2019-01-08

Master's thesis project in computer modelling and simulation of freeze drying processes at Novo Nordisk

Introduction and purpose

Lyophilisation, or freeze drying, removes water by sublimation at low temperature and pressure and is widely used in the biopharmaceutical industry to stabilise and maintain the biological activity of active pharmaceutical protein or peptide molecules.

The freeze drying process contains three steps including freezing, followed by primary and secondary drying as shown in Figure 3. To better understand the freezing step and the behaviour of the aqueous formulation containing the active ingredients, a mathematical model – as a simplified representation of the freezing process – would be highly valuable.

The aim of this project is to develop a deterministic model of the super cooling and freezing behaviour of the aqueous formulation at different cooling rates based on temperature measurement data obtained in a full scale freeze drier system at Novo Nordisk.

Freeze drying of a biopharmaceutical product

General background

Biopharmaceutical products are freeze dried in vials filled with an aqueous formulation of the active proteins and excipients that protect the proteins during the freeze drying process. An example is shown in Figure 1.

Figure 1. Vials with content before freezing.

In the present case we are working with a biopharmaceutical product containing the active protein in a formulation of different salts and sugars, including sucrose, which protects the protein.

The vials are placed on shelves in a freeze drying chamber with regulated shelf temperature, where the solution is cooled from the bottom of the vial via cooling coils in the shelf, and the water in the vials is frozen, as shown in Figure 2.

Figure 2. Vials with content after freezing.

The ice formed in the vials is sublimated at low pressure in the subsequent primary drying step. When the ice has evaporated the process continues to a secondary dying step at higher temperatures where the residual moisture is removed from the product to obtain a final water content of typically below 2%. The steps in a typical freeze drying process are shown in Figure 3.

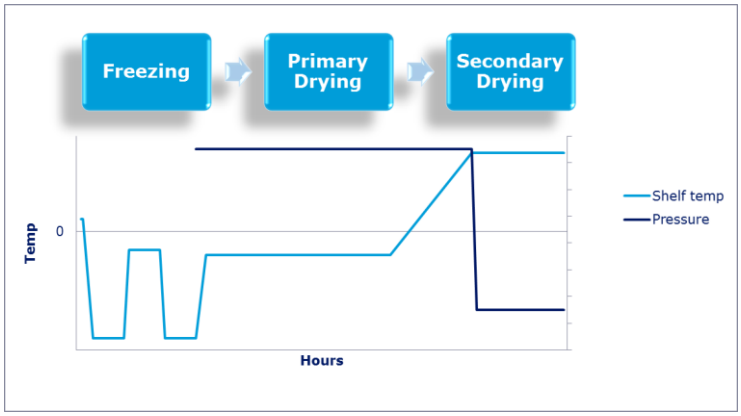
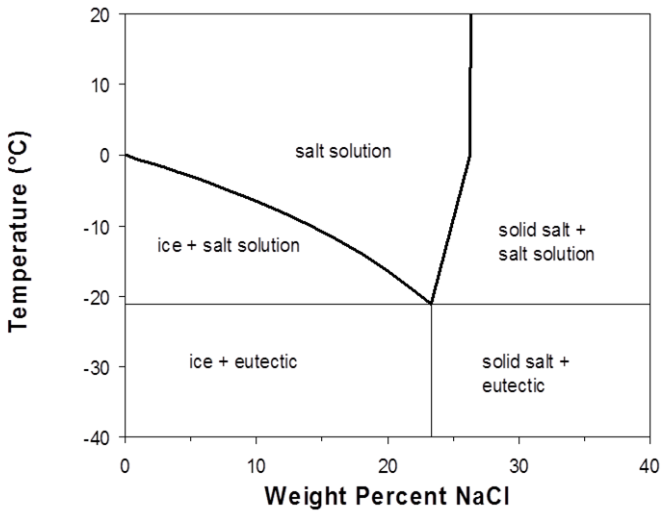


Figure 3. Freeze drying process including freezing, annealing (thermal treatment), primary drying and secondary drying.

The freezing step of the process

The freezing step defines the microstructure of the solidified solution and therefore also that of the finished freeze dried product and is very important to the quality of the finished freeze dried product.

In the freezing step of the process the aqueous solution is cooled from typically +5°C to below -45°C. At the freezing point, water crystalizes into ice and the system separates into different phases. This introduces a pronounced stress to the active molecules due to the dehydration effect of ice formation that removes bulk water from the protein phase. The formation of ice also results in an enormous increase in the salt concentrations. The unfrozen water in the concentrated salt solution freezes when the solution is cooled below the eutectic temperature of the specific salt (for example, for sodium chloride the eutectic temperature is -21°C). An example of a phase diagram for sodium chloride is shown in Figure 4.



Felix Franks. Freeze-drying of Pharmaceutical and Biopharmaceuticals. The Royal Socity of Chemistry 2007.

Figure 4. Freezing behaviour: temperature composition phase diagram for sodium chloride and water.

The formulation, including excipients, and design of the freezing process therefore play very important roles in protein stabilization. The freezing and formation of ice also defines the microstructure and hence the drying properties of the product.

As a rule of thumb, a fast cooling rate results in a high-degree of super cooling which means that ice is formed at low temperatures. A high degree of super cooling leads to small ice crystals and a compact ice structure that can be difficult to freeze dry. On the other hand, a slow cooling rate results in a low degree of super cooling, large ice crystals and a less compact ice structure.

Cooling and heating curves for a 5 % sodium chloride solution in a vial are shown in Figure 5 and Figure 6, respectively. In Figure 5, the solution is cooled below 0°C with no change in state. The chilling of the solution continues until a sudden phase change is observed as ice is formed – in this case at about -16°C (the super cooling temperature). The crystallization of pure water into ice is an exothermic process and the heat released is warming the vial to almost 0°C. Sodium chloride is excluded from the solution and concentrated. The freeze concentrated sodium chloride solution remains unfrozen at this point. After the water has crystalized into ice, cooling continues. It is observed that the temperature in the vial drops as the shelf is cooled further down. Another exothermic event is observed in the form of heat release at -30°C. At this point water in the freeze concentrated sodium chloride solution crystallizes. After this, cooling of the crystalline substance continues and the temperature is approaching the final set-point of -50°C for the shelf temperature.

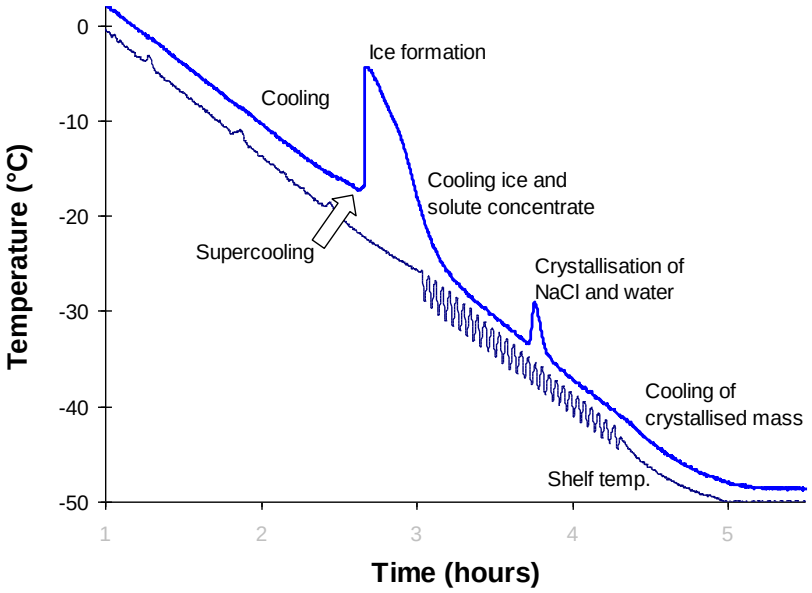


Figure 5. Product temperature against time during freezing of 5% NaCl (crystalline solute).

The heating of the same vial is illustrated in Figure 6. An endothermic event is observed at -21° as the sodium chloride ice substance is melting. The heat is used to melt the concentrated sodium chloride and the temperature therefore remains constant until the crystals have melted.

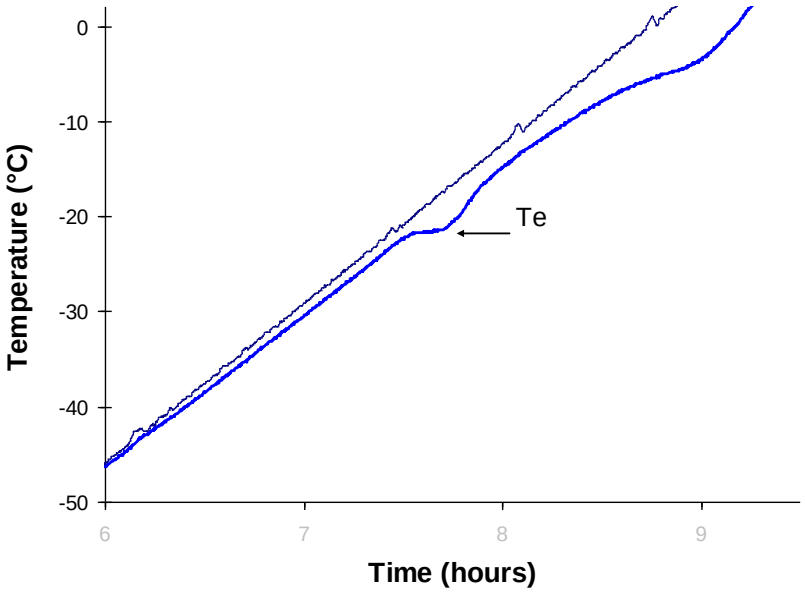


Figure 6. Product temperature against time during thawing of 5% NaCl.

Mathematical model of the freezing process

To better understand the freezing behaviour of the formulation, a mathematical model as a simplified representation of the freezing process, summarising available process knowledge, is desirable. In this project, the aim is to establish a deterministic model,

that is, a completely mechanistic model, derived from first principles like mass and energy balances.

The deterministic model should describe the super cooling and freezing behaviour of the formulation at different cooling rates based on temperature measurement data obtained in a full scale freeze drier system.

Basic definitions

Cooling: Refers to the reduction of temperature.
Freezing: Refers to the phase transformation where liquid water in a solution is converted into solid ice.
Freezing point: Note that freezing at thermodynamic equilibrium does not necessarily occur at the nucleation temperature.
Nucleation: Clustering of water molecules to form nuclei, which act as seeds for ice crystal growth.
Super cooling: Liquids cooled below the freezing/melting temperature but above the homogeneous nucleation temperature (lowest possible pure substance freezing temperature) are super cooled. Normally, freezing due to super cooling occurs at -15 to -25°C before ice is formed for sterile filtered DP solutions (pure solutions with few particles).
Eutectic temperature, Te: The lowest temperature in which the residual liquid phase and solid phase (crystalline) are in equilibrium.
Glass transition temperature, Tg´ (cold area): Temperature at which maximum concentration of amorphous component can be obtained during freezing. This implies a reversible transition from a rubber-like state to a hard and relatively brittle glass state. The glass transition is not in itself a phase transition, but relates to the mobility of the component.

Suggested reading

Start to read the PhD thesis from DTU and the papers with high citation numbers in the literature reference list below.

[1] Teresa Melo de Carvalho. *Consistent scale-up of the freeze drying process.* PhD thesis, Department of Chemical and Biochemical Engineering, Technical University of Denmark. October 2018.

Freeze drying introduction

[2] X. C. Tang and M. J. Pikal. Design of Freeze-Drying Processes for Pharmaceuticals: Practical Advice. *Pharm. Res.* 21: 191–200 (2004).
Times cited: 378

Freezing

[3] R Esfandiary et al. Effect of Freezing on Lyophilization Process Performance and Drug Product Cake Appearance. *Journal of Pharmaceutical Sciences* 105: 1427-1433 (2016).
Times cited: 3 (from industry)
[4] J. C. Kasper and W Friess. The freezing step in lyophilization: Physico-chemical fundamentals, freezing methods and consequences on process performance and quality attributes of biopharmaceuticals. *European Journal of Pharmaceutics and Biopharmaceutics* 78: 248–263 (2011).
Times cited 160 (University of Munich)

Modelling review

[5] W Chen et al. Application of Mechanistic Models for Process Design and Development of Biologic Drug Products. *J Pharm Innov.* 11:200–213 (2016).
Times cited: 0 (from industry)

Specific for freezing and freeze drying processes

[6] T Zue et al. Predictive models of lyophilization process for development, scale-up/tech transfer and manufacturing. *European Journal of Pharmaceutics and Biopharmaceutics* 128: 363–378 (2018).
Times cited: 0 (Purdue Univ and AbbVie)

[7] L. C. Capozzi and R. Pisano. Looking inside the 'black box': Freezing engineering to ensure the quality of freeze-dried biopharmaceuticals. *European Journal of Pharmaceutics and Biopharmaceutics* 129: 58–65 (2018).
Times cited: 0 (University of Turin, Itally)

[8] K. Nakagawa et al. Modeling of freezing step during freeze-drying of drugs in vials. *AIChE Journal,* Vol. 53, No. 5, May 2007.
Times cited: 50 (University of Lyon and Hyogo in Japan)

[9] C R Muzzio and NG Dini. Simulation of freezing step in vial lyophilization using finite element method. *Computers and Chemical Engineering* 35: 2274– 2283 (2011).
Times sited: 4 (University of Buenos Aires)

[10] Hottot et al. Freeze-Drying of Pharmaceutical Proteins in Vials: Modeling of Freezing and Sublimation Steps. *Drying Technology* 24: 561–570 (2006).
Times cited 41 (University of Lyon)