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# In-line prediction of secondary drying time from measured ice nucleation temperature during lyophilization process

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## ABSTRACT

**Purpose:** To propose an approach to find optimal secondary drying time ( $t^{SD}$ ) specifically for each lyophilized batch by predicting residual moisture at the beginning of secondary drying ( $C_s^0$ ) using measured ice nucleation temperatures ( $T_N$ ) obtained in-line. **Materials and Methods:** Value of  $C_s^0$  was determined by minimizing the residual error between measured ( $T_p^{SD,mea}$ ) and calculated ( $T_p^{SD,cal}$ ) product temperatures during secondary drying. Value of  $T_N$  was determined by using product temperatures during freezing ( $T_p^{Fr}$ ). Then, the correlation between  $C_s^0$  and  $T_N$  was conducted and resulted in the  $C_s^0$  prediction equation. This equation was used with developed mathematical simulation to propose  $t^{SD}$  prediction model for each lyophilized batch. **Results:**  $C_s^0$  and  $T_N$  of training lyophilized batches were successfully determined by using  $T_p^{SD}$  and  $T_p^{Fr}$ , respectively. Linear relationship was assumed and used to predict  $C_s^0$  and  $T_N$  of testing lyophilized batch. The accuracy of  $t^{SD}$  prediction model was confirmed by comparing calculated and measured final product residual moisture ( $C_s^t$ ) of testing lyophilized batch. Then, the optimum  $t^{SD}$  for each targeted  $C_s^t$  was predicted from given range of  $T_N$ . **Conclusion:** Measured  $T_N$  could be used to predict the optimum  $t^{SD}$  for each lyophilization batch. Nevertheless, further study is required before applying this approach in pharmaceutical industry scale.

**Keywords:** Ice nucleation temperature, lyophilization, prediction, secondary drying, secondary drying time

## ABBREVIATIONS

### Symbols

$A_v$	Cross-sectional area of the vial, m <sup>2</sup>
$c_{p,p}$	Specific heat of the product, J kg <sup>-1</sup> K <sup>-1</sup>
$C_s$	Residual moisture, kg <sub>water</sub> kg <sup>-1</sup> dried product
$C_s^0$	Residual moisture at the beginning of secondary drying phase, kg <sub>water</sub> kg <sup>-1</sup> dried product
$C_s^t$	Final product residual moisture, kg <sub>water</sub> kg <sup>-1</sup> dried product
$H_{des}$	Heat of desorption, J kg <sup>-1</sup> water
$K_v$	Overall heat transfer coefficient, WK <sup>-1</sup> m <sup>2</sup>
$k_d$	Desorption kinetics constant, kg <sup>-1</sup> dried product s <sup>-1</sup> m <sup>2</sup>

$m_d$	Mass of dried product, kg <sub>dried product</sub>
$n$	Number of secondary drying product temperature data used
$t$	Time interval, s
$t$	Time, s
$t^{SD}$	Secondary drying time, hrs.
$T_{shelf}$	Shelf temperature, K
$T_N$	Ice nucleation Temperature, K
$T_p$	Product temperature, K
$T_p^{Fr}$	Product temperature during freezing phase, K
$T_p^{PD}$	Product temperature during primary drying phase, K
$T_p^{SD}$	Product temperature during secondary drying phase, K

## Superscripts

<i>cal</i>	calculated values of parameter
<i>est</i>	Estimated values of parameter
<i>i</i>	Values of parameter at interval $t=t_i$
<i>mea</i>	Measured values of parameter
<i>prd</i>	Predicted values of parameter
<i>tar</i>	Targeted values of parameter

## INTRODUCTION

Lyophilization is a dehydration process that physically converts liquid products from solution state to solids. This process is applied in the pharmaceutical industry to overcome instability problems or extend the shelf-lives of perishable pharmaceutical products, such as proteins in solution.<sup>[1,2]</sup>

In general, lyophilization process consists of three consecutive dehydration phases, i.e., freezing, primary drying, and secondary drying.<sup>[3]</sup> The freezing phase begins after vials are loaded into the lyophilization chamber, and the shelf temperature is then reduced to freeze and crystallize water, as well as, the product. In the primary drying phase, the chamber pressure is reduced to initiate ice crystal sublimation. As the shelf temperature gradually increases while holding vacuum condition in the secondary drying phase, residual moisture is further removed to reach targeted moisture that assures the stability and quality of the product.<sup>[4]</sup>

To obtain the desired lyophilized product within targeted moisture level, all processing conditions must be carefully determined and controlled. Many studies have suggested that the secondary drying phase is critical to obtain the targeted moisture.<sup>[5]</sup> Among the critical processing conditions during secondary drying, researchers were focusing on the relationships between shelf temperature and cycle time while chamber pressure was found to exert little or no effect on the residual moisture of the final products.<sup>[5]</sup> Furthermore, procedures to determine the above optimal combinations usually based on trial-and-error experiments. By setting the shelf temperature as high as possible without initiating product collapse, the cycle time is determined by sampling products periodically to measure residual moistures offline through Karl Fischer titration experiments or NIR spectroscopy<sup>[6,7]</sup> until residual moisture reaches the targeted values.

In addition, some researchers have proposed a more systematic approach to obtain the desired combination between shelf temperature and cycle time with mathematical simulations. These simulations use equations to model heat and mass transfer events occurred during the secondary drying phase. Heat transfer equation describes the energy balance between amount of heat transferred into the product and amount of heat used to remove water and raise the lyophilized product temperature. Mass transfer equation describes the rate of water desorption which is usually referred to water desorption, from lyophilized product which is considered as a rate-limiting step.<sup>[5]</sup> Various equations were proposed to model this process. Pisano *et al.*<sup>[8]</sup> proposed the

first-order mass transfer rate equation between the desorption rate and residual moisture content. Whereas Kodama *et al.*<sup>[9]</sup> proposed another approach using a modified Fick's Second Law to describe the water desorption step. Results of the above approaches<sup>[8,9]</sup> were able to identify the best combination of secondary drying processing conditions by using the optimal range of shelf temperatures and cycle time without a non-systematic trial-and-error experiments.

These previous mathematical simulation approaches were shown to have some drawbacks as their efficiency is highly influenced by the initial conditions of the lyophilized product, especially the moisture level at the beginning of secondary drying ( $C_s^0$ ). Moreover, the values of  $C_s^0$  are greatly dependent on the properties of ice crystal structures. Lyophilized product's drying behavior is shown to be significantly affected by the stochastic nature of ice nucleation, resulting in unpredicted heterogeneity of drying behavior from batch-to-batch or even within batch.<sup>[10-12]</sup>

For this reason, the optimized secondary drying processing conditions could be achieved if the processing condition, especially secondary drying time ( $t^{SD}$ ), is determined based on the value of  $C_s^0$  obtained for each lyophilized batch. This study extends the application of previous mathematical simulation approach by predicting the values of  $C_s^0$  based on the properties of ice crystal structures. According to previous researches,<sup>[13,14]</sup> varying ice nucleation temperatures ( $T_N$ ) can be used as representative for different ice crystal structures. Therefore, the objective of this study is to propose an approach to predict  $C_s^0$  using  $T_N$  obtained in-line and apply it to a developed mathematical simulation to find optimal  $t^{SD}$  specifically for each lyophilized batch.

## MATERIALS AND METHODS

### Preparation of Omeprazole Sodium Solutions

Omeprazole sodium was selected as a model drug for the study. Water for injection (WFI), omeprazole sodium (USP), Type I clear glass vials (10 ml), and gray bromo butyl rubber stopper (20 mm) were supplied by Biolab Co. Ltd. The solution was prepared by mixing omeprazole sodium and WFI to make 2% w/w aqueous solution. Then, fill 2 ml of the solution into each glass vial and insert stoppers in a semi-stoppering position appropriate for further lyophilization.

### Lyophilization of Omeprazole Sodium Solution

Lyophilization was done utilizing laboratory freeze dryer (LYO-0.5 m<sup>2</sup>, Tofflon). Each batch consists of four trays with 212 vials per tray distributed hexagonally. Lyophilization cycle was configured using standard processing condition as shown in Table 1.

### Approach to Build In-line $t^{SD}$ Prediction Model for Each Lyophilized Batch

The approach to build in-line  $t^{SD}$  prediction model for each lyophilized batch is shown as the Figure 1. Each block in the diagram represents the steps in building and validating

prediction model from measured data of training and testing lyophilized batches, respectively.

#### Lyophilization data collection

During the lyophilization cycle, product temperatures, which included product temperature profile during freezing ( $T_p^{Fr}$ ), primary drying ( $T_p^{PD}$ ), and secondary drying ( $T_p^{SD}$ ), were recorded throughout the experiment for every 60 s intervals. The product temperatures of each batch were monitored by inserting thermocouples at the center of the product solution in 4 vials situated on the second tray at a fixed position.

After the experiment was completed, three vials surrounding each monitored vial were selected to evaluate for  $C_s^t$  using the Karl-Fischer titration method. Then, the average  $C_s^t$  values of these three vials will be defined as the measured  $C_s^t$  ( $C_s^{t,mea}$ ) of that particular product.

#### Determination method of $C_s^0$

As conventional methods to determine the value of  $C_s^0$ , such as sampling products at the end of the primary drying process of every batch, are not feasible for industrial application. This study proposed the determination method based on the assumption that  $T_p^{SD}$  could be calculated by the function of  $C_s^0$ . Consequently, estimated value of  $C_s^0$  ( $C_s^{0,est}$ ) of each lyophilized product could be determined by minimizing the residual errors

between measured  $T_p$  ( $T_p^{SD,mea}$ ) and calculated  $T_p$  ( $T_p^{SD,cal}$ ) by Levenberg-Marquardt algorithm as described in Equation 1.

$$C_s^{0,est} = \arg \min_{C_s^0} \sum_{i=1}^n (T_p^{SD,i,mea} - T_p^{SD,i,cal})^2$$

$$= \arg \min_{C_s^0} \sum_{i=1}^n (T_p^{SD,i,mea} - f(i, C_s^0))^2 \quad (1)$$

To obtain the function described, the correlation of  $T_p^{SD,i,cal}$  and  $C_s^0$  at any given time point during secondary drying, heat, and mass transfer equations for secondary drying proposed by Pisano et al.<sup>[8]</sup> (Equation 2) was applied and derived into mathematical equation depicting the relationship between  $T_p^{SD,i,cal}$  and  $C_s^0$ .

$$m_d c_{p,p} \frac{dT_p^{SD}}{dt} = K_v A_v (T_{shelf} - T_p^{SD}) - m_d k_d C_s^0 \left( \prod_{i=1}^n e^{-k_d(dt)} \right) \Delta H_{des} \quad (2)$$

Where  $m_d$  is the mass of the dried product.  $c_{p,p}$  is the specific heat of the product.  $t$  is time.  $K_v$  is an overall heat transfer coefficient.  $A_v$  is the cross-sectional area of a vial.  $T_{shelf}$  is the shelf temperature.  $k_d$  is the desorption kinetics constant.  $\Delta H_{des}$  is the heat of desorption.

#### Determination method of $T_N$

As ice nucleation is an exothermic event that occurs during freezing phase,<sup>[12]</sup>  $T_N$  can be determined by detecting the trough just before the rapid rise in  $T_p^{Fr}$ .

#### Correlation between $T_N$ and $C_s^0$ and build $C_s^0$ prediction model

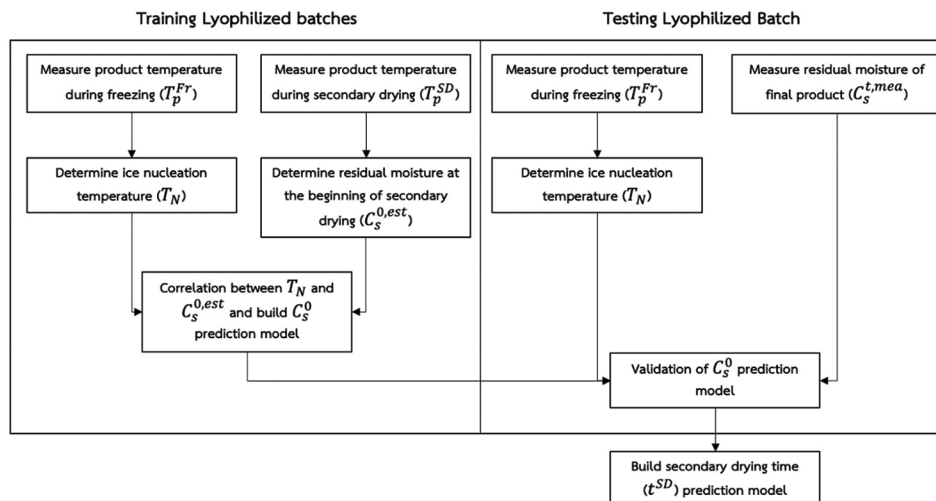
After obtaining the values of  $T_N$  and  $C_s^{0,est}$  for all training lyophilized products, the scattered plots are used to define the relationship of both parameters. In addition, the linear relationship is assumed from the previous relationship between  $T_N$  and other parameters.<sup>[13,14]</sup> Then, the linear equation can be further used as prediction model to predict  $C_s^0$  ( $C_s^t$ ) for any given  $T_N$ .

#### Validation of $C_s^0$ prediction model

To validate accuracy of the  $C_s^0$  prediction approach, measured data of testing lyophilized products were used in order to

**Table 1:** Processing condition of lyophilization cycles

Lyophilized phase	Processing condition		
	Temperature (K)	Pressure (mbar)	Time (h)
Freezing	269.15	-	1.50
	229.15	-	5.00
Primary drying	253.15	0.50	13.00
	263.15	0.50	8.00
	278.15	0.50	2.75
Secondary drying	303.15	0.00	5.00



**Figure 1:** Block diagram of the approach to build  $t^{SD}$  prediction model for each lyophilized batch

calculate  $C_s^{t,cal}$ . First,  $T_p^{Fr}$  of testing lyophilized product was used to obtain  $T_N$ . Then,  $T_N$  was used to predict  $C_s^0$  with prediction model. After that,  $C_s^{t,cal}$  can be calculated using the mathematical equation proposed by Fissore *et al.*<sup>[15]</sup> as depicted in Equation 3.

$$C_s^{t,cal} = C_s^{0,prd} \prod_{i=1}^n e^{-k_d^{est}(t^i - t^{i-1})} \quad (3)$$

Where  $k_d^{est}$  is estimated desorption kinetics constant of all available training lyophilized vials. Then, the comparison of the means of  $C_s^{t,cal}$  and  $C_s^{t,mea}$  of each testing lyophilized product was performed by Welch's *t*-test to validate the accuracy of this approach.

*Prediction model of  $t^{SD}$  for each lyophilized batch*

Predicted  $t^{SD}$  ( $t^{SD,prd}$ ) for each lyophilized batch could be predicted by using  $t^{SD}$  prediction model obtained by combining  $C_s^0$  prediction model with Fissore's mathematical equation.<sup>[15]</sup> By using this  $t^{SD}$  prediction model,  $t^{SD,prd}$  for each lyophilized batch to obtain targeted residual moisture ( $C_s^{t,tar}$ ) could be optimized by measuring the value of  $T_N$ .

## Data Processing

All calculations, including minimization of residual values by the Levenberg-Marquardt algorithm, were performed using R/ RStudio.<sup>[16]</sup>

## RESULTS AND DISCUSSION

Four batches of omeprazole lyophilized products were obtained using identical equipment and processing conditions. Three batches were randomly chosen and served as training batches to develop a  $C_s^0$  prediction model, whereas one remaining batch was used to validate the efficacy of our prediction approach.

### Determination of $C_s^0$ of Lyophilized Omeprazole Training Batches

In order to estimate  $C_s^{0,est}$  with Equation 1 of training lyophilized batches, mathematical equation describing the correlation of  $T_p^{SD,i,cal}$  and  $C_s^0$  at any given time point during secondary drying need to be defined by the derivation of Equation 2, where the following assumptions should be clearly stated:

- The amount of heat transferred into vials depended on the differences between the final shelf temperature and the final product temperature
- Because lyophilization process was performed utilizing identical formulation and equipment, parameters relating to the product and equipment, ( $m_d, c_{p,p}, A_v, \Delta H_{des}, K_v$ ) were assumed to be constants
- As secondary drying phase was completed at constant shelf temperature, the value of  $k_d$  for each vial can be viewed as a constant.
- Product temperatures are monitored at constant time interval ( $\Delta t$ ).

Based on these assumptions, Equation 2 could be rearranged to a more simpler equation of product temperature ( $T_p^{SD,1}$ ) at first interval,  $t = t_1$  compared to starting product temperature ( $T_p^{SD,0}$ ) at  $t = t_0$ , as shown in Equation 4.

$$T_p^{SD,1} = \alpha T_p^{SD,0} + \beta T_{shelf} (1) - \gamma C_s^0 (e^{-k_d(\Delta t)}) \quad (4)$$

Where  $\alpha$  is  $\frac{m_d c_{p,p}}{m_d c_{p,p} + K_v A_v (\Delta t)}$   $\beta$  is  $\frac{K_v A_v (\Delta t)}{m_d c_{p,p} + K_v A_v (\Delta t)}$   $\gamma$  is

$$\frac{m_d k_d \Delta H_{des}}{m_d c_{p,p} + K_v A_v (\Delta t)}$$

. Similarly, the equations of  $T_p^{SD,2}$  and  $T_p^{SD,3}$  can

be rearranged in a similar fashion and are shown in Equations 5 and 6, respectively.

$$T_p^{SD,2} = \alpha^2 T_p^{SD,0} + \beta T_{shelf} (\alpha + 1) - \gamma C_s^0 (\alpha e^{-k_d(\Delta t)} + e^{-2k_d(\Delta t)}) \quad (5)$$

$$T_p^{SD,3} = \alpha^3 T_p^{SD,0} + \beta T_{shelf} (\alpha^2 + \alpha + 1) - \gamma C_s^0 (\alpha^2 e^{-k_d(\Delta t)} + \alpha e^{-2k_d(\Delta t)} + e^{-3k_d(\Delta t)}) \quad (6)$$

With common constants, the mathematical equation depicted the relationship between  $T_p^{SD,i,cal}$  and  $C_s^0$  at any specific time during secondary drying can be generalized as shown in Equation 7.

$$T_p^{SD,n} = \alpha^n T_p^{SD,0} + \beta T_{shelf} - \gamma C_s^0 \sum_{i=1}^n (\alpha^{n-i}) e^{-ik_d \Delta t} \quad (7)$$

Then, Equation 7 was used as function of  $C_s^0$  for  $T_p^{SD,i,cal}$  in Equation 1 and the value of  $C_s^{0,est}$  for each lyophilized training product is determined by minimizing the residual error by the Levenberg-Marquardt algorithm. However, this determination method is an unconstrained optimization and the number of secondary drying temperature data used (*n*) effect the result. The suitable value of *n* must first be determined in order to accurately estimate a realistic value of  $C_s^{0,est}$ .

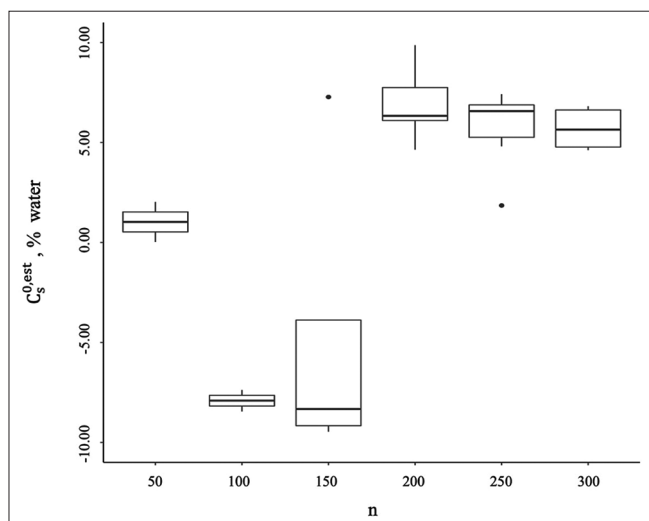
In this study, six different value of *n* were used to test the effect of *n* on the value of  $C_s^{0,est}$ . Then, the suitable value of *n* was selected based on two selection criteria. First, all value of  $C_s^{0,est}$  estimated using suitable value of *n* must not be negative value. Second, the variance of suitable value of *n* should be the lowest. The comparison range of  $C_s^{0,est}$  was defined as  $C_s^0$  is usually less than 10.0 % and average  $C_s^{t,mea}$  of all training lyophilized batches is approximately 3.0 %. Therefore, the data of  $C_s^{0,est}$  between 3.0 and 10.0% are valid for the variance calculation.

The result suggested that a suitable value of *n* should be between 200 and 300 records because the determination method encountered a break-down of negative values if below 200 data records were chosen as shown in Figure 2. In addition, variance of 250 records ( $s^2 = 0.882$ ) is the lower than those of 200 records ( $s^2 = 2.147$ ) and 300 records ( $s^2 = 1.130$ ). Hence, this study selected  $n = 250$  records as a suitable number of secondary drying temperature data for further determination method. Values of  $C_s^{0,est}$  of all training lyophilized batches with  $n = 250$  records are shown in Table 2.

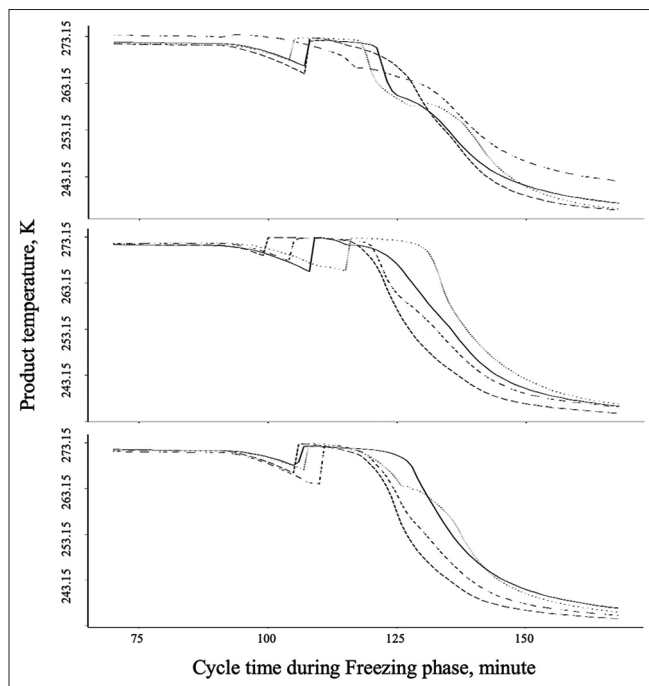
### Determination of $T_N$ of Lyophilized Omeprazole Training Batches

This study was done to determine  $T_N$  by detecting the trough just before the rapid rise in product temperatures when shelf

temperatures were decreased during the freezing phase, as shown in Figure 3. Out of twelve training lyophilized omeprazole products, only eleven products could clearly identify  $T_N$  as the trough in the profile. Even though product at position number four in training lyophilized Batch 01 was completely dried as others, the rapid rise could not be detected. We assume that this error occurred due to probe positioning error which may move off-centered while loading the product into freeze dryer. Therefore, only eleven  $T_N$  were collected to generate the appropriate relationship with  $C_s^{0,est}$ .



**Figure 2:** Optimization of  $n$  to estimate probable value of  $C_s^{0,est}$



**Figure 3:** Change in product temperatures during freezing phase of all lyophilized omeprazole training batches. (a) Training lyophilized Batch 01, (b) training lyophilized Batch 02, (c) training lyophilized Batch 03. Each line represents vial position which solid line is position 1, dash line is position 2, dotted line is position 3 and dotted-dash line is position 4

## Correlation between $T_N$ and $C_s^0$ of Lyophilized Omeprazole Training Batches and Build $C_s^0$ Prediction Model

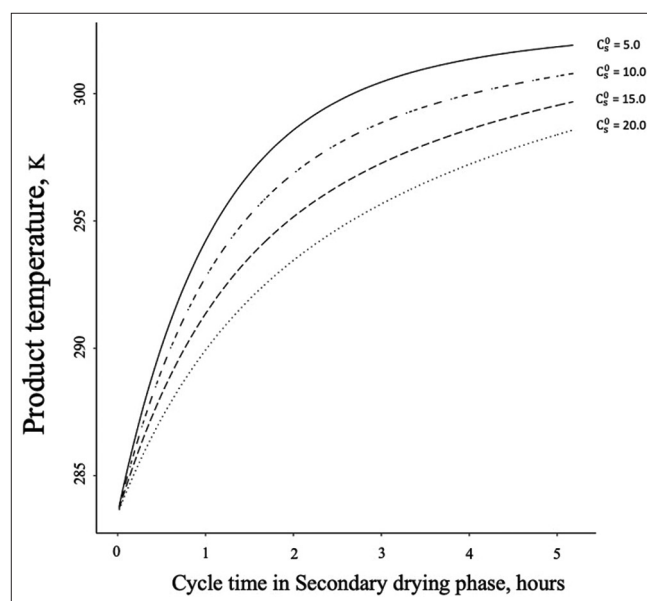
In each training lyophilized batch, before mapping values of  $C_s^{0,est}$  with values of measured  $T_N$ , the outlier data need to be determined and eliminated. Based on Pisano's heat and mass transfer equation (8), the higher value of  $C_s^0$  could result in the slower development of product temperature as more heat energy is used for the water desorption process. In this study, the outlier data were justified based on the equation and its pattern as shown in Figure 4.

Based on this pattern, value of  $C_s^{0,est}$  of product in position two of training batch 03 (21.49 %w/w), which is the highest, was eliminated as outlier because the development of  $T_p^{SD,mea}$  of this position is not the slowest as expected from the pattern. Therefore, we assumed that this  $C_s^{0,est}$  value is an abnormal case initiated from our calculation and should be removed. Then, the relationship between these two parameters was created as shown in Figure 5.

This relationship aligned with previous studies as lower nucleation temperature linearly increases product resistance<sup>[13]</sup> and slows down the drying process<sup>[14]</sup> during the primary drying phase which both lead to a higher value of  $C_s^0$ . Moreover, this linear relationship was proved by using Pearson's product-moment correlation ( $r = -0.659$ ,  $P = 0.0382$ ). Then, a linear relationship between both parameters was derived as Equation 8.

$$C_s^{0,prd} = -1.23T_N + 336.49, \quad r^2 = 0.434 \quad (8)$$

Even though  $r^2$  of Equation 8 is only 0.434, the significance of the overall model was proven using F test ( $P = 0.0381$ ) and the significance of individual terms, both slope, and intercept, were proven using  $t$ -test ( $P = 0.0382$  and  $0.0352$ ). Therefore, there is no lack of fit in this linear prediction model and could be used for  $C_s^0$  prediction.



**Figure 4:** Simulation of calculated product temperature with different values of  $C_s^0$

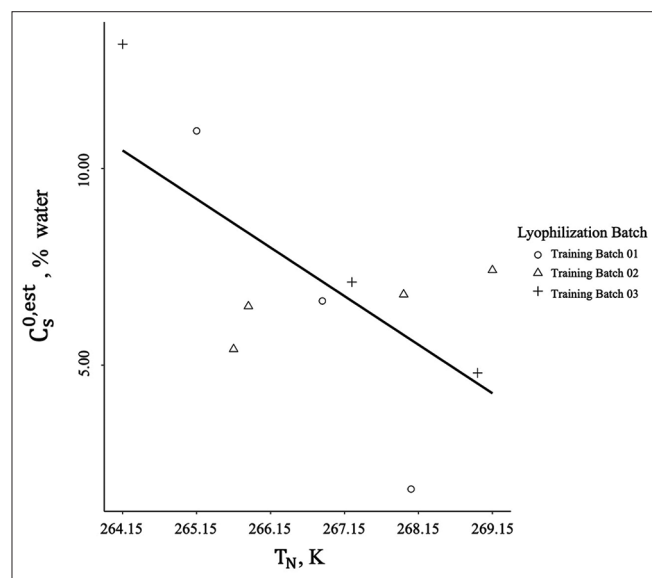
## Validation of $C_s^0$ Prediction Model with Lyophilized Omeprazole Testing Batches

The application of Equation 8 leads to the prediction of  $C_s^{0,prd}$  from any  $T_N$  found and eventually to improve the efficacy in  $C_s^{t,cal}$  calculation. To validate the  $C_s^{t,cal}$  accuracy, the data of lyophilized omeprazole testing batch were applied to compare between  $C_s^{t,cal}$  and  $C_s^{t,mea}$ .

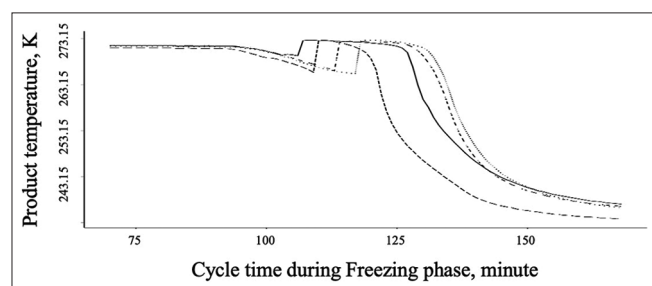
Value of  $T_N$  of lyophilized omeprazole testing batch were determined by the same method as the previous lyophilized omeprazole training batches. However, as shown in Figure 6, the pattern of rapid rise in  $T_p^{Fr}$  of product at position one (solid line) is abnormal as it has two turning points before sharp rise instead

**Table 2:** Estimated values of  $C_s^{0,est}$  of all training lyophilized batches with  $n=250$

Lyophilized batch	Position of vial			
	1	2	3	4
Training Batch 01	6.63	10.96	1.85	12.28
Training Batch 02	5.41	7.42	6.50	6.80
Training Batch 03	4.80	21.49	7.11	13.16



**Figure 5:** Relationship between  $T_N$  and  $C_s^{0,est}$  of training lyophilized batches



**Figure 6:** Change in product temperatures during freezing phase of lyophilized omeprazole testing batch. Each line represents vial positions which solid line is position 1, dash line is position 2, dotted line is position 3 and dotted-dash line is position 4

of one turning point like others. We suggest that the abnormal pattern of  $T_N$  could be resulted by too early primary nucleation in product which affected ice crystal structure and resulted in much higher value of  $T_N$  (269.45 K vs. 266.5 K, 265.85 K, 266.15 K) and could interfere validation result. Therefore, this study excluded data from vial position 1 in the calculation. Thus,  $T_N$  of 3 remaining vials were used and is shown in Table 3.

$C_s^{0,prd}$  could be predicted by using Equation 8 and the prediction results are shown in Table 3. Then,  $C_s^{t,cal}$  was calculated by the mathematical Equation 3.<sup>[15]</sup>

Constant  $k_d^{est}$  is estimated and obtained from previous training lyophilized products used in  $C_s^{0,est}$  determination approach. Therefore, for each testing vial,  $C_s^{0,prd}$  is predicted from  $T_N$  and  $k_d^{est}$  were values obtained from ten training lyophilized products. Resulting in ten  $C_s^{t,cal}$  per each  $C_s^{0,prd}$ . Then, the comparison between mean of  $C_s^{t,cal}$  and  $C_s^{t,mea}$  was performed by Welch's t-test and the result was shown in Table 3.

The mean of  $C_s^{t,cal}$  and  $C_s^{t,mea}$  of all testing product were not significantly different. Therefore, the accuracy in predicting  $C_s^{0,prd}$  with  $T_N$  was proven to be valid.

## Prediction Model of $t^{SD}$ for Each Lyophilized Omeprazole Batch

As  $C_s^{0,prd}$  predicting equation and  $C_s^{t,cal}$  calculating equation were validated in the previous section,  $t^{SD}$  predicting equation could be obtained by combining both equation 3 and equation 8 as shown in Equation 9.

$$t^{SD,prd} = \frac{(\ln C_s^{t,tar} - \ln(-1.23T_N + 336.49))}{-k_d * 60} \quad (9)$$

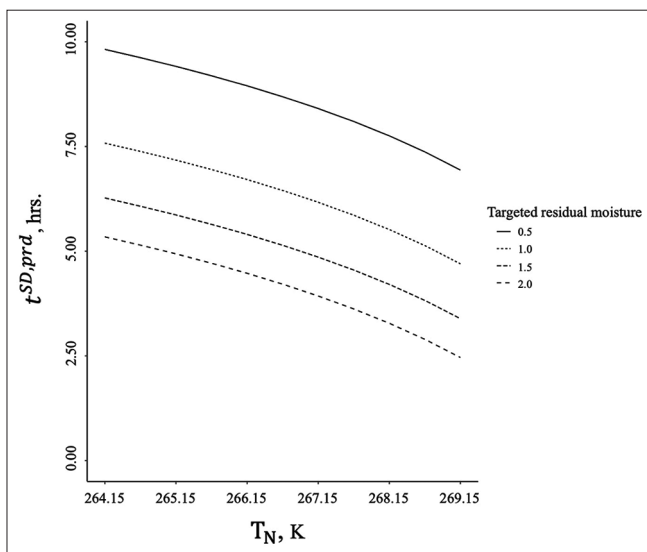
Where  $t^{SD,prd}$  is predicted secondary drying time.  $C_s^{t,tar}$  is targeted residual moisture. Then, the optimal  $t^{SD}$  for each lyophilized batch could be predicted by defining targeted residual moisture and measuring ice nucleation temperature as shown in Figure 7.

In Figure 7, optimal  $t^{SD}$  of the same  $C_s^{t,tar}$  for each lyophilized batch could be different due to ice nucleation temperature of each batch. For example, in case of  $C_s^{t,tar} = 2.0\%$ ,  $t^{SD}$  can be varied from 2.5 h for product with  $T_N = 269.15$  to 5.0 h for product with  $T_N = 264.15$  K. Therefore, the result in this study highlights the importance of finding optimal  $t^{SD}$  specifically for each lyophilized omeprazole batch.

This study has limitations due to limited access to resource and facility. As research materials, equipment, and supporting facilities were provided by sponsor and lyophilization process is time-consuming technique, the number of experimental batches is depended on the available of equipment and time on sponsor side. Therefore, only 4 lyophilized batches could be done in this study which resulted in 3 training lyophilized batches and 1 testing lyophilized batches. The result in this study shows the potential to use  $T_N$  as an in-line lyophilization process prediction method to find optimal  $t^{SD}$  specifically for each lyophilized batch. However, it remains true that our experimental batches are too small to propose the validated model for  $t^{SD}$  prediction of the lyophilization

**Table 3:** Comparisons between  $C_s^{t, cal}$  and  $C_s^{t, mea}$  of lyophilized omeprazole testing batch

Position of Vial	$T_N$ , K	$C_s^{O,prd}$	Residual Moisture, % of water $\pm$ SD		
			$C_s^{t, cal}$	$C_s^{t, mea}$	P-value
2	265.85	8.36	2.26 $\pm$ 0.72	2.07 $\pm$ 0.06	0.427
3	265.55	8.73	2.36 $\pm$ 0.75	2.07 $\pm$ 0.21	0.298
4	266.15	7.99	2.15 $\pm$ 0.69	2.20 $\pm$ 0.10	0.851

**Figure 7:** Optimal  $t^{SD}$  to obtain  $C_s^{t, tar}$  for each lyophilized batch based on  $T_N$ 

process at the industrial scale. Future study with more experimental batches is required before this approach can be effectively applied.

## CONCLUSION

Previously, optimized secondary drying time may be calculated by the mathematical model from the value of moisture level at the beginning of secondary drying obtained by lab-scale batches. However, the product's ice crystal structures formed during the freezing phase are shown to affect values of moisture level at the beginning of secondary drying and the accuracy of the mathematical model developed. As a result, the residual moisture of the final lyophilized product with processing conditions resulting from such mathematical model can deviate from the targeted moisture level.

Therefore, we extend the application of the mathematical model and proposed an approach to first predict secondary drying time using the ice nucleation temperature measured from each lyophilized batch. The comparison of calculated residual moisture and measured residual moisture of the product yielded acceptable outcome. Nevertheless, further study is required before this approach can be effectively applied as an in-line monitoring method in lyophilization product manufacturing.

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