Journal of Chromatography A, 1459 (2016) 47–56

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Similar interaction chromatography of proteins: A cross interaction chromatographic approach to estimate the osmotic second virial coefficient

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ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 15 June 2016
Accepted 15 June 2016
Available online 16 June 2016

Keywords:
Self-interaction chromatography
Cross-interaction chromatography
Virial coefficients
Protein aggregation
B_{22}

ABSTRACT

Self-interaction chromatography (SIC) has established itself as an important experimental technique for the measurement of the second osmotic virial coefficients B_{22}. B_{22} data are critical for understanding a range of protein solution phenomena, particularly aggregation and crystallisation. A key limitation to the more extensive use of SIC is the need to develop a method for immobilising each specific protein of interest onto a chromatographic support. This requirement is both a time and protein consuming constraint, which means that SIC cannot be used as a high throughput method for screening a wide range of proteins and their variants. Here an experimental framework is presented for estimating B_{22} values using Similar Interaction Chromatography (SimIC). This work uses experimental B_{23} and B_{32} data for lysozyme, lactoferrin, catalase and concanavalin A to reliably estimate B_{22} using arithmetic mean field approximations and is demonstrated to give good agreement with SIC measurements of B_{22} for the same proteins. SimIC could form the basis of a rapid protein variant screening methods to assess the developability of protein therapeutic candidates for industrial and academic researchers with respect to aggregation behaviour by eluting target proteins through a series of well-characterised protein immobilized reference columns.
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1. Introduction

Protein therapeutics have become a potent and versatile approach to treat a broad range of diseases from breast cancer to rheumatoid arthritis. Protein-based pharmaceuticals now represent a significant proportion of drugs currently under development, and the market share for these medicines has burgeoned in recent years and is expected to reach 400 billion USD by 2019 [1]. While considerable advances have been made in the design and production of biotherapeutics, downstream manufacturing remains a challenging and costly endeavour that represents a key bottleneck for the industry. Given the greater complexity of protein therapeutic molecules compared to their small molecule counterparts in terms of both molecular mass as well as secondary and tertiary structures, biotherapeutics are far more susceptible to a range of physical and chemical instabilities. In particular, the successful development of protein therapeutics can be limited by their aggregation and solubility behaviour. In order to select the best biotherapeutic candidate for production it is necessary to determine which molecules possess the optimal properties including stability and solubility but also activity and affinity [2].

The high risk involved in biopharmaceutical development has led to a shift in focus towards methodologies which facilitate the "win quick, fail fast" [3] model so as to minimise potential losses of biotherapeutic development. Particular emphasis is being placed on predictive technologies, scale down models and product characterisation studies earlier in the development cycle [4,5]. The aim is to not just identify candidates with the greatest levels of activity and affinity but also to determine which of those will also meet later required standards for manufacturing and stability. One area of study gaining a lot of attention is the early phase study of protein aggregation. Protein aggregation poses a significant challenge because it can arise during any stage of the bio-manufacturing process and can affect the quality of the final product in terms of both efficacy and potential immunogenicity [6]. Aggregation is particularly problematic because it is influenced by a huge array of factors from the protein polypeptide sequence itself, to the host organism used in fermentation, the steps used in the downstream purification and filling process as well as the final formulation and storage conditions [7].

Current techniques to predict and optimise the long-term stability of protein therapeutics require the generation of relatively large

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http://dx.doi.org/10.1016/j.chroma.2016.06.048
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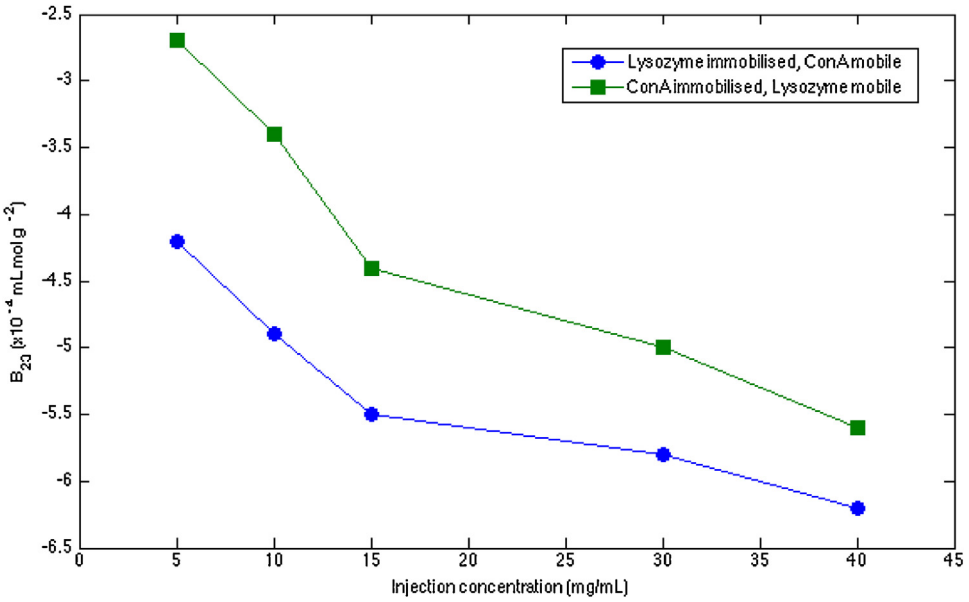

Fig. 1. Dependence of B_{23} on injection concentration for lysozyme-concanavalin A system (20 mM sodium phosphate pH 7.0 0.05 M NaCl, 10 μL injection 0.5 mL/min).

quantities of purified protein sample that are often not available, particularly at the discovery stage, and as such this requirement severely limits the number of test conditions that can be assessed with these methods [8]. Ideally, in the future computational models will be developed that can predict which combinations of candidates and solution conditions produce the optimal level of stability. However, at present the existing computational tools are limited by gaps in our fundamental understanding and knowledge of the precise mechanisms of protein aggregation, the role of the solvent conditions and the molecular interactions between protein, excipient molecules as well as salt/buffer species. Consequently, empirical methods are vital tools for the prediction of protein aggregation propensity in early phase studies. Many computational tools exist that predict protein stability from protein sequence information such as PASTA, TANGO and AGGRESCAN [9–11] as well as experimental methods such as the use of intrinsic protein fluorescence or differential scanning calorimetry (DSC) which can determine protein thermal unfolding or melting temperature (T_M) [8,12] and measurement of the osmotic second virial coefficient (B_{22}). These techniques cannot replace the real-time stability screening of protein candidates and formulations that are necessary to meet regulatory standards set by bodies such as the FDA or EMA, but they do have the potential to markedly reduce the probability that those investigations will fail. In this paper we describe how Similar Interaction Chromatography based on principles of self- and cross-interaction chromatography could potentially be utilised as a one of those tools to aid in the selection of optimal protein candidates and solution conditions during the early stages of biotherapeutic development.

The measurement of osmotic second virial coefficients, B_{22}, has garnered attention in recent years as a method of considerable potential for the quantification of protein self-interactions in

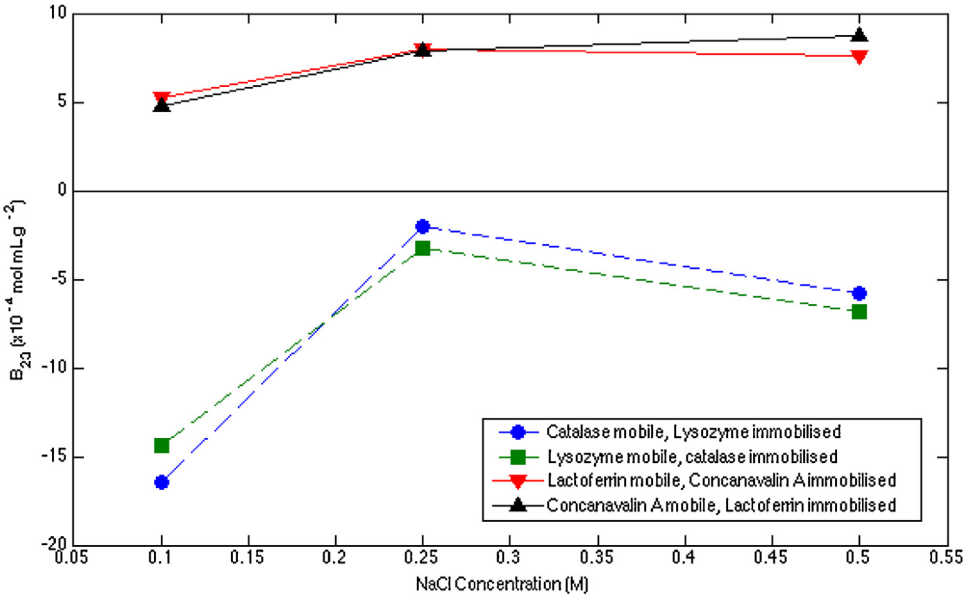

Fig. 2. The effect of interchanging the immobilised protein on virial cross coefficient values for catalase-lysozyme systems and lactoferrin- concanavalin A for various NaCl concentrations (20 mM sodium phosphate buffer pH 7.0, 10 μL injection, 0.5 mL/min).

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