Report on the Use of Poly(organophosphazenes) for the Design of Stimuli-Responsive Vesicles

ARTICLE *in* BIOMACROMOLECULES · NOVEMBER 2004

Impact Factor: 5.75 · DOI: 10.1021/bm0400527 · Source: PubMed

2 AUTHORS, INCLUDING:

Anne-Claude Couffin

Atomic Energy and Alternative Energies Com…

21 PUBLICATIONS **368** CITATIONS

Report on the Use of Poly(organophosphazenes) for the Design of Stimuli-Responsive Vesicles

Anne-Claude Couffin-Hoarau† and Jean-Christophe Leroux*

Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, C.P. 6128 Succ. Centre-ville, Montreal (Qc) Canada H3C 3J7

Received June 7, 2004; Revised Manuscript Received September 21, 2004

A novel family of amphiphilic temperature- and pH-sensitive poly(organophosphazenes) with varying ratios of ethylene oxide, alkyl chains and free acid units was synthesized by living cationic polymerization. Depending on their composition, these poly(organophosphazenes) exhibited lower critical solution temperatures ranging from 32 to 44°C, which were pH-dependent for copolymers bearing carboxylic acid groups. The alkylated copolymers were then anchored into phospholipid bilayers to obtain stimuli-responsive liposomes that released their content upon a change in temperature or pH. Such polymer/vesicle complexes could find practical applications for site-specific and intracellular drug delivery.

Introduction

Polyphosphazenes (PPZ) are inorganic polymers with backbones consisting of alternating nitrogen and phosphorus atoms linked by alternating single and double bonds. The phosphorus atoms can each accommodate 2 organic or organometallic side groups. These polymers are of interest as fire retardants,[1,2] low-temperature elastomers,[3] and fuel cell membranes.[4] They are also under development as solid polymer lithium ion conductors[5,6] and as biomedical materials.[7,8] A distinctive feature of PPZ is the ease with which polymer properties can be tuned by attaching appropriate pendant groups to the phosphorus atoms. Additionally, the phosphorus−nitrogen backbone can be rendered hydrolytically unstable so as to generate phosphate and ammonia as nontoxic degradation products. Biodegradability is generally achieved by introducing low amounts of hydrolytically labile substituents such as amino acid esters.[9−11] Thus, because of their versatility in terms of chemical and physical properties, PPZ offer tremendous potential for pharmaceutical applications.

It has been shown that poly(organophosphazenes), substituted with oligo-ethylene oxide segments, exhibit a lower critical solution temperature (LCST) in aqueous solution. Recently, a series of biodegradable PPZ bearing methoxy-poly(ethylene glycol) and amino acid esters as side groups was synthesized by Sohn and co-workers[12,13] and presented phase transition temperatures ranging from 30 to 80 °C. These polymers were exploited in the design of "intelligent" drug delivery systems, such as temperature-responsive, in situ forming hydrogels.[14] Of particular interest is the preparation of stimuli-sensitive phospholipid vesicles. These vesicles can be readily obtained by complexation with a polymer undergoing coil-to-globule phase transition upon a change in environmental conditions. For instance, temperature-sensitization of liposomes can be achieved by decorating the vesicles with a polymer exhibiting a LCST. At temperatures above the LCST, the polymer collapses, creating defects in the lipid bilayers and triggering the release of its encapsulated cargo. Indeed, it has been demonstrated that liposomes coated with *N*-isopropylacrylamide (NIPAM) copolymers bearing long alkyl chains acquire temperature-responsive properties.[15−17] Temperature-responsive vesicles hold great promise in cancer chemotherapy to selectively deliver drugs at tumoral sites where mild hyperthermia can be applied.[18] Over the last 5 years, we have been interested in pH-sensitive liposomal formulations based on copolymers of NIPAM and methacrylic acid (MAA).[19,20] pH-sensitive liposomes have been proposed to facilitate the delivery of drugs to the cytoplasm following receptor-mediated endocytosis.[21] After cell uptake, the liposomes transit via endosomes, where the acidic pH can induce their destabilization. Although interesting results have been obtained so far with NIPAM copolymer/liposome complexes, the nonbiodegradability of the polymer may be a serious limitation to their widespread use in the biomedical field. We therefore investigated whether PPZ could be employed for the preparation of stimuli-sensitive vesicles. In this communication, we report the synthesis and characterization of stimuli-responsive PPZ grafted with up to three different substituents, namely, ethoxyethoxyethoxy (EEE) groups, stearyl chains, and amino butyric acid (ABA). Such copolymers were complexed to liposomes for the first time and used to trigger content release as a function of temperature and pH.

Experimental Section

Materials and Equipment for Polymer Synthesis. Tetrahydrofuran (THF) and diethyl ether (Aldrich) were distilled over sodium-benzophenone ketyl under dry nitrogen atmosphere. Dichloromethane (DCM) and triethylamine

* To whom correspondence should be addressed. Phone: (514) 343-6455. Fax: (514) 343-7738. E-mail: Jean-Christophe.Leroux@umontreal.ca.

† Current address: Lysac Technologies Inc., 75 J-Armand-Bombardier, Boucherville (Qc) Canada J4B 8P1.

10.1021/bm0400527 CCC: $27.50
Published on Web 10/15/2004

Table 1. Characteristics of Poly(organophosphazenes)[a]

#	formula	M_w[b]	PDI	acid (mol %)	LCST (°C)	NMR ^{31}P δ (ppm)
1	$[NP(EEE)_2]$	20 280[c]	1.04		32.1	−7.9
2	$[NP(EEE)_{1.94}(C_{17}C(O)(EO)_5)_{0.06}]$	44 650[c]	1.04		33.1	−5.5
3	$[NP(EEE)_{1.9}(C_{17}C(O)(EO)_5)_{0.1}]$	30 030[c]	1.04		33.9	−6.1
4	$[NP(EEE)_{1.8}(C_{17}C(O)(EO)_5)_{0.2}]$	37 850[c]	1.12		42.5	−5.7
5	$[NP(MPEG350)_2]$	26 660[c]	1.55		n/d	−5.6
6	$[NP(MPEG350)_{1.9}(C_{17}C(O)(EO)_5)_{0.1}]$	60 620[c]	1.03		n/d	−5.6
7	$[NP(EEE)_{1.89}(ABA)_{0.11}]$	35 800[d]	1.08	5.7	31.3[e]	−6.1
8	$[NP(EEE)_{1.82}(ABA)_{0.18}]$	40 200[d]	1.05	9.4	44.2[e]	−6.1
9	$[NP(EEE)_{1.71}(ABA)_{0.19}(C_{18}(EO)_2)_{0.1}]$	36 730[d]	1.09	9.7	30.7[e]	−7.9
10	$[NP(EEE)_{1.64}(ABA)_{0.26}(C_{18}(EO)_{10})_{0.1}]$	n/a	n/a	13.1	34.2[e]	−6.5; 1.2
11	$[NP(EEE)_{1.72}(ABA)_{0.18}(C_{18}(EO)_{10})_{0.1}]$	38 000[d]	1.05	9.2	32.4[e]	−7.0

[a] n/d: not detected. n/a: not available. $C_{18}(EO)_2$ = Brij 72. $C_{18}(EO)_{10}$ = Brij 76. [b] Polymers were not necessarily prepared using the same poly(dichlorophosphazene) backbone. [c] Trizma (50 mM), pH 8 was the GPC solvent. [d] DMF, 10 mM LiBr was the GPC solvent. [e] LCST values were determined at pH 7.4 in HEPES-saline buffer (20 mM HEPES, 140 mM NaCl).

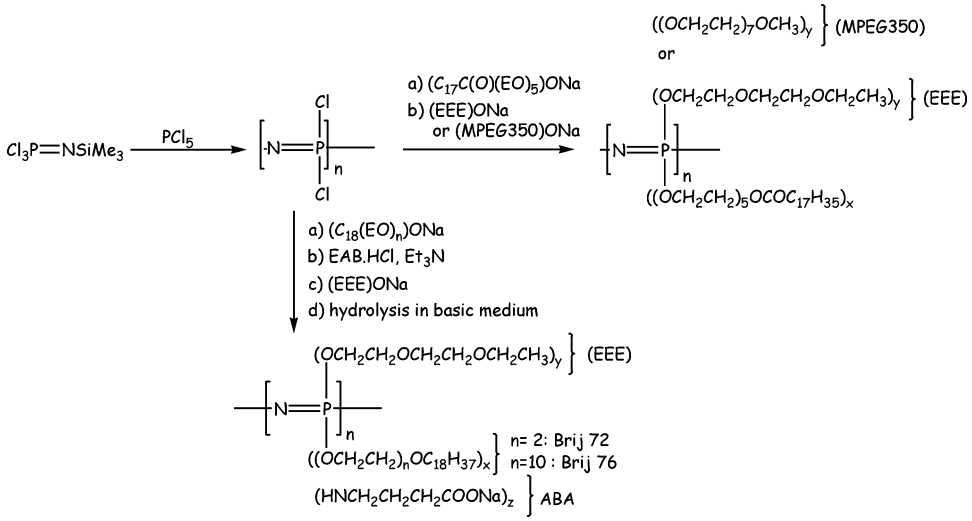
(Aldrich) were dried and distilled over CaH_2 and KOH, respectively. Phosphorus pentachloride (PCl_5) (Aldrich) was sublimed under vacuum. Phosphorus trichloride, di(ethylene glycol) ethyl ether, and sulfuryl chloride (Aldrich) were distilled under argon before use. Methoxypoly(ethylene glycol) 350 (MPEG 350), polyoxyethylene 5 stearyl ester ($C_{17}C(O)(EO)_5$), polyoxyethylene 2 stearyl ether ($C_{18}(EO)_2$) (Brij72), and polyoxyethylene 10 stearyl ether ($C_{18}(EO)_{10}$) (Brij76) were purified by azeotropic distillation in toluene or dried overnight under vacuum over P_2O_5. Ethylaminobutyrate hydrochloride (EAB) (Aldrich) was used as received. All glassware was flame-dried under vacuum or dried overnight in an oven. The reactions were performed by standard Schlenk techniques or in a dry argon atmosphere glovebox. ^{1}H and ^{31}P NMR spectra were recorded on a Brücker ARX400 spectrometer operating at 400 and 121.4 MHz, respectively. The solvents used for all spectra were either $CDCl_3$ or D_2O, and chemical shifts were referenced internally to these deuterated solvents.

Synthesis and Characterization of PPZ Derivatives. Trichloro(trimethylsilyl)-phosphoranimine ($Cl_3P{=}NSiMe_3$) was prepared by oxidizing chlorophosphine, $Cl_2PN(SiMe_3)_2$, with sulfuryl chloride in diethyl ether at 0°C, as described elsewhere.[22] The resultant crude oil was purified by distillation under static vacuum (ca. 27 °C, 0.1 mmHg, yield 75%) giving the monomeric precursor $Cl_3P{=}NSiMe_3$ as a colorless liquid. Poly(dichlorophosphazenes) were synthesized via controlled cationic polymerization of $Cl_3P{=}NSiMe_3$ at room temperature, as reported previously.[23,24] Briefly, a solution of 3.66 g (16.3 mmol) $Cl_3P{=}NSiMe_3$ in 15 mL of DCM was added to 0.084 g (0.4 mmol) of PCl_5 in 30 mL of DCM and stirred at room temperature. The progression of polymerization was monitored by ^{31}P NMR spectroscopy. The reaction was stopped after complete disappearance of the −54 ppm peak of $Cl_3P{=}NSiMe$, and the appearance of a new peak at −17 ppm for $[N{=}PCl_2]_n$. Volatiles were removed at reduced pressure, and the resultant colorless oil was stored at −80 °C until substitution of chlorine atoms. Stimuli-sensitive polymers were prepared by adapting the procedure described by Song et al.[13] The preparation conditions of a typical PPZ derivative (polymer 9, Table 1) are given below. The sodium salt of 2-(2-ethoxyethoxy)ethanol was prepared by reacting a solution of di(ethylene glycol) ethyl ether (1.95 mL, 14.4 mmol) in THF (20 mL) with a suspension of NaH (0.328 g, 13 mmol in 50 mL THF) overnight at room temperature. Similarly, the sodium salt of $C_{18}(EO)_2$ was obtained by adding a solution of $C_{18}(EO)_2$ (0.301 g, 0.42 mmol in 10 mL THF) to a suspension of NaH (0.011 g, 0.42 mmol in 15 mL THF). Alcoholate solutions were passed through a metallic filter before being added to the polymer solution to remove excess NaH. A $(C_{18}(EO)_2)$-ONa solution was added dropwise over 1 h to a solution of poly(dichlorophosphazene) (0.49 g, in 20 mL THF). The resulting mixture was stirred for 6 h at room temperature. Subsequently, a suspension of EAB hydrochloride (0.142 g, 0.85 mmol) in 20 mL of THF containing 0.26 mL (1.87 mmol, 2.2 eq.) of freshly distilled triethylamine was transferred to the polymer solution. The reaction was stirred overnight at 50 °C and allowed to proceed for an additional 24 h at room temperature. The reaction mixture was filtered. Finally, sodium EEE was added dropwise, and the third substitution was undertaken with stirring for 24 h. The mixture was concentrated under vacuum, and the polymer was purified by successive dialyzes against a THF/water solution and water for 2 days. The copolymer was isolated by freeze-drying to yield a pale yellow oil (yield 75%). The free acid derivative (ABA) was generated by overnight alkaline hydrolysis of the EAB moiety. After hydrolysis and neutralization, the polymer was dialyzed against water for 24 h and freeze-dried.

Elemental analysis. Polymer 1: Anal. Calcd.: C, 46.31; H, 8.42; N, 4.50. Found: C, 46.26; H, 8.95; N, 4.49. Polymer 2: Anal. Calcd.: C, 47.97; H, 8.62; N, 4.20. Found: C, 45.21; H, 8.66; N, 4.54. Polymer 3: Anal. Calcd.: C, 48.97; H, 8.74; N, 4.02. Found: C, 45,29; H, 8.70; N, 4.42. Polymer 4: not available. Polymer 5: Anal. Calcd.: C, 49.79; H, 8.63; N, 1.94. Found: C, 48.85; H, 9.29; N, 1.95. Polymer 6: Anal. Calcd.: C, 50.80; H, 8.87; N, 1.90. Found: C, 48.38; H, 9.08; N, 2.07. Polymer 7: Anal. Calcd.: C, 45.59; H, 8.26; N, 5.01. Found: C, 43.59; H, 8.53; N, 5.19. Polymer 8: not available. Polymer 9: Anal. Calcd.: C, 47.79; H, 8.60; N, 5.02. Found: C, 44.87; H, 8.73; N, 5.31. Polymer 10: not available. Polymer 11: Anal. Calcd.: C, 49.04; H, 8.76; N, 4.55. Found: C, 47.08; H, 9.01; N, 5.16.

Weight-average molecular weights (M_w) and polydispersity indices (PDI) were ascertained by gel permeation chromatography (GPC), using a Waters 1525 unit equipped with Ultrahydrogel (aqueous conditions) and Styragel HT

Scheme 1. Polymerization and Macromolecular Substitution of Poly(dichlorophosphazenes)



(organic conditions) columns connected to a Waters 2410 refractive index detector and a PD 2000DLS dynamic light scattering detector. The samples were eluted (flow rate = 1 mL/min) at room temperature in either Trizma (50 mM), pH 8, or DMF, 10 mM LiBr. The LCST of each polymer solution (5 mg/mL) was determined by differential scanning calorimetry (DSC) (VP-DSC, Microcal, LLC) at a heating rate of 1°C/min. The LCST values were taken as the maximum of the endothermic peak from the thermograms. DSC measurements were undertaken in citric acid buffers (55 mM, pH 3 or 4), in 2-*N*-(morpholino)ethanesulfonic acid (MES)-saline buffers (200 mM, 50 mM NaCl, pH 5 or 200 mM, 20 mM NaCl, pH 6) or in 2-*N*-hydroxyethylpiperazine-2-*N*′-ethanesulfonic acid (HEPES)-saline buffers (40 mM, 140 mM NaCl, pH 6.8 to 9.4). pH-sensitive PPZ were titrated with an automatic titrator (775 Dosimat, Metrohm) and an Accumet AP61 pH-meter equipped with an Accumet AP50 electrode. Experiments were performed in triplicate.

In Vitro Release Kinetics. Lipid films composed of egg phosphatidylcholine (EPC) and cholesterol (Chol) (Northen Lipids) (3:2 mol/mol) were hydrated in an isotonic aqueous solution of trisodium 8-hydroxypyrene trisulfonate (HPTS) (35 mM) and *p*-xylene-bis-pyrimidium bromide (50 mM) (Molecular Probes), used as fluorescent marker and quencher, respectively. The liposomes were subsequently extruded through polycarbonate filters. To prepare liposome−polymer complexes, the polymer (0.01−0.3% *w/w* vs lipids) was either incorporated during the liposome preparation procedure or incubated with preformed liposomes overnight at 4 °C, as described previously.[20,25] Nonencapsulated dye and free polymer were separated from the liposomes by GPC over a Sepharose 2B column (Sigma) in HEPES buffer (20 mM, 140 mM NaCl, pH 7.4). Liposome hydrodynamic diameter was determined by dynamic light scattering at 25 °C and at a fixed scattering angle of 90° using a Malvern Autosizer 4800 equipped with an uniphase argon-ion laser. The extent of content release was recorded on a SAFIRE microplate reader (Tecan GmbH) by monitoring the increase in fluorescence intensity of HPTS (λ_{ex}= 413 nm, λ_{em}= 512 nm) after incubation of the vesicles at different temperatures or pH values (37 °C). One hundred % release was obtained after sample lysis with 10% (*w/v*) Triton X-100. The effect of serum on the release kinetics was assessed by incubating selected formulations in 75% (*v/v*) human serum for 1 h at 37 °C. Excess serum components were removed by GPC prior to content release evaluation. Temperature-dependent release kinetics were performed in HEPES buffer (20 mM, 140 mM NaCl, pH 7.4). pH-dependent release kinetics were carried out in MES buffers (200 mM, 50 mM NaCl, pH 5 or 200 mM, 20 mM NaCl, pH 6) or HEPES-saline buffers (40 mM, 150 mM NaCl, pH 7.4 or 7.8).

Results and Discussion

Synthesis and Characterization. Poly(dichlorophosphazene) was synthesized by living cationic polymerization and then substituted as illustrated in Scheme 1. Up to three different side groups were introduced in the polymer chain. The EEE groups were grafted to provide PPZ with a LCST close to human body temperature. Stearyl ester ($C_{17}C(O)(EO)_5$) or ether ($C_{18}(EO)_2$ or $C_{18}(EO)_{10}$) were chosen as C_{18}-bearing chains to allow anchoring of the polymers into the lipid membranes. The hydrophobic anchor chain content was kept below 10 mol % to ensure adequate hydration of the copolymers at room temperature (data not shown). pH-sensitive polymers were prepared by reacting EAB with poly(dichlorophosphazene) via its amino function. Hydrolysis of the ester-ending moiety of EAB in alkaline media generated free acid units (ABA side chains).

Several PPZ with a low PDI (Figure 1) were prepared, and their physicochemical characteristics are presented in Table 1. All copolymers yielded pale yellow, viscoelastic solids. Their absolute molecular weight values, determined by GPC with light scattering detection, corroborated those calculated on the basis of monomer-to-initiator ratios. However, in aqueous solutions, the GPC chromatograms of most copolymers bearing alkyl substituents displayed a second peak, which corresponded to the presence of large size aggregates (>700 000). This finding suggests the formation of supramolecular assemblies that was probably driven by hydrophobic interactions between the alkyl substituents. For copolymers bearing ABA, the proportion of carboxylic acid units was evaluated by reverse-pH titration and ranged between 5 and 13 mol % (Table 1). The presence

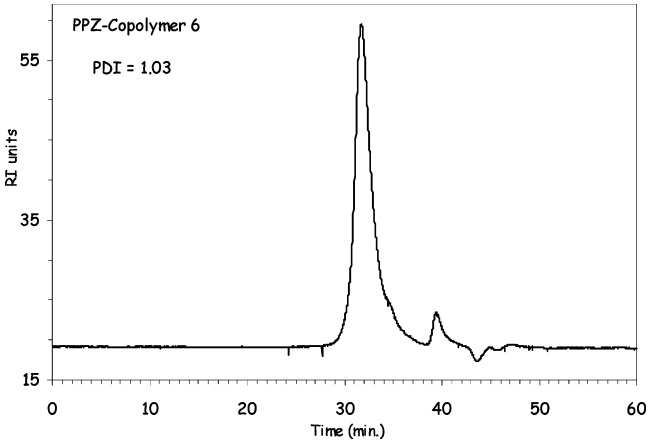


Figure 1. GPC trace of polymer 6 in Trizma (50 mM), pH 8.

of C_{18} chains was confirmed by ^{1}H NMR, with a small peak at 0.9 ppm that could be assigned to the terminal methyl group of the stearyl chain.

Thermal and pH Sensitivity. Thermosensitive PPZ bearing EEE and C_{18} chains as side groups exhibited a LCST ranging between 32 and 43 °C (Table 1, copolymers 1 to 4). Polymer collapse and phase separation at the LCST have been described as an entropy-driven process implicating hydrophobic interactions.[26] At low temperatures, owing to their three oxygen atoms, the EEE groups develop strong H-bonding interactions with water and thus ensure polymer hydrosolubility. As the temperature is raised, the H-bonds weaken, while hydrophobic interactions increase and become predominant above the LCST. Incorporation of more hydrophilic substituents is generally known to increase the LCST, whereas hydrophobic substituents have the opposite effect.[12,26] In our study, a LCST shift toward higher temperatures was observed with the incorporation of alkyl chains. The LCST values increased from 32.1 °C for copolymer 1 (no hydrophobic moiety) to 42.5 °C for copolymer 4 (10 mol % of C_{18} side groups). It should be pointed out that, in this case, the grafted alkyl chains are linked to the backbone through an oligo(ethylene oxide) spacer. Being hydrophilic, the effect of this spacer on the LCST might predominate over that of the stearyl chain. Furthermore, in water, the segregation of hydrophobically modified polymers allows the protection of alkyl substituents from the aqueous environment and, therefore, prevents their hydrophobic contribution to the LCST.[27]

Copolymers 5 and 6, which are substituted with MPEG 350 alone or in combination with C_{18} chains, did not display any LCST. This can be explained by the high hydrophilicity of PEG units with a molecular weight of 350. These results are consistent with previous studies demonstrating the absence of cloud points for PPZ containing PEG with molecular weights of 350 and 750.[13,28,29]

All synthesized copolymers bearing ABA units exhibited a pH-dependent LCST (Table 1, copolymers 7 to 11). At pH 7.4, the effect of ABA content on the PPZ LCST is exemplified by copolymers 7 and 8. As the ABA content increased from 5.7 to 9.4 mol %, the LCST rose from 31.3 to 44.2 °C, reflecting the hydrophilic contribution of the ionizable substituent.[30,31] Figure 2 shows typical thermograms of a copolymer bearing ABA units (copolymer 7) in aqueous

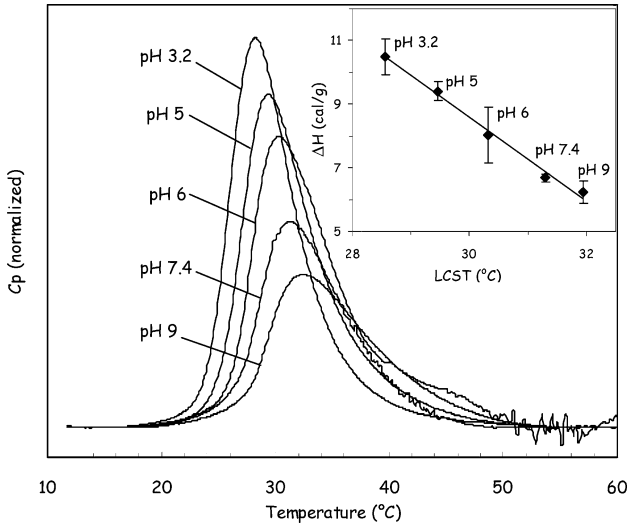


Figure 2. DSC thermograms of polymer 7 at varying pH values (3.2−9). The inset represents the enthalpies of phase transition (ΔH) as a function of LCST at different pH (Mean ± SD, $n = 3$).

solution at different pH values. Phase transition temperature was heightened with increasing pH. This can be explained by the copolymer deprotonation and solubility increments seen at elevated pH values. Also, the endothermic peaks broadened with rising pH. Such broadening of the endothermic peaks has been observed with NIPAM copolymers bearing MAA units[32,33] and has been attributed to the segmentation of cooperative domains by ionized carboxylic acid units in a molecule that undergoes an all-or-none phase transition.[26,32,34] As illustrated in Figure 2 (inset), the heat of phase transition (ΔH) decreased linearly vs the LCST ($r^2 = 0.991$). A similar trend has been reported for NIPAM copolymers[26,35,36] and alkyl ether-based PPZ.[37] The ΔH of NIPAM copolymers is mainly attributed to breakage of water−water hydrogen bonds in the hydration shell surrounding the isopropyl hydrophobic groups. The decrease of ΔH at higher LCST reflects the progressive loss of structured water molecules surrounding the hydrophobic groups as the temperature rises.[26]

In Vitro Release Kinetics. The thermosensitive copolymers were evaluated for their ability to destabilize EPC/Chol liposomes above 37 °C. Polymer insertion into the lipid bilayer did not trigger polymer aggregation (data not shown). Figure 3 shows the amount of HPTS released as a function of temperature for different liposome/polymer formulations. As reported previously for NIPAM copolymers,[19] it can be seen that polymer anchoring into the lipid bilayer is essential for effective membrane destabilization. Indeed, copolymer 1, which is devoid of hydrophobic alkyl chains, was unable to trigger the release of more than 10% of the encapsulated dye above its LCST (>32 °C). Conversely, copolymer 3, which bears 5 mol % stearyl moieties interacted with the phospholipid membrane and triggered the release of up to 80% of liposomal content upon an increase in temperature. However, the proportion of anchor chain should be kept low, given that copolymer 4, which contains 10 mol % of stearyl moieties, was poorly efficient. Polymers with high hydrophobic content may strongly self-associate through intra/intermolecular interactions and not anchor well into the lipid bilayer. Polymer binding studies will be undertaken in the

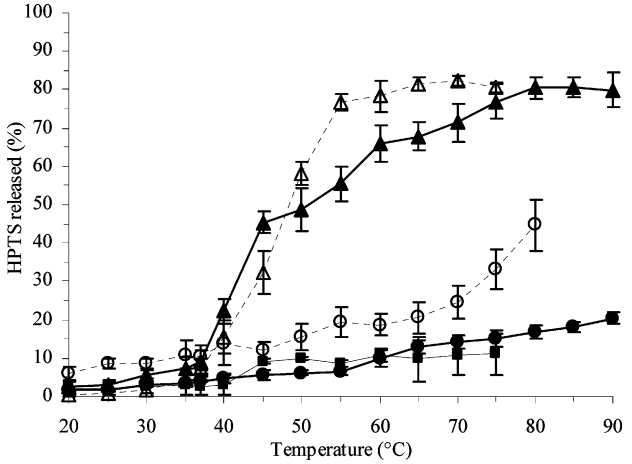


Figure 3. Percent HPTS released from EPC/Chol (3:2 mol/mol) liposomes after 8-min incubation (polymer/lipid = 0.3% *w/w*) at pH 7.4 (20 mM HEPES, 140 mM NaCl). Polymers 1 (squares), 3 (triangles), and 4 (circles) were either incubated with preformed liposomes (closed symbols) or incorporated during the liposome preparation procedure (open symbols). Mean liposome diameter: 150 nm. Mean ± SD ($n = 3$).

future to clarify this issue. As shown in Figure 3, copolymer addition during the liposome preparation procedure elicited slightly higher content leakage than copolymer insertion via incubation with preformed vesicles. In the former procedure, the polymer is located in both monolayers (vs selective inclusion in the outer leaflet with the post-insertion method) and could destabilize the liposomal membrane more efficiently. In addition, incorporation of the polymer during the preparation step may also result in better insertion efficiency.

As depicted in Figure 4, pH-sensitive vesicles were obtained by adding an ionizable PPZ (copolymer 11, 0.05−0.1% *w/w*) to liposomes during their preparation. At 37 °C and at pH values >7.4, slow but continuous leakage was observed irrespective of the polymer concentration. This may be explained by the fact that the experiments were carried out at body temperature (37 °C), which is slightly greater than the polymer's LCST measured by DSC, even under near neutral conditions (data not shown). Lowering the pH to mildly acidic values (pH 5−6) increased polymer dehydration and triggered, within 1 min, virtually complete release of entrapped HPTS for polymer/lipid mass ratios of 0.05 and 0.1. The drastic enhancement of content release observed at pH 5−6 is thought to be related to the high cooperativity of the phase transition, which favors polymer collapse.[33] The short time span over which release occurred is an important feature of the system since, upon endocytosis, the transit time

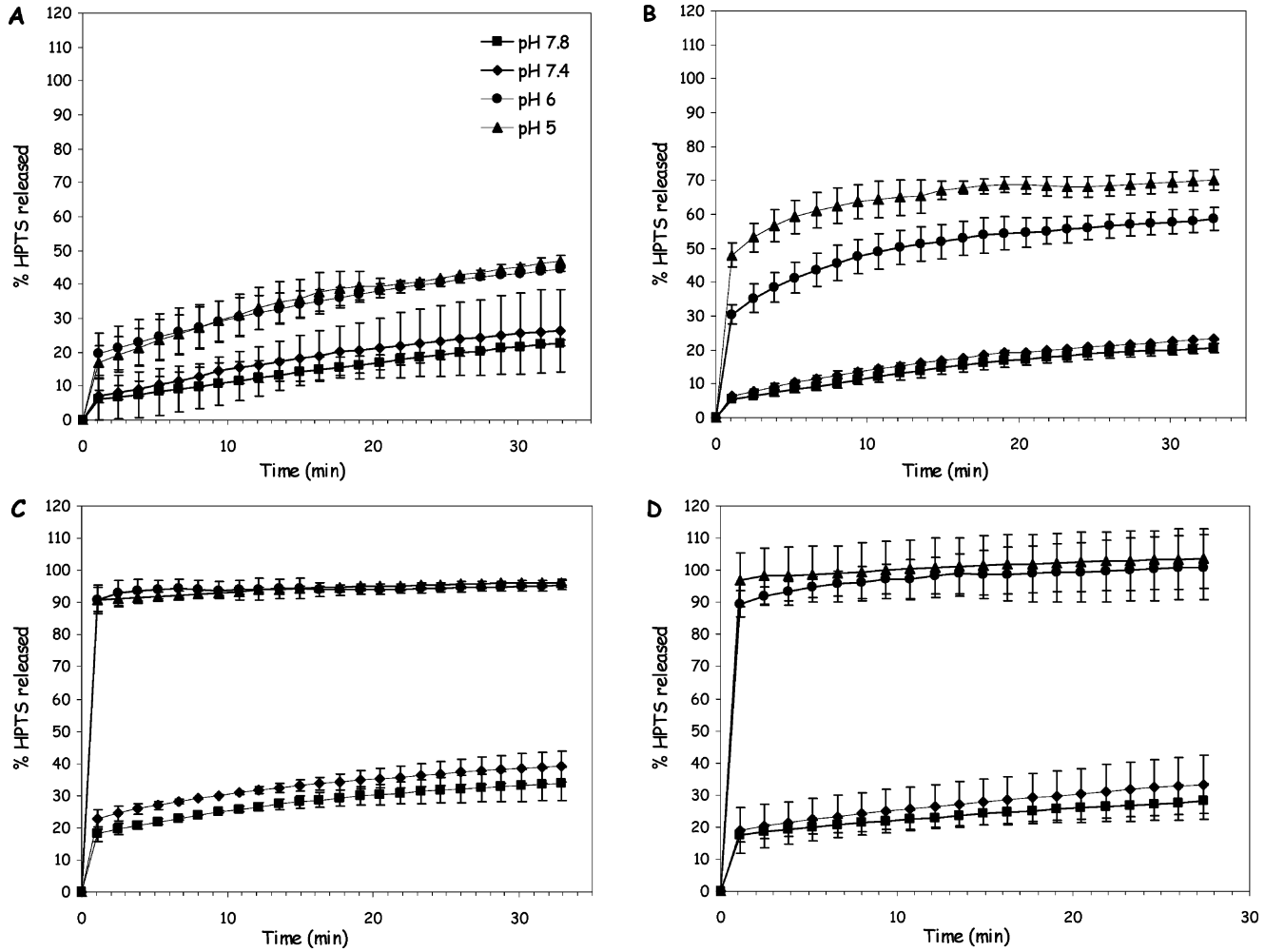


Figure 4. Percent HPTS released from pH-sensitive EPC/Chol (3:2 mol/mol, polymer 11) liposomes at 37 °C and pH values ranging from 5 to 7.8. Polymer/lipid mass ratios: (A) 0.01, (B) 0.02, (C) 0.05, and (D) 0.1. Polymers were dissolved in MES-saline buffers (200 mM, 50 mM NaCl, pH 5 and 200 mM, 20 mM NaCl, pH 6) or in HEPES-saline buffers (40 mM, 150 mM NaCl, pH 7.4 and 7.8). Mean liposome diameter: 150 nm. Mean ± SD ($n = 3$).

from uptake to degradation in lysosomes is generally about 30 min.[38] Moreover, pH-responsiveness was generally preserved after incubation in human serum, with approximately 10% loss in the amount of HPTS released after 30 min at pH 5 (data not shown). Such formulations still require optimization to minimize leakage at pH 7.4 as vesicles would otherwise empty their content in plasma before reaching their target. Nevertheless, these preliminary results suggest that stimuli-responsive PPZ/liposome systems could provide a valuable biodegradable alternative to current polymer-based vesicles for drug delivery to endosomal compartments or to tumoral sites where external heat can be applied.

Acknowledgment. The authors express their gratitude to Prof. Michel Lafleur for his assistance with the DSC experiments and to David Ghattas and Anand Dhanikula for their critical reading of the manuscript. Financial support from the Canadian Institutes of Health Research and the Canada Research Chair program is acknowledged.

References and Notes

(1) Taylor, J. P.; Allcock, H. R. *Polym. Eng. Sci.* **2000**, *40*, 1177−1189.
(2) Reed, C. S.; Taylor, J. P.; Guigley, K. S.; Coleman, M. M.; Allcock, H. R. *Polym. Eng. Sci.* **2000**, *40*, 465−472.
(3) Allcock, H. R. *Adv. Mater.* **1994**, *6*, 106−115.
(4) Guo, Q. H.; Pintauro, P. N.; Tang, H.; O'Connor, S. *J. Membr. Sci.* **1999**, *154*, 175−181.
(5) Allcock, H. R.; Kellam, E. C. *Solid State Ionics* **2001**, *143*, 297−308.
(6) Blonsky, P. N.; Shriver, D. F.; Austin, P.; Allcock, H. R. *J. Am. Chem. Soc.* **1984**, *106*, 6854−6855.
(7) Luten, J.; van Steenis, J. H.; van Someren, R.; Kemmink, J.; Schuurmans-Nieuwenbroek, N. M. E.; Koning, G. A.; Crommelin, D. J. A.; van Nostrum, C. F.; Hennink, W. E. *J. Controlled Release* **2003**, *89*, 483−497.
(8) Qiu, L. *J. Appl. Polym. Sci.* **2003**, *87*, 986−992.
(9) Allcock, H. R.; Fuller, T. J.; Mack, D. P.; Matsumura, K.; Smeltz, K. M. *Macromolecules* **1977**, *10*, 824−830.
(10) Allcock, H. R.; Hymer, W. C.; Austin, P. E. *Macromolecules* **1983**, *16*, 1401−1406.
(11) Allcock, H. R.; Pucher, S. R.; Scopelianos, A. G. *Biomaterials* **1994**, *15*, 563−569.
(12) Lee, S. B.; Song, S.-C.; Jin, J. I.; Sohn, Y. S. *Macromolecules* **1999**, *32*, 7820−7827.
(13) Song, S.-C.; Lee, S. B.; Jin, J. I.; Sohn, Y. S. *Macromolecules* **1999**, *32*, 2188−2193.
(14) Lee, B. H.; Lee, Y. M.; Sohn, Y. S.; Song, S.-C. *Macromolecules* **2002**, *35*, 3876−3879.
(15) Kono, K.; Hayashi, H.; Takagishi, T. *J. Controlled Release* **1994**, *30*, 69−75.
(16) Hayashi, H.; Kono, K.; Takagishi, T. *Biochim. Biophys. Acta* **1996**, *1280*, 127−134.
(17) Kim, J. C.; Bae, S. K.; Kim, J. D. *J. Biochem.* **1997**, *121*, 15−19.
(18) Needham, D.; Dewhirst, M. W. *Adv. Drug Delivery Rev.* **2001**, *53*, 285−305.
(19) Meyer, O.; Papahadjopoulos, D.; Leroux, J. C. *FEBS Lett.* **1998**, *42*, 61−64.
(20) Zignani, M.; Drummond, D. C.; Meyer, O.; Hong, K.; Leroux, J. C. *Biochim. Biophys. Acta* **2000**, *1463*, 383−394.
(21) Drummond, D. C.; Zignani, M.; Leroux, J. C. *Prog. Lipid. Res.* **2000**, *39*, 409−460.
(22) Wang, B.; Rivard, E.; Manners, I. *Inorg. Chem.* **2002**, *41*, 1690−1691.
(23) Honeyman, C. H.; Manners, I. *J. Am. Chem. Soc.* **1995**, *117*, 7035−7036.
(24) Allcock, H. R.; Crane, C. A.; Morrissey, C. T.; Nelson, J. M.; Reeves, S. D.; Honeyman, C. H.; Manners, I. *Macromolecules* **1996**, *29*, 7740−7747.
(25) Roux, E.; Francis, M.; Winnik, F. M.; Leroux, J. C. *Int. J. Pharm.* **2002**, *242*, 25−36.
(26) Feil, H.; Bae, Y. H.; Feijen, J.; Kim, S. W. *Macromolecules* **1993**, *26*, 2496−2500.
(27) Ringsdorf, H.; Venzmer, J.; Winnik, F. M. *Macromolecules* **1991**, *24*, 1678−1686.
(28) Allcock, H. R.; Pucher, S. R.; Scopelianos, A. G. *Macromolecules* **1994**, *27*, 1071−1075.
(29) Vandorpe, J.; Schacht, E. *Polymer* **1996**, *37*, 3141−3145.
(30) Hirotsu, S.; Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *87*, 1392−1395.
(31) Chen, G.; Hoffman, A. S. *Nature* **1995**, *373*, 49−52.
(32) Maeda, Y.; Yamamoto, H.; Ikeda, I. *Langmuir* **2001**, *17*, 6855−6859.
(33) Roux, E.; Lafleur, M.; Lataste, E.; Moreau, P.; Leroux, J. C. *Biomacromolecules* **2003**, *4*, 240−248.
(34) Tiktopulo, E. I.; Bychkova, V. E.; Ricka, J.; Ptitsyn, B. *Macromolecules* **1994**, *27*, 2879−2882.
(35) Otake, K.; Inomata, H.; Konno, M.; Saito, S. *Macromolecules* **1990**, *23*, 283−289.
(36) Maeda, Y.; Higuchi, T.; Ikeda, I. *Langmuir* **2001**, *17*, 7535−7539.
(37) Allcock, H. R.; Dudley, G. K. *Macromolecules* **1996**, *29*, 1313−1319.
(38) Mukherjee, S.; Ghosh, R.; Maxfield, F. *Physiol. Rev.* **1997**, *77*, 759−803.

BM0400527