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SYNTHESIS AND CHARACTERIZATIONS OF COPOLYMERS CONTAINING BOTH PHOSPHATIDYLCHOLINE ANALOGUES AND POLY(ETHYLENE GLYCOL) IN THE SIDE CHAINS

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ABSTRACT

New phospholipid analogous polymers were prepared by radical copolymerizations of 2-(methacryloyloxy)ethyl-2-(trimethylammonio)-ethyl phosphate and poly(ethylene glycol)monomethylether methacrylates (PEGMM) at room temperature, using $(NH_4)_2S_2O_8$ as the initiator and pure water as the solvent. The copolymers obtained were characterized based on their IR, ^{1}H-, and ^{13}C-NMR spectral data and melting point measurements. The molecular weights of these copolymers decrease as the length of the PEGMM side chain increases. These new polymers, which contain phosphatidylcholine analogous groups in their side chains, show viscosity properties similar to typical polyelectrolytes.

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INTRODUCTION

Phospholipids are found in high concentration in various cellular membranes where they perform many different functions such as serving as structural components of membranes and performing some physiological actions [1–5]. Phospholipids are polar, ionic lipids composed of 1,2-diacylglycerol and a phosphodiester bridge that links the glycerol backbone to some base. The most abundant phospholipids in human tissues are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. The behaviors of synthetic monomeric and polymeric phospholipid analogues seem attractive [6–13]. Recently, considerable attention has been paid to polymers modified by phosphatidylcholine moieties or analogues (which exist on the surface of the phospholipid bilayer) concerning their biocompatibility and other properties [14–16].

On the other hand, the fusion of biological membranes is a widely applicable technique in the fields of biology, immunology, cell engineering, etc. For almost twenty years much effort has been made to induce membrane fusion by chemicals instead of viruses [17–21]. Polyethylene glycol (PEG), a water-soluble synthetic polymer, is generally used as a cell fusion agent because 1) it can be utilized not only for animal cells but also for plant protoplasts, 2) the procedure is very simple, and 3) PEG is relatively stable and safe compared with viruses. Therefore, it would be very interesting to synthesize new types of poly(ethylene glycol)s modified by a phosphatidylcholine moiety.

Since 2-(methacryloyloxy)ethyl-2-(trimethylammonio)ethyl phosphate (MTP) was first synthesized by our research group in 1982 [22], a great amount of interest has been shown in its properties and applications [22–27]. In the present work we have prepared some water-soluble copolymers containing both MTP groups and poly(ethylene glycol) parts in the side chains by radical polymerization in water using $(NH_4)_2S_2O_8$ as the initiator. The structures of these copolymers were investigated through their IR and NMR spectral data and their elemental analysis. The viscosity behaviors of these copolymers are discussed.

EXPERIMENTAL

Materials

Acetonitrile was distilled over phosphorus pentoxide. Tetrahydrofuran (THF) was distilled from lithium aluminium hydride. Anhydrous methanol was obtained by distillation in the presence of magnesium and iodine. Acetone was dried by distillation from anhydrous potassium carbonate. Poly(ethylene glycol)monomethylether methacrylates (PEGMM) were obtained from Toure Co., Japan. All other solvents and chemicals were extra pure grade reagents and were used without further purification.

The synthesis and characterization of 2-(methacryloyloxy)ethyl-2-(trimethylammonio)ethyl phosphate (MTP) has been described in detail elsewhere [22].

Polymerization Procedure

The copolymerizations of monomers of PEGMM with monomer MTP were carried out in sealed glass tubes as noted below. The monomers and ammonium peroxodisulfate $(NH_4)_2S_2O_8$ were dissolved in a sealable glass tube at a certain

concentration (see Table 1) in distilled water replaced by nitrogen for 1 hour prior to use. Then known amounts of sodium hydrogen sulfite ($NaHSO_3$) as a promoter was rapidly added to the same tube. Before the tube was sealed, it was degassed repeatedly by the freeze-thaw technique. These solutions were kept at room temperature for 1 week. Colorless viscous solutions were obtained after polymerization. These solutions were poured into excess chloroform to give crude polymers as white solids. The crude products were purified by dissolving them in methanol and then reprecipitated twice in dry chloroform. The precipitates obtained were dried in vacuum to give the corresponding copolymers as white solids. The copolymers were confirmed based on their IR and NMR spectral data.

IR of copolymers PEGMM4-MTP, PEGMM9-MTP, and PEGMM28-MTP: 2850-2915 (s, $-(CH_2CH_2O)_n-$, ν_{C-H}), 1720 (vs, $-COO-$, $\nu_{C=O}$), 1480 (s, $-(CH_2CH_2O)_n-$, δ_{C-H}), 1230-1240 (vs, $O^- - P=O$, $\nu_{P=O}$), 1140 (vs, $-(CH_2OCH_2)_n-$, ν_{C-O-C}), 1050-1080 cm^{-1} (vs, $-P-O-C-$, ν_{C-O}).

^1H (400 MHz) and ^{13}C (100.4 MHz) NMR in CD_3OD:

1. The ^1H- and ^{13}C-NMR spectra of copolymer PEGMM4-MTP are shown in Fig. 1.
2. Copolymer PEGMM9-MTP, ^1H NMR: δ 4.31 (bs, $-COOCH_2CH_2OP-$), 4.17 (bs, $-OCH_2CH_2OP-$ and $-COOCH_2CH_2OC-$), 4.06 (bs, $-OCH_2CH_2N^+$), 3.77 (bs, $-COOCH_2CH_2OC-$), 3.68 (s, $-(CH_2CH_2O)_7-$), 3.61 (bs, $-CH_2OCH_3$), 3.38 (s, $-OCH_3$), 3.27 (s, $N^+(CH_3)_3$, 1.90 (bs, $-CH_2-$ in the main chain), 1.05 and 0.90 ppm (bs, $-C-CH_3$). ^{13}C NMR: δ 179.28 and 178.49 ($-COO-$), 71.13 ($-COOCH_2CH_2OC-$), 69.80 ($-(CH_2CH_2O)_7-$), 68.12 ($-CH_2CH_2OCH_3$), 66.05 ($-CH_2N^+$), 65.32 ($-OCH_2CH_2OP-$), 64.75 ($-CH_2OCH_3$), 63.18 ($-OCH_2CH_2OP-$), 59.41 ($-OCH_2CH_2N^+$), 58.18 ($-OCH_3$), 54.00 ($N^+(CH_3)_3$), 44.77 (>C< in the main chain), 18.15 ($-CH_2-$ in the main chain), 16.91 ppm ($-C-CH_3$).
3. Copolymer PEGMM28-MTP, ^1H NMR: δ 4.33 (bs, $-COOCH_2CH_2OP-$), 4.20 (bs, $-COOCH_2CH_2OP-$ and $-COOCH_2CH_2OC-$), 4.11 (bs, $-POCH_2CH_2N^+$), 3.71-3.76 (s, $-(CH_2CH_2O)_{26}-$ and $-COOCH_2CH_2OC-$), 3.63 (bs, $-CH_2OCH_3$), 3.39 (s, $-OCH_3$), 3.28 ppm (s, $N^+(CH_3)_3$). ^{13}C NMR: δ 179.13 and 178.32 ($-COO-$), 70.98 ($-COOCH_2CH_2OC-$), 69.57 ($-(CH_2CH_2O)_7-$), 68.10 ($-CH_2CH_2OCH_3$), 65.98 ($-CH_2N^+$), 64.83 ($-OCH_2CH_2OP-$), 64.71 ($-CH_2OCH_3$), 63.23 ($-OCH_2CH_2OP-$), 59.38 ($-OCH_2-$

TABLE 1. Copolymerization Conditions[a]

Copolymer	Monomer weights (g) of PEGMM, MTP	H_2O, mL	$(NH_4)_2S_2O_8$, mg[b]	$NaHSO_3$, mg
PEGMM4-MTP	0.24, 0.25	5.0	2.50	1.25
PEGMM9-MTP	0.42, 0.25	7.0	3.35	1.68
PEGMM28-MTP	1.13, 0.25	14.0	6.90	3.45

[a]The copolymerizations were carried out at 25°C for 1 week.
[b]The initiator was used as 0.5 wt% of the monomers.

CH_2N^+), 58.05 ($-OCH_3$), 54.03 ($N^+(CH_3)_3$), 45.06 (>C< in the main chain), 18.16 ($-CH_2-$ in the main chain), 17.01 ppm ($-C-CH_3$).

The elemental analyses of the copolymers obtained were as follows: PEGMM4-MTP: C, 50.22; H, 8.12; N, 2.51. PEGMM9-MTP: C, 51.11; H, 8.30; N, 2.03. PEGMM28-MTP: C, 52.43; H, 8.70; N, 1.12.

Characterizations

Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a JEOL α-400 FT-NMR spectrometer at 400 and 100.40 MHz, respectively. Proton and carbon chemical shifts, reported in parts per million, were referenced to tetramethylsilane (TMS) directly as the internal standard, and deuteromethanol was used as the solvent. Infrared (IR) spectra were obtained with a Jasco A 202 spectrometer using potassium bromide pellets and were reported in wavenumbers (cm^{-1}). The melting points of the polymers were measured by a Micro Melting Point Apparatus (Yanaco MP-J3). The viscosity determinations were performed by diluting the same sample with water and using an Ubbelohde-type viscometer at 25 ± 0.01°C. For different concentrations, reduced specific viscosities (η_{sp}/C) were established and extrapolated to zero concentration.

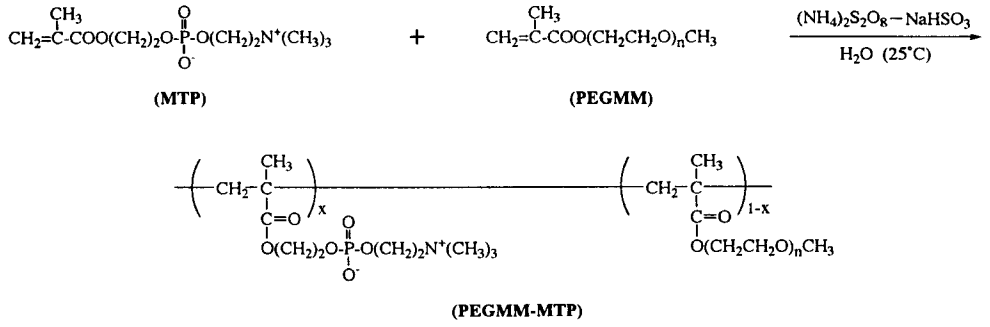
RESULTS AND DISCUSSION

Phospholipid analogous methacrylate monomer, 2-(methacryloyloxy)ethyl-2-(trimethylammonio)ethyl phosphate (MTP), was synthesized and characterized following the method described previously [22]. In brief, 2-chloro-2-oxo-1,3,2-dioxaphospholane was reacted with 2-hydroxyethyl methacrylate to give a colorless viscous liquid. Then this product was ring opened by trimethylamine, and monomer MTP as a hygroscopic white solid was obtained in good yield.

The copolymerizations of monomer MTP with monomers PEGMM4, PEGMM9, or PEGMM28 were carried out in pure water at room temperature by using $(NH_4)_2S_2O_8$ as an initiator and aging for a week. The resulting polymers were purified by reprecipitation in dry chloroform two times. Both the monomers MTP and PEGMM are soluble in chloroform, but their copolymers are almost insoluble. Then pure copolymers PEGMM4-MTP, PEGMM9-MTP, and PEGMM28-MTP as hygroscopic white solids were obtained. The polymerization procedures are shown in Scheme 1.

The melting points and yields of all copolymers are listed in Table 2, while the solubilities in various solvents are summarized in Table 3. From these tables we can see that:

1. The copolymerizations for all polymers were carried out under similar concentrations of polymeric monomers and initiator, but the copolymers PEGMM4-MTP and PEGMM9-MTP were obtained with comparable yields, and their yields are higher than that of PEGMM28-MTP. It was revealed that the monomers PEGMM4 and PEGMM9 are more reactive than monomer PEGMM28 in polymerization processes.



n	PEGMM	Copolymers	x
4	PEGMM4	PEGMM4-MTP	0.517
9	PEGMM9	PEGMM9-MTP	0.549
28	PEGMM28	PEGMM28-MTP	0.594

SCHEME 1. The copolymerizations of MTP with PEGMM4, PEGMM9, and PEGMM28.

TABLE 2. Properties of Synthesized Copolymers

Copolymer	State	Melting point, °C	Yield, %
PEGMM4-MTP	White solid	>250	79.3
PEGMM9-MTP	White solid	>250	78.0
PEGMM28-MTP	White solid	125	70.4

TABLE 3. Solubilities of Synthesized Copolymers in Various Solvents[a]

	Solvent					
Copolymer	Water	Methanol	Acetone	Benzene	Chloroform	THF
PEGMM4-MTP	++	++	−	−	−	−
PEGMM9-MTP	++	++	−	−	+−	−
PEGMM28-MTP	++	++	+	−	+−	−

[a](++)Soluble at room temperature, (+) soluble on heating, (+−) swelling, (−) insoluble.

2. No melting points were observed for copolymers PEGMM4-MTP and PEGMM9-MTP until they were heated up to 250°C, while PEGMM28-MTP was observed to melt at about 125°C. It may be considered that molecular weights of the two former copolymers are relatively large because they contain the short ethylene glycol group in their side chains.
3. All copolymers are very soluble in polar solvents such as water and methanol, but insoluble in unpolar solvents like benzene and THF. Copolymers PEGMM4-MTP and PEGMM9-MTP are insoluble in acetone, but PEGMM28-MTP is soluble in acetone because its poly(ethylene glycol) side chain (soluble in acetone) is larger than the phosphatidylcholine analogous side chain (insoluble in acetone).

We further confirmed the formation of these copolymers by their IR and NMR spectral data. The IR spectra of these copolymers show two strong absorption bands around 1230 and 1080 cm^{-1}, which can be attributed to the existence of the phosphatidylcholine moiety. Moreover, a peak at 1140 cm^{-1} due to the stretching vibration of the $-CH_2OCH_2-$ group of PEGMM is also observed. These spectral data show that copolymerizations of MTP and PEGMM were indeed achieved.

In the NMR spectra of all copolymers, the peaks of the C=C double bond disappeared. As an example, the ^1H- and ^{13}C-NMR spectra of PEGMM4-MTP are shown in Fig. 1. From ^1H NMR (Fig. 1(A) it can be seen that:

1. The characteristic peak of $-OCH_3$ at the end of PEGMM4 appears at δ 3.39 ppm, and the peaks of $-(OCH_2CH_2)_4-$ are displayed at δ 3.65 ppm, corresponding to the existing of PEGMM group.
2. The characteristic peak of the trimethylammonio group can be observed at δ 3.27 ppm, which results from the introduction of the MTP group.

On the basis of ^1H-NMR spectral data, it is proved that the proposed copolymer PEGMM4-MTP was obtained with high purity. Figure 1(B) shows the ^{13}C-NMR spectrum of copolymer PEGMM4-MTP. The components of these copolymers were calculated from the integrated areas of their characteristic peaks in their ^1H NMR. The calculated results agree with results based on elemental analyses data.

In previous work we found that vinyl polymers having phosphatidylcholine groups or analogues in their side chains or in their main chains show the properties of polyelectrolytes by their viscosity behavior in aqueous solution [15, 28, 29]. This is because the PO_4^- group dissociates as a weak acid and the $N^+(CH_3)_3$ group dissociates as a strong base [27]. Therefore, in this study the viscosity measurements of these copolymers were performed at 25°C in the presence or absence of sodium nitrate using pure water as the solvent. Figures 2–4 show plots of reduced viscosity (η_{sp}/C) vs concentration (C) for PEGMM4-MTP, PEGMM9-MTP, and PEGMM28-MTP, respectively. In the presence of sodium nitrate (1.0 N aqueous solution), the η_{sp}/C values of all copolymers are found to decrease linearly with a decrease of polymer concentration, and their inherent viscosities [η] are found to be 0.342, 0.355, and 0.106 dL/g for PEGMM4-MTP, PEGMM9-MTP, and PEGMM28-MTP, respectively. These results reveal that the relative molecular weights of the copolymers is PEGMM4-MTP $\approx$ PEGMM9-MTP $\gg$ PEGMM28-MTP. It may be because of different reactivities in polymerizations of PEGMMs which contain different ethylene glycol group lengths.

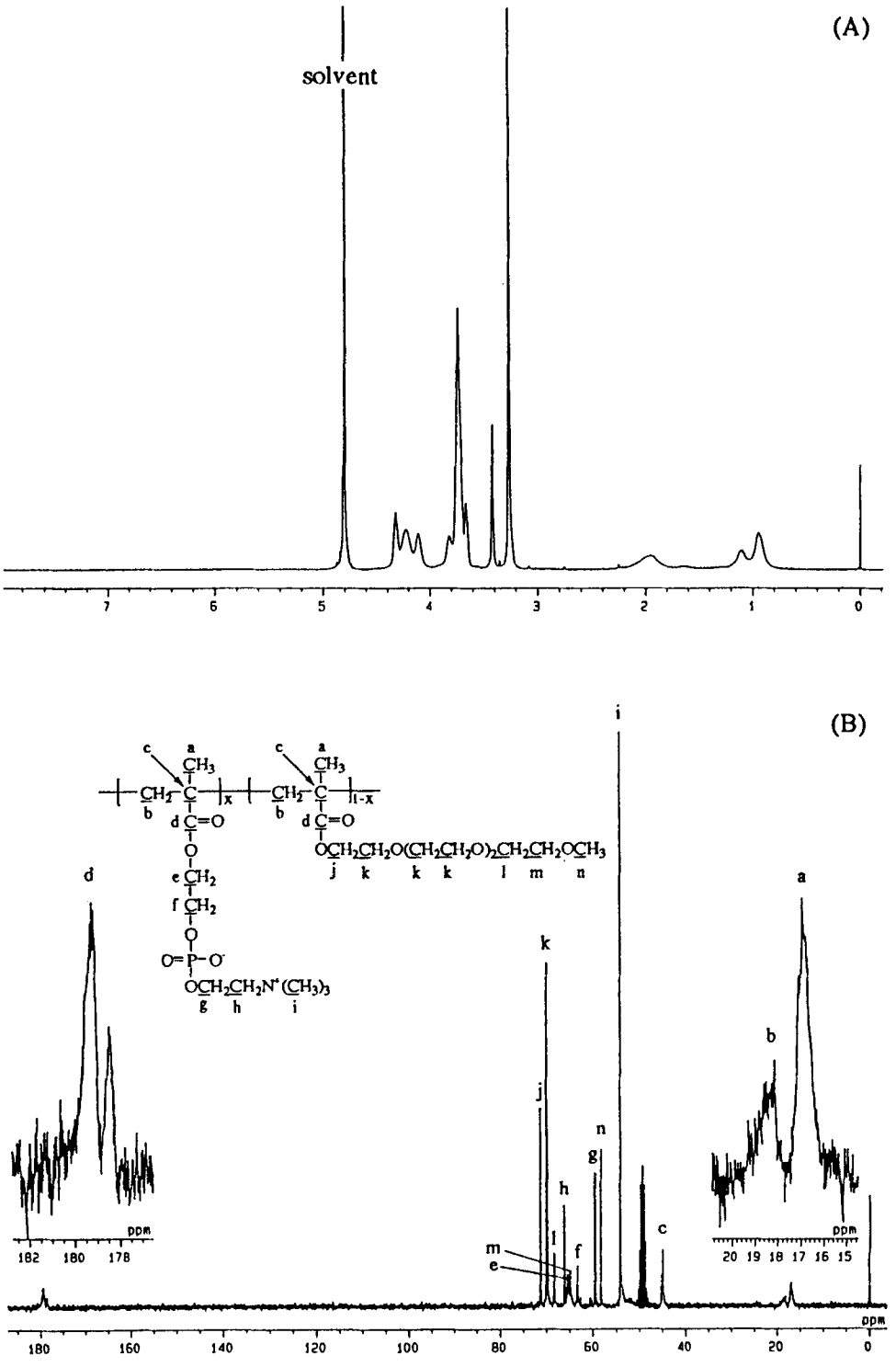


FIG. 1. ^{1}H (A) and ^{13}C (B) NMR spectra of copolymer PEGMM4–MTP in CD_3OD with TMS as the internal standard.

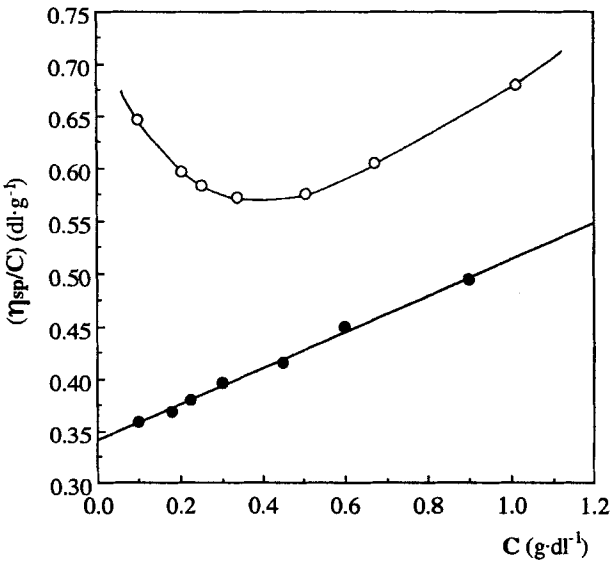


FIG. 2. Reduced viscosity of copolymer PEGMM4-MTP in pure water (○) and in aqueous 1 N $NaNO_3$ (●).

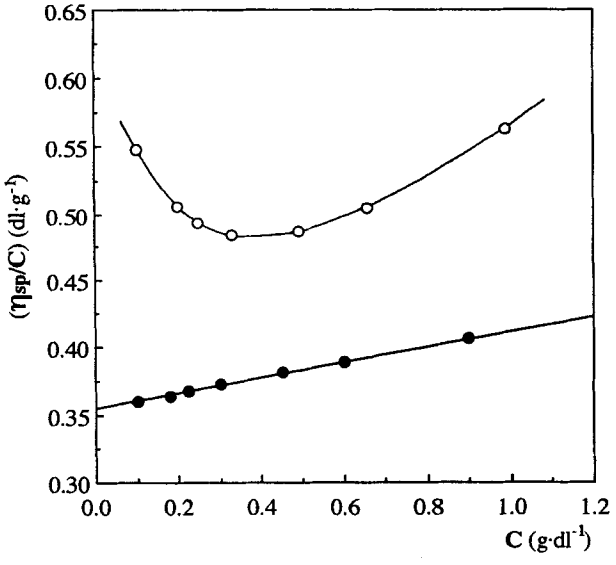


FIG. 3. Reduced viscosity of copolymer PEGMM9-MTP in pure water (○) and in aqueous 1 N $NaNO_3$ (●).

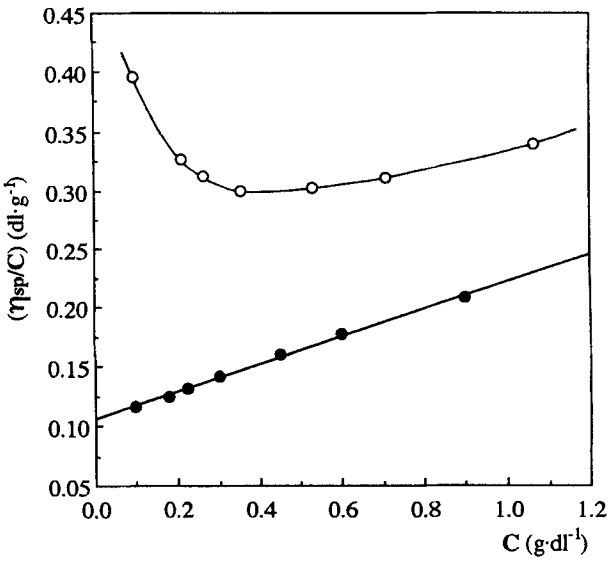


FIG. 4. Reduced viscosity of copolymer PEGMM28-MTP in pure water (○) and in aqueous 1 N $NaNO_3$ (●).

In the cases without $NaNO_3$, the reduced viscosities do not change linearly with copolymer concentrations. These η_{sp}/C were found to decrease with concentration dilution in the 0.35 ~ 1.00 g/dL range, but increase following further dilution ($C < 0.35$ g/dL). The reversal of this phenomenon of η_{sp}/C at higher dilutions resembles the typical behavior of a polyelectrolyte solution. Therefore, based on these results, it is proposed that copolymers containing analogous phosphatidylcholine moieties show properties similar to normal polyelectrolytes. This may result from the mutual repulsion of $N^+(CH_3)_3$ and $N^+(CH_3)_3$, particularly possible chain expansion at low concentrations. A similar observation was reported for a polymerizable zwitterionic betaine surfactant in acidic conditions [30]. Moreover, in our early work the η_{sp}/C of some analogous phospholipid homopolymers containing the phosphatidylcholine moiety or analogue in the side chains [15] or in the main chain [29] was found to increase monotonously with a dilution of concentrations. The reversed variations of η_{sp}/C for these present copolymers may come from the weak electric charge density in the side chains.

CONCLUSION

A series of new phosphatidylcholine analogous poly(methacrylate)s were synthesized by radical copolymerizations of 2-(methacryloyloxy)ethyl-2-(trimethylammonio)ethyl phosphate (MTP) and poly(ethylene glycol)monomethylether methacrylates (PEGMM) at room temperature, using $(NH_4)_2S_2O_8$ as an initiator and pure water as a solvent. The copolymers obtained were characterized based on their IR, NMR spectra, and melting point measurements. The molecular weights of these copolymers decrease as the lengths of the PEGMM side chains increase. Based on the results of viscosity measurements in aqueous $NaNO_3$ or pure water, it was

found that these novel copolymers, which contain both biocompatible poly(ethylene glycol) and phosphatidylcholine analogous groups in their side chains, show the viscosity properties of typical polyelectrolytes. The present copolymers could be used as biomaterials (for example, in cell fusions).

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