Keyphrases

Stilbene isothiocyanates—synthesis
Fluorescent tagging, antibodies—stilbene isothiocyanates
Quinine reference units—fluorescence determination
Fluorometry—analysis

Dissolution Behavior and Solubility of Anhydrous and Trihydrate Forms of Ampicillin

By JOHN W. POOLE and CHANDER KANTA BAHAL

Anhydrous ampicillin and ampicillin trihydrate were compared for solubility and relative rates of dissolution in distilled water at temperatures ranging from 7.5 to 50°. Differences were noted in the physical-chemical properties of these two forms of ampicillin. The thermodynamic properties of these compounds have been experimentally evaluated. The properties noted for the two forms of the antibiotic are consistent with the observed differences in the biological utilization of the two forms after oral administration to laboratory animals and human subjects.

MANY ORGANIC medicinal compounds are capable of existing in more than one crystalline form having different physical-chemical properties. The resulting variation in the thermodynamic properties associated with differences in crystal form may be of considerable pharmaceutical importance as pointed out previously by Higuchi (1). The present report is concerned with studies conducted to determine the differences in some of the physical-chemical properties of two forms of ampicillin, a semisynthetic penicillin. Specifically, the solubilities and relative rates of dissolution in distilled water of anhydrous ampicillin and ampicillin trihydrate were determined and the thermodynamic properties of these crystal forms were experimentally evaluated.

Most of the past work reported on the physical-chemical properties of crystalline hydrates has been concerned with inorganic compounds. The studies of Taylor and Henderson (2) on the various hydrates of calcium nitrate and of Hill (3) on calcium sulfate are examples of such studies. More recently several investigations concerned with studies of organic molecules in the anhydrous and hydrated forms have been reported. An anhydrous form of phenobarbital and two of its hydrates were examined by Eriksson (4) for apparent solubility in water as a function of time. The relative dissolution rates of solvated and nonsolvated crystal forms of several types of compounds of pharmaceutical interest, including steroids and xanthines were reported by Shefter and Higuchi (5). These workers also determined the thermodynamic properties of several of these crystal systems.

EXPERIMENTAL

Apparatus—A constant-temperature water bath equipped with Unitherm Haake constant-temperature circulator[1] and a rotating-bottle apparatus,[2] Swinney hypodermic adaptor,[3] Millipore filters[3] (pore size 0.45 μ), amber bottles, 120 ml. with polyseal caps.[4]

Compounds—In all the experiments anhydrous ampicillin, (Wyeth Laboratories batch C-10575, m.p. 203–204°) was used. The trihydrate form of ampicillin was prepared from the anhydrous form by the method of Austin *et al.* (6). IR spectra and differential thermal analysis curves were obtained for this material.

Procedure—An excess of drug, 2 g., in the appropriate form was added to 100 ml. of distilled water previously equilibrated to the desired temperature.

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[1] Brinkmann Instruments, Westbury, N. Y.
[2] E. D. Menold Sheet Co., Lester, Pa.
[3] Millipore Corp., Bedford, Mass.
[4] Erno Products, Philadelphia, Pa.

The bottles were rotated in a constant-temperature water bath maintained at the indicated temperature. Samples withdrawn at definite intervals were filtered through a Millipore filter and diluted immediately to avoid any precipitation of ampicillin in the filtered samples due to supersaturation. The penicillin content was determined by means of an iodometric titration procedure as described below. To 2.0-ml. aliquots containing 1 to 3 mg. of ampicillin, 2.0 ml. of 1 *N* sodium hydroxide was added and samples were allowed to stand at room temperature for 15 min. At the end of this time, 2.0 ml. of 1.2 *N* HCl was added followed by 10 ml. of 0.01 *N* iodine. After 15 min., the excess of iodine was titrated using 0.01 *N* sodium thiosulfate. For the blank determinations, to a 2.0-ml. sample, 10 ml. of 0.01 *N* iodine was added and titrated immediately.

RESULTS AND DISCUSSION

The solubility and dissolution behavior of the anhydrous and trihydrate forms of ampicillin at 7.5, 20, 30, and 40° are shown in Fig. 1. Similar data for the study conducted at 50° are shown in Fig. 2. These figures show the concentration of the antibiotic attained in solution as a function of time in the presence of an excess of the solid phase in the appropriate form and under essentially constant agitation. One interesting feature of these studies is the inverse relationship noted between temperature and solubility for the anhydrous form of the drug. The apparently greater dissolution rate observed for the anhydrous form at the lower temperatures cannot be attributed solely to the higher free energy content of this species since no serious attempt was made to maintain equal specific surface areas of the two forms. However, microscopic examination of the materials employed showed the anhydrous and trihydrate forms to be substantially the same with regard to particle size and shape and since the anhydrous form is significantly more soluble than the trihydrate form, the dissolution rate of the two forms is in the direction that would be expected on solubility considerations alone. In addition, the study at 50°, which is above the transition temperature of this system, shows the trihydrate to have an apparently greater dissolution rate than the anhydrous form. This supports the contention that solubility is the dominant factor in this system.

The dissolution behavior in water noted for the two forms of ampicillin suggest that the equilibrium

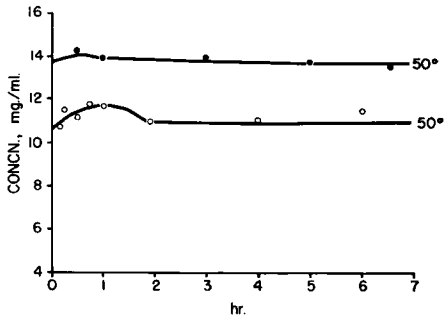

Fig. 2—The dissolution behavior of anhydrous and trihydrate crystalline forms of ampicillin in water at 50°. Key: ○, anhydrous; ●, trihydrate.

solubility observed are good approximations of the true solubility of these crystals. Consequently the measurements made at the several temperatures permit calculation of the thermodynamic quantities involved in the transition of the anhydrous form to the trihydrate. An extensive treatment of the thermodynamic relationship involving polymorphism and solubility is presented in reports by Shefter and Higuchi (5) and Higuchi *et al.* (7).

The apparent equilibrium solubilities observed over the temperature range 20 to 50° when plotted in the classical van't Hoff fashion gave a reasonably good linear relationship for both forms of the antibiotic as shown in Fig. 3.

The transition temperature for the trihydrate-anhydrous crystal system corresponds to the temperature at which the solubility of the two forms is equal. The transition temperature for this system is 42° as shown in Fig. 3. This plot also points up the fact noted earlier that the solubility of the anhydrous form decreases with an increase in temperature whereas that of the trihydrate exhibits the usual temperature-solubility relationship.

The values of the heat of solution for each of the crystal forms was calculated from the slopes of the van't Hoff-type plot (Fig. 4) and were determined to be −1000 and 5400 cal./mole for the anhydrous and trihydrate forms, respectively. The enthalpy of hydration ($\Delta H_{A,H}$), the heat of solution of the anhydrous form minus the heat of solution of the hydrated species, was determined to be −6400 cal./mole.

At constant temperature and pressure the free energy difference ΔF_T, between the anhydrous and

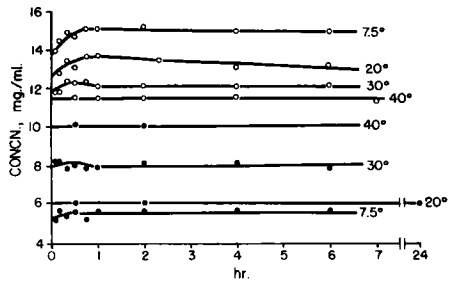

Fig. 1—The dissolution behavior of anhydrous and trihydrate crystalline forms of ampicillin in water at temperatures ranging from 7.5 to 40°. Key: ○, anhydrous; ●, trihydrate.

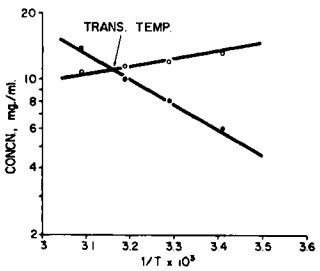

Fig. 3—The van't Hoff-type plot for the anhydrous and trihydrate forms of ampicillin in water. Key: ○, anhydrous; ●, trihydrate.

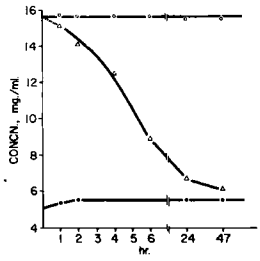

Fig. 4—The influence of seeding anhydrous ampicillin with 1% trihydrate crystals on the dissolution behavior in water at 10°. Key: ○, anhydrous; ●, trihydrate; Δ, anhydrous seeded with 1% trihydrate.

hydrated forms is determined by Eq. 1.

$$\Delta F_T = RT \ln \frac{C_s \text{ (anhydrous)}}{C_s \text{ (trihydrate)}} \qquad \text{(Eq. 1)}$$

where C_s is the solubility of the form under consideration at a particular temperature T, and R is the gas constant. This ΔF_T is a measure of the free energy change involved in conversion of the anhydrous crystal to the trihydrate crystal. The ΔF_T at 25 and 37° (corresponding to room and body temperatures) have been determined to be −430 and −140 cal./mole, respectively.

The entropy change, ΔS_T, for the reaction involved in hydrate formation can be calculated by Eq. 2.

$$\Delta S_T = \frac{\Delta H_{A,H} - \Delta F_T}{T} \qquad \text{(Eq. 2)}$$

The values computed for the hydration of the anhydrous to trihydrate ampicillin crystals at 25° and 37° were −20.0 e.u. and −20.2 e.u., respectively. At the transition temperature of the anhydrous-trihydrate crystal system ΔF is equal to zero and the entropy change can be calculated by Eq. 3.

$$\Delta S_{\text{trans.}} = \frac{\Delta H_{A,H}}{T_{\text{trans.}}} \qquad \text{(Eq. 3)}$$

For ampicillin $\Delta S_{\text{trans.}}$ was determined to be −20.3 e.u. The hydrated species in this system contains three molecules of water and the possible intramolecular hydrogen bond formation between these associated water molecules may account for the relatively large entropy change noted. It has been suggested that a hydrated ampicillin complex of this type may be responsible to some extent for the relative stability of this compound in acidic solution (8).

The thermodynamic values calculated for the anhydrous-trihydrate ampicillin system are summarized in Table I.

As noted earlier the equilibrium solubilities observed in these experiments apparently correspond to the solubilities of the anhydrous and trihydrate crystalline phases for the ampicillin molecule. At the temperatures utilized there was no evidence of conversion of the more soluble anhydrous form to the less soluble trihydrate species as would be expected from the thermodynamic considerations. This may be due in part to the steric factors involved in the association of three molecules of water in the crystal system.

However, at lower temperatures (10°) the seeding of the anhydrous form with trihydrate crystals resulted in a relatively rapid and complete conversion of the anhydrous to the trihydrate form, as shown by the decrease in solubility. These data are shown in Fig. 4.

TABLE I—THERMODYNAMIC VALUES CALCULATED FOR THE ANHYDROUS-TRIHYDRATE AMPICILLIN SYSTEM

Temp., °C.	ΔH, cal./mole Anhydrous	ΔH, cal./mole Trihydrate	ΔF_T, cal./mole[a]	ΔS_T, e.u.[a]
	(−1000)	(5400)		
25			−430	−20.0
37			−140	−20.2
42			0	−20.3

[a] Calculated for the conversion from the anhydrous to the trihydrate form.

The relative ease of conversion of anhydrous to trihydrate ampicillin at the lower temperatures is to be expected, since the rate of phase transformation in a given system depends on the solubilities of the forms at that temperature, the rates of solution, and the diffusion rates of the molecules in solution. The higher the solubility and the greater the difference in solubilities of the two phases the greater will be the rate of transformation. At the lower temperatures the anhydrous form exhibits an increased solubility due to its negative heat of solution. The trihydrate form shows a decrease in solubility at lower temperatures (positive heat of solution) which results in a relatively large difference in the solubilities for the two forms.

Higuchi (1) pointed out that the physiological activity and availability of a drug is often directly related to its thermodynamic activity in a system of this type. Recently Aguiar *et al.* (9) reported on the effect of polymorphism on the absorption of chloramphenicol from chloramphenicol palmitate. From the data presented in the present investigation it would be reasonable to expect an enhanced biological utilization of the anhydrous form of ampicillin compared to the trihydrate form of this agent. In addition, since the anhydrous system is apparently stable to conversion at room and body temperature, these differences should be maintained in the clinical situation. That this is the case was shown in a recent report by Poole *et al.* (10). In these investigations, various pharmaceutical formulations of the drug in each form were administered to laboratory animals (beagle dogs) and to human subjects in a series of crossover experiments. The formulations containing the anhydrous form of the penicillin resulted in blood serum levels of the antibiotic which were consistently earlier and significantly higher than those observed after administration of similar formulations containing the hydrated material. In every instance the area under the serum level-time curves was greater for the anhydrous form of the drug than for the hydrated substance indicating a more efficient biological utilization of this form of the medicinal agent. The results of the *in vivo* evaluation of the oral suspensions of the two forms of ampicillin are summarized in Table II.

SUMMARY

The solubility and relative rates of dissolution of anhydrous ampicillin and ampicillin trihydrate

TABLE II—PEAK SERUM LEVEL AND AREA UNDER THE BLOOD LEVEL-TIME CURVE AFTER ORAL ADMINISTRATION OF SUSPENSIONS OF ANHYDROUS AMPICILLIN AND AMPICILLIN TRIHYDRATE

Form of Ampicillin	Test Species	Peak Serum Level, mcg./ml.	Peak Time, min.	Area Under Curve (mcg./ml. × hr.)
Anhydrous[a]	Dog	20.6	45	36.6
Trihydrate[b]	Dog	11.0	90	22.8
Anhydrous[a]	Human	2.2	60	6.9
Trihydrate[b]	Human	1.7	120	5.7

[a] Administered as Omnipen for oral suspension, Wyeth Laboratories, Inc., Radnor, Pa. [b] Administered as Polycillin for oral suspension, Bristol Laboratories, Syracuse, N. Y.

in distilled water have been determined over a temperature range of 7.5 to 50°. Below the transition temperature, 42°, the anhydrous form was found to be significantly more water soluble than the trihydrate. In addition, the solubility of the anhydrous crystal was shown to be inversely related to temperature.

The thermodynamic values have been calculated for the anhydrous-trihydrate ampicillin system. The greater thermodynamic activity of the anhydrous form correlates with the observed enhanced biological availability noted with this crystal form of the antibiotic.

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Keyphrases

Ampicillin, anhydrous, trihydrate—thermodynamic properties
Dissolution rate—ampicillin, anhydrous, trihydrate
Solubility—ampicillin, anhydrous, trihydrate
Blood serum levels—ampicillin, anhydrous, trihydrate
Iodometric titration—analysis

Potential Antitumor Agents III

Sodium Salts of α-[*N*]-Heterocyclic Carboxaldehyde Thiosemicarbazones

By KRISHNA C. AGRAWAL and ALAN C. SARTORELLI

Sodium salts of four of the most active antineoplastic agents in a series of α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones have been prepared as a means of solubilizing for parenteral administration these extremely insoluble compounds. The sodium salt of 1-formylisoquinoline thiosemicarbazone (II) is soluble in nonaqueous vehicles for injection such as propylene glycol, whereas the sodium salts of 5-hydroxy-1-formylisoquinoline thiosemicarbazone (III), 3-hydroxy-2-formylpyridine thiosemicarbazone (IV), and 5-hydroxy-2-formylpyridine thiosemicarbazone (V) are readily soluble in water. Compounds III and IV, at the optimum effective dosage regimens, caused a greater prolongation of the survival time of mice bearing the L1210 lymphoma than did the parent derivatives, while II and V produced antineoplastic activity against sarcoma 180 and the L1210 lymphoma, respectively, equivalent to that of the parent compounds.

A VARIETY OF thiosemicarbazones of α-(*N*)-heterocyclic carboxaldehydes has been prepared and tested for antineoplastic activity (1–7). Several of these derivatives, especially 1-formylisoquinoline thiosemicarbazone (2, 3), its 5-hydroxy derivative (4), and both 3-hydroxy-2-formylpyridine thiosemicarbazone and 5-hydroxy-2-formylpyridine thiosemicarbazone (5, 6), have demonstrated pronounced antineoplastic activity when tested against a relatively wide spectrum of transplanted rodent tumors. To

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