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Short communication

Effect of bore fluid composition on microstructure and performance of a microporous hollow fibre membrane as a cation-exchange substrate

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ABSTRACT

Micro-capillary film (MCF) membranes are effective platforms for bioseparations and viable alternatives to established packed bed and membrane substrates at the analytical and preparative chromatography scales. Single hollow fibre (HF) MCF membranes with varied microstructures were produced in order to evaluate the effect of the bore fluid composition used during hollow fibre extrusion on their structure and performance as cation-exchange adsorbers. Hollow fibres were fabricated from ethylene-vinyl alcohol (EVOH) copolymer through solution extrusion followed by nonsolvent induced phase separation (NIPS) using bore fluids of differing composition (100 wt.% *N*-methyl-2-pyrrolidone (NMP), 100 wt.% glycerol, 100 wt.% water). All HFs displayed highly microporous and mesoporous microstructures, with distinct regions of pore size <1 μm, 5–15 μm and up to 50 μm in diameter, depending upon proximity to the bore fluid. Scanning electron microscopy (SEM) revealed skins of pore size <1 μm at the inner surface of HFs produced with water and glycerol, while NMP bore fluid resulted in a skinless inner HF surface. The HFs were modified for chromatography by functionalising the polymer surface hydroxyl groups with sulphonic acid (SP) groups to produce cation-exchange adsorbers. The maximum binding capacities of the HFs were determined by frontal analysis using lysozyme solutions (0.05–100 mg ml^{-1}) for a flow rate of 1.0 ml min^{-1}. The NMP–HF–SP module displayed the largest maximum lysozyme binding capacity of all the fibres produced (40.3 mg lysozyme/ml adsorbent volume), a nearly 2-fold increase over the glycerol and 10-fold increase over the water variants at the same sample flow rate. The importance of NMP as a bore fluid to hollow fibre membrane performance as a result of inner surface porosity was established with a view to applying this parameter for the optimisation of multi-capillary MCF performance in future studies.

1. Introduction

Ion-exchange chromatography is widely used in the downstream processing of biopharmaceuticals as it provides high-resolution separation of biomolecules from mixtures based on net charge. The two most commonly used approaches in ion-exchange chromatography, packed bed columns and membrane adsorbers, have high binding capacities and good separation characteristics [1]. However, packed bed columns usually operate under low column flow rates due to bed compression, high pressure drops and low mass transfer rates as diffusion is the primary mode of analyte transport to binding sites within the bed pores [2]. In order to overcome this limitation, membrane adsorbers have been developed in which analyte transport to binding sites depends primarily on convection, thus allowing separations to run at higher flow rates without compromising performance. Typically, membranes have lower pressure drops and offer independence of binding capacity from flow rate [3], although generally exhibit lower binding capacities than packed beds [2].

Micro-capillary film (MCF) membranes have been demonstrated to be effective platforms for bioseparations and viable alternatives to established chromatography substrates at the analytical and preparative scales, as they offer good binding capacities with high superficial flow rates, low pressure drops and do not involve column packing operations [1,4,5]. MCFs are microstructured membranes containing continuous capillaries embedded within a flat polymer film, with the number of capillaries depending on the die used during the manufacturing process. Both non-porous MCFs (NMCFs) and microporous MCFs (MMCFs) have been produced from ethylene-vinyl alcohol copolymer (EVOH) through extrusion processes [6,7]. Taking advantage of the hydrophilic nature of EVOH and its exposed hydroxyl group, the MCF

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membrane surfaces can be functionalised into adsorbent surfaces via conjugation of functional ligands to the polymer [4]. Previously, the nonporous NMCF was used as a cation-exchange membrane adsorber for lysozyme purification [4] and as an anion-exchange membrane adsorber for lentivirus capture from cell culture [1]. The use of porous MMCF as a cation-exchange adsorber for bioseparations has also been demonstrated [5].

MMCFs offer distinct advantages over NMCFs. Due to their porous structure, the MMCF surface area available for functionalisation is greatly increased, resulting in a 10^4-fold higher binding capacity compared to NMCF in membranes functionalised for cation-exchange chromatography [5]. In addition, bioseparations can be run at high superficial flow velocities while withstanding low pressure drops. It has been demonstrated that the binding capacity of MMCF is comparable to commercially available packed bed and membrane adsorbers, while providing a sharp breakthrough and higher throughput and pressure tolerance than that of currently available preparative scale purification substrates [5]. The potential of MMCF in terms of binding capacity can be further enhanced by altering key parameters in the membrane manufacturing process. Parameters such as dope composition, bore fluid composition, polymer and bore fluid flow rate, air gap distance to coagulant, take-up rate, and extrusion temperature have been shown to affect membrane characteristics [8–10]. In particular, the composition of the bore fluid has been shown to affect membrane morphology [11] and adsorption performance [12].

Previously, MMCF has been proven to be a suitable chromatography substrate [5]. However, MMCF manufacturing conditions have yet to be optimised for improved chromatographic performance. The main objective here is to evaluate the effect of bore fluid composition during MMCF fabrication on the morphology and performance of an EVOH hollow fibre MMCF as a cation-exchange adsorber. The single hollow fibre (HF) membrane variant of MMCF was chosen as a test system due to its symmetry and relative geometrical simplicity compared to the multi-capillary MMCF extruded with a 19-nozzle die used in the studies described previously [5]. Since HFs were produced through a nonsolvent induced phase separation process (NIPS), the varying solubility of the bore fluid used to form the central capillary of the hollow fibre affected the speed of polymer phase separation into this coagulant. This in turn altered the morphology and porous microstructure that was observed with SEM, as it changed the rate of polymer precipitation at the coagulant interface. The extruded HFs were then functionalised into cation-exchange chromatography modules and their performance as cation-exchange adsorbers was evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Ethylene-vinyl alcohol copolymer (EVOH) containing 32 mol% ethylene was supplied by Kuraray (Hattersheim, Germany). *N*-Methyl-2-pyrrolidone (NMP), polyvinylpyrrolidone (PVP, avg. mol wt. 360,000), glycerol, anhydrous NaOH, cyanuric chloride, acetone, Na_2HPO_4, 3-amino-1-propanesulphonic acid, crystalline tris(hydroxymethyl)aminomethane (Tris), HCl and crystalline chick-egg lysozyme were supplied by Sigma–Aldrich (St. Louis, MO, USA). All chemicals and biochemicals used were of analytical grade.

2.2. Extrusion and assembly of hollow fibre module

Hollow fibre (HF) membranes were extruded from EVOH via nonsolvent induced phase separation (NIPS) according to a protocol described by Bonyadi and Mackley [7]. Briefly, polymer solutions containing 15/10/75 wt.% EVOH/PVP/NMP were extruded (polymer solution flow rate of 1.5 ml min^{-1}) at ambient temperature through an annular die into a water coagulation bath along with one of three different entrained bore fluids (bore fluid flow rate 0.5 ml min^{-1}). An air gap of 0.5 cm and take-up speed of 75.4 cm min^{-1} were used. 100 wt.% bore fluids of water, glycerol and NMP were used to manipulate fibre microstructure by altering polymer precipitation rates across the membranes (Fig. 1). NMP and water are similar in viscosity and density [11], while glycerol is denser and more viscous. At the concentrations used in this study, EVOH was completely soluble in NMP, sparingly soluble in glycerol and insoluble in water, based on each compound's Hansen solubility parameters. The HFs were adapted into chromatography columns by encasing 10 cm long sections of HF within 6.35 mm diameter FEP plastic tubing (Kinesis Ltd., St. Neots, UK) using epoxy glue purchased from Huntsman (Araldite®, Cleveland, OH, USA). Upchurch 1/4 inch HPLC connectors were placed at the column ends and were attached to an ÄKTA FPLC system (GE Healthcare Life Sciences, Uppsala, Sweden). The ÄKTA FPLC was used to pump various buffers and protein solutions axially through the HF module lumen. There was no radial flow through the fibres as they were encased in epoxy.

2.3. Scanning electron microscopy (SEM) and mercury intrusion porosimetry

SEM samples were fractured in liquid nitrogen, freeze-dried starting at −90 °C in a Quorum K775X freeze dryer (Laughton, UK) and sputter coated in platinum in order to obtain sharp membrane cross-sections for imaging. Surface and cross-sections of HF samples were imaged using an FEI Verios 460 scanning electron microscope (FEI, USA) operated at 5 kV. Membrane pore surface area was measured by mercury intrusion porosimetry using a Micromeritics AutoPore IV 9500 porosimeter (Norcross, GA, USA).

2.4. Surface modification of HF modules with SP groups

The surface of the HF modules was adapted into a cation-exchange chromatography adsorber using protocols established by Darton [4] and McCreath [13]. Briefly, the nucleophilicity of the HF membrane surface was increased by flowing 30 ml of 1 M NaOH for 30 min through the module using a Knauer Smartline 100 HPLC pump (Berlin, DE). Next, a linker group was added by flowing 20 ml of 50 mM cyanuric chloride in acetone solution for 20 min. After a 10 min wash with 10 ml of MilliQ water, sulphonic acid (SP) groups were covalently attached to the linker by recirculating 20 ml of a 1 M solution of Na_2HPO_4 containing 1 g of 3-amino-1-propanesulphonic acid overnight in a 60 °C water bath. Finally, the column was washed with 20 ml MilliQ water for 20 min, 20 ml NaOH in water (0.4 M) for 20 min, and again 20 ml MilliQ water for 20 min. Hereafter, the SP functionalised HFs produced with the three bore fluids water, glycerol and NMP will be referred to as water–HF–SP, glycerol–HF–SP and NMP–HF–SP.

2.5. Frontal analysis study and equilibrium binding capacity analysis

Binding capacity analysis was carried out based on a methodology developed by Darton et al. [4]. The HF modules were first pre-equilibrated with running buffer, 20 mM Tris–HCl pH 7.2, for at least two column volumes (column volume (CV), is defined as the total volume within the microporous walls and the central capillary, 1 CV = 0.14 ml). The modules were then continuously loaded with lysozyme (C_{inj} = 5.0 mg ml^{-1}) in 20 mM Tris–HCl pH 7.2 using a Knauer Smartline 100 HPLC pump until the column was saturated based on UV absorbance as measured by the ÄKTA FPLC system. When nearly 100% lysozyme breakthrough was reached, the saturation UV absorbance height in optical density units at 280 nm