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Surface extenders and an optimal pore size promote high dynamic binding capacities of antibodies on cation exchange resins

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ABSTRACT

Increased recombinant protein expression yields and a large installed base of manufacturing facilities designed for smaller bulk sizes has led to the need for high capacity chromatographic resins. This work explores the impact of three pore sizes (with dextran distribution coefficients of 0.4, 0.53, and 0.64), dextran surface extender concentration (11–20 mg/mL), and ligand density (77–138 μmol H+/mL resin) of cation exchange resins on the dynamic binding capacity of a therapeutic antibody. An intermediate optimal pore size was identified from three pore sizes examined. Increasing ligand density was shown to increase the critical ionic strength, while increasing dextran content increased dynamic binding capacity mainly at the optimal pore size and lower conductivities. Dynamic binding capacity as high as 200 mg/mL was obtained at the optimum pore size and dextran content.

1. Introduction

As our understanding of fundamental cellular processes (e.g. gene expression, apoptosis, and metabolism) continues to grow, so does our ability to manipulate these processes as they relate to production of therapeutic proteins [1]. The use of stably transfected cell lines [2] in conjunction with cellular engineering [3] has increased recombinant protein expression yields to the multiple gram per liter range in recent years [4]. For therapeutic proteins, such as monoclonal antibodies at the industrial scale; this has created a need for increased product capacity in purification unit operations. Chromatography has been a key unit operation in the purification of therapeutic proteins since the inception of genetic engineering [5]. This work aims to increase the dynamic binding capacity of therapeutic antibodies on cation exchange resins by exploring three physical properties of these resins, namely apparent pore size, ligand density, and dextran surface extender content.

It is important to consider charge on the antibody [6,7], ligand density [8], solution conductivity [9], pore size [10] and mass transport to maximize dynamic binding capacity. Therapeutic antibodies have been shown to be excluded from ion-exchange resin pores at low conductivities and pH values that correspond to high protein net charge through confocal microscopy and dynamic binding capacity measurements [9]. At these conditions the antibodies bind to the outer part of the beads and exclude additional antibody from entering the bead by charge repulsion and steric exclusion. This reduces intraparticle transport, and ultimately the dynamic binding capacity. Conversely, the dynamic binding capacity is traditionally known to decrease at higher conductivities due to reduced equilibrium capacity [15]. Thus the highest dynamic binding capacity is obtained at an intermediate conductivity resulting from a trade off between exclusion (reduced mass transport) and the equilibrium capacity.

Prior work on ion exchangers without surface extenders has shown that the conductivity at which the maximum dynamic binding capacity occurs (i.e. critical conductivity) is a function of the ligand density on Sepharose™ 6 Fast Flow and Sepharose™ 4 Fast Flow resins, however the maximum dynamic binding capacity value does not vary to a great extent with ligand density for these resins [11]. This work also demonstrated that for the studied system, ligand densities below 50 μmol/mL resin, reduced maximum dynamic binding capacities of therapeutic antibodies relative to higher ligand density resins because of the insufficient number

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Table 1
Properties of strong cation exchange resin prototypes.

Resin identifier	Distribution coefficient of 110 kDa dextran	Ligand density [μmol/mL]	Dextran concentration [mg/mL]	Mean particle size [μm]
I	0.40	90	12	89
II	0.40	80	15	89
III	0.40	108	15	89
IV	0.40	138	15	89
V	0.40	97	20	89
VI	0.53	91	12	77
VII	0.53	92	20	77
VIII	0.64	77	11	86

of binding sites. Dynamic binding capacity was seen to be independent of pore size when comparing Sepharose™ 6 Fast Flow (small pores) and Sepharose™ 4 Fast Flow (larger pores).

Dextran is a preferred surface coating because it is hydrophilic, has few non-specific interactions with proteins and is stable in alkali solution. It has also been shown to enhance protein mass transport in porous resins [12]. The resins examined in this work utilize dextran as a surface extension of the base matrix, and thus have the potential to expand the three dimensional spacing between ligands relative to typical cation exchange resins with the same ligand density. This work examines dynamic binding capacity as a function of pore size, ligand density and dextran content for eight cation exchange resins sharing the same type of base matrix chemistry. Solution conductivity is typically used to describe the salt concentrations of complex ionic solutions, such as feedstocks. Since only monovalent electrolytes were used in equilibrating these resins, dynamic binding capacity trends will be examined as a function of ionic strength to emphasize the salt concentration. Critical ionic strength will refer to the ionic strength at which the maximum dynamic binding capacity occurs.

2. Experimental

Tricorn™ 5/100 columns packed with 2 mL bed volumes (0.5 cm column diameter, 10 cm bed height) of eight cation exchange resin prototypes (Table 1) were supplied by GE Healthcare (Uppsala, Sweden). The base-matrices of these resins were highly cross-linked agarose with mean particle sizes of 77–89 μm. Resins designated as I–V used the smallest pore size base matrix, resins VI–VII used the medium pore size, and resin VIII used the largest pore size base matrix. Briefly, dextran of 40 kDa molecular weight was attached to the base matrix, and a sulfonate functional group was subsequently introduced to both the agarose and the dextran. Chromatographic experiments were performed on an ÄKTAexplorer™ 100 (GE Healthcare, Uppsala, Sweden) at room temperature. All chemicals were obtained from Angus Buffers and Biochemicals (Buffalo Grove, IL, USA) and used as received, unless otherwise specified. Each buffer and feedstock was filtered with Millipore Stericup™-GP vacuum filtered units (Billerica, MA, USA).

Feedstocks of a full-length monoclonal antibody (Genentech Inc., South San Francisco, CA, USA) with an isoelectric point of 9.2 (net protein charge at experimental conditions = +64.8), and a molecular weight of 160 kDa were adjusted to pH 5 with 1.5 M TRIS base and the corresponding conductivity (2, 4, 6, 8, or 10 mS/cm) with 5 M NaCl. The 2, 4, 6, 8, and 10 mS/cm conductivities correspond to ionic strengths of 0.03, 0.05, 0.07, 0.09, 0.11 in the same order. It should be noted that this antibody has been denoted as Mab1 in prior publications [9,11]. The load concentrations post-filtration were 4–6 mg/mL, except in the case of the 2 mS/cm feedstock where it was diluted to 2–3 mg/mL with ultrapure water. Antibody concentration was determined based on absorbance at 280 nm with subtraction of absorbance at 320 nm with a Shimadzu 1601 UV–vis spectrophotometer (Shimadzu, Kyoto, Japan) at a path length of 1.0 cm. Columns were equilibrated with 5 column volumes (CV) of 25 mM sodium acetate adjusted to pH 5 with a fixed volume of glacial acetic acid (Mallinckrodt, Phillipsburg, NJ, USA) based on the Henderson-Hasselbach equation.

Feedstocks were loaded to 20% of total breakthrough (BT) based on UV spectroscopic absorbance at 280 nm. Since absorbance curves did not depict sharp breakthroughs at 10% BT, the resins were loaded to 20% BT where sharp breakthroughs occurred (Fig. 1). Upon 20% BT, the column was washed with 5 CV of equilibration buffer. A 15 CV gradient elution was performed with 25 mM MES pH 5.5 from 50 to 500 mM sodium acetate. Columns were regenerated with 4 CV of 1 M NaCl, 25 mM MES pH 5.5, sanitized for 30 min with 0.5 N NaOH, and stored in 0.1 N NaOH. Experiments were performed at a flow rate of 20 CV/h except for the load phase at 3 CV/h (20 min residence time). The flow rates selected fit with Genentech's facility and capacity requirements.

Dynamic binding capacities ($Q_{B20\%}$) were calculated according to the following equation:

$$Q_{B20\%} = \left(\frac{V_P - V_H}{V_B}\right) C_P \quad (1)$$

where V_P is the volume of feedstock loaded to 20% breakthrough based on absorbance, V_H is the total holdup volume within the system (i.e. volume occupying deadspace from feedstock container tubing to the UV detector flow cell), V_B is the volume of the resin bed, and C_P is the antibody concentration in the feedstock.

The volume of feedstock loaded onto each column, V_P, was determined through the ÄKTAexplorer™ 100 software, UNICORN™ v5.01 (GE Healthcare, Uppsala, Sweden) by measuring the volume from the onset of loading to 20% breakthrough with the UV absorbance at 280 nm. The system holdup volume was determined

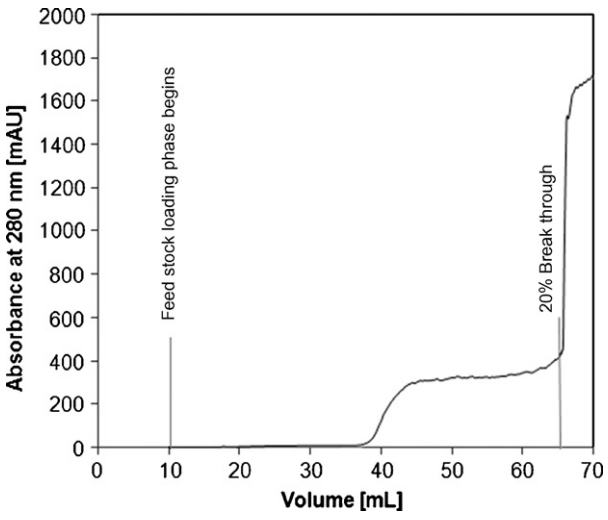

Fig. 1. Breakthrough curve of feedstock at 0.05 ionic strength from resin I. The absorbance at 280 nm of this feedstock was 2090 mAU at breakthrough.