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Optimization of lactoferrin and bovine serum albumin separation using ion-exchange membrane chromatography

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ABSTRACT

Lactoferrin (LF), which is a high value minor whey protein, has recently received extensive attention from research scientists and industry due to its multifunction and potential therapeutic applications. In this study, the separation of two similar-sized proteins: bovine serum albumin (BSA) and LF was investigated using strong cation and anion exchange membrane chromatography (MC). Single protein and BSA–LF mixture adsorption were performed on Sartobind Q75 and S75 at pH between the LF and BSA isoelectric points. Identical breakthrough curves were obtained for both single protein and binary protein mixture, which suggests that there is no protein adsorption competition at the binding sites. The process optimization was further studied to yield optimum buffer and operating conditions. The highest BSA flux per membrane area (728.00 $gm^{-2}\,h^{-1}$) was obtained using 100 mM phosphate buffer solution at pH 6.0 on the cation exchange membrane, whereas LF was bound to the membrane with the dynamic binding capacity at 10% breakthrough ($DBC_{10\%}$) of about 60 mg. On the anion exchange membrane, LF was collected in the effluent at the flux of 287.46 $gm^{-2}\,h^{-1}$ using 5 mM phosphate buffer at pH 6.0, while BSA was retained on the membrane with $DBC_{10\%}$ equal to 60.96 mg. The combination effect between pH change and hydrophobic interaction improved the eluted protein mass for both anion and cation exchangers. Furthermore, the completed separation cycle was operated with the Sartobind S75 device with a short process time of 34.19 min and optimal LF productivity over 2628.84 mg $mL^{-1}\,h^{-1}$. This study confirms the advantage of MC for the separation of biomolecules with similar molecular weight and different isoelectric points, such as BSA–LF mixture separation. This fast and effective protein separation method could be applied at an industry scale.

1. Introduction

Lactoferrin (LF) is a metal-binding glycoprotein, which was discovered over 50 years ago as red protein in bovine milk. The isolation of this molecule from both human and bovine milk was first achieved using cation exchange chromatography on traditional resin-based column [1,2]. LF is a well-known multifunctional or multi-tasking protein. Many important roles such as immunoregulatory, anti-bacterial, anti-virus, anti-parasitic and anti-inflammatory activity have been reported [3–5]. The wound healing, which is a complex biological process can be promoted using this protein [6]. In addition, LF has been proved to function as an anti-infective agent and prevent the outbreak of infections. It makes this molecule and its derivatives very promising tools for health or nutritional applications [7]. LF is found in whey as a high value minor protein with bovine serum albumin (BSA) and immunoglobulins [8,9].

Membrane separation and chromatography are the most widely used methods for LF isolation from bovine milk and whey. Membrane separation processes provide key advantages for whey fractionation as they do not include adsorption and elution steps, and avoid costs for chromatographic material, buffers and effluent disposal. However, membrane filtration is usually not effective in separating similar size proteins, such as LF and BSA, and other whey proteins. Several authors have reported the separation of LF from other proteins and whey. For example, Nyström et al. [10] investigated the fractionation of several proteins with molecular weight between 15 kD and 80 kD. The best pH value for fractionation was such that one protein had its isoelectric point at this pH, and passed through the membrane, while the other one was held back in the retentate because of charge repulsion with the membrane. In particular, LF was purified at low pressures while BSA was totally retained. However, at higher pressures, the selectivity was low. Similarly, Almécija et al. [11] reported LF isolation

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from whey using a 300 kDa tubular ceramic membrane. The effect of pH on LF selectivity was investigated in a continuous diafiltration mode. The best resolution was achieved at pH 5 and 10, where LF was obtained in the permeate and in the retentate, respectively, with α-lactalbumin and β-lactoglobulin. Other strategies have been investigated to overcome the limitations associated with LF separation by membrane filtration. For example, Brisson et al. [12] used charged membranes and electrically-enhanced cross-flow microfiltration. The electrical field played an important effect on protein transmission. However, electrolytic reactions occurring at the electrodes/solution interface had a negative impact on the protein separation. Ndiaye et al. [13] evaluated the feasibility of separating LF from whey solution using electrodialysis with an ultrafiltration membrane of 500 kDa. The highest LF migration rate was obtained at pH 3.0 with a migration yield of 15%. However, the selectivity of the technique decreased in whey solution due to simultaneous migration of β-lactoglobulin and other whey proteins. Valiño et al. [9] investigated the separation of BSA and LF using charged ultrafiltration membranes. Using an unmodified neutral membrane at pH 5.0 (isoelectric point of BSA), LF was completely retained, and BSA passed in the permeate at a maximum flux of 30.31 g m^{-2} h^{-1}. By contrast, BSA was completely retained by the negatively charged membrane at pH 9.0 (isoelectric point of LF), and LF was recovered at a maximum flux of 1.07 g m^{-2} h^{-1}.

Conventional chromatography is the most widely used method for protein recovery and purification as it is a robust and efficient technique. Many studies have reported LF isolation using micro-sized resins as a stationary phase. Different chromatographic modes have been tested such as cation exchange [14–18], affinity [19] and hydrophobic interaction [20]. However, conventional chromatographic processes show several disadvantages, since large volumes and high protein concentrations in whey may cause fouling of columns, long cycle times, large pressure drops and complicated process control [21,22]. Other stationary phases have therefore been tested as possible alternatives to resins such as mixed-matrix membranes (MMMs), monolithic columns, and chromatographic membranes.

MMMs are prepared by incorporating an adsorptive resin into a membrane polymer solution prior to membrane preparation [23]. The polymer/resin suspension is then cast as a flat sheet membrane or spun into a hollow fiber membrane. The MMM concept has been successfully applied to the preparation of anionic, cationic, anionic/cationic hybrid membranes for protein separation. MMM combines the properties of membrane techniques (easy scale-up, low pressure drop) with column chromatography (high binding capacity, high recovery) [23]. Several MMMs have been applied to the recovery of LF from whey. For example, Saufi and Fee [24] developed a cationic MMM for recovery of LF from bovine whey by embedding SP Sepharose™ cation exchange resin into an ethylene vinyl alcohol polymer based membrane. The separation was operated in cross-flow mode and recycling both permeate and retentate into the feed, to minimize fouling and enhance LF binding capacity. The system resulted in a constant permeate flux equal to 100 L m^{-2} h^{-1} and a high LF recovery of 91%, with high purity. A disadvantage of MMM could be the negative effect of flow rate on separation. For example, Avramescu et al. [23] reported a lower separation factor of 30 between BSA and bovine hemoglobin using MMM, when the filtration flux per membrane area increased from 10 to 20 L m^{-2} h^{-1}. This effect was due to the decrease of the efficiency of adsorptive sites with flow velocity.

Another development in chromatography is the use of macroporous monolith columns. A monolith is a single piece of highly porous material characterized by a highly interconnected network of channels with a diameter in the range of 10–4000 nm [25]. Therefore, mass transport in monoliths is mainly based on convection. The use of short monolithic columns enhances the speed of the separation process and reduces the backpressure, unspecific binding and product degradation, without reducing resolution. In addition, the lack of void volume eliminates the turbulent void flow that contributes to molecular shear in conventional resin columns. Some studies have reported the use of monolithic columns for LF and whey protein separation. For example, Noppe et al. [26] covalently coupled phage clones expressing a peptide with high binding affinity for LF to a macroporous poly(dimethylacrylamide) monolithic column. The large pore size of the macroporous monolith makes it possible to couple the long phages as ligands without any risk of blocking the pores. Using this affinity support, LF was purified from human skimmed milk with purity higher than 95%, in one step. In another application, Etzel and Bund [27] purified whey protein-dextran conjugates from a feed solution also containing un-reacted protein and dextran using either a cation exchange packed bed column or a tube monolith. Binding capacities were similar for both monolith and beaded column (4–6 mg mL^{-1}). However, the monolith was operated at a 48-fold higher flow rate, which gave a 42-fold higher productivity, at the expense of a somewhat lower conjugate purity.

Membrane chromatography (MC) is a well-established technique for protein purification [22,23,28]. It is based on the integration of membrane filtration and liquid chromatography into a single-step operation. The advantage of MC over conventional resin chromatography is mainly attributed to the shorter diffusion times, as the interactions between molecules and active sites in the membrane occur in convective through-pores rather than in stagnant fluid inside the pores of the adsorbent particles. Therefore, MC has the potential to operate both at high flow rates and for use of large biomolecules with small diffusivities, reducing biomolecules degradation and denaturation. Low pressure drops associated with high flow rates, as compared to packed bed chromatography, reduce buffer usages [22]. Fractionation of whey proteins by MC has been reported in several studies. For example, LF and lactoperoxidase (LP) were isolated from sweet cheese whey using cation exchange MC in an axial flow configuration [21]. LF was eluted in a three-step elution process (0.1 M NaCl, 0.2 M NaCl, 1 M NaCl), which led to a LF fraction of about 95% purity. The cationic MC was then upscalable from 15 cm^2 to 4 m^2 scale with a recovery yield for LF of more than 90%. However, it was observed that when increasing the flow rate from 3 to 15 mL min^{-1}, the binding capacity decreased from 0.6 to 0.3 mg cm^{-2}. Similarly, Chiu and Etzel [28] extracted LF and LP from whey using cation exchange MC devices with surface area of 100 cm^2 and 790 cm^2. The purification process was operated repeatedly with 12 cycles consisting of loading of whey, washing, stepwise elution and washing. Recovery was unaffected by scale-up and repeated cycling, and was 50.0% and 73.0% for LF and LP, respectively. For the complete fractionation of whey proteins in a two-step process, Voswinkel and Kulozik [29] used ion-exchange radial flow devices with improved fluid distribution (anion and cation exchanger Sartobind Nano and Sartobind 150-mL). First, β-lactoglobulin and BSA were bound to the anion exchanger at pH 7.0. Second, the permeate obtained in the first step was introduced into the cation exchanger at pH 4.8. LF, LP and immunoglobulin G bound while β-lactalbumin passed through the membrane. The scalability of the process was investigated with the radial flow column and 50-fold membrane area. At lab scale, 97% LF purity was obtained with a yield of 66%. However, at pilot scale, LF purity and yield decreased to 89% and 39%, respectively. The authors concluded that further investigations were needed to increase the LF yield in the cation exchanger step. Affinity MC has also been tested for LF purification from bovine whey and colostrum. For example, Wolman et al. [30] modified a hollow fiber polysulfone membrane by grafting a glycidyl methacrylate/dimethyl acrylamide copolymer and attaching the