공학석사학위논문

pH-Sensitive Polymeric Micelles Based on PEG-Oligo(L-Lysine)-PCL System

폴리에틸렌글라이콜-라이신-폴리카프로락톤 블록공중합체 기반의 pH 민감성 마이셀 시스템

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이 논문을 공학석사 학위논문으로 제출함

2012년 6월

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강 정 규 의 공학석사 학위논문을 인준함

2012년 6월

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ABSTRACT

Methoxy poly(ethylene glycol) amine with a molecular weight of 5K and ε-caprolactone with a molecular weight of 3K were conjugated to five lysine residues with a molecular weight and M_w/M_n of 9.6K and 1.04. The shift of peak molecular weight and narrow molecular weight distribution in GPC trace without any noticeable shoulder as well as ^{1}H NMR analysis confirmed the successful synthesis of the copolymer. Polymeric micelles, size around 60 nm, were formed by dialysis and crosslinked micelles were prepared by adding crosslinker, terephthalaldehyde, to generate weak acid labile benzoic-imine bond in the interface of the micelle-forming amphiphilic copolymer. The critical micelle concentration of non-crosslinked micelle and crosslinked micelle was determined to be 4.26×10^{-2} mg/mL and 7.01×10^{-3} mg/mL, respectively. The hydrolysis rate of the crosslinked micelles is highly pH-dependent and much more rapid at mild acid than physiological conditions. Doxorubicin was successfully loaded into the crosslinked micelles and a controlled pH-dependent release behavior was observed. The enhanced micelle stability opens a way for preparing long circulating delivery systems encapsulating poorly water

soluble drugs.

Keywords: Micelle, pH-sensitive, crosslinking, lysine, poly(ethylene glycol), poly(ε-caprolactone)

Student number: 2010-23176

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1. INTRODUCTION

In drug delivery system, formulation of poorly water-soluble drugs has been one of the most important problems.[1] Micelles formed by the self-assembly of amphiphilic block copolymers are promising tools for drug delivery applications.[2] The amphiphilic nature of copolymer micelles, composed of hydrophobic core and hydrophilic outer shell, have advantages of encapsulating hydrophobic drugs.[3-5] The hydrophobic nature of the micelle makes them efficiently load hydrophobic drugs through hydrophobic-hydrophobic interaction, meanwhile the hydrophilic shell can stabilize the aggregates and effectively reduce the micelle clearance through the reticuloendothelial system.[6,7] Normally, poly(β-amino ester), poly(lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL) are considered for the hydrophobic core according to the delayed hydrolysis depending on the degree of hydrophobicity and enzyme-catalyzed degradation. For the hydrophilic shell, the most widely used polymer is poly(ethylene glycol) (PEG) because of its excellent solubility in organic solvent as well as in water and non-toxic characteristics.[8,9] Due to smaller size (<200nm) and presence of PEG chains on the surface of the micelles, these carriers can

have a longer blood circulation and ability to accumulate passively in the tumor site, which is mediated by an enhanced permeability and retention (EPR) effect. Various copolymer micelles have been developed as potential carriers for antitumor drugs with poor water solubility.[10, 11]

One major problem of polymeric micelles for drug delivery systems is the low structural stability in the blood stream due to the concentration-dependant characteristics represented by the critical micelle concentration (CMC), which has limited its applications as an *in vivo* drug carrier. Thermodynamically, micelles are disassembled into unimers at a concentration lower than the CMC. The injection of a micelle-based delivery system into the blood stream meets such a diluted condition.[12] Stabilization of micelles via shell crosslinking and core crosslinking strategies have been considered to overcome this problem. Wooley's group reported the first example of shell crosslinking micelles in 1996.[13] In the same year, Liu report the first method to make core crosslinking micelles.[14] Especially, shell crosslinking is an excellent approach for stabilization of micelles and generation of hollow nanocarriers. These shell crosslinked nanostructures are expected to have greater application than their core stabilized counterparts, due to their higher core mobility, greater versatility

in the core composition and properties, and membrane-like characteristics of the crosslinked shell layer, which improves their encapsulation and surface binding potential.[15] Therefore, the crosslinked micelles are stable at high dilution compared to the conventional micelles and this robustness makes the application of micelles *in vivo* possible.[16]

Recently, responsive crosslinking has been reported by the application of external stimuli such as pH, temperature, enzyme or hypoxia condition.[17-19] Among these various stimuli-responsive systems, pH-responsive amphiphilic block copolymer micelles which is capable of releasing drugs in a pH-dependent manner at slightly acidic pHs are promising for antitumor chemotherapy. Since the extracellular environment of solid tumors is slightly acidic (pH ~6.8) compared with the physiological pH 7.4, degradation of a drug carrier in response to the change of external pH around 6.8-7.4 would especially help to gain smart antitumor drug delivery systems.[20] There are lots of examples of pH-sensitive crosslinked micelles. Wooley reported pH-responsive shell crosslinked nanoparticle-based acetaldehyde conversion.[21] Bulmus reported a core crosslinked micelle with an acid-labile crosslinker di(2-acryloyloxy ethoxy)-[4-hydroxyphenyl] methane via reversible addition-fragmentation chain transfer (RAFT)

polymerization *in situ*.[2] Yang and coworkers prepared degradable micelles poly(ethylene glycol)-b-(n-octadecane amine) with benzoic-imine linker for tumor specific uptake and enhanced intracellular drug delivery.[22] Among these, benzoic-imine bond was significantly influenced by the solution pH within a very narrow pH interval (7.4-5.0). The imine bond is not frequently used for the synthesis of delivery vehicles because it is less stable under physiological conditions.[26] However in Yang's earlier study showed that an inclusion of conjugate structure could efficiently stabilize the linkage at physiological pH whereas the linker hydrolyzed at very slight acidic condition, i.e., pH ~6.8, near the extracellular pH of solid tumors. The benzoic-imine bond will be promising in building delivery systems responsive to small pH fluctuations under physiological conditions.

Inspired by the extremely pH-sensitive system, we designed and synthesized an inner shell crosslinked micelle with acid-labile crosslinker. The copolymer consisted of biocompatible PEG, cationic oligo amino acid and PCL. Terephthalaldehyde was used as crosslinker. The stability of micelles and pH dependent release behavior were investigated in the presence and the absence of crosslinker. The schematic strategy of such a system is shown in Figure 1.

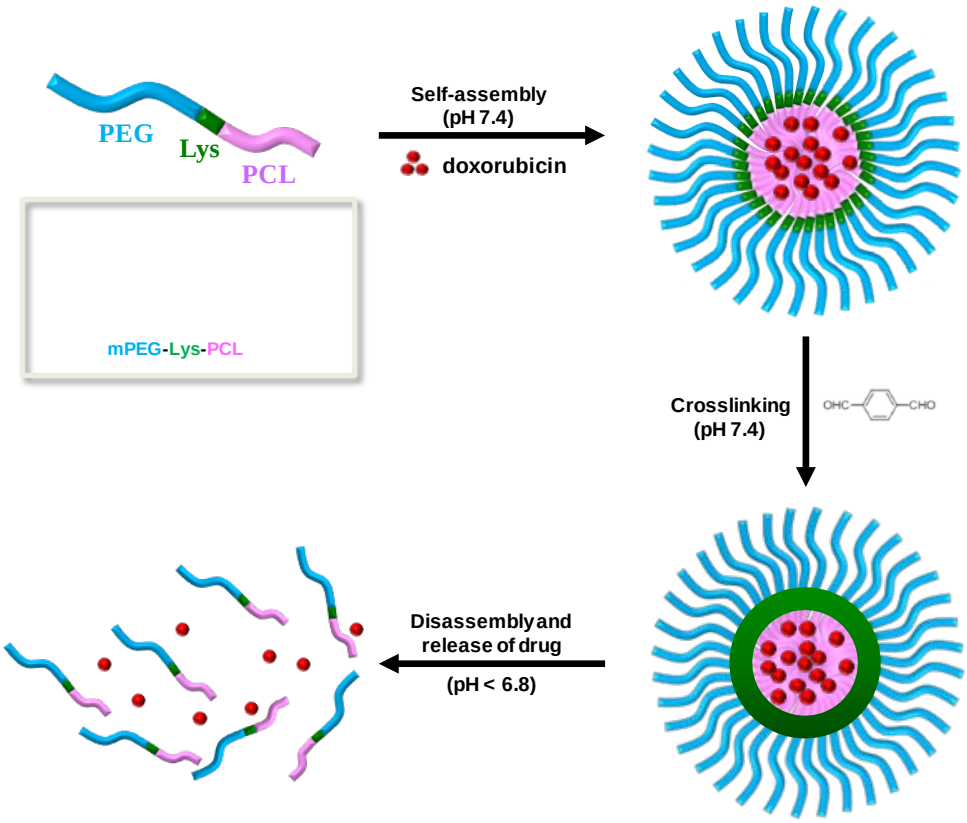


Figure 1. The concept of designing proposed pH-sensitive micelle.

2. EXPERIMENTS

2.1 Materials

HCl (2M) in diethyl ether, ε-caprolactone (99.0%), anhydrous methanol (99.8%), triphosgene, triethylamine (TEA), 4-nitrophenyl chloroformate (NPC), formic acid, palladium activated carbon (Pd/C, 10wt%), terephthalaldehyde (TPA) and N,N-dimethylformamide (anhydride, 99.8%) (DMF) were purchased from Sigma-Aldrich (St. Louis, MO). (N^{ε}-benzyloxycarbonyl)-L-lysine (CBZ-L-lysine) was commercially available from Bachem Bioscience (King of Prussia, PA). Methoxy PEG amine (MW: 5K, 95+ %) (mPEG-NH_2) were obtained from Sunbio (Korea). Sodium bicarbonate, dichloromethane, tetrahydrofuran (THF), n-hexane, diethyl ether and methanol were purchased from Daejung Chemicals and Metals Co. (Korea). Dichloromethane and TEA were dried over calcium hydride. THF and n-hexane were freshly distilled over sodium under nitrogen atmosphere. All other chemicals were used as received.

2.2 Instruments

^{1}H-NMR analysis was performed using Bruker Avance 300 MHz spectrometer in $CDCl_3$, DMSO-d_6 or D_2O at room temperature. Molecular weight and its distribution were determined by gel permeation chromatography using Shimadzu RID-10A refractometer detector Styragel HR 3, HR 4 and HR 4E columns. DMF was used as an eluent with the flow rate of 1.0 mL/min and PEG standards were used for calibration. Measurement of particle size was carried out using Otsuka ELS-Z size analyzer equipped with He-Ne laser at a wavelength of 630nm. UV-vis spectra were recorded from UV-2450 by Shimadzu (Tokyo, Japan) scanned over the range of 200~700 nm. Fluorescent spectra were obtained by Shimadzu RF-500 spectrofluorophotometer.

2.3 Synthesis of amino acid N-carboxy anhydride (NCA)

N^{ε}-CBZ-L-lysine (1.00 g, 3.57 mmol) in anhydrous THF (10.0 mL) were suspended into an 100 mL 2-neck round bottom flask equipped with a condenser and N_2 inlet-outlet and the temperature of the reaction mixtures was gradually increased to 55 ^{o}C. Calculated amount of triphosgene (0.44 g, 1.48 mmol), dissolved in 5 mL anhydrous THF, was added dropwise into the

solution. After all the suspensions of amino acids disappeared, the reaction mixture was stirred for an additional 1 h to ensure the formation of N-carboxy anhydrides. The reaction mixture was condensed and poured into a 10 fold excess amount of anhydrous n-hexane to precipitate NCA. N^{ε}-CBZ-L-lysine NCA was obtained by filtration and dried in vacuum oven for 24 h (0.92 g, 92 %). Characterizations of the synthesized amino acid NCAs by ^{1}H NMR spectrometer are as follow. N-CBZ-lysine NCA (DMSO-d_6, ppm): 7.38 (m, 5H, Ar*H* in CBZ), 5.01 (s, 2H, ArCH_2O), 4.43 (t, 1H, α-C*H*), 2.96-3.00 (m, 2H, ε-CH_2), 1.39-1.46 (m, 2H, β-CH_2), 1.02-1.33 (m, 4H, γ- and δ-CH_2).

2.4 Ring-opening polymerization and activation of ε-caprolactone

Poly(ε-caprolactone) (PCL) was synthesized using metal-free cationic ring opening polymerization as reported earlier.[23] Anhydrous methanol (0.02 g, 0.67 mmol) and 2 M HCl in diethyl ether (1.0 equiv. mol to methanol) were added into anhydrous dichloromethane (MC) (10.0 mL) in a 2-neck round bottom flask under nitrogen atmosphere using microsyringe. ε-Caprolactone (2.00 g, 17.52 mmol) was dissolved in 5 mL MC and

introduced to the reaction mixture. Polymerization was continued at room temperature for 24 h. After the reaction was completed, the mixture was precipitated 2 times in cold methanol and the obtained PCL was dried under vacuum for 24 h (1.96 g, 98 %).

PCL (1.50 g, 0.50 mmol), 4-nitrophenyl chloroformate (0.40 g, 2.00 mmol) and 15 mL anhydrous MC introduced into a 100 mL 2-neck round bottom flask and anhydrous TEA (0.20 g, 2.00 mmol) was added under nitrogen atmosphere. The reaction mixture was stirred for 24 h at room temperature, and extracted with water to remove excess NPC. The organic layer was separated, dried over magnesium sulfate, and then precipitated into cold methanol. Activated NPC was obtained by filtration and dried in vacuum at 25 ^{o}C for 24 h (1.31 g, 87 %). Molecular weight was 3K by ^{1}H NMR and 2.1K by GPC with M_w/M_n, of 1.08. Characterizations of the synthesized activated PCL by ^{1}H-NMR spectrometer are as follows. PCL-NPC ($CDCl_3$, ppm): 8.27 (d, 2H, Ar*H* in NPC), 7.38 (d, 2H, ArH in NPC), 4.29 (t, 2H, $CH_2CH_2CH_2CH_2CH_2$COO from PCL reacted with NPC), 3.95-4.19 (m, 26H, $CH_2CH_2CH_2CH_2CH_2$COO from PCL), 3.63 (t, 3H, CH_3COO), 2.28-2.49 (m, 26H, $CH_2CH_2CH_2CH_2CH_2$COO), 1.62-1.79 (m, 52H, $CH_2CH_2CH_2CH_2CH_2$COO), 1.47-1.60 (m, 26H, $CH_2CH_2CH_2CH_2CH_2$COO)

2.5 Synthesis of mPEG-Lys-PCL

In 100mL 2-neck round bottom flask, mPEG-NH_2 (1.00 g, 0.20 mmol) was dissolved in 10 mL of anhydrous DMF. Lysine NCA (0.28 g, 1.00 mmol), dissolved in 5.0 mL anhydrous DMF, was added into the flask to initiate ring-opening polymerization and the reaction was continued for 24 h at room temperature under nitrogen atmosphere. Aliquots were taken for GPC analysis to produce the molecular weight of 6.4K with M_w/M_n value of 1.02. mPEG-Lys(Z) which has five amino acid units on average was used for the following experiments.

Activated PCL (0.66g, 0.22 mmol, 1.1 equiv) was dissolved in 5 mL anhydrous DMF and added into the reaction mixture of mPEG-Lys(Z). After additional 24 h reaction, the mixture was precipitated in 500 mL of cold diethyl ether and filtered to produce mPEG-Lys(Z)-PCL. The product was dried in vacuum at 25 ^{o}C for 24 h (1.40 g, 74 %, M_n = 8.1K, M_n/M_w = 1.04 by GPC).

The obtained copolymer (1.00 g, 0.11 mmol) was then dissolved in 20.0 mL DMF with 4.70 g of palladium catalyst for the deprotection reaction of CBZ groups at the side chains of lysine blocks. With vigorous stirring, 45.0 mL of formic acid was slowly added to the reaction mixture and the

deprotection reaction continued at room temperature for 12 h. The palladium catalyst was filtered and the filtrate was washed with 35.0 mL of 1 N HCl to replace the formate salt by hydrochloric acid. The solution was condensed and precipitated into cold diethyl ether and filtered. Obtained mPEG-Lys-PCL was dried under vacuum for 24 h at 25 ^{o}C (0.64 g, 64 %).

2.6 Preparation of micelle and crosslinking

mPEG-Lys-PCL (25.0 mg) was dissolved in 5.0 mL DMF and stirred for 12 h at room temperature. Micellization was induced by addition of phosphate buffer, at pH 7.4 (volume ratio of DMF to buffer solution: 1:4). After ultrasonication for 2 h in a bath sonicator, the solution was stirred overnight The solution was passed through a 0.22 um filter to produce nano-sized micelles.

The crosslinking reaction of micelles was initialized by adding accurately weighed terephthalaldehyde (TPA) to the precursor micelle solution. The ratio of copolymer to crosslinker was 1/20 (mol/mol). The reaction solution was allowed to stir for 12 h at 40 ^{o}C and then dialyzed (3.5K cut off) against 1.0 L of phosphate buffer (pH = 7.4) with eight changes within 24 h until the free TPA was not detected by the UV spectrometer.

2.7 Determination of the Critical Micelle Concentration (CMC)

The critical micellar concentration (CMC) was determined by using pyrene as a fluorescence probe. The concentration of block copolymer was varied from 1.0×10^{-3} to 1.0 mg/ mL and the concentration of pyrene was fixed at 1.2×10^{-6} M. The CMC values were estimated as the first inflection point of the evolution of the ratio I_{337}/I_{334} nm vs. concentration obtained from pyrene excitation spectra.

2.8 Stability of micelles in the presence of SDS and at different pH values

The stability study was performed to monitor the change in particle size of non-crosslinked micelle (NCM) and crosslinked micelle (CM) in the presence of sodium dodecyl sulfate (SDS), which was reported to be able to efficiently break down polymeric micelles. An SDS solution (7.50 mg/mL) was added to aqueous solutions of micelles (1.50 mg/mL). The final SDS concentration was 2.50 mg/mL and the micelle concentration was kept at 1.0 mg/mL. The size and size distribution of the micelle solutions were monitored at predetermined time intervals and pH.

2.9 Drug loading and release test

25.0 mg mPEG-Lys-PCL and 10.0 mg doxorubicin were dissolved in 5.0 mL DMF and self-aggregates encapsulating drugs were formed using the same procedure as described above. The amount of doxorubicin was determined using UV absorbance measurement at the wavelength of 498 nm. For determining of drug loading amount, 1.0 mL doxorubicin loaded non-crosslinked micelles and crosslinked were lyophilized for 2 days. The obtained powder was dissolved in 5.0 mL DMSO and analyzed by UV-Vis spectroscopy.

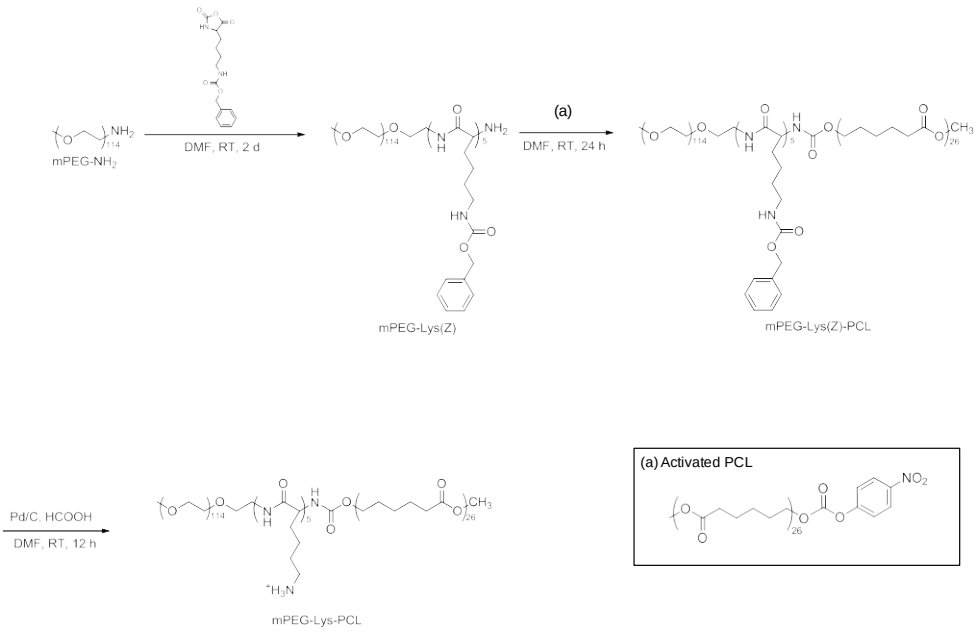
The release profiles of doxorubicin from NCM and CM were obtained by dialysis method using dialysis membrane with the molecular weight cut off of 3.5K at 37 ^{o}C. 2.0 mL doxorubicin encapsulated micelle solution was dialyzed against 40.0 mL fresh medium (phosphate buffer, pH 7.4) and 10.0 mL aliquot was taken from medium for analysis at predetermined time intervals. After 24 h, the medium was changed to acidic medium (pH 6.5). The acidic medium was prepared by titration of phosphate buffer using 1 N hydrochloric acid. The amount of released doxorubicin was determined by UV absorbance measurement at the wavelength of 498 nm. Average value of the released drug was reported with the data from three runs.

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization of block copolymer

An amphiphilic copolymer comprising amino acid oligomers between hydrophilic PEG and hydrophobic PCL was synthesized by the ring-opening polymerization of Z-Lysine NCA with amino methoxy PEG macroinitiators with a molecular weight of 5K, followed by coupling reaction with activated PCL with a molecular weight of 3K. The reaction was carried out *in situ* in order to minimize the oxidation of amino groups at the distal end of mPEG-Lys(Z). Scheme 1 shows the synthetic procedure to produce mPEG-Lys-PCL.

As shown in Figure 2(a) the Z-Lysine NCAs were characterized by ^{1}H NMR. Prepared Z-Lysine NCAs were polymerized by ring opening polymerization of α-amino acid NCA with polymerization initiator. The primary amine group containing mPEG-NH_2 attacks the carbonyl carbon of the NCA ring to initiate the polymerization, which results in the formation of polymer that contains amino acid and obtained polymer named mPEG-Lys(Z). The number of amino acid, Lysine(Z), in the copolymer was determined by ^{1}H NMR spectroscopy based on the relative peak integration



Scheme 1. Synthetic procedure of mPEG-Lys-PCL.

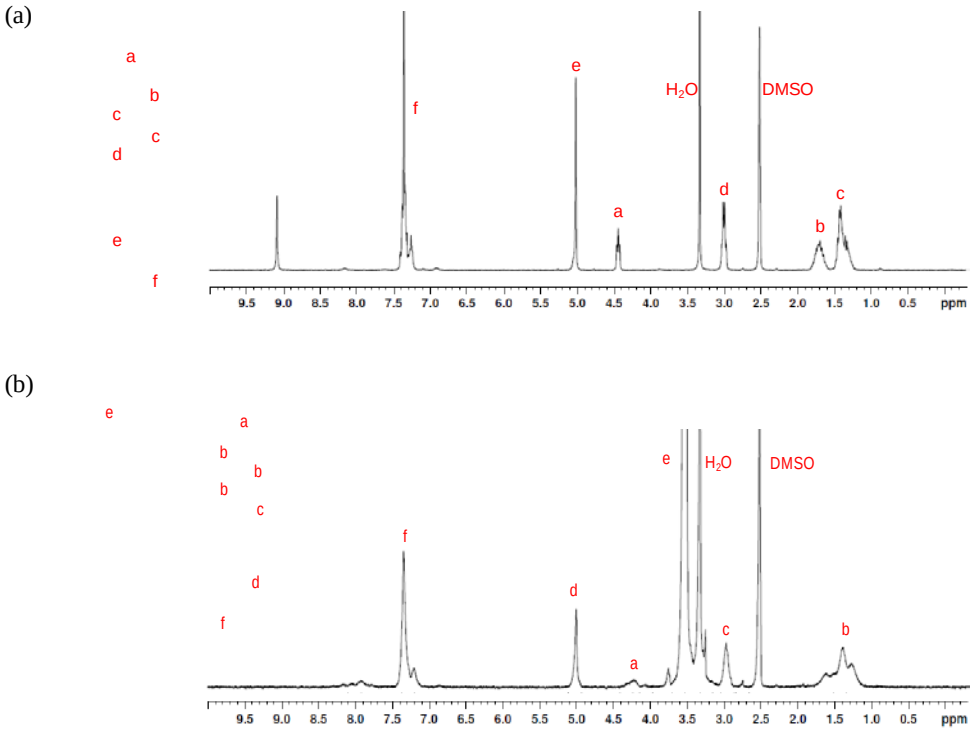


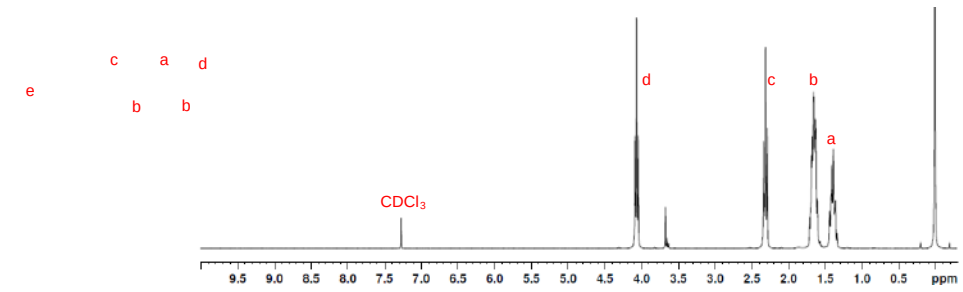
Figure 2. ^{1}H-NMR spectra of (a) Z-Lysine NCA and (b) mPEG-Lys(Z) in DMSO-d_6.

ratio between the methylene protons of mPEG around 3.5 ppm and those of benzyl carbamate at 5.2 ppm as shown in Figure 2(b). The polymer has five amino acid units on average.

ε-Caprolactone was polymerized with methanol as an initiator and 2 M HCl in ether as a catalyst to produce PCL with molecular weight of 3K by ^{1}H NMR.[23] PCL is consecutively activated with NPC. ^{1}H NMR spectra for PCL before and after activation are respectively shown in Figure 3.

The activated PCL was conjugated to the mPEG-Lys(Z) and the amphiphilic copolymers with amine groups at the junction between the hydrophilic PEG and hydrophobic PCL blocks were obtained by selectively cleaving the CBZ protecting groups in the lysine units without degrading the ester bonds in the PCL blocks.[24] Palladium, 10 wt% on activated carbon, was used as catalyst for hydrogenolysis, and the selective cleavage was confirmed by the disappearance of the benzyl carbamate peak at 7.2-7.4 ppm and intact integration ratio of the other groups. Figure 4 shows ^{1}H NMR spectrum of the polymers mPEG-Lys-PCL before and after deprotection. The GPC traces in Figure 5 support the successful oligomerization, coupling and deprotection reactions. A shift of the peak molecular weight before and after the coupling reaction without noticeable

(a)



(b)

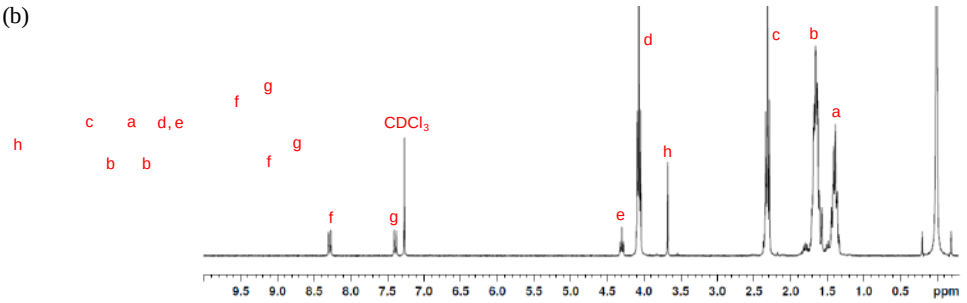


Figure 3. ^{1}H-NMR spectra of (a) PCL and (b) activated PCL in $CDCl_3$.

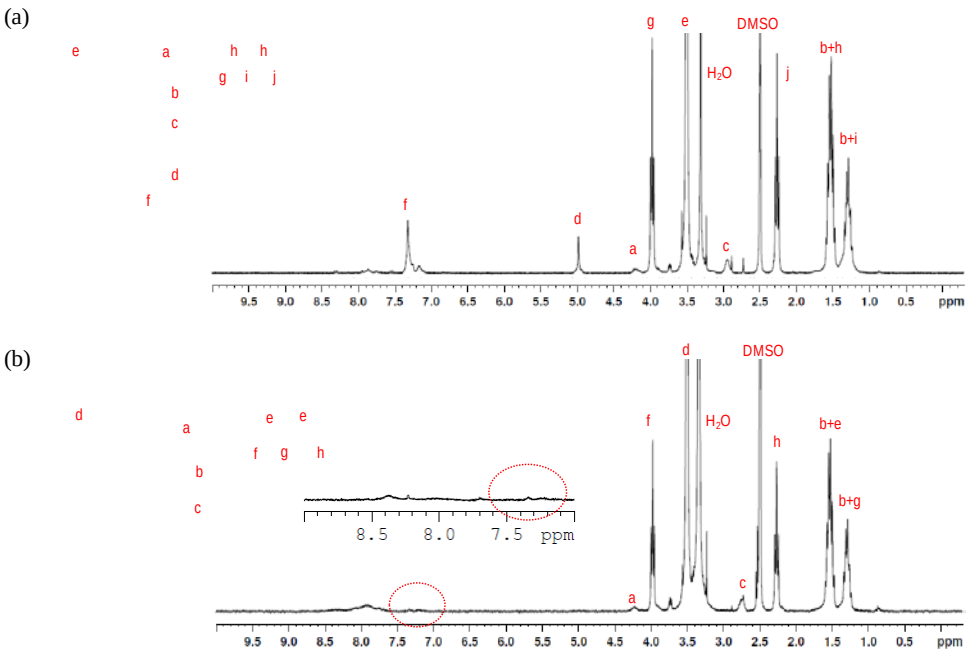


Figure 4. ^{1}H -NMR spectra of mPEG-Lys-PCL (a) before deprotection and (b) after deprotection in DMSO-d_6.

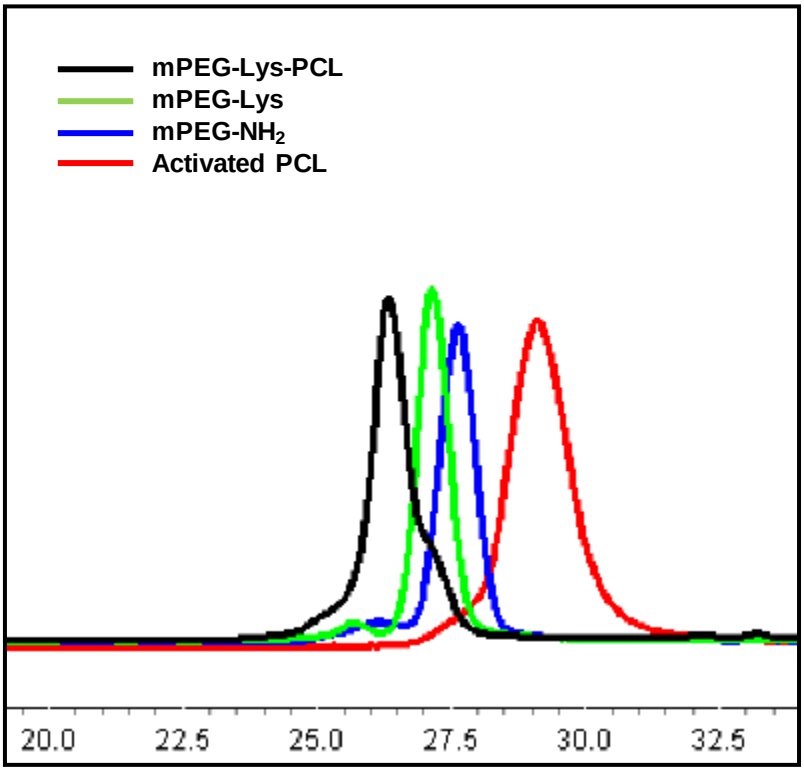


Figure 5. GPC traces of PCL, mPEG-NH_2, mPEG-Lys and mPEG-Lys-PCL using DMF as an eluent.

shoulders in the trace indicated successful formation of a block copolymer and the complete separation of the unreacted starting polymers.

3.2 Micelle preparation and crosslinking

Micelles were conveniently prepared by adding phosphate buffer into polymer solution in DMF followed by dialysis 24 h against phosphate buffer (pH 7.4). Figure 6(a) shows the size and its distributions determined by light scattering. DLS measurements revealed that non-crosslinked micelle had average particle size of 66.0 ± 44.2 nm.

Crosslinking procedures were carried out after the micelle formation. The crosslinker, terephthalaldehyde, were added to the micelle solution in DMF/H_2O (v/v = 1/4). After 12 h of reaction at 40 ^oC, the unreacted crosslinker and DMF were removed by dialysis against phosphate buffer (pH 7.4). The hydrodynamic diameters of crosslinked micelles were also measured by DLS. The result showed that the particle sizes of crosslinked micelles decreased to 60.0 ± 30.2 nm as shown in Figure 6(b). This result is coincident with Wooley's earlier report that the nanostructures underwent "shrinking" after the crosslinking. [25]

The UV-vis spectrum of a crosslinked micelle through the addition of

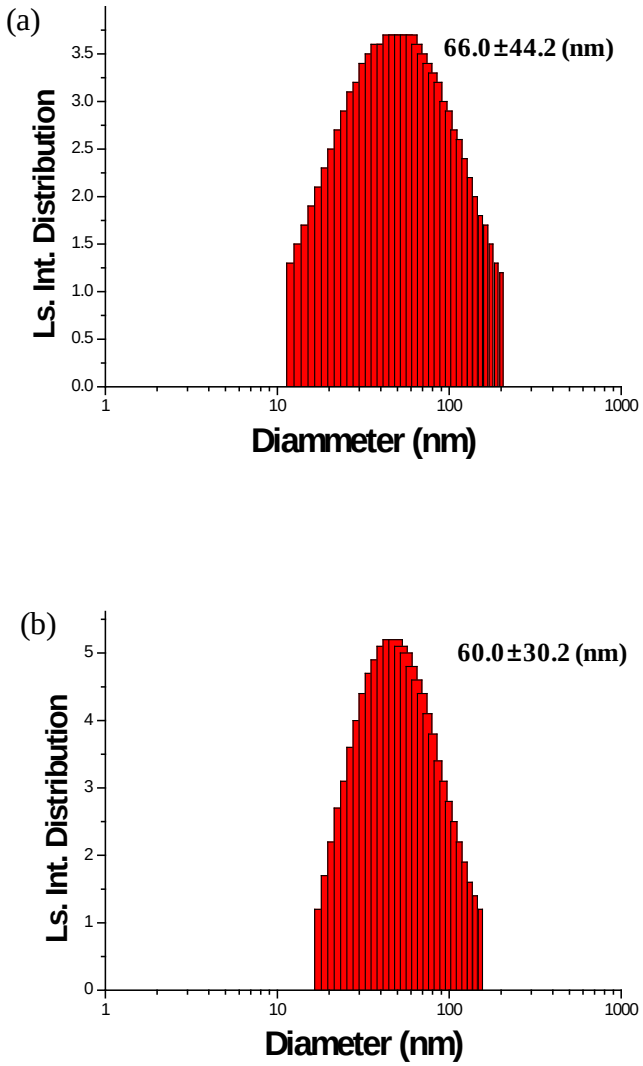


Figure 6. Size distribution of (a) non-crosslinked micelles and (b) crosslinked micelles in phosphate buffer at pH 7.4.

terephthalaldehyde is consistent with the formation of the benzoic-imine bond as it shows increased absorption at 260-280 nm. (Figure 7) This result also suggests that the benzoic-imine bond formation is an event that occurs within micelles. The PEG outer corona confines the crosslinking reaction intra-micellarly, preventing the formation of inter-micellar aggregates.

3.3 Critical micelle concentrations (CMC)s before and after crosslinking

The critical micelle concentration (CMC) is an implication of the relative thermodynamic stability of the micelles in solution.[28, 29] The CMC of the mPEG-Lys-PCLs before and after crosslinking was measured through fluorescence spectra by using pyrene as a hydrophobic fluorescent probe and found to be 4.26×10^{-2} mg/mL and 7.01×10^{-3} mg/mL , respectively. (Figure 8) After crosslinking, the CMC of crosslinked micelles decreased about 6 times lower than that of non-crosslinked micelles. This observation for the crosslinked micelles is consistent with the reported crosslinked Pluronic L121 micelles.[30] This result also suggests that the crosslinked micelles have enhanced stability in the diluted conditions.

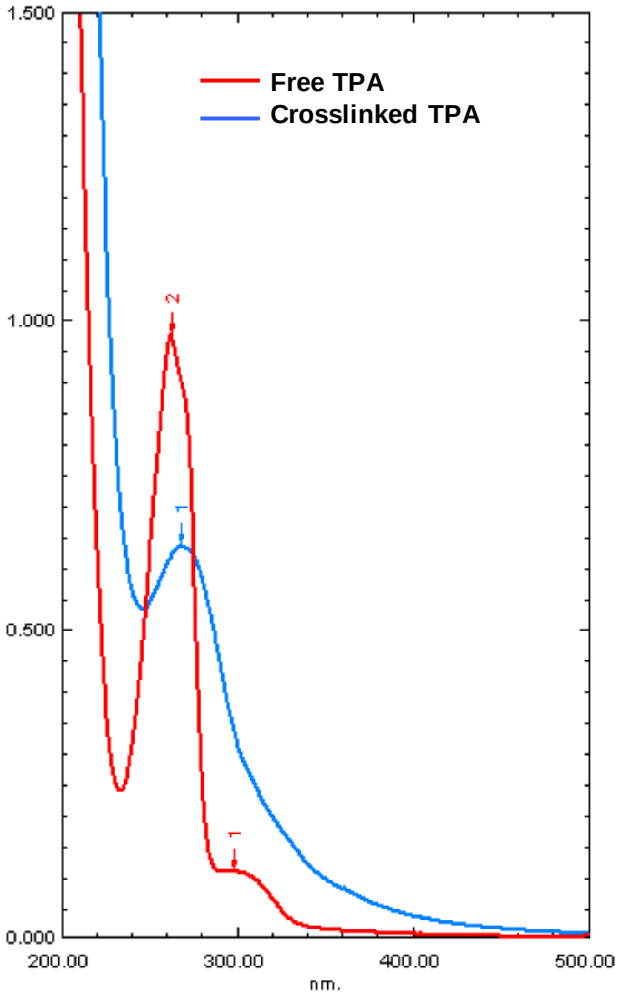


Figure 7. UV absorption spectrum of free TPA and crosslinked TPA.

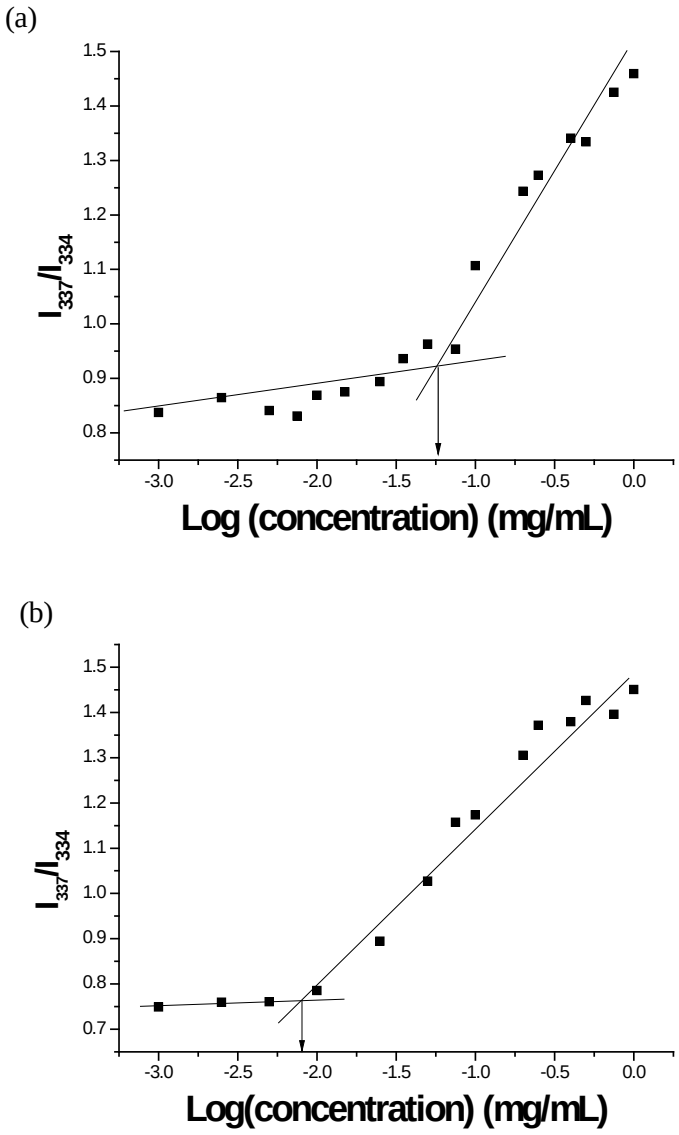


Figure 8. The critical micelle concentration (CMC) of (a) non-crosslinked micelle and (b) crosslinked micelle intensity ratio (I_{337}/I_{334}) from pyrene excitation spectra as a function of polymer concentration in PBS (pH 7.4).

3.4 Stability study of the micelles

Sodium dodecyl sulfate (SDS), a strong ionic detergent, has been reported to be able to efficiently break down polymeric micelles.[31] The exchange rate between polymeric micelles and unimers is accelerated by low concentrations of SDS while at higher concentrations, the presence of SDS micelles solubilize the amphiphilic block copolymers resulting in destabilization of the polymeric micelles.[32] The stability of non-crosslinked micelles and crosslinked micelles was tested in the presence of the reported micelle-disrupting SDS concentration of 2.5 mg/mL.[31] After each micelle solution (1.0 mg/mL, pH 7.4) was added with an aqueous solution of SDS (2.5 mg/mL), the particle size was monitored at various time points. The immediate aggregates appeared and consequent disappearance of particle size signal of the non-crosslinked micelles reflects the distinct dynamic association-dissociation property of non-crosslinked micelles as shown in Figure 9. The new larger aggregates appeared in the presence of SDS appears that the SDS molecules interacted with the block copolymer micelles to destroy the micellar structure of mPEG-Lys-PCL, leading to formation of large aggregates with a diameter up to 1 μm.

To monitor the stability and pH-dependent hydrolysis of the benzoic-

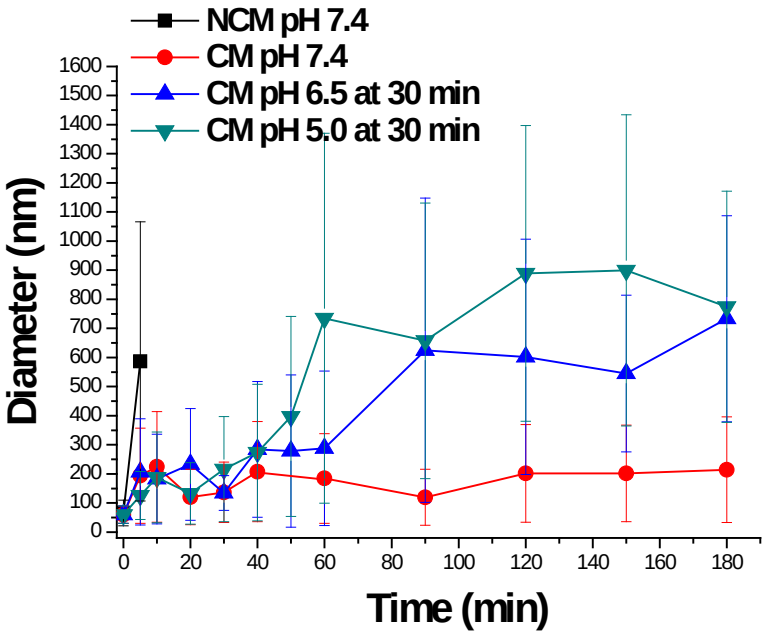


Figure 9. The stability in particle size of NCMs and CMs in the presence of 2.5 mg/mL SDS measured by DLS.

imine bond between mPEG-Lys-PCL polymers, the crosslinked micelles solutions (pH 7.4) were prepared. Addition of SDS to crosslinked micelles the particles did not fell apart at pH 7.4, but an increase in size from 60 to 180 nm was observed (Figure 9). DLS analysis did not detect particles in a micellar SDS dispersion (the size of SDS-micelles is below the detection limit). The particle size increase might be ascribed to absorption of SDS by the micelles.[33] On the other hand, when the pH of micelle solutions were adjusted to pH 6.5 and pH 5.0 respectively, it was shown that the hydrolysis extent of benzoic-imine bond increased with the decrease of solution pH. In addition, the hydrolysis reaction was rapid and reached equilibrium within 30 min. This result demonstrates that the crosslinked micelles have an excellent physical stability at physiological pH 7.4 and can be rapidly cleaved at both extracellular pH 6.8 of the tumor and the endosomal pH 5.0.[22]

3.5 Drug loading and *in vitro* release

The acid-labile behavior of the crosslinked micelles was investigated directly by measuring the pH-dependent release of doxorubicin, a poorly water-soluble and widely used antitumor drug, from the crosslinked micelles

formed from mPEG-Lys-PCL. The drug was loaded into the micelles during the micellization and subsequent crosslinking process. Doxorubicin loading amount and efficiency of non-crosslinked micelles were 3.4 % and 20.7 %, respectively. Crosslinked micelles also had similar loading amount and efficiency of 3.9 % and 23.5 %. The effect of crosslinking on the drug loading was not obvious but its effect on the release behavior was clear as shown in Figure 10.

The micelles from mPEG-Lys-PCL copolymer meet the conditions of pH-sensitive and effective targeting drug delivery system by sudden burst at the extracellular environment of solid tumors, while the delivery system maintains its stability during circulation in the bloodstream. Release profile of doxorubicin was monitored with three samples, which were prepared and stabilized at the physiological pH 7.4. As it can be seen in Figure 10, the drug release from the crosslinked micelles was found to be slower than that from the non-crosslinked micelles when the medium was maintained at pH 7.4. After 24 h, one of the dialysis tubes of crosslinked micelles was placed and incubated in pH 6.5 phosphate buffer solution, where the weak acid cleaved the benzoic-imine bond. The condition of weak acid destabilized the micelles via hydrolysis of benzoic-imine bond and the following

disassembly of the micelles triggered a burst release of doxorubicin, whereas the sustained release profile was maintained from the crosslinked micelles encapsulating doxorubicin in PBS at pH 7.4. Improved structural stability and burst release of encapsulated drugs responding to the external stimulus of weak acid assess the successful development of a pH-sensitive and specifically targeted drug delivery system.[34,35]

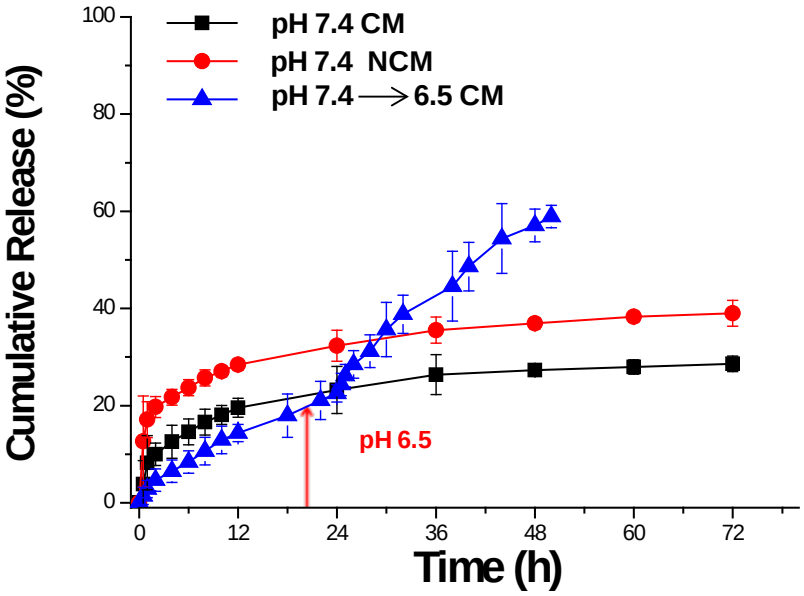


Figure 10. *In vitro* doxorubicin release patterns from micelles formed from mPEG-Lys-PCL in PBS medium.

4. CONCLUSION

An amphiphilic copolymer mPEG-Lys-PCL was synthesized and crosslinked via a novel pH-responsive benzoic-imine bond. The benzoic-imine linker was stable at physiological pH and cleaves with the increasing magnitude following the decrease of pH progressively to tumor and endosomal pHs. The crosslinked micelles maintained the stability of the micellar structure at high dilution and against good solvents. We have also shown the potential of the system as a drug carrier controlling the release of an antitumor drug in a pH-dependent manner. The release of doxorubicin from the crosslinked micelles was slower than that from the non-crosslinked micelles and can be gradually facilitated in a weak acidic environment. Stabilized micelles with benzoic-imine bond will be promising material for the development of polymeric nanocarriers that are applicable to *in vivo* drug delivery systems.

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요약문

항암제를 효과적으로 전달하기 위한 약물 전달체를 만들기 위해 마이셀 구조를 형성할 수 있는 폴리에틸렌글라이콜-라이신-폴리카프로락톤 블록공중합체를 합성한 후 크로스링커를 도입하여 마이셀의 생체내 안정성 및 약물 방출 효과를 높이를 연구를 수행하였다. 핵자기 공명 스캐너와 젤 투과 크로마토그래피 분석을 통해 블록공중합체가 성공적으로 합성된 것을 확인하였다. 사이즈 60 나노미터의 마이셀을 만든 후 크로스링커인 테레프탈알데하이드를 넣어 주어 마이셀을 형성하는 양쪽 친매성 고분자의 인터페이스 부분에 산에 약한 벤조익 이민 본드를 형성하였다. 크로스링킹 된 마이셀의 분해 속도는 pH에 의존적이었고 생리학적 환경의 pH에서보다 약산성의 환경에서 훨씬 빠르게 분해되었다. 독소루비신은 크로스링킹 된 마이셀에 성공적으로 탑재되었고 pH에 따라 약물이 방출되는 속도를 조절할 수 있었다. 이처럼 안정성이 증대된 마이셀은 물에 잘 녹지 않는 항암제를 탑재하여 오랜 시간 동안 몸 안을 순환하며

향상된 항암치료 효과를 기대할 수 있다.

주요 어: 폴리에틸렌글라이콜, 폴리카프로락톤, 라이신, 마이셀, pH 민감성, 크로스링커

학번: 2010-23176