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A new multimodal membrane adsorber for monoclonal antibody purifications

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ABSTRACT

This contribution describes research on the use of a newly developed multimodal membrane (MMM) adsorber that can be used as a chromatographic stationary phase in bioseparation processes. Compared with commercial cationic multimodal adsorbers, this MMM has superior static binding capacity (SBC = 180 mg IgG/ml), dynamic binding capacity ($DBC_{10\%}$ = 60 mg IgG/ml), and load productivity (> 10 mg/ml/min). Furthermore, the incorporation of functional groups that provide orthogonal modes of interactions increases the range of ionic strength for operation of the MMM relative to conventional ion-exchange and hydrophobic interaction chromatography media. The effects of different salt types (kosmotropic, neutral, chaotropic salts) and ionic strength on IgG binding were investigated. To further understand the protein adsorption on the MMM, a thermodynamic model was employed to describe IgG adsorption isotherms on the MMM by providing a unique set of physically meaningful parameters for each salt type. The model was also a precise predictor of the adsorption isotherms under non-test conditions. A breakthrough analysis was used to determine dynamic binding capacities. The MMM maintained 70% DBC as ionic strength increased from 0 to 300 mM NaCl. Finally, a range of flow rates was used to study the effect of volumetric throughput on DBC. Because DBC was insensitive to flow rate, process productivity increased with flow rate nearly linearly up to high linear velocity (535 cm/h). A kinetic study indicated that the rate limiting step of IgG binding on the MMM was the adsorption rate, not the convective mass transport of protein molecules to binding sites.

1. Introduction

Excellent separation resolution and mild operating conditions make chromatographic processes particularly useful in downstream protein purification operations. However, substantial improvements in downstream manufacturing processes are necessary to increase production capacities and meet the large, rapidly increasing demand for protein therapeutics. While the conventional chromatographic operations are designed to purify protein based on a single interaction mode such as affinity interaction, Coulombic interaction, hydrophobic interaction and size exclusion [1,2], multimodal chromatographic operations are used to purify protein through two or more orthogonal modes of interaction. Multimodal adsorbents have been shown to improve product quality by removal of high molecular weight aggregates and can improve process efficiency in industrial-scale mAb drug manufacturing [3–5]. Specifically, the advantage of multimodal operations is that they may decrease the number of purification steps, thereby shortening purification times and increasing the overall protein yields. These factors are paramount in controlling overall manufacturing capacity and protein product quality.

Most commercial multimodal chromatographic media comprise resin beads functionalized with ligands that exhibit both hydrophobic and Coulombic properties. Exceptions are the commercial membranes from EMD-Millipore (Chromasorb) and Sartorius (Sartobind STIC® PA Nano); however, these are limited to anionic MMMs. The use of resin beads as packing media results in low productivity [6–8]. Consequently, the use of membranes has become a viable option for membrane chromatography, particularly now that strategies have been developed by our group and others to increase the binding capacities of membrane adsorbers to values that meet or exceed the corresponding resins [9–11]. Just recently, we described the use of surface-initiated atom transfer polymerization to prepare the first cationic multimodal membrane [10].

Given the promising application of multimodal membrane adsorbers for protein bind-and-elute purifications, detailed theoretical simulations of protein binding on multimodal membrane adsorbents are needed to reduce the time and cost spent on

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process development. While the well-known Langmuir isotherm model used to describe convex isotherms does provide the apparent maximum binding capacity and association coefficient, it does not provide mechanistic insights on the influence of different interaction types and the mobile phase conditions on protein binding, and its constants must be adjusted for each new set of operating conditions. In attempts to overcome such limitations, Brooks and Cramer [12] developed the Steric Mass Action (SMA) formalism model, which entails the use of three parameters for determining the non-linear adsorption in an ion-exchange system. This SMA model considers the steric hindrance of salt counterions, but not the protein–protein interactions. Mollerup et al. [13] extended SMA to develop a thermodynamic model that includes interaction-type parameters, and applies to both hydrophobic interaction chromatography and ion exchange. Using the generalized framework developed by Mollerup et al., Ottens and coworkers [14] developed a model for protein adsorption on multimodal adsorbents functionalized with ligands carrying both hydrophobic and groups. This model has not yet been applied to membrane adsorbers.

The objectives of this study were to apply the thermodynamic model described by Ottens and coworkers [11] to analyze immunoglobulin G (IgG) adsorption on our newly developed multimodal membrane adsorbers and to evaluate membrane performance under static and dynamic protein binding conditions. Fitted model parameters were used to elucidate the protein adsorption mechanism(s) under different salt conditions (type and ionic strength). IgG dynamic binding capacities and load productivities were measured for comparison with commercial products. Using column studies, we determined the effects of flow rate and ionic strength on the dynamic binding capacities, identified the rate limiting factors for protein binding on the new MMM, and developed an effective elution strategy.

2. Theory

Ottens and coworkers [14] developed a thermodynamic model for protein adsorption on multimodal adsorbents and an approach for estimating the unknown model parameters. The stoichiometric exchange of protein and hydrophobic ligands with salt counterions is represented by the following reaction:

$$P + \nu SL + nL \Leftrightarrow PL_n + \nu S$$

where a protein molecule P is adsorbed to a stationary phase with n hydrophobic ligands and simultaneously exchanges with ν salt counter-ions to form the protein–ligand complex PL_n. ν is defined as the ratio of the binding charge of the protein z_p to the charge of the salt counterion z_s: $\nu = z_p/z_s$.

In multimodal exchange chromatography, the general form of the single component isotherm is given by [14]

$$\frac{q_p}{c_p} = \tilde{K}_{eq}\left(\frac{\Lambda_{IEX}}{z_s c_s}\right)^{\nu}\left(\frac{\Lambda_{HIC}}{c}\right)^{n}\left(1-\frac{q_p}{q^{max}_{p,\,IEX}}\right)^{\nu}\left(1-\frac{q_p}{q^{max}_{p,\,HIC}}\right)^{n}\tilde{\gamma}_p \tag{1}$$

where q_p and c_p are the protein concentrations in the adsorbed phase and liquid phase, respectively; $q^{max}_{p,\,IEX}$ and $q^{max}_{p,\,HIC}$ are the maximum binding capacities achieved through Coulombic interaction and hydrophobic interaction, respectively; $\tilde{K}_{eq}$ is the thermodynamic equilibrium constant; c_s is the salt concentration in the liquid phase; c is the molarity of the solution in the pore volume; $\tilde{\gamma}_p$ is the normalized activity coefficient; Λ_{IEX} is the ionic-exchange ligand density; and Λ_{HIC} is the hydrophobic ligand density.

Ottens and coworkers [14] point out for the case of multimodal adsorbents bearing the same number of Coulombic interaction groups and hydrophobic interaction groups that $\Lambda_{IEX} = \Lambda_{HIC} = \Lambda$, and the adsorption isotherm simplifies to

$$\frac{q_p}{c_p} = \tilde{K}_{eq}(\Lambda)^{\nu+n}\left(\frac{1}{z_s c_s}\right)^{\nu}\left(\frac{1}{c}\right)^{n}\left(1-\frac{q_p}{q^{max}_{p,MM}}\right)^{\nu+n}\tilde{\gamma}_p \tag{2}$$

The asymmetric activity coefficient is expressed by the following activity coefficient model:

$$\tilde{\gamma}_p = \frac{\gamma_p}{\gamma_p^{\infty,w}} = \exp(K_s c_s + K_p c_p) \tag{3}$$

where K_s and K_p are interaction constants. K_s is proportional to the difference of intermolecular attractive forces between protein–water and protein–salt, and K_p is proportional to the difference of intermolecular attractive forces between protein–water and protein–protein [10].

Combining Eqs. (2) and (3) yields the single component adsorption isotherm:

$$\frac{q_p}{c_p} = A\left(1-\frac{q_p}{q^{max}_{p,MM}}\right)^{\nu+n} \tag{4}$$

where A is the initial slope of the isotherm or the partition coefficient in the limit $q_p \to 0$.

$$A = \tilde{K}_{eq}(\Lambda)^{\nu+n}\left(\frac{1}{z_s c_s}\right)^{\nu}\left(\frac{1}{c}\right)^{n}\exp(K_s c_s + K_p c_p) \tag{5}$$

In previous studies of resin columns [13–15], the parameter A was estimated from isocratic retention data under varying salt concentrations. This method is based upon Ettres definition of the retention factor [16], which considers that part of the solute retention time is due to pore diffusion within the resin beads. This method is limited to the estimation of A for salts that can be used as elution modulators. However, in the case of our MMM adsorbers, not all salt types can be used as elution modulators, and convection dominates the mass transport of protein within the MMM. To overcome this limitation, we used the Langmuir isotherm model to estimate parameter A:

$$q_p = \frac{q_p^{max} K_a c_p}{1 + K_a c_p} \Rightarrow \frac{q_p}{c_p} = q_p^{max} K_a\left(1-\frac{q_p}{q_p^{max}}\right) = A\left(1-\frac{q_p}{q_p^{max}}\right) \tag{6}$$

where A represents the initial slope of the isotherm.

Ottens and coworkers provide a detailed account of their methods to fit the isotherm parameters, which include linear regression and constrained minimization [14]. Here, however, we used minimization algorithms from the pyOpt 1.1.0 optimization tool to estimate the parameters providing the best fit of the models to the data [17].

3. Experimental

3.1. Materials

In our experiments we used regenerated cellulose membranes with a 1.0 μm average effective pore size, a 70 μm thickness and a 47 mm diameter, which we purchased from Whatman, Inc. The following chemicals were purchased from Sigma-Aldrich with purities given in weight percentage: 2-bromoisobutyryl bromide (2-BiB, 98%), copper(I) chloride (CuCl, 99.99%), glycidyl methacrylate (GMA, 97%), guanidine hydrochloride (Gua-HCl, ≥99.9%), hydrochloric acid (HCl, 37%), hydrochloric acid 0.01 M standard solution, 4-mercaptobenzoic acid (99%), N,N,N′,N″,N″-pentamethyldiethyldiethylenetriamine (PMDETA, 99%), phenolphthalein (0.5% solution), sodium chloride (NaCl, ≥99%), sodium citrate (≥99%), sodium hydroxide 0.01 M standard solution, sodium thiocyanate (NaSCN, ≥98%), tetrahydrofuran (THF, anhydrous, ≥99.9%), urea (≥98%). The following chemicals were obtained