Synthesis of Alkyds Involving Regioselective Lipase-Catalyzed Transesterification in Organic Media

G. SUDESH KUMAR,[1,*] A. GHOGARE,[2] and D. MUKESH[2]

[1]Shriram Institute for Industrial Research, 14 & 15 Sadarmangala Indl. Area, Whitefield Road, Bangalore-560 048, India, and [2]Alchemie Research Centre, P.O. Box 155, Thane-Belapur Road, Thane-400601, Maharashtra, India

SYNOPSIS

Porcine pancreatic lipase (PPL) is established as an effective biocatalyst for the selective transesterification of triglycerides and diols in organic medium at room temperature yielding primarily 2-monoglyceride mixtures. Molecular modelling simulations of repeating units of alkyd resin incorporating one- and two-monoglycerides show end-to-end distance of 9.76 and 12.82 Å, respectively. The modeling studies on the trimers of these repeating units also reveal more extended configuration for the structures based on two-monoglycerides. A comparative study of alcoholysis by conventional base-catalyzed process and this novel biocatalytic process was carried out with coconut oil triglycerides and a series of diols, followed by polymerization. The rates of transesterification were dependent on the nature of triglyceride, diol, concentration of the enzyme, and temperature. The 1,3 specificity of lipase produced 2-monoglycerides as determined by periodic acid method, resulting in uniform distribution of fatty acids along the alkyd polyester backbone. Comparisons are made between those alkyds prepared by base catalysis and biocatalytic method. © 1997 John Wiley & Sons, Inc.

INTRODUCTION

An important target of biocatalysis in polymer science is exploitation of nonaqueous enzyme chemistry for macromolecular design. Polymer synthesis involving biocatalytic steps[1] is an attractive route to chiral polymers, stereoregular polymers, etc. Recently, we have reported a lipase-mediated acylation methodology for the preparation of chiral acrylate monomers and polymers.[2-3] Vegetable oils play an important role in surface coatings because of their availability as a renewable source and then variety and versatility. These triglycerides are commonly incorporated into alkyd structure by a base-catalyzed transesterification with desired polyol at 220°C (Fig. 1).[4]

The degree of alcoholysis has an important bearing on the properties of the final resin.[4] Differences between alkyds prepared by differing monoester compositions have been studied by a number of authors.[5-6] Practically, the reaction does not go to completion, and an equilibrium of species is present, including unreacted oil, polyol and monodiesters of glycerol, and added polyol. The alcoholysis reaction is complicated by the interetherification of polyols at higher temperatures, adding to the complexity of final alkyd structure. In order to design and develop tailor-made alkyd resins for high solids coating formulations, there is a need for a more predictive, planned approach involving molecular simulations of to-be synthesized macromolecular framework.

Molecular modelling techniques are widely applied to derive many structural features, such as the chain conformation, chain packing, and local motions of the functional groups in polymers.[7-10] It allows for varying monomer arrangements, given a monomer composition, and generates energetically valid statistical chains, estimating the mean squared end-to-end distance. For example, in all conventional alkyd resin preparations, monoglyceride compositions are known to influence the performance of the final resin. However, no attempts have

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 63, 35–45 (1997)
 CCC 0021-8995/97/010035-11

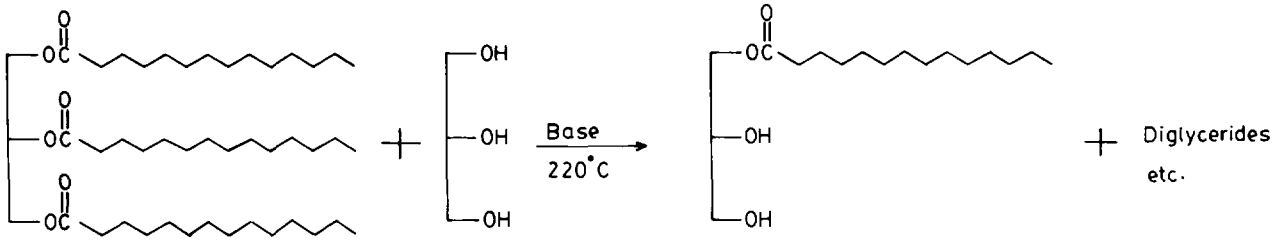
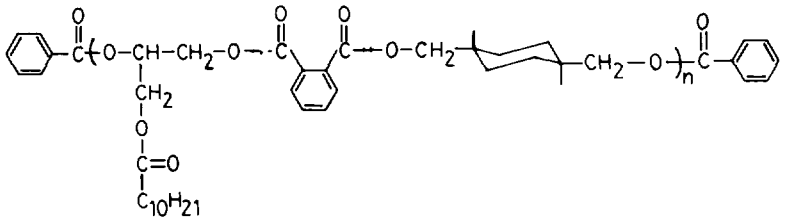


Figure 1 Base-catalyzed transesterification of a triglyceride with glycerol.

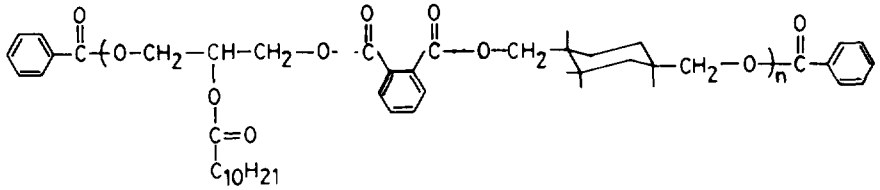
been made to develop designer alkyds based on the computer simulations of monomeric and oligomeric units. Likewise, few attempts have been made to selectively synthesize alkyds with two-monoglyceride repeating units and to evaluate their performance. In this context, we have initiated a program with the following objectives:

1) to evolve appropriate chemoenzymic routes to two-monoglyceride-based polymer/monomer structures,
2) to perform molecular modelling studies on monomeric/oligomeric building blocks representing alkyd chains, and
3) to evaluate the performance of these novel materials vis-a-vis their conventional analogs.

A major development in tailor-made alkyd resins requires stereoselective transesterification of triglycerides with polyols under mild conditions followed by a step-growth polymerization step.[6] In this context, we have investigated the use of porcine pancreatic lipase (PPL) as a biocatalyst for the alcoholysis of triglycerides. In the past, lipases were discounted in the coating area because alkyd reactions are nonaqueous in nature (the presence of water will result in hydrolysis over transesterification, resulting in monofunctional fatty acids, and therefore, not very useful in polymerizations), while lipase activity depends on oil–water interface. Of late, the



(I)



(II)

Figure 2 Model structures representing repeating units of alkyds: (I) 1-MG/PA/CHDM, and (II) 2-MG/PA/CHDM (end-capped with benzoic acid). n = 1 in (I) and (II), and n = 3 in (III) and (IV).

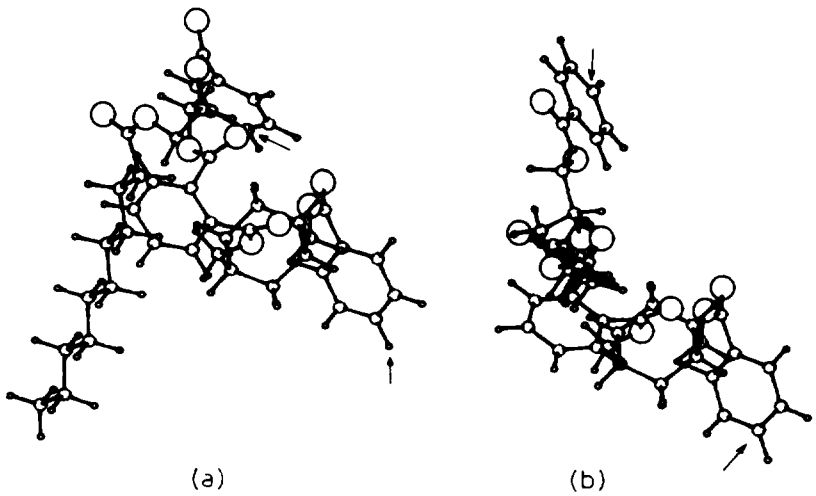


Figure 3 Computer models of (a) 1-MG/PA/CHDM (I) and (b) 2-G/PA/CHDM (II). End-to-end distance (marked by arrows): 9.76 Å for (I) and 12.82 Å for (II).

synthetic potential of lipases has been broadened by using them in organic media.[11-12] Consequently, exploitation of varying specificities of different lipases should permit the synthesis of alkyds with defined molecular architecture. Lipase-catalyzed transesterifications of triglycerides with polyols in organic media and subsequent step-growth polymerization to alkyds of defined structure is unprecedented. In this paper, we report the efficacy of PPL for the synthesis of coconut-oil-based alkyds and molecular modeling studies of alkyd repeating units. This biotechnological process offers new dimensions in the design of alkyd resins.

EXPERIMENTAL

Molecular Modelling

The molecular modelling studies were carried out on an IRIS 3100 workstation using QUANTA2/CHARMm softwares (Polygen Corp. UK). Quanta is a comprehensive program that enables the construction graphical modelling and analysis of molecular structures. It accepts input and can generate structural data in all standard molecular file formats. Interactive access is provided to the CHARMm program and its extensive set of minimization and molecular dynamics simulation functions.

The methodology adopted to calculate the conformational energy of polymers is similar to the molecular force field techniques developed by Levitt.[13] The actual molecular force fields used in the empirical energy calculations are a set of analytical expressions to derive individual energy terms in the following equations:

$$E_{\text{total}} = E_{\text{angle}} + E_{\text{dihedral}} + E_{\text{improper}} + E_{\text{elec}} + E_{\text{vdw}}$$

Each of the terms in the above equation is calculated using the procedure reported by Levitt.[13] Steepest descent method was used to remove bad contacts, and, finally, Powell's method was adopted for the local minimization of the energy of polymer chains. Indeed, macromolecules are known to have multiple minima, so the minimum energy value obtained by the above method need not necessarily be the global minimum.

Materials

Porcine pancreatic lipases (EC 3.1.1.3) was obtained from Sigma Chemical Co. Its specific activity was 11 units/mg of solid and was used straight from the bottle. The lipase used in this work was a crude preparation.

Vegetable oils employed were of refined grade, which are commercially available. The fatty acid composition of these triglycerides is as follows.[14] Coconut oil (CNO): lauric, 44–51%; palmitic, 8–10%; myristic, 13–18%. Castor oil: ricinoleic, 87%; oleic, 7%; linoleic, 3%; palmitic, 2%. Safflower oil: palmitic, 6.4%; stearic, 3.1%; oleic, 13.4%; linoleic, 76–79%. Tobacco seed oil: palmitic, 22%; oleic, 10–20%; linoleic, 68–75%; stearic, 2%. Phthalic anhydride, glycerol, trimethylolpropane (TMP), neopentyl glycol (NPG), 1,6 hexane diol (HD), and *trans-*

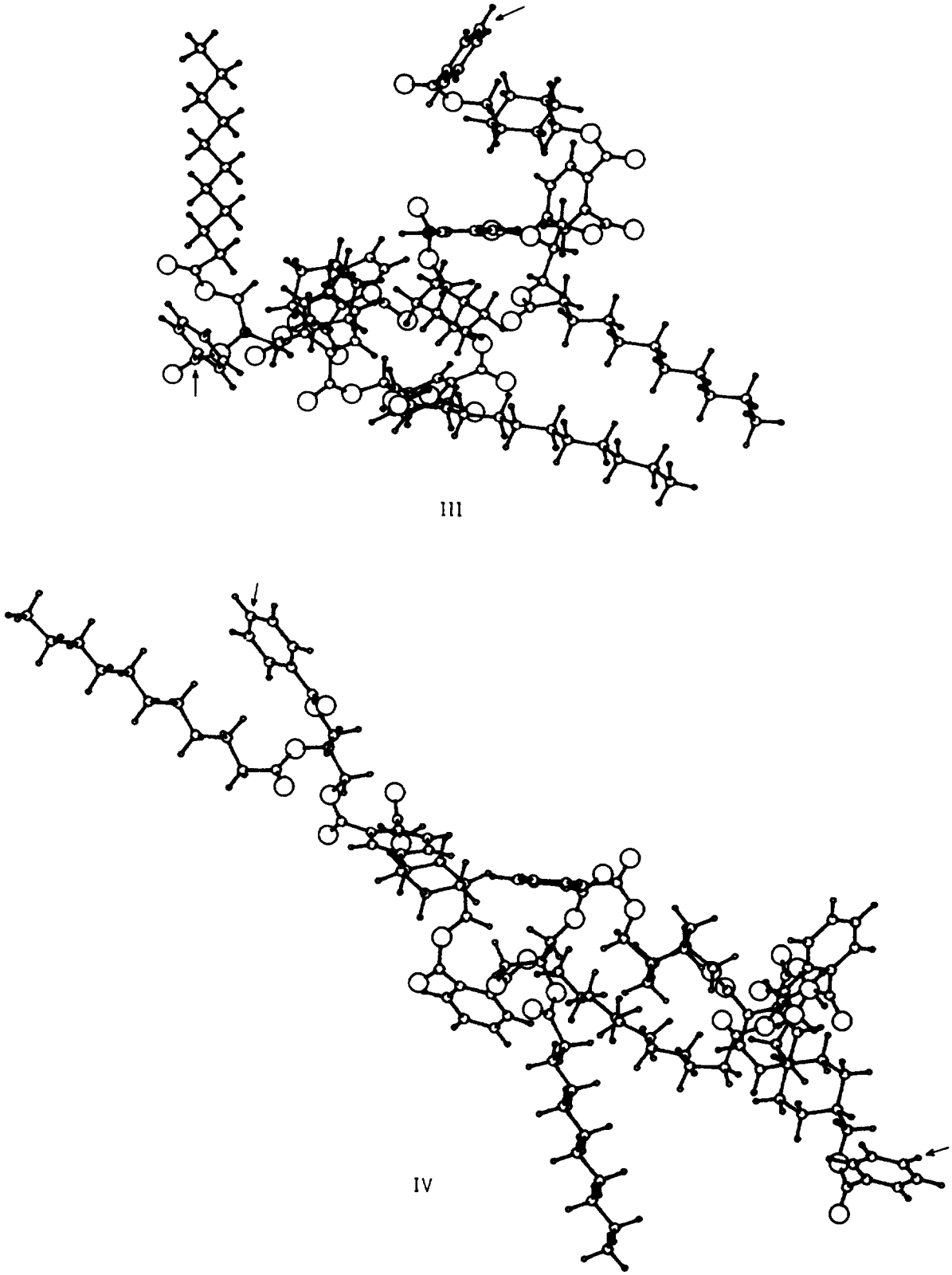


Figure 4 Computer-generated trimer models of (a) 1-MG/PA/CHDM and (b) 2-MG/PA/CHDM. End-to-end distance: 22.06 Å for (III) and 36.56 Å for (IV).

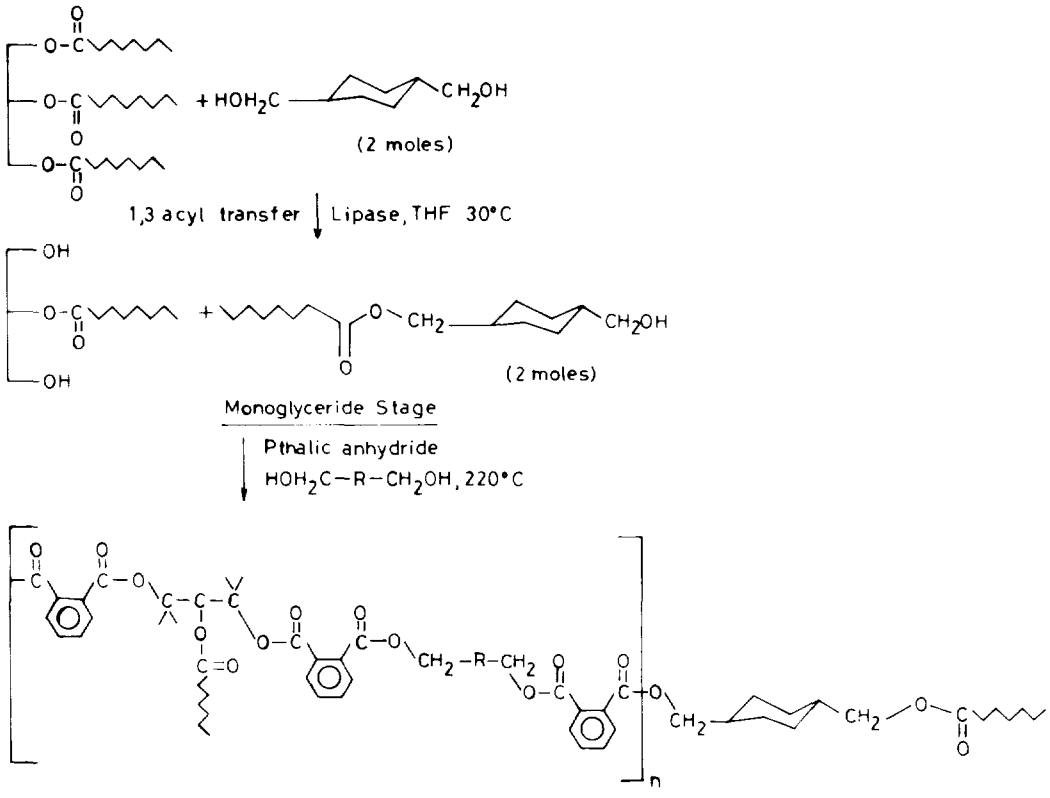


Figure 5 Lipase-assisted route to the synthesis of oil-based polyester resins.

1,4 cyclohexane dimethanol (CHDM) were all from Aldrich.

Procedure for Lipase-Catalyzed Transesterification of Triglycerides in Organic Solvents

A typical experimental protocol for this biocatalytic transesterification is as follows: Triglyceride[10] (0.15 mol) and a diol (such as CHDM) (0.35 mol) were stirred in 30 mL of THF with 10 g of PPL at room temperature (32°C) or at required temperatures in a thermostat. At the end of the reaction, suspended lipase was filtered out, and the solvent evaporated. The progress of alcoholysis was followed by taking all of the reaction mixture and analyzing by gel permeation chromatography (GPC).

Procedure for Base-Catalyzed Transesterification of Triglycerides in Organic Solvents

Vegetable oil triglyceride (0.15 mol) and a diol (0.35 mol) and 0.05 g of calcium acetate in 3 g of xylene were heated at 240°C under N2 with stirring for 1 h.[15] The alcoholysis mixture was analyzed as follows.

Analysis of Alcoholysis Mixtures from Steps (1) and (2)

Each of the above alcoholysis mixtures was analyzed by GPC, methanol solubility test, and periodic oxidation, as follows. (It was not possible to estimate the amounts of glycerol monoesters and diol monoesters separately.)

Gel Permeation Chromatography

The alcoholysis mixture (containing monoglyceride, diglyceride, and mono- and fatty esters of added diol) was analyzed on Waters GPC instrument using styragel (500, 500, 100, and 100) with THF as the solvent (1.5 mL/min). (Although the term monoglyceride is valid only when the alcoholysis is carried out with glycerol, it is generally used to represent the mixture of monoesters formed after alcoholysis with any diol or polyol.)

Methanol Solubility Test

The progress of alcoholysis was ascertained by checking the solubility of the alcoholysis mixture in methanol in the ratio of 1 : 2. The total disappearance of the oil phase with complete solubility in 1 :

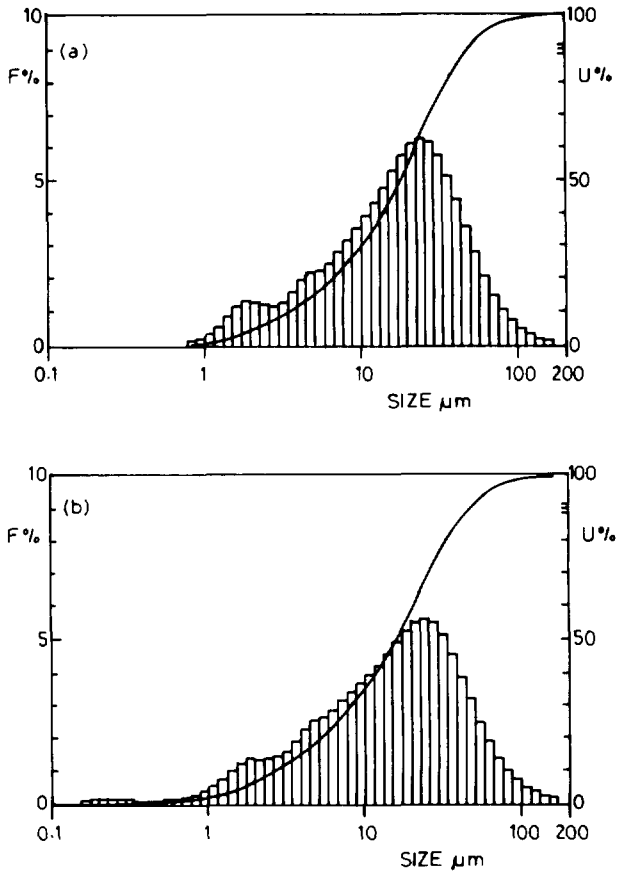


Figure 6 Particle size distribution of lipase suspension in (a) isopropanol and (b) hexane. (a): Median = 18.40 μm; sp. area = 6868 cm^2/cm^3; % on DIA = 9.1 μm = 26.6%; DIA on % = 90.0% = 50.08 μm. (b): Median = 16.21 μm; sp. area = 10076 cm^2/cm^3; % on DIA = 9.1 μm = 32.1%; DIA on % = 90.0% = 49.08 μm.

2 methanol at 30°C represented the completion of alcoholysis reaction.[14]

Periodic Acid Oxidation

Periodic acid oxidation experiments have been carried out according to the method of Pohle and Mehleubacher[16] to differentiate one- and two-monoglyceride composition in both cases.

Preparation of Alkyd Resins by Polyesterification

The alkyd resin synthetic method employed is essentially an extention of the standard method.[15] To each of the above monoester compositions, 0.72 mol of phthalic anhydride (PA), 0.72 mol of polyol, and 17 g of xylene were added and placed in a 1 L, three-necked round bottom flask equipped with a Dean–Stark trap, a cold water condenser, a nitrogen inlet, and a mechanical stirrer. The condensation polymerization was carried out at 210°C for $1\frac{1}{2}$ h, and about 15 mL water was collected until an acid value of approximately 14–15 was attained. After allowing the mixture to cool to 90°C, xylene was added to make 60% solid content.

Preparation of Alkyd–Urethane Resins with Diisocyanates

To the alcoholysis mixture of step (1) or (2), 0.245 mol toluene diisocyanate (TDI) was added in 200 g of dry THF (Tetrahydrofuran) with stirring. The reaction was carried out for 12 h at room temperature, and the molecular weights were determined by GPC.

Particle Size Measurements

Particle size distribution of PPL suspended in organic solvent was measured on Horiba Lasersize 500 using isopropanol or hexane as the solvent. Infrared spectra of polyester and polyurethanes were measured on Perkin-Elmer infrared spectrophotometer.

The acid values of the resins were determined by titration following the standard procedure. The molecular weights of the above resin samples were determined by standard GPC calibration method.

RESULTS AND DISCUSSION

Coatings based on alkyd resins retain a large market share because of their low cost, versatility, and ease of application. These resins are largely synthesized from three basic components—triglycerides, polybasic acids, and polyols—but yield ill-defined and rather complex structures. The nature, composition, sequence, and stereochemistry of these building blocks control the physical properties of the final resin. The introduction of a number of new technologies, all derived from the demand to reduce or eliminate solvent emissions from coating formulations, have given a new impetus to the design and development of tailor-made alkyds resins. Modern research tools, such as molecular modelling coupled with selective chemical/chemoenzymic synthetic routes, are capable of yielding insights into the structure-property relationships of these complex materials.

Molecular graphic representations of the repeating units I and II incorporating one- and two-mono-

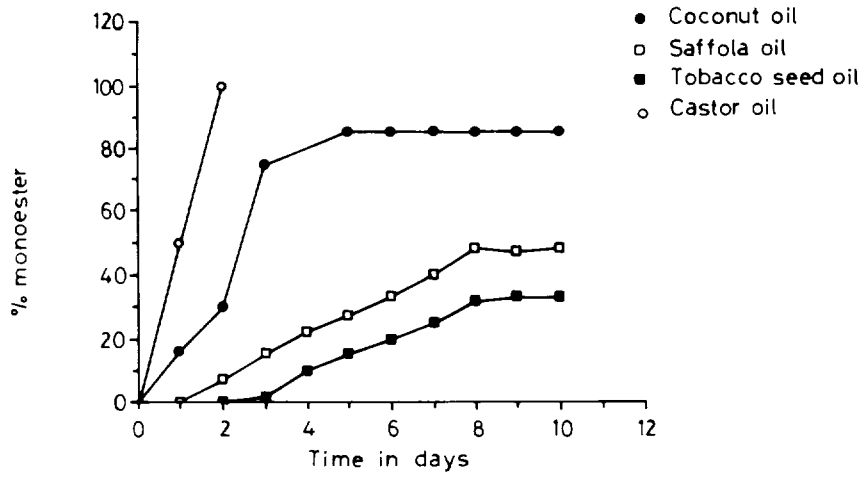


Figure 7 Rates of transesterification of various triglycerides with CHDM. Experimental conditions are as follows: triglyceride, 0.15 mol; CHDM, 0.35 mol; solvent (THF), 30 mL; temperature, 32°C; lipase, 10 g.

glycerides are very revealing and are shown in Figure 2. The monoglyceride selected for modelling is monoester of lauric acid, a predominant fatty acid in CNO. Other fatty acids present in CNO differ only in chain length and are not expected to be very different. Model structures comprising of PA/1-MG/CHDM (I) and PA/2 MG/CHDM (II) are the two basic structures considered in this investigation. The CHARMm has several built-in energy minimization techniques available for determining the minimum energy conformation of the molecule. For small molecules, a global minimum energy conformation can be found; but for large macromolecular

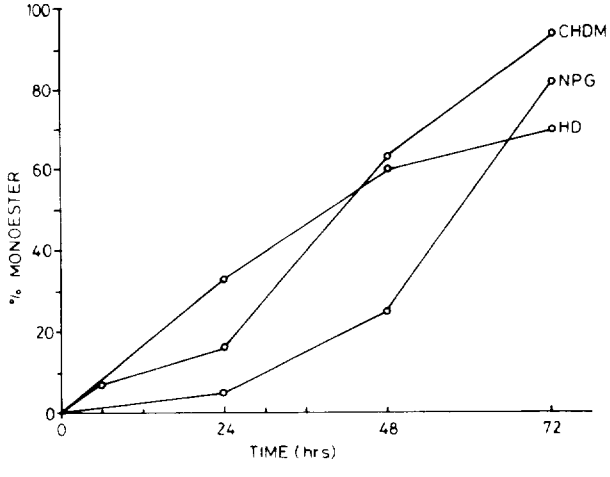


Figure 8 The rates of transesterification of CNO with various diols in THF. Experimental conditions are as follows: CNO, 0.15 mol; diol, 0.35 mol; THF, 30 g; lipase, 10 g; temperature, 32°C; time, 72 h.

structures, energy minimization allows one to examine the local minimum around a particular conformation. The energy functions described in the experimental section are applied in vacuum. The final energy minimized conformation of these repeating units consisting of three monomeric residues are distinctly different. We have also constructed models of corresponding trimers III and IV (nine monomeric residues) and minimized their total energy with respect to the geometry. The modelling studies show end-to-end distances of 9.76 and 12.82 Å for I and II (Fig. 3) and 22.06 and 36.56 Å for III and IV trimers (Fig. 4), respectively. These results suggest more extended chains for polyesters based on 2-monoglyceride units. Likewise, the distances between the fatty acids chain ends also are shorter in III (4.52, 29.37, and 27.91 Å) than in IV (12.1, 28.6, and 37.83 Å). Two terminal phenyl groups project away from each other in II, while they face each other in I. The extended chain conformation is expected to influence the viscosity behavior in solutions and also enhance the glass transition temperature. Likewise, the proximity of the fatty acid pendant units may influence the drying properties of the resins. It is our objective to synthesize alkyd resins incorporating these features and find out if they exhibit better performance characteristics.

Lipases are a class of triglyceride-hydrolyzing enzymes whose activity depends on the occurrence of an oil–water interface.[17]

Different lipases show differing specificities as far as the cleavage of various ester bonds, chain length, and structure of fatty acid are concerned. PPL hy-

Table I Polymerization Data

	Monoglyceride Stage		Alkyd Resin[b]			Alkyd Urethane[c]	
Diol[a] Catalyst	Monoglyceride	Diglyceride	Acid Value	M_w	M_n	M_w	M_n
Lipase	90.00	10.00	4.2	2538	2229	3607	2921
CHDM Ca $(OAC)_2$	62.0	38.0	4.06	2499	2215	3958	3100
Lipase	82.0	18.00	9.58	2001	1838	2958	2548
NPG Ca $(OAC)_2$	67.66	32.3	9.23	1976	1812	4002	3016
Lipane	75.5	24.5	8.38	2206	1999	3618	2988
Hexane Diol Ca $(OAC)_2$	56.4	43.5	5.14	2467	2211	4010	3163

[a] Diol is used in alcoholysis of triglyceride.
[b] During the polymerization added diol was the same as the one used during alcoholysis.
[c] TDI was the diisocyanate used. Experimental conditions are as follows: 10 g of PPL in 20 mL, THF for 72 h; 0.05 g of Ca $(OAC)_2$ in 3 g; Xylene for 1 h; Triglyceride, 0.15 mol; Diol, 0.35 mol.

drolyses fatty acids regiospecifically from one and three positions and is ineffective in exchanging at the two position.[18] In commercial preparations, all monoglycerides are usually 1-MG and are easily analyzed by the periodic acid method. 1-MG have one primary and one secondary hydroxyl (1,2) available for reaction with phthalic anhydride, while 2-monoglycerides would have two primary (1,3) hydroxyls, allowing higher polymerization rates and uniform distribution of fatty acids at two position along the polyester chain. If no randomization takes place during polymerization, a more ordered structure of the alkyd is expected from 2-monoglycerides. Figure 5 illustrates the enzyme-assisted route to oil-based polyester resins.

To date, the general consensus seems to be that high biocatalytic activity is favored in relatively hydrophobic solvents and that none or low activity is observed in relatively hydrophilic solvents. It is also generally agreed that log P as a measure of hydrophobicity of a solvent is preferred over all other parameters.[19] Since PPL is dispersed in a solvent, the active surface area available to the substrate is determined by the particle size and distribution of these lipase particles. Although the differences of lipase activity in different solvents were attributed to the retention or removal of water layer surrounding the enzyme molecule,[11] we found some differences in the particle size distributions (Fig. 6). The number average diameter of the lipase particles in isopropanol and hexane were 21.0 and 19.3 μ, and the specific areas in the same were 6868 and 10076 cm^2/cm^3, respectively. The enzyme particles in polar media seem to aggregate into larger particles, thus offering less surface area available for enzyme-substrate binding. The size of the lipase particle is larger, and the surface area available is less in polar isopropanol than in nonpolar hexane. In our investigations, the selection of solvent was limited by the mutually exclusive solubilities of the substrates, i.e., triglycerides and diol in organic solvents. As a result, all the transesterifications were carried out in THF. No alcoholic solvent could be used since it would participate in transesterifications. Hexane and other hydrocarbon solvents could not dissolve the diols and triols to maintain a homogenous phase. Unfortunately, the particle size measurements in THF could not be carried out because of the susceptibility of flow tubes, but the values are expected to fall within the limits of these two solvents.

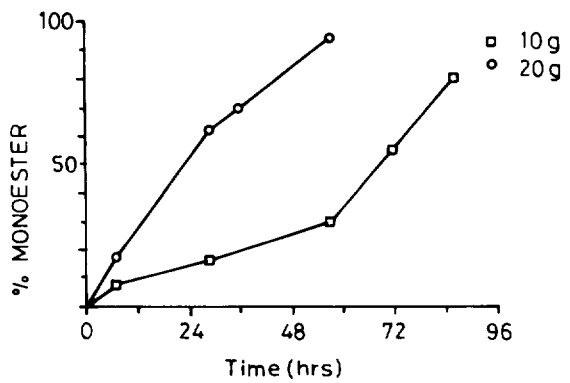


Figure 9 The effect of lipase concentration on the transesterification of CNO with CHDM. Experimental conditions are as follows: CNO, 0.15 mol; CHDM, 0.35 mol; THF, 30 g; temperature, 32°C; time, 72 h.

Figure 7 shows the rates of transesterification of various triglycerides with 1,4-cyclohexane dimethanol in THF at room temperature. In all the

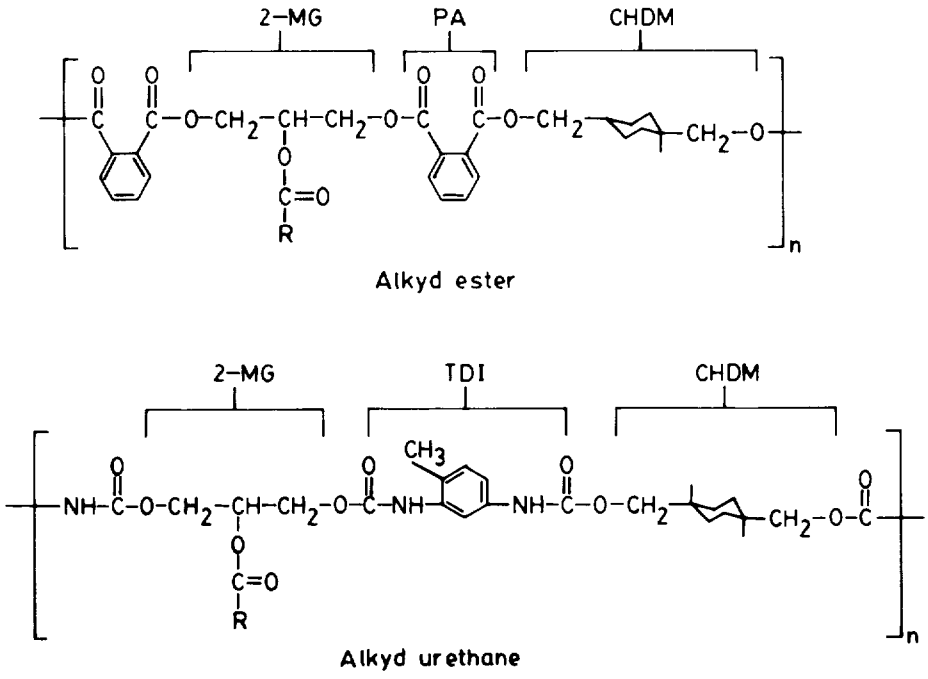


Figure 10 Structures of alkyd resin (a) and alkyd urethanes (b).

experiments, diol/oil ratio was maintained at 2.3 to 2.4, which is close to that required to give monoesters. Separate control experiments have also been carried out with oil/diol and oil/lipase to make sure that the transesterification is indeed enzyme-mediated. There was no reaction in each case. The rate of transesterification with a given diol seems to be dependent on the nature and composition of triglyceride. In the case of castor oil, the situation is more complicated because of the presence of hydroxy fatty acids. While coconut oil contains predominantly C_{11} lauric acid, other oils contain longer C_{16}–C_{18} fatty acids. With tobacco seed and saffola oils, the reaction could not be carried to completion even after one week at room temperature. About 90% of CNO fatty acids are saturated, and they differ only in molecular weight. Safflower oil has the highest linoleic acid content of any known oil, and its glycerides necessarily consist of dilinoleoglycerides and trilinolein. The glyceride composition of TSO resembles that of safflower oil and is very rich in linoleic acid. Although no specific studies are available on the fatty acid specificity of lipases toward triglycerides investigated in our work, the position of the double bond and the nature of unsaturation are reported to be decisive factors in the rates of transesterification. Transesterifications are reported to be lower with unsaturated triacyl glycerol when compared to their saturated counterpart. Berger et al.[20] in a detailed comparative study on the rates of transesterifications of triglycerides demonstrated that unsaturated triglycerides, such as triolein, undergo slow reaction in comparison to their saturated analogs, such as tripalmolein. The differences observed between CNO, on one hand, and TSO and safflower, on the other, can directly be attributed to

Table II Differences in CNO-based Monoglyceride Processes

Base Catalysis	Lipase Catalysis
Low levels of monoesters (65%)	Higher levels of monoesters (75–90%)
1-monoglycerides	2-monoglycerides
One primary and one secondary OH	Primary (1 and 3) hydroxyls available for polymerization
Coiled conformation	Extended chain conformation
220°C, 1 h	Ambient, two to three days

the high levels of unsaturated triglycerides in the latter and their resistance to enzymatic transesterification.

Figure 8 shows the reactivity of various diols with CNO under identical conditions (Table I). The extent of base-catalyzed alcoholysis can be related to the chemical reactivity of diols. Increased reactivity of CHDM hydroxyls over other primary hydroxyls may be attributed to the presence of ring, which acts as a semirigid spacer and prevents conformations, which would hinder reactions of the hydroxy group. The neopentyl-like structure is believed to be less reactive because of steric reasons. In all the cases, the disappearance of CNO triglyceride peak (or less than 5%) in GPC coincided with the total solubility of reaction mixture in 1 : 2 methanol.

The rates of reaction increased, marginally going from room temperature 35 to 50°C, but the lipase was found to be inactive above 60°C. Our observations are in conformity with the reported instability of lipases at higher temperature in polar solvents.[21] The amount of lipase has an effect on the completion of transesterification reaction, as shown in Figure 9. The recovered lipase was active and was used two to three times without loss of activity. Detailed studies on the reuse of lipase are in progress.

Another significant aspect of this biocatalytic transesterification is the positional specificity. PPL showed preference for one and three positions of a triglyceride molecule. Periodic acid experiments with CNO and each of the diols tested have confirmed the absence of any vicinal hydroxyl groups, indicating the absence of any one-monoglyceride. However, the base-catalyzed alcoholysis mixture showed 30% of vicinal hydroxyl experiments could not be carried out with saffola and TSO because of their incomplete alcoholysis. The differences between these processes are summarized in Table II.

The monoglyceride mixtures from both base catalysis and biocatalysis were used to prepare two classes of condensation polymers: (1) alkyd polyesters by polyesterification with phthalic anhydride at 220°C, (2) alkyd urethanes by polycondensation reaction with toluene diisocyanate (TDI) at room temperature. In case of alkyd resin synthesis, randomization is still possible because of high temperature polymerization. Representative GPC results of the alcoholysis mixtures and the corresponding alkyd resin molecular weights are listed in Table I. The molecular weights of alkyds or alkyd urethanes are not expected to follow any trend because of following reasons. The number of hydroxyl equivalents available for polymerization are the same, although the hydroxyls may be of diol or that of glycerol monoester. In lipase-catalyzed process, which results in higher MG monglyceride formation, added diol content in the final mixture is correspondingly low. Likewise, when the MG formation is lower, unreacted diol content is correspondingly higher. The interpretation of molecular weight is further complicated because the monoester of added diol acts as a chain terminator in condensation polymerization and will also influence the molecular weight. The use of triols or polyols will eliminate this possibility. We are also investigating the use of other lipases, such as from *Geotrichum candidum*, which are reported to be selective towards triacylglycerols containing unsaturated fatty acids.[20] It is implied that the presence of two monoglycerides as a repeating unit, as evident from periodic acid experiments, would make the alkyd structure resemble those of II and IV (Figs. 3 and 4). Attempts are underway to screen a wide range of triglycerides and to investigate the structure–property relationships of alkyd resins. The scope of the work is vast in view of differing selectivities of lipases and a wide range of monoglycerides and polyols, which can be used as building blocks in alkyd design.

CONCLUSION

PPL is established as an effective and selective biocatalytic aid in the computer-assisted CNO-based alkyd resin synthesis. Molecular modelling studies of alkyd repeating units incorporating two monoglycerides show extended chain conformations. Lipase-catalyzed transesterifications of CNO triglycerides with diols yield two-monoglyceride monoester in higher yields, thus allowing the synthesis of more controlled alkyd resin structures. The performance characteristics of these novel alkyd structures will be investigated.

The authors thank Dr. K. Srinivasan for helpful discussions and R. Kelkar for analytical services. Financial support was provided by ICI India Limited.

REFERENCES

1. G. Sudesh Kumar, *Biodegradable Polymers: Prospects and Progress*, Marcel Dekker, New York, 1986, p. 11.
2. A. Ghogare and G. Sudesh Kumar, *J. Chem. Soc., Chem. Commun.*, 1533 (1989).

3. A. Ghogare and G. Sudesh Kumar, *J. Chem. Soc. Chem. Commun.*, 135 (1990).
4. A. Ghogare and G. Sudesh Kumar, in *Polymer Science: Contemporary Themes*, S. Sivaram, Ed., Tata-McGraw Hill, New Delhi, 1991, p. 263.
5. S. Paul, *Surface Coatings—Science and Technology*, Wiley, New York, 1985, p. 415.
6. K. Holmberg and J. Johansson, in *Organic Coatings: Science & Technology*, Vol. 6, G. D. Parfit and A. V. Patsis, Eds., Marcel Dekker, New York, 1984, p. 23.
7. R. Potenzone and D. C. Doherty, in *ACS Symposium Series 313*, T. Provder, Ed., American Chemical Society, Washington, D.C., 1986, p. 31.
8. R. Potenzone and A. J. Hopfinger, *Polymer*, **10,** 181 (1978).
9. P. Coulter, S. Hanna, and A. H. Windle, *Polym. Prepr.*, **30,** 49 (1989).
10. E. Helfand, Z. R. Wasserman, and T. A. Weber, *Macromolecules*, **13,** 526 (1980).
11. A. M. Klibanov, *CHEMTECH*, **16,** 354 (1986).
12. M. Therisod and A. M. Klibanov, *J. Am. Chem. Soc.*, **109,** 3977 (1987).
13. M. Levitt, *J. Mol. Biol.*, **82,** 393 (1974).
14. D. Swern, Ed., *Bailey's Industrial Oil and Fat Products, 3rd ed.* Wiley, New York, 1964.
15. S. R. Sandler and W. Karo, *Polymer Synthesis—II*, Academic Press, New York, 1977, p. 140.
16. W. D. Pohole and V. C. Mehleubacher, *J. Am. Oil. Chem. Soc.*, **27,** 54 (1950).
17. A. E. Macrae, *J. Amer. Oil. Chem. Soc.*, **60,** 291 (1983).
18. R. D. Schmid, *J. Amer. Oil. Chem. Soc.*, **64,** 563 (1987).
19. C. Laane, S. Boeren, and C. Veeger, in *Biocatalysis in Organic Media*, C. Laane, J. Tramper, and M. D. Lilly, Eds., Elsevier, Amsterdam, 1987, p. 65.
20. M. Berger and M. Schneider, *Biotech. Lett.*, **13,** 641, (1990).
21. A. Zaks and A. M. Klibanov, *Science*, **224,** 1249 (1984).

Received October 3, 1995
Accepted June 10, 1996