Crystalline to Amorphous Transition of Disodium Hydrogen Phosphate during Primary Drying

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Purpose. To monitor the phase transitions during freeze-drying of disodium hydrogen phosphate.

Methods. The variable temperature sample stage of the X-ray diffractometer (XRD) was attached to a vacuum pump, which enabled the entire freeze-drying process to be carried out in the sample chamber. The phase transitions during the freeze-drying cycle were monitored in real time by XRD. Aqueous buffer solution (containing disodium hydrogen phosphate and sodium dihydrogen phosphate) was cooled at 2°C/min from room temperature to –70°C. It was then heated to –25°C and subjected to primary drying for 2 h at a chamber pressure of ~100 mTorr, followed by secondary drying at –10°C.

Results. In the frozen solution, disodium hydrogen phosphate had crystallized as the dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) as was evident from its characteristic lines at ~5.37, 4.27, and 2.81 Å. Primary drying for 2 h resulted in ice sublimation, and the complete disappearance of the dodecahydrate peaks.

Conclusion. The dehydration of the crystalline dodecahydrate resulted in an amorphous anhydrate. Thus the amorphous nature of the end product is a result of phase transitions *during* the process and do not reflect the solid-state of the ingredients during the entire process.

KEY WORDS: sodium phosphate buffers; freeze-drying; crystallization; dehydration.

Freeze-dried formulations are typically multicomponent systems, and often contain excipients such as stabilizers, buffers, and bulking agents. The desired solid-state of these ingredients, in the final lyophile, is often dictated by their function. For example, stabilizers should be retained amorphous while bulking agents are preferred in the crystalline state. In case of the buffering agents, solute crystallization in the frozen solution, can lead to significant pH shifts in the freeze-concentrated amorphous phase (1). For active ingredients that exhibit pH-dependent degradation, such pH shifts might accelerate chemical decomposition. Conclusions regarding solute crystallization during freeze-drying are usually based on physical characterization of the final lyophile. If the lyophile is amorphous, it is presumed that there was no solute crystallization during freeze-drying. The implicit assumption here is that once a solute crystallizes, it does not undergo a crystalline-amorphous transition during the freeze-drying cycle. In this report, we show that such an assumption can lead to erroneous conclusions when the crystallizing phase is a hydrate.

It is well known that sodium and potassium salts of phosphate buffer crystallize during cooling and in frozen aqueous solutions (2–4). This may lead to a decrease in pH of up to 4 pH units (4). Our discussion is restricted to sodium phosphate, wherein, it is only the disodium hydrogen phosphate that readily crystallizes as the dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) while sodium dihydrogen phosphate (NaH_2PO_4) does not crystallize and forms an amorphous freeze-concentrate (3). Earlier studies in our laboratory, using low temperature X-ray powder diffractometry (XRD), showed that the crystallization of disodium hydrogen phosphate was completely inhibited only in the presence of high concentrations of the sodium dihydrogen phosphate (5). We are reporting the crystallization of disodium hydrogen phosphate as a dodecahydrate and its subsequent dehydration during primary drying to yield an amorphous lyophile.

The phase transitions during the entire process of freeze-drying were monitored by *in situ* X-ray diffractometry. An X-ray powder diffractometer (Model XDS 2000, Scintag) with a variable temperature stage (Micristar, Model 828D, R. G. Hansen & Associates; working temperature range of –190° to –300°C) was used. The sample stage of the XRD was attached to a vacuum pump. As a result it was possible to carry out the entire freeze-drying process in the sample chamber of the XRD. Approximately 100 mg of 200 mM aqueous buffer solution (disodium hydrogen phosphate and sodium dihydrogen phosphate buffered to pH 7.4) was filled into a copper sample holder and cooled at 2°C/min from room temperature to –70°C. It was held for 20 min, heated to a shelf temperature of –25°C and subjected to primary drying at a chamber pressure of ~100 mTorr. Primary drying was conducted for 2 h after the disappearance of the characteristic XRD peaks of ice. The temperature was then increased to –10°C, where it was subjected to secondary drying.

Figure 1 shows the XRD patterns that were obtained during the various stages of the freeze-drying process. In the solution cooled to –70°C, in addition to the characteristic peaks of hexagonal ice, crystallization of disodium hydrogen phosphate dodecahydrate was evident from the peaks observed at ~16.5 (5.37 Å), 20.8 (4.27 Å), and 31.8°2θ (2.81 Å). Primary drying resulted in ice sublimation, which is evident from the progressive decrease in the intensities of the ice peaks. After the ice peaks had almost completely disappeared, the peaks of the buffer salt decreased in intensity signifying dehydration during primary drying. Though disodium hydrogen phosphate crystallized in the frozen solution, it dehydrated during drying and the resulting anhydrous phase was X-ray amorphous.

Based on crystal lattice studies, three cases have been distinguished following dehydration of hydrates: (i) the residue is amorphous or poorly crystalline, (ii) the residue recrystallizes with a different crystal lattice, and (iii) the crystal lattice of the residue is nearly identical to that of the original hydrate (6). Under the dehydration conditions of this experiment, disodium hydrogen phosphate hydrate belongs to the first category. There are numerous other examples of crystalline hydrates, which on dehydration, yielded amorphous anhydrates (7,8). It has been reported for example, that raffinose pentahydrate when stored in a vacuum oven at 60°C for 24 h, undergoes dehydration to yield an amorphous anhydrate (8). In an investigation currently in progress, the authors observed that raffinose was retained amorphous in frozen aqueous solutions. Annealing caused solute crystalliza-

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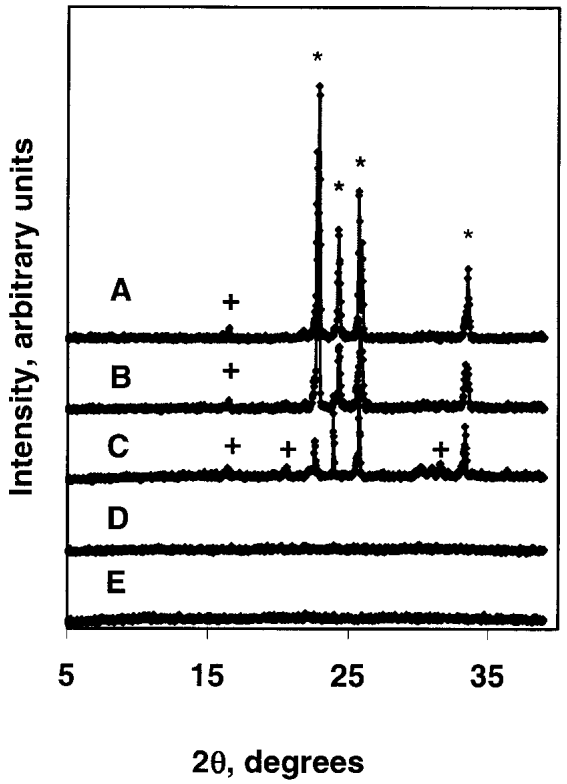

Fig. 1. *In situ* X-ray powder diffractometry during freeze-drying of aqueous solution of 200 mM sodium phosphate buffer. The solution was cooled from room temperature to –70°C at 2°C/min and then heated to the primary drying temperature of –25°C at 5°C/min. Secondary drying was carried out at –10°C. The characteristic peaks of ice (*) and disodium hydrogen phosphate dodecahydrate (+) are pointed out. (A) XRD pattern at –70°C, showing presence of hexagonal ice and dodecahydrate salt. (B) Start of primary drying at –25°C. (C) XRD pattern after 30 min of primary drying; intensities of ice peaks have reduced substantially. (D) Amorphous pattern at the end of primary drying, showing no evidence of ice or the dodecahydrate salt. (E) Amorphous pattern at the end of secondary drying.

tion, and then dehydrated to an amorphous anhydrate during primary drying (9). In a recent publication, we reported that cefazolin sodium crystallized as a pentahydrate in frozen aqueous solutions and then transformed to a poorly crystalline anhydrate during primary drying (10).

Observations, mentioned earlier, draw attention to the importance of physical characterization during all the stages of freeze-drying. If physical characterization is limited to the final product then phase transitions during the freezing or drying processes may not be detected. In the situation described in this report, crystallization during freezing followed by dehydration during drying led to an amorphous product. The amorphous nature of the end product is a result of the phase transitions that have occurred during the process and do not reflect the solid-state of the ingredients during the entire process.

At lower buffer salt concentrations (8 and 100 mM), Na_2HPO_4 existed as a crystalline anhydrate in the final freeze-dried cake indicating dehydration of the hydrate to the crystalline anhydrate (11). Because our experiments were carried out using higher salt concentrations of 200 mM, it seems that the amorphous dihydrogen sodium phosphate inhibited the crystallization of the disodium hydrogen phosphate anhydrate formed during drying.

Our observations also reveal the importance of baseline solid-state characterization of the active ingredient and the excipients in a formulation. If a solute is capable of existing as a hydrate and it crystallizes in frozen aqueous solutions, then the possibility of a hydrate crystallizing from solution is high. It is then important to determine whether dehydration during primary drying results in an amorphous or a crystalline anhydrate. The degree of crystallinity of the product phase can be influenced by the dehydration conditions. Thus, even subtle changes in the dehydration conditions could have an impact on the solid-state of the product phase and lead to batch-to-batch variations in the product.

There are analytical challenges in the characterization of phase transitions during pharmaceutical processing. While differential scanning calorimetry is an excellent technique to detect, and possibly quantify crystallization, it does not reveal the identity of the crystallizing phases. The *in situ* XRD technique enables identification of the crystallizing phases in real time. Moreover, the sequence of water loss was also revealed. Ice sublimation was followed by dehydration of the hydrate (Fig. 1).

The intention of this article is to shed light on the possibilities of phase transitions *during* freeze-drying and to speculate on their potential implications. The concept of "function-specific solid-state" of components (both active and excipients) may form the basis for the rational selection of the formulation ingredients and the development of the lyophilization cycle.

REFERENCES

1. J. F. Carpenter, M. J. Pikal, B. S. Chang, and T. W. Randolph. Rational design of stable lyophilized protein formulations: some practical advice. *Pharm. Res.* **14**:969–975 (1997).
2. L. Van den Berg and D. Rose. Effect of freezing on the pH and composition of sodium and potassium phosphate solutions; the reciprocal system KH_2PO_4-Na_2HPO_4-H_2O. *Arch. Biochem. Biophys.* **81**:319–329 (1959).
3. N. Murase and F. Franks. Salt precipitation during the freeze-concentration of phosphate buffer solutions**.** *Biophys. Chem.* **34**: 293–300 (1989).
4. G. Gomez, M. J. Pikal, and N. Rodriguez–Hornedo. Effect of initial buffer composition on pH changes during far-from-equilibrium freezing of sodium phosphate buffer solutions. *Pharm. Res.* **18**:90–97 (2001).
5. R. K. Cavatur and R. Suryanarayanan. Characterization of frozen aqueous solutions by low temperature X-ray powder diffractometry. *Pharm. Res.* **15**:194–199 (1998).
6. W. E. Garner. The kinetics of endothermic solid reactions. In W. E. Garner (ed.), *Chemistry of the Solid State,* Academic Press, New York 1955 pp. 213–231.
7. Y. Li, J. Han, G. G. Z. Zhang, D. J. W. Grant, and R. Suryanarayanan. *In situ* dehydration of carbamazepine dihydrate: a novel technique to prepare amorphous anhydrous carbamazepine. *Pharm. Dev. Technol.* **5**:257–266 (2000).
8. A. Saleki–Gerhardt, J. G. Stowell, S. R. Byrn, and G. Zografi. Hydration and dehydration of crystalline and amorphous forms of raffinose. *J. Pharm. Sci.* **84**:318–323 (1995).
9. K. Chatterjee, E. Y. Shalaev, and R. Suryanarayanan. Phase transitions in raffinose solutions in frozen state and during freeze-drying: practical implications. *Poster presented at the Annual AAPS meeting*, Toronto, 2002.
10. A. Pyne and R. Suryanarayanan. The effect of additives on the crystallization of cefazolin sodium during freeze-drying. *Pharm. Res.* **20**:280–288 (2003).
11. G. Gomez. Crystallization-related pH changes during freezing of sodium phosphate buffer solutions. *PhD Thesis,* University of Michigan, 1995.