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Evaluation of an anion-exchange hollow-fiber membrane adsorber containing γ-ray grafted glycidyl methacrylate chains

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ABSTRACT

It is widely recognized that membrane adsorbers are powerful tools for the purification of biopharmaceutical protein products and for this reason a novel hollow-fiber AEX type membrane adsorber has been developed. The membrane is characterized by grafted chains including DEA ligands affixed to the pore surfaces of the membrane. In order to estimate the membrane performance, (1) dynamic binding capacities for pure BSA and DNA over a range of solution conductivity and pH, (2) virus reduction by flow-through process, and (3) HCP and DNA removal from cell culture, are evaluated and compared with several other anion-exchange membranes. The novel hollow-fiber membrane is tolerant of high salt concentration when adsorbing BSA and DNA. When challenged with a solution containing IgG the membrane has high impurity removal further indicating this hollow-fiber based membrane adsorber is an effective tool for purification of biopharmaceutical protein products including IgG.

1. Introduction

In the 1990s numerous studies on membrane chromatography [1–6] expected functionalized membranes to have many applications based on the possibility of high flow rates and the lack of column packing required of traditional resin based chromatography. The use of membrane chromatography in biopharmaceutical manufacturing processes was, however, not widely accepted due to the relatively low binding capacity of membrane adsorbers and low resolution during elution [7]. Eventually, it was recognized that membrane chromatography is feasible when operated not in bind-and-elute mode, but rather in flow-through mode where the molecule of interest remains unbound and impurities are adsorbed.

In 2001, Knudsen et al. [8] reported that anion-exchange membranes in flow-through mode may provide a reasonable alternative to packed bed columns for the removal of low levels of impurities such as DNA, host cell protein (HCP) and virus in process-scale antibody purification. They also suggested that, due to economic and process restrictions, cation-exchange membranes may not be advantageous for process-scale antibody purification in a bind-and-elute mode. In 2006, Zhou and co-workers [9,10] thoroughly examined the advantages and disadvantages of using anion-exchange membrane chromatography as a purification unit operation and showed that anion-exchange membrane chromatography was a viable alternative to Q column chromatography as a polishing step in process-scale antibody production when operated in flow-through mode. Zhou also showed that using anion-exchange membranes in antibody manufacturing processes was cost effective. After their work, the use of anion-exchange membranes in the purification process became more common in bio-pharmaceutical production.

Although all the market leading anion-exchange adsorbers are flat-sheet membranes, hollow-fiber membrane adsorbers are widely thought of as potentially advantageous because of their high membrane area to housing volume ratio. Preparation and application of hollow-fiber type anion-exchange membranes have been studied rigorously [11–18]. The characteristic property of this novel membrane is the addition of grafted chains fixed on the pore surface to which the ligands are attached. The advantage is that the grafted chain enhances the accessibility of the ligand to the binding site on the protein or contaminant thus increasing the binding capacity. A similar three-dimensional adsorption is reported by Janzen et al. [19] and Muller and Klein [20].

The grafted chain hollow-fiber membrane was developed by Asahi Kasei Medical under the name QyuSpeed™ D (hereafter "QSD"). In order to estimate the membrane performance, (1) dynamic binding capacities for pure BSA and DNA over a range of solution conductivity and pH, (2) virus reduction by flow-through process, and (3) HCP and DNA removal from cell culture, were evaluated and compared with several other anion-exchange membranes.

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2. Materials and methods

2.1. Materials

2.1.1. Materials for the anion-exchange hollow-fiber membrane

A porous polyethylene (PE) hollow-fiber membrane is the base material for grafting. The hollow-fibers have an inner and outer diameter of 2.0 and 3.0 mm, respectively. The average maximum pore diameter is 0.3 μm, determined by bubble point method [21]. The porosity is approximately 70%. The monomer material for the graft chains is technical grade glycidyl methacrylate (GMA, CH_2=$CCH_3COOCH_2CHOCH_2$) purchased from Tokyo Kasei (Tokyo, Japan) and used without further purification. The ligands are comprised of diethylamine (DEA, $NH((CH_2CH_3)_2)$ purchased from Wako Pure Chemical Industry (Tokyo, Japan).

2.1.2. Materials for dynamic binding capacity evaluation and impurity removal test

Bovine serum albumin (BSA) was purchased from Sigma–Aldrich (Albumin, from bovine serum >98% (agarose gel electrophoresis) powder) for the evaluation of the protein dynamic binding capacity (DBC). DNA was purchased from Invitrogen (Salmon Sperm DNA Solution, 10 mg/mL, <2000 bp). Serum free CHO cell culture with pH 7.5 and conductivity of 9.8 mS/cm was kindly provided by Asahi Kasei Pharma Co., Ltd. γ-Globulins (Sigma, γ-globulins, Human: From Cohn Fraction II, III Approx. 99% (electrophoresis)) was added to the CHO cell culture as antibody protein.

2.2. Preparation

2.2.1. Preparation of anion-exchange porous hollow-fiber membrane

The porous hollow-fiber membrane containing grafted DEA ligands is prepared by a γ-ray grafting technique and subsequent chemical modification as shown in Fig. 1. This preparation process is based on the method reported by Saito and co-workers [11]. A vinyl monomer containing an epoxy group (GMA) is grafted onto the membrane after radicals have been generated by γ-ray irradiation. In this case, the intensity of γ-ray radiated from Cobalt 60 equipment was ca. 10 kGy/h and irradiation time was 20 h, i.e., the total dose of γ-ray was ca. 200 kGy. After the irradiation, the hollow-fiber membranes are immersed in a GMA solution (GMA: methanol = 5:95 in volume) for 10 h at 313 K. The amount of GMA graft polymerized (degree of grafting) is defined as [(weight gain)/(weight of PE membrane) × 100 (%)] and averaged 70%. The DEA group is added by exposing the epoxy group to diethylamine solution [diethylamine:H_2O = 1:1 in volume (pH 13.2)] for 12 h at a temperature of 303 K. The degree of substitution was 86–96%, determined from the weight change before and after exposure to diethylamine solution. A schematic illustration of a micropore of the membrane with grafted chains, and the chemical structure of the grafted chains are shown in Fig. 2. Unreacted epoxy groups turn to diol groups (from –CH–CH_2O to –CH(OH)–CH_2OH) under alkaline conditions for 12 h. FTIR and solid state NMR methods confirmed no unreacted epoxy groups remained after substitution. After grafting the hollow-fibers were found to have expanded to 3.6 mm OD and 2.2 mm ID. Scanning electron microscopy (SEM) images of the cross-section for the hollow-fiber membrane are shown in Fig. 3.

The length and the density of GMA graft chains are considered to be controlled by irradiation dose and degree of grafting [22]. The length and density were not evaluated directed since any chemical or physical methods would not separate the chain from the base polymer. Alternatively, Lee et al. measured the density of free radicals generated by the irradiation by electron spin resonance (ESR) and evaluated the length and density of GMA graft chains from radical density and degree of grafting [23]. Applying this principle to QSD, where the degree of grafting was 70% and 200 kGy of total irradiation was dosed, the length and density of GMA graft chains was estimated as ca. Mn = 3×10^5 and 2×10^{19} (brush/g-BP), respectively. Where, brush/g-BP is the number of graft chains per 1 g of PE base polymer.

In order to evaluate the change in permeability due to the addition of the grafted chain and ligand, pure water was pumped through the hollow-fibers (effective length: 9 cm, inner diameter: 2.0 mm for unreacted PE hollow-fiber and 2.2 mm for grafted QSD) from inside to outside at the flow rate of 2 mL/min. The trans membrane pressure at 2 mL/min flow was 0.015 MPa for the PE hollow-fiber and 0.013 MPa for QSD indicating that the permeability was unchanged as a result of the grafting process. Additionally, the pressure at 2 mL/min flow through QSD was 0.02 MPa for both buffer alone (20 mM Tris–HCl pH 8.0) and with buffer including 1 M NaCl. Although it was observed that the filter pressure increased from 0.025 MPa to 0.045 MPa when pure water was filtered after a solution of 20 mM Tris–HCl pH 8.0, 1 M NaCl. When 20 mM Tris–HCl pH 8.0 was used instead of pure water the pressure remained constant 0.025 MPa. The pressure increase is likely due to the swelling of the membrane by high concentration salt solution.

2.2.2. Fabrication of the hollow-fiber membrane filter

A schematic illustration of the hollow-fiber membrane filter is shown in Fig. 4. One hollow-fiber (outer diameter of 3.6 mm) is housed in the cartridge with inner diameter of 5.0 mm and potted by sealant at both ends using epoxy resin. The cartridge material is polysulfone. The effective length of the hollow-fiber between both sealant surfaces is 9.3 cm. Four openings are present in the cartridge, i.e., two inlets to the fiber (A and D) and two outlets (B and C) on the shell side. For dead-end filtration use, flow direction is arranged from A to C as permeating from the inside of the hollow-fiber to the outside. The effective membrane area, defined by the inner surface area of the fiber is 6.3 cm^2. The effective membrane volume is 0.6 mL.

2.3. Instrument and membranes

All the measurements for the evaluations of the dynamic binding capacity and impurity removal test from cell culture are carried out using an ÄKTA Explorer 100 (GE Healthcare). The adsorption performance of the membrane is evaluated in dead-end mode at a defined flow rate normalized for each filters membrane volume. The normalized flow rate is given in the units mL/min/mL-ad where the flow rate (mL/min) divided by the membrane volume (mL-ad). In order to also normalize the volume of fluids used including load volume, membrane volumes (MV) are used where the volume applied (mL) is divided by the membrane volume (mL-ad). For example, in the case of QSD: 100 MV = 60 mL applied volume/0.6 mL-ad membrane volume.

Four different types of membranes were used: QSD (DEA group, membrane volume = 0.6 mL-ad), membrane A (Sartorius stedim, Sartobind Q15, Q amine group, 0.41 mL-ad), membrane B (Pall Corporation, Mustang Q Acrodisc 25 mm, Q amine group, 0.18 mL-ad) and membrane C (CUNO, Zeta Plus 90ZA, Zeta potential type, >5 mL-ad). QSD is a hollow-fiber membrane with long graft chains, membranes A and B are flat sheet type membranes, and membrane C is depth filter with an inorganic filter aid, cellulose and a proprietary positive charge.

2.4. Dynamic binding capacity

In order to compare the flow rate dependence of membranes and packed bed column, dynamic binding capacity (DBC) for QSD,