Facilitated Permeation of Myoglobin Through Porous Membranes: Different Permeation Behavior Through Membranes of Polyoxyethylene- and Dextran-Conjugated Polyamides Prepared by Quasi-Living Polymerization of a Bicyclic Lactam

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SYNOPSIS

In the ultrafiltration test of a myoglobin solution through porous membranes of the ABA-type block copolymers composed of polyamide as outer segments and polyoxyethylene (M_n = 1.9–2.0 × 10^4) as an inner segment, in which the values of weight fraction of the polyamide segments (W) were 0.90, 0.84, 0.82, 0.81, and 0.73, the concentration of the permeate was found to be much higher than that in feed under the pressure difference of 1–2 kg/cm^2. Such singularly facilitated permeation was observed also in the test through the dense membranes of the polyamide–polyoxyethylene block copolymer with W of 0.81 and 0.73. On the other hand, neither porous nor dense membranes of the graft copolymer (W = 0.83) having a dextran stock (M_n = 1.8 × 10^4) and 2.6 pieces of polyamide branches showed similar facilitated permeation, although dextran was soluble in water as well as polyoxyethylene. The apparent interaction of myoglobin with dextran may be stronger than that with polyoxyethylene. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

General ultrafiltration and reverse osmosis through membranes give the permeate with lower concentration of the solutes than that in feed, and the residue with their higher concentration.[1] However, the higher concentration solutions of several solutes are known to permeate through some membranes by different additional driving forces, for example, phenols through acetylated cellulose membranes,[2–5] metal ions through mosaic membranes,[6–11] and other membranes,[12–19] and hydrophobic or amphiphilic compounds through perfluoropolymer membranes.[20,21] The conspicuous permeation behavior is inferred to arise from some weak interaction between the solutes and the polymeric materials forming the membranes, such as electronic and hydrophobic interactions and so on.

We have also found similar behavior in the ultrafiltration test of proteins such as myoglobin and cytochrome C through porous membranes of an ABA-type block copolymer (**5**) having polyamide **2** as outer segments and polyoxyethylene (**3**, M_n = 1.9 × 10^4) as an inner segment, in which the weight fraction of **2** (W) was 0.82 (see Fig. 1).[22,23] Such a novel function may generate in the pores covered with hydrogel of **3** in the membrane, but its mechanism was not clear. The facilitated permeation will be dependent not only upon the permeation conditions but also upon the kinds of copolymer membranes. Other porous membranes having hydrogel pores may also be able to show an efficient permeation of proteins.

The polyamide **2** having an alternating arrangement of a tetrahydropyran ring and amide bond can be easily prepared by the quasi-living anionic ring opening polymerization of a bicyclic lactam (**1**), and

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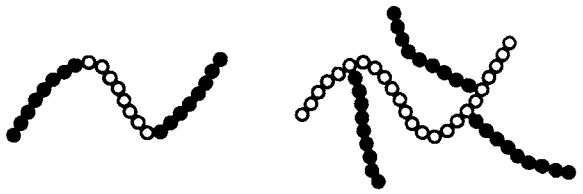

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Figure 1 Schematic representation of block copolymer (polyamide-block-polyoxyethylene-block-polyamide, **5**) and graft copolymer (dextran-graft-polyamide, **6**) composed of polyamide and water-soluble segments. (●●●), polyamide segment; (○○○), water-soluble segment.

cast from the chloroform-methanol mixed solvent to give hydrophilic membranes.[24–28] Therefore **2** is a convenient outer segment in the block and graft copolymers, which can be cast to porous membranes. On the other hand, a biomedically useful polysaccharide, dextran **4** will be a water-soluble inner segment as well as polyoxyethylene **3.** Recently not only the polyamide–polyoxyethylene block copolymer **5**[22,29,30] but also the graft copolymer **6** having a dextran stock (**4**) and 2.6 pieces of polyamide branches (**2**)[31–33] have been synthesized.

The present article describes the permeation behavior of myoglobin through different porous membranes having hydrophilic microdomains of water-soluble polymeric segments prepared from the polyoxyethylene-conjugated polyamide **5** and the dextran-conjugated polyamide **6.**

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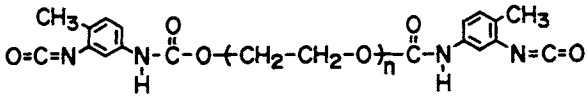

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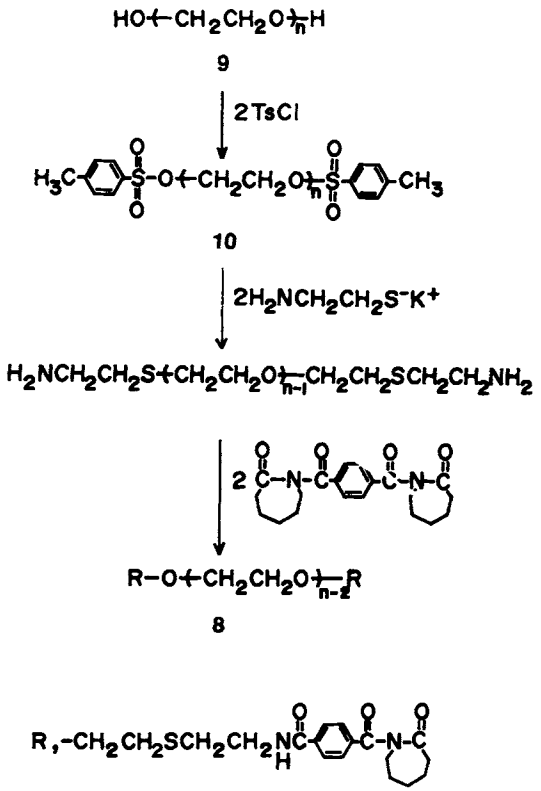
RESULTS AND DISCUSSION

Polyoxyethylene- and Dextran-Conjugated Polyamides (5 and 6)

In the previous work,[22,29,30] the ABA-type block copolymer (polyamide-block-polyoxyethylene-block-polyamide, **5**) in which the weight fraction of the polyamide segments (W) was 0.82, had been prepared by the anionic polymerization of **1** activated with the polyoxyethylene having isocyanate groups at both chain ends (**7**).

In the work, all procedures including the preparation of **7**, its purification, and the polymerization of **1** using **7** as an activator had been carried out in



Scheme 1

a high vacuum line, because the isocyanate groups in **7** were very sensitive to humidity.[29,30] For simpler treatment, another macromolecular activator, the polyoxyethylene telechelated with acyllactam groups (**8**), which can be easily handled in air, was prepared from polyoxyethylene glycol (M_n = 2.0 × 10^4) in the present work (see Scheme 1).

After purification, **8** was dried in vacuo and used as a macromolecular activator. By using different amounts of **8** as an activator, **1** was polymerized in dimethyl sulfoxide at room temperature by the same method as used in the case of **7**[30] to yield the ABA-type block copolymers **5** with different compositions. The values of W in **5** used for preparation of membranes were estimated by ^{1}H-NMR spectroscopic and elemental analyses to be 0.90, 0.84, 0.81, and 0.73.

The graft-type dextran-conjugated polyamide (**6**, W = 0.83) having a dextran stock (**4**, M_n = 1.8 × 10^4) and 2.6 pieces of polyamide branches (**2**) was also prepared as described in the literature.[33]

Different Dense and Porous Membranes

The porous membranes of **5** and **6** were prepared by casting the chloroform–methanol (10 : 1 w/w)

Table I Characterization of Various Dense and Porous Membranes

Membrane No.[a]	W[b]	R[c]	H[d]	Thickness (mm)	K_w × 10^9 (mol/cm atm s)[e]	$\frac{c_m}{c_0}$[f]	Adsorbed Myoglobin (mg/g)
2-100-0	1.00	0	0.32	29	0.38	0.1	0.2
5-90-0	0.90	0	0.42	43	1.2	0.4	3.8
5-90-0.5	0.90	0.5	0.64	67	1.7	0.4	2.5
5-90-0.65	0.90	0.65	0.70	160	12	0.2	1.8
5-90-0.8	0.90	0.8	0.66	76	32	1.8	6.8
5-90-1.0	0.90	1.0	0.73	92	58	1.0	5.3
5-84-0	0.84	0	0.54	90	1.6	0.4	2.8
5-84-0.2	0.84	0.2	0.58	100	5.5	2.7	4.3
5-84-0.3	0.84	0.3	0.61	77	4.6	3.8	6.3
5-84-0.5	0.84	0.5	0.62	61	18.1	1.8	4.0
5-84-1.0	0.84	1.0	0.71	86	—	—	2.6
5-82-0	0.82	0	0.58	120	1.7	0.1[g]	0.1
5-82-0.1	0.82	0.1	0.66	170	3.9	0.1[g]	—
5-82-0.3	0.82	0.3	0.74	87	10	5.7[g]	15
5-82-0.5	0.82	0.5	0.69	170	5.3	4.6[g]	—
5-82-1.0	0.82	1.0	0.76	220	36	2.1[g]	4.3
5-81-0	0.81	0	0.56	103	3.1	1.5	3.2
5-81-0.2	0.81	0.2	0.64	76	7.0	3.1	5.6
5-81-0.3	0.81	0.3	0.66	110	25	2.6	3.8
5-81-0.5	0.81	0.5	0.70	67	94	1.8	4.1
5-73-0	0.73	0	0.67	49	63	1.7[h]	8.2
5-73-0.3	0.73	0.3	0.70	68	296	2.1[h]	4.1
5-73-0.5	0.73	0.5	0.79	70	454	—	4.7
6-83-0	0.83	0	0.40	140	1.0	0.2	3.6
6-83-0.5	0.83	0.5	0.42	110	3.0	0.3	—
6-83-0.9	0.83	0.9	0.61	120	3.1	0.3	3.6
6-83-1.5	0.83	1.5	0.66	82	12	0.7	2.9
6-83-1.8	0.83	1.8	0.71	120	59	1.1	4.2

[a] **2**, Polyamide; **5**, polyamide-block polyoxyethylene-block-polyamide; **6**, dextran-graft-polyamide.
[b] Weight fraction of polyamide segment in copolymer.
[c] Weight fraction of polyoxyethylene glycol (M_n, 1.9–2.0 × 10^4) or ethylene glycol to copolymer on casting.
[d] Degree of hydration.
[e] 13.9 cm^2, 25°C, 1–2 kg/cm^2.
[f] Feed, 0.02–0.03 wt %; 25°C; 2 kg/cm^2; 450 rpm.
[g] Data published in the previous article.[22]
[h] 1 kg/cm^2.

solution of the mixture of **5** with polyoxyethylene glycol ($M_n = 2.0 \times 10^4$) and the hexafluoroisopropanol solution of the mixture of **6** with ethylene glycol, respectively, in various ratios (weight ratio of the glycols to the copolymer, R = 0–1.8) and drying up at room temperature, followed by immersing and rinsing in water. Their dense membranes were obtained by casting without addition of the glycols ($R = 0$). The detailed casting conditions and the properties of the membranes are listed in Table I.

The dense membranes were transparent, but the opacity of the porous membranes increased with the amount of the glycols added in the copolymer solution on casting, in other words, with their porosity. Because both glycols have higher compatibility with the corresponding water-soluble polymer segments than the polyamide segments, the domains formed by the former segments are speculated to be more porous than the latter ones. Figure 2 shows the scanning electron micrographs of the surfaces of the typical dense and porous membranes (membrane No. 5-81-0 and 5-81-0.5).

The degrees of hydration (H) and the hydraulic permeability (K_w) of all the copolymer membranes were higher than those of the polyamide dense membrane, and roughly speaking, they were apt to increase with the weight fraction of the water-soluble polymer segment in the copolymer. In the membranes of the copolymers having relatively high content of water-soluble polymer segments, the water-soluble polymer segments might form the phase developed along the direction of the membrane. In addition, the values of H and K_w of the membranes of **5** with any composition were apt to increase with their porosity, namely with the content of glycols added in the copolymer solution on casting.

Permeation of Myoglobin Through Membranes of Block-Type Polyoxyethylene-Conjugated Polyamide 5

The membranes were used for the ultrafiltration test of 0.02–0.03 wt % aqueous solution of myoglobin at 25°C under the pressure difference of 1–2 kg/cm^2 with mechanical stirring. In the case of the porous membrane of **5**, in which the value of W was 0.84 (membrane No. 5-84-0.3), the concentration of the permeate (c) was much higher than that in feed (c_0), while the dense membrane (membrane No. 5-84-0) rejected myoglobin molecules, as shown in Figure 3.

During the permeation through the porous membranes, the apparent concentration ratio expressed by the value of c/c_0 increased progressively and revealed a maximum followed by a gradual decrease. The value of the apparent concentration ratio varied

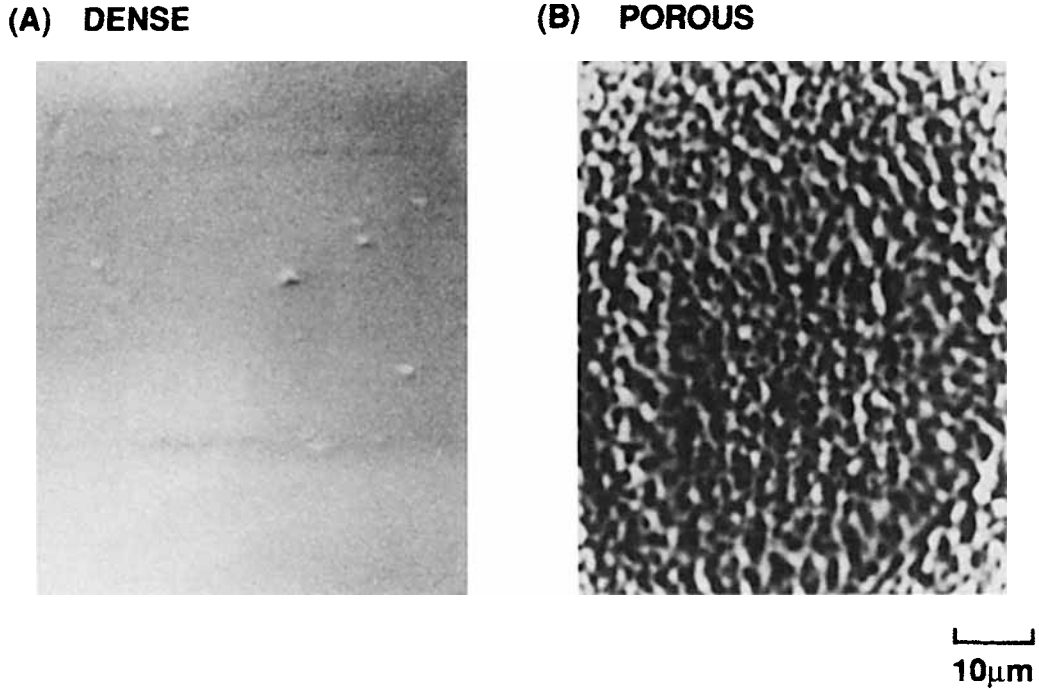


Figure 2 Scanning electron micrographs of the surfaces of the membranes prepared from copolymer **5**. (A) Membrane No. 5-81-0; (B) No. 5-81-0.5.

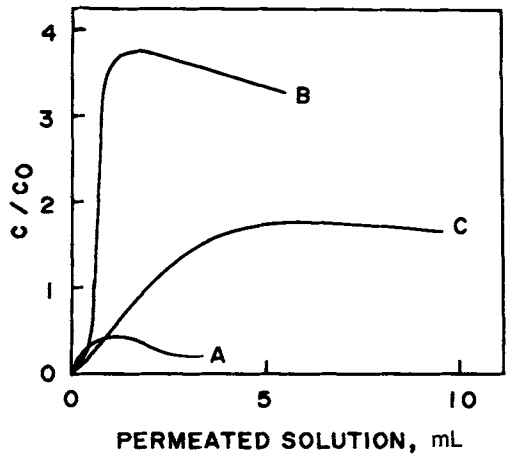


Figure 3 Permeation of an aqueous solution of myoglobin through copolymer membranes (**5**; W, 0.84). (A) Membrane No. 5-84-0 in Table I; (B) No. 5-84-0.3; (C) No. 5-84-0.5.

with their porosity. The concentration ratio at the maximum concentration of the permeate (c_m/c_0) was estimated to be 3.8.

From the relationship between the amounts of solute and solution permeated through the porous membrane (No. 5-84-0.3) as shown in Figure 4, about 40% of myoglobin molecules in the feed were found to have permeated during the permeation of 15% of the solution.

Previously similar behavior had been observed in the case of the porous membrane of **5** with 0.82 of W (membrane No. 5-82-0.3), although the value of c_m/c_0 was 5.7 and not equal to the present one.[22] Nakayama et al.[34] also reported that the ethylene-vinyl alcohol copolymer membrane permeated cytochrome C singularly, which corresponded to 1.2–2.2 of c_m/c_0. Therefore such facilitated permeation may not be unusual.

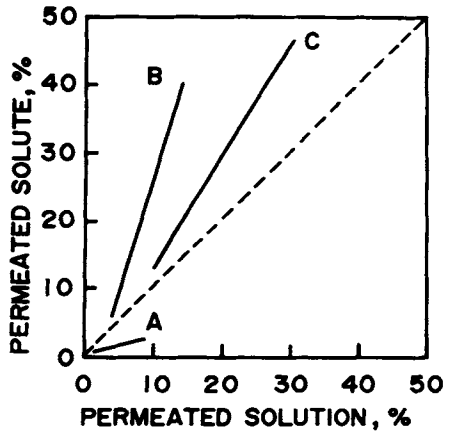


Figure 4 Relationship between amounts of permeated solute and solution. (A) Membrane No. 5-84-0 in Table I; (B) No. 5-84-0.3; (C) No. 5-84-0.5.

Porous membranes of **5** with 0.90 of W (membrane No. 5-90) also showed similar facilitated permeation of myoglobin and their dense ones did not, as summarized in Table I. On the other hand, not only the porous membranes but also the dense ones prepared from **5**, in which the values of W were 0.81 and 0.73, showed similar facilitated permeation. The phase formed by the polyoxyethylene segment across the membranes may be sufficiently large to permeate myoglobin molecules quickly even in the dense membranes.

Table I shows that the facilitated permeation was observed more clearly through porous membranes than through the corresponding dense ones. But the value of c_m/c_0 for the membranes having too high hydraulic permeability were found to lower gradually with the increase of their porosity. As shown in Figure 5, roughly speaking the most efficient condensation of myoglobin solution was attained on the permeation through the membranes having about 10^{-8} mol/cm atm s of K_w in each copolymer composition.

In the previous article, the extent of the condensation of myoglobin has been reported to depend upon several operating conditions such as mechan-

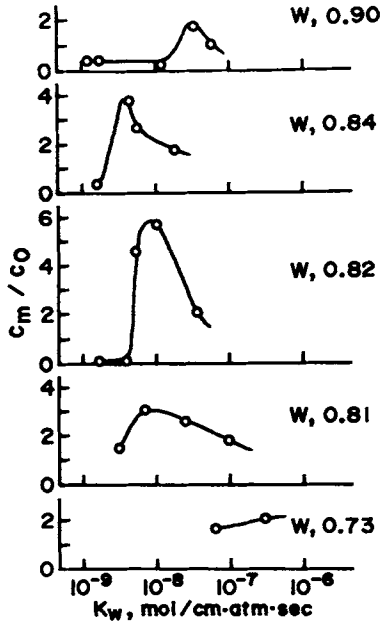


Figure 5 Relationship between hydraulic permeability and facilitated permeation of myoglobin solution through various membranes prepared from copolymers **5** having different compositions.

ical stirring of the fed solution, the operating pressure difference, the concentration of myoglobin, and so on (the best conditions for the membrane (No. 5-82-0.3): ≥ 450 rpm, 2 kg/cm^2, 0.02–0.03 wt %).[22] In addition, the porous membranes of **5** for the efficient facilitated permeation of myoglobin solution were found to adsorb a large amount of the protein molecules apparently as shown in Table I. From these results, the adsorption during the permeation with stirring is inferred to be rapid, and the desorption by the low pressure difference to be easy for the present novel transport system. Such rapid but weak adsorption of protein molecules on the surface and on the inner wall of the porous membranes covered with the hydrogel phase of polyoxyethylene segments (see Fig. 6) should be necessary and the high concentration phase of myoglobin formed on the surface is speculated to be moved out through the pores in the membrane by the low pressure difference.

Permeation of Myoglobin Through Membranes of Graft-Type Dextran-Conjugated Polyamide 6

In order to discuss in detail the influence of the chemical structure of the water-soluble segments in the copolymers upon the permeability through the copolymer membranes, the block-type dextran-conjugated polyamide should be supplied for casting the membranes and their permeability should be compared with that through the membranes of **5**. However the block-type dextran-conjugated polyamide with high molecular weight having film-forming ability has not been prepared efficiently yet.[32] Therefore the graft-type dextran-conjugated polyamide (**6**) was instead cast to the membranes because **6** had only 2.6 pieces of grafting points, in other words, it had relatively long segments as well as the block copolymer **5**.

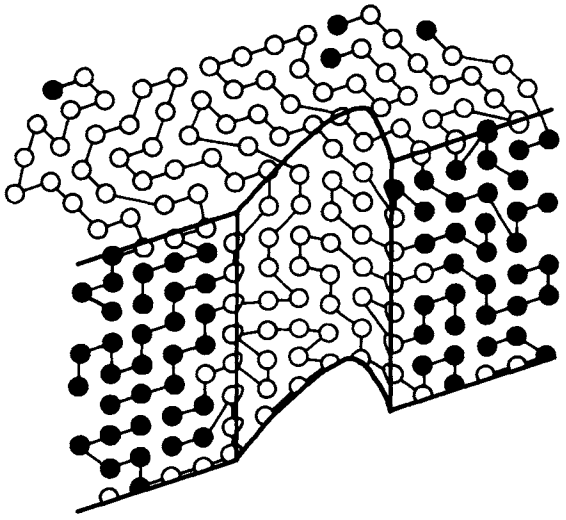


Figure 6 Schematic representation of a side view of porous membranes prepared from **5**.

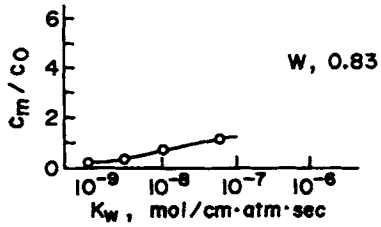


Figure 7 Relationship between hydraulic permeability and facilitated permeation of myoglobin solution through various membranes prepared from copolymer **6**.

Because the water-soluble dextran segment in the copolymer **6** was soluble in ethylene glycol, the porous membranes of **6** could be prepared by casting the mixture of **6** and ethylene glycol dissolved in hexafluoroisopropanol. As shown in Table I, the membranes have high degrees of hydration and high hydraulic permeability. In addition, a significant amount of myoglobin was found to adsorb on the membranes. However, the facilitated permeation of myoglobin through the porous membranes of **6** was not observed in the ultrafiltration test, and the membranes rejected myoglobin molecules more or less (Fig. 7).

Polyoxyethylene glycol and dextran are used in the aqueous polymer two phase method (APTP method) for the separation and purification of a large amount of biological macromolecules such as native proteins and nucleic acids.[35,36] The partition coefficient of myoglobin is known to be 0.48 in the system,[36] which suggests that the affinity between dextran and myoglobin is higher than that between polyoxyethylene glycol and the protein. In addition the mobility of the dextran segment containing a pyranose skeleton in each repeating unit should be lower than that of the linear and flexible polyoxyethylene segment in the hydrogel phase, even if the molecular weights of the segments were not so different from each other. Therefore, myoglobin molecules may adsorb in the hydrogel phase formed by dextran segments on the surface of the porous membrane more strongly than in the hydrogel phase formed by polyoxyethylene segments, and their desorption and permeation driven by the low pressure difference may be harder.

The detailed morphology of the membranes prepared from **6** may be different from **5**, which has

not been investigated yet. Even if they were different from each other, the difference between the present systems should be caused mainly by the chemical structure of the water-soluble segments, but not by the linkage-type of the segments in the copolymers, because the graft copolymer **6** has only a few pieces of relatively long branches.

In summary, the facilitated permeation of proteins described herein results from the combination of the interaction between the protein molecules and the membrane covered with the polyoxyethylene hydrogel and the transport driven by the pressure difference. The delicate balance between them is necessary for the effective facilitated permeation.

EXPERIMENTAL

Preparation of Tosyl-Telechelated Polyoxyethylene 9

According to the literature,[37] a solution of 2.8 g (0.14 mmol) of polyoxyethylene glycol ($M_n = 2.0 \times 10^4$) in 13 mL of dichloromethane was added dropwise to a mixture of 1.6 g (8.3 mmol) of tosyl chloride and 0.9 mL of pyridine in 14 mL of dichloromethane under dry nitrogen, and the solution was stirred at 4°C for 9 days. After the reaction mixture was condensed, the viscous residue was poured into 180 mL of diethyl ether and the precipitate was collected by filtration. The recovered polymer was precipitated from the ethanol solution, and dried *in vacuo:* yield of **9**, 1.0 g (64%). The functionality defined as the number of tosyl groups per polyoxyethylene chain was estimated to be 1.97 by using the extinction coefficient (ε_{max}, 12600) at the maximum wave length of β-methoxyethyl *p*-toluenesulfonate (225 nm) in methanol.

Conversion of Tosyl Groups to Amine Groups in 9

In a 100-mL three-necked round-bottom flask equipped with a condenser, a mixture of 0.68 g (6.1 mmol) of potassium *t*-butoxide and 0.40 g (3.5 mmol) of 2-mercaptoethylammonium chloride was dissolved in 37.5 mL of *t*-butanol-benzene (2 : 1 v/v) mixed solvent, and the solution was refluxed with stirring until it became turbid. After a solution of 3.0 g of tosyl-telechelated polyoxyethylene (**9**) in 12.5 mL of benzene was added dropwise to the system, the mixture was refluxed for 5 h and subsequently stirred at room temperature for 15 h. The resulting precipitate was removed by centrifugation and the supernatant solution was condensed under reduced pressure. The residue was dissolved in hot ethanol and the solution was kept at room temperature to obtain the yellowish precipitate, which was purified by reprecipitation using dichloromethane and diethyl ether as a solvent and a precipitant, respectively. The resulting colorless powder was dried *in vacuo:* yield of amine-telechelated polyoxyethylene (**10**), 1.73 g (59%).[37]

Preparation of Polyoxyethylene Telechelated With Acyllactam Groups (8)

Under dry nitrogen, a solution of **10** (1.38 g, 0.068 mmol) in 27 mL of benzene was added slowly to a hot solution of *N,N*-telephthaloylbis(ε-caprolactam) (1.96 g, 5.5 mmol) in 50 mL of benzene and refluxed for 40 h. After the reaction mixture was cooled, the precipitate was filtrated out. The condensed filtrate was poured into 50 mL of deionized water and stirred for 1 h. The precipitate was also removed by centrifugation and the supernatant was lyophilized. The resulting yellowish powder was purified by reprecipitation in the benzene-diethyl ether system and dried *in vacuo:* yield of **8** (colorless powder), 0.96 g (68%). The functionality of acyllactam group was estimated to be 2.00 by UV spectroscopy, in which *N*-(*p*-(*n*-butylcarbamoyl)benzoyl)-ε-caprolactam was used as a model compound (ε_{max}, 13600 at 237 nm).

Preparation of Porous Membranes

In order to obtain the membranes of copolymers **5**, the solution of **5** and polyoxyethylene glycol ($M_n = 2.0 \times 10^4$) in various ratios at a concentration of 3% in chloroform–methanol (10 : 1 w/w) mixed solvent was cast on glass plates and dried at room temperature. After the plates were repeatedly immersed and rinsed in water at room temperature for several days, the resulting porous membranes were peeled from the plate.

The porous membranes of copolymer **6** were also prepared in a similar method from the mixtures of **6** and ethylene glycol in various ratios in hexafluoroisopropanol.

Characterization of Membranes

The permeation rate of water through the porous membranes was determined by using a commercial ultrafiltration cell, of which the effective membrane area was 13.9 cm^2, at 25°C under the pressure difference of 1–2 kg/cm^2.

The permeation test of the solution of myoglobin (Sigma Chemicals) through the membranes was conducted using the same ultrafiltration cell, which was filled with 50 mL of the 0.02–0.03 wt % aqueous solution, at 25°C under the pressure difference of 1–2 kg/cm^2 with mechanical stirring of 450 rpm. The solute concentration in the permeate was measured by UV spectrometry.

The amount of proteins adsorbed in the membrane was estimated from the decrease of the concentration of a certain amount of the aqueous solution, in which a certain size of the membrane was dipped.

Scanning electron micrographs of surfaces of the membranes were measured with a WET-SEM type WS-250 scanning electron microscope (Akashi Beam Technology Co., Japan).

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