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Phenylboronic acid chromatography provides a rapid, reproducible and easy scalable multimodal process for the capture of monoclonal antibodies

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ABSTRACT

This work addresses the feasibility of scaling-up phenylboronic acid (PBA) chromatography for the purification of human mAbs directly from Chinese Hamster Ovary cell culture supernatants. Column performance optimization regarding superficial velocity and feed volume was performed with the best results being achieved with superficial velocities of 5.1, 15.3 and 25 cm/min in the adsorption, wash and elution steps, respectively. The column volume was scale-up 100 times from 0.4 cm^3 (laboratory scale) to 4, 16 and 40 cm^3 (preparative scale). This 100-fold scale-up was successfully achieved with a recovery yield of 97.7% and a protein purity of 82.6%. Following purification, purified IgG fractions were characterized in terms of isoelectric point, size, CHO proteins and genomic DNA content. Overall results suggest that using ProSep®-PB for mAbs purification is simple, reproducible, robust and scalable without compromising the target molecule integrity and purity.

1. Introduction

Monoclonal antibodies (mAbs) currently represent one of the leading families of the biopharmaceutical industry in terms of therapeutic and market potential, with total sales expected to reach a value of $70 billion in 2015 [1]. Their tremendous potential has derived from the recent advances in molecular biology, genetics and protein engineering that have allowed the production of low immunogenic and highly effective mAbs [2]. By February 2014, 43 mAbs had been approved by the US Food and Drug Agency (FDA) for the treatment of different diseases, including cancer, autoimmune and inflammatory disorders. The combination of an ever-growing patient population with the relatively low potency of mAbs has led to the demand for large quantities of pharmaceutical-grade mAbs [3]. This increasing market demand has foster the development of new and more efficient processes for the production and purification of mAbs. If in early years, upstream processes were responsible for the majority of the production bottlenecks, nowadays with new cell culture media formulations based on chemically defined serum-free media and improved cell lineages, these problems have been overcome and shifted towards the downstream processes where the capture is the critical step. Due to the conserved domains of mAbs, a general purification process based on a common sequence of unit operations is currently employed by many companies [4]. The heart of the process is the protein A chromatography capture, which despite being highly stable, reliable, and reproducible, is considered to be a productivity bottleneck and especially expensive, representing up to 25% of the total mAbs manufacturing process [5,6].

We have recently proposed the use of phenylboronic acid (PBA) multimodal chromatography as an alternative capture step for the direct purification of mAbs from clarified cell culture supernatants, with phenylboronate (PB) immobilized in controlled porous glass (CPG) beads – ProSep®-PB [7]. Phenylboronate was first reported as an affinity ligand able to selectively bind to 1,2-*cis*-diol containing molecules by *cis*-diol esterification [4]. MAbs bear two N-linked oligosaccharide chains at the 297-asparagine residue of the CH2 domain of the Fc region, and despite some heterogeneity found in the terminal sugars attached, mannose, galactose, fucose and N-acetylneuraminic acid typically present contain *cis*-diol groups, thus making PB ligands suitable for mAbs purification [8,9]. Nonetheless, it has been reported that proteins can interact by non-specific interactions [10] which motivated the development of a wash step where impurity proteins, that co-adsorbed with mAbs were washed out. The nature of the interactions between cell culture supernatants and ProSep®-PB matrix, wash and elution buffer composition, as well as mAb integrity after purification has been thoroughly analyzed [7]. Those studies led to the

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definition of the optimized conditions to purify mAbs using ProSep®-PB, namely 20 mM HEPES, pH 7.5 with 150 mM NaCl as adsorption buffer, 100 mM D-sorbitol in 10 mM Tris–HCl, pH 7.5 as wash buffer and 0.5 M D-sorbitol with 150 mM NaCl in 10 mM Tris–HCl, pH 7.5 as elution buffer [7].

Nevertheless, in order to promote the use of PBA multimodal chromatography in mAbs purification process, the efficacy and consistency of the method at a process scale needs to be evaluated. Therefore, in this work we have assessed the effect of the working superficial velocity on process performance, and have implemented a series of loading and scale-up studies in order to evaluate the feasibility of using PBA multimodal chromatography for the purification of mAbs at a preparative scale and in a prospective industrial setting.

Multimodal chromatography has been receiving considerable attention for protein purification in the last years. Compared with other types of chromatography, multimodal chromatography is particular advantageous in its salt-independent adsorption and simple elution by charge repulsion. Multimodal ligands, such as Capto MMC and MEP-Hypercel have been tested for mAbs purification, as an alternative to the affinity-based capture platform [11,12]. Capto MMC presents similar characteristics to ProSep-PB in terms of recovery yield, purity and also dynamic binding capacity (≈30 mg IgG/cm^3 resin) [13]. However, the cost of the PBA silica-based resins is much lower than that of the Capto MMC (agarose-based). The physical bed stability is also higher using silica-based resins than agarose-based. Regarding the MEP-Hypercel, both PBA and Capto MMC present much higher recovery yields (>97% *vs* 86%) [14].

2. Materials and methods

2.1. Chemicals

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium chloride (NaCl), D-sorbitol and 2-amino-2-hydroxyme thyl-propane-1,3-diol (Tris) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium phosphate monobasic anhydrous and sodium phosphate dibasic were obtained from Panreac Quimica Sau (Barcelona, Spain), and hydrochloric acid from Fluka (Buchs, Switzerland). All other chemicals were of analytical or HPLC grade. All solutions were prepared using purified water from a Milli-Q® system (Millipore, Bedford, MA, USA).

2.2. IgG production

Anti-human interleukin-8 (IL-8) monoclonal antibodies were produced by CHO DP-12 clone#1934 (ATCC CRL-12445) using DHFR minus/methotrexate selection system. CHO DP-12 cells were grown in serum-free media formulated with 0.1% Pluronic® F-68 and without L-glutamine, phenol red, hypoxanthine, or thymidine (ProCHO™5, Lonza Group Ltd, Belgium). The cell culture media was supplemented with 4 mM L-glutamine (Gibco®, Carlsbad, CA), 2.1 g/dm^3 $NaHCO_3$ (Sigma–Aldrich), 10 mg/dm^3 recombinant human insulin (Lonza), 0.07% (v/v) lipids (Lonza),1% (v/v) antibiotics (100 U/cm^3 penicillin and 100 μg/cm^3 streptomycin) (Gibco®) and 200 nM methotrexate. The cultures were carried out in T-175 flasks (BD Falcon, Franklin Lakes, NJ) at 37 °C and 5% CO_2 with an initial cell density of 2.8×10^4 cells/cm^2. Cell passages were performed every 6 days. Cell supernatants were centrifuged at 1250 rpm for 7 min, collected and storage at −20 °C [15]. This culture was maintained for several months and the mAb concentration varied between 48 and 74 mg/dm^3.

2.3. Monoclonal antibody purification

Purification of mAbs was performed using (i) controlled porous glass (CPG) beads functionalized with 3-aminophenyl boronate (ProSep®-PB media, Millipore) purchased from Fisher Scientific (Leicestershire, UK) and (ii) CPG beads functionalized with recombinant native protein A (ProSep® ultra plus chromatography media, Millipore). All chromatographic runs were performed in ÄKTA Purifier 10 and 100 systems from GE Healthcare (Uppsala, Sweden), both equipped with a Unicorn 5.1 data acquisition and processing software. Conductivity and UV absorbance at 280 nm of the outlet sample were continuously measured.

2.3.1. Phenylboronic acid chromatography

All chromatographic runs were performed following a binding-washing-elution approach and using a Tricorn™ 5/20 empty glass column (GE Healthcare, Uppsala, Sweden) (0.5 cm internal diameter (i.d.)) packed with 0.4 cm^3 of resin, except otherwise stated. All runs were performed at pH 7.5 using (i) 20 mM HEPES with 150 mM NaCl as adsorption buffer, (ii) 100 mM D-sorbitol in 10 mM Tris–HCl as washing buffer, and (iii) 0.5 M D-sorbitol with 150 mM NaCl in 10 mM Tris–HCl as elution buffer. The purification methodology applied was previously developed by our group, as described in [7], except when a new approach is presented.

2.3.1.1. Superficial velocities studies. Different superficial velocities were tested along the chromatographic run. The velocities evaluated were 5.1, 15.3 and 25.5 cm/min. As a first approach, all the chromatographic steps – adsorption, wash and elution – were performed at the same velocity. Then, in a second study, the adsorption superficial velocity was set at 5.1 cm/min and the superficial velocity of the wash and elution steps were varied. Samples were analyzed for IgG and protein content.

2.3.1.2. Loading studies. Different loading volumes of clarified CHO supernatants (5, 12.5 and 25 CV) were injected at the optimal superficial velocities determined: adsorption at 5.1 cm/min, wash at 15.3 cm/min and elution at 25.5 cm/min. A 50 cm^3 Superloop (GE Healthcare) was used for the 25 CVs loading assays. Collected samples were analyzed for IgG, protein and gDNA content.

2.3.1.3. Scale-up. The scale-up of PBA multimodal chromatography was performed in three steps. In the first step, a Tricorn™ 5/200 glass columns (GE Healthcare) was packed with 4 cm^3 of resin; in the second step, a Tricorn™ 10/200 glass column (GE Healthcare) was packed with 16 cm^3 of resin; and in the last step a HiScale 16 20BH column (GE Healthcare) was packed with 40 cm^3 of resin. All chromatographic runs were performed in ÄKTA Purifier 100 system from GE Healthcare.

In the first scale-up step, the column height was increased from 2 to 20 cm, while the i.d. was maintained at 0.5 cm. The column volume was increased 10 times from an initial volume of 0.4 cm^3 to a final volume of 4 cm^3 [16]. The flow rates of each chromatographic step were maintained at 1, 3 and 5 cm^3/min. In the second scale-up step, the column height was maintained at 20 cm, while the i.d. and final volume were increased to 1 cm and 16 cm^3, respectively [16]. The working flow rates were also increased to 4, 12 and 20 cm^3/min, respectively, for the adsorption, wash and elution step. The final scale-up, was performed maintaining the column height at 20 cm and increasing the i.d. to 1.6 cm, to a resin volume of 40 cm^3, accomplishing a final 100-fold scale-up. The working flow rates were increased accordingly to 10.2, 30.7 and 51.2 cm^3/min, respectively, for the adsorption, wash and elution steps [17].

Flow-through, wash and elution fractions were collected using Frac-920 fraction collector from GE Healthcare, and further ana-