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Ion exchange chromatography of monoclonal antibodies: Effect of resin ligand density on dynamic binding capacity

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ABSTRACT

Dynamic binding capacity (DBC) of a monoclonal antibody on agarose based strong cation exchange resins is determined as a function of resin ligand density, apparent pore size of the base matrix, and protein charge. The maximum DBC is found to be unaffected by resin ligand density, apparent pore size, or protein charge within the tested range. The critical conductivity (conductivity at maximum DBC) is seen to vary with ligand density. It is hypothesized that the maximum DBC is determined by the effective size of the proteins and the proximity to which they can approach one another. Once a certain minimum resin ligand density is supplied, additional ligand is not beneficial in terms of resin capacity. Additional ligand can provide flexibility in designing ion exchange resins for a particular application as the critical conductivity could be matched to the feedstock conductivity and it may also affect the selectivity.

1. Introduction

Ion exchange chromatography is a common and powerful purification technique in the production of many proteins. In ion exchange chromatography, charged solute molecules are reversibly adsorbed onto oppositely charged porous resin particles. Separation of target molecules from other species present in the mobile phase depends on attraction of these target molecules to the chromatographic resin relative to that of the other species present, as well as the capacity of the resin for the target molecule. Interaction strength and resin capacity have long been known to depend on the ionic charge of the solute molecules as well as buffer ionic strength [1,2]. Recent work has shown that the performance of an ion exchange separation is a complex function of mobile phase conditions, protein properties, and chromatographic support properties [3–13].

Mobile phase conditions affect the maximum binding capacity of the chromatographic support as well as the surface properties of macromolecule solutes, and thus both buffer conductivity and pH are important parameters in determining the behavior of an ion exchange separation [4,10,11,13]. In general, the capacity of a resin for a given protein is expected to decrease with increasing buffer conductivity and with decreasing protein charge [14]. Recently published work, however, has identified the existence of two mechanisms in ion exchange chromatography of monoclonal antibodies; exclusion and equilibrium [13]. The exclusion mechanism occurs due to antibodies binding to the outer part of the resin beads and excluding additional antibody from entering the bead by charge repulsion and steric exclusion. An increase in conductivity and pH (decrease in protein net charge) leads to an increase in DBC until a maximum is achieved after which the equilibrium mechanism dominates, where DBC decreases with increasing conductivity and pH. This maximum DBC is a consequence of the trade off between the exclusion and equilibrium mechanisms. The critical conductivity is defined as the conductivity at which the maximum DBC occurs and depends on the buffer pH, and hence the charge of the protein. Under strong binding conditions of low conductivity and high protein net charge, the protein has been shown by confocal microscopy to bind in a narrow region near the surface of the resin while the interior region remains unoccupied. More moderate conditions of slightly increased ionic strength or decreased protein net charge allow increased pore accessibility.

The work presented herein investigates the effect of ligand density, apparent pore size and protein charge on the DBC. To that end, the dynamic binding capacity of a range of strong cation exchange resins for a monoclonal antibody is presented under conditions of varying pH and conductivity. The resins utilized in this study vary

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Table 1
Resin ligand densities tested.

Backbone	Sepharose 4 Fast Flow	Sepharose 6 Fast Flow
Ligand	Sulfopropyl	Sulfopropyl
K_d 110 kDa dextran	0.60	0.42
Ligand density (μmol/mL)	55, 130, 240	25, 60, 110, 190, 240

Ligand densities are expressed as μmol ligand per mL resin.

by apparent pore size as well as surface ligand density, or total ionic capacity.

2. Methods and materials

2.1. Proteins, chemicals and resins

Eight strong cation exchange resin prototypes based on the base matrices Sepharose™ 6 Fast Flow and Sepharose™ 4 Fast Flow resin chemistry and backbone were obtained from GE Healthcare (Uppsala, Sweden). The distribution coefficients (K_d) of dextran standards were determined for each of the unmodified base matrices with inverse size-exclusion chromatography (ISEC) utilizing refractive index to monitor elution [15–17]. The distribution coefficient corresponding to 110 kDa dextran standard was estimated by interpolation for the two base matrices used for the eight resins. The cation exchange resins were manufactured to have a variety of ligand densities varying from 25 to 240 μmol/mL as shown in Table 1. The resins were packed in columns (Biochem Valve/Omnifit™, Boonton, NJ) with packed bed volumes of 3.4 mL (0.66 cm column diameter, 10 cm bed height). A full-length monoclonal antibody (160 kDa, *p*I 9.2) from Genentech, Inc. (South San Francisco, CA) was utilized, and is designated Mab1 in this study as well as in a previous related study [13]. Protein net charge versus pH, as calculated from the amino acid sequence, is shown in Fig. 1.

For pH 4 and 5 studies, 15 mM sodium acetate buffer (Fisher Scientific, Hampton, NH) was used. pH adjustment was accomplished by addition of glacial acetic acid (Mallinckrodt, Phillipsburg, NJ). For the pH 6 studies, 14 mM MES (Angus Buffers and Biochemicals, Buffalo Grove, IL) and 10 mM sodium MES (Angus Buffers and Biochemicals) was used. The buffers were pH adjusted with 50% sodium hydroxide (J.T. Baker, Phillipsburg, NJ). Conductivity was adjusted by the addition of 5 M NaCl stock solution. Protein concentration of the load was adjusted to 8 mg/mL (determined by absorption at 280 nm) by ultrafiltration with a 10 kDa composite regenerated cellulose membrane in a Pellicon™ XL cassette (Millipore, Bedford, MA) using a Labscale™ TFF system (Millipore) and then diafiltered into the appropriate load buffer solution. Protein solution conductivity was adjusted by the addition of a stock solution of 5 M NaCl solution to the desired buffer conductivity. A six-column volume (CV) gradient elution was performed with load and elution buffer (load buffer with 1.5 M NaCl).

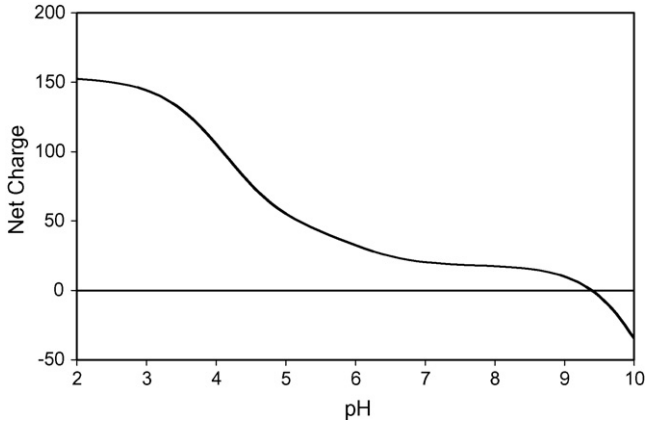

Fig. 1. Mab1 net charge as a function of pH. Protein net charge is calculated based on amino acid sequence.

2.2. Dynamic binding capacity experiments

Dynamic binding capacity was determined for Mab1 at pH 4, 5 and 6 and at conductivities of 1, 5, 10, 15, and 25 mS/cm on the resins listed in Table 1. In some cases, very little change in DBC was observed over the range of conductivity values, and only selected buffer conductivities were tested to conserve time and material. The resins were equilibrated with 5 CV of load buffer, which was sufficient to achieve the target pH and conductivity. Protein was loaded onto the resin to 10% breakthrough ($Q_{B10\%}$) based on absorbance at 280 nm (A_{280}). Unbound protein was washed from the resin with 5 CV of load buffer. Bound protein was eluted using 6 CV gradient with load buffer and elution buffer (load buffer with 1.5 M NaCl), followed by 6 CV of elution buffer. Dynamic binding capacity was calculated according to Eq. (1).

$$Q_{B10\%} = C_p \frac{V_p - V_h}{V_b} \quad (1)$$

where $Q_{B10\%}$ is the dynamic binding capacity (mg/mL), C_p the concentration of protein loaded (mg/mL), V_P the volume of protein solution loaded at 10% breakthrough (mL), V_h the holdup volume of the system (mL), and V_b is the packed bed volume of the resin (mL).

Each condition was run in duplicate, and averages of the capacities are reported. All experiments were conducted at ambient temperature (25–27 °C) and a flow rate of 100 cm/h corresponding to a residence time of 6 min. In all studies, the resins were regenerated and stored using 0.5N NaOH and 0.1N NaOH, respectively.

3. Results

DBC trends were obtained as a function of mobile phase pH or protein net charge, mobile phase conductivity, and resin ligand density for resins based on Sepharose 6 Fast Flow and Sepharose 4 Fast Flow (Figs. 2 and 3). The unfunctionalized base matrix Sepharose 6 Fast Flow has a distribution coefficient (K_d) corresponding to 110 kDa dextran standard of 0.42. With few exceptions such as low resin ligand density, the resins based on this base matrix (Fig. 2) consistently demonstrate an exclusion mechanism, as demonstrated by the discernible optimum in the DBC curves. The Sepharose 4 Fast Flow base matrix by contrast have larger pores (K_d = 0.60) and resins based on this base matrix (Fig. 3) demonstrate minimal exclusion only with high resin ligand density and high charge conditions (low pH and low conductivity).

Both ligand density and protein net charge can be seen to have an effect on performance of the resin. The extent to which these system variables affect the critical conductivity, defined as the conductivity at which the maximum DBC occurs, depends on the resin pore size (Fig. 4). For the smaller pore size resins (Fig. 4a), the critical conductivity increases with increasing resin ligand density and protein net charge. The larger pore size resins (Fig. 4b), however, do not demonstrate this same dependence, with a critical conductivity less than 5 mS/cm for all resins tested. The increased differentiation