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Fractionation Characteristics of Binary Protein Mixtures by Ultrafiltration

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ABSTRACT

Ultrafiltration (UF) of mixtures of bovine serum albumin (BSA) and egg white lysozyme was conducted using membranes which were almost completely retentive for BSA but permeable for lysozyme. The experimental data have clearly demonstrated that the separation properties of binary protein mixtures are largely influenced by the hydrodynamics above the membrane, the solution environment, and the adsorption of the protein solutes within the membrane. In particular, it must be noted that the increase of the shear stress acting on the membrane surface improves the filtration rate, but causes the increase of the lysozyme rejection, resulting in a reduction in the fractionation efficiency. Furthermore, the effects of ultrasonic irradiation on the UF properties have been tested experimentally. Although the filtration rate was enhanced significantly by ultrasonic irradiation, the lysozyme rejection remained unchanged. The results showed that ultrasonic irradiation is quite effective for protein fractionation.

Key Words. Ultrafiltration; Binary protein mixture; Fractionation; Hydrodynamics; Ultrasonic irradiation

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INTRODUCTION

In recent years ultrafiltration (UF) technology has attracted a considerable amount of attention because of many industrial advantages, and consequently it has been applied in widely diversified commercial processes such as biotechnology, biomedicine, and food and beverage processing. The control of such filtration properties as filtration rate and rejection (or transmission) of the solutes in the UF process is of great interest in both industry and academia. While there exist many factors influencing the filtration behaviors, the underlying phenomena are currently not well understood.

It is well known that the separation behaviors of UF of proteinaceous solutions can be strongly affected by such factors of the solution environment as the pH and the ionic strength, which control the electrostatic charge on the macromolecules involved and consequently the solute/solute interactions (1–4). The effects of the solution environment on dead-end UF of bovine serum albumin (BSA) solutions have been reported in detail in recent studies (5, 6) by the present authors. The hydrodynamics above the membrane and the surface characteristics of the membrane also have a marked influence on the filtration rate and the solute rejection (7).

Especially when more than one type of solute is present in solutions, the situation is more complicated. Although the UF characteristics of multicomponent protein mixtures are of great interest in industrial applications, so far there have been few fundamental studies (7–9).

In a previous paper (10) the effects of pH and the presence of salts on the separation behaviors of upward dead-end UF were investigated by using an aqueous solution of the mixtures of BSA and lysozyme. From the experimental results it was 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 UF. Also, the properties of the filter cake formed on the retentive membranes in downward dead-end UF of binary protein mixtures have been studied (11). It was found that the structure of the filter cake plays a vital role in determining the filtration rate in UF of binary protein mixtures.

Recently it has been reported that ultrasonic irradiation decreases markedly the permeation resistance caused by a so-called gel layer formed during UF of ovalbumin solutions (12). However, no research has been done concerning the application of ultrasonic irradiation to fractionation of the proteins by UF.

The major target of the work presented in this paper is to investigate the influence of such factors as the hydrodynamics above the membrane, the solution environment, the protein adsorption within the membrane,

and ultrasonic irradiation on the filtration rate and the solute rejection of UF of binary protein mixtures. The filtration rate and the solute rejection have been examined under various experimental conditions in order to develop a strategy for conducting the efficient fractionation of binary protein mixtures by UF.

EXPERIMENTAL

BSA (Fraction V Powder, Katayama Chemical Ind. Corp., Japan) and egg white lysozyme (Nagase Biochemical Ind. Corp., Japan), with significant differences in their molecular weights and isoelectric points, were used as the model proteins. Their important physicochemical characteristics are summarized in Table 1. Each single protein solution was prepared by dissolving a preweighed amount of powder in 10 mol/m^3 phosphate buffer solution (pH 7) or 10 mol/m^3 acetate buffer solution (pH 4) with gentle agitation for a sufficient time (2 hours for all runs) to insure homogeneity. Subsequently, a mixed protein solution was prepared by mixing single protein solutions with gentle agitation for 30 minutes and it was used as a sample solution. The weight fraction of each component was kept at 5×10^{-4} for all runs. In some experiments the ionic strength of the protein solutions was adjusted by the addition of NaCl (c_s = 300 mol/m^3). Ultrapure, deionized water for solution make-up was prepared by a ultrapure water system for laboratory use (Puric-R, Olgano Corp., Japan). The resistivity of this ultrapure, deionized water was 18 MΩ·cm. Two types of membranes, YM30 and PM30 (both supplied by Amicon Division, W. R. Grace Corp., USA), were tested to examine the effects of the membrane properties. The former is a hydrophilic polysaccharide membrane displaying very low adsorptivity for proteins; the latter is a hydrophobic polysulfone membrane exhibiting significant protein adsorptivity.

TABLE 1
Physicochemical Properties of Proteins Used

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

Both are claimed to display a nominal molecular weight cutoff of 30,000 Dalton. Theoretically, these membranes enable lysozyme to permeate through while retaining BSA on the feed side of the membranes.

UF experiments were performed by means of the various filtration modes. One of them was an unstirred upward filtration mode in which the filtrate flow was opposite to the direction of gravity. A schematic layout of the experimental apparatus was shown in our previous paper (10). In addition, unstirred downward UF in which the filtrate flow was in the same direction as gravity was performed. A specially designed unstirred batch cell with an effective membrane area of 12.6 cm^2 was used for these UF experiments. Some UF experiments were also carried out in a standard stirred ultrafilter (Model UHP-62, Advantec Toyo Corp., Japan) with an effective membrane area of 28.0 cm^2 under various stirring conditions. The impeller and the membrane were 6 mm apart. In order to assess the effectiveness of the ultrasonic irradiation, an ultrasonic cleaning bath (LTH 610-6, Branson Corp., USA) with the ultrasonic transducer champed to its base was used, and the whole filter was kept immersed in the bath full of water during the course of filtration. The filter was set up so that the membrane surface was perpendicular to the propagation direction of the ultrasonic wave. The oscillating frequency of the ultrasonic irradiation was 25 kHz.

After both a filter and a feed reservoir were charged with the protein solutions, they were pressurized by applying compressed nitrogen gas via a reducing valve (p = 98 kPa for all runs) and the filtrate was collected in several triangular flasks over measured time intervals. The filtrate weight was measured using an electronic balance and a printer with a timer, and converted to volume using density correlations. The concentration of lysozyme in the filtrate was measured spectrophotometrically by reading the absorbance at the wavelength of its maximum absorbance, approximately 280 nm. The measured absorbance can be considered to be equal to that of only lysozyme, since the filtrate does not contain BSA solutes due to the retentivity of the membrane used.

RESULTS AND DISCUSSION

Influences of Hydrodynamics above Membrane

In Fig. 1 the flux behaviors in the unstirred downward and upward and the stirred downward UF of binary BSA/lysozyme mixtures are shown in the form of the reciprocal filtration rate ($d\theta/dv$) versus the cumulative filtrate volume v per unit effective membrane area collected in the filtration time θ. This plot is well known as the Ruth plot (13) in conventional cake filtration of particulate suspensions. Experiments were performed using

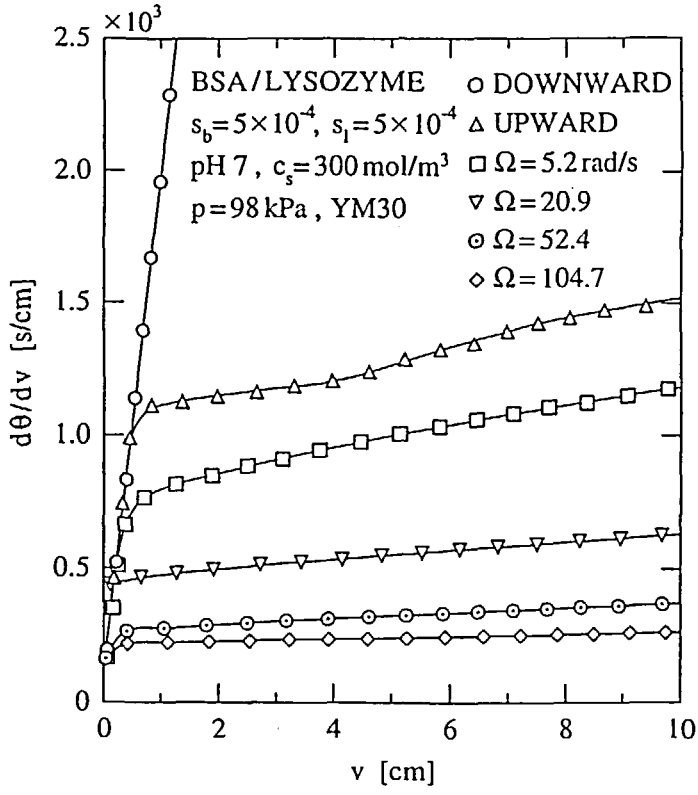

FIG. 1 Effect of hydrodynamics above membrane on filtration rate.

YM30 membranes with a very low adsorptivity for proteins, at pH 7, and at a high salt concentration where electrostatic interactions between BSA and lysozyme weakened comparatively (10). For conventional unstirred downward UF, the plot is virtually linear throughout the course of filtration due to the continuous formation of the filter cake on the membrane in accordance with the compressible cake resistance model (5). For unstirred upward UF, the flux decline is suppressed because the filter cake is exfoliated continuously by the effect of gravity with the progress of filtration (10). This phenomenon is different from sedimentation because a linear relationship in accordance with the plot of unstirred downward UF appears in the initial period of filtration (14). The increase in the shear stress acting on the membrane surface by stirring leads to the suppression of the cake deposition, and consequently a marked increase in the filtration

rate. Since the filtration rate in upward UF is slightly lower than that in stirred UF with stirring of 5.2 rad/s, the effect of gravity acting on the filter cake in upward UF corresponds to that of very low shear stress.

Figure 2 shows the solute rejection behavior of the experiment described in Fig. 1 in the form of the apparent rejection $R_{obs,l}$ of lysozyme versus the filtrate volume v per unit membrane area. The apparent lysozyme rejection $R_{obs,l}$ 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 feed solution, respectively. Filtration of binary protein mixtures with this membrane resulted in complete retention of BSA. The figure indicates that the lysozyme rejection is high in the incipient stages of filtration, probably because of protein adsorption within the membrane (10). Afterward the lysozyme solutes pass almost completely through the membrane in unstirred downward UF and are only a little retained by the membrane

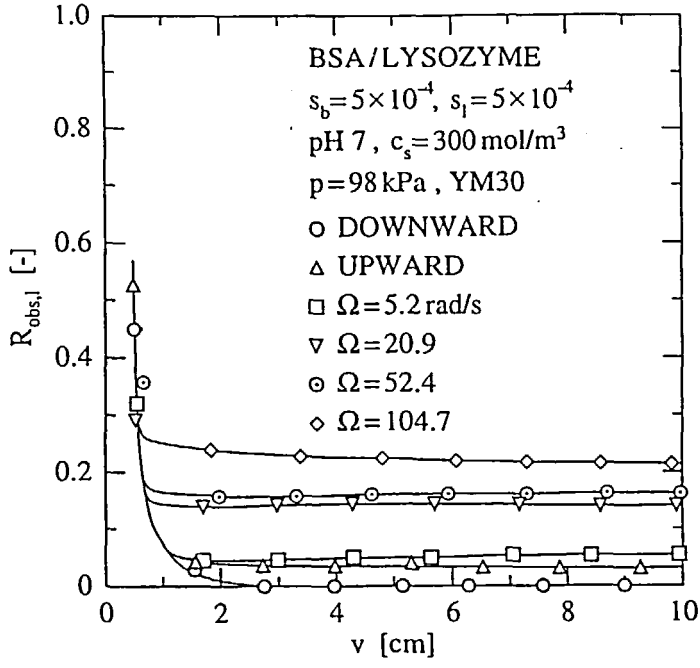

FIG. 2 Effect of hydrodynamics above membrane on apparent lysozyme rejection.

in upward UF. It has been reported that in single protein solutions the stirring resulted in increasing the solute rejection (7). It should also be emphasized that in binary protein mixtures the higher stirring speed causes a higher rejection of the solutes. This is because the concentration of lysozyme near the membrane decreases with the increase of the shear stress acting on the membrane. It is important to note that the existence of the filter cake of BSA on the membrane surface does not substantially retard the passage of lysozyme molecules because the filter cake is quite porous (11). Figures 1 and 2 suggest that it is necessary to control the hydrodynamics above the membrane in order to obtain high lysozyme transmission simultaneously with high filtration rate. While hard stirred UF, which produces the high filtration rate and solute rejection, is very effective for concentrating protein solutions, it is not suitable for fractionation of binary protein mixtures. It can be concluded from the figures that unstirred upward or mildly stirred UF is more advantageous for the efficient fractionation of binary protein mixtures.

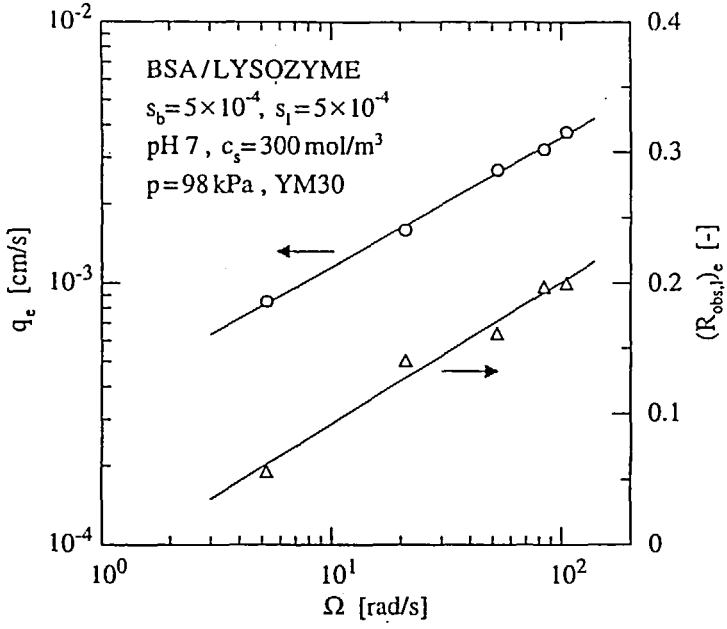

FIG. 3 q_e and $(R_{obs,l})_e$ as function of Ω.

In order to evaluate the effect of stirring on the filtration behaviors quantitatively, the logarithm of the filtration rate q_e and the rejection of lysozyme $(R_{obs,l})_e$ at the filtrate volume v of 10 cm are plotted against the logarithm of Ω in Fig. 3. The logarithm of q_e increases linearly with the increase in log Ω, and the plots of $(R_{obs,l})_e$ versus log Ω are also roughly linear.

Influences of pH and Protein Adsorption within Membrane

The solution environment (for instance, pH and the presence of salts) is one of the principal factors governing UF behaviors. In this study the effects of pH have been investigated by using BSA/lysozyme mixtures in a salt-free solution and YM membranes with very low adsorptivity for proteins for both unstirred upward and stirred downward UF, as shown in Figs. 4 and 5. Both the BSA and lysozyme molecules have net positive charges at pH 4, and consequently an electrostatic repulsive force acting between the macromolecules makes the formed filter cake loose and wet.

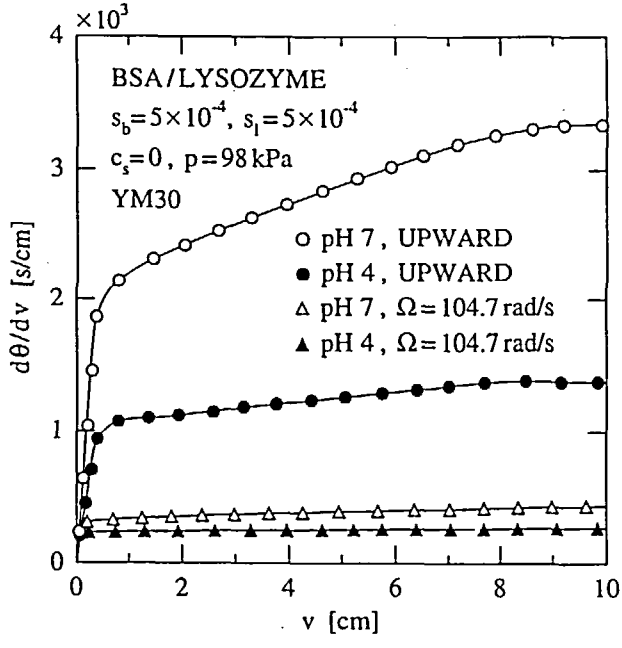

FIG. 4 Effect of pH on filtration rate.

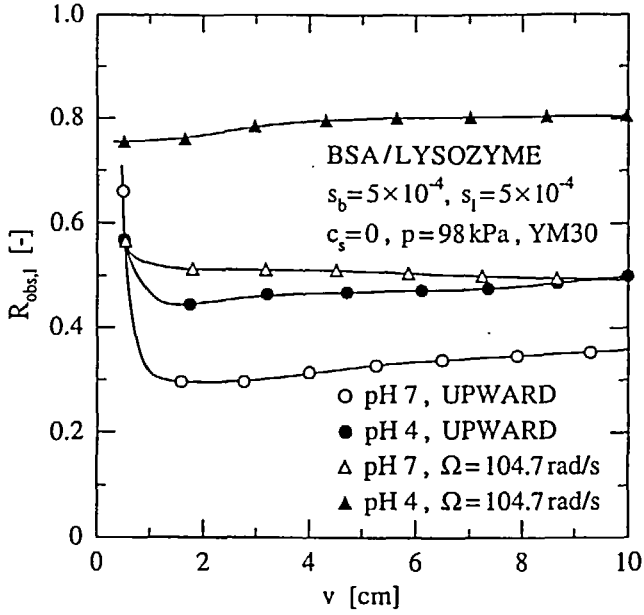

FIG. 5 Effect of pH on apparent lysozyme rejection.

Therefore, as shown in Fig. 4, the high filtration rate is obtained at pH 4 regardless of the filtration mode as compared with that at pH 7 where the dense filter cake is formed due to a coulombic attractive force acting between the molecules of negatively charged BSA and positively charged lysozyme. On the other hand, because the repulsion exerted by the filter cake of BSA at pH 4 prevents lysozyme molecules from passing through the cake, the lysozyme rejection at pH 4 is higher than that at pH 7 for every filtration mode, as shown in Fig. 5. The compactness of the cake was judged on the basis of the measurement of the cake porosity in our previous paper (11).

Figure 6 illustrates the filtration rate and the lysozyme rejection in upward UF of BSA/lysozyme mixtures at pH 7 and high salt concentration where electrostatic interactions between BSA and lysozyme weaken comparatively (10). The figure shows a comparison between a protein adsorptive PM30 membrane and a nonadsorptive YM30 membrane. No significant difference in the filtration rate was noticed between two different

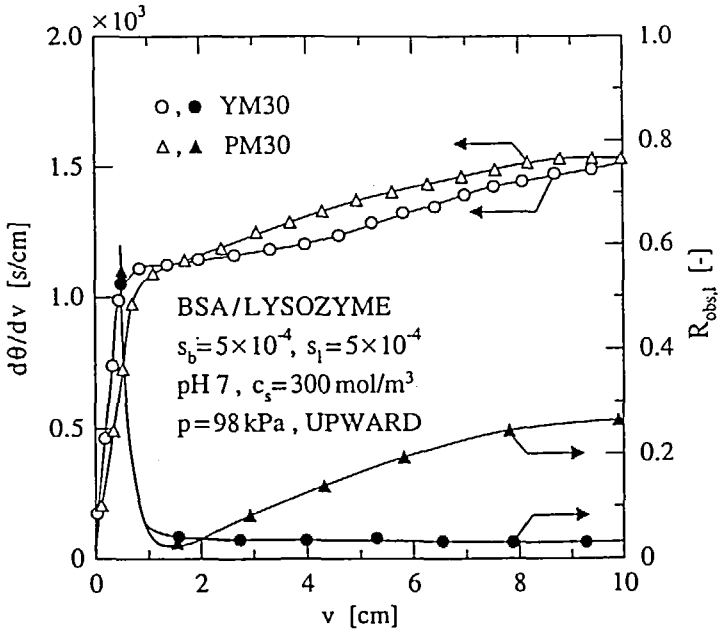

FIG. 6 Comparison between protein adsorptive and nonadsorptive membranes on filtration rate and apparent lysozyme rejection.

membranes although it may be expected that the adsorption of protein solutes occurs within the PM membrane much more significantly than within the YM membrane. This is probably because the influence of the filter cake formed on the membrane surface may be dominant over the flux decline rather than that of the adsorptive property of the membrane. In contrast, it is of interest to note that the rejection behaviors exhibit a great difference between two membranes. In the YM membrane the transmission of lysozyme remains high except that lysozyme molecules may be adsorbed within the membrane in the initial stage of filtration. In contrast, in the PM membrane the lysozyme rejection inclines to increase gradually as filtration proceeds, probably because of the progress of the adsorption of lysozyme molecules within the membrane.

Influences of Ultrasonic Irradiation

In this study the effects of ultrasonic irradiation on the separation properties of UF of binary BSA/lysozyme mixtures by use of the YM mem-

brane with very low adsorptivity for proteins at pH 7 were explored. The comparative data for the filtration rate in upward UF experiments are shown in Fig. 7. The output power of the ultrasonic irradiation was adjusted to 180 W. It is evident that the ultrasonic fields can contribute to the remarkable improvement in the filtration rate. In Fig. 8 the dynamic variations of the lysozyme rejection of the experiment described in Fig. 7 are shown. As shown in Figs. 1 and 2, although the observed filtration rate becomes high by increasing the shear stress on the membrane, the permeation of the lysozyme molecules becomes less. In contrast, of particular importance is the surprising observation that the ultrasonic irradiation does not lower the lysozyme transmission regardless of involving a marked increase in the filtration rate. One possible explanation for this result is that sonication supplies vibrational energy to the filter cake to keep the solutes partly suspended and therefore leaves more free channels for filtrate flow. However, the underlying basis for the result clearly requires further investigation. Also, Figs. 7 and 8 show that the addition of

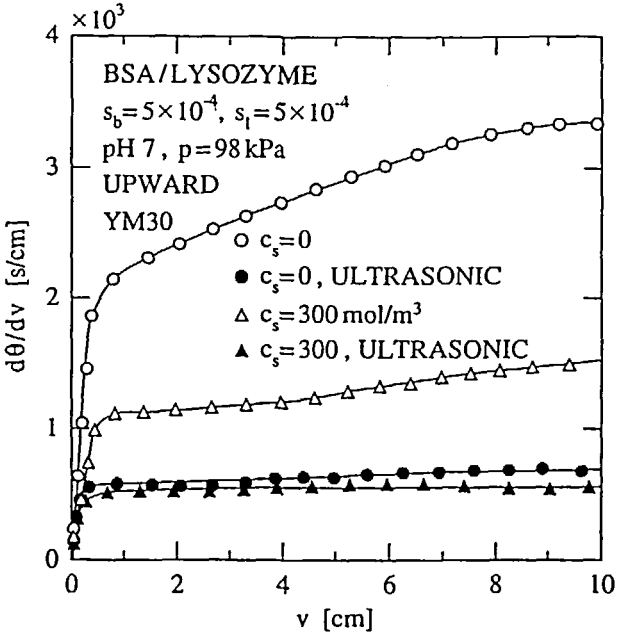

FIG. 7 Effect of ultrasonic irradiation on filtration rate in upward UF for different NaCl concentrations.

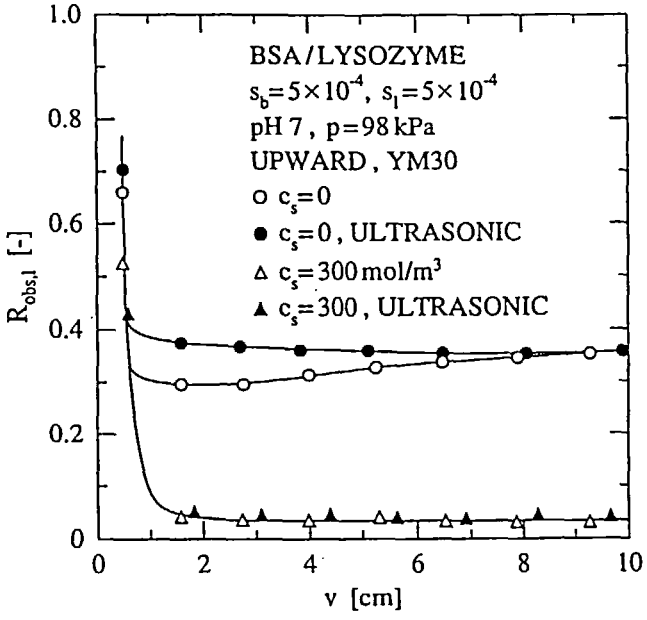

FIG. 8 Effect of ultrasonic irradiation on apparent lysozyme rejection in upward UF for different NaCl concentrations.

salts at this pH is effective for obtaining a high filtration rate and the transmission of lysozyme because the charge effect weakens.

Comparison between upward and downward UF experiments as carried out under conditions with and without ultrasonic irradiation of 180 W is shown in Figs. 9 and 10. Ultrasonic irradiation plays a significant role in the improvement of the filtration rate. The filtration rates observed in upward UF experiments are higher than those in downward UF experiments, whether the ultrasonic is irradiated or not. Meanwhile, the ultrasonic fields hardly alter the rejection behaviors, and sufficient transmission of lysozyme is maintained in either filtration mode. While there is very little difference in the protein fractionation level between the downward and upward UF modes, the upward UF is considered to be more effective because a higher filtration rate can be acquired.

To clarify the effects of ultrasonic irradiation, Fig. 11 compares the lysozyme rejections between cases with and without ultrasonic irradiation

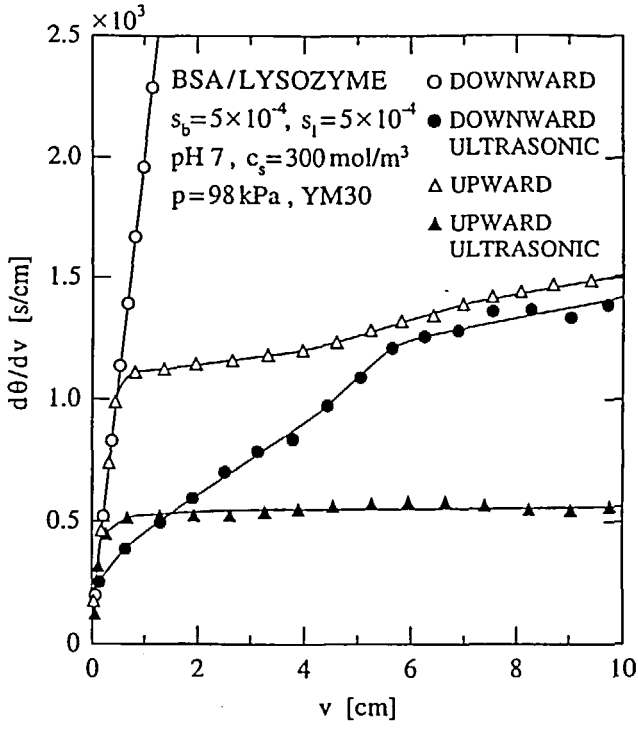

FIG. 9 Comparison of filtration rate between downward and upward UF with and without ultrasonic irradiation.

in which similar filtration rates are observed. The figure shows that the filtration rate in the ultrasonic upward UF irradiated under an output power of 180 W is substantially similar to that in downward UF stirred with a rotational speed of 20.9 rad/s without ultrasonic irradiation. However, it is important to note that there is a marked difference in lysozyme rejection. The lysozyme transmission in ultrasonic upward UF is much higher than that in stirred UF. Therefore, it is believed that ultrasonic irradiation is quite effective not only for the concentration of proteins by UF (12) but also for fractionation of binary protein mixtures by UF.

In order to evaluate the effects of the ultrasonic intensity on the filtration behaviors quantitatively, log q_e and $(R_{obs,l})_e$ in upward UF are plotted against the ultrasonic output power P in Fig. 12. It appears that q_e in-

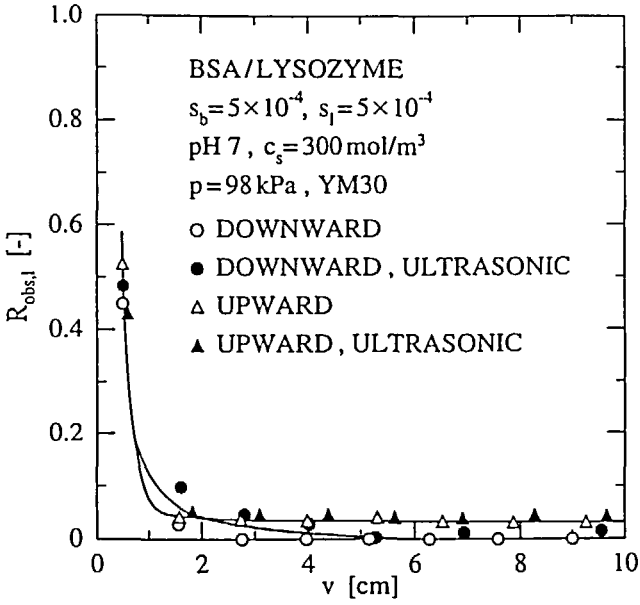

FIG. 10 Comparison of apparent lysozyme rejection between downward and upward UF with and without ultrasonic irradiation.

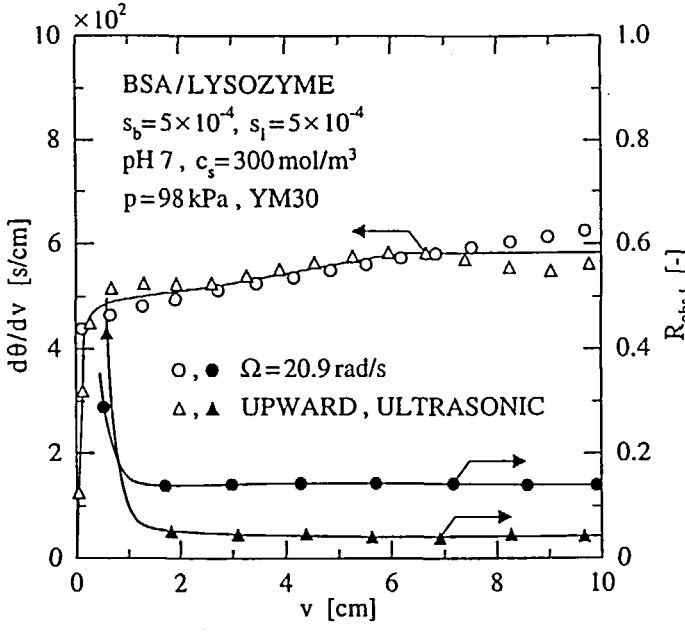

FIG. 11 Comparison of apparent lysozyme rejection between cases with and without ultrasonic irradiation in which similar filtration rates are observed.

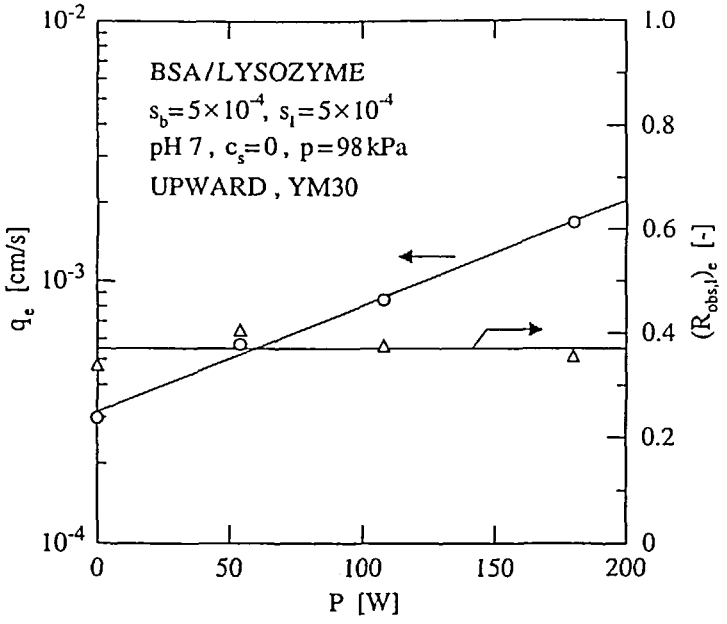

FIG. 12 q_e and $(R_{obs,l})_e$ as function of output power of ultrasonic.

creases linearly with increasing P from 0 to 180 W, while $(R_{obs,l})_e$ is independent of the ultrasonic intensity.

CONCLUSIONS

The separation properties of UF of binary protein mixtures consisting of BSA and lysozyme through BSA-impermeable, lysozyme-permeable membrane have been examined for various filtration modes, solution environments, and membranes. It was found that the filtration rate and the apparent rejection of lysozyme are strongly influenced by the hydrodynamics above the membrane, the solution pH, and the protein adsorption within the membrane. In particular, it is of interest and significance that the increase in the shear stress acting on the membrane surface is effective for obtaining a high filtration rate but otherwise obstructs the passage of lysozyme solutes through the membrane, thereby reducing the fractionation efficiency. The effects of ultrasonic irradiation during UF of binary protein mixtures on the separation properties were also explored. The experimental results revealed that ultrasonic irradiation was quite effec-

tive for fractionation of the binary protein mixtures because the ultrasonic fields led to a remarkable improvement in the filtration rate and did not lower the lysozyme transmission. We believe that these results serve as a valuable aid for achieving more efficient fractionation 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	output power of ultrasonic (W)
p	applied filtration pressure (Pa)
q_e	filtration rate at v of 10 cm (m/s)
$R_{obs,l}$	apparent rejection of lysozyme defined by Eq. (1)
$(R_{obs,l})_e$	apparent rejection of lysozyme at v of 10 cm
s_b	mass fraction of BSA in bulk feed fluid
s_l	mass fraction of lysozyme in bulk feed fluid
v	cumulative filtrate volume per unit effective membrane area (m)

Greek Symbols

θ	filtration time (s)
Ω	angular velocity of stirring (rad/s)

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REFERENCES

1. A. G. Fane, in *Progress in Filtration and Separation 4, Ultrafiltration: Factors Influencing Flux and Rejection* (R. J. Wakeman, Ed.), Elsevier, Netherlands, 1986, pp. 134–139.
2. V. G. Rodgers and R. E. Sparks, "Effects of Solution Properties on Polarization Redevelopment and Flux in Pressure Pulsed Ultrafiltration," *J. Membr. Sci.*, *78*, 163–180 (1993).
3. S. P. Palacek and A. L. Zydney, "Intermolecular Electrostatic Interactions and Their Effect on Flux and Protein Deposition during Protein Filtration," *Biotechnol. Prog.*, *10*, 207–213 (1994).
4. W. R. Bowen and F. Jenner, "Dynamic Ultrafiltration Model for Charged Colloidal Dispersions: A Wigner-Seitz Cell Approach," *Chem. Eng. Sci.*, *50*, 1707–1736 (1995).
5. E. Iritani, S. Nakatsuka, H. Aoki, and T. Murase, "Effect of Solution Environment

on Unstirred Dead-End Ultrafiltration Characteristics of Proteinaceous Solutions," *J. Chem. Eng. Jpn.*, *24*, 177–183 (1991).
6. E. Iritani, T. Watanabe, and T. Murase, "Effects of pH and Solvent Density on Dead-End Upward Ultrafiltration," *J. Membr. Sci.*, *69*, 87–97 (1992).
7. S. Nakatsuka and A. S. Michaels, "Transport and Separation of Proteins by Ultrafiltration through Sorptive and Non-Sorptive Membranes," *Ibid.*, *69*, 189–211 (1992).
8. A. Higuchi, Y. Ishida, and T. Nakagawa, "Surface Modified Polysulfone Membranes: Separation of Mixed Proteins and Optical Resolution of Tryptophan," *Desalination*, *90*, 127–136 (1993).
9. S. Najarian and B. J. Bellhouse, "Effect of Liquid Pulsation on Protein Fractionation Using Ultrafiltration Processes," *J. Membr. Sci.*, *114*, 245–253 (1996).
10. E. Iritani, Y. Mukai, and T. Murase, "Upward Dead-End Ultrafiltration of Binary Protein Mixtures," *Sep. Sci. Technol.*, *30*, 369–382 (1995).
11. E. Iritani, Y. Mukai, and T. Murase, "Properties of Filter Cake in Dead-End Ultrafiltration of Binary Protein Mixtures with Retentive Membranes," *Trans. IChemE, Part A, Chem. Eng. Res. Des.*, *73*, 551–558 (1995).
12. T. Kokugan, Kaseno, S. Fujiwara, and M. Shimizu, "Ultrasonic Effect on Ultrafiltration Properties of Ceramic Membrane," *Membrane*, *20*, 213–223 (1995).
13. B. F. Ruth, "Studies in Filtration. III. Derivation of General Filtration Equations," *Ind. Eng. Chem.*, *27*, 708–723 (1935).
14. E. Iritani, T. Watanabe, and T. Murase, "Upward and Inclined Ultrafiltration under Constant Pressure by Use of Dead-End Filter," *Kagaku Kogaku Ronbunshu*, *17*, 206–209 (1991).

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