Journal of Molecular Catalysis B: Enzymatic 57 (2009) 34–39

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

# Immobilized lipase-catalysed synthesis of cinnamyl laurate in non-aqueous media

Ganapati D. Yadav*, Shrikant B. Dhoot

*Department of Chemical Engineering, University Institute of Chemical Technology (UICT), University of Mumbai, Nathalal Parekh Road, Matunga, Mumbai, Maharashtra 400019, India*

ARTICLE INFO

*Article history:*
Received 11 October 2007
Received in revised form 17 June 2008
Accepted 17 June 2008
Available online 27 June 2008

*Keywords:*
Immobilized lipase
Non-aqueous media
Kinetics
Mechanism
Esterfication
Cinnamyl laurate

ABSTRACT

Esters of cinnamyl alcohol find many applications in food, cosmetic and pharmaceutical industries as flavor and fragrance compounds. The current work focuses on the synthesis of cinnamyl laurate from cinnamyl alcohol and lauric acid, including screening of various immobilized lipases and optimization of reaction conditions such as catalyst loading, speed of agitation, mole ratio and temperature. Among different lipases screened such as Novozym 435, Lipozyme RM IM and Lipozyme TL IM, Novozym 435 was found to be the best catalyst with 60% conversion in 2 h at 30 °C for equimolar quantities of the reactants using 0.33% (w/v) of catalyst and toluene as solvent. An ordered bi–bi mechanism with dead-end complex of lauric acid was found to represent the kinetic data.



## 1. Introduction

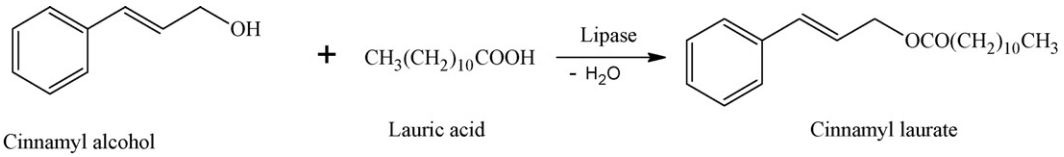
Phenylpropanoids, a class of common phenolic compounds found in plants, may potentially be useful as pest repellents. Particularly their esters have more repellent action than the alcohol [1]. Also these esters belong to a very important class of chemicals having applications as intermediates in the synthesis of fine chemicals, drugs, plasticizers, perfumes, cosmetics and pharmaceuticals and as intermediates to produce chiral auxiliaries [2–4]. In chemical industry the esterifications are generally carried out in the presence of hazardous, toxic and corrosive sulfuric acid and expensive ion exchange resins. The drawbacks in the above processes are longer reaction times, higher reaction temperatures, lower conversions and poor yields [5]. These esters can also be produced by fermentation, which require a lot of purification steps, which are often expensive for commercial production [6–8]. Although enzymes are used for catalysing reactions of non-natural compound, reactions of natural precursors are also an important aspect particularly in the production of esters of natural aroma compounds. Enzymes have been used mostly for aqueous phase reactions. However, non-aqueous enzymology has potential applications in synthetic chemistry as studied, for instance, by our research group [9–13]. It is of particular relevance when reactants are poorly soluble in aqueous media and the hydrolysis reaction is to be suppressed. In nature, enzymes function in aqueous solutions. However, from the biotechnological standpoint there are numerous advantages of conducting immobilized enzymatic conversions in organic solvents as opposed to water, such as: (i) high solubility of most organic compounds in non-aqueous media; (ii) ability to carry out new reactions impossible in water because of kinetic or thermodynamic restrictions; (iii) greater stability of enzymes; (iv) relative ease of product recovery from organic solvents as compared to water [14,15]. Synthesis of cinnamyl laurate in non-aqueous media by using immobilized lipase at room temperature has been considered for the present study. Cinnamyl laureate has applications in pharmaceuticals and fine chemicals industries.

Several mechanisms have been proposed to explain lipase-catalysed reactions. In the ping-pong bi–bi mechanism, a product is released between additions of two substrates. There are good deal of publications on ordered bi–bi mechanism with a dead-end complex of the substrate in non-aqueous media [16] including fatty acid esterifications [17–19]. The kinetic model of Yadav and Lathi [20] for butyl isobutyrate with *n*-butanol considers ping-pong bi–bi mechanism with inhibition by *n*-butanol whereas that for the synthesis of perlauric acid and citronellol laurate is an ordered bi–bi mechanism [21,22].

Although several publications have appeared on enzyme-catalysed reactions in non-aqueous media, there is a dearth of

* Corresponding author. Tel.: +91 22 24102121/24145616; fax: +91 22 24145614.
*E-mail addresses:* gdyadav@udct.org, gdyadav@yahoo.com (G.D. Yadav).

1381-1177/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.molcatb.2008.06.013




**Scheme 1.** Preparation of cinnamyl laureate in non-aqueous media.

quantitative information on kinetics and modeling of some of the industrially relevant reactions. In the light of all these aspects, the current work emphasizes on the synthesis of cinnamyl laurate by using various commercially available immobilized lipases and includes kinetics and mechanism of the reaction.

## 2. Materials and methods

### 2.1. Enzymes

Novozym 435, Lipozyme RM IM and Lipozyme TL IM are gift samples from Novo Nordisk, Denmark. Novozym 435 is the component B of the lipase from *Candida antarctica*, immobilized on a macroporous polyacrylic resin. Lipozyme RM IM is *Rhizomucor miehei* lipase immobilized on an anionic resin. Lipozyme TL IM is *Thermomyces lanuginosus* lipase immobilized on silica. *T. lanuginosus* lipase is produced by genetically modified *Aspergillus oryzae*.

### 2.2. Chemicals

All chemicals were procured from firms of repute and used without any further purification. Lauric acid, toluene, 1,4-dioxane, tetrahydrofuran, *t*-butanol and other analytical reagents were obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, India. Cinnamyl alcohol was purchased from Sisco Chem Pvt. Ltd., Mumbai, India.

### 2.3. Experimental setup

The experimental setup consisted of a 3 cm i.d. mechanically agitated glass reactor of 50 $cm^3$ capacity, equipped with four baffles and a six-bladed turbine impeller. The entire reactor assembly was immersed in a thermostatic water bath, which was maintained at a desired temperature with an accuracy of $\pm 1\,^{\circ}C$.

### 2.4. Enzymatic reaction

A typical reaction mixture consisted of equimolar quantities of cinnamyl alcohol and lauric acid (0.005 mol each) diluted to 15 $cm^3$ with toluene as solvent. Then 50 mg of immobilized enzyme was added to initiate the reaction. The reaction mixture was agitated at 30 °C at a speed of 200 rpm. Liquid samples were withdrawn periodically from the reaction mixture and analyzed on gas chromatograph.

### 2.5. Analysis

Analysis of reaction mixture was carried out on gas chromatograph (Chemito 8610) equipped with flame ionization detector using 4 m × 3.2 mm stainless steel column packed with 10% OV-101 stationary phase. Formation of cinnamyl laurate was confirmed by GC–MS analysis (Clarus 500 GC/MS, PerkinElmer, USA, India).

## 3. Results and discussion

The effects of various parameters on conversion and rate of reaction were studied systematically. The reaction is shown by Scheme 1.

### 3.1. Efficacy of various catalysts

Different lipases were used to evaluate their efficacy. Lipozyme RM IM, Lipozyme TL IM and Novozym 435 were screened under otherwise similar conditions. The conversion profiles are presented in Fig. 1. The conversion varied markedly with the type of lipase. The order of activity was as follows: Novozym 435 > Lipozyme RM IM > Lipozyme TL IM, with final conversion of 60, 16.5 and 9% in 2 h, respectively. Lipozyme TL IM is mainly used for interesterification of bulk fats in the synthesis of frying fats [23]. Novozym 435 and Lipozyme RM IM were effective in catalysing the synthesis of cinnamyl laurate. Novozym 435 is a thermostable lipase and mainly useful for the synthesis of esters and amides [24]. Novozym 435, being the most active among the screened lipases, was selected for all further experiments.

### 3.2. Effect of different solvents

A number of solvents such as tetrahydrofuran (THF), toluene, 1-4 dioxane, *t*-butanol and hexane were used to study their effect on reaction rate and conversion (Fig. 2). The activity of Novozym 435 was maximum in toluene with a conversion of 60%. The conversion in hexane was slightly lower (57%) than that in toluene. The conversion was very low in *t*-butanol (24%) whereas conversions of 31 and 35% were obtained in 1-4 dioxane and THF, respectively.

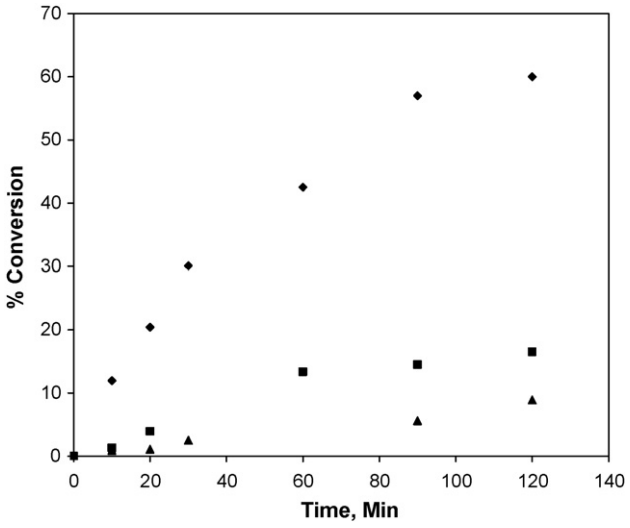



**Fig. 1.** Screening of different catalyst. Reaction conditions—cinnamyl alcohol: 0.33 mol $l^{-1}$, lauric acid: 0.33 mol $l^{-1}$, toluene up to 15 $cm^3$, speed of agitation: 200 rpm, temperature: 30 °C, catalyst: 0.0033 g/$cm^3$, time: 120 min. (♦) Novozym 435; (■) Lipozyme RM IM; (▲) Lipozyme TL IM.

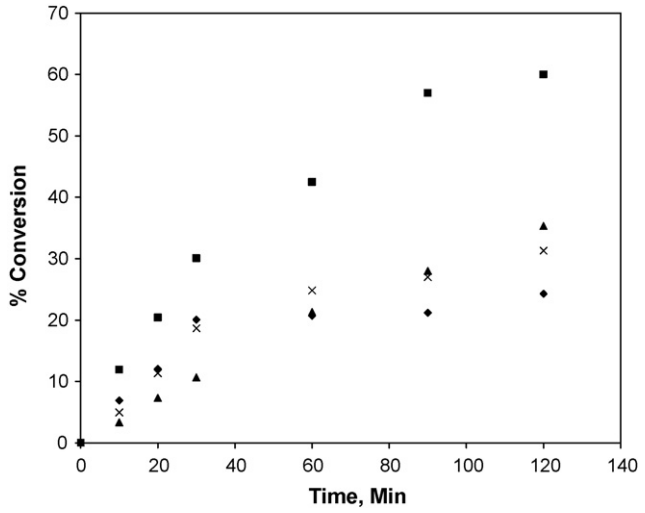



**Fig. 2.** Screening of different solvents. Reaction conditions—cinnamyl alcohol: 0.33 mol $l^{-1}$, lauric acid: 0.33 mol $l^{-1}$, solvent up to 15 $cm^3$, speed of agitation: 200 rpm, temperature: 30 °C, catalyst: 0.0033 g/$cm^3$, time: 120 min, (♦) *t*-butanol; (■) toluene; (▲) THF; (×) 1,4 dioxane.

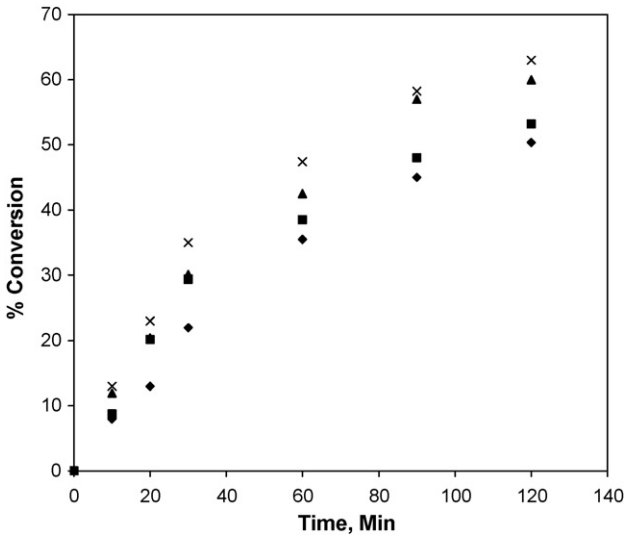



**Fig. 3.** Effect of speed of agitation on the formation of cinnamyl laurate. Reaction conditions—cinnamyl alcohol: 0.33 mol $l^{-1}$, lauric acid: 0.33 mol $l^{-1}$, toluene up to 15 $cm^3$, speed of agitation: 100–250 rpm, temperature: 30 °C, catalyst: 0.0033 g/$cm^3$, time: 120 min, (♦) 100 rpm; (■) 150 rpm; (▲) 200 rpm; (×) 250 rpm.

In general, enzymes are more stable when suspended in non-polar solvents that have low solubility in water [19]. Naturally lipases catalyse the hydrolysis of esters of triacid glycerol. Shift in their catalytic activity from hydrolysis to esterification of natural as well as non-natural compounds is the result of medium engineering. Thus, the overall efficiency of the enzyme changes dramatically when the reaction medium is changed from water to an organic solvent. Further choosing the appropriate reaction media for the enzymatic reaction is very important because many organic solvents are known to inactivate and denature them. Apolar solvents with high log *P* do not distort the essential water layer around the particle, thereby leaving the enzyme in an active state [25]. Therefore toluene offered maximum conversion. All further experiments were conducted in toluene.

### 3.3. Effect of speed of agitation

The effect of speed of agitation was studied in the range of 100–250 rpm by taking 0.005 mol cinnamyl alcohol and lauric acid each and 50 mg Novozym 435 made up to 15 $cm^3$ with toluene (Fig. 3). It was observed that the conversion increased with an increase of speed of agitation from 100 to 200 rpm. There was no further change in conversion up to 250 rpm. This indicated that there was no external mass transfer limitation above 200 rpm. Hence, a speed of agitation 200 rpm was chosen for further studies. At higher speed of agitation it was observed that substantial catalyst particles were thrown outside the liquid phase at higher speed, sticking to the wall of the reactor, which would thereby reduce the effective catalyst loading. Also with increasing speed shearing of the enzyme molecule occur. So if the speed of agitation is increased beyond 250 rpm, the reaction rate goes down, because of the enzyme loss and not because of the mass transfer influence.

### 3.4. Analysis of mass transfer and internal diffusion effects

The observed linearity between initial rate and enzyme concentration alone does not rule out the possibility of mass transfer limitations, because a reaction that is controlled by mass transfer would produce a linear dependence of rate upon substrate concentration. To ascertain the role of mass transfer and intra-particle diffusion, it is useful to compare the time constants for reaction ($t_r$) and diffusion ($t_d$). These are defined as follows: $t_r = C_0/r(C_0)$ and $t_d = D_S/(k_{SL})^2$, where $C_0$ is the substrate concentration in bulk organic phase (mol $l^{-1}$), $r_0$ the initial rate of reaction (mol $l^{-1}$ $s^{-1}$), $D_S$ the diffusivity of substrate in organic phase ($m^2$ $s^{-1}$) and $k_{SL}$ is the solid–liquid mass transfer coefficient in organic phase (m $s^{-1}$). $C_0$ and $r_0$ are determined experimentally. The mass transfer coefficient could be estimated from the Sherwood number and thus $k_{SL} = 2D_S/d_p$, where $d_p$ is the diameter of the support particle. If $t_r \gg t_d$, it implies that the reaction is not influenced by mass transfer. The diffusivity of the substrate in organic solvent could be estimated from the Siebel equation [26]. It was observed that when $C_0$ was 0.33 mol $l^{-1}$, $r_0$ was $4.33 \times 10^{-5}$ mol $l^{-1}$ $s^{-1}$. Diffusivity of lauric acid was calculated as $2.2 \times 10^{-9}$ $m^2$ $s^{-1}$. The average diameter of the support particle was taken as $6 \times 10^{-4}$ m since the particle size ranged between $3 \times 10^{-4}$ and $9 \times 10^{-4}$ m. The value of $k_{SL}$ was calculated from the Sherwood number correlation as $0.73 \times 10^{-5}$ m $s^{-1}$. Thus, $t_r$ and $t_d$ were calculated as 7621 and 40 s, respectively, indicating $t_r \gg t_d$.

Further, it is necessary to compare the rate of substrate diffusion per unit interfacial area ($k_{SL}C_0$) with the reaction rate per unit area $\varphi r_0/a$. $\varphi$ is the phase volume ratio and $a$ is the interfacial area per volume of organic phase. Since the particle is spherical, $\varphi/a = R_p/3$, where $R_p$ is the radius of the particle, which is $3 \times 10^{-4}$ m in this case. $k_{SL}C_0$ was found to be $0.2409 \times 10^{-5}$ mol $m^{-2}$ $s^{-1}$ and $\varphi r_0/a$ was found to be $4.33 \times 10^{-9}$ mol $m^{-2}$ $s^{-1}$. Since the rate of substrate diffusion per unit area is greater than the reaction rate per unit area, it is obvious that intra-particle diffusion does not influence the reaction rate and it is solely controlled by intrinsic enzyme kinetics [26].

### 3.5. Effect of catalysts loading

The effect of catalyst loading was studied from 12.5 to 75 mg under otherwise similar conditions. The rate of reaction increased with increasing catalyst loading and the overall conversion also increased from 42 to 65% (Fig. 4). A linear relationship was obtained when the initial rates were plotted against catalyst loading, which indicated that the reaction was kinetically controlled (Fig. 5). Since there was not significant increase in the final conversion with

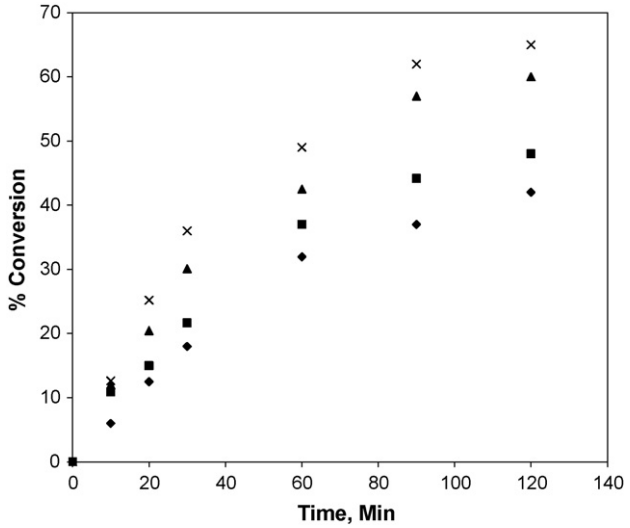



**Fig. 4.** Effect of catalyst loading. Reaction conditions—cinnamyl alcohol: 0.33 mol $l^{-1}$, lauric acid: 0.33 mol $l^{-1}$, toluene up to 15 $cm^3$, speed of agitation: 200 rpm, temperature: 30 °C, catalyst: 0.00083–0.005 g/$cm^3$, time: 120 min, (♦) 12.5 mg; (■) 25 mg; (▲) 50 mg; (×) 75 mg.

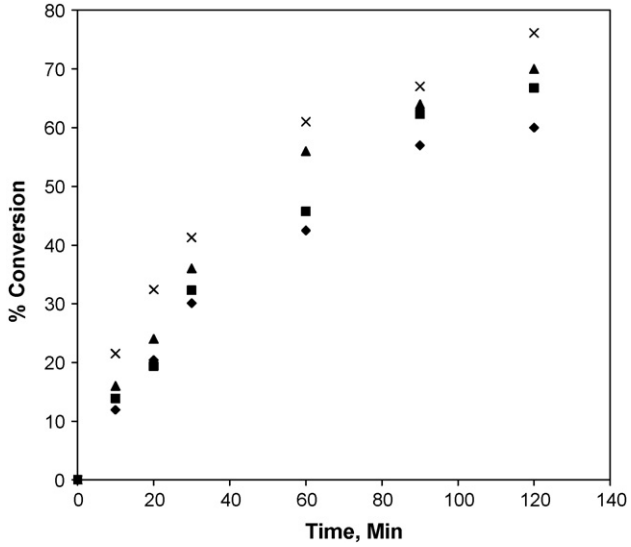



**Fig. 6.** Effect of temperature. Reaction conditions—cinnamyl alcohol: 0.33 mol $l^{-1}$, lauric acid: 0.33 mol $l^{-1}$, toluene up to 15 $cm^3$, speed of agitation: 200 rpm, temperature: 30–60 °C, catalyst: 0.0033 g/$cm^3$, time: 120 min, (♦) 30 °C; (■) 40 °C; (▲) 50 °C; (×) 60 °C.

increased catalyst loading from 50 to 75 mg, further parameters were studied using 50 mg catalyst loading.

### 3.6. Effect of temperature

The effect of temperature was studied in the range of 30–60 °C (Fig. 6). The initial rate and the conversion increased with an increase in temperature from 30 to 60 °C. The final conversions after 2 h were 60, 66, 70 and 76% at 30, 40, 50 and 60 °C, respectively. It is well known that Novozym 435 is thermally stable at 60 °C and hence there was no deactivation of the enzyme at 60 °C. This further supported the previous finding that the reaction was intrinsically kinetically controlled. Elevated temperature reduces density of solvent. Pressure and temperature also directly affect enzyme activity and reaction parameters including the rate constant. Especially the rise in temperature is responsible for the activation of Novozym. Also the enthalpic contribution to the enzymatic rate enhancement suggests that there are important electrostatic and hydrogen-bonding interactions in the transition state of the enzymatic reaction, which are responsible for increased rate with temperature.

### 3.7. Effect of concentration of lauric acid

The amount of lauric acid was varied in the range of 0.005–0.02 mol, holding constant the amounts of other components of the reaction mixture: cinnamyl alcohol (0.005 mol), Novozym 435 (50 mg), toluene (to make the volume to 15 $cm^3$). Increasing the concentration of lauric acid decreased the rate of reaction and conversion (Fig. 7) which is discussed with an appropriate model later. It could be due to the substrate getting strongly adsorbed on the enzyme active site or inhibition of enzyme due to excess substrate.

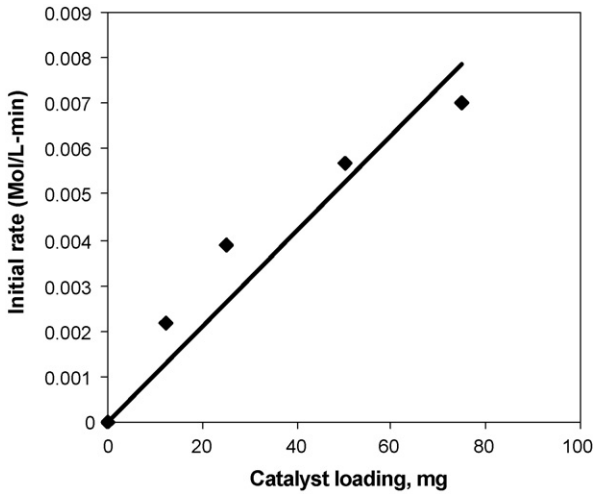



**Fig. 5.** Initial rate vs. catalyst loading. Reaction conditions—cinnamyl alcohol: 0.33 mol $l^{-1}$, lauric acid: 0.33 mol $l^{-1}$, toluene up to 15 $cm^3$, speed of agitation: 200 rpm, temperature: 30 °C, catalyst: 0.00083–0.005 g/$cm^3$, time: 120 min, (■) initial rate (mol $l^{-1}$ $min^{-1}$).

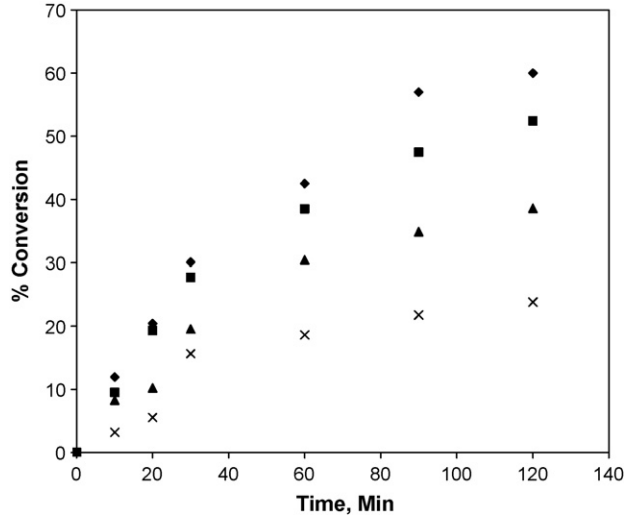



**Fig. 7.** Effect of lauric acid concentration. Reaction conditions—cinnamyl alcohol: 0.33 mol $l^{-1}$, lauric acid: 0.33–1.33 mol $l^{-1}$, toluene up to 15 $cm^3$, speed of agitation: 200 rpm, temperature: 30 °C, catalyst: 0.0033 g/$cm^3$, time: 120 min, (♦) 1:1; (■) 1:2; (▲) 1:3; (×) 1:4.

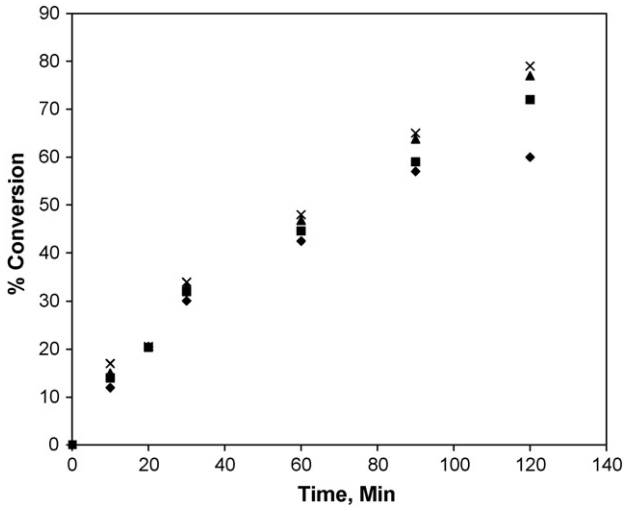



**Fig. 8.** Effect of concentration of cinnamyl alcohol. Reaction conditions—lauric acid: 0.33 mol $l^{-1}$, cinnamyl alcohol: 0.33–1.33 mol $l^{-1}$, toluene up to 15 $cm^3$, speed of agitation: 200 rpm, temperature: 30 °C, catalyst: 0.0033 g/$cm^3$, time: 120 min, (♦) 1:1; (■) 1:2; (▲) 1:3; (×) 1:4.

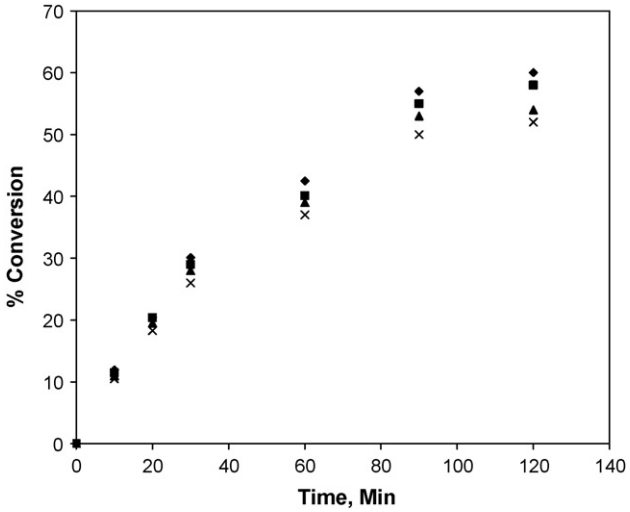



**Fig. 9.** Reusability of the catalyst. Reaction conditions—cinnamyl alcohol: 0.33 mol $l^{-1}$, lauric acid: 0.33 mol $l^{-1}$, toluene up to 15 $cm^3$, speed of agitation: 200 rpm, temperature: 30 °C, catalyst: 0.0033 g/$cm^3$, time: 120 min, (♦) fresh; (■) 1st reuse; (▲) 2nd reuse; (×) 3rd reuse.

### 3.8. Effect of concentration of cinnamyl alcohol

The effect of concentration of cinnamyl alcohol was studied in the range of 0.005–0.02 mol under otherwise similar conditions. It was found that increasing the concentration of cinnamyl alcohol increased the reaction rate (Fig. 8). On increasing cinnamyl alcohol concentration the overall conversion increased from 60 to 79%.

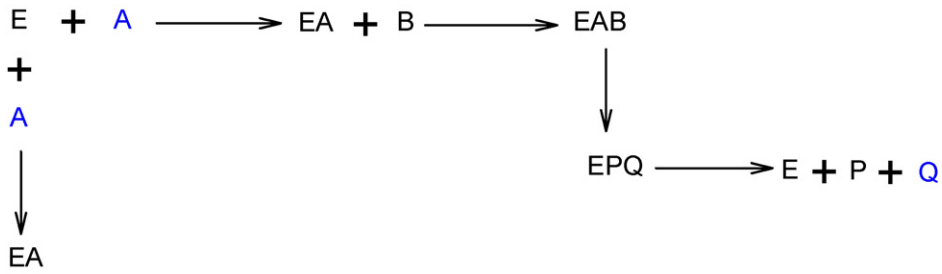
### 3.9. Reusability of the catalyst

Novozym 435 reusability was studied out to ascertain its stability during the reaction. The catalyst was filtered after each use and washed with *tert*-butanol and dried at room temperature. The reusability was examined under otherwise similar conditions. It was found that there was a marginal decrease in activity from 60 to 52% after three reuses (Fig. 9) which might be due to the loss of catalysts during handling.

## 4. Kinetic model

It was observed that the initial reaction rate had increased as the concentration of cinnamyl alcohol ($B$) was increased. A further observation was that when lauric acid ($A$) concentration was increased, initial reaction rate decreased. The effect of substrate inhibition by lauric acid was notable which caused the reaction rate to fall. The Lineweaver–Burk double inversion plot ($1/r_0$ vs. $1/[B]$) shows that the lines do not cross at the same point (Fig. 10). It suggests that both slope and intercepts are affected in a noncompetitive inhibition [27]. The plot also shows that as the concentration of lauric acid is increased, the slope increases and intercepts on $1/r_0$ axis decrease. The inhibition is due to the formation of a dead-end complex with lauric acid.

From the initial rate studies, a complete rate equation was proposed. A ternary complex mechanism was used to analyze the experimental data. A typical reaction sequence is shown below. According to it, the lipase (E) first binds with the lauric acid ($A$) to form the lauric acid enzyme complex (AE). The second reactant cinnamyl alcohol ($B$) then combines with (AE) to form ternary complex ABE. This ternary complex then isomerises to another ternary complex EPQ, which releases the product cinnamyl laurate (P) and water (Q) and frees the enzyme E.




The final equation for the above reaction sequence is [24]:

$$\frac{r_0}{r_{max}} = \frac{[A][B]}{K_{iA} + K_{mB} + K_{mA}[B] + K_{mB}[A] + [A][B]} \quad (1)$$

where $r_0$ is the initial rate of reaction (mol $l^{-1}$ $min^{-1}$); $r_{max}$ is maximum rate of reaction (mol $l^{-1}$ $min^{-1}$); $[A]$ is initial concentration of lauric acid (mol $l^{-1}$); $[B]$ is initial concentration of cinnamyl alcohol (mol $l^{-1}$); $K_{mA}$ is Michaelis constant for lauric acid (mol $l^{-1}$); $K_{mB}$ is Michaelis constant for cinnamyl alcohol (mol $l^{-1}$); $K_{iA}$ is inhibition constant for lauric acid (mol $l^{-1}$) [28].

The data from initial rate measurement were used for the optimization of parameters by least square error estimation using the software Polymath 5.1. Plots of simulated rate versus experimental rate show that the proposed model fits the data very well (Fig. 11). The values of the kinetic parameters obtained are given in Table 1.

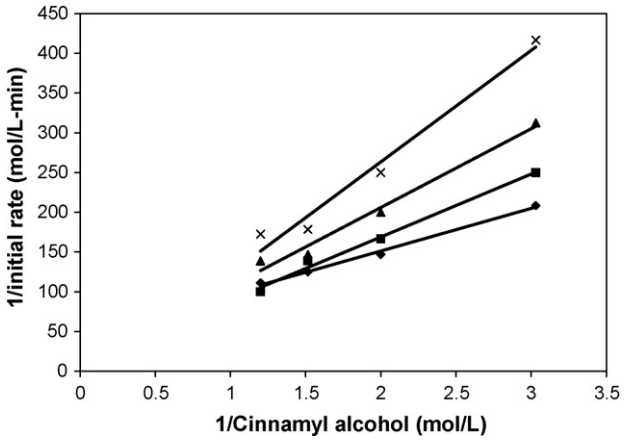



**Fig. 10.** Lineweaver–Burk double inversion plot for reaction between cinnamyl alcohol ($B$) and lauric acid ($A$) for different concentrations of $B$ at 30 °C. The initial rate of reaction is (mol $l^{-1}$ $min^{-1}$) for a fixed catalyst loading of 50 mg. [$A$] = (♦) 0.33; (■) 0.66; (▲) 1; (×) 1.33 mol $l^{-1}$.

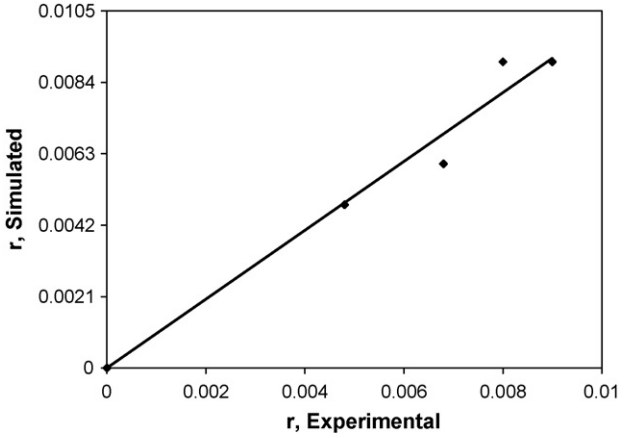



**Fig. 11.** Parity plot for reaction between cinnamyl alcohol and lauric acid (The rates are in mol $l^{-1}$ $min^{-1}$.).

**Table 1**
Values of the kinetic parameters for ternary complex mechanism with dead-end inhibition by lauric acid

| Kinetic parameters | Value |
|---|---|
| $V_{max}$ (mol $l^{-1}$ $min^{-1}$) | 2.0099 |
| $K_{mA}$ (mol $l^{-1}$) | 0.9102 |
| $K_{mB}$ (mol $l^{-1}$) | 1.3833 |
| $K_{iA}$ (mol $l^{-1}$) | 0.0633 |

## 5. Conclusions

The synthesis of cinnamyl laurate from cinnamyl alcohol and lauric was studied systematically including screening of various immobilized lipases and optimization of reaction conditions such as catalyst loading, speed of agitation, effect of mole ratio and temperature. Among different lipases screened such as Novozym 435, Lipozyme RM IM and Lipozyme TL IM, Novozym 435 was found to be the best catalyst with 60% conversion in 2 h at 30 °C for equimolar quantities of the reactants using 0.33% (w/v) of catalyst and toluene as solvent. The kinetic model was built using initial rate data and it follows the ordered bi–bi mechanism with inhibition by lauric acid. The simulated and experimental data agree very well.

## Acknowledgement

This work was supported by Department of Biotechnology, Govt. of India under a project titled, 'Synthesis of chiral drugs through biotransformation', under which SBD received a SRF. GDY also acknowledges the Darbari Seth Professorship Endowment for financial support. Also the authors thank Novo Nordisk, Denmark for the gift samples of enzymes.

GDY also thanks the Purdue University for inviting him as Distinguished Visiting Scholar under the President's Asia Initiative Program, which allowed him to indulge into creative pursuits.

## References

[1] W.J. Jakubas, P.S. Shah, J.R. Mason, D.M. Norman, Ecol. Appl. 2 (1992) 147–156.
[2] H. Stamatis, P. Christakopoulos, D. Kekos, B.J. Macris, F.N. Kolisis, J. Mol. Catal. B: Enzym. 4 (1998) 229–236.
[3] N.N. Gandhi, J. Am. Oil Chem. Soc. 74 (1997) 621–634.
[4] C.C. Akoh, L.N. Yee, J. Mol. Catal. B: Enzym. 4 (1998) 149–153.
[5] B.M. Choudary, V. Bhaskar, L.M. Kantam, K.K. Rao, V.R. Kondapuram, US Patent 6,472,555 B2 (2002).
[6] C. Marlot, G. Langrand, C. Triantaphylides, J. Baratti, Biotechnol. Lett. 9 (1985) 647–650.
[7] Y. Wang, Y.-Y. Linko, J. Fermentation Bioeng. 80 (1995) 473–477.
[8] W.W. Welsh, W.D. Murry, R.E. Williams, Crit. Rev. Biotechnol. 9 (1989) 105–169.
[9] G.D. Yadav, P. Sivakumar, Biochem. Eng. J. 19 (2004) 101–107.
[10] G.D. Yadav, I.V. Borkar, AIChE J. 52 (2006) 1235–1247.
[11] G.D. Yadav, S.S. Joshi, P.S. Lathi, Enzyme Microb. Technol. 36 (2005) 217–222.
[12] G.D. Yadav, P.S. Lathi, J. Mol. Catal. B: Enzym. 32 (2005) 107–113.
[13] G.D. Yadav, K. Manjula Devi, JAOCS 78 (2001) 347–351.
[14] A. Zaks, A.M. Klibanov, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 3192–3196.
[15] H. Hirakawaa, N. Kamiyab, Y. Kawarabayashic, T. Nagamunea, Biochim. Biophys. Acta 1748 (2005) 94–99.
[16] J. Tramper, M.H. Vermuë, H.H. Beeftink, U. Von Stockar, Biocatalysis in Non-Conventional Media, Elsevier Science, Amsterdam, The Netherlands, 1992.
[17] T. Garcia, A. Coteron, M. Martinez, J. Aracil, Chem. Eng. Sci. 55 (2000) 1411–1423.
[18] D. Mukesh, S. Jadhav, A.A. Banerji, K. Thakkar, H.S. Bevinakatti, J. Chem. Technol. Biotechnol. 69 (1997) 179–186.
[19] J.A. Arcos, C.G. Hill Jr., C. Otero, Biotechnol. Bioeng. 73 (2001) 104–110.
[20] G.D. Yadav, P.S. Lathi, Biochem. Eng. J. 16 (2003) 245–252.
[21] G.D. Yadav, K. Manjula Devi, Biochem. Eng. J. 10 (2002) 93–101.
[22] G.D. Yadav, P.S. Lathi, J. Mol. Catal. B: Enzym. 27 (2004) 113–119.
[23] Novo Nordisk Product Sheet for Lipozyme TL IM.
[24] Novo Nordisk Product Sheet for Novozym 435.
[25] C. Laane, S. Boeren, K. Vos, C. Veeger, Biotechnol. Bioeng. 30 (1987) 81–87.
[26] D.W. Green (Ed.), Perry's Chemical Engineers Handbook, McGraw-Hill, New York, 1984, p. 3-226.
[27] P.D. Boyer (Ed.), The Enzyme Kinetic and Mechanism, vol. 2, 3rd ed., Academic Press, New York, 1970, p. 18.
[28] I.H. Segel, Enzyme Kinetics, Wiley, New York, 1975, p. 309.