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Liquid poly(ethylene glycol) and supercritical carbon dioxide as a biphasic solvent system for lipase-catalyzed esterification

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The biphasic solvent system composed of poly(ethylene glycol) (PEG) and supercritical carbon dioxide ($scCO_2$) is ideally suited for the lipase-catalyzed acylation of alcohols; batch or continuous flow acylations are possible, $scCO_2$ being used to extract the products.

Lipase-catalyzed acylation of achiral and chiral alcohols in organic solvents[1] is a standard procedure in synthetic organic chemistry, kinetic resolution of racemates and desymmetrization of meso-type compounds often being the focus of interest.[2] In order to replace toxic organic solvents by environmentally benign media, these reactions have been carried out in supercritical carbon dioxide ($scCO_2$)[3] and in ionic liquids (ILs).[4,5] However, in many cases organic solvents were in fact used in the work-up in order to isolate the products, which defeats the original purpose. The combination of an IL as the reaction medium and $scCO_2$ as the extractive agent for product isolation in a biphasic system led to further progress,[6] including selective extraction of chiral esters and alcohols in kinetic resolutions.[7] However, the disadvantages of ILs are the relatively high price and the problem of their final disposal following the gradual accumulation of side or decomposition products. As an alternative to ILs, Jessop recently demonstrated that the combination of poly(ethylene glycol) (PEG) and $scCO_2$ constitutes a cheap and benign biphasic solvent system in homogeneous transition-metal catalysis.[8] We now report that this biphasic system is also well suited for biocatalytic transformations, specifically in lipase-catalyzed processes. PEG has been used previously as a stabilizing additive in lipase-catalyzed reactions,[9] but not as a solvent.

As the model reaction we chose the acylation of 2-phenylethanol by vinyl acetate, catalyzed by commercially available lipase B from *Candida antarctica* (CAL B) (Fig. 1). In these and later experiments PEG having a molecular weight of 1500 was used, because this particular form is a liquid under the reaction conditions (50 °C/150 bar) and (like ILs) essentially insoluble in $scCO_2$.

In an exploratory experiment using this biphasic system the acylation of 2-phenylethanol (1 part) by vinyl acetate (1.5 parts) was terminated after 2 h and the product was extracted using $scCO_2$.† This afforded full conversion and the ester in 74% yield, in spite of the fact that PEG 1500 itself contains two terminal alcohol moieties which can be acylated. However, this process is considerably slower, requiring longer reaction times. For example, the use of vinyl acetate, propionate, butyrate or laureate at 50 °C/100 bar/48 h results in 85–92% yields of the corresponding PEG-diesters, which themselves can serve as solvents in biphasic systems (PEG-diester/$scCO_2$).[10]

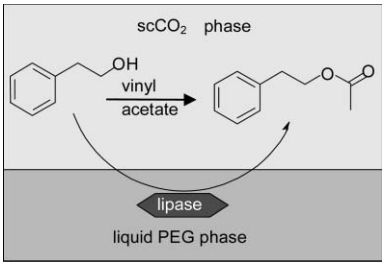


Fig. 1 General scheme for biphasic lipase-catalysis.

We then performed the same reaction in a continuous process using a reaction/extraction apparatus previously described for our IL/$scCO_2$ system.[6a] Fig. 2 shows that excellent conversion is maintained for at least 25 h. After a short induction period, the amount of product is almost identical to the amount of substrate and the system operates with a constant high activity of 5700 μmol min^{-1} g^{-1} corresponding to a space time yield of 0.1 kg l^{-1} h^{-1}. Since an excess of vinyl acetate was used, all of the PEG 1500 was acylated as shown by ESI-MS and NMR analyses.[10]

In further experiments the kinetic resolution of *rac*-1-phenylethanol was carried out using the biphasic system PEG 1500/$scCO_2$. In a batch reaction an ideal conversion of 50.4% was achieved, affording (*R*)-**3** (ee = 98.1%) and (*S*)-**1** (ee = 99.7%). In order to test recyclability, the reaction was repeated using the lipase-containing PEG after $scCO_2$ extraction (55 °C/140 bar) of the products, a process which was performed a total of 11 times. Table 1 shows the excellent performance of the system.§

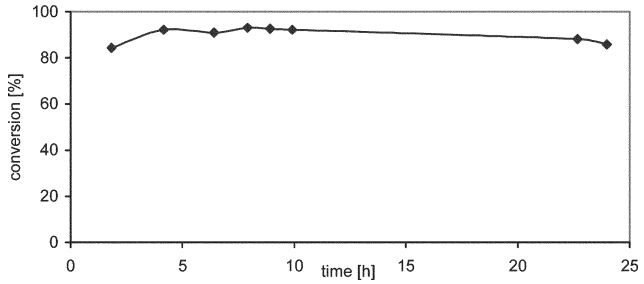
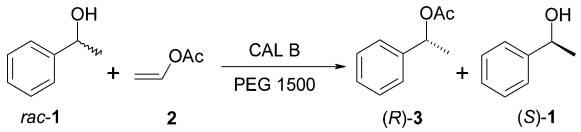


Fig. 2 Lipase-catalyzed acylation of 2-phenylethanol in a continuous flow apparatus.‡

Table 1 Kinetic resolution of the lipase-catalyzed acylation of 1-phenylethanol in a biphasic system (PEG/$scCO_2$)



rac-**1** **2** (*R*)-**3** (*S*)-**1**

Cycle number	Reaction time/h	Amount of **1** + **3**/g	ee of (*R*)-**3** (%)	ee of (*S*)-**1** (%)	Conversion (%)
1[a]	5.0	0.47	98.1	99.7	50.4
2	14.0	0.55	95.8	98.3	50.6
3	3.0	0.39	98.7	98.8	50.0
4	3.0	0.64	97.9	91.9	48.4
5	13.0	0.62	96.6	>99.9	50.9
6	3.0	0.75	97.5	93.6	49.0
7	3.0	0.68	97.1	95.3	49.5
8	17.0	0.95	96.7	99.8	50.8
9	3.0	0.80	98.9	87.5	46.9
10	39.0	0.91	93.6	>99.9	51.6
11	3.0	0.88	97.4	97.9	47.9

[a] Additional vinyl acetate (2.5 mmol) was added.

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Finally, a preliminary experiment was carried out directed towards testing potential compound selectivity in the extractive step ((*R*)-**3** *vs.* (*S*)-**1**). For this purpose the kinetic resolution of *rac*-1-phenylethanol was repeated, but the conditions of $scCO_2$-mediated extraction were changed (50 °C/80 bar corresponding to lower CO_2 density). Under these conditions a selectivity of 73% in favor of the (*R*)-ester was observed, which is considerably higher than in the case of the IL/$scCO_2$ system under similar conditions (56%) reported previously.[7] Upon raising the pressure to 190 bar all of the products (ester and alcohol) were extracted. Higher degrees of extraction selectivity are likely in optimized versions using acylating agents such as vinyl laureate, as previously shown in the IL/$scCO_2$ system.[7] It is also possible to perform kinetic resolution of chiral acids in PEG/$scCO_2$, the PEG serving as a polymeric tag.[10]

In summary, we have demonstrated for the first time that enzyme catalysis is possible in a biphasic system composed of poly(ethylene glycol)/$scCO_2$. In order to illustrate the principle, a lipase was suspended in the PEG part of the PEG/$scCO_2$ biphasic system in which the acylation of alcohols occurs, followed by extraction of the products by $scCO_2$. Batch and continuous flow processes are possible, even in the case of kinetic resolution of chiral alcohols. The advantages of this process relative to other biphasic systems[6,7,11] include the low cost of PEG and its simple waste disposal if needed. It remains to be seen if other enzymes can also be used in this system.

Notes and references

† **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.[3a]

‡ Procedure for continuous-flow operation: *Candida antartica* lipase B (CAL B, lyophilyzed, obtained from Roche as Chirazyme L-2, lyo, 10 mg) and PEG 1500 (2.5 g) were added to a window-equipped stainless-steel high pressure reactor (V = 10 cm^3) equipped with a magnetic stirring bar, a thermocouple, a pressure sensor, and an inlet and outlet valve. The reactor was heated to at 50 °C and CO_2 was flashed through the IL *via* a capillary at p = 150 bar using a compressor. The substrates 2-phenylethanol 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 1.5 cm^{-3} h^{-1}. The outlet flow-rate was adjusted to approximately 6 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 Fig. 2 and the contents analyzed by GC and ^{1}H NMR spectroscopy.

§ Procedure for batch-wise lipase-catalyzed kinetic resolution: *Candida antartica* lipase B (CAL B, 100 mg) and PEG 1500 (3 g) were added in the same reactor as described above. The reactor was heated to 50 °C and CO_2 (p = 60 bar) was introduced into the autoclave using a compressor. The substrates *rac*-(**1**) and **2** were then introduced as a solution (molar ratio of 1:1.5) *via* a HPLC pump (1.5 ml per batch). The reaction mixture was stirred for the time given in Table 1. The products were extracted by flashing CO_2 *via* a capillary through the PEG at T = 50 °C and p = 150 bar. The outlet flow-rate was adjusted to approximately 20 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 60 bar and a new charge of substrates was introduced for the next cycle. The recovered products were analyzed by ^{1}H NMR spectroscopy (conversion) and GC (ee).

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