Synthesis of Terpolymers Having a Phospholipid Polar Group and Poly(oxyethylene) in the Side Chain and Their Protein Adsorption–Resistance Properties

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ABSTRACT: Terpolymers having phospholipid polar groups were synthesized from 2-methacryloyloxyethyl phosphorylcholine (MPC), methacryloyl or acryloyl poly(oxyethylene) macromonomers (POEM) [$(CH_2CH_2O)_n$ (where n = 2–23); PEOM(2), PEOM(23), ME(9), Ph(6)], and n-butyl methacrylate (BMA). The characteristics of these terpolymer membranes were investigated by water content (H) and X-ray photoelectron spectroscopy. The content of water in the terpolymer increased with increasing content of MPC and length of oxyethylene units. The membranes of terpolymers were found to adsorb bovine serum albumin much less than those of poly(methyl methacrylate) and poly(BMA). Even though the contents of MPC in the terpolymer were 5 to 25 mol %, the terpolymer depressed BSA adsorption more than poly(MPC-*co*-BMA) consisting of 29 mol % of MPC. The use of terpolymer with POEM can decrease the amount of MPC in the polymer. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 1092–1105, 2002

Key words: biocompatibility; X-ray photoelectron spectroscopy; radical polymerization; phospholipids; biomembrane

INTRODUCTION

The most important property for medical materials is blood compatibility and nonthrombogenicity. To obtain polymers with these characteristics, numerous investigators reported on synthesis and biocompatibility of polymers containing a phosphatidylcholine moiety.[1] Phosphorylcholine is the most important moiety because biomembrane consists of phospholipids and lecithin having a phosphorylcholine moiety. A vinyl monomer with a phosphorylcholine group, that is, 2-methacryloyloxyethyl phosphorylcholine (MPC) (**Scheme 1**), which is particularly interesting, and the compatibility of the copolymer was systematically investigated.[2–4] In addition, its derivatives and relative compounds such as 2-acryloyloxyethyl phosphorylcholine (APC),[5] poly(fumaramate)[6] bearing a phosphorylcholine moiety, and methacrylate[7] bearing a phosphorylcholine analogous group, 2-[3'-(trimethylammonium)propylphosphoryl]ethyl methacrylate were newly synthesized and the compatibility of their copolymers was investigated. More recently, a macromonomer bearing oligo(MPC) was also prepared.[8–10] These

results suggest that the introduction of phosphorylcholine groups into a polymer surface is useful for improvement of protein adsorption–resistance properties.

Ishihara and coworkers reported on the synthesis of polymers having a phospholipid polar group connected to a poly(oxyethylene) chain (**Scheme 1**), and their protein adsorption–resistance properties.[11] Introduction of poly(oxyethylene) into the copolymer effectively led to depression of bovine serum albumin (BSA) adsorption and exhibited biocompatible character, given poly(oxyethylene)'s many oxygen elements.[11] With regard to synthesis of the monomers, however, it was relatively difficult to introduce poly(oxyethylene) units into a monomer bearing a phosphorylcholine group. MPC monomer has been widely investigated for application to biocompatible materials. It is comparatively easy to introduce oxyethylene units into the polymers using a macromonomer bearing poly(oxyethylene), compared with synthesis of the monomer bearing oxyethylene units.

The present study describes the radical terpolymerizations of MPC, n-butyl methacrylate (BMA), and methacryloyl or acryloyl poly(oxyethylene) macromonomers (POEM) [$(CH_2CH_2O)_n$; n = 2: PEOM(2), n = 4: PEOM(4), n = 9: PEOM(9), n = 23: PEOM(23), n = 9: ME(9), n = 6: Ph(6)], as shown in Scheme 2. The terpolymer membranes were characterized by water fraction (H) and X-ray photoelectron spectroscopy (XPS). The adsorption behavior of BSA onto the ter-

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$$n = 1 \quad : \textbf{MPC}$$
$$n = 2 \quad : \textbf{MEO2PC}$$
$$n = 3 \quad : \textbf{MEO3PC}$$
$$n = 9 \quad : \textbf{MEO9PC}$$
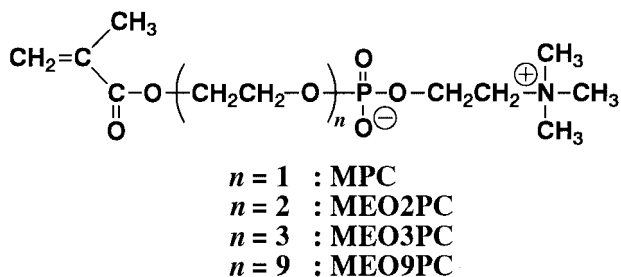
Scheme 1

polymer was investigated, compared with that of poly(MPC-*co*-BMA), poly(methyl methacrylate) (MMA), and poly(BMA). The objective of this work was to decrease the amount of MPC in the copolymer by use of POEM because MPC is still very expensive.

EXPERIMENTAL

Materials

2-Methacryloyloxyethyl phosphorylcholine (MPC) was supplied by Nippon Oil and Fats Co. (Tokyo, Japan), and lyophilized for 1 day before use. POEMs [PEOM(2)–PEOM(23), ME(9), Ph(6)] were supplied by Shin-Nakamura Chemical Co. (Japan). PEOM(2) and PEOM(4) were purified by distillation under reduced pressure and other POEMs were used without further purification. BMA was purified by vacuum distillation according to the conventional method.[12] α,α'-Azobisisobutyronitrile (AIBN) was purified from methanol. Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Other special grade reagents and solvents were used without further purification. Distilled water was used throughout the experiments.

Synthesis of terpolymers having a phospholipid polar group and POEM

Terpolymers with a phospholipid polar group, consisting of MPC, POEM, and BMA were synthesized from radical polymerizations in solvents. Structures of POEM are shown in Scheme 2. Desired amounts of MPC (M_1), one of POEM (M_2), and BMA (M_3) were dissolved in ethanol. A fixed amount of AIBN was added to the solution and the solution was put into a sealed glass tube with a stirrer and degassed with dry nitrogen gas three times to remove oxygen in the solution. The polymerization was carried out at 60°C for 6 h with stirring.

In the use of POEM with the methacryloyl group (M_2), the reaction mixture was poured into excess *n*-hexane to precipitate terpolymers. The terpolymers were twice purified by reprecipitation from the ethanol solution to excess *n*-hexane. In the use of POEM with the acryloyl group (M_2), the reaction mixture was

poured into an excess amount of water to precipitate terpolymers. The terpolymer was dissolved in methanol and poured into excess diethyl ether to precipitate pure terpolymer, which was dried *in vacuo* at 30°C for 2 days.

The chemical structure of the terpolymer was confirmed by FTIR analysis and ^{1}H-NMR measurement in CDCl$_3$.
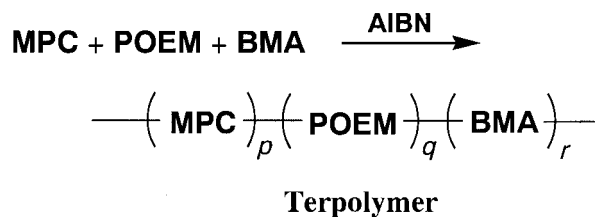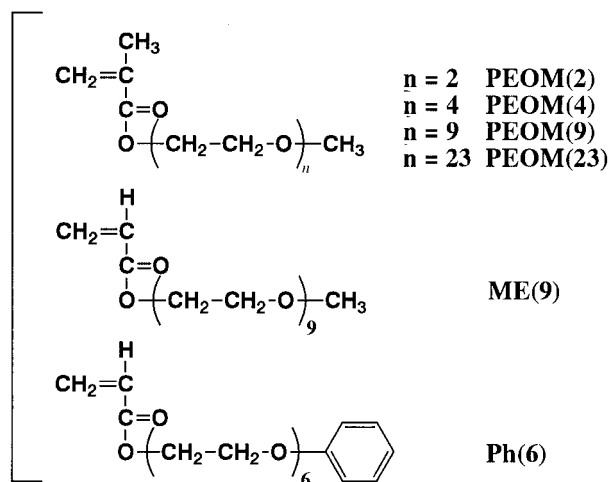
Preparation of polymer membrane

Polymer membrane was prepared from a casting method, as follows. The obtained polymer (0.5 g) was dissolved in 5 mL of ethanol as a solvent. The polymer solution was spread onto a Teflon plate to allow the solvent to evaporate at room temperature. In poly(BMA), tetrahydrofuran (THF) was used as solvent instead of ethanol. Every membrane obtained was dried *in vacuo* at 30°C overnight to eliminate residual solvent. The polymer membranes obtained were used for measurement of equilibrium water content (*H*), scanning electron microscopy (SEM), XPS, and the amount of adsorbed BSA.

Measurement of water content (*H*)

The polymer membrane was immersed in water to equilibrate at 30°C. The saturated polymer membrane,

POEM :

$$n = 2 \quad \textbf{PEOM(2)}$$
$$n = 4 \quad \textbf{PEOM(4)}$$
$$n = 9 \quad \textbf{PEOM(9)}$$
$$n = 23 \quad \textbf{PEOM(23)}$$

ME(9)

Ph(6)

MPC + POEM + BMA $\xrightarrow{\text{AIBN}}$

$$\left(\!\!\!-\; \textbf{MPC} \;\right)_{\!p}\!\!\left(\!\!\!-\; \textbf{POEM} \;\right)_{\!q}\!\!\left(\!\!\!-\; \textbf{BMA} \;\right)_{\!r}\!\!\!-$$

Terpolymer

Scheme 2

from which excess water was removed by wiping with a dry filter paper, was weighed every hour until the weight remained constant. Water content (H) was calculated from the following equation:

$$H = \frac{\text{weight of hydrated membrane} - \text{weight of dry membrane}}{\text{weight of hydrated membrane}}$$

XPS analysis

Atomic contents on the surface of polymer membrane were measured by XPS analysis with a Shimadzu ESCA-3300 electron spectrometer (Shimadzu, Kyoto, Japan). XPS analysis was carried out for lyophilized membrane after swelling in water for 24 h. Atomic contents on the surface of dried polymer membrane were calculated from the intensity ratio of detection peak for the following: carbon (C_{1P}), phosphorus (P_{2P}), nitrogen (N_{1S}), and oxygen (O_{1S}). The composition of each polymer film surface was determined by the ratio of N_{1S}/C_{1P}, P_{2P}/C_{1P}, and O_{1S}/C_{1P}.

Adsorption procedure of BSA

The sample polymer was coated on a disposable cell made of poly(MMA) for spectrometry (semimicro type, $10 \times 20 \times 4$ mm), by a solvent evaporation method as follows. An ethanol solution (2 mL) containing 0.5 wt % of the test polymer was poured into a disposable cell and kept at 30 min. After the residue of the polymer solution was removed from the disposable cell, the cell was dried at room temperature for 6 h and then dried *in vacuo* at 40°C overnight. The amount of BSA adsorbed onto the polymer membrane was determined by the micro-BCA method. The amount of adsorbed BSA was calculated from the content of BSA solution eluted from the surface of the disposable cell in sodium dodecyl sulfate (SDS) aqueous solution. To calculate the amount of BSA in the SDS solution, the protein assay kit (micro-BCA protein assay reagent kit 23235; Pierce, Rockford, IL) was used. The amount of adsorption of BSA onto the polymer surface was calculated by measuring the absorbance at 562 nm based on BSA by UV–Vis measurement with a Shimadzu UV-2200 spectrophotometer. The calibration curve was constructed from this value and the BSA concentration, which deducted blank values from the absorbance of each sample.

Other measurements

^{1}H-NMR spectra were recorded on a JEOL NMR EX-270 instrument (JEOL, Peabody, MA). Chemical shifts were reported with tetramethyl silane (0 ppm) as the internal standard. The surface of the membrane was observed by SEM with a Hitachi S-2300 instrument (Hitachi, Tokyo, Japan) after Pt sputtering on the membrane surface. The reduced viscosity (η_{SP}/C) was measured using an Ostwald viscometer (no. 3) (Sibata Hario Glass Co., Ltd., Tokyo, Japan) in ethanol at 30°C.

RESULTS AND DISCUSSION

Radical terpolymerization

Terpolymers having phospholipid polar groups were synthesized from MPC (M_1), POEM [PEOM(2), PEOM(23), ME(9), Ph(6)] (M_2), and BMA (M_3). M_3 was fed in mole fractions (mol %) of 70 or 80 as a hydrophobic monomer. Other monomers of M_1 and M_2 were of hydrophilic character. These terpolymerizations proceeded homogeneously throughout. Tables I–VI summarize the results of the terpolymerizations.

Figure 1 shows the ^{1}H-NMR spectrum for poly(MPC-co-PEOM(9)-co-BMA). The compositions of comonomers in the terpolymers were calculated from the integrated value of ^{1}H-NMR peaks corresponding to a $-N^+(CH_3)_3$ group for a MPC unit and a $-CH_2CH_2-$ group for M_2. The contents of hydrophilic monomers of both M_1 and M_2 in terpolymer slightly decreased with increasing the ratio of hydrophobic monomer M_3 in the feed. This suggests that the degree of polymerization did not increase with increase of composition of hydrophobic M_3 because ethanol with high polarity was used as a solvent. In the terpolymerizations the contents of M_2 decreased with increase of alkyl chain length because of steric hindrance of the long chains. In general, the compositions of the terpolymers were similar. According to the report by Akashi,[13] in the polymerization process, the hydrophilic portion of MPC and PEOM(2)–PEOM(23) may orient around hydrophobic BMA and thus the polymerization would proceed like a soap-free emulsification copolymerization in homogeneous solvent.

Terpolymers were obtained in high yield in all systems. However, when the feed of BMA was high and when the MPC units were not contained in the polymer, yields were relatively low. This supports the polymerization mechanism of the orientation around hydrophobic BMA, as described above. Originally, the MPC has micelle formation ability.[3] However, when the concentration of BMA with hydrophobic nature was high in the feed, MPC could not form micelles in polar solvent. Thus, the obtained polymer was dissolved in *n*-hexane of precipitant because the polymerization of MPC produced only polymers with low molecular weight. Accordingly, yields were relatively low. In additions, when the concentration of MPC in the feed was zero, the yields were low (Table I, runs 1-1 and 1-8).

The reduced viscosity of the terpolymer increased with increase of the MPC (M_1) composition and the number of oxyethylene units of M_2.

TABLE I
Radical Terpolymerizations of MPC (M_1), PEOM(2) (M_2), and BMA (M_3) in Ethanol at 60°C[a]

Run	M_1 in monomer (mol %)	M_2 in monomer (mol %)	M_3 in monomer (mol %)	Polymerization time (h)	M_1 in terpolymer[b] (mol %)	M_2 in terpolymer[b] (mol %)	M_3 in terpolymer[b] (mol %)	Yield (%)	η_{sp}/C[c]	H[d]	Adsorbed BSA ($\mu g/cm^2$)
1-1	—	30.0	70.0	6	—	26.3	73.7	91.2	0.16	0.10	1.03 ± 0.19
1-2	5.0	25.0	70.0	6	4.1	22.9	73.0	94.6	0.19	0.24	0.66 ± 0.09
1-3	10.0	20.0	70.0	6	6.6	18.0	75.4	97.2	0.23	0.24	0.48 ± 0.14
1-4	15.0	15.0	70.0	6	13.2	11.7	75.1	99.8	0.23	0.35	0.46 ± 0.08
1-5	20.0	10.0	70.0	6	18.8	7.4	73.8	95.4	0.21	0.37	0.42 ± 0.03
1-6	25.0	5.0	70.0	6	23.6	3.1	73.3	96.5	0.28	0.60	0.32 ± 0.06
1-7	30.0	—	70.0	6	28.8	—	71.2	97.4	0.45	0.75	0.36 ± 0.10
1-8	—	20.0	80.0	6	—	17.9	82.1	71.2	0.13	0.04	0.56 ± 0.09
1-9	5.0	15.0	80.0	6	3.0	17.0	80.0	84.8	0.16	0.12	0.60 ± 0.09
1-10	10.0	10.0	80.0	6	8.3	11.6	80.1	97.0	0.20	0.24	0.51 ± 0.08
1-11	15.0	5.0	80.0	6	14.3	5.6	80.1	99.8	0.22	0.37	0.63 ± 0.10
1-12	20.0	—	80.0	6	18.4	—	81.6	97.4	0.35	0.35	0.44 ± 0.11

[a] AIBN: α,α'-azobisisobutyronitrile, [AIBN]/[total monomer] = 2 mol %, [total monomer] = 1.25×10^{-2} mol; PEOM(2): methoxypolyethyleneglycol methacrylate; ethanol (4 mL).
[b] Calculated by ^{1}H-NMR.
[c] Measured in ethanol at 30°C.
[d] Equilibrium water fraction, H = [(weight of hydrated membrane) − (weight of dry membrane)]/(weight of hydrated membrane).

Water content of the terpolymer

Results of the water content measurement for terpolymer membranes are shown in Tables I–VI. In the M_2 monomer bearing relatively short oxyethylene groups, the water content (H) increased with an increase of the content of MPC unit in the terpolymer. Values of H increased with an increase of the number of oxyethylene groups in the side chain. The value of H was the highest ($H > 0.60$) for PEOM(9). The reason may be as follows. It was reported[14] that three water molecules can combine on one unit of oxyethylene. Thus, the number of the combined water molecules increases because of the increase of oxyethylene unit length. The value of H for ME(9) was equal to that for PEOM(9), as shown in Tables III and V, suggesting that no difference between methacryl and acryl groups was observed. As shown in Table VI, in the BMA monomer feed of more than 80 mol %, the H value for terpolymer consisting of Ph(6) was less than that for the terpolymer from PEMO(4), even though the number of repeating units of oxyethylene was 6. This may result from the fact that the terpolymer consisting of Ph(6) cannot take in many water molecules because hydrophobic parts of the polymer main chain exist in the vicinity of phenoxy groups with hydrophobic nature at the end of the side chain.

XPS analysis of terpolymers

Table VII summarizes the results of XPS measurements for terpolymers and copolymers. The signals for each C_{1S}, N_{1S}, P_{2P}, and O_{1S} atom were assigned to binding energies at 287.7, 405.0, 135.4, and 535.0 eV, respectively. The values of N/P, P/C, and O/C were calculated from the ratios of C_{1S}, N_{1S}, P_{2P}, and O_{1S} atoms determined from the peak intensity ratio of XPS spectrum in each polymer film. In Table VII, the ratios calculated from the ^{1}H-NMR spectra of terpolymers are also cited for comparisons.

Generally, the intensity ratio of each atom becomes larger as the atom is closer to the surface of the polymer film. Compared with the values of N/C and P/C before and after swelling, the value of N/C changed to 23.1×10^{-3} from 32.3×10^{-3}, which was about 0.7 times, whereas the value of P/C and O/C increased about 3.9 and 3.2 times, respectively. This suggests that the comonomer side chain was oriented in aqueous solution and that the anion position adjacent to the P atom was located in the upper part in aqueous solution more than was the cation site of the N atom. The P atom increased with an increase of the value of P/C, suggesting that P atoms of phosphorylcholine unit were oriented in phase of distilled water, whereas in runs 7-2 to 7-11 in Table VII, the value of O/C increased for all polymers. The value of O/C significantly changed when the chain length of the polymer

TABLE II
Radical Terpolymerizations of MPC (M_1), PEOM(4) (M_2), and BMA (M_3) in Ethanol at 60°C[a]

Run	M_1 in monomer (mol %)	M_2 in monomer (mol %)	M_3 in monomer (mol %)	Polymerization time (h)	M_1 in terpolymer[b] (mol %)	M_2 in terpolymer[b] (mol %)	M_3 in terpolymer[b] (mol %)	Yield (%)	η_{sp}/C[c]	H[d]	Adsorbed BSA ($\mu g/cm^2$)
2-1	—	30.0	70.0	6	—	33.1	66.9	92.9	0.20	—[e]	0.64 ± 0.10
2-2	5.0	25.0	70.0	6	6.0	23.7	70.3	91.8	0.26	0.36	0.49 ± 0.10
2-3	10.0	20.0	70.0	6	8.3	23.1	68.6	98.2	0.27	0.57	0.37 ± 0.10
2-4	15.0	15.0	70.0	6	15.5	14.2	70.3	94.2	0.18	0.58	0.34 ± 0.08
2-5	20.0	10.0	70.0	6	18.7	10.3	71.0	90.1	0.20	0.61	0.31 ± 0.01
2-6	25.0	5.0	70.0	6	26.5	5.4	68.1	92.4	0.30	0.64	0.33 ± 0.08
1-7[f]	30.0	—	70.0	6	28.8	—	71.2	97.4	0.45	0.75	0.36 ± 0.10
2-8	—	20.0	80.0	6	—	22.5	77.5	86.8	0.16	—[e]	0.75 ± 0.04
2-9	5.0	15.0	80.0	6	2.4	13.9	83.7	86.1	0.19	0.40	0.47 ± 0.01
2-10	10.0	10.0	80.0	6	6.9	8.0	85.2	92.5	0.22	0.62	0.38 ± 0.08
2-11	15.0	5.0	80.0	6	12.3	6.5	81.2	96.3	0.24	0.42	0.34 ± 0.05
1-12[f]	20.0	—	80.0	6	18.4	—	81.6	63.8	0.35	0.35	0.44 ± 0.11

[a] AIBN: α,α'-azobisisobutyronitrile, [AIBN]/[total monomer] = 2 mol %, [total monomer] = 1.25×10^{-2} mol; PEOM(4): methoxypolyethyleneglycol methacrylate; ethanol (4 mL).
[b] Calculated by ^{1}H-NMR.
[c] Measured in ethanol at 30°C.
[d] Equilibrium water fraction, H = [(weight of hydrated membrane) − (weight of dry membrane)]/(weight of hydrated membrane).
[e] No measurement.
[f] Table I.

TABLE III
Radical Terpolymerizations of MPC (M_1), PEOM(9) (M_2), and BMA (M_3) in Ethanol at 60°C[a]

Run	M_1 in monomer (mol %)	M_2 in monomer (mol %)	M_3 in monomer (mol %)	Polymerization time (h)	M_1 in terpolymer[b] (mol %)	M_2 in terpolymer[b] (mol %)	M_3 in terpolymer[b] (mol %)	Yield (%)	η_{sp}/C[c]	H[d]	Adsorbed BSA ($\mu g/cm^2$)
3–1	—	30.0	70.0	6	—[e]	—[e]	—[e]	—[e]	—[e]	—[e]	0.46 ± 0.08
3–2	5.0	25.0	70.0	6	4.8	23.3	71.9	76.4	0.74	—[e]	0.34 ± 0.01
3–3	10.0	20.0	70.0	6	7.6	17.9	74.5	72.0	0.70	0.66	0.28 ± 0.03
3–4	15.0	15.0	70.0	6	12.1	15.8	72.1	80.1	0.61	0.64	0.26 ± 0.03
3–5	20.0	10.0	70.0	6	17.0	8.0	75.0	75.8	0.45	0.72	0.33 ± 0.03
3–6	25.0	5.0	70.0	6	24.0	4.7	71.3	71.6	0.45	0.69	0.39 ± 0.03
1–7f	30.0	—	70.0	6	28.8	—	71.2	97.4	0.45	0.75	0.36 ± 0.10
3–8	—	20.0	80.0	6	—	19.4	80.6	87.8	—[e]	—[e]	—[e]
3–9	5.0	15.0	80.0	6	5.5	13.9	80.6	87.0	0.33	0.44	0.43 ± 0.04
3–10	10.0	10.0	80.0	6	7.4	9.7	82.9	63.7	0.30	0.75	0.50 ± 0.04
3–11	15.0	5.0	80.0	6	13.8	1.4	84.8	57.3	0.28	0.35	0.32 ± 0.04
1–12f	20.0	—	80.0	6	18.4	—	81.6	63.8	0.29	0.35	0.44 ± 0.11

[a] AIBN: α,α'-azobisisobutyronitrile, [AIBN]/[total monomer] = 2 mol %, [total monomer] = 1.25×10^{-2} mol; PEOM(9): methoxypolyethyleneglycol methacrylate; ethanol (4 mL).
[b] Calculated by ^{1}H-NMR.
[c] Measured in ethanol at 30°C.
[d] Equilibrium water fraction, H = [(weight of hydrated membrane) − (weight of dry membrane)]/(weight of hydrated membrane).
[e] No measurement.
[f] Table I.

TABLE IV
Radical Terpolymerizations of MPC (M_1), PEOM(23) (M_2), and BMA (M_3) in Ethanol at 60°C[a]

Run	M_1 in monomer (mol %)	M_2 in monomer (mol %)	M_3 in monomer (mol %)	Polymerization time (h)	M_1 in terpolymer[b] (mol %)	M_2 in terpolymer[b] (mol %)	M_3 in terpolymer[b] (mol %)	Yield (%)	η_{sp}/C[c]	H[d]	Adsorbed BSA ($\mu g/cm^2$)
4–1	—	30.0	70.0	6	—e	—e	—e	—e	—	—e	—e
4–2	5.0	25.0	70.0	6	5.6	16.0	78.4	86.8	—	—e	—e
4–3	10.0	20.0	70.0	6	14.5	22.7	62.8	90.9	—	—e	—e
4–4	15.0	15.0	70.0	6	14.3	18.3	67.4	75.8	—	—e	—e
4–5	20.0	10.0	70.0	6	20.3	10.5	69.2	83.8	—	—e	—e
4–6	25.0	5.0	70.0	6	19.5	7.2	73.3	93.3	—	—e	—e
1–7f	30.0	—	70.0	6	28.8	—	71.2	97.4	—	0.75	0.36 ± 0.10
4–7	—	20.0	80.0	6	—	14.7	85.3	93.0	—	—e	—e
4–8	5.0	15.0	80.0	6	3.7	16.1	80.2	86.1	—	0.62	—e
4–9	10.0	10.0	80.0	6	4.5	8.0	87.5	89.2	—	0.65	—e
4–10	15.0	5.0	80.0	6	8.3	5.3	86.4	87.8	—	0.71	—e
1–12f	20.0	—	80.0	6	18.4	—	81.6	63.8	—	0.35	0.44 ± 0.11

[a] AIBN: α,α'-azobisisobutyronitrile, [AIBN]/[total monomer] = 2 mol %, [total monomer] = 1.25×10^{-2} mol; PEOM(23): methoxypolyethyleneglycol methacrylate; ethanol (4 mL).
[b] Calculated by ^{1}H-NMR.
[c] Not measured.
[d] Equilibrium water fraction, H = [(weight of hydrated membrane) − (weight of dry membrane)]/(weight of hydrated membrane).
[e] No measurement.
[f] Table I.

TABLE V
Radical Terpolymerizations of MPC (M_1), ME(9) (M_2), and BMA (M_3) in Ethanol and THF at 60°C[a]

Run	M_1 in monomer (mol %)	M_2 in monomer (mol %)	M_3 in monomer (mol %)	Polymerization time (h)	M_1 in terpolymer[b] (mol %)	M_2 in terpolymer[b] (mol %)	M_3 in terpolymer[b] (mol %)	Yield (%)	η_{sp}/C[a]	H[d]	Adsorbed BSA ($\mu g/cm^2$)
5-1	—	30.0	70.0	6	—	36.8	63.2	771	—[e]	0.41	—[e]
5-2	5.0	25.0	70.0	6	3.8	23.1	73.1	47.1	0.67	0.70	0.43 ± 0.10
5-3	15.0	15.0	70.0	6	19.1	13.3	67.6	45.7	0.39	0.75	0.46 ± 0.13
5-4	25.0	5.0	70.0	6	29.2	3.7	67.1	81.5	0.82	0.77	0.29 ± 0.10
1-7[f]	30.0	—	70.0	6	28.8	—	71.2	97.4	0.45	0.75	0.36 ± 0.10
5-5	—	20.0	80.0	6	—	29.1	70.9	28.7	0.20	0.43	0.44 ± 0.14
5-6	10.0	10.0	80.0	6	13.0	5.2	81.8	61.8	0.22	0.34	0.50 ± 0.13
1-12[f]	20.0	—	80.0	6	18.4	—	81.6	97.4	0.35	0.35	0.44 ± 0.11

[a] AIBN: α,α'-azobisisobutyronitrile, [AIBN]/[total monomer] = 5 mol %; ME(9): methoxypolyethyleneglycol acrylate; ethanol/THF = 80/20 (v/v).
[b] Calculated by ^{1}H-NMR.
[c] Measured in ethanol at 30°C.
[d] Equilibrium water fraction, H = [(weight of hydrated membrane) − (weight of dry membrane)]/(weight of hydrated membrane).
[e] No measurement.
[f] Table I.

TABLE VI
Radical Terpolymerizations of MPC (M_1), Ph(6) (M_2), and BMA (M_3) in Ethanol and THF at 60°C[a]

Run	M_1 in monomer (mol %)	M_2 in monomer (mol %)	M_3 in monomer (mol %)	Polymerization time (h)	M_1 in terpolymer[b] (mol %)	M_2 in terpolymer[b] (mol %)	M_3 in terpolymer[b] (mol %)	Yield (%)	η_{sp}/C[c]	H[d]	Adsorbed BSA ($\mu g/cm^2$)
6-1	—	30.0	70.0	6	—	36.8	63.2	33.6	—[e]	—[e]	—[e]
6-2	5.0	25.0	70.0	6	3.6	18.3	62.8	57.1	—[e]	0.54	—[e]
6-3	15.0	15.0	70.0	6	14.8	11.2	74.0	45.5	0.24	0.61	0.31 ± 0.08
1-7	30.0	—	70.0	6	28.8	—	71.2	97.4	0.45	0.75	0.36 ± 0.10
6-4	—	20.0	80.0	6	—	12.3	87.7	30.9	—[e]	0.22	—[e]
6-5	10.0	10.0	80.0	6	8.7	17.2	74.1	74.8	—[e]	0.30	—[e]
1-12	20.0	—	80.0	6	18.4	—	81.6	97.4	0.35	0.35	0.44 ± 0.11

[a] AIBN: α,α'-azobisisobutyronitrile, [AIBN]/[total monomer] = 5 mol %; Ph(6): phenoxypolyethyleneglycol acrylate; ethanol/THF = 80/20 (v/v).
[b] Calculated by ^{1}H-NMR.
[c] Measured in ethanol at 30°C.
[d] Equilibrium water fraction, H = [(weight of hydrated membrane) − (weight of dry membrane)]/(weight of hydrated membrane).
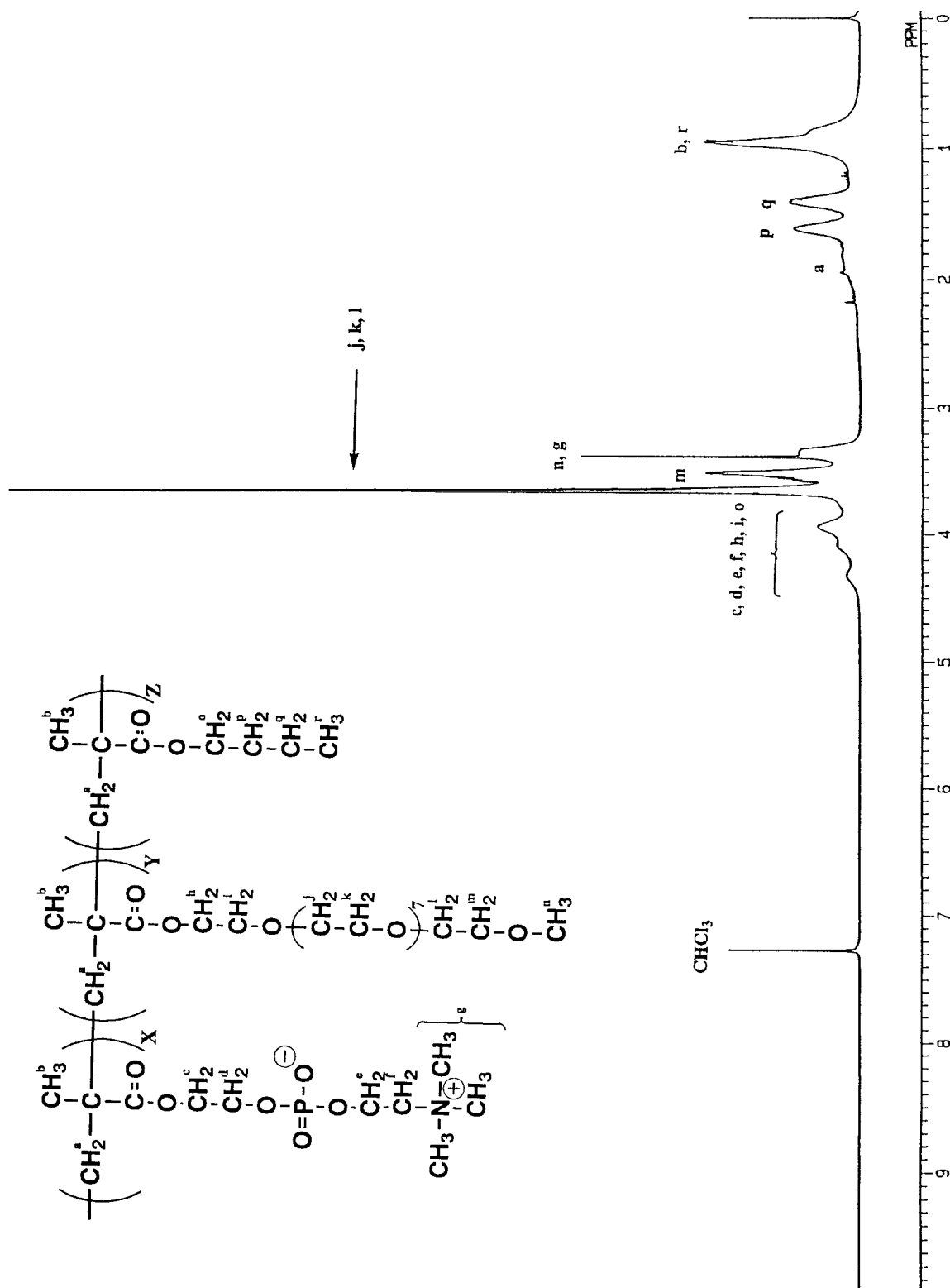[e] No measurement.
[f] Table I.

Figure 1 ^{1}H-NMR spectrum for poly(MPC-*co*-PEOM(9)-*co*-BMA) (Table III, run 3-4).

TABLE VII
X-ray Photoelectron Spectroscopy (XPS) Analysis for Poly(MPC-*co*-M$_2$-*co*-BMA) Membrane[a]

Run	Sample (run no. in Tables I–VI)	M$_2$	NMR[b]			XPS[c]			
			$(\times 10^3)$N/C	$(\times 10^3)$P/C	$(\times 10^3)$O/C	$(\times 10^3)$N/C	$(\times 10^3)$P/C	$(\times 10^3)$O/C	N/P
7–1	run1–7	None	32.3	32.3	178	23.1	124.9	574	0.18
7–2	run1–6	PEOM(2)	27.1	27.1	176	24.5	127.5	599	0.19
7–3	run2–4	PEOM(4)	16.9	16.9	205	11.8	22.6	318	0.52
7–4	run2–5	PEOM(4)	21.0	21.0	197	16.1	81.9	454	0.20
7–5	run3–3	PEOM(9)	7.0	7.0	250	5.3	9.4	304	0.56
7–6	run3–4	PEOM(9)	11.3	11.3	248	11.5	16.3	345	0.71
7–7	run5–5	ME(9)	0	0	341	0	0	281	—[d]
7–8	run5–2	ME(9)	3.3	3.3	278	21.7	33.2	313	0.65
7–9	run5–3	ME(9)	18.3	18.3	259	23.3	33.0	338	0.71
7–10	run5–4	ME(9)	31.1	31.1	204	9.3	7.2	316	1.29
7–11	run6–3	Ph(6)	15.0	15.0	199	10.4	27.1	319	0.38

[a] Hydrated membrane in water.
[b] N/C and P/C are the ratios of atom for N and P to C calculated from NMR.
[c] Values in parentheses are XPS analysis for poly(MPC-*co*-M$_2$-*co*-BMA).
[d] Not determinate.

was short. This may result from the fact that a short side chain of the M$_2$ comonomer is mainly localized in the polymer surface because of slight steric hindrance. In the case of Ph(6) the value of O/C did not increase compared with that of other polymers because of a phenyl group with strong hydrophobicity at the end of oxyethylene units. The N/P value is important in examining the structure of the phosphorylcholine unit in aqueous solution, and the theoretical value of N/P is 1, calculated from the number of N and P atoms in the polymer. However, the N/P values showed deviation from theoretical values calculated from XPS, and obtained smaller values. The reason for the deviation may be ascribed to the fact that the cationic part of N atom interacted with the oxygen atom of oxyethylene chain and that the anion part adjacent to the P atom was oriented. For example, the value of N/P increased in the low content of oxyethylene units with anionic character (runs 7-4, 7-6, and 7-10 in Table VII).

Assay of BSA-absorbed amount using micro-BCA method

The results of the BSA adsorption test for terpolymer from PEOM(2) as M$_2$ comonomer are shown in Table I. In the case of the terpolymer obtained from PEOM(2), the BSA adsorption amount decreased as the content of MPC increased in the copolymer. The amount of BSA adsorbed on poly(MPC-*co*-PEOM(2)-*co*-BMA) (run 1-6 in Table I) was almost equal to that on poly(MPC-*co*-BMA) (run 1-7 in Table I). This results from the fact that BSA adsorption cannot effectively be suppressed by the short side chain of PEOM(2) and that protein suppression ability is attributed solely to the MPC content, as reported by Hayward and Chapman.[15]

The results of the BSA adsorption test for the terpolymer from PEOM(4) as M$_2$ comonomer are shown in Table II. With the increase of MPC contents, the BSA absorbed amount decreases, as well as the terpolymers from PEOM(2). Terpolymers from POEM(4) compared to poly(MPC-*co*-BMA), poly(MPC-*co*-PEOM(4)-*co*-BMA)s having less than 29 mol % MPC contents (runs 2-4 to 2-6, 2-10, and 2-11 in Table II) depressed BSA adsorption more effectively than did poly(MPC-*co*-BMA) (run 1-7).

Table III depicts the results of the BSA adsorption on the terpolymer from PEOM(9). Similar results were also observed in this case. The minimum value (0.26 μg cm^{-2}) was observed (run 3-4 in Table III). Kajiyama et al.[16] determined the number of thromboses by the contact of human platelet multiblood plasma (HPRP), using various PEG containing poly(urethane) as a soft segment. Their result indicated that the number of platelet adhesions was influenced by the molecular weights of PEG and that the adhesion quantity had the minimum value on a suitable chain length of PEG. Cocke et al.[17] used thermodynamic examination to clarify the mechanism of the blood plasma protein adsorption for PEG. They concluded that the polymer chain length of PEG significantly influenced the amount of blood plasma protein adsorption.

The absorbed amount of the protein for our terpolymers showed a tendency that was identical to the results of Kajiyama et al.[16] and Cocke et al.[17] That is, the protein adsorption was depressed, even if the M$_1$ content decreased with an increase of chain length of the M$_2$ monomer, given that the conformational change is scarcely received by the entanglement of the polymer chain of the M$_2$ monomer. Terpolymers from POEM(9) compared to poly(MPC-*co*-BMA), in terpoly-
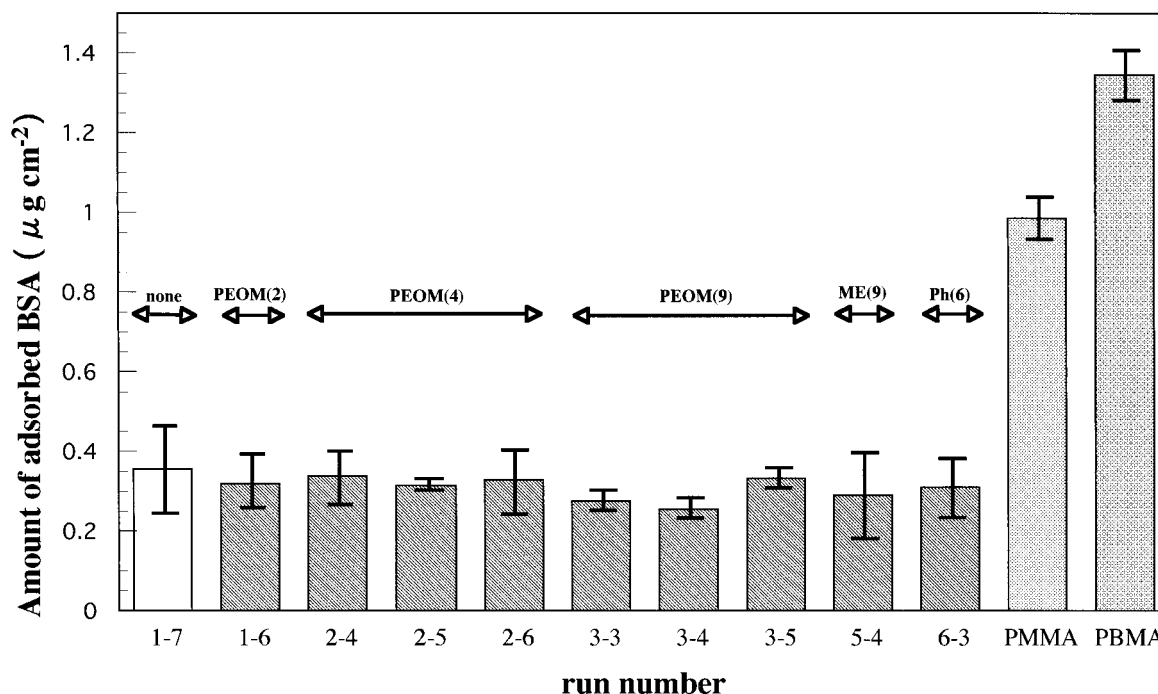
Figure 2 Amount of BSA adsorbed onto poly(MMA) cell coated with poly(MPC-*co*-M$_2$-*co*-BMA) after contact for 60 min.

mers consisting of 5–25 mol % MPC content (runs 3-2 to 3-6, 3-9, and 3-11 in Table III), can reduce the BSA adsorption more effectively than can poly(MPC-*co*-BMA) consisting of 29 mol % MPC content. This suggests that the use of terpolymers consisting of MPC, BMA, and POEM can reduce the amount of expensive MPC.

Tables V and VI show the results of BSA adsorption on the terpolymers from ME(9) or Ph(6). A significant difference of the BSA adsorption on the terpolymer from ME(9) and Ph(6) was not observed, although the amount of the adsorption tended to be slightly different. This may result, not from the main chain structure, but rather from conformation changes of the side chain between methacrylate and acrylate. The BSA adsorption was carried out for terpolymer from Ph(6) of M$_2$ comonomer (run 6-3 in Table VI) because it was soluble in ethanol for the BSA adsorption test. Compared to poly(MMA) and poly(BMA), the terpolymer from Ph(6) clearly suppressed the BSA adsorption amount.

Figure 2 depicts relationships between the amount of adsorbed BSA and the number of repeating units in poly(oxyethylene), together with results of poly(MMA) and poly(BMA). The data of better values were selected in each system. The bar graphs depict the average of the absorbed amount obtained from five measurements.

BSA adsorption distribution of the polymer film surface

Figure 3 shows SEM micrographs of polymer films of (A) poly(BMA), (B) poly(MPC-*co*-BMA), (C) poly(MPC-

co-PEOM(9)-*co*-BMA), (D) poly(MPC-*co*-ME(9)-*co*-BMA), and (E) surface of poly(MPC-*co*-ME(9)-*co*-BMA) before BSA adsorption. The surfaces of all polymers were extremely clean before the BSA adsorption, as shown in Figure 3(E). However, the BSA adsorption was widely observed on the poly(BMA). The protein adsorbed amount on poly(MPC-*co*-BMA) was clearly depressed compared with that on poly(BMA), supporting the result of the BSA absorbed amount. Poly(MPC-*co*-PEOM(9)-*co*-BMA) and poly(MPC-*co*-ME(9)-*co*-BMA) did not show any significant difference in BSA adsorption, compared with that of poly(MPC-*co*-BMA). However, BSA adsorption distributions of these polymers were clearly different from that of poly(BMA).

Relationship between the amount of BSA absorbed and the water content behavior

Figure 4 depicts plots of the amount of BSA absorbed versus water contents (*H*) of terpolymers and copolymers with respect to each M$_2$ comonomer. The water content generally showed low values for the terpolymer from PEOM(2), and the BSA absorbed amount decreased with increase of the water content. In terpolymers from ME(9), PEOM(4), and PEOM(9) as M$_2$, the BSA-absorbed amounts remained low, even though the value of *H* was low (40–50%). The BSA-absorbed amount decreased with increase of the *H* for the terpolymer from PEOM(2) because of the increase of M$_1$ contents. It is difficult for the terpolymer from PEOM(2) to obtain high water content and to attain
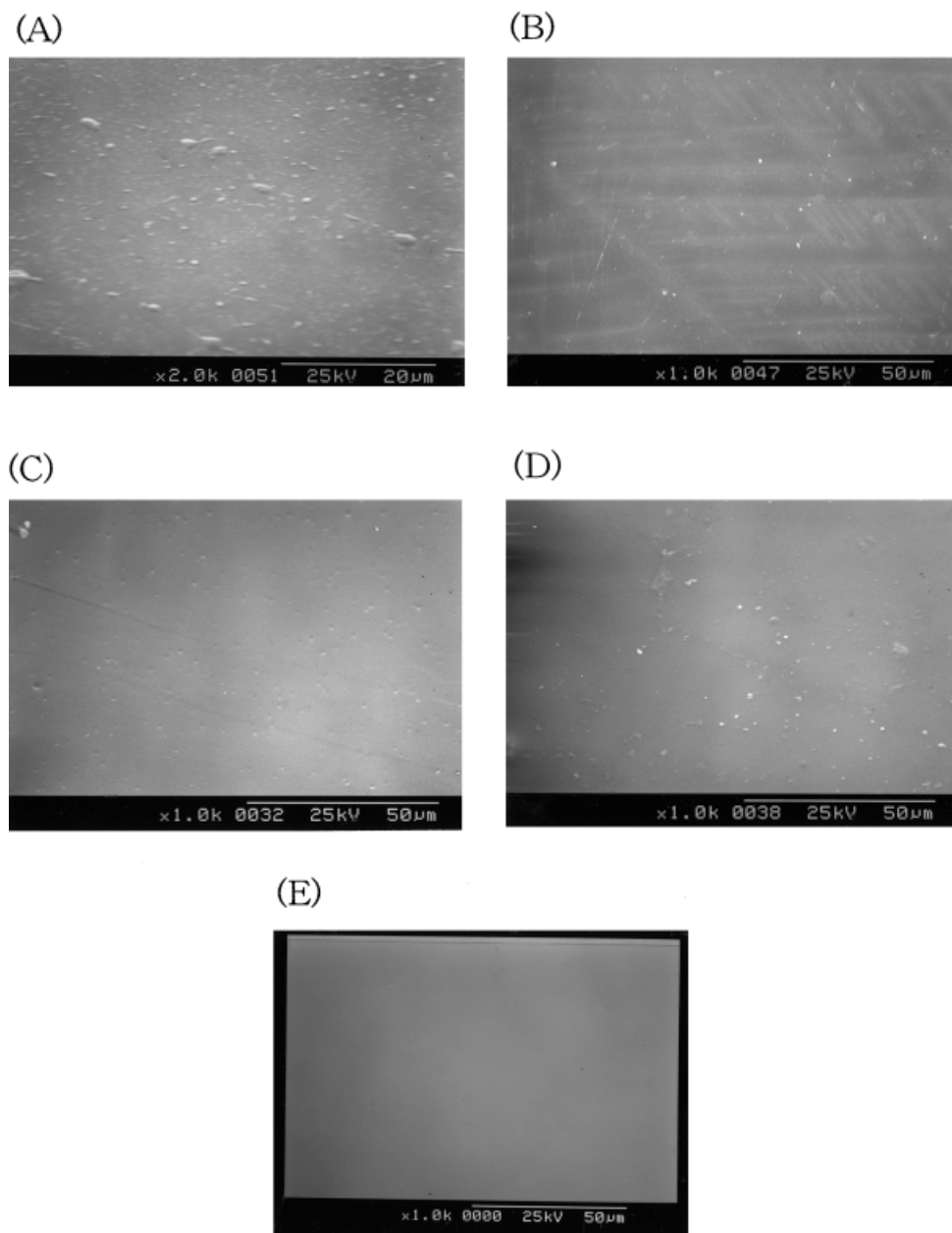
Figure 3 SEM micrographs of polymer surface after contact with BSA for 180 min: (A) poly(BMA); (B) poly(MPC-*co*-BMA) (Table I, run 1-7); (C) poly(MPC-*co*-PEOM(9)-*co*-BMA) (Table III, run 3-4); (D) poly(MPC-*co*-ME(9)-*co*-BMA) (Table V, run 5-3); (E) surface of poly(MPC-*co*-ME(9)-*co*-BMA) before BSA adsorption.

effective depression of protein adsorption because of short oxyethylene chains and side-chain entanglement. Consequently, the increase of water content was attributed to the increase of MPC (M_1) content in the polymer. The MPC (M_1) content played an important role in depression of the BSA-absorbed amount.

The water content showed generally high values because the side chain becomes relatively longer in the terpolymers obtained from PEOM(4), PEOM(9), and ME(9). In these cases, the polymer chains were not affected by conformation because of the entanglement

of the side chain, even if BSA approached polymer film. Accordingly, those terpolymers effectively depressed the BSA adsorption even with the low content of MPC (M_1). In the case of the terpolymers with oxyethylene chains in the side chain, protein adsorption was effectively depressed not by high water content but by the entangled structure of the oxyethylene side chain.

In this study, it was found that the terpolymers consisting of 5–25 mol % MPC content can more effectively depress BSA adsorption than can the copolymer consisting of 29 mol % MPC content.
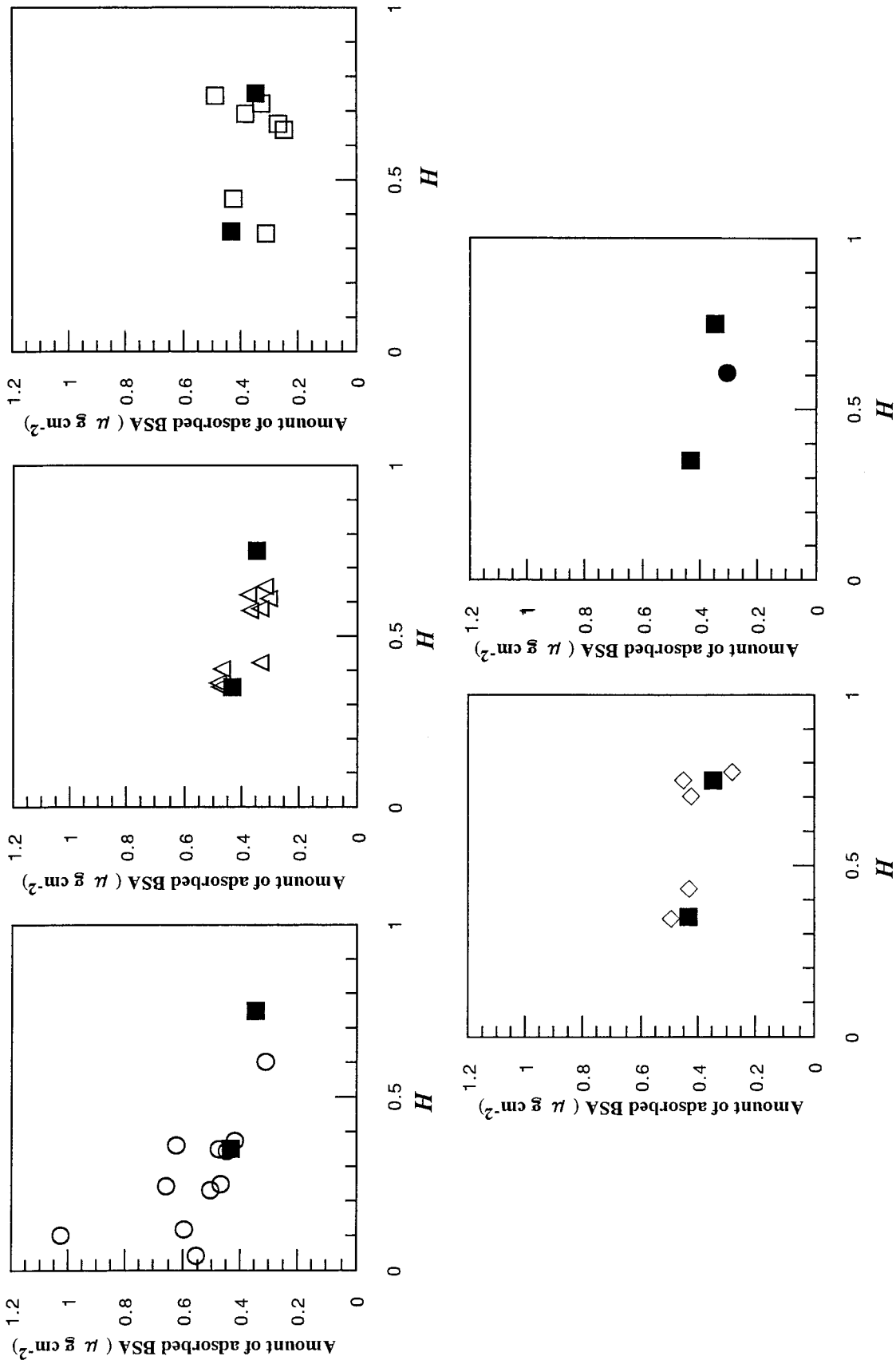
Figure 4 Relationships between the amount of adsorbed BSA and the equilibrated degree of hydration of the polymer membrane saturated with water: poly(MPC-*co*-M$_2$-*co*-BMA): M$_2$ = (○) PEOM(2), (△) PEOM(4), (□) PEOM(9), (◇) ME(9), (●) Ph(6), and (■) poly(MPC-*co*-BMA).

CONCLUSIONS

1. Terpolymers with phospholipid polar groups were synthesized from 2-methacryloyloxy-ethyl phosphorylcholine (MPC), methacryloyl or acryloyl poly(oxyethylene)s [PEOM(2), PEOM(23), ME(9), Ph(6)], and *n*-butyl methacrylate (BMA).
2. The content of water in the terpolymers increased with an increase of the content of MPC and the length of oxyethylene units.
3. Phosphorylcholine and oxyethylene units were not localized on the surface of membrane, as determined by XPS.
4. The membranes of terpolymers were found to adsorb bovine serum albumin to a much lesser extent than those of poly(MMA) and poly(BMA).
5. Even though the contents of MPC in the terpolymer were 5 to 25 mol %, the terpolymer can depress BSA adsorption to a much greater extent than can poly(MPC-*co*-BMA) consisting of 29 mol % MPC content.
6. When the numbers of the oxyethylene units in POEM with a methacryloyl group were 4 and 9, the amounts of adsorbed BSA were relatively lower.

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