Polymer 50 (2009) 3706–3713

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Synthesis and self-assembly of amphiphilic poly(acrylic acid-*b*-DL-lactide) to form micelles for pH-responsive drug delivery

Ya-Nan Xue, Zhen-Zhen Huang, Jian-Tao Zhang, Min Liu, Min Zhang, Shi-Wen Huang*, Ren-Xi Zhuo*

Key Laboratory of Biomedical Polymers, Ministry of Education, Department of Chemistry, Wuhan University, Wuhan 430072, PR China

ARTICLE INFO

Article history:
Received 9 March 2009
Received in revised form
5 May 2009
Accepted 19 May 2009
Available online 27 May 2009

Keywords:
Amphiphilic diblock copolymer
pH-responsive micelle
Drug delivery

ABSTRACT

An amphiphilic diblock copolymer of poly(acrylic acid-*b*-DL-lactide) (PAAc-*b*-PDLLA) was synthesized by ring-opening polymerization of DL-lactide initiated by hydroxyl-terminated polyacrylic acid (PAAc-OH). The critical micelle concentration (CMC) of PAAc-*b*-PDLLA in aqueous solution, determined by fluorescence spectroscopy using pyrene as a probe, was found about 80 mg L^{-1}. A solution of PAAc-*b*-PDLLA in tetrahydrofuran (THF) was dialyzed against pure water to form pH-responsive micelles. Transmission electron microscopy (TEM) measurement showed that the micelles exhibited regular spherical morphology and the diameters of particles were in the range from 40 to 90 nm. The micelles were stable at a pH above 3 or at an ionic strength below 1.0, however, they aggregated and precipitated in the solutions when further decreasing pH or increasing ionic strength. Prednisone acetate, as a model hydrophobic drug, was loaded into the polymeric micelles. *In vitro* release of prednisone acetate from polymeric micelles showed that the release kinetics was strongly pH-dependent. Hydrophobic drug displayed "burst" release at pH 7.4, while only a small part of loaded drug released at pH 1.4. This provides a new choice to design delivery system for the gastrointestinal tract (GI tract), where the pH environment is strongly acidic in stomach and basic in intestine. The cytotoxicity measurement by MTT assay indicated that PAAc-*b*-PDLLA was low toxic in HeLa cells with an IC50 value of 2.8 mg mL^{-1}, which suggests that PAAc-*b*-PDLLA could be used as a safe candidate for pH-responsive drug delivery.

1. Introduction

Drug delivery systems (DDSs) have evoked researchers' interests in the past three decades. The ideal DDSs should be biocompatible and nontoxic, which have high loading capacity, suitable size and sufficient stability to prevent from uptake by reticuloendothelial system and excretion from body [1,2]. It is also desirable for DDSs to reduce the side effect of drugs on healthy cells and tissues. Therefore, in the field of drug delivery, one current tendency is to design intelligent nanoparticles, which exhibit the ability to target specific sites [3–7] and tunable release kinetics in response to the environmental stimuli, such as temperature [8–11,16,17], pH [12–23] and photodynamic [24], etc.

Core–shell polymeric micelles prepared by the self-assembly of stimuli-responsive block copolymers in aqueous solution have been extensively investigated and applied to develop targeting DDSs [9,15–19,25]. The inner hydrophobic core of the micelles can enhance the loading efficiency of hydrophobic drugs and the outer hydrophilic shell can provide a stabilizing interface between the hydrophobic core and the aqueous medium, which inhibits intermicellar aggregation of particles, protects drugs from inactivating under the biological environment, and reduces the side effect of drugs on healthy cells and tissues. By selecting suitable hydrophilic segments which are resistible towards blood or tissues as the outer shell, polymeric micelles cannot be recognized by certain proteins or phagocytic cells and achieve long-circulation in the blood or tissues [3].

Polymeric micelles with environment-responsive hydrophilic outer shells have been extensively studied in recent years. Thermal responsive polymeric micelles composed of poly(*N*-isopropylacrylamide) (PNIPAAm) segment for biomedical applications have achieved significant progress [8–11,16,17]. Okano et al. [8] have reported a thermally responsive block copolymer micelles consisting of a biodegradable poly(DL-lactide) (PLA) core and a thermally responsive PNIPAAm shell. The diameters of the micelles could respond to temperature changes around the lower critical solution temperature (LCST). Poly(butyl methacrylate) has also been used as the hydrophobic core to prepare the reversible thermally responsive on–off switching micelles, where the release

* Corresponding authors.
E-mail addresses: swhuang@whu.edu.cn (S.-W. Huang), pibmp@public.wh.hb.cn (R.-X. Zhuo).

0032-3861/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2009.05.033

of anticancer drug adriamycin could be modulated by tuning the temperature [9].

Although thermally responsive polymeric micelles have achieved significant progress, the temperature change has to be altered externally in most cases, which limits its use in the human body. However, the pH value in different parts or tissues in the body is greatly different. As along the gastrointestinal tract (GI tract), it is acidic in the stomach with pH 1–2 and basic in the intestine with pH 5–8. Therefore, many researchers have developed various pH-sensitive drug carriers for DDSs to facilitate the delivery of drugs to target tissues or organs [12–23]. Ionized polymers with pK_a value between 3 and 10 are valuable candidates for pH-responsive systems [26]. Ouchi et al. [12] prepared biodegradable and biocompatible polymeric micelles via amphiphilic poly(aspartic acid-*b*-lactide) (PAsp–PLA) diblock copolymer. The diameters of formed micelles in aqueous solution increased responding to the change of pH from 4 to 9, which was caused by the deionization of the carboxylic acid groups in PAsp segments. Block copolymer poly(acrylic acid-*b*-4-vinyl pyridine) (PAAc-P4VP) containing both weakly acidic PAAc chains and weakly basic P4VP chains formed micelles with different structures at different pH. At low pH, polymer was protonated, micelles formed with hydrophobic PAAc core and cationic polyelectrolyte P4VP shell. While at high pH, protons were released, micelles formed with hydrophobic P4VP core and anionic polyelectrolyte PAA shell [13]. Bae's group [14,15] designed a series of polymers based on poly(L-histidine-*b*-ethylene glycol) diblock copolymer. The formed micelles showed pH-dependent stability, that is, micelles were stable above critical pH, while dissociated below it, which induced the drug release from the micelles.

Polyacrylic acid (PAAc) is a biocompatible material, which has been widely used as pH-responsive drug carriers [13,17]. The pendant carboxylic groups can accept protons at low pH and release protons at high pH, as a result, the balance between hydrogen bonds and electrostatic repulsion forces causes the change of hydrophobic/hydrophilic characteristics of PAAc at different pH. Furthermore, the pendant carboxylic groups could form multiple hydrogen bonds with the glycoprotein presented in the mucus, which can generate a bioadhesive polyelectrolyte outer shell of micelles [27,28].

The aim of this work is to develop a novel carrier for DDSs with pH-responsibility and low toxicity. An amphiphilic block copolymer of PAAc-*b*-PDLLA was first synthesized by ring-opening polymerization of DL-lactide using the terminal hydroxyl group of PAAc-OH as the initiator. Then, nano-sized micelles with anionic PAAc shells and PDLLA cores were prepared by self-assembly. The formed micelles showed small sizes with regularly spherical appearance. The critical micelle concentration (CMC) of PAAc-*b*-PDLLA in aqueous solution was low, which would provide good stability during circulation in the body. Hydrophobic prednisone acetate as a model drug was incorporated into micelle cores during the formation of micelles by dialysis. *In vitro* drug release kinetics showed that almost no drug released at pH 1.4, however, most of drug diffused out at pH 7.4 within 10 h. This pH-responsive release characteristic of PAAc-*b*-PDLLA micelles would provide a pH inducible release profile for the GI tract and have the potential to be a candidate for the on–off controlled release carriers.

2. Experimental

2.1. Materials and methods

DL-Lactide was synthesized from DL-lactic acid (Analytical reagent, 85–90%) using zinc powder as the catalyst and purified by repeated crystallization in ethyl acetate for five times. (m.p. 126.3 ± 0.3 °C). Acrylic acid (Chemically pure, ≥98%) was distilled in vacuum. Stannous octoate [$Sn(Oct)_2$] (Aldrich) was vacuum-distilled and dissolved in freshly dried toluene prior to use. Toluene and 1,4-dioxane were dried over sodium. Prednisone acetate was extracted and purified from prednisone acetate tablets, which were purchased from Zhejiang Xianju Pharmaceutical Co. Ltd. of China. Dialysis tube with a molecular weight cut-off of 8000 and other chemicals were purchased from Shanghai chemical Reagent Company and used directly. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazoplium bromide (MTT) was purchased from Sigma. Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine and ampicillin were purchased from Gibco BRL (Grand Island, NY). Fetal bovine serum (FBS) was from Hyclone. HeLa cells were from Chinese Typical Culture Center (CTCC) (Wuhan University).

Fourier transform infrared (FT-IR) spectra were recorded on a PerkinElmer-2 spectrometer. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Mercury VX-300 spectrometer at 300 MHz, using DMSO-d_6 or $CDCl_3$ as solvents and tetramethyl silane (TMS) as an internal standard. Gel permeation chromatographic (GPC) measurement was carried out by using a Waters-2690D HPLC equipped with Styragel columns. Samples were detected with a Wyatt multiangle light scattering detector and a Waters 2410 differential refractive index detector. THF was used as the mobile phase at a flow rate of 0.3 mL/min. The column temperature was 25 °C.

2.2. Synthesis of hydroxyl-terminated poly(acrylic acid) (PAAc-OH)

Hydroxyl-terminated polyacrylic acid (PAAc-OH) was synthesized by free-radical polymerization using benzoyl peroxide (BPO) as an initiator and 2-mercaptoethanol as a chain transfer agent. Briefly, AAc monomer (14 mL, 0.2 mol), 2-mercaptoethanol (140 μL, 2.0 mmol) and BPO (0.242 g, 1.0 mmol) were dissolved in 1,4-dioxane (140 mL). The solution was bubbled with nitrogen at room temperature for 30 min to remove the oxygen. Polymerization was carried out at 80 °C for 4 h under nitrogen. After evaporation of most of the 1,4-dioxane, the polymer was precipitated in diethyl ether and further purified by repeated dissolved in 1,4-dioxane and precipitated in diethyl ether for three times and dried in vacuum. Yield: 96.8%. FT-IR (KBr, cm^{-1}): 3439, 2964, 1721, 1456, 1384, 1258, 1165, 1117, 1080. ^{1}H NMR (d_6-DMSO, ppm) δ: 1.2–1.8 (2H, d, C**H**$_2$); 2.1–2.3 (1H, m, C**H**); 12.2 (1H, s, COO**H**). $M_w = 7.30 \times 10^3$, $M_w/M_n = 1.2$.

2.3. Synthesis of poly(acrylic acid-*b*-DL-lactide) block copolymer (PAAc-*b*-PDLLA)

AB type diblock copolymer of PAAc-*b*-PDLLA was synthesized by ring-opening polymerization of DL-lactide initiated by terminal hydroxyl groups of PAAc-OH using $Sn(Oct)_2$ as the catalyst. PAAc-OH (1.5 g), DL-lactide (1.5 g, 0.01 mol), and 0.1 mol L^{-1} of $Sn(Oct)_2$ in anhydrous toluene solution (0.1 mL) were added into a dried silanized glass flask with a magnetic stirring bar. The flask was degassed by several vacuum-purge cycles to remove the solvent introduced in the catalyst solution, and then vacuum-sealed. Copolymerization was carried out at 140 °C for 24 h. The cool reaction mixture was dissolved in methanol and precipitated in diethyl ether. After dried in vacuum, PAAc-*b*-PDLLA diblock copolymer was obtained as white powder. Yield: 64.3%. FT-IR (KBr, cm^{-1}): 3445, 2993, 2942, 1733, 1455, 1384, 1197, 1097. ^{1}H NMR (d_6-DMSO, ppm) δ: 1.2–1.8 (5H, m, C**H**$_2$ + C**H**$_3$); 2.1–2.3 (1H, m, C**H**); 4.9–5.2 (1H, m, C**H**); 12.3 (1H, s, COO**H**). $M_w = 9.7 \times 10^3$, $M_w/M_n = 1.3$.

2.4. Preparation of polymeric micelles

Polymeric micelles were prepared from PAAc-*b*-PDLLA block copolymer by a dialysis method. Briefly, a solution of copolymer (6 mg) in 2 mL of water-soluble organic solvent (ethanol, DMF, DMSO, THF, etc.) was separately dialyzed against distilled water for 24 h at room temperature using a dialysis tube (MWCO 8000). The external distilled water was refreshed every 4 h. The obtained polymeric micelle solution was directly used for the measurement of size and zeta potential.

2.5. Determination of the critical micelle concentration (CMC)

CMC was determined using pyrene as a fluorescence probe. A solution of pyrene in acetone was added to a vial and the solvent was allowed to evaporate to form a thin film at the bottom of the vial. Polymer micelle solutions at different concentrations were added to the vials and the final pyrene concentration was 6×10^{-7} mol L^{-1} in water. The concentrations of polymer micelles varied from 6.7×10^{-4} to 1.4 mg mL^{-1}. The solutions were kept on a shaker at room temperature for 24 h to reach equilibrium prior to fluorescence measurement. Fluorescence spectra were recorded on an LS55 luminescence spectrometer (Perkin–Elmer) at room temperature.

The excitation spectra were scanned from 320 to 350 nm at the emission wavelength of 373 nm. Excitation and emission bandwidths were 5 nm and 10 nm, respectively. The fluorescence intensity ratio of I_{338}/I_{333} was analyzed as a function of micelle concentration.

2.6. Transmission electron microscopy (TEM)

A drop of micelle solution was dropped on a copper grid with Formvar film and stained with a 0.2% (w/v) solution of phosphotungstic acid. Measurement was carried out under JEM-100CXII TEM at an acceleration voltage of 100 KV.

2.7. Size and zeta-potential distribution measurements

The sizes and zeta-potentials of the freshly prepared micelles in aqueous solution were measured using Zetasizer Nano ZS (Malvern Instruments Ltd., UK) with a He–Ne laser beam at 633 nm at 25 °C. An average value was obtained from three repeated measurements for each sample.

2.8. In vitro cytotoxicity assay

HeLa cells were seeded into a 96-well plate at a density of 5000 cells/well in 50 μL complete DMEM containing 10% FBS, 4 mM L-glutamine, 100 μg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin and cultured for 1 day at 37 °C in 5% CO_2 atmosphere. The polymer was dissolved in complete DMEM medium, sterile-filtered and then serially diluted in complete DMEM to different concentrations ranging from 0.2 mg mL^{-1} to 10.6 mg mL^{-1}. Polymer solutions at different concentrations (50 μL) were separately added to the wells, and then the cells were cultured for further 24 h at 37 °C. Then MTT solution (5 mg mL^{-1}, 25 μL) in PBS buffer (pH 7.4) was added to each well except that PBS buffer (25 μL) was added to control wells. After incubated at 37 °C for 2 h, the medium was replaced by 150 μL of DMSO to dissolve the formazan blue crystal. The absorbance of the solution was measured using microplate reader (Bio-Rad 550, Hercules, CA) at 570 nm. The percentage relative viability in reference to control wells containing complete DMEM without the added polymer was calculated by the following equation:

$$\text{Relative cell viability}\,(\%) = 100 \times (A_{\text{test}} - A_0)/(A_{\text{control}} - A_0)$$

where A was the absorbance at 570 nm and A_0 was the absorbance of the solution containing cells and complete DMEM without MTT and polymer.

2.9. Drug loading

Prednisone acetate loaded micelles were prepared by the dialysis method similar to polymeric micelle formation mentioned above. Briefly, 6 mg of copolymer and 1 mg of prednisone acetate were dissolved in 2 mL of THF. Then the solution was dialyzed against distilled water for 24 h using a dialysis tube (MWCO 8000). After dialysis, the final solution was freeze dried and drug loaded micelles were obtained in the form of white powder. The yield, drug loading content and entrapment efficiency of micelles were calculated by the following equations:

$$\text{Micelle yield}\,(\%) = 100 \times W_{\text{micelle}}/(W_{\text{polymer}} + W_0)$$

$$\text{Drug loading content}\,(\%) = 100 \times W_{\text{drug}}/W_{\text{micelle}}$$

$$\text{Entrapment efficiency}\,(\%) = 100 \times W_{\text{drug}}/W_0$$

where W_{polymer} was the weight of polymer fed initially and W_{micelle} was the weight of drug loaded micelle. W_0 was the weight of drug fed initially and W_{drug} was the weight of drug loaded into the micelle which was regarded as the totally released weight of the drug from micelle.

2.10. In vitro drug release

The release of prednisone acetate from drug loaded micelles was separately measured in two buffer solutions at pH values of 1.4 and 7.4 (pH 1.4: KCl–HCl, pH 7.4: NaH_2PO_4–Na_2HPO_4, Ionic strength: 0.1 M). 5 mg of drug loaded micelles powder was dissolved in 5 mL of buffer solution and put into dialysis tube. The drug release was conducted in 15 mL of buffer solution at 37 °C in a shaker. At predetermined intervals, 3 mL of buffer solution was taken out and equal amount of fresh pH buffer solution was added back. The released amount of prednisone acetate was measured with an ultraviolet–visible (UV) spectrophotometer (Perkin Elmer Lambda Bio 40) using the characteristic UV absorbance of prednisone acetate at 243 nm. The cumulative drug release was calculated from the following relationship:

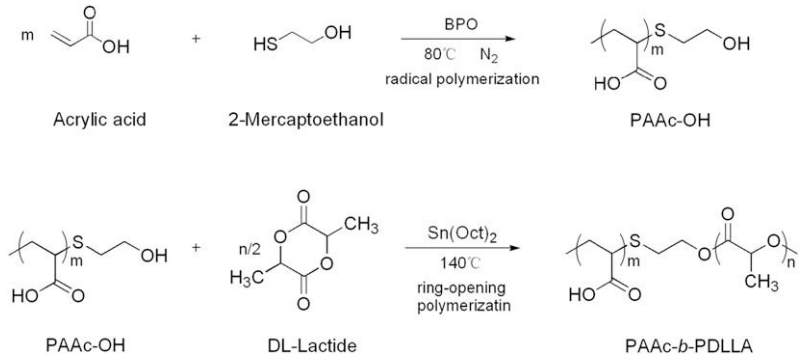
$$\text{Cumulative release}\,(\%) = 100 \times W_t/W_{\text{drug}}$$

where W_t was the weight of drug released from micelle at time t and W_{drug} was the same as mentioned above.

3. Results and discussion

3.1. Synthesis and characterization of polymers

PAAc-*b*-PDLLA, anionic amphiphilic block copolymer, was synthesized by a two-step synthesis as shown in Scheme 1. First, the macromolecular initiator, PAAc-OH, was prepared by free-radical polymerization of AAc in dry 1,4-dioxane, using BPO as the initiator and 2-mercaptoethanol as a chain transfer agent. The degree of polymerization of PAAc-OH can be tailored by controlling the feed ratio of AAc to 2-mercaptoethanol. In this work, PAAc-OH with a weight-average molecular weight of 7300 was used.



Scheme 1. Synthesis of PAAc-*b*-PDLLA diblock copolymer.

Next, block copolymer PAAc-*b*-PDLLA was synthesized by ring-opening polymerization of DL-lactide using PAAc-OH as the initiator and $Sn(Oct)_2$ as the catalyst. The previous work has demonstrated that the ring-opening polymerization of cyclic esters was initiated only by hydroxyl groups in the presence of both hydroxyl groups and carboxyl groups [29–32]. In these cases, the carboxyl groups do not initiate but accelerate ring-opening polymerization of cyclic esters. PAAc-*b*-PDLLA was characterized by SEC-MALLS, FT-IR and ^{1}H NMR. The SEC-MALLS chromatographs in Fig. 1 shows unimodal molecular weight distributions which confirmed the successful conversion of PAAc-OH to PAAc-*b*-PDLLA block copolymer without graft structure. Fig. 2 shows the ^{1}H NMR spectra of polymers. The peaks at δ1.2–1.8 ppm, δ2.2 ppm and δ12.3 ppm were attributed to the protons of –C**H**$_2$–, –C**H**– and –COO**H** in PAAc block. The peaks at δ1.6 ppm and δ5.0–5.2 ppm were due to the protons of –C**H**$_3$ and –C**H**– in PDLLA block. The combination of PDLLA with PAAc led to an increased relative intensity of the peak at δ1.6 ppm. The molar ratio of AAc units to DLLA units was calculated based on the integrated intensity of signal *b* (δ2.2 ppm) from PAAc and signal *d* (δ5.0–5.2 ppm) from PDLLA, which was determined to be 1.0:0.6.

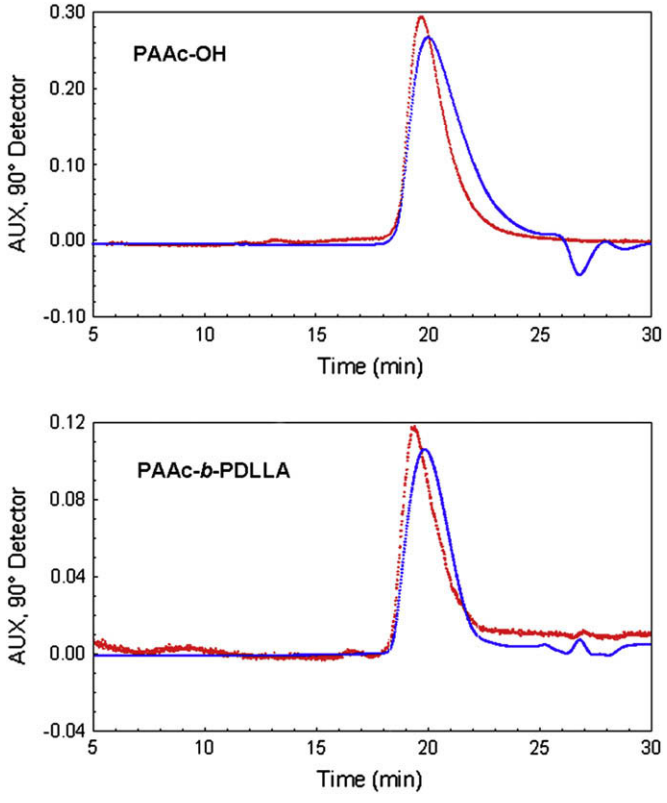


Fig. 1. SEC-MALLS chromatographs of PAAc-OH (top) and PAAc-*b*-PDLLA (bottom) using THF as the eluent (red curve: laser signal, blue curve: GPC trace). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. CMC determination

The CMC of amphiphilic diblock PAAc-*b*-PDLLA was determined by fluorescence technique based on selective partition of fluorescence probe in hydrophobic phase against aqueous phase. It has been reported that fluorescence spectra of pyrene solutions contain a vibrational band exhibiting high sensitivity to the polarity of the pyrene environment [29]. When polymeric micelles formed, pyrene was preferentially distributed in the hydrophobic micelle core instead of in the hydrophilic outer shell, thus the environment of pyrene was turned from polar to non-polar. Accordingly, an increase in the fluorescence intensity of pyrene and a red shift of the (0, 0) band in the emission spectra was observed, which is shown in Fig. 3a. The apparent CMC can be obtained from the plot of the fluorescence of pyrene, the I_{338}/I_{333} ratio from excitation spectra, against concentration of polymer: a major change in the slope indicates the onset of micellization. This concentration is defined as the CMC [1,33]. From Fig. 3b, the CMC of PAAc-*b*-PDLLA copolymer is determined to be 80 mg L^{-1}. Due to the low CMC value, it is suggested that micelle formed from PAAc-*b*-PDLLA would provide good stability in solution, even after extreme dilution by the much larger volume of systemic circulation in the body.

3.3. Preparation and characterization of PAAc-b-PDLLA micelles

Polymeric micelles were prepared from PAAc-*b*-PDLLA block copolymer by a dialysis method. A solution of copolymer (6 mg) in 2 mL of water-soluble organic solvent was dialyzed against distilled water for 24 h at room temperature using a dialysis tube (MWCO 8000).

TEM was used to characterize the morphology of micelles prepared from the solution of PAAc-*b*-PDLLA in THF. From the image shown in Fig. 4a, the self-assembled micelles from PAAc-*b*-PDLLA are well dispersed as individual nanoparticles with regularly spherical shape and have a diameter about 50 nm. The size distribution of these micelles in aqueous solution was measured using Nano ZS and shown in Fig. 4b. Micelles showed mono size distribution with PdI 0.125 and the mean size was determined to be 187 nm. The mean size of polymeric micelles observed by the laser diffraction method in aqueous solution is much larger than that obtained from TEM in dry state. This may be caused by the collapse of the free hydrophilic segments of the particles in the process of drying [20].

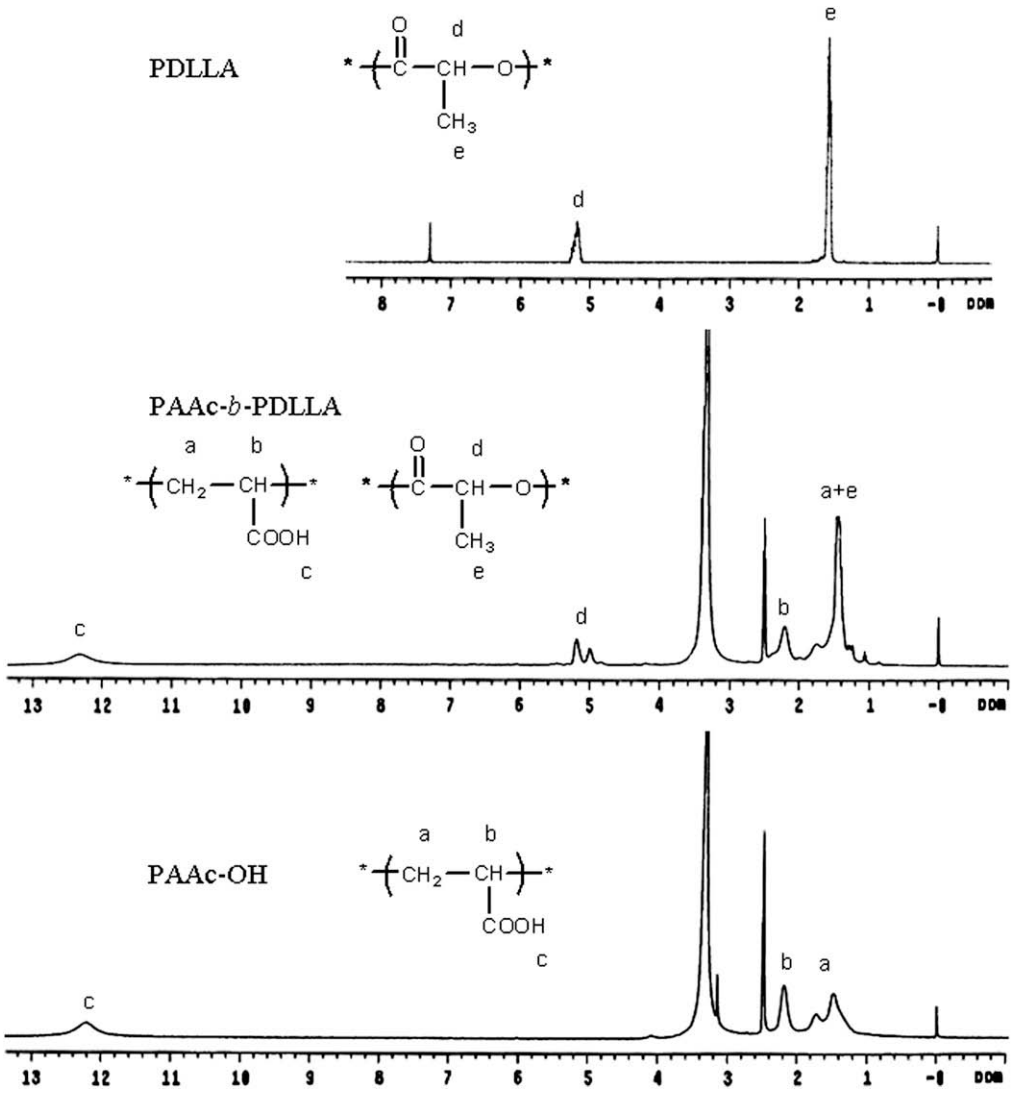


Fig. 2. ^{1}H NMR spectra of PAAc-OH, PAAc-*b*-PDLLA and PDLLA (DMSO-d_6 for PAAc-OH and PAAc-*b*-PDLLA, $CDCl_3$ for PDLLA).

The results of sizes and zeta-potentials of micelles separately prepared from the solution of PAAc-*b*-PDLLA in ethanol, DMF, DMSO and THF are shown in Table 1. It is found that the zeta-potentials of the micelles prepared from four organic solvents are about −30 mV, which suggests that the shell of micelles was composed of ionized carboxyl groups. The average sizes of the micelles formed from different organic solvent are almost same (~200 nm), which indicates that there is little effect of the solvent

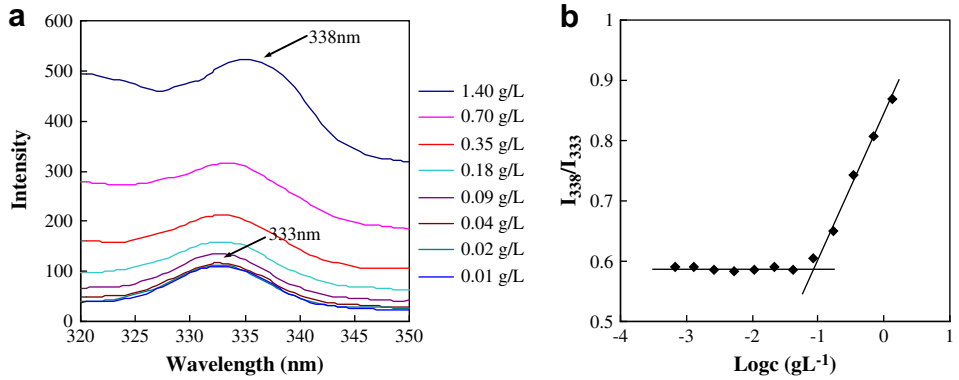


Fig. 3. (a) Excitation spectra of pyrene ($\lambda_{em} = 373$ nm) (b) Plot of the fluorescence intensity ratio of I_{338}/I_{333} from excitation spectra, as a function of PAAc-*b*-PDLLA concentration ($\lambda_{em} = 373$ nm, concentration of pyrene $= 6 \times 10^{-7}$ mol L^{-1}).

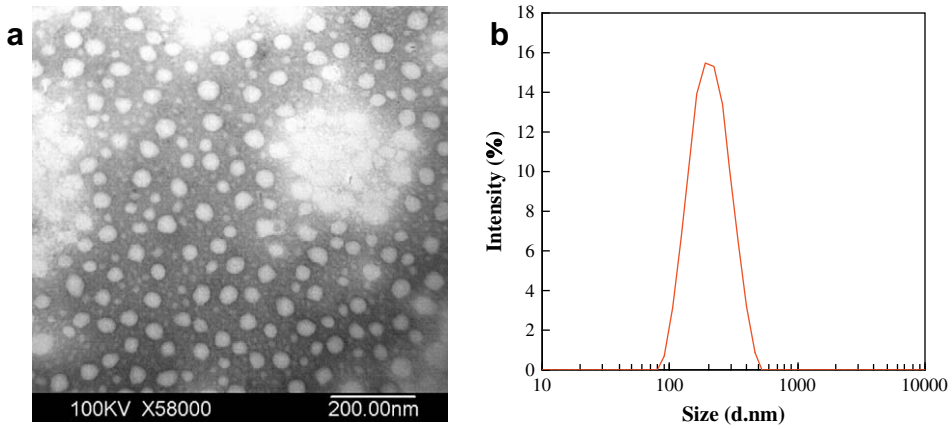


Fig. 4. (a) TEM image of PAAc-*b*-PDLLA micelles stained with 0.2% (w/v) phosphotungstic acid (b) size distribution of PAAc-*b*-PDLLA micelles assembled in aqueous solution (measured by Zetasizer Nano ZS).

Table 1
Size and zeta-potential distributions of PAAc-*b*-PDLLA micelles formed from various organic solvents.

Solvent	Ethanol	DMF	DMSO	THF
Mean size (nm)	248 ± 26	246 ± 19	186 ± 7	187 ± 20
Zeta-potential (mV)	−34 ± 6	−38 ± 2	−34 ± 1	−31 ± 3

of the original solution for dialysis on the sizes and zeta-potentials of the micelles formed from different solutions.

3.4. The stability of the micelles prepared from PAAc-b-PDLLA

The micelles prepared from the solution of PAAc-*b*-PDLLA in THF were chosen to study the stability of the micelles at different pH and ionic strength. As shown in Fig. 5a, at pH 3, the average size of the micelles is 180 nm. With decreasing pH, the micelles easily aggregated to form microparticles; while with increasing pH, the size of micelles increased gradually and reached a platform of 250 nm above pH 5. Below pH 3, the carboxyl groups of the PAAc segments are highly protonated and the effects of hydrogen bond between hydroxyl in the outer shells are dominant, which reduce the hydrophilicity of PAAc and induce micelles to become unstable and seriously aggregate to form microparticles. Above pH 3, the effect of hydrogen bond becomes weak and stable nano-sized micelles are attained. The size of the micelles is the smallest around pH 3. The sizes of the micelles increase gradually with the increase of pH from 3 to 5.

The effect of ionic strength on the size of micelles is shown in Fig. 5b. When ionic strength is below 1.0, the micelles are stable with the size of 200 nm, while with increasing the ionic strength, micelles become unstable and aggregated to microparticles. At lower ionic strength, micelles are stable and well dispersed due to the electrostatic repulsion forces between the ionized carboxyl groups on the outer shells. While at high ionic strength, the charges on the micelle outer shells are neutralized by the electrolytes, which decrease the electrostatic repulsion forces between the ionized carboxyl groups and the aggregation of micelles occur. According to the results, it is suggested that micelles be stable with nano sizes when exposed to saline solution at ionic strength of 0.15 M.

The zeta-potentials of PAAc-*b*-PDLLA micelles in buffer solutions at different pH are shown in Fig. 6. The results indicated that the zeta-potentials of micelles decreased with the increase of pH from 1.5 to 12. With the increase of pH, the carboxyl groups in PAAc-*b*-PDLLA on the surface of micelles released protons and possessed more negative charges.

3.5. In vitro cytotoxicity

Cytotoxicity is an important factor for the application of drug carriers in human body. Fig. 7 shows the cytotoxicity of PAAc-*b*-

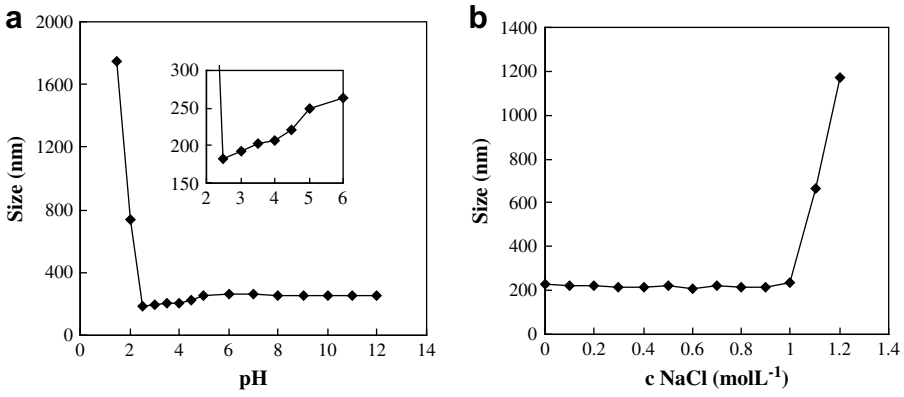


Fig. 5. Effects on the sizes of PAAc-*b*-PDLLA micelles: (a) pH (b) ionic strength (reflect as concentration of NaCl).

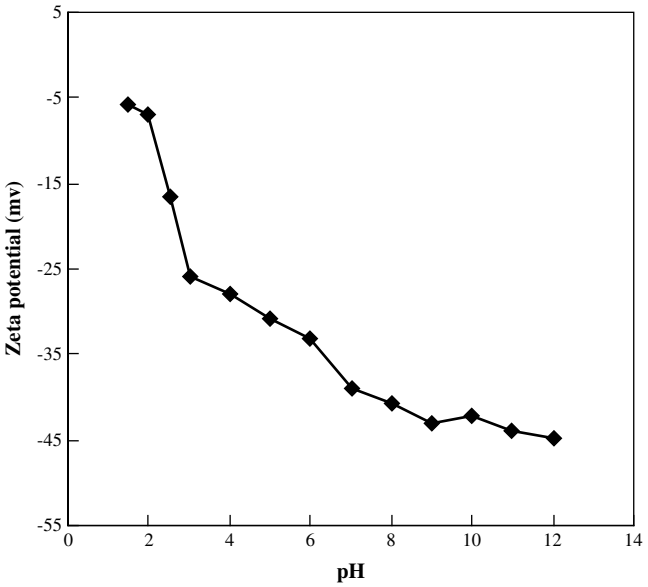


Fig. 6. Effect of pH on the zeta-potentials of PAAc-*b*-PDLLA micelles.

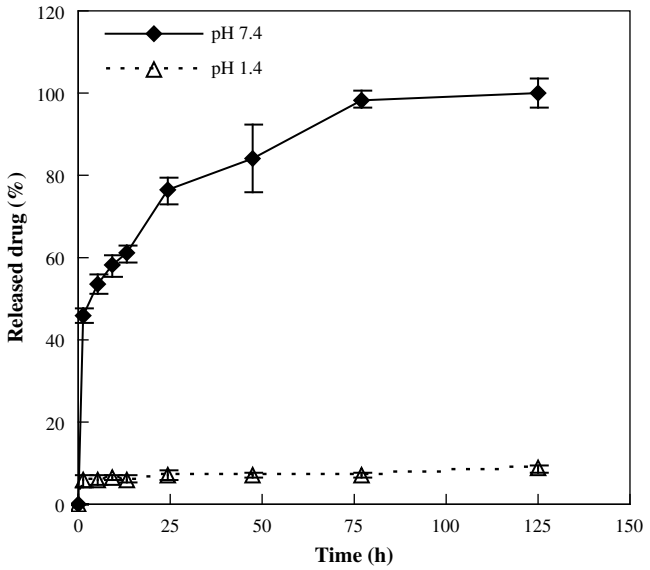


Fig. 8. Drug release behavior of prednisone acetate from PAAc-*b*-PDLLA micelles in different pH buffer solutions at 37 °C (pH 1.4: HCl–KCl buffer solution, ionic strength 0.1 M; pH 7.4: Na_2HPO_4–NaH_2PO_4 buffer solution, Ionic strength 0.1 M).

PDLLA copolymer in HeLa cells. The IC50 is 2.8 mg mL^{-1}, which indicates low cytotoxicity of PAAc-*b*-PDLLA copolymer in HeLa cells.

3.6. *pH-sensitive release of drug from PAAc-*b*-PDLLA micelles*

Prednisone acetate loaded micelles were prepared by the dialysis of the mixed solution of PAAc-*b*-PDLLA and prednisone acetate in THF against water. The yield, drug loading content and encapsulation efficiency of the polymeric micelles are found 77.7%, 6.6% and 39.6%, respectively.

The release kinetics of drug loaded PAAc-*b*-PDLLA micelle is shown in Fig. 8. It is clear that a remarkable on–off release behavior in different pH buffer solutions is obtained. As shown in Fig. 8, at pH 1.4, which is close to the pH of stomach, little amount (6%) of drug released. In contrast, at pH 7.4, around the intestinal pH, 60% of drug released within 10 h, and then all loaded drug continuously released within 100 h. At lower pH, the pendant carboxyl groups of the PAAc segments in the outer shells were protonated to cause that the shells become hydrophobic and gathered together. As a result, the inner cores were tightly wrapped by the outer shells and the encapsulated drug was difficult to release from the inner cores. While at higher pH, the protons of carboxyl groups in the PAAc segments released and the shells transformed into polyelectrolyte, therefore the electrostatic repulsion forces between PAAc segments became dominant, which initiated the stretch of PAAc chains and loose of outer shells, accordingly the drug in the inner cores easily released. It is suggested that the drug loaded in the micelles be difficult to release in the stomach while diffuse out in the intestine.

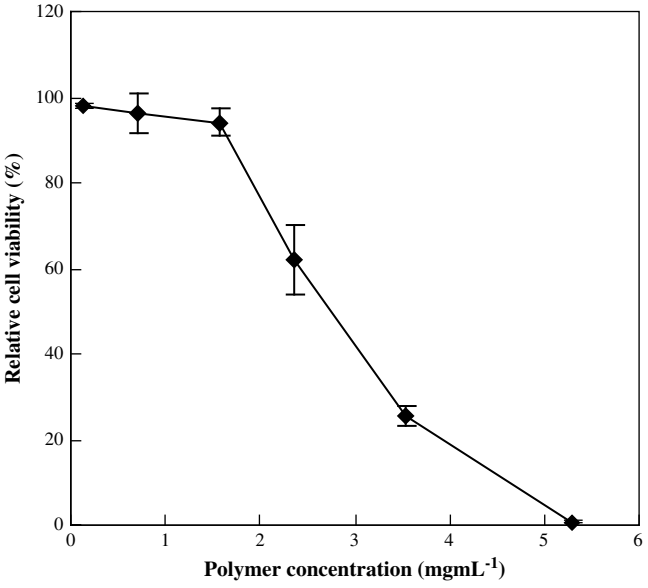


Fig. 7. *In vitro* cytotoxicity of PAAc-*b*-PDLLA copolymer in HeLa cells.

4. Conclusion

Amphiphilic diblock copolymer PAAc-*b*-PDLLA with low cytotoxicity was successfully synthesized by ring-opening polymerization of DL-lactide using hydroxyl-terminated polyacrylic acid as the initiator. pH-Responsive micelles were prepared by self-assembly of diblock copolymer using membrane dialysis technique. The properties of micelles in aqueous solution were influenced by the balance among electrostatic repulsion, hydrogen bond of PAAc segments and hydrophobic interactions between PDLLA segments. The micelles remained stable with the size around 200 nm at pH above 3 or ionic strength below 1.0. The release kinetics of hydrophobic drug from the micelles exhibited pH dependence. PAAc-*b*-PDLLA micelles are anticipated to be a promising carrier for pH-responsive drug delivery systems, especially for targeted delivery to intestine in GI tract.

Acknowledgements

This work was financially supported by National Basic Research Program of China (2009CB930300, 2005CB623903), National Natural Science Foundation of China (20674058, 20874076) and Ministry of Education of China (Program for New Century Excellent Talents in University, Cultivation Fund of Key Scientific and Technical Innovation Project 707043).

References

[1] Torchilin VP. J Controlled Release 2001;73:137–72.
[2] Kwon GS, Kataoka K. Adv Drug Deliv Rev 1995;16:295–309.
[3] Kwon GS, Okano T. Adv Drug Deliv Rev 1996;21:107–16.
[4] Vinogradov S, Batrakova E, Li S, Kabanov A. Bioconjugate Chem 1999;10:851–60.
[5] Yasugi K, Nakamura T, Nagasaki Y, Kato M, Kataoka K. Macromolecules 1999;32:8024–32.
[6] Katayama Y, Sonoda T, Maeda M. Macromolecules 2001;34:8569–73.
[7] Kakizawa Y, Harada A, Kataola K. J Am Chem Soc 1999;121:11247–8.
[8] Kohori F, Sakai K, Aoyagi T, Yokoyama M, Sakurai Y, Okano T. J Controlled Release 1998;55:87–98.
[9] Chung JE, Yokoyama M, Yamato M, Aoyagi T, Sakurai Y, Okano T. J Controlled Release 1999;62:115–27.
[10] Chen WQ, Wei H, Li SL, Feng J, Nie J, Zhang XZ, et al. Polymer 2008;49:3965–72.
[11] Li YY, Zhang XZ, Kim GC, Cheng H, Cheng SX, Zhuo RX. Small 2006;2:917–23.
[12] Arimura H, Ohya Y, Ouchi T. Biomacromolecules 2005;6:720–5.
[13] Bo Q, Zhao Y. J Polym Sci Part A Polym Chem 2006;44:1734–44.
[14] Lee ES, Oh KT, Kim D, Youn YS, Bae YH. J Controlled Release 2007;123:19–26.
[15] Lee ES, Gao Z, Kim D, Park K, Kwon IC, Bae YH. J Controlled Release 2008;129:228–36.
[16] Wei H, Zhang XZ, Cheng H, Chen WQ, Cheng SX, Zhuo RX. J Controlled Release 2006;116:266–74.
[17] Li GY, Song S, Guo L, Ma SM. J Polym Sci Part A Polym Chem 2008;46:5028–35.
[18] Koa J, Park K, Kim YS, Kim MS, Han JK, Kim K, et al. J Controlled Release 2007;123:109–15.
[19] Liu SQ, Wiradharma N, Gao SJ, Tong YW, Yang YY. Biomaterials 2007;28:1423–33.
[20] Soppimath KS, Tan DCW, Yang YY. Adv Mater 2005;17:318–20.
[21] Soppimath KS, Liu LH, Seow WY, Liu SQ, Powell R, Chan P, et al. Adv Funct Mater 2007;17:355–62.
[22] Gillies ER, Frechet JMJ. Bioconjugate Chem 2005;16:361–8.
[23] Guo M, Yan Y, Zhang H, Yan H, Cao Y, Liu K, et al. J Mater Chem 2008;18:5104–12.
[24] Nostrum CF. Adv Drug Deliv Rev 2004;56:9–16.
[25] Kataoka K, Kwon GS, Yokoyama M, Okano T, Sakutai Y. J Controlled Release 1993;24:119–32.
[26] Schmaljohann D. Adv Drug Deliv Rev 2006;58:1655–70.
[27] Serra L, Domenech J, Peppas NA. Eur J Pharm Biopharm 2006;63:11–8.
[28] Park H, Robinson JR. Pharm Res 1987;4:457–64.
[29] Hang L, Guo R, Yang M, Jiang X, Liu B. Adv Mater 2007;19:2988–92.
[30] Zou T, Cheng SX, Zhang XZ, Zhuo RX. J Biomed Mater Res Part B Appl Biomater 2007;82B:400–7.
[31] Wan T, Liu Y, Yu JQ, Chen S, Li F, Zhang XZ, et al. J Polym Sci Part A Polym Chem 2006;44:6688–96.
[32] Fu HL, Yu L, Zhang H, Zhang XZ, Cheng SX, Zhuo RX. J Biomed Mater Res Part A 2007;81A:186–94.
[33] Kalyanasundaram K, Thomas JK. J Am Chem Soc 1977;99:2039–44.