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Separation of Polyunsaturated Fatty Acids with Silver Nitrate Using a Hollow-Fiber Membrane Extractor

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ABSTRACT

Solvent extraction of ethyl esters of polyunsaturated fatty acid such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was performed with an aqueous silver nitrate solution using a microporous hydrophobic hollow-fiber membrane extractor. The mixture of four kinds ethyl esters of fatty acids involved in fish oil was employed as a model solution of fish oil. Also studied was the extraction equilibria of these fatty acids at various conditions. It was demonstrated from the experimental results that EPA and DHA ethyl esters could be satisfactorily separated from ethyl esters of lower fatty acids such as oleic and palmitic acids. In addition, the effects of silver and ester concentrations in aqueous and organic feed solutions on the apparent permeabilities of fatty acids ethyl esters were investigated in extraction and stripping stages, respectively. It was concluded that the permeation rate was controlled by the diffusion across the membrane and the laminar boundary layer in the inner and outer sides of the hollow fiber.

INTRODUCTION

A series of ω-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid and docosahexaenoic acid, are now in great demand for

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medicines and food additives because of their excellent physiological functions such as the suppression of cardiovascular disease and cancer and the enhancement of the brain activity, etc. (1). At present, a main source of PUFAs is fish oil derived from sardines, tuna fish, bonito, mackerels, etc., containing PUFAs to some extent (1, 2). However, the isolation of the desired fatty acid is very difficult because the source fish oil consists of a mixture of various saturated or unsaturated fatty acids whose physical properties are very similar. Various techniques have been developed for the separation and purification of individual fatty acid such as urea crystallization (2–4), fractional vacuum distillation (5), high-performance liquid chromatography (6), and supercritical fluid extraction (7). However, these techniques require an elaborate apparatus and a large number of stages to obtain a high-purity product or, in certain cases, high temperature which causes the decomposition of PUFAs. Further, these techniques are not suitable for large-scale production. Therefore, a more simple technique is desired for the present purpose.

Solvent extraction has been widely used for the separation and concentration of metals in hydrometallurgical processes. Its application to the purification of the ethyl ester of PUFA (PUFA-Ets) based on the complexation between double bonds of PUFA-Et and silver ions by solvent extraction was recently reported as a new method (1). Since the separation is performed exclusively via chemical reaction, this technique is very simple and would be a promising method for the purification of PUFAs. Teramoto et al. studied the extraction equilibria of esters of PUFAs systematically (8) and demonstrated the facilitated transport of EPA-Et through a bulk and supported liquid membrane containing silver nitrate as a carrier (9). In contrast, solvent extraction of PUFAs through a solid membrane has not been reported. Membrane-based solvent extraction with a hollow-fiber membrane has been developed as an alternative to conventional solvent extraction with a liquid–liquid dispersion from the viewpoint of energy efficiency (10, 11). A hollow-fiber module can eliminate the problems caused by the mixing of aqueous and organic phases such as loading, flooding, and emulsification. Further, it is possible to provide a large contact area per unit equipment volume by a simple device.

The purpose of this study is to apply this novel membrane extraction method to the separation of PUFAs from fish oil. The extraction and stripping of ethyl esters of DHA, EPA, and two other lower fatty acids involved in fish oil was performed with $AgNO_3$ as an extractant in a hollow-fiber membrane extractor. The permeation behavior of PUFA-Ets through the membrane was investigated to find the optimal conditions and to discuss the possibility of isolation of DHA and EPA.

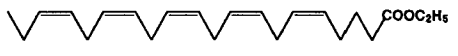
EXPERIMENTAL

Reagents

The structures and abbreviations of fatty acid ethyl esters (FA-Ets) used in this work are shown in Fig. 1. DHA-Et and EPA-Et were from Harima Chemicals Co., Ltd. (Lot No. 930628 and 930421, respectively). OA-Et and PA-Et were purchased from Tokyo Chemical Inc. Silver nitrate was from Shoei Chemical Inc. *n*-Heptane of commercial GR grade was used as the organic solvent without further purification. All other inorganic reagents used were GR grade.

An organic solution containing these fatty acid ethyl esters was prepared by dissolving them in *n*-heptane. An aqueous solution containing Ag^+ was prepared by dissolving silver nitrate in deionized water. An aqueous solution containing silver complexes of FA-Ets used in the stripping study was prepared by contacting an aqueous silver nitrate solution with an organic FA-Ets solution. The ionic strength of an aqueous solution was adjusted with sodium nitrate. The concentration of Ag^+, C_{Ag}, in the aqueous phase was determined by atomic absorption spectrophotometry (Seiko Model SAS-760).

docosahexaenoic acid ethyl ester (DHA-Et)



eicosapentaenoic acid ethyl ester (EPA-Et)

oleic acid ethyl ester (OA-Et)

palmitic acid ethyl ester (PA-Et)

FIG. 1 The structure and the abbreviations of fatty acid ethyl esters.

Measurement of Extraction Equilibrium

A mixture of an appropriate volume ratio of aqueous and organic solutions in a sealed vial was shaken about 18 hours at constant temperature (303 K) to attain equilibrium. The concentration of FA-Ets distributed into the aqueous phase was determined by measuring its concentration after backextracting into *n*-heptane phase as follows. Deionaized water was first added into a known volume of the aqueous phase to decrease C_{Ag}. Then FA-Ets released from the complex were backextracted to an appropriate volume of *n*-heptane. The concentration of FA-Ets in the organic phase was determined by gas chromatography (HP5890, Hewlett-Packard).

Measurement of Extraction and Stripping Rates

The extraction and stripping rates were measured at 303 K with the membrane extractor shown schematically in Fig. 2. The extractor is composed of a glass tube and a microporous hollow fiber made of polytetrafluoroethylene. The specifications of the hollow fiber are shown in Table 1. The aqueous solution containing Ag^+ and/or silver complex of PUFA-Ets was fed along the inner side of the fiber, and the organic solution containing FA-Ets or none was fed along the outer side of the fiber cocurrently by a microtube pump (Tokyo Rika MP1001). The pores of the membrane were filled with the organic solution because the fiber was hydrophobic. At the steady state, the concentration of FA-Ets in the effluent solutions was determined by the same manner mentioned above.

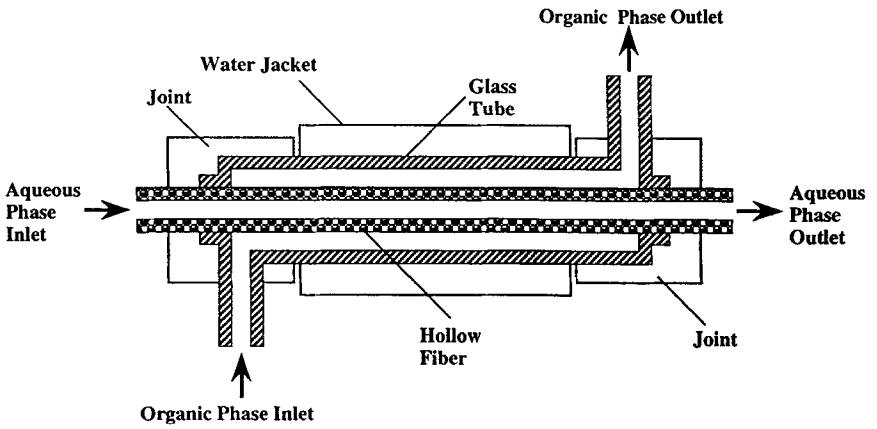


FIG. 2 Schematic diagram of membrane extractor using a hollow fiber.

TABLE 1
Specifications of the Hollow Fiber Membrane Extractor

Inner diameter of membrane (d_i): 1.02×10^{-3} m
Outer diameter of membrane (d_o): 1.86×10^{-3} m
Pore size: 2.0 μm
Porosity: 0.43
Tortuosity factor: 1.14
Inner diameter of extractor (D): 2.4×10^{-3} m
Length (L): 0.25 m

The apparent permeabilities for the extraction and stripping of FA-Et, P and P', respectively, were evaluated as follows:

$$P\ (= J_{FA}/C_{FA0}) = EQ_{org}/(\pi d_i L) \quad (1)$$

$$P'\ (= J'_{FA}/C_{comp0}) = E'Q_{aq}/(\pi d_i L) \quad (2)$$

where J_{FA} and J'_{FA} are the average extraction and stripping rates, Q_{aq} and Q_{org} are the volumetric flow rates of the aqueous and organic solutions, and E and E' are the extents of FA-Et extracted and stripped, respectively. The subscript "comp" denotes the complex of FA-Et with silver.

RESULTS AND DISCUSSION

Extraction Equilibrium

Effect of Ionic Strength in Aqueous Phase on Extraction Equilibrium

Since the extraction of FA-Ets is performed by employing an aqueous silver nitrate solution of fairly high concentration, it is presumed that the extraction equilibrium is influenced by the ionic strength of the aqueous phase. In order to reveal this effect, the distribution ratio D of EPA-Et, defined by $C_{EPA\text{-}Et,aq}/C_{EPA\text{-}Et,org}$, was measured at various ionic strengths while keeping the concentration of silver nitrate at 1.0 or 2.0 kmol/m^3. As shown in Fig. 3, D decreased with increasing ionic strength. In the range of low ionic strength, D decreased sharply, whereas it was almost constant in the rage of high ionic strength.

Extraction Equilibria of Fatty Acids

Figure 4 shows plots of D for four fatty acids against C_{Ag0} at ionic strengths, higher than 4 kmol/m^3 where D was independent on ionic

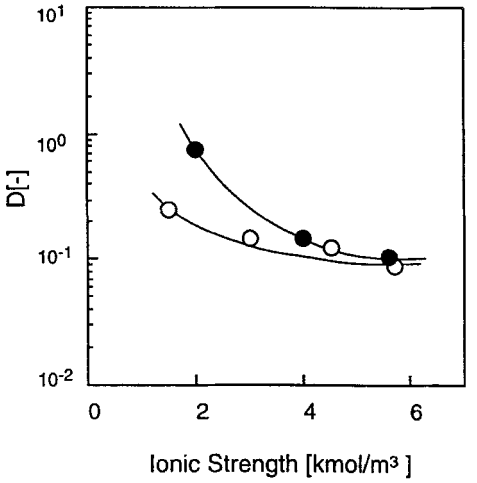


FIG. 3 Relation between D and ionic strength. $C_{EPA\text{-}Et,0}$ = 10 mol/m^3, (○) C_{Ag0} = 1 kmol/m^3, (●) C_{Ag0} = 2 kmol/m^3.

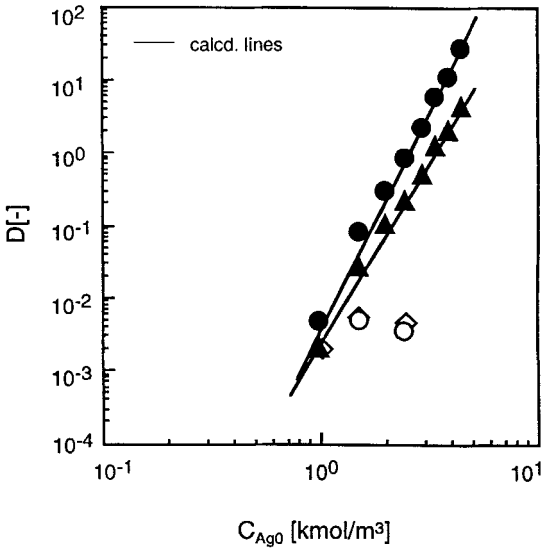


FIG. 4 Relation between D and C_{Ag0}. C_{FAi0} = 10 mol/m^3, (●) DHA-Et, (▲) EPA-Et, (○) OA-Et, (◇) PA-Et.

strength. OA-Et and PA-Et were scarcely extracted even at high C_{Ag0}. This finding demonstrates that DHA-Et and EPA-Et are readily separated from lower fatty acids ethyl esters in a source oil with the solvent extraction method using Ag^+ as an extractant. The slopes of the linear correlation for DHA-Et and EPA-Et were nearly equal to 6 and 5, respectively, which corresponds to the number of carbon—carbon double bonds in each PUFA-Et, n, as reported by Teramoto et al. (8). PUFA-Et is extracted to the aqueous solution by forming a 1:1 complex between each double bond and Ag^+ as follows:

$$\text{PUFA-Et}_{org} + n\text{Ag}^+_{aq} = (\text{PUFA-Et}\cdot n\text{Ag})^{n+}_{aq}; \qquad K_{ex} \tag{3}$$

The extraction equilibrium constant, K_{ex}, is written as

$$K_{ex} = C_{(\text{PUFA-Et}\cdot n\text{Ag})^{n+}_{aq}} / C_{\text{PUFA-Et}_{org}} \cdot C^n_{\text{Ag}_{aq}} \tag{4}$$

By rewriting Eq. (4) with D, the following equation is obtained:

$$\log D = \log K_{ex} + n \log C_{Ag} \tag{5}$$

The values of K_{ex} determined from Fig. 4 with Eq. (5) is 3.3×10^{-21} $(m^3/mol)^6$ and 2.3×10^{-18} $(m^3/mol)^5$ for DHA-Et and EPA-Et, respectively. The solid lines in Fig. 4 are the calculated results from using these values.

Extraction and Stripping Rates

Effect of Ionic Strength in Aqueous Phase on Extraction Rate

The results in the equilibrium study evidently show that the ionic strength affects the extraction behavior of PUFA-Et with silver nitrate. In order to determine the ionic strength suitable for the study of the permeation mechanism through the membrane, the extraction rates of EPA-Et were measured under several ionic strengths. Figure 5 shows the effect of ionic strength on the relation between the permeability of EPA-Et, $P_{EPA\text{-}Et}$, and C_{Ag0}. The ionic strength of the solution noted as "not adjusted" is equal to the silver nitrate concentration. $P_{EPA\text{-}Et}$ decreased remarkably with increasing ionic strength from 2 to 3 kmol/m^3. In fact, at high ionic strength, a measurable amount of EPA-Et was not extracted in the low C_{Ag} range. In the range of ionic strength higher than 3 kmol/m^3, D was almost independent of ionic strength. Also, $P_{EPA\text{-}Et}$ was not affected by ionic strength at high C_{Ag0}. From these results, the extraction rate of PUFA-Et was measured in the range of ionic strength higher than 4 kmol/m^3.

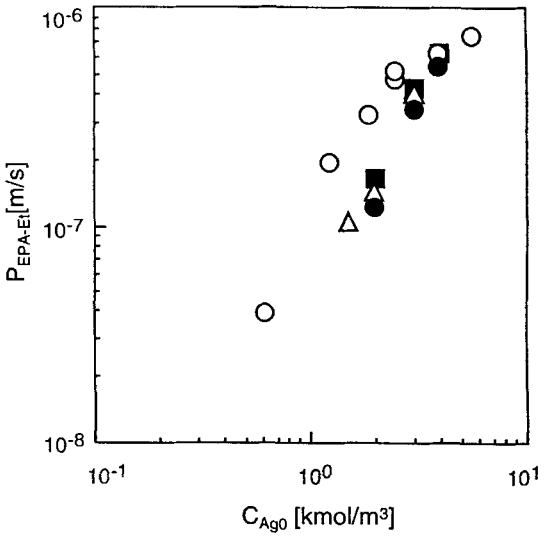


FIG. 5 Effect of ionic strength on the relation between the permeability of EPA-Et and C_{Ag0}. $C_{EPA-Et,0}$ = 10 mol/m^3, ionic strength; (○) not adjusted, (△) 3 kmol/m^3, (●) 4 kmol/m^3, (■) 5 kmol/m^3.

Extraction and Stripping Rates of PUFA-Et

Figure 6 shows the relation between P and the concentration of EPA-Et in the organic feed solution, C_{FA0}. P was not affected by C_{FA0} under the present experimental conditions, hence the extraction rate was proportional to C_{FA0}. Figure 7 shows the relation between P and C_{Ag0} in the aqueous solution. P increased with increasing C_{Ag0} and approached a constant value at high C_{Ag0}. OA-Et and PA-Et were scarcely extracted in all runs, although they were involved simultaneously in the organic feed solution. This suggests DHA-Et and EPA-Et can easily be separated by the membrane extraction method from lower fatty acids in the feed solution prepared from fish oil. In the low C_{Ag0} range the slopes of linear correlations in Fig. 7 were nearly equal to 6 and 5 for DHA-Et and EPA-Et, respectively. Consequently, the suitable condition for mutual separation of DHA-Et and EPA-Et in the extraction stage seems to be in a moderate range of C_{Ag0}. Figure 8 shows the relation between P' and C_{Ag0} in the aqueous feed solution. At low C_{Ag0}, P' was scarcely affected by C_{Ag0}, while it decreased sharply with increasing C_{Ag0} at high C_{Ag0}. There-

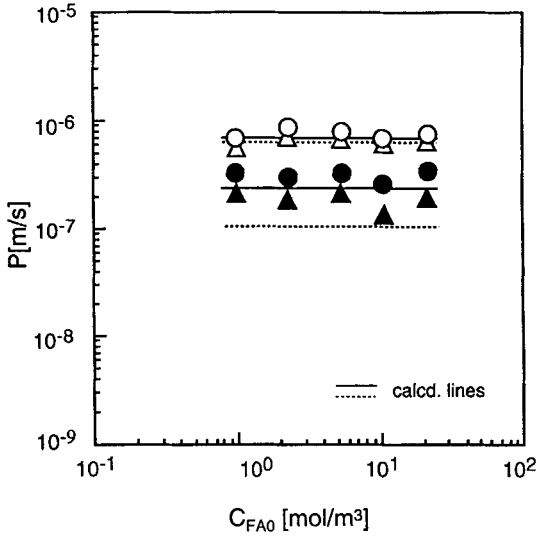


FIG. 6 Relation between P and C_{FA0}. (○) DHA-Et, (△) EPA-Et; C_{Ag0} = 4.9 kmol/m^3. (●) DHA-Et, (▲) EPA-Et; C_{Ag0} = 2.2 kmol/m^3.

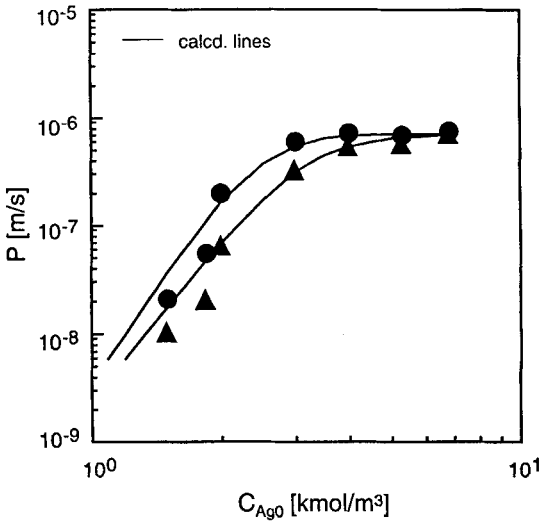


FIG. 7 Relation between P C_{Ag0}. C_{FAi0} = 10 mol/m^3, (●) DHA-Et, (▲) EPA-Et.

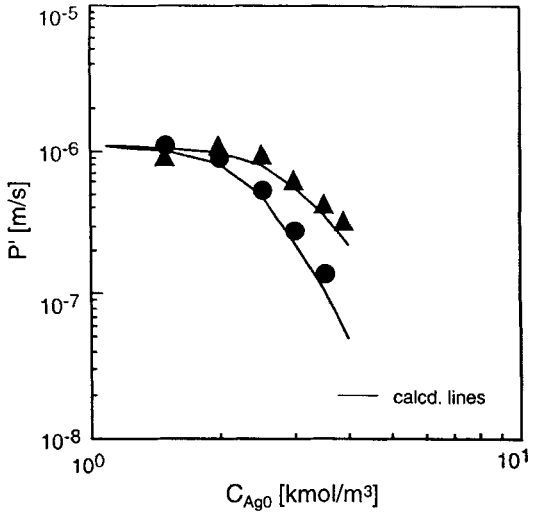


FIG. 8 Relation between P' and C_{Ag0}. C_{FAi0} = 10 mol/m^3, (●) DHA-Et, (▲) EPA-Et.

fore, higher C_{Ag0} is desirable in the stripping stage to accomplish better mutual separation of two PUFA-Ets.

As noted above, the desirable silver concentration of the aqueous phase for the permeation rate and selectivity is different in the extraction and stripping stages. Therefore, it is necessary for the best setup of the recovery system to change the silver concentration between the extraction and stripping stages. This is not practical because the dilution and concentration steps are introduced in the recovery system. Consequently, determining the optimum silver concentration reguires taking into account the recovery rate and the selectivity. At this point we believe that operation at a moderate silver concentration of 2–3 kmol/m^3 is desirable in the present case based on the difference of permeabilities between DHA-Et and EPA-Et.

Permeation Mechanism of PUFA-Et

The characteristics shown in Figs. 6 to 8 are typical of metal extraction and stripping kinetics in a hollow-fiber membrane extractor as shown in a previous paper (12). According to the permeation mechanism of metals reported so far, the extraction of PUFA-Et is considered to proceed as follows. PUFA-Et diffuses from the bulk organic phase to the outer sur-

face of the hollow fiber and penetrates across the membrane pore filled with organic solution. Then it reaches the aqueous/organic phase interface at the inner surface of the hollow fiber. PUFA-Et distributed to the aqueous phase reacts with the extractant Ag^+ near the interface to form the complex which diffuses into the aqueous bulk phase. Figure 9 shows the schematic concentration profile of PUFA-Et along the raidal direction in a hollow-fiber extractor.

By assuming that the interfacial chemical reaction is in an equilibrium state, the extraction rate at steady state is derived in each region of the extractor as follows:

$$\begin{aligned} J_{FA} &= d_o/d_i \cdot k_o(C_{FA,b} - C'_{FA}) \\ &= d_{lm}/d_i \cdot k_m(C'_{FA} - C_{FA,i}) \\ &= k_a(C_{comp,i} - C_{comp,b}) \end{aligned} \tag{6}$$

where k_o and k_m are the mass transfer coefficients of PUFA-Et in the organic and membrane phases, respectively, and k_a is that of the complex in the aqueous phase. d_{lm} is the logarithmic mean of diameters d_i and d_o. From Eqs. (4) and (6), the extraction rate is derived as

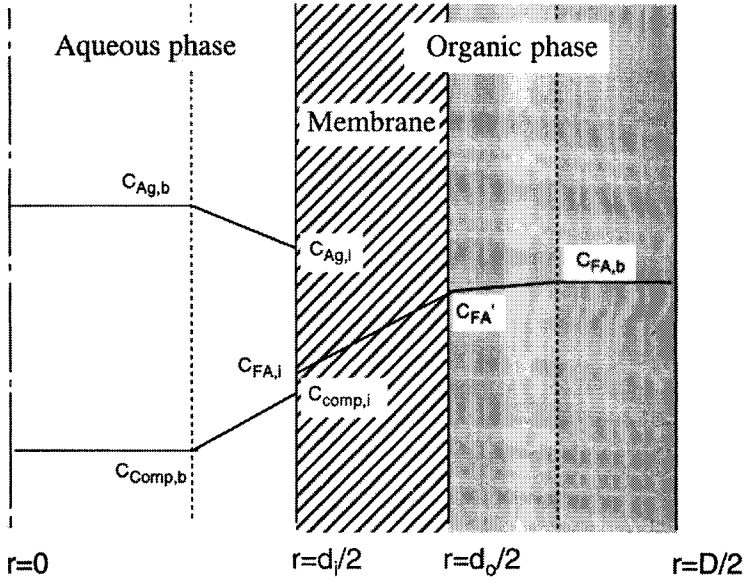


FIG. 9 Schematic concentration profile of PUFA-Et along the raidal direction in a hollow fiber extractor.

$$J_{FA} = K_w(C_{FA,b} - C_{comp,b}/(K_{ex} \cdot C_{Ag}^n)) \quad (7)$$

where K_w, the overall mass transfer coefficient of PUFA-Et, is represented as

$$1/K_w = 1/K_{ex}C_{Ag}^n k_a + d_i/d_{lm}k_m + d_i/d_o k_o \quad (8)$$

Similarly, the stripping rate is derived as

$$J'_{FA} = K'_w(C_{comp,b} - K_{ex} \cdot C_{Ag}^n \cdot C_{FA,b}) \quad (9)$$

where

$$1/K'_w = 1/k_a + K_{ex}C_{Ag}^n d_i/d_{lm}k_m + K_{ex}C_{Ag}^n d_i/d_o k_o \quad (10)$$

Equations (8) and (10) indicate that the total mass transfer resistance is the sum of the individual resistances of each region of the extractor. In the organic phase resistances, the third term on the right-hand side of each equation is considered to be negligibly small compared to the second term, since the boundary layer in the outer side of the membrane is much thinner than the wall thickness of the membrane in the organic phase. P and P', obtained from experimental data according to Eqs. (1) and (2), are approximately equal to the overall mass transfer coefficients K_w and K'_w defined by Eqs. (7) and (9), respectively.

From Fig. 7 it is evident that the second term of Eq. (8) predominantly controls the extraction rate of PUFA-Et at high C_{Ag0} where P is almost constant. Similarly, the stripping rate is exclusively controlled by the first term of Eq. (10) in the low C_{Ag0} region of Fig. 8. Therefore, the following equations hold at the plateau region.

$$1/P = d_i/d_{lm}k_m \quad (11)$$

$$1/P' = 1/k_a \quad (12)$$

From the experimental results, $k_m = 5.2 \times 10^{-7}$ m/s and $k_a = 1.0 \times 10^{-6}$ m/s were determined. The solid lines in Figs. 7 and 8 were calculated from Eqs. (8) and (10), respectively, by using the estimated mass transfer coefficients. Satisfactory agreement between the experimental and calculated results was obtained, although a deviation of data in the estimated permeability caused by the low extent of extraction in the lower C_{Ag0} range was observed in Fig. 7.

CONCLUSION

In order to develop a novel membrane extraction technique for the separation of PUFAs from lower fatty acids involved in fish oil, the extraction and stripping of ethyl esters of DHA, EPA, OA, and PA were investi-

gated with silver nitrate as an extractant in a hollow-fiber membrane extractor. Also studied was the extraction equilibria of these fatty acids. It was demonstrated that the hollow-fiber system was suitable for separating PUFAs from lower fatty acids, since DHA-ET and EPA-Et were selectively extracted to the aqueous solution by forming a complex with the silver ion. Further, the permeation behavior of DHA-Et and EPA-Et through the membrane was investigated to determine the possibility of mutual separation of these PUFA-Ets. The suitable concentration of Ag^+ to separate DHA-Et and EPA-Et was in moderate range in the extraction stage. Separation was enhanced in the stripping stage with increasing C_{Ag0}. The observed permeation rate of PUFA-Et was well explained by the diffusion model accompanied with an instantaneous complexation or decomplexation reaction between PUFA-Et and Ag^+ at the aqueous/organic phase interface.

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