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Modified Polyethersulfone (PES) Ultrafiltration Membranes for Enhanced Filtration of Whey Proteins

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Modified Polyethersulfone (PES) Ultrafiltration Membranes for Enhanced Filtration of Whey Proteins

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Abstract: Application of membrane technology to whey protein separation is an interesting development that has seen growth in recent years. In particular, modification of existing membranes to impart charge properties on the membrane surface or in the pores has been shown to improve membrane selectivity, product purity, and throughput of protein solutions. This paper focuses on exploring the effects of membrane charge and solution pH on filtration of the major whey proteins α-lactalbumin (14.1 kDa) and β-lactoglobulin (18.4 kDa) using functionalized PES membranes. The membranes have an open pore structure containing charged sulfonated grafted polymer chains that allows for greater protein retention. The modified membranes were synthesized by polymerization of styrene in the membrane pores followed by sulfuric acid treatment of the resulting polystyrene grafts. The charged membrane gave a calculated selectivity of five times better than the raw membrane at pH 7.2 based on data from single protein transmission experiments. The enhanced selectivity of the tailor-made membrane was due to increased retention of β-lactoglobulin due to a reduction in molecular sieving combined with electrostatic repulsion between negatively charged β-lactoglobulin and the negatively charged membrane.

Keywords: Polyethersulfone membrane, whey protein separation, protein filtration, selectivity, α-lactalbumin, β-lactoglobulin

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INTRODUCTION

There has been considerable interest in the separation of whey proteins into individual components, as each whey protein possesses unique functionalities and properties which are of value for the food, the pharmaceutical, and other related industries (1–4). Table 1 provides a summary of some important physical properties of the major whey proteins found in milk. The most abundant whey protein, β-Lg, is well-suited to confection production because of its good gel formation properties. The whey protein α-La has been found to be an important ingredient of infant formula because of its high tryptophan content.

Various methods have been studied for whey protein fractionation, including selective precipitation using salts, pH, and/or high temperatures (5–10), ion-exchange chromatography (11–13), and affinity binding (14–16). Ion-exchange chromatography is one of the more advanced fractionation techniques, and is currently the technique of choice for large-scale purification of whey protein isolate when paired with ultrafiltration (17). It is capable of producing fairly good separation and protein purity if problems associated with resin regeneration, and water and chemical consumption can be controlled or minimized. Other techniques have limited implementation on a commercial scale due to factors such as high cost, protein contamination and degradation, and poor selectivity. In recent times considerable interests have focused on the development of membrane systems as a potential alternative for fractionation of complex protein mixtures encountered in many biotechnological, food processing, and biomedical applications (18–20).

Commercially, membrane processes have been successfully used in dairy processes and applications to reduce the fat and lactose content of milk (2). Use of membrane technology for fractionation of whey proteins has been studied by several authors (21–26). For example, Roger et al. (25) patented a two-stage ultrafiltration process for obtaining an α-La enriched product from raw acid whey or mixed whey. However, the final product only had an α-La to β-Lg ratio of 2:1. In later studies, Cheang and Zydney (24) were able to obtain an α-La purification greater than 10-fold for whey protein isolate using two-stage high performance tangential flow filtration (HPTFF).

Table 1. Characteristics of major whey proteins (2)

Protein	Concentration [g/L]	Molecular weight [g/mol]	Isoelectric pH
β-Lactoglobulin (β-Lg)	2.7	18,362	5.2
α-Lactalbumin (α-La)	1.2	14,147	4.5–4.8
Bovine serum albumin (BSA)	0.4	69,000	4.7–4.9
Immunoglobulin (Ig)	0.65	150,000–1,000,000	5.5–8.3

For the most part, low molecular weight cut-off (MWCO) ultrafiltration (UF) membranes are used for whey protein separation (α-La and β-Lg). The molecular weights (α-La = 14.1 kDa and β-Lg = 18.4 kDa) are quite close thus it is difficult to effectively separate them based on size exclusion alone. Therefore, another characteristic of the proteins must be used to enhance separation. For example, there is some variation in the isoelectric points (pI) of these proteins (recall Table 1). The values are quite close; however, careful manipulation of the solution pH would permit enhanced electrostatic repulsion of the more negatively charged β-Lg by a negatively charged membrane.

A number of authors have examined the effect of protein isoelectric points on protein separation. For example, Saskena and Zydney (18) used an unmodified polyethersulfone membrane to obtain a 20-fold increase in selectivity for the filtration of BSA and IgG by simple adjustment of pH and ionic strength resulting in electrostatic contributions to both bulk and membrane transport. The observed protein selectivity was thus a function not only of the membrane pore size but also of the filtrate flux and solution condition. Additionally, modified membranes possessing an overall charge have been shown to enhance protein selectivity when used in conjunction with the appropriate selection of pH and ionic strength of the protein solution. For example, Lucas et al. (26) achieved a selectivity close to 10 for α-La separation from whey protein concentrate using a chemically modified inorganic membrane bearing positive charges. The pores of a 150 kD MWCO inorganic membrane were coated with polyethyleneimine (PEI) to impart positively charged sorption groups to the material. The observed improvement in selectivity was due to adsorption of the more highly charged β-Lg and less hindered transport of α-La.

In this work, polyethersulfone (PES) ultrafiltration membranes are rendered negatively charged by the formation of sulfonated polystyrene grafted chains within the membrane pores as illustrated in Fig. 1. Functionalization of the membrane in this manner not only results in a decrease in

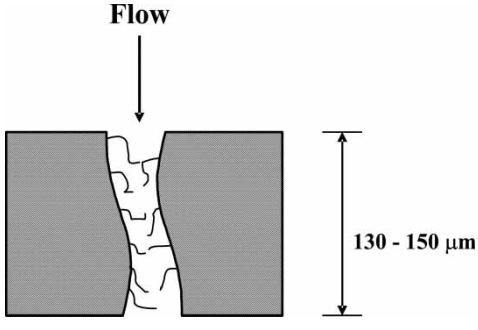

Figure 1. Schematic showing polymer grafted chains in a 100 kDa PES ultrafiltration membrane pore. The grafted polymer chains contain immobilized sulfonic acid ion-exchange groups.

membrane pore size, and hence molecular weight cut-off, but also can result in rejection of negatively charged proteins due to charge repulsion. For our studies, the modified membrane is used to examine the transmission/rejection of charged whey protein in solution and is quantified by the sieving coefficient. The sieving coefficient is affected by the relative size of the molecules with respect to the size of the membrane pores, as well as the protein and membrane charge.

EXPERIMENTAL

Materials

Commercially available polyethersulfone (PES) ultrafiltration membrane (100 kDa) from Millipore Corporation (Bedford, MA) was used as the starting material. Sulfuric acid solutions of 0.5 N were prepared from 99% analytical grade sulfuric acid (Fisher) and deionized water. The 0.4 N sodium hydroxide solutions were prepared from 98% laboratory grade sodium hydroxide crystals and deionized water. The monomer-inhibited styrene (99.8% purity) from Fisher Scientific was converted into an inhibitor-free styrene monomer by adsorption of the inhibitor on alumina powder, supported on glass wool, in a chromatographic column. Toluene was used as the solvent for styrene polymerization. Methanol was used as the sample diluter for analysis of styrene-toluene permeates by UV-Visible Spectroscopy. All solvents were analytical grade. The whey protein samples, BioPURE α-La (90.8%) and BioPURE β-Lg (95%), were supplied by Davisco Foods International Inc. (Eden Prairie, MN) and were used as is with no further purification.

Preparation of Modified Membrane

Functionalized membranes were prepared using a similar approach to Shah et al. (27). Sulfonic acid groups served as the initiator for cationic polymerization of styrene. The sulfonic acid groups were formed by convective permeation of 0.5 N H_2SO_4 through a 47 mm diameter PES membrane for 3 hr. A constant pressure drop of 1 bar was maintained across the membrane. The membrane was rinsed with deionized water and allowed to dry in air for 1 hr before permeation with 0.4 N NaOH to determine the extent of sulfonation. The ion-exchange capacity of the membrane was quantified by the amount of regenerated sodium ions in acid solution by atomic absorption spectroscopy.

The polymerization reaction was carried out by permeation of inhibitor-free styrene (5v%) in toluene at a constant pressure drop of 1 bar for approximately 3.5 hr including one recycle of the permeate. The feed cell was rinsed

thoroughly with methanol and water to remove residual styrene and toluene. The immobilized polystyrene grafts formed in the membrane pores were activated by convective permeation of 0.5 N H_2SO_4 solution for three hours. All reaction solutions were fed from a nitrogen-pressurized feed cell into a stainless steel membrane holder. Permeate was collected at atmospheric pressure, and all experiments were performed at room temperature.

Ion-exchange Capacity Study

The ion-exchange capacities of the raw and functionalized membranes were quantified by elemental analysis of regenerated sodium ions in sulfuric acid solution using atomic absorption spectroscopy (Varian 220 FS). In each case, the membrane was treated with 0.4 N NaOH by convection for approximately 3 hr at a pressure drop of 1 bar. It was then rinsed with deionized water to remove any non-specificially bound sodium in the membrane pores. Finally, the membrane was retreated with 0.5 N H_2SO_4 for 3 hr to regenerate sodium ions from the pores. The standard calibration curve was prepared by dilutions of a 2 mg/L reference standard of Na^+ ions in de-ionized water. The amount of sodium recovered is used to determine the number of available sulfonic acid groups in the membrane.

UV-Visible Spectroscopy Analysis

Samples of styrene-toluene solution were diluted 1:1250 with pure methanol in order to analyze the styrene concentrations in the feed and permeate after polymerization. The styrene concentration of each sample was quantified by UV-Visible spectrophotometry at its characteristic peak wavelength of 291 nm. The standard calibration curve for styrene was obtained by analysis of different styrene concentrations in the photometric mode.

Protein Filtration

Individual solutions of α-La and β-Lg were prepared by dissolving 1 g of the protein powders in 1 mM NaH_2PO_4 buffer solution. The pH of the solutions was adjusted to the desired value by addition of 0.1 M HCl or NaOH as required. The pH was measured with an Acumet AR15 pH meter (Fisher Scientific, Pittsburgh, PA) to within 0.01 units.

Experiments were conducted using a 50 ml Amicon stirred ultrafiltration cell (model 8050, Amicon Beverly, MA) with protein solution of known concentration and pH. The transmembrane pressure (ΔP) was set by pressurizing the cell with nitrogen gas. A schematic of the apparatus set-up is shown in Fig. 2. A pressure drop of approximately 0.7 bar was used and a permeate volume of 45 ml was collected from the 50 ml feed. In each case, the time

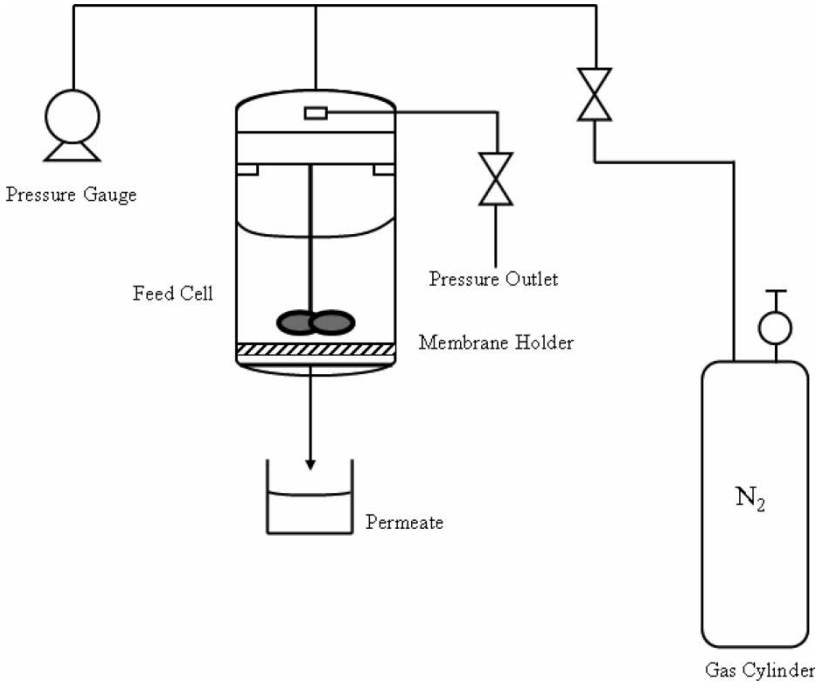

Figure 2. Membrane cell for whey protein filtration.

required to permeate the required volume was noted. All permeate and retentate protein concentrations were evaluated by UV-Visible spectrophotometry at 280 nm. The experiments were run using pH values of 3.2 and 7.2 for each protein. The membranes were cleaned by washing the surface with 25 ml 0.1 M NaCl followed by filtration with 50 ml NaCl then 50 ml deionized water. Standard calibration curves for each protein were obtained by analysis of different protein concentrations in the photometric mode, also at 280 nm.

RESULTS AND DISCUSSION

Ion-exchange Capacity

The ion-exchange capacity of the modified membrane at various stages of preparation is shown in Fig. 3. The raw PES membrane has a low ion-exchange capacity which is due to limited sulfonic acid groups. The ion-exchange capacity increased 3-fold when the raw membrane was activated with dilute sulfuric acid. Assuming that each repeat unit of the PES is associated with two sulfonic acid groups, and based on the projected area of a PES repeat

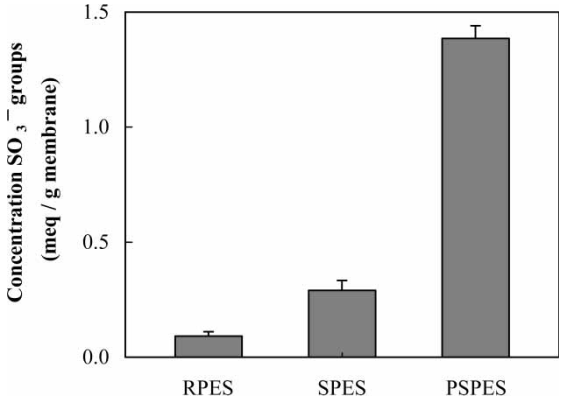

Figure 3. Ion-exchange capacity for 100 kD membrane. Abbreviations include: raw PES (RPES), sulfonated PES (SPES), and polymerized sulfonated PES (PSPES).

unit (approximately 0.3 nm^2), the theoretical surface density is 6 ion-exchange groups per nm^2 of internal surface area. A surface density of about 2 SO_3^- groups/nm^2 was obtained experimentally.

These sites promote polymerization of styrene in the pores of the membrane, which can be subsequently activated by acid treatment. The theoretical ion-exchange capacity of sulfonated polystyrene is 5.43 meq/g. The observed ion-exchange capacity of the functionalized membrane was 1.4 meq/g. Given that initiator sites are associated with the polymer grafts, all ion-exchange capacity in the functionalized membranes is due to sulfonated polystyrene. This number is equivalent to an ion-exchange capacity of roughly 4 meq/g of grafted polymer, or a sulfonation efficiency of 73%. Therefore, in addition to a high negative charge, the grafted chains should extend into the pore, resulting in reduced permeability and protein sieving based on size exclusion.

Modified Membrane Water Flux Studies

Pure water flux studies carried out on raw and modified membranes showed successive decreases in water permeability at each step from the raw to the sulfonated polymerized membrane. Membrane permeabilities were determined by taking the gradient of the water flux vs. pressure graphs. As shown in Fig. 4, the permeability of the raw membrane was 1,936 L/(m^2 hr bar) and that of the functionalized membrane was found to be 772 L/(m^2 hr bar). Fully sulfonated PES is water soluble, and therefore sulfonation of the raw membrane may have resulted in swelling of the membrane and constriction of the flow path. Subsequent polymerization of styrene would partially fill the pores with globular polymer grafts, and result in decreased permeability.

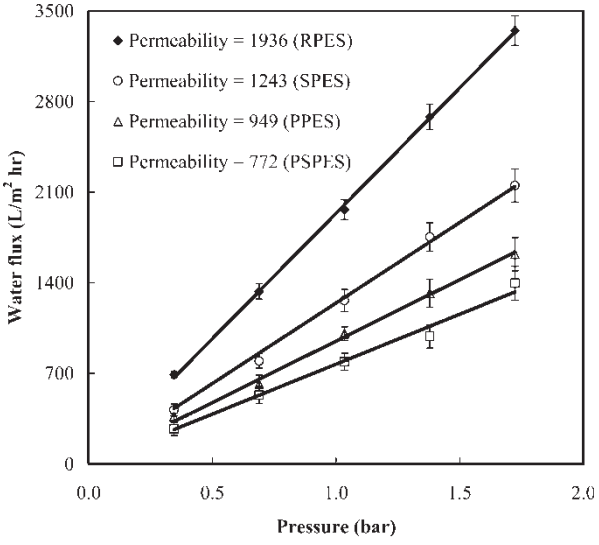

Figure 4. Water flux for the raw (RPES), sulfonated (SPES), polymerized (PPES), and polymerized sulfonated (PSPES) 100 kDa membrane.

Finally, sulfonated polymer grafts would extend into the membrane pores, leading to a reduction in the membrane permeability. This last observation confirmed the hypothesis described in the previous section on ion-exchange capacity.

Protein Filtration Study

Filtration of individual solutions of α-La and β-Lg through raw and modified PES membranes was examined in combination with changes in solution pH. Protein species were made to be positively or negatively charged by adjusting the pH of the solution to 3.2 or 7.2, respectively. The raw membrane filtration was used as a benchmark for comparative study with the modified membranes.

Protein transmission through the membrane was quantified in terms of the sieving coefficient which is defined by equation (1),

$$S = 1 - R \tag{1}$$

where R is the protein rejection coefficient. The rejection coefficient for a batch process, as was used in our work, can be calculated from the average protein rejection given by equation (2).

$$R = \frac{\ln[VCF - (C_p/C_f)(VCF - 1)]}{\ln(VCF)} \tag{2}$$

Parameters in equation (2) include: volume concentration factor (VCF = volume feed/volume permeate), permeate concentration (C_p), and feed concentration (C_f). The sieving coefficient is a function of the relative size of the molecules with respect to the membrane pore size, as well as the protein and membrane charge. Additionally, the sieving coefficient is used to determine the selectivity of the membrane.

The membrane selectivity (ψ) for α-La is defined as the ratio of the experimental sieving coefficients for α-La to β-Lg protein as given by equation (3),

$$\psi = \frac{S_{\alpha\text{-La}}}{S_{\beta\text{-Lg}}} \tag{3}$$

Note that the sieving coefficients used in the equation are based on single protein transmission studies.

Raw Membrane Filtration

The sieving coefficients of α-La and β-Lg through the raw membrane at a filtrate flux of 136 L/(m^2 hr) for pH values of 3.2 and 7.2 are illustrated in Figs. 5 and 6. In general, the observed sieving coefficient for α-La was higher than that of β-Lg because of its smaller size (Table 1). Studies with α-La at different values of pH showed negligible difference in sieving. Similar behavior was observed for β-Lg, and was most likely due to the relatively low ion-exchange capacity of the raw membrane. A lack of membrane charge would provide insufficient electrostatic repulsion or attraction with any one particular charged protein species. The relatively large

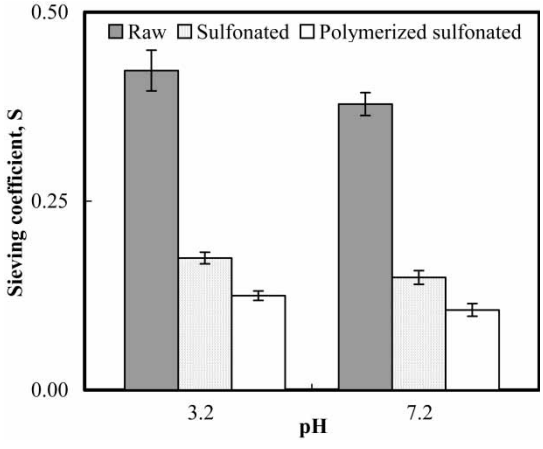

Figure 5. Sieving coefficient of 1 g/l α-La through the raw, sulfonated, and polymerized sulfonated 100 kDa PES membrane.

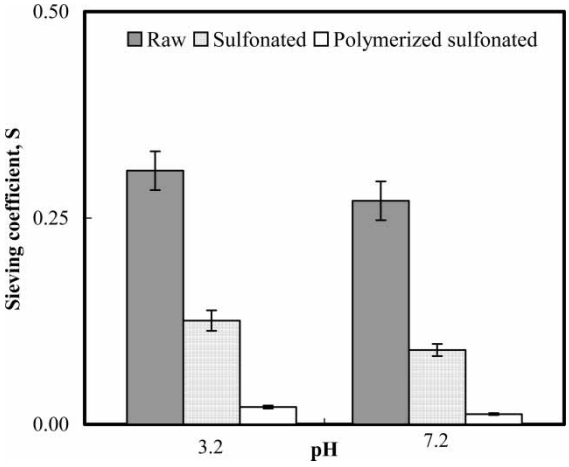

Figure 6. Sieving coefficient 1 g/l β-Lg through the raw, sulfonated, and polymerized sulfonated 100 kDa PES membrane.

sieving coefficients for the raw membrane are likely due to the large membrane pore size compared to the protein molecules.

Sulfonated Membrane Filtration

The observed protein sieving using the sulfonated PES membrane at a filtrate flux of 102 L/(m^2 hr) is shown in Figs. 5 and 6. A decrease in sieving was observed for both α-La and β-Lg, and may be due to a combination of decreased effective pore size and some amount of protein-membrane electrostatic interactions. The observed reduction in solvent flux for the functionalized membrane (Fig. 4) is evidence of reduced pore size. A reduction in protein sieving of over 58% was also observed for both proteins at pH 3.2, with a further 6% reduction at pH 7.2. The rejection of both α-La and β-Lg were slightly higher at higher pH. The proteins are positively charged at pH 3.2, and negatively charged at pH 7.2. The interaction between the protein and the membrane switches from electrostatic attraction to repulsion as the pH increases. The β-Lg carries a significant negative charge at pH 7.2, but its rejection was only slightly higher compared to that at pH 3.2. This would suggest that the observed sieving was a stronger function of size exclusion than electrostatic repulsion.

Functionalized Membrane Filtration

A further decrease in sieving coefficient was observed with the polymerized sulfonated membrane for a filtrate flux of 93 L/(m^2 hr) (Figs. 5 and 6). Polymer grafts in the membrane pores resulted in reduced membrane pore size and

membrane porosity. The pure water permeability was 40% of the corresponding value for the raw membrane. A reduction in protein sieving was also observed for both proteins, including an order of magnitude decrease for β-Lg at pH 7.2 compared to the raw membrane. In both cases, these observations might be due to reduced pore size and porosity. Increased rejection of β-Lg may also be due to increased repulsive effects with the charged membrane.

Membrane Selectivity

The data in Figs. 5 and 6 show average values for sieving, and therefore a difference in electrostatic repulsion was not apparent. However, when expressed as membrane selectivity, differences in protein sieving at pH 3.2 and 7.2 were obvious. The data in Fig. 7 show the comparison of selectivity values for the raw and modified membranes for single protein transmission studies. The raw membrane shows fairly low selectivity (1.38–1.42) between α-La and β-Lg. The low selectivity is due to the large difference between protein molecular weight and the membrane pore size. The insignificant number of charged groups in the membrane did not result in pore narrowing or electrostatic repulsion, as shown by the negligible difference in selectivity at pH 3.2 and 7.2. Therefore, it would be difficult to obtain good fractionation of these proteins using the raw ultrafiltration membrane.

Sulfonation of the membrane resulted in some increase in membrane selectivity (1.41–1.66). The improvement was due to reduced pore size based on a reduction in the membrane permeability (recall Fig. 4). Minor differences in membrane selectivity at different values of pH indicate some potential improvement in electrostatic repulsion. However, this change was only 17.7%, and was not much larger than the standard deviation of the data.

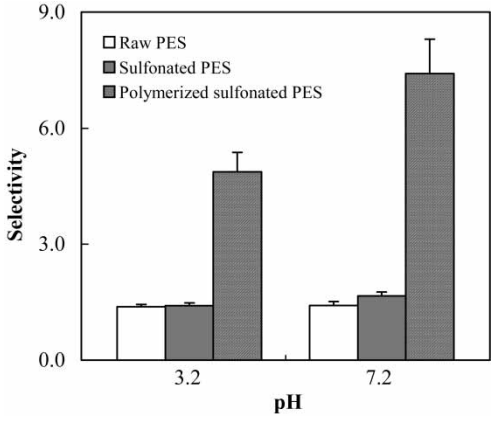

Figure 7. Selectivity for α-La and β-Lg as a function of pH for filtration through the raw, sulfonated, and polymerized sulfonated 100 kDa membrane.

The formation of sulfonated polystyrene grafted chains in the membrane pores had a substantial impact on membrane selectivity. At a pH of 3.2, the functionalized membrane showed a four-fold enhancement in selectivity compared to the raw or sulfonated membranes. The enhancement was primarily attributed to size exclusion of the β-Lg. At a pH of 7.2, however, the impact of the highly-charged membrane becomes evident. The functionalized membrane showed a 50% improvement in selectivity compared to separation at pH 3.2. The increase in selectivity results from a decrease in membrane pore size and improved electrostatic repulsion as well, given that β-Lg is approximately 4 times more negatively charged at pH 7.2 than α-La (26).

CONCLUSION

Ultrafiltration membranes have been used to separate various whey proteins, including α-La and β-Lg. Protein fractionation based solely on size exclusion is more effective at low membrane permeability. In this research, a larger pore size ultrafiltration membrane was functionalized with a charged polymer graft to achieve similar protein selectivity at higher flux. The functionalized PES ultrafiltration membrane was produced by cationic polymerization of styrene in the pores of the membrane followed by activation of the formed polystyrene grafts with sulfuric acid. The modified PES membranes were tested for sieving of α-La and β-Lg.

Protein sieving data obtained in this study demonstrate that it is possible to increase the selectivity of α-La through a combination of effects, including reduced membrane pore size and enhanced electrostatic repulsion between the charged membrane and protein species. Results for the raw membrane showed limited selectivity for α-La, and no significant change in selectivity as a function of pH. Sulfonation of the raw membrane gave a slight improvement in selectivity, independent of pH. However, functionalization of the membrane caused changes in selectivity and electrostatic repulsion. On average, the selectivity improved by 5 times compared to the raw membrane at pH 7.2. This value was also 50% greater than the selectivity at pH 3.2, demonstrating some enhancement due to electrostatic repulsion.

Although the application of these functionalized membranes for enhancement of protein filtration has promise, future work should be done to further explore the potential of these membranes for protein separation. For example, the effect of electrostatic interactions on the observed protein sieving is unclear. Thus studies will be done using a combination of pH and high ionic strength where electrostatic charges would be shielded by the high salt concentration. Further studies using an unmodified 30 kD membrane would reveal any advantage of the modified membrane in terms of selectivity for a given pore size/permeability.

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