Journal of Applied Polymer Science

Poly(methyl methacrylate)/Poly(ethylene glycol)/Poly(ethylene glycol dimethacrylate) Micelles: Preparation, Characterization, and Application as Doxorubicin Carriers

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ABSTRACT: A crosslinked amphiphilic copolymer [poly(ethylene glycol) (PEG)–poly(methyl methacrylate) (PMMA)–ethylene glycol dimethacrylate (EGDM)] composed of PMMA, PEG, and crosslinking units (EGDM) was synthesized by atom transfer radical polymerization to develop micelles as carriers for hydrophobic drugs. By adjusting the molar ratio of methyl methacrylate and EGDM, three block copolymer samples (P0, P1, and P2) were prepared. The measurement of gel permeation chromatography and ^{1}H-NMR indicated the formation of crosslinked structures for P1 and P2. Fluorescence spectroscopy measurement indicated that PEG–PMMA–EGDM could self-assemble to form micelles, and the critical micelle concentration values of the crosslinked polymer were lower than those of linear ones. The prepared PEG–PMMA–EGDM micelles were used to load doxorubicin (DOX). The drug-loading efficiencies of P1 and P2 were higher than that of P0 because the crosslinking units enhanced the micelles' stability. With increasing drug-loading contents, DOX release from the micelles *in vitro* was decreased, and in the crosslinked formulations, the release rate was also slower. An *in vitro* release study indicated that DOX release from the micelles for the linear samples was faster than that for crosslinked micelles. The drug feeding amount increased and resulted in an increase in the drug-loading content, and the loading efficiency decreased. These PEG–PMMA–EGDM micelles did not show toxicity *in vitro* and could reduce the cytotoxicity of DOX in the micelles; this suggested that they are good candidates as stable drug carriers. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39623.

KEYWORDS: crosslinking; drug-delivery systems; micelles

Received 15 January 2013; accepted 3 June 2013
DOI: 10.1002/app.39623

INTRODUCTION

Self-assembly linear amphiphilic copolymers composed of two chemically different homopolymers have been attractive as carriers for drug and gene encapsulation for decades.[1–3] They can be used to carry lipophilic drugs that can dissolve in the core of the micelles[4] and transport them to the target site effectively.[5,6] However, the thermodynamic transition of linear regimes is comparatively weak.[7] The assemblies may provoke disruption, or the copolymers may even partially hydrolyze in the enzyme environment.[8] The drugs encapsulated in micelles may leak out before arrival at the target site. So an ideal drug carrier has to combine the properties of target ability and excellent stability.

To this end, in the most recent several years, a variety of research efforts have been directed to the design of some novel amphiphilic copolymers and the addition of some functional groups to create more stability assemblies, which can maintain the original structure much longer in the process of transportation.[9,10] The normal idea is the use of three block methodologies or shell-functionalized copolymers to self-assemble the micelles, and these methods are also effective.[11] Jeong et al.[12] obtained a multiblock copolymer composed of two hydrophilic blocks and a hydrophobic one to prepare flower-type polymeric micelles in aqueous solution, and they found that the novel micellar aggregates had a much tighter hydrophobic core. This also improved its stability. Giacomelli et al.[13] prepared a self-assembled copolymer consisting of a dense, hydrophobic, PS-based core stabilized by a thin hydrophilic Beta-Cyclodextrin shell. However, among these methodologies, the linear amphiphilic copolymers were the easiest to synthesize. Hong et al.[14] proposed a shell-crosslinked micelle and found that the drug-release behavior of the shell-crosslinked micelles was successfully modulated at a controlled rate compared with that of noncrosslinked micelles, which showed a burst release of drug within a short time.

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Inspired by Hong et al.'s[14] work to stabilize the encapsulation of guest molecules, a crosslinker was introduced into the micelle core. In this study, we proposed a new strategy to design crosslinked copolymers with the addition of a crosslinker [ethylene glycol dimethacrylate (EGDM)] during atom transfer radical polymerization (ATRP) on the basis of poly(ethylene glycol) (PEG)–poly (methyl methacrylate) (PMMA), as shown in Figure 1. The copolymers were assembled in an aqueous solution. The physicochemical characteristics of the micelles were also investigated.

EXPERIMENTAL

Materials

All of the commercially obtained solvents and reagents were used without further purification, except as noted below. Tetrahydrofuran (THF), methylene chloride (CH_2Cl_2), and triethylamine were purified by distillation from calcium hydride and stored in solvent storage flasks before use. Poly(ethylene glycol) monomethyl ether (PEG-OH; molecular weight = 1900 g/mol) and 2-bromoisobutyryl bromide were purchased from Alfa Aesar and were used as received. *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (PMDETA), methyl methacrylate (MMA), EGDM, and Nile Red (NR) were purchased from Aladdin and stored in a refrigerator. CuBr (98%, Aladdin) was washed with acetic acid and ethanol repeatedly and stored in a desiccator. Doxorubicin hydrochloride (DOX·HCl) was purchased from J&K.

Synthesis of the Macroinitiator PEG-Br (a linear bromine-terminated PEG)

PEG-OH (19 g, 10 mmol) was distilled with an azeotropic adsorbent with THF with a rotary evaporator to remove the trace amount of water. After dehydration, PEG-OH was dissolved in CH_2Cl_2 (80 mL) in a 100-mL flask, and the solution was brought to 0°C. Then, 2-bromoisobutyryl bromide (2.6 mL, 15.4 mmol) was added dropwise into the flask under N_2. The reaction mixture was stirred for about 20 h at room temperature (RT), after which the reaction was quenched by the addition of $NaHCO_3$ solution (5%, 40 mL). Then, the mixture was extracted with CH_2Cl_2 (30 mL × 3). The organic layers were dried over anhydrous Na_2SO_4 for 24 h and concentrated by the use of an evaporator. The concentrated liquid was dissolved in CH_2Cl_2 (5 mL) and precipitated in ether (150 mL) at 5°C to remove the unreacted PEG-OH. The white precipitate was collected with vacuum filtration and dried *in vacuo* at 35°C to give 8 g of PEG-Br as a white powder.

^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.33 (*t*, 2H, —OCH_2COO^-), 3.73 (*m*, —CH_2O^-), 3.38 (*s*, 3H, —OCH_3), 1.90 [*s*, 6H, —$CBr(CH_3)_2$].

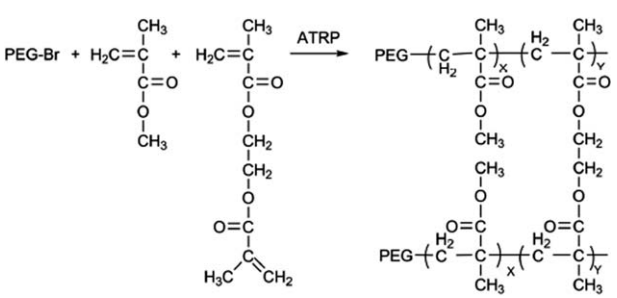

Figure 1. Schematic illustrations of PEG–PMMA–EGDM.

Synthesis of the Crosslinked Amphiphilic Block Copolymer (PEG–PMMA–EGDM) and the Contrast Sample (PEG–PMMA)

ATRP of MMA was carried out with PEG-Br as the initiator, CuBr and PMDETA as the catalysts, and EGDM as the crosslinker at 60°C under N_2. The polymerization scheme is shown in Figure 1. Briefly, PEG-Br (190 mg, 0.1 mmol), CuBr (29 mg, 0.2 mmol), monomer (MMA, 0.5 mL, 4.5 mmol), and a certain amount of EGDM were dissolved in freshly distilled THF (3 mL) and mixed in a 10-mL flask, which was filled with N_2. Then, PMDETA (42 μL, 0.2 mmol) was added, and the mixture was degassed three times with the freeze–pump–thaw procedure and sealed in N_2. After 30 min of stirring at RT, the mixture was placed in an oil bath at 60°C, and the polymerization was allowed to proceed for 14 h. Then, the mixture was passed through a neutral Al_2O_3 column with dried CH_2Cl_2 as the eluent to remove the catalysts, and a milky mixture was obtained.

The mixture contained unreacted MMA, EGDM, and the desired crosslinked amphiphilic block copolymer (PEG–PMMA–EGDM). The copolymer was purified by precipitation from a mixture of CH_2Cl_2 and petroleum ether. Through the addition of a large excess of petroleum ether to the mixture, the copolymer precipitate was collected by centrifugation for 5 min, whereas the unreacted MMA and EGDM were removed as a supernatant. PEG–PMMA–EGDM was dried *in vacuo* at 35°C to give 205 mg of a milky powder.

The linear PEG–PMMA, used as contrast sample, was prepared and purified the same way.

The compositions of the copolymers were determined by ^{1}H-NMR spectra ($CDCl_3$, 400 MHz), and the molecular weights of copolymer were determined by gel permeation chromatography (GPC) with freshly distilled THF as an eluent.

Preparation of the Micelles

Preparation of the Blank Micelles. Typically, the blank micelles were prepared under stirring by the dropwise addition of water to the block copolymer solution in THF at RT. THF and most of water were then concentrated by an evaporator at 40°C until the solution regained the initial polymer concentration. In the micelles, the hydrophobic PMMA block formed the inner core, and the hydrophilic PEG formed the outer shell (Figure 2).

Preparation of the NR-Incorporated Micelles and Determination of the Critical Micelle Concentration (cmc). The cmc values of the copolymers were estimated to prove the potential of

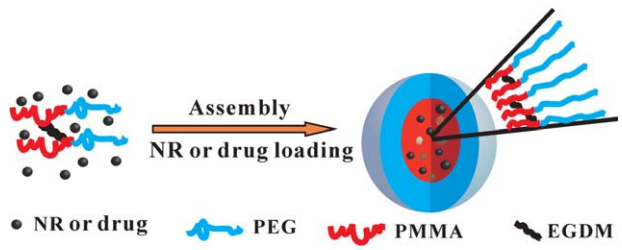

Figure 2. Formation of the core crosslinked polymeric micelles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

micelle formation by the measurement of fluorescence spectroscopy with NR, a hydrophobic dye, as a probe[15–17] (Figure 2). Here, NR was first dissolved in THF, after which the water was added to form the micelles, and NR was encapsulated in the hydrophobic core of the micelles. After a short interval of stirring, a fourfold volume of water was added to quench the micelles and precipitate the unloaded NR. The precipitated NR was removed by filtration through a 0.45-μm membrane, and then, the solution was concentrated at 40°C until it regained the initial polymer concentration.

Preparation of the Doxorubicin (DOX)-Incorporated Micelles. The DOX-incorporated micelles were prepared by a similar method from the literature.[18]

First, 10 mg of PEG–PMMA–EGDM or PEG–PMMA was dissolved in a dimethylformamide (DMF)–THF mixed solvent (250 μL DMF and 750 μL THF). A certain amount of DOX·HCl (0.5, 1.25, or 2.5 mg) was dissolved in 250 μL of DMF, and one drop of triethylamine was add to neutralize the HCl. DOX in a DMF solution was mixed with the polymer solution and stirred for 1 h at RT. Then, to form the polymeric micelles, the mixture solution was dropped slowly in 5 mL of water and stirred for 30 min. THF was removed with a rotary evaporator under reduced pressure for 20 min. DMF was removed by dialysis against 500 mL of water, which was exchanged every 2 h. After 12 h, the dialyzed solution was adjusted to 10 mL.

A 100-μL sample was concentrated and dissolved in 1.2 mL of DMF, and the precipitated polymer was removed by centrifugation for 10 min. The DOX contents were determined by UV spectrophotometry at 485 nm, and the measurements were performed in triplicate. The equations used to calculate the drug-loading content and loading efficiency are as follows:

$$\text{Drug-loading content} = \frac{\text{Amount of DOX in the micelles}}{\text{Weight of the copolymer}} \times 100\% \quad (1)$$

$$\text{Loading efficiency} = \frac{\text{Amount of DOX in the micelles}}{\text{Amount of DOX added}} \times 100\% \quad (2)$$

Scanning Electron Microscopy (SEM) Observation

The sample of micelles was filtered through a 0.45-μm membrane, and a small amount of filtrate (10 μL) was suspension in deionized water (1:100). Then, a drop of the diluted micelles was placed on a silicon wafer. The sample was allowed to dry overnight at RT in N_2. The samples on silicon wafers were coated with 5 nm of Au/Pd before imaging. Observation was done at 2 kV.

***In Vitro* Release Studies**

The drug release *in vitro* was studied as follows: a 4-mL aliquot of DOX-incorporated micelles was introduced into a dialysis bag with a molecular weight cutoff of 14,000. The bag was then placed into 21 mL of release medium (phosphate-buffered saline, 20 m*M*, pH 7.4, containing 0.5% Tween80) at 37°C with mild magnetic stirring. An amount of 2 mL of release medium was exchanged with fresh phosphate-buffered saline at a certain time intervals. The concentration of DOX in the exchanged release medium was determined by UV spectrophotometry at 485 nm. The experiment was performed in triplicate.

Measurement of *In Vitro* Cytotoxicity[19]

The cytotoxicities of the blank micelles, DOX, and DOX-incorporated micelles was evaluated with Lewis lung carcinoma cells (LLCs). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS), 3.5 g of glucose/L, and 2.5 mL of penicillin–streptomycin/L in 96-well plates at 37°C. Then, the medium was exchanged by medium-containing blank micelles, free DOX, and DOX-incorporated micelles (P0 and P2) for 24, 48, and 72 h. The cell viability was determined by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method.

RESULTS AND DISCUSSION

Characterization of the Copolymers

The reaction schemes of PEG–PMMA–EGDM were prepared by ATRP polymerization with a PEG-Br macroinitiator and a EGDM crosslinker in THF at 60°C. By adjusting the molar ratio of MMA to EGDM (1:0, 26:1, and 16:1), three-block copolymer samples (P0, P1, and P2, respectively) with various molecular weights, compositions, and structures were prepared.

With the assumption that the hydrophobic part was all composed of the MMA units, the number-average molecular weights of the linear structure ($M_{n,\text{H-NMR}}$'s) and the unit ratio ($n_{\text{PEG}}/n_{\text{MMA}}$) were calculated from the methylene proton signal (3.68 ppm, s, —CH_2OCH_2—) of the PEG block and the peak intensities of the —OCH_3 proton signal (3.61 ppm, s, —OCH_3) of the PMMA block in the ^{1}H-NMR spectrum (Table I and Figure 3). By comparing the integrals at $\delta = 3.68$ and 3.61, we preliminarily determined the linear structure's molecular weight ($M_{n,\text{H-NMR}}$) of P0, P1, and P2 were 6500, 4520, and 3850, respectively.

The actual number-average molecular weight of the amphiphilic copolymers was determined by GPC ($M_{n,\text{GPC}}$; Table I and Figure 4). As shown in Table I, the $M_{n,\text{GPC}}$ values of P1 and P2 were about twice that of $M_{n,\text{H-NMR}}$; this demonstrated that there were more than two linear structures in a macromolecule. Also, the linear structures in the molecule were linked by the crosslinking agent. However, $M_{n,\text{GPC}}$ of P0 was approximated with those determined by ^{1}H-NMR analysis (Table I) because no crosslinker was introduced. So, we demonstrated that the

Table I. Characterization Data for PEG–PMMA–EGDM and PEG–PMMA

	Sample	$M_{n,\text{H-NMR}}$	$M_{n,\text{GPC}}$	$n_{\text{PEG}}/n_{\text{MMA}}$	$M_{n,\text{GPC}}/M_{n,\text{H-NMR}}$
P0	PEG–PMMA	6,500	6,678	1:45.0	1.02
P1	PEG–PMMA–EGDM	4,520	11,160	1:25.2	2.47
P2	PEG–PMMA–EGDM	3,850	9,743	1:18.5	2.53

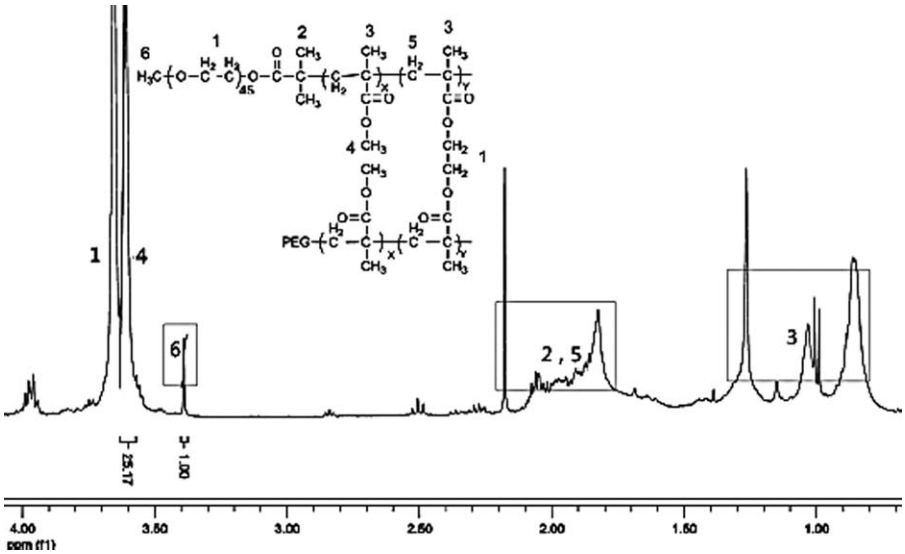

Figure 3. ^{1}H-NMR of PEG–PMMA–EGDM (P1).

crosslinker could link two or more linear amphiphilic copolymers for P1 and P2, whereas P0 preferred the linear structure.

cmc and Micelle Formation

Micelles are aggregates of amphipathic molecules with nonpolar portions in the interior and polar portions at the exterior surface in aqueous solution.[20] Compounds that have an amphiphilic structure (hydrophilic and hydrophobic portions) inherently have the capacity to form micelles in aqueous solutions. Amphiphilic molecules form micelles above a particular concentration, the cmc.[21] Therefore, the cmc value is a significant feature in the formation of micelles.

NR displays a higher fluorescence activity in the nonpolar environments, and its fluorescence can be quenched very quickly by polar solvents such as water. Therefore, we used NR as the fluorescence probe to measure the cmc of the copolymers in aqueous media. The concentration of the block copolymers was varied, and the concentration of NR was fixed at 0.04 mg/mL. The fluorescence spectra were recorded under an excitation wavelength of 550 nm at 25°C.

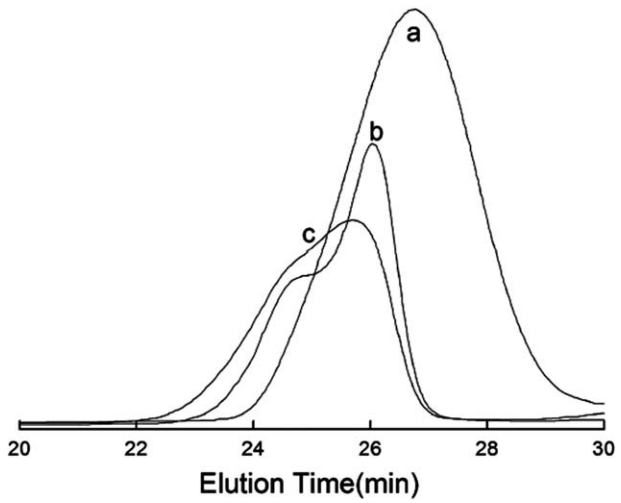

Figure 4. GPC curves of the copolymers (a) P0, (b) P1, and (c) P2. For P0, $M_{n,GPC} = 6678$ and weight-average molecular weight (M_w)/number-average molecular weight (M_n) = 1.21. For P1, $M_{n,GPC} = 11{,}160$ and $M_w/M_n = 1.28$. For P2, $M_{n,GPC} = 9743$ and $M_w/M_n = 1.31$.

The fluorescence intensities at 615 nm increased slowly, whereas the concentration was below a certain value when no micelle formed and then increased sharply when the concentration exceeded this value, after which micelles were generated and the NR was dissolved in the hydrophobic core region of the micelles. This value was called the cmc. The results show that the fluorescence intensity of the solution changed with the concentration. However, this change was not significant enough to determine the cmc. Thus, to obtain an observable turning point, a serial plot of the fluorescence intensity versus log C (the concentration of the block copolymers) was made, and cmc was determined by the intersection of the two straight lines (Figure 5). Figure 5 shows that the cmc values for P1 and P2 were 0.178 and 0.167 mg/mL, respectively, values that were lower than that of P0 (0.214 mg/mL). We supposed that when the crosslinking agent was introduced into the copolymers' hydrophobic segments, the self-assembly ability was significantly improved. Thereby, the ability to form micelles for P1 and P2 was also improved.

To prove the formation of micelles, we prepared a polymer (PMMA–EGDM) without PEG, which was a hydrophobic polymer, and studied the cmc value of PMMA–EGDM. The results show that there was no significant cmc value, which was because that there were no micelles in the aqueous phase and most of the NR in PMMA–EGDM was removed by the 0.45-μm membrane. In addition, because the thermodynamic stability of the micelles was much higher than that of the nanoparticles. We prepared two DOX-incorporated samples formed by P2 and PMMA–EGDM. After 20 min of centrifugation (4000 rpm), the absorbance (485 nm) of the PMMA–EGDM sample was decreased by 50%, whereas for the P2 sample, the variation

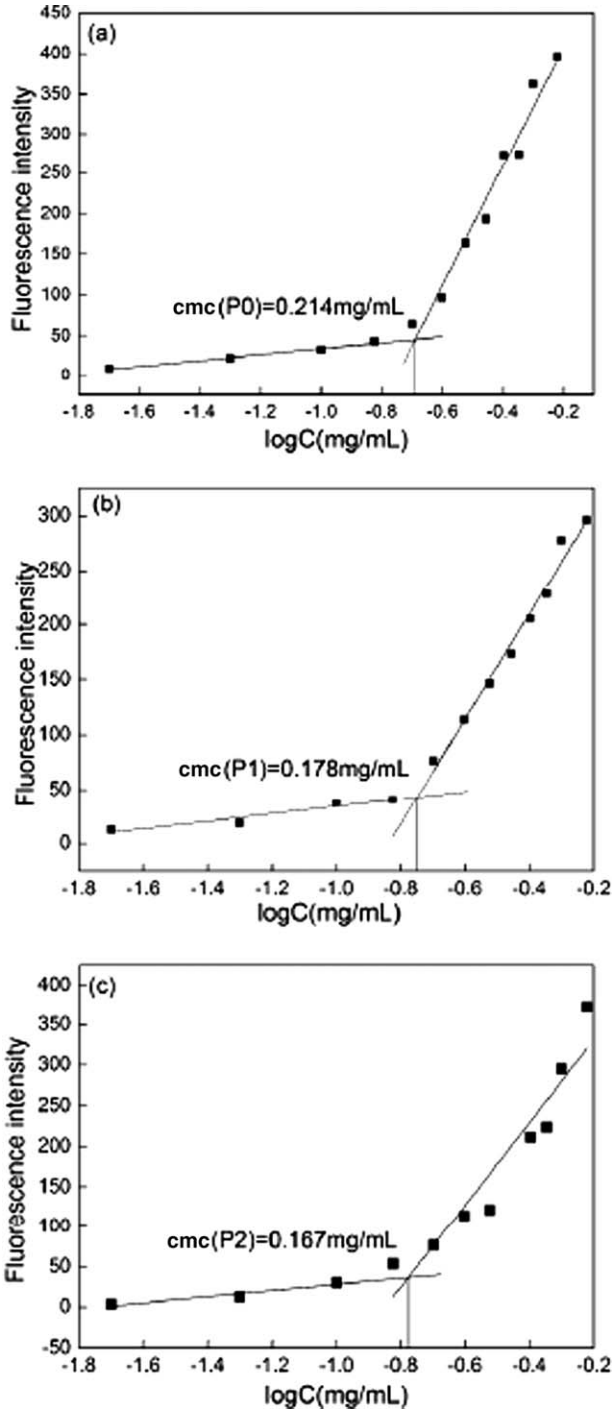

Figure 5. cmc values of P0, P1, and P2 in aqueous media.

was only 5%; this was because the micelles were not separated under low-speed centrifugation, and the nanoparticles or microparticles without any hydrophilic structure were separated easily.

Characterization of the DOX-Incorporated Micelles

Table II shows the drug-loading characterization of the DOX-incorporated micelles. As shown in Table II, for all of the crosslinked formulations (P1 and P2), the drug-loading efficiency was higher than 42% (w/w), whereas the drug-loading efficiency was only 28.27% for linear formulation (P0). This was because the crosslinking unit in the micelles' hydrophobic core segment strengthened the association between the hydrophobic segments and improved the tight degree of the micelles' core. So for P1 and P2, the drug loss during dialysis could be reduced, and the drug efficiency was improved.

Table II. DOX Loading Content and Loading Efficiency of the Polymeric Micelles

	Drug feeding amount (mg)	Drug loading content (w/w %)	Loading efficiency (w/w %)
P0	2.5	7.07	28.27
P1	2.5	10.53	42.15
P2	0.5	2.63	52.54
	1.25	6.03	48.23
	2.5	10.83	43.32

The influence of the drug feeding amount is also shown in Table II; when the drug feeding amount was increased from 0.5 to 1.25 mg, the drug-loading contents for P2 increased, and the loading efficiency decreased.

Figure 6 shows the morphologies of the DOX-incorporated micelles of P2 as observed by SEM. From the image, we observed that the micelles had a near-spherical shape, and their particle sizes were around 100–200 nm. The more exact particle size was measured by DLS and is shown in Figure 7. According to the literature,[12] the particle sizes of the micelles were related to their drug loading. The higher loading efficiency induced larger particle sizes. Therefore, the particle sizes of the DOX-incorporated micelles for all of the crosslinked formulations were about 150 nm; this was much higher than those of the linear formulation (110 nm) with a lower loading efficiency. For

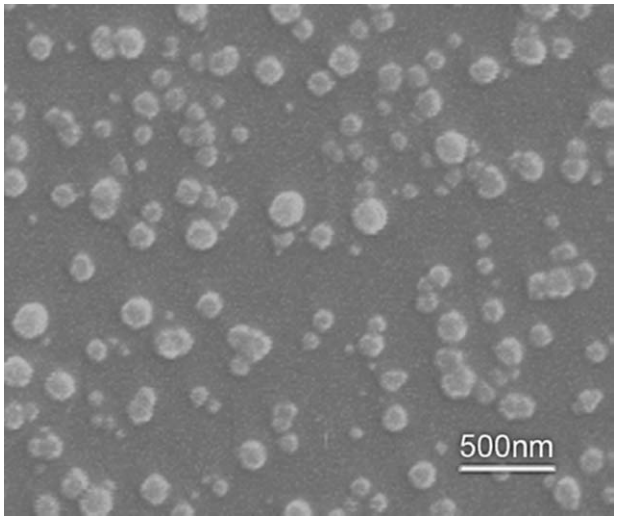

Figure 6. SEM images of the DOX-incorporated micelles (7.07% drug-loading content in P2).

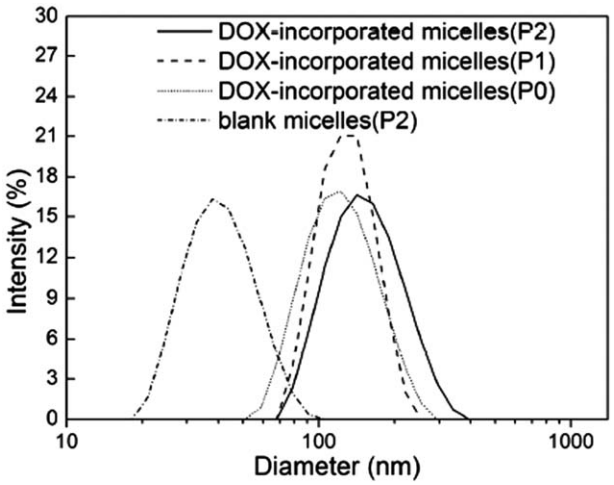

Figure 7. Dynamic light-scattering measurements of the micelles of the copolymers. Particle sizes of the DOX-incorporated micelles: P0 = 116.5 nm, PDI = 0.107; P1 = 148.1 nm, PDI = 0.263; P2 = 149.2 nm, PDI = 0.273. Particle sizes of the P2 blank micelles: P2 = 42.2 nm, PDI = 0.268.

all of the DOX-incorporated micelles, the particle sizes were higher than those of the blank ones (ca. 50 nm).

In Vitro Drug-Release Behavior and Measurement of the *In Vitro* Cytotoxicity

Figure 8 shows the release behavior of DOX from the DOX-incorporated micelles into the release medium. As shown in Figure 8, the release of DOX in the medium from the crosslinked micelles (P1 and P2) was much slower than that from the uncrosslinked micelles (P0); this was attributed to the much tighter core of the crosslinked micelles. So the crosslinking structures improved the stability of the micelles and prolonged the drug release.

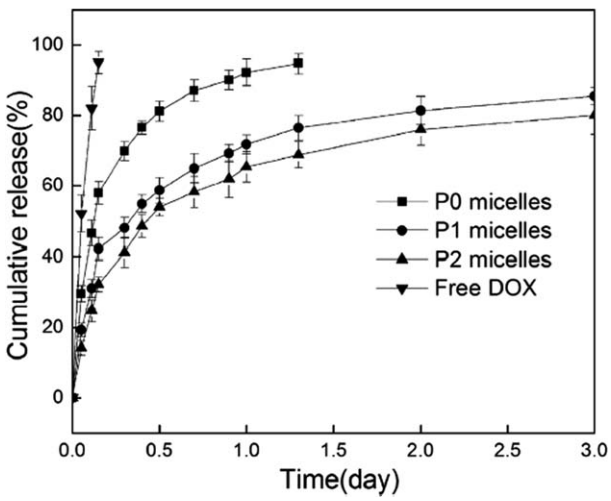

Figure 8. Release of DOX from the micelles. The drug-loading contents of P0, P1, and P2 were 7.07, 10.53, and 10.83%, respectively. The data is displayed as the mean plus or minus the standard deviation with a sample size of 3.

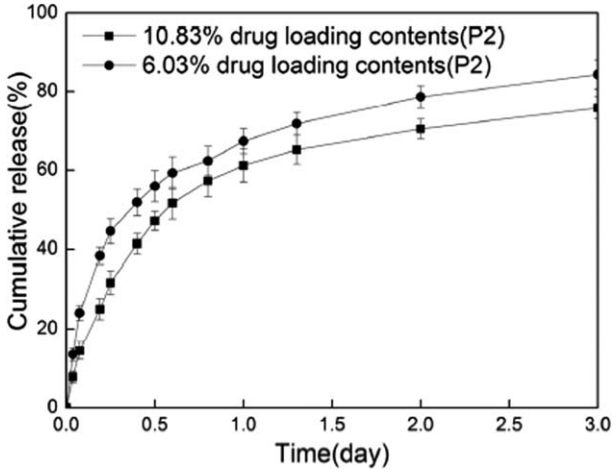

Figure 9. Release of DOX from the P2 polymeric micelles. The data is displayed as the mean plus or minus the standard deviation with a sample size of 3.

Also, there was another significant phenomenon for the release behavior of both the crosslinked and uncrosslinked micelles: The release of DOX was related to the drug-loading contents (Figure 9). The drug-release rate decreased as the drug-loading content increased. Similar results were reported by several researchers.[22,23] This phenomenon may be supposed to be explained as follows: when a hydrophobic drug, such as DOX, was incorporated into the micelles' hydrophobic core segment, crystallization may occur at higher drug-loading contents, whereas DOX can be present as a dispersed state at lower loadings. Generally, the drug solubility of the crystalline state was lower than that of the dispersed state. Therefore, the rate that DOX released from the micelles with a higher drug-loading contents was much slower. To improve the stability of the micelles and slow down the drug-release kinetics, the drug feeding amount could be increased moderately.

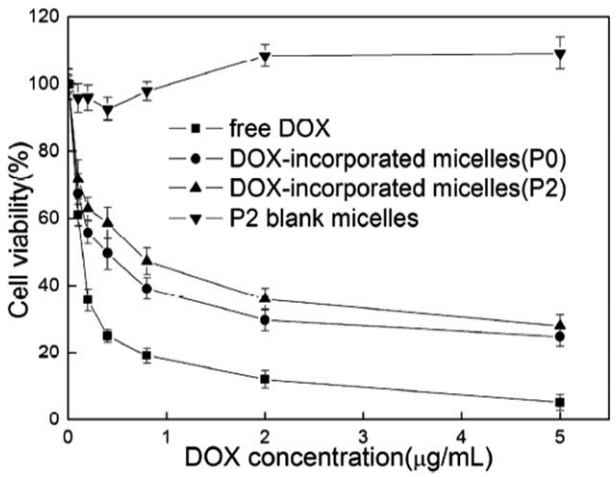

Figure 10. Percentage relative viability of LLC after 72 h of exposure to free DOX, blank micelles, and DOX-incorporated micelles. The data is displayed as the mean plus or minus the standard deviation with a sample size of 4.

The cytotoxicity of the DOX-loaded P2 micelles was compared with that of both the free DOX and DOX-loaded P0 micelles *in vitro*. To compare the cytotoxicity, the LLCs were incubated in a series of blank P2 micelles and in equivalent concentrations of DOX-incorporated micelles (P0 and P2) or free DOX for 72 h, and the percentage of viable cells was detected by the MTT method. The results are shown in Figure 10. The IC_{50} (the half maximal inhibitory concentration) values (marking 50% cell death) for the free DOX, DOX-incorporated P0 micelles, and DOX-incorporated P2 micelles were 0.144, 0.394, and 0.702 μg/mL, respectively, as shown in Figure 10. This result implied that the encapsulation of DOX in the P2 micelles and P0 ones reduced the cytotoxicity of DOX; this was because DOX release from the micelles was much slower than that of free DOX, as shown in Figure 8. In addition, the blank micelles (P2) are displayed as a nontoxic drug carrier for the LLCs.

With the equivalent copolymer concentration (2.5 mg/mL) and DOX concentration (20 μg/mL), we also compared the final percentage of viable cells for 24 h with for 48 h, and the results are shown in Figure 11. As shown in Figure 11, for 24 h, the percentage of viable cells of DOX in the P2 micelles was the highest; this was followed by the P0 samples, and free DOX had the lowest percentage. The concentration of free DOX in the P2 cells' micelles was less than that of the P0 micelles for 24 h in that the release of DOX from the P2 micelles was slower. However, for 48 h, the percentage of viable cells of DOX in P2 micelles was almost the same as that of the P0 micelles; this implied an equivalent release amount for these two samples. Thus, the P2 micelles required a longer DOX release process and maintained a much higher stability.

CONCLUSIONS

In this study, a crosslinked amphiphilic copolymer (PEG–PMMA–EGDM) was successfully prepared by ATRP, and we developed micelles to load DOX by an easy procedure. Three block copolymer samples (P0, P1, and P2) with various molecular weights, compositions, and structure were prepared. From the measurement of GPC, the molecular weights of P0, P1, and P2 were 6678, 11,160, and 9743, respectively. However, the linear molecular weights of P0, P1, and P2 calculated from ^{1}H-NMR were 6500, 4520, and 3850. These results indicate the formation of crosslinked structures for P1 and P2. The cmc values of P1 and P2 were lower than that of P0. Thus, the presences of crosslinking units were conducive for micelle formation. The drug-loading efficiencies of P1 and P2 were higher than that of P0, as the crosslinking units enhanced the micelles' stability. The drug feeding amount increased resulted in an increase in drug-loading content, and the loading efficiency decreased. The release of DOX from the micelles *in vitro* was slower than that of the crosslinked micelles and slower at a higher drug feeding amount. These PEG–PMMA–EGDM micelles did not show toxicity *in vitro* and reduced the cytotoxicity of DOX. For 24 h, the cytotoxicity of DOX in the P2 micelles was lower than that in the P0 micelles, whereas for a longer interval (48 h), the cytotoxicity of two samples were almost the same; this was attributed to the high stability and the longer DOX release process of the P2 micelles. So, we suggest the PEG–PMMA–EGDM micelles as a good candidate as a stable drug carrier.

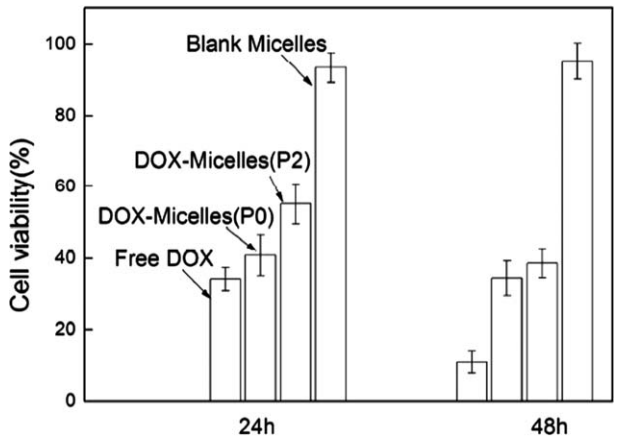

Figure 11. Percentage of viable cells of exposure to DOX, blank micelles, and DOX-incorporated micelles for 24 and 48 h. The data is displayed as the mean plus or minus the standard deviation with a sample size of 4.

ACKNOWLEDGMENTS

This work was financially supported by the State Key Laboratory of Natural and Biomimetic Drugs (contract grant number K20120212), the Scientific Research Program of the Shaanxi Provincial Education Department (contract grant numbers 11JK0694 and 2013JK0759), the Scientific Research Program of Xi'an Medical University (contract grant number 12FZ04), and the Scientific Research Foundation of Xi'an Medical University for Ph.D. studies (contract grant number 2011DOC05).

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