mAb formulation development and stability studies through complementary and orthogonal analytical methods

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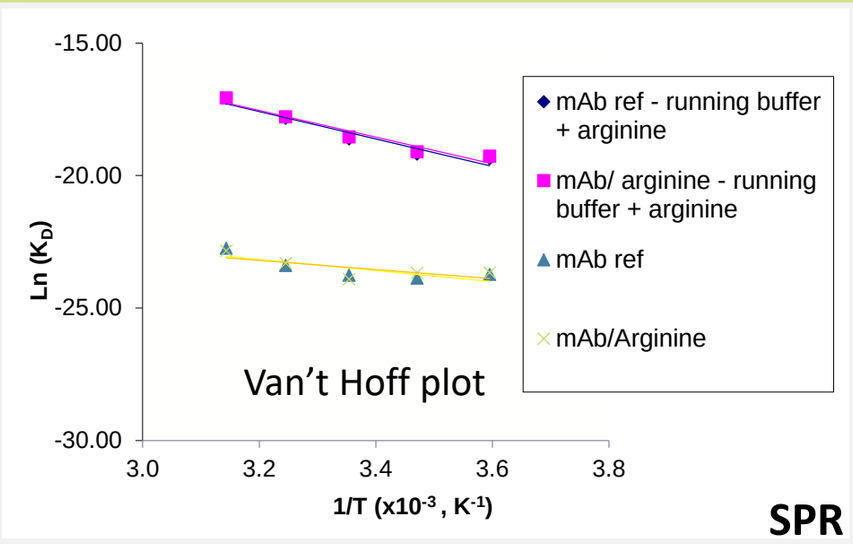
1 CONTEXT & OBJECTIVES

Biologics such as monoclonal antibodies (mAbs) are much more complex than small-molecule drugs, which raise challenging questions for their development. In order to develop a specific ELISA with homemade reagents, monoclonal antibodies (C003M) were produced by immunization of rabbits, sera were purified and mAbs were characterized. The study's aim was the development of a liquid formulation with selected excipients to maximize the stability and keep mAbs properties (specificity and affinity).
Two excipients, **Arginine** and **Trehalose**, were used in formulation development as protein stabilizing agents. To study the formulation impact of these additives, several stability studies were performed with orthogonal analytical methods such as:

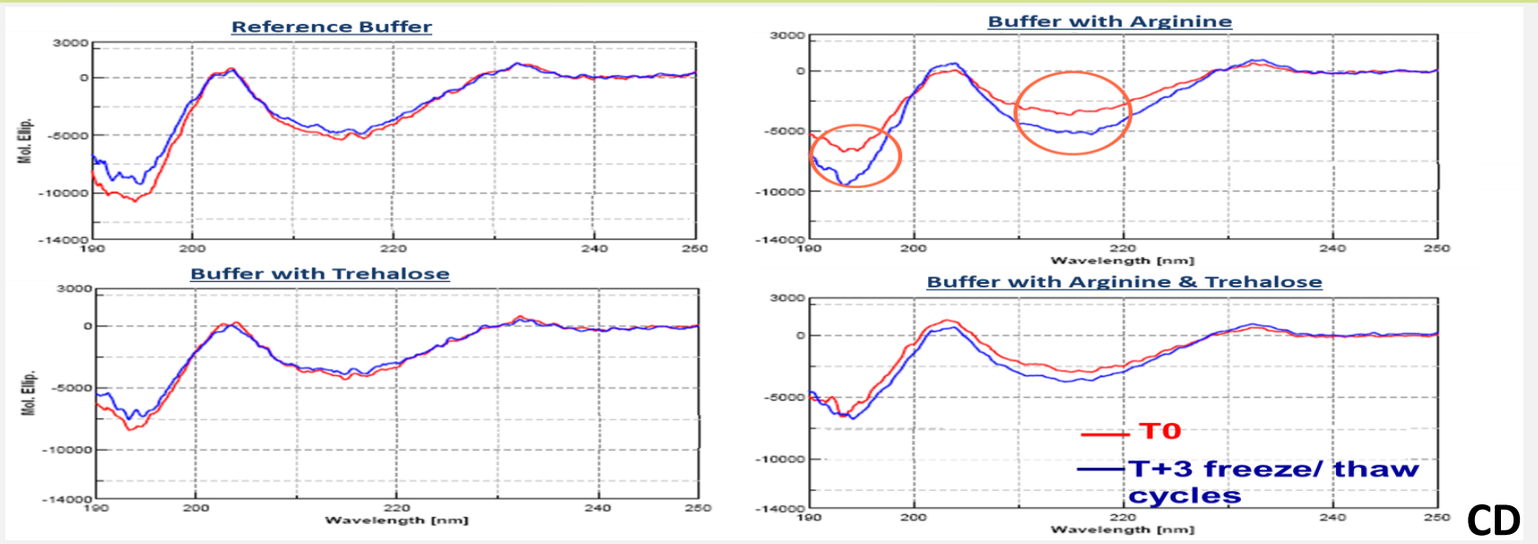
-Circular Dichroism (CD) as absorption spectroscopy technique for the characterization of biomolecules and the analysis of chiral substances.
-Differential Scanning Calorimetry (DSC) as thermal analysis used to measure thermodynamics of solid or liquid phase transitions that produce or absorb heat.
-Size Exclusion Chromatography coupled to Multi Angle Light Scattering (SEC-MALS) as absolute biophysical characterization (molecular weight, size, conformation, degree of conjugation, aggregation and complex-forming interactions)
-Dynamic Light Scattering (DLS) as non-invasive technique for measuring particle size of sub-micron particles, even lower than 1 nm.
-Surface Plasmon Resonance (SPR) as platform technology providing high-quality molecular interaction data to guide any biotherapeutic development program.

2 RESULTS



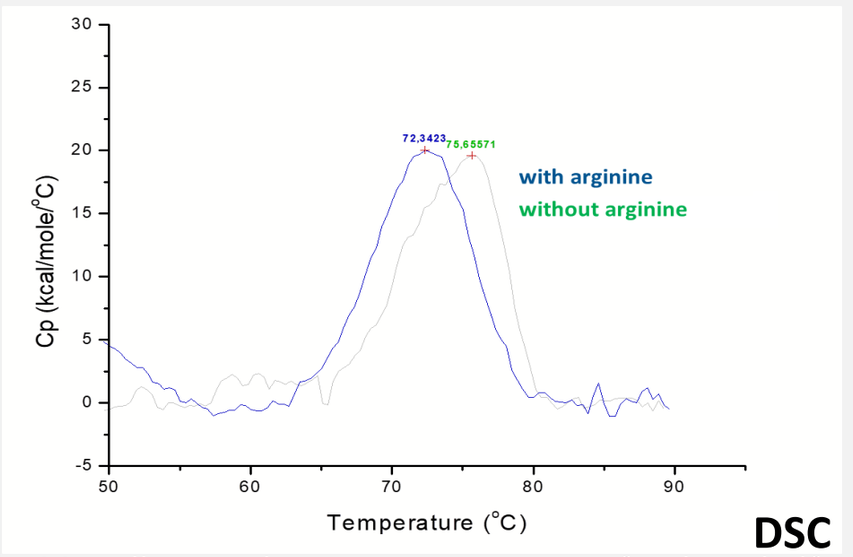
SPR

To study the excipients impact on affinity constant (K_D) between specific Ag (protein Y) and mAb, affinity is analysed with **SPR** (Biacore T200)[1]. In the graph above, kinetic and thermodynamic values obtained for these two proteins confirm their mutual similarity.



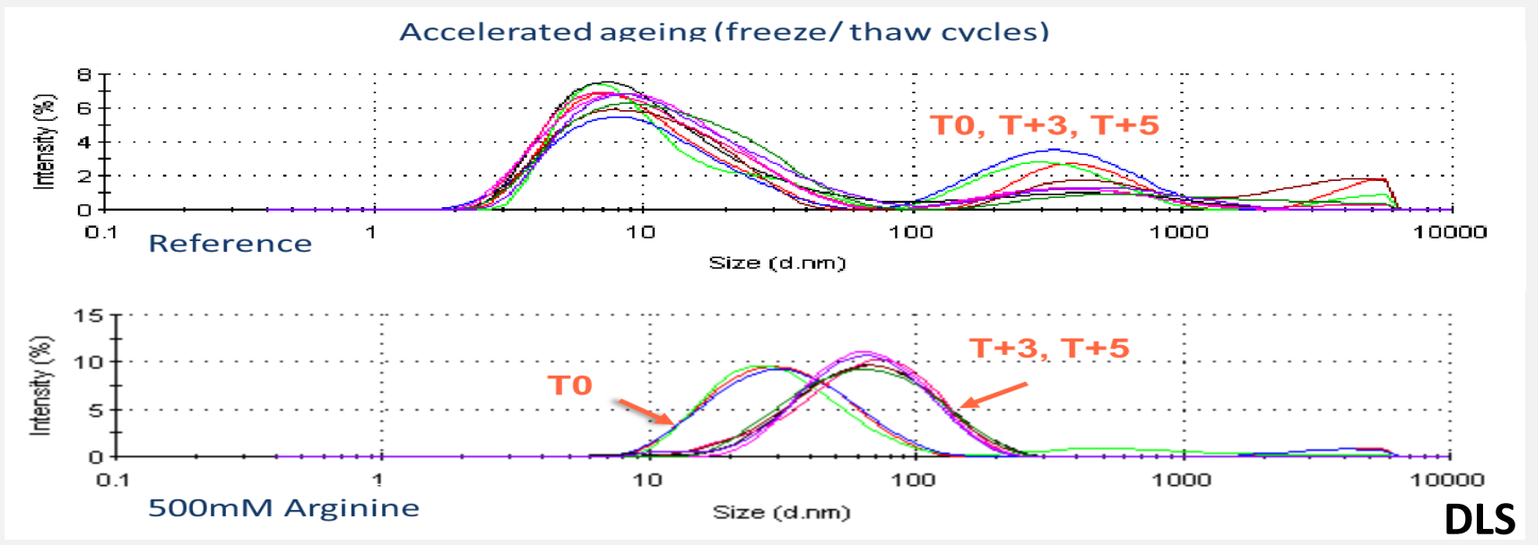
CD

Circular dichroism (CD) is used to observe the effect of excipients on the protein's structure relative to the reference buffer. Impact of stabilizing agents (Arginine[2] and/or Trehalose[3]) was evaluated on the secondary structure of the protein (Far-UV CD region: 250-190 nm) according to freeze/thaw cycles effects. Presence of Trehalose, cryoprotectant in formulation, allows to offset the effect of Arginine on protein structure after 3 freeze/thaw cycles.



DSC

DSC allows the measurement of the half-denaturation T°C (50% of the protein in native form and 50% in unfolded form). This determination of the half-denaturation T°C is able to show the impact of the stabilizing agent (Arginine) added to the formulation.



DLS

Dynamic light scattering (DLS) is a technique where the Brownian motion of particles or molecules in suspension causes laser light to be scattered at different intensities. Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship. The polydispersity of a solution can be assessed. On the top graphs, the effect of the stabilizing agents added to the formulation after accelerated ageing cycles was assessed. A significant decrease of polydispersity in solution was observed with Arginine in formulation, even after repeated freeze-thaw cycles.

3 CONCLUSION

The use of **physico-chemical analytical methods** allows to **develop a liquid formulation of mAbs**. Trehalose, owing to its protein stabilizing quality and Arginine, an amino acid used to suppress protein aggregation were added on buffer to **prevent instabilities**[4].
Some accelerated ageing (freeze/thaw cycles) was monitored with **CD** and **DLS** to study **excipient impact** on **protein conformation** and on **polydispersity** (aggregates). Thermodynamic and affinity measures (**SPR** and **DSC**) allowed to complete this study by showing that use of these excipients do not modify mAb specificities.
Then, these two excipients, Trehalose and Arginine, were selected to stabilize mAbs for **extended shelf-life.** This developed liquid formulation is compatible with the use of this mAb like specific ELISA's reagent.

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