Enzyme-Catalyzed Copolymerization of Oxiranes with Dicarboxylic Acid Anhydrides

Ritimoni Rajkhowa,[1] I. K. Varma,[1] Ann-Christine Albertsson,[2] Ulrica Edlund[2]

[1]*Centre for Polymer Science and Engineering, Indian Institute of Technology, Delhi, Hauz Khas, New Delhi, India*
[2]*Department of Fibre and Polymer Technology, Royal Institute of Technology, Stockholm, Sweden*

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ABSTRACT: Ring-opening copolymerizations of the oxiranes glycidyl phenyl ether (GPE) and diglycidyl ether of bisphenol A (DGEBA) with a dicarboxylic acid anhydride [methyl hexahydrophthalic anhydride, nadic anhydride, maleic anhydride (MA), or itaconic anhydride (IA)] were carried out with the lipases *Candida cylindracea* (CCL), Lipozyme TL-IM (LIM), and Novozyme 435 (N435) as catalysts. The CCL-catalyzed reaction of DGEBA with MA or IA (at a 1:2 molar ratio) at 80°C resulted in only partial curing. We monitored the reactions by Fourier transform infrared spectroscopy and by following the changes in the intensities of carbonyl stretching frequencies of the anhydride and ester groups. The reactivity of the oxirane group in GPE was higher than that in DGEBA; this may have been due to the higher viscosity of DGEBA. The reactivities of the enzymes for the copolymerization of the oxiranes and dicarboxylic acid anhydride were in the order LIM > CCL > N435. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 697–704, 2005

Key words: catalysis; curing of polymers; enzymes

INTRODUCTION

The reactions of diacid anhydrides with oxiranes are well documented in the literature and have been commercially exploited for the curing of epoxy resins. Anhydrides are low-cost hardeners that produce ester-like structures in cured resins. Commonly used anhydrides include phthalic anhydride (PA), tetrahydrophthalic anhydride, hexahydrophthalic anhydride, pyromellitic dianhydride, chlorendic anhydride, and methylnadic anhydride.[1] The anhydrides react slowly with glycidyl ether resins [e.g., the commonly used diglycidyl ether of bisphenol A (DGEBA)], and the curing schedules are generally long and require high temperatures (>150°C). To cure the resins at a low temperature, a catalyst, such as a tertiary amine, is required. In the first step, the amine associates with the anhydride to produce a quaternary ammonium ion and a carboxylate ion. The nucleophilic attack of the carboxylate ion on the 2 carbon of the oxirane leads to the formation of an ester and an alkoxide ion. The alternation of attack of alkoxide ion on anhydride and carboxylate ion on oxirane results in the crosslinking of the epoxy resin without the generation of any hydroxyl groups. The absence of hydroxyl groups in the cured resins is advantageous for the application of epoxy resin laminates in electrical fields.

Aliphatic polyesters have been synthesized by the ring-opening copolymerization of an aliphatic acid anhydride [succinic anhydride (SA)] and oxiranes (ethylene oxide, propylene oxide, or styrene oxide).[2,3] Diethyl zinc, aluminum isopropoxide, and bimetallic (Al, Zn) μ-oxobutoxide, magnesium ethoxide, $SnCl_2$, $ZnCl_2$, $AlCl_3$, and other compounds have been used as catalysts in these copolymerizations. Poly(ethylene succinate), with a number-average molecular weight of 1.3×10^4, has been prepared by the reaction of SA and ethylene oxide at 100°C in toluene with magnesium ethoxide as a catalyst.[2] High-molecular-weight polyesters have been obtained by a chain-extension reaction with tetraisopropyl titanate.[3] Such chemical methods for the ring-opening copolymerization of oxiranes with an acid anhydride require extremely pure monomers, anhydrous conditions, and an organometallic catalyst, which must be completely removed before use, if these polyesters are intended for medical applications.

Biodegradable aliphatic polyesters have also been prepared by the *in vitro* use of enzymes. Lipases catalyze the ring-opening polymerization of lactones (small to large rings), cyclic diesters (lactides), and cyclic carbonates to produce biodegradable polyesters and polycarbonates. Enzyme-catalyzed polymerizations are eco-friendly techniques for the preparation of biodegradable polyesters.[4–7]

Few reports are available on the enzyme-catalyzed copolymerization of oxiranes with dicarboxylic anhy-

Correspondence to: A.-C. Albertsson (aila@polymer.kth.se).
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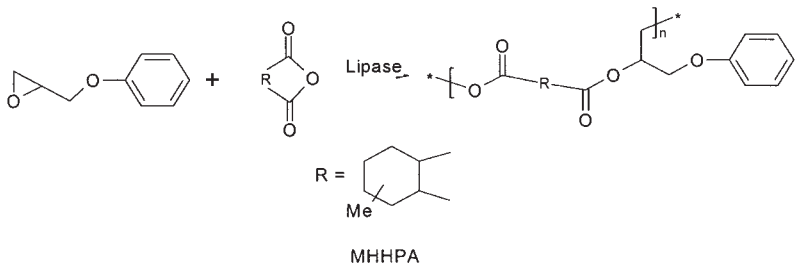


Scheme 1 Lipase-catalyzed reaction of GPE and cycloaliphatic anhydride (molar ratio = 1:1).

drides. Matsumura and coworkers[8–10] reported the synthesis of polyesters by the bulk copolymerization of benzyl glycidate, glycidyl phenyl ether (GPE), glycidyl methyl ether, and styrene oxide with anhydrides such as SA, maleic anhydride (MA), and PA in the presence of lipases from different sources. This was a stepwise reaction that led to the formation of either a carboxy or hydroxy end group. The molecular weight increased with the duration of the reaction[10] and with the addition of more monomer mixture after the depletion of the initial monomer mixture. The highest molecular weight (13,500, with a polydispersity index of 1.4) was obtained when the bulk polymerization of GPE and SA was carried out for 9 days at 80°C with porcine pancreatic lipase as a catalyst. However, no conclusions regarding the relative reactivities of the different anhydrides and oxiranes in the enzyme-catalyzed polymerization could be drawn from these studies.

The enzyme-catalyzed bulk copolymerization of oxiranes and anhydrides is a heterogeneous reaction and may be a diffusion-controlled process. The use of liquid anhydrides may facilitate the reaction. Therefore, we were interested in studying the copolymerization of GPE with a cycloaliphatic anhydride, that is, methyl hexahydrophthalic anhydride (MHHPA), in the presence of lipases of different origin (Scheme 1).

No studies have been reported on the anhydride curing of DGEBA in the presence of lipases. Our objective was to study the curing of DGEBA with aliphatic anhydrides [e.g., itaconic anhydride (IA), MA] and cycloaliphatic anhydrides [nadic anhydride (NA) or MHHPA] in bulk with different lipases (Scheme 2).

EXPERIMENTAL

Materials

DGEBA (Sigma-Aldrich, Stockholm, Sweden), MHHPA (Sigma-Aldrich), GPE (Acros), MA (Sigma-Aldrich), and NA (Labora AB, Upplands Väsby, Sweden), were used as received. IA was prepared by the dehydration of itaconic acid. The lipases *Candida cylindracea* (CCL; 50 IU/mg), Lipozyme TL-IM (LIM; immobilized lipase enzyme *Thermomyces lanuginosa,* silica granulated), and Novozyme 435 (N435; Novo Nordisk, Bagsvaerd, Denmark) were used as received. Acetone (Aldrich, Stockholm, Sweden) was dried over K_2CO_3 and distilled before use.

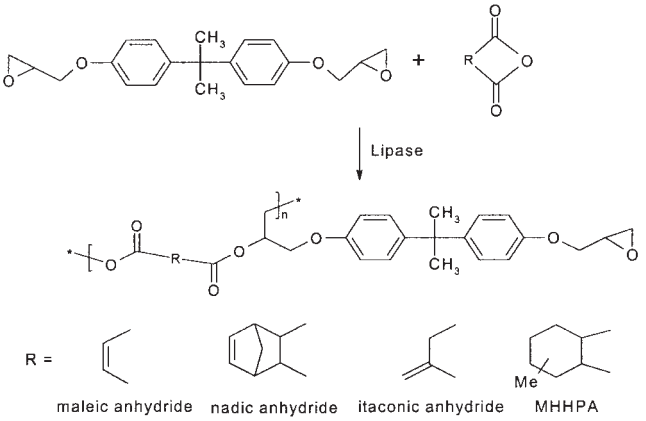
Copolymerization

Equimolar ratios of OPE and MHHPA were mixed thoroughly with 5 or 10 wt % enzyme (with respect to the weight of GPE resin). The polymerization was carried out in capped vials at 80°C for different time intervals. After the reaction, the reaction mixture was dissolved in acetone, and the insoluble enzyme was removed by filtration. The acetone was then removed, and the polymer was collected. Blank experiments without enzymes were also carried out at 80°C for the same durations.

Three different molar ratios of DGEBA and anhydrides (1:1, 1:1.5, and 1:2) were taken, and the reaction was carried out in the presence of enzymes in a similar way as described for GPE.

Characterization

Monitoring of reaction of DGEBA with anhydrides (at a 1:2 molar ratio) was done by solubility measure-



Scheme 2 Lipase-catalyzed reaction of DGEBA and dicarboxylic acid anhydrides. The reaction was monitored by FTIR.

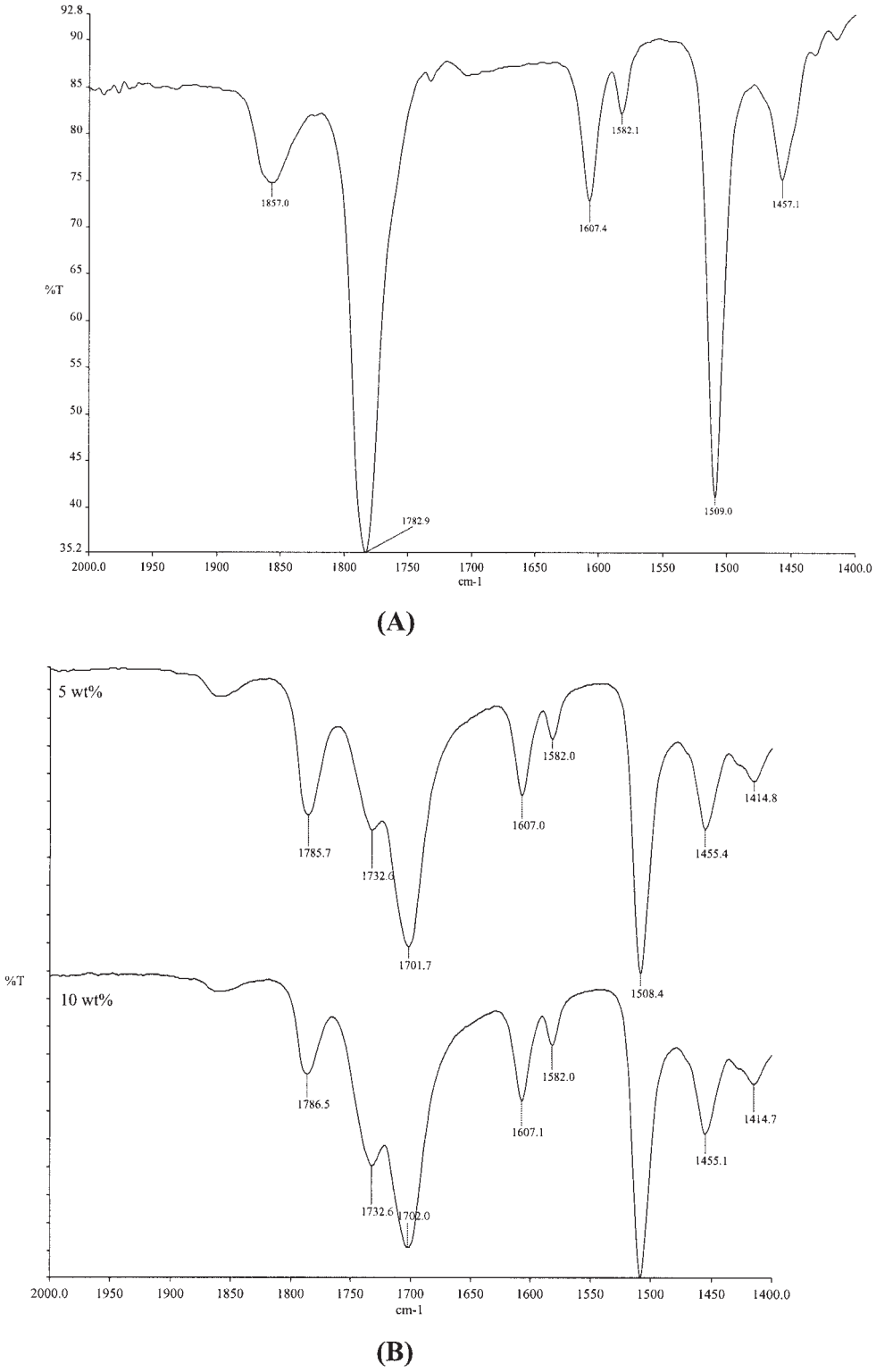


Figure 1 FTIR spectra of DGEBA/MHHPA (1:1 molar ratio) after 24 h (A) without CCL and with (B) 5 and 10 wt % CCL.

ments of the polymer in organic solvents such as acetone and dimethylformamide (DMF) at regular intervals of time. The formation of completely insoluble product was taken as an indication of the completion of the reaction.

Fourier transform infrared (FTIR) analysis was performed on a PerkinElmer model Spectrum 2000 equipped with a Golden Gate Attenuated Total Reflectance holder with a diamond FTIR crystal (P/N 10500 series, Graseby Spectra, Weisbaden, Germany).

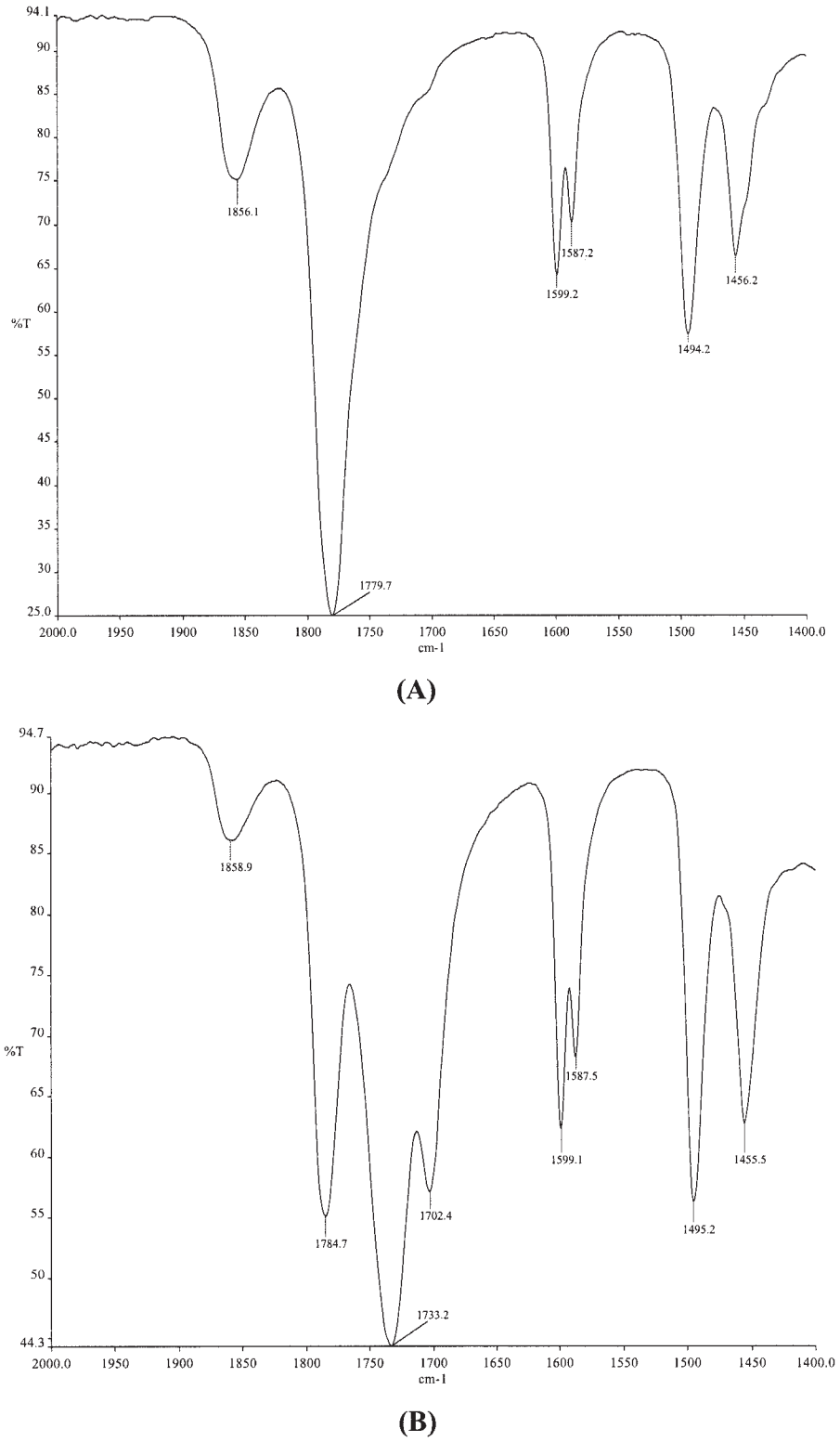


Figure 2 FTIR spectra of GPE/MHHPA after 48 h (A) without CCL and with (B) 10 wt % CCL.

Size exclusion chromatography (SEC), with a Waters (Sollentuna, Sweden), 6000A pump, a PL-EMD 960 light scattering evaporative detector (Labora AB, Upplands Väsby, Sweden), two Polymer Laboratories gel 10-mm mixed B columns (300 × 7.5 mm) from Polymer Laboratories (Sollentuna, Sweden), and one Ultrahydrogel linear column (300 × 7.8 mm) from Waters, was used for the molecular weight determinations of the polymers. DMF (70°C) was used as the mobile phase at a flow rate of 1 mL/min. Calibration was done with polystyrene

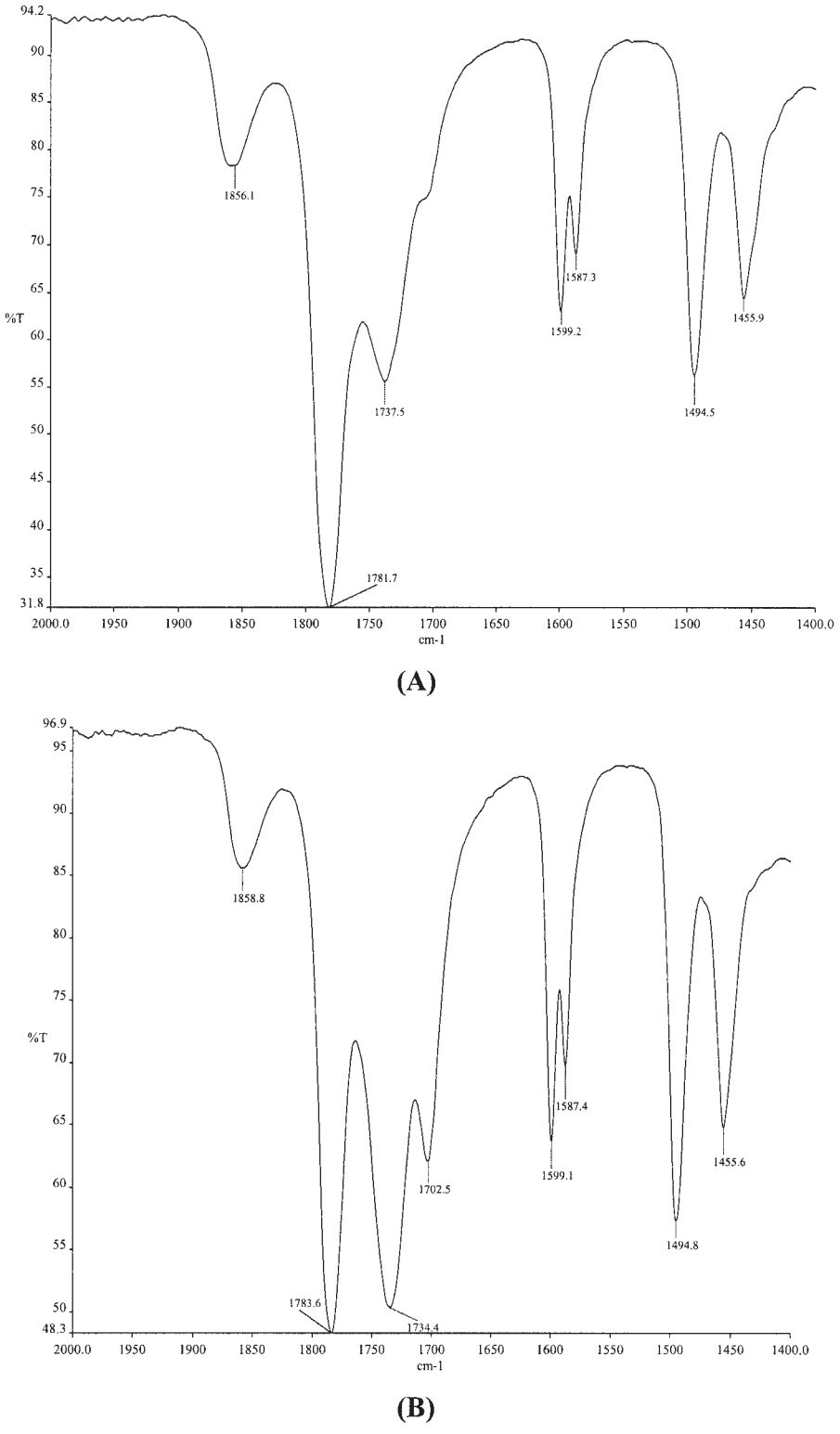
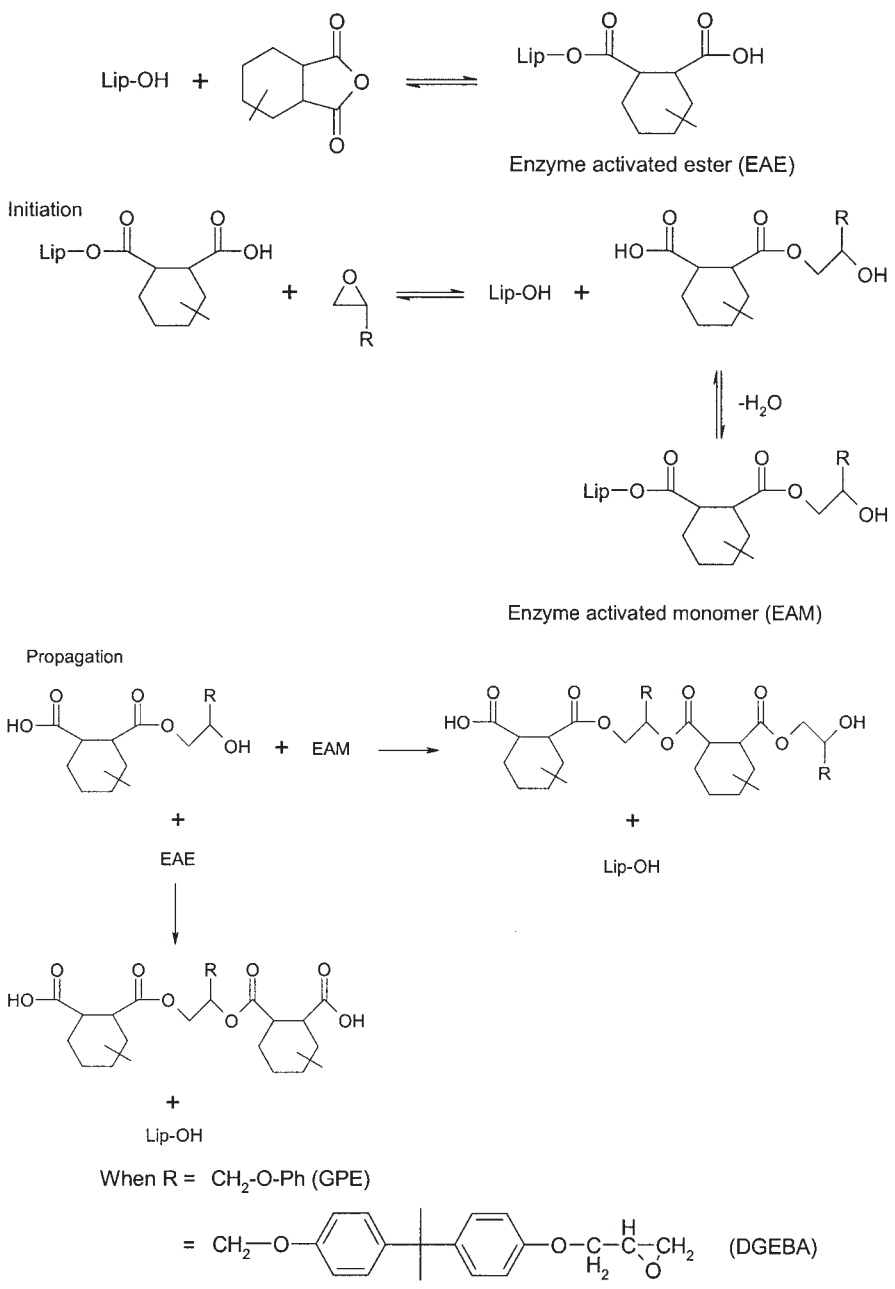


Figure 3 FTIR spectra of GPE/MHHPA after 13 h in the presence of 10 wt % (A) LIM and (B) CCL.

standards. Peak molecular weights were calculated with Waters Empower software.

RESULTS AND DISCUSSION

The curing of DGEBA with structurally different anhydrides (at a molar ratio of 1:2) was undertaken initially to assess the reactivity of various anhydrides. The mixing of MHHPA (liquid anhydride) with DGEBA was done easily, whereas with other anhydrides, it was difficult to mix highly viscous DGEBA and solid anhydrides and prepare a homogeneous solution. The addition of enzymes further increased



Scheme 3 Mechanism of polymerization of MHHPA and oxirane in the presence of enzymes.

the heterogeneity of the system. Insoluble crosslinked products were obtained after the polymerization of DGEBA was carried out at 80°C for 3 days with NA or MHHPA. The curing of DGEBA with IA or MA was incomplete, and a sticky polymer that was partially soluble in organic solvents was obtained after a 5-day reaction at 80°C. This indicated the incomplete curing of DGEBA in the presence of the aliphatic anhydrides (MA or IA). Similar results were reported earlier for the *Candida antartica* lipase-catalyzed polymerization of α,ω-alkylene glycols with dicarboxylic anhydrides in toluene.[11] Polymer formation was not observed when MA or PAs were used. We therefore decided to use only the liquid anhydride (MHHPA) for further studies.

The duration of the reaction, the molar ratios of anhydride and oxirane, and the concentration of enzyme and its origin may affect the extent of reaction of oxiranes with anhydrides. The effect of these parameters on the curing of DGEBA and the copolymerization of GPE with anhydrides was, therefore, investigated. The initial viscosity of GPE was significantly lower than that of DGEBA. As the polymerization proceeded, the viscosity increased significantly, and a solid polymer soluble in DMF was obtained after 6 days, whereas for DGEBA, a solid polymer was obtained after 2 days of reaction at 80°C.

The reaction was monitored by IR spectroscopy. In the enzyme-catalyzed reactions, the anhydride peaks

TABLE I
Effect of Reaction Parameters on the Ratio of Ester to Anhydride Peaks in IR Spectra (Epoxy: MHHPA Ratio = 1:1)

No.	Epoxy resin	Enzyme (wt %)	Ratio of 1735 to 1785cm^{-1} peaks (ester:anhydride)[a]
1	GPE	LIM (5)	0.29 (3), 0.34 (6), 0.45 (13), 0.58 (24)
2	GPE	CCL (5)	0.25 (13), 0.39 (24), 0.55 (48), 0.68 (72)
3	DGEBA	LIM (5)	0.36 (3), 0.46 (6), 0.55 (9), 0.58 (12), 0.75 (24), 0.88 (42)
4	DGEBA	CCL (5)	0.58 (12), 1.00 (24), 0.85 (48)
5	GPE	LIM (10)	0.39 (3), 0.6 (13), 0.68 (28), 0.9 (37)
6	GPE	CCL (10)	0.39 (13), 0.6 (24), 0.96 (48), 1.3 (72)
7	DGEBA	LIM (10)	0.19 (6), 0.43 (15), 0.52 (18), 0.53 (24), 0.6 (27), 0.85 (30), 0.87 (36)
8	DGEBA	CCL (10)	1.62 (12), 1.92 (24), 2.04 (48)
9	DGEBA[b]	CCL (5)	0.43 (12), 0.6 (24), 1.25 (48)
10	DGEBA[b]	LIM (10)	0.3 (2), 0.34 (4), 0.64 (24), 0.72 (37)
11	DGEBA[b]	N435 (10)	0.35 (3), 0.4 (13), 0.55 (24)

[a] The values in parentheses in this column represent time (h).
[b] DGEBA/MHHPA ratio = 1:1.5.

at 1783 ± 2 and 1858 ± 2 cm^{-1} decreased in intensity, and new peaks at l732 ± 2 and 1702 ± 2 cm^{-1} appeared due to the formation of ester and acid groups, respectively. In blank samples containing DGEBA and MHHPA (1:1) without lipase, no change in the characteristic absorption peaks was observed (Fig. 1). An increase in the concentration of enzyme (5–10 wt %) or in the duration of the reaction resulted in a significant increase in the ester carbonyl stretching frequency. Similar results were obtained with GPE and MHHPA (Fig. 2). The absorption peak due to the carbonyl stretching of acid groups at 1702 ± 2 cm^{-1} was significantly higher in the copolymers of DGEBA/MHHPA compared to those of GPE/MHHPA. The concentration of acid groups was higher in the copolymers of GPE/MHHPA prepared with CCL than in those prepared with LIM (Fig. 3).

The first step in the polymerization of oxiranes with anhydrides is the formation of an enzyme-activated ester (EAE) with a free carboxylic group (Scheme 3) as an intermediate. The hydroxyl group attached to the serine residue of enzyme is believed to participate in this reaction. Further reaction of this intermediate with oxirane monomer leads to the formation of an enzyme-activated monomer with a free hydroxyl group. The concentration of carboxyl groups will be higher if the EAE reacts with the growing oligomeric chain to produce an oligomeric species with an anhydride-derived carboxyl group at both ends.[10]

To quantify the FTIR results and to determine the relative rates of reaction under different conditions, the ratio of intensity of the 1732 ± 2 and 1783 ± 2 cm^{-1} absorption bands was taken with a base line from 1600 to 1800 cm^{-1}. As the reaction progressed, the intensity of the carbonyl stretching of ester (1732 ± 2 cm^{-1}) increased, whereas the intensity of the anhydride carbonyl group (1783 ± 2 cm^{-1}) decreased (Table I). These ratios were plotted as a function of time, and the slopes of such plots (Fig. 4) were determined. The initial slopes (up to the first 10 h) of these plots were significantly higher, but later, a linear dependence on time was observed. The higher reactivity in the initial stages may have been due to the low viscosity of the system. Later, the viscosity buildup may have affected the diffusion of the reactants to the active site of the enzyme. The rates reported in Table II were obtained from the linear portions.

The data given in Table II reveals that the reactivity of the oxirane was slightly higher in GPE compared to DGEBA when the same enzyme and same weight percentages of enzyme were used (see samples 1 and 3, 2 and 4, and 6 and 8). This may have been due to the lower viscosity of GPE compared to DGEBA.

The rates of reaction were found to be dependent on the weight percentage and type of lipase (Table II). An increase in the weight percentage of the enzyme from 5 to 10 increased the rate of reaction in both GPE and DGEBA with MHHPA (samples 1 and 5, 2 and 6, 3 and 7, and 4 and 8). The enzyme activity of LIM was higher than CCL, whereas N435 was least reactive (samples 10 and 11).

Molecular weight measurements were done by SEC with styrene as standards (Table III). The presence of high- and low-molecular-weight products was indi-

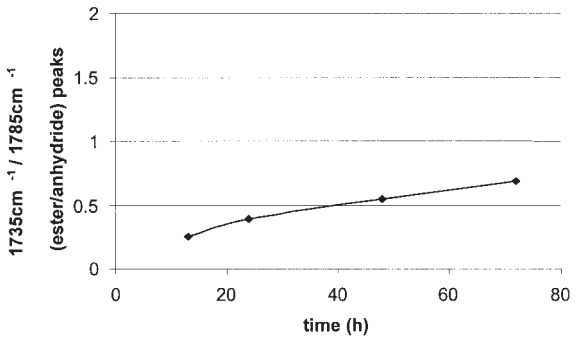


Figure 4 Plot of the ratio of ester to anhydride peaks (1735/1785 cm^{-1}) for GPE/MHHPA containing 5 wt % CCL versus time.

TABLE II
Effect of the Reaction Parameters on the Rate of Polymerization

No.	Oxirane	Epoxyanhydride ratio	Enzyme	Enzyme (wt %)	Rate of polymerization[a]
1	GPE	1:1	LIM	5	0.0138
2	GPE	1:1	CCL	5	0.0071
3	DGEBA	1:1	LIM	5	0.012
4	DGEBA	1:1	CCL	5	0.0057
5	GPE	1:1	LIM	10	0.0153
6	GPE	1:1	CCL	10	0.0151
7	DGEBA	1:1	LIM	10	0.016
8	DGEBA	1:1	CCL	10	0.0107
9	DGEBA	1:1.5	CCL	5	0.0233
10	DGEBA	1:1.5	LIM	10	0.0123
11	DGEBA	1:1.5	N435	10	0.0098

[a] Increase of 1732 ± 2:1783 ± 2 cm^{-1}/hour.

cated in the GPC trace. An increase in the weight percentage of the enzyme resulted in a decrease in the molecular weight of the polymer. In GPE, where the polymerizations were carried out for several days (6–7 days), a decrease in the molecular weight was observed at later stages (Table III). This may have been due to the lipase-catalyzed hydrolytic degradation of the polyester.

TABLE III
Molecular weight of the Polymers and Oligomers Obtained by Enzymatic Polymerization of Oxiranes and Dicarboxylic Acid Anhydrides (Molar Ratio = 1:1)

Oxirane	Enzyme	Enzyme (wt %)	Time (h)	Molecular weight (g/mol)	
GPE	CCL	5	72	5687	293,0571
			96	6630	2,658,582
			120	6592	1,933,943
GPE	CCL	10	72	7898	1,010,464
			96	7201	704,143
			120	8940	730,428
GPE	LIM	5	37	478	15,730
			48	543	14,812
			54	617	19,700
GPE	LIM	10	13	—	6,849
			24	386	13,469
			37	548	10,535
			48	674	11,002
			54	817	8,625
			240	2531	20,287
DGEBA	LIM	5	3	—	10,622
			6	593	11,699
			9	764	13,712
			12	664	10,258
			15	788	11,713
			18	1024	10,174
			24	1052	15,457
DGEBA	LIM	10	42	1016	3,760
			48	1087	6,775
			52	1008	12,317

CONCLUSIONS

The oxiranes DGEBA and GPE could be readily copolymerized with MHHPA in the presence of the enzymes CCL, LIM, and N435. The reactivity needed to catalyze the process was highest in LIM followed by CCL; N435 was least reactive. Very-high-molecular-weight polymers were obtained with liquid anhydride, which was readily miscible with the oxiranes.

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