

1 **The development of a weak anion micro-capillary film for protein** 2 **chromatography**

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8

9 *Abstract*

10 In this study, the surface of a microporous walled micro-capillary film (MMCF) was
11 modified into a weak anion exchanger by coupling cyanuric chloride and 2-
12 diethylaminoethylamine (DEAE) to the ethylene-vinyl alcohol (EVOH) matrix. Fourier
13 transform infrared spectroscopy (FTIR) measurements of modified and unmodified MMCFs
14 confirmed the addition of a triazine ring and DEAE onto the membrane. Binding of bovine
15 serum albumin (BSA) at pH 7.2 was found to follow a Langmuir isotherm with a maximum
16 equilibrium binding of 12.4 mg BSA/mL adsorbent and 8.2 mg BSA/mL adsorbent under
17 static and flow conditions, respectively. The ion exchange capacity, determined by Mohr's
18 titration of chlorine atoms displaced from the functionalised surface, was found to be $195 \pm$
19 $21 \mu\text{mol Cl}^-/\text{mL}$ of adsorber, comparable to commercial ion exchangers. BSA adsorption
20 onto the ion exchanger was strongly pH-dependant, with an observed reduction in binding
21 above pH 8.2.

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

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

32 **1.**

33 **Introduction**

34 The pharmaceutical industry has progressively shifted its focus from small chemical drugs
35 towards the use of large biomolecules such as antibodies. In order to scale-up the
36 manufacture of biopharmaceuticals and produce them at a greater efficiency, product-specific
37 titres have increased steadily over the past three decades from less than 0.5 g/L in the early
38 1990s to values in excess of 3 g/L for newer processes, with 7 g/L and above being the new
39 top-end industry target [1]. This order of magnitude increase has moved the production
40 bottleneck downstream, towards the product purification stage where technologies with
41 greater throughput and faster separation capabilities are needed.

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

51 Micro-capillary films (MCFs) aim to provide a low-cost technology for protein separations
52 [7]. Composed of a continuous capillary array embedded into an ethylene-vinyl alcohol
53 (EVOH) film matrix, these membranes have the potential for use in direct capture of proteins
54 from unfiltered cell lysate. MCFs can be extruded as a non-porous (NMCFs) film using melt
55 extrusion [8] or a porous (MMCFs) film using non-solvent induced phase separation (NIPS)
56 [9]. Benefits of this technology include its ease of manufacture and scale-up (tubular
57 configuration), its low cost (~ 50 pence/metre for MCF manufacture) [7] and the high
58 superficial velocities through the membrane lumen which can be attained (greater than 5000
59 cm/h) [10]. Moreover, the hydroxyl-rich nature of the polymer allows for a wide-range of
60 ligands to be coupled to MCFs for chromatography applications. Q-functionalised MCFs
61 have been successfully used to remove lentivirus from unfiltered culture media containing
62 suspended solids [11] and strong cation SP- membranes have been used to separate

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

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

72 **2.**

73 **Materials and methods**

74 *2.1*

75 *Chemicals used*

76 Ethylene vinyl alcohol (EVOH) copolymer with 68 mol% vinyl alcohol was purchased from
77 Kuraray (Hattersheim, Germany). N-methyl-2-pyrrolidone (NMP), polyvinyl-pyrrolidone
78 (PVP, average molecular weight 360 kDa), glycerol, bovine serum albumin (BSA, pI 5.3,
79 MW 66.4 kDa), tris(hydroxy- methyl)aminomethane (Tris), hydrochloric acid (HCl), sodium
80 hydroxide (NaOH), sodium chloride (NaCl), crystalline chick-egg lysozyme (pI 11, MW 14.3
81 kDa), sodium phosphate monobasic (NaH₂PO₄), 2-diethylaminoethylamine (DEAE),
82 Bradford reagent and cyanuric chloride were purchased from Sigma Aldrich (St. Louis, MO,
83 USA).

84 *2.2*

85 *Membrane manufacture*

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

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

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

97 2.3

98 *BET and mercury intrusion porosimetry*

99 N₂ adsorption was measured at 77.4 K using a Micromeritics ASAP 2020 instrument
100 (Norcross, GA, USA) to determine the membrane surface area using Brunauer-Emmett-Teller
101 (BET) theory. The membrane pore surface area and pore size distribution (PSD) was
102 measured using a Micromeritics AutoPore IV 9500 porosimeter (Norcross, GA, USA) up to a
103 final pressure of 2000 bar.

104 MMCF samples were vacuum dried overnight at 150°C prior to N₂ adsorption and
105 porosimetry measurements.

106 2.4

107 *Chemical modification of MMCFs with DEAE*

108 The coupling of 2-diethylaminoethylamine (DEAE) onto the MMCF backbone was achieved
109 using a modified batch protocol from McCreath *et al.* [13]. Briefly, a MilliQ washed
110 membrane (25 cm, dry weight 205 ± 6 mg) was placed in 50 mM ice cold cyanuric chloride
111 in acetone (40 mL) solution under constant agitation for 30 minutes. DEAE (2.48 g, 0.02
112 moles) was added to a 0.5 M NaH₂PO₄ aqueous solution (36 mL), the final solution was at
113 pH 9.6. Cyanuric chloride activated MMCF was added to the DEAE solution, heated
114 overnight at 40°C then at 60°C for 5 hours. The membrane was subsequently washed with
115 MilliQ (50 mL) for 30 minutes and reactivated with 0.5 M NaOH (40 mL) for 30 minutes.

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

117 2.5

118 *Fourier transform infrared spectroscopy of MMCFs*

119 Fourier transform infrared spectroscopy (FTIR) was used to determine the presence of a
120 cyanuric chloride ring and DEAE on the MMCF membrane. Spectra were collected using a
121 Thermo Nicolet Nexus 870 spectrometer (Waltham, MA, USA) as the average of 32 scans
122 with a wavenumber resolution of 4 cm⁻¹ in the 600-4000 cm⁻¹ range. As a control, unmodified
123 MMCF was compared to MMCF-DEAE and spectra were normalised to the common CH₂
124 vibration peak at 2852 cm⁻¹.

125 2.6

126 *Ion exchange capacity measurement*

127 The ion exchange capacity was determined using a modified protocol from Karas *et al.* [14].
128 Briefly, 20 sections of 1 cm MMCF-DEAE (dry weight 164 ± 5 mg) were regenerated in 1
129 M NaOH (40 mL) for 30 minutes prior to a 1 hour step in 1 N HCl (50 mL). After two MilliQ
130 wash steps (30 minutes each), the MMCF-DEAE segments were placed overnight in 0.1 M
131 NaNO₃ (40 mL) solution under agitation to displace Cl⁻ ions from the modified membrane
132 into the supernatant. Mohr's titration was used to titrate the Cl⁻ in solution with 0.1 M AgNO₃
133 and to determine the ion exchange capacity of the membrane. 0.25 M K₂CrO₄ (1 mL) was
134 used to indicate when all the Cl⁻ had been exhausted from the supernatant.

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

136 2.7

137 *Adsorption isotherm of BSA onto the membrane*

138 Stock solutions of BSA at concentrations 0.25, 0.5, 1, 2, 3, 5 and 10 mg/mL were prepared in
139 20 mM Tris-HCl pH 7.2 buffer to determine the adsorption behaviour of protein onto the
140 membrane.

141 4 sections of 1 cm MMCF-DEAE (dry weight 33 ± 1 mg) were left for 48 hours under
142 agitation in 5 mL protein solution to reach equilibrium. To remove unbound protein from the
143 membrane surface, the supernatant was replaced with 3 mL of buffer and the membrane was
144 centrifuged at 5000g for 12 minutes. Both supernatants were then combined and a Bradford
145 assay was used following the protocol provided by the supplier. Measurements of the
146 remaining BSA in the supernatant were performed at UV 595 nm using a BMG Labtech
147 SPECTROstar Nano (Allmendgruen, Germany). The amount of protein bound onto the
148 MMCFs was determined by mass balance and, all experiments were repeated in triplicate.

149 2.8

150 *Time course binding studies*

151 4 sections of 1 cm MMCF-DEAE membranes were immersed in 1 mg/mL BSA in Tris-HCl
152 pH 7.2 (5 mL) and were used to determine the binding of BSA onto the membrane as a
153 function of time. 50 μ L samples were taken at $t = 0, 0.5, 1, 2, 3, 6, 8, 12, 16, 24, 32$ and 48
154 hours and quantified using a Bradford assay.

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

156 2.9

157 *Column fabrication*

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

164 2.10

165 *Equilibrium binding studies in flow operation*

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

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

$$172 \text{ Mass eluted (mg)} = \frac{C_{inj} (\text{mg mL}^{-1}) \times \text{Elution Area (mA U mL)}}{100\% \text{ Saturation height (mA U)}} \quad (1)$$

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

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

$$176 q = q_{\max} \frac{\alpha C_{inj}}{1 + \alpha C_{inj}} \quad (2)$$

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

179 2.11

180 *Effect of pH and flowrate on binding*

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

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

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

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

$$193 \text{ Capture (\%)} = \frac{\text{Elution Area (mA U mL)}}{\text{Elution Area (mA U mL)} + \text{Flowthrough Area (mA U mL)}} \times 100 \quad (3)$$

194 Flowrates between 0.1 mL/min and 10 mL/min were tested to keep the pressure drop below ~
195 1.5 MPa which is the limiting upper pressure for the column adaptors.

196 2.12

197 *Lysozyme and BSA separation*

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

202 Fractions (diluted tenfold) were loaded onto NuPAGE Novex 4-12% Bis-Tris pre-cast gels
203 (Invitrogen, Paisley, UK), per the manufacturer's instructions. Novex Sharp pre-stained
204 protein standards (Invitrogen, Paisley, UK) were used as molecular weight (MW) markers.
205 The XCell SureLock Mini-Cell electrophoresis system (Invitrogen, Paisley, UK) was used at
206 200 V for 35 min with MES SDS running buffer. Coomassie staining was performed using
207 SimplyBlue SafeStain (Invitrogen, Paisley, UK) following the manufacturer's protocol.

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

210 2.13

211 *Herring sperm DNA and BSA separation*

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

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

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

220 3.

221 Results and Discussions

222 3.1

223 *Surface characterisation of MMCFs: Nitrogen adsorption, mercury porosimetry and*
224 *FTIR results.*

225 Nitrogen adsorption onto an MMCF sample exhibited a type III isotherm [15] as shown in
226 **Fig. 3A** and the BET surface area was calculated to be 8.95 m²/g. The total pore area of 8.6
227 m²/g obtained from mercury porosimetry was found to agree well with N₂ adsorption results
228 and, a bimodal pore size distribution (PSD) centred at 1000 nm and 100 000 nm in diameter
229 (see **Fig. 3B**) was observed. **Fig. 2A** suggests that, the larger “pores” corresponded to the
230 central capillaries, defects and macrovoids whereas the pores between 150 nm and 3000 nm
231 were representative of the PSD within the membrane film.

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

237 3.2

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

240 The ion exchange capacity of the modified membrane was found to be 195 ± 21 μmol Cl⁻
241 /mL, twice the reported value of McCreath et al [13] and comparable to commercial
242 exchangers (0.11 to 0.16 mmol Cl⁻ /mL medium for DEAE Sepharose Fast Flow [18] and
243 0.29 to 0.35 mmol Cl⁻ /mL medium for GE Capto DEAE [19]).

244 Static binding data fit the Langmuir model well ($R^2 = 0.98$), suggesting monolayer binding of
245 protein to active sites (see **Fig. 5A**). The equilibrium binding capacity was measured to be
246 $q_{\max} = 12.4$ mg of BSA/mL of adsorbent, similar to the value reported in McCreath *et al.* of
247 9.7 mg/mL adsorbent for human serum albumin (HSA) onto perfluoropolymers [13] but more
248 than an order of magnitude lower than that achieved with commercial membranes [20].

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

254 3.3

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

257 By loading a 20 cm MMCF-DEAE column with BSA at varying concentrations from 1
258 mg/mL to 40 mg/mL, an equilibrium dynamic binding profile was obtained and fitted with a
259 Langmuir equation (see **Fig. 5B**). The Langmuir model was found to fit the data well ($R^2 =$
260 0.99) but showed a 30% binding reduction compared to the equilibrium binding capacity
261 (EBC) obtained under static condition, 8.2 mg BSA/mL adsorber compared to 12.4 mg
262 BSA/mL adsorber. This has been observed previously [13] and was most likely a result of the
263 larger mass transfer resistance present in small pores: under flow conditions, some binding
264 sites were inaccessible to proteins due to the shorter residence times available for the analytes
265 to diffuse into the membrane matrix.

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

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

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

297 3.4

298 *Proof-of-concept separations of cationic-anionic and anionic-anionic protein mixtures* 299 *using MMCF-DEAE*

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

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

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

321 The mass of BSA recovered in the first elution peak was 0.38 mg of BSA corresponding to a
322 77% capture. The low capture of BSA could be improved by loading the column at a lower
323 flowrate, using longer MMCF columns, increasing the salt concentration in the first elution or
324 reducing the injected protein concentrations.

325 **4.**

326 **Conclusions**

327 In this study, the versatility of MMCFs was demonstrated by successful modification of the
328 matrix with a new DEAE chemistry for protein chromatography. A simple two-step chemical
329 modification resulted in a weak anion exchanger, as verified by FTIR analysis.

330 Characterisation of the membrane showed an ion exchange capacity of 195 ± 21 $\mu\text{mol Cl}^-$
331 /mL of adsorber, significantly higher than that reported in previous studies and comparable to
332 commercial exchangers. However, the binding capacity of MMCF-DEAE of 12.4 mg
333 BSA/mL of adsorber was found to be significantly lower than that of commercial columns.
334 This indicates that the binding behaviour will have to be improved for any preparative use to
335 be achieved. Increasing the number of capillaries within the membrane film or improving the
336 chemical modification used could result in improved binding.

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

340 compounds using this system. 77% of the BSA injected was recovered free of detectable
341 impurity despite the large DNA load present in the feed mixture..
342 These results suggest that with proper optimisation, MMCFs could be used as low-cost
343 residual DNA clearance devices.

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389 **5.**

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460 6. Figure captions

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

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

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

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

478 **Fig. 5:** (A) Static adsorption isotherm of BSA onto MMCF-DEAE. (B) Adsorption isotherm of BSA
479 on MMCF-DEAE in flow (dynamic) condition. Langmuir fit for (A) $q_{\text{max}} = 12.4\text{ mg/mL}$ adsorbent
480 and $\alpha = 0.36$. $R^2 = 0.98$. Langmuir fit for (B) $q_{\text{max}} = 8.2\text{ mg/mL}$ adsorbent and $\alpha = 0.34$. $R^2 =$
481 0.99 . Experiments were repeated in triplicate and the standard deviation is shown. The binding
482 reported was normalised to the column volume of 1.06 mL.

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

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

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

496 **Fig. 9:** (A) Frontal loading of BSA (5 mg/mL) and lysozyme (5 mg/mL). The running buffer was 20
497 mM Tris-HCl at pH 7.2 and elution was done using 1M NaCl in running buffer. The experiment was
498 repeated in triplicate and fractions were collected for SDS-PAGE purity assessment. (B) SDS-PAGE
499 gel of the fractions collected tenfold diluted. Lanes M on either side of the gel correspond to the
500 molecular weight markers. Lane A is the pure lysozyme control (0.1 mg/mL), lane B the BSA control
501 (0.1 mg/mL) and lane AB is a mixture of both. Lanes 1 and 2 are fractions collected during the load
502 step. Lane 3 has a sample of the wash step and lanes 4-7 correspond to the elution of bound protein.

503 **Fig. 10:** 100 μ L injection of proteins onto an MMCF-DEAE column at a flowrate of 1 mL/min. A
504 two-step elution is specified at 0.25 M NaCl and 1 M NaCl. (A) BSA injection (5 mg/mL) (B)
505 Injection of herring sperm DNA (0.25 mg/mL), (C) Mixture of BSA (5 mg/mL) and fish DNA (0.25
506 mg/mL). Experiments were repeated in triplicate. UV measurements were made at a wavelength of
507 254 nm.