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A microporous walled micro-capillary film module for cation-exchange protein chromatography

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ABSTRACT

Opportunities exist in preparative chromatography for alternative chromatography media that possess high binding capacity and throughput, but are also economically feasible for single use disposability and avoid column packing. An ion-exchange functionalised, microporous walled micro-capillary film (MMCF), has been developed as a module for cation-exchange separation of proteins. A MMCF module has been operated on a standard AKTA chromatography system at pressures up to 1.5 MPa and superficial flow velocities up to 54,000 cm h^{-1}. The dynamic binding capacity of the MMCF module at 10% breakthrough was 13.8 mg lysozyme/ml adsorbent volume, which is comparable to the capacity of current commercial adsorbents. Frontal analysis studies using a mixture of lysozyme and bovine serum albumin (BSA) have shown that lysozyme can be isolated free of BSA to the limit of detection of the SDS gel assay used. 98.8% of the total sample eluted was the target protein lysozyme with only 1.2% BSA impurity. MMCF may thus be a viable chromatographic medium for preparative protein chromatography.

1. Introduction

Bio-manufacturers seek new downstream processing techniques that match the purification ability and reliability of current chromatographic media but which provide significantly higher throughput, ease of use and economy [1–7]. Techniques are particularly sought that avoid the need for end-user column packing and qualification to remove cost, effort and reduce batch-to-batch variations leading to single-use and disposable systems. Comparisons of current techniques (packed bed columns, perfusion and monolith-based chromatography, and membrane chromatography) with earlier methods (ultracentrifugation, precipitation) highlight the importance of materials science. Extending the approach to materials science, the concept demonstrated here implements high throughput microchannels in an extruded porous polymer film.

The micro-structured film material, microporous walled micro-capillary film (MMCF), contains 19 parallel micro-capillaries, each of approximately 200 μm equivalent diameter, within a flat polymer film (variable cross section of 5.5 mm by 1.4 mm). Any number of capillaries can be incorporated into a film by simply changing the extruder die. They can be fabricated from low-cost polymers (polysulfone and EVOH blends) to make microflow devices that can be exploited for the capture and adsorptive purification of a biological target by conjugation of a functional ligand to the surface of the polymer.

Previously, nonporous micro-capillary films (NMCFs) had been melt extruded from ethylene-vinyl alcohol (EVOH) copolymer [8]. EVOH is widely used in packaging industry since it is recyclable as a post-consumer plastic waste [9,10]. The hydrophilic EVOH NMCF was chemically modified and developed as a chromatography device [11]. The NMCF device with cation-exchange chemistry withstood high superficial flow velocities with a low pressure drop. However the binding capacity obtained was relatively low for applications in preparative scale chromatography.

Bonyadi et al. [12] extruded a microporous walled MCF geometry, also made of EVOH, by a non-solvent induced phase separation process (NIPS). MMCF has pores in the range of 0.1–3.0 μm across its capillary walls [12,13]. This study examines whether the microporous nature of the MMCF provides a higher protein binding capacity compared to NMCFs, whilst maintaining the advantages of high superficial flow velocities and low pressure drop across the medium.

Here, MMCF has been chemically functionalised into a chromatography medium and its separation performance has been characterised for the cation-exchange purification of a model mixture of lysozyme and bovine serum albumin (BSA). The MMCF was functionalised and processed to form a cation-exchange chromatography module which was characterised for its dynamic binding capacity (DBC) at 10% breakthrough and its equilibrium

Abbreviations: MCF, micro-capillary film; NMCF, nonporous micro-capillary film; MMCF, microporous walled micro-capillary film; NMP, N-methyl-2-pyrrolidone; NIPS, non-solvent induced phase separation process; PVP, polyvinyl-pyrrolidone

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binding capacity (EBC). The chromatography parameters of the MMCF module are compared with a NMCF device that had been similarly functionalized, as well as commercially available media.

2. Materials and methods

2.1. Chemicals

MMCF was made from EVOH copolymer supplied by Kuraray (Hattersheim, Germany). N-methyl-2-pyrrolidone (NMP) was used as a solvent for EVOH. NMP is commonly used in solution polymer processing due to its ability to solubilise polymers, its water miscibility and low environmental impact [12]. Polyvinyl-pyrrolidone (PVP) was used as a processing aid and pore forming agent [12]. Glycerol was used as the bore fluid and for post-processing storage [12]. NMP, PVP and glycerol were purchased from Sigma Aldrich (St. Louis, Missouri). Epoxy glue for coating the MMCF in a module was purchased from Huntsman (Araldite®, Redcar, Cleveland). Module fittings, adaptors and tubing were purchased from Kinesis (St. Neots, Cambridgeshire). Sodium hydroxide (NaOH), cyanuric chloride, acetone, sodium phosphate (Na_2HPO_4), 3-amino-1-propanesulphonic acid (SP), tris(hydroxy-methyl)aminomethane (Tris), hydrochloric acid (HCl), chick-egg lysozyme (pI 11, MW 14.3 kDa), BSA (pI 4.9, MW 67.0 kDa) were all purchased from Sigma Aldrich (St. Louis, Missouri).

2.2. Extrusion and construction of the MMCF module

MMCF (Fig. 1(a)) was extruded in a previously developed NIPS system [12]. Briefly, a polymer solution containing 15/10/75 wt% EVOH/PVP/NMP was used. The extrusion was conducted through a 19 nozzles die of capillary size 0.5 mm with pure glycerol as the bore fluid. MMCF module was developed (Fig. 1(b)) for comparability in length with commercial packed bed columns which usually have a 200 mm bed height. Approximately 250 mm of the extruded MMCF was cut and washed with distilled water to remove surface impurities. The MMCF was placed in an 8 mm diameter plastic tube and coated with epoxy glue and dried overnight. Care was taken to minimalise bubbles or air gaps. The ends were trimmed to give a final MMCF length of 200 mm and module adaptors were fitted. A water leak test was conducted using a high pressure liquid chromatography (HPLC) pump (Knauer, St. Neots, Cambridgeshire).

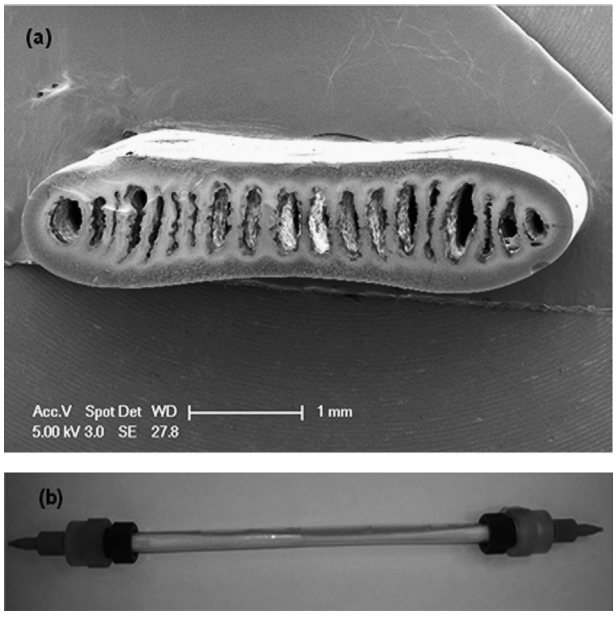

Fig. 1. Microporous walled micro-capillary film (MMCF) constructed into a module for chromatography. (a) Scanning electron micrograph of a transverse cross section of a 19 capillary MMCF. (b) Photograph of a 200 mm MMCF module filled with epoxy glue coating and assembled with module adaptors.

2.3. Surface modification of MMCF module with SP groups

The MMCF module was functionalised for cation-exchange functionality by methods developed by McCreath et al. and Darton et al. [11,14]. Briefly, the MMCF module was first activated by flowing 30 ml of ice cold NaOH (1 M) in an ice bath for 30 min using a HPLC pump. Subsequently, 20 ml of ice cold cyanuric chloride (50 mM) in acetone was passed through the MMCF module in an ice bath for 20 min to attach the linker onto the activated EVOH surface. The MMCF module was then washed with 10 ml of ice cold deionised water in an ice bath for 10 min. For the covalent attachment of SP groups to the activated surface of MMCF module, 20 ml of Na_2HPO_4(1 M) containing 1 g 3-amino-1-propanesulphonic acid in a 40 °C water bath was left circulating through the MMCF module overnight. Following this step the waterbath temperature was increased to 60 °C for 5 h, after which 20 ml of deionised water was flowed through the SP modified MMCF module for 20 min. Finally the module was washed with 20 ml of NaOH (0.4 M) for 20 min followed by 20 ml deionised water for another 20 min. The modified MMCF module was stored at 4 °C in 20 mM Tris–HCl pH 7.2 or 20% ethanol for long term storage [11,14].

2.4. Frontal analysis study and dynamic binding capacity analysis

Frontal analysis was carried out according to methods described by Darton et al. [11]. Briefly, the MMCF module was connected to an AKTA FPLC liquid chromatography system (GE Healthcare Bioscience, Uppsala, Sweden) as illustrated in the experimental setup in Fig. 2. Using this configuration it was possible to control the continuously fed sample volume and buffer gradients for different salt concentrations. The 200 mm MMCF module has a capillary volume of 1.55 ml. The module was first pre-equilibrated with running buffer 20 mM Tris–HCl pH 7.2 for at least two capillary volumes. 5.0 mg ml^{-1} lysozyme was continuously fed into the MMCF module using a sample pump C till near 100% breakthrough was reached. The module was then washed with running buffer for 40 capillary volumes. An elution buffer consisting of 0.5 M NaCl in running buffer was passed through for 50 capillary volumes to elute bound protein completely. The module was re-equilibrated using seven capillary volumes of running buffer.

The equilibrium binding capacity (EBC) represents the total theoretical amount of protein that can be bound per unit volume of the medium. However, in downstream bioprocessing under flow conditions a significant proportion of the theoretical capacity is not accessed. The dynamic binding capacity (DBC), the capacity of the medium during flow conditions, is regarded as of a greater practical value to the end user [11]. For the purposes of this paper, DBC is taken to be the apparent capacity at 10% column breakthrough.

DBC tests were carried out at flow rates of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml min^{-1} corresponding to mean superficial flow velocities of 5400, 10,800, 21,600, 32,400, 43,200, 54,000 cm h^{-1} respectively. These tests were conducted in duplicate. DBC at 10% breakthrough was calculated using [15]:

$$DBC_{10\%} = \int_{V_0}^{V_{10\%}} (C_0 - C)\, dV \quad (1)$$