Freeze-Drying of Cephalothin Sodium: Granularly Agglomerated Crystallization during Freezing. I

Yusuke SUZUKI,*a Toyohiko TAKEDA,b Kunihei INAZUT and Teruo SAKAMOTOb

Research Laboratories, Shionogi & Co., Ltd.* and Manufacturing Division, Shionogi & Co., Ltd.,b 1-3, Kaise-Terajima 2-chome, Amagasaki, Hyogo 660, Japan. Received August 5, 1992

Optimal conditions for the crystallization and subsequent freeze-drying of an aqueous cephalothin sodium (CET-Na) solution of supersaturated concentrations to obtain granular crystalline CET-Na have been discussed, as have the qualities of the product thus obtained.

In general, CET-Na in supersaturated aqueous solution is barely recrystallized, even when in its frozen stage. Our previous report revealed that when the solution is kept at low temperatures for a long duration, the molecules begin to change structurally to as condensed a state as that of liquid crystals, and such change facilitates a spontaneous nucleation and seed-independent crystal growth in the frozen solution.

These findings prompted the authors to investigate optimal CET-Na concentrations and thermal histories during the crystallization and subsequent freeze-drying process without seeding.

It has subsequently been found out that optimization of the latter conditions gives granular agglomerated crystalline CET-Na contaminated with neither the amorphous nor the quasi-crystalline form. The optimized conditions are: 25–28% CET-Na concentrations; storage before the freezing process at 0°C for 2 h, and subsequent storage at 20–25°C for 1 h; cooling in the freezing process at a rate not faster than 0.5°C/min; warming of the solution for facilitating crystallization prior to vacuum application for drying at −4°C.

Under these conditions, the freeze-dried product of CET-Na in granular form has been successfully obtained in a shorter freeze-drying cycle, exhibiting a faster reconstitution time than those of CET-Na prepared according to seeded crystallization followed by a conventional freeze-drying.

Keywords freeze-drying; cephalothin sodium (CET-Na); supersaturated solution; crystallization; granulation; thermal history

Introduction

Freeze-drying is frequently applied to the preparation of sterile solid injections to be reconstituted before use1–8); however, the application to cephalothin sodium (CET-Na) in an aqueous solution induces the formation of amorphous CET-Na to cause a decrease in potency and marked color development during storage of the product.9)

Improvements have been made to obtain crystalline CET-Na, where the aqueous solution was frozen and then warmed at a fixed temperature for facilitating the crystallization prior to vacuum application for drying10); however, the product thus obtained was found to unavoidably contain traces of amorphous and quasi-crystalline CET-Na.11) These findings have been developed to another method of obtaining a product of high crystallinity by seeding the supersaturated aqueous CET-Na solution.12)

In general, CET-Na is only slightly recrystallized from its supersaturated solution, even when treated in a frozen state. It has been demonstrated, however, that prolonged storage at a lower temperature or excess mechanical stress given to the solution causes a structural change into as condensed a state as that of liquid crystals, and that the change invites spontaneous nucleation and a seed-independent crystal growth.13,14)

Further investigation has been carried out on the above non-seeding technique, where different structural changes were observed with varying thermal histories of the solution. During the course of the investigation, the authors have determined the formation of a granular mass of crystalline CET-Na which has never been reported anywhere up to the present.

This paper deals with two conditions appropriate to producing the granular mass of CET-Na with high crystallinity: CET-Na concentrations, thermal histories during the course of freeze-drying. Pharmaceutical qualities of the product have also been discussed.

Experimental

Materials CET-Na was purchased from Eli Lilly & Co., Ltd., U.S.A., and purified for the present studies by the authors’ hands. Crystalline CET-Na seeds were used in the form of slurry in ethanol; the size was about 1 × 0.3 μm.

Preparation of Supersaturated Aqueous CET-Na Solution Powdered CET-Na was milled with a hammer mill, sieved with a 120-mesh ss screen, and dispersed in Water for Injection (JPXI grade) of below 5°C. The slurry was then quickly made into a solution at 70°C and immediately cooled to temperatures below 5°C, where a high speed dissolution device designed by the authors was used for the purpose of obtaining CET-Na solutions of various concentrations.

Storage of the Solution before Freezing Solutions prepared as above were subjected to filtration through a filter membrane of 0.22 μm in pore size and stored in an air-tight vessel first at 0°C for 2 h and then at temperatures of 0, 10, 20, 25 and 30°C, each for 1 h.

Preparation of the Freeze-Dried CET-Na A freeze-dryer (Ulvac & Co., Ltd., Japan) of 1.5 kI in chamber size was used to treat all samples according to the programs shown in Fig. 1. Sample solutions were transferred to 14 ml glass vials, each with 3.3 ml, with or without seeding at a ratio of 5 × 10−5%. The vials were semi-stoppered with rubber closures for freeze-drying.

In the first freezing process of (a) in Fig. 1, the sample vials were loaded on shelves at 0°C and subsequently cooled to −40°C at specified cooling rates; in the crystallization process of (b) in Fig. 1, the sample was maintained at varying levels of temperature between −6 to −1°C for 2 h. All of the samples thus treated were dried under 0.1 to 0.6 mbar of vacuum at 30°C in the drying process of (d) in Fig. 1.

Microscopic Study The morphology of the crystallized and subsequently freeze-dried product was examined by scanning electron microscopy (SEM, SEM-2, JEOL & Co., Ltd., Japan).

Powder X-ray Diffraction Analysis (PXDA) Powder X-ray diffraction patterns were obtained by using a Rigaku Denki 2013 diffractometer. An X-ray generator (CuKα (λ = 1.5405 Å), Ni-filtered, 40 kV, 20 mA) and a Geiger-Mullern counter were used. Scanning speed was 7°/min. The
crystallite size was obtained by measuring a half width of the diffraction peak at a point of 12.1° of 2θ for calculation according to the Sherrer's equation (Eq. 1).

\[ D = \frac{K \lambda}{\beta \cos \theta} \]  

(1)

\[ \beta, \text{ half width of the diffraction peak; } K, \text{ shape constant; } \lambda, \text{ wave length of X-ray, 1.5404 } \AA; \theta, \text{ Bragg's angle, 6.57° (Fig. 5); } D, \text{ crystallite size in. Relative crystallite size } (D/D_0) \text{ has been defined as a ratio of the } D \text{ value from the freeze-dried product prepared without seeding to that from the product prepared with seeding)}^{132} \]

Thermogravimetry (TG) 5mg of sample taken from the freeze-dried plug surface was examined with a Rigaku Denki TG-DSC. Individual weight loss due to the pyrolysis of amorphous, of quasi-crystalline and of crystalline CET-Na, were thermogravimetrically checked using an open aluminum pan at a heating rate of 1.25 °C/min under N2 gas flow.\(^{111}\) Non-crystallinity was calculated according to Eq. 2.\(^{111}\)

\[ \beta_{nc} = (d_{nc} + d_{oc}) \sum d_{oc} \]  

(2)

\[ \beta_{nc}, \text{ non-crystallinity; } d_{nc}, d_{oc}, \text{ weight loss due to the pyrolysis of amorphous and of quasi-crystalline CET-Na; } \sum d_{oc}, \text{ total weight loss due to the pyrolysis of all the amorphous, quasi-crystalline and crystalline CET-Na.} \]

Measurement of Reconstitution Time Time for reconstitution was measured in such a manner that 4.4 ml of distilled water was transferred to a sample vial for manual shaking at a rate of once a second till its complete dissolution.

Measurement of Drying Rate in Freeze-Drying An electric balance, Shimazu Corporation LABOR, type LU-T1100, was equipped to the inside of the freeze-drying chamber for measurement. The drying rate was determined by measuring the whole weight of 50 sample vials at every 5 min under 0.1, 0.2, 0.4 and 0.6 mbar of vacuum in the drying process (d) of Fig. 1. Concurrently, product temperature in a vial \(T_p\) and the vacuum level in the freeze-drying chamber were recorded with C. thermocouples and a Pirani gauge, respectively. The conductance of water vapor flux \(C_{wv} \) was calculated according to Eq. 3.\(^{151}\) and the relationship between mean water vapor pressure and \(C_{wv} \) was investigated using Eq. 4.\(^{151}\)

\[ dW/dt = C_{wv} (P_v - P_a) \]  

(3)

\[ dW/dt, \text{ drying rate; } C_{wv}, \text{ conductance of water vapor flux; } P_v, \text{ water vapor pressure at the sublimation interface of a sample at } T_p; P_a, \text{ water vapor pressure in the freeze-drying chamber, that is approximately replaceable by a vacuum level in the chamber.} \]

\[ C_{wv} = \frac{\eta a^2}{8 \eta L} \]  

(4)

\[ P_v, \text{ mean water vapor pressure, } (P_v + P_a)/2; \eta, \text{ viscosity coefficient of gas; } a, \text{ radius of a conduit tube; } L, \text{ length of a conduit tube; } \alpha, \text{ constant.} \]

Equation 4 explains an approximate relationship between \(C_{wv} \) and \(P_v \), in the freeze-drying process, when a water vapor flux is assumed as a simplified model in which the water vapor is eliminated through a cylindrical conduit tube in a viscous flow, as shown in Fig. 2 (II).\(^{152}\) A larger slope of a straight line of \(C_{wv} \) against \(P_v \) can be interpreted as indicating that freeze-dried CET-Na crystals become more loosely aggregated.

Symbols used in this paper, except for those given above, are: \( Y_c \), yield of granulated portion in a freeze-dried CET-Na plug; \( C_{nc} \), CET-Na concentration in aqueous solution; \( T_s \), storage temperature of CET-Na aqueous solution before freezing; \( dT_s/dt \), cooling rate in first freezing process; \( T_c \), holding temperature in crystallization process (crystallization temperature); \( \beta_{nc} \), reconstitution time.

Results and Discussion

Formation of Granular Freeze-Dried Product When sample solutions stored at 0°C for 2h were frozen on a -40°C shelf in the first freezing process (----; Fig. 1(a)) and subsequently freeze-dried, the plugs were found to contain a large amount of the amorphous material. In another case, when those sample solutions were chilled gradually from 0°C to -40°C at a rate of -4°C/min (-----; Fig. 1(a)), partial granulation was found to occur, as seen in Fig. 3.

Figure 4 shows the appearance and scanning electron micrographs of two types of freeze-dried preparations: (A) is a granular product obtained from a non-seeding technique and (B) is a normal crystalline plug obtained from a seeding one. Each of the granules is constructed with fine CET-Na crystals which agglomerate like a
Fig. 4. Appearance and Scanning Electron Micrograph of Freeze-Dried CET-Na
A. product from granularly agglomerate crystallization; B. product from normal crystallization with seeding.

Fig. 5. Powder X-Ray Diffractions of Freeze-Dried CET-Na
A, B. same procedures were followed as in Fig. 4.

Table 1. Relative Crystallite Size (D/Dₐ) and Non-crystallinity (βₜ) of Freeze-Dried Cephalothin Sodium

<table>
<thead>
<tr>
<th>Sample</th>
<th>D/Dₐ</th>
<th>βₜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granular CET-Na</td>
<td>1.01</td>
<td>0.22</td>
</tr>
<tr>
<td>Normal CET-Na</td>
<td>1.00</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* a, b) Freeze-dried CET-Na from granularly agglomerated crystallization without seeding a), and from normal crystallization with seeding b). c) Data from Sherrer's method; powder X-ray diffraction peak (θ = 6.57°); D and Dₐ are the crystallite sizes of sample and standard sample (normal CET-Na), respectively. d) Data from TG curves of freeze dried CET-Na.

chestnut bur, the crystal size being the same size as that of crystals found in the normal plug. Both types of samples have the same patterns in powder X-ray diffractometry, as seen in Fig. 5.

Table 1 lists the non-crystallinity and relative crystallite size of these freeze-dried samples. In Table I, the non-crystallinity of the granular portion is as small as the relative crystallite size is as large as that of a conventionally freeze-dried plug mass.

Optimal Conditions for the Formation of Granular Crystallized Products Yield of the granulated portion (Yₙ) was defined as the ratio of the sum weight of the portion to the whole weight of the freeze-dried plug. The granulated portion was easily separable upon slight impact of the freeze-dried sample vial. Factors influencing Yₙ have been revealed to include: 1) CET-Na concentration, 2) thermal history of supersaturated CET-Na solutions before freezing, 3) cooling rate of the shelf temperature in the first freezing.

Fig. 6. Dependence of CET-Na Concentration (Cₙ) in Aqueous Solution on the Yield of Granulated Portion (Yₙ) in Freeze-Dried Plug
Samples were obtained through holding at 25°C for 1 h after storage at 0°C for 2 h, freezing from 0°C to -40°C at a rate of -0.5°C/min. crystallization (-4°C, 2 h), and subsequent freeze-drying.

Fig. 7. Dependence of Storage Temperature (Tₛ) of Aqueous CET-Na Solution on the Yield of Granulated Portion (Yₙ) in Freeze-Dried Plug
Same procedures were followed as in Fig. 6, except 30% of Cₙ and 0-30°C of Tₛ.

Fig. 8. Dependence of Cooling Rate (dTₛ/dt) of Aqueous CET-Na in First Freezing Process on the Yield of Granulated Portion (Yₙ) in Freeze-Dried Plug
Same procedures were followed as in Fig. 6, except for 30% of Cₙ and 0.25-5°C/min of dTₛ/dt.
Fig. 9. Dependence of Crystallization Temperature ($T_c$) of Aqueous CET-Na Solution on the Yield of Granulated Portion ($Y_g$) in Freeze-Dried Plug

Same procedures were followed as in Fig. 6, except for 30% of $C_g$ and $-6-0^\circ C$ of $T_c$.

Table II. Optimal Conditions for Production of Freeze-Dried CET-Na from Granular Crystallization

<table>
<thead>
<tr>
<th>Items</th>
<th>Optimal condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CET-Na concentration ($C_g$)</td>
<td>28—35% (30%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Storage temp. of soln. ($T_s$)</td>
<td>20—25°C (25°C)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooling rate in the first freezing process ($dT_f/df$)</td>
<td>Not more than $-0.5^\circ C/min$&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crystallization temp. ($T_c$)</td>
<td>$-4^\circ C (\text{or} -4^\circ C)$&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Experimental conditions.

Figures 6—9 demonstrate these four factors, when CET-Na solutions of varying concentrations ($C_g$) were stored at different temperatures ($T_s$) for 1 h after make-up and subsequent cooling at $0^\circ C$ for 2 h, followed first by freezing under various cooling rates ($dT_f/df$) and secondly by crystallization at a fixed crystallization temperature ($T_c$) for freeze-drying. These figures illustrate individual results from experiments where only one of the four factors were changed at a time.

With CET-Na solutions of 28—35%, the plugs were 100% granulated in all cases; however, when the concentration dipped to 26%, or became higher than 37%, the yield of the granulated portion ($Y_g$) decreased markedly (Fig. 6).

When the CET-Na solutions were stored at 20—25°C of $T_s$, the plugs were 100% granulated in all cases; however, when $T_s$ lowered to $0^\circ C$, or became higher than 28°C, $Y_g$ markedly decreased (Fig. 7).

When CET-Na solutions were frozen under a cooling rate of 0.25—0.5°C/min, the plugs were 100% granulated in all cases; however, when the cooling rate was faster than 0.5°C/min, $Y_g$ decreased markedly (Fig. 8).

When frozen CET-Na solutions were rewarmed at a crystallization temperature ($T_c$) of $-4^\circ C$, the plugs were 100% granulated in all cases; however, when $T_c$ decreased to $-5^\circ C$, or became higher than $-3^\circ C$, $Y_g$ decreased (Fig. 9).

From the above findings, the optimal conditions for preparing granular crystallized product have been clarified as summarized in Table II.

Reconstitution Time of the Freeze-Dried Product

Table III compares the reconstitution time ($t_r$) of the granular crystallized product with that of normally crystallized product from a seeding technique: $t_r$ of the granular crystallized product is $1-2$s, notably faster than that of the normally crystallized product ($20-60$ s). In another case, when the yield of granulated portion was less than 80%, the reconstitution time exceeded 120 s.

Drying Rate in Primary Drying Process

Figure 10 shows the change in product temperature and in the level of remaining water of two sample types: the granular crystallized product and the normally crystallized one. The water retained was found to decrease in both cases at a constant rate in the primary drying process, where the product temperature was on an equilibrium level; however, the time required for primary drying of the granular crystallized samples was shorter by about 60% compared with that of the normally crystallized samples.

Water in a frozen mass can usually be expected to sublime as a viscous flow. A mean free path of water vapor molecules is much smaller than a conduit tube inside the crystallized and freeze-dried product during the primary drying process. According to the conductance of water vapor flux ($C_w$) increases in proportion to a mean water vapor pressure ($P_v$), as given in Eq. 4. Table IV shows the water vapor flux parameters concerning two types of crystallized samples in the primary drying process. Figure 11 shows the relationship between $C_w$ and $P_v$.

In Fig. 11, $C_w$ increased in proportion to $P_v$ with both samples, and the slope in the case of the granularly...
TABLE IV. Parameters of Water Vapor Flux from Frozen Aqueous
CET-Na Solution in Primary Drying Process

<table>
<thead>
<tr>
<th>Sample</th>
<th>$P_c$ (mbar)</th>
<th>$T_e$ (°C)</th>
<th>$P_e$ (mbar)</th>
<th>$\frac{dW}{dt} f$ (g/h cm²)</th>
<th>$C_w$ (g/W cm²)</th>
<th>$P_w$ (mbar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granular CET-Na</td>
<td>0.1</td>
<td>-16.2</td>
<td>1.40</td>
<td>0.119</td>
<td>0.098</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>-15.0</td>
<td>1.59</td>
<td>0.159</td>
<td>0.113</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>-14.5</td>
<td>1.73</td>
<td>0.207</td>
<td>0.140</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>-13.0</td>
<td>1.96</td>
<td>0.253</td>
<td>0.150</td>
<td>1.28</td>
</tr>
<tr>
<td>Normal CET-Na</td>
<td>0.1</td>
<td>-10.1</td>
<td>2.44</td>
<td>0.093</td>
<td>0.039</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>-8.2</td>
<td>2.90</td>
<td>0.113</td>
<td>0.040</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>-7.0</td>
<td>3.28</td>
<td>0.137</td>
<td>0.043</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>-6.0</td>
<td>3.75</td>
<td>0.158</td>
<td>0.046</td>
<td>2.09</td>
</tr>
</tbody>
</table>

a, b) Same procedures were followed as in Table I. c) The water vapor pressure in chamber, that was assumed as equal to each programed vacuum level; d) equilibrated product temperature; e) equilibrated water vapor pressure on sublimation interface at $T_e$; f) water vapor flux; g) conductivity of water vapor flux, h) mean water vapor pressure.

Fig. 11. Water Vapor Flux Conductivity ($C_w$) Dependence on Mean Water Vapor Pressure ($P_w$)

C, product from granularly agglomerated crystallization; ●, product from normal crystallization with seeding at 30°C of shelf temperature.

agglomerated samples was exceedingly larger than that in the case of the normally crystallized samples. This finding demonstrates that the radius of a conduit tube (a) in Fig. 2, namely void ratio in the former dried plug, is larger than that in the latter.

Conclusion

Granularly agglomerated crystallization during freeze-drying of CET-Na has been found to give a unique dosage form of injections that can be produced in a shortened freeze-drying cycle and can also be used with a shortened time for reconstitution, when compared with the freeze-dried product prepared by seeded crystallization and subsequent freeze-drying. Also, the product obtained from granularly agglomerated crystallization during freeze-drying has been demonstrated to show no difference in non-crystallinity from the product obtained from seeded crystallization and freeze-drying, so that a long shelf life of chemical stability can be expected.

The newly defined technical term “granularly agglomerated crystallization” is derived from the expression “agglomerated crystallization” which appears in Kawashima’s report 17) meaning that crystals are agglomerated in the form of spherical granules.

References