

# Synthesis of Poly(D,L-lactic acid) Modified by Cholic Acid via Direct Melt Copolycondensation and Its Characterization

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**ABSTRACT:** From D,L-lactic acid and the natural functional molecule cholic acid (CA), the biodegradable material poly(D,L-lactide–cholate) was synthesized via direct copolycondensation. For the CA/lactic acid (LA) molar feed ratio of 1/64, the optimal synthesis conditions were as follows: a prepolymerization time of 8 h, 0.3 wt % SnO catalyst, and melt copolycondensation for 8 h at 160°C, which gave a novel star-shaped poly(D,L-lactic acid) (PDLLA) modified by CA with the maximum weight-average molecular weight of 5600 Da at a yield of 51.9%. The copolymer poly(D,L-lactide–cholate) at different molar feed ratios was characterized by Fourier transform infrared spectroscopy, proton nuclear magnetic resonance, gel permeation chromatography, differential scanning calorimetry, thermogravimetry, and X-ray diffraction. Decreasing the molar feed ratio of CA/LA from 1/15 to 1/128 reduced the average number of CA units embedded in the copolymer from 4 to 1. With 1/15 CA/LA, the copolymer was not a star-shaped polymer, and its weight-average

molecular weight was the biggest (weight-average molecular weight = 12,700 Da, weight-average molecular weight/number-average molecular weight = 1.68). With 1/32 CA/LA, the copolymer with two CA units was not a star-shaped polymer either. With 1/64, 1/100, and 1/128 CA/LA, the copolymer mainly had one CA unit core embedded as a normal star-shaped PDLLA with four arms, and certain crystallinity could be detected. The novel direct copolycondensation method was simple and practical for the synthesis of the asymmetrical star-shaped PDLLA material, and it was advantageous for this PDLLA material embedded in the special bioactive molecule CA to be applied in the field of drug delivery and tissue engineering. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 117: 1405–1415, 2010

**Key words:** biodegradable; copolymerization; drug delivery systems; polycondensation; synthesis

## INTRODUCTION

As a kind of important biodegradable aliphatic polyester, poly(lactic acid) (PLA) is wholly environmentally friendly. Its excellent biocompatibility and biological resorbability afford it extensive applications in biomedical fields, including sutures, bone fixation materials, drug-delivery microspheres, and tissue engineering.<sup>1–6</sup> To improve the performance of PLA, especially to promote its cell adhesion, many PLA copolymers, such as poly(lactic acid–glycolic acid),<sup>7</sup> PLA–poly(ethylene glycol),<sup>8</sup> PLA–poly(phosphate ester),<sup>9</sup> and poly(lactic acid–amino

acid),<sup>10</sup> have been developed via the copolymerization of other functional molecules with lactic acid (LA) or lactide.

Cholic acid (CA), a naturally occurring chiral biomolecule with important biological activity and high biocompatibility, has been applied in the synthesis of polymeric biomaterial,<sup>11</sup> including PLA copolymers.<sup>12,13</sup> CA-functionalized star-shaped oligo/poly(D,L-lactide)s with different molecular weights have been applied in drug-release microspheres and tissue engineering.<sup>12,13</sup> However, the ring-opening polymerization of D,L-lactide initiated by CA uses only its three OH groups in the reaction and gives a star-shaped poly(D,L-lactic acid) (PDLLA) with three arms. More importantly, lactide is often prepared from LA through a troublesome and low-yield process, and the purification of lactide by repetitious crystallization consumes large amounts of organic solvents. Higher molecular weight polymers require a higher purity lactide and increasing numbers of crystallization times.<sup>1</sup>

With the advent of the direct synthesis of PLA with LA as a starting material,<sup>2–4,14–22</sup> increasing

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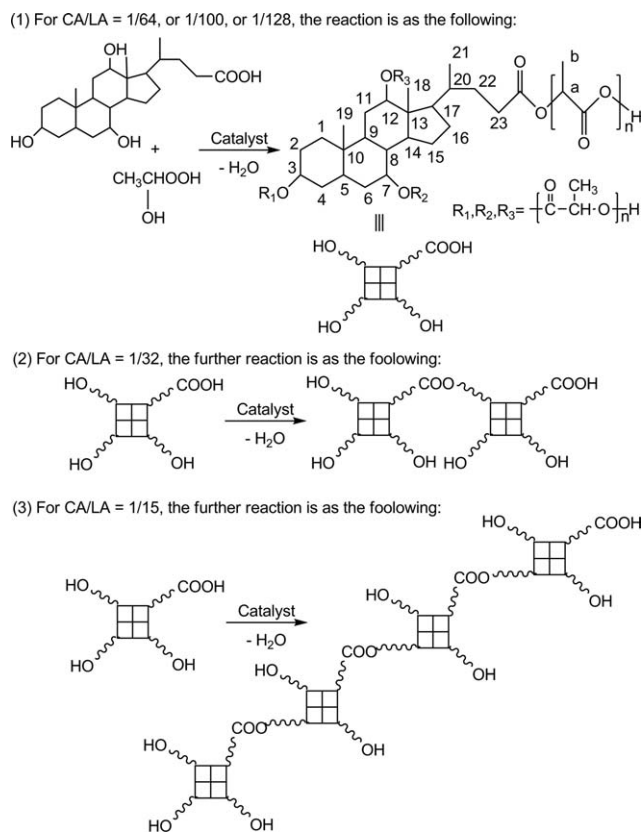
importance has been attached to the direct copolycondensation of LA, a novel one-step method, instead of the traditional two-step method that uses lactide as an intermediate.<sup>7,8,10,23–28</sup> Therefore, it is necessary to investigate the direct synthesis of the biodegradable material copolymer poly(D,L-lactide-cholate) to reduce its synthesis costs and extend its application. Furthermore, when CA is copolymerized with LA, both OH groups and COOH groups could be used in the reaction, which would probably yield a star-shaped PDLLA with four arms and thereby increase the weight-average molecular weight ( $M_w$ ) of the polymer.<sup>29–31</sup> At the same time, because star-shaped polymers have typically been synthesized from symmetrical cores or at least cores with the same reactive groups, such as pentaerythritol<sup>29</sup> and hydroxyl-terminated cyclotriphosphazene,<sup>30</sup> glycerol,<sup>31</sup> new investigation would provide an important method for the synthesis of a novel, asymmetric star-shaped polymer, especially with an asymmetrical core with different reactive groups.

Thus, in this article, on the basis of our previous work on the simple direct synthesis of PDLLA,<sup>2,4</sup> poly(lactic acid–glycolic acid),<sup>7</sup> PLA–poly(ethylene glycol),<sup>8</sup> and poly(lactic acid–amino acid)<sup>10</sup> via melt polycondensation and the application of PDLLA in drug delivery,<sup>2,4</sup> inexpensive D,L-lactic acid (D,L-LA) and CA were used as starting materials for copolycondensation, and a novel biodegradable material, PDLLA modified by CA, was synthesized (Scheme 1). The structure of the copolymer, relative molecular weight and its distribution, thermal properties, and crystallinity were characterized by Fourier transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectroscopy, gel permeation chromatography (GPC), differential scanning calorimetry (DSC), thermogravimetry (TG), and X-ray diffraction (XRD), respectively. The optimal synthetic conditions for a star-shaped PDLLA with four arms, including the prepolymerization time, catalyst type and quantity, polymerization time, and temperature and the influences of different molar feed ratios are discussed in detail later.

## EXPERIMENTAL

### Materials

D,L-LA, and SnO were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). CA was purchased from Alfa Aesar China Co., Ltd. (Tianjin, China). Other chemical reagents, including CHCl<sub>3</sub> and CH<sub>3</sub>OH, were purchased from Tianjin Damao Chemical Factory (Tianjin, China). All of these materials were analytical reagent grade and were used without further purification.



Scheme 1 Synthesis route of poly(D,L-lactide-cholate).

### Instrumental analysis and measurements

<sup>1</sup>H-NMR spectra were recorded with a DRX-400 NMR spectrometer (Bruker Instruments, Billerica, MA) with CDCl<sub>3</sub> as the solvent and an internal standard. IR spectra were obtained from an FTIR spectrometer (Bruker Vector 33, Ettlingen, Germany) by the KBr salt slice method.

The intrinsic viscosity ( $[\eta]$ ) of PDDL A modified by CA was determined with an Ubbelohde viscometer (Cannon-Ubbelohde, State College, PA) with CHCl<sub>3</sub> as the solvent at 25°C. According to the literature,<sup>32–34</sup> the relative molecular weight and molecular weight distribution of modified PLA were determined by GPC (Waters 1515 pump, Torrance, CA) with tetrahydrofuran as the solvent and with polystyrene as the reference at 40°C and with a flow velocity of 1 mL/min.

As an important comparative reference, the molecular weights were also tested via the titration method (TM).<sup>35</sup> In a typical procedure, the sample was dissolved in CHCl<sub>3</sub> (20 mL) at 25°C. With phenolphthalein as the indicator, the solution was titrated by 0.01 mol/L standard alcoholic potash. When the solution became red and the color did not fade within 30 s, it had reached the titration end point. After blank titration, the number-average molecular weight ( $M_n$ ) was calculated with the following equation:

$$M_n = [(m \times 1000)]/[0.01 \times (V_b - V_a)] \quad (1)$$

where  $m$ ,  $V_b$ , and  $V_a$  are the sample weight (g), actual volume of standard alcoholic potash titrated to the sample (mL), and volume of blank analysis (mL), respectively.

DSC and TG were performed with a PerkinElmer DSC7 thermal analyzer (PerkinElmer, Cetus Instruments, Norwalk, CT) at a heating rate of 10°C/min under a nitrogen atmosphere (flow velocity = 20 mL/min). The crystallinity of PDLLA modified by CA was investigated on a Rigaku D/max-1200X X-ray diffractometer (Rigaku Co., Tokyo) with Cu K $\alpha$  radiation with a wavelength of  $1.5406 \times 10^{-10}$  m, and a scanning range of  $2\theta = 1\text{--}40^\circ$  at a scanning speed of 0.3°/min.

### Prepolymerization

According to previous work on the melt homopolymerization or copolymerization of LA,<sup>2,4,7,8,10,15</sup> LA and CA should be prepolymerized before copolymerization. After LA and CA were uniformly mixed at a preplanned molar feed ratio, the mixture was directly dehydrated for a preplanned time at 140°C under 4000 Pa in a flask equipped with a mechanical stirrer and thermometer.

### Melt copolymerization

After prepolymerization, the selected catalyst was added in according to the weight percentage of the dehydrated reactants. The melt copolymerization was carried out at a certain temperature (130–180°C) and at an absolute pressure of 70 Pa for 2–10 h. When the reaction finished, purification via dissolution in CHCl<sub>3</sub> and subsequent precipitation by CH<sub>3</sub>OH/H<sub>2</sub>O (or CH<sub>3</sub>COCH<sub>3</sub>/H<sub>2</sub>O) ordinarily produced a white powder after drying *in vacuo*.

## RESULTS AND DISCUSSION

For the direct synthesis of poly(D,L-lactide–cholate), both the reactive groups OH and COOH of CA took part in the copolycondensation with LA to produce

a star-shaped polymer with four arms, but sometimes, they possibly produced a copolymer with multiple CA units. Therefore, it was necessary to evaluate the influences of the synthetic conditions and molar feed ratio through the characterization of the resulting copolymer by  $[\eta]$ , FTIR, <sup>1</sup>H-NMR, GPC, DSC, TG, and XRD.

### Influence of different synthetic conditions

According to Moon et al.'s<sup>15</sup> report, during the melt polymerization of LA, prepolymerization was very important for dehydration. The influences of different prepolymerization times on the reaction are shown as Table I. For the prepolymerization times between 7 and 9 h,  $[\eta]$  was the largest of all runs. When the prepolymerization time was less than 7 h, the prepolymerization was insufficient. Once the time was more than 9 h, the total polycondensation time was too long to obtain a larger  $[\eta]$  because of the thermal decomposition of the product. Thus, for the following experiments, the prepolymerization time was maintained at 8 h.

The catalyst is key for the direct melt polycondensation of LA, and usually, tin catalysts give the best effects for their good dispersability in the reaction system.<sup>15–17</sup> In our experiments, the influences of different catalysts on the reaction are shown in Table II, and the results were similar to those in the reported literature.<sup>2,7,8,15–17</sup> Although the reaction catalyzed by *p*-toluenesulfonic acid (TSA) gave the biggest yield (76.8%),  $[\eta]$  was smaller than that in the reaction catalyzed SnO. Thus, SnO was selected for the following experiments as the catalyst most likely to generate a bigger molecular weight.

The influences of different catalyst quantities on the reaction are also shown in Table II. Usually, metallic catalysts, including SnO, have double side effects: they accelerate polymerization, but they also catalyze the thermal decomposition of the product.<sup>2,7,8</sup> Therefore, neither too much nor too little are suitable for the catalyst concentration, and for this reaction, the best quantity of SnO was 0.3%.

The influences of different melt polymerization temperatures on the reaction are shown in Table III.

TABLE I  
Influence of Different Prepolymerization Times on the Reaction

Run	Prepolymerization time (h)	Appearance of product	Yield (%)	$[\eta]$ (dL/g)
1	3	Off-white, viscous solid	51.9	0.46
2	5	Yellowish solid	54.0	0.55
3	7	Yellowish solid	54.0	0.87
4	9	Yellowish powder	41.5	0.87
5	11	Yellowish, viscous solid	37.4	0.44

All runs were polymerized with a CA/LA molar feed ratio of 1/64, a prepolymerization temperature of 140°C, a polycondensation temperature of 160°C, a polycondensation time of 8 h, and 0.3% SnCl<sub>2</sub> as the catalyst.

**TABLE II**  
Influence of the Catalyst on the Reaction

Run	Catalyst	Catalyst quantity (%)	Appearance of product	Yield (%)	$[\eta]$ (dL/g)
1	SnCl <sub>2</sub>	0.3	Yellowish, viscous solid	53.9	0.87
2	SnO	0.3	Yellowish, viscous solid	53.2	1.16
3	ZnO	0.3	Yellowish, viscous solid	64.3	0.58
4	ZnCl <sub>2</sub>	0.3	Yellowish, viscous solid	56.0	0.70
5	TSA	0.3	Yellowish powder	76.8	0.92
6	SnO	0.1	Yellowish, viscous solid	56.0	0.51
7	SnO	0.5	Yellowish, viscous solid	60.2	0.95
8	SnO	0.7	White, viscous solid	56.0	0.90
9	SnO	0.9	Yellowish, viscous solid	41.5	0.85

All runs were polymerized with a CA/LA molar feed ratio of 1/64, a prepolymerization time of 8 h, a prepolymerization temperature of 140°C, a polycondensation time of 8 h, and a polycondensation temperature of 160°C.

When the temperature was 130°C, the lower temperature was disadvantageous for polycondensation to remove the produced water from the reaction system, and the reaction gave a viscous product with the smallest  $[\eta]$ . Increasing the temperature to 160°C yielded the biggest  $[\eta]$  (1.25 dL/g), and the product was a white powder. However, at higher temperature, the  $[\eta]$  decreased, and the product even became brown because of side reactions, such as thermal decomposition and oxidation (Table III, run 6). Thus, the most suitable temperature was 160°C.

The influences of different melt polymerization times on the reaction are also shown in Table III. Obviously, the most suitable time was 8 h. The reason was similar to that discussed previously, mainly because of the influence of the balance between the polycondensation and thermal decomposition. Therefore, when the molar feed ratio of CA/LA was 1/64, the optimal synthetic conditions were as follows: a prepolymerization time of 8 h, the catalyst SnO at 0.3%, and the melt copolycondensation at 160°C for 8 h.

### Structural characterization of poly(D,L-lactide-cholate)

Under the previously discussed optimal synthetic conditions, the structure of PDLLA modified by CA

was characterized by FTIR and <sup>1</sup>H-NMR (Fig. 1). Combined with the results from GPC, we confirmed that, when the molar feed ratio of CA/LA was 1/64, the novel copolymer had only one CA unit embedded as the core, and it was indeed a star-shaped PDLLA with four arms [Scheme 1(1)].

FTIR (KBr,  $\nu$ , cm<sup>-1</sup>): 1186.2, 1089.8 (strong, absorption of C—O—C); 1758.0 (strong, absorption of C=O); 2997.4 (weak, absorption of saturated C—H). <sup>1</sup>H-NMR (CDCl<sub>3</sub> as the solvent with a chemical shift of 7.28 as the internal standard,  $\delta$ , ppm): 0.81–0.91 (*m*, H<sub>18</sub>, H<sub>19</sub>, H<sub>21</sub>), 1.49–1.64 (*m*, H<sub>b</sub> and other H in the CA unit), 2.14 (*t*, H<sub>23</sub>), 4.33–4.39 (*m*, H<sub>3</sub>, H<sub>7</sub>, H<sub>12</sub>), 5.00–5.30 (*m*, H<sub>a</sub>).

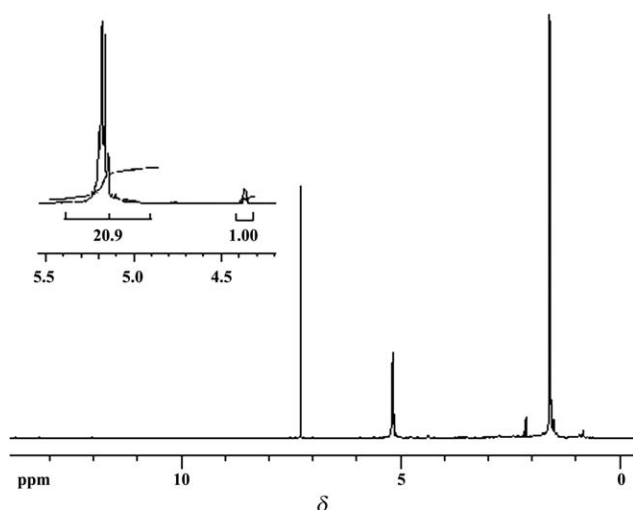
These data indicated that the structure of polymer was that illustrated in Scheme 1(1).

The ratio of H atom number (H<sub>3</sub>, H<sub>7</sub>, and H<sub>12</sub> to H<sub>a</sub>) was calculated from the ratio of the H peak integral at 4.33–4.39 to that at 5.00–5.30, and the data was about 1/21 (Fig. 1). Thus, the composition ratio of the structural unit (CA/LA) was 1/63. When the copolymer poly(D,L-lactide-cholate) only had one CA core, its molecular weight ( $M_n$  tested by <sup>1</sup>H-NMR) should have been as follows: 408 (formula weight of CA unit) + 72 (formula weight of LA chain unit)  $\times$  63 = 4944 Da, or approximately 5000 Da.

**TABLE III**  
Influence of Different Melt Polymerization Temperatures and Times on the Reaction

Run	Temperature (°C)	Time (h)	Appearance of product	Yield (%)	$[\eta]$ (dL/g)
1	130	8	Yellowish, viscous solid	60.2	0.73
2	140	8	Yellowish, viscous solid	53.2	1.16
3	150	8	White powder	68.5	0.87
4	160	8	White powder	51.9	1.25
5	170	8	White powder	62.2	1.03
6	180	8	Brown powder	41.5	1.00
7	160	2	Off-white, viscous solid	58.1	0.95
8	160	4	White powder	53.2	1.14
9	160	6	White powder	58.1	1.18
10	160	10	White powder	64.3	1.23

All runs were polymerized with a CA/LA molar feed ratio of 1/64, a prepolymerization time of 8 h, a prepolymerization temperature of 140°C, and 0.3% SnO as the catalyst.



**Figure 1**  $^1\text{H-NMR}$  spectrum of poly(D,L-lactide-cholate) synthesized with the CA/LA molar feed ratio of 1/64.

Similarly, when the CA/LA molar feed ratio was 1/64, when it only had one CA core, the copolymer poly(D,L-lactide-cholate) should only have had a terminal COOH because of the structure of the CA core [Scheme 1(1)]. Therefore,  $M_n$  was tested by TM.<sup>35</sup>  $M_n$  by TM was about 4300 Da, which was very close to the  $M_n$  determined by GPC.

#### GPC characterization of poly(D,L-lactide-cholate)

As a relative reference, the molecular weight and molecular weight distribution of PDLLA modified by CA were characterized by GPC according to the reports on star-shaped PLA,<sup>32–34</sup> although the copolymer and standard (polystyrene) had different structures and probably different conformations in solution.

When the CA/LA molar feed ratio was 1/64, the GPC flow curve of the product had a single symmetrical peak.  $M_w$  was 5600 Da, and the polydispersity index ( $\text{PDI} = M_w/M_n$ ) was 1.37, which was less than 2 but slightly bigger than that of PDLLA ( $\text{PDI} = 1.25$ ,  $M_w = 17,800$  Da, and  $M_n = 14,200$  Da).<sup>4</sup> The single peak of the GPC curve and lower PDI indicated that the direct copolycondensation of LA and

CA indeed only gave the copolymer poly(D,L-lactide-cholate) and that no homopolymer was produced.<sup>7,8,10</sup>

When PLA biodegradable materials were applied to drug delivery, poly(lactic acid-glycolic acid) with a molecular weight greater than 900 Da was reported,<sup>36,37</sup> and it has appeared that a suitable  $M_w$  for PLAs should be in the range 1800–30,000 Da.<sup>2,4,38</sup> In this study, all poly(D,L-lactide-cholate)s directly synthesized were within the desired  $M_w$  range (when the molar feed ratio was different, and the  $M_w$  is shown in Table IV). At the same time, the biodegradable material poly(D,L-lactide-cholate) had a special affinity to cells; the use of PDLLA modified by CA as drug-delivery material was advantageous for the improvement of the medicinal effect.<sup>12,13</sup> Therefore, the direct synthesis of poly(D,L-lactide-cholate) is practically valuable.

Combining the  $M_n$  determined by  $^1\text{H-NMR}$  with the  $M_n$  determined by GPC (4100 Da) and the  $M_n$  by TM (4300 Da), we calculated the average number of CA units in the polymer:  $4100/5000$  (or  $4300/5000$ )  $\approx 1$ . These indicated that, when the molar feed ratio of CA/LA was 1/64, the copolymer synthesized may have just contained a CA unit as the core. Therefore, in the previous experiments on the investigation of the optimal synthetic conditions for the star-shaped PDLLA with four arms, the use of the CA/LA molar feed ratio of 1/64 as an example was very suitable.

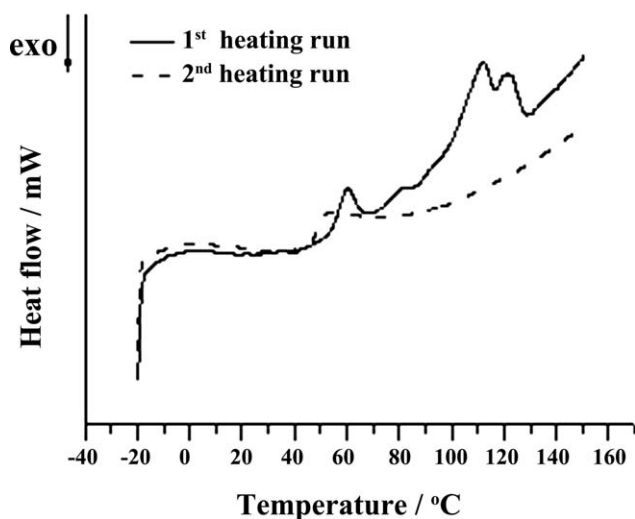
#### Thermal properties and crystallinity of poly(D,L-lactide-cholate)

The thermal properties of PDLLA modified by CA were characterized by DSC and TG. When the molar feed ratio of CA/LA was 1/64, the DSC curve of the polymer was shown in Figure 2. In the first heating run curve, it was obvious that the glass-transition temperature ( $T_g$ ) was between 40.7 and 67.7°C, and a double-head endothermal peak was found between 84.6 and 130.4°C. These indicated that two aggregate morphologies, especially different crystalline forms,<sup>39–41</sup> coexisted in the copolymer [LA had a

**TABLE IV**  
Influence of Different Molar Feed Ratios on the Reaction

Run	Feed molar ratio (CA/LA)	Appearance of product	Yield (%)	$[\eta]$ (dL/g)	Retention time (min)	$M_n$ (Da)	$M_w$ (Da)	$M_w/M_n$
1	1/15	Off-white powder	47.5	1.30	25.139	7,500	12,700	1.69
2	1/32	Off-white powder	50.8	0.87	26.783	3,300	4,500	1.36
3	1/64	White powder	51.9	1.25	26.374	4,100	5,600	1.37
4	1/100	White powder	45.3	1.34	27.176	3,300	4,700	1.42
5	1/128	White powder	41.6	1.40	27.086	3,300	4,400	1.31

All runs were polymerized with a prepolymerization time of 8 h, a prepolymerization temperature of 140°C, a polycondensation time of 8 h, a polycondensation temperature of 160°C, and 0.3% SnO as the catalyst.

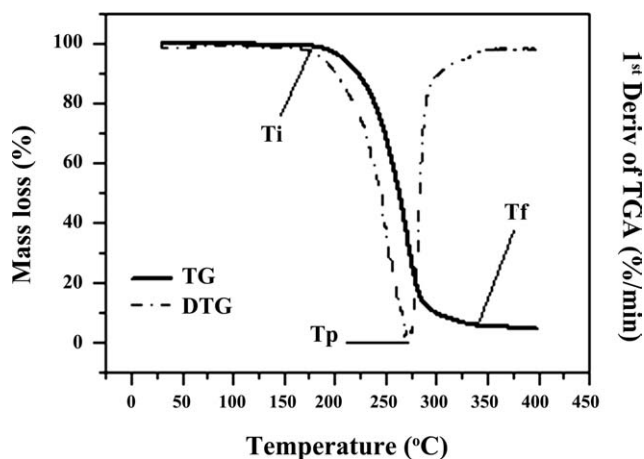


**Figure 2** DSC curves of poly(D,L-lactide-cholate) synthesized with the CA/LA molar feed ratio of 1/64.

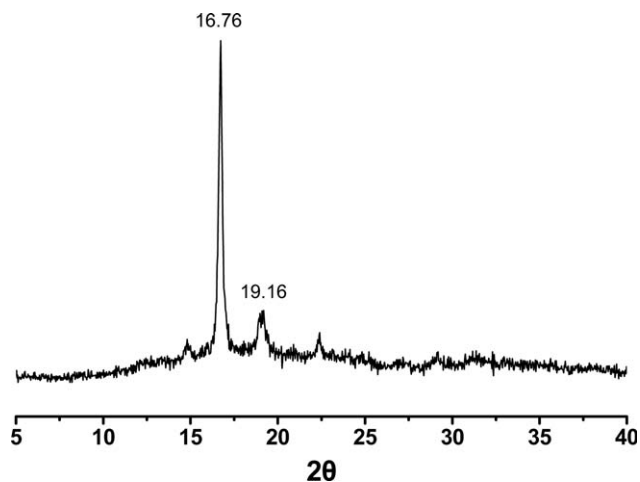
chiral center like 3-hydroxybutyric acid; therefore, the DSC curves of PLA may have had similar phenomena, including the double-head endothermal peak to the DSC curves of poly(3-hydroxybutyrate)].<sup>42–44</sup> However, after the second heating run, there was only a glass transition, which indicated that the certain crystalline copolymer wholly became amorphous.

The TG and differential thermogravimetry (DTG) curves for the product are shown in Figure 3. There was a single and acute thermal decomposition peak in the DTG curve. This indicated that, once the thermal decomposition started, the entire polymeric chain instantaneously split into small molecular fragments.<sup>45</sup>

The certain crystallinity of PDLLA modified by CA was further characterized by XRD. When the



**Figure 3** TG and DTG curves of poly(D,L-lactide-cholate) synthesized with the CA/LA molar feed ratio of 1/64 ( $T_f$  = final temperature; TGA = thermogravimetric analysis;  $T_i$  = initial temperature;  $T_p$  = polymerization temperature.).



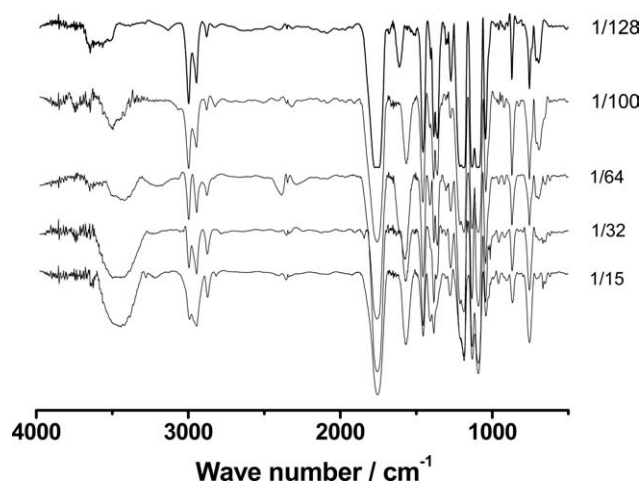
**Figure 4** XRD spectrum of poly(D,L-lactide-cholate) synthesized with the CA/LA molar feed ratio of 1/64.

molar feed ratio of CA/LA was 1/64, the XRD scan for the polymer is shown in Figure 4. It was determined that diffraction peaks existed at  $2\theta = 16.8$  and  $19.2^\circ$ . Calculations gave the crystallinity (18.7%) and the crystallite size ( $D_{110} = 208.9 \times 10^{-10}$  m). Compared with PDLLA (crystallinity = 20.8%,  $D_{110} = 154.4 \times 10^{-10}$  m),<sup>4</sup> the peak positions were similar, and the crystallinity decreased, but the crystallite size increased. These changes probably resulted from the formation of a star-shaped structure. However, when the molar feed ratio was different, the polymer properties may have been different, and the copolymer may not have been a star-shaped PDLLA with four arms again for the existence of multiple CA units per molecule for certain molar feed ratios.

#### Influence of different molar feed ratios on $[\eta]$ and $M_w$

Under the optimal synthetic conditions described previously, the influences of different molar feed ratios on  $[\eta]$ , yield, and  $M_w$  are provided in Table IV. The yield remained close to 50% for all of the molar feed ratios investigated. The GPC results indicate that  $M_w$  did not increase with increasing  $[\eta]$ ; this implied that the molar feed ratio influenced the microstructure of the CA-modified PDLLA. For the star-shaped polymer, including the star-shaped PLA,<sup>29–31,46–49</sup>  $[\eta]$  was smaller than that of the linear polymer with the same molecular weight; for the same  $[\eta]$ ,  $M_w$  for the star-shaped polymer was bigger than that of the corresponding linear polymer, and the polymer with more arms had a bigger  $M_w$ .

Therefore, although  $[\eta]$  for 1/32 CA/LA was markedly smaller than that for 1/100 or 1/128 CA/LA, the  $M_n$  values of the two polymers were close to each other. Combined with the previous analysis of the  $^1\text{H-NMR}$  and GPC results for 1/64 CA/LA,



**Figure 5** FTIR spectra of poly(D,L-lactide-cholate) synthesized with different molar feed ratios.

these indicated that for 1/32 CA/LA, the microstructure of the CA-modified PDLLA with multiple arms had more than one CA unit embedded. Thus, for 1/15 CA/LA, although the  $[\eta]$  was not the biggest of all, its  $M_w$  and  $M_n$  were the biggest of all (Table IV, run 1), only because the copolymer had the most embedded CA units [Scheme 1(3)]. This conclusion was further confirmed by the following series of characterizations.

#### Influence of different molar feed ratios on the structure

The variation of the molar feed ratios gave the IR spectra of the resulting polymers, which were basically similar to each other, and the terminal OH absorptions at  $3500\text{ cm}^{-1}$  were all weak (Fig. 5). However, for 1/15 or 1/32 CA/LA, the terminal OH absorption peaks were strengthened to a level similar to that of the weak C—H absorption peak near  $3500\text{ cm}^{-1}$ ; this indicated that, the polymer synthesized as the CA/LA molar feed ratio of 1/15 or 1/32 had relatively more terminal OH, as a result of having more CA units in the polymer molecule [Scheme 1(2,3)].

The chemical shift data of the polymers synthesized at different molar feed ratio were basically similar to each other, but the integral data were different.  $^1\text{H-NMR}$  analysis gave the estimated  $M_n$  and the number of CA units in the polymer; the results are shown in Table V. Compared with the results of GPC provided in Table IV, especially the  $M_n$  from GPC, when the molar feed ratios were 1/15, 1/32, 1/64, 1/100, and 1/128 in turn, the number of CA units in the copolymer molecule should have averaged 4, 2, 1, 1, and 1, respectively (Scheme 1).

This deduction also could be calculated from the  $M_n$  tested by TM (also provided in Table V). When there were multiple CA units per molecule for certain molar feed ratios, mutual esterification between the terminal COOH and OH of different arms made the polymer have only a terminal COOH left in the end [Scheme 1(2,3); the polymerization mechanism is proposed in detail in the following discussion]. Therefore, it was possible to test the  $M_n$  for the polymers with multiple CA units per molecule by the same TM. Also, on the basis of the results of TM, the previous conclusion was further confirmed.

However, this was only for the most microstructure of the polymer. For example, for  $\text{CA/LA} \leq 1/64$ , most copolymers contained a single CA unit, but the microstructure with multiple CA units still existed; only its content in all of the microstructure was decreased with the increase of LA feed. Therefore, as shown in Table IV, although  $[\eta]$  increased slightly,  $M_w$  and  $M_n$  were smaller.

In addition, Table V also shows that, for 1/64 CA/LA, the practical component molar ratio of CA/LA computed from the  $^1\text{H-NMR}$  test was 1/63 (Table V, run 3), which was the closest to the theoretical data of all of the molar feed ratios. This indicated the biggest reactant conversion, in accordance with the maximum yield (Table IV, run 3). In other cases, because not all CA reacted (Table V, run 1) or because LA left the reaction system as lactide, the yield was lower.

The influences of different molar feed ratios on the structure of the copolymers, especially the

**TABLE V**  
 **$^1\text{H-NMR}$  Test Results for Poly(D,L-lactide-cholate) Copolymers**

Run	CA/LA		$\text{H}_{3,7,12}/\text{H}_a$		$M_n$ from $^1\text{H-NMR}$ (Da) <sup>a</sup>				$M_n$ (Da)	
	Feed	Test	Feed	Test	1 CA unit	2 CA units	3 CA units	4 CA units	GPC	TM <sup>b</sup>
1	1/15	1/20	3/15	3/19.2	1,900	3,700	5,600	7,400	7,500	7,100
2	1/32	1/22	3/32	3/21.2	2,000	4,000	6,000	8,000	3,300	3,600
3	1/64	1/63	3/64	3/62.7	5,000	9,900	14,900	19,800	4,100	4,300
4	1/100	1/38	3/100	3/37.5	3,200	6,300	9,500	12,600	3,300	3,100
5	1/128	1/41	3/128	3/40.7	3,400	6,800	10,100	13,500	3,300	3,100

<sup>a</sup>When the number of CA units was different, different  $M_n$  values of  $^1\text{H-NMR}$  were estimated, and they are listed. All  $M_n$  values are based on 100 Da as the unit.

<sup>b</sup>All  $M_n$  values are based on 100 Da as the unit.

**TABLE VI**  
Influence of Different Feed Molar Ratios on  $T_g$  and  $T_m$

Run	Feed molar ratio (CA/LA)	$T_g$ (°C)	$T_{m1}$ (°C)	$T_{m2}$ (°C)
1	1/15	66.2	— <sup>a</sup>	— <sup>a</sup>
2	1/32	47.5	— <sup>a</sup>	— <sup>a</sup>
3	1/64	47.8	111.9	121.0
4	1/100	39.9	107.7	119.0
5	1/128	43.3	116.5	125.0
6	PDLLA <sup>b</sup>	54.6	111.3	120.0

<sup>a</sup> Not detected.

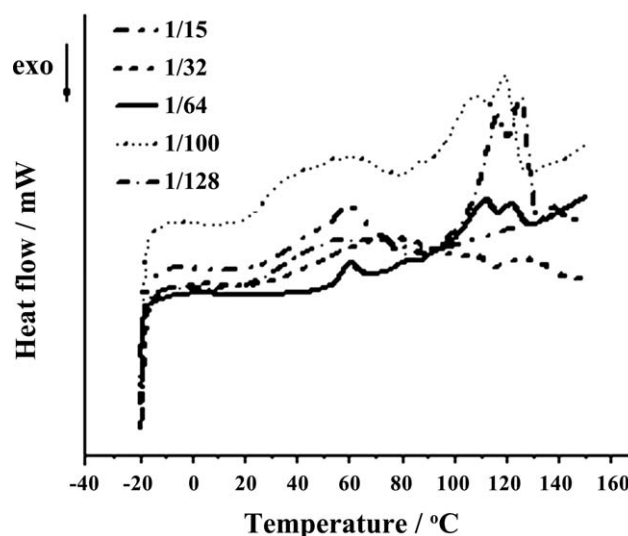
<sup>b</sup> Data from previous experiments.<sup>4</sup>

existence of multiple CA units per molecule for certain molar feed ratios (Scheme 1), were further confirmed in the following characterizations of DSC, TG, and XRD.

#### Influence of different molar feed ratios on the thermal properties

The  $T_g$  data and melting behavior of different poly(D,L-lactide-cholate) copolymers were investigated by DSC, and the results are shown in Table VI. Usually, the star-shaped PLA had a lower  $T_g$  than that of linear PLA,<sup>50</sup> but run 1 was an exception because of its microstructure [Scheme 1(3)]. For 1/15 CA/LA, the basic microstructure of the copolymer had about four CA units embedded. Because of the structure of CA, the normal star-shaped PDLLA modified by CA should have had only one CA unit core and four PDLLA chain arms: three arms terminated with OH and one terminated with COOH. Therefore, one more than one CA unit existed, and the arm terminated with OH could be easily reacted with another arm terminated with COOH via esterification. The more CA units per molecule there was, the greater was the chance for crosswise esterification. The crosswise esterified structure increased  $T_g$ ; even in the end, it was higher than that of linear PDLLA (Table VI, runs 1 and 6). However, for 1/32 CA/LA, the effect of the crosswise esterified structure was not very obvious for the less crosswise esterification [Scheme 1(2)], so its  $T_g$  data was still less than that of linear PDLLA and even similar to that of the case with 1/64 CA/LA (Table VI, runs 2, 3, and 6).

The influences of different molar feed ratios on the melting temperature ( $T_m$ ) of the copolymers are shown as Figure 6; the corresponding data are listed in Table VI. For 1/15 or 1/32 CA/LA, when there were multiple CA units per polymer molecule, the melting peak was not visible. On the contrary, when the copolymers mainly had one CA unit as the core, the melting peak was visible (a double-head melting peak probably indicated the existence of two distinct



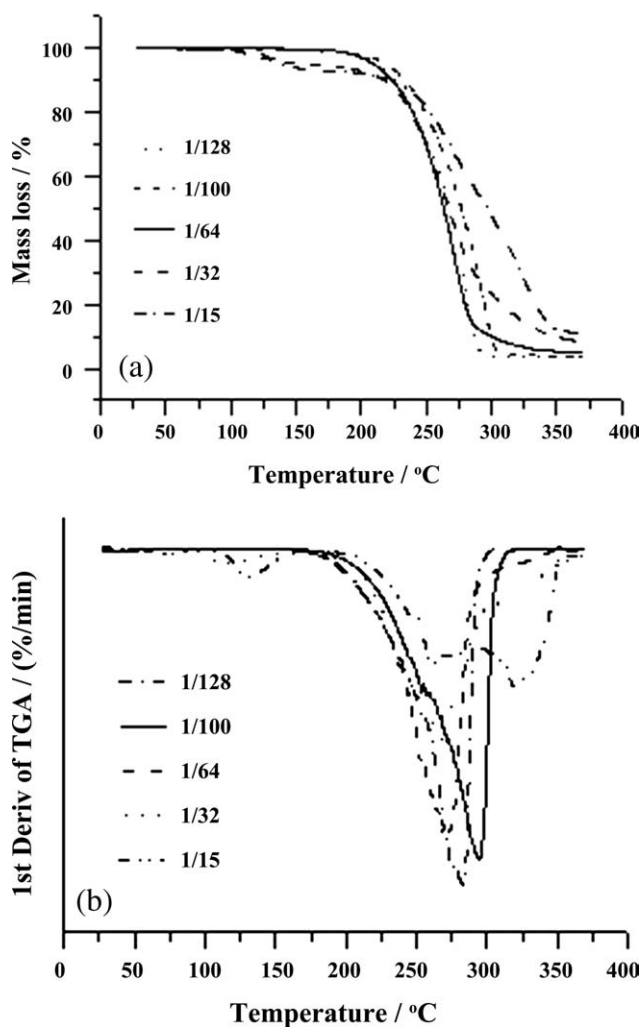
**Figure 6** DSC curves of poly(D,L-lactide-cholate) synthesized with different molar feed ratios.

crystalline morphologies, or it was a consequence of a complex interplay between melting and recrystallization),<sup>39–44,51</sup> and both the peak shape and the corresponding  $T_m$  data were similar to those for linear PDLLAs.<sup>4</sup> These indicated that the microstructure with multiple CA units was not conducive to crystallization of the polymer, and the crystallization behavior of star-shaped PLA was mainly due to the PLA chain (arms).<sup>50</sup>

The TG and DTG curves for various molar feed ratios are shown in Figure 7; the important data are presented in Table VII. The TG curves for 1/15 and 1/32 CA/LA were similar to each other, and the thermal decompositions of these polymers with more than one CA unit per molecule occurred in two steps and, mainly, during the second stage at the higher temperature. However, for the polymers mainly with one CA unit, similar TG curves all indicated that the thermal decomposition occurred in one step, and their corresponding DTG curve had a single peak. For 1/32 CA/LA, the DTG curve had two peaks. For 1/15 CA/LA, the DTG curve became more complex as a result of its multiple CA units microstructure [Scheme 1(3)].

In Table VII, the initial temperature and the final temperature of the thermal decomposition showed that when the polymers mainly had one CA unit the initial temperatures were all about 175°C and the thermal decomposition mainly occurred in the range 175–340°C (with a mass loss close to 95%). For 1/15 and 1/32 CA/LA, the initial temperature in stage I was lower (ca. 110°C), and the mass loss was lower. This was due to the transition from a microstructure with multiple CA units to a single CA unit microstructure involving the loss of LA units as lactide. According to the method reported by Yuan,<sup>52</sup> the





**Figure 7** (a) TG and (b) DTG curves of poly(D,L-lactide-cholate) synthesized with different molar feed ratios (TGA = thermogravimetric analysis).

number of LA units lost could be calculated as follows:

$$M_n \times \text{Mass loss in the first stage}(\%) / 72 = \text{Number of LA units lost} \quad (2)$$

where 72 is the formula weight of the LA chain unit ( $C_3H_4O_2$ ). For 1/15 CA/LA

$$7500 \times 5.6\% / 72 = 5.8 \approx 6 \quad (3)$$

For 1/32 CA/LA

$$3300 \times 3.9\% / 72 = 1.8 \approx 2 \quad (4)$$

These were in excellent agreement with the theoretical analysis as follows: for 1/15 CA/LA, the microstructure averaged four CA units per molecule [Scheme 1(3)] and, therefore, needed to lose three lactide molecules at least to form a microstructure of only one CA unit per molecule; for 1/32 CA/LA, the microstructure averaged two CA units per molecule [Scheme 1(2)] and, thus, only needed to lose one lactide molecule (formula:  $C_6H_8O_4$ , equivalent to 2 LA units). Therefore, the characterization of the thermal properties reinforced the concept of multiple CA units per molecule for certain molar feed ratios.

#### Influence of different molar feed ratios on the crystallinity

When the molar feed ratio was different, the crystallinity of the copolymers was different. For 1/15 or 1/32 CA/LA, because of the formation of more or less crosswise esterified microstructure [Scheme 1(2,3)], the copolymers were amorphous. For the copolymers mainly or only with a CA unit per molecule, the partial crystallinity was detected (Table VIII).

Obviously, both the peak position and crystallinity were similar to those of the linear PDLLA, but the crystallite size increased markedly after modification by CA. Especially with the increase of LA molar feed ratio, the simpler the structure of copolymer became, the more evident the tendency was. Thus, the XRD results further demonstrate the complexity of the copolymer structure resulting from the variation in the molar feed ratio.

#### Reaction mechanism for the modification of PDLLA by CA via direct melt polymerization

Direct melt polymerization in the syntheses of PLA biodegradable materials involves repeated condensation (esterification) reactions between COOH and

**TABLE VII**  
Influence of Different Feed Molar Ratios on Thermal Degradation

Run	Feed molar ratio (CA/LA)	Stage I				Stage II			
		$T_i$ (°C)	$T_f$ (°C)	Mass loss (%)	$T_p$ (°C)	$T_i$ (°C)	$T_f$ (°C)	Mass loss (%)	$T_p$ (°C)
1	1/15	112.3	161.8	5.6	130.5	206.9	357.8	80.3	321.9
2	1/32	102.8	152.6	3.9	114.4	179.2	387.3	86.7	269.9
3	1/64	175.7	343.9	93.6	274.0			Not detected	
4	1/100	174.8	317.9	94.3	295.9				
5	1/128	179.2	302.3	95.0	284.4				

$T_f$  = final temperature;  $T_i$  = initial temperature;  $T_p$  = maximum temperature of thermal weight.

TABLE VIII  
XRD Test Results for the Poly(D,L-lactide–cholate) Copolymers

Run	Feed molar ratio (CA/LA)	2θ (°)		Crystallinity (%)	Crystallite size (10 <sup>-10</sup> m)	
		Face 110	Face 020		D <sub>110</sub>	D <sub>020</sub>
1	1/64	16.8	19.2	18.7	208.9	120.0
2	1/100	16.7	19.1	18.8	290.7	118.5
3	1/128	16.7	19.1	20.5	322.6	169.8
4	PDLLA <sup>a</sup>	16.7	19.1	20.8	154.4	83.9

<sup>a</sup> Data from previous experiments.<sup>4</sup>

OH. The reaction mechanism for the modification of PDLLA by CA via direct melt polymerization is similar to this.

First, the COOH and OH in the CA molecule react with the OH and COOH in the LA molecule via dehydration (condensation/esterification). With excess LA, a star-shaped polymer was synthesized, which was composed of an embedded CA core and four arms [PDLLA chains with different terminal groups; Scheme 1(1)]. For small CA/LA ratios, the formation probability of the crosswise esterified microstructure between the PLA arms with the different terminal group via the esterification of COOH and OH was less. Thus, each polymer molecule averaged a single CA unit as the core; this led to a relatively uniform molecular weight distribution and, hence, a relatively small PDI.

However, for 1/15 or 1/32 CA/LA, the additional CA molecules increased the formation probability of the crosswise esterified microstructure [Scheme 1(2,3)], which was disadvantageous for the copolymer to make the  $M_w$  averaged and aggregate together. Thus, the copolymer synthesized at a CA/LA molar feed ratio of 1/15 had a broader molecular weight distribution and, hence, the largest PDI of all (Table IV, run 1) and different properties.

## CONCLUSIONS

Starting from D,L-LA, an inexpensive material, and CA, we directly synthesized a natural functional molecule, the biodegradable material poly(D,L-lactide–cholate), via melt copolycondensation. When the CA/LA molar feed ratio was suitably controlled (CA/LA ≤ 1/64), a novel star-shaped PDLLA with four arms was obtained. Because of the PLA arms with different reactive terminal groups and the specificity of CA, the CA-modified PDLLA may provide greater cell affinity and, hence, advantages for applications in drug-release microspheres and tissue engineering.<sup>12,13</sup>

The novel direct copolycondensation method is simple and effective. More importantly, this research on the synthesis and characterization of PDLLA modified by CA via direct melt copolycondensation

provides a good example of the synthesis of an asymmetrical star-shaped PLA material, especially starting from an asymmetrical core with different reactive groups, which is advantageous for the simple and practical syntheses of more biodegradable PLA materials and their more extensive applications in biomedicine.

At the same time, the influences of different molar feed ratios showed that when the CA/LA molar feed ratio is increased, the number of CA units embedded per polymer molecule may be increased. For 1/15 CA/LA, the copolymer averaged four embedded CA units per molecule, both its  $M_w$  (12700 Da) and PDI (1.69) were the biggest, and the thermal properties and crystallinity of the copolymer were wholly different from the normal star-shaped PDLLA modified by CA. These findings and the discussion on the reaction mechanism will be helpful for further research on the direct melt copolycondensation of LA with other multifunctional monomers.

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