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Synthesis and polymerization of methacrylate bearing a phosphorylcholine, analogous moiety

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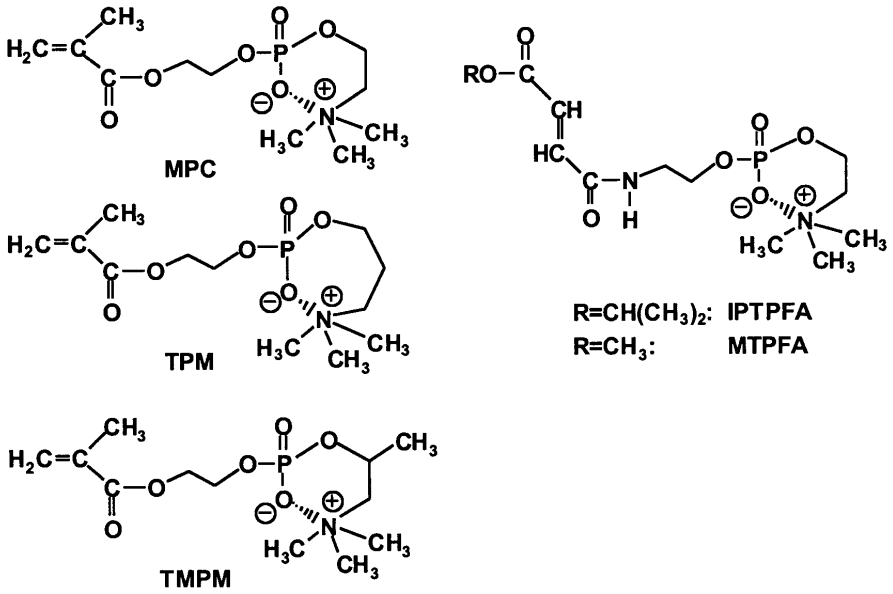
Summary

A new type of methacrylate bearing a phosphorylcholine analogous moiety, 2-[2'-(trimethylammonium)-1′-methylethyl-phosphoryl]ethyl methacrylate (TMPM) was synthesized. TMPM was radically homopolymerized and copolymerized with BMA to obtain the polymers. The critical micelle concentration (CMC) of TMPM was 4.9 X 10^{-2} mol/l according to the fluorescent probe method.

Introduction

Lately, 2-methacryloyloxyethyl phosphorylcholine (MPC, Scheme 1) was synthesized as a monomer having a phospholipid polar group [1,2]. MPC was copolymerized with various methacrylates and styrene, and the obtained copolymers showed biocompatibility with excellent antithrombogenesity [2-7]. There is no doubt the fact that the polymers obtained from MPC bearing a phosphorylcholine group exhibit excellent biocompatibility and depress both activation of the thrombogenicity and adsorption of the blood plasma protein. We reported that the synthesis of alkyl-2-[2'-(trimethylammonium)ethyl phosphoryl]ethyl fumaramate [RTPFA: alkyl = isopropyl (IPTPFA, Scheme 1) and methyl (MTPFA, Scheme 1)] and the emulsifier-free emulsion copolymerization of methyl methacrylate (MMA) with RTPFA, and the obtained copolymers depressed adsorption of bovine serum albumin (BSA) [8]. By addition of a methylene unit to an ethylene unit in the phosphorylcholine group, a novel type of methacrylate bearing a phosphorylcholine analogous group, 2-[3'-(trimethylammonium)propyl phosphoryl]ethyl methacrylate (TPM, Scheme 1) was synthesized and the copolymers consisting of TPM with butyl methacrylate (BMA) showed decrease of adsorption of BSA, too [9].

The present paper describes the synthesis of 2-[2'-(trimethylammonium)-1'-methyl-ethylphosphoryl]ethyl methacrylate (TMPM, Scheme 1) and the radical solution homopolymerization and copolymerization of BMA with TMPM in order to investigate the balance between hydrophilic and hydrophobic groups influencing BSA adsorption. Critical micelle concentration (CMC) was determined for TMPM because TMPM has both hydrophilic and hydrophobic character.



Scheme 1. Structure of phospholipid derivatives

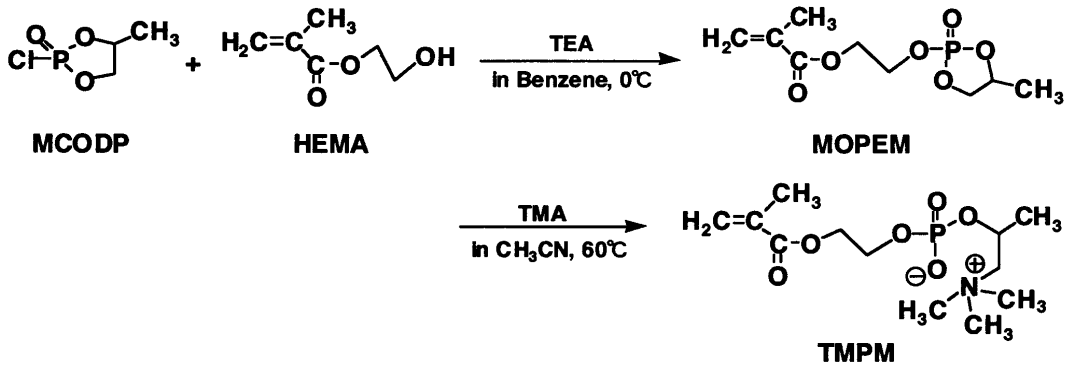
Experimental

Materials

Anhydrous trimethylamine (TMA) (99%) purchased a gas bomb from Aldrich Co., Ltd and used to reaction through a potassium hydroxide trap for removing the remained water. Potassium persulfate (KSP) and 2,2'-azobis(isobutyronitrile) (AIBN) were purified by ordinary methods. Phosphorus trichloride, propylene glycol, 2-hydroxyethyl methacrylate (HEMA) and butyl methacrylate (BMA) were purified by distillation. Tetrahydrofuran (THF) and all other solvents were purified by the method in the literature. Other reagents were used without further purification. Distilled water after the ion exchange was used throughout the experimental.

Synthesis of 2-{2'-(trimethylammonium)-1'-methyl-ethyl phosphoryl}} ethyl methacrylate (TMPM)

TMPM monomer was synthesized from 4-methyl-2-chloro-2-oxo-1,3,2-dioxaphospholane (MCODP), HEMA and TMA, as shown in Scheme 2.



Scheme 2. Synthesis of TMPM

MCODP: Phosphorus trichloride (90.2 g, 0.66 mol) in dichloromethane (50 ml) was dropped into propylene glycol (50.0 g, 0.66 mol) in dichloromethane (100 ml) with stirring at room temp. for 4 h. After the stirring was kept until the generation of the hydrogen chloride stopped, the reaction solution was removed the solvent and the residue was distilled *in vacuo*, and then 4-methyl-2-chloro-1,3,2-dioxaphospholane (MCDP) of the colorless liquid was obtained. MCODP was synthesized from MCDP (72.2 g, 0.51 mol) in benzene (300 ml) and oxygen by bubbling with stirring at room temp. for 16 h. After removed benzene from the reaction solution, the residue was distilled *in vacuo* and colorless liquid MCODP was obtained (b.p. 75-78°C/4.4 X 10^{-2} mmHg, Yield 68.0% (54.7 g, 0.35 mol)). ^{1}H NMR (δ in ppm from TMS in $CDCl_3$): 4.47-4.97 (m, 2H, -OCHC$\underline{H}_2$O-), 4.02-4.18 (m, 1H, -OC$\underline{H}$C$\underline{H}_2$O-), 1.56-1.52 (t, 3H, -OCHC$\underline{H}_3$).

TMPM: To obtain 2-(4-methyl-2-oxo-1,3,2-dioxaphospholane-2-yloxy) ethylmethacrylate (MOPEM), MCODP (10.0 g, 6.39 X 10^{-2} mol) in benzene (70 ml) was added dropwise to a mixture of HEMA (8.31 g, 6.39 X 10^{-2} mol) and triethylamine (TEA) (6.46 g, 6.39 X 10^{-2} mol) in benzene (130 ml) with stirring under the nitrogen atmosphere at 0°C for 2 h. After the reaction mixture gradually rose to room temp. then it was kept with stirring at room temp. for 5 h. Triethylamine hydrochloride deposited with the reaction was filtered off from the reaction mixture. The residue was evaporated and then used in the next reaction without purification. Acetonitrile (70 ml) was cooled to −40°C and bubbled dried TMA (22.3 g, 0.38 mol) to dissolve. The acetonitrile solution was placed in a 250 ml-glass pressure-resistance-reaction bottle with prepared MOPEM and a magnetic stirrer. The bottle was immediately sealed and stirred at 60°C for 36 h. The bottle was raised to room temp. and transferred to the freezing chamber. The precipitate formed was filtered off quickly and purified by silica gel column chromatography (chloroform / methanol (MeOH) / H_2O = 65 / 25 / 4 (vol/vol/vol)), and freeze drying for one day to give white crystalline TMPM after distilled the solvent under reduced pressure. Since the deliquescence of TMPM is very high, the melting point has not been decided. (Yield 8.1% (1.6 g, 5.17 X 10^{-3} mol)). ^{1}H NMR (δ in ppm from TMS in $CDCl_3$): 6.09 (s, 1H, C$\underline{H}$=, *cis*), 5.56 (s, 1H, C$\underline{H}$=, *trans*), 4.90-4.91 (m, 1H, -POC$\underline{H}$-), 4.30-4.32 (d, 2H, -COOC$\underline{H}_2$-), 4.02-4.13 (m, 2H, -C$\underline{H}_2$OP-), 3.65 (bs, 2H, -C$\underline{H}_2$N-), 3.49 (s, 9H, -N(C$\underline{H}_3$)$_3$), 1.92 (s, 3H, =CC$\underline{H}_3$) 1.29-1.31 (d, 3H, -POCHC$\underline{H}_3$); ^{13}C NMR (δ in ppm from TMS in $CDCl_3$): 167.27 (-$\underline{C}$=O), 136.15 (=$\underline{C}$CH$_3$), 125.78 ($\underline{C}$H$_2$=C-), 70.74 (-PO$\underline{C}$HCH$_2$N-), 66.32 (-COO$\underline{C}$H$_2$-), 64.38 (-$\underline{C}$H$_2$OP-), 63.03 (-$\underline{C}$H$_2$N-), 54.37 (-N($\underline{C}$H$_3$)$_3$), 20.00 (-POCH$\underline{C}$H$_3$), 18.25 (=C$\underline{C}$H$_3$); IR (KBr disk, cm^{-1}): 1720 (C=O), 1630 (C=C), 1300 (P=O), 1240 and 1080 (PO-O$^-$). $C_{12}H_{24}NO_6P$: Calc. H 7.82, C 46.6, N 4.53. Found H 7.51, C 46.9, N 4.42.

Radical solution polymerization

Homopolymerization of TMPM: Radical solution homopolymerizations of TMPM were carried out in a polymerization tube with an initiator at 60°C and 70°C for 24 h. To obtain polymerized precipitate, in the case of water used as a solvent, the polymerized solution was poured into acetone (100 ml), in the case of MeOH used as a solvent, it was poured into chloroform (100 ml). The obtained polymer was purified by reprecipitation from the MeOH solution to excess chloroform. The purified polymer was lyophilized *in vacuo* for 2 days.

Copolymerization of TMPM with BMA: Radical solution copolymerizations of TMPM (M_1) with BMA (M_2) were performed with AIBN (5×10^{-3} mol) as an initiator in THF / MeOH = 1 / 1 (ml/ml) at 60°C for 2 h in a polymerization tube. The polymerized solution was poured into excess *n*-hexane to precipitate the polymer. The obtained polymer was purified by reprecipitation from THF / MeOH = 1 / 1 (ml/ml) solution to a large excess of water. The purified copolymer was lyophilized *in vacuo* for 1 day. The composition of the copolymer obtained was calculated by ^{1}H NMR spectra.

Determination of critical micelle concentration (CMC)

Several concentrations ($1.0 \times 10^{-6} \sim 1.0 \times 10^{-1}$ mol/l) of TMPM solutions 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 ($1.0 \times 1.0 \times 4.0$ cm^3) and excitation wavelength at 342 nm at a scanning rate at 50 nm/min. CMC was determined from variation in (I_3/I_1) of the first signal (I_1) at 372-373 nm and the third signal (I_3) at 383-385 nm, according to the method by Thomas [11].

Measurement

Chemical structures of the monomer and polymers were determined by the ^{1}H and ^{13}C NMR spectra with a JEOL EX-270 apparatus (JEOL Co., Ltd.) using $CDCl_3$ and D_2O as solvents. FT-IR spectra were obtained using a Shimadzu FT-IR-8100A spectrophotometer. The composition of the monomer was determined by the elemental analysis using Yanagimoto CHN-Corder.

Results and discussion

Synthesis of TMPM

TMPM was synthesized according to Scheme 2, and identified from the ^{1}H, ^{13}C NMR, FT-IR spectra and elemental analysis, as described in experimental section. A racemic propylene glycol was used, consequently the obtained TMPM was also a racemic compound. Purified TMPM used as a white powder showed remarkable deliquescence as well as MPC. In the preparation of TMPM dry atmosphere and dry solvent were always needed. Because MCDP and MCODP were five-membered phosphorus compounds and very unstable and easy to decompose. It was possible to purify by recrystallization in acetonitrile in case of MPC. But it was difficult to purify by recrystallization in acetonitrile or other organic solvents in case of TMPM. It is suggested that TMPM became low crystalline comparison of MPC by changed balance of between hydrophobic and hydrophilic group introducing a methyl group onto a phosphorylcholine group.

Radical solution homopolymerization of TMPM

Radical solution homopolymerizations of TMPM were carried out in water at 70°C for 24 h or in MeOH at 60°C for 24 h. The results are summarized in table 1.

Table 1. Radical homopolymerization of TMPM

Run	TMPM $\times 10^3$ (mol)	Solvent (ml)	Initiator[a] $\times 10^5$ (mol)	Polym. temp. (°C)	Polym. time (h)	Yield (%)
1-1	0.970	MeOH (1.0)	AIBN(4.81)	60	24	trace
1-2	0.971	MeOH (1.0)	AIBN(4.85)	60	24	trace
1-3	1.62	MeOH (2.0)	AIBN(1.02)	60	24	13.3
1-4	0.971	H_2O(1.0)	KPS(4.86)	70	24	16.7
1-5	1.36	H_2O(2.0)	KPS(1.01)	70	24	22.5

[a] AIBN: 2,2'-azobis(isobutyronitrile); KPS: potassium persulfate

Polymerization proceeded homogeneously throughout. ^{1}H NMR spectrum of the TMPM homopolymer (run 1-3) obtained from reprecipitation is shown in Figure 1. It seems that the ordinary radical addition polymerization took place because signals assigned to vinyl protons disappeared in the ^{1}H NMR spectrum. Yield decreased in comparison to MPC and TPM [9]. The filtrate after reprecipitation contained a lot of the TMPM monomer and a slight amount of oligomers. The reason may result from the fact that TMPM has a methyl group at the 1' position of the phosphorylcholine analogous unit, while each phosphorylcholine analogous unit of TPM and phosphorylcholine unit of MPC has a straight chain structure. The obtained polymers were purified by the reprecipitation and then the polymer was lyophilized *in vacuo* for 2 days. The lyophilized polymer had soluble and insoluble parts in water and MeOH. This suggests that ion stabilization between trimethylammonium and phosphate unit was inhibited by introduction of a methyl group and became insoluble in organic solvents and water since similar ternary network structure is formed by the electrostatic interaction between polymer chains.

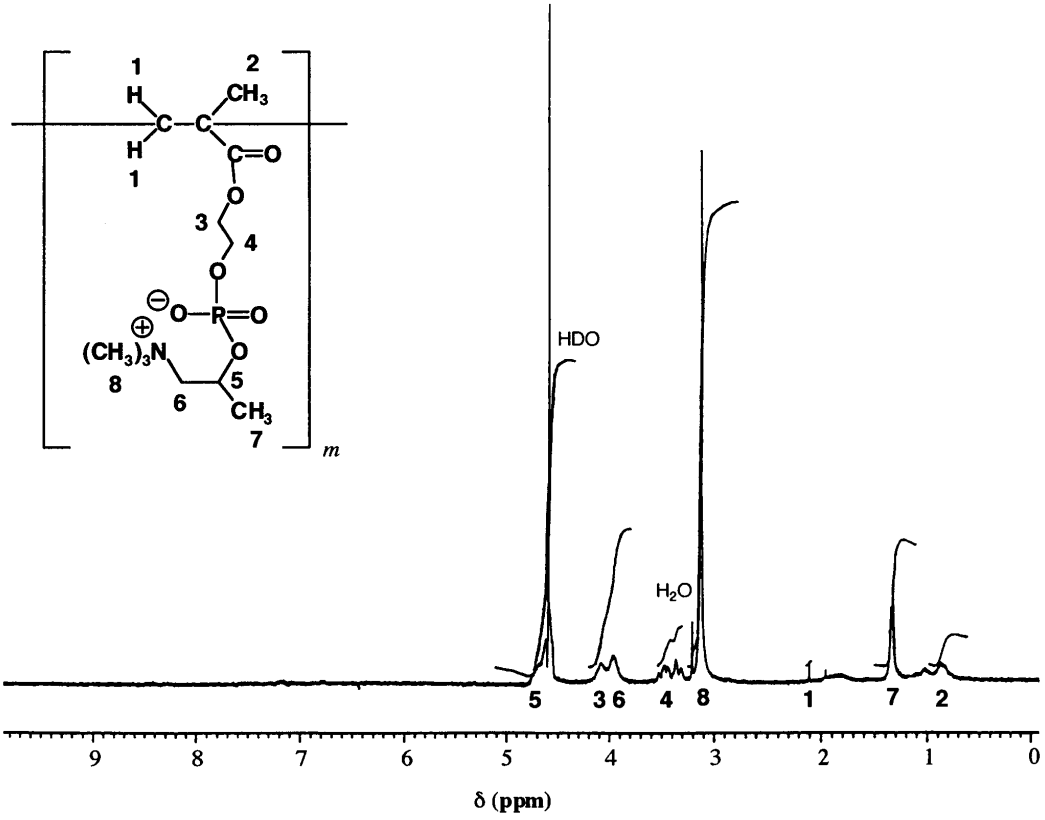


Figure 1. ^{1}H NMR spectrum for poly(TMPM) in D_2O

Radical solution copolymerization of TMPM with BMA

TMPM was radically copolymerized with BMA in the mixture of MeOH and THF at 60°C for 2 h. TMPM of the concentration of 10, 20, 30, 50 and 70 (mol%) were used in monomer feed. The results are summarized in table 2.

Table 2. Radical copolymerizations of TMPM (M_1) with BMA (M_2) in a mixture of THF and MeOH[a] at 60°C[b]

Run	M_1 in monomer (mol%)	Polym. time (h)	M_1 in copolymer[c] (mol%)	Yield (%)
2-1	9.4	2.0	18.0	20.3
2-2	18.6	2.0	13.4	36.5
2-3	28.9	2.0	-[d]	46.2
2-4	48.8	2.0	-[d]	55.2
2-5	67.9	2.0	-[d]	84.3

[a] THF/ MeOH = 1.0 ml/1.0 ml; [b] [AIBN] = 5.0 X 10^{-3} mol/l, $M_1 + M_2 = 0.5$g; [c] Calculated by ^{1}H NMR; [d] No measurement

All copolymerizations proceeded homogeneously throughout. The copolymer composition was calculated from ^{1}H NMR spectra and elemental analysis. ^{1}H NMR spectrum of the obtained copolymer poly(TMPM-*co*-BMA) (run 2-2) is shown in Figure 2. Polymer yield increased with the TMPM content in the copolymer in all the copolymerization systems. The copolymerizations of BMA with TMPM easily proceeded. The copolymers obtained from over 30 mol% of the TMPM monomer feeds (runs 2-3 ~ 2-5) were insoluble in organic solvents and water after the purification by reprecipitation. It seems that ion stabilization between trimethylammonium group and phosphate unit was inhibited by introduction of a methyl group. That is, the copolymer became insoluble in organic solvents and water, since similar ternary network structure was taken by the electrostatic interaction between polymer chains.

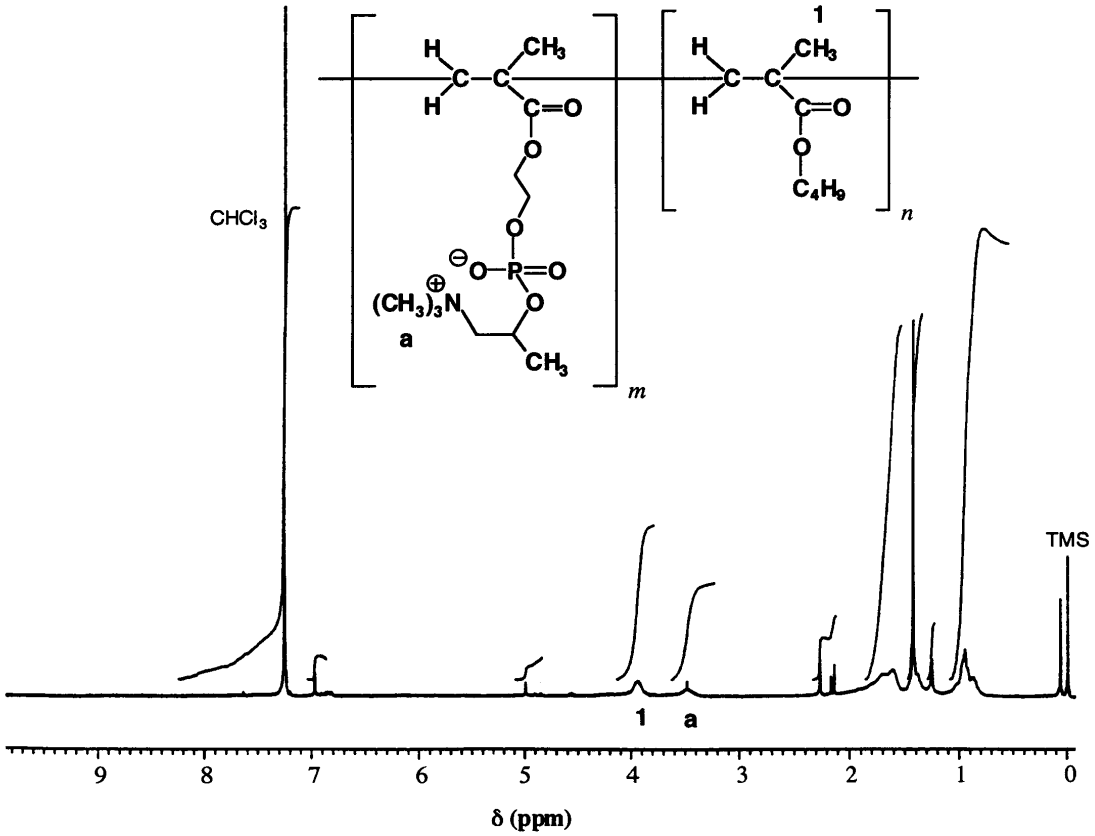


Figure 2. ^{1}H NMR spectrum for poly(TMPM-*co*-BMA) in $CDCl_3$

Molecular aggregation of TMPM in water

Molecular aggregation of TMPM was investigated according to the fluorescence probe of Thomas et al. [11,12]. Pyrene using as a fluorescence probe in the measurement of CMC depicts high hydrophobic

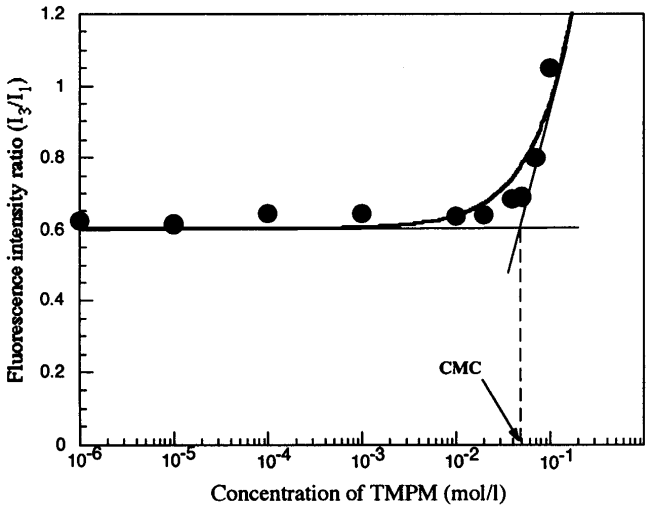


Figure 3. Fluorescence intensity ratio vs. TMPM concentration in distilled water

nature and its solubility in water is very low. Fluorescence spectrum of pyrene was measured in distilled water at excitation wavelength of 342 nm. Fluorescence spectra of pyrene in various concentrations of TMPM aqueous solution were measured and fluorescence intensity (I_3/I_1) were calculated. Relationship between fluorescence intensity ratio (I_3/I_1) and the TMPM concentration in distilled water shows Figure 3. Signal intensity (I_3/I_1) remarkably changed at 4.9×10^{-2} mol/l in water, which indicating that the CMC of TMPM was 4.9×10^{-2} mol/l. That is, TMPM gathered in the aqueous solution over this concentration and then formed micelles. The CMC values of MPC and TPM were measured in our laboratory as 1.3×10^{-3} mol/l and 3.6×10^{-3} mol/l, respectively [9].

The hydrophilic nature of TMPM became lower than those of both MPC and TPM by introduction of a hydrophobic methyl group to a hydrophilic phosphorylcholine group, therefore CMC of TMPM in water may become higher.

Biocompatibility of the copolymers obtained from TMPM is examined at present.

Conclusions

1. A new type of methacrylate bearing a phosphorylcholine analogous moiety, 2-[2'-(trimethylammonium)-1'-methylethyl-phosphoryl]ethyl methacrylate (TMPM) was synthesized.
2. TMPM was radically homopolymerized and copolymerized with BMA to obtain the polymers.
3. The critical micelle concentration (CMC) of TMPM was 4.9×10^{-2} mol/l according to the fluorescent probe method.

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