Journal of Chromatography A, 1468 (2016) 64–72

Contents lists available at ScienceDirect

Journal of Chromatography A

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

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

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 31 August 2016
Accepted 2 September 2016
Available online 3 September 2016

Keywords:
Weak anion-exchange
Chromatography
Micro-capillary films
Membranes
DEAE

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 ± 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 sulphate 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.

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/m for MCF

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http://dx.doi.org/10.1016/j.chroma.2016.09.006
0021-9673/

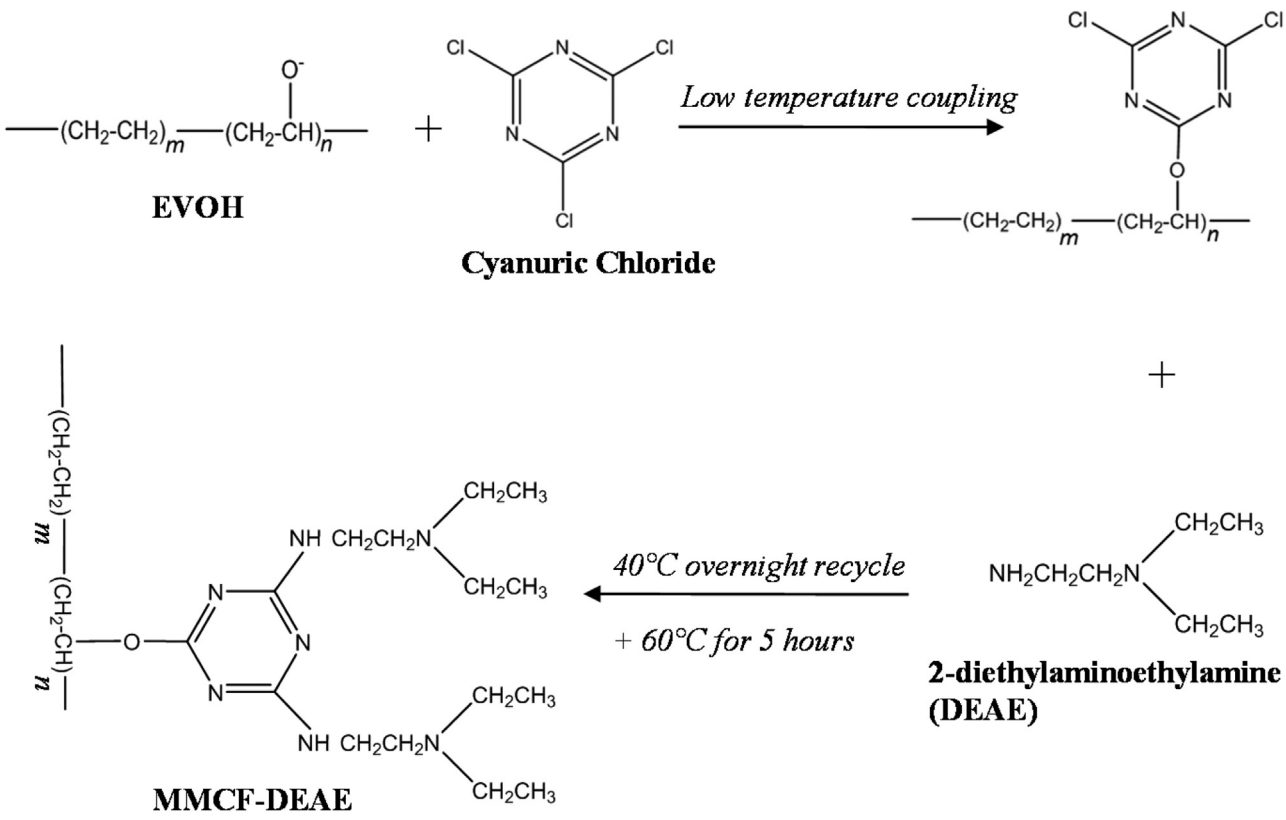

Fig. 1. Chemical route to modify MMCFs into weak anion exchangers using DEAE as the chemical ligand. Coupling of cyanuric chloride to the membrane was performed and maintained in ice cold condition until addition of DEAE.

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 (*N*MP), 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