Characterization of Frozen Aqueous Solutions by Low Temperature X-ray Powder Diffractometry

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Purpose. A low temperature X-ray powder diffractometric (XRD) technique has been developed which permits *in situ* characterization of the solid-state of solutes in frozen aqueous solutions.

Methods. A variable temperature stage, with a working temperature range of –190 to 300°C, was attached to a wide-angle XRD. The stage was calibrated with a sodium chloride-water binary system.

Results. When aqueous nafcillin sodium solution (22% w/w) was frozen, eutectic crystallization of the solute was not observed. However, annealing at –4°C, caused crystallization of the solute. With increasing annealing time, there was a progressive increase in the crystallinity of the solute. Studies were carried out with sodium nafcillin solutions ranging in concentration from 20 to 50% w/w. The solid-state of the phase crystallizing from solution was independent of the solute concentration. Next, solutions of mono- and disodium hydrogen phosphate were individually frozen. Only the latter crystallized as the dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$). However when an aqueous buffer mixture of mono- and disodium hydrogen phosphate was frozen, the former inhibited the crystallization of the latter.

Conclusions. Since freezing of solutions is the first step in lyophilization, the XRD technique can provide a mechanistic understanding of the alterations in solid-state that occur during freeze-drying. DSC has so far been the technique of choice to study frozen systems. The advantage of XRD is that it not only permits unambiguous identification of the crystalline solid phase(s), but it also provides information about the degree of crystallinity. While overlapping thermal events are difficult to interpret in DSC, XRD does not suffer from such a limitation.

KEY WORDS: low temperature X-ray powder diffractometry; sodium nafcillin; monosodium hydrogen phosphate; disodium hydrogen phosphate.

INTRODUCTION

Some antibiotics, when formulated as solutions, have unacceptably short shelf-life (1). Under such circumstances, there are two approaches to prepare parenteral products with adequate shelf-life. (i) Formulation as a 'premixed frozen solution'. These solutions are prepared, immediately frozen and stored at ≤ –20°C. The product is thawed to room temperature at the time of administration. (ii) Formulation as a freeze-dried sterile powder which is reconstituted before administration (1,2).

When an aqueous antibiotic solution is cooled, the first event usually observed is the primary crystallization of ice. This results in freeze concentration of the solute. The secondary crystallization of solute can then occur. However, in some instances, the solute remains in a supercooled amorphous state (3,4). The solid-state of the active ingredient in the frozen formulation can profoundly influence the stability of the dosage form. When frozen ampicillin solutions were stored at –20°C, significant drug decomposition occurred in as little as 48 hours (5). This instability was attributed to the existence of the drug in a freeze concentrated state since it had not crystallized from solution.

Differential scanning calorimetry (DSC) has so far been the most popular technique for the characterization of frozen systems. However, the technique suffers from some drawbacks. (i) It does not permit direct identification of crystalline solid phase(s). Moreover, it is difficult to draw conclusions about the degree of crystallinity. (ii) When there is significant overlap of thermal events, interpretation of the DSC curves becomes difficult if not impossible. Therefore, DSC alone is often unsuitable for the unequivocal characterization of the solid-state of solutes in frozen systems.

Low temperature X-ray powder diffractometry (XRD) is an ideal complement to DSC for the characterization of frozen systems. It is possible to subject samples to controlled temperature programs and obtain X-ray powder diffraction patterns as a function of temperature. In these systems, the temperature can range from –190 to +300°C and powder patterns can be obtained while heating or cooling the sample. This provides direct and unambiguous identification of any crystalline solid phases in the system. Simultaneous identification of multiple crystalline phases is possible, because each phase is characterized by its unique XRD pattern. This feature was exploited for simultaneous identification of hexagonal ice and two hydrates of ethanol in frozen water-ethanol systems (6). In addition, the polymorphic transition of cubic to hexagonal ice was detected only by low temperature XRD (7). It was not detected by calorimetry because of the small enthalpy change associated with this transition (8 $J \cdot g^{-1}$) coupled with the slow rate of transition (7,8). An added advantage of XRD is that the intensities and the widths of the diffraction peaks provide insight into the crystallinity of the sample. Low temperature XRD was found to be an excellent complement to differential thermal analysis in the characterization of water-glycine-sucrose ternary systems (9).

Low temperature XRD has found limited application in the study of pharmaceutical systems. Our first object therefore was to develop a low temperature XRD method to characterize the solid-state of frozen aqueous solutions consisting of one or more solutes. The second object was to use this technique to monitor the crystallization induced by annealing in an amorphous freeze-concentrate.

MATERIALS AND METHODS

Materials

Sodium nafcillin monohydrate ($C_{21}H_{21}N_2NaO_5S \cdot H_2O$; Sigma, St Louis, MO), sodium chloride (Sigma), disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$; Mallinckrodt, Paris, KY) and sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$; J.T. Baker; Phillipsburg, NJ) were used as received.

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X-ray Powder Diffractometry

Instrumentation

A variable temperature powder X-ray diffractometer (Model XDS 2000, Scintag) was used to characterize the system at different temperatures. A HIGH-TRAN SYSTEM, which included a heat and temperature controller (Micristar®, Model 828D, R. G. HANSEN & ASSOCIATES) was used to control the sample temperature. The working temperature range of the variable temperature XRD is –190°C to 300°C. The samples to be analyzed were usually solutions at room temperature. They were filled into the copper holder and cooled at an approximately constant rate to the desired temperature. XRD patterns were obtained by exposing them to CuKα radiation (45 kV × 40 mA), wherein the scanning speed was 5°2θ·min^{-1} and the step size was 0.03°2θ. The samples were then heated at the desired rate and XRD patterns were obtained at different temperatures. However, during the XRD runs, the samples were maintained under isothermal conditions at the selected temperatures.

Calibration of the Variable Temperature Stage

The International Centre for Diffraction Data (ICDD) maintains a collection of single phase X-ray powder patterns. Once an XRD pattern is experimentally obtained, it is possible to objectively compare it with that reported in the ICDD database on a line by line basis. Since there are few literature reports on low temperature XRD, it was necessary to calibrate the variable temperature stage. Since the phase equilibria of sodium chloride–water binary system has been characterized in detail, it was used to calibrate the variable temperature stage of the powder X-ray diffractometer (10). An aqueous sodium chloride solution (20% w/w) was cooled from room temperature to –50°C, at ~20°C/min in the XRD holder. The sample was then warmed to –30°C at 10°C/min and its diffraction pattern was obtained over the angular range of 10 to 50°2θ. The heating was continued at 1°C/min up to –22°C and then at 0.5°C/min up to –20°C. XRD patterns were obtained at –22°C and at –20°C. The heating was continued at 1°C/min up to –10°C and XRD patterns were obtained at several temperatures in this range. A second composition, wherein the sodium chloride concentration was 80% w/w, was also evaluated. As before, the sample was cooled to –50°C, at ~20°C/min in the XRD holder. The sample was then warmed to –10°C at 10°C/min and then the heating was continued at 1°C/min up to –2°C and then at 0.5°C/min up to +2°C. XRD patterns were obtained at –2 and at +2°C.

Sodium Nafcillin Solutions

An aqueous solution of sodium nafcillin (22% w/w) was prepared at room temperature, cooled to –20°C in the XRD holder at ~20°C/min and its diffraction pattern was obtained over the angular range of 5 to 40°2θ. The sample was then heated to –4°C at 0.6°C/min and XRD patterns were obtained at –10 and at –4°C. These experiments were repeated with several solutions, wherein the sodium nafcillin concentration ranged from 20 to 50% w/w.

One of these samples (22% w/w) was also subjected to annealing. In this case, the solution was first cooled to –20° and then heated to –4° at 0.6°C/min. The sample was annealed at this temperature for 2 hours and XRD patterns were obtained at 5 min intervals. The integrated intensity (after appropriate background subtraction) of the 9.54 Å line (peak at 9.28°2θ) was obtained as a function of annealing time.

The effect of heating rate on the crystallization of sodium nafcillin was also examined. After cooling the solution to –20°C, the samples were heated to –4°C at heating rates of 0.2 and 0.1°C/min. XRD patterns were obtained at –10°C and at –4°C.

Sodium Phosphate Buffer Solutions

Aqueous sodium dihydrogen phosphate solutions of three different concentrations (0.36, 0.73 and 1.45 M) were cooled to –40°C in the XRD holder at ~15°C/min and their diffraction patterns were obtained over the angular range of 10 to 50°2θ. The samples were then heated to –3°C at 10°C/min and XRD patterns were obtained at –20°, –10° and –3°C. Identical experiments were carried out with a 0.19 M solution of disodium hydrogen phosphate.

Buffer solutions containing a mixture of sodium dihydrogen phosphate and disodium hydrogen phosphate were also examined. In these solutions, the concentration of disodium hydrogen phosphate was maintained constant at 0.19 M. The concentrations of sodium dihydrogen phosphate were 0.36 (pH 6.19), 0.73 (pH 5.65) and 1.45 M (pH 5.05). The buffer solutions were cooled to –40°C in the XRD holder and their diffraction patterns were obtained over the angular range of 10 to 50°2θ. The samples were then heated to –3°C at 10°C/min and XRD patterns were obtained at –20°, –10° and at –3°C.

RESULTS AND DISCUSSION

Calibration of the Low Temperature Stage of the Powder X-ray Diffractometer

The eutectic temperature of the sodium chloride–water system is –21.2°C and the eutectic composition is ~22% w/w sodium chloride (10). Sodium chloride exists as a dihydrate ($NaCl·2H_2O$) at this temperature. The XRD pattern of an aqueous sodium chloride solution (20% w/v) cooled to –30°C is presented in Fig. 1. As expected from the phase diagram, it contains peaks due to sodium chloride dihydrate as well as ice. Some of the peaks attributed to sodium chloride dihydrate are marked with an asterisk (for example at 15.0, 17.4 and 27.3°2θ) while those at 22.5, 24.0, 25.6° and 33.3°2θ were due to the hexagonal form of ice based upon a comparison with their respective powder patterns reported in the powder diffraction files of the ICDD (11). From Fig. I it is apparent that crystalline sodium chloride is present at –22°C while it disappears at –20°C. Thus, this technique permitted us to bracket the eutectic temperature between –20 and –22°C and this was in excellent agreement with the reported value of –21.2°C. The next XRD pattern was obtained at –18°C which is close to the liquidus phase boundary. When the temperature was raised to –16°C, no crystalline phases were present in the sample (Fig. 1). Thus the liquidus phase boundary was bracketed between –18 and –16°C, which is in good agreement with the reported value of –17°C.

According to the H_2O-NaCl phase diagram, when the wt. % of NaCl > 38, the anhydrous sodium chloride, sodium chloride

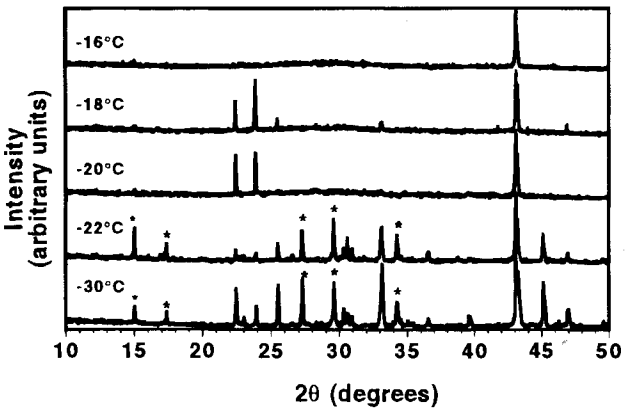

Fig. 1. XRD patterns of a frozen aqueous solution of sodium chloride (20% w/w) at different temperatures. The sample was cooled from room temperature to –50°C. It was then heated and XRD patterns were obtained at the desired temperatures. Some of the characteristic peaks of sodium chloride dihydrate are marked with an asterisk. These peaks have disappeared at –20°C which is above the eutectic temperature. The characteristic peaks of ice at 22.5 and 24.0°2θ are absent at –16°C which is above the liquidus phase boundary. The peak at 43.0°2θ is due to the copper sample holder.

dihydrate and brine will be in equilibrium at 0°C. Therefore, when the 80% w/w NaCl sample was heated, the sodium chloride dihydrate was expected to disappear at 0°C. The characteristic peaks of sodium chloride dihydrate at 15.0, 17.4 and 27.3°2θ were observed at –2°C, while they all disappeared at +2°C (Fig. 2). At this temperature, the observed XRD pattern is that of anhydrous sodium chloride. Therefore, we were able to bracket the phase boundary between –2 and +2°C which is in good agreement with the reported temperature of 0°C.

Sodium Nafcillin

Characterization

The XRD pattern of the 'as is' material matched that of sodium nafcillin monohydrate reported in the literature (12). The DSC curve (sample heated in a nonhermetically crimped aluminum pan under nitrogen purge) revealed an endotherm at ~105°C which has been attributed to dehydration and vaporization of water. When heated in the TGA (sample heated in a platinum pan under nitrogen purge), a weight loss of ~4.2% was observed over the temperature range of 100 to 140°C. This is close to the stoichiometric water content of 4.0% w/w in nafcillin monohydrate. However, the TGA profile revealed a continuous weight loss from ~105°C suggesting that the dehydration might be immediately followed by decomposition. The water content determined by Karl Fischer titrimetry was 4.8% w/w suggesting the existence of some sorbed water in the sample.

Frozen Sodium Nafcillin Solutions

The XRD patterns of frozen sodium nafcillin solutions (22% w/w) are presented in Fig. 3. When the solution was cooled to –20°C, only the presence of ice was evident from the intense peaks observed at 22.5, 24.0, 25.6 and 33.5°2θ (Fig. 3). Heating to –10°C and then on to –4° did not result in any changes in the XRD pattern and there was no evidence of crystallization of sodium nafcillin. When Gatlin and Deluca (13) performed DSC studies on frozen sodium nafcillin solutions, a reversible endotherm was observed at –7°C. They freeze-dried aqueous sodium nafcillin solutions (33.3% w/w) under two conditions, (i) below –10°C and (ii) warmed to –5°C, held for a short time and then cooled and dried. In the former case, the lyophile was X-ray amorphous while it was crystalline in the latter case. Based on this observation, it is possible that the endotherm observed at ~–7°C is due to combination of a glass transition and a reversible first order transition. When a similar system was studied by Milton and Nail (12), they observed an endotherm at –5.5°C followed by an exotherm which were respectively attributed to the melting of a lyotropic liquid crystalline phase followed by the crystallization of sodium nafcillin.

The unique advantage of XRD is that it provides direct evidence of the crystallization process and permits identification of the crystallized phase. Since heating to –4°C did not

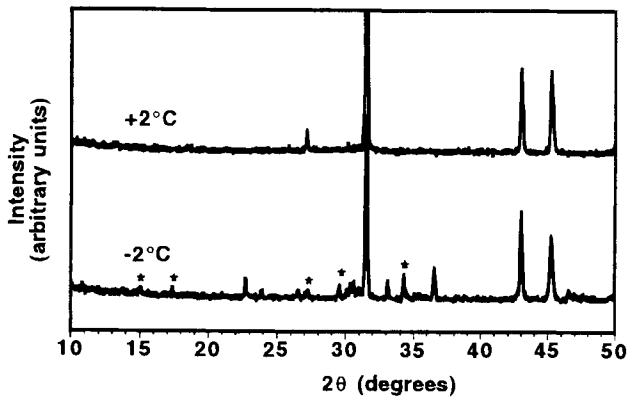

Fig. 2. XRD pattern of a frozen aqueous solution of sodium chloride (80% w/w). The characteristic peaks of sodium chloride dihydrate (marked with an asterisk) disappeared when the sample was heated to +2°C. Anhydrous sodium chloride is characterized by peaks at 27.3, 31.7 and 45.5°2θ (11).

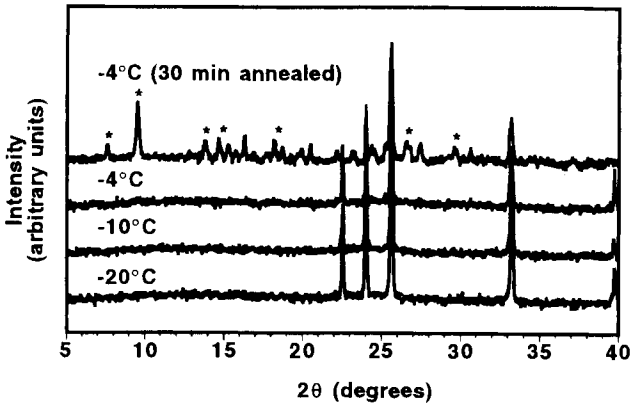

Fig. 3. XRD patterns of a frozen aqueous solution of sodium nafcillin (22% w/w) at different temperatures. The intense peaks observed at 22.5, 24.0, 25.6 and 33.5°2θ are attributed to hexagonal ice. While heating to –4°C did not result in crystallization of sodium nafcillin, annealing for 30 min caused its crystallization (characteristic peaks marked with an asterisk).

result in crystallization, the sample was annealed at this temperature for 30 min. This annealing temperature was based on the observations of Gatlin and DeLuca (13) discussed in the previous paragraph. Several new peaks appeared and these are attributed to crystallization of sodium nafcillin (Fig. 3). However, the solid-state of sodium nafcillin crystallizing from solution was different from that of the 'as is' sodium nafcillin (Fig. 3). We postulate that a higher hydrate is crystallizing from the frozen solution. The characterization of this phase is outside the scope of this work and will be the subject of a different publication.

We were interested in determining the effect of annealing time on the amount of sodium nafcillin crystallizing from solution. Therefore a solution of sodium nafcillin was first cooled to –20°C and then warmed to –4°C and held at this temperature for 1 hour. XRD patterns were obtained at regular intervals. The integrated intensity of one characteristic peak of sodium nafcillin (at 9.28°2θ) was plotted as a function of annealing time (Fig. 4). The plot revealed an initial dramatic increase in the amount of sodium nafcillin crystallizing from frozen solution.

Sodium nafcillin solutions ranging in concentration from 20 to 50% w/w were studied similarly. The XRD pattern of the solid crystallizing from solution was superimposable on the powder pattern of the annealed sample presented in Fig. 3 (XRD patterns not shown). This indicates that the solid-state of the phase crystallizing from solution was independent of the solute concentration. However, when the solute concentration was high (>30% w/w), the crystalline sodium nafcillin phase was detected as soon as the sample was warmed to –4°C (XRD patterns not shown). In these cases, annealing was not required to induce crystallization. However, annealing increased the amount of solute crystallizing from solution.

Crystallization was also achieved by altering the heating rates of the frozen solutions. When a 30% w/w solution was heated at slower heating rates of 0.2 and 0.1°C/min, crystallization of sodium nafcillin was observed at –4°C (data not shown).

From the chemical stability viewpoint, it is desirable to have crystallization of drug in frozen formulations. The stability of the solute existing in the amorphous state is expected to be less than that of its crystalline counterpart. In formulations prepared by freeze-drying, crystallization of drug in the frozen solution *may* be desirable. Crystallization of the active ingredient in the frozen solution will result in a crystalline freeze-dried product. Since crystallization can be achieved by annealing, low temperature XRD permitted real time study of the amount crystallizing as a function of annealing time. It will therefore be possible to determine the annealing time required so as to cause maximum crystallization of the active ingredient before the commencement of the primary drying cycle. There is one other advantage if the solute exists in the crystalline state. Primary drying is carried out below the eutectic temperature if the solute is crystalline. However, if the solute exists in the amorphous state, the primary drying is carried out below the T'_g. The eutectic temperature is expected to be substantially higher than the T'_g even in the maximally freeze-concentrated system (14). For example, the eutectic temperature and T'_g of mannitol are reported to be –0.5 and –29°C respectively (15). Therefore the existence of crystalline solute will permit primary drying at substantially higher temperatures than in the case of amorphous solutes.

For the sake of completeness, we wish to point out the potential disadvantages if the lyophile is crystalline. The reconstitution time of a crystalline lyophile is expected to be longer than that of its amorphous counterpart. It also appears that some lyoprotectants have to exist in the amorphous state in order to be effective.

The solid-state of 'as is' sodium nafcillin (sodium nafcillin monohydrate) was different from that of the sodium nafcillin crystallized in the frozen solution (Fig. 5a & 5b). In order to observe whether freeze-drying would bring about any changes in the solid-state of sodium nafcillin, a 40% w/w solution was first cooled to –40°C and annealed at –4°C for 1 hour. The annealed sample was then freeze-dried (Labconco® bench top freeze-dryer.) The freeze-dried material was identified to be sodium nafcillin hemihydrate, the XRD pattern of which (Fig. 5c) is different from that of 'as is' sodium nafcillin and the sodium nafcillin crystallized in the frozen state.

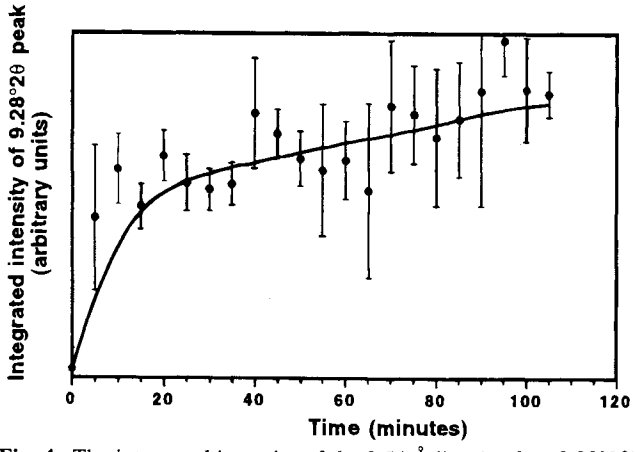

Fig. 4. The integrated intensity of the 9.54 Å line (peak at 9.28°2θ) of sodium nafcillin as a function of annealing time. Error bars represent standard deviations (n = 3). The curve is drawn to assist in visualizing the trends.

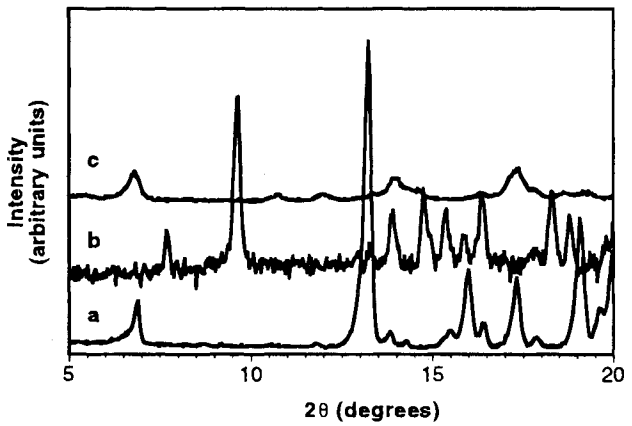

Fig. 5. The XRD patterns of (a) 'as is' sodium nafcillin, (b) frozen solution of sodium nafcillin (40% w/w) annealed at –4°C for one hour and (c) after freeze-drying solution (b).

Sodium Phosphate Solutions

Based on DSC studies, Murase and Franks (16) had concluded that sodium dihydrogen phosphate (NaH_2PO_4) does not crystallize and forms an amorphous freeze-concentrate whereas disodium hydrogen phosphate readily crystallizes as the dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$). Low temperature XRD permitted us to detect and identify phases crystallizing from solution. When aqueous sodium dihydrogen phosphate solutions, ranging in concentration from 0.36 to 1.45 M were frozen, there was no crystallization of the solute (Fig. 6a). However, when an aqueous solution of disodium hydrogen phosphate was frozen, the solute crystallized as a dodecahydrate (Fig. 6b; peaks at ~16.1, 20.2, 29.9, 30.7 and 31.3°2θ). When the system was warmed, the characteristic peaks of the dodecahydrate persisted up to –3°C (XRD patterns not shown). This is in agreement with the reported eutectic temperature of –0.5°C for the $Na_2HPO_4 \cdot 12H_2O$–water system (16).

When frozen ternary buffer systems containing both monosodium and disodium hydrogen phosphate were studied by DSC, unambiguous interpretation of the DSC curves was not possible. Therefore DSC was of limited utility in studying such systems. In order to evaluate the usefulness of XRD in the characterization of such complex systems, powder patterns of frozen buffer solutions which contained both disodium and monosodium hydrogen phosphate were next obtained (Fig. 6). In these solutions, the concentration of disodium hydrogen phosphate was maintained constant at 0.19 M while the sodium dihydrogen phosphate concentration ranged between 0.36 and 1.45 M. Sodium dihydrogen phosphate appears to be effective in inhibiting the crystallization of disodium hydrogen phosphate. At a low sodium dihydrogen phosphate concentration (0.36 M), crystallization of disodium hydrogen phosphate was not completely inhibited (Fig. 6c). However, this was accomplished at higher concentrations of sodium dihydrogen phosphate (Fig. 6d & 6e). As mentioned in the previous paragraph, the eutectic temperature of the $Na_2HPO_4 \cdot 12H_2O$–water system was –0.5°C. Eutectic crystallization is governed by nucleation as well as the growth of the crystalline phase from the freeze-concentrated solution. Inhibition of either of these processes is possible by an additive that forms an amorphous freeze-concentrate. As the solution is cooled, the nucleation inhibition coupled with the pronounced increase in viscosity inhibits crystal growth particularly as the temperature approaches T'_g (17). Hence monosodium hydrogen phosphate which forms an amorphous freeze concentrate effectively inhibited the crystallization of disodium hydrogen phosphate.

van den Berg and Rose (18) studied the effect of freezing on the pH and composition of sodium phosphate solutions. They prevented supercooling and supersaturation by using the seeding technique. The ternary eutectic temperature was determined to be –9.9°C and the eutectic mixture composition was 3.42 M monosodium and 0.06 M disodium hydrogen phosphate. Therefore the ratio of the monosodium to the disodium salt is 57 (18). Even at a much lower monosodium to disodium salt ratio of ~4, we observed complete inhibition of crystallization (Fig. 6d). Our results strongly suggest that the system has a pronounced tendency to supercool. Therefore, we did not deem it necessary to evaluate the eutectic mixture. However, in an effort to induce crystallization, the frozen solutions were heated from –40°C up to –3°C. When the monosodium hydrogen phosphate concentration was 0.36 or 0.73 M, crystallization of disodium hydrogen phosphate was observed. Fig. 7 is a representative example, wherein there is evidence of crystallization of disodium hydrogen phosphate (as the dodecahydrate) at –20°C. However, when the sodium dihydrogen phosphate concentration was increased to 1.45 M, crystallization was not observed.

Phosphate buffers, consisting of a mixture of monosodium and disodium hydrogen phosphate, are routinely used in freeze-dried pharmaceutical formulations (19). The selective crystallization of disodium hydrogen phosphate can result in a dramatic shift in the pH of the solution and this has been elegantly demonstrated by Gomez (19). Using XRD, we have

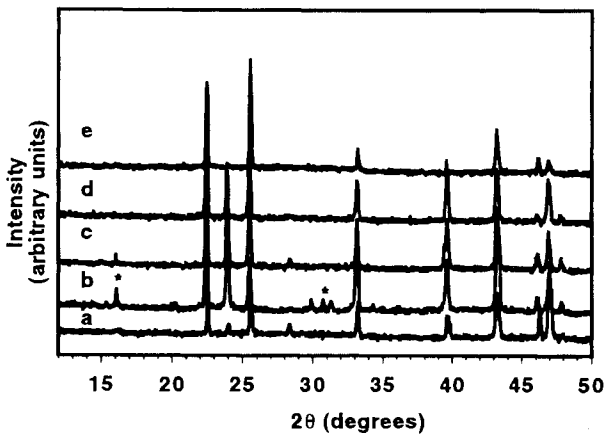

Fig. 6. XRD patterns of frozen aqueous solutions of, (a) sodium dihydrogen phosphate (1.45 M), (b) disodium hydrogen phosphate (0.19 M) and (c) to (e) mixtures of monosodium and disodium hydrogen phosphate. In these mixtures, the concentration of disodium hydrogen phosphate was maintained constant (0.19 M) while the concentration of monosodium hydrogen phosphate were, (c) 0.36 M, (d) 0.73 M and (e) 1.45 M. All the XRD patterns were obtained at –40°C. Some characteristic peaks of disodium hydrogen phosphate dodecahydrate are marked with an asterisk.

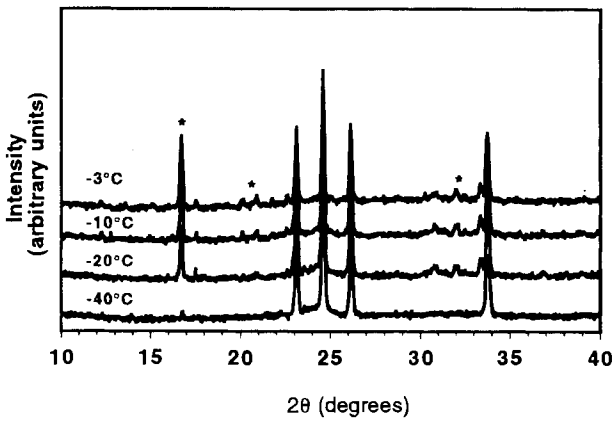

Fig. 7. XRD pattern of a frozen aqueous solution containing disodium hydrogen phosphate (0.19 M) and sodium dihydrogen phosphate (0.36 M) at different temperatures. Some characteristic peaks of disodium hydrogen phosphate dodecahydrate are marked with an asterisk.

determined the concentration ratios of monosodium to disodium hydrogen phosphate at which crystallization of neither phase occurs from solution. By inhibiting the crystallization of disodium hydrogen phosphate, changes in pH could be minimized. We believe that low temperature XRD will be an excellent complement to the *in situ* pH measurement techniques developed by Gomez *et al* (20).

Problems Encountered

During the development of the low temperature XRD method, some problems were encountered, which appear to be unique to this technique. As mentioned in the Experimental section, solutions at room temperature were placed in the XRD holder and cooled to the desired temperature. Freezing resulted in a change in the volume and as a result, the sample surface and the holder surface were no longer coplanar. This is the possible reason for the slight shift in the peak positions that were sometimes observed. We minimized this problem by filling the holder with an accurately weighted amount of solution which allowed us to account for the expansion in volume encountered during freezing. The second problem is concerned with the XRD pattern of ice. Only the hexagonal form of ice was observed in all the frozen solutions. However, the intensities of the peaks of ice sometimes exhibited pronounced run-to-run variation. The three intense peaks of ice in the angular range of 22 to 26°2θ in Fig. 1, is one representative example. We do not have an explanation for this observation.

CONCLUSIONS

Two types of formulations are subjected to freezing: (i) premixed frozen solutions (which are the focus of this research project) and (ii) freeze-dried formulations. Numerous studies suggest that the performance of a frozen formulation (stability, ease of reconstitution) is dependent on the solid-state of the solute(s) in the frozen state. The solid-state of the solute(s) can be influenced by the nature and concentration of the additives, and processing related parameters (cooling rate, annealing temperature, time of annealing). So far, differential scanning calorimetry (DSC) has been the predominant analytical technique to characterize frozen systems. The limitations of this technique were earlier discussed. We propose low temperature diffractometry as a complement to DSC in the study and characterization of frozen systems. Using this technique, we have successfully characterized some simple frozen systems. While sodium nafcillin did not crystallize on freezing, crystallization was induced by annealing. It was also possible to monitor the amount of sodium nafcillin crystallizing as a function of the annealing time. Moreover, the solid-state of the crystallizing phase was different from that of the 'as is' sodium nafcillin. XRD is the only technique that permits such detailed *in situ* characterization of the solid-state in frozen systems. Some solutes do not crystallize when their aqueous solutions are frozen. We demonstrated that the presence of such a solute can inhibit the crystallization of a second solute. While disodium hydrogen phosphate crystallized when frozen, the presence of monosodium hydrogen phosphate effectively inhibited the crystallization of the former. Again XRD provided direct evidence of these events.

Though we have focused on frozen systems, the X-ray diffractometric technique has great potential utility in the characterization of lyophilized systems. By appropriate modification of the instrument, *in situ* characterization of alterations during the primary and secondary drying is possible. This will permit a mechanistic understanding of the alterations in solid-state during lyophilization.

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