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pH-dependent self-assembly of amphiphilic poly(L-glutamic acid)-block-poly-(lactic-co-glycolic acid) copolymers

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ABSTRACT

A novel biodegradable AB-type diblock copolymer poly(L-lactic- co-glycolic acid)-block-poly(L-glutamic acid) (PLGA-b-PGA) was synthesized by a macromolecular coupling reaction between carboxyl-terminated PLGA and amino-terminated poly(γ-benzyl-glutamate) (PBLG) and the subsequent elimination of the protecting benzyl group. The structures of PLGA–PGA and its precursors were confirmed by Fourier transform infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy and gel permeation chromatography (GPC). This synthetic strategy simplified a former synthesis process of polypeptide-poly(L-lactic acid)(PLA); by using this new synthetic route the molecular weight and block ratio of PLGA–PGA could be easily controlled by adjusting the chain length of PLGA/PGA. The pH sensitivity and self-assembly behavior of PLGA–PGA copolymer were investigated by environmental scanning electron microscopy (ESEM), transmission electron microscopy (TEM) and dynamic light scattering (DLS). The results showed that the copolymer exhibited high pH responses, and the morphologies of the copolymer aggregates underwent four stages orderly with the pH increase (pH = 3–9): a disorganized form, micelles, semi-vesicles with thick walls and vesicles. Such a pH-dependent self-assembly process of the copolymer is promising for drug control release and bio-applications.

1. Introduction

Aliphatic polyesters and their copolymers have received great interest in pharmaceutical and biomedical applications such as drug delivery systems, sutures, artificial skins, orthopedics, and scaffold for tissue engineering because of their excellent biodegradability and biocompatibility [1–8]. Poly[(L-lactic acid)-co-(glycolic acid)] (PLGA), a typical biodegradable polyester, has been widely used in surgical repair, carriers in drug deliver and temporary matrices in tissue engineering due to its biodegradability, biocompatibility and good shaping and molding properties[9–12]. However PLGA is hydrophobic and lacks reactive main or side chains, making it difficult to interact and to be modified with biologically active moieties, and restricting its application in fields such as self-assembly, biological sensors, biodegradable macromolecular drug and so on [3,6,13,14].

To improve the hydrophilicity of PLGA and to introduce functional groups on it, many efforts were made, including the synthesis of random, block, and graft copolymers. Among all the copolymers, AB-type diblock or ABC-type triblock copolymers composed of hydrophobic polyesters and hydrophilic soft segments or some bioactive moieties have been widely investigated. Poly (ethylene glycol) is a common polymer to be introduced because of its hydrophilicity, non-toxicity, biocompatibility and nonimmunogenicity [15–18].

Polypeptides consisting of α-amino acids are very important biological macromolecules and suitable for biomedical applications such as sutures, artificial tissues, implants and drug delivery because of their hydrophilicity, pH sensitivity, biocompatibility and biodegradability [19–21]. Therefore, introducing polypeptides into polyesters to form block copolymers has been an intriguing field [22–24]. However, the reported methods of this block copolymerization are often complicated and uncontrollable. As an example, the preparation of poly(L-lactide)-b-poly(aspartic acid) [25] is very complicated, which is through the polymerization of b-benzyl-L-aspartate-N-carboxyanhydride [Asp(OBzl)- NCA] with amino-terminating polylactide (NH_2–PLA) as a macroinitiator and the removal of the benzyl group protecting the side carboxyl group under the treatment of trifluoromethanesulfonic acid (TFMSA)/thioanisole/trifluoro- acetate (TFA). However, the synthesis of NH_2–PLA itself is also complicated, which makes this preparation more complicated. Briefly, the hydroxyl group of Boc-aminoethanol [the amino group is protected by a t-butoxy carbonyl group (Boc)] is converted into the corresponding potassium alkoxide by using

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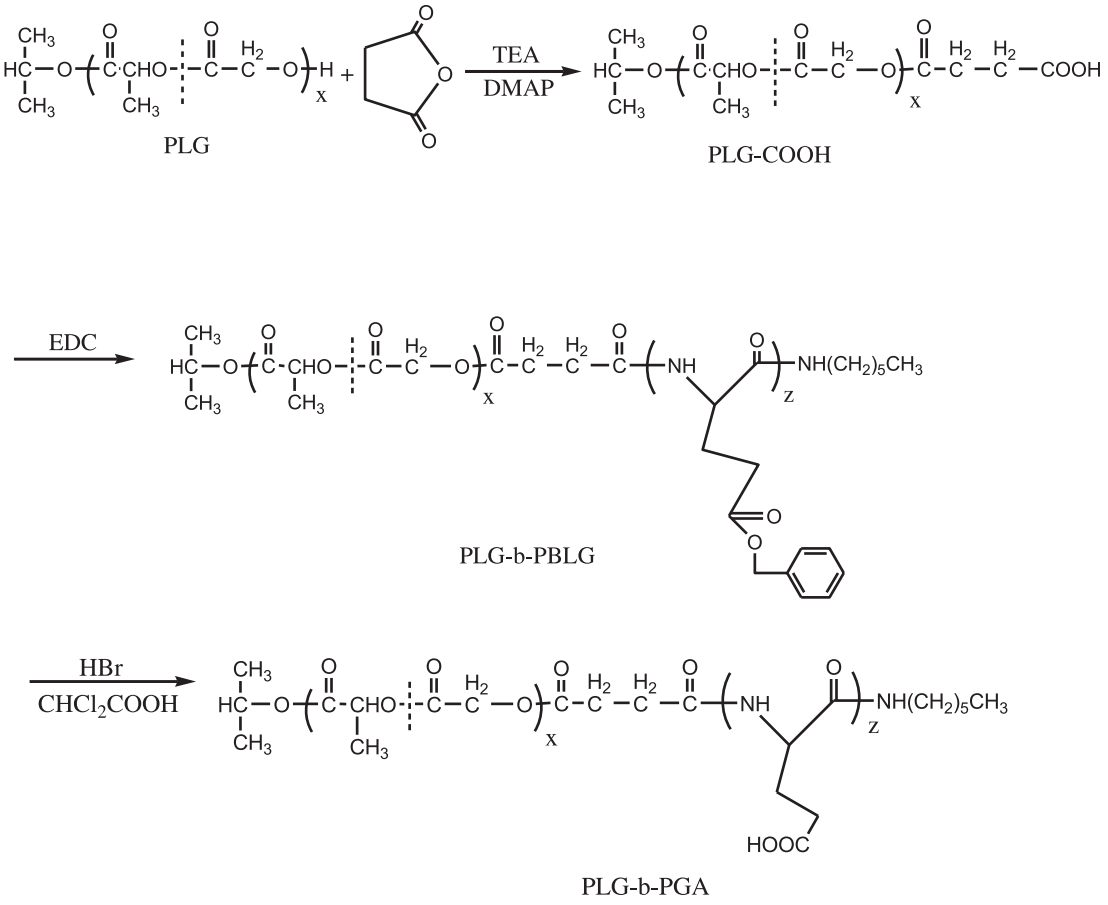


Scheme 1. Synthetic route to PLG-block-PGA.

potassium naphthalene in tetrahydrofuran (THF). This alkoxide group is then used as an initiation site for the polymerization of L-lactide (L-LA) to give polylactide with protected amino groups at the terminals. The Boc groups are subsequently removed by treatment with a 25%-HBr/AcOH solution followed by desalination to get the desired NH_2—PLA. From this example we can see that to find a convenient synthesis is essential.

In this study, we report a novel synthesis method to prepare poly(L-lactic-co-glycolic acid)-block-Poly (L-glutamic acid) (PLGA-b-PGA) AB-type diblock copolymer through a macromolecular coupling reaction between carboxyl-terminated PLGA and amino-terminated PBLG, and the subsequent elimination of the benzyl groups. The copolymer has a hydrophobic PLGA segment and PGA block whose charge state and solubility are pH-dependent. Although many studies on polypeptide-PLA block copolymers have been reported, there has hardly any research done on polypeptide-PLGA diblock copolymers and their self-assembly behavior and mechanism with pH variation in aqueous solution. The effects of pH on the morphology and structure of the copolymer aggregates in aqueous solution are investigated by environmental scanning electron microscopy (ESEM), transmission electron microscopy (TEM) and dynamic light scattering (DLS), and the self-assembly process at different pH values is discussed. Such pH sensitivity and self-assembly behavior of PLG-b-PGA is promising for applications in targeting or environmental drug delivery, gene delivery and other bio-applications.

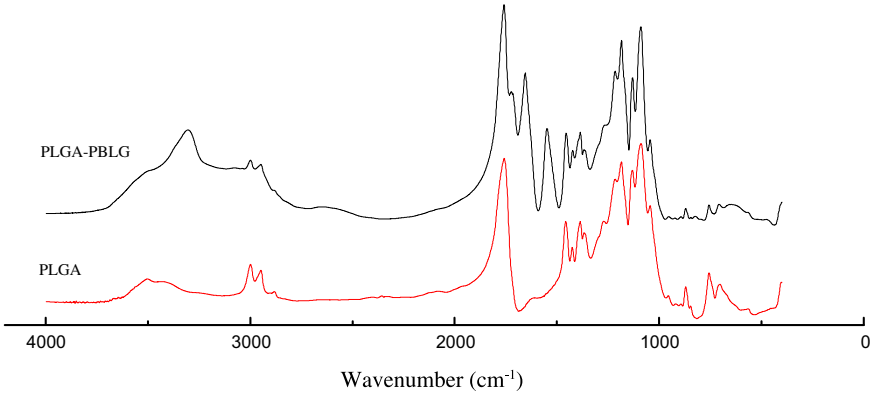


Fig. 1. The IR spectra of PLGA and PBLG.

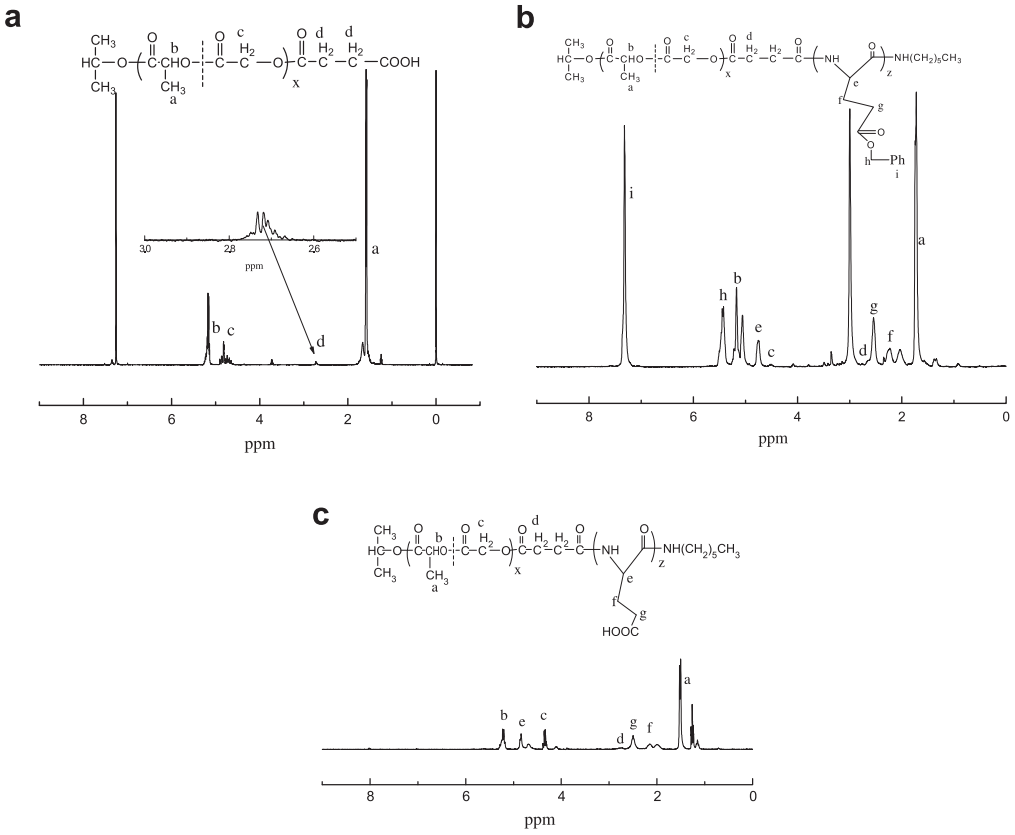


Fig. 2. The ^{1}H NMR spectra of (a) PLGA–COOH, (b) PLGA–PBLG, (C) PLGA–PGA.

2. Experimental section

2.1. Materials

Poly (lactic-co-glycolic acid) (PLGA) and BLG–NCA were prepared via the methods reported by Wu X. [26] and Daly WH et al. [27] respectively. PBLG was synthesized by the ring opening polymerization of BLG–NCA using hexylamine as the initiator in chloroform. Succinic anhydride, 4-(dimethylamino) pyridine (DMAP), dicyclohexylcarbodiimide (DCC), and 1-ethyl-(3-dimethyl aminopropyl) carbodiimide (EDC) were purchased from GL Biochem (Shanghai) Ltd. Dimethyl sulfoxide (DMSO), Triethylamine (TEA) and dimethyl formamide (DMF) were dried over CaH_2 and distilled prior to use. An HBr solution of 33°wt.% in acetic acid (Acros), dichloroacetic acid (Shanghai Laize Chemical Co., China), ethanol and aether (Beijing Chemical Co) were used as received.

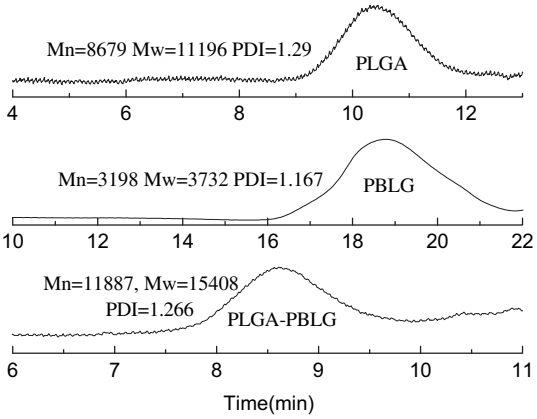


Fig. 3. The GPC traces of PLGA, PBLG and PLGA–PBLG.

2.2. Polymer synthesis

PLGA-b-PGA copolymers were synthesized according to Scheme 1, including the following three-step synthetic route.

(1) Synthesis of monocarboxy poly(lactic-co-glycolic acid) (PLG-COOH) [28]: PLGA (Mn = 8679), succinic anhydride, TEA and DMAP were dissolved in 1, 4-dioxane and reacted in an atmosphere of nitrogen for 24°h at room temperature. The product was precipitated and washed in cooled ethanol, and then dried under vacuum for 2 days at room temperature. A typical ^{1}H NMR spectrum of PLGA–COOH is displayed in Fig. 1a. The peaks marked with letters from a to d could be assigned to the characteristic signals of protons in PLGA–COOH repeat-units.

(2) Synthesis of PLGA-block-PBLG: The reaction of PLGA–COOH 0.88°g(0.1 mmol) and lightly excessive PBLG (Mn = 3198) 0.48°g (0.15 mmol) using EDC (0.1 mmol) as the catalyzer was carried out in 15°mlDMSO at 30 °C for 24°h, and the mixed solution was dialyzed against DMSO (dialysis membrane: molecular weight cut off: 7000 g mol^{-1}; type: R-315-44-7K; source: Greenbird Company, Shanghai, China) to remove the unreacted PBLG homopolymer and EDC; the remainder solution was precipitated in cool aether and dried under vacuum at room temperature for 24 h. The composition of the copolymer was analyzed by ^{1}H NMR (Fig. 1b).

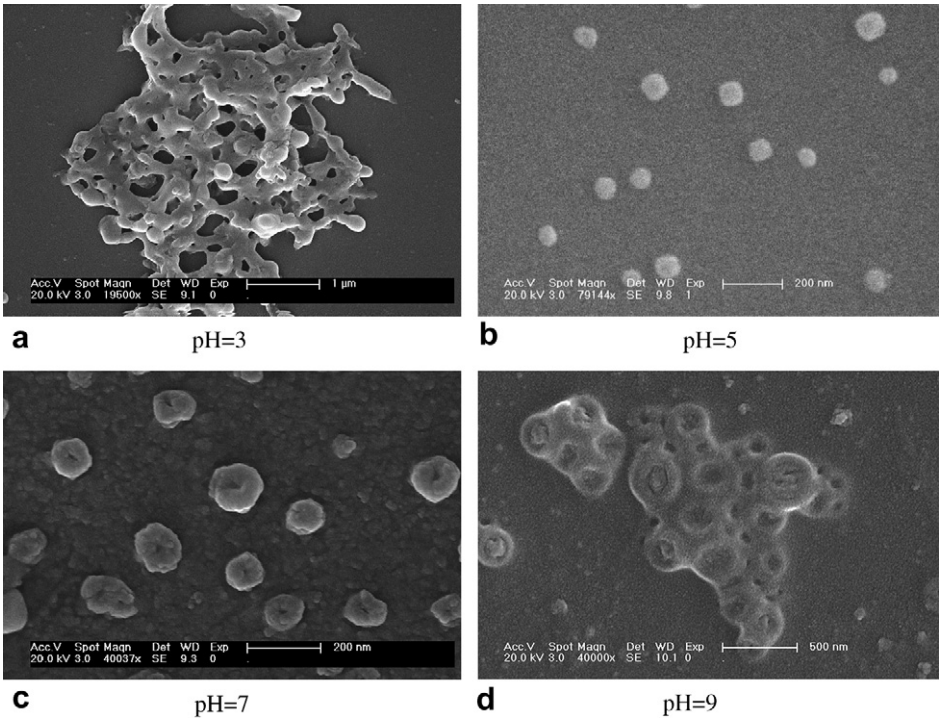


Fig. 4. ESEM images of the aggregates at different pH values.

(3) Deprotection: The Boc groups of the obtained copolymer (PLGA–PBLG) were subsequently removed by treatment with a 33% HBr/$CHCl_2$·COOH to get the desired poly (lactic-co-glycolic acid)-b-poly (L-glutamic acid) (PLGA-b-PGA). Briefly, the 1 g PLGA-b-PBLG was dissolved in 10 ml dichloroacetic acid, followed by addition of 33% HBr 8 ml. The mixture was gently stirred at ambient temperature for 1 h, and then was transferred to a cooled diethyl ether bath to get the precipitate, which was dried under vacuum. The removal of the benzyl groups was confirmed by ^{1}H NMR (Fig. 1c).

2.3. Aggregate preparation at different pH values

The copolymer 5 mg was dissolved in 5 ml DMF (1 mg/mL), which was a common solvent for the diblocks, and placed in a dialysis bag (molecular weight cut off: 3500). The dialysis bag was immersed in aqueous solution of which the pH value was controlled to increase gradually from 3 to 9 with an increment of 2 units under gentle stirring at room temperature. The solution in the dialysis bag at different pH values (3, 5, 7 and 9) was collected for the characterization.

2.4. Characterizations

FT-IR spectra were recorded on a Bio-Rad Win-I instrument. ^{1}H NMR spectra were measured in D_2O and in a mixture solvent of $CDCl_3$ and CF_3COOD (TFA-*d*; 1/1, v/v) at 20 °C using an AV-400 NMR spectrometer. Gel permeation chromatography (GPC) measurements were conducted on a Waters 410 Gel permeation chromatograph with THF as the fluent (flow rate: 1 ml/min, at 35 °C). The molecular weights were calibrated against polystyrene (PS) standards.

The field emission scanning electron microscopy (FESEM) images of the specimens treated at different pH values were observed with a model XL 30 ESEM FEG from Micro FEI Philips. The dilute aggregate solution was dropped onto a freshly cleaved silicon wafer to form a very thin layer and dried at room temperature. Then the samples were coated with a thin layer of Au before observation.

Transmission electron microscopy (TEM) measurements were performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. The samples for TEM observation were prepared by spreading a drop of aggregate solution on a copper grid coated with a carbon film and then air drying at room temperature before the measurements.

Dynamic light scattering (DLS) measurements were carried out with a DAMN EOS instrument equipped with a He–Ne laser at the scattering angle of 108°. The aggregate solutions of about 0.38 mg/mL were passed through a 0.45 μm filter before the measurements except for sample 1 (pH = 3).

3. Results and discussion

3.1. Polymer synthesis

The AB-type diblock copolymer PLGA–PBLG was synthesized by a macromolecular coupling reaction between carboxyl-terminated PLGA and amino-terminated PBLG using EDC as catalyzer according to Scheme 1.

The IR spectra of PLGA and PLGA–PBLG were shown in Fig. 1. The absorption peak at 3301 cm^{-1} was assigned to ν_{NH} stretch vibration and the peaks at 1655 cm^{-1}(ν_{CO}) and 1548 cm^{-1}(ν_{CO-NH}) were attributed to the amide group, indicating the formation of the polypeptide block. The peak at 1759 cm^{-1} (ν_{CO}) was characteristic of the PLGA block. The absorptions at 698 and 754 cm^{-1} from the phenyl group were characteristic of PBLG block carrying protection groups. The peak at 1091 cm^{-1}(ν_{C-O-C}) was corresponding to PLGA block.

The structures of the copolymers (PLGA–PBLG, PLGA–PGA) were also confirmed by the ^{1}H NMR spectra (Fig. 2). According to the ^{1}H NMR spectra (Fig. 2a), PLGA–COOH was synthesized successfully because new methylene protons in PLGA–COOH appeared at the location of δ = 2.72 ppm (d), which had a slight shift from δ = 2.62 ppm in succinic anhydride after the reaction

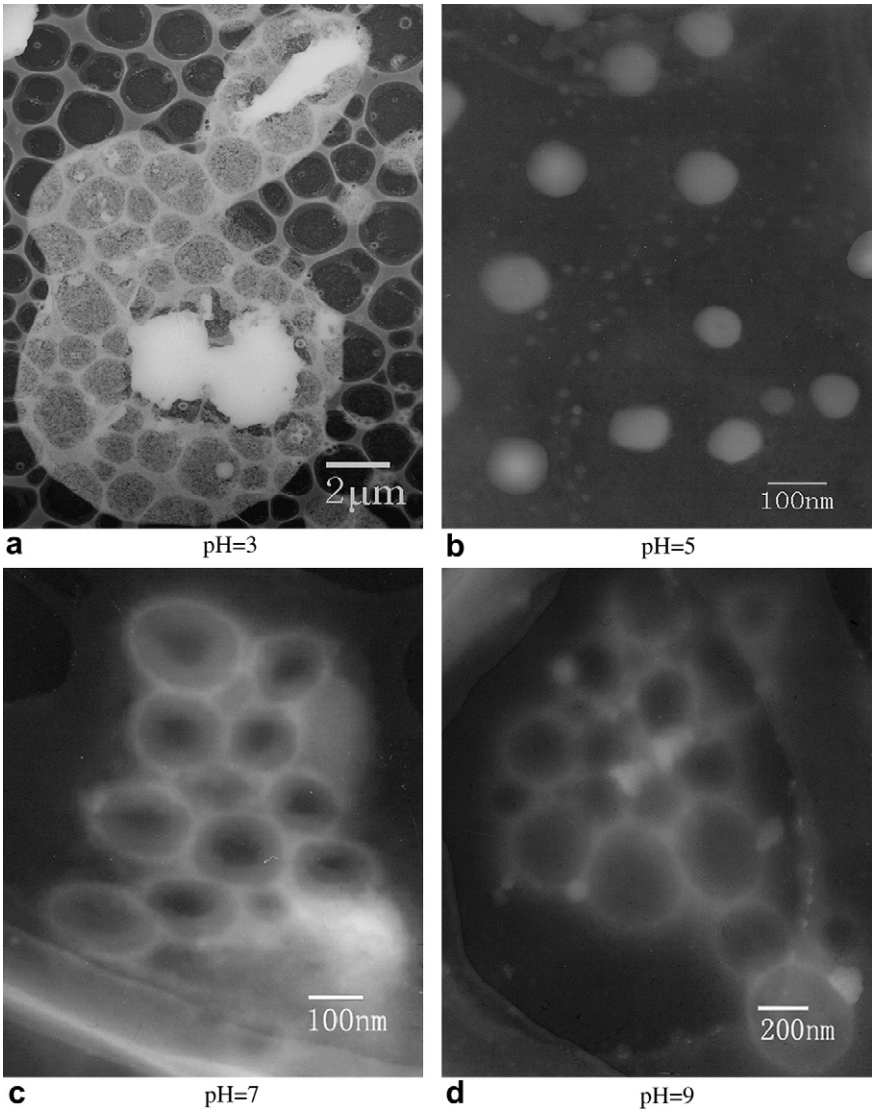


Fig. 5. TEM micrographs of the aggregates formed by PLGA-b-PGA at di pH values.

between PLGA and succinic anhydride, indicating that succinic anhydride was introduced into PLGA through an ester linkage. The PLGA-b-PBLG diblock copolymers were prepared by the macromolecular coupling reaction between PLGA–COOH and PBLG–NH_2. Furthermore, the chain length of both PLGA and PBLG could be controlled by adjusting the molecular weights of PLGA or PBLG. The typical ^{1}H NMR spectrum of PLGA-b-PBLG was shown in Fig. 2b. The new peaks at $\delta = 7.27$ ppm (5H, Ar–H, i), $\delta = 5.41$ ppm (2H, Ar–CH_2–, h), $\delta = 4.75$ ppm (1H, –NH–CH–CO–, e), $\delta = 2.52$ ppm (2H, –CH_2CH_2–CO–, g), $\delta = 2.23$ and 2.03 ppm (2H, –CH_2 CH_2CO–, f) in PLGA-b-PBLG could be ascribed to the protons of PBLG segments. The GPC trace of the copolymer PLGA–PBLG (Fig. 3) in THF also showed a single peak. These results indicated that the obtained product was the copolymer PLGA-b-PBLG instead of the blend of PLGA and PBLG. These results also proved that this synthetic strategy of macromolecular reaction was feasible and simplified the former synthetic strategy of PLA–PBLG. The removal of the benzyl group was confirmed by the ^{1}H NMR spectrum (Fig. 2c). Peaks derived from the benzyl group (i, h) disappeared, indicating that benzyl groups were removed successfully. In addition, other peaks assigned to the protons of PLGA segments and PGA backbone exhibited no change except for slight shifts. This suggested that the removal of benzyl groups was selective without obvious cleavage of the PLGA chain and PGA backbone.

3.2. pH-dependent self-assembly

It was reported [29] that in an acidic solution, the PGA chain was neutralized, and its secondary conformation formed a compact α-helical structure, resulting in the hydrophobicity of PGA. While in an aqueous neutral or basic solution, the PGA chain exhibited hydrophilic nature because of the ionized carboxylic groups. The structure variation was accompanied with an increase in solubility with the increase of pH. In our research, this hydrophilic variation of PGA chain with the pH increase was the driving force for self-assemble of the copolymer PLGA-b-PGA. The influence of pH value on self-assemble aggregates images was observed by ESEM (Fig. 4) and TEM (Fig. 5), which showed similar self-assembly trends that with the increase of pH value, the morphologies of aggregates underwent four stages orderly: a disorganized form (Figs. 4a and 5a, pH = 3), micelles (Figs. 4b and 5b, pH = 5), semi-vesicles with thick walls (Figs. 4c and 5c, pH = 7), vesicles (Figs. 4d and 5d, pH = 9). These phenomena indicated that the AB-type diblock copolymer PLGA-b-PGA was sensitive to pH and could self-

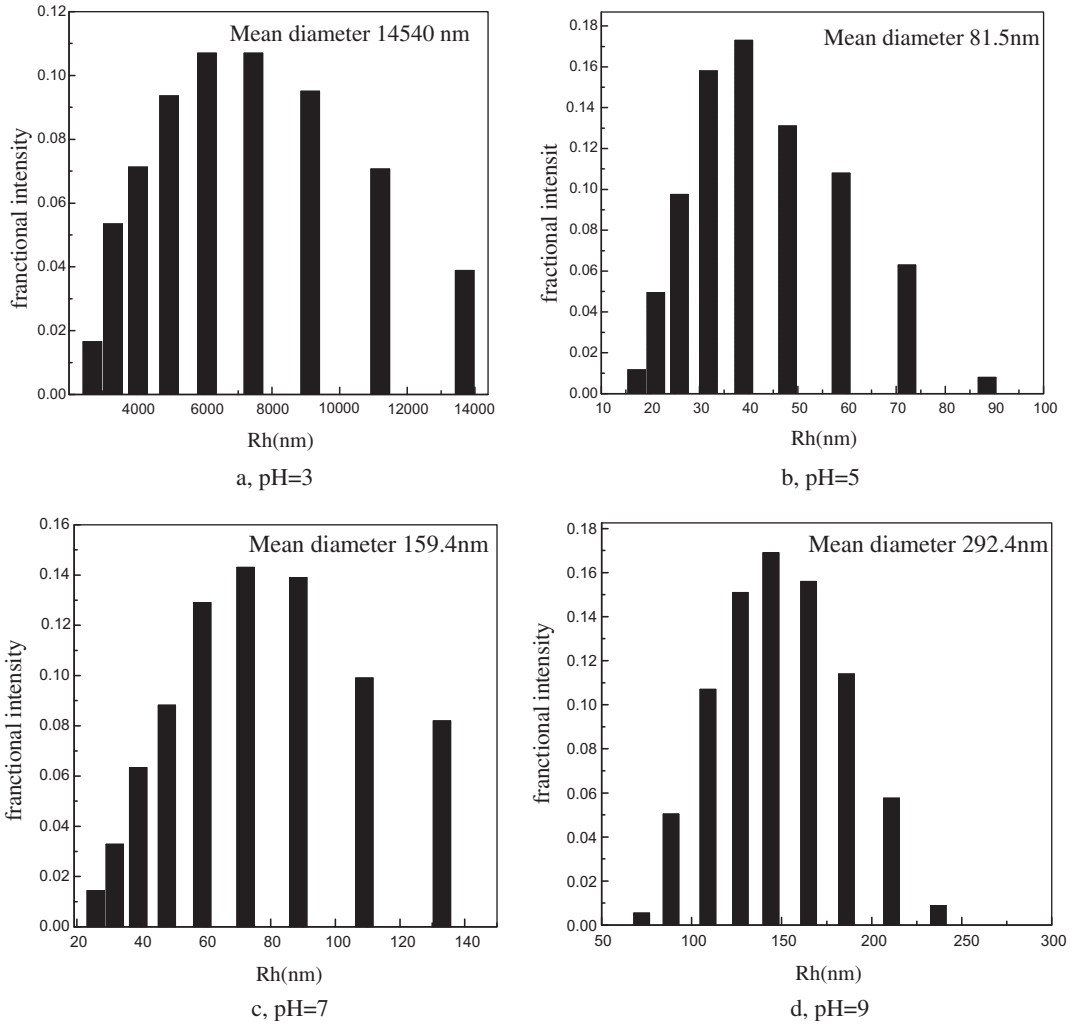
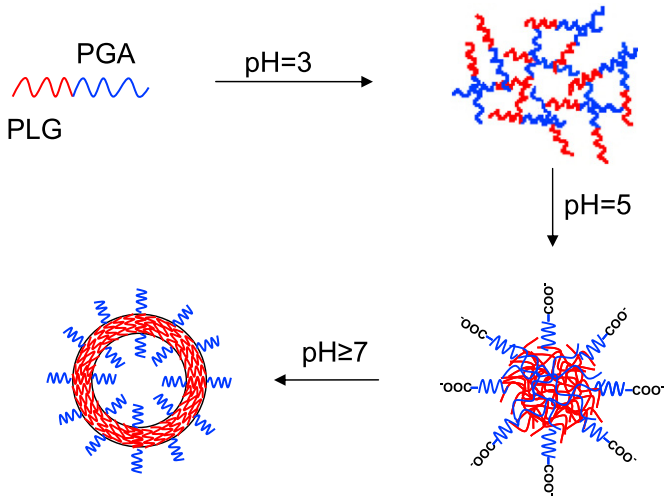


Fig. 6. Size distribution and Mean diameter of PLGA-b-PGA copolymer aggregates at different pH values measured by DLS.

assemble into different morphologies from micelles to vesicles with the pH increase.

The dependence of aggregates size and size distribution on pH value was investigated by DLS (Fig. 6). In all cases, the particles showed single peak distribution, and the particle size varied considerably with the pH value increase. The mean diameter obtained at pH = 5 was more than 14,000 nm (Fig. 6a); while the pH value was increased to 5, the mean size decreased drastically to 81.5 nm (Fig. 6b); the mean size increased again with further increasing in pH (Fig. 6c, d). The remarkable results show a good correspondence with the pH value as well as the morphology variation. From this it can be seen that pH values played a key role in the aggregate self-assembly.



Scheme 2. Schematic illustration of aggregates conversion formed by PLGA-b-PGA at different pH values.

The self-assembly process of the aggregates with pH variation could be described as Scheme 2. The whole copolymer PLGA-b-PGA exhibited hydrophobic nature for PGA (α-helical structure) being insoluble in low pH aqueous solutions, so that the molecular chains of copolymer aggregated together to form large aggregates. With the increase of pH value (pH = 5), carboxylic groups of the PGA block were ionized partly and exhibited hydrophilicity to some extent, and the amphiphilic nature of the diblock copolymer led to the formation of micelles with the ionized PGA as the shell and PLGA and unionized PGA as the core, which caused particle size to decrease sharply. With a further increase of pH value, more PGA blocks in the core moved into the shell, because more carboxylic groups were ionized and got solvated. At the same time, the carboxylic groups located inside the core attracted the solvent into the cores of the micelles, resulting in the increase of inner aqueous phase and the expansion of particle size, finally in the formation of

vesicles consisting of double-decked amphiphilic diblock copolymer PLGA-b-PGA.

4. Conclusion

In this work, a AB-type diblock copolymer PLGA-b-PBLG was prepared through a macromolecular coupling reaction between carboxyl-terminated PLGA and NH_2-terminating PBLG and the subsequent elimination of benzyl groups by treatment with 33% $HBr/CHCl_2 \cdot COOH$. It was found that this synthetic strategy simplified a former synthesis process of polypeptide-PLA, and the molecular weight and block ratio of PLGA-b-PGA could be easily controlled by adjusting the chain length of PLGA/PGA. In addition, the copolymer exhibited high pH response and distinct self-assembly property. With a pH increase, the morphology of the copolymer aggregates underwent four stages orderly: a disorganized form, micelles, semi-vesicles with thick walls and vesicles. This variation was caused by the ionization of carboxylic groups on the PGA block of the copolymer, the indiffusion of alkaline water (solvent) into the core and by the solvation of the inner carboxylic groups in the core. Such a pH-dependent self-assembly process of the copolymer could be very useful for drug control release and bioapplication [30–40]. A detailed investigation of drug loading and control release properties of the PLGA–PGA vesicles will be reported else where.

Acknowledgement

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