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Drug Release Behavior of Poly(ε-caprolactone)-*b*-Poly(acrylic acid) Shell Crosslinked Micelles below the Critical Micelle Concentration

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Abstract: To explore the potential of shell crosslinked micelle (SCM) as a drug carrier, the drug release behavior of poly(ε-caprolactone)-*b*-poly(acrylic acid) (PCL-*b*-PAA) SCMs was investigated. PCL-*b*-PAA was synthesized by ring opening polymerization of ε-caprolactone and atom transfer radical polymerization of *tert*-butyl acrylate, followed by selective hydrolysis of *tert*-butyl ester groups to acrylic acid groups. The resulting amphiphilic polymer was used to prepare SCMs by crosslinking of PAA corona via amidation chemistry. The drug release behavior of the SCMs was studied, using pyrene as a model drug, and was compared with that of non-crosslinked micelles, especially below the critical micelle concentration (CMC). When the shell layers were crosslinked, the drug release behavior of the SCMs was successfully modulated at a controlled rate compared with that of the non-crosslinked micelles, which showed a burst release of drug within a short time.

Keywords: PAA, PCL, release, shell crosslinked micelles.

Introduction

Amphiphilic block copolymers in a solvent that is selective for one of the block self-assemble into polymeric micelles.[1] These micelles have a fairly narrow size distribution and are characterized by their unique core-shell architecture.[2] A number of studies have shown that water-insoluble drugs can be physically encapsulated and solubilized in the hydrophobic core of micelles due to hydrophobic-hydrophobic interaction. Although the usefullness of such self-assembled micelle as a drug delivery material has been demonstrated over the past decades,[2-9] there is a need to further improve the performance of these polymeric micelle systems in terms of both temporal and distribution control.[10] Here, temporal control refers to the ability to adjust the period of time over which drug release takes place or to the possibility to trigger the release process at a specific time during treatment. Distribution control means the ability to precisely direct the drug delivery system to the desired site of activity. An important point is that both controls are directly connected with the stability of micelles. Generally, micelles from amphiphilic block copolymers have thermodynamically self-assembled structures. Since the critical micelle concentration (CMC) is defined as the concentration of polymeric solution when block copolymers begin to self-assemble into thermodynamically stable micelles, the micellar structure becomes unstable and finally dissociates into free diblock copolymer chains below the CMC. For control of drug delivery performance of micelles, it is important that drug loaded micelles show sustained release profiles even under an infinitely diluted condition. If the drug loaded micells are injected into human body, the micellar concentration goes down to infinitely low value and finally drops far below the CMC. In this condition, micelles are disintegrated and the drugs loaded into micelles show the burst release behavior instead of the sustained release. As a result, it is difficult to achieve an improved temporal and distributional control without enhanced stability of micelles.

A challenging approach to overcome the problems mentioned above is to enhance the stability of micelles by introducing crosslinkages into the micelle. Shell crosslinked micelles (SCMs) are new class of nanomaterials, which resemble polymeric micelles in their amphiphilic core-shell morphology and at the same time are similar to dendrimers in being covalently bound and stable structures.[11-13] In addition, the SCMs have both hydrogel properties and conventional properties of polymeric micelles, which makes it possible to be used for various applications such as specific binding and molecular recognition, controlled release of drugs, gene therapy, water-borne coatings, pollutant removal, catalysis, and sensors. However, to our best knowledge, a detailed investigation of SCMs for drug delivery applica-

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tions has not been well reported. For success of such pharmaceutical applications, it is essential to exploit the relationship between the structure of SCMs and their drug release behavior.

The primary objective of this paper is to gain a deeper knowledge of the effect of shell crosslinking on the drug release behavior of SCMs, focusing on the stability of SCMs particularly below the CMC as compared with non-crosslinked conventional micelles. The effect of the crosslinking density of SCMs on the drug release behavior is also analyzed.

Experimental

Materials. All reagents were purchased from Sigma-Aldrich unless otherwise noted. ε-Caprolactone, *tert*-butyl acrylate (*t*BA) (98%), isopropyl alcohol (iPA) (99.9%, Mallinckrodt), *N,N',N',N'',N''*-pentamethyldiethylenetriamine (PMDETA) (98%), and triethylamine (TEA) (99.5%) were dried over CaH_2 and distilled under reduced pressure. Chloroform (99%, Daejung Chemicals & Metals), methylene chloride (CH_2Cl_2) (99%, Daejung Chemicals & Metals), toluene (99%, Daejung Chemicals & Metals) were dried over CaH_2 and distilled under atmospheric pressure. Tetrahydrofuran (THF) (Daejung Chemicals & Metals) was dried over sodium and distilled under atmospheric pressure. Dimethylaminopyridine (DMAP) (98%) was recrystallized from toluene. Stannous 2-ethylhexanoate ($SnOct_2$) (98%), 2-bromoisobutyryl bromide (BIB) (98%), copper (I) bromide (99.999%), neutral alumina oxide, celite (Daejung Chemicals & Metals), trifluoroacetic acid (99%), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDACH) (98+%, TCI), 2,2'-(ethylenedioxy)bis(ethylamine) (EDBEA) (99%), pyrene (98%), methanol (99%, Daejung Chemicals & Metals) were used as received.

Synthesis of Poly(ε-caprolactone) (PCL). PCL was synthesized via ring opening polymerization of ε-caprolactone. $SnOct_2$ (0.00395 g) was added to a three-neck round-bottom flask, and the flask was dried in vacuum at 80 °C for 2 h to completely remove water. N_2 gas was purged after cooling to room temperature. iPA (0.285 mL, 3.09×10^{-3} mol) was added to the flask via a micro syringe, followed by addition of ε-caprolactone (15 mL, 0.135 mol) via a syringe. The flask was placed in a PEG bath at 110 °C for 15 h to allow polymerization. For purification of PCL, bulk PCL was first dissolved in a small amount of CH_2Cl_2 and then precipitated in cold methanol. The precipitated PCL was then dried in vacuum at 50 °C.

Synthesis of PCL Macroinitiator for Atom Transfer Radical Polymerization (ATRP). PCL (5 g) and DMAP (0.964 g, 2.63×10^{-3} mol) were put in a three-neck round-bottom flask, and the flask was vacuum dried at 80 °C for 2 h to completely remove water. CH_2Cl_2 (50 mL) was added to the flask via a syringe after purging with N_2 gas. TEA (0.244 mL, 1.75×10^{-3} mol) was added to the flask via a micro syringe, followed by cooling to 0 °C. BIB (0.542 mL, 4.39×10^{-3} mol) was then added via a micro syringe and the temperature of the flask was slowly raised to room temperature. The reaction was continued for 24 h under stirring. The solution was filtered and precipitated in cold methanol to purify PCL macroinitiator. PCL macroinitiator was then dried in vacuum at 50 °C.

Synthesis of Poly(ε-caprolactone)-*b*-Poly(*t*-butyl acrylate) (PCL-*b*-P*t*BA). PCL-*b*-P*t*BA was synthesized via ATRP of *t*BA using PCL macroinitiator. PCL macroinitiator (1 g) and CuBr (0.0252 g, 1.75×10^{-4} mol) was put in a three-neck round-bottom flask, and the flask was degassed and backfilled with N_2 gas repeatedly three times. Toluene (10 mL) was added to the flask via a syringe, followed by addition of PMDETA (0.0366 mL, 1.75×10^{-4} mol) to the flask via a micro syringe. After three freeze-and-thaw cycles for removal of oxygen, *t*BA (2 mL, 1.37×10^{-2} mol) was added via a syringe. The flask was then placed in a PEG bath thermostated at 100 °C for 18 h to allow ATRP. For purification of PCL-*b*-P*t*BA, a solution of the crude product was passed through a alumina/celite column to remove the catalyst and precipitated in 1/1 (v/v) methanol/water mixture. PCL-*b*-P*t*BA was then dried in vacuum at 50 °C.

Synthesis of Poly(ε-caprolactone)-*b*-Poly(acrylic acid) (PCL-*b*-PAA). For conversion of PCL-*b*-P*t*BA to PCL-*b*-PAA, PCL-*b*-P*t*BA (1 g) was dissolved in CH_2Cl_2 (10 mL), followed by addition of trifluoroacetic acid (0.0229 mL, 2.98×10^{-4} mol) via a micro syringe. The reaction was continued for 18 h at room temperature under stirring to transform *t*-butyl acrylate groups to acrylic acid groups. The resulting PCL-*b*-PAA was purified by precipitating in cold ether and then the precipitate was dried in vacuum. For further purification, PCL-*b*-PAA was dissolved in THF, and the solution was dialyzed against distilled water for 72 h using cellulose dialysis membrane (molecular weight cut-off: 3,500, Membrane Filtration Products, Inc.). The final solution was dried in vacuum.

Preparation of PCL-*b*-PAA Micelles. 100 mL of THF solution of PCL-*b*-PAA (5.00×10^{-2} mg/mL) was dropwise added to distilled water (400 mL). The solution was then slowly evaporated at 40 °C until THF was completely evaporated. After removal of THF, an extra amount of distilled water was added to obtain a desired micellar concentration (5.00×10^{-3} mg/mL). The final micellar solution was sonicated for 12 h at room temperature.

Preparation of PCL-*b*-PAA Shell Crosslinked Micelles (SCMs). 1 mL of an aqueous solution of EDACH (3.36×10^{-7} mol/mL, 1.00 equivalent to carboxyl acid groups of PAA blocks) was added to 25 mL of the stock solution of PCL-*b*-PAA (5.00×10^{-3} mg/mL) for activation of carboxylic acid groups. The mixture was stirred for 30 min until the mixture became homogeneous. 1 mL of an aqueous solution of EDBEA was then gradually added (0.1 mL/min) for cross-

linking the corona PAA blocks of micelles via formation of amide bonds. The extent of crosslinking was varied by controlling the amount of EDBEA added.

Preparation of Pyrene-Loaded PCL-*b*-PAA Micelles and SCMs. Pyrene was loaded in PCL-*b*-PAA micelle according to the o/w emulsion method.[3] 10 mL of a chloroform solution of pyrene (5.00×10^{-2} mg/mL) was dropwise added to 100 mL of an aqueous solution of PCL-*b*-PAA micelles (5.00×10^{-3} mg/mL). The mixture solution was then slowly stirred at room temperature for several days until chloroform is completely evaporated. Free pyrene in solution was removed by ultrafiltration using a membrane with the molecular weight cut-off of 3,000 (Stirred Cells 8000, Millpore). The amount of pyrene loaded into PCL-*b*-PAA micelles was photometrically determined by measuring the fluorescence of pyrene. Pyrene-loaded PCL-*b*-PAA SCMs were prepared using the solution of pyrene-loaded micelles by the same crosslinking method described above.

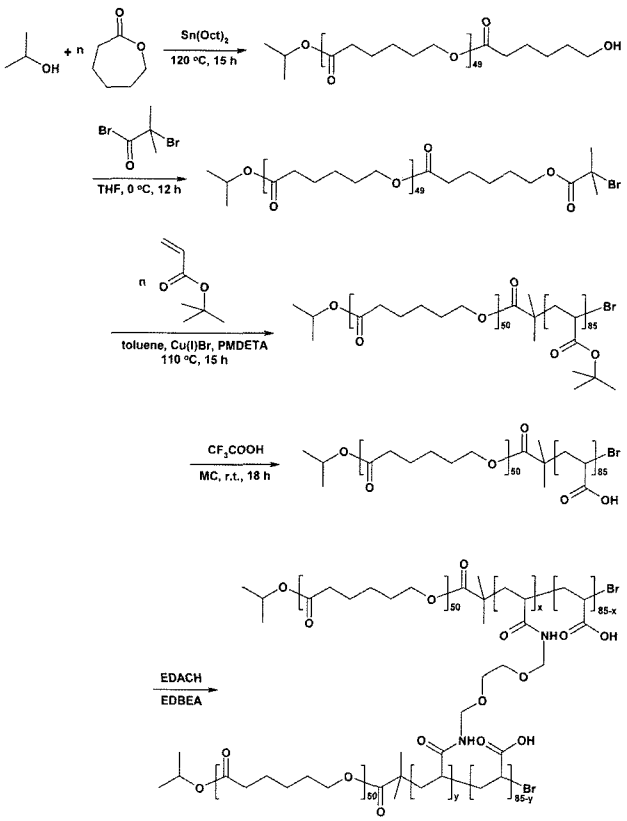
Release of Pyrene from PCL-*b*-PAA Micelles and SCMs below CMC. To achieve a condition below the CMC, both solutions of pyrene-loaded PCL-*b*-PAA micelles and pyrene-loaded PCL-*b*-PAA SCMs were diluted to 5.00×10^{-4} mg/mL. A dialysis membrane bag with the molecular weight cut-off of 3,500 (Membrane Filtration Products, Inc.) containing 1 mL of diluted solution of pyrene-loaded PCL-*b*-PAA micelles or pyrene-loaded PCL-*b*-PAA SCMs was put into a conical tube filled with 40 mL of PBS solution. The tube was shaken at 37 °C in a shaking water bath (model ESH-304, Science Community) at 100 rpm. At an appropriate time interval, the solution in the tube was sampled. The total volume of solution in the tube was held constant by adding an equal volume of PBS solution after each sampling. The amount of pyrene released was measured using a fluorescence spectrometer. All data points were reported by averaging two measurements.

Characterization. The composition and the number-average molecular weight of polymers were determined by 300 MHz ^{1}H NMR (Avance DPX-300, Bruker) and gel permeation chromatography equipped with styragel® HR 5E columns (Waters) and 2487 dual λ absorbance detector (Waters), respectively. Size distributions of PCL-*b*-PAA micelles and PCL-*b*-PAA SCMs were characterized by dynamic light scattering using the Lexel 95 Ion Laser (Lexel Laser) as a laser source and PD2000 as a detector (Precision Detectors). The critical micellar concentration, pyrene loading efficiency, and pyrene release behavior were determined using a fluorescence spectrometer (RF 5301, Shimadzu).

Results and Discussion

Synthesis of Copolymers. The overall scheme for synthesis of PCL-*b*-PAA is shown in Scheme I. First, PCL was prepared by ring opening polymerization of ε-caprolactone

Scheme I



using iPA as an initiator. For synthesis of PCL macroinitiator for ATRP, the terminal hydroxyl group of PCL was conjugated with BIB to introduce an alkyl halide group. The resulting PCL macroinitiator was then used to polymerize *t*BA with CuBr as a catalyst and PMDETA as a ligand, for preparation of PCL-*b*-P*t*BA diblock copolymer. PCL-*b*-P*t*BA was converted to an amphiphilic diblock copolymer, PCL-*b*-PAA, by treating PCL-*b*-P*t*BA with trifluoroacetic acid to cleave *tert*-butyl acrylate groups into acrylic acid groups.

The structure of PCL macroinitiator, PCL-*b*-P*t*BA, and PCL-*b*-PAA are identified by ^{1}H NMR and their molecular weights are determined by GPC. The NMR spectra of PCL macroinitiator, PCL-*b*-P*t*BA, and PCL-*b*-PAA are shown in Figure 1, where all the peaks are assigned. When Figure 1(a) is compared with Figure 1(b), the new peaks at 1.43-1.49 ppm and 2.15-2.30 ppm corresponding to protons of *tert*-butyl group and methylene protons in P*t*BA are observed in Figure 1(b), indicating that PCL-*b*-P*t*BA diblock copolymer is successfully synthesized. As *tert*-butyl acrylate groups are selectively hydrolyzed, the characteristic peaks of *tert*-butyl group completely disappeare and a new peak at 12 ppm corresponding to proton of carboxyl acid group in PAA is observed, as shown in Figure 1(c). This provides on evidence that PCL-*b*-P*t*BA is completely hydrolyzed to give PCL-*b*-PAA. GPC also confirms the synthesis of PCL-*b*-

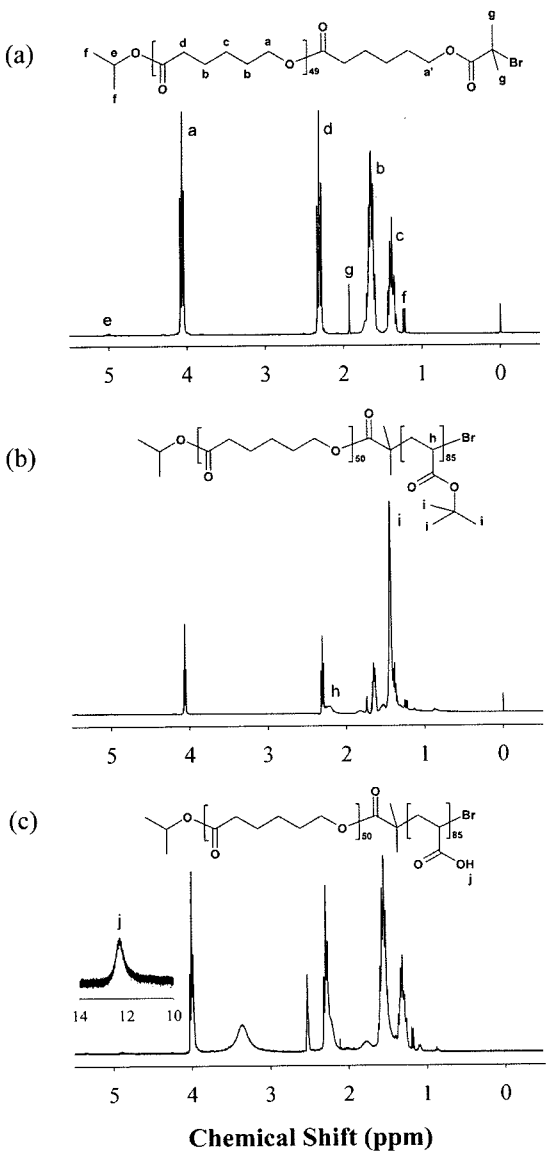


Figure 1. ^{1}H NMR spectra of (a) PCL macroinitiator in $CDCl_3$, (b) PCL-*b*-P*t*BA in $CDCl_3$, and (c) PCL-*b*-PAA in DMSO-d_6.

P*t*BA and PCL-*b*-PAA. When Figure 2(a) is compared with Figure 2(b), the molecular weight increases without significant change in polydispersity index (PDI) as the block copolymer of PCL-*b*-P*t*BA is formed, indicating that the block copolymerization proceeds in a controlled manner. Since the PDI of PCL-*b*-PAA was nearly eaqul to that of PCL-*b*-P*t*BA, it is realized that PCL backbone is essentially intact without noticeable degradation during selective cleavage of *tert*-butyl groups under acidic condition. Table I lists the molecular weights of polymers and their distribution.

Micellization Behavior. The formation of micelles from amphiphilic PCL-*b*-PAA was verified by fluorescence spectroscopy using pyrene as a probe. When the intensity ratio

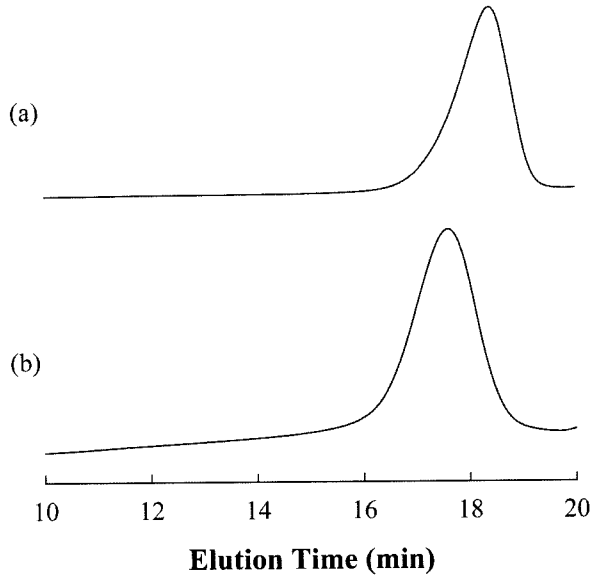


Figure 2. GPC traces of (a) PCL macroinitiator determined by $CHCl_3$ based GPC and (b) PCL-*b*-P*t*BA determined by $CHCl_3$ based GPC.

Table I. Molecular Weight and Molecular Weight Distribution of PCL Macroinitiator, PCL-*b*-P*t*BA, and PCL-*b*-PAA by ^{1}H NMR and GPC

Polymer	$M_{n,\text{calc.}}$	$M_{n,\text{NMR}}$	$M_{n,\text{GPC}}$	PDI_{GPC}
PCL maroinitiator	5,000	5,700	12,000[a]	1.33[a]
PCL-*b*-P*t*BA	15,700	16,800	25,700[a]	1.31[a]
PCL-*b*-PAA	12,000	10,400	10,400[b]	1.41[b]

[a]Determined by $CHCl_3$ based GPC.
[b]Determined by THF based GPC.

of I_{339}/I_{334} from pyrene excitation spectra is plotted against the concentration of PCL-b-PAA, the intensity remains nearly unchanged at lower concentration and then starts to increase steeply at a certain concentration, where the critical micelle concentration (CMC) is determined from the intersection of two straight lines, as shown in Figure 3.[15] The CMC of PCL-*b*-PAA determined from the intersection was 0.001 mg/mL. Here, it is noted that pyrene itself does not affect the CMC value significantly because the total amount of pyrene in solution is extremely small.

Preparation of SCMs. SCMs were prepared from PCL-*b*-PAA micelles by following the method reported previously.[14] The carboxylic acid groups of corona PAA block were first activated by addition of EDACH. As a crosslinking agent, EDBEA, is added to the micelle solution of PCL-*b*-PAA, the shell composed of PAA blocks is crosslinked by formation of amide bonds between carboxylic group of PAA and amine groups of EDBEA, as shown in Scheme I. The crosslinking density is controlled by changing the stoichiometric equivalence of amine group to carboxylic group.

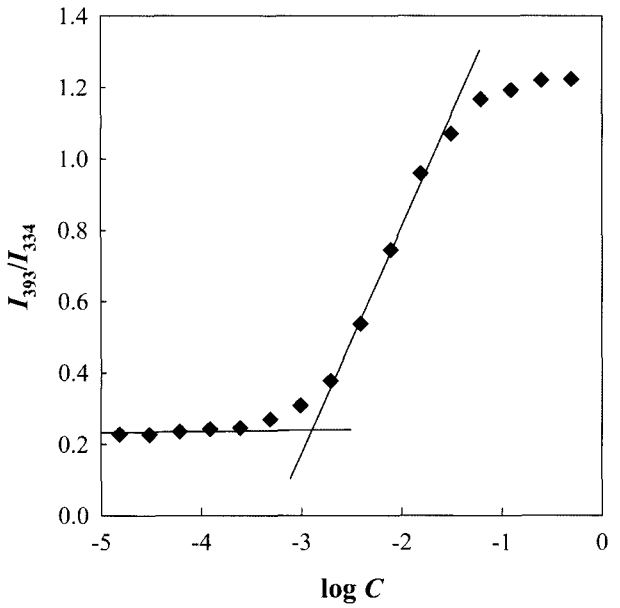


Figure 3. Plot of the intensity ratio of I_{339}/I_{333} from pyrene excitation spectra as a function of PCL-*b*-PAA concentration.

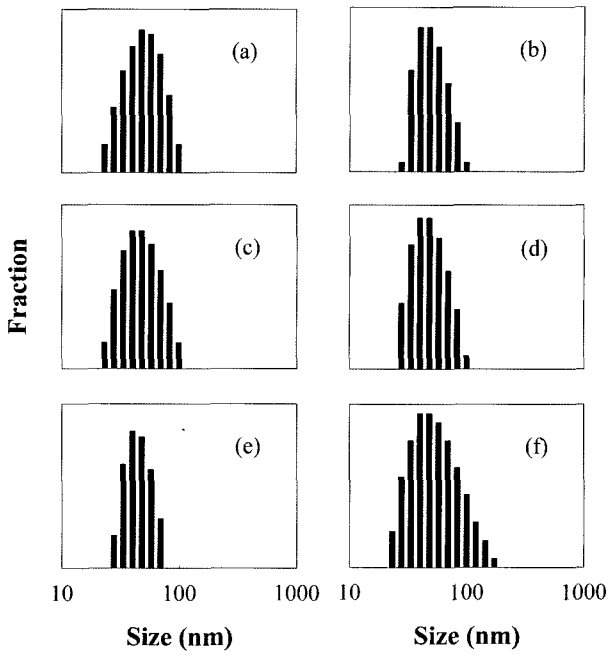


Figure 4. Fractional size distributions of SCMs measured by dynamic light scattering: (a) CA00, (b) CA20, (c) CA40, (d) CA60, (e) CA80, and (f) CA100.

Table II. Characterization Data for SCMs with Different Shell Crosslinking Densities

Sample Designation	Crosslinking Density (%)[a]	D_h (nm)[b]	Pyrene Loading Efficiency (%)[c]
CA00	0	52.1	41.0 %
CA20	20	51.6	-
CA40	40	50.2	41.0 %
CA60	60	50.2	-
CA80	80	45.7	41.0 %
CA100	100	59.5	-

[a]Theoretical percent crosslinking density based on the stoichiometric ratio of amine groups of crosslinking agents to carboxyl acid groups of PAA block segments.
[b]Hydrodynamic diameter measured by dynamic light scattering in aqueous solution.
[c]Determined after removal of free pyrene by ultrafiltration.

Detailed experimental conditions and sample designations are listed in Table II, where the percentage of crosslinking density is the maximum crosslinking based upon the stoichiometry.[14,16] When the hydrodynamic diameters of SCMs prepared with different crosslinking densities are compared with each other, it is realized that the diameters do not show a significant difference between them, as can be seen in Table II. However, Figure 4 shows that CA100 shows much broader size distribution than CA00 and other SCMs. Indeed, the size distribution of micelle becomes slightly narrower as the crosslinking density increases and then becomes broader at CA100. Close examination of Figure 4(f) reveals that CA100 shows a bimodal distribution with a small shoulder at larger sizes. This leads us to assume that the inter-micellar crosslinking reaction takes place at high crosslinking densities.

Release Behavior. Micelles are known to encapsulate and solubilize hydrophobic drugs in the hydrophobic core due to the hydrophobic-hydrophobic interaction. Drug encapsulation efficiency of PCL-*b*-PAA micelles and SCMs prepared from them are investigated by the o/w emulsion method using pyrene as a model drug, where pyrene is chosen due to its high fluorescence sensitivity and its simplicity for quantitative analysis. After removal of free pyrene by ultrafiltration, the pyrene loading efficiency is determined by the weight ratio of encapsulated pyrene in PCL-*b*-PAA micelles to PCL-*b*-PAA micelles. The weight ratio is calculated on the basis of the intensity of fluorescence emission spectra of pyrene at 393 nm. The loading percent of pyrene within PCL-*b*-PAA micelle and SCMs are listed in Table II. This indicates that pyrene molecules are not released from PCL-*b*-PAA micelles during formation of SCM.

To investigate the effect of shell crosslinking on the release behavior of pyrene below the CMC, CA00 is used as a non-crosslinked conventional micelle, and CA40 and CA80 are used as SCMs with different crosslinking densities. It should be noted that the solubility of pyrene is 0.135 mg/L (taken from International Chemical Safety Cards), which means that pyrene is still soluble in the release medium under our experimental condition. Figure 5 compares the cumulative release profiles of CA00, CA40, and CA80.

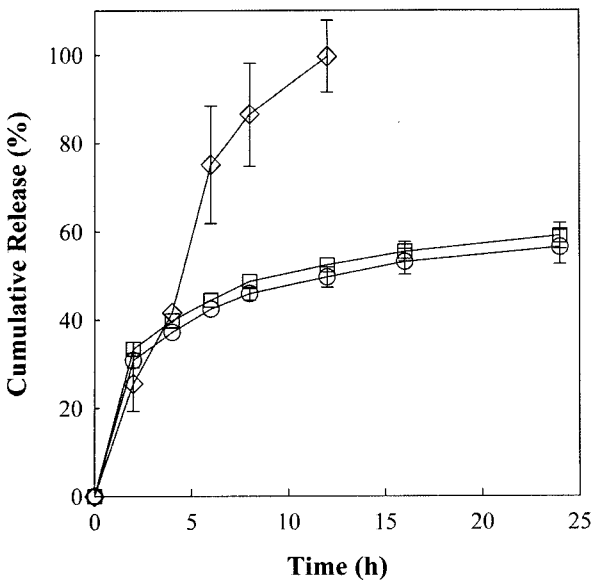


Figure 5. Pyrene release profiles below the CMC from CA00 (◇), CA40 (○), and CA80 (□).

Profiles of both CA40 and CA80 indicate that both SCMs show sustained release behavior for a prolonged time and residual pyrenes were not released for further incubation of a few days, whereas CA00 released pyrene rapidly with the initial burst phenomenon as compared to CA40 and CA80. This is because the conventional micelles dissociate into free diblock copolymer chains below the CMC whereas SCMs maintain their micellar structures even below the CMC due to chemical crosslinks in the shell of SCM. It is easily expected that CA20 with a mild crosslinking density also shows a sustained release behavior similar to CA40 and CA80, because CA20 does not dissociate into free unimers below the CMC. Here the crosslinked shell acts as a physical barrier or as a membrane to gate the diffusion of small molecules into and out of the micelles.[17] It is noted that the release behavior of CA40 is not significantly different from that of CA80, indicating that the crosslinking density does not affect significantly the release behavior. This might be attributed to relatively small molecular size of pyrene as compared to the mean distance between crosslinking points. We believe that the release behavior would be more significantly affected by the crosslinking density when a larger size of molecule is used as a model drug. Indeed, Wooley and her coworkers have reported that the released amount and the release rate of polystyrene (PS) chains are altered by the shell crosslinking density.[18] It should be noted that the molecular weight of PS chain used as a model drug in their study is much larger (M_w=14,100) than that of pyrene.

Conclusions

As a potential drug delivery system, SCMs with different shell crosslinking densities are synthesized and their drug release behavior is compared with non-crosslinked conventional micelle. SCMs exhibit the unique release behavior of guest molecules due to the crosslinked shell layer. The hydrogel-like properties of the crosslinked shell layer enable SCMs to control the rate of the drug release. Below the CMC, SCMs show sustained release behavior, whereas conventional micelles exhibit burst release mainly due to dissociation of micelles into free diblock chains. It is also found that the crosslinking density does not affect significantly the release behavior when small molecular size of probe such as pyrene is used as a model drug.

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