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A Parametric Study on Biphasic Medium Conditions for the Enantioselective Production of Naproxen by *Candida rugosa* Lipase

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Abstract

A parametric study to increase the enantioselectivity of *Candida rugosa* lipase (CRL) toward S-Naproxen production by the hydrolysis of racemic Naproxen methyl ester in an aqueous-organic biphasic batch system was carried out. Effects of organic solvent type, aqueous phase/organic solvent volume ratio, agitation rate, concentrations of the substrate and the enzyme, pH of the aqueous phase, and temperature on the enantiomeric excess for the product (ee_p), on the enantiomeric ratio (E) and on the conversion (x) were evaluated. Employing isooctane as the solvent resulted in higher ee_p, E, and x than those obtained in hexane, cyclohexane, and toluene. The higher the volume ratio of aqueous phase/organic solvent employed, the higher the conversion and enantioselectivity achieved. The increase in agitation rate increased the hydrolysis rate. Higher concentration of racemic Naproxen methyl ester than 10 mg/mL decreased both the conversion and enantioselectivity. The increase in crude CRL concentration resulted in enhancement of x, but the decrease of ee_p and E. Acidic pH led to higher conversion and enantioselectivity than the medium and alkaline pH values. A further increase in temperature to over 45°C decreased the conversion and enantioselectivity. The highest enantiomeric ratio achieved in the S-Naproxen production was E = 171.1, with x = 49.8% and ee_p = 95.7%.

Index Entries: Biphasic systems; *Candida rugosa* lipase; enantioselectivity, Naproxen methyl ester; S-Naproxen.

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Introduction

Candida rugosa lipase (CRL; E.C.3.1.1.3) is widely used in the resolution of racemic mixtures due to its high specificity toward stereoisomers. A nonsteroidal, anti-inflammatory drug S-Naproxen [(S)-(+)-2-(6-Methoxy-2-naphthyl) propionic acid] and its ester pro-drugs are pharmaceuticals that are marketed as single S-enantiomers. Therefore, they have generated a great interest in their production by the catalytic activity of CRL through hydrolysis, esterification, or transesterification reactions. The limited productivity of hydrolysis in an aqueous system due to the low solubility of Naproxen esters and the difficulty to work up the reaction product from an aqueous system have favored the employment of organic solvents to form an aqueous-organic biphasic system in such reactions. The literature on the production of S-Naproxen by hydrolysis mainly covers either the improvement of the enantioselectivity of CRL by purifying the enzyme (1) or the enhancement of the productivity by developing the operational conditions (2–8). However, among them, the number of studies employing the two-liquid phase concept for the development of enantioselectivity of crude CRL in the hydrolysis of racemic Naproxen methyl ester in batch systems is few.

The studies on the hydrolysis of racemic Naproxen methyl ester by CRL have principally reported the effect of solvents not in aqueous-organic biphasic but in water-saturated organic solvent systems in which isooctane was the most employed (3,7,9). Xin et al. (5) investigated the effect of organic solvents on the hydrolysis of racemic Naproxen methyl ester in a trapped aqueous-organic solvent biphasic system by using immobilized CRL in a continuous reactor. In that study, organic solvents of different log P values were used and the highest conversion of racemic mixture as well as the enantiomeric excess for S-Naproxen was obtained in isooctane. A series of comparative studies on the effect of solvents in the hydrolysis of racemic mixtures by CRL in several biphasic systems can be found in the reports of Cipiciani and co-workers. The authors reported that the increase in log P of the organic solvent increased the activity and enantioselectivity of CRL toward the hydrolysis of several racemic substrates (10,11).

Xin et al. (5) investigated the effect of Naproxen methyl ester concentration between 5 and 20 mg/mL on the hydrolysis progress in a continuous system using a trapped aqueous-organic biphasic system and immobilized CRL and found that the maximum yield occurred at 10 mg/mL concentration. Ten milligrams per milliliter racemic Naproxen methyl ester was the most employed concentration in the hydrolysis reactions to produce S-Naproxen in aqueous phase-isooctane systems (4,7). However, Lee et al. (1) reported the utilization of 100 mM (24.4 mg/mL) concentration of racemic Naproxen methyl ester in the hydrolysis carried out in MES buffer. The same authors carried out the hydrolysis of racemic Naproxen methyl ester at the temperatures between 20 and 45°C and found that 37°C was optimal with respect to conversion and enantiomeric excess for the product by

crude CRL. Capiciani et al. (12) investigated the effect of temperature on the enantioselectivity of propan-2-ol-treated CRL in the kinetic resolution of (±)-4-acetoxy-[2,2]-paracyclophane in aqueous medium in the range of 22–60°C and reported that the enantioselectivity was higher at higher temperatures than 30°C. The studies on the alteration of enantioselectivity with the pH of CRL solution in biphasic systems are very few (1,12–14). Lee et al. (1) altered the pH between 3.0 and 8.0 in the hydrolysis of racemic Naproxen methyl ester in MES buffer solution. When crude CRL was used, the highest conversion was obtained at pH 6.0 whereas the highest enantioselectivity was at pH 3.0 and 8.0, respectively. However, when two-step acetone-treated CRL was employed, the conversion and enantioselectivity did not change considerably with pH.

Despite numerous studies on the employment of CRL in the production of S-Naproxen from its racemic esters, the effects of aqueous-organic biphasic conditions on the enzyme enantioselectivity in batch systems are few. The present paper reports, for the first time, a parametric study on the influences of biphasic system properties on the enantioselectivity in the hydrolysis of racemic Naproxen methyl ester by crude CRL in a batch reaction system. The results were explained in terms of two-liquid phase notion complying with the interfacial activation of the lipase enzyme.

Materials and Methods

Materials

Optically pure S-Naproxen and *Candida rugosa* lipase (Type VII, Lot: 033K0612) were purchased from Sigma. The solvents used in high-performance liquid chromatography (HPLC) analyses were HPLC-grade (Merck). All other solvents were of the highest purity (Merck and Aldrich). The chemicals were of reagent grade and purchased from commercial suppliers (Merck, Sigma and Aldrich). Racemic Naproxen was produced in our laboratory by the racemization of optically pure S-Naproxen as described by Wu and Liu (15). Racemic Naproxen methyl ester was produced by dissolving racemic Naproxen in methanol with catalytic amount of H_2SO_4. After refluxing the mixture for 1 h, methanol was removed and the pH was adjusted to 9.0. The precipitate was then washed with 1 M $NaHCO_3$ solution and distilled water subsequently. After being dried, racemic Naproxen methyl ester powder was used in the hydrolysis reactions.

Enzyme Preparation and Assay

Crude CRL was dissolved in a buffer solution (0.01 *M* pH = 7.5 phosphate buffer except for the experiments designed for the effect of aqueous phase pH) to give the desired enzyme concentration, stirred for 2 h at 4°C and centrifuged at 12,000*g* for 30 min at 4°C. The supernatant was used in the hydrolysis reactions as biocatalyst after assaying its activity and protein content.

The enzyme activity was measured spectrophotometrically (Shimadzu 1601A) by following the increase in the absorbance at 404 nm due to the liberation of *p*-nitrophenol in the hydrolysis of 0.005 *M p*-nitrophenylacetate in 0.1 *M* sodium phosphate buffer at pH 7.5 and 25°C. One unit enzyme activity (U) was defined as the amount of enzyme necessary to produce 1 μmol *p*-nitrophenol per min under the conditions mentioned above. The initial activity of CRL used in the study was $A_{CRLo} = 2.09$ U unless otherwise noted.

The protein concentration was measured spectrophotometrically (Shimadzu 1601A) at 750 nm by the Lowry's method using bovine serum albumin as the protein standard *(16)*. The initial protein concentration of crude CRL used in the study was $C_{Po} = 0.99$ mg/mL unless otherwise noted.

Hydrolysis of Racemic Naproxen Methyl Ester

Hydrolysis reactions were carried out in aqueous phase-organic solvent biphasic batch reaction systems of 10-mL volumes. The reaction medium was consisted of a solvent (isooctane except for the experiments designed for the effect of organic solvent) dissolving racemic Naproxen methyl ester and a buffer solution (pH = 7.5, 0.1 *M* phosphate buffer except for the experiments designed for the effect of aqueous pH) including enzyme and recovering reaction products. The reactions were carried out in an orbital shaker (Buhler) under different medium conditions; and samples drawn separately from two phases were analyzed to calculate ee, E, and x values *(17)*.

Analyses

The concentrations of S- and R- enantiomers of Naproxen methyl ester were measured with HPLC (Waters Alliance) by using Chiralcel OD-H column at the temperature of 25°C. In the analyses, n-hexane (98%): 2-propanol (2%) was used as the mobile phase at the flow rate of 1 mL/min; and UV detection at 254 nm was done. The enantiomers of Naproxen were also measured with HPLC by using Chirex R-NGLY&DNB column at the temperature of 25°C. In the analyses, methanol (100 mM ammonium acetate) solution was used as the mobile phase at the flow rate of 0.7 mL/min where the ultraviolet detection was done at 254 nm. Each analysis was conducted in duplicate.

Results and Discussion

Effect of Organic Solvent Type

Isooctane, hexane, cyclohexane, and toluene, of which the log P values are 4.7, 3.5, 3.2, and 2.5, respectively *(18)*, were employed in the hydrolysis of racemic Naproxen methyl ester by CRL. The enantiomeric excess, enantiomeric ratio and conversion obtained at the reaction time of 120 h are given in Table 1. The conversion decreased in the solvents following the order: isooctane > cyclohexane > toluene > hexane, whereas ee_p and E decreased in the order: isooctane > cyclohexane > hexane > toluene.

Table 1
Effects of Solvent Type, Aqueous Phase/Organic Solvent Volume Ratio, and Agitation Rate on the Enantiomeric Excess for the Product (ee_p), on the Enantiomeric Ratio (E) and on the Conversion (x) in the Hydrolysisof Racemic Naproxen Methyl ester

Effect of solvent type A/O (v/v)=1/2, T=37°C, N = 200 rpm, C_{S0} = 8.3 mg/mL, C_{CRL} = 0.04 g/mL, pH = 7.5, t = 120 h			
	ee_p (%)	E	x (%)
Isooctane (log P = 4.7)	95.2	130.3	48.9
Cyclohexane (log P = 3.2)	96.1	92.7	39.2
Hexane (log P = 3.5)	93.6	27.9	20.2
Toluene (log P = 2.5)	66.6	6.5	29.6
Effect of aqueous phase/organic solvent volume ratio (A/O) T = 37°C, N = 200 rpm, C_{S0} = 8.3 mg/mL, C_{CRL} = 0.04 g/mL, pH = 7.5, t = 120 h			
	ee_p (%)	E	x (%)
A/O (v/v) = 2	91.5	94.9	51.5
A/O (v/v) = 1	94.4	133.5	50.4
A/O (v/v) = 1/2	95.2	130.3	48.9
A/O (v/v) = 1/8	89.8	51.1	48.9
Effect of agitation rate Solvent = isooctane, A/O (v/v) = 1/2, T = 37°C, C_{S0} = 8.3 mg/mL, C_{CRL} = 0.02 g/mL, pH = 7.5, t = 72 h			
	ee_p (%)	E	x (%)
N = 200 rpm	95.7	127.6	47.2
N = 250 rpm	94.6	120.2	48.4
N = 300 rpm	95.6	160.6	49.7

These results showed that the effect of organic solvents on the activity and enantioselectivity of CRL toward S-Naproxen production was not fully in proportion to the log P of solvents. Isooctane, the solvent of the highest log P, led to the highest conversion and selectivity; however, hexane with higher log P than cyclohexane, led to lower enantioselectivity than cyclohexane. The similar result was also reported by Shang et al. *(19)* for the enantioselective esterification of S-Naproxen to give hydroxyalkyl esters. The higher activity of lipase in cycloalkanes compared with the corresponding linear alkanes is explained by Nakamura et al. *(20)* through the compactness of cycloalkanes, which provides them to incorporate more easily into enzyme molecules than the corresponding normal alkanes. On the other hand, the conversion and enantiomeric ratio obtained in the presence

of toluene, which has the lowest log P among the organic solvents used, was higher than it was in hexane. Organic solvents facilitate the substrate to access the catalytic center of CRL by opening the polypeptide lid that covers the active site *(21,22)*. However, the results of the present study indicate that the hydrophobicity of organic solvents is not sufficient alone to explain the enantioselective feature of CRL.

Effect of Aqueous Phase/Organic Solvent Volume Ratio

Lipase-catalyzed reactions at interfaces proceed through the adsorption of a water-soluble enzyme into an interface, the formation of an interfacial enzyme-substrate complex and finally the liberation of the product to the aqueous or organic phase *(23)*. When a water-immiscible organic solvent is added to the system, the substrate may partition in the aqueous phase, at the interface and in the organic phase. CRL shows its catalytic activity at the interface between aqueous buffer solution and organic solvent. The solubility of Naproxen methyl ester is low in aqueous medium *(24)*; therefore, the hydrolysis reaction takes place at the interface.

The interfacial area is an important parameter governing the flux of the substrate to the enzyme active sites. Taking into account that this area depends on the volume of the droplets surrounding and enclosing the enzyme molecules, the hydrolysis of racemic Naproxen methyl ester was carried out at the aqueous phase/organic solvent volume ratios (A/O; v/v) of 1/8, 1/2, 1, and 2 by using isooctane as organic solvent. The enantiomeric excess, enantiomeric ratio and conversion obtained at the reaction time of 120 h are given in Table 1 where x decreased in the order: A/O = 2 > 1 > 1/2 > 1/8. The ee_p values followed the order: A/O = 1/2 ≅ 1 > 2 > 1/8. On the other hand, the highest and the lowest E values were obtained at the volume ratios of A/O = 1 and A/O = 1/8, respectively.

These results showed that the high volume fraction of isooctane in the biphasic reaction medium decreased the activity and enantioselectivity of CRL in the hydrolysis of racemic Naproxen methyl ester. Capiciani et al. *(10)* similarly carried out the hydrolysis of (±) methyl 2-(2,4-dichlorophenoxy) propionate by CRL in two-phase aqueous organic media employing solvents of which the log P values between –0.33 and 4.00 at different organic solvent/phosphate buffer volume ratios. The authors found that the rate of hydrolysis decreased as the volume percent of organic solvent increased whereas the enantiomeric ratio was mainly depended on the organic solvent type. They achieved the best yield and enantiomeric excess in benzene/water with the volume ratio of 1/9 by using 2-propanol-treated CRL. The differences between the reactivity and enantioselectivity conducted in pure water or in benzene/water were explained by the conformational change of the enzyme. Cipiciani et al. *(11)* subjected racemic methyl 2-aryloxypropionates to hydrolysis in water and in two-phase aqueous-organic media by using alcohol treated-CRLs. The authors used the organic solvents of which log P values varied between –0.23 and 3.20; and reported that water/benzene medium improved the enantioselectivity of

CRL with respect to water and to other media. Cipiciani et al. *(11)* also reported that they carried out the hydrolysis at 10/1 (v/v) water/organic solvent ratio, since the E value did not change while the rate of hydrolysis decreased beyond 10% of the amount of organic fraction.

The increase in interfacial area with the volume fraction of the organic phase in lipolytic hydrolysis reactions occurred in biphasic aqueous-organic systems was reported by Tsai and Chan *(23)*. The authors also suggested that there was a competition between the enhancement of interfacial area and the decrease of total enzyme amount, when the volume fraction of the lipid phase was increased. In the present study, when the A/O ratio increased, the decrease in the enzyme amount might have dominated over the increase in the interfacial area; and, therefore, a decrease in conversion was observed. Besides, the denaturation or inhibition effect of high concentrations of solvents on the enzyme molecules might have caused the decrease in the conversion and enantioselectivity.

Effect of Agitation Rate

Small droplets of organic phase provided by vigorous agitation in biphasic reaction systems give high areas for mass transfer of molecules to the interface. In this study, the extent of mass transfer resistance was investigated by employing the agitation rates of 200, 250, and 300 rpm. The enantiomeric excess, enantiomeric ratio and conversion obtained at the reaction time of 72 h are given in Table 1. The increase in agitation rate increased the E and x values; however, did not change ee_p considerably. These results show that high agitation rate is necessary to eliminate the mass transfer resistance to contact the enzyme and substrate molecules.

Effect of Racemic Naproxen Methyl Ester Concentration

The effect of substrate concentration in the organic phase on the hydrolysis of racemic Naproxen methyl ester was investigated for the initial concentrations of 5, 7.5, 10, and 15 mg/mL. The enantiomeric excess, enantiomeric ratio and conversion obtained at the reaction time of 72 h are given in Table 2. Increasing substrate concentration between 5 and 10 mg/mL increased the ee_p, E, and x values; however, a further increase decreased both the enantioselectivity and conversion indicating an inhibition occurred at high substrate concentrations. The result of the present study is similar to that reported by Xin et al. *(5)* for the hydrolysis of Naproxen methyl ester in a trapped aqueous-isooctane biphasic system. Because the hydrolysis reaction takes place at the interface, the enzyme can be exposed to both substrate and product molecules. Although the inhibitory effect of methanol, which is the co-product of the enantioselective hydrolysis, on CRL is well known *(1,7)*, the influence of high concentrations of racemic Naproxen methyl ester and S-Naproxen is not definite. Therefore, the origin of the inhibition cannot be ascertained without doing further experiments specifically designed for the inhibition phenomenon.

Table 2
Effects of Initial Substrate and Crude CRL Concentrations on the Enantiomeric Excess for the Product (ee_p), on the Enantiomeric Ratio (E) and on the Conversion (x) in the Hydrolysis of Racemic Naproxen Methyl Ester

Effect of initial substrate concentration
Solvent = isooctane, A/O (v/v) = 1/2, T = 37°C, N = 200 rpm, C_{CRL} = 0.02 g/mL, pH = 7.5, t = 72 h

	ee_p (%)	E	x (%)
Cso = 5 mg/mL	92.7	54.9	42.7
Cso = 7.5 mg/mL	92.6	102.6	51.0
Cso = 10 mg/mL	94.1	124.2	51.0
Cso = 15 mg/mL	94.0	104.7	42.4

Effect of crude CRL concentration
Solvent = isooctane, A/O (v/v) = 1/2, T = 37°C, N = 200 rpm, C_{S0} = 8.3 mg/mL, pH = 7.5; t = 72 h

	ee_p (%)	E	x (%)
C_{Eo} = 0.01 g/mL	96.1	110.4	37.2
C_{Eo} = 0.02 g/mL	95.5	120.0	47.3
C_{Eo} = 0.04 g/mL	85.8	42.9	51.5
C_{Eo} = 0.06 g/mL	89.7	65.3	51.2

Effect of Crude CRL Concentration

Hydrolysis of racemic Naproxen methyl ester in phosphate buffer-isooctane biphasic reaction system was carried out for the crude CRL concentrations of 0.01, 0.02, 0.04, and 0.06 g/mL to determine the effect of enzyme concentration on the time course of hydrolysis.

The enantiomeric excess, enantiomeric ratio and conversion obtained at the reaction time of 72 h are given in Table 2. In general, the increase in enzyme concentration resulted in enhancement of x; however, decrease of ee_p and E. The variation in the hydrolysis rate with total enzyme concentration is consistent with the kinetic model proposed by Tsai and Chang *(23)*. According to the model, an increment of the total amount of enzyme initially present in the aqueous medium increases the amount of enzyme at interfaces. On the other hand, the decrease in enantioselectivity observed at 0.04 and 0.06 g/mL CRL concentrations is due to the fact that the conversion reached 50% at the reaction time of 48 h (data are not shown). Therefore, a particular decrease was seen in ee_p and E after that time in the hydrolysis carried out at high enzyme concentrations.

0.02 g/mL crude CRL concentration was convenient to be used in the hydrolysis to produce S-Naproxen in terms of both conversion and enantioselectivity.

Table 3
Effects of pH and Temperature on the Enantiomeric Excess for the Product (ee_p), on the Enantiomeric Ratio (E) and on the Conversion (x) in the Hydrolysis of Racemic Naproxen Methyl Ester

Effect of pH
Solvent = isooctane, A/O (v/v) = 1/2, T = 37°C, N = 200 rpm, C_{S0} = 8.3 mg/mL, C_{CRL} = 0.02 g/mL, C_{Po} (mg protein/mL); 0.87 (pH = 4), 0.82 (pH = 6), 0.96 (pH = 7.5), 0.87 (pH = 9), A_{CRLo} (U); 2.32 (pH = 4), 2.03 (pH = 6), 2.09 (pH = 7.5), 1.22 (pH = 9), t = 96 h

	ee_p (%)	E	x (%)
pH = 4.0	95.7	171.1	49.8
pH = 6.0	93.9	120.5	50.3
pH = 7.5	95.3	112.4	47.2
pH = 9.0	96.9	145.2	43.4

Effect of temperature
Solvent = isooctane, A/O (v/v) = 1/2, N = 200 rpm, C_{So} = 8.3 mg/mL, C_{CRL} = 0.02 g/mL, pH = 7.5, t = 96 h

	ee_p (%)	E	x (%)
T = 30°C	85.5	37.5	50.2
T = 37°C	95.3	112.1	47.2
T = 45°C	95.4	131.2	49.9
T = 50°C	85.7	93.0	50.0

Effect of Aqueous Phase pH

Hydrolysis of racemic Naproxen methyl ester was carried out in the aqueous phase /isooctane biphasic reaction system (A/O = 2) for the aqueous phase pH values of 4.0, 6.0, 7.5 and 9.0. The pH of the aqueous phase was adjusted to 4.0 with acetate buffer, to 6.0 and 7.5 with phosphate buffer, and to 9.0 with Tris-HCl buffer. The same buffer solutions were used in the preparation of CRL for the hydrolysis reaction. The enantiomeric excess, enantiomeric ratio and conversion obtained at the reaction time of 96 h are given in Table 3. The acidic pH values were more favorable for conversion than the milder and alkaline pHs; however, enantioselectivity for S-Naproxen (ee_p) was higher in the alkaline pH. The x values decreased in the order pH = 4.0 > 6.0 > 7.5 > 9.0; whereas ee_p values decreased in the order: pH = 9.0 > 4.0 > 7.5 > 6.0. E values decreased in the order: pH = 4.0 > 9.0 > 6.0 > 7,5. pH = 4.0 seemed to be more favorable than other pH values with respect to high enantiomeric ratio.

The effect of pH on the conversion and enantioselectivity in the hydrolysis of racemic Naproxen methyl ester can be explained through different ways:

1. The ionization state of the enzyme changes with the change in pH, which affects its activity and selectivity *(25)*.

2. The enantioselectivity of CRL may change through a conformational change with pH as a consequence of the displacement of its polypeptide lid that makes the access of S-enantiomer of the racemic mixture to the active center easier than the R-form *(12,13)*.
3. The type of buffer may influence the selectivity of a given reaction *(25)*. The ions of buffer solutions that may alter the solubility of proteins and make them more hydrophobic or more hydrophilic *(26,27)* or that may change their configuration through attraction and repulsion forces also have a considerable influence on the enzyme performance. The utilization of different buffer solutions in the preparation of CRL to be used in hydrolysis reactions might have caused the removal of impurities, opened the polypeptide lid, or converted the isoforms of CRL into each other, which consequently leads to different activity and enantioselectivity.
4. It is noteworthy that pH = 4.0, which is very close to the isoelectric point of CRL *(28)*, is optimal for high conversion and enantioselectivity. The reason for this can be addressed to the fact that the pH of the enzyme solution influences the level of adsorption onto the interface, with maximum adsorption at the enzyme's isoelectric point. The maximal adsorption at isoelectric point is a consequence of a minimum in electrostatic repulsions between the enzyme molecules, allowing them to pack closely together *(29)*.

Effect of Temperature

The temperature dependence of the enantioselectivity of CRL in the hydrolysis of racemic Naproxen methyl ester in phosphate buffer-isooctane biphasic medium was investigated for T = 30, 37, 45, and 50°C and the enantiomeric excess, enantiomeric ratio, and conversion obtained at the reaction time of 96 h are given in Table 3. The increase in temperature increased both the conversion and enantioselectivity; however, a decrease was seen at 50°C.

The increase in hydrolysis rate with temperature up to 45°C might be attributed to the increase in collision of the substrate and enzyme molecules where the decrease observed at 50°C was due to the inactivation of the enzyme. The alteration of physicochemical properties, such as solubility of reaction components and pH, with temperature is another parameter that affects the hydrolysis. However, because the substrate initially introduced into the medium dissolved in isooctane totally in the range of temperatures studied, the assist of temperature on the diffusion of substrate molecules to enzyme surface by increasing the solubility was not considered in the present study.

Conclusions

The present paper reports the results of a parametric study on the alteration of CRL enantioselectivity with biphasic batch reaction system conditions in the hydrolysis of racemic Naproxen methyl ester that favor

S-Naproxen production. Biphasic medium properties and reaction conditions that exceed the enantiomeric ratio of 100 with 50% conversion in the production of S-Naproxen were found. The changes in conversion and enantioselectivity based on the parameters investigated shows that the type of the solvent type leads to the highest variations (with the standard deviation of 12.4% in conversion and that of 57.2% in enantiomeric ratio), which is followed by crude enzyme concentration (with the standard deviation of 6.7% in conversion and that of 42% in enantiomeric ratio). The results also show that the enantioselectivity is more affected than the conversion from the system parameters investigated. The hydrophobic character of the solvent is the major contributor to molecular interactions that alters the activity and enantioselectivity of CRL. The second significant parameter is the enzyme concentration, which reflects the feature of the reactions occurred at interfaces.

Besides the parameters investigated in the present study, the alteration of the physicochemical properties at the interface, i.e., lipase concentration, substrate concentration, interfacial area, and even lipase conformation must be taken into account as another important parameter that affects the activity and enantioselectivity of CRL.

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