Biodegradable Polylactide/Poly(ethylene glycol)/Polylactide Triblock Copolymer Micelles as Anticancer Drug Carriers

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ABSTRACT: Adriamycin (ADR) was selected as a model drug to evaluate the potential applications of polylactide/poly(ethylene glycol)/polylactide (PLA/PEG/PLA) micelles as drug carriers in parenteral delivery systems. The PLA/PEG/PLA triblock copolymer micelles were characterized by dynamic light scattering and transmission electron microscopy. It was found that the micelle size increased with the increasing of the PLA chain length. The average size of ADR-loaded micelles was 143.2 nm. The histogram analysis showed that the ADR-loaded micelles possessed a narrow unimodal size distribution. The ADR loading contents of the micelles and ADR entrapment efficiency were dependent on the PLA chain length and PEG chain length in the copolymer. They increased with the increase of the PLA chain length, but the PEG chain length was identical and decreased with the increase of the PEG chain length; the length of the PLA block was similar. The initial amount of ADR also influenced the drug contents and entrapment efficiency (i.e., the more the initial amount added, the more the drug contents and the higher encapsulation efficiency). The drug release experiments indicated that the ADR-loaded micelles possessed sustained release characteristics. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 1976–1982, 2001

Key words: biodegradable; polylactide; poly(ethylene glycol); micelles; drug delivery

INTRODUCTION

Polymeric micelles formed from amphiphilic block copolymers have attracted much interest as drug carriers for delivery systems. The use of block copolymers in drug delivery was first proposed by Pratten et al. in the early 1980s.[1] The work of several groups greatly promoted the development of block copolymer micelles as drug delivery vehicles.[2–5] Amphiphilic block copolymers are assembled in the aqueous medium to form polymeric micelles with a core–shell structure; the hydrophobic core is surrounded by a hydrophilic corona.[6] The hydrophilic corona provides a stabilizing interface between the micelle core and the aqueous environment. Moreover, the size and the morphology of the micelles can be easily controlled. Drug carriers formed from block copolymers have some advantages, including reduced toxic side effects of anticancer drugs and selective targeting, stable storage, a long blood circulation time, and lower interactions with reticuloendothelial system (RES).[7–9] Thus, these core–shell type micelles may be used as appropriate vehicles for drug delivery.

Recently, linear ABA triblock copolymers were prepared from a biodegradable polyester and

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poly(ethylene glycol) (PEG).[10–14] The copolymerization of PEG and lactide or lactide/glycolide is now regarded as a suitable method to achieve new polymeric materials with novel physical, chemical, and biological properties adaptable to specific uses.

We previously described the micellar formation of the biodegradable triblock copolymer polylactide/PEG/polylactide (PLA/PEG/PLA) in an aqueous milieu.[15] Here we selected the anticancer agent adriamycin (ADR) as a model drug to investigate the potential of the PLA/PEG/PLA micelles as drug carriers in delivery systems. This article reports the efficacy of the polymeric micelles for the entrapment and controlled release of ADR.

EXPERIMENTAL

Materials

The PLA/PEG/PLA triblock copolymers were prepared by ring-opening polymerization of D,L-lactide. The PEG, D,L-lactide, and stannous octoate were introduced into a dried polymerization tube. The mixture was kept under a high vacuum at 80°C for 2 h to remove all volatiles. Afterward the tube was purged with nitrogen 3 times and sealed under a vacuum. The reaction was carried out at 130°C for 30 h without stirring. The product was dissolved in dichloromethane, precipitated in cold ether, and washed with distilled water. The copolymer was dried in a vacuum oven at 60°C for 3 days.

The compositions of the copolymers were determined by ^{1}H-NMR (Varian UNITY plus-400 NMR spectrometer, *d*-chloroform), and the molecular weight of the PLA and PLA/PEG/PLA copolymers was calculated by the following equation:

$$M_{n\mathrm{PLA}} = \mathrm{LA/EO} \times 144/44 \times M_{n\mathrm{PEG}}$$

$$M_n = M_{n\mathrm{PLA}} + M_{n\mathrm{PEG}}$$

where LA/EO is the molar ratio of the lactide to ethylene oxide in the copolymer determined by ^{1}H-NMR and $M_{n_{\mathrm{PEG}}}$ is the number-average molecular weight of PEG. The molecular weight distributions of the copolymers were determined by gel-permeation chromatography on a Waters 510 high-performance liquid chromatograph using chloroform as the eluent solvent. The peaks were detected using a refractive index detector. Polystyrene standards were used to calibrate the system.

The anticancer agent ADR was produced by Haimen Pharmaceutical Factory (Zhejiang, P.R.C.). All the other solvents used were analytical grade.

Preparation of PLA/PEG/PLA Triblock Copolymer Micelles

The copolymer micelles were prepared by a solvent diffusion method. A given amount of triblock copolymer was dissolved in acetone. Then a measured amount of copolymer solution was added dropwise into 10 mL of distilled water under magnetic agitation to form the micelles. The acetone was removed under a vacuum, and the micelle aqueous solution was obtained.

Preparation of ADR-Loaded Micelles

Three milliliters of copolymer solution in acetone (3 mg/mL) was added to a solution of 1 mL of ADR in H_2O. The solution containing the copolymer and the drug was added dropwise under agitation to 20 mL of water. The resulting micelle solution was continuously stirred at room temperature to evaporate the solvent.

Determination of Micelle Size

Dynamic light scattering (DLS) experiments were performed to measure the hydrodynamic diameter of the micelles. Before measurements, all samples were purified by filtering them through a 0.45-μm filter (Millipore). The DLS measurements were made using an argon ion laser with a wavelength of 514.5 nm and an output power of 10–200 mW. All the DLS was measured at 90° and collected on a Brookhaven BI-9000AT correlator at 25°C. The measurement for each solution was repeated at least 3 times.

The translational diffusion coefficient D and the hydrodynamic radius R_h were obtained from the average decay rate Γ and the Stokes–Einstein formula

$$D_T = \Gamma/q^2 \quad (1)$$

and

$$R_h = kT/6\pi\eta D_T \quad (2)$$

where the scattering vector

Table I Characteristics of PLA/PEG/PLA Triblock Copolymers

Copolymer	M_w/M_n	M_n	PEG Content (%)	L_{PLA}	L_{PEG}
$PLA_{246}/PEG_{45}/PLA_{246}$	1.24	38,000	5.2	246	45
$PLA_{247}/PEG_{90}/PLA_{247}$	1.73	39,600	10	247	91
$PLA_{115}/PEG_{23}/PLA_{115}$	1.29	18,000	5.6	115	23
$PLA_{50}/PEG_{23}/PLA_{50}$	1.35	8,200	12.1	50	23
$PLA_{26}/PEG_{23}/PLA_{26}$	1.25	4,700	21	26	23

L_{PLA}, the average chain length of the PLA block; $L_{PLA} = M_{n_{PLA}}/72$; L_{PEG}, the average chain length of the PEG block, $L_{PEG} = M_{n_{PEG}}/44$.

$$q = 4\pi n/\lambda \sin(\theta/2) \quad (3)$$

In eqs. (1)–(3) the θ, λ, k, T, η, and n are the scattering angle, the incident wavelength in a vacuum, the Boltzmann constant, temperature, solvent viscosity, and solvent refractive index, respectively. The hydrodynamic diameter $2R_h$ can be calculated using eqs. (1)–(3). The polydispersity of the micelles (μ/Γ^2, where μ is the second cumulant of the decay function) can be obtained using CONTIN V5.0 software supplied by Brookhaven.

Transmission Electron Microscopy

Transmission electron microscopy was used to observe the morphology of the micelles. Specimens were prepared by dropping the sample solution into a copper grid. The grid was held horizontally for 20 s to allow the molecular aggregates to settle and then at 45° to allow excess fluid to drain. The grid was returned to the horizontal position and a drop of phosphotungstic acid was added to give a negative stain. The grid was then left to stand for 30 s before removing excess stain as above. Specimens were air dried before examination using a Philips EM400ST transmission electron microscope at an accelerating voltage of 80 kV.

Evaluation of ADR Loading Capacity of Micelles

The solution containing ADR-loaded micelles and free ADR was dialyzed against deionized water over 24 h. The concentration of free ADR in the dialysis solution was measured by UV spectrophotometry at λ = 485 nm. The extinction coefficient of ADR is 18.5 L/cm g at 485 nm.[16] A blank sample was used as a reference.

The ADR entrapment efficiency (EE) and the ADR loading content (LC) of the micelle were calculated from eqs. (4) and (5):

$$\text{EE} = \frac{\text{total amount ADR} - \text{free amount ADR}}{\text{total amount ADR}} \times 100\% \quad (4)$$

$$\text{LC} = \frac{\text{total amount ADR} - \text{free amount ADR}}{\text{polymer weight}} \times 100\% \quad (5)$$

In Vitro Release Studies

Ten milliliters of ADR-loaded micelle solution was placed into the dialysis tube and incubated at 37°C in 20 mL of phosphate-buffer saline (PBS, pH 7.4). At appropriate intervals, 5 mL of the dialysis solution was taken out and replaced by 5 mL of fresh PBS. The concentration of ADR in the medium was evaluated by the analytical method described above. At each time interval, 5 mL of dialysis solution of the nonloaded micelles was also taken as a reference.

RESULTS AND DISCUSSION

Various PLA/PEG/PLA triblock copolymers were prepared by the ring-opening polymerization of D,L-lactide in the presence of α,ω-dihydroxy-terminated PEG and a small amount of stannous octoate. For the sake of clarity, the different copolymers were donated as $PLA_x/PEG_y/PLA_x$, where x and y are the average chain length of PLA and PEG blocks, respectively. The characteristics of the triblock copolymers are listed in Table I.

Micelles of PLA/PEG/PLA Triblock Copolymers in Water

It is well known that amphiphilic block copolymers with a suitable hydrophilic/hydrophobic balance can form a micellar structure when exposed to a

Table II Size and Polydispersity Factor of PLA/PEG/PLA Micelles

Copolymer	L_{PEG}	L_{PLA}	D (nm)	Polydispersity (μ/Γ^2)
$PLA_{246}/PEG_{45}/PLA_{246}$	45	246	124.9	0.13
$PLA_{247}/PEG_{90}/PLA_{247}$	91	247	129.5	0.11
$PLA_{115}/PEG_{23}/PLA_{115}$	23	115	134.9	0.06
$PLA_{50}/PEG_{23}/PLA_{50}$	23	50	123.5	0.08
$PLA_{26}/PEG_{23}/PLA_{26}$	23	26	120.4	0.14

selective solvent.[6] The amphiphilic nature of the PLA/PEG/PLA triblock copolymers, consisting of hydrophilic PEG and hydrophobic PLA blocks, provided an opportunity to form micelles in water.

The size and size distribution of the obtained micelles were measured by DLS. The micellar size and polydispersity factor determined by a cumulant method are summarized in Table II. The mean diameters of the micelles were 120–140 nm. The mean diameter data in Table II show that the micelle sizes are dependent on the PLA chain length. When the chain lengths of the PLA blocks are 115, 50, and 26, the mean diameters of the micelles are 134.9, 123.5, and 120.4, respectively. This result indicates that if the length of the hydrophilic PEG block is identical, the micelle sizes get larger as the hydrophobic PLA block increases. Balsara et al.[17] pointed out that for a BAB triblock copolymer in the solvent that preferentially dissolves the A block, the increase in the molecular weight of the poorly solvated block tends to favor micelle formation. The number of block copolymer chains in a micelle and the average radius of the micelles both increase with the increasing of the molecular weight of the B block. The polydispersity factors (μ/Γ^2) of the micelles were fairly low (0.06–0.14), suggesting a narrow size distribution.[4,5,18,19]

The morphology of the micelles was investigated by the transmission electron microscopy technique. Figure 1 shows the transmission electron micrograph of the $PLA_{246}/PEG_{45}/PLA_{246}$ triblock copolymer micelle. The picture clearly indicates the presence of micelles as shown by light spherical entities surrounded by the dark staining.

Entrapment of ADR within PLA/PEG/PLA Triblock Copolymer Micelles

The anticancer agent ADR was selected as a model drug to evaluate the feasibility of using

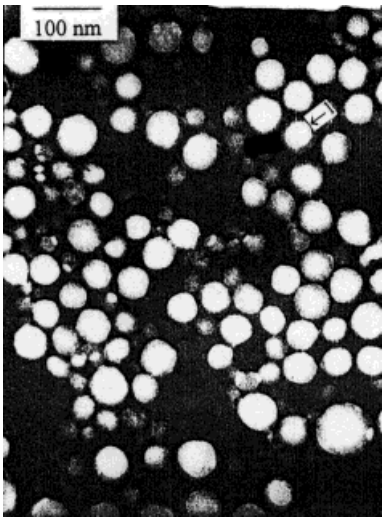


Figure 1 Transmission electron microscopy photographs of $PLA_{246}/PEG_{45}/PLA_{246}$ triblock copolymer micelles.

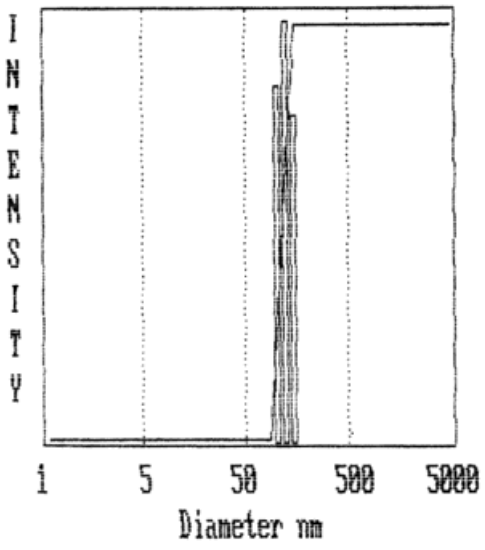


Figure 2 The size distribution of ADR-loaded micelles.

Table III Effects of PEG and PLA Block Chain Length on ADR Loading Content (LC) and Entrapment Efficiency (EE)

Copolymer	ADR/Copolymer (mg/mg)	EE (%)	LC (%)
$PLA_{246}/PEG_{45}/PLA_{246}$	0.72/20	70	3.1
$PLA_{247}/PEG_{90}/PLA_{247}$	0.72/20	48	2.4
$PLA_{123}/PEG_{45}/PLA_{123}$	0.72/20	59	3.3
$PLA_{115}/PEG_{23}/PLA_{115}$	0.72/20	70	3.9
$PLA_{50}/PEG_{23}/PLA_{50}$	0.72/20	57	3.2
$PLA_{26}/PEG_{23}/PLA_{26}$	0.72/20	53	3.0

PLA/PEG/PLA micelles as carriers. The average size of ADR-loaded $PLA_{246}/PEG_{45}/PLA_{246}$ micelles was 143.2 nm, and the size of the blank copolymer micelles was 124.9 nm. This result indicated that after incorporation of ADR into the $PLA_{246}/PEG_{45}/PLA_{246}$ micelle, the average particle size became larger. The size distribution of ADR-loaded $PLA_{246}/PEG_{45}/PLA_{246}$ micelles is shown in Figure 2. It can be seen from Figure 2 that the ADR-loaded micelles possess a narrow unimodal distribution in the histogram analysis.

The ADR loading contents and entrapment efficiency into the PLA/PEG/PLA triblock copolymer micelles are shown in Table III. We found that the ADR loading contents and entrapment efficiency were dependent on both the PEG and PLA block chain lengths. Increasing the chain length of the PEG block from 45 to 90 caused the entrapment efficiency to decrease from 70 to 48% and the ADR loading content to decrease from 3.1 to 2.4%. The entrapment efficiency and drug content increased with the increase of the PLA block chain length, but the chain length of the PEG blocks was identical (L_{PEG} = 23). The entrapment efficiency increased from 53 to 70% and the drug loading content increased from 3.0 to 3.9% when the PLA chain length was increased from 26 to 115.

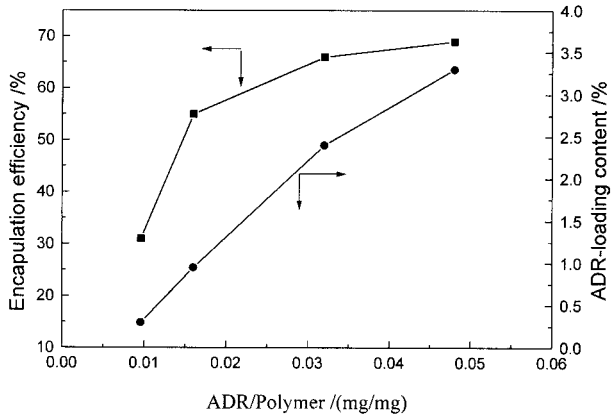
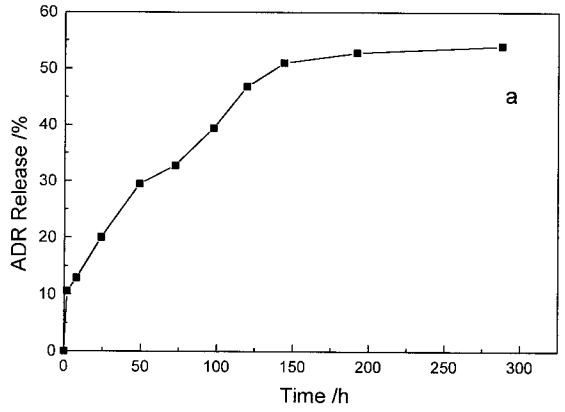


Figure 3 The effect of the initial ADR addition amount on the ADR entrapment efficiency and ADR-loaded content of micelles.



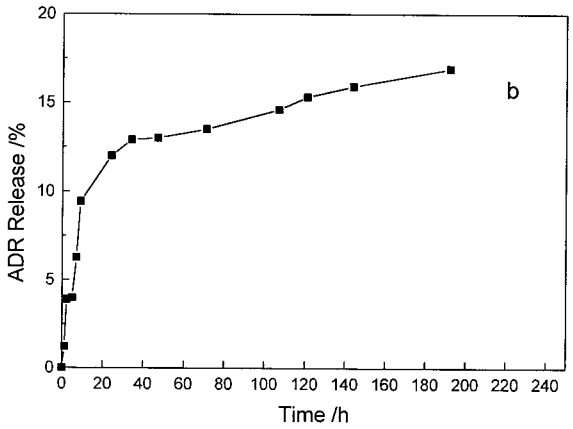


Figure 4 The *in vitro* release curves of ADR from different triblock copolymer micelles: (a) $PLA_{247}/PEG_{91}/PLA_{247}$, 1% drug content; (b) $PLA_{246}/PEG_{45}/PLA_{246}$, 3% drug content.

Figure 3 shows the effect of the ADR addition amount on the entrapment efficiency and drug loading content. When the initial added amount of ADR versus the constant amount of polymer (20 mg) was increased, we found that the greater the amount of ADR added, the higher the entrapment efficiency and ADR loading content in the micelles.

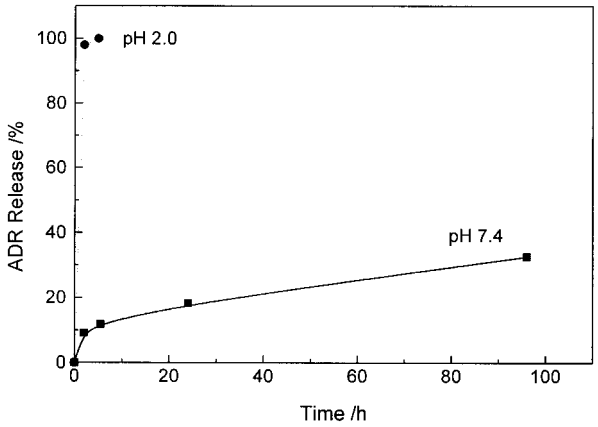


Figure 5 The effect of the medium pH on the release of ADR from the $PLA_{247}/PEG_{91}/PLA_{247}$ micelles (1% drug content).

In Vitro Release of ADR from Micelles

Figure 4 shows ADR release curves from $PLA_{247}/PEG_{91}/PLA_{247}$ and $PLA_{246}/PEG_{45}/PLA_{246}$ micelles in pH 7.4 PBS. The direct comparison of the release rate between $PLA_{247}/PEG_{91}/PLA_{247}$ and $PLA_{246}/PEG_{45}/PLA_{246}$ micelles cannot be carried out because of the different drug loading contents. The profile of ADR release from the $PLA_{247}/PEG_{91}/PLA_{247}$ micelle presents three phases: an initial burst release phase in which 13% of the entrapped ADR was released during 12 h, a sequential slow release phase in which 50% of the ADR was released in a continuous way during 6 days, and a plateau region in which very little ADR was released. The initial burst release may be due to the ADR adsorbed on the shell of the micelles. The mechanism of slow release may involve the following aspects: diffusion of ADR molecules through the micelles and degradation of the PLA block. However, it is very interesting that the release profile of ADR from the $PLA_{246}/PEG_{45}/PLA_{246}$ micelle shows only two phases, which is different from the release of ADR from the $PLA_{247}/PEG_{91}/PLA_{247}$ micelle. Release of ADR from the $PLA_{246}/PEG_{45}/PLA_{246}$ micelle revealed an initial burst over 24 h and thereafter a slow release. From these results it can be concluded that the control of drug release can be achieved by the adjustment of the PLA/PEG/PLA triblock copolymer compositions.

The release medium pH had a remarkable influence on the release rate of ADR from the micelles. Figure 5 shows the release curves of ADR from $PLA_{247}/PEG_{91}/PLA_{247}$ micelles in different medium pH. In the medium of pH 2.0, a significant burst release was observed and ~ 95% of the ADR encapsulated in the micelle was released within 2 h. However, in the medium of pH 7.4, a small initial release was observed followed by slow release, which lasted about 4 days. From these results it seems that the micelle is less stable in the more acidic condition and some breakdown of micelles occurs, which causes the fast release of ADR from the micelles.

CONCLUSION

With the potential usage as drug carriers for PLA/PEG/PLA triblock copolymer micelles in parenteral delivery systems, the efficacy of the micelles for the entrapment and controlled release of ADR were investigated. The ADR-loaded micelles prepared by the solvent diffusion method possessed a narrow unimodal size distribution. The studies showed that the ADR loading contents of the micelles and ADR entrapment efficiency depended on the PLA and PEG chain lengths in the copolymer. The drug content and entrapment efficiency increased with the increasing of the initial ADR addition amount. The drug release experiments showed that the ADR-loaded micelles had sustained release characteristics. This feature makes the PLA/PEG/PLA triblock copolymer micelles a promising carrier for drug delivery systems.

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