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Polymorphism in Pharmaceutical Solids
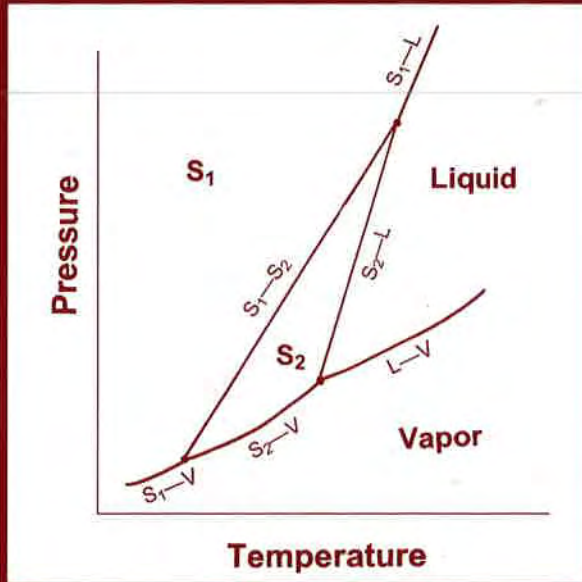
edited by
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Polymorphism in Pharmaceutical Solids

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Theory and Origin of Polymorphism

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I. INTRODUCTION

Many pharmaceutical solids exhibit *polymorphism*, which is frequently defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the mol-
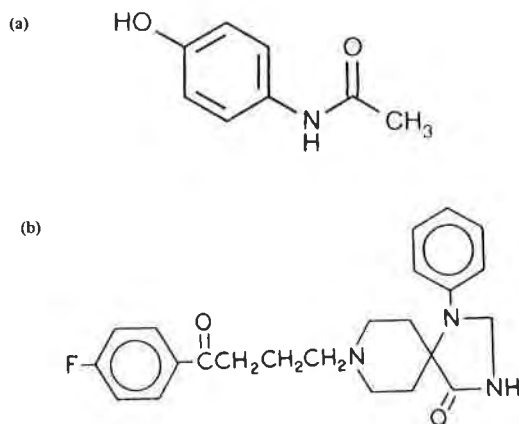
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(a)

(b)

Fig. 1 Molecular structure of (a) acetaminophen and (b) spiperone.

ecules in the crystal lattice [1–3]. Thus, in the strictest sense, polymorphs are different crystalline forms of the same pure substance in which the molecules have different arrangements and/or different conformations of the molecules. As a result, the polymorphic solids have different unit cells and hence display different physical properties, including those due to packing, and various thermodynamic, spectroscopic, interfacial, and mechanical properties, as discussed below [1–3].

For example, acetaminophen (paracetamol, 4-acetamidophenol, 4-hydroxyacetanilide, shown in Fig. 1a) can exist as a monoclinic form, of space group $P2_1/n$ [4], which is thermodynamically stable under ambient conditions. The compound can also be obtained as a less stable orthorhombic form, of space group $Pbca$, and which has a higher density indicative of closer packing [5–7]. The unit cells of these two forms are compared in Fig. 2 and Table 1. The molecule of acetaminophen is rigid on account of resonance due to conjugation involving the hy-

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Fig. 2 View of the unit cell contents for two polymorphs of acetaminophen: (a) orthorhombic form (b) monoclinic form [4,5,7]. (Reproduced with permission of the copyright owner, the American Crystallographic Association, Washington, DC.)

(a)

(b)
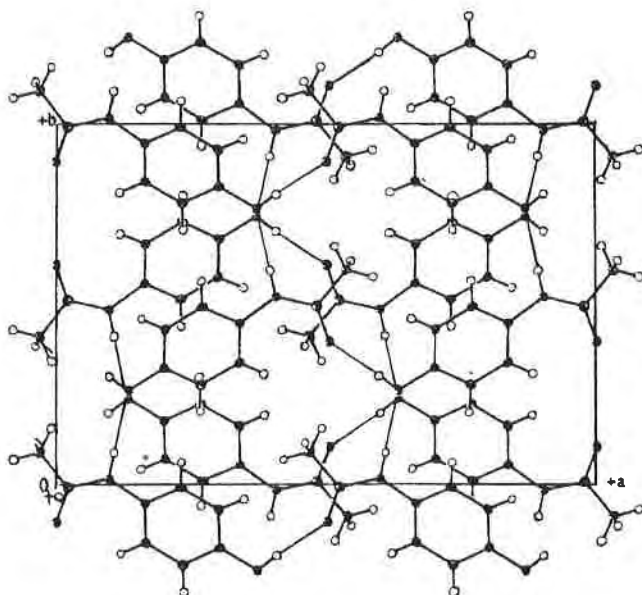
Table 1 Crystal Data for Two Polymorphs of Acetaminophen

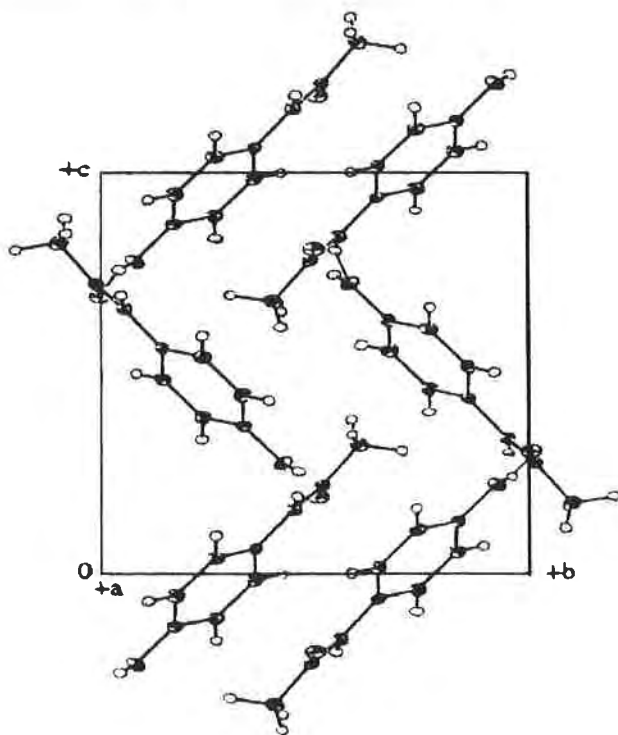| Crystal data and structure refinement	Orthorhombic phase	Monoclinic phase
Empirical formula	$C_8H_9NO_2$	$C_8H_9NO_2$
Formula weight	151.16	151.16
Crystal system	Orthorhombic	Monoclinic
Space group	*Pbca*	*P2₁/n*
Unit cell dimensions	$a = 17.1657(12)$ Å	$a = 7.0941(12)$ Å
	$b = 11.7773(11)$ Å	$b = 9.2322(11)$ Å
	$c = 7.212(2)$ Å	$c = 11.6196(10)$ Å
	$\alpha = 90.000°$	$\alpha = 90.000°$
	$\beta = 90.000°$	$\beta = 97.821(10)°$
	$\gamma = 90.000°$	$\gamma = 90.000°$
Volume	1458.1(4) Å³	753.9(2) Å³
Z	8	4
Density (calculated)	1.377 g/cm³	1.332 g/cm³
Crystal size	0.28 × 0.25 × 0.15 mm	0.30 × 0.30 × 0.15 mm
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Hydrogen bond lengths and angles		
H(5)O(2)	1.852(26) Å	1.772(20) Å
H(6)O(1)	2.072(28) Å	2.007(18) Å
O(1)—H(5)O(2)	170.80(2.35)°	166.15(1.75)°
N(1)—H(6)O(1)	163.52(2.19)°	163.93(1.51)°

Source: Refs. 4, 5, and 7. Reproduced with permission of the copyright owner, the American Crystallographic Association, Washington, DC.

droxyl group, the benzene ring, and the amido group. Therefore the conformation of the molecule is virtually identical in the two polymorphs of acetaminophen. On the other hand, the spiperone molecule (8-[3-(p-fluorobenzoyl)-propyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one, shown in Fig. 1b) contains a flexible -CH₂-CH₂-CH₂- chain and is therefore capable of existing in different molecular conformations [8]. Two such conformations, shown in Fig. 3, give rise to two different conformational polymorphs (denoted Forms I and II), which have different unit cells (one of which is shown in Fig. 4) and densities, even

Form I
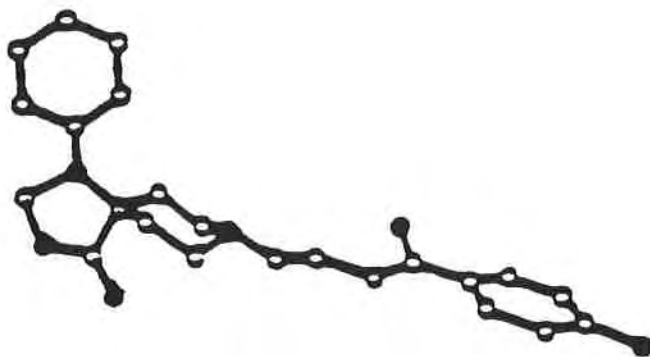
Form II
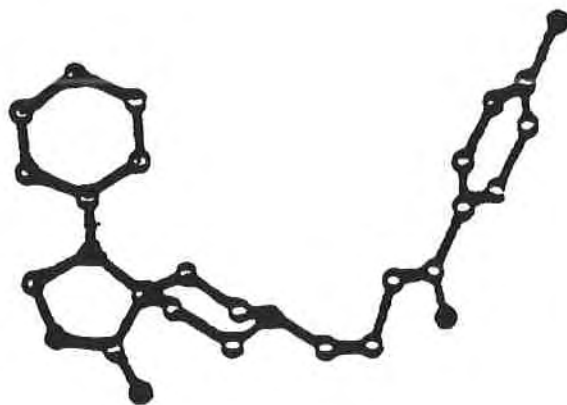
Fig. 3 The molecular conformations of the spiperone molecule in polymorphic forms I and II [8]. (Reproduced with permission of the copyright owner, the American Pharmaceutical Association, Washington, DC.)

though their space groups are the same, both being $P2_1/n$, monoclinic, as shown in Table 2 [8].

As mentioned above, the various polymorphs of a substance can exhibit a variety of different physical properties. Table 3 lists some of the many properties that differ among different polymorphs [1–3,9]. Because of differences in the dimensions, shape, symmetry, capacity
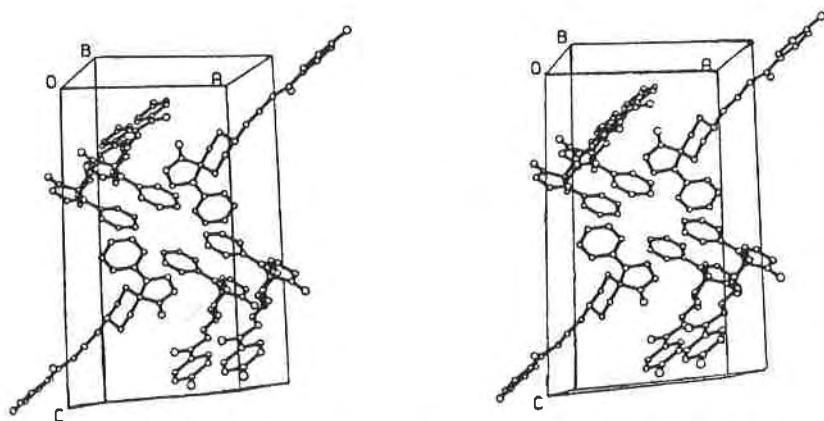
Fig. 4 View of the unit cell contents for the form I polymorph of spiperone [8]. (Reproduced with permission of the copyright owner, the American Pharmaceutical Association, Washington, DC.)

Table 2 Crystal Data for Two Polymorphs of Spiperone

	Form I	Form II
Empirical formula	$C_{23}H_{26}FN_3O_2$	$C_{23}H_{26}FN_3O_2$
Molecular weight	395.46	395.46
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/a$	$P2_1/c$
Unit cell dimensions	$a = 12.722$ Å	$a = 18.571$ Å
	$b = 7.510$ Å	$b = 6.072$ Å
	$c = 21.910$ Å	$c = 20.681$ Å
	$\alpha = 90.00°$	$\alpha = 90.00°$
	$\beta = 95.08°$	$\beta = 118.69°$
	$\gamma = 90.00°$	$\gamma = 90.00°$
Unit cell volume	2085.1 Å^3	2045.7 Å^3
Z	4	4

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Table 3 List of Physical Properties that Differ Among Various Polymorphs

1. Packing properties
   a. Molar volume and density
   b. Refractive index
   c. Conductivity, electrical and thermal
   d. Hygroscopicity
2. Thermodynamic properties
   a. Melting and sublimation temperatures
   b. Internal energy (i.e., Structural energy)
   c. Enthalpy (i.e., Heat content)
   d. Heat capacity
   e. Entropy
   f. Free energy and chemical potential
   g. Thermodynamic activity
   h. Vapor pressure
   i. Solubility
3. Spectroscopic properties
   a. Electronic transitions (i.e., ultraviolet–visible absorption spectra)
   b. Vibrational transitions (i.e., infrared absorption spectra and Raman spectra)
   c. Rotational transitions (i.e., far infrared or microwave absorption spectra)
   d. Nuclear spin transitions (i.e., nuclear magnetic resonance spectra)
4. Kinetic properties
   a. Dissolution rate
   b. Rates of solid state reactions
   c. Stability
5. Surface properties
   a. Surface free energy
   b. Interfacial tensions
   c. Habit (i.e., shape)
6. Mechanical properties
   a. Hardness
   b. Tensile strength
   c. Compactibility, tableting
   d. Handling, flow, and blending

(number of molecules), and void volumes of their unit cells, the different polymorphs of a given substance have different physical properties arising from differences in molecular packing. Such properties include molecular volume, molar volume (which equals the molecular volume multiplied by Avogadro's number), density (which equals the molar mass divided by the molar volume), refractive index in a given direction (as a result of the interactions of light quanta with the vibrations of the electrons in that direction), thermal conductivity (as a result of the interaction of infrared quanta with the intramolecular and intermolecular vibrations and rotations of the molecules), electrical conductivity (as a result of movement of the electrons in an electric field), and hygroscopicity (as a result of access of water molecules into the crystal and their interactions with the molecules of the substance). Differences in melting point of the various polymorphs arise from differences of the cooperative interactions of the molecules in the solid state as compared with the liquid state. Differences in the other thermodynamic properties among the various polymorphs of a given substance are discussed below. Also involved are differences in spectroscopic properties, kinetic properties, and some surface properties. Differences in packing properties and in the energetics of the intermolecular interactions (thermodynamic properties) among polymorphs give rise to differences in mechanical properties.

Many pharmaceutical solids can exist in an amorphous form, which, because of its distinctive properties, is sometimes regarded as a polymorph. However, unlike true polymorphs, amorphous forms are not crystalline [1,2,10]. In fact, amorphous solids consist of disordered arrangements of molecules and therefore possess no distinguishable crystal lattice nor unit cell and consequently have zero crystallinity. In amorphous forms, the molecules display no long-range order, although the short-range intermolecular forces give rise to the short-range order typical of that between nearest neighbors (see Fig. 5). Thermodynamically, the absence of stabilizing lattice energy causes the molar internal energy or molar enthalpy of the amorphous form to exceed that of the crystalline state. The absence of long-range order causes the molar entropy of the amorphous form to exceed that of the crystalline state. Furthermore, the lower stability and greater reactivity of the amorphous form indicates that its molar Gibbs free energy exceeds that of the crys-

(a)

(b)

Fig. 5   Schematic diagram showing the difference in long-range order of silicon dioxide in (a) the crystalline state (crystobalite) and (b) the amorphous state (silica glass) [2]. The two forms have the same short-range order. (Reproduced with permission of the copyright owner, the American Pharmaceutical Association, Washington, DC.)

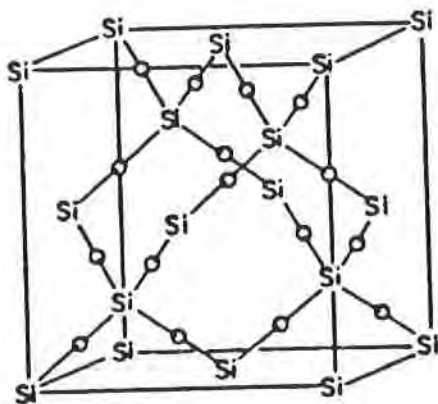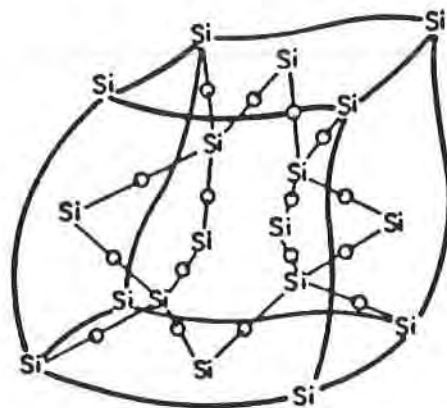talline state. This observation implies that the increased molar enthalpy of the amorphous form outweighs the $T\Delta S$ term that arises from its increased molar entropy.

II. THERMODYNAMICS OF POLYMORPHS

The energy of interaction between a pair of molecules in a solid, liquid, or real gas depends on the mean intermolecular distance of separation according to the Morse potential energy curve shown in Fig. 6 [11,12]. For a given pair of molecules, each polymorph, liquid or real gas has its own characteristic interaction energies and Morse curve. These intermolecular Morse curves are similar in shape but have smaller energies and greater distances than the Morse potential energy curve for the interaction between two atoms linked by a covalent bond in a diatomic molecule or within a functional group of a polyatomic molecule. The Morse potential energy curve in Fig. 6 is itself the algebraic sum of a curve for intermolecular attraction due to van der Waals forces or hydrogen bonding and a curve for intermolecular electron–electron and nucleus–nucleus repulsion at closer approach. The convention employed is that attraction causes a decrease in potential energy, whereas repulsion causes an increase in potential energy. At the absolute zero of temperature, the pair of molecules would occupy the lowest or zero point energy level. The Heisenberg uncertainty principle requires that the molecules have an indeterminate position at a defined momentum or energy. This indeterminate position corresponds to the familiar vibration of the molecules about the mean positions that define the mean intermolecular distance. At a temperature T above the absolute zero, a proportion of the molecules will occupy higher energy levels according to the Boltzmann equation:

$$\frac{N_1}{N_0} = \exp\left(\frac{-\Delta\varepsilon_1}{kT}\right) \tag{1}$$

where N_1 is the number of molecules occupying energy level 1 (for which the potential energy exceeds the zero point level by the energy difference $\Delta\varepsilon_1$), N_0 is the number of molecules occupying the zero point
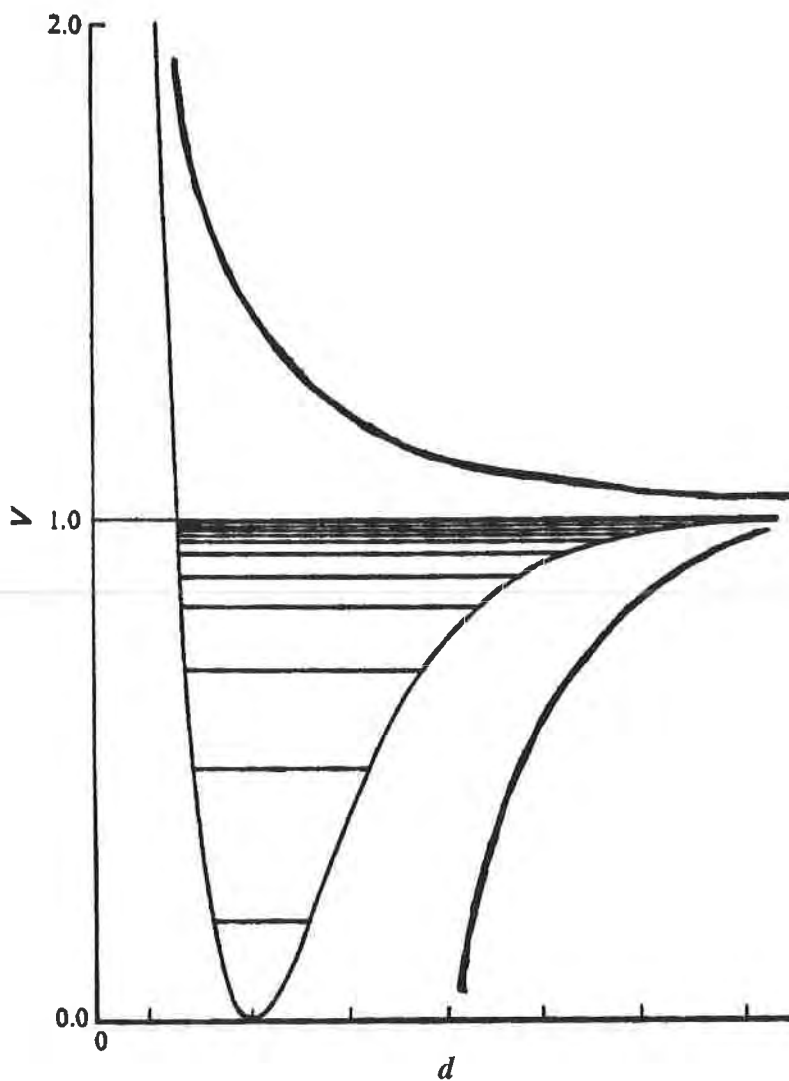
Fig. 6 Morse potential energy curve of a given condensed phase, solid or liquid [11]. The potential energy of interaction V is plotted against the mean intermolecular distance d. (Reproduced with permission of the copyright owner, Oxford University Press, Oxford, UK.)

level, and k is the Boltzmann constant (1.381×10^{-23} J/K, or 3.300×10^{-26} cal/K, i.e. the gas constant per molecule).

With increasing temperature, increasing numbers of molecules occupy the higher energy levels so that the distribution of the molecules among the various energy levels (known as the Boltzmann distribution) becomes broader, as shown in Fig. 7. At any given temperature, the number of distinguishable arrangements of the molecules of the system among the various energy levels (and positions in space) available to them is termed the thermodynamic probability Ω. With increasing temperature, Ω increases astronomically. According to the Boltzmann equation,

$$S = k \cdot \ln \Omega \tag{2}$$

where the entropy S is a logarithmic function of Ω, so increasing temperature causes a steady rise, though not an astronomical rise, in the entropy. In a macroscopic system, such as a given polymorph, the product $T \cdot S$ represents the energy of the system that is associated with
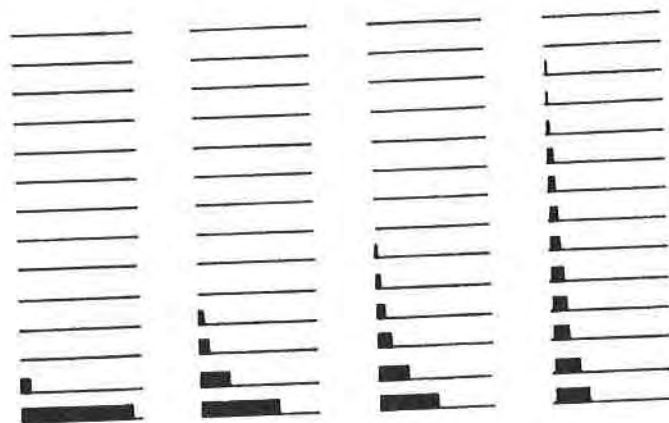
Fig. 7 Populations of molecular states at various temperatures [11]. The temperature is increasing from left to right. (Reproduced with permission of the copyright owner, Oxford University Press, Oxford, UK.)

the disorder of the molecules. This energy is the bound energy of the system that is unavailable for doing work.

The sum of the individual energies of interaction between nearest neighbors, next nearest neighbors, and so on, throughout the entire crystal lattice, liquid, or real gas can be used to define the internal energy E (i.e., the intermolecular structural energy) of the phase. Normally the interactions beyond next nearest neighbors are weak enough to be approximated or even ignored. For quantitative convenience one mole of substance is considered, corresponding to molar thermodynamic quantities. At constant pressure P (usually equal to atmospheric pressure), the total energy of a phase is represented by the enthalpy H:

$$H = E + P \cdot V \tag{3}$$

where V is the volume of the phase (the other quantities have already been defined). With increasing temperature, E, V, and H tend to increase.

Figure 8 shows that the enthalpy H and the entropy S of a phase
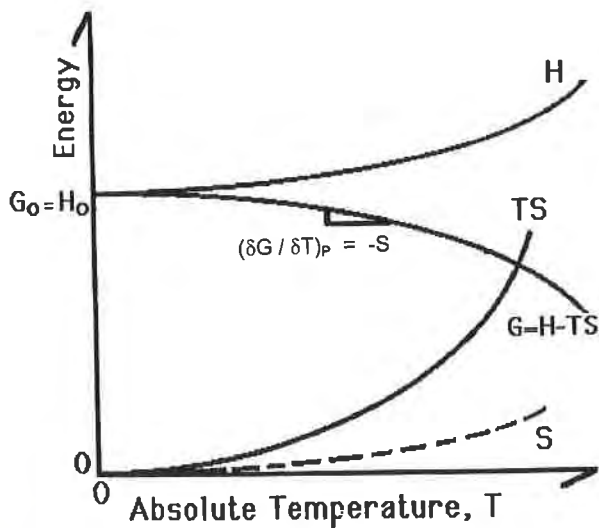
Fig. 8   Plots of various thermodynamic quantities against the absolute temperature T of a given solid phase (polymorph) or liquid phase at constant pressure. H = enthalpy, S = entropy, and G = Gibbs free energy.

tend to increase with increasing absolute temperature T. According to the third law of thermodynamics, the entropy of a perfect, pure crystalline solid is zero at the absolute zero of temperature. The product $T \cdot S$ increases more rapidly with increasing temperature than does H. Hence the Gibbs free energy G, which is defined by

$$G = H - T \cdot S \qquad (4)$$

tends to decrease with increasing temperature (Fig. 8). This decrease also corresponds to the fact that the slope $(\delta G / \delta T)$ of the plot of G against T is negative according to the equation

$$\left(\frac{\delta G}{\delta T} \right)_{p} = -S \qquad (5)$$

As already stated, the entropy of a perfect, pure crystalline solid is zero at the absolute zero of temperature. Hence the value of G at $T = 0$ (termed G_0) is equal to the value of H at $T = 0$, termed $H_0$ (Fig. 8). Each polymorph yields an energy diagram similar to that of Fig. 6, although the values of G, H, and the slopes of the curves at a given temperature are expected to differ between different polymorphs.

Because each polymorph has its own distinctive crystal lattice, it has its own distinctive Morse potential energy curve for the dependence of the intermolecular interaction energies with intermolecular distance. The liquid state has a Morse curve with greater intermolecular energies and distances, because the liquid state has a higher energy and molar volume (lower density) than does the solid state. Figure 9 presents a series of Morse curves, one for each polymorph (A, B, and C) and for the liquid state of a typical substance of pharmaceutical interest. The composite curve in Fig. 9 is the algebraic sum of the Morse curves for each phase (polymorph or liquid). The dashed line corresponds to the potential energy of the separated, noninteracting molecules in the gaseous state. The increase in potential energy from the zero point value of a given polymorph to the dashed line corresponds to the lattice energy of that polymorph or energy of sublimation (if at constant pressure, the enthalpy of vaporization). For the liquid state the increase in potential energy from the average value in the liquid state to the dashed line for the gaseous molecules corresponds to the energy of vaporiza-
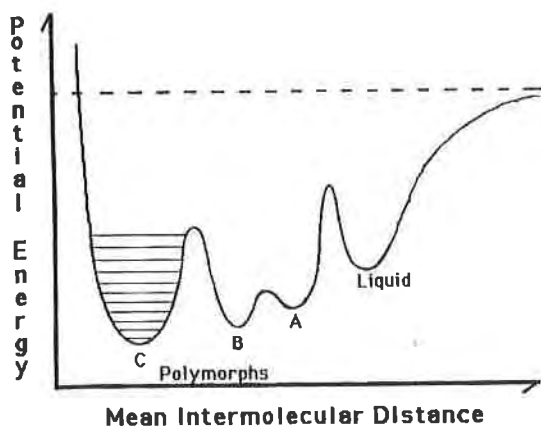
Fig. 9 Composite Morse potential energy curve of a series of polymorphs, A, B, and C, and of the corresponding liquid phase.

tion (if at constant pressure, the enthalpy of vaporization). The increase in potential energy from the zero point value of a given polymorph to the average value for the liquid state corresponds to the energy of fusion (if at constant pressure, the enthalpy of fusion).

When comparing the thermodynamic properties of polymorph 1 and polymorph 2 (or of one polymorph 1 and the liquid state 2) the difference notation is used:

$$\Delta G = G_2 - G_1 \tag{6}$$

$$\Delta S = S_2 - S_1 \tag{7}$$

$$\Delta H = H_2 - H_1 \tag{8}$$

$$\Delta V = V_2 - V_1 \tag{9}$$

In discussions of the relative stability of polymorphs and the driving force for polymorphic transformation at constant temperature and pressure (usually ambient conditions), the difference in Gibbs free energy is the decisive factor and is given by

$$\Delta G = \Delta H - T \Delta S \tag{10}$$
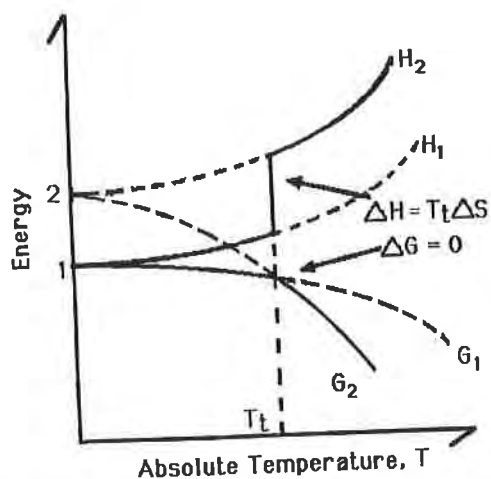
Fig. 10 Plots of the Gibbs free energy G and the enthalpy H at constant pressure against the absolute temperature T for a system consisting of two polymorphs, 1 and 2 (or a solid, 1, and a liquid, 2). T_t is the transition temperature (or melting temperature) and S is the entropy.

Figure 10 shows the temperature dependence of G and H for two different polymorphs 1 and 2 (or for a solid 1, corresponding to any polymorph, and a liquid 2) [13]. In Fig. 10 the free energy curves cross. At the point of intersection, known as the transition temperature T_t (or the melting point for a solid and a liquid), the Gibbs free energies of the two phases are equal, meaning that the phases 1 and 2 are in equilibrium (i.e., $\Delta G = 0$). However, at T_t Fig. 10 shows that polymorph 2 (or the liquid) has an enthalpy H_2 that is higher than that of polymorph 1 (or the solid), so that $H_2 > H_1$. Equations 10 and 6 show that, if $\Delta G = 0$, polymorph 2 (or the liquid) also has a higher entropy S_2 than does polymorph 1 (or the solid), so that $S_2 > S_1$. Therefore according to Equation 10, at T_t,

$$\Delta H_t = T_t \, \Delta S_t \tag{11}$$

where $\Delta H_t = H_2 - H_1$ and $\Delta S_t = S_2 - S_1$ at T_t. By means of differential scanning calorimetry, the enthalpy transition ΔH_t (or the enthalpy of fusion ΔH_f) may be determined. For a polymorphic transition, the rate

of temperature increase must be slow enough to allow polymorph 1 to change completely to polymorph 2 over a few degrees. Because in Fig. 10, $H_2 > H_1$, ΔH is positive and the transition is endothermic in nature.

Figure 10 shows that, below T_t, polymorph 1 (or the solid) has the lower Gibbs free energy and is therefore more stable (i.e., $G_2 > G_1$). On the other hand, above T_t, polymorph 2 (or the liquid) has the lower Gibbs free energy and is therefore more stable (i.e., $G_2 < G_1$). Under defined conditions of temperature and pressure, only one polymorph can be stable, and the other polymorph(s) are unstable. If a phase is unstable but transforms at an imperceptibly low rate, then it is sometimes said to be metastable.

The Gibbs free energy difference ΔG between two phases reflects the ratio of "escaping tendencies" of the two phases. The escaping tendency is termed the fugacity f and is approximated by the saturated vapor pressure, p. Therefore

$$\Delta G = RT \ln \left(\frac{f_2}{f_1}\right) \tag{12}$$

$$\sim RT \ln \left(\frac{p_2}{p_1}\right) \tag{13}$$

where the subscripts 1 and 2 refer to the respective phases, R is the universal gas constant, and T is the absolute temperature. The fugacity is proportional to the thermodynamic activity a (where the constant of proportionality is defined by the standard state), while thermodynamic activity is approximately proportional to the solubility s (in any given solvent) provided the laws of dilute solution apply. Therefore

$$\Delta G = RT \ln \left(\frac{a_2}{a_1}\right) \tag{14}$$

$$\sim RT \ln \left(\frac{s_2}{s_1}\right) \tag{15}$$

in which the symbols have been defined above. Hence, because the most stable polymorph under defined conditions of temperature and pressure has the lowest Gibbs free energy, it also has the lowest values

of fugacity, vapor pressure, thermodynamic activity, and solubility in any given solvent. During the dissolution process, if transport-controlled under sink conditions and under constant conditions of hydrodynamic flow, the dissolution rate per unit surface area J is proportional to the solubility according to the Noyes–Whitney [14] equation; therefore

$$\Delta G = RT \ln \left(\frac{J_2}{J_1} \right) \tag{16}$$

According to the law of mass action, the rate r of a chemical reaction (including the decomposition rate) is proportional to the thermodynamic activity of the reacting substance. Therefore

$$\Delta G = RT \ln \left(\frac{r_2}{r_1} \right) \tag{17}$$

To summarize, the most stable polymorph has the lowest Gibbs free energy, fugacity, vapor pressure, thermodynamic activity, solubility, and dissolution rate per unit surface area in any solvent, and rate of reaction, including decomposition rate.

III. ENANTIOTROPY AND MONOTROPY

If as shown in Fig. 10 one polymorph is stable (i.e., has the lower free energy content and solubility over a certain temperature range and pressure), while another polymorph is stable (has a lower free energy and solubility over a different temperature range and pressure), the two polymorphs are said to be enantiotropes, and the system of the two solid phases is said to be enantiotropic. For an enantiotropic system a reversible transition can be observed at a definite transition temperature, at which the free energy curves cross before the melting point is reached. Examples showing such behavior include acetazolamide, carbamazepine, metochlopramide, and tolbutamide [9,14,15].

Sometimes only one polymorph is stable at all temperatures below the melting point, with all other polymorphs being therefore unstable. These polymorphs are said to be monotropes, and the system of the two solid phases is said to be monotropic. For a monotropic system

the free energy curves do not cross, so no reversible transition can be observed below the melting point. The polymorph with the higher free energy curve and solubility at any given temperature is, of course, always the unstable polymorph. Examples of this type of system include chloramphenicol palmitate and metolazone [9,14,15].

To help decide whether two polymorphs are enantiotropes or monotropes, Burger and Ramberger developed four thermodynamic rules [14]. The application of these rules was extended by Yu [15]. The most useful and applicable of the thermodynamic rules of Burger and Ramberger are the heat of transition rule and the heat of fusion rule. Figure 11, which includes the liquid phase as well as the two polymorphs, illustrates the use of these rules. The heat of fusion rule states that, if an endothermic polymorphic transition is observed, the two forms are enantiotropes. Conversely, if an exothermic polymorphic transition is observed, the two forms are monotropes.

The heat of fusion rule states that, if the higher melting polymorph has the lower heat of fusion, the two forms are enantiotropes. Conversely, if the higher melting polymorph has the higher heat of fusion, the two forms are monotropes. Figure 11, which includes the liquid phase as well as the two polymorphs, is necessary to illustrate the heat of fusion rule.

The above conditions, that are implicit in the thermodynamic rules, are summarized in Table 4. The last two rules in Table 4, the infrared rule and the density rule, were found by Burger and Ramberger [14] to be significantly less reliable than the heat of transition rule and the heat of fusion rule and are therefore not discussed here.

IV. KINETICS OF CRYSTALLIZATION

Among the various methods for preparing different polymorphs are sublimation, crystallization from the melt, crystallization from supercritical fluids, and crystallization from liquid solutions. In the pharmaceutical sciences, different polymorphs are usually prepared by crystallization from solution employing various solvents and various temperature regimes, such as initial supersaturation, rate of de-supersaturation, or final supersaturation. The supersaturation of the solution

(a)
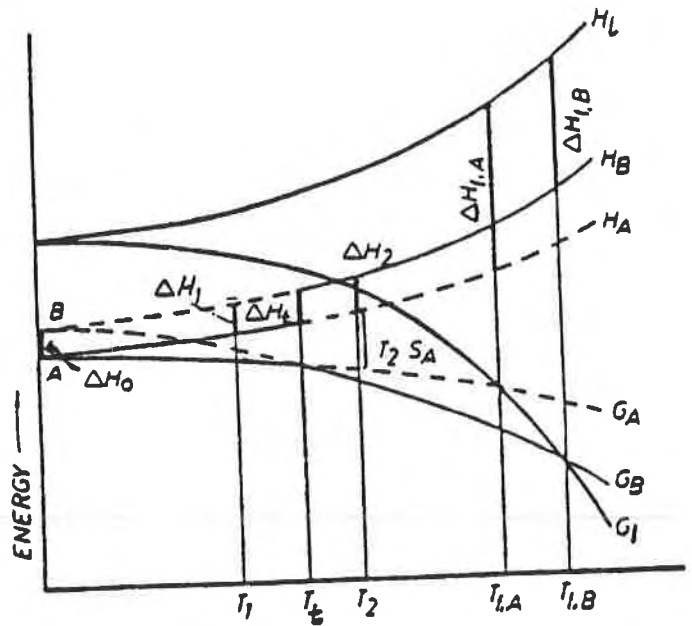
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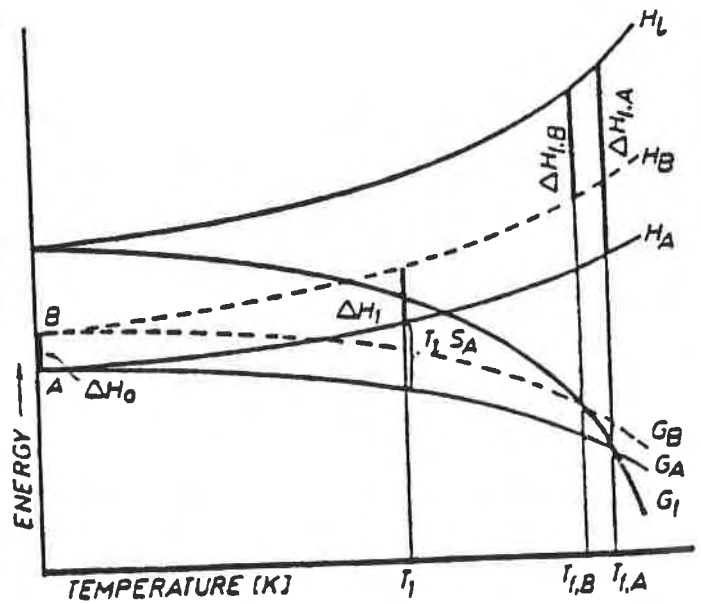
Table 4 Thermodynamic Rules for Polymorphic Transitions According to Burger and Ramberger [14], Where Form I is the Higher-Melting Form

Enantiotropy	Monotropy
Transition < melting I	Transition > melting I
I Stable > transition	I always stable
II Stable < transition	
Transition reversible	Transition irreversible
Solubility I higher < transition	Solubility I always lower than II
Solubility I lower > transition	
Transition II → I is endothermic	Transition II → I is exothermic
$\Delta H_f^I < \Delta H_f^{II}$	$\Delta H_f^I > \Delta H_f^{II}$
IR peak I before II	IR peak I after II
Density I < density II	Density I > density II

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that is necessary for crystallization may be achieved by evaporation of the solvent (although any impurities will be concentrated), cooling the solution from a known initial supersaturation (or heating the solution if the heat of solution is exothermic), addition of a poor solvent (sometimes termed a precipitant), chemical reaction between two or more soluble species, or variation of pH to produce a less soluble acid or base from a salt or vice versa (while minimizing other changes in composition).

During the 19th century, Gay Lussac observed that, during crystallization, an unstable form is frequently obtained first that subsequently transforms into a stable form [13]. This observation was later explained thermodynamically by Ostwald [13,16–19], who formulated the law of successive reactions, also known as Ostwald's step rule. This

Fig. 11 Plots of the Gibbs free energy G and the enthalpy H at constant pressure against the absolute temperature T for a system consisting of two polymorphs, A and B, and a liquid phase, 1 [14]. T_t is the transition temperature, T_f is the melting temperature, and S is the entropy for (a) an enantiotropic system and (b) a monotropic system. (Reproduced with permission of the copyright owner, Springer Verlag, Vienna, Austria.)

rule may be stated as, "In all processes, it is not the most stable state with the lowest amount of free energy that is initially formed, but the least stable state lying nearest in free energy to the original state [13]."

Ostwald's step rule [13,16–19] is illustrated by Fig. 12. Let an enantiotropic system (Fig. 12a) be initially in a state represented by point X, corresponding to an unstable vapor or liquid or to a supersaturated solution. If this system is cooled, the Gibbs free energy will de-

(a)

(b)

Fig. 12 Relationship between the Gibbs free energy G and the temperature T for two polymorphs for (a) an enantiotropic system and (b) a monotropic system in which the system is cooled from point X [9]. The arrows indicate the direction of change. (Reproduced with permission of the copyright owner, Elsevier, Amsterdam, The Netherlands.)

crease as the temperature decreases. When the state of the system reaches point Y, form B will tend to be formed instead of form A, because according to Ostwald's step rule Y (not Z) is the least stable state lying nearest in free energy to the original state. Similarly, let a monotropic system (Fig. 12b) be initially in a state represented by point X, corresponding to an unstable vapor or liquid or to a supersaturated solut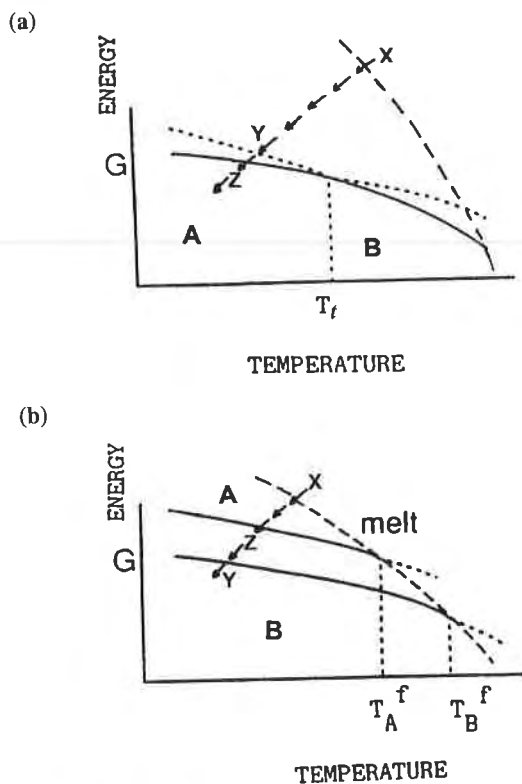ion. If this system is cooled, the Gibbs free energy will decrease as the temperature decreases. When the state of the system reaches point Z, form A will tend to be formed instead of form B, because according to Ostwald's step rule Z (not Y) is now the least stable state lying nearest in free energy to the original state. This rule is not an invariable thermodynamic law but a useful practical rule that is based on kinetics, and it is not always obeyed.

An understanding of the kinetics of the crystallization process involves consideration of the various steps involved. In the first step (termed nucleation) tiny crystallites of the smallest size capable of independent existence (termed nuclei) are formed in the supersaturated phase. Molecules of the crystallizing phase then progressively attach themselves to the nuclei, which then grow to form macroscopic crystals in the process known as crystal growth, until the crystallization medium is no longer supersaturated because saturation equilibrium has now been achieved. If the crystals are now allowed to remain in the saturated medium, the smaller crystals, which have a slightly greater solubility according to the Thomson (Kelvin) equation [11,20], tend to dissolve. At the same time, the larger crystals, which consequently have a lower solubility, tend to grow. This process of the growth of larger crystals at the expense of smaller crystals is sometimes termed Ostwald ripening.

The nucleation step is the most critical for the production of different polymorphs and is therefore discussed in some detail below. Nucleation may be primary (which does not require preexisting crystals of the substance that crystallizes) or secondary (in which nucleation is induced by preexisting crystals of the substance). Primary nucleation may be homogeneous, whereby the nuclei of the crystallizing substance arise spontaneously in the medium in which crystallization occurs, or heterogeneous, whereby the nuclei comprise foreign solid matter, such as particulate contaminants (including dust particles or the walls of the container).

Heterogeneous (i.e., spontaneous) nucleation is a stochastic process that is governed by the algebraic opposition of a volume term that favors the accretion of additional molecules from the supersaturated medium and a surface term that favors the dissolution of the molecular aggregates that would otherwise form nuclei. The resulting curve (Fig. 13) resembles an inverted Morse curve [21]. The molecules of the crystallizing substance tend to aggregate in the supersaturated medium under the influence of the volume term that tends to reduce the Gibbs free energy of the system. The prenuclear aggregates, termed embryos, are relatively small and have a high ratio of surface area to volume. The smaller the embryos, the larger will be the surface-to-volume ratio, and the more effective is the surface term in causing the embryos to
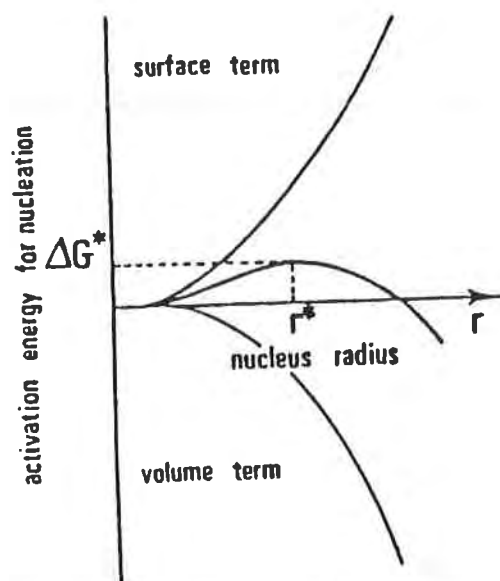
Fig. 13 Plot of the Gibbs free energy G of molecular aggregates (embryos) that are capable of forming nuclei against the size (mean radius r) of the aggregates [21]. ΔG^* is the activation energy for the formation of a nucleus of critical size r^* at which the nucleus can spontaneously grow (G decreases as r increases) or dissolve (G decreases as r decreases) by addition or removal of a single molecule. (Reproduced with permission of the copyright owner, Academic Press, New York, NY.)

dissolve. The resultant free energy curve in Fig. 13 has a maximum corresponding to the critical nuclear aggregate of critical radius r^* and representing an activation energy barrier ΔG^*. Embryos of smaller radius than r^* tend to dissolve, whereas those larger than r^* are true nuclei that tend to grow to form macroscopic crystals [21].

V. NUCLEATION OF POLYMORPHS

For a substance capable of existing in two or more polymorphic forms, each polymorph has its own characteristic curve typified by Fig. 13, each with its own characteristic value of r^* and ΔG^*. Within the limits imposed by their characteristic curves, the aggregates or embryos of the various polymorphs compete for molecules as depicted in Fig. 14 [22]. Depending on the nature of its curve, the aggregate present at the highest concentration (or for which the critical activation energy is the lowest) will form the first nucleus leading to the crystallization of that particular polymorph [22]. This mechanism explains the usual situation in which one polymorph crystallizes depending on the conditions that exist. However, examples are known in which more than one polymorph is obtained in the crystallization process. In these cases, conditions presumably exist whereby more than one type of nucleus is formed in the supersaturated medium at about the same time.

The formation of prenuclear molecular aggregates or embryos in a supersaturated solution can be studied by various physical methods, such as laser Raman spectroscopy [23], a technique that is especially
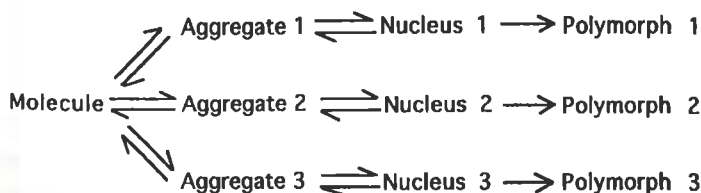
Fig. 14 Nucleation of polymorphs. The aggregate present at the highest concentration, or for which the critical activation energy is lowest, will form the first nucleus leading to the crystallization of that particular polymorph. (Reproduced with permission from Ref. 22.)

useful for aqueous solutions. The vibrational spectra of the aggregates contain peaks that are characteristic of some of the intermolecular interactions (such as hydrogen bonding) that are present in the solid phase that ultimately crystallizes. By appropriate examination of the supersaturated solution, perhaps by spectroscopic methods such as laser Raman spectroscopy [23], it may be possible to identify the intermolecular interaction in the aggregates and hence to identify the nature of the polymorph that will form before it actually crystallizes.

The foregoing theoretical discussion on nucleation, and on the factors that influence nucleation, readily explains why and how the following factors determine the polymorph that crystallizes out: solvent medium, supersaturation, temperature, impurities or additives dissolved, surface of the crystallization vessel, suspended particles, and seed crystals.

Under appropriate thermodynamic conditions discussed at the beginning of this chapter, a less stable polymorph may be converted into a more stable polymorph. The rate of conversion to the more stable polymorph is often rapid, if mediated by the solution phase or vapor phase. In these phases the less stable polymorph (having the greater solubility or vapor pressure) dissolves or sublimes, while the more stable polymorph (having the lower solubility or vapor pressure) crystallizes out. The rate of conversion to the more stable polymorph is usually slower, if the transformation proceeds directly from one solid phase to another. In this case, the mechanism of interconversion is likely to involve the following three steps: (1) loosening and breaking of the intermolecular forces (not covalent bonds) in the less stable polymorph, (2) formation of a disordered solid, similar to a localized amorphous form, and (3) formation of new intermolecular forces leading to crystallization of the more stable polymorph as the product phase [24].

We have seen earlier in this chapter that for an enantiotropic system, one polymorph may transform to another polymorph on the appropriate side of the transition temperature. Figure 15 [9] shows a plot of the rate of polymorphic change as a function of temperature. Close to the transition temperature, the rate is minimal but increases at higher temperatures, at which I → II, or at lower temperatures, at which II → I. If the temperature is lower than a certain optimal value, the rate of polymorphic change of II → I decreases based on the rules of chemical
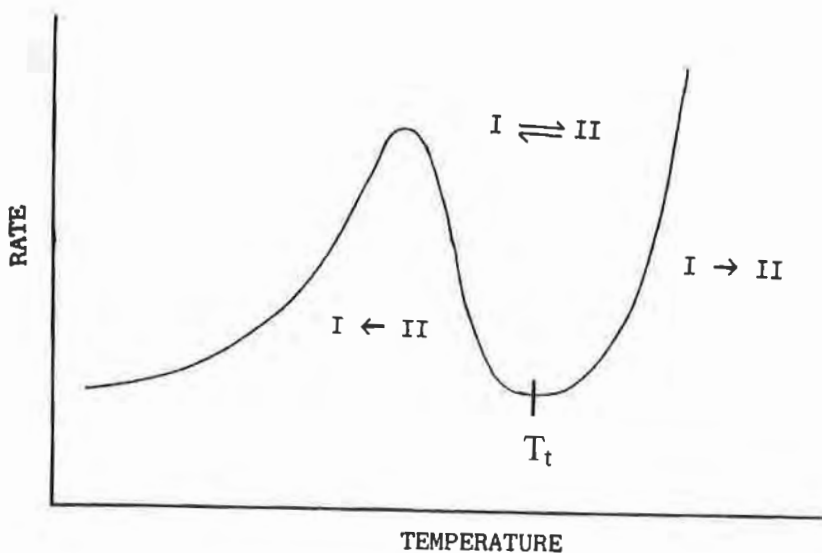
Fig. 15 Temperature dependence of the rates of transformation for a typical first-order transition between a low-temperature polymorph (I) and a high temperature polymorph (II) in an enantiotropic system for which T_t is the transition temperature [9]. (Reproduced with permission of the copyright owner, Elsevier, Amsterdam, The Netherlands.)

kinetics. At temperatures much lower than the transition temperature, the rate of change II → I may be negligible, explaining the observation that the higher temperature polymorph II is metastable at sufficiently low temperatures [9].

VI. NEW OR DISAPPEARING POLYMORPHS

We have seen that the nature of the polymorph that crystallizes depends on the relative rates of nucleation of the polymorphs. These kinetic factors also explain why solid state transformations in molecular crystals often display pronounced hysteresis [25]. For example, to induce the transition of the low-temperature enantiotrope to the high-temperature form, the former may have to be heated well above the transition temperature. Analogously, the absence of a solid state transformation

of the lower melting form below the melting point may not necessarily indicate monotropy but could merely arise from slow nucleation of an enantiotropic transition. Similarly, on cooling the high-temperature form, transformations to the low-temperature form are usually associated with hysteresis. Thus X-ray diffraction studies of crystals have been achieved at 100K, well below (by more than 200K) the temperature range of thermodynamic stability. For example, single-crystal X-ray structural analysis was performed at 98K on the white high-temperature polymorph of dimethyl-3,6-dichloro-2,5-dihydroxyterephthalate, although this polymorph is thermodynamically unstable below 340K [26,27]. Thus a metastable high-temperature form can sometimes remain kinetically stable well below the transition point.

There are several documented examples of the inability to obtain a previously prepared crystal form [27,28]. Dunitz and Bernstein [25] quoted the following passage by Webb and Anderson [29], "Within the fraternity of crystallographers anecdotes abound about crystalline compounds which, like legendary beasts, are observed once and then never seen again." Similar anecdotes have been recounted by some industrial pharmaceutical scientists prior to 1970, but published reports relating to drugs and excipients are exceedingly difficult to find, undoubtedly because they would indicate a lack of process control. Most crystallographers and preformulation scientists recognize the role of seeding in initiating nucleation, and many consider the disappearance of a metastable form to be a local and temporary phenomenon. Jacewicz and Nayler [30] concluded that "any authentic crystal form should be capable of being re-prepared, although selection of the right conditions may require some time and trouble."

The chemical and pharmaceutical literature documents a number of examples of crystal forms that were apparently displaced by a more stable polymorph. One example is benzocaine picrate, for which a crystal form melting at 129–132°C was referred to in the 1968 edition of the *Pharmacopoeia Nordica* as one of the identification tests for the local anesthetic. The 8th edition of the *Merck Index* (1968) gives 134°C as the melting point [31]. Nielsen and Borka [32] described a more stable polymorph melting at 162–163°C, which can be obtained by drying the original lower-melting form at 105°C for two or more hours or by vacuum drying at 100°C and 0.1 mmHg with or without sublima-

tion. On a hot stage under a microscope, the phase-pure polymorphs melt at 132–133°C or 162–163°C, respectively. A partially transformed sample that contains both polymorphs partially melts at 132°C, whereupon the molten benzocaine picrate resolidifies within seconds [32]. The new resolidified crystals that grow from the liquid phase are found to melt at 162–163°C, typical of the more stable polymorph. The infrared spectra of the two polymorphs differ mainly around 3500 cm^{-1} and in the 1500–1700 cm^{-1} region [32]. The authors report [32] that, once the stable (higher-melting) form had been obtained in either of the two laboratories, the metastable (lower-melting) polymorph could no longer be isolated. Most significantly, it was reported that the lower-melting polymorph could be isolated again after discarding all the samples, washing the equipment and laboratory benches, and waiting for 8–12 days. This cleansing procedure had been repeated several times in the laboratories of the above authors, who commented ''Obviously, the seeding effect during the formation of the primary crystals (or during the very procedure of determination of the melting point) is exceptionally strong'' [32]. After these findings the monograph in the 1973 edition of the *Pharmacopoeia Nordica* was modified, stating that benzocaine picrate has a melting point between 161°C and 164°C and may be formed as a metastable modification with melting point between 129°C and 132°C, which will not in every case be transformed into the higher-melting modification during the determination of the melting point [32].

Another example of the displacement of a metastable polymorph by a stable polymorph is xylitol (the RS or meso form), which is used as a sweetening agent in tablets, syrups, and coatings and as an alternative to sucrose in foods, confectionery, and toiletries [33–35]. Xylitol is also described in a NF monograph [36]. In the early 1940s, two polymorphs of xylitol were described. One of these is a metastable, hygroscopic, monoclinic form, melting at 61–61.5°C [37] and the other a stable orthorhombic form melting at 93–94.5°C [38]. After a sample of the orthorhombic form was introduced into a laboratory in which the monoclinic polymorph had been prepared, the latter ''changed in a few days into the high-melting and stable form on exposure to the air of the laboratory'' [38]. Later, Kim and Jeffrey determined the crystal structure of the stable orthorhombic polymorph [39]. These authors

stated, ''Attempts to obtain the lower melting monoclinic form from alcoholic solutions either at room temperature or close to 0°C have hitherto been unsuccessful. We invariably grow the orthorhombic crystals. It is interesting to note that although xylitol was first prepared as a syrup in 1891 there was no report of crystallization until fifty years later, when it was the metastable hygroscopic form that was prepared first. Having now obtained the stable form, it is difficult to recover the metastable crystals . . . The availability of appropriate nuclei in the laboratory is clearly a determining factor, as is well known to carbohydrate chemists [39].''

Since the late 1980s, solid state chemists and pharmaceutical scientists have increasingly recognized the possibility of regulating the processes of nucleation and growth of different polymorphs by careful control of the environmental conditions. One interesting approach is to suppress the growth of a particular crystal form and thereby to promote the growth of the other forms, or at least to present them with a competitive advantage, by addition of ''tailor-made'' additives or impurities [40]. In this example, certain polypeptides can preferentially induce the crystallization of one of the homochiral crystals of histidine hydrochloride (R- or S-His·HCl·H$_2$O) at 25°C instead of the racemic compound (R, S-His·HCl·2H$_2$O), which is the thermodynamically more stable form below 45°C. In the absence of the additives, the racemic compound crystallizes below 45°C, whereas the homochiral crystals form above 45°C. Other examples of the use of tailor-made additives to direct the crystallization of one crystal form at the expense of another crystal form have been reported, and the number of examples is increasing. In many of these examples the additive preferentially blocks the growth of certain faces of the crystal form that is being suppressed, as in the just-discussed case of histidine hydrochloride [40].

Dunitz and Bernstein [25] pointed out that their examples of disappearing polymorphs involve molecules that can adopt different shapes (i.e., conformational polymorphism). These molecules often possess conformational freedom, or different configurations (epimers, such as α and β sugars), or different arrangements of their parts (e.g., benzocaine picrate) [25]. When present in solution or in the liquid state, the different conformations will exist in a dynamic equilibrium. The most stable conformer in the solution may not necessarily be that pres-

ent in the thermodynamically most stable crystal form. Dunitz and Bernstein, supporting the scheme presented in Fig. 14 by Etter [22], argue that the rate of formation of nuclei of a stable polymorph could be significantly reduced by a low concentration of the required conformer, while another conformer could be incorporated into the nuclei of a less stable polymorph, which then grows rapidly leading to a metastable crystal [25]. Of course, a polymorph that is metastable at or above ambient temperature might be obtained as the thermodynamically stable form at a lower temperature below the transition point. In preformulation studies of pharmaceutical compounds it is usually, if not always, important to resolve these kinetic and thermodynamic issues. Dunitz and Bernstein [25], echoing Jacewicz and Nayler [30], state that it should always be possible to prepare a previously known polymorph again, although the repreparation will require the appropriate experimental conditions, which might be found quickly or only after some effort. This statement probably expresses the prevailing view and emphasizes the importance of initiating and carrying out a comprehensive screening procedure for polymorphic forms appropriate to the drug substance under consideration [41].

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5

Generation of Polymorphs, Hydrates, Solvates, and Amorphous Solids

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I. METHODS EMPLOYED TO OBTAIN UNIQUE POLYMORPHIC FORMS

Organic medicinal agents that can exist in two or more solid phases often can provide some distinct advantages in particular applications. The metastable solid may be preferred in those instances where absorption of the drug is dissolution rate dependent. The stable phase may be less susceptible to chemical decomposition and may be the only form that can be used in suspension formulations. Often a metastable polymorph can be used in capsules or for tableting, and the thermodynamically stable form for suspensions. Factors related to processing, such as powder flow characteristics, compressibility, filterability, or hygroscopicity, may dictate the use of one polymorph in preference to another. In other cases, a particular form may be selected because of the high reproducibility associated with its isolation in the synthetic procedure.

It is essential to ascertain whether the crystalline material that results from a synthetic procedure is thermodynamically stable before conducting pivotal trials, since a more stable form may be obtained subsequently, and it may be impossible to produce the metastable form in future syntheses. Conversion from one polymorph to another can occur during processing or upon storage. An additional incentive for

isolating and identifying polymorphs that provides certain advantages is the availability of subsidiary patents for desirable polymorphic forms, or for retaining a competitive edge through unpublished knowledge. In 1990 Byrn and Pfeiffer found more than 350 patents on crystal forms granted on the basis of an advantage in terms of stability, formulation, solubility, bioavailability, ease of purification, preparation or synthesis, hygroscopicity, recovery, or prevention of precipitation [1].

One question that is likely to arise during the registration process is ''What assurance can be provided that no other crystalline forms of this compound exist?'' It is incumbent on the manufacturer of a new drug substance to show that due diligence has been employed to isolate and characterize the various solid-state forms of a new chemical entity. This may seem to be a daunting task, particularly in light of the widely quoted statement by Walter C. McCrone [2] that ''Those who study polymorphism are rapidly reaching the conclusion that all compounds, organic and inorganic, can crystallize in different crystal forms or polymorphs. In fact, the more diligently any system is studied the larger the number of polymorphs discovered.'' On the other hand, one can take comfort from the fact that some important pharmaceuticals have been in use for many years and have, at least until now, exhibited only one stable form. Indeed, it seems to this author that there must be particular bonding arrangements of some molecules that are so favorable energetically as to make alternate arrangements unstable or nonisolatable.

In the future, computer programs using force-field optimization should be perfected to the point where it will be possible to predict, with confidence, that a particular crystalline packing arrangement is the most stable that is likely to be found. These programs also may make it possible to predict how many alternate arrangements having somewhat higher energy can potentially be isolated [3,4]. Until that time, the developmental scientist is handicapped in attempting to predict how many solid forms of a drug are likely to be found. The situation is further complicated by the phenomenon of ''disappearing polymorphs'' [5], or metastable crystal forms that seem to disappear in favor of more stable ones.

Some polymorphs can be detected, but not isolated. Hot stage microscopy has been used extensively to study polymorphic transfor-

mations. The microscopist can detect numerous polymorphic transformations, but the individual polymorphs often prove to be so unstable that they cannot be isolated by the usual methods. An excellent example of this is the work of Grießer and Burger on etofylline [6]. These authors identified five polymorphic forms by thermomicroscopy, but only stable Modification I could be obtained by recrystallization, even when seed crystals from the hot stage were used. Similarly, Kuhnert-Brandstätter, Burger, and Völlenklee [7] described six polymorphic forms of piracetam, only three of which could be obtained by solvent crystallization. All the others were found only by crystallization from the melt. What, then, is a careful investigator to do?

In this chapter, the various methods used to isolate polymorphs, hydrates, and solvates will be described. As Bernstein [8] has observed, ''The conditions under which different polymorphs are obtained exclusively or together also can provide very useful information about the relative stability of different phases and the methods and techniques that might be necessary to obtain similar structures of different chemical systems.'' In this context, it is hoped that the following information will prove useful in devising a ''screening'' protocol for the preparation of the various solid state forms of pharmaceuticals. While one cannot be absolutely certain that no additional forms will be identified in the future, this approach should provide some assurance that ''due diligence'' has been exercised to isolate and identify crystalline forms that are likely to arise during the normal course of drug development and storage.

A. Sublimation

On heating, approximately two-thirds of all organic compounds are converted partially from the solid to the gaseous state and back to solid, i.e., they sublime [9]. While strictly speaking the term sublimation refers only to the phase change from solid to vapor without the intervention of the liquid phase, it is often found that crystals are formed on cooler surfaces in close proximity to the melt of organic compounds when no crystals were formed at temperatures below the melting point. The most comprehensive information concerning sublimation temperatures of compounds of pharmaceutical interest can be found in tables

in the textbook of Kuhnert-Brandstätter [9]. While the information in these tables is designed primarily for the microscopic examination of compounds, it is also possible to utilize it to determine which compounds might be susceptible to the application of techniques (such as vacuum sublimation) that can be carried out on larger scales and at lower temperatures.

The sublimation temperature and the distance of the collecting surface from the material undergoing sublimation have a great influence on the form and size of the crystals produced. The occurrence of polymorphic modifications depends on the temperature of sublimation. In general, it may be assumed that unstable crystals form preferentially at lower temperatures, while at higher temperatures stable forms are to be expected. Nevertheless, mixtures consisting of several modifications are frequently found together. This is the case for barbital and for estradiol benzoate. It should be obvious that the sublimation technique is applicable only to those compounds that are thermally stable.

A simple test can be used to determine if a material sublimes. A small quantity (10–20 mg) of the solid is placed in a petri dish that is covered with an inverted watch glass. The petri dish is heated gently on a hot plate and the watch glass is observed to determine if crystals are growing on it. According to McCrone [2], one of the best methods for obtaining a good sublimate is to spread the material thinly over a portion of a half-slide, cover with a large cover glass, and heat slowly using a Kofler block. When the sublimate is well formed, the cover glass is removed to a clean slide for examination. It is also possible to form good crystals by sublimation from one microscope slide to a second held above it, with the upper slide also being heated so that its temperature is only slightly below that of the lower slide. Cooling of the cover slip by placing drops of various low-boiling solvents on the top surface will cause condensation of the more unstable forms, the lower temperatures leading to the most unstable forms. On a larger scale, a glass cold finger or a commercial sublimator can be employed. Once crystals of various modifications have been obtained, they can be used as seeds for the solution phase crystallization of larger quantities.

Form I of 9,10-anthraquinone-2-carboxylic acid was obtained as needle-like crystals upon sublimation at temperatures exceeding 250°C [10]. Fokkens et al. have used sublimation to purify theophylline for

vapor pressure studies [11]. Sakiyama and Imamura found that stable phases of both 1,3-dimethyluracil and malonamide could be prepared by vacuum sublimation [12].

B. Crystallization from a Single Solvent

Slow solvent evaporation is a valuable method for producing crystals. Solutions of the material being crystallized, preferably saturated or nearly so, are filtered to remove most nuclei and then left undisturbed for a reasonable period of time. The rate of evaporation is adjusted by covering the solution with aluminum foil or Parafilm® containing a few small holes. For a solvent to be useful for recrystallization purposes, the solubility of the solute should be on the order of 5–200 mg/mL at room temperature. If the solubility exceeds 200 mg/mL, the viscosity of the solution will be high, and a glassy product is likely to be obtained. A useful preliminary test can be performed on 25–50 mg of sample, adding a few (5–10) drops of solvent. If all the solid dissolves, the solvent will not be useful for recrystallization purposes. Similarly, highly viscous solvents, and those having low vapor pressures (such as glycerol or dimethylsulfoxide) are not usually conducive to efficient crystallization, filtration, and washing operations. The solvents selected for recrystallization should include any with which the compound will come into contact during synthesis, purification, and processing, as well as solvents having a range of boiling points and polarities. Examples of solvents routinely used for such work are listed in Table 1 together with their boiling points.

The process of solution mediated transformation can be considered the result of two separate events, (a) dissolution of the initial phase, and (b) nucleation/growth of the final, stable phase. If crystals do not grow as expected from a saturated solution, the interior of the vessel can be scratched with a glass rod to induce crystallization by distributing nuclei throughout the solution. Alternatively, crystallization may be promoted by adding nuclei, such as seed crystals of the same material. For example, Suzuki showed that the α-form of inosine could be obtained by crystallization from water, whereas isolation of the β-form required that seeds of the β-form be used [13].

If two polymorphs differ in their melting point by 25–50°C, for

Table 1 Solvents Often Used in the
Preparation of Polymorphs

Solvent	Boiling point (°C)
Dimethylformamide	153
Acetic acid	118
Water	100
1-Propanol	97
2-Propanol	83
Acetonitrile	82
2-Butanone	80
Ethyl acetate	77
Ethanol	78
Isopropyl ether	68
Hexane	69
Methanol	65
Acetone	57
Methylene chloride	40
Diethyl ether	35

monotropic polymorphs the lower melting, more soluble, form will be difficult to crystallize. The smaller the difference between the two melting points, the more easily unstable or metastable forms can be obtained.

A commonly used crystallization method involves controlled temperature change. Slow cooling of a hot, saturated solution can be effective in producing crystals if the compound is more soluble at higher temperatures; alternatively, slow warming can be applied if the compound is less soluble at higher temperatures. Sometimes it is preferable to heat the solution to boiling, filter to remove excess solute, then quench cool using an ice bath or even a dry ice–acetone bath. High boiling solvents can be useful to produce metastable polymorphs. McCrone [2] describes the use of high boiling solvents such as benzyl alcohol or nitrobenzene for recrystallization on a hot stage. Behme et al. [14] showed that when buspirone hydrochloride is crystallized above 95°C the higher melting form is obtained; below 95°C the lower

melting form is obtained. Thus the lower melting polymorph could be converted to the higher melting polymorph by recrystallizing from xylene (boiling point 137–140°C).

To understand how temperature influences the composition of crystals that form, it is useful to examine typical solubility–temperature diagrams for substances exhibiting monotropic and enantiotropic behavior [15]. In Fig. 1a, Form II, having the lower solubility, is more stable than Form I. These two noninterchangeable polymorphs are monotropic over the entire temperature range shown. For indomethacin, such a relationship exists between Forms I and II, and between Forms II and III.

In Fig. 1b, Form II is stable at temperatures below the transition temperature T_t, and Form I is stable above T_t. At the transition temperature the two forms have the same solubility, and reversible transformation between enantiotropic Forms I and II can be achieved by temperature manipulation. The relative solubility of two polymorphs is a
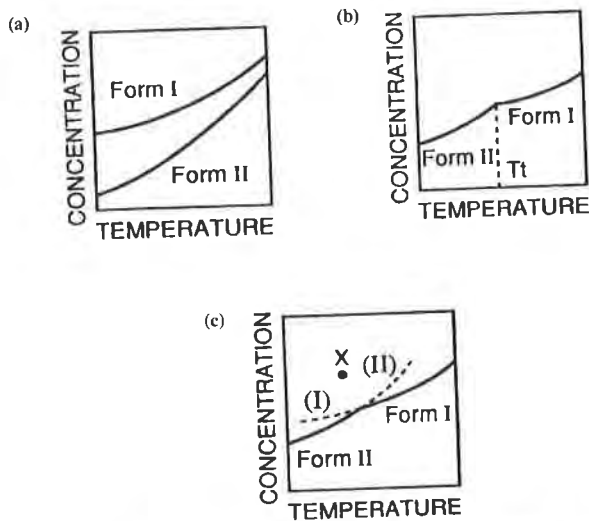
Fig. 1 Solubility curves exhibiting (a) monotropy, (b) enantiotropy, and (c) enantiotropy with metastable phases. (Reprinted with permission of the copyright holder [15].)

convenient measure of their relative free energies. The polymorph having the lower solubility is the more thermodynamically stable form, i.e., the form with the lower free energy at the temperature of the solubility measurement. At room temperature, carbamazepine Form I (m.p. 189°C) is more soluble than is Form III (m.p. 174°C), so the form with the higher melting point is more soluble. The polymorphs are enantiotropic with respect to each other [16].

There are situations in which kinetic factors can for a time override thermodynamic considerations. Figure 1c depicts the intervention of metastable phases (the broken line extensions to the two solubility curves). If a solution of composition and temperature represented by point X (supersaturated with respect to both I and II) is allowed to crystallize, it would not be unusual if the metastable Form I crystallized out first even though the temperature would suggest that Form II would be the more stable (i.e., less soluble) form. This is an extension of Ostwald's law of stages [17], which states that "when leaving an unstable state, a system does not seek out the most stable state, rather the nearest metastable state which can be reached with loss of free energy." This form then transforms to the next most soluble form through a process of dissolution and crystallization. Crystallization of Form I when Form II is more stable would be expected if Form I had the faster nucleation and/or crystal growth rate. However, if the crystals of Form I were kept in contact with the mother liquor, transformation could occur as the more soluble Form I crystals dissolve and the less soluble Form II crystals nucleate and grow. For crystals that exhibit this type of behavior, it is important to isolate the metastable crystals from the solvent by rapid filtration so that phase transformation will not occur.

In the general case, if there are any other polymorphic forms with solubilities below that of Form II, the above-described process will continue between each successive pair of forms until the system finally contains only the most stable (the least soluble) form. The implication of this hypothesis is that, by controlling supersaturation and by harvesting crystals at an appropriate time, it should be possible to isolate the different polymorphic forms. Furthermore, the theory predicts that at equilibrium the product of any crystallization experiment must be the stable form, regardless of the solvent system. It is apparent, however,

from the literature that for some solutes it is the choice of solvent rather than the effects of supersaturation that determines the form that crystallizes [18].

Crystallization of mannitol as a single solute was found to be influenced by both the initial mannitol concentration and by the rate of freezing [19]. In the range of 2.5% to 15%, the δ-polymorph is favored by higher concentrations, whereas the β-polymorph is favored at lower concentrations. At constant mannitol concentration (10%), the α-polymorph is favored by a slow freezing rate, whereas the δ-polymorph is favored by a fast freezing rate.

Kaneko et al. [20] observed that both the cooling rate and the initial concentration of stearic acid in *n*-hexane solutions influenced the proportion of polymorphs A, B, C, and E that could be isolated. Garti et al. [21] reported that for stearic acid polymorphs crystallized from various organic solvents, a correlation was observed between the polymorph isolated and the extent of solvent–solute interaction.

The reason for using crystallization solvents having varying polarities is that molecules in solution often tend to form different types of hydrogen-bonded aggregates, and that these aggregate precursors are related to the crystal structures that develop in the supersaturated solution [22]. Crystal structure analysis of acetanilide shows that a hydrogen-bonded chain of molecules is aligned along the needle axis of the crystals. This pattern is characteristic of secondary amides that crystallize in a trans conformation so that the carbonyl acceptor group and the –NH hydrogen bond donor are anti to one another. The morphology of acetanilide crystals can be controlled by choosing solvents that promote or inhibit the formation of this hydrogen-bond chain. Hydrophobic solvents such as benzene and carbon tetrachloride will not participate in hydrogen-bond formation, so they will induce the formation of rapidly growing chains of hydrogen-bonded amides. Crystals grown by evaporation methods from benzene or carbon tetrachloride are long needles. Solvents that are proton donors or proton acceptors inhibit chain formation by competing with amide molecules for hydrogen-bonding sites. Thus acetone inhibits chain growth at the –NH end, and methanol inhibits chain growth at the carbonyl end of the chain. Both solvents encourage the formation of rod-like acetanilide crystals, while

mixtures of benzene and acetone give hybrid crystals that are rod-shaped, with fine needles growing on the ends [23].

Some solvents favor the crystallization of a particular form or forms because they selectively adsorb to certain faces of some polymorphs, thereby either inhibiting their nucleation or retarding their growth to the advantage of others. Among the factors affecting the types of crystal formed are (a) the solvent composition or polarity, (b) the concentration or degree of supersaturation, (c) the temperature, including cooling rate and the cooling profile, (d) additives, (e) the presence of seeds, (f) pH, especially for salt crystallization, and (g) agitation [22].

Martínez-Ohárriz et al. [24] found that Form III of diflunisal is obtained from polar solvents, whereas Forms I and IV are obtained from nonpolar solvents. Likewise, Wu et al. [25] observed that when moricizine hydrochloride is recrystallized from relatively polar solvents (ethanol, acetone, and acetonitrile), Form I is obtained, whereas nonpolar solvents (methylene chloride or methylene chloride/ethyl acetate) yield Form II.

In determining what solvents to use for crystallization, one should be careful to select those likely to be encountered during formulation and processing. Typically these are water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, and hexane. Matsuda employed 27 organic solvents to prepare two polymorphs and six solvates of piretanide [26].

According to McCrone [27], in a poor solvent the rate of transformation of a metastable to a more stable polymorph is slower. Hence a metastable form once crystallized can be isolated and dried before it is converted to a more stable phase by solution phase mediated transformation. In some systems the metastable form is extremely unstable and may be prepared only with more extreme supercooling. This is usually performed on a very small scale with high boiling liquids so that a saturated solution at a high temperature that is suddenly cooled to room temperature will achieve a high degree of supersaturation [28].

There are many examples in the literature of the use of single solvents as crystallization screens. Slow crystallization from acetone, acetonitrile, alcohols, or mixtures of solvents yields the Form A of

fosinopril sodium, but rapid drying of a solution of this compound yields Form B, sometimes contaminated with a small amount of Form A [29]. A rotary evaporator can be used to maintain a solution at the appropriate temperature as solvent is being removed.

Form I of dehydroepiandrosterone was obtained by recrystallization from warm ethyl acetate, acetone, acetonitrile, or 2-propanol. Form II was obtained by rapid evaporation, using a vacuum from solutions in dioxane, tetrahydrofuran, or chloroform (which are higher boiling, less polar solvents) [30].

C. Evaporation from a Binary Mixture of Solvents

If single-solvent solutions do not yield the desired phase, mixtures of solvents can be tried. Multicomponent solvent evaporation methods depend on the difference in the solubility of the solute in various solvents. In this approach, a second solvent in which the solute is sparingly soluble is added to a saturated solution of the compound in a good solvent. Often a solvent system is selected in which the solute is more soluble in the component with the higher vapor pressure. As the solution evaporates, the volume of the solution is reduced and, because the solvents evaporate at different rates, the composition of the solvent mixture changes.

Occasionally, crystals are obtained by heating the solid in one solvent and then pouring the solution into another solvent or over cracked ice. Otsuka et al. [31] obtained phenobarbital Form B by adding dropwise a saturated solution of the compound in methanol to water at room temperature. Form E was obtained by the same technique, but by using a saturated solution of phenobarbital in dioxane.

Kitamura et al. have shown that the fraction of Form A of *L*-histidine decreases quickly when the volume fraction of ethanol in an ethanol–water solvent system increases above 0.2, and that pure Form B is obtained at a 0.4 volume fraction of ethanol [32]. The transformation rate for conversion of Form B to Form A decreases with ethanol concentration. The authors postulated that the concentration of the conformer that corresponds to Form A decreases more with ethanol concentration than that of Form B, and so the growth rate of Form A will also decrease.

An example of precipitation in the presence of a second solvent is seen in the case of indomethacin. The γ-crystal form of indomethacin can be obtained by recrystallization from ethyl ether at room temperature, but the α-form is prepared by dissolution in methanol and precipitation with water at room temperature [33]. Precipitation can also result from the addition of a less polar solvent. Form II of midodrine hydrochloride, metastable with respect to Form I, can be prepared by precipitation from a methanolic solution by means of a less polar solvent such as ethyl acetate or dichloromethane [34].

In Fig. 2, three crystalline modifications of thalidomide are illustrated. These were obtained by solvent recrystallization techniques and differ both in crystal habit and in crystal structure. Two of the forms were obtained from a single solvent, and one from a binary mixture.

D. Vapor Diffusion

In the vapor diffusion method, a solution of the solute in a good solvent is placed in a small, open container that is then stored in a larger vessel containing a small amount of a miscible, volatile nonsolvent. The larger vessel (often a desiccator) is then tightly closed. As solvent equilibrium is approached, the nonsolvent diffuses through the vapor phase into the solution, and saturation or supersaturation is achieved. The solubility of the compound in a precipitant used in a two-solvent crystallization method such as vapor diffusion should be as low as possible (much less than 1 mg/mL), and the precipitant (the solvent in which the compound is poorly soluble) should be miscible with the solvent and the saturated solution. The most frequent application of this technique is in the preparation of single crystals for crystallographic analysis. An illustration of the technique is provided in Fig. 3 [35].

E. Thermal Treatment

Frequently when using differential scanning calorimetry as an analysis technique, one can observe an endothermic peak corresponding to a phase transition, followed by a second endothermic peak corresponding to melting. Sometimes there is an exothermic peak between the two endotherms, representing a crystallization step. In these cases it is often
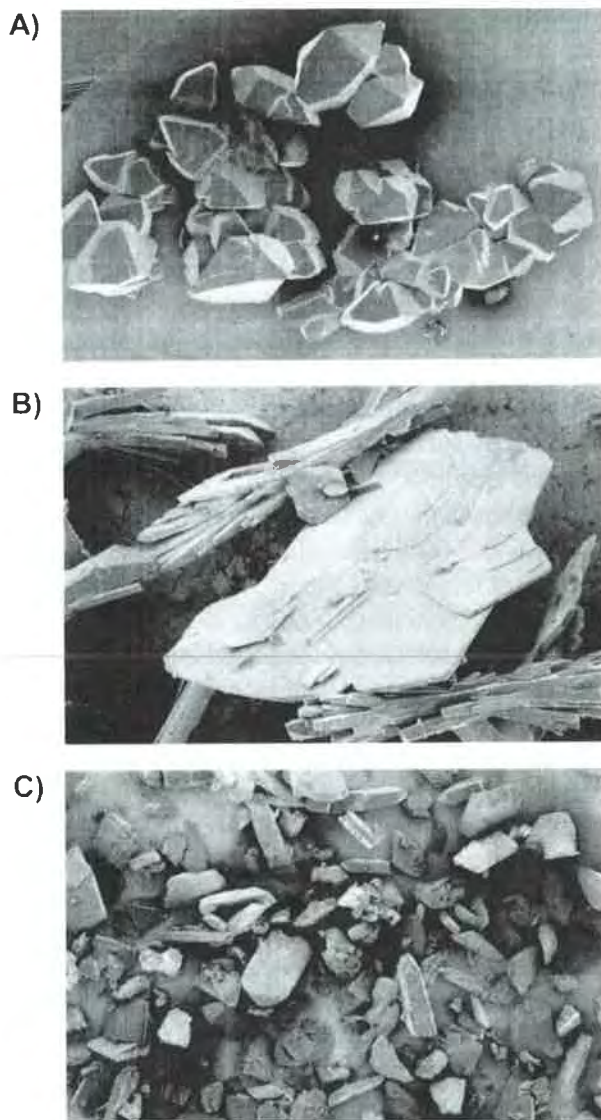
Fig. 2 Three crystalline modifications of thalidomide obtained by solvent recrystallization. (A) Form I obtained as bipyramids by slow crystallization of thalidomide in 1:1 dimethylformamide:ethanol at room temperature. (B) Form II obtained by immersing a saturated solution of thalidomide in acetonitrile in an ice bath. (C) Form III prepared as tabular crystals from a solution in boiling 1,4-dioxane, filtered, then allowed to cool to room temperature. (Photomicrographs courtesy of Dr. S. A. Botha, the University of Iowa.)
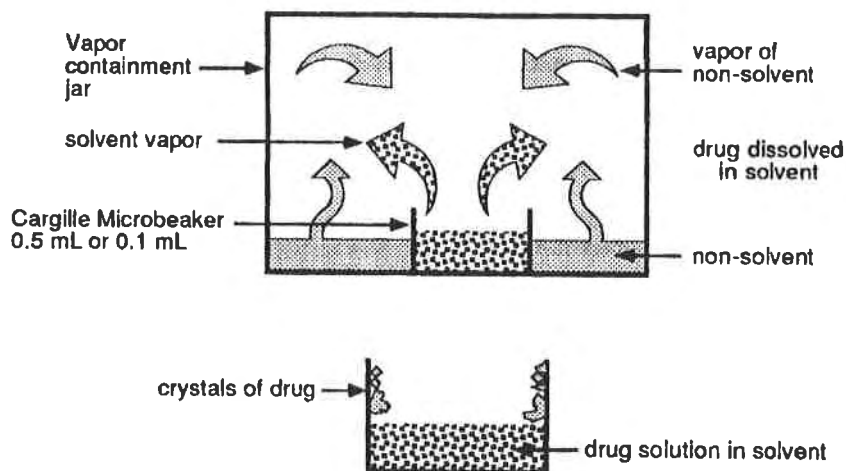
Fig. 3 Crystallization by vapor diffusion. (Reproduced with permission of the author [35] and the copyright holder, Pfizer, Inc.)

possible to prepare the higher melting polymorph by thermal treatment. Thus chlorpropamide Form A is obtained by recrystallization from ethanol solution, but Form C is obtained by heating Form A in an oven maintained at 100°C for 3 hours [36]. While the β-form of tegafur is obtained by the evaporation of a saturated methanol solution, the γ-form is obtained by heating the β-form at 130°C for one hour [37]. Form II of caffeine is prepared by recrystallization from distilled water, but Form I is prepared by heating Form II at 180°C for 10 hours [38].

F. Crystallization from the Melt

In accordance with Ostwald's rule [17], the cooling of melts of polymorphic substances often first yields the least stable modification, which subsequently rearranges into the stable modification in stages. Since the metastable form will have the lower melting point, it follows that supercooling is necessary to crystallize it from the melt. After melting, the system must be supercooled below the melting point of the metastable form, while at the same time the crystallization of the more stable form or forms must be prevented. Quench cooling a melt can

sometimes result in formation of an amorphous solid that on subsequent heating undergoes a glass transition followed by crystallization [39].

On a somewhat larger scale, one can use a vacuum drying pistol and a high boiling liquid such as chlorobenzene to achieve the desired end. Form II of p-(1R,3S)-3-thioanisoyl-1,2,-2-trimethylcyclopentane carboxylic acid was obtained by recrystallization from a 50:50 v/v benzene:petroleum ether mixture. Form I then was obtained by melting Form II in the vacuum drying pistol [40]. Caffeine Form I is prepared by heating Form II at 180°C for 10 hours [38]. Yoshioka et al. [41] observed that when the amorphous solidified melt of indomethacin was stored at 40°C, it partly crystallized as the thermodynamically stable γ-form. Yet at 50°C, 60°C, and 70°C, mixtures of the α- and the γ-form were obtained. Sulfathiazole Form I is obtained by heating Form III crystals (grown from a dilute ammonium hydroxide solution at room temperature) at 170°C for 30–40 minutes [42].

G. Rapidly Changing Solution pH to Precipitate Acidic or Basic Substances

Many drug substances fall in the category of slightly soluble weak acids, or slightly soluble weak bases, whose salt forms are much more soluble in water. Upon addition of acid to an aqueous solution of a soluble salt of a weak acid, or upon addition of alkali to an aqueous solution of a soluble salt of a weak base, crystals often result. These crystals may be different from those obtained by solvent crystallization of the weak acid or weak base. Nucleation does not necessarily commence as soon as the reactants are mixed, unless the level of supersaturation is high, and the mixing stage may be followed by an appreciable time lag before the first crystals can be detected. Well-formed crystals are more likely to result in these instances than when rapid precipitation occurs.

Form I of the x-ray contrast agent iopanoic acid was prepared [43] by dissolving the acid in 0.1 N NaOH, adjusting the pH to 12.5, bubbling nitrogen into the solution, and adding 0.1 N hydrochloric acid until the pH reached 2.15. The resulting precipitate was vacuum filtered

and stored *in vacuo* (380 torr) for 12 hours at 35°C. Similarly, Form III of hydrochlorothiazide was precipitated from sodium hydroxide aqueous solution by the addition of hydrochloric acid [44].

When piretanide was dissolved in 0.1 N NaOH at room temperature and acid was added in a 1:1 ratio (to pH 3.3), piretanide Form C precipitated. However, when the base:acid ratio used was 1:0.95, a mixture of amorphous piretanide and Form C precipitated [45].

H. Thermal Desolvation of Crystalline Solvates

The term ''desolvated solvates'' has been applied to compounds that were originally crystallized as solvates but from which the solvent has been removed (generally by vaporization induced by heat and vacuum). Frequently, these ''desolvated solvates'' retain the crystal structure of the original solvate form and exhibit relatively small changes in lattice parameters. For this reason, these types have been referred to as pseudopolymorphic solvates. However, in instances where the solvent serves to stabilize the lattice, the process of desolvation may produce a change in lattice parameters, resulting in the formation of either a new crystal form or an amorphous form. These solvates have been referred to as polymorphic solvates. Byrn [46] has characterized the desolvation of polymorphic solvates as occurring in four steps, (a) molecular loosening, (b) breaking of the host–solvent hydrogen bonds (or other associations), (c) solid solution formation, and (d) separation of the product phase.

The process of desolvating pseudopolymorphic solvates is simpler, involving only the two steps of (a) molecular loosening and (b) breaking of host–solvent hydrogen bonds or associations. Byrn [46] has summarized the desolvation studies performed on caffeine hydrate, theophylline hydrate, thymine hydrate, cytosine hydrate, dihydrophenylalanine hydrate, dialuric acid hydrate, cycloserine hydrate, erythromycin hydrate, fenoprofen hydrate, manganous formate dehydrate, bis(salicylaldehyde) ethylenediamine cobalt (II) chloroformate, cephatoglycine hydrates and solvates, and cephalexin solvates and hydrates. Among factors that influence the desolvation reaction are the appearance of defects, the size of tunnels in the crystal packing arrange-

ment, and the strength of hydrogen bonding between the compound and its solvent of crystallization [46].

Rocco et al. [47] obtained Form II of zanoterone by recrystallization from ethanol and vacuum drying at 45°C. Form III was isolated by desolvating the acetonitrile solvate form at 80°C under vacuum, and this was the form chosen for use in the clinical drug product due to the high reproducibility of its isolation during manufacture. Similarly, Forms I and II of stanozolol were obtained by heating solvates of the compound to 205°C and 130°C, respectively [48].

The benzene solvate of iopanoic acid was prepared by rapidly freezing a warm benzene solution of iopanoic acid in a dry ice–acetone mixture [43]. The solid obtained was permitted to melt at room temperature, yielding crystals of the solvate suspended in benzene. When these were vacuum filtered and stored *in vacuo* (380 torr) for 12 hours at 70°C, Form II was obtained free of benzene.

Dehydration of hydrates can also lead to the formation of unique crystals. Caffeine Form II was prepared by recrystallizing caffeine from water, drying for 8 days at 30°C, and then heating for 4 hours at 80°C [38]. Chloroquine diphosphate 3:1 hydrate was converted to the anhydrous form at temperatures above 188°C [49]. Etoposide Form I (a monohydrate) was found to undergo a dehydration reaction in the temperature range of 85–115°C to yield etoposide Form 1a. This form could be melted at 198°C and transformed to etoposide Form IIa, which itself melted at 198°C and crystallized to still another polymorph, etoposide Form IIa at 206°C. Etoposide Form IIa was found to melt at 269°C and convert to its hydrated form, etoposide Form II, when exposed to the atmosphere at room temperature. This hydrate was also found to undergo a dehydration reaction at 90–120°C to yield etoposide Form IIa [50].

Differential scanning calorimetry (DSC) curves of levofloxacin hemihydrate measured under various conditions showed different thermograms. This behavior was attributed to the dehydration process that resulted in a multiple-phase transition. Dehydration at higher temperatures (above 70°C) gave a sharp endothermic peak in the DSC thermogram due to the melting of the γ-form, and at a lower temperature (50°C) it led to the observation of a sharp endothermic peak due to the

melting of the α-form. In contrast, the thermal behavior of levofloxacin monohydrate was not affected by dehydration [51].

I. Growth in the Presence of Additives

The presence of impurities can have a profound effect on the growth of crystals. Some impurities can inhibit growth completely, and some may enhance growth. Still others may exert a highly selective effect, acting only on certain crystallographic faces and thus modifying the crystal habit. Some impurities can exert an influence at very low concentrations (less than 1 part per million), whereas others need to be present in fairly large amounts to have any effect [15].

Additives can be designed to bind specifically to the surfaces of particular polymorphs and so inhibit their achieving the critical size for nucleation, allowing a desired phase to grow without competition [52]. Lahav and coworkers have shown that additives at levels as low as 0.03% can inhibit nucleation and crystal growth of a stable polymorph, thus favoring the growth of a metastable polymorph [53]. They also showed that it is possible to design crystal nucleation inhibitors to control polymorphism.

Davey et al. found that Form I crystals of terephthalic acid could be obtained by crystallization only in the presence of *p*-toluic acid [54]. Form II, the more stable polymorph at ambient temperatures, was recovered from a hydrothermal recrystallization experiment.

Ikeda et al. [55] determined that indomethacin can exist in three different crystal forms, denoted α-, β-, and γ-, with the α-form possessing a higher solubility than the γ-form. On recrystallization, crystals of the α-form were the first to be deposited, but these converted gradually to the less soluble γ-form. However, in the presence of hydroxypropyl methylcellulose, conversion from the α-form to the γ-form was inhibited, leading to an increase in the solubility of indomethacin.

While the α-form of glycine normally is obtained by recrystallization from water, 3% of racemic hexafluorovaline leads to the precipitation of the γ-polymorph as trigonal pyramids [56]. This additive was designed to be strongly adsorbed at the four {011} crystal faces of the α-form and to bind at only one pole of the polar crystal, thus leaving

the crystal free to grow at the opposite pole. Since it is bound at the slow growing NH_3^+ end of the polar axis, it does not interfere with the fast growing CO_2^- end.

J. Grinding

Polymorphic transformations have been observed to occur on grinding of certain materials, such as sulfathiazole, barbital, phenylbutazone, cephalexin, chloramphenicol palmitate, indomethacin, and chlorpropamide. Byrn [46] has stated that polymorphic transformations in the solid state require the three steps of (a) molecular loosening (nucleation by separation from the lattice), (b) solid solution formation, and (c) separation of the product (crystallization of the new phase). Depending on the material and the conditions employed, grinding can result in conversion to an amorphous substance. With the exercise of care, different polymorphic forms can be obtained. Otsuka et al. [57] showed that metastable Forms B and C of chloramphenicol palmitate were transformed into stable Form A upon grinding at room temperature. Indomethacin was transformed into a noncrystalline solid during grinding at 4°C, and into metastable Form A by grinding at 30°C. Caffeine Form II is converted into Form I with grinding, and a 95% phase conversion was obtained following 60 hours of grinding time [38].

II. METHODS EMPLOYED TO OBTAIN HYDRATE
FORMS

Pharmaceutical solids may come into contact with water during processing steps, such as crystallization, lyophilization, wet granulation, aqueous film-coating, or spray-drying. Moreover, they may be exposed to water during storage in an atmosphere containing water vapor, or in a dosage form consisting of materials that contain water (e.g., excipients) and are capable of transferring it to other ingredients. Water may be adsorbed onto the solid surface and/or may be absorbed in the bulk solid structure. When water is incorporated into the crystal lattice of the compound in stoichiometric proportions, the molecular adduct or adducts formed are referred to as hydrates [58]. More than 90 hydrates

are described in various USP monographs. Hydrates can be prepared by recrystallization from water or from mixed aqueous solvents. They can also result, in some instances, from exposure of crystal solvates (such as methanolates or ethanolates) to an atmosphere containing water vapor.

Crystalline substances often form with water molecules located at specific sites in the crystal lattice, which are held in coordination complexes around lattice cations. This type of water is denoted as water of crystallization and is common for inorganic compounds. For example, nickel sulfate forms a well-defined hexahydrate, where the waters of hydration are bound directly to the Ni(II) ion. Extraneous inclusion of water molecules can occur if a coprecipitated cation carries solvation molecules with it. Water also can be incorporated into random pockets as a result of physical entrapment of the mother liquor. Well-defined multiple hydrate species can also form with organic molecules. For example, raffinose forms a pentahydrate.

Although most hydrates exhibit a whole-number-ratio stoichiometry, an unusual case is the metastable hydrate of caffeine, which contains only 0.8 moles of water per mole of caffeine. Only in a saturated water vapor atmosphere will additional amounts of water be adsorbed at the surface of the 4/5-hydrate to yield a 5/6 hydrate [59].

In some instances, a compound of a given hydration state may crystallize in more than one form, so that the hydrates themselves exhibit polymorphism. One such example is nitrofurantoin, which forms two monohydrates that have distinctly different temperatures and enthalpies of dehydration. The monohydrates have quite different packing arrangements, with Form I possessing a layer structure and Form II exhibiting a herringbone motif. The included water molecules play a major role in stabilizing the crystal structures. Whereas water molecules are contained in isolated cavities in Form II, in Form I they are located in continuous channels, and this apparently facilitates the escape of water when these crystals are heated [60].

Another example of hydrate polymorphism is amiloride hydrochloride [61], which can be obtained in two polymorphic dihydrate forms. These forms are indistinguishable by techniques other than x-ray powder diffraction.

It is interesting that scopolamine hydrobromide has been reported

to exist as the anhydrous form, a "hemihydrate," a sesquihydrate, and a trihydrate [62], while the unit cell parameters and the molecular geometry of these are all the same as those of the hemihydrate. This finding suggests that the "hemihydrate" is actually a partially desolvated sesquihydrate.

Ouabaine is another example of a compound that exhibits many different hydration levels, the most hydrated form being stable at the lowest temperature. Thus the nonahydrate phase of ouabaine is obtained from water at 0–15°C, the octahydrate phase at 15–28°C, and the dihydrate phase at 28–90°C. In addition, ouabaine phases corresponding to 4.5 H_2O, 4 H_2O, and 3 H_2O may be obtained from mixtures of water with other solvents. The anhydrous phase of ouabaine anhydrate is crystallized from ethanol at high temperatures [63].

Typically, hydrates are obtained by recrystallization from water. For example, trazodone hydrochloride tetrahydrate was prepared by dissolving the anhydrate in hot distilled water, allowing the solution to remain at room temperature overnight, and storing the collected crystals at 75% relative humidity and 25°C until they reached constant weight [64].

Hydrates can sometimes be obtained by simply suspending the anhydrous material in water, whereupon a form of Ostwald ripening occurs. For instance, aqueous suspensions of anhydrous metronidazole benzoate are metastable, and storage at temperatures lower then 38°C leads to monohydrate formation accompanied by crystal growth [65]. Sorbitol provides another example of this behavior, where slow cooling of a saturated aqueous solution yields long thin needles of sorbitol hydrate [66]. When suspended in water, anhydrous carbamazepine is transformed to carbamazepine dihydrate [67]. In other instances, hydrates can be obtained from mixed solvent systems. Acemetacin monohydrate can be obtained by slow evaporation from a mixture of acetone and water at room temperature [68].

Simply exposing an anhydrous powder to high relative humidity can often lead to formation of a hydrate. On exposure to a relative humidity of 100%, dexmedetomidine hydrochloride is converted to a monohydrate [69]. Droloxifene citrate is an example of a compound that is not very hygroscopic and yet forms a hydrate. Only after storage of the anhydrous form at 85% relative humidity does some sorption of

water occur. The monohydrate phase can be formed by exposing the anhydrous form to 98% relative humidity for ten days at 24°C [70].

III. METHODS EMPLOYED TO OBTAIN SOLVATE FORMS

Often, when solvents are employed in the purification of new drug substances by recrystallization, it is observed that the isolated crystals include solvent molecules, either entrapped within empty spaces in the lattice or interacting via hydrogen bonding or van der Waals force with molecules constituting the crystal lattice. Solvent molecules also can be found in close association with metal ions, completing the coordination sphere of the metal atom. Coordinated solvent molecules are considered as part of the crystallized molecule. A crystal with large empty channels or cavities is not stable because of packing demands. The size and chemical environment of the cavity or channel determine what kind of solvent molecule can be included in the structure and what kind of interaction occurs between solvent and structure.

Depending on the nature of molecular packing arrangements, it may happen that the inclusion of solvent is necessary to build a stable crystal structure. van Geerestein et al. [71] found during numerous crystallization attempts of 11β-[4-(dimethylamino)phenyl]-17β-hydroxy-17α-(I-propynyl) estra-4,9-diene-3-one] that crystals were only obtainable in the presence of *n*-butyl acetate or *n*-propyl acetate. The crystal structure of the compound crystallized from *n*-butyl acetate/methylcyclohexane was solved, and one solvent molecule was found in the crystal structure that showed no strong interactions with the rest of the structure. Apparently, this solvent molecule was necessary to fill empty space resulting after the molecular packing. Solvates in which the solvent fills empty space are generally nonstoichiometric, such as the nonstoichiometric solvates formed by droloxifene citrate with acetonitrile, 2-propanol, ethanol, 1-propanol, and 1-butanol. Typically such solvates exhibit the same x-ray diffraction pattern as does the nonsolvated compound.

When solvent molecules increase the strength of the crystal lattice, they can affect the stability of the compound to solid-state decom-

position. It has been observed that the four solvated and one nonsolvated structures of prenisolone *tert*-butyl acetate affect the flexibility of the steroid nucleus and the structure-dependent degradation of the compound when exposed to air and light [72].

van der Sluis and Kroon found 1,247 different compounds with cocrystallized solvents in the Cambridge Crystallographic Database [73]. Out of 46,460 total structures, they found 9,464 solvate structures, and 95% of these contained one of the 15 solvents given in Table 2.

The most commonly encountered solvates among pharmaceuticals are those of 1:1 stoichiometry, but occasionally mixed solvate species are encountered. For structures containing more than one solvent type, one generally finds nonpolar solvents crystallizing together on the one hand and polar solvents on the other. For example, the most common solvents found cocrystallizing with water are (in order of im-

Table 2   Distribution of the 15 Most Abundant Solvents in the Cambridge Crystallographic Database, as the Percentage of Solvate Structures

Solvent	Occurrence (%)
Water	61.4
Methylene dichloride	5.9
Benzene	4.7
Methanol	4.1
Acetone	2.8
Chloroform	2.8
Ethanol	2.6
Tetrahydrofuran	2.3
Toluene	2.2
Acetonitrile	1.9
N,N-dimethylformamide	0.9
Diethyl ether	0.9
Pyridine	0.7
Dimethyl sulfoxide	0.5
Dioxane	0.5

Source: From Ref. 73. Reproduced with permission of the copyright owner.

portance) ethanol, methanol, and acetone. An interesting example of a structure containing a polar and a nonpolar solvent is the sodium salt of the antibiotic K-41, p-bromobenzoate monohydrate n-hexane solvate [74], which is crystallized from n-hexane saturated with water. Perhaps the best known mixed solvate is doxycycline hyclate: (doxycycline · HCl)$_2$C$_2$H$_6$O · H$_2$O. Triamterene also forms a mixed solvate, containing one N,N-dimethylformamide molecule and one water molecule within the crystal lattice [75].

The techniques used to obtain solvates are generally similar to the solvent methods used to obtain polymorphs, i.e. crystallization from a single solvent, from mixed solvents, or by vapor diffusion. Sometimes, it is possible to exchange one solvent within the crystal structure for another. When one recrystallizes a hydrate from dry methanol, in most cases one is left with either a methanol solvate or an anhydrous, unsolvated form of the compound.

A large number of solvates have been reported, especially for steroids and antibiotics. It has been observed that cortisone acetate and dexamethasone acetate can be crystallized as 10 different solvates. Dirithromycin, a semisynthetic macrolide antibiotic, crystallizes in two anhydrous polymorphic forms and in at least nine stoichiometric solvate forms. Six of the known solvates are isomorphic, having nearly identical x-ray powder diffraction patterns [76]. In addition to the anhydrate and dihydrate, erythromycin also forms solvates with acetone, chloroform, ethanol, n-butanol, and i-propanol [77].

It may be instructive to consider some examples of solvate formation. The compound 5-methoxysulphadiazine forms 1:1 host–guest solvates with dioxane, chloroform, and tetrahydrofuran [78]. These were prepared by heating to boiling a solution of the sulfonamide in the appropriate solvent, followed by slow cooling to obtain large crystals. Spironolactone forms 1:1 solvates with methanol, ethanol, ethyl acetate, and benzene. It also forms a 2:1 spironolactone–acetonitrile solvate [79,80]. The spironolactone solvates were prepared by crystallization in a refrigerator from solutions that were nearly saturated at room temperature.

Another steroid that forms solvates is stanozolol [81]. Solvates having 1:1 stoichiometry were prepared by recrystallization from methanol, ethanol, and 2-propanol, by heating the compound in the

appropriate solvent to 60–70°C and then cooling to 0°C in an ice bath to induce crystallization. The compound also forms a monohydrate and two polymorphs. The polymorphs were prepared by heating the solvates to either 130°C (Form II) or 205°C (Form I).

Mefloquine hydrochloride is an interesting case of a compound that forms stoichiometric 1:1 solvates on cooling hot (50°C) saturated acetone solutions (Form B, acetone solvate 1:1), hot (50°C) saturated isopropanol (Form I, isopropanol solvate 1:1), and a nonstoichiometric ethanol solvate (2.12% ethanol) from hot (50°C) saturated ethanol, Form E, whose x-ray powder pattern does not change following heating to 80°C, in spite of a decrease in the ethanol level to 0.12%. Mefloquine hydrochloride can also be obtained in a nonsolvated form from hot (70°C) saturated acetonitrile (Form A) and as two hemihydrates from water (Forms D and C) prepared at room temperature and at 30°C [82].

IV. METHODS EMPLOYED TO OBTAIN AMORPHOUS MATERIALS

Solids can exist in crystalline or amorphous form. Crystalline materials have defined structures, stoichiometric compositions, and melting points and are characterized by their chemical, thermal, electrical, optical, and mechanical properties [83]. By contrast, amorphous materials have no clearly defined molecular structure and no long-range order, so their structure can be viewed as being similar to that of a frozen liquid but without the thermal fluctuations observed in the liquid phase. As a result, amorphous materials exhibit the classical diffuse ''halo'' x-ray powder diffraction pattern rather than the sharp peaks observed in the pattern of a crystalline substance. When the halo is broad, it is often difficult to distinguish between a material that is truly amorphous (e.g., a true glass) and one that is merely microcrystalline. This situation exists because when microcrystallites have diameters less than about 50 Å in diameter, a similar ''halo'' effect is observed.

While crystalline solids offer the advantages of chemical and thermodynamic stability, amorphous solids are occasionally preferred because they undergo dissolution at a faster rate. Rapid dissolution is desirable in the case of solids, which must be dissolved prior to paren-

teral administration. Faster dissolution is also important for poorly sol-
uble compounds administered orally, since there is often a correlation
between dissolution rate and bioavailability. In fact, there are instances
in which only the amorphous form has adequate bioavailability.

Amorphous solids can be precipitated from solution or obtained
from melts of compounds by carrying out the solidification in such a
way as to avoid the thermodynamically preferred crystallization pro-
cess. They also can be prepared by disrupting an existing crystal struc-
ture. Excess free energy and entropy are incorporated into solids as
they are converted into the amorphous state, since solidification occurs
without permitting the molecules to reach their lowest energy states.

A.   Solidification of the Melt

Amorphous solids are often created by rapidly cooling a liquid so that
crystallization nuclei can neither be created nor grow sufficiently,
whereupon the liquid then remains in the fluid state well below the
normal freezing point. In principle, a liquid should freeze (crystallize)
when cooled to a temperature below its freezing point. However, if the
rate of cooling is high relative to the rate of crystallization, then the
liquid state can persist well below the normal freezing point. As cooling
continues there is a rise in the rate of increase of the viscosity of the
supercooled liquid per unit drop in temperature. The initially mobile
fluid turns into a syrup, then into a viscoelastic state, and finally into
a brittle glass. A glass is, therefore, a supercooled liquid, and is charac-
terized by an extremely high viscosity (typically of the order of 1014
Pa · s). Mechanically, if not structurally, glasses can be regarded as
solids.

The characteristic temperature below which melted solids must
be cooled to form a glass is the glass transition temperature T_g. The
glass transition is a dynamic event that occurs at a temperature below
which coordinated molecular motion becomes so slow that a liquid can
be considered to take on the properties of a solid. While the exact value
of this transition temperature depends on the heating rate, the glass
transition temperature is generally found to be about two-thirds that of
the melting temperature T_m. Glass transition temperatures reported for
pharmaceuticals also follow this general rule, as can be seen in the

listing of ten pharmaceuticals that form glasses (Table 3). It is often found that the presence of impurities that facilitate glass formation increases the ratio T_g/T_m either by raising T_g or by lowering T_m. Hence one might wonder if some of the high values in the last column of Table 3 are due to partial decomposition of the drug substance upon melting. Of course, this is an important concern when employing the melt solidification procedure for the preparation of amorphous materials.

There are many examples given in the monograph *Thermomicroscopy in the Analysis of Pharmaceuticals* [9] of other compounds that solidify on the microscope hot stage to form glasses. However, Table 4 contains examples from the literature in which solidification from the melt (either by slow cooling to room temperature or by quench cooling with liquid nitrogen) has been employed as the specific method for obtaining amorphous material.

B. Reduction of Particle Size

Reduction of the particle size of crystalline materials to the microcrystalline level can yield a material incapable of exhibiting an x-ray pow-

Table 3 Pharmaceuticals Forming Glasses above Room Temperature

Compound	T_g(K)	T_m(K)	T_g/T_m
Cholecalciferol	296	352	0.84
Sulfisoxazole	306	460	0.67
Stilbestrol	308	439	0.70
Phenobarbital	321	443	0.72
Quinidine	326	445	0.73
Salicin	333	466	0.71
Sulfathiazole	334	471	0.71
Sulfadimethoxine	339	465	0.73
Dehydrocholic acid	348	502	0.69
17-β-Estradiol	354	445	0.80

Source: Ref. 84.

Table 4 Amorphous Pharmaceuticals Obtained by Solidification from the Melt

Compound	Method used	Reference
Phenylbutazone	Solidification from the melt	[85]
Indomethacin	Quench cooling using liquid nitrogen or slow cooling from the melt over 30 min	[86,87]
Felodipine	Cooling of the melt in liquid nitrogen or at ambient temperature	[88,89]
Nifedipine	Melting at 180°C followed by immersion in liquid nitrogen	[90]
Benperidol	Melt in an oven at 277°C then cool to room temperature	[91]
Acetaminophen	Solidification of the melt at −5°C/min	[92]
Sulfapyridine	Melting any crystalline form and slowly cooling the melt	[93]
Lovostatin	Melting under nitrogen, rapid cooling to 20°C below the glass transition point	[94]

der diffraction pattern. Dialer and Kuessner [95] found that when sucrose was milled in a vibratory ball mill, the ordered crystal was transformed into a glass-like structure. The increase in surface energy of milled sucrose, as measured by heat of solution, could not be accounted for by an increase in surface area alone. Hence milling disrupts the crystal lattice and imparts the excess free energy and entropy associated with amorphous substances.

Particle size reduction can be achieved using a variety of methods. Sometimes it is helpful to carry out the particle size reduction at reduced temperatures, such as at 4°C or at liquid nitrogen temperature, −196°C. In other instances, grinding with an excipient has been employed as a means of obtaining amorphous materials. Cyclodextrins and microcrystalline cellulose have been used for this purpose. It is also possible that the use of polymeric excipients may inhibit crystal growth when the amorphous solid is dissolved in water. Table 5 contains a list of compounds that have been obtained in amorphous, or partly amorphous, form by milling.

Table 5   Amorphous Pharmaceuticals Obtained by Milling

Compound	Method used	Reference
Cimetidine	Milling	[96]
FR76505	Grinding in a ball mill	[97]
Cephalexin	Grinding in an agate centrifugal ball mill for 4 hours	[98]
Indomethacin	Grinding for 4 hours at 4°C in a centrifugal ball mill; grinding the γ-form at 4°C	[57,99]
(E)-6-(3,4-Dimethoxyphenyl)-1-ethyl-4-mesitylimino-3-methyl-3,4-dihydro-2(1H)-pyrimidinone	Grinding in a stainless steel shaker ball mill for 60 minutes	[100]
9,3″-Diacetyl-midecamycin	Mixed grinding with polyvinylpyrrolidone or polyvinylpyrrolidone + hydroxypropylmethylcellulose for 9 hours	[101]
Chloramphenicol stearate	Milling in a Pulverisette 5 grinder (Fritsch) (agate mortar and balls) with colloidal silica or microcrystalline cellulose	[102,103]
Calcium gluceptate	Milling in a Pulverisette 2 grinder (Fritsch) (agate mortar and balls) for 4 hours	[104]
Chloramphenicol palmitate	Milling in a Pulverisette 0 grinder (Fritsch) (agate mortar and balls) for 85 hours	[105]
Aspirin	Grinding with adsorbents under reduced pressure	[106]
	Grinding with β-cyclodextrin	[107]
Ibuprofen	Roll mixing with β-cyclodextrin	[108]
Hydrocortisone acetate	Grinding with crystalline cellulose	[109]

Table 5 Continued

Compound	Method used	Reference
Digoxin	Milling in a Glen Creston Model M270 ball mill for 8 hours	[110]
	Comminution of 1 g at 196°C for 15 minutes in a freezer mill	[111]
Amobarbital	Ball-milling with methylcellulose, microcrystalline cellulose, or dextran 2000	[112,113]
Acetaminophen	Ball milling for 24 hours with α- and β-cyclodextrin	[114]
6-Methyleneandrosta-1, 4-diene-3,17-dione	Co-grinding with β-cyclodextrin for 2 hours	[115]

C. Spray-Drying

In the pharmaceutical industry, spray-drying is used to dry heat-sensitive pharmaceuticals, to change the physical form of materials for use in tablet and capsule manufacture, and to encapsulate solid and liquid particles. This methodology is also used extensively in the processing of foods [116]. In the spray-drying process, a liquid feed stream is first atomized for maximal air spray contact. The particles are then dried in the airstream in seconds owing to the high surface area in contact with the drying gas. Spray-drying can produce spherical particles that have good flow properties, and the process can be optimized to produce particles of a range of sizes required by the particular application. The process can be run using either aqueous or nonaqueous solutions. Examples of pharmaceuticals obtained in the form of amorphous powders by spray-drying are found in Table 6.

D. Lyophilization

Lyophilization (also known as freeze-drying) is a technique that is widely employed for the preparation of dry powders to be reconstituted at the time of administration. It is a particularly useful technique in the

Table 6 Amorphous Pharmaceuticals Obtained by Spray-Drying

Compound	Method used	Reference
YM022	Spray-drying a methanol solution	[117]
α-Lactose monohydrate	Spray-drying in a Buchi 190	[118]
	Spray-drying a solution or suspension	[119]
4″-O-(4-methoxy-phenyl) acetyltylosin	Spray drying a dichloromethane solution	[120]
Salbutamol sulfate	Spray-drying of an aqueous solution in Buchi 90 spray dryer	[121]
Lactose	Spray-drying an aqueous solution	[118,122]
Furosemide	Spray-drying from a 4:1 chloroform: methanol solution at 50 and 150°C inlet temperature	[123,124]
Digoxin	Spray-drying an aqueous solution containing hydroxypropyl methylcellulose	[125]
Cefazolin sodium	Spray-drying from a 25% aqueous solution with an inlet temperature of 150°C and an outlet temperature of 100°C	[126]
9,3″-Diacetyl-midecamycin	Spray-drying of aqueous solution in the presence and absence of ethylcellulose	[127]

case of compounds that are susceptible to decomposition in the presence of moisture but that are more stable as dry solids. The physical form, chemical stability, and dissolution characteristics of lyophilized products can be influenced by the conditions of the freeze-drying cycle. In most pharmaceutical applications, lyophilization is performed on aqueous solutions containing bulking agents, and these often are chosen so as to form a coherent cake after completion of the freeze-drying process. However, lyophilization also can be employed to convert crystalline materials into their amorphous counterparts. The lyophilization process usually consists of the three stages of freezing, primary drying,

and secondary drying. For the preparation of amorphous materials, rapid freezing is employed so as to avoid the crystallization process. Both aqueous solutions and solutions containing organic solvents have been lyophilized. The primary drying phase involves sublimation of frozen water or vaporization of another solvent. This step is carried out by reducing the pressure in the chamber and supplying heat to the product. The secondary drying phase consists of the desorption of moisture (or residual solvent) from the solid.

Recently, excipients of various types have been employed in frozen solutions so as to inhibit crystallization. Cyclodextrins appear to be particularly useful for this purpose, although it is generally necessary to employ rapid freezing to liquid nitrogen temperatures to ensure that the freeze-dried product is noncrystalline. When α-cyclodextrin, which has a larger cavity than does β-cyclodextrin, is frozen at a relatively slow rate, it will cocrystallize with compounds such as benzoic acid, salicylic acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, and methyl p-hydroxybenzoate [128]. However, rapid freezing of a methyl p-hydroxybenzoate solution containing α-cyclodextrin at a benzoate/cyclodextrin ratio of 0.33 yields an amorphous solid after freeze-drying [29].

β-Cyclodextrin and its derivatives have been shown to form amorphous lyophilized products with a number of compounds, principally nonsteroidal antiinflammatory agents. Examples from the literature of excipients and pharmaceuticals prepared as amorphous materials by lyophilization are given in Table 7.

E. Removal of Solvent from a Solvate or Hydrate

Solids can sometimes be rendered amorphous by the simple expedient of allowing solvent molecules of crystallization to evaporate at modest temperatures. If the solvent merely occupies channels in the crystal structure, the structure often remains intact, but when the solvent is strongly bonded to molecules of the host, the structure frequently will collapse when the solvent is removed and one obtains an amorphous powder. A few examples of amorphous solids obtained in this manner are found in Table 8.

Table 7 Amorphous Pharmaceuticals Obtained by Lyophilization

Compound	Method used	Reference
Lactose	Lyophilization of a 5% Aqueous Solution	[130]
MK-0591	Lyophilization	[131]
Raffinose	Lyophilization of a 10% aqueous solution frozen at −45°C	[132]
Sucrose	Lyophilization of 10% aqueous solutions	[133]
Dirithromycin	Freeze-drying from methylene chloride solution	[134]
Cefalexin	Aqueous solution frozen at −196°C, then freeze-dried	[135]
	Lyophilization of a saturated aqueous solution	[136]
Calcium gluceptate	Freeze-drying from 2% aqueous solution	[137]
Griseofulvin	Freeze-drying of solutions of griseofulvin or of solutions of mixtures of griseofulvin and mannitol in dioxane or 1:1 dioxane-water with fast freezing in liquid nitrogen	[138]
Tolobuterol hydrochloride	Freeze-drying of aqueous solution	[139]
E1040	Freeze-drying of aqueous solution	[140]
Glutathione	Freeze-drying of a 5% aqueous solution	[141]
Aspirin	Freeze drying of an aqueous solution in the presence of 1.0% hydroxypropyl-β-cyclodextrin	[142]
Ketoprofen	Freeze-drying in the presence of heptakis-(2,6-O-dimethyl)-β-cyclodextrin	[143]
	Freeze-drying with β-cyclodextrin (rapid freezing with liquid nitrogen)	[144]
Glibenclamide	Freezing at liquid nitrogen temperature, freeze-drying over 24 hours	[145]

Table 7 Continued

Compound	Method used	Reference
Naproxen	Colyophilization (223K and 0.013 torr) of naproxen and hydroxyethyl-β-cyclodextrin, or hydroxypropyl-β-cyclodextrin	[146]
Sodium ethacrynate	Rapid freezing of an aqueous solution to −50°C, followed by freeze-drying	[147]
p-Aminosalicylic acid	Colyophilization of p-aminosalicylic acid in aqueous solution with pullulan	[148]
Ceftazidime	Freeze-drying a nearly saturated aqueous solution of the free acid	[149]
Cefaclor	Freeze-drying from a nearly saturated aqueous solution	[149]
Cephalothin sodium	Freeze-drying from a 25% aqueous solution	[149]
Cefamandol sodium	Freeze-drying from a 25% aqueous solution	[149]
Cefazolin sodium	Freeze-drying an aqueous solution at low temperature	[149]
Nicotinic acid	Freeze-drying in the presence of β-cyclodextrin (fast-freezing); and heptakis (2,6-O-dimethyl)-β-cyclodextrin	[150]

F. Precipitation of Acids or Bases by Change in pH

If the level of supersaturation is carefully controlled, it is often possible to avoid crystallization when a water-soluble salt of a weak acid is precipitated with a base, or when a water-soluble salt of a weak base is precipitated with an acid. When crystalline iopanoic acid is dissolved in 0.1 N NaOH, and 0.1 N HCl is added, an amorphous powder is precipitated [43]. A similar phenomenon is observed in the case of the precipitation of piretanide [155]. Another example in this genre is the

Table 8 Amorphous Pharmaceuticals Obtained by Solvent Removal

Compound	Method used	Reference
Tranilast anhydrate	Dehydration of the monohydrate over P_2O_5	[151]
Raffinose	Lyophilization and heat drying of the pentahydrate	[132]
Erythromycin	Heating the dihydrate for 2 hours at 135°C in an oven, and then cooling to room temperature	[152,153]
Calcium DL-pantothenate	Drying the methanol:water 4:1 solvate *in vacuo* at 50–80°C	[154]

precipitation of amorphous calcium carbonate, which occurs when a calcium chloride solution is combined with a sodium carbonate solution at 283K [156].

G. Miscellaneous Methods

Earlier during the discussion on the preparation of polymorphs, the doping of crystals was mentioned as a technique for encouraging the formation of one type of polymorph over another. Similarly, if a dopant is employed at levels that will disrupt the crystal lattice, the substance can be made to solidify as an amorphous material. Duddu and Grant [157] observed changes in the enthalpy of fusion of (−)-ephedrinium 2-naphthatenesulfonate when the opposite enantiomer, (+)-ephedrinium 2-naphthalenesulfonate, was added as a dopant.

When *m*-cresol was added to a suspension of insulinotropin crystals grown from a normal saline solution, the crystals were immediately rendered amorphous. It was postulated [158] that the *m*-cresol molecules diffused into the crystals through solvent channels and disturbed the lattice interactions that ordinarily maintained the integrity of the crystal. When zinc acetate or zinc chloride was added to the suspension, the zinc ion stabilized the crystal lattice so that the subsequent addition of *m*-cresol did not alter the integrity of the crystals.

Sometimes solvents exert a similar effect. When a small amount of ethyl acetate is added to a calcium chloride solution prior to addition

of sodium fenoprofen, the calcium fenoprofen that precipitates has a low degree of crystallinity [159]. Similarly, when calcium DL-pantothenate is precipitated from methanol or ethanol solution by the addition of acetone, ether, ethyl acetate, or other solvents, the precipitate obtained is found to be amorphous [154].

V. SUMMARY

The pharmaceutical development scientist who is assigned the task of demonstrating that a substance exhibits only one crystalline form, or that of discovering whether additional forms exist, can utilize the techniques outlined in this chapter as a starting point. Upon completion of this program, one can certainly conclude that due diligence has been employed to isolate and characterize the various solid-state forms of any new chemical entity. One should always be aware that nuclei capable of initiating the crystallization of previously undiscovered forms might be lurking around the laboratory, ready to confound the investigator should their effects become known. In addition, the phenomenon of ''disappearing polymorphs'' can come into play, and techniques that formerly yielded the same crystals every time may subsequently yield crystals of another, more stable form. In the future, the use of computer simulations of alternative crystallographic structures will suggest how much laboratory work might be required to isolate the polymorphs or solvates of a given compound. Until then, the empirical approach remains superior.

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6

Methods for the Characterization of Polymorphs and Solvates

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I. INTRODUCTION

Certainly the most important aspect relating to an understanding of polymorphic solid and solvate species is the range of analytical methodology used to perform the characterization studies [1–3]. The importance of this area has been recognized from both scientific and regulatory concerns, so the physical methods have begun to come under the same degree of scrutiny as have the traditional chemical methods of analysis. Byrn et al. have provided a series of useful definitions that concisely give the characteristics of the various solid forms that can be found for a given drug substance [4] and that will be used throughout this chapter. Compounds may be *polymorphs* (forms having the same chemical composition but different crystal structures), *solvates* (forms containing solvent molecules within the crystal structure), *desolvated solvates* (forms when the solvent is removed from a specific solvate while still retaining the original crystal structure), or *amorphous* (solid forms that have no long-range molecular order).

Of all the methods available for the physical characterization of solid materials, it is generally agreed that crystallography, microscopy, thermal analysis, solubility studies, vibrational spectroscopy, and nuclear magnetic resonance are the most useful for characterization of polymorphs and solvates. However, it cannot be overemphasized that the defining criterion for the existence of polymorphic types must always be a nonequivalence of crystal structures. For compounds of pharmaceutical interest, this ordinarily implies that a nonequivalent x-ray

powder diffraction pattern is observed for each suspected polymorphic variation. All other methodologies must be considered as sources of supporting and ancillary information; they cannot be taken as definitive proof for the existence of polymorphism by themselves alone.

In the present work, the practice of the most commonly encountered techniques performed for the solid-state characterization of polymorphic or solvate properties will be reviewed. No attempt will be made to summarize every recorded use of these methodologies for such work, but selected examples will be used to illustrate the scope of information that can be extracted from the implementation of each technique.

II. CRYSTALLOGRAPHY: X-RAY DIFFRACTION

The x-ray crystallography technique, whether performed using single crystals or powdered solids, is concerned mainly with structural analysis and is therefore eminently suited for the characterization of polymorphs and solvates. An external examination of crystals reveals that they often contain facets, and that well-formed crystals are completely bounded by flat surfaces. Planarity of this type is not commonly encountered in nature, and it was quickly deduced that the morphological characteristics of a crystal are inherent in its interior structure. In fact, the microscopic form of a crystal depends critically on structural arrangements at the atomic or molecular level; the underlying factor controlling crystal formation is the way in which atoms and molecules can pack together.

A. Single Crystal X-Ray Diffraction

Every crystal consists of exceedingly small fundamental structural units that are repeated indefinitely in all directions. In 1830, Hessel conducted a purely mathematical investigation of the possible types of symmetry for a solid figure bounded by planar faces and deduced that only 32 symmetry groups were possible for such objects. The same conclusion was reached by Bravais in 1949 and Gadolin in 1867. These 32 crystallographic point groups are grouped into six crystal systems,

denoted triclinic, monoclinic, orthorhombic, tetragonal, trigonal, hexagonal, and cubic. Each crystal system is characterized by unique relationships existing among the crystal axes and the angles between these, and this information is summarized in Table 1.

   One of the characteristics of many crystals is their ability to be split along certain directions, yielding fragments containing smooth faces along the direction of the break. The angular relations between these cleavage planes were found to be the same in every fragment. Ultimately, it was learned that crystal cleavage planes corresponded to planes of atoms or molecules in the crystal, which in turn resulted from the repetition of unit cells. This three-dimensional pattern of atoms in a crystalline solid was shown to be capable of acting as a diffraction grating to light having wavelengths of the same order of magnitude as the translational repeat period of the molecular pattern. This period is

Table 1   Characteristics of the Six Crystal Systems

System	Description
Cubic	Three axes of identical length (identified as a_1, a_2, and a_3) intersect at right angles.
Hexagonal	Four axes (three of which are identical in length, denoted a_1, a_2, and a_3) lie in a horizontal plane and are inclined to one another at 120°. The fourth axis, c, is different in length from the others and is perpendicular to the plane formed by the other three.
Tetragonal	Three axes (two of which are denoted a_1 and a_2 and are identical in length) intersect at right angles. The third axis, c, is different in length with respect to a_1 and a_2.
Orthorhombic	Three axes of different lengths (denoted a, b, and c) intersect at right angles. The choice of the vertical c axis is arbitrary.
Monoclinic	Three axes (denoted a, b, and c) of unequal length intersect so that a and c lie at an oblique angle and the b axis is perpendicular to the plane formed by the other two.
Triclinic	Three axes (denoted a, b, and c) of unequal length intersect at three oblique angles.

of the order of 10^{-10} meters (i.e., angstrom units), and light having wavelengths of this magnitude is called x-ray radiation. The discovery that x-rays could be diffracted by crystalline solids was made by von Laue and his collaborators, and the method was quickly improved by Bragg and subsequently developed by countless others. It must be emphasized, however, that it is the electron density about an atom that is responsible for the scattering of x-rays by matter.

All x-ray diffraction techniques are ultimately based on Bragg's law, which describes the diffraction of a monochromatic x-ray beam impinging on a plane of atoms [5]. Parallel incident rays strike the crystal planes at an angle θ and are then diffracted at the same angle. The observation of reinforcement requires that the path difference of the impinging beam (i.e., the distance between molecular planes) be equal to a whole number of wavelengths. The scattering angles are therefore correlatable to the spacings between planes of molecules in the lattice by means of Bragg's law:

$$n\lambda = 2\,d\,\sin\theta \tag{1}$$

where

  n = order of the diffraction pattern
  λ = wavelength of the incident beam
  d = distance between the planes in the crystal
  θ = angle of beam diffraction

It should be noted that the Bragg equation yields only the scattering angles with respect to the incident x-ray beam and has nothing to say about the relative intensities of diffracted radiation. To describe scattered intensities, one uses the concept of the scattering power of a sample. This is equal to the number of free and independent electrons, scattering according to Thompson's law governing the scattering by a free electron, which would be required to replace the object in order to obtain the same scattered intensity.

A determination of the internal structure of a crystal requires the specification of the unit cell dimensions (axis lengths, and angles between these) and measurement of the intensities of the diffraction pattern of the crystal. For a given lattice, regardless of the content of the

unit cell, the directions of reflection are the same. The experimental determination of these directions is used to deduce the reciprocal lattice of the crystal, which unambiguously yields the crystal lattice. In addition, the relative intensities diffracted by different planes depend on the contents of the unit cell. Their measurement leads to the determination of the crystal structure factor, and these data permit the determination of the atomic structure of crystals. More detailed expositions of the procedures used to obtain the structures of single crystals are available in the literature [5–9] and are beyond the scope of this article.

Genuine polymorphism ordinarily arises either from differences in the packing of conformationally equivalent molecules or from different modes of assembly of conformationally inequivalent molecules. The former situation is well-known for inorganic and geological crystals [10], and the latter has been discussed in detail [11]. One of the best known instances of packing polymorphism are the allotropes of carbon, graphite and diamond. As shown in Fig. 1, in diamond each carbon atom is tetrahedrally surrounded by four equidistant neighbors, and the tetrahedra are arranged to give a cubic unit cell. Graphite is composed of planar hexagonal nets of carbon atoms, which can be arranged to yield either a hexagonal unit cell (the α-form) or a rhombohedral unit cell (the β-form).

Two anhydrous polymorphs of nitrofurantoin have been reported [12], as well as two polymorphic monohydrate forms [13]. In the triclinic α-anhydrate form, the nitrofurantoin molecules were found to be associated in a head-to-head manner, forming centrosymmetric dimer units through two identical intermolecular hydrogen bonds, with these dimer units being further linked into sheets by a system of weaker hydrogen bonds. The hydrogen bonding existing in the molecular planes of the monoclinic β-anhydrate form was found to differ in symmetry, where the key hydrogen bond was seen to link nitrofurantoin molecules by a twofold screw-axis [12]. In each of the two monohydrate forms, the conformations of the nitrofurantoin molecules are essentially equivalent to each other and not significantly different from the conformation observed for the anhydrate forms. However, the hydrogen bonding pattern induced by the presence of lattice water molecules yields two very different packing modes for the two monohydrate polymorphs. In the monoclinic form, virtually all of the atoms lie in
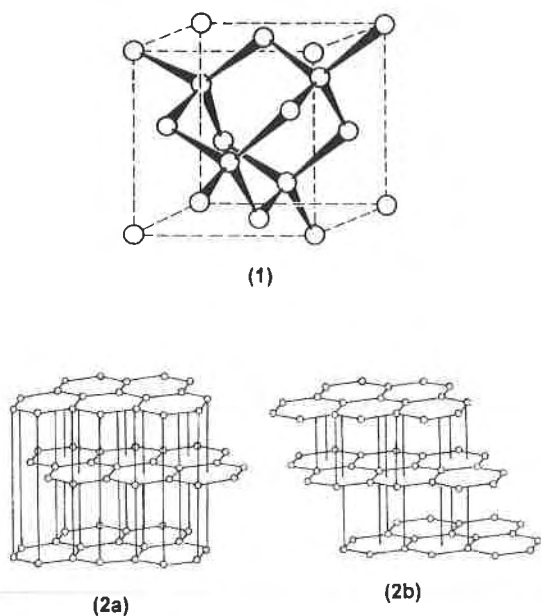
(1)

(2a)   (2b)

Fig. 1 Crystal structure of (1) diamond, showing the tetrahedral coordination of each carbon atom. Also shown are the crystal structures of the two polymorphs of graphite, specifically (2a) the hexagonal α-form and (2b) the rhombohedral β-form.

a single plane, giving rise to a completely layered structure. In the orthorhombic form, layers of molecules are arranged parallel to different planes, so the overall packing is that of a herringbone arrangement [13].

Probucol is an example of a compound where the polymorphism arises from the packing of different conformers [14]. Although both forms were found to be monoclinic, the unit cells belonged to different space groups and the molecular conformations of the title compound were quite different (Fig. 2). In Form II, the C–S–C–S–C chain is extended, and the molecular symmetry approximates C_{2v}. This symmetry is lost in Form I, where the torsion angles about the two C–S bonds deviate significantly from 180°. The extended conformer was shown to be less stable relative to the bent conformer, as simple grinding was sufficient to convert Form II into Form I.
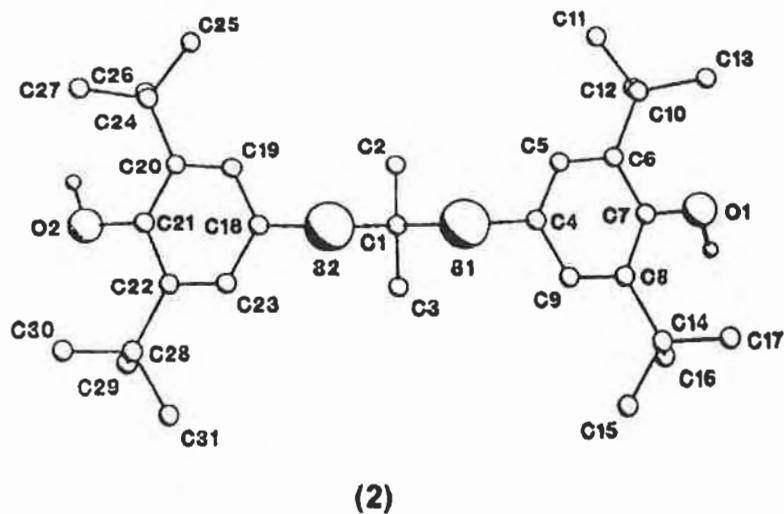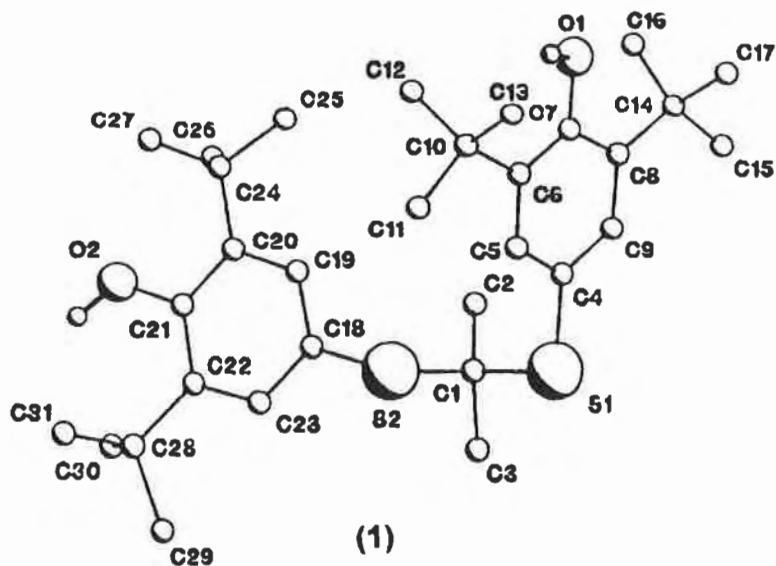
Fig. 2 Conformation of the probucol molecule existing in (1) Form I and (2) Form II. (The figure was adapted from data contained in Ref. 14).

Not all instances of conformational polymorphism are as dramatic as that just described, and often different conformers of a single side chain are able to pack into different crystalline arrangements. For instance, the two polymorphs of p-(1R,3S)-3-thioanisoyl-1,2,2-trimethyl-cyclopentane carboxylic acid were found to be associated with different conformations of the carboxylate group [15]. Torsion about a single C–N bond was shown to be the origin of the polymorphism detected for lomeridine dihydrochloride [16]. Finally, relatively small differences in molecular conformation were detected for the two polymorphic and four solvated crystalline forms of spironlactone [17].

B. X-Ray Powder Diffraction

Although the solving of a crystal structure provides the greatest understanding of polymorphic solids, the necessity for obtaining suitable single crystals and the degree of complexity associated with the data analysis preclude this technique from being used on a routine basis for batch characterization. In fact, most drug substances are obtained as microcrystalline powders, from which it is often fiendishly difficult to obtain crystallographically adequate crystals. Furthermore, during the most common evaluation of drug substances, it is usually sufficient to establish only the polymorphic identity of the solid and to verify that the isolated compound is indeed of the desired structure. For these reasons, and to its inherent simplicity of performance, the technique of x-ray powder diffraction (XRPD) is the predominant tool for the study of polycrystalline materials [18] and is eminently suited for the routine characterization of polymorphs and solvates.

A correctly prepared sample of a powdered solid will present an entirely random selection of all possible crystal faces at the powder interface, and the diffraction off this surface provides information on all possible atomic spacings in the crystal lattice. To measure a powder pattern, a randomly oriented sample is prepared so as to expose all the planes of a sample and is irradiated with monochromatic x-ray radiation. The scattering angle θ is determined by slowly rotating the sample and using a scintillation counter to measure the angle of diffracted x-rays with respect to the angle of the incident beam. Alternatively, the angle between sample and source can be kept fixed, and the detector

moved along a proscribed path to determine the angles of the scattered radiation. Knowing the wavelength of the incident beam, the spacing between the planes (identified as the d-spacings) is calculated using Bragg's law.

The XRPD pattern will therefore consist of a series of peaks detected at characteristic scattering angles. These angles, and their relative intensities, can be correlated with the computed d-spacings to provide a full crystallographic characterization of the powdered sample. After indexing all the scattered lines, it is possible to derive unit cell dimensions from the powder pattern of the substance under analysis [18]. For routine work, however, this latter analysis is not normally performed, and one typically compares the powder pattern of the analyte to that of reference materials to establish the polymorphic identity. Since every compound produces its own characteristic powder pattern owing to the unique crystallography of its structure, powder x-ray diffraction is clearly the most powerful and fundamental tool for a specification of the polymorphic identity of an analyte. The USP general chapter on x-ray diffraction states that identity is established if the scattering angles of the ten strongest reflections obtained for an analyte agree to within ±0.20 degrees with that of the reference material, and if the relative intensities of these reflections do not vary by more than 20 percent [19].

The power of XRPD as a means to establish the polymorphic identity of an analyte can be illustrated by considering the case of the anhydrate and trihydrate phases of ampicillin. The crystal structures of both phases have been obtained, and they differ in the nature of the molecular packing [20]. The amino group in the monoclinic anhydrate is hydrogen bonded to the ionized carboxyl groups of two molecules, while the amino group of the orthorhombic trihydrate is hydrogen bonded to a single carboxylate group and to the waters of hydration that link other molecules in the structure. The powder patterns of these two materials are shown in Fig. 3 and are seen to be readily distinguishable from each other. Amoxycillin trihydrate has been found to crystallize in the same space group as does ampicillin trihydrate, and it exhibits a very similar pattern of hydrogen bonding [21]. However, the dimensions of the two unit cells differ significantly, and this fact is

Scattering Angle (degrees 2-θ)
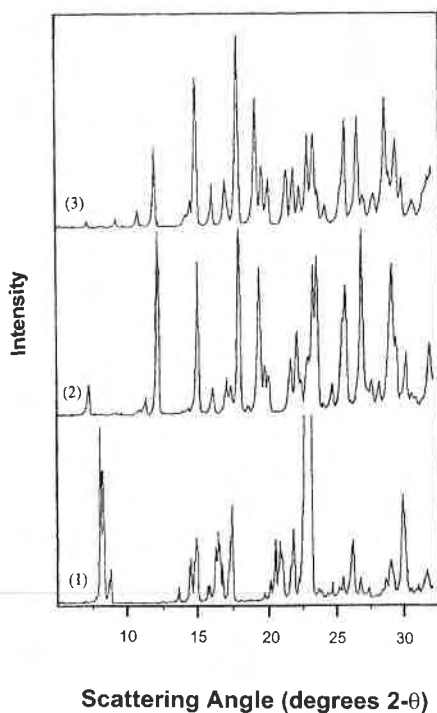
Fig. 3 X-ray powder diffraction patterns of (1) ampicillin anhydrate, (2) ampicillin trihydrate, and (3) amoxicillin trihydrate.

reflected in the differences among the relative intensities of the corresponding peaks contained in Fig. 3. Even though the two structures would be considered as being isostructural, the XRPD patterns of the two trihydrate phases readily permit an unambiguous identification and distinction between these.

X-ray powder diffraction can also be used for the quantitative determination of phase composition, and this approach has been discussed in detail [22]. In one particularly well-developed example, XRPD was used to quantitate the relative amounts of the anhydrate and dihydrate phases existing in carbamazepine samples [23]. The method was based on the observation that the XRPD of each phase

featured a scattering peak unique to each form, which was noted at a scattering angle where no scattering was observed for the other phase. Unlike loose powders, compressed samples yielded highly reproducible intensity values, so pelletized materials were used for the data acquisition. Good correlation between sample composition and scattering intensities was obtained in standard materials, permitting the generation of analytical relations suitable for the analysis of analyte samples.

The degree of crystallinity associated with a sample can often be established using powder x-ray diffraction. If the patterns of 100% crystalline and 100% amorphous material can be established, then the integrated peak intensity of the analyte is used to deduce the percent crystallinity. Such methodology has been used to measure the crystallinity of digoxin [24] and calcium gluceptate [25]. The XRPD method is extremely important during the characterization of lyophilized materials, since the stability of a crystalline solid is expected to exceed that of an amorphous or disordered solid. For instance, the technique has been used to study the properties of lyophilized imipenem [26].

III. MORPHOLOGY: MICROSCOPY

An extremely important tool for the characterization of polymorphs and solvates is that of microscopy, since the observable habits of differing crystal structures must necessarily be different and therefore useful for the characterization of such systems [27]. Common sense would dictate that the visual observation of such materials would immediately follow an x-ray crystallographic study, which would in principle make the science of optical crystallography [28–30] an essential aspect of any program of study. A review of crystallography from the pharmaceutical viewpoint is available [31].

As stated in an earlier section, a crystal is a polyhedral solid, bounded by a number of planar faces that are normally identified using the Miller indices. The arrangement of these faces is termed the *habit* of the crystal, and the crystal is built up through the repetition of the unit cell. The three-dimensional basic pattern of molecules in a solid

form the space lattice, and the application of simple geometry has shown that only 14 different types of simple space lattices are possible. By taking combinations of the various lattices possible for each crystallographic system, it has also been determined that all solids must belong to one of 230 space groups [28–30].

Both optical and electron microscopies have found widespread use for the characterization of polymorphs and solvates. Although optical microscopy is more limited in the range of magnification suitable for routine work (working beyond 600× being difficult when observing microcrystalline materials), the use of polarizing optics introduces enormous power into the technique not available with other methods. Electron microscopy work can be performed at extraordinarily high magnification levels (up to 90,000× on most units), and the images that can be obtained contain a considerable degree of three-dimensional information. The two methods are complementary in that each can provide information not obtainable by the other. With judicious use of these techniques, one can obtain substantial characterization of a polymorphic system. These data are extremely useful during the early stages of drug development, since often only a limited amount of the drug candidate is available at that time.

The literature pertaining to microscopy and its applications is quite large but fortunately is updated periodically [32]. A number of texts and review articles have been written that cover light or electron microscopy or some combination of the two [33,34]. It is beyond the scope of this chapter to provide a mechanical description of the various instruments; readers are referred to representative references for light microscopy [35–40] and electron microscopy [41–44].

McCrone has provided an excellent discussion of the synergistic aspects of optical and electron microscopies [45]. He concludes that electron microscopy yields excellent topographic and shape information and is most useful in forensic situations involving trace evidence characterization and identification. When polarizing optics are used during a light microscopy study, the optical properties of the crystals under investigation can also be determined. This latter aspect is extremely useful in the characterization of polymorphs and solvates, and consequently polarizing optical microscopy is an extremely important tool for the study of such systems.

A. Polarizing Optical Microscopy

The polarizing microscope is essentially a light microscope equipped with a linear polarizer located below the condenser and an additional polarizer mounted on top of the eyepiece. Full performance of an optical crystallographic study requires the additional use of a rotating stage. Application of this method can yield parameters such as the sign and magnitude of any observed birefringence, knowledge of the refractive indices associated with each crystal direction, what the axis angles are, and what are the relations among the optical axes. To conduct the analysis, the light from the source is rendered linearly polarized by the initial polarizer. The second polarizer mounted above the sample (the analyzer) is oriented so that its axis of transmission is orthogonal to that of the initial polarizer. In this condition of "crossed polarizers," no transmitted light can be perceived by the observer. The passage (or lack thereof) of light though the crystal as a function of the angle between crystal axes and the direction of polarization is of key importance to the method.

The refractive index of light passing through an isotropic crystal must be identical along each of the crystal axes, and such crystals therefore possess *single refraction*. By virtue of their symmetry, crystals within the cubic system are isotropic along all three crystal axes. This property is to be contrasted with that associated with anisotropic substances, which exhibit different refractive indices for light polarized with respect to the crystal axes. This latter property results in the property of *double refraction*, or birefringence. Crystals within the hexagonal and tetragonal systems possess one isotropic direction and are termed *uniaxial*. Anisotropic crystals possessing two isotropic axes are termed *biaxial* and include all crystals belonging to the orthorhombic, monoclinic, or triclinic systems. Biaxial crystals will exhibit unequal indices of refraction along each of the crystal axes.

Isotropic samples will have no effect on the polarized light no matter how the crystal is oriented, since all crystal axes are completely equivalent. This effect is known as complete or *isotropic extinction*. Noncrystalline, amorphous samples yield the same behavior.

When the sample is capable of exhibiting double refraction, the specimen will then appear bright against a dark background. For exam-

ple, when a uniaxial crystal is placed with the unique c axis horizontal on the stage, it will appear to be alternately dark and bright as the stage is rotated. Furthermore, the crystal will be completely dark when the c axis is parallel to the transmission plane of the polarizer or analyzer. If the crystal has edges or faces parallel to the c axis, then it will be extinguished when such an edge or face is parallel to one of the polarizer directions. This condition is called *parallel extinction*. At intermediate positions, the crystal will appear light and will exhibit colors depending on the thickness of the crystal. A rhombohedral or pyramidal crystal will be extinguished when the bisector of a silhouette angle is parallel to a polarization direction, and this type of extinction is termed *symmetrical extinction*. However, when a uniaxial crystal is mounted with the c axis vertical on the stage, it is found that the crystal remains uniformly dark as the stage is rotated (another case of isotropic extinction).

For biaxial crystals, similar results are obtained to those with uniaxial crystals. The exception to this rule is that in monoclinic and triclinic systems, the polarization directions need not be parallel to faces or to the bisectors of face angles. If the prominent faces or edges of an extinguished crystal are not parallel to the axes of the initial polarizer, the extinction is said to be *oblique*.

A summary of the extinction characters covering biaxial and uniaxial crystals is provided in Table 2. Knowledge of the type of extinction permits a determination of the system to which a given crystal belongs, and such information can be extremely important during the initial evaluation of crystal polymorphs or solvates. For example, identification of the polymorphic or solvated forms of thiamine hydrochloride, bromvalerylurea, ampicillin [46], and the polymorphs of sulfamethoxydiazine [47] is easily done using optical microscopic methods. Application of this procedure requires that one determine the refractive indices along each of the crystal axes, which is done using immersion oils of known refractive indices [48].

Optical microscopy can be a powerful tool in the study of processes associated with phase conversion of one crystal form that is metastable with respect to another form in the presence of an appropriate solvent. For instance, Form II of sulfathiazole can be converted to Form I when suspended in glycerin at 90°C, while at 100°C the

Table 2   Differentiation of Crystal Systems by the Character of Their Extinction

System	Character of observed extinction
Cubic	Isotropic or complete extinction.
Hexagonal	Parallel or symmetrical extinction. Can be isotropic if viewed down the c axis, but then a six-sided silhouette should be observed.
Tetragonal	Parallel or symmetrical extinction. Can be isotropic, but then a four-sided silhouette should be observed.
Orthorhombic	All crystals will show parallel or symmetrical extinction.
Monoclinic	Some crystals will show parallel or symmetrical extinction, and others oblique extinction.
Triclinic	All crystals will show oblique extinction.

reverse process has been found to take place [49]. During the development of a method to obtain solubility data for rapidly reverting solid states, optical microscopy proved to be a useful tool for study of the phase conversion.

The anhydrate/monohydrate equilibrium of theophylline has received a great deal of attention, especially since the monohydrate phase is considerably less soluble than the anhydrate phase [50,51]. This situation has implications for the production and storage of theophylline solid dose forms, since phase interconversion may take place either during processing [52–54] or as a result of environmental exposure [55–56]. Optical microscopy has been used to study the anhydrate-to-monohydrate phase conversion that takes place when the drug substance is exposed to bulk water, and scanning electron microscopy was used to study the surfaces of compressed tablets when these were exposed to high degrees of relative humidity [57].

It has been found that (S)-4-[[[1-(4-fluorophenyl)-3-(1-methyl-ethyl)-1H-indol-2-yl]-ethynyl]-hydroxyphosphinyl]-3]-hydroxybutanoic acid, disodium salt, is capable of existing in three crystalline hydrate and one liquid crystalline phase depending on the relative humidity to which the compound is exposed [58]. Among other things, these have been found to exhibit varying fluorescence properties in their respective solid states [59]. As shown in Fig. 4, the particle morphology of these
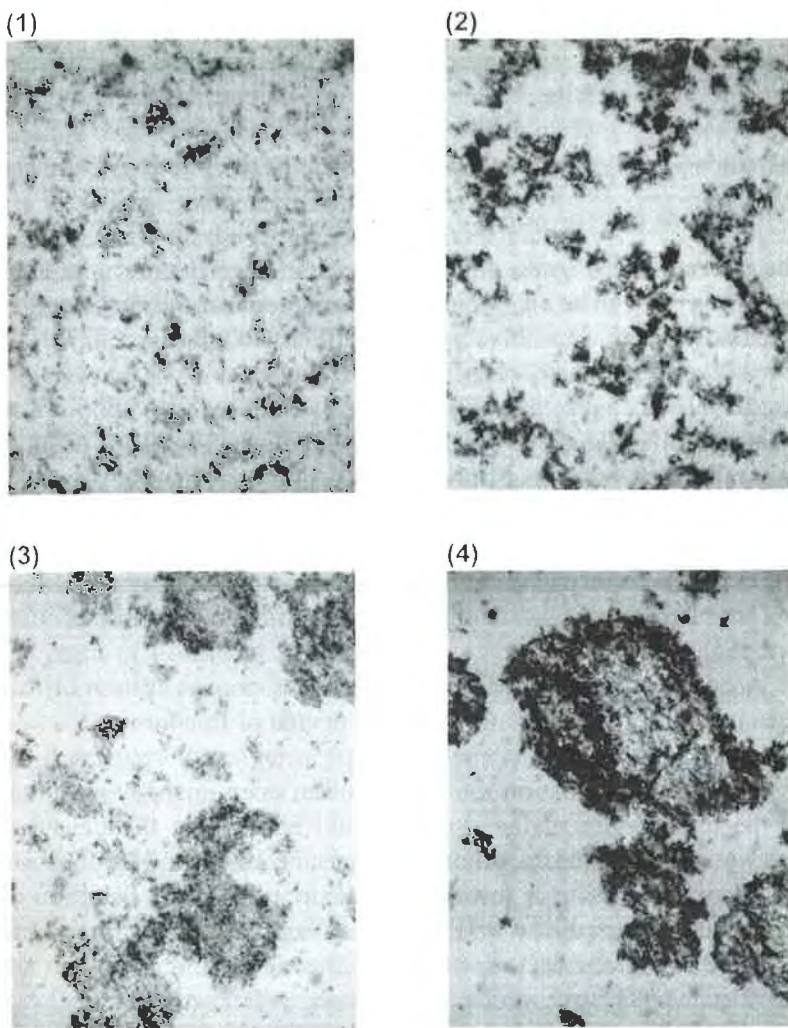
Fig. 4 Particle morphology of (S)-4-[[[1-(4-fluorophenyl)-3-(1-methyl-ethyl)-1H-indol-2-yl]-ethynyl]-hydroxyphosphinyl]-3]-hydroxybutanoic acid, disodium salt, after equilibration at relative humidity values of (1) 11%, (2) 60%, (3) 75%, and (4) 84%. All photomicrographs were taken at a magnification of 100×.

materials is profoundly affected by the hydration state of the drug substance. The birefringence associated with the liquid crystalline state was particularly striking and useful for qualitative identification purposes.

B. Thermal Microscopy

Often referred to as fusion microscopy or hot-stage microscopy, thermal microscopy can be an extremely valuable tool for the characterization of polymorphic or solvate systems. The technique requires that one make observations during the heating and cooling of a few milligrams of substance on a microscope slide, as well as observations made on the crystallized material [60,61]. It is therefore possible to conduct a very rapid analysis using only small quantities of material, and the entire phase diagram of a drug material can be deduced upon the conduct of suitably designed experiments. The most widely used device in the conduct of thermal microscopic studies is the hot stage of Kofler and Kofler [62], which has facilitated an extraordinary number of studies [63,64].

According to the phase rule, a single component system of two phases in equilibrium can only have one degree of freedom and is said to be univariant. If the experiments are conducted at constant and fixed pressure (as would exist on the surface of an open microscope slide), then the temperature of the system would be fixed at the transition point. Nevertheless, the transition point must be influenced by pressure, which may either raise or lower the transition point. The direction of the effect can be predicted on the basis of Le Chatelier's principle, if the change of volume accompanying the passage of one form into the other is known. The magnitude of the pressure effect can be calculated by the Clapeyron equation, and one finds that since the change in volume accompanying a phase transition is small, pressure can only exert a minor effect on the transition point.

The transition point at which a phase transition will take place is defined as the intersection of the pressure–temperature curves associated with each phase. Clearly, the transition point (at atmospheric pressure) can exist at a temperature that either exceeds or is less than the melting point. The former situation is termed *monotropy*, while the

latter is termed *enantiotropy*. An enantiotropic substance is distinguished by its ability to change reversibly into one phase or another, while the transformation of a monotropic material is irreversible. The thermal microscope can be used to determine melting points with great accuracy, and through studies where the heating and cooling processes are cycled one can usually deduce the nature of a polymorphic transition. In addition, the loss of solvent molecules from a solvate species can easily be detected by the processes that take place when the sample is immersed in a suitable fluid.

The conduct of a thermal microscopic study may be illustrated through the work conducted by Kuhnert-Brandstätter on the polymorphs and solvates of a series of steroid hormones [65]. For example, if Form II of corticosterone-21-acetate is preheated to 140°C on a hot stage, the solid is observed to melt at 145–148°C. Further heating of the system results in a solidification of the melt, to yield Form I, which then melts at 153–155°C. Similarly, Form II of prednisone acetate is isolated from most solvents and melts over the range of 225–228°C. With continued heating, the melt solidifies, and one observes the melting point of Form I at 232–241°C.

IV. PHASE TRANSITIONS: THERMAL METHODS OF ANALYSIS

Thermal analysis methods are defined as those techniques in which a property of the analyte is determined as a function of an externally applied temperature. Regardless of the observable parameter measured, the usual practice requires that the physical property and the sample temperature be recorded continually and automatically, and that the sample temperature be altered at a predetermined rate. Measurements of thermal analysis are conducted for the purpose of evaluating the physical and chemical changes that may take place in a heated sample, requiring that the operator interpret the events noted in a thermogram in terms of plausible reaction processes. Thermal reactions can be endothermic (melting, boiling, sublimation, vaporization, desolvation, solid–solid phase transitions, chemical degradation, etc.) or exothermic (crystallization, oxidative decomposition, etc.) in nature.

Such methodology has found widespread use in the pharmaceutical industry for the characterization of compound purity, polymorphism, solvation, degradation, and excipient compatibility [66,67]. However, given the utility that thermal microscopy has shown for the characterization of polymorphic systems, it is not surprising that the quantitative applications of thermal analysis have proven to be even more useful. Although a large number of techniques have been developed, the most commonly applied are those of thermogravimetry (TG), differential thermal analysis (DTA), and differential scanning calorimetry (DSC).

A. Thermogravimetry

Thermogravimetry (TG) is a measure of the thermally induced weight loss of a material as a function of the applied temperature [68]. TG analysis is restricted to transitions that involve either a gain or a loss of mass, and it is most commonly used to study desolvation processes and compound decomposition. TG analysis is a useful method for the quantitative determination of the total volatile content of a solid and can be used as an adjunct to Karl Fischer titrations for the determination of moisture. As such it readily permits the distinction between solvates and the anhydrous forms of a given compound.

TG analysis also represents a powerful adjunct to the other methods of thermal analysis, since a combination of either a DTA or a DSC study with a TG determination can be used in the assignment of observed thermal events. Desolvation processes or decomposition reactions must be accompanied by weight changes, and they can be thus identified by a TG weight loss over the same temperature range. On the other hand, solid–liquid and solid–solid phase transformations are not accompanied by any loss of sample mass and would not register in a TG thermogram.

When a solid is capable of decomposing by means of several discrete, sequential reactions, the magnitude of each step can be separately evaluated. The TG analysis of compound decomposition can also be used to compare the stability of similar compounds. In general, the higher the decomposition temperature of a given compound, the greater would be its stability.

The measurement of thermogravimetry is simple in principle; it consists of the continual recording of the mass of the sample as it is heated in a furnace. The weighing device most used is a microbalance, which permits the characterization of milligram quantities of sample. The balance chamber itself is constructed so that the atmosphere may be controlled, which is normally accomplished by means of a flowing gas stream. The furnace must be capable of being totally programmable in a reproducible fashion, and its inside surfaces must be resistant to the gases evolved during the TG study. It is essential in TG design that the temperature readout be that of the sample and not that of the furnace. Thus the thermocouple or resistance thermometer must be mounted as close to the sample pan as possible.

Although the TG methodology is conceptually simple, the accuracy and precision associated with the results are dependent on both instrumental and sample factors [69]. The furnace heating rate used for the determination will greatly affect the transition temperatures, while the atmosphere within the furnace can influence the nature of the thermal reactions. The sample itself can play a role in governing the quality of the data obtained; factors such as the sample size, the nature of the evolved gases, the particle size, the heats of reaction, the sample packing, and the thermal conductivity all influence the observed thermogram.

Other than its ability to demonstrate the anhydrous nature of genuine polymorphic materials, the entire utility of TG analysis is in the differentiation and characterization of solvate species. The multitude of solvate species formed by the disodium salt of (S)-4-[[[1-(4-fluorophenyl)-3-(1-methylethyl)-1H-indol-2-yl]-ethynyl]-hydroxyphosphinyl]-3]-hydroxybutanoic acid was most effectively characterized by TG analysis of materials exposed to various relative humidity values [58]. Representative thermograms of the different hydrates are shown in Fig. 5, where it is evident that the well-defined plateaus observed upon completion of the individual dehydration steps were ideally suited for the evaluation of the hydration state of an isolated material.

Thermogravimetric analysis played an important role during the characterization of the insoluble adducts formed by 5-nitrobarbituric acid (dilituric acid) with alkali metal (Group IA) and alkaline earth (Group IIA) cations [70]. This study was performed to understand the
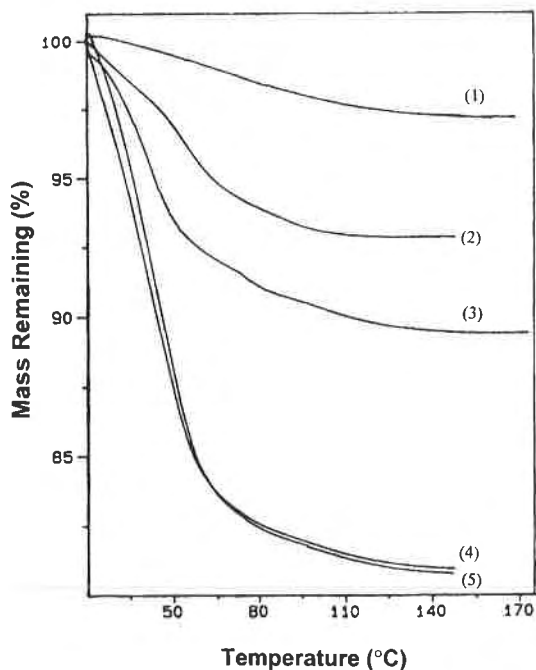
Fig. 5 Thermogravimetric analysis of (S)-4-[[[1-(4-fluorophenyl)-3-(1-methylethyl)-1H-indol-2-yl]-ethynyl]-hydroxyphosphinyl]-3]-hydroxybutanoic acid, disodium salt. Shown are the thermograms for (1) the initial material containing approximately 3% water, and materials obtained after exposure to relative humidities of (2) 33%, (3) 52%, (4) 60%, and (5) 75%.

scientific foundations that permitted this particular compound to function as a useful analytical reagent for Group IA and IIA cations, which were identified on the basis of the characteristic crystal morphologies associated with the adduct compounds. As is evident in the data collected in Table 3, the origin of the different crystal morphologies associated with each adduct species began with the formation of numerous, crystallographically inequivalent, hydrate species.

B.   Differential Thermal Analysis

In the differential thermal analysis (DTA) technique, one monitors the difference in temperature existing between a sample and a reference

Table 3 Thermogravimetric Analysis of the Adducts of Dilituric Acid with Group IA and IIA Cations [70]

Cation	Volatile content, measured at the 150°C plateau (%)	Theoretical volatile content for the n-hydrate species	Deduced hydration state (n)
Free acid	23.8	23.9	3
Lithium	12.3	12.2	2
Sodium	0	0	0
Potassium	0	0	0
Rubidium	0	0	0
Cesium	0	0	0
Beryllium	22.9	23.0	3
Magnesium	12.1	12.1	1.5
Calcium	3.9	4.1	0.5
Strontium	0	0	0
Barium	2.5	2.8	0.50

Source: Ref. 70.

as a function of temperature. Nonequivalences in temperature between the sample and the reference are observed when a process takes place that requires a finite heat of reaction. Typical solid-state changes of this type would be phase transformations, structural conversions, decomposition reactions, or desolvation processes. These processes may require either the input or the release of energy in the form of heat, which in turn translates into events that affect the temperature of the sample relative to a nonreactive reference. The primary applicability of DTA analysis to the study of polymorphs and solvates has been with respect to its ability to yield information about the phase transformations that take place as a function of temperature.

Although it is possible to use DTA as a quantitative tool, such applications require extensive calibration and care in data interpretation. For this reason, DTA has historically been mostly used in a quantitative sense as a means to determine the temperatures at which thermal events take place. Owing to the fortuitous combination of experimental conditions normally used for its measurement, the technique can be

used for the characterization of materials that evolve corrosive gases during the heating process. DTA analysis is highly useful as a means for the determination of compound melting points, although in systems capable of undergoing phase changes the analyst must always be aware of such concerns.

Methodology appropriate for the measuring of DTA profiles has been extensively reviewed [71,72] and need only be outlined here. Both the sample and the reference materials are contained within the same furnace, whose temperature program is externally controlled. The outputs of the sensing thermocouples are amplified, electronically subtracted, and finally shown on a suitable display device. If the observed change in enthalpy ΔH is positive as in the case of endothermic reactions, the temperature of the sample will lag behind that of the reference. If the ΔH is negative (exothermic reaction), the temperature of the sample will exceed that of the reference.

Wendlandt has provided an extensive compilation of conditions and requirements that influence the shape of DTA thermograms [73]. These can be divided into instrumental factors (furnace atmosphere, furnace geometry, sample holder material and geometry, thermocouple details, heating rate, and thermocouple location in the sample) and sample characteristics (particle size, thermal conductivity, heat capacity, packing density, swelling or shrinkage of sample, mass of sample taken, degree of crystallinity). A sufficient number of these factors are under the control of the operator, thus permitting selectivity in the methods of data collection. The ability to correlate an experimental DTA thermogram with a theoretical interpretation is profoundly affected by the details of heat transfer between the sample and the calorimeter [74].

The calibration of DTA systems is dependent on the use of appropriate reference materials rather than on the application of electrical heating methods. The temperature calibration is normally accomplished with the thermogram being obtained at the heating rate normally used for analysis [75], and the temperatures known for the thermal events are used to set temperatures for the empirically observed features. Recommended reference materials that span melting ranges of pharmaceutical interest include benzoic acid (melting point 122.4°C), indium (156.4°C), and tin (231.9°C).

The simplest and most straightforward application of DTA analy-

sis is concerned with studies of the relative stability of polymorphic forms. For example, DTA thermograms enabled the deduction that one commercially available form of chloroquine diphosphate was phase pure while another consisted of a mixture of two polymorphs [76]. DTA analysis was used to demonstrate that even though different crystal habits of sulfamethazine could be obtained, these in fact consisted of the same anhydrous polymorph [77]. In a study aimed at profiling the dissolution behavior of the three polymorphs and five solvates of spironlactone, DTA analysis was used in conjunction with powder x-ray diffraction to establish the character of the various materials [78].

In one study, it was found that three different crystalline forms of phenylbutazone could be obtained by the spray-drying of methylene chloride solutions at different temperatures, and that neither of these corresponded to the stable form that was obtained at the highest drying temperatures [79]. In fact, one of these could not be obtained by any conventional crystallization process but was only detected under conditions involving a slower solvent evaporation rate. As shown in Fig. 6, the DTA thermograms of each form are distinct and permit a facile distinction among the isolated forms.

Prior to the widespread use of differential scanning calorimetry, DTA analysis was the only method whereby one could obtain heats of transition or fusion. For example, sulfathiazone was found to undergo a transition from Form I to Form II at 161°C, for which the heat of transition was determined to be 1420 cal/mol [80]. The heat of fusion directly obtained for Form II was found to be 5970 cal/mol, which compared favorably with the heat of fusion determined for the material resulting from the conversion of Form I (5960 cal/mol). In another study, heats of fusion were determined for sixteen sulfonamides, some of which exhibited polymorphism and some of which did not [81]. In this work, a fuller understanding of the thermodynamics was provided, where the entropies as well as the enthalpies of the various processes were deduced.

C. Differential Scanning Calorimetry

In many respects, differential scanning calorimetry (DSC) is similar to the DTA method, and analogous information about the same type of thermal events can be obtained. However, DSC is far easier to use
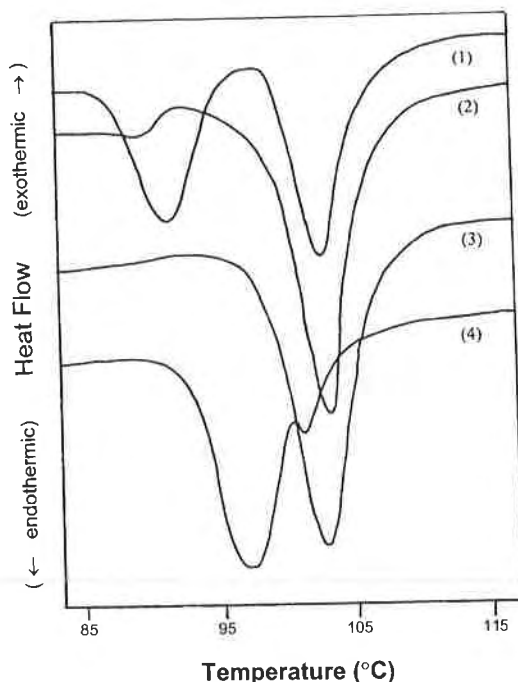
Fig. 6 Differential scanning calorimetry study of various spray-dried forms of phenylbutazone. Shown are the thermograms of (1) Form α, (2) Form β, (3) Form δ, and (4) Form ε. (The figure was adapted from data contained in Ref. 79.)

routinely on a quantitative basis, and for this reason it has become the most widely accepted method of thermal analysis for the pharmaceutical industry. The relevance of the DSC technique as a tool for pharmaceutical scientists has been amply discussed in numerous reviews [66,83–86], and a general chapter on DSC is documented in the *United States Pharmacopoeia* [87].

In the DSC method, the sample and reference materials are maintained at the same temperature, and the heat flow required to keep the equality in temperature is measured. DSC plots are therefore obtained as the differential rate of heating (in units of W/s, cal/s, or J/s) against temperature [88]. The area under a DSC peak is directly proportional to the heat absorbed or evolved by the thermal event, and integration

of these peak areas yields the heat of reaction (in units of cal/s · g or J/s · g).

Two types of DSC measurements are possible, which are usually identified as power-compensation DSC and heat-flux DSC, and the details of each have been fully described [88,89]. In power-compensated DSC, the sample and reference materials are kept at the same temperature by the use of individualized heating elements, and the observable parameter recorded is the difference in power inputs to the two heaters. In heat-flux DSC, one simply monitors the heat differential between the sample and the reference materials, with the methodology not being much different from that used for DTA.

In the DTA measurement, an exothermic reaction is plotted as a positive thermal event, while an endothermic reaction is usually displayed as a negative event. Unfortunately, the use of power-compensation DSC results in endothermic reactions being displayed as positive events, a situation counter to the latest IUPAC recommendations [90]. When the heat-flux method is used to detect the thermal phenomena, the signs of the DSC events concur with those obtained using DTA and also agree with the IUPAC recommendations.

The calibration of DSC instruments is normally accomplished through the use of compounds having accurately known transition temperatures and heats of fusion. A list of the standards currently supplied by the National Technical Information Service (NTIS) [91] is provided in Table 2. Once the DSC system is properly calibrated, it is trivial to obtain the melting point and enthalpy of fusion data for any compound upon integration of its empirically determined endotherm and application of the calibration parameters. The current state of methodology is such, however, that unless a determination is repeated a large number of times, the deduced enthalpies must be regarded as being accurate only to within approximately 5%.

As has been noted for DTA analysis, differential scanning calorimetry can also be used to establish the melting points of polymorphic species. For example, gepirone hydrochloride has been obtained in three polymorphic forms, which were found to melt at 180°C, 200°C, and 212°C [92]. In this work, it was shown that Forms I and II, and Forms I and III, were enantiotropic pair systems, but that Forms II and III were monotropic with respect to each other. The two polymorphs of phenylephrine oxazolidine each exhibited well-defined melting

points and could easily be distinguished on the basis of their thermograms [93].

Some of the most interesting DSC studies have been conducted on metastable phases that undergo a phase transformation to a more stable phase during the lifetime of the thermal analysis experiment. It was found that Form I of iopanoic acid yielded a single melting endotherm at 154°C, but that the thermogram obtained on Form II was much more complicated [94]. As shown in Fig. 7, Form II exhibits one endotherm at 133°C (the melting transition of Form I), an exotherm at 141°C (crystallization to Form II), and another endotherm at 153°C (melting of the recrystallized Form II). The literature abounds with similar examples where metastable polymorphs have been observed to convert
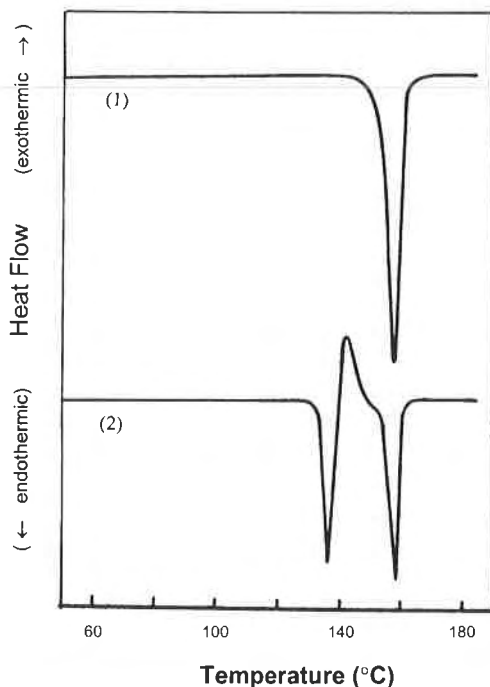

Fig. 7 Differential scanning calorimetry thermograms obtained for the (1) Form I and (2) Form II polymorphs of iopanoic acid. (The figure was adapted from data contained in Ref. 94.)

into more stable forms, with carbamazepine [95], piroxicam [96], and pireanide [97] being quoted as recent examples of work in this genre.

When studying the stability relationships between different polymorphic forms, the use of temperature cycling experiments has often proven to be very useful. Samples are heated to a preset temperature (but not high enough to induce a thermal decomposition event), cooled back to a lower temperature, and then reheated. Alterations in the recorded thermograms resulting from the cycling process can provide information about the ease of phase conversion. For example, such work enabled deductions to be made regarding the relative stabilities of the polymorphs of 1,2-dihydro-6-neopentyl-2-oxonicotinic acid [98]. The polymorphism associated with glyceryl monostearate was found to exert a profound effect upon the stability of formulations containing raw materials obtained from different sources, but the system could be understood in terms of phase transformations brought about by repeated melting and congealing cycles [99]. In fact, the use of cycling experiments may be essential to eliminate artifacts from entering into the construction of phase diagrams [100].

DSC techniques may be used to determine the kinetics of solid-state transformations as well. The kinetics associated with the transformation of disopyramide Form I to Form II were followed by changes in the DSC thermograms, and an activation energy of 144 kJ/mol was calculated for the system [101]. According to this work, the model proposed by Prout and Tompkins [102] for decomposition in solid phases (with no prior melting or liquefaction) appears to be appropriate for solid-state polymorphic transitions. As anticipated for any solid-state reaction, the phase change would initiate at defect sites, producing energetically favorable configurations that would serve as templates for continued phase transformation. In a similar work, a combination of DSC and XRPD studies was used to evaluate the kinetics associated with the various polymorphic transitions of phenylbutazone [103].

DSC analysis is often used in conjunction with structural techniques during the characterization of hydrate and solvate systems, with the thermal method being used to pinpoint the transition temperature range over which the bound water or solvent can be liberated. For instance, although a number of solvate forms could be crystallized for ethynylestradiol, the different solvent molecules were found incapable of exerting any effect on the conformation of the drug [104]. DSC

studies permitted the deduction of the relative stabilities of the three solvates of alprazolam [105] and contributed to the characterization of the solvate systems identified for norfloxacin [106] and dehydroepian-drosterone [107].

The character of the anhydrous phase resulting after the desolvation process has taken place can be effectively understood using DSC analysis. In many cases, loss of solvation or hydration molecules leads to the formation of an amorphous material, but this is not always the case. It was established that the acetone, isopropanol, and ethyl acetate solvates of cyclopenthiazide all reverted to Form I (a known anhydrous phase) above the endotherm corresponding to loss of solvent [108]. In other systems, loss of solvation results in the formation of a metastable phase, which undergoes a phase conversion to the stable anhydrate phase before overall melting takes place. Such behavior is illustrated in Fig. 8, where it was reported that the *tert*-butanol and dioxane solvates of piretanide exhibited a recrystallization after desolvation, but that the propylene glycol and dimethylformamide solvates did not [109].

V. MOLECULAR MOTION: VIBRATIONAL SPECTROSCOPY

The energies associated with the vibrational modes of a chemical compound lie within the range of 400–4000 cm^{-1}. These modes can be observed directly through their absorbance in the infrared region of the spectrum, or through the observation of the low-energy scattered bands that accompany the passage of an intense beam of light through the sample (the Raman effect). In either case, the use of Fourier-transform methodology has vastly improved the quality of data that can be obtained [110]. Most workers are familiar with the use of mid-infrared spectra for identity purposes, where the pattern of absorption bands is taken to be diagnostic for a given compound. However, it has come to be recognized that the vibrational spectra of solid materials will reflect details of the crystal structure, and hence these methods can be used in the spectroscopic investigation of polymorphs and solvates [111,112].
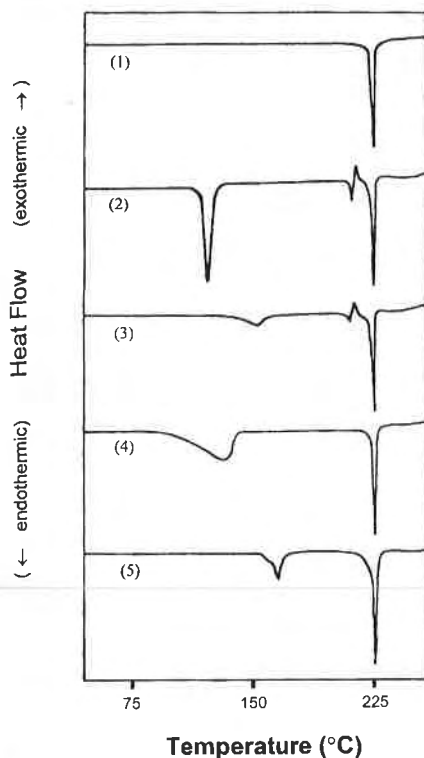
Fig. 8 Differential scanning calorimetry study of various solvates of piretan-
ide. Shown are the thermograms of (1) the anhydrate phase, (2) the *tert*-butanol
solvate, (3) the dioxane solvate, (4) the propylene glycol solvate, and (5) the
dimehylformamide solvate. (The figure was adapted from data contained in
Ref. 109.)

    In the best-designed studies of polymorphic or solvate systems,
the purpose of the vibrational spectroscopy investigation should be to
gather information from the observed pattern of vibrational frequencies
and to use these data to understand the structural aspects that yield
crystallographic differences. Once suitable spectral features are identi-
fied from this work, they can be used to develop easily performed meth-
ods for the quantitative analysis of one polymorph (or solvate) in the
presence of the other. Unfortunately, all too many workers are merely

satisfied to obtain the spectra of the various polymorphs and/or solvates and simply to display the differences. In doing so, they miss a great opportunity to gain additional understanding about the system they are trying to characterize.

A. Infrared Absorption Spectroscopy

The acquisition of high-quality infrared absorption spectra appropriate for the characterization of polymorphs and solvates is best performed using Fourier transform technology (the FTIR method), since this approach minimizes transmission and beam attenuation problems. Essentially all FTIR spectrometers use a Michelson interferometer. Radiation entering the interferometer is split into two beams by means of a beam splitter. One beam follows a path of fixed distance before being reflected back into the beam splitter, while the other beam travels a variable distance before being recombined with the first beam. The recombination of these two beams yields an interference pattern, and the time-dependent constructive and destructive interferences have the effect of forming a cosine signal.

Each component wavelength of the source will yield a unique cosine wave having a maximum at the zero path length difference (ZPD) that decays with increasing distance from the ZPD. The detector is placed so that radiation in the central image of the interference pattern will be incident upon it, and therefore intensity variations in the recombined beam are manifest as phase differences. The observed signal at the detector is a summation of all the cosine waves, having a maximum at the ZPD that decays rapidly with increasing distance from the ZPD. If the component cosine waves can be resolved, then the contribution from individual wavelengths can be observed. The frequency domain spectrum is obtained from the interferogram by performing the Fourier transformation mathematical operation.

The solid-state FTIR spectra of many polymorphic systems often are found to be only slightly different, indicating that the pattern of molecular vibrations is not grossly affected by differences in crystal structure. Examples of this type of behavior are typified in studies conducted on etoposide [113], tegafur [114], lomeridine dihydrochloride [115], and carbamazepine [116]. In other instances, the FTIR spectra

of polymorph systems differ substantially, permitting the ready identification of a given form. For instance, the two forms of ranitidine hydrochloride yielded spectra that differed in the region above 3000 cm^{-1} and in the regions spanning 2300–2700 cm^{-1} and 1570–1620 cm^{-1} [117].

Mexiletine hydrochloride has been found to crystallize in six polymorphic forms, each of which yields a characteristic IR spectrum [118]. As shown in the data collected in Table 4, the very intense bands due to the C–N(H) stretching mode (1020–1060 cm^{-1}) and the aromatic C–H out-of-plane deformation mode (760–780 cm^{-1}) were found to be particularly sensitive to the structural differences. In fact, the splitting of the latter mode observed in four of the six polymorphs suggests the existence of different types of molecules within the unit cell. The data in Table 4 also illustrate the typical magnitudes in band energy differences encountered within the vibrational spectra of polymorphic systems.

When solvent molecules are incorporated in a crystal lattice, the new structure is often sufficiently different from that of the anhydrous phase so that many of the molecular vibrational modes are altered [119]. Not surprisingly, the most pronounced effects are associated with modes expressing the motion of atoms involved in hydrogen bonding with the solvent. For this reason, studies carried out in the high-

Table 4 Effect of Crystal Polymorphism on Selected Infrared Bands of Mexiletine Hydrochloride

Polymorph	Energy of band(s) associated with the C–N(H) stretching mode (cm^{-1})	Energy of band(s) associated with the aromatic C–H out-of-plane deformation mode (cm^{-1})
I	1037	772
II	1033	770, 765
III	1039	769, 760
IV	1027	775, 760
V	1047	769, 760
VI	1052, 1034	766

Source: Ref. 118.

energy region of the infrared spectrum (2000–4000 cm $^{-1}$) often yield the most striking spectral differences between solvate phases and the corresponding anhydrous phase.

When water acts as the solvation agent, observations within the -OH stretching region (3100–3600 cm^{-1}) are most fruitful for identification purposes. For example, the anhydrate and hydrate phases of oxyphenbutazone [120], digoxin [121], trazodone hydrochloride [122], and ampicillin [123] are readily distinguished on the basis of their IR absorption spectra. Organic solvents are equally amenable to study within the high-energy spectral region. As illustrated in Fig. 9, the - OH stretch of bound methanol in the solvate of 9,10-anthraquinone is observed at 3440 cm^{-1} [124], which represents a shift from the frequency of 3400 cm^{-1} noted for the same mode in liquid methanol [125].
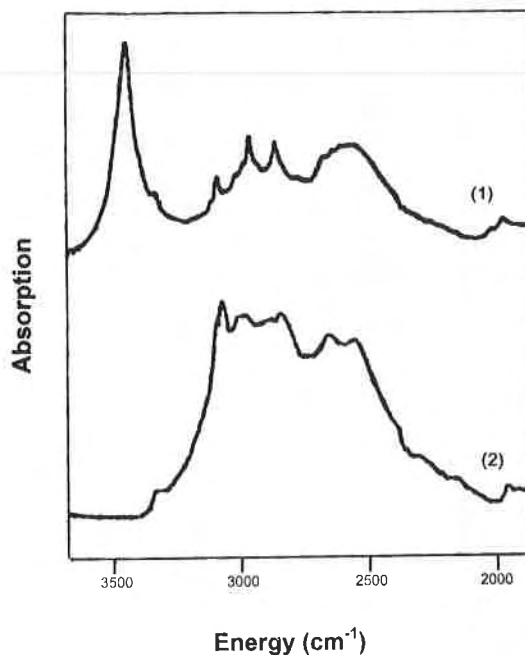

Fig. 9 Infrared absorption spectra obtained within the high-energy vibrational region for the (1) methanol solvate and (2) anhydrous Form I polymorphs of 9,10-anthraquinone. (The figure was adapted from data contained in Ref. 124.)

When systems can form multiple anhydrate, hydrate, and solvate phases, the use of infrared spectroscopy can be extremely valuable. For example, depending on the recrystallization solvent, delavirdine mesylate has been found to form two anhydrous phases, two hydrates, an ethanol solvate, and an acetonitrile solvate, as well as six other phases resulting as the products of solid-state transformations of the hydrated and solvated phases [126]. In this work, FTIR spectroscopy played an important role in working out the characterization of this system.

However, it is clear that vibrational spectroscopy has considerable use beyond the identification of polymorphs and solvates. The infrared spectra obtained on the polymorphs of acetohexamide and selected derivatives has been used to study the tautomerism of the drug compound [127]. It was deduced in this work that Form A existed in the enol form, stabilized by the intramolecular bonding between the O—H and S=O groups that produces a six-membered ring. Form B was characterized by the existence of the keto form, with the urea carbonyl group being intermolecular bonding to a sulfonamide N—H functionality. This behavior can be contrasted with that noted for spironolactone, where no evidence was found for the existence of enolic tautomers in any of the four polymorphs [128].

Variable-temperature vibrational spectroscopy can be a powerful tool for the study of phase transitions and/or desolvation processes. The technique has been combined with factor analysis to deduce the three phase transitions and four conformational changes associated with pentaerythritol tetrastearate [129]. In this particular work, each thermal event was substantiated by analogous DSC studies. The dihydrates prepared from the two polymorphs of carbamazepine were also studied using variable-temperature techniques [130].

When a nonequivalence in absorption bands is identified for a polymorphic or solvate system, the band intensities can be used for a quantitative analysis of mixtures containing the various phases. Such work requires the nontrivial preparation of homogeneous calibration samples containing known amounts of the phases in question, but most workers underestimate the difficulty associated with mixing powders of potentially different morphologies. For example, the monohydrate phase of cefepime dihydrochloride consists of rods, while the dihydrate phase consists of needles, and the mixing of these could only be prop-

erly effected using a solvent slurrying technique [131]. But with this superior method of mixing the two phases, workers were able to obtain superior analytical performance parameters for their validated assay method. Quantitative analytical methods have also been reported for sulfamethoxazole [132], chlorpropamide [133], and 7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)-propoxy-3,4-dihydro-8-propyl-2H-1-benzo-pyran-2-carboxylic acid [134], illustrating the generality of the approach.

B. Raman Spectroscopy

The vibrational modes of a compound may also be studied using Raman spectroscopy, where one measures the inelastic scattering of radiation by a nonabsorbing medium [135]. When a beam of light is passed through a material, approximately one in every million incident photons is scattered with a loss or gain of energy. The inelastically scattered radiation can occur at lower (Stokes lines) and higher (anti-Stokes lines) frequencies relative to that of the incident (or elastically scattered) light, and the energy displacements relative to the energy of the incident beam correspond to the vibrational transition frequencies of both mediums. The actual intensities of the Stokes and anti-Stokes lines are determined by the Boltzmann factor characterizing the vibrational population. For high-frequency vibrations, the Stokes lines are intense relative to the anti-Stokes lines, so conventional Raman spectroscopy makes exclusive use of the Stokes component.

The Raman effect originates from the interaction of the oscillating induced polarization or dipole moment of the medium with the electric field vector of the incident radiation. Raman spectra are measured by passing a laser beam through the sample and observing the scattered light either perpendicular to the incident beam or through backscatter detection. The scattered light is analyzed at high resolution by a monochromator and ultimately detected by a suitable device. One way of obtaining good spectra is to use a notch filter that will eliminate the exciting line, since this is required to obtain acceptable signal-to-noise ratios.

Although both infrared absorption and Raman scattering yield information on the energies of the same vibrational bands, the different

selection rules governing the band intensities for each type of spectros-
copy can yield useful information. For the low-symmetry situations
presented by the structures of molecules of pharmaceutical interest,
every vibrational band will be active to some degree in both infrared
absorption and Raman scattering spectroscopies. The relative intensi-
ties of analogous bands will differ, however, when observed by infrared
absorption or Raman spectroscopy. In general, symmetric vibrations
and nonpolar groups yield the most intense Raman scattering bands,
while antisymmetric vibrations and polar groups yield the most intense
infrared absorption bands. Both types of vibrational spectroscopy were
used to investigate the polymorphism of nimodipine, and the data were
contrasted in a particularly illustrative series of figures [136]. It was
evident from the intensity relations that although each technique
yielded a summary of the vibrational transitions, substantial differences
in band intensity were readily discernible.

Raman scattering bands are often quite sharp, and consequently
Raman spectra often contain significantly less spectral overlap than in-
frared absorption spectra. As shown in Fig. 10, the high-energy spectral
region obtained for the two polymorphs of fluconazole consists of a
series of well-resolved spectral features, which permits a more facile
characterization of the structural differences between the two systems
[137]. Raman spectra are generally not complicated by contributions
from adventitious moisture, owing to the weak scattering nature of the
water molecule.

Even when Raman spectroscopy is used primarily as a means for
the differentiation of polymorphs or solvates, the degree of spectral
simplification associated with Raman data permits a more facile gener-
ation of band assignments. This feature has been successfully exploited
in the cases of spironolactone [128], losartan [138], 3-(p-thioanisoyl)-
1,2,2-trimethyl-cyclopentanecarboxylic acid [139], and *trans*-3,4-
dichloro-N-methyl-N-[1,2,3,4-tetrahydro-5-methoxy-2-(pyrrolidin-1-
yl)]-naphth-1-ylbenzeneacetamide [140].

Owing to its ability to provide data on very low frequency vibra-
tional modes, Raman spectroscopy can yield information on the lattice
vibrations of a crystal. Such work has been performed on the various
crystal forms of ampicillin and griseofulvin [141,142], and useful infor-
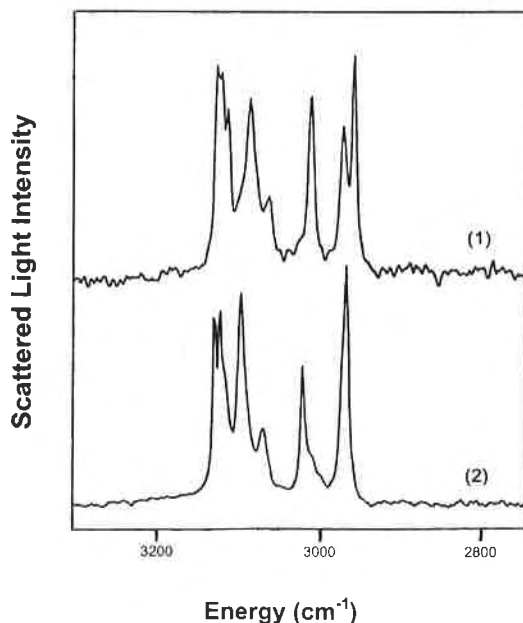mation has been obtained on the nature of the solvate species formed.

Fig. 10   Raman spectra obtained within the high-energy vibrational region for the (1) Form I and (2) Form II polymorphs of fluconazole. (The figure was adapted from data contained in Ref. 137.)

Most interesting was the use of variable-temperature Raman studies, where the characteristics of the crystal lattice were followed during the desolvation process [142]. Variable-temperature Raman spectroscopy was also used to study the phase transformations of 2-(S)-hydroxy-3-(R)-(2-carboxyethyl)thiol-3-[2-(phenyloctyl)-phenyl]-propionic acid [143].

VI.   CHEMICAL ENVIRONMENT: NUCLEAR MAGNETIC RESONANCE SPECTROMETRY

One technique that is becoming increasingly important for the characterization of materials is that of solid-state nuclear magnetic resonance (NMR) spectroscopy [144]; the application of this methodology to topics of pharmaceutical interest has been amply demonstrated [112,145–146]. Although any nucleus that can be studied in the solution phase

can also be studied in the solid state, most of the work has focused on ^{13}C studies. As mentioned in the case of vibrational spectroscopy, the ability of solid-state NMR to differentiate between a system of polymorphs or solvates requires that individual nuclei exist in nonequivalent magnetic environments within the two crystal structures. If the structural variations do not lead to a magnetic nonequivalence for a given nucleus, then the resonances obtained for the nucleus will not differ. Powerful as the technique has proven to be, one must remember that the ultimate arbiter of polymorphism is crystallography and not spectroscopy.

^{1}H-NMR remains an extremely difficult measurement in the solid state, and the data obtained from such work can only be obtained at medium resolution. The field acting at the nucleus is affected by the magnetic dipoles of neighboring nuclei, and the local fields thus generated are sensitive to both the internuclear distances and their orientation relative to the external field. Since protons are abundantly present in organic compounds, the removal of proton–proton dipolar interactions is necessary to obtain high-resolution ^{1}H spectra in solids. Although this is possible, the resulting ^{1}H-NMR spectra are still inferior to those obtained in the solution phase. The primary reason for this is that ^{1}H-NMR has one of the smallest isotropic chemical shift ranges (12 ppm), but with peak broadening effects that can span several ppm in magnitude. Other nuclei yield far better data, with ^{13}C and ^{31}P solid-state NMR studies being very useful to the physical characterization of all pharmaceutical solids.

The local magnetic field B_{loc} at a ^{13}C nucleus in an organic solid is given by

$$B_{loc} = \pm \left\{ \frac{h\gamma_H}{4\pi} \right\} \left\{ \frac{3\cos^2 \theta - 1}{r^3} \right\} \tag{2}$$

where γ_H is the magnetogyric ratio of the proton, r is the internuclear C–H distance to the bonded proton, and θ is the angle between the C–H bond and the external applied field (B_0). The $\pm$ sign indicates that the local field may add to or subtract from the applied field depending on whether the neighboring proton dipole is aligned with or against the direction of B_0. In a microcrystalline organic solid, there is a summation over many values of θ and r, resulting in a proton dipolar broad-

ening of many kilohertz. A rapid reorientation of the C–H internuclear vectors (such as those associated with the random molecular motions that take place in the liquid phase) would result in reduction of the dipolar broadening. In solids, such rapid isotropic tumbling is not possible, but since $3 \cos^2 \theta - 1$ equals zero if θ equals $\cos^{-1} 3^{-1/2}$ (approximately $54°44'$), spinning the sample at the so-called magic angle of $54°44'$ with respect to direction of the applied magnetic field results in an averaging of the chemical shift anisotropy. In a solid sample, the anisotropy reflects the chemical shift dependence of chemically identical nuclei on their spatial arrangement with respect to the applied field. Since it is this anisotropy that is primarily responsible for the spectral broadening associated with ^{13}C samples, spinning at the magic angle makes it possible to obtain high-resolution ^{13}C-NMR spectra of solid materials.

An additional method for the removal of ^{13}C–^{1}H dipolar broadening is to use a high-power proton decoupling field. This is often referred to as dipolar decoupling. One irradiates the sample using high power at an appropriate frequency, which results in the complete collapse of all ^{13}C–^{1}H couplings. With proton dipolar coupling alone, the resonances in a typical solid-state ^{13}C spectrum will remain very broad (on the order of 10–200 ppm). This broadening arises because the chemical shift of a particular carbon is directional, depending on the orientation of the molecule with respect to the magnetic field.

Even though high-resolution spectra can be obtained on solids using the magic angle spinning (MAS) technique, the data acquisition time is lengthy due to the low sensitivity of the nuclei and the long relaxation times exhibited by the nuclei. This problem is circumvented through the use of cross polarization (CP), where spin polarization is transferred from the high-abundance, high-frequency nucleus (^{1}H) to the rare, low-frequency nucleus (^{13}C). This process results in up to a fourfold enhancement of the normal ^{13}C magnetization and permits a shortening of the waiting periods between pulses. The CP experiment also allows the measurement of several relaxation parameters that can be used to study the dynamic properties of the solid under investigation.

It is often observed that the NMR spectra of compound polymorphs or solvates contain nonequivalent resonance peaks for analogous nuclei. This effect arises because the intimate details of the molec-

ular environments associated with differing crystal structures can yield a nonequivalent relationship with respect to the applied magnetic field of the NMR experiment, which in turn causes the analogous nuclei to resonate at different energies. As has been already noted for the infrared spectra of polymorphs or solvates, it is not uncommon for certain resonance peaks to be observed at identical chemical shifts, while other resonances are significantly shifted [145]. Since it is not difficult to assign organic functional groups to observed resonances, solid-state NMR spectra can be used to deduce the nature of polymorphic variations. This technique is especially valuable when the crystal polymorphism is conformational in origin. Such information is extremely valuable at the early stages in drug development when solved single crystal structures for each polymorph or solvate may not be available.

In their simplest application, solid-state NMR spectra can be used to differentiate qualitatively between polymorphs or solvates, much in the manner described for vibrational spectroscopy. Such data have been reported for the polymorphs of sulfathiazole [147], cyclopentthiazide [148], and indomethacin [149]. The technique can also be profitably used to differentiate between anhydrate and solvate phases, as has been reported for ampicillin [123], androstanolone [150], and dirithromycin [151].

When more detailed interpretation of the results is required, solution-phase NMR spectra can often be useful in the assignment of resonances observed in the solid-state NMR spectrum of the same compound. At the same time, the effects of magnetic nonequivalence associated with details of the crystallography can also be evaluated. This situation has been illustrated in Fig. 11, where the solid-state spectrum reported for benoxaprofen Form I is found to exhibit both similarities and differences relative to the solution-phase spectrum [152]. Contrasts between solution-phase and solid-state NMR spectra have also been drawn for mofebutazone, phenylbutazone, and oxyphenbutazone [153].

When detailed assignments of solid-state spectra have been made, the technique can be used to deduce differences in molecular conformation that cause crystallographic variations to exist. During the development of fosinopril sodium, a crystal structure was solved for the most stable phase, but no such structure could be obtained for its metastable phase [154]. The compound contains three carbonyl groups, but the
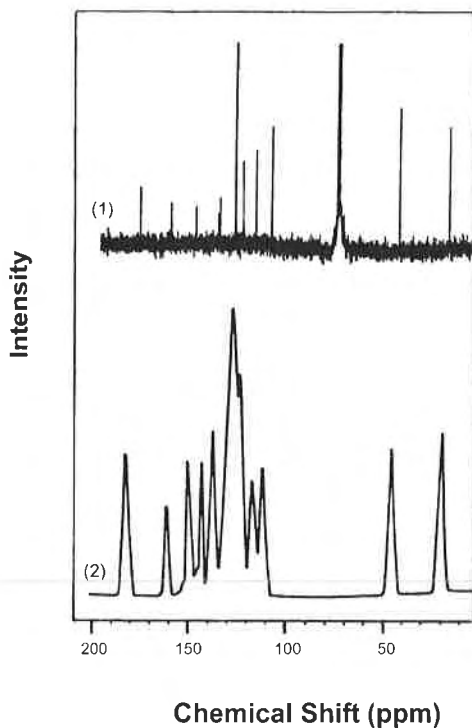
Chemical Shift (ppm)

Fig. 11   (1) Completely decoupled solution-phase and (2) solid-state ^{13}C nuclear magnetic resonance spectra obtained for benoxaprofen. The solution-phase spectrum is compared with the solid-state spectrum of Form I. (The figure was adapted from data contained in Ref. 152.)

solid-state ^{13}C-NMR spectra of two of these were essentially equivalent. The third carbonyl, located on the acetal side chain, was found to resonate at different chemical shifts in the two structures. When combined with the observations obtained using vibrational spectroscopy, these results permitted the deduction that the solid-state polymorphism was associated with different conformations of this side chain. The NMR data also suggested that additional conformational differences between the two polymorphs were associated with cis–trans isomerization along the peptide bond, which in turn results in the presence of nonequivalent molecules existing in the unit cell. In the absence of

solved crystal structures for the two polymorphs, this information would not have been otherwise obtainable.

The acquisition of solid-state ^{13}C-NMR spectra at various temperatures can be a powerful approach to the study of molecular motion in solids and for the study of phase conversion. Such methodology was used to study the two polymorphs of 2,2-bis(p-acetoxyphenyl)propane, where markedly different molecular mobilities were found to exist in the two crystal structures [155]. Below the glass transition temperature, the important active mode correlates with the flipping of the phenylene rings, and this effect was evident in the NMR spectra obtained at different temperatures. Some of the resonance bands associated with Form II were found to coalesce upon sample heating from $-10°C$ to ambient probe temperature.

The solid-state NMR technique can be used to deduce quantitative measurements of phase composition, as has been reported for the anhydrate and dihydrate phases of carbamazepine [156]. In the solid-state NMR spectra of delavirdine mesylate, Form VIII shows a unique resonance at 17.3 ppm and Form XI a unique resonance at 20.2 ppm, while a resonance at 23.9 ppm is shared by both forms [157]. These spectral characteristics have been exploited for the development of a method to determine the Form VIII content in bulk Form XI. As evident from the spectra shown in Fig. 12, the empirical limit of detection for the determination of Form VIII was approximately 2%.

The applications of solid-state ^{13}C-NMR spectra for the study of polymorphs and solvates can go beyond evaluations of resonance band positions and make use of additional spectral characteristics. For instance, studies of T_{1p} relaxation times of furosemide polymorphs were used to show the presence of more molecular mobility and disorder in Form II, while the structure of Form I was judged to be more rigid and uniformly ordered [158]. The analysis of the solid-state ^{13}C-NMR spectra of ($1R,3S$)-3-p-thioanisoyl)-1,2,2-trimethylcyclopentanecarboxylic acid was facilitated by the J-modulated spin-echo technique, which was used to deduce the number of protons bound to each carbon atom [159]. Differences in the dipolar dephasing behavior between the two polymorphs of ($\pm$)-$trans$-3,4-dichloro-N-methyl-N-[1,2,3,4-tetrahydro-5-methoxy-2-(pyrrolidin-1-yl)]naphth-1-yl-benzeneacetamide were noted and ascribed to motional modulation of the carbon–hydro-

Chemical Shift (ppm)
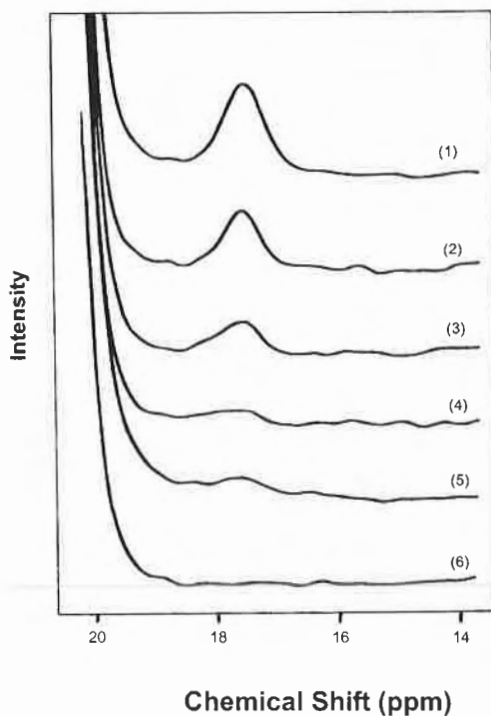
Fig. 12   Solid-state ^{13}C nuclear magnetic resonance spectra obtained for Form XI of delavirdine mesylate, spiked with various levels of Form VIII. Spectra are shown for spiking levels of (1) 1%, (2) 2%, (3) 3%, (4) 5%, (5) 10%, and (6) 15%. (The figure was adapted from data contained in Ref. 157.)

gen dipolar interaction [160]. This added degree of molecular motion was used to deduce a loosely packed crystal structure for Form II of this compound.

VII.   SUMMARY

The study of polymorphs and solvates begins with the methods used to elucidate the nature of the system in question. Beginning with the basic arsenal of crystallography, microscopy, thermal analysis, solubil-

ity studies, vibrational spectroscopy, and nuclear magnetic resonance, one can do significant work on the characterization of polymorphs and solvates. In spite of the power associated with the thermal or analytical techniques, one must always remember that the defining criterion for the existence of different crystal forms is a nonequivalence of crystal structures. All other methodologies must be considered as sources of supporting and ancillary information; they cannot be taken as definitive proof for the existence of polymorphism by themselves.

It is hoped that the range of studies discussed in the present work sheds sufficient light onto the practice of the most commonly encountered techniques for the solid-state characterization of polymorphic or solvate properties. Every system presents its own range of challenges, requiring an entire program of study to comprehend the system. Through suitably designed multidisciplinary work, any clever investigator will be able to obtain information at whatever level of complexity might be required. It is also anticipated that continued developments in methodology will enable the design of even better studies and ultimately result in the generation of even more useful data.

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7

Effects of Polymorphism and Solid-State Solvation on Solubility and Dissolution Rate

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I. INTRODUCTION

Earlier chapters have amply demonstrated that the different lattice energies (and entropies) associated with different polymorphs or solvates give rise to measurable differences in the physical properties (density, color, hardness, refractive index, conductivity, melting point, enthalpy of fusion, vapor pressure, etc.—see Chapter 1, Table 3). Even the explosive power of cyclotetramethylene-tetranitramine depends on which of its four polymorphs is being used [1]. We have seen in Chapter 1 that the different lattice energies of polymorphs or solvates give rise to different solubilities and dissolution rates. If the solubilities of the various solid forms are sufficiently different, they can be very important during the processing of drug substances into drug products [2] and may have implications for the adsorption of the active drug from its dosage form [3]. These concerns have led to an increased regulatory interest in the solid-state properties and behavior of drug substances and in their characterization [4].

    That the crystal structure can have a direct effect on the solubility

of a solid can be understood using a simple model. For a solid to dissolve, the forces of attraction between solute and solvent molecules must overcome the attractive forces holding together the solid and the liquid solvent. In other words, for the process to proceed spontaneously, the solvation free energy released upon dissolution must exceed the sum of the lattice free energy of the solid plus the free energy of cavity formation in the solvent. The balance of the attractive and disruptive forces will determine the equilibrium solubility of the solid in question (which is an exponential function of the free energy change of the system—see Chapter 1, Equation 15). The enthalpy change and the increase in disorder of the system (i.e., the entropy change) determine the Gibbs free energy change. Since different lattice energies (and enthalpies) characterize different crystal structures (as discussed in Chapter 1), the solubility of different crystal polymorphs (or solvate species) must differ as well. Finally, the act of dissolution may be endothermic or exothermic in nature, so that measurements of solution calorimetry can be used to provide important information about the substance under study. The most common solvent media used in the characterization of polymorphs or solvates are liquids or liquid mixtures that give rise to liquid solutions of the solute [5], and which constitute the focus of this chapter.

As explained in Chapter 1, the solubility differences between polymorphs or solvates enable a less stable form to convert to the most stable form. When such a conversion can take place, the measured solubility of each form will approach a common value, namely that of the most stable form at the temperature of measurement.

The effect of polymorphism on solubility becomes especially critical because the rate of compound dissolution must also be dictated by the balance of attractive and disruptive forces existing at the crystal–solvent interface. A solid having a higher lattice free energy (i.e., a less stable polymorph) will tend to dissolve faster, because the release of a higher amount of stored lattice free energy will increase the solubility and hence the driving force for dissolution. At the same time, each species would liberate (or consume) the same amount of solvation energy, because all dissolved species (of the same chemical identity) must be thermodynamically equivalent. The varying dissolution rates possible for different structures of the same drug entity can in turn lead to varying degrees of bioavailability for different polymorphs or solvates.

To achieve bioequivalence for a given drug compound usually requires equivalent crystal structures in the drug substance, although exceptions are known to exist.

The dissolution rate and solubility in a solvent medium are two of the most important characteristics of a drug substance, because these quantities determine the bioavailability of the drug for its intended therapeutic use. Solubility is defined as the equilibrium concentration of the dissolved solid (the solute) in the solvent medium and is ordinarily a function of temperature and pressure.

II. EQUILIBRIUM SOLUBILITY

The capacity of any system to form solutions has limits imposed by the phase rule of Gibbs:

$$F + P = C + 2 \qquad (1)$$

where F is the number of degrees of freedom in a system consisting of C components with P phases. For a system of two components and two phases (e.g., solid and liquid) under the pressure of their own vapor and at constant temperature, F equals zero. If one of the phases consists solely of one component (a pure substance), the equilibrium solubility at constant temperature and pressure is a fixed quantity that is given as the amount of solute contained in the saturated solution in a unit amount of the solvent or solution.

For any case in which F is zero, a definite reproducible solubility equilibrium can be reached. Complete representation of the solubility relations is accomplished in the phase diagram, which gives the number, composition, and relative amounts of each phase present at any temperature in a sample containing the components in any specified proportion. Solubilities may therefore be expressed in any appropriate units of concentration, such as the quality of the solute dissolved (defined mass, number of moles) divided by the quantity either of the solvent (defined mass, volume, or number of moles) or of the solution (defined mass, volume, or number of moles). Jacques et al. have provided a compilation of the expressions for concentration and solubility [6].

A. Determination of Equilibrium Solubility

Methods for the determination of solubility have been thoroughly reviewed [5,7,8], especially with respect to the characterization of pharmaceutical solids [9]. Solubility is normally highly dependent on temperature, so the temperature must be recorded for each solubility measurement in addition to the precise nature of the solvent and the solid phase at equilibrium. Plots of solubility against temperature are commonly used for characterizing pharmaceutical solids and have been extensively discussed [5,10]. Frequently (especially over a relatively narrow temperature range), a linear relationship can be given either by a van't Hoff plot:

$$\ln X_2^{sat} = \frac{-a}{RT} + c' \qquad (2)$$

or by a Hildebrand plot:

$$\ln X_2^{sat} = \frac{b}{R} \ln T + c'' \qquad (3)$$

In Eqs. (2) and (3), X_2^{sat} is the mole fraction solubility of the solid solute at an absolute temperature T, a is the apparent molar enthalpy of solution, b is the apparent molar entropy of solution, and c' and c'' are constants. The combined equation, attributed to Valentiner, has been used by Grant et al. [10] in the form

$$\ln X_2^{sat} = \frac{-a}{RT} + \frac{b}{R} \ln T + c''' \qquad (4)$$

This three-parameter equation enables solubility to be simulated and correlated quite accurately over a wide temperature range (e.g., 60 K).

As implied in the previous paragraph, the validity of the aforementioned equations requires that each crystal phase be stable, as indicated by the absence of any phase conversion during the determination of equilibrium solubility. Systems characterized by the presence of metastable phases constitute special cases that have been discussed in Chapters 1 and 2.

Two general methods, the analytical method and the synthetic method [9], are available for determining solubility. In the analytical method, the temperature of equilibration is fixed, while the concentra-

tion of the solute in a saturated solution is determined at equilibrium by a suitable analytical procedure. The analytical method can be either the traditional, common batch agitation method, or the more recent flow column method. In the synthetic method, the composition of the solute–solvent system is fixed by appropriate addition and mixing of the solute and solvent, and then the temperature at which the solid solute just dissolves or just crystallizes is carefully bracketed.

It is usually not difficult to determine the solubility of solids that are moderately soluble (greater than 1 mg/mL), but the direct determination of solubilities much less than 1 mg/mL is not straightforward. Problems such as slow equilibrium resulting from a low rate of dissolution, the influence of impurities, and the apparent heterogeneity in the energy content of the crystalline solid [11] can lead to large discrepancies in reported values. For example, reported values of the aqueous solubility of cholesterol range from 0.066 to 3000 mg/L at 30°C [12].

B. Studies of the Equilibrium Solubility of Polymorphic and Solvate Systems

The thermodynamic relationships examined in Chapter 1 involving polymorphism and solubility have been applied to the methylprednisolone system [13]. The solubilities of the two polymorphs of this steroid were determined at various temperatures in water, decyl alcohol, and dodecyl alcohol. Because the chemical potential and thermodynamic activity of the drug in the solid state and in each saturated solution are constant, the solubility ratios for the two forms (which can be found in Table 1) were found to be independent of the solvent. The enthalpy, entropy, and temperature of transition calculated from the data were 1600 cal/mol, 4.1 cal/K · mol, and 118°C, respectively.

Solubility determinations were used to characterize the polymorphism of 3-(((3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)phenyl)-((3-dimethylamino-3-oxopropyl)thio)methyl)thio)propanoic acid [14]. The solubility of Form II was found to be higher than that of Form I in both isopropyl alcohol (IPA, solubility ratio approximately 1.7 from 5 to 55°C) and in methyl ethyl ketone (MEK, solubility ratio approximately 1.9 from 5 to 55°C), indicating that Form I is the thermodynamically stable form in the range of 5 to 55°C. An analysis of the entropy contributions to the free energy of solution from the solubility results

Table 1 Equilibrium Solubility and Solubility Ratios of the Polymorphs of Methylprednisolone in Different Solvent Systems

Temperature (°C)	Solubility in water (mg/mL)		
	Form I	Form II	Solubility ratio, II/I
30	0.09	0.15	1.67
39	0.12	0.20	1.67
49	0.16	0.26	1.63
60	0.21	0.33	1.57
72	0.30	0.43	1.43
84	0.43	0.55	1.28

Temperature (°C)	Solubility in decyl alcohol (mg/mL)		
	Form I	Form II	Solubility ratio, II/I
30	2.9	4.8	1.66
39	3.5	5.7	1.63
49	4.3	6.9	1.60
60	5.5	8.6	1.56
72	8.3	11.9	1.43

Source: Ref. 13.

implied that the saturated IPA solutions were more disordered than were the corresponding MEK solutions, in turn indicating the existence of stronger solute–solvent interactions in the MEK solution. This finding corroborated results determined for the enthalpy with respect to the deviations of the saturated solutions from ideality.

Phenylbutazone has been found capable of existing in five different polymorphic structures, characterized by different X-ray powder diffraction patterns and melting points [15]. The equilibrium solubilities of all five polymorphs in three different solvent systems are summarized in Table 2. Form A exhibits the highest melting point (suggesting the least energetic structure at the elevated temperature), while its solubility is the lowest in each of the three solvent systems studied (actually demonstrating the lowest free energy). These findings indicate that Form A is the thermodynamically most stable polymorph both at room

Table 2 Equilibrium Solubility of Phenylbutazone Polymorphs at Ambient Temperature in Different Solvent Systems

Solvent system	Solubility (mg/mL)				
	A	B	D	E	C
pH 7.5 phosphate buffer	4.80	5.10	5.15	5.35	5.9
Above buffer with 0.05% Tween 80	4.50	4.85	4.95	5.10	5.52
First buffer with 2.25% PEG 300	3.52	5.77	5.85	6.15	6.72

Note: The polymorphs are listed in order of increasing free energy at ambient temperature.
Source: Ref. 15.

temperature and at the melting point (105°C). However, identification of the sequence of stability for the other forms at any particular temperature was not straightforward. Following one common convention, the polymorphs were numbered in the order of decreasing melting points, but the solubility data of Table 7 did not follow this order. This finding implies that the order of stability at room temperature is not the same as that at 100°C and emphasizes that only measurements of solubility can predict the stability order at room temperature. If different polymorphs are not discovered in the same study, they are ordinarily numbered according to the order of discovery to avoid renumbering those discovered earlier. Ostwald's rule of stages, discussed in Chapter 1, explains why metastable forms are often discovered first.

Gepirone hydrochloride was found to exist in at least three polymorphic forms, whose melting points were 180°C (Form I), 212°C (Form II), and 200°C (Form III) [16]. Forms I and II, and Forms I and III, were deduced to be enantiotropic pairs in the sense that their G vs. T curves crossed. Form III was found to be monotropic with respect to Form II, since the G vs. T curves did not cross below their melting points, and since there was no temperature at which Form III was the most stable polymorph. The solubility data illustrated in Fig. 1 were used to estimate a transition temperature of 74°C for the enantiotropic Forms I and II, while the reported enthalpy difference was 4.5 kcal/mol at 74°C and 2.54 kcal/mol at 25°C. The most stable polymorph below 74°C was Form I, whereas Form II was the most stable above 74°C.
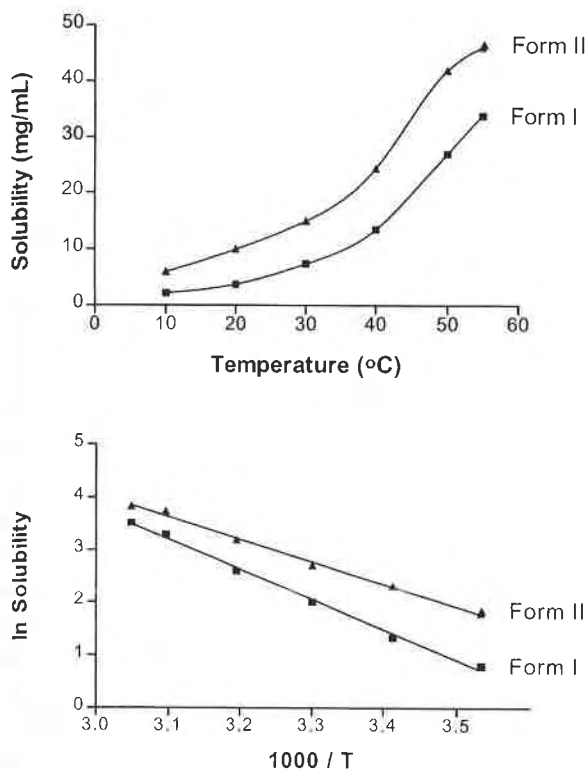
Fig. 1. Temperature dependence of the equilibrium solubilities of two poly-
morphic forms of gepirone hydrochloride. (The plots were adapted from data
originally presented in Ref. 16.)

The effect of solvent composition on the solubility of polymorphs
was investigated with cimetidine [17]. Both forms exhibited almost
identical melting points, but Form B was found to be less soluble than
Form A, identifying it as the most stable polymorph at room tempera-
ture. The two forms were more soluble in mixed water–isopropanol
solvents than in either of the pure solvents, reflecting the balance be-
tween the solvation of the molecules by water and isopropanol in de-
termining the activity coefficient of the solute and hence the solubility.
At constant temperature, the difference in the Gibbs free energy and
the solubility ratio were constant, independent of the solvent system.
The equilibrium solubilities of two polymorphs of an experimen-

tal antiviral compound were used to verify the results of solubility ratio predictions made on the basis of melting point and heat of fusion data [18]. Even though the solubilities of Forms I and III were almost equal in three different solvent systems, the theoretically calculated solubility ratio agreed excellently with the experimentally derived ratios in all of the solvent systems studied. The highest melting form (Form I) was found to be more soluble at room temperature, indicating that an enantiotropic relationship existed between Forms I and III.

It is well established that the temperature range of thermodynamic stability (and certain other quantities) can be determined from measurements of the equilibrium solubilities of the individual polymorphs [19]. In one such study, the two polymorphic forms of 2-[[4-[[2-(1H-tetrazol-5-ylmethyl)phenyl]methoxy]phenoxy]methyl]quinoline were found to exhibit an enantiotropic relationship, because their G vs. T curves intersected with Form I melting at a lower temperature than did Form II [20]. Form I was determined to be the more thermodynamically stable form at room temperature, although the solubility of the two forms was fairly similar. The temperature dependence of the solubility ratio of the two polymorphs afforded the enthalpy of transition (Form II to Form I) as $+0.9$ kcal/mol, while the free energy change of this transition was -0.15 kcal/mol.

When the hydrates or solvates of a given compound are stable with respect to phase conversion in a solvent, the equilibrium solubility of these species can be used to characterize these systems. For instance, the equilibrium solubility of the trihydrate phase of ampicillin at 50°C is approximately 1.3 times that of the more stable anhydrate phase at room temperature [21]. However, below the transition temperature of 42°C, the anhydrate phase is more soluble and is therefore less stable. These relationships are illustrated in Fig. 2.

Amiloride hydrochloride can be obtained in two polymorphic dihydrate forms, A and B [22]. However, each solvate dehydrates around 115–120°C, and the resulting anhydrous solids melt at the same temperature. However, form B was found to be slightly less soluble than form A between 5 and 45°C, indicating that it is the thermodynamically stable form at room temperature. The temperature dependencies of the solubility data were processed by the van't Hoff equation to yield the apparent enthalpies of solution of the two polymorphic dihydrates.

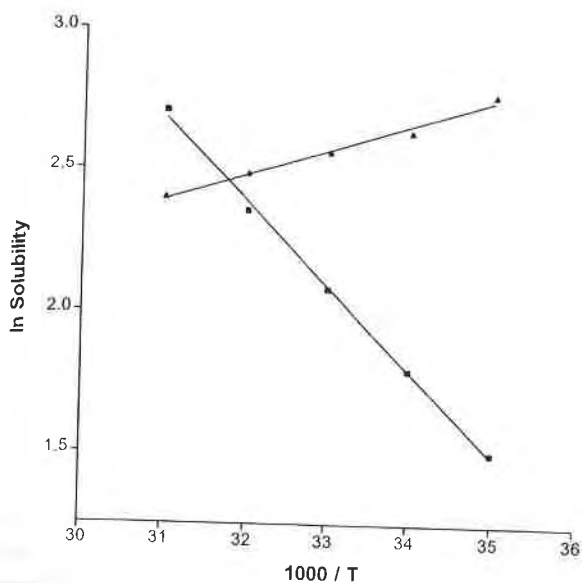The solubility of polymorphic solids derived from the anhydrate

Fig. 2. The van't Hoff plot for the anhydrate (-▲-) and trihydrate (-■-) phases of ampicillin in water. (The relations were adapted from data originally presented in Ref. 21.)

and monohydrate phases of tranilast crystals were evaluated, as were materials processed from them to enhance in vitro availability and micromeritic properties [23]. Agglomerates of monohydrate phases I, II, or III were produced using different crystallization solvents and procedures. Monohydrate Form I transformed directly to the stable α-form upon dehydration, while Forms II and III dehydrated to the amorphous and β phases, respectively. The apparent equilibrium solubilities of monohydrate Form II and the amorphous form were much higher than those of the α and β forms due to their high surface energies. The solubilities of tranilast hydrate phases exceeded those of the anhydrate phases, which runs counter to the commonly observed trend and suggests that the anhydrate/hydrate transition temperatures are below the temperature of measurement. An analogous situation applies to the anhydrate and trihydrate phases of ampicillin [21] discussed above. The trihydrate phase is more soluble than the anhydrate phase at 50°C, because the transition temperature (42°C) is lower.

C.   Metastable Solubility

Because only one member of a family of polymorphs or solvates can be the most thermodynamically stable form under a given set of environmental conditions defined by the phase rule, one frequently finds that one form spontaneously converts to another form during the time required to establish an equilibrium solubility. The existence of an unexpected metastable solubility can lead to important (and possible undesirable) consequences. For digoxin, unexpectedly high solubility values and abnormally high dissolution rates have resulted in the overdosing of patients before the phenomenon of its solid-state phase conversion was properly understood and controlled [24]. This phenomenon was caused by higher-energy crystals resulting from a greater density of crystal defects rather than by polymorphism.

Any metastable phase will have a higher free energy than would a thermodynamically more stable phase, and it will undergo a phase transformation to the more stable phase once the activation energy barrier is overcome. Often the barrier to phase transformation is merely the improbability of a suitable nucleation step. Hence, only fortuitously unfavorable kinetics permitted a determination of the equilibrium solubility of the various higher-energy phases discussed in the preceding section.

Conversions of a metastable phase into a more stable phase may include the transformation of one polymorphic phase into another, the solvation of an anhydrous phase, the desolvation of a solvate phase, the transformation of an amorphous phase into a crystalline anhydrate or solvate phase, the degradation of a crystalline anhydrate or solvate phase to an amorphous phase, or in the case of digoxin, the conversion of imperfect (less crystalline, more amorphous) crystals with a high density of defects into more perfect (more crystalline) crystals with a lower density of defects. While it is straightforward to determine the equilibrium solubility of a phase that is stable with respect to conversion, the measurement of solubilities of metastable phases that are susceptible to conversion is not a trivial matter.

Because determinations of the solubility of solid materials are often made by suspending an excess of the compound in question in the chosen solvent or other dissolution medium, the application of this

equilibrium method to a metastable phase will result in a determination of the solubility of the stable phase. One of the attempts to measure the solubility of a metastable polymorph was made by Milosovich, who developed a method based on the measurement of the intrinsic dissolution rates (IDR) and used it to deduce the relative solubilities of sulfathiazole Forms I and II [25]. This method assumes that the IDR is proportional to the solubility, the proportionality constant being the transport rate constant, which is constant under constant hydrodynamic conditions in a transport-controlled dissolution process.

Ghosh and Grant have developed an extrapolation technique to determine the solubility of a crystalline solid that undergoes a phase change upon contact with a solvent medium [26]. They proposed a thermodynamic cycle analogous to Hess's law, but based on free energies, and used this cycle to predict the theoretical solubility of solvates in water. In the model systems to which the technique was applied, good agreement was obtained between the solubility values measured by equilibration (and derived from an extrapolation method in a mixed solvent system) and those derived from the extrapolation method and calculated by means of the thermodynamic cycle.

A light scattering method has recently been described for the determination of the solubility of drugs, and its application to solubility evaluations of metastable phases has also been demonstrated [27]. Using this technique to deduce solubility data for theophylline anhydrate (metastable with respect to the monohydrate phase in bulk water at room temperature), agreement with the most reliable literature data was excellent. The light scattering method appears to be most useful in the determination of solubility data for metastable crystal phases in dissolution media in which they spontaneously and rapidly convert into a more stable crystal phase.

D. Studies of the Solubility of Metastable Polymorphic and Solvate Phases

Aqueous suspensions of tolbutamide were reported to thicken to an unpourable state after several weeks of occasional shaking, while samples of the same suspensions that were not shaken showed excellent stability after years of storage at ambient and elevated temperature [28].

Examination by microscopy revealed that the thickening was due to partial conversion of the original platelike tolbutamide crystals to very fine needle-shaped crystals. The new crystals were identified as a different polymorphic form and did not correspond either to a solvate species or to crystals of a different habit. The crystalline conversion was observed to take place in a variety of solvents, the rate of conversion being faster in solvents where the drug exhibited appreciable solubility. Because the conversion rate in 1-octanol was relatively slow, use of this solvent permitted an accurate solubility ratio of 1.22 to be obtained (Form I being more soluble than Form III).

The polymorphism and phase interconversion of sulfamethoxydiazine (sulfameter) have been studied in detail [29]. This compound can be obtained in three distinct crystalline polymorphs, with the metastable Form II being suggested for use in solid dosage forms on the basis of its greater solubility and bioavailability [30]. However, the formulation of Form II in aqueous suspensions was judged inappropriate because of the fairly rapid rate of transformation to Form III. This behavior is illustrated in Fig. 3, which shows that seeding of a Form II suspension with Form III crystals greatly accelerates the phase conversion. It was subsequently learned that phase conversion could be retarded by prior addition of various formulation additives, possibly permitting the development of a suspension containing the metastable Form II [31]. Although there are many examples of the conversion of a metastable polymorph to a stable polymorph during the dissolution process, some of them seminal [32], the use of tailor-made additives to inhibit the crystallization of a more stable polymorph is relatively recent [33].

Carbamazepine is known to exist in both an anhydrate and a dihydrate form, with the anhydrate spontaneously transforming to the dihydrate upon contact with bulk liquid water [34]. The anhydrous phase is reported to be practically insoluble in water, but this observation is difficult to confirm owing to its rapid transition to the dihydrate phase. The rates associated with the phase transformation process have been studied and appear to follow first-order kinetics [35]. Interestingly, the only difference in pharmacokinetics between the two forms was a slightly higher absorption rate for the dihydrate [36]. The slower absorption of anhydrous carbamazepine was attributed to the rapid trans-
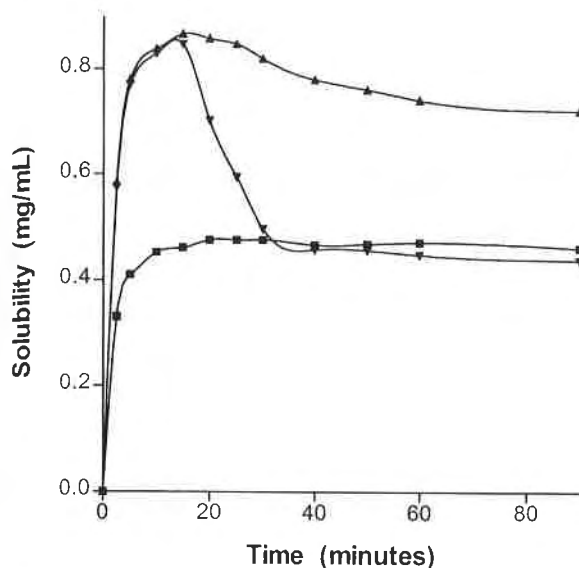
Fig. 3. Effect on the solubility of sulfamethoxydiazine Form II by seeding with crystals of Form III. Shown are the dissolution profiles of Form II (-▲-), Form III (-■-), and Form II seeded with Form III after 20 minutes elapsed time (-▼-). (The plots were adapted from data originally presented in Ref. 31.)

formation to the dihydrate, accompanied by a fast growth in particle size. Comparison of the bioavailabilities of different polymorphs of a given drug suggest that significant differences are found only when the polymorphs differ significantly in Gibbs free energy deduced from the ratio of solubilities or intrinsic dissolution rates, as in the case of chloramphenicol palmitate.

A monohydrate phase of metronidazole benzoate exhibited solubility properties different from those of the commercially available anhydrous form [37]. The monohydrate was found to be the thermodynamically stable form in water below 38°C. The enthalpy and entropy changes of transition for the conversion of the anhydrate to the monohydrate were determined to be -1200 cal/mol and -3.7 cal/K · mol, respectively. This transition was accompanied by a drastic increase in particle size and caused physical instability of oral suspension formula-

tions. These findings were taken to imply that any difference in bio-availability between the two forms could be attributed to changes in particle size distribution and not to an inherent difference in the in vivo activity at body temperature.

Recognizing that the hydration state of a hydrate depends on the water activity in the crystallization medium, Zhu and Grant investigated the influence of solution media on the physical stability of the anhydrate, trihydrate, and amorphous forms of ampicillin [38]. The crystalline anhydrate was found to be kinetically stable in the sense that no change was detected by powder x-ray diffraction for at least 5 days in methanol+water solutions over the whole range of water activity ($a_w = 0$ for pure methanol to $a_w = 1$ for pure liquid water). However, addition of trihydrate seeds to ampicillin anhydrate suspended in methanol+water solutions at $a_w \geq 0.381$ resulted in the conversion of the anhydrate to the thermodynamically stable trihydrate. The trihydrate converted to the amorphous form at $a_w \leq 0.338$ in the absence of anhydrate seeds, but it converted to the anhydrate phase at $a_w \leq 0.338$ when the suspension was seeded with the anhydrate. These trends are illustrated in Fig. 4. The metastable amorphous form took up water progressively with increasing a_w from 0 to 0.338 in the methanol+water mixtures. The most significant finding of this work was that water activity was the major thermodynamic factor determining the nature of the solid phase of ampicillin that crystallized from methanol+water mixtures.

Perhaps the most studied example of phase conversion in the presence of water concerns the anhydrate-to-monohydrate transition of theophylline. It had been noted in a very early work that the anhydrous phase would convert to the monohydrate phase within seconds of exposure of the former to bulk water [39]. The conversion to the monohydrate phase was also demonstrated to take place during wet granulation [40] and could even occur in processed tablets stored under elevated humidity conditions [41]. The difficulty in determining the equilibrium solubility of theophylline anhydrate is evident in the literature, which reports a wide range of values [42–44]. Better success has been obtained in mixed solvent systems, as in the work of Zhu and Grant [45], analogous to the experiments with ampicillin [39]. However, the data obtained in water-rich solutions was distorted by the formation of the monohydrate phase [46,47].
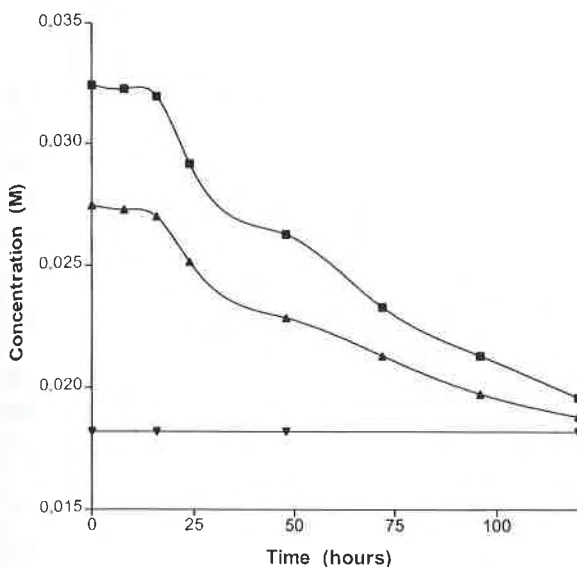
Fig. 4. Conversion of ampicillin anhydrate to the trihydrate phase at various water activities after seeding with the trihydrate. Shown are the concentration-time data at $a_w = 1.0$ (-■-), $a_w = 0.862$ (-▲-), and $a_w = 0.338$ (-▼-). (The curves were adapted from data originally presented in Ref. 38.)

III. SOLUTION CALORIMETRY

The practice of thermochemistry involves measurement of the heat absorbed or evolved during a chemical reaction or physical process. Such a measurement determines the amount of heat q according to the first law of thermodynamics:

$$\Delta E = q + w \tag{5}$$

where w is the work done on the system (negative for work done by the system), and ΔE is the corresponding change in internal energy of the system. Under conditions of constant volume, $\Delta V = 0$, so no mechanical work is done, $w = 0$, and $q = \Delta E$ is the heat of the reaction (or process) at constant volume. Calorimeters, such as bomb calorimeters, that operate under constant volume conditions are not commonly used for studies of polymorphs or solvates. The usual practice for solu-

tion calorimetry is to conduct the studies at constant pressure, p, so that the only work done on or by the system is that due to the change in volume, ΔV. The heat of the reaction (or process) at constant pressure is the enthalpy change, ΔH, which is positive for heat absorbed (an endothermic change) and negative for heat evolved (an exothermic change). The following equation shows the relationship between the heat of reaction at constant pressure, ΔH, and the heat of reaction at constant volume, ΔE.

$$\Delta H = \Delta E + p \cdot \Delta V \tag{6}$$

The principal requirement for calorimetry is that the measured heat change must be assignable to a definite process, such as the dissolution of a solute in a solvent medium.

A.   Enthalpies of Solution

When a solute is dissolved in a solvent to form a solution, there is almost always absorption or evolution of heat. According to the principle of Le Chatelier, substances that absorb heat as they dissolve must show an increase in solubility with an increase in temperature. Those which evolve heat upon dissolution must become less soluble at higher temperatures.

The heat change per mole of solute dissolved varies with the concentration c of the solution that is formed. It is useful to plot the total enthalpy change ΔH at constant temperature against the final molar concentration. This type of curve increases rapidly at low solute concentrations but levels off at the point when the solution is saturated at the temperature of the experiment. The magnitude of the enthalpy change at a given concentration of solute divided by the corresponding number of moles of that solute dissolved represents the increase in enthalpy per mole of solute when it dissolves to form a solution of a particular concentration. This quantity is called the *molar integral heat of solution* at the given concentration. The integral heat of solution is approximately constant in dilute solution but decreases as the final dissolved solute concentration increases.

For hydrated salts and salts that do not form stable hydrates, the integral heat of solution is ordinarily positive, meaning that heat is ab-

sorbed when these substances dissolve. When the anhydrous form of a salt capable of existing in a hydrated form dissolves, there is usually a liberation of heat energy. This difference in behavior between hydrated and anhydrous forms of a given salt is attributed to the usual negative change in enthalpy (evolution of heat) associated with the hydration reaction.

Because the heat of solution of a solute varies with its final concentration, there must be a change of enthalpy when a solution is diluted by the addition of solvent. The *molar integral heat of dilution* is the change in enthalpy resulting when a solution containing one mole of a solute is diluted from one concentration to another. According to Hess's law, this change in enthalpy is equal to the difference between the integral heats of solution at the two concentrations.

The increase of enthalpy that takes place when one mole of solute is dissolved in a sufficiently large volume of solution (which has a particular composition), such that there is no appreciable change in the concentration, is the *molar differential heat of solution*. When stating a value for this quantity, the specified concentration and temperature must also be quoted. Because the differential heat of solution is almost constant in very dilute solutions, the molar differential and integral heats of solution are equal at infinite dilution. At higher concentrations, the differential heat of solution generally decreases as the concentration increases.

The *molar differential heat of dilution* can be defined as the heat change when one mole of solvent is added to a large volume of the solution at the specified concentration. The difference between the integral heats of solution at two different concentrations corresponds to the heat of dilution between these two concentrations. The heat of dilution at a specified concentration is normally obtained by plotting the molar heat of solution at various concentrations against the number of moles of solvent associated with a definite quantity of solute and finding the slope of the curve at the point corresponding to that particular concentration. Because of the approximate constancy of the molar integral heat of solution at small concentrations, such a curve flattens out at high dilutions, and the differential heat of dilution then approaches zero.

The molar differential heats of solution and dilution are examples of partial molar quantities, which are of such importance that they must

be used whenever systems of variable composition, such as solutions, are involved.

B. Principles Underlying Partial Molar Quantities

A solution is deduced to be ideal if the chemical potential μ_i of every component is a linear function of the logarithm of its mole fraction X_i according to the relation

$$\mu_i = \mu_i^* + RT \ln X_i \tag{7}$$

where μ_i^* is the (hypothetical or actual) value of μ_i when X_i equals unity and is a function of temperature and pressure. A solution is termed ideal only if Eq. (7) applies to every component in a given range of composition (usually corresponding to dilute solutions), but it is not necessary that the relation apply to the whole range of composition. Any solution that is approximately ideal over the entire composition range is termed a perfect solution, although relatively few such solutions are known. However, because a given solution may approach ideality over a limited composition range, it is worthwhile to develop the equations further.

When substance i is present both as a pure solid and as a component of an ideal solution, the condition of equilibrium may be stated as

$$\mu_i^s = \mu_i^* + RT \ln X_i \tag{8}$$

where μ_i^s is the chemical potential of the pure solid and X_i is the mole fraction in the solution. Rearranging, one finds

$$\ln X_i = \frac{\mu_i^s}{RT} - \frac{\mu_i^*}{RT} \tag{9}$$

According to the phase rule, this two-component, two-phase system is characterized by two degrees of freedom. One concludes that both the temperature and the pressure of the solution can be varied independently. Since the pressure on the system is normally held fixed as that of the atmosphere during solubility studies, differentiation of Eq. (9) yields

$$\left(\frac{\delta \ln X_i}{\delta T}\right)_p = \frac{H_i - H_i^s}{RT^2} \tag{10}$$

H_i is the partial molar enthalpy of the component in the ideal solution, and H_i^s is its enthalpy per mole as the pure solid. The equation can therefore be rewritten as

$$\left(\frac{\delta \ln X_i}{\delta T}\right)_p = \frac{\Delta H_i}{RT^2} \tag{11}$$

where ΔH_i is the heat absorbed (at constant temperature and pressure) when one mole of the component dissolves in the ideal solution. As stated above, this quantity is the differential heat of solution and is given by

$$\Delta H_i = H_i - H_i^s \tag{12}$$

Provided that the solution remains ideal up to $X_i = 1$, and because H_i is independent of composition in the region of ideality, H_i is the same as the enthalpy per mole of the pure liquid component. ΔH_i is equal to its molar heat of fusion, which was formerly termed the molar latent heat of fusion. It is noted, however, that these quantities refer to the temperature at which the solution having mole fraction X_i is in equilibrium with the pure solid.

If one now assumes that ΔH_i is independent of temperature over a narrow temperature range, then Eq. (11) can be integrated at constant pressure to yield

$$\ln \frac{X_1}{X_2} = \frac{\Delta H_i}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{13}$$

where X_1 and X_2 refer to the solubilities (expressed as mole fractions) of the solute at temperatures T_1 and T_2, respectively. If Eq. (13) remains approximately valid up to a mole fraction of unity, this situation corresponds to that where pure liquid solute is in equilibrium with its own solid at the melting point. In that case, Eq. (13) yields

$$\ln X = \frac{\Delta H_i}{R} \left(\frac{1}{T_m} - \frac{1}{T} \right) \tag{14}$$

where X is the solubility at temperature T, and T_m is the melting point of the solute. ΔH_i is the heat of solution but, by the nature of the assumptions that have been made, it is also equal to the latent heat of fusion (ΔH^f) of the pure solute.

Because the number of energy levels available to take up thermal energy is greater in the liquid state than in the solid state, the heat capacity of a liquid frequently exceeds that of the same substance in the solid state. As a result, ΔH^f must be assumed to be a function of temperature. If one assumes the change in heat capacity to be constant over the temperature range of interest, then one can use the relation

$$\Delta H_i = \Delta H_R + \Delta C_p (T_i - T_R) \tag{15}$$

where ΔH_R is the heat of fusion at some reference temperature T_R and ΔC_p is the difference in heat capacity between the liquid and the solid state. This situation has been treated by Grant and coworkers [10], who have provided the highly useful Eq. (4) for the treatment of solubility data over a wide range of temperature values. Equation (4) may be derived by substituting the expression for ΔH_i of Eq. (15) into the differential form of the van't Hoff Eq. (11) and integrating. In Eq. (4), a is equal to ΔH_R when T_R equals 0 K, while b is equal to ΔC_p.

The determination of solubility data over a defined temperature range can therefore be used to calculate the differential heat of solution of a given material. For instance, the data illustrated in the bottom half of Fig. 1 indicate that Eq. (14) can be used to deduce a value for the molar differential heat of solution of gepirone. In addition, the fact that forms I and II yield lines of different slopes indicates the existence of unique values of the molar differential heat of solution for the two polymorphs. One can subtract the differential heats of solution obtained for the two polymorphs to deduce the heat of transition ΔH_T between the two forms:

$$\Delta H_t = \Delta H_s^B - \Delta H_s^A \tag{16}$$

where ΔH_s^A and ΔH_s^B denote the differential heats of solution for polymorphs A and B, respectively. For gepirone, A $\equiv$ I, B $\equiv$ II.

The validity of the assumption regarding constancy in the heats of solution for a given substance with respect to temperature can be made by determining the enthalpy of fusion ΔH_f for the two forms and then taking the difference between these:

$$\Delta H_t' = \Delta H_f^B - \Delta H_f^A \tag{17}$$

where $\Delta H_t'$ represents the heat of transition between forms A and B at the melting point. The extent of agreement between ΔH_t and $\Delta H_t'$ can be used to estimate the validity of the assumptions made.

For example, the heats of fusion and solution have been reported for the polymorphs of auranofin [48], and these are summarized in Table 3. The similarity of the heats of transition deduced in 95% ethanol (2.90 kcal/mol) and dimethylformamide (2.85 kcal/mol) with the heat of transition calculated at the melting point (3.20 kcal/mol) indicates that the difference in heat capacity between the two polymorphs is relatively small.

Because of the temperature dependence of the various phenomena under discussion, and because of the important role played by entropy, discussions based purely on enthalpy changes are necessarily incomplete. One can rearrange Eq. (7) to read

Table 3 Heats of Solution and Fusion for the Polymorphs of Auranofin

	Heat of solution, 95% ethanol (kcal/mol)	Heat of solution, dimethylformamide (kcal/mol)
Form A	12.42	5.57
Form B	9.52	2.72
Difference	2.90	2.85

	Heat of fusion (kcal/mol)
Form A	9.04
Form B	5.85
Difference	3.20

Source: Ref. 48.

$$\mu_i^* - \mu_i^s = -RT \ln X_i \tag{18}$$

where the left hand side of the equation represents the difference in chemical potential between the chemical potential of i in its pure solid state and the chemical potential of this species in the solution at a defined temperature and pressure. This difference in chemical potential is by definition the molar Gibbs free energy change associated with the dissolution of compound i, so one can write

$$\Delta G_s = -RT \ln X_i \tag{19}$$

where ΔG_s is the molar Gibbs free energy of solution. By analogy with Eq. (16), the molar Gibbs free energy associated with the transformation of polymorph A to B is given by

$$\Delta G_t = \Delta G_s^B - \Delta G_s^A \tag{20}$$

$$= RT \ln \frac{X_A}{X_B} \tag{21}$$

where X_A and X_B are the equilibrium solubilities of polymorphs A and B, respectively, expressed in units of mole fraction.

Finally, the entropy of solution ΔS_s is obtained from the relation

$$\Delta S_s = \frac{\Delta H_s - \Delta G_s}{T} \tag{22}$$

For basic thermodynamic understanding of the solubility behavior of a given substance, ΔG_s, ΔH_s, and ΔS_s must be determined. Similarly, a basic thermodynamic understanding of a polymorphic transition requires an evaluation of the quantities ΔG_t, ΔH_t, and ΔS_t associated with the phase transition.

To illustrate the importance of free energy changes, consider the solvate system formed by paroxetine hydrochloride, which can exist as a nonhygroscopic hemihydrate or as a hygroscopic anhydrate [49]. The heat of transition between these two forms was evaluated both by differential scanning calorimetry ($\Delta H_t' = 0.0$ kJ/mol) and by solution calorimetry ($\Delta H_t = 0.1$ kJ/mol), which indicates that these two forms are almost isoenthalpic. However, the free energy of transition (-1.25 kJ/mol) favors conversion of the anhydrate to the hemihydrate, and

such phase conversion can be initiated by crystal compression or by seeding techniques. Since the two forms are essentially isoenthalpic, the entropy increase that accompanies the phase transformation is responsible for the decrease in free energy and can therefore be viewed as the driving force for the transition.

C. Methodology for Solution Calorimetry

Any calorimeter with a suitable mixing device and designed for use with liquids can be applied to determine heats of solution, dilution, or mixing. To obtain good precision in the determination of heats of solution requires careful attention to detail in the construction of the calorimeter. The dissolution of a solid can sometimes be a relatively slow process and requires efficient and uniform stirring. Substantial experimental precautions are ordinarily made to ensure that heat input from the stirrer mechanism is minimized.

Most solution calorimeters operate in the batch mode, and descriptions of such systems are readily found in the literature [50,51]. The common practice is to use the batch solution calorimetric approach, in which mixing of the solute and the solvent is effected in a single step. Mixing can be accomplished by breaking a bulb containing the pure solute, by allowing the reactants to mix by displacing the seal separating the two reactants in the calorimeter reaction vessel, or by rotating the reaction vessel and allowing the reactants to mix [51]. Although the batch calorimetric approach simplifies the data analysis, there are design problems associated with mixing of the reactants. Guillory and coworkers have described the use of a stainless steel ampoule whose design greatly facilitates batch solution calorimetric analyses [52]. This device was validated by measurement of the enthalpy of solution of potassium chloride in water, and the reproducibility of the method was demonstrated by determination of the enthalpy of solution of the two common polymorphic forms of chloramphenicol palmitate in 95% ethanol.

D. Applications of Solution Calorimetry

Solution calorimetric investigations can be classified into studies that focus entirely on enthalpic processes and studies that seek to under-

stand the contribution of the enthalpy change to the free energy change of the system. Although the former can prove to be quite informative, only the latter permit the deduction of unequivocal thermodynamic conclusions about relative stability.

While heats of solution data are frequently used to establish differences in enthalpy within a polymorphic system, they cannot be used to deduce accurately the relative phase stability. According to Eq. (16), the difference between the differential heats of solution of two polymorphs is a measure of the heat of transition ΔH_t between the two forms. Because enthalpy is a state function (Hess's law), this difference must necessarily be independent of the solvent system used. However, conducting calorimetric measurements of the heats of solution of the polymorphs in more than one solvent provides an empirical verification of the assumptions made. For instance, ΔH_t values of two losartan polymorphs were found to be 1.72 kcal/mol in water and 1.76 kcal/mol in dimethylformamide [53]. In a similar study with moricizine hydrochloride polymorphs, ΔH_t values of 1.0 kcal/mol and 0.9 kcal/mol were obtained from their dissolution in water and dimethylformamide, respectively [54]. These two systems, which show good agreement, can be contrasted with that of enalapril maleate, where ΔH_t was determined to be 0.51 kcal/mol in methanol and 0.69 kcal/mol in acetone [55]. Disagreements of this order (about 30%) suggest that some process, in addition to dissolution, is taking place in one or both solvents.

In systems characterized by the existence of more than one polymorph, the heats of solution have been used to deduce the order of stability. As explained above, the order of stability cannot be deduced from enthalpy changes but only from free energy changes. If the enthalpy change reflects the stability, then the polymorphic change is not driven by an increase in entropy but by a decrease in enthalpy. The heat of solution measured for cyclopenthiazide Form III (3.58 kcal/mol) was significantly greater than the analogous values obtained for Form I (1.41 kcal/mol) or Form II (1.47 kcal/mol); thus Form III is the polymorph with the lower enthalpy but not necessarily the most stable polymorph at ambient temperature [56]. Other examples follow.

In the case of the anhydrate and hydrate phases of norfloxacin [57], the dihydrate phase was found to exhibit a relatively large endothermic heat of solution relative to either the anhydrate or the sesquihy-

drate. Both urapidil [58] and dehydroepiandrosterone [59] were found to exhibit complex polymorphic/solvate systems, but the relative enthalpy of these could be deduced through the use of solution calorimetry. As an example, the data reported for urapidil [58], which have been collected into Table 4, show that the form with the lowest heat of solution consequently has the highest enthalpy. In this particular case, the rank order of enthalpy changes corresponds to that of the free energy changes.

It is invariably found that the amorphous form of a compound is less stable than its crystalline modification, in the sense that the amorphous form tends to crystallize spontaneously, indicating that the amorphous form has the greater Gibbs free energy. As discussed in Chapter 1, the amorphous form is more disordered and must therefore have a greater entropy than does the crystalline form. Hence the enthalpy of the amorphous form is also greater. The heat of solution of amorphous piretanide in water was found to be 12.7 kJ/mol, while the heat of solution associated with Form C was determined to be 32.8 kJ/mol [60]. The authors calculated the heat of transformation associated with the amorphous-to-crystalline transition to be −20.1 kJ/mol. Any facile transformation of the two phases was obstructed by the significant activation energy (145.5 kJ/mol).

As emphasized above, a basic thermodynamic understanding of

Table 4 Heats of Solution in Water for the Various Polymorphs and Solvates of Urapidil

Crystalline form	Heat of solution (kJ/mol)
Form I	21.96
Form II	24.26
Form III	22.98 (estimated)
Monohydrate	44.28
Trihydrate	53.50
Pentahydrate	69.16
Methanol solvate	48.39

Source: Ref. 58.

a polymorphic system requires a determination of the free energy difference between the various forms. The two polymorphs of 3-amino-1-(*m*-trifluoromethlyphenyl)-6-methyl-1H-pyridazin-4-one have been characterized by a variety of methods, among which solubility studies were used to evaluate the thermodynamics of the transition from Form I to Form II [61]. At a temperature of 30°C, the enthalpy change for the phase transformation was determined to be -5.64 kJ/mol. From the solubility ration of the two polymorphs, the free energy change was then calculated as -3.67 kJ/mol, which implies that the entropy change accompanying the transformation was -6.48 cal/K·mol. In this system, one encounters a phase change that is favored by the enthalpy term but not favored by the entropy term. However, since the overall free energy change ΔG_T is negative, the process takes place spontaneously, provided that the molecules can overcome the activation energy barrier at a significant rate.

A similar situation has been described for the two polymorphic forms of 2-[[4-[[2-(IH-tetrazol-5-ylmethyl)phenyl]methoxy]phenoxy]methyl]quinoline [20]. The appreciable enthalpic driving force for the transformation of Form II to Form I (-0.91 kcal/mol) was found to be partially offset by the entropy of transformation (-2.6 cal/K · mol), resulting in a modest free energy difference between the two forms (-0.14 kcal/mol).

In other instances, an unfavorable enthalpy term was found to be compensated by a favorable entropy term, thus rendering negative the free energy change associated with a particular phase transformation. Lamivudine can be obtained in two forms, of which one is a 0.2-hydrate obtained from water or from methanol that contains water, and the other nonsolvated and obtained from many nonaqueous solvents [62]. Form II was determined to be thermodynamically favored in the solid state. Solubility studies of both forms as a function of solvent and temperature were used to determine whether entropy or enthalpy was the driving force for solubility. Solution calorimetric data indicated that Form I would be favored in all solvents studied on the basis of enthalpy alone (see Table 5). In higher alcohols and other organic solvents, Form I exhibited a larger entropy of solution than did Form II, compensating for the unfavorable enthalpic factors and yielding an overall negative free energy for the phase change.

Table 5 Thermodynamic Quantities of Solution for Lamivudine in Various Solvents

	Solvent = water	
	Form I	Form II
ΔG_{Sol}(cal/mol)	2990	2950
ΔH_{Sol}(cal/mol)	5720	5430
ΔS_{Sol}(cal/K · mol)	9.2	8.3
	Solvent = ethanol	
	Form I	Form II
ΔG_{Sol}(cal/mol)	3180	3460
ΔH_{Sol}(cal/mol)	5270	4740
ΔS_{Sol}(cal/K·mol)	7.0	4.3
	Solvent = n-propanol	
	Form I	Form II
ΔG_{Sol}(cal/mol)	3120	3610
ΔH_{Sol}(cal/mol)	5350	5000
ΔS_{Sol}(cal/K·mol)	7.5	4.7

Source: Ref. 62.

Shefter and Higuchi considered the thermodynamics associated with the anhydrate/hydrate equilibrium of theophylline and glutethimide [40]. For both compounds, the free energy change for the transformation from the anhydrate to the hydrate was negative (hence indicating a spontaneous process), the favorable enthalpy changes being mitigated by the unfavorable entropy changes. In this work, the free energy was calculated from the solubilities of the anhydrate and hydrate forms, while the enthalpy of solution was calculated from the temperature dependence of the solubility ratio using the van't Hoff equation. The entropy of solution was evaluated using Eq. (22).

A similar conclusion was reached regarding the relative stability of the monohydrate and anhydrate phases of metronidazole benzoate [37]. The enthalpy term (-1.20 kcal/mol) favored conversion to the monohydrate, but the strong entropy term (-3.7 cal/K · mol) essen-

tially offset this enthalpy change. At 25°C, the overall ΔG_t of the transition was still negative, favoring the monohydrates, but only barely so (-0.049 kcal/mol). This difference was judged to be too small to result in any detectable bioavailability differences.

IV. KINETICS OF SOLUBILITY: DISSOLUTION RATES

Evaluation of the dissolution rates of drug substances from their dosage forms is extremely important in the development, formulation, and quality control of pharmaceutical agents [9,63–65]. Such evaluation is especially important in the characterization of polymorphic systems owing to the possibility of bioavailability differences that may arise from differences in dissolution rate that may themselves arise from differences in solubility [4]. The wide variety of methods for determining the dissolution rates of solids may be categorized either as batch methods or as continuous flow methods, for which detailed experimental protocols have been provided [66].

A. Factors Affecting Dissolution Rates

The dissolution rate of a solid may be defined as dm/dt, where m is the mass of solid dissolved at time t. To obtain dm/dt, the following equation, which defines concentration, must be differentiated:

$$m = Vc_b \tag{23}$$

In a batch dissolution method the analyzed concentration c_b of a well-stirred solution is representative of the entire volume V of the dissolution medium, so that

$$\frac{dm}{dt} = V \frac{dc_b}{dt} \tag{24}$$

In a dissolution study, c_b will increase from its initial zero value until a limiting concentration is attained. Depending on the initial amount of solute presented for dissolution, the limiting concentration will be at the saturation level, or less than this.

Batch dissolution methods are simple to set up and to operate,

are widely used, and can be carefully and reproducibly standardized. Nevertheless, they suffer from several disadvantages [9]. The hydrodynamics are usually poorly characterized, a small change in dissolution rate will often create an undetectable and immeasurable perturbation in the dissolution time curve, and the solute concentration may not be uniform throughout the solution volume.

In a continuous flow method, the volume flow rate over the surface of the solid is given by dV/dt, so that differentiation of Eq. (23) leads to

$$\frac{dm}{dt} = c_b \frac{dV}{dt} \tag{25}$$

where c_b is the concentration of drug dissolved in the solvent that has just passed over the surface of the solid drug.

Continuous flow methods have the advantages that sink conditions can be easily achieved, and that a change in dissolution rate is reflected in a change in c_b [9]. At the same time, they require a significant flow rate that may require relatively large volumes of dissolution medium. Should the solid be characterized by a low solubility and a slow dissolution rate, c_b will be small, and a very sensitive analytical method would be required.

The diffusion layer theory is the most useful and best known model for transport-controlled dissolution and satisfactorily accounts for the dissolution rates of most pharmaceutical solids. In this model, the dissolution rate is controlled by the rate of diffusion of solute molecules across a thin diffusion layer. With increasing distance from the surface of the solid, the solute concentration decreases in a nonlinear manner across the diffusion layer. The dissolution process at steady state is described by the Noyes–Whitney equation:

$$\frac{dm}{dt} = k_D A (c_s - c_b) \tag{26}$$

where dm/dt is the dissolution rate, A is the surface area of the dissolving solid, c_s is the saturation solubility of the solid, and c_b is the concentration of solute in the bulk solution. The dissolution rate constant k_D

is given by D/h, where D is the diffusivity. The hydrodynamics of the dissolution process have been fully discussed by Levich [67].

It has been shown [9] that the dissolution rates of solids are determined or influenced by a number of factors, which may be summarized as follows:

1. Solubility of the solid, and the temperature
2. Concentration in the bulk solution, if not under sink conditions
3. Volume of the dissolution medium in a batch-type apparatus, or the volume flow rate in a continuous flow apparatus
4. Wetted surface area, which consequently is normalized in measurements of intrinsic dissolution rate
5. Conditions in the dissolution medium that, together with the nature of the dissolving solid, determine the dissolution mechanism

The conditions in the dissolution medium that may influence the dissolution rate can be summarized as

1. The rate of agitation, stirring, or flow of solvent, if the dissolution is transport-controlled, but not when the dissolution is reaction-controlled.
2. The diffusivity of the dissolved solute, if the dissolution is transport-controlled. The dissolution rate of a reaction-controlled system will be independent of the diffusivity.
3. The viscosity and density influence the dissolution rate if the dissolution is transport-controlled, but not if the dissolution is reaction-controlled.
4. The pH and buffer concentration (if the dissolving solid is acidic or basic), and the pK_a values of the dissolving solid and of the buffer.
5. Complexation between the dissolving solute and an interactive ligand, or solubilization of the dissolving solute by a surface-active agent in solution. Each of these phenomena tends to increase the dissolution rate.

B. Applications of Dissolution Rate Studies to Polymorphs and Hydrates

Historically, batch-type dissolution rate studies of loose powders and compressed discs have played a major role in the characterization of essentially every polymorphic or solid-state solvated system [13,21,39]. Stagner and Guillory used these two methods of dissolution to study the two polymorphs and the amorphous phase of iopanoic acid [68]. As is evident in the loose powder dissolution data illustrated in the upper half of Fig. 5, the two polymorphs were found to be stable with respect to phase conversion, but the amorphous form rapidly converted to Form I under the dissolution conditions. In the powder dissolution studies, the initial solubilities of the different forms followed the same rank order as did their respective intrinsic dissolution rates, but the subsequent phase conversion of the amorphous form to the stable Form I appeared to change the order. The amorphous form demonstrated a 10-fold greater intrinsic dissolution rate relative to Form I, while the intrinsic dissolution rate of Form II was 1.5 times greater than that of Form I.

The nature of the dissolution medium can profoundly affect the shape of a dissolution profile. The relative rates of dissolution and the solubilities of the two polymorphs of 3-(3-hydroxy-3-methylbutylamino)-5-methyl-as-triazino[5,6-b]indole were determined in USP artificial gastric fluid, water, and 50% ethanol solution [69]. In the artificial gastric fluid, both polymorphic forms exhibited essentially identical dissolution rates. This behavior has been contrasted in Fig. 6 with that observed in 50% aqueous ethanol, in which Form II has a significantly more rapid dissolution rate than Form I. If the dissolution rate of a solid phase is determined by its solubility, as predicted by the Noyes–Whitney equation, the ratio of dissolution rates would equal the ratio of solubilities. Because this type of behavior was not observed for this triazinoindole drug, the different effects of the dissolution medium on the transport rate constant can be suspected.

The solubilities of the two polymorphs of difenoxin hydrochloride have been studied, as well as the solubility of tablets formed from mixtures of these polymorphs [70]. Form I was found to be more soluble than was Form II, and the solubilities of materials containing known
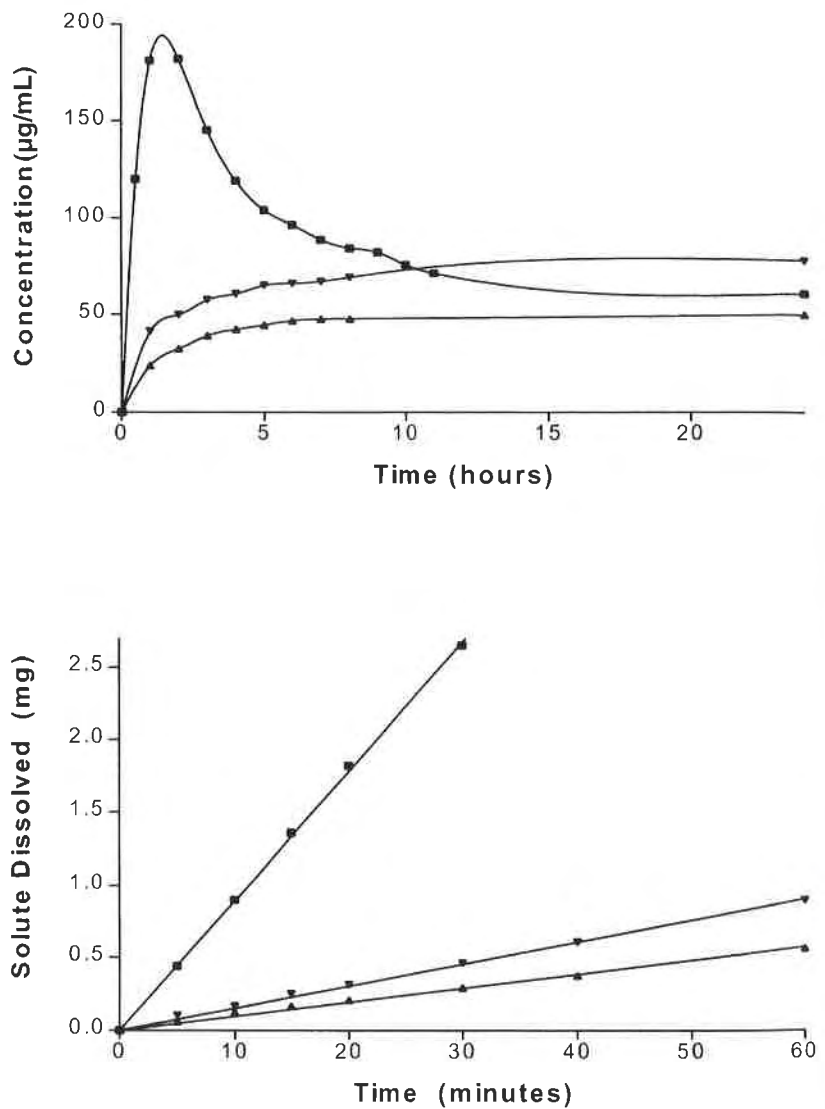
Fig. 5. Loose powder dissolution (upper family of traces) and intrinsic dissolution (lower family of traces) profiles of iopanoic acid. Shown are the profiles of Form I (-▲-), Form II (-▼-), and the amorphous form (-■-). (The plots were adapted from data originally presented in Ref. 68.)
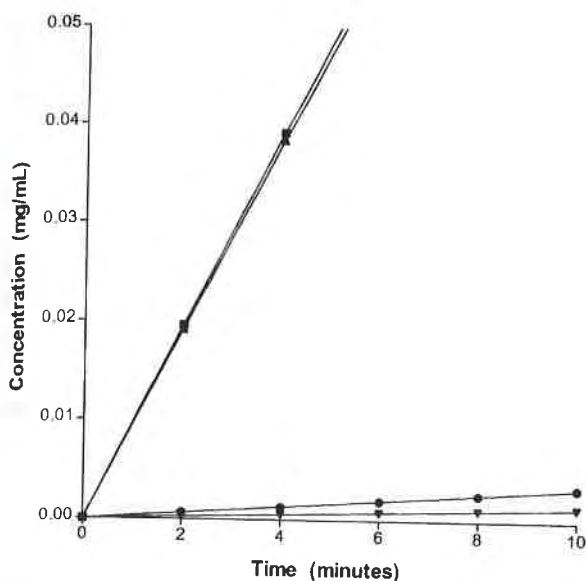
Fig. 6. Initial stages of the dissolution of the two polymorphs of 3-(3-hy-droxy-3-methylbutylamino)-5-methyl-*as*-triazino[5,6-*b*]indole in different media. Shown are the profiles obtained for Form I (-■-) and Form II (-▲-) in simulated gastric fluid, as well as the profiles of Form I (-○-) and Form II (-▼-) in 50% aqueous ethanol. (The traces were adapted from data originally presented in Ref. 69.)

proportions of Form I and II reflected the differences in the solubilities of the pure forms. Likewise, the dissolution rate of difenoxin hydro-chloride from tablets was determined by the ratio of Form I to Form II. In these studies, no solid-state transformation of the more soluble form to the less soluble form was observed. In addition, micronization proved to be a successful method for improving the dissolution of tab-lets prepared from the less soluble polymorph.

Stoltz and coworkers have conducted extensive studies on the dis-solution properties of the hydrates and solvates of oxyphenbutazone [71,72]. They compared the dissolution properties of the benzene and cyclohexane solvates with those of the monohydrate, hemihydrate, and anhydrate forms, and then compared their findings with results reported

in the literature. The powder dissolution rates of the solvates proved to be comparable with those of the hemihydrate and the anhydrate but superior to that of the monohydrate. This trend is illustrated in Fig. 7, which confirms the usual observation that increasing degrees of hydration result in slower dissolution rates. This observation differed from that previously described by Matsuda and Kawaguchi who reported powder dissolution rates in simulated intestinal fluid that were in the sequence: hemihydrate > monohydrate > anhydrate [73]. The reversed order in the dissolution rates of the former work [71] was attributed to the presence of a surfactant in the dissolution medium, which apparently overcame the hydrophobicity of the crystal surfaces of the anhydrate form. In terms of the Noyes–Whitney equation, these results can
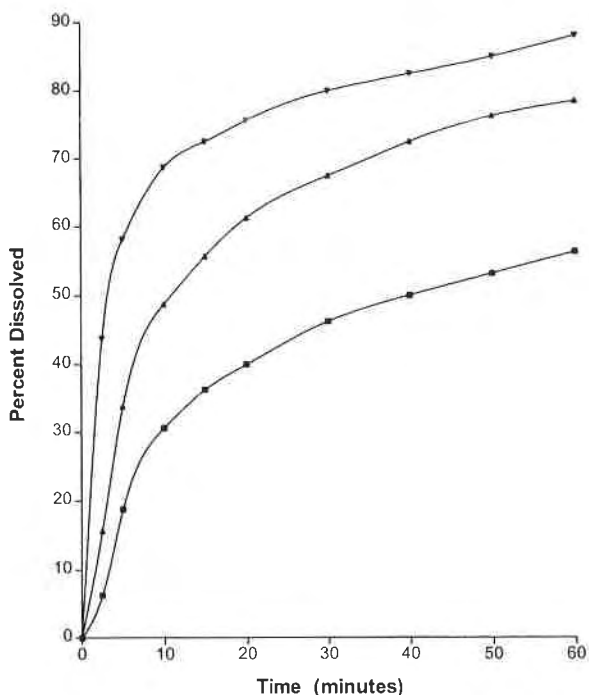

Fig. 7.   Powder dissolution profiles obtained for oxyphenbutazone anhydrate (-▼-), hemihydrate (-▲-), and monohydrate (-■-). (The curves were adapted from data originally presented in Ref. 71.)

be explained by the influence of the surface active agent in increasing either the wetted surface area, or the transport rate constant, or both quantities.

It has been noted from the earliest dissolution work [39] that, for many substances, the dissolution rate of an anhydrous phase usually exceeds that of any corresponding hydrate phase. These observations were explained by thermodynamics, where it was reasoned that the drug in the hydrates possessed a lower activity and would be in a more stable state relative to their anhydrous forms [74]. This general rule was found to hold for the previously discussed anhydrate/hydrate phases of theophylline [42,44,46], ampicillin [38], metronidazone benzoate [37], carbamazepine [34,36], glutethimide [75], and oxyphenbutazone [72], as well as for many other systems not mentioned here. In addition, among the hydrates of urapidil, the solubility decreases with increasing crystal hydration [58].

Since the mid-1970s, a number of exceptions to the general rule have been found. For example, Fig. 8 shows that the hydrate phases of erythromycin exhibit a reverse order of solubility where the dihydrate phase exhibits the fastest dissolution rate and the highest equilib-
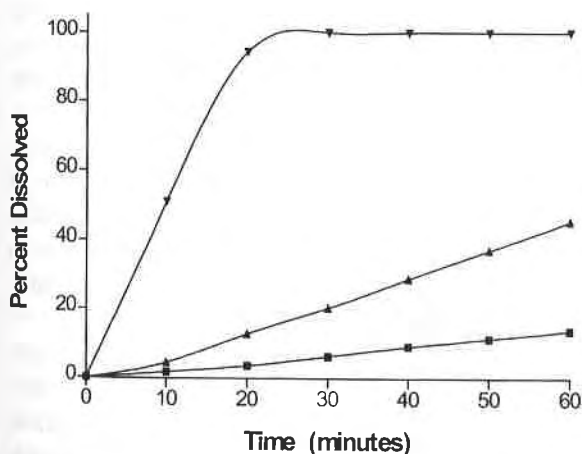

Fig. 8. Dissolution profiles of erythromycin anhydrate (-■-), monohydrate (-▲-), and dihydrate (-▼-). (The plots were adapted from data originally presented in Ref. 76.)

rium solubility [76]. More recent examples include the magnesium, zinc, and calcium salts of nedocromil, for which the intrinsic dissolution rate increases with increasing water stoichiometry of their hydrates [77]. The explanation for this behavior is that the transition temperatures between the hydrates are below the temperature of the dissolution measurements and decrease with increasing water stoichiometry of the hydrates. Consequently, the solubilities, and hence the intrinsic dissolution rates, increase with increasing stoichiometry of water in the hydrates. Acyclovir was recently found to be capable of forming a 3:2 drug/water hydrate phase that exhibited an almost instantaneous dissolution relative to the more slowly dissolving anhydrous form [78]. This latter finding implies a substantial difference in Gibbs free energy between the two forms.

C.   Intrinsic Dissolution Rates: Principles and Practice

It should be recognized that the final concentration measured using the loose powder dissolution method is the equilibrium solubility, and that the initial stages of this dissolution are strongly affected by the particle size and surface area of the dissolving solids. For this reaction, many workers have chosen to study the dissolution of compacted materials, by which the particle size and surface area are regulated by the process of forming the compact.

In the disc method for conducting intrinsic dissolution studies, the powder is compressed in a die to produce a compact. One face of the disc is exposed to the dissolution medium and rotated at a constant speed without wobble. The dissolution rate is determined as for a batch method, while the wetted surface area is simply the area of the disc exposed to the dissolution medium.

It is good practice to compare the powder X-ray diffraction patterns of the compacted solid and of the residual solid after the dissolution experiment with that of the original powder sample. In this manner, one can test for possible phase changes during compaction or dissolution.

The dissolution rate of a solid from a rotating disc is governed by the controlled hydrodynamics of the system and has been treated

theoretically by Levich [67]. In this system, the intrinsic dissolution rate J can be calculated using either of the following relations:

$$J = 0.620\ D^{2/3}\nu^{-1/6}(c_s - c_b)\omega^{1/2} \qquad (27)$$

or

$$J = 1.555\ D^{2/3}\nu^{-1/6}(c_s - c_b)W^{1/2} \qquad (28)$$

where D is the diffusivity of the dissolved solute, ω is the angular velocity of the disc in radians per second (W revolutions per second, Hz), ν is the kinematic viscosity of the fluid, c_b is the concentration of solute at time t during the dissolution study, and c_s is the equilibrium solubility of the solute. The dependence of J on $\omega^{1/2}$ has been verified experimentally [79].

Equations (27) and (28) enable the diffusivity of a solute to be measured. These relations assume the dissolution of only one diffusing species, but since most small organic molecules exhibit a similar diffusivity (of the order 10^{-5} cm^2/s in water at 25°C), it follows that J depends on the 2/3 power of D. Consequently, the errors arising from several diffusing species only become significant if one or more species exhibit abnormal diffusivities. In fact, diffusivity is only weakly dependent on the molecular weight, so it is useful to estimate the diffusivity of a solute from that of a suitable standard of known diffusivity under the same conditions. In most cases, the diffusivity predictions agree quite well with those obtained experimentally [80].

D. Intrinsic Dissolution Rate Studies of Polymorphic and Hydrate Systems

Under constant hydrodynamic conditions, the intrinsic dissolution rate is usually proportional to the solubility of the dissolving solid. Consequently, in a polymorphic system, the most stable form will ordinarily exhibit the slowest intrinsic dissolution rate. For example, a variety of high-energy modifications of frusemide were produced, but the commercially available form was found to exhibit the longest dissolution times [81]. Similar conclusions were reached for the four polymorphs of tegafur [82] and (R)-N-[3-[5-(4-fluorophenoxy)-2-furanyl]-1-methyl-2-propynyl]-N-hydroxyurea [83]. However, it is possible that

one of the less stable polymorphs of a compound can exhibit the slowest dissolution rate, as was noted in the case of diflunisal [84].

Intrinsic dissolution rate studies proved useful during the characterization of the two anhydrous polymorphs and one hydrate modification of alprazolam [85]. The equilibrium solubility of the hydrate phase was invariably less than that of either anhydrate phase, although the actual values obtained were found to be strongly affected by pH. Interestingly, the intrinsic dissolution rate of the hydrate phase was higher than that of either anhydrate phase, with the anhydrous phases exhibiting equivalent dissolution rates. The IDR data of Table 6 reveal an interesting phenomenon, where discrimination between some polymorphs was noted at slower spindle speeds, but not at higher rates. Thus if one is to use IDR values as a means to determine the relative rates of solution of different solids, the effect of stirring speed must be investigated before the conclusions can be judged genuine.

Intrinsic dissolution rate investigations can become complicated when one or more of the studied polymorphs interconverts to another during the time of measurement. Sulfathiazole has been found to crystallize in three distinct polymorphic forms, two of which are unstable in contact with water [86] and convert only slowly to the stable form (i.e., are kinetically stable) in the solid state. As can be seen in Fig. 9, the initial intrinsic dissolution rates of these are all different, but as Forms I and II convert into Form III, the dissolved concentrations converge. Only the dissolution rate of Form III remains constant, which suggests that it is the thermodynamically stable form at room tempera-

Table 6   Intrinsic Dissolution Rates (IDR) for the Various Polymorphs of Aprazolam at Two Different Spindle Speeds

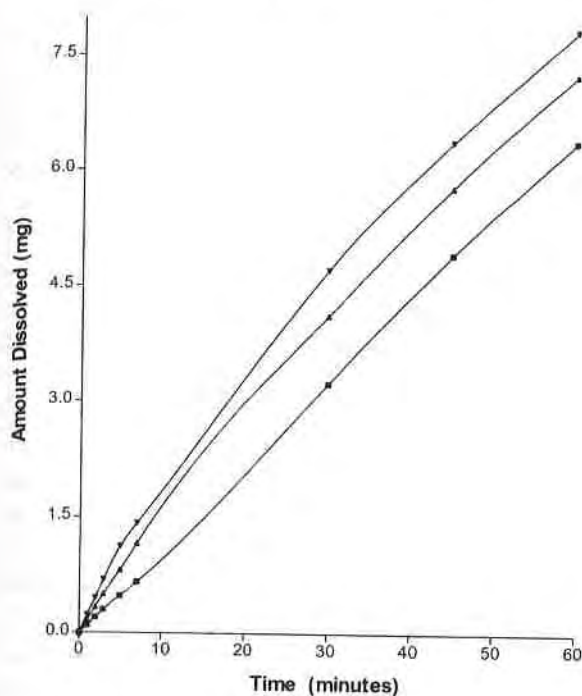| Crystalline form	IDR, 50 RPM (μ/min $\cdot$ cm^2)	IDR, 75 RPM (μ/min $\cdot$ cm^2)
Form I	15.8	21.8
Form II	18.4	21.9
Form V	20.7	27.3

Source: Ref. 85.

Fig. 9. Dissolution profiles obtained for sulfathiazole Form I (-▲-), Form II (-▼-), and Form III (-■-) in water at 37°C. (The figure has been adapted from data originally presented in Ref. 86.)

ture. Aqueous suspensions of Forms I or II each converted into Form III over time, supporting the conclusions of the dissolution studies.

Suitable manipulation of the dissolution medium can sometimes inhibit the conversion of one polymorph to another during the dissolution process, thus permitting the measurement of otherwise unobtainable information. In studies on the polymorphs of sulfathiazole and methylprednisolone, Higuchi, who used various alcohols and additives in the dissolution medium to inhibit phase transformations, first employed this approach [87]. Aguiar and Zelmer were able to characterize thermodynamically the polymorphs formed by chloramphenicol palmitate and mefenamic acid by means of dissolution modifiers [88]. Furthermore, the use of an aqueous ethanol medium containing 55.4%

v/v ethanol yielded adequate solubility and integrity of the dissolving disc during studies conducted on digoxin [89].

One area of concern associated with intrinsic dissolution measurements is associated with the preparation of the solid disc by compaction of the drug particles. If a phase transformation is induced by compression, one might unintentionally measure the dissolution rate of a polymorph different from the intended one. This situation was encountered with phenylbutazone, where Form III was transformed to the most stable modification (Form IV) during the initial compression step [90].

One interesting note concerns the aqueous dissolution rates of solvate forms, where the solvent bound in the crystal lattice is not water. As noted earlier, the dissolution rate of an anhydrous phase normally exceeds that of any corresponding hydrate phase, but this relation is not usually applicable to other solvate species. It has been reported that the methanol solvate of urapidil exhibits a heat of solution approximately twice that of any of the anhydrate phases and that it also exhibits the most rapid dissolution rate [91]. Similarly, the pentanol and toluene solvates of glibenclamide (glyburide) exhibit significantly higher aqueous dissolution rates and aqueous equilibrium solubility values when compared to either of the two anhydrous polymorphs [92]. The acetone and chloroform solvates of sulindac yielded intrinsic dissolution rates that were double those of the two anhydrate phases [93]. These trends would imply that a nonaqueous solvate phase could be considered as being a high-energy form of the solid with respect to dissolution in water.

The most usual explanation of these phenomena is that the negative Gibbs free energy of mixing of the organic solvent, released during the dissolution of the solvate, contributes to the Gibbs free energy of solution, increasing the thermodynamic driving force for the dissolution process [39]. This explanation, due to Shefter and Higuchi, was originally derived from observations on the higher dissolution rate of the pentanol solvate of succinylsulfathiazole than of the anhydrate [39]. Prior addition of increasing concentrations of pentanol to the aqueous dissolution medium reduced the initial dissolution rate of the pentanol solvate. This reduction was attributed to a less favorable (less negative) Gibbs free energy of mixing of the released pentanol in the solution that already contained pentanol. In this way, the Gibbs free energy

of solution was rendered less favorable (less negative), reducing the thermodynamic driving force for dissolution of the solvate. Thermodynamic characterization of the various steps in the dissolution of solvates and evaluation of their respective Gibbs free energies (and enthalpies) has been carried out by Ghosh and Grant [94].

V. CONSEQUENCES OF POLYMORPHISM AND SOLVATE FORMATION ON THE BIOAVAILABILTY OF DRUG SUBSTANCES

In those specific instances where the absorption rate of the active ingredient in a solid dosage form depends upon the rate of drug dissolution, the use of different polymorphs would be expected to affect the bioavailability. One can imagine the situation in which the use of a metastable polymorph would yield higher levels of a therapeutically active substance after administration owing to its higher solubility. This situation may be either advantageous or disadvantageous depending on whether the higher bioavailability is desirable or not. On the other hand, unrecognized polymorphism may result in unacceptable dose-to-dose variations in drug bioavailability and certainly represents a drug formulation not under control.

The trihydrate/anhydrate system presented by ampicillin has received extensive attention, with conflicting conclusions from several investigations. In one early study, Poole and coworkers reported that the aqueous solubility of the anhydrate phase was 20% higher than that of the trihydrate form at 37°C [95]. They also found that the time for 50% of the drug to dissolve in vitro was 7.5 and 45 minutes for the anhydrate and trihydrate forms, respectively [96]. Using dogs and human subjects, these workers then determined in vivo blood levels, following separate administration of the two forms of the drug in oral suspensions or in capsules. The anhydrous form produced a higher maximum concentration of ampicillin C_{max} and an earlier time to reach maximum concentration T_{max} in the blood serum relative to the trihydrate form. This behavior was more pronounced in the suspension formulations. In addition, the area under the curve (AUC) was found to

be greater with the anhydrous form, implying that the anhydrous form was more efficiently absorbed.

Since the early works just discussed, an interesting discussion on the comparative absorption of ampicillin has arisen. Some workers have concluded that suspensions and capsules containing ampicillin anhydrate exhibit superior bioavailabilities to analogous formulations made from the trihydrate [97,98]. For instance, in a particularly well-controlled study, Ali and Farouk [98] obtained the clear-cut distinction between the anhydrate and the trihydrate that is illustrated in Fig. 10. However, others have found that capsules containing either form of ampicillin yielded an essentially identical bioavailability [99–101]. These conflicting observations indicate that the problem is strongly affected by the nature of the formulation used, and that the effects of compounding can overshadow the effects attributed to the crystalline state.
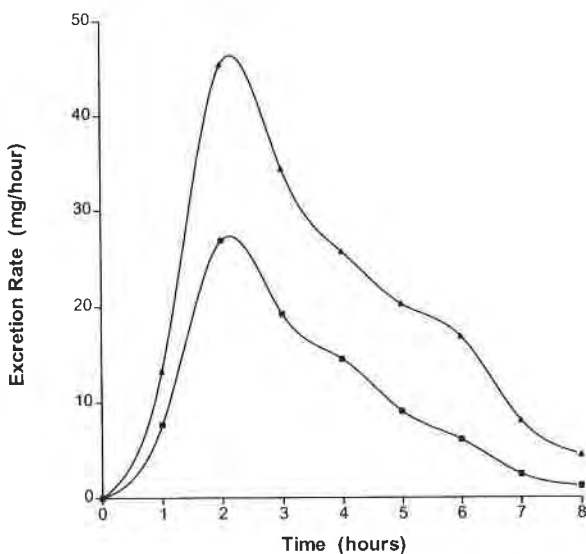

Fig. 10. Ampicillin urinary excretion rates at various times after separate administration of the two forms. Shown are the profiles obtained for the anhydrate (-▲-) and trihydrate phases (-■-). (The figure has been adapted from data provided in Ref. 98.)

Chloramphenicol palmitate has been shown to exist in four crystal modifications, and the effect of two of these on the degree of drug absorption has been compared [102]. After oral ingestion of Forms A and B, the highest mean blood levels were obtained with suspensions containing only Form B. In mixed dosage forms, the blood levels of the drug were found to bear an inverse relationship with the fraction of Form A. This finding explained the previous report, which noted that a particular suspension formulation of chloramphenicol palmitate exhibited an unsatisfactory therapeutic effect [103]. A study of various commercial products indicated that the polymorphic state of the drug in this formulation was uncontrolled, consisting of mixtures of the active polymorph B and the inactive polymorph A.

Sulfamethoxydiazine has been shown to exist in a number of polymorphic forms, which exhibit different equilibrium solubilities and dissolution rates [104]. Form II, the polymorph with the greater thermodynamic activity, was found to yield higher blood concentrations than Form III which is stable in water [105]. This relationship is illustrated in Fig. 11. Although the urinary excretion rates during the absorption phase confirmed the different drug absorption of the two forms as previously observed, the extent of absorption (as indicated by 72-hour excretion data) of the two forms was ultimately shown to be equivalent [106].

Fluprednisolone has been shown to exist in seven different solid phases, of which six were crystalline and one was amorphous [107]. Of the crystalline phases, three were anhydrous, two were monohydrates, and one was a *tert*-butylamine solvate. The in vitro dissolution rates of the six crystalline phases of fluprednisolone were determined and compared with in vivo dissolution rates derived from pellet implants in rats [108]. The agreement between the in vitro and in vivo dissolution rates was found to be quite good, but the correlation with animal weight loss and adrenal gland atrophy was only fair. These results can be interpreted to indicate that, for fluprednisolone, differences in dissolution rates of the drug did not lead to measurable biological differences.

Erythromycin base is reported to exist in a number of structural forms, including an anhydrate, a dihydrate, and an amorphous form [109, 110]. The commercially available product appears to be a partially crystalline material, containing a significant amount of amor-
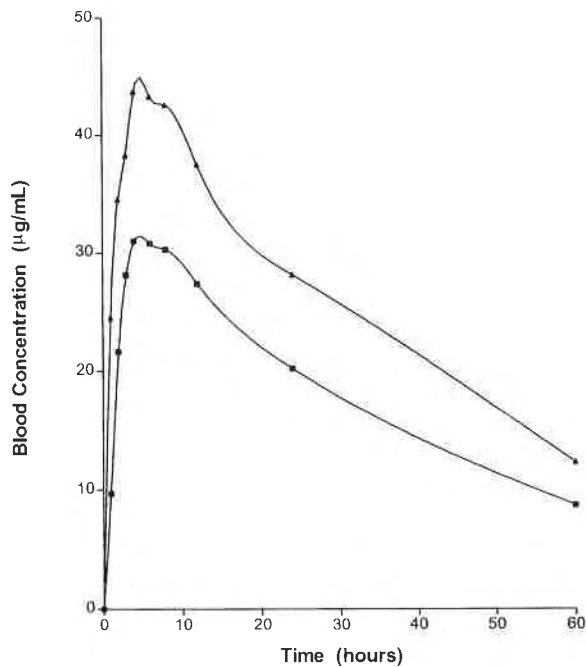
Fig. 11. Mean concentrations of sulfamethoxydiazine in blood as influenced by the polymorphic form of the drug substance. Shown are the profiles of Form II (-▲-) and Form III (-■-). (The figure has been adapted from data provided in Ref. 105.)

phous drug [111]. From studies conducted in healthy volunteers, it was learned that the anhydrate and dihydrate phases were absorbed faster and more completely than was either the amorphous form or the commercially available form [112]. These observations were reflected in the two pharmacokinetic parameters (C_{max} and AUC).

Azlocillin sodium can be obtained either as a crystalline form or as an amorphous form, depending on the solvent and method used for its isolation [113]. The antibacterial activity of this agent was tested against a large number of reference strains, and, in most cases, the crystalline form exhibited less antibacterial activity than did the amorphous form. Interestingly, several of the tested microorganisms also proved to be resistant to the crystalline form.

Whether the different polymorphs or solvates of a given drug substance will lead to the existence of observable differences in the adsorption, metabolism, distribution, or elimination of the compound clearly cannot be predicted a priori at the present time. It is certainly likely that different crystal forms of highly soluble substances ought to be roughly bioequivalent, owing to the similarity of their dissolution rates. An effect associated with polymorphism that leads to a difference in bioavailability would be anticipated only for those drug substances whose absorption is determined by the dissolution rate. However, the literature indicates that, even in such cases, the situation is not completely clear. Consequently, when the existence of two or more polymorphs or solvates is demonstrated during the drug development process, wise investigators will determine those effects that could be associated with the drug crystal form and will modify their formulations accordingly.

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8

Effects of Pharmaceutical Processing on Drug Polymorphs and Solvates

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I. INTRODUCTION

In the previous chapters, the structural origin, energetics, and thermodynamics of polymorphs and solvates have been largely described for pure chemical entities. In most of the studies reported, the compounds were intentionally converted among various polymorphic forms for the purpose of study. In the present chapter, we will discuss the *unintentional* conversion of polymorphs and the desolvation of hydrates upon exposure to the energetics of pharmaceutical processing. Environments as harsh as 80°C and 100% RH for up to 6 h are not unusual during the routine manufacture of dosage forms. As previously noted, the various crystalline polymorphs frequently differ in their heats of fusion by as little as 1 kcal/mol, with the transition temperature being well below the boiling point of water. In the case of hydrates, removal of water from the crystal lattice requires more energy but is very much dependent on the temperature and humidity history of the sample.

In this chapter we will discuss the effects of pharmaceutical processing upon the crystalline state of polymorphic and solvate systems. Given the degree of attention lavished on drug substances that is required by solid-state pharmaceutical [1] and regulatory [2] concerns, it is only logical that an equivalent amount of attention be paid to processing issues. A variety of phase conversions are possible upon exposure to the energetic steps of bulk material storage, drying, milling, wet granulation, oven drying, and compaction. In this setting, an environment as harsh as 80°C and 100% RH for up to 12 h is not unusual,

and the mobility of water among the various components must be considered.

II. PRODUCTION AND STORAGE OF BULK DRUG SUBSTANCE

The first processing opportunity to effect a change in polymorphic form or solvate nature is with the final crystallization step in the synthesis of the bulk drug substance. Crystallization is thought to occur by first forming hydrogen-bonded aggregates in the solution state, followed by the buildup of molecules to produce a crystal nucleus. A number of parameters are known to affect the crystallization process, including solvent composition and polarity, drug concentration and degree of supersaturation, temperature and cooling rate during the crystallization process, presence of seed crystals and/or nucleation sites, additives that influence crystal habit or add strain to the crystal lattice, agitation, pH, and the presence of a salt-forming molecule. It is evident that ample opportunity exists for the appearance of a polymorphic change when a process is scaled up, or moved to a new site, or run by a new operator.

Since the discovery chemist would have been able to make gram quantities of a quasi-crystalline drug substance, logic holds that the process chemist should be able to make kilograms of the same substance in a GMP manufacturing setting. While Mother Nature and equilibrium may have been fooled at the bench-top (where reaction steps are short and yield is improved through the use of anti solvents), longer processing times and improvements in purity usually mean that thermodynamic equilibrium will be achieved for the first time at the scale-up stage. On the other hand, the need for high yield frequently is the chief motivating force early in a development program, so even the first scale-up phase often fails to produce the thermodynamically preferred polymorph. Eventually, either at the first scale-up site or when the process is moved to a new site, the thermodynamically preferred form will appear. This observation has been attributed to Gay-Lussac, who noted that unstable forms are frequently obtained first, and that these subsequently transform to stable forms.

Eventually, either at the first scale-up site or when the process is moved to a new site, the thermodynamically preferred form will appear.

When this change occurs late in development, retesting of the substance is required, to correct the analytical profile of the compound, as well as expensive clinical or toxicology testing. In the competitive environment of today, time is money, and delaying an NDA can be as costly as the expense of additional clinical or toxicological testing. It is the appearance of a thermodynamically preferred polymorph late in the development cycle that often requires the use of seed crystals during processing. One occasionally runs into legends of "whiffle dust," or seed crystals circulating in the air-handling system, which lead to the occurrence of the disappearing polymorphs discussed earlier in Chapter 1. Experienced process chemists who have tried to generate an unstable polymorph for an analytical standard generally support the tenets of this legend and contribute to its dissemination.

III. EFFECTS OF PARTICLE SIZE REDUCTION

The last processing step in the production of bulk drug substances usually involves milling to reduce the particle size distribution of the material. This is ordinarily conducted using the mildest conditions possible to render a sample homogeneous, or through the use of more rigorous milling to reduce the primary particle size in an effort to improve formulation homogeneity or dissolution and bioavailability. In the latter process, a substantial amount of energy is used to process the substance, which can result either in a polymorphic conversion or in the generation of an amorphous substance. The formation of an amorphous material is highly undesirable, since it will often be hygroscopic and more water soluble than any of the crystalline forms. The amorphous material must also be considered as being a thermodynamically metastable state of high energy, and a variety of pathways exist that can result in a back-conversion to a crystalline form of the material. This will certainly alter the dissolution characteristics and possibly even the bioavailability of the drug. In any case, the use of any rigorous milling process requires careful analysis for consequent changes in crystallinity or crystal form.

Hancock and Zografi have reviewed the characteristics of the amorphous state and methods whereby this form of a given material may be obtained [4], and this information is also covered in the final chapter of this book. Amorphous materials are disordered, have the

highest free energy content, have the highest water solubility, and are usually hygroscopic. The effect of amorphous materials or amorphous regions within pharmaceutical solids is to convert the normal solid properties of high elasticity and brittleness to varying degrees of visco-elasticity that allow the materials to flow under the mechanical stress of milling or tableting. Upon heating, amorphous materials pass through a glass transition temperature and convert to a crystalline state. The effect of adsorbed moisture from the atmosphere or excipients is to act as a plasticizer, lowering the glass transition temperature, increasing molec-ular mobility in the solid, and allowing crystallization to occur at a lower temperature. Thus any rigorous milling of a drug substance re-quires careful analysis and monitoring for subsequent changes in crys-tallinity.

Much of the literature on the milling of polymorphs involves long grinding or ball-milling times, which impart unusual thermodynamic stress to the material and possibly lead to confusion about the real ef-fects of milling on polymorphism. In practice, drugs are typically milled in a Bantam Mill or a Fitzpatrick Mill (alone or with excipients) where the exposure time of the material to stress is very short. One would predict that the thermodynamic impact would be very small, and therefore one might expect to observe little or no change in polymor-phism arising from effects of industrial-scale milling.

Nevertheless, one can glean an understanding as to the effect of milling on polymorphism from ball-milling studies, such as those con-ducted by Miyamae et al. on (E)-6-(3,4-dimethoxy-phenyl)-1-ethyl-4-mesitylimino-3-methyl-3,4-dihydro-2(1H)-pyrimidinone [5]. In this work, the title compound was milled for up to 60 min and then stored at ambient conditions for up to 2 months. It was found that the unstable Form A (melting point 118°C) was converted to a noncrystalline solid as a result of the milling process but then crystallized upon storage to the stable Form B (melting point 141°C).

Fostedil exists in two polymorphic forms, characterized by melt-ing points of 95.3°C (Form I) and 96.4°C (Form II), a free energy difference of only 71.8 cal/mol at 37°C, and distinctly different infrared absorption and x-ray powder diffraction patterns [6]. Solubility studies suggested that Form I was more stable than Form II. Mechanical mix-ing in an automated mortar showed that complete conversion of Form II to Form I occurred in 2 h. Milling fostedil in an industrial fluid energy

mill and a hammer mill resulted in a similar conversion of Form II to Form I, presumably through the generation of nuclei on the surface of the crystal. The hammer milled material was exposed to less energy, which resulted in fewer seed nuclei and therefore less conversion to Form I even when exposed to elevated temperature and humidity. In contrast, since the fluidized energy mill provides more energy, its use generated more surface defect nuclei and greater conversion to the Form I polymorph. The presence of excipients also was found to exert a strong perturbation on the kinetics of the phase transformation. As illustrated in Fig. 1, the presence of microcrystalline cellulose retarded the conversion of Form II into Form I with grinding.

Chloramphenicol palmitate Form B was found to transform to the less therapeutically desirable Form A during grinding [7]. The transformation could be accelerated by the presence of appropriate seed crystals, and it was also found that the least stable Form C could be progressively converted from Form B and then to Form A if the grinding times were sufficiently long. In another study, it was shown that the temperature increases that accompanied the grinding process could accelerate
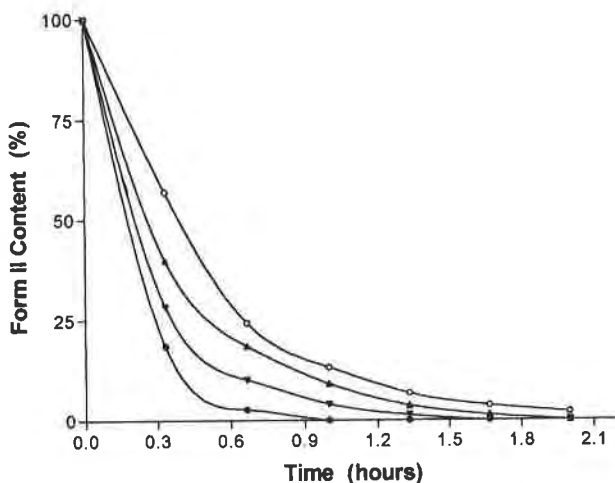

Fig. 1. Effect of grinding time on the Form-II-to-Form-I phase transformation composition of pure fostedil (○), and also for fostedil/microcrystalline cellulose mixtures having ratios of 3:1 (▲), 1:1 (▼), and 1:3 (●). (The figure is adapted from data presented in Ref. 6.)

the phase conversion [8]. In fact, when one grinds chloramphenicol stearate for a sufficiently long period of time, Form III first turns into Form I, and ultimately into an amorphous state [9]. One interesting note of this latter study was the finding that the rate of phase conversion was accelerated by the presence of microcrystalline cellulose.

Clearly, the temperature maintained during the milling process can influence the degree of any polymorphic transitions. The α-and γ-forms of indomethacin could be converted to an amorphous solid during grinding at 4°C, but at 30°C the γ-form converted to the α-form, which could not be further transformed [10,11]. The authors concluded that although the noncrystalline form was stable at 4°C, it evidently was unstable with respect to crystallization at 30°C. An illustration of the crystal form present in samples of indomethacin as a function of grinding time is provided in Fig. 2.

The transformation behavior of phenylbutazone polymorphs during grinding at 4 and 35°C, and the solid-state stability and dissolution behavior of the ground materials, was investigated [12]. The α, β, and δ forms were transformed to the new ζ-form, which in turn was transformed to the ϵ-form (which was stable at 4°C). On the other hand,
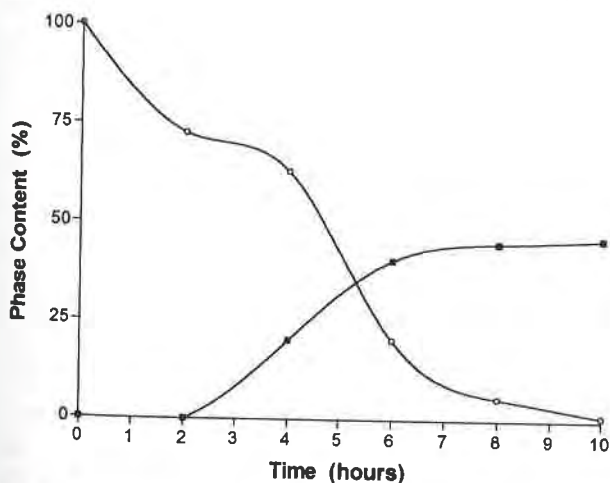

Fig. 2. Effect of grinding time (conducted at 30°C) on the phase composition of γ-indomethacin (O), showing also the α-phase (■) formed. (The figure is adapted from data presented in Ref. 11.)

during grinding at 35°C, the δ-form was not changed, while the α-form was transformed to the δ-form by way of the ζ-form. The β-form was apparently transformed directly to the δ-form. Once obtained by grinding, the ε-form was transformed to the δ-form under 0% relative humidity at various storage temperatures after an induction time of a few hours.

A common occurrence during grinding is the formation of an amorphous, noncrystalline phase. For instance, the grinding of cephalexin has been found to decrease the degree of crystallinity [13] as well as a wide range of physical properties that depend on the crystalline content of the material [14]. When the material becomes less crystalline, its stability decreases, and the hydrate forms become easier to dehydrate. Similar conclusions were reached with respect to the strength of the crystal lattice when the effect of grinding on the stability of cefixime trihydrate was studied [15]. The degree of interaction between the waters of hydration and the cefixime molecules was weakened by grinding, negatively affecting the solid-state stability.

Four modifications of cimetidine were prepared as phase-pure materials, and each was found to be stable during dry storage [16]. The milling process enabled the conversions of Forms B and C to Form A, while Form A transformed into Form D only upon nucleation. In all cases, the particle size reduction step resulted in substantial formation of the amorphous phase. Compression of the various forms into compacts did not yield any evidence for phase conversion.

The pitfalls that can accompany the development of the amorphous form of a drug substance were shown for the capsule formulation of (3R,4S)-1,4-bis(4-methoxy-phenyl)-3-(3-phenylpropyl)-2-azetidinone [17]. A variety of spectroscopic techniques were used to study the amorphous-to-crystalline phase transition, and it was determined that problems encountered with dissolution rates could be related to the amount of crystalline material generated in the formulation.

One general finding of milling studies conducted on hydrate species is that the grinding process serves to lower the dehydration temperatures of ground materials, facilitating the removal of lattice water and formation of an amorphous product. This behavior has been noted for cephalexin [13,14], cefixime [15], cyclophosphamide [18], 2-[(2-methylimidazoyl-1-yl)-methyl]-benzo[f]thiochromen-1-one [19], and lactitol [20], to name a few representative examples.
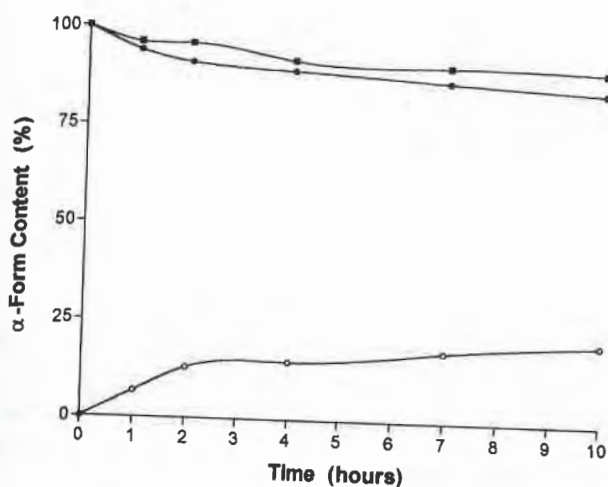
Fig. 3. Changes in α-form content for the α-monohydrate (■), α-anhydrate (●), and β-anhydrate (○) modifications of lactose with grinding time. (The figure is adapted from data presented in Ref. 22.)

Although most studies have been concerned with the state of the drug entity in either bulk or dosage form, milling can also affect the crystalline state of excipients. For example, isomerization was found to take place during the grinding of the α-monohydrate, α-anhydrate, and β-anhydrate forms of lactose [21]. The crystalline materials were all transformed into amorphous phases upon grinding, but these materials quickly re-sorbed water to approach the equilibrium composition ordinarily obtained in aqueous solutions. This behavior has been illustrated in Fig. 3. In a subsequent study [22], these workers determined the degree of crystallinity during various stages of the grinding process and concluded that while the isomerization rate of the α-monohydrate phase depended on the crystallinity, the rates for the two anhydrate phases depended on the content of absorbed water.

IV. EFFECTS DUE TO GRANULATION

When the requirements of a given formulation require a granulation step, this will represent an opportunity for a change in crystal form of

the drug to take place. The problem will be most acute when a wet granulation step is used, since a potential transforming solvent is added to the blend of drug and excipients. The mixture is physically processed in the wet stage until homogeneity is obtained and then dried at a fairly significant temperature. The rationale for including a granulation step is to improve flow and blend homogeneity, permitting the high-speed compression of tablets. The granulation process is used to maintain the distribution of the drug throughout the formulation even during free flow, thus blend homogeneity.

Clearly, one can consider a wet granulation to be equivalent to a suspension of the drug entity in a mixture of solvent and excipients. Since the usual solvent is water, one can encounter a variety of inter-conversions between anhydrates and hydrates, or between hydrates and hydrates, which are mediated by the presence of the solvent. It is equally clear that one should not expect to be able to wet-granulate the metastable phase of a particular compound if that metastable phase is capable of transforming into a more stable form. A discussion of solvent-mediated phase transformations has been given in an earlier chapter and need not be repeated here.

It has been noted that chlorpromazine hydrochloride Form II exhibits severe lamination and capping when compressed, and that wet granulation with ethanol and water significantly improves the tableting characteristics [23]. The reason for this process advantage was shown to entail a phase change of the initial Form II to the more stable Form I during the granulation step. It was concluded that the improvements in tabletability and tablet strength that followed the use of wet granulation were due to changes in lattice structure that facilitated interparticulate bonding on compaction.

The effects of granulation solvent on the bulk properties of granules prepared using different polymorphic forms of carbamazepine have been studied [24]. It was found that although the anhydrate phase of the drug transformed to the dihydrate phase in the presence of 50% aqueous ethanol, the phase transformation did not take place when either pure water or pure ethanol was used as the granulating solution. This unusual finding was attributed to the fact that the solubility of the drug in 50% ethanol was 37 times higher than that in pure water. Granules processed using 50% ethanol were harder than those processed with pure solvents and led to the production of superior tablets. This

work demonstrates the extreme need to evaluate the robustness of the granulation step during process development.

The crystal characteristics of a drug substance can undergo a sequence of reactions during wet granulation and subsequent processing. When subjected to a wet granulation step where 22% w/w water was added to dry solids, and the resulting mass dried at 55°C, an amorphous form of (S)-4-[[[1-(4-fluorophenyl)-3-(1-methylethyl)-1H-indol-2-yl]-ethynyl]-hydroxyphosphinyl]-3]-hydroxybutanoic acid, disodium salt, was obtained [25]. However, this amorphous gradually became crystalline upon exposure to humidity conditions between 33 and 75% RH. Depending on the exact value of the applied humidity, different hydrates of the drug substance could be obtained in the formulation.

V. EFFECTS DUE TO DRYING

Whenever a wet suspension containing a drug substance is dried, the possibility exists that a change in crystal state will take place. It has been amply shown in earlier chapters that anhydrates or amorphates can be produced by the simple desolvation of a solvate species, and such reactions are always possible during any drying step. The production of anhydrate phases by simple drying is one example of a reaction that can accompany the drying process, and this reaction type has been fully discussed in an earlier chapter.

In the simplest type of drying procedure, moist material from the wet granulation step is placed in coated trays and the trays are placed in drying cabinets with a circulating air current and thermostatic heat control. Historically, the tray drying method was the most widely used method, but more recently, fluid-bed drying is now widely used. In drying tablet granulations by fluidization, the material is suspended and agitated in a warm air stream. The chief advantages of the fluid-bed approach are the speed of drying and the degree of control that can be exerted over the process. The wet granulations are not dried to zero moisture; for a variety of reasons one seeks to maintain a residual amount of moisture in the granulation. It is evident from these simple considerations that the combination of solvent and drying conditions provides a suitable environment for the generation of new polymorphs or solvates when such conversion routes are available.

During ordinary manufacturing processes, crystals of the drug substance are dried under vacuum or through the circulation of warm dry air. Evaluation of wet and dried samples of the bulk substance by thermogravimetric analysis and hygroscopicity studies usually yields the necessary insight into the limiting parameters of the drying process. Since drying times can be relatively long and the environmental temperature mild (such as 24 to 48 h at 60°C), partial or total polymorphic conversion is possible. The likelihood that phase conversion will take place becomes increasingly less as the solvent is removed during drying. This area has been thoroughly covered earlier in Chapter 5, so only a few illustrative examples will be quoted at this time.

Shefter et al. used x-ray diffraction analysis to show that overdrying ampicillin trihydrate resulted in an unstable amorphous product, and that the drying of theophylline hydrate yielded a crystalline anhydrous form [26]. Kitamura et al. dehydrated (with vacuum) cefixime trihydrate to obtain a crystalline anhydrous form, which also proved to be unstable [27].

Otsuka et al. studied the effect of humidity and drying on the polymorphic transformation rate at 45°C for six forms of phenobarbital [28]. Figure 4 shows that the monohydrate (Form C) and the hemihydrate (Form E) slowly convert to the anhydrous Form B under high-humidity stress conditions (45°C and 75% RH), while the conversion is very fast at low humidity (45°C and 0% RH). This is reasonable, since the driving force to form anhydrous material is a dry environment. The key point of Figure 4 is the rapid conversion of phenobarbitol in less than 12 at 45°C and 0% RH, which represents very reasonable processing conditions for an industrial drying oven.

The sodium salt of 3-(((3-(2-(7-chloro-2- quinolinyl)ethenyl)phenyl)((3-dimethylamino)-3-oxo-propyl)thio)propanoic acid represents an unusual case of a hygroscopic drug substance, where the surface-active substance (in both crystalline and lyophilized forms) produced nonflowing, semisolid masses with exposure to increasing relative humidity [29]. The substance was shown to form first an amorphous material and then a mesomorphic phase, as the moisture sorption increased with exposure to relative humidity.

The favorite endpoint of the process chemist is to ''dry to constant weight,'' but this laudable goal can lead to the production of a desol-
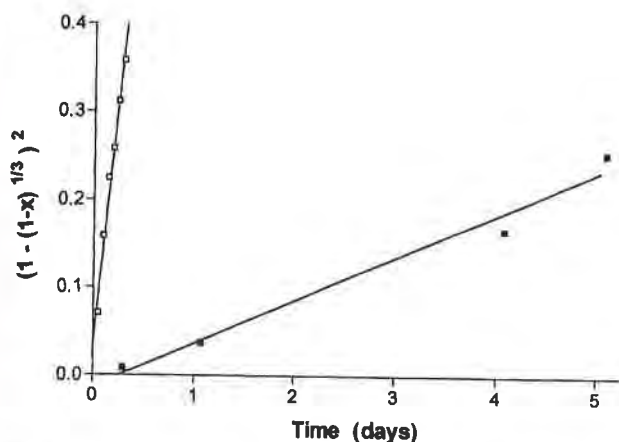
Fig. 4. Jander plots of one polymorphic transformation of phenobarbital. Illustrated are data for the conversion at 45°C of Form C (monohydrate phase) to Form B (anhydrate phase). The 0% relative humidity atmosphere points are given by the open symbols, and the 75% relative humidity atmosphere points are given by the closed symbols. (The figure is adapted from data presented in Ref. 28.)

vated product. Without doubt, the overdrying process has produced many anhydrous lots of bulk drug substance, which unfortunately are eventually found to be unstable.

Since the effects of simple drying have been more fully discussed in detail in Chapter 5 of this book, we will turn to case studies that illustrate how the use of two specialized drying methods can lead to phase interconversions.

A. Changes in Crystalline Form Accompanying the Spray-Drying Process

The spray-drying process first requires the formation of a slurry to be sprayed, which can be a concentrated solution of the agent to be dried or a dispersion of the agent into a suitable nondissolving medium. The dispersion is then atomized into droplets, which are exposed to a heated atmosphere to effect the drying process. Completion of the process yields a dry, freeflowing powder that ordinarily consists of spherical

particles of relatively uniform particle size distribution. For instance, the morphology of spray-dried lactose has been contrasted with that of the monohydrate and anhydrous phases of lactose, with significant differences being reported [30].

One interesting feature of spray-drying is the possibility of controlling the final crystal form through manipulation of the drying conditions. For instance, three different crystalline forms of phenylbutazone were prepared from methylene chloride solution by varying the drying temperature of the atomized droplets between 30 and 120°C [31]. One of the isolated polymorphs could not be obtained by any crystallization procedure and could only be produced using extremely slow solvent evaporation rates.

Using phenobartitone and hydroflumethiazide as examples, it has been shown that high-energy solids can be produced through the use of spray-drying [32]. Commercially available phenobartitone is obtained as Form II, but with spray-drying the authors were able to obtain Form III having a large specific surface area. The analogous spray-drying of hydroflumethiazide yielded an amorphous solid. The spray drying of indomethacin produced a viscous glassy phase, which was found to be physically unstable with time [33]. Upon storage, the glassy solid converted into a mixture of crystalline Forms I and II. Representative x-ray powder diffraction patterns of these materials are shown in Fig. 5.

Spray-drying is often used to encapsulate drug substances into excipient matrices, but phase interconversions are still possible in such situations. Sulfamethoxazole Form I was microencapsulated with cellulose acetate phthalate and talc, colloidal silica, or montmorillonite clay by a spray-drying technique, and the drug was found to convert to Form II during the process [34]. Increasing the amount of cellulose acetate phthalate in the formulation led to increased amorphous drug content, but increasing the talc level yielded more polymorphic conversion. In a subsequent study, the authors showed that no phase conversion took place in formulations containing colloidal silica [35].

Frequently, the spray drying process yields amorphous materials, which undergo crystallization upon storage or exposure to suitable environmental conditions. The effect of various additives on the recrystallization of amorphous spray-dried lactose has been studied [36]. The
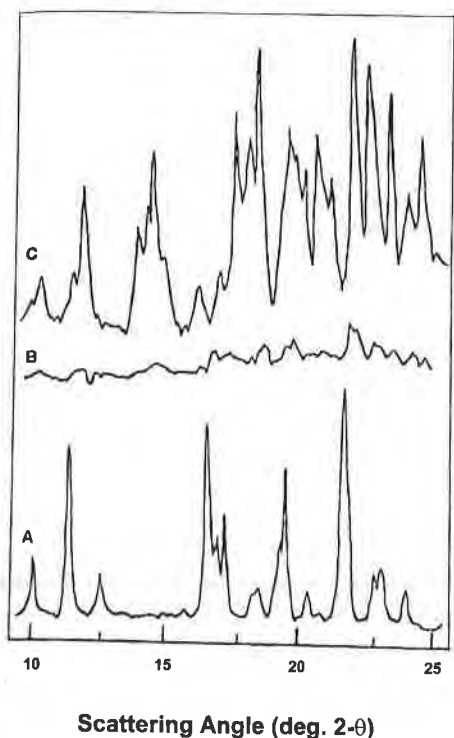
Scattering Angle (deg. 2-θ)

Fig. 5. X-ray powder diffraction patterns for (A) crystalline indomethacin, (B) spray-dried indomethacin, and (C) indomethacin spray-dried with 5% polyvinylpyrrolidone and stored for two months. (The figure is adapted from data presented in Ref. 32.)

presence of magnesium stearate was found to inhibit the recrystallization process, while layers of microcrystalline cellulose in the spray-dried products could result in long lag times prior to recrystallization. It was deduced that the onset of crystallization was critically related to the mobility of water in the formulations.

B. Changes in the Crystalline State of Lyophilized Products

Another drying procedure that can yield changes in drug crystal form is that of freeze-drying, or lyophilization. In this approach, the material

to be dried is prepared as an aqueous solution or suspension and then frozen rapidly and cooled to a temperature below its eutectic point. The frozen formulation is then exposed to vacuum and the ice removed by sublimation. Since lyophilized products are often dried to moisture levels less than 1%, the materials are ordinarily hygroscopic and must be protected from adventitious water to prevent any unwanted phase transformation steps. In usual practice, the product is produced in its amorphous state, which is favorable for its subsequent solubilization at the time of its intended use. Consequently, the study of any possible moisture-induced crystallization is important to the characterization of a lyophilized product.

The effect of storage conditions on the crystalline nature of lyophilized ethacrynate sodium has been reported [37]. Samples of the drug substance were dried to various moisture levels and then stored at either 60°C or 30°C/75% RH. It was found that crystalline drug substance was obtained after short periods of time as long as sufficient levels of water were either present in the initial lyophilized solid or introduced by adsorption of humidity. The crystalline form of the drug was identified as a monohydrate phase.

Cefazolin sodium is capable of existing in a number of crystalline modifications, but the amorphous state is produced by the lyophilization process [38]. The amorphous form retained its nature at relative humidities less than 56% but converted completely to the pentahydrate above 75% RH. Interestingly, the hygroscopicity of the amorphous form was essentially similar to that exhibited by the dehydrated monohydrate or dehydrated α-form. Lamotrigine mesylate was also found to exhibit a moisture-dependent amorphous-to-crystalline transition [39]. The transition was found to be independent of the presence of mannitol, whether the amorphous solid was produced by lyophilization or by spray-drying.

Excipients in a lyophilized product can also undergo amorphous-to-crystalline phase transitions. The transformation of amorphous sucrose to its crystalline phase was studied as a function of applied relative humidity [40]. As illustrated in Fig. 6, the amorphous material gains up to 6% water when exposed to a relative humidity of 33%, but continued storage leads to crystallization of the anhydrous crystalline phase and a consequent expulsion of the initially adsorbed water. It
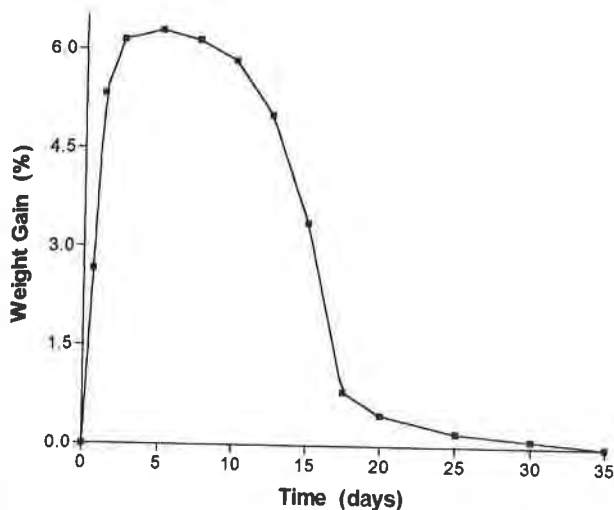
Fig. 6. Moisture update curve for amorphous sucrose, obtained at 23°C and a relative humidity of 33%. (The figure is adapted from data presented in Ref. 40.)

was hypothesized that the lag time that accompanied the amorphous-to-crystalline phase transition was required for the random motion of molecules to rotate and translate into the proper orientation to form a nucleus of sufficient size to allow crystallization.

Mannitol is commonly used as a bulking agent in lyophilization, owing to its tendency to freeze-dry into a mixture of crystalline and amorphous solids [41]. However, since mannitol can exist in a number of crystalline modifications, slight variations in the exact details of the lyophilization procedure can lead to the production of differing solids [42]. The effect of surface-active agents on the composition of lyophilized mannitol formulations has been studied, and it was reported that polysorbate 80 affected both the polymorphic state and the degree of crystallinity of freeze-dried mannitol [43]. When lyophilizing pure mannitol, one obtains a mixture consisting of the α, β, and δ forms in various ratios. In the presence of polysorbate 80, however, production of the δ-phase is favored.

VI. EFFECTS DUE TO COMPRESSION

It is often assumed that the crystalline form of a drug entity will remain unchanged during the compression steps associated with the manufacture of tablets. The reason for this line of thought is that under the applied load of tableting, the softer excipients should deform preferentially to the relatively hard crystalline drug substance. As tableting speeds increase towards commercial production, exposure times to stress decrease and one would anticipate even less chance for crystalline conversion. For the production of many substances, this situation is certainly true. There are numerous examples, however, which do not follow the general rule, and where changes in polymorph or solvate form accompany the compression step.

Effects due to compression can be further divided two categories. One of these concerns the phase transformations that accompany the compaction and consolidation steps of tablet manufacture. The other relates to the effect on tablet properties that arise from the use of different crystal forms in a direct compression tablet formulation. Case studies pertaining to each of these categories will be discussed in turn.

A. Changes in Crystal Form Effected by Compaction

In one study of wide scope, 32 drugs known to exist in different polymorphic states were subjected to a trituration test for possible phase transitions [44]. Out of 11 transforming substances, detailed studies of tableting were conducted on caffeine, sulfabenzamide, and maprotiline hydrochloride. Tablets were produced and sectioned, so that thermal analysis could be used to investigate polymorphic changes at the upper and lower surfaces, middle region, and sides of the compacts. Relationships were examined between the extent of transformation and the compression pressure and energy, as well as the effect of drug particle size. It was deduced that the form with the lowest melting point would have the least degree of intermolecular attractive forces and probably the least yield stress values at a given temperature. This conclusion was supported by the observation that the compression of metastable phases brought about a transformation to the most stable phase. As illustrated for the particular example of caffeine in Fig. 7, the extent of transforma-
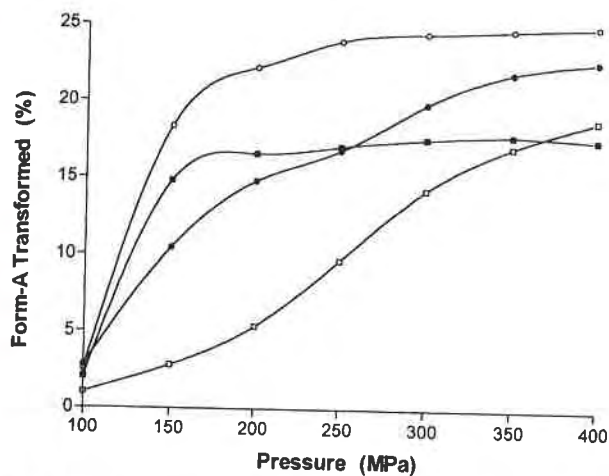
Fig. 7. Percentage of caffeine Form A transformed into Form B as a function of applied pressure. Phase composition data are shown for the upper surface (○), lower surface (□), middle region (■), and side (●) of compressed tablets. (The figure is adapted from data presented in Ref. 44.)

tion was found to depend on the zone of the tablet, the pressure applied, and the particle size of the powder.

Carbamazepine is a drug where irregular plasma levels can be obtained owing to differences in dissolution rates that are associated with the production of differing polymorphs by tablet manufacturing operations. The behavior of the α, β, and dihydrate crystalline forms during compression has been investigated, as well as the possibility of crystalline structural changes accompanying grinding and tableting conditions [45]. Grinding was performed in a ball mill for 15 and 60 minutes, while compression was carried out using an instrumented single punch machine. It was found that although the dihydrate form exhibited the best compressibility characteristics, it was not phase-stable with respect to compression. The α-form exhibited the best stability, but the use of this phase induces sticking in the tablets. It appears that the commercially available β-form represents the best compromise between performance and stability, and it was found to remain stable under normal conditions of fabrication and storage.

The effect of tablet compression mechanical energy on the polymorphic transformation of chlorpropamide has been examined [46]. A single-punch eccentric tableting machine with a load cell and a noncontact displacement transducer was used to measure compression stress, distance, and energy. Both the stable Form A and the metastable Form C were compressed at a stress of 196 MPa at room temperature. The compression cycle was repeated from 1 to 30 times, and the phase composition of deagglomerated tablets was measured using powder x-ray diffraction. It was found that each form could be transformed into the other by the mechanical energy input during tableting, with the composition reaching equilibrium above 100 J/g of compression energy after more than 10 cycles. It was hypothesized that the phase transformation required the conversion of the crystalline forms to an amorphous intermediate, which then transformed into the equilibrium ratio of forms A and C.

The effect of compressional force on the polymorphic transition in piroxicam has been examined for phase-pure materials, where the needle-like α-phase was found to convert to the cubic β-phase during compression [47]. The phase transformation took place only at higher applied forces, such that formation of the α-phase could only be observed after compression to a tablet hardness of 9 kg/cm^2. Compression of the β-phase yielded no changes in its crystalline form.

The extent of the polymorphic transformation of anhydrous caffeine has been studied as a function of grinding time and compression pressure [48]. It was found that both grinding and compression induced the transformation from the metastable Form I into the stable Form II. The transformation could be observed even after only 1 min of grinding, or by using a tableting compression pressure of about 50 MPa. Quantitative measurements of phase conversion indicated that the degree of transformation was greater near the surface of the tablet than in its interior.

The effect of temperature on polymorphic transformations that take place during compression has been studied. Tableting temperature was found to exert a definite effect on the polymorphic transformation of chlorpropamide during compression, and on the physical properties of the produced tablets [49]. Compression stress, distance, and energy were measured using a noncontact displacement transducer mounted

within a temperature-controlled single punch eccentric tableting machine with two load cells (upper and lower punches). The phase composition of deagglomerated compacts was subsequently measured using powder x-ray diffraction to calculate the polymorphic content. It was learned that the amount of Form C produced at 45°C by the transformation of Form A was about twice that produced at 0°C using the same compression energy. The amount of Form A transformed from Form C by compression at 45°C was almost the same as that at 0°C. This suggested that the compressional effect on Form A depended on the compression temperature, while the effect on Form C was independent of temperature. The crushing strength of tablets produced using Form A was about twice that obtained for tablets produced from Form C, even when they were compressed at the same porosity.

A study of the effect of temperature on the polymorphic transformation and compression of chlorpropamide Forms A and C during tableting has been reported [50]. With the aid of apparatus similar to that previously described [49], these authors used multitableting at room temperature and single tableting at 0–45°C to effect compression. In the first method, the stable Form A or the metastable Form C was loaded into the die and the sample was compressed with a compression stress of 196 MPa. It was found that both forms were mutually transformed, and as evident in Fig. 8, an equilibrium ratio of Forms A and C was attained above roughly 200 J/g of compression energy. In the second method, the amount of Form C transformed from Form A at 45°C was about two times larger than that at 0°C at the same compression energy. The crushing strength of tablets prepared from Form A was about twice that of tablets manufactured using Form C as the source of drug substance.

A further study was conducted to study the effect of compression temperature on the consolidation mechanism of the polymorphs of chlorpropamide [51]. The effect of environmental temperature on the compression mechanism of the A and C forms of chlorpropamide was investigated with an eccentric type tableting machine with two load cells and a noncontact displacement transducer. The temperature of the sample powders was controlled at 0 and 45°C, and these were compressed at almost 230 MPa. The tableting dynamics were evaluated by Cooper and modified Heckel analyses. The results suggest that particle
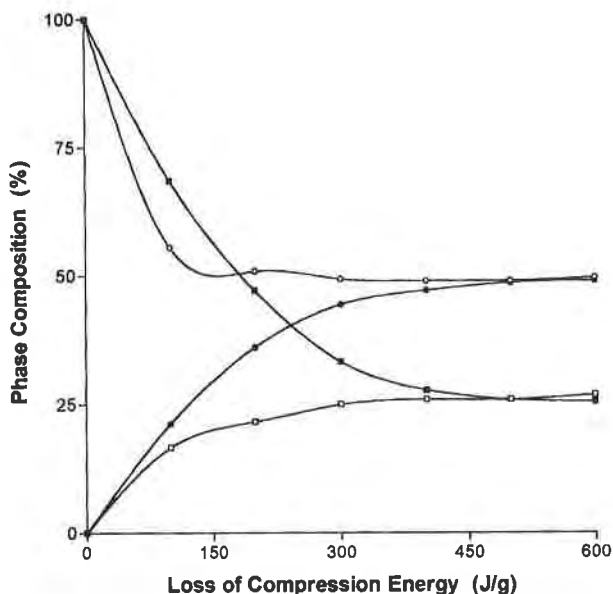
Fig. 8. Relationship between phase composition and compression energy during the compaction of different polymorphs of chlorpropamide. Shown are data for the residual content of Form A during the compression of Form A (○), the amount of Form C formed during the compression of Form A (□), the residual content of Form C during the compression of Form C (■), and the amount of Form A formed during the compression of Form C (●). (The figure is adapted from data presented in Ref. 50.)

brittleness or plasticity was affected by compression at different temperatures. The higher tablet hardness of Form A tablets produced at 45°C was thought to be caused by the increased plasticity of primary particles, whereas the hardness of tablets containing Form C (and also produced at 45°C) was ascribed to the decreased size of the secondary particles. Part of the reasoning behind this conclusion was the observation that Form A melted approximately 8°C lower than did Form C and would therefore be more plastic with respect to deformation.

Sebhatu et al. studied the tableting characteristics of spray-dried lactose [52], which typically contains 15% amorphous material. The spray-dried material is known to absorb moisture when exposed to high

levels of relative humidity, which lowers the glass transition temperature of dry material from 104°C to 37°C (at moisture content 7.17%). It was found that tablets made from spray-dried lactose that had been stored for less than 4 h at 57% RH before compaction showed an increased in postcompaction tablet hardness when stored at 57% RH. This effect was ascribed to crystallization of the amorphous regions brought into close contact by compression. On the other hand, spray-dried lactose stored at 57% RH for more than 6 h prior to compaction showed no change in postcompression hardness for tablets stored at 57% RH because the amorphous regions had completed crystallization prior to compaction. One concludes that any tableted product initially containing a large portion of amorphous material (either active drug or inactive excipient) may be subject to changes in tablet hardness upon storage in high humidity environments.

B. Effects on Tablet Properties Associated with the Use of Different Crystal Forms

The dissolution rate of phenylbutazone from disintegrated tablets has been used to determine whether the drug particles underwent crushing or bonding during compression [53]. By using two polymorphic forms of the drug substance, it was shown that the predominant effect during compression in formulations containing high drug concentrations was dependent upon the original particle size of the active and on its polymorphic state. In formulations having a low drug concentration, the excipients effectively prevented the drug particles from bonding together. Lactose was found to exert a more abrasive action (relative to Avicel) on Form A but had little effect on the more ductile Form B. This effect is illustrated in Fig. 9, where the dissolution rates of tablets produced from the two polymorphs are contrasted. The effect of using differing particle size ranges of input material is particularly evident in the dissolution data of tablets prepared using the finest grade of drug substance. A second aspect of this work concerns the analysis of how the rate of compression during tableting affects the dissolution time. For larger particle sizes of raw material, no difference was observed on passing from 0.26 to 29 of compression time. For the smallest grade of input material, a significant difference was observed. The authors
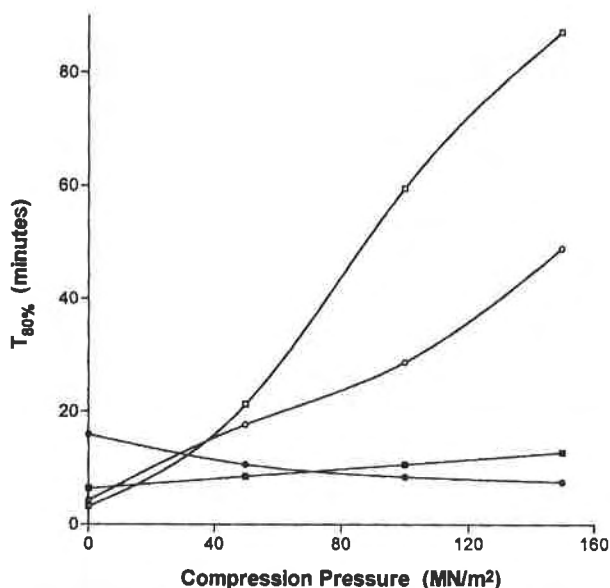
Fig. 9.   Change in dissolution rate of disintegrated tablets of phenylbutazone as a function of particle size, compression pressure, and crystal form. Shown are the profiles for tablets prepared from Form A of 6 μm particle size (○), tablets prepared from Form B of 6 μm particle size (□), tablets prepared from Form A of 137 μm particle size (●), and tablets prepared from Form B of 146 μm particle size (■). (The figure is adapted from data presented in Ref. 53.)

concluded that a decrease in the rate of compression from 29 s to 0.26 s resulted in less bond formation within the tablet matrix and a more rapid dissolution.

The work of Tuladhar et al. [53] is significant because industrial tableting machines operate typically at 200 revolutions per minute. This means 0.3 seconds per revolution (i.e., the exposure time to compression), which is far less than the prolonged exposure on a manual Carver Press (on the order of minutes) or on some of the single-station tablet machines presented earlier (3 revolutions per minute). Prolonged exposure during compression may lead to erroneous conclusions about the significance of polymorphic transformation during tableting. Neverthe-

less, some interesting information can be derived from such studies if they are of proper design.

Others have sought to develop new methods for a better evaluation of the relation between the mechanical strength of solids and the polymorphic form used to produce these [54]. In order to evaluate the role played by polymorphism in the mechanical strength of solid dosage forms, and to minimize the influence of other factors, a melted disc technology was proposed. Tablet-shaped discs of zero porosity were prepared by melting powder and subsequent crystallization in the desired modification. Using phenobartitone as a model drug, discs consisting of Forms I, II, and III, as well as the amorphous form, were prepared. An evaluation of the mechanical properties of the discs was conducted, primarily through determinations of bending strength. It was found that the amorphous form and Form III yielded the strongest discs and would therefore probably be the most suitable materials for tablet manufacture.

The poor compression of paracetamol was addressed through the preparation of a new orthorhombic form [55]. Owing to its well-known poor compressional properties, commercially available paracetamol materials for direct compression are compounds of paracetamol with gelatin, polyvinylpyrrolidone, starch, or starch derivatives. Since a chemically pure paracetamol that could be used for direct compression would constitute a better compendial article, a new polymorph was produced. The new form was recrystallized from dioxane, and its crystals were found to consist of sliding planes that led to good compressibility. However, the orthorhombic form is metastable with respect to the monoclinic form; phase conversion was observed if the raw material contained greater than 20% of the monoclinic phase. However, the dissolution rates for the two forms were found to be similar, and therefore any questions as to the relative bioavailability of the two forms would probably be meaningless.

Compressive deformation studies were conducted on single crystals of α-lactose, using mechanical strength and acoustic emission analyses to deduce any possible differences in the deformation behavior of α-lactose monohydrate and anhydrous α-lactose [56]. The α-lactose monohydrate crystals were found to exhibit greater mechanical strength

than did the anhydrous α-lactose crystals. The acoustic emission data showed that the fragmentation process of the monohydrate phase was acoustically more active and energetic. Amplitude distribution analysis of the acoustic signals further confirmed that the nature of fragmentation during the deformation of the two types of lactose was different. This was attributed to fundamental differences in the internal crystal structure of the two lactose types. This work showed that mechanical strength and acoustic emission analyses are able to provide significant insights into the fundamental deformation characteristics of different polymorphic or solvate species.

The compressional behavior of four polymorphs of mannitol (the α, β, and δ forms, as well as one unidentified phase) has been studied [57]. It was found that the compressibility of the α-phase is superior, and fortunately this phase is the major form in most commercial products. The particle shape was found to exert an influence upon the compressibility properties; granulated powders show better behavior than native crystalline powders. It was reported that no polymorphic transitions took place under the compression stresses used during the tableting process.

VII. SUMMARY

The key parameters to consider at the bulk drug substance production stage are prolonged processing times due to batch size, improved product purity, a lengthy final crystallization step to improve purity, improve yield, or increase particle size, drying conditions for the product, and milling to improve homogeneity or reduce particle size. In all these steps, the small energy barriers between crystalline forms are easily overcome, resulting in the wrong polymorphic form, a desolvated product, or an amorphous product.

Once the kilogram bulk has been produced in its preferred polymorphic form, or salt form, or solvate form, it will proceed to the pharmaceutics department, where it will be mixed with excipients, exposed to processing conditions, and converted to a marketable dosage form. The pharmaceutical scientist must formulate this material into a dosage form that is homogeneous, scalable, stable, and bioavailable. Since

most dosage forms are solid dosage forms, capsules or tablets, this effort will focus primarily on those processing parameters that are operative in the production of solid dosage forms.

Solution dosage forms are ordinarily independent of polymorphic problems, but they require one warning. If there should happen to exist a less soluble form, it will appear upon temperature cycling stability testing. The reason for this solution state warning is that temperature cycling is the most severe challenge to solubility, and if one should generate seed crystals of a less soluble form of a compound during cooling, then equilibrium will rapidly be established. The final stage of this scenario will be precipitation or crystal growth. The classic cases in the industry are the soluble anhydrous material converting to an insoluble hydrate upon stability testing, or moisture leakage into a delivery system.

In solid dosage forms, the first opportunity for a change in crystallinity will be in the blending and milling of drug with excipients to produce a homogeneous blend. The excipients in a formulation can exert a strong influence on polymorphic conversion and may create new pathways that did not exist for the pure drug substance. One should expect polymorphs with similar thermodynamic energies to be prone to substantial conversion during a milling operation.

The key parameters that cause polymorphic conversion or dehydration of hydrates at the formulation stage are milling and granulation wetting. Other factors that can be important are drying, tableting, and drying of the film coating. In addition, one can have migration of water between drug substance, excipients, and capsule shells. All these parameters can become more important during prolonged processing times should the batch size become sufficiently large.

During mechanical treatment, the most likely mechanism for polymorphic interconversion is that of nucleation and growth of a second phase within the original phase. Nucleation proceeds from dislocation sites in a crystal, since their higher free energy ensures that the energy needed for transformation is lower at these sites. In the majority of cases, metastable phases transform to the most stable form. The degree of polymorphic conversion will depend on the relative stability of the phases in question, and on the type and degree of mechanical processing applied. These phase conversions are generally modulated

by the presence of excipients in the formulation, and consequently they must be investigated in the dosage form as well as in the bulk drug substance.

The goal in any industrial pharmaceutical organization is to have the thermodynamically preferred polymorph or solvate present in the first scaled-up batch of drug substance. If this situation is achieved, then all toxicology, pharmacokinetic, and clinical studies will be conducted with the crystalline form that is likely to be the commercial form of the drug substance. This will eliminate expensive retesting should a more stable but previously unknown polymorph appear. Prudent drug development programs will identify the preferred crystalline form early in development, with a polymorph/salt group working in close conjunction with process chemists to make the thermodynamically preferred polymorphic form the initial kilogram-scale batch. They will also identify and define the physicochemical boundaries of that polymorph.

In the end, however, there is no substitute for multidisciplinary studies whose goal is to determine the likelihood of polymorphic interconversions at any time during the handling of the drug substance. Fortunately, the wide variety of available characterization methods makes it possible to detect virtually any problem that could be encountered during the course of drug development. The successful approach to problem solving will require the full collaboration of process, formulation, and analytical scientists and the complete sharing of information. Although problems in development invariably occur at the worst possible moment, the judicious use of information previously gathered from properly designed studies should minimize their impact and lead to ready deductions of methods for their elimination.

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