Synthesis and Characterization of Thermosensitive Poly(organophosphazene) Gels with an Amino Functional Group

Bae Hoon Lee, Soo-Chang Song

Division of Life Science, Korea Institute of Science and Technology, Seoul 130-650, Korea

Received 27 April 2010; accepted 14 August 2010
DOI 10.1002/app.33181
Published online 8 November 2010 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Thermosensitive poly(organophosphazene) gels have been synthesized with a host of side groups, including α-amino-ω-methoxy-poly(ethylene glycol), hydrophobic amino acid esters (PheOEt, LeuOEt, and IleuOEt), depsipeptide ethyl ester (GlyGlycOEt), and lysine ethyl ester (lysOEt). The fraction of the last side group, lysOEt, which possesses two amine functional groups, was designed to be in the range of 0.1–0.3 mol per polymer unit. The poly(organophosphazenes) have been characterized via ^{1}H- and ^{31}P-NMR spectroscopies, GPC, and elemental analysis. The phase transition behavior of the poly(organophosphazenes) in aqueous solution has been determined via viscometry. Some of the poly(organophosphazenes) with amino functional groups exhibit reversible sol–gel transitions at temperatures near those of the human body, when in aqueous solution. These polymers form a sol at lower temperatures, and become gels at higher temperatures. Also, these polymer solutions have been found to behave generally like Newtonian fluids in the sol state, but appear to exhibit pseudoplastic qualities in the gel state. The polymers possessing depsipeptide ethyl esters (ethyl-2-(*O*-glycyl)glycolate) as a side group tend to exhibit much higher degradation rates under physiological conditions than do those which lack the depsipeptide ethyl ester group. J Appl Polym Sci 120: 998–1005, 2011

Key words: polyphosphazenes; gels; biomaterials; biodegradable; stimuli-sensitive polymers

INTRODUCTION

Thermosensitive biodegradable polymers that exhibit sol–gel properties have been intensively studied for their efficacy as injectable or implantable drug delivery systems.[1–5] Such systems would possess clear advantages over the previous implantable system. There is no need for the use of harmful chemical initiators or crosslinking reagents for the effective use of such systems. There is also no need for a surgical procedure for the insertion and removal of the above polymer gels. They can be prepared in aqueous solutions and can be readily sterilized via simple filtration. Typical examples of such applications are as follows: Jeong et al.[1,3,6] have reported the use of poly(ethylene glycol)/poly(lactic acid-*co*-glycolic acid) based block, triblock, and graft copolymers in a series of injectable drug delivery systems, and have also reported their use in tissue engineering. Chenite et al.[7] have characterized a chitosan-glycerolphosphate based gel, which is a sol at room temperature, but becomes a gel at body temperature. Behravesh et al.[8] studied biodegradable themosensitive triblock copolymers composed of methoxy-poly(ethylene glycol) and poly(propylene fumarate).

Poly(organophosphazenes) substituted with amino acid esters have a potential as polymeric carriers for biologically active agents. The advantages of these polymers come mainly from a wide variety of properties that can be designed and synthesized by the reaction of poly(dichlorophosphazene) with various amino acid esters. Allcock et al.[9,10] have described amino acid ester functionalized polyphosphazenes, which are potentially biocompatible and biodegradable. Most of these polymers posses tunable degradation rates and readily degrade in aqueous media to products nontoxic phosphates, ammonium salts, amino acid, and ethanol. Therefore, these polymers have been studied as matrices or particles for controlled drug release application.[11,12]

We have also conducted investigations into the properties of biodegradable thermosensitive poly(organophosphazenes) containing α-amino-ω-methoxy-PEG (AMPEG) and hydrophobic amino acid esters, including isoleucine ethyl ester (IleOEt), leucine ethyl ester (LeuOEt), and valine ethyl ester (ValOEt), as well as one which contained depsipetide ethyl ester (ethyl-2-(*O*-glycyl)glycolate, GlyGlyOEt).[13,14] These

Correspondence to: S.-C. Song (scsong@kist.re.kr).
Contract grant sponsor: Ministry of Science and Technology in Korea; contract grant number: M104140300001-05N1403-00110.

polymers all, to varying degrees, exhibited sol–gel transition properties in aqueous solutions: It was inferred that the gelation of such polymers was attributable to hydrophobic interactions occurring between the side chain fragments of the hydrophobic amino acids, such as $-CH(CH_3)CH_2CH_3$ of IleOEt, and the $CH_2CH(CH_3)_2$ contained in LeuOEt. Our polymers also degraded under physiological conditions. Their byproducts, which included poly(ethylene glycol) (PEG), amino acids, ethanol, phosphate, and ammonium salts, are all known nontoxic materials.

In this article, we show a cyclic phosphazene trimer with an amino group as a preliminary model for the production of poly(organophosphazenes) possessing an amino group and then report the synthesis of several poly(organophosphazenes), which contain AMPEGs, some hydrophobic amino acid esters (LeuOEt, PheOEt, and IleOEt), and a depsipeptide ethyl ester (ethyl-2-(*O*-glycyl)glycolate) as hydrolytic labile moieties, and L-lysine ethyl ester (lysOEt) with two amino groups as the final substitute. So far, there have been only a few reports of biodegradable thermonsensitive polymers containing functional groups. These amino group-containing thermosensitive polymer gels are expected to prove useful in the construction of a bioactive molecule-conjugated carrier and gene delivery system. Here, we report the synthesis, gelation properties, and hydrolytic behavior of these amino functional group-containing poly(organophosphazenes).

EXPERIMENTAL

Materials

Hexachlorocyclotriphosphazene (Aldrich) was purified by sublimation at 55°C under vacuum (about 0.1 mm Hg). The ethyl esters of amino acids were prepared according to the literature.[15] Ethyl-2-(*O*-glycyl)-glycolate (GlyGlycOEt) was prepared as described before by Crommen et al.[16] Tetrahydrofuran (THF) was dried by reflux over sodium metal and distilled, and triethylamine was distilled over BaO under dry nitrogen. Methoxy-poly(ethylene glycol) (MPEG) with molecular weights of 350 were dried azeotropically with benzene, followed by vacuum drying, and then stored over molecular sieve 4A. α-Amino-ω-methoxy-poly(ethylene glycols) (AMPEGs) with molecular weights of 350, 550, and 750 were prepared by a published method.[17]

Synthesis of trimer and poly(organophosphazenes)

Synthesis of trimer $[N_3P_3(MPEG350)_3(L\text{-}LysOEt)_3]$ [Fig. 1(a)]. The sodium salt of polyethylene glycol 350 monomethyl ether (MPEG350) was prepared by reaction of MPEG350 (6.1 g, 17.4 mmol) with 1.5 equiv of sodium metal in THF (230 mL) at refluxing temperature for 24 h. After the resultant solution was filtered to remove excess sodium, the filtrate solution was added slowly to hexachlorocyclotriphosphazene (2.0 g, 5.8 mmol) dissolved in THF (50 mL). The reaction mixture was stirred for 5 h at −60°C and then 12 h at room temperature. Meanwhile, lysine ethyl ester dihydrochloride (21.5 g, 87.0 mmol) was suspended in dry THF (100 mL) containing triethyl amine(48.5 mL, 348.0 mmol), which was stirred for 6 h at 50°C to deprotect dihydrochloride and cooled to the room temperature. The trimer solution was added to the lysine ethyl ester solution, which was stirred for 2 days at 50°C. The reaction mixture was filtered, and after the filtrate was concentrated, it was poured into *n*-hexane to obtain a precipitate, which was reprecipitated twice in the same solvent system. The product was dissolved in a small amount of ethyl acetate, which was washed twice by water. The ethyl acetate solution was concentrated, purified by cloud point separation in water and freeze-dried to obtain the trimer.[18]

Yield: 66%. ^{31}P-NMR (D_2O), δ (ppm): 42.42. ^{1}H-NMR (D_2O), δ (ppm): 1.3(t, 3H, ester CH_3); 1.4 (br m, 2H, lysine γ CH_2); 1.6 (br m, 2H, lysine β CH_2); 1.7 (br m, 2H, lysine δ CH_2); 2.9 (br m, 2H, lysine ε CH_2); 3.4 (s, 3H, methoxy CH_3); 3.6 (t, 1H, lysine α CH); 3.7 (br m, 26H, MPEG CH_2); 4.1 (m, 2H, MPEG CH_2); 4.2 (q, 2H, ester CH_2).

Synthesis of $[NP(LeuOEt)_{0.90}(AMPEG350)_{0.95}(LysOEt)_{0.15}]_n$ (polymer 1)

Poly(dichlorophosphazene) was prepared as described previously.[19] L-leucine ethyl ester hydrochloride (3.0 g, 15.5 mmol) suspended in dry THF (100 mL) containing triethylamine (6.3 g, 62.0 mmol) was added slowly to poly(dichlorophosphazene) (2.0 g, 17.3 mmol) dissolved in dry THF (100 mL). The reaction mixture was stirred for 4 h in dry ice bath and then for 20 h at room temperature. To this mixture, triethylamine (0.3 g, 3.2 mmol) and AMPEG350 (12.1 g, 34.5 mmol) dissolved in THF (50 mL) were added, and the reaction was allowed to be reacted for 24 h at room temperature. After LysOEt (12.1 g, 34.5 mmol) dissolved in dry THF (100 mL) containing triethylamine (7.0 g, 69.0 mmol) was added to the polymer solution, the reaction mixture was stirred for 2 days in ice bath. The reaction mixture was filtered. After the filtrate was concentrated, it was poured into *n*-hexane to obtain a precipitate, which was reprecipitated twice in the same solvent system. The polymer product was further purified by dialysis in methanol for 2 days and then in distilled water for 2 days at 4°C. The final dialyzed solution was freeze-dried to obtain polymer **1**. Yield: 68%. ^{31}P-NMR (acetone d^6), δ (ppm): 24.1. ^{1}H-NMR

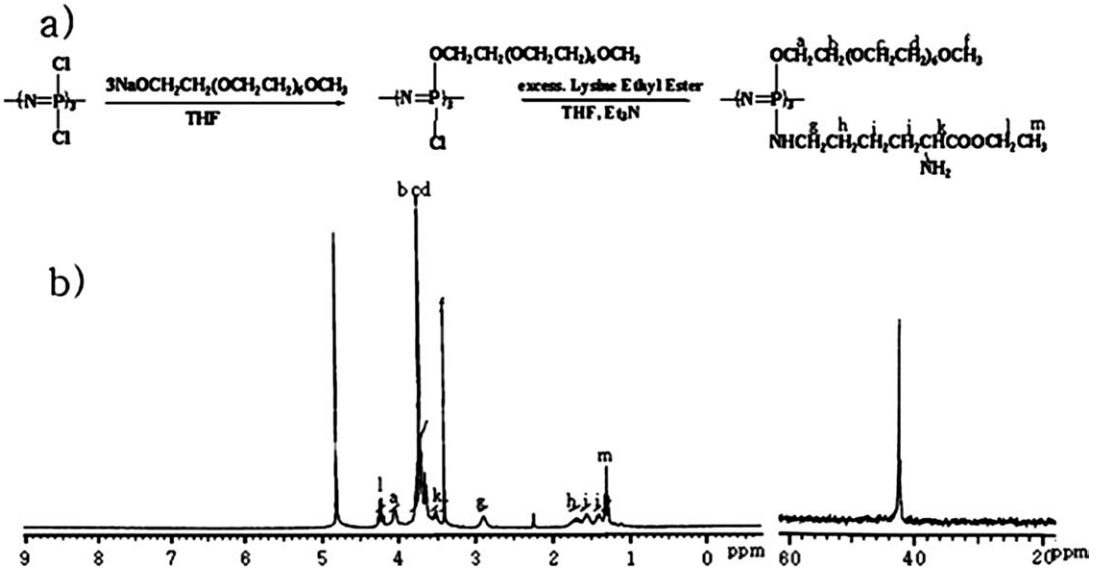


Figure 1 Cyclic trimeric phosphazene with an amino group as a preliminary model: (a) synthetic procedure, (b) ^{1}H-NMR and ^{31}P-NMR spectra of the trimer.

(acetone d^{6}), δ (ppm): 0.9–1.2 (b, 6H), 1.2–1.4 (b, 6H), 1.4–2.2 (b, 9H), 3.0–3.3 (b, 4H), 3.4 (s, 3H), 3.5–3.9 (b, 27H), 3.9–4.1 (b, 1H), 4.1–4.3 (b, 4H). Elem. anal. (%) calcd:. C, 49.00; H, 8.73; N, 9.84. found: C, 49.40; H, 8.55; N, 9.73.

The other polymers were prepared analogously using different mole ratios and molecular weights of Ampeg.

$[NP(PheOEt)_{1.03}(AMPEG350)_{0.84}(LysOEt)_{0.13}]_n$ (polymer 2)

PheOEt (17.8 mmol), AMPEG 350 (14.5 mmol), and LysOEt (30.0 mmol). Yield: 65%. ^{31}P-NMR ($CDCl_3$), δ (ppm): 17.9. ^{1}H-NMR (acetone d^{6}), δ (ppm): 0.8–1.2 (b, 3H), 1.2–1.4 (b, 3H), 1.4–2.1 (b, 6H), 2.9–3.2 (b, 6H), 3.4 (s, 3H), 3.5–4.0 (b, 28H), 4.1–4.4 (b, 4H), 7.0–7.3 (b, 5H). Elem. anal. (%) calcd: C, 53.76; H, 7.86; N, 7.86. found: C, 53.40; H, 7.65; N, 7.80.

$[NP(IleOEt)_{0.86}(AMPEG350)_{0.85}(LysOEt)_{0.29}]_n$ (polymer 3)

IleOEt (14.87 mmol), AMPEG 350 (14.7 mmol), and LysOEt (51.9 mmol). Yield: 75%. ^{31}P-NMR ($CDCl_3$), δ (ppm): 19.3 ^{1}H-NMR ($CDCl_3$), δ (ppm): 0.8–1.0 (b, 6H), 1.1–1.3 (b, 6H), 1.4–2.0 (b, 9H), 2.8–3.1 (b, 4H), 3.4 (s, 3H), 3.5–3.9 (b, 27H), 3.9–4.1(b, 1H), 4.1–4.3 (b, 4H). Elem. anal. (%) calcd: C, 49.22; H, 8.74; N, 10.09. found: C, 49.00; H, 8.62; N, 9.90.

$[NP(IleuOEt)_{1.01}(AMPEG550)_{0.82}(LysOEt)_{0.17}]_n$ (polymer 4)

IleOEt (17.3 mmol), AMPEG 550 (13.8 mmol), and LysOEt (34.6 mmol). Yield: 70%. ^{31}P-NMR ($CDCl_3$), δ (ppm): 19.2. Elem. anal. (%) calcd: C, 49.67; H, 8.60; N, 6.49. found: C, 49.61; H, 8.66; N, 6.52.

$[NP(IleuOEt)_{1.10}(GlyGlycOEt)_{0.16}(AMPEG550)_{0.57}(LysOEt)_{0.16}]_n$ (polymer 5)

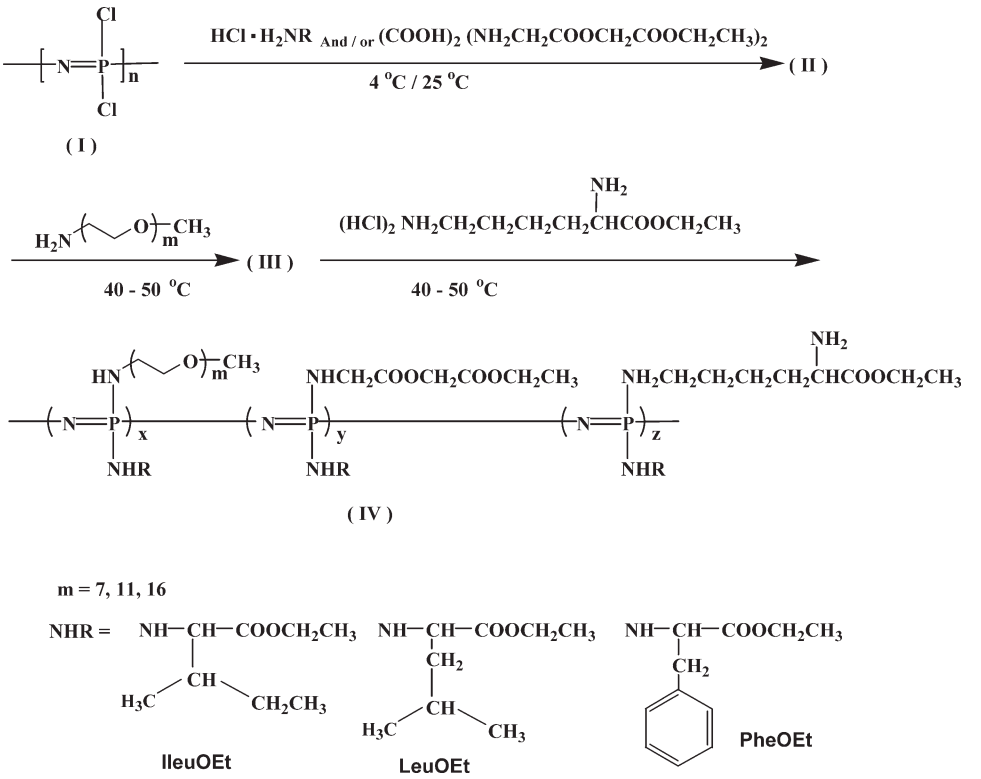
IleOEt (19.0 mmol), GlyGlyOEt (3.46 mmol), AMPEG 550 (10.4 mmol), and LysOEt (34.6 mmol). Yield: 65%. ^{31}P-NMR ($CDCl_3$), δ (ppm): 19.3. ^{31}P-NMR ($CDCl_3$), δ (ppm): 19.3 ^{1}H-NMR ($CDCl_3$), δ (ppm): 0.8–1.0 (b, 6H), 1.1–1.3 (b, 9H), 1.4–2.0 (b, 9H), 2.8–3.1 (b, 4H), 3.4 (s, 3H), 3.5–3.9 (b, 44H), 3.9–4.1 (b, 1H), 4.1–4.4 (b, 6H), 4.5–4.7 (b, 2H). Elem. anal. (%) calcd: C, 49.57; H, 8.49; N, 8.49. found: C, 49.75; H, 8.63; N, 8.00.

$[NP(IleuOEt)_{1.10}(AMPEG750)_{0.68}(LysOEt)_{0.22}]_n$ (polymer 6)

IleOEt (19.0 mmol), AMPEG 750 (11.8 mmol), and LysOEt (34.6 mmol). Yield: 75%. ^{31}P-NMR ($CDCl_3$), δ (ppm): 18.9. Elem. anal. (%) calcd: C, 50.20; H, 8.81; N, 5.83. found: C, 50.00; H, 8.66; N, 6.12.

Instruments and measurements

All reactions were carried out under an atmosphere of dry nitrogen using standard Schlenk-line techniques. Elemental analysis was carried out with Fisons 1108 CHNS Microanalizer and Polyscan 61E ICP. ^{1}H-NMR measurements were made with a Varian Gemini-300 spectrometer operating at 300 MHz in the Fourier transform mode. Proton-decoupled ^{31}P-NMR spectra were measured with the same spectrometer operating at 121.4 MHz using triphenyl phosphate as an external standard. Gel-permeation chromatography



Scheme 1 Synthesis of thermosensitive poly(organophosphazenes) with an amino group.

was carried out using a GPC system (Waters 1515) with a refractive index detector (Waters 2410) and two styragel columns (Waters styragel HR 5E) connected in line at a flow rate of 0.8 mL/min at 35°C. Polystyrenes (M_w: 1140, 3570, 14,100, 28,700, 65,300, 181,000, 613,000, 1,010,000, 2,660,000) were used as standards to calibrate the column. The viscosity of the aqueous solutions of polymers was measured as a function of temperature: viscosity measurements on polymer solutions were carried out on a Brookfield RVDV-III+ viscometer with small sample adaptor using the spindle SC 4-15 between 5 and 80°C with a heating rate of 0.25°C/min and under shear rate of 0.07–20.4 s^{-1}.

In vitro hydrolytic degradation of polymers

Time-dependant degradation of the polymers was examined in pH 7.4 buffer solutions at 37°C. The polymers were dissolved in the buffered solutions (10 wt %) of pH 7.4 (0.1 m*M* phosphate buffered saline), which were incubated in shaking water bath at 37°C. Samples were taken every day and lyophilized. The samples were redissolved in THF, and the molecular weights of the polymer residues were determined by GPC.

RESULTS AND DISCUSSION

Initially, a cyclic phosphazene trimer was used as a preliminary model for the production of poly(organophosphazenes) possessing an amino group. Hexachlorocyclotriphosphazene was initially allowed to react with the sodium salt of MPEG 350, and the resultant solution was then allowed to react with an excess of lysine ethyl ester, thereby yielding the final trimer.[18] The synthetic procedure, as well as the ^{1}H-NMR and ^{31}P-NMR data, are shown in Figure 1. The sharp single ^{31}P-resonance located at 42.4 ppm confirms that the final trimer is the ci-nongeminal isomer, $N_3P_3(MPEG350)_3(lysOEt)_3$, as was reported in a previous study.[18] Also, the triplet located at 3.6 ppm was assigned to the α-CH of lysine ethyl ester in the ^{1}H-NMR spectrum, suggesting that $N_3P_3(MPEG350)_3(Cl)_3$ reacted completely with ε-amine, rather than α-amine, in the lysine ethyl ester. Crosslinking occurring during the reaction of lysine ethyl ester with Cl-P-N was prevented, as the p*K*a (10.53) of ε-amine in lysine is considerably higher than that (8.95) of α-amine in lysine, and also because excess lysine ethyl ester was used in this reaction.[18]

On the basis of the above-mentioned result, we were able to synthesize poly(organophosphazenes) possessing amino function groups. Scheme 1 represents the procedure by which these polymers were synthesized. In brief, poly(dichlorophosphazene) (I) dissolved in THF was allowed to react with hydrophobic amino acids, including IleOEt, LeuOEt, and PheOEt, and/or GlyGlycOEt, to yield the partially substituted polymer (II), which was then reacted with α-amino-ω-methyl-PEG. This resulted in the

TABLE I
Characteristics of Poly(organophosphazenes)

Polymer	Structure	T_{ass} (°C)[a]	T_{max} (°C)[b]	T_{lcst} (°C)[c]	V_{max} (Pa S)[d]	M_w ($\times 10^4$)[e]
1	$[NP(LeuOEt)_{0.90}(AMPEG350)_{0.95}(LysOEt)_{0.15}]_n$	19	48	65	1.8	6.2
2	$[NP(PheOEt)_{1.03}(AMPEG350)_{0.84}(LysOEt)_{0.13}]_n$	7	31	55	25.5	3.4
3	$[NP(IleuOEt)_{0.86}(AMPEG350)_{0.85}(LysOEt)_{0.29}]_n$	18	45	60	15.4	2.5
4	$[NP(IleOEt)_{1.01}(AMPEG550)_{0.82}(LysOEt)_{0.17}]_n$	32	64	85	5.0	0.9
5	$[NP(IleOEt)_{1.10}(GlyGlycOEt)_{0.15}(AMPEG550)_{0.57}(LysOEt)_{0.16}]_n$	16	35	43	279.5	3.2
6	$[NP(IleOEt)_{1.10}(AMPEG750)_{0.68}(LysOEt)_{0.22}]_n$	42	78	>100	168.0	1.5

[a] The association temperature at which the viscosity of the polymer solutions (10 wt %) begin to increase sharply.
[b] The temperature at which the polymer solutions (10 wt %) reach their maximum viscosity.
[c] The LCST was identified as the temperature at which the polymer solutions (10 wt %) became turbid.
[d] The viscosity of the polymer solutions at T_{max}.
[e] The weight average (M_w) molecular weight of the polymers was measured by GPC using THF solutions containing 0.1 % (w/v) TBAB(tetrabutylammonium bromide).

generation of the intermediate (III). This intermediate (III) was in a state with a small quantity of unreacted chlorine (Cl-P-N). Finally, the intermediate (III) was allowed to react with excess lysine ethyl ester, thereby generating the final polymer products (IV). Different copolymers were obtained by varying the amino acid esters used, as well as the length of the α-amino-ω-methyl-PEG, and the mole ratios of the substituents. The polymer products generated were then characterized via multinuclear NMR spectroscopies, GPC, and elemental analysis.

The synthesized polymers are summarized in Table I. All of these polymers appeared as pale yellow viscoelastic solids. They were all soluble in cold water, as well as in several organic solvents, including chloroform, tetrahydrofuran, and methyl alcohol. The average molecular weight of these compounds was between $0.9 \times 10^4 - 6.2 \times 10^4$.

Figure 2 shows the changes in the ^{31}P-NMR spectra during the process of synthesizing polymer **1**. When poly(dichlorophosphazene) (I) was allowed to react with LeuOEt, the intermediate (II) exhibited several primary peaks. After the intermediate (II) was reacted with AMPEG 350, only two major peaks remained, at about 10 and 19 ppm, which were assigned to *N*-P-N and *N*-P-Cl, respectively. Finally, after intermediate (III) was allowed to react with the excess LysOEt, the peak located at 10 ppm (*N*-P-Cl) disappeared, and the final product (IV) was obtained, which exhibited one major peak, located at 19 ppm. This confirmed that the chlorine (Cl) atoms had been completely replaced by subsequent reaction with ε-amine lysine ethyl ester.

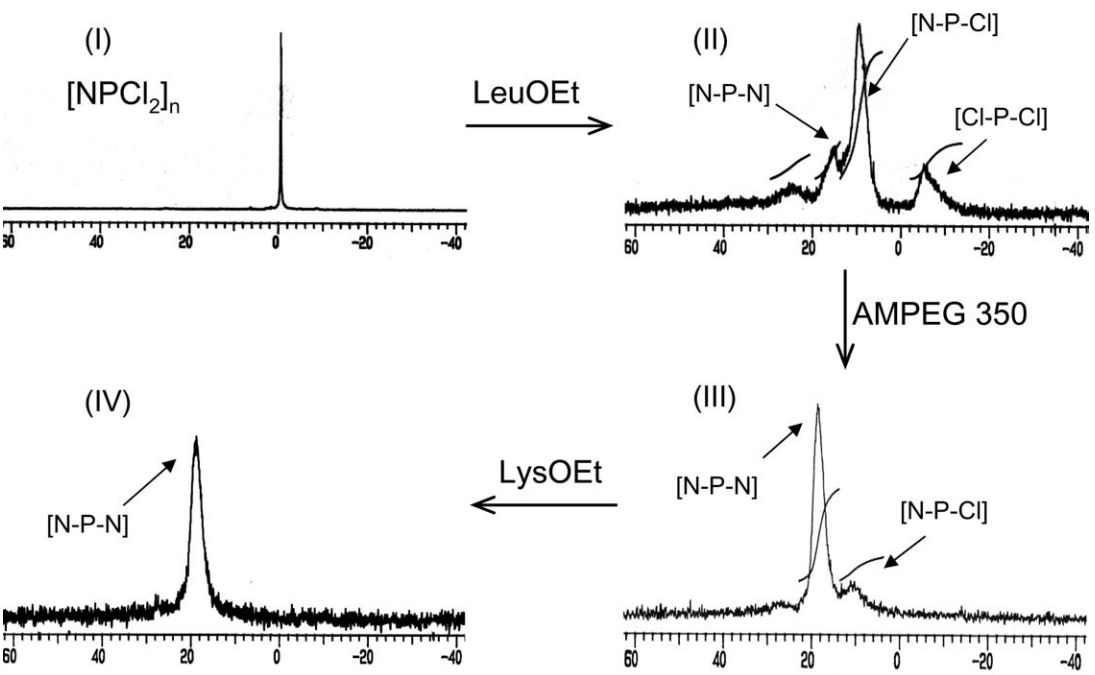


Figure 2 ^{31}P-NMR spectral change monitored during the process of synthesizing polymer **1**.

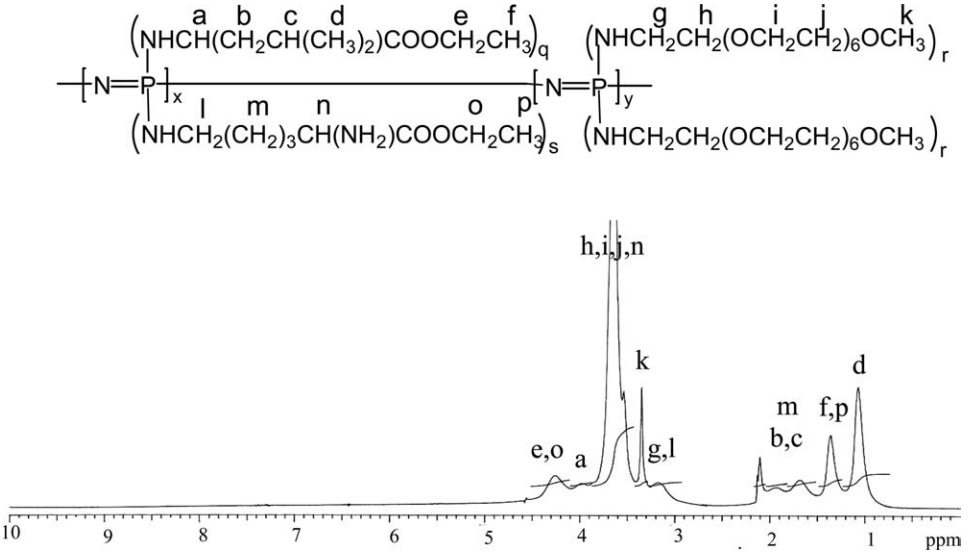


Figure 3 ^{1}H-NMR spectrum of polymer **1**.

The ^{1}H-NMR spectra of polymer **1** are shown in Figure 3. Most of the peaks representing the lysine ethyl ester on polymer side chains were found to overlap with other peaks of AMPEG350 and LeuOEt, owing to a small amount of remaining lysine ethyl ester. However, the mole ratio of the MPEG 350, LeuOEt, and LysOEt of polymer **1** was calculated from the integration among the methoxy protons (3H, —NH(CH_2CH_2O)$_7$CH_3) of the MPEG 350, the methyl protons (3H, —NHCH($CH_2CH(CH_3)_2$)COOCH$_2$CH_3, —NH(CH_2)$_4$CH(NH_2)COOCH$_2$CH_3) of the LeuOEt and LysOEt, and the methyl protons (6H, —NHCH(CH_2CH(CH_3)$_2$)COOCH$_2$CH$_3$) of the LeuOEt, which appeared at 3.4, 1.3, and 1.1 ppm, respectively. The mole ratios of the substituents in the other polymers were estimated in a similar way.

The poly(organophosphazenes) possessing the amino groups exhibited reversible sol–gel transitions. Figure 4 represents the temperature-dependent viscosity changes of the 10 wt % solutions of polymer **2** by heating, and by cooling. The polymer solution exhibits sol–gel–sol phase transition due to heating: The polymer solution (10 wt %) is a clear sol until about 9°C (T_{ass}), and exhibits a sol–gel phase transition from T_{ass} to T_{max} (31°C). As the temperature rises from T_{max} to 55°C, the polymer gel gradually becomes opaque, finally becoming a turbid solution probably owing to destruction of hydrogen bonding between polymer chains and water at approximately 55°C. When this turbid solution was cooled under the same conditions, the polymer solution exhibited a curve from 59 to 33°C which was quite similar to the curve drawn by heating and a similar curve with a few degrees left shift from 31 to 9°C. This data indicates that polyphosphazene gels which possess amino groups exhibit reversible gelation properties.

In general, alkyl, perfluoroalkyl, or aromatic groups in polymers can function as junction points via hydrophobic interactions in aqueous solution. It is believed that the present polymer gels are formed not by covalent bonding, but rather by physical association: The gelation of the present polymer appears to be attributable to hydrophobic interactions occurring between side chain fragments, such as the —CH(CH_3)CH_2CH_3 of IleOEt, the —CH_2CH(CH_3)$_2$ of LeuOEt, and the —CH_2C$_6$H$_5$ of PheOEt. The gelation properties of the polymers assessed in the present study were determined to be dependent on the types of amino acid esters used, as well as the molecular weight of AMPEG, as is shown in Table I. The more hydrophobic the amino acid side groups were, the lower T_{max} was, and the stronger the gelation

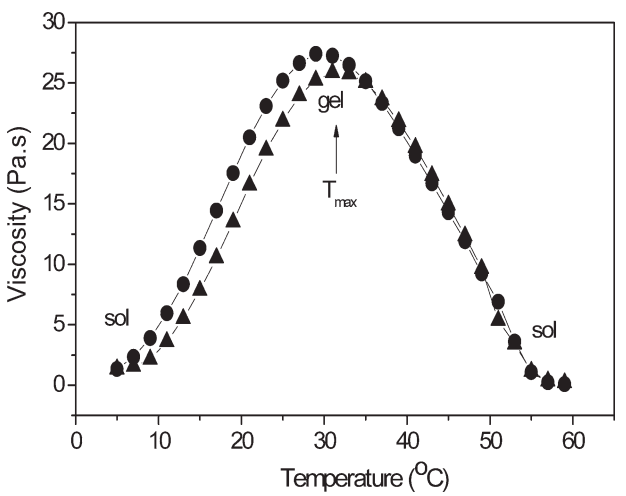


Figure 4 Temperature-dependent viscosity changes of the 10 wt % solutions of polymer **2** by heating (▲), and by cooling (●) under shear rate 1.7 s^{-1}.

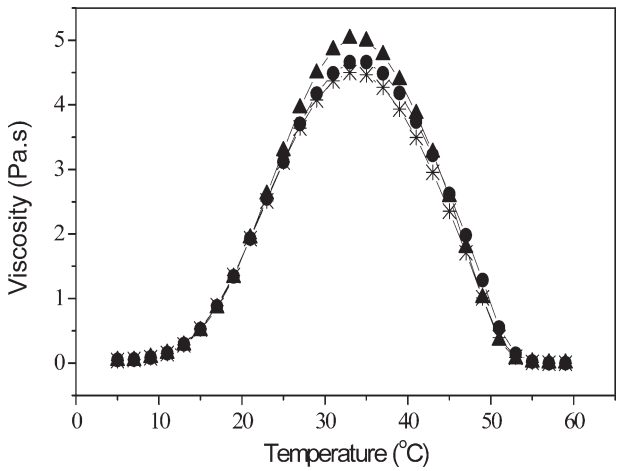


Figure 5 Viscosity changes of the 7.5 wt % solution of polymer **2** with a variation of shear rates: 20.4 s^{-1} (✳), 13.6 s^{-1} (●), and 6.8 (▲) s^{-1}.

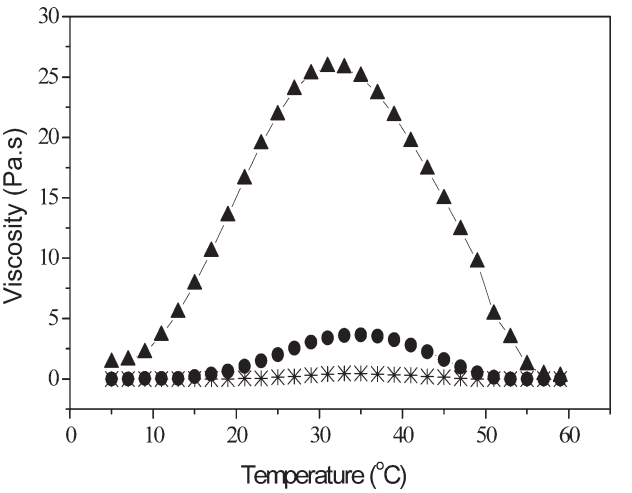


Figure 6 Temperature-dependent viscosity changes of the 5 (✳), 7.5 (●), and 10 wt % (▲) solutions of polymer **2**.

properties exhibited by the polymers were. For example, the T_{ass} and T_{max} values of polymer **1** with LeuOEt (0.90 mol) were 19 and 48°C, respectively, and those of polymer **2** with PheOEt (1.03 mol) were 7 and 31°C, respectively. The Vmax values of polymers **1** and **2** were 1.8 and 25.5 Pa S, respectively. These results may be related to the fact that phenylalanine is more hydrophobic than leucine. The increase chain length of AMPEG results in an increase in the T_{ass}, T_{max}, and V_{max} values of the polymer gels. For example, the T_{ass}, T_{max}, and V_{max} values of polymer **4** with AMPEG 550 and polymer **6** with AMPEG 750 were 32°C, 64°C, 5.0 Pa.s, 42°C 78°C, and 168 Pa.s, respectively.

Usually the viscosity exhibited by thermothickening polymer solutions depends heavily on the shear rates at around the gelling temperature.[20] A similar trend was observed in our polymer system. The dependence of viscosity on the shear rate for polymer **2** as a function of temperature is shown in Figure 5. In low temperature regions, at below approximately 21°C, the viscosity of the polymer solution appeared to be independent of the shear rates, implying that the polymer solution remained in sol state, and behaved similarly to Newtonian fluid. At around T_{max}, the polymer's viscosity was affected by the shear rates to a substantially higher degree: The viscosity of the polymer increased as the shear rate decreased, indicating that the polymer solution was pseudoplastic. Similar results were found in studies of other thermogelling materials.[20,21]

The temperature-dependent changes in the viscosity of 5, 7.5, and 10 wt % solutions of polymer **2** are shown in Figure 6.Thermosensitive polymers can, in general, exhibit sol–gel phase transitions at sufficient concentrations above the critical gelling concentration (CGC). A similar phenomenon was also observed in the polymers evaluated in this study. As is shown in Figure 6, the viscosity of these polymer solutions was significantly affected by the concentrations of the polymer solutions. The magnitude of the viscosity of the polymer solutions decreased as the concentration of the polymer solutions was reduced: the V_{max} of the 10 wt % polymer solution was substantially higher than that of the 7.5 wt % polymer solution; the 5 wt % polymer solution exhibited no thermothickening properties whatsoever. This indicates that the gelation properties exhibited by the poly(organophosphazenes) possessing amino groups are similar to those of the previously-characterized poly(organophosphazene) gels.[13]

The time-dependent degradation behavior of the amino group-containing thermosensitive poly(organophosphazenes) was determined with regard to the reductions in the molecular weight of the polymers, as measured by GPC. Figure 7 shows the profiles of

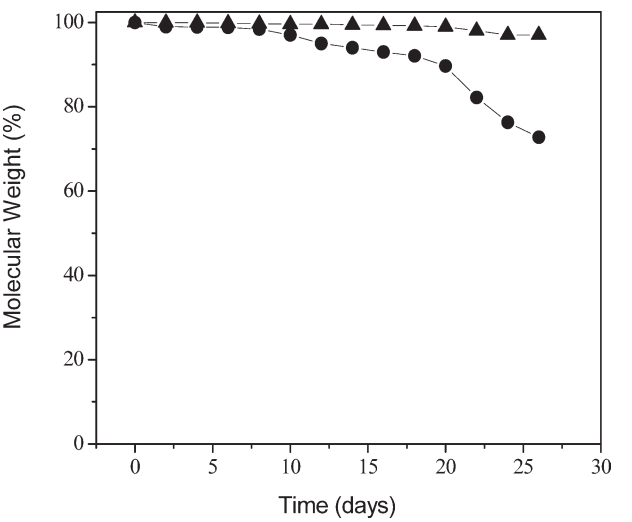


Figure 7 Time-dependent hydrolytic degradation of polymers **4** (▲) and **5** (●) in 10 m*M* phosphate buffered saline solution at pH 7.4 and 37°C.

time-dependent molecular weight reductions occurring in polymers **4** and **5** in pH 7.4 buffer solutions, at 37°C. For the *in vitro* degradation study, polymers **4** and **5** were dissolved in buffer solutions (10 wt %) at a pH of 7.4 (10 m*M* phosphate buffered saline). They were then incubated at 37°C for 26 days, in a shaking water bath. Samples were collected every day. The samples were then lyophilized and dissolved in THF, and the molecular weights of the polymer residues were determined via GPC. The degradation rate of the studied polymer solution was substantially affected by a hydrolytic sensitive moiety, namely, a depsipeptide ethyl ester (GlyGlycOEt). Less than a 3% molecular weight loss was observed for polymer **4**, which lacked GlyGlycOEt. A molecular weight loss of approximately 30% was observed for polymer **5**, which possessed GlyGlycOEt. The hydrolysis rate of depsipeptide ethyl esters is known to be much faster than that of amino acid esters, and our data confirms that an increase in the content of depsipeptide ethyl esters results in an acceleration of the degradation of the polymer solution.[14,22]

CONCLUSIONS

A series of thermosensitive poly(organophosphazenes) have been synthesized, which harbor AMPEG and hydrophobic amino acid esters such as isoleucine ethyl ester (IleOEt), leucine ethyl ester (LeuOEt), and phenylalaine ethyl ester (PheOEt), and a depsipeptide ethyl ester (GlyGlycOEt), and lysine ethyl ester (lysOEt) as side groups. The poly(organophosphazenes) which possessed amino groups were also observed to exhibit sol–gel–sol phase transitions in aqueous solution as the temperature was raised. This polymer solution behaved similarly to a Newtonian fluid in its sol state, but appeared to exhibit pseudoplastic qualities in its gel state. The degradation rate of the amino group-containing poly(organophosphazenes) also appeared to depend on the content of hydrolytically labile depsipeptide ethyl ester, GlyGlycOEt, in the polymer. The present biodegradable thermosensitive amino group-containing poly(organophosphazene) gels are expected to prove useful in the formulation of injectable drug delivery systems, and as carriers for bioactive molecules. Further study on addition of anticancer drugs and bioactive molecules to polyphosphazene gels is on-going.

References

1. Jeong, B.; Bae Y. H.; Lee, D. S.; Kim, S. W. Nature 1999, 388, 860.
2. Hatefi, A.; Amsden, B. J Control Rel 2002, 80, 9.
3. Jeong, B.; Bae, Y. H.; Kim, S. W. Adv Drug Delivery Rev 2002, 54, 37.
4. Hoffman, A. S. Adv Drug Delivery Rev 2002, 43, 3.
5. Gutowska, A.; Jeng, B.; Jasionowski, M. Anat Rec 2001, 263, 342.
6. Jeong, B.; Lee, K. M.; Gutowska, A.; An, Y. H. Biomacromolecules 2002, 3, 865.
7. Behravesh, E.; Shung, A. K.; Jo, S.; Mikos, G. Biomacromolecules 2002, 3, 153.
8. Chenite, A.; Chaput, C.; Wang, D.; Combes, C.; Buschmann, M. D.; Hoemann, C. D.; Leroux, J. C.; Atkinson, B. L.; Binette, F.; Selmani, A. Biomacromolecules 2000, 21, 2155.
9. Allcock, H. R.; Singh, A.; Ambrosio, A. M. A.; Laredo, W. R. Biomacromolecules 2003, 4, 1646.
10. Laurencin, C. A.; Norman, M. E.; Elgendy, H. M.; EL-Amin, S. F.; Allcock, H. R.; Pucher, S. R.; Ambrosio, A. A. J Biomed Mater Res 1993, 27, 963.
11. Ibim, S. M.; Ambrosio, A. M. A.; Larrier, D.; Allcock, H. R.; Laurencin, C. A. J Control Release 1996, 40, 31.
12. Verones, F. M.; Marsilio, F.; Calciceti, P.; Filippis, P. D.; Giunchedi, P.; Lora, S. J. Control Release 1998, 52, 227.
13. Lee, B. H.; Lee, Y. M.; Sohn, Y. S.; Song, S.-C. Macromolecules 2002, 35, 3876.
14. Lee, B. H.; Song, S.-C. Macromolecules 2004, 37, 4533.
15. Greenstein, J. D.; Winitz, M. Chemistry of the Amino Acids; Wiley: New York, 1961; p 925.
16. Crommen, J.; Schacht, E. Bull Soc Chim Belge 1991, 100, 747.
17. Bromberg, L.; Temchenko, M. Langmuir 1999, 15, 8633.
18. Lee, S. H.; Song, S.-C.; Jin, J.-I.; Sohn, Y. S. J. Am Chem Soc 2000, 122, 8315.
19. Sohn, Y. S.; Cho, Y. H.; Baek, H.-G.; Jung, O.-S. Macromolecules 1995, 28, 7566.
20. Cabana, A.; Ait-Kadi, A.; Juhasz, J. Colloid Interface Sci 1997, 190, 307.
21. Durand, A.; Hourdet, D. Polymer 1999, 40, 4941.
22. Schacht, E.; Vandorpe, J.; Dejardin, S.; Lemmouchi, Y.; Seymour, L. Biotechnol BioEng 1996, 52, 102.