Formation and Characterization of Chitosan Formed-in-Place Ultrafiltration Membranes

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ABSTRACT: Formed-in-place (FIP) ultrafiltration (UF) membranes were formed from dilute solutions of chitosans with different molecular weights in 1% acetic acid on a macroporous titanium dioxide substrate. The ultrafiltration properties were characterized by investigating the rejection and permeability of a 1.0 g/L bovine serum albumin (BSA) solution at various pH and ionic strength conditions. The membrane stability to the crossflow shear and to the ionic strength was investigated. There was very little dependence of the membrane-formation capability and the membrane properties on the chitosan molecular weight. In contrast, pH had a marked effect on membrane surface properties, membrane stability, and membrane morphology. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci **67:** 513–519, 1998

Key words: chitosan UF membrane; dynamic formation; BSA rejection; ionic strength effects

INTRODUCTION

A formed-in-place (FIP) membrane is dynamically created by depositing organic and inorganic polymers on the surface or at the entrance of the pores of macroporous substrates that are usually made of materials having excellent chemical and mechanical stability, such as stainless steel, ceramics, plastics, or carbon. FIP membranes, partly due to their reformation capabilities, have attracted attention for nanofiltration, ultrafiltration, and microfiltration applications involving separation, concentration, and purification in textile, biotechnology, food processing, and pharmaceutical fields.[1–7]

In this research, chitosan is deposited to form membranes on a macroporous TiO_2 layer sintered on the inner surface of a porous stainless-steel support tube. Chitosan is a natural biopolymer obtained by deacetylation of chitin, which is produced from marine shellfish, such as crab, shrimp, and krill.[8] Chemically, it is a linear polymer of 2-amino-2-deoxy-D-glucopyran connected by β-1,4-linkages.[9] Since there is an amino group on each glucose ring, chitosan can be dissolved in acidic solutions and is a weakly basic polyelectrolyte which possesses positive charges at low pH. Therefore, chitosan can be easily removed from the TiO_2 substrate whenever desired by circulating an acid solution through the module at low pressure and high crossflow velocity.

The purpose of the present work was to form chitosan membranes on the TiO_2 substrate, to study their ultrafiltration properties, such as the rejection of BSA under different ionic strength and pH conditions, and their stability, and to investigate the dependence of membrane stability and retentive ultrafiltration properties on the molecular weight of chitosan.

EXPERIMENTAL

Membrane Formation

The chitosan FIP membranes were formed using dilute solutions of chitosan on a macroporous tita-

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Table I Chemical Characterization of the Chitosan Samples

Sample	Molecular Weight (kDa)	Degree of Acetylation (%)
LM	70	15.0
MM	720	13.5
HM	2000	13.5

nium dioxide (TiO_2) layer sintered on the inner surface of a stainless-steel porous tube provided by Du Pont Separation Systems, Inc., Seneca, SC. The tubular substrate was 0.61 m in length and 0.016 m in the inner diameter, with a membrane area of 0.030 m^2. The chitosans used in the experiments were obtained from Fluka Chemical Corp. (Ronkonkoma, NY) purified by washing in 0.01*M* ethylenediaminetetraacetic acid disodium salt (EDTA), rinsing with ethanol, and drying at 60°C overnight. The molecular weight determined by viscometry and the degree of acetylation by a spectrophotometric method to an accuracy of 1% were provided by the manufacturer for each sample and are listed in Table I.

The membrane formations were performed under a constant pressure (P) of 1.7 bar (25 psi), temperature of 25 ± 1°C, and crossflow velocity (u) of 0.32 ± 0.02 m/s in a filtration system described previously.[10] The formation solution was prepared by first dissolving 1.0 g of chitosan in 500 mL 2% HOAc (acetic acid) solution and then diluting with a 2% HOAc solution and water in the feed tank to 10 L. The concentration of the formation solution was finally 0.1 g/L of chitosan and 1.0% of HOAc. The volume flux, J, was measured before the formation, during the period of the formation, at the end of formation, and after two crossflow rinses with water at a higher crossflow velocity, u = 2.5 m/s.

Ultrafiltration of Dilute BSA Solutions and Membrane Cleaning

The ultrafiltration properties of the membranes were investigated by measuring the volume flux, J, and the solute rejection r(BSA) of a 1.0 g/L BSA solution as a function of ionic strength and pH. The bovine serum albumin fraction V (BSA) was obtained from the United States Biochemical Corp. (Cleveland, OH). The molecular weight of BSA obtained by a laser matrix oblation time-of-flight mass spectrometer method in our laboratory was 66 kDa. The ionic strengths of the solutions used in the ultrafiltration experiments were adjusted by adding a 2*M* KCl solution and the pH was adjusted by adding a minimum amount of 2*M* HCl or KOH solution. The ultrafiltration experiments were run at a complete recycle of permeate and concentrate under a constant pressure of 1.9 bar (27 psi) and a temperature of 25 ± 1°C at pH 3.6 ± 0.2, 6.0 ± 0.2, and 8.2 ± 0.2. BSA rejections were determined from the concentrations of the protein in the permeate and in the feed obtained using a UV-20101 PC, UV-VIS scanning spectrophotometer (Shimadzu) at the wavelength of 280 nm. The solution flux was measured at regular intervals during each ultrafiltration experiment and water flux was determined after two crossflow rinses with water following the discharge of the BSA solution. All the crossflow velocities in the ultrafiltration tests and water rinses were 2.5 m/s.

After BSA filtration, the membrane was cleaned by circulating a mixture of 1% (w/v) NaOH and 1% (v/v) of 30% H_2O_2 10–15 L for 6–12 h and then 1% (v/v) of concentrated HNO_3 for 1–4 h at 40°C. The crossflow velocity and transmembrane pressure were changed occasionally from 3.0 to 0.3 m/s and 1.1 to 2.2 bar in order to clean both the membrane surface and membrane pores. Two 10 min rinses with water followed these cleaning steps, and water permeability was determined to check the cleaning sequence efficiency. An additional cleaning cycle was executed whenever needed. The cleaning procedure was always concluded with the HNO_3 rinse to ensure that the substrate surface was in the same state for each experiment. All the water used in the experiments was deionized and then filtered through an FIP ultrafiltration membrane.

RESULTS AND DISCUSSION

Determination of the deposition model describing the formation of the chitosan membranes by examining the functional dependence of the flux, J, on time, t, during the deposition of the chitosan was not possible with the experimental setup because the major portion of the flux decline occurred too rapidly to obtain interpretable plots.[11,12] The $(P/J)^2$ vs. t plots in Figure 1 show that the membrane formation was completed in less than 5 min under the formation conditions. The resistance of the membranes increased mod-

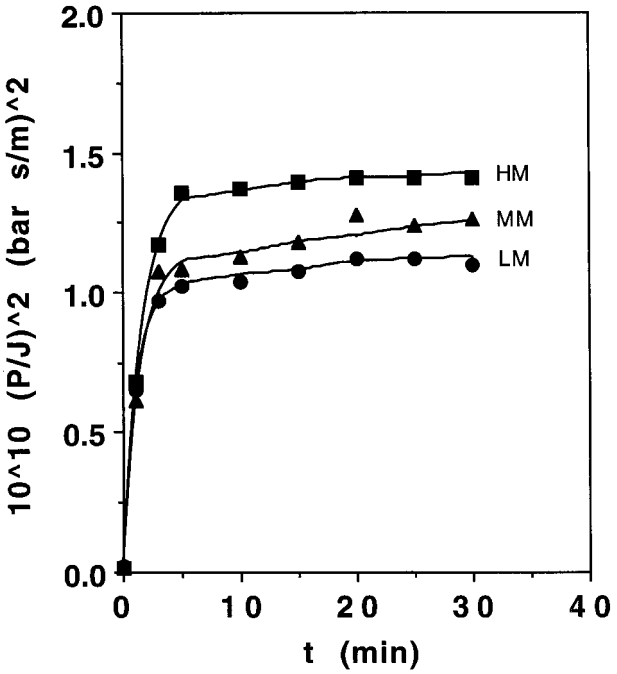

Figure 1 Plots of $(P/J)^2$ vs. t during the membrane formation of chitosans with different molecular weights, (HM) 2000 kDa, (MM) 720 kDa, and (LM) 70 kDa, with the following formation conditions: 1.0 g/L chitosan in 1% acetic acid; $P = 1.7 \pm 0.1$ bar; $T = 25 \pm 1$°C; $u = 0.32 \pm 0.02$ m/s.

estly with the molecular weight of the chitosan used in the formations.

The results of the steady-state rejection of BSA, r(BSA), vs. $[KCl]^{1/2}$ obtained in the ultrafiltration experiments on the chitosan FIP membranes and the TiO_2 microfiltration substrate using 1.0 g/L BSA at pH 6.0 ± 0.2 are shown in Figure 2. The chitosan membranes showed high BSA rejection at very low ionic strength, whereas the substrate without the chitosan coating had almost no BSA rejection at all. The BSA rejection by the chitosan membranes declined with the addition of KCl and was almost identical for the three membranes formed with different molecular weight chitosans. However, the ultrafiltration properties were sensitive to the changes in pH as shown in Figures 3 and 4. At pH 3.6, where both the protein and membrane possess positive charges, high BSA rejection and solution permeability were observed at low ionic strength, possibly due to the electrostatic repulsive forces between BSA and the membrane as well as between the BSA molecules. However, the rejection declined rapidly as the ionic strength increased.

There were probably at least two contributions to the decline: The first was the decrease of the electrostatic repulsive force with increasing ionic strength that made the passage of the BSA molecules or its aggregates easier. The second was the loss or rearrangement of the membrane during the test. The chitosan gel formed on the substrate was not stable to the crossflow current under this acidic condition, possibly due to the repulsive force between the chitosan molecules in the gel and the high solubility of the chitosan in the acidic solution. The volume flux increased as the ionic strength increased as shown in Figure 4, but remained much smaller than the flux through the titania microfiltration substrate using the same BSA solution and operating conditions. On the other hand, the BSA rejections showed a much smaller decline with increasing ionic strength in the ultrafiltration at pH 6.0 or 8.2. At these pH conditions, the BSA carried negative charges and the membrane was nearly uncharged or carried a very low density of positive charges. The chitosan gels were crosslinked enough by the intermolecu-

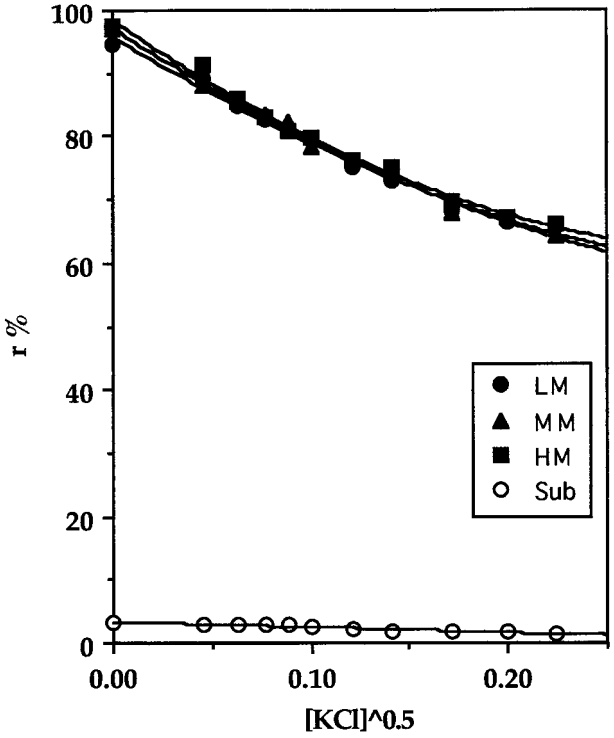

Figure 2 Dependence of steady-state rejection of 1.0 g/L BSA solutions on the concentration of added KCl at pH 6.0 by membranes formed from chitosan with different molecular weights, (HM) 2,000 kDa, (MM) 720 kDa, and (LM) 70 kDa, and the substrate (Sub). Experimental conditions: $P = 1.9 \pm 0.1$ bar; $T = 25 \pm 1$°C; $u = 2.5 \pm 0.1$ m/s.

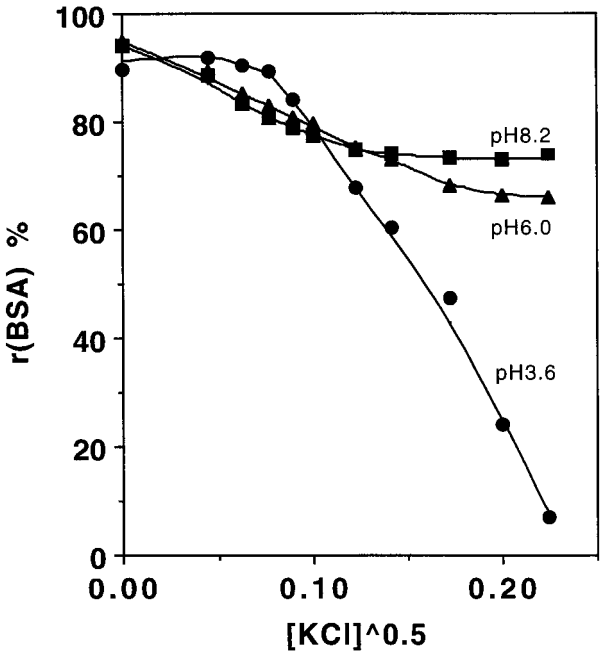

Figure 3 Dependence of steady-state rejection of 1.0 g/L BSA solutions on the concentration of added KCl at pH 8.2, 6.0, and 3.6 by a membrane formed with low molecular weight (70 kDa) chitosan. Experimental conditions: $P = 1.9 \pm 0.1$ bar; $T = 25 \pm 1$°C; $u = 2.5 \pm 0.1$ m/s.

lar forces, such as hydrogen bonding and van der Waals forces, to exhibit little alteration of the flux in the higher crossflow velocity and the high ionic strength as shown in Figure 4. The membrane tested at pH 8.2 displayed even weaker ionic strength effects on the BSA rejection and on the membrane stability than that at pH 6.0. At pH 8.2, the chitosan is essentially uncharged, so that the effects of ionic strength observed at this pH should be determined by its effect on the BSA–BSA interaction.

To evaluate the membrane stability, the volume flux of water was determined at selected steps in the experimental sequences and used to calculate the membrane resistances as described previously.[10] The membrane resistances for each formation and ultrafiltration experiment are summarized in Table II. The values of $P/\eta J_{wbf}$ indicate that the resistance of the substrate upon which the various membranes were formed were similar: $1.5 \pm 0.1 \times 10^{12}$ m^{-1}. Despite the substantial deposition of chitosan on the substrate [shown by the large $P/(\eta J_f)$ and $N(R)$ values], all the chitosan membranes showed a dramatic flux increase after the water crossflow rinses ($u = 2.5$ m/s) following the formation [shown by the small $P/(\eta J_{waf})$ and $N(R_m)$ values]. This was not caused only by the desorption of the chitosan from the substrate or the elimination of the osmotic pressure across the membrane during the crossflow water rinse. Actually, the main cause of the flux increase was the constriction of the membrane layer by raising the pH. When the membrane was rinsed with water, the pH increase resulted in the reduction of the number of the positively charged amine groups ($—NH_3^+$) in the deposition layer. Therefore, the chitosan gel tended to constrict during the procedure of water rinses due to the reduction of the repulsive force between the chitosan molecule chains.

This mechanism was further demonstrated by testing the membrane with solutions not containing BSA or chitosan at different pH shown in Figure 5. After forming the membrane, instead of rinsing with water, the system was rinsed with 1.0% HOAc solution (the formation solution without chitosan), then rinsed with water of pH 7–8. The immediate small flux increase should be caused by the loss of some deposited chitosan and/or the elimination of the osmotic pressure. The large flux increase was caused by the polymer gel

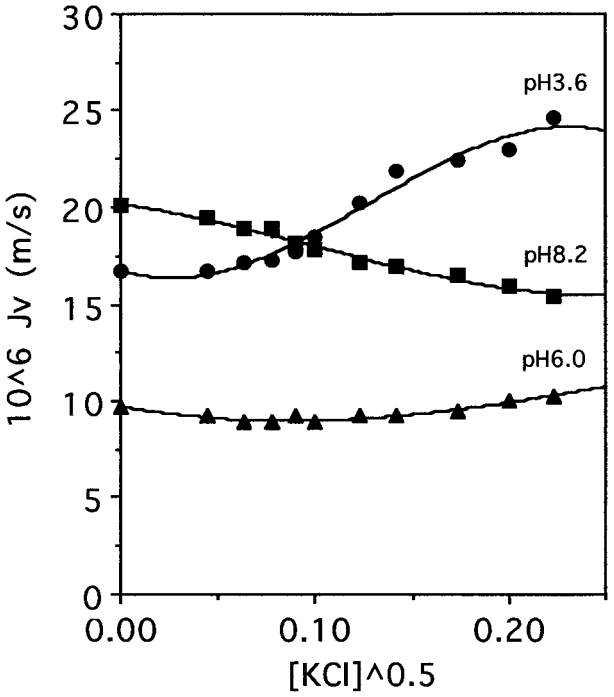

Figure 4 Dependence of steady-state flux of 1.0 g/L BSA solutions on the concentration of added KCl at pH 8.2, 6.0, and 3.6 by a membrane formed with low molecular weight (70 kDa) chitosan. Experimental conditions: $P = 1.9 \pm 0.1$ bar; $T = 25 \pm 1$°C; $u = 2.5 \pm 0.1$ m/s.

Table II Experimental and Normalized Characteristic Resistance for the Chitosan Membrane of Different Molecular Weights and BSA Ultrafiltration at Different pH Conditions

Alginate	$\frac{P}{\eta J_{wbf}}$	$\frac{P}{\eta J_f}$	$\frac{P}{\eta J_{waf}}$	$\frac{P}{\eta J_{wa}}$	$N(R)$	$N(R_m)$	$N(R_a)$
LM (pH 3.6)	1.6	18.1	3.3	5.1	11.3	1.1	1.1
LM (pH 6.0)	1.4	18.1	3.6	13.8	12.9	1.6	7.3
LM (pH 8.2)	1.5	17.2	3.5	6.8	11.5	1.3	2.2
MM (pH 6.0)	1.5	18.2	4.0	13.6	12.1	1.7	6.4
HM (pH 6.0)	1.5	18.8	4.2	14.3	12.5	1.8	6.7

The unit of the resistance: 10^{12}/m.

shrinkage which increased the pore size of the membrane. This result suggested that the chitosan gel or adsorbed layer was formed in the pores of the substrate and the membrane retained a pore morphology. The experiment also indicated that the constriction and swelling of the chitosan layer were reversible. Therefore, it is possible to control the pore size of the membrane by simply adjusting the pH condition of the system according to the separation requirement.

Based on the pore morphology of the membranes, the mean pore sizes (d_{pore}) of the membranes were estimated by[13]

$$r = 1 - H_f S_f$$

where $H_f = 1 + (16/9)q^2$, $S_f = (1 - q)^2(1 + 2q - q^2)$, $q = d_{BSA}/d_{pore}$, and r is the BSA rejection at high crossflow velocity and ionic strength ([KCl] = 0.05M) sufficiently high to make r(BSA) independent of the ionic strength. The Debye length for a 1 : 1 electrolyte is small enough that the effects of the charges on the BSA and the membranes could be ignored and d_{BSA} is the average spherical diameter of the BSA aggregates at that condition. The d_{BSA} was determined by a quasi-elastic light scattering instrument (Brookhaven Instruments Corp., Model BI-8000AT Digital Correlator) operating at 90°, wavelength 632.8 nm, 25.0 ± 0.2°C, at the same pH and ionic strength as used in the experimental determination of r. The estimated mean pore size for the membrane near neutral conditions (pH 6.0 and 8.2) was about 17 nm, and for the membrane at pH 3.6, it was 55 nm, which approaches the minimum pore size of the substrate. This result is also consistent with a loss of the chitosan membrane in the acidic solution.

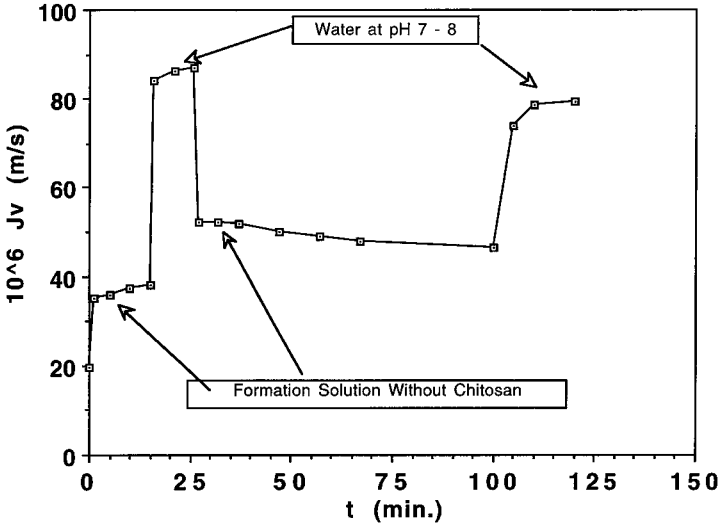

Figure 5 Flux of 1.0% acetic acid solution at pH 3.0 and flux of water at pH 7.5 ± 0.5 by a membrane formed with low molecular weight (70 kDa) chitosan. Experimental conditions: P = 1.9 ± 0.1 bar; T = 25 ± 1°C; u = 2.5 ± 0.1 m/s.

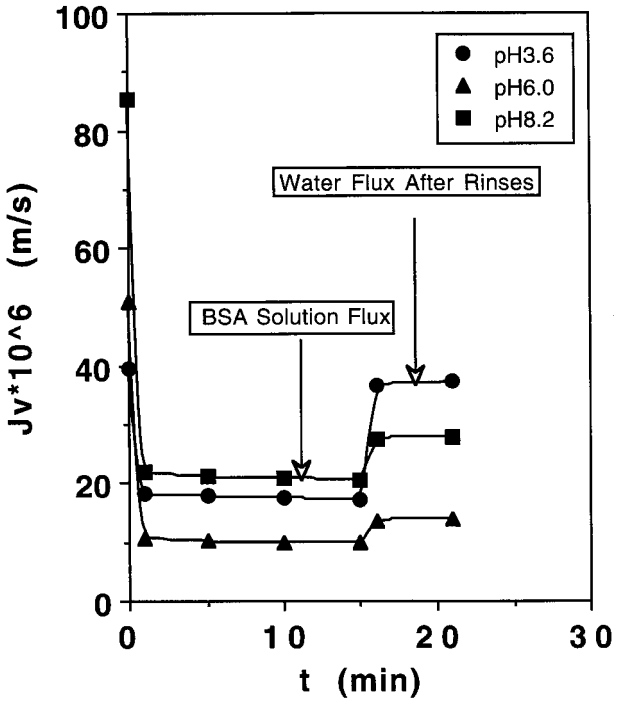

Figure 6 Flux decline of a 1.0 g/L BSA solution at pH 8.2, 6.0, and 3.6 vs. time and water flux after water washing. Experimental conditions: $P = 1.9 \pm 0.1$ bar; $T = 25 \pm 1$°C; $u = 2.5 \pm 0.1$ m/s.

Generally, BSA fouled the chitosan membrane, especially at neutral pH. The large $P/(\eta J_{wa})$ value in Table II for the membrane tested at pH 6.0 was primarily due to the BSA fouling. At this pH, the charges on the membrane and BSA were of opposite signs, although the density of charge on the chitosan gel would be small. Figure 6 shows that the membrane flux declined dramatically in the BSA solution and the loss of the flux could not be recovered effectively by water washing. The greater flux decline for the solution containing BSA and the smaller flux recovery in water washing for the membranes used at pH 6.0 and 8.2 compared with the result at pH 3.6 indicated greater fouling in a near neutral pH solution than at pH 3.6, perhaps because there was no electrostatic repulsive force between the chitosan membrane and BSA molecules at the higher pH. The small $N(R_a)$ value for the membrane used at pH 3.6 was caused by both low BSA fouling and membrane destruction or loss. The high affinity of chitosan for proteins suggests that the chitosan membrane may be suitable for enzyme immobilization in bioreactors.

Despite the fouling by BSA in near neutral and weakly basic solutions, the chitosan membrane exhibited approximately the same flux and a smaller decline in the rejection of BSA with increasing ionic strength at pH 6.0 than did sodium alginate membranes formed at pH 6.5 on the same type of substrate. In addition, the flux through the chitosan membrane was approximately 40% higher than the alginic acid membranes formed at pH 3.6. However, the alginic acid membrane retained a high rejection even at KCl concentrations of $0.04M$.[10]

CONCLUSIONS

The membranes were formed by the deposition of chitosan on a TiO_2 substrate in dilute acetic acid. However, the mechanism was not identified by the simple models relating the flux dependence on time to mechanisms. All the membranes formed exhibited more than 90% BSA rejection at low ionic strength and there was very little dependence of the membrane formation rate or the membrane ultrafiltration properties on the chitosan molecular weight.

The water flux of the initially formed membranes decreased with decreasing pH. The ultrafiltration properties of the membrane in dilute BSA solutions were also dependent on the pH of the solution. The flux of BSA solutions without added electrolyte was lowest at pH 6.0. The flux at pH 3.6 increased while the flux at 6.0 and 8.2 remained constant or decreased with increasing ionic strength. Membrane loss apparently occurred at pH 3.6 and high ionic strength, but not at pH 6.0 and 8.2 even at high ionic strength. Estimation of the pore diameter after use in the BSA ultrafiltration experiments including high ionic strength gave approximately 55 nm at pH 3.6 and 17 nm at pH 6.0 and 8.2, consistent with membrane loss only at the low pH. Fouling by BSA could also contribute to the smaller pore diameter determined after exposure to the BSA at pH 6.0 and 8.2. The decrease in water flux obtained after the ultrafiltration of BSA at pH 6.0 and 8.2 indicated that the membranes exhibited a high affinity for BSA at these pH conditions. However, the extent of fouling could not be indicated by flux measurements after the ultrafiltration experiment at pH 3.6 because membrane loss apparently occurred. The flux of the BSA solution through the chitosan membranes at pH 6.0 was approximately 40% higher than the flux obtained in similar ultrafiltration experiments with membranes formed with alginic acid at low pH and

approximately the same as the flux obtained with sodium alginate membranes formed at neutral pH. The decline in BSA rejection with increasing ionic strength at pH 6.5 was smaller for the chitosan membranes than for the sodium alginate membranes, but greater than for the membranes formed at low pH as alginic acid.

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