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Use of mep HyperCel for polishing of human serum albumin

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ABSTRACT

The manufacture of human serum albumin by chromatographic procedures involves gel filtration chromatography as a final polishing step. Despite this step being essential to remove high molecular weight impurity proteins and thus ensure a stable and safe final product, it is relatively inefficient. This paper explores the use of hydrophobic charge induction chromatographic media, MEP HyperCel as an alternative to Sephacryl S200HR gel filtration for the polishing of human serum albumin derived by ion exchange chromatographic purification of Cohn Supernatant I. The use of MEP HyperCel results in a product with a higher purity than achieved with gel filtration and in a less time consuming manner and with potential resource savings. MEP HyperCel appears to have great potential for incorporation into downstream processes in the plasma fractionation industry as an efficient means of achieving polishing of intermediates or capture of proteins of interest.

1. Introduction

Commercial human serum albumin is predominantly manufactured using ethanol precipitation methods based on those originally developed by Cohn et al. (1946) [1] and the subsequent modifications of Kistler & Nitschmann (1962) [2]. The continued purification of albumin by ethanol precipitation can be attributed to the fact that these methods allow the efficient processing the large volumes encountered in a modern plasma fractionation process. However, smaller but increasing amounts of albumin are fractionated using chromatographic processes, introduced either as a final polishing step following initial ethanol fractionation [3] or as fully integrated chromatographic processes [4–6].

Chromatographic purification of albumin has a number of benefits including higher process yields, higher purity [3,5,7] and improved tolerability and clinical safety of the product [8]. CSL Behring (Australia) have manufactured human serum albumin (Albumex) using a chromatography method based on that developed by Berglof et al. (1983) for almost two decades [9]. This chromatographic process, which includes two ion exchange and a size exclusion columns, was initially applied to delipidated Supernatant II + III, but was subsequently adapted to the processing of a delipidated Supernatant I feedstream to allow the complete chromatographic purification of IgG for the Intragam P process [5,10].

Despite the above mentioned benefits, the widespread adoption of chromatographic processes for fractionation of human albumin has been limited due to cost and inefficiencies associated with the processes at industrial scale, with respect to the large number of chromatographic cycles required to process a batch. This is particularly the case in the fully chromatographic processes, with the Sephacryl S200HR gel filtration step for final polishing to remove high molecular weight impurity proteins. The efficiency of a chromatographic process for the purification of albumin could be greatly enhanced if an alternative mode to Sephacryl S200HR was developed as a final polishing step.

There have been a number of reports that hydrophobic interaction chromatography (HIC) offers sufficient selectivity to allow separation of plasma proteins based on differences in the surface hydrophobicity of the proteins [11,12]. This has allowed HIC to be used successfully for the purification of albumin from human plasma [13], human placenta [14] and of recombinant human serum albumin from *Pichia pastoris* [15].

Despite HIC offering an orthogonal mode of separation to the ion exchange steps used for chromatographic purification of albumin, these resins have not been used by any of the commercial plasma fractionators. Their use at large-scale has been limited by the fact that HIC-based chromatography requires use of significant amounts of lyotropic salts in order to achieve the binding and fractionation of proteins [16]. An alternative to conventional HIC is hydrophobic charge induction chromatography (HCIC). One such resin for HCIC is 4-mercaptoethylpyridine (MEP) HyperCel [16]. The

Abbreviations: ID, Internal diameter; ELISA, Enzyme linked immunosorbent assay; DEAE, Diethylaminoethyl; CM, Carboxymethyl; pI, Isoelectric point; HCIC, Hydrophobic charge induction chromatography.

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MEP ligand permits separation based on the surface hydrophobicity of proteins but does not require the addition of lyotropic salts [17]. MEP HyperCel has been shown to have a high selectivity for immunoglobulins and for this reason there has been a major focus on the use of this media for the isolation of monoclonal antibodies [17–20]. In addition, MEP HyperCel has been proven to be effective for the separation of penicillin acylase [21] and Fc-fusion proteins [22].

In this paper an alternative method for the polishing of human serum albumin using MEP HyperCel is investigated. IgG is one of the major residual proteins present in chromatographically purified albumin. Therefore the optimal conditions for MEP HyperCel chromatography for the partitioning of albumin and IgG were initially evaluated using purified samples of albumin and IgG. The defined conditions were then verified by examining the capability of MEP HyperCel to polish the ion exchange purified albumin process intermediate. The impact of flow rate on the achieved separation and optimal protein sample loading was also determined. After successful demonstration of the new process at small-scale, the process was evaluated at Pilot-scale and the final product was compared to the albumin produced at industrial-scale.

2. Materials and methods

2.1. Protein solutions

Process intermediates including Cohn Supernatant I (SNI) depleted of lipids and euglobulins, albumin eluate from CM Sepharose-FF chromatography, purified human serum albumin (HSA) and purified human immunoglobulin G (IgG) and purified albumin from Sephacryl S200HR chromatography were obtained from CSL Behring (Broadmeadows, Victoria, Australia). All protein solutions were filtered through a 0.22 μm membrane (Durapore®, Millipore Corporation, Bedford, MA, USA) prior to chromatography.

2.2. Antibodies and ELISA reagents

Antibodies against the human plasma proteins of transferrin, α_2-macroglobulin, α_1-glycoprotein, α_1-antitrypsin, haptoglobin and ceruloplasmin were obtained from DakoCytomation Denmark A/S (Glostrup, Denmark), inter α trypsin inhibitor antibodies were obtained from The Binding Site (Birmingham, England), and apolipoprotein A1 and apolipoprotein B antibodies were obtained from Siemens Healthcare Diagnostics Inc. (Tarrytown, NY, USA). ELISA kits for IgG and IgM were obtained from Bethyl Laboratories Inc. (Montgomery, TX, USA) and the ELISA kit for α_2-macroglobulin was obtained from GenWay Biotech. Inc. (San Diego, CA, USA). Antibodies for the IgA ELISA were obtained from DakoCytomation Denmark A/S (Glostrup, Denmark) and the IgA standard was obtained from Calbiochem-Novabiochem Corporation (San Diego, CA, USA). Antibodies against the human plasma proteins of albumin, IgG, IgA and IgM, which were used for nephelometry analysis, were obtained from Beckman-Coulter Inc. (Fullerton, CA, USA).

2.3. Chromatographic resins

MEP HyperCel resin was obtained from Pall Life Sciences (East Hills, NY, USA). DEAE Sepharose-FF, Capto DEAE, CM Sepharose-FF and Sephacryl S200HR resins were obtained from GE Healthcare BioSciences AB (Uppsala, Sweden).

2.4. Characterisation of Sephacryl S200HR process

The albumin intermediates before and after Sephacryl S200HR chromatography were characterised using nephelometry (IMMAGE Immunochemistry system, Beckman-Coulter Inc., Fullerton, CA, USA) to determine the levels of albumin, IgG, IgA and IgM, and immunoelectrophoresis to determine the levels of transferrin, α_2-macroglobulin, α_1-glycoprotein, α_1-antitrypsin, inter α trypsin inhibitor, haptoglobin, apolipoprotein A1, apolipoprotein B and ceruloplasmin.

2.5. Impact of heating on turbidity of albumin solutions

The excellent viral safety record of commercial HSA solutions is attributed largely to the pasteurisation step (60 °C for 10 h) [3]. The albumin is stabilised by addition of sodium caprylate, however, contaminant proteins present not protected by the caprylate are susceptible to heat induced denaturation. Thus evaluation of the turbidity of an albumin solution following pasteurisation is a good indication of the presence of residual proteins. A control sample of albumin eluate from CM Sepharose-FF chromatography and samples of purified albumin derived from Sephacryl S200HR chromatography were concentrated to 15–20% w/v protein by ultrafiltration (10 kDa OMEGA membrane; Pall Corporation, Hauppauge, NY, USA) and formulated with 32 mM sodium caprylate. The samples were pasteurised by heating at 60 °C for 10 h. At the completion of pasteurisation the turbidity of the samples was assessed using a Hach 2100AN Turbidimeter (Hach Company, Loveland, CO, USA).

2.6. Determination of MEP HyperCel operating conditions for partitioning of albumin and IgG

The operating parameters for MEP HyperCel chromatography were explored using purified samples of albumin and IgG. The binding and elution of albumin and IgG was determined separately using a range of equilibration conditions including; 50 mM sodium phosphate (pH 7.0); 50 mM sodium acetate (pH 5.5); 50 mM sodium acetate (pH 5.5) + 150 mM NaCl; 50 mM sodium acetate (pH 5.5) + 300 mM NaCl; 50 mM sodium acetate (pH 7.0); and 110 mM sodium acetate (pH 7.0), shown in Table 3. The pure IgG and pure albumin samples were prepared in the appropriate equilibration buffer at a concentration of approximately 5-10 mg/mL and filtered through a 0.22 μm membrane (Durapore®, Millipore Corporation, Bedford, MA, USA) prior to chromatography. The samples were loaded on to an equilibrated MEP HyperCel column (17.5 cm × 1.6 cm ID; GE Healthcare BioSciences AB, Uppsala, Sweden) at 20 g per litre of resin. The unbound protein was recovered during the sample loading period and during the subsequent post-sample loading wash with equilibration buffer. Any bound protein was eluted using sodium acetate buffer at pH 3.0. All chromatography steps were conducted at a flow rate of 100 cm/h and monitored at 280 nm using an AKTA Explorer 100 equipped with Unicorn software (GE Healthcare BioSciences AB, Uppsala, Sweden). The amount of protein recovered in the flow through and eluate fractions was determined using nephelometry.

The most suitable equilibration buffer conditions defined from the above experiment was subsequently verified using a sample containing a 9:1 mixture of albumin and IgG. The pure albumin and pure IgG was mixed in 110 mM sodium acetate (pH 7.0) at a ratio of 9:1 to give a final protein concentration of approximately 5–10 mg/mL. The sample was loaded on to the equilibrated MEP HyperCel column at protein loadings of 100, 200 and 300 g per litre of resin. The unbound protein was recovered using 110 mM Sodium acetate (pH 7.0) equilibration buffer and the bound protein was eluted using sodium acetate buffer at pH 3.0. The amount of albumin and IgG in the flow through and eluate fractions was determined using nephelometry.