

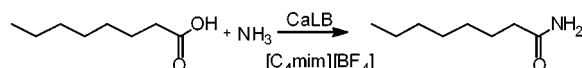
Lipase-Catalyzed Reactions in Ionic
LiquidsR. Madeira Lau,[†] F. van Rantwijk,[†] K. R. Seddon,[‡] and R. A. Sheldon*[†]

Laboratory of Organic Chemistry and Catalysis, Delft University of Technology,
Julianalaan 136, 2628 BL Delft, The Netherlands, and The Quill Centre, The Queen's
University of Belfast, Stranmillis Road, Belfast BT9 5AG, Northern Ireland, U.K.

r.a.sheldon@tnw.tudelft.nl

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ABSTRACT



Candida antarctica lipase was shown to catalyze alcoholysis, ammoniolysis, and perhydrolysis reactions using the ionic liquids 1-butyl-3-methylimidazolium tetrafluoroborate or hexafluorophosphate as reaction media. Reaction rates were generally comparable with, or better than, those observed in organic media.

Following the pioneering studies of Klivanov and co-workers,^{1–3} the use of hydrolytic enzymes in anhydrous organic media has become a valuable addition to the synthetic repertoire. Reactions can be performed that are impossible in water, and the enzyme can even show enhanced thermostability.^{4,5} A serious drawback of conventional organic media is, however, the substantial reduction in reaction rate, which is mainly caused by altered partitioning of the reactant between the solvent and the active site.⁶ Moreover, environmental problems are associated with the use of many volatile organic solvents, e.g., chlorinated hydrocarbons. Ionic liquids, formerly called molten salts, are liquids that are composed entirely of ions (see Figure 1). They are liquid

cal window; and are extremely good solvents for a wide range of organic, inorganic, and polymeric compounds. Moreover, ionic liquids are simple and inexpensive to manufacture and easy to recycle, and their properties can be fine-tuned by changing the anion or the R group of the cation.^{7–13} Ionic liquids, however, have scarcely been used in combination with enzyme catalysis although they may combine the advantages of a nonaqueous medium while being moderately polar.¹⁴ Cull et al. reported a two-phase biotransformation where the ionic liquid acts as a reservoir for the substrate while the biocatalyst, whole cells of *Rhodococcus* R312, is present in the aqueous phase.¹⁵ The

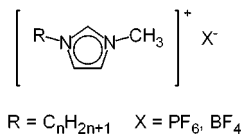


Figure 1. Example of ionic liquids: 1-alkyl-3-methylimidazolium salt (for R = Bu, X = BF₄ the ionic liquid is miscible with water and for R = Bu, X = PF₆ it is immiscible with water).

over a broad temperature range; have essentially no vapor pressure, a high ionic conductivity, and a large electrochemi-

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[†] Delft University of Technology.[‡] The Queen's University of Belfast.

thermolysin-catalyzed synthesis of Z-aspartame in ionic liquids containing 5% (v/v) water has been reported by Erbeltinger et al.¹⁶ Herein we report the first example of the use of a free enzyme in an ionic liquid in the absence of water.

Lipases tolerate nonnatural reaction conditions surprisingly well and hence are ideal candidates for exploring the possibilities of ionic liquids. Lipases, which in nature catalyze the hydrolysis of triacyl glycerides, readily catalyze related reactions such as esterification or transesterification in anhydrous organic media.

The ionic liquids 1-butyl-3-methylimidazolium tetrafluoroborate ([C₄mim][BF₄])⁶ or hexafluorophosphate ([C₄mim][PF₆])¹² were used as solvent. The former salt was used when the effects of water were to be studied, since this compound is miscible with water in all proportions, whereas the latter is hydrophobic.

The transesterification of ethyl butanoate with butanol in both salts of [C₄mim] was studied using *Candida antarctica* lipase B (Novozym 435) as catalyst (Figure 2). The butyl

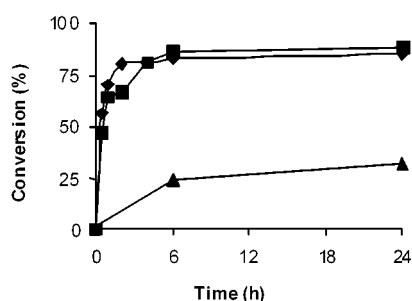


Figure 2. Transesterification of ethyl butanoate with butan-1-ol catalyzed by Novozym 435 in different ionic liquids: (◆) [C₄mim][BF₄]; (■) [C₄mim][PF₆]; (▲) [C₄mim][BF₄]/H₂O (9:1, v/v). Reaction conditions: 40 mM ethyl butanoate, 200 mM butan-1-ol, and 25 mg of enzyme in 1 mL of solvent were stirred at 40 °C.

ester was formed in more than 80% yield when the reaction was carried out in an anhydrous ionic liquid (83% yield is expected at equilibrium, on the basis of the molar ratio of ester and alcohol). The anion had only a small effect on the reaction rate; the initial rate was slightly lower in the [PF₆]⁻ salt. In the presence of water (10% v/v), butanoic acid was formed, as would be expected, in 62% yield, resulting in a correspondingly lower yield of butyl ester.

For comparison, the reaction was also performed in *tert*-butyl alcohol and butan-1-ol (Table 1). The transesterification was slightly faster in the ionic liquids than in *tert*-butyl alcohol. The somewhat lower reaction rate in butan-1-ol is to be ascribed to inhibition by the solvent.¹⁷

Table 1. Transesterification of Ethyl Butanoate with Butan-1-ol Catalyzed by Free and Immobilized *Candida antarctica* Lipase B^a

solvent	lipase	time (h)	conv (%)
[C ₄ mim][BF ₄]	CaLB (Nov 435)	4	81
[C ₄ mim][PF ₆]	CaLB (Nov 435)	4	81
<i>t</i> -BuOH	CaLB (Nov 435)	4	74
1-BuOH	CaLB (Nov 435)	4	66
[C ₄ mim][BF ₄]	CaLB (SP 525)	24	79
[C ₄ mim][PF ₆]	CaLB (SP 525)	24	78

^a Reaction conditions: 40 mM ethyl butanoate, 200 mM butan-1-ol, and 25 mg of enzyme in 1 mL of solvent were stirred at 40 °C.

The transesterification was also performed with free CaLB (Novo SP525) suspended in the ionic liquid. A slower reaction was observed, compared with Novozym 435, although the active protein content of SP525 is much higher. This is consistent with the results obtained in organic media where suspensions of “free” lipases are generally less effective than immobilized lipases.¹⁸

The transesterification of ethyl octanoate with butan-1-ol and propan-2-ol was performed in the presence of Cal B (Novozym 435) (Table 2). With butan-1-ol a higher conver-

Table 2. Transesterification of Ethyl Octanoate Using Novozym 435^a

solvent	alcohol	conv (% , 24 h)
[C ₄ mim][PF ₆]	butan-1-ol	81
<i>t</i> -BuOH	butan-1-ol	69
[C ₄ mim][PF ₆]	propan-2-ol	56
<i>t</i> -BuOH	propan-2-ol	81

^a Reaction conditions: 10 mM ethyl octanoate, 120 mM alcohol, and 25 mg of Novozym 435 in 0.5 mL of solvent were stirred at 40 °C.

sion was observed in [C₄mim][PF₆], whereas with propan-2-ol the conversion was higher when *tert*-butyl alcohol was used as solvent.

Primary fatty acid amides are important commodities that can be prepared under mild conditions via a lipase-catalyzed reaction of the carboxylic ester and ammonia.¹⁹ We compared the ammoniolysis of ethyl octanoate in an ionic liquid medium with that in *tert*-butyl alcohol under otherwise identical reaction conditions (Table 3). The conversion into octanamide was markedly lower in [C₄mim][BF₄] than in *tert*-butyl alcohol. The several preparations of Cal B (immobilized on Lewatit E, free and immobilized on EP100¹⁸) show different performances, the free enzyme showing the lowest activity in this system (38% conversion after 24 h, whereas Novozym 435 shows 40% and 57% for SP525 on EP100).

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Table 3. Ammoniolysis of Ethyl Octanoate with *Candida antarctica* Lipase B^a

solvent	lipase	conv (% , 24 h)	
		octanamide	octanoic acid
<i>t</i> -BuOH	CaLB (Nov 435)	99	0.5
[C ₄ mim][BF ₄]	CaLB (Nov 435)	40	5
<i>t</i> -BuOH	CaLB (SP525)	57	6
[C ₄ mim][BF ₄]	CaLB (SP525)	38	2
<i>t</i> -BuOH	CaLB (SP525/EP100)	100	0
[C ₄ mim][BF ₄]	CaLB (SP 525/EP100)	57	0

^a Reaction conditions: 300 mM ethyl octanoate in 0.5 mL of solvent and 0.1025 KLU of enzyme were stirred at 40 °C.

It has recently been shown that, contrary to expectations, the condensation of a carboxylic acid and ammonia in the presence of a lipase is feasible, albeit with very long reaction times: 90–100% conversion in 17 days using ammonium carbamate in methylisobutyl ketone.²⁰

The reaction of octanoic acid with ammonia was carried out in ionic liquid in the presence of Novozym 435, by bubbling ammonia through the reaction mixture (Figure 3). A quantitative conversion was obtained after 4 days.

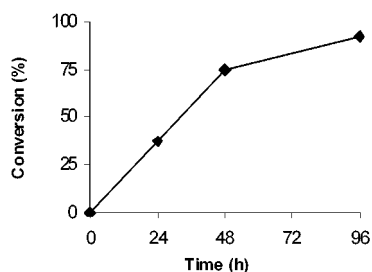


Figure 3. Time-course of the condensation of octanoic acid and ammonia catalyzed by Novozym 435 in [C₄mim][BF₄]. Reaction conditions: 300 mM octanoic acid in 0.5 mL of solvent and 25 mg of Novozym 435 were stirred at 40 °C.

Peroxy-carboxylic acids are commonly used oxidants in organic synthesis. Their application is hampered, however, by the hazards involved in their handling, especially on a

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large scale. Hence, generating them *in situ* would be an attractive option.^{21,22} The epoxidation of cyclohexene by peroctanoic acid, generated *in situ* by Novozym 435–catalyzed reaction of octanoic acid with 60% aqueous hydrogen peroxide, proceeded smoothly in [C₄mim][BF₄] as solvent. A yield of 83% was observed in 24 h compared to 93% in acetonitrile, which was previously shown to be the optimum organic solvent for this reaction¹⁷ (Figure 4).

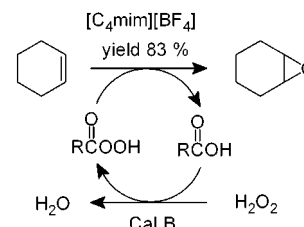


Figure 4. Epoxidation of cyclohexene by *in situ* generation of peroctanoic acid. Reaction conditions: 0.4 M octanoic acid, 3 M cyclohexene in 0.5 mL solvent, and 10 mg of Novozym 435 were stirred at room temperature. H₂O₂ 60% (1.1 equiv) was added in several portions.

We have shown, for the first time, that *Candida antarctica* lipase catalyzes a number of elementary reaction types—alcoholysis, ammoniolysis, and perhydrolysis—in ionic liquid media. Reaction rates were comparable with or better than those observed in conventional organic reaction media. We envisage that ionic liquids could have added benefits for performing biotransformations with highly polar substrates, e.g., carbohydrates, which are sparingly soluble in common organic solvents. We are currently investigating the scope of enzymatic processes in ionic liquids with regard to type of enzyme, transformation and the effect of the structure of the ionic liquid and will report our results in due course.

Acknowledgment. Generous donations of *C. antarctica* lipase by Novo Nordisk A/S (Bagsvaerd, Denmark) are gratefully acknowledged.

Supporting Information Available: Description of experimental procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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