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Stereoselective synthesis of (*R*)-1-chloro-3(3,4-difluorophenoxy)-2-propanol using lipases from *Pseudomonas aeruginosa* in ionic liquid-containing system

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ABSTRACT

Direct transesterification of (*R,S*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol (*rac*-CDPP) (a key intermediate in the synthesis of the chiral drug (*S*)-lubeluzole) with vinyl butyrate by lipases from *Pseudomonas aeruginosa* (*P. aeruginosa*) MTCC 5113 was performed in hexane with ionic liquids (ILs) 1-butyl-3-methyl imidazolium hexafluorophosphate [BMIm][PF_6] and 1-butyl-3-methyl imidazolium tetrafluoroborate [BMIm][BF_4] as co-solvents. The maximum conversion (>49%) and enantiomeric excess (ee > 99.9%) was achieved in 6 h of incubation at 30 °C with [BMIm][PF_6] as co-solvent in a two-phase system. The enzyme was able to perform with the same specificity even at 60 °C in the presence of ILs. It was possible to use lipases repeatedly for more than 10 times while still maintaining absolute enantioselectivity and reactivity. Stability studies on lipases from *P. aeruginosa* in ILs revealed the fact that the enzyme constancy and the reactivity in catalyzing transesterification of *rac*-CDPP into (*S*)-1-chloro-3-(3,4-difluorophenoxy)-2-butanoate was of the order of [BMIm][PF_6] > [BMIm][BF_4] in two-phase system.

1. Introduction

Lipases (EC 3.1.1.3) are considered the most ubiquitous and valuable enzymes for asymmetric synthesis. Lipase applications include kinetic resolution of racemic alcohols, acids, esters or amines [1], as well as desymmetrization of prochiral compounds [2]. Biocatalysis in non-aqueous media has been extensively used for the resolution of alcohols by lipase catalyzed transesterification reactions [3]. But the use of solvents has certain disadvantages, such as being volatile and toxic to environment, particularly when they are used on a large scale [4].

The use of ionic liquids (ILs) has contemporarily emerged in organic synthesis and in some cases can be of immense efficacy in biocatalysis. These are salts that have organic cation and an inorganic anion. They are often fluid at room temperature with boiling temperature less than 100 °C [5]. It constitutes an alternative for carrying out the processes that present serious difficulties in organic solvents or water. In an enzymatic system ionic liquids can be used in three different ways: as co-solvent in the aqueous phase, as a pure solvent and as a two-phase system together with other solvents. These solvents possess several interesting properties, such as ease of preparation, reuse, high thermal stability and low vapour pressure [6–8]. They have widely regulated assets with regard to polarity, hydrophobicity and solvent miscibility behaviour by means of appropriate modification of the cation and anion [9,10]. The first example of ionic liquids as solvent in biocatalysis was stated in the year 2000, when the synthesis of octanamide from octanoic acid and ammonia using CALB was carried out by Sheldon and co-workers [11]. The most frequently used ILs is 1-butyl-3-methyl imidazolium hexafluorophosphate ([BMIm][PF_6]) and tetrafluoroborate ([BMIm][BF_4]). Subsequently, other groups have investigated lipases in ionic liquids, due to the widespread use of these enzymes in industrial processes [12–15]. Lipases have been shown to keep their activity and enantioselectivity in some ILs. Moreover, in some cases they are higher than in common organic solvents. For both synthetic and analytical applications, enantiomerically pure racemic alcohols are vital synthetic intermediates and are also valuable chiral auxiliaries. Lubeluzole [(*S*)-4-(2-benzothiazolylmethylamino)-*a*-((3,4-difluorophenoxy)methyl)-1-piperidine ethanol] is a novel benzothiazole compound that has revealed neuroprotective activity in preclinical models of ischemic stroke [16].

Earlier, we successfully employed lipases from *Pseudomonas aeruginosa* MTCC 5113 in regioselective transesterification (*R,S*)-1-chloro-3(3,4-difluorophenoxy)-2-propanol (*rac*-CDPP) [a key intermediate of drug lubeluzole] in hexane [17]. Extending our study on lipases from *P. aeruginosa*, we carried out the transesterification reactions (*rac*-CDPP) in presence of vinyl butyrate

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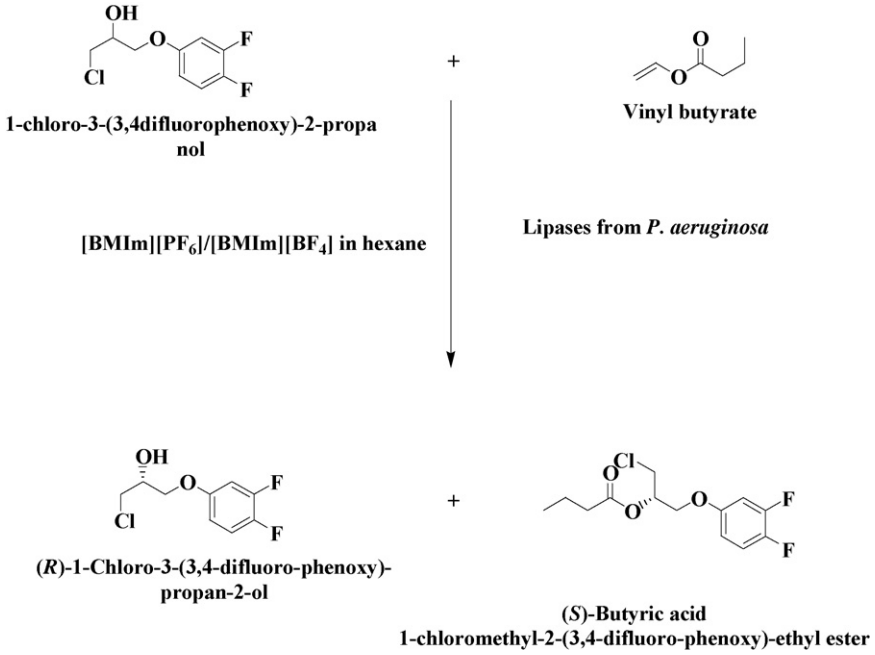

Fig. 1. Schematic representation of transesterification of *rac*-CDPP to (*S*)-butyric acid 1-chloromethyl-2-(3,4-difluoro-phenoxy)-ethyl ester using ionic liquids.

as the acetylating agent and hexane as solvent and ionic liquids as co-solvent and compared the results of reaction in pure ILs. Herein, we report for the first time, the performance of *P. aeruginosa* lipases in the enantioselective transesterification of *rac*-CDPP with vinyl butyrate in two-phase system containing hexane and ILs ([BMIm][PF_6] and [BMIm][BF_4]) with the result compared with pure hexane (Fig. 1). The reaction was optimized with respect to the type of ILs, ratio between hexane and [BMIm][PF_6] and reaction time.

2. Materials and methods

2.1. Microorganisms and chemicals

The organism *P. aeruginosa* MTCC 5113 obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh was grown on nutrient agar (peptone (0.5%), beef extract (0.5%), yeast extract (0.1%), NaCl (0.5%) and agar (1.5%)) plate (pH 8) for 24 h. Single colony was then inoculated in 20 ml nutrient broth (peptone (0.5%), beef extract (0.5%), yeast extract (0.1%), NaCl (0.5%), pH 8) and incubated in an orbital shaker (200 rpm) at 30 °C for 6 h. This seed culture (1%, v/v) was used as an inoculum to carry out the enzyme production in 100 ml nutrient broth (pH 8). The flasks were incubated for 96 h (200 rpm, 30 °C). Since the enzyme (lipases) produced is extracellular in nature, the cells were centrifuged out at 10,000 × *g* for 10 min (Sigma 6K15, Germany) and supernatant was concentrated to obtain a cell free extract. This was used as enzyme source. Triton X-100, Tween-80, *p*-nitrophenyl palmitate and *p*-nitrophenol were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Several components of growth media were obtained from Himedia (Mumbai, India). Vinyl butyrate and ionic liquids ([BMIm][PF_6] and [BMIm][BF_4]) used in all the transesterification reactions were procured from Fluka chemicals. Various solvents used in the study were of HPLC grade and were obtained from J.T Baker (Phillipsburg, USA) and Ranbaxy Fine Chemical Limited (New Delhi, India). (*R*,*S*)-1-Chloro-3(3,4-difluorophenoxy)-2-propanol was synthesized from 3,4-difluorophenol, epichlorohydrine, lithium chloride and acetic acid according the reported procedure [18]. All other chemicals were purchased from various commercial sources and were of analytical grade.

2.2. Biocatalytical reaction

In a typical experimental procedure, 5 mM (*R*,*S*)-1-chloro-3(3,4-difluorophenoxy)-2-propanol and 150 mM vinyl butyrate were stirred (~1000 rpm) in 10 ml capacity round bottom flask (RBF) containing 5 ml solvent (hexane:ILs::50:50). The constant temperature (20–70 °C) was maintained by incubating the reaction mixture in an incubator (Kuhnur, Switzerland). At regular time intervals (2 h), 500 μl samples were taken by adding 500 μl hexane each time for 6 h. This was done to maintain the volume, till the end of the reaction. Hexane was evaporated from the samples on rotavapor (Buchi, Switzerland). Samples for Chiral HPLC were prepared by adding 900 μl hexane and 100 μl of isopropyl alcohol (IPA). The conversion and enantiomeric excess of the substrate and product was analyzed. After completion of the reaction the unreacted substrate and product were separated from the reaction mixture and authenticated by NMR, GCMS, LCMS, polarimeter, etc. and the results were compared with earlier reported data [33] not shown here.

2.3. Analytical techniques

2.3.1. Enzyme assay for the lipase stability experiment in hexane and ILs

Winkler and Stuckmann method with slight modifications was applied for the quantification of lipase activity of *P. aeruginosa* [19]. An aqueous solution (9 ml) containing gum Arabic (0.11%, w/v) and Triton X-100 (0.44%, w/v) was prepared. The substrate for lipase (*p*-nitrophenyl palmitate) was dissolved in isopropanol (3 mg/ml) and this was mixed with aqueous solution using intense agitation (vortex mixture) to form emulsion. This emulsion (0.9 ml) was mixed with 1.5 ml Tris–HCl buffer (50 mM, pH 8) and 0.5 ml $CaCl_2$ (75 mM). After 5 min incubation at 60 °C, 100 μl appropriately diluted (in 50 mM Tris–HCl buffer, pH 8.0) enzyme solution was added to it. Incubation was continued for a further 10 min. The reaction was

stopped by putting the test tubes in ice and the optical density was measured spectrophotometrically at 410 nm against substrate blank. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μmol *p*-nitrophenol per minute under the above specified assay conditions.

The stability experiments were performed in stoppered flask containing extracellular broth from *P. aeruginosa* in solvent (hexane, [BMIm][PF_6] and [BMIm][BF_4]) in the ratio of 1:1. The mixture was stirred on a magnetic stirrer for 12 h at 30 °C. ILs were separated from the mixture by filtration using Whatman filter paper no. 1 and the hexane was evaporated from the aqueous phase containing lipase by rotavaporation. The lipase activity was determined in the aqueous phase by using above-mentioned approach.

2.3.2. Chromatography analysis

High-performance liquid chromatography (HPLC) was performed using Shimadzu 10AVP instrument equipped with a UV detector on a Chiracel ODH column (0.46-mm diameter, 250-mm long, 5 μm, Chiracel). The mobile phase consisted of hexane–isopropyl alcohol at 95:05 (v/v) with a flow rate of 0.5 ml/min. The conversion and enantiomeric excess (ee) were quantified at 220 nm. The retention time of (*S*)-ester was 10.2 min and (*S*)- and (*R*)-alcohol enantiomers were eluted simultaneously at 20 and 22.7 min on the Chiracel ODH column. The ee was defined as the ratio of [R] − [S]/[R] + [S] × 100%, where [R] and [S] are the concentrations of [*R*]- and [*S*]-alcohol, respectively. The remaining substrate and the product formed were authenticated by ^1H and ^{13}C NMR spectra recorded on a Bruker Advance DPX 300 NMR spectrometer and the optical rotation was obtained on Rudolph polarimeter (Rudolph Research Autopol IV).

2.4. Consecutive use of enzyme and IL in transesterification of rac-CDPP

One of the most imperative properties of ionic liquids is their reusability, which determines their environmental friendly character and potential industrial applications. The reaction was carried out in 10 ml flask containing *rac*-CDPP, vinyl butyrate and solvent system (ILs and hexane) at 30 °C on a magnetic stirrer at the rate of ~1000 rpm. After each cycle of reaction, the upper layer was removed and the lower IL phase containing the enzyme was used again up to 10 cycles.

3. Results

3.1. Time course for transesterification in ionic liquids

Fig. 2 shows the conversion and enantiomeric excess (ee) of the product formed as a function of time reaction. The reaction was catalyzed by lipases from *P. aeruginosa* in a solvent system composed of hexane and IL ([BMIm][PF_6]) as co-solvent (50:50), respectively. Previously, we have reported that maximum conversion (49%) and ee (>99%) of *rac*-CDPP was achieved in 24 h when hexane was used as solvent at 30 °C [16]. Interestingly, with [BMIm][PF_6] as co-solvent in hexane reaction gets completed in 6 h of incubation. Therefore, all the subsequent reactions were carried for 6 h.

3.2. Selection of ionic liquid

The two ILs employed for the current study [BMIm][PF_6] and [BMIm][BF_4] are distinctly different in their properties, such as hydrophobicity, polarity, anion nucleophilicity, hydrogen-bond basicity and viscosity [4]. The [BMIm][PF_6] is highly hydrophobic

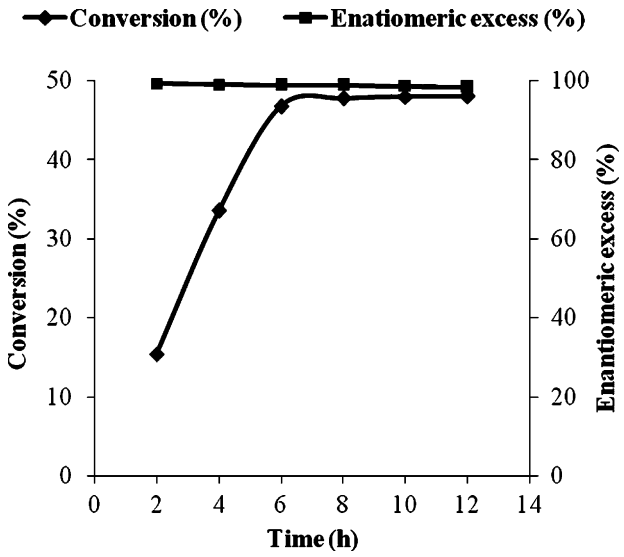

Fig. 2. Time-course of conversion and enantioselectivity of (*R,S*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol by crude lipase of *P. aeruginosa* in a two-phase system containing hexane and [BMIm][PF_6] (1:1). (Reaction conditions: 5 mM *rac*-CDPP, 150 mM vinyl butyrate, 5 ml ILs and hexane (1:1) and 5 ml lipase containing broth from *P. aeruginosa* (app. 7500 U). The reaction was carried out at 30 °C).

in nature due to the different anions associated with the common organic cation. In comparison, the [BMIm][BF_4] is highly hydrophilic. Since these properties influence the conformation of the lipases and consequently their reactivity, therefore, these properties are of primary concern. Table 1 shows the effect of addition of [BMIm][PF_6] and [BMIm][BF_4] as co-solvent in six different organic solvents for the transesterification of *rac*-CDPP. It was found that the best solvent was hexane with [BMIm][PF_6] as the co-solvent. Exciting results were seen when tetrahydrofuran was used as solvent, the selectivity of the enzyme changed in the presence of ionic liquids. The enzyme became (*R*) selective, while in pure solvent lipases from *P. aeruginosa* were specific for (*S*)-CDPP. These results were authenticated by polarimetry and comparing the data with the standards [16,20]. Similar observations were made by Hirose et al. where a variation of solvent induces a striking effect on lipase enantioselectivity [21]. By changing the solvent from water–saturated cyclohexane to water–saturated isopropyl ether a reversed enantiomer preference is observed in a *Pseudomonas* lipase-catalyzed hydrolysis reaction of a prochiral diester. Explanation for the change in enantioselectivity may be due to the solvent molecules coordinated to the active site interfering differently with the substrate enantiomers [21]. A similar reversal of enantioselectivity for other substrates by changing the solvent is also reported for *Candida*

Table 1
Effect of solvent type on the transesterification reaction of *rac*-CDPP by *P. aeruginosa* lipase at 30 °C in ionic liquids

Solvent	Ionic liquids		
	[BMIm][PF6], conversion and ee (%)	[BMIm][BF4], conversion and ee (%)	Selectivity, *R*/*S*
Acetonitrile	6.89 (50)	4.23 (48)	*S*
Toluene	3.78 (73)	3.04 (70)	*S*
Heptane	24.06 (93)	21.04 (88)	*S*
Hexane	40.32 (97)	35.25 (96)	*S*
Tetrahydrofuran	5.46 (83)	3.57 (79)	*R*
Benzene	3.05 (49)	2.35 (43)	*S*

All reaction mixtures had 50% (v/v) of organic solvent and ILs, the initial substrate concentrations were 5 mM (*rac*-CDPP) and 150 mM (vinyl butyrate).

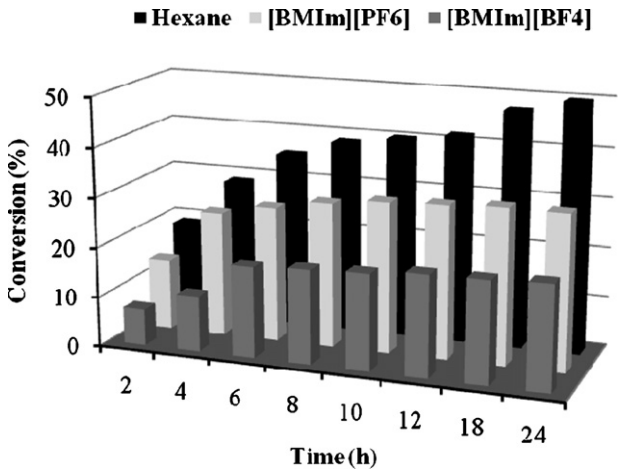

Fig. 3. Comparison of conversion rate of *rac*-CDPP in hexane (■), [BMIm][PF_6] (■) and [BMIm][BF_4] (■). (The experiment was carried out using 5 mM *rac*-CDPP, 150 mM vinyl butyrate in a 10 ml RBF containing 5 ml of the three solvents. All the reactions were carried out at 30 °C).

rugosa, *Pseudomonas cepacia* and for protease lipases [22–24]. It was also reported that salvation of a phenyl group exposed to the solvent is thermodynamically more favorable in solvents of a high log *P* [25]. In our system with both the ILs, in the presence of tetrahedrofuran the reversal of enantioselectivity was observed. Due to the highly polar nature of THF the structure of the enzyme protein molecule gets distorted and stabilized by the ILs in such a way that (*R*)-isomer gets the preference as compared to (*S*)-isomer which is more prevalent with other co-solvents.

Influence of hexane, [BMIm][PF_6] and [BMIm][BF_4] as solvent for the transesterification was investigated separately. Fig. 3 suggests that the conversion was highest for hexane when the reaction was carried out for 24 h and [BMIm][PF_6] is the best one out of the two ionic liquids taken. The hydrophilicity of the co-solvent in two-phase reaction system is important as it allows interaction and breaking of hydrogen bonds, which stabilizes the tertiary structure of the protein [5]. Such interactions are likely to occur with ILs.

3.3. Optimization of co-solvent concentration (influence of ratio between hexane and [BMIm][PF_6])

It has previously been studied that ILs as pure solvents and in a two-phase system with another solvent such as organic solvent and with supercritical carbon dioxide increased the conversion rate and enantioselectivity [26,27]. Ganske and Bornscheuer reported that lipase from *Candida antarctica* do not show activity in the synthesis of sugar esters in pure ionic liquids. However, the reaction is possible in biphasic system containing ILs ([BMIm][PF_6] and [BMIm][BF_4]) and *t*-butanol as solvent [28]. Therefore, the transesterification was carried out in biphasic system and in the reaction mixture the amount of [BMIm][PF_6] was progressively increased from 10 to 100% and the conversion and ee were monitored after 6 h. The results indicated that the resolution of *rac*-CDPP occurred effectively when the solvent system is biphasic with hexane and [BMIm][PF_6] and is in the ratio of 50:50 (Table 2). Moreover, using ILs in excess, the upper hexane phase disappeared in the beginning of the reaction and the sampling becomes impossible. As indicated by Contesini [29], the presence of both the solvents in the reaction mixture causes the solubility of the substrate to increase. This may be the reason for the decrease in reaction time from 12 to 6 h for the conversion of *rac*-CDPP into (*S*)-1-chloro-3-(3,4-difluorophenoxy)-2-butanoate.

Table 2
Influence of hexane to [BMIm][PF_6] ratio on the transesterification of *rac*-CDPP

Ratio ([BMIm][PF_6]:hexane)	Conversion (%)	Enantiomeric excess (%)
10:90	25.47	98.23
20:80	28.38	98.92
30:70	32.67	98.99
40:60	40.32	99.02
50:50	48.75	99.6
60:40	42.78	99.1
70:30	35.99	99
80:20	30.54	98.98
90:10	28	98.85

The experiment was carried out in a100 ml flask on a magnetic stirrer using different volume ratios of hexane to [BMIm][PF_6]. The initial concentration of *rac*-CDPP was 5 mM and vinyl butyrate was 150 mM.

3.4. Stability of lipase from P. aeruginosa in hexane and ionic liquids

The objective of this study was to compare the stability of lipases from *P. aeruginosa* in pure solvent (hexane) and ILs ([BMIm][PF_6] and [BMIm][BF_4]). The enzyme containing broth was stirred with all the three solvents separately for 48 h at 30 °C. Residual lipase activity in ionic liquids was much higher than that obtained in *n*-hexane demonstrating the greater suitability of these solvents for the proposed reaction. The sequence of enzyme activity observed was the following: [BMIm][PF_6] > [BMIm][BF_4] > *n*-hexane (Fig. 4). Extraction of water from the vicinity of the lipase may be the reason for the sharp decrease of activity in pure hexane. The activity of lipase decreased in both ILs, however, the fall is more in the case of [BMIm][BF_4]. The reason behind this may the hydrophobic nature of [BMIm][PF_6] as compared to [BMIm][BF_4].

3.5. Reuse of lipases from P. aeruginosa in ionic liquids for the synthesis of (R)-1-chloro-3(3,4-difluorophenoxy)-2-propanol

The reusability of the lipases from *P. aeruginosa* was investigated for the transesterification of *rac*-CDPP with vinyl butyrate (as acyl donor) in ILs and hexane. In all the cases residual substrate and product formed were extracted each time from the reaction

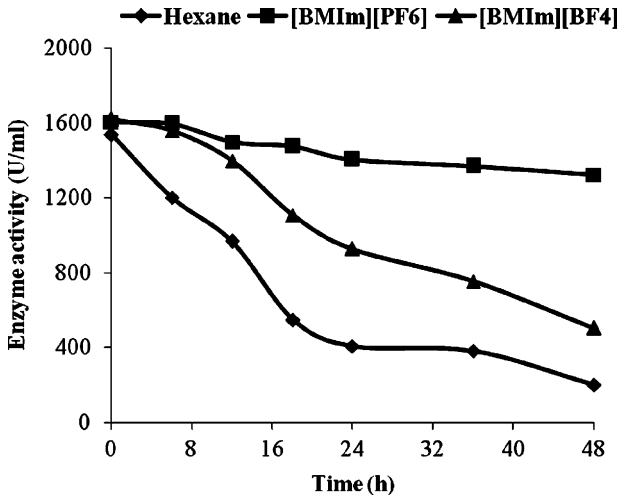

Fig. 4. Influence of ionic liquids and hexane on the hydrolytic activity (enzyme activity) of extracellular lipase from *P. aeruginosa*. (The experiment was performed in a 50 ml-stoppered flask containing lipase broth and solvent in the ratio of 1:1. After adding lipase mixture to the solvent, the reaction was stirred on a magnetic stirrer for 48 h. Samples were taken at regular interval of time. The hydrolytic activity of lipase was verified using *p*-nitrophenol palmitate as substrate at 60 °C).

Table 3
Simultaneous reuse of lipases from *P. aeruginosa* in two-phase system (hexane and [BMIm][PF_6])

No. of cycles	Conversion (%)	Enatiomeric excess (%)
1	49.3	99.4
2	49.2	99.3
3	49	99.4
4	48.3	99
5	48.7	98.9
6	48.4	99.1
7	48.1	99
8	47.1	99.2
9	46.8	99.1
10	46.1	98.3

The experiment was carried out in a100 ml flask on a magnetic stirrer using 1:1 ratio of hexane to [BMIm][PF_6]. After the completion of reaction the solvent phase was removed from the ILs and enzyme. To start the reaction again, a fresh volume of *rac*-CDPP and vinyl butyrate was added in to ILs containing enzyme.

mixture. The great advantage of the two-phase system in ILs was the easy removal of upper hexane phase containing product and unreacted substrate, where the lower phase containing the enzyme and IL was used again in 10 cycles. The lipases from *P. aeruginosa* kept its activity and selectivity almost at the same level even after 10 cycles of repeated use, based on the conversion and enantiomeric excess. The stabilizing properties of [BMIm][PF_6] on CALB enzyme have been reported earlier, which explains the compact confirmation of enzyme in the presence of ILs [30]. We also found the similar stabilizing effect of [BMIm][PF_6] on the activity of lipases from *P. aeruginosa*. After 10 cycles of repeated reactions, there was no loss of enzyme activity and ILs volume (Table 3). It has already been reported by Itoh et al. the repeated use of another ionic liquid, 1-butyl-2,3-dimethylimidazolium tetrafluoroborate ([bdmim]BF_4) in the transesterification reaction using vinyl acetate as acyl donor [31].

The reaction rate in [BMIm][PF_6] solvent system was superior than in [BMIm][BF_4], which is superior than hexane (Fig. 5). It has been found out that [BMIm][PF_6] is the best solvent for the reuse of enzyme under standard reaction condition for the transesterification of *rac*-CDPP. Possibly, the hydrophilic ionic head of the ILs in comparison to the pure hydrophobic solvents like hexane is important, which stabilizes the tertiary structure of the protein. The difference in reactivity of two ILs taken was probably

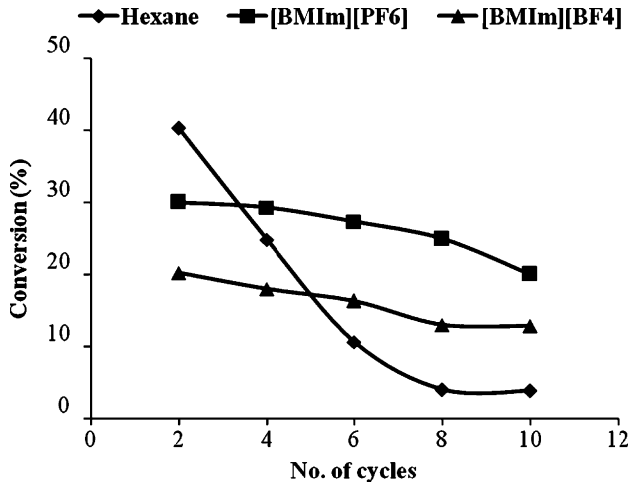

Fig. 5. Simultaneous reuse of lipases from *P. aeruginosa* and IL in transesterification reaction of *rac*-CDPP. (The experiment was performed using 5 mM *rac*-CDPP, 150 mM vinyl butyrate in 5 ml solvent (2.5 ml ILs and 2.5 ml hexane). After every cycle, solvent was removed from the ILs containing enzyme and analyzed for the product).

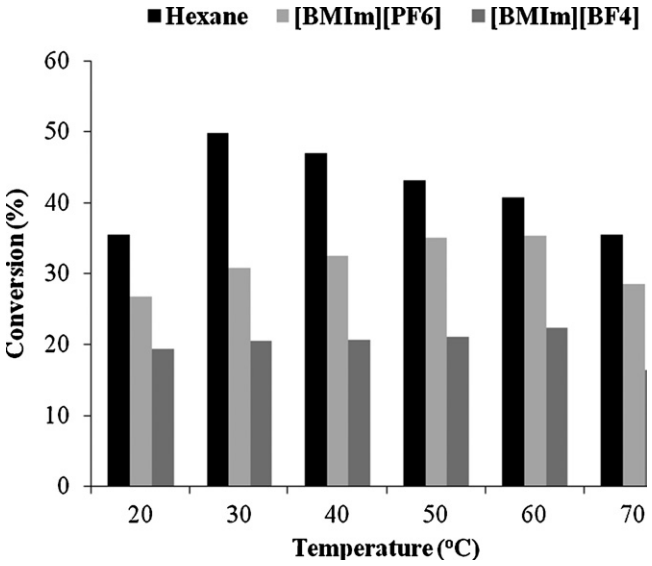

Fig. 6. Influence of reaction temperature on the synthesis of (*S*)-butyric acid 1-chloromethyl-2-(3,4-difluoro-phenoxy)-ethyl ester. (The reaction was carried out in hexane (■), [BMIm][PF_6] (■) and [BMIm][BF_4] (■) separately for 24 h and the temperature was varied from 20 to 70 °C).

due to the increase in solubility of the substrate in hydrophobic solvent.

3.6. Effect of reaction temperature on the transesterification of *rac*-CDPP

Lipase catalyzed synthesis of (*S*)-1-chloro-3-(3,4-difluorophenoxy)-2-butanoate was carried out at constant temperature ranging between 20 and 70 °C in hexane, [BMIm][PF_6] and [BMIm][BF_4]. Earlier, Ema et al. studied the lipase-catalyzed kinetic resolution of 5-[4-(1-hydroxyethyl)phenyl]-10,15,20-triphenylporphyrin in ionic liquids at various temperatures (30–80 °C) to obtain high *E* values [32]. The effect of temperature on the ester synthesis is shown in Fig. 6. In hexane, the reaction rate decreased with the increase in temperature, however, it increased in both ILs. Remarkable results were obtained when the reaction was carried out in two-phase system (hexane + [BMIm][PF_6]) where the reaction rate increased with the increase in temperature (Fig. 7) and the maximum conversion was achieved at 60 °C. The results were in contrast with the neat solvent (hexane) where the reaction rate started falling above 30 °C. We have reported earlier that the lipases from *P. aeruginosa* MTCC 5113 are highly thermostable [33]. Most likely the stability of enzyme has been maintained by the ILs in the reaction medium at high temperatures and the reduction in enzyme activity might be due to the presence of neat solvent (hexane).

4. Discussion

Although ILs has many applications, however, their environmental fate and potential toxicity is still unknown. Rogers et al. reported that the ILs like 1-butyl-3-methylimidazolium hexafluorophosphate [C_4mim][PF_6] produces volatile fumes of HF, POF_3, etc. during the synthesis of ILs [34]. Other researchers have also reported about the instability of hexafluorophosphate containing ILs during their synthesis [35,36], however, we have used the ionic liquid ([BMIm][PF_6] and [BMIm][BF_4]) as co-solvent along with organic solvent, where the possibility of generation of HF fumes is much less as the reactions are carried out at ambient temperature.

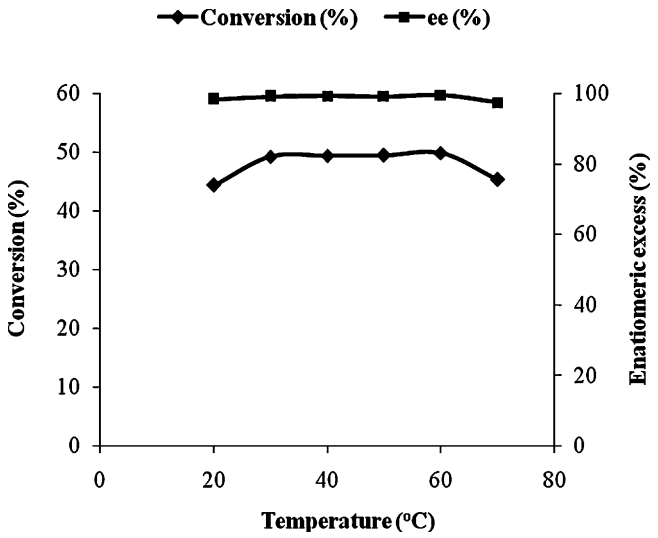

Fig. 7. Influence of reaction temperature (20–70 °C) on the transesterification of *rac*-CDPP (conversion and enantiomeric excess) using lipases from *P. aeruginosa*. (The reaction was conducted in two-phase system containing hexane and [BMIm][PF_6] in the ratio of 1:1).

Ionic liquids are not fundamentally green; therefore, precautions may be taken during their synthesis and use. ILs can be designed to be environment-friendly, with large possible benefits for sustainable chemistry [37]. Rapid lipase-catalyzed transesterification reaction was also reported by Itoh et al. using 2-methoxyethyl (tri-*n*-butyl) phosphonium bis(trifluoromethanesulfonyl) imide ([$MEBu_3P$][NTf_2]) which is a clean alternate to PF_6 containing ILs [38]. Keeping in view the convincing assets of ionic liquids, we proceeded with the transesterification of *rac*-CDPP. Lipases from *P. aeruginosa* were effectively used as catalyst for the synthesis of (*R*)-1-chloro-3(3,4-difluorophenoxy)-2-propanol from *rac*-CDPP and vinyl butyrate in biphasic system containing hexane and [BMIm][PF_6]. Studies illustrated that [BMIm][PF_6] is an excellent co-solvent and the use of ILs in the reaction substantially decreased the time of transesterification, catalyzing *rac*-CDPP into (*S*)-1-chloro-3-(3,4-difluorophenoxy)-2-butanoate. Results suggest that the hydrophobic IL ([BMIm][PF_6]) in two-phase system with hexane (1:1) provided most suitable solvent for the reaction at 30 °C. We have demonstrated the stability of the ILs with lipases from *P. aeruginosa* and had shown that they can be recycled over prolonged periods of time under standard reaction conditions. The findings obtained here, with the existing knowledge, revealed the potentiality of lipases at high temperature (60 °C) and the effect of ILs to increase thermostability for the transesterification reaction [16,32].

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