The development of a weak anion micro-capillary film for protein chromatography

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Abstract

In this study, the surface of a microporous walled micro-capillary film (MMCF) was modified into a weak anion exchanger by coupling cyanuric chloride and 2-diethylaminoethylamine (DEAE) to the ethylene-vinyl alcohol (EVOH) matrix. Fourier transform infrared spectroscopy (FTIR) measurements of modified and unmodified MMCFs confirmed the addition of a triazine ring and DEAE onto the membrane. Binding of bovine serum albumin (BSA) at pH 7.2 was found to follow a Langmuir isotherm with a maximum equilibrium binding of 12.4 mg BSA/mL adsorbent and 8.2 mg BSA/mL adsorbent under static and flow conditions, respectively. The ion exchange capacity, determined by Mohr's titration of chlorine atoms displaced from the functionalised surface, was found to be 195 $\pm$ 21 μmol Cl^- /mL of adsorber, comparable to commercial ion exchangers. BSA adsorption onto the ion exchanger was strongly pH-dependant, with an observed reduction in binding above pH 8.2.

Frontal experiments of a BSA (5 mg/mL) and lysozyme (5 mg/mL) mixture demonstrated successful separation of BSA from lysozyme at more than 97% purity as verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Separation between similarly charged anionic molecules was also achieved using BSA (5 mg/mL) and herring sperm DNA (0.25 mg/mL). BSA was extracted at 100% purity, demonstrating the ability of MMCF-DEAE to remove significant DNA contamination from a protein solution. These experiments highlight the potential for MMCFs to be used for fast protein purification in preparative chromatography application.

Key words: weak anion-exchange, chromatography, micro-capillary films, membranes, DEAE

1. Introduction

The pharmaceutical industry has progressively shifted its focus from small chemical drugs towards the use of large biomolecules such as antibodies. In order to scale-up the manufacture of biopharmaceuticals and produce them at a greater efficiency, product-specific titres have increased steadily over the past three decades from less than 0.5 g/L in the early 1990s to values in excess of 3 g/L for newer processes, with 7 g/L and above being the new top-end industry target [1]. This order of magnitude increase has moved the production bottleneck downstream, towards the product purification stage where technologies with greater throughput and faster separation capabilities are needed.

Membranes have been demonstrated to be viable chromatography support for rapid protein purification on account of the high superficial velocities that can be attained without performance penalties [2]. As convective mass transport is the dominant mode by which separations occur, flowrate independent binding can be achieved. Membranes have been used in a wide range of chromatographic operations such as the purification of plasmid DNA using anion exchange [3], the capture of IgG using Protein A affinity chromatography [4] and the purification of retroviruses [5]. Membrane adsorbers still suffer from several disadvantages with respect to packed beds including their historically poor binding capacity [6] which limit their use in industry.

Micro-capillary films (MCFs) aim to provide a low-cost technology for protein separations [7]. Composed of a continuous capillary array embedded into an ethylene-vinyl alcohol (EVOH) film matrix, these membranes have the potential for use in direct capture of proteins from unfiltered cell lysate. MCFs can be extruded as a non-porous (NMCFs) film using melt extrusion [8] or a porous (MMCFs) film using non-solvent induced phase separation (NIPS) [9]. Benefits of this technology include its ease of manufacture and scale-up (tubular configuration), its low cost (~ 50 pence/metre for MCF manufacture) [7] and the high superficial velocities through the membrane lumen which can be attained (greater than 5000 cm/h) [10]. Moreover, the hydroxyl-rich nature of the polymer allows for a wide-range of ligands to be coupled to MCFs for chromatography applications. Q-functionalised MCFs have been successfully used to remove lentivirus from unfiltered culture media containing suspended solids [11] and strong cation SP- membranes have been used to separate

cytochrome-c and lysozyme [7], lysozyme from BSA [10] and to monitor at-line IgG aggregates in bioreactors [12].

The objective of this paper was to develop and demonstrate a weak MMCF ion exchanger to complement existing MMCF chemistries. The MMCF was modified with 2-diethylethylenediamine (DEAE) and the performance of the exchanger was characterised. The separation of BSA from lysozyme was used to determine the ability of MMCFs to separate model proteins of opposite charge at pH 7.2 and, the separation of BSA from herring sperm DNA was performed to highlight the potential use of MMCF-DEAE for residual DNA clearance.

2. Materials and methods

2.1 Chemicals used

Ethylene vinyl alcohol (EVOH) copolymer with 68 mol% vinyl alcohol was purchased from Kuraray (Hattersheim, Germany). N-methyl-2-pyrrolidine (NMP), polyvinyl-pyrrolidone (PVP, average molecular weight 360 kDa), glycerol, bovine serum albumin (BSA, pI 5.3, MW 66.4 kDa), tris(hydroxy- methyl)aminomethane (Tris), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl), crystalline chick-egg lysozyme (pI 11, MW 14.3 kDa), sodium phosphate monobasic (NaH_2PO_4), 2-diethylaminoethylamine (DEAE), Bradford reagent and cyanuric chloride were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2 Membrane manufacture

MMCFs were produced using protocols described previously [9] [10]. Briefly, polymer solutions containing 15/10/75 wt.% EVOH/PVP/NMP were wet extruded through a 19-nozzle die of capillary size 0.5 mm, followed by non-solvent induced phase separation (NIPS) in a water bath. Membranes were then stored in a 50/50 v/v glycerol-water solution for long term processing.

Gravimetric analysis was used to determine the column volume (CV) of the membranes so that the binding capacities obtained could be normalised and compared with commercial columns. The column volume, was defined as the total volume from the microporous walls and central capillaries of the film and was found to be 1.06 ± 0.09 mL for a 20 cm MMCF

section (dried weight, 164 ± 5 mg). A 20 cm MMCF length was used for this work as it had a convenient column volume ~ 1 mL.

2.3 BET and mercury intrusion porosimetry

N_2 adsorption was measured at 77.4 K using a Micromeritics ASAP 2020 instrument (Norcross, GA, USA) to determine the membrane surface area using Brunaeur-Emmett-Teller (BET) theory. The membrane pore surface area and pore size distribution (PSD) was measured using a Micromeritics AutoPore IV 9500 porosimeter (Norcross, GA, USA) up to a final pressure of 2000 bar.

MMCF samples were vacuum dried overnight at 150°C prior to N_2 adsorption and porosimetry measurements.

2.4 Chemical modification of MMCFs with DEAE

The coupling of 2-diethylaminoethylamine (DEAE) onto the MMCF backbone was achieved using a modified batch protocol from McCreath *et al.* [13]. Briefly, a MilliQ washed membrane (25 cm, dry weight 205 ± 6 mg) was placed in 50 mM ice cold cyanuric chloride in acetone (40 mL) solution under constant agitation for 30 minutes. DEAE (2.48 g, 0.02 moles) was added to a 0.5 M NaH_2PO_4 aqueous solution (36 mL), the final solution was at pH 9.6. Cyanuric chloride activated MMCF was added to the DEAE solution, heated overnight at 40°C then at 60°C for 5 hours. The membrane was subsequently washed with MilliQ (50 mL) for 30 minutes and reactivated with 0.5 M NaOH (40 mL) for 30 minutes.

The chemical modification is summarised in **Fig. 1**.

2.5 Fourier transform infrared spectroscopy of MMCFs

Fourier transform infrared spectroscopy (FTIR) was used to determine the presence of a cyanuric chloride ring and DEAE on the MMCF membrane. Spectra were collected using a Thermo Nicolet Nexus 870 spectrometer (Waltham, MA, USA) as the average of 32 scans with a wavenumber resolution of 4 cm^{-1} in the 600-4000 cm^{-1} range. As a control, unmodified MMCF was compared to MMCF-DEAE and spectra were normalised to the common CH_2 vibration peak at 2852 cm^{-1}.

2.6

Ion exchange capacity measurement

The ion exchange capacity was determined using a modified protocol from Karas *et al.* [14]. Briefly, 20 sections of 1 cm MMCF-DEAE (dry weight 164 ± 5 mg) were regenerated in 1 M NaOH (40 mL) for 30 minutes prior to a 1 hour step in 1 N HCl (50 mL). After two MilliQ wash steps (30 minutes each), the MMCF-DEAE segments were placed overnight in 0.1 M $NaNO_3$ (40 mL) solution under agitation to displace Cl^- ions from the modified membrane into the supernatant. Mohr's titration was used to titrate the Cl^- in solution with 0.1 M $AgNO_3$ and to determine the ion exchange capacity of the membrane. 0.25 M K_2CrO_4 (1 mL) was used to indicate when all the Cl^- had been exhausted from the supernatant.

Experiments were repeated in triplicate with unmodified MMCF used as a control.

2.7

Adsorption isotherm of BSA onto the membrane

Stock solutions of BSA at concentrations 0.25, 0.5, 1, 2, 3, 5 and 10 mg/mL were prepared in 20 mM Tris-HCl pH 7.2 buffer to determine the adsorption behaviour of protein onto the membrane.

4 sections of 1 cm MMCF-DEAE (dry weight 33 ± 1 mg) were left for 48 hours under agitation in 5 mL protein solution to reach equilibrium. To remove unbound protein from the membrane surface, the supernatant was replaced with 3 mL of buffer and the membrane was centrifuged at 5000g for 12 minutes. Both supernatants were then combined and a Bradford assay was used following the protocol provided by the supplier. Measurements of the remaining BSA in the supernatant were performed at UV 595 nm using a BMG Labtech SPECTROstar Nano (Allmendgruen, Germany). The amount of protein bound onto the MMCFs was determined by mass balance and, all experiments were repeated in triplicate.

2.8

Time course binding studies

4 sections of 1 cm MMCF-DEAE membranes were immersed in 1 mg/mL BSA in Tris-HCl pH 7.2 (5 mL) and were used to determine the binding of BSA onto the membrane as a function of time. 50 µL samples were taken at t = 0, 0.5, 1, 2, 3, 6, 8, 12, 16, 24, 32 and 48 hours and quantified using a Bradford assay.

Experiments were repeated in triplicate and unmodified MMCF was used as a control.

2.9 Column fabrication

Extruded membranes were encased in 8 mm FEP tubing (Kinesis Ltd, St. Neots, UK) using epoxy glue (Araldite®, Cleveland, OH, USA), trimmed to 20 cm in length and fitted with Upchurch 1/4 inch HPLC connectors to be attached to an ÄKTA FPLC system (GE Healthcare Life Sciences, Uppsala, Sweden) as described by Mandal *et al*. [10]. **Fig. 2** shows a schematic representation of the MMCF column module and a SEM image of an MMCF cross-section.

2.10 Equilibrium binding studies in flow operation

BSA solutions of 1, 5, 10, 20 and 40 mg/mL in 20 mM Tris-HCl pH 7.2 (running buffer) were loaded to saturation onto the membrane to determine the binding profile of MMCF-DEAE under flow (dynamic) condition. The flowrate was chosen to be 1 mL/min (~ 56.6 CV/h), flowing through the lumen of the membrane. Elution was performed with a step gradient of 1 M NaCl in running buffer.

Eqs. (1) was used to calculate the mass of BSA eluted at increasing loading concentration.

$$Mass\ eluted\ (mg) = \frac{C_{inj}\ (mg\ mL^{-1} \times Elution\ Area\ (mA\ U\ mL))}{100\%\ Saturation\ height\ (mA\ U)} \quad (1)$$

Where C_{inj} is the concentration of protein used at injection.

The data was fitted to a Langmuir isotherm adsorption model, described according to Eqs. (2) derived from adsorption-desorption kinetics:

$$q = q_{max} \frac{\alpha C_{inj}}{1+\alpha C_{inj}} \quad (2)$$

With q being the mass bound at all C_{inj}, q_{max} the equilibrium binding capacity and α the Langmuir adsorption constant.

2.11 Effect of pH and flowrate on binding

Frontal analysis experiments using BSA were conducted from pH 6.2 to pH 9.2 to determine pH dependency of the membrane in flow condition. A 20 mM Tris-HCl running buffer was used and samples were eluted in running buffer containing 1 M NaCl.

The column was equilibrated for 5 CV at a flowrate of 1 mL/min, then continuously loaded through the lumen of the membrane with BSA (10 mg/mL) until 100% column saturation as determined by UV absorbance measurements at 280 nm. The column was washed with running buffer for 15 CV followed with a step elution over 10 CV. The column was re-equilibrated for 10 CV between each run.

Experiments were repeated in triplicate and the mass of BSA eluted was calculated using Eqs. (1).

The effect of flowrate on binding was determined using 100 μL pulse injections of BSA (5 mg/mL) onto MMCF-DEAE. The capture (%) was calculated using Eqs. (3):

$$Capture\ (\%) = \frac{Elution\ Area\ (mA\ U\ mL)}{Elution\ Area\ (mA\ U\ mL) + Flowthrough\ Area\ (mA\ U\ mL)} \times 100 \quad (3)$$

Flowrates between 0.1 mL/min and 10 mL/min were tested to keep the pressure drop below ~ 1.5 MPa which is the limiting upper pressure for the column adaptors.

2.12

Lysozyme and BSA separation

A subtractive separation of BSA from lysozyme was tested on MMCF-DEAE using 5 mg/mL lysozyme and 5 mg/mL BSA loaded through the membrane lumen at 1 mL/min until 100% column saturation. 1 mL fractions of the flowthrough and elution were collected to assess peak purity using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Fractions (diluted tenfold) were loaded onto NuPAGE Novex 4-12% Bis-Tris pre-cast gels (Invitrogen, Paisley, UK), per the manufacturer's instructions. Novex Sharp pre-stained protein standards (Invitrogen, Paisley, UK) were used as molecular weight (MW) markers. The XCell SureLock Mini-Cell electrophoresis system (Invitrogen, Paisley, UK) was used at 200 V for 35 min with MES SDS running buffer. Coomassie staining was performed using SimplyBlue SafeStain (Invitrogen, Paisley, UK) following the manufacturer's protocol.

Experiments were repeated on an unmodified MMCF as a control to determine the presence of non-specific protein binding to the membrane matrix (see supplementary materials).

2.13

Herring sperm DNA and BSA separation

The ability to separate two anionic molecules was tested using 100 μL injections of BSA (5 mg/mL) and herring sperm DNA (0.25 mg/mL) onto a 20 cm MMCF-DEAE column. A two-

step elution at 0.25 M NaCl and 1 M NaCl was used to separate BSA from DNA. UV measurements were performed at 254 nm and 20 mM Tris-HCl pH 7.2 was used as the running buffer at 1 mL/min.

To assess the purity of the elution peaks, the elution fractions were injected onto a commercial Mono Q 5/50 GL high-resolution Tricorn column (GE Healthcare Life Sciences, Uppsala, Sweden) following the protocol described in the supplementary materials.

3. Results and Discussions

3.1 Surface characterisation of MMCFs: Nitrogen adsorption, mercury porosimetry and FTIR results.

Nitrogen adsorption onto an MMCF sample exhibited a type III isotherm [15] as shown in **Fig. 3A** and the BET surface area was calculated to be 8.95 m^2/g. The total pore area of 8.6 m^2/g obtained from mercury porosimetry was found to agree well with N_2 adsorption results and, a bimodal pore size distribution (PSD) centred at 1000 nm and 100 000 nm in diameter (see **Fig. 3B**) was observed. **Fig. 2A** suggests that, the larger "pores" corresponded to the central capillaries, defects and macrovoids whereas the pores between 150 nm and 3000 nm were representative of the PSD within the membrane film.

FTIR spectra of unmodified MMCF and MMCF-DEAE revealed the presence of two additional peaks at 1578 cm^{-1} and 1547 cm^{-1} for MMCF-DEAE (see **Fig. 4**). These correspond to in-plane vibration of a triazine [16] and secondary amine N-H bend [17] respectively. This indicates that both triazine and DEAE were successfully coupled to the membrane.

3.2 Characterisation of MMCF-DEAE in static conditions: ion exchange capacity and protein binding isotherm.

The ion exchange capacity of the modified membrane was found to be 195 $\pm$ 21 μmol Cl^- /mL, twice the reported value of McCreath et al [13] and comparable to commercial exchangers (0.11 to 0.16 mmol Cl^- /mL medium for DEAE Sepharose Fast Flow [18] and 0.29 to 0.35 mmol Cl^- /mL medium for GE Capto DEAE [19]).

Static binding data fit the Langmuir model well (R^2 = 0.98), suggesting monolayer binding of protein to active sites (see **Fig. 5A**). The equilibrium binding capacity was measured to be q_{max} = 12.4 mg of BSA/mL of adsorbent, similar to the value reported in McCreath *et al.* of 9.7 mg/mL adsorbent for human serum albumin (HSA) onto perfluoropolymers [13] but more than an order of magnitude lower than that achieved with commercial membranes [20].

The binding of BSA onto MMCF-DEAE was found to increase linearly during the first 8 hours (see **Fig. 6**) prior to reaching a plateau at the 12 hour mark due to binding site saturation. After 24 hours, a maximum binding was reached of 10 mg BSA/mL adsorbent, in-line with the previously observed maximum binding of 10 mg BSA/mL adsorbent at 1 mg BSA/mL loading (**Fig. 5A**).

3.3 Performance of MMCF-DEAE under flow conditions: dynamic protein binding capacity and the effect of pH and flowrate on binding

By loading a 20 cm MMCF-DEAE column with BSA at varying concentrations from 1 mg/mL to 40 mg/mL, an equilibrium dynamic binding profile was obtained and fitted with a Langmuir equation (see **Fig. 5B**). The Langmuir model was found to fit the data well (R^2 = 0.99) but showed a 30% binding reduction compared to the equilibrium binding capacity (EBC) obtained under static condition, 8.2 mg BSA/mL adsorber compared to 12.4 mg BSA/mL adsorber. This has been observed previously [13] and was most likely a result of the larger mass transfer resistance present in small pores: under flow conditions, some binding sites were inaccessible to proteins due to the shorter residence times available for the analytes to diffuse into the membrane matrix.

The effect of pH on binding under flow condition is shown in **Fig. 7**. As the pH was increased from pH 6.2 to 9.2, a sharper breakthrough curve (**Fig. 7A**) during the loading step was obtained indicating that less mass was bound to the column. This was verified in the elution step (**Fig. 7B**) where, the largest elution area was obtained at pH 6.2 corresponding to a binding of 7.7 mg BSA/mL adsorbent. Further increase in pH resulted in diminished binding and, at pH 9.2 the elution peak was below detection limit **(Fig. 7C).** This behaviour was in-line with what was expected from a weak anion exchanger and the known pKa value of the tertiary amine group of DEAE ~ 7.1 [21]. At pH > pKa, the protonation of the binding site was diminished and loss of charge occurred.

The breakthrough curves at all pHs had a characteristic S-shape corresponding to non-ideal protein breakthrough. The elution peaks of **Fig. 7B** show an asymmetrical elution profile with a long tail (greater than 5 CV) which would suggest that long diffusion times are needed to elute analytes trapped within the smaller pores of the matrix and within the membrane dead volume. It is likely that this non-ideality in flow was a result of the non-uniform pore size within the membrane (see **Fig. 3B**) and channelling down the membrane bore which have been shown to increase peak broadening [22]. The pore size distribution introduces a range of different solute path length and a varying diffusion rate (eddy diffusion) from the stationary phase to the mobile phase resulting in band broadening [23].

The effect of flowrate on binding was determined using 100 μL injections of BSA (5 mg/mL) applied onto an MMCF-DEAE column at flowrates ranging from 0.1 mL/min to 10 mL/min (superficial velocities between 330 –33 000 cm/h through the MMCF lumen). **Fig. 8** shows that increasing the flowrate led to an increase in flowthrough in the loading stage and a decrease in mass eluted. The capture decreased from 90% at 0.1 mL/min to 63% at 10 mL/min with a sharper drop between 0.1 mL/min and 1 mL/min (**Fig. 8C**). Increasing the flowrate above 1 mL/min did not significantly reduce the capture of BSA but introduced peak broadening. Although membranes typically have flowrate independent binding [24], this result was not surprising on account of the flow operation chosen. By flowing the protein mixture in the axial direction, through the membrane lumen, instead of as a radial transmembrane flow, radial mass transport to the membrane was slow (the Reynolds number is <<1) and, at high flowrates, binding was reduced due to the decreased residence time between the mobile phase and stationary phase.

3.4 Proof-of-concept separations of cationic-anionic and anionic-anionic protein mixtures using MMCF-DEAE

The separation of BSA from a lysozyme containing mixture is shown in **Fig. 9**. It can be seen that BSA was successfully recovered at high purity as determined by SDS-PAGE analysis of the elution (**Fig. 9B**). A faint lysozyme band below the limit of quantification was observed in the elution suggesting non-specific protein binding. From the control experiment using unmodified MMCF (see supplementary materials), it is likely that the non-specific lysozyme was bound to the MMCF backbone and was estimated to be ~ 0.2 mg of lysozyme/mL of adsorber. Therefore, with the same amount of non-specific binding, a purity greater than 97% BSA (on a weight basis) was obtained using MMCF-DEAE. The high

molecular weight species observed in **Fig. 9B** were neglected in this analysis and most likely corresponded to dimers and trimers of BSA which have been found to persist even under denaturing conditions [25].

The separation of two anionic molecules, fish sperm DNA and BSA, onto an MMCF-DEAE column was tested using a two-step NaCl elution. As can be observed in **Fig. 10C**, two elution peaks were obtained. From the controls, **Fig. 10A** and **Fig. 10B**, the first elution corresponded to a BSA dominant peak and that the second elution was DNA dominant. Further analysis of the fractions collected with a commercial Mono Q 5/50 GL high-resolution Tricorn column (see supplementary materials) indicated that the first peak was pure BSA and the second elution peak was 81% herring sperm DNA with a 19% BSA contamination. The detection limit of herring sperm DNA was estimated to be ~ 16 ng of DNA (see supplementary materials), of similar order of magnitude with the regulatory requirements of less than 10 ng of genomic DNA per therapeutic dose [26].

The mass of BSA recovered in the first elution peak was 0.38 mg of BSA corresponding to a 77% capture. The low capture of BSA could be improved by loading the column at a lower flowrate, using longer MMCF columns, increasing the salt concentration in the first elution or reducing the injected protein concentrations.

4. Conclusions

In this study, the versatility of MMCFs was demonstrated by successful modification of the matrix with a new DEAE chemistry for protein chromatography. A simple two-step chemical modification resulted in a weak anion exchanger, as verified by FTIR analysis. Characterisation of the membrane showed an ion exchange capacity of 195 ± 21 μmol Cl^- /mL of adsorber, significantly higher than that reported in previous studies and comparable to commercial exchangers. However, the binding capacity of MMCF-DEAE of 12.4 mg BSA/mL of adsorber was found to be significantly lower than that of commercial columns. This indicates that the binding behaviour will have to be improved for any preparative use to be achieved. Increasing the number of capillaries within the membrane film or improving the chemical modification used could result in improved binding.

The proof-of-concept separation of BSA from lysozyme revealed that recovery of BSA at more than 97% purity could easily be achieved. The more complex separation of BSA and herring sperm DNA demonstrated future potential of resolving two negatively charged

compounds using this system. 77% of the BSA injected was recovered free of detectable impurity despite the large DNA load present in the feed mixture..

These results suggest that with proper optimisation, MMCFs could be used as low-cost residual DNA clearance devices.

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6. Figure captions

Fig. 1: Chemical route to modify MMCFs into weak anion exchangers using DEAE as the chemical ligand. Coupling of cyanuric chloride to the membrane was performed and maintained in ice cold condition until addition of DEAE.

Fig. 2: (A) SEM image of an MMCF cross section taken at the Cambridge Advanced Imaging Centre (CAIC) at an acceleration voltage of 5.0 kV using FEI Verios 460. Membranes were freeze dried with liquid nitrogen in a Quorum K775X freeze dryer (Laughton, UK), fractured to obtain clean edges and gold coated. The average capillary bore diameter was calculated assuming a circular cross-section of same area using Image J and found to be 351 ± 42 μm. (B) Cross-sectional representation of an MMCF column module. (C) Schematic representation of an MMCF column encased in a FEP 8 mm tube fitted with PTFE adaptors.

Fig. 3: (A) Nitrogen adsorption isotherm at 77.4 K of MMCF membrane sample of mass 0.09 g.(B) Mercury porosimetry pore size distribution of a sample of mass 0.36 g. Smaller pores were attributed to the pores within the matrix of the membrane whereas the larger apparent pores corresponded to the capillary bores, macrovoids and other membrane defects.

Fig. 4: FTIR spectra of unmodified and DEAE modified MMCFs normalised to the common CH_2 vibrational peak at 2852 cm^{-1} and offset vertically. Triazine in-plane ring vibrations (1578 cm^{-1}) and the secondary N-H amine bond (1547 cm^{-1}) of DEAE coupled to the MMCF are shown in bold.

Fig. 5: (A) Static adsorption isotherm of BSA onto MMCF-DEAE. (B) Adsorption isotherm of BSA on MMCF-DEAE in flow (dynamic) condition. Langmuir fit for (A) q_{max} = 12.4 mg/mL adsorbent and $\alpha = 0.36$. $R^2 = 0.98$. Langmuir fit for (B) q_{max} = 8.2 mg/mL adsorbent and alpha = 0.34. R^2 = 0.99. Experiments were repeated in triplicate and the standard deviation is shown. The binding reported was normalised to the column volume of 1.06 mL.

Fig. 6: (A) Time course experiment measuring the mass of BSA bound onto an MMCF-DEAE membrane with an initial loading solution of 1 mg/mL BSA solution in Tris-HCl. (B) First 8 hours of the loading. A linear fit was performed and found to agree well with experimental data, $R^2 = 0.98$. Experiments were repeated in triplicate and the standard deviation is shown.

Fig. 7: Effect of buffer pH on the binding of BSA onto an MMCF-DEAE column. (A) Breakthrough curves during continuous loading of BSA (10 mg/mL). (B) Elution profile of the bound protein. (C) Mass of BSA eluted normalised to column volume. Experiments were repeated in triplicate and the standard deviation is reported.

Fig. 8: The effect of flowrate on the binding of BSA onto an MMCF-DEAE column. Experiments were repeated in triplicate at flowrates between 0.1 mL/min and 10 mL/min. Only representative chromatograms are shown in (A) and (B). (A) corresponds to the sample loading flowthrough and (B) to the associated elution step. (C) shows the captured amount of BSA in the elution peak relative to the injected amount of BSA (0.5 mg). The standard deviation is reported.

Fig. 9: (A) Frontal loading of BSA (5 mg/mL) and lysozyme (5 mg/mL). The running buffer was 20 mM Tris-HCl at pH 7.2 and elution was done using 1M NaCl in running buffer. The experiment was repeated in triplicate and fractions were collected for SDS-PAGE purity assessment. (B) SDS-PAGE gel of the fractions collected tenfold diluted. Lanes M on either side of the gel correspond to the molecular weight markers. Lane A is the pure lysozyme control (0.1 mg/mL), lane B the BSA control (0.1 mg/mL) and lane AB is a mixture of both. Lanes 1 and 2 are fractions collected during the load step. Lane 3 has a sample of the wash step and lanes 4-7 correspond to the elution of bound protein.

Fig. 10: 100 µL injection of proteins onto an MMCF-DEAE column at a flowrate of 1 mL/min. A two-step elution is specified at 0.25 M NaCl and 1 M NaCL. (A) BSA injection (5 mg/mL) (B) Injection of herring sperm DNA (0.25 mg/mL), (C) Mixture of BSA (5 mg/mL) and fish DNA (0.25 mg/mL). Experiments were repeated in triplicate. UV measurements were made at a wavelength of 254 nm.