Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Salgın, Sema , Takaç, Serpil and Özdamar, Tunçer H.(2005) 'A Parametric Study on Protein-Membrane-Ionic Environment Interactions for Membrane Fouling', Separation Science and Technology, 40: 6, 1191 — 1212
To link to this Article: DOI: 10.1081/SS-200052202
URL: http://dx.doi.org/10.1081/SS-200052202

Separation Science and Technology, 40: 1191–1212, 2005

ISSN 0149-6395 print/1520-5754 online
DOI: 10.1081/SS-200052202

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A Parametric Study on Protein-Membrane-Ionic Environment Interactions for Membrane Fouling

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Abstract: This work reports on protein-membrane-ionic environment interactions on the basis of chemical and electrochemical features of ultrafiltration membranes and the protein in the solution that affects the extent of protein adsorption onto the membrane, which is a measure of membrane-fouling. Bovine serum albumin (BSA) was chosen as the model protein; and 10 kDa of hydrophobic polyethersulfone (PES) and hydrophilic cellulose triacetate (CTA) ultrafiltration membranes at the solution pH values of 3.78, 4.78, and 6.80, and ionic-strengths of 0.01 M and 0.1 M were employed. Isotherms for BSA adsorption on both types of membranes were correlated by the Freundlich equation. More BSA was adsorbed on hydrophobic PES membranes than was adsorbed on hydrophilic CTA membranes. The highest degree of adsorption on PES membranes was obtained at pH = 3.78 whereas the minimum adsorption occurred at the isoelectric point (IEP) (pH = 4.78) of BSA. With increasing ionic strength, the adsorbed protein on both membranes decreased. The zeta-potentials of the membranes and protein were determined by streaming potential measurements and theoretical calculations, respectively; and the electrostatic interactions and van der Waals energies between the membranes and the protein were calculated using the Deryagin-Landau/Verivey-Overbeek (DVLO) theory. To detect the structural changes that occurred, membrane surfaces were analyzed by Fourier transform infrared-attenuated total reflectance (FTIR-ATR) measurements, and scanning electron microscopy (SEM) and atomic force microscope (AFM) images.

Keywords: Ultrafiltration membranes, protein, BSA, adsorption, membrane fouling, protein-membrane interactions, interaction energy, DLVO theory

Received 6 July 2004, Accepted 24 November 2004
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INTRODUCTION

Ultrafiltration membranes are widely used in the separation of proteins in biotechnological processes; however, membrane fouling, which is mainly caused by adsorption and/or aggregation, is an influential phenomenon in separation and purification of macro-biomolecules using ultrafiltration systems. Although adsorption often has been explained through complicated interactions among the membrane, protein, and solution components, an in-depth insight related to the interfacial forces that control the protein adsorption onto the membrane surface is required in order to clarify the origin of this phenomenon. In the present study, we carried out systematic and comparative research to meet the requirement of a more detailed understanding of the interfacial forces occurring between two types of ultrafiltration membranes and protein bovine serum albumin (BSA) under the physicochemical influence of its microenvironment.

Several research results have been published to describe the adsorption characteristics of BSA on different types of membranes over a range of pH and ionic strengths. Although the literature has a consensus that the ionic environment strongly affects the extent of adsorption, the results reported on the effect of pH on membrane fouling are not fully converged. Matthiasson (1) studied the adsorption kinetics of BSA on different kinds of membranes as well as the amount adsorbed and the hydraulic resistance of the adsorbed layer, reported that the adsorption increased with decreasing pH of the solution, and hydrophilic cellulose acetate membrane adsorbed less protein than did hydrophilic polyamide and hydrophobic polysulfone membranes Aimar et al. (2), reported that the amount of adsorbed BSA on polyacrylonitrile membranes increased with decreasing pH, with increasing bulk concentration, and contact time. Burns and Zydney (3) investigated the effect of solution pH on the transport of globular proteins with different surface-charge characteristics through polyethersulfone (PES) membranes and obtained the maximum protein-sieving coefficient near the protein isoelectric point. The authors postulated that the nonlinear dependence of the sieving coefficient on pH was due to the nonlinear dependence of the BSA charge on solution pH. Moreover, attractive electrostatic interactions occurred when the protein and membrane had large opposite charges, causing a second maximum in transmission at a pH at the isoelectric-points of the protein and membrane. Möckel et al. (4) studied the influence of membrane hydrophilicity, pH, and ionic strength on the static adsorption of *cys*-BSA, protein ultrafiltration performance, and cleanability. The authors found that BSA adsorption showed a maximum at its IEP and the increase in ionic strength led to stronger static adsorption and greater flux reduction for pH values on either side of the IEP. It was asserted that besides the electrostatic aspects, hydrophilicity played an important role on the static adsorption; and ultrafiltration flux reduction decreased and cleanability increased with

increasing level of functionality. Menon and Zydney (5) investigated the effect of specific ionic composition on the rate of BSA transport through PES membranes over a range of solution pH and salt concentrations; and they emphasized significant impact of electrostatic interactions on protein transmission. The effects of specific ions on BSA sieving were attributed to differences in net protein charge arising from differences in ion-binding affinity to protein surface and to differences in electrostatic shielding associated with differences in ion valence. Huisman et al. (6) studied protein-protein and protein-membrane interactions to explain the membrane fouling during the ultrafiltration of BSA solution over PES membranes. The experiments were carried out at pH values within a wide range using membranes of different cut-off values. The authors reported that protein-membrane interactions influenced the fouling behavior in the initial stages of the filtration; however, in the later stages of the process, protein-protein interactions, which dictated the overall performance as the less retentive membranes, resulted in weaker interactions. The extent of coverage of membrane with protein was analyzed by the measurement of the streaming potential and by using atomic force microscopy (AFM), and the structure of the fouling layer was found to depend strongly on pH. Recently, Xu et al. (7) investigated the adsorption of BSA on porous polyethylene membrane as a function of the pH of the solution in addition to several parameters such as concentration, time, and agitation speed. They observed the maximum adsorption at the isoelectric point of the protein since the molecular size of a disordered protein was at its lowest value.

The studies on the intermolecular interaction theories, which examined particularly in-flow systems, also gain attention in the literature. Zydney and Pujar (8) discussed the application of hard-sphere, electrical, and van der Waals interaction theories to membrane systems; and they presented new calculations for the transport of charged solute through the membrane pores based on the charge regulation model. The authors suggested that long-range colloidal interactions could have a dramatic effect on the rate of protein transport through porous membranes. Thereafter, Burns and Zydney (9) studied the rate of protein transport through porous charged membrane taking into consideration both thermodynamic (solute partitioning) and hydrodynamic (frictional) interactions; they quantitatively compared predictions of theoretical models with experimental data for ovotransferrin transport through charged PES membranes over a range of solution conditions. The authors asserted that the model predicted the complex effects of solution pH and ionic strength on protein transmission in the presence of both attractive and repulsive electrical interactions. Although the nature of interaction forces causing protein adsorption and eventual fouling is discussed in the literature, there is no general insight into interfacial forces that are dominant in adsorption. The aim of this work is to examine the membrane fouling in terms of intermolecular interactions between the membrane and the protein, with the

adsorption data complemented by scanningelectron microscopy (SEM), atomic force microscopy (AFM), and Fourier transform-infrared (FTIR-ATR) measurements. In the parametric experimental study, BSA as the model protein and two different kinds of ultralfiltration membranes, i.e. hydrophobic PES and hydrophilic cellulose triacetate (CTA) membranes were used. Based on the hydrophobic and hydrophilic characters of the membranes, the influences of the properties of protein solution such as pH and ionic strength on the degree of static adsorption were investigated. Moreover, the qualitative dependence of the membrane fouling on ionic environment was explained by showing that the changes occurred on the membrane surface after the adsorption using FTIR-ATR spectra, SEM, and AFM images; and the quantitative dependence of interfacial interactions on adsorption was explained by calculating the interaction energies between the membrane and the protein.

INTERACTION ENERGIES: THEORY

Various types of interactions occur between the membrane and the protein, and specific contributions such as polarization may be important besides the nonspecific dispersion and repulsion interactions (10). In the present study, electrostatic and van der Waals interaction energies were considered as the major nonspecific interaction energies and calculated using the DVLO theory developed for colloids. According to the theory, electrostatic effects are governed by the interactions between the diffuse ion atmosphere outside the charged surfaces (11). The electrostatic energy between an infinitely thick flat surface, i.e., membrane, and a sphere, i.e., protein, can be evaluated by Eq. (1); and, the van der Waals energy between a membrane and a protein molecule can be evaluated by Eq. (2).

$$E_e = \varepsilon_r \varepsilon_o r\left(\zeta_1^2 + \zeta_2^2\right)\left\{\frac{2\zeta_1\zeta_2}{\zeta_1^2 + \zeta_2^2}\ln\left[\frac{1+\exp(-\kappa H)}{1-\exp(-\kappa H)}\right] + \ln[1-\exp(-2\kappa H)]\right\} \tag{1}$$

$$E_w = -\frac{A}{6}\left[\frac{2r(H+r)}{H(H+2r)} - \ln\left(\frac{H+2r}{H}\right)\right] \tag{2}$$

Interaction energies can be either a repulsive or an attractive one depending on chemical structure, medium properties, and surface potential. Therefore, the sum of the electrostatic energy and van der Waals energy predicts whether repulsion or attraction forces are dominant between the membrane and protein.

The Debye screening length, κ^{-1}, is calculated by using Eq. (3) [Ref. (3)]:

$$\kappa^{-1} = \left(\frac{F^2}{\varepsilon_o \varepsilon_r RT} \sum z_i^2 C_i \right)^{-1/2} \tag{3}$$

The membrane zeta potential (ζ_1) to be used in Eq. (1) can be calculated by inserting experimentally evaluated streaming potentials into the Helmholtz-Smoluckowski equation, Eq. (4) [Ref. (3)]:

$$\frac{dE_z}{d\Delta P} = \frac{\varepsilon_o \varepsilon_r \zeta}{\eta \Lambda_o} \tag{4}$$

On the other hand, the zeta potential at the BSA surface (ζ_2) can be calculated by Eq. (5) [Ref. (5)]:

$$\zeta = \frac{eZ}{4\Pi\varepsilon\varepsilon_o r(1 + \kappa r)} \tag{5}$$

The net BSA charge, Z, is evaluated by taking into account the effects of both charge regulation and chloride ion binding. The calculations include the dissociation equilibria for six distinct types of amino acid residues (12) and three specific Cl^- binding sides (13). The net charge on BSA can be calculated from the differences in H^+ and Cl^- binding as described by Pujar and Zydney (14) [see Eq. (6)]:

$$Z = 96 - \sum_i \frac{n_i K_i^{\text{int}}}{K_i^{\text{int}} + \left[H_b^+\right] \exp(-e\zeta/kT)} - \sum_j \frac{m_j K_j \gamma [Cl^-] \exp(e\zeta/kT)}{1 + K_j [Cl^-] \exp(e\zeta/kT)} \tag{6}$$

In Eq. (6), γ is the activity coefficient of Cl^- and the value of 96 is the net charge on BSA at very low pH where all sites are protonated (14). The number (n_i) and intrinsic equilibrium constants (K_i^{int}) of titratable amino acids on BSA as well as the parameter values for Cl^- binding (m_j and K_j) are also given by Pujar and Zydney (14) By solving Eq. (5) and Eq. (6) together, the actual zeta potential on BSA is calculated.

EXPERIMENTAL

Materials

The PES (pH = 2–14) and CTA (pH = 4–8) ultrafiltration membranes (Sartorious, Germany) with nominal cut-off values of 10 kDa and diameters of 25 mm were used in the experiments. Before all experiments, the membranes were rinsed with MilliQ (Millipore, USA) deionized distilled water to remove preservatives. All solutions were prepared by using deionized water with the resistivity of 18 MΩcm. BSA (Sigma: A5674)

with the molecular weight of 67 kDa and IEP of 4.7–4.9 was used as the model protein. The BSA solutions of different ionic strengths were prepared using different concentrations of KCl solution. The pH and conductivity of solutions were measured with a Sartorius PP-50 Ion-meter, and pH of the medium was adjusted with either 0.01 M HCl or 0.01 M KOH solutions.

Static Adsorption Experiments

Static adsorption experiments of BSA on PES and CTA membranes were carried out in 150 mL Erlenmeyer flasks in an orbital shaker (Gallenkamp, UK) operated at 200 strokes min^{-1} and 30°C. Membranes were brought into contact with BSA solution until equilibrium was reached. The BSA concentration in the solution was determined with a spectrophotometer (Shimadzu UV-160A) at 277 nm and the amount of adsorbed protein per unit of membrane mass was calculated by the protein mass balance. Adsorption experiments were performed for the concentrations of 0.1–2.8 g dm^{-3} BSA. After adsorption, membranes were rinsed with distilled water to remove unbounded BSA and dried at room temperature for 24 h.

Streaming Potential Measurements

Experiments to characterize the membrane charge streaming potential measurements, which depend on the measurement of the electrical potential between two surfaces provided by moving the electrolyte solution through the porous membrane by an external pressure (3), were performed. These experiments were accomplished by means of a device of two chambers in which Ag/AgCl electrodes were inserted through the ends. The membranes were first stabilized with KCl solutions to be used and then the streaming potentials were measured as a function of the pressure difference. The membrane zeta potentials were calculated by the Helmholtz-Smoluckowski equation, Eq. (4), using the measured streaming potential values.

FTIR-ATR Measurement, SEM, and AFM Images

The surface of ultrafiltration membranes was characterized by FTIR-ATR spectroscopy (Perkin-Elmer 040520) to detect chemical changes after protein adsorption. The clean and used membranes were rinsed with deionized water three times and completely dried at room temperature before analyses. The ATR accessory of the FTIR contained a ZnSe crystal. The surface of membranes was also examined with a scanning electron microscope (SEM; LeoVP35) before and after protein adsorption. The AFM images

of the clean and used membranes were obtained using an atomic a force microscope (AFM Nanoscope III-a, Digital Instruments, Inc., Santa Barbara, CA, USA) in tapping mode.

RESULTS

Effect of pH and Ionic Strength on Adsorption

Isotherms for BSA adsorption on hydrophobic PES membranes were obtained at the pH values of 3.78, 4.78 (isoelectric point, pI), and 6.80 in 0.01 M KCl solution; and at the pH values of 4.78 and 6.80 in 0.1 M KCl solution (Fig. 1). The amount of BSA adsorbed was the lowest at its IEP where the maximum adsorption was obtained below the IEP of the protein. The increase in ionic strength provided by KCl solutions decreased the amount of BSA adsorbed. The equilibrium data fit the Freundlich isotherm (15). The parameters of the isotherm were determined by a nonlinear regression program and given in Table 1.

Isotherms for BSA adsorption on hydrophilic CTA membranes were obtained at the pH values of 4.78 (pI) and 6.80 in 0.01 M and 0.1 M KCl solutions (Fig. 2). More BSA was adsorbed above the IEP of the protein;

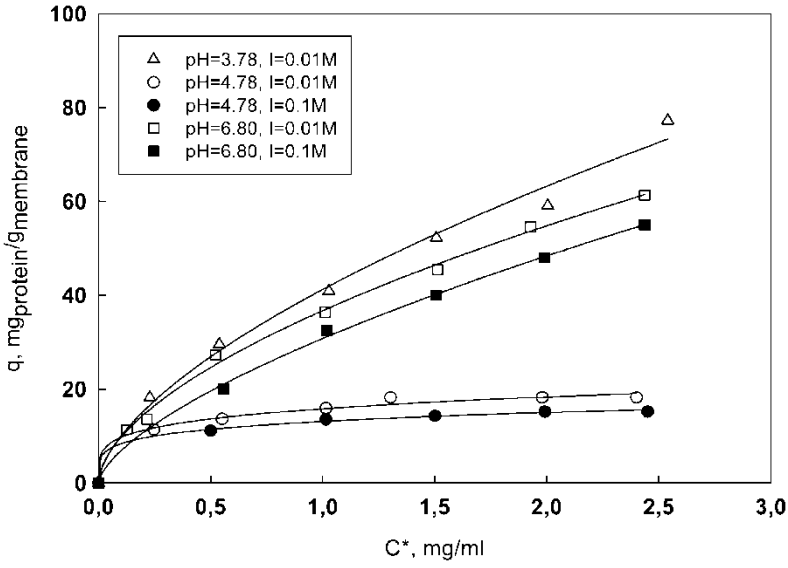

Figure 1. Adsorption isotherms for BSA on PES membranes at different pH and ionic strength values.

Table 1. Freundlich equations for BSA adsorption on ultrafiltration membranes

		Adsorption isotherms	
	pH	0.01M KCl	0.1M KCl
PES	3.78	$q = 41.21(C^*)^{0.62}$ $R^2 = 0.99$	—
	4.78	$q = 15.84(C^*)^{0.26}$ $R^2 = 0.98$	$q = 13.09(C^*)^{0.19}$ $R^2 = 0.99$
	6.80	$q = 36.74(C^*)^{0.58}$ $R^2 = 0.99$	$q = 30.76(C^*)^{0.65}$ $R^2 = 0.99$
CTA	4.78	$q = 14.52(C^*)^{0.32}$ $R^2 = 0.98$	$q = 13.75(C^*)^{0.36}$ $R^2 = 0.99$
	6.80	$q = 24.27(C^*)^{0.57}$ $R^2 = 0.99$	$q = 16.59(C^*)^{0.37}$ $R^2 = 0.99$

and the decrease in the ionic strength of the protein solution increased the adsorption (15). The isotherms showed a good agreement with the Freundlich equation (Table 1).

Interaction Energies During Adsorption

The pH and ionic strength dependences of the zeta potentials for PES and CTA membranes found by Eq. (4) are given in Table 2. It was found that PES and CTA membranes were both negatively charged under all the conditions

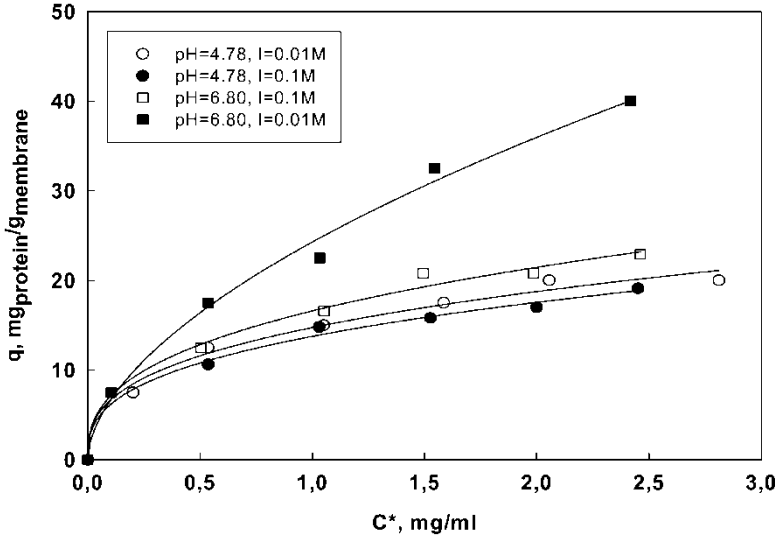

Figure 2. Adsorption isotherms for BSA on CTA membranes at different pH and ionic strength values.

Table 2. Zeta potentials for ultrafiltration membranes at different pH and ionic strength values

	10 kDa PES		10 kDa CTA	
pH	0.01M KCl ζ (mV)	0.1M KCl ζ (mV)	0.01M KCl ζ (mV)	0.1M KCl ζ (mV)
3.78	−34.40	−20.99	—	—
4.78	−43.66	−23.07	−18.20	−13.65
6.80	−48.33	−33.71	−25.9	−19.83

applied; and, the zeta potential of hydrophobic PES membrane was higher than was that of hydrophilic CTA membrane. The increase in pH increased the zeta potential of the membranes whereas the increase in ionic strength decreased the zeta potential. The zeta potentials for BSA at different pH and ionic strength values were calculated by Eqs. (5) and (6) (Table 3). In the calculations, the radius of BSA, **r**, was taken as 3×10^{-9}m (16) and H was assumed to be 1×10^{-9}m. The zeta potential of the protein was minimum at the IEP, negative at the higher pH than at the IEP; contrariwise, positive at the lower pH than at the IEP. The van der Waals interaction energy was calculated by Eq. (2) taking Hamaker coefficients for PES and CTA membranes to be A = 0.18 kT and A = 1.96 kT, respectively (8).

The calculated electrostatic energy, the van der Waals energy, and total interaction energy between the protein and the membranes are given in Tables 4 and 5. The results revealed that the electrostatic energy was the minimum at the IEP of the protein for both membranes. At pH = 3.78 and 4.78, the attraction forces were dominant; however, at pH = 6.80, the repulsion forces were dominant, except for CTA membranes at high ionic strength.

Structural Changes on Membrane Surfaces after Adsorption

The PES and CTA membrane surfaces were analyzed using the FTIR-ATR spectra to detect the structural chemical changes originated by the protein adsorption. The FTIR-ATR spectra of PES and CTA membranes at different pH values are shown in Fig. 3 and Fig. 4, respectively. Two

Table 3. The zeta potentials of BSA at different pH and ionic strength values

	pH = 3.78	pI = 4.78	pH = 6.80
Zeta potential (V)-0.01M	0.0394	0.0052	−0.0283
Zeta potential (V)-0.1M	0.0264	−0.0001	−0.0236

Table 4. The electrostatic, the van der Walls, and total interaction energies between BSA and PES membranes

	pH		
	3.78	4.78	6.80
Ee (J) 0.01 M KCl	-1.45×10^{-20}	-4.69×10^{-21}	5.58×10^{-21}
Ee (J) 0.1 M KCl	-2.04×10^{-21}	-1.43×10^{-22}	1.99×10^{-21}
Ew (J)		-8.86×10^{-22}	
Total energy (J; DLVO theory)			
0.01 M KCl	-1.54×10^{-20}	-5.58×10^{-21}	3.69×10^{-21}
0.1 M KCl	-1.33×10^{-20}	-1.03×10^{-21}	1.10×10^{-21}

characteristic peaks were inspected as the evidence of the adsorption of BSA on the membrane surfaces, the amide I band, which was observed at $1650\,cm^{-1}$, and the amide II band, which was seen at $1540\,cm^{-1}$. The spectra of the two membranes at all pH values revealed the presence of the amide I band. However, the amide II band appeared only on PES membrane at pH = 3.78 where the highest extent of protein adsorption was obtained (Fig. 3a). The lowest intensity of the amide I band was observed on the membrane fouled at the IEP (4.78) (Fig. 3c), where the adsorption degree was the lowest (Fig. 1). On the other hand, the intensity of the amide I peak was lower on the CTA membranes than that observed on PES membranes related to less adsorption. The lowest intensity of the amide I band on CTA membranes was observed at the IEP of the protein (Fig. 4a).

The SEM images of clean and protein-fouled PES and CTA membranes are given in Figs. 5 and 6, respectively. More membrane fouling was seen on PES membrane surfaces than was obtained on CTA membranes, which coincided with the adsorption results. The AFM images of the PES

Table 5. The electrostatic, the van der Walls, and total interaction energies between BSA and CTA membranes

	pH	
	4.78	6.80
Ee (J) 0.01 M KCl	-1.27×10^{-21}	3.31×10^{-21}
Ee (J) 0.1 M KCl	-4.85×10^{-23}	1.19×10^{-21}
Ew (J)		-2.00×10^{-21}
Total energy (J; DLVO theory)		
0.01 M KCl	-3.27×10^{-21}	1.31×10^{-21}
0.1 M KCl	-2.05×10^{-21}	-8.10×10^{-22}

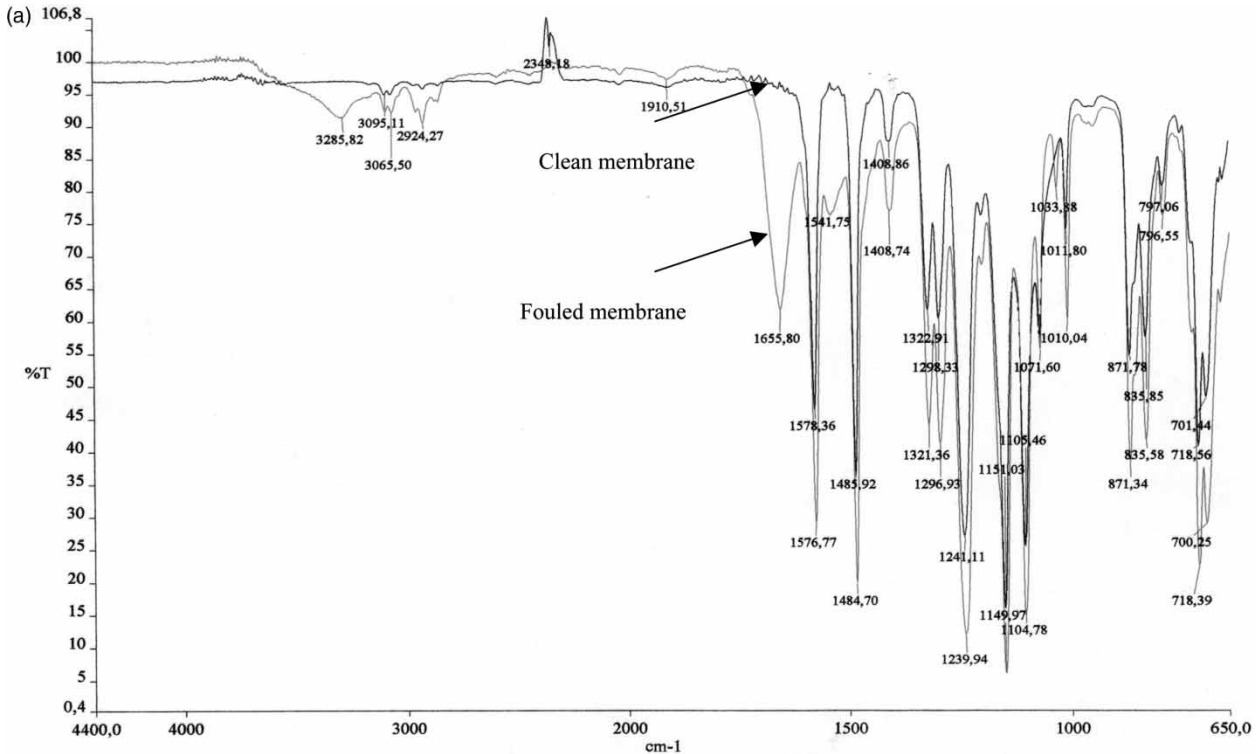

Figure 3. FTIR-ATR spectra of clean and protein-fouled PES membranes in 0.01 M KCl at a) pH = 3.78; b) pH = 4.78; c) pH = 6.80. *(continued)*

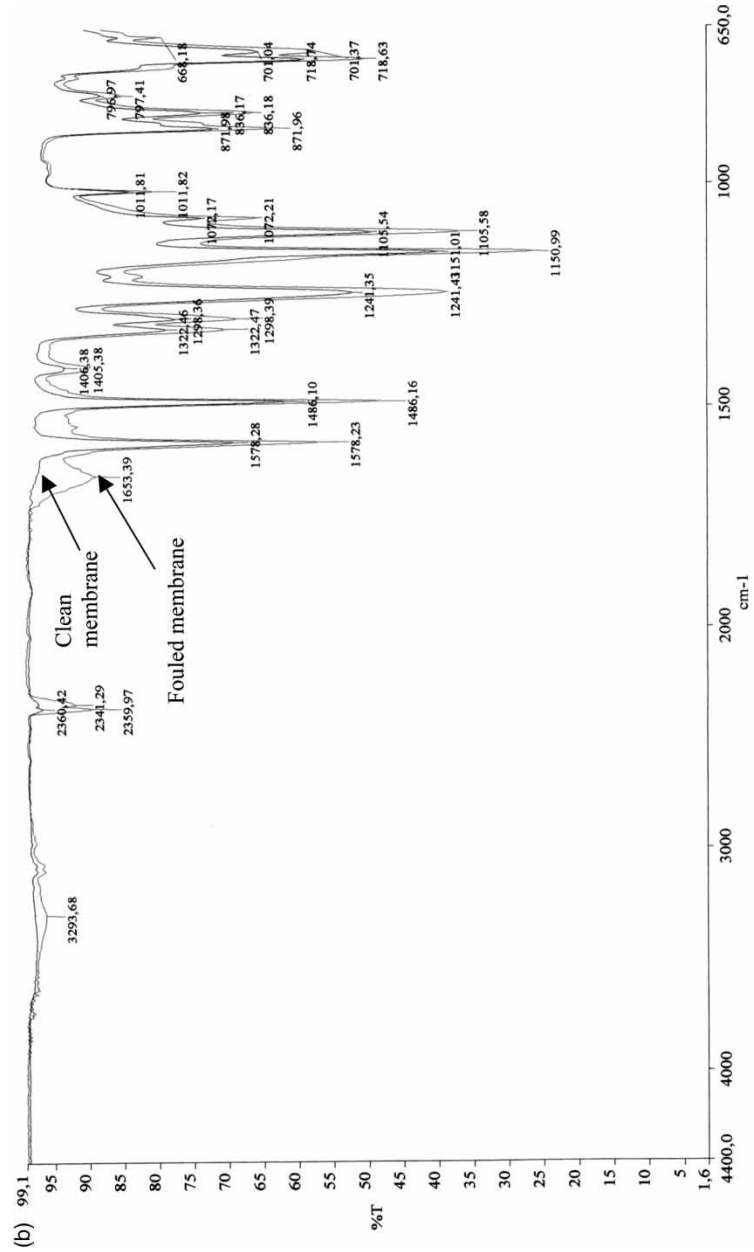

Figure 3. Continued.

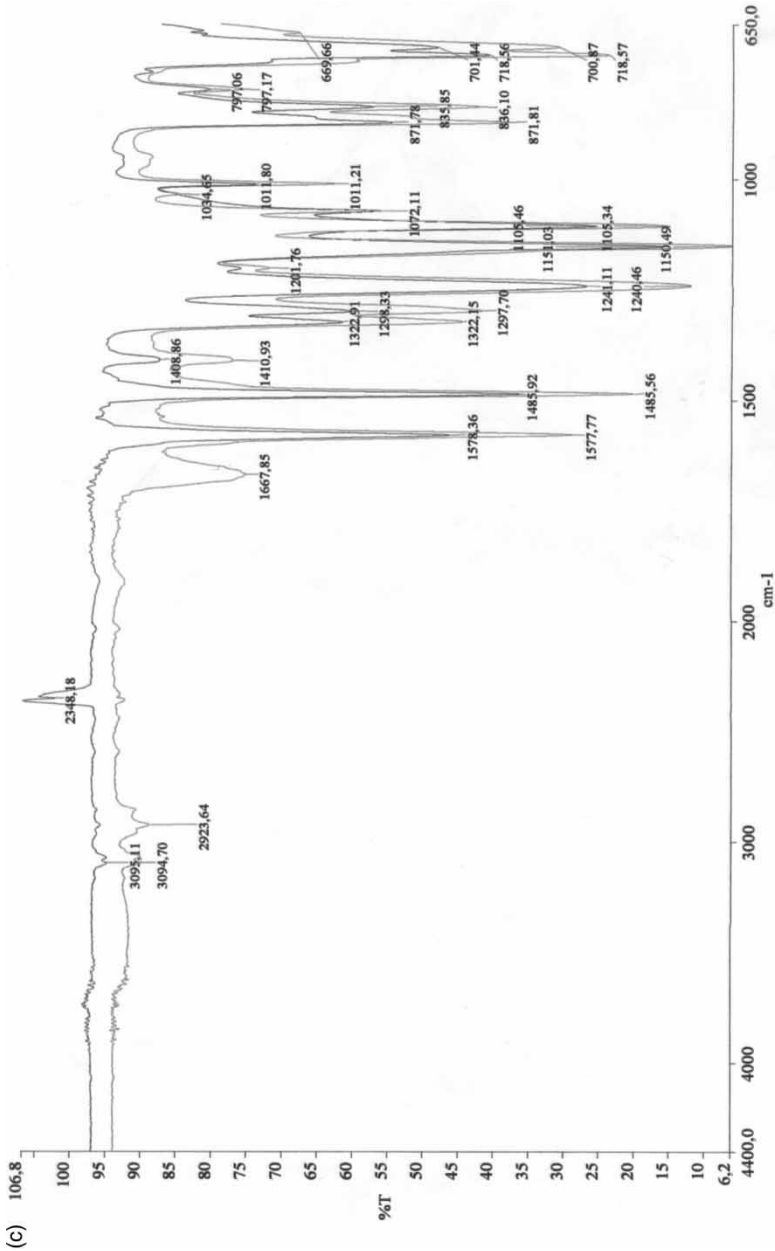

Figure 3. Continued.

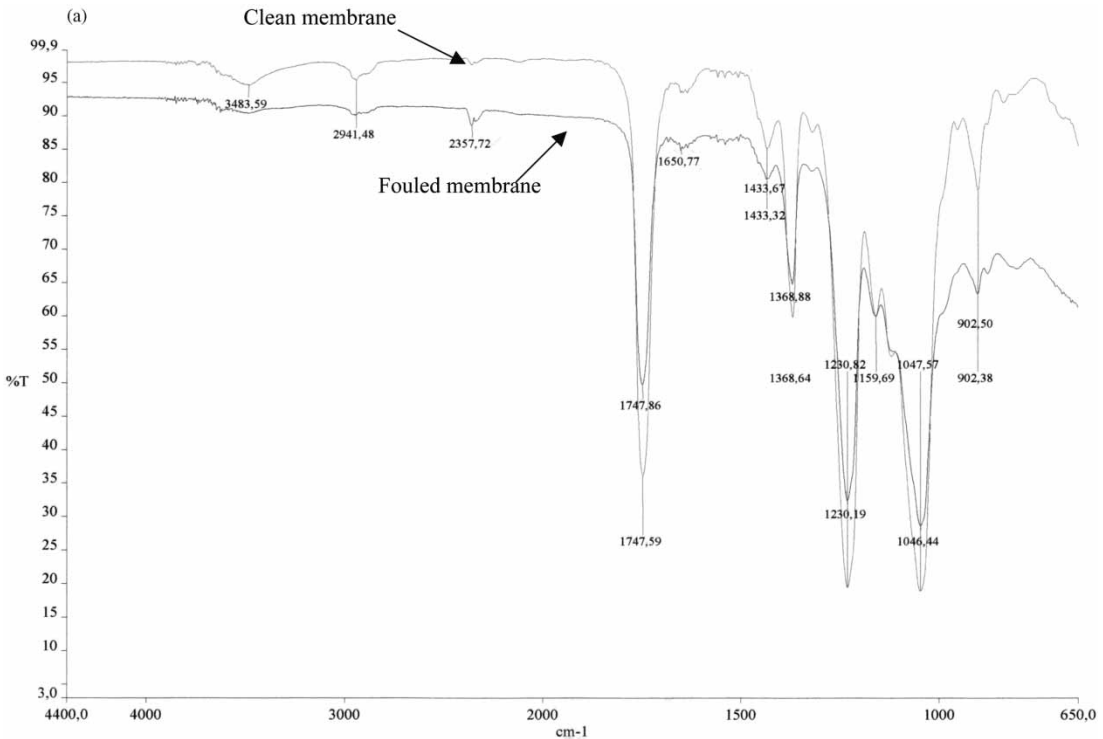

Figure 4. FTIR-ATR spectra of clean and protein-fouled CTA membranes in 0.01 M KCl at a) pH = 4.78 b) pH = 6.80.

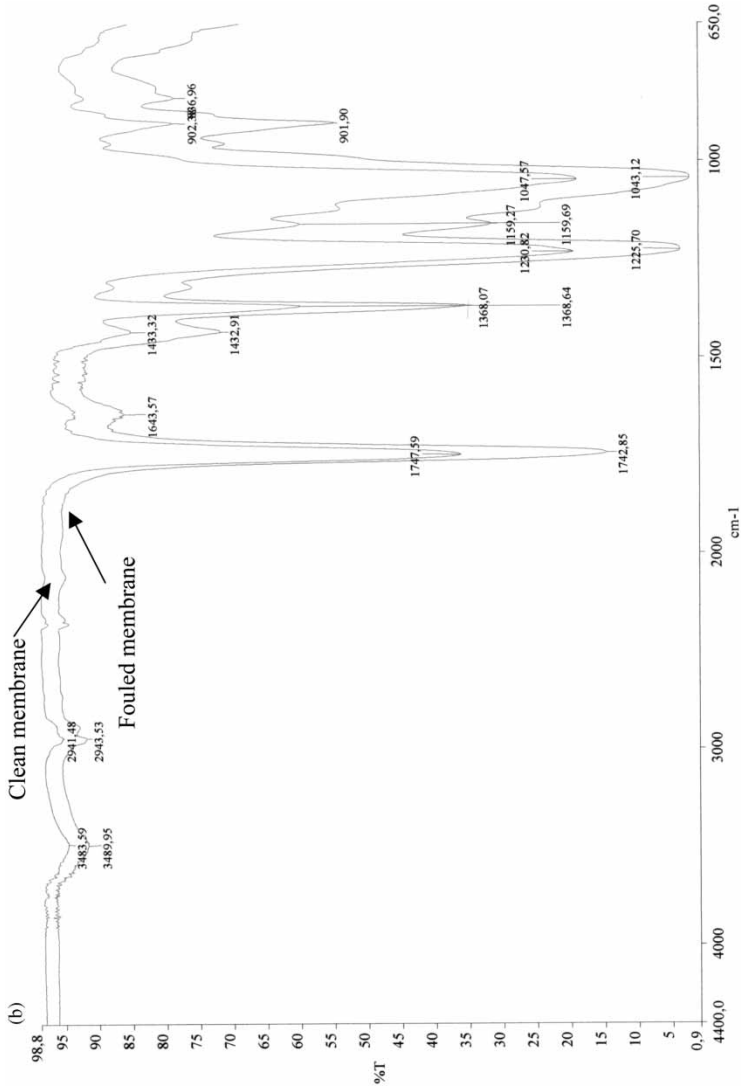

Figure 4. Continued.

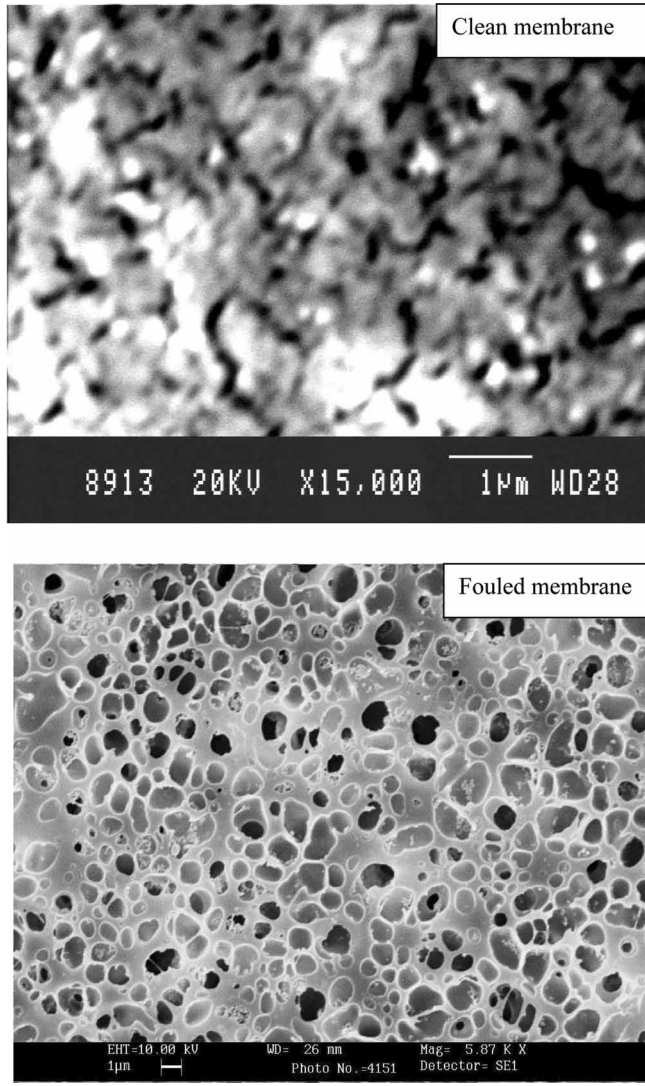

Figure 5. SEM images of clean and protein-fouled PES membranes in 0.01M KCl at pH = 3.78.

membrane surfaces taken after protein fouling are shown in comparison with the clean membrane surfaces in Fig. 7. The AFM images also showed that the membranes were covered by a different extent of protein layers depending on the solution pH during adsorption. The lowest and the highest protein coverage were seen on the membranes fouled at pH = 4.78 and pH = 3.78, respectively, which agreed well with the adsorption data.

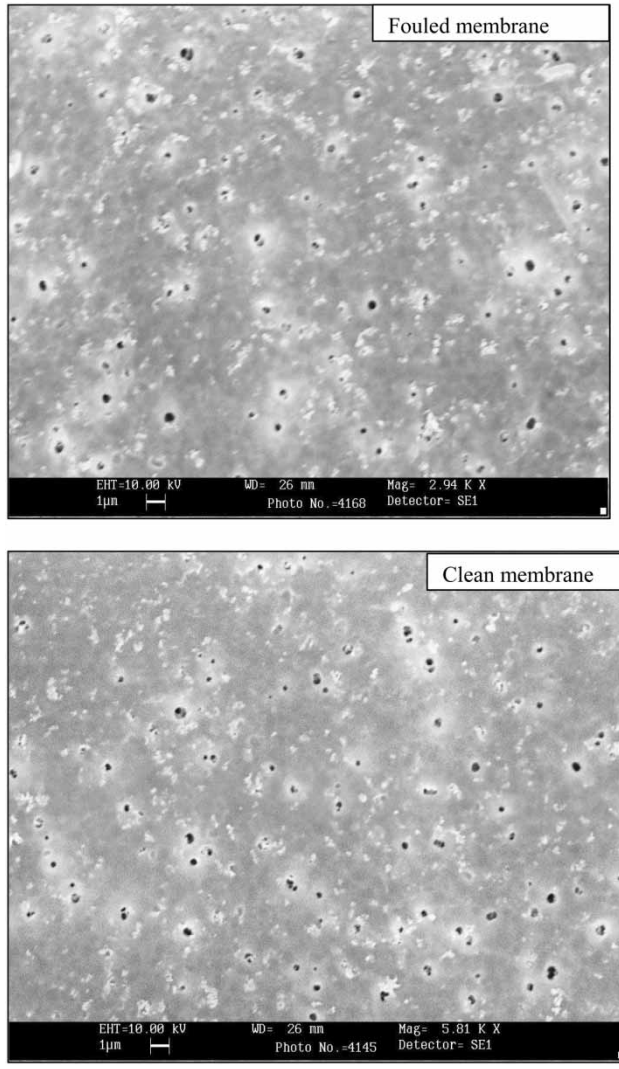

Figure 6. SEM images of clean and protein-fouled CTA membranes in 0.01 M KCl at pH = 6.80.

DISCUSSION AND CONCLUSIONS

Since adsorption is one of the characteristics of membrane fouling, static adsorption of BSA on PES and CTA membranes was investigated at different ionic conditions. Hydrophobic forces were the first determinative factor for the dissimilarity in protein adsorption on different membrane

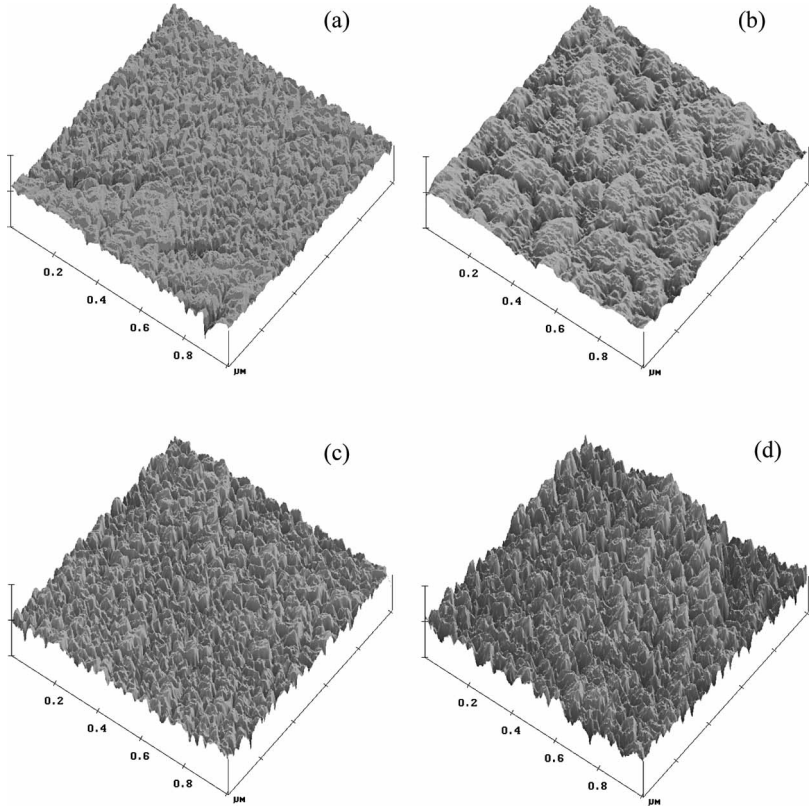

Figure 7. AFM images of PES membranes in 0.01 M KCl at a) clean membrane b) protein-fouled at pH = 3.78, c) protein-fouled at pH = 4.78, d) protein-fouled at pH = 6.80.

surfaces. More BSA was adsorbed on hydrophobic PES membranes than it was on hydrophilic CTA membranes at all the pH and ionic strength values studied. The hydrophobic interactions between the PES membrane and protein surfaces resulted in a high degree of adsorption. In addition, protein-membrane interactions could cause changes in the structure of adsorbed molecules. The globular structure of BSA adsorbed on the surface of hydrophilic membranes changes little according to the native state in free solution. However, on the surface of hydrophobic membranes, the protein appears long and filamentous, more open and denaturated (17), which can increase the extent of adsorption. Another reason for the higher adsorption on PES membranes was the higher surface charges of hydrophobic PES membranes compared with those of hydrophilic CTA membranes (Table 2).

The PES and CTA membranes used in this study are asymmetric membranes, which are characterized by a thin skin on the surface. Protein adsorption on asymmetric membranes is reported to occur only at the membrane surface and maybe at the pore entrance, because of their ultrastructure (17). On the other hand, since the molecular weight of BSA was higher than was the MWCO (molecular weight cut-off) value of the membranes, the adsorption within the membrane pores seems to be difficult. Therefore, it can be concluded that the adsorption occurs mainly on the membrane surface.

Membranes gain charge due to the ionization of polar groups at their surfaces or due to the adsorption of electrolyte ions from the surrounding solution onto the membrane surface. Therefore, besides the protein charge that is dependent on pH and ionic strength of the solution, the charge and density of the membrane surface affect the extent of adsorption. The zeta potentials of hydrophobic PES and hydrophilic CTA membranes showed that both membranes were negatively charged at all pH and ionic strength values; moreover, the zeta potential of the membranes increased with increasing pH (Table 2). Since the increased absolute value of zeta potential of the membrane decreases the hydrophobicity (6), less adsorption was observed on PES membranes at pH = 6.80 compared with the adsorption at pH = 3.78 (Fig. 1). On the other hand, the sign of zeta potential of the protein surface changed with the solution pH (Table 3). Below the IEP, i.e., at pH = 3.78, BSA had a positive charge; contrariwise, PES membrane had a negative charge. Therefore, below the IEP for PES membrane, attraction forces were dominant over repulsion forces and the protein showed a tendency to be adsorbed. Above the IEP of the protein, i.e., at pH = 6.80, the surfaces of the PES and CTA membranes as well as the protein had negative charges. Although less adsorption was expected at this pH in comparison with pH = 4.78 due to the repulsion forces; a considerable amount of adsorption was observed. Robertson and Zydney (18) also reported that proteins could be adsorbed quite strongly even to surfaces with the same charge as the protein. In addition, at the pH values above IEP, BSA is reported to undergo an expansion due to intramolecular electrostatic repulsion between electrical charges of the same sign. Less of this larger BSA can fit on the membrane surface (19). This behavior shows that the adsorption is not only due to electrostatic and van der Waals interactions that is considered by DLVO theory; but it is also due to hydrophobic, hydrophilic, structural, and steric interactions between the protein and the membrane as well as between protein molecules. The unexpected attraction forces found at pH = 6.80 and 0.1 KCl conditions for CTA membranes (Table 5) are therefore, due to the lack of such interactions in the DLVO theory. At the IEP of BSA, the degree of adsorption was minimum related to mainly low electrostatic interactions.

The ionic strength of the medium also affects the extent of adsorption. With increasing ionic strength, the adsorbed protein on both membranes

decreased as the membrane and protein surface charges became more shielded by the counterions in the solution resulting in a decrease in electrostatic interactions. The decrease in total interaction energy with increasing ionic strength is seen in Tables 4 and 5.

The comparison of the membrane surfaces using FTIR-ATR spectra before and after adsorption showed the presence of the amide-I and amide-II bands on the fouled membranes, which originates from the C═O stretching vibration of the peptide groups and is a characteristic of the bending of N-H groups of the protein in the plane, respectively. The variation in the intensity of the amide-I peak with the solution pH was in agreement with the variation in the degree of adsorption (Figs. 3 and 4). On the other hand, the amide-II band was not seen at all the conditions. The intensities of the bands observed on hydrophobic PES membranes were higher than were those observed on hydrophilic CTA membranes, as the result of more fouling on the PES membranes. The SEM images of the membranes also showed that the deposition of protein molecules on the PES membrane surfaces were higher than they were on the CTA membranes. In addition, AFM images of the PES membranes fouled at different solution pH values clearly showed that the adsorbed protein layer was higher at the pH values above and below the IEP of BSA than the adsorbed protein layer that was observed at the IEP; and the highest protein layer was observed at pH = 3.78.

The results of the present work are evidence that electrostatic forces are as important as hydrophobic interactions in membrane fouling with proteins. Protein-membrane interaction was found to be a strong function of the solution pH and ionic strength for both hydrophobic and hydrophilic membranes since protein molecules and membrane surfaces change their charged states with alterations in their ionic environment. The results also revealed that the adsorption data did not agree entirely with the calculated interactions energies under all the conditions studied and that the intermolecular interactions of protein should be included in the energy calculations.

ABBREVIATIONS

A	Hamaker coefficient of system that describes the net van der Waals interactions between the protein, membrane surface and solvent
BSA	Bovine serum albumin
Ci	the concentration of ionic species
CTA	cellulose triacetate
e	the electronic charge
E_e	electrostatic interaction energy (J)
E_W	van der Waals interaction energy (J)
E_z	streaming potential (V)
F	Faraday constant (96,500 C mol^{-1})

H	separation distance (m)
$[H_b^+]$	bulk hydrogen ion concentration (M)
IEP	isoelectric point
K	a parameter value for chloride ion binding
K^{int}	intrinsic equilibrium constant of the titratable amino acids on BSA
m	a parameter value for chloride ion binding
n	the number titratable amino acids on BSA
PES	polyether sulfone
r	the radius of the protein (m)
R	gas constant (8.314 J $mol^{-1}K^{-1}$)
T	absolute temperature (K)
z_i	the valance of ion
ϵ_o	permittivity of vacuum (8.85×10^{-12} $CV^{-1}m^{-1}$)
ϵ_r	dielectric constant of the solution
κ	the inverse Debye screening length (m^{-1})
ζ_1	the zeta potential of the membrane (mV)
ζ_2	the zeta potential of the protein (mV)
ΔP	applied pressure difference (N m^{-2})
η	viscosity of the solution (kg m^{-1} s^{-1})
Λ	conductivity of the solution (S m^{-1})
γ	the activity coefficient of chloride ion

ACKNOWLEDGMENTS

The authors acknowledge the financial support provided by The Scientific and Technical Research Council of Turkey (TUBITAK-MISAG 225), and by the Ankara University (Scientific Research Project No: 2001-07-05-061).

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