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Biocatalysis in ionic liquids: batchwise and continuous flow processes using supercritical carbon dioxide as the mobile phase

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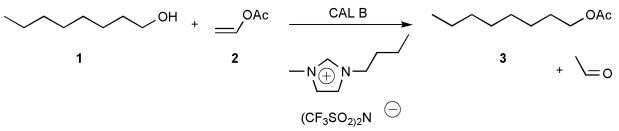
A new and environmentally benign protocol for enzymatic reactions in ionic liquids is described using supercritical CO_2 as the mobile phase; the products are obtained in solvent-free form and the enzyme/ionic liquid mixture can be recycled in batchwise or continuous flow operations.

Ionic liquids (ILs) are emerging as environmentally friendly solvents for a variety of reactions,[1] as they combine good and tuneable solubility properties with the absence of a measurable vapour pressure and excellent thermal and chemical stabilities (including air and H_2O tolerance in favourable cases). In the case of enzyme-catalysed reactions[2] the prospect of replacing volatile and toxic organic solvents is of considerable interest, and indeed, a number of studies concerning enzymatic reactions in ionic liquids have appeared recently.[3] In these reports additional benefits were described to arise from the use of ILs in certain cases, including enhanced stability, activity and stereoselectivity of the enzyme. However, although such reactions occur in the absence of conventional organic solvents, the latter are in fact usually added during work-up which defeats part of the original purpose. Here we describe a general solution to this problem based on the use of supercritical carbon dioxide ($scCO_2$) as the mobile phase to isolate the products in continuous or discontinuous process schemes.[4,5]

The acylation of octan-1-ol (**1**) by vinyl acetate (**2**) catalysed by lipase B from *Candida antarctica* (CAL B) was studied as a model reaction[2] to demonstrate the viability of the concept. The reactants **1** (8.3 mmol) and **2** (17 mmol) were added directly to the suspension of the lipase (10 mg) in 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonimide) [BMIM][BTA][1,6] (2 cm^3) as the ionic liquid.

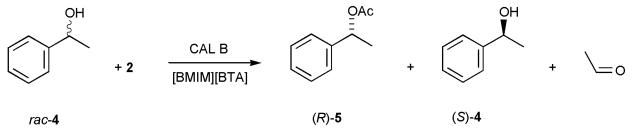


After a reaction time of 0.5 h, the autoclave was connected to a CO_2 compressor and extracted for 1 h at T = 39 °C and p = 9.5 MPascal. A mixture of **3**, acetaldehyde and excess **2** was collected in a cold trap from the gas stream upon venting. The yield of **3** amounted to 92% as shown by ^{1}H-NMR analysis. The process of reaction and extraction was repeated three times using the same ionic liquid–lipase mixture remaining in the reactor. Conversion was complete in all cases and no trace of **1** were observed in the isolated products. Product **3** was recovered with yields as high as 97, 98 and 98% in the three runs, respectively, demonstrating constant performance and no loss of enzyme activity.

Encouraged by these promising results, the development of a continuous process was envisaged using the apparatus shown in Fig. 1. The reactor (V = 10 cm^3) was charged with 4 ml of the IL and 40 mg of CAL B and flushed with CO_2 at T = 45 °C and p = 10.5 MPascal. The substrates **1/2** (1 : 2) were introduced to the CO_2 stream with an HPLC pump. After passing through the reactor, the CO_2 stream was depressurised and the organic components were collected in a cold trap. Table 1 documents the excellent performance of this continuous flow enzymatic reaction.

The first 10 min after substrate addition are required to saturate the IL with the organic components and consequently the amount of recovered material is fairly poor at this stage. After this induction period, the output is almost identical to the input and the system operates with a constant high activity corresponding to a space time yield of 0.1 kg per litre reactor volume and hour. A total yield of **3** of 93.9% is obtained after 24 h based on the amount of **1** introduced with the HPLC pump.

As a second reaction type, the lipase-catalysed kinetic resolution of 1-phenylethanol (**4**) was studied in the same IL



using $scCO_2$ in a batchwise process. Again, the activity of the enzyme was fully retained and the enantiomeric discrimination remained uniformly high in four consecutive runs (Table 2).

In summary, we have demonstrated a new and highly efficient methodology to carry out enzyme-catalysed reactions

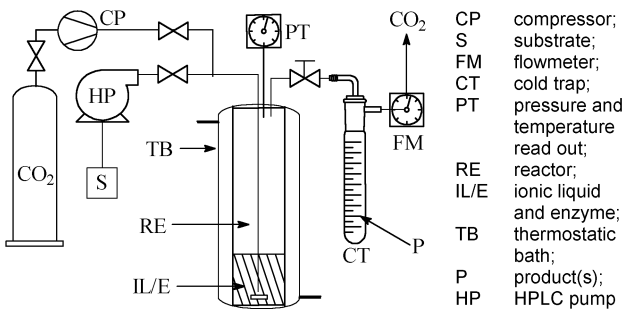


Fig. 1 Apparatus for continuous-flow biocatalysis in IL–$scCO_2$.

Table 1 Continuous-flow lipase-catalyzed acylation of octan-1-ol (**1**) in [BMIM][BTA] using $scCO_2$ as the mobile phase†

Cold trap	Changed after (h)	Total mass rcovered (g)	Amount of **3** (g)	Conv. (%)	Activity (μmol per min g)
1	0.7	0.16	0.12	89.1	428
2	2.3	2.22	1.62	94.4	2455
3	3.8	2.10	1.70	94,0	2754
4	6.3	3.40	2.72	92.6	2636
5	15.5	13.56	10.10	95.4	2790
6	21.1	8.56	6.21	96.4	2774
7	24.0	5.06	3.81	95.0	2929
Total/ average	24	35.07	26.29	95.1	2620

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Table 2 Batchwise lipase-catalysed kinetic resolution of 1-phenylethanol (*rac*-**4**) in [BMIM][BTA] using $scCO_2$ as the mobile phase‡

Cycle	Reaction time (h)	Total mass recovered (g)	Conv. of **4** (%)	*ee* (*S*)-**4** (%)	*ee* (*R*)-**5** (%)
0	1	1.66	44.6	97.9	99.5
1	0.8	1.57	43.1	83.0	98.7
2	1.2	1.50	46.7	95.2	99.4
3	12	1.58	51.4	99.6	98.6

in ionic liquids combined with isolation of the products using $scCO_2$ as the mobile phase in batchwise or continuous flow operation. The enzyme used here, a lipase, is completely stable under the reaction conditions and can be re-used many times without loss of activity. Given the large body of enzymatic reactions that can be expected to be compatible with the use of compressed CO_2,[7] we believe that this environmentally benign protocol for enzymatic catalysis has very general implications for the development of sustainable synthetic chemistry.[8]

Safety warning: Experiments using large amounts of compressed gases such as supercritical fluids are potentially hazardous and must only be carried out using appropriate equipment and safety precautions.[4]

Notes and references

† Procedure for continuous-flow operation: A suspension of *Candida antartica* Lipase B (CAL B, 40 mg) in [BMIM][BTA] (4 cm^3) was added to a window-equipped stainless-steel high pressure reactor equipped with a magnetic stirring bar, a thermocouple, a pressure sensor, and an inlet and outlet valve. The reactor was heated at 45 °C and CO_2 was flashed through the IL *via* a capillary at p = 10.5 MPascal using a compressor. The substrates **1** and **2** were introduced in a molar ratio of 1 : 2 into the CO_2 stream *via* a T-joint just before the inlet valve using a HPLC pump at a flow-rate of 2.25 $cm^3\ h^{-1}$. The outlet flow-rate was adjusted to approximately 11 $l\ h^{-1}$ (gas at ambient conditions) through a heated needle valve and the organic material was collected from the gas stream in a cold trap at −30 °C. The cold trap was changed after the time intervals given in Table 1 and the contents analysed by GC and ^{1}H-NMR spectroscopy.‡ Procedure for batchwise lipase-catalysed kinetic resolution: A suspension of *Candida antartica* Lipase B (CAL B, 100 mg) in [BMIM][BTA] (2 cm^3) was added in the same reactor as described above. The substrates *rac*-(**4**) (10 mmol) and **2** (5.5 mmol) were then introduced. The reaction mixture was stirred at rt for the time given in Table 2. The products were extracted by flashing CO_2 *via* a capillary through the IL at T = 40 °C and p = 11 MPascal. The outlet flow-rate was adjusted to approximately 30 $l\ h^{-1}$ (gas at ambient conditions) through a heated needle valve and the organic material was collected from the gas stream in a cold trap at 30 °C. After 1 h, the reactor was depressurised to ambient pressure and a new charge of substrates was introduced for the next cycle. The recovered products were analysed by ^{1}H-NMR spectroscopy (conversion) and GC (*ee*).

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6 This ionic liquid is commercially available from Solvent Innovation GmbH, Köln, (http://www.solvent-innovation.com).

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