Fluorinated Polyphosphazene Polyelectrolytes

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ABSTRACT: Polyphosphazene polyelectrolytes containing various amounts of hydrophobic fluorinated moieties and ionic carboxylic acid groups were synthesized. Polymer compositions and molecular weights were characterized by NMR and gel permeation chromatography. Interestingly, poly[(carboxylatophenoxy)(trifluoroethoxy)phosphazene] containing 60 mol % fluorinated groups was found to be soluble in aqueous solutions. The behavior of fluorinated polyelectrolytes in reactions of ionic complexation with multivalent and monovalent salts was studied in aqueous solutions and ethanol–water mixtures. Such reactions led to the formation of ionotropic hydrogels under mild conditions and, thus, are of importance to the development of microencapsulation processes and controlled release formulations. All of the synthesized polymers underwent phase separation in the presence of multivalent ionic crosslinkers, such as spermine and calcium chloride. This included a water-soluble polyelectrolyte containing 40 mol % ionic groups and hydrophobic polymer with only 3 mol % carboxylic acid groups. J Appl Polym Sci 103: 53–58, 2007

Key words: biological applications of polymers; fluoropolymers; polyelectrolytes; polyphosphazenes; water-soluble polymers

INTRODUCTION

Polyphosphazenes, polymers with phosphorus and nitrogen backbone and organic side groups, present significant interest for life sciences applications. These macromolecules have been advanced in the development of biodegradable materials, immunomodulators, microencapsulation systems, and biocompatible coatings.[1]

Polyphosphazenes containing ionic moieties have been investigated as materials for controlled release formulations and multilayer coatings.[2–5] These polymers show activity in ionic complexation reactions and are capable of forming ionically crosslinked hydrogels under mild conditions. A water-soluble polymer containing carboxylic acid groups, poly[di(carboxylatophenoxy)phosphazene] (PCPP) has been extensively studied in hydrogel-based microencapsulation systems and controlled release formulations.[4–6]

Previous studies have also demonstrated that hydrophobic fluorinated polyphosphazenes are interesting as biocompatible materials.[7,8] Specifically, polyphosphazenes containing trifluoroethoxy side groups showed promising results as stent coatings and were capable of significantly reducing the restenosis rates.[8]

Thus, it was of interest to investigate the properties of polyphosphazenes containing both ionic and fluorinated moieties and to study their potential both as hydrophilic and hydrophobic materials for biological applications. This article describes synthesis and characterization of polyphosphazenes containing various amounts of trifluoroethoxy and carboxylatophenoxy side groups and the evaluation of the behavior of these polymers in reactions of ionic complexation.

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EXPERIMENTAL

Materials

Hexachlorocyclotriphosphazene trimer (Nippon Fine Chemicals, Japan) was used as received. The macromolecular precursor polydichlorophosphazene (PDCP) was synthesized with the ring-opening polymerization of hexachlorocyclotriphosphazene in a titanium pressure reactor, as described previously.[9] Propyl 4-hydroxybenzoate (propyl paraben; ≥ 99.5%, Aldrich Chemical Co., Inc., Milwaukee, WI) was dried before use in a vacuum oven at 80°C for 2 h. 2-Methoxyethyl ether (diglyme; anhydrous, 99.5%) and sodium hydride (95%; Aldrich Chemical Co.), 2,2,2-trifluoroethanol and tetra-*n*-butylammonium bromide (Alfa Aesar, Ward Hill, MA), and tetrahydrofuran (THF; anhydrous) and *N*,*N*-dimethylacetamide (EMD Chemicals, Inc., Gibbstown, NJ) were used as received. Spermine, tetrahydrochloride (*N*,*N*′-bis(3-aminopropyl)-1,4-butanediamine tetrahydrochloride, was purchased from Sigma Chemical Co. (St. Louis, MO) and was used as received. Poly[bis(trifluoroethoxy)phosphazene] and PCPP were synthesized as described previously.[1,6]

Analytical methods

^{1}H-NMR spectra in *N,N*-dimethylformamide (DMF)-d_7 were recorded with a Bruker 400 NMR spectrometer (Billerica, MA) with magnet operated at 400 MHz. The chromatographic system was equipped with a Waters 510 high performance liquid chromatography pump (Milford, MA), a Waters 717 plus autosampler, and a Waters 410 refractive-index detector. A Waters Styragel HT6E column was used with a Waters Styragel guard column; *N,N*-dimethylacetamide containing 0.1% tetra-*n*-butyl ammonium bromide was used as a mobile phase, and the flow rate was 0.8 mL/min. Molecular weights were calculated on the basis of polystyrene standards. The results were processed with Waters Millenium software.

Synthesis of poly[(2,2,2-trifluoroethoxy)(4-carboxylatophenoxy)phosphazenes]: Copolymers I, II, and III

Polyphosphazene copolymers containing trifluoroethoxy and carboxylatophenoxy side groups were synthesized as exemplified in the following procedure for polymer **II** (Table I).

A suspension of sodium hydride (0.214 g, 8.48 mmol) in 3 mL of diglyme was slowly added to a solution of propyl 4-hydroxybenzoate (1.83 g, 10.1 mmol) in 5 mL of diglyme under nitrogen to form sodium propyl 4-hydroxybenzoate. Sodium 2,2,2-trifluroethoxide was prepared by adding the suspension of sodium hydride (0.64 g, 25.4 mmol) in 1.3 mL of THF to a solution of 2,2,2-trifluoroethanol (3.0 g, 30 mmol) in 5 mL of THF. The solution of sodium propyl 4-hydroxybenzoate was added slowly to a solution of 1.4 g (24.2 mequiv) of poly(dichlorophosphazene) in 25 mL of diglyme at 50°C under nitrogen. The temperature was increased to 90°C, and the reaction was continued at this temperature for an additional 3 h while stirring. The reaction mixture was then cooled to ambient temperature and diluted with 50 mL of THF. Then, the solution of sodium 2,2,2-trifluroethoxide was added slowly to the reaction mixture. The reaction was continued at room temperature for additional 24 h. After the completion of the reaction, THF was evaporated, the reaction temperature was increased to 90°C, and 15 mL of 12.7*N* aqueous potassium hydroxide solution was slowly added. The reaction was continued with stirring for 1 h, and then, the polymer was recovered by precipitation in 165 mL of 0.4*N* aqueous hydrochloric acid. The polymer was then purified two times by redissolution in 50 mL of THF and precipitation in 210 mL of 0.2*N* aqueous hydrochloric acid. The yield after drying *in vacuo* was 2.32 g.

Copolymer compositions

The composition was determined with ^{1}H-NMR and HPLC. (1) It was calculated on the basis of the ratio between the peak areas of the ethylene protons of 2,2,2-trifluoethoxy side group and the aromatic protons of the carboxylatophenoxy side group in ^{1}H-NMR spectra. (2) The composition was determined with HPLC based on the difference in refractive index signals of poly[bis(trifluoroethoxy)phosphazene] (negative peak) and PCPP (positive peak) in *N,N*-dimethylacetamide. We plotted the calibration curves by plotting HPLC peak areas of PCPP, poly[bis(trifluoroethoxy)phosphazene], and their mixtures versus mixture composition. The polymer concentration was 3 mg/mL in the mobile phase.

Water sorption

Polymer films were prepared by a solvent casting method. The polymer (23 mg) was dissolved in 0.4 mL of a solvent. THF was used for the acidic and salt forms of polymer **I**, DMF was used for the acidic and salt forms of polymer **II** and the acidic forms of polymer **III** and PCPP, and water was used for the salt form of polymer **III** and PCPP. These solutions were placed in glass dishes with a flat bottoms (i.d. = 23 mm). The samples were first kept at ambient

TABLE I
Polymer Characterization Data

Polymer	Content of —COOH (mol %)[a]	Yield (%)	$M_w \times 10^{-3}$ (g/mol)	^{1}H-NMR (ppm)
I	3	53	180	(a) 6.9–7.4, (b) 7.8–8.0, (c) 4.2–4.9
II	14	72	295	(a) 6.7–7.3, (b) 7.5–7.9, (c) 4.1–4.6
III	40	52	246	(a) 6.6–7.3, (b) 7.5–7.9, (c) 3.8–4.6

M_w = weight-average molecular weight.
[a] Calculated on the basis of ^{1}H-NMR data.

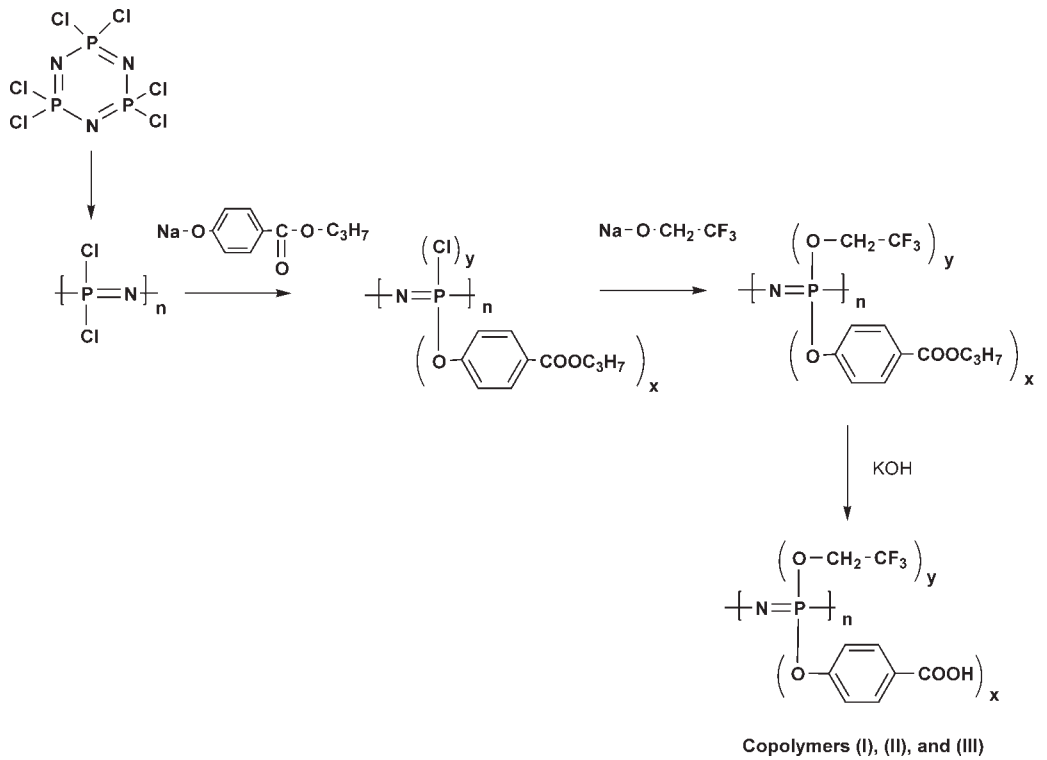

Figure 1 Synthesis of fluorinated polyphosphazene polyelectrolytes.

temperature for 3 days to remove the solvent and then dried in a vacuum oven at 35°C for 2 days. The thickness of the films was estimated to be 55 μm.

Water sorption was studied by the incubation of polymer films in saturated water vapors in a desiccator containing water on the bottom at 22°C. The amount of water absorbed by the sample was calculated gravimetrically and expressed as a percentage by weight (weight of water in the sample per dry weight of polymer).

Turbidimetric titration

The turbidimetric titration of polymers with spermine, sodium chloride, and calcium chloride was conducted by the measurement of the turbidity of their aqueous solutions with an ultraviolet–visible spectrophotometer (Genesys 2, Spectronic Instruments, Inc., Rochester, NY) at 500 nm. The solutions of 0.35% w/v of spermine, 30% w/v of sodium chloride, and 0.5% w/v of calcium chloride were used for the titration of 1 mL of 0.08% w/v polymer solution. After the addition of titrant, the solutions were mixed by hand and analyzed immediately.

RESULTS AND DISCUSSION

Polymer synthesis and characterization

Polymers containing various amounts of trifluoroethoxy and carboxylatophenoxy side groups were synthesized with the macromolecular substitution reaction of polydichlorophosphazene (Fig. 1). Chlorinated polyphosphazene was first modified with the desired amount of sodium propyl 4-hydroxybenzoate and then treated with an excess of sodium trifluoroethoxide (Fig. 1). These substitution reactions were followed with the alkaline hydrolysis step to yield carboxylic acid functions on the polymer.

Polymer characterization data is shown in Table I. All of the synthesized polyphosphazenes had molecular weights in excess of 100,000 and relatively high yields, which suggested the absence of significant degradation processes during the substitution reaction. The content of carboxylic acid groups varied in the range of 3–40 mol % (Table I and Fig. 2). Figure 2 also demonstrates good agreement between the two methods used for the determination of copolymer composition: ^{1}H-NMR and HPLC (on the basis of the differences in the refractive index increments for two polymers). As shown in Figure 2, the composition of the synthesized copolymers could be controlled through the amount of sodium propyl 4-hydroxybenzoate in the reaction mixture, although the content of carboxylic acid groups in the copolymers was noticeably lower than expected. Extending the reaction time beyond 3 h can probably lead to a more effective incorporation of propyl 4-hydroxybenzoic groups in the polymer, however, this can also lead to a higher probability of crosslinking and hydrolytic reactions involving chlorine atoms of the polymer.[6]

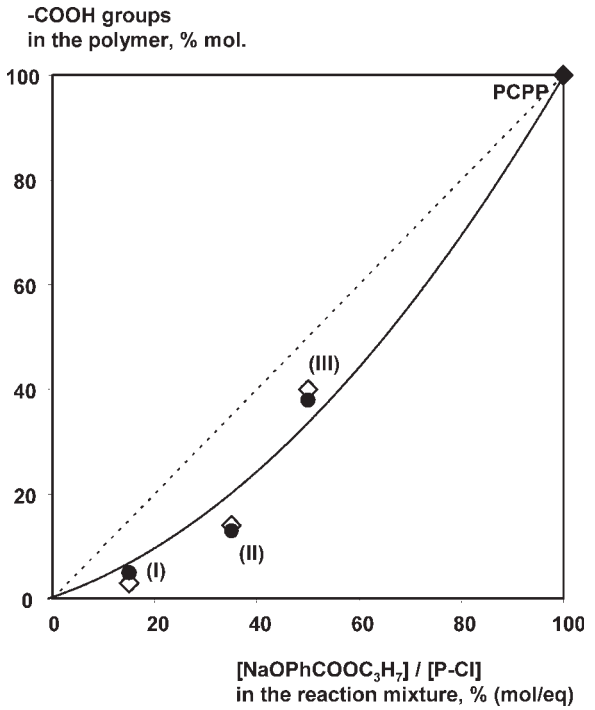

Figure 2 Content of carboxylic acid groups in copolymers **I**, **II**, and **III** and PCPP versus the ratio of sodium propyl 4-hydroxybenzoate and chlorine atoms in (—) polydichlorophosphazene (PDCP) (mol/mequiv). The polymer composition was determined by (◇) ^{1}H-NMR and (●) HPLC. (- - -) Expected content of carboxylic acid groups in copolymers based on the amount of propyl 4-hydroxybenzoate in the reaction mixtures.

Studies of polymer solubility and water sorption

One of the important characteristics of fluorinated polyelectrolytes is the ease of manipulating them in common solvents.[10] Table II shows solubility data for the synthesized copolymers. Interestingly, copolymer **III**, containing the highest content of carboxyl groups, was found to be soluble in water when converted to a sodium salt form with the equivalent amount of sodium hydroxide. The water solubility of this hydrophobic polyphosphazene, which contained approximately 60 mol % hydrophobic trifluoroethoxy groups, makes it compatible with previously described aqueous-based protein encapsulation methods.[4] All of the synthesized polymers were soluble in a mixture of 25% (v/v) water and 75% (v/v) ethanol.

The water sorption characteristics of fluorinated polyelectrolytes were also studied. Polymer films were cast on glass supports, and samples were exposed to the air saturated with water vapors at 22°C. Water sorption was monitored gravimetrically. Figure 3 shows the water uptake for fluorinated polyelectrolytes and PCPP, both in the sodium salt and acid forms, as a percentage of the initial sample's weight after 5 h of exposure. As expected on the basis of the solubility data, maximum swelling was achieved

TABLE II
Solvents (Ss) and Nonsolvents (NSs) for Fluorinated Polyphosphazene Polyelectrolytes

	Polymer		
	I	**II**	**III**
Water	NS	NS	S[a]
DMF and dimethyl sulfoxide	S	S	S[b]
Ethanol and methanol	S	S	NS
THF and diglyme	S	S[b]	NS
Hexane and heptane	NS	NS	NS

[a] Soluble in a sodium salt form only.
[b] Soluble in an acid form only.

for the sodium salt of **III** and PCPP. For these samples, there was also a much higher water uptake by polymeric salts compared to acids, which also followed from the differences in solubility. There was no distinction, however, in the swelling of the salt and acid forms of **I** and **II**. This can be probably explained either by the rigidity of the polymer matrix or by the differences in sodium ion density in the vicinity of carboxylic acid groups, which can lead to the modulation in the hydrophobicity of PCPP copolymers.[6] The water uptake of polymers **I** and **II** after 5 h of exposure (4.5–5%) was only slightly higher than that of the fluorinated homopolymer poly[bis(trifluoroethoxy)phosphazene] (0.5%), whereas the salt form of polymer **III** showed 55% swelling under the same conditions. A longer incubation of the polymers did not result in a significant increase in swelling, except for coatings based on water-soluble polymers: the salt forms of PCPP and **III**. Thus, it appears that fluorinated polyelectrolytes can be used for the preparation of both hydrophilic and hydrophobic coatings.

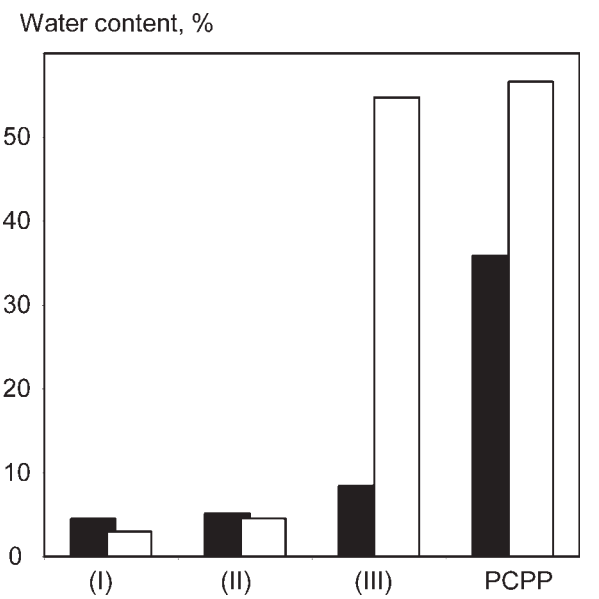

Figure 3 Percentage water uptake for polymers **I**, **II**, and **III** and PCPP in the (□) sodium salt form and (■) acid form (saturated water vapor, 22°C, 5 h).

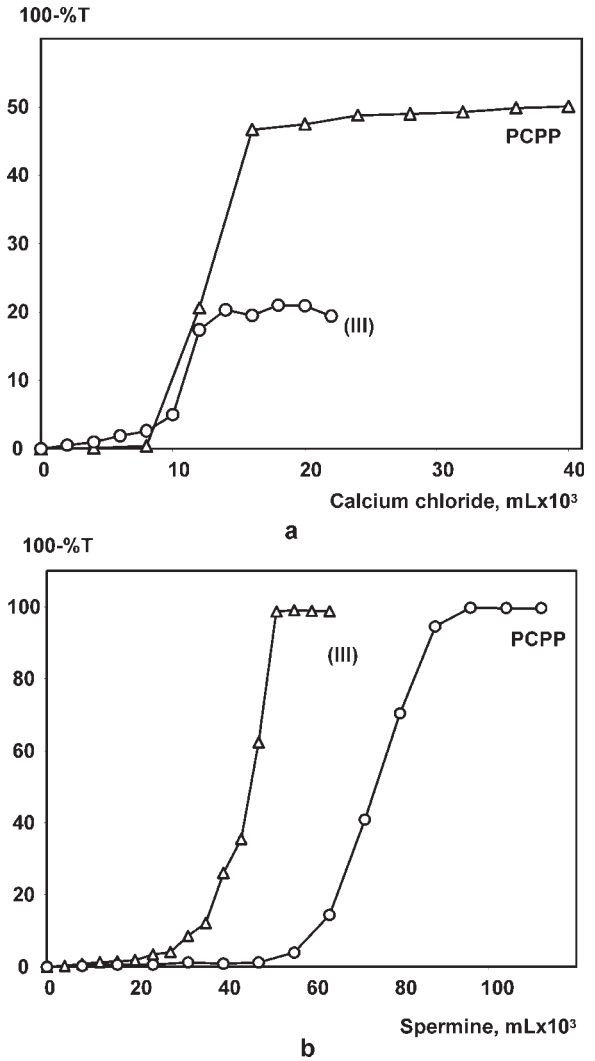

Figure 4 Turbidimetric titration of polymer **III** and PCPP with (a) calcium chloride and (b) spermine in water (solutions: 0.08% w/v polymer, 0.17*M* calcium chloride, and 0.01*M* spermine; 20°C).

Ionic complexation in aqueous solutions

Reactions of ionic complexation are of practical importance for polyelectrolytes because they lead to the formation of ionotropic hydrogels under mild conditions, which allows the convenient preparation of hydrophilic microspheres and biocompatible coatings.[2,11] Salts of multivalent ions, such as calcium chloride or spermine tetrachloride are typically used as crosslinking agents in such systems. Ionic complexation also offers a simple method for the production of microparticulates, for example, through the coacervation of polyphosphazene polyelectrolytes with sodium chloride.[4,12] Thus, it was of interest to evaluate the interactions of the synthesized fluorinated polyphosphazene polyelectrolytes with these ions. Because polymer **III** was water soluble, it was possible to investigate such reactions in aqueous solutions, which are common media for the encapsulation of proteins. In our studies, the ionic sensitivity of copolymer **III** was compared to the behavior of the most studied polyphosphazene microencapsulating agent, PCPP.[4]

Figures 4 and 5 show the results of the turbidimetric titration of PCPP and copolymer **III** with calcium chloride, spermine, and sodium chloride in aqueous solutions. Both PCPP and copolymer **III** interacted with aqueous calcium chloride with the formation of a precipitate, which is in accordance with previous findings.[4] Titration experiments showed no major differences in the reactivity of these polymers with the divalent ion [Fig. 4(a)]. However, it appeared that polymer sensitivity toward spermine significantly increased with the introduction of hydrophobic trifluoroethoxy groups in the macromolecular structure. The amount of spermine required for the onset of a phase separation for copolymer **III** was lower than that for PCPP [Fig. 4(b)]. The results demonstrate that both spermine and calcium ions can be used for the formation of hydrogels with fluorinated polymer **III**. This combination of water solubility, high content of the fluorinated side groups, and reactivity of carboxylic acid groups can open new opportunities in the design of novel polymer-based drug delivery systems.

The results of turbidimetric titration with aqueous sodium chloride demonstrate that contrary to PCPP, copolymer **III** is not sensitive to the presence of sodium ions in solution (Fig. 5). This correlates with previous findings showing that the phase separation

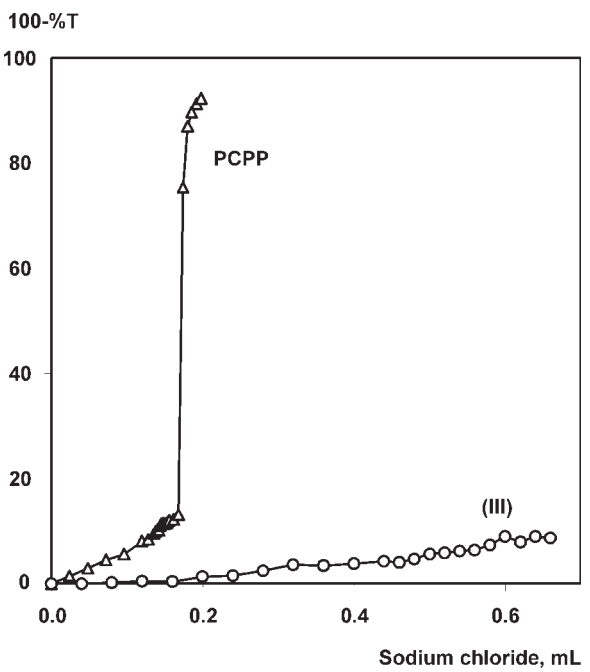

Figure 5 Turbidimetric titration of polymer **III** and PCPP with sodium chloride (solutions: 0.08% w/v polymer, 5.13*M* sodium chloride, and water; 20°C). T is transmittance.

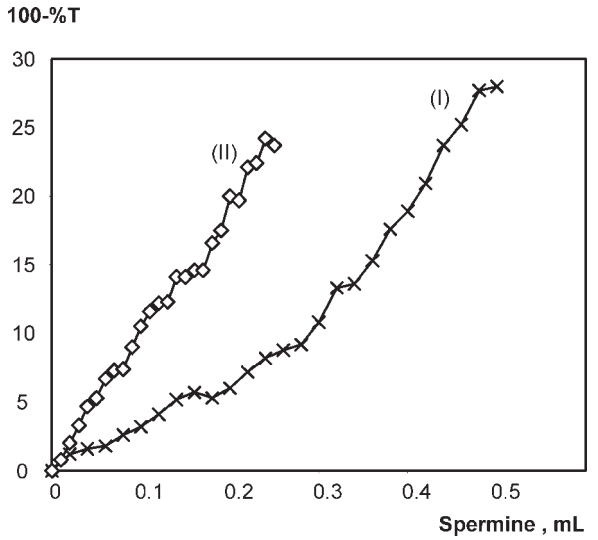

Figure 6 Turbidimetric titration of polymers **I** and **II** with spermine in an ethanol–water mixture (75 : 25 vol %; solutions: 0.08% w/v polymer and 0.02*M* spermine; 20°C).

threshold in such systems is a function of carboxylic acid content in the polymer but not the polymer hydrophobicity.[6] The results, once again, emphasize the importance of the steric component in PCPP–sodium salt interactions, where even minimal distortions in PCPP structure lead to a loss of sodium sensitivity.[6]

Ionic complexation in ethanol–water mixtures

The ionic complexation reactions of polymers **I** and **II** were also studied. Because these polymers were not soluble in water, the evaluation was conducted in an ethanol–water mixture (75/25 vol %), where clear solutions were obtained. Both polymers did not show any signs of phase separation in the presence of sodium chloride, and there were only minor changes in the turbidity after the addition of calcium chloride. However, the formation of heterogeneous systems was observed on the addition of spermine (Fig. 6). Remarkably, polymer **I**, which contained only 3 mol % carboxylic acid groups, demonstrated the ability to interact with the ionic crosslinker.

As mentioned previously, polyphosphazenes containing trifluoroethoxy side groups were investigated as stent coatings and showed promising results in the reduction of restenosis rates.[8] Further improvements require the development of drug eluting coatings on the basis of this polymer.[8] These results demonstrate that the introduction of even 3 mol % carboxylic acid groups in such polymers can generate an ionically crosslinkable polymer, which can potentially provide a powerful tool for the modulation of drug release profiles.

CONCLUSIONS

Novel polyphosphazene polyelectrolytes containing fluorinated side groups were synthesized and characterized. Their behavior in reactions of ionic complexation was studied. Both spermine and calcium ions can be used for the formation of ionotropic hydrogels with fluorinated polyphosphazene polyelectrolytes. Interestingly, polyphosphazene containing 60 mol % trifluoroethoxy moieties demonstrated solubility in aqueous solutions despite the high content of fluorinated side groups. Fluorinated polyphosphazene with only 3 mol % carboxylic acid groups were active in the reactions of ionic complexation. These results are interesting for the development of both hydrophilic and hydrophobic fluorinated drug delivery systems, such as microspheres or coatings.

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