Journal of Membrane Science 444 (2013) 157–163

Contents lists available at SciVerse ScienceDirect

Journal of Membrane Science

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

Mixed matrix membrane chromatography based on hydrophobic interaction for whey protein fractionation

Syed M. Saufi [a,*], Conan J. Fee [b]

[a] Faculty of Chemical and Natural Resource Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Kuantan, Pahang, Malaysia
[b] Biomolecular Interaction Centre and Department of Chemical and Process Engineering, University of Canterbury, Private Bag 4800, Christchurch 8020, New Zealand

ARTICLE INFO

Article history:
Received 31 January 2013
Received in revised form
2 May 2013
Accepted 7 May 2013
Available online 16 May 2013

Keywords:
Membrane chromatography
Hydrophobic interaction
Whey protein
Mixed matrix membrane

ABSTRACT

The mixed matrix membrane (MMM) concept is extended, for the first time, to produce a hydrophobic interaction chromatography (HIC) membrane using crushed Phenyl Sepharose™ (GE Healthcare Technologies, Uppsala, Sweden) resin and tested for use in whey protein fractionation. The HIC MMM had static binding capacities (membrane volume basis) of 18.419 ± 0.605 mg mL^{-1} for β-lactoglobulin, 45.850 ± 2.753 mg mL^{-1} for α-lactalbumin, 41.106 ± 9.004 mg mL^{-1} for bovine serum albumin and 42.467 ± 4.130 mg mL^{-1} for lactoferin in individual protein solutions. For flowthrough whey fractionation, the adsorption performance of the HIC MMM was similar to a HiTrap™ Phenyl (GE Healthcare Technologies) HIC column. However, the well-known high processing speeds and inherently low pressure drops of MMM chromatography may offer benefits over a conventional packed bed column.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Protein separation by packed bed column chromatography is common in downstream processing. However, limitations of packed bed column chromatography include a high pressure drop, relatively slow intra-bead mass transport, difficulty in column packing and complicated scale up procedures [1–5]. These limitations have led to the development of membrane chromatography.

Membrane chromatography, also known as membrane adsorption, uses an adsorptive membrane that carries specific functionality similar to a chromatography resin. Thus, it combines the principles of chromatography and membrane filtration in a single separation device. Membrane chromatography may be superior to column chromatography in terms of its low pressure drop, operation at high flow rate while maintaining adsorptive capacity, convective mass transfer properties, low clogging tendency and the ease of column packing and scale up [1–6].

According to the literature, to date the main separation chemistry utilized in membrane chromatography has been ion exchange, followed by affinity interactions [1] and there have been few reports on hydrophobic interaction-based membrane chromatographic separations. Alkyl (e.g. butyl and octyl) and aryl (e.g. phenyl) groups are representative ligands for hydrophobic interaction chromatography (HIC). The number of hydrophobic amino acid side chains on the protein surface (such as valine, tryptophan, phenylalanine, leucine), the ligand–polymer structure, the surface ligand density, the type and concentration of salt, pH of the medium and temperature influence the hydrophobic interaction separation of proteins. By manipulating the separation process parameters, it is possible to enhance the hydrophobic interaction between the protein and hydrophobic ligand to affect the separation of protein molecules [7]. In addition, HIC is an ideal "next step" after protein precipitation with ammonium sulfate ($(NH_4)_2SO_4$) or elution by high salt concentrations during ion exchange chromatography.

Kubota's group, [8–10] was among the first groups involved in preparing hydrophobic interaction membrane chromatography, using a series of polyethylene-based hollow fiber HIC membranes. In their work, a polyethylene base membrane was modified by radiation-induced graft polymerization with glycidyl methacrylate monomer and various types of hydrophobic ligands were attached. HIC membranes were prepared at different ligand density and reaction conditions. Phenyl [8,9] and butyl amine [9] membranes were prepared and tested with a single bovine serum albumin (BSA) protein. A binding capacity as high as 30 mg BSA g^{-1} membrane [9] was achieved, with repeated cycles of adsorption and elution being possible when 1 M NaOH regeneration was included between each cycle [9].

Arica et al. [11] prepared HIC membranes with phenylalanine as a hydrophobic ligand. Two main reactions were involved. In the first reaction, phenylalanine reacted with 2-methacrylochloride to produce a co-monomer of methacrylamidophenyalanine (MAPA). Then, a co-monomer of MAPA reacted with 2-hydroxyethelmethacrylate (HEMA) monomer by UV-initiated photopolymerization at different HEMA/MAPA ratios to produce a flat sheet HIC membrane. The HIC membrane was tested for γ-globulins adsorption and showed a maximum adsorption capacity of 2.37 mg γ-globulins g^{-1} dry membrane.

* Corresponding author. Tel.: +60 9 549 2823; fax: +60 9 549 2889.
E-mail address: smsaufi@gmail.com (S.M. Saufi).

0376-7388/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.memsci.2013.05.007

Suen's group prepared an inorganic HIC membrane based on an alumina membrane [12], modified with C8 and C18 alkyl chains, and a glass fiber membrane [13] modified with a few short-chain organosilicon derivatives. The alumina HIC membrane however, had a low protein elution recovery using a salt-free elution buffer, as normally applied to recover bound protein from HIC. To recover the bound lysozyme (LZY) and conalbumin from the alumina HIC membrane, 70% acetonitrile and 70% isopropanal, respectively, were necessary, which are harsh elution buffers not usually used in hydrophobic interaction chromatography because of their denaturing effects on the eluted proteins. Normally such buffers would only be used in reverse-phase chromatography, indicating that the membranes prepared were more hydrophobic than is normally consistent with HIC.

Commercial polyvinylidene fluoride (PVDF) microfiltration (MF) membranes may also function as HIC media in the presence of a high concentration $(NH_4)_2SO_4$ buffer, as demonstrated by Ghosh's group [14,15]. A PDVF MF membrane was able to bind monoclonal antibody at 2 M $(NH_4)_2SO_4$ [14] and human immunoglobulin (HIgG) at 1.5 M $(NH_4)_2SO_4$ [15]. More recently, it was found that this PVDF based HIC membrane could also be used to separate monoclonal antibody aggregates (i.e. monomer, dimer, trimer, etc.) very efficiently compared with size exclusion chromatography [16,17]. Based on the fact that the hydrophobicity of monoclonal antibodies increases with the degree of aggregation, a linear salt gradient elution in HIC membrane was able to discriminate different types of monoclonal antibody aggregates. Sartorius expanded its membrane adsorber product range recently by introducing a new Sartobind Phenyl™ membrane [18] as a hydrophobic interaction membrane.

The concept of mixed matrix membrane (MMM) has recently been recognized as a simple method to prepare adsorptive membranes [19–21]. The chemical activation of the base membrane and the coupling of ligands to the activated membrane, as normally practiced in preparing a membrane chromatography material, can be avoided using this method. Undesirable and irreversible changes in the membrane structure can thus be prevented. MMM chromatography is prepared by incorporating an adsorptive resin into a membrane polymer solution prior to membrane fabrication. The polymer/resin suspension can then be cast as a flat sheet membrane or spun into a hollow fiber membrane.

The MMM concept has successfully been applied to make a variety of anionic [20,21] and cationic [19,21,22] membranes for protein separation. However, to date, no one has reported the incorporation of a hydrophobic resin using this technique. Therefore, in the current study a hydrophobic interaction MMM using a commercial Phenyl Sepharose™ (GE Healthcare Technologies, Uppsala, Sweden) resin was developed. The static binding capacity of the resultant HIC-MMM was measured for the major whey protein components and tested for the feasibility of whey protein recovery. Based on the isocratic elution strategy, we were able to produce whey protein isolates depleted in β-Lac using HIC MMM. Other elution strategies could be developed to isolate different proteins but this was beyond the scope of the current work. The performance of HIC MMM was compared with a 1 mL HiTrap™ Phenyl FF (GE Healthcare Technologies) HIC column for whey protein fractionation and showed a comparable performance.

2. Materials and methods

2.1. Chemicals

Ethylene vinyl alcohol (EVAL) (a random copolymer of ethylene and vinyl alcohol) with an average ethylene content of 44 mol% was purchased from Sigma (St. Louis, MO, USA) and used without chemical modification to cast membranes. Dimethysulfoxide (DMSO) (Fluka, Steinheim, Germany) was employed as the EVAL solvent and 1-octanol (Fluka) as a non-solvent additive in casting solutions. Phenyl Sepharose Low Substitution (GE Healthcare) was used as an adsorptive particle in MMM.

β-Lactoglobulin (β-Lac), α-lactalbumin (α-Lac) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis) and used without further purification. LF of known purity was sourced from Tatua Co-operative Dairy Company Ltd., Morrinsville, New Zealand.

Sodium phosphate binding buffers were prepared from sodium phosphate dibasic heptahydrate (Sigma), sodium dihydrogen orthophosphate 1-hydrate (BDH Chemicals, Poole, England) and NaCl (BDH Chemicals). $(NH_4)_2SO_4$ for hydrophobic interaction buffer was purchased from BDH Chemicals. All buffer solutions were prepared using deionized (DI) water.

2.2. Preparation of mixed matrix membranes

Flat sheet mixed matrix membranes were prepared using a similar technique to that described in a previous publication [20] but using Phenyl Sepharose resin as the embedded adsorbent. The resin was ground and screened to obtain a particle fraction that passed through a 38 μm stainless steel mesh. Ground resin was added to the prepared polymer solution (15 wt% EVAL and 15 wt% 1-octanol in DMSO) to make a 20 wt% (relative to the EVAL content in the polymer solution) homogenous casting slurry solution. Conventional dry–wet casting process was then applied to make a flat sheet membrane. The membrane thickness after solvent exchange and the drying process was about 200 μm. The structure of the Phenyl Sepharose MMM was very similar to that described previously for our anion exchanger MMM [20].

2.3. Preparation of whey

Skim (0.05% fat) milk was purchased from a retail store and heated to 40 °C in a water bath. Casein was precipitated by adjusting the pH between 4.6 and 4.8 with 0.5 M HCl under stirring. The precipitated casein was discarded and the whey supernatant was centrifuged at 17,902 × *g* at 4 °C for 20 min using an Eppendorf Centrifuge Model 5810R. Whey was adjusted to pH 6 and $(NH_4)_2SO_4$ salt was added into this whey solution to give a salt concentration of 2 M. After the salt was completely dissolved in the solution, whey was centrifuged one more time at 17,902 × *g* at 4 °C for 20 min.

2.4. Binding capacity of phenyl Sepharose resin at different ammonium sulfate concentrations

About 30 mg (wet mass) of intact Phenyl Sepharose resin was used in scouting experiments to determine the optimum salt concentration in the binding buffer. $(NH_4)_2SO_4$ salt concentration varied from 0 to 2.5 M in sodium phosphate buffer pH 6 at 0.5 M interval, to give a set of six experiments. Each experiment was repeated for the individual proteins α-Lac, β-Lac, BSA and LF.

Pre-equilibrated resin was incubated with 1 mL of 1 mg mL^{-1} of individual protein solution in small Eppendorf tubes. Resin and liquid in these tubes were gently mixed by inversion for at least 12 h at room temperature, 22 °C. After binding, the tubes were spun in a centrifuge at 16,100 × *g* for 5 min and the equilibrium protein concentrations in the supernatants were assayed by spectrophotometer as described below. The bound protein was calculated by the difference between the initial protein content and the protein content in the equilibrium solution after binding.

Download English Version:

https://daneshyari.com/en/article/7022465

Download Persian Version:

https://daneshyari.com/article/7022465

Daneshyari.com