Dissolution Behavior of Crystalline Solvated and Nonsolvated Forms of Some Pharmaceuticals

By ELI SHEFTER† and TAKERU HIGUCHI

Relative rates of dissolution of several crystalline steroids, xanthines, and other solid drugs have been measured to determine the effect of solvate formation on this property. Equations have been derived relating the solubility products and diffusional constants to rates of solution of organic solvates. The results suggest that the tendency of many drugs to form such adducts provide pharmaceutical investigators a powerful tool in effecting rapid dissolution of highly insoluble substances.

MANY ORGANIC and inorganic compounds are capable of existing in more than one crystalline form having different physical properties. In some cases these states are the result of solvate formation; in others the molecular arrangement within the crystal lattice is responsible for the differences in the properties of crystal modifications. The pharmaceutical importance of variations in the thermodynamic properties associated with the differences in crystal forms have been recently pointed out. It has been suggested by Higuchi (1) that the differences in the free energy of polymorphs, for example, can be an extremely important factor in determining the stability and availability of certain pharmaceuticals. The present report is concerned with the results of theoretical and experimental studies conducted to determine the magnitude of the differences in the thermodynamic and dissolution properties arising from the formation of such crystalline variants of a drug.

Organic medicinal compounds appear to be particularly prone to both formation of polymorphs and solvates and to have large differences in energy associated with such crystalline modifications. This can be ascribed to the size and general complexity of molecules having medicinal value and to their polyfunctionality. Such structures do not usually favor rapid crystalline nucleation and growth. These forms which are favored from the rate of crystal growth viewpoint often turn out to be metastable compared to more difficultly produced polymorphs. It is apparent that the appropriate selection of the most suitable crystalline modification, whether arising from polymorphic differences or as a result of solvate complex formation, can often significantly increase the medicinal value of a given drug in a particular dosage form.

Specifically, this study was concerned with a theoretical and experimental investigation of the relative dissolution rates of some crystalline solvates and polymorphs in aqueous solutions. The thermodynamic properties of these crystal systems also have been experimentally determined in several instances.

The six crystalline systems whose dissolution behaviors were studied are cholesterol, forms N_1 and H_1; caffeine, forms N_1 and H_1; theophylline, forms N_1 and H_1; glutethimide, forms N_1 and H_1; succinyl sulfathiazole, forms N_1, H_1, H_2, and P_1; and 9α fluorohydrocortisone acetate (fludrocortisone acetate), forms N_1, N_2, P_1, and E_1, where N_1 represents a nonsolvated crystalline form and H_1, P_1, and E_1, represent a specific hydrate, pentanol solvate, and ethyl acetate solvate, respectively, for each compound. Polymorphic and additional variations are noted by appropriate subscripts. The thermodynamic differences between the various solvated and nonsolvated crystal forms of theophylline, glutethimide, fludrocortisone acetate, and succinyl sulfathiazole were evaluated. In addition, studies were undertaken to determine the effect of certain protective colloids on prolongation of a metastable phase.

PAST WORK ON CRYSTALLINE HYDRATES AND SOLVATES

Past studies on the physical chemical properties of crystalline hydrates appear to have been mainly limited to inorganic compounds. The phase solubility method was utilized, for example, by many workers to obtain the solution properties of inorganic hydrate systems in aqueous solutions. The investigation of Taylor and Henderson (2) on the various hydrates of calcium nitrate is a good example of the type of results obtained by this method.

Hill (3), in a similar type study on calcium sulfate, was able to determine accurately the transition temperature between an anhydrous form and a dihydrated form. Kuznetsov and co-workers (4) determined some of the factors involved in the con-

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version of an anhydrous form of calcium sulfate to the dihydrate at 18°.

Eriksson (5) recently examined the apparent solubility of an anhydrous form and two hydrates of phenobarbital as a function of time in water. The concentration of drug in solution was measured on an hourly basis, assuming no immediate conversion of the metastable phases in the solution. In this investigation the solubility of the anhydrous form was greater than the two hydrates below 50°. No attempt was made to evaluate the thermodynamic properties or dissolution rates of these crystalline modifications. This study seems to be the only investigation reported in the literature which compares the solubilities of hydrated and anhydrous forms of organic compounds.

There are many references in the literature on the formation of crystalline solvates of organic compounds. These references are usually in connection with organic synthesis, a particular compound being reported as crystallizing from an organic solvent with molecules of the solvent attached. No previous investigations seem to have been reported on the properties of behavior of these types of solvates in aqueous media.

THEORETICAL CONSIDERATIONS

A general discussion of the theories basic to the equilibrium solubility (thermodynamic) and rates of solution for the various types of crystal systems studied is presented.

Thermodynamic Considerations

The equilibrium solubility of a nonsolvated form of a crystalline, nondissociating organic compound, A, in water may be represented by equilibrium 1 (Eq. 1) (6). This equilibrium is not only in-

$$A_{solid} \overset{Ks}{\rightleftharpoons} A_{aqueous} \qquad \text{(Eq. 1)}$$

fluenced by the parameters of temperature and pressure, but also by the crystalline state. Because organic compounds are able to exist in more than one crystalline state (polymorphism), it is necessary to identify the solid state in this equilibrium as a parameter.

The equilibrium constant, Ks, at a particular temperature and pressure may be defined as the solubility of a particular polymorph and is approximately proportional to the thermodynamic activity of the solid. An extensive treatment of the thermodynamic differences in the properties of polymorphs can be found in a recent report by Higuchi, *et al.* (7).

Equilibrium 2 (Eq. 2) represents the solid-solution

$$A:xH_2O_{solid} \overset{Ks}{\rightleftharpoons} A_{aqueous} + xH_2O \qquad \text{(Eq. 2)}$$

equilibrium for a hydrate in water. This system is analogous to the equilibrium for the nonsolvated form; consequently, the equilibrium constant, Ks, is essentially the solubility of the hydrate.

The hydration of an anhydrous crystal modification in water is represented by Eq. 3. The free

$$A_{solid} + xH_2O_{liquid} \rightleftharpoons A:xH_2O_{solid} \qquad \text{(Eq. 3)}$$

energy change for this process may be computed from Eq. 4

$$F_T = RT \ln \frac{(Ks) \text{ Hydrated form}}{(Ks) \text{ Anhydrous form}} \qquad \text{(Eq. 4)}$$

This energy difference can easily be obtained from solubility data for the two crystal forms at a particular temperature. Since the solubilities can be readily measured at several temperatures, the enthalpy and entropy changes corresponding to this process can also be determined. These thermodynamic data are of some theoretical interest relative to the information they provide concerning the molecular bonding of water in these solids.

An equilibrium model for the crystalline solvates of the type studied is shown in Eq. 5. This model assumes that in solution A and B exist as separate

$$A:nB_{solid} \overset{Ksp}{\rightleftharpoons} A_{aqueous} + nB_{aqueous} \qquad \text{(Eq. 5)}$$

species whose concentrations are determined by the solubility product, Ksp.

The free energy change corresponding to the process shown by Eq. 5 could be evaluated from the solubility of the solvated A in pure B, the solubility of unsolvated A in B, the solubility of the same unsolvated A in water, and the activity coefficient of B in water. It suffices to say at this point that for the solvates with which this study is concerned, Eq. 5 usually represents a far greater free energy difference than Eqs. 1 and 2.

Dissolution Rate (General Discussion)

Noyes and Whitney (8) have shown that the rate of dissolution of solids is directly proportional to the concentration gradient when the surface area of the dissolving material changes negligibly for systems yielding only a single species in solution.

In their equation, Eq. 6, Cs is the concentration of the saturated solution and Ct is the amount dis-

$$\frac{dC}{dt} = k(Cs - Ct) \qquad \text{(Eq. 6)}$$

solved at time t. The k in this equation has been shown to be dependent on many factors—on the surface area of exposed solid (9), the intensity of agitation (10), the temperature (11, 12), the size and shape of the particles (9), the apparatus, and the diffusion constant of the dissolved material. It is evident from the equation that for dissolution processes occurring in media where $Ct/Cs \ll 1$, such as may be found during the absorption of uncharged, nearly insoluble drugs *via* the oral route, the solubility term Cs is the major determining factor. This same relationship holds whether anhydrous forms or hydrates of drugs are being dissolved in water.

For systems which yield more than a single species in solution, such as the case with solvates of the type $A:nB$, the Noyes-Whitney equation cannot be used to predict their dissolution rate. The dissolution rate of a solvated organic solid in water seems never to have been treated mathematically. Since it appears that these crystalline addition complexes may possess such significantly high rates of solution as to be of pharmaceutical importance, equations relating some of the factors involved have been derived.

For a crystalline substance which dissociates as follows in water, and which follows the solubility

$$A{:}nB_{solid} \rightleftharpoons A_{aqueous} + nB_{aqueous}$$

product principle of the rate of dissolution under

$$[C_A]\ [C_B]^n = Ksp$$

constant stirring and geometric conditions may be written as

Rate of Dissolution =

$$G = \frac{dC_{A^*}}{dt} = kD_A\,(C_A - C_{A^*})$$

$$= \frac{1}{n}\frac{dC_{B^*}}{dt} = \frac{kD_B}{n}\,(C_B - C_{B^*}) \quad \text{(Eq. 7)}$$

C_{A^*} and C_{B^*} refer to the concentration of the two species in the bulk of the solution, C_A and C_B their respective concentrations at the immediate crystal surface, D_A and D_B the respective diffusitivity, and k the combined geometric and agitation factor. For our thinking, A can be considered to be the drug component (*e.g.*, steroid or sulfa drug) and B the organic solvent adduct (*e.g.*, amyl alcohol).

For the case $n = 1$ and $D_A = D_B$ the above relationships yield a very simple solution which is

$$G = \frac{dC_{A^*}}{dt} = kD_A(\sqrt{Ksp} - C_{A^*}) \quad \text{(Eq. 8)}$$

similar in form to Eq. 6. It is evident that C_{A^*} may be able to build well above the solubility of A itself in water.

Other simple relationships can be derived from the above equations when $n = 1$ but $D_A \neq D_B$. Thus from Eq. 7 and the solubility product, we have

$$G = kD_A\left(\frac{Ksp}{C_B} - C_{A^*}\right) \quad \text{(Eq. 9)}$$

And since $G = kD_B\,(C_B - C_{B^*})$

$$C_B = \frac{G}{kD_B} + C_{B_*} \quad \text{(Eq. 10)}$$

By substituting Eq. 10 into Eq. 9, the quadratic in Eq. 11 is obtained.

$$G^2 + G(kD_BC_{B^*} + kD_AC_{A^*}) - k^2D_BD_AKsp + k^2D_BD_AC_{B^*}C_{A^*} = 0 \quad \text{(Eq. 11)}$$

When $C_{A^*} = C_{B^*}$, the quadratic Eq. 11 is easily solved to yield Eq. 12

$$G = \frac{dC_{A^*}}{dt} = \frac{kC_{A^*}}{2}\times \left[\sqrt{(D_B - D_A)^2 + \frac{4KspD_AD_B}{(C_{A^*})^2}} - (D_A + D_B)\right] \quad \text{(Eq. 12)}$$

This equation leads to a zero rate only when $(C_{A^*})^2 = Ksp$ as expected and does not represent a first-order approach to saturation as is the case in both Eqs. 6 and 7.

If the concentration of C_{B^*} is increased much more than C_{A^*} by addition of the solvating solvent to the dissolution media, a binomial expansion can be used to simplify Eq. 11.

$$G = \frac{KD_AKsp - kD_AC_{A^*}}{C_{B^*}} \quad \text{(Eq. 13)}$$

which indicates that the dissolution rate for the solvate will decrease with an increase of B in solution.

Equations for solvates of higher order than one-to-one become more difficult to solve. In general the dissolution rates for these solvates are also dependent on their Ksp values.

EXPERIMENTAL

Apparatus

A glass-jacketed, 250-ml. Erlenmyer flask, constant temperature bath equipped with circulating pump,[1] magnetic stirrer, 1 1/2 in. flurocarbon-covered stirring bar, timer,[1] latex rubber tubes plugged with glass wool; Swinney hypodermic adaptor,[2] Millipore filters[2] (pore size 0.45 μ), Cary recording spectrophotometer (model 11 ms.), and Kofler melting-point apparatus[3] comprised the apparatus utilized.

Compounds

Cholesterol.—The hydrate (H_1) was obtained by crystallization of cholesterol (Eastman Kodak White Label) from an ethyl alcohol–water solution. Titration of these crystals with Karl Fisher reagent showed that 1.0 ± 0.2 molecule of water was bound to each cholesterol molecule.

Determination of the true melting point of this hydrate was not possible because of the rapid transformation of it to a nonsolvated form at elevated temperatures. This type of transformation also hindered the determination of the true melting behavior than the other crystalline forms of a compound and for the relatively stable nonsolvated forms.

The anhydrous form (N_1) (m.p. 149°) was formed by heating the hydrate above 80° for 48 hours.

Glutethimide.—The method of Neveu and Legagneur (13) was used to prepare the anhydrous and hydated forms of this compound. The hydrate (H_1) (m.p. 68°) was obtained by recrystallization of glutethimide (supplied by Ciba Pharmaceutical Co.) from water. The anhydrous crystals (N_1) (m.p. 83°) were prepared by recrystallization of the hydrate from anhydrous ethyl ether.

Theophylline.—The monohydrate (H_1) was obtained by using water to recrystallize theophylline (U.S.P. grade). The anhydrous form (N_1) (m.p. 272°) was prepared from the hydrate by heating it at 100° for 24 hours (14). Characteristic X-ray powder diffraction patterns (Debeye-Scherrer) for these two forms have been obtained.

Caffeine.—The monohydrate (H_1) and an anhydrous form (N_1) (m.p. 238°) of this compound were prepared in the same manner as that used for preparing the two forms of theophylline. Characteristic X-ray powder diffraction patterns (Debeye-Scherrer) of these two crystal forms have been obtained.

Fludrocortisone Acetate.—The crystalline pentanol solvate (P_1) and ethyl acetate solvate (E_1) of this compound were obtained by recrystallization of this steroid from the respective solvent. A nonsolvated crystal form (N_1, form 1) (m.p. 218–222°) was prepared by crystallization from a dilute ethyl alcohol solution.

The amount of solvent bound in each crystal form was determined by comparing the molar absorptivities of the different forms. The pentanol solvate

[1] Obtained from Precision Scientific Co., Chicago, Ill.
[2] Obtained from Millipore Corp., Bedford, Mass.
[3] Obtained from Arthur H. Thomas Co., Philadelphia, Pa.

contained 1.1 molecules of pentanol for each molecule of steroid. The ethyl acetate solvate was found to have 0.5 molecule of solvent bound to each molecule of fludrocortisone acetate.

A nonsolvated form (N_2, form 2) was isolated after the normal amyl alcohol solvate was agitated in an aqueous solvent for 24 hours and had the same molar absorptivity as form 1.

Characteristic X-ray powder patterns for each of these crystal forms have been obtained.

Succinyl Sulfathiazole.—A monohydrate (H_2, form 2) was obtained by recrystallization of succinyl sulfathiazole (obtained from National Biochemical Corp.) from a 25% ethyl alcohol–water solution. In the dissolution studies on this hydrate, another hydrate (H_1, form 1) was isolated. An anhydrous form (N_1) (m.p. 188°) of this compound was prepared by drying the crystals of form 2 to 100° *in vacuo* for 24 hours.

The pentanol solvate (P_1) (partial melting with subsequent resolidification between 127 and 136°, followed by complete melting at 191°) was obtained by recrystallization of the anhydrous form 1 from hot pentanol. By comparison of the molar absorptivity of this crystalline form with that of the nonsolvated form (N_1) it was found that 0.9 molecule of pentanol was bound to each molecule of the sulfa drug.

The distinctly different X-ray diffraction patterns for these four crystalline modifications have been obtained.

General Procedure in Dissolution Studies

The general procedure used to study the dissolution properties of all the crystal systems was similar. For each series of dissolution studies at a particular temperature for a drug, the same weight of each crystalline modification was used. The amount of the solid used was approximately in fourfold excess of that necessary to saturate the selected solvent with the most soluble form. The weighed sample was added rapidly to exactly 200 ml. of the solvent in a thermostated 250-ml. Erlenmyer flask subjected to agitation as described below. At measured time intervals small samples were withdrawn from the system and filtered. These samples were then analyzed for the concentration of drug present by an appropriate method.

Several of the specific procedures describing the individual steps in the above procedure are given in more detail in the following sections.

Agitation

The solution in the flask was agitated by a fluorocarbon-covered magnetic stirring bar rotating at a high speed. Although no serious attempt was made to maintain the intensity of agitation exactly constant in all the dissolution experiments, the simple procedure appeared to have given surprisingly good reproducibility of the dissolution curves for a given system.

Crystal Size

The size of the crystals in all the dissolution experiments was not controlled. Microscopic examination of many of the crystal samples showed that the initial particle size was well above 5 μ. Particles in this range should not show a solubility effect due to surface free energy (15).

Sampling

The samples from the dissolution system were withdrawn directly through a latex tube filled with glass wool. This produced a particle-free sample for analysis.

In the dissolution studies on fludrocortisone acetate and succinyl sulfathiazole, the crystal size of the various forms became smaller with prolonged agitation and were not able to be filtered by the above method. It was necessary to use a filter with a fine pore size to get good filtration. Millipore filters, pore size 0.45 μ, were used.

In the latter cases a syringe was used to withdraw the sample from the dissolution flask. A Swinney hypodermic adaptor with a Millipore filter inside was then placed on the syringe and the sample was pushed through the filter. The filtrate obtained in this manner was very clear.

Analytical Methods

The solutions of all the compounds except cholesterol were analyzed spectrophotometrically in the ultraviolet region. The concentration of cholesterol in solution was determined by a colorimetric assay (16), based on its color formation with iron in sulfuric acid.

Solvents Used

The dissolution experiments on the various crystalline modifications were generally performed in aqueous media. Since some of the compounds have a solubility in water too low to permit convenient determination of the solution concentration, ethyl alcohol–water solutions were used. In the case of cholesterol a glycerin–ethyl alcohol mixture containing approximately 5% water was used as the solvent.

In the dissolution studies on the various forms of succinyl sulfathiazole an acidic solution was used so that the ionization of this compound in water was held to a minimum.

Following is a list of the compounds studied with the solvents used, enclosed in the brackets: theophylline [water], caffeine [water], cholesterol [50% solution of glycerin in ethyl alcohol (contains approximately 5% water), fludrocortisone acetate [4.5% (v/v) ethyl alcohol in water and 15% (v/v) ethyl alcohol in water], glutethimide [13.4% (w/v) ethyl alchol in water], and succinyl sulfathiazole [~0.001 *N* sulfuric acid solution].

RESULTS AND DISCUSSION

The results of these investigations are considered in two parts. The first is concerned with the studies on the relative dissolution properties of hydrated and nonsolvated crystal systems. The second section presents the dissolution behaviors of crystalline solvates other than hydrates.

Comparison of Crystal Hydrates with Their Nonsolvated Forms

Dissolution Behavior.—The dissolution behaviors of hydrated and nonsolvated forms of cholesterol, theophylline, caffeine, glutethimide, and succinyl sulfathiazole are shown in Figs. 1–5, respectively. These figures show the concentration for each crystalline form attained in solution as a function of time

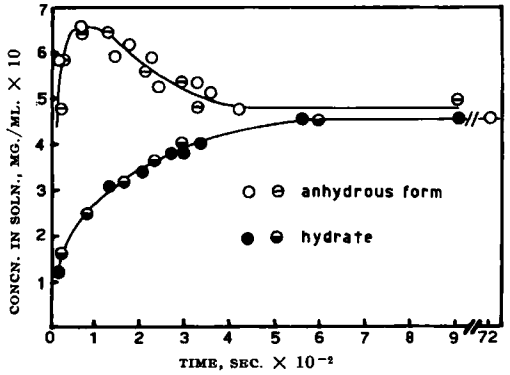

Fig. 1.—The dissolution curves for the anhydrous (N_1) and hydrated (H_1) crystalline forms of cholesterol in a 50% glycerin in ethanol solution at 25°C. The two different types of circles for each form represent successive experimental runs.

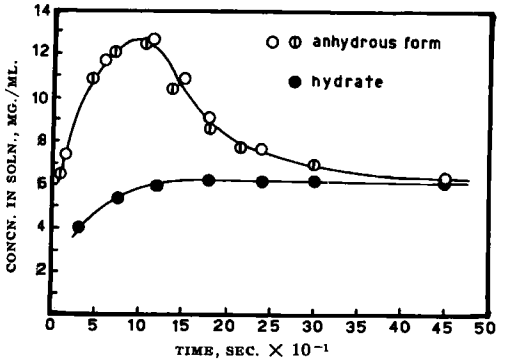

Fig. 2.—The dissolution curves for the anhydrous (N_1) and hydrated (H_1) crystalline forms of theophylline in water at 25°C. The two types of circles for the anhydrous form represent successive experimental runs.

in the presence of an excess of the solid phase and under essentially constant agitation. As indicated in some of these plots, each curve is drawn through points obtained during more than one run. Despite the relative simplicity of the dissolution technique employed, the results indicate that the procedure yielded a surprisingly reproducible rate value for each system.

It would be quite improper, of course, to attribute the apparently greater dissolution rates observed for the anhydrous forms entirely to the higher free energy contents of these species. In these as well as other dissolution experiments performed, no serious attempts were made to obtain samples possessing comparable specific surface areas, although gross microscopic examination showed no substantial differences in the size between the crystal systems of any one compound. It is evident from Eq. 6 that the ratio of the dissolution rates equals

$$\frac{\text{Dissolution Rate for Anhydrous form}}{\text{Dissolution Rate for Hydrate}} = \frac{k_A(Cs - Ct)_A}{k_H(Cs - Ct)_H}$$

where subscripts A and H represent the anhydrous and hydrated species of a particular compound. It is obvious from this ratio that some part of the observed enhancement may be due to contributions from the geometric factors k_A and k_H. It should be noted, however, that since the solubilities of the anhydrous forms in these particular instances were apparently substantially greater than those of the hydrates, the ratio of the dissolution rates of the two forms are always in the same direction as those predicted on solubility alone.

From a practical viewpoint it is evident that the rates of dissolution of a variety of crystalline drugs can be greatly influenced by the selection of the more energetic species. As seen in Figs. 1–5 the anhydrous samples dissolve much faster than the corresponding hydrates and in all cases yield concentrations substantially supersaturated with respect to the stable form. The maximal values observed in some instances may correspond to the solubility of the anhydrous crystalline phase; in others they probably represent a short-term steady-state phase situation involving equal rates of dissolution of the metastable form and crystallization of the stable hydrate.

From Fig. 1, for example, it would appear that the initial rate of dissolution of the anhydrous form, N_1, of cholesterol was very much greater than that for the hydrate, H_1, at 25°. The particular study was carried out in a 50% glycerin in ethyl alcohol solution containing approximately 5% water since cholesterol was extremely insoluble in water and because the high viscosity produced by glycerin reduced the initial dissolution rate sufficiently to permit its ready measurement. Although the maxi-

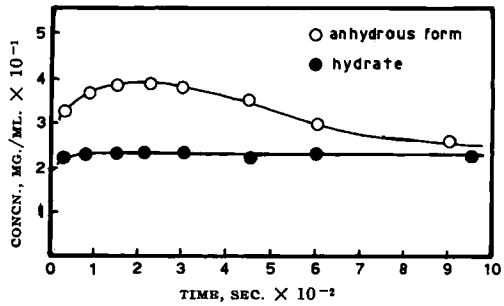

Fig. 3.—The dissolution curves for the anhydrous (N_1) and hydrated (H_1) crystalline forms of caffeine in water at 28°C.

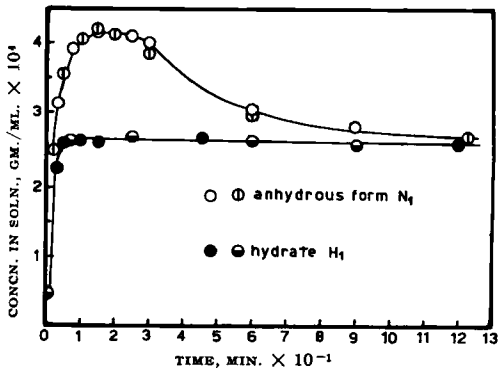

Fig. 4.—The dissolution of anhydrous and hydrated glutethimide in a 13.4% ethanol solution at 25°C. The different circles for each form represent successive experimental runs.

mum concentration reached with the nonsolvated form was only 1.4 times as great as the solubility of the hydrate, the initial rate appears to be at least three times that for the hydrate. The dissolution behavior of the anhydrous material suggests that nucleation and formation of the more stable hydrate prevented realization of the true solubility of the metastable form. It would appear that if this had not occurred, a markedly higher concentration would have been reached with the anhydrous crystals.

Qualitatively, both the dissolution and crystal growth phases appear from these diagrams to be kinetically controlled by the concentration gradient near the dissolving or growing crystalline surfaces. This is particularly evident for theophylline in Fig. 2. If the data shown are plotted as a first-order approach to equilibrium (concentration gradient controlled process) in the usual logarithmic fashion, a fairly respectable straight-line relationship is obtained. Apparently for this system the maximum exhibited by the anhydrous form, N_1, closely approaches the true solubility.

The apparent dissolution rate of anhydrous theophylline again appears to be much greater than that for the hydrate. The maximum concentration value attained with the nonsolvated crystals was twice the solubility of the hydrate at 25° in water. The higher thermodynamic activity associated with the anhydrous form apparently was the major contributing factor causing the initially greater dissolution rate observed for this modification. After the maximum concentration peak was reached with the anhydrous crystals, there is an apparent first-order decline in the amount of drug dissolved. The limiting value of this decrease was found to be the solubility of the hydrate, H_1.

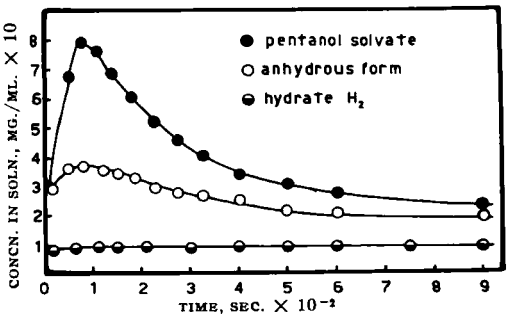

Fig. 5.—The dissolution behavior of anhydrous (N_1), hydrated (H_2), and pentanol solvated (P_1) forms of succinyl sulfathiazole in ~ 0.001 *N* sulfuric acid solution at 20°C.

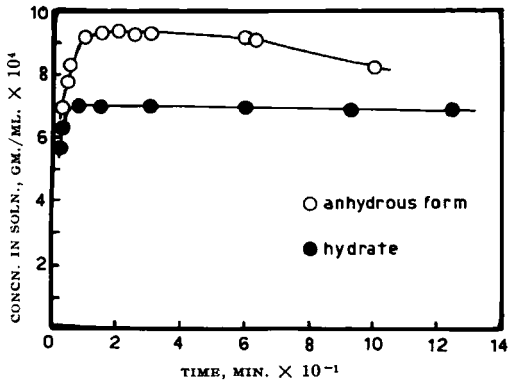

Fig. 6.—The dissolution behavior of anhydrous (N_1) and hydrated (H_1) forms of glutethimide in 13.4% ethanol solution at 40°C. Compare with Fig. 4.

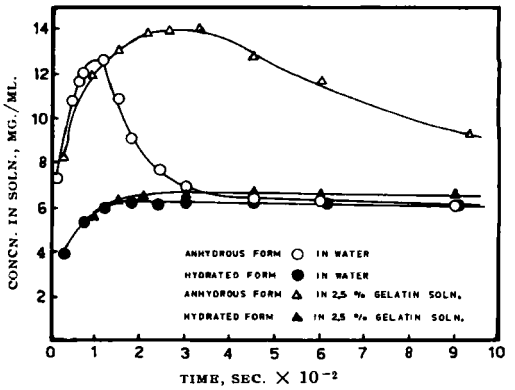

Fig. 7.—The influence of gelatin on the dissolution behaviors of the anhydrous (N_1) and hydrated (H_1) forms of theophylline at 25°C.

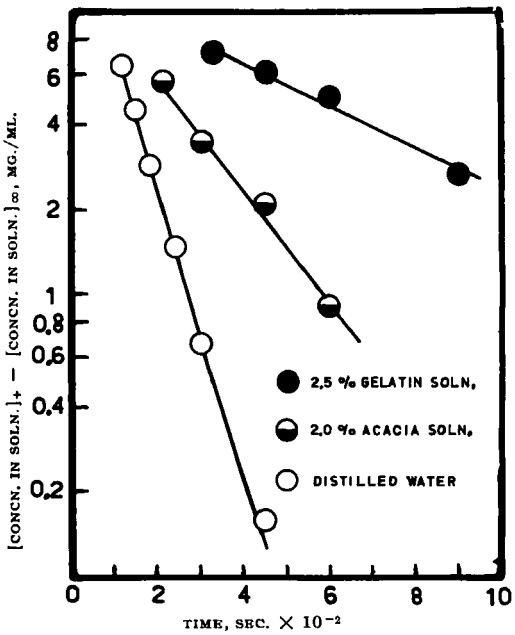

Fig. 8.—The influence of the acacia and gelatin solutions on the observed first-order transformation of anhydrous (N_1) theophylline to the hydrate (H_1) at 25°C.

Anhydrous caffeine, N_1, apparently also exhibits a greater initial dissolution rate than the hydrated form, H_1, in water. Figure 3 shows that the maximum concentration of caffeine achieved in solution with the nonsolvated form is 1.7 times the solubility

TABLE I.—INFLUENCE OF ADDITIVES ON RATE OF CRYSTALLIZATION OF THEOPHYLLINE HYDRATE

Solvent System	Transformation Rate, sec.$^{-1}$ (obtained from Fig. 9)	Water at 25°C.
Water	-5.3×10^{-3}	1.0
Acacia soln., 2.0%	-1.8×10^{-3}	1.6
Gelatin soln., 2.5%	-0.6×10^{-3}	2.5

of the hydrate at 28°. The higher concentration level reached with the anhydrous form was apparently sustained longer than that observed for many of the other metastable systems, suggesting an equilibrium saturation state over this interval.

The dissolution curves (Fig. 4) for the two crystalline forms of glutethimide also show that the initial dissolution rate of the nonsolvated form is greater than that for the hydrate. The dissolution study on this system was performed in a 13.4% (w/v) ethyl alcohol–water solution because glutethimide had a very limited solubility in pure water. The maximum concentration level attained with the anhydrous form, N_1 (1.6 times greater than the solubility of the hydrate at 25°) has a flatness similar to that observed for anhydrous caffeine. This suggests that the transformation process may occur in this system at a slower rate than, for example, cholesterol N_1. If this were the case, the plateau concentration level achieved with the anhydrous crystals might very well be its equilibrium solubility. This equilibrium effect is even more pronounced at higher temperatures as is evident in Fig. 6 for glutethimide at 40°. The observed increase in stability of the anhydrous material in solution is probably due to the reduction in the free energy difference between the two solid phases, as will be discussed later.

Succinyl sulfathiazole appears to form several hydrates and polymorphs (17). Results of the dissolution studies carried out on a few species of the sulfonamide are shown in Fig. 5. These measurements were performed in a ~0.001 *N* sulfuric acid solution to limit the ionization of the drug. The behavior of the drug in suspension strongly suggests formation of at least three hydrates. The lowest plot shows the dissolution behavior of hydrate H_2, (form 2) over a period of 15 minutes. Although an apparent equilibrium concentration is reached well within this time, storage of the suspension over several days, under the same conditions, results in a drop in the dissolved drug concentration of approximately 30% to another stable level corresponding to formation of a structurally different hydrate (H_1, form 1). The dissolution curve for the anhydrous form, N_1, shows that after the initial peak concentration value is reached, there is an apparent first-order decline in concentration to a lower constant value, but which is substantially higher than the solubility of the other two hydrates. After a few days, the

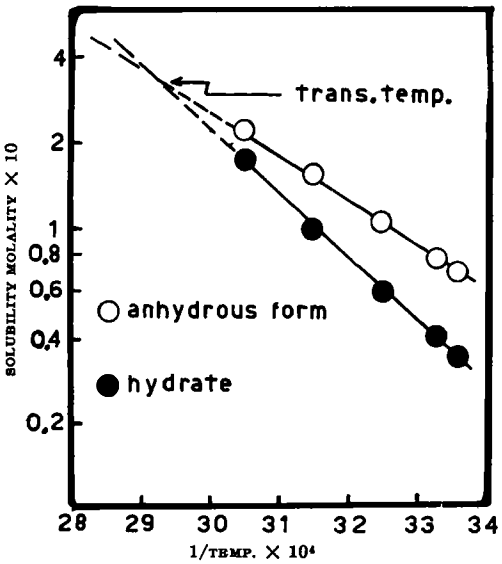

Fig. 9.—The van't Hoff-type plot for the anhydrous (N_1) and hydrated (H_1) forms of theophylline in water.

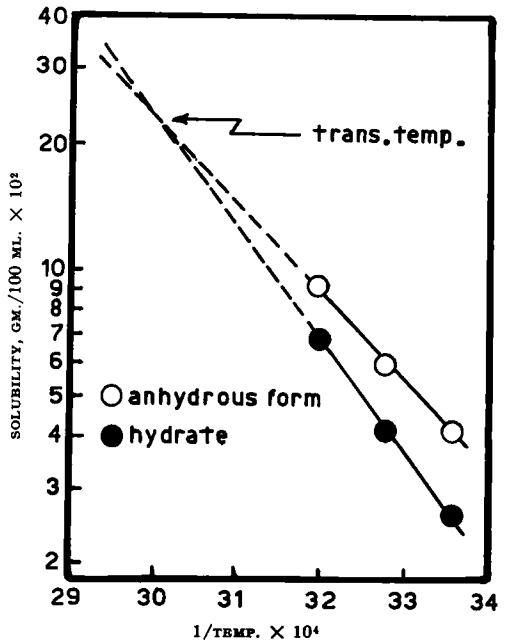

Fig. 10.—The van't Hoff-type plot for the anhydrous (N_1) and hydrated (H_1) forms of glutethimide in a 13.4% (w/v) aqueous ethanol solution.

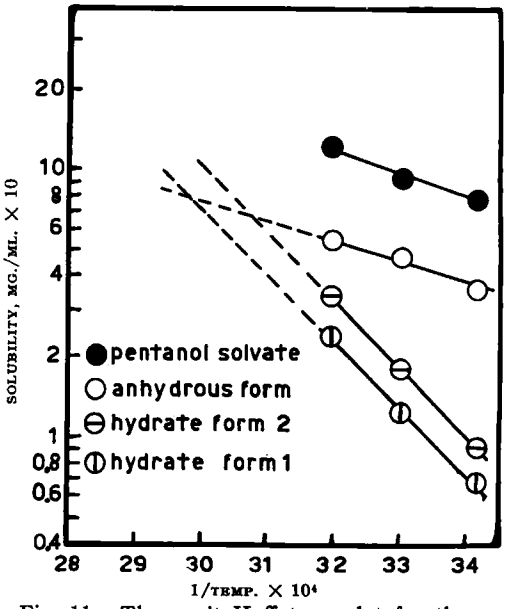

Fig. 11.—The van't Hoff-type plot for the anhydrous (N_1), hydrated (H_1), and hydrated (H_2) forms of succinyl sulfathiazole, in a ~ 0.001 *N* sulfuric acid solution. The maximal concentration of the sulfa drug attained in solution with the pentanol solvate (P_1) is included.

TABLE II.—THERMODYNAMIC VALUES CALCULATED FOR ANHYDROUS-HYDRATED SYSTEMS OF GLUTETHIMIDE AND THEOPHYLLINE

Compound	Transition Temp., °C.	ΔH, cal./mole		ΔF_{298}, cal./mole	ΔS_{298}, e.u.	$\Delta S_{Tran.\ T}$, e.u.
		Hydrate	Anhydrous			
Glutethimide	52	11,700	9700	−280	−5.8	−6.1
Theophylline	73	10,700	7400	−410	−10	−9.5

TABLE III.—THERMODYNAMIC VALUES CALCULATED FOR ANHYDROUS-HYDRATED SYSTEM OF SUCCINYL SULFATHIAZOLE

Crystal Form	ΔH, cal./mole	ΔF_{298}, cal./mole[a]	ΔS_{298}, e.u.[a]	Transition Temp. with anhydrous form, °C.
Form 1 hydrate	12,000	...	...	60
Form 2 hydrate	11,800	−210	Small + value	51
Anhydrous form (N_1)	3,400	−880	−26	...

[a] Calculated for the conversion to the most stable hydrate, form 1.

concentration level of this system decreased until it reached the solubility value of form 1 hydrate.

The results obtained for the crystalline forms of succinyl sulfathiazole are interesting from a practical viewpoint, since the compound is particularly useful in medicine because its low solubility limits its absorption in the intestinal tract. In Fig. 5 it is evident that not only is the rate of dissolution of the anhydrous form much greater than that of the hydrate form 2, but also the maximum concentration value attained is 3.9 times higher than that of the solubility of the hydrate. This may be of some practical pharmaceutical importance if the least soluble form is desired.

Effect of Protective Colloids on the Nucleation Process.—Since protective colloids are known to retard various nucleation phenomena, their effect on the transformation occurring in the present systems was investigated. Studies were carried out with gelatin and acacia to determine their influence on the rate of transformation of anhydrous theophylline to the hydrate. The study was performed at 25° in the previously described manner using water, a 2.5% gelation solution, and a 2.0% acacia solution as solvents.

In Fig. 7 the overall dissolution process observed for the two forms of theophylline in water and in 2.5% gelatin solution are compared. It is apparent that the dissolution rates of both forms were slower in the gelatin solution than in water. The slightly higher solubility of theophylline observed in the presence of gelatin may be due to an interaction with the protein. The main point of interest in Fig. 7 is that the area under the curve for the anhydrous form dissolving in the gelatin solution was much greater than observed in water.

The relative rates of formation of the hydrate species in the several solution vehicles can be best compared by plotting the approach to equilibrium as a first-order process. In Fig. 8, the solution concentrations experimentally observed minus the concentration at infinite time have been plotted as logarithmic functions against time for the precipitation phases of these measurements in water, 2.0% acacia in water, and 2.5% aqueous gelatin solution. The slopes of these lines serve as convenient measures of the relative rates of nucleation and growth of the stable phase in the several vehicles. Although the reduction in the rate of solution depletion agrees qualitatively with the increase in viscosity as measured in a capillary viscometer, quantitative fit is rather poor as is evident from Table I. It is quite likely that the effect of the protective colloid arises as the result of nucleation inhibition of both the two and three dimensional variety.

Thermodynamic Analysis for the Hydrate Systems.—The dissolution behaviors of certain of the anhydrous crystalline forms in water suggested that the maximum values obtained were good approximations of the true solubility of these crystals. It is apparent that if this were the case, that measurements made at several temperatures would permit calculations of the thermodynamic quantities involved in the transitions of the anhydrous form to the hydrate.

Dissolution plots for theophylline, succinyl sulfathiazole, and, in particular, glutethimide seem to give fairly reliable approximations of the solubility of their respective anhydrous species. Measurements over the temperature range 20 to 50° when plotted in the classical van't Hoff fashion gave reasonably good linear relationships for the anhydrous species as shown in Figs. 9–11. Similar data for the stable hydrates are also given in the same figures. The values of the heat of solution for each crystalline form, calculated from the slopes of the latter plots, appear in Tables II and III.

Enthalpy of Hydration (18).—The change in heat content for the hydration of the anhydrous crystals in water (Eq. 17) was calculated from the enthalpy changes of the following reactions.

$$A_{solid} \rightleftharpoons A_{aqueous} \qquad \text{(Eq. 15)}$$

$$A:H_2O_{solid} \rightleftharpoons A_{aqueous} + H_2O_{liquid} \qquad \text{(Eq. 16)}$$

Equation 15 minus Eq. 16 gives Eq. 17

$$A_{solid} + H_2O_{liquid} \rightleftharpoons A:H_2O_{solid} \qquad \text{(Eq. 17)}$$

The heats of reactions of Eqs. 15 and 16 are obviously the heats of solution of the anhydrous and hydrated crystals of compound A. The enthalpy change for Eq. 17, $\Delta H_{A,H}$ is therefore the heat of solution of the anhydrous form minus the heat of solution for the hydrate. $\Delta H_{A,H}$ was utilized to calculate the entropy change involved in the hydration process of Eq. 17.

The values of $\Delta H_{A,H}$ for theophylline and glutethimide were found to be −3300 and −2000 cal./-

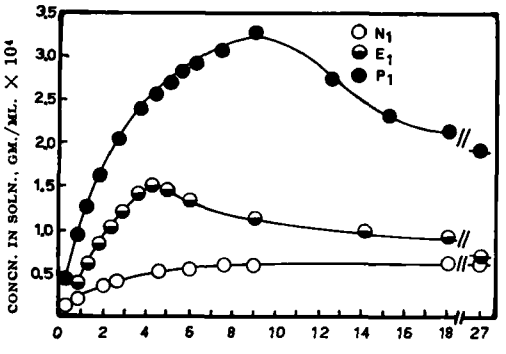

Fig. 12.—The dissolution behaviors of the nonsolvated form (N_1), pentanol solvate (P_1), and ethyl acetate solvate (E_1) of fludrocortisone acetate in an aqueous ethanol solution (15% v/v) at 20°C.

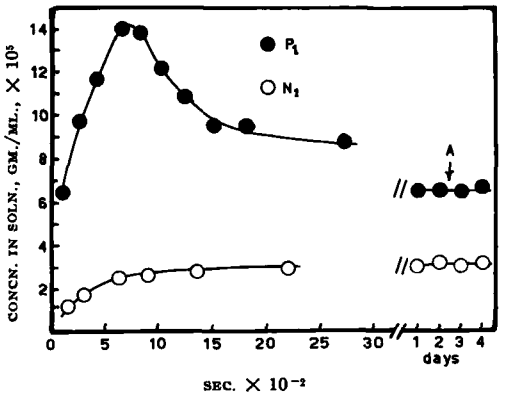

Fig. 13.—The dissolution behaviors of the pentanol solvate (P_1) and nonsolvated form (N_1) of fludrocortisone acetate in a 4.5% aqueous ethanol solution at 20°C. At the *A* concentration level, P_1 was found to be converted to a nonsolvated form (N_2).

mole, respectively. The $\Delta H_{A,H}$ value for the conversion of the anhydrous form of succinyl sulfathiazole to form 1 hydrate was −8600 cal./mole.

Free Energy of Hydration.—At constant temperature and pressure the free energy difference between the anhydrous and hydrated forms is determined by Eq. 18

$$\Delta F_T = RT \ln \frac{(Cs)\ \text{anhydrous}}{(Cs)\ \text{hydrate}} \quad \text{(Eq. 18)}$$

This equation relates the solubility, *Cs*, of the two forms at a particular temperature, *T*, to the free energy difference. This ΔF_T corresponds to the free energy change involved in Eq. 17, since the activity of water is approximately unity in the present systems. These values are listed in Tables II and III for glutethimide, theophylline, and succinyl sulfathiazole systems at 25°.

Entropy Change.—The entropy change for the hydration reaction in Eq. 17 can be calculated at a particular temperature, *T*, by Eq. 19

$$\Delta S_T = \frac{\Delta H_{A,H} - \Delta F_T}{T} \quad \text{(Eq. 19)}$$

The entropy changes for the hydration of the anhydrous crystals of glutethimide, theophylline, and succinyl sulfathiazole to their respective stable hydrate were computed by Eq. 19 at 25°. These values appear in Tables II and III.

The entropy change involved in the fusion of water at 25° is approximately −6 e.u. This decrease in entropy associated with the formation of ice is approximately the same entropy change obtained for the hydration of glutethimide and theophylline. It may be likely, therefore, that the energy involved in the transformation of the dehydrated form of these compounds to the hydrate is related mainly to the decrease in the entropy of water molecules in the hydrate structure.

The structure of theophylline hydrate was elucidated by Sutor (19). This structure analysis showed that the water molecules form a chain network throughout the lattice. One water molecule is hydrogen bonded to two other waters and to one theophylline molecule. This type of structure could be responsible for the larger decrease in entropy associated with the hydration of theophylline than with the fusion of pure water.

At the transition temperature of the anhydrous and hydrated crystalline forms, $\Delta F_{\text{Tran. } T}$ is equal to zero. Equation 19 then reduces to the simple form shown in Eq. 20. The entropy change calculated at

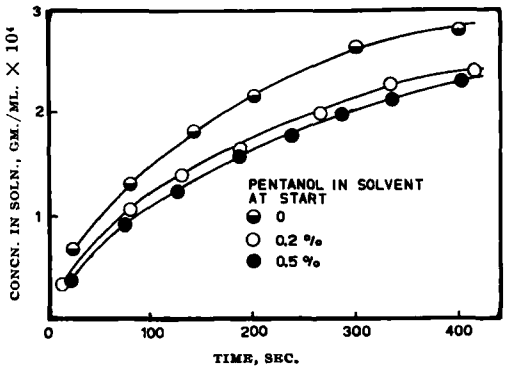

Fig. 14.—The influence of the addition of pentanol to the 15% aqueous ethanol solution on the dissolution of the pentanol solvate of fludrocortisone acetate at 20°C.

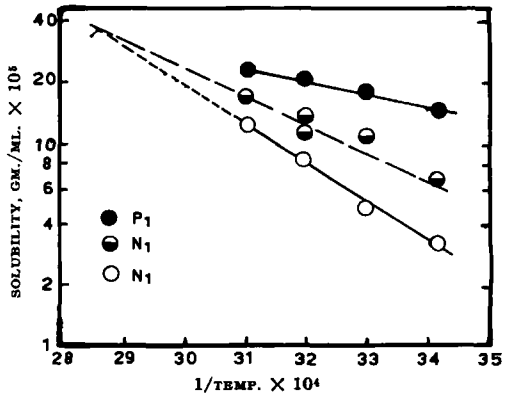

Fig. 15.—The van't Hoff-type plot for the nonsolvated forms N_1 and N_2 of fludrocortisone acetate in a 4.5% aqueous ethanol solution. The maximal concentration of steroid attained in solution with the pentanol solvate is also plotted in the same fashion.

$$\Delta S_{\text{Tran. } T} = \frac{\Delta H_{A,H}}{\text{Tran. } T} \qquad \text{(Eq. 20)}$$

the transition temperature for the theophylline and glutethimide systems are shown in Table II.

Transition Temperature.—The transition temperature for the hydrate-anhydrous crystal systems corresponds to that temperature at which the fugacity of the anhydrous form is equal to that of the hydrated form, *i.e.*, the temperature at which the solubility of the two forms are equal. The transition temperatures for the theophylline, glutethimide, and succinyl sulfathiazole systems were obtained directly from Figs. 9, 10, and 11 in that order.

Glutethimide hydrate was reported (13) by Legagneur and Neveu to decompose at 53° and melt at 68°. The transition temperature derived from the solubility data of the two crystal forms is 52°. This latter temperature could correspond to the "decomposition" temperature of the above workers.

Theophylline hydrate imbedded in paraffin and heated on a Kofler melting apparatus started to release bubbles at approximately 70°. These bubbles may indicate that water vapor is being given off by the crystals. Using the solubility of the anhydrous and hydrated forms of theophylline, a transition temperature of 73° was found.

In Table III the transition temperature for each hydrate of succinyl sulfathiazole is presented. Since form 1 hydrate seemed to be the most stable hydrate, it correspondingly had a lower transition temperature than form 2 hydrate.

Crystalline Solvate Systems

Dissolution Behavior.—The dissolution behaviors of the mono-amyl alcohol solvates of succinyl sulfathiazole and fludrocortisone acetate are recorded along with their stable modifications in Figs. 5 and 12, respectively. In the latter figure, the dissolution of the ethyl acetate solvate of fludrocortisone acetate also appears. The dissolution studies on the steroid were carried out in ethanol-water solutions because of the compounds' limited solubility in water.

These dissolution curves seemed to confirm the theoretical prediction that the concentration of drug attainable in solution with a solvate may be many times greater than that with a stable crystalline form. The pentanol solvates of the steroid and the sulfa drug produce drug concentrations five and ten times greater than the solubility of their respective stable crystalline forms. A similar measurement of the ethyl acetate adduct of fludrocortisone acetate showed that it was able to achieve more than twice the concentration in solution as did the stable form (form 1). It is apparent from the initial slopes of these dissolution curves that the solvates have, as expected, a much greater rate of solution than the stable forms.

The overall solution behavior of the pentanol solvate of the steroid is plotted in Fig. 13 at 20° in a 4.5% (v/v) ethyl alcohol–water solution. A decrease in the solubility was observed after the initially high concentration peak is reached with the solvate. This decrease corresponded to the conversion of the solvated crystals to a nonsolvated species, form 2. This was attested by X-ray analysis of the crystalline material within the dissolution flask. It appeared to have a solubility of more than twice that of form 1 at 20°. It also appears to be stable in solution at 20° for at least 5 days without subsequent transformation.

In Fig. 14, the effect on the dissolution rate of the pentanol solvate of fludrocortisone acetate by varying concentrations of pentanol is shown. The limited solubility of pentanol in the aqueous solvent allowed a maximum concentration of only 0.5% (v/v) to be used in this study. It is evident from Fig. 14 that the dissolution rate is retarded by the addition of pentanol, in qualitative agreement with Eq. 12. This equation for the generalized monosolvate indicates that the rate of solution decreases with the concentration of the solvating solvent in solution when it is present in greater concentration than the drug. An interesting result from this experiment that does not appear in Fig. 14 is that the maximum equilibrium concentration reached was the same for the three solvent systems, probably corresponding to the solubility of a nonsolvate, but was attained at different rates in the three systems. The maximum concentration peaks were attained in the following sequence: first the solvent without *n*-amyl alcohol present at 900 seconds, then the system with 0.2% (v/v) *n*-amyl alcohol at approximately 1250 seconds, and lastly the 0.5% (v/v) solution at 1800 seconds.

Temperature Dependence.—Although it was not possible to calculate the thermodynamic quantities of the solvate systems from the data obtained directly from the dissolution experiments, it is interesting to note the effect of temperature on the dissolution behaviors of the pentanol solvates. In Fig. 15, the solubility obtained for the nonsolvated polymorphs (forms 1 and 2) of fludrocortisone acetate along with the peak drug concentration attained in solution with the pentanol solvate (P_1) are shown as a function of temperature. A van't Hoff–type plot was used because it gave a relatively linear relationship for the different crystalline forms. The solubility of nonsolvate, form 2, was taken as the plateau concentration level reached after 24 hours of agitation of the pentanol solvate in solution. In Fig. 11, the maximum concentration attained with the pentanol solvate of succinyl sulfathiazole in solution is shown as a function of temperature together with the van't Hoff–type plots for the other forms of this compound that were studied.

GENERAL DISCUSSION

Experimental observations and the theoretical analysis presented above again strongly emphasize the importance of the role of the crystalline state of solid drugs. It is apparent that since the thermodynamic activity of these systems is often directly related to the physiological activity and availability, the choice of the proper crystalline form is of vital pharmaceutical concern.

We would like, in particular, to call attention to the possible broader utilization of organic solvates and complexes in dosage form development. Since the apparent free energy change associated with their dissolution in aqueous media can be vastly greater than that exhibited by an unstable polymorph relative to the stable form, much higher temporary solution concentrations and rates of solution

can be obtained by their use than from purely crystalline modification. This is because the system utilizes, in effect, the free energy of dilution of the complexing agent to raise the solubility of the drug. Since molecular complexes of this type are readily produced, particularly by relatively insoluble drugs, this approach may often provide the answer for those products which are poorly available because of slow rates of dissolution.

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Drug Standards

Determination of Amphetamine in Dosage Forms by Partition Chromatography

By JOSEPH E. MOODY, JR.

A procedure is presented for the assay of preparations of amphetamine and several other sympathomimetic amines. The amine is isolated by extraction from the stationary aqueous phase of a partition column with a chloroform solution of triethylamine. The chloroform solution is then extracted with aqueous acid and the amine determined spectrophotometrically. Data obtained on several commercial samples of dextro-amphetamine sulfate tablets by three different assay procedures are reported, and the inapplicability of the U.S.P. XVI assay procedure to some of the commercial products is demonstrated. An analysis by the proposed column-ultraviolet procedure can be completed in about 1 hour.

MANY METHODS of analysis of sympathomimetic amines have been reported (1). They include direct titration of free bases, residual titration of free bases, extraction of bases combined in salts, distillation and titration of volatile bases, nonaqueous titration, gravimetric methods, ultraviolet absorption, and various chromatographic techniques. The widespread use of these preparations in medical practice and their extensive distribution in illegal channels creates a necessity for a simple and rapid method of assay.

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This assay procedure was submitted to the Committee on Revision of the "United States Pharmacopeia" for consideration as a substitute for the currently official method. Subsequent to the preparation of this manuscript, the committee expressed a preference for the modification, using ammonium hydroxide rather than triethylamine as reagent. We have concurred that this modification is preferable for an official assay method.

The proposed procedure is designed to permit the assay of small samples, thus making it applicable to the analysis of a single dosage unit. In this procedure, the extraction of the amphetamine from the dosage form is achieved by elution from an immobile aqueous phase of a column with a solution of triethylamine in chloroform. The eluate is collected in a separator containing an accurately measured quantity of sulfuric acid solution (2 in 100) into which the amphetamine is then extracted. It is quantitatively determined by ultraviolet absorption of the acid solution.

In the development of the method, several other elution procedures were investigated. In a published report of an extraction procedure for alkaloids (2), *p*-toluenesulfonic acid and the alkaloid are incorporated in an aqueous solution as the stationary phase on a partition column. Ether is used to elute the acidic and neutral