Selective Binding of Docosahexaenoic Acid Ethyl Ester to a Silver-Ion-Loaded Porous Hollow-Fiber Membrane

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ABSTRACT: Silver ions were loaded at a density of 1 mmol/g onto a sulfonic acid group-containing porous hollow-fiber membrane prepared by radiation-induced graft polymerization of an epoxy group-containing monomer with subsequent modification by reaction with sodium sulfite. The permeability (i.e., permeation flow rate per inside surface area of the hollow fiber) of 4.6% wt/vol bonito oil ethyl ester solution in water/ethanol (7.5:92.5, vol/vol) was 1.7 m/h at a permeation pressure of 0.1 MPa. Breakthrough curves (i.e., concentration changes of the effluent with increasing effluent volume) obtained with docosahexaenoic acid ethyl ester (DHA-Et) overlapped, irrespective of the permeation flow rate. This indicates that a higher rate of DHA-Et adsorption onto the silver ions on the membrane was attained with increasing permeation flow rate. DHA-Et, which was selectively bound to the membrane, was quantitatively eluted with acetonitrile as an eluent. The adsorption characteristics (i.e., binding rate, selectivity and durability for repeated use) of DHA-Et using the silver ion loaded porous hollow-fiber membrane were demonstrated. Feasibility studies will enable comparison of the purification cost of DHA-Et among the other purification techniques.

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KEY WORDS: Bonito oil, DHA, permeation flow, porous hollow-fiber membrane, silver ion binding.

Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are representative long-chain n-3 fatty acids. Since EPA and DHA are powerful competitive inhibitors of lipoxygenases and cyclooxygenases, the intake of these polyunsaturated fatty acids (PUFA) may have fundamental consequences for cellular and tissue functions. DHA is considered to be a promising pharmaceutical because of its beneficial effects on the retina and brain (1).

Conventionally, DHA has been purified by vacuum distillation, column chromatography using octadecyl-group-containing silica beads, and supercritical fluid extraction. Stein and Slawson (2) used silicic acid-silver nitrate adsorbents for chromatography, based on a weak interaction between silver ions and the double bonds of PUFA. Recently, liquid extraction using a silver nitrate aqueous solution has been proposed (3). A liquid membrane using the silver nitrate aqueous solution as the water phase was applied for the extraction of EPA (4) and PUFA (5). Moreover, a silver ion-loaded cation-exchange membrane exhibited considerable differences in the permeability rates of PUFA with different numbers of double bonds (6). A silver ion-sandwiched clay material has been suggested for DHA purification (7).

We have prepared porous membranes containing functional groups: chelating porous membranes (8) for collecting metal ions, and ion-exchange (9), hydrophobic (10) and affinity (11,12) porous membranes for collecting proteins. These membranes realize a high collection rate because of convection-aided transport of target ions and proteins to the functional groups. Membrane chromatography using porous functional membranes is advantageous over column chromatography using functional beads because of high throughput (13) and linear scale-up (14); therefore, the loading of silver ions to the porous functional membranes will lead to a potential application in high-performance recovery of DHA.

The objectives of this study were: (i) to prepare a silver-ion-loaded porous hollow-fiber membrane, (ii) to examine the specific binding of DHA ethyl ester (DHA-Et) to other ethyl esters, and (iii) to demonstrate the repeated adsorption and elution capability of DHA-Et.

EXPERIMENTAL PROCEDURES

Materials. A commercially available porous hollow-fiber membrane, manufactured by Asahi Chemical Industry Co., Ltd. (Tokyo, Japan), was used as a trunk polymer for grafting. This hollow fiber made of polyethylene (PE) is designed for microfiltration of suspended solids and bacteria, and has inner and outer diameters of 1.9 and 3.0 mm, respectively. The average pore diameter and porosity were 0.3 μm and 70 %, respectively.

Glycidyl methacrylate (GMA, CH_2=$CCH_3COOCH_2CHOCH_2$) was purchased from Tokyo Chemical Co. (Tokyo, Japan) and used without further purification. Other chemicals were of analytical grade.

Bonito oil was converted into a corresponding mixture of ethyl esters by ester exchange with ethyl alcohol using the oil

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processing line of Maruha Co. (Tokyo, Japan). The composition of the bonito oil ethyl esters was as follows: C22:6 (DHA-Et), 70.3 wt%; C18, 3.1 wt%; C20, 13.3 wt%; C22, 9.8 wt%; C24, 1.7 wt%; other, 1.8 wt%.

Immobilization of silver ion onto porous hollow-fiber membrane. The porous silver ion-loaded hollow-fiber membrane was prepared *via* the following four steps, as illustrated in Scheme 1: (i) radiation-induced grafting of GMA onto a PE porous membrane, (ii) conversion of the produced epoxy group to a sulfonic acid (SA) group by reaction with sodium sulfite, (iii) addition of water to the remaining epoxy group to form a diol group adjacent to the SA group, and (iv) immobilization of silver ion onto the SA by ion-exchange interaction.

The first three steps were completed according to the procedure reported by Tsuneda *et al.* (15). The resultant SA group-containing porous hollow-fiber membrane was referred to as a GMA-SA membrane. The SA group density, which is equivalent to the ion-exchange capacity of the GMA-SA membrane, was determined from the weight gain as:

$$\text{SA group density} = (W_2 - W_1)/82/W_2 \qquad [1]$$

where W_1 and W_2 are the weights of the GMA-grafted and GMA-SA membranes, respectively. The figure 82 is the mass of H_2SO_3. The GMA-SA membrane was immersed in 0.1 M silver nitrate aqueous solution at 303 K with an excess molar ratio of silver ions to SA groups. The immersion time was 24 h. The resultant silver ion-loaded porous hollow-fiber membrane is referred to as the GMA-SA-Ag membrane. The silver ion density was evaluated from the weight gain *via* the ion exchange of silver ions with hydrogen ions as:

$$\text{silver ion density} = (W_3 - W_2)/107/W_3 \qquad [2]$$

where W_3 is the weight of the GMA-SA-Ag membrane. The figure 107 is the mass value of silver minus that of hydrogen.

Permeation of bonito oil ethyl esters through porous silver ion-loaded membrane. The 10-cm-long GMA-SA-Ag membrane was positioned was positioned as shown in Scheme 2. A 4.6% wt/vol bonito oil ethyl ester solution, the solvent of which was a 7.5% vol/vol water/ethanol mixture, was forced to permeate radially outward through the hollow-fiber membrane at a constant permeation flow rate ranging from 0.3 to 3.0 mL/min. The effluent penetrating the outside surface of the membrane was continuously sampled using a fraction collector. The composition of each fraction was determined by gas chromatography. The permeation flux of the solution based on the inside surface area of the hollow-fiber membrane, the amount of ethyl esters bound to the membrane, and the equilibrium binding capacity were calculated as follows:

$$\text{permeation flux} = (\text{permeation flow rate})/(\pi d_i L) \qquad [3]$$

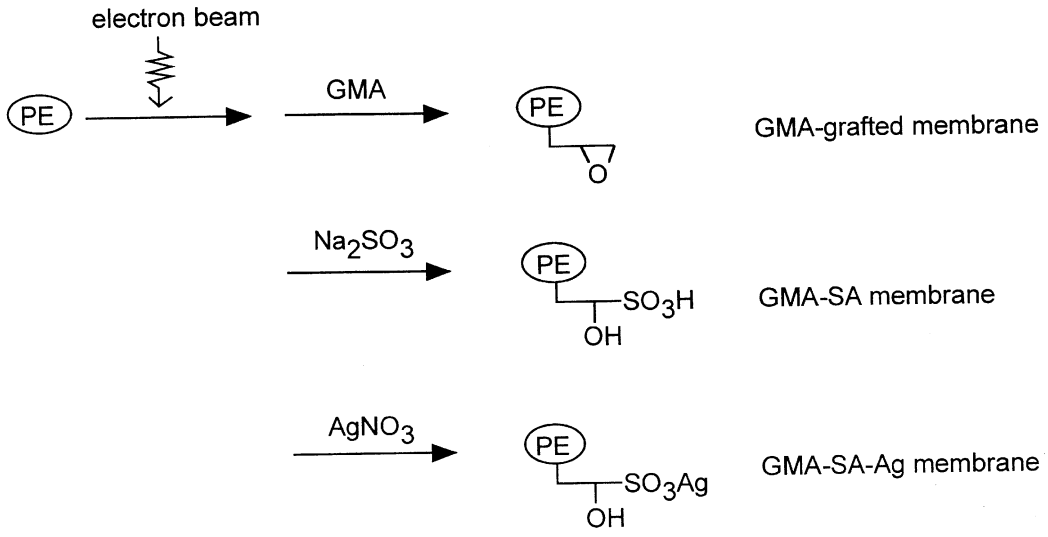
$$\text{amount of ethyl ester bound} = \int_0^V (C_0 - C)dV/W_3 \qquad [4]$$

$$\text{equilibrium binding capacity} = \int_0^{V_e} (C_0 - C)dV/W_3 \qquad [5]$$

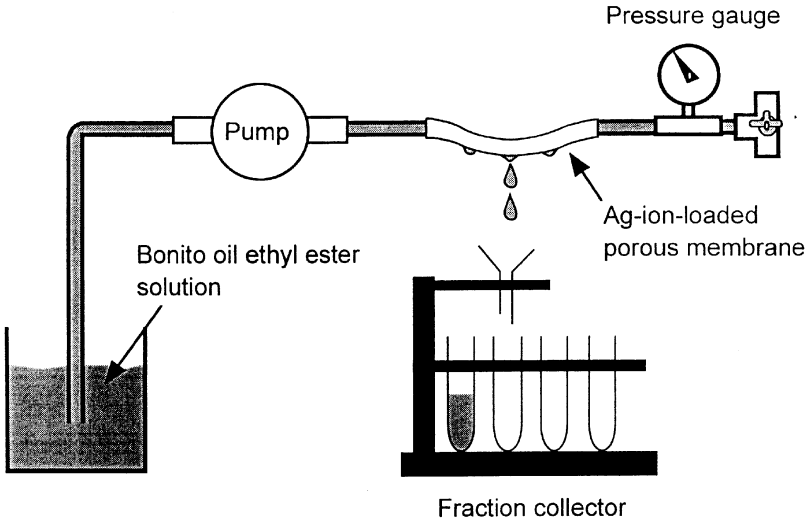
Here, d_i and L are the inner diameter and length of the hollow fiber, respectively. C_0 and C are the ethyl ester concentrations of the feed and effluent, respectively. V and V_e are the effluent volume and the effluent volume when the effluent concentration reached the feed concentration, respectively. Initially, the effluent volume corresponding to the void volume of the GMA-SA-Ag membrane was subtracted.

For comparison, a similar experiment was performed using a GMA-SA membrane.

Elution of bonito oil ethyl esters and repeated use. After washing with 20 mL of the solvent, an eluent of either isopropyl alcohol or acetonitrile was permeated to elute the ethyl



SCHEME 1



SCHEME 2

esters bound to the GMA-SA-Ag membrane. The elution percentage was defined as

$$\text{elution percentage} = (\text{amount eluted})/[(\text{amount bound}) - (\text{amount washed})] \quad [6]$$

Prior to the next adsorption procedure, 20 mL of the solvent was permeated to wash the membrane pores. The washing, adsorption, washing, and elution procedures were repeated five times. The amounts of DHA-Et and their elution percentages were determined after each cycle.

RESULTS AND DISCUSSION

Silver ion density and liquid permeability of porous membrane. The properties of the GMA-SA-Ag membrane are summarized in Table 1. The silver ion density of 5.1 mol per kg of the trunk porous hollow-fiber membrane is comparable to the silver ion concentration of silver nitrate aqueous solution used in solvent extraction (16). The silver ions were bound to the SA groups at a molar ratio of 0.70. The permeation flux of the bonito oil ethyl ester solution for the GMA-SA-Ag membrane, calculated by Equation 3, of 1.7 m/h at a permeation pressure of 0.1 MPa, was 70% the permeation flux of the trunk porous hollow-fiber membrane, which was favorable for obtaining a high permeation flow rate of the feed. Volume swelling accompanied by graft polymerization was observed; this swelling enhanced the retention of the porous structure of the membrane.

TABLE 1
Properties of Ag-Ion-Loaded (GMA-SA-Ag) vs. Unmodified (trunk) Porous Hollow-Fiber Membranes

	GMA-SA-Ag membrane	Trunk membrane (PE)
Degree of grafting (%)	141	—
Conversion (%)	73	—
Inner diameter (mm)	2.3	1.9
Outer diameter (mm)	4.0	3.0
Flux (m/h)[a]	1.7	2.4
Apparent density (kg-fiber/L[b]-fiber)	0.47	0.33
Ag ion density (mol/kg-PE)	5.1	—
(mol/kg-fiber)	1.4—	

[a]Solvent = 7.5% vol/vol water/ethanol, permeation pressure = 0.1 MPa.
[b]Volume = $1/4\pi(d_o^2 - d_i^2) \times$ length. GMA, glycidyl methacrylate; SA, sulfonic acid; PE, polyethylene.

Fast recovery of DHA-Et during permeation through the GMA-SA-Ag membrane. Breakthrough curves of DHA-Et and other ethyl esters for the GMA-SA-Ag membrane are shown in Figure 1 as a function of the permeation flow rate, ranging from 0.3 to 3.0 mL/min. The abscissa is the dimensionless effluent volume (DEV) defined as:

$$\text{DEV} = 4(\text{effluent volume})/\pi L(d_o^2 - d_i^2) \quad [7]$$

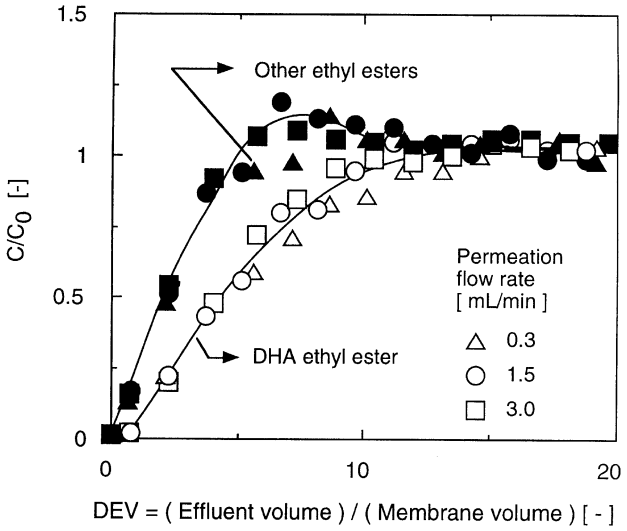


FIG. 1. Breakthrough curves of fatty-acid ethyl esters for silver-ion-loaded porous hollow-fiber membrane as a function of permeation flow rate. DEV = dimensionless effluent volume, C_o and C or the ethyl ester concentrations of the feed and effluent, respectively.

The ordinate is the dimensionless effluent concentration defined as the concentration ratio of the effluent to the feed. The breakthrough curves overlapped irrespective of the permeation flow rate (i.e., the residence time of the solution across the membrane). The residence time ranged from 12 to 120 s, as calculated from

$$\text{residence time} = \pi\varepsilon L(d_o^2 - d_i^2)/4(\text{permeation flow rate}) \quad [8]$$

where is the porosity of the membrane. This result demonstrated that the diffusional mass-transfer resistance of DHA-Et and other ethyl esters to the loaded silver ions was negligible owing to the convective flow of the solution through the pores. This favorable characteristic is also observed for other combinations of the target molecule and functional moiety: bovine γ-globulin/hydrophobic amino acid ligand (12), bovine serum albumin/diethylamino group (17,18), and cobalt ion/iminodiacetate group (19). As a result, fast recovery of DHA-Et in a permeation mode was demonstrated.

Selective binding of DHA ethyl esters onto the membrane. As shown in Figure 1, ethyl esters other than DHA-Et appeared in the effluent at DEV = 1, and DHA-Et broke through at DEV = 2, where the breakthrough point is defined as C/C_0 = 0.1. Then, at DEV > 6, the concentration of other ethyl esters exceeded the feed concentration.

To elucidate selective adsorption, the amounts of ethyl esters bound to the membrane, calculated using Equation 4, are shown in Figure 2 as a function of DEV. Up to DEV = 6, both DHA-Et and other ethyl esters were adsorbed, whereas above DEV = 6, only DHA-Et continued to adsorb by displacing the other adsorbed ethyl esters; the loaded silver ions were found to exhibit stronger interaction with DHA-Et than with other ethyl esters. This phenomenon is known as displacement adsorption and was observed during adsorption by a chelating porous hollow-fiber membrane during permeation of a binary metal ion solution through the membrane (20).

The molar ratio of DHA-Et to silver ion and the molar ratio of other ethyl esters to silver ion were calculated as 0.07 and 0.002, respectively, at equilibrium with the feed concentration. This value agreed with that reported for the solvent extraction between organic and silver nitrate aqueous solutions (16).

Elution of DHA-Et and repeated use. Acetonitrile was a more effective eluent than isopropyl alcohol because it provided a higher peak concentration of DHA-Et in the elution curves. This is because acetonitrile forms a complex with the silver ion (21) and displaces fatty acid ethyl esters bound to the silver ion. An example of adsorption, washing, and elution is shown in Figure 3.

After elution with isopropyl alcohol or acetonitrile and subsequent washing with the solvent, permeation of bonito

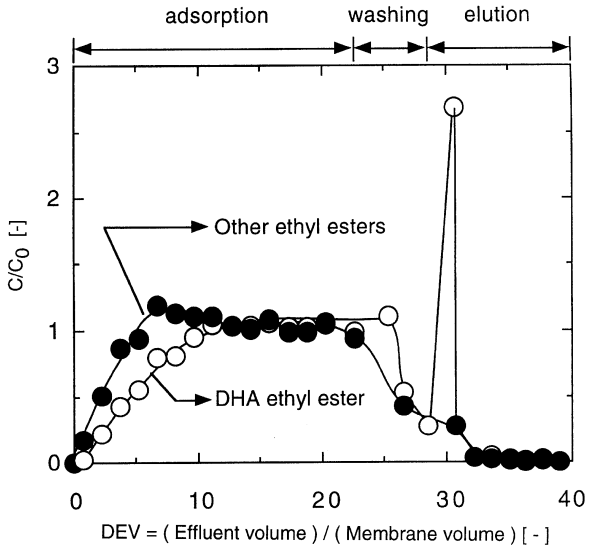


FIG. 3. An example of adsorption, washing, and elution during permeation of bonito oil ethyl ester solution through the GMA-SA-Ag membrane. Adsorption and washing solvent = 7.5 vol/vol water/ethanol; elution solvent = acetonitrile. Permeation flow rate = 1.5 mL/min.

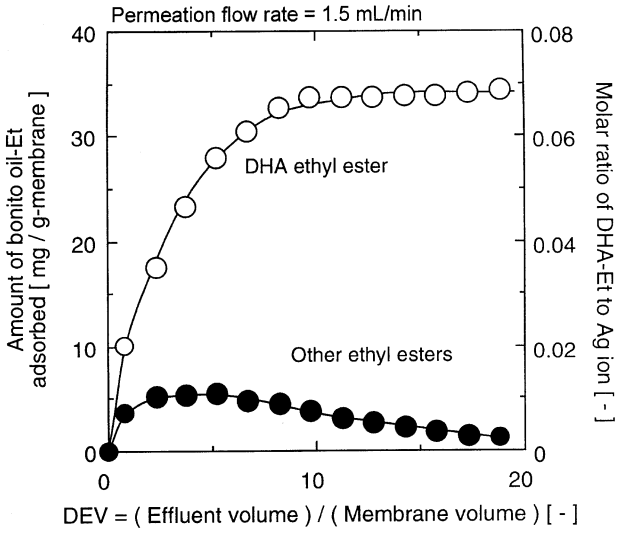


FIG. 2. Selective binding of fatty-acid ethyl esters onto the silver-ion-loaded porous hollow-fiber membrane.

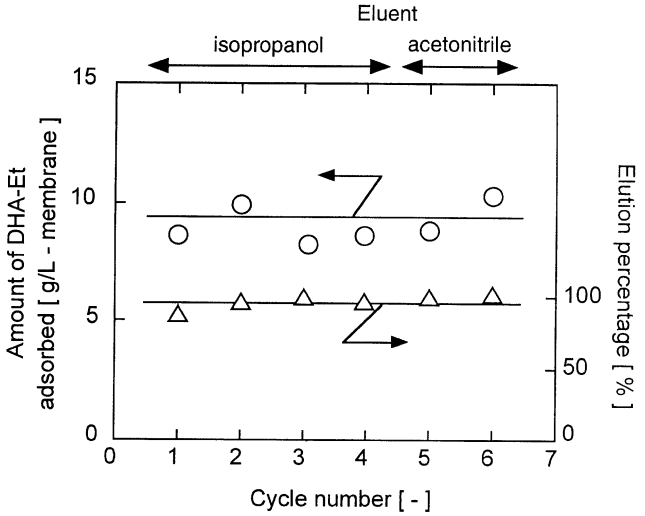


FIG. 4. Amount of DHA-Et bound to the GMA-SA-Ag membrane and elution percentage vs cycle number. GMA, glycidyl methacrylate; SA, sulfonic acid; for other abbreviations see Figs. 1 and 3.

oil ethyl esters was repeated. The amount of DHA-Et bound to the GMA-SA-Ag membrane remained constant after six cycles with elution percentages of almost 100%, as shown in Figure 4. This is indicative of negligible leakage of silver ions from the membrane.

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