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Effects of resin ligand density on yield and impurity clearance in preparative cation exchange chromatography. I. Mechanistic evaluation

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ABSTRACT

The effects of resin ligand density on cation exchange chromatography performance in preparative monoclonal antibody purification processes were evaluated. A set of agarose-based cation exchange resins spanning a relatively wide range of ligand densities was tested using three different monoclonal antibodies with unique impurity profiles. Experiments were performed in bind-and-elute mode using gradient elution at both intermediate protein loadings and near saturation capacity. Ligand density did not affect clearance of high molecular weight variants under any of the conditions tested; however, ligand density did affect resolution of a basic charge variant in one case and changed host cell protein clearance in another instance. In general, the results indicate that the relationships between ligand density, retention, and resolution are affected by both characteristic charge and protein surface charge distribution.

1. Introduction

Cation exchange chromatography is commonly used as a downstream polishing step in recombinant monoclonal antibody purification processes. Requirements for this unit operation can include removal of product-related impurites such as aggregates and non-product-related impurities such as host cell proteins (HCP) and leached Protein A [1–5]. While upstream affinity chromatography steps often remove non-product-related impurities to trace levels, preparative purification processes usually rely on the cation exchange step to remove aggregates and other high molecular weight (HMW) species to levels that are safe for human therapeutic applications [6]. Process developers are faced with the challenge of designing downstream processes that provide robust impurity clearance while maximizing product yield, minimizing raw material cost, controlling cycle time, and operating within plant constraints.

There are an increasing number of commercial cation exchange resins available for use in large scale purification processes. These resins span a wide range of physical characteristics such as particle size, pore size and geometry, ligand density, and base matrix chemistry [7,8]. While the diverse nature of these products offers opportunity to screen resins for improved performance in specific applications, the relationships between resin properties and performance are not well understood. In particular, the effects of ligand density on impurity clearance have not been studied in a systematic manner for large scale monoclonal antibody purification processes.

Ligand density has been shown to influence both protein retention and mass transfer characteristics in ion exchange chromatography. Wu and Walters observed a switch in the elution order of lysozyme and cytochrome c on a set of five silica-based cation exchange resins with different ligand densities; cytochrome c eluted first on the lower ligand density resins, while lysozyme eluted first on the higher ligand density resins. They also noted pronounced band broadening at lower ligand densities which negatively impacted resolution of the two proteins [9]. Langford and co-workers used confocal microscopy techniques to show that resin ligand density can affect the ionic strength at which intraparticle transport switches from a pore diffusion to a homogenous diffusion mechanism [10]. Franke et al. found that ligand density affected resin porosity and the corresponding pore diffusivities [11]. These studies imply that the resolution of target protein and impurities could be significantly impacted by resin ligand density in preparative cation exchange processes; however, there is a lack of direct evidence to support this conclusion.

High molecular weight species such as protein aggregates can pose a particularly interesting challenge during process development because they may have similar tertiary structure and isoelectric point (pI) as the monomeric target protein [12]. Suda et al. showed that the characteristic charge of monomer and

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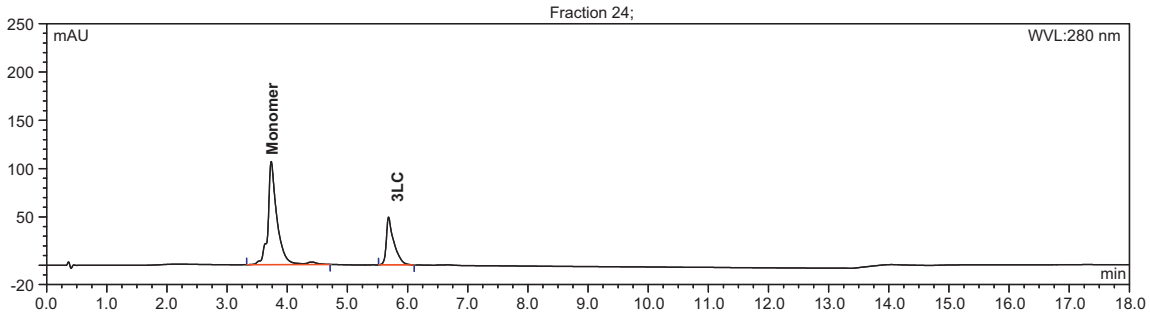

Fig. 1. mAb A HPLC-HIC chromatogram.

aggregate on cation exchange resins is significantly different, an indication that the mechanism which drives resolution of these two species is primarily electrostatic. However, a comparative study of SP Sepharose™ FF and SP Sepharose™ XL revealed that while the difference in characteristic charge (also known as the protein effective binding charge) between monomeric and aggregated forms of a recombinant monoclonal antibody was much larger on SP Sepharose™ XL, the separation performance of the two resins was similar [5]. This is consistent with the work of Wu and Walters [9] who concluded that retention is not perfectly correlated with characteristic charge and must be influenced by other factors. DePhillips and Lenhoff compared the retention of several model proteins on a range of commercial resins and concluded that ligand type (sulfopropyl versus carboxylate) and pore size were the primary determinants of protein retention in cation exchange chromatography [13]. Clearly there exists a complicated relationship between resin properties and resin performance; understanding the role of individual characteristics such as ligand density or pore geometry in actual separation processes can be difficult without the use of sophisticated modeling techniques or resin prototypes that vary in only one aspect of their design.

The objective of this work was to elucidate the effects of ligand density in cation exchange chromatography operations. This was accomplished using a series of prototype preparative resins differing only in ligand density. The resins were packed into columns with representative bed heights and experiments were run in bind-and-elute mode. Three monoclonal antibody feedstocks with relatively high levels of product-related impurities and host cell proteins were used in an attempt to identify changes in yield and impurity clearance caused by ligand density variation. In all cases, the resins were operated at intermediate load densities and also near capacity to obtain a meaningful assessment of resin performance at conditions relevant to large scale biochemical manufacturing processes.

2. Materials and methods

2.1. Resins and columns

Experiments were performed on five cation exchange (CIEX) resins provided by GE Healthcare Bio-Sciences (Uppsala, Sweden). These resins consisted of sulphopropyl (SP) ligands coupled to the same type of cross-linked agarose base matrix as that used in Capto™ ImpRes. Mean particle size was approximately 46 μm. Ligand densities were as follows: 0.045 eq/L (45 meq/L), 0.064 eq/L (64 meq/L), 0.103 eq/L (103 meq/L), 0.141 eq/L (141 meq/L), 0.154 eq/L (154 meq/L). This range of ligand densities extends far below the commercial specification of 0.13–0.16 eq/L for Capto™ SP ImpRes.

All resins were packed into 0.66 cm i.d. × 20 cm Omnifit columns (Bio-Chem Valve, Inc., Cambridge, England). All column chromatography experiments were performed on an ÄKTA™ Explorer 100 FPLC system (GE Healthcare, Piscataway, NJ) with a 2 mm pathlength UV cell.

2.2. Feedstock

Full-length monoclonal antibodies (mAbs) were expressed in Chinese hamster ovary (CHO) cells in 2000 l bioreactors at Genentech (South San Francisco or Oceanside, CA). Cell culture fluid was harvested using a combination of continuous centrifugation and depth filtration. Harvested cell culture fluid was initially purified using Protein A affinity chromatography with low pH elution in 0.10–0.15 M acetic acid. Protein A eluate was adjusted to pH 5.5 with 1.5 M Tris base and loaded onto the lab scale cation exchange columns without further adjustment.

Table 1 lists the antibodies used in this work. mAb A was engineered with an unpaired cysteine residue on each heavy chain. This resulted in a covalent "triple light chain" (3LC) high molecular weight variant which comprised approximately 10% of the cation exchange load by mass (see Section 2.3). mAb B cation exchange load was comprised of approximately 11% aggregate by mass; in this case, the high molecular weight species were primarily covalent dimers and trimers as verified by high performance size exclusion chromatography (see Section 2.4) and SDS-PAGE. mAb C had nearly undetectable levels of high molecular weight variants, but the cation exchange load had relatively high levels of host cell protein (HCP) impurities (approximately 10-fold higher than other mAbs employed in this study).

2.3. High performance hydrophobic interaction chromatography (HPLC-HIC)

An HPLC-HIC assay was used to quantify the amount of 3LC in the cation exchange load and eluate for mAb A. The assay used a TSKgel Butyl-NPR (4.6 mm × 35 mm) column (cat. No. 14947) from Tosoh Bioscience (Montgomeryville, PA). The column was run at 0.8 mL/min for 18 min. Buffer A was 1.5 M ammonium sulfate, 25 mM sodium phosphate, pH 6.95. Buffer B was 25 mM sodium phosphate, pH 6.95 with 25% isopropyl alcohol by volume. Protein was eluted with a gradient of 0–100% B in 12 min with UV

Table 1
Monoclonal antibody feedstock.

Property	mAb A	mAb B	mAb C
Framework	IgG1	IgG1	IgG1
Theoretical pI	9.4	8.9	8.5
pH of cation exchange load	5.5	5.5	5.5
Conductivity of cation exchange load (mS/cm)	4.3	4.0	4.1
HMW species in protein A pool (%)	10	11	<1
Approximate HCP in protein A pool (ng/mg[a])	2000	8000	40,000

[a] ng of HCP/mg antibody.