Examining the effect of protein size on transport and adsorption in polymer-modified ion-exchange media

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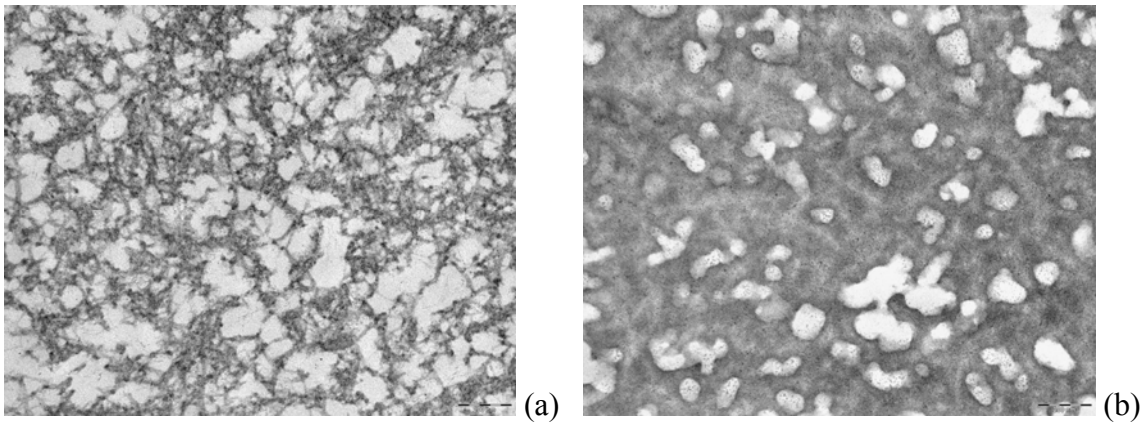

Figure 1: TEM images of (a) Q Sepharose FF and (b) Q Sepharose XL sections at high adsorbed protein concentrations. The dark regions correspond to stained protein and the light regions correspond to void.

Ion-exchange particles in which functionalized secondary polymers have been grafted onto the base matrix have been shown to offer significant performance advantages over traditional particles, especially with respect to increases in dynamic binding capacity (DBC). However, the mechanistic origins of these advantages are not clearly understood. A number of studies have found that, depending on pH and flow rate, a maximum in the DBC can exist as a function of ionic strength, suggesting that the key lies in the balance between improved transport at higher ionic strengths and improved static capacity at lower ionic strengths. The broad goal of this work is to gain a better understanding of this interplay between protein transport and adsorption that accounts for observed column breakthrough performance in both traditional and polymer-modified particles. To this end, the model proteins lysozyme, lactoferrin, and a monoclonal antibody (mAb) are used to examine the role of protein size in column breakthrough performance on the traditional resin SP Sepharose FF and on the dextran-modified resins SP Sepharose XL and Capto S, each having an agarose base matrix. The base matrices for FF and XL are similar, providing an especially good basis for examining the role of the dextran.

In this study, a variety of techniques spanning length scales from the column to local properties within the individual chromatography particle are used to rationalize observed differences in breakthrough for the various protein-resin pairings. Isocratic retention experiments show that the retention mechanism for the three resins is quite similar for the small protein lysozyme. However, the larger proteins can be almost completely excluded from the pores of the polymer-modified resins at higher ionic strengths, which may result from a combination of steric and electrostatic interactions. This exclusion could be important industrially in ion-exchange steps where the ionic strength must be kept relatively high for economic reasons (i.e. minimization of buffer consumption in dilution or dialysis), as the polymer-modified resins may give poor performance, despite their clear advantages at lower ionic strengths. Adsorption isotherms are used to establish the static binding capacities of the resins and knowledge of resin and protein

properties is used to interpret observed behavior. The isotherms generally reflect higher static capacities for the dextran-modified resins, except at higher ionic strengths where the exclusion behavior observed in isocratic retention experiments is presumably a limiting factor. Transmission electron microscopy (TEM) images of particles with adsorbed protein, such as those in Figure 1, show that the dextran modification gives a larger available binding volume, providing convincing visual support for the higher static capacities of XL and Capto under most conditions. Manipulation of previously published inverse size exclusion chromatography (ISEC) data allows estimation of the binding volume afforded by the dextran.

The static capacity of a resin sets an upper limit on its dynamic capacity. Comparison of these two capacities nicely demonstrates that, while the dynamic capacity can approach the static capacity at high ionic strengths, transport limitations can be quite significant at lower ionic strengths. In this work, confocal microscopy is used to examine the uptake of protein into individual particles. Observations reveal significant changes with protein size and, as is evident from the representative images in Figure 2, also reflect consistently faster uptake in the dextran-modified resins, which can be understood in terms of the comparative ability of protein to move in a three-dimensional dextran layer and on a two-dimensional agarose surface. The faster transport in XL and Capto shifts the optimum operation to lower ionic strengths where static capacities are higher, resulting in greatly improved dynamic binding capacities.

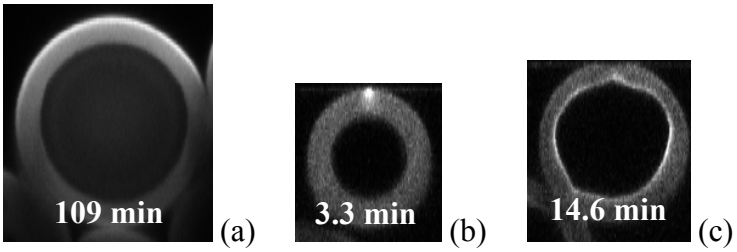

Figure 2: Confocal images showing times to reach similar fractional uptake of pH 7, 20 mM total ionic strength lactoferrin into (a) SP Sepharose FF, (b) Capto S, and (c) SP Sepharose XL. Uptake is clearly much faster in the two dextran-modified resins, even accounting for particle size differences, and is particular quick in Capto S.

The results of this work nicely address some of the broad questions regarding the advantages of the dextran-modified materials in terms of adsorption and transport properties, accounting for improvements in column performance. This work should be helpful in the design of ion-exchange steps and may help in the design of new resins. There are more subtle points that have yet to be adequately addressed to be considered for future work. For example, better understanding of the exclusion of larger proteins from the dextran-modified pores at high ionic strength could help to minimize such exclusion when selecting operating conditions for an ion-exchange step or when developing new particles, or perhaps this understanding could be used to tune the exclusion to actually enhance a separation. Also, improved knowledge of the how the physical and chemical properties of a resin affect the various aspects of its chromatographic performance could help guide the eventual development of a model that provides an appropriate mechanistic description of adsorption and transport in polymer-modified media.