Polymerization of Trimethylene Carbonate with High Molecular Weight Catalyzed by Immobilized Lipase on Silica Microparticles

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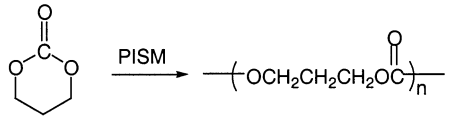
Introduction. Worldwide potential demands for biodegradable polymer materials are quite significant from environmental and biomedical viewpoints. Aliphatic polycarbonates have become one of the most promising biodegradable materials for their biocompatibility, low toxicity, and biodegradability as well as excellent mechanical properties.[1−3] The homopolymerization and copolymerization of aliphatic cyclic carbonates such as trimethylene carbonate (TMC) have been extensively studied.[4−8]

In the past decade, enzyme-catalyzed polymerization has been of increasing importance as a new trend in macromolecular science. Among them, enzymatic ring-opening polymerization has received much attention as a new methodology of biodegradable polymer synthesis for lactones, cyclic carbonates, and other cyclic monomers.[9−15] Compared with it, chemical methods need extremely pure monomers and anhydrous conditions as well as metallic initiators which must be completely removed because of its possible toxicity, especially for medical application. Although the environmental benefits of the process and products may be clear, a number of demands still exist in enzymatic polymerization methods from the laboratory to industrial processes: (I) improvement of the catalytic activities displayed by enzymes; (II) development of highly stable catalysts, suitable supports, and processes that facilitate the recycling of the enzymes; (III) decrease of the amount and cost of the enzyme catalysts.[16]

It was found that some immobilized lipase enzymes showed better catalytic efficacy than naked lipase enzymes due to their thermal activation and thermal stability, and they could be recycled for the reutilization.[11,13] Porous silica beads, activated by methanesulfonic acid, are effective and economic inorganic carriers for enzyme immobilization.[17] We have reported the preparation of PPL immobilized on porous silica beads (120−160 mesh) and its application in ring-opening polymerization of cyclic phosphates.[13] It was found that immobilized PPL lipase would exhibit higher activity than naked enzymes and much higher activity when recovered immobilized PPL was used again in the polymerization. In the present paper, we first prepared immobilized PPL using silica microparticles (narrow distributed micron glass beads) as carrier and took it as catalyst for the enzymatic ring-opening polymerization of TMC (Scheme 1). The higher catalytic activity and recycled lipase used for many times are expected to obtain so as to help resolve the mentioned problems.

Experimental Section. a. Materials. Porcine pancreas lipase (PPL) was purchased from Sigma with an activity of 198.90 u/mg protein (at pH 6.0 using olive oil as substrate) and used without further purification. Silica microparticles (narrow distributed micron glass beads) were obtained from Dupont Co. Glutaraldehyde (25% (w/v)) was purchased from Merck. 3-Aminopropyltriethoxysilane was obtained from the Chemical Plant of Wuhan University of China and redistilled before use (bp 213−216 °C). Olive oil was of chemical grade and without any treatment before use. TMC was synthesized as described previously.[18] After recrystallization in acetyl ester for three times, TMC was obtained by distillation under reduced pressure. The melting point was 43−44 °C. All other reagents used in this study were of analytical grade.

Scheme 1. Polymerization of TMC Catalyzed by PISM



b. Enzyme Immobilization. Silica microparticles were treated as the similar method described by Liu and Zhuo.[17] Enhanced surface activity of silica microparticles was achieved by treatment with methanesulfonic acid instead of concentrated hydrochloric acid. The silanization of the activated silica microparticles by 3-aminopropyltriethoxysilane was carried out in two steps: first at room temperature and then at reflux temperature. Lipase PPL was covalently immobilized on these functional silica microparticles by cross-linking with glutaraldehyde. A 1.0 g sample of functional silica microparticles was mixed with 77 mg of lipase and 0.375% (w/v) glutaraldehyde solution in a 0.1 mol/L phosphate buffer, pH 8.0. After the reaction was maintained with slow magnetic stirring for 12 h at 25 °C, the lipase immobilized on silica microparticles was filtered, washed thoroughly with redistilled water, and dried in vacuo at 25 °C. A good coupling yield was achieved, which was equivalent to 53.2 mg of native lipase/1 g of silica microparticles while it was 41.88 in the liter using silica beads (120−160 mesh).[13] It indicated that silica microparticles improved immobilization efficiency much more.

c. Measurement. FTIR spectra were obtained on a Nicolet-170SX FTIR spectrophotometer. Thin films were cast from chloroform solutions onto a NaCl plate. ^{1}H NMR and ^{13}C NMR spectra were recorded in a solution of $CDCl_3$ on a Varian Mercury-VX 300 apparatus and TMS as internal standard. M_n was measured by gel permeation chromatography (GPC). GPC was carried out on a Waters HPLC system equipped with a Model 2690D separation module, a Module 2410 refractive index (RI) detection system, and a Shodex K803 column. Chloroform was used as eluent at a flow rate of 1.0 mL/min. Waters MILLIENIUM^{32} module software was used to calculate molecular weights based on a universal calibration curve generated by narrow molecular weight distribution polystyrene standards.

d. Enzymatic Polymerization of TMC. All reactions were carried out in bulk at determined temperature. The monomer and lipase were dried (40 Pa, 24 h, room temperature) with anhydrous phosphorus pentoxide as desiccant before use. A typical preparation of PTMC with an M_n of 14 700 (entry 4 in Table 1) was carried out as follows. A mixture of TMC (1.02 g) and

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Table 1. Ring-Opening Polymerization of TMC by Using Immobilized PPL on Silica Microparticles

entry	conc (wt %)	temp (°C)	time (h)	M_n	M_w/M_n	yield (%)
1	0.1	100	24	48 000	2.00	66
2	0.25	100	24	25 800	1.93	70
3	0.5	100	24	21 800	1.78	56
4	1.0	100	24	14 700	1.92	70
5	2.0	100	24	9 700	2.12	58
6	1.0	60	24	*a*		
7	1.0	80	24	7 300	2.26	81
8	1.0	120	24	8 700	1.81	80
9	0.5	100	12	15 800	1.98	60
10	0.5	100	36	19 900	1.86	68
11	0.5	100	48	19 800	2.20	77
12	0.5	100	60	15 300	1.84	81
13[b]	1.0	80	24	12 100	1.50	79
14[b]	1.0	100	24	25 400	1.56	75
15[b]	1.0	120	24	15 800	1.76	70

[a] After precipitation we could not obtain the corresponding polymer. [b] Recovered enzymes from entries 7, 4, and 8 were used for the polymerization in entries 13, 14, and 15, respectively.

PPL immobilized on silica microparticles (PISM) (10.2 mg) was introduced to a thoroughly cleaned and dried glass flask with a magnetic stirring bar. The vessel was vacuumed and then purged with argon several times. The flask was then closed with a glass stopper and immersed in an oil bath and held at 100 °C for 24 h. The resulting polymers were dissolved in dichloromethane (10 mL) and filtered to remove the lipase enzymes. Then the filtrate was concentrated under reduced pressure to quantity to obtain the crude polymer and precipitated in menthol (75 mL) as a poor solvent. The polymers were reprecipitated for two times and dried in vacuo to constant weight and stored under dry conditions.

e. Recycling of PISM. After the polymerization of TMC, the product was dissolved in dichloromethane (10 mL) and then transferred into a centrifuge tube. The solution and insoluble enzyme were obtained after separating them by a centrifuge. The recycled enzyme was then washed with dichloromethane, and the separation process was repeated five times to thoroughly isolate PISM and polymer solution. The recycled PISM was then dried in vacuo to constant weight.

Result and Discussion. It was found that TMC (trimethylene carbonate) might be polymerized at the presence of immobilized PPL on silica microparticles (PISM). The corresponding PTMC obtained had a M_n of up to 87 400 with a M_w/M_n within 1.5−2.26. ^{1}H NMR and ^{13}C NMR confirmed the structure of the PTMC. ^{1}H NMR of PTMC ($CDCl_3$): δ = 2.0−2.1 (m, $-OCH_2CH_2-$), 4.2−4.3 (t, $-OCH_2-$). ^{13}C NMR of PTMC: δ = 28.017 ($-OCH_2CH_2-$), 64.272 ($-OCH_2-$), 154.908 (C=O). The ^{1}H NMR spectra of PTMC obtained offered no evidence of decarboxylation occurring during the polymerization because no signals at 3.4 ppm characteristic of an ether group ($-CH_2-O-CH_2-$) could be detected.

Table 1 showed the typical ring-opening polymerization of TMC catalyzed by PISM. The polymerization was carried out at different temperatures (60, 80, 100, and 120 °C) as well as concentration ranging from 0.1 to 2.0 wt % for the determined polymerization time.

It was found that no polymer was obtained by precipitation using dichloromethane as a good solvent and methanol as a poor solvent when the polymerization was carried out at 60 °C in the presence of PISM. The most preferable temperature for the polymerization of TMC catalyzed by PISM would be 100 °C. Compared with the polymerization at 80 and 120 °C, the higher M_n and similar yield were obtained at 100 °C for both the native and recovered PISM.

Table 2. Variation of *Mn* and Yield Catalyzed by Recycled Lipase for Many Times at 100 °C for 24 h

entry	time	conc (wt %)	M_n	M_w/M_n	yield (%)
1	I[a]	1.0	14 700	1.92	70
2	II[b]	1.0	25 400	1.77	75
3	III	1.0	30 800	2.11	81
4	IV	1.0	45 200	2.01	78
5	VI	1.0	61 700	1.94	83
6	VII	1.0	63 000	2.53	81
7	VIII	1.0	64 000	2.20	72
8	VIII[c]	0.1	87 400	2.06	69

[a] The immobilized lipase PPL was used for the first time. [b] The recycled immobilized lipase PPL was use for the second time. The rest is the same as this method. [c] The polymerization was carried out at concentration 0.1 wt % by using recycled immobilized PPL which was the seventh used.

The enzyme concentration had much influence on the M_n of PTMC polymerized at 100 °C for 24 h polymerization. The M_n of the PTMC decreased with the increasing lipase concentration from 0.1% to 2.0%. The maximum M_n of PTMC 48 000 was obtained at a lipase concentration of around 0.1%. This might be ascribed to the number variation of the initiating species by increasing the enzyme concentration. On the other hand, the yield was almost invariable with the increasing of lipase concentration. These results were in agreement with the polymerization of TMC catalyzed by native PPL.[11]

The time course of the ring-opening polymerization of TMC catalyzed by PISM was studied. The experiments were carried out at 100 °C with the enzyme concentration of 0.5%. The result data indicated that the M_n of the polymers was increased to the maximum 21 837 after 24 h polymerization and then gradually decreased. On the other hand, the yield was slightly increased with the increasing polymerization time. This was probably caused by the degradation also catalyzed by PISM.

It was obvious that the activity of recovered PISM was increased when they were used again. The polymerization of TMC was performed at 80, 100, and 120 °C catalyzed by recovered PISM for the first use. The M_n of the polymers was significantly increased compared with that of the polymers catalyzed by native PISM while the yield had no marked change. In other words, the recovered PISM again worked more actively for the polymerization of TMC.

On the basis of the above experiments, we polymerized TMC using recycled PISM as catalyst for repeated uses (Table 2). It was found that, with the increase of the repeated use times, the catalytic activity would increase and tend to keep constant. The polymerization was carried out at the concentration of 1.0 wt % and 100 °C. The M_n of the polymers was increased from 14 700 to 61 700 and kept unchanged around 63 000 (Figure 1). This was probably due to the number of the initiating species in IPSM which was constant after several repeated uses. On the other hand, it seemed that the yields of the polymers were not changed on the whole. In continuation to the experiments concerning enzyme concentration effects, PTMC was synthesized at 100 °C for 24 h polymerization by using recovered PISM for the seventh use at 0.1% concentration. The

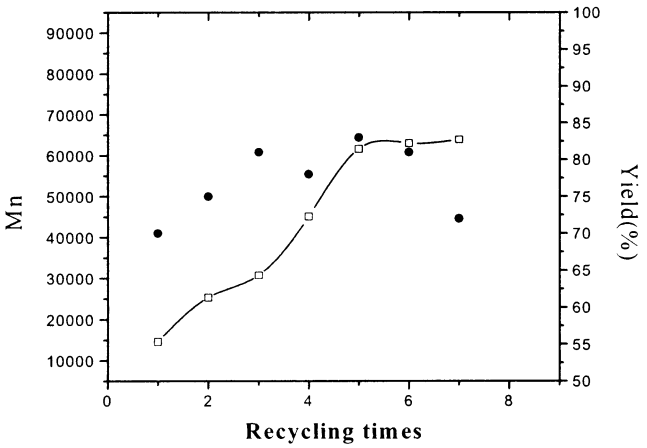

Figure 1. Variation of M_n and yield vs the used time of the recovered PISM: ●, yield; □, M_n.

same result was found that the very high M_n of 87 400 with a yield of 69% could be obtained.

It was known that TMC could be polymerized using enzymes as catalysts. Up to date, the highest M_n is 48 000 with the M_w/M_n of 3.5 by enzymatic ring-opening polymerization.[11] In the present paper, we adopt silica microparticles which possessed much bigger surface as carrier. Compared with other observed enzymes, PISM showed much better catalytic activity in the polymerization of TMC in bulk. The highest M_n of the polymer was increased to 87 400 with the M_w/M_n of 2.06. Furthermore, the PISM could be recycled for many times and showed much higher activity compared with the native PISM and naked PPL. All these might provide favorable foreground for its wide use.

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