Journal of Chromatography A, 1217 (2010) 216–224

Contents lists available at ScienceDirect

Journal of Chromatography A

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

The distinctive separation attributes of mixed-mode resins and their application in monoclonal antibody downstream purification process

Jie Chen*, Jen Tetrault, Yanyu Zhang, Andy Wasserman, Greg Conley, Mike DiLeo, Elliot Haimes, Andrew E. Nixon, Arthur Ley

Process Sciences and Discovery Research, 300 Technology Square, Dyax Corp., Cambridge, MA, 02139, USA

ARTICLE INFO

Article history:
Available online 23 September 2009

Keywords:
Mixed-mode chromatography
MEP
CHT
Capto adhere
Aggregates removal
Monoclonal antibody purification process

ABSTRACT

Increased upstream productivity and the continuous pressure to deliver high quality drug product have resulted in the development of new separation technologies and platform strategies for downstream purification processes of monoclonal antibodies (mAb). In this study, the separation attributes of three mixed-mode resins, Mercapto-Ethyl-Pyridine (MEP) hydrophobic charge induction resin, Capto adhere multi-modal anion exchange resin, and ceramic hydroxyapatite/fluoroapatite (CHT/CFT) resins, were investigated to define their roles in monoclonal antibody purification processes. We demonstrated that the multi-modal nature of ligands on mixed-mode resins allows the separation resolution to be honed, either through a single dominant mechanism or through mix-modal balanced purification strategies. In addition, the three mixed-mode resins present different purification powers for different types of impurities. We also demonstrated that besides enhancing chromatography separation and improve product quality, especially for high molecular weight (HMW) aggregate reduction, mixed-mode resins can also help to improve process efficiency in industrial-scale mAb drug manufacturing. Our results underscore the importance of selecting appropriate chromatography resins during DSP design to obtain the best overall process outcome.

1. Introduction

Increases in upstream mAb production levels result in downstream purification challenges in the scale up efficiency and cost-effectiveness of Protein A (ProA) affinity chromatography based processes [1–4]. The physical constraint of processing increasingly large batches of monoclonal antibodies in existing manufacturing plants demands less sample manipulation and process volume control. It is not preferred and sometimes is not practical to further dilute the large ProA elution pool derived from small ProA column-multiple run capture steps if the subsequent ion exchange column demands lower conductivity for loading [5–7]. Therefore, in-process ultrafiltration/diafiltration (UF/DF) is often added to the process scheme to reduce tank size demand, loading process time, and/or to condition the load for the next ion exchange column. Such in-process UF/DF of ProA elution pools often faces a problem with turbidity which can be either product-related, contaminant-related, or both. The degree of the turbidity problem varies from product to product, and from process to process. In some cases, when in-process UF/DF cannot be developed into a robust process, an alternative approach is required [8–10]. In addition, the increasing emphasis from regulatory authorities on decreasing aggregate levels and improving product purity has influenced the goals of the downstream purification process [11]. ProA affinity and ion exchange separation techniques in the common generic purification platform cannot always handle the purification of all mAb products from discovery pipelines [8,12]. When faced with challenges such as high levels of aggregation from mammalian cell culture, other chromatographic techniques are often necessary to reduce aggregates. Hydrophobic interaction chromatography (HIC) and several newly evolved mixed-mode chromatography techniques have been reported as good options for aggregate reduction [13–17,29]. In a previous study, we have presented the advantages of mixed-mode resins in facilitating process step transitions without complex sample manipulation [18]. In this study, three mixed-mode resins commonly used in mAb DSP, Mercapto-Ethyl-Pyridine (MEP) hydrophobic charge induction resin, Capto adhere multi-modal anion exchange resin, and ceramic hydroxyapatite/fluoroapatite (CHT/CFT) resins, were investigated for their separation attributes and for their distinctive contributions to overall mAb purification process outcomes.

* Corresponding author.
E-mail address: jchen@dyax.com (J. Chen).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.09.047

2. Materials and methods

2.1. Chromatography purification process

The test proteins are three human monoclonal antibodies (hMab) IgG1 selected from Dyax antibody phage libraries. The hMab products were expressed by Chinese Hamster Ovary (CHO) cell lines. CHO fermentation cultures were clarified by depth filtration using Millipore HC pod depth filters (Billerica, MA, USA) and the clarified supernatant materials (Dyax, Cambridge, MA, USA) were subjected to downstream purification by defined processes described below.

Capture step Protein A resin is Mabselect ProA affinity resin from GE Healthcare (Piscataway, NJ, USA). The three mixed-mode resins used in these studies are Hydrophobic Charge Induction (HCIC) resin: MEP (Mercapto-Ethyl-Pyridine) HyperCel from Pall Life Sciences (East Hills, NY, USA); Capto adhere multi-modal anion exchange resin from GE Healthcare (Piscataway, NJ, USA), and Ceramic Hydroxyapatite and Fluoroapatite resins from Bio-Rad Laboratories (Hercules, CA, USA). In addition, other ion exchange resins including Capto Q anion exchange resin from GE; UNO-Q anion exchange resin from Bio-Rad Laboratories; Gigacap Q anion exchange resin from Tosoh Bioscience (Montgomeryville, PA, USA); Poros HS cation exchange and Porous HQ anion exchange resins from Applied Biosystems (Bedford. MA, USA); Fractogel TMAE(M) and TMAEhicap(M) anion exchange resins from EMD Chemicals Inc. (Gibbstown, NJ, USA), were also used in the studies.

All chemical reagents were from Sigma (St. Louis, MO, USA) and J.T. Baker (MallinkrodtBaker, Phillipsburg, NJ, USA) unless otherwise noted. Chromatography separations were operated on AKTA LC systems from GE Healthcare using AP columns from Waters (Millford, MA, USA), XK and BPG100 columns from GE Healthcare.

All chromatography studies used constant residence time across scale up based on capacity study results. Columns were packed to bed heights of 10–20 cm following manufacturer's recommendations and packing efficiency was ensured by preset criteria of HETP (Height Equivalent to a Theoretical Plate) and peak Asymmetry (As) factor. Each chromatographic column separation was operated under the conditions described in the results section or in figure legend. The process yield was measured by analytical ProA-HPLC and A280. Product purity was evaluated by analytical SEC-HPLC and impurity-specific ELISA assays.

2.2. Analytical HPLC

The analytical ProA-HPLC assay was performed using a 30 mm × 2.1 mm Poros ProA column on a Waters 2695 Alliance system. Standard and test samples were injected onto pre-equilibrated ProA column with 50 mM sodium phosphate, 150 mM NaCl, pH 7.0 buffer. IgG in the samples that had bound to the column was eluted by applying an acidic solution, 12 mM hydrochloric acid, 150 mM NaCl, pH 2.0. The amount of sample IgG injected was adjusted to be within the standard curve range of the assay (2–100 μg). The IgG protein concentration in the test sample was determined from the elution peak area using a reference standard curve.

The analytical SEC-HPLC assay was performed using a 7.8 mm × 30 mm SW3000 column on a Waters 2695 Alliance system. Molecular weight standards, reference IgG standards and test samples were injected onto the pre-equilibrated SEC column using phosphate buffer with 300 mM NaCl at pH 7.0. IgG monomer, together with impurities and contaminants in the samples were separated based on their molecular size. Product purity in a test sample is represented as the ratio of monomer peak area to total peak area at 280 nm.

2.3. Analytical ProA-ELISA and CHO HCP ELISA

The ProA-ELISA was performed with rProtein A^{TM} ELISA kit from Repligen (Waltham, MA, USA). Standard and test samples were diluted with special sample diluent and added to the wells of a pre-coated microtiter plate according to manufacturer's instructions. Protein A in the sample was detected by specific anti-rProtein A antibodies and measured by an enzyme–substrate colorimetric reaction. ELISA assays plates were read on SpectraMax M2 Plate Reader from Molecular Devices (Sunnyvale, CA, USA). Concentrations of rProtein A in each of the test samples were calculated based on the standard curve.

The CHO Host Cell Protein (HCP) ELISA was performed using the CHO HCP ELISA kit F015 from Cygnus Technologies (Southport, NC, USA). Standard and test samples were diluted with the specified sample diluent and incubated 2 h at 37 °C with alkaline phosphatase labeled anti-CHO HCP antibodies. After incubation, samples were added to wells of a microtiter plate that had been pre-coated with anti-CHO antibodies and treated according to manufacturer's instructions. CHO HCP in the sample, detected by specific anti-CHO HCP antibodies, was measured by an enzyme–substrate colorimetric reaction. ELISA plates were read out as described above. The resulting color intensity is proportional to the concentration of CHO HCP. CHO HCP levels in each of test sample were calculated based on the standard curve.

2.4. A280 adsorption and insoluble aggregation index (AI) measurement

Measurement of adsorption was carried on spectrophotometer of DU800 UV–vis from Beckman Coulter (Fullerton, CA, USA) or Cary50 UV–vis from Varian Inc. (Palo Alto, CA, USA). Adsorption at 280 nm was used for IgG concentration quantification, while adsorption at 280 and 350 nm were used for calculation of an insoluble aggregate index (AI) based on the formula below [19]:

$$\mathrm{AI} = \left(\frac{100 \times \mathrm{OD}_{350}}{\mathrm{OD}_{280} - \mathrm{OD}_{350}}\right)$$

2.5. Imaged capillary electrophoresis (iCE)

Samples were prepared with ampholyte from GE Healthcare, methyl cellulose from Convergent, and pI markers from Fluka. After pre-focusing for 1.0 min at 1500 V and focusing for 8.0 min at 3000 V, a UV light absorption image of the entire capillary is captured with a CCD camera of Convergent iCE$_{280}$ Analyzer (Con-

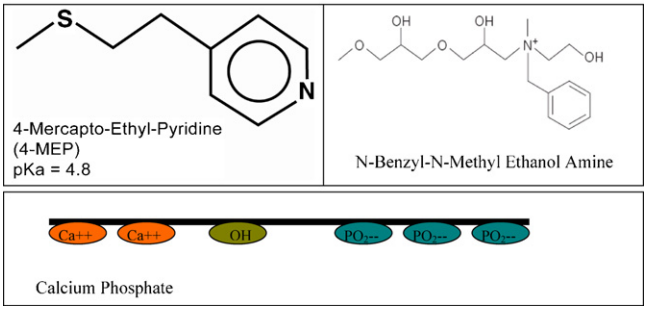

Fig. 1. MEP ligand, 4-Mercapto-Ethyl-Pyridine (A), is a mixed-mode resin with hydrophobic and electrostatic interaction mechanisms. Captoadhere ligand, N-benzyl-N-methyl ethanol amine (B), is a multi-modal strong anion exchanger. The most pronounced functionalities reported are ionic interaction, hydrogen bonding and hydrophobic interaction. Ceramic hydroxyapatite ligand, unique form of calcium phosphate (C), is a mixed-mode resin consisting of electrostatic and metal affinity interaction mechanisms.