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Synthesis, degradation and in vitro controlled drug release of novel copolymers of 5-methyl-5-methoxycarbonyl-1,3-dioxan-2-one and caprolactone

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Abstract

Novel biodegradable copolymers of 5-methyl-5-methoxycarbonyl-1,3-dioxan-2-one (MMTC) and caprolactone (CL) were synthesized under different conditions by ring-opening polymerization. The structure of the resultant copolymers was characterized by IR, ^{1}H NMR and ^{13}C NMR methods. Their molecular weight was measured by gel permeation chromatography (GPC). Incorporating the MMTC units into PCL main chain results in the great enhancement of hydrolytic degradation rate and the effective retardance of release rate of Tegafur in comparison with the PCL homopolymer. The enzymatic degradation rate increases with increasing the molar content of PCL, and the copolymers degrade faster in the presence of pseudomonas (PS) lipase than that in the absence of PS lipase. Some mechanical properties of the copolymers were tested and showed that they can be adjusted by varying the composition of the copolymers.

Keywords: Biodegradable; Controlled release; Copolymerization

1. Introduction

Since the past three decades, biodegradable polymers have attracted much attention in the field of biomedical applications such as drug delivery system, surgery and tissue engineering [1,2]. Among them, polycaprolactone (PCL) is one of the most important biodegradable polymers due to its biodegradability, biocompatibility, non-toxicity and good permeability to drug [3–5]. However, PCL has slow degradation rate because of its poor hydrophilicity and high crystallinity that limit its applications in the biomedical fields. Modification via copolymerization is an effective approach to obtain the materials with desirable properties, so many copolymers of CL with other monomers such as lactide (LA) [6,7], 5-methyl-5-benzyloxycarbonyl-1,3-dioxane-2-one (MBC) [8,9], 1,3-dioxane-2-one (TMC) [10–12], glycolide (GA) [13,14] and poly(ethylene glycol) (PEG) [15,16] have been extensively investigated in order to improve degradation rate and expand applications of PCL.

Aliphatic polycarbonates have also gained considerable interest because they are biocompatible, biodegradable and non-toxic [17–20]. However, most of them show slow degradation rate, such as poly(1,3-dioxan-2-one) (PTMC) and poly(5,5-dimethyl-1,3-dioxan-2-one) (PDTC). Albertsson et al. reported that introducing CL units into PTMC main chain would lead to an enhancement of the degradation rate of the resultant polymers in comparison with the polycarbonate homopolymer, and both PTMC and poly(TMC-*co*-CL) had slight weight loss (less than 5%) in pH 7.4 phosphate buffer solution for 500 days at 37 °C [11].

In our earlier work, we reported the synthesis and properties of poly(5-methyl-5-methoxycarbonyl-1,3-dioxan-2-one) (PMMTC) which is an aliphatic polycarbonate with pendent methyoxycarbonyl groups [21,22]. The experimental result of the hydrolytic degradation in phosphate buffer solution at 37 °C showed that the degradation rate of PMMTC is faster than that of PTMC [11,17,21]. In this article, the copolymers of CL with MMTC were synthesized in order to disturb the regular structure of PCL chain, consequently, enhance its degradation rate and improve its thermal and mechanical

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properties. Furthermore, the hydrolytic and enzymatic degradation of the copolymers with different compositions were investigated at 37 °C in pH 7.4 phosphate buffer solution with or without PS lipase. Their in vitro controlled drug release and some mechanical properties were also preliminarily studied.

2. Experimental

2.1. Materials

CL was purchased from Fluka and was dried over CaH_2 for 2 days and distilled under reduced pressure. The cyclic carbonate monomer, 5-methyl-5-methoxycarbonyl-1,3-dioxan-2-one (MMTC) was synthesized according to the published procedure [21], stannous 2-ethylhexanoate [$Sn(Oct)_2$] was purchased from Shanghai Chemical Reagent Co. and purified by distillation under reduced pressure. Toluene was freshly distilled over sodium metal before use. Pseudomonas lipase (EC 3.1.1.3, 40 U/mg) was purchased from Fluka. 5-Fluoro-1-(tetrahydro-2-furyl)uracil (Tegafur) was recrystallized with ethanol. All other reagents were of analytic reagent grade.

2.2. Characterization

IR spectra were recorded on a Perkin–Elmer-2 spectrometer. ^{1}H NMR spectra were measured on a Mercury VX-300 spectrometer at 300 MHz. Deuterated chloroform ($CDCl_3$) was used as solvent and tetramethylsilane (TMS) used as internal standard. ^{13}C NMR spectra were recorded on a Mercury VX-300 spectrometer at 75.5 MHz using $CDCl_3$ as solvent. Melting points were determined on a microscope (20×10) melting point apparatus. The tensile testing was performed at room temperature, using a universal testing machine (CMT6503, Shenzhen SANS Test Machine Co., Ltd, China). The concentration of Tegafur was determined by Shimadzu UV-240. Number- and weight-average molecular weight (M_n and M_w) and polydispersity index (M_w/M_n) of the polymers were determined by gel permeation chromatography (GPC) using a Waters 2690-D liquid chromatograph equipped with Shodex K803 and K805 gel columns and an internal waters 2410 refractive index detector. Chloroform was used as eluent at a flow rate of 1.0 ml/min. Polystyrene standard with a narrow distribution were used to generate a calibration curve.

2.3. Copolymerization of MMTC and CL

A single-necked round bottom flask, charged with given amounts of MMTC, CL and $Sn(Oct)_2$ solution in dry toluene, was sealed under reduced pressure. After the mixture was stirred with a magnetic stirrer in vacuum at 80 °C for predetermined time, the polymerization was quenched by immersing the flask in a cool water bath. The resultant polymer was dissolved in chloroform and precipitated with methanol, the product was dried in vacuum. The polymer was analyzed by IR, NMR and GPC. The following data are of poly(MMTC-*co*-CL) (10/90), IR (KBr, cm^{-1}): 2950, 2865 (CH), 1735 (COO), 1252 (C–O–C); ^{1}H NMR ($CDCl_3$, ppm) δ: 1.30–1.24 (s, CH_3, 3H), 1.44–1.36 (m, CH_2, 2H), 1.78–1.58 (m, CH_2, 4H), 2.38–2.28 (t, $COCH_2$, 2H), 3.78–3.72 (s, OCH_3, 3H), 4.16–4.02 (m, CH_2O, 2H), 4.32–4.24 (s, OCH_2C, 4H).

2.4. Preparation of polymer films

The polymer films were prepared by casting a chloroform solution of the polymer (120 mg/ml) onto Teflon board at room temperature, then were dried in vacuum until a constant weight was achieved. The size and weight of the films used were about $24\times10\ mm^2$ and 17–21 mg, respectively.

2.5. Hydrolytic/enzymatic degradation of polymers

The in vitro hydrolytic degradation experiment was carried out at 37 °C by immersing polymer films in 5 ml phosphate buffer solution (pH 7.4, 0.1 M). In the case of enzymatic degradation, the experiment was performed at 37 °C by immersing copolymer films in 5 ml pH 7.4 phosphate buffer solution in the presence of 0.5 mg PS lipase and 0.5 mg sodium azide, and the buffer-enzymatic solution was changed every 24 h to maintain the enzymatic activity. These films were taken out from the solution over predetermined time intervals, then washed with distilled water and dried in vacuum. The degradation rate was determined by the weight loss and the molecular weight change. Weight loss is defined as weight loss(%)$=[(W_0-W_d)/W_0]\times100\%$, where W_0 is initial weight and W_d is weight after degradation at different time intervals.

2.6. In vitro controlled drug release of polymers

A mixture of 60 mg polymer and 3 mg Tegafur was dissolved in 0.5 ml $CHCl_3$, then the solution was cast onto Teflon board at room temperature, the film was dried in vacuum until a constant weight was achieved. The sample was immersed in 50 ml phosphate buffer solution (pH 7.4, 0.1 M) at 37 °C, 10 ml solution was taken off for released Tegafur content measurement and the same volume of fresh buffer solution was added at predetermined time intervals. The concentration of Tegafur was determined by UV spectroscopy at maximal absorption wavelength ($\lambda_{max}=$ 270.8 nm). The rate of controlled drug release was measured by accumulatively released weight of Tegafur according to the calibration curve of Tegafur.

3. Results and discussion

3.1. Synthesis and characterization of the copolymers

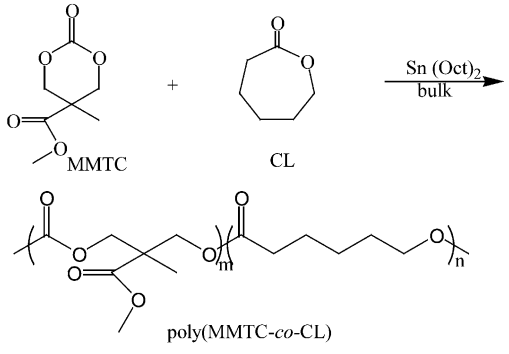
Novel biodegradable copolymers were synthesized by ring-opening polymerization of MMTC and CL, using $Sn(Oct)_2$ as catalyst in bulk (Scheme 1). The copolymerization was carried out under different conditions, such as different reaction times, different monomer/catalyst molar ratios and different molar feed ratios of MMTC/CL. The results are listed in Tables 1 and 2.

Copolymers with different compositions were synthesized by varying the comonomer feed ratio at 80 °C for 24 h when the [monomer]/[catalyst] was 100, the results summarize in Table 1. It can be seen that the molar ratio of MMTC in the corresponding copolymers is lower than that in feeds, and that the M_n and yield of the copolymers obtained decrease with increasing the MMTC content, which indicates that CL has higher reactivity than MMTC in the copolymerization. GPC results show that all of the polymers obtained have unimodal-molecular-weight distributions, so it can be preliminarily concluded that the two monomers of CL and MMTC have copolymerized.

To study the effect of reaction conditions on the copolymerization of MMTC with CL, the copolymerization was examined at 80 °C when the molar content of MMTC in feed was 30%. It can be seen from Table 2 that when the copolymerization time varies from 16 to 36 h and the molar ratio of monomer to catalyst is 100 (entries 8,10 and 11), the M_n of the copolymers increases from 24,100 to 38,000 with increasing reaction time. When the copolymerization time increases to 48 h, almost all the reaction product forms gel, the similar phenomenon has also been observed by an earlier finding [8]. The gel-like cross-linked copolymer is insoluble in water and organic solvents, but they can swell in some organic solvents such as $CHCl_3$, CH_2Cl_2 or DMSO. When the molar ratio of monomer to catalyst increases from 50 to 500 and the reaction time is 24 h (enties 7–9), the M_n of the copolymers increases from 14,300 to 28,200.

The copolymers were characterized by IR and ^{1}H NMR. In the IR spectra, there was only single ester carbonyl absorption band at 1735 cm^{-1}, which might indicate the formation of random copolymers [23]. The characteristic absorption band at 1253 cm^{-1} was the C–O stretching vibration. The characteristic absorption bands at 2950 and 2865 cm^{-1} were assigned to the stretching vibration of $–CH_3$ and $–CH_2–$. In the ^{1}H NMR spectra, the peaks at 4.29 ppm belonged to the main chain methylene protons of MMTC units, the peaks at 3.72 and 1.27 ppm were assigned to methyl protons of MMTC units. The chemical shift at 4.06 ppm belonged to the characteristic peaks of $–COCH_2CH_2CH_2CH_2\mathit{CH_2}O–$ of CL units, 2.31 ppm to $–CO\mathit{CH_2}CH_2CH_2CH_2CH_2O–$, 1.66 ppm to $–COCH_2\mathit{CH_2}$-$CH_2CH_2CH_2O–$ and 1.39 ppm to $–COCH_2CH_2\mathit{CH_2}CH_2$-$CH_2O–$ of CL units. ^{1}H NMR was also used to measure the copolymer compositions by comparing the relative areas of peaks corresponding to the protons of the CL and MMTC repeat units.

The ^{13}C NMR spectrum of the copolymer with the molar feed ratio of 50/50 MMTC/CL is shown in Fig. 1. Signals from the MMTC units and the CL units can be clearly observed. The detailed assignments are shown in Fig. 1. It can be seen that the carbonyl carbon resonance of the MMTC units in the copolymer splits into several peaks in the range of 154.61–155.10 ppm, which is due to the different chemical environments caused by the different sequences in the copolymer chain. Therefore, from the ^{13}C NMR spectra, we can further confirm that the two comonomers are copolymerized by random copolymerization.



Scheme 1. Ring-opening copolymerization of MMTC and CL.

3.2. Mechanical properties estimate

The mechanical properties of poly(MMTC-*co*-CL) were measured and the results are listed in Table 3. We can see that the tensile strength and the Young modulus decrease greatly with increasing MMTC content, and that the elongation of poly(MMTC-*co*-CL) is greatly higher than that of the PCL homopolymer. When the molar ratio of MMTC in feed is higher than 30%, the corresponding copolymer is so flexible that the mechanical property data are not obtained. Furthermore, when the MMTC content is less than 10%, the copolymer films are opaque; however, when the MMTC content is more than 30%, the copolymers are transparent. It is probably because that the PCL regular structure is disturbed, and its crystallization is retarded by introducing the MMTC units into the PCL main chain. Thus, the copolymer mechanical properties can be adjusted by varying the comonomer feed ratio in order to adapt to the different applications.

3.3. Hydrolytic/enzymatic degradation of copolymers

The hydrolytic/enzymatic degradation properties of poly(MMTC-*co*-CL) copolymers were evaluated by the weight loss at different degradation time, the results are presented in Figs. 2 and 3.

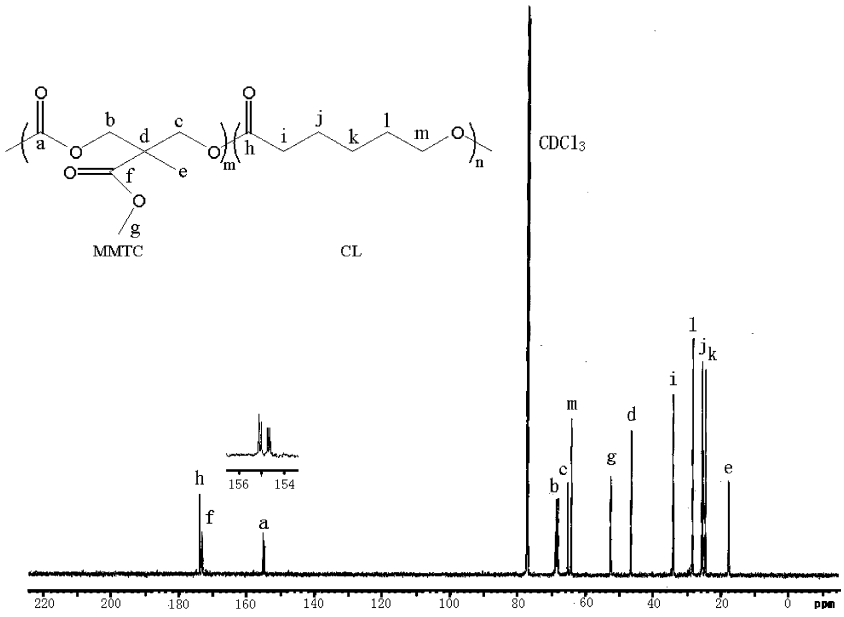


Fig. 1. ^{13}C NMR spectrum of poly(MMTC-*co*-CL) (50/50) in $CDCl_3$.

In the hydrolytic degradation experiment (Fig. 2), the weight loss of the PCL homopolymer is only 0.9% in pH 7.4 phosphate buffer solution for 49 days at 37 °C, after incorporating the MMTC units into the PCL chain, the resultant copolymers have higher weight loss than the PCL homopolymer. It can be attributed to the facts that the regular structure of PCL chain is disturbed by random copolymerization with MMTC and the PMMTC homopolymer have faster degradation rate than the PCL homopolymer in pH 7.4 phosphate buffer solution at 37 °C. When the molar ratio of MMTC in feed is higher than 30%, the corresponding copolymer is so flexible and the film cannot be formed as usual.

In the enzymatic degradation experiment, it can be seen from Fig. 3 that the degradation rate increases greatly with increasing the molar content of PCL. In comparison with Figs. 2 and 3, the copolymer with the same composition degrades faster in the presence of PS lipase than that in the absence of PS lipase. The result confirms again that the PS lipase can accelerate the PCL degradation [24–26].

The degradation of poly(MMTC-*co*-CL) (30/70) in phosphate buffer solution with PS lipase (pH = 7.4, 37 °C)

Table 1
The copolymerization of MMTC and CL with different compositions

Entry	F_{MMTC} (%)[a]	f_{MMTC} (%)[b]	M_n	M_w/M_n	Yield
1	0	0	48,000	1.33	99
2	10	9	38,500	1.17	86
3	30	23	28,100	1.53	78
4	50	43	26,600	1.87	65
5	70	59	22,300	1.40	43
6	90	89	13,100	1.35	54

The copolymerization conditions: [monomer]/[catalyst] = 100, 24 h, 80 °C, in bulk.
[a] The molar fraction of MMTC in feed.
[b] The molar content of MMTC in copolymer determined by ^1H NMR (solvent: $CDCl_3$).

Table 2
Effect of reaction conditions on the copolymerization of MMTC and CL

Entry	Time (h)	[Monomer]/[catalyst]	M_n	M_w/M_n	Yield (%)
7	24	50	14,300	1.20	48
8	24	100	28,100	1.53	78
9	24	500	28,200	1.83	31
10	16	100	24,100	1.22	26
11	36	100	38,000	1.94	72

The molar fraction of MMTC in feed was 30%, $Sn(Oct)_2$ as catalyst, 80 °C, in bulk.

Table 3
The mechanical properties of poly(MMTC-*co*-CL) with different compositions

MMTC content (mol%)	Tensile strength (MPa)	Breaking strength (MPa)	Elongation %	Young modulus (MPa)
0	17	15	14	332
10	7	6	500	103
30	1	0	229	11

was also evaluated by the molecular weight change at different time, the result is shown in Fig. 4. It can be seen that the molecular weight of the copolymer decreases obviously with increasing degradation time. At the same time, the polydispersity index (M_w/M_n) increases gradually from 1.53 to 2.88.

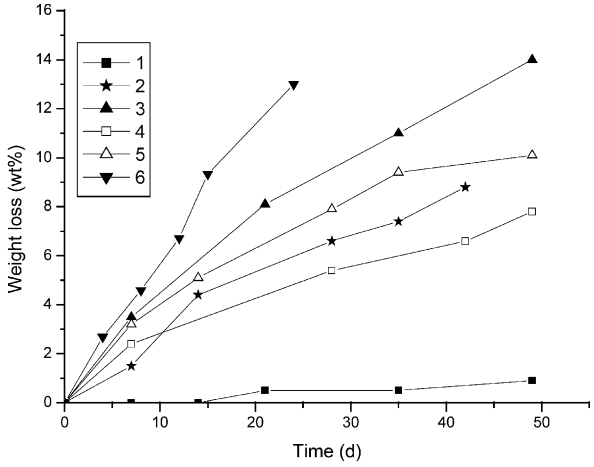


Fig. 2. Hydrolytic degradation of poly(MMTC-*co*-CL) copolymers in phosphate buffer solution (pH=7.4, 37 °C): MMTC/CL: (1) 0/100; (2) 10/90; (3) 30/70; (4)50/50; (5)90/10; (6)100/0.

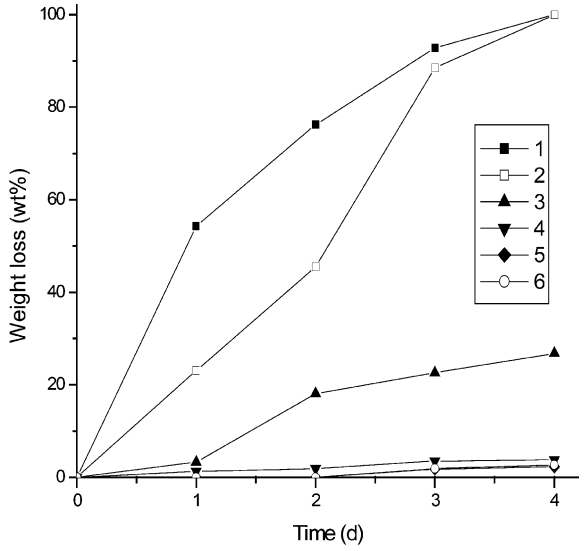


Fig. 3. Enzymatic degradation of poly(MMTC-*co*-CL) copolymers in phosphate buffer solution with PS lipase (pH=7.4, 37 °C): MMTC/CL: (1) 0/100; (2) 10/90; (3) 30/70; (4) 50/50; (5) 70/30; (6) 100/0.

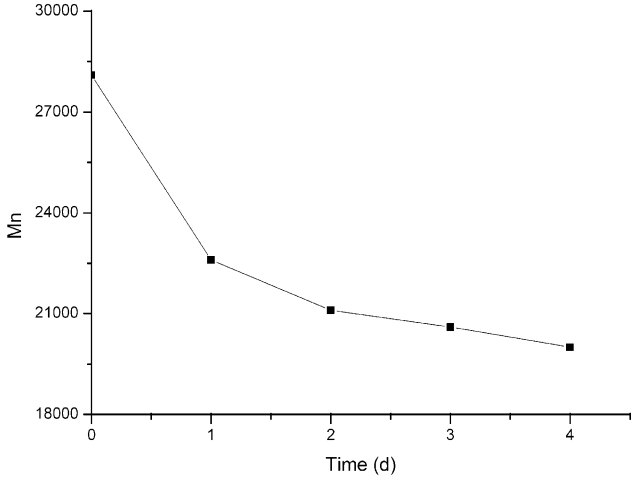


Fig. 4. The molecular weight change of poly(MMTC-*co*-CL) (30/70) in phosphate buffer solution (pH=7.4, 37 °C) and in the presence of PS lipase.

3.4. In vitro controlled drug release of copolymers

Anti-tumor drug Tegafur was selected as a model drug to investigate the in vitro controlled drug release property of the poly(MMTC-*co*-CL) copolymers. The release rate was monitored by determining the concentration of accumulatively released Tegafur. The results are shown in Fig. 5. It can be seen that the rate of controlled drug release decreases with the increase of the MMTC content, and that the initial burst release increases with increasing the CL content, which may be caused by the good drug permeability of PCL. It demonstrates that the rate of release drug can be controlled by varying the content of MMTC incorporated into the PCL main chain.

4. Conclusion

Novel biodegradable copolymers of MMTC and CL

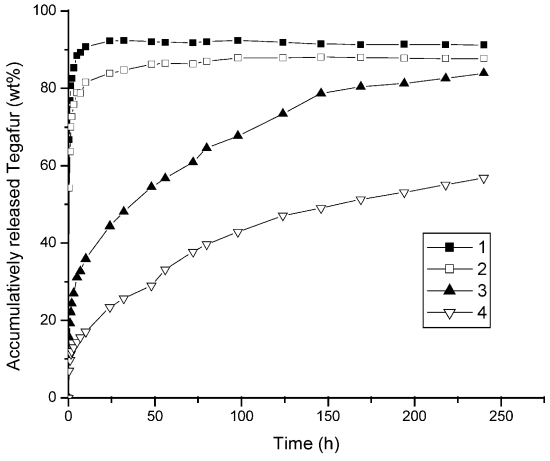


Fig. 5. Release profile of Tegafur from poly(MMTC-*co*-CL) copolymers in phosphate buffer solution (pH=7.4, 37 °C). MMTC/CL: (1) 0/100; (2) 30/70; (3) 50/50; (4) 70/30.

were synthesized under different conditions by ring-opening polymerization. The results show that CL has higher reactivity than MMTC. Incorporating the MMTC units into PCL main chain results in the great enhancement of hydrolytic degradation rate and the effective retardance of release rate of Tegafur in comparison with the PCL homopolymer. The enzymatic degradation rate increases with increasing the molar content of PCL, and the copolymers degrade faster in the presence of PS lipase. The copolymer mechanical properties can be adjusted by varying the composition of the copolymers.

Acknowledgements

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