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Enhancement of phase separation using a drop coalescer in an aqueous two-phase system

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Abstract

The effect of a drop coalescer on phase separation in a PEG/salt aqueous two-phase system (ATPS) in the absence and presence of protein has been investigated. Raschig rings of ceramic, PTFE and glass were used as a drop coalescer in order to separate the mixture into two phases. Among the three materials PTFE is the most effective in coalescing the dispersed drops, with the throughput with PTFE being twice that without the coalescer. Random packing gives good results for phase separation. Two types of fiber mesh coated with PTFE were also used as drop coalescers, one in a spirally folded form and the other in a three-dimensional lattice-form. Throughput in the PEG/salt system with the three-dimensional lattice-form is 1.2 times as high as that with the spirally folded form. Throughput with the coalescer formed by compiling PTFE Raschig rings and fiber mesh in lattice form is 1.6 and 1.2 times as high as the case of separate use of the fiber mesh and the PTFE Raschig rings, respectively. The hydrophobic surface of PTFE in the compiled coalescer has no significant effect on the recovery fraction of the protein in ATPS.

Keywords: Aqueous two-phase systems; Drop coalescer; Proteins

1. Introduction

Separation of dispersed liquids into two phases is still an important problem in the extraction process. Industrial gravity settlers are usually horizontally cylindrical or rectangular tanks which contain inclined plates or impingement baffles [1–3]. In the settler the liquid–liquid dispersion is continuously fed from one end and the separated phases are drawn out from the other end. In these settlers the flow of the dispersion is essentially horizontal. Vertically cylindrical or rectangular gravity settlers are used in pilot plant experiments [1], in which dispersions are continuously fed from the midpoint of a side surface and the separated phases are drawn out from the top and bottom. The flow of dispersion in such settlers is essentially vertical. Takahashi et al. [4] found in their work on mixer–settler extraction columns that a drop coalescer with a hydrophobic surface is most suitable for stable operation, especially at a large flow-rate of dispersed phase, and that a large throughput is achieved by the suction pressure induced by a lifter-turbine impeller.

Aqueous two-phase systems (ATPS) have been applied for the separation of bioproducts. The systems are usually composed of polyethylene glycol (PEG) and salt (e.g. sulfate or phosphate) or PEG

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and dextran. These systems are suitable for biological materials such as proteins because each phase consists essentially of hydrophilic material. The PEG/salt systems have been used for large scale extraction in industry, since the chemicals are inexpensive [5]. The rate of phase separation in ATPS has been studied from their relation to the physico–chemical properties of the phase [6,7]. However, studies related to the mechanical and structural characteristics of the settler are scare. This work concerns the elucidation of this point. In addition, the same coalescer was used for continuous protein extraction, and determination of the fraction recovered.

2. Experimental

2.1. Reagents

PEG 8000 (8 wt.%)/potassium phosphate (10 wt.%) aqueous solutions separated into two phases were used. Potassium phosphate was prepared as an equimolar mixture of potassium dihydrogenphosphate and dipotassium hydrogenphosphate. Lipase and lysozyme (Sigma Chemical Co., USA) were used as model proteins in the continuous protein extraction experiments.

2.2. Preliminary experiment

The upper phase in ATPS is the PEG-rich phase and the lower phase is the salt-rich phase. The effect of temperature on volume ratio of upper to lower phase in ATPS was investigated for the above mentioned system as shown in Fig. 1. The volume ratio of the mixture is unity at 293 K. The ATPS becomes homogeneous at temperatures lower than 283 K. Thus, separation experiments were carried out at 293 K.

2.3. Continuous operation

The apparatus used in the separation experiments is shown in Fig. 2. The ATPS was agitated at 150 rpm in a mixer and then was introduced into the separator using a peristaltic pump. The installed baffles within the mixer vessel satisfy the fully

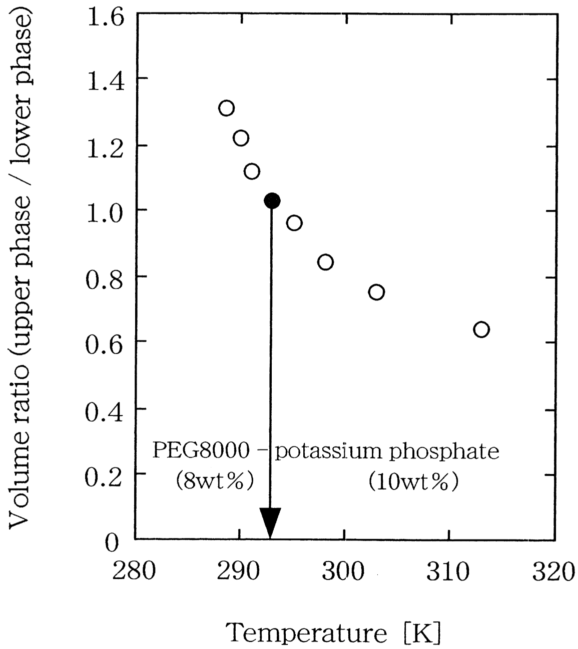

Fig. 1. Effect of temperature on the volume ratio in PEG/phosphate system.

baffled condition. The dispersed droplets coalesce in the period of their passing through the drop coalescer, then enter the settler. Part of the mixture was drawn out from the top of the settler and the remaining part from the bottom with the same volume flow-rate for the top and bottom. Samples were taken from both drawn-out flows and were settled for phase separation. Times necessary for settling of the samples were determined by visual inspection. After complete separation, both of the phase ratios of the drawn-out mixtures were determined. All experimental data are based on one trial.

2.4. Drop coalescer

Takahashi et al. [4] found that a drop coalescer having a hydrophobic surface enhances the coalescence of droplets produced by vigorous agitation and leads to an increase in the maximum throughput for aqueous/organic phase systems. Thus coalescers having a hydrophobic surface as well as a hydrophilic surface were examined for the ATPS system. Columns of the separator are 16.5 mm in inside

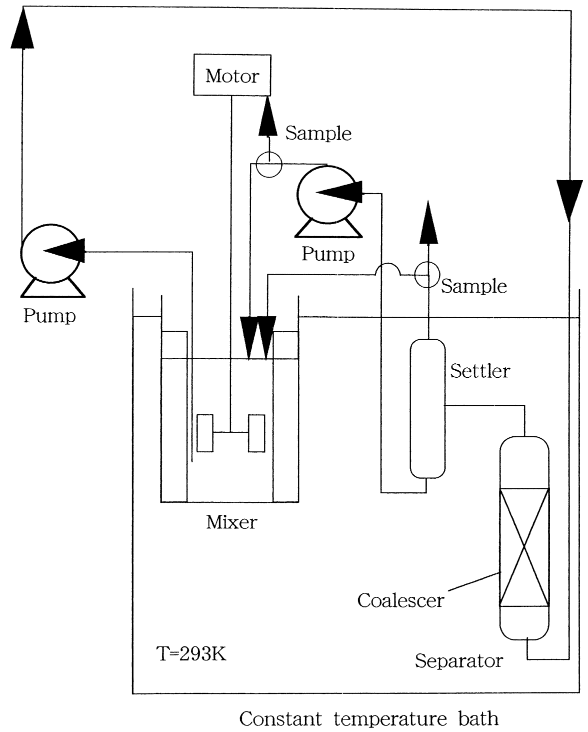

Fig. 2. Experimental apparatus.

diameter and 18, 27, 48 cm^3 in volume, and can be separated into bottom and cap parts. Details of packings in the drop coalescers used in this work are illustrated in Fig. 3. Fiber mesh coated with PTFE and Raschig rings were used as packings. The Raschig rings are made of PTFE, glass, or ceramic. Though both phases of ATPS are hydrophilic, the upper phase with a high PEG concentration is relatively more hydrophobic than the lower phase. The hydrophobic upper phase in ATPS wets the coalescer surface and the mixture is separated into two phases. The meshes are shaped into the three-dimensional lattice-form or the spirally folded form. A separation experiment was similarly conducted for the coalescer obtained by compiling the PTFE Raschig rings and the fiber mesh in lattice form.

2.5. Definition of holdup, ϕ

In this work the holdup, ϕ, is defined as the volume ratio of the upper phase to the total volume of the mixture and the degree of phase separation is

	Mesh		Raschig rings
Shape	• Spiral • Lattice	Material	• Ceramic • Glass • PTFE

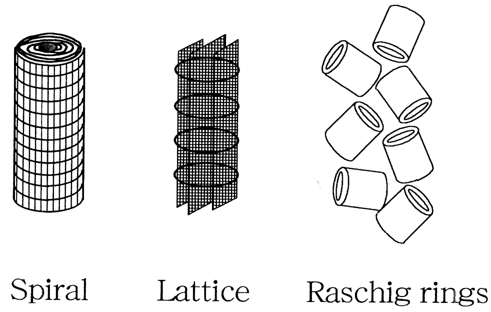

Fig. 3. Shape and material of coalescers.

evaluated in terms of ϕ. As illustrated in Fig. 4, the mixtures are drawn from the top and the bottom of the settler at the same flow-rate. If the drop coalescer set preceding the settler works satisfactorily, the mixture from the top of the settler is wholly composed of the upper phase, while the mixture from the bottom of the settler consists only of the lower phase. When the flow-rate of mixture becomes higher, not only the upper phase but also some of the lower phase is contained in the mixture drained out from the top of the settler and vice versa. After attainment of complete separation of the mixture into two phases at 293 K, the total volume of mixture, $V_{tu}+V_{tl}$, and the upper phase volume, V_{tu}, are mea-

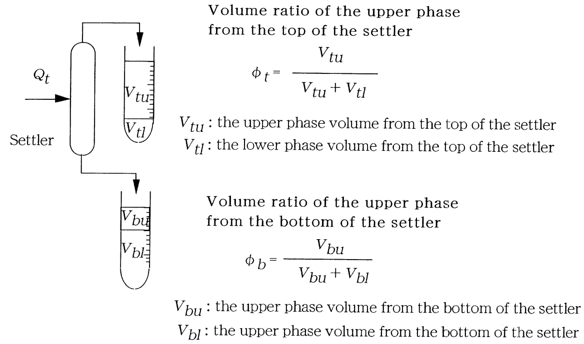

Fig. 4. Definition of holdup.

sured, where V_{tu} and V_{tl} are the volumes of the upper and the lower phase in the mixture drawn from the top of the settler. The volume ratio of the upper phase to the total volume in the mixture is defined as ϕ_t, while the volume ratio in the mixture drawn from the bottom of settler is defined as ϕ_b. If the phase separation is completely done, then $\phi_t = 1$ and $\phi_b = 0$.

2.6. Application of the coalescer to protein extraction

The effect of the coalescer on the fraction of protein recovered was investigated in continuous extraction operation. The coalescer formed by compiling PTFE Raschig rings and fiber mesh in a lattice shape was used for this operation. Protein adsorption on the surface of the coalescer may decrease the fraction of protein recovered. Lysozyme and lipase were used as hydrophilic and hydrophobic proteins, respectively, and the concentrations in both phases were determined by a spectrophotometer (Shimadzu Co., type UV-160).

3. Results and discussion

The effect of separator volume on throughput in the PEG 8000/phosphate system is shown in Fig. 5, where the separators used are hollow tubes without any packings. As the total flow-rate increases, the lower phase appears in the mixture drawn from the top of separator and simultaneously the upper phase appears in that from the bottom of separator. Both of these fractions increase from 0 to 0.5. The maximum throughput is defined as a flow-rate where ϕ_t and ϕ_b deviate from $\phi_t = 1$ and $\phi_b = 0$, respectively. The maximum throughput increases somewhat with the increase in the separator volume, as can be seen in Fig. 5. The upper phase within the bottom mixture decreases with the increase in separator volume from 18 to 27 cm^3, but increases with the volume change from 27 to 48 cm^3. It should also be mentioned that the experimental data of holdup plotted versus residence time for the 18 and the 27 cm^3 separator fall on the same curve except the case of the 48 cm^3 separator. This indicates that the drop coalescence behavior is not determined only by the residence time. In what follows, discussion is limited to the separator of total volume 27 cm^3 which shows the highest performance.

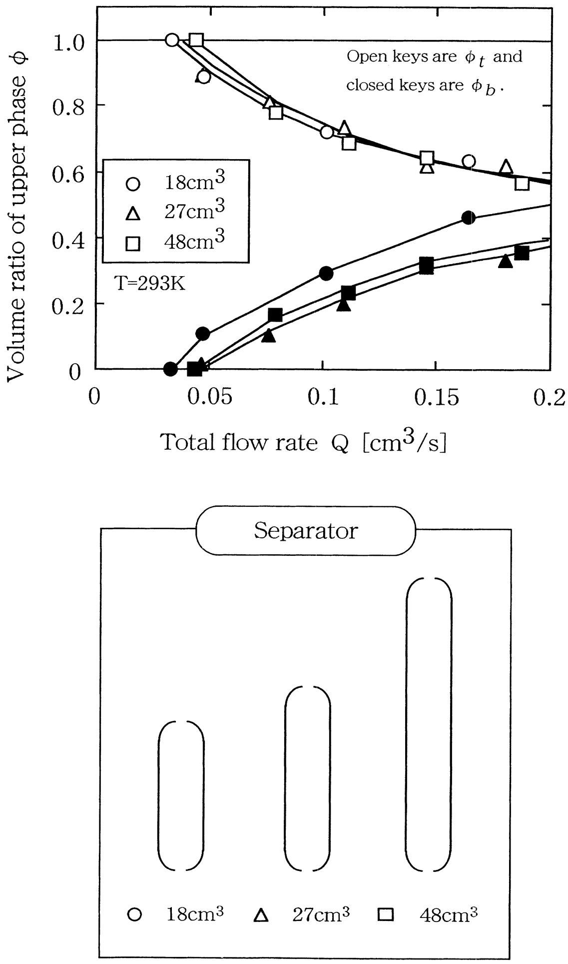

Fig. 5. Effect of separator volume on throughput.

To increase available surface area for drop coalescence, Raschig rings were set in the separator and the coalescence behavior was observed. If some of each phase wet the surface and flew along it, the layer would catch more drops and thus coalescence would be enhanced. Such a packing also gives narrow and irregular flow paths to the drops. Collision of drops could be increased by the flow within the packing layer. This flow effect can also improve the drop coalescence. Fig. 6 shows the effect of volume of PTFE Raschig rings on throughput. The

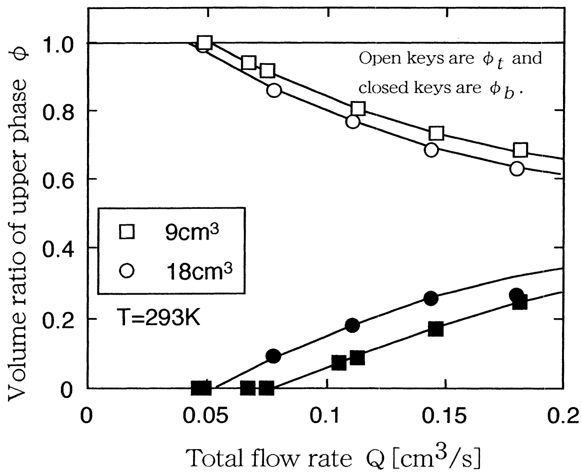

Fig. 6. Effect of volume of Raschig rings on throughput.

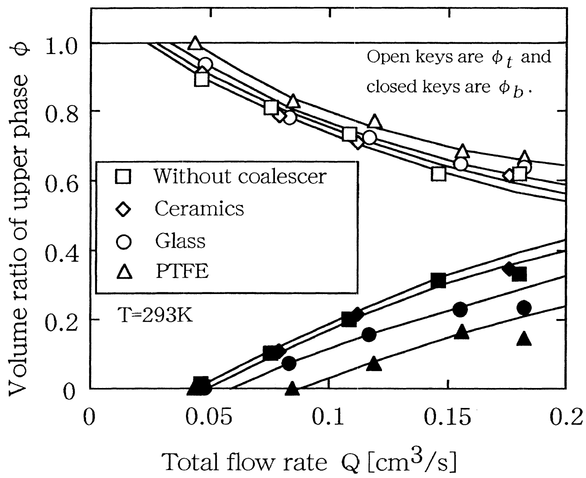

Fig. 7. Effect of the materials of the packings on throughput.

diameter and the length of Raschig rings are 2 and 6 mm, respectively. As can be seen in Fig. 6, smaller volumes of packing give better results for phase separation than larger ones. Although a better result was expected for the packing of larger volume due to a longer residence time of the liquid in packing layer, the result differs from our prediction. A quite similar result was obtained for Raschig rings of 3 mm in length. Presently we have no definite idea on these observations. Results described below refer to the packing volume 9 cm^3 which shows better separation ability.

Fig. 7 shows the effect of the surface material on throughput. The packings are in shape of Raschig ring of 4 mm in diameter and 6 mm in length. It is evident that all the packings enhance phase separation, and that PTFE Raschig rings show the best coalescence performance. The upper phase droplets in the bottom liquid (closed key) coalesce more effectively than the lower phase droplets in the top liquid (open key) in the presence of the packings. The contact angles of each material in an air–water system measured by Nishii [8] are given in Table 1. The value for the PTFE Raschig rings is the highest among the three, which indicates the highest hydrophobicity. This may be the reason for the fact that PTFE Raschig ring separates ATPS most efficiently.

The effect of the diameter of PTFE Raschig rings on the throughput is shown in Fig. 8. The length of Raschig rings is constant (6 mm) and the Raschig rings are packed at random in the separator. The effect of the packing's diameter is minor. Though the fraction of upper phase in the bottom mixture for the packings diameter of 4 mm is somewhat smaller than those for other cases, the throughput seems to increase with the decrease in the packing's diameter. The decrease in diameter results in decreases in the opening between the packings. Thus the narrow flow path causes a large velocity gradient near the surface of packings which increases the chance of drop contact.

Fig. 9 shows the effect of differences in packing mode of the Raschig rings on the throughput. Irregular packing denoted by the key ○ in Fig. 9 refers to random packing of the Raschig rings, while regular packing is related to the four-staged structure of Raschig rings aligned vertically. Partitions of PTFE mesh are set between the stages. Raschig rings used in this experiment were 2 mm in diameter and 6 mm in length, and the number of the Raschig ring for the regular packing is the same as that for the random packing. The regular packing gives little effect on the drop coalescence behavior, while the

Table 1
Contact angles for materials in air–water system

Ceramic	10°
PTFE	97°
Glass	12°

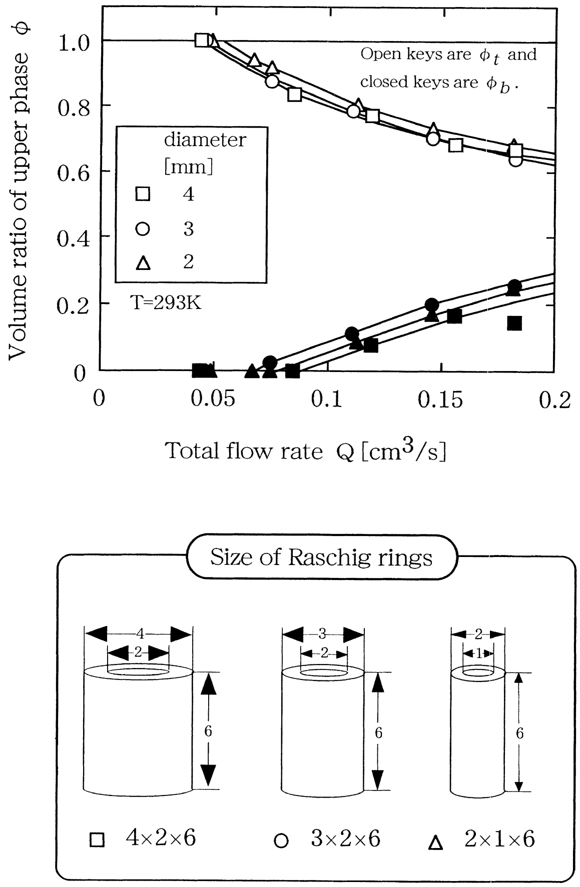

Fig. 8. Effect of diameter of Raschig rings on throughput.

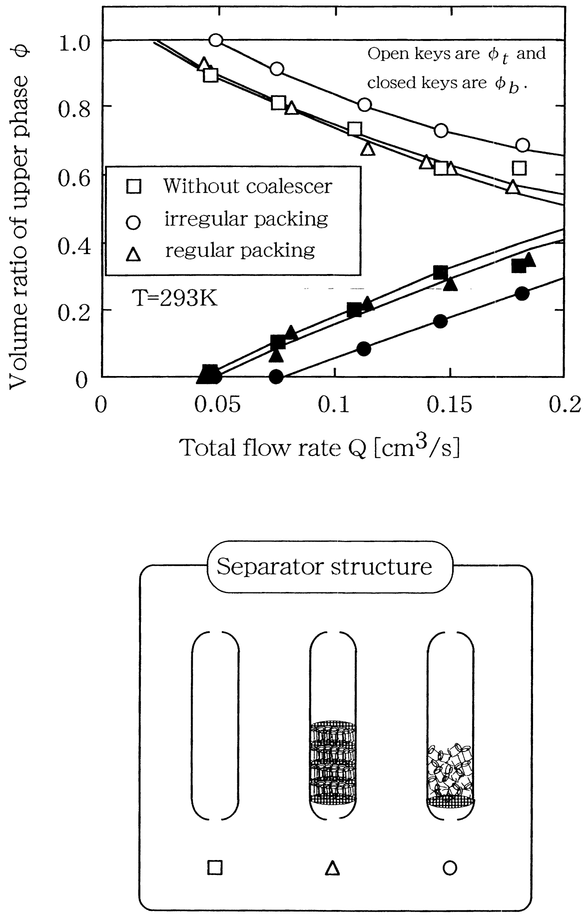

Fig. 9. Effect of packing mode of the Raschig rings on the throughput.

throughput increases significantly for the irregular packings. This implies that the changes in flow direction and flow velocity within the random packing are highly useful to promote drop coalescence.

Fig. 10 shows the results obtained using coalescers prepared with PTFE coated mesh of 2.5×2.3 mm rectangular pitch. Two types, the spiral type and the lattice type, were used. The height of these coalescers is 8 cm. The mesh sheet is rolled up around the core stick in the spiral type, and the mixture flows along the mesh sheet. Its surface area is 290 cm^2 and void fraction is 0.53. The lattice type is composed of sheets parallel in the direction of the mixture flow and sheets perpendicular to the flow. The lattice is a coarse structure. It has a large void fraction of 0.92 and 60 cm^2 surface area. Though the spiral type can catch the upper phase drops effectively at small flow-rate, the total performance of the lattice type is superior to that of the spiral one. The lower phase drops coalesce effectively in the lattice type coalescer. The increase in the number of sheets perpendicular to the flow pushed the phase separation efficiency higher and lead to large throughput.

As can be seen in Fig. 6, an increase in packing volume, i.e. the surface area, is not necessary for promotion of drop coalescence. Thus, a phase separation experiment was carried out, combining the Raschig ring packing and mesh lattice. In Fig. 11 is shown phase separation behavior for various coalescers, mesh lattice, Raschig ring packing and combined type of the Raschig ring packing and mesh lattice. The most remarkable enhancement in

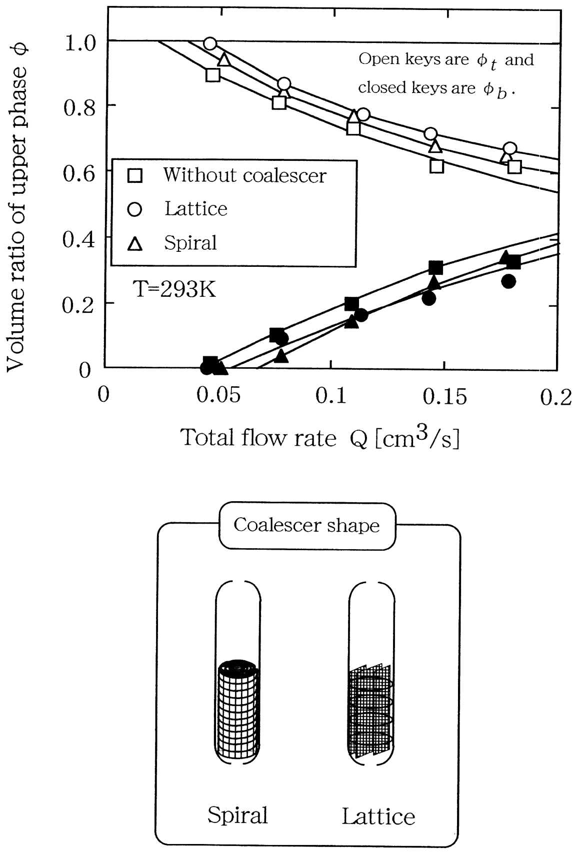

Fig. 10. Effect of coalescer shape on throughput.

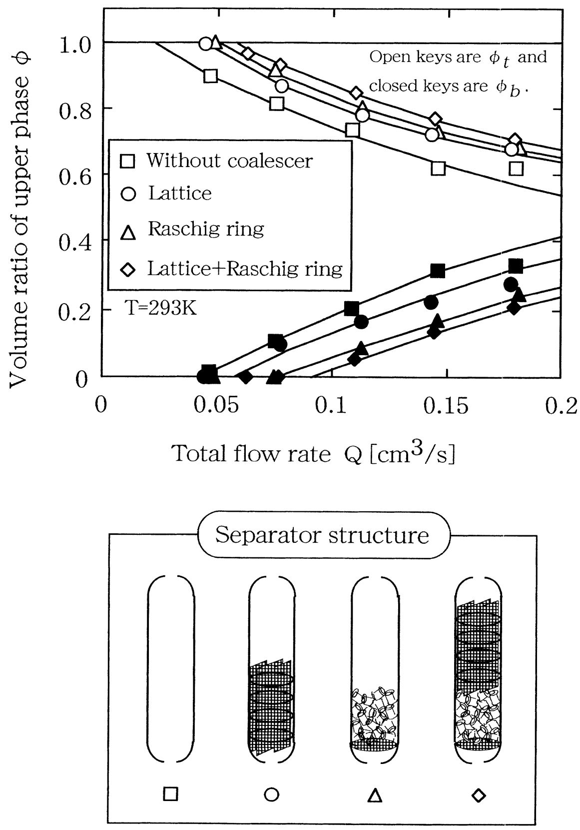

Fig. 11. Effect of various coalescers on throughput.

coalescence behavior is observed for the combined type. The maximum throughput of the bottom liquid with combined type approximately equals to the sum of the maximum throughput with either packing alone, while the maximum throughput of the top liquid with combined type is somewhat smaller than the sum of the maximum throughput with either packing alone. However, using the combined type large throughput, which could not be obtained by increasing the packing volume, are achieved. The addition of the lattice to the Raschig ring gives additional surface area without adding flow resistance because of the coarse structure. The lattice also provides a disturbance of flow by the mesh placed perpendicular to the flow. The combined type gives two kinds of different disturbance to flow of drops. Such different flow effects and a large surface area work synergistically to improve phase separation.

The effect of protein adsorption on the surface of the coalescer on the fraction of protein recovered was investigated for continuous extraction operation. The coalescer having PTFE Raschig rings and fiber mesh in a lattice shape was used for this purpose. Lysozyme and lipase were selected as hydrophilic and hydrophobic proteins, respectively. First, the distribution ratio of protein was measured. For the present ATPS the distribution ratio of lipase in upper phase to lower phase was 0.71, which is larger than the value of 0.54 for lysozyme. This observation is reasonable in view of much higher hydrophobicity of lipase than lysozyme. Second, continuous extraction experiments of proteins using the apparatus shown in Fig. 2 were carried out to measure the change in amounts of the proteins included in the whole ATPS before and after extraction. The results show that lysozyme is not adsorbed on the surface of packings in the combined type separator. However, a decrease

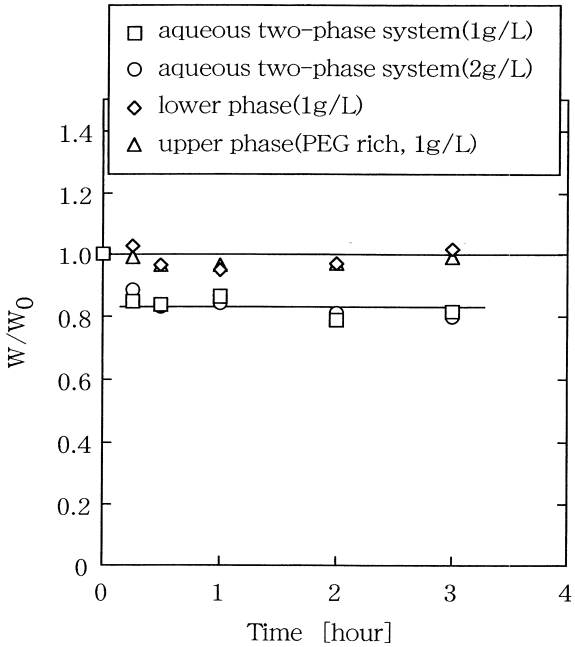

Fig. 12. Time course of ratio between amounts of lipase in outlet and feed solutions for two concentrations of lipase.

in amounts of lipase was observed. Fig. 12 shows the time course of the ratio between amounts of lipase in outlet and feed solutions for two concentrations of lipase. The amounts in the outlet solutions are smaller by 20% than in the feed amounts for both concentrations. Third, an experiment in a batch system without coalescer was conducted and a similar decrease in the lipase amount was observed. This indicates that lipase is not adsorbed on the coalescer but at the interface between the upper and lower phases. Continuous flow experiments with the coalescer were carried out for samples prepared wholly from either the upper or lower phase liquid. In Fig. 12 are shown the results obtained by operating with lower phase or upper phase. The decrease in amounts of lipase is not observed in these cases, which supports the above fact that lipase does not adsorb on the surface of packings. The amount of lipase lost in the two-phase system in the continuous or the batch experiments may be concentrated at the interface between the upper and the lower phases.

4. Conclusion

In continuous operation, drop coalescence behavior is not determined only by the separation volume (i.e. residence time). PTFE, among the three materials studied, most strongly enhances the phase separation, and throughput with PTFE is about twice as high as that without a coalescer, and throughput in a random packing of PTFE Raschig rings is about 1.6 times as high as that in a regular packing of PTFE. These facts suggests the surface wettability and the flow in narrow and irregular paths are important for phase separation. Both the effects of diameter and volume of PTFE Raschig ring packings are minor. Throughput with a three-dimensional lattice-form is 1.2 times as high as that with a spirally folded form. The coalescer obtained by compiling PTFE Raschig rings and fiber mesh in a lattice form works more satisfactorily than the case of separate use of the PTFE Raschig rings or the fiber mesh. Throughput with the coalescer formed by compiling PTFE Raschig rings and fiber mesh in the lattice form is 1.6 and 1.2 times as high as the case of separate use of the fiber mesh and the PTFE Raschig rings, respectively.

During continuous extraction experiments of lipase and lysozyme in the ATPS, no protein loss due to unspecific adsorption on the coalescer surface was observed.

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