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PAPER

Solvent-free lipase-mediated synthesis of six-membered cyclic carbonates from trimethylolpropane and dialkyl carbonates†

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Six-membered cyclic carbonates with hydroxyl and/or alkoxycarbonyloxy groups, as potential monomers for polyurethanes and polycarbonates, were prepared by the reaction between trimethylolpropane (TMP) with dimethyl carbonate (DMC) or diethylcarbonate (DEC) mediated by immobilized *Candida antarctica* lipase B, Novozym® 435 in a solvent-free medium. The dialkyl carbonate served as a solvent for the reaction. The solubility of TMP at 50 °C was 565 mg mL^{-1} in DMC, and 64.8 mg mL^{-1} in DEC. Reactions using biocatalyst concentrations of 10, 20 and 40% (w/w of TMP) showed similar profiles, with a linear increase in the conversion of TMP to 90% within 24 h, and complete conversion within 48 h. At biocatalyst concentrations of 2.5 and 5% (w/w of TMP), the main products were mono-carbonated TMP (**3**) and/or cyclic carbonate (**4**), while di and tri-carbonated TMP (**5**, **7**) were obtained at lipase concentrations of 20 and 40%. The reactivity of DEC was lower than that of DMC, but led to higher selectivity in production of **3** or **4**. A large fraction of the linear carbonates in the product mixture were cyclized by disproportionation involving heating at 60–80 °C without any catalyst. The total yield of cyclic carbonates was about 85% after thermal treatment at 80 °C. This process, consisting of lipase-catalyzed transesterification and thermal disproportionation, provides a novel and more environmentally friendly approach for the synthesis of cyclic carbonates without using toxic organic solvents, phosgene or isocyanate.

Introduction

The synthesis of carbonate analogues, especially five- and six-membered cyclic carbonates, has received much attention lately due to their potential application as monomers for environmentally benign production of polycarbonates and polyurethanes.[1,2] These types of polymers are mainly produced using toxic phosgene and/or isocyanates.[3–5]

Aliphatic polycarbonates are tough, dimensionally stable thermoplastics widely used in engineering and optical applications. Aliphatic polycarbonates and their copolymers are biodegradable and recyclable,[6,7] and are expected to find use in the biomedical field because of their biocompatibility and low toxicity.[8,9] Polyurethanes are widely used in a variety of applications such as foams, seals, and high-performance coatings and adhesives. In recent years, a variety of biomedical polyurethane elastomers exhibiting improved hydrolytic stability have been developed. Due to their toughness, durability, biocompatibility, and improved biostability, they have been incorporated into a wide variety of implantable biomedical devices.[10] A demand has now emerged for new types of polyurethanes providing unaltered properties, produced without the use of toxic starting materials.

Among the alternative greener routes that have been proposed, a popular way to synthesize polycarbonate is the ring-opening polymerization (ROP) of cyclic carbonates in bulk or solution, usually using metallic compounds as catalysts.[11] Use of metal-free or low-toxicity catalyst/initiators systems for the ROP of carbonates, such as alcohols/diols, and acid, have increasingly attracted attention. The ROP initiated by alcohols in the absence of a catalyst has been extensively investigated, since the use of hydroxyl groups (OH) in the alcohols as initiators allows for control of the molar mass, improvement in the hydrophilicity/degradability, and further functionalization of the resulting biodegradable polyesters and polycarbonate.[11]

Lately, a number of reports have appeared on the synthesis of five-membered and six-membered cyclic carbonates by a phosgene-free route.[12–17] For use in the ROP process, however, six-membered cyclic carbonates are preferred to five-membered carbonates because they are less thermodynamically

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stable than their ring-opened polymers, and thus retain CO_2 during the polymerization process.[2] Synthesis of six-membered trimethylene carbonate is traditionally achieved by reacting 1,3-propanediol with phosgene or its derivatives. Among the other reactions studied, metal-catalysed coupling of oxetanes such as trimethylene oxide with carbon dioxide has given high yields of trimethylene carbonate.[2] Endo *et al.*[18] have reported a method of cyclic carbonate synthesis from propane-1,3-diols and ethyl chloroformate in the presence of a stoichiometric amount of triethylamine. Transesterification of propane-1,3-diols with dialkyl carbonate catalyzed by metal or organo-catalysts has been proposed as a more environmentally benign procedure.[19,20] The lipase-catalysed transesterification reaction between dialkyl carbonate and 1,3-diol in an acetonitrile–toluene solvent system for the synthesis of a cyclic trimethylene carbonate (1,3-dioxane-2-one) monomer with/without a methyl substituent has also been reported using very high concentration of the biocatalyst (900% w/w of the diol).[21]

However, the syntheses of six-membered cyclic carbonates with functional groups from poly-functional alcohols such as trimethylolpropane (TMP) or pentaerythritol (PE) have required more complicated methods with low yields.[22,23] Polycyclic six-membered carbonates could be prepared by radical polymerization of acrylic monomers with pendant cyclic carbonate groups.[22] As a different approach, tris- or tetrakis(alkoxycarbonyloxy) derivatives, obtained from catalytic transesterification of the polyol, TMP and diethylcarbonate (DEC), have been subjected to thermal disproportionation using Aerosil 200 at 200–220 °C followed by distillative depolymerisation under reduced pressure, to give the cyclic product 5-ethyl-5-ethoxycarbonyloxymethyl-1,3-dioxan-2-one with a low yield.[23]

This paper presents a study showing the possibility of the synthesis of six-membered cyclic carbonates with functional groups by lipase-mediated reaction between trimethylolpropane (TMP) with dimethyl carbonate (DMC) or DEC in a solvent-free medium followed by thermal disproportionation without any catalyst.

Results and discussion

Solubility/miscibility of TMP in DMC and DEC

Solvents such as THF, acetonitrile and toluene have been employed to enhance co-solubilization of substrates in biocatalytic syntheses of cyclic carbonates.[15,19] Although some environmentally benign alternatives to the use of organic solvents in organic syntheses, *e.g.* ionic liquids, are proposed for enzymatic reactions,[24] the most desirable strategy from environmental and economic perspectives is to carry out the reaction without a solvent. In the present system, the dialkyl carbonate can serve as a solvent due to a relatively low boiling point (DMC: 90 °C and DEC: 126 °C) allowing easy removal and recycling. The solubility of TMP in DMC and DEC was thus investigated at different temperatures. DMC is an inexpensive, environmentally benign chemical having interesting solvating properties, low toxicity and high biodegradability.[25]

As seen in Fig. 1, the TMP solubility increased at temperatures higher than 30 °C, and the TMP was fully soluble in DMC at 50 °C at concentrations up to 565 mg mL^{-1} (1.3 g TMP added to 1 mL DMC), and in DEC up to 64.8 mg mL^{-1}. At 60 °C there was a phase change of TMP (melting point 57 °C), which was completely miscible with DMC (2 g TMP added to 1 mL DMC) but formed a biphasic system with DEC. The polyol concentration in the upper DEC phase was 138.3 mg mL^{-1}. The solubility of TMP was thus sufficient for reaction at 50–60 °C, which is an optimum temperature range for the activity of immobilized lipase.[26]

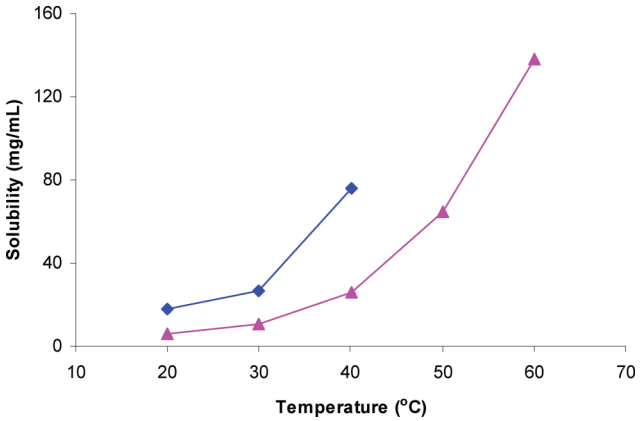

Fig. 1 Solubility of TMP in DMC (◆) and DEC (▲) with increase in temperature. Experimental details are stated in the text. TMP was soluble in DMC at 50 °C up to a concentration of 565 mg mL^{-1}.

The stability of a biocatalyst is usually higher in more hydrophobic solvents (*i.e.* those with higher log*P* – the logarithm of the partition coefficient of solvent between water and octanol), such as toluene (2.5) and n-hexane (3.5). In contrast, solvents with lower log*P* values such as pyridine, dimethyl formamide (DMF, –1.0), dimethyl sulfoxide (DMSO, –1.3) and acetonitrile (–0.33) can solubilise many polar molecules like TMP, but often inactivate the enzyme by their ability to remove water molecules of hydration and promote accumulation of water in the reaction medium.[27–29] Hence, the balance between hydrophobicity and solubility needs to be considered in enzymatic reactions. The log*P* values of DMC and DEC were calculated as 0.23 and 1.22, respectively, according to Parham's method.[30] A stability test of immobilized *Candida antarctica* lipase B, Novozym® 435 (N435), in DMC, DEC, acetonitrile and n-hexane at 60 °C showed the residual (esterification) activity after 72 h incubation to be 67.6, 77.6, 63.1 and 82.4%, respectively. The biocatalyst was more stable in DEC than in DMC and acetonitrile. As expected, the highest stability was obtained in n-hexane, a solvent that did not dissolve TMP and provided poor conditions for the enzymatic reaction.

Structure elucidation of the products and proposed reaction pathway

Due to the unavailability of standard compounds, TMP was initially reacted with excess of DMC or DEC at 60 °C using N435 as catalyst, to determine the identity of the products formed. All the reaction components were analysed by gas chromatography, and identified by GC-MS and ^{1}H-NMR (Table S1†).

GC analysis showed the presence of several peaks (Fig. 2A). All product peaks on GC chromatograms were purified by silica flash chromatography, and the chemical shifts of these products

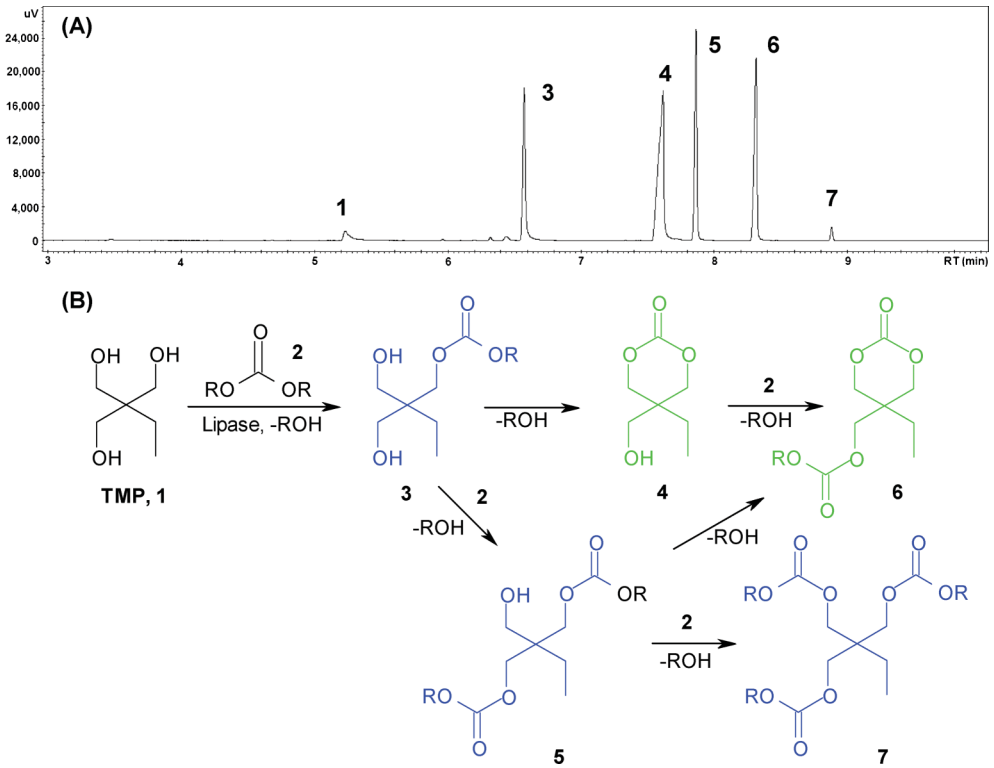

Fig. 2 Gas chromatogram (A) and scheme of the possible reaction pathway (B) for lipase-catalyzed reaction of TMP and dialkyl carbonate. R = CH_3 (DMC) or CH_2CH_3 (DEC).

were elucidated by ^{1}H-NMR (Table S1), which agreed with the structures shown in Fig. 2B. A possible reaction pathway was thus proposed. A lipase-catalysed transesterification reaction between the TMP (**1**) hydroxyl groups and the dialkyl carbonate (**2**) would result in the formation of linear carbonates, which appeared as products **3** and **5** (TMP mono- and dicarbonate, respectively). The transesterification products were further converted to the corresponding cyclic carbonates **4** and **6** (Fig. 2). **7** (TMP-tricarbonate) was formed in extremely small amounts.

In contrast to the GC results, GC–MS data did not reveal any masses corresponding to the linear carbonates, but confirmed the presence of cyclic carbonates. This implies that the GC–MS data of the linear carbonates, **3**, **5** and **7**, gave masses of demethylated and decarbonated products, 160.9 ($M-32$ (–$HOCH_3$)), 219.0 ($M-32$(–$HOCH_3$)) and 233.0 ($M-76$ (–$HOCOOCH_3$)), respectively. This was probably due to exposure of the product samples to the high temperature in the injection port. Masses of 160.9 and 219.0 correspond to that of cyclic carbonates, **4** and **6**, respectively, obtained from reaction of TMP with DMC and DEC, respectively (Table S1†).

Effect of Novozym® 435 concentration on the reaction between TMP and DMC

N435 was used at concentrations of 2.5, 5, 10, 20 and 40% (w/w of TMP) for reaction with DMC at 60 °C. As shown in Fig. 3, reactions employing higher biocatalyst amounts (10, 20 and 40%) showed nearly identical time courses, with a linear increase of the conversion of TMP to reach 90% within 24 h, and continued reaction leading to complete conversion (98.5% for

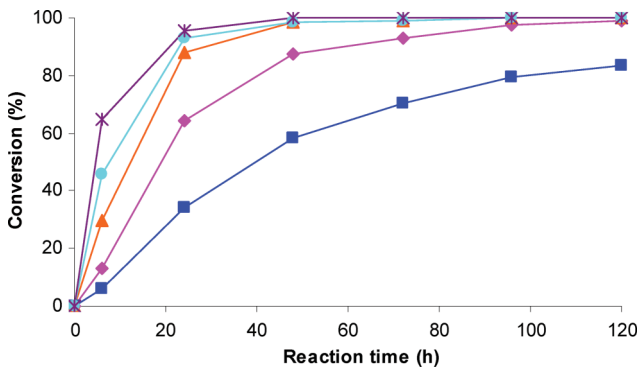

Fig. 3 Effect of Novozym® 435 concentration on conversion of TMP during reaction with DMC at 60 °C. The biocatalyst was used at 2.5% (■), 5% (◆), 10% (▲), 20% (●) and 40% (✱) concentration (w/w), respectively, with respect to TMP.

10% (w/w) biocatalyst, and 100% for 20 and 40% biocatalyst) within 48 h. Conversions of 98.9% and 83.3% were achieved with 5% (w/w) and 2.5% N435, respectively, at 120 h. Excellent reproducibility of the results was demonstrated.

The proportions of products formed varied depending on the biocatalyst concentration and reaction time (Table S2†). In the reaction with 2.5 and 5% N435, the TMP-monocarbonate **3** was the predominant product (62%) along with other products in the order **4** > **5** > **6** until 48 h reaction time, and subsequently the amount of **3** decreased due to conversion to the above products. Cyclic carbonate formation seemed to be preferred rather than further transesterification of **3**. Compound **7** (TMP-tricarbonate) was not detected except in the sample from 120 h

reaction with 5% N435. With 10% N435, concentrations of the products formed at 48 h varied in the order **5** > **3** > **6** > **4** > **7**, indicating increased activity of the biocatalyst for transesterification (Table S2†). With continued incubation, the levels of **5**, **6** and **7** increased, while that of **3** decreased (Table S2,† Fig. 4). With further increase in the biocatalyst concentration (20, 40%), the main product was TMP-dicarbonate **5**, along with all the other products. **7** was formed in significant amounts, and levels of both **5** and **7** increased when the biocatalyst concentration was increased from 20 to 40%, indicating that the transesterification activity was dominating over the subsequent cyclic carbonate formation. Among the cyclic carbonates, the levels of **6** were higher than that of **4** due to higher proportion of **5** formed.

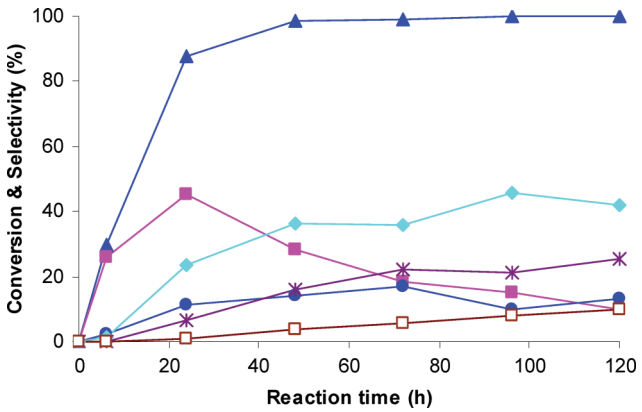

Fig. 4 Conversion of TMP (▲) and formation of products, **3** (■), **4** (●), **5** (◆), **6** (✱), and 7 (□), respectively, during the reaction of the polyol with DMC catalysed by 10% (w/w of TMP) Novozym® 435 at 60 °C.

The immobilized lipase concentrations used here are much lower than that used in the earlier reports of the synthesis of six-membered trimethylene carbonate (1,3-dioxane-2-one) monomer (900% w/w of 1,3-diol)[21] and five-membered glycerol carbonate (55% w/w of glycerol).[15] In the former case, oligomers were formed as side-products, which were recovered for further transformation to cyclic carbonate, obtained in a final yield of 53%.[21]

Effect of reaction temperature on reaction yield and product selectivity

The optimum temperature for the activity of N435 is reported to be around 70–80 °C, although lower temperatures are recommended due to the risk of thermal inactivation.[26] However, the optimum reaction rate varies depending on the kind of reaction, properties of substrates and solvents used. The reaction efficiency and product profile were investigated for the reaction of TMP with DMC using 10% (w/w) N435 at 50, 60 and 70 °C, respectively. The reaction rate was slightly higher at 60 °C than at the other temperatures (Fig. S1†). Irrespective of the temperature, a maximum TMP conversion of 98% was obtained, although with significantly different proportions of products (Table S3†). The product profile obtained at 50 °C was in the order **3** > **5** > **4** > **6**, while at 60 °C, higher concentrations of **5** compared to **3** were obtained due to higher enzyme activity. The levels of the cyclic carbonates were still in a lower range, with **6** being greater than **4**. Meanwhile, cyclic carbonates **4** and **6** were obtained as the main products (about 75% of total) during the reaction at 70 °C. This was an indication that high temperature is important for decarbonation of the linear carbonates to cyclic carbonates. Hence, the optimum temperature is 50 °C to produce linear mono-carbonated product, and 70 °C to produce cyclic carbonates. It may be possible to utilize the entire product mixture containing high proportion of cyclic carbonates for a coating formulation, but when there is a need for high-purity cyclic carbonates the components of the product mixture can most probably be separated by distillation or crystallization.

Reaction between TMP and DEC catalysed by Novozym® 435

Although both DMC and DEC can undergo transesterification with TMP, they have different physicochemical properties. DEC is more hydrophobic, larger in size, and dissolves TMP to a lower extent. As shown in Fig. 5, DEC had lower reactivity than DMC. The conversion of TMP during reaction with DEC using 20% (w/w) N435 reached 98.7% at 120 h, while reaction under similar conditions with DMC using 5–10% (w/w) lipase reached same conversion at 48 h. Nevertheless, the trend of product formation was almost the same as for DMC (Table S4†, Fig. 6).

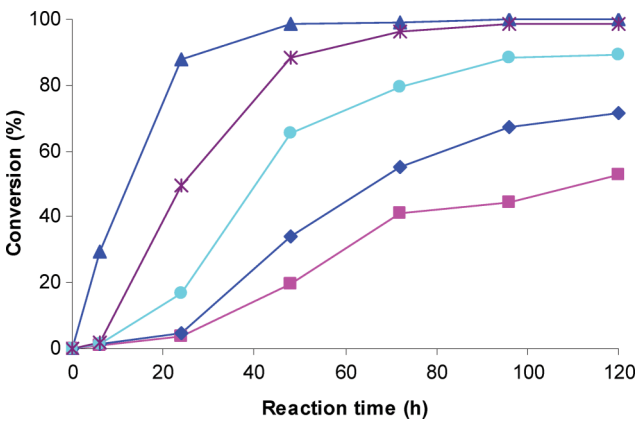

Fig. 5 Comparison of the reaction profiles of TMP with DEC using 2.5% (■), 5% (◆), 10% (●), and 20% (w/w of TMP) (✱), respectively, of Novozym® 435 with the reaction between TMP and DMC using 10% N435 (▲) at 60 °C.

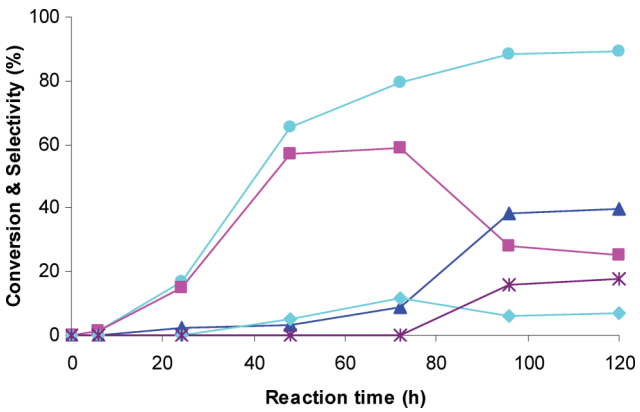

Fig. 6 Conversion of TMP (●) and formation of products, **3′** (■), **4** (▲), **5′** (◆), and **6′** (✱), respectively, during the reaction of the polyol with DEC using 10% (w/w of TMP) Novozym® 435 at 60 °C. The marks (′) indicate that the products were from reaction with DEC (R=CH_2CH_3).

Table 1 Disproportionation of carbonates by thermal treatment

Run	Reaction temp. (°C)	Reaction time (h)	TMP	Carbonate products				
				Cyclic (%)		Linear (%)		
				4	**6**	**3**	**5**	**7**
1[a]	Starting Material		2.6	35.3	22.7	17.1	21.0	1.3
2	60	48	3.9	41.4	23.6	13.1	16.6	1.4
3	70	48	4.3	49.6	24.5	10.6	9.4	1.6
4	80	48	3.9	55.3	23.3	8.2	7.6	1.7
5	80	144	3.1	63.6	21.7	5.3	4.6	1.7

[a] Starting material (Fig. 2A) was prepared from 5 g TMP in 40 mL DMC using 10% Novozym® 435 at 60 °C and used for thermal treatment.

Compound **3′** (mono(ethoxycarbonyloxy)-TMP) was the main product (>87%) with DEC using 2.5–10% (w/w) N435 until 48 h, which on prolonged reaction was converted mainly to **4** (72–44%). No **7′** (tris(ethoxycarbonyloxy)-TMP) was detected. With 20% biocatalyst, besides **3′** (75%), a significant amount of **5′** (bis(ethoxycarbonyloxy)-TMP) (21%) was also formed, and on prolonged incubation more **6′** (mono(ethoxycarbonyloxy)-TMP cyclic carbonate) than **4** was formed. Increasing the temperature to 70 °C for the reaction with 10% N435, the extent of TMP conversion was reduced to 44% at 48 h, perhaps due to deactivation of the biocatalyst, and the product was composed of almost equal amounts of **3′** and **4** (Table S4†). These results point towards the possibility of achieving higher selectivity for cyclic carbonate **4**, although at the expense of lower substrate conversion. The selectivity could be related to a lower reaction rate for the enzyme with DEC, at the same time providing more time for cyclic carbonate formation than with DMC.

Disproportionation of carbonates by thermal treatment

As the reaction of TMP with the dialkyl carbonates produced several products including the desired cyclic carbonate, it was of interest to study if the linear carbonates present in the product mixture could be converted to the cyclic forms. Conversion of 1,1,1-tris(ethoxycarbonyloxymethyl)propane to the cyclic carbonate, 5-ethyl-5-ethoxycarbonyloxymethyl-1,3-dioxan-2-one, by thermal disproportionation with Aerosil 200 at 200–220 °C followed by distillative depolymerisation under reduced pressure, reported earlier, is energy-intensive and not suitable for polyfunctional alcohols because of possible crosslinking during transesterification with carbonic acid esters.[23]

In the present study, disproportionation by thermal treatment without any catalyst was tested for the formation of cyclic carbonates. The starting material for this was the reaction mixture shown in Fig. 2A, prepared from reaction between 5 g TMP and 40 mL DMC using 10% (w/w) N435 for 72 h reaction at 60 °C, followed by filtration to remove solids including the immobilized lipase and molecular sieves, and evaporation. It contained a higher content of cyclic carbonates than preparations on a small scale. Disproportionation was performed by heating with shaking at 60–80 °C under atmospheric pressure to allow free evaporation of the resulting methanol. At the higher temperature (80 °C), a significant decrease in the content of linear carbonates **3** and **5** with a simultaneous increase in cyclic carbonate **4** was observed, while the content of **6** was maintained nearly constant (Fig. 7, Table 1). After 48 h at 80 °C, the total cyclic carbonate (**4** and **6**) yield was about 79% (**4** being 55%) and was increased further to 85% with prolonged incubation. This reaction was focused on equilibrium shift to cyclic carbonate **4**, which has a minimum content of the ester (–CO–OCH_3), and this was achieved by continuous removal of the resulting methanol by evaporation, which prevented the reverse reaction from taking place. These results are a significant improvement over the earlier report on the synthesis of six-membered cyclic carbonate at lower yields (53%), which used large amounts (900% w/w of the diol) of the biocatalyst.[15,21]

Conclusions

Synthesis of six-membered cyclic carbonates with functional groups was achieved at high yields by a lipase-catalyzed reaction between TMP and dialkyl carbonate combined with thermal disproportionation. TMP is currently produced industrially from fossil-based butyraldehyde and formaldehyde,[32] while DMC is produced by oxidative carbonylation of methanol (and DEC by transesterification of DMC with ethanol).[31,32] These substrates could potentially be produced from bio-based butanol and methanol, once these chemicals are available in larger quantities. This biocatalytic reaction, which does not use any solvent, or other toxic materials such as phosgene and isocyanate that are generally used for the production of polycarbonate and polyurethane, constitutes a green process for the synthesis of cyclic carbonates.

A strategy to be considered for achieving higher productivity and selectivity would be to reach high yields of **3** (or **5**) in a short time (as in Fig. 4) followed by subjecting the product to high temperature. Further studies on improving the productivity of both the lipase-catalysed reaction and disproportionation are ongoing to make it an economically attractive process. Designing lipase mutants with higher activity and selectivity with polyol substrates is also planned.

Experimental

Materials

TMP was a product of Perstorp AB (Sweden). Immobilized lipase B from *Candida antarctica* (Novozym® 435) was a generous gift from Novozymes A/S (Bagsvaerd, Denmark). Dimethyl carbonate (97%), diethyl carbonate (97%), and molecular sieves

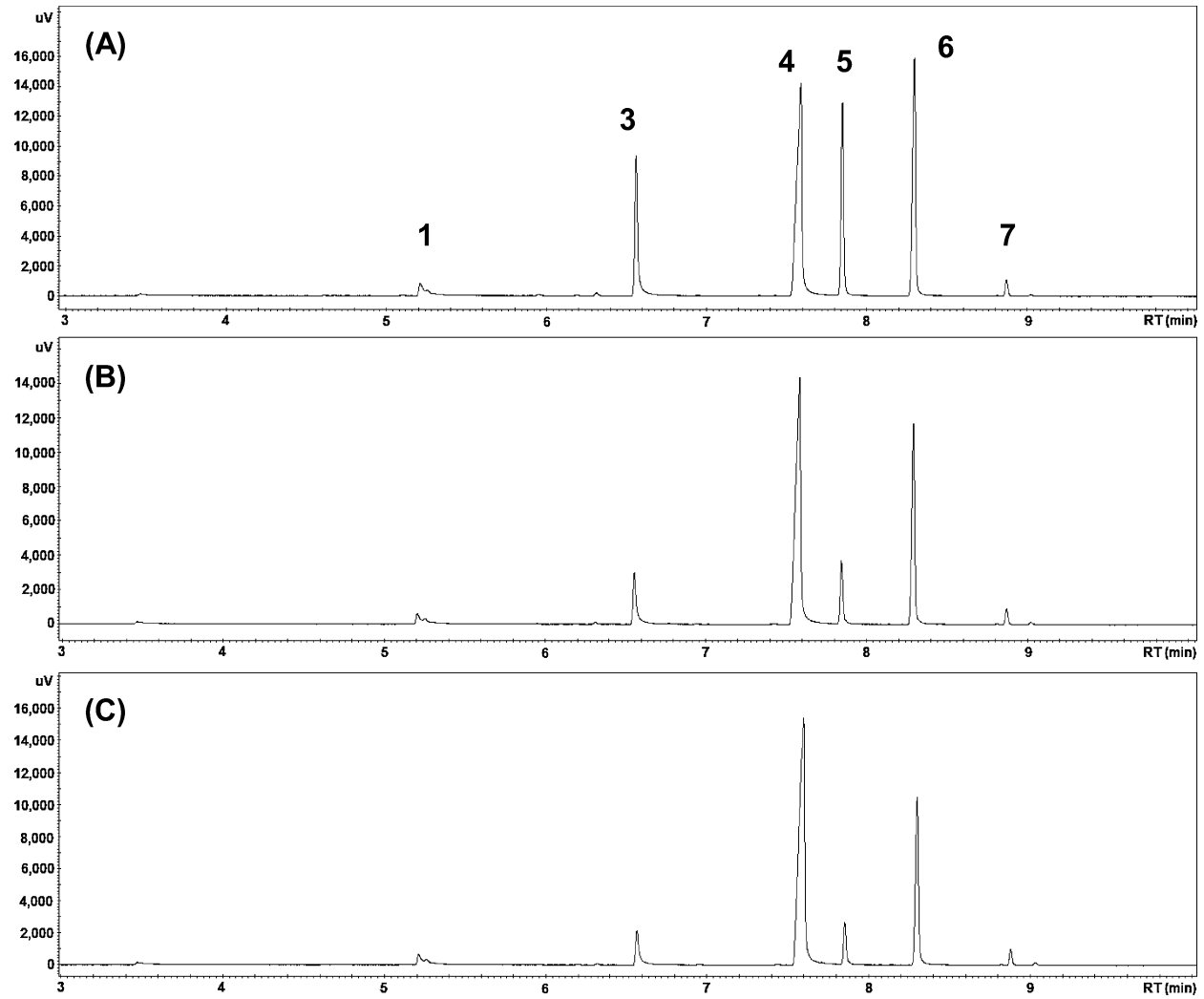

Fig. 7 Chromatograms for disproportionation of carbonates by thermal treatment at 80 °C at reaction time of 15 h (A), 48 h (B), and 144 h (C). For reaction time 0 h (starting material), see Fig. 2A.

(4 Å) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). HPLC-grade acetonitrile, ethyl acetate and dichloromethane were purchased from Merck (Germany). All chemicals were used without further treatment.

Determination of TMP solubility in dialkyl carbonate

TMP (0.5 g, 3.7 mmol) was added to 1 mL DMC in a 5 mL capped vial and shaken for 30 min at 700 rpm and 20 °C on a ThermoMixer (MKR 13, HLC Biotech, Germany). After separation of the residual solid TMP by standing at 20 °C for 20 min, a small aliquot of the supernatant was withdrawn for analysis. Then incubation of the mixture was repeated at 30 and 40 °C as above. TMP was freely soluble at 50 °C at the above concentration, hence 1–1.3 g was added to 1 mL DMC for testing the solubility. In DEC, the solubility measurement was performed in a similar manner (using 0.5 g TMP per 1 mL DEC) at 20, 30, 40 and 50 °C. At 60 °C, separate phases of DEC and melted TMP were generated.

Lipase-catalysed reaction between TMP and dialkyl carbonate

TMP (50 mg, 0.37 mmol) was dissolved in 1.5 mL (17.8 mmol) DMC in a 5 mL vial by shaking at 700 rpm at the required temperature on a thermomixer. The reaction was started by adding 150 mg molecular sieves and N435 and continued for several days. Aliquots were withdrawn at different time intervals for analysis of reaction components. Effects of biocatalyst concentration and reaction temperature on TMP conversion and product profile were investigated. The reactions in DEC (1.5 mL, 12.4 mmol) were carried out in the same manner.

The amount of immobilized lipase used is always stated as weight/weight of TMP.

Quantitative analysis and structure elucidation

Quantitative analyses of reaction components were performed using gas chromatography (GC, Varian 430-GC, Varian, USA) equipped with a FactorFour Capillary column, VF-1 ms (Varian, 15 m × 0.25 mm) and a flame ionization detector. The initial column oven temperature was increased from 50 to 250 °C at

a rate of 20 °C min^{-1}. The samples, diluted with acetonitrile at a concentration of 0.1–0.5 mg mL^{-1}, were injected in a split injection mode of 10% at 275 °C. The conversion of TMP and ratio of products were calculated by comparison of peak areas on the gas chromatograms.

The molecular masses of products were measured by GC–MS (Varian 431-GC, Varian 210-MS) equipped with a FactorFour Capillary column, VF-5 ms (Varian, 30 m × 0.25 mm). The initial column oven temperature was increased from 50 °C to 275 °C at a rate of 15 °C min^{-1}. The samples diluted as above were injected at 275 °C.

For structure elucidation, the products were isolated from the reaction mixtures by flash chromatography. The reaction mixture was loaded onto a silica column (25 mm i.d. × 250 mm), which was equilibrated with dichloromethane–ethyl acetate (2 : 1, v/v). The elution was performed using 3 : 1 dichloromethane–ethyl acetate. The structures of the purified products were then determined by ^{1}H-NMR using 400 MHz NMR (Bruker, UltraShield Plus 400, Germany).

Determination of residual activity of Novozym® 435

The residual enzyme activity of N435 after incubation in DMC, DEC, acetonitrile and n-hexane, respectively, was evaluated by esterification of caprylic acid with ethanol.[33] Ten milligrams of the biocatalyst was incubated in the solvents up to 72 h at 400 rpm and 60 °C on a ThermoMixer. The biocatalyst was washed using 90% ethanol after removal of the solvent, and then reacted with 200 mM caprylic acid in ethanol (90%) at 40 °C. The initial esterification rate was measured over 15 min by GC analysis as reported earlier,[33] and compared with the fresh biocatalyst treated using the same procedure.

Acknowledgements

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References

1 B. Barkakaty, K. Morino, A. Sudo and T. Endo, *Green Chem.*, 2010, **12**, 42.
2 G. T. Howard, *Int. Biodeterior. Biodegrad.*, 2002, **49**, 245.
3 S. Matsumura, Y. Soeda and K. Toshima, *Appl. Microbiol. Biotechnol.*, 2006, **70**, 12.
4 *An Ullmann Encyclopedia: Industrial Organic chemicals: Starting Materials and Intermediates*, Wiley-VCH: New York, 1999, vol. 2, 1045.
5 D. J. Darensbourg, A. Horn Jr and A. I. Moncada, *Green Chem.*, 2010, **12**, 1376.
6 T. Suyama and Y. Tokiwa, *Enzyme Microb. Technol.*, 1997, **20**, 122.
7 T. Suyama, H. Hosoya and Y. Tokiwa, *FEMS Microbiol. Lett.*, 1998, **161**, 255.
8 Z. Zhang, R. Kuijer, S. K. Bulstra, D. W. Grijpma and J. Feijen, *Biomaterials*, 2006, **27**, 1741.
9 Y. Yamamoto, S. Kaihara, K. Toshima and S. Matsumura, *Macromol. Biosci.*, 2009, **9**, 968.
10 S. A. Guelcher, *Tissue Eng., Part B: Rev.*, 2008, **14**, 3.
11 L. Liao, C. Zhang and S. Gong, *Eur. Polym. J.*, 2007, **43**, 4289.
12 G. Rokicki, P. Rakoczy, P. Parzuchowski and M. Sobiecki, *Green Chem.*, 2005, **7**, 529.
13 Y. Patel, J. George, S. M. Pillai and P. Munshi, *Green Chem.*, 2009, **11**, 1056.
14 J. Sun, S.-I. Fujita and M. Arai, *J. Organomet. Chem.*, 2005, **690**, 3490.
15 S. C. Kim, Y. H. Kim, H. Lee, D. Y. Yoon and B. K. Song, *J. Mol. Catal. B: Enzym.*, 2007, **49**, 75.
16 A. J. George, Y. Patel, S. M. Pillai and P. Munshi, *J. Mol. Catal. A: Chem.*, 2009, **304**, 1.
17 B. C. Vieville, J. W. Yoo, S. Pelet and Z. Mouloungui, *Catal. Lett.*, 1998, **56**, 245.
18 J. Matsuo, K. Aoki, F. Sanda and T. Endo, *Macromolecules*, 1998, **31**, 4432.
19 W. H. Carothers and F. J. Van Natta, *J. Am. Chem. Soc.*, 1930, **52**, 314.
20 A.-C. Albertsson and M. J. Sjoeling, *J. Macromol. Sci., Part A: Pure Appl. Chem.*, 1992, **29**, 43.
21 H. Tasaki, K. Toshima and S. Matsumura, *Macromol. Biosci.*, 2003, **3**, 436.
22 K. Miyazaki, T. Endo, F. Sanda, O. Moriya, T. Fukushima and J. M. Antonucci, *Polym. Prepr., ACS Div. Polym. Chem.*, 1997, **38**, 165.
23 G. Rokicki, T. Kowalczyk and M. Glinski, *Polym. J.*, 2000, **32**, 381.
24 R. A. Sheldon, R. M. Lau, M. J. Dorgedrager, F. van Rantwijk and K. R. Seddon, *Green Chem.*, 2002, **4**, 147.
25 R. Bernini, E. Mincione, M. Barontini, F. Crisante, G. Fabrizi and A. Gambacorta, *Tetrahedron*, 2007, **63**, 6895.
26 Novozymes A/S, Product Sheet, Biocatalysis/2003-03782-01.pdf.
27 S. H. Pyo and D. G. Hayes, Synthesis of saccharide–fatty acid ester biosurfactants catalyzed by lipase, *Biobased Surfactants and Detergents: Synthesis, Properties, and Applications*, AOCS Press: Champaign, IL, 2009, p. 323.
28 P. Villeneuve, *Biotechnol. Adv.*, 2007, **25**, 515.
29 D. G. Hayes, *J. Am. Oil Chem. Soc.*, 2004, **81**, 1077.
30 M. Regel-Rosocka and J. Szymanowski, *Chemosphere*, 2005, **60**, 1151.
31 P. Werle, M. Morawietz, S. Lundmark, K. Sörensen, E. Karvinen and J. Lehtonen, *An Ullmann Encyclopedia of Industrial Chemistry: Alcohols, Polyhydric*: Wiley-VCH: Weinheim, 2008, 1.
32 P. Tundo, F. Aricò, A. E. Rosamilia and S. G. L. Rossi, *Green Chemical Reactions*, Springer Science: Heidelberg, 2008, p. 213.
33 A. E. V. Hagström, M. Nordblad and P. Adlercreutz, *Biotechnol. Bioeng.*, 2009, **102**, 693.

