Synthesis of Poly(4-methylphenoxyphosphazene)-*graft*-poly(2-methyl-2-oxazoline) Copolymers and Their Micelle Formation in Water

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ABSTRACT: Poly(4-methylphenoxyphosphazene)-*graft*-poly(2-methyl-2-oxazoline) was prepared, and micelle formation of the copolymer in water was studied. Poly(dichlorophosphazene) of a low molecular weight ($<10^4$) was obtained by the thermal polymerization of hexachlorocyclotriphosphazene in the presence of aluminum chloride (10 wt %). The chloro groups were replaced by 4-methylphenoxy groups, and then methyl groups were brominated. The brominated unit content was controlled to be 5% and 20%. The brominated polymers were used as macroinitiators for polymerization of 2-methyl-2-oxazoline to give amphiphilic graft copolymers. Micelle formation of the copolymers in water was investigated by measuring surface tensions and a fluorescence study. The micelles were found to solubilize hydrophobic pyrene in water effectively in the fluorescence study and expected to use as drug carriers.

Introduction

The micelles formed from amphiphilic polymers in an aqueous solution are of much interest due to their potential application as a drug carrier.[2−4] Although considerable amounts of study has been made on the synthesis and structures of micelles from amphiphilic block copolymers,[4−9] only a few graft copolymers have been reported to shape micelles.[10−14] Moreover, most graft copolymers reported for micelle formation have hydrophilic backbones such as polyacrylate or poly(acrylic acid) and hydrophobic grafts such as polystyrene and thus poor solubilities in pure water. This is probably due to synthetic difficulties of amphiphilic graft copolymers with controlled structures.

As main chains, poly(organophosphazene)s are very suitable for the synthesis of graft copolymers because of the flexible synthetic methodology developed for their structural modification.[15−18] Several organic polymer/polyphosphazene graft copolymers have been reported. These polymers have attracted considerable attention due to their unique chemical structures, i.e., inorganic polymer backbones with organic polymer side chains. So far three types of grafting methods have been used, which are radical, anionic, and stepwise grafting reactions. Gleria and co-workers reported the grafting of polystyrene by radical initiation.[19] This method was expected to apply for the grafting of other vinyl polymers. Anionic grafting reaction of polystyrene was also reported by Wisian-Neilson and Schaefer.[20] Allcock and Chang reported the grafting of oligopeptides by stepwise coupling reactions that allowed facile control over amino acid sequences.[21]

For delivery, a hydrophobic drug should be coupled to the polymer covalently or incorporated within a polymeric micelle physically. From this viewpoint, the polyphosphazene system has advantages over the block copolymers. A drug can be attached to the phosphazene backbone relatively easily by usual substitution reactions. More facile incorporation of a hydrophobic drug inside of a micelle is also possible if a side group interacting with the drug is coupled to the phosphazene backbone. In the present work, we prepared amphiphilic graft copolymers by cationic grafting reaction of 2-methyl-2-oxazoline onto hydrophobic poly(4-methylphenoxyphosphazene). Since the polymerization of 2-methyl-2-oxazoline follows living polymerization pathway,[22] the lengths of the grafts could be controlled by varying the monomer feed ratio. The graft copolymers were soluble in water and formed micelles, which solubilized hydrophobic pyrene effectively.

Experimental Section

Materials and Instrumentation. Hexachlorocyclotriphosphazene (Aldrich) was purified by fractional vacuum sublimation at 60 °C in 0.5 mmHg. ^{1}H NMR spectra were recorded on a Varian 200 spectrometer. Gel permeation chromatography (GPC) was carried out with a Waters 150C GPC fitted with a M410 refractive index detector and Waters Styragel columns. Chloroform was used as the eluent. Approximate calibration of the column was accomplished by means of narrow molecular weight polystyrene standards obtained from Waters. The surface tension of the polymers in water was measured by a Fisher tensiometer at 22 °C. For fluorescence measurement, the stock solution of the copolymer in doubly distilled water was prepared. Pyrene (2.4 mg) was dissolved in THF (2 mL). To the solution was added 100 mL of doubly distilled water, and then THF was removed by evaporation under reduced pressure. The concentration of pyrene in each sample solution was made to 6×10^{-7} M. Fluorescence spectra were obtained by a Kontron SFM-25 spectrometer.

Poly(dichlorophosphazene). Poly(dichlorophosphazene) of high molecular weights ($M_w > 10^5$) was prepared by the thermal polymerization of hexachlorocyclotriphosphazene at 250 °C. An average of 30−40% conversion to the linear polymer was obtained. Poly(dichlorophosphazene) of low molecular weights ($M_w < 10^4$) was prepared by the thermal polymerization of hexachlorocyclotriphosphazene at 250 °C in the pres-

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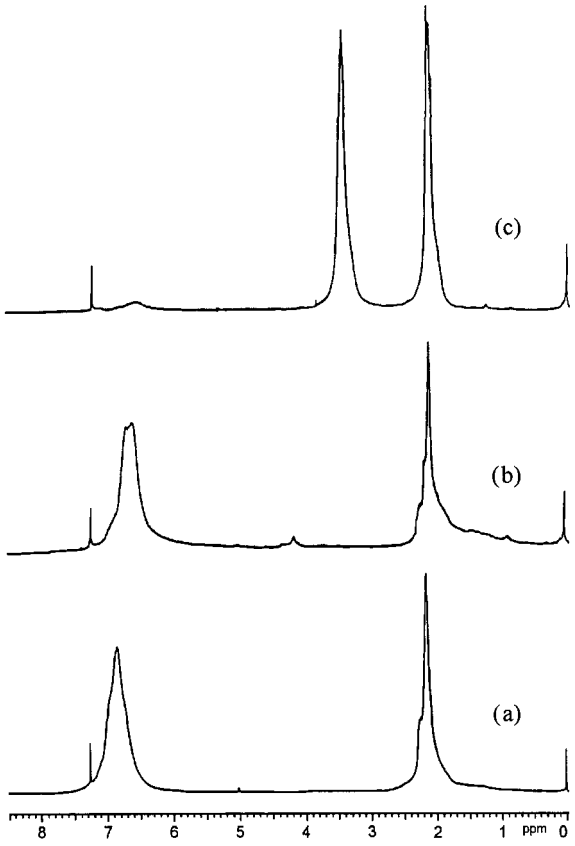


Figure 1. ^{1}H NMR spectra of (a) poly(4-methylphenoxyphosphazene), (b) the 5% brominated polymer, and (c) the 5% grafted copolymer taken in $CDCl_3$.

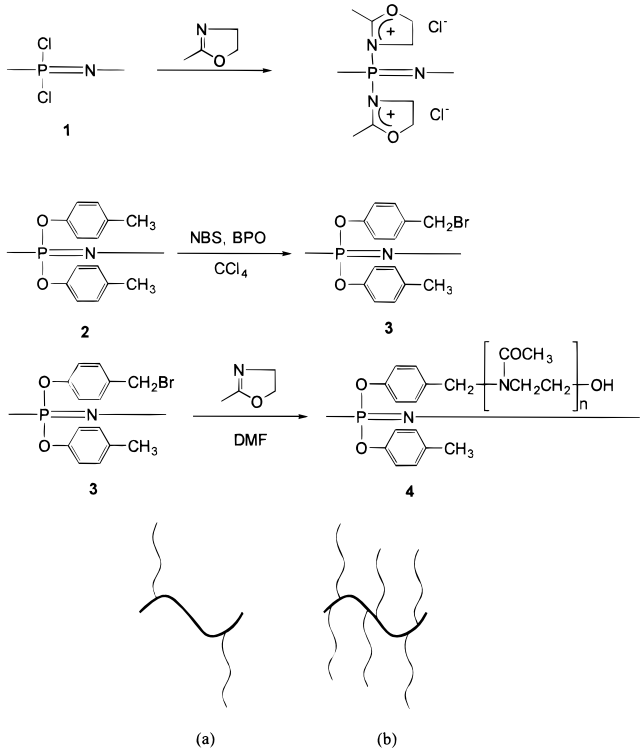
ence of 10 wt % $AlCl_3$ for 2 h. Conversion to the polymer was higher than 80%.

$[NP(OC_6H_4$-*p*-$CH_3)_2]_n$ (2). The procedure in the literature was followed.[23] A mixture of *p*-cresol (10 g, 92.5 mmol) and sodium hydride (2.3 g, 95.8 mmol) in 1,4-dioxane (100 mL) was stirred for 5 h at room temperature. To the solution was added dropwise a solution of poly(dichlorophosphazene) (2.5 g) in 1,4-dioxane (50 mL), and the reaction mixture was stirred at refluxing temperature for 3 days. After concentration to 30 mL under reduced pressure, the polymer was precipitated in hexane (200 mL) and further purified by precipitation from the polymer solution in THF into water. The ^{1}H NMR spectrum of polymer **2** is shown in Figure 1.

$[NP(OC_6H_4$-*p*-$CH_3)_x(OC_6H_4$-*p*-$CH_2Br)_y]_n$ (3). The procedure in the literature was followed.[23] To a solution of polymer **2** (2 g) in carbon tetrachloride (100 mL) was added *N*-bromosuccinimide (3 g, 16.9 mmol) and benzoyl peroxide (0.2 g, 0.8 mmol). The solution was shielded from light and stirred at refluxing temperature until bromination reaction occurred to 5−20% of methyl groups. After concentration to 30 mL under reduced pressure, the polymer was precipitated in hexane (200 mL) and further purified by precipitation from the polymer solution in THF into water. The ^{1}H NMR spectrum of polymer **3** is shown in Figure 1.

Graft Copolymerization of 2-Methyl-2-oxazoline onto Polymer 3. A typical experiment was as follows. Polymer **3** (0.2 g) was dissolved in DMF (10 mL), and the appropriate amount of 2-methyl-2-oxazoline was added to the solution under nitrogen. The polymerization was carried out with stirring at 80 °C. After 10 h, the reaction mixture was added dropwise to hexane/methanol (100 mL, 5:1), and the precipitated polymer was collected by filtration. The polymer was further purified by precipitation twice from the polymer solution in DMF into diethyl ether and dialysis against water with a cellulose membrane (M_w cutoff 1000). The ^{1}H NMR spectrum of polymer **4** is shown in Figure 1.

Scheme 1



Results and Discussion

Synthesis of Polymers. Hexachlorocyclotriphosphazene was polymerized thermally in the usual way at 250 °C to give the polymers with molecular weights of higher than 10^5, when measured after the substitution reaction with sodium trifluoroethoxide. It is well-known that phosphorus on poly(dichlorophosphazene) is sufficiently electrophilic to react with various nucleophiles such as alkoxides and amines. Since 2-methyl-2-oxazoline can be polymerized by initiation with an electrophile, poly(dichlorophosphazene) itself was employed in initiating the polymerization of 2-methyl-2-oxazoline in DMF. At the beginning of the reaction, however, all the polymer chains precipitated probably because of the formation of insoluble salts (Scheme 1).

Benzyl bromide compounds are good initiators for ring-opening polymerization of cyclic iminoethers.[22,24,25] Poly(dichlorophosphazene) was reacted with sodium 4-methylphenoxide in 1,4-dioxane to give polymer **2**. The bromination reaction was performed with *N*-bromosuccinimide and benzoyl peroxide in CCl_4.[23] Although radical species were involved in the reaction, no cross-linking was observed below 30% bromination.

The polymerization of 2-methyl-2-oxazoline by initiation with the 20% brominated polymer was carried out in DMF at 80 °C. In an hour, however, the polymer became gellike and insoluble either in polar solvents or in nonpolar solvents. It was unlikely that covalent cross-linking occurred. Presumably, the coexistence of hydrophobic groups and hydrophilic groups on the polymer chain made it insoluble in both nonpolar and polar solvents by physical cross-linking. Physical gelation by self-association of block copolymers in solutions have been reported.[7,26] We tried to find homogeneous polymerization conditions by varying the reaction temperature, solvent, monomer concentration, and number of grafting points but were unable to obtain the soluble polymers. High molecular weights of the phosphazene

backbone seemed to be in part responsible for this result and such a physical gelation during the grafting reaction was expected to be avoided by using the polymer initiator of a low molecular weight.

Sohn et al. reported poly(dichlorophosphazene) of a low molecular weight by thermal polymerization of hexachlorocyclotriphosphazene in the presence of Lewis acids.[27] They found the molecular weights of the polymers were dependent on the nature of Lewis acids as well as their contents. In this work, we used 10% of $AlCl_3$ by weight as a catalyst. The conversion of hexachlorocyclotriphosphazene to the polymer was higher than 80% in 2 h, and cross-linking was not observed. The mechanism of this polymerization is not clear, and no true initiating species were confirmed. One plausible guess is that aluminum trichloride was self-ionized to be $AlCl_2^+$ and $AlCl_4^-$, which might be involved in initiating or terminating the polymerization. Chloro groups of poly(dichlorophosphazene) were replaced by 4-methylphenoxy groups in a usual manner. The number (M_n) and weight-average molecular weights (M_w) of the resulting polymer measured by GPC (chloroform, polystyrene standards) were 3800 and 6200, respectively. Bromination reaction was performed with *N*-bromosuccinimide and benzoyl peroxide in CCl_4.[23] The reaction was monitored by taking the ^{1}H NMR spectrum of the reaction mixture in CCl_4. The brominated methyl group content was controlled to be 5−20% (Figure 1).

The polymerization of 2-methyl-2-oxazoline by initiation with the 5% brominated polymer proceeded homogeneously in DMF at 80 °C. The polymer was isolated by precipitation in a mixture of hexane and methanol. Ungrafted poly(2-methyl-2-oxazoline), if any, was removed by dialysis against water. The polymerization of 2-methyl-2-oxazoline is known to follow living polymerization pathway.[22] We controlled the length of the side chain and the number of graft points by varying the mole ratio of monomer to the polymer initiator and degree of bromination, respectively. The number-average degree of polymerization (DP) per a branch was calculated from the peak area ratio of phenyl protons at 6.4−7.2 ppm to methylene protons at 3.1−3.8 ppm in the ^{1}H NMR spectrum (Figure 1). The average degree of polymerization was determined by ^{1}H NMR spectroscopy to be 56. The polymerization with the 20% brominated polymer also yielded a soluble polymer. The average degree of polymerization of a branch was 39. Because of the hydrophilicity of the poly(2-methyl-2-oxazoline) side chains, the graft copolymers became soluble in water, and they showed quite different solubilities in organic solvents compared with poly(4-methylphenoxyphosphazene). They were soluble in methanol, chloroform, and DMF but insoluble in tetrahydrofuran, benzene, and acetone, which were good solvents for poly(4-methylphenoxyphosphazene). Figure 2 shows GPC elution curves of polymer **2** and the 5% grafted polymer. After grafting reaction, weight-average molecular weights of the 5% and 20% brominated polymers increased to 11 000 (M_n 6500) and 23 000 (M_n 14000), respectively. These values were lower than the calculated ones based on DPs of side chains due to branched structures of the polymers.

Micelle Formation in Water. Since M_n of polymer **2** was 3800, the average number of grafting points of the 5% brominated polymer was calculated to be 1.5. This means that the graft polymer has a structure similar to that of AB or ABA type block copolymer (a in Scheme 1), where A is a polyoxazoline side chain and B is a polyphosphazene backbone. Figure 3 shows concentration and surface tension relationships of the 5% and 20% grafted copolymers in water. As expected from its structure, the 5% grafted polymer showed a well-defined plot, showing the micelle formation. The critical micelle concentration (cmc) was found to be 0.025 wt %. Above cmc, the polymer solution showed surface tension of 46 dyn/cm.

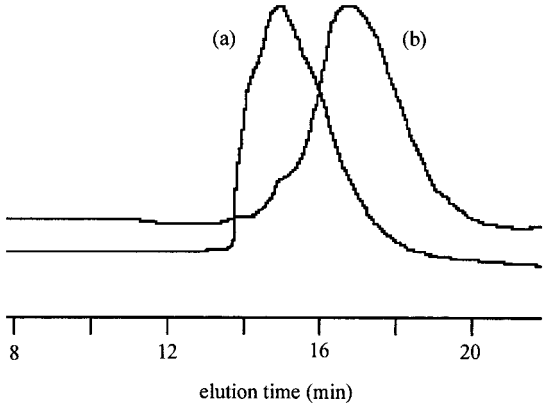


Figure 2. GPC curves of (a) the 5% grafted copolymer and (b) poly(4-methylphenoxyphosphazene).

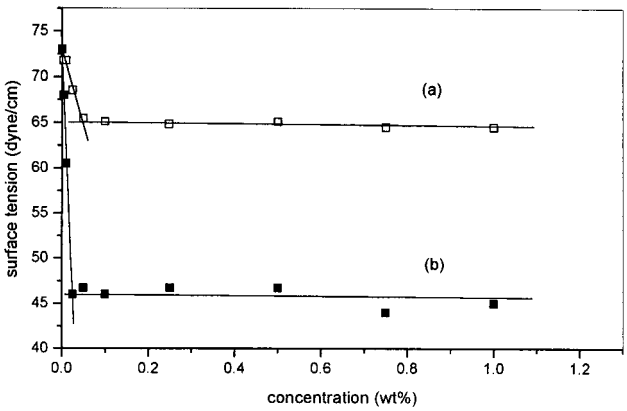


Figure 3. Concentration−surface tension relationships for (a) the 20% grafted copolymer and (b) the 5% grafted copolymer.

In the case of the 20% grafted polymer, each chain has six grafting points on average, having a brushed type structure (b in Scheme 1). The polymer also formed micelles but showed poor surfactant behavior with high surface tension (65 dyn/cm) above cmc (0.05 wt %). Micelle formation was also confirmed by fluorescence spectroscopy. The solutions of the 20% grafted copolymer with different concentrations were prepared, and the fluorescence spectra were obtained after exciting the sample solutions at 342 nm (Figure 4). An increase in the total fluorescence intensity was observed by increasing concentrations of the polymer, notably with sharp increase above the concentration of 0.1 g/L. Apparently this seems to be related to micelle formation of the polymer chains although the origin of strong fluorescence is not fully understood at this point.[28] One possible explanation is that excimer of pendent aromatic rings was easily formed in a hydrophobic core of the micelle.

Pyrene Solubilization. Hydrophobic drugs can be delivered in an aqueous environment by dissolving in micelles. We studied the solubilization of a hydrophobic compound in micelles of polymer **4** by using pyrene. The fluorescence of pyrene is known to be sensitive to

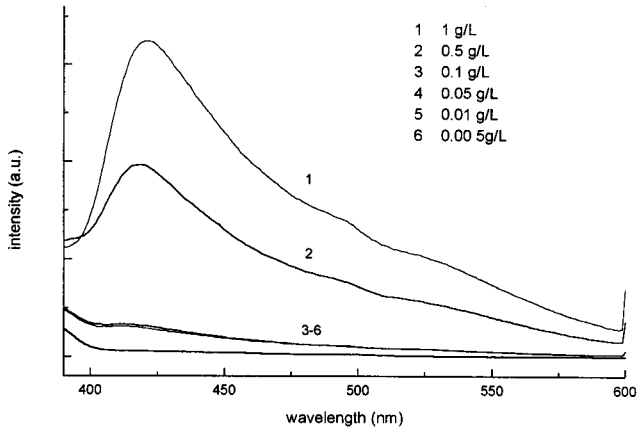


Figure 4. Fluorescence emission spectra (λ_{ex} = 342 nm) of the 20% grafted copolymer in water.

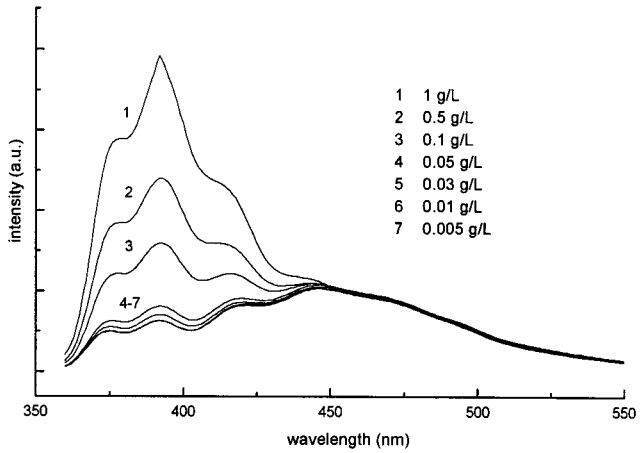


Figure 5. Fluorescence emission spectra (λ_{ex} = 336 nm) of pyrene in water in the presence of the 5% grafted copolymer.

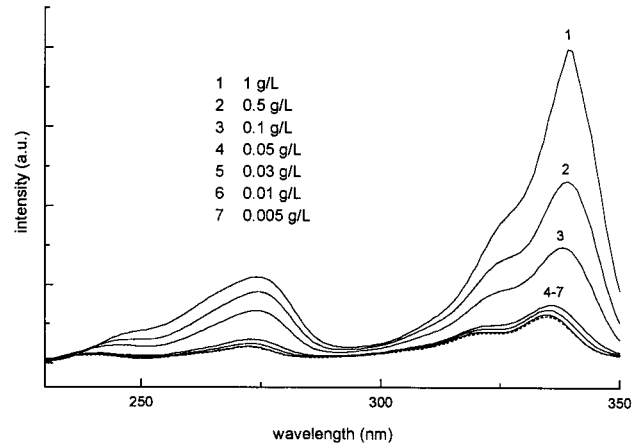


Figure 6. Excitation spectra (λ_{em} = 392 nm) of pyrene in water in the presence of the 5% grafted copolymer.

changes in the microenvironment.[4,9,28,29] Figure 5 shows the fluorescence spectra of pyrene in water in the presence of the 5% grafted copolymer. Pyrene concentration was 6×10^{-7} M. Excitation was carried out at 336 nm. Above the cmc, the fluorescence intensity values increased substantially, indicating pyrene transferring into the hydrophobic micelle domain. In the excitation spectra monitored at the wavelength of 392 nm, the same result was observed (Figure 6). Absorptions near 275 and 339 nm increased significantly as

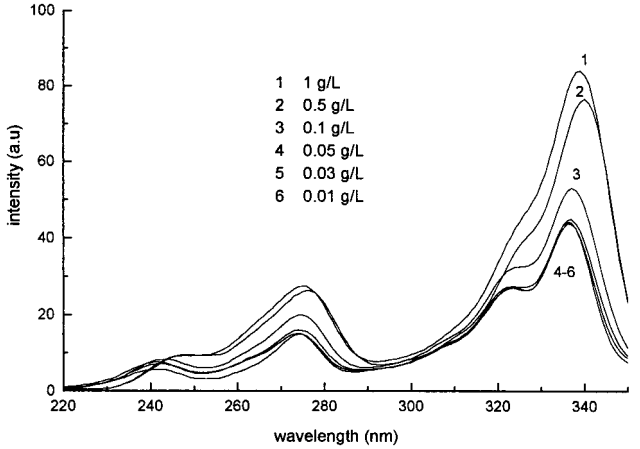


Figure 7. Excitation spectra (λ_{em} = 390 nm) of pyrene in water in the presence of the 20% grafted copolymer.

pyrene was transferred into the less polar micelle domain. A red shift was also observed as the polymer concentration increased. Figure 7 shows the excitation spectra of pyrene in water in the presence of the 20% grafted copolymer. The concentration at which the intensity began to increase considerably was below cmc determined by the surface tension measurement.

In conclusion, we prepared water-soluble graft copolymers with polyphosphazene backbones and polyoxazoline side chains. By using a polyphosphazene as a macroinitiator, the number of grafting points as well as the length of a side chain could be controlled. The graft copolymers formed micelles in water and solubilized pyrene effectively.

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References and Notes

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