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Lipase-catalysed transesterification in ionic liquids and organic solvents: a comparative study

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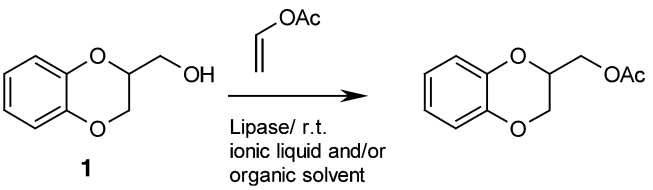
Abstract—The lipase-catalysed transesterifications of 2-hydroxymethyl-1,4-benzodioxane in two different ionic liquids, 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim]PF_6 and 1-butyl-3-methylimidazolium tetrafluoroborate, [bmim]BF_4 and different organic solvents was studied. The hydrophobic and hydrophilic properties of ionic liquids and organic solvents do influence the lipase activity as illustrated from the results. The influence of the ionic liquid as an additive in an organic solvent on this reaction has been demonstrated. The enzymes in ionic liquids in combination can be recycled for several runs without substantial diminution in the lipase activity. © 2002 Elsevier Science Ltd. All rights reserved.

Enzymes are well recognised as remarkable catalysts, capable of accepting a wide array of complex molecules as substrates, and exquisitely selective, catalysing reactions with unparalleled enantio- and regioselectivities. As a result, the biocatalysts can be recruited to perform a myriad of chemical transformations without the need for the tedious blocking and deblocking steps that are common in enantio- and regioselective organic synthesis. Such high selectivity also affords efficient reactions with few by-products under ambient conditions, thereby making enzymes satisfy increasingly stringent environmental constraints. In view of increasing environmental and economic pressure to use renewable sources of energy and chemical feedstocks in industry, biocatalysts are potentially attractive technological tools. The versatility of the enzymes to work well with comparable efficiency in aqueous environments, organic solvents,[1] supercritical fluids[2] and even in the gas phase[3] has widened the scope of enzyme-catalysed reactions enormously in organic synthesis.

Studies spanning over the last decade have brought to light several important features of organic solvents as the media for enzyme-catalysed reactions. The data indicates that enzymes are not only extremely thermally stable in anhydrous organic solvents owing to their conformational rigidity in the dehydrated state, but also there is considerable enhancement in their catalytic activity, stability and in certain cases even selectivity.[4]

Despite these merits, the disadvantages associated with organic solvents have raised several questions of environmental concern, particularly when they are employed on a preparative scale. In contrast to molecular organic solvents, the new generation solvent-ionic liquids are relatively safe owing to their negligible vapour pressure. Apart from this, the remarkable feature possessed by the ionic liquids is our ability to manipulate at will the structure (with respect to the organic cation, inorganic anion and the length of the side chain attached to the organic cation) and consequently their properties. This feature carries the prospect of applications previously not considered in the realm of biocatalysts such as large-scale chemical production. Their intrinsic ability to solvate substrates of wide diversity ranging in nature from organic to organometallic to inorganic, and the ease of recyclabilty adds ionic liquids to the chemists' arsenal for the execution of diverse processes. All these properties[5] possessed by the ionic liquids in an ensemble make them useful alternatives to conventional organic solvents.



Scheme 1. The transesterifications of 2-hydroxymethyl-1,4-benzodioxane catalysed by lipases using vinyl acetate in ionic liquids and organic solvents.

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Recently, the field of ionic liquids has been escalating at an overwhelming rate. These liquids have captivated us for quite sometime, owing to their remarkable properties. In continuation of our quest to explore different reactions in ionic liquids,[6] we thought it would be worthwhile to employ these liquids as solvents in biocatalytic transformations. As an extension of our ongoing research program on enzyme-catalysed reactions,[7] we herein report transesterifications of 2-hydroxymethyl-1,4-benzodioxane catalysed by several lipases in ionic liquids using vinyl acetate as an irreversible acyl donor (Scheme 1). Earlier reports on the biotransformations in ionic liquids have demonstrated that both cell-free enzymes[8] and whole cells[9] not only work well in these new environments, but also can exhibit enhancement in enantioselectivity.[10] However, since the area is new, much needs to be explored and unravelled. We have carried out a comprehensive comparative study of lipase-catalysed transesterifications in ionic liquids and organic solvents and the effect of the ionic liquid as an additive is also studied. The lipases from *Pseudomonas cepacia*, both supported (PS-D, immobilised on diatomite particles) and free (PS) were used to study the influence of such polar media on the catalytic activity and the stability of enzymes. The ionic liquids employed for the current study are 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim]PF_6 and 1-butyl-3-methylimidazolium tetrafluoroborate, [bmim]BF_4. These two liquids are distinctly different in their properties in the sense that the former is hydrophobic and the latter is highly hydrophilic in nature, due to the different anions associated with the common organic cation. These properties are of primary concern in enzyme-catalysed reactions, since they are capable of influencing the conformation of the enzymes and consequently their activity.

The solvent dichloromethane works well as a reaction medium for the transesterification of **1**, moreover, it is also miscible with [bmim]PF_6 in all proportions. The substrate **1** happens to be an important synthon widely used in the design of therapeutic agents with α-adrenergic blocking, antigastric, spasmolytic, antipsychotic and anxiolytic properties. To study the influence of the ionic liquid as the reaction medium on the PS-D-catalysed transesterification of **1**, we designed an experiment wherein [bmim]PF_6 was added as an additive to the solvent dichloromethane. The amount of [bmim]PF_6 in dichloromethane was progressively increased from 0 to 90% v/v in the reaction mixture and the initial conversion after 10 min was monitored.[11] The study revealed that as the proportion of ionic liquid was increased, a slight increase in initial rates was observed as illustrated in Fig. 1.

The ability of lipases PS and PS-D to catalyse the transesterification of **1** in different reaction media was gauged in terms of the extent of transesterification. The lipase PS initially exhibited a relatively high extent of transesterification in [bmim]PF_6 in comparison to [bmim]BF_4 and dichloromethane (Fig. 2). The substantial difference in initial rates of reaction observed in [bmim]PF_6 and [bmim]BF_4 can be rationalised by the hydrophilic nature of [bmim]BF_4. The influence of the hydrophilicity of [bmim]BF_4 was studied on the lipase activity in an extremely dry ionic liquid.[12] The rate of transesterification reduced drastically in anhydrous [bmim]BF_4 probably due to the stripping of the essential water of the lipase. However, when [bmim]BF_4 was used without subjecting it to a rigorous drying procedure, the initial extent of transesterification was marginally greater than that observed in dichloromethane. Thus, it seems that the chief factor contributing to the relatively reduced enzymatic activity in an apolar organic media is the highly restricted conformational mobility. In the case of lipase PS-D, the initial extent of transesterification is highest in [bmim]PF_6, followed by dichloromethane and [bmim]BF_4, respectively. However, owing to the conformational rigidity of the supported lipase PS-D, the solvent effects are not as pronounced as seen in the case of PS, which is reflected in the results illustrated in Fig. 3.

The polarity of the medium is known to influence dramatically the catalytic activity of enzymes.[13] Earlier endeavours to study the influence of the nature of the solvent on the catalytic activity of enzymes have revealed that hydrophobic solvents favour rapid reactions[14,15] and that hydrophilic solvents impede the rate of enzyme-catalysed reactions to a certain extent irrespective of the parameters[16] used to describe the solvent quantitatively. The rationale for this observed phenomenon has been substantiated by several

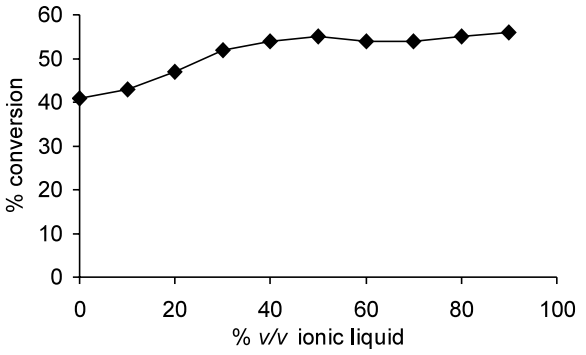

Figure 1. The lipase PS-D-catalysed transesterification of 2-hydroxymethyl-1,4-benzodioxane in [bmim]PF_6 and dichloromethane mixtures.

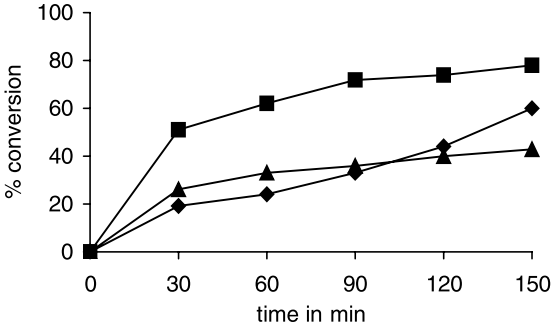

Figure 2. The transesterification of 2-hydroxymethyl-1,4-benzodioxane catalysed by PS in dichloromethane (◆), [bmim]PF_6 (■) and [bmim]BF_4 (▲).

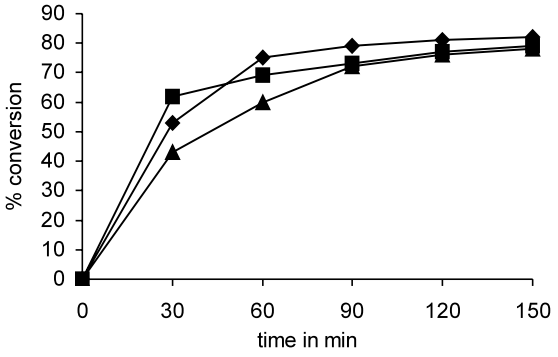

Figure 3. The transesterification of 2-hydroxymethyl-1,4-benzodioxane catalysed by PS-D in dichloromethane (◆), [bmim]PF_6 (■) and [bmim]BF_4 (▲).

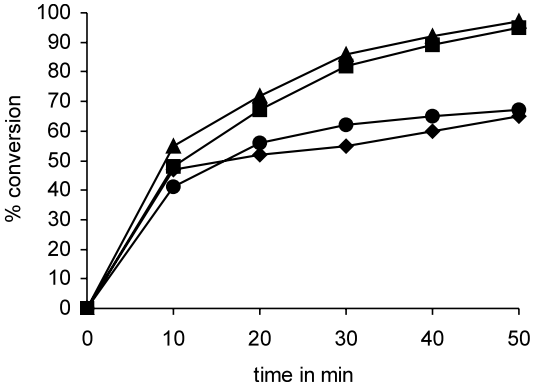

Figure 4. The transesterification of 2-hydroxymethyl-1,4-benzodioxane catalysed by PS-D in dichloromethane (◆), benzene (■), hexane (▲) and [bmim]PF_6 (●).

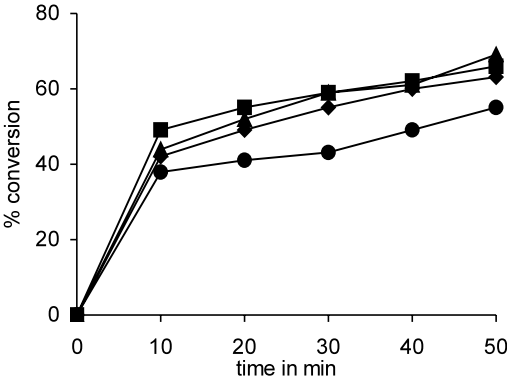

Figure 5. The transesterification of 2-hydroxymethyl-1,4-benzodioxane catalysed by PS-D in tetrahydrofuran (◆), acetonitrile (■), dioxane (▲) and [bmim]BF_4 (●).

reports.[17] The extremely polar organic solvents interact with the absolute amount of water which is indispensable for acquisition and maintenance of the catalytic conformation of the enzyme. These polar solvents being hydrophilic in nature, strip off the tightly bound water molecules resulting in either alteration or sometimes distortion of the catalytic conformation and consequently result in deactivation of the enzyme. Apart from their hydrophilicity, extremely polar solvents may interact with the secondary structure of the functional protein via multiple hydrogen bonding or other strong interactions and may lead to unfolding resulting in their deactivation.[18]

The ionic liquid [bmim]PF_6, in particular, despite being polar due to its ionic nature, is hydrophobic, being contrary to what is observed in the cases of most of the common polar organic solvents. This feature of [bmim]PF_6 made us inquisitive about the behavior of lipases in such a medium. A comparative study of the extent of the transesterification of **1** as a function of time in hydrophobic as well as hydrophilic media (organic solvents and ionic liquids) with lipase PS-D was carried out. The results are illustrated in Figs. 4 and 5. Thus, analogous to the hydrophobic organic solvents in [bmim]PF_6, lipase PS-D exhibited rates of transesterification much better than those observed in [bmim]BF_4, a hydrophilic ionic liquid. However, the lack of physical data (the parameters[16] employed commonly to describe solvents quantitatively) for the ionic liquids rendered it difficult to arrive at a clear consensus on the influence of the reaction media on enzyme activity.

The potential of supported enzymes as practical biocatalysts in several biochemical applications has been utilised over years. Their behavior in terms of catalytic activity in different media in comparison with their free or unsupported counterparts is equally important. Apart from the advantage of recyclability, immobilisation of the enzymes makes them more robust and stable due to conformational rigidity. The supported enzyme PS-D gave results better than the unsupported counterpart PS in terms of the initial rates of transesterification in all the reaction media under investigation as illustrated in Figs. 2 and 3. The differences in the initial rates are substantial in dichloromethane and [bmim]BF_4, but comparable in [bmim]PF_6. Thus, [bmim]PF_6 served as a relatively better medium for both PS-D- and PS-catalysed transesterifications.

Supported enzymes are a commercially viable option for large-scale synthesis. However, if the enzyme-containing medium were recyclable then it would be an added advantage. We carried out several experiments wherein the lipase was taken in [bmim]PF_6 and charged with substrate, **1** and vinyl acetate. The reaction was quenched after 30 min by the removal of vinyl acetate under reduced pressure and the products were extracted using diethyl ether. The same reaction medium containing lipase was recharged with **1** and vinyl acetate. Similarly, the recyclability of the medium containing the enzyme was studied for five consecutive runs. Thus, ionic liquids can act as a medium as well as a reservoir of enzymes, which can be recycled for several runs without substantial diminution in lipase activity. The results of the study on the resolution of this substrate using various lipases in organic solvents and using whole cells will be submitted elsewhere.

In conclusion, ionic liquids serve as an alternative, recyclable media for lipase-catalysed transesterifications. The lipases exhibit better or comparable catalytic activity in these unconventional media.

Acknowledgements

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11. In a typical experimental procedure, to the solvent ([bmim]PF_6 or [bmim]BF_4 or organic solvent or the mixture of ionic liquid and organic solvent, 1.9 mL), 2-hydroxymethyl-1,4-benzodioxane **1** (0.30 mmol), lipase (PS-D, 25 mg, 8 U/mg or PS, 2.5 mg, 40 U/mg) and vinyl acetate (1.16 mmol) were added. The reaction mixture was stirred at room temperature and the aliquots were withdrawn after fixed intervals of time. The volatile organic solvents were removed under reduced pressure and the products were extracted using diethyl ether. The ether extracts were assayed on GC. The product was characterised by IR, NMR and physical constants. The extent of transesterification in all cases was monitored on GC with respect to the relative areas of **1** and its acetate ester. A Nucon 5700 chromatograph equiped with a FID was employed for the analysis. The detector temperature was maintained at 270°C. The column was programmed with an initial temperature of 180°C and was increased thereafter to 270°C at the rate of 10°C min^{-1}. The column used was liquid phase ov-17 (length 6′).
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