PREDICTING THE STABILITY OF BIOTHERAPEUTICS IN FORMULATION

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Biotherapeutics, especially monoclonal antibodies (mAbs), is one of the fastest growing groups of pharmaceuticals, corresponding to a treatment option to life-threatening conditions, such as cancer, autoimmune diseases and cardiovascular diseases. A key barrier in the production of these pharmaceuticals is the formation of protein aggregates, which can lead to increased production costs, loss of the biological function, and immunogenic responses from patients. To detect the presence of aggregates and ensure protein stability upon storage, efficient formulation screening methods are required.

Self-Interaction Chromatography (SIC) has previously been shown to be an accurate, automated and high-throughput technique to predict protein aggregation [1]. This study is adapting SIC into Interaction Chromatography (IC), which takes the interactions between two different proteins or species into account. The aim of the first case study was to analyse different formulations containing histidine buffer, phosphate-citrate buffer and sodium citrate buffer at 25 mM, and using a therapeutic mAb. Additionally, the stabilising effects of NaCl, polysorbate and L-arginine were examined in the range of 0-250 mM, 0-0.1% v/v and 0-200 mM, respectively. The protein-protein interactions for each formulation condition were quantified by calculating the osmotic second virial coefficient (B_{22}), a fundamental physicochemical property that describes protein-protein interactions in solution. These results were also compared with aggregation data and aggregation kinetics using size-exclusion chromatography and dynamic light scattering.

In a second case study bovine serum albumin (BSA) was mixed in formulation buffers in combination with sucrose and trehalose, where the protein-protein interactions were studied by determining B_{22} and the aggregation propensity measured. Later these conditions were lyophilised, which is a common way to formulate many biotherapeutics. The aim was to see if the results from the initial screenings also could predict the stability of the freeze-dried cakes.

The first case study showed that IC was a suitable technique to detect small changes in formulation conditions as it managed to predict the increase in stability of the mAb with the ionic strength and arginine concentration, but with no major effect with the addition of polysorbate. The second case study showed that BSA exhibited repulsive protein-protein interactions in all the range of concentrations analysed, as expected due to its high stability in solution, which could be increased with the addition of stabilisers, especially trehalose. Even in the freeze-dried cakes higher concentration of trehalose led to an increased Young's modulus, and therefore higher mechanical stability. Based on these results it could be seen that a protein-protein interaction technique such as IC could be used as an early predictor for formulation behaviour both in liquid and solid states.

References

1. Hedberg, S.H.M., Lee, D., Mishra, Y., Haigh, J.M., and Williams, D.R. (2018) Mapping the Mab Aggregation Propensity Using Self-Interaction Chromatography as a Screening Tool*. Analytical Chemistry*. *90*, 3878-3885.