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Synthesis and properties of poly(fumaramate) bearing a phosphorylcholine moiety

Tsutomu Oishi*, Tsuyoshi Fukuda, Hironobu Uchiyama, Fumio Kondou, Hideki Ohe and Hiromori Tsutsumi

Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Yamaguchi University, 2557 Tokiwadai, Ube, Yamaguchi 755, Japan
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Novel types of fumaramate bearing a phosphorylcholine group, alkyl-2-[2′-(trimethylammonium)ethyl phosphoryl]ethyl fumaramate [alkyl = isopropyl (IPTPFA) and methyl (MTPFA)] were synthesized. IPTPFA and MTPFA were polymerized and copolymerized with methyl methacrylate (MMA) in the presence of radical initiators. Critical micelle concentrations (CMC) of IPTPFA and MTPFA were 1.7×10^{-3} and 1.0×10^{-3} mol l^{-1}, respectively. Three types of copolymer microspheres of MMA with comonomers such as IPTPFA, MTPFA and 2-methacryloyloxyethyl phosphorylcholine (MPC) were prepared from the emulsifier-free emulsion copolymerizations over the CMC. An addition of small amounts of IPTPFA or MTPFA resulted in an increase of the rate of polymerization of MMA. Amount of bovine serum albumin adsorption decreased in the following order: poly(MMA) > poly(MTPFA-*co*-MMA) > poly(IPTPFA-*co*-MMA) = poly(MPC-*co*-MMA) microspheres. © 1997 Elsevier Science Ltd.

(Keywords: phosphorylcholine; fumaramate; emulsion copolymerization)

INTRODUCTION

There have been many reports on biocompatibility and other properties of copolymers containing a phosphorylcholine moiety[1–7]. Nakaya *et al.* reported on various polymers bearing a phosphatidylcholine moiety[8–10]. Nakabayashi and Ishihara and co-workers reported that the copolymers of 2-methacryloyloxyethyl phosphorylcholine (MPC)[11,12] (*Scheme 1*) with n-butyl methacrylate (BMA) showed excellent blood compatibility, i.e. suppression of protein adsorption and platelet adhesion[13–16]. Sugiyama *et al.* clarified that introduction of a very small amount of MPC moiety (even at lower than 0.05 mol% in feed) onto poly(methyl methacrylate) (PMMA) microspheres results in a drastic decrease in bovine serum albumin (BSA) adsorption[17,18]. This strongly suggests that introduction of phosphorylcholine containing a zwitterionic head group into a polymer is useful for improvement of non-thrombogenicity.

There have been some reports on the polymerization of fumaric acid derivatives, i.e. fumaramate[19–21]. However, there have been no reports on the synthesis and emulsion copolymerizations of fumaramate bearing a phosphorylcholine moiety. Fumaramate has a 1,2-disubstituted ethylene type structure, and differs from MPC which has a 1,1-disubstituted ethylene structure. Sugiyama *et al.* reported that MPC can form micelles in water and give microspheres by emulsifier free emulsion copolymerizations of MPC with MMA[17,18]. Thus, we were interested in the possible micelle formation of fumaramate-bearing bulky substituents such as the phosphorylcholine moiety and alkyl groups. In addition, possible microspheres formation by the emulsifier-free emulsion polymerization and adsorption properties of BSA onto microspheres are of interest.

The present paper describes the synthesis of alkyl-2-[2′-(trimethylammonium) ethyl phosphoryl]ethyl fumaramate [RTPFA: alkyl = isopropyl (IPTPFA) and methyl (MTPFA)] and emulsifier-free emulsion copolymerization of MMA with RTPFA. The poly(RTPFA-*co*-MMA) microspheres obtained were characterized, and the adsorption behaviour of BSA onto them was investigated, as compared with poly(MPC-*co*-MMA).

EXPERIMENTAL

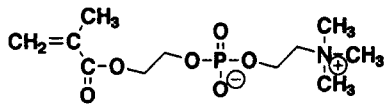
RTPFA monomers

RTPFA monomers were synthesized from maleic anhydride, the corresponding alcohol, 2-chloro-2-oxo-1,3,2-dioxaphospholane (CODP), and trimethylamine (*Scheme 2*).

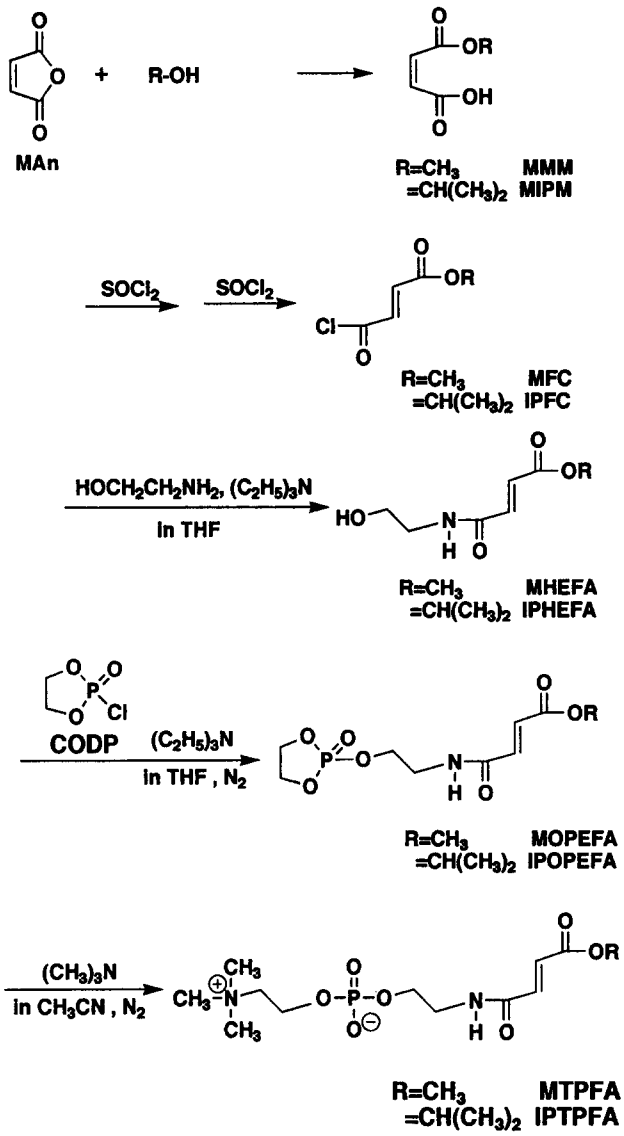
CODP. CODP was synthesized according to the method of Edmundson[22] and purified by distillation under reduced pressure, bp 92–93°C/0.8 mmHg (lit.[22] bp 79°C/0.4 mmHg).

Monoisopropyl maleate (MIPM). A mixture of maleic anhydride (100.0 g, 1.02 mol) and isopropyl alcohol (61.3 g, 1.02 mol) was stirred at 50°C and dissolved to homogeneous solution. The mixture was stirred at 65°C for 2 h, at 80°C for 2 h, and finally at 90°C for 1 h. MIPM was obtained quantitatively and

* To whom correspondence should be addressed



Scheme 1



Scheme 2

used for the following reaction. ^{1}H n.m.r. (δ in ppm from TMS in $CDCl_3$): 10.13 (bs, 1H, COOH), 6.38 (d, $J = 4.95$ Hz, 2H, CH=CH), 5.22–5.13 (m, 1H, COO–CH), 1.34 (d, $J = 6.27$ Hz, 6H, $CH(C\underline{H}_3)_2$).

Monoisopropyl fumarate (MIPF) and isopropyl fumaroyl chloride (IPFC). Thionyl chloride (2.03 g, 0.017 mol) was slowly added to MIPM with stirring at 80°C for isomerization. After the reaction heats decreased, thionyl chloride (158.4 g, 1.33 mol) was added dropwise to the mixture with stirring at 80°C for 4 h. After cooling, excess thionyl chloride was immediately evaporated under reduced pressure to obtain crude IPFC, which was purified by distillation under reduced pressure: yield 59.5%, bp 85–90°C/20 mmHg. ^{1}H n.m.r. (δ in ppm from TMS in $CDCl_3$): 6.90 (d, $J = 2.64$ Hz, 2H, CH=CH), 5.19–5.09 (m, 1H, –COO–CH–), 1.33 (d, $J = 6.27$ Hz, 6H, $-CH(C\underline{H}_3)_2$).

Isopropyl-2-hydroxyethyl fumaramate (IPHEFA). Into a 500 ml three-necked round-bottomed flask, equipped with a mechanical stirrer, dropping funnel and thermometer, were placed 8.02 g (0.13 mol) monoethanolamine and 9.2 g (0.091 mol) of triethylamine in 250 ml dry THF. After the solution was cooled below −20°C, 16.0 g (0.091 mol) of IPFC was added dropwise to the stirred solution over a period of 6 h. The reaction was continued for another 30 h at −20°C. Then, the precipitate (triethylammonium chloride) was filtered off. The filtrate was evaporated under reduced pressure. The residue was distilled under reduced pressure to obtain pure IPHEFA: yellow viscous liquid, yield 66.7%, bp 175–180°C/3.6×10^{-2} mmHg. ^{1}H n.m.r. (δ in ppm from TMS in $CDCl_3$): 7.45 (bs, 1H, CONH), 6.78 (d, $J = 15.18$ Hz, 2H, CH=CH), 5.13–5.03 (m, 1H, –COO–CH–), 4.37 (bs, 1H, $\underline{H}O-CH_2-$), 3.75 (t, $J = 5.61$ Hz, 2H, $N-CH_2-C$), 3.51 (t, $J = 5.28$ Hz, 2H, $HO-C\underline{H}_2-$), 1.28 (d, $J = 6.27$ Hz, 6H, $-CH(C\underline{H}_3)_2$); ^{13}C n.m.r. (δ in ppm from TMS in $CDCl_3$): 165.25 and 164.74 (–C=O), 136.14 and 130.62 (CH=CH), 68.75 (–COO–$\underline{C}$H), 61.10 ($HO-CH_2-$), 42.41 ($-CONH-\underline{C}H_2-$), 21.28 ($-CH(\underline{C}H_3)_2$).

Isopropyl-2-(2-oxo-1,3,2-dioxaphospholane-2-yloxy) ethyl fumaramate (IPOPEAFA). Into a 500 ml four-necked round-bottomed flask, equipped with a mechanical stirrer, dropping funnel, thermometer and N_2 inlet tube, were placed 9.6 g (0.048 mol) IPHEFA and 4.8 g (0.048 mol) triethylamine in 180 ml dry THF. After the solution was cooled at −15°C, 6.8 g (0.048 mol) CODP was added dropwise to the stirred solution over a period of 3.5 h. The precipitate (triethylammonium chloride) was filtered off, and the filtrate evaporated under reduced pressure. After being dried at r.t., it was used for following reaction without further purification because the product was decomposed by treatment.

Isopropyl-2-[2′-(trimethylammonium)ethyl phosphoryl] ethyl fumaramate (IPTPFA). 14.7 g IPOPEFA and 150 ml dry acetonitrile were placed into a 200 ml glass pressure resistance bottle. After the bottle was cooled below −35°C, 15.9 g (0.27 mol) of trimethylamine was dissolved in the mixture by bubbling. The closed glass bottle was stirred at 40°C for 24 h. After cooling, the solution was evaporated under reduced pressure. The residue was recrystallized from a large amount of chloroform to obtain pure IPTPFA: yield 33.5%, mp 240–244°C. ^{1}H n.m.r. [δ in ppm from 3-(trimethylsilyl)-1-propane-sulfonic acid, sodium salt (TMSPS) in D_2O]: 6.80 (d, $J = 15.84$ Hz, 2H, CH=CH), 5.08–4.99 (m, 1H, –COO–CH–), 4.20 (bs, 2H, $(CH_3)_3N^+-CH_2-C\underline{H}_2-$), 3.92 (t, $J = 5.28$ Hz, 2H, $POO-C\underline{H}_2-CH_2-NHCO$), 3.56 (m, 2H, $-CONH-C\underline{H}_2-$), 3.48 (t, $J = 5.28$ Hz, 2H, $(CH_3)_3N^+-C\underline{H}_2-$), 3.14 (s, 9H, $(CH_3)_3N^+$), 1.23 (d, $J = 6.27$ Hz, 6H, $-CH(C\underline{H}_3)_2$); ^{13}C n.m.r. (δ in ppm from TMSPS in D_2O): 167.79 and 167.31 (–C=O), 136.74 and 131.58 (CH=CH), 71.62 (–COO–$\underline{C}$H–), 66.90 ($POO-\underline{C}H_2-CH_2-NHCO$), 64.97 ($(CH_3)_3N^+-CH_2-\underline{C}H_2-$), 60.41 ($(CH_3)_3N^+\underline{C}H_2$), 54.87 ($(\underline{C}H_3)_3N^+$), 41.15 ($-\underline{C}H_2-NHCO-$), 21.74 ($-CH-(\underline{C}H_3)_2$).

Elemental analysis. Found: H 8.22%, C 46.09%, N 7.89% Calc. for $C_{14}H_{27}O_7N_2P$: H 7.43%, C 45.90%, N 7.65%.

Monomethyl maleate (MMM). A mixture of maleic anhydride (150.0 g; 1.53 mol) and methanol (51.6 g; 1.61 mol) was stirred at 75°C for 2 h. The excess methanol was evaporated under reduced pressure. The yield was quantitative, and the product was used for following reaction without further purification. ^{1}H n.m.r. (δ in ppm from TMS in $CDCl_3$): 7.57 (bs, 1H, –COOH), 6.36 (d, $J = 0.99$ Hz, 2H, CH=CH), 3.84 (s, 3H, –COO–CH_3).

Monomethyl fumarate (MMF) and methyl fumaroyl chloride (MFC). Into a 300 ml two-necked round bottomed flask, equipped with a dropping funnel and thermometer, was placed 140.0 g (1.08 mol) of MMM. After the solution was heated at 150°C, thionyl chloride (1.12 ml, 0.016 mol) was added dropwise to the stirred solution to form an isomer of MMF. After the reaction heat decreased, 167.0 g (1.40 mol) of thionyl chloride was added dropwise to the stirred solution at 150°C over a period of 5 h. The reaction was continued at 150°C for another 0.5 h. Then, byproduct was filtered off and the excess thionyl chloride evaporated under reduced pressure. The residue was distilled under reduced pressure to obtain pure MFC, bp 72–76°C/20 mmHg. ^{1}H n.m.r. (δ in ppm from TMS in $CDCl_3$): 6.92 (d, $J = 3.95$ Hz, 2H, CH=CH), 3.79 (s, 3H, –COO–CH_3).

Methyl-2-hydroxyethyl fumaramate (MHEFA). MHEFA was synthesized according to a method similar to that of IPHEFA syntheses. The crude MHEFA was purified by recrystallization in a mixture of n-hexane and chloroform (1 vol%/4 vol%): yield 70.5%, mp 70–75°C. ^{1}H n.m.r. (δ in ppm from TMS in $CDCl_3$): 7.44 (bs, 1H, –CONH–), 6.90 (d, $J = 15.18$ Hz, 2H, CH=CH), 3.79 (s, 3H, –COO–CH_3), 3.75 (t, $J = 5.28$ Hz, 2H, N–CH_2–C), 3.63 (bs, 1H, HO–CH_2–), 3.51 (t, $J = 5.28$ Hz, 2H, HO–CH_2–); ^{13}C n.m.r. (δ in ppm from TMS in $CDCl_3$): 166.20 and 164.55 (–C=O), 136.55 and 129.88 (CH=CH), 61.35 (HO–CH_2–), 52.24 (–COO–CH_3), 42.50 (–CONH–CH_2).

Methyl-2-(2-oxo-1,3,2,-dioxaphospholane-2-yloxy)ethyl fumaramate (MOPEFA). MOPEFA was prepared according to a procedure similar to that of IPOPEFA. After MOPEFA was dried at r.t., it was used for the following reaction without further purification.

Methyl-2-{2′-(trimethylammonium)ethyl phosphoryl} ethyl fumaramate (MTPFA). MTPFA was synthesized according to a method similar to that of IPTPFA synthesis. When the reaction mixture was cooled in a refrigerator, MTPFA precipitated. The precipitation was filtered off and purified by recrystallization in acetonitrile: yield 32.8%, mp 188–193°C. ^{1}H n.m.r. (δ in ppm from TMSPS in D_2O): 6.76 (d, $J = 15.84$ Hz, 2H, CH=CH), 4.14 (bs, 2H, $(CH_3)_3N^+$–CH_2CH_2–), 3.85 (q, 2H, POO–CH_2–CH_2–NHCO), 3.68 (s, 3H, –COO–CH_3), 3.50 (t, $J = 4.62$ Hz, 2H, –CONH–CH_2–), 3.42 (t, $J = 5.28$ Hz, 2H, $(CH_3)_3N^+$–CH_2–), 3.06 (s, 9H, $(CH_3)_3N^+$); ^{13}C n.m.r. (δ in ppm from TMSPS in D_2O): 168.4 and 167.11 (–C=O), 137.24 and 131.15 (CH=CH), 66.99 (POO–CH_2CH_2–NHCO–), 64.99 ($(CH_3)_3N^+$–CH_2CH_2–), 60.39 ($(CH_3)_3N^+$–CH_2–), 54.96 ($(CH_3)_3N^+$–), 53.76 (–COO–CH_3), 41.36 (–CH_2–NHCO–).

Elemental analysis: Found: H 6.33%, C 41.57%, N 8.12%; Calc. for $C_{12}H_{23}O_7N_2P$: H 6.85%, C 42.60%, N 8.28%.

Other materials

MPC synthesis was reported by Imoto[8], Nakabayashi[11,12] and Sugiyama[18]. MPC used in this experiment was synthesized by the method described by Nakabayashi *et al.*[12]: yield 55.5%, mp 138–140°C. MMA, di-*t*-butyl peroxide (DTBP) and potassium persulfate (KPS) were purified by the ordinary methods[23]. Dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), and all other solvents were purified by the methods described in the literature[24].

Polymerization procedure

Radical solution polymerizations of IPTPFA and MTPFA were carried out in a sealed glass tube with the initiator at 70 and 120°C. After polymerization for a given time, the solution was evaporated under reduced pressure and the residue solved in methanol. The solution was poured into a large amount of methylene chloride to precipitate the polymer. The polymer was purified by reprecipitation from the methanol solution to excess methylene chloride, and dried under reduced pressure for a day.

Radical copolymerizations of IPTPFA and MTPFA with MMA were performed with DTBP in DMF (IPTPFA system) or DMSO (MTPFA system) at 120°C in a sealed tube. After the polymerization of IPTPFA for a given time, precipitating product was solved in a small amount of methanol. The solution was poured into an excess solution of diethyl ether/methanol (9/1, v/v) to precipitate the copolymer.

Determination of critical micelle concentration (CMC)

Solutions of several concentrations (1×10^{-6}–1×10^{-2} mol l^{-1}) of IPTPFA and MTPFA were prepared in distilled water saturated with pyrene. Fluorescence spectra from 352 to 500 nm were measured with a Jasco FP-777 spectrofluorometer using a quartz cell (1 cm) and excitation wavelength at 342 nm. The CMC was determined from variation of the ratios (I_3/I_1) of the first signal (I_1) at 372–373 nm and the third signal (I_3) at 272–273 nm, according to the method reported by Thomas[25].

Preparation of polymer microspheres

Poly(IPTPFA-*co*-MMA), poly(MTPFA-*co*-MMA), poly(MPC-*co*-MMA) and poly(MMA) microspheres were prepared by emulsifier-free emulsion polymerizations. Constant amounts of MMA and RTPFA (or MPC) were placed into a separable round bottomed flask (500 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, then the required amount of KPS aqueous solution was added at once. After the mixture was stirred for another 55–100 min, it was cooled in an ice bath and then poured into a large amount of methanol. The methanol solution was filtered through a glass filter (1G1) to remove coarse particles and coagulated polymer. The filtrate was centrifuged,

decanted, and redispersed in distilled water. This procedure was repeated three times. Using methanol instead of water, the same procedure was repeated three times. The obtained polymer microsphere was dried, and the conversion was calculated from this weight of the polymer.

Adsorption procedure

The amount of BSA adsorbed on polymer microsphere was determined by the Lowry method[26]. The amount of BSA adsorbed was calculated from the content of free BSA in the buffer solution (pH 6.6), by measuring the absorbance at 750 nm based on BSA by u.v.–v.i.s. measurements with a Shimadzu UV-1200 spectrophotometer.

Measurements

Gel permeation chromatographic analysis was carried out with polystyrene gel HGS-10-15-20-40, using a Shimadzu LC-3A GPC apparatus. The 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 a constant volume adsorption apparatus according to the BET equation[27,28].

RESULTS AND DISCUSSION

Radical solution homopolymerization of RTPFA

The results of radical solution homopolymerization of RTPFA are summarized in *Table 1*. Both monomers, IPTPFA and MTPFA were insoluble in organic solvents but soluble in polar solvent such as water, DMSO and DMF, which were used as polymerization solvents. The polymerization in distilled water proceeded homogeneously throughout. The polymerization of IPTPFA in DMF proceeded homogeneously in the beginning but heterogeneously in the course of polymerization process. That is, polymers precipitated in solvent.

As can be seen from *Table 1*, in all systems the reduced viscosities were relatively low. This suggests that polymerization reactivity of RTPFA was very low because RTPFA is a 1,2-disubstituted ethylene type derivative and has steric hindrance due to a bulky phosphorylcholine group. In addition, chain transfer to solvents such as water and DMSO may not be negligible. Even for the polymerization in DMF, high molecular weight polymer could not be obtained because of the precipitation of polymer during the polymerization.

Otsu *et al.* reported that bulk polymerizations of dialkyl fumarate gave high molecular weight polymers even when having a relatively bulky ester substituent such as isobutyl and *t*-butyl (e.g. refs 29, 30). In our IPTPFA and MTPFA, both the polymerization reactivities were similar. This suggests that the polymerization reactivities were more influenced by the bulky phosphorylcholine moiety than by branched alkyl groups.

Radical copolymerization of RTPFA with MMA

The results of radical copolymerizations of RTPFA with MMA are summarized in *Table 2*. In the copolymerization of IPTPFA, the polymers precipitated in the course of the polymerization process. The copolymerization of MTPFA in DMSO proceeded homogeneously throughout. The composition of the copolymer was calculated from the ratios of signals due to the trimethyl (9H) of phosphorylcholine and methyl (3H) of MMA in the ^{1}H n.m.r. spectra and elemental analysis. The copolymerization reactivity of MTPFA was superior to that of IPTPFA. This is attributable to the lower steric hindrance of MTPFA than that of IPTPFA. In all systems the reduced viscosities of the copolymers were higher, as compared with homopolymers.

Molecular aggregation of RTPFA in water

Figure 1 shows the fluorescence intensity ratio vs

Table 1 Radical polymerization of IPTPFA and MTPFA

Run	Monomer $\times 10^3$ (mol)	Initiator[a] $\times 10^4$ (mol)	Polym. solvent[b] (ml)	Polym. temp. (°C)	Polym. time (h)	Convn (%)	η_{sp}/c[c] (dl g^{-1})
1	IPTPFA (0.87)	$K_2S_2O_8$ (0.87)	H_2O (0.5)	70	72	39.2	0.04
2	IPTPFA (0.82)	DTBP (1.94)	DMF (1.0)	120	24	37.0	0.04
3	MTPFA (0.89)	$K_2S_2O_8$ (0.89)	H_2O (0.5)	70	72	58.1	0.04
4	MTPFA (0.89)	DTBP (1.77)	DMSO (1.0)	120	24	18.6	0.03

[a] DTBP: di-*t*-butylperoxide
[b] DMF: *N*,*N*-dimethylformamide; DMSO: dimethyl sulfoxide
[c] Measured in CH_3OH at 30°C

Table 2 Radical copolymerization of IPTPFA (M_1) and MTPFA (M_1) with MMA (M_2) at 120°C for 24 h[a]

Run	M_1	M_1 in monomer (mol%)	Initiator[b] $\times 10^2$ (g)	Polym. solvent[c] (ml)	M_1 in copolymer (mol%)	Convn (%)	η_{sp}/c[d] (dl g^{-1})
1	IPTPFA	9.9	11.5	DMF (1)	2.7	64.0	0.06
2	IPTPFA	19.8	9.7	DMF (1)	4.2	64.9	0.05
3	MTPFA	10.0	11.8	DMSO (8)	27.0	14.8	0.07
4	MTPFA	20.0	9.9	DMSO (8)	25.9	49.7	0.04

[a] $M_1 + M_2 = 0.5$ g
[b] DTBP: di-*t*-butylperoxide
[c] DMF: *N*,*N*-dimethylformamide; DMSO: dimethyl sulfoxide
[d] Measured in THF/CH_3OH = 1/1 (vol%/vol%) at 30°C

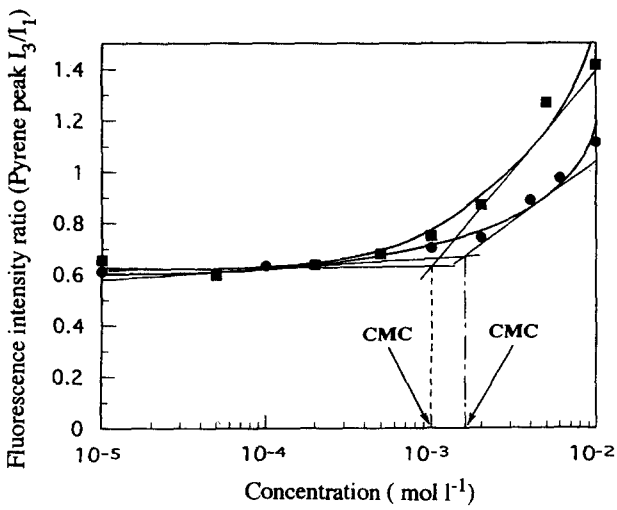


Figure 1 Fluorescence intensity ratio vs IPTPFA and MTPFA concentration in distilled water: (●) IPTPFA and (■) MTPFA

RTPFA concentration in distilled water. Fluorescence intensity (I_3/I_1) represents the ratios of signals at 372–373 and 383–384 nm in fluorescence spectra measurements. Pyrene used as a fluorescence probe shows a highly hydrophobic nature. The fluorescence signal intensity for pyrene in water, i.e. under a hydrophilic atmosphere, is different from that in micelle formation, i.e. it has a hydrophobic nature[25]. As can be seen from *Figure 1*, the signal intensity ratio (I_3/I_1) changed remarkably at 1.7×10^{-3} mol l^{-1} (IPTPFA) and 1.0×10^{-3} mol l^{-1} (MTPFA), according to the fluorescence probe method reported by Thomas *et al.*[25] This result confirmed that the environment for pyrene changed from a hydrophilic to a hydrophobic one. It was found that IPTPFA and MTPFA can form micelle in water and create a hydrophilic atmosphere. Thus the concentration described above was determined as a CMC of RTPFA in water.

A typical emulsifier such as 1-dodecanesulfonic acid sodium salt has a hydrophilic group at the head of long methylene units. However, it was found that even a structure without long alkyl chains like IPTPFA and MTPFA can form micelles.

Preparation of polymer microsphere

Table 3 shows conditions and results of preparation of poly(IPTPFA-*co*-MMA), poly(MTPFA-*co*-MMA), poly(MPC-*co*-MMA) and poly(MMA). Sugiyama *et al.* reported that poly(MPC-*co*-MMA) microspheres could be obtained in the best yield when the concentration of MPC was 1 mol% of MMA in the feed[18] and that many aggregation polymers can be obtained under other conditions. Thus, according to this suggestion[18], the concentration of our RTPFA was also determined to be 1 mol% of MMA in the feed. In this case, the concentration of RTPFA was 1×10^{-2} mol l^{-1}, which is over the CMC of RTPFA in water described above. The conversions in *Table 3* are yields of microsphere insoluble in water, which excluded crude particles, aggregating microspheres and microspheres soluble in water. The yields and reduced viscosities of the copolymers containing a phosphorylcholine group (*Table 3* runs 1, 2 and 3) were higher than those of poly(MMA). This may result from micelle formation in the RTPFA and MPC systems. Comparing MPC with RTPFA microspheres, the reduced viscosity of MPC microsphere was highest. This could be ascribed to steric hindrance due to the 1,2-disubstituted ethylene type structure and to amide groups bearing a bulky phosphorylcholine moiety.

Characterization of particles

Table 3 shows the surface area of the microspheres. *Figure 2* shows a typical scanning electron micrograph (SEM) of the microspheres. TEM pictures for poly(MPC-*co*-MMA) and poly(MMA) were reported previously by Sugiyama *et al.*[18]. The average diameter of poly(IPTPFA-*co*-MMA) and poly(MTPFA-*co*-MMA)

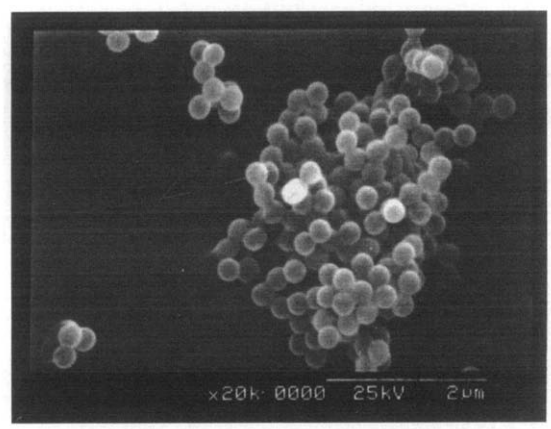


Figure 2 SEM picture of poly(MTPFA-*co*-MMA) microspheres

Table 3 Emulsifier-free emulsion copolymerization of MTPFA (M_1), IPTPFA (M_1) and MPC (M_1) with MMA (M_2)

Run	M_1 $\times 10^3$ (mol)	M_2 $\times 10^3$ (mol)	Initiator[a] $\times 10^3$ (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 (nm)	η_{sp}/c[e] ($dl\ g^{-1}$)
1	MTPFA (1.0)	100	0.40	100	350	70	90	91.2	13.16	310	2.00
2	IPTPFA (1.0)	100	0.40	100	430	70	90	73.5	17.20	460	1.72[f]
3	MPC (1.0)	100	0.40	100	450	70	55	59.3	11.00	–[h]	4.54
4	None	70	0.53	70	350	70	100	35.2	17.86	–[h]	0.94[g]

[a] $K_2S_2O_8$
[b] H_2O
[c] Except coarse particles, coagulated polymer and water soluble polymer
[d] Calculated by the BET equation
[e] Measured in THF at 30°C
[f] M_n 169 000; $M_w/M_n = 1.59$
[g] M_n 24 000; $M_w/M_n = 4.90$
[h] No measurements

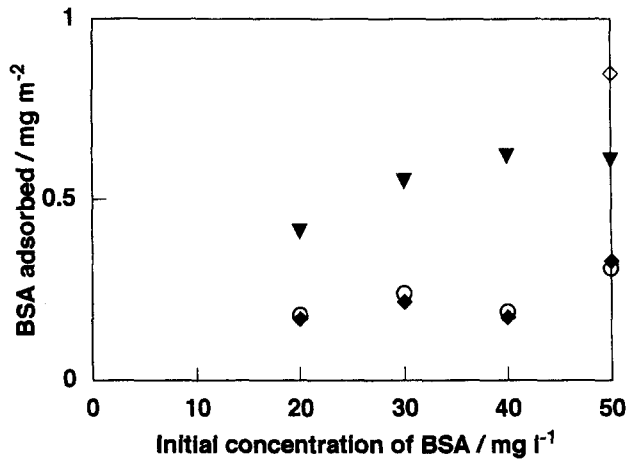


Figure 3 Adsorption of BSA onto various microspheres in the buffer solution (pH 6.6) at 25°C for 2 h: (◇) poly(MMA); (◆) poly(IPTPFA-*co*-MMA); (▼) poly(MTPFA-*co*-MMA) and (○) poly(MPC-*co*-MMA)

particles was 460 and 310 nm, respectively, determined from the SEM pictures in *Figure 2*. The surface area of poly(IPTPFA-*co*-MMA) and poly(MTPFA-*co*-MMA) particles was 17.20 and 13.16 $m^2 g^{-1}$, respectively, according to the BET equation[27,28] using a nitrogen and helium gas atmosphere in constant volume adsorption apparatus.

Adsorption of BSA onto microspheres

Figure 3 shows adsorption of BSA onto various microspheres measuring in pH 6.6 at 25°C for 2 h. Poly(IPTPFA-*co*-MMA) depressed adsorption of BSA more strongly than poly(MMA). The depression of BSA adsorption by poly(IPTPFA-*co*-MMA) was similar to that by poly(MPC-*co*-MMA), which was the best depression of BSA adsorption reported so far[18]. The amount of BSA adsorption decreased as follows: poly(MMA) > poly(MTPFA-*co*-MMA) > poly(IPTPFA-*co*-MMA) = poly(MPC-*co*-MMA) microspheres. The fact that the amount of BSA adsorption for poly(MTPFA-*co*-MMA) was greater than that for poly(IPTPFA-*co*-MMA) may be ascribed to lower contents of the MTPFA unit in the copolymer. The structure of the rigid polymer main chain bearing an amide group at the side chain could not prevent depression of BSA adsorption.

CONCLUSIONS

IPTPFA and MTPFA were synthesized from maleic anhydride, the corresponding alcohol, monoethanolamine, CODP and trimethylamine. The homopolymers were prepared with radical initiator ($K_2S_2O_8$ in water and DTBP in DMF or DMSO). The yields of polymers were relatively low (18–58%) and reduced viscosities were very low (0.04). IPTPFA and MTPFA were copolymerized with MMA in DMF or DMSO as solvent at 120°C using DTBP. The reduced viscosity of the copolymer was higher than that for the homopolymer.

CMC of IPTPFA and MTPFA were determined to be 1.7×10^{-3} and 1.0×10^{-3} mol l^{-1}, respectively, according to the fluorescence probe method using pyrene. Three types of copolymer microspheres were prepared from emulsifier-free emulsion copolymerizations of MMA (99 mol%) with comonomer (1 mol%) such as IPTPFA, MTPFA or MPC, and conditions over the CMC of the comonomer. The yields of poly(MTPFA-*co*-MMA) and poly(IPTPFA-*co*-MMA) were relatively high (91 and 74%) and reduced viscosities (η_{sp}/c) were much higher (2.00 and 1.72) than that of poly(MMA) ($\eta_{sp}/c = 0.94$).

The number average molecular weight and polydispersity for poly(IPTPFA-*co*-MMA) were 1.69×10^5 and 1.59, respectively. The addition of small amounts of IPTPFA or MTPFA increased the rate of copolymerization of MMA with IPTPFA or MTPFA. The average diameter of the particle was calculated to be 310–460 nm from SEM pictures. The surface area was estimated to be 13.16–17.20 $m^2 g^{-1}$ from the BET equation.

Adsorption properties of BSA on these microspheres were investigated by the Lowry method. The amount of BSA adsorption decreased as follows: poly(MMA) > poly(MTPFA-*co*-MMA) > poly(IPTPFA-*co*-MMA) = poly(MPC-*co*-MMA) microspheres. The structure of the rigid polymer main chain bearing an amide group at the side chain did not affect BSA adsorption.

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