Preformulation

When the first quality sample of the new drug becomes available, probing experiments should be conducted to determine the magnitude of each suspected problem area. If a deficiency is detected, then the project team should decide on the molecular modification(s) that would most likely improve the drug's properties. Salts, prodrugs, solvates, polymorphs, or even new analogs may emerge from this modification effort.

While salt formation is limited to molecules with ionizable groups, prodrugs may be formed with any organic molecule having a chemically reactive functional group. Prodrugs are synthetic derivatives (e.g., esters and amides) of drug molecules that may have intrinsic pharmacologic activity but usually must undergo some transformation in vivo to liberate the active drug molecule. Through the formation of a prodrug, a variety of side chains or functional groups may be added to improve the biologic and/or pharmaceutical properties of a compound.[2] Some of the biologic response parameters that may be altered by prodrug formation are absorption due to increased lipophilicity or increased water solubility, duration of action via blockade of a key metabolic site, and distribution to organs due to changes in lipophilicity. Examples of biologic improvements are abundant in the steriod and prostaglandin prodrug literature.[3] Pharmaceutical improvements resulting from prodrug formation include stabilization, an increase or decrease in solubility, crystallinity, taste, odor, and reduced pain on injection.

Erythromycin estolate is an example of a prodrug with improved pharmaceutical properties (Fig. 8-3). In aqueous solutions, protonated erythromycin is water-soluble, has a bitter taste, and is rapidly hydrolyzed in gastric acid ($t_{10\%}$ = 9 sec) to yield inactive decay products. To overcome this problem, the water-insoluble lauryl sulfate salt of the propionate ester prodrug (estolate) was formed for use in both suspension and capsule dosage forms. Erythromycin propionate is inactive as an antimicrobial and must undergo ester hydrolysis to yield bioactive erythromycin. In an oral q.i.d. bioavailability comparison between Upjohn's enteric coated tablet formulation of erythromycin base E-Mycin and Dista's nonenteric Ilosone capsule formulation of erythromycin estolate (Fig. 8-4), the lipophilic ester prodrug was absorbed four times more efficiently than the formulated free base, but hydrolyzed only 24% in serum to produce equivalent plasma levels of bioactive erythromycin base.[4,5] Thus, a prodrug was used to overcome a pharmaceutical formulation problem without compromising bioavailability.

To date, most prodrugs have been esters or amides designed to increase lipophilicity. Unfortunately, this type of modification often decreases water solubility and thus decreases the concentration gradient across the cell membrane, which controls the rate of drug absorption. This trade-off between lipophilicity and concentration gradient is generally assumed to result in a net improvement in absorption. In 1980, Amidon suggested the making of water-soluble prodrugs by adding selected amino acids that are substrates for enzymes located in the intestinal brush border.[6] Assuming that enzyme cleavage was not rate-limiting, and that the liberated drug molecule would remain in the lipophilic membrane, then the resulting membrane transport of the parent compound should be very rapid, owing to the large concentration gradient of liberated drug across the membrane, as illustrated in Figure 8-5. Using the lysine ester pro-

drug of estrone, a potential increase of five orders of magnitude in adsorption rate was found in vivo using perfused rat intestines.

Although any of the modifications discussed may provide an increase in bioavailability, chemical instability or a lack of synthetic feasibility may prohibit the commercial development of a modified drug molecule. Whatever the case, the molecular form of the drug advancing from this preliminary evaluation should have a sub-

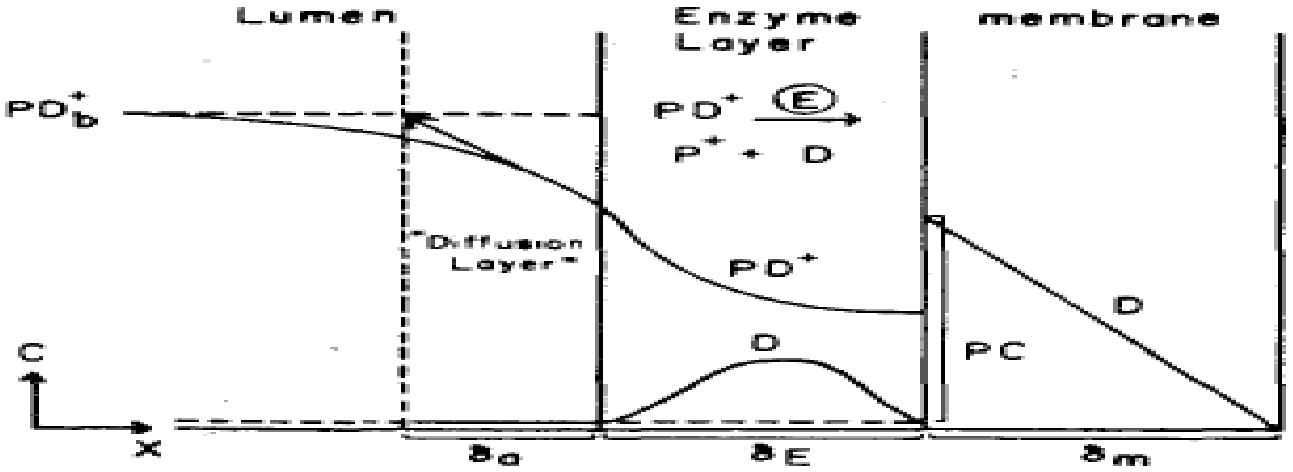


FIG. 8-5. *Concentration (C) versus distance (X) profile for the absorption of water-soluble prodrugs (PD^+), which are enzymatically (E) hydrolyzed in the intestinal brush border to liberate the lipophilic parent compound (D). Key: δ_o, thickness of aqueous diffusion layer; δ_E, enzyme layer thickness; δ_m, membrane thickness; and PC membrane-enzyme layer partition coefficient. (From Amidon, G. L., et al.: J. Pharm. Sci., 69:1363, 1980. Reproduced with permission of the copyright owner.)*

I. Bulk Characterization

Crystallinity and Polymorphism
Hygroscopicity
Fine Particle Characterization
Bulk Density
Powder Flow Properties

II. Solubility Analysis

Ionization Constant – pKa
pH Solubility Profile
Common Ion Effect – K_{sp}
Thermal Effects
Solubilization
Partition Coefficient
Dissolution

III. Stability Analysis

Stability in Toxicology Formulations
Solution Stability
    pH Rate Profile
Solid State Stability
    Bulk Stability
    Compatibility

Crystallinity and Polymorphism

Crystal habit and the internal structure of a drug can affect bulk and physicochemical properties, which range from flowability to chemical stability. *Habit* is the description of the outer appearance of a crystal whereas the *internal structure* is the molecular arrangement within the solid. Several examples of different habits of

The internal structure of a compound can be classified in a variety of ways, as shown in Figure 8-8. The first major distinction is whether the solid is crystalline or amorphous. Crystals are characterized by repetitious spacing of con-

stituent atoms or molecules in a three-dimensional array, whereas amorphous forms have atoms or molecules randomly placed as in a liquid. Amorphous forms are typically prepared by rapid precipitation, lyophilization, or rapid cooling of liquid melts. Since amorphous forms are usually of higher thermodynamic energy than corresponding crystalline forms, solubilities as well as dissolution rates are generally greater. Upon storage, amorphous solids tend to revert to more stable forms. This thermodynamic instability, which can occur during bulk processing

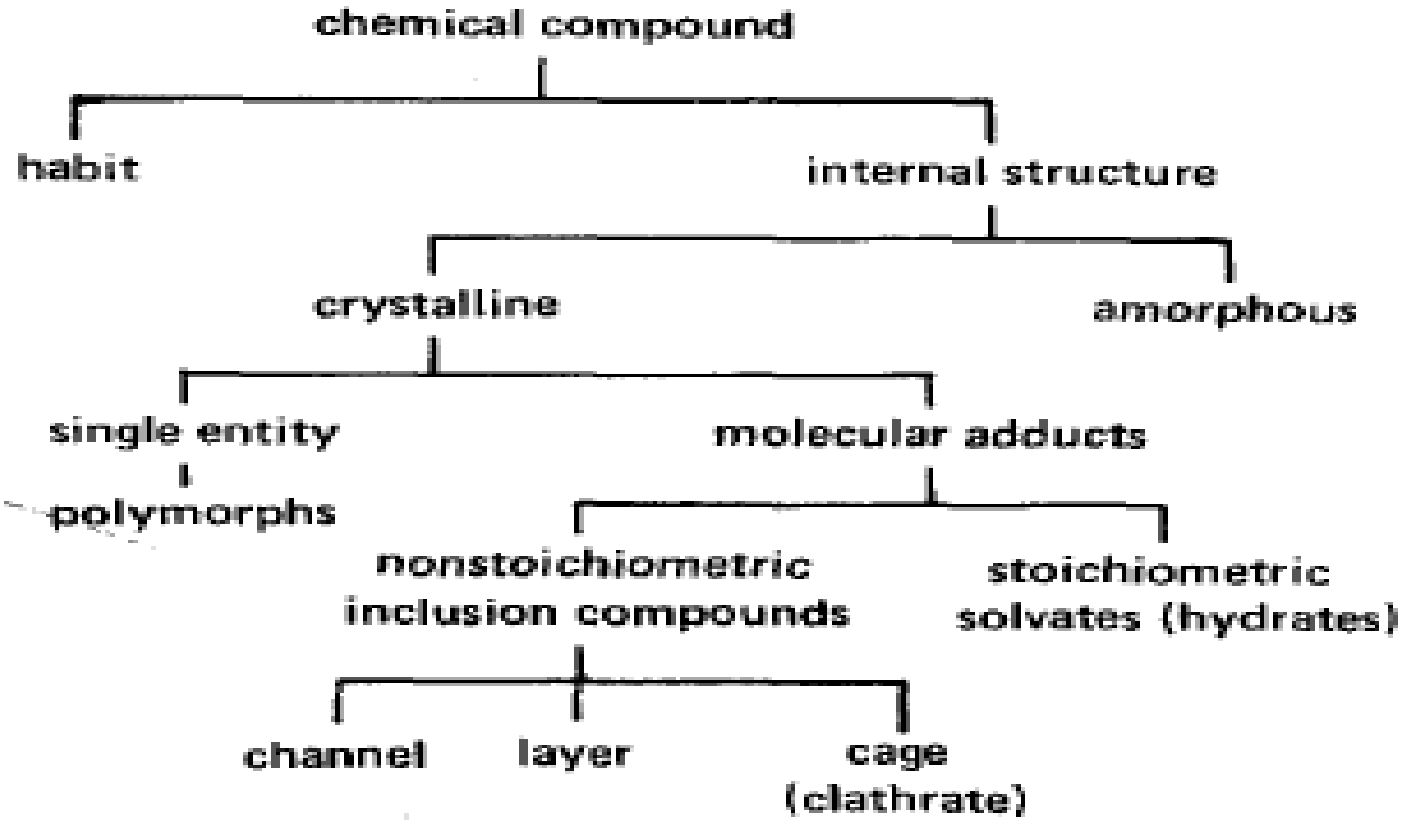


FIG. 8-8. *Outline of differentiating habit and crystal chemistry of a compound. (From Haleblian, J. K.: J. Pharm. Sci., 64:1269, 1975. Reproduced with permission of the copyright owner.)*

or within dosage forms, is a major disadvantage for developing an amorphous form.

A crystalline compound may contain either a stoichiometric or nonstoichiometric amount of crystallization solvent.[7] Nonstoichiometric adducts, such as inclusions or clathrates, involve entrapped solvent molecules within the crystal lattice. Usually this adduct is undesirable, owing to its lack of reproducibility, and should be avoided for development. A stoichiometric adduct, commonly referred to as a solvate, is a molecular complex that has incorporated the crystallizing solvent molecules into specific sites within the crystal lattice. When the incorporated solvent is water, the complex is called a hydrate, and the terms hemihydrate, monohydrate, and dihydrate describe hydrated forms with molar equivalents of water corresponding to half, one, and two. A compound not containing any water within its crystal structure is termed anhydrous.

Identification of possible hydrate compounds is important since their aqueous solubilities can be significantly less than their anhydrous forms. Conversion of an anhydrous compound to a hydrate within the dosage form may reduce the dissolution rate and extent of drug absorption.

An example of the in vivo importance of solvate forms is shown in Figure 8-9, where the

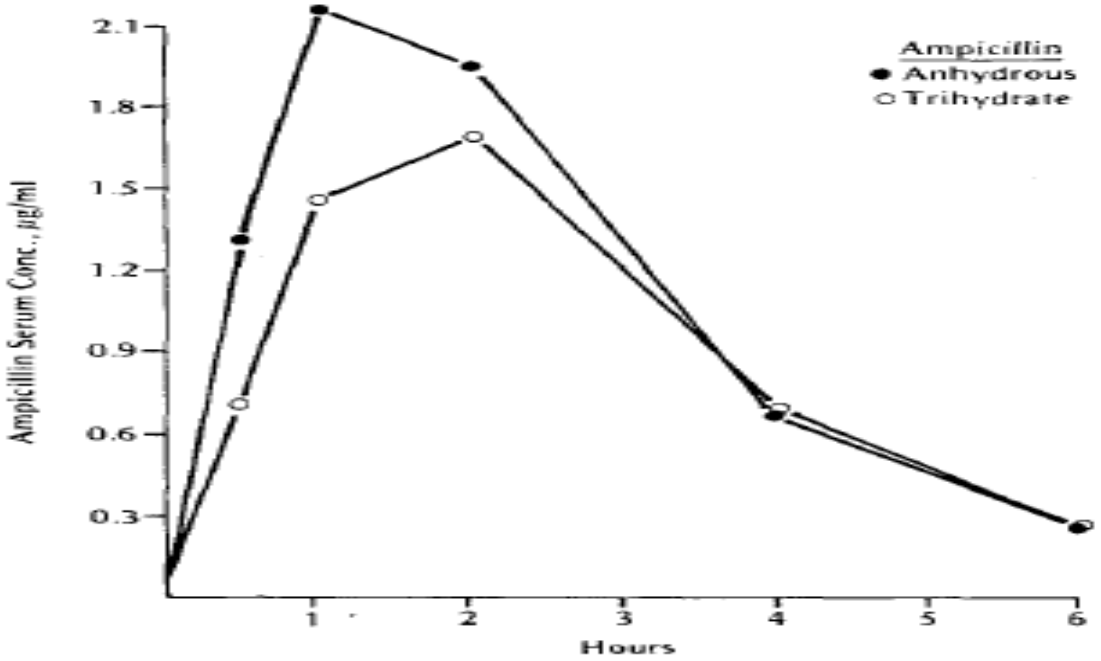


FIG. 8-9. *Mean serum concentrations of ampicillin in human subjects after oral administration of 250-mg doses of two solvate forms of the drug in suspension. Key: ●, anhydrous; and ○, trihydrate. (From Poole, J., et al.: Current Therapeutic Research, 10:292, 1968. Reproduced with permission of the copyright owner.)*

anhydrous and trihydrate forms of ampicillin were administered orally as a suspension to human subjects.[8] The more soluble anhydrous form (10 mg/ml) produced higher and earlier peaks in the blood serum levels than the less soluble trihydrate form.

Polymorphism is the ability of a compound (or element) to crystallize as more than one distinct crystalline species with different internal lattices. Chemical stability and solubility changes due to polymorphism can have an impact on a drug's bioavailability and its development program. Chloramphenicol palmitate exists in three crystalline polymorphic forms (A, B, and C) and an amorphous form.[9] Aguiar and co-workers investigated the relative absorption of polymorphic forms A and B from oral suspensions administered to human subjects.[10] As summarized in Figure 8-10, "peak" serum levels increased substantially as a function of the percentage of form B polymorph, the more soluble polymorph.

Many physicochemical properties vary with the internal structure of the solid drug, including melting point, density, hardness, crystal shape, optical properties, and vapor pressure.[11] Characterization of polymorphic and solvated forms involves quantitative analysis of these differing physicochemical properties. Several

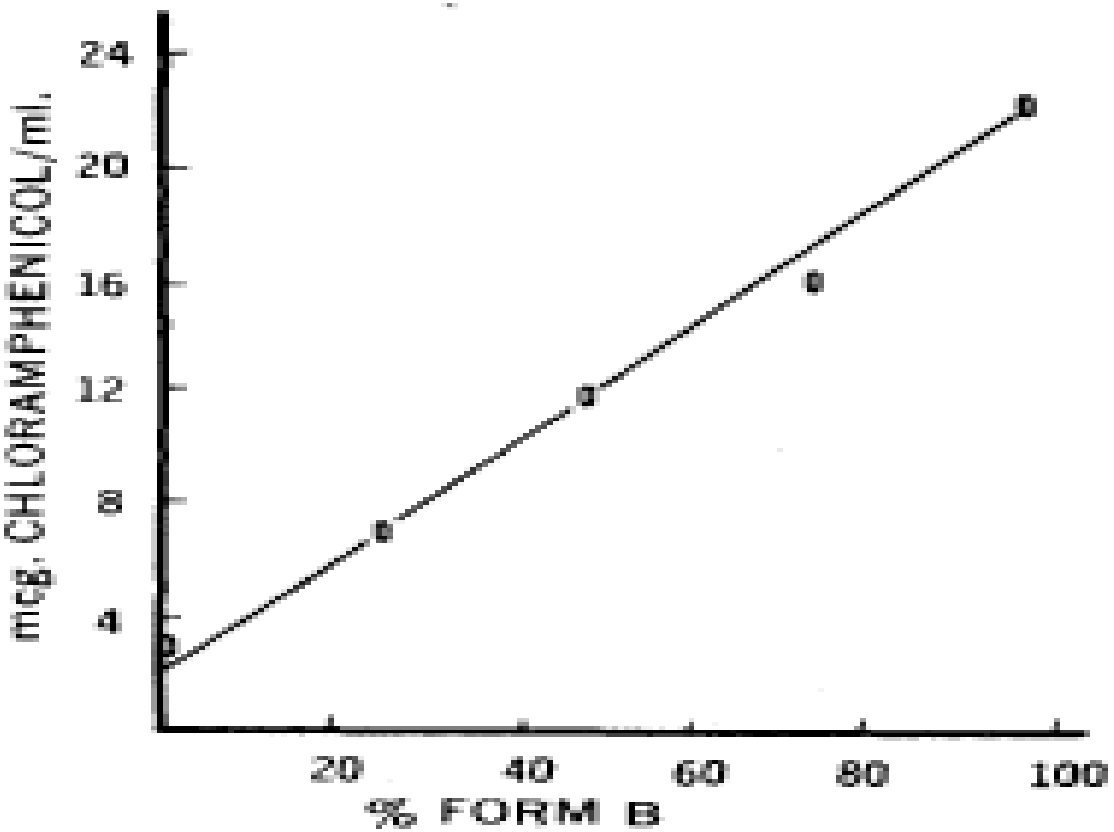


FIG. 8-10. *Correlation of "peak" blood serum levels (2 hr) of chloramphenicol vs. percentage of concentration of polymorph B. (From Agatar, A. J., et al.: J. Pharm. Sci., 56:847, 1967. Reproduced with permission of the copyright owner.)*

TABLE 8-3. *Analytic Methods for Characterization of Solid Forms*

Method	*Material Required per Sample*
Microscopy	1 mg
Fusion methods (hot stage microscopy)	1 mg
Differential scanning calorimetry (DSC/DTA)	2–5 mg
Infrared spectroscopy	2–20 mg
X-ray powder diffraction	500 mg
Scanning electron microscopy	2 mg
Thermogravimetric analysis	10 mg
Dissolution/solubility analysis	mg to gm

Microscopy. All substances that are transparent when examined under a microscope that has crossed polarizing filters are either isotropic or anisotropic. Amorphous substances, such as supercooled glasses and noncrystalline solid organic compounds, or substances with cubic crystal lattices, such as sodium chloride, are isotropic materials, which have a single refractive index. With crossed polarizing filters, these isotropic substances do not transmit light, and they appear black. Materials with more than one refractive index are anisotropic and appear bright with brilliant colors (birefringence) against the black polarized background. The interference colors depend upon the crystal thickness and the differences in refractive indices. Anisotropic substances are either uniaxial, having two refractive indices, or biaxial, having three principal refractive indices.

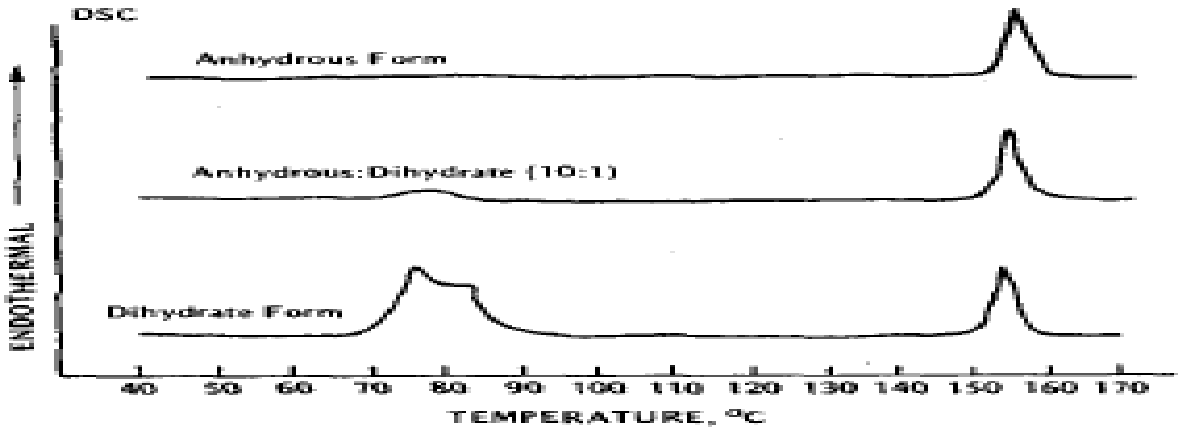
even simple biaxial systems. Crystal morphology differences between polymorphic forms, however, are often sufficiently distinct that the microscope can be used routinely by the less experienced microscopist to describe polymorphic

The polarizing microscope fitted with a hot stage is a useful instrument for investigating polymorphism, melting points, transition temperatures, and rates of transition at controlled heating rates. In addition, the hot-stage microscope facilitates differentiation of DSC endotherms (explained in the next section) for polymorphic transitions from desolvation processes (when the sample is heated in degassed immersion oil). A problem often encountered during thermal microscopy is that organic molecules can degrade during the melting process, and recrystallization of the melt may not occur, because of the presence of contaminant degradation products.

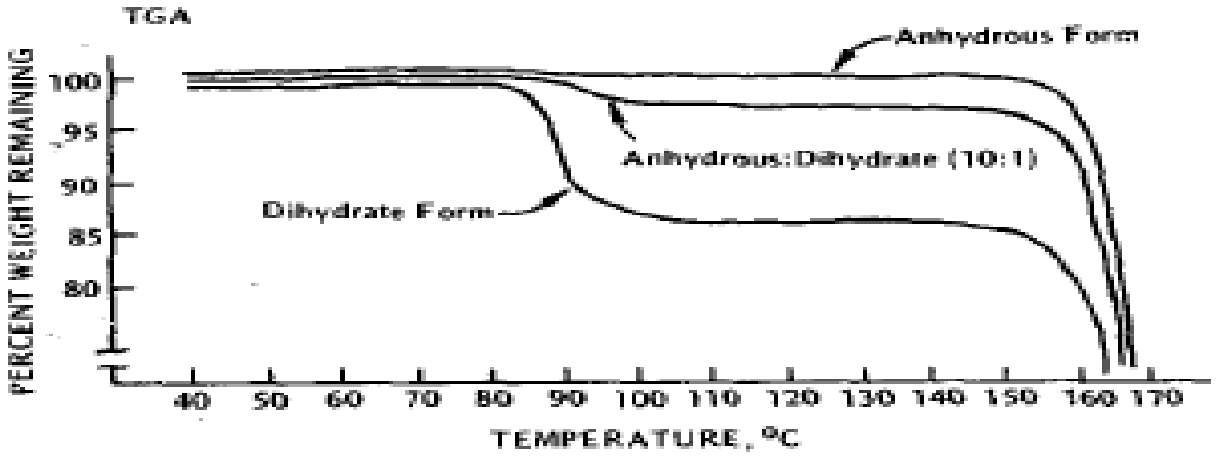
Thermal Analysis. Differential scanning calorimetry (DSC) and differential thermal analysis (DTA) measure the heat loss or gain resulting from physical or chemical changes within a sample as a function of temperature. Examples of endothermic (heat-absorbing) processes are fusion, boiling, sublimation, vaporization, desolvation, solid-solid transitions, and chemical degradation. Crystallization and degradation are usually exothermic processes. Quantitative measurements of these processes have many applications in preformulation studies including purity,[12] polymorphism,[13] solvation,[14] degradation, and excipient compatibility.[15,16]

For characterizing crystal forms, the heat of fusion, ΔH_f, can be obtained from the area-under-the-DSC-curve for the melting endotherm. Similarly, the heat of transition from one polymorph to another may be calculated as shown by Guillory for several sulfonamides.[13] A sharp symmetric melting endotherm can indicate relative purity, whereas broad, asymmetric curves suggest impurities or more than one thermal process. Heating rate affects the kinet-

A variable with DSC experiments is the atmosphere in contact with the sample. Usually, a continual nitrogen purge is maintained within the heating chamber; however, the loss of a volatile counter ion such as ethanolamine or acetic acid during a polymorphic transition may produce misleading data unless the transition occurs within a closed system. In contrast, desolvation of a dihydrate-species, as shown in Figure 8-11, releases water vapor, which if unvented can generate degradation prior to the melting point of the anhydrous form. During initial testing, a variety of atmospheres should be tried until the observed thermal process becomes fully understood.



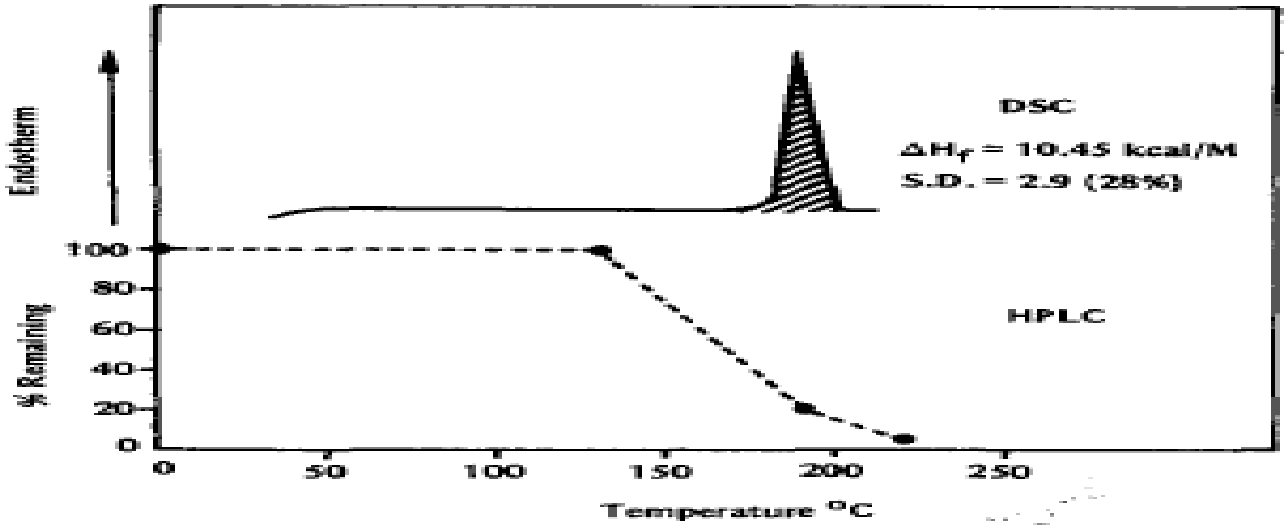
Thermogravimetric analysis (TGA) measures changes in sample weight as a function of time (isothermal) or temperature. Desolvation and decomposition processes are frequently monitored by TGA. Comparing TGA and DSC data recorded under identical conditions can greatly aid in the interpretation of thermal processes. In Figure 8-11, the dihydrate form of an acetate salt loses two moles of water via an endothermic transition between 70° and 90°C. The second endotherm at 155°C corresponds to the melting process, with the accompanying weight loss due to vaporization of acetic acid as well as to decomposition.



TGA and DSC analysis can also be used to quantitate the presence of a solvated species within a bulk drug sample. For the above example, 10% of the dihydrate form was easily detected by both methods (Fig. 8-11).

ple preparation. Degradation during thermal *analysis may provide misleading results, but*

may be detected by high-performance liquid chromatography (HPLC) analysis of samples heated under representative conditions for retention of drug or appearance of decay products (Fig. 8-12).



X-Ray. An important technique for establishing the batch-to-batch reproducibility of a crystalline form is x-ray powder diffraction. Random orientation of a crystal lattice in a powder sample causes the x-rays to scatter in a reproducible pattern of peak intensities at distinct angles (θ) relative to the incident beam. Each diffraction pattern is characteristic of a specific crystalline lattice for a given compound.[17] An amorphous form does not produce a pattern. Mixtures of different crystalline forms can be analyzed using normalized intensities at specific angles, which are unique for each crystalline form. A typical

Polymorphism

Polymorphs can be classified as one of two types: *enatiotropic* (one polymorph can be reversibly changed into another by varying temperature or pressure, e.g., sulfur) or *monotropic* (one polymorphic form is unstable at all temperatures and pressures, e.g., glyceryl stearates). There is no general way of relating enatiotrophy and monotrophy to the properties of the polymorphs, except by locating the transition temperature or the lack of one. At a specified pressure, usually 1 atmosphere, the temperature at which two polymorphs have identical free energies is the transition temperature, and at that temperature, both forms can coexist and have identical solubilities in any solvent as well as identical vapor pressures. Below the solid melting temperatures, the polymorph with the lower free energy, corresponding to the lower solubility or vapor pressure, is the thermodynamically stable form.

During preformulation, it is important to identify the polymorph that is stable at room temperature and to determine whether polymorphic transitions are possible within the temperature range used for stability studies and during processing (drying, milling, etc.). As discussed by Haleblian and McCrone, a polymorphic compound is best characterized by a complete pressure-temperature phase diagram showing melt-vapor, solid-vapor, and solid-melt curves.[11] A free energy-temperature curve at 1 atmosphere should be constructed since temperature is usually a more critical variable than pressure in pharmaceutics. As previously discussed, chloramphenicol palmitate has three known polymorphic forms, which are thermodynamically described by a van't Hoff plot of free energy (as determined from solubility measurements) versus temperature (Fig. 8-14). Transition temperatures are shown by intersection of the extrapolated lines; 50°C for forms A and C, and 88°C for forms A and B. Form A is the stable form at temperatures less than 50°C.[10]

Transition temperatures obtained by extrapolation of van't Hoff plots are susceptible to large errors. Direct measurements of transitions are preferred to support the extrapolated intersection points in the solubility-temperature diagrams. The most direct means for determining transition temperatures is microscopic observation of samples held at constant temperatures. Unfortunately, these solid-solid or solid-vapor-solid transitions usually occur slowly, owing to large activation energies and slow nucleation. To facilitate the conversion rate, a single polymorph or a mixture of forms can be granulated in a "bridging" solvent at various temperatures. The drug should be only sparingly soluble in the bridging solvent, and solvate formation should not occur. These experiments can be conducted quickly with a polarizing microscope, or samples can be stored in sealed containers at controlled temperatures and periodically examined by other suitable analytic methods.

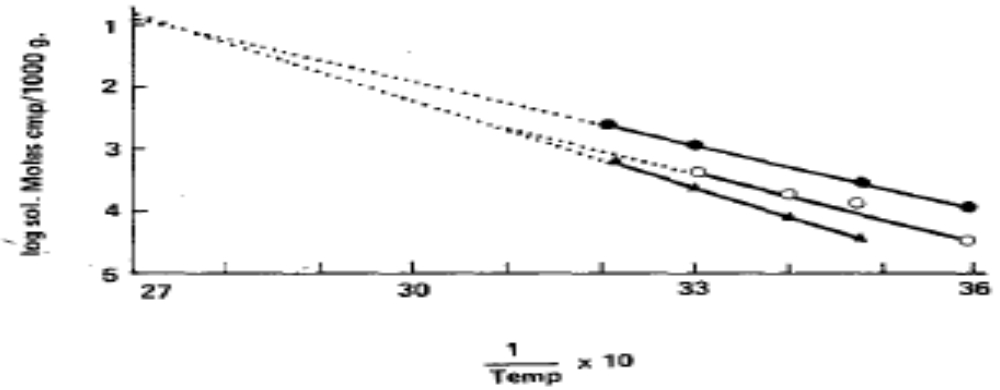


FIG. 8-14. *The van't Hoff plot of solubility vs. reciprocal absolute temperature for polymorphs A, B, and C of chloramphenicol palmitate. Key: Polymorphs A (►—►); B (●—●); and C (○—○). (From Aguiar, A. J., et al.: J. Pharm. Sci., 58:983, 1969. Reproduced with permission of the copyright owner.)*

morphism is determination of the relative stability of metastable polymorph and prediction of its rate of conversion within a dosage form. For suspension dosage forms, the rate of conversion can depend on several variables, including drug solubility within the vehicle, presence of nucleation seed for the stable form, temperature, agitation, and particle size. Solid dosage forms such as capsules and tablets have similar complications due to the influence of particle size, moisture, and excipients. In short, the most effective

Hygroscopicity

Many drug substances, particularly water-soluble salt forms, have a tendency to adsorb atmospheric moisture. Adsorption and equilibrium moisture content can depend upon the atmospheric humidity, temperature, surface area, exposure, and the mechanism for moisture uptake, as described by Van Campen and coworkers.[18] Deliquescent materials adsorb sufficient water to dissolve completely, as is observed with sodium chloride on a humid day. Other hygroscopic substances adsorb water because of hydrate formation or specific site adsorption. With most hygroscopic materials, changes in moisture level can greatly influence many important parameters, such as chemical stability, flowability, and compactibility.

To test for hygroscopicity, samples of bulk drug are placed in *open* containers with a thin powder bed to assure maximum atmospheric exposure. These samples are then exposed to a range of controlled relative humidity environments prepared with saturated aqueous salt solutions.[19] Moisture uptake should be monitored

Fine Particle Characterization

Bulk flow, formulation homogeneity, and surface-area controlled processes such as dissolution and chemical reactivity are directly affected by size, shape, and surface morphology of the drug particles. In general, each new drug candidate should be tested during preformulation with the smallest particle size as is practical to facilitate preparation of homogeneous samples and maximize the drug's surface area for interactions.

A light microscope with a calibrated grid usually provides adequate size and shape characterization for drug particles.[20] Sampling and prepa-

In conjunction with light microscopy, stream counting devices, such as the Coulter counter and HIAC counter, often provide a convenient method for characterizing the size distribution of a compound. Samples are prepared for analysis by the Coulter counter by dispersing the material in a conducting medium such as isotonic saline with the aid of ultrasound and a few drops of surfactant. A known volume (0.5 to 2 ml) of this suspension is then drawn into a tube through a small aperture (0.4 to 800 microns in diameter), across which a voltage is applied. As each particle passes through the hole, it is counted and sized according to the resistance

generated by displacing that particle's volume of conducting medium. Given that the instrument has been calibrated with standard spheres, the counter provides a histogram output (frequency versus size) within the limits of that particular aperture tube. Several different sizes of aperture tubes should be used to assure accurate counting of single particles. Other stream counters are based on the principles of light blockage or laser light scattering for sizing each particle.[20]

Although the Coulter method is quick and statistically meaningful, it assumes that each resistance arises from a spherical particle; thus, nonspheres are sized inaccurately. Other limitations with the Coulter counter are the tendency of needle-shaped crystals to block the aperture hole, the dissolution of compound in the aqueous conducting medium, and stratification of particles within the suspension. Additional methods of particle size analysis are image analysis and sieve analysis. Sieve methods are used primarily for large samples of relatively large particles (~100 microns). Computer interfacing

Kinetic processes involving drug in the solid state, such as dissolution and degradation, may be more directly related to available surface area than to particle size. If drug particles have a shape that can be defined mathematically, then light microscopy size analysis or Coulter counter analysis with appropriate geometric equations may provide a reasonable estimation of surface area.

A more precise measurement of surface area is made by Brunauer, Emmett, and Teller (BET) nitrogen adsorption, in which a layer of nitrogen molecules is adsorbed to the sample surface at −196°C. Once surface adsorption has reached equilibrium, the sample is heated to room temperature, the nitrogen gas is desorbed, and its volume is measured and converted to the number of adsorbed molecules via the ideal gas law. Since each nitrogen molecule (N_2) occupies an area of $16A^2$, one may readily compute the surface area per gram for each preweighed sample. By determining the surface area at several partial pressures of nitrogen (5% to 35% N_2 in He), extrapolation to zero nitrogen partial pressure yields the true monolayer surface area. While BET measurements are usually precise and quickly obtained with current commercial equipment, errors may arise from the use of impure gases and volatile surface impurities (e.g., hydrates).

Surface morphology may be observed by scanning electron microscopy (SEM), which serves to confirm qualitatively a physical observation

During preparation for SEM analysis, the sample is exposed to high vacuum during the gold coating process, which is needed to make the samples conductive, and concomitant removal of water or other solvents may result in a false picture of the surface morphology. Variable vacuum treatment of an identical sample prior to the gold coating step may confirm the effects of sample preparation on surface morphology. Most modern SEM instruments also provide energy dispersive x-ray spectroscopy analysis of surface metal ions, which may prove beneficial in deciphering an instability or incompatibility problem.

Bulk Density

Bulk density of a compound varies substantially with the method of crystallization, milling, or formulation. Once a density problem is identified, it is often easily corrected by milling, slugging, or formulation. Usually, bulk density is of great importance when one considers the size of a high-dose capsule product or the homogeneity of a low-dose formulation in which there are large differences in drug and excipient densities.

Apparent bulk density (g/ml) is determined by pouring presieved (40-mesh) bulk drug into a graduated cylinder via a large funnel and measuring the volume and weight "as is." Tapped density is determined by placing a graduated cylinder containing a known mass of drug or formulation on a mechanical tapper apparatus, which is operated for a fixed number of taps (~1000) until the powder bed volume has reached a minimum. Using the weight of drug in the cylinder and this minimum volume, the tapped density may be computed. Knowing the anticipated dose and tapped formulation density, one may use Figure 8-15 to determine the appropriate size for a capsule formulation.

In addition to bulk density, it is frequently desirable to know the true density of a powder for computation of void volume or porosity of packed powder beds. Experimentally, the true density is determined by suspending drug particles in solvents of various densities and in which the compound is insoluble. Wetting and pore penetration may be enhanced by the addition of a small quantity of surfactant to the solvent mixtures. After vigorous agitation, the samples are centrifuged briefly and then left to stand undisturbed until floatation or settling has reached equilibrium. The sample that remains suspended corresponds to the true density of the material. Density of the test solution corre-

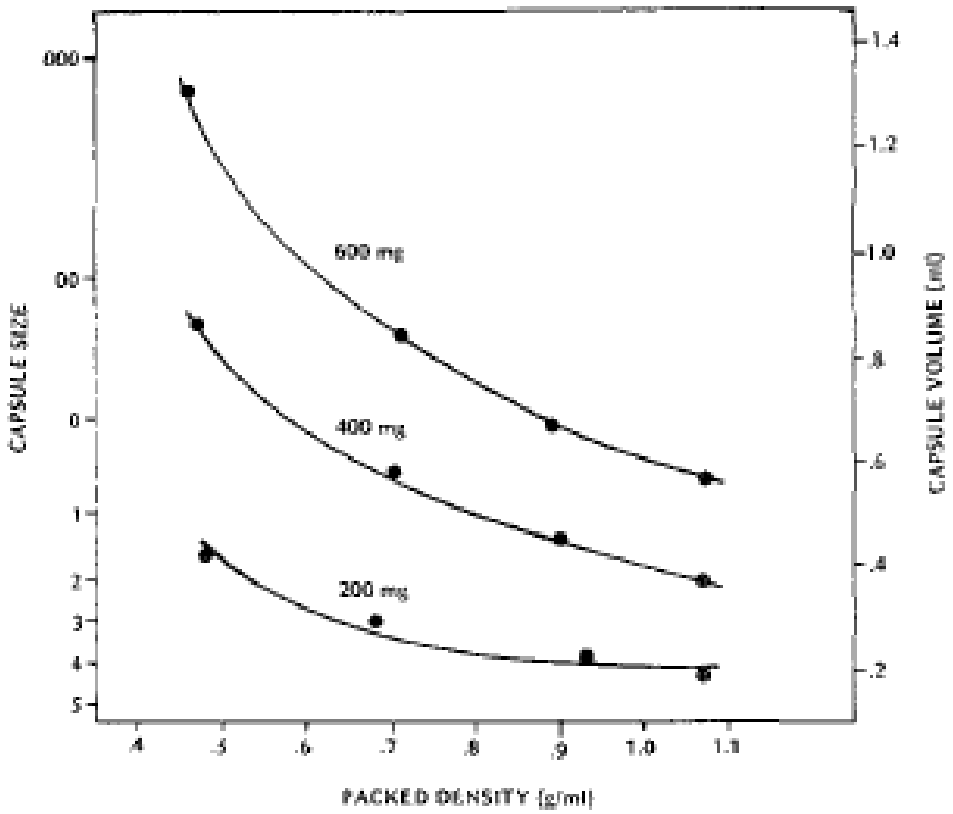


FIG. 8-15. *Correlation between capsule size and packed density for different fill weights (200–600 mg).*

Powder Flow Properties

Pharmaceutical powders may be broadly classified as free-flowing or cohesive (non-free-flowing). Most flow properties are significantly affected by changes in particle size, density, shape, electrostatic charge, and adsorbed moisture, which may arise from processing or formulation.[22] As a result, a free-flowing drug candidate may become cohesive during development, thus necessitating an entirely new formulation strategy. Preformulation powder flow investigations should quantitatively assess the pharmaceutical consequences of each process improvement and provide direction for the formulation development project team. This direction may consist of a formulation recommendation such as granulation or densification via slugging, the need for special auger feed equipment, or a test system for evaluating the improvements in flow brought about by formulation. This subject be-

Another measurement of a free-flowing powder is compressibility, as computed from powder density, equation (2):

$$\% \text{ Compressibility} = \left(\frac{\rho_t - \rho_o}{\rho_t}\right) \times 100 \quad (2)$$

where ρ_t is the tapped bulk density and ρ_o is the initial bulk density. Table 8-4 lists compressibility data and flowability characterization for several pharmaceutical excipients.

While angle of repose determinations are usually useless because of their lack of precision, observation of powder flow from a glass funnel and then a grounded metal funnel provides insight into the drug's flow properties, electrostatic properties, and tendency to brige in a cone-shaped hopper.[24]

Table 8-4. *Compressibility and Flowability of Pharmaceutical Excipients.*

% Compressibility	*Flowability*
5–15	Excellent
12–16	Good
18–21	Fair—passable
23–35	Poor
33–38	Very poor
<40	Very, very poor

Solubility Analysis

pKa Determinations

Determination of the dissociation constant for a drug capable of ionization within a pH range of 1 to 10 is important since solubility, and consequently absorption, can be altered by orders of magnitude with changing pH. The Henderson-Hasselbalch equation provides an estimate of the ionized and un-ionized drug concentration at a particular pH.

For acidic compounds:

$$\text{pH} = \text{pKa} + \log \frac{[\text{ionized drug}]}{[\text{un-ionized drug}]}$$

For basic compounds:

$$\text{pH} = \text{pKa} + \log \frac{[\text{un-ionized drug}]}{[\text{ionized drug}]} \qquad (3)$$

A pKa value can be determined by a variety of analytic methods. Buffer, temperature, ionic strength, and cosolvent affect the pKa value and should be controlled for these determinations. The preferred method is the detection of spectral shifts by ultraviolet (UV) or visible spectroscopy since dilute aqueous solutions can be analyzed directly. A second method, potentiometric titration, offers maximum sensitivity for compounds with pKa values in the range of 3 to 10 but is often hindered by precipitation of the un-ionized form during the titration since a high drug concentration is usually required to obtain a significant titration curve. To prevent precipitation, a cosolvent such as methanol or dimethylsulfoxide can be incorporated to maintain sufficient solubility for the un-ionized species, and the pKa value is extrapolated from titration data collected for various cosolvent concentrations. As shown in Figure 8-16, dependence of the dissociation constant on cosolvent can be highly significant, and extrapolations provide only an estimate of the pKa value. In general, the use of

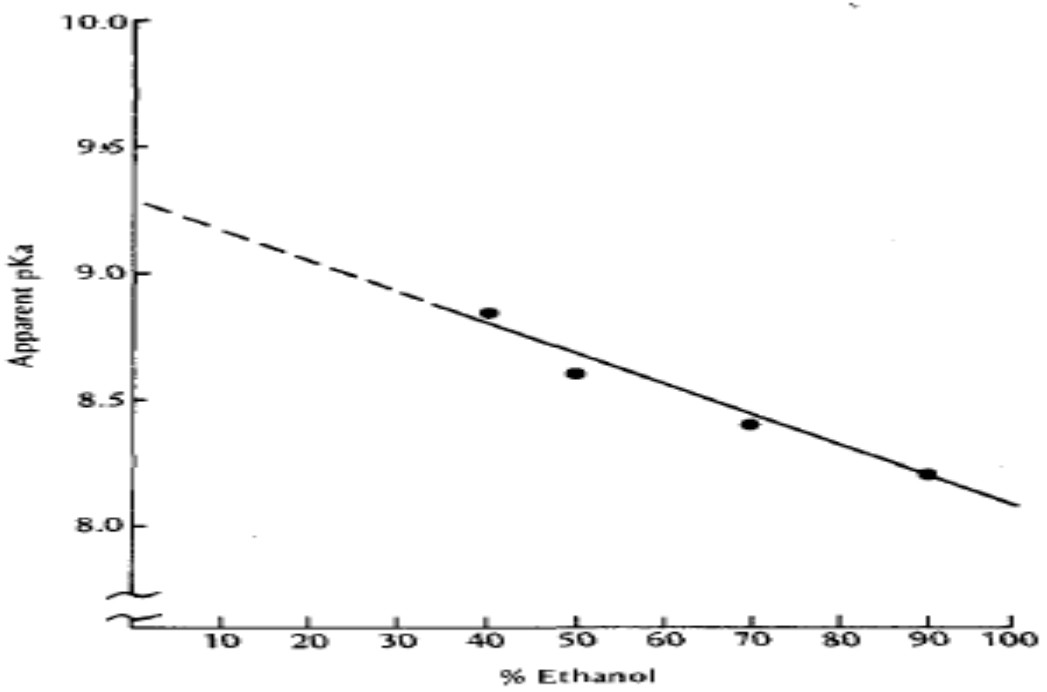


FIG. 8-16. *The pKa determination for an organic amine drug candidate whose un-ionized form is exceedingly insoluble in water.*

pH Solubility Profile and Common Ion Effects

The pH solubility profile for doxycycline (pKa 3.4) reported by Bogardus and Backwood illustrates a common ion effect for an amine hydrochloride salt.[37] As shown in Figure 8-17, the solubility in aqueous medium with pH 2 or less logarithmically decreased as a function of pH (which was adjusted with hydrochloric acid) because of corresponding increases in the chloride ion concentration. In gastric juice, where the pH can range from 1 to 2 and the chloride ion concentration is between 0.1*M* and 0.15*M*, doxycycline hydrochloride dihydrate has a solubility of ~4 mg/ml, which is a factor of 7 less than its solubility in distilled water. For the hydrochloride salts of chlortetracycline, demeclocycline, and methacycline, the apparent dissolution rates and solubilities were even less than their respective free base forms in media containing chloride ion.[38] Consequently, the solu-

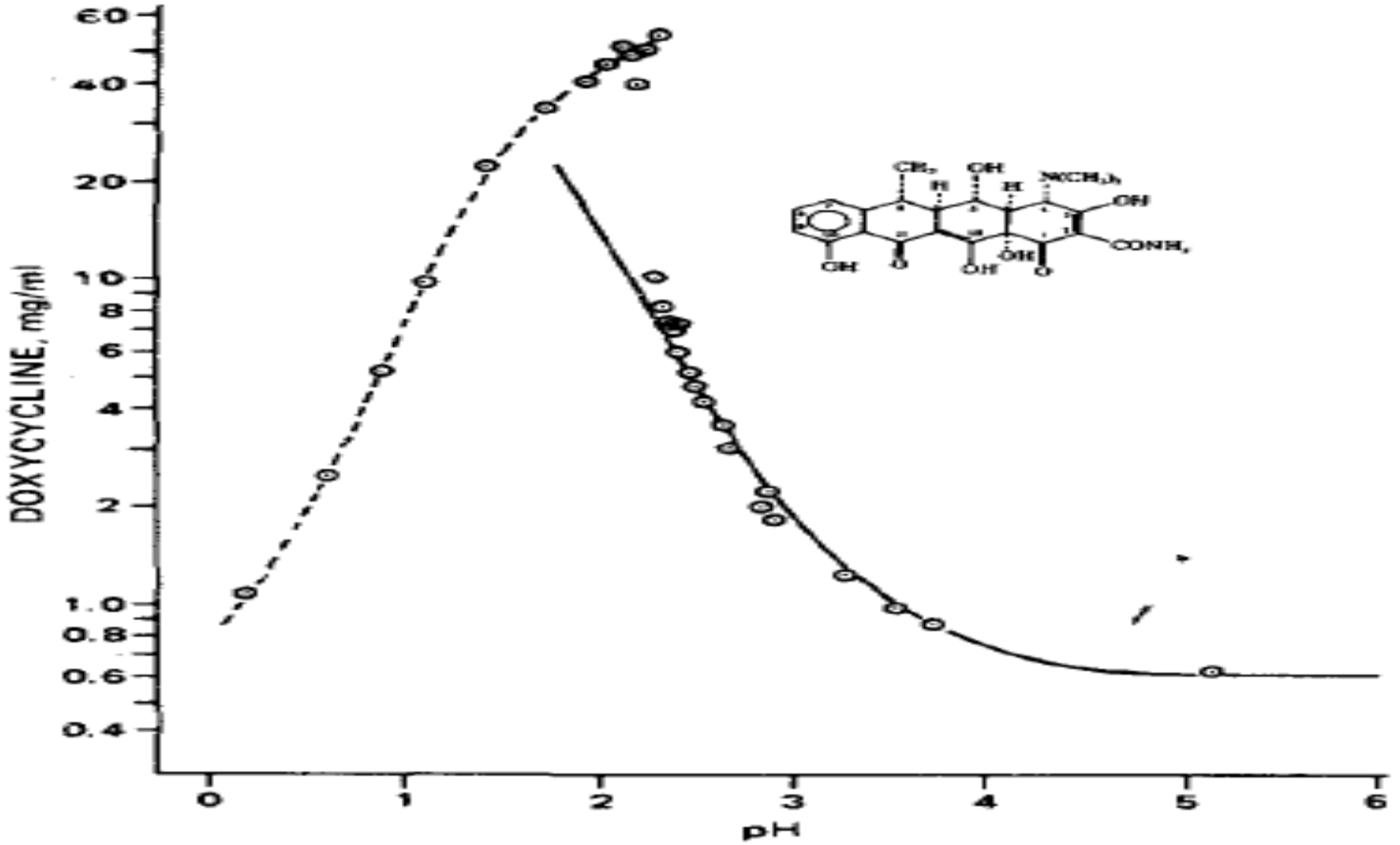


FIG. 8-17. *The pH-solubility profile for doxycycline in aqueous hydrochloric acid at 25°C. At pH = 2.16, both doxycycline monohydrate and doxycycline hydrochloride dihydrate were in equilibrium with solution. Theoretic curves are detailed by authors.* (*From Bogardus, J. B., and Blackwood, R. K.: J. Pharm. Sci., 68:188, 1979. Reproduced with permission of the copyright owner, the American Pharmaceutical Association.*)

Another point illustrated with doxycycline is that nonideal behavior of a solution species can dramatically affect the solubility at certain pH values. Doxycycline was shown to form dimeric species involving self-association of the protonated form. This mechanism accounted for the large positive deviation from ideal behavior, in which actual solubility values are a factor of 10 higher at pH 2.0. Therefore, actual solubility profiles should be experimentally determined within the pH region of interest.

Effect of Temperature

The heat of solution, ΔH_s, represents the heat released or absorbed when a mole of solute is dissolved in a large quantity of solvent. Most commonly, the solution process is endothermic, or ΔH_s is positive, and thus increasing the solution temperature increases the drug solubility. For such solutes as lithium chloride and other hydrochloride salts that are ionized when dissolved, the process is exothermic (negative ΔH_s) such that higher temperatures suppress the solubility.

Heats of solution are determined from solubility values for saturated solutions equilibrated at controlled temperatures over the range of interest. Typically, the temperature range should include 5°C, 25°C, 37°C, and 50°C. The working equation for determining ΔH_s is:

$$\ln S = \frac{-\Delta H_s}{R}\left(\frac{1}{T}\right) + C \qquad (13)$$

where S is the molar solubility at temperature T (°kelvin) and R is the gas constant. Over limited temperature ranges, a semilogarithmic plot of solubility against reciprocal temperature is linear, and ΔH_s is obtained from the slope. For nonelectrolytes and un-ionized forms of weak acids and bases dissolved in water, heats of solution are usually in the range of 4 to 8 kcal/mole. Salt forms of drugs are often less sensitive to temperature and may have heats of solution between −2 and 2 kcal/mole.

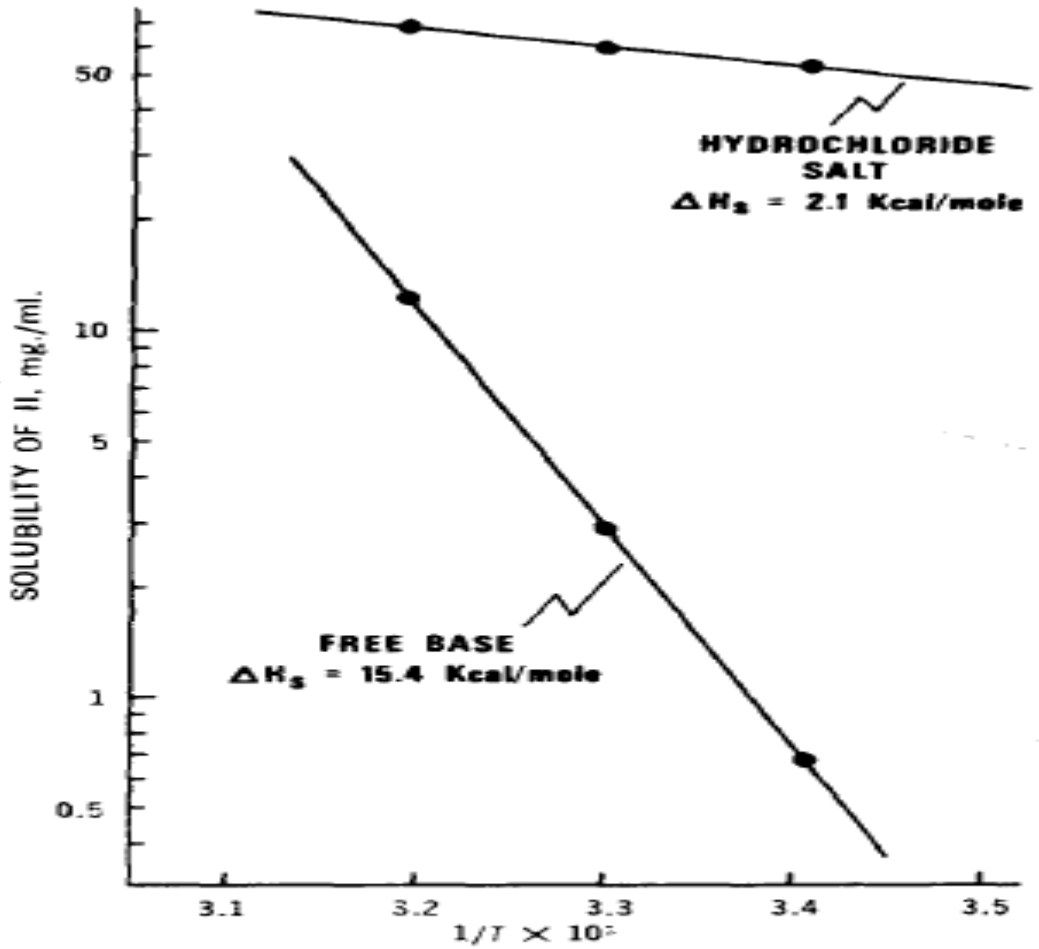


FIG. 8-18. *Plot of hydrochloride and free base solubilities for etoxadrol, an organic amine, against reciprocal temperature. (From Kramer, S. F., and Flynn, G. L.: J. Pharm. Sci., 61:1896, 1972. Reproduced with permission of the copyright owner.)*

Solubilization

For drug candidates with either poor water solubility or insufficient solubility for projected solution dosage forms, preformulation studies should include limited experiments to identify possible mechanisms for solubilization. A general means of increasing solubility is the addition of a cosolvent to the aqueous system. The solubility of poorly soluble nonelectrolytes can often be improved by orders of magnitude with suitable cosolvents such as ethanol, propylene glycol, and glycerin.[39] These cosolvents solubilize drug molecules by disrupting the hydrophobic interactions of water at the nonpolar solute/water interfaces. The extent of solubilization due to the addition of cosolvent depends on the chemical structure of the drug, that is, the more nonpolar the solute, the greater is the solubilization achieved by cosolvent addition. This relationship is illustrated in Figure 8-19 for hydrocortisone and hydrocortisone 21-heptanoate.[40] The lipophilic ester is solubilized to a greater extent by additions of propylene glycol than by the more polar parent compound.

Cosolvent effects for dissociated drug molecules are usually much less, as shown by Kramer and Flynn.[36] Some poorly soluble drugs can be solubilized in micellar solutions such as 0.01M Tween 20, or via molecular complexes as with caffeine.[41,42] These specific formulations are usually not developed during the preformulation phase, however.

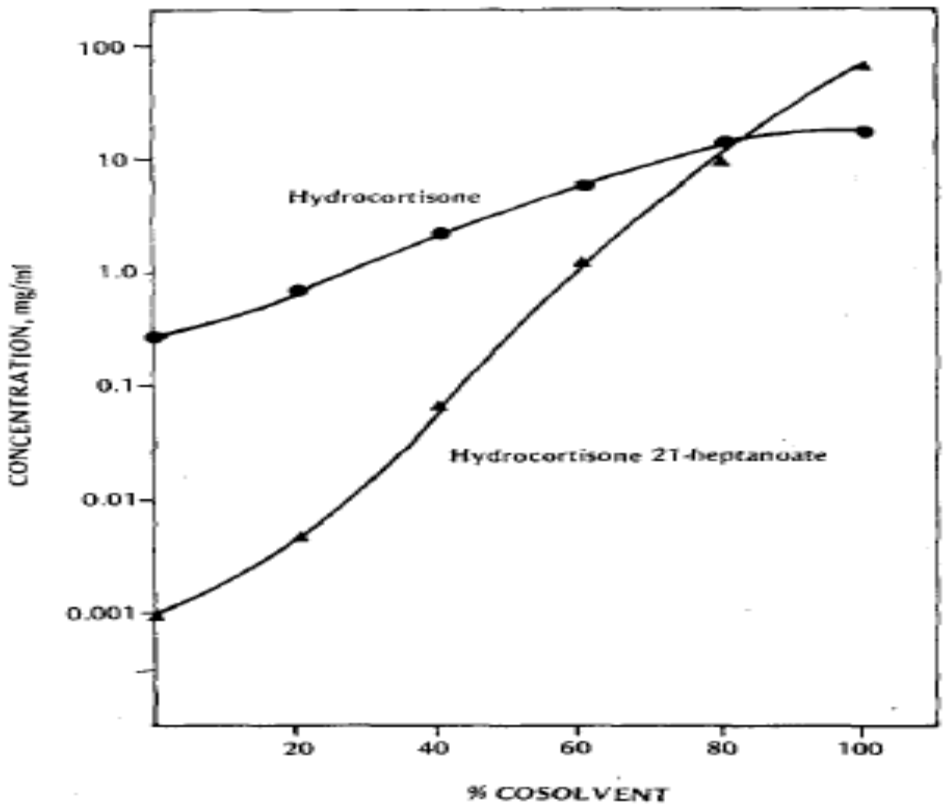


FIG. 8-19. *Solubility of hydrocortisone and hydrocortisone 21-heptanoate in propylene glycol-water mixtures. (From Hagen, T. A.: Ph.D. dissertation, University of Michigan, Ann Arbor, Michigan, 1979.)*

Partition Coefficient

A measurement of a drug's lipophilicity and an indication of its ability to cross cell membranes is the oil/water partition coefficient in systems such as octanol/water and chloroform/water. The partition coefficient is defined as the ratio of un-ionized drug distributed between the organic and aqueous phases at equilibrium.

$$P_{o/w} = \left(\frac{C_{oil}}{C_{water}}\right)_{equilibrium} \qquad (14)$$

Dissolution

Dissolution of a drug particle is controlled by several physicochemical properties, including chemical form, crystal habit, particle size, solubility, surface area, and wetting properties. When coupled with equilibrium solubility data, dissolution experiments can help to identify potential bioavailability problem areas. For example, dissolution of solvate and polymorphic forms of a drug can have a significant impact on bioavailability and drug delivery.

The dissolution rate of a drug substance in which surface area is constant during dissolution is described by the modified Noyes-Whitney equation:

$$\frac{dC}{dt} = \frac{DA}{hV}(C_s - C) \qquad (15)$$

where D is the diffusion coefficient, h is the thickness of the diffusion layer at the solid-liquid interface, A is the surface area of drug exposed to dissolution media, V is the volume of media, C_s is the concentration of a saturated solution of the solute in the dissolution medium at the experimental temperature, and C is the concentration of drug in solution at time t. The dissolution rate is given by dC/dt. If the surface area of the drug is held constant and $C_s >> C$, then equation (15) can be rearranged and integrated to give the working equation:

$$\frac{W}{A} = k\,t \tag{16}$$

where the constant k is defined as:

$$k = \frac{D}{h} C_s \tag{17}$$

and W is the weight (mg) of drug dissolved in time t.

A plot of W versus t gives a straight line with the slope equal to the intrinsic dissolution rate constant k,[46,47] usually expressed in units of mg/cm^2/min.

Experimentally, a constant surface area is obtained by compressing powder into a disc of known area with a die and punch apparatus. Either of the two systems shown in Figure 8-20 can be used to maintain uniform hydrodynamic conditions (k constant). The rotating disc method or Wood's apparatus permits the hydrodynamics of the system to be varied in a mathematically well-defined manner.[48] The static disc method is used because it is conveniently available, but it contains an element of undefined turbulence, which necessitates calibration with standards. Potential problems with this method are transformations of the crystal form, such as polymorphic transformations or desolvation, during its compression into a pellet or during the dissolution experiment. Since many drug candidates are weak acids and bases, pH and common ion gradients at the solid-liquid interface can lead to erroneous conclusions, as discussed by Mooney and co-workers.[49,50]

Dissolution experiments with drug suspensions are further complicated by changing surface area, changing surface crystal morphology, and interstitial wetting.[51] However, dissolution profiles with excess drug can be used to characterize metastable polymorphs or solvates. In Figure 8-21, the conversion of the metastable form II to form I is shown to occur in an organic solvent medium, which clearly depicts form I as the thermodynamically stable form at room temperature. Static pellet dissolution rates also substantiated that form II was the higher energy form since its dissolution rate was significantly greater (Table 8-5).

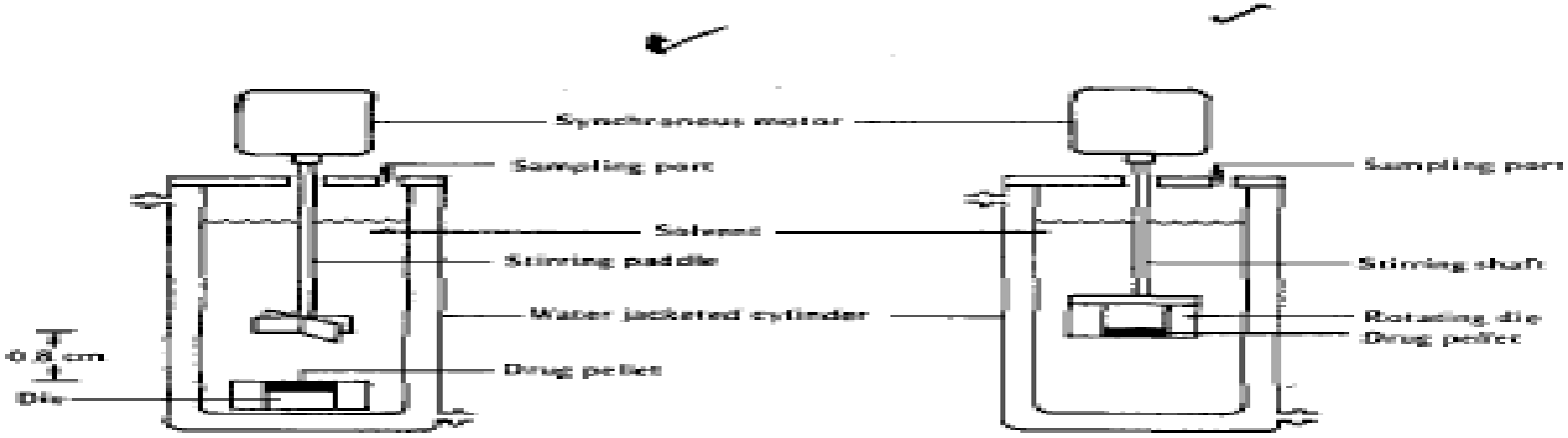


FIG. 8-20. *Constant surface area dissolution apparatus. Left: static disc dissolution apparatus. Right: rotating disc apparatus.*

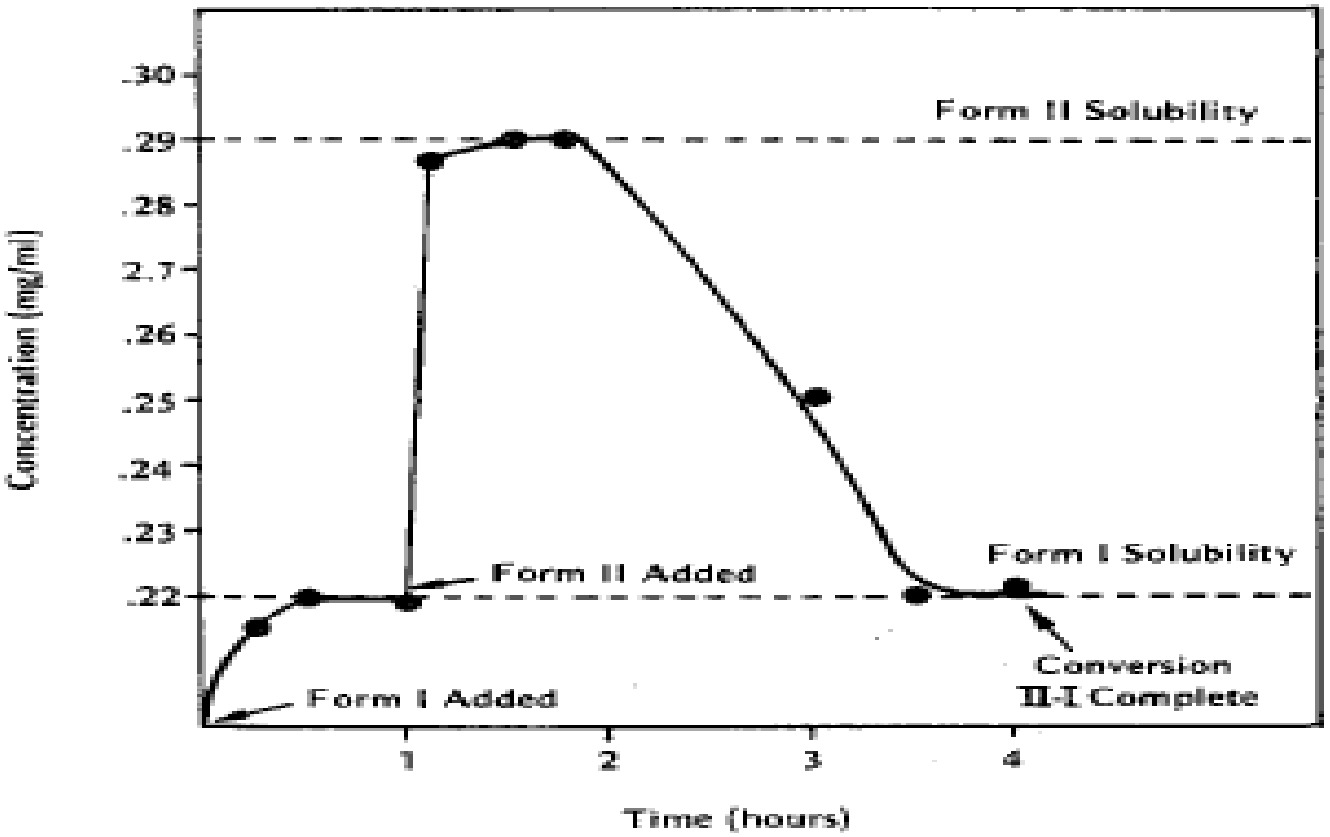


FIG. 8-21. *Powder dissolution profiles for two polymorphic forms of an organic acetate salt in acetonitrile at 25°C.*

TABLE 8-5. *Comparison of Dissolution Rates* and Solubility for Two Polymorphic Forms of an Organic Acetate Salt.*

	Form I	*Form II*	*Ratio I/II*
Dissolution rate in ethanol, $mg/cm^2/min$	0.57	0.69	.83
Dissolution rate in Acetonitrile, $mg/cm^2/min$	0.017	0.027	.62
Solubility in Acetonitrile, mg/ml	.22	.29	.75

**Dissolution rates measured with a static-disc apparatus.*

Stability In Toxicology Formulations

Since toxicology studies typically commence early in drug development, it is often advisable to evaluate samples of the toxicology preparations for stability and potential homogeneity problems. Usually, a drug is administered to the animals in their feed, or by oral gavage of a solution or suspension of the drug in an aqueous vehicle.

Solution Stability

The primary objective of this phase of preformulation research is identification of conditions necessary to form a stable solution. These studies should include the effects of pH, ionic strength, cosolvent, light, temperature, and oxygen.

Solution stability investigations usually commence with probing experiments to confirm decay at the extremes of pH and temperature (e.g., 0.1N HCl, water, and 0.1N NaOH all at 90°C). These intentionally degraded samples

To generate a pH-rate profile, stability data generated at each pH and temperature condition are analyzed kinetically to yield the apparent decay rate constants. All of the rate constants at a single temperature are then plotted as a function of pH as shown in Figure 8-22. The minimum in this curve is the pH of maximum stability. Often, this plot, as it approaches its limits, provides insight into the molecular involvement of hydrogen or hydroxide ions in the decay mechanism.[59]

An Arrhenius plot is constructed by plotting the logarithm of the apparent decay rate con-

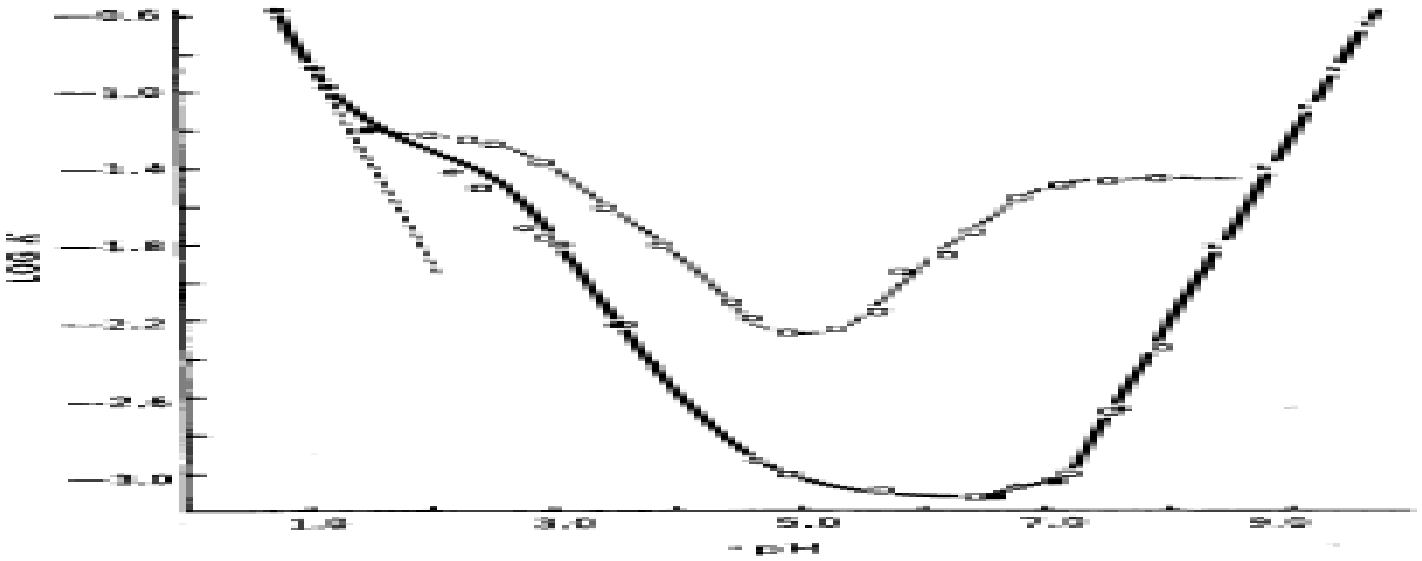


FIG. 8-22. *The pH-rate profiles for ampicillin degradation in solution at 35°C and constant ionic strength (μ = 0.5). Dotted line is the apparent rate profile in the presence of buffer, while the solid line is the theoretic rate profile at zero buffer concentration. (From Hou, J. P., and Poole, J. W.: J. Pharm. Sci., 58:447, 1969. Reproduced with permission of the copyright owner.).*

stant versus the reciprocal of the absolute temperature at which each particular buffer solution was stored during the stability test. To justify extrapolation to "use" conditions, stability storage temperatures should be selected that incrementally (Δt = 10°C) approach the anticipated "use" temperature. If this relationship is linear, one may assume a constant decay mechanism over this temperature range and calculate an activation energy (Ea) from the slope (−Ea/R) of the line described by:

$$\ln k = \frac{-E_a}{R}\left(\frac{1}{T}\right) + C \qquad (19)$$

where C is a constant of integration and R is the gas constant.

A broken or nonlinear Arrhenius plot suggests a change in the rate-limiting step of the reaction or a change in decay mechanism, thus making extrapolation unreliable. In a solution-state oxidation reaction, for example, the apparent decay rate constant decreases with elevation of temperature because the solubility of oxygen in water decreases. At elevated temperatures, ex-

cipients or buffers may also degrade to give products that are incompatible with the drug under study. Often, inspection of the HPLC chromatograms for decay products confirms a change in the decay mechanism.

Shelf-life ($t_{10\%}$) for a drug at "use" conditions may be calculated from the appropriate kinetic equation, and the decay rate constant obtained from the Arrhenius plot. For a first-order decay process, shelf-life is computed from:

$$t_{10\%} = \frac{-\ln 0.90}{k_1} = \frac{0.105}{k_1} \qquad (20)$$

where $t_{10\%}$ is the time for 10% decay to occur with apparent first-order decay constant k_1. Frequently, it is useful to present the pH-rate profile as a plot of pH versus $t_{10\%}$ shelf-life data.

Solid State Stability

structed; if linear, it may be extrapolated to "use" conditions for predicting a shelf-life. If humidity directly affects drug stability, the concentration of water in the atmosphere may be determined from the relative humidity and temperature by using psychrometric charts.[63] Stability data obtained at various humidities may be linearized with respect to moisture using the following apparent decay rate constant:

Another useful relationship for analyzing solid state stability data assumes that a compound must partially liquefy prior to decomposition. Given that the mole fraction of the solid that has liquefied (F_m) is directly proportional to its decay rate, then:

$$\ln k_{app} \propto \ln F_m = \frac{-\Delta H_{fusn}}{R}\left[\frac{1}{T} - \frac{1}{T_m}\right] \qquad (22)$$

A list of the most common excipients is created along with the hypothetic formulations utilizing these excipients. Usually, the approximate dose of the drug is known; thus, each excipient can be blended with the drug at levels that are realistic with respect to a final dosage form (e.g., 10:1 drug to disintegrant and 1:1 drug to filler such as lactose). Each blend is then divided into weighed aliquots, which are tested for stability at some elevated temperature (50°C) that is lower than the melting point of the ingredients. Early inspection ($\Delta T \sim 2$ days) of these stability samples may allow culling of those samples with a phase change and allow for retesting at a lower temperature. If possible, pellets should be formed from the drug excipient blends to increase drug–excipient contact and accelerate testing.

In addition to excipient compatibility testing, small batches of hypothetic capsule or tablet formulations (2 or more) should be prepared and tested in the same stability protocol to check for possible incompatibilities arising from a multicomponent formulation.