SUPERCRITICAL CARBON DIOXIDE/IONIC LIQUID SYSTEM FOR SEPARATION OF BIOTRANSFORMATION PRODUCTS

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INTRODUCTION

The need of technologies that allow the separation of enantiomers is increasing. Enzymes can distinguish substances on the molecular level very efficiently. They can catalyse a biotransformation in which one of the enantiomers of a racemic mixture is preferentially modified. As a result, the reaction products differ in their physical behaviour from the substrates. This difference can be adjusted to the needs of subsequent separation processes by choosing appropriate reaction schemes.

There are many reports in the literature on the resolution of secondary alcohols in ionic liquids (ILs).[1-3] The use of enol-esters as acylating agents has the advantage of being irreversible but the aldehyde formed can decrease enzyme activity. To overcome this problem, succinic anhydride as an acylating agent might be applied.[1] It has since been used for the enzymatic resolution of a wide range of substrates.[3, 4] Recently Rasalkar et al. have shown that the resolution of aryl alcohols with succinic anhydride is feasible in 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4mim][PF_6]$) using an immobilised lipase.[2] We decided to apply the same approach for the resolution of the model substrate, (R,S)-2-octanol, catalysed by Novozym 435.

The enzymatic reaction was part of an overall scheme involving the use of environmentally friendly supercritical CO_2 ($scCO_2$) for post-reaction separation. $ScCO_2$ can dissolve significantly in ionic liquids, but the reverse is not true. VLE measurements are a helpful tool for quantifying the behaviour of these systems.

The partitioning between an IL and CO_2, of 2-octanol, hemiester and a diester produced in the course of the acylation of (R,S)-2-octanol with succinic anhydride based on the VLE measurements extraction has been performed. These experiments were followed by the examinations of ternary systems included all possible combination of all five considered compounds.

MATERIALS AND METHODS

Materials

Immobilised *Candida antarctica* lipase B (Novozym 435) was a gift from Novo Nordisk Bioindustrial, Spain. (R,S)-2-octanol (> 97 % purity), acetonitrile (> 99 % purity), Hydranal Coulomat A and C Karl-Fischer reagents were from Riedel de Häen. (R,S)-2-butanol (> 98 % purity) was from Fluka. Succinic anhydride (> 99 % purity), tridecane (> 99 % purity) and tetrahydrofuran (> 99 % purity) freshly distilled from sodium/benzophenone, were from Aldrich. Di-isopropyl ether (> 98 % purity) was supplied by Merck. Ionic liquids: ECOENG^{TM}21M (98 % purity), ECOENG^{TM}11O (99 % purity) and ECOENG^{TM}212 were gifts from Solvent Innovation GmbH, Cologne, Germany. Ethyl-(2-hydroxyethyl)-dimethylammonium bis(trifluoromethylsulfonyl)imide, (purity: ≥ 99 %) was synthesised by us. $[Bmim][BF_4]$ (≥ 98 % purity), $[bmim][NTf_2]$ (≥ 98 % purity), $[bmim][PF_6]$ (≥ 98 % purity), $[bmim][N(CN)_2]$ (≥ 98 % purity), $[aliquat][N(CN)_2]$ (≥ 98 % purity), $[omim][PF_6]$ (≥ 98 % purity) and $[omim][N(CN)_2]$ (≥ 98 % purity) were supplied by/gifts from Solchemar Portugal, Lisbon, Portugal. To reduce the content of volatile compounds to negligible values, vacuum (0.1 Pa) and moderate temperature (50 °C) were applied to the IL samples for several days always immediately prior to their use. Carbon dioxide was supplied by Air Liquide, with a stated purity of 99.998 mol%.

N_2 for GC was supplied by Linde Gas AG and has purity 99.999%. Hemiester and diester were obtained by us according to the procedure described in literature.[3]

Methods

Reactions were performed in plastic vials (reaction volume up to 1 mL) placed in an orbital shaker (400 rpm, 35 °C).Solvent (750 μl) was added to succinic anhydride (800 mM), followed by addition of the amount of water required to reach $a_w = 0.1$, enzyme (40 g L^{-1}) and, finally, (R,S)-alcohol (400 mM) to start the reaction. Tridecane (2.91 mM) in acetonitrile was used as external standard for GC analysis. Scales of a_w vs. water concentration were built by equilibrating solvents with saturated salt solutions or with water at 25 °C for a number of days, to achieve values 0.22 (potassium acetate) and 0.75 (sodium chloride), taken from literature.[5] Water concentration was measured by Karl-Fisher titration.

Apparatus for VLE measurements was previously described in the literature.[6] The main part of the phase equilibrium apparatus is a sapphire cylindrical tube placed inside an air bath and initially connected to the vacuum pump, to facilitate loading of sample. Stirring of the contents was performed and temperature and pressure were measured. In each experiment, the time allowed for phases to reach equilibrium was about 2 hours. A sample from either the top or the bottom phase was taken through a HPLC valve, into a sample loop. This was followed by an expansion of the loop contents into calibrated volume (474.9 mL). Measurement of pressure in this volume allowed the calculation of the CO_2 quantity in the sample using equation of state for CO_2 presented in literature.[7]

Extraction apparatus was previously described by Serbanovic et al.[8] A sapphire-windowed cell is the core of this installation. The reaction mixture after the enzymatic reaction was introduced into cell placed into a water-bath. Temperature was controlled (35 °C) and post-reaction mixture was stirred. The pressure in the system was controlled with accuracy 0.1 %. CO_2 was initially pumped into the cell and the pressurised cell was left for 1 hour for mixing. Afterwards, by careful opening venting valves, the extract started to be collected in two glass vessels placed in a liquid nitrogen bath. The drop of pressure was continuously compensated by the introduction of fresh CO_2 from pressure generator. The samples collected in the glass vessels were analysed by GC.

Experiments were followed by GC analysis performed with a VARIAN CHROMPACK CP-3800 gas chromatograph equipped with a 30 m x 0.32 mm i.d. fused silica capillary column, coated with a 0.25 μm thickness film of 5 % phenyl groups dissolved in dimethylpolysiloxane polymer, from Chrompack Company. Oven temperature program: 50 °C hold for 1 min, 50 - 240 °C ramp at 5 °C min^{-1}. Injection temperature: 250 °C. Flame ionisation detection (FID) temperature: 275 °C. Carrier gas: helium at 10.5 psi. Split ratio: 1:20. The data given are the average of at least two measurements. Tridecane (2.91 mM) in acetonitrile was used as external standard for GC analysis.

RESULTS

Enzymatic reactions

The formation of an acidic hemiester, easy to separate from the reaction mixture, was one of the main reasons for using succinic anhydride as an acylating agent in the reports available in the literature. In the present case, this reaction pathway can be represented as shown (Figure 1).

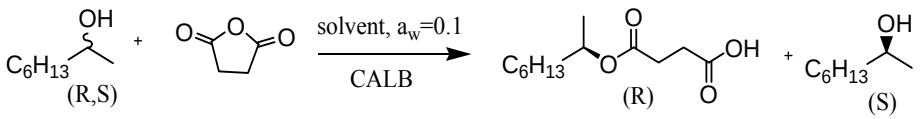

Figure 1. Formation of an hemiester from (R,S)-2-octanol and succinic anhydride.

Reactions were performed in eleven different imidazolium and ammonium ionic liquids and also in the organic solvents such as hexane, acetonitrile, di-isopropyl ether and tetrahydrofuran.

Novozym 435 was highly enantioselective (ee_p > 0.99) in the acylation of (R,S)-2-octanol with succinic anhydride, except in the less polar solvents. It was in the latter solvents that the equilibrium conversion of (R)-2-octanol was highest (Figure 2).

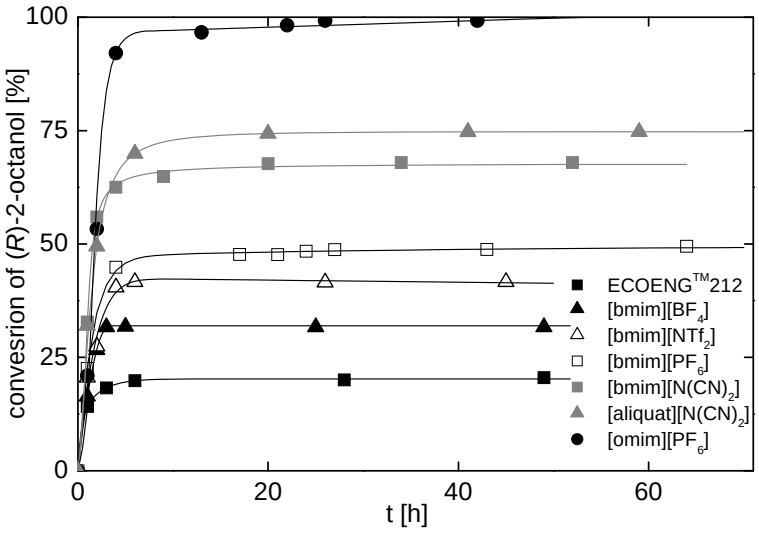

Figure 2. Reaction progress for (R)-2-octanol in some of the solvents tested, at 35 °C and a_w = 0.1.

As with the equilibrium conversion, amounts of hemiester and diester formed also depended on the polarity of the solvent (Figure 3). In the more polar solvents, only the diester was obtained. In all the other solvents, the reaction yielded a mixture of hemiester and diester.

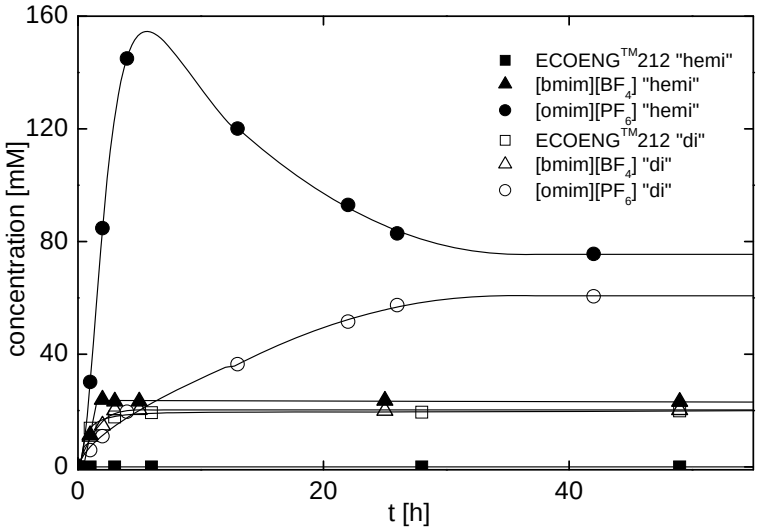

Figure 3. Reaction progress for the (R)-hemiester and the (R,R)-diester in some of the solvents tested, at 35 °C and a_w = 0.1.

A more detailed analysis of the reaction profiles led to the realisation of the important role of succinic acid whose low solubility and hence partial precipitation in the less polar solvents could be a driving force towards the conversion of the hemiester into (diester + succinic acid). Indeed, we found that at a_w = 0.1, the hemiester yielded stoichiometric amounts of diester and succinic acid. Succinic acid, formed via the hydrolysis of succinic anhydride, was also shown to react readily with 2-octanol, yielding both the hemiester and the diester. The overall process should be a combination of several reaction pathways (Figure 4).

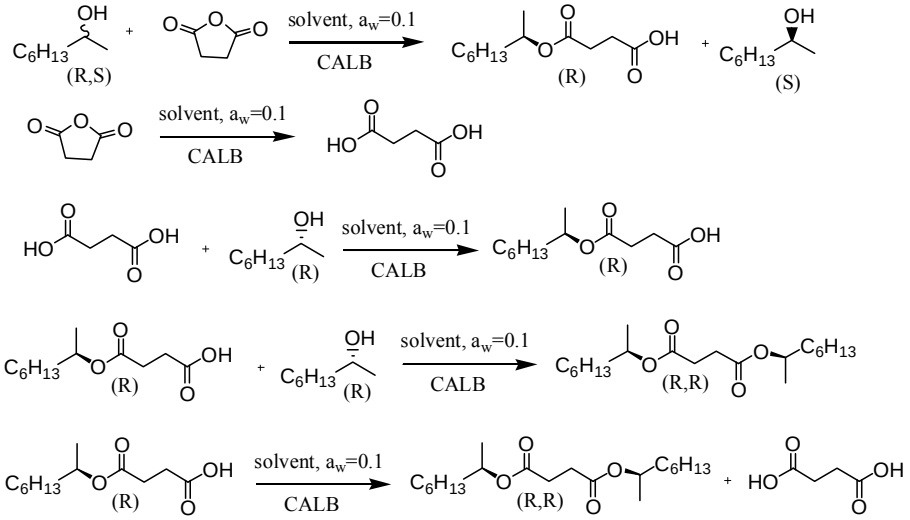

Figure 4. Reaction pathways leading to the formation of both an hemiester and a diester.

Separation study

Vapour-liquid equilibrium measurements were performed for ternary systems containing ionic liquids ([omim][PF_6], or [omim][$N(CN)_2$] + CO_2 + an alcohol, or hemiester, or diester). The concentrations of solutes in ionic liquids were selected according to typical concentrations of these solutes during the enzymatic reactions. Vapour-liquid equilibrium measurements were performed at four different pressures and at a temperature of 35°C. The water activity, of samples used for experiments, was controlled and equals 0.1, the value used in the enzyme-catalysed reactions.

The concentration of IL in the vapour phase was not detectable at the experimental conditions. Moreover the solubility of 2-octanol in the CO_2-rich phase is more than one order of magnitude higher than solubilities of hemiester or diester. This means that 2-octanol can be extracted easily from the mixture of enzymatic reaction products. The composition of the vapour phase, varies in a very narrow range for different initial concentrations of solute in ionic liquid, however, the solubility of solute in vapour phase increases with an increase in the initial concentration of solute.

Vapour-liquid equilibrium experiments were also carried out on five component mixtures containing all three solutes (2-octanol, hemiester and diester). The initial concentrations of solutes in the ionic liquid samples, before introduction of carbon dioxide, were identical to the concentrations of solutes from enzymatic reaction. VLE data allowed the calculation of partition coefficients (Figure 5) and separation factors.

Separation factors (α) are defined as ratios of partition factors for different solutes. Separation factors calculated from the VLE experiments showed that α for 2-octanol/hemiester, and 2-octanol/diester are dependent on CO_2 pressures only up to moderate pressure, when for higher pressures they remain constant. Separation factors for 2-octanol/hemiester in both ionic liquids are very similar and equal to 11.5.

An extraction study was carried out on a mixture resulting from lipase-catalysed reaction carried out in [omim][PF_6]. This sample was selected due to the higher solubility of CO_2 in this ionic liquid

and to the higher ee_s achieved. The initial partial mole fraction of 2-octanol was 0.569 when after extraction increased to 0.981, with high enantiomeric excess (ee_s = 98.42 %).

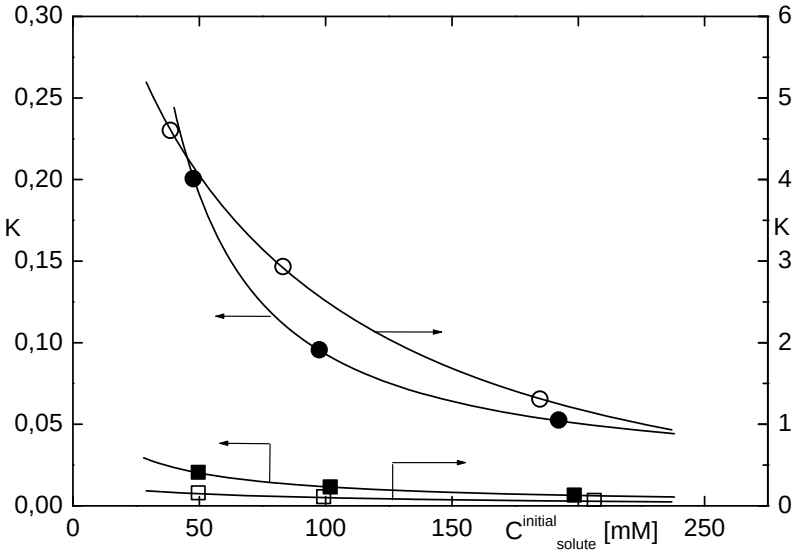

Figure 5. Partition coefficients as a function of initial concentrations of 2-octanol (black-white symbols, right scale) and hemiester (black symbols, left scale) in [omim][PF_6] before adding CO_2 at □, ■ - 5 MPa and ○,● - 11MPa.

The extraction performed at 11 MPa and at 35 °C allowed to recover > 99.99 %mol of 2-octanol, with low co-extraction of other products (3.69 %mol of hemiester and 0.73 %mol of diester). Complete recovery of 2-octanol was achieved for a mole ratio of (CO_2 flow / initial 2-octanol) ≈ 1000 (Figure 6). The other two solutes are very difficult to extract, even at 3-5 times higher molar ratios of CO_2 to hemiester, or CO_2 to diester.

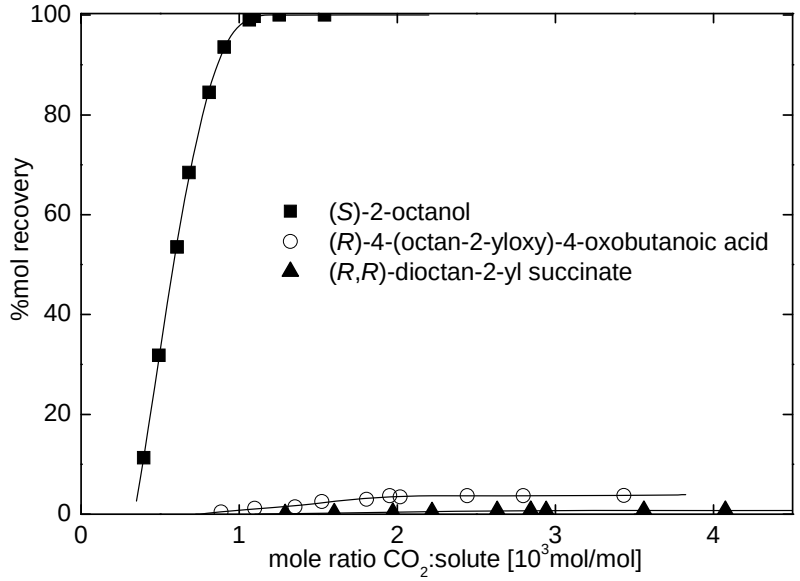

Figure 6. Extraction of biotransformation products.

CONCLUSIONS

Novozym 435 used in the acylation of (R,S)-2-octanol with succinic anhydride was highly enantioselective (ee_p > 0.99). In most cases, both ester (hemiester and diester) were produced. When the reaction yielded one ester only, as in the case of the more polar ILs, it was the diester. The explanation of these results based on the differences in solvation of the intervening species seems to be truly possible. However, lack of thermodynamics data for quantifying solvation effects prevents a full elucidation of the correlation obtained between solvent polarity and additionally the equilibrium conversion and process selectivity.

Moreover, we derived the vapour-liquid equilibrium data for investigated system. VLE data was used to calculate separation factors for analysed solutes. Later on, the extraction was performed and 99.99 %mol of unreacted (S)-2-octanol with minimal co-extraction of other solutes was recovered.

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