Articles

Enzymatic Synthesis and Characterization of Novel Biodegradable Copolymers of 5-Benzyloxy-trimethylene Carbonate with 1,4-Dioxan-2-one

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Enzymatic ring-opening copolymerization of 5-benzyloxy-trimethylene carbonate (BTMC) and 1,4-dioxan-2-one (DON) was investigated for the first time. Immobilized porcine pancreas lipase (IPPL) on silica particles was selected to perform the copolymerization. A series of novel biodegradable copolymers with different compositions were characterized by ^{1}H NMR, ^{13}C NMR, and GPC. The influences of reaction conditions such as polymerization time and catalyst concentration on the yield and molecular weight of the copolymers were also studied. The copolymerizations of different monomer feed ratios were carried out in bulk at 150 °C with 4.5 wt ‰ IPPL as a catalyst for 24 h. With the increase of the BTMC molar feed ratio from 20% to 79%, the M_n of the resulting copolymers increased from 5600 to 63400. Water uptake and static contact angle experiments showed that the hydrophilicity of copolymers could be improved with increasing DON content in the copolymers. Moreover, the in vitro drug release rate (ibuprofen as the model drug) of the resulting copolymers also increased along with the DON content in the copolymers.

Introduction

Biodegradable polymers are receiving more and more attention for their wide application in biomedical uses, such as drug carriers, matrixes in tissue engineering, surgical sutures, etc.[1] Among them, aliphatic polycarbonates are a class of surface erosion biodegradable polymers attracting great interest due to their good biocompatibility, favorable mechanical properties, and low toxicity.[2,3] However, the degradation rate of aliphatic polycarbonate is much slower than that of PLA and some other conventional biodegradable polymers, which limits its wide application.[3] Therefore, studies on the biodegradable polycarbonates have been focused on the improvement of their degradation properties. One useful strategy for modifying the properties of aliphatic polycarbonate is copolymerization.[4,5] Another effective method is to introduce pendant functional groups to aliphatic polycarbonate, which can be used to not only adjust the properties of polycarbonates but also facilitate further modification.[6,7]

Poly(1,4-dioxan-2-one) (PDON), a well-known aliphatic polyester with outstanding biocompatibility, bioabsorbability, and good flexibility,[8] has received the approval of the Food and Drug Administration (FDA) to be used as suture material in gynecology.[9] PDON has been viewed as a candidate not only for medical use but also for general uses such as films, molded products, laminates, foams, nonwovens, and coatings.[10] Because of the poor solubility of PDON with high molecular weight, copolymers containing different DON contents have been synthesized to meet different needs.[11−14]

Tin(II) 2-ethylhexanoate, which has been approved for surgical and pharmacological applications by the FDA, is generally employed as the catalyst for the synthesis of biomedical polymers. However, it has been reported that tin(II) 2-ethylhexanoate cannot be removed by a purification process such as the dissolution/precipitation method; thus, the residual Sn may be concentrated within matrix remnants after hydrolytic degradation.[15] To avoid the potential harmful effects of metallic residues in biomedical polymer materials, enzymatic polymerization is one of the powerful candidates for polymer synthesis.[16] Enzymes, natural kinds of protein without toxicity, have remarkable properties such as high catalytic abilities and high selectivity under mild reaction conditions. Up to now, various kinds of polyesters[17−19] and polycarbonates[20,21] and their copolymers[22,23] have been synthesized by enzymatic polymerization. However, to our knowledge, most previous studies of enzyme-catalyzed polymerizations have avoided temperatures >90 °C, which is likely due to thermal deactivation of the enzyme catalyst.[24−28] It has been found that enzyme immobilization can improve the stability and recyclability of a native enzyme.[29,30] In our previous studies, we have confirmed that a silica particle is a good carrier for enzyme immobilization, and we also successfully use immobilized porcine pancreas lipase (IPPL) on silica particles for ring-opening (co)polymerization of cyclic phosphates[30] and cyclic carbonates.[20,31]

The present paper deals with a series of novel biodegradable copolymers of 5-benzyloxytrimethylene carbonate (BTMC) with DON synthesized by ring-opening polymerization in bulk using IPPL as the catalyst at high temperature. DON content was introduced to adjust the flexibility and degradation rate of aliphatic polycarbonates. On the other hand, BTMC content was employed to improve the solubility of PDON and to introduce

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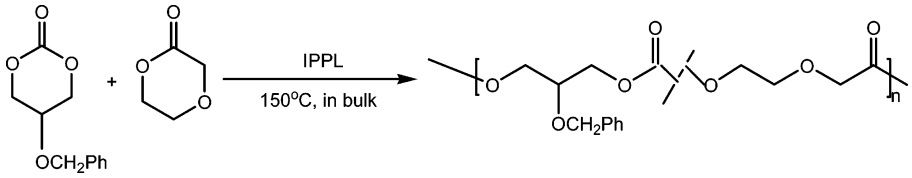
Scheme 1. Ring-Opening Copolymerization of BTMC with DON Catalyzed by IPPL



functional side groups for further modifications. It is expected a new biodegradable copolymer with an improved degradation property will be obtained that can be further applied in drug delivery systems.

Experimental Section

Materials. BTMC and DON were synthesized according to the literature.[7,32] BTMC was recrystallized several times before use (mp 140 °C). DON was redistilled over CaH_2 just before use (50 Pa, 54−56 °C). IPPL was prepared according to He.[30] Tetrahydrofuran was purified by distillation over sodium, and triethylamine was distilled over CaH_2. Ethyl chloroformate was redistilled prior to use. All other reagents were of analytical grade.

Characterization. ^{1}H NMR and ^{13}C NMR spectra were performed on a Mercury VX-300 spectrometer using tetramethylsilane (TMS) as an internal reference and $CDCl_3$ as solvent.

The molecular weights of the polymers were measured by GPC. GPC analysis was performed on a Waters HPLC system equipped with a model 2690D separation module and a 2410 refractive index detector. Chloroform was used as eluent at a flow rate of 1.0 mL/min. Sample solutions (20 μL, 1.0% (w/v)) were injected for each analysis. Waters Millennium module software was used to calculate the molecular weights on the basis of a universal calibration curve generated by narrow molecular weight distribution polystyrene standards.

The static contact angle against water of the film was determined on a contact angle system machine (OCA20, Dataphysics). Polymer films were cast from 10% (w/v) solution in methylene chloride at room temperature on a silanized glass microscopy slide. All films were dried at room temperature under atmospheric pressure for 5 days to remove residual chloroform. Angles were measured on five different regions of each polymer sample at 25 °C and the results averaged.

Enzymatic Ring-Opening Copolymerization of BTMC with DON. All reactions were carried out in bulk at 150 °C. BTMC and IPPL were dried (40 Pa, 24 h, room temperature) with anhydrous phosphorus pentoxide as desiccant before use. The mixture of BTMC, DON, and IPPL was placed in a thoroughly dried glass flask with a magnetic stirring bar. Then the flask was evacuated, purged with N_2 three times, sealed, and immersed in an oil bath at 150 °C with stirring for a predetermined time. After the reaction was completed, the reaction mixture was dissolved in dichloromethane, and the insoluble immobilized enzyme was removed by filtration. Then the solvent was concentrated under reduced pressure to obtain the crude polymer, which was further purified by reprecipitation (dichloromethane as a good solvent and methanol as a poor solvent). The copolymer was dried in vacuo at room temperature to constant weight.

For comparison, PBTMC and PDON homopolymers were also synthesized by enzymatic ring-opening polymerization under the same conditions.

Preparation of Copolymer Samples with/without Drug. The copolymer disk-shaped samples with 1 cm diameter and 1 mm thickness with/without drug were prepared by the solvent-casting method. The samples were dried at room temperature under atmospheric pressure for 3 days and then in vacuo for 24 h till a constant weight was achieved.

Hydrophilicity of Copolymers. The water uptake and static contact angle were used to evaluate the hydrophilicity of the resulting polymers. The samples were immersed in distilled water at room temperature for 2 days and weighed. After the polymers were dried under vacuum at room temperature, the weight of the dry samples was determined. The water uptake was defined as follows: water uptake (wt %) = $100(W_w - W_d)/W_d$, where W_w represents the weight of the wet sample after immersing and W_d represents the weight of the sample after drying.

In Vitro Degradation of Copolymers. Each sample was placed in an individual test bottle and incubated at 37 °C in phosphate buffer solution (PBS; pH 7.4, 0.1 M). PBS was changed at each analysis point. The degradation rate was determined by the weight loss over predetermined time intervals. Weight loss was defined as follows: weight loss (%) = $(W_o - W_t)/W_o \times 100\%$, where W_o represents the weight of the dry sample before degradation and W_t represents the weight of the dry sample after degradation at different time intervals.

In Vitro Drug Release Rate of Copolymers. Each sample containing 10 wt % ibuprofen was placed in an individual test bottle containing 2 mL of PBS at 37 °C. A 2 mL sample of solution was taken off for released ibuprofen content measurement, and the same volume of fresh PBS was added at predetermined time intervals. The concentration of ibuprofen was determined with a UV−vis spectrophotometer (Perkin-Elmer Lambda Bio 40) at the maximal absorption wavelength (λ_{max} = 264 nm). The rate of drug release was measured by the released concentration of ibuprofen at predetermined time intervals according to the calibration curve of ibuprofen.

Results and Discussion

Enzymatic Copolymerization of BTMC with DON. Copolymers were synthesized by the copolymerization of BTMC with DON at 150 °C in bulk using IPPL as the catalyst (shown in Scheme 1). The polymerization temperature was kept at 150 °C because BTMC could melt at that temperature. Under the same conditions, BTMC and DON were allowed to polymerize in the absence of enzyme for the control. After precipitation, no corresponding copolymers could be obtained, which indicates that the lipase enzymes actually catalyze the copolymerization of BTMC and DON.

The influences of polymerization time and catalyst concentration on polymerization were investigated. Table 1 shows the effect of reaction time on the copolymerization of BTMC with DON. As the polymerization time varied from 12 to 72 h, the M_n of the copolymers ranged from 12000 to 20200 with M_w/M_n between 1.82 and 2.53. M_n increased obviously when the reaction time increased from 12 to 24 h. Additional increases in the reaction time to 48 and 72 h resulted in a decrease of M_n, which might be due to thermal degradation and enzymatic

Table 1. Effect of the Reaction Time on the Enzymatic Copolymerization of BTMC with DON

entry[a]	time (h)	M_n[b]	M_w/M_n[b]	yield[c] (%)
1	12	15300	2.32	78
2	24	20200	1.82	80
3	48	12700	1.57	79
4	72	12000	2.53	72

[a] Reactions were carried out in bulk with equal monomer feed ratio at 150 °C using 4.5 wt ‰ IPPL as the catalyst. [b] Determined by GPC. [c] Calculated according to weight of the resulting polymer/(weight of BTMC + weight of DON) × 100%.

Table 2. Effect of IPPL Concentration on the Enzymatic Copolymerization of BTMC with DON

entry[a]	IPPL concn (wt ‰)	M_n[b]	M_w/M_n[b]	yield[c] (%)
5	10	10300	2.40	78
6	4.5	20200	1.82	80
7	2	10500	2.24	77
8	1	5500	1.90	73

[a] Reactions were carried out in bulk for 24 h at 150 °C with equal monomer feed ratio. [b] Determined by GPC. [c] Calculated according to weight of the resulting polymer/(weight of BTMC + weight of DON) × 100%.

Table 3. Synthesis of Poly(BTMC-*co*-DON) with Different Compositions

entry[a]	monomer ratio (feed), BTMC:DON[b]	copolymer molar composition, BTMC:DON[c]	yield[d] (%)	M_n[e]	M_w/M_n[e]
8	100:0	100:0	84	83100	1.51
9	79:21	86:14	80	63400	1.85
10	64:36	78:22	64	22300	1.84
11	50:50	54:46	80	20200	1.82
12	33:67	40:60	51	9400	1.71
13	20:80	29:71	42	5600	1.52

[a] Reactions were carried out in bulk for 24 h at 150 °C using 4.5 wt ‰ IPPL as the catalyst. [b] Monomer feed ratio, mol/mol. [c] Copolymer molar composition measured by ^{1}H NMR. [d] Calculated according to weight of the resulting polymer/(weight of BTMC + weight of DON) × 100%. [e] Determined by GPC.

degradation. Similar results were also reported by Madras[18] and Matsumura[23] in the case of enzymatic synthesis of polyesters.

The catalyst concentration had great influence on the molecular weight of poly(BTMC-*co*-DON). The results are shown in Table 2. With the increase of the IPPL concentration from 1 to 4.5 wt ‰, the M_n of the copolymer increased quickly from 5500 and 20200, while the yield also increased from 73% to 80%. However, a marked decrease in M_n with the continued increase of the IPPL concentration to 10 wt ‰ was observed. The tendency followed the same trend as that described in some other publications on enzyme catalysis.[23] The number of the initiating species increased along with the increase of the enzyme concentration, while an amount of enzyme was necessary to initiate the polymerization.

On the basis of the above results, a series of poly(BTMC-*co*-DON) copolymers with different compositions were synthesized in bulk at 150 °C for 24 h using 4.5 wt ‰ IPPL as the catalyst. When the molar ratio of BTMC increased from 20% to 79%, the M_n of poly(BTMC-*co*-DON) increased from 5600 to 63400, as shown in Table 3. It can be seen that the composition of poly(BTMC-*co*-DON) was relevant to the monomer feed ratio, while the DON incorporation into the copolymers was less than that in the monomer feed. This result suggests that the reactivity of DON in IPPL-catalyzed ring-opening polymerization was less than that of BTMC.

On the other hand, PBTMC and PDON homopolymers were also synthesized under the same conditions. Haruo Nishida[33] reported that the M_n of PDON was 1800 with PPL as the catalyst. In our studies, only some powders could be obtained (about 6.5%), which cannot be dissolved in common solvents and could not be further analyzed.

Characterization of Poly(BTMC-*co*-DON). The resulting copolymers were characterized by ^{1}H NMR, ^{13}C NMR, and GPC. The ^{1}H NMR of PBTMC was consistent with the references.[7] The typical ^{1}H NMR spectrum of poly(BTMC-*co*-DON) is shown in Figure 1. Signals from both the BTMC units and DON units can be clearly observed in the spectra of the copolymers. ^{1}H NMR ($CDCl_3$, ppm): δ = 3.84 (BTMC, CH_2CHCH_2), 4.10−4.30 (BTMC, OCH_2CH), 4.63−4.64 (BTMC, CH_2Ph), 7.32 (BTMC, aromatic), 3.74−3.78 (DON, OCH_2CH_2-OCO) (shifted downfield with the DON content increase in the copolymer), 4.12 (DON, OCH_2CO), 4.2−4.3 (DON, OCH_2CH_2-OCO). In the ^{1}H NMR spectra, the signal intensity at 3.74−3.78 (H_d), 4.12 (H_e), and 4.2−4.3 (H_c) ppm increased along with the DON content in the copolymers (shown in Figure 2). There is no evidence for decarboxylation occurrence during the polymerization because no methylene protons of ether-linked repeated units could be detected (δ = 3.4 ppm).

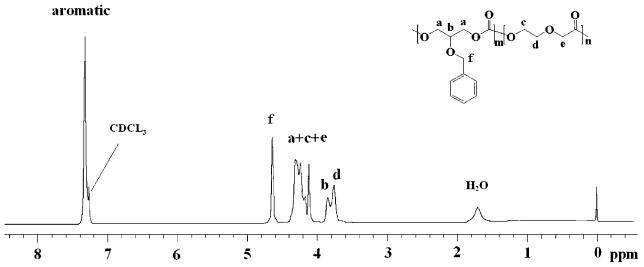


Figure 1. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of poly(BTMC-*co*-DON) (entry 11, Table 3).

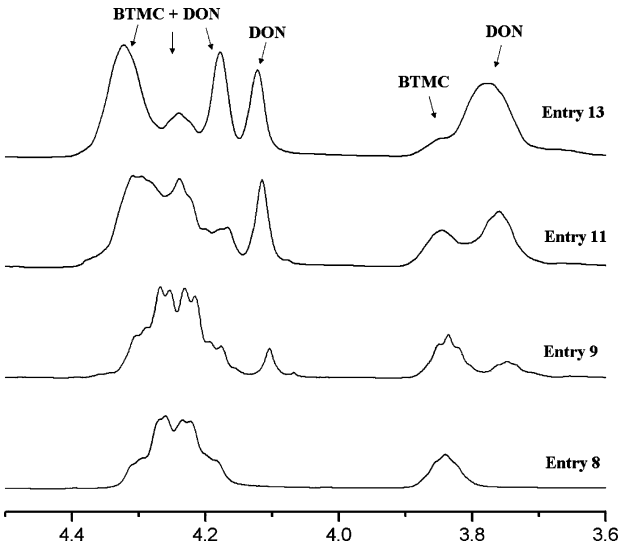


Figure 2. Expanded ^{1}H NMR spectrum of poly(BTMC-*co*-DON) with different molar compositions: PBTMC (entry 8, Table 3); 86:14 BTMC−DON (entry 9, Table 3); 54:46 BTMC−DON (entry 11, Table 3); 29:71 BTMC−DON (entry 13, Table 3).

The BTMC:DON molar ratio in the copolymers was determined from ^{1}H NMR spectra by comparing the integration of signals from the BTMC segment at 4.63−7.64 ppm and from the DON segment at 3.74−3.78 ppm.

In addition, ^{13}C NMR spectroscopy was employed to study the sequence distribution of two kinds of monomeric units and to further confirm the success of the copolymerization. Figure 3 shows the ^{13}C NMR spectrum of poly(BTMC-*co*-DON). The signals at 155.10 and 154.98 ppm are assigned to the C═O in BTMC units of BTMC−DON and BTMC−BTMC, while signals at 170.29 and 170.01 ppm are assigned to the C═O in DON units of DON−DON and DON−BTMC. These splits are due to the different chemical environments caused by the different sequences in the copolymer chain.

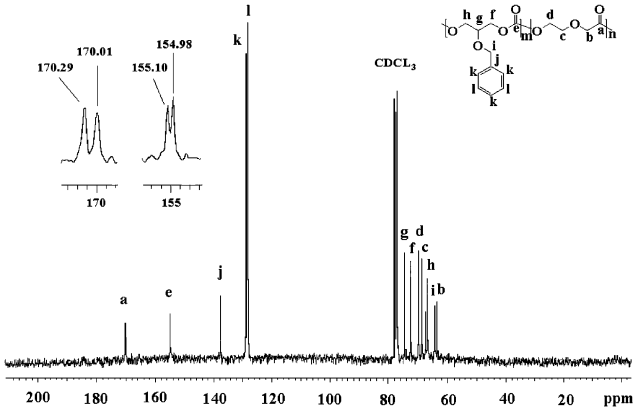


Figure 3. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of poly(BTMC-*co*-DON) (entry 11, Table 3).

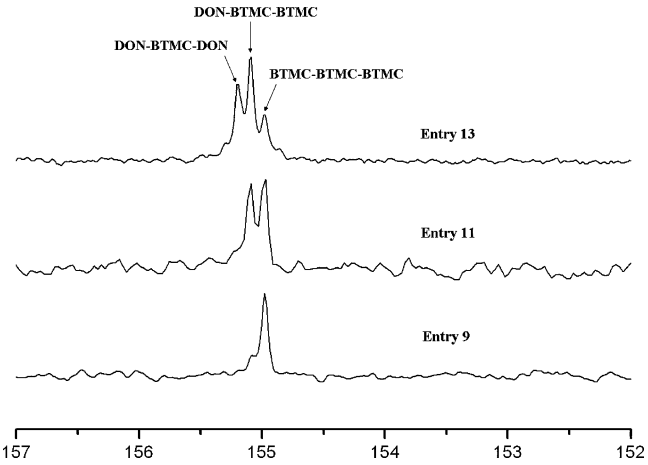


Figure 4. Expanded ^{13}C NMR carbonyl region of poly(BTMC-*co*-DON) with different molar compositions: 86:14 BTMC−DON (entry 9, Table 3); 54:46 BTMC−DON (entry 11, Table 3); 29:71 BTMC−DON (entry 13, Table 3).

The expanded BTMC carbonyl group region (152−157 ppm) of the ^{13}C NMR spectrum of the poly(BTMC-*co*-DON) with three different compositions is shown in Figure 4. As mentioned above, at monomer compositions of 54:46 BTMC−DON (entry 11, Table 3) the BTMC carbonyl resonance separates into two peaks. Changing the composition to 86:14 in favor of BTMC (entry 9, Table 3) increased the signal intensity at 154.98 ppm while significantly diminishing the intensity at 155.10 ppm. However, changing the composition to 29:71 in favor of DON (entry 13, Table 3) caused the BTMC carbonyl resonance to separate into three peaks. It could be due to the lower polymerizability of DON. Only the copolymers with richer DON contents can lead to the triad sequences. Therefore, the signals at 155.19, 155.09, and 154.97 ppm (entry 13, Table 3) are assigned to DON−BTMC−DON, DON−BTMC−BTMC, and BTMC−BTMC−BTMC, respectively. The ^{13}C NMR spectra suggested that the enzymatic copolymerizations of BTMC with DON catalyzed by IPPL on silica particles resulted in the formation of random copolymers.

For all the copolymers, GPC chromatograms showed symmetric and narrow molecular weight distributions. There was no peak in the zone of low molecular weights, thus indicating the absence of residual BTMC or DON monomer.

Hydrophilicity. The hydrophilicity can be determined by static contact angles. The results were shown in Figure 5. PBTMC has the largest contact angle of about 112.1°. With the increase of DON content from 14% to 71%, the static contact angle of the copolymers decreases from 93.3° to 71.4°.

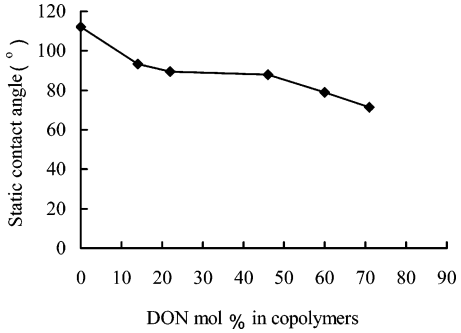


Figure 5. Correlation between the static contact angle and DON molar fraction in the copolymer.

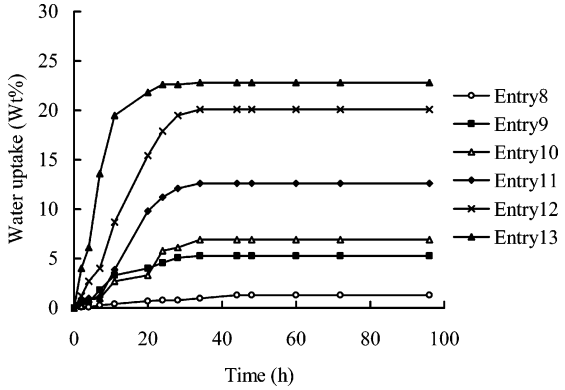


Figure 6. Water uptake of PBTMC and poly(BTMC-*co*-DON) as a function of the immersion time in water.

On the other hand, the water uptake experiments were also investigated (shown in Figure 6). The water uptake increased with immersion time and reached a plateau after 44 h. PBTMC also showed a very low hydrophilicity with the water uptake of 1.2. Incorporation of DON in the copolymers enhanced the hydrophilicity of the copolymers markedly. Increasing the DON content in the copolymers from 14% to 71% resulted in the increase of the water uptake from 5.3% to 23.2%. Furthermore, the time required to reach equilibrium water uptake decreased with richer DON content in the copolymers. The hydrophilicity of the copolymers could be adjusted by varying the DON content.

In Vitro Degradation. The in vitro degradation experiment of copolymers was carried out by immersion of the copolymer samples in PBS (pH 7.4, 0.1 M) at 37 °C. The degradation rate was evaluated by the weight loss of the polymers over predetermined time intervals (shown in Figure 7).

After 20 days of degradation in PBS at 37 °C, the weight losses of poly(BTMC-*co*-DON) are all below 8.5%. As shown in Figure 7, the copolymer compositions have some influence on their hydrolytic degradation rate. PBTMC degrades slowly because of its high hydrophobicity. With the increase of the DON content in the copolymers, the degradation rate of poly(BTMC-*co*-DON) increased, which was in agreement with the hydrophilicity of the copolymers. The reason may be that the resulting copolymers containing more DON have more hydrophilicity and water diffusion is relatively easy.

The copolymer with a composition of 54:46 BTMC−DON (entry 11, Table 3) was selected as a model to study the M_n changes during the degradation process. The results showed that only a 3% M_n decrease could be observed after 20 days of degradation.

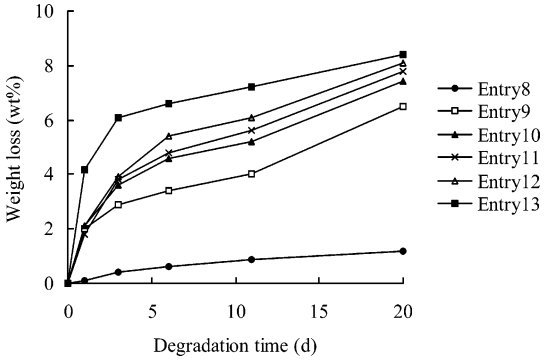


Figure 7. In vitro degradation of poly(BTMC-*co*-DON) (PBS, 0.1 M, pH 7.4; 37 °C).

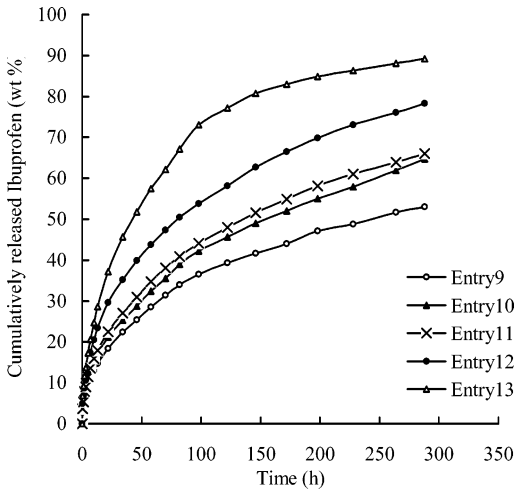


Figure 8. Release profile of ibuprofen from poly(BTMC-*co*-DON) (PBS, 0.1 M, pH 7.4; 37 °C).

In Vitro Drug Release. Ibuprofen was selected as the model drug to investigate the drug release property of poly(BTMC-*co*-DON) in vitro. The release rate was monitored by determining the concentration of released ibuprofen at predetermined time intervals. The results are shown in Figure 8. Although the in vitro degradation rate was slow, the rate of ibuprofen release from the copolymer film was quite different, which indicated the drug release occurred by diffusion. With the increase of the DON content in poly(BTMC-*co*-DON), the ibuprofen release rate increased markedly, which was consistent with the hydrophilicity of the copolymers. After 12 days, the amount of ibuprofen release could reach 89% in the case of poly(BTMC-*co*-DON) (29:71, entry 13 in Table 3) while only 52% in the case of poly(BTMC-*co*-DON) (86:14, entry 9 in Table 3). Therefore, the degradation rate as well as drug release rate of poly(BTMC-*co*-DON) could be controlled by adjusting the copolymer compositions.

Conclusions

A series of poly(BTMC-*co*-DON) copolymers with different compositions were successfully synthesized in bulk at 150 °C by ring-opening polymerization using IPPL on silica particles as the catalyst. The BTMC monomer had higher reactivity in comparison with the DON monomer, which led to higher BTMC contents in the copolymers than that in the feed. The hydrophilicity of poly(BTMC-*co*-DON) increased along with the DON content. Both the degradation rate and drug release rate of poly(BTMC-*co*-DON) can be tailored by adjusting the copolymer compositions.

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