

Diblock Copolymers for Magnetically Triggered Drug Delivery Systems

Sarah M. Nikles¹, Jacqueline A. Nikles, Ph.D.², Jason S. Hudson², and *David E. Nikles, Ph.D.¹

*Faculty Sponsor

1. Department of Chemistry, University of Alabama, Tuscaloosa, Alabama 35487-0336, USA
2. Department of Chemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294-1240, USA

Our interest in building a magnetically triggered delivery vehicle for drug delivery has led to this study of the thermally triggered release of pyrene from the core of polymer micelles. Poly(ethylene glycol)-b-polycaprolactone diblock copolymers were dissolved in water at concentrations above the critical micelle concentration. DSC curves showed a melting endotherm peaking near 41°C, indicating the polycaprolactone core was crystalline. Pyrene added to the micelle core did not interfere with the crystallization. At temperatures below the melting point of the core, the pyrene was trapped in the core. When heated above the melting point of the core, the pyrene was released from the core to enter the continuous aqueous phase. This showed the potential for thermally triggered release from polymer micelles.

Introduction

Diblock and triblock copolymers containing hydrophilic blocks, e.g., poly(ethylene glycol) and hydrophobic blocks, either poly(propylene glycol) (PEO-PPO-PEO) [1], poly(L-histidine) (His-PEG) [2], polylactic acid (PEG-PLA) [3-6], poly(D,L-lactide-co-glycolide) (PEG-PLGA) [7,8], poly(β -benzyl-L-aspartate) (PEG-PBLA) [9], or polycaprolactone (PEG-PCL) [10-16] form micelles in aqueous solution at concentrations above the critical micelle concentration (CMC). The micelle cores can be loaded with a hydrophobic cancer drug, such as taxol [3, 5], or doxorubicin [10-12]. The drug can be released by a change in pH for pH responsive poly(L-histidine)-PEG polymer micelles [2]. For polymer micelles containing polyester blocks (i.e. PEG-PLA [3], PLA-PEG-PLA [5, 6], PEG-PLGA [8], or PEG-PCL [10-16]), hydrolytic degradation of the ester block erodes the polymer core, effecting drug release.

We seek to build a magnetically triggered drug delivery system for cancer chemotherapy based on PEG-PCL polymer micelles (Figure 1). The micelles, containing a payload of cancer drugs, will be injected into the blood stream and travel to the cancer site. The targeting moiety will allow the micelle to specifically bind to the surface of the cancer cell, thereby immobilizing the micelle at the cancer site. An external AC radio frequency magnetic field pulse will heat the magnetic nanoparticle, which in turn heats the crystalline core to its melting point. When the micelle core is melted, the cancer drug is free to

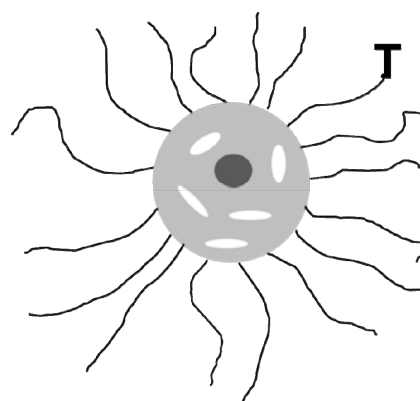


Figure 1. Schematic of a magnetically triggered drug delivery vehicle. The core contains a crystalline polymer (gray), a magnetic nanoparticle (black) and a trapped cancer drug (white). The micelle is dispersed in aqueous media by a hydrophilic polymer block (black curved lines). The micelle has a targeting moiety (black T) that specifically binds to cancer cells.

move from the core, leaving the micelle and attacking the cancerous tissue. As long as the micelle core is melted, we expect the cancer drug to set up a dynamic equilibrium between being in the core and in the continuous aqueous phase outside the micelle. When the magnetic field pulse is discontinued, the micelle core will cool and crystallize, trapping any remaining cancer drug in the core. The drug would remain in the core until the next magnetic field pulse, when it would be free to leave the core again. This magnetically triggered drug delivery system would

provide the oncologist an unprecedented spatial and temporal control of drug delivery, a powerful new tool for cancer therapy.

To create this drug delivery system, we must design polymer micelles with crystalline cores that can trap the cancer drug at physiological temperature (37°C). We chose polycaprolactone as the hydrophobic block because it is biocompatible, and its use *in vivo* is widely accepted [17]. Furthermore, polycaprolactone crystallizes with a melting point in the range of 40 to 50°C, just above physiological temperature. The cancer drug can be immobilized in between the crystallites in the micelle core and then be released when the core is melted. The enthalpy of melting would provide a thermal energy barrier against release at low temperature. Melting the polycaprolactone core would trigger the drug release. In this paper, we determined whether the polycaprolactone core in the micelles does indeed crystallize. We examined the effect of the incorporation of a small molecule on the ability of the polycaprolactone to crystallize. We also demonstrated release of the small molecule when the micelles were heated to temperatures above the melting point of the polycaprolactone core.

Experimental

Materials

ϵ -Caprolactone and dibutyltin dilaurate were purchased from Aldrich Chemical Company and used as received. Poly(ethylene glycol) monomethylether ($M_n \sim 2,000$) was also purchased from Aldrich Chemical Company. It was dried at 60°C in the vacuum oven overnight, allowed to cool to room temperature, and then stored in a dessicator over calcium chloride until used. The solvents were reagent grade and purchased from Fisher Scientific.

Synthesis of MeO-(EG)₃₆-(CL)₁₆-OH

A 250 mL three necked round bottom flask was dried in a glass drying oven overnight at 110°C. The flask was allowed to cool and was equipped with a reflux condenser, a thermometer, magnetic stirrer and a nitrogen atmosphere. Poly(ethylene glycol) monomethyl ether, $M_n \sim 2,000$ (20.00 g, 10.00 mmol), ϵ -caprolactone (22.80 g, 200.0 mmol) and dibutyltin dilaurate (0.10 mL) were added to the reactor and the reaction mixture was heated to 140 to 150°C using a

silicon oil bath. The reaction mixture was allowed to stir at this temperature under nitrogen for 2.5 hours to give a viscous liquid. Afterwards, it was allowed to cool to room temperature whereupon the polymer solidified. The solid was taken up in a minimum amount of acetone, and enough hexane was added to the point of imminent precipitation. The solution was chilled in the refrigerator at 4°C overnight. The next day, the polymer had precipitated and was isolated by vacuum filtration. The polymer was rinsed with hexane and allowed to dry. Yield 28.53 g, 67%.

The other polymers reported here were synthesized using the same procedure, except the amount of ϵ -caprolactone was varied. For the case of MeO-(EG)₄₃-(CL)₅-OH, 5.70 g of ϵ -caprolactone (50.0 mmol) was used, while 11.40 g (100.0 mmol) was used for MeO-(EG)₄₀-(CL)₉-OH

Instrumentation

¹H NMR spectra of the polymers in CDCl₃ solution (Aldrich) were obtained on a Bruker Advance 360 Digital NMR. The degree of polymerization of the two blocks was determined for the integrated intensity of the resonances for selected peaks in the spectra. The critical micelle concentration was determined from the concentration dependence of the surface tension measured by the deNouy ring method using a Fisher manual model 20 surface tensiometer.

Results and Discussion

Diblock Copolymers

The diblock copolymers were made by the tin-catalyzed ring opening polymerization initiated from the alcohol terminus of poly(ethylene glycol) monomethylether. The molecular weight of the poly(ethylene glycol) was nominally 2,000, leading to the expectation that the degree of polymerization was 45. Integration of the peak for the terminal methyl group at 3.33 ppm (integral 3.00) and the methylene groups at 3.59 ppm (integral 138.7) gave a degree of polymerization of 35, indicating a molecular weight of 1570. The methylene groups in the polycaprolactone block had peaks at 1.37, 1.63, 1.52, 2.30 and 4.25 ppm, with the peaks at 1.63 and 1.52 ppm overlapping. The methylene group from the PCL block that links to the PEG block had a unique resonance at 4.17 ppm, thereby confirming the PCL

block was linked to the PEG block. As with the poly(ethylene glycol) monomethylether precursor, the PEG block had a resonance at 3.32 ppm for the terminal methyl group and a resonance at 3.59 ppm for the methylene groups. The integrated intensities of the peaks in the NMR were used to determine the degree of polymerization for each block (Table 1). The degree of polymerization for the PCL block was expected to be 5 for the first polymer, 10 for the second polymer, and 20 for the third polymer. Indeed, the degree of polymerization for the PCL block was 5 for the first polymer (MeO-(EG)₄₃-(CL)₅-OH), but was less than the expected degree of polymerization for the other two polymers. Notice that the degree of polymerization for the PEG block varied even though each used the same PEG starting material. We speculate that the purification procedure fractionated the polymers.

Table 1. PEG-PCL diblock copolymers.

| Block Copolymer | M _n (g/mol) | CMC (mg/L) | CMC (M) |
|------------------------------------------------|------------------------|------------|------------------------|
| MeO-(EG) ₄₃ -(CL) ₅ -OH | ~2,500 | 160 | 6.4 × 10 ⁻⁵ |
| MeO-(EG) ₄₀ -(CL) ₉ -OH | ~2,800 | 46 | 1.6 × 10 ⁻⁵ |
| MeO-(EG) ₃₆ -(CL) ₁₆ -OH | ~3,400 | 112 | 3.2 × 10 ⁻⁵ |

The surface tension of aqueous solutions of the block copolymers was measured as a function of polymer concentration to give CMC values (Table 1), reported either as mg polymer per liter of solution or as moles per liter. The CMC of the diblocks was somewhat dependent on the length of the CL block.

MeO-(EG)₃₆-(CL)₁₆-OH was dissolved in water at a concentration of 2 mg/mL, well above the CMC. The solid, black DSC curve in Figure 2 shows a sharp melting endotherm that peaks at 41°C. This concentration is well above the CMC for this diblock. This provides strong evidence that PCL blocks in the core of the micelles crystallized.

Pyrene (0.010 mM) was added to an aqueous solution of 2 mg/mL MeO-(EG)₃₆-(CL)₁₆-OH (0.59 mM). This is a hydrophobic probe molecule that resides in the hydrophobic core of the micelle. The dashed DSC

curve in Figure 2 shows the same sharp melting endotherm seen in the DSC curve for the micelles in the absence of pyrene. Indeed, the curves were almost superimposed. Clearly, the pyrene did not interfere with the ability of the polycaprolactone core to crystallize. This leads us to expect that a cancer drug would also not interfere with the ability of the polycaprolactone core to crystallize.

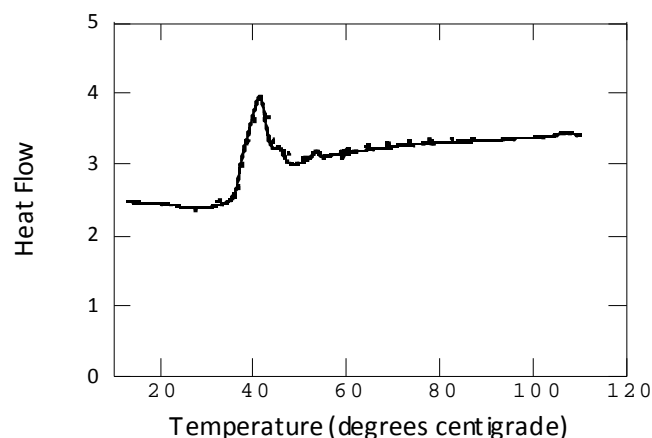


Figure 2. DSC curve for a 0.59 mM aqueous solution of MeO-(EG)₃₆-(CL)₁₆-OH, solid curve. The dotted curve is the DSC for the 0.59 mM MeO-(EG)₃₆-(CL)₁₆-OH solution containing 0.010 mM pyrene.

The capacity of the micelles to release a drug when heated was tested using pyrene (0.010 mM), a fluorescent probe, which was added to an aqueous solution of MeO-(EG)₃₆-(CL)₁₆-OH (0.59 mM). The fluorescence spectrum was measured during a 10°C/min ramp, with the fluorescence intensity at 374 nm shown as a function of temperature in Figure 3. In hydrophobic environments (e.g., inside the micelle), pyrene has a high fluorescence intensity, while in hydrophilic environments the fluorescence intensity decreases [18]. In the diblock copolymer, the pyrene was largely in the micelle core. However, as the temperature was increased above the melting point of the micelle core, the fluorescence intensity decreased, indicating that the pyrene had moved to a hydrophilic environment, the aqueous medium. Due to the temperature ramp used in this experiment, the release of pyrene happened quite fast as well. Similar results were obtained for the other polymers.

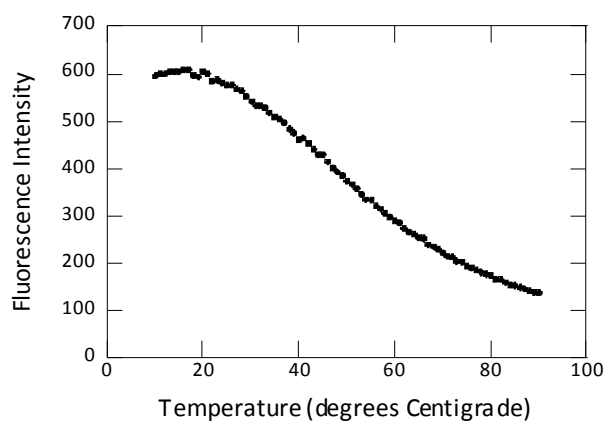


Figure 3. Fluorescence intensity at 374 nm as a function of temperature for pyrene in the presence of aqueous MeO-(EG)₃₆-(CL)₁₆-OH micelles.

Conclusion

Polymer micelles made from poly(ethylene glycol)-*b*-polycaprolactone diblock copolymers show promise for a magnetically triggered drug delivery vehicle. The polycaprolactone core was crystalline at temperatures below the melting point of the polycaprolactone. The DSC showed a sharp melting endotherm with a peak near 41°C. A small molecule was incorporated into the core, and its presence had little effect on the ability of the polycaprolactone to crystallize. The small molecule was released from the micelle core when the micelles were heated above the melting point of the core.

Our next task is to include magnetic nanoparticles into the core and determine whether they interfere with the ability of the core to crystallize. The last stage of our project will be to incorporate a cancer drug into the core and demonstrate magnetically triggered release.

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Sarah Nikles is a senior from Tuscaloosa, Alabama, majoring in biochemistry with a minor in mathematics. She works in the laboratory of Dr. David Nikles in the Department of Chemistry, where she conducted all of the research for this paper. Sarah has received a President's Cabinet Scholarship, was named a Semi-Finalist for the Siemens Westinghouse Science and Technology Competition for 2004-2005, and is a member of the Gamma Sigma Epsilon Chemistry Honor Society.