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Use of an ionic liquid in a two-phase system to improve an alcohol dehydrogenase catalysed reduction†

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Received (in Cambridge, UK) 22nd January 2004, Accepted 5th March 2004
First published as an Advance Article on the web 30th March 2004

Due to favourable partition coefficients the highly enantioselective reduction of 2-octanone, catalysed by an alcohol dehydrogenase from *Lactobacillus brevis*, is faster in a biphasic system containing buffer and the ionic liquid [BMIM][$(CF_3SO_2)_2N$] compared to the reduction in a biphasic system containing buffer and methyl *tert*-butyl ether.

Recently, it was reported that alcohol dehydrogenases (ADH) can be used in biphasic systems containing an aqueous and an organic phase to catalyse reductions of prochiral ketones to enantiopure alcohols.[1,2] While the enzyme is responsible for the high enantioselectivity, the use of an organic phase suppresses limitations caused by poor substrate-solubility in the aqueous phase leading to higher volumetric productivities. Among further advantages of this biphasic approach are an easy work-up by phase separation and the "free immobilisation" of the enzyme and charged cofactor NAD(P)H in the aqueous phase allowing a re-use of enzyme and cofactor.[3–6] However, the drawback of this system is an unfavourable shift in the thermodynamics of the substrate-coupled cofactor regeneration, which will be discussed in detail later.[7]

In this paper we report the use of an ionic liquid as a second phase in an ADH-catalysed reduction to make use of the advantages of biphasic reaction conditions, but simultaneously to avoid thermodynamic limitations.

Ionic liquids are defined as salts, which are liquid at temperatures below 100 °C.[8] Several groups have already shown the use of ionic liquids as solvents in biocatalytic applications, which have recently been reviewed.[9,10] In some cases good activities, enantioselectivities and stabilities of isolated enzymes and whole cells have been reported. In biocatalysis ionic liquids have been used as pure solvents, as additives and even in combination with supercritical carbon dioxide.[11,12] However, the use of an ionic liquid in a two-phase system for a biocatalytic application has only been described once so far.[13]

The partition coefficients of 2-propanol and acetone in a two-phase system containing buffer and ionic liquid [BMIM][$(CF_3SO_2)_2N$][14] significantly differ from their partitioning behaviour in buffer/organic solvent systems (Table 1). This remarkable fact has drawn our attention to its potential in an ADH-catalysed reduction.

Table 1 Partition coefficients for 2-propanol and acetone[a]

	Partition coefficients *m* between the two phases	
Substance	MTBE/buffer	[BMIM][$(CF_3SO_2)_2N$][14]/buffer
2-propanol	1.0	0.4
acetone	1.1	2.0

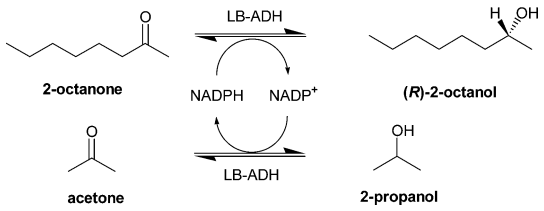
[a] Conditions: 50 mM phosphate buffer (pH 7.0); the ionic liquid was prepared referring to Bonhôte *et al.* and Eckstein *et al.*;[15,16] measurements at 25 °C; analysis by GC-FID.

† Electronic supplementary information (ESI) available: experimental section. See http://www.rsc.org/suppdata/cc/b4/b401065e/

In this study we have used the alcohol dehydrogenase from *Lactobacillus brevis* (LB-ADH), which catalyses the enantioselective reduction of prochiral ketones to (*R*)-alcohols. Thereby the enzyme requires the cofactor NADPH. Since the cofactor NADPH is expensive, it is beneficial to regenerate the cofactor during the reaction.[4,17,18] In the investigated reduction of 2-octanone the cofactor regeneration was realised by a substrate-coupled approach, whereby 2-propanol is oxidised to acetone yielding the reduced cofactor NADPH (Scheme 1). In a two-phase system the aqueous phase contains the LB-ADH and the cofactor NADP(H), while the organic phase can be seen as a reservoir for the poorly water-soluble substrate 2-octanone (~ 1 g/l = 7 mM). During the reaction the substrate (2-octanone), the co-substrate (2-propanol), the product (2-octanol) and the co-product (acetone) will partition between the two phases. The partitioning behaviour of substrates and products is of great importance for the thermodynamics of reactions in two-phase systems.[7,19,20]

Since the partition coefficients of 2-propanol and acetone are approximately equal ($P \sim 1$) in a two-phase system consisting of buffer and MTBE (Table 1), their partition behaviour will not lead to any shift in the thermodynamics of the cofactor regeneration. The partition coefficients of the same substances conspicuously change when MTBE is replaced by the ionic liquid [BMIM][$(CF_3SO_2)_2N$]. While 2-propanol preferably remains in the aqueous phase (m = 0.4), acetone is rather removed by the ionic liquid (m = 2.0). In this case the partition behaviour of the co-substrate and the co-product will lead to a positive shift in the thermodynamics of cofactor regeneration. The partition coefficients for 2-octanone and 2-octanol were measured in a range > 100 for both two-phase systems.

Figure 1 demonstrates the advantages of the two-phase system combining buffer and ionic liquid for the investigated reaction system. The LB-ADH-catalysed reduction of 2-octanone was monitored in a biphasic system of [BMIM][$(CF_3SO_2)_2N$] and buffer and compared to the same reaction in the biphasic system consisting of MTBE and buffer. The figure shows the conversion of 2-octanone as function of reaction time. Within the first 180 min the reduction is much faster in the biphasic system containing the ionic liquid, leading to a conversion of 88%, while the reduction reaches a conversion of only 61% in the presence of MTBE.



Scheme 1 Reduction of 2-octanone catalysed by alcohol dehydrogenase from *Lactobacillus brevis* (LB-ADH). Substrate-coupled NADPH-regeneration with 2-propanol.

DOI: 10.1039/b401065e

The cofactor regeneration is the rate-limiting step of the investigated reduction. This means: the more effective the regeneration step, the faster the main reaction. The regeneration equilibrium can be shifted using a higher concentration of 2-propanol and/or by removing acetone.[21] In this biphasic approach acetone is continuously removed by the ionic liquid due to its partition coefficient of 2.0, which leads to a very fast regeneration. Additionally, 2-propanol is permanently available in the aqueous phase (P = 0.4).

Acetone is known as an inhibitor for the alcohol dehydrogenase. Using the ionic liquid as a second phase, the extraction of acetone from the buffer phase leads to a lower concentration of acetone in the buffer phase. Thus, the inhibiting effect of acetone can be reduced.

As confirmed by GC-analysis for the product (*R*)-2-octanol on a chiral stationary phase, we could not detect any (*S*)-2-octanol in any of the two reaction systems (ee > 99%).

Villela *et al.* have reported that the alcohol dehydrogenase from *Lactobacillus brevis* shows high stabilities in biphasic systems. Thereby, the LB-ADH reaches half life times of ~ 10 hours in the presence of aliphatic hydrocarbons. Using ethers like MTBE in a two-phase system, the LB-ADH reaches highest stabilities with half life times > 1000 h (stabilities were measured at 4 °C).[2] Under the investigated reaction conditions (30 °C) the LB-ADH showed good stabilities with half life times of about 50 h in the presence of the ionic liquid [BMIM] [$(CF_3SO_2)_2N$].

Further investigations will be necessary to understand the development of the partitioning behaviour of the substrates and products throughout the whole reaction. Another interesting future aspect is the recycling of the enzyme and cofactor by replacing the organic phase respectively the ionic liquid phase with fresh substrate solution. Furthermore, the applicability of the investigated system for other substrates is of interest.

In summary, we were able to show that the NADPH-dependent alcohol dehydrogenase from *Lactobacillus brevis* catalyses the reduction in a biphasic system consisting of buffer and the ionic liquid [BMIM][$(CF_3SO_2)_2N$]. Due to the favourable partition coefficients of 2-propanol and acetone, this reaction shows higher reaction rates compared to the same reaction in the presence of methyl *tert*-butyl ether (MTBE). To the best of our knowledge, this is the first report of an alcohol dehydrogenase being used in a two-phase system with an ionic liquid. Therefore, these results might represent a novel route to biphasic reaction media, not only for biocatalysis.

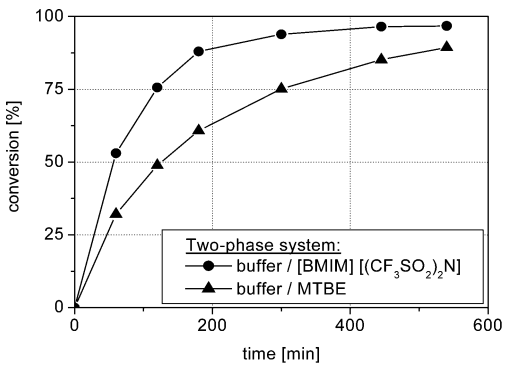


Fig. 1 Reduction of 2-octanone to (*R*)-2-octanol catalysed by alcohol dehydrogenase from *Lactobacillus brevis*. Substrate-coupled NADPH-regeneration.

We thank Thomas Daußmann from JFC – Jülich Fine Chemicals for the alcohol dehydrogenase from *Lactobacillus brevis* (www.juelich-chemicals.de). We also thank Heike Offermann and Ursula Mackfeld for their skilful help in the laboratory and Christine Fischer (IfOK; Rostock) for her help with the GC analysis.

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