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Research Article

Adsorption behaviour of β-lactoglobulin onto polyethersulphone membrane surface

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Abstract

The adsorption of dairy protein onto PES membrane was investigated by static adsorption experiments to understand the fouling mechanism and optimize the process conditions to minimize membrane fouling. Based on these adsorption experiments, the major interactions to increase the protein adsorption were the hydrophobic interaction and electrostatic interactions. Especially, the hydrophobic interaction by dissociation of dimer structure of β-lactoglobulin to monomer structure at acidic and alkaline conditions resulted in greatly increasing the amount of protein adsorption by the protein and membrane interaction which might form the strong and rigid protein layer on the polymeric membrane surface. Increase in hydrophilicity of membrane surface generally reduces protein adsorption regardless of modification methods. β-lactoglobulin adsorption onto the polyethersulphone (PES) surface was mainly affected by the hydrophobic interaction and electrostatic interaction between protein and PES membrane; any approach to reducing these interactions would reduce protein adsorption on membrane surface, thus alleviating membrane fouling.

Keywords: protein adsorption, dairy, membrane fouling, membrane surface modification, whey protein, PES, Korea.

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* Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Introduction

Membrane filtration is an important technology in food processing and has many advantages; the major limitation of its applications is membrane fouling, which is defined as a decrease in permeation flux over processing time by the accumulation of solute and/or the formation of fouled layer onto the membrane surface and inside pores. Membrane fouling occurring in the filtration of protein solutions can result in a significant loss of filtration performance such as selectivity and permeation flux thus could have a serious impact on the efficiency and economics of the whey protein recovery process. The adsorption of protein molecules onto the membrane surface and the entrapment of protein and protein aggregates inside the pores are known as the major factor to induce membrane fouling [1]. The fouling mechanism has been investigated in order to improve permeation flux and reduce membrane fouling [1, 2, 3, 4]. Based on previous research, hydrophobic interactions and electrostatic interactions have been identified to interpret the fouling mechanisms [5, 6, 7, 8]. These interactions are generally affected by the membrane properties such as hydrophilicity, surface topography, roughness, pore size and pore distribution and solution properties such as solute type, concentration, pH and ionic strength.

Although it is possible to delay the onset and reduce the amount of fouling by controlling the processing conditions and/or the modification of membrane surface, fouling is an inevitable problem in the membrane filtration process. In order to maintain the permeation flux and selectivity of membrane processes, various cleaning methods are commonly used in the food industry [9, 10]. For example, in the dairy industry employing the membrane filtration system, membranes are daily cleaned in an empirical way until the final flux is higher than 90% of the initial one [11]. Membrane cleaning is usually performed by physical, chemical and enzymatic methods [12]. As one physical cleaning method, ultrasonic technique has been widely used to remove the foulants of ultrafiltration and microfiltration membranes [13, 14, 15, 16]. Chemical cleaning is the most common method to clean membranes. Alkaline solutions, acids, metal chelating agents and surfactants have been used as the cleaning agents [17, 18, 19]. Enzymes have also used for removing adsorbed proteins from the membrane surface [19]. However, the physical and chemical cleaning methods may significantly reduce the lifetime of the membrane which in turn increases the replacement cost.

Strictly speaking, membrane fouling is a process phenomenon that manifests itself as a decline of flux with time during the operation. Its underlying source of flux decline in membrane separations is obstruction of flow through the membrane layer by proteins on the surface of, and in the pores of, the membrane. The obstruction of flow through membrane pores are caused by adsorption of protein onto membrane surfaces, both inside and outside of pores. Many factors in the membrane process contribute to membrane fouling; membrane properties, solute properties and process engineering are the most critical of all [20]. The membrane-solute interaction, in the case of food processing involving proteins, encompasses all the above-mentioned critical factors. The hydrophobicity of commercial ultrafiltration and nanofiltration and its effect on membrane interaction with dairy proteins (i.e., the adsorption of dairy proteins onto an ultrafiltration membrane surface) is most likely the culprit of irreversible membrane fouling in the membrane operation, whether it is concentration of proteins or fractionation of proteins. The adsorption of dairy proteins onto a commercial ultrafiltration membrane involves many dynamic steps before it reaches equilibrium.

Adsorption equilibrium is the fundamental property of the solute-surface interaction because the adsorption process will continue until the thermodynamic equilibrium of the solute concentration is reached. The equilibrium at a given temperature is usually presented as an isotherm which is a plot of the amount of the adsorbed solute as a function of the solute concentration. The adsorption isotherm is useful for predicting the performance of adsorption systems. In this current study, β-lactoglobulin was chosen as the model dairy protein as it is the main protein in whey protein recovered from a membrane ultrafiltration system and is available in highly purified form, readily soluble in water. Polyethersulphone (PES) membrane was used in this study as the family of polysulphone related membranes (polyethersulphone and polysulphone) is one of the most widely used membrane materials for ultrafiltration operations [19]. These membranes can be operated in a wide range of temperatures ranging from -100ºC to 150ºC and pH tolerance ranging from pH 1 to pH 13, which is advantageous in a commercial operation as membranes are subject to frequent washing and chemicals such as chlorine and alkaline solution. The main disadvantage of this family of membranes is their hydrophobicity that makes them prone to fouling in protein processing compared to the more hydrophilic polymeric membranes.

This investigation was designed to study the adsorption isotherms and the effects of protein solution, pH and ionic strength in the form of NaCl concentration in the protein solution. The effect of hydrophilic surface modification through polymer surface grafting and interfacial polymerization methods on protein adsorption was also examined.

Materials and Methods

Materials

Polyethersulphone (PES) polymer was kindly provided by Solvay Advanced Polymers, L.L.C. (Alpharetta, GA, USA) under the trade name of RADEL^{RM} H-2000P NT. Flat sheet PES membrane was purchased from Sterlitech (Kent, WA, USA). MWCO of flat sheet PES membrane is 20,000. Bovine milk β-lactoglobulin, which is a mixture of A and B variants crystallized and lyophilized three times (Lot No. 033K7003), phosphate-buffered saline (PBS) and *N, N*-dimethylformamide (DMF) were purchased from Sigma-Aldrich (St. Louis, MO). Deionized water was obtained from a Millipore Milli-Q filtration system (Millipore Corporation, MA, USA) with a resistivity of 18.2 MΩ cm. Terephthaloyl chloride (TC), *m*-phenylenediamine, polyethylene glycol (PEG) 2000 and poly(vinyl alcohol) (PVA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitosan (MW 300,000, degree of deacetylation 90%) was purchased from Kunpoong Bio Co., Ltd. (Jeju, South Korea).

Experimental procedure

Adsorption of protein onto PES membranes

Static adsorption experiments of β-lactoglobulin on virgin (unmodified) PES membranes and hydrophilically modified PES membranes were carried out by shaking 2 x 2 cm^2 pieces of membrane with 50 ml aqueous solution of β-lactoglobulin of desired concentrations (0.5 to 2.5 mg/ml) and pHs (pH 3.0, 5.2, 7.0 and 9.0) at room temperature for 24 hr to reach the equilibrium state. At the end of the adsorption period, the concentration of the residual protein solutions was determined with a UV-Vis spectrophotometer (UV-1700, Shimadzu) via the absorbance at 280 nm. By comparing the initial and final concentrations, the adsorption amounts were calculated. The calibration curves and equations of absorbance versus β-lactoglobulin concentrations from 0.5 mg/ml to 2.5 mg/ml in working condition were obtained by linear regression.

The adsorption isotherms of β-lactoglobulin on virgin PES membranes were obtained at pH 3.0, 5.2 (isoelectric point), 7.0 and 9.0. During the protein adsorption process on the polymeric membrane surface, many interactions between the membrane and the protein, as well as among proteins themselves, might affect the amount of protein adsorption. Hydrophobic PES membrane is negatively charged in aqueous solution due to the ionization of polar groups at the membrane surface and/or the selective adsorption of anions from the surrounding solution onto the membrane surface [21]. Therefore, the protein adsorption can be affected by the charge and density of the polymeric membrane surface, as well as the protein charge which is dependent on the pH of the protein solution. The native structure of β-lactoglobulin is predominantly a dimer structure at room temperature and at neutral pH but it can be dissociated into the monomer structure depending on the pH of the solution. Below pH 3.5 and above pH 7.5 the dimer structure of β-lactoglobulin can be usually dissociated into the monomer structure [22, 23].

The adsorptions of proteins on hydrophilically modified membranes and on a virgin PES membrane were carried out using UV-Vis spectrophotometer via the absorbance at 280 nm to determine the decrease of the amount of protein adsorption by hydrophilic surface modification. The concentration of β-lactoglobulin was 2.5 mg/mL and solution pH was 3.0, where the maximum amount of protein adsorption was observed.

Grafting polymerization using UV/ozone

UV/ozone treatment was carried out to initiate and activate the PES membrane surface for grafting of hydrophilic polymers. The dried small piece of PES flat membrane was exposed to UV/ozone treatment for 1 min to 30 min. Peroxide groups were formed on the activated membrane surface by ozone treatment. The peroxide groups generated on the membrane surface reacted with hydrophilic polymers such as PVA, PEG 2000 and chitosan. 20% (w/v) PVA, 20% (w/v) PEG 2000 and 1% (w/v) chitosan solutions were used to react with the activated PES membranes. The grafting reaction was performed in a glass ampoule at 70°C for 2 hr. After the grafting reaction with hydrophilic polymers, the PES membranes were rinsed with distilled water for 24 hrs to remove excess hydrophilic polymers from the PES membrane surface. The hydrophilic polymer grafted PES membranes were subsequently dried at room temperature under vacuum. Detailed information regarding reaction pathway and characterization of the modified membrane surface are described in a previous publication [24].

Thin film composite by interfacial polymerization

The polymeric membranes were immersed in 1% (w/v) TC in benzene for 20 min. After 20 min, the membranes were exposed in an aerator for one minute to remove excess TC solution from the membrane surface. Membranes were then immersed into the aqueous solution containing 1% *m*-phenylenediamine and 1% hydrophilic polymers such as PVA, PEG 2000 and chitosan to form a composite membrane surface layer by interfacial polymerization. The fabricated PES membranes were rinsed with deionized water and then dried at room temperature for 24 hrs. Detailed information regarding reaction pathway and characterization of the modified membrane surface can be found in a previous paper [25].

Results and Discussion

Adsorption isotherm

Figure 1 shows typical adsorption isotherms: linear, Langmuir and Freundlich. Linear isotherm is an ideal but unrealistic description of equilibrium adsorption; Langmuir and Freundlich isotherms are widely used to interpret equilibrium adsorption data. Langmuir isotherm assumes adsorbed layer is monolayer and there are finite numbers of identical adsorption sites and the adsorption ability of a solute (in this case, protein molecule) to the each of these sites is independent of the occupation of neighbouring sites. Clearly, the aforementioned assumptions do not reflect the kind of protein adsorption occurring on the surface of PES membrane. Unlike Langmuir isotherm, Freundlich isotherm is not restricted by these assumptions, allowing for interactions involving adsorbed molecules and thus more useful as an empirical equation. The Freundlich equation is commonly given by

$$q_e = K_F C_e^{1/n} \tag{1}$$

with the linear form,

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \tag{2}$$

where K_F is a constant for the Freundlich system, related to the bonding energy. K_F can be defined as the adsorption or distribution coefficient and represents the quantity of the adsorbate on the surface for a unit equilibrium concentration (C_e = 1 mol/L). The slope $1/n$, ranging between 0 and 1, is a measure of adsorption intensity or surface heterogeneity, becoming more heterogeneous as its value gets closer to zero. A plot of $ln\ q_e$ versus $ln\ C_e$ enables the empirical constants K_F and $1/n$ to be determined from the intercept and slope by the linear regression.

Effect of pH on protein adsorption

As shown in Figure 2, the amount of β-lactoglobulin adsorbed on the PES membrane surface was the lowest at the neutral pH (pH 7.0). The maximum adsorption of β-lactoglobulin on the PES membrane surface was obtained at the acidic condition (pH 3.0), which was below the isoelectric point (pH 5.2) of β-lactoglobulin. In this acidic condition, the protein adsorption might be affected by the electrostatic attraction between the protein and the PES membrane surface because β-lactoglobulin had positive charges below its isoelectric point (pI) and hydrophobic PES membrane had negative charges in an aqueous solution due to the selective adsorption of anions from the surrounding water or due to the ionization of polar groups at the membrane surface. In addition, the hydrophobic interaction between protein and membrane can increase the amount of the protein adsorption. At pH 3.0, the dimer structure of β-lactoglobulin can be dissociated into the monomeric units which expose the hydrophobic sites as shown in Figure 3. These monomeric units are inclined to interact with the hydrophobic PES surface. Although some other interactions might affect the protein adsorption, both hydrophobic interaction by the dissociation of dimer structure of β-lactoglobulin and electrostatic attraction between protein and PES membrane are the principal factor to show the higher adsorption than at higher pHs.

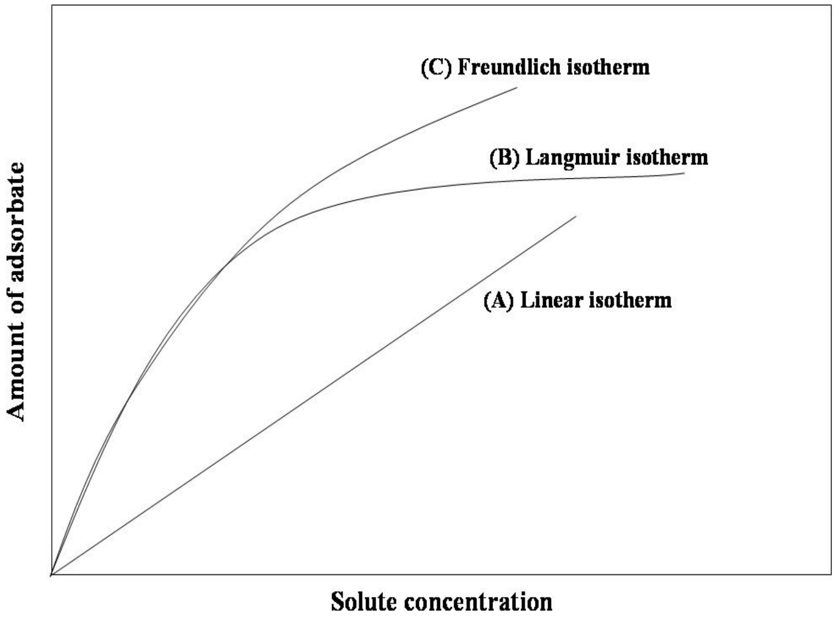

Figure 1. Typical adsorption isotherms.
(A) linear isotherm, (B) Langmuir isotherm and (C) Freundlich isotherm.

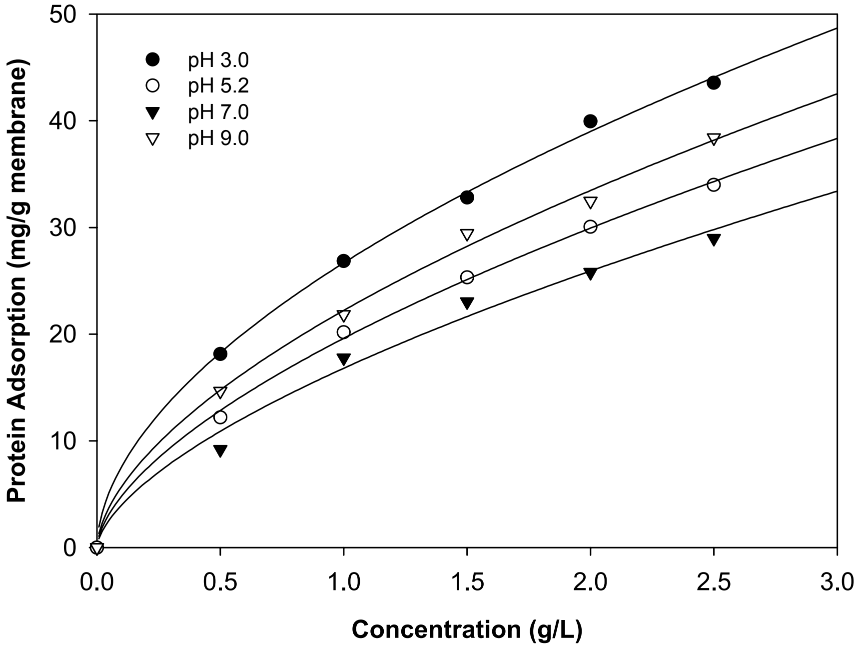

Figure 2. Adsorption isotherm for β-lactoglobulin on PES membranes at different pH.
pH 3.0, 5.2, 7.0 and 9.0

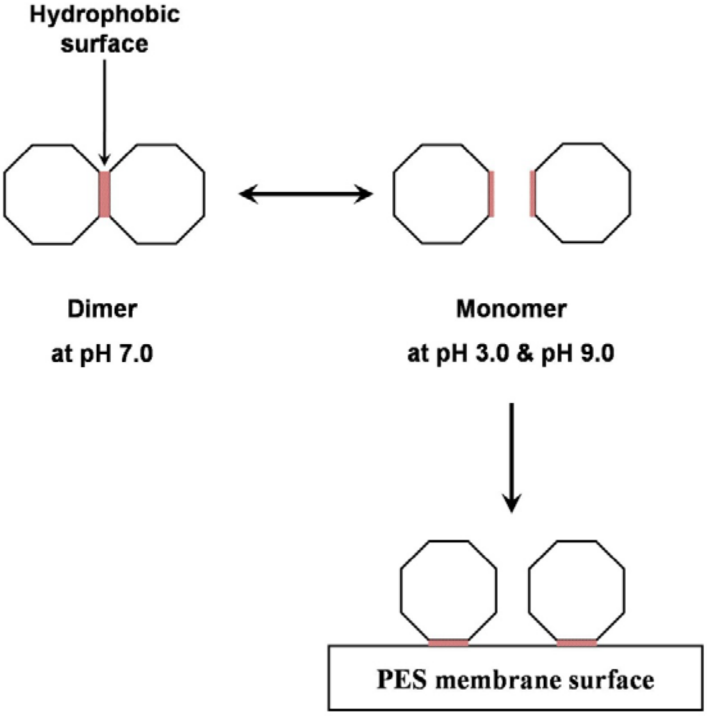

Figure 3. Dissociation of dimer structure of β-lactoglobulin to monomer structure depending on pH of protein solution.

At the pI (pH 5.2) where β-lactoglobulin structure was more hydrophobic and compact because β-lactoglobulin aggregates strongly to a tetramer which has a cyclic structure and involving four bonds between pH 3.7 to pH 5.2, the protein adsorption occurred due to the mainly hydrophobic interaction between protein and PES membrane surface [26]. The amount of β-lactoglobulin adsorbed at the pI was higher than that at the neutral pH where the electrostatic repulsion existed because both β-lactoglobulin and the PES membrane surface had negative charges above the pI of the protein solution. Also, hydrophobic interaction at pH 7.0 might be weaker than at the pI because β-lactoglobulin is less hydrophobic at neutral pH.

The higher adsorption of β-lactoglobulin, however, was observed at pH 9.0, compared with pH 5.2 and pH 7.0, even if the electrostatic repulsion was much stronger in this alkaline condition than in the neutral pH. Because β-lactoglobulin also could be dissociated into monomeric units above pH 7.5, the hydrophobic interaction between the PES surface and the monomeric units of β-lactoglobulin might increase the amount of adsorption at pH 9.0. This hydrophobic interaction might be much stronger than the electrostatic repulsion so the protein adsorption is much higher at pH 9.0 than at pH 7.0 and 5.2. These results support the earlier explanation that the protein adsorption is not only due to the electrostatic interactions but also due to the change of protein structure and hydrophobic interaction between the protein and the PES membrane, as well as among the proteins themselves. Also, hydrophobic interaction by dissociation of dimer structure to monomer structure shows more adsorption than the electrostatic interactions. From these observations, it is apparent that the concentration of β-lactoglobulin of 2.5 mg/mL and solution pH of 3.0 produced the maximum amount of protein adsorption on virgin PES membranes.

The adsorption equilibrium curves of β-lactoglobulin on the PES membrane surface were obtained by plotting ln q_e versus ln C_e. The parameters of the Freundlich isotherm were determined from the intercept and slope of a linear regression and the results are given in Table 1. It can be seen clearly that the Freundlich constants such as adsorption capacity, K_F and adsorption intensity, 1/n, for β-lactoglobulin were dependent on the pH of the protein solution. The adsorption capacity, K_F, which is the measure of bonding energy and indicates the quantity of the adsorbate on the adsorbant, increased further away from the neutral pH (pH 7.0). The K_F values increased about 65% at pH 3.0, 20% at pH 5.2 and 38% at pH 9.0 compared with that at pH 7.0. This result supports the view that the pH of protein solution is one of the important factors for the protein adsorption process and the amount of protein adsorption would be higher at the acidic (pH 3.0) and the alkaline (pH 9.0) conditions where the hydrophobic interaction by dissociation of dimer to monomer structure is occurred. The intensity constants, 1/n, were just opposite to the trend of K_F. The intensity constants (1/n) decrease with the increase in adsorption capacity value (K_F). The lower intensity constant means that the protein adsorbed surface is more heterogeneous. As shown in Table 1, the more protein adsorbed on the membrane surface (increase the adsorption capacity, K_F), the more heterogeneous surface can be formed on the membrane surface.

Table 1. Freundlich constants of β-lactoglobulin adsorbed on PES membrane at different pH.

pH	K_F	*1/n*	R^2
3.0	.64	0.551	0.998
5.2	19.41	0.634	0.996
7.0	16.16	0.709	0.972
9.0	22.16	0.596	0.995

Effect of ion strength (NaCl concentration) on protein adsorption

Figure 4 shows the salt effects on the β-lactoglobulin adsorption on the PES membrane at pH 3.0 where the maximum adsorption was obtained. The increase in ionic strength provided by NaCl reduced the amount of adsorption for β-lactoglobulin on PES membrane at pH 3.0. Although the hydrophobic interaction by the change of β-lactoglobulin structure from dimer to monomer increases the amount of adsorption, the electrostatic attraction between positive charged protein and negative charged PES membrane in acidic condition is also the major factor in the increase of β-lactoglobulin adsorption on the PES membrane surface. The salt ions, however, can interact with the charged protein molecules and PES membrane surface so the electrostatic attractions between protein molecules and membrane surface could be reduced by the salt addition. Therefore the amount of β-lactoglobulin adsorption on the PES membrane surface was reduced with salt concentration. As shown in Table 2, the adsorption capacity (K_F) decreases with NaCl concentration. Although the amount of β-lactoglobulin adsorption decreases with salt concentration, the intensity constants (1/n) also decrease with salt concentration. This result reveals that the protein adsorbed layers are more heterogeneous with salt concentration.

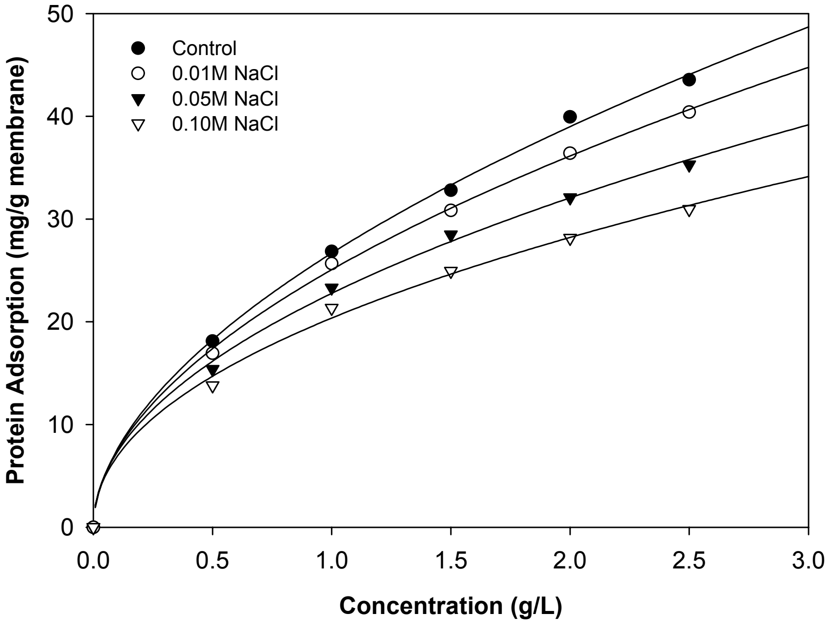

Figure 4. NaCl effect on the adsorption isotherm of β-lactoglobulin at pH 3.0.

Table 2. Freundlich constants of β-lactoglobulin adsorbed on PES membrane at pH 3.0 depending on the concentration of NaCl.

NaCl	K_F	*1/n*	R^2
Control	.64	0.551	0.998
0.01 M	24.96	0.538	0.997
0.05 M	22.55	0.516	0.993
0.10 M	20.15	0.496	0.987

Effect of surface modification on adsorption

The adsorption of β-lactoglobulin on the modified PES membrane surface was studied to compare the decrease in amount of adsorption on the membrane. As shown in Figure 5, the amount of β-lactoglobulin adsorbed on the PES membrane was reduced from 20% to 60% by surface modification. Among the modified PES membranes, PEG grafted PES membrane using UV/ozone showed the lowest protein adsorption. Chitosan-grafted PES membrane using UV/ozone showed the highest protein adsorption on modified membrane surface, though still lower than that of the virgin commercial PES membrane. The reason that the chitosan modified PES membrane was susceptible to protein adsorption, despite improvement in hydophilicity of the membrane surface, is that chitosan grafted PES membrane could increase the peptide bond between free amine groups in chitosan and hydroxyl groups in protein. Also the protein adsorption could be increased if more chitosan molecules were grafted on the PES membrane.

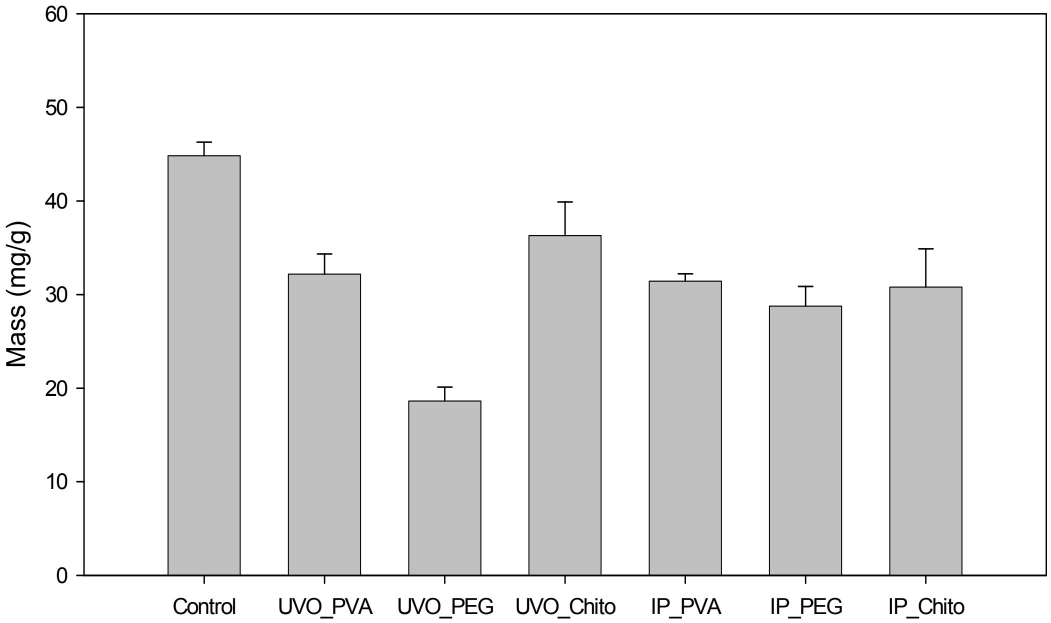

Figure 5. Mass of the β-lactoglobulin adsorbed on the unmodified and modified PES membranes by static adsorption experiment.

The concentration of β-lactoglobulin was 2.5 mg/mL and the solution pH was 3.0.

Conclusions

Static adsorption of β-lactoglobulin on PES surface was investigated using UV-spectrophotometer. In these adsorption experiments, Freundlich isotherm model was used to fit the data and to obtain the isotherm parameters such as K_F and 1/n. Based on the data of the static adsorption process, the amount of adsorption was highest at the acidic pH (pH 3.0) which is below the isoelectric point of β-lactoglobulin due to the electrostatic attraction and the interactions by the dissociation of β-lactoglobulin from dimer to monomeric unit. On the other hand, the amount of adsorption was lowest at the neutral pH (pH 7.0) due to the electrostatic repulsion. Although the electrostatic repulsion is still strong, the adsorption at the alkaline condition (pH 9.0) was higher than that at the isoelectric point. This result supports the theory that the interactions by the dissociation of β-lactoglobulin are higher than the electrostatic repulsion so the adsorption of β-lactoglobulin increased in the alkaline condition. The addition of salt reduced the adsorption of β-lactoglobulin on PES because the salt ions interact with the charged protein molecules.

In order to reduce the protein adsorption on polymeric membrane, PES membranes were modified by two surface modification methods: hydrophilic polymer grafting using UV/ozone and thin film composite by interfacial polymerization. These modification methods are powerful techniques to improve hydrophilicity of polymeric membranes. The adsorption of protein on the modified PES membranes was compared and it was found that hydrophilic modification of PES membranes reduces the amount of β-latoglobulin adsorption by 20% to 60%.

References

1. D'Souza, N.M. and Mawson, A.J. (2005). Membrane cleaning in the dairy industry: a review. ***Critical Reviews in Food Science and Nutrition,*** 45, 125-134.

2. Jim, K.J., Fane, A.G., Fell, C.J.D. and Joy, D.C. (1992). Fouling mechanisms of membranes during protein ultrafiltration. ***Journal of Membrane Science.*** 68, 79-91.

3. Jonsson, G., Prádanos, P. and Hernández, A. (1996). Fouling phenomena in microporous membranes: flux decline kinetics and structural modifications. ***Journal of Membrane Science.*** 112, 171-183.

4. Costa, A.R., de Pinho, M.N. and Elimelech, M. (2006). Mechanisms of colloidal natural organic matter fouling in ultrafiltration. ***Journal of Membrane Science.*** 281, 716–725.

5. McDonogh, R.M., Bauser, H., Stroh, N. and Chmiel, H. (1990). Concentration Polarisation and Adsorption Effects in Cross-Ultrafiltration of Proteins. ***Desalination,*** 79: 217-231.

6. Nystrom, M., Pihlajamaki, A. and Ehsani, N. (1994). Characterization of Ultrafiltration Membranes by Simultaneous Streaming Potential and Flux Measurements. ***Journal of Membrane Science,*** 87: 245-256.

7. Kelly, S.T. and Zydney, A.L. (1995). Mechanisms for BSA fouling during microfiltration. ***Journal of Membrane Science.*** 107, 115-127.

8. Ricq, L., Narcom, S., Reggiani, J.C. and Pagetti, J. (1999). Streaming Potential and Protein Transmission Ultrafiltration of Single Proteins and Proteins in Mixture: B-lactoglobulin and Lysozyme. ***Journal of Membrane Science,*** 156: 81-96.

9. Bartlett, M., Bird, M.R. and Howell, J. (1995). An experimental study for the development of a qualitative membrane cleaning model. ***Journal of Membrane Science.*** 105, 147-157.

10. Muthukumaran, S., Kentish, S., Lalchandani, S, Ashokkumar, M., Mawson, R., Stevens, G.W. and Grieser, F. (2005). The Optimisation of Ultrasonic Cleaning Procedures for Dairy Fouled Ultrafiltration Membranes. ***Ultrasonics Sonochemistry,*** 12: 29–35.

11. Delaunaya, D., Rabiller-Baudrya, M., Paugama, L., Pihlajamäkib, A. and Nyström, M. (2006). Physicochemical characterisations of a UF membrane used in dairy application to estimate chemical efficiency of cleaning. ***Desalination.*** 200, 189–191.

12. Trägårdh, G. (1989). Membrane Cleaning. ***Desalination,*** 71: 325-335.

13. Muthukumarana, S., Yanga, K., Seuren, A.S., Kentish, S., Ashokkumarb, M., Stevens, G.W. and Grieser, F. (2004). The Use of Ultrasonic Cleaning for Ultrafiltration Membranes in the Dairy Industry. ***Separation and Purification Technology,*** 39: 99–107.

14. Simon, A., Gondrexon, N., Taha, S., Cabon, J. and Dorange, G. (2000). Low-Frequency Ultrasound to Improve Dead-End Ultrafiltration Performance. ***Separation Science and Technology,*** 35: 19-37.

15. Li, J., Sanderson, R.D. and Jacobs, E.P. (2002). Ultrasonic cleaning of nylon microfiltration membranes fouled by Kraft paper mill effluent. ***Journal of Membrane Science.*** 205, 247-257.

16. Kobayashi, T., Hosaka, Y. and Fujii, N. (2002). Ultrasound-enhanced membrane-cleaning processes applied water treatments: influence of sonic frequency on filtration treatments. ***Ultrasonics.*** 41, 185-190.

17. Liikanen, R., Yli-Kuivila, J. and Laukkanen, R. (2002). Efficiency of various chemical cleanings for nanofiltration membrane fouled by conventionally-treated surface water. ***Journal of Membrane Science.*** 195, 5–276.

18. Madaeni, S.S., Mohamamdi, T. and Moghadam, M.K. (2001). Chemical Cleaning of Reverse Osmosis Membranes. ***Desalination.*** 134, 77-82.

19. Sadhwani, J.J. and Veza, J.M. (2001). Cleaning Tests for Seawater Reverse Osmosis Membranes. ***Desalination,*** 139: 177-182.

20. Cheryan, M. (1998). Ultrafiltration and Microfiltration Handbook. Technomic Publishing CO, Inc. Lancaster, PA, USA, pp 245-6.

21. Pujar, N.S. and Zydney, A.L. (1997). Charge Regulation and Electrostatic Interactions for a Spherical Particle in a Cylindrical Pore. ***Journal of Colloid and Interface Science,*** 192: 338–349.

22. Verheul, M., Pedersen, J.S., Roefs, S.P.F.M. and de Fruif, K.G. (1999). Association Behavior of Native β-Lactoglobulin. ***Biopolymers,*** 49: 11-20.

23. McKenzie, H.A. and Sawyer, W.H. (1967). Effect of pH on β-Lactoglobulins. ***Nature,*** 214: 1101-1104.

24. Liu, S.X., Kim, S., Kim, J.-T. and Singh, M. (2009). The effect of polymer surface modification on polymer-protein interaction via interfacial polymerization. ***Journal of Applied Polymer Science.*** 112, 1704-1715.

25. Liu, S.X., Kim, S. and Kim, J.-T. (2007). The effect of polymer surface modification on polymer- protein interaction via hydrophilic polymer grafting. ***Journal of Food Science.*** 73(3), E143-E150.

26. Timasheff, S.N. and Towned, R. (1964). Structure of the β-Lactoglobulin Tetramer. ***Nature,*** 203: 517-519.