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## Effects of controlled nucleation on freeze-drying lactose and mannitol aqueous solutions

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### ABSTRACT

The lyophilization of lactose and mannitol aqueous solutions was investigated with an emphasis on analyzing the effects of controlled nucleation, temperature of nucleation, and pore size distribution on the freeze-drying process. The experimental procedure involved the depressurization technique of controlled nucleation, in-vial temperature measurements as well as measurements of the chamber pressure, which allowed the analysis of the product batch, loaded in the laboratory lyophilizer. The average pore enlargement was 93 and 58% with the incorporation of the controlled nucleation step in the lyophilization of 6 wt% lactose and 6 wt% mannitol solutions, respectively. Consequently, the primary drying times were lowered from 450 to 500 min in both cases. The pore sizes were determined to be as important as the solid material itself in the scope of the sublimation rates. Namely, the average equivalent diameter of the pores was larger in the dried mannitol cake compared to the lactose cake. However, despite the higher porosity of the dried mannitol cake, the end of the sublimation in the primary drying step was observed approximately 500 min earlier during the lyophilization of the lactose solution with the same initial concentration as the mannitol solution in a comparable freeze-drying protocol. In addition, an increase in mannitol concentration from 3 to 12 wt% was found to substantially extend the time required for the sublimation phase of the lyophilization.

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depressurization technique;  
freeze-drying; pore size

### Introduction

The freeze-drying process is widely used in pharmaceutical applications due to the low-cost storage, simple reconstitution of the product and long-term stability.<sup>[1]</sup> The process is comprised from three phases: freezing, primary drying, and secondary drying. The research and development of lyophilization is in great extent engaged in process control and optimization,<sup>[2–4]</sup> which involves reduction of the overall process time, energy consumption, and production costs. Although, typical process improvements involve the optimization of primary drying, we have focused on the freezing step of lyophilization, as it affects the morphology of the dried product and hence all the subsequent steps of the freeze-drying process.<sup>[5–9]</sup>

The most important event in the freezing step of a lyophilization cycle is the nucleation of ice crystals. The size, shape, and homogeneity of the frozen structure are highly dependent on (i) the number of nuclei, (ii) the nucleation temperature, and (iii) the crystal growth rate. One of the main factors influencing the growth rate of the crystals is the cooling rate of the product, which is mostly controlled with the temperature of the lyophilizer's shelves. Lower supercooling and lower cooling rate

result in the formation of larger ice crystals, which is beneficial during the drying process. Rambhatla et al.<sup>[10]</sup> confirmed that the control of the nucleation temperature is crucial for establishing a desired flow resistance of the product. The controlled nucleation presents several benefits: better intra- and inter-vial homogeneity of the product; shorter primary drying times, lower energy consumption, and reduced costs.

A thorough literature review of the freezing step and its possible improvements was covered by Kasper and Friess.<sup>[8]</sup> Several techniques of controlled nucleation ranging from the ice fog technique to the mechanical agitation of the shelves were presented by Geidobler and Winter<sup>[11]</sup> along with their advantages, disadvantages, and suitability for the lyophilization process. Recently, a review of the alternative methods for ice nucleation control was published by Dalvi-Isfahan et al.<sup>[12]</sup> Two techniques for controlled nucleation are used vastly more often than others due to their convenient implementation<sup>[4]</sup>: ice fog and depressurization technique. Our focus is on the depressurization method of controlled nucleation, which involves high pressures of up to 300 kPa with the addition of argon or nitrogen.

After the pressurized product reaches the desired temperature, the nucleation of ice is triggered by depressurization of the lyophilization chamber to the atmospheric pressure. Konstantinidis et al.<sup>[13]</sup> stated that at this moment three possible mechanisms for the nucleation exist. The first potential origin of the nucleation is the cooling of the liquid surface of the product due to the gas expansion during depressurization. The second mechanism is a mechanical pressure wave, which disturbs the meta-stable subcooled product and initiates the nucleation. The third possible source is due to the dissolved gasses in the liquid product, which could form bubbles after depressurization. The presence of a bubble in the liquid product presents a nucleation site for ice crystals. Currently, there is no consent yet, which of the mechanisms (or a combination of them) is the true origin for the formation of ice crystals. The pressure rise and depressurization method have been validated in laboratory lyophilizers as well as in small-scale freeze dryers with the shelf area of 5 m<sup>2</sup> by Bursac et al.<sup>[14]</sup> The benefits of the controlled nucleation was further validated by Konstantinidis et al.<sup>[13]</sup> with an approximately 40% shorter primary drying time when using depressurization method in aqueous solutions of mannitol and sucrose. Similarly, the controlled nucleation was assessed by Awotwe-Otoo et al.<sup>[15]</sup> and confirmed a more efficient lyophilization cycle with the incorporation of the controlled ice nucleation of the freezing step. Recently, a successful attempt of incorporating controlled ultrasound-assisted nucleation to freeze-drying of solid samples was presented by Dai et al.<sup>[16]</sup> The benefits of controlled nucleation include shorter time of the primary drying, improved cake appearance and morphology, better vial-to-vial homogeneity, etc. Another possible improvement of the freeze-drying process is complementary to the controlled nucleation in the form of annealing step, during which the ice crystals grow to their final size, as it was shown by Searles et al.<sup>[17]</sup>

The aim of this study is the employment of the controlled nucleation method in a lyophilization protocol and the analysis of its effect. In addition, the comparative analysis of two different products at various concentrations will be performed on the basis of in-vial and batch measurements. The dried product porosity, which was assessed from Scanning Electron Microscopy (SEM) photographs, was also considered in the comparative analysis of lactose and mannitol aqueous solutions.

## Experimental setup and procedure

The freeze-drying experiments were performed in a laboratory lyophilizer SP Scientific LyoStar 3

enhanced with a Praxair controlled nucleation system. The freeze dryer has four heated/cooled shelves with the combined loading area of 0.43 m<sup>2</sup>, whereas the condenser chamber is designed for loads up to 30.l. The Praxair's ControLyo system triggers a simultaneous nucleation of ice crystals in all the vials on the shelves by elevating the pressure to approximately 200 kPa and afterward a fast depressurization slightly below 100 kPa during the freezing step, which consequently leads to the formation of crystals at a designated time and temperature. The nucleation temperature is crucial for achieving the desired cake porosity, as higher temperatures result in a smaller number of larger crystals and lower temperatures of nucleation lead to a large number of smaller crystals. The size of the ice crystals affects the porosity and the permeability of the product, which is the most influential parameter in the scope of the primary and secondary drying times.

The experimental work was focused on aqueous solutions of lactose and mannitol, which were filled in 13.5-mL vials with an outside diameter of 24 mm. The filling volume was 4 mL in all conducted experiments. The total amount of the product in the lyophilization chamber was 100 mL during the local in-vial product temperature measurements, while the total loaded product was 3,000 mL, when the process was monitored at the batch level with the chamber pressure measurements. The temperature measurements were performed with *T*-type thermocouples, which are suitable for monitoring low temperatures. The freeze dryer was equipped with a capacitance manometer and a Pirani gauge, which were used to determine the pressures lower than 100 Pa. The pressures from 100 Pa to 200 kPa were measured with two additional pressure sensors, which are suitable for measuring higher pressures. All the signals were acquired and recorded at a minute interval.

Similar lyophilization protocols were used for the in-vial measurements of 6 wt% lactose and 6 wt% mannitol aqueous solutions. The lactose solution was freeze-dried with five different protocols: UN-A(−5°C)—uncontrolled nucleation with annealing at −5°C; CN—controlled nucleation at −10°C without annealing, CN-A(−5/−7/−10°C)—controlled nucleation at −5, −7, or −10°C with annealing at the same temperature. The annealing step is important, as it allows a preset and controlled shelf temperature for all the vials during the growth of the ice crystals. Consequently, the homogeneity of the batch is improved and the size of the crystals can be more accurately predicted. The CN protocol is 120 min shorter compared to other protocols with the full length of 4,860 min, due to the exclusion of the annealing step. Similar but not exactly the same protocols were used with

the mannitol solution: UN—uncontrolled nucleation; UN-A( $-5^{\circ}\text{C}$ )—uncontrolled nucleation with annealing at  $-5^{\circ}\text{C}$ ; CN-A( $-3/-5/-7^{\circ}\text{C}$ )—controlled nucleation at  $-3$ ,  $-5$ , or  $-7^{\circ}\text{C}$  with annealing at the same temperature. The full length of all the mannitol solution protocols was equalized to 4,880 min. The 6 wt% solutions of lactose and mannitol were filled to 25 vials, thus the total amount of the product in the lyophilization chamber reached 100 mL. The positioning of the vials was at the front of the bottom shelf during all the freeze-drying cycles.

The freeze-drying protocol with and without the controlled nucleation step was also used for the aqueous mannitol solutions of 3 and 12 wt%, which were used in the batch measurements of the freeze-drying process. The volume of the product in the lyophilization chamber was 3,000 mL, which was distributed to 750 vials. The initial thermal stabilization steps as well as the primary and secondary drying steps were kept the same during the lyophilization. However, the freezing step was prolonged in the controlled nucleation cycle to enable a predetermined moment of nucleation in all the vials in the chamber after depressurization and also to allow the growth of the nucleated ice crystals. Consequently, the freezing step and also the full length of the controlled nucleation lyophilization cycle was 240 min longer compared to the uncontrolled nucleation cycle.

## Results and discussion

### Homogeneity of the process

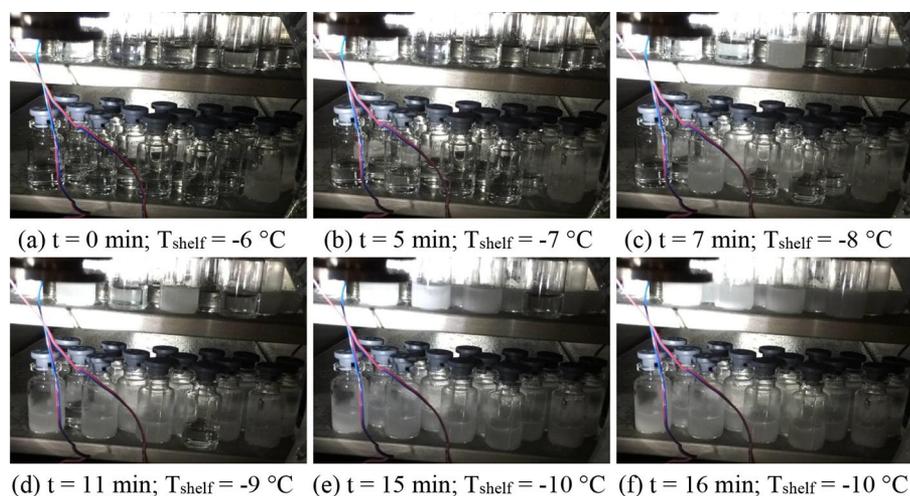
The freezing step is crucial for establishing a homogeneous freeze-drying process in all the vials, as the time and temperature of the ice nucleation are very

uncertain and it has a large impact on the successive drying steps. On the one hand, the heterogeneity of the uncontrolled nucleation is seen in Fig. 1, as the total time needed for nucleation in the observed vials was longer than 15 min. The initial recorded nucleation of ice is seen in Fig. 1a, and the last nucleation took place in Fig. 1f, which was more than 16 min after the first observed nucleation. The temperature of the shelf as well as the temperature of the product was lowered during this time period, which leads to the inter-vial heterogeneity. The loading of the chamber was very small (25 vials), therefore we should expect an even more distinctive time and temperature differences at a fully loaded chamber.

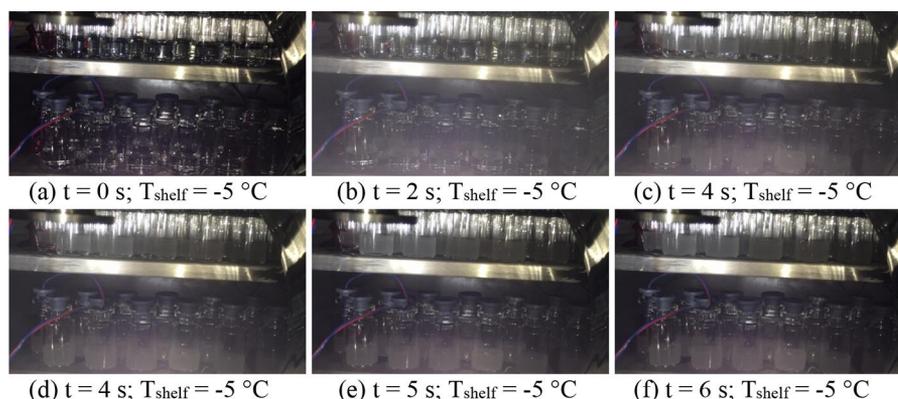
On the other hand, the controlled nucleation cycle led to a synchronized nucleation of ice crystals in all the filled vials, as presented in Fig. 2. Depressurization was initiated in Fig. 2b and all the visible vials contain solely ice crystals after a mere 4 s, which is presented in Fig. 2f. The set of photographs in Fig. 2 also confirms that the initial crystallization occurs at the surface of the liquid product, which is followed by nucleation and crystal growth toward the bottom of the vial. The nucleation was triggered at almost the same moment in Fig. 2b and more importantly at the same temperature of the product, which promotes the formation of similarly sized ice crystals. Consequently, the inter-vial porosity and permeability are much more constant compared to the batch of vials from the uncontrolled nucleation cycle. Improved vial-to-vial homogeneity leads to a better uniformity of the primary drying.

### In-vial monitoring

The freeze-drying of aqueous solution of lactose was performed with five different lyophilization protocols,



**Figure 1.** The uncontrolled nucleation of ice crystals.

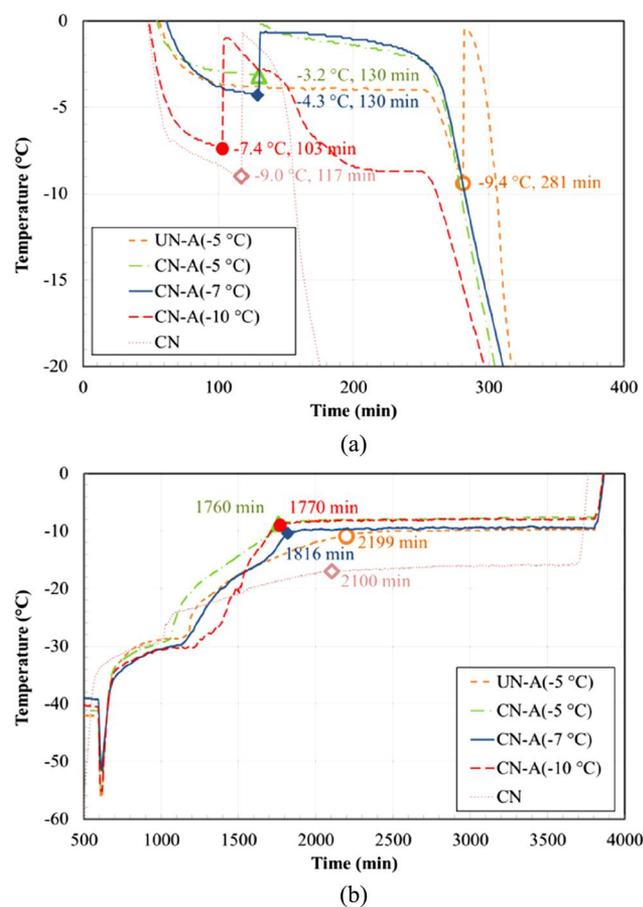


**Figure 2.** The controlled nucleation of ice crystals.

in which solely the freezing step was modified. Temperatures of the product as well as the process variables (chamber pressure, shelf temperature, condenser temperature and pressure) were recorded. The comparison of the product temperatures measured just above the vial's bottom during the freezing step is presented in Fig. 3a. The product temperature during the uncontrolled nucleation with annealing denoted with UN-A(5°C) clearly indicates that the annealing step was not successful as the product nucleated after the annealing step at 281 min and  $-9.4^{\circ}\text{C}$ . Similar nucleation temperature of  $-9.0^{\circ}\text{C}$  was measured in the controlled nucleation cycle without annealing CN; however, the moment of nucleation is seen at 117 min. The controlled nucleation cycles CN-A( $-5^{\circ}\text{C}$ ) and CN-A( $-7^{\circ}\text{C}$ ) initiated the nucleation of ice crystals at 130 min with slightly higher temperatures measured in the product in comparison with the shelf temperature. Although, the temperature of the product did not reach the prescribed shelf temperature of  $-5$  and  $-7^{\circ}\text{C}$  at the moment of depressurization, the nucleation of ice was successful at  $-3.2$  and  $-4.3^{\circ}\text{C}$ , respectively. The nucleation of crystals during the lyophilization protocol CN-A( $-10^{\circ}\text{C}$ ) was spontaneous at  $-7.4^{\circ}\text{C}$  before the moment of depressurization at 130 min. Therefore, the controlled nucleation at such a low temperature of  $-10^{\circ}\text{C}$  is not suitable for the used lactose solution, as there is a high chance that the product will spontaneously nucleate before the depressurization occurs.

The product temperature measurements during the freeze-drying are an adequate indicator for the end of the primary drying. The initial temperature rise above the sublimation temperature, which is approximately  $-30^{\circ}\text{C}$  for the aqueous lactose solution, denotes the exact moment when the sublimation front passes by the temperature sensor. Afterward, the temperature of the product at the sensor's location rises, due to the

higher temperature of the shelves and the vapor passing through the porous cake. After there is no more sublimation present in the product, the vapor ceases to cool down the sensor, which is seen as the temperature stabilization in Fig. 3b. It is difficult to unambiguously define the exact end of sublimation for different vials and products, as the temperature gradually stabilizes in a certain time period. Therefore, we have determined



**Figure 3.** (a) The freezing step and (b) the primary drying step of the 6% aqueous lactose solution lyophilization.

the end of the primary drying as the moment when the temperature reaches the value, which is 1°C lower than the steady-state temperature

$$t_{p.end} = t_{T_{steady-state}-1} \quad (1)$$

To assure comparable results, we have defined the steady-state temperature as the average temperature in the range from 3,000 to 3,500 min for all the experimental runs

$$T_{steady-state} = \frac{1}{n_2 - n_1 + 1} \sum_{n_1=3000}^{n_2=3500} T_i \quad (2)$$

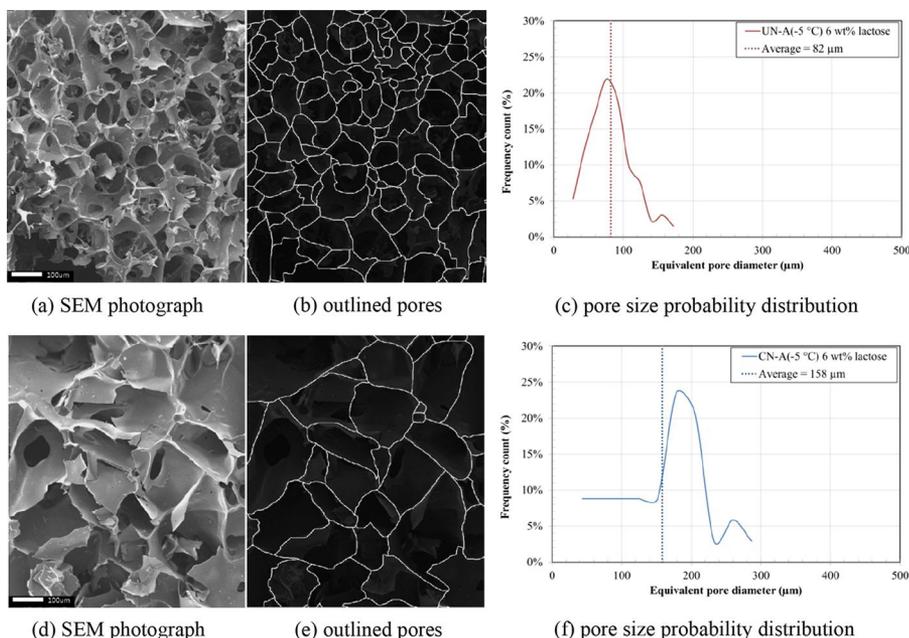
The used method of determining the end of primary drying is suitable for the performed analysis, as the determined times are comparable among different used protocols.

The end of primary drying was first observed at 1,760 min for the CN-A(-5°C) cycle, which was closely followed by a vial from the CN-A(-10°C) cycle and a vial from the CN-A(-7°C) protocol, which concluded the primary drying at 1,770 and 1,816 min, respectively. The longest time needed for the end of primary drying is seen at 2,199 min, which corresponds to the vial from the uncontrolled nucleation cycle with annealing UN-A(-5°C). The controlled nucleation cycle without annealing CN required 2,100 min for the end of sublimation; however, this protocol's freezing step was 120 min shorter. Therefore, if we compare only the time of the primary drying step, it becomes evident that the sublimation process took the longest time during

the controlled nucleation without annealing. If we compare only the successful controlled nucleation protocols CN-A(-5°C) and CN-A(-7°C) with the uncontrolled nucleation protocol UN-A(-5°C) it becomes evident, that the primary drying time was shortened for approximately 400 min, which is a significant reduction of the freeze-drying process.

The origin of the improved lyophilization process with controlled nucleation is the lower vapor flow resistance of the dried cake, which is to some degree proportional to the cake's porosity. Therefore, we have obtained SEM images of the lyophilized product after the secondary drying to obtain the product's morphology.<sup>[18]</sup> Image analysis was made using the Matlab numerical computing environment. First, the original SEM photographs, seen in Figs. 4a and 4d, were artificially darkened to obtain greater contrast between the pore outlines and pores, thus enabling further analysis. The darkened images were afterward processed with an image editing tool to enhance the pore outlines with white borders, which is given in Figs. 4b and 4e. The images with outlined pore borders were analyzed to obtain the surface area of each individual pore. A comparable parameter was needed for all the acquired pores, although the pores have various geometries. Therefore, the equivalent pore diameters were chosen similarly as by Hottot et al.<sup>[9,19]</sup> The diameters were determined as:

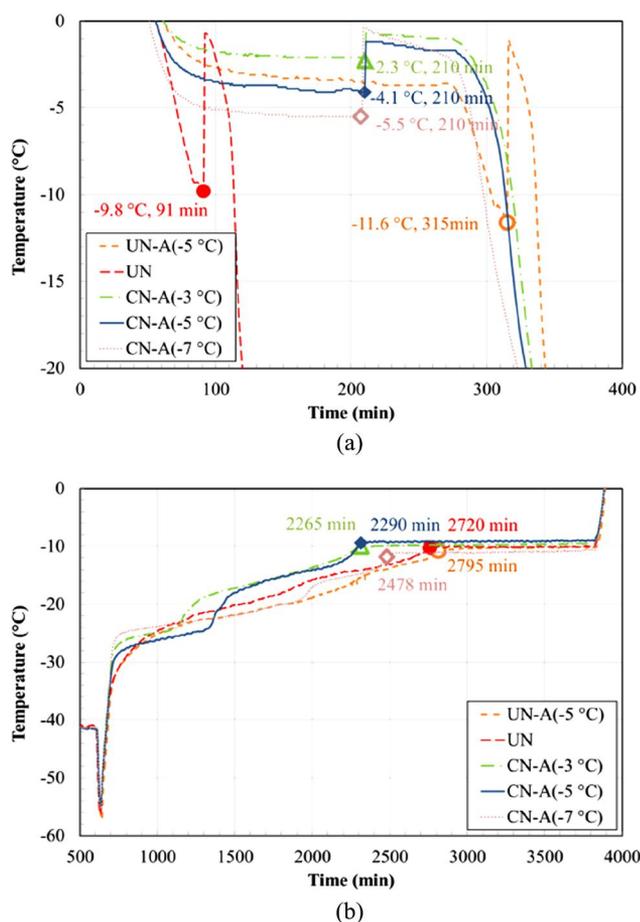
$$d_{eq} = 2\sqrt{\frac{A_{pore}}{\pi}} \quad (3)$$



**Figure 4.** The SEM photographs and the pore size analysis of the freeze-dried 6% lactose with (a–c) uncontrolled nucleation UN-A(-5°C) and (d–f) controlled nucleation CN-A(-5°C).

The probability distributions of the calculated equivalent pore diameters are presented in Fig. 4c for the freeze-drying of 6 wt% lactose solution with an average equivalent pore diameter of 82  $\mu\text{m}$  for the UN-A(-5°C) protocol. Similar analysis was performed on the lactose solution lyophilized in accordance with the CN-A(-5°C) cycle and the results presented in Fig. 4f show that the average equivalent pore diameter was 158  $\mu\text{m}$ , when the controlled nucleation protocol was used. The pore diameters were in average enlarged for almost 93%, if we compare the selected lyophilization cycles. The origin of the shortened primary drying time are the larger pore diameters, which were achieved with the introduction of controlled nucleation and annealing to the freeze-drying process.

The freeze-drying cycles were slightly altered with the transition from the lactose solution to the 6 wt% solution of mannitol. Figure 5a shows the product temperature during the freezing step of five different protocols, used with the mannitol solution. The controlled nucleation cycles were programmed

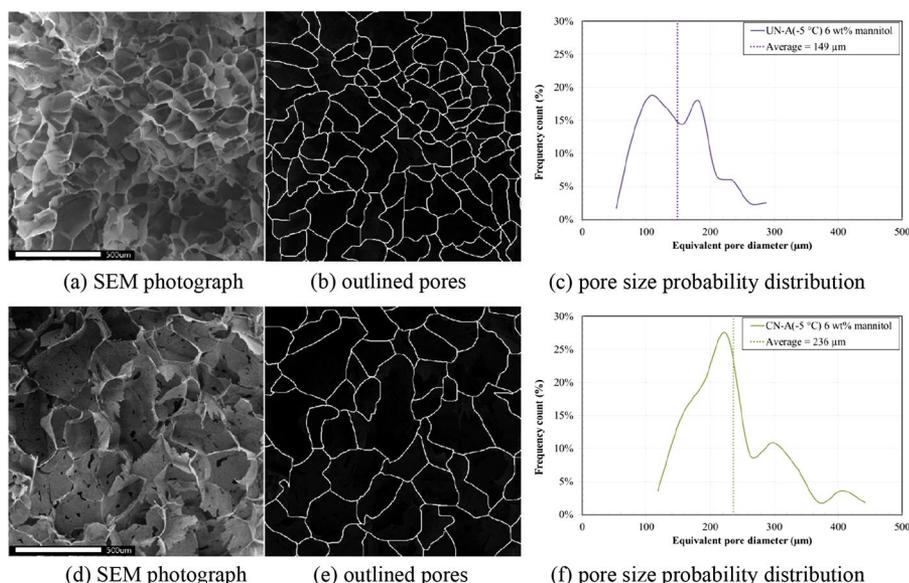


**Figure 5.** (a) The freezing step and (b) the primary drying step of the 6% aqueous mannitol solution lyophilization.

to depressurize the chamber from approximately 190 to 100 kPa at 210 min. The results of the temperature measurements confirm that all the three controlled nucleation protocols were successful at triggering the crystallization at -2.3, -4.1, and -5.5°C for the CN-A(-3°C), CN-A(-5°C), and CN-A(-7°C) cycles, respectively. The temperatures of the product are slightly above the controlled shelf temperature, which was expected. Two protocols with uncontrolled nucleation were also carried out. The temperature of nucleation was -9.8°C for the uncontrolled nucleation without annealing UN, whereas the temperature of crystallization reached -11.6°C during the controlled nucleation with annealing at -5°C UN-A(-5°C). The annealing step was not efficient in the uncontrolled nucleation protocols, as the annealing phase occurred before nucleation and was therefore useless.

The primary drying step for all the protocols tested with the 6 wt% mannitol solution is presented in Fig. 5b. The end of the primary phase was determined in accordance with Eq. (1). The shortest primary drying phase is seen with the CN-A(-3°C) protocol, as the sublimation ends at 2,265 min. The second best protocol is the CN-A(-5°C) with the end of the primary drying at 2,290 min and the third best protocol is the CN-A(-7°C) with the end of sublimation at 2,478 min. The results are consistent with the fact that the porous structure of the cake is more permeable at higher temperatures of ice crystal nucleation. The uncontrolled nucleation protocols with or without the annealing step have similar times of the primary drying end. Sublimation ended in the monitored vial at 2,720 min during the UN protocol and at 2,795 min during the UN-A(-5°C) cycle. The controlled nucleation protocols CN-A(-3°C) and CN-A(-5°C) shortened the primary drying time for approximately 500 min, which is an even better improvement compared to the lactose solutions.

The same procedure of the SEM photograph analysis as for the lactose solution was used also for the 6 wt% mannitol solution, which is presented in Figs. 6a-f. The compared lyophilization protocols were UN-A(-5°C) and CN-A(-5°C). Both cycles have an annealing step with the main distinction in the uncontrolled or controlled nucleation of ice crystals during the freezing step. The uncontrolled nucleation cycle is presented in Figs. 6a-c and the calculated average equivalent pore diameter was 149  $\mu\text{m}$ . The dried cake structure of the controlled nucleation protocol is presented and analyzed in Figs. 6d-f with the average equivalent pore diameter of 236  $\mu\text{m}$ . The increase in the average pore diameter with the incorporation of controlled nucleation was 58%. The pore enlargement was substantial, which corresponds to the improved



**Figure 6.** The SEM photographs and the pore size analysis of the freeze-dried 6% mannitol with (a) uncontrolled nucleation UN-A(-5°C) and (b) controlled nucleation CN-A(-5°C).

freeze-drying cycle, in which the time of primary drying was also considerably shortened.

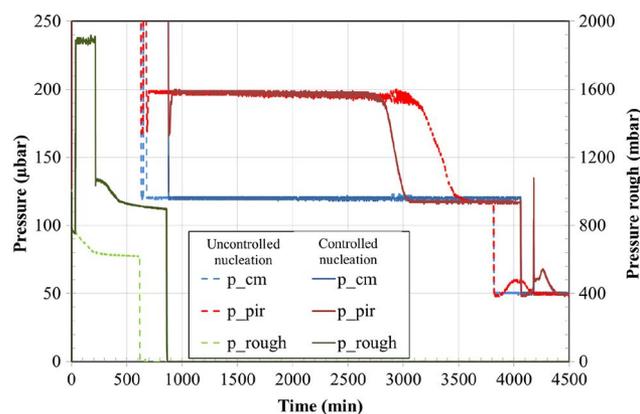
### Batch monitoring

Although the presented results of the in-vial temperature measurements show a substantially improved freeze-drying process with the implementation of the controlled nucleation, we have made additional experimental measurements on a fully loaded chamber (750 vials containing 3,000 mL) with the focus on monitoring the conditions of the whole batch of vials. Therefore, the comparative analysis is based on the measured pressures in the lyophilization chamber. Four different pressure sensors are located in the lyophilizer LyoStar 3. Two sensors are measuring higher values from 100 Pa to 200 kPa and are depicted in Figs. 7 and 8 with “p\_rough,” whereas the capacitance

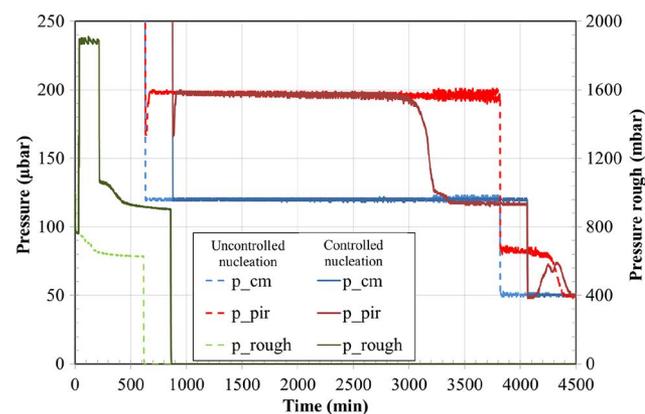
manometer and the Pirani gauge measure low pressures up to 100 Pa and are presented in Figs. 7 and 8 with “p\_cm” and “p\_pir,” respectively. The capacitance manometer is suitable for controlling the process, while the Pirani gauge indicates the end of the primary drying quite accurately.

The 3 wt% mannitol solution was used with controlled (CN) and uncontrolled nucleation (UN) protocols to allow the comparative analysis of the drying process and drying times. The diagram in Fig. 7 depicts the pressures during CN and UN freeze-drying cycles. The duration of the freezing step was 240 min longer, when the pressurize/depressurize method of controlled nucleation was used. The end of primary drying was defined as:

$$|p_{\text{pir}}(t_{\text{p.end}}) - p_{\text{cm}}(t_{\text{p.end}})| \leq 1 \mu\text{bar} \quad (4)$$



**Figure 7.** Freeze-drying of 3% aqueous mannitol solution.



**Figure 8.** Freeze-drying of 12% aqueous mannitol solution.

The sublimation of the mannitol aqueous solution was concluded at 3,019 min for the controlled nucleation cycle and at 3,464 min for the uncontrolled cycle, which results in roughly 450 min time savings with the incorporation of the CN protocol. In addition, the freezing step of the CN cycle was longer, therefore the comparison of only the primary drying times of the CN and UN cycles results in an even larger time saving of 690 min. The controlled nucleation at  $-5^{\circ}\text{C}$  was highly effective at improving the time needed for primary drying, which was shortened for approximately 24% as it was reduced from 2,854 to 2,169 min.

The same lyophilization cycles were used also for a 12 wt% solution of mannitol and the experimental results are presented in Fig. 8. The time of the end of the primary drying was determined at 3,318 min for the CN protocol, whereas the UN cycle was unsuccessful at concluding the sublimation process in the predetermined primary drying phase. The end of primary drying occurs only after the process enters the secondary drying phase, therefore we can confirm that the end of the sublimation exceeded the 3,820 min boundary between the primary and secondary drying phases. An accurate estimation of the moment of the end of sublimation is not possible from this experiment. Nonetheless, it can be concluded that the time savings were at least 500 min. If we compare solely the primary drying phases, the controlled nucleation shortened the process from at least 3,210 to 2,468 min, which corresponds to a minimal 23% improvement.

The effect of mannitol concentrations in aqueous solutions is also seen from the comparison of the freeze-drying processes, presented in Figs. 7 and 8. The lower 3 wt% concentration of mannitol dried substantially faster compared to the 12 wt% solution during the uncontrolled as well as the controlled nucleation cycle. The time savings were 300 min for the CN cycle and at least 350 min for the UN cycle. The higher concentration of mannitol resulted in slower sublimation rates during the primary drying, which was based on the lower permeability and porosity of the dried cake.

## Conclusion

Initially, the favorable effect of the pressurization/depressurization method of controlled nucleation was confirmed. On the one hand, the nucleation of ice crystals occurred spontaneously in a time period of 16 min, during which the temperature of the shelves lowered from  $-6$  to  $-10^{\circ}\text{C}$  when uncontrolled nucleation protocol was used. On the other hand,

during the controlled nucleation cycle the crystallization took place momentarily, and the ice growth was finished in all the vials in 4 s. The used controlled nucleation method was successful at triggering the nucleation in all the vials in the lyophilization chamber.

The in-vial monitoring of temperature was performed with a single thermocouple inserted in the product. Different lyophilization protocols were used to analyze the effect of controlled nucleation, temperature of nucleation, and annealing on the freezing step and on the primary drying, during which the sublimation of ice occurs. A 6 wt% lactose aqueous solution was used in the first set of experiments. The controlled nucleation step commenced the crystallization and afterward the annealing step allowed the further growth of the ice crystals. The drying cycles with controlled nucleation included had shorter primary drying times compared to the uncontrolled nucleation cycles. The time savings were approximately 400 min. The SEM photograph analysis was performed for the controlled and uncontrolled nucleation protocols denoted with CN-A( $-5^{\circ}\text{C}$ ) and UN-A( $-5^{\circ}\text{C}$ ). The results unambiguously show an average pore enlargement of 93% with the incorporation of depressurization method of controlled nucleation.

Another fluid was used to allow an assessment of the product's effect on the freeze-drying cycle. A 100 mL of the 6 wt% mannitol aqueous solution was evenly filled to 25 vials, which was the same as with the lactose solution. The results depict a successful triggering of the simultaneous nucleation in the controlled nucleation protocols. The end of sublimation in the primary drying phase was observed approximately 500 min earlier in the protocols with the controlled nucleation. The experimental results are also indicating the importance of the nucleation temperature, as the shortest drying time was recorded in the protocol with the highest temperature of nucleation. The analysis of the SEM photographs showed a 58% increase in the average pore size with the inclusion of the controlled nucleation step in the lyophilization protocol.

The drying times of the 6 wt% lactose solution are in average approximately 500 min shorter compared to the 6 wt% mannitol solution. The two fluids used differ quite substantially regarding the time needed for the end of sublimation. The difference was approximately the same in all comparable protocols. The size of the pores in the dried product was actually larger in the mannitol solution, which could indicate a lower flow resistance and shorter drying times. However, the pore size and distribution are good parameters of evaluating the permeability only with similar characteristics of the pore's walls. Another possible origin of the lactose

solution's shorter drying times is the shrinkage of the amorphous lactose cake, which resulted in a small gap between the product and the glass wall. Additional surface was therefore available for drying of the lactose solution, which could accelerate the drying process compared to the mannitol solution, as there was no gap observed between the mannitol cake and the vial's walls. Concluding points regarding the primary drying times based on the porosity are therefore possible only with the use of the same or very similar products.

Batch monitoring of the freeze-drying process has some advantages over the in-vial monitoring with contact temperature sensors, as it is not interacting and thus interfering with the lyophilization process in any manner. The pressure measured with the Pirani gauge is a suitable and widely used indicator of the existence of the sublimation flow and is therefore used to determine the end of the primary drying. A comparison of lyophilization cycles with and without controlled nucleation was made and the results of 3 wt% mannitol aqueous solution show a reduction of 450 min of the time needed for sublimation end with the addition of the pressurization/depressurization step. Moreover, if we compare only the primary drying times, the time savings reach 690 min which corresponds to 24%, as the freezing step of the controlled nucleation cycle was 240 min longer. Similar results were gathered from the freeze-drying of 12 wt% mannitol solution; however, the sublimation during the uncontrolled nucleation cycle ended only when the lyophilizer entered the secondary drying stage, which makes the determination of the exact time of the end of sublimation impossible. Nonetheless, we can conclude that we have shortened the time needed for primary drying for at least 500 min with the incorporation of the controlled nucleation step. The comparison of the primary drying phases shows at least a 740 min or 23% reduction of the time needed for the end of the ice sublimation. The transition from the 3 to 12 wt% mannitol solution was followed by an approximately 300 min and at least 350 min longer drying times for the controlled and uncontrolled nucleation protocols, respectively.

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