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APPLICATION OF XRD-DSC SYSTEM TO THE OPTIMIZATION OF MANUFACTURING PROCESS FOR THE FREEZE-DRIED PHARMACEUTICALS

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We have developed the vacuum dryer attached XRD-DSC system and monitored manufacturing process of freeze-dried pharmaceutical product. The aim of this study is to apply the XRD-DSC system for the preformulation of freeze-dried injections. Gabexate mesilate was used as a model drug. Drug solution was frozen then heated to annealing temperature according to the process-controlling program. The XRD-DSC analyses were performed to monitor the crystallized spicies and their crystallinity of sample. When the solution was cooled slowly, peaks of gabexate mesilate and mannitol polymorph had been already observed during the cooling process while those crystallinity were low at fast cooling rate. As the drying underwent, intensity of ice peaks were getting weaker. At the cooling rate of 0.1°C min^{-1}, the XRD profile of final product was revealed that the characteristic peaks of gabexate mesilate, mannitol δ-form and β-form were appeared. When the cooling rate was increased, the crystallinity of final products was decreased. From these results, it was confirmed that the XRD profiles during freeze-drying process significantly related to the final freeze-dried product. It is obvious that monitoring by XRD-DSC system is a quite effective way to simulate the manufacturing process and to optimize the qualified product.

Keywords: *freeze-dry, gabexate mesilate, pre-formulation, XRD-DSC*

Introduction

Freeze-drying technology is usually used for injections, which formulate chemically unstable drug in solution [1]. However, the quality of freeze-dried product is significantly affected by manufacturing process [2–4], and hence it is very important to monitor the manufacturing process of freeze-dried product. Optimal conditions for the freeze-drying process for pharmaceutical injections have been best determined by trial and error using DSC, and dielectric property measurement, so far. Therefore, in situ measurement for the freeze-drying process was required. Temperature controlled XRD techniques have been used for studying phase change of organic substances. Simultaneous XRD and DSC (XRD-DSC) measurements have been also reported [5–8]. XRD-DSC measurements can overcome problems arising from separate measurements of XRD and DSC, for example, rapid phase transition of substance due to poor stability. The XRD-DSC instrument would be a suitable system for monitoring structural and thermal information of material at the same time. By attaching a vacuum pump to XRD-DSC system, we made it possible to monitor the freeze-drying process in situ.

Gabexate mesilate having ε-guanidino-fatty acid structure is one of the protease inhibitor. This drug is used for pancreatitis and disseminated intravascular coagulation (DIC) as intravenous injections. Because the rapid dissolution in normal saline solution and high stability are required for injection, this drug is manufactured as a crystalline freeze dried product.

The aim of this study is to apply the XRD-DSC system for controlling the quality of freeze-dried injections. The XRD-DSC analyses were performed to investigate the effect of cooling rate on crystallinity of products.

Experimental

Materials and methods

Gabexate mesilate was purchased from Takata Seiyaku Co., Ltd. Mannitol, and N-methyl glucamin were used as a reagent grade. Table 1 shows the formulation of gabexate mesilate injection. Mannitol (stabilizer), N-methyl glucamin (buffer) and distilled water were used for this product.

Table 1 Formulation of gabexate mesilate injection

	Amount	Effect
Gabexate mesilate/g	2.0 g	–
Mannitol/g	1.0 g	excipient
N-methyl glucamin/g	0.05 g	buffer
Purified distilled water/mL	Total 10 mL	–

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XRD-DSC measurement

According to the formulation, sample solution was prepared and 75 μg of sample was filled to the plastic cell. Figure 1 shows the sample filling and sealing procedure for a plastic cell. Generally, original solution for freeze-dried product contains various salts which cause the sample cell to corrode aluminum pan. Therefore, we selected plastic cell for the measurement of the sample solution. Advantages of the plastic cells are (1) sealable to prevent drying solution while cooling, (2) have smooth surface, which enable to prevent from nucleation, and (3) applicable to strong salts. Calibration for the sample temperature had been made before the measurements using gallium (29.7°C), cyclohexane (6.5°C), undecane (–25.6°C), mercury (–34.6°C) and water (0°C). Sample was cooled with 4 different cooling rates according to the programs as shown in Fig. 2. Decompression was achieved from definite time ✯, which revealed the starting point for sample drying. The heating rate was controlled to 1°C min^{-1} for all samples.

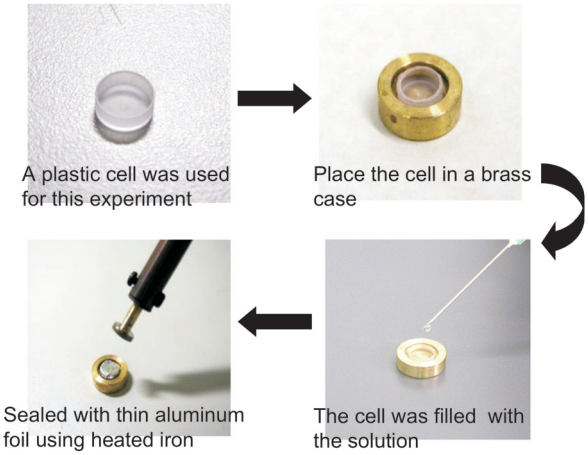

Fig. 1 Sample filling and sealing procedure

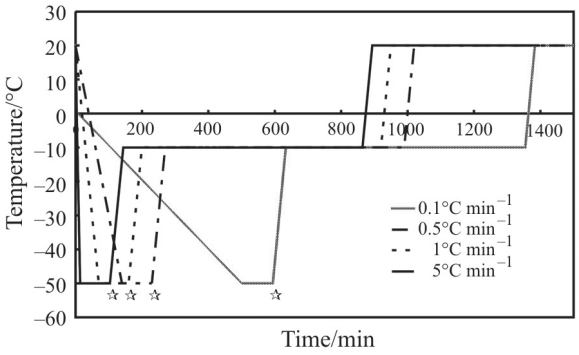

Fig. 2 Experimental condition for XRD-DSC measurement

DSC measurement

PerkinElmer DSC7 was used for the measurement of glass transition temperature of samples. Samples were cooled to –60°C at each cooling rates and hold for 60 min. Then, the samples were heated to room temperature at a heating rate of 20°C min^{-1}.

Results and discussion

Determination of solidification and annealing temperature

In general, the manufacturing process of freeze-dried product consists of three main steps, (1) freezing (solidification) process at ordinary pressure, (2) primary drying where free water is sublimed, and (3) secondary drying where sorbed water to solute is removed (Fig. 3). In the solidification process (1), sample solution was cooled to below its glass transition temperature. For the primary drying (2), the sample chamber was evacuated and heated to below the eutectic melting temperature. Exceeding this temperature will cause the sample to melt during processing. For the efficiency of the process, it is required that drying temperature keep as high as possible. Every formulation will have a characteristic temperature, above which it will suffer processing defects during freeze

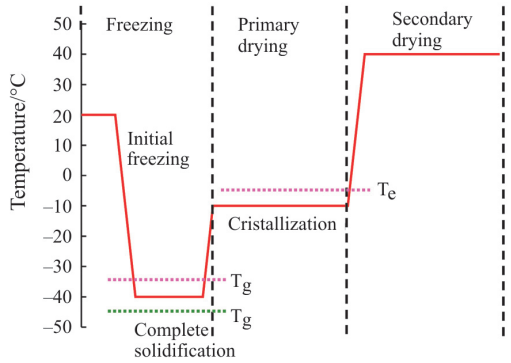

Fig. 3 Process temperature for freeze-drying T_g, glass transition temperature of frozen solution; T_e, eutectic melting temperature

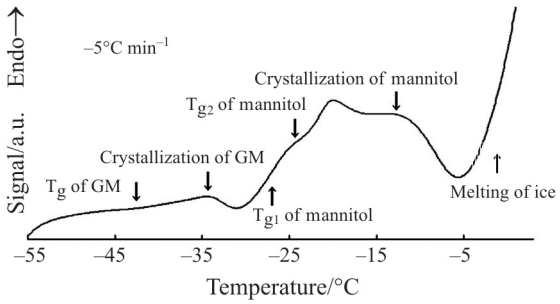

Fig. 4 DSC curves of sample solution frozen at a cooling rate of –5°C min^{-1}

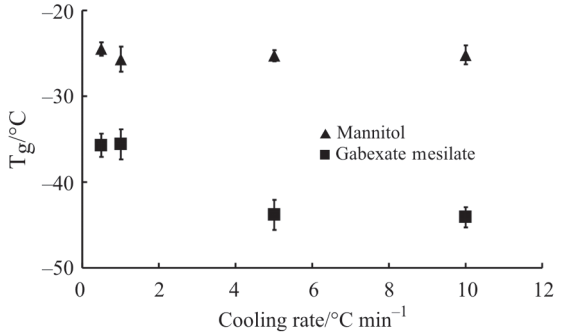

Fig. 5 Relationship between T_{gs} and cooling rate for the sample solution

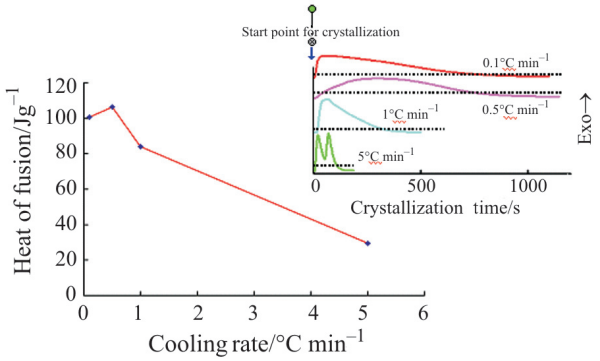

Fig. 6 Heat of fusion of ice at various cooling rate in the sample solution

drying. Therefore, it is important to maintain the temperature below this until it is dried; however, maintaining the product too far below this temperature will lead to the drying process becoming unacceptably slow, since the kinetics of the drying process are temperature dependent. Therefore it is valuable, if not essential, to know the critical temperature of a formulation prior to freeze drying, in order that suitable conditions can be adopted for its successful and safe processing in a reasonable timeframe. Glass transition temperature of gabexate mesilate and mannitol were measured by DSC to determine the solidification and annealing temperature of sample solution. Figure 4 shows the typical DSC curve for the sample with a cooling rate of 5°C min^{-1}. Glass transition temperature of gabexate mesilate was observed at –42°C, and it was crystallized at –34°C. On the other hand, mannnitol showed two glass transition temperatures at –30 and –25°C, and the crystallization of mannitol was occurred at –10°C. Multiple glass transitions in frozen aqueous solutions have been the subject of several discussions [9, 10]. Therefore, annealing temperature was decided to –10°C. Figure 5 shows the relationship between glass transition temperatures and cooling rate for the samples. Although, glass transition temperatures of mannitol did not show any difference as a function of cooling rates, those of gabexate mesilate showed good relation such as glass transition

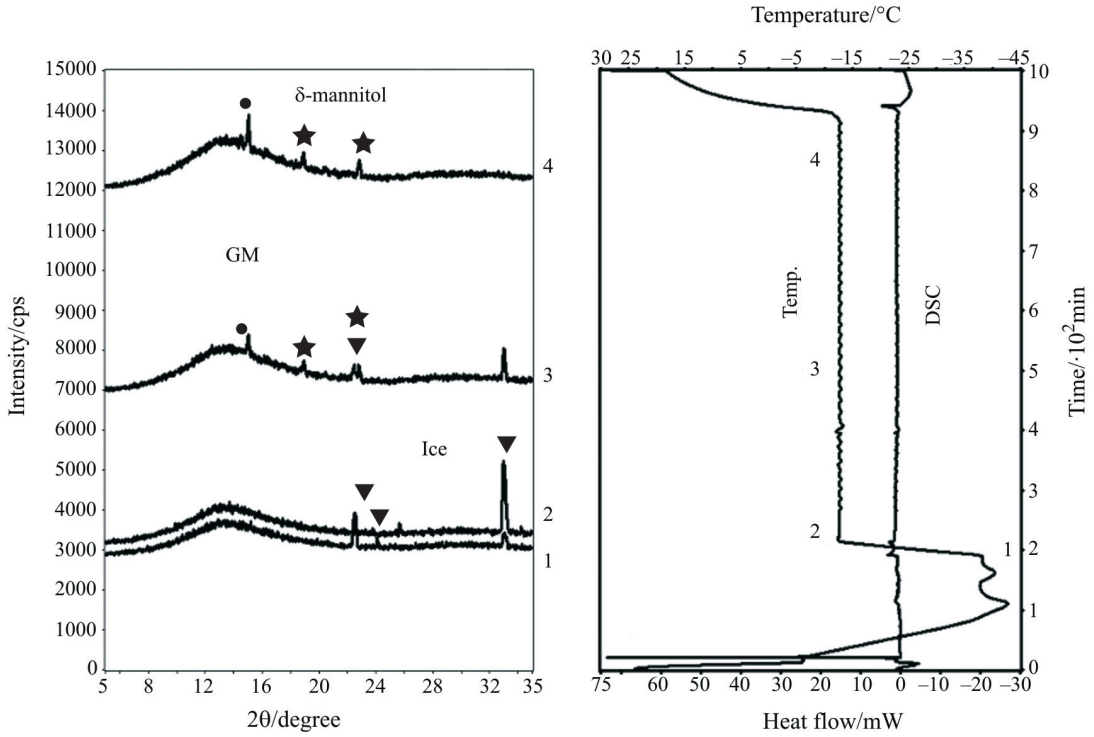

Fig. 7 XRD-DSC profile for freeze-drying process of sample. ● – GM, ★ – mannitol, ▼ – ice
1 – at the point of completely frozen; 2 – at the point of annealing was started; 3 – 450 min passed since measurement was started; 4 – 800 min passed since measurement was started

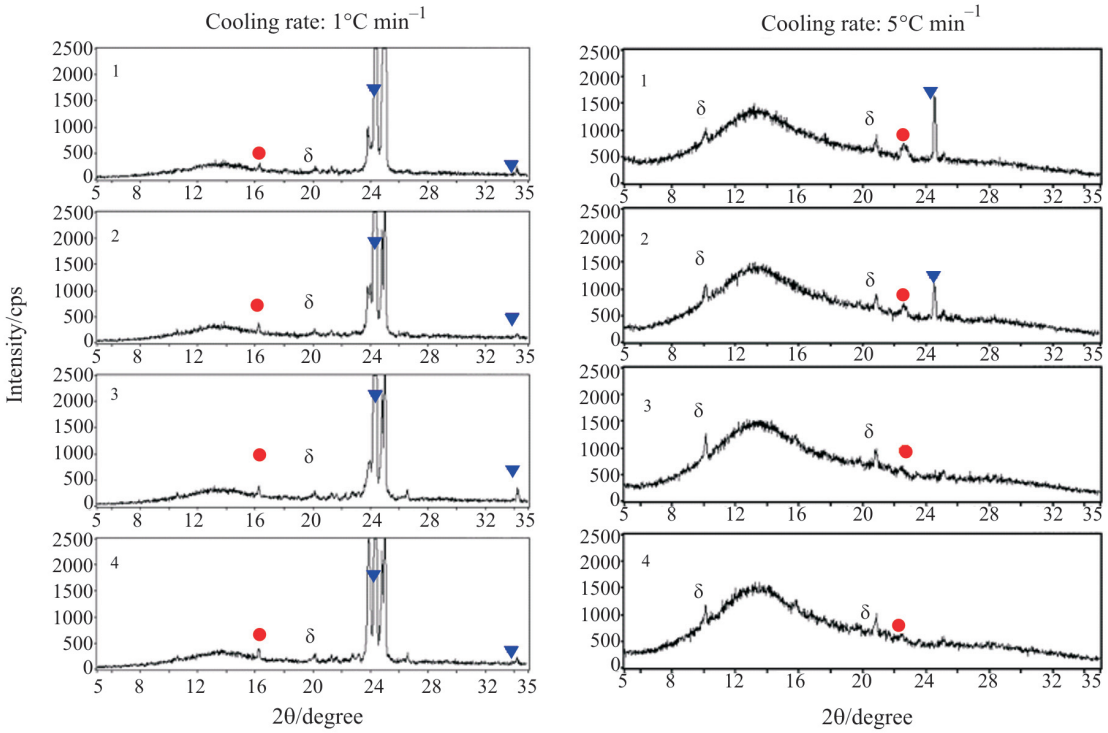

Fig. 8a XRD profiles of samples cooled at slower cooling rates ● – GM, δ – δ- mannitol, ▼ – ice
1 – at the point of completely frozen; 2 – at the point of annealing was started; 3 – 450 min passed since measurement was started; 4 – 800 min passed since measurement was started

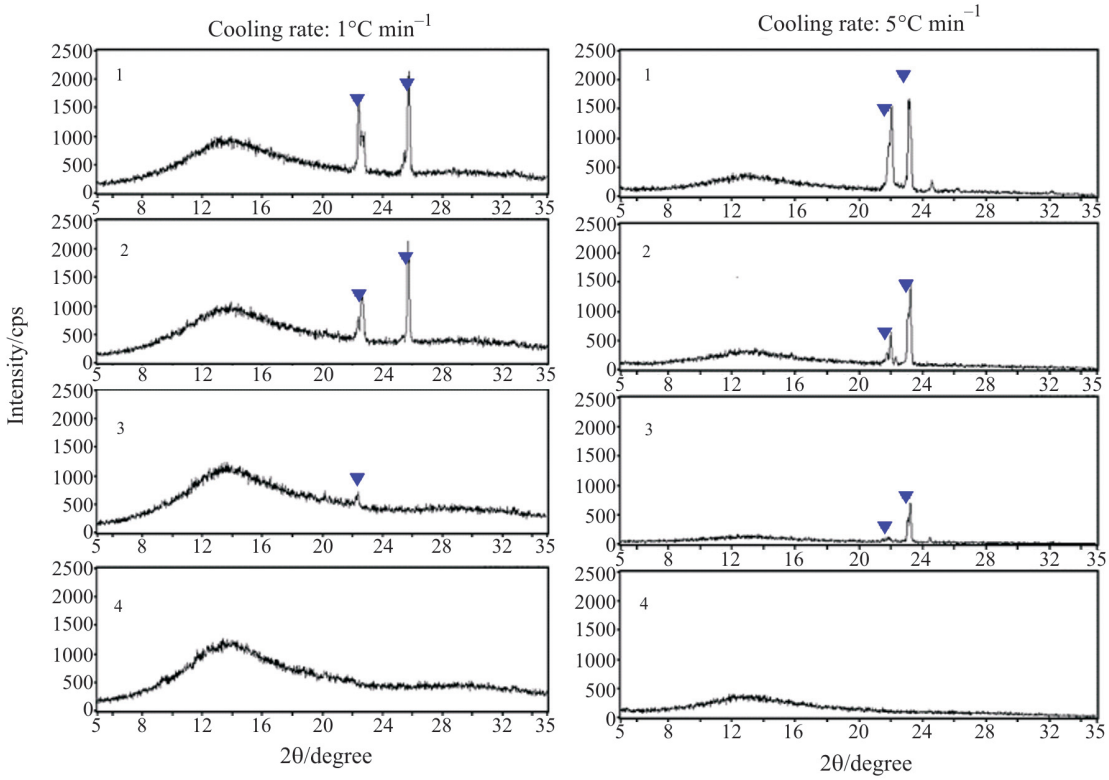

Fig. 8b XRD profiles of samples cooled at faster cooling rates ▼ – ice, 1 – at the point of completely frozen; 2 – at the point of annealing was started; 3 – 450 min passed since measurement was started; 4 – 800 min passed since measurement was started

temperature got lower as cooling rates are faster. From this result, the freezing temperature for the process program was determined to –50°C.

Effect of cooling rate on the XRD profiles of sample

Figure 6 shows the heat of fusion of ice at various cooling rates, and upper figure shows the heat flow of ice freezing measured by DSC at various cooling rates. Start point for crystallization was indicated by arrow. The time for their peak areas were plotted at the bottom figure. The heat flows were significantly affected by the cooling rate. They were greater as the cooling rates were slower. They showed good correlation with the intensity of ice peaks obtained by XRD measurements (as shown in Figs 8 and 9).

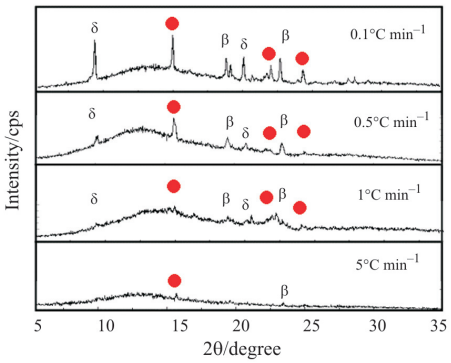

Fig. 9 XRD profiles of final products ● – GM, δ – δ-mannitol β – β-mannitol

Whole process of freeze-drying obtained by XRD-DSC measurement is shown in Fig. 7. Left part shows the XRD patterns at various conditions, and the right part shows the DSC curve and sample temperature. While annealing was achieved at –10°C (XRD patterns are shown from 1 to 4), ice peaks shown as a triangle '▼' at 22.8, 25.9 and 33.6° gradually got weaker, while characteristic peaks of gabexate mesilate shown as a circle '●' appeared at 15.7°, δ-form of mannitol shown as 'δ' appeared at 19.5°, and β-form at 18.6 and 23.0° got stronger as the time goes by, indicating drying process was surely undergo.

Figures 8a and b show the XRD profiles of samples cooled at various cooling rates. Morphology of crystal ice was dependent on the cooling rates. When the solution was cooled slowly, peaks of gabexate mesilate and mannitol polymorph had been already observed during the cooling process. As the drying underwent, intensity of ice peaks shown as a triangle '▼' were getting weaker and weaker at those of the cooling rates were 0.5, 1 and 5°C min^{-1}. The alternations of peak intensity of ice crystal during drying process were observed successively by XRD-DSC.

Figure 9 shows the XRD profiles of final products. When the cooling rates were increased, the crystallinity of final products were decreased. High crystallinity of product was observed at the slower cooling rates. Although, the stable form of mannitol is β-form, some characteristic peaks of δ-form were observed at a final product. The characteristic peaks of gabexate mesilate was observed at 15.7, 22.3 and 24.8°. Peaks due to mannitol δ-form was appeared at 9.6, 19.5, 21.3 and 23.8°, also β-form was appeared at 18.6 and 23.0° at the cooling rate of 0.1°C min^{-1}.

Conclusions

By attaching a vacuum pump to XRD-DSC, we made it possible to simulate the freeze-drying process in situ in the sample chamber. It enabled real-time monitoring of the solid state of the solutes during the process. Monitoring by XRD-DSC system is quite effective to simulate the manufacturing process and to optimize the qualified product.

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