Synthesis and Properties of Poly(methacrylate) Bearing a Phosphorylcholine Analogous Group

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ABSTRACT: A novel type of methacrylate bearing a phosphorylcholine analogous group, 2-[3'-(trimethylammonium)propylphosphoryl]ethyl methacrylate (TPM) was synthesized. TPM was polymerized and copolymerized with vinyl monomers in the presence of radical initiators. Monomer reactivity ratios and Q and e for TPM were determined. A critical micelle concentration (CMC) of TPM was $3.6 \times 10^{-3}\,\mathrm{mol\,l^{-1}}$. Two types of copolymer microspheres of methyl methacrylate (MMA) with TPM or 2-methacryloyloxyethyl phosphorylcholine (MPC) were prepared from emulsifier-free emulsion copolymerizations over the CMC. The suppression of bovine serum albumin (BSA) adsorption onto poly(TPM-*co*-MMA) microsphere was similar to that onto the poly(MPC-*co*-MMA). The length of three methylene units between zwitter-ions in the phosphorylcholine group could not prevent depressing BSA adsorption.

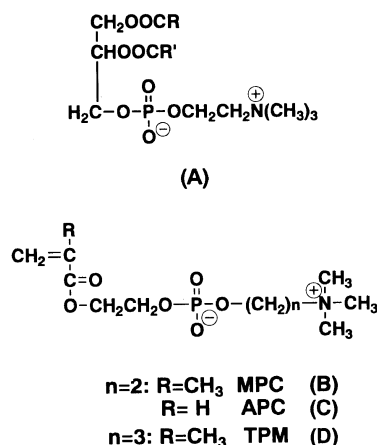KEY WORDS Phosphorylcholine / Methacrylate / Polymer Microsphere / Adsorption of albumin /

A biomembrane mainly consists of phospholipids and lecithin [Scheme 1, A] containing a phosphorylcholine moiety. Synthetic polymers containing a phosphatidylcholine moiety have been extensively studied from several viewpoints of polymer chemistry, biological, medical and life science.[1-15] Lately polymers obtained from a vinyl monomer, 2-methacryloyloxyethyl phosphorylcholine (MPC) [Scheme 1, B] are particularly interesting. Nakabayashi, Ishihara and co-workers reported that MPC copolymers with *n*-butyl methacrylate (BMA) possess outstanding blood compatibility, *i.e.*, suppression of protein adsorption and platelet adhesion.[16-21] Sugiyama *et al.* reported that microspheres composed of MPC and alkyl methacrylate decrease bovine serum albumin (BSA) adsorption compared with poly(methyl methacrylate) (PMMA) microspheres. That is, the introduction of a very small amount of MPC moiety (even at lower than 0.05 mol% in feed) onto PMMA microspheres results in drastic reduction in BSA adsorption.[22,23] More recently, they reported that copolymer microspheres composed of 2-acryloyloxyethyl phosphorylcholine (APC) [Scheme 1, C] with alkyl methacrylate superior to the MPC copolymer microspheres with respect to suppression of protein adsorption.[24] Other groups report that fibrinogen adsorption and platelet adhesion are reduced on the poly(MPC-*co*-BMA) and poly(MPC-*co*-*n*-dodecyl methacrylate).[25] These results strongly suggest that the introduction of phosphorylcholine groups into a polymer surface is useful for improvement of protein adsorption-resistance properties.

We also reported the synthesis and brief study on biocompatibility of poly(fumaramate) bearing a phosphorylcholine moiety.[26] Microspheres composed of the fumaramate with methyl methacrylate (MMA) reduced BSA adsorption as well as microspheres composed of MPC with MMA. This suggests that the structure of the rigid polymer main chain bearing an amide group at the side chain is not concerned with BSA adsorption.

There is no doubt of the excellent biocompatibility of a phosphorylcholine moiety. We added a methylene to an ethylene unit in the phosphorylcholine group and synthesized a novel type of methacrylate bearing a phosphorylcholine analogous group, 2-[3'-(trimethylammonium)propylphosphoryl]ethyl methacrylate (TPM) [Scheme 1, D].

The present paper describes the synthesis of TPM, polymerizability and emulsifier free emulsion copolymerizations of MMA with TPM. The poly(TPM-*co*-MMA) microspheres obtained were characterized, and adsorption behavior of BSA was investigated, and compared with poly(MPC-*co*-MMA) microspheres.


$$CH_2OOCR$$
$$CHOOCR'$$
$$H_2C-O-\overset{\overset{O}{\|}}{\underset{\underset{O^{\ominus}}{}}{P}}-OCH_2CH_2\overset{\oplus}{N}(CH_3)_3$$

(A)

$$CH_2=\overset{\overset{R}{|}}{C}$$
$$\overset{\overset{C=O}{|}}{O}-CH_2CH_2O-\overset{\overset{O}{\|}}{\underset{\underset{O^{\ominus}}{}}{P}}-O-(CH_2)n-\overset{\oplus}{N}\overset{CH_3}{\underset{CH_3}{-CH_3}}$$

n=2: R=CH₃ MPC (B)
R= H APC (C)
n=3: R=CH₃ TPM (D)

Scheme 1.

EXPERIMENTAL

TPM Monomer

TPM monomer was synthesized from 2-hydroxyethyl methacrylate (HEMA), 2-chloro-2-oxo-1,3,2-dioxaphosphorinane (CODPR), and trimethylamine, as illustrated

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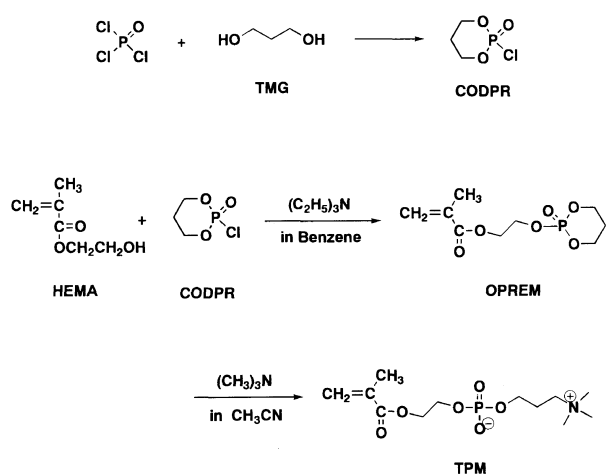
Scheme 2. Synthetic scheme for TPM.

in Scheme 2.

Preparation of TPM

2-Chloro-2-oxo-1,3,2-dioxaphosphorinane (CODPR).
CODPR was synthesized according to the method of
Lanham *et al.*[27] and purified by distillation under re-
duced pressure, bp $113°C/4.0 \times 10^{-2}$ mmHg, mp $39°C$
(lit.[27] mp $39°C$). ^{13}C NMR (δ in ppm from tetramethyl-
silane (TMS) in CDCl$_3$): 70.33 (–OCH$_2$CH$_2$CH$_2$O–),
25.65 (–OCH$_2$CH$_2$CH$_2$O–).

*2-(2-Oxo-1,3,2-dioxaphosphorinane-2-yloxy)ethyl Me-
thacrylate (OPREM).* HEMA (7.5 g, 0.058 mol), triethyl-
amine (TEA; 5.0 g, 0.058 mol), and benzene (120 ml)
were placed in a 300 ml round bottom flask. After the
solution was heated at $60°C$ with a water bath, 9.0 g of
CODPR in 70 ml of benzene were added to the stirred
solution. After being kept at $60°C$ for 2.5 days, the
precipitate (triethylammonium chloride) was filtered off.
The filtrate was extracted with water and evaporated
under reduced pressure. OPREM was obtained as a
colorless liquid. Yield 10.6 g (73.6%). ^1H NMR (δ in
ppm from TMS in CDCl$_3$): 6.18 (s, 1H, CH =, *cis*), 5.62
(s, 1H, CH =, *trans*), 4.32—4.44 (m, 4H, –OCH$_2$CH$_2$-
CH$_2$O–), 2.23—2.31 (m, 2H, –OCH$_2$CH$_2$CH$_2$O–), 1.97
(s, 3H, –CH$_3$), 1.78—1.80 (m, 2H, –OCH$_2$CH$_2$CH$_2$O–);
^{13}C NMR (δ in ppm from TMS in CDCl$_3$): 166.86
(–C=O), 135.69 (CH$_2$ = C), 126.13 (CH$_2$ = C), 68.64
(–OCH$_2$CH$_2$CH$_2$O–), 64.89 (COOCH$_2$–), 63.02
(–CH$_2$OP), 25.88 (–OCH$_2$CH$_2$CH$_2$O–), 18.10 (–CH$_3$).

*2-[3'-(Trimethylammonium)propylphosphoryl]ethyl
Methacrylate (TPM).* OPREM (6.5 g, 0.026 mol) and
90 ml of dry acetonitrile were placed in a 200 ml glass
pressure resistance bottle. After the bottle was cooled
below $-30°C$, 17.8 g (0.30 mol) of trimethylamine were
dissolved in the mixture by bubbling. The closed glass
bottle was stirred at $60°C$ for 46 h. The precipitate formed
was filtered off and purified by recrystallization in
acetonitrile to give white crystalline TPM. Yield 3.1 g
(38.5%). ^1H NMR (δ in ppm from 3-(trimethylsilyl)-1-
propane-sulfonic acid, sodium salt (TMSPS) in D$_2$O):
6.08 (s, 1H, CH =, *cis*), 5.64 (s, 1H, CH =, *trans*), 4.26
(br s, 2H, COOCH$_2$–), 4.02 (br s, 2H, –CH$_2$OP), 3.84 (q,
2H, POCH$_2$–), 3.36 (t, $J = 8.25$ Hz, 2H, –CH$_2$N$^+$), 3.03
(s, 9H, –N(CH$_3$)$_3$), 2.01 (br s, 2H, PO–CH$_2$CH$_2$CH$_2$N$^+$),
1.84 (s, 3H, –CH$_3$); ^{13}C NMR (δ in ppm from TMSPS

in D$_2$O): 170.58 (–C=O), 136.80 (CH$_2$ = C), 128.06
(CH$_2$ = C), 65.53 (COOCH$_2$–), 65.42 (–CH$_2$OP), 63.34
(–CH$_2$N$^+$), 53.94 (–N(CH$_3$)$_3$), 24.92 (PO–CH$_2$CH$_2$-
CH$_2$–N$^+$), 18.36 (–CH$_3$).

Elemental Analysis. Found: H, 7.65%; C, 47.96%; N,
4.51%. Calcd for C$_{12}$H$_{24}$O$_6$NP: H, 7.82%; C, 46.60%;
N, 4.53%.

Other Materials

MPC was synthesized essentially according to the
method by Nakaya.[2] HEMA and MMA were purified
by distillation. Potassium persulfate (KPS) and 2,2'-
azobisisobutyronitrile (AIBN) were purified by ordinary
methods.[28] Tetrahydrofuran (THF) and all other sol-
vents were purified by the method in the literature.[29]
Distilled water was used throughout the experimental.
BSA was purchased from Sigma Chemical Co., Ltd.

Polymerization Procedure

Radical solution polymerizations of TPM were carried
out in a sealed glass tube with the initiator at 60 and
$80°C$. After polymerization for a given time, the solution
was poured into a large amount of acetone to precipi-
tate the polymer. The obtained polymer was purified
by reprecipitation from the methanol solution to excess
chloroform three times. The polymer was dried *in vacuo*
for 2 days.

Radical copolymerizations of TPM with MMA and
styrene (ST) were performed with AIBN in THF/metha-
nol (1 ml/1 ml) at $60°C$ in a sealed tube. After polym-
erization for a given time, the solution was poured into
excess diethyl ether/methanol (4 vol/1 vol) to precipitate
the polymer. The polymer obtained was dried *in vacuo*
for 2 days. The composition of the copolymer obtained
was calculated by ^1H NMR spectra and elemental
analysis.

Determination of Critical Micelle Concentration (CMC)

Solutions of several concentrations (1.0×10^{-4} to
1.0×10^{-1} mol l^{-1}) of TPM were prepared in distilled
water saturated with pyrene. Fluorescence spectra from
352 to 500 nm were measured with a FP-777 Spec-
trofluorometer (JASCO) using a quartz cell (1 cm) and
excitation wavelength at 342 nm. CMC was determined
from variation in (I_3/I_1) of the first signal (I_1) at
372—373 nm and third signal (I_3) at 272—273 nm, ac-
cording to the method by Thomas.[30]

Preparation of Polymer Microspheres

Poly(TPM-*co*-MMA), poly(MPC-*co*-MMA), and
PMMA microspheres were prepared by emulsifier free
emulsion polymerizations. Constant amounts of MMA
and TPM (or MPC) were placed into a separable round
bottomed flask (200 ml), equipped with a mechanical
stirrer, condenser and N$_2$ inlet tube. The solution was
stirred at a rate of 350—450 rpm at $70°C$ for 1 h, and
the required amount of KPS aqueous solution was added
at once. After the mixture was stirred for another 48 min
to 100 min, it was cooled with ice bath. The solution was
filtered through glass filter (1G3) to remove coarse par-
ticles and coagulated polymer. The filtrate was centri-
fuged, decanted, and redispersed in distilled water. This
procedure was repeated three times. Using methanol in-

stead of water, the same procedure was repeated three times. The obtained polymer microsphere was dried, and conversion was calculated from this weight of the polymer.

Measurements of ζ Potentials

ζ potentials of polymer microspheres were measured at pH 5.9 at 0.001 of ionic strength adjusted by the addition of aq HCl to aq NaCl solution at room temperature using a Particle Electrophoresis Apparatus Model Mark II (Rank Brother Co.).

Adsorption Procedure

The amount of BSA adsorbed on polymer microsphere was determined by the Lowry method.[31] The amount of BSA adsorbed was calculated from the content of free BSA in water, by measuring absorbance at 750 nm based on BSA by UV-VIS measurements with a Shimadzu UV-1200 spectrophotometer.

Measurements

Particle size was determined by a scanning electron microscope (SEM) with a Hitachi S-2300. The surface area of the particle was calculated using nitrogen and helium gas atmosphere with constant volume adsorption apparatus according to the BET equation.[32,33]

RESULTS AND DISCUSSION

Synthesis of TPM

TPM was synthesized according to Scheme 2, and identified from the ^{1}H and ^{13}C NMR spectra, as described in the experimental section, and elemental analysis. The ^{31}P NMR spectrum showed only a singlet peak assigned to P. Purified TPM showed strong deliquescence as well as MPC. Compared to the precursor of a five-membered phosphoryl compound, 2-chloro-2-oxo-1,3,2-dioxaphosphorane (CODP), used in the preparation of MPC, CODPR was relatively stable to water. CODPR did not decompose even on contact with water. In the use of CODPR dry atmosphere was not always needed. Intermediate OPREM was very stable to water. Thus OPREM can be polymerized to obtain polymers bearing a six-membered ring. Synthesis and its characterization of OPREM polymers will be reported in the near future.

Radical Solution Homopolymerization of TPM

The results of radical solution homopolymerizations of TPM are summarized in Table I. The polymerizations were performed at TPM monomer concentration over CMC. When TPM concentration was less than CMC, appreciable polymers could not be obtained. TPM monomer indicated solubility in polar solvents such as water and methanol because of the possession of a phosphorylcholine analogous group at the side chain end.

Table I. Radical polymerizations of TPM

Run	Monomer (mol L^{-1})	Initiator (×10^{-3} mol L^{-1})	Polym. solvent ml	Polym. temp °C	Polym. time h	Convn. %	η_{sp}/C[a] dl g^{-1}
1	0.86	K$_2$S$_2$O$_8$ (5.1)	H$_2$O (2)	80	0.3	77.3	1.76[b]
2	0.85	K$_2$S$_2$O$_8$ (5.0)	H$_2$O (2)	80	0.1	72.1	0.79
3	0.84	K$_2$S$_2$O$_8$ (5.0)	H$_2$O (2)	80	2	76.2	0.32
4	0.85	AIBN (5.0)	CH$_3$OH (2)	60	2	44.2	0.46

[a] Measured in CH$_3$OH at 30°C.   [b] 2.12 when measured in H$_2$O at 30°C.

Table II. Radical copolymerizations of TPM (M$_1$) with ST, MMA, or BMA (M$_2$) in a mixture of THF and CH$_3$OH[a] at 60°C[b]

Run	M$_1$	M$_2$	M$_1$ in monomer mol%	Polym. time h	M$_1$ in copolymer mol%	Convn. %	η_{sp}/C[c] dl g^{-1}	T_g[d] °C
1	TPM	ST	4.9	14.0	8.4	4.5	0.13	—[e]
2	TPM	ST	19.8	12.0	35.0	23.2	0.10	—[e]
3	TPM	ST	30.2	12.0	39.2	34.7	0.10	—[e]
4	TPM	ST	50.1	9.5	58.0	30.9	0.09	—[e]
5	TPM	ST	0.0	7.0	59.9	36.5	0.08	—[e]
6	TPM	MMA	10.0	2.0	9.6	24.7	1.05	—[e]
7	TPM	MMA	20.1	2.0	19.2	28.3	0.91	—[e]
8	TPM	MMA	29.9	1.2	40.1	20.8	0.57	—[e]
9	TPM	MMA	49.0	0.8	43.8	23.6	0.47	—[e]
10	TPM	MMA	68.5	0.5	73.7	22.8	0.40	—[e]
11	TPM	BMA	10.1	15.0	12.8	78.5	0.31	40.5
12	TPM	BMA	19.8	15.0	19.8	98.2	0.24	35.2

[a] THF/CH$_3$OH = 1.0 ml/1.0 ml (run 1—10, 12) or THF/CH$_3$OH = 1.4 ml/0.6 ml (run 11).   [b] [AIBN] = 5.0 × 10^{-3} mol L^{-1}; M$_1$ + M$_2$ = 0.5 g.   [c] Measured in THF/CH$_3$OH = 1:1 (vol% : vol%) (run 1—10) or THF/CH$_3$OH = 7:3 (vol% : vol%) (run 11, 12) at 30°C.   [d] Determined by DSC.   [e] No measurements.

Table III. Copolymerization data

M$_1$	M$_2$	Solvent	Temp °C	r_1	r_2	Q_1	e_1	Ref
TPM	MMA	THF–CH$_3$OH (=1:1)	60	1.05	0.91	0.87	0.59	This work
TPM	ST	THF–CH$_3$OH (=1:1)	60	0.52	0.37	0.97	0.49	This work
MPC	ST	CH$_3$COOC$_2$H$_5$–CH$_3$OH (=8:3)	60	0.39	0.46	0.76	0.51	36
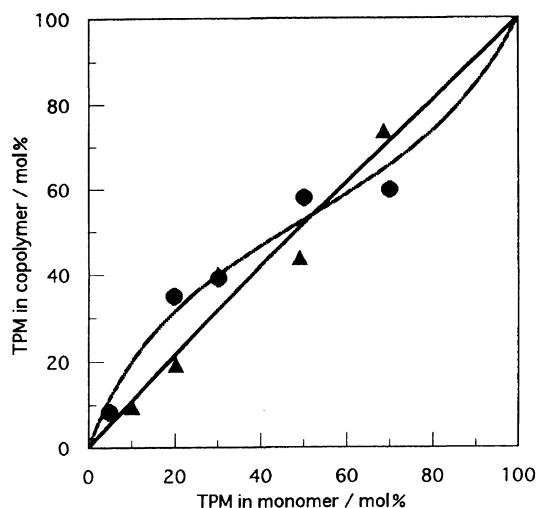
Figure 1. Copolymer–composition curves for (●) TPM–ST and (▲) TPM–MMA systems.

Table IV. Solubility of TPM copolymers

Run	M$_1$	M$_2$	M$_1$ in copolymer mol%	Solvent H$_2$O	Solvent CHCl$_3$
1	TPM	ST	8.4	×	○
2	TPM	ST	35.0	×	△
3	TPM	ST	39.2	×	×
4	TPM	ST	58.0	○	×
5	TPM	ST	59.9	○	×
6	TPM	MMA	9.6	×	○
7	TPM	MMA	19.2	△	△
8	TPM	MMA	40.1	△	×
9	TPM	MMA	43.8	○	×
10	TPM	MMA	73.7	○	×
11	TPM	BMA	12.8	×	○
12	TPM	BMA	19.8	×	○

○, soluble; ×, insoluble; △, swelling.

All polymerizations proceeded homogeneously throughout. In the polymerization in distilled water, yields and reduced viscosities were relatively higher in spite of short polymerization time. This tendency was similar to that for MPC.[26] That is, the length of a methylene unit in the phosphorylcholine group does not influence the polymerization reactivity. However, the specific rotations were very different among the polymers (run 1 to 3) in spite of almost the same conversion. The reason is not clear at the present time. The poly(TPM)s obtained were soluble in water and methanol, but insoluble in chloroform and THF. In addition, the polymer exhibited deliquescence because of a phosphorylcholine analogous group having hydrophilic nature as well as poly(MPC). It seems that the ordinary radical addition polymerization took place because the signals assigned to vinyl protons disappeared in the ^{1}H NMR spectrum.

Radical Copolymerization of TPM with ST, MMA, or BMA

TPM was radically copolymerized with ST, MMA, or BMA in a mixture of methanol and THF at 60°C. The results are summarized in Table II. All copolymerizations proceeded homogeneously throughout. Copolymer composition was calculated from ^{1}H NMR spectra and elemental analysis.

In all copolymerization systems, polymer yield increased with TPM content in the copolymer. It seems that the yield is concerned with solubility of the copolymer obtained. Reduced viscosities decreased with increase of TPM content in the copolymer. That is, molecular weight decreased with increase of the TPM content because a bulky phosphorylcholine group re-

duced the polymerizability of TPM. Reduced viscosity for the TPM–MMA copolymers (run 6—10) and TPM–BMA copolymers (run 11, 12) were much higher than those for the TPM–ST copolymers (run 1—5). Glass transition temperatures were determined as 40.5°C and 35.2°C according to DSC.

Figure 1 shows copolymer–composition curves for the copolymerization of TPM (M$_1$) with ST (M$_2$), or MMA (M$_2$). The curve for the TPM–MMA system is equal to almost ideal copolymerization along the azeotropic line.

The monomer reactivity ratios calculated by the high conversion method reported by Kelen and co-workers,[34] and Alfrey–Price[35] Q and e values are shown in Table III. In both systems, r_1 were little larger than r_2, which suggests that the polymerization reactivity of TPM is higher than that of M$_2$ monomer. The copolymerization parameter for TPM was similar to those for MPC reported by Sato *et al.*[36]

Table IV shows solubility of the TPM copolymers. The copolymer consisting of the 20 mol% TPM exhibited no complete solubility in chloroform and THF but swelling. Solubility increased in water but decreased in chloroform as the content of TPM increased. The copolymers containing the TPM of 19 to 40 mol% swelled in water and had hydrogel structures containing much water.

Molecular Aggregation of TPM in Water

Molecular aggregation of TPM was investigated according to the fluorescence probe method of Thomas *et al.*[30] Fluorescence spectra for pyrene were measured in distilled water at 342 nm excitation wavelength. Pyrene using as a fluorescence probe depicts high hydrophobic

Table V. Emulsifier-free emulsion copolymerizations of TPM (M_1) and MPC (M_1) with MMA (M_2)

Run	M_1 × 10³ (mol)	M_2 × 10³ (mol)	Initiator[a] × 10³ (mol)	Dispersion medium[b] (ml)	Speed of agitation (rpm)	Polym. temp (°C)	Polym. time (min)	Convn.[c] (%)	Surface area[d] ($m^2 g^{-1}$)	Diameter[e] (nm)	η_{sp}/C[f] (dl g⁻¹)	ζ-Potential[g] (mV)
1	TPM (1.0)	100	0.10	100	450	70	48	46.6	21.62	321	11.9	−55.3
2	TPM (1.0)	100	0.30	100	350	70	30	41.5	—[j]	336	—[j]	—[j]
3	TPM (1.0)	100	0.10	100	350	70	60	63.8	—[j]	—[j]	—[j]	—[j]
4	MPC (1.0)	100	0.40	100	450	70	55	59.3	11.00	356	4.54[h]	−54.1
5	None	70	0.53	70	350	70	100	35.2	17.86	326	0.94[i]	−54.6

[a] $K_2S_2O_8$. [b] H_2O. [c] Except coarse particles, coagulated polymer and water soluble polymer. [d] Calculated by BET equation. [e] Calculated from SEM pictures. [f] Measured in THF at 30°C. [g] Measured at pH 5.9 at r.t. [h] $M_n = 74,000$ $M_w/M_n = 2.69$. [i] $M_n = 24,000$ $M_w/M_n = 4.90$. [j] No measurements.
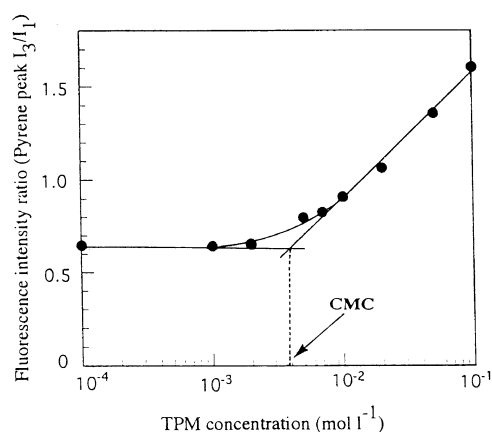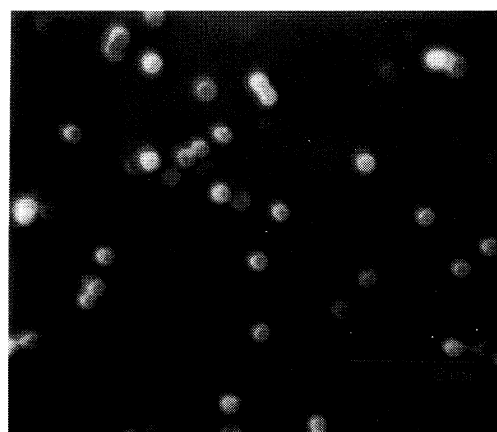

Figure 2. Fluorescence intensity ratio vs. TPM concentration in distilled water.

Figure 3. SEM picture of poly(TPM-co-MMA) microspheres.

nature. In the TPM aqueous solution, pyrene molecules tend to aggregate with hydrophobic site of TPM to avoid hydrophilic sites of TPM, i.e., the phosphorylcholine group. Consequently, phosphorylcholine sites tend to orient toward the aqueous layer. Accordingly, TPM monomer can play a role of an emulsifier itself to form a micelle. The fluorescence signal intensity for pyrene in water, i.e., under hydrophilic atmosphere, is different from that after micelle formation, i.e., under hydrophobic atmosphere.[30] That is, the fluorescence intensity of peak I_1 at 383—384 nm and peak I_3 at 372—373 nm changes.[30]

Figure 2 shows the relationship between fluorescence intensity (I_3/I_1) and TPM concentrations in distilled water. Signal intensity (I_3/I_1) remarkably changed at 3.6×10^{-3} mol l⁻¹, which suggests that the field for pyrene changed from hydrophilic environment to hydrophobic one at the concentration. The concentration was determined as a critical micelle concentration (CMC) of TPM in water. TPM formed micelles with comonomers having hydrophobic nature such as MMA in water over the CMC as well as MPC to obtain polymer microspheres.

Preparation of Polymer Microsphere

Table V summarizes the results of emulsifier free emulsion copolymerizations of TPM (M_1) with MMA (M_2), including data for the poly(MPC-co-MMA) and PMMA. Sugiyama et al. reported that poly(MPC-co-MMA) microsphere could be obtained in best yield when the concentration of MPC was 1 mol% of MMA in feed[23] and that many aggregation polymers can be obtained

under other conditions. Thus, according to the suggestion for the MPC concentration reported by Sugiyama,[23] TPM concentration was determined to be 1 mol% of MMA in feed. The concentration of TPM in the solution was 1×10^{-2} mol l⁻¹, which was over the CMC (3.6×10^{-3} mol l⁻¹) of TPM in water. The conversions in Table V are yields of microsphere insoluble in water, which exclude crude particles, aggregating microspheres and microspheres soluble in water. The yields and reduced viscosities of the copolymers containing a phosphorylcholine and its analogous group (Table V run 1 to 4) were higher than those of PMMA, which suggests that micelles are formed in the TPM and MPC systems. The copolymerization of TPM proceeded with MMA in hydrophobic micelles prepared in the TPM aqueous solution. Thus polymerization and molecular weight of the copolymers obtained increased. The reduced viscosity (11.9 dl g⁻¹) of the TPM microsphere was higher than that (4.54 dl g⁻¹) of MPC. The emulsifier free copolymerization of TPM with MMA indicated higher reactivity as well as solution polymerization, as shown in Table II.

Characterization of Particles

Surface microsphere area is shown in Table V. Figure 3 displays a typical SEM of the microsphere. The average diameter of a poly(TPM-co-MMA) particle was 321 nm. Surface area of a poly(TPM-co-MMA) particle was 21.62 $m^2 g^{-1}$, according to the BET equation.[33]

Adsorption of BSA onto Microspheres

Figure 4 shows the results of adsorption of BSA onto various microspheres measuring in pH 6.6 at 25°C for
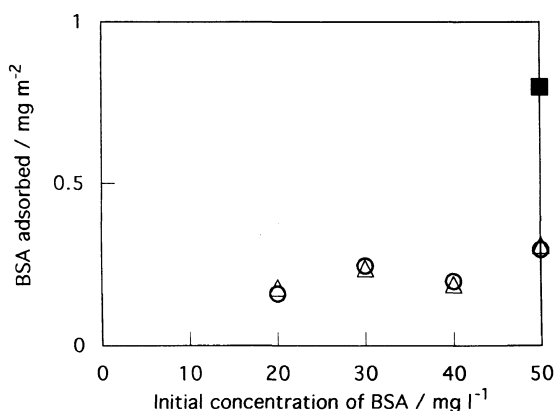
Figure 4. Adsorption of BSA onto various microspheres in the buffer solution (pH 6.6) at 25°C for 2 h: (○), poly(TPM-*co*-MMA); (■), poly(MMA); (△), poly(MPC-*co*-MMA).

2 h. Poly(TPM-*co*-MMA) more strongly depressed adsorption of BSA than PMMA. The depression of BSA adsorption by poly(TPM-*co*-MMA) was similar to that by poly(MPC-*co*-MMA), which was the best depression of BSA adsorption reported so far.[22] The structure of three methylene units between zwitter-ions could not prevent depression of BSA adsorption.

CONCLUSIONS

(1) A new methacrylate bearing a phosphorylcholine analogous moiety, TPM was synthesized, homopolymerized and copolymerized with vinyl monomers.

(2) Monomer reactivity (r_1, r_2) in the copolymerizations of TPM (M_1) and methyl methacrylate (MMA, M_2) or styrene (ST, M_2) and Alfrey–Price Q, e were determined as follows:

$r_1 = 0.52, r_2 = 0.37, Q_1 = 0.97, e_1 = 0.49$ in the TPM–ST system and $r_1 = 1.05, r_2 = 0.91, Q_1 = 0.87, e_1 = 0.59$ in TPM–MMA system.

(3) CMC of TPM was determined to be 3.6×10^{-3} mol l^{-1} by the fluorescent probe method.

(4) Poly(TPM-*co*-MMA) microspheres could be prepared by emulsifier-free emulsion copolymerizations of TPM with MMA.

(5) Adsorption property of BSA on these microspheres was investigated. Poly(TPM-*co*-MMA) more strongly depressed adsorption of BSA than poly(MMA). The depression of BSA adsorption by poly(TPM-*co*-MMA) was similar to that by poly(MPC-*co*-MMA). The structures of three methylene units between zwitter-ions could not prevent depression of BSA adsorption.

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