Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Characterizing ice nucleation sites in pharmaceutical freezing

Topic:	**Experimental evaluation and comparison of ice nucleation behavior during freezing**
Type:	Semester Project / Research Assistantship
Starting date:	Anytime, preferably from July 2022
Advisor:	Prof. Dr. Marco Mazzotti, ML G27, mazzotti@ipe.mavt.ethz.ch
Supervisor:	Leif-Thore Deck, ML G16, deckl@ipe.mavt.ethz.ch

Freezing and freeze-drying are widely used to improve the stability of biopharmaceutical drug products such as vaccines [1]. At production scale, tens of thousands of vials containing the drug product are densely packed together. For such system, properties of the frozen products such as the time and temperature of nucleation inherently vary among vials [2,3,4]. This variability presents a major challenge for process design and optimization: the stability, and thus shelf life, of the drug products strongly depend on the nucleation behavior [5].

A rational freezing process design should therefore ensure that all vials freeze in a way that not only preserves their activity in general, but also minimizes variability among vials [6]. Since ice nucleation, i.e., the first step of freezing, is a stochastic process, this variability cannot be mitigated entirely [2]. This has motivated the development of technical solutions to control the nucleation process, e.g., by inducing nucleation with artificial means.

These *controlled* nucleation methods include seeding with ice fog, application of ultrasound, as well as fast cooling via evaporation; they have in common that they add significant complexity to the process design, limiting their application mainly to academic settings. The need to control nucleation in industrial settings still has to be addressed [7].

To do so, we have to study the mechanism of nucleation during freezing. Nucleation is generally assumed to occur heterogeneously, i.e. on a surface; such surface may either be provided by the container or by impurities within the drug product [2,4,7]. Under sterile manufacturing conditions in industry where only miniscule amounts of impurities are present, the vial surface is considered to play a major role in nucleation [7].

This work aims to study the effect of vial surface and impurity content on nucleation; specifically, we will study to which extent different vial materials and coatings effect the nucleation behavior. Furthermore, we will quantify the differences in nucleation behavior among standard laboratory conditions and sterile conditions.

Scope of the Project:

This project aims at deepening the understanding of ice nucleation in complex aqueous systems during freezing. To do so, the student will carry out a comprehensive experimental campaign using the methodology recently developed in our lab. The campaign will involve various vial types made of different materials and coatings, as well as different environmental conditions (sterile conditions, standard lab environment). The experimental findings will be analyzed in detail in order to identify promising vial specifications, and potentially to develop novel vial coatings with superior nucleation properties.

Proposed Outline of the Main Project

- Short literature review on the research topic and existing works in this field
- Familiarization with the setup available in the lab as well as with the methodology for nucleation kinetics measurements
- Carrying out an experimental campaign in the setup
- Analyzing the outcome of the experimental campaign and developing best-practice guidelines regarding the choice of vial material in the context of freezing

Requirements

- Solid background in engineering subjects
- Creative, highly motivated and independent attitude
- Frustration tolerance (Experiments are always challenging… ☺)

Deliverables

Short reports of what has been done during the week should be submitted to the supervisors every Friday. The work carried out and the results obtained will be presented in two oral presentations, one about halfway through the project and one at the end.

References

[1] J Wang, Y Peng, H Xu, Z Cui and RO Williams: The COVID-19 Vaccine Race: Challenges and Opportunities in Vaccine Formulation. *AAPS PharmSciTech 21 (2020) 225.* https://doi.org/10.1208/s12249-020-01744-7

[2] LT Deck, DR Ochsenbein and M Mazzotti: Stochastic Shelf-Scale Modeling Framework for the Freezing Stage in Freeze-Drying Processes, *Int. J. Pharm.* (2021) in press: https://doi.org/10.1016/j.ijpharm.2021.121276

[3] LT Deck, DR Ochsenbein and M Mazzotti: SNOW – Stochastic Nucleation of Water, (2021), GitHub Repository, https://github.com/SPLIfA/snow/

[4] GM Maggioni, L Bosetti, E dos Santos and M Mazzotti: Statistical analysis of series of detection time measurements for the estimation of nucleation rates, *Cryst. Growth Des.* (2017), 17, 5488−5498, https://doi.org/10.1021/acs.cgd.7b01014

[5] LC Capozzi, R Pisano: Looking inside the 'black box': Freezing engineering to ensure the quality of freeze-dried biopharmaceuticals. *European Journal of Pharmaceutics and Biopharmaceutics 129 (2018) 58–65.* https://doi.org/10.1016/j.ejpb.2018.05.020

[6] S Ehlers, R Schroeder and W Friess: Trouble with the Neighbor during Freeze-Drying: Rivalry About Energy (2021), *J. Pharm. Sci. 110, pp. 1219-1226,* https://doi.org/10.1016/j.xphs.2020.10.024

[7] G Assegehegn, E de la Fuente, J Franco and C Gallegos: The Importance of Understanding the Freezing Step and Its Impact on Freeze-Drying Process Performance (2019), *J. Pharm. Sci. 108, pp. 1378-1395,* https://doi.org/10.1016/j.xphs.2018.11.039