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Micro scale self-interaction chromatography of proteins: A mAb case-study

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ABSTRACT

Self-interaction chromatography is known to be a fast, automated and promising experimental technique for determination of B_{22}, but with the primary disadvantage of needing a significant amount of protein (>50 mg). This requirement compromises its usage as a technique for the early screening of new biotherapeutic candidates. A new scaled down SIC method has been evaluated here using a number of micro LC columns of different diameters and lengths, using typically 10 times less stationary phase than traditional SIC. Scale-down was successfully accomplished using these micro-columns, where the SIC results for a range of differing columns sizes were in agreement, as reflected by k', B_{22} and column volumes data. The results reported here demonstrate that a scaled down version of SIC can be easily implemented using conventional liquid chromatography system where the final amount of mAbs used was 10 times less than required by conventional SIC methodologies.

1. Introduction

1.1. Background

Monoclonal antibodies (mAbs) are playing an increasingly important role as a treatment for cancer, infectious and autoimmune diseases, and are therefore an important class of products in the biopharmaceutical industry [1,2]. However, they are also some of the most expensive drugs on the market and the ultimate success for the next generation of mAb therapeutics depends on economic factors with the motto “fail fast, fail cheap” [3]. The selection of the most stable mAb formulation as a part of the preformulation stages in drug development is a very time-consuming and expensive process and therefore the availability of experimental techniques using only a small amount of protein are highly desirable [4]. There are still significant concerns and problems in terms of aggregation propensity in biological formulations that affects manufacturing, delivery, commercial packaging and shipping [5]. In order to minimize these aggregation problems within bioprocess formulations, the availability of experimental or theoretical tools that can facilitate the proper understanding and prediction of protein aggregation would be a useful advance in bioprocess development [6].

Protein-protein intermolecular interactions are known to be fundamentally related to the aggregation behavior between proteins in solution [7]. The osmotic second virial coefficient, B_{22}, derived from the virial equation of state, is a fundamental physiochemical property that describes molecular interactions between proteins in solution. These interactions can be useful for understanding and predicting protein aggregation behavior. B_{22} has been shown to correlate well with protein aggregation behavior for a number of proteins across a wide range of solution conditions [8,9].

There are a number of different experimental techniques that have been employed to determine B_{22}. Membrane osmometry was the first technique used to determine B_{22} and is based on the theory by McMillan and Mayer [10]. However, the technique has many disadvantages including membrane fouling, leakage and solute contamination as well as being time-consuming due to the long equilibration times encountered. Static light scattering (SLS) has become most recently the reference technique for the determination of B_{22} and the technique is still widely used today. However, there are still some disadvantages associated with SLS such as long experimental times and the relatively large amount of protein required for reliable estimates as several solution concentrations are needed for one B_{22} measurement [11]. Only some types of molecules are suitable for the technique, with small biomolecules

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and large macromolecular solution species having been shown to be unsuitable [12,13].

One of the newest techniques to determine B_{22} is self-interaction chromatography (SIC), first described by Tessier et al. [14], which offers scope as a higher throughput way of estimating B_{22}. SIC is a relatively new technique and was first introduced by Patro and Przybycien [15] and then developed further by Tessier et al. [14]. This method involves immobilizing a target protein onto a solid state material which is usually a chromatographic stationary phase. The same protein will then be used within the mobile phase as well, and a pulse of this protein (together with buffer) will be injected and eluted through the packed column which contains the immobilized protein. The elution volume for the protein which passes through the column is measured, normally using a UV–vis LC detector, and is an indication of the protein–protein interactions between the immobilized protein and the 'free' protein. This analysis assumes that the immobilized protein molecules are randomly orientated [14–16] though later work by Rakel et al. [17] reported that some orientations were more common than others, but in general are quite consistent across a range of pH's.

However, the major limitation with the SIC technique involves the large amount of proteins needed for the immobilization process. Tessier et al. [14] use 65 mg of lysozyme and Binabaji et al. [18] use 50 mg of mAbs for the immobilization process. The extensive amounts of proteins needed may therefore limit the use of SIC in pre-formulation for certain therapeutic proteins. SIC has recently shown to be able to be scaled-down to a microfluidic chip for the model proteins catalase and lysozyme, though this method of course requires very specialized microfluidic apparatus [19,20]. These studies have not been applied to therapeutic proteins such as mAbs or using conventional experimental methodologies.

1.2. Scale-up/scale-down

Scale-up and scale-down in chromatography for bioprocess separation can be quite a challenge and is commonly driven by both empirical studies and prior experience. The scale-up technique that has been traditionally employed industrially is to keep the bed height fixed between laboratory and pilot plant columns, and increase the bed diameter. This allows the linear operating velocity and the feed load to be kept the same [21]. However, this approach is quite a non-flexible way of scale-up and scale-down. Columns of the required dimensions may not be available whilst the use of fixed columns can lead to producing over- or under-capacity in production scale or differences in packing densities with suitable dimensions [22]. There will also be an additional challenge when scaling up from micro-scale columns to laboratory scale columns with different bed heights. Kidal and Jensen [23] have presented a simple model that would allow chromatography scale-up and scale-down to be more flexibly achieved, where the column volumes per hour would be kept the same between two columns of different bed heights and diameters. This approach would also allow a constant residence time and number of theoretical plates thus ensuring that the resolution stayed the same. This method is based on combining the chromatography plate equation and a modified van Dempter equation [24], which does not consider the longitudinal chromatogram broadening:

$$H = A + CV, \quad N = \frac{L}{H} \tag{1}$$

where N is the plate number, H is the plate height, L is the bed height, V is the velocity in cm/h and A, C are constants (axial dispersion, mass transfer).

$$N = \frac{1}{\frac{A}{L} + CQ} \tag{2}$$

where Q is the flowrate in column volumes (cv)/h. This scale-up concept describes the influence of the bed height on the flowrate [22,23].

1.3. Determination of the second virial coefficient

The second virial coefficient can be determined by the following equation:

$$B_{22} = \frac{N_A}{M_W^2}\left(B_{HS} - \frac{k'}{\rho_s \Phi}\right) \tag{3}$$

where B_{HS} is the excluded volume or the hard sphere contribution defined by $16/3\pi r^3$ using the protein radius, r, calculated from molecular volume [25], ρ_s the amount of protein immobilized per unit pore surface area and Φ is the effective phase ratio, that is the total accessible surface area available for the mobile phase protein that can be interpolated from the work performed by DePhillips and Lenhoff [26]. k' is the chromatographic retention factor which is defined below:

$$k' = \frac{V_p - V_0}{V_0} \tag{4}$$

where V_p is retention volume for the protein and V_0 is the dead volume, the retention volume of a non-interacting molecule the same size as the protein. V_0 was traditionally determined by preparing a column the same way as the SIC column but without protein immobilized on it; the dead column. However, this approach has limitations as some dead columns have been found to strongly interact with certain proteins [18,27], so the best practice recommended for this procedure is to use a packed bed of resin less likely to cause interactions with the proteins or using a dextran standard the same effective size as the protein [18,27].

The retention times for the protein and acetone injections on all columns were determined here using the first moment (centre of mass) approach recommended by Quigley et al. [28]. In cases where the chromatograms were Gaussian shaped the first moment analysis and peak maximum retention times/volumes will be coincident. For acetone injections the chromatograms were almost completely Gaussian, which resulted in similar results for both methodologies. However, for protein injections many chromatograms showed tailed peaks, confirming the importance of a first moment analysis as used here.

This paper investigates the specific experimental requirements for the scale-down of SIC when using therapeutic proteins such as mAbs, whether it is to scale-down to a micro column or a microfluidic chip. For example, establishing the flowrate where the system is in equilibrium is a critical aspect for accurately measuring accurately the protein–protein interactions. Faster flowrates will lead to lower residence times and potentially non equilibrium data that in turn can result in incorrect estimates of k' and B_{22}.

2. Materials and methods

2.1. Materials and equipment

Experiments with the model proteins were performed with a monoclonal antibody supplied by FUJIFILM Diosynth Biotechnologies which was highly purified (pI of 8.6 and molecular weight 144.5 kDa). Potassium phosphate, $NaBH_3CN$, dibasic and monobasic sodium phosphate, ethanolamine, HCl and NaOH were all purchased from Sigma–Aldrich (ACS grade). NaCl, Sodium acetate trihydrate, glacial acetic acid and acetone were obtained from Fisher Scientific (AR grade). Toyopearl AF-Formyl-650 M (08004) was purchased from Tosoh Bioscience. For buffer preparation ultrapure deionized water was used. The pH of the buffers were adjusted with HCl or NaOH and monitored using a Mettler Toledo FiveEasy