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Notes

pH-Induced Micellization of Sulfamethazine-Coupled MPEG-PCLA Block Copolymer

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Introduction

With the growing interest for drug delivery systems, amphiphilic block copolymers have attracted considerable attention as drug carriers in pharmaceutical and biomedical applications.[1-3] The enormous potential of amphiphilic copolymers in these fields is originated from their micellar structure, consisting of hydrophobic inner core and hydrophilic outer corona. The inner core serves as a depot for water-insoluble drugs and regulates the retention as well as the release of them. Meanwhile the exterior corona with appreciable biocompatibility has the role of a palisade, protecting from protein adsorption and the subsequent non-specific uptake by mononuclear phagocytic systems (MPS) after intravenous injection. More recently polymeric micelles have been developed as gene carriers in multiple pharmaceutical applications and carriers for various contrasting agents in diagnostic imaging. To improve the delivery efficiency, the micelles have been required to response to the environment such as stimuli, pH, temperature, electric field and so on.[4,5] The control of micellization behavior in response to the exterior stimuli would make it possible to administrate drugs selectively on a targeted site.

In the human body, each organ has its own pH condition, for example, pHs of 1.3, 5~6, 6.6~7.5, and 6.4~7.0 in the stomach, mouth, small intestine, and colon, respectively. Also, it was found that the tumor cells have a lower extracellular pH than normal cells.[6] On the basis of these backgrounds, the pH-sensitive micelle has been proposed as a promising material for site-targeted carrier. Ionizable groups, i.e., carboxylic group as polyacids and amine group as polybases, are essential ingredients for pH-sensitive behavior of polymeric micelle. On the addition of ionizable groups in the block copolymer, the reversible micellization-demicellization might be controlled by adjusting the pH condition. When the ionization takes place in micelle structure, the charged moieties generally induce electrostatic repulsion and/or suppress the hydrophobic interaction. This phenomenon causes the change of hydrophilic-hydrophobic balance and solubility of the block copolymer, resulting in so-called demicellizaiton.

In this study, we synthesized a novel block copolymer, MPEG-PCLA-CSM-PCLA-MPEG, which is composed of hydrophilic methoxy poly(ethylene glycol) (MPEG), biodegradable hydrophobic poly(ε-caprolactone-*co*-D,L-lactide) (PCLA) and carboxylated sulfamethazine (CSM). It was deliberately designed to bear intrinsic biodegradability, amphiphilicity, and pH-sensitivity. Sulfamethazine was introduced to endow the pH sensitivity, which has two ionizable groups, an acidic sulfonamide group and a basic amine group.[7-9] The micellization-demicellization behavior was investigated at various pH conditions by fluorescence spectroscopy.

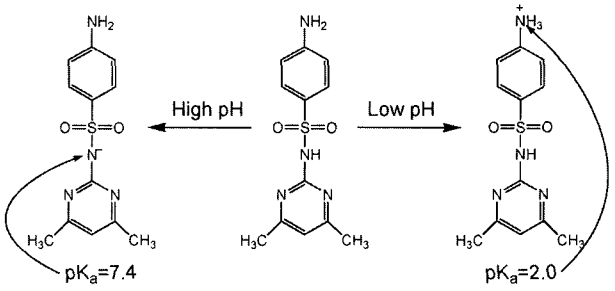

Figure 1. Ionization mechanism of sulfamethazine.

Experimental

Materials. The monomethoxy poly(ethylene glycol) (MPEG, M_n=750 g/mol), D,L-lactide (LA), ε-caprolactone (CL) and sulfamethazine were purchased from Aldrich. All other reagents and solvents were purchased from Sigma-Aldrich.

Carboxylation of Sulfamethazine. The carboxylated sulfamethazine (CSM) was obtained by carboxymethylation of the amine group of sulfamethazine.[10] For the carboxylation, first, the potassium salt of bromoacetic acid was prepared. Bromoacetic acid (70 mmol, 9.98 g) was dissolved in 20 mL of water. Then, 20 mL aqueous solution of potassium

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carbonate (35 mmol, 4.96 g) was added drop-wise into prepared bromoacetic acid solution under vigorous stirring at 0 °C. The resulting solution was added to the 250 mL round-bottom flask charged with sulfamethazine (18 mmol, 5 g) and water (60 mL), and then stirred at 80 °C for 12 hrs. The reaction mixture was filtered, dried, dissolved in THF, and filtered again. The filtrate was precipitated in diethyl ether and dried under vacuum at 60 °C for 48 hrs. The yield of CSM product was around 50%.

Synthesis of Biodegradable MPEG-PCLA Diblock Copolymer. The preparation of MPEG-PCLA diblock copolymers was carried out through one step ring opening polymerization. The ring opening polymerization for MPEG (M_n of 750 g/mol)-PCLA (MPEG/PCLA=1/0.8 wt/wt, CL/LA=1/1 mol/mol) is as follows: A 100 mL round-bottom flask was charged with MPEG (13 mmol, 10 g) and stannous 2-ethyl-hexanoate ($Sn(Oct)_2$) (0.05 wt%), and dried under vacuum at 80 °C for 2 hrs. The solution was then charged with nitrogen gas and cooled. CL (52 mmol, 5.70 mL) and LA (26 mmol, 3.87 g) were added, and the reaction mixture was stirred for 30 min at 80 °C. The temperature was then gradually increased to 135 °C and maintained for 24 hrs. Finally, the contents was cooled to room temperature, diluted with methylene chloride and then poured into hexane (500 mL) to precipitate the diblock copolymer. The hexane was decanted off and the obtained polymer was dried under vacuum for 12 hrs at room temperature.

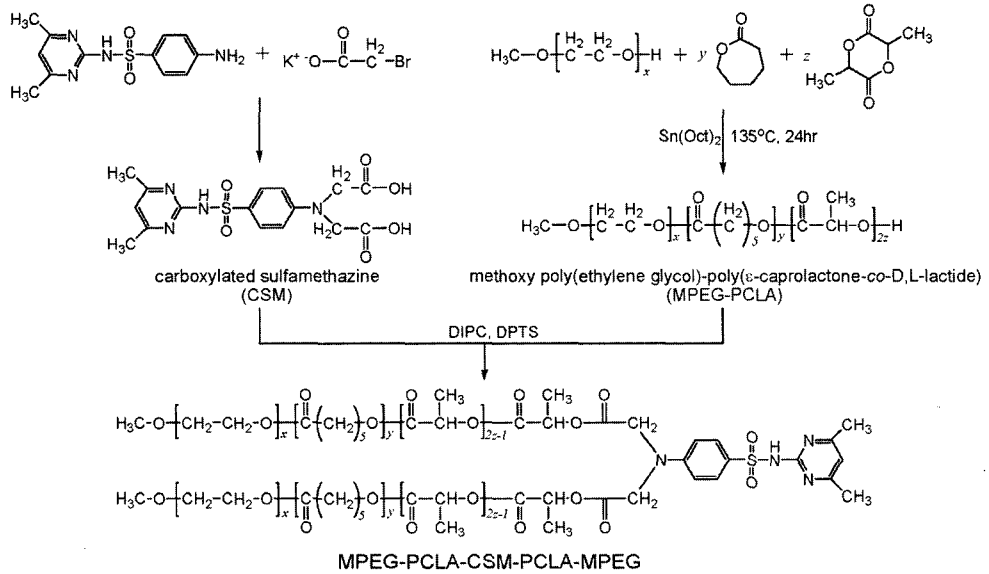
Synthesis of pH-Sensitive MPEG-PCLA-CSM-PCLA-MPEG Block Copolymer. The synthesis of the pH-sensitive block copolymer, MPEG-PCLA-CSM-PCLA-MPEG, was achieved by coupling reaction of the MPEG-PCLA and CSM. In this study, a coupling catalyst, 4-(dimethylamino) pyridinium 4-toluenesulfonate (DPTS), was prepared by the reaction of 4-(dimethylamino) pyridine (DMAP) and *p*-toluene sulfonic acid (PTSA).[11] The MPEG-PCLA, CSM, and DPTS were placed in a two-necked flask, and dried under vacuum at 70 °C for 2 hrs. The mixture was then dissolved in methylene chloride at room temperature.

Diisopropyl carbodiimide (DIPC) was added to the solution and the solution was stirred at room temperature for 24 hrs. The resulting content was filtered, evaporated and dissolved in THF. Then the solution was filtered again to eliminate the urea, then precipitated in diethyl ether, and dried under vacuum at 40 °C for 48 hrs. Overall synthetic scheme is shown in Scheme I.

Measurements. ^{1}H-NMR spectra were recorded on a Varian-Unity Inova 500NB, operated at 500 MHz, with DMSO-d_6 and $CDCl_3$ as the solvents. LC/MSD trap (Agilent-1100) and gel permeation chromatograpy (GPC) with styragel columns (Shodex-KF802.5, KF-803L) were used to determine the molecular weight. The fluorescence spectroscopy (AMINCO · BOWMAN® Series2) was performed using pyrene as a probe.

Results and Discussion

Synthesis of Carboxylated Sulfamethazine (CSM). The amine group of the sulfamethazine was reacted with bromoacetic acid to yield the carboxymethyl sulfamethazine. As shown in Figure 2, after reaction, the absorption for carboxyl group (1730 cm^{-1}) could be detected while the sharper absorption for primary amine at 3300~3500 cm^{-1} disappeared. We confirmed from FT-IR spectrum that the primary amine was successfully converted to the tertiary amine. In ^{1}H-NMR spectrum, the peak of the amino group of sulfamethazine appeared at 6 ppm, but this peak could not be



Scheme I. Synthetic scheme of the pH-sensitive MPEG-PCLA-CSM-PCLA-MPEG block copolymer.

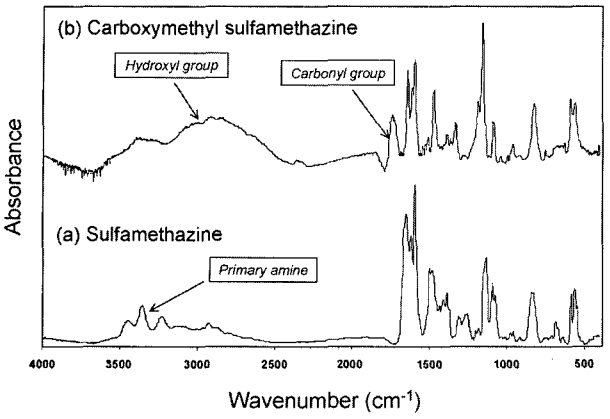


Figure 2. FT-IR spectra of sulfamethazine (a) and carboxylated sulfamethazine (b).

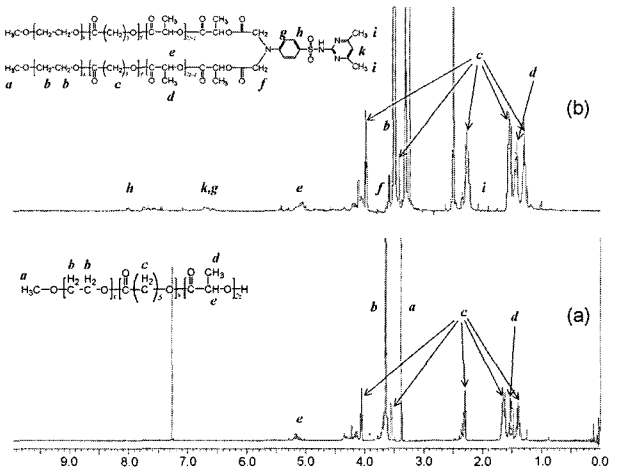


Figure 3. ^{1}H-NMR of the MPEG-PCLA diblock copolymer (a) and MPEG-PCLA-CSM-PCLA-MPEG copolymer (b).

observed for CSM (data not shown). The molecular weights of the SM and CSM were determined, using LC/MSD trap, to be 278.8 and 392.9, respectively.

Synthesis of MPEG-PCLA Diblock Copolymer. The MPEG-PCLA diblock copolymer, with a hydroxyl group, was prepared by ring opening polymerization. The synthesized diblock copolymer was characterized by ^{1}H-NMR (Figure 3(a)). The characteristic peak of the methyl protons (CH_3O-) (protons of a in Figure 3(a)) of MPEG in the copolymer appeared at 3.5 ppm in the ^{1}H-NMR spectrum. The peaks at 5.1 and 2.2 ppm were assigned to the protons bound to the carbons in the backbone of LA and CL, respectively. The MPEG and PCLA weight ratio was calculated based on the molecular weights of the MPEG and the MPEG-PCLA diblock copolymers obtained from GPC. The ratio of MPEG/PCLA and CL/LA is around 0.6 and 1, respectively. And the molecular weight of MPEG-PCLA block copolymer is about 1,200 g/mol.

Synthesis of Copolymer with MPEG-PCLA and CSM. The coupling reaction of MPEG-PCLA and CSM was conducted at room temperature to prevent the degradation of PCLA on moisture and heat. In general, dicylcohexyl carbodiimide (DCC) and DMAP are required for the coupling of acid and hydroxyl groups to form an ester group. However, these reagents were not suitable for our esterification reaction. This can be explained that the short distance between two acid groups of CSM impedes the approach of bulky DCC, resulting in the failure in formation of DCC-acid complex due to steric hindrance. To overcome this problem, DIPC was selected as a coupling reagent, where the aliphatic alkyl chain instead of cyclic group in DCC would help to facilitate the complex formation between DIPC and CSM. The synthesized triblock copolymer was verified by ^{1}H-NMR (Figure 3(b)) and GPC analysis. After the coupling reaction, the peaks for methyl protons in the benzene ring of the sulfamethazine were detected at around 7.6 ppm and the molecular weight of the final block polymer was found to be around 1,900 g/mol.

Micellization-Demicellization Behavior. The critical micelle concentration (CMC) was determined by a fluorescence probe technique using pyrene. The emission spectra of pyrene were measured from 350 to 440 nm at a fixed excitation wavelength of 334 nm. It is known that the fluorescence properties of pyrene largely depend on its microenvironment. The ratio of the intensity of the third vibrational peak (III, λ=384 nm) to that of the first vibrational peak (I, λ=374 nm) of emission spectrum of pyrene is generally employed to detect the micelle formation, because III/I value increases abruptly when pyrene is partitioned from a hydrophilic to a more hydrophobic environment, which indicates the micelle formation.[12] As shown in Figure 4, CMC values at pH 5.0 and 7.0 solutions were approximately 0.32 and 0.39 mg/mL, respectively. In the solution

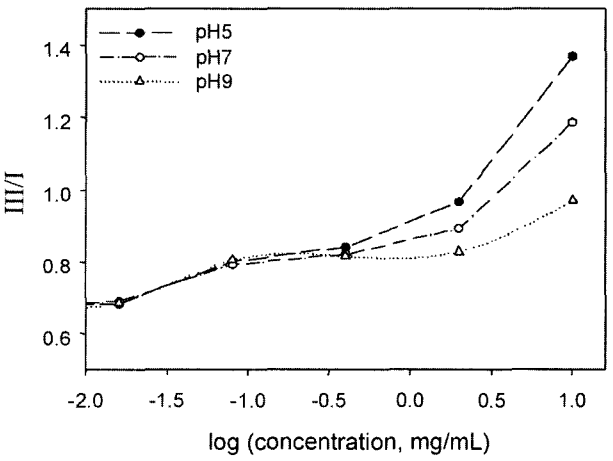


Figure 4. Plots of the intensity ratio III/I as a function of concentration of the MPEG-PCLA-CSM-PCLA-MPEG copolymer in various pH conditions.

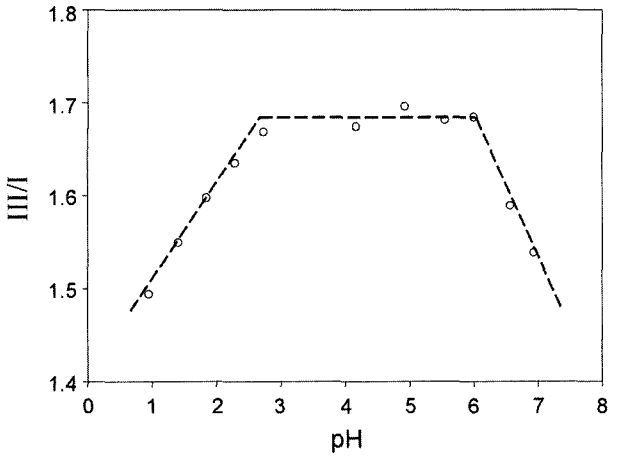


Figure 5. Plot of the intensity ratios III/I as a function of pH of the polymer solution.

of pH 9.0, however, the inflection point for determination of CMC value is very unclear. As the solution became basic, that is, the pH value is increased, the degree of the protonation of the sulfonamide group in the hydrophobic segment would increase, and thus the hydrophobic interaction and micelle formation were interfered due to the electrostatic repulsion and increased hydrophilicity.

Figure 5 shows the pH dependence of the micelle. The intensity ratio of III/I decreased below pH 2.6 and above pH 6.0 while the band ratio between pH 2.6 and 6.0 was nearly constant. It indicates that, at below pH 2.6 and above pH 6.0, micelle structure is unstable due to the ionization of the amine group and sulfonamide group in sulfamethazine as mentioned above (Figure 1).

Conclusions

The MPEG-PCLA-CSM-PCLA-MPEG block copolymer, with sulfamethazine as a pH-sensitive group, was synthesized, and the pH-sensitive micellization behavior was investigated. After coupling of the sulfamethazine with amphiphilic MPEG-PCLA block copolymer, the micellization-demicellization transition occurred at pH 6.0 and 2.6, due to the ionization of sulfonamide group and amine group, respectively. It is confirmed that the disassociation of micelle structure could be induced by the pH change, indicating that the targeted release would be possible. These micelles may be applicable to drug delivery system, diagnostic imaging as well as other potential applications.

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