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Pseudomonas cepacia lipase catalyzed esterification and transesterification of 3-(furan-2-yl) propanoic acid/ethyl ester: A comparison in ionic liquids vs hexane

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ABSTRACT

A comparison of the *Pseudomonas cepacia* lipase (lipase *PS*) catalyzed esterification of 3-(furan-2-yl) propanoic acid and transesterification of ethyl 3-(furan-2-yl) propanoate with six straight chain alcohols (propanol to octanol) in ionic liquids and hexane was carried out. The ionic liquids selected, [Bmim]BF_4, [Bmim]PF_6, and [Bmim]Tf_2N, consisted of an identical cation and different anions. This is the first report on the biocatalyzed synthesis of these esters. In all the media, lipase *PS* catalyzed esterification of 3-(furan-2-yl) propanoic acid resulted in high yields of the esters compared to the transesterification of ethyl 3-(furan-2-yl) propanoate. [Bmim]Tf_2N proved to be the best; yielding 98–67% of the product by lipase *PS* catalyzed esterification. The lipase *PS*–[Bmim]Tf_2N and lipase *PS*–[Bmim]PF_6 mixture was recycled five times without any decrease in the yields of the products and was found to be operationally stable up to 10 months at room temperature.

1. Introduction

The use of biocatalysis in non-aqueous environments has the advantages of increasing the solubility of organic substrates, the possibility of carrying out processes which are thermodynamically unfavorable in water (e.g. esterification and transesterification) and facilitating enzyme and product recovery [1]. Among these non-conventional media, organic solvents are widely used with enzymes to improve solubility of hydrophobic reactants and/or products and to shift reaction equilibrium from hydrolysis toward synthesis [2]. In recent years ionic liquids (ILs) have attracted attention because, as opposed to organic solvents, they are non-volatile, non-flammable and have high thermal stability [2–4]. Ionic liquids are substances that are completely composed of ions and are liquids at or close to room temperature [4]. One of the most promising advantages of ionic liquids is the biocatalytic preparation of compounds that can be used as food additives or in pharmaceutical and cosmetic formulations because of their reduced toxicity. Toxicological tests of a series of ionic liquids with imidazolium cations and various anions (including PF_6^-, TF_2N^- and BF_4^-) showed that most of the ionic liquids were less toxic than organic solvents by several orders of magnitude [5]. Enzymatic reactions carried out in ILs have shown high activity, good enantioselectivity, high reaction rates, high thermal and operational stability [6–9]. Another important feature of ionic liquids is that their solvent properties can be finely tuned by selecting appropriate combinations of cations and anions [2] thus allowing ionic liquids to be specifically designed for different reaction conditions. For the same cation, properties of ILs such as hydrophobicity, miscibility with organic solvents, capacity to dissolve organic and inorganic solutes vary widely depending on the anion [10–14]. The use of ILs as reaction media can thus greatly expand the repertoire of enzyme catalyzed transformations. Consequently, a number of potential applications of enzymes that are either impossible or marginal in organic media become quite feasible and commercially attractive in these media [2].

As part of our ongoing research on enzyme catalysis in non-conventional media, we used ionic liquids as a reaction medium for lipase *PS* catalyzed synthesis of 3-(furan-2-yl) propanoic acid esters, which are used as flavors for food and in the fragrance industry [15]. The chemical methods to synthesize these esters have the common disadvantage of high temperature requirements, harsh conditions and the use of carcinogenic organic compounds [16]. In this paper we report the lipase *PS* catalyzed esterification of 3-(furan-2-yl) propanoic acid and transesterification of ethyl 3-

Abbreviations: ILs, ionic liquids; [Bmim]BF_4, 1-butyl-3-methylimidazolium tetrafluoroborate; [Bmim]PF_6, 1-butyl-3-ethylimidazolium hexafluorophosphate; [Bmim]Tf_2N, 1-butyl-3-methyl imidazolium triflamide; lipase *PS*, *Pseudomonas cepacia* lipase; ser, serine; his, histidine; asp, aspartic acid; glu, glutmic acid; gln, glutamine; CAL-B, *Candida antarctica* lipase B; [Emim]Tf_2N, 1-ethyl-3-methylimidazolium bis(trifluoromethyl-sulfonyl)imide; [Bm_3N]Tf_2N, butyl trimethyl ammonium bis(trifluoromethyl-sulfonyl)imide; EDCI, 1-[3-(dimethylamino)propyl]3-ethylcarbodiimide hydrochloride; DCC, dicyclohexyl carbodiimide.

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(furan-2-yl) propanoate with a variety of alcohols in ionic liquids and hexane. A thorough literature survey showed that there is no 'best' ionic liquid for performing biotransformation just as there is no 'best' organic solvent in general for carrying out biocatalysis. As in our previous study [9] in order to evaluate the influence of anions on the catalytic performance of lipase *PS*, three ILs, [Bmim]BF_4, [Bmim]PF_6 and [Bmim]Tf_2N, with an identical cation [Bmim] and different anions [BF_4^-, PF_6^- and Tf_2N^-] were selected as reaction media. To the best of our knowledge this is the first report on the biocatalytic synthesis of these esters. In order to understand the role of ILs, the impact of their physicochemical properties on the following: (a) stabilization/desatbilization effect on the transition state, (b) micro-aqueous environment of the enzyme and (c) charged groups of that enzyme can be taken into account. In this paper we discuss the high yields of esterification formed over transesterification in hydrophobic ILs, taking into account the impact of ILs on all the above-mentioned factors, especially on the structure and function of lipase *PS*. The long-term stability of the enzyme in hydrophobic ILs up to a period of 10 months was also examined. The recyclability of lipase *PS*–hydrophobic IL mixture was also investigated.

2. Experimental procedures

2.1. Materials

ILs [Bmim]BF_4, [Bmim]PF_6 and [Bmim]Tf_2N were prepared as reported earlier [17]. They were purified according to a published procedure [18] by filtration through silica gel to remove halide impurities and then washed with sodium carbonate to remove acidic impurities. Lipase *PS* was bought from Amano Pharmaceuticals Co., Nagoya, Japan. All other chemicals were obtained from commercial sources locally and were of analytical grade. The conversions were determined by HPLC analysis using a Jasco PU-1580 liquid chromatograph with a PDA detector using a Sil-C18 column (size 4.6 mm × 250 mm). The eluent used was acetonitrile and water (85:15) at a flow rate of 1 ml min^{-1}. Column chromatography was done on silica gel (100–200 mesh). ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL GSX-400 MHz spectrometer. Chemical shifts are expressed in ppm values using TMS as an internal standard. IR spectra were recorded on a Shimadzu IR 470 Instrument. Mass spectra were recorded on a Q TOF micromass spectrometer. TLC was done using Kieselgel 60 F_{254} aluminium sheets (Merck 1.05554). The water content in the ILs was determined with an 831 Metrohm Karl–Fischer coulometer.

2.2. Preparation of standard esters

3-(Furan-2-yl) propanoic acid was synthesized according to the published procedure [19] and esterified as per reported procedure for esterification [20] replacing DCC with EDCl. All the 3-(furan-2-yl) propanoic acid esters, except hexyl, heptyl, and octyl (Table 1 entries 4, 5 and 6) are reported earlier. These were characterized by ^1H and ^{13}C NMR and compared with literature data [21]. Hexyl 3-(furan-2-yl) propanoate was obtained as a colorless liquid in 90% yield after silica gel column purification using hexane as solvent. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 7.22 (s, 1H, CH), 6.22 (s, 1H, CH), 5.94 (s, 1H, CH), 4.02–3.99 (t, 2H, CH_2, J = 6.8 Hz), 2.90–2.87 (t, 3H, CH_2, J = 7.2 Hz), 2.59–2.55 (t, 3H, CH_2, J = 7.2 Hz), 1.54–1.51 (m, 2H, CH_2), 1.22–1.19 (m, 6H, CH_2), 0.81–0.80 (t, 3H, CH_2, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 172.5, 154.1, 141.1, 110.1, 105.2, 64.7, 32.7, 31.3, 28.3, 25.5, 23.4, 22.4, 13.9; IR (neat, cm^{-1}): 2986, 1736, 1507, 1479, 1163; HRMS(EI): calculated for $C_{13}H_{20}O_3Na$ $(M+Na)^+$, 247.1310; observed 247.1310.

Table 1
Esterification of 1 in ILs/hexane at 50 °C catalyzed by lipase *PS*.

Entry	ROH	Yield % of ester formed in			
		[Bmim]BF_4	[Bmim]PF_6	[Bmim]Tf_2N	Hexane
1	$CH_3(CH_2)_2OH$	9	58	98	52
2	$CH_3(CH_2)_3OH$	8	56	98	57
3	$CH_3(CH_2)_4OH$	7	48	96	60
4	$CH_3(CH_2)_5OH$	7	22	82	51
5	$CH_3(CH_2)_6OH$	8	17	73	45
6	$CH_3(CH_2)_7OH$	7	28	67	49

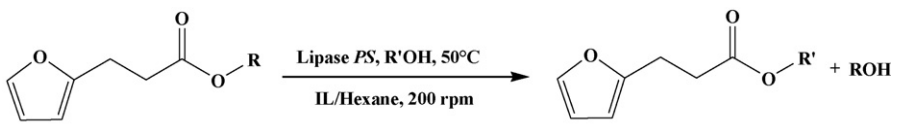
Heptyl 3-(furan-2-yl) propanoate was obtained as a colorless liquid in 85% yield after silica gel column purification using hexane as solvent. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 7.34 (s, 1H, CH), 6.31 (s, 1H, CH), 5.06 (s, 1H, CH), 4.14–4.11 (t, 2H, CH_2, J = 6.8 Hz), 3.03–2.99 (t, 3H, CH_2, J = 7.2 Hz), 2.71–2.68 (t, 3H, CH_2, J = 7.2 Hz), 1.75–1.64 (m, 2H, CH_2), 1.35–1.33 (m, 8H, CH_2), 0.94–0.92 (t, 3H, CH_2, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 172.5, 154.1, 141.1, 110.1, 105.1, 64.6, 32.7, 31.6, 28.8, 28.5, 25.8, 23.4, 22.5, 13.9; IR (neat, cm^{-1}): 2927, 1735, 1467, 1161; HRMS(EI): calculated for $C_{14}H_{23}O_3$ $(M+H)^+$, 239.1644; observed 239.1647.

Octyl 3-(furan-2-yl) propanoate was obtained as a colorless liquid in 87% yield after silica gel column purification using hexane as solvent. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 7.32 (s, 1H, CH), 6.29 (s, 1H, CH), 6.04 (s, 1H, CH), 4.12–4.09 (t, 2H, CH_2, J = 6.8 Hz), 3.01–2.97 (t, 3H, CH_2, J = 7.2 Hz), 2.69–2.65 (t, 3H, CH_2, J = 7.2 Hz), 1.65–1.62 (m, 2H, CH_2), 1.46–1.30 (m, 10H, CH_2), 0.93–0.90 (t, 3H, CH_2, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 172.5, 154.1, 141.1, 110.1, 105.1, 64.7, 32.7, 31.8, 31.5 29.4, 29.6, 28.5, 14.03; IR (neat, cm^{-1}): 2985, 1736, 1505, 1479, 1162; HRMS(EI): calculated for $C_{15}H_{25}O_3$ $(M+H)^+$, 253.1796; observed 253.1804.

The conversion was determined by HPLC using a Sil-C18 column by comparing the area under the peak of the product obtained with that of the reactant, 3-(furan-2-yl) propanoic acid or ethyl 3-(furan-2-yl) propanoate. The retention time (in minutes) for the acid and esters were as follows: 3-(furan-2-yl) propanoic acid, 2.80; ethyl 3-(furan-2-yl) propanoate, 4.10; propyl 3-(furan-2-yl) propanoate, 4.54; butyl 3-(furan-2-yl) propanoate, 4.93; pentyl 3-(furan-2-yl) propanoate, 5.69; hexyl 3-(furan-2-yl) propanoate, 6.85; heptyl 3-(furan-2-yl) propanoate, 7.68; and octyl 3-(furan-2-yl) propanoate, 9.80.

2.3. Typical procedure for lipase PS catalyzed esterification/transesterification

To screw capped test tubes of 3 ml capacity 3-(furan-2-yl) propanoic acid (20 mg, 0.14 mmol) or ethyl 3-(furan-2-yl)



R' = $CH_3(CH_2)_2$, $CH_3(CH_2)_3$, $CH_3(CH_2)_4$, $CH_3(CH_2)_5$, $CH_3(CH_2)_6$, $CH_3(CH_2)_7$.

IL = Bmim] BF_4, [Bmim] PF_6, [Bmim] Tf_2N

Scheme 1. (a) R = H, lipase *PS* catalyzed esterification of 3-(furan-2-yl) propanoic acid (1) in IL/hexane (b) R = CH_3CH_2, lipase *PS* catalyzed transesterification of ethyl-3-(furan-2-yl) propanoate (2) in IL/hexane.

Table 2
Transesterification of 2 in ILs/hexane at 50 °C catalyzed by lipase *PS*.

Entry	ROH	Yield % of ester formed in			
		[Bmim]BF_4	[Bmim]PF_6	[Bmim]Tf_2N	Hexane
1	$CH_3(CH_2)_2OH$	5	6	18	2
2	$CH_3(CH_2)_3OH$	4	9	21	2
3	$CH_3(CH_2)_4OH$	7	8	22	6
4	$CH_3(CH_2)_5OH$	5	10	20	4
5	$CH_3(CH_2)_6OH$	6	12	22	2
6	$CH_3(CH_2)_7OH$	5	9	16	2

propanoate (20 mg, 0.12 mmol), alcohol (0.42 mmol), 20 mg of lipase *PS* and 500 μl of IL/hexane were added. This mixture was kept in an orbital shaker at 50 °C at 200 rpm for 48 h (Scheme 1). At intervals of 8 h, aliquots of 20 μl were taken in 1 ml of hexane–ethyl acetate (90:10). For, ILs this biphasic mixture was vortexed for 2 min to extract all the reactants and products to the organic layer. The organic layers were analyzed by HPLC to monitor the time course of the reaction (extraction efficiency was >99%). After the completion of the reaction, the reaction mixture was extracted into hexane–ethyl acetate (90:10, 2 ml × 3), concentrated, purified by column chromatography and characterized as given in Section 2.2.

2.4. Lipase PS–IL/hexane mixture long-term stability and recyclability for transesterification

To screw capped test tubes of 3 ml capacity, 20 mg of lipase *PS* and 500 μl of [Bmim] PF_6, [Bmim]Tf_2N or hexane were added. The resulting mixture was vortexed for 2 min and kept at room temperature. At selected intervals of time, i.e. every 60 days up to 10 months, a fresh set of substrates (**1** and alcohol) were added to each lipase *PS*–IL/hexane mixture and the esterification reaction was carried out as given in Section 2.3.

3. Results and discussion

3.1. A comparison of the lipase PS catalyzed esterification and transesterification

The results for lipase *PS* catalyzed esterification of 3-(furan-2-yl) propanoic acid with six alcohols (propanol to octanol) in ionic liquids and hexane are summarized in Table 1 and that for the transesterification of ethyl 3-(furan-2-yl) propanoate are summarized in Table 2. The yields for the lipase *PS* catalyzed esterification of 3-(furan-2-yl) propanoic acid were found to be 98–67% for [Bmim]Tf_2N, 58–17% for [Bmim]PF_6, 9–7% for [Bmim]BF_4 and 60–45% for hexane (Table 1), while for the lipase *PS* catalyzed transesterification of ethyl-3-(furan-2-yl) propanoate the yields were found to be quite low in all the media (22–16% for [Bmim]Tf_2N, 12–6% for [Bmim]PF_6, 7–4% for [Bmim]BF_4 and 6–2% for hexane as in Table 2). In both the media, ILs and hexane, for the lipase *PS* catalyzed synthesis of 3-(furan-2-yl) propanoic acid esters, esterification is a better choice than transesterification. The yields in [Bmim]Tf_2N were the highest while in [Bmim]PF_6 and hexane were lower but comparable, while [Bmim]BF_4 showed very low yields. Fig. 1 shows the time course of overall conversion of 3-(furan-2-yl) propanoic acid catalyzed by lipase *PS* with propanol, pentanol and octanol in [Bmim]PF_6, [Bmim]Tf_2N, and hexane. Fig. 2 shows overall conversion of 3-(furan-2-yl) propanoic acid catalyzed by lipase *PS* with butanol, hexanol and heptanol in the above said media.

As found for the whole family of serine proteases a highly polar tetrahedral intermediate (an oxyanion) is formed in the transition state of this reaction [22]. The transition state formed with the 3-(furan-2-yl) propanoic acid would be relatively more polar than that formed with ethyl 3-(furan-2-yl) propionate. Hence it can be

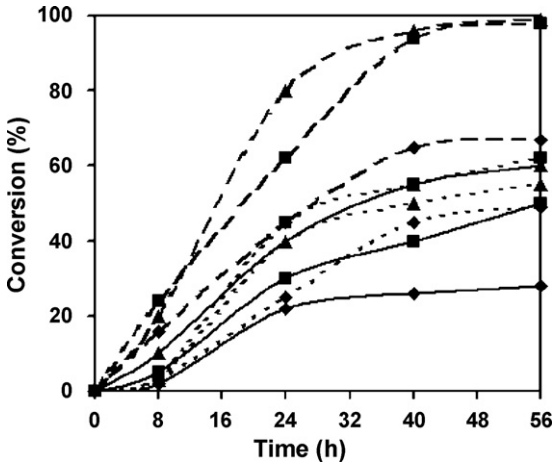


Fig. 1. Time course of 3-(furan-2-yl) propanoic acid conversion by lipase *PS* to produce esters with propanol (▲), pentanol (■), and octanol (♦) in [Bmim] PF_6 (—), [Bmim] Tf_2N (- -) and hexane (. . .).

hypothesized that the more polar transition state formed by the 3-(furan-2-yl) propanoic acid is stabilized by polar nature of ionic liquids. For esterification, one of the products, water can act as a nucleophile and can compete with the alcohol as a nucleophile for the acyl enzyme intermediate. The attack of water as a nucleophile results in hydrolysis (backward reaction) forming the acid (substrate) and the attack by the alcohol leads to the ester product (forward reaction). As water is a better nucleophile than all the alcohols tried, hydrolysis could be expected to dominate over esterification. But contrary to this, high yields were obtained for esterification (Table 1). A reasonable explanation is that the biotransformation is conducted at 50 °C (Section 2.3) in 3 ml screw capped vials. Most of the water formed (0.14 mmol or 2.52 mg in case of 100% biotransformation as in Section 2.3) as a byproduct during the esterification may evaporate into the free space of the vial during the course of time, and is thus unattainable to act as a nucleophile for the reverse reaction to occur, leading to high yields of the product ester. The possibility of evaporation of low boiling alcohols can be excluded since as discussed in Section 2.3, excess alcohol (acid:alcohol, 1:3 molar ratio) was used for the biotransformation.

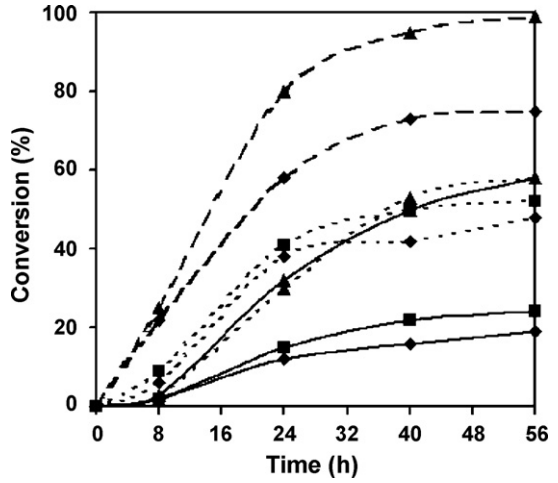


Fig. 2. Time course of 3-(furan-2-yl) propanoic acid conversion by lipase *PS* to produce esters with butanol (▲), hexanol (■), and heptanol (♦) in [Bmim]PF_6 (—), [Bmim]Tf_2N (- -) and hexane (. . .).

3.2. Role of reaction medium on biotransformation

The yields for lipase *PS* catalyzed esterification (Table 1) in all the three ILs and hexane were found to be higher than the yields of tranesterification (Table 2). The results for the lipase *PS* catalyzed esterification of 3-(furan-2-yl) propanoic acid (Table 1), demonstrate that there is a remarkable dependence of the nature of the reaction media on the yields of the products formed. Among all the three ILs tried, [Bmim]BF_4, [Bmim]PF_6, and [Bmim]Tf_2N, the highest yields were obtained in [Bmim]Tf_2N (98–67%). Lipase *PS* was found to be catalytically more active in the ionic liquids containing 1-alkyl-3-methylimidazolium cation in combination with anions such as PF_6^- and Tf_2N^- [10,11,23–26]. The yields for the esterified products were comparable in hexane and [Bmim] PF_6 the yields were found to be very low in [Bmim]BF_4.

Room temperature ILs (molten salts) are simply a composition of ions and remain liquids because the anions and cations do not pack well [27]. In this context it is important to mention that the effect of individual ions of inorganic salts on protein stabilization is well known which follows a recurring sequence known as the Hofmeister series [28]. According to Hofmeister phenomena, the ions present in the aqueous solution of a protein induces changes in the bulk water structure thus affecting the enzyme performance. Kosmotropic anions and chaotropic cations stabilize proteins, while chaotropic anions and kosmotropic cations destabilize them [28,29]. The kosmotropicity of the anions in this study follows the order $BF_4 > PF_6 > Tf_2N$, but the yields of the esters formed in this study (Tables 1 and 2) are completely contrary to this. The enzyme was found to be most active in [Bmim]Tf_2N followed by [Bmim]PF_6 and was least active in [Bmim] BF_4. Earlier studies also found the enzyme to be more active and stable in ILs containing anions such as PF_6^- and Tf_2N^- [10,11,23–26]. It is a matter of debate whether Hofmeister effects on proteins result from direct interactions of ions with the enzymes or indirectly with the bulk water [30]. Hence the results obtained in this study could not be explained by Hofmeister phenomena.

It is evident that enzymes in ILs basically follow the same catalytic mechanism as in organic solvents [31–33]. Therefore, an ionic liquid may play the same role as an organic solvent and depending upon its physicochemical properties, affect the enzyme performance by (i) stripping off the essential water associated with the enzyme, (ii) interacting with charged groups of the enzyme and (iii) stabilize/destabilize the transition state. The crystal structure determinations of lipase *PS* has shown that it contains the α/β hydrolase fold, a structural motif common to a wide variety of hydrolases [34]. In the 'closed' structures, both, a large deep active site cleft and the catalytic residues are buried underneath a helical segment, called a 'lid' or a 'flap'. In the case of lipase *PS* a relatively large number of residues (37 residues) out of 320 residues, are involved in the opening of the lid. As a consequence of such a large-scale movement, the active site of lipase *PS* becomes highly accessible to the solvent exposing the active site cleft and the hydrophobic inside surface. The resolved structure reveals a highly open conformation with solvent-accessible active site. The loop regions are rearranged in such a way that tight intra molecular contacts are possible in the new environment after opening of the lid [34]. It can be suggested that lipase *PS* will be more active and stable in a media which stabilizes this loop region. The excellent activity in [Bmim]Tf_2N could be partly attributed to its two opposing properties, high hydrophobicity and high polarity. Due to its high hydrophobicity, [Bmim]Tf_2N possibly stabilizes the active site cleft and the hydrophobic inside surface of lipase *PS* in the active site, at the same time its high polar nature may be stabilizing the highly polar tetrahedral intermediate (an oxyanion) formed in the transition state of this reaction [22]. [Bmim]PF_6 and hexane do not exhibit the same kind of stabilizing effect and [Bmim]BF_4 due to its hydrophilic nature lacks this stabilization effect on the hydrophobic inside surface of active site. The nucleophilicity of these ions are in the order of $F_4^- > PF_6^- > Tf_2N^-$. Another reason for the high activity in [Bmim]Tf_2N could be that, the enzyme compatible anions exhibit lower nucleophilicity and thus would show lower tendency to change the enzyme's conformation by interacting with the positively charged sites in the enzyme structure [35].

It is reported that lipase *PS* needs calcium ion for its activity and stability [36,37]. There are six oxygen atoms from six calcium ion ligands which stabilize the calcium binding [38]. Ionic liquids, in particular their anions that form strong hydrogen bonds with these ligands may result in loss of calcium binding and thus destabilize the enzyme structure thereby reducing its catalytic activity. Since ability of these anions to form hydrogen bonds are in the order, $BF_4^- > PF_6^- > Tf_2N^-$, [Bmim]$BF_4$ due to its strong hydrogen bonding ability may be reducing the calcium binding thus leading to poor activity. BF_4^- can also react readily with the internal hydrogen bonds of the enzyme thus reducing its activity [9,39]. Moreover, this esterification reaction was carried out under anhydrous conditions (since, only IL was used as the reaction medium, without any addition of water as a co-solvent), it is possible that [Bmim]BF_4 due to its high water affinity removes the tightly bound water from the enzyme molecule causing its deactivation while the hydrophobic ILs preserve the critical water molecules in the microenvironment of the enzyme, thus preventing its deactivation due to the loss of the essential water shell [40]. All these stabilizing/destabilizing effects are absent in hexane since it is a very apolar molecule.

The experiments conducted (Section 2.4) clearly demonstrated that while the lipase *PS*–[Bmim]Tf_2N and lipase *PS*–[Bmim]PF_6 were operationally stable up to 10 months at room temperature, the lipase *PS*–hexane mixture was found to be completely inactive after 2 months. Recyclability of the lipase *PS*–hydrophobic IL/hexane mixture (Section 2.4) showed that both the lipase *PS*–IL mixtures can be reused five times without any decrease in the yields. In accordance with our observation earlier, [Bmim]PF_6 and [Bmim]Tf_2N function as excellent stabilizing agents for lipase *PS* [9]. It should be kept in mind, however, that the hydrophobic effect, which significantly contributes to protein stabilization, does not exist in organic solvents [4]. The water content in the ILs for this transesterification reaction, as determined by Karl–Fischer titration was found to be in the range 0.72–0.77% for [Bmim]Tf_2N and 1.09–1.13% for [Bmim]PF_6. Hence lipase *PS* in these water-immiscible ILs at saturation of water ('wet IL') can be considered as existing as an aqueous solution, in which there are free water clusters which preserve the critical water molecules in the microenvironment of the enzyme, thus preventing its deactivation due to the loss of the essential water shell [2,9]. The thermal stability of CAL-B at 50 °C over 4 days was found to be 3–4 times higher in [Emim]Tf_2N and [Bm_3N]Tf_2N than in water or hexane [41]. The storage stability of α-chymotrypsin in the ionic liquid [EmIm]Tf_2N was compared with that in water, 3 M sorbitol, and 1-propanol. The enzyme's lifetime in [EmIm]Tf_2N at 30 °C was more than two and six times as long as those in 3 M sorbitol and water, respectively, and 96 times longer than that in 1-propanol [2]. The activity of thermolysin was well-retained after incubation in [BmIm][PF_6] at 37 °C for 144 h, whereas the same treatment in ethyl acetate resulted in the loss of almost half of the enzyme activity [42].

3.3. Effect of alcohols on the lipase PS catalyzed esterification of **1**

The effect of alcohols on conversion yields for lipase *PS* catalyzed esterification of 3-(furan-2-yl) propanoic acid (Table 1) in hydrophobic ILs, [Bmim]Tf_2N and [Bmim]PF_6 present some interesting observations. The yields for all the alcohols are higher in [Bmim]Tf_2N [98–67%] than in [Bmim]PF_6 [58–17%] but the pattern was found to be the same. The yields were high for propanol,

butanol and pentanol in both media, 98–96% in the [Bmim]Tf_2N and 58–48% in [Bmim]PF_6. The yields decrease as the number of carbon atoms increases, 82–67% in [Bmim]Tf_2N and 22–17% in [Bmim]PF_6, except for octanol in [Bmim]PF_6 (28%). This difference in yields due to change in number of carbon atoms is not found to be much pronounced in hexane, which yielded 60–45% of products with all the alcohols.

4. Conclusion

We have here compared the behavior of lipase *PS* for the esterification of 3-(furan-2-yl) propanoic acid and transesterification of ethyl 3-(furan-2-yl) propanoate with six straight chain alcohols (propanol to octanol) in three ionic liquids and hexane. In order to evaluate the influence of anions on the catalytic performance of lipase *PS*, three ILs, [Bmim]BF_4, [Bmim]PF_6 and [Bmim]Tf_2N, with identical cation and different anions were selected as reaction media for this biotransformation. This study showed that for the preparation of these esters, lipase *PS* catalyzed esterification is better choice than transesterification. [Bmim]Tf_2N markedly enhanced the yields of the products for esterification (98–67%), while in [Bmim]PF_6 and in hexane the yields (60–17%) were comparable. The lipase *PS*–hydrophobic IL mixture was found to be operationally stable up to 10 months and was recycled five times. It has therefore become clear that with the advent of ionic liquids the choice of medium for carrying out biotransformation and thus the chance to find one that is satisfactory has increased enormously.

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