Journal of Membrane Science 345 (2009) 177–182

Contents lists available at ScienceDirect

Journal of Membrane Science

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

Chromatographic separation of proteins using hydrophobic membrane shielded with an environment-responsive hydrogel

R. Huang, K.Z. Mah, M. Malta, L.K. Kostanski, C.D.M. Filipe, R. Ghosh*

Department of Chemical Engineering, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4L7, Canada

ARTICLE INFO

Article history:
Received 17 July 2009
Received in revised form 26 August 2009
Accepted 28 August 2009
Available online 4 September 2009

Keywords:
Hydrophobic interaction
Membrane chromatography
Hydrogel
Environment responsive
Monoclonal antibody

ABSTRACT

Environment-responsive membranes, suitable for carrying out hydrophobic interaction membrane chromatography (HIMC) were prepared by modification of a commercial hydrophobic microporous polyvinylidene fluoride (PVDF) membrane with a salt-responsive hydrogel composed of poly(N-vinyl lactam) cross-linked with bisacrylamide. These modified membranes were moderately hydrophobic in the presence of lyotropic salts but were quite hydrophilic in their absence. The membranes were characterized in terms of their mass gains after modification as well as their contact angles. These new membranes were found to be suitable for carrying out monoclonal antibody purification.

1. Introduction

Membrane chromatography which has shown significant promise in the purification of biopharmaceuticals gives fast separation due to the predominance of convective solute transport [1–6]. It is particularly suitable where large target molecules present at low concentration in the feed solution have to be purified [7,8]. The purification of monoclonal antibodies (mAbs) from cell culture media is an important potential application area for membrane chromatography [9–11].

Hydrophobic interaction membrane chromatography (HIMC) relies on the binding of proteins on synthetic environment-responsive membranes [12]. The media used for HIMC is different from that used in conventional hydrophobic interaction chromatography (HIC). HIC media is prepared by grafting hydrophobic ligands such as phenyl, butyl and octyl on a hydrophilic support material such as cellulose, agarose or cross-linked dextran [13–15]. HIMC media on the other hand consists of an environment-responsive membrane with tunable hydrophobicity, prepared by grafting smart polymers on a hydrophobic surface. In the presence of lyotropic salts, the polymer exists in a collapsed state and the membrane surface is largely hydrophobic whereas in their absence the polymer exists in an extended state and the membrane surface is therefore hydrophilic. In HIMC, the membrane being hydrophilic during elution, high recovery of bound protein can be obtained [16]. Moreover, the hydrophobic–hydrophilic change with these membranes in response to decrease in salt concentration is graded in nature and hence high-resolution separation can be anticipated [16,17]. The membranes used for HIMC in earlier studies, i.e. [12,16] were not specifically developed for chromatographic applications but were microfiltration membranes, surface pretreated by polymer grafting to make them less prone to fouling. While these membranes performed satisfactorily, it may be anticipated that membranes specifically designed and developed for chromatographic applications would perform better.

This paper discusses the development of environment-responsive hydrogel-coated membranes for HIMC. A hydrophobic microporous polyvinylidine fluoride (PVDF) membrane was coated with a hydrogel prepared by cross-linking vinyl-lactam monomers with bisacrylamide (see Fig. 1). In an earlier paper we discussed the coating of glass fiber micro-filters using similar hydrogels for preparation of membranes with tunable hydraulic permeability [17]. The presence of salt decreases the lower critical solution temperature (LCST) of the hydrogel leading to its collapse accompanied by expulsion of surrounding water molecules. The net surface hydrophobicity of the resultant membranes can be attributed to the collapsed hydrogel as well as to the patches of the supporting PVDF which get exposed in the process. Proteins such as antibodies can therefore bind on the membrane. When the salt is removed, the LCST of the hydrogel is increased, resulting in a hydrophilic membrane surface. The protein molecules bound to the hydrogel are desorbed and those bound to the previously exposed PVDF are pushed away by the swelling hydrogel. When the hydrogel is in its extended configuration, protein molecules are prevented from

* Corresponding author. Tel.: +1 905 525 9140x27415; fax: +1 905 521 1350.
E-mail address: rghosh@mcmaster.ca (R. Ghosh).

0376-7388/$ – see front matter.
doi:10.1016/j.memsci.2009.08.043

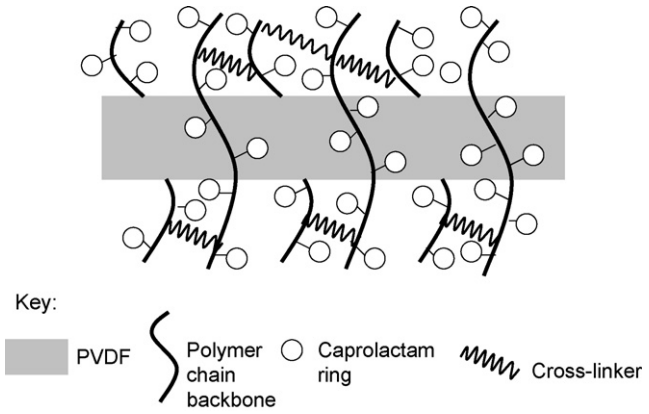

Fig. 1. Cartoon image of the hydrogel coated environment-responsive adsorptive membrane.

approaching the PVDF membrane due to entropic penalty. Fig. 2 shows how such switch between hydrophobic and hydrophilic states is utilized in HIMC.

The hydrogel-coated membranes were characterized in terms of their mass gains following modification and contact angle in the presence and absence of salt. Based on these, appropriate membranes were selected for HIMC. The binding of monoclonal antibody hIgG1-CD4 on the selected membranes was compared with that on commercial hydrophilized PVDF membranes, similar to those reported in [12,16]. hIgG1-CD4 is a humanized IgG1 type anti-CD4 mAb which has been shown to be effective in the treatment of refractory psoriasis and rheumatoid arthritis [18]. The effects of solution conditions on antibody binding were systematically examined. The feasibility of mAb purification from simulated mammalian cell culture media supernatant was also examined.

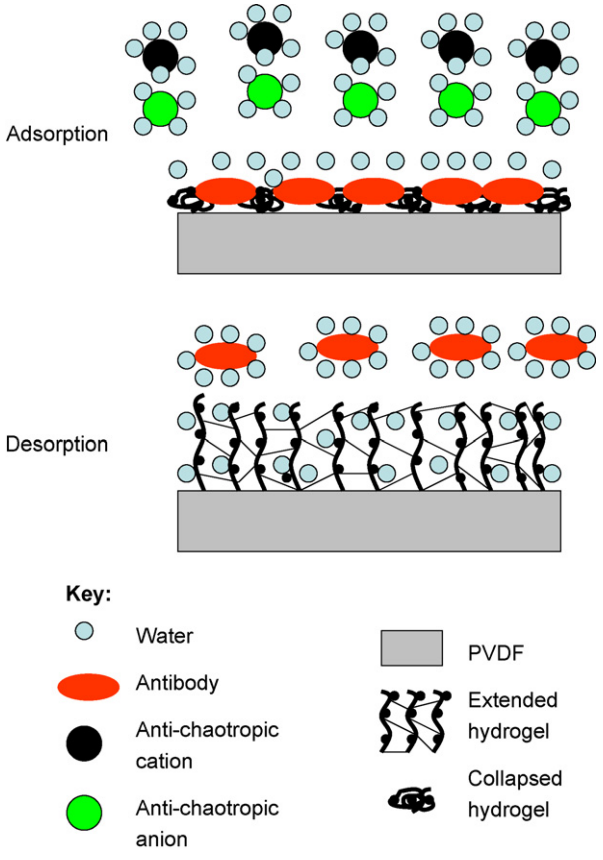

Fig. 2. Mechanism of antibody binding and desorption from the hydrogel coated environment-responsive membrane.

2. Experimental

2.1. Materials

Hydrophobic PVDF membrane (GVHP, 0.22 μm pore size, catalogue # GVHP04700) was purchased from Millipore, MA, USA. Hydrophilized PVDF membrane (GVPP, 0.22 μm pore size, catalogue # GVWP10050) was kindly donated by Millipore. Monomers N-vinyl-caprolactam (catalogue # 415464) and 1-vinyl-2-pyrrolidone (catalogue # V3409), cross-linker bisacrylamide (catalogue # 146072), photo-initiators diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide/2-hydroxy-2-methylpropiophenone (catalogue # 405663), serum-free CHO cell culture media (catalogue # C1707) and other laboratory reagents such as sodium chloride, sodium phosphate and acetone were purchased from Sigma–Aldrich, Oakville, ON, Canada. Isopropanol (catalogue # A451) used as liquid media for polymerization and cross-linking was purchased from Fisher Scientific, Ottawa, ON, Canada. Laminating pockets (product # 3202002) used to sandwich the membranes during polymerization were purchased from Grand & Toy, Vaughan, ON, Canada. Monoclonal antibody hIgG1-CD4 (batch # 10) was kindly donated by the Therapeutic Antibody Centre, University of Oxford, UK and was used as obtained. All buffers and solutions were prepared using high quality deionised water (18.2 MΩ cm) obtained from a Diamond NANOpure water purification unit (catalogue # D11931, Barnstead, Dubuque, IA, USA).

2.2. Membrane modification procedure

The GVHP membrane sheets were first soaked in acetone overnight to remove all organic extractables that could potentially affect membrane modification. The sheets were then dried at constant relative humidity (50%) and temperature (25 °C) for 8 h followed by soaking in a solution of the monomers, cross-linker and initiator in isopropanol for 10–15 min. The soaked membranes were taken out of the solution, placed inside individual laminating pouches and then put through a laminating machine (EAGLE35, General Binding Corporation, Don Mills, ON, Canada) to ensure that no air bubbles (which would potentially inhibit the polymerization reaction) were present. After lamination, the closed laminating pouches with the membranes sealed inside were placed in a UV chamber and irradiated with 360 W/m^2 UV light for 1 h to carry out polymerization. After this, the membranes were taken out of their respective pouches and extracted with deionised water at 70 °C for 3 days to remove un-reacted monomers and other leachables. The membranes were then washed with deionised water and left to dry at constant temperature (25 °C) and relative humidity (50%).

2.3. Membrane characterization

The membranes were weighed prior to and after modification and the percentage mass gain in each case was determined. The contact angle at different buffer conditions was measured by the sessile drop method with a contact angle goniometer (Model 100-00, Rame-Hart, Netcong, NJ, USA). The membrane samples were positioned on the measuring stage of the apparatus, and a 0.5 pL sessile drop of appropriate liquid was placed on the membrane followed by measurement of the contact angle. The angles on both sides of each drop were measured to check for symmetry. Candidate membranes for HIMC were selected based on their contact angles. The expansion and collapse of the hydrogel loaded on the membrane was demonstrated by change in transmembrane pressure in