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Use of mobile phase additives for the elution of bispecific and monoclonal antibodies from phenyl based hydrophobic interaction chromatography resins

Troii Hall*, Gerard M. Kelly, Warren R. Emery

Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46285-0001, USA

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ABSTRACT

Hydrophobic interaction chromatography (HIC) is routinely used in the purification of biopharmaceuticals such as antibodies. However, hydrophobic proteins can be difficult to elute resulting in low recovery of product thereby complicating early phase process development and potentially excluding the use of HIC resins for their manufacture. Mobile phase additives such as hexylene glycol and arginine facilitate protein elution from resins including HIC; therefore, these additives were evaluated toward the recovery and purification of bispecific and monoclonal antibodies from Phenyl Sepharose HP and Capto Phenyl ImpRes resins. The influences of gradient versus step elution as well as superficial linear velocity on product quality were evaluated. Improved protein recovery and reduction of both soluble product aggregate and host cell protein were observed for the tested antibodies with both hexylene glycol and arginine. Furthermore, the impact of salt removal from the HIC load on protein-resin binding was examined providing opportunities to minimize processing time. This method was successfully scaled using a Phenyl Sepharose HP (5 cm i.d. × 20.0 cm) and Capto Phenyl ImpRes (3.2 cm i.d. × 21.4 cm) column demonstrating potential for manufacturing purposes.

1. Introduction

Biopharmaceutical companies continually search for opportunities to accelerate the development and purification of bio-therapeutics using cost effective processes [1–4]. In particular, minimizing the time dedicated to early phase purification development and material generation would provide a competitive commercial advantage [5]. For example, most antibody purification processes start with a templated capture step using Protein A chromatography [6,7]. However, the development of robust polishing chromatography steps for removing soluble product aggregate and host cell protein (HCP) is more ambiguous and time consuming.

Resins commonly used for polishing chromatography include ion-exchange, mixed-mode and hydrophobic interaction chromatography (HIC) with each providing unique attributes [8]. HIC has been widely used for antibody production because of its consistent aggregate reduction [9]. Moreover, HIC can be performed using flow through or traditional bind-and-elute chromatography [10]. In traditional bind-and-elute mode, protein-resin interactions are promoted by incorporating salts such as ammonium sulfate during loading and then elution is performed by decreasing the salt concentration [11]. Furthermore, salt type and concentration, temperature, pH and ligand choice can each be manipulated to influence protein-resin interactions to improve selectivity, making HIC a powerful purification tool [12,13]. Conversely, HIC resins are prone to lower binding capacity and linear velocity compared to other modes of commercially viable chromatography; additionally, protein elution and thus recovery can sometimes be unacceptably low [14].

Strong protein-resin interaction can result in the partial or total loss of protein recovery during HIC separations. Less hydrophobic ligands (for example butyl rather than octyl) may facilitate better yields, but empirically identifying the optimal ligand(s) as well as resin type, salt, pH, etc. is often a time-consuming process [12,13]. High throughput screening technologies enable faster investigation of these variables, yet this approach still requires materials, time and resources [15]. Therefore, identifying an approach that lessens the influence of HIC resin hydrophobicity while achieving satisfactory yield and product quality would be highly desirable from a process development perspective. One approach toward this goal would be the incorporation of mobile phase additive(s) to elute proteins [16,17].

Abbreviation: HIC, hydrophobic interaction chromatography; HCPs, host cell proteins

* Corresponding author.
E-mail addresses: hall_troii@lilly.com (T. Hall), kelly_gerard_m@lilly.com (G.M. Kelly), emery_warren_r@lilly.com (W.R. Emery).

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Many mobile phase additives have been shown to improve protein elution and recovery from various chromatographic resins including HIC [16–24]. For example, detergents including Triton X-100 have been previously described for improving protein recovery. However, it requires demonstration of adequate removal of the detergent used. In addition, because certain detergents pose environmental hazards it necessitates special disposal protocols. Solvents including methanol, isopropanol and ethanol have been used to improve protein recovery from HIC, but solvents pose a significant flammable hazard necessitating specific handling and safety measures. Chaotropic agents such as urea and guanidine tend to be less active toward protein elution, may potentially denature and/or modify the bio-therapeutic and are also of concern with respect to disposal [24]. Amino acids including arginine have been described in the facilitation of proteins from various resins as well [21,22]. Ethylene glycol and glycerol are polyols that have been extensively described for their ability to function as mobile phase modifiers including their use in different HIC methods [18]. However, most of these reported methods focus on recovery of non-antibody proteins and while some reports describe antibody recovery, few detail the ability to improve purity by demonstrating aggregate and HCP reduction.

Another less described polyol, hexylene glycol, has been used as a substitute for the flammable solvent, acetonitrile, in the purification of human insulin-like growth factor-1 using reversed phase chromatography [25]. However, hexylene glycol has not been rigorously examined for its ability to improve product yield and quality of various antibodies from HIC resins. The amino acid arginine has been successfully tested with multiple resins including HIC for improving protein quality and recovery [26]. But, to our knowledge, arginine has not yet been investigated for aggregate and HCP reduction for bispecific and monoclonal antibodies using Phenyl based HIC resins. Furthermore, hexylene glycol and arginine use in HIC have not been compared using Phenyl Sepharose HP or Capto Phenyl ImpRes resins.

This work describes the effect of mobile phase additives, hexylene glycol and arginine, on antibody recovery and purity (aggregate and HCP reduction) using Phenyl based HIC resins. Three antibodies, including two bispecific and one IgG4 monoclonal, differing in structure and strength of hydrophobic interaction were used to investigate the robustness of these additives. Furthermore, the methods described herein were examined using semi-preparative chromatography to support possible implementation at a manufacturing scale.

2. Materials and methods

2.1. Materials

Hexylene glycol, trizma base, sodium chloride, sodium sulfate and arginine HCl were purchased from Sigma Chemical Company, St. Louis, MO. The HIC HiScreen columns (Phenyl Sepharose HP and Capto Phenyl ImpRes), Phenyl Sepharose HP and Capto Phenyl ImpRes resin were purchased from GE Healthcare, Uppsala, Sweden. The TSK gel G3000 SWXL analytical size-exclusion column and TSK gel Butyl-NPR columns were purchased from TOSOH Bioscience (Tokyo, Japan). Antibodies were produced in Chinese hamster ovary (CHO) cells at Eli Lilly (Indianapolis, IN). Reagents used in the CHO HCP assay were generated at Eli Lilly (Indianapolis, IN).

2.2. Expression of antibodies in CHO cells

Antibodies (Mab 1, Mab 2 and Mab 3) were expressed in CHO cells at Eli Lilly (Indianapolis, IN). Cell culture fluid was harvested by depth filtration and antibodies initially purified using Protein A affinity chromatography. Protein A eluate was adjusted to pH 5.0 with Tris base and sterile filtered prior to use.

2.3. Analytical size-exclusion chromatography

Relative soluble product aggregate content was estimated by analytical size exclusion using a Waters 2790 Alliance HT HPLC instrument (Waters Corporation, Milford, MA) and absorbance detected with a Waters 2487 dual wavelength absorbance detector at 280 nm. Fifty microliters of sample (0.5–1.0 mg/mL) was loaded onto a TOSOH TSK gel G3000 SWXL (7.8 mm × 30 cm, 5 μm) column and operated at 0.50 mL/min in isocratic mode using 50 mM potassium phosphate, 350 mM NaCl, pH 7.0.

2.4. ELISA quantification of host cell proteins

Residual host cell proteins were quantitated using an ELISA assay developed at Eli Lilly and Company.

2.5. Incubation of antibodies (Mab 1, Mab 2 and Mab 3) with hexylene glycol or arginine

The effect of additive (hexylene glycol and arginine) on reversible aggregation was determined by incubating Mab 1, Mab 2 and Mab 3 with varying concentrations of hexylene glycol or arginine. Antibodies (5–10 mg/mL) were spiked with hexylene glycol (10%), arginine (1 M) or no additive (control) and allowed to incubate at room temperature for 5 h. Relative aggregate content was estimated by analytical size exclusion chromatography.

2.6. Analytical HIC to characterize protein hydrophobicity

Analytical HIC separations using a TSK gel Butyl-NPR column and Waters 2790 Alliance HT HPLC instrument were performed to determine relative hydrophobicity for Mab 1, 2 and 3. Antibodies (0.5–1.0 mg/mL) were prepared in 100 mM sodium phosphate, pH 7.0, 1 M ammonium sulfate solution. Fifty microliters of protein sample was injected onto the 4.6 mm × 10 cm, 2.5 μm column. A gradient including

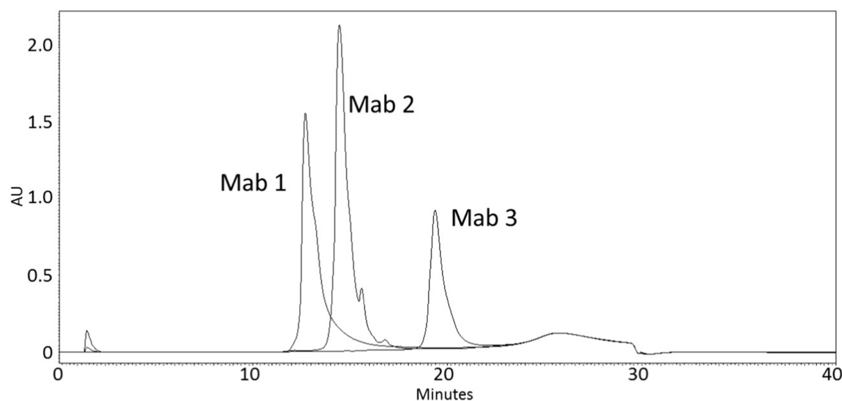

Fig. 1. Analytical HIC assay ranking the hydrophobicity of Mab 1, Mab 2 and Mab 3. Antibodies (0.5–1.0 mg/mL) were injected (50 μL) onto the TSK gel Butyl-NPR column (4.6 mm i.d. × 10 cm, 2.5 μm) and separated using a decreasing ammonium sulfate gradient (40–100% B) over 20 min run at 0.5 mL/min while detecting at A214 nm. Buffer A was 100 mM Sodium Phosphate, pH 7.0, 1 M $(NH_4)_2SO_4$ and Buffer B was 20 mM Tris, pH 7.

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