Journal of Membrane Science 466 (2014) 123–129

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

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

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ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form
17 April 2014
Accepted 27 April 2014
Available online 9 May 2014

Keywords:
Cation-exchange
Chromatography
Protein
Separations
Preparative

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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http://dx.doi.org/10.1016/j.memsci.2014.04.049

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).

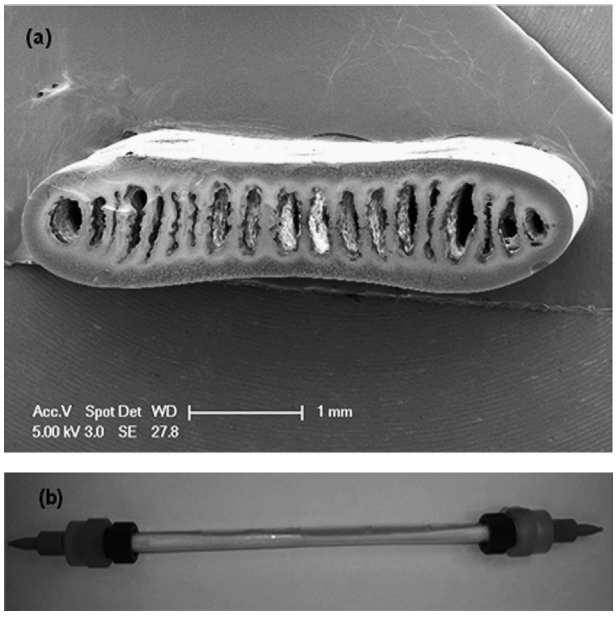

Fig. 1. Microporous walled micro-capillary film (MMCF) constructed into a module for chromatography. (a) Scanning electron micrograph of a transverse cross section of a 19 capillary MMCF. (b) Photograph of a 200 mm MMCF module filled with epoxy glue coating and assembled with module adaptors.

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]:

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

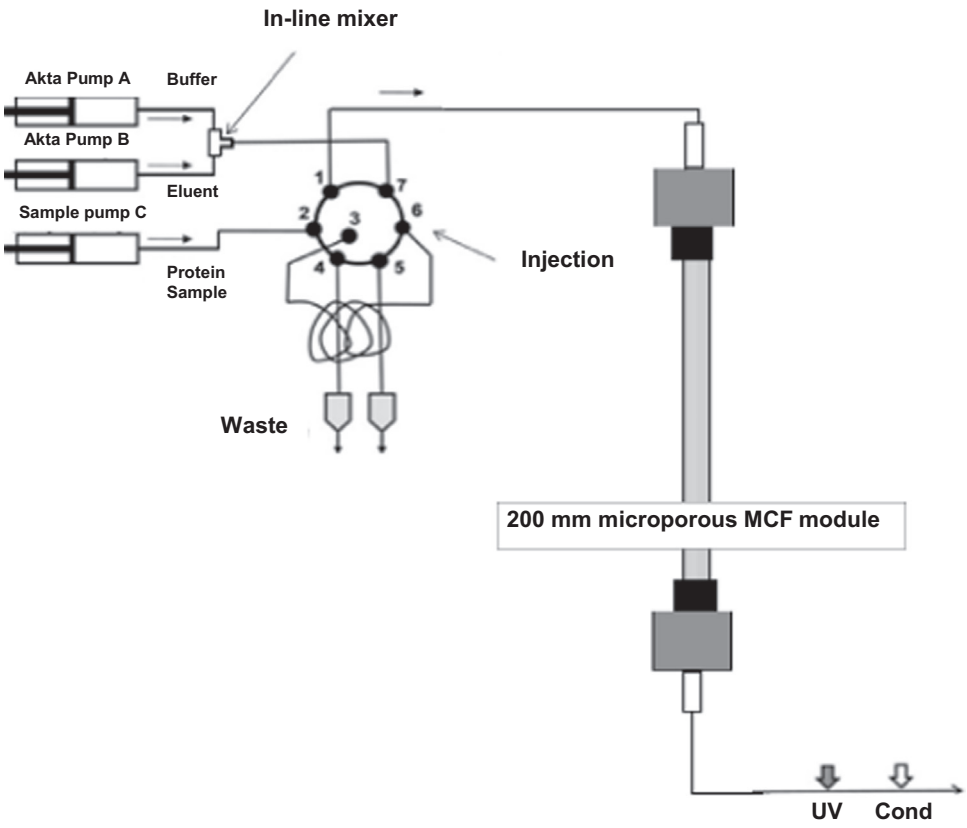

Fig. 2. Experimental setup used for frontal analysis experiments on AKTA FPLC liquid chromatography system with a 200 mm MMCF module. A HPLC sample pump C was used for continuously feeding protein samples into the MMCF module. UV absorbance was measured at 280 nm. Conductivity was recorded in mS/cm.

C_0 and C are protein concentrations in the feed and at a specific retention volume V. V_0 is the dead volume and $V_{10\%}$ is the volume of the loaded protein solution at 10% breakthrough. The integrals were obtained by numerical integration of the data using the trapezoid approximation in MATLAB®.

2.5. Equilibrium binding capacity analysis

The mass of lysozyme bound at different flow velocities was calculated from previous frontal analysis studies. To calculate the mass of protein bound, the module was continuously loaded with a lysozyme concentration, C_{inj} of 5.0 mg ml^{-1} in running buffer with a sample pump C. The adsorbent surface was loaded with protein till it was near 100% breakthrough and the saturation height in optical density units was recorded. After washing out any non-specifically bound protein, the module was eluted under high salt conditions and the area under the elution peak was integrated. The mass of lysozyme bound was calculated by

$$\text{Mass bound (mg)} = \frac{C_{inj}\ (\text{mg/ml}) \times \text{Elution area (mA U ml)}}{100\%\ \text{Saturation height (mA U)}} \quad (2)$$

The superficial flow velocity with the highest mass bound was selected for EBC studies with a range of lysozyme concentrations and this data was then modelled for the protein adsorption nature of the MMCF module. Lysozyme solutions with concentrations between 1 and 120 mg ml^{-1} were used to saturate the MMCF module at a mean superficial flow velocity of 10,800 cm h^{-1}. The experimental setup and run methodology was the same as the frontal analysis studies as shown in Fig. 2. These tests were also conducted in duplicate.

The Langmuir isotherm is one of the models for adsorption on a ligand surface [16] and is described by

$$\theta = \frac{(\alpha C_{inj})}{(1+\alpha C_{inj})} \quad (3)$$

where θ is the fractional coverage of the surface, α is the Langmuir adsorption constant and C_{inj} is the lysozyme concentration used at injection.

The Langmuir equation is based on three assumptions: (i) no protein is adsorbed on the adsorbent surface free from a ligand, (ii) one ligand site can only be occupied by one protein molecule and (iii) individual ligand sites are independent from each other, i.e. bound molecules do not affect adjacent sites. If the mass bound at different protein concentrations at a particular superficial flow velocity fits the Langmuir isotherm, it can be assumed that the medium fulfils the assumptions stated above.

Eq. (4) below relates the amount of protein at a certain loading concentration (q) to the maximum binding capacity of the medium (q_{max}) [11].

$$q = \theta q_{max} \quad (4)$$

Substituting in Eq. (4), Eq. (3) can be rewritten as:

$$q = q_{max}\frac{(\alpha C_{inj})}{(1+\alpha C_{inj})} \quad (5)$$

q_{max} represents the binding capacity of the medium at equilibrium i.e. EBC.

2.6. Cation-exchange studies via frontal analysis

Bio-separation tests using frontal analysis were conducted in the same experimental setup as shown in Fig. 2. The experiment was conducted at a mean superficial flow velocity of 10,800 cm h^{-1}.

The running buffer 20 mM Tris–HCl pH 7.2 was pre-equilibrated through the module for at least two capillary volumes. 15 ml of protein sample was continuously fed using HPLC Pump C. Protein solutions used were 5.0 mg ml^{-1} lysozyme, 5.0 mg ml^{-1} BSA and a mixture of 5.0 mg ml^{-1} lysozyme and 5.0 mg ml^{-1} BSA, all dissolved in running buffer. The module was then washed with running buffer for 40 capillary volumes. A 0.5 M NaCl in running buffer was passed through for 50 capillary volumes for eluting bound protein. The module was re-equilibrated using seven capillary volumes of running buffer. These tests were conducted in duplicate.

2.7. SDS-PAGE and Coomassie staining

The sample fractions collected from the frontal analysis experiment of the mixture of 5.0 mg ml^{-1} lysozyme and 5.0 mg ml^{-1} BSA were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The samples were diluted 10 times, with 1.3 μl of original fraction, prepared with denaturing agent and sample buffer and loaded in NuPAGE Novex Bis–Tris 4–12% Pre-Cast Gel (Invitrogen, Paisley, UK) using instruction guide recommendations. Invitrogen's Novex Sharp Pre-Stained Protein Standard was used for molecular weight markers. The gel was run on Invitrogen's XCell SureLock™ Mini-Cell electrophoresis system using appropriate running buffers at 200 V for 35 min. The gel was then Coomassie stained using SimplyBlue SafeStain™ (Invitrogen, Paisley, UK) using instruction guide recommendations. Gels were photographed on G:BOX Chemi XT4 (Syngene, Cambridge, UK).

3. Results and discussion

3.1. MMCF module development and characterisation

MMCF (Fig. 1(a)) was extruded through a 19 capillary die of nozzle size 0.5 mm diameter with a polymer composition of 15/10/75 wt% EVOH/PVP/NMP and pure glycerol as the bore fluid. The optimised polymer flow rate used was 1.4 g min^{-1}, bore fluid rate was 0.5 ml min^{-1} and the air gap size was 8 mm. Pure glycerol gave a uniform distribution of the bore fluid across the 19 injector nozzles. Bonyadi et al. [12] had previously characterised the porosity of the capillary wall. From scanning electron micrographs the pores in the vicinity of the inner capillary sized were 0.3–0.5 μm size, in the middle region between 2 and 3 μm, and close to the outer capillary wall between 0.1 and 0.5 μm [12]. The total internal surface area of a 200 mm length of MMCF was measured hereby BET (Brenauer, Emmett and Teller) surface area analysis and was 18.0 ± 0.1 m^2. This is ~425 fold higher surface area than a 5 m NMCF, which has a surface area of 0.042 m^2 [11]. A module (Fig. 1(b)) was constructed around the MMCF using epoxy glue coating and adaptors. By constructing the module in this manner, column packing as in traditionally bead based media, was avoided.

3.2. Breakthrough curve and dynamic binding capacity analysis on SP MMCF module

A series of frontal analysis experiments using the model protein lysozyme allowed the DBC of SP MMCF module to be determined for a range of superficial velocities 5400–54,000 cm h^{-1}. The high capacity of the MMCF module required an increase in protein loading concentration from 0.2 mg ml^{-1} lysozyme used for NMCF device capacity studies to 5.0 mg ml^{-1} lysozyme to reach near 100% breakthrough [11]. The breakthrough curve for the MMCF module (Fig. 3(a)) shows the binding and elution profile using 5.0 mg ml^{-1} lysozyme solution at 10,800 cm h^{-1}. The MMCF module displays a sharp breakthrough even though it has a microporous wall. The DBC of MMCF module at 10% breakthrough was 18.4 mg lysozyme bound. When loaded to near saturation 41.4 mg lysozyme was bound and on subsequent elution 40.1 mg lysozyme was recovered. This corresponds to a binding density of 228 ng lysozyme/cm^2 adsorbent surface area, in the same magnitude but lower than NMCF device at 314 ng lysozyme/cm^2 adsorbent surface area [11]. In terms of length this corresponds to

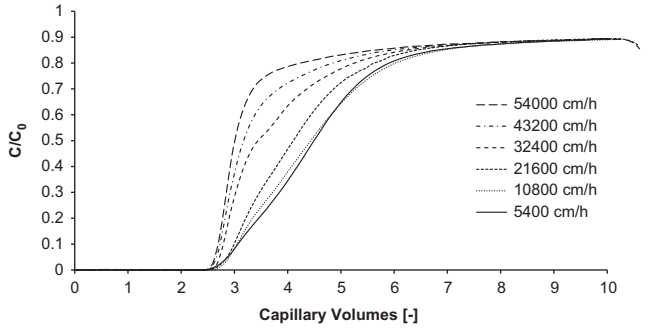

Fig. 4. Zoomed frontal analysis breakthrough curves using 5.0 mg ml^{-1} lysozyme to near 100% breakthrough at flow rates of 5400 cm h^{-1} to 54,000 cm h^{-1} through 200 mm MMCF module. 1 capillary volume of the MMCF module is 1.55 ml. C/C_0 is the relative UV absorbance of the running lysozyme concentration by the initial feed lysozyme concentration.

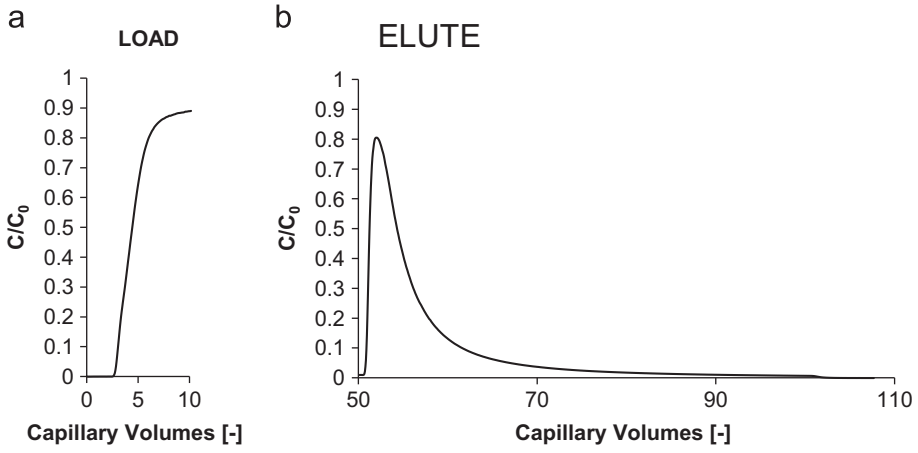

Fig. 3. Lysozyme loading and elution profile on MMCF. Frontal analysis experiment with 5.0 mg ml^{-1} lysozyme solution loaded onto 200 mm MMCF module at a superficial flow velocity of 10,800 cm h^{-1}. On the *X* axis one capillary volume of the MMCF module is 1.55 ml. On the *Y* axis C/C_0 is the relative UV absorbance of the running lysozyme concentration by the initial feed lysozyme concentration. The experiment was carried according to the frontal analysis study methodology described in Section 2.4. (a) Lysozyme loading profile showing a sharp near 100% breakthrough in a short time scale. (b) The elution profile shows mass eluted is 40.1 mg lysozyme.

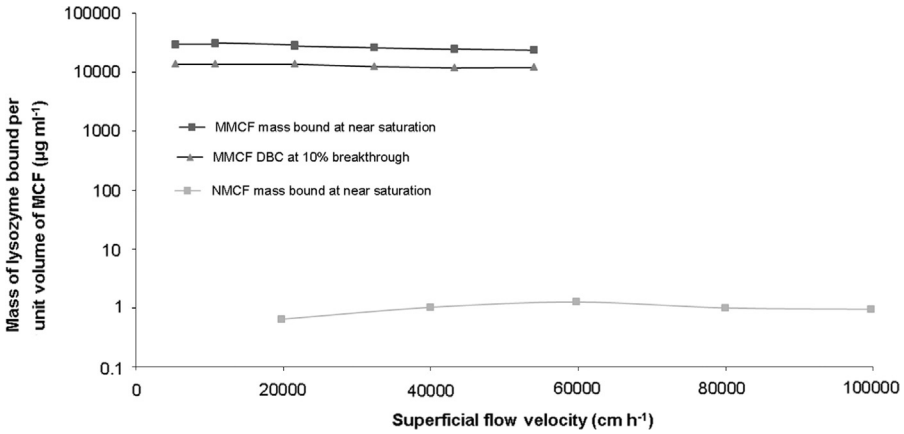

Fig. 5. Effect of superficial flow velocity on binding capacity of MMCF module (black lines) and NMCF device (grey line). The mass of lysozyme bound is shown as per unit adsorbent volume of MCF. These tests were conducted in duplicate and the data points indicate averages.

Table 1
Comparison of MMCF module against NMCF device and current commercial packed bed and membrane media.

	Chromatography medium			
	Microporous beads (GE SP Sepharose XL®)	Membrane (Pall Mustang S modified Supor®)	Nonporous MCF device [11]	Microporous MCF module
Packing requirement	Yes	No	No	No
Superficial flow velocity	300–500 cm h^{-1}	N/A	19,800–99,720 cm h^{-1}	5400–54,000 cm h^{-1}
Volumetric flow rate	2–10 ml min^{-1}	1–4 ml min^{-1}	1–5 ml min^{-1}	1–5 ml min^{-1}
Maximum pressure	0.15 MPa	0.55 MPa	2.0 MPa	> 1.5 MPa
Typical operating dynamic binding capacity	160 mg lysozyme/ml adsorbent volume	47 mg lysozyme/ml adsorbent volume	0.001 mg lysozyme/ml capillary volume	13.8 mg lysozyme/ml adsorbent volume, 11.8 mg lysozyme/ml capillary volume

2.05 mg lysozyme per cm of MMCF, much higher than 266 ng lysozyme per cm of NMCF.

Fig. 4 shows the breakthrough curves for various superficial flow velocities through the MMCF module. A sharper breakthrough is seen at higher superficial flow velocities. Superficial flow velocities also have an effect on the pressure drop across the module and dynamic binding capacities. As superficial flow velocity increases, pressure drop across the MMCF module also increases. For the highest superficial flow velocity tested (54,000 cm h^{-1}) the pressure drop across the module was 0.65 MPa. Hence as with the NMCF device, the MMCF module can operate at high superficial flow velocities experiencing low pressure drops, concluding its high throughput behaviour.

Results of the binding capacity analyses with lysozyme for a range of superficial flow velocities are shown in Fig. 5. The binding capacity at near saturation of MMCF module is 10^4 times higher than the NMCF device. The dynamic binding capacity of MMCF module 10% breakthrough is 13.8 mg lysozyme/ml adsorbent volume is of a similar magnitude to commercial packed bed columns (160 mg lysozyme/ml adsorbent volume GE SP Sepharose XL®) and membranes (47 mg lysozyme/ml adsorbent volume Pall Mustang S modified Supor®) as compared in Table 1.

With conventional packed bed chromatography, the superficial flow velocities that can be used are limited by relatively low intraparticle mass transfer rates for adsorbates [6] and by the compressibility of gels that causes pressure drops to increase. Commonly, DBC's fall with increasing flow velocities for protein adsorption in conventional beads [11]. By contrast, the DBC for the MMCF is relatively invariant with superficial flow velocity (Fig. 5). Similarly, pressure drop increased but was well within the normal operating range of the AKTA chromatography system.

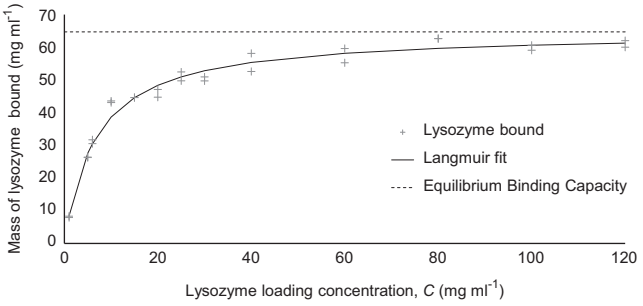

Fig. 6. Ligand density model of MMCF module. The values of lysozyme mass bound was measured at different lysozyme loading concentrations, *C*, have been plotted (crosses). The experiments were conducted in duplicate. The solid line represents the Langmuir isotherm equation and has a good fit with the data (R^2 = 0.9503) and gives an equilibrium binding capacity of 64.7 mg lysozyme/ml adsorbent volume (shown as dotted line).

3.3. Equilibrium binding capacity studies and protein adsorption model

A flow rate of 1.0 ml min^{-1} corresponding to a superficial flow velocity of 10,800 cm h^{-1} was used for subsequent surface ligand binding density studies. Fig. 6 shows the mass of lysozyme bound calculated from frontal analysis experiments at different protein concentrations (*C*). The solid black line models the Langmuir isotherm equation which fits the data with a R^2 value of 0.9503. The fit of the Langmuir plot with the experimental data indicates that the lysozyme binds to the MMCF in a monolayer. Fig. 6 shows the mass of lysozyme bound at equilibrium per unit volume of MMCF as a fraction of lysozyme concentration in the supernatant.

The EBC of the MMCF module was 479 ng lysozyme/cm^2 adsorbent surface area and 64.7 mg lysozyme/ml adsorbent volume. The EBC is limited by the surface area of the resin that is sterically available via the SP ligand density and contact with the solute [6].

3.4. Cation-exchange studies via frontal analysis and SDS-PAGE

Fig. 7 shows frontal analysis plots for lysozyme (5.0 mg ml^{-1}), BSA (5.0 mg ml^{-1}) and mixtures of these two proteins. Comparison between the lysozyme plot and BSA plot indicate that the BSA front was less steep as it reached saturation, indicating non-specific adsorption. From the mixed protein samples, 41.0 mg of lysozyme and 0.5 mg of BSA were eluted. Hence at elution 98.8% of the total sample eluted was the target protein lysozyme with only 1.2% impurity BSA. SDS-PAGE analysis (Fig. 8) of 1.0 ml sample fractions collected from Fig. 7(c) confirm that both lysozyme and BSA flow through the saturated MMCF module at the frontal

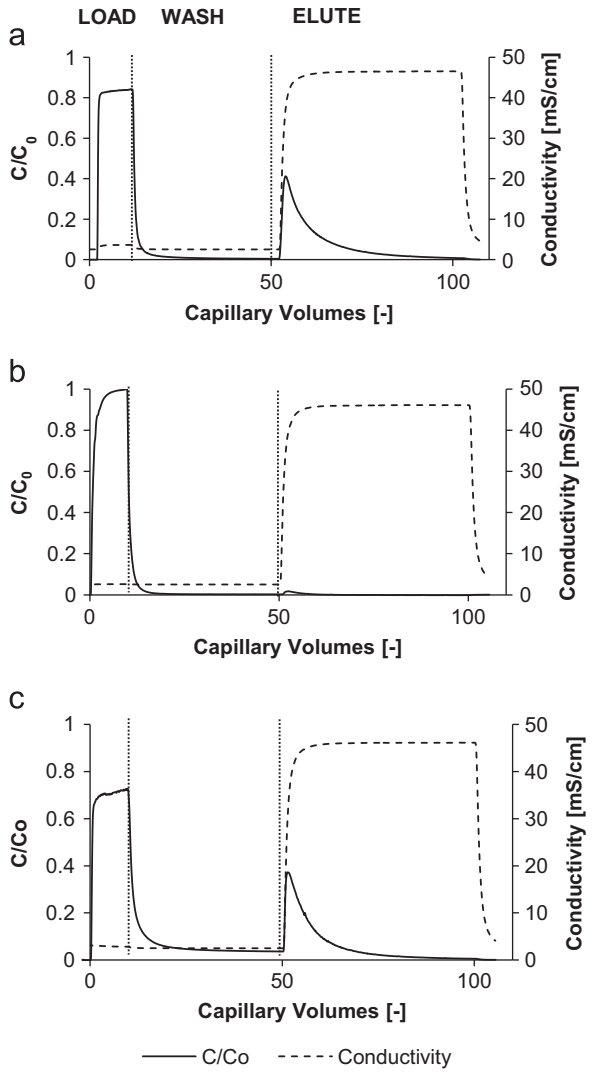

Fig. 7. Application of MMCF module as a cation-exchange medium. Frontal analysis loading experiments of lysozyme, BSA and mixture were tested at a superficial flow velocity of 10,800 cm h^{-1}. On the *X* axis one capillary volume of the MMCF module is 1.55 ml. On the *Y* axis C/C_0 is the relative UV absorbance of the running protein concentration by the initial feed protein concentration. The experiments were carried out as per methodology described in Section 2.6. (a) Frontal analysis study of 5.0 mg ml^{-1} lysozyme, 41.0 mg lysozyme was eluted. (b) Frontal analysis study of 5.0 mg ml^{-1} BSA, 0.49 mg BSA was eluted. (c) Frontal analysis study of a mixture of 5.0 mg ml^{-1} lysozyme and 5.0 mg ml^{-1} BSA, samples from this experiment were analysed on SDS-PAGE in Fig. 8.

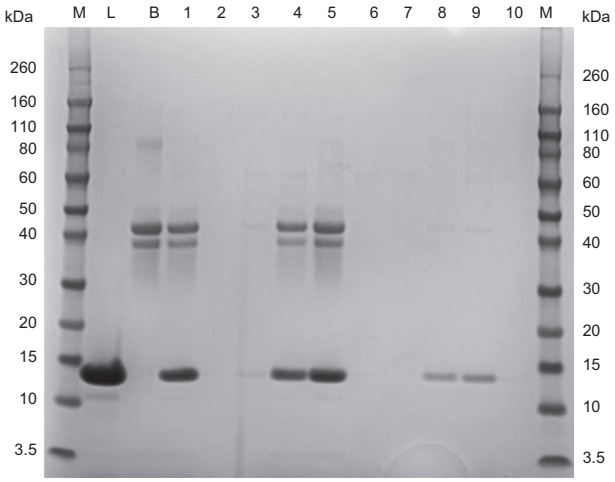

Fig. 8. Coomassie stained SDS-PAGE analysis of 1.3 μl samples from Fig. 7(c) frontal analysis experiment mixture of 5.0 mg ml^{-1} lysozyme and 5.0 mg ml^{-1} BSA, loaded in a NuPAGE Novex Bis–Tris 4–12% Pre-Cast Gel. Lane M on the far left and right marks the Novex Sharp Pre-Stained Protein Standard ladder. Lane L consists of pure 5.0 mg ml^{-1} lysozyme. Lane B consists of pure 5.0 mg ml^{-1} BSA. Lane 1 has the loading mixture of 5.0 mg ml^{-1} lysozyme and 5.0 mg ml^{-1} BSA, lane 2 has a sample of the fraction from the equilibrium step. Lanes 3, 4 and 5 have samples from the fractions of the sample loading step. Lanes 6 and 7 have samples from the fractions of the wash step. Lanes 8 and 9 have samples from the fractions of the elution step. Lane 10 has a sample of the fraction from the re-equilibrium step.

analysis loading step (lanes 3, 4 and 5) and the elution fractions consist of lysozyme predominantly (lanes 8 and 9).

Scale-up of MMCF module separations is similar to the linear scale-up of membrane processes. Whereas conventional columns are operated with low superficial liquid velocities between 300 and 500 cm h^{-1}, MMCF modules can be run with superficial velocities up to 180-fold higher since no matrix compression occurs. High dynamic binding capacities are obtainable when operated at high throughput. The material chemicals cost for the MMCF used in this work is estimated to be only a few pence as the cost of extrusion is low. A major proportion of scale-up cost is likely to lie in the materials and labour expenses for the chemical functionalization and other pilot scale-up production requirements.

Table 1 compares typical operating parameters for a conventional packed bed adsorber and a membrane adsorber with NMCF and MMCF. The capillary volume for the MCF is defined as the internal volume of all the capillaries. Adsorbent volume in packed beds is the total volume of the column excluding inter-particle volume, whilst the adsorbent volume of MMCF is the total volume of the MMCF excluding the capillary volume.

Notably, Table 1 shows that MMCF's can be operated at much higher superficial flow velocities than packed beds, which reduces film mass transfer resistance. MMCF's require no packing which avoids validation of packing integrity and can be operated up to higher pressures. The DBC of the prototype MMCF is about 12-fold lower than the conventional packed bed comparator, but only about 4-fold lower than that of the membrane adsorber. Compared with NMCF, the MMCF has substantially higher DBC yet retains similar ability to operate at high superficial velocities and pressures.

4. Conclusions

In this paper we have tested an ion-exchange functionalised microporous walled micro-capillary film (MMCF) for protein chromatography and compared it with other chromatography

media. The application of MMCF module for the chromatographic separation of model proteins was successfully demonstrated using a superficial velocity 180-fold higher that of conventional packed bead columns, of flow velocities up to 54,000 cm h^{-1}, 1.5 MPa pressure tolerance, and high dynamic binding capacity at 10% column breakthrough of 13.8 mg lysozyme/ml adsorbent volume. The results presented here indicate that the MMCF module may provide a high capacity, higher throughput, sharp breakthrough and pressure tolerant alternative to current preparative scale purification media, additionally avoiding the need for column packing.

Acknowledgement

Ipshita Mandal would like to thank EPSRC (Grant no. 63832) for their financial support. The authors would also like to thank Dr. David McNally for experimental design advice, Radu Lazar for aiding in software analysis and Dr. Duncan Sharp for advice on experimental statistics. We would also like to thank Dr. Bart Hallmark and Dr. Simon Butler for advising on the hydrodynamic pressure experiments.

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