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Investigation of optimal manufacturing process for freeze-dried formulations: Observation of frozen solutions by low temperature X-ray diffraction measurements

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Abstract

Freeze-drying is used for the production of sterile injections in the pharmaceutical industry. However, most pharmaceutical compounds are obtained as less stable amorphous form. Freeze crystallization by annealing is an effective method for pharmaceutical compounds that fail to crystallize in the freeze-drying process. Crystallization occurs in the frozen solution during the thermal treatment. In order to establish suitable annealing conditions efficiently, it is important to observe the crystallization process directly in the frozen solution. Recently, low temperature X-ray diffraction has been used to observe frozen solutions. In order to investigate the crystallization process kinetically, the temperature of the low temperature X-ray diffraction instrument must be accurately controlled.

We calibrated the temperature of X-ray diffraction instrument by measuring eutectic temperatures of solutions for a series of compounds. Each eutectic crystal was observed in frozen solution with ice crystal below the eutectic temperature. Eutectic temperatures were detected by the decrease in diffraction intensity associated with heating from below the eutectic temperature. Good correlation was obtained between values in the literature and experimental values.
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Keywords: Low temperature X-ray diffraction; Calibration of temperature measurement; Eutectic temperature; Freeze-dried formulations

1. Introduction

Freeze-drying is widely used to preserve microorganisms, foodstuffs, and biological products, as well as in the pharmaceutical industry for production of sterile injections. However, in these freeze-dried formulations, most pharmaceutical compounds are obtained as a less stable amorphous form rather than as crystalline form. Due to the instability of amorphous compounds, crystalline compounds are preferred when long shelf life is required. Several methods of crystallizing compounds in the freeze-dried formulations are reported [1–4]. More stable products with higher crystallinity are generally manufactured by these methods, the method of thermal treatment being among them.

The freeze-drying process is divided into three stages (Fig. 1): the solution is first cooled until it is completely frozen (freezing); the chamber pressure is then reduced and water is removed by the sublimation of ice (primary drying); the shelf temperature is then raised to remove the moisture by desorption (secondary drying). Thermal treatment is used to raise the shelf temperature to a specific level before the primary drying, at which it is held without apparent melting of the frozen solution for a specified period. Crystallization of the compound is induced in the frozen solution during the treatment. After the crystallization, water is removed by sublimation and desorption and freeze-dried formulations are obtained as crystalline form.

The chemical stability of the freeze-dried formulation depends on the crystallinity of the compound, which results from the crystallization in the frozen solution; therefore, in the manufacturing of stable crystalline freeze-dried

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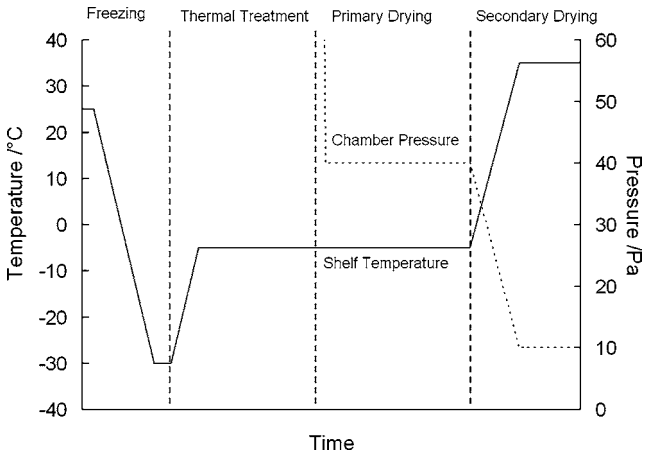

Fig. 1. Freeze-drying process.

formulations, it is desirable to finish the crystallization in the frozen solution completely before beginning the sublimation process. To establish suitable thermal treatment conditions, it is important to understand the process of crystallization, and thus the crystallization in the frozen solution was observed and evaluated using such techniques as electrical conductivity [5–7], differential scanning calorimetry (DSC) [1,8], and microscopy [9–12]. These measurements can detect changes in physicochemical properties, but can not detect changes in crystal structure.

X-ray diffractometry is the best method known for investigating changes in crystal structure, and therefore it is used to examine the crystallinity of the freeze-dried samples. In many cases, several samples taken at various intervals during the thermal treatment are freeze-dried. This method is cumbersome for many X-ray measurements or experiments at multiple temperatures. The crystallization should be measured dynamically in situ and in real time by X-ray diffraction. In many X-ray diffraction studies of crystal transformation, the thermal analysis is linked to the equipment temperature control [13–15], since investigation of the kinetics of dehydration by X-ray diffractometry is conducted with a heater [16]; however, these transformations are examined at temperatures above room temperature. To our knowledge, there are few reports regarding investigation of frozen solution of compounds below 0 °C by X-ray diffractometry. Suzuki et al. [17] studied the size reduction by freeze-drying and measured the X-ray diffraction patterns of sodium chloride dihydrate while cooling with dry ice. Shalaev et al. [18] studied the water–glycine–sucrose system and reported that they detected the hexagonal ice and β-glycine in the solution by low temperature X-ray diffraction. Cavatur and Suryanarayanan [19] recently monitored the crystallization of sodium nafcillin in the frozen solution by the same method. It is hoped that this method will it make it possible to determine the rate of crystallization and establish suitable thermal treatment conditions without need for multiple freeze-drying steps.

To investigate the crystallization kinetically, the temperatures of the low temperature X-ray diffraction equipment must be accurately controlled. This study aimed to calibrate the equipment even though the properties of standard materials below 0 °C are not well known. We calibrated the equipment temperature measurement by measuring eutectic temperatures of solutions for a series of compounds.

2. Experimental methods

2.1. Materials

Potassium chloride, sodium chloride, sodium acetate anhydrate, sodium acetate trihydrate, sodium salicylate, and β-alanine were used. All materials were reagent grade. Water for injection was also used.

2.2. X-ray diffraction measurement

X-ray diffraction patterns were measured from 5° to 40° (=2θ) with a RAD-IIC X-ray diffractometer (Rigaku Denki Tokyo Japan). The measurement conditions were as follows: CuKα radiation; voltage 40 kV; current 40 mA; scanning rate 3° (=2θ)/min. A graphite monochromator was used to measure powder samples at room temperature.

Low temperature equipment (Rigaku Denki Tokyo Japan) was attached to the X-ray diffractometer for measurements. The temperature was controlled by liquid nitrogen and the supplied heater. Approximately 0.8 mL of the sample solution was filled into the holder (ϕ15 mm) at room temperature. The holder was covered with polymer film and was placed vertically into the equipment chamber. The sample was then cooled to freeze the solution. Using the vacuum pump, the chamber was evacuated to defrost the sample surface. The sample temperature was maintained isothermally at the desired temperature during the X-ray diffraction measurements. A Ni filter was used to monochromize the X-ray beam.

2.3. Calibration of the temperature measurement

The low temperature equipment was calibrated by measuring a range of eutectic temperatures for a series of compounds. Potassium chloride, sodium chloride, sodium acetate, and sodium salicylate were used since their eutectic temperatures were well known. Aqueous solutions were prepared. The samples were cooled down from room temperature to −30 °C and the X-ray diffraction patterns were measured. The goniometer was fixed at the peak angle of each sample and the diffraction intensity was measured at this angle while heating. A correlation curve of diffraction intensity versus temperature was obtained. The eutectic temperature was determined from the decrease of diffraction intensity. The sample was then cooled to −30 °C again, then heated to 3 °C below the eutectic temperature, and maintained isothermally at that temperature for 30 min. This annealing process was important for the complete crystallization in the frozen solution. The accurate eutectic temperature was taken

as the extrapolated onset defined by the intersection of two tangents (reference to a figure suggested). The cooling and heating rates were 1 °C/min as not to overshoot the desired temperature.

3. Results and discussion

3.1. Observation of frozen solutions

3.1.1. Potassium chloride

Fig. 2(a) shows the X-ray diffraction pattern for the frozen solution of potassium chloride (10%, w/w). Sharp peaks were observed at 22.7°, 24.2°, 25.8°, 33.5°, and 39.8° (=2θ). Fig. 2(b) shows the pattern for ice. These peaks well agreed with those from the hexagonal ice [20]. A peak different from the ice peaks was observed at 28.5° (=2θ) and correlated with that of solid-state potassium chloride (Fig. 2(c)). Below the eutectic temperature, potassium chloride–water exists as a eutectic mixture of ice and potassium chloride. Potassium chloride was confirmed to crystallize as an anhydrate below the eutectic temperature in the frozen solution.

3.1.2. Sodium chloride

Fig. 3(a) shows the pattern for the frozen solution of sodium chloride (10%, w/w). Aside from ice peaks, peaks were also observed at 15.3°, 17.6°, 30.6°, 31.0°, 35.3°, and 36.8° (=2θ). These peaks were different from those of solid-state sodium chloride (27.4° and 31.7° (=2θ)) (Fig. 3(b)). It is well known that sodium chloride–water exists as a eutectic mixture of ice and sodium chloride dihydrate below the eutectic temperature. Sodium chloride dihydrate has the space group $P21/c$, with $a = 6.3313$ Å, $b = 10.1178$ Å, $c = 6.5029$ Å; $\beta = 114.407°$; and $Z = 4$ at −168 °C. These peaks obtained at Fig. 3(b) were assigned to be sodium chloride dihydrate [21,22].

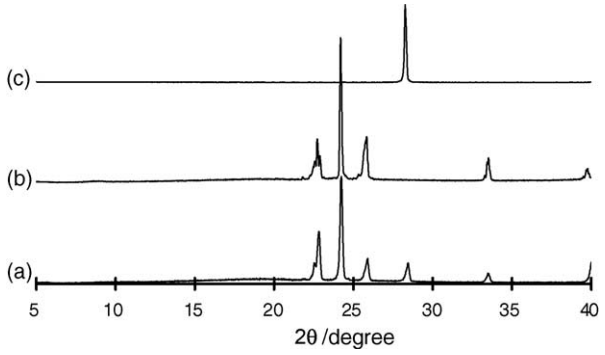

Fig. 2. X-ray diffraction patterns of potassium chloride: (a) 10% frozen solution of at −30 °C; (b) ice; and (c) potassium chloride.

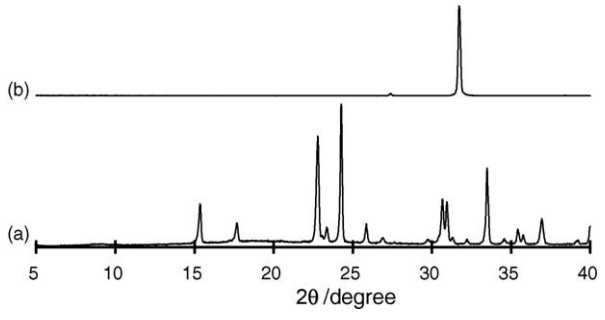

Fig. 3. X-ray diffraction patterns of sodium chloride: (a) 10% frozen solution of sodium chloride at −30 °C; (b) sodium chloride.

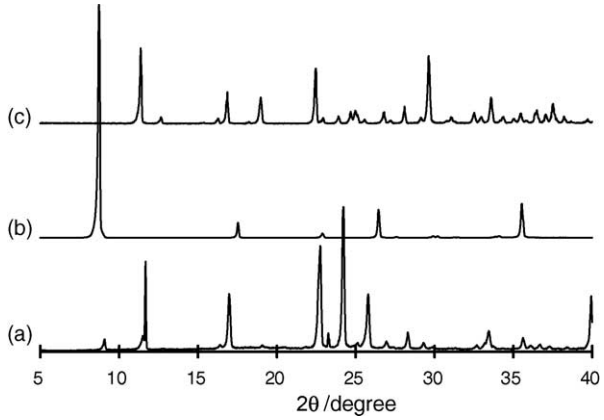

Fig. 4. X-ray diffraction patterns of sodium acetate: (a) 10% frozen solution of sodium acetate at −30 °C; (b) sodium acetate anhydrate; (c) sodium acetate anhydrate.

3.1.3. Sodium acetate

Fig. 4(a) shows the pattern for the frozen solution of sodium acetate (10%, w/w). The diffraction peaks were observed at 9.1°, 11.7°, and 17.0° (=2θ) in addition to ice peaks. Sodium acetate exists as anhydrate and trihydrate at room temperature, and the two forms are distinguishable from one another. Peaks observed in the frozen solution were different from the anhydrate form (8.72°, 17.54°, 26.44°, and 35.54°(=2θ)) (Fig. 4(b)). It was found that sodium acetate–water exists as a eutectic mixture of ice and sodium acetate trihydrate below the eutectic temperature. [23]. Some peaks observed in the frozen solution agreed with trihydrate form (11.36°, 16.86°, 18.98°, 29.62°, and 35.58°(=2θ)) (Fig. 4(c)). Sodium acetate was supposed to crystallize as a trihydrate below the eutectic temperature in the frozen solution.

3.1.4. Sodium salicylate

Fig. 5(a) shows the powder X-ray pattern for the frozen solution of sodium salicylate (5%, w/w). The diffraction peaks were observed at 12.1°, 16.1°, and 30.2° (=2θ), in addition to ice peaks. These peaks were different from those of solid-state sodium salicylate (6.3°, 12.6°, 25.4°, and 32.0° (=2θ)) (Fig. 5(b)). Sodium salicylate was observed to crystallize in a different form from sodium salicylate anhydrate. It was suggested that sodium salicylate crystallized as a

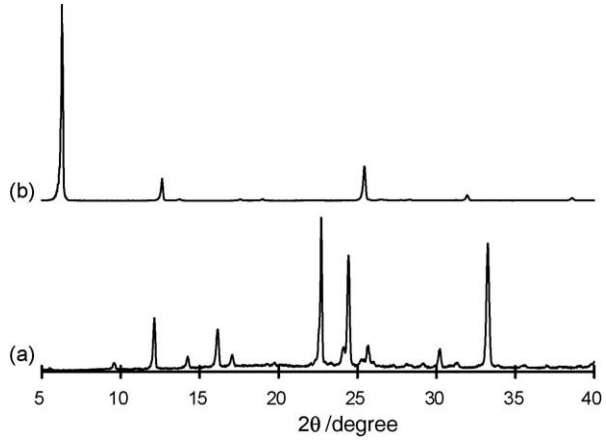

Fig. 5. X-ray diffraction patterns of sodium salicylate: (a) 5% frozen solution of sodium salicylate at −30 °C; (b) sodium salicylate.

hydrate in the frozen solution. The characterization of the hydrate was not a part of the focus of this paper. We utilized the hydrate for calibration of temperature as described below.

3.2. Observation of the eutectic melting

The diffraction intensity of the peak attributed to the eutectic crystal was measured with heating from below the eutectic temperature. Fig. 6 shows the change of diffraction intensity from 10% frozen solution of sodium chloride during heating process. Diffraction intensity decreased at −29 °C. After decrease of diffraction intensity, peaks attributed to the eutectic crystal were not observed. Melting of the eutectic crystal was observed at the eutectic temperature. For potassium chloride, sodium acetate, and sodium salicylate, eutectic melting was observed. We detected eutectic temperatures for all four compounds by the decrease in diffraction intensity.

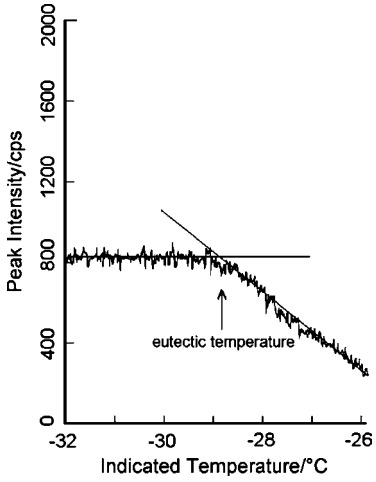

Fig. 6. The change of diffraction intensity from 10% frozen solution of sodium chloride by heating.

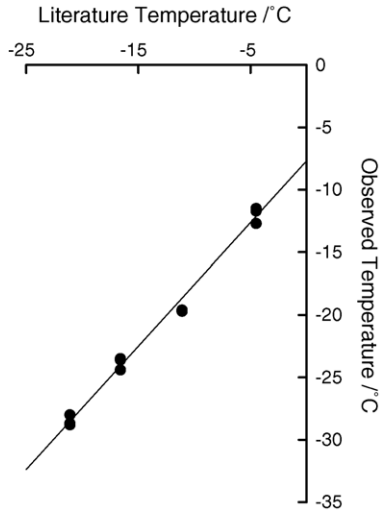

Fig. 7. Correlation between literature temperatures and observed temperatures.

3.3. Calibration of temperature measurements

These all of observed temperatures were lower than those described in the literature. So, the observed eutectic temperatures were plotted against those temperatures obtained by literature. Fig. 7 shows the correlation between literature eutectic temperatures [5,9] and observed temperatures. The calibration curve was obtained by the least-squares method:

$$Y = 0.988182X - 7.68137 \quad (r = 0.996198)$$

X: literature temperature, Y: observed temperature.

The data fit linearly and good correlation was obtained. The slope was nearly 1.0 and the Y-intercept was about −7.7 °C.

After calibration of temperature measurements, the eutectic temperature of β-alanine was measured. The eutectic melting of β-alanine (10%, w/w) was observed to be -13.9 ± 0.3 °C ($n = 3$). This temperature was in agreement with the value (−14 to −13 °C) reported by Ito et al. [9]. The calibration of the equipment temperature measurement was thus validated.

3.4. Discussion

The calibration of temperature of instrument is important for the measurement with temperature sensitive research. For these cases, the standard samples should be used for the calibration; however there are few suitable calibration compounds below 0 °C. Some pharmaceutical compounds crystallize independently of concentration in the frozen solution at the eutectic temperature. Instead of melting temperatures of standard samples, eutectic temperatures were utilized in the pharmaceutical field. Nail and co-workers [11,12]

validated the microscopy by measuring eutectic melting of several compounds. Cavatur and Suryanarayanan [19] used a sodium chloride–water system to calibrate the temperature measurement of a low temperature X-ray diffractometer. They detected the eutectic temperature between −20 and −22 °C and the melting point of ice between −18 and −16 °C from an aqueous solution of sodium chloride (20% w/v), and they detected the melting point of sodium chloride dihydrate between −2 and +2 °C from an aqueous solution of sodium chloride (80%, w/w). They also detected the eutectic melting and two points of phase boundaries in the range of 2–4 °C. Accurate equipment temperature measurements is necessary for pharmaceutical manufacturing because the rate of crystallization changes drastically by the annealing temperature in the frozen solution of the drug. Dowell and Rinfret measured the rate of ice transformation by setting the diffractometer at the correct angle and observing the diffraction intensity [20]. By the same method, we observed the change in the diffraction intensity of the eutectic crystal while heating. We detected the eutectic temperature sensitively, from the decrease of the intensity by the melting of the eutectic crystal.

In this study the temperature difference was about 7.7 °C. The thermocouple should be located close to the sample if possible; however, in our case the location of the thermocouple was not changeable. Because of good linearity, we supposed the sample temperature could be calculated from the calibration curve over the range of these data.

We utilized eutectic temperatures of four salts: −21.1 °C (sodium chloride), −16.6 °C (sodium acetate), −11.1 °C (potassium chloride), and −4.5 °C (sodium salicylate), which are melting temperatures of cryohydrates [24]. A cryohydrate is a eutectic mixture that freezes as a pure substance at the eutectic temperature. When we measured the diffraction intensity of the frozen solution at the beginning of the study, we observed that the intensity increased just below the eutectic temperature. This effect is due to crystallization. It was revealed that some part of the frozen solution remained amorphous only when freezing below the eutectic temperature. It was supposed that the crystallization was delayed because of this amorphous component [24]. To achieve complete crystallization, it is necessary to induce crystallization by annealing. Unless cryohydrate crystallizes completely, the eutectic temperature will not be detected accurately as the intersection of two tangents of the peak intensity, because of the increase of the peak intensity below the eutectic temperature. We emphasized complete annealing below the eutectic temperature for the temperature calibration. Therefore, eutectic crystal was induced to crystallize by thermal treatment and not by heating, and the diffraction intensity from the crystal was monitored while heating at 1 °C/min. The intensities were observed to decrease suddenly at a certain temperature. No peaks due to eutectic crystals were observed after this event. This temperature was considered as the measured eutectic temperature. Eutectic temperatures of the other salts were similarly estimated.

4. Conclusion

We measured the frozen solution of compounds by the low temperature X-ray diffraction. We calibrated the equipment temperature monitoring by the measurement of eutectic temperatures.

Crystal forms of several compounds were observed with ice crystal in frozen solution below the eutectic temperature. Eutectic temperatures were detected by the decrease of diffraction intensity during the heating from below to above the eutectic temperature. A good correlation was obtained between the observed values and values obtained from the literature. We found that calibration of the low temperature X-ray diffraction equipment can be successfully done by measurement of eutectic temperatures.

We concluded that eutectic temperatures are useful experimental tools for temperature calibration. The calibrated low temperature X-ray diffraction measurements will contribute to the establishment of the optimal method for freeze-dried manufacturing process.

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