Injectable and Thermosensitive Poly(organophosphazene) Hydrogels for a 5-Fluorouracil Delivery

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ABSTRACT: The drug solubility and its release profiles of an anticancer drug from an injectable thermosensitive poly(organophosphazene) hydrogel bearing hydrophobic L-isoleucine ethyl ester and hydrophilic α-amino-ω-methoxy-poly(ethylene glycol) with and without hydrolysis-sensitive glycyl lactate ethyl ester or functional glycyl glycine have been investigated. 5-Fluorouracil (5-FU) was used as a model anticancer drug. The aqueous solutions of 5-FU incorporated poly(organophosphazenes) were an injectable fluid state at room temperature and formed a transparent gel at body temperature. The poly(organophosphazene) solution could enhance the solubility of 5-FU and its solubility (34.26 mg/mL) was increased up to 10-fold compared to that in phosphate-buffered saline (3.39 mg/mL, pH 7.4, 4°C). The *in vitro* drug release profiles from poly(organophosphazene) hydrogels were established in phosphate-buffered saline at pH 7.4 at 37°C and the release of 5-FU was significantly affected by the diffusion-controlled stage. The results suggest that the injectable and thermosensitive poly(organophosphazene) hydrogel is a potential carrier for 5-FU to increase its solubility, control a relatively sustained and localized release at target sites and thus decrease systemic side effects. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 3831–3839, 2009

Key words: polyphosphazene; biodegradable; drug delivery system; thermosensitive hydrogels; injectable

INTRODUCTION

Hydrogels have been gaining acceptances in a wide variety of biological applications such as drug carriers and artificial organs because of their high water content and tissue-like physical and mechanical properties.[1] Hydrogels can be formed by a variety of methods, including a crosslinking by physical, ionic, and covalent interactions.[1] Thermosensitive polymers respond to small changes in the temperature stimuli and form physically crosslinked hydrogels by a sol–gel phase transition. These thermosensitive polymers have become increasingly attractive as carriers for injectable drug delivery systems during the past decade.

Recently, many studies of injectable drug delivery systems with thermosensitive polymers such as poly(DL-lactide-*co*-glycolide-*b*-ethylene glycol-*b*-DL-lactide-*co*-glycolide) (PLGA-PEG-PLGA) triblock copolymers,[2] poly(*N*-acryloylglycine-chitosan),[3] poly(*N*-isopropylacrylamide),[4] and gelatin–dextran[5] have been reported. Representatively, the triblock copolymers composed of poly(ethylene glycol-*b*-propylene glycol-*b*-ethylene glycol), known as Pluronics or Poloxamers, exhibit a reversible sol–gel transition behavior in aqueous solution.[6] However, poloxamers are not biodegradable, and the formed hydrogels are dissolved at the injection site in a few days at most. Another thermosensitive polymer is poly(*N*-isopropyl acryl amide), poly-NIPAAm, which exhibits a sharp lower critical solution temperature of approximately 32°C.[7] Unfortunately, poly-NIPAAm is not suitable for biomedical applications because of its well-known cytotoxicity. Moreover, poly-NIPAAm is nonbiodegradable. A chitosan-based thermosensitive hydrogel for the local and sustained delivery of drugs also has been reported.[8,9]

Thus, new injectable biodegradable polymers possessing reversible gelation properties have been reported.[10–17] One of these polymers is ABA and BAB triblock copolymer, Regel, in which A denotes the hydrophobic poly(DL-lactide-*co*-glycolide) and B denotes the hydrophilic poly(ethylene glycol).[10–13] However, the Regel has been shown a gelation at body temperature when highly concentrated polymer solutions (more than 23%, w/w) were injected.[18]

The other of these polymers is thermosensitive and biodegradable poly(organophosphazene) hydrogel,

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bearing a hydrophobic and hydrophilic property.[14–17] Their various hydrophobic, hydrophilic, and other functional substituents could be easily introduced to the polymer backbone, which provides easy controlling of the hydrogel characteristics. They are soluble in water at or below room temperature and become hydrogels at the injection site at body temperature, forming depots that can slowly be degraded over the course of 4 weeks.[15,17] Because the polymers are biodegradable, they obviate the need for removal of the carrier after the drug depot exhausted because it is known that the poly(organophosphazene) hydrogels produce nontoxic biodegradable products such as H_3PO_4, NH_4^+, PEG and amino acids, and have good biocompatible properties. In the recent study, we described the drug release profiles from the poly(organophosphazene) hydrogels including doxorubicin and protein drugs.[19,20]

5-Fluorouracil (5-FU) is one of the antitumor agents most frequently used for treating solid tumors, such as breast, colorectal, and gastric cancers.[21] 5-FU has been widely developed to implant with a simple surgery.[22] The locally injectable delivery system of 5-FU may overcome certain limitations associated with oral and parenteral administrations. After an oral administration, 5-FU is poorly absorbed with serious variations in the bioavailability ranging between 0 and 80%.[23] After the parenteral administration of 5-FU, there is a rapid elimination of the drug with an apparent terminal half-life of approximately 8–20 min.[23] Thus, the 5-FU is an appropriate candidate for the local injectable delivery system to enhance the bioavailability and control a relatively sustained release at target sites. This local delivery system can be used as injectable fluids of thermosensitive polymers that can be injected into the body without a surgical procedure.

In this study, we synthesized injectable and thermosensitive poly(organophosphazene) hydrogels bearing hydrophobic L-isoleucine ethyl ester (IleOEt), hydrophilic α-amino-ω-methoxy-poly(ethylene glycol) with molecular weight of 550 Da (AMPEG 550) along with hydrolysis-sensitive glycyl lactate ethyl ester (GlyLacOEt) or functional glycyl glycine (GlyGlyOH). The gelation and hydrolytic degradation properties of the hydrogels, and the release profile of 5-FU from the hydrogel and solubility of 5-FU in the polymer solution were observed for the development of local drug-delivery systems.

EXPERIMENTAL

Materials

Hexachlorocyclotriphosphazene was acquired from Aldrich and purified by sublimation at 55°C under vacuum (about 0.1 mmHg). Poly(dichlorophosphazene) was prepared as described previously.[24] The L-isoleucine ethyl ester (IleOEt) was prepared by a previously reported method.[25] α-Amino-ω-methoxy-poly(ethylene glycol) (AMPEG) with a molecular weight of 550 Da was prepared as described by Bromberg and Temchenko.[26] Ethyl-2-(*O*-glycyl)lactate (GlyLacOEt) was prepared following the method described by Crommen et al.[27] Glycylglycine allyl ester (GlyGlyOAll) was prepared by a modified procedure in the previous manner.[28] 5-FU was purchased from Sigma (St. Louis, MO). Tetrahydrofuran (THF) was dried by a reflux system over sodium metal and distilled under the nitrogen atmosphere.

Instruments and measurements

^{1}H-NMR measurements were made with a Varian Gemini-300 spectrometer operating 300 MHz in the Fourier transform mode with $CDCl_3$ as a solvent and tetramethylsilane as an internal reference. 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 was performed with a GPC system (Waters 1515) with a refractive index detector (Waters 2410) and two stryagel columns (Waters styragel HR 5E) connected in line at a flow rate of 0.8 mL/min at 35°C. THF containing 0.11 wt % of tetrabutylammonium bromide was used as the solvent. Polystyrenes (MW: 1140; 3570; 14,100; 28,700; 65,300; 181,000; 613,000; 1,010,000; 2,660,000 Da) were used as standards to calibrate the column. The electrical charge (ζ-potential) of a prepared aqueous polymer solution was determined using a particle electrophoresis instrument (ZEM5002, Zetamaster, Malvern Instruments, Worcestershire, UK). An individual ζ-potential measurement was determined from the average of 10 readings taken on the same sample. The viscosities of aqueous polymer solutions were measured as a function of temperature using a Brookfield RVDV-III+ rheometer at the temperature range of 5–70°C with a heating rate of 0.34 °C/min and a fixed shear rate of 0.1 s^{-1}.

Synthesis of $[NP(IleOEt)_{1.20}(AMPEG550)_{0.80}]_n$ (Polymer 1)

Polymer **1** was synthesized similarly by the procedure in our previous report.[14] L-Isoleucine ethyl ester hydrochloride (IleOEt·HCl; 8.11 g, 41.43 mmol) suspended in distilled THF (100 mL) containing triethylamine (8.41 g, 82.86 mmol) was added slowly to poly(dichlorophosphazene) (4.0 g, 34.52 mmol) dissolved in distilled THF (200 mL). The reaction mixture was stirred at −60°C for 24 h and then at room temperature for 24 h. After AMPEG550 (37.97 g,

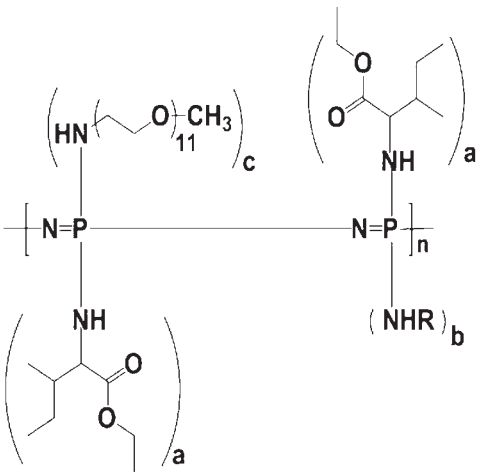
69.04 mmol) dissolved in distilled THF (150 mL) containing triethylamine (10.51 g, 103.56 mmol) was added to the reaction polymer solution, the reaction mixture was stirred at room temperature for 24 h and then at 50–55°C for 24 h. The reaction mixture was filtered. After the filtrate was concentrated, it was poured into *n*-hexane to obtain a precipitate, which was reprecipitated twice in the same solvent system. The polymer product was dialyzed with a dialysis membrane (MW 10,000–12,000 cutoff) against methanol for 4 days and distilled water at 4°C for 4 days, and the finally dialyzed solution was subsequently freeze-dried to obtain the polymer **1**. Yield: 70%. ^{31}P-NMR ($CDCl_3$), δ (ppm): 19.9. ^{1}H-NMR ($CDCl_3$), δ (ppm): 0.8–1.0 (s, 6H, —**CH_3** and —CH_2**CH_3** of IleOEt), 1.1–1.3 (b, 3H, —OCH_2**CH_3** of IleOEt), 1.3–1.6 (b, 2H, —**CH_2**CH_3 of IleOEt), 1.6–1.9 (b, 1H, —**CH**(CH_3)CH_2CH_3 of IleOEt), 3.4 (s, 3H, —**CH_3** of AMPEG), 3.5–3.9 (b, 44H, —(**CH_2CH_2**O)$_{11}$— of AMPEG), 3.9–4.1 (b, 1H, —NH**CH**— of IleOEt), 4.1–4.3 (b, 2H, —O**CH_2**CH_3 of IleOEt).

Synthesis of [NP(IleOEt)$_{1.23}$(AMPEG550)$_{0.56}$(GlyGlyOH)$_{0.21}$]$_n$ (Polymer 2)

Polymer **2** was synthesized similarly by the modified procedure in our previous report.[28] L-Isoleucine ethyl ester hydrochloride (IleOEt·HCl; 8.31 g, 42.46 mmol) suspended in distilled THF (100 mL) containing triethylamine (12.92 g, 127.38 mmol) was added slowly to poly(dichlorophosphazene) (4.0 g, 34.52 mmol) dissolved in distilled THF (200 mL). The reaction mixture was stirred at −60°C for 24 h and then at room temperature for 24 h. To this mixture, glycylglycine allyl ester trifluoroacetic acid salt (GlyGlyOAll·TFA; 2.47 g, 8.63 mmol) and triethylamine (2.62 g, 25.89 mmol) dissolved in distilled THF (100 mL) were added, and the reaction mixture was stirred at room temperature for 8 h. After AMPEG550 (26.58 g, 48.33 mmol) dissolved in distilled THF (150 mL) was added to the reaction polymer solution, the reaction mixture was stirred at room temperature for 24 h and then at 50–55°C for 24 hr. 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 dialyzed with a dialysis membrane (MW 10,000–12,000 cutoff) against methanol for 4 days and distilled water at 4°C for 4 days, and the finally dialyzed solution was subsequently freeze-dried to obtain the pre-polymer **2**. A solution of pre-polymer **2** (10 g, 17.5 mmol) in distilled THF (100 mL) was stirred at room temperature under nitrogen atmosphere, and then tetrakis(triphenylphosphine)palladium(0) (2.02 g, 1.75 mmol) and morpholine (15.24 g, 175 mmol) were added subsequently to the pre-polymer **2** solution. After stirring at room temperature for 8 h, the solvent was evaporated and the residue was dialyzed with a dialysis membrane (MW 6000–8000 cutoff) against methanol for 4 days and distilled water at 4°C for 4 days, and the finally dialyzed solution was subsequently freeze-dried to obtain the polymer **2**. Yield: 76%. ^{31}P-NMR ($CDCl_3$), δ (ppm): 19.9. ^{1}H-NMR ($CDCl_3$), δ (ppm): 0.8–1.0 (s, —**CH_3** and —CH_2**CH_3** of IleOEt), 1.1–1.3 (b, —OCH_2**CH_3** of IleOEt), 1.3–1.6 (b, —**CH_2**CH_3 of IleOEt), 1.6–1.9 (b, —**CH**(CH_3)CH_2CH_3 of IleOEt), 3.2 (s, —NH**CH_2**CONH— of GlyGlyOH), 3.3 (s, —**CH_3** of AMPEG), 3.4–3.8 (b, —(**CH_2CH_2**O)$_{11}$— of AMPEG), 3.9 (s, —NH**CH_2**COO— of GlyGlyOH), 4.0–4.1 (b, —NH**CH**— of IleOEt), 4.1–4.3 (b, —O**CH_2**CH_3 of IleOEt).

Synthesis of [NP(IleOEt)$_{1.18}$(AMPEG550)$_{0.80}$(GlyLacOEt)$_{0.02}$]$_n$ (Polymer 3)

Polymer **3** was synthesized similarly by the procedure in our previous report.[18] L-Isoleucine ethyl ester hydrochloride (IleOEt·HCl; 7.97 g, 40.73 mmol) suspended in distilled THF (100 mL) containing triethylamine (12.40 g, 122.19 mmol) was added slowly to poly(dichlorophosphazene) (4.0 g, 34.52 mmol) dissolved in distilled THF (200 mL). The reaction mixture was stirred at −60°C for 24 h and then at room temperature for 24 h. To this mixture, ethyl-2-(*O*-ethyl) lactate ammonium oxalate (GlyLacOEt·OA; 0.46 g, 1.73 mmol) and triethylamine (0.53 g, 5.18 mmol) dissolved in distilled acetonitrile (50 mL) were added, and the reaction mixture was stirred in an ice bath for 19 h. After AMPEG550 (37.97 g, 69.04 mmol) dissolved in distilled THF (150 mL) was added to the reaction polymer solution, the reaction mixture was stirred at room temperature for 24 hr and then at 50–55°C for 24 h. 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 dialyzed with a dialysis membrane (MW 10,000–12,000 cutoff) against methanol for 4 days and distilled water at 4°C for 4 days, and the finally dialyzed solution was subsequently freeze-dried to obtain the polymer **3**. Yield: 80%. ^{31}P-NMR ($CDCl_3$), δ (ppm): 19.6. ^{1}H-NMR ($CDCl_3$), δ (ppm): 0.8–1.0 (s, 6H, —**CH_3** and —CH_2**CH_3** of IleOEt), 1.1–1.3 (b, 9H, —OCH_2**CH_3** of IleOEt and —OCH_2**CH_3** and —OCH(**CH_3**)— of GlyLacOEt), 1.3–1.6 (b, 2H, —**CH_2**CH_3 of IleOEt), 1.6–1.9 (b, 1H, —**CH**(CH_3)CH_2CH_3 of IleOEt), 3.4 (s, 3H, —**CH_3** of AMPEG), 3.5–3.8 (b, 44H, —(**CH_2CH_2**O)$_{11}$— of AMPEG), 3.8 (b, 2H, —O**CH_2**CH_3 of GlyLacOEt), 3.9 (s, 2H, —NH**CH_2**— of GlyLacOEt), 4.1 (s, 1H, —NH**CH**— of IleOEt), 4.2–4.3 (b, 2H, —O**CH_2**CH_3



Polymer 1: NHR = IleOEt (a+b=1.2 , c= 0.80)
Polymer 2: NHR = GlyGlyOH (a=1.23, b= 0.21, c= 0.56)
Polymer 3: NHR = GlyLacOEt (a=1.18, b= 0.02, c= 0.80)

Figure 1 The chemical structures of poly(organophosphazenes).

of IleOEt), 5.0–5.1 (b, 1H, —O**CH**(CH_3)— of GlyLacOEt).

Solubility of 5-fluorouracil in poly(organophosphazene) solution

An excess amount of 5-FU was mixed with the 10% (w/w) aqueous poly(organophosphazene) solutions by vortex mixing for 30 s and stirred at 4°C for 3 days. The suspensions were filtrated through a membrane filter (Whatman®, 0.45 μm) and the concentration of the filtrate was analyzed using a high-performance liquid chromatography (HPLC). The HPLC system was equipped with a C_{18} column (Hyperbond C_{18}, Thermo Fisher Scientific, Waltham, MA), P580 pump, UV D170S detector, and ASI-100 automated sample injector (DIONEX, Sunnyvale, CA). The mobile phase was a methanol–water system with a ratio of 5 : 95 (v/v) for 5-FU. The UV detector was read at 265 nm for maximum absorption wavelength for the 5-FU analysis. The flow rate of the mobile phase was at 0.8 mL/min.

In vitro 5-fluorouracil release study

To evaluate the release of 5-FU from the poly(organophosphazene) hydrogel, 0.4 g of poly(organophosphazenes) was dissolved in 3.6 g of 0.01*M* phosphate-buffered saline (PBS) at pH 7.4. The 5-FU weighed at a pre-determined concentration was fully dissolved in the polymer PBS solutions (10 wt %) by vortex mixing for 30 s and stirring at 4°C for a couple of hours. A 0.5 mL of the polymer solutions incorporated with 5-FU was transferred to millicells (Ø: 12 mm, Millipore) and the millicells were incubated at 37°C for 30 min, in order to transform the solutions into hydrogels. The millicells containing hydrogels were soaked in 10 mL of 0.01*M* PBS solution (pH 7.4) preheated to 37°C under the mild constant shaking (50 rpm). At sampling times, the released medium was replaced with the same amount of the fresh PBS buffer in order to maintain the sink conditions and subjected to be analyzed with the HPLC system previously.

In vitro hydrolytic degradation

The time-dependent hydrolytic degradation behavior was evaluated in terms of residual mass of poly(organophosphazene) hydrogel by mass measurement. The preparation of hydrogel (10 wt%) and the incubation condition (0.01*M* PBS, pH 7.4 and 37°C) were the same manner and the same condition of *in vitro* 5-FU release study noted previously. The mass of residuary hydrogel of the poly(organophosphazenes) was determined by weighing the lyophilized polymers after samples were taken at predetermined time intervals and lyophilized.

TABLE I
The Thermosensitive Characterization of Prepared Poly(organophosphazenes)

Polymer	Formula	T_{ass} (°C)[a]	T_{max} (°C)[b]	$V_{37°C}$ (Pa s)[c]	V_{max} (Pa s)[d]	M_w ($\times 10^4$)[e]	PDI
1	$[NP(IleOEt)_{1.20}\ (AMPEG550)_{0.80}]_n$	17.6	33.6	10.0	790	1.6	3.5
2	$[NP(IleOEt)_{1.23}\ (AMPEG550)_{0.56}\ (GlyGlyOH)_{0.21}]_n$	5.0	31.8	37.5	1573	9.5	3.7
3	$[NP(IleOEt)_{1.18}\ (AMPEG550)_{0.80}\ (GlyLacOEt)_{0.02}]_n$	5.0	36.8	304	308	4.4	2.6

[a] The association temperature at which the viscosity of the polymer solutions (10 wt %) began to increase sharply.
[b] The temperature at which the polymer solutions (10 wt %) reached their maximum viscosity.
[c] The viscosity of the polymer solutions at 37°C.
[d] The viscosity of the polymer solutions at T_{max}.
[e] The molecular weight of the polymers was measured by GPC using THF solutions containing 0.1% (w/v) tetrabutylammonium bromide (TBAB).

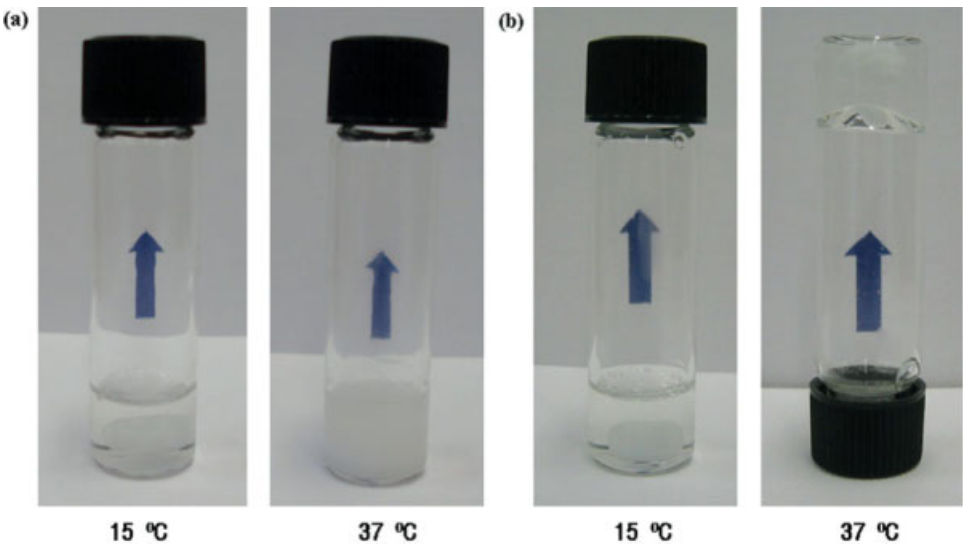


Figure 2 Thermosensitive phase transitions of (a) the aqueous solution of polymer **1**: transparent (15°C) and turbid solution (37°C); (b) the aqueous solution of 0.1% 5-FU incorporated polymer **1**: transparent solution (15°C) and transparent gel (37°C).

RESULTS AND DISCUSSION

Characterization of poly(organophosphazenes)

In this study, we provided the three types of poly(organophosphazenes) bearing a hydrophobic L-isoleucine ethyl ester (IleOEt) and a hydrophilic α-amino-ω-methoxy-poly(ethylene glycol) (AMPEG). As shown in Figure 1, the difference of prepared poly(organophosphazenes) was the existence of a hydrolysis-sensitive glycyl lactate ethyl ester (GlyLacOEt) and a functional glycyl glycine (GlyGlyOH). The polymers were identified by means of ^{1}H- and ^{31}P-NMR spectroscopy, GPC and elemental analysis. The numerical subscriptions used in the formula are the mole ratios of substitutes in poly(organophosphazenes) calculated from their integration ratios of the representative ^{1}H-NMR peaks between methyl protons (6H) of IleOEt, methine proton (1H) of GlyLacOEt, ethylene protons (44H) of AMPEG, and allyl protons (2H) of GlyGlyOAll which generated GlyGlyOH through the cleavage reaction of allyl ester. The polymers were soluble in cold water as well as in several organic solvents such as THF, methanol, and chloroform. The weight average molecular weights of the polymers determined by GPC were in the range from 16,000 to 95,000 and polydispersity indices (PDIs) were less than 3.7.

Thermosensitive properties of poly(organophosphazenes)

The thermosensitive properties of prepared poly(organophosphazenes) were measured by the viscosity as a function of temperature and listed in Table I. The gelation mechanism of thermosensitive poly(organophosphazenes) was reported in the previous reports.[14–17,28] In brief, the gelation of the polymers seems to be attributed to the hydrophobic interactions between the side chain fragments of isoleucine ethyl ester which acts as a physical junction in the polymer solution whereas the hydrogen-bonding interactions between hydrophilic segments of the poly(ethylene glycol) and water molecules are preserved. Also, the thermosensitive properties of poly(organophosphazenes) were affected by the types and ratios of the substitutes.

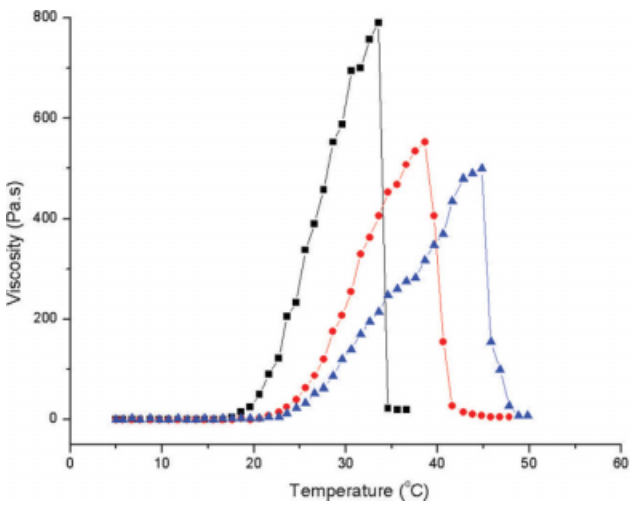


Figure 3 Amount-dependent viscosity variations of 5-FU incorporated polymer **1** solution in 0.01*M* phosphate-buffered solution at pH 7.4 and 37°C. The concentrations of 5-FU: 0% (■); 0.1% (●); 0.75% (▲). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
The Solubility of 5-Fluorouracil in Several Aqueous Polymer Solutions[a]

Polymer	Structure	Solubility (mg/mL) at 4°C	Potential values (mV)
Control	Phosphate-buffered saline	3.39 ± 0.19	–
1	$[NP(IleOEt)_{1.20}(AMPEG550)_{0.80}]_n$	7.66 ± 0.17	2.2
2	$[NP(IleOEt)_{1.23}(AMPEG550)_{0.56}(GlyGlyOH)_{0.21}]_n$	1.70 ± 0.02	−8.8
3	$[NP(IleOEt)_{1.18}(AMPEG550)_{0.80}(GlyLacOEt)_{0.02}]_n$	34.26 ± 2.75	11.5

[a] Concentration of polymer in the aqueous solutions was 10 wt % in pH 7.4 PBS.

Figure 2 photographically demonstrated the thermosensitive phase transitions of the polymer **1** and 5-FU incorporated polymer **1** solutions. The aqueous solution (10%, w/w) of the polymer **1** showed a fluid phase at low temperature and started to become a viscous phase as the temperature was raised to about 17.6°C (T_{ass}). Its viscosity reached to maximum at 33.6°C (T_{max}) and maintained the transparent gel phase until T_{max}. When the temperature was more increased to 37°C, the transparent gel turned into a turbid gel and finally showed a turbid solution at body temperature [Fig. 2(a) and Fig. 3]. After loading of 5-FU in the aqueous poly(organophosphazenes) solution (10%, w/w), the polymer **1** solution incorporated with 0.1% 5-FU maintained the gel phase from 22.8 to 37.8°C and showed the transparent gel at body temperature [Figs. 2(b) and 3].

The thermosensitive gelation properties of the poly(organophosphazenes) also were affected by the amount of 5-FU loaded in the polymer solution as shown in Figure 3. As the amount of 5-FU in the polymer solution was increased, the increase in T_{max} and the decrease in V_{max} were observed. Because the addition of the hydrophilic 5-FU to the poly(organophosphazenes) solution might increase a hydrophilic property of the polymer solution, the 5-FU incorporated polymer solution showed the decrease of V_{max} and the increase of T_{max}.

Solubility of 5-FU

The solubility of 5-FU in PBS at 4°C is 3.39 mg/mL. The solubility of 5-FU in poly(organophosphazene) solutions was improved 1.70–34.26 mg/mL according to the types of poly(organophosphazenes). Polymer **2** solution containing a dipeptide, GlyGlyOH, showed lower solubility than PBS. Polymer **1** and polymer **3** solutions without/with a depsipeptide, GlyLacOEt, showed greater solubility than PBS. In case of the polymer **3**, the solubility of 5-FU in the polymer **3** solution was increased to 10-fold compared with that in PBS. This behavior is probably due to the salt formation of 5-FU because of the pH dependent on the external environment. The aqueous solubility of 5-FU has been reported to be greater at greater pH compared with at lower pH due to its salt formation.[29] We measured the ζ-

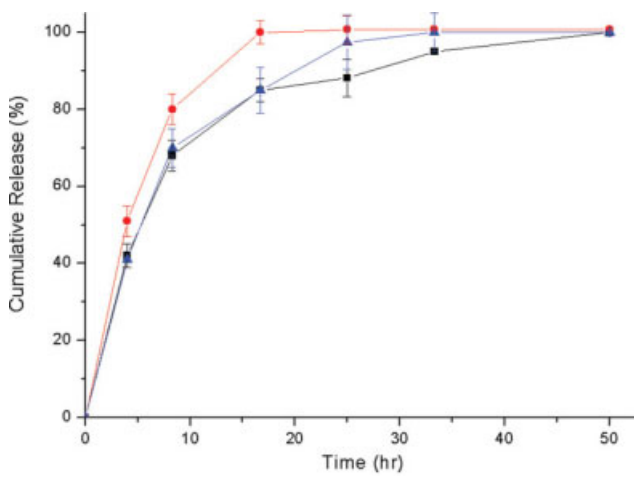


Figure 4 Release profiles from 0.1% 5-FU incorporated polymer gels. Polymer **1** (■); Polymer **2** (●); Polymer **3** (▲). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

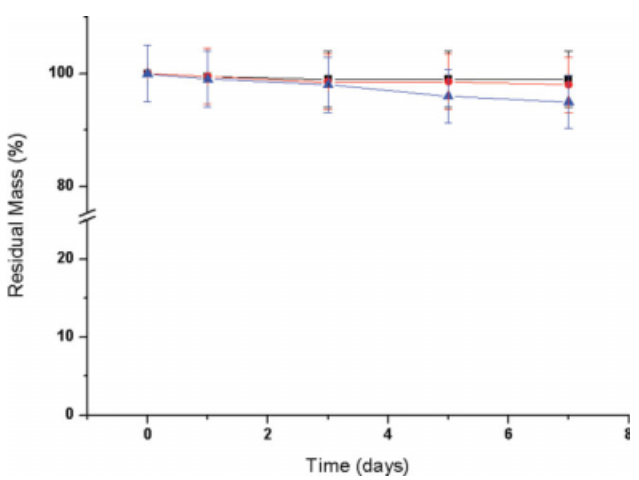


Figure 5 Time-dependent mass decreases of polymer gels. Polymer **1** (■); Polymer **2** (●); Polymer **3** (▲). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

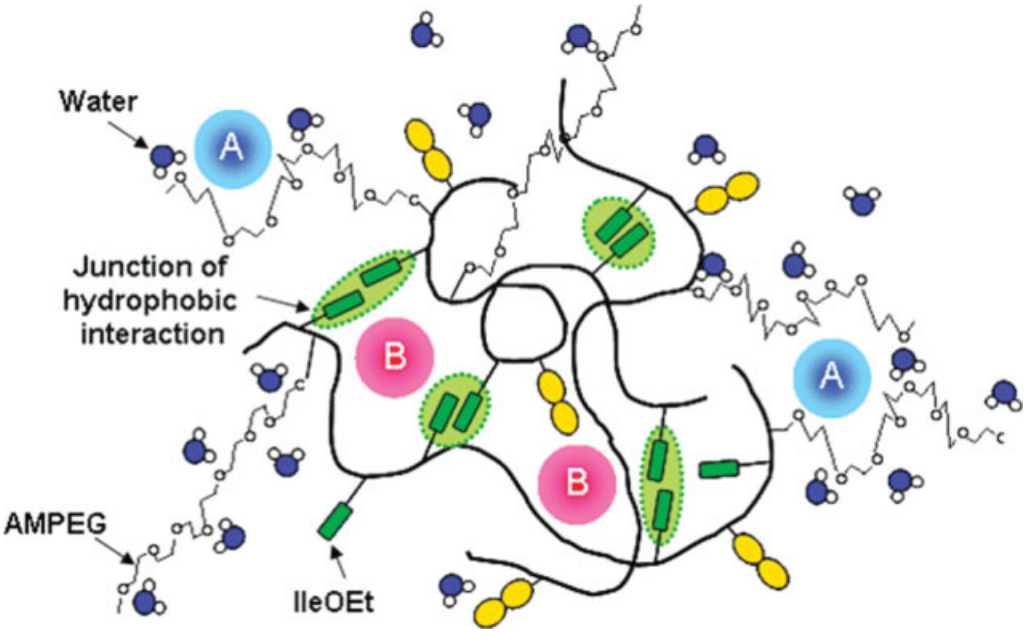


Figure 6 A schematic diagram of the drug distributing in the hydrogel. (A) Hydrophilic domains and (B) hydrophobic domains. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

potential of different types of poly(organophosphazene) solution in distilled water. The results of 5-FU solubility and ζ-potential of the gel solutions are summarized in Table II. The polymer **2** with the lowest solubility of 5-FU showed the ζ-potential was −8.8 mV. The polymer **3** with 11.5 mV of the ζ-potential showed the solubility of 5-FU was the greatest in the polymer solutions. Poly(organophosphazene) solutions with the higher ζ-potential values might easily form the salt formations of 5-FU and showed the higher solubility of 5-FU.

In vitro release studies of 5-FU

The drug release study was performed in 0.01*M* PBS of pH 7.4 at 37°C under mild constant shaking of 50 rpm. The release profiles of 5-FU are shown in Figure 4. We used the three types of poly(organophosphazenes) bearing a hydrophobic group: IleOEt and a hydrophilic group, AMPEG with/without a hydrolysis-sensitive group, and GlyLacOEt or a functional group, GlyGlyOH. These polymer solutions incorporated with 0.1% 5-FU showed a transparent solution at low temperature and turned into a transparent gel at body temperature.

In general, the drug release from the hydrogels occurs by two principal mechanisms drug diffusion from the hydrogel during the initial release phase and release of drug by the erosion of the hydrogel matrix during the later release phase.[30] The time-dependent mass decrease of poly(organophosphazene) hydrogels was showed in Figure 5. In the fully released period of 5-FU, these poly(organophosphazene) hydrogels used in this study lost less than 2% of the total mass of the polymer hydrogels with a minute difference. Thus these results showed that a hydrophilic 5-FU release mechanism from poly(organophosphazene) hydrogels would be only a diffusion-controlled drug release.

As shown in Figure 4, the 5-FU release profile of polymer **2** with strong gel strength showed faster than those of polymer **1** and polymer **3** with weak gel strength. Thus, the strength of poly(organophosphazene) hydrogel had less effect on the drug

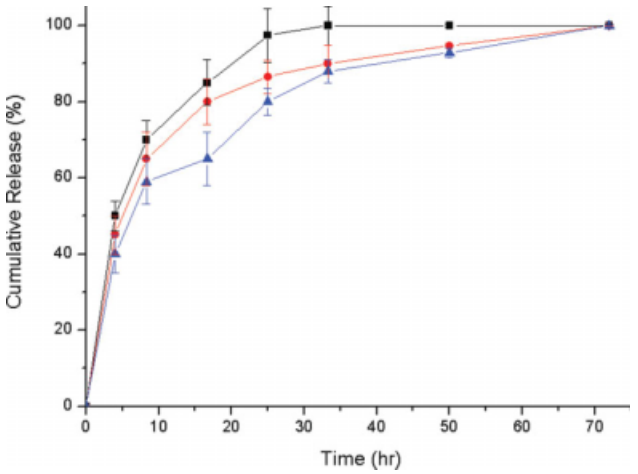


Figure 7 Incorporated 5-FU concentration-dependent release profiles from polymer **3** hydrogel in 10 m*M* PBS at pH 7.4 and 37°C. 0.1% 5-FU incorporated polymer gel (■); 1% 5-FU incorporated polymer gel (●); 3% 5-FU incorporated polymer gel (▲). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

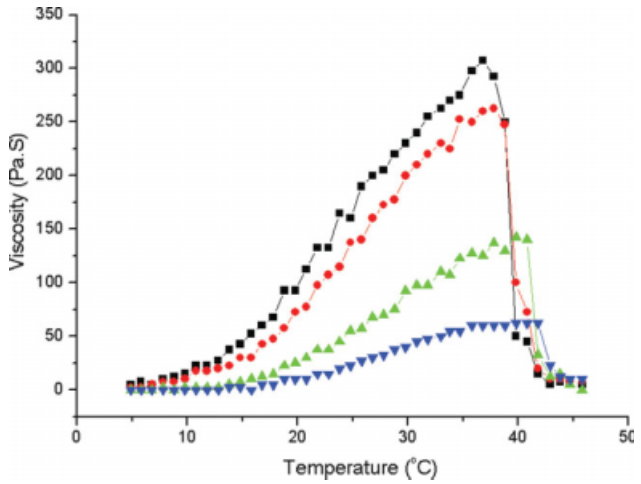


Figure 8 Viscosity changes of polymer **3** solution with varied 5-FU concentrations in 10 m*M* PBS at pH 7.4. The concentrations of 5-FU: 0% of 5-FU (■) 0.1% 5-FU incorporated polymer gel (●); 1% 5-FU incorporated polymer gel (▲); 3% 5-FU incorporated polymer gel (▼). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

release, which is dominated by diffusion. Poly(organophosphazene) hydrogel constituted of a hydrophobic isoleucine network domain and a hydrophilic poly(ethylene glycol) domain as shown in Figure 6. An interaction of 5-FU in the poly(organophosphazene) hydrogel might be a hydrophilic interaction, which the hydrophilic 5-FU tends to partition into the hydrophilic domain. Thus, the release behavior of 5-FU in poly(organophosphazene) hydrogel could be affected by the amount of the hydrophilic moiety of PEG. The polymer **1** and polymer **3** with a greater amount of PEG (0.8, molar ratio) showed slower release profiles than the polymer **2** with lower amount of PEG (0.56, molar ratio) in spite of its strong gel strength. We have been known that the release of 5-FU was affected in the presence of PEG and with a varied molecular weight of PEG from several previous reports.[31,32]

The incorporation of 5-FU has been achieved by the use of dendrimers of poly(amidoamine) modified with mPEG-500. The hydrophilicity of the 5-FU allowed it to complex with the dendrimers after simply incubating the polymer with the drug. For *in vitro* studies, PEGylated formulations showed their releases over 144 h (6 days) while non-PEGylated formulations had completed their releases within 1 day.[31] In case of phosphoester linkage-containing hydrogels based on PEG, the load and release of 5-FU could be controlled by the PEG segments of the varied molecular weights.[32]

The incorporated 5-FU concentration in the polymer solution showed an effect on the release behaviors as shown in Figure 7. The diffusion rate of 5-FU from poly(organophosphazenes) hydrogel was similar to the independent drug diffusion rate. The results indicated that the more amount of 5-FU in the incorporated polymer showed slower release rate than the less amount of 5-FU. It might be explained that during the gelation process some portions of 5-FU could be participated into the junction of hydrophobic interactions or newly formed network systems at the higher concentration of 5-FU. The 5-FU into the junction of hydrophobic interactions at higher concentration might be released a little bit slowly. The higher viscosity level of poly(organophosphazene) hydrogel as shown in Figure 8 have also had less effect on the drug release, which is dominated by diffusion only.

CONCLUSIONS

In this study, we attempted to create a locally injectable drug-delivery system of an anticancer drug, 5-FU, using thermosensitive poly(organophosphazene) hydrogels, which had hydrophobic and hydrophilic substitutes along with a hydrolysis sensitive or functional group. The solubility of 5-FU in the poly(organophosphazene) solution with the greater ζ-potential was increased and the substitute types of poly(organophosphazenes) showed the great effect on solubilization efficiency. The release of 5-FU from poly(organophosphazene) hydrogels was affected by the diffusion controlled during the initial release phase and by the amount of PEG, one of the substitutes in the poly(organophosphazene) hydeogels. The results indicate that the injectable and biodegradable thermosensitive poly(organophosphazene) hydrogel is a promising carrier for 5-FU to increase its solubility, control a relatively sustained and localized release at target sites and thus decrease systemic side effects.

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