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Microwave-assisted rapid synthesis, characterization and application of poly (D,L-lactide)-graft-pullulan

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ABSTRACT

A novel microwave-assisted method was developed to synthetize amphiphilic copolymer poly (D,L-lactide)-graft-pullulan (PL) in a monomode microwave reactor. The effects of microwave power, ratio of catalyst/lactide, ratio of lactide/hydroxyl group of pullulan (lactide/OH-P) and solvent on the synthesis were further investigated. Three samples (designated as PL 8, 9, and 6), characterized by FT-IR and NMR, were applied to form nanoparticles and microparticles investigated by dynamic light scattering, fluorescence spectroscopy and transmission electron microscopy. PL9 and PL6 were used for loading model drug curcumin. The results indicated that microwave-assisted synthesis shortened the copolymerization of PL, with higher yield and lactide conversion, from 24 h to 5 min and showed some specific microwave effects compared with conventional oil heating. PL with a relative higher substitution degree gave nanoparticles with smaller sizes and critical aggregation concentrations. The solubility of curcumin was increased to 1.97 mg mL^{-1} as the forms of nanoparticles.

1. Introduction

Amphiphilic copolymers, composed of hydrophilic and hydrophobic moieties, can form micelles spontaneously or other self-aggregates in an isotropic aqueous solution. These polymeric self-aggregates have been recognized as a promising delivery carrier because the hydrophobic core can serve as a reservoir for various hydrophobic bioactive agents. These amphiphilic copolymers, when intended for use in drug delivery, generally consist of biocompatible, biodegradable hydrophobic polymer blocks such as polyesters or poly(amino acids) covalently bonded to a biocompatible hydrophilic block such as polyethylene glycol or natural polysaccharides (Letchford & Burt, 2007).

Pullulan has been studied as a potential drug delivery carrier because it is non-toxic, non-mutagenic, non-irritant, blood compatible and biodegradable (Leathers, 2003). Some groups have taken its advantage of targeting liver to develop liver target-oriented drug delivery systems (Hosseinkhani, Aoyama, Ogawa, & Tabata, 2002; Kang et al., 2010; Rekha & Sharma, 2009, 2011). A lot of work have been done to render pullulan to be hydrophobic by grafting it with some components, like cholesterol, poly (lactic-co-glycolic acid) (PLGA), sulfonamide, dodecanoic acid and perfluoroalkyl carboxylic acid (Akiyoshi et al., 1998; Jeong et al., 2006; Na & Bae, 2002; Sallustio, Galantini, Gente, Masci, & La Mesa, 2004).

Polylactic acid (PLA) has been widely used as the hydrophobic moieties to form the amphiphilic copolymers for drug delivery (Jiang, Wu, He, & Nie, 2009; Kohori et al., 1998; Kwon & Forrest, 2006), owing to its good mechanical property, biodegradability and non-toxic degradation products to the living organisms (Tsuji, 2005). Therefore, the physical–chemical properties, biodegradation of pullulan and PLA can be enhanced by grafting PLA to pullulan. The optimum hydrophilic-hydrophobic balance, the solvation and degradation of the liver-target amhiphilic copolymers can be controlled by managing the ratio of lacide/pullulan. Only limited work has been reported about the copolymerization of pullulan and lactide. Lactide was firstly successfully grafted onto pullulan via one-step ring-opening polymerization (ROP), using Oct_2Sn as a catalyst in dimethyl sulfoxide (DMSO). But this method consumed up to 6 days for synthetizing the copolymer (Donabedian & McCarthy, 1998). Other researchers adopted a trimethylsilyl (TMS) protection method with three steps to synthetize this copolymer with a large number of PLA side chains (Ohya, Maruhashi, & Ouchi, 1998). Later, they synthetized poly (L-lactide)-grafted pullulan through coupling reaction between amino group end-capped poly (L-lactide)

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and carboxymethyl pullulan and studied its aggregation behavior in water. The new method can control the number of PLA side chains and the chain length, but it still required three steps (Ouchi, Minari, & Ohya, 2004). Afterwards, Cho et al. successfully synthesized water-insoluble pullulan-g-poly (L-lactide) via a one-pot method in the presence of triethylamine in DMSO, in an effort to design a novel anticancer agent carrier of doxorubicin (Cho, Park, & Na, 2009). They further investigated thermo-responsive nanogels derived from pullulan-g-poly (L-lactide) and accomplished the triggered drug release (Seo, Lee, Jung, & Na, 2012). However, this method also took up to 12 h to synthetize this copolymer.

Microwave-assisted synthesis, compared with conventional techniques, can not only shorten reaction time from hours to minutes but also reduce side reactions, increase yields and improve reproducibility. It also arouses the interests of polymer chemists and gets broad applications in polymer synthesis, covering ROP, radical polymerization as well as step-growth process. But these microwave-assisted syntheses of polymers were bulk polymerization, conducted without solvent.

Hitherto, there is no report about PL synthesis under microwave irradiation through solution polymerization. In this work, a new method, assisted by microwave, was developed to synthetize PL and compared with conventional oil heating. In order to get the desired PL, the effects of microwave power, ratio of catalyst/lactide, ratio of lactide/OH–P and solvent on the copolymerization were further investigated. Structural properties of this copolymer were characterized by NMR, FT-IR. Then the effects of degree of substitution (DS) on the properties of the copolymers and on the formation of nanoparticles were investigated in details. The properties of nanoparticles were further studied through dynamic light scattering (DLS), fluorescence spectroscopy and transmission electron microscope (TEM). And PL was also first applied to form microparticles, a new application of PL as drug delivery system.

2. Experiments

2.1. Chemicals and reagents

Pullulan (Mw:1.2×10^5 g/mol) was purchased from Puao biomaterial Co., Ltd. (Shanghai, China) and dried under a vacuum of 30 mmHg at 80 °C for 24 h. D,L-lactide (DLLA) was supplied by Daigang Biomaterial Co., Ltd. (Jinan, China) and recrystallized three times using dry toluene. Oct_2Sn was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shenyang, China). DMSO, dimethyl formamide (DMF) and dimethylacetamide (DMAC) were distilled under reduced pressure over CaH_2 to remove water. All the other reagents were analytical grade and used as received.

2.2. Synthesis of PL

2.2.1. Microwave-assisted synthesis of PL

The copolymerization was carried out in a monomode microwave reactor (Discover-SP, CEM, USA). Typically, 1.0 g dried pullulan was introduced into glass flask with an internal volume 100 mL. The 8 mL solvent was injected into the glass flask to make a 12.5% (w/v) solution. DLLA and Oct_2Sn were introduced into the flask, which then was closed and purged with nitrogen. The flask was subjected to microwave irradiation at 80 °C for a period of 5 min. When the irradiation was complete, non-solvent (90 mL), a mixture of dichloromethane–ethyl acetate (1:3, v/v), was used to precipitate the copolymer PL. These materials were further collected by filtration and dried under a vacuum of 30 mmHg at 40 °C for 24 h. This reaction was conducted under different conditions, such as various microwave power, ratio of catalyst/lactide, ratio of lactide/OH–P and solvents.

2.2.2. Synthesis of PL under conventional oil heating

Pullulan initiated ring opening polymerizations of lactide under conventional heating was conducted in an oil bath at 80 °C for 24 h and 36 h respectively with DMSO as the solvent. The ratios of catalyst/lactide and of lactide/OH–P were fixed at 85:0.1 and 85:100. All the other procedures were the same as those of microwave-assisted synthesis of PL.

2.3. Characterization of PL

2.3.1. NMR studies

^{1}H NMR spectra were recorded on a spectrometer (AVIII 600 NMR, Bruker, Germany) at 600 MHz. Chemical shifts in parts per million (ppm) were reported using DMSO-d_6 and TMS as a solvent and an internal reference respectively. The DS of the PL was determined by comparing the integration at 5.2 ppm (3H) to the methyl (6H) protons (Cho et al., 2009; Donabedian & McCarthy, 1998).

2.3.2. FT-IR analysis

Fourier transform infrared (FT-IR) spectra were recorded on a spectrometer (VERTEX 70 V FT-IR, Bruker, Germany) at 25 °C. The spectral position of the sample IR absorbance was given in units of reciprocal centimeters (cm^{-1}).

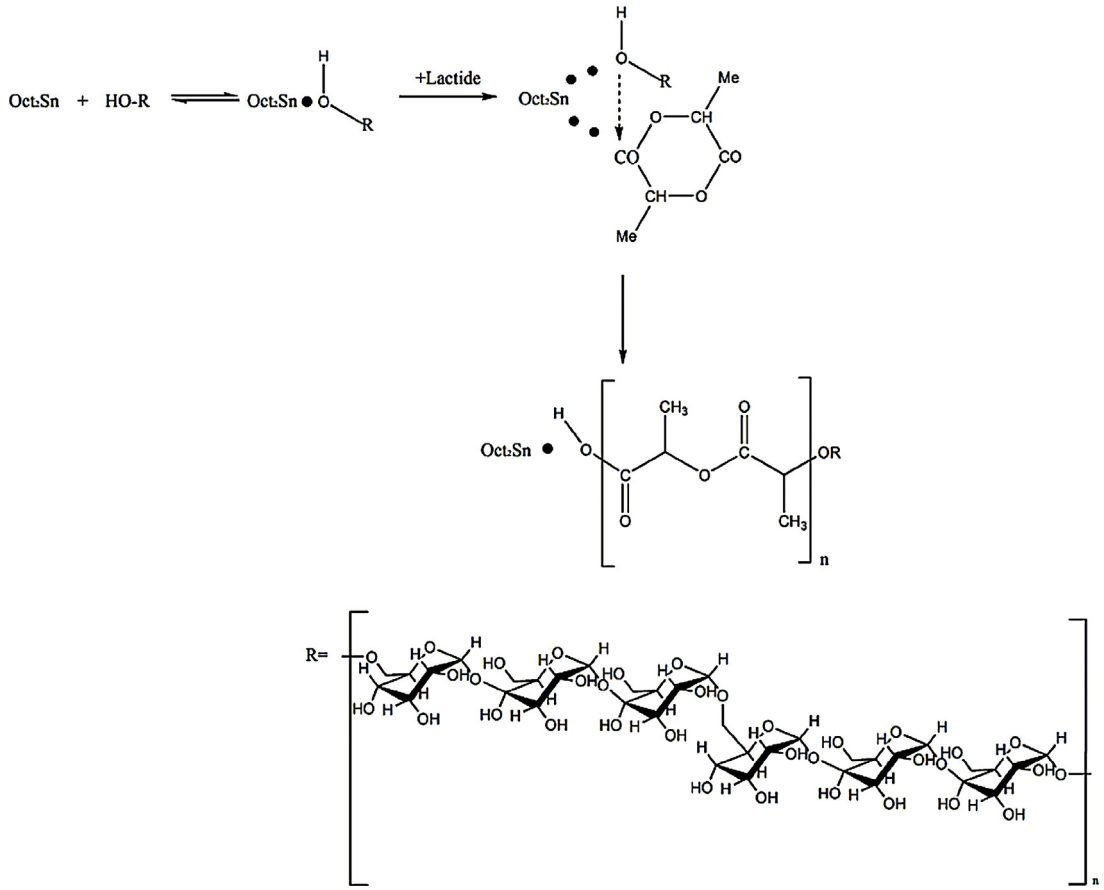
2.3.3. Critical aggregation concentration of PL in aqueous medium

The critical aggregation concentration (CAC) of PL was estimated by the fluorescence probe technique (Cho et al., 2009; Zhu, Chan-Park, Dai, & Li, 2005). Briefly 55 μL pyrene solutions (6.67×10^{-4} mol mL^{-1}) in acetone were added to a series of vials and followed by drying with N_2 to remove the acetone. Then, a series of the PL suspensions in the range from 0.1 to 200 μg mL^{-1} were added to each vial. The final concentration of pyrene in each sample was 6.67×10^{-7} mol mL^{-1}. Then the vials were sonicated in an ultrasonic bath (SK2200H, KUDOS, China) for 1 h for equilibrium, and then left overnight at room temperature. The solution was mildly shaken for 2 h to reach equilibrium. The emission spectra of pyrene was performed at a range from 350 to 500 nm using a fluorescence spectrophotometer (Spectra Max M3, Molecular Devices, USA) at the excitation wavelength of 335 nm.

2.4. Preparation and characterization of nanoparticles from PL

Self-assembled blank nanoparticles were prepared via the thin-film hydration method (Zhang, Jackson, & Burt, 1996). Briefly, 100 mg of PL was dissolved in 10 mL methanol in a flask. The solvent was evaporated by rotary evaporation at 40 °C for 1 h to obtain a solid copolymer film. Then the film was vacuum desiccated overnight to remove any traces of remaining methanol. To form spontaneous self-assembly nanoparticles, the resultant thin polymeric film was hydrated in 5 mL 5% glucose solution and incubated at 37 °C under stirring for 0.5 h, which was then filtrated through 0.2 μm filter membrane. The resultant nanoparticles were stored at 4 °C until characterization. The particle sizes and distribution were determined via DLS analysis conducted on Malvern Nano ZS (Nano-ZS90, Malvern, UK) at 25 °C. The morphology of the nanoparticles was studied using TEM (JEM-1400, JEOL, Japan). The nanoparticles loaded with curumin were prepared by dissolving curcumin in 1% PL methanol solution. Then all the other procedures were the same as above.

In order to detect the curcumin solubility as the forms of nanoparticles, an aqueous solution of curcumin nanoparticles was diluted 500-fold with DMSO. Then the diluent was analyzed at 437 nm using UV-spectroscopy (UV-2000, UNICO, China).



Scheme 1. Schematic representation of the synthesis of PL copolymer.

2.5. Preparation and characterization of microparticles from PL

PL was dissolved in 10 mL methanol to provide concentration of 0.5% (w/v). The PL solution was sprinkled into a 50 mL 0.5% poly (vinyl alcohol) aqueous solution. The solution was stirred on a magnetic stirrer and continued for 2 h at room temperature. Then the solution was rotary evaporated to form microparticles suspension. Microparticles were collected through centrifugation washed with distilled water and dried under a vacuum of 30 mmHg at 50 °C for 24 h. The morphology of the microparticles was studied using TEM. The microparticles loaded with curumin were first to dissolve curcumin in PL 0.5% methanol solution. All the other procedures were the same as above. And then the microparticles were further washed with acetone in order to remove curcumin unloaded in microparticles.

3. Results and discussion

3.1. Synthesis of PL

3.1.1. Microwave-assisted synthesis of PL

In this study, a monomode microwave reactor, which gives wave focusing (reliable homogeneity in electric field) and accurate control of the temperature, was used for microwave-assisted synthesis of PL. And considering the thermal stability of DMSO, synthesis of PL was not conducted at higher temperature than 80 °C. The effect of irradiation time on the microwave-assisted synthesis of PL was not investigated because when the temperature reached the set point 80 °C, in order to maintain the set temperature, there was a sharp drop at irradiation power which was close to 0 W. A schematic representation of grafting of DLLA onto pullulan in the presence of Oct_2Sn was shown in Scheme 1.

3.1.1.1. Influence of irradiation power on the microwave-assisted synthesis of PL. Four microwave power levels (50, 100, 150 and 200 W) were applied to examine the effect of microwave power on the synthesis of PL. Our study showed that the temperature of the reaction cannot be kept at 80 °C when the power reached to higher than 200 W. Higher powers were not applied to synthetize PL. According to the study done by Luckachan et al. (Luckachan & Pillai, 2006), the results from ^{1}H NMR correlated well with the results of gravimetric method, the molar ratio of lactide to pullulan of PL ($F_{LLA}/F_{pullulan}$) was calculated. As shown in Table 1, in generally, the yield and $F_{LLA}/F_{pullulan}$ increased with the increase of microwave power. When the microwave power was adjusted from 50 to 200 W, the yield and $F_{LLA}/F_{pullulan}$ of PL increased from 65.10% and 2.50–85.79% and 4.02, respectively. The conversion of lactide reached to 79.50% when synthesis was conducted at 200 W (Fig. 1(a)). It is presumed that higher power could provide more energy for polymerization and more opportunity for chain propagation of PLA. There might be more specific microwave effects accompanying with higher microwave power as well.

3.1.1.2. Influence of ratio of catalyst/lactide on the microwave-assisted synthesis of PL. The influence of the ratio of catalyst/lactide on yield and $F_{LLA}/F_{pullulan}$ was also investigated. The plots of yield and $F_{LLA}/F_{pullulan}$ versus the ratio of catalyst/lactide were shown in Fig. 1(b). In the case of Oct_2Sn as a catalyst, whose ratio to lactide (mol/mol) was selected as 0.06, 0.12, 0.24 and 0.48%, the reaction mixture was irradiated for 5 min at 150 W and at 80 °C. PL with different $F_{LLA}/F_{pullulan}$ was obtained. An increase in $F_{LLA}/F_{pullulan}$ of PL

Table 1
Effects of various reaction conditions on the synthesis of PL.

Conditions		PL	Solvent	Microwave power (W)	OH–P:lactide:cat (mol:mol:mol)	Yield%[a]	Grafting%[b]	$F_{LLA}/F_{pullulan}$[c]	Conversion%[d]
Microwave irradiation	Power	1	DMSO	50	100:85:0.1	65.10	112.14	2.50	49.65
		2	DMSO	100	100:85:0.1	70.27	129.08	2.89	57.12
		3	DMSO	150	100:85:0.1	74.45	144.28	3.23	63.25
		4	DMSO	200	100:85:0.1	85.79	179.65	4.02	79.50
	Catalyst:lactie	5	DMSO	150	100:85:0.05	69.37	126.07	2.82	55.81
		3	DMSO	150	100:85:0.1	74.45	144.28	3.23	63.25
		6	DMSO	150	100: 85:0.2	89.73	192.98	4.30	85.20
		7	DMSO	150	100: 85:0.4	81.93	167.63	3.75	73.95
	Lactide:OH–P	8	DMSO	150	100:60:0.1	83.33	115.92	2.59	72.86
		3	DMSO	150	100:85:0.1	74.45	144.28	3.23	63.25
		9	DMSO	150	100:100:0.1	65.93	141.26	3.16	53.12
		10	DMSO	150	100:150:0.1	49.19	146.20	3.27	36.50
	Solvent	3	DMSO	150	100:85:0.1	74.45	144.28	3.23	63.25
		11	DMF	150	100:85:0.1	56.19	83.43	1.87	36.84
		12	DMAC	150	100:85:0.1	54.52	78.32	1.75	34.49
Conventional oil heating		13	DMSO	–	100:85:0.1	70.21	129.07	2.89	57.04
		14	DMSO	–	100:85:0.1	71.62	133.20	2.98	59.04

"–" Synthesis conducted under conventional oil heating.
[a] Yield% = $W_{PL} \times 100/(W_L + W_P)$; W_{PL}, W_L and W_P denote the weight of the grafted copolymer PL and initial weight of lactide and pullulan.
[b] Grafting% = $(W_{PL} - W_P) \times 100/W_P$.
[c] Molar composition of DLLA in graft copolymer = grafting% × 161/72.
[d] Conversion% = $(W_{PL} - W_P) \times 100/W_L$.

was observed with the ratio of Oct_2Sn increased from 0.06 to 0.24%, and the highest value of $F_{LLA}/F_{pullulan}$ was 4.3 by 0.24%. However, a higher concentration of Oct_2Sn (0.48%) led to a lower $F_{LLA}/F_{pullulan}$ (3.75) (Fig. 1(b)). The dependence between $F_{LLA}/F_{pullulan}$ and the catalyst concentration was similar to the reports (Jing, Peng, Tong, & Baoxiu, 2006; Liao et al., 2002). When the ratio of catalyst/lactide was adjusted from 0.06 to 0.24%, there was a significant increase both in yield and $F_{LLA}/F_{pullulan}$. The possible reason was that the initial increase in catalyst improved the active species of lactide for copolymerization with pullulan. The catalyst accelerated not only the rate of polymerization but also the rate of the decomposition. When the catalyst concentration increased up to a certain value, the catalysis rate to decomposition was faster than the rate of polymerization (Jing et al., 2006). This caused that when the concentration of Oct_2Sn reached to 0.48%, decomposition of PLA grafting on pullulan was faster than its polymerization, so accompanied with the decrease of $F_{LLA}/F_{pullulan}$ and yield of PL.

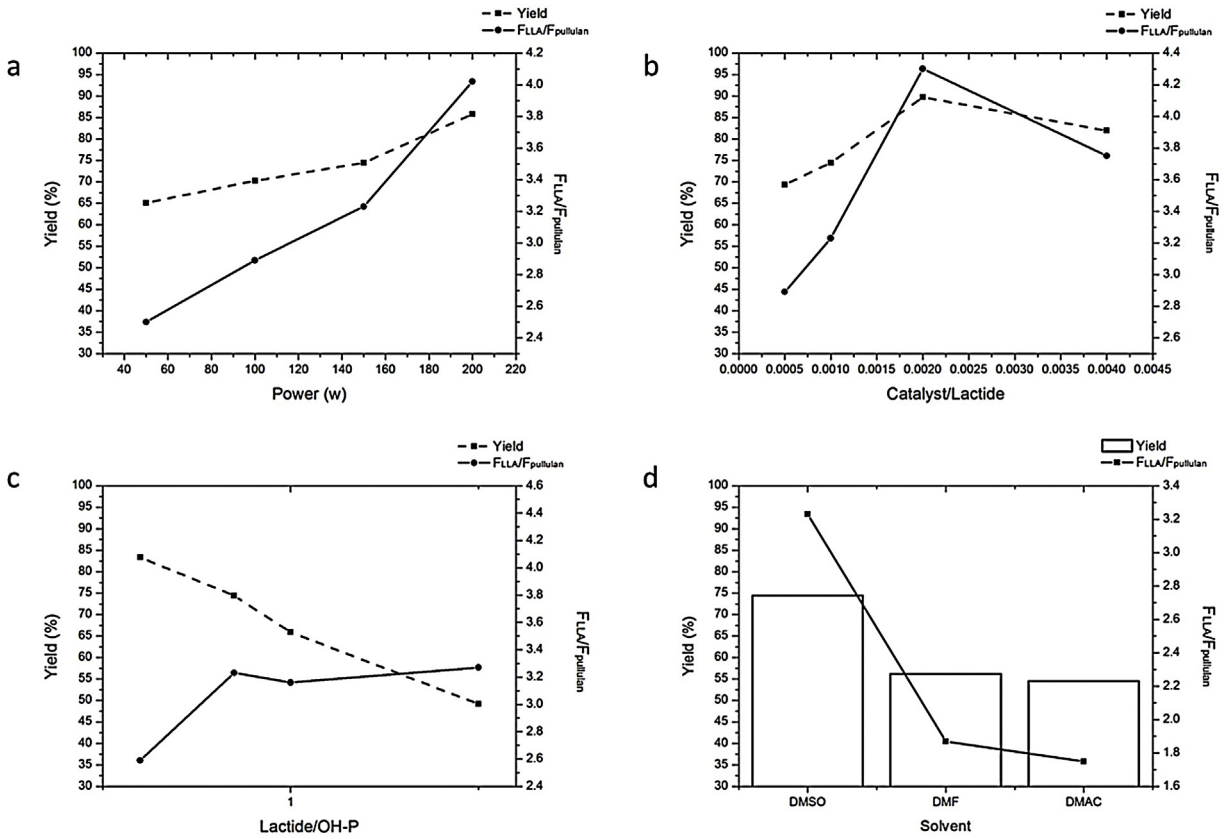


Fig. 1. Effects of microwave power (a), ratio of catalyst/lactide (b), ratio of lactide/OH–P (c) and solvent (d) on yield and $F_{LLA}/F_{pullulan}$ of PL.

3.1.1.3. Influence of ratio of lactide/OH-P on the microwave-assisted synthesis of PL. Four lactide/OH–P feed ratios (0.6, 0.85, 1, and 1.5) were used to study the influence of the ratio on the microwave-assisted synthesis of PL comb-shaped copolymers. The effect of lactide/OH–P ratio on the percentage of grafting of lactide onto pullulan was given in Table 1 and shown in Fig. 1(c). When the molar ratio of the lactide/OH–P increased from 0.6 to 0.85, the grafting percentage rose from 115.92 to 144.28, the molar ratio of lactide to pullulan in the graft copolymer from 2.59 reached to 3.23. But when the ratio of lactide/OH–P continued to increase, even to 1.5, there was no remarkable increase in both grafting percentage and $F_{LLA}/F_{pullulan}$. While the conversion of lactide decreased almost linearly with the ratio of lactide/OH–P. This phenomenon might ascribe to that more lactide can provide more opportunity for chain propagation of PLA, so when the lactide/OH–P ratio increased from 0.6 to 0.85, there was also an increase in $F_{LLA}/F_{pullulan}$ of PL. But the amount of Oct_2Sn was limited and the chain growth center was numbered, so when the lactide continued to increase, there was no more chain growth center for them to polymerize, which resulted in the incomplete conversion of lactide, the decrease of PL yield and little change in $F_{LLA}/F_{pullulan}$ of PL. Otherwise the self-polymerization of PLA rather than copolymerization with pullulan might also make a contribution to this phenomenon.

3.1.1.4. Influence of solvent on the microwave-assisted synthesis of PL. In light of that the solvents which can solve pullulan was limited, only three kinds of solvent, DMSO, DMF and DMAC, were selected to investigate the effect of solvent on the microwave-assisted synthesis of PL. The yield, grafting, $F_{LLA}/F_{pullulan}$ and conversion of lactide were listed in Table 1.

Synthesis of PL was carried out in DMSO, DMF and DMAC at 80 °C and 150 W for 5 min of microwave irradiation. The $F_{LLA}/F_{pullulan}$ and yield of the PL were shown in Fig. 1(d). Resulted products had yield of 74.45, 56.19 and 54.52%, the $F_{LLA}/F_{pullulan}$ of them were 3.23, 1.87 and 1.75, respectively.

The heating characteristics of a system under microwave irradiation conditions are dependent on its dielectric properties. The ability of a specific substance to convert electromagnetic energy into heat at a given frequency and temperature is determined by the so-called loss factor $\tan\delta$. This loss factor is expressed as the quotient $\tan\delta = \varepsilon''/\varepsilon'$, where ε'' is the dielectric loss, which is indicative of the efficiency with which electromagnetic radiation is converted into heat, and ε' is the dielectric constant describing the ability of molecules to be polarized by the electric field. A reaction system with a high $\tan\delta$ value is required for efficient absorption and, consequently, for rapid heating (Kappe, 2004; Lidström, Tierney, Wathey, & Westman, 2001). In the synthesis of PL, the reaction system was composed of pullulan, lactide and solvent, the only difference was the solvent. The $\tan\delta$ of DMSO is 0.825, which is higher than that of DMF ($\tan\delta$ 0.161) (Hayes, 2002), but the $\tan\delta$ of DMAC was not obtained. This suggests that the system with DMSO as solvent can absorb microwave more efficiently and convert it into heating energy more rapidly. But in this study, all the synthesis of PL was controlled at 80 °C, consuming almost equivalent energy. While, the ε' of DMSO is 48.9 higher than those of DMF (36.7) and DMAC (37.78) (Cheng & Hu, 2002), suggesting that DMSO has more

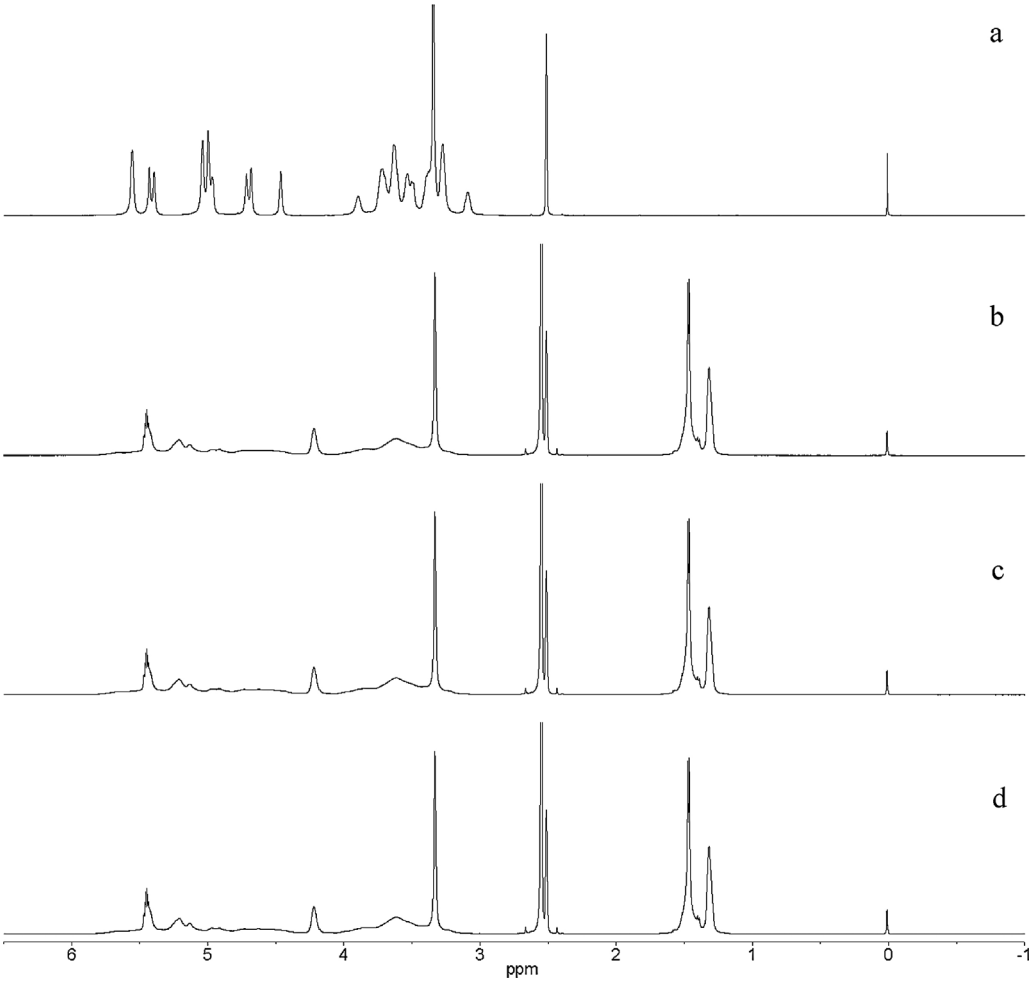


Fig. 2. ^{1}H NMR of pullulan (a), PL6 (b), PL8 (c) and PL9 (d), respectively.

ability to be polarized by the electric field and more microwave specific effects of highly polarizing radiation, so give PL with higher yield and lactide conversion.

3.1.2. Synthesis of PL under conventional oil heating

Synthesis of PL by conventional heating was carried out using oil heating at 80 °C for 24 and 36 h, respectively. The data in Table 1 showed that synthesis of PL assisted by microwave got higher yield 85.79% with corresponding $F_{LLA}/F_{pullulan}$ 4.02 and lactide conversion 79.5% than the corresponding of PL synthesized by conventional oil heating either for 24 h or for 36 h. With their counterparts under oil heating, the yield, $F_{LLA}/F_{pullulan}$ of PL and lactide conversion were 70.21, 2.89, 57.04% and 71.62, 2.98, 59.04% for 24 and 36 h, respectively. With the increase of reaction time for oil heating, the yield and $F_{LLA}/F_{pullulan}$ reached little higher levels. It should be emphasized that the synthesis of the PL by microwave heating was much faster than that via conventional heating in terms of yield and lactide conversion. Microwave irradiation shortened the polymerization time to 5 min.

In some cases, it was considered that the enhanced polymerization rate originated not only from thermal effect but also from specific microwave effects in microwave-irradiated ROP of DLLA (Jing et al., 2006). But a latest study indicated that non-thermal microwave effects do not exist in microwave-assisted ROP of D,L-lactide (Ramier, Renard, & Grande, 2012). There is a report that if polar solvents are concerned the main interaction may occur between microwave and polar molecules of the solvent. Energy transfer is from the solvent molecules to the reaction mixtures and the reactants, and it would be expected that any specific microwave effects on the reactants would be masked by solvent absorption of the field. The reaction rates should therefore be nearly the same as those under conventional heating (Perreux & Loupy, 2001). But the results of this study showed that the reaction rate under microwave was faster than that under conventional heating and that solvent with higher dielectric constant had higher reaction rate. And considering that the reaction system including not only aprotic solvent DMSO, but also reactants and catalyst, all of them can also interact with microwave, it is deduced that microwave accelerates the copolymerization and improves the lactide conversion and yield of PL might not only ascribe to its high energy transformation but also to some specific microwave effects for the synthesis of PL. And microwave irradiation raises the temperature of the whole reaction volume simultaneously, whereas in the oil heated tube, the reaction mixture in contact with the vessel wall is heated first (Schanche, 2003). Homogeneous nature of microwave heating can eliminate local overheating at the reaction walls, which lead to side products. Therefore, microwave-assisted reactions can

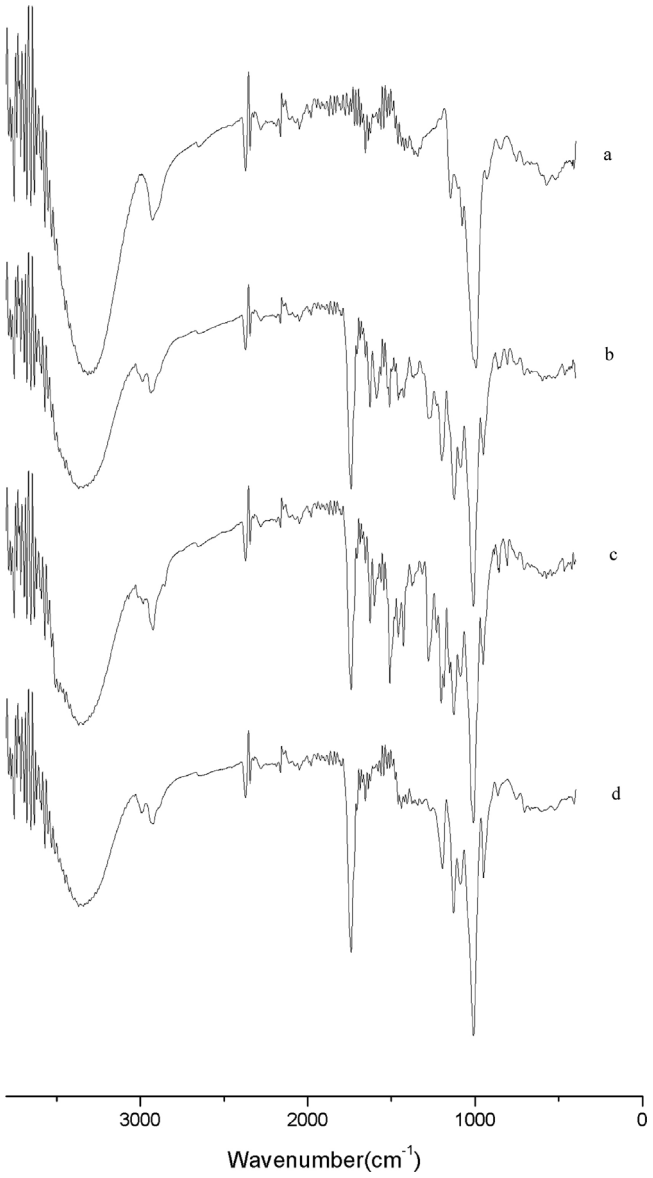


Fig. 3. FT-IR spectra of pullulan (a), PL6 (b), PL8 (c) and PL9 (d), respectively.

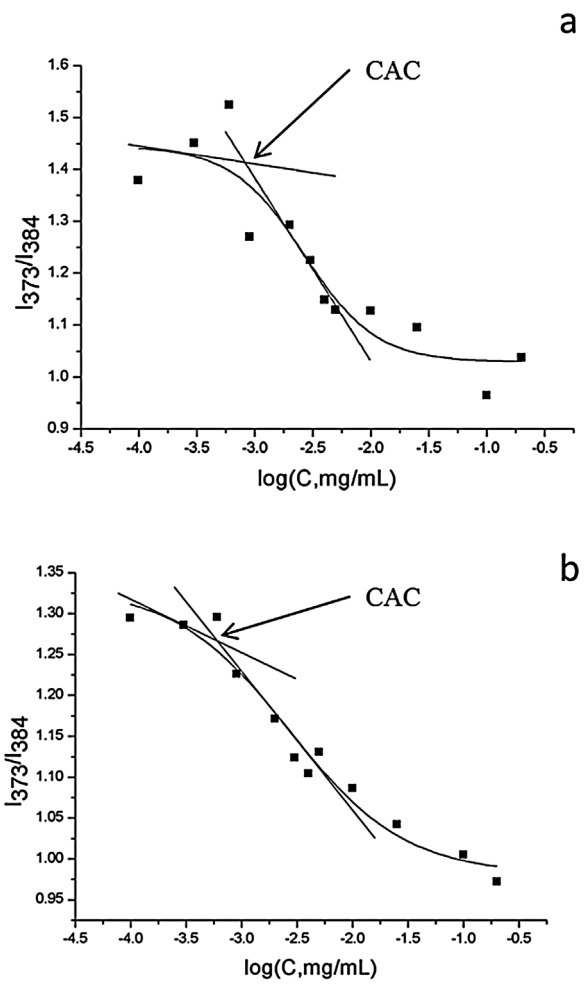


Fig. 4. I_{373}/I_{384} ratios from pyrene emission spectra as a function of CAC in aqueous solution. The CAC of PL8 (a) and PL9 (b) were obtained from the intersection of the two tangent lines shown by the arrows.

not only increase the reaction rate, but also provide higher purity and higher yields (Nikolic et al., 2010).

3.2. Characterization of PL

Three samples, PL 8, 9 and 6 were discussed in detail because they had interesting and representative properties. Unlike the parent polysaccharide, pullulan, these three samples were soluble in methanol and PL 8 and 9 were still soluble in water, while PL 6 was no longer soluble in water. All of them were insoluble in chloroform and dichloromethane.

3.2.1. NMR studies

The ^{1}H NMR of pullulan and PL in DMSO-d_6 were shown in Fig. 2. Typically distinctive peaks of pullulan appeared at 3.6–3.9, 4.5–5.0 and 5.3–5.6 ppm, as shown in Fig. 2(a). Upon the grafting of PLA on to pullulan, new peaks relevant to PLA appeared at 1.5, 4.2 and 5.2 ppm, as shown in Fig. 2(b–d). The appearance of a doublet at 1.5 ppm was indicative of the methyl group of the lactide moiety. The methine proton for the lactide was normally found in this region as a quartet. The appearance at 4.2 and 5.2 ppm were indicative of the lactide CH-end group and lactide central CH-groups (Cho et al., 2009; Donabedian & McCarthy, 1998; Wittmar, 2004). These results indicated that the PL graft copolymers contained PLA side chains. To determine the DS of these polymers, integration at 1.3–1.5 ppm was compared to integration at 5.0–5.5 ppm. The DS for three derivatives ranged from 0.9 to 1.1 per residue. The characteristic peaks observed at 1.66 and 5.09 ppm of monomer (D,L-lactide) were not observed in Fig. 2(b–d), indicating that the unreacted monomer in the product was not remained.

3.2.2. FT-IR analysis

The FT-IR spectroscopy of pullulan and the series of PL were shown in Fig. 3. There was a decrease in the OH stretch at 3400 cm^{-1}, along with a strong absorption band in the region 1745–1749 cm^{-1} indicative of carbonyl absorption of an ester group. It was reported that the carbonyl absorption of the pure PLA was generally observed at 1759 cm^{-1} (Kricheldorf & Serra, 1985), which was not observed in Fig. 3(b–d), thus indicating that the self-polymerized PLA did not remain. By comparing the spectra of pullulan and PL, it was verified the successful synthesis of the PL copolymers.

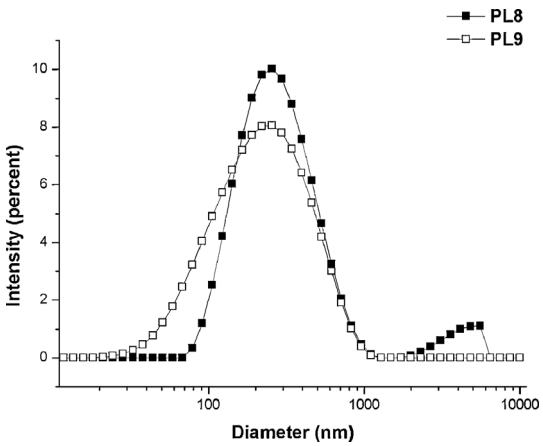


Fig. 5. Particle size distributions of PL nanoparticles determined by dynamic light scattering at 25 °C.

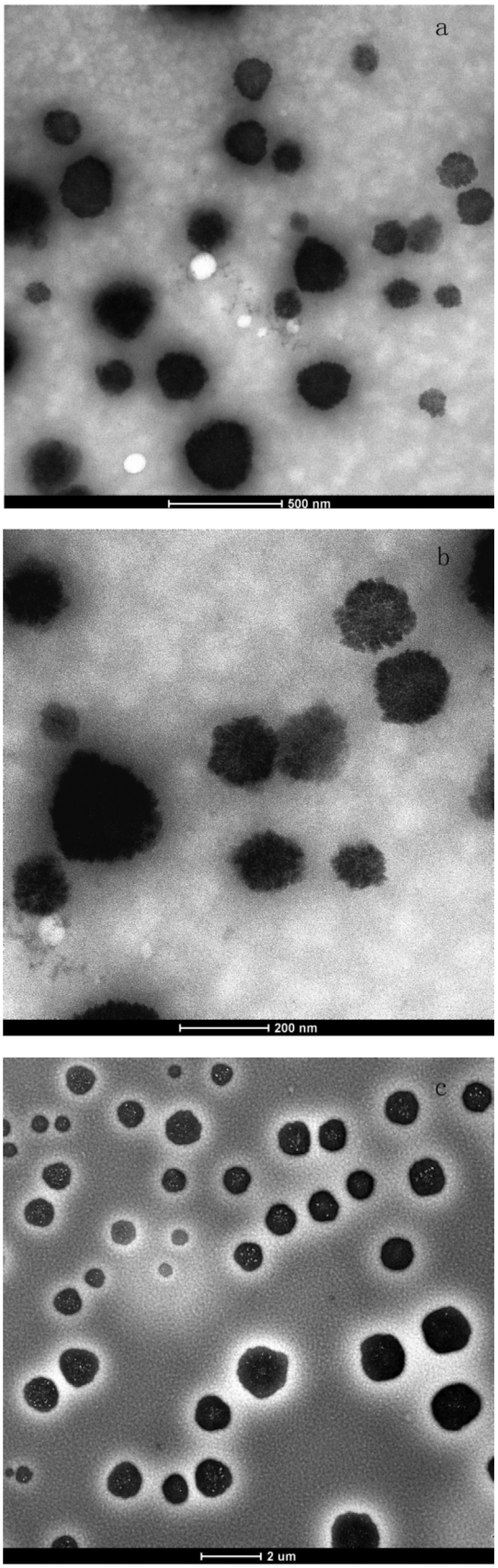


Fig. 6. TEM micrographs of PL8 (a), PL9 (b) nanoparticles and PL6 (c) microparticles.

3.2.3. Critical aggregation concentration of PL in aqueous medium

Pyrene as a fluorescence probe was used to characterize the self-organizing behavior of PL. As the concentrations of the polymer solution increased, increase of the total fluorescence intensity of pyrene in the excitation spectra was observed, which showed that the pyrene was transferred from the aqueous polar environment to the less polar micellar domain and suggested the self-assembly of the PL copolymer in aqueous media. The hydrophilic pullulan domain formed a flexible shell and the hydrophobic PLA domain formed the rigid inner core of self-assembling nanoparticles in the aqueous environment. The intensity ratios of I_{384}/I_{373} versus log C of PL copolymer for the pyrene emission spectra were shown in Fig. 4. There were a flat region and a sigmoid change in the crossover region at low concentrations, indicating that the signal change in the crossover region which could be related to the CAC value of PL copolymer. The CAC values were 7.9 and 5.8 mg/L for PL8 and PL9 respectively. The low CAC value of the PL as compared with low molecular weight surfactants could be one of the important characteristics of polymeric amphiphiles suggesting the stability of self-aggregates in the dilute condition (Lee, Kwon, Kim, Jo, & Jeong, 1998). This stability may be explained by the increased hydrophobic interaction and rigidity of PLA chains in PL as a function of the hydrophobic segment (Cho et al., 2009). The results showed that PL9, the DS of which was relatively higher than PL8, had a smaller CAC and indicated that micelle formed from PL9 with higher DS was more stable than that formed from PL8. So the PL with relative high DS may be better for drug delivery.

3.3. Preparation and characterization of nanoparticles from PL

The nanoparticles were prepared via the thin-film hydration method using PL copolymers with relative low substitution degree (PL 8 and 9). The hydrodynamic diameters of the nanoparticles and their distribution in an aqueous environment were analyzed using DLS technology at 25 °C. As shown in Fig. 5, the diameters have been found to be 215.8 nm and 173.6 nm for nanoparticles respectively from PL8 and PL9. The polydispersity indices of nanoparticles were 0.391 and 0.294, indicating a narrow size distribution for nanoparticles. With the increase of DS, there was a decrease in the nanoparticles sizes which was consistent with the report proposing that higher lactide contents provided a better chance for hydrophobic interaction between the lactide portions of the PL copolymers in the interior structure, thus resulting in a squeezing of nanoparticles size (Cho et al., 2009).

The morphology of the PL nanoparticles has been observed by TEM (Fig. 6(a and b)). It showed that with low polydispersity spherical nanoparticles are formed. The solubility of curcumin, completely insoluble in water (Mohanty & Sahoo, 2010), was improved to 1.97 mg mL^{-1} by the curcumin nanoparticles.

3.4. Preparation and characterization of microparticles from PL

Microparticles were made from PL 6 with relative high DS by solvent precipitation. The morphology of the PL microparticles had been observed by TEM. TEM micrographs showed the microparticles were regularly spherical and with particle size around 2 μm (Fig. 6(c)). PL microparticles were successfully applied to load curumin.

4. Conclusions

In this study, we developed a new method, microwave-assisted synthesis, to synthetize PL, with different characteristics and applications, rapidly and effectively. Compared with conventional oil heating, this method showed not only rate enhancement but also benign conditions. PL with different hydrophobic–hydrophilic balance was successfully applied to load anti-cancer drug curcumin as the forms of nanoparticles and microparticles. Further investigations, thermo-stimuli drug release and liver target, are in progress.

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