Biosensor and chromatography based strategies for high throughput measurement of protein self-association during formulation development

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Self-Interaction Chromatography

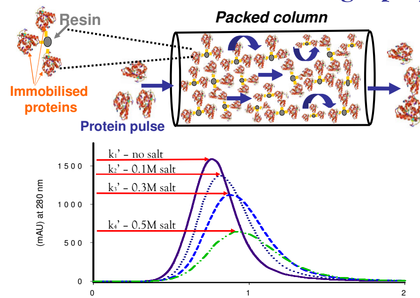

Fig 1. Toyopearl SIC column at pH3 using lysozyme. The retention volume change (k`) reflects the average strength of the interaction. Lysozyme and Mab (1mg/ml) were studied in buffers between pH3-9 with salt concentration of 0 to 0.5M on an AKTA system.

Abstract

In protein formulation, solution conditions and high protein concentrations are responsible for the self-association that leads to aggregation. By detecting self-association behaviour using the second virial coefficient (B22), process parameters can be modified in order to avoid aggregation. The task of B22 determination is made all the more challenging by the weak interactions responsible for self-association. In the literature, the most common approach to B22 measurement is static light scattering (SLS), which is currently not amenable to rapid screening. To this end, self-interaction chromatography (SIC) and a biosensor system (SPR – surface plasmon resonance) were investigated. Both techniques could be adapted to a high throughput approach and required limited sample volume. The challenges and value that each technique offered to quantification of self-association is discussed.

Surface Plasmon Resonance

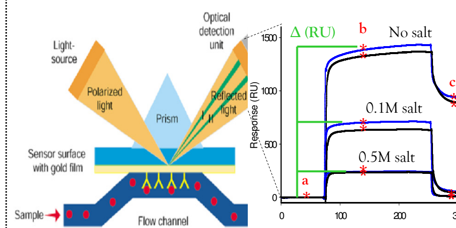

Fig. 2 Target protein, (lysozyme, Mab 1mg/ml) was immobilised onto the sensor surface and the same protein was injected through the flow cell. The accumulation of protein on the active surface (F2) results in an increase in the refractive index. Reference surface (F1) was used to monitor non-specific binding. The runs used buffer conditions of pH3-9 with salt concentration of 0 to 0.5M using Biacore X100 system.

Resin Selection

SIC performance and the effect of non-specific binding to three chromatography matrices, Toyopearl AF-Formyl 650 (methacrylic polymer), Sepharose 4FF (agarose) and Cellufine Formyl (cellulose) were investigated. CNBr protein immobilisation was used to create 15% surface coverage on the resins. The base material of Toyopearl has a negative charge[2], while Sepharose is positive due to the groups being created during immobilization[3] while Cellufine is neutral. The non-specific binding effect was monitored using control resins with ethanolamine immobilised in place of a protein.

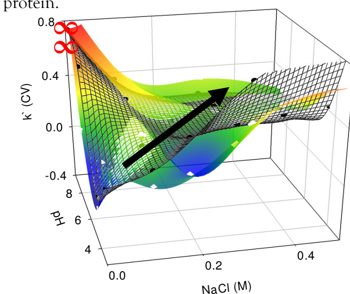

Fig 3. Sepharose *resin study using* ***lysozyme*** *– control surface (mesh) and SIC (coloured) surface with immobilized protein, ∞ peak retained on the column*

The two surfaces show similar trends with retention time increase at low pH, high salt and at high pH, low salt condition including very strong non-specific binding at pH9 on both columns. The effect was due to the increased attraction between the matrix and the lysozyme.

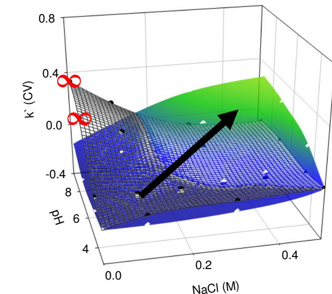

Fig 4. Toyopearl *resin study using* ***lysozyme*** *– control surface (mesh) and SIC (coloured) surface with immobilized protein, ∞ peak retained on the column*

Self-interaction is seen at high pH and salt, in these conditions, lysozyme is close to its *pI* and the salt masks remaining charge favouring this phenomena. This same trend was found in the literature[2,3], although control experiments were not reported.

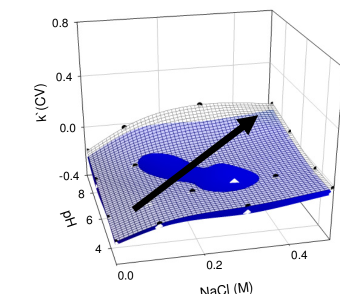

Fig 5. Cellufine *resin study using* ***lysozyme*** *– control surface (mesh) and SIC surface with immobilized protein*

Minimal differences between the two surfaces were observed due to low retention time measurements.

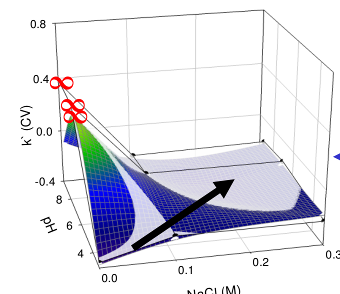

Fig 6. Toyopearl *resin study using* ***Mab*** *– control surface (mesh) and SIC surface with immobilized, ∞ peak retained on the column*

The two surfaces show similar trends with strong binding at pH7 on both control and protein covered surface and at pH9 on the control column when no salt was present; the pI of mAb is close to 7, which could lead to the exposure of negatively charged groups leading to attraction to the positive surface.

general trend in k` and Response as a function of pH and NaCl concentration response

Feasibility Study

The SPR experiments were performed using C1 (carboxymethylated) chips in order to mimic the SIC model of direct immobilisation onto the a surface and SA (carboxymethylated dextran with pre-immobilized streptavidin for biotinylated protein capture) to increase surface coverage and to attempt to eliminate protein - surface interaction. The traditional method of SPR operation was modified to be able to measure self-interaction behaviour of the target molecule using a wide range of buffer conditions.

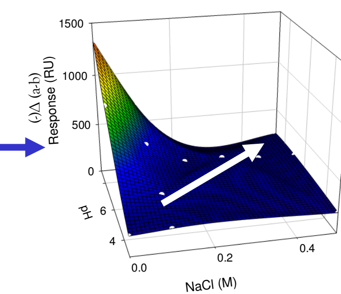

Fig 7. Carboxymethyl chip *(F2) with 35RU* ***lysozyme***

Due to the strong negative charge of the carboxymethylated surface, similar non-specific binding can be observed at pH 7-9, when no salt is present as during the SIC experiments. This strong charge over shadows any detectable self-interaction forces on the protein covered surface (F2) leading the two (F1 and F2) responses over lapping each other.

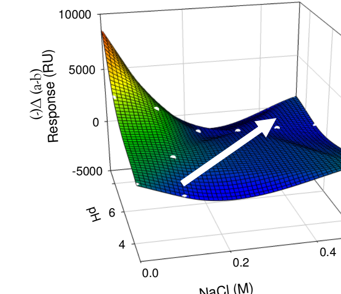

Fig 8. Streptavidin chip *(F2) with 500RU* ***biotinylated lysozyme***

The same non-specific interaction and accumulation of the target protein can be observed on the SA chip, due to its strong negatively charged dextran base. Difference between F1 and F2 surfaces cannot be distinguished as they behaved the same way.

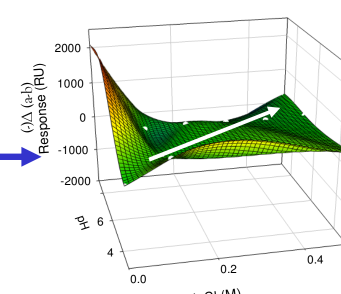

Fig 9. Carboxymethyl chip *(F2) with 1200RU* ***Mab***

Even stronger interactions were detected when Mab was used, being complex molecules, the charge distribution can be disturbed easily due to buffer conditions change.

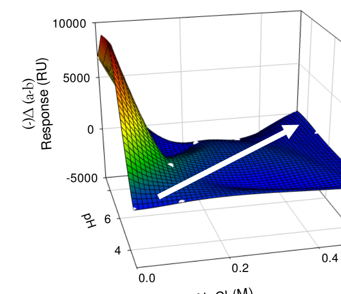

Fig 10. Streptavidin chip *(F2) with 1300RU* ***biotinylated Mab***

Using Mab, the pH7 and 9 trends are very similar to the C1 chip behaviour (Fig 9).

Discussion

The protein self-interactions of interest are relatively weak forces which are challenging to quantify. The initial findings of this research agrees with the SIC literature[4,5]. However, the observed non-specific binding on various resins can lead to misinterpretation of the results particularly at low salt where non-specific binding can dominate. To compensate for this effect, minimal levels of NaCl are needed. Further investigation of the immobilization technique, surface coverage and resin type is required to further control and understand these effects if high throughput industrial application is to be considered. The data suggests different proteins will require different resins / surfaces in order to minimise there differing propensity for non-specific binding.

The SIC and SPR data highlight similar issues – that extensive non-specific surface binding occurs at no salt or low salt conditions and that proteins adsorb to surfaces especially closer to their *pI* value. During formulation screening, no salt conditions are often used to control for the confounding effects of buffer salts, which means, the desired measurement technique should be able to function in these areas..

Future Work

As industry moves towards high concentration formulations, there is a need for high throughput methods to screen molecules in this environment. This will allow the prediction of their association/aggregation behaviour at the beginning of the process - and product development. At the moment, no such technique has been fully developed

Ideally, the target molecules should be kept in solution without immobilizing them onto surfaces, eliminating non-specific surface interactions and allowing investigation in solution. One possibility would be to employ capillary electrophoresis under conditions that do not require physical tethering of the target molecule.

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