TYPE II:
IMMOBILIZED LIPASE CATALYSED KINETIC RESOLUTION OF SELECTED RACEMIC SECONDARY ALCOHOLS

Chapter 5
Lipase catalyzed kinetic resolution of (±)-1-(2-furyl) ethanol in non-aqueous media
5.1 INTRODUCTION

Enantiomerically pure alcohols are one of the most attractive building blocks for active pharmaceutical intermediate (API) syntheses and are also used as chiral auxiliaries. In biocatalysis, both whole cell and isolated enzymes are used as catalysts (Carvalho, 2011). Highly pure enantiomer of secondary alcohol can be prepared by adopting different methods such as resolution of exiting racemic mixture via enzymatic kinetic resolution, reduction of pro-chiral compounds by asymmetric reduction using whole cell catalysts, and C-C bond formation with lyases (Clapes and Garrabou, 2011; Fatima et al., 2007; Yadav and Devendran, 2012b). Among them, enzymatic method has been successfully applied. Especially, lipase catalyzed kinetic resolution has been well adapted for preparation of single isomeric compounds such as secondary alcohol, acid and amine through esterification/transesterification and hydrolytic reactions, respectively. Lipases offer high chemo-, regio-, and stereo selectivity, accept a repertoire of compounds, function without expensive co-factors and are commercially prepared from different species like microbes, animals and plants (Fernandez et al., 2006; Pilissao et al., 2009). Further the use of organic and neoteric solvent as reaction media for lipase catalyzed reaction widens the scope including greater solubility of organic molecules, enhanced stability of enzymes, ease recovery of product and catalyst as compared to aqueous media (Cantone et al., 2007; Yadav and Devendran, 2012a).

Optically active S-enantiomer of 1-(2-furyl) ethanol used as an important building block for the synthesis of various natural products such as flavonoids, polyketide antibiotics and carbohydrate derivatives (Borisova et al., 2010; Shan and O’Doherty, 2006; Zamojski and Gryniewics, 1984). Kobayashi et al., 2001 reported the enantioselective synthesis of natural product macrophelides A and B, which act as anti-adhesion compounds and effectively inhibit the adhesion of human leukemia HL-60 cells to human-umbilical-vein endothelial cells, using S-1-(2-furyl) ethanol as starting precursor. There are a few studies reported on the synthesis of the single enantiomer of 1-(2-furyl) ethanol by asymmetric reduction using baker’s yeast, asymmetric chemical catalysts or procine pancreatic lipase catalyzed kinetic resolution (Fantin et al., 1994; Li et al., 2004; Mamaghani et al., 2008). All these
reported processes require longer time and there is no information on kinetics of the reaction, which is required for reactor design and scale-up the process. In the current work, lipase catalyzed transesterification of (R/S) -1-(2-furyl) ethanol with various acyl donors was conducted in non-aqueous media (Scheme 5.1). The effect of various parameters such as different commercially available immobilized lipases, acyl donors, solvents, agitation speed, temperature, catalyst loading, and acyl donor concentration was studied systematically by varying one parameter at a time. Finally the mechanism and kinetics are also developed. The results are new and have potential for scale up and industrial application.

Scheme 5.1: Lipase catalyzed kinetic resolution of (±) 1- (2-furyl) ethanol.

5.2 MATERIALS AND METHODS

5.2.1 Enzymes

The following enzymes were received as gift samples from M/s Novozymes A/S (Bagsvaerd, Denmark): (i) Novozym 435: Lipase B from Candida antarctica, supported on a macroporous acrylic resin with a water content of 1–2% (w/w) and enzyme activity 10,000 PLU/g, (ii) Lipozyme RM-IM: Lipase from Rhizomucor miehei, supported on a macroporous anion exchange resin with a water content of 2–3% (w/w) and enzyme activity 6 BAU/g, (iii) Lipozyme TL IM: Lipase from Thermomyces lanuginosus, supported on porous silica granulates with water content 1-2 % and enzyme activity 175 IU/g.
5.2.2 Chemicals

Iso-octane, n-heptane, toluene, cyclohexane, vinyl acetate, methyl acetate, acetic anhydride, ethyl acetate and other analytical and HPLC grade reagents were purchased from M/s S.D. Fine Chemicals Pvt. Ltd., Mumbai, India. (±)-1-(2-Furyl) ethanol, vinyl butyrate and vinyl laurate were purchased from Sigma-Aldrich India Pvt. Ltd., Bangalore, India. All chemicals and enzymes were used without any further modification/purification.

5.2.3 Experimental setup

The experimental set-up consisted of a 3 cm i.d. mechanically agitated glass reactor of 50 cm³ 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 predetermined temperature with an accuracy of ±1 °C. A typical reaction mixture consisted of 0.001 mol racemic alcohol and 0.001 mol vinyl ester diluted to 20 cm³ with n-heptane as a solvent. The reaction mass was agitated at 60 °C for 15 min at a speed of 300 rpm and then 5 mg of enzyme was added to start the reaction.

5.2.4 Analysis

Reaction progress and enantiomeric excess (ee) were monitored by periodical withdrawal of clear liquid samples from the reaction mixture which were analyzed by high performance liquid chromatography (HPLC) (1260 infinity series, Agilent technologies, CA, USA) equipped with chiralpak-IB analytical column (250 x 4.6mm ID) (Daicel Corporation, Japan and; particle size 5μm). Samples (10 μl) were injected via auto-sampler. The mobile phase consisted of n-hexane and isopropyl alcohol (99:1) and the flow rate was maintained at 0.75 ml.min⁻¹. A DAD detector was used at a wavelength of 230 nm. The retention time of (R) and (S) alcohol were 23.1 and 23.9 min, respectively.

The enantioselectivity ratio (E) and conversion (c, %), were calculated from the enantiomeric excess of the substrate (eeₛ, %) and product (eeₚ, %) based on the following equation.
\[ E = \frac{\ln \left( \frac{1-c}{1-ee} \right)}{\ln \left( \frac{1-c}{1+ee} \right)} \]  \hspace{1cm} \text{... (5.1)}

Where,

\[ c = 1 - \frac{B_{(R)} + B_{(S)}}{B_{(R)} + B_{(S)}} \]  \hspace{1cm} \text{... (5.2)}

\[ ee_t = \frac{B_{(S)} - B_{(R)}}{B_{(S)} + B_{(R)}} \] \text{ and } \hspace{1cm} \text{... (5.3)}

\[ ee_p = \frac{Q_{(S)} - Q_{(R)}}{Q_{(S)} + Q_{(R)}} \] \hspace{1cm} \text{... (5.4)}

Where, \( B_{(R)}, B_{(S)}, B_{(R)}, B_{(S)}, Q_{(R)} \) and \( Q_{(S)} \) denote area under the curve of \((R)-1-(1\text{-furyl})\) ethanol, \((S)-1-(1\text{-furyl})\) ethanol and their corresponding esters.

### 5.3 RESULTS AND DISCUSSION

#### 5.3.1 Effect of catalysts

Lipases from different origin such as \emph{Candida antarctica} lipase B (Novozym 435), \emph{Rhizomucor miehei} lipase (Lipozyme RMIM) and \emph{Thermomyces lanuginosus} lipase (Lipozyme TLIM) were evaluated for resolution of \((\pm)-1-(2\text{-furyl})\) ethanol under similar condition. The reactions were carried out using n-heptane as solvent. Figure 5.1 shows conversion at the end of 2 h for each enzyme. Lipase B from \emph{Candida antarctica} (Novozym 435) had given maximum conversion compared to other immobilized catalysts. The order of activity is as follows Novozym 435 > Lipozyme RMIM > Lipozyme TLIM. With Novozym 435, conversion of 47.04\% was obtained compared to Lipozyme RMIM (1.8 \%) and Lipozyme TLIM (1.53 \%). It is reported that both Lipozyme RM IM and Lipozyme TL IM are mainly used to interesterify bulk molecules such as fatty acid derivatives and they have less activity in the resolution of racemic molecules (Sontakke and Yadav, 2011). However, Novozym 435 is a well-known and commercially available lipase for both chiral resolution and
synthesis of small molecules esters (Yadav and Sivakumar, 2004). Hence, further study was carried out using Novozym 435 as catalyst.

**Figure 5.1:** Effect of catalysts for kinetic resolution of \(\pm\)-1-(2-furyl) ethanol

1-(2-furyl) ethanol – 0.001 mol; vinyl acetate – 0.001 mol; n-heptane up to 20 cm³; speed of agitation – 300 rpm; catalyst loading – 5 mg; temperature – 60 °C, ee, Conversion, CAL-B – *Candida antarctica* lipase B, RMIM - *Rhizomucor miehei* lipase, TLIM - *Thermomyces lanuginosus* lipase

### 5.3.2 Effect of acyl donors

The selection of suitable acyl donor is primarily important for lipase catalyzed kinetic resolution of secondary alcohols. It has been well reported that the type of acyl donor and its chain length can influence both the reaction rate and enantioselectivity of enzyme in transesterification/interesterification reactions (Hoffmann et al., 2011). In order to examine the effect of acyl donors on kinetic resolution of \(\pm\)-1-(2-furyl) ethanol, initially we had taken different alkyl and vinyl esters such as acetic anhydride, methyl acetate, ethyl acetate, vinyl acetate as model acylating agents (Figure 5.2). The reactions were performed under similar conditions. At end of 2h, it
was observed that vinyl acetate gave higher conversion (47.04%) and enantioselectivity (88.82%) with $E > 200$ compared to the other alkyl esters. Among alkyl esters, acetic anhydride had given good conversion (39.26%) and enantioselectivity (64.64%) than other esters. It has well reported that vinyl esters are good acylating agents than alkyl esters (Dhake et al., 2012). The liberated co-product is vinyl alcohol (enol form) which is unstable and easily tautomerized to acetaldehyde (aldehyde form) and it is no longer a substrate for lipase. So, the reaction equilibrium shifts to the forward direction which leads to the formation of the chiral ester. Further the effect of acyl chain length was evaluated by using three different vinyl esters such as vinyl acetate, butyrate and laurate. Increasing chain length has lead to decrease in enantioselectivity and conversion. The highest conversion (47.04%) with $ee_s$ of 88.82% was obtained with vinyl acetate as the acyl donor whereas low conversion (38.21%) with $ee_s$ of 61.84% was obtained with vinyl laurate. Hence further study was carried out using vinyl acetate as the acyl donor.

**Figure 5.2:** Effect of acyl donors for kinetic resolution of ($\pm$)-1- (2-furyl) ethanol

1-(2-furyl) ethanol – 0.001 mol; acyl donor – 0.001 mol; n-heptane up to 20 cm$^3$; speed of agitation – 300 rpm; catalyst loading – 5 mg; temperature – 60 °C, $ee_s$, Conversion, AA – Acetic anhydride, EA – Ethyl acetate, MA – Methyl acetate, VA – Vinyl acetate, VB – Vinyl butyrate, VL – Vinyl laurate
5.3.3 Effect of solvents

Solvent plays significant role in any enzymatic reactions. The important criteria for proper choice of organic media are substrate solubility, product recovery and enzyme stability (Silva and Nascimento, 2012). It has been reported well in literature that solvent can alter the enzyme activity, reaction rate and enantioselectivity by modifying aqueous layer around the enzyme particle or changing the enzyme conformational structure (Pan et al., 2010). Several experiments were performed to evaluate the effect of chosen solvents such as iso-octane (logP – 4.5), n-heptane (logP – 4), cyclohexane (logP – 3.2) and toluene (logP - 2.5) on transesterification reaction (Figure 5.3). The experiments were carried out by using 1:1 mole ratio of alcohol to ester (each 1mmol), with 5 mg enzyme loading at 60°C, and the liquid phase volume was made up to 20 cm$^3$. Both conversion and enantioselectivity were found to increase as logP value increased from 2.5 to 4 (toluene to n-heptane). The highest conversion (47.04%) and ee, (88.82%) were obtained when n-heptane employed as solvent, whereas low conversion (22.24 %) and ee, (28.59%) were obtained with toluene. Due to low solubility of racemic alcohol in iso-octane, moderate conversion and enantioselectivity were observed. It clearly correlated with the reported literature that solvents having high log P are hydrophobic in nature but do not strip the water layer around enzyme particle which is essential for maintaining the conformation structure (Yadav and Borkar, 2009b). Thus, they show high enzyme activity. Further work was carried out using n-heptane as the solvent.
5.3.4 Effect of speed of agitation

In immobilized catalysts, reactants have to pass from bulk liquid phase to enzyme particle’s surface, and then diffuse from external surface to enzyme active site. The external mass transfer resistance and intra-particle diffusion rate play significant roles during the reaction. It must be overcome to study the intrinsic kinetics of lipase catalyzed transesterification reaction. Several experiments were performed in the range of 100 to 400 rpm by taking 0.001 mol racemic alcohol and vinyl acetate each at 60 °C with 5 mg Novozym 435 and volume made up to 20 cm³ using n-heptane as solvent. From progress curve (Figure 5.4), the conversion is seen to increase with increase in agitation speed from 100 to 300 rpm and beyond this value; there was no significant difference in conversion. This implies that, there is no external mass transfer resistance and intra-particle diffusion above 300 rpm. The external mass transfer and intra-particle diffusion were further evaluated by comparing the time constants for reaction ($t_r$) and diffusion ($t_d$) using theoretical
calculations (Perry and Green, 1984). These are defined as follows: \( t_r = \frac{C_0}{r(C_0)} \) and \( t_d = \frac{D_S}{(k_{SL})^2} \). If \( t_r >> t_d \) means that the reaction was not mass transfer controlled. Both \( C_0 \) and \( r(C_0) \) were determined experimentally and their values were as 0.05 mol.dm\(^{-3}\) and \( 9.833 \times 10^{-6} \) mol.dm\(^{-3}\).s\(^{-1}\). Diffusivity of racemic alcohol at 60 °C was calculated using Siebel equation as \( 4.513 \times 10^{-5} \) cm\(^2\).s\(^{-1}\). The average diameter of the support particle was taken as 0.06 cm since the particle size ranged between 0.03 and 0.09 cm. The value of mass transfer coefficient of liquid phase was calculated by using Sherwood number \( (k_{SL} = 2D/d_p) \) to be \( 1.504 \times 10^{-3} \) cm.s\(^{-1}\). From these values, the calculated time constants for reaction and diffusion were \( 5.084 \times 10^3 \) s and 19.94 s. The time constant for the reaction was much higher than diffusion indicating that there was no external mass transfer resistance.

Further, it is necessary to rule out the intra-particle diffusion. It could be done by comparing the rate of substrate diffusion per unit interfacial area \( (k_{SL}C_0) \) with the reaction rate per unit area \( (\phi r_0/a) \), where \( \phi \) is the phase volume ratio and ‘a’ is the interfacial area per volume of organic phase (Perry and Green, 1984). As particle was spherical, \( \phi/a = R_p/3 \), where \( R_p \) is the radius of the particle. It was found that the value of \( k_{SL}C_0 = 7.521 \times 10^{-5} \) mol.cm\(^{-2}\).s\(^{-1}\) and \( \phi r_0/a = 9.833 \times 10^{-8} \) mol.cm\(^{-2}\).s\(^{-1}\). It indicated that the rate of substrate diffusion per unit interfacial area was much higher than the reaction rate per unit area. This suggested that there was no intra-particle diffusion limitation. Thus the reaction was controlled by intrinsic enzyme kinetics. Therefore, further experiments were performed at a speed of 300 rpm.
5.3.5 Effect of temperature

Temperature is one of the important parameters which affect the reaction rate and enantioselectivity in lipase catalyzed kinetic resolution of secondary alcohols (Jin et al., 2011). In order to study the effect of temperature, a number of experiments were performed in the range of 40 °C to 70 °C under similar conditions (Figure 5.5). It was observed that reaction rate, enantioselectivity and conversion increased with increase in temperature up to 60 °C. Above this value, there was no significant change in conversion. It has reported that the enzyme is thermally stable at 60 °C (Yadav and Devendran, 2012b). This would further confirm the previous finding that the reaction is intrinsically kinetically controlled. The elevated temperature reduces the viscosity of reaction mixture; increases the collusion frequency between reactants and enzyme particles that lead to increase in reaction rate. Since both conversion and enantioselectivity were found to be maximum at 60 °C and also maintained the
enzyme activity. The temperature of 60 °C was selected as the optimum. Thus further experiments were conducted at 60 °C.

![Graph showing conversion vs time](image)

**Figure 5.5:** Effect of temperature for kinetic resolution of (±) 1-(2-furyl) ethanol

1-(2-furyl) ethanol – 0.001; vinyl acetate – 0.001 mol; n-heptane up to 20 cm³; speed of agitation 300 rpm; catalyst loading – 5 mg; temperature – 40 - 70 °C, ▲ 40 °C, ■ 50 °C, ▲ 60 °C, × 70 °C

The Arrhenius plot was made on the basis of ln(initial rates) vs reciprocal of temperature (Figure 5.6). The apparent activation energy was calculated from the observed initial rates at different temperature under otherwise similar conditions. The activation energy was found to be 8.57 kcal/mol. This value lies within the typical value for enzyme catalytic reaction (Segel, 1975).
5.3.6 Effect of enzyme loading

The addition of enzyme loading has considerable effect on reaction rate and enantioselectivity (Yadav and Sivakumar, 2004). In order to study the effect of catalyst loading, several experiments were performed by keeping the mole ratio constant while enzyme loading was changed in the range from $1.75 \times 10^{-4}$ to $4 \times 10^{-4}$ g.cm$^{-3}$ under similar reaction conditions (Figure 5.7). It was observed that the reaction rate had a linear dependence on enzyme loading. The overall conversion was increased with increase in enzyme loading from $1.75 \times 10^{-4}$ to $2.5 \times 10^{-4}$ g.cm$^{-3}$. Above this value, there was no significant change in substrate conversion. The increase of enzyme loading increases number of active sites proportionately and facilitates the interaction between enzyme active sites and reactant that further enhance the reaction rate and conversion (Figure 5.8). However, at high enzyme concentration, the available enzyme sites are much larger than the substrate used in reaction and it will not further improve the conversion. Thus $2.5 \times 10^{-4}$ g.cm$^{-3}$ was taken as optimum and further studies were carried out with this loading.

![Figure 5.6: Arrhenius plot for kinetic resolution of (±) 1- (2-furyl) ethanol](image)

1-(2-furyl) ethanol – 0.001; vinyl acetate – 0.001 mol; n-heptane up to 20 cm$^3$; speed of agitation 300 rpm; catalyst loading – 5 mg; temperature – 40 - 70 °C

\[ y = -4321.5x + 6.6353 \]
\[ R^2 = 0.9741 \]
Figure 5.7: Effect of enzyme loading for kinetic resolution of (±)-1-(2-furyl) ethanol. 1-(2-furyl) ethanol – 0.001; vinyl acetate – 0.001 mol; n-heptane up to 20 cm³; speed of agitation 300 rpm; catalyst loading – 3.5 - 8 mg; temperature – 60 °C, 3.5 mg, 5 mg, 6.5 mg, × 8 mg

Figure 5.8: Initial rate versus enzyme loading for kinetic resolution of (±)-1-(2-furyl) ethanol

\[ y = 1.6655x \]
\[ R^2 = 0.9718 \]
1-(2-furyl) ethanol – 0.001; vinyl acetate – 0.001 mol; n-heptane up to 20 cm$^3$; speed of agitation 300 rpm; catalyst loading – 3.5 - 8 mg; temperature – 60 °C

5.3.7 Effect of vinyl acetate concentration

In order to study the effect of acyl donor concentration on reaction rate and conversion of kinetic resolution of ($\pm$) 1-(2-furyl) ethanol, a number of experiments were performed at 60 °C with 5 mg of enzyme loading in n-heptane as solvent. The concentration of ($\pm$) 1-(2-furyl) ethanol was kept constant (0.001 mol), and vinyl acetate concentration was varied from 0.001 to 0.004 mol; the mixture volume was kept constant at 15 cm$^3$ by adjusting the addition of n-heptane (Figure 5.9). It was found that the conversion and rate of reaction had decreased with increase in concentration of vinyl acetate. The overall conversion decreased from 47.04% to 41.78%. At high concentration, vinyl acetate acts as an inhibitor and this will be explained later.

**Figure 5.9:** Effect of vinyl acetate concentration for kinetic resolution of ($\pm$) 1- (2-furyl) ethanol

1-(2-furyl) ethanol – 0.001; vinyl acetate – 0.001 – 0.004 mol; n-heptane up to 20 cm$^3$; speed of agitation 300 rpm; catalyst loading – 5 mg; temperature – 60 °C, × 1:1, 1:2, 1:3, × 1:4

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5.3.8 Catalyst reusability

Catalyst reusability is very important in order to make process is more viable at industrial level and also to examine the stability of catalyst after each run. Initial experiments were carried out using 5 mg of catalyst loading under similar conditions. After completion of each run, the catalyst particles were filtered by membrane filter. Multiple washes were given to the catalyst with fresh solvent-heptane, dried at room temperature for 12 h and reused. It was found that there was a marginal decrease in conversion from 47.04 to 44.58% after three reuses, which was due to loss of enzyme during filtration and drying. No make-up quantity was added (Figure 5.10).

**Figure 5.10:** Catalyst reusability for kinetic resolution of (±) 1-(2-furyl) ethanol
1-(2-furyl) ethanol – 0.001 mol; vinyl acetate – 0.001 mol; n-heptane up to 20 cm$^3$; speed of agitation 300 rpm; catalyst loading – 5 mg; temperature – 60 °C
5.4 KINETIC MODEL

Two model have been proposed for two substrate based enzymatic reaction, one is random model, where order of binding of substrate to enzyme active site is random and second model is bi-bi model, where the first step is formation of acyl-enzyme Complex (Krishna et al., 2001a). It has been well reported that the first step of lipase-catalyzed reaction involves the formation of an acyl enzyme complex with the acyl donor and will rules out a random mechanism (Yadav and Borkar, 2009b). As a result, it can only be bi-bi model, wherein two mechanisms have been proposed, namely, ordered bi – bi mechanism or ternary complex mechanism and ping – pong bi-bi mechanism. In the former mechanism, both reactants bind to enzyme and form ternary and subsequently the products are released. In the latter mechanism, the product is released between the additions of substrate (Yadav and Borkar, 2009a; Yadav and Trivedi, 2003).

An intricate kinetic analysis was carried out in order to develop the appropriate mechanism for kinetic resolution of (±) 1-(2-furyl) ethanol. A number of experiments were performed to investigate the effect of substrate concentration on the rate of reaction over a wide range using 5 mg Novozym 435 under similar conditions and the volume was made up to 20 cm-3 with n-heptane. For determination of initial rates, the concentration of vinyl acetate (0.025 - 0.1M) was varied over a wide range of (±) 1-(2-furyl) ethanol concentration (0.025 – 0.1M).
Initial rates were calculated systematically from the linear portion of the concentration-time profiles. It was observed that the rate increased with increasing concentration of alcohol. However, the rate of reaction decreased with increasing concentration of vinyl acetate, suggesting that vinyl acetate acts as reversible inhibitor at high concentration. The Lineweaver–Burk plot (Figure 5.11) with reciprocal of initial rate and concentration of alcohol shows that the lines are not parallel, ruling out the possibility of a ping pong bi-bi mechanism. The slope and intercept changed linearly with increased concentration of acyl donor. The lines were intersecting at certain point suggesting ternary complex mechanism (Fig. 10). According to this, the lipase (E) will react with acyl donor (A) to form a complex (EA). The second reactant 1-(2-furyl) ethanol (B) then reacts to form a ternary complex (EAB). This ternary complex then isomerizes to another ternary complex EPQ, which releases the product ester (P) and vinyl group (Q) and frees the enzyme E. At higher concentrations of
vinyl acetate, reversible dead end complex is formed. A typical reaction sequence is shown below.

\[ E + A \rightarrow EA + B \rightarrow EAB \]

\[ \downarrow \]

\[ EPQ \rightarrow E + P + Q \]

The equation obtained with the above mechanism is:

\[
v = \frac{v_m [A][B]}{K_{iA} K_m^A + K_m^A [B] + K_m^B [A] + [A][B]} \quad \ldots 5.5
\]

whereas \( v \) is the rate of reaction, \( v_m \) the maximum rate of reaction, \( [A] \) the initial concentration of vinyl acetate, \( [B] \) the initial concentration of 1-(2-furyl) ethanol, \( K_{mA} \) the Michaelis constant for vinyl acetate, \( K_{mB} \) the Michaelis constant for 1-(2-furyl) ethanol, \( K_{iA} \) the inhibition constant for vinyl acetate. The initial rate data were used to determine the kinetic parameters of above mechanism by non-linear regression analysis using the software package Polymath 5.1 and their values were tabulated (Table 5.1). The rates of reaction for different reactant concentration were stimulated using above equation to verify the proposed kinetic model. The parity plot between stimulated rate and experimental rate suggested that the proposed mechanism was valid for this reaction (Figure 5.12).
Table 5.1: Values of kinetic parameters for kinetic resolution of (±)-1-(2-furyl) ethanol

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_m ) (mol.dm(^{-3}).s(^{-1}).g(^{-1}) of enz)</td>
<td>4.67( \times )10(^{-3})</td>
</tr>
<tr>
<td>( K_{mA} ) (mol.dm(^{-3}))</td>
<td>0.0261</td>
</tr>
<tr>
<td>( K_{mB} ) (mol.dm(^{-3}))</td>
<td>0.0313</td>
</tr>
<tr>
<td>( K_i ) (mol.dm(^{-3}))</td>
<td>0.0585</td>
</tr>
</tbody>
</table>

Figure 5.12: Parity plot of experimental versus simulated rates for kinetic resolution of (±)-1-(2-furyl) ethanol

5.5 CONCLUSION

In this work, the kinetic resolution of (±)-1-(2-furyl) ethanol was carried out using different immobilized catalysts. Among which, *Candida antarctica* lipase B (Novozym 435) was found to be the best catalyst in n-heptane as solvent. The effect of acyl donor was compared by employing both alkyl and vinyl esters. The highest
conversion and initial rate was obtained with vinyl acetate as the acyl donor. The effect of kinetic parameters on reaction conversion and initial rate was analyzed systematically over a wide range. Maximum conversion of 47.04% was obtained in 2 h using 5 mg enzyme loading with equimolar concentration of alcohol and ester at 60 °C. The progress curve and initial rate data were used to predict the suitable model and then various kinetic parameters were estimated using non-linear regression. The ordered bi bi mechanism with acyl donor inhibition was found to be fit the initial rate data. This model was used to simulate the rate data, which were in excellent agreement with experimental values. The optimized values and kinetic mechanism for resolution of (±) 1-(2-furyl) ethanol is very useful and gives new insight for scale up the enzymatic process at industrial level.