INDUSTRIAL PILOT PROJECT

Role of water on protein thermal stability in dry protein formulations: probing the structural transition using SAXS/WAXS

THE INDUSTRIAL CHALLENGE

The stability of biologically produced pharmaceuticals is a limiting factor for commercialization. This can be improved by production in solid state, mostly via lyophilization. Solid formulations involve stages where they are exposed to low levels of hydration. The presence and behavior of residual water after drying are however not well understood and yet they most likely affect the physical and chemical stability of the biological drug. Understanding the mechanisms of destabilization of proteins in the dry/semi dry state is essential to the development of more stable and effective drugs.

WHY USING A LARGE SCALE FACILITY

The task requires synchrotron instrumentation for several reasons. Firstly, the scattering of proteins in a solid or semisolid matrix of excipients is characterised by low X-ray contrast, thus high flux is required to achieve good statistics. Secondly, the sample environment required for the experiment, the Linkam heating stage, is not widely available outside synchrotron facilities. Finally, the high flux of the synchrotron source allowed to investigate these systems at realistic heating rates, which would not be possible on low-flux instruments.

HOW THE WORK WAS DONE

Small and Wide-Angle X-ray scattering (SAXS/WAXS) was used to investigate the mechanisms of thermal destabilization of both a therapeutic protein (palifermin) and a model protein (lysozyme) in the dry/semi dry state. Measurements were conducted at different hydration levels for different heating conditions up to protein denaturation temperature. Testing were performed both at the P12 beamline of Petra III in Hamburg and at the NCD-SWEET beamline of the ALBA synchrotron in Barcelona.The team consisted of different partners from industry (Swedish Orphan Biovitrum) and public sector (Malmö University, MAX IV and RISE), providing expertise in scattering and diffraction ,thermodynamics and dynamics of solid state formulation and freeze drying.

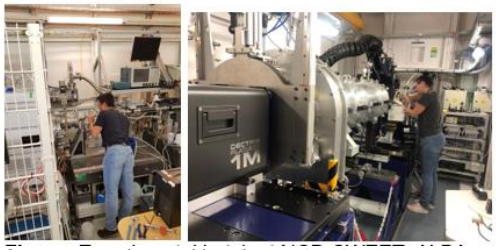

Figure. Experimental hutch at NCD-SWEET, ALBA (left) and P12, Petra III (right)

THE RESULTS AND EXPECTED IMPACT

The findings indicate that protein molecules adopt structures that can continuously fill the space to remove the destabilizing protein-air interface that may be formed upon dehydration. In the presence of excipients, the native structure of protein is preserved and thus the structure adopted at low water content is absent. This change in structure could be linked to the stability of protein upon rehydration.

The results enable us to explore the relationship between protein structure, excipients, and moisture in relation to the protein's thermal stability. Thus, understanding the mechanism of protein stabilisation under these conditions is essential to the development of more stable drugs.

"With the innovative use of SAXS/WAXS we can now better predict protein stability in lyophilized formulations"
/Jonas Fransson, Swedish Orphan Biovitrum

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