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Influence of protein adsorption kinetics on breakthrough broadening in membrane affinity chromatography

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ABSTRACT

Existing mathematical models developed to describe membrane affinity chromatography are unable to match the complete breakthrough curve when a single Langmuir adsorption isotherm is used, because important deviations from the observed behavior are systematically encountered in the simulation of breakthrough broadening near saturation. The relevant information required to overcome that limitation has been obtained by considering simultaneously both loading and washing curves, thus evaluating the adsorption data at equilibrium and recognizing what are the appropriate adsorption mechanisms affecting the observed behavior. The analysis indicates that a bi-Langmuir binding kinetics is essential for a correct process description up to the saturation of the stationary phase, together with the use of the relevant transport phenomena already identified for the experimental system investigated. The input parameters used to generate the resulting simulations are evaluated from separate experiments, independent from the chromatographic process. Model calibration and validation is accomplished comparing model simulations with experimental data measured by feeding pure human immunoglobulin G (IgG) solutions as well as a cell culture supernatant containing human monoclonal IgG_1 to B14-TRZ-Epoxy2 bio-mimetic affinity membranes. The simulations obtained are in good agreement with the experimental data over the entire adsorption and washing stages, and breakthrough tailing appears to be associated to the reversible binding sites of the bi-Langmuir mechanism. Remarkably, the model proposed is able to predict with good accuracy the purification of IgG from a complex mixture simply on the basis of the results obtained from pure IgG solutions.

1. Introduction

Therapeutic antibodies manufacture is a key issue in the biotechnology industry, and their production at a large scale has become increasingly important with the recent approval of several drugs of this class for different critical illnesses [1,2]. Downstream processing is recognized as the bottleneck in current antibody production platforms [3,4] and its optimization is a prerequisite for important reductions of antibodies production costs.

At present, antibody capture with Protein A resins is the most expensive step among the unit operations involved in downstream processing, which can contribute up to 50–80% of the total purification costs [5]. However, a huge optimization potential is expected [6] as a result of the increasing efforts devoted to the development of possible alternatives to the canonical bead based Protein A affinity chromatography.

Mimetic affinity membrane chromatography is particularly attractive to that aim, because it combines the advantages of mimetic ligands, in terms of antibody specificity and lower manufacture costs [7–9], with membrane technology, which introduces its superior mass transport characteristics, high throughput and absence of pressure drop issues [10–13]. In order to pursue an industrial application of that technique, an effective modeling tool is needed to predict reliably the process performance also in large-scale modules, as required for scale-up design and optimization purposes.

The modeling and simulation of an affinity chromatographic cycle has been considered in several works. The basic approach is the combination of a species mass balance equation coupled with a kinetic equation to represent the protein adsorption/desorption mechanism on the surface active sites [14,15]. The binding kinetics generally adopted to describe the protein–ligand interaction is represented by a simple reversible Langmuir model [16,17], even though that is unable to reproduce the complete experimental breakthrough, especially near membrane saturation where a typical broadening is frequently observed [18–20].

Different binding kinetics have been proposed in the literature in order to accurately describe breakthrough broadening close to

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saturation conditions: Shi et al. proposed a kinetic equation based on Freundlich model [21], while Yang and Etzel considered the possibility of a steric hindrance resistance at high surface coverages, as well as the conformational changes that a protein may experience when bound to a surface [22]. The simulation results presented in those works are fairly adequate to approximate the onset of the experimental breakthrough curves, but the simulations still do not match the tailing behavior close to membrane saturation. The goal to represent well the entire breakthrough curve up to saturation might be considered an unnecessary refinement since in actual practice breakthrough point is well below complete column saturation and usually does not exceed 10% of feed concentration. However, the initial layers of the stationary phase, encountered close to feed entrance, may have already reached their saturation while subsequent layers still remain with little loading. Thus, a better understanding of the membrane behavior up to saturation appears indeed important also for the actual chromatography practice.

Based on the analysis of both adsorption and washing stages, in the present work the origin of breakthrough broadening is ascribed to the co-existence of two different and independent binding sites, with two different binding kinetics, which leads to a bi-Langmuir adsorption mechanism. This heterogeneous binding kinetics is similar to the one used by Wang and Carbonell for staphylococcal enterotoxin B adsorption onto a bio-mimetic affinity resin [23] and by Boi et al. for IgG adsorption on mimetic A2P affinity membranes [24]. The above binding kinetics is used in the general simulation model for membrane chromatography which has been described in detail in a recent work [25], obtaining simulations suitable to describe the entire chromatographic cycle, including the behavior observed in breakthrough curves close to saturation. Interestingly, the resulting complete model presented in this work is characterized by two main advantages in comparison to the previous version based on a single (reversible or irreversible) Langmuir model: (i) it is not limited to describe breakthrough curves up to 80% saturation (which still is an appreciable result), and consequently does not require to estimate the proper reduction of the maximum binding capacity of the membrane, as discussed in Ref. [25] for the previous model; (ii) in addition, use of the bi-Langmuir kinetics allows to overcome conceptual inconsistency of the previous models where a reversible Langmuir kinetics was used during adsorption stage, while no reaction was considered during washing, even if no buffer changes were introduced in the washing stage.

The experimental reference system considered for model validation is the purification of human IgG through mimetic B14-TRZ-Epoxy2 membranes, which have been produced and studied in view of the actual interest on mimetic ligands [26]. This material consists of a highly interconnected porous matrix, where mimetic ligand B14 is immobilized onto the pore surface through a triazole ring (TRZ) spacer. These new affinity membranes combine the highly accessible internal structure of Epoxy2 membranes with the benefits of B14 ligand, represented by high specificity towards IgG and pluronic F68 tolerance. In addition, an industrial application of this chromatographic medium results particularly promising as significant improvements in its binding capacity are expected [26].

Main contributions of the present work are represented by the use of a proper adsorption kinetic expression, obtained from simultaneous analysis of experimental observations during loading and washing stages. That leads (a) to a rather satisfactory model simulations of the chromatographic cycles up to complete membrane saturation, and (b) to a rather satisfactory description of the washing stage, with no need to artificially change binding/unbinding kinetics in this stage, in absence of changes in the buffer used. In fact, the washing stage is often disregarded because of its minor importance in process practice, but its accurate analysis was found very useful as many important information on binding kinetics and thermodynamics can be extracted from the washing profile.

2. Experimental

The equipment and materials used in the experiments performed are the same as those reported in Refs. [25] and [26]; for clarity sake the experimental set up and procedures adopted to evaluate numerical values of model parameters are briefly recalled in the following.

The experimental system considers the purification of human IgG_1 from a cell culture supernatant by using B14-TRZ-Epoxy2 affinity membranes. A layered stack of 5 membranes with total thickness of 0.1 cm and a diameter of 2.5 cm, was allocated into an appropriate cartridge and connected to an FPLC Akta Purifier 100 (GE Healthcare, Milan, Italy). The IgG_1 concentration in cell culture supernatant is 0.11 mg/ml, while the investigated flow rates range from 1 to 5 ml/min, corresponding to linear velocities of 29 and 145 cm/h, respectively.

Prior to feeding the complex medium, membranes were preliminarily tested with pure polyclonal IgG solutions under a broad range of different operating conditions. In experiments with pure IgG, 0.1 M phosphate buffered saline (PBS) pH 7.4 was used as loading and washing buffer. Experiments were carried out at four different flow rates, i.e. 1, 2, 5 and 10 ml/min, corresponding to linear velocities of 29, 58, 145 and 290 cm/h, respectively; ten different IgG feed concentrations ranging from 0.14 to 2.15 mg/ml were tested in the chromatographic cycles.

Physical properties of the membranes were obtained with pulse experiments as described in a previous work [25]. The membrane void fraction, ε, is equal to 0.545, while the measured dispersivity coefficient, α, is 0.104 cm. From experiments performed in non adsorbing conditions, the volumes of CSTR and PFR required to describe system dispersion (see Section 4) have been determined as 0.69 and 1.75 ml, respectively [25].

3. Relevant binding mechanisms

Several important information on binding mechanism can be obtained from an accurate study of equilibrium adsorption data derived from experimental breakthrough and washing curves measured under adsorbing and non adsorbing conditions.

The curves shown in Fig. 1 qualitatively represent loading and washing stages, reporting protein concentration in the effluent solution versus the sample volume fed to the column. The different areas identified in this plot have all a precise physical meaning in terms of protein amounts. In particular, the area below the saturation horizontal line and above the breakthrough curve in non adsorbing conditions ($A_{HOLD\ UP}$) represents the amount of protein

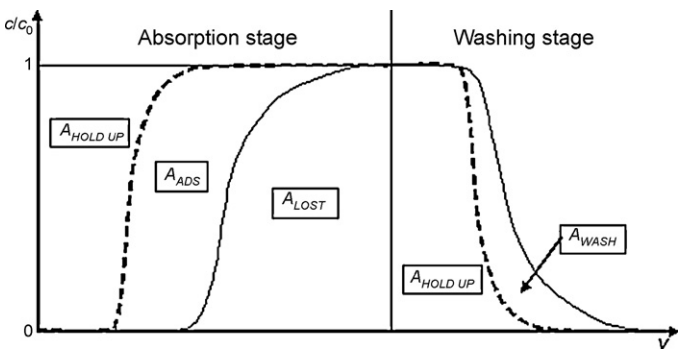

Fig. 1. Qualitative example of the adsorption and washing curves measured under adsorbing (solid line) and non adsorbing (dashed line) conditions. The figure highlights the physical meaning of the different areas in the plot.