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Upward Dead-End Ultrafiltration of Binary Protein Mixtures

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ABSTRACT

Upward dead-end ultrafiltration of aqueous solutions of mixtures of bovine serum albumin (BSA) and egg white lysozyme was conducted using membranes which are almost completely retentive for BSA but permeable for lysozyme. The dependence of lysozyme rejection and the filtration flux rate on pH and the addition of salts has been investigated. The experimental data obtained in this study clearly demonstrate that the electrostatic interactions between dissimilar molecules may control the solute rejection and the filtration rate in upward ultrafiltration of binary protein mixtures. For instance, the BSA and lysozyme molecules have opposite electric charges at pH 7. Consequently, lysozyme rejection is large because both molecules within the filter cake pack more tightly due to heterocoagulation. On the other hand, the BSA and lysozyme molecules have the same electric charge at pH 4. Thus, lysozyme is rejected by the filter cake of the retained BSA molecules due to repulsive electrostatic interactions between the positively charged proteins. However, these charge effects weaken in the presence of salts. This study revealed that the solution environment can have profound effects upon solute and solvent transport in the ultrafiltration of binary protein mixtures.

INTRODUCTION

Ultrafiltration for the bioseparation of proteins has become increasingly attractive in recent years in such widely diversified fields as biotechnology, biomedicine, and food and beverage processing because of the com-

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paratively mild conditions required. It is well known that the ultrafiltration properties of proteinaceous solutions are dramatically influenced by the solution pH and the ionic environment, which control the electrostatic charge on the macromolecules involved (1–9).

Recent studies (7–9) by the present authors focused on the effect of the solution environment on the upward and downward ultrafiltration of bovine serum albumin (BSA) solution. We observed that the filtration rate obtained a distinct minimum around the isoelectric point, and it tended to increase substantially away from the isoelectric point. These behaviors appear to be primarily influenced by the structure of the filter cake formed on the membrane surface. For instance, as all solutes have the same electric charge at pHs both above and below the isoelectric point, they will repel one another more or less strongly in the filter cake. Thus, the filter cake becomes loose and wet due to electrostatic repulsion between the charged BSA molecules. As a result, it is believed that the filtration rate will show a large increase away from the isoelectric pH.

So far we have considered only the interaction between solutes which are identical in nature, size, and surface potential. When more than one type of solute is present, the situation becomes more complex. The ultrafiltration characteristics of multicomponent protein mixtures are of great interest in both laboratory and industrial practice. Solute–solute interaction plays an important role in both the filtration flux rate and the solute rejection when two or more proteins are ultrafiltered (10–12). It is generally accepted that solutes whose molecular dimensions are clearly small enough to permeate the membrane are substantially retained by that same membrane when larger solutes are present (13). Despite considerable progress, precisely how the solution properties control the filtration behavior of binary protein mixtures remains to be demonstrated and clarified.

Dead-end upward ultrafiltration, where the filtrate flow is in the opposite direction to gravity, is a membrane separation technique newly developed by the present authors to suppress cake formation on the membrane surface and to achieve a sustained permeation rate (8, 14). Upward ultrafiltration is more suitable for processes involving biological molecules that may be denatured by the generation of heat or air bubbles caused by the use of a circulating pump. The objective of this study has been to observe and interpret the effects of the solvent environment, such as pH and the ionic strength, on the filtration rate and the solute rejection in upward dead-end ultrafiltration of the admixture of two well-characterized proteins of different molecular dimensions and isoelectric points.

EXPERIMENTAL

BSA (Fraction V Powder, Katayama Chemical Ind. Corp.) and egg white lysozyme (Nagase Biochemical Ind. Corp.) were used as the model proteins for investigating protein–protein interactions in ultrafiltration. Their important physicochemical characteristics are summarized in Table 1 (15–17). All other reagents were analytical grade. Proteinaceous solutions were prepared by dissolving preweighed amounts of the powder in 10 mM phosphate buffer solution (pH 7) or 10 mM acetate buffer solution (pH 4) with gentle agitation for a sufficient time (2 hours for all runs) to insure homogeneity. Experiments were completed using single protein solutions and solutions containing mixtures of the two proteins. The mixed protein solutions were prepared by mixing single protein solutions with gentle agitation for 30 minutes. The ionic strength of the protein solutions was adjusted by the addition of NaCl. Ultrapure, deionized water for solution make-up was prepared by a ultrapure water system for laboratory use (Puric-R, Olgano Corp.). The resistivity of this ultrapure, deionized water was 18 megohm. Hydrophobic polysulfone membranes (PTTK, Millipore Corp.) were employed for all studies. The membranes are claimed to display a nominal molecular weight cutoff of 30 kDa, making it essentially impermeable to BSA but permeable to lysozyme.

A schematic layout of the experimental setup is shown in Fig. 1. Both a filter with an effective membrane area of 12.6 cm^2 and a feed reservoir were charged with the protein solutions. The filter was placed on the angled plate so that the filtrate flow was opposite to the direction of gravity. Upward ultrafiltration experiments were performed under constant pressure by applying compressed nitrogen gas via a reducing valve. Transmembrane pressure for all the runs was set at 98 kPa. The filtrate was collected in triangular flasks over measured time intervals. The filtrate

TABLE 1
Physical Properties of Proteins Used

Property	BSA	Lysozyme
Molecular weight (Dalton)	67,000	14,300
Molecular dimensions (nm)	14 × 4	4.5 × 3
Isoelectric point	4.9	11.0
Stokes-Einstein radius (nm)	3.64	2.00
Partial specific volume (cm^3/g)	0.733	0.726

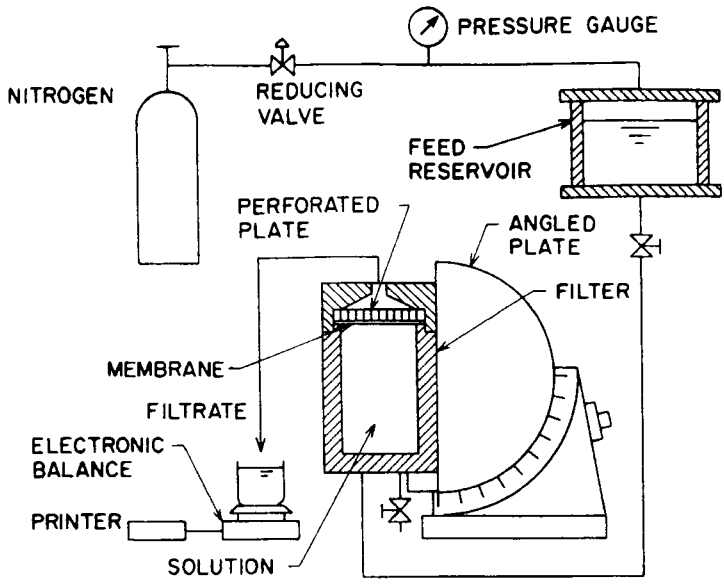

FIG. 1 Schematic diagram of experimental apparatus.

weight was measured using an electronic balance and a printer with a timer. The weights were converted to volumes using density correlations. The concentration of the protein in the single protein solutions was measured spectrophotometrically by reading the absorbance at the wavelength of their maximum absorbance, approximately 280 nm. Each concentration of the two proteins in the mixed protein solutions was determined by high-performance liquid chromatography (HPLC). For the analytical separations, a gel permeation column (Asahipak GS-520), supplied by Asahi Chemical Ind. Corp., was employed. The filtration rate and the apparent solute rejection were calculated as functions of time and cumulative filtrate volume per unit membrane area.

RESULTS AND DISCUSSION

In Fig. 2 the solute rejection behavior following ultrafiltration of binary BSA/lysozyme mixtures containing equal amounts of each protein is shown in the form of the apparent rejection $R_{obs,l}$ of lysozyme versus the cumulative filtrate volume v per unit effective membrane area collected in the filtration time θ. Solution pH was adjusted to 7 (pH between the

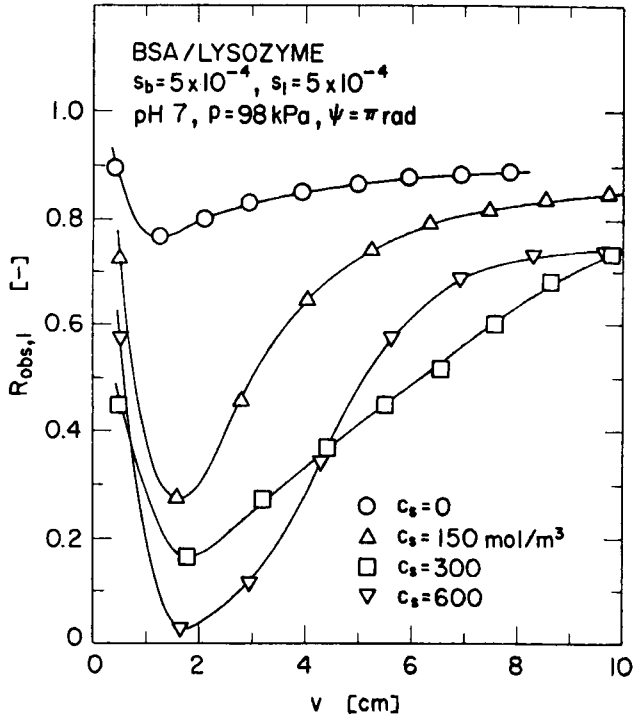

FIG. 2 Apparent lysozyme rejection variation with permeate volume per unit membrane area at pH 7.

isoelectric points of both proteins). Each experiment was performed with a different membrane. The apparent rejection $R_{obs,l}$ of lysozyme can be defined by

$$R_{obs,l} = 1 - c_l/s_l \tag{1}$$

where c_l and s_l are the mass fractions of lysozyme in the filtrate and bulk upstream solution, respectively. In the figure, s_b and c_s denote the mass fraction of BSA and NaCl concentration in the bulk upstream solution, respectively, and ψ is the angle between the filtrate flow and the direction of gravity. Filtration of binary protein mixtures with this membrane resulted in nearly complete retention of BSA. The lysozyme rejection varies with time or the filtrate volume. In the incipient stages of filtration, the membrane exhibits a high lysozyme rejection which may be attributed to adsorption of the lysozyme solutes on the hydrophobic surface of the

membrane (18, 19). The rejection $R_{obs,1}$ is high during ultrafiltration in the absence of NaCl ($c_s = 0$). The BSA molecule is negatively charged at pH 7, while the lysozyme molecule, which is a basic protein, has a net positive charge at this pH value. Consequently, heterocoagulation does occur due to BSA–lysozyme attraction because the coulombic attractive force associated with oppositely charged solutes dominates. Such protein–protein interactions in solution may result in the formation of association complexes of dimensions larger than those of the smaller primary species present. The resulting aggregation process produces dramatic changes in the properties of the filter cake formed on the membrane surface. The cake consists of the protein aggregates very well packed to form a compact layer, resulting in high rejection of lysozyme. However, the lysozyme rejection is significantly altered when salts are present in the solution. It can be seen that the lysozyme rejection decreases by the increase of the addition of NaCl. In the case of a charge-stabilized colloid such as protein, solute interactions depend on the magnitude of the surface charge and/or on the extent of the electrical double layer, and this depends on the total electrolyte concentration. When salts are added, this leads to a less extensive diffuse double layer. Such charge-shielding between the protein molecules due to the existence of salts would reduce electrostatic attraction between BSA and lysozyme molecules. Thus, the solute rejection at first declines to a minimum value. However, as filtration proceeds, the compressible filter cake of retained BSA solutes provides a barrier to transport of the smaller lysozyme solutes, and hence the rejection of the lysozyme solutes rises dramatically.

In Fig. 3 the flux behavior of the experiment described in Fig. 2 is shown in the form of the reciprocal filtration rate ($d\theta/dv$) versus the filtrate volume v per unit membrane area. This plot is well known as the Ruth plot (20, 21) in the classical cake filtration of particulate suspensions. The plot shows a linear relationship in downward dead-end ultrafiltration in which the solutes are completely retained by the membrane (22). In principle, the value of $d\theta/dv$ is directly proportional to the hydraulic resistance due to solute accumulation on or in the membrane. It would be expected that a rather dense, finely porous filter cake of retained BSA/lysozyme mixtures forms on the membrane surface during the early stages of filtration without added salt. Since the hydraulic resistance of this filter cake is remarkably large, the value of $d\theta/dv$ rises rapidly to a near-asymptotic value with increasing filtrate volume. Increase in the thickness of the filter cake is suppressed by effects of upward ultrafiltration as filtration proceeds, similar to ultrafiltration of single protein solutions described previously by us (8, 14). The filtration rate increases markedly upon addi-

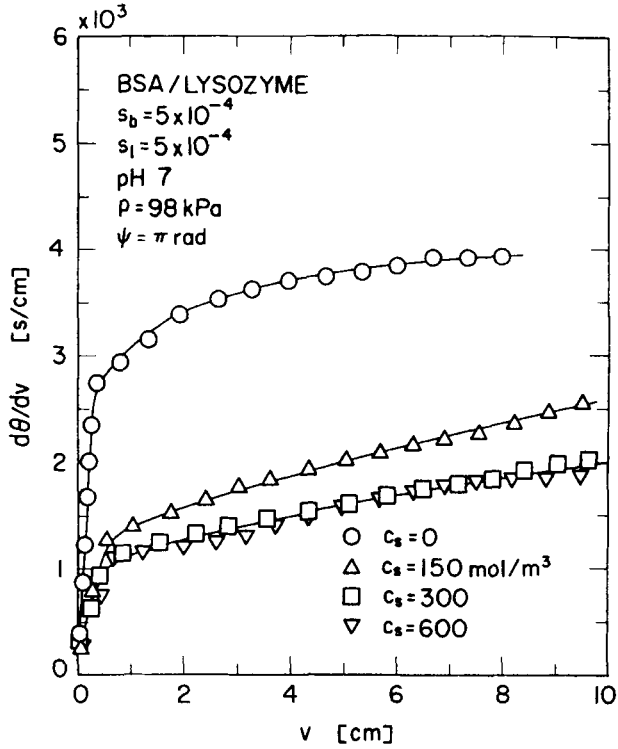

FIG. 3 Filtration rate variation with permeate volume per unit membrane area at pH 7.

tion of NaCl because of the formation of a filter cake which is substantially free from lysozyme molecules. However, as filtration proceeds, the filtration rate decreases gradually because the lysozyme molecules are trapped in the pores of the filter cake. The very same filtration rate was observed with NaCl concentrations above 300 mol/m^3.

Figure 4 shows the comparative data for the filtration rate of both single BSA solutions and BSA/lysozyme mixtures at pH 7. In the salt-free solutions it is clear that the BSA/lysozyme mixture exhibits a significantly lower filtration rate than does the BSA solution alone. This means that BSA and lysozyme solutes form a mixed protein filter cake of greater density and smaller pore size in the filtration of BSA/lysozyme mixtures

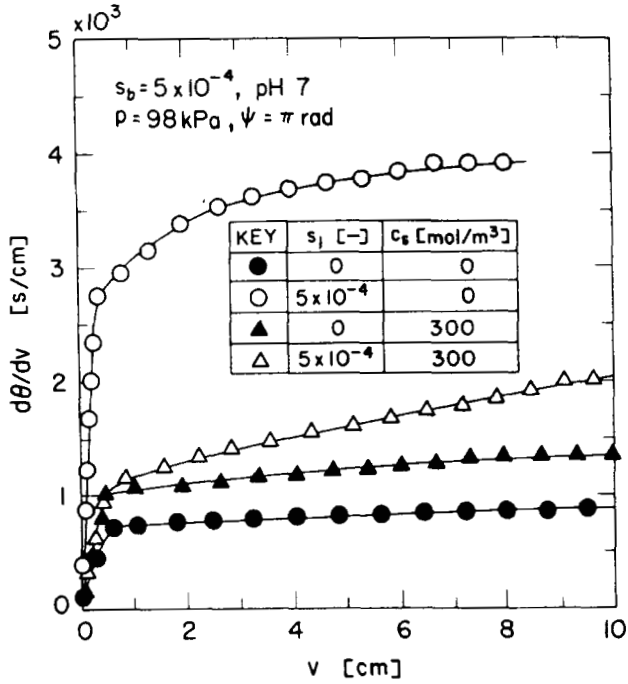

FIG. 4 Effect of lysozyme concentration on filtration rate variation at pH 7.

than does a filter cake comprised solely of BSA molecules. On the other hand, with the elevation of ionic strength due to the addition of salts, in the initial stages of filtration, the filtration rate for the binary mixture is virtually identical to that observed for the single solute solution. This is probably because the filter cake is expected to be composed initially only of BSA solutes. However, as filtration proceeds, the filtration rate decreases gradually in filtration of the BSA/lysozyme mixture because the lysozyme molecules are trapped in the pores of the filter cake comprised of BSA molecules.

Figures 5 and 6 show, respectively, the lysozyme retention and the reciprocal filtration rate at pH 7 with no salts added under conditions of constant lysozyme concentration and variable BSA concentrations. It is clear from the experiment using the lysozyme solution alone that the ap-

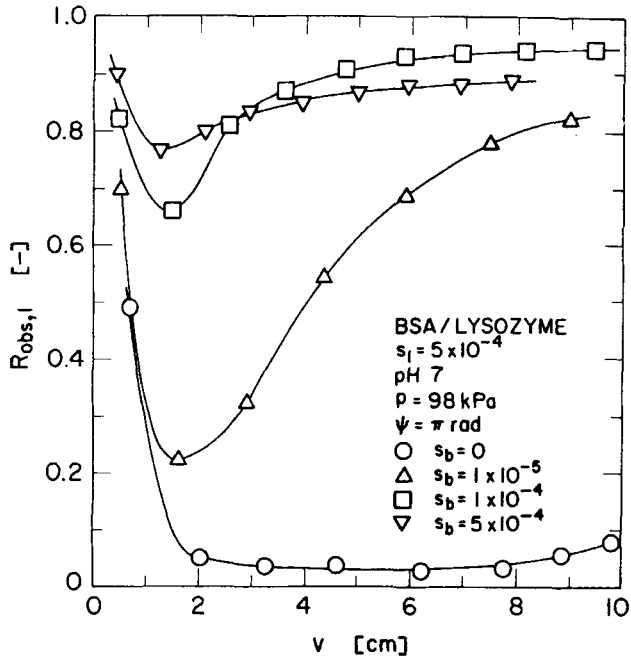

FIG. 5 Effect of BSA concentration on apparent lysozyme rejection variation at pH 7.

parent lysozyme rejection by this membrane is very low as could be expected from its nominal molecular weight cutoff. When the feed BSA concentration is one-fiftieth of the lysozyme concentration, the lysozyme passes through the membrane after the very early stages of filtration. However, the rejection of lysozyme increases markedly as filtration proceeds. This is because the filter cake formed on the membrane surface creates a secondary membrane barrier to the passage of a much smaller macrosolute which would otherwise freely permeate the membrane. As a result, the filtration rate decreases gradually. It is of great interest to note that the lysozyme molecules can be retained eventually even in a BSA concentration which is extremely small compared with the lysozyme concentration. As the BSA concentration in solution is increased, the rejection of the lysozyme molecules increases substantially. This would suggest that the rejection of the smaller of two macrosolutes is increased

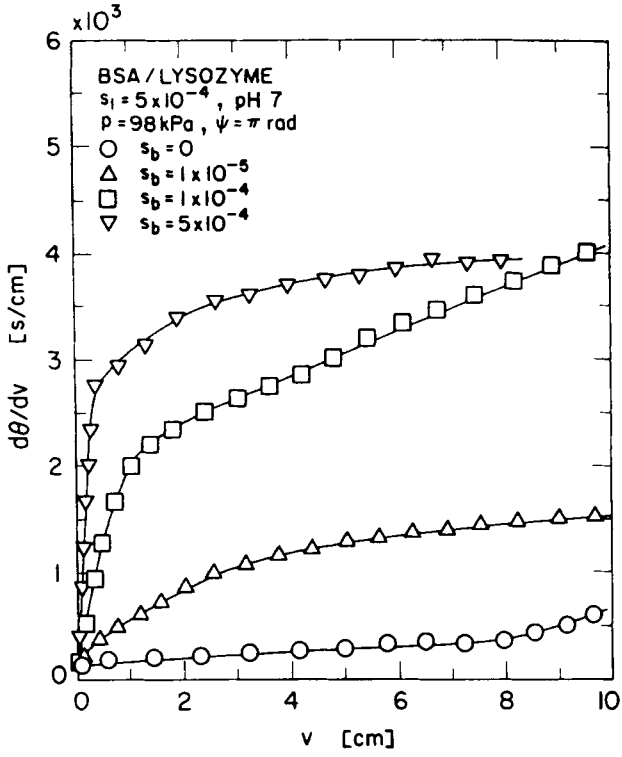

FIG. 6 Effect of BSA concentration on filtration rate variation at pH 7.

in the presence of the larger species. Consequently, the filtration rate decreases remarkably with an increase of the BSA concentration in the initial stages of filtration.

The lysozyme rejection and filtration rate variation with permeate volume in ultrafiltration of BSA/lysozyme mixtures at pH 4 are shown in Figs. 7 and 8, respectively. Without the addition of salts, the apparent rejection of lysozyme is surprisingly high for a nominal 30 kDa cutoff membrane. It should be noted that both BSA and lysozyme are electro-

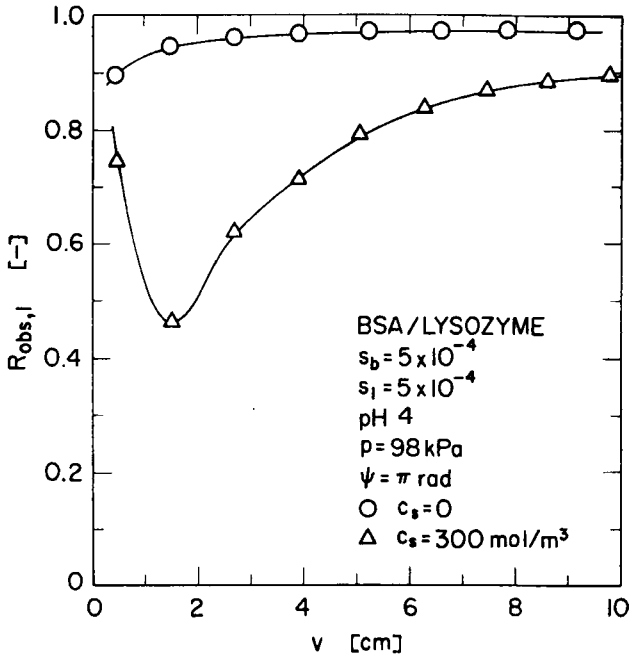

FIG. 7 Apparent lysozyme rejection variation with permeate volume per unit membrane area at pH 4.

positive at pH 4. Consequently, an electrostatic repulsive force acts between the BSA and lysozyme molecules. The lysozyme molecules do not pass through the filter cake of the retained BSA molecules due to electrostatic repulsion. Consequently, the filter cake is substantially free from lysozyme molecules, and then a decrease in the filtration rate is suppressed. But, with the addition of salts, the charge of the BSA and lysozyme molecules may be considerably shielded. As a result, the rejection of the lysozyme molecules decreases. However, the filtration rate declines considerably during operation because of the build-up of the filter cake consisting not only of BSA molecules but also of lysozyme molecules.

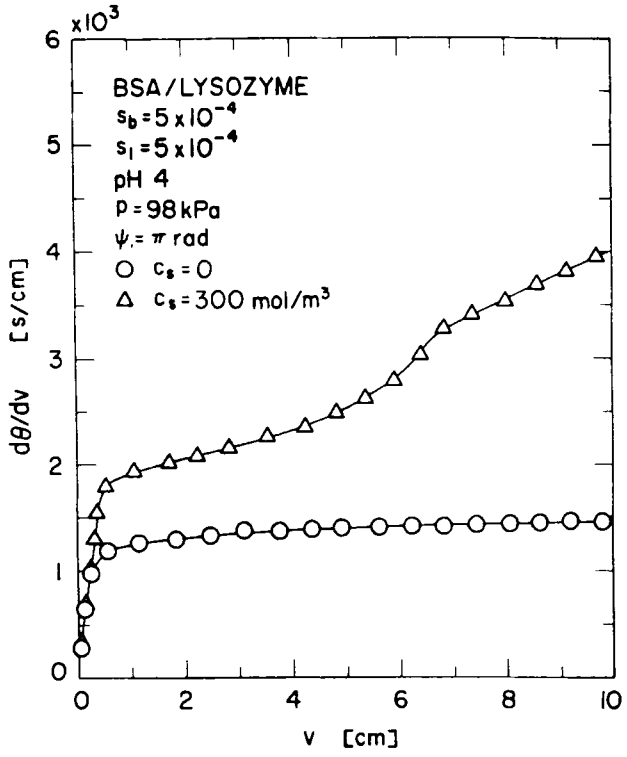

FIG. 8 Filtration rate variation with permeate volume per unit membrane area at pH 4.

CONCLUSIONS

The characteristics of upward dead-end ultrafiltration of binary protein mixtures through BSA-impermeable, lysozyme-permeable membrane have been investigated by using an aqueous solution of mixtures of BSA and lysozyme. The effects of pH and the addition of salts on the apparent rejection of lysozyme solutes and the filtration flux rate were explored. The experimental results clearly demonstrated that inducing protein–protein interactions in a solution by changing the solution environment may cause changes in the retention and the filtration rate during ultrafiltration. The BSA and lysozyme molecules have opposite electric charges at pH 7. Thus, the lysozyme rejection became high because heterocoagulation occurred and a cake composed of very densely packed molecules formed.

On the other hand, the BSA and lysozyme molecules have the same electric charge at pH 4. Therefore, lysozyme did not pass through the filter cake of BSA. However, both the lysozyme rejection and the filtration rate were significantly altered when salts were added.

Additional experimentation will be required in order to develop a more fundamental understanding of the mechanisms governing the ultrafiltration properties of binary mixtures. Specifically, measurement of such properties of the filter cake as the porosity and the specific filtration resistance should provide important additional insight into these phenomena. Work is in progress in our laboratory to investigate the properties of the filter cake in the ultrafiltration of binary protein mixtures.

NOMENCLATURE

c_l	mass fraction of lysozyme in filtrate
c_s	NaCl concentration of bulk feed fluid (mol/m^3)
p	applied filtration pressure (Pa)
$R_{obs,l}$	apparent rejection of lysozyme defined by Eq. (1)
s_b	mass fraction of BSA in bulk feed fluid
s_l	mass fraction of lysozyme in bulk feed fluid
v	filtrate volume per unit membrane area (m^3/m^2)

Greek

θ	filtration time (s)
ψ	angle between filtrate flow and direction of gravity (rad)

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