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Original article

Synthesis of novel copolymer: Poly(*p*-dioxanone-co-L-phenylalanine)

Bing Wang [a,b], Chi Ma [a,b], Zuo-Chun Xiong [a], Cheng-Dong Xiong [a], Quan-Hua Zhou [c], Dong-Liang Chen [a,*]

[a] Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Sichuan Staff University of Science and Technology, Chengdu 610041, China

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ABSTRACT

In order to expand the application of poly(*p*-dioxanone) or PPDO in biomedical area, a series of novel copolymers were synthesized successfully by one-step, melted copolymerization of *p*-dioxanone (PDO) and L-phenylalanine *N*-carboxyanhydride (L-Phe-NCA) monomers. With the in-feed molar ratio of L-Phe-NCA/PDO equal to 1/20, the conversions of the two kinds of monomers were calculated from ^{1}H NMR. The average molecular weight and polydispersity of the copolymer increase with the increasing reaction time and catalyst concentration. However, the conversions of the two kinds of monomers did not change with the reaction conditions. A three-step mechanism is presented and proved by high resolution ^{1}H NMR and IR spectrums.

1. Introduction

Poly(*p*-dioxanone) (PPDO), as an aliphatic polyester, has been used in biomedical applications, such as tissue engineering, bone fracture fixation and controlled drug delivery, due to its outstanding biodegradability, biocompatibility and flexibility [1–5]. However, the combination of high crystallinity, intrinsic hydrophobicity of PPDO and the absence of biological properties have limited its further application [6]. The most frequently used method to improve the performances of PPDO, such as crystallinity and hydrophobicity of PPDO, is copolymerization [7–12].

In recent years, functionalized polyesters, especially polyesters containing α-amino acid, have attracted much attention. Many papers have reported on the synthesis of α-amino acid containing polyesters and revealed that, as copolymerization components, amino acids could alter not only the physical properties, but also the biological properties of polyesters [13]. However, there have been only a limited number of published reports regarding the copolymers of *p*-dioxanone (PDO) and amino acids [14] and therefore, the aim of our research was to synthesize copolymers composed of PDO and L-phenylalanine (L-Phe) by a new, convenient method. L-Phe was chosen as the copolymerization component because its rigid benzyl group may alter the flexibility of the copolymer chains and decrease the crystallinity, along with specific inhibitions on cell proliferation [15,16].

In this work, copolymers of poly(*p*-dioxanone-co-L-phenylalanine) were synthesized using different polymerization conditions by a one-step, melted reaction of PDO and L-phenylalanine *N*-carboxyanhydride (L-Phe-NCA). A three-step mechanism was provided and proven by FTIR and ^{1}H NMR.

2. Experimental

2.1. Synthesis of L-Phe-NCA [17]

The L-Phe-NCA was synthesized from L-Phe (5.0 g) and triphosgene (3.7 g) in dry THF (50 mL) at 50 °C for 4 h under N_2 atmosphere. After completion of the reaction, the mixture was concentrated by rotary evaporator at 30 °C and dry hexane was added to precipitate the crude product. The crude product was purified by recrystallization from diethyl ether/hexane (1/2, v/v) twice to yield L-Phe-NCA monomer of high purity. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.19–7.41 (m, 5H, ArH), 5.60 (s, 1H, NH), 4.55 (m, 1H, CH), 2.94–3.35 (m, 2H, CH_2). FTIR (KBr, cm^{-1}): ν 3351 (N–H), 3031 (C–H from phenyl), 2905 (C–H), 1849 (C=O), 1779 (C=O).

2.2. Synthesis of poly(p-dioxanone-co-L-phenylalanine)

To a 100-mL round-bottom flask containing PDO (5.0 g), calculated quantities of L-Phe-NCA and stannous octoate were added. The flask was sealed using a long-neck piston connected to an oil pump to remove moisture, oxygen, and solvent until the pressure was reduced to below 10^{-3} Pa. The polymerization was carried out under vacuum at 95 °C with magnetic stirring.

* Corresponding author.
E-mail address: cdl921@yahoo.com.cn (D.-L. Chen).

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Table 1
Synthesis of PDO and L-Phe-NCA copolymers.

Entry	[PDO]:[Phe] (mol/mol)	T (h)	$[M]_0$:$[C]_0$ (mol/mol)	Conversion of PDO%[a]	Conversion of Phe-NCA%[b]	M_n[c] (g/mol)	M_w[c] (g/mol)
1	20	72	3500	86	76	2800	3841
2	20	96	3500	82	100	6784	11,455
3	20	120	3500	76	88	7747	15,002
4	20	144	3500	81	90	12,899	26,147
5	20	168	3500	82	88	8716	17,964
6	20	72	2000	75	94	5457	9141
7	20	96	2000	79	100	8707	15,932
8	20	120	2000	79	84	9009	19,274
9	20	144	2000	79	82	19,443	37,292
10	20	168	2000	81	79	6163	12,069
11	10	120	2000	80	98	7868	14,513
12	6.67	120	2000	84	100	3071	4830

[a] Calculated from ^{1}H NMR of crude products of copolymerization.
[b] Calculated from mol comparison of PPDO to L-Phe from ^{1}H NMR of copolymer after purification and the in-feed molar ratio of the two kinds of monomers.
[c] Calculated by GPC using a system consisting of a Waters 1515 isocratic HPLC pump, Styragel HT 4 or HT 5 column, and Waters 2414 refractive index detector. Chloroform was the eluent at a flow rate of 0.5 mL/min. Narrow molecular weight distribution polystyrene was used as the molecular weight basis.

The crude product was dissolved in chloroform and precipitated with diethyl ether, then redissolved in chloroform again and precipitated with ethanol. The purified product was filtered and dried under vacuum at room temperature for 24 h. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18–7.22 (d, NH), 7.26 (m, C_6H_5), 4.87 (m, CH of L-Phe structure units), 4.34 (t, $COCH_2OCH_2C\underline{H}_2O$), 4.17 (s, $COC\underline{H}_2OCH_2CH_2O$), 3.79 (t, $COCH_2OC\underline{H}_2CH_2O$), 3.16 (m, CH_2 of L-Phe structure units). FTIR (KBr, cm^{-1}): ν 3413 (N-H), 2980–2881 (C–H), 1749 (C=O), 1673 (amide I), 1525 (amide II), 1137(O–C–O).

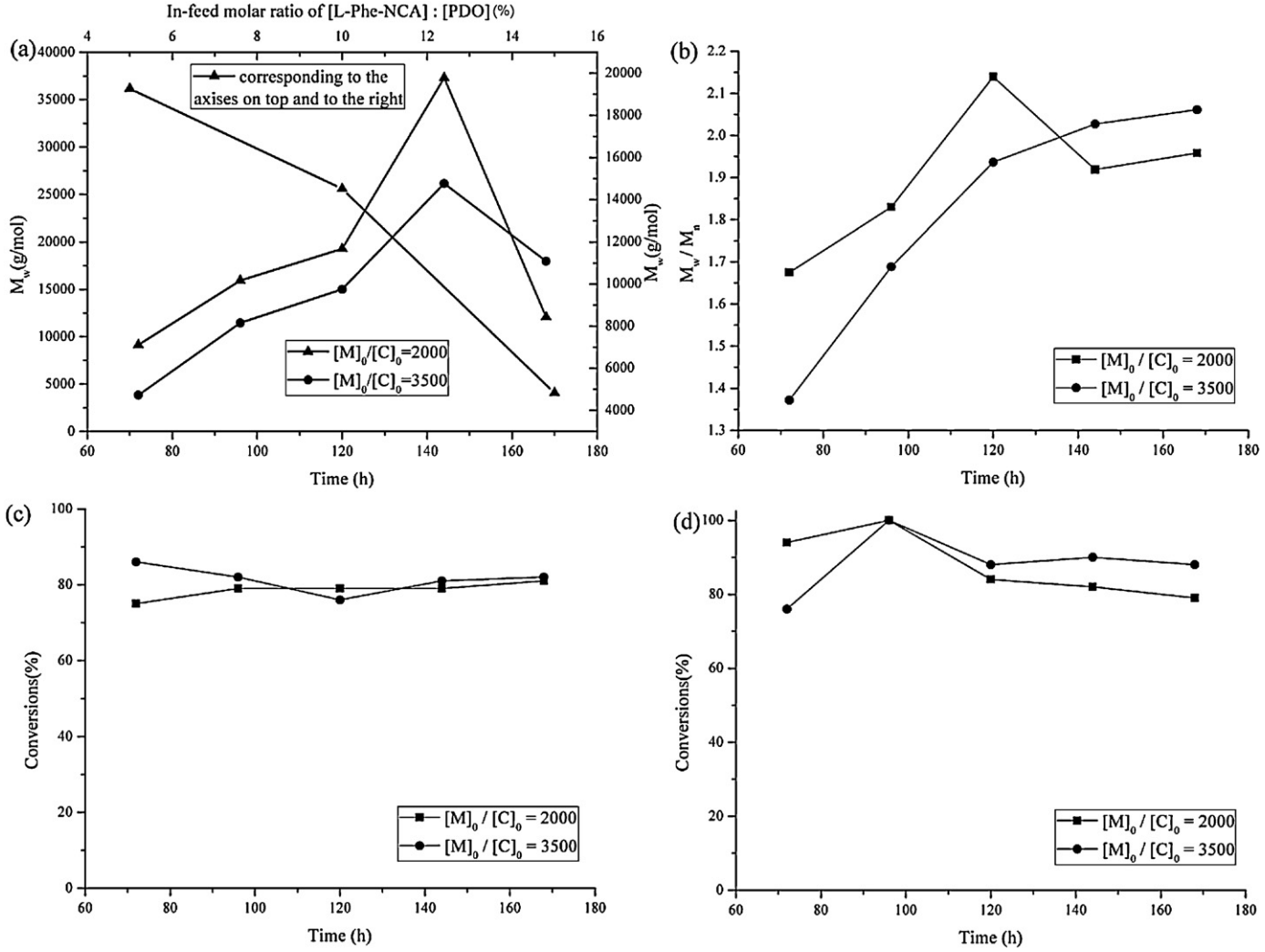
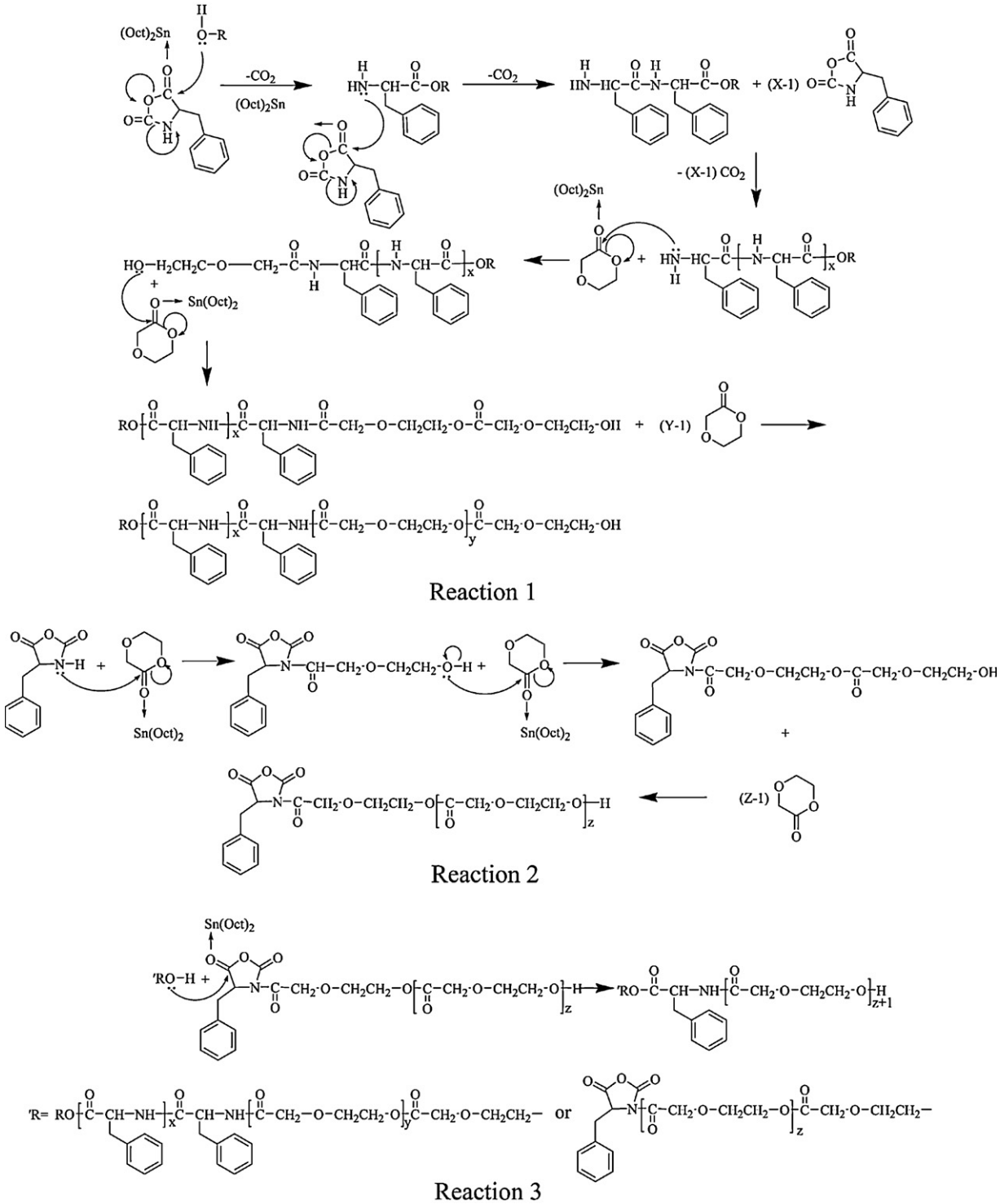


Fig. 1. (a) Weight-average molecular weight of poly(*p*-dioxanone-co-L-phenylalanine) with different reaction times and catalyst concentration, weight-average molecular weight of poly(*p*-dioxanone-co-L-phenylalanine) with different in-feed molar ratios of [L-Phe-NCA]:[PDO] (%) and same reaction time and catalyst concentration; (b) Polydispersity of poly(*p*-dioxanone-co-L-phenylalanine); (c) Conversion of PDO monomer; (d) Conversion of L-Phe-NCA monomer evolutions with polymerization time.

3. Results and discussion

The synthesis of poly(*p*-dioxanone-co-L-phenylalanine) was carried out by a one-step copolymerization of L-Phe-NCA and PDO. All subsequent polymerization experiments were performed at 95 °C. This temperature was chosen because higher temperature (105 °C) was found to result in low conversions of L-Phe-NCA, an indication of deactivation, whereas a lower temperature (85 °C) was not sufficient for synthesis of the copolymers.

The structure of poly(*p*-dioxanone-co-L-phenylalanine) was confirmed by ^{1}H NMR and FTIR spectra. The triplet at 4.34 ppm belonged to the $COCH_2OCH_2C\underline{H}_2O$ protons in PPDO segments, the single peak at 4.17 ppm was attributed to the $CO\underline{CH}_2OCH_2CH_2O$ protons in PPDO segments, and the triplet at 3.79 ppm was attributed to the $COCH_2OC\underline{H}_2CH_2O$ protons in PPDO segments. These characteristic peaks are in accordance with literature reports of PPDO [1]. The multiplet at 7.26 ppm belonged to the benzyl protons in the phenylalanine unit, and the peaks at 4.87 ppm and



Scheme 1. Mechanism of PDO and L-Phe-NCA copolymerization reaction.

3.16 ppm were attributed to the protons of the methenyl groups and methylene groups in the phenylalanine unit, respectively. The characteristic peaks at 7.18 ppm and 7.22 ppm were assigned to the protons of the amide group in poly(*p*-dioxanone-co-L-phenylalanine). All of the characteristic peaks indicate that the copolymers were successfully obtained. In addition, the absorptions at 1849 cm^{-1} and 1779 cm^{-1} in FTIR spectrum, which are characteristics of L-Phe-NCA acid anhydride groups, disappeared after copolymerization. The amide I and amide II absorptions appeared at 1673 cm^{-1} and around 1525 cm^{-1}, respectively, indicating that the polymerization of L-Phe-NCA monomers occurred.

The influences of reaction conditions on copolymerization were studied by changing the monomer-to-catalyst ratio ($[M]_0/[C_0]$), where C is stannous octoate, and reaction time. With the fixed in-feed molar ratio of [PDO]/[L-Phe-NCA] equal to 20, a series of polymerization experiments were carried out in which the $[M]_0/[C_0]$ ratio and reaction times were systematically varied. Copolymerization reactions of PDO and L-Phe-NCA with different in-feed molar ratios were also carried out. The results are summarized in Table 1. The increase of polymer molecular weight during polymerization was monitored using GPC. PDO conversions were determined using ^{1}H NMR spectroscopy by comparison of integrations of the $COCH_2OC\underline{H}_2CH_2O$ protons of the PDO residue of the monomer and polymer. L-Phe-NCA conversions were determined using ^{1}H NMR spectroscopy (in deutero-dichloromethane) by comparison of the benzene proton integrations of phenylalanine in the polymer to the $COCH_2OC\underline{H}_2CH_2O$ protons of the PDO in the polymer after purification. The results showed that the $[M]_0/[C]_0$ ratio and reaction time can affect the molecular weights of the copolymers. Together with the information provided in Table 1, plots a–d of Fig. 1 show the evolution of monomer conversion, M_w, polydispersity with polymerization time and the evolution of M_w with in-feed molar ratio of PDO to L-Phe-NCA. From Fig. 1, M_w increased with increasing polymerization time and catalyst quantity before 144 h. The polydispersity of the copolymers also increased with increasing polymerization time. However, the conversions of the two monomers did not change with reaction time. It also could be concluded from Fig. 1(a) that the M_w decreased with increasing L-Phe-NCA content while the reaction times and catalyst concentrations are constant.

In order to explain these phenomena, a possible mechanism is proposed in Scheme 1 [1,18]. Initially, when the temperature of the polymerization system becomes higher than the melting point of PDO but lower than the temperature at which PDO can polymerize successfully, L-Phe-NCA dissolves in the melted PDO, and then the trace amount of OH groups in the system initiates the polymerization of L-Phe NCA. Next, the oligopeptide can initiate the polymerization of PDO monomers along with the increasing temperature to yield the copolymers. This step of the copolymerization is shown as Reaction 1 of Scheme 1. Meanwhile, the residual L-Phe-NCA monomers can also initiate the polymerization of PDO monomers as the temperature reaches the proper value, yielding the L-Phe-NCA end-capped PPDOs. This step of the copolymerization is shown as Reaction 2 of Scheme 1. Finally, for the reactions among macromolecules, the hydroxyl end caped macromolecules react with L-Phe-NCA end caped macromolecules, which lead to an obvious increase in the molecular weight, as shown in Reaction 3 of Scheme 1. This mechanism indicates that the extent of Reaction 3 increases with increasing reaction time, so the M_w increased with the reaction time before 168 h when depolymerization occurred. At the same time, the inhomogeneity of the length of copolymer chains increases with the extent of Reaction 3, leading to the increase of polydispersity. Use of more catalyst resulted in reaction of larger amounts of L-Phe-NCA *via* Reaction 1 and less reaction *via* Reaction 2. This means that the active centers for PDO polymerization decrease with the catalyst amount, and M_w increases with the catalyst amount. According to the mechanism mentioned

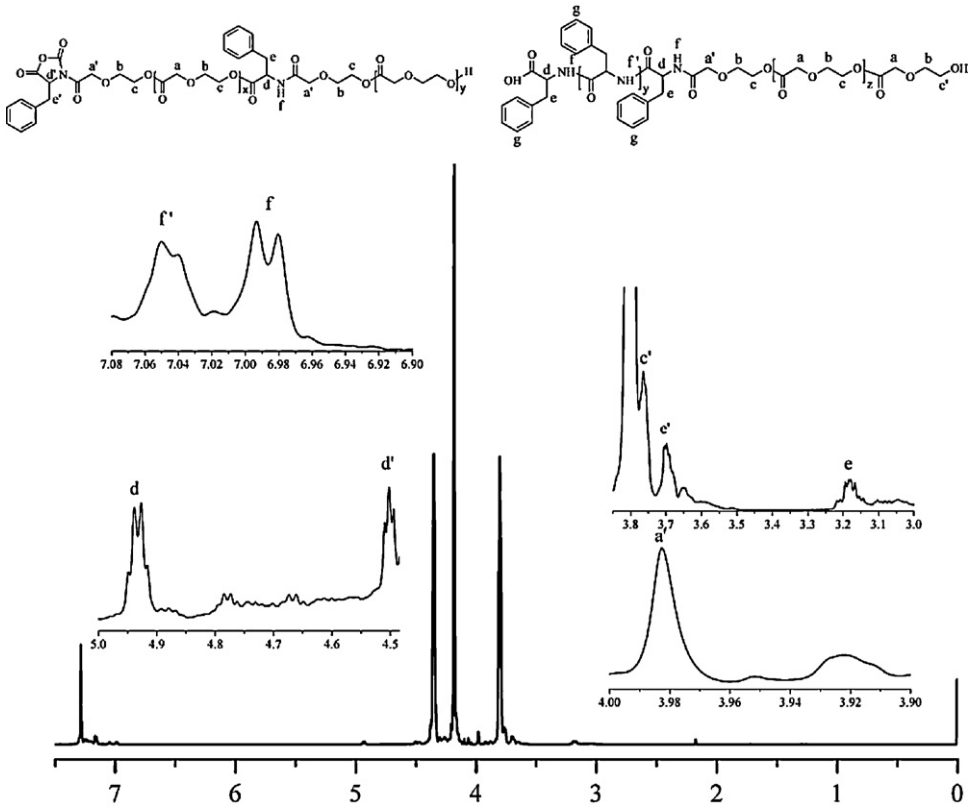


Fig. 2. 600 MHz ^{1}H NMR of poly(*p*-dioxanone-co-L-phenylalanine) (entry 8, Table 1) in $CDCl_3$.

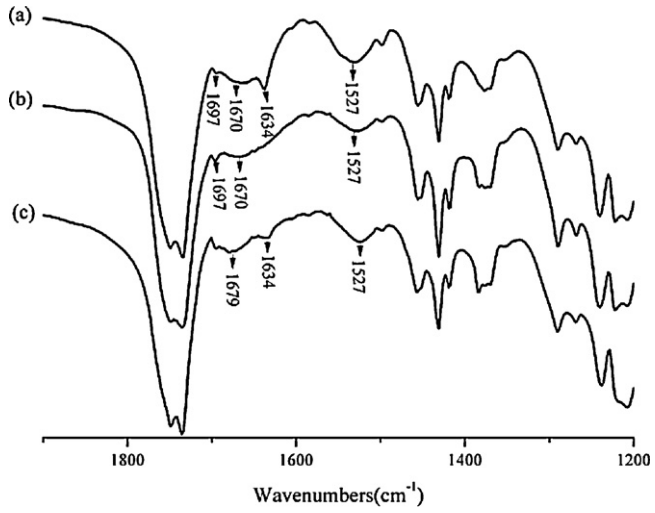


Fig. 3. IR (KBr) of (a) poly(*p*-dioxanone-co-L-phenylalanine) with highest L-Phe contents (entry 12, Table 1), (b) poly(*p*-dioxanone-co-L-phenylalanine) with smallest weight-average molecular weight (entry 1, Table 1), (c) poly(*p*-dioxanone-co-L-phenylalanine) with largest weight-average molecular weight (entry 10, Table 1).

above, when the copolymerization proceeds under same conditions, more L-Phe-NCA content as in-feed, means more active centers for the monomers to polymerize and then lower M_w of copolymers would result in accordance with Fig. 1(a).

In order to verify the mechanisms, the copolymers were analyzed by 600 MHz ^{1}H NMR and IR spectroscopy, and the results are illustrated in Figs. 2 and 3. Fig. 2 affirms the structures in Scheme 1: The α-CH protons and β-CH_2 protons of phenylalanine in the copolymer chains labeled d and e appear at 4.95 ppm and 3.16 ppm, respectively, and those in the L-Phe-NCA end group labeled d′ and e′ appear at 4.48 ppm and 3.70 ppm, respectively. The IR spectrum of poly(*p*-dioxanone-co-L-phenylalanine) is also consistent with the mechanism. Fig. 3(b) and (c) shows the IR spectra of the poly(*p*-dioxanone-co-L-phenylalanine) with the smallest and largest average molecular weights corresponding to entries 1 and 9 in Table 1. It is worth noting the absorption at 1697 cm^{-1} attributable to the imide groups, appears in the spectrum of the poly(*p*-dioxanone-co-L-phenylalanine) with the smallest M_w (spectrum b, Fig. 3), but not in the largest M_w (spectrum c, Fig. 3). This can be explained by the mechanism mentioned above, *i.e.*, as the average molecular weight decreases, the number of PPDO structural units with L-Phe-NCA end caps increases. In other words, the number of imide groups increases. Furthermore, the absorption at 1634 cm^{-1}, which belongs to the associated amide groups, appears in the spectrum of the poly(*p*-dioxanone-co-L-phenylalanine) with the largest M_w. According to the proposed mechanism, there would be increased, and longer phenylalanine segments if the average molecular weight of the poly(*p*-dioxanone-co-L-phenylalanine) sustained increase. Thus, the associated amide absorption peaks appear in the spectrum of the copolymer with the largest M_w, but not in that with the smallest M_w. Fig. 3(a) shows the IR spectrum of copolymer with highest phenylalanine content (entry 12, Table 1), with both absorptions at 1697 cm^{-1} and 1634 cm^{-1}. Although the two copolymers in Fig. 3(a) and (b) have similar M_w, the associated amide groups absorption at 1634 cm^{-1} still appears. As confirmation of the above mechanism, similar M_w means similar numbers of active centers, so the length of phenylalanine segments increase with the initial L-Phe-NCA content. The differences of M_w and structures among copolymers with different initial L-Phe-NCA contents could be seen as further evidence for the proposed mechanism.

4. Conclusion

The poly(*p*-dioxanone-co-L-phenylalanine)s were successfully synthesized using PDO and L-Phe-NCA as copolymerization monomers. The structure of the copolymer was characterized using ^{1}H NMR spectra from which characteristic peaks were easily identified. The average molecular weight and polydispersity of the copolymer increased with increasing reaction time, though the conversions of the two types of monomers remained almost unchanged. The average molecular weight of the copolymers increased with increasing quantities of catalyst when reaction time remained the same. With fixed reaction conditions, the average molecular weights of the copolymers decreased with increasing in-feed L-Phe-NCA content. A three-step reaction mechanism for the polymerization was also proposed on the basis of high resolution ^{1}H NMR and IR analysis. Further investigations of the copolymer and its application in the biomedical area where cell proliferation should be inhibited, such as anti-adhesion, are planned in the immediate future.

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