Neutrons and acoustic levitation offer clues into freeze drying processes

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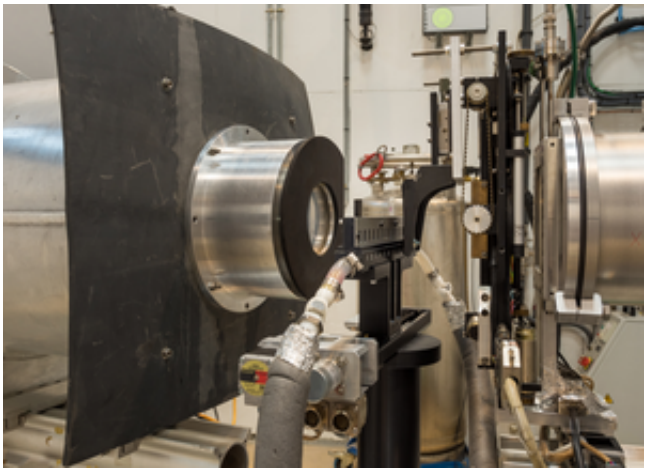

D16. Credit: Ecliptique

The drying process is a critical final stage in various manufacturing processes – it influences the quality of many a product and has many industrial applications, particularly in the food and pharmaceutical sectors. Freeze drying (lyophilization) is a drying method where the solvent is frozen prior to drying and is then sublimed. In addition to providing an extended shelf-life, successful freeze-drying should yield a product that has a short reconstitution time with acceptable potency levels. The process should be reproducible with well defined temperature, pH and time parameters for each step. Visual and functional characteristics of the dried product are also important for many applications.

In pharmaceuticals, freeze drying is commonly used to preserve the integrity and bioactivity of protein drugs, minimising chemical and physical degradation during their shelf life. Unfortunately for such an integral process, storage in freeze-dried states does not guarantee long-term stability, and aggregation is often observed after thawing or reconstitution of freeze-dried powder samples. A particular issue is the formation of ice which is known to have a destabilising effect on protein molecules during freezing. Therefore an essential step in optimising the conservation and stabilisation of samples is to understand the biophysical mechanisms involved during the freeze-drying process – a knowledge gap that a recent a Institut Laue Langevin (ILL) study addressed. This work was a collaboration between ILL, Soleil, CEMHTI laboratory in Orleans and the University of Palermo.

The study focused on the structural evolution of protein solutions up to supersaturation conditions, created by partial evaporation of the solvent, to mimic drying process conditions. This enabled structural analysis to be obtained at a series of time intervals at different drying conditions of the sample, therefore enabling data to be collected during the process itself and mimicking realistic lab conditions. Container interactions and contamination of the sample can affect greatly the freeze-dried process and thereby, the quality of the final product – there is no universal 'safe' choice of container. Therefore, in order to ensure optimum understanding of the freeze-drying process, container-free or 'contactless' techniques must be used. In this study, acoustic levitation was the chosen technique – solid and liquid samples positioned in the surrounding medium (ambient air or defined gas) by means of a stationary ultrasonic field creating a pressure gradient in the medium.

A single droplet is held in a node of a standing acoustic wave, avoiding any contact with a thermally-conducting holding-device. The droplet size can be controlled in a wide range up to 5 mm in diameter. The surrounding drying-gas can be conditioned such that its temperature,

relative humidity, and flow rate past the droplet are accurately controlled. Evaporation of the solvent during levitation gradually decreases the volume of the droplet and therefore increases the corresponding concentration of the solute. Thus, formation of aggregates and processes of crystallisation could be followed in situ in order to identify suitable crystallising conditions.

This paper shows the capabilities of the levitation technique once integrated into small angle neutron scattering (SANS) and x-ray scattering (SAXS) beamlines. Despite the small quantities of sample that it is possible to suspend (just few tens of nanoliters in volume), the high intensity of neutron instruments make possible in-situ monitoring of fast containerless reactions, providing detailed molecular and structural information.

Changes in the SANS and SAXS signal intensities provide information on particle distances and morphology of proteins, possible formation of larger domains, as clusters or heterogeneities, directly linked to the drying condtions. It was observed that, considering a lysozyme dissolved in D2O solution at low concentration, the center-to-center distances between proteins become smaller due to the evaporation inducing an increase in concentration. For high concentration solutions, the distance between molecules changes little during evaporation.

The acoustic levitation device shows its potential when used in combination with synchrotron radiation circular dichroism. Preliminary results on myoglobin in aqueous solution allow to follow up the evolution of the secondary structure of the protein as a function of concentration, revealing an increase of α-helices content and the full loss of parallel β-sheets. The results prove that the acoustic levitator can be used as a tool for structure analysis and that it easily permits the contactless study of many kinds of samples.

Neutrons are the ideal tool for this type of experiment due to their non-destructive properties so data can be collected at room temperature, closer to physiological temperatures and resulting in the determination of 'damage-free' structures – essential for studying biological molecules. SAXS experiments were carried out at the SOLEIL synchrotron's SWING beamline while SANS was conducted on the ILL's D16 and D33 instruments.

This study allows us to validate levitation methodology for investigating bio-based materials during the drying process. To our knowledge, this SANS investigation combined with acoustic levitation is the first study of its kind.

Using these advanced analytical methods for characterisation of various pharmaceuticals including small molecules and proteins, drug substances and products, we open up the way for novel insights into aggregation and crystallisation phenomena. A better understanding of the biochemical mechanisms of these classes of bio-materials is necessary to improve the long-term stability of pharmaceutical formulations. These studies could underpin improvements in industrial processes such as spray-drying and spray-freeze-drying and the process development of pharmaceuticals and biopharmaceuticals.

The behaviour of protein solutions at low temperature and the role of cryo- and lyo- protectant agents added to a protein solution using neutron (SANS) techniques will be the main topic of future work in this area.

More information: Viviana Cristiglio et al. Combination of acoustic levitation with small angle scattering techniques and synchrotron radiation circular dichroism. Application to the study of protein solutions, *Biochimica et Biophysica Acta (BBA) - General Subjects* (2016). DOI: 10.1016/j.bbagen.2016.04.026

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