The lyophilization process that is commonly used consists of three stages: (1) freezing, (2) primary drying, and (3) secondary drying. If water is used as a solvent, then during the freezing stage, water will change to ice, separated from other solute components, and freezing will typically be completed within a few hours.1,2 When water is cooled by atmospheric pressure, it does not voluntarily freeze at the equilibrium freezing temperature (0°C), and it continues to maintain its liquid form below 0°C. This is termed supercooling. In the case of purified water free of foreign particles or impurities, it can continuously supercool to approximately −48°C.3 Because injectable products are manufactured in a dust-free environment, they generally can continuously supercool up to approximately −20°C.4 The degree of supercooling is dependent on the characteristics of the formulation and freezing conditions.

The freezing stage, which determines the degree of variations in productivity and product quality, is among the most critical stages during the lyophilization process. Because water does not voluntarily freeze and maintains its supercooled state, the freezing temperature cannot be directly controlled. When the freezing temperature is high (a lower degree of supercooling), the size of ice crystals formed increases; when the freezing temperature is low (higher degree of supercooling), the size of ice crystals formed decreases. The larger the size of ice crystals, the higher the primary drying efficiency. A study reported that vials with product temperature sensors tend to have a higher freezing temperature than those without sensors, and therefore, their sublimation rate will accelerate, causing variations in the drying endpoint.5 According to another study, if the freezing temperature determines the sublimation rate, and if the freezing temperature can be increased by 1°C, the primary drying time can be shortened by 3%.6 However, the size of ice crystals determines the size of the specific surface area. In addition, the size of the specific surface area determines the diffusion and desorption rate during the secondary drying stage.6,7 If the freezing temperature is high, the size of the ice increases, and the specific surface area decreases. A study reported that this causes the secondary drying efficiency to decrease, increasing the moisture residue in the finished product.1 From the aforementioned discussion, it can be concluded that controlling the freezing temperature during the freezing stage is the key to designing a robust drying process.

In recent years, various ice nucleation techniques have been developed, and there are some scientific reports that have evaluated the advantages and disadvantages of these techniques.5,9 The pressurization and depressurization technique is a promising ice nucleation control method. With pressurization and depressurization, the lyophilizer is pressurized to 0.28–0.29 MPa and quickly depressurized to 0.11 MPa (within 3 s) to form an ice nucleus on the surface of the liquid in vials.10 For this technique, nitrogen or argon gas is used for pressurization. The mechanism of ice nucleation has not yet been clarified; however, it has been reported that the main driving forces for ice nucleation are considered to be the vibrational disturbance caused by sudden depressurization, the cooling of the liquid surface by cold gas contact, and local evaporation on the liquid surface during the sudden depressurization.11

Once the vial heat transfer coefficient (which is dependent on the dry chamber pressure) and the drying resistance ($R_p$) are determined, both the sublimation interface temperature and the drying time (sublimation rate) during the primary drying stage can be predicted.12–14 The region where the product quality is not damaged, and at the same time, where stable manufacturing can be performed is expected to be established. The regions constructed in line with the aforementioned idea are termed design spaces. However, stable operation has been
performed at a practical level to tolerate the quality variations that occur during the freezing stage. Accordingly, a larger design space has been used to afford excess safety factors. The wide range of both the sublimation interface temperature and the drying time (sublimation rate) often causes variations in the size of ice crystals. If the ice nucleation can be controlled during the primary drying stage of the lyophilization process, the area of the practical design space would be more robust.

The major objective of this study was to verify the efficacy of the improved design space combined with the controlled nucleation of ice crystals. Using the pressurization and depressurization technique, we controlled the ice nucleation of target formulation during the freezing stage. We investigated the effect of the ice nucleation control on the robust design space during the primary drying stage. Finally, a verification study was performed.

**Experimental**

**Materials** Flomoxef sodium solution for injection (molecular weight: 518.45, CAS No. 92823-03-5) was used for the investigation. The formulation included sodium chloride as a stabilizing agent. The total solid content of the solution was 31% (w/w, liquid density: 1.156 g/mL), with all solid material dissolved in the water for injection. The 14-mL vials were manufactured from clear, colorless, round borosilicate glass tubing that met United States Pharmacopeia (USP) criteria for Type I glass and stoppers suitable for lyophilization were manufactured from chlorinated butyl elastomer and were used during the investigation. The physical properties of the Flomoxef sodium bulk solution are as follows: freezing temperature: −3.3°C; glass-transition temperature (Tg): −31°C; and cake collapse temperature: −28°C.

**Analytical Procedure** The water content of the lyophilized cakes was determined using the Karl Fischer (Kyoto Electronics Manufacturing, MKS-510N) coulometric titration method. Three samples of each lot were used for the evaluation. The specific surface area (SSA) of the lyophilized samples was obtained from Brunauer–Emmett–Teller (BET) specific surface area analysis. A BET surface area analyzer (TriStar3000, Micromeritics Instrument Corporation, U.S.A.) was used to measure the SSA. Outgassing of the samples was performed by heating the sample on a heating mantle at 40°C for 1 h under reduced pressure. Nitrogen gas was introduced into the sample as the adsorbate. The equilibration interval was set as 5 s. Three samples of each lot were used for the evaluation. A scanning electron microscope (SEM; VE-8800, KEYENCE Corporation, Japan) was used to examine the morphologies of the lyophilized samples. The microscope scanned across the surface of the samples using an ultrafine beam of electrons at an acceleration voltage of 2–20kV. The images of the sample surfaces were displayed at a magnification of 100 times.

**Theory–Design Space** Heat transfer to the product during the primary drying consists of three types of heat transfer.12–17) The first is contact heat transfer from the surface that directly comes into contact with the shelf as well as the bottom of the vial. The second is gas heat transfer through the gas between the shelf surface and the bottom of the vial. The third is radiant heat transfer. When a vial is used as a container, the gas heat transfer is estimated as the main heat transfer. However, compared to the vial that is placed in the center of the lyophilizer, the vial placed at the edge of the lyophilizer has a faster sublimation rate.12–17) This indicates that the effects of radiation heat transfer cannot be ignored.17) In addition, the gas heat transfer depends on the chamber pressure. When the chamber pressure decreases, the gas heat transfer increases. When the chamber pressure is greater than 13.3 Pa, gas heat transfer becomes the most dominant heat transfer of the three.18) Accordingly, we estimated the gas heat transfer by using the vial heat transfer coefficient (Kv) as follows.

Heat transfer (dQ/dt) caused by the temperature difference between the shelf surface temperature (Tc) and the product temperature (Ts) is related to Kv and A (cm²), i.e., the cross-sectional area of the vial calculated from the vial outer diameter as follows:

\[
\frac{dQ}{dt} = K_v A_v (T_c - T_s)
\]

The relationship between the heat transfer and the material transfer via sublimation (dm/dt) is as follows:

\[
\frac{dQ}{dt} = \Delta H_s \frac{dm}{dt}
\]

where \(\Delta H_s\) (cal/g) is the latent heat of sublimation. Both Eqs. 1 and 2 yielded Eq. 3 to determine the \(K_v\) value as follows:

\[
K_v = \frac{\Delta H_s (dm/dt)}{A_v (T_c - T_s)}
\]

This heat transfer coefficient of the contact heat, gas heat, and radiant heat transfer were defined as \(K_v\), \(K_p\), and \(K_r\), respectively. According to previous reports,\(^{19}\) \(K_v\) and \(K_p\) do not depend on the chamber pressure (Pc) and the \(K_r\) value depends on Pp as is described by the function \(K_r = b P_p (1 + c P_p)\) (b and c are the positive constant). Then, \(K_v = K_v + K_p + K_r\) can be represented as follows:

\[
K_v = a + \frac{b P_p}{1 + c P_p}
\]

This relationship between \(K_v\) and \(P_p\) has been used in the operational design of lyophilization.\(^{12–14}\) The mass transfer is generated from the difference between the equilibrium vapor pressure of the ice on the sublimation interface (Pice) and Pp in the lyophilizer, and the resistance of the dried layer on the sublimation interface (Rp) determines the sublimation rate.\(^{15}\) In addition, the resistance of a rubber stopper, which is extremely small compared to the drying resistance, is negligible. Accordingly, the relational expression is shown using Eq. 5:

\[
\frac{dm}{dt} = \frac{A_p (P_{ice} - P_p)}{R_p}
\]

From Eq. 5, the drying resistance (Rp) is obtained as Eq. 6.

The required drying time can be calculated from the integration of Eq. 6.

\[
R_p = \frac{A_p (P_{ice} - P_p)}{(dm/dt)}
\]

The conversion factor between the heat flow (dQ/dt) and the mass of substance (m) can be expressed using Eq. 7. The conversion factor used herein is 0.1833 as previously reported.\(^{19}\) Term dm/dt is the sublimation rate in g/h, and the coefficient 0.1833 is the factor to convert the sublimation rate of the pure
water from g/h to cal/s as follows:

$$\frac{dQ}{dt} = 0.1833 \frac{dm}{dt}$$  \hspace{1cm} (7)

The thickness of the maximum frost layer (corresponding to the mass of water $\Delta m_{H,O}$) is defined as $L_{max}$. Thereby, the thickness of the frost layer ($L_{ice}$) can be expressed as Eq. 8:

$$L_{ice} = L_{max} \left(1 - \frac{\Delta m}{\Delta m_{H,O}}\right)$$  \hspace{1cm} (8)

Assuming the percentage of the ice deposit in solutes is $\varepsilon$, $L_{max}$ can be defined as follows:

$$L_{max} = \frac{\Delta m_{H,O}}{\rho_{ice} \varepsilon}$$  \hspace{1cm} (9)

Because the heat quantity ($dQ/dt$) that was supplied from the shelf surface to the product is transferred to the sublimation interface via the frost layer, the sublimation interface temperature ($T_{ice}$) can be expressed by Eq. 10 as follows:

$$T_{ice} = T_b - \frac{dQ}{dt} \frac{L_{ice}}{A_{v} K_{ice}}$$  \hspace{1cm} (10)

Furthermore, from Eqs. 1 and 10, the sublimation interface temperature ($T_{ice}$) can be expressed as Eq. 11 as follows:

$$T_{ice} = T_b - \frac{1}{A_v} \frac{dQ}{dt} \left(\frac{1}{K_v} + \frac{L_{ice}}{K_{ice}}\right)$$  \hspace{1cm} (11)

$\Delta T (= T_b - T_{ice})$ is defined similar to Eq. 12. Its substitution into Eq. 10 yields Eq. 13. Furthermore, the substitution of Eqs. 5 and 13 into Eq. 2 provides Eq. 14, in which the value of 3600 originates from the conversion of seconds to hours as follows:

$$\Delta T = T_b - T_{ice}$$  \hspace{1cm} (12)

$$\frac{dQ}{dt} = \frac{\Delta TL_{ice}}{A_v K_{ice}}$$  \hspace{1cm} (13)

$$\Delta T = \frac{\Delta H_{v,A_P} L_{ice} (P_{ice} - P_e)}{3600 A_v K_{ice} R_p}$$  \hspace{1cm} (14)

where $T_{ice}$ is related to $P_{ice}$ as shown in Eq. 15:

$$T_{ice} = \frac{-6144.96}{\ln(P_{ice}) - 24.01849}$$  \hspace{1cm} (15)

Eventually, $P_{ice}$ is expressed as Eq. 16, by substituting this formula into Eq. 2, the drying resistance ($R_p$) at a specific time can be calculated as follows:

$$P_{ice} = 2.69 \times 10^{19} \cdot \exp\left(-\frac{6144.96}{263.15 + T_{ice}}\right)$$  \hspace{1cm} (16)

In addition, the use of Eqs. 1, 5, and 7 yields Eq. 17 as follows:

$$K_v A_v (T_e - T_b) = 0.1833 \frac{A_v (P_{ice} - P_e)}{R_p}$$  \hspace{1cm} (17)

Furthermore, the substitution of both Eqs. 12 and 16 into Eq. 17 provides Eq. 18 as follows:

$$K_v A_v (T_e - T_b + \Delta T) = \frac{0.1833 A_v}{R_p} \left(2.69 \times 10^{19} \cdot \exp\left(-\frac{6144.96}{273.15 + T_{ice}}\right) - P_e\right)$$  \hspace{1cm} (18)

When the $R_p$ value is known, the design of $T_e$ and $P_e$ can provide $T_{ice}$ and $T_b$ values.

**Operation of LyoStar 3** Lyophilizer LyoStar 3 (total shelf area of 0.46 m²), manufactured by SP Scientific (Stone Ridge and Gardiner, NY, U.S.A.), was utilized during this investigation. The maximum allowable vial number of LyoStar 3 is 726 vials for a 14-mL vial. We used this equipment in the following five manners.

To Estimate the Vial Heat Transfer Coefficient
First, 5 mL of water for injection was filled into 242 vials to be placed fully on one shelf in the lyophilizer for this evaluation, and the mass before lyophilization was measured. The vials were tightly packed on the shelf (hexagonal arrangement). The thermocouples were installed in the vials and placed in the center as well as the edge of the shelf to monitor the product temperature during lyophilization. In addition, to monitor the temperature of the shelf surface, the thermocouples were taped onto the shelf surfaces at the inlet as well as the outlet of the heat medium. For the container, 14-mL glass vials were used and filled with 5 mL of water for injection, and then lyophilized. The freezing procedure was performed at −40°C, and the primary drying was performed at −5°C under three pressure conditions: 5, 13, and 20 Pa. The mass after the lyophilization was measured and the amount of water used for sublimation was determined. From the shelf surface temperature, product temperature, and sublimation amount during lyophilization, the vial heat transfer coefficient was then calculated using Eq. 3.

To Estimate the Water Vapor Transfer Resistance of the Dried Layer
Prior to lyophilization, Flomoxef sodium bulk solution was filtered through a 0.2-µm filter. Then, 3.15 mL of filtered Flomoxef sodium bulk solution was filled into 242 vials to be placed fully on one shelf in the lyophilizer. After filling, the vials were semi-stoppered and loaded into the lyophilizer and lyophilized. The detailed lyophilization conditions are presented in Table 1. Thermocouples were installed in the vials filled with the Flomoxef sodium solution in such a manner that the end part of the thermocouple is in the center of the bottom of the vials. If the sensor touches the inside wall of the vial, the vial temperature will be measured, instead of the product temperature. The thermocouples were taped onto the shelf surfaces at the inlet as well as the outlet of the heat medium. During the lyophilization, the shelf temperature, product temperature, and pressure were monitored. The point at which the product temperature sharply increases toward the established shelf temperature was determined as the drying endpoint for analysis. From the shelf surface temperature, product temperature, and pressure profile, the water vapor transfer resistance of the dried layer ($R_p$) and the drying time were calculated using Eq. 6.

For Lyophilization Procedures with a Normal and Annealed Freezing Step
Lyophilizer LyoStar 3 (total shelf area of 0.46 m²), manufactured by SP Scientific, was utilized for the experiments. Three lots (Lots 01, 02, and 03) of manufacturing were performed.
Prior to lyophilization of each lot, Flomoxef sodium bulk solution was filtered through a 0.2-µm filter. Then, 3.15 mL of filtered Flomoxef sodium bulk solution was filled into 242 vials to be placed fully on one shelf in the lyophilizer. After filling, the vials were semi-stoppered and loaded into the lyophilizer and lyophilized.

The detailed lyophilization conditions for Lot 01 to Lot 03 are presented in Table 1. In short, Lot 01 of the Flomoxef sodium bulk solution was cooled to 5°C for 1 h, and then frozen. The freezing procedure was performed at −41.5°C for 2 h. The primary drying was performed at −25°C at 6.7 Pa. The secondary drying was then performed at 50°C at 2 Pa. Lot 02 of the bulk solution was cooled to 5°C for 1 h and then cooled to −5°C for 1 h to improve the homogeneity of the ice crystalization. The freezing, primary drying, and secondary drying procedures were the same as those of Lot 01. The freezing drying cycle for Lot 03 was the same as that of Lot 02 except for the annealing step. The annealing step was designed at 0°C for 0.5 h to keep the product temperature below the freezing temperature, which was −3.3°C.

For Lyophilization Procedures with a Temperature-Controlled Nucleation Step

Lyophilizer LyoStar 3 (total shelf area of 0.46 m²), manufactured by SP Scientific, was utilized for the experiments. Three lots (Lots 04, 05, and 06) of manufacturing were performed. Prior to lyophilization of each lot, Flomoxef sodium bulk solution was filtered through a 0.2-µm filter. Then, 3.15 mL of filtered Flomoxef sodium bulk solution was filled into 242 vials to be placed fully on one shelf in the lyophilizer. After filling, the vials were semi-stoppered and loaded into the lyophilizer.

The detailed lyophilization conditions for Lot 04 to Lot 06 are presented in Table 1. Each lot of Flomoxef sodium bulk solution was cooled to 5°C for 1 h and then cooled to −5°C for 1.5 h without ice formation. After the completion of the precooling, the chamber was pressurized with nitrogen gas from 0.28 to 0.29 MPa, and then the chamber was depressurized to 0.11 MPa in 3 s or less. The shelf temperature was maintained at −25°C at 1°C/min and held for 2 h, and the primary and secondary drying were performed at −10°C under 6.7 Pa of pressure and at 50°C under 2 Pa of pressure, respectively. Lot 02 of the Flomoxef sodium bulk solution was cooled to 5°C for 1 h and then cooled to −5°C for 1.5 h without ice formation. Following the completion of the precooling, the chamber was pressurized with nitrogen gas from 0.28 to 0.29 MPa, and then the chamber was depressurized to 0.11 MPa in 3 s or less. The shelf temperature was maintained at −25°C at 1°C/min and held for 2 h, and the primary and secondary drying were performed at −10°C under 6.7 Pa of pressure and at 50°C under 2 Pa of pressure, respectively.

For Verification Study for the Primary Drying Conditions Calculated Using the Design Space

Two lots (Trials 1 and 2) of manufacturing were performed to verify the primary drying conditions calculated using the design space. Prior to lyophilization of each lot, Flomoxef sodium bulk solution was filtered through a 0.2-µm filter. Then, 3.15 mL of filtered Flomoxef sodium bulk solution was filled into 726 vials to be placed fully on three shelves in the lyophilizer. After filling, the vials were semi-stoppered and loaded into the lyophilizer.

The detailed lyophilization conditions for Trials 01 and 02 are presented in Table 1. Trial 01 of the Flomoxef sodium bulk solution was cooled to 5°C for 1 h and then cooled to −5°C for 1.5 h without ice formation. Following the completion of the precooling, the chamber was pressurized with nitrogen gas from 0.28 to 0.29 MPa, and then the chamber was depressurized to 0.11 MPa in 3 s or less. The shelf temperature was maintained at −25°C at 1°C/min and held for 2 h, and the primary and secondary drying were performed at −10°C under 6.7 Pa of pressure and at 50°C under 2 Pa of pressure, respectively.

Results and Discussion

Evaluation of the Vial Heat Transfer Coefficient \( K_v \). The dependency of the chamber pressure on \( K_v \) was first determined. The sublimation rate \( dm/dt \) at different \( P_e \) values was measured to provide the \( K_v \) value using Eq. 3. The resulting \( K_v \) values are summarized in Table 2. At each \( P_e \), the \( K_v \) value at the edge position was higher than that at center position. Thus, the \( K_v \) value depended on the position of the vials on
the shelf in agreement with previous reports.\textsuperscript{12,14} The higher $K_v$ value of the vials at the edge position relative to the vials at the center position originated from the contribution of radiant heat transfer from the wall to the vial. At both positions, the $K_v$ value increased with increasing $P_c$. This resulted from the gas heat transfer through the gas between the bottom of the vial and the surface of the shelf.

These data were then analyzed using a nonlinear regression analysis with Eq. 4.\textsuperscript{15,19} The results of the analysis are shown in Fig. 1. The regressed parameters $a$, $b$, and $c$ indicated a positive value in agreement with the definition of the three parameters. Based on the results of this analysis, the $K_v$ value under each $P_c$ value can be predicted.

**Lyophilization Cycle with a Normal and Annealing Freezing Step** To discuss the ice crystal size, the SSA and water content were examined. This is because larger ice crystals form in the larger pores of the dried cakes and the larger pores can reduce the resistance to flow of the water vapor during the primary drying stage. The larger pores of the dried cakes result in a smaller SSA. The SSA value and the water content of Lots 01 to 03 after their lyophilization are summarized in Table 3. The SSA value of Lot 02 was smaller than that of Lot 01. It was considered that the precooling of Lot 02 resulted from the annealing above $T_g$ that caused growth in the ice crystals. In contrast to the SSA value, there was no significant difference in the water content (0.10±0.00 to 0.12±0.01). Notably, the water content is the remaining water in the lyophilized Flomoxef. The residual water was sublimated from the Flomoxef. Therefore, the SSA value increased under the same water content, implying the generation of small ice in Lot 01 relative to Lot 03. An SEM observation was then performed to confirm the microscopic structure of the ice after lyophilization. The SEM image indicated the mass of Flomoxef after the lyophilization, strongly indicating the formation of micropore structures of Flomoxef via the sublimation of ice of a small size (Fig. 2(a)).

The product temperature $T_b$ was then monitored from the initial to the final freezing temperature ($−41.5°C$). Figure 3(a) shows the typical profile of the $T_b$ value of Lot 03 during the freezing stage. The freezing temperature of the product is $−3.3°C$. However, the further decrease in $T_b$ to $−10°C$ or lower was observed after the $T_b$ value reached $−3.3°C$, which corresponded with the supercooling. Supercooling during the freezing stage to $−10°C$ or lower was observed in both vials at the center and edge in the lyophilizer. Following the freezing stage, annealing was performed such that the product temperature could be in a range between the freezing temperature and the glass-transition temperature.

**Lyophilization with a Temperature-Controlled Nucleation Step** After equilibration of the vials on the shelf at $−5°C$, the pressurization and depressurization of the chamber was conducted to control the ice nucleation. Figure 3(b) shows the typical profile of the $T_b$ value of Lot 04 during the freezing stage. Freezing at $5°C$ was observed in both vials placed at the center and edge of the lyophilizer after depressurization and supercooling was not found when the shelf temperature was reduced to $−41.5°C$. The difference between Lot 03 (Fig. 3(a)) and Lot 04 (Fig. 3(b)) was the addition of the pressurization and depressurization. It was, therefore, considered that the dissipation of supercooling might be a result of the addition of pressurization and depressurization. Moreover, the SEM image of Lot 4 indicated the formation of large micropores in the Flomoxef, as compared to the case of Lot 03. This result suggested the addition of the pressurization and depressurization induced the formation of large ice in the Flomoxef.

As another factor to control the size of ice, the freezing rate was maintained from 0.1 to 1°C/min (Lots 04–06). Both the SSA value and water content of Lot 04 to Lot 06 were investigated (Table 3). The SSA value decreased from 0.14±0.01 to 0.04±0.01. Moreover, the water content increased

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**Table 2. Analysis of the Vial Heat Transfer Coefficient with the Lyophilizer**

| Chamber pressure $P_c$ (Pa) | $10^4 K_v$ (cal/s·cm$^2$·°C) |
|-----------------------------|-------------------------------|
| 5                           | 2.28                          |
| 13                          | 3.45                          |
| 20                          | 4.14                          |

| Quality attributes          | SSA (m$^2$/g) | Water content (%) |
|-----------------------------|---------------|-------------------|
| Lot 01                      | 0.64±0.05     | 0.10±0.00         |
| Lot 02                      | 0.50±0.01     | 0.11±0.04         |
| Lot 03                      | 0.40±0.01     | 0.12±0.01         |
| Lot 04                      | 0.14±0.01     | 0.21±0.04         |
| Lot 05                      | 0.10±0.01     | 0.24±0.00         |
| Lot 06                      | 0.04±0.01     | 0.41±0.03         |
from 0.21±0.04 to 0.41±0.03 in accordance with the decreasing freezing rate. The ice of Lot 06 showed the smallest SSA value and the highest water content. In contrast, the ice of Lot 04 showed the opposite values, which decreased the water desorption rate during the secondary drying stage and led to increased residual water content. In addition, the cooling rate for Lot 06 was less than that for Lot 04. Therefore, the slower cooling rate was considered to cause growth of ice crystals. A comparison of SEM images between Lots 04 and 06 showed the coarsely pored structure of the ice crystals in Lot 06 as shown in Fig. 2(a), demonstrating the formation of large ice in the Flomoxef of Lot 06 relative to Lot 04. Thus, the control of ice size via the freezing rate can be termed ice nucleation control.

Both the growth of ice crystals and their size should be related to the resistance of the water vapor to the frozen Flomoxef cake. The average \( R_p \) value during the primary drying stage was calculated using Eq. 6 as shown in Table 4. The \( R_p \) value of Lots 04 to 06 decreased with a decrease in the freezing rate as shown in Fig. 2(b). This was because slower cooling was confirmed to cause growth of ice crystals as previously discussed. The \( R_p \) values with ice nucleation control became lower than those of the product (Lot 03) without any ice nucleation control (i.e., annealing). This demonstrated that the ice nucleation control contributed to a reduction in the drying resistance.

The water content of the products and their SSA are presented in Table 3 and again summarized in Fig. 2c. The increase in the SSA value induced reduction in the water content and increase in the \( R_p \) value. The smaller ice crystals such as those of Lot 01 were disadvantageous for the sublimation of water. Meanwhile, the larger ice crystals appeared to induce rapid sublimation under low water vapor resistance. Therefore, the ice nucleation control enabled shortening of the primary drying time because of the formation of large ice in the Flomoxef.

**Calculation of the Design Space for the Primary Drying Stage** The sublimation interface temperature during primary drying was established using the drying resistance (\( R_p = 4.0 \)) with ice nucleation control and the drying resistance (\( R_p = 6.3 \)) without ice nucleation control, as listed in Table 5. With ice nucleation control, when both the \( T_s \) and \( P_c \) values...
were designed at $-10^\circ C$ and 6.7Pa, respectively, it was predicted that the sublimation temperature of the vials placed at both the center and edge positions in the lyophilizer during the primary drying stage can be controlled at a temperature lower than the cake collapse temperature ($T_c$). In contrast, without ice nucleation control, the sublimation temperature of the vials placed at the center position in the lyophilizer can be controlled at a temperature lower than $T_c$, but the sublimation temperature of the vials placed at the edge position in the lyophilizer was higher than $T_c$. Operating conditions (chamber pressure and primary drying time) that result in the shelf temperature increase from $-25$ to $0^\circ C$ and those resulting in the product temperature increase from $-33$ to $-26^\circ C$ are summarized in Fig. 4. Because the product temperature during the primary drying should preferably be from 2 to 5$^\circ C$ lower than the collapse temperature, the acceptable region of the product temperature would be from $-33$ to $-30^\circ C$ considering the $T_c$ of the Flomoxef sodium bulk solution which is $-28^\circ C$.

As illustrated in Figs. 4(a) and (b), the product temperature with ice nucleation control during the primary drying stage was confirmed to be within the acceptable region. In contrast, as illustrated in Fig. 4(d), the product temperature without ice nucleation control in the edge position in the lyophilizer during the primary drying stage was confirmed to be outside the acceptable region, although the product temperature with ice nucleation control at the center position was within the acceptable region (Fig. 4(c)).

**Verification Study for the Primary Drying Conditions Based on the Design Space** Two lots (Trials 01 and 02) of manufacturing were performed to verify the primary drying conditions calculated using the design space.

Trial 01 was manufactured with ice nucleation control and visual inspection was conducted for all 726 vials after the completion of the lyophilization. Consequently, there were no collapsed cakes. In contrast, Trial 02 was manufactured without ice nucleation control. As predicted in the previous design space, some collapsed cakes were observed in the vials placed at the edge position in the lyophilizer. The defective rate of the collapsed cake was 18%. It may be concluded that the $R_p$,

![Fig. 3. Product Temperature Profile during the Freezing Stage (a) without and (b) with Ice Nucleation Control](image)

(a) Lots 03 and 04 were used. Solid line: The vial placed at the center position in the lyophilizer; dotted line: the vial placed at the edge position in the lyophilizer.

Table 4. Average Resistance of the Dried Product Layer during Primary Drying Stage

| Lot       | Freezing condition                      | Freezing rate (°C/min) | Trial number ($n$) | Water vapor transfer resistance of the dried layer ($R_p$) (Torr·cm²·h/g) |
|-----------|-----------------------------------------|------------------------|-------------------|--------------------------------------------|
| Lot 03    | Non-nucleation control annealing: 0°C for 0.5h | 1                      | 4                 | 6.3±1.0                                    |
| Lot 04    | Nucleation controlled at $-5^\circ C$    | 0.5                    | 3                 | 4.0±0.5                                    |
| Lot 05    | Nucleation controlled at $-5^\circ C$    | 0.5                    | 3                 | 3.0±0.5                                    |
| Lot 06    | Nucleation controlled at $-5^\circ C$    | 0.1                    | 3                 | 2.4±0.4                                    |

*a* Average±S.D. The values of the parameters to estimate $R_p$ value are as follows: $W_{wv}=3.64g$, $\rho_{wv}=0.918g/mL$, $\rho=1.156g/mL$, $C_C=0.31g/g$, $A_v=3.84cm^2$, $A_e=4.71cm^2$, $L_{max}=0.73cm$, $\Delta n_{H2O}=2.51g/vial$, $\Delta H=669cal/g$, and $10^4 K_v$ (at 6.7Pa)=$2.57 cal/(s·cm^2·°C)$.

Table 5. Predicted Sublimation Interface Temperature and Primary Drying Time (Calculated Using the Drying Resistance) under Ice Nucleation Control under a Condition of a Shelf Temperature of $-10^\circ C$ and a Chamber Pressure of 6.7Pa

| Ice nucleation control | Drying resistance $R_p$ (Torr·cm²·h/g) | Product temperature ($T_p$) (°C) | Sublimation interface temperature ($T_{sub}$) (°C) |
|------------------------|----------------------------------------|----------------------------------|--------------------------------------------------|
| With control           | 4.0                                    | Center                           | $-32.8$                                          | $-33.1$                                    |
|                        |                                        | Edge                             | $-30.0$                                          | $-30.5$                                    |
| Without control        | 6.3                                    | Center                           | $-30.3$                                          | $-30.6$                                    |
|                        |                                        | Edge                             | $-27.5$                                          | $-27.9$                                    |

The values of the parameters to calculate $R_p$ value are as follows: $W_{wv}=3.64g$, $\rho_{wv}=0.918g/mL$, $\rho=1.156g/mL$, $C_C=0.31g/g$, $A_v=3.84cm^2$, $A_e=4.71cm^2$, $L_{max}=0.73cm$, $\Delta n_{H2O}=2.51g/vial$, $\Delta H=669cal/g$, $10^4 K_v$ (center)=$2.57 cal/(s·cm^2·°C)$, and $10^4 K_v$ (edge)=$4.11 cal/(s·cm^2·°C)$. LyoStar 3 as a lyophilizer was used to estimate the $K_v$ value.
value of Trial 01 and the variation with ice nucleation control became lower than those of Trial 02, which was the product without ice nucleation control. The ice nucleation control enables a robust design space for the primary drying stage to be established with high productivity.

**Conclusion**

Our study demonstrated that the ice crystal size has an impact on the product quality and productivity. The pressurization and depressurization technique was combined by varying the freezing rate to avoid supercooling of the solution and control the size of the ice formed in the drug product during the freezing stage, which contributed to a reduction in $R_p$ during the primary drying stage. This approach was termed ice nucleation control, which was advantageous in shortening the primary drying time. The reduced $R_p$ made it possible to set the robust design space for the primary drying stage to achieve uniform products with higher productivity (no collapsed cakes in 726 vials).

Thus, our study emphasizes the impact of ice nucleation control on the quality and productivity of a small-molecule pharmaceutical product. However, the increase in the residual water content of the lyophilized cake may affect the solid stability. A stability test to determine the maximum allowable water content is needed, which will be the topic of our future investigation.

**Conflict of Interest** The authors declare no conflict of interest.

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