Journal of Chromatography A, 1506 (2017) 55–64

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Full length article

Ionic strength-dependent changes in tentacular ion exchangers with variable ligand density. II. Functional properties

Rahul Bhambure[a,b], James M. Angelo[a], Christopher M. Gillespie[c], Michael Phillips[c], Heiner Graalfs[d], Abraham M. Lenhoff[a,*]

[a] Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE, 19716, USA
[b] National Chemical Laboratory, Pune, India
[c] EMD Millipore Corporation, Bedford, MA, 01730, USA
[d] Merck KGaA, Darmstadt, Germany

ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form 5 May 2017
Accepted 7 May 2017
Available online 10 May 2017

Keywords:
Ligand density
Binding capacity
Isocratic retention
Uptake and elution
Monoclonal antibody
Lysozyme

ABSTRACT

The effect of ligand density was studied on protein adsorption and transport behavior in tentacular cation-exchange sorbents at different ionic strengths. Results were obtained for lysozyme, lactoferrin and a monoclonal antibody (mAb) in order to examine the effects of protein size and charge. The combination of ligand density and ionic strength results in extensive variability of the static and dynamic binding capacities, transport rate and binding affinity of the proteins. Uptake and elution experiments were performed to quantify the transport behavior of selected proteins, specifically to estimate intraparticle protein diffusivities. The observed trend of decreasing uptake diffusivities with an increase in ligand density was correlated to structural properties of the ligand-density variants, particularly the accessible porosity. Increasing the ionic strength of the equilibration buffer led to enhanced mass transfer during uptake, independent of the transport model used, and specifically for larger proteins like lactoferrin and mAb, the most significant effects were evident in the sorbent of the highest ligand density. For lysozyme, higher ligand density leads to higher static and dynamic binding capacities whereas for lactoferrin and the mAb, the binding capacity is a complex function of accessible porosity due to ionic strength-dependent changes. Ligand density has a less pronounced effect on the elution rate, presumably due to ionic strength-dependent changes in the pore architecture of the sorbents.

1. Introduction

Cation-exchange chromatography in bind-and-elute mode plays a major role in purification of biopharmaceutical proteins. Typical examples are the use of cation-exchange chromatography in separation of mono-PEG variants in PEGylated biopharmaceuticals [1] and separation of charge variants and aggregates in mAb therapeutics [2–4]. With existing high-titer upstream processes, high-capacity chromatographic purification steps can facilitate cost-effective manufacturing of biopharmaceutical proteins [5]. Polymer-derivatized chromatographic sorbents offer benefits such as higher dynamic binding capacities and enhanced selectivity. Within this class of stationary phases, tentacular adsorbents [6], in which the polymer extenders are end-grafted to the base matrix, allow flexibility of the adsorbent in its interactions with protein adsorbates, resulting in benefits attributed to the wider class of polymer-derivatized ion exchangers [7]. The ligand density of a tentacular sorbent impacts its performance in terms of the adsorption capacity and transport characteristics of proteins. However, in view of the structural complexity of these materials, there is a complex interplay among the ligand density, pore size distribution and operating conditions via the effect on conformational changes in the grafted polyelectrolyte layer, all affecting the functional performance of the sorbent.

The role of ligand density and its impact on functional characteristics has been investigated for different types of chromatographic sorbents, such as ion-exchange [8–14], affinity [15,16], and hydrophobic interaction chromatography [17,18]. This prior work focused on functional characterization in terms of static and dynamic binding capacities of proteins, with limited understanding of structural features affecting these capacities and relevant transport behavior. In the case of tentacular sorbents, Wrzosek et al. [19] found that the interplay of the ligand density and ionic strength led to sometimes-anomalous trends in the static binding capacities of

* Corresponding author.
E-mail address: lenhoff@udel.edu (A.M. Lenhoff).

http://dx.doi.org/10.1016/j.chroma.2017.05.021
0021-9673/

immunoglobulin G and human serum albumin. For the high ligand density variant, higher binding capacities were seen at higher NaCl concentrations. A systematic study of the pore diffusivity and dynamic binding capacity of immunoglobulin G as a function of ligand density indicated that an optimal ligand density arises as a trade-off between higher ligand densities to enhance equilibrium capacity and lower ligand densities for higher mass transfer rates [20].

From a physical perspective, tentacular adsorbents mimic a system of polyelectrolyte brushes grafted inside the porous particle. There is a limited understanding of the physics underlying salt-dependent conformational changes of the brush (tentacles). The impact of these salt-dependent structural changes on interactions with proteins of different sizes affects binding affinity and transport behavior during uptake as well as in elution.

This investigation focuses on the systematic determination of the effect of ligand density on functional properties of a set of tentacular sorbents, including static binding capacity, dynamic binding capacity, binding affinity, and transport rates during uptake and elution. This was accomplished using three model proteins differing in their size and charge distribution. Observed trends in the functional characteristics of the tentacular sorbents are explained using the structural features of the sorbents, described in a companion study [21].

2. Materials and methods

2.1. Stationary phases

Three prototype tentacular cation exchangers differing in ligand density were provided by Merck KGaA (Darmstadt, Germany): prototypes A (ligand density 395 μeq/g), B (478 μeq/g) and C (645 μeq/g). All resins were synthesized from the same cross-linked polymethyl methacrylate matrix as a support and strong cation-exchange sulfoisobutyl groups as ligands. The average particle size was approximately 65 μm for all three resins. The base matrix and ligand type of the prototypes are the same as for the commercially available cation-exchange resin Fractogel® EMD SO_3^- (M) (Merck KGaA, Darmstadt, Germany).

2.2. Proteins and solutions

Monobasic sodium phosphate (NaH_2PO_4) and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ) and used to prepare buffer solutions at pH 7.0. At this pH, a total ionic strength (TIS) of 20 mM was achieved using 10 mM sodium phosphate and ionic strengths of 50, 100 and 300 mM were reached by adding appropriate amounts of sodium chloride. Acetic acid and sodium acetate were purchased from Fisher Scientific (Fair Lawn, NJ) and used to prepare buffer solutions at pH 5.0. All buffers were filtered using 0.22 μm filters prior to use. For breakthrough experiments column packing was performed using 10 mM sodium phosphate buffer, pH 7.0, containing 150 mM NaCl (column packing buffer).

Hen egg white lysozyme was purchased from Sigma-Aldrich (St. Louis, MO). Bovine lactoferrin was provided by DMV-International (Veghel, The Netherlands). A monoclonal antibody (mAb) was provided by Millipore Sigma (Bedford, MA). Both the lysozyme and lactoferrin solutions were prepared by dissolving the protein in buffer, while the mAb was provided in a concentrated solution. Initial purification of lactoferrin was achieved by cation-exchange chromatography on an SP Sepharose® XL column at pH 7.0 (GE Healthcare, Uppsala, Sweden) [22].

Protein solutions were prepared by performing buffer exchange into the appropriate buffer using a Sephadex® G-25 prepacked column. Amicon® centrifugal filters from Millipore Sigma (Billerica, MA) were used for concentrating protein solutions. Solutions were filtered using 0.22 μm filters and concentrations were determined using UV spectrophotometry (Nanodrop® 2000, Thermo Scientific, Wilmington, DE) with extinction coefficients at 280 nm of 2.64, 1.51 and 1.47 cm^2/mg for lysozyme [23], lactoferrin [24] and the mAb [25], respectively. Protein characteristics used in the interpretation of adsorption behavior are included in Table 1.

Table 1
Properties of model proteins.

Property	Lysozyme	Lactoferrin	mAb
Isoelectric point (pI)	11.4	8.8	≈8.0
Molecular weight M_w (kDa)	14.3	78.0	144.0
Hydrodynamic radius (nm)	2.01 [45]	3.33 [46]	4.50 [47]

2.3. Resin volume calibration

The hydrated particle volume was used as a measure of the amount of resin and was determined by exclusion of blue dextran [22] in a Waters AP mini glass column of 0.5 cm i.d. (Waters Corporation, Milford, MA, USA). For packing resin particles in the column, a slurry of particles in DI water was allowed to gravity-settle for 45 min in Wiretrol® 200 μL capillary tubes (Drummond Scientific Company, Broomall, PA). The settled height was measured using a Vernier caliper and the particles were added to the column, where they were again allowed to gravity-settle. The top flow adaptor of the column was adjusted to touch the top of the settled particle bed without any compression.

Experiments were performed on a Waters 2695 chromatography workstation (Waters Corporation, Milford, MA) using a Waters 2996 photodiode array detector. Triplicate injections of blue dextran (1 mg/mL) were run at 0.1 mL/min, this low flow rate having been chosen to avoid compression of the resin bed. The retention volume, V_R, was measured using the first moment of the elution peak, and the system dead volume, V_d, was determined by injecting the blue dextran without the column in place. The hydrated particle volume, V_{hyd}, was calculated from

$$V_{hyd} = V_c - (V_R - V_d) \quad (1)$$

where V_c is the total column volume. The quantity in parentheses is the interparticle void volume of the column. A resin-specific calibration factor was determined by dividing the hydrated particle volume by the settled capillary tube height, allowing for accurate addition of resin particles with the capillary tubes in subsequent experiments.

2.4. Adsorption isotherm measurements

Resin particles were equilibrated in the desired buffer solution by packing into a Waters AP mini glass column. The column was then unpacked and the equilibrated resin particles were collected. Wiretrol® II 100 and 200 μL capillary tubes were used for adding known volumes of the equilibrated particles to Eppendorf tubes containing known amounts of protein in a buffer of the same composition. The small volume of interstitial liquid in the capillary tubes, which affects the initial protein concentration, was also accounted for. The Eppendorf tubes were rotated slowly for five days to allow protein adsorption to proceed to equilibrium, after which the tubes were centrifuged and the protein concentration in the supernatant, C, was measured. The adsorbed protein concentration, q, which includes unadsorbed protein within the pores of the particles, was determined from a mass balance. All adsorbed protein concentrations refer to the mass of protein per hydrated particle volume.