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Polishing of monoclonal antibodies streams through convective flow devices

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ABSTRACT

Monoclonal antibodies (mAbs) are the class of biopharmaceuticals showing the fastest growth in the pharmaceutical market. Therefore, an efficient manufacture process must be designed to meet the actual market needs. Diffusion-based devices such as packed-bed resins are traditionally used for polishing steps with some drawbacks, the most important of which is the low process throughput. A promising alternative is the use of convective flow devices, such as membranes and monoliths. In line with this, the performances of Sartobind® Q SingleStep Nano membrane and of CIM® QA monolithic column were evaluated for the polishing of mAbs from different cell fluids and their mimetics and compared with that of HiTrap Q XL packed-bed. Concerning recovery and purification, best working pH values were found in the range 7.5–8.0, and the best performing buffer was phosphate, opposed to HEPES and Tris–HCl. Two different polishing scenarios were evaluated, with and without a previous mAbs capture step, both studied with convective flow and diffusion-based devices. All three-used anion-exchange supports originated yields around 98% and purity higher than 99%. Monoliths outperformed the other two media, in both IgG recovery and impurities removal. However, data reported herein strengthens the choice of using both monoliths and membranes at higher flow rates to overcome the bottlenecks currently found in the purification of mAbs.

1. Introduction

In the pharmaceutical industry the pressure to bring new drugs into the market is enormous. During the last two decades, recombinant monoclonal antibodies (mAbs) have emerged as a very successful class of biological drugs [1]. These recombinant proteins are one of the most representative biopharmaceutical products, being responsible for an important sector of the biopharma industry and market [1]. The demand to efficiently supply the biopharmaceutical market with mAbs, led to the development of cellular processes that rapidly produce large quantities of mAbs at moderate costs and in a consistent and reproducible manner [2]. The dramatic increase in cell culture productivity, at the upstream process, has increased the amount of expectations for faster and reliable purification processes. However, the downstream process has failed to keep up with the upstream productivity boost [3,4], causing critical bottlenecks in mAbs purification stages, that are affecting the overall production efficiency and threatening the economic viability of this specific pharmaceutical industry [5,6].

In order to make processes faster and avoid downstream bottlenecks, new alternatives must be found to foster more cost-effective processes. Different alternatives to the traditional protein A chromatography have been described for the capture of mAbs, including aqueous two-phase extraction [7,8], magnetic fishing [9], and monoliths [10], among others. However, process intensification can also arise from the final purification or polishing stage [11], designed to remove trace amount of impurities, like DNA, host cell proteins, virus, and other impurities, in compliance to the quality demands of regulatory agencies [12]. In a polishing step, capacity does not need to be extremely high, as in a capture step, and thus traditional resin-based chromatography can be replaced by alternative chromatographic formats using convective flow devices, such as membranes and monoliths [13]. These devices combine the possibility of working at higher flow rates with a sufficient capacity for removing low levels of impurities present after a robust capture step [14]. Similar to packed-bed resins used for polishing purposes, membranes and monoliths used at this stage are typically anion exchangers operating in flow-through mode, where the desired product is washed out, while the impurities are retained.

Recently, membrane technologies have been subjected to a tremendous evolution, being this related with the use of different membrane material, housings and manufacturing process [15,16].

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Membranes have larger pore sizes than traditional chromatographic beads and are typically used for polishing steps, offering the ideal conditions to eliminate trace amounts of large particles, like DNA [16] and viruses [17], that are still present after a first purification stage. Anion exchange membranes have seen improvements in the last years and have already been applied in flow-through polishing steps in the manufacture of biopharmaceuticals [18,19]. However, in the specific case of mAbs, there are still some doubts concerning its efficiency. Regarding the monoliths, these are typically used to purify large biomolecules such as virus and plasmids [20], being their use in the polishing of antibodies never been subjected to a very thorough study. Nevertheless, the few already published data and the theory behind monoliths, suggest that this type of devices could be a valuable solution for this purpose [21,22].

The work described herein aims to elucidate the efficiency of convective flow devices for the polishing of monoclonal antibodies under several ionic microenvironments, by comparing the performances of commercially available anion-exchange membranes, monoliths and resins through which simulated and real anti-human interleukin 8 (IL8) monoclonal antibody from CHO cell supernatants have been processed. Different cell culture fluids were investigated in order to assess the influence of process design in the mAbs quality parameters and robustness of polishing technology.

2. Materials and methods

2.1. Chemicals

Sodium phosphate monobasic anhydrous (Na_2HPO_4), tris (hydroxymethyl)aminomethane (Tris), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium chloride (NaCl), and phosphate buffered saline (PBS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Acetic acid 100% was obtained from VWR (West Chester, PA, USA). Sodium dodecyl sulfate (SDS) and glycine were obtained from Bio-Rad (Hercules, CA, USA). Silver nitrate, sodium thiosulfate, formaldehyde and sodium carbonate were obtained from Merck (Darmstadt, Germany). Coomassie Phast Blue R was obtained from Pharmacia (Uppsala, Sweden). Water used in all experiments was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Biologics

Human immunoglobulin G (IgG) for therapeutic administration (product name: Gammanorm) was obtained from Octapharma (Lachen, Switzerland). Low IgG Fetal Bovine Serum (FBS) was obtained from Life Technologies (Grand Island, NY/USA). The anti-human interleukin-8 (IL8) antibody-producing CHO DP-12 clone#1934 (ATCC CRL-12445) with a DHFR expression system was purchased from the American type culture collection (LGC Standards, Middlesex, UK).

2.2.1. Artificial IgG cell culture supernatant

An artificial cell culture supernatant was used to simulate a serum-dependent cell culture media and was prepared by mixing human IgG (1 g/L) with 10% FBS prepared in seven different buffers, including 20 mM acetate at pH 5 and 6, 20 mM phosphate at pH 7, 7.5 and 8, 20 mM HEPES at pH 7.5, and 20 mM Tris–HCl at pH 7.5.

2.2.2. Chinese Hamster Ovary cell cultures

Two methodologies for cell culture were used in this work and these are indicated below.

2.2.2.1. Cell culture-A. CHO DP-12 cells previously adapted to serum-free media were grown in ProCHO™5 (Lonza, Basel, Switzerland). ProCHO™5 was supplemented with 4 mM L-glutamine (Gibco, Life Technologies), 2.1 g/L $NaHCO_3$ (Sigma–Aldrich), 200 nM Methotrexate, MTX (Sigma–Aldrich), 10 mg/L recombinant human insulin (Sigma–Aldrich), 0.07% (v/v) lipids (Lonza) and 1% (v/v) antibiotics (50 U/mL penicillin and 50 μg/mL streptomycin, both from Gibco). The cultures were carried out in T-75 or T-25 flasks (BD Falcon, Franklin Lakes, NJ/USA) 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. In each passage, cells were washed with phosphate buffered saline (PBS, Gibco) and detached from the flask by adding Accutase solution (Sigma–Aldrich) for 3 min at 37 °C. CHO cell suspensions were then centrifuged (12 °C for 8 min at 1250 rpm) for medium clarification.

2.2.2.2. Cell culture-B. CHO DP-12 cells were grown using a mixture of 25% (v/v) ProCHO™5 and 75% (v/v) of Dulbecco's modified Eagle's medium (DMEM). DMEM was formulated to contain 4 mM L-glutamine, 4.5 g/L D-glucose, 1.5 g/L $NaHCO_3$, 200 nM MTX, 2 mg/L recombinant human insulin, 35 mg/L L-proline, all from Sigma–Aldrich; 0.1% (v/v) of trace element A and B (Cellgro, Mediatech) and 1% (v/v) antibiotics (50 U/mL penicillin and 50 μg/mL streptomycin). DMEM was further supplemented with 10% ultra-low IgG FBS. ProCHO™5 was supplemented as described above.

The cultures were also carried out as previously described for the serum-free culture with cell passages performed every 4 days.

2.2.3. Serum-free anti-human IL8 mAb diafiltrated cell culture supernatant

Clarified supernatants from cell culture-A were subsequently diafiltrated for buffer exchange using a QuixStand benchtop system from GE Healthcare, in which an ultrafiltration hollow fiber cartridge (Xampler Ultrafiltration Cartridge UFP-10-E-3MA from GE Healthcare) was installed. The cartridge, with 3 mL void volume and 110 cm^2 effective filtration area, was composed by 13 polysulfone hollow fiber membranes of 1 mm inner diameter and 10 kDa nominal molecular weight cut-off. Serum-free supernatant aliquots (200 mL) were buffer exchanged to (i) 20 mM sodium phosphate at pH 7.5 and (ii) 20 mM sodium phosphate at pH 8.0, using 4 diafiltration volumes. The final conductivity of these supernatants were around 3 mS/cm (ECTestr Low conductivity tester, OAKTON Instruments, Vernon Hills, IL/USA). The diafiltrated supernatants had a mAb concentration in the range of 90–110 mg/L.

2.2.4. Partially purified anti-human IL8 mAb cell culture supernatant

The cell culture-B broths containing an anti-human IL8 mAbs were subject to a previous capture step by phenyl boronate affinity chromatography (ProSep-PB from Millipore) using 20 mM HEPES with 150 mM NaCl at pH 7.5 as adsorption buffer and 10 mM of Tris–HCl with 500 mM of Sorbitol, 150 mM NaCl at pH 7.5 as elution buffer. The eluted pool had a mAb concentration of 113 mg/L.

2.3. Preparative chromatographic methods

2.3.1. Chromatographic media

Three different anion-exchanger media were used: (i) Sartobind® Q SingleStep Nano, obtained from Sartorius-Stedim Biotech (Goettingen, Germany), herein referred as Sartobind Q (ii) HiTrap Q XL obtained from GE Healthcare (Uppsala, Sweden), herein referred as HiTrap Q, and (iii) CIM® QA monolithic column obtained from BIA Separations (Ljubljana, Slovenia) herein referred as CIM Q monolith (equipped with two CIM Q DISKS). The main characteristics of the three used systems are summarized in Table 1.