Phase Equilibria of Nafcillin Sodium–Water

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Abstract □ The phase diagram for the binary system nafcillin sodium–water, was determined using differential scanning calorimetry (DSC) and polarized light microscopy. In the temperature range of −20–30°, three crystalline forms and an amphiphilic liquid crystalline phase were detected. The stable crystalline form of nafcillin sodium (form α) and water exhibit a eutectic mixture containing 28% drug (w/w) at −1°. In more dilute solutions a lower temperature eutectic (−5°) occurs. The composition and form of nafcillin sodium in this eutectic are not known. The form in the −5° eutectic was found to be metastable above 28% concentration and converted to form α. Two other crystalline forms were observed at 9° (form β) and 22° (form γ) at concentrations above 40%. The crystalline forms could not be further characterized due to their transient nature and existence in highly concentrated mixtures. A lamellar mesophase is present near ambient temperature in mixtures containing more than 55% nafcillin sodium. The phase equilibria were highly susceptible to supercooling. Temperature cycling methods were devised which gave reproducible DSC data and allowed construction of the phase diagram.

Keyphrases □ Nafcillin sodium—phase equilibria in water □ Phase diagram—nafcillin sodium–water binary system □ Crystals—forms of nafcillin sodium in aqueous solutions, determined by differential scanning calorimetry and polarized light microscopy □ Differential scanning calorimetry—determination of phase diagram for nafcillin sodium–water system □ Polarized light microscopy—determination of phase diagram for nafcillin sodium–water system

In the drug phase, polymorphic forms and solvates can have a large influence on solubility and dissolution properties (1). Liquid crystalline phases can also occur in pharmaceutical systems, although few examples of mesophase formation by drug compounds have been reported (2, 3). In the solution phase, the influence of salt forms and ionic equilibria on solubility is well understood (4). The difficulties involved in solubility determination for highly insoluble substances was recently studied (5). For such compounds the dissolution rate may strongly retard the approach to equilibrium.

Problems also occur in determining the solubility of compounds which are moderately to highly soluble in water. Nucleation and crystallization of metastable solutions are often inhibited by high solute concentrations or the presence of impurities. The potential for solute-solute interactions becomes greater as drug concentration increases. For example, doxycycline hydrochloride forms a dimer in moderately concentrated solutions which accounts for approximately half of the apparent solubility of the compound in water (6).

In the present investigation phase equilibria of a water-soluble antibiotic, nafcillin sodium (I), with water were studied. Preliminary observations using the polarizing microscope indicated that highly concentrated I–water mixtures formed an amphiphilic liquid crystalline phase. The present study was initiated to further investigate this unusual phase behavior and to determine the phase diagram.

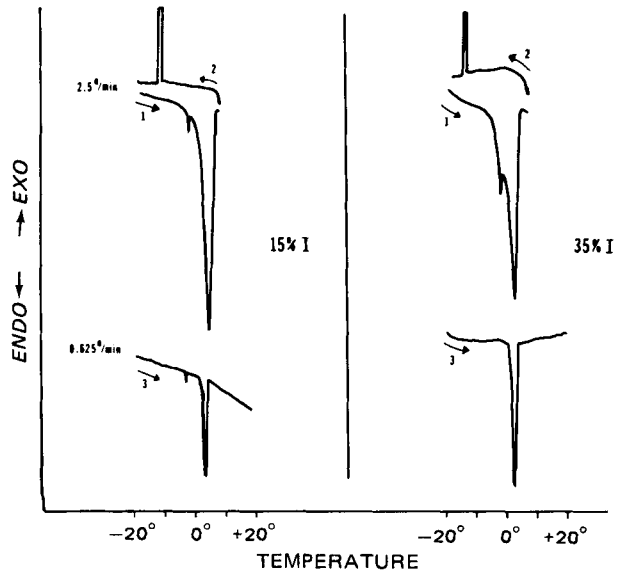

Figure 1—*DSC scans for 15 and 35% nafcillin sodium–water mixtures. Scans 1 and 2 were run at 2.5°/min while scan 3 was obtained at 0.625°/min. Prior to scan 1 the samples were cooled from ambient temperaure to −20° at 2.5°/min.*

EXPERIMENTAL

Transition temperatures were determined using a differential scanning calorimeter[1]. Solutions of I and water were prepared by weight at room temperature and one drop was placed in the aluminum sample pan using a capillary pipet. After sealing, the pan was placed in the sample holder and an empty pan was used as the reference. The low-temperature cell cover supplied with the instrument was filled with dry ice and acetone for cooling the detector cell, which was continuously purged with dry nitrogen. Samples were used within 4 hr to avoid possible effects due to penicillin hydrolysis.

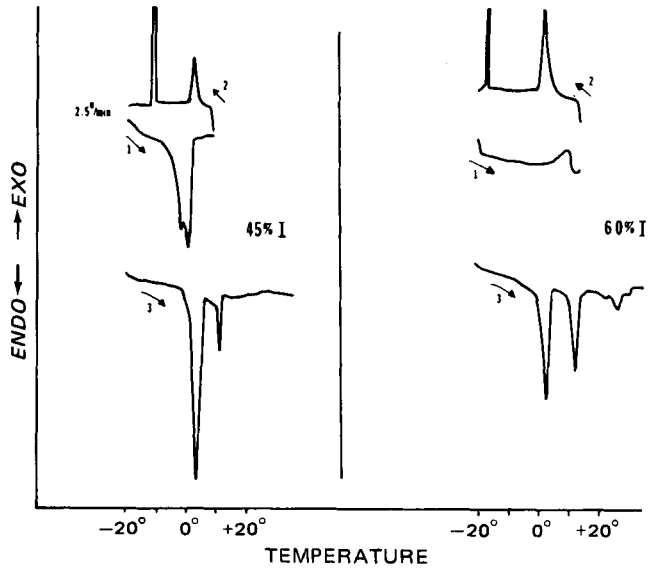

Figure 2—*DSC scans for 45 and 60% nafcillin sodium–water mixtures. All scans were run at 2.5°/min. Prior to scan 1 the samples were cooled from ambient temperature to −20° at 2.5°/min.*

[1] DSC-1B, Perkin-Elmer, Norwalk, Conn.

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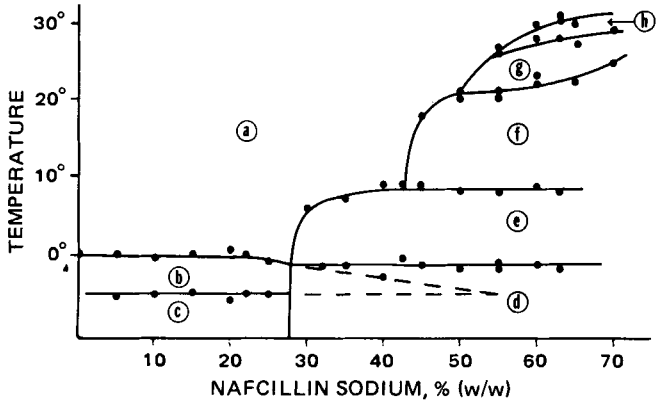

Figure 3—*Phase diagram for nafcillin sodium–water. The dashed line indicates a possible pathway for formation of the −5° eutectic. Phase regions are:* a, *isotropic solution;* b, *ice and solution;* c, *eutectic mixture of ice and low temperature form of nafcillin sodium;* d, *eutectic mixture of ice and crystalline Form α;* e, *solution and Form α;* f, *solution and Form β;* g, *solution and Form γ;* h, *solution and lamellar mesophase.*

Temperature calibration was checked using water, cyclohexane (mp 6.5°), benzene (mp 5.5°), and indium (mp 157°). At the slowest scanning rate, 0.625°/min, the melting transition of water occurred over a 3° range. This behavior was attributed to inefficiency of heat transfer in the system and the large heat of fusion of water. Under the same conditions, the hydrocarbons and indium gave much narrower peaks. The transition temperature was chosen to be the temperature at which ~10% of the total peak height of a transition had occurred. This convention was adopted to allow comparison of large and small transitions in a single scan and to account for variation in sample weight. This method gave melting points for the standards equal to literature values (7). The instrument output was plotted using an X–Y recorder.

The DSC measures the differential heat (millicalories per second) required to keep the sample and reference at the same temperature. Thus, less heat per unit time is required when scanning slowly than when scanning rapidly. The scanning rate is not indicated on the X–Y recorder but the longer time required to scan a peak at a slower scan rate will cause the apparent peak area to be smaller.

A polarizing microscope[2] with a camera attachment was used to observe and photograph the phases. Samples were either equilibrated in a test tube and quickly transferred to a slide for observation or cooled directly on the microscope slide. Although the temperature could not be closely controlled using these methods, the crystalline forms and phase transitions were clearly and reproducibly observed.

Nafcillin sodium monohydrate was used as received[3]. Distilled water which had been redistilled from an all-glass apparatus was used.

RESULTS

The thermal behavior of I–water mixtures was investigated up to 70% I (w/w). At this concentration high viscosity and poor reproducibility prevented further study. Figures 1 and 2 show representative DSC scans over the concentration range studied. Considerable difficulty was encountered in obtaining reproducible data due to the tendency of cooled solutions to supersaturate. The repetitive scanning sequence shown in the figures induced crystallization and provided reproducible DSC data. The transition temperatures from the equilibrium data (scan 3) were used to construct the phase diagram shown in Fig. 3.

After the outline of the phase diagram was established the phases were examined microscopically to determine the nature of the physical changes associated with the thermal processes. Several phase regions were photographed under polarized light and the results are shown in Figs. 4–9. Further identification, such as isolation and spectrometric analysis, was not possible due to the narrow temperature range of existence for the phases.

DISCUSSION

DSC Analysis of Nafcillin Sodium–Water Mixtures—The 15% I scans (Fig. 1) are representative of data obtained in the 5–28% concentration range. A small sharp endotherm occurred at −5° on the shoulder of a large broad endotherm at 0° (scan 1). At the upper temperature limit (6°) the scanning direction was reversed and the sample recooled to −20° (scan 2). This cooling resulted in an exothermic transition between −10 and −20°. The exact temperature of the exotherm varied between samples and was concentration dependent. In some runs a small shoulder was detected on the low temperature side of the exotherm indicating the reversibility of the −5° transition.

Upon warming the sample a second time (scan 3) the −5° endotherm was unchanged although the large endotherm occurred over a narrower temperature range. The apparently smaller area of the transition in scan 3 is due to a fourfold reduction in scanning rate compared to scans 1 and 2. The thermal data at this concentration are typical of a simple eutectic transition followed by melting of crystalline ice at 0°. The melting of an eutectic in a binary system at constant pressure is invariant according to the Gibbs Phase Rule. In DSC this results in an endothermic transition during heating which proceeds until one of the three phases disappears. Solubility equilibrium of the remaining phases, ice and solution, is mo-

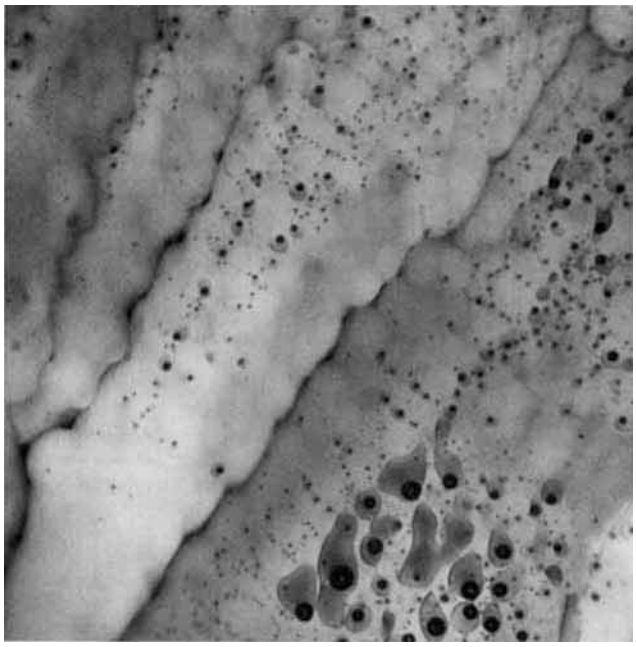

Figure 4—*Region* c, *eutectic mixture of ice and low temperature form of I. Long axis ≃ 400 μm (Initial concentration = 30%).*

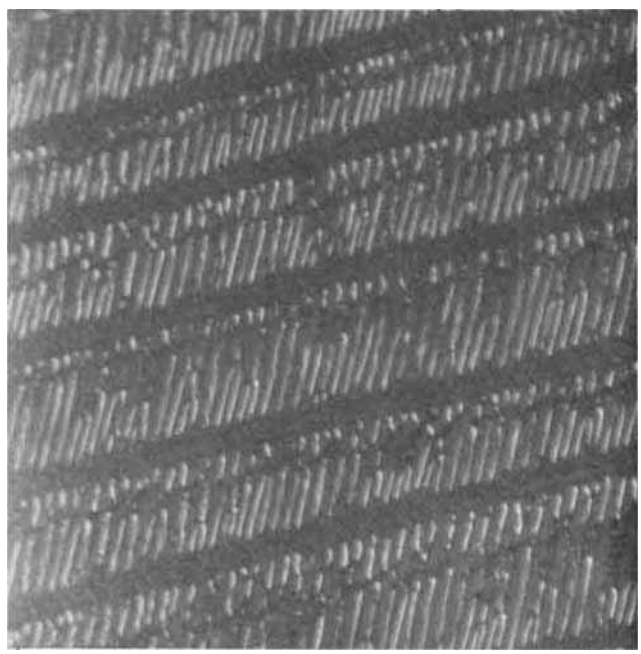

Figure 5—*Region* b, *ice and solution. Long axis ≃ 400 μm (Initial concentration = 25%).*

[2] Zetopan, Reichert, Vienna, Austria.
[3] Wyeth, Philadelphia, Pa.

Figure 6—*Region* e, *form* α *and solution. Long axis* $\simeq$*400* μ*m (Initial concentration = 60%).*

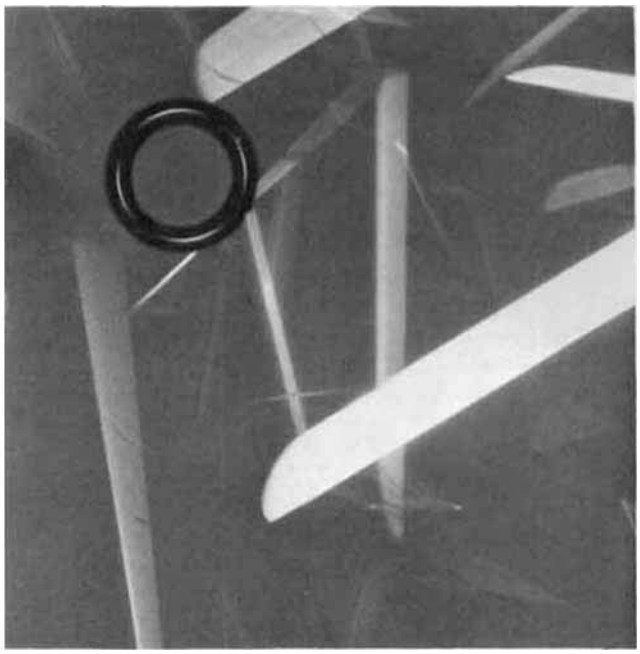

Figure 8—*Region* g, *solution and Form* γ *(needles). Long axis* $\simeq$*400* μ*m (Initial concentration = 60%).*

novariant at constant pressure resulting in a gradual endothermic transition over a wider range of temperature.

The lack of significant freezing point depression in this system is due to the surface activity of I. The critical micelle concentration of I is approximately 20–30 mg/ml (8). Thus, under the conditions of the present study, I is largely present in the system as micelles resulting in minimal freezing point depression of water.

At 35% I (Fig. 1, right side), the data in scans 1 and 2 are qualitatively similar to the 15% mixture. The temperature of the ice-melting endotherm in scan 1 is slightly depressed due to the higher concentration. A major difference exists between the two concentrations. Although present in scan 1, the −5° eutectic point is absent in scan 3. These data indicate that at 35% I the −5° eutectic is metastable since it occurs in the first warming cycle (scan 1) but not in the second (scan 3).

The method employed for scans 1 and 2 (warming to 6–8° and refreezing) was found to be necessary to eliminate the −5° endotherm in scan 3. If the sample is warmed to room temperature (instead of 6–8°) and refrozen, a profile similar to scan 1 is repeatedly obtained. In some runs at this concentration, a small endotherm at 7–8° can be detected. This endotherm becomes prominent at higher concentrations, as shown in Fig. 2. These data can only be explained by the existence of a less soluble (more stable) form of I, different from that present in the −5° eutectic.

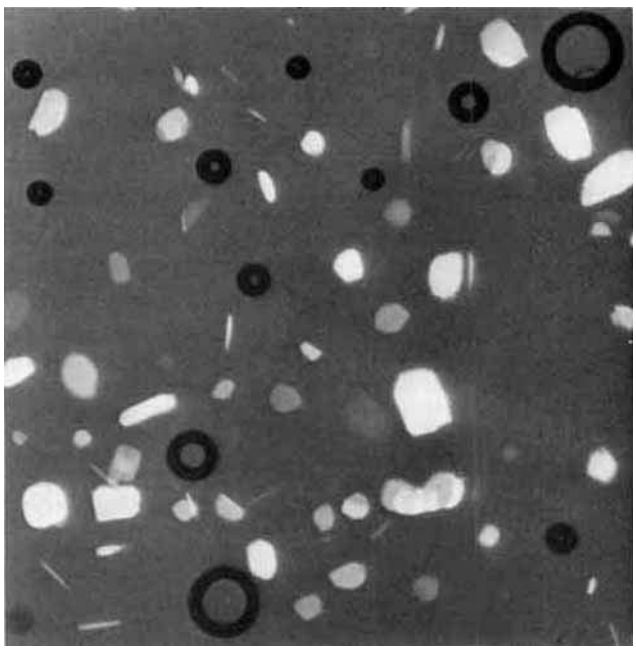

Figure 7—*Region* f, *form* β *(plates) and solution contaminated with Form* γ *(needles). Long axis* $\simeq$*400* μ*m (Initial concentration = 60%).*

The thermal behavior of 45% I solutions is shown in Fig. 2. Scan 1 is again similar to that found at lower concentrations having an apparent −5° eutectic closely followed by the apparent ice-melting endotherm. In the cooling cycle, scan 2, an exotherm occurs at about 2° in addition to the exotherm at −12°. In scan 3 the system is quite different from the lower concentrations. The −5° metastable eutectic is eliminated during scans 1 and 2 and endotherms at −1° and 9° are observed.

At this concentration the −1° transition is due to the dissolving of the eutectic mixture of ice and I. The peak areas indicate that most of the sample is present as the eutectic mixture. Microscopic studies show that the form of I in this eutectic is not the same as that found below 28% concentration. The two-phase region above the eutectic temperature contains crystalline I (denoted form α) and solution.

At 9° the undissolved form α undergoes an endothermic transformation to another crystalline phase, form β. The newly formed crystals of form β at 45% I gradually dissolve and the system becomes an isotropic solution above 18°. These relationships may be seen more clearly in the phase diagram, Fig. 3.

At 60% I (Fig. 2, right side), transitions in scan 1 are absent below 5°. At this temperature, a broad exotherm–endotherm pair occurs due to crystallization and subsequent dissolution of form α I. The cooling process (scan 2) shows a large exotherm due to crystallization of form α in addition to the exotherm for formation of the ice–form α eutectic at −17°. The effect of scan 1 was to induce crystallization of form α allowing the stable eutectic to deposit upon cooling. The warming curve (scan 3) contains the eutectic transition at −1° followed by conversion of form α to form β at 9°. The amount of form α converted to form β is larger at 60% than it was as 45% based on the peak areas. These processes are followed by three small endotherms attributed to the conversion at 22° of form β to a third crystalline phase, form γ, the possible transformation of form γ to a lamellar liquid crystalline phase at 27°, and the dissolution of the liquid crystalline phase to form isotropic solution at 30°.

The small size of the higher temperature endotherms is due to the lower concentrations of crystalline or liquid crystalline material remaining in the systems, since most of the sample is present as an isotropic solution. These conclusions were confirmed microscopically.

Phase Diagram—The various regions of the phase diagram are identified in the legend for Fig. 3.

Regions b *and* c—Freezing of dilute (<28%) aqueous I solutions first causes ice to crystallize below approximately 0° and the system (region

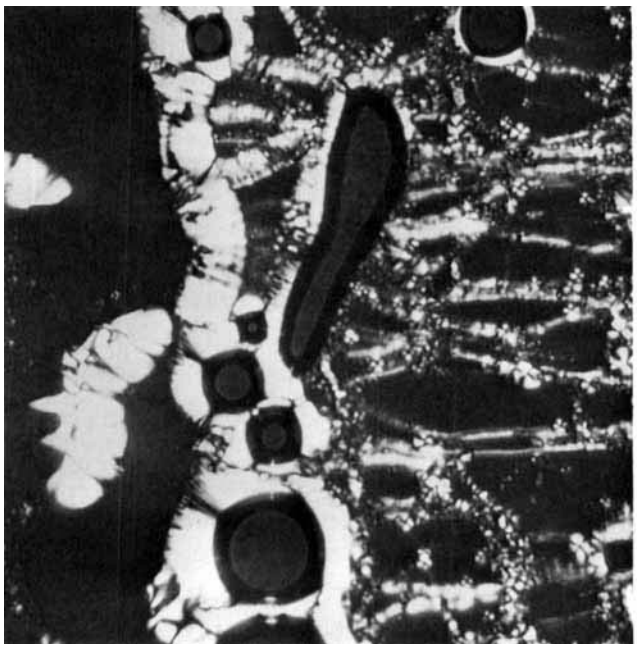

Figure 9—*Region* h, *solution and lamellar mesophase. Long axis* ≃*400 μm (Initial concentration = 60%).*

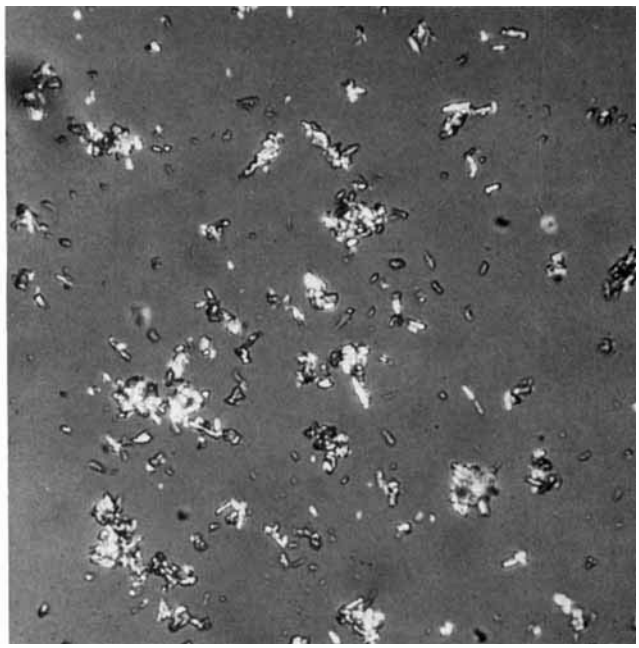

Figure 10—*Nafcillin sodium monohydrate used to prepare all samples. Long axis* ≃*400 μm.*

b) contains ice and solution. Further cooling leads to the −5° eutectic (region *c*), which is stable below 28% I. Above this concentration, the −5° eutectic is easily formed on cooling, indicated by the horizontal dashed line. But between −5 and −1°, the system is not stable and form α crystallizes. The diagonal dashed line shows a possible metastable extension of the ice–solution equilibrium line to the eutectic temperature. The eutectic is expected to contain at least 50% I.

Attempts were made to determine the nature of I in the −5° eutectic. Region *c*, shown in Fig. 4, has a silvery appearance. Figure 5 is a photograph of region *b*, ice and solution, taken just after melting of the eutectic. In the herringbone pattern, the dark channels are solution and the bright lines are ice.

The form of I in this eutectic has the thermodynamic properties of a phase. The sharp and constant eutectic temperature over a wide concentration range indicates that the phase has a fixed composition independent of the initial concentration. If I were in a glassy or amorphous state, no eutectic transition would occur. Only a broad endotherm for melting of ice would be expected (9). Possible explanations for the nature of I in this eutectic are that it is a liquid crystalline mesophase, crystalline forms β or γ, or an unknown crystalline form.

The first explanation, a liquid crystalline phase, is thought to be most consistent with the physical chemical properties of the system. Microscopy does not show discrete crystals, but instead a silvery appearance is observed. During crystallization of ice, the micelles are concentrated into increasingly smaller volumes of solution. The limit of condensation of the micelles would occur at some critical point and the result would probably be a liquid crystalline phase. This hypothesis is the basis of the "R" theory of Winsor (10) in which micellar and liquid crystalline structural features are unified into an order of progression from dilute to concentrated systems. At saturation, micellar solutions usually form amphiphilic liquid crystalline phases (10). Thus, the crystallization of ice from solution follows the horizontal 0° line up to 28% I, as shown in Fig. 3. Instead of forming form α and the eutectic at −1°, the crystallization of ice proceeds and the system continues to be two phases indicated by the diagonal dashed line in the phase diagram. The eventual limit is the formation of the −5° eutectic, which is expected to occur above 50% I.

The existence of the −5° eutectic may be a consequence of the micellar structures in solution. The shape and size of the micelles can change as a function of concentration or ionic strength (11). It is possible that at low concentrations, crystallization of form α would not be favorable because of incompatible micellar structure. At higher concentrations the micelles may become more similar in structure to the packing in form α allowing crystallization to take place.

Regions d *and* e—The eutectic temperature of water–form α mixtures is −1°, shown by the horizontal boundary of region *d* from 28 to 65% I (Fig. 3). It is the point of intersection of the ice–solution and form α–solution phase boundaries. Thus, at concentrations below 28%, ice crystallizes first on cooling the isotropic solution, whereas form α crystallizes first between 28 and 40%.

The crystalline nature of form α is shown in Fig. 6. The sample in Fig. 6 was equilibrated by immersing a test tube containing isotropic solution in an ice–water bath which caused the sample temperature to be in region *e*. Stirring induced crystallization and at this temperature the sample is a thick paste. A small amount of sample was then applied to a precooled slide and photographed immediately.

Crystals of form α are typically splinter-like with high birefringence. The rounded edges are due to dissolution of the crystals into the surrounding isotropic solutions as the sample becomes warmer.

The upper boundary temperature for region *e* (form α and solution) is 9° from 35 to 65% I. Between 28 and 43% I, equilibrium of form α with isotropic solution occurs. At 9° and 43% I, however, an intersection point occurs and the system is invariant. The three phases in equilibrium are form α, form β, and solution. The remaining fraction of form α in suspension is unstable and upon warming to 9°, it converts endothermically to form β. The system is invariant at this temperature until all form α is converted to form β. Such a transformation is called an incongruent melting point (12).

The phenomena of incongruent melting points is common in binary alloys as well as in aqueous solutions of hydrated salts. In phase equilibria, an incongruent melting point occurs when a compound is physically unstable and decomposes (transforms) at a temperature below its melting point; that is, it has no true melting point. Common salts with incongruent melting points are sodium chloride, calcium chloride, and sodium sulfate.

Sodium sulfate decahydrate forms a simple eutectic with water at −1.3° (12). The hydrated salt is in equilibrium with saturated solution up to 32.4°. At this temperature (the incongruent melting point), the decahydrate is unstable and converts to the anhydrous crystalline form. Thus, the invariant system contains the decahydrate, anhydrate, and solution. Thermal arrest occurs until the transformation is complete.

The analogous phase behavior of I and inorganic salts suggests that the observed crystalline forms differ in the degree of hydration in the crystalline state. This proposal is consistent with the small range of temperature in which the forms are stable. The similar crystal energies can be rationalized by small changes in spatial arrangement or solvation resulting in different crystalline forms. The alternate explanation of polymorphism cannot be ruled out at present.

Regions f *and* g—Form β is in equilibrium with solution in region *f* (9–22°, <43% I). The crystal habit of this form (Fig. 7) consists of brightly colored, reflective plates with rectangular faces. The rounded edges are due to dissolution as the slide warmed under the microscope. Samples

of this form are usually contaminated with needle-shaped crystals, which are the higher temperature form γ. This was shown by the following experiment. A tube containing 60% I at 15° containing needles and plates was slowly warmed in a water bath. As the temperature increased, samples were periodically taken and observed with the microscope. The sample temperature was directly monitored with a thermometer and a thermal arrest occurred at 22°, consistent with the DSC data. At this temperature, the plates gradually disappeared and the number of needles increased due to the transformation of form β to form γ. These forms are also related by an incongruent melting point.

When a sample at ambient temperature is cooled to 10–20° to induce crystallization of form β, a small amount of metastable form γ is often obtained, since the temperature region for form γ is passed during cooling. The high viscosity of the system and small difference in stability of the forms allows the metastable form γ to remain in suspension at a temperature where form β is the stable crystalline form. Form γ can be removed from mixtures by warming to near 22° and recooling. If samples of form β are rapidly warmed, the crystals dissolve directly to isotropic solution without the intermediacy of form γ or the mesophase.

Figure 8 shows the acicular habit of form γ which is present between 22 and 26°, above 50% I. These crystals are difficult to grow and were prepared by repeatedly cooling the sample below 22° and warming to obtain the large crystals shown. Form γ is a small fraction of the total sample which is largely isotropic solution.

Region h—When I and water are mixed on a microscope slide at room temperature, a layer of liquid crystalline phase forms on the interface between solid and saturated solution. The mesophase is also easily formed by peripheral evaporation of a drop of solution placed between slide and cover slip. Figure 9 shows the liquid crystalline phase formed from a 60% I sample. Its microscopic texture clearly identifies it as a lamellar mesophase (13). It is indistinguishable from authentic samples prepared with lecithin or docusate sodium. Growth tends to occur on the surfaces of bubbles causing the high birefringence (10). The oily streak texture shown in Fig. 9 is also characteristic of lamellar forms (13). Light pressure on the cover slip causes the viscous phase to flow showing that it is not a solid crystalline phase.

The thermal characteristics of the mesophase could only be approximated by DSC. The slow and erratic nature of its formation and the small thermal changes involved make the boundaries of region *h* uncertain. In addition, the relationship between form γ and the mesophase is not clear since it is difficult to obtain reliable data at such high concentrations. Microscopic samples have been observed in which the mesophase and needles of form γ are present simultaneously so that the conditions for formation of these phases must be quite similar.

In a lamellar mesophase, the amphiphilic molecules are arranged in bilayers with polar groups oriented to the aqueous regions separating the layers. This arrangement was proved for a number of substances using small angle X-ray scattering data (10). The hydrophobic portions form the interior of the bilayers and the thickness of the bilayer is about 40 Å. The lamellar mesophase commonly appears in aqueous systems containing fatty acid soaps, surfactants, and phospholipids. It is only one of several known amphiphilic liquid crystalline phases.

Relationship of the Low Temperature Forms with the Monohydrate—The form of I in commercial use is a monohydrate. Figure 10 shows the bulk form of I used to prepare all samples. It would be reasonable to expect that this crystalline form would be the same as one of the forms identified in this study. Seeding experiments, however, indicate that they are not related. Small amounts of monohydrate were seeded into supersaturated solutions at the temperatures of the three other forms. In each case the seed dissolved, although it did so more slowly at the lower temperatures. Crystallization eventually occurred but control samples indicated that seeding had no effect. The monohydrate appears to be a higher energy form (more soluble) than forms α, β, or γ.

Implications in Freeze-Drying—Although nafcillin sodium is not supplied in a freeze-dried form, several comments should be made regarding the importance of such equilibria in freeze-drying processes. Simple freezing may cause the drug to be present in a metastable state. This may lead to difficulties in the drying process as well as poor physical properties of the dried material (9, 14). These problems are especially serious when the drug is present at high concentration. Gatlin and DeLuca (14) have shown that the physical state of cefazolin sodium and nafcillin sodium in the dried product could be changed by thermal treatment prior to freeze-drying. If processed directly, the frozen drug solutions led to amorphous products, whereas if the frozen solutions were warmed to near the eutectic temperature and refrozen the resulting products were crystalline. The warming–cooling cycle in the frozen state induced crystallization of the drug which was amorphous after the initial freezing.

A single crystalline drug form should not always be expected in frozen or subambient systems. Equilibria beyond the usual eutectic state can occur with drugs in a small temperature range. The nafcillin sodium–water phase diagram shows that the initial drug concentration is an important factor. The form of I in the −5° eutectic (0–28% I) is different from the form α crystals found at higher concentrations. It is expected that this would lead to differences in the respective freeze-dried products.

Depending upon the cooling conditions above 0° the crystalline form of I can easily be altered. Forms β or γ can crystallize and allow little or no form α, the stable form at low temperatures, to be present in the frozen state. This is possible because the forms are similar in energy. In some cases, thermal treatments of the solution may be necessary in the 0–20° range to initiate crystallization of the form desired for freeze-drying.

REFERENCES

(1) E. Shefter and T. Higuchi, *J. Pharm. Sci.*, **52,** 781 (1963).

(2) A. R. Mlodozeniec, *J. Soc. Cosmet. Chem.*, **29,** 659 (1978).

(3) J. S. G. Cox, G. D. Woodward, and W. C. McCrone, *J. Pharm. Sci.*, **60,** 1458 (1971).

(4) S. F. Kramer and G. L. Flynn, *ibid.*, **61,** 1896 (1972).

(5) T. Higuchi, F. L. Shih, T. Kimura, and J. H. Rytting, *ibid.*, **68,** 1267 (1979).

(6) J. B. Bogardus and R. K. Blackwood, Jr., *ibid.*, **68,** 188 (1979).

(7) "The Merck Index," 8th ed., Merck and Co., Rahway, N.J., 1968.

(8) S. C. Penzotti, Jr., and J. W. Poole, *J. Pharm. Sci.*, **63,** 1803 (1974).

(9) L. R. Rey, *Ann. N.Y. Acad. Sci.*, **85,** 510 (1960).

(10) P. A. Winsor, *Chem. Rev.*, **68,** 1 (1968).

(11) F. M. Menger, *Acc. Chem. Res.*, **12,** 111 (1979).

(12) S. Glasstone, "Textbook of Physical Chemistry," 2nd ed., Van Nostrand, New York, N.Y., 1946, chap. 10.

(13) F. B. Rosevear, *J. Am. Oil Chem. Soc.*, **31,** 628 (1954).

(14) L. Gatlin and P. P. DeLuca, *J. Parenter. Drug Assoc.*, **34,** 398 (1980).

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