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High-speed protein purification by adsorptive cation-exchange hollow-fiber cartridges

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ABSTRACT

Novel cation-exchange adsorptive membranes were assessed according to their protein adsorption capacity and permeation flowrate. Maximum static adsorption capacities for the three main egg-white proteins, lysozyme, ovoalbumin and conalbumin, were 140, 88 and 66 mg/ml, respectively. However, membranes showed an inverse relationship between permeation flowrate and static protein adsorption capacity. Two size cartridges (membrane volume of 0.42 and 3.5 ml) were built using the selected membrane. An adsorptive cross-flow cartridge was tested to recover and purify lysozyme from an egg-white solution. Breakthrough curves developed using a pure lysozyme solution showed a dynamic-to-static capacity ratio of 0.6, which was reduced to 0.4 during lysozyme recovery from egg-white solution in cross-flow mode. Total process cycle for the enzyme recovery and purification was in the range of 10–15 min for both cartridges. In both cases high-purity lysozyme (95%) was recovered with a productivity of 150 g/(l h) and no size-exclusion effect was detected.

1. Introduction

Liquid-chromatography is the most widely used method for protein recovery and purification as it is a robust and efficient technique. Nowadays, affinity and pseudo-affinity adsorbents are increasingly applied due to their high selectivity [1]. However, different factors such as ligand stability, cost, toxicity and adsorptive capacity, reduce their use in protein purification at process scale. As a consequence, ion-exchange chromatography is still extensively applied in large-scale protein purification.

In addition to the development of new synthetic adsorptive ligands, improvement in liquid chromatography is also coming from new supports. In this area, adsorptive membranes and monoliths are able to transport solutes by convection or facilitated diffusion, thus cutting down process time [2,3]. In many cases, the fast separation process can improve yield by reducing the chance of proteolysis of the target protein.

Since the original work of Brandt et al. [4], affinity membranes have shown high process fluxes and mechanical resistance for capturing target proteins from highly diluted feedstocks [5–7]. Ion-exchange adsorptive membranes were further developed and commercialized but their utilization on protein recovery is highly dependent of further improvement in their protein adsorption capacity and hydrodynamics [8]. Recently they were successfully applied to remove contaminants, like DNA, host cell proteins, protein A leakage, where binding capacity is not a limiting factor [9].

However, high capacity membranes are still required in order to be a competitive technique for protein recovery and purification steps. One way to improve adsorptive capacity is through the making of a three-dimensional hydrogel on the internal membrane surface, in the sub-micron size range, where ligands are immobilized. To reach this high capacity property, two main different approaches were described: (a) anchorage of pending polymers [10,11] and (b) in situ grafting polymerization techniques [12,13]. Grafting polymer chains allow multilayer protein adsorption, as it has been demonstrated in several reports [13,14]; i.e. poly(ethylene)-DEA hollow-fiber membranes prepared by radiation-induced graft polymerization can be loaded with up to 37-folds higher capacity than the monolayer adsorption capacity of the enzyme urease [15].

Therefore, macroporous ion-exchange adsorptive membrane could be an alternative to packed beaded chromatography for proteins adsorption. These are directly transported by convection to the ligand onto the inner surface of the membrane at low operating pressure, making the adsorption rates faster while tentacle configuration ensures a high protein capacity. However, methacrylate-based grafted membranes are highly sensitive to environmental conditions, such as pH or ion strength, changing

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drastically their permeation flux properties [16]. Up to this moment, chemical control of the grafted chain shrinking and stretching motion was not possible.

In order to reduce this hydrodynamic drawback, flat-shape adsorptive membranes have the chance to stack one on top of each other, as is the case of commercial ion-exchange modules. Nevertheless, membrane module is much closer to a monolithic column than to a cross-flow membrane cartridge. In the case of hollow-fiber shape membranes, stable permeation flux property is a critical parameter for the successful use of this technology in downstream processing of proteins.

In this study adsorptive hollow-fiber membranes, synthesized by grafting copolymerization of methacrylic monomers, were characterized considering their hydrodynamic and adsorptive properties. Adsorptive cartridges of two sizes were built and used to purify lysozyme from a real feedstock such as egg-white protein solution. Considering lysozyme is only 3% of the total protein amount in egg-white, its purification is a good example of protein recovery process in view of the fact that a large amount of proteinaceous raw material has to be processed in order to get a small amount of product.

2. Experimental

2.1. Materials

Polysulfone hollow-fiber microfiltration membranes were a kind gift from A/G Technology Co., Needham, MA, USA, (currently GE Healthcare) and grafted with methacrylate-based monomers according to the simultaneous grafting technique previously described [13]. Glycidyl methacrylate (GMA) and diethyleneglycol dimethacrylate (DEGMA) were purchased at Sigma–Aldrich Co., St. Louis, USA, and used as received. GMA and DEGMA were used as monomers (reactive and cross-linker monomer, respectively) in the grafting polymerization step. Monomer solutions with 1% (v/v) GMA and increasing DEGMA concentration from 20 to 80% respect to GMA were used for the synthesis of grafted membrane (type A: 80%; type B: 60%; type C: 40%; type D: 20%). Plain membrane has a nominal 0.65 μm internal pore diameter and a nominal 80% porosity. Inner and outer diameters were 0.75 and 1.25 mm, respectively.

Lysozyme (L-6876), ovoalbumin (A-2512) and conalbumin (C-0755) were from Sigma–Aldrich Co., St. Louis, USA. All other reagents were AR grade.

Cation-exchange hollow-fiber membranes were synthesized from the epoxy-grafted membranes by ring-opening reaction with sodium sulfite as previously described [17]. Briefly, hollow fibers were incubated in sodium sulfite/isopropyl alcohol/water (10/15/75; w/w/w) at 37 °C overnight. A following treatment with sulfuric acid 0.5 M at 80 °C 2 h was done to hydrolyze the residual epoxy groups. Sulfonic group density was measured by titration.

Two cartridge sizes were build: Size 1, eight cation-exchange hollow-fiber membranes were potted in a housing laboratory cartridge (effective membrane length was 6.75 cm and total membrane volume, 0.42 ml; and Size 2, seventh five cation-exchange hollow-fiber membranes were potted in a housing laboratory cartridge (effective membrane length was 6 cm and total membrane volume, 3.5 ml). Four openings, two on the lumen side and two on the shell side were made with tubing nipples.

Measurement of the static maximum protein capacity was carried out by soaking small pieces of membrane into tubes containing 1 ml of protein solution (1–2 mg/ml) in the adsorption buffer. A 30 mM sodium phosphate buffer, pH 7.0, was used as adsorption buffer for lysozyme, and a 50 mM sodium acetate, pH 4.0, for ovoalbumin and conalbumin. The suspension was gently shaken overnight at 20 °C to enable the system to reach its equilibrium. After incubation, protein concentration was quantified spectrophotometrically at 280 nm. Protein concentrations were calculated using the adsorption coefficients 2.42, 0.628 and 0.936 ml/(g cm) for lysozyme, ovoalbumin and conalbumin, respectively. Protein adsorption capacity was determined from the difference between initial and final protein concentrations and referred per membrane volume.

Pure water flux of the sulfonic membrane was measured with a dead-end constant pressure apparatus similar to one previously described [12]: A 10-cm long hollow fiber was positioned in a U-shaped configuration and filtered water or sodium chloride solutions were forced to permeate outwards at a constant pressure of 1 bar. Hydraulic permeability was calculated as the flowrate divided by the membrane area.

Adsorption breakthrough (BT) experiments were performed with a Gilson ProTech (Villiers le Bel, France) low-pressure system. The cartridge was fed with a 1 mg/ml lysozyme solution, in the dead-end flow mode at 10 ml/min. The lumen side was used as an inlet and the shell sides as an outlet for permeate solution. The permeate absorbance at 280 nm was measured on-line.

Capacity under dynamic conditions ($Qdin^{10\%}$) is defined as the amount of lysozyme adsorbed per membrane volume when the permeate solution reaches the 10% of the concentration of feed solution (as was determined from BT experiments). BT curves with lysozyme solutions were normalized using the T parameter. T is defined as the ratio between the mass of lysozyme in the feed loaded at time t and the mass of lysozyme eluted from the full saturated cartridge.

The egg-white solution was prepared according to the following procedure [18]: fresh egg-white (approximately 30 ml) was diluted with 6 volume 20 mM sodium acetate buffer, pH 4.8. After an overnight incubation at 5 °C, the pH of the solution was adjusted to 6.5 by using a 3 M sodium phosphate buffer, pH 9.0. Then the solution was filtered to remove the insoluble.

Lysozyme concentration in egg-white solutions was determined by measuring its lytic activity on *Micrococcus lysodeikticus* cells [19]. One lysozyme unit is defined as the amount of enzyme that causes a decrease of 0.001 units/min in absorbance at 540 nm, at pH 7.0 and 30 °C, by using a suspension of *M. lysodeikticus* (1 mg/ml) as substrate, in 1 ml of reaction mixture.

The purification process was performed in four sequential steps: loading, washing, elution and regeneration, using a Watson-Marlow 501/R (Falmouth, Cornwall, UK) peristaltic pump. Absorbance at 280 nm and lysozyme activity were measured off-line.

2.1.1. Loading

Before loading, the membrane was equilibrated by passing 10 ml of 30 mM sodium phosphate buffer, pH 6.5. Egg-white solution was processed in cross-flow mode. Approximately 1/10 of the fluid was filtered while the solution that had failed to pass through the membrane (9/10) was recycled.

2.1.2. Washing

Washing was carried out in one step by pumping distilled water in cross-flow mode until permeate absorbance at 280 nm was under 0.01.

2.1.3. Elution

For protein elution, a 30 mM sodium phosphate buffer, pH 6.5, 1 M NaCl was pumped through the membrane in the dead-end mode.

The regeneration step was performed in a similar way to the washing step.