) are not possible, and where the lipid solubility of the drug molecule is high. Other advantages include the potential to reduce local pain and irritation, improved drug stability, and the availability of large-scale methods of production such as homogenization. Disadvantages include the inherent thermodynamic instability of lipid emulsions, the limited number of lipids and emulsifiers that have been approved for parenteral use, and the possibility of low drug loading (Mirtallo et al., 2010). Parenteral administration in a lipid emulsion can also alter clearance and biodistribution profiles (Hippalgaonkar et al., 2010; Caliph et al., 2012), and these effects require consideration in both the clinical and preclinical setting.

Parenteral lipid emulsions generally consist of 10–30% triglyceride (long chain and/or medium chain), emulsifiers, and an aqueous phase. LC triglycerides approved for clinical use include triolein, soybean oil, safflower oil, sesame oil, and castor oil, whereas MC triglycerides include fractionated castor oil, Miglyol 810 and 812, Neobee M5 (Stepan Company, Deerfield, IL), and CapteX 300. Drug solubility (mg/ml) in MC triglycerides is usually higher than in LC triglycerides, and MC triglycerides typically exhibit greater oxidative stability than LC triglycerides (as a result of the presence of fewer unsaturated fatty acid chains). The choice of emulsifier is driven by toxicity, the need for all components in parenteral formulations to be metabolized or excreted, the potential for effective emulsion stabilization, and the need for stability during sterilization. Natural lecithin, from either animal (egg yolk) or vegetable (soybean) origin, has been used as an emulsifier in almost all marketed products. Lecithin-stabilized emulsions require high-pressure colloid milling to facilitate emulsification, but the resulting oil-in-water dispersions are remarkably stable owing to strong adsorption of the lecithin at the oil-water interface. The aqueous phase of parenteral emulsions contains ionic or osmotic agents, antioxidants, buffers, and preservatives (Floyd, 1999; Hippalgaonkar et al., 2010). Oils exert no osmotic effects, so isotonic adjustment (to 280–300 mosm/kg) is typically achieved via the addition of glycerol, sorbitol, or xylitol to commercial preparations. Buffering agents are generally not used as they may catalyze hydrolysis of TG and PL and reduce emulsion stability. A small amount of sodium hydroxide is often added to adjust to a slightly alkaline pH (~8.0) as emulsion pH decreases on sterilization and storage because of the small amounts of hydrolysis of TG and PL to free FA.

Parenteral emulsions are typically formulated by dissolving drug in the oil phase before emulsification with the aqueous phase (i.e., de novo emulsion formation) or via the addition of drug to preprepared commercial parenteral emulsions. The latter approach is a relatively simple way to prepare poorly water-soluble drugs for i.v. administration in preclinical studies. In this case, the drug is predissolved in a cosolvent and slowly added to a commercial lipid emulsion under conditions of agitation (e.g., ultrasonication or homogenization). The de novo method is more commonly used for clinical formulations, but drug may also be incorporated into preprepared i.v. emulsions with the aid of a cosolvent, via direct incorporation of drugs, which are liquid at room temperature (e.g., propofol), or via direct incorporation of nanomilled drug powders or nanocrystals followed by homogenization. De novo preparation is achieved by first dissolving (usually with heating) all water-soluble and oil-soluble ingredients in the aqueous or lipid phase, respectively. Emulsifiers are then added to either the oil or aqueous phase. The lipid phase is mixed with the aqueous phase under controlled conditions (temperature, agitation, rate of addition) to form a coarse emulsion (droplet size <20 μm). The droplet size of the coarse emulsion is subsequently reduced (to a maximum mean droplet size <500 nm) via homogenization or microfluidization. The emulsion is ultimately filtered to remove any large particulates. Sterilization is achieved through aseptic preparation.
or terminal heat sterilization or filtration. Glass containers are preferred as poorly water-soluble drugs and lipophilic components may adsorb to plastic and because plastics are more permeable to oxygen and susceptible to leaching oil-soluble plasticizers.

Characterization of the resulting emulsion involves the examination of visual appearance (creaming, coalescence, oil separation, color change), chemical analysis (drug and excipient and degraded product content, e.g., FA), droplet size distribution and zeta potential, viscosity, pH, preservative tests, sterility, and pyrogen testing (Driscoll, 2006b). For preclinical studies, preparation under as near as possible to aseptic conditions followed by filtration before administration is usually sufficient, along with verification of drug content after preparation and filtration. Mean droplet size is an important indicator of emulsion stability, and the presence of larger droplets (>5 μm) is likely to lead to fat embolism and toxicity. The US Pharmacopeia limits for lipid injectable emulsions include pH between 6.0 and 9.0, mean droplet size ≤500 nm, volume proportion of larger fat globules (>5 μm) ≤0.05%, and free fatty acids ≤0.07 mEq/g (Driscoll, 2006b).

2. Lipid-Based Formulations for Oral Administration. For oral administration, LBF may be liquid, semisolid, or solid formulations at room temperature and comprise lipid solutions, emulsions, microemulsions, micellar solutions, emulsion preconcentrates, liposomes, and lipid nanoparticles. Liquid formulations are useful in the preclinical setting as they are relatively quick to develop and can be gavaged at a range of doses (Li and Zhao, 2007; Shah and Agnihotri, 2011). They may also be of benefit in select patient groups (pediatrics or those with swallowing difficulties), and a limited number of liquid LBF have been marketed (Strickley, 2004, 2007).

In most clinical applications, however, solid dose forms are preferred. To facilitate this, liquid LBF such as lipid solutions and self-emulsifying drug delivery systems (SEDDS) are filled into hard or soft gelatin capsules. SEDDS form emulsions with particle sizes in the nanometer to micrometer size range, and the terminology that has been used to describe these systems is complex. Initially, second-generation SEDDS, which dispersed to form optically clear emulsions with low particle sizes (typified by the cyclosporine Neoral formulation), were classified as self-microemulsifying drug delivery systems (SMEDDS) with the understanding that what was produced was a microemulsion. However, the definition of a microemulsion is thermodynamic (i.e., microemulsions are thermodynamically stable, unlike all other emulsions) and does not relate to particle size. Indeed, most pharmaceutically relevant microemulsions have particle sizes in the nanometer size range. This has led to the use of SNEDDS as a descriptor based on particle size rather than thermodynamic stability (which is inherently difficult to measure). Perhaps the more flexible definition is to use the generic description of SEDDS formulations and subsequently to quote a particle size to define more accurately the dispersion obtained. Regardless of definition, the feasibility of solid dosage forms containing liquid LBF is evident in the availability of several marketed products (Strickley, 2007).

Solid and semisolid LBF are also being developed with greater frequency (Vasanthavada and Serajuddin, 2007; Cole et al., 2008; Jannin et al., 2008; Tang et al., 2008; Shah and Serajuddin, 2012; Tan et al., 2012), and a number of lipid formulation excipients are solid or semisolid at room temperature. Examples include hydrogenated vegetable oils, saturated fatty acids, and glycerides and semisynthetic glycerides based on hydrogenated vegetable oils (e.g., Gulecires). A more exhaustive list of excipient melting point and physical state data are available in Gibson (2007). Solid and semisolid lipid fill materials minimize the chance of capsule leakage and incompatibilities on storage (Cole et al., 2008) and may provide for some degree of sustained release (Vasanthavada and Serajuddin, 2007; Jannin et al., 2008; Tang et al., 2008). However, drugs are typically incorporated into solid or semisolid lipidic vehicles by mixing under elevated temperature before capsule filling, and drug or lipid may phase-separate and crystallize when the vehicle solidifies. More stable systems have been described in which solid SEDDS are prepared by dissolving drug in a melt containing SEDDS excipients and high-molecular-weight PEG or poloxamer (to promote solidification) followed by hot filling into capsules and cooling (Li et al., 2009b; Shah and Serajuddin, 2012). A range of novel formulation techniques have also been used to transform liquid or semisolid formulations into solid particles (powders, granules, or pellets) that can be filled into capsules or sachets or compressed into tablets. These techniques have been reviewed in detail (Vasanthavada and Serajuddin, 2007; Jannin et al., 2008; Tang et al., 2008) and may be used to enhance drug solubility and to promote absorption or to control drug release. Commonly used techniques include spray cooling, spray drying, adsorption onto solid carriers, melt granulation, melt extrusion, high-pressure homogenization (to produce solid lipid nanoparticles or nanostructured lipid carriers), and a range of supercritical fluid–based methods.

In spray cooling, a melt is sprayed into a cooled chamber in which the molten droplets congeal and recrystallize into spherical solid particles. Polyoxylglycerides, particularly stearoyl polyoxyglycerides (Gelucire 50/13) (Cavallari et al., 2005; Passerini et al., 2006), are frequently used for spray-cooled products. In contrast, spray drying involves spraying a liquid solution into a heated rather than a cooled chamber to evaporate the solvent (organic solvent or water), yielding solid microparticles. In this way, LBF excipients (e.g., lauroyl
or stearoyl polyoxylglycerides) either alone or in combination with a solid carrier may be solubilized in organic solvent and spray dried to remove the solvent (Chauhan et al., 2005). Alternatively, oil-in-water emulsions may be spray dried to prepare dry emulsions. Medium-chain triglycerides and oleyl polyoxylglycerides have been used as the lipophilic phase in dried emulsions of this type (Christensen et al., 2001; Dollo et al., 2003; Hansen et al., 2004; Jang et al., 2006; Yi et al., 2008; Oh et al., 2011; Kang et al., 2012). To generate adsorbed solid LBF, liquid lipid formulations such as SEDDS are simply adsorbed to a carrier (e.g., calcium silicate, magnesium aluminometasilicate, silicon dioxide, or carbon nanotube) under mixing to form a free-flowing powder (Ito et al., 2005; Patil and Paradkar, 2006; Abdalla et al., 2008; Agarwal et al., 2009; Tan et al., 2009; Dixit and Nagarsenker, 2010; Wang et al., 2010c; Tan et al., 2011; Van Speybroeck et al., 2012).

Solid or semisolid lipids (e.g., polyglycerol esters, lecithin, partial glycerides, polysorbates) (Seo et al., 2003; Shimpi et al., 2005; Vihelmersen et al., 2005) have also been used to create solid SEDDS via traditional melt granulation, effectively forming an emulsifiable solid dispersion (see Section X). Melt granulation may be used further to adsorb semisolid self-emulsifying systems onto solid carriers (Gupta et al., 2001, 2002). In contrast, melt extrusion techniques force molten lipid-based formulations through a die under controlled conditions to produce a product that subsequently solidifies to form extrudates of uniform shape and density. Lipid-based excipients, such as sucrose monopalmitate (Surhype D-1616; Halim Biotech, Singapore, Singapore), lauroyl polyoxylglycerides (Gelucire 44/14), and polysorbate 80 (Tween 80) have also been included as additives in traditional melt extrusion formulations (e.g., those containing microcrystalline cellulose) to enhance the dissolution of poorly water-soluble drugs (Hulsmann et al., 2000; Serratoni et al., 2007). More complex extrusion techniques have described the specific introduction of lipidic excipients (such as Gelucire 44/14) into the core of sustained release formulation matrices (Mehuys et al., 2004, 2005; Windbergs et al., 2010).

Solid lipid nanoparticles (SLNs, which possess a solid core) or nanostructured lipid carriers (NLCs, which possess a liquid core) may be produced by high-pressure homogenization (Müller et al., 2000; Mehnert and Mader, 2001; Hu et al., 2004c; Puri et al., 2009; Bunjes, 2010). SLNs typically consist of a solid matrix of high melting lipids such as glyceryl dibehenate (Compritol 888 ATO; Gattefossé) and surfactants such as poloxamer 188 (Pluronic F68) or Tween 80. NLCs generally contain a liquid lipid excipient such as MC triglycerides in addition to the components of SLNs. Supercritical fluid–based methods have also been used to generate SLNs where the drug and lipidic excipients are dissolved in a supercritical fluid, followed by a gradual decrease in temperature and pressure to promote phase separation and the generation of lipid-coated drug particles or solid dispersions containing lipids. Lipid excipients that have been used during the production of supercritical fluid–based SLNs include glyceryl trimyristate (Dynasan 114; Sasol), stearoyl polyoxylglycerides (Gelucire 50/13), vitamin E TPGS, and lauroyl polyoxylglycerides (Gelucire 44/14) (Sethia and Squillante, 2002; Ribeiro Dos Santos et al., 2003; Thies et al., 2003). Techniques for formulating solid dispersions containing lipids and SLN/NLC are described in more detail in Sections X and XII.B, respectively.

3. The Lipid Formulation Classification System. Pouton (2000) introduced the Lipid Formulation Classification System (LFCS) in 2000 to describe and classify LBF according to their composition and behavior on dispersion and digestion. The LFCS was subsequently updated in 2006 to include an additional classification (Pouton, 2006). There are currently four classes of LBF listed in the LFCS. The advantage of the LFCS lies in its simplicity; however, there are also limitations when trying to classify all possible combinations of excipient into four categories and more inclusive, albeit more complex, classifications can be envisaged (Müllertz et al., 2010). The lipid composition of the current LFCS classifications and a comparison of their behavior on dispersion and digestion are listed in Table 28.

Type I LBF comprise drugs dissolved in a digestible oil and may contain triglycerides, diglycerides, monoglycerides, or mixtures thereof. Type I formulations are therefore most appropriate for drugs with high solubility in simple glyceride lipids. Type I formulations have the advantage of simplicity and are resistant to precipitation on capsule rupture. The major complexity of type I formulations is the requirement for digestion to promote formulation dispersion and the limited solvent capacity for many drugs.

Type II formulations are isotropic mixtures of lipids and lipophilic surfactants (HLB < 12) that self-emulsify to form 200 nm–1 μm particle size emulsions on contact with aqueous media (i.e., typical SEDDS behavior). Self-emulsification occurs when the surfactant concentration exceeds 25% w/w, and the optimum concentration is often 30–40%. Viscous liquid crystalline phases may occur in formulations with higher surfactant concentrations (i.e., >50%). Type II systems have the advantage of low hydrophile content (and therefore a reduced likelihood of drug precipitation on dispersion), coupled with effective self-emulsification. Although some of the first publications to describe SEDDS formulations centered around type II systems (Charman et al., 1992), more recently the popularity of type III and type IV LBF and the limited number of approved lipophilic surfactants that support self-emulsification have limited the application of type II systems.
Type III formulations include lipids, hydrophilic surfactants (HLB > 12), and cosolvents and self-emulsify very effectively to form emulsions with small particle sizes (usually <250 nm). Type III LBF have therefore been described as self-microemulsifying drug delivery systems (SMEDDS) (based on thermodynamic expectations of the dispersion) or self-nanoemulsifying drug delivery systems (SNEDDS) (based on particle size). Type III formulations contain long- or medium-chain glycerides, high HLB surfactants (e.g., Cremophor EL, Cremophor RH40, Tween 80, Gelucire), and cosolvents. There is a somewhat arbitrary division between type IIIA and IIIB formulations to distinguish those that contain greater quantities of hydrophilic components (type IIIB). Key considerations to formulation design include protection against drug precipitation on dispersion and digestion of the formulation. In vitro testing of the potential for precipitation on dispersion and digestion is therefore crucial to development and assessment (see Sections XI.C.1 and XI.C.2). Most currently marketed LBF are type III formulations.

Type IV formulations were more recently added to the LFCS (Pouton, 2006) and comprise mixtures of surfactant and cosolvent in the absence of traditional lipids. The attraction of type IV formulations is the high solvent capacity of cosolvents and surfactant compared with lipids, which usually permits higher drug loading in type IV formulations compared with types I, II, and III. The disadvantage of type IV formulations is that the high quantity of watermiscible excipients renders them highly susceptible to drug precipitation on dispersion. Combinations of cosolvents and surfactants are used in an attempt to reduce the extent of precipitation on dilution of pure cosolvent solutions and to increase the dispersion of pure surfactant solutions (which often gel on contact with aqueous fluids). The lack of traditional lipids in type IV formulations restricts the impact of digestion on formulation performance; however, surfactant digestion is likely and in some cases may alter formulation properties. Assessment of behavior under simulated dispersion and digestion conditions is therefore recommended.

### 4. Selection of Lipid-Based Formulation Excipients

Many of the lipidic excipients that are commonly used in LBF have been tabulated in detail elsewhere (Strickley, 2004; Gibson, 2007; Strickley and Oliyai, 2007). The FDA maintains a list of excipients that have been previously approved and incorporated in marketed products (the Inactive Ingredient Guide, or IIG). The IIG provides a useful database of approved excipients and the maximum current use levels by route of administration (http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm). Table 29 gives a very brief summary of the most common excipients employed in LBF.

A flowchart providing some general guidance for excipient selection in the assembly of LBF is provided.
in Scheme 4. Generally, LBF development is initiated via a solubility screen to identify excipients capable of dissolving the required dose, followed by dispersion and digestion testing to identify combinations that most readily prevent drug precipitation during GI processing. Additional factors to consider when selecting excipients for LBF and in selecting the type of glyceride are summarized in Tables 30 and 31 and in the sections that follow. Considerations surrounding encapsulation (Cole et al., 2008), stability (Cannon, 2008), scale-up (Sirois, 2007), and regulatory approval (Chen, 2008) of LBF have been reviewed and are not addressed here.

a. Solvent Capacity. With the exception of highly lipophilic drug molecules, triglycerides are relatively poor solvents for poorly water-soluble drugs; as such, most LBF also contain more polar oils, surfactants, or cosolvents. Care needs to be taken, however, to balance the inclusion of hydrophilic excipients to increase solvent capacity with the propensity for drugs to precipitate on dispersion of formulations containing large quantities of surfactants and cosolvents. To facilitate more effective measurement of solubility in lipidic vehicles, in vitro solubility screening methods have been described that are rapid; inexpensive; minimally labor-intensive; amenable to high-throughput automation, parallel processing, and miniaturization; and require only small quantities of drug (milligram or submilligram) (Dai et al., 2008b).

b. Mutual Miscibility. Excipient miscibility in LBF is required to ensure stability and drug content homogeneity. Ternary or pseudoternary phase diagrams are commonly used to identify regions in which excipients are miscible. These phase diagrams allow easy representation of phase behavior and miscibility as a function of the concentration of excipients in LBF plotted on three sides of a triangular phase diagram. Immiscibility may not be apparent immediately, and therefore physical stability should be assessed over time. Particular care should be taken when waxy excipients are heated before or during blending since this may lead to supersaturation on cooling to room temperature. In general, type I and type IV LFCS formulations are most readily miscible, whereas type III systems containing LC oils and hydrophilic surfactants or cosolvents present the most problems with respect to miscibility. Type III systems usually require the presence of polar oils, such as mixed glycerides, to promote mutual miscibility. An additional benefit of the addition of mixed glycerides is the potential for these more polar oils to promote formulation dispersion. In contrast to type III systems, type II systems are readily miscible as both MC triglyceride and LC triglyceride are miscible with lipophilic surfactants. Mixed glycerides are therefore not a requirement in type II systems, but they may be added to optimize performance.

c. Toxicity/Irritancy. In general, glycerides are regarded as nontoxic as they are a common dietary component. Intravenous administration of large quantities (>2.5 g/kg/day) of lipid, however, can lead to excessive lipid load (Mirtallo et al., 2010). After oral administration, toxicity and irritancy are more likely to be issues with surfactants that may penetrate, fluidize, or solubilize biologic membranes. As such, surfactant inclusion needs to be considered carefully, particularly where products are expected to be used chronically. Notable trends in surfactant toxicity include toxicity of ionic surfactants typically > nonionic surfactants; cationic > anionic surfactants; single-chain > bulky surfactants (e.g., polysorbates or polyethoxylated vegetable oils) and others > esters (which are digestible).

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TABLE 29

Excipients commonly used in oral lipid-based formulations

<table>
<thead>
<tr>
<th>Excipient Class</th>
<th>Examples</th>
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</thead>
<tbody>
<tr>
<td>Medium-chain triglycerides</td>
<td>Coconut or palm seed oil, Miglyol, Captex</td>
</tr>
<tr>
<td>Long-chain triglycerides</td>
<td>Soybean, sesame, safflower, sunflower, corn, cottonseed, olive, palm, peanut (arachis), rapeseed oils, and hydrogenated vegetable and soybean oils</td>
</tr>
<tr>
<td>Medium- and/or long-chain monoglycerides, diglycerides, or mixed glycerides; Low HLB surfactants (HLB &lt;8)</td>
<td>Medium chain: Capmul, Imwitor, Labrafil, Capmul GMO</td>
</tr>
<tr>
<td>Water-insoluble, lipophilic surfactants (HLB 8-12)</td>
<td>Predominantly olate esters and include polysorbate 85 (TWEEN 85) and Tagat TO</td>
</tr>
<tr>
<td>Water-soluble surfactants (HLB &gt; 12)</td>
<td>Include products synthesized by reacting alcohols with ethylene oxide to produce alkyl ether ethoxylates (e.g., Brij, cethamorcelogol); sorbitan esters with ethylene oxide to form sorbitan ester ethoxylates (e.g., Tween); ethylene oxide with castor oil to produce castor oil ethoxylates (e.g., Cremophor EL, RH40); and vegetable oils with PEG to produce mixtures of monoglycerides, diglycerides, and triglycerides and PEG esters of fatty acid (Labrasol, Labrafil, Gelucire)</td>
</tr>
</tbody>
</table>

**Excipient Miscibility**

- **Type I:** systems containing LC oils and hydrophilic surfactants or cosolvents are most readily miscible; type II systems are readily miscible as both MC triglyceride and LC triglyceride are miscible with lipophilic surfactants. Mixed glycerides are therefore not a requirement in type II systems, but they may be added to optimize performance.
- **Type II:** systems containing LC oils and hydrophilic surfactants or cosolvents are most readily miscible, whereas type III systems containing LC oils and hydrophilic surfactants or cosolvents present the most problems with respect to miscibility. Type III systems usually require the presence of polar oils, such as mixed glycerides, to promote mutual miscibility. An additional benefit of the addition of mixed glycerides is the potential for these more polar oils to promote formulation dispersion. In contrast to type III systems, type II systems are readily miscible as both MC triglyceride and LC triglyceride are miscible with lipophilic surfactants. Mixed glycerides are therefore not a requirement in type II systems, but they may be added to optimize performance.
- **Type III:** systems containing LC oils and hydrophilic surfactants or cosolvents present the most problems with respect to miscibility. Type III systems usually require the presence of polar oils, such as mixed glycerides, to promote mutual miscibility. An additional benefit of the addition of mixed glycerides is the potential for these more polar oils to promote formulation dispersion. In contrast to type III systems, type II systems are readily miscible as both MC triglyceride and LC triglyceride are miscible with lipophilic surfactants. Mixed glycerides are therefore not a requirement in type II systems, but they may be added to optimize performance.

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*HLB, hydrophilic-lipophilic balance.*

*The distinction between polar oils and low HLB surfactants is difficult, and glycerides, especially monoglycerides of medium-chain lipids, might be classified as either.*
Nonionic surfactants are therefore more usually included in products for oral use. After parenteral administration, toxicity is more likely due to direct introduction into the systemic circulation. For this reason, emulsifiers based on dietary lipids such as lecithin are used in most parenteral products (see Section XI.B.1).

d. Purity and Chemical Complexity. Peroxides and aldehydes are present in trace quantities in many LBF excipients and have the potential to impact negatively on product stability. Excipients with low levels of trace contaminants are therefore preferred, and consistency in trace contaminant profiles between batches should be confirmed. Furthermore, many surfactants are obtained via hydrolysis and esterification of glycerides with PEG or ethylene oxide. This leads to the generation of a series of esterification products and a lack of explicit molecular characterization of the product. Potential batch-to-batch variation in surfactant properties is therefore particularly evident for surfactants of this type where variations in the quantities of each product are likely as a function of batch.

e. Capsule Compatibility. Compatibility of LBF with hard and soft gelatin capsules was reviewed in detail by Cole et al. (2008). In general, low-molecular-weight polar molecules such as cosolvents (e.g., propylene glycol), surfactants, and water are most likely to interact with capsule shells, although differences are evident between hard and soft gelatin capsules. Newer capsule materials such as hydroxypropyl methylcellulose hard gelatin capsules and plant polysaccharide-based soft gel capsules provide additional options with
respect to capsule compatibility and may provide advantage in some cases. Capsule manufacturers are an excellent source of information regarding excipient compatibility and should be contacted for further information.

f. Polymers. Polymeric precipitation inhibitors (PPIs) may be included in formulations to reduce drug precipitation during formulation dispersion and digestion in the GI tract (see Section XI.A.2) (Erlich et al., 1999; Gao et al., 2004; Gao and Morozowich, 2006; Warren et al., 2010; Gao et al., 2011a). PPIs aim to maintain the drug in a supersaturated, thermodynamically unstable (metastable) state (where drug is solubilized at concentrations above equilibrium solubility) for a period that is sufficient to facilitate absorption. Solubilization at supersaturated concentrations may also provide additional benefits via an increase in thermodynamic activity. Polymers that are commonly used in LBF are listed in Table 29.

C. Assessment of Lipid-Based Formulations

1. In Vitro Dispersion Testing. Dispersion testing is conducted to identify formulations that disperse slowly or those that lead to drug precipitation during the dispersion process. Many different methods have been described, but a typical dispersion test involves simple dilution (1:50, 1:100, or 1:250) of the formulation into biorelevant media (Jantratid and Dressman, 2009; Di Maio and Carrier, 2011) and subsequent assessment of the extent of precipitation. The choice of media depends on whether behavior under gastric or intestinal conditions or under fasted or postprandial conditions is required. The formulation may be added dropwise as a liquid or, preferably, where a solid dosage form is required. The formulation may be added dropwise as a liquid or, preferably, where a solid dosage form is required.

2. In Vitro Digestion Models. In vitro digestion testing is used to examine the potential for drug precipitation from LBF during in vivo digestion. Furthermore, when coupled with biophysical methods (e.g., dielectric viscosity and conductivity measurements, scattering techniques such as dynamic light scattering (DLS), small-angle neutron scattering (SANS), or small-angle X-ray scattering (SAXS) (Fatouros et al., 2007b), NMR, raman spectroscopy, and electron microscopy (Fatouros et al., 2007a), electron paramagnetic resonance (EPR) (Abdalla and Mäder, 2009), multiplex coherent antistoked Raman scattering microspectroscopy (CARS) (Day et al., 2010) atomic force microscopy and cryo-TEM (Müllertz et al., 2012), the in vitro and precipitated drug via centrifugation, and analysis of drug content in the precipitate or solubilized phase. Formulations that disperse slowly or form coarse emulsions can usually be identified by visual inspection, although particle size can be quantified more specifically with Fraunhofer diffraction sizers and photon correlation spectrometers. Although complete dispersion to form monodisperse colloidal systems provides for rapid processing in vivo, particle size provides a poor indicator of absolute in vivo performance since the properties of most formulations are changed dramatically by dilution and digestion before drug absorption. For example, systems containing high proportions of surfactant or cosolvent commonly produce emulsions with very small particle sizes but may rapidly lose solvent capacity on further dilution or digestion leading to drug precipitation (Porter et al., 2004a; Sek et al., 2006; Cuiné et al., 2007; Mohsin et al., 2009; Prajapati et al., 2012). Assessment of solubilization behavior is therefore more critical than particle size characterization.

### Table 30

<table>
<thead>
<tr>
<th>Factors affecting the choice of excipients for lipid-based formulations</th>
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<tbody>
<tr>
<td>Solvent capacity</td>
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<tr>
<td>Miscibility</td>
</tr>
<tr>
<td>Regulatory issues: irritancy, toxicity, knowledge, and experience</td>
</tr>
<tr>
<td>Morphology at room temperature (i.e., melting temperature)</td>
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<tr>
<td>Self-dispersibility and role in promoting self-dispersion of the formulation</td>
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<tr>
<td>Digestibility, and fate of digested products</td>
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<tr>
<td>Capsule compatibility</td>
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<tr>
<td>Purity, chemical stability</td>
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<tr>
<td>Cost of goods</td>
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### Table 31

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<th>Comparative properties of glyceride lipids in oral lipid-based formulations</th>
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<td>Triglycerides vs. monoglycerides, diglycerides, or mixed glycerides</td>
</tr>
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<td>Partial glycerides are often better solvents than triglycerides (unless the drug is highly lipophilic), and their increased amphiphilicity typically results in improved emulsification properties.</td>
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<td>LBF based on LC glycerides are often more effective at maintaining solubilization capacity on dispersion and digestion. LC glyceride more effectively promote lymphatic transport of highly lipophilic drugs compared with MC glycerides.</td>
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<td>LC lipids are typically less stable to oxidation. Unsaturated glycerides vs. saturated glycerides</td>
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digestion model may also be useful in determining the nature of the colloidal phases formed, as well as the fate of a drug (in particular, crystallinity/amorphous character) (Sassene et al., 2010), during or after digestion of different LBF. Several variations of the in vitro digestion model have been described, and differences in experimental conditions are detailed elsewhere (Porter et al., 2007, 2008; Dahan et al., 2008; Fatouros and Mullertz, 2008; Larsen et al., 2011). However, efforts to develop standardized in vitro tests for LBF have led to the Lipid Formulation Classification System (LFCS) Consortium, which is an industry-academic consortium that aims to identify consistent and appropriate in vitro digestion conditions (Williams et al., 2012a,b).

Typical in vitro digestion conditions are described in Fig. 66. The conduct of an in vitro digestion test usually involves the dispersion of a LBF in temperature-controlled (37°C) media consisting of buffer, bile salt, and phospholipid, followed by the initiation of digestion via the addition of a source of pancreatic enzymes. Samples of the digest are then taken throughout the experiment and centrifuged to separate an undigested oil phase, an aqueous phase containing mixed micelles and vesicles, and a pellet phase. Drug that precipitates during digestion is contained within the pellet phase and is thought to represent drug that is poorly available for absorption since redissolution is required and the dissolution of poorly water-soluble drugs from crystalline solids is poor. In contrast, drug that remains solubilized within the aqueous phase is expected to be in rapid equilibrium with drug in free solution and to provide a reservoir of drug that is highly available for absorption. Using variations of this general protocol, several groups have demonstrated rank-order correlations between drug solubilization in the aqueous phase on in vitro digestion and drug bioavailability in animals (Porter et al., 2004a,b; Dahan and Hoffman, 2006, 2007; Cuiné et al., 2007, 2008; Fatouros et al., 2008; Larsen et al., 2008a; Han et al., 2009; Tan et al., 2011; Ahmed et al., 2012). In vitro digestion testing has also been reported to provide an indication of in vivo formulation behavior in humans (Taillardat et al., 2007).

**Fig. 66.** Experimental setup for an in vitro digestion experiment. (A) Lipid formulations are introduced into a temperature-controlled reaction vessel (37°C), which contains media consisting of buffer, bile salt, and phospholipid concentrations designed to simulate fasted or fed intestinal conditions. The contents of the reaction vessel are stirred throughout experiments. Either at the time of introduction of the formulation or after a period of dispersion (to enable assessment of drug precipitation upon dispersion), a source of pancreatic enzyme(s) is added to the system to initiate the process of lipid digestion. Digestion of glycerides liberates FAs, which causes a transient drop in pH. The pH is maintained at a setpoint (B) by the addition of NaOH via an autoburette coupled to a pH-stat meter controller. Maintenance of constant pH is important to mimic in vivo conditions and to enable digestion to continue. The rate of NaOH addition is recorded via the computer. This enables the calculation of the extent of digestion as digestion of a molecule of triglyceride liberates a molecule of monoglyceride and 2 free FAs and OH ions react with H ions from liberated free FA in a 1:1 stoichiometric ratio (assuming complete ionization). (C). Samples can be taken throughout the digestion process, digestion stopped via the addition of the lipase inhibitor 4-bromophenylboronic acid (BPBA), and samples centrifuged to obtain three separate phases: an undigested oil phase, an aqueous phase, and a pellet phase. Precipitated drug is present in the pellet phase, whereas drug solubilized in micellar structures within the aqueous phase is believed to represent drug that is available for absorption. Adapted from Porter et al. (2007).
As an example, Fig. 67 shows data obtained after in vitro dispersion and digestion of MC SEDDS and LC SEDDS containing danazol and the corresponding in vivo data obtained after fasted administration of the same formulations to beagle dogs. Also shown are exposure profiles obtained after fasted administration of a LC triglyceride solution of danazol and fasted and postprandial administration of a micronized powder formulation of danazol to the beagles. Both the MC and LC SEDDS formulations supported solubilization of danazol after in vitro dispersion under nondigesting conditions, with <12% of the drug present in the pellet phase (Fig. 67A). However, after digestion, there was significant precipitation of danazol from the MC SEDDS, whereas danazol remained predominantly (>95%) solubilized in the aqueous phase after 30 min of in vitro digestion of the LC SEDDS (Fig. 67B). These results were reflected in the in vivo performance of the SEDDS where exposure to danazol was significantly greater after administration of the LC SEDDS compared with the MC SEDDS (Fig. 67C). Indeed, exposure after administration of the SEDDS was similar to that seen after postprandial administration of a traditional micronized powder formulation and was significantly greater than that seen after administration of the micronized powder formulation of danazol to fasted beagles. These results demonstrate the utility of LBF in supporting exposure of lipophilic poorly water-soluble drugs, the potential for LBF to overcome food effects, and the potential benefits of the in vitro digestion model in identifying formulations that are likely to perform poorly in vivo.

3. In Vivo Studies. In vivo evaluation of the performance of LBF is performed in a similar manner to that used for most other formulations. Unlike many traditional formulation approaches, however, the
quantity of formulation administered (on a milliliters per kilogram basis) is likely to play a significant role in ongoing drug solubilization and should therefore be controlled. Formulations may be administered via oral gavage (either directly or after dispersion in a small volume of water) or after encapsulation. Capsules can be hand-filled for large animals, and minicapsules can be used for administration to small animals (Li and Zhao, 2007; Shah and Agnihotri, 2011). Intravenous formulations may be administered via i.v. bolus or infusion into a catheter or cannula.

The importance of formulation digestion by gastric and pancreatic enzymes in the performance of LBF and the potential role of bile salt solubilization in the prevention of drug precipitation dictate that differences in physiology associated with bile salt release and digestion profile may affect product performance. Notable differences are evident, for example, in biliary secretory patterns in rats (where bile flow is continuous) and in dogs or humans, where bile is stored in the gallbladder and released as a bolus in response to lipid sensing in the GI tract. A consensus view as to whether these differences lead to higher absorption in dogs or rats, however, has yet to emerge, although apocryphal evidence suggests that for poorly water-soluble drugs, absorption may be higher in dogs than in rats.

A second significant variable in the in vivo evaluation of drug absorption from LBF is the potential for intestinal lymphatic drug transport. For highly lipophilic drugs, assessment of the extent of lymphatic transport may be important, as small changes in formulation components and the presence of food may change the extent of lymphatic transport and in doing so impact drug exposure, activity, and toxicity via changes to drug clearance and distribution (Porter and Charman, 2001c,d; O’Driscoll, 2002; Porter et al., 2007; Trevaskis et al., 2010b). To this point, intestinal lymphatic drug transport has not been measured directly in humans as the surgical cannulation of the mesenteric lymph duct is highly invasive (and typically nonreversible). However, several animal models have been described for the assessment of lymphatic drug transport after oral administration. These models involve the collection of lymph flowing from the intestine via insertion of a cannula into either the mesenteric or the thoracic lymph duct and subsequent assessment of drug uptake into the lymph after oral or intestinal administration to conscious or anesthetized animals. Studies have been conducted in rats (Edwards et al., 2001), dogs (Khoo et al., 2001, 2003; Lespine et al., 2006), pigs (White et al., 1991), sheep (Onizuka et al., 1997; Segrave et al., 2004), and rabbits (Bocci et al., 1986). The advantages and disadvantages and variations in conduct of lymphatic drug transport studies in animals are summarized by Edwards et al. (2001) and have been discussed previously (Porter and Charman, 2001b,c; O’Driscoll, 2002; Porter et al., 2007; Trevaskis et al., 2008).

In addition to assessment in lymph cannulated animal models, interest has increased in alternate (and less surgically complex) animal models and in vitro predictive models. These include bioavailability studies in animals in which intestinal chylomicron formation and lymphatic transport is blocked via preadministration of small molecular inhibitors of chylomicron assembly (Dahan and Hoffman, 2005), assessment of drug secretion within lipoproteins on incubation with caco-2 cells (Seeballuck et al., 2003, 2004; Karpf et al., 2006), in vitro assessment of drug affinity for collected intestinal triglyceride-rich lipoproteins (Gershkovich and Hoffman, 2005; Trevaskis et al., 2010b), and in silico models that rely on simple molecular descriptors such as log P, long-chain TG solubility, and molecular weight (Holm and Hoest, 2004).

D. Summary

Lipids may be formulated into a range of delivery systems for oral or parenteral administration (solutions, suspensions, emulsions, microemulsions, nanoemulsions, micellar solutions, liposomes, lipid nanoparticles, and emulsion preconcentrates). LBF provide a relatively facile means of generating liquid formulations that can be gavaged at varying doses during preclinical development and that provide for significant increases in exposure for many poorly water-soluble drugs. In addition, LBF have the advantage that essentially the same fill material can be subsequently filled into soft or hard gelatin capsules to facilitate clinical development. LBF can also be transformed into solid dosage forms directly via the use of high melting point lipids or polymers or via adsorption onto high-surface-area carriers.

Oral LBF commonly include combinations of glyceride lipids, lipophilic or hydrophilic surfactants, and cosolvents, resulting in formulations that spontaneously emulsify on contact with GI fluids (so called self-emulsifying drug delivery systems, or SEEDS). In the preferred embodiment of the technology, drug is present in a dissolved form within a nonaqueous solution in the fill material. Under these circumstances, traditional dissolution is circumvented and drug absorption proceeds via dispersion of the dose form in the GI content and integration into endogenous lipid digestion and processing pathways. Alternatively, where drug dose requirements exceed the solubility limit in the formulation, lipid suspensions may be used, although this provides additional complexity with respect to content uniformity and reinstalls the need to overcome crystal lattice energy limitations to solubility.

LBF have the potential to support oral bioavailability via several mechanisms—including enhancements...
Aqueous Solubility of Poorly Water-Soluble Drugs

A number of strategies have emerged in recent years with the potential to enhance the delivery of poorly water-soluble drugs, but these strategies have yet to progress to broad clinical application. These are described in brief in the sections below.

A. Drug Adsorption to Microporous Adsorbents

The use of microporous adsorbents has found increasing recent interest as a mechanism to facilitate the oral delivery of amorphous drug. The earliest reports of an improved dissolution rate of poorly soluble drugs using adsorbents such as fumed silica dioxide emerged in the 1970s (Monkhouse and Lach, 1972a,b). More recent studies have extended these early concepts to focus on drug adsorption to materials with increasingly high surface areas, such as microporous silica, and have shown significant in vitro utility (Salonen et al., 2005; Ambrogi et al., 2007; Pan et al., 2008; Van Speybroeck et al., 2009; Wang et al., 2010a; Vialpando et al., 2011; Ambrogi et al., 2012). Enhancements in oral bioavailability have been described after oral administration of adsorbed microporous systems compared with unmodified crystalline material with model compounds, including itraconazole (Mellaerts et al., 2008; Van Speybroeck et al., 2010b), fenofibrate (Van Speybroeck et al., 2010a; Jia et al., 2011), glibenclamide (Van Speybroeck et al., 2011), indomethacin (Wang et al., 2010a), and K-832 (Miura et al., 2010). Substantial increases in bioavailability have been described, and in the latter example, a ~5-fold enhancement in the oral bioavailability of the development candidate K-832 (under development for immune, inflammatory, and ischemic diseases) was evident after oral administration of the drug adsorbed to a porous silica substrate compared with the unmodified crystal form (Miura et al., 2010).

The ability of silica carriers to increase drug dissolution (and therefore oral bioavailability) has been suggested to reflect their capacity to stabilize drug in the more rapidly dissolving amorphous form (Mellaerts et al., 2007; Prestidge et al., 2007; Van Speybroeck et al., 2009). Silica carrier systems are therefore analogous to solid dispersion technologies (see Section X) that derive benefit via delivery of drug in the amorphous form. For this reason, silica carrier systems have been described as “surface solid dispersions” (Kerc et al., 1998). The mechanisms of amorphous drug stabilization across the two delivery technologies, however, are different: solid dispersion formulations typically contain drug molecularly dispersed or suspended throughout an inert carrier (usually a high-molecular-weight polymer), which slows drug recrystallization via an increase in viscosity and a decrease in drug mobility within the matrix. In contrast, amorphous drug adsorbed to the surfaces of a porous carrier is stabilized via spatial confinement to small pores or capillaries that restrict drug nucleation and crystal growth as a result of the overall small size of these pores (Mellaerts et al., 2007; Wang et al., 2010a; Bras
et al., 2011). Drug crystallization tendency is also decreased by the formation of drug-carrier interactions (Gupta et al., 2003; Van Speybroeck et al., 2009; Bras et al., 2011), although the formation of such interactions is dependent on drug positioning within the pore/capillary (Bras et al., 2011) and the chemical surface of the silica carrier (Madieh et al., 2007; Qian and Bogner, 2012).

The most effective carriers for amorphous drug delivery provide an extensive network of nanosized or microsized capillaries or pores, resulting in high surface areas (usually >500 m²/g) and high pore volumes (usually around 1 cm³/g); properties that maximize drug loading in the amorphous form (Vialpando et al., 2011; Qian and Bogner, 2012). Drug is commonly loaded onto the carrier via solvent impregnation, where a known volume of a concentrated solution of drug in organic solvent is added to the carrier before mixing and solvent evaporation at elevated temperatures. This provides a dry, free-flowing powder of known drug loading (Mellaerts et al., 2007; Van Speybroeck et al., 2009). Alternatively, drug-carrier physical mixtures may be dry milled (Gupta et al., 2003; Bahl and Bogner, 2006) or drug loaded via vapor adsorption (Qian and Bogner, 2011; Qian et al., 2011). The advantages and disadvantages of alternative drug-loading approaches have been described by Qian and Bogner (2012).

Silica-based carriers constitute the most widely investigated porous materials in drug delivery. Commercial examples include magnesium alumino-metasilicate (Neuselin; Fuji Chemical Industry Co., Ltd., Tokyo, Japan), calcium silicate (Florite; Tokuyama Corporation, Tokyo, Japan), and ordered mesoporous silicon dioxide (Solustia; Formac Pharmaceuticals, Heverlee, Belgium). Ordered mesoporous silicas (OMS), including SBA-15 and MCM-41, show a collection of unique properties that are especially attractive to drug delivery, such as very high specific surface areas (up to 1500 m²/g), large pore volumes (up to 1.5 g/cm³), a tunable pore diameter (for variable drug release rates), and functionalized surfaces (Van Speybroeck et al., 2009; Popovici et al., 2011). Polymeric precipitation inhibitors (HPMC and HPMCAS) may also be used in combination with OMS to slow the rate of recrystallization of the supersaturated drug solutions that form on dissolution of the amorphous drug load (Van Speybroeck et al., 2010b).

Although there is significant interest in the use of microporous materials as carriers for the delivery of poorly water-soluble drugs, in particular, to stabilize amorphous drug content, the feasibility of manufacturing silica-based formulations at larger production scales is not yet known (Qian and Bogner, 2012). The processability of formulations that contain high (usually >80% w/w) quantities of silica-based materials are also not fully described, and recent work suggests that diluents and disintegrants may be necessary to ensure rapid drug release from compressed silica formulations (Vialpando et al., 2011).

The physical stability challenges associated with high-energy formulations must also be considered when using porous silica carriers containing amorphous drug. In particular, the potential for recrystallization of the stable crystal form over time, during processing, and on exposure to moisture and after dissolution. These physical stability considerations are analogous to those described for solid dispersions (see Section X).

### B. Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) consist of a lipidd core that is solid at both room temperature and physiologic temperatures, and a surfactant-stabilized outer surface (Mehnert and Mader, 2001; Bummer, 2004; Muller et al., 2011b). The concept of using solid particulate lipid vehicles for drug delivery emerged more than 20 years ago (Eldem et al., 1991), and a significant level of interest has subsequently focused on the use of SLN formulations for 1) improved oral bioavailability of poorly water-soluble drugs (Hu et al., 2004c; Luo et al., 2006; Muller et al., 2006), 2) parenteral administration of poorly water-soluble drugs (Wissing et al., 2004; Manjunath and Venkateswarlu, 2005; Reddy et al., 2005), 3) enhanced topical drug delivery, 4) controlled drug release, and 5) targeted drug delivery. Descriptions of SLN formulations for dermal application or sustained and targeted drug release are beyond the scope of the present review, but they have been extensively reviewed (zur Mühlen et al., 1998; Boyd, 2008; Pardeike et al., 2009).

SLNs combine the advantages of colloidal delivery systems such as liposomes, lipid emulsions, and polymeric nanoparticles (Müller et al., 2000; Wissing et al., 2004), with the potential for facile large-scale production and a favorable tolerability profile for most core lipid components (Müller et al., 2000; Bummer, 2004). The drug-loading capacity of SLNs for lipid-soluble drugs may be high (Schwarz and Mehnert, 1999); however, for less lipid-soluble compounds, low drug loading may limit applicability (Mehnert and Mader, 2001). Second-generation SLNs, usually termed nanostructured lipid carriers (NLCs), have subsequently been developed. NLCs incorporate blends of solid and liquid lipids (but retain solid properties at room temperature) that increase the drug-loading capacity of the lipid core (Müller et al., 2002). For example, the addition of liquid oils to an SLN formulation of retinol increases the maximum drug loading from 1% w/w (using only glyceryl behenate) to 5% (using a combination of glyceryl behenate and Miglyol 812) (Jenning and Gohla, 2001).

SLN formulations contain drug molecularly dispersed within the lipidic vehicle. Drug delivery via SLN formulations, therefore, circumvents the potentially
slow rate of dissolution of poorly water-soluble drugs from crystalline solids (Muller et al., 2006). Cyclosporine (Muller et al., 2006), nitrendipine (Manjunath and Venkateswarlu, 2006), puerarin (Luo et al., 2011), quercetin (Li et al., 2009a), and lovastatin (Suresh et al., 2007) all show increases in oral bioavailability when delivered in SLN formulations compared with the administration of more traditional suspension and nanosuspension formulations. In some cases, SLN formulations have also been directly compared with alternate solubilization techniques such as cosolvent-surfactant solutions (Hu et al., 2004c; Luo et al., 2006) and lipid emulsions (Hu et al., 2004c) and shown to provide for superior bioavailability after oral administration. SLN formulations, like traditional lipid-based drug delivery formulations, are expected to be digested in the small intestine (Muller et al., 2011b). It seems likely, therefore, that a better understanding of the factors that influence in vivo performance of SLNs may be obtained via the application of in vitro lipid digestion models (similar to those described in detail in the lipid formulation section of this review; see Section XI) to evaluate better the impact of digestion on drug solubilization.

In addition, SLN formulations may be used to facilitate parenteral delivery of poorly water-soluble drugs (Wissing et al., 2004; Reddy et al., 2005; Muller et al., 2011b) and in a manner analogous to nanocrystal suspensions (see Section IX), aqueous suspensions may be lyophilized for convenient transport and storage and later reconstituted before use (Zimmermann et al., 2000). Recent interest in the parenteral utility of SLN formulations has focused largely on use as a controlled-release platform (Cavalli et al., 1999; Mehnert and Mader, 2001; Jia et al., 2010). In this application, drug partitioning from the oil phase into the surrounding aqueous environments is expected to be hindered compared with liquid nanoemulsion oil droplets (of equal diameter) (zur Mühlen et al., 1998; Mehnert and Mader, 2001) by virtue of the lower mobility of the drug within the lipid component of the formulation. SLN formulations also provide opportunities for targeted therapies; however, these lie beyond the scope of this review [see Muller et al. (2011b) and Joshi and Muller (2009) for details].

Lipidic excipients widely used in SLN formulations are triglycerides (e.g., tricaprin and tristearin), mixtures of partially digested glycercides (e.g., glyceryl mono-oleate or dioleate or stearate), fatty acids (e.g., stearic acid), cholesterol, and typical pharmaceutical waxes (e.g., cetyl palmitate). Since the core excipients are usually GRAS-status lipids, SLN formulations offer toxicological advantages over solubilization techniques that require more complex components, particularly in parenteral drug delivery. However, whereas the components of the lipid core may be GRAS, the pharmacological side effects from the surfactant stabilizers should also be considered. Similar to drug nanoparticle formulations (see section Section IX), stabilizers are required to facilitate the production of nanosized material and to maintain the long-term physical stability of SLN formulations (Mehnert and Mader, 2001; Bummer, 2004). Combinations of different stabilizers are often used to stabilize SLN formulations against potential agglomeration. Common emulsifiers include polysorbate surfactants (e.g., Tweens), poloxamers (e.g., Pluronics), lecithin, and various bile salts.

Usually, SLNs are produced via high-pressure homogenization techniques (Mehnert and Mader, 2001). These are summarized in Section IX. During “hot” homogenization, drug/lipid melts are mixed with a hot aqueous surfactant solution and then passed through a homogenizer while the temperature is maintained approximately 5°C higher than the melting temperature of the lipid component(s). The resultant nanoemulsion is cooled to room temperature, causing the lipid matrix to crystallize and form solid nanoparticles. “Cold homogenization” techniques have been developed to minimize potential temperature-induced drug degradation and drug partitioning from the oil phase into surrounding aqueous phase (Mehnert and Mader, 2001). In this method, the drug-lipid melts are rapidly cooled with liquid nitrogen, and particles are milled to form coarse lipid microparticles. The microparticles are subsequently dispersed in cold surfactant solution and subjected to high-pressure homogenization to reduce particle size (Muller et al., 2000). SLNs can be manufactured in large scale using high-pressure homogenization (Muller et al., 2011b).

In addition, SLNs may be isolated by solvent evaporation; lipids are dissolved in water-immiscible organic solvents, mixed with an aqueous surfactant solution, and the solvent evaporated (Sjostrom and Bergenstahl, 1992); and by combined ultrasonication-solvent evaporation techniques. Ultrasonication-solvent techniques mix drug, lipid, and organic solvent at high temperature before solvent evaporation and drop-wise addition of an aqueous surfactant solution to the warm lipid phase. Ultrasonication of the subsequent coarse emulsions and dispersion in water results in the formulation of nanoparticles (Luo et al., 2006). These techniques have been reviewed elsewhere (Mehnert and Mader, 2001; Bummer, 2004).

Since delivery of drug in a noncrystalline, molecularly dispersed form is critical to the utility of SLNs (at least in applications in the area of low drug solubility), evidence of drug crystallinity in SLNs after manufacture or prolonged periods of storage is routinely assessed by DSC.

XIII. Conclusions

Although major progress has been made in the application of “developability” criteria to support
Understanding of the principles that underpin these with continued improvement in the depth of understanding of the principles that underpin these technologies.

The determinants of low aqueous solubility are poor solvation (i.e., unfavorable solute-solvent interactions in solution) and high crystal lattice energy (i.e., strong solute-solute interactions in the solid state). Approaches to address apparent solubility therefore seek to enhance solute-solvent interactions or to reduce solute-solute interactions in the solid state. Considerable progress has been made in the application of a wide range of technologies to address these issues. Increasingly flexible and robust formulation (solid dispersions, lipid-based formulations, nanocrystals, cyclodextrins) and chemical form (salts, co-crystals) approaches are apparent, most of which have been applied to clinically and commercially successful products.

Progress has also been made in understanding fundamental solute-solvent interactions, and de novo prediction of indicators of lipophilicity (log P) and ionization (pK_a) are now widespread. An ability to predict crystal lattice energy from first principles, however, is still elusive and continues to hamper predictions of utility for approaches predicated on changes to solid-state properties. These include a priori predictions of K_sp (solubility product constant) for different salt forms or cocrystals and the likely impact of solid-state properties on solid cyclodextrin complexes or solid dispersion formation. Under these circumstances, high-throughput screening approaches are used to select the most advantageous approach rather than the application of bottom-up rational design.

The inherent advantage of high-energy solids (e.g., the amorphous form) in enhancing solubility, at least over short periods, has long held appeal. Practical application of these approaches, however, has been limited by concerns over prediction of the kinetics of recrystallization and, therefore, the approaches that might be taken to slow recrystallization over pharmaceutically relevant time frames. Advances in understanding of the drivers of recrystallization and correlation with readily measurable thermal properties (notably T_m) have significantly increased confidence with these approaches, and increasing numbers of marketed products provide evidence that the formulation of drugs in the amorphous state is a practical option. Concerns remain, however, over the importance of microstructure, how best to fingerprint, and the difficulties in predicting stability in systems that do not follow Arrhenius kinetics.

Finally, a distinction should be made between hydrophobic drugs (typically high log P and low lipid solubility) and truly lipophilic drugs (high log P and high lipid solubility). This difference may also be couched in terms of solvation and solid-state properties, where poorly water-soluble hydrophobic drugs (colloquially referred to as “brick dust”) show both solvation and solid-state property limited solubility and poorly water-soluble lipophilic drugs (“grease balls”) are typically solvation-limited in water, but the lack of solid-state limitations and good solvation in nonaqueous vehicles promote lipid solubility. Lipophilic compounds are more readily delivered using formulations typically grouped as lipid-based formulations, which may contain a wide range of nonaqueous and usually liquid vehicles, including glyceride lipids, surfactants, and cosolvents. When delivered as a nonaqueous solution filled into gelatin capsules, LBF have the advantage of circumventing traditional dissolution processes and integrating into endogenous (and highly efficient) lipid absorption pathways. However, LBF must be carefully assessed to understand changes in properties as the formulation is processed by digestion in the GI tract and are most effective for drugs where lipid solubility is high.

In summary, it seems likely that the challenge of low water solubility in drug hits, leads, and development candidates will continue for the foreseeable future and, therefore, that strategies to address these issues will remain a critical determinant of product success (or failure). The techniques overviewed here provide a framework from which to build formulation approaches at all stages of development, and such approaches have been applied in tried and tested drug products. Nonetheless, challenges remain, and many highly potent and highly selective molecules still have not progressed to development because of their low solubility. Better tools are required to ensure reproducible exposure, especially during toxicity testing, where very large doses are required. To this end, considerable effort within the pharmaceutical sciences remains focused on better understanding the relationships between molecular structure and solubility and on the development of novel approaches to overcome the barriers to solubility and to facilitate clinically useful exposure.

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