

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

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## Abstract

Micro-capillary film (MCF) membranes are effective platforms for bioseparations and viable alternatives to established packed bed and membrane substrates at the analytical and preparative chromatography scales. Single hollow fibre (HF) MCF membranes with varied microstructures were produced in order to evaluate the effect of the bore fluid composition used during hollow fibre extrusion on their structure and performance as cation-exchange adsorbers. Hollow fibres were fabricated from ethylene-vinyl alcohol (EVOH) copolymer through solution extrusion followed by nonsolvent induced phase separation (NIPS) using bore fluids of differing composition (100 wt.% N-methyl-2-pyrrolidone (NMP), 100 wt.% glycerol, 100 wt.% water). All HFs displayed highly microporous and mesoporous microstructures, with distinct regions of pore size  $<1\ \mu\text{m}$ ,  $5\text{--}15\ \mu\text{m}$  and up to  $50\ \mu\text{m}$  in diameter, depending upon proximity to the bore fluid. Scanning electron microscopy (SEM) revealed skins of pore size  $<1\ \mu\text{m}$  at the inner surface of HFs produced with water and glycerol, while NMP bore fluid resulted in a skinless inner HF surface. The HFs were modified for chromatography by functionalising the polymer surface hydroxyl groups with sulphonic acid (SP) groups to produce cation-exchange adsorbers. The maximum binding capacities of the HFs were determined by frontal analysis using lysozyme solutions ( $0.05\ \text{mg ml}^{-1}$  to  $100\ \text{mg ml}^{-1}$ ) for a flow rate of  $1.0\ \text{ml min}^{-1}$ . The NMP-HF-SP module displayed the largest maximum lysozyme binding capacity of all the fibres produced ( $40.3\ \text{mg lysozyme/ml adsorbent volume}$ ), a nearly 2-fold increase over the glycerol and 13-fold increase over the water variants at the same sample flow rate. The importance of NMP as a bore fluid to hollow fibre membrane performance as a result of inner surface porosity was established with a view to applying this parameter for the optimisation of multi-capillary MCF performance in future studies.

## Keywords

Cation-exchange, Ion-exchange, Chromatography, Hollow fibre, Microporous, Separation

## 1. Introduction

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

Micro-capillary film (MCF) membranes have been demonstrated to be effective platforms for bioseparations and viable alternatives to established chromatography substrates at the analytical and preparative scales, as they offer good binding capacities with high superficial flow rates, low pressure drops and do not involve column packing operations [1,4,5]. MCFs are microstructured membranes containing continuous capillaries embedded within a flat polymer film, with the number of capillaries depending on the die used during the manufacturing process. Both nonporous MCFs (NMCFs) and microporous MCFs (MMCFs) have been produced from ethylene-vinyl alcohol copolymer (EVOH) through extrusion processes [6,7]. Taking advantage of the hydrophilic nature of EVOH and its exposed hydroxyl group, the MCF membrane surfaces can be functionalised into adsorbent surfaces via conjugation of functional ligands to the polymer [4]. Previously, the nonporous NMCF was used as a cation-exchange membrane adsorber for lysozyme purification [4] and as an anion-exchange membrane adsorber for lentivirus capture from cell culture [1]. The use of porous MMCF as a cation-exchange adsorber for bioseparations has also been demonstrated [5].

MMCFs offer distinct advantages over NMCFs. Due to their porous structure, the MMCF surface area available for functionalisation is greatly increased, resulting in a  $10^4$  fold higher binding capacity compared to NMCF in membranes functionalised for cation-exchange chromatography [5]. In addition, bioseparations can be run at high superficial flow velocities while withstanding low pressure drops. It has been demonstrated that the binding capacity of

MMCF is comparable to commercially available packed bed and membrane adsorbers, while providing a sharp breakthrough and higher throughput and pressure tolerance than that of currently available preparative scale purification substrates [5]. The potential of MMCF in terms of binding capacity can be further enhanced by altering key parameters in the membrane manufacturing process. Parameters such as dope composition, bore fluid composition, polymer and bore fluid flow rate, air gap distance to coagulant, take-up rate, and extrusion temperature have been shown to affect membrane characteristics [8,9,10]. In particular, the composition of the bore fluid has been shown to affect membrane morphology [11] and adsorption performance [12].

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

## **2. Materials and methods**

### *2.1 Chemicals and reagents*

Ethylene-vinyl alcohol copolymer (EVOH) containing 32 mol% ethylene was supplied by Kuraray (Hattersheim, Germany). N-methyl-2-pyrrolidone (NMP), polyvinylpyrrolidone (PVP, avg. mol wt. 360,000), glycerol, anhydrous NaOH, cyanuric chloride, acetone, Na<sub>2</sub>HPO<sub>4</sub>, 3-amino-1-propanesulphonic acid, crystalline tris(hydroxymethyl)aminomethane (Tris), HCl and crystalline chick-egg lysozyme were supplied by Sigma Aldrich (St. Louis, MO, USA). All chemicals and biochemicals used were of analytical grade.

### *2.2 Extrusion and assembly of hollow fibre module*

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

### *2.3 Scanning electron microscopy (SEM) and mercury intrusion porosimetry*

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

### *2.4 Surface modification of HF modules with SP groups*

The surface of the HF modules was adapted into a cation-exchange chromatography adsorber using protocols established by Darton [4] and McCreath [13]. Briefly, the nucleophilicity of the HF membrane surface was increased by flowing 30 ml of 1 M NaOH for 30 min through the module using a Knauer Smartline 100 HPLC pump (Berlin, DE). Next, a linker group was added by flowing 20 ml of 50 mM cyanuric chloride in acetone solution for 20 min. After a 10 min wash with 10 ml of MilliQ water, sulphonic acid (SP) groups were

covalently attached to the linker by recirculating 20 ml of a 1 M solution of Na<sub>2</sub>HPO<sub>4</sub> containing 1 g of 3-amino-1-propanesulphonic acid overnight in a 60°C water bath. Finally, the column was washed with 20 ml MilliQ water for 20 min, 20 ml NaOH in water (0.4 M) for 20 min, and again 20 ml MilliQ water for 20 min. Hereafter, the SP functionalised HF produced with the three bore fluids water, glycerol and NMP will be referred to as water-HF-SP, glycerol-HF-SP and NMP-HF-SP.

## 2.5 Frontal analysis study and equilibrium binding capacity analysis

Binding capacity analysis was carried out based on a methodology developed by Darton et al. [4]. The HF modules were first pre-equilibrated with running buffer, 20 mM Tris-HCl pH 7.2, for at least 2 column volumes (column volume, CV, is defined as the total volume within the microporous walls and the central capillary, 1 CV = 0.14 ml). The modules were then continuously loaded with lysozyme ( $C_{inj} = 5.0 \text{ mg ml}^{-1}$ ) in 20 mM Tris-HCl pH 7.2 using a Knauer Smartline 100 HPLC pump until the column was saturated based on UV absorbance as measured by the ÄKTA FPLC system. When nearly 100% lysozyme breakthrough was reached, the saturation UV absorbance height in optical density units at 280 nm was noted. The module was then washed with running buffer and then eluted using a step gradient of 1 M NaCl in 20 mM Tris-HCl pH 7.2 solution to strip any bound protein. The module was then re-equilibrated with running buffer. The absorbance during loading and elution at 280 nm was monitored and integrated to calculate the amount of lysozyme bound and eluted. Tests were conducted in triplicate at a sample flow rate of 1 ml min<sup>-1</sup>. Pressure drops and binding capacities across the modules were measured at flow rates of 1, 2, 3, 4 and 5 ml min<sup>-1</sup>, corresponding to superficial flow velocities through the fibre lumens ranging from 6,000 cm h<sup>-1</sup> to 175,000 cm h<sup>-1</sup> (0.017 m s<sup>-1</sup> to 0.486 m s<sup>-1</sup>). The mass of protein eluted was then calculated by:

$$\text{Mass eluted (mg)} = \frac{C_{inj} \left( \frac{\text{mg}}{\text{ml}} \right) \times \text{Elution Area (mA.U.ml)}}{100 \% \text{ saturation height (mA.U.)}} \quad (1)$$

To study the equilibrium binding capacity of the HF, the above protocol was repeated at a flow rate of 1 ml min<sup>-1</sup> using lysozyme solutions with concentrations between 0.05 and 100 mg ml<sup>-1</sup>. The equilibrium binding capacity was calculated using the Langmuir isotherm model for adsorption on a ligand surface [14], which states:

$$\theta = \frac{\alpha C_{inj}}{1 + \alpha C_{inj}} \quad (2)$$

where  $\theta$  is the fractional coverage of the surface,  $\alpha$  is the Langmuir adsorption constant and  $C_{inj}$  is the lysozyme concentration used at injection. The amount of protein being loaded,  $q$ , is related to the maximum equilibrium amount bound,  $q_{max}$ , by [4]

$$q = \theta q_{max} \quad (3)$$

Combining Eqs. (2) and (3):

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

allows  $q_{max}$  to be calculated. This represents the maximum equilibrium binding capacity of the HF membranes.

### 3. Results and Discussion

HF membrane cross-sections and internal surfaces were observed by SEM to identify differences in microstructure caused by the bore fluid used. All membranes displayed a sponge-like, macrovoid-free structure characteristic of phase separation by spinodal decomposition [15], as seen in **Fig. 2**. The membranes showed ring-like bands of distinct pore sizes within their cross-sections, suggesting regio-specific precipitation rates based on proximity to the coagulant, either in the coagulation bath or bore fluid, and the type of coagulant used. Dense skins of small pores (<1  $\mu\text{m}$  diameter) were found in regions where phase separation occurred fastest, such as in **Fig. 2(A)** at the inner and outer membrane surfaces where the polymer dope came into contact with nonsolvent (water) as soon as it was exposed to both the bore fluid and the coagulant bath. The largest pores, up to 50  $\mu\text{m}$  diameter, were found in regions where phase separation occurred slowest [16], as in **Fig. 2(C)** at the inner surface where the polymer contacted solvent NMP. At this surface, large pores formed due to the presence of NMP in the bore fluid reducing the flux of solvent out of the polymer solution [12]. Regions of intermediate pore size (5-15  $\mu\text{m}$ ) were found within the membrane cross-sections where phase separation occurred at intermediate rates. At the inner membrane surface, skins of pores <1  $\mu\text{m}$  formed when glycerol and water were used (**Fig. 2(A) & (B)**), whereas no skin was formed when NMP solvent was used, with pores reaching 50  $\mu\text{m}$  in diameter (**Fig. 2(C)**). The membrane pore surface areas available for functionalisation with SP groups were similar for all three HFs (water, 6.07  $\text{m}^2 \text{g}^{-1}$ ; glycerol, 6.62  $\text{m}^2 \text{g}^{-1}$ ; NMP, 6.84  $\text{m}^2 \text{g}^{-1}$ ).

The NMP-HFs and glycerol-HFs displayed fully formed central circular capillaries. The highly irregular inner membrane surface for water-HF seen in **Fig. 2(A)** may be attributable to a buckling of the innermost region due to firstly, a rapid precipitation of this region caused by

exposure to water bore fluid prior to the HF entering the coagulation bath, and then further volume shrinkage of the polymer-rich phase caused by precipitation of the remaining membrane once the HF passed into the coagulation bath [11,17].

To test the effect of HF microstructure on membrane binding capacity performance as cation-exchange adsorbers, lysozyme frontal analysis and Langmuir isotherm binding capacity studies were conducted on the three HF-SP variants. Blank runs with HF modules without surface modifications showed no protein adsorption. No membrane blocking or fouling was observed. The pressure drop across the modules increased linearly with flow rate (**Fig. 3(A)**). All pressure drops were below 0.35 MPa in the flow rate range (1-5 ml min<sup>-1</sup>) typically used for commercial chromatography membranes (Pall Mustang® Q and S membranes). However, in terms of HF membrane operation, these correspond to much higher lumen superficial flow velocities (6,000 cm h<sup>-1</sup> to 175,000 cm h<sup>-1</sup>). Low pressure drops at high superficial flow velocities indicate that HF MMCFs can be used for high throughput studies. As expected, equilibrium lysozyme binding capacities were independent of the flow rates tested (**Fig. 3(B)**).

A characteristic normalised breakthrough curve for lysozyme frontal analysis can be seen in **Fig. 4**. The NMP-HF-SP module was able to bind and elute the largest amount of lysozyme, while the water-HF-SP module bound and eluted the least. The NMP-HF-SP module displayed the best performance as it bound the greatest mass of lysozyme (19.6 ± 2.64 mg ml<sup>-1</sup> adsorbent) at equilibrium among the modules tested when loaded with a 5 mg ml<sup>-1</sup> lysozyme solution, as shown by its large elute peak in **Fig. 4(B)**. When loaded to near saturation and on subsequent elution, 88% of the protein bound was recovered in the eluent. The water-HF-SP module bound the lowest amount of lysozyme (1.99 ± 0.17 mg ml<sup>-1</sup> adsorbent), while the glycerol-HF-SP module bound an intermediate amount of protein (9.73 ± 0.87 mg ml<sup>-1</sup> adsorbent).

**Fig. 5** shows the mass of lysozyme bound to the HF modules based on different protein loading concentrations. The NMP-HF-SP module displayed the largest lysozyme maximum binding capacity ( $q_{max} = 40.3$  mg ml<sup>-1</sup> adsorbent), while the glycerol and water modules bound less, 21.2 mg ml<sup>-1</sup> adsorbent and 3.9 mg ml<sup>-1</sup> adsorbent respectively. The Langmuir isotherms fit the data well ( $R^2$  values > 0.90), indicating that the lysozyme binds to all HF membranes as a monolayer. All three HF modules had a lower lysozyme maximum binding capacity than the 19-capillary MMCFs produced with glycerol bore fluid (64.7 mg ml<sup>-1</sup> adsorbent) [5] and might be attributed to the geometry of the MMCF module whose 19 parallel capillaries may provide better analyte access to the functionalised membrane



surface. In addition, the NIPS process during MMCF manufacture would be expected to produce different microstructures depending on the membrane geometry used. The size and shape of the membranes greatly affect phase separation kinetics within the membrane. Compared to commercial membrane substrates whose binding capacities typically exceed 50 mg ml<sup>-1</sup> adsorbent (Pall Mustang® S), the HF membranes also have lower binding capacities. However, commercial membrane substrates are typically operated in a trans-membrane flow configuration, which may increase binding capacity.

In membrane adsorbers, the accessibility of the functionalised pore surface to analytes is key for good binding performance [18]. Since only axial convective flow is present in the HF module configuration tested, a more open pore structure at the inner membrane surface near the lumen is expected to provide better analyte access into the remaining porous mass. Smaller porosity near the lumen would reduce this access. The NMP-HF-SP module displayed the most open structure with the largest pores at the lumen surface. This module also had the highest equilibrium binding capacities at all tested loading concentrations, likely due to the NMP-HF-SP membrane presenting more accessible functional sites to lysozyme as a result of large porosity near the lumen. The small pores in the dense inner membrane surface of the water-HF-SP and glycerol-HF-SP modules are potentially the reason for the lower binding capacity evaluated. These dense skins offered a greater barrier to the analyte reaching and interacting with a large portion of the adsorptive surface.

#### **4. Conclusions**

In this study we have shown the importance of the bore fluid composition used during EVOH hollow fibre membrane manufacture to membrane microstructure and performance as a cation-exchange adsorber. Three bore fluids with varying solubility were used to produce HFs which were then functionalised into cation-exchange adsorbers. SEM micrographs revealed that NMP bore fluid produced a membrane with a skinless inner surface populated by large pores up to 50 µm in diameter. Glycerol and water bore fluids produced dense skins with much smaller pores at this inner membrane surface (<1 µm). Results of binding capacity studies indicated that NMP as an internal coagulant produced modules with the largest lysozyme maximum binding capacity, which corresponded with the fibres displaying the most open microstructure near the fibre lumen. This open microstructure caused by NMP bore fluid provided more accessible functional sites to lysozyme as an analyte. These findings can be further applied to optimise the inner surface microstructure in multi-capillary MMCFs by increasing bore fluid NMP concentration in order to obtain higher binding capacities and enhanced performance as chromatography substrates.

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## References

- [1] N.J. Darton, D. Darling, M.J. Townsend, D.J. McNally, F. Farzaneh, N.K.H. Slater, Lentivirus capture directly from cell culture with Q-functionalised microcapillary film chromatography., *Journal of Chromatography. A.* 1251 (2012) 236–9.
- [2] R. Ghosh, Protein separation using membrane chromatography: opportunities and challenges., *Journal of Chromatography. A.* 952 (2002) 13–27.
- [3] C. Charcosset, Review: purification of proteins by membrane chromatography, *Journal of Chemical Technology and Biotechnology.* 71 (1998) 95–110.
- [4] N.J. Darton, N.M. Reis, M.R. Mackley, N.K.H. Slater, Fast cation-exchange separation of proteins in a plastic microcapillary disc., *Journal of Chromatography. A.* 1218 (2011) 1409–15.
- [5] I. Mandal, M.J. Townsend, N.J. Darton, S. Bonyadi, N.K.H. Slater, A microporous walled micro-capillary film module for cation-exchange protein chromatography, *Journal of Membrane Science.* 466 (2014) 123–129.
- [6] B. Hallmark, F. Gadala-Maria, M.R. Mackley, The melt processing of polymer microcapillary film (MCF), *Journal of Non-Newtonian Fluid Mechanics.* 128 (2005) 83–98.
- [7] S. Bonyadi, M. Mackley, The development of novel micro-capillary film membranes, *Journal of Membrane Science.* 389 (2012) 137–147.
- [8] J. Qin, T. Chung, Effect of dope flow rate on the morphology, separation performance, thermal and mechanical properties of ultrafiltration hollow fibre membranes, *Journal of Membrane Science.* 157 (1999) 35–51.
- [9] J. Ren, T. Chung, D. Li, R. Wang, Y. Liu, Development of asymmetric 6FDA-2,6 DAT hollow fiber membranes for CO<sub>2</sub>/CH<sub>4</sub> separation: 1. The influence of dope composition and rheology on membrane morphology and separation performance, *Journal of Membrane Science.* 207 (2002) 227–240.
- [10] S. Deshmukh, K. Li, Effect of ethanol composition in water coagulation bath on morphology of PVDF hollow fibre membranes, *Journal of Membrane Science.* 150 (1998) 75–85.
- [11] S. Bonyadi, T.S. Chung, W.B. Krantz, Investigation of corrugation phenomenon in the inner contour of hollow fibers during the non-solvent induced phase-separation process, *Journal of Membrane Science.* 299 (2007) 200–210.

- [12] M. Rahbari-sisakht, a. F. Ismail, T. Matsuura, Effect of bore fluid composition on structure and performance of asymmetric polysulfone hollow fiber membrane contactor for CO<sub>2</sub> absorption, *Separation and Purification Technology*. 88 (2012) 99–106.
- [13] G. McCreath, R. Owen, D. Nash, H. Chase, Preparation and use of ion-exchange chromatographic supports based on perfluoropolymers, *Journal of Chromatography A*. 773 (1997) 73–83.
- [14] I. Langmuir, The Constitution and Fundamental Properties of Solids and Liquids, *J. Am. Chem. Soc.* 38 (1916) 2221–2295.
- [15] R.W. Baker, *Membrane Technology and Applications*, John Wiley & Sons, Ltd, Chichester, UK, 2004.
- [16] G.R. Guillen, Y. Pan, M. Li, E.M. V. Hoek, Preparation and Characterization of Membranes Formed by Nonsolvent Induced Phase Separation: A Review, *Industrial & Engineering Chemistry Research*. 50 (2011) 3798–3817.
- [17] H. Tanaka, T. Araki, Viscoelastic phase separation in soft matter: Numerical-simulation study on its physical mechanism, *Chemical Engineering Science*. 61 (2006) 2108–2141.
- [18] M. Ulbricht, Advanced functional polymer membranes, *Polymer*. 47 (2006) 2217–2262.

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**Fig. 1.** Schematic diagram of the NIPS extrusion platform used to fabricate hollow fibre membranes.

**Fig. 2.** SEM images showing cross-sectional microstructure in hollow fibre membranes manufactured with three bore fluids: (A) water, (B) glycerol and (C) NMP

**Fig. 3.** Effect of flow rate on (A) pressure drop,  $\Delta P$ , across the HF MMCF modules and (B) binding capacity of three HF-SP modules loaded to equilibrium. The mass of lysozyme bound per unit adsorbent volume is shown. Error bars represent the standard deviation of three replicates.

**Fig. 4.** Lysozyme breakthrough curves for HF membranes produced with three bore fluids (water, glycerol, NMP) and functionalised into cation-exchange chromatography modules via sulphonic acid surface modification (HF-SP). The modules were loaded to near saturation (A) with 5 mg ml<sup>-1</sup> lysozyme in 20 mM Tris-HCl buffer (pH 7.2) for at least 15 column volumes (column volume, CV, is defined as the total volume within the microporous walls and the central capillary, 1 CV = 0.14 ml), washed with Tris-HCl running buffer to remove unbound protein and eluted (B) with 1 M NaCl in Tris-HCl running buffer for 80 column volumes to remove mass adsorbed to the membrane surface. All flow rates used were 1 ml min<sup>-1</sup>. Protein absorbance at 280 nm and NaCl conductivity were measured.  $C/C_0$  represents

the UV absorbance of the running lysozyme concentration,  $C$ , normalised by the feed concentration,  $C_0$ .

**Fig. 5.** Ligand density model of HF modules. Lysozyme mass bound was measured at different lysozyme loading concentrations,  $C$ , and binding data was fitted to a standard Langmuir isotherm model. The dotted lines represent Langmuir isotherm equation fits and give the following maximum equilibrium binding capacities ( $q_{max}$ ): 40.3 mg ml<sup>-1</sup> adsorbent volume (NMP-HF-SP), 21.2 mg ml<sup>-1</sup> adsorbent volume (glycerol-HF-SP) and 3.9 mg ml<sup>-1</sup> adsorbent volume (water-HF-SP). Error bars represent the standard deviation of three replicates.











