Synthesis and Characterization of Diblock and Gel-Forming Pentablock Copolymers of Tertiary Amine Methacrylates, Poly(ethylene glycol), and Poly(propylene glycol)

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ABSTRACT: Novel pH-sensitive gel-forming pentablock copolymers based on commercially available Pluronic (poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide), PEO-*b*-PPO-*b*-PEO) triblock copolymers and cationic diblock copolymers based on poly(ethylene glycol) methyl ether (PEGME) were synthesized by oxyanionic polymerization. Polymerization of the cationic moiety, poly((diethylamino)-ethyl methacrylate), PDEAEM, was initiated by a difunctional potassium alcoholate of the triblock Pluronic copolymer F127 (PEO_{106}−PPO_{69}−PEO_{106}) or PEGME. The difunctionality of the initiation using the triblock macroinitiator, indicating formation of a pentablock copolymer rather than a tetrablock copolymer, was verified by functionalized termination of the living polymer chains. Critical micellization temperatures (cmt) of the synthesized polymers were obtained from differential scanning calorimetry for the pentablock materials. The pentablock copolymers retained the thermoreversible gel-forming properties of Pluronic F127 as well as similar cmt values. The polydispersity of both the diblock and pentablock copolymers was similar to the macroinitiators, indicating a very low polydispersity associated with the addition of the cationic PDEAEM blocks. Both of the materials show pH-sensitive release behavior, whereas the native polymers do not.

Introduction

Interest in the development of novel environmentally sensitive biomaterials for drug delivery applications has grown in the past several years. Cross-linked hydrogels have been developed that incorporate characteristics such as pH and/or temperature sensitivity for stimuli-sensitive release.[1,2] It has been established that the incorporation of monomeric units containing tertiary amines introduces pH-dependent swelling in cross-linked polymeric membranes.[3−5] Many of the studies involving cross-linked copolymer membranes of tertiary amines and other materials focus on the use of these materials for glucose-sensitive insulin delivery.[4,5] With the incorporation of the enzyme glucose oxidase, materials that swell under low-pH conditions will swell under conditions of high glucose concentration[6] and have been explored for use in self-regulating systems for insulin delivery. However, there have been very few studies that attempt to exploit these pH-dependent functionalities in non-cross-linked injectable systems. Such systems would have advantages over cross-linked systems as they can be simply injected into the body to form solid non-cross-linked gels that will eventually dissolve and be excreted.

The triblock copolymer Pluronic (poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)) has distinct amphiphilic properties and the ability to form non-cross-linked gels. Under the appropriate concentration and thermal conditions, aqueous solutions of this polymer form micellar systems consisting of dehydrated poly(propylene oxide) cores surrounded by solvated poly-(ethylene oxide) coronas.[6−8] The segregated lipophilic nanophase can increase the total aqueous solution solubility of small organic molecules like naphthalene[9] and ibuprofen,[10] molecules that are relatively insoluble in nonmicellar aqueous solutions.

At sufficient polymer concentrations, these materials undergo a sol−gel transition at temperatures slightly higher than the critical micellization temperature (cmt).[11] This non-cross-linked gel is soluble in an aqueous medium as water penetrates the gel, lowering the total concentration of polymer at the gel interface below a concentration sufficient to maintain the gel state at that temperature. This thermoreversible gelation property has been investigated for use as a controlled release delivery device both in vitro[12−14] and in vivo.[15,16] Aqueous polymer/drug solutions can be injected intramuscularly[15] or intraperitoneally[16] to produce non-cross-linked matrix delivery devices that do not require surgical insertion or removal. Drug release is controlled by the dissolution of the polymer gel as water penetrates the device at the polymer/tissue interface.[14]

However, Pluronics are not sensitive to pH, and typical in-vitro dissolution times for such these devices are on the order of 5−6 h.[12,14] Although in-vivo release times are slightly longer, on the order of 10−20 h,[15−16] Pluronic polymers, on their own, may not be extremely useful for controlled drug or bioactive molecule delivery. Because of the availability of orally administered controlled released tablets, injectable devices must release their dosage over a time period much longer than that available with Pluronic-based devices in order to compete with the available technology.

The incorporation of stimuli-sensitive functionality, for example pH sensitivity, into a Pluronic-based deliv-
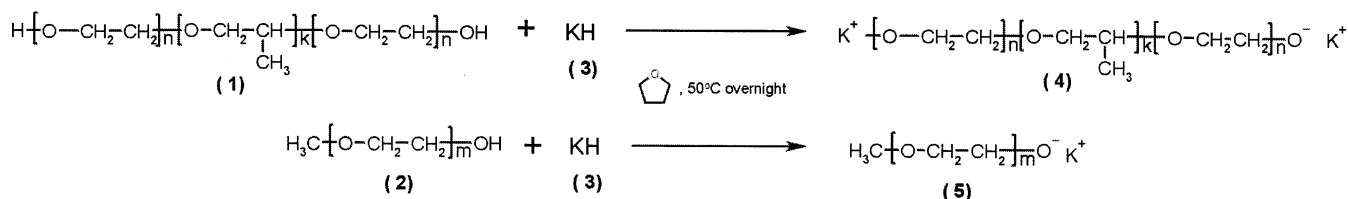
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Scheme 1. Reaction Scheme for Initiator Formation Reactions

ery device could, however, provide modulated delivery over a time period similar to or longer than the current release devices based on Pluronics. In the past, research on self-monitoring modulated delivery included cross-linked pH-sensitive polymers or implanted electronic microsensors and micropumps;[17,18] however, current research has focused on devices that do not have to be implanted. An injectable system would be superior to implantable technologies from an administration standpoint. Noninvasive delivery, for example stimuli-sensitive transdermal patches, often lack the ability to be environmentally responsive, due to their lack of direct contact with most body fluids. Our work has focused on the synthesis of novel materials that can be used as self-regulating injectable and water-soluble delivery devices to rectify these shortcomings of existing systems.

Recently, homopolymers and random copolymers of 2-(*N,N*-diethylamino)ethyl methacrylate (DEAEM) and poly(ethylene glycol) methyl ether methacrylate (PEG-MEM) have been synthesized using an oxyanion-initiated anionic route.[19] Ni et al. have reported the synthesis of triblock materials based on a poly(propylene glycol) (PPG)-based macroinitiator using a similar synthesis route.[20] There is no verification, however, that the materials synthesized are truly triblock or are in fact diblock materials.

Our approach involves utilizing a difunctional potassium alcoholate initiator based on the Pluronic triblock copolymer formulation F127, as well as monomethyl ether terminated poly(ethylene oxide) (PEGME), to produce pentablock and diblock copolymers of DEAEM, PEG, and PPG to be used for pH-sensitive drug delivery. The pentablock materials (PDEAEM-*b*-PEO-*b*-PPO-*b*-PEO-*b*-PDEAEM) retain the thermodynamic phase transitions present in the triblock base polymer while providing a stimuli-sensitive release profile suitable for self-regulated drug release. The diblock materials (PEG-*b*-PDEAEM) do not form gels like their pentablock counterparts; however, they do supply environment-sensitive release of small molecules in dehydrated tablet form. We have used benzyl end-caps to quantify the average functionality of the synthesized pentablock materials to verify the presence of pentablock rather than tetrablock materials.

Experimental Section

Materials. 2-(*N,N*-Diethylamino)ethyl methacrylate (DEAEM, Sigma-Aldrich, St. Louis, MO) was dried over calcium hydride and purified by distillation under reduced pressure. Tetrahydrofuran (THF, Sigma-Aldrich Co., St. Louis MO) was dried by passing through solvent purification columns of alumina and Q5 copper/silica/alumina catalyst (columns, Solv-Tek, Berryville, VA; Q5, Engelhard Corp., Iselin, NJ). Poly-(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (Pluronic F127, $\bar{M}_n$ = 12 600, 70% w/w PEG, Sigma-Aldrich Co., St. Louis, MO) and poly(ethylene glycol) methyl ether (PEGME, $\bar{M}_n \sim$ 5000, Polysciences Inc., Warrington, PA) were dried by heating under vacuum. Sodium phosphates were obtained from Fisher Scientific. Nile blue chloride (NBCl,

visible absorbance maximum at 636 nm, MW = 375.0) was obtained from Sigma-Aldrich. All other materials were purchased from Sigma-Aldrich Co. and used as received.

Techniques. *Polymerization.* All flasks and magnetic stir bars used were either flame-dried and cooled under an inert atmosphere or heated overnight at 180 °C and cooled under an inert atmosphere. Flasks were sealed with metal-tied rubber septa to allow for argon pressurization. Potassium hydride, stored under mineral oil, was washed with THF in an inert atmosphere in a round-bottom flask. Enough dry THF was added to completely submerse the solid potassium hydride.

F127 **(1)** or PEGME **(2)** was dissolved in THF in a round-bottom flask. It was necessary to heat the THF and F127/PEGME to slightly above room temperature in order to dissolve the polymer. The solvated polymer was transferred via canula into a flask containing potassium hydride **(3)** to form either the monofunctional alcoholate **(4)** or the difunctional alcoholate **(5)** (Scheme 1).

An appropriate amount of DEAEM **(6)** was added via air-free syringe or canulla to the solution of either **4** or **5** while stirring at 400 rpm at room temperature for 20 min, followed by 50 °C for 20 min (Scheme 2). The living polymers **7** and **8** were terminated with an injection of methanol **(9)** or benzyl bromide **(10)** (Scheme 3). The resulting polymers **11**, **12**, and **13** were precipitated in −78 °C *n*-hexane and dried under vacuum for at least 24 h. The polymer was then characterized, and its pH sensitivity was tested using the following techniques.

NMR Characterization. ^{1}H NMR data were collected on Varian VXR400 (400 MHz) and Varian VXR300 (300 MHz) spectrometers. Chloroform-*d* was used as the solvent for most samples. For samples in which phenyl protons were used as a functionality marker, acetone-*d*_6 was used to avoid peak overlap.

Gel Permeation Chromatography. GPC was used to obtain the polydispersity index of the polymer. THF was used as the mobile phase with a sample injection volume of 100 μL. The system was equipped with three PLgel columns (Polymer Laboratories, Amherst, MA) heated to 40 °C. An Optilab inline refractive index detector (Wyatt Corp., Santa Barbara, CA) was used as the detector for retention times of the synthesized polymers relative to poly(methyl methacrylate) and polystyrene standards.

Differential Scanning Calorimetry. Differential scanning calorimetry measurement of the critical micellization temperature was performed on a DSC7 (Perkin-Elmer, Shelton, CT). Samples were cooled to −10 °C and held at this temperature for 15 min before beginning a temperature scan from −10 to 35 °C at a rate of 5 °C/min under a nitrogen purge. The critical micellization temperature was determined as the onset of the deviation of the endothermic micellization transition peak from the baseline.

Buffer Preparation. Sodium phosphate buffers were prepared by adding the appropriate amount of anhydrous monobasic sodium phosphate (NaH_2PO_4) and anhydrous dibasic sodium phosphate (Na_2HPO_4) to deionized water. The total ionic strength of the solutions was 0.5 M. These buffers were used to test the pH sensitivity of the polymers that were synthesized.

Release Studies. The dye Nile blue chloride (NBCl) was used as a model drug for all release studies. Its moderate water solubility and molecular weight of 375.0 g/mol make it a suitable model drug for many small molecules that do not partition exclusively into lipophilic or aqueous phases. The

Scheme 2. Reaction Scheme for Polymerization Reactions
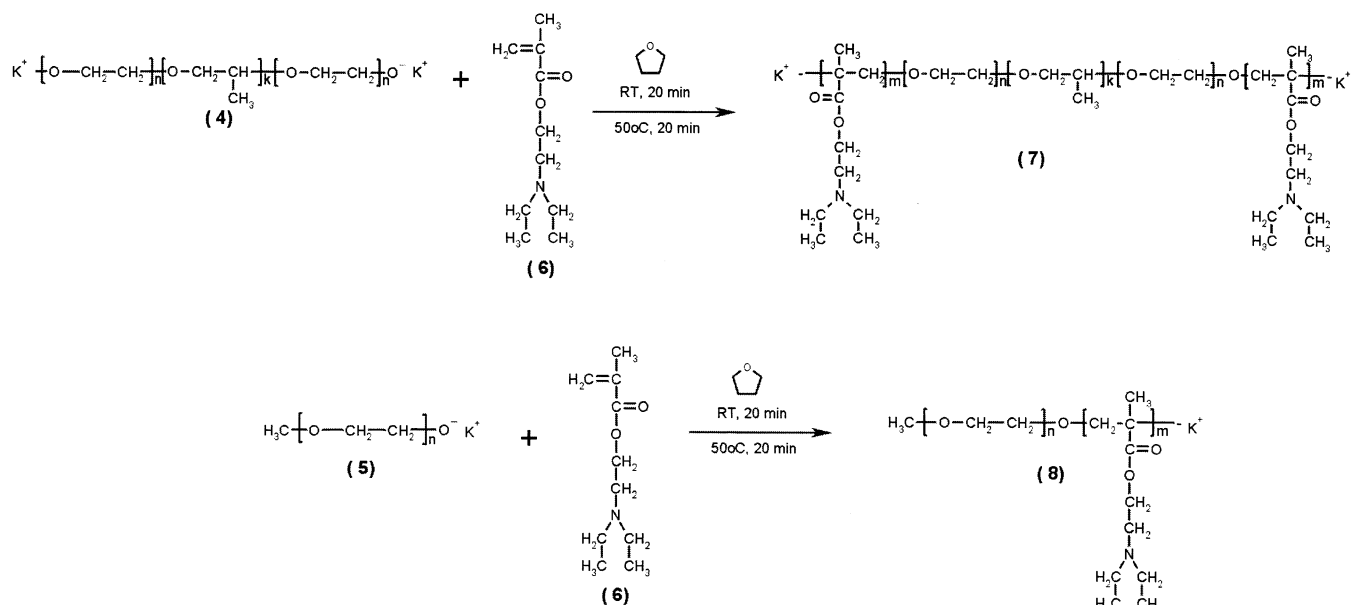
Scheme 3. Reaction Scheme for Termination Reactions
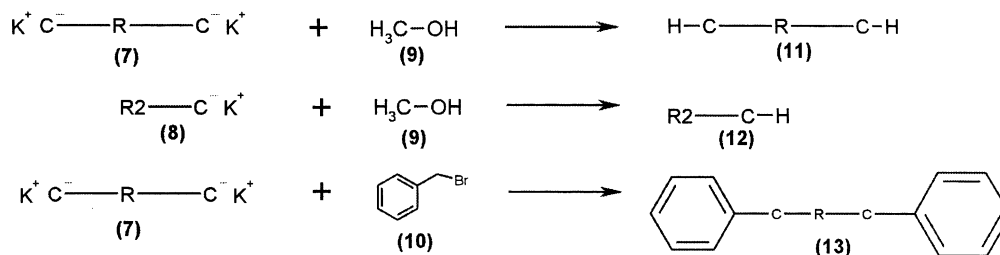
R=PDEAEM-b-PEO-b-PPO-b-PEO-b-PDEAEM living polymer
R2=PEGME-b-PDEAEM living polymer

absorbance maxima of NBCl in the visible spectra at 636 nm make release rates easy to measure without interference from the dissolved polymer.

Dissolution of polymer samples was tested using two methods: one for the diblock copolymer tablets and another for the pentablock copolymer gels. For the pentablock materials, a 10:1 polymer to dye solution was prepared in ethanol. The ethanol was evaporated leaving a homogeneous polymer/dye solid. Cold aqueous solutions were prepared from this material as reported in other studies.[14,21] The samples were then placed in appropriate containers, typically glass dishes with a radius of 14 mm and height of 10 mm, and were placed in a 37 °C oven where they formed non-cross-linked hydrogels. These samples were tested in a stirred dissolution tank at 37 °C with 800 mL of buffer solution and allowed to dissolve over a period of time. Samples were removed from the dissolution tanks at various intervals and tested for dye concentration using visible wavelength spectrophotometry. The agitation rate used for the tests was 60 rpm with a 10:1 F127:NBCl solution as a control. Release from Pluronic gels served as a control for these release experiments.

For the diblock materials, tablets were prepared from a similar homogeneous polymer/dye solid by compression-molding at 7000 psi for 5 min. The tablets were placed in a dissolution testing apparatus and tested for NBCl concentration in a manner similar to the gel-forming polymers. For both types of materials various pH values were investigated, and measurements were performed in triplicate. Poly(ethylene glycol) with $\bar{M}_n$ values of 5000 and 8000 g/mol were used as nonionic controls and were used as received from Sigma-Aldrich (St. Louis, MO).

Cytotoxicity Testing. The cytotoxicity of the materials was determined using an elution-type test reported in our previous work.[22] Briefly, approximately 30 mg of the polymers to be tested was dissolved in 100 mL of low-glucose Dulbecco's modified eagle medium (DMEM, Sigma) with 10% fetal bovine serum (FBS, Sigma), 10 μg/mL insulin (Sigma), 10 units/mL penicillin/streptomycin (Sigma), and 100 μg/mL L-ascorbic acid (Sigma). This solution was diluted to achieve the desired polymer concentration for all tests.

NIH/3T3 mouse fibroblasts were grown in polystyrene flasks until reaching confluence at 150 cells/mm². The growth media was removed from the flasks and replaced with one of the following: DMEM (negative control), DMEM with phenol (positive control), DMEM with the pentablock material. The concentrations of the pentablock material and phenol were 3, 0.3, and 0.03 mg/L.

After 24 h of incubation in a humidified incubator with 5% CO$_2$ at 37 °C, the samples were removed and the media was replaced with Karnovsky's fixative (2.5% glutaraldehyde, 2.0% paraformaldehyde, 0.1 M sodium cacodylate) for 12 h. The samples were then stained with a 20% crystal violet dye (CVD) solution in ethanol for 6 h followed by dehydration with ethanol. The cell layer was then inspected for a cytotoxic response by noting changes in cell density, morphology, and adherence relative to the positive and negative control samples.

Results and Discussion

Molecular Weight. All the samples prepared showed PDI values similar to the macroinitiators used, indicating very little added polydispersity due to the PDEAEM blocks (Table 1). The relative amount of PDEAEM is reported as percent mass of the methacrylate blocks relative to the total weight of the copolymer. The
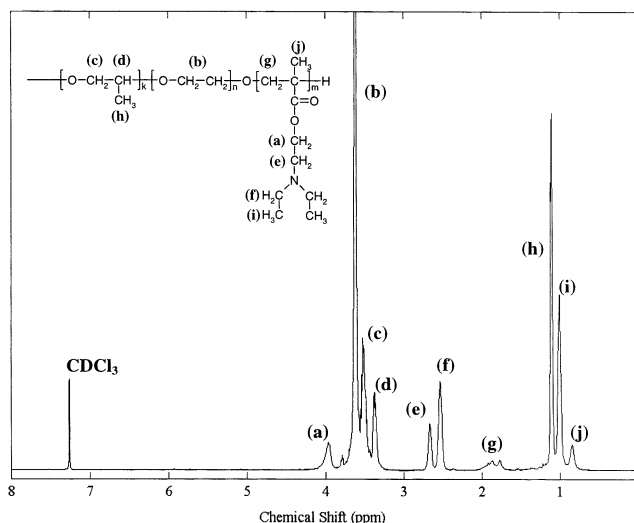
Table 1. Sample Polymerizations of F127-Initiated Pentablock Copolymers and PEGME-Initiated Diblock Copolymers

sample ID	initiator	target $\bar{M}_n$	$\bar{M}_n$(NMR)	PDI(GPC)	DEAEM (%)
A	F127	19 810	19 730	1.20	36.2
B	F127	16 930	15 670	1.19	19.6
C	F127	15 600	13 890	1.19	9.3
D	F127	14 530	13 330	1.18	5.4
E	F127	13 930	12 840	1.20	1.9
F	PEGME	8 150	7 970	1.08	37.2
G	PEGME	9 190	9 140	1.06	43.8
H	F127		12 600[a]	1.23	0
I	PEGME		5 000[a]	1.10	0

[a] $\bar{M}_n$ values obtained from manufacturer for the macroinitiator polymers.
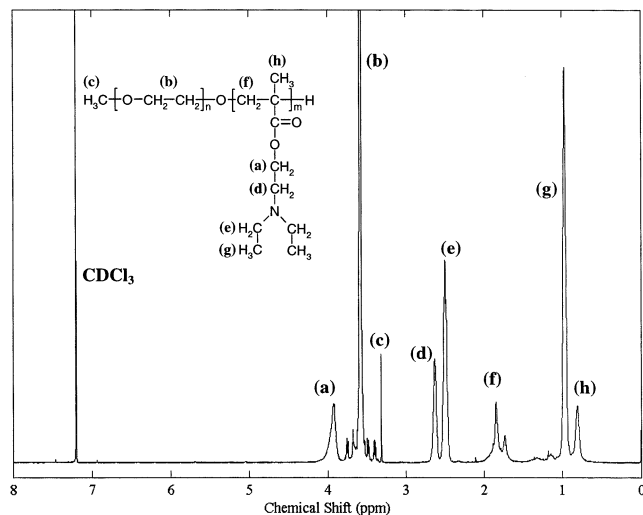
Figure 1. ^{1}H NMR analysis of PEGME-*b*-PDEAEM. Integration of peaks (a), (b), (c), (d), and (e) were used in $\bar{M}_n$ calculations.

Figure 2. ^{1}H NMR analysis of PDEAEM-*b*-PEO-*b*-PPO-*b*-PEO-*b*-PDEAEM. Integration of peaks (a), (b), (c), (d), (e), and (f) were used in $\bar{M}_n$ calculations.

Table 2. Thermodynamic Properties of Polymer Gels Obtained from Pentablock Materials

sample ID	% polymer	% DEAEM	T_m (°C)	ΔH (J/g)	ΔS (J/(g K))
B	28	19.6	−0.73 (4.19)	4.38 (0.79)	16.1 (3.16)
C	28	9.3	0.18 (0.64)	5.55 (0.38)	20.3 (1.38)
D	28	5.4	1.90 (0.65)	5.17 (0.17)	19.3 (0.57)
E	28	1.9	2.12 (1.00)	5.32 (0.79)	18.8 (0.27)
H	28	0	2.61 (0.10)	6.01 (0.19)	21.8 (0.69)
A	27	36.2	1.92 (2.15)	3.56 (0.30)	13.0 (1.19)
H[a]	25.2	0	9.00 (0.28)	5.28 (0.52)	18.7 (1.83)
A[a]	25.2	36.2	7.83 (0.36)	3.35 (0.68)	11.9 (2.43)

[a] Samples contained 2.8% NBCl dye and 25.2% polymer. Numbers in parentheses are the sample standard errors for the measurements.

apparent slight decrease in PDI from the macroinitiator (samples H and I) to the block copolymers, especially for the pentablock copolymer, is assumed to be due to a higher reactivity of the lower molecular weight initiators relative to the higher molecular weight initiators. This is more evident in the case of the pentablock, due to the bimodal nature of the Pluronic copolymer macroiniator.[23] For Pluronic F127, the lower molecular weight mode is on the order of $\bar{M}_p$ = 6000 g/mol, whereas the upper mode has an $\bar{M}_p$ of approximately 14 000 g/mol. The distance between the modes appears to get smaller as DEAEM is added to the polymers, resulting a slightly lower PDI. As appears to be the case in other studies with PDEAEM, GPC is not always an accurate measure of $\bar{M}_n$ or $\bar{M}_w$ most likely because of binding of the DEAEM moiety with the column packing and the high molecular weight of the DEAEM pendent groups. Often NMR values are used for $\bar{M}_n$ and the PDI is approximated from GPC.[24] A sample NMR of a pentablock material with peak assignments is given as Figure 1, and a sample NMR of a diblock material is given as Figure 2. The $\bar{M}_n$ values for the DEAEM blocks for both materials can cover a wide range; however, our release studies focused on a specific range of molecular weights. Simple dissolution and gelation tests indicated the pentablock material A (Table 1) and the diblock material F appeared to be in a molecular weight range and DEAEM/initiator ratio that produced interesting pH-sensitive behavior while maintaining the properties of PEG and F127 that were desirable. Because of this,

these two materials were used for the bulk of the release studies. However, materials with customized DEAEM block lengths and mass fractions can easily be prepared by the addition of slightly more or less of the cationic moiety.

Differential Scanning Calorimetry. DSC was used to evaluate two thermodynamic properties of the pentablock materials. First, the onset of the micellization temperature, T_m, was determined as reported in the literature for triblock materials.[21] Second, the endothermic enthalpy (ΔH) of the micellization phase transition was measured by integrating the micellization peak. Values for ΔH, T_m, and ΔS are given for 28% w/w aqueous samples over a wide range of PDEAEM block lengths in Table 2. Samples at lower polymer concentrations are also reported for the 36.2% DEAEM pentablock copolymer and the Pluronic triblock copolymer.

The trend seen in the data presented in Table 2 indicates that the addition of the PDEAEM blocks slightly depresses T_m and reduces the magnitude of the endothermic ΔH. The magnitude of this depression is not great for the smaller PDEAEM block lengths, namely 10% and less; however, the magnitude increases for the larger PDEAEM block lengths. Although the trend is clear, a Tukey multiple comparison test (α = 0.05) indicated that only the extreme samples, 0%−36.2% and 0%−20%, are statistically significant for ΔH and ΔS. For T_m, all samples were statistically the same at a 0.05 level due to the large variance in measured
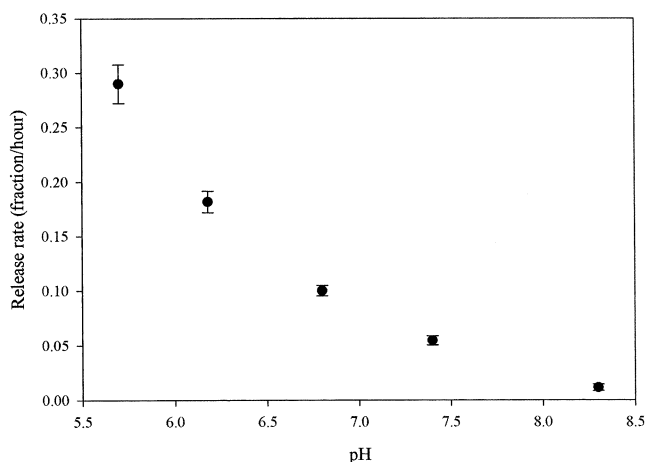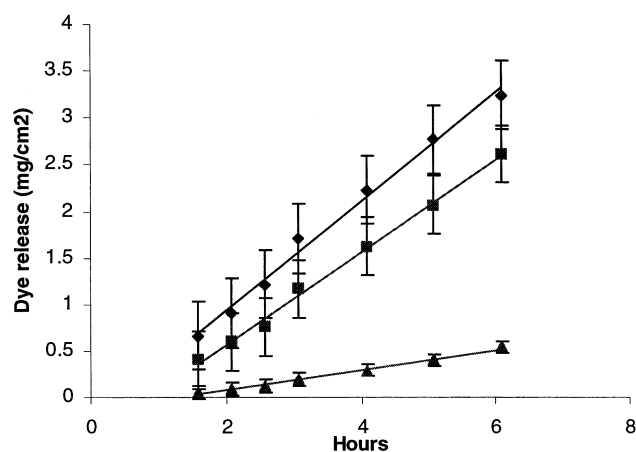
Figure 3. Release rate of Nile blue chloride from PEGME-*b*-PDEAEM diblock copolymer tablets, sample F, as a function of pH. Error bars represent standard errors of the parameter estimate of a linear fit to release data. Dye loading is 30 mg/cm^3.

values. However, the values for samples H[a] and A[a] are a good example of the T_m and ΔH depression. The differences between these samples, as seen in a *t*-test for different means, are statistically significant to a $p < 0.01$ level for both T_m and ΔH.

The reason for the ΔH depression is an apparent reduction in the entropic driving force for micellization. The PPO core of the micelles is the influential factor for micellization.[8] It is assumed that the PDEAEM portions of the pentablock material partition into the hydrophobic micelle core due to the fact PDEAEM is quite hydrophobic and would at least partially be solvated by the PPO nanophase. This would lead to a reduction in entropic advantage to micellization and thus the observed change in enthalpy and entropy of micellization. In addition, limited hydrogen bonding with the methacrylate at temperatures below the cmt may partially disrupt the hydrophobic effect, the entropic driving force for micellization.[25] The depression in T_m with increasing PDEAEM block length is most likely due to an increase in the amount of hydrophobic characteristic of the polymer. The more monomeric units of hydrophobic species, the lower the micellization temperature.

Pentablock Functionality. For pentablock materials terminated with benzyl bromide, the phenyl peaks were integrated relative to the known PEG Pluronic peaks at ~3.7 ppm to determine the average number of benzyl termini per molecule. The terminal signals integrated against the PEG peak divided by the number of equivalent PEG protons in the initiator showed a ratio of 10:1, or two benzyl groups, per Pluronic initiator molecule. This indicates that, according to our procedure, we are able to prepare materials that are fully pentablock in nature. Whether the block lengths are identical cannot be verified; however, this benzyl termination procedure allows some insight into the material's molecular structure.

Release Studies. The tablet dissolution studies of the diblock materials revealed a dramatic pH dependence on the release rate of dye from the polymer tablets. The specific material reported here has a $\bar{M}_n$ of 8120 g/mol, or 38.4% PDEAEM. The release rates were calculated from the slope of the zero-order release curves at various pH values. At the higher pH values, specif-


Figure 4. Release of Nile blue chloride from PDEAEM-*b*-PEO-*b*-PPO-*b*-PEO-*b*-PDEAEM pentablock copolymer gels, sample A, at a pH of (♦) 6.2, (■) 7.4, and (▲) 8.2. Error bars represent standard errors.
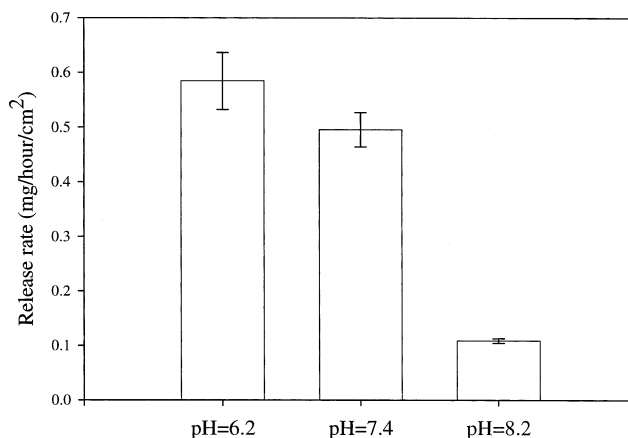
Figure 5. Release rate of Nile blue chloride from PDEAEM-*b*-PEO-*b*-PPO-*b*-PEO-*b*-PDEAEM pentablock copolymer gels, sample A, as a function of pH. Error bars represent standard errors of the parameter estimate of a linear fit to release data.

ically 7.4 and 8.2, the release rate is markedly slower than the lower pH values (Figure 3). The release rate at a pH value of 5.7 (0.290 fraction/h) is over an order of magnitude faster than at the pH value of 8.2 (0.021 fraction/h). The tablets submerged in the lower pH buffers dissolved at the polymer/buffer interface and released the contained dye. At the higher pH values, the tablets merely broke apart into small fragments that did not dissolve. The entrapped dye released very slowly, probably from dye dissolving at the fragment interfaces. In the high pH buffers, the PDEAEM portion of the diblock material remains insoluble, as we have reported previously.[23] At the low pH values, however, the materials become charged by protonation of the tertiary amine methacrylate. The increased water solubility of the charged amine and electrostatic repulsion of the protonated pendent groups allow the tablets to dissolve at low pH values.

As a comparison to the diblock materials, two controls, PEG $\bar{M}_n = 5000$ and PEG $\bar{M}_n = 8000$, were used. The PEG$_{5000}$ was used because it is the starting material for the polymerization, and the PEG$_{8000}$ was used because it mimics a material of similar molecular weight to the specific diblock material tested without the PDEAEM block. A lack-of-fit test was used to determine that the PEG$_{5000}$ and PEG$_{8000}$ release rates are statistically the same for all pH values and the release rate is the same
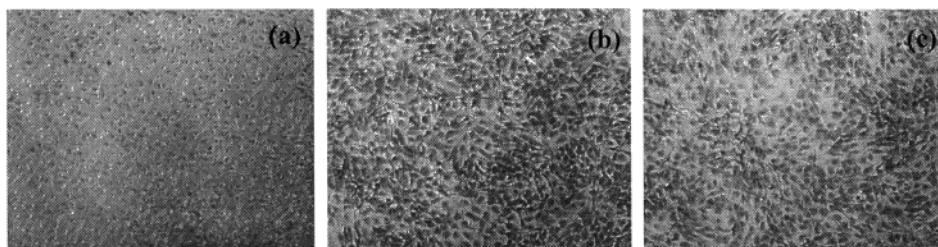
Figure 6. Cytotoxicity of (a) positive control, phenol, (b) negative control, growth media, and (c) PDEAEM-*b*-PEO-*b*-PPO-*b*-PEO-*b*-PDEAEM, 32% w/w PDEAEM. Polymer and phenol concentrations are all 0.3 mg/L.

for both materials. A regression fit data for all pH values and both non-ionic materials vs regression fits assuming the release rates are different at different pH values yielded a *p* value of 0.89 from an *F*-statistic value of 0.47. On the other hand, a similar test for the diblock material yielded a *p* value of <0.0001 from an *F*-statistic value of 135.5, indicating that there is a significant difference between the pH values. The regression for the pH-independent PEG release data gave an estimate of 0.91 fraction/h, a much faster release rate than the pH-sensitive diblock material of a similar molecular weight.

The release of dye from 28% w/w gels of the pentablock materials also displayed pH sensitivity for similar reasons. Once the material sets into a non-cross-linked gel, the release of molecules is dependent on the pH of the buffer (Figure 4). As water penetrates the gel, as described in previous work[14] for Pluronic systems, protons are carried into the interfacial area of the gel. It has been shown that when cross-linked membranes containing PDEAEM become protonated, they swell due to electrostatic interactions of the charged cations.[5] The same is true in the non-cross-linked case; however, swelling leads to dissolution of the gel and thus release of the entrapped molecules.

At the higher pH values, the gel is relatively insoluble. In lower pH buffers, the gel is soluble with a rate of dye release more than 5 times the rate at higher pH values (Figure 5). The release rates were computed from the slope of the release plots in Figure 4. Again, the nonionic control material, the Pluronic, proved to be pH-insensitive in its release profile and had a release rate similar to the pentablock copolymer at low pH values. The release from Pluronic F127 gels occurred at a rate of 0.57 mg/(cm^2 h) for a loading of 30 mg/cm^3. A lack-of-fit test for these data indicated a *p* value of 0.54 from an *F*-statistic value of 0.898.

Cytotoxicity Testing. Elution tests were performed on one sample of the pentablock material to assess the cytotoxic properties of the block copolymers. The results of the tests were compared to a negative control and a positive control. The negative control (Figure 6b), pure growth media, was taken as the result expected for a noncytotoxic material. The positive control (Figure 6a), phenol laced media, was taken as the result expected for a cytotoxic material. The pentablock material (Figure 6c), at the same concentration as the phenol positive control, led to results similar to the negative control. The fibroblast cells used in the tests showed good adhesion to the polystyrene cell culture substrate, and the cells remained confluent after the 24 h test period, neither of which is true for the positive control.

Conclusions

Novel pentablock and diblock materials were synthesized that possess a variety of properties applicable to environment-sensitive drug or biomolecule release. The pentablock materials synthesized maintain the properties of thermoreversible gelation as well as thermally induced micellization in aqueous solutions, two properties the macroinitiator possesses that have been studied for their application to injectable drug delivery systems. In addition to these thermodynamic properties, the materials also exhibit pH-dependent release profiles for entrapped molecules by virtue of the added cationic moiety.

The diblock materials show a dramatic increase in the release rate of small molecules when tested in tablet as the pH of the tablet dissolution medium decreases. There is an order of magnitude change in the release rate of Nile blue chloride between a pH 6.2 phosphate buffer solution and a pH 8.2 phosphate buffer solution. This increase in release rate over a rather small range of pH values that are only slightly more acidic or alkaline than physiological pH has the potential to be useful for pH-sensitive drug release.

The pentablock materials have a direct biomedical application, as the material mimics the pH-sensitive release behavior of extensively studied cross-linked polycation systems while adding the benefits of device injectability. Initial cytotoxicity tests have shown that these materials are not cytotoxic.

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