Characterization of Cross-Linked Cellulosic Ion-Exchange Adsorbents: 2. Protein Sorption and Transport

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Abstract

Adsorption behavior in the HyperCel family of cellulosic ion-exchange materials (Pall Corporation) was characterized using methods to assess, quantitatively and qualitatively, the dynamics of protein uptake as well as static adsorption as a function of ionic strength and protein concentration using several model proteins. The three exchangers studied all presented relatively high adsorptive capacities under low ionic strength conditions, comparable to commercially available resins containing polymer functionalization aimed at increasing that particular characteristic. The strong cation- and anion-exchange moieties showed higher sensitivity to increasing salt concentrations, but protein affinity on the salt-tolerant STAR AX HyperCel exchanger remained strong at ionic strengths normally used in downstream processing to elute material fully during ion-exchange chromatography. Very high uptake rates were observed in both batch kinetics experiments and time-series confocal laser scanning microscopy, suggesting low intraparticle transport resistances relative to external film resistance, even at higher bulk protein concentrations where the opposite is typically observed. Electron microscopy imaging of protein adsorbed phases provided additional insight into particle structure that could not be resolved in previous work on the bare resins.

Keywords: Cellulosic materials, ion-exchange chromatography, salt-tolerant adsorbents, protein adsorption, confocal laser scanning microscopy, electron microscopy

1. Introduction

Cellulosic ion-exchange (IEX) materials exhibit several advantages for the adsorption of proteins during chromatographic processes. The hydrophilic nature of cellulose minimizes non-specific binding (Peterson, 1970) and the intricate, yet open, microstructure provides a large surface area for protein adsorption (Angelo et al., 2013; Graham and Fook, 1982; Peterson and Sober, 1956). These characteristics give rise to transport rates higher than commonly observed in traditional, macroporous adsorbents under favorable binding conditions, which help facilitate rapid diffusion along with high adsorptive capacities. In this regard, cellulosic media have characteristics similar to those of polymer-modified adsorbents, namely high binding capacities and rates of uptake (Bowes and Lenhoff, 2011a; Bowes et al., 2009; Fernandez and Carta, 1996; Lewus et al., 1998; Wang and Sun, 2007; Weaver and Carta, 1996), which typically exceed those of related conventional chromatographic media. That cellulosic media may be able to achieve comparable results without the use of end-grafted polymers (Wang and Sun, 2007) or covalently attached extenders (Bowes and Lenhoff, 2011b; Stone and Carta, 2007) makes them functionally attractive while being more homogeneously structured than conventional media.

Here we describe functional characterization of a family of cellulosic ion exchangers in order to elucidate the principal features of their chromatographic performance. This work complements the previously-reported structural characterization of the same group of materials (Angelo et al., 2013), allowing any clear structure–function relationships to be identified. The present work also includes additional structural characterization of the protein-loaded media.

The functional characteristics are studied via several experimental and analytical techniques. Adsorption isotherm measurements provide static binding capacities of each resin-protein system over a range of total ionic strength (TIS) values. They are complemented by isocratic retention experiments to allow determination of protein binding affinity at different solution conditions. Apart from their value in interpreting adsorption behavior, the relative values of the retention factor for any pair of solutes provide a direct measure of selectivity.

In chromatographic practice, the dynamic binding capacity is of greater interest than the static binding capacity, making measurement of protein transport rates essential (Müller, 2005; Shi et al., 2010). These measurements are confounded by the coupling between adsorption and transport, so mechanistic studies are of value. We pursue these here qualitatively, and to some extent quantitatively, using time-series confocal laser scanning microscopy (CLSM) and quantitatively using batch uptake kinetic measurements in an agitated bath. The determination of intraparticle resistances encountered by solutes being taken up into these adsorbents is important in the predictive modeling of behavior, and the overall efficiency of processes in scaled-up applications.

As with many chromatographic systems with unconventional bead morphologies, structure often dictates functional characteristics, specifically with regard to transport and protein localization. Electron microscopy imaging of protein-adsorbed phases can help elucidate aspects of this behavior, as has been illustrated previously for agarose-

based IEX materials (Bowes et al., 2009; Koku, 2011). Comparison of stationary phases with and without protein provides a qualitative understanding of how the particle structure, and therefore the space available for protein movement and binding within these materials, changes under different loading and operating conditions.

In this work, these techniques are put to use to characterize the adsorption of protein on three adsorbents. The roles of protein size, TIS of the solution, functional moieties, protein concentrations, and other operational factors are examined in order to assess qualities of adsorptive behavior. The adsorbents are based on the HyperCel base matrix: S, Q and STAR AX HyperCel. The first two are standard strong cation- and anion-exchange media respectively, while STAR AX is a salt-tolerant anion exchanger developed to allow for high protein affinities and capacities under moderate to high conductivities (Champagne et al., 2013). This allows for processing of feedstocks with high salt concentrations without the need for buffer exchange or dilution, resulting in more efficient downstream separations. Mechanisms of protein transport within these types of materials and comparisons with strong IEX counterparts have yet to be studied extensively.

2. Materials and Methods

2.1. Buffers, Protein Solutions and Stationary Phases

Monobasic sodium phosphate (NaH_2PO_4) and bis-tris were purchased from Fisher Scientific (Fair Lawn, NJ) and Sigma-Aldrich (St. Louis, MO), respectively, and used to prepare 10 mM sodium phosphate (for S HyperCel) and 50 mM bis-tris (for Q and STAR AX HyperCel) buffer solutions at pH 7. The TIS of solutions was adjusted using NaCl. Each HyperCel resin was washed successively with DI water and 1 M NaCl and then equilibrated with the desired TIS-controlled buffer.

Hen egg white lysozyme, BSA and bovine β -lactoglobulin were purchased from Sigma-Aldrich and bovine lactoferrin was obtained from DMV-International. Solutions were prepared by dissolving lyophilized protein in the desired TIS-controlled buffer and concentrated using 10K Amicon centrifugal filters. Concentrated samples were rediluted with buffer and reconcentrated three times, and the final protein solutions were filtered using 0.22 μm syringe filters. Lactoferrin was first purified to remove impurities by using a sodium chloride gradient on a 56 mL SP Sepharose FF cation-exchange column and collecting the primary elution peak. Protein concentrations were determined using UV spectrophotometry on a Shimadzu UV-1700 or a ThermoScientific NanoDrop 2000. Target protein concentrations for stock solutions were 4-6 mg/mL. Protein properties, including isoelectric point, molecular weight, effective radius and extinction coefficient at 280 nm, are shown in Table 1. Properties of the S, Q and STAR AX HyperCel resins were listed previously (Angelo et al., 2013).

2.2. Capillary Tube Calibration

Resin was drawn into Wiretrol II 100 and 200 μL capillary tubes to measure a given hydrated particle volume. For the AEX materials, capillary tubes were silanized using 4% 3-aminopropyltriethoxysilane (Fisher Scientific) in acetone in order to reduce

electrostatic interactions between the positively-charged resin and the inherently negatively-charged glass surfaces. Tubes were treated in an agitated vessel of aminosilane solution for 2.5 hours before being generously washed with pure acetone, followed by DI water three times. Tubes were then sonicated in DI water for 30 minutes to remove loosely physisorbed species and dried using high-pressure air. The procedure for determining factors relating resin height to hydrated particle volume has been described previously (Bowes et al., 2009). The capillary tube calibration factors are shown in Table 2.

2.3. Adsorption Isotherms

A series of protein dilutions were prepared in 1.6 mL Eppendorf tubes at concentrations ranging from 100% to 6.25% of that of the protein stock; the dilutions used the same TIS-controlled buffer in which the protein solutions were prepared. Small amounts of resin were dispensed into each tube via Wiretrol II capillary tubes after the gravity-settled height of the resin and the additional buffer volume added in each capillary tube were recorded. Samples were allowed to equilibrate by rotation for 5 days (for S and Q HyperCel) to 14 days (for STAR AX HyperCel). Final protein concentrations in the supernatant were determined by absorbance measurements at 280 nm. The amount adsorbed at the end of each experiment is determined by mass balance where the adsorbed concentration, q , is expressed in terms of mass per unit volume of hydrated particle volume, determined using the factors in Table 2. Points were fit to the Langmuir isotherm model in order to obtain maximum static binding capacities ($q_{\max}$) and affinity coefficients that characterize the softness of the isotherm. The Langmuir isotherm was used purely to obtain a curve to describe the data, with no assumptions made regarding the consistency of adsorption on HyperCel media with the assumptions underlying the derivation of the Langmuir isotherm.

2.4. Isocratic Retention

Isocratic retention measurements were made in 10 mM sodium phosphate buffer, pH 7, for S HyperCel and 50 mM bis-tris buffer, pH 7, for Q and STAR AX HyperCel, with NaCl added to adjust the TIS of the solution. A TIS of 1.5 M was used to obtain retention times of protein under unretained conditions. Various protein concentrations were used between roughly 0.25 mg/mL and 5 mg/mL for higher and lower TIS runs, respectively, in order for elution peaks of sufficient height to be developed. The measured retention time was not found to depend on the injection concentration for any of the cases examined. Twenty μ L samples were injected into 1 mL prepacked PRC HyperCel columns (Pall Life Sciences) at a flow rate of 0.2 mL/min (interstitial velocity ~ 60 cm/hr). Samples were run in triplicate, and the first moments of the resulting peaks were used to determine the retention times. The retention factor, k' , was calculated by

$$k' = \frac{t_r - t_{hs}}{t_{hs} - t_d} \quad (1)$$

where t_r is the retention time of the sample of interest, t_{hs} is the retention of that sample under unretained conditions (1.5 M TIS), and t_d is the system dead time without a

column in place. While t_{hs} as used here provides a functional measure of the retention time in the absence of binding to the resin, there is some uncertainty regarding the mechanistic interpretation because of the questions regarding partitioning of the protein into the resin in the absence of binding. However, our principal interest is in strongly retained conditions, where k' as calculated in Equation 1 provides an adequate measure.

2.5. Batch Uptake Kinetics

Batch uptake experiments were performed in a 100 mL baffled beaker agitated at 450 rpm by an impeller with a blade radius of 1 cm. The supernatant solution was continuously pumped at 20 mL/min, using an ÄKTA P960 sample pump (GE Healthcare), via an intake filter placed in the beaker directly above the impeller, through a UV detector on a GE Healthcare ÄKTA Explorer system, and back into the base of the beaker through a side port. The protein concentration was measured by UV absorbance at 280 nm and the dead volume of the loop was less than 2.5 mL.

Concentrated protein solutions were prepared in a similar fashion to those in the adsorption isotherm experiments. Aliquots of a concentrated protein solution and a buffer solution were then added to the beaker to bring the final volume to 60-70 mL at the desired initial protein concentration. Enough resin was measured using Wiretrol II capillary tubes to adsorb approximately 50% of the total protein concentration in the beaker. The resin was quickly added and the protein concentration was recorded until there was little additional protein depletion. The remaining solution and resin were collected into Nalgene bottles and rotated for 24-48 hours in order to measure the final protein concentration after allowing a much longer time for adsorption to be complete.

To estimate the uptake rate parameters, the experimental data were fit to different analytical relations describing transport into a spherical particle using the nonlinear least-squares regression routine, 'nlinfit', in Matlab. The simplest transport model is for external mass transfer control, in which the intraparticle resistance is neglected and the uptake rate depends on an external mass transfer coefficient, k_f , via (Carta and Jungbauer, 2010; Weaver and Carta, 1996)

$$\frac{C - C_f}{C_0 - C_f} = \exp\left(-\frac{3k_f V_m t}{R_p V}\right) \quad (2)$$

for the bulk solution protein concentration, or

$$\frac{\bar{q}}{q_{\max}} = \frac{V C_0}{V_m q_{\max}} \left[1 - \exp\left(-\frac{3k_f V_m t}{R_p V}\right) \right] \quad (3)$$

for the adsorbed protein concentration, where C_f is the final concentration in the bath once uptake is complete, $\bar{q}$ is the average protein concentration adsorbed within the material, R_p is the particle radius and V and V_m are the volumes of the bulk solution and the resin respectively. If intraparticle transport is primarily by homogeneous diffusion with a near-rectangular isotherm, this limit would be expected to apply in the limit of low initial protein concentration. Conversely, in the case of high initial protein concentrations, the intraparticle resistance would be expected to be controlling

(Fernandez and Carta, 1996; Helfferich, 1962; Weaver and Carta, 1996), and in the asymptotic limit of negligible external resistance, the homogeneous intraparticle diffusion model may be expressed as (Carta and Jungbauer, 2010; Weaver and Carta, 1996)

$$\frac{\bar{q}}{q_{\max}} = 1 - \frac{6}{\pi^2} \sum_{i=1}^{\infty} \frac{1}{i^2} \exp\left(-\frac{i^2 \pi^2 D_h t}{R_p^2}\right) \quad (4)$$

where D_h is the homogeneous diffusivity. Alternatively, for the case of dominant intraparticle resistance where transport is by pore diffusion, the analytical solution for the high-affinity limit (rectangular isotherm) is the shrinking-core model (Dziennik et al., 2005; Teo and Ruthven, 1986; Weaver and Carta, 1996).

Numerical solutions to the models were employed along with analytical solutions for purely external mass transfer and homogeneous diffusion resistances in order to compare fit transport parameters obtained assuming complete dominance of a singular transport regime. The numerical solution for homogeneous diffusion incorporated the external mass transfer resistance. The numerical solutions used the partial differential equation solver routine within Matlab, 'pdepe', to solve the conservation equations and boundary conditions for one-dimensional homogeneous diffusion within a spherical geometry. The concentration at the solution-particle interface is determined by the adsorption isotherm, for which the Langmuir isotherm was used for simplicity and its accurate description of the equilibrium concentrations.

2.6. Protein Uptake Profiles via Confocal Microscopy Imaging

Particles were packed into a flow cell designed for use in a confocal microscope (Dziennik et al., 2003). Reservoirs of equilibration buffer, protein solution, and high-salt buffer (1 M NaCl) were connected to an LKB Bromma positive displacement pump equipped with a multi-port valve to allow switching among buffer/protein solutions to be pumped through the flow cell. Flow rates used ranged from 0.25 to 1 mL/min, corresponding to linear velocities of 525-2100 cm/hr. The proteins used were fluorescently labeled with DyLight 650 NHS Ester (Thermo Scientific, 62266: amine-reactive dye packs) in a 1-2% labeling ratio following protocols supplied by the manufacturer. Particles were imaged using a Zeiss 5 LIVE DUO high-speed confocal microscope equipped with a 40x C-Apochromat (NA 1.2) water-immersion lens (Carl Zeiss), with images collected approximately every 5 seconds. The microscope was also equipped with a piezoelectric motor for fast scanning in the z (vertical) direction. In order to allow comparison of intensities, the laser intensity and gain were kept constant for each experiment involving uptake of the same protein and were set to the highest setting possible without saturating the detector once nearly full saturation was achieved.

Packed particles were washed with high-salt buffer and equilibrated at 1 mL/min before imaging began. A single particle was selected for continuous observation so as to capture the uptake and elution profiles in the xz plane using the z-stack functionality of the microscope. The time point at which feeding of the protein solution through the flow cell was started was marked in order to correct for the system dead volume (< 0.75 mL), and the protein solution was allowed to flow until apparent full particle saturation was achieved. The valve was then switched to feeding of high-salt buffer in order to

observe elution behavior. Imaging was performed until no fluorescently-labeled protein remained visible inside the particle or no appreciable decrease in fluorescence was observed.

2.7. Electron Microscopy

2.7.1. Sample Preparation

Particle samples for SEM and TEM were prepared using similar methods to those described previously (Angelo et al., 2013). Samples of resin with adsorbed lysozyme (for S HyperCel) or β -lactoglobulin (for Q and STAR AX HyperCel) were prepared in the same manner as for the adsorption isotherms (Section 2.3). A target loading of 50% of the maximum static binding capacity for each resin was used.

2.7.2. Scanning Electron Microscopy

Samples were temporarily fixed with 1% glutaraldehyde and 1% paraformaldehyde before being chemically fixed with 1% osmium tetroxide (OsO_4) in DI water. Samples were then washed with DI water and dehydrated in ethanol before the same processing method as for the samples without adsorbed protein (Angelo et al., 2013) was followed. Several samples without adsorbed protein were also chemically fixed using this procedure to allow a qualitative comparison between phases. All particle samples were sputter-coated with palladium-gold for 4 minutes at a current of 30 mA and an argon pressure of 65 mtorr. SEM imaging was performed using a Hitachi S4700 FESEM operated in secondary electron mode with a voltage of 2.0 kV and a working distance of 2-3 mm.

2.7.3. Transmission Electron Microscopy

All samples were prepared using 10 mM sodium phosphate buffer at pH 7 for the initial fixation, as amine groups in bis-tris cause undesired imaging artifacts (Angelo et al., 2013; Hayat, 2000). Samples were temporarily fixed using 1% glutaraldehyde and 1% paraformaldehyde before being chemically fixed using 1% osmium tetroxide in DI water. Samples were then washed with DI water and dehydrated in acetone before the same processing method was followed as for the samples without adsorbed protein (Angelo et al., 2013). Q and STAR AX HyperCel materials were also stained using 1% glutaraldehyde and 1% tannic acid, followed by the remainder of the standard sample processing; this is the so-called TAGO fixation method (tannic acid-glutaraldehyde-osmium tetroxide) (Hayat, 2000). Tannic acid acts as a mordant for the OsO_4 , binding to the cellulosic backbone of the material as opposed to only the functionalized ligands of the ion exchangers. This increases contrast provided by the OsO_4 as well as allowing the staining of material without a favorable affinity to the heavy metal stain (e.g., sulfonated resins). All particle samples were microtomed into sections approximately 50 nm in thickness and imaging was performed on a Zeiss LIBRA 120 transmission electron microscope operated at 120 kV.

3. Results and Discussion

3.1. Adsorption Isotherms

Adsorption isotherms of β -lactoglobulin and BSA on STAR AX and Q HyperCel are shown in Figure 1. Isotherms at 20, 50, and 100 mM TIS were measured for both resins, but only for STAR AX at 200 mM TIS since the 100 mM TIS data set on the Q resin revealed relatively low static capacity. The typical hyperbolic shape is seen for all the plots, and the fits to the Langmuir isotherm model are reasonable; values of the maximum static binding capacity and the affinity coefficient from the fits are given in Table 3. These results verify that the ligand chemistry of the STAR AX material allows a significantly increased maximum static binding capacity compared to that on Q HyperCel with increasing TIS, with STAR AX having more than double the capacity of Q at 100 mM TIS. Examination of these results also suggests that protein size is not a significant factor affecting adsorptive capacity for Q HyperCel, since β -lactoglobulin and BSA have molecular weights that differ by approximately 50 kDa. However, STAR AX HyperCel shows higher sensitivity to TIS of the static capacity for BSA compared to β -lactoglobulin. The increased scatter in the data at lower TIS values for STAR AX HyperCel may be due to variability in measuring resin volumes in the capillary tubes, where increased adhesion of STAR AX to the tube wall was noted.

Isotherms of lysozyme and lactoferrin on S HyperCel are shown in Figure 2 at 20, 50 and 100 mM TIS. Despite the difference in the protein sizes, capacities for both proteins at 20 mM TIS are very similar. The 2-lobed structure of lactoferrin may contribute to this, as the highly positively charged lobe could orient the protein in the adsorbed state (Bowes et al., 2009). Another noteworthy feature is the sharper decline of the maximum capacity for lactoferrin with increasing salt. This may reflect an exclusion effect similar to that seen on polymer-modified media for larger proteins (Bowes and Lenhoff, 2011a) or could be due, as above, to the surface charge distribution on lactoferrin, which may make it more susceptible to electrostatic screening (Kisley et al., 2014).

The relatively high binding capacities across all three materials are particularly noteworthy, as capacities of these magnitudes have, for the most part, been achievable only on materials with polymer extenders used to expand the adsorptive surface area into a three-dimensional volume (Bowes and Lenhoff, 2011a, 2011b; Bowes et al., 2009; Stone and Carta, 2007; Wang and Sun, 2007; Weaver and Carta, 1996; Yu and Sun, 2013; Yu et al., 2013). Our investigation into the pore structure of HyperCel resins (Angelo et al., 2013) discussed how these materials exhibit characteristics similar to polymer-modified stationary phases, but are not polymer-modified themselves, as the base matrix does not undergo polymer grafting or covalent attachment during manufacturing. The gel-like quality of the cellulosic beads, narrow pore space and high pore interconnectivity permitted by the carbohydrate structure may provide a large enough surface area for adsorption to be comparable to that on this class of adsorbents.

3.2. Isocratic Retention

Results of the isocratic retention experiments for the three HyperCel materials are shown in Figure 3 for lysozyme and lactoferrin on S HyperCel and for β -lactoglobulin and BSA on Q and STAR AX HyperCel, as a function of TIS. Retention factors below 0.1 were truncated because of the uncertainties in these values under weakly-retained conditions, while retention factors greater than about 100 are difficult to determine because of the very long retention times and broad peaks.

Retention was found to be strongest on STAR AX HyperCel and weakest on Q HyperCel. This result was expected for the salt-tolerant primary amine ligand chemistry at higher TIS values and is consistent with the high static capacities that were determined from the adsorption isotherms. The high retention factors are also consistent with the highly-favorable near-rectangular isotherms that were obtained, as k' values are directly related to the slope of the linear portions of the corresponding isotherms. Despite the differences between the k' values on the two anion exchangers, the slopes of the respective curves for the two proteins are very similar. This is consistent with previous results (Bowes et al., 2009; DePhillips and Lenhoff, 2001; Traylor et al., 2014) that show the slope to be a strong function of the protein, independent of the adsorbent, at a given pH.

Retention data on S HyperCel show stronger retention of lactoferrin than of lysozyme. This may be related, in addition to the higher net charge on lactoferrin, to its charge distribution, which exhibits a dense cluster of positively-charged residues (Bowes et al., 2009). This attribute seems to have an effect on the strength of adsorption, and possibly thereby the transport characteristics, differentiating it from the other model proteins that were investigated.

3.3. Uptake Kinetics

Batch uptake kinetics were measured for lysozyme and lactoferrin on S HyperCel, β -lactoglobulin on Q and STAR AX HyperCel and BSA on Q HyperCel. Experiments were performed at 20 and 100 mM TIS for β -lactoglobulin and lysozyme and at 20 mM TIS for BSA and lactoferrin, all at pH 7. Two initial protein concentrations were also used in all cases, 2 ± 0.2 mg/mL and 0.2 ± 0.02 mg/mL, in order to exploit regimes where intraparticle and external mass transfer resistances respectively are expected to be dominant. Judgment of the applicability of particular mass transfer models was aided by CLSM observations of intraparticle intensity profiles (Section 3.4), with diffuse profiles indicating a homogeneous diffusion mechanism and sharp uptake fronts a pore diffusion mechanism (Bowes and Lenhoff, 2011a; Hubbuch et al., 2003; Tao et al., 2011; Yang et al., 2006). The former are generally seen at high TIS and the latter more often at low TIS, but the distinction may sometimes be uncertain. Similarly, distinguishing whether the dominant resistance is intraparticle or extraparticle may also be uncertain.

Figure 4 shows the uptake of lysozyme and lactoferrin on S HyperCel. For a high concentration of lysozyme, the added particles reach full saturation in less than three minutes, which is very rapid when compared to other conventional materials (Dziennik et al., 2003; Weaver and Carta, 1996) and even to polymer-modified adsorbents

(Bowes and Lenhoff, 2011a; Shi et al., 2010; Weaver and Carta, 1996). Fits to the rapid uptake are shown to both the homogeneous diffusion model and the external mass transfer model, as uptake this fast indicates very low intraparticle resistance. There is much better agreement with the homogeneous diffusion model than the pure external control model, but both give reasonable descriptions of the data. The numerical solution of the homogeneous diffusion model that was utilized in these cases includes the external mass transfer resistance in the boundary conditions, which explains its closer fit than the analytical form of the external control model. The value of k_f was obtained from the experiments at the lower initial concentration, in which the external mass transfer control model is more appropriately applied.

Lysozyme uptake at the lower initial protein concentration requires an increased time to achieve saturation under the stronger binding condition (20 mM TIS), and the external mass transfer control model adequately describes the uptake behavior. The lower protein concentration in the bulk solution would decrease the extraparticle flux but not affect the intraparticle transport rate appreciably if this latter contribution is predominantly homogeneous. In this case, the bulk protein concentration becomes the driving force for the mass transfer as opposed to the adsorbed concentration in the case of homogeneous diffusion. Extremely good agreement between the external control model and the data was observed whenever it was applied to uptake curves obtained using a lower initial protein concentration.

Lactoferrin uptake was much slower than that of lysozyme (note the differences in time scale), indicating significant intraparticle resistance. The data were fit to the numerical solution of the homogeneous diffusion model and the external resistance model for the high and low initial protein concentrations, respectively. Both models provide good fits to their respective data sets. The increased time for apparent full saturation to occur for this system could be due to several factors, including steric hindrance for the larger lactoferrin molecule or the higher adsorption affinity and hence lower contribution from homogeneous diffusion inferred for lactoferrin under strong-binding conditions (see Section 3.2). The propensity for homogeneous diffusion is thought to be related to the ability of adsorbed protein to diffuse and hence to the protein-surface affinity (Lenhoff, 2008; Wesselingh and Bosma, 2001). The analytical form of the pore diffusion model (i.e., the shrinking-core model) also provides a good fit to the high initial protein concentration data (not shown), indicating some uncertainty in the transport mechanism for lactoferrin; this is addressed further in section 3.4.

The external mass transfer coefficients and diffusivities gathered from these fits are shown in Tables 4 and 5, respectively, along with the results for the anion-exchange adsorbents discussed further below. Fitted k_f values are comparable to those predicted by standard correlations for mass transfer coefficients in stirred vessels (Armenante and Kirwan, 1989; Levins and Glastonbury, 1972). Homogeneous diffusivities (found numerically) are given for only the high initial protein concentration systems ($C_0 = 2 \pm 0.2$ mg/mL) as external mass transfer control is dominant for low initial protein systems and homogeneous diffusivity values could misrepresent the resistances present. However, the external mass transfer resistance was accounted for in all the systems studied in view of the rapid uptake and hence relatively low intraparticle resistance. The fact that there are only small differences in the fitted values of k_f between low and high initial protein concentrations for similar systems indicates that the external mass transfer

resistance is a significant factor regardless of bulk solution concentration, and despite the fairly vigorous agitation used experimentally. The homogeneous diffusivities are, as expected, orders of magnitude lower than for the free-solution diffusivities (Chang and Lenhoff, 1998; Chen et al., 2002), and the generally increased values at higher salt concentrations are consistent with the relationship to strength of adsorption noted above (Lenhoff, 2008; Wesselingh and Bosma, 2001).

Discrepancies between the plateau concentrations in the uptake experiments and capacities found from the adsorption isotherms may be due to errors in measuring resin volumes in the capillary tubes, the inability to remove all fluid and resin material from the agitation vessel, leading to an improper final mass balance, or to a slow mode of uptake in the isotherm measurements. However, even the largest discrepancies in these values did not exceed 15% error, and capacities obtained for each individual uptake experiment were used for model fitting where appropriate.

Figure 5 shows protein uptake on Q HyperCel, and much as in the case of lysozyme on S HyperCel, all plots reflect very fast uptake and good fits to the external resistance model. That this is the case for both β -lactoglobulin and BSA indicates that protein size is not as significant in determining the mechanism of transport as protein affinity, with only lactoferrin uptake reflecting a dominant intraparticle resistance. Transport parameters for the β -lactoglobulin data are of the same order of magnitude as values for the lysozyme data, albeit somewhat slower transport is reported. The BSA data present even slower uptake, but only marginally. While this can again be inferred as a function of protein size relating to its mobility, it is also dependent on the protein-surface affinity for each protein-adsorbent system.

Figure 6 shows the uptake of β -lactoglobulin on STAR AX HyperCel. Unlike uptake in the other two adsorbents, STAR AX exhibits a counterintuitive trend in the apparent capacity between 20 and 100 mM TIS. For both the high and low initial bulk concentrations, the uptake of β -lactoglobulin at 100 mM TIS overtakes the uptake at 20 mM TIS, although both have similar maximum static binding capacities based on isotherm measurements (Figure 1). However, the kinetic experiments were carried out for a period no longer than 45-75 minutes for either case, while the isotherm measurements had up to 14 days to equilibrate (which was the case for protein adsorption on STAR AX HyperCel at low TIS).

While uptake remains fairly rapid using a high bulk protein concentration, the apparent equilibrium reached was far below the maximum static capacity for each system, suggesting bimodal uptake that includes a period of slow uptake beyond the time range shown. This bimodal uptake behavior could be explained by a rapid superficial loading of protein that saturates particles to one- to two-thirds of the total capacity, followed by a slower contribution that may reflect protein rearrangement within the particle. This type of uptake has been modeled conceptually using a partial shrinking-core model (Bowes and Lenhoff, 2011a), characterized by rapid but incomplete shrinking-core uptake preceding a backfilling of additional free capacity over a longer time scale. During normal operation in a downstream purification setting, a nominal salt concentration (greater than 20 mM TIS, approximately a 2 mS/cm conductivity) may be present, in which case this unfavorable uptake behavior would become negligible.

It has been shown that the ligand chemistry of STAR AX has some effect on the nature of the pore architecture and may hinder fast transport of protein to some extent (Angelo et al., 2013). The structural differences in conjunction with the much higher affinity for β -lactoglobulin on the salt-tolerant material could be what slows initial uptake to only a fraction of the total adsorptive capacity. Previous studies have shown that small relative pore sizes and strong retention contribute to hindering the diffusion of adsorbed species with slow kinetic off-rates, especially at higher loadings (Traylor et al., 2014). However, over longer time scales this behavior becomes less significant.

A mechanistic model that describes transport of this type is difficult to specify in detail, as the diffusion of protein is slowed by unfavorable binding kinetics and/or steric hindrance in the pore; an alternative interpretation is in terms of single-file diffusion in multicomponent systems (Perez-Almodovar et al., 2012; Tao et al., 2012). However, the initial uptake (< 200 seconds) is still very rapid for high bulk protein concentrations, where it can be assumed that external mass transfer resistance is still dominant in the case of low bulk solution concentrations.

3.4. Protein Uptake Profiles via Confocal Microscopy Imaging

Confocal microscopy was used for real-time observation of intensity profiles of fluorescently-labeled protein in order to obtain information on the mechanism of protein transport within the stationary phase (Bowes and Lenhoff, 2011a; Bowes et al., 2012; Dziennik et al., 2005, 2003; Ljunglöf and Hjorth, 1996; Ljunglöf and Thömmes, 1998; Tao et al., 2011). Batch uptake experiments allowed a quantitative analysis of uptake rates as well as determination of the appropriate rate model to apply to the data. The qualitative form of the concentration profile that may be seen from confocal microscopy can reveal two limiting cases, one consistent with homogeneous diffusion and the other consistent with pore diffusion, in the shrinking-core limit (see Section 2.5). A homogeneous uptake mechanism exhibits concentration profiles that are diffuse, and a shrinking-core mechanism gives rise to a sharp protein front that moves toward the center of the particle (Bowes and Lenhoff, 2011a; Dziennik et al., 2005, 2003; Hubbuch et al., 2003; Tao et al., 2011; Yang et al., 2006).

The very fast uptake seen in HyperCel under most conditions (section 3.3) leads to the hypothesis that HyperCel displays a homogeneous diffusion mechanism; this is consistent with its similarities to hydrogels (Lewus and Carta, 1999; Weaver and Carta, 1996) and other natural polymers. Figure 7 shows the uptake of DyLight 650-labeled lysozyme in S HyperCel resin at 20 and 100 mM TIS. The value shown in the lower left of each image indicates the time elapsed after switching to the feed stream, corrected for the system dead time. Uptake is qualitatively homogeneous, with the images presenting no sharp uptake front that would indicate pore diffusion being the dominating mechanism of protein transport. Apparent full saturation of the particle was achieved before three minutes had elapsed, in agreement with the batch uptake data for lysozyme on S HyperCel under similar conditions.

The differences in fluorescence intensity between 20 and 100 mM TIS correspond to the lower overall binding capacities that are seen at higher TIS values, as with the batch uptake and adsorption isotherms. Relative adsorbed concentrations can be

inferred from the differences in intensity, as laser power and gain settings were kept the same across runs at different operating conditions.

Much slower uptake is observed for lactoferrin uptake in S HyperCel (Figure 8), again consistent with the batch data. The fluorescence intensity profiles in Figure 8 are sharp, consistent with a shrinking-core mechanism, indicating a strong pore-diffusion contribution. As noted in section 3.3, the batch uptake curves could be adequately described by either a pore- or a homogeneous-diffusion model, so the confocal images highlight the significance of obtaining real-time profiles to allow for more informed model discrimination.

The fitted homogeneous diffusivity for lactoferrin in S HyperCel is reported in Table 5 for comparison with the other uptake data, while application of the shrinking-core model to the batch uptake data gives an effective pore diffusivity of $1.5 \times 10^{-7} \text{ cm}^2/\text{s}$. The pore diffusivity value obtained by fitting the shrinking-core model to the radial position of the sharp uptake front (Bowes and Lenhoff, 2011a; Dziennik et al., 2005) is $4.9 \times 10^{-7} \text{ cm}^2/\text{s}$. Both pore diffusivity values are of the same order of magnitude as that for lactoferrin uptake on Capto S (Bowes and Lenhoff, 2011a), a dextran-functionalized agarose-based material that presents higher uptake rates than its agarose-based counterparts (SP Sepharose XL and SP Sepharose FF) under similar conditions. The discrepancy between the two pore-diffusivity values reflect a faster apparent saturation observed via confocal microscopy than the overall uptake seen by batch uptake. This discrepancy is consistent with a partial shrinking-core mechanism, in which the initial rapid shrinking-core uptake does not saturate the adsorbent and a significant portion of the particle saturation takes place after the uptake front reaches the particle center (Bowes and Lenhoff, 2011a). A gradual increase in the fluorescent intensity throughout the particle after the sharp front reaches the center also validates the presence of a backfilling mechanism. This mechanism may reflect slow rearrangement of initially sorbed protein, with the rate limited by the protein size or the strong sorption at low salt.

The uptake of DyLight 650-labeled β -lactoglobulin in Q HyperCel resin at 20 and 100 mM TIS presented results very similar to those for lysozyme uptake in S HyperCel at the same operating conditions, so they are not shown. The rapid apparent full particle saturation again suggests low intraparticle resistance and a homogeneous diffusion mechanism, as with lysozyme in S HyperCel.

Figure 9 shows uptake of DyLight 650-labeled β -lactoglobulin in STAR AX HyperCel at 20 and 100 mM TIS. The uptake front, while still diffuse, appears slightly sharper than for the other cases apart from lactoferrin, suggesting a reduced contribution from homogeneous diffusion. Rapid saturation, as with the S and Q moieties, is still observed, however. The lower fluorescence intensity at 20 mM TIS than at 100 mM TIS is consistent with the batch uptake results at the same operating conditions. For the time scale of these uptake experiments, STAR AX HyperCel has a higher capacity for protein at higher TIS and shorter time scales, presumably caused by attenuation of homogeneous diffusion caused by the strong affinity for protein at low salt conditions. Despite the strong attraction for protein on this salt-tolerant resin, regeneration at 1 M NaCl was adequate to remove all protein from the system (not shown).

All the confocal microscopy figures show uptake under a high linear flow rate of 2100 cm/hr (1 mL/min). This flow rate was required in order to reduce particle

movement within the flow cell, as lower flow rates could allow particles to shift during elution, thereby providing inadequate axial profile data. Flow packing using both high-salt and equilibration buffer was used to ensure optimal packing. Comparison of intensity plots between uptake profiles at high and low superficial velocities was used to determine that the intraparticle transport of protein was independent of the flow rates used for these systems (data not shown).

3.4.1. Intraparticle Voids

Intraparticle voids were visible in sections of all HyperCel materials when imaged using different microscopy techniques. The presence of the voids has been discussed previously (Angelo et al., 2013), mostly with regard to electron microscopy of these adsorbents. In this work, the voids could also be seen during the uptake of protein during real-time confocal imaging. During uptake, the voids are much lower in fluorescence intensity than the rest of the cross-section of the particle, most likely due to lower ligand density, as the cellulosic density is also assumed to be lower in these regions. Conversely, during protein elution, these spots have a higher fluorescence intensity than the rest of the particle, presumably because the lower backbone density facilitates pooling of fluorescently-labeled protein before it fully leaves the particle. The narrow pore size of these materials acts as a bottleneck, retarding full elution of protein by some degree when compared to other, more open, stationary phases, but the overall effect is minimal (Angelo et al., 2013).

3.5. Electron Microscopy

3.5.1. Scanning Electron Microscopy

Figure 10 shows whole-particle SEM images of Q and STAR AX HyperCel containing adsorbed protein. β -lactoglobulin was loaded to 57% of the maximum static binding capacity on Q HyperCel and to 45% of the maximum static binding capacity on STAR AX HyperCel. It is apparent that the protein gives rise to globular structures on the surfaces of the material, giving it a rough topography as opposed to the open fibrous network described previously for phases devoid of protein (Angelo et al., 2013). The globular formations on Q HyperCel appear too large to be individual protein molecules or even small clusters, but the texture is much finer on the STAR AX resin, where the formations could be single protein molecules or clusters of molecules.

Comparison of these images to SEM micrographs of other stationary phases on carbohydrate base matrices (i.e., Sepharose materials) (Bowes et al., 2009; Koku, 2011) sheds light on what can be inferred about the protein localization. These images are qualitatively similar to those of dextran-modified Sepharose XL, which presents a volumetric layer engulfed with protein molecules under similar loading conditions. Sepharose FF, an agarose base structure lacking polymer extenders, does not exhibit any sort of volumetric swelling when loaded with protein.

HyperCel may have cellulosic extenders akin to a polymer-grafted resin, suggesting that the HyperCel materials exhibit structural characteristics similar to those of polymer-modified stationary phases. Small-angle neutron scattering on these

materials suggests that protein adsorbs fairly uniformly within the particle (Koshari et al., 2015), including the possibility of protein partitioning throughout most of the particle volume. The globular formations that can be seen in the electron micrographs makes the determination of other structural characteristics somewhat difficult. The use of a lower protein loading may be better for distinguishing the presence of extenders.

3.5.2. Transmission Electron Microscopy

TEM images of Q HyperCel with and without adsorbed protein chemically fixed to the material are shown in Figure 11. The protein loading on the protein-adsorbed samples was 52% of the maximum static binding capacity. Each figure shows the results of the two methods of fixation as well as the protein-free and protein-adsorbed phases. With both methods of fixation, the protein-loaded phases seem to be less fibrous and open, suggesting that protein is filling the negative space and swelling the material. The TAGO fixation method presents a more disconnected pore network within the material without protein adsorbed, but does allow for sharper resolution and clearer delineation between the positive and negative spaces in the protein-adsorbed phase. The dark spots in the TAGO fixed samples are most likely deposits of OsO_4 that had aggregated during osmication. It was verified that these were not artifacts from the post-staining procedure by imaging sections that had undergone fixation but not post-staining.

STAR AX HyperCel (Figure 12) differs from Q HyperCel in that the structure seems to be more homogeneous, with poorer discrimination between pore space and the cellulose backbone. The protein loading on the protein-adsorbed samples was 49% of the maximum static binding capacity. The TAGO fixation method does not seem to change the result much from the standard aldehyde fixation, albeit the contrast in these samples is better since tannic acid acts as a mordant for the OsO_4 . In the case of the protein-free samples, there is little to no heterogeneity in the structure, implying a much smaller and complex pore network. When protein is adsorbed, the presence of pores becomes more apparent, especially in the TAGO-fixed case. Protein seems to localize in homogeneous pockets, and the fibrous nature of cellulose becomes more apparent, as may be seen on the edges of these areas; it would seem that there are areas for protein localization interspersed with more vacant areas, which could be the pores. The dark spots are most likely OsO_4 deposits as seen in Q HyperCel and do not signify any particular structural characteristic or protein cluster.

S HyperCel (Figure 13) was stained using standard aldehyde fixation only. Because OsO_4 has an affinity for positive charges, such as amines, it did not stain the protein-devoid S HyperCel phase very well since not much definition in the image is apparent. The lysozyme itself in the protein-adsorbed phase acted as a mordant for the OsO_4 , resulting in a much sharper image of the intraparticle morphology. Much like Q HyperCel, it shows a fibrous pore architecture with definite vacant pore spaces interspersed between regions of denser protein localization. This result suggests that the alternative ligand chemistry in STAR AX HyperCel in some way alters the pore structure and adsorption behavior within the material, even though the materials are based on the same cellulosic base matrix.

4. Conclusions

This work presents the adsorptive characteristics of protein onto both strong ion-exchange cellulosic adsorbents and a salt-tolerant moiety using changes in total ionic strength of solution as a variable condition in all cases. The cellulosic HyperCel materials allowed for a high static binding capacity of protein, especially in strong binding conditions (i.e., low salt concentrations), as well as very rapid uptake of protein, apparent via both batch kinetics and confocal microscopy. Analysis of the adsorptive protein uptake indicated that the intraparticle diffusion resistances, which are normally rate-controlling in most porous beaded materials, were near-negligible in comparison to external film transfer resistances. This exceptionally fast uptake occurs by a homogeneous diffusion mechanism that is aided by protein-surface attraction that is not excessively high. The very fast uptake allows very efficient column loading and suggests that particles of the same composition but larger in total diameter would be feasible without a significant loss in uptake rate, allowing reduced pressure drop and the possibility of larger packed beds.

While the structure of these adsorbents has been studied previously (Angelo et al., 2013), observing changes in the surface topography and TEM micrographs under protein-adsorbed conditions helps to elucidate the nature of the pore microstructure. Further evidence that HyperCel displays polymer-modified-like characteristics is apparent from small-angle neutron scattering (Koshari et al., 2015) and from SEM imaging of adsorbed phases, in which the pore architecture could be more clearly seen in STAR AX HyperCel when protein was adsorbed, a system where definite structural characteristics could not be resolved via TEM in a phase devoid of protein. The work presented provides a good complement to the investigation of the structural characteristics for the same materials, as adsorption of protein has a definite impact on the native architecture of the beads, and by extension, the transport mechanisms that govern the movement of solutes through these stationary phases.

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Figure captions

- Figure 1: Adsorption isotherms for β -lactoglobulin (a, b) and BSA (c, d) on Q (a, c) and STAR AX (b, d) HyperCel in 50 mM bis-tris, pH 7, at 20 (■), 50 (●), 100 (▲), and 200 (▼) mM TIS. Data are shown with fits to the Langmuir isotherm.
- Figure 2: Adsorption isotherms for lysozyme (top) and lactoferrin (bottom) on S HyperCel in 10 mM sodium phosphate, pH 7, at 20 (■), 50 (●), and 100 (▲) mM TIS. Data are shown with fits to the Langmuir isotherm.
- Figure 3: Isocratic retention data for HyperCel resins: BSA (□) and β -lactoglobulin (○) on Q HyperCel, BSA (■) and β -lactoglobulin (●) on STAR AX HyperCel, lactoferrin (▽) and lysozyme (△) on S HyperCel. Measurements were performed using 1 mL prepacked PRC columns operated at 60 cm/hr in 10 mM sodium phosphate, pH 7, for S HyperCel and 50 mM bis-tris, pH 7, for Q and STAR AX HyperCel; injection volume was 20 μ L.
- Figure 4: Batch uptake of lysozyme (a, b) and lactoferrin (c) on S HyperCel in 10 mM sodium phosphate, pH 7. a) $C_0 = 2$ mg/mL; TIS of solution as shown. Data fit to external mass transfer (—) and homogeneous diffusion (---) models. b) $C_0 = 0.2$ mg/mL; TIS of solution as shown. Data fit with external mass transfer control model. c) C_0 are shown on plot; data fit to external mass transfer (—) and homogeneous diffusion (---) models for low and high initial protein concentrations, respectively. Note different time scales.
- Figure 5: Batch uptake of β -lactoglobulin (a, b) and BSA (c) on Q HyperCel in 50 mM bis-tris, pH 7. a) $C_0 = 2$ mg/mL; TIS of solution as shown. Data fit to external mass transfer (—) and homogeneous diffusion (---) models. b) $C_0 = 0.2$ mg/mL; TIS of solution as shown. Data fit to external mass transfer model. c) C_0 as shown on plot; data fit to external mass transfer (—) and homogeneous diffusion (---) models. Note different time scales.
- Figure 6: Batch uptake of β -lactoglobulin on STAR AX HyperCel in 50 mM bis-tris, pH 7. a) $C_0 = 2$ mg/mL; TIS of solution as shown. Data fit to external mass transfer (—) and homogeneous diffusion (---) models. b) $C_0 = 0.2$ mg/mL; TIS of solution as shown. Data fit to external mass transfer control model. Note different time scales.
- Figure 7: Uptake of Dylight 650-labeled lysozyme on S HyperCel at 20 mM (top) and 100 mM (bottom) TIS (10 mM sodium phosphate, pH 7). Feed solution contained 2 mg/mL protein flowing at 2100 cm/hr.

- Figure 8: Uptake of Dylight 650-labeled lactoferrin on S HyperCel at 20 mM TIS (10 mM sodium phosphate, pH 7). Feed solution contained 2 mg/mL protein flowing at 2100 cm/hr.
- Figure 9: Uptake of Dylight 650-labeled β -lactoglobulin on STAR AX HyperCel at 20 mM (top) and 100 mM (bottom) TIS (50 mM bis-tris, pH 7). Feed solution contained 2 mg/mL protein flowing at 2100 cm/hr.
- Figure 10: SEM images of Q (a, b) and STAR AX (c, d) HyperCel at low (a, c) and high (b, d) magnification. Both materials were loaded to approximately 50% of the maximum capacity of β -lactoglobulin and chemically fixed with OsO_4 .
- Figure 11: TEM images of Q HyperCel prepared using standard aldehyde fixation (a, b, e, f) and TAGO fixation (c, d, g, h); no protein adsorbed (a, c, e, g) and loaded to approximately 50% of maximum capacity of β -lactoglobulin (b, d, f, h). Low (a, b, c, d) and high (e, f, g, h) magnification are shown.
- Figure 12: TEM images of STAR AX HyperCel prepared using standard aldehyde fixation (a, b, e, f) and TAGO fixation (c, d, g, h). No protein adsorbed (a, c, e, g) and loaded to approximately 50% of maximum capacity of β -lactoglobulin (b, d, f, h). Low (a, b, c, d) and high (e, f, g, h) magnification are shown.
- Figure 13: TEM images of S HyperCel with (a) no protein adsorbed and (b) loaded to approximately 50% of maximum capacity of lysozyme.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

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Figure 10

Figure 11

Figure 12

Figure 13

Table 1: Characteristics of proteins used in adsorption experiments; r = radius of sphere of equivalent volume; ε = absorbance.

Protein	pI	M _w (kDa)	r (nm)	ε (cm ² /mg)
Lysozyme	11.4	14.3	1.6	1.64
Lactoferrin	8.8	78	2.8	1.51
β-lactoglobulin	5.4	18.3	1.75	0.962
BSA	4.7	66.5	2.7	0.659

Table 2: Calibration factors to convert resin height in Wiretrol II capillary tubes into hydrated particle volume

	Calibration factor (mL hyd. part. vol./mm)
S HyperCel	0.00182
Q HyperCel	0.00197
STAR AX HyperCel	0.00182

Table 3: Langmuir isotherm parameters for HyperCel adsorbents

Resin	Protein	TIS (mM)	q _{max} (mg/mL)	b (mL/mg)
S HyperCel	lysozyme	20	250	37
		50	197	28
		100	170	2
	lactoferrin	20	247	41
		50	109	25
		100	23	2
Q HyperCel	β-lactoglobulin	20	326	28
		50	261	7
		100	144	1
	BSA	20	283	173
		50	176	10
		100	86	1
STAR AX HyperCel	β-lactoglobulin	20	338	121
		50	335	80
		100	307	30
		200	160	121
	BSA	20	244	1479
		50	260	333
		100	202	215
		200	157	3

Table 4: External mass transfer coefficients for HyperCel materials (10^{-3} cm/s); 95% confidence intervals are shown for all values

Resin	Protein	$C_0 = 2 \pm 0.2$ mg/mL		$C_0 = 0.2 \pm 0.02$ mg/mL	
		20 mM TIS	100 mM TIS	20 mM TIS	100 mM TIS
S HyperCel	lysozyme	3.02 ± 0.03	1.49 ± 0.05	3.40 ± 0.04	1.49 ± 0.03
	lactoferrin	n/a	n/a	0.71 ± 0.01	n/a
Q HyperCel	β -lactoglobulin	2.03 ± 0.04	1.02 ± 0.05	2.67 ± 0.03	1.03 ± 0.04
	BSA	1.73 ± 0.08	n/a	1.76 ± 0.07	n/a
STAR AX HyperCel	β -lactoglobulin	1.53 ± 0.04	1.45 ± 0.03	2.51 ± 0.07	2.07 ± 0.05

Table 5: Homogeneous diffusivities for HyperCel materials (10^{-8} cm²/s); 95% confidence intervals are shown for all values

Resin	Protein	$C_0 = 2 \pm 0.2$ mg/mL	
		20 mM TIS	100 mM TIS
S HyperCel	lysozyme	4.72 ± 0.01	7.92 ± 0.18
	lactoferrin	$0.18 \pm .0001$	n/a
Q HyperCel	β -lactoglobulin	2.68 ± 0.002	5.77 ± 0.01
	BSA	1.44 ± 0.03	n/a
STAR AX HyperCel	β -lactoglobulin	2.87 ± 0.001	1.88 ± 0.001