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Ionic strength-dependent changes in tentacular ion exchangers with variable ligand density. I. Structural properties

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Abstract

The ligand density critically affects the performance of ion-exchange resins in such measures as the adsorption capacity and transport characteristics. However, for tentacular and other polymer-modified exchangers, the mechanistic basis of the effect of ligand density on performance is not yet fully understood. In this study we map the ionic strength-dependent structural changes in tentacular cation exchangers with variable ligand densities as the basis for subsequent investigation of effects on functional properties. Inverse size-exclusion chromatography (ISEC), scanning electron microscopy (SEM) and small-angle x-ray scattering (SAXS) were used to assess the effect of ionic strength on the pore size and intraparticle architecture of resin variants with different ligand densities. Comparison of ISEC and cryo-SEM results shows a considerable reduction in average pore size with increasing ligand density; these methods also confirm an increase of average pore size at higher ionic strengths. SAXS analysis of ionic strength-dependent conformational changes in the grafted polyelectrolyte layer shows a characteristic ionomer peak at values of the scattering vector q ($0.1 < q < 0.2 \text{ \AA}^{-1}$) that depend on the ligand density and the ionic strength of the solution. This peak attribution reflects nanoscale changes in the structure of the grafted polyelectrolyte chains that can in turn be responsible for observed pore-size changes in the tentacular resins. Finally, salt breakthrough experiments confirm a stronger Donnan exclusion effect on pore accessibility for small ions in the high ligand density variant.

Keywords

Ligand density; cation-exchange chromatography (CEX); small-angle x-ray scattering (SAXS); inverse size-exclusion chromatography (ISEC); scanning electron microscopy (SEM); salt exclusion

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1. Introduction

Ion-exchange chromatography, a workhorse for downstream processing associated with biotherapeutic proteins, is crucial for removal of various product-related and unrelated impurities [1–5]. The particle size, pore size, type of ligand, ligand density and non-specific interactions of analytes with the resin base matrix are some of the critical factors that affect the chromatographic performance (selectivity, yield and throughput). In conventional ion-exchange resins, the resin is typically a cross-linked polymer network that forms an insoluble phase to which proteins are bound primarily by electrostatic interactions, whereas new-generation polymer-derivatized ion-exchange media comprise a rigid base matrix within which polymeric extenders (e.g., end-grafted tentacles) are attached [6]. Ion-exchange functionalities in polymer-derivatized media may be present on the added polymer layer as well as on the base matrix [6].

The ligand density plays a key role in the performance of various types of chromatographic media, including affinity chromatography [7–8], hydrophobic interaction chromatography [9–10], hydrophobic charge induction chromatography [11], ion-exchange electrochromatography [12] and ion-exchange chromatography [13–19]. A principal objective of this work was to identify the effect of changes in ligand density on the binding capacity as well as the transport behavior for proteins of various sizes. Earlier investigations into the role of ligand density in ion-exchange chromatography reported significant differences in retention characteristics of selected model proteins [20–21]. More recent studies have focused on the impact of ligand density on impurity resolution in biotherapeutic protein purification. Fogle et al. observed that ligand density in Capto^{TM}® SP ImpRes resin variants does not have any significant impact on resolution of high molecular weight aggregates associated with monoclonal antibody therapeutics, although improved resolution was observed for one of the charge variants [14]. Subsequent high-throughput measurements of the effect of the ligand density of SP Sepharose® FF resin on the purification of monoclonal antibody therapeutics showed no effect on yield, host-cell protein (HCP) clearance or aggregate clearance [15].

For the particular case of tentacular ion exchangers [22], in which polymer chains are end-grafted to a cross-linked polymethyl methacrylate base matrix, Wrzosek et al. [23] found the effect of ligand density on the static binding capacity of immunoglobulin G and human serum albumin to depend significantly on ionic strength, with a surprising observation being that higher binding capacities were observed at higher ionic strengths. Franke et al. [24] and Neuville et al. [25] focused more on structural effects in using inverse size-exclusion chromatography (ISEC) to show that increases in ligand density induce size-exclusion selectivity in tentacular ion exchange sorbents. This effect was associated with conformational changes in the tentacle structure, leading to larger pore sizes, induced by changes in the salt concentration.

Although these investigations into the effects of ligand density focused more on performance measures than on underlying mechanisms, observed relationships such as those of pore size changes to the binding capacity and transport rates for proteins of various sizes have started to expand mechanistic understanding. However, the complexity of physical phenomena in

tentacular adsorbents, e.g., conformational changes in the grafted polyelectrolyte layer that in turn are responsible for the observed pore size changes, are likely to require more concerted efforts. Indeed, the effects of the ligand density are likely to involve a complex interplay among the ligand density, pore size and operating condition-dependent conformational changes in the grafted polymer that determine the key parameters defining the overall performance of the ion-exchange media. As a result, there is some ambiguity in the term “change in ligand density” for polymer-grafted ion exchangers. For tentacular ion exchangers, i.e., with end-grafted polymer extenders, a change in ligand density may result from a change in the number of ion-exchange groups per polymer chain, a change in the number of grafted polymer chains per unit area or a change in the chain length of the grafted polymer.

The objective of the present work was to understand the ionic strength-dependent changes in pore architecture in tentacular sorbents using a series of prototype tentacular resins differing in their ligand densities; associated functional studies are reported in a companion paper [26]. The measurements made include those of pore dimensions under different ionic strength conditions by inverse size-exclusion chromatography (ISEC) as well as insights into changes in pore architecture and geometry by scanning electron microscopy (SEM). More global measures of conformational changes in the grafted polyelectrolyte layer were obtained using small-angle x-ray scattering (SAXS). The extent of salt exclusion within the pore space at low ionic strength was determined using salt accessibility studies. By using this combination of experimental techniques we have sought to understand in a concerted fashion the changes in pore size, pore architecture and the conformation of the grafted polyelectrolyte chains. This improved knowledge and the associated functional measurements can aid in elucidating the nanoscale implications of the concept of “change in ligand density” for tentacular adsorbents.

2. Materials and methods

2.1. Stationary phases

Prototype A (ligand density 395 $\mu\text{eq/g}$), prototype B (478 $\mu\text{eq/g}$) and prototype C (645 $\mu\text{eq/g}$) were provided by Merck KGaA (Darmstadt, Germany). All resins were synthesized using a Fractogel® base matrix (cross-linked polymethyl methacrylate) as a support and sulfoisobutyl groups as strong cation-exchange ligands. The average particle diameter is on the order of 65 μm for all three resins. Toyopearl® HW 65 F (lot no. 65HWFB22D) and Toyopearl® SP-650 M (lot no. 65SPMB06K) were purchased from Tosoh Biosciences LLC (Montgomeryville, PA, USA) and used as controls. Toyopearl® HW 65 F is the base matrix for the prototype variants without grafted polymer and sulfonate groups, and Toyopearl® SP-650 M has sulfonate groups attached to HW 65 F without grafted polymer.

2.2. Buffers

Monobasic sodium phosphate (NaH_2PO_4) and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ) and used to prepare pH 7.0 buffer solution. ISEC, SEM and SAXS experiments were performed using 10 mM sodium phosphate buffer, pH 7.0, with NaCl added to 0 mM, 100 mM or 1000 mM total ionic strength (TIS). For ISEC and salt-

exclusion experiments column packing was performed using 10 mM sodium phosphate buffer, pH 7.0, containing 150 mM NaCl (column packing buffer).

2.3. Inverse size-exclusion chromatography (ISEC)

A set of dextran standards ranging from 180 to 3,000,000 Da was purchased from Polymer Standards Service (Silver Spring, MD); characteristics of the dextran standards used are shown in Table 1. Standard solutions of dextran markers were prepared by dissolving dextran in equilibration buffer to a concentration of 1 mg/mL and storing them at 4 °C for at least 24 h prior to their use in ISEC experiments. All samples were filtered through 0.22 µm syringe filters prior to injection to remove any impurities [27].

2.3.1. ISEC procedures—Prior to resin packing, the storage solution was removed by washing the resin slurry with deionized water. Resin was slurried in a 75/25 resin-to-buffer ratio and poured into a graduated cylinder to a volume of 15–20 mL. Packing buffer was added and decanted a total of three times. Packing buffer was then added to produce a suspension of 60–75% (v/v) resin. The suspension was poured along the inside wall of vertical Omnifit® (Diba Industries, Inc., Danbury, CT, USA) chromatography columns (6.6 × 400 mm). The column was topped off with packing buffer and the top flow adapter attached. The flow of packing buffer was initiated and gradually increased to 1.71 mL/min (300 cm/h). The top flow adapter was repeatedly lowered to the top of the bed every 15 minutes while packing continued until no noticeable further drop in bed height was observed. The final packed bed height was 30 ± 1.0 cm in all cases (total column volume 10.2 ± 0.5 mL).

Chromatography was performed using a Waters® 2695 separation module equipped with a Waters® 2414 RI detector. Column qualification was performed using an injection of 1% (v/v) acetone. For all packed columns asymmetry (A_s) values were between 0.8 and 1.2. For each column the intraparticle void fraction, ϵ_p , was determined based on the retention of glucose injections and the interstitial void fraction, ϵ , was determined based on the retention of dxt 3000k (3,000,000 Da dextran) injections. For the ISEC experiments, injections of solutes (dextran markers) of 100 µL were used at a flow rate of 0.25 mL/min (45 cm/h) [27–28].

2.3.2. ISEC analysis—For extraction of pore size and other structural parameters data, ISEC results were organized in the form of a calibration curve of distribution coefficients (K_d) [27],

$$K_d = \frac{V_R - V_0}{V_T - V_0} \quad (1)$$

where V_R is the elution volume for the dextran probe, V_0 is the interparticle void volume and V_T is the total mobile-phase volume, determined using a glucose injection.

Rapid changes in solvent composition and sample carry-over sometimes lead to false additional peaks due to sensitivity of the refractive index (RI) detector. To minimize such interference of ghost peaks in accurate determination of V_R and hence of the K_d values of the dextran probes, we used the multiple peak-fit tool of OriginLab®. An appropriate fitting function (Gaussian, modified Gaussian, bi-Gaussian, Lorentzian or log normal) was selected to fit the required peak shape and minimize ghost-peak interference. Fig. 1 illustrates the use of the bi-Gaussian peak-fitting function to appropriately define the peak for a 5.2 kDa dextran probe in prototype C resin. The ghost peak could be distinguished from the true peak by the dependence of the signal in the latter on injection concentration.

The ISEC calibration curve, which expresses K_d as a function of the size of the dextran probes, was used to estimate the pore-size distribution of the resin, from which the mean pore size and the accessible pore area were estimated. The dextran probe size is most often expressed as the viscosity radius, R_η , based on the empirical correlation [27]

$$R_\eta = 0.0271 M_p^{0.498} \quad (2)$$

K_d for a dextran probe of viscosity radius r_m is related to the pore-size distribution $f(r)$ via

$$K_d = \frac{\int_{r_m}^{\infty} f(r) [1 - (\frac{r_m}{r})]^2 dr}{\int_0^{\infty} f(r) dr} \quad (3)$$

which reflects the physical significance of K_d as a ratio of accessible pore volume to total pore volume, with partitioning of the dextran probe represented as that of a sphere in a cylindrical pore. A log normal pore-size distribution function

$$f(r) = \frac{1}{r} \exp \left[-\frac{1}{2} \left(\frac{\log \frac{r}{r_p}}{s_p} \right)^2 \right] \quad (4)$$

was used, where r_p and s_p represent the mode and the width respectively of the pore-size distribution. The quadgk function in MATLAB was used to evaluate the integrals and the nlinfit function was used to fit the experimental K_d data to the pore-size distribution parameters using nonlinear least-squares regression [27–28].

2.4. Electron microscopy

2.4.1. Scanning electron microscopy (SEM)—Sample preparation for SEM included qualitative assessment of the effect of fixation on resin samples. Fixed samples were prepared by temporary fixation with 1% glutaraldehyde and 1% paraformaldehyde before chemical fixation with 1% osmium tetroxide (OsO_4) in DI water. After fixation, samples were washed with DI water and dehydrated in ethanol, similar to samples that were not

chemically fixed; dehydration was performed using series of washes with ethanol solutions (25, 50, 75, 95 and 100% v/v), each for 10 minutes. Resin samples were transferred to 30 μm microporous capsules for critical-point drying, which was performed using a Tousimis Autosamdri®-815B critical-point drier for 2 hours, after which samples were mounted on aluminum stubs using double-sided carbon tabs [28].

Samples were sputter-coated with gold–palladium in a Denton Bench Top Turbo III coater for 30 min at a current of 30 mA and an argon pressure of 65 mtorr. Several samples were left uncoated in order to observe the effects of electron charging on the materials while imaging was performed. All SEM imaging was performed using a Hitachi S-4700 field-emission scanning electron microscope (FESEM) operated in secondary-electron mode with a voltage of 3.0 kV and a working distance of 2–3 mm [28].

2.4.2. Cryo-scanning electron microscopy (cryo-SEM)—Two different sample-freezing approaches were examined. For plunge freezing, a drop of a 50–70% (v/v) resin slurry was placed on a SEM sample holder that was then plunged into liquid nitrogen slush. A vacuum was drawn, allowing sample transfer to a Gatan Alto 2500 cryo-chamber at a temperature of $-125\text{ }^{\circ}\text{C}$. Samples were fractured, and then sublimated for 10 minutes at $-90\text{ }^{\circ}\text{C}$ followed by cooling to $-125\text{ }^{\circ}\text{C}$ for imaging. The samples were then transferred uncoated into a Hitachi S-4700 FESEM for imaging. Several samples were left unsectioned to check the effect of sectioning in cryo-SEM.

High-pressure freezing was performed using a Leica EM PACT high-pressure freezer for vitrification of a 50–70% (v/v) resin slurry resin sample using a jet of liquid nitrogen at 2100 bar. A cryo-SEM sample holder was then carried in liquid nitrogen and a vacuum was drawn, allowing sample transfer to the Gatan Alto 2500 cryo-chamber at a temperature of $-125\text{ }^{\circ}\text{C}$. Samples were fractured and then sublimated for 10 minutes at $-90\text{ }^{\circ}\text{C}$, followed by cooling to $-125\text{ }^{\circ}\text{C}$ for imaging. The samples were then transferred uncoated into a Hitachi S-4700 FESEM for imaging.

All cryo-SEM imaging was performed using a Hitachi S-4700 FESEM operated in secondary electron mode with a voltage of 3.0 kV and a working distance of 6–8 mm.

The porosity changes in tentacular sorbents were quantitatively assessed using SEM image analysis. Pores in the SEM image were selected by using manual thresholding and binarization in ImageJ [29]. The pore fraction was then calculated as a percentage of the total image area.

2.5. Small-angle x-ray scattering (SAXS)

SAXS was performed on the G1 beamline of the Biological Small-Angle X-Ray Solution Scattering (BioSAXS) facility at MacCHESS, the Cornell High-Energy Synchrotron Source. The x-ray characteristics used were: energy (E), 9.968 keV; wavelength (λ), 1.257 Å; beam diameter, 250 μm $\times$ 250 μm ; photon flux, 1.6×10^{11} photons/s; detector, dual Pilatus 100K-S SAXS/WAXS. Resin slurries (50% v/v) in suitable equilibration buffers at different ionic strengths were used in the experiments. Sample exposures of 30 frames for 2 seconds each (60 s total) were recorded. The forward scattering was recorded on a CCD detector and

circularly averaged using the program BioXTAS RAW to yield one-dimensional intensity profiles as a function of $q = 4\pi (\sin \theta)/\lambda$, where 2θ is the scattering angle. Scattering from an equilibration buffer solution was subtracted from the data, and data were corrected for the incident intensity of the x-rays. Replicate exposures were examined carefully for evidence of radiation damage by Guinier analysis before averaging and merging. Silver behenate powder was used to locate the beam center and to calibrate the sample-to-detector distance.

2.6. Salt exclusion measurements

Salt breakthrough measurements [28] were performed to determine the extent of Donnan exclusion. Resin variants were packed in Omnifit® (Diba Industries, Inc, Danbury CT, USA) chromatography columns (6.6×250 mm) with a bed height of 12 ± 2.0 cm. An ÄKTA® Purifier system equipped with a conductivity meter was used to measure the salt breakthrough profiles. Pump A was used for feeding the low-salt buffer whereas pump B was used for feeding the high-salt buffer (0–200 mM TIS). Starting at 10% B, the concentration of high-salt buffer was increased stepwise by 5% every 20 min until 70% B was reached. All steps were performed at a linear velocity of 120 cm/h. The dead volume for each step was calculated using a blank experiment without the column in place. Column properties were determined in a similar manner to that used in the ISEC experiments. The data were analyzed in terms of apparent volumetric partitioning [30].

3. Results and discussion

3.1. Inverse size-exclusion chromatography

The ISEC analysis was based on experimental calibration curves such as that shown in Fig. 2 for prototype C at various ionic strengths. A representation of the pore-size distribution was obtained for each case by fitting the ISEC calibration curves to the log normal distribution, assuming applicability of the sphere-in-cylinder model. The calculated pore size distributions for the base matrix, HW 65F, and the three prototype resins at 20 mM (Fig. 3) show that the polymer grafting leads to a significant loss of large pores in the prototype resins. The overall trend in pore size is HW 65F > prototype A > prototype B > prototype C, so that the extent of pore constriction is correlated with the ligand density. This trend is preserved with increasing ionic strength as well, as reflected in the mean pore radii for all the sorbents and HW 65F, at various ionic strengths (Fig. 4). The considerable reduction in mean pore size due to polyelectrolyte grafting seen at 20 mM TIS in Fig. 4 is attenuated at higher ionic strengths as a result of salt-induced collapse in the grafted polyelectrolyte chains [24, 31]. Electrostatic repulsion within and between the grafted polyelectrolyte chains determines the three-dimensional arrangement of intrapore polymer and hence the pore size of the tentacular sorbent. Charge screening at higher salt concentrations leads to decreased repulsion among the grafted polyelectrolyte chains and hence chain collapse, which in turn may lead to increased pore sizes as observed. That the increase in mean pore radius is noticeably greater for prototype A than for prototypes B and C may reflect steric limitations on polyelectrolyte chain collapse in the latter variants, which have higher ligand densities. The reason for the small increase in mean pore radius of the base matrix, which is nominally uncharged, is unclear.

Fig. 5, which compares the phase ratios for the three prototypes and HW 65F, adds alternative quantitative insights into the structural differences among them; the phase ratio here is defined as the surface area per unit accessible pore volume of the sorbent. Compaction and collapse of the grafted polyelectrolyte layer at higher ionic strength leads to an increase in pore size and subsequent decrease in surface area for all the variants, so these trends are effectively captured. However, the phase ratio as defined here requires careful interpretation overall. The phase ratio decreases as the probe size increases, reflecting solute exclusion from more restricted regions, which have a higher specific surface area. Compared to the base matrix HW 65F, the phase ratio accessible to smaller dextran probes ($R_h < 1.77$ nm) in the prototype sorbents is increased approximately 3–4 fold. This is due in part to the restricted space within the polymer that is added by the tentacles, but the change in the accessible volume also plays a role. This is because all but the smallest probes are presumably excluded from the polymer layer, so both the surface area and the accessible pore volume are calculated based on the residual pore lumen. Since the prototypes are all derived from the same base matrix but with the pore cross-sections constricted, solutes that are excluded from the polymer layer have access to a more limited surface area. However, because the accessible pore volume is also smaller, the nominal phase ratio increases with increasing pore constriction, as a result of which the phase ratio increases with increasing ligand density. An alternative measure is a phase ratio based on the pore volume of the base matrix, which is the same for all the variants.

3.2. Scanning electron microscopy

A key objective of the SEM imaging was to visualize the microstructure of the sorbents and to track the ionic strength-dependent changes in the sorbent structure. However, a critical challenge was to assess the effect of complex sample preparation steps on the native microstructure. Results from conventional and cryo-SEM were compared to determine which was better able to show the effect of changes in ionic strength on sorbent microstructure.

3.2.1. Effect of fixation and coating—Fig. 6 summarizes the effect of chemical fixation and coating on the microstructure of prototype B. These results show that coating of the adsorbent with gold-palladium alloy significantly alters the pore structure, so further effects were examined in samples without chemical fixation and coating. The effect of ionic strength on the pore structure was studied by equilibrating samples of prototype B in 20 and 100 mM TIS phosphate buffer at pH 7.0. Fig. 7 shows a significant increase in pore size at the higher ionic strength, but there is some ambiguity regarding the reasons for the observed effect. A principal reason may be ionic strength-dependent collapse of the grafted polyelectrolyte layer [24,31], but sample processing steps in conventional SEM, such as dehydration using ethanol and critical-point drying, may lead to conformational changes in the layer and hence to the observed pore size changes.

3.2.2. Cryo-SEM—To minimize the impact of sample processing on sorbent microstructure, imaging was also performed using cryo-SEM. Samples were fixed using two methods, flash-freezing in liquid nitrogen slush and high-pressure freezing. Based on the results from conventional SEM, resin samples were left uncoated in cryo-SEM as well. For

resin samples without sectioning, ice deposition on the resin surface makes pore space visualization difficult (Fig. 8), so resin samples were sectioned in the cryo state for accurate visualization. Comparison of samples prepared by plunge freezing and high-pressure freezing shows only slight differences in the pore structure (Fig. 9), so to keep sample processing as simple as possible and to minimize the impact of high-pressure conditions we used plunge freezing to study the effect of changes in ligand density. Fig. 10 summarizes the effect of changes in ligand density on the pore fraction. At 20 mM TIS comparable pore sizes are observed for the sorbents with low and intermediate ligand densities (prototypes A and B), but there is a significant reduction in pore size for the high ligand density variant (prototype C).

Fig. 11 shows the effect of ionic strength on the microstructure of prototype C. Equilibration at higher ionic strength leads to an increase in the apparent pore size, in good agreement with ISEC observations (Fig. 4). This qualitative observation was quantified using image processing by analyzing the pore fraction for all three variants using ImageJ [29] on results at two different magnifications (10x and 50x) (Fig. 12). A significant increase in pore size is observed at higher ionic strengths for all variants, with the most significant changes for the high ligand density variant (prototype C). Although comparison of the pore fractions determined using ISEC and cryo-SEM shows meaningful differences, both techniques accurately capture the dependence of pore fraction on ionic strength (Fig. 13).

3.3. SAXS

Small-angle x-ray scattering (SAXS) was performed to probe the nanoscale changes in the grafted polyelectrolyte layer responsible for the observed pore size changes. We are not aware of previous use of SAXS to characterize such sorbents, although neutron scattering has recently been used to characterize lysozyme adsorption on cellulosic adsorbents [32].

SAXS determines the scattering intensity as a function of the magnitude of the scattering vector, q . The q range ($0.006 - 0.8 \text{ \AA}^{-1}$) of the data provides information on length scales $d = 2\pi/q$ that include the approximate thickness of the grafted polyelectrolyte layer ($100 \text{ \AA}$) [22]. The measured SAXS intensity profile (Fig. 14A) shows two limiting ranges of q . The low- q region shows q^{-4} dependence, reflecting the surface fractal nature of the base matrix on length scales $> \sim 200 \text{ \AA}$ [33–34], while the high- q region shows different characteristics in the presence and absence of the grafted polyelectrolyte layer. Scattering from the base matrix, HW 65F, and SP 650M, a strong cation exchanger without grafted polymer, show roughly q^{-2} dependence at high q , indicating a Gaussian chain network [33–34]. However, addition of the polyelectrolyte layer in the low ligand density variant, prototype A, gives rise to a characteristic “ionomer peak” at $0.1 < q < 0.2 \text{ \AA}^{-1}$ [35–38]. Equilibrating the tentacular sorbent in solutions differing in ionic strength gives rise to signature changes in the ionomer peak, with increasing ionic strength leading to decreasing ionomer peak intensity and a shift in the ionomer peak maximum towards high q (Fig. 14B).

The term “ionomer” has been used to describe polymers containing hydrophobic repeat units with a fraction of monomers containing ionic functionalities either on pendant groups or on the main chain [35]. Ionomer peaks are generally recognized as reflecting the existence of ionic aggregates, but there is a lack of clarity about the sizes of these ionic clusters and their

internal structure and distribution inside the material [35]. Commercial examples of ionomers are Surlyn® and Nafion®, both of which are membranes with immobilized ionic functionalities [39–40]. However, we are not aware of prior reports of ionomers in chromatographic particles, and we provide a more detailed investigation of the effect of the length of grafted polyelectrolyte chains on the ionomer peak position and intensity in a companion paper [41]. This observation indicates significant changes in the characteristic length scale ($< 100 \text{ \AA}$) associated with the grafted polyelectrolyte layer.

3.4. Salt exclusion

Complementing the earlier results (section 3.1) on the steric exclusion of dextran solutes, we have also investigated the effect of ligand density on the electrostatic exclusion of small ions, which is governed mainly by the Donnan equilibrium effect [42–44]. Fig. 15 shows the data collected from salt step gradient experiments. Salt exclusion was apparent in all three prototypes up to about 90 mM TIS. The salt exclusion effect is most pronounced for the high ligand density variant (prototype C), consistent with the theory. In the case of other polymer-modified resins such as SP Sepharose® XL, salt exclusion is noticeable up to about 300 mM TIS [44]. These observed differences suggest significant differences in the three-dimensional arrangement of the grafted polyelectrolyte layer in tentacular sorbents.

4. Conclusions

A critical objective of this work was to understand the structural changes defining the term “increase in ionic capacity or ligand density” in tentacular sorbents and how these changes are correlated to operating conditions such as the ionic strength of the equilibration buffer. The picture that emerges from the results reflects an association of the increase in ionic capacity/ligand density with an increase in polyelectrolyte chain length, which in turn leads to a significant reduction in pore size. The ionic strength of the equilibration buffer is a key parameter that determines the conformation of the grafted polyelectrolyte layer and hence the pore structure of a tentacular sorbent. Ligand density-dependent intensity differences in the ionomer peak indicate the possibility of ionic cluster regions of variable size. Further investigation and modeling of SAXS data will be helpful in understanding the structural and thermodynamic origins of the ionomer peak and in determining the grafted polyelectrolyte thickness.

The relevance of the structural characteristics reported here can best be appreciated within the context of the implications for functional properties. These are addressed in a companion article [26].

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Highlights

- Ligand density can appreciably affect the structure and properties of ion exchangers
- Effects of ligand density on structure of tentacular resins were investigated
- Increasing ligand density was found to result in pore constriction
- SAXS measurements indicate the presence of ionomers (ion clusters)

Fig. 1. Use of bi-Gaussian function (....) to minimize the interference of ghost peak in ISEC analysis of 5.2 kDa dextran probe in prototype C resin at 100 mM TIS. A: Dextran 5.2 kDa marker peak; B: ghost peak.

Fig. 2. Dextran calibration curves for prototype C resin equilibrated at various ionic strengths (20, 100 and 1000 mM TIS) in phosphate buffer, pH 7.0.

Fig. 3. Pore size distributions for base matrix HW 65F and prototype sorbents at 20 mM TIS in phosphate buffer, pH 7.0.

Fig. 4. Trends in mean pore radii of prototype tentacular sorbents and the base matrix, determined by ISEC at different ionic strengths in phosphate buffer, pH 7.0.

Fig. 5.

Trends in phase ratios for prototype sorbents at different ionic strengths (20, 100 and 1000 mM TIS, phosphate buffer, pH 7.0). a) Base matrix HW 65F; b) prototype A; c) prototype B; d) prototype C.

Fig. 6. Effect of chemical fixation and coating on SEM images of prototype B. A and B: chemically fixed and coated samples at 1100x and 35000x magnification respectively; C and D: no chemical fixation and coating at 1100x and 35000x magnification.

Fig. 7. Effect of ionic strength on prototype B microstructure in conventional SEM (without chemical fixation and coating). A and B: 20 mM TIS, 35000x and 70000x magnification respectively; C and D: 100 mM TIS, 35000x and 70000x magnification respectively.

Fig. 8.

Effect of particle sectioning in cryo-SEM of base matrix (HW 65F) at 20 mM TIS. A and B: 1100x and 10000x magnification respectively without sectioning; C and D: 1100x and 10000x magnification respectively with sectioning.

Fig. 9.

Effect of sample processing step (freezing method) in cryo-SEM imaging of prototype B at 20 mM TIS. A and B: effect of plunge freezing, 1000x and 10000x magnification respectively; C and D: effect of high-pressure freezing, 1000x and 10000x magnification respectively.

Fig. 10. Effect of ligand density on pore microstructure of tentacular sorbents at 20 mM TIS. Labels indicate prototypes.

Fig. 11. Effect of ionic strength on pore microstructure of prototype C equilibrated at different ionic strengths. Total ionic strengths: A: 20 mM; B: 100 mM; C: 1000 mM.

Fig. 12.

Pore fraction analysis using image processing. A: image processing technique used for determination of pore fraction for prototype B at 100 mM TIS; B: comparison of pore fraction for different variants. Prototypes A (∇), B ($\blacklozenge$), C ($\blacktriangle$); magnification: 10x, filled symbols; 50x, open symbols.

Fig. 13.
Comparison of pore fraction for prototype C using ISEC and SEM analysis.

Fig. 14. Effect of ligand density and change in ionic strength on ionomer peak position. A: differences in SAXS profiles for prototypes and the base matrix; B: ionic strength-dependent behavior of ionomer peak in prototype C.

Fig. 15.
Salt exclusion effect in prototype resins.

Table 1

Dextran characteristics, including weight-average molecular weight (M_w), number-average molecular weight (M_n), molar mass at peak maximum (M_p), polydispersity index (PDI), as reported by Polymer Standards Service, and viscosity radius (R_η , derived from Eq. (2) [27].

Dextran	M_w (kDa)	M_p (kDa)	M_n (kDa)	PDI	R_η (nm)
dxtp1	0.180	0.180	0.180	1.00	0.36
dxtp2	0.342	0.342	0.342	1.00	0.50
dxtl1n	1.350	1.080	1.160	1.16	0.88
dxt5	5.200	4.400	3.300	1.58	1.77
dxtl12	11.600	9.900	8.100	1.43	2.65
dxt25	23.800	21.400	18.300	1.30	3.89
dxt50	48.600	43.500	35.600	1.37	5.53
dxtl50	148.00	124.00	100.00	1.48	9.32
dxt270	273.00	196.00	164.00	1.66	11.71
dxt410	410.00	277.00	236.00	1.74	13.91
dxt3000K	3000.00	2800.00	1230.00	2.44	44.02

Table 2

Pore-size distribution parameters and mean pore radii for all prototype resins determined using ISEC based on the log normal distribution applied to the sphere-in-cylinder model.

Resin	20 mM TIS			100 mM TIS			1000 mM TIS		
	r_p	s_p	Mean pore radius (nm)	r_p	s_p	Mean pore radius (nm)	r_p	s_p	Mean pore radius (nm)
HW 65F	25.87	2.05	33.54	26.11	2.34	37.52	25.71	2.55	39.57
Prototype A	12.24	2.10	16.09	15.90	2.71	26.18	18.15	2.48	27.42
Prototype B	9.74	0.57	11.35	10.79	2.02	13.84	14.85	2.17	20.05
Prototype C	7.26	0.64	8.02	9.62	2.04	12.41	12.40	2.56	19.28