

# Lipase-Catalyzed Resolution of (*R,S*)-1-Phenylethanol and Phase Equilibrium Measurements of (*R,S*)-1-Phenylethanol and Vinyl Acetate in Supercritical Carbon Dioxide

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Ionic liquids represent an exciting new class of reaction solvents for catalysis, which have been used successfully for enzyme-catalyzed reactions. To ascertain the influence of ionic liquids on the immobilized lipase B activity, three different ionic liquids, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium hexafluorophosphate, and 1-ethyl-3-methylimidazolium bis[trifluoromethyl)sulfonyl]amide, were investigated as potential media for transesterification of (*R,S*)-1-phenylethanol with vinyl acetate. Reactions were performed in a batch stirred-tank reactor at atmospheric pressure. The highest conversion of (*R*)-1-phenylethanol to (*R*)-1-phenylethyl acetate (99.3 %) was obtained when ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate was used as a solvent after 5 hours of reaction performance.

To design supercritical carbon dioxide/substrate or supercritical carbon dioxide/product separation processes, one must first characterize the phase behaviour of the solute in the supercritical carbon dioxide. Therefore, phase equilibrium measurements for the (*R,S*)-1-phenylethanol/CO<sub>2</sub> and vinyl acetate/CO<sub>2</sub> systems were performed, as well. Bubble points of binary mixtures of (*R,S*)-1-phenylethanol/CO<sub>2</sub> and vinyl acetate/CO<sub>2</sub> were determined at 40 °C, 60 °C, 80 °C, 100 °C, and 120 °C and pressures up to 23.74 MPa for (*R,S*)-1-phenylethanol/CO<sub>2</sub> systems and up to 12.22 MPa for vinyl acetate/CO<sub>2</sub> systems.

## INTRODUCTION

Ionic liquids (ILs), a novel class of green solvents, have recently emerged as interesting non-aqueous reaction media for enzymatic reactions [1]. They are organic salts made of cations, which are generally bulk, organic with low symmetry, e.g. derivatives of imidazolium, pyridinium, pyrrolidinium, ammonium, sulfonium, phosphonium, ..., and anions, which are either organic or inorganic, such as tetrafluoroborate, hexafluorophosphate, bis[trifluoromethyl)sulfonyl]amide, nitrate, acetate, trifluoroacetate, methylsulfate ... The possible choices of cation and anion that will result in the formation of ILs with different physico-chemical properties are numerous [2]. The most important physical property of ILs is their negligibly small vapor pressure. As a result, ILs do not evaporate, even when exposed to vacuum and are easy to contain [3]. A diverse range of organic, inorganic and organometallic compounds [3] is soluble in ILs. The solubility of gases such as oxygen, carbon dioxide, carbon monoxide and hydrogen [4] is also good. Furthermore, ILs tend to have good thermal stability and can be liquid over a range of 300 °C, thereby making it feasible to carry out reactions requiring high temperature conveniently in ILs [4]. Their unique properties have

made them an environmentally attractive alternative to conventional organic solvents. ILs are being used in a wide variety of applications. The applications areas of ILs can be expressed as electrolytes, lubricants, surfactants, as solvents for organic and organometallic synthesis, for extractions and separation, for biocatalysis, and also in analytics [5].

Like ILs, SC CO<sub>2</sub> has several advantages over organic solvents since it is less expensive than conventional organic solvents and highly available, is inert, non-flammable, non-toxic, has near-ambient critical temperature (31.1 °C) and moderate critical pressure (7.3 MPa). Because of low critical temperature, SC CO<sub>2</sub> is suitable for processing most biochemicals [6].

In present research, lipase-catalyzed transesterification of (*R,S*)-1-phenylethanol was performed in a batch stirred-tank reactor at atmospheric pressure. For the transesterification, immobilized lipase B from *Candida antarctica* was used as biocatalyst, vinyl acetate as acyl donor, and three different ILs (1-butyl-3-methylimidazolium tetrafluoroborate [bmim][BF<sub>4</sub>], 1-butyl-3-methylimidazolium hexafluorophosphate [bmim][PF<sub>6</sub>], and 1-ethyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]amide [emim][NTf<sub>2</sub>]) were used as reaction media. The highest conversion of 99.3 % after 5 hours of reaction performance was achieved in the case when hydrophilic ionic liquid [bmim][BF<sub>4</sub>] was used as a solvent. Therefore, the influence of reaction parameters on lipase-catalyzed transesterification of (*R,S*)-1-phenylethanol was studied in [bmim][BF<sub>4</sub>].

Knowledge of phase equilibrium data of binary mixtures of reactants and CO<sub>2</sub> at high pressure is important, because a good solubility of the substances in a wide range of pressure and temperature is desired so that the parameters for the reaction can be varied [2]. Therefore, phase equilibrium measurements for the (*R,S*)-1-phenylethanol/CO<sub>2</sub> and vinyl acetate/CO<sub>2</sub> systems were performed, as well. Bubble points of binary mixtures of (*R,S*)-1-phenylethanol/CO<sub>2</sub> and vinyl acetate/CO<sub>2</sub> were determined at 40 °C, 60 °C, 80 °C, 100 °C, and 120 °C and pressures up to 23.74 MPa for (*R,S*)-1-phenylethanol/CO<sub>2</sub> systems and up to 12.22 MPa for vinyl acetate/CO<sub>2</sub> systems.

## MATERIALS AND METHODS

### Enzymes and chemicals

Immobilized lipase B from *Candida antarctica* (Novozym 435) was kindly donated from Novo Nordisk AS (Copenhagen, Denmark). (*R,S*)-1-Phenylethanol (≥98 %) and vinyl acetate (≥99 %), were supplied from Fluka (Buchs, Switzerland). Decane – Reagent Plus<sup>®</sup> (≥99 %) was provided from Aldrich Chemical Co. (Diesenhofen, Germany). *n*-Heptane (≥99 %) was purchased from Merck (Darmstadt, Germany). Carbon dioxide 4.5 (purity 99.995 vol. %) was supplied from Messer MG (Ruše, Slovenia) and helium 6.0 from Linde plin (Celje, Slovenia).

### Enzyme-catalyzed transesterification of (*R,S*)-1-phenylethanol, performed in batch stirred-tank reactor at atmospheric pressure

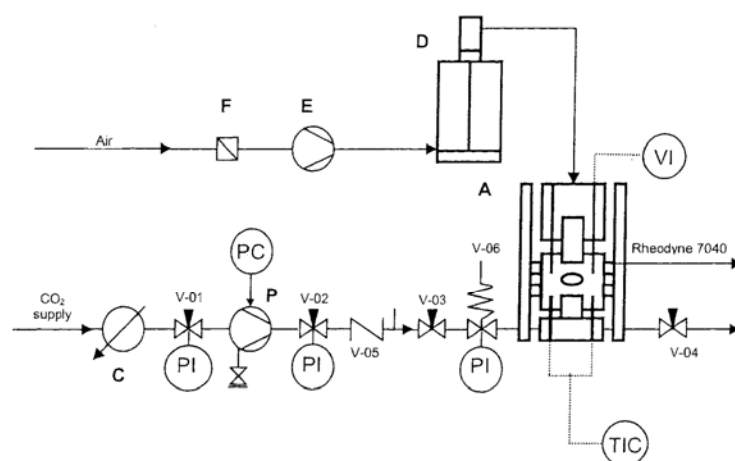
Transesterification of (*R,S*)-1-phenylethanol with vinyl acetate, catalyzed with immobilized lipase B from *Candida antarctica*, was performed in a batch stirred-tank reactor at atmospheric pressure. (*R,S*)-1-Phenylethanol and vinyl acetate were dissolved in ionic liquid, which was used as a reaction medium. The reactor filled up with substrates, was immersed in a water bath, heated to the desired operating temperature and stirred with a magnetic stirrer. The reaction was started by addition of the immobilized lipase. Aliquots of the sample were periodically withdrawn from the reaction mixture at fixed time intervals, suspended in decane (internal standard, IS) solution in *n*-heptane and 1 μL of the resulting solution was analyzed by gas chromatograph.

## Gas chromatography analysis

Enantiomers content during the reaction time course was monitored using an HP 5890 series A gas chromatograph equipped with a flame-ionisation detector (FID), using helium as carrier gas and a  $\beta$ -cyclodextrin capillary column ( $\beta$ -DEX 120) with the dimension length  $\times$  I.D. 30 m  $\times$  0.25 mm with 0.25  $\mu$ m film thickness (Supelco, Schnelldorf, Germany), at following temperature program: 100  $^{\circ}$ C hold for 5 min, rise up to 120  $^{\circ}$ C at rate of 5  $^{\circ}$ C/min and hold for 11 min; the temperature of injector and detector were maintained at 220  $^{\circ}$ C and 250  $^{\circ}$ C, respectively.

## Phase equilibrium measurements

Bubble points of binary mixtures of (*R,S*)-1-phenylethanol/ $\text{CO}_2$  and vinyl acetate/ $\text{CO}_2$  were determined at 40  $^{\circ}$ C, 60  $^{\circ}$ C, 80  $^{\circ}$ C, 100  $^{\circ}$ C, and 120  $^{\circ}$ C in the variable-volume view cell apparatus (Figure 1). The cell was loaded with known amount of selected compound and afterwards the  $\text{CO}_2$  from a gas cylinder was cooled to a liquid state and compressed into the cell by a high-pressure pump up to the desired pressure. The binary mixture was heated and mixed with a blade-turbine stirrer until the operating temperature was reached. Bubble points were measured at constant temperature by decreasing the volume of the cell and consecutively the system pressure was increased. The volume of the cell was decreased until homogeneous phase was observed. After measurement, the pressure was decreased via increasing the volume. Finally, the volume of  $\text{CO}_2$  was measured by volumetric method.



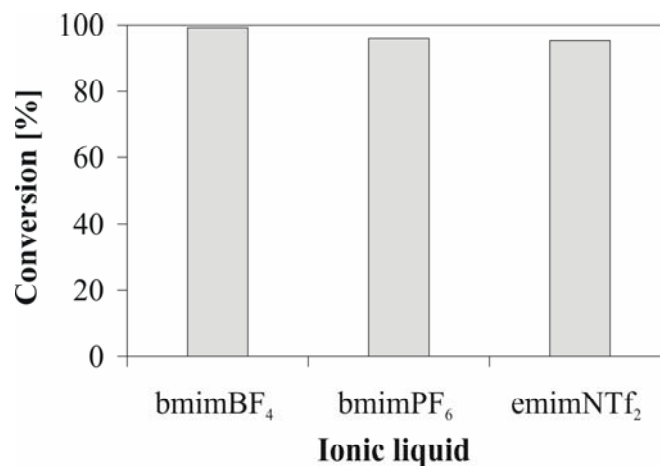
**Figure 1:** Sketch of the variable-volume view cell apparatus: A – variable-volume view cell; C – cooler, D – hydraulic oil system; E – air compressor; F – filter, P – high-pressure membrane pump, V01-04 – high-pressure needle valves; V05 – one-way valve; V06 – safety valve; PI – pressure indicator; VI – voltage indicator; PC – pressure controller; TIC – temperature indicator controller.

## RESULTS AND DISCUSSION

### Screening of ionic liquids for transesterification of (*R,S*)-1-phenylethanol with vinyl acetate

In biotechnology ILs can be used for enzymatic reactions as co-solvents in aqueous phase, as pure solvents and as two-phase systems together with other solvents [7]. Some potential advantages of enzymatic reactions in ILs, such as high activity, thermal and operational

stability of biocatalysts, and good enantioselectivity of biotransformation in comparison with conventional media have been reported [8]. Therefore, screening of [bmim][BF<sub>4</sub>], [bmim][PF<sub>6</sub>], and [emim][NTf<sub>2</sub>] for transesterification of (*R,S*)-1-phenylethanol with vinyl acetate was studied (Figure 2). Reactions were performed in batch stirred-tank reactor at 40 °C and atmospheric pressure.



**Figure 2:** Influence of three different ILs on transesterification of (*R,S*)-1-phenylethanol with vinyl acetate after 5 hours of reaction performance.

The assayed ILs, [bmim][BF<sub>4</sub>], [bmim][PF<sub>6</sub>] and [emim][NTf<sub>2</sub>], proved to be adequate reaction media for lipase-catalyzed acylation of (*R,S*)-1-phenylethanol with vinyl acetate. Obtained conversions for reactions performed in [bmim][BF<sub>4</sub>], [bmim][PF<sub>6</sub>] and in [emim][NTf<sub>2</sub>] were 99.3 %, 95.9 % and 95.3 %, respectively. There was almost no difference in conversion obtained after 5 hours when reaction was performed in [bmim][PF<sub>6</sub>] and [emim][NTf<sub>2</sub>]. A slightly increase in conversion was observed when the reaction was carried out in [bmim][BF<sub>4</sub>].

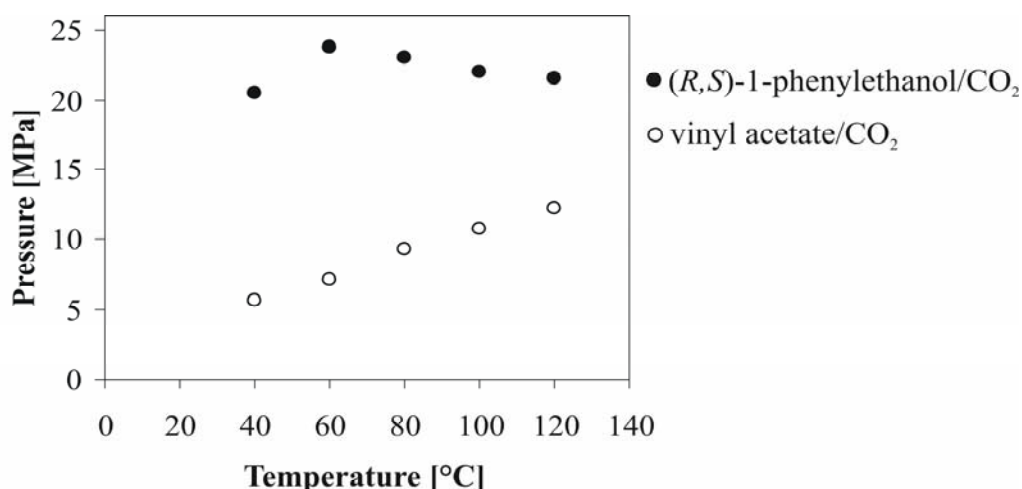
ILs are much more viscous than conventional organic solvents. Viscosity of the ILs can control the enzyme activity by affecting the mass-transfer limitations and consecutively, a lower reaction rate would be expected in an ionic liquid with higher viscosity [9]. When reaction was performed in [bmim][BF<sub>4</sub>] and [bmim][PF<sub>6</sub>] with viscosities of 154 cP and 330 cP, respectively, a reduction in the enzyme activity could correspond to an increase in the viscosity of the ILs. Furthermore, according to visual observations, [bmim][PF<sub>6</sub>] formed a layer around the enzyme. Therefore, the contact between substrate and the active site of the lipase was limited, what resulted in lower conversion of (*R*)-1-phenylethanol to (*R*)-1-phenylethyl acetate.

In the researches published to date, ILs based on [NTf<sub>2</sub>] anion have been considered as a suitable reaction media for kinetic resolution of (*R,S*)-1-phenylethanol [1, 10, 11]. In the present work, higher suitability of IL [bmim][BF<sub>4</sub>] for transesterification of (*R,S*)-1-phenylethanol with vinyl acetate was demonstrated. Therefore, all further studies were done using [bmim][BF<sub>4</sub>] as a reaction media.

### Phase equilibrium measurements

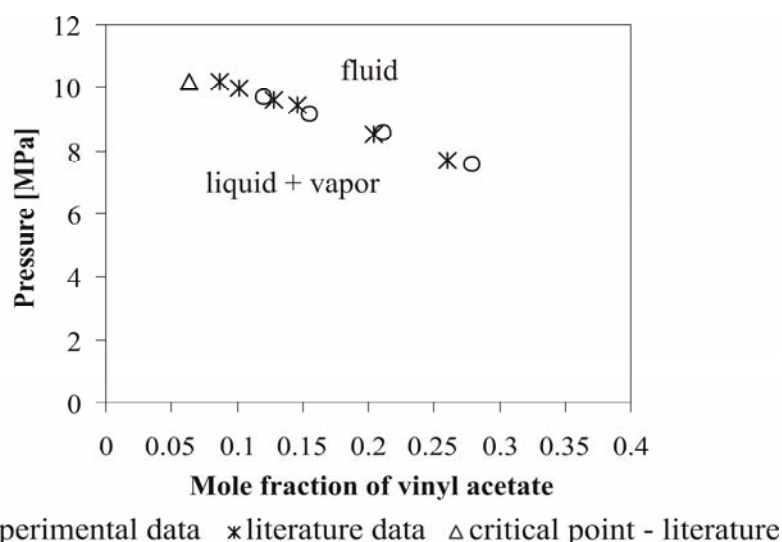
The phase behaviour data of binary mixtures of reactants and CO<sub>2</sub> at high pressure are required for design of reaction and subsequent separation processes [12]. Bubble points for both the (*R,S*)-1-phenylethanol/CO<sub>2</sub> and vinyl acetate/CO<sub>2</sub> systems were measured at 40 °C, 60 °C, 80 °C, 100 °C, and 120 °C and pressures up to 23.74 MPa for

(*R,S*)-1-phenylethanol/ $\text{CO}_2$  systems and up to 12.22 MPa for vinyl acetate/ $\text{CO}_2$  systems by using variable-volume view cell apparatus. Figure 3 shows the bubble points for assayed binary systems.



**Figure 3:** Bubble points for (*R,S*)-1-phenylethanol/ $\text{CO}_2$  ( $x_{\text{VA}} = 0.297 - 0.316$ ) with and vinyl acetate/ $\text{CO}_2$  systems ( $x_{1\text{-PE}} = 0.410 - 0.483$ ).

As shown in Figure 3, phase transition pressure of vinyl acetate/ $\text{CO}_2$  system ( $x_{\text{VA}} = 0.297 - 0.316$ ) increases with increasing temperature from 40 °C to 120 °C, while for the (*R,S*)-1-phenylethanol/ $\text{CO}_2$  system ( $x_{1\text{-PE}} = 0.410 - 0.483$ ) phase transition pressure slowly decreases with increasing temperature above 60 °C. Bubble points of vinyl acetate/ $\text{CO}_2$  system were measured at 60 °C with different mixture compositions, as well. The obtained experimental results for the vinyl acetate/ $\text{CO}_2$  system are in good agreement with data from literature [12] (Figure 4).



**Figure 4:** Pressure-composition experimental and literature data [12] for the vinyl acetate/ $\text{CO}_2$  system obtained at 60 °C.

From the comparison of experimentally obtained bubble points of binary mixture vinyl acetate/CO<sub>2</sub> with the available literature data [12], it can be clearly seen that the experimentally measured bubble points are comparable to the literature data.

## CONCLUSION

Ionic liquids are promising media for enzymatic reactions. Besides potential environmental benefits, ionic liquids permit enzyme-catalyzed reactions in a solvent polarity range that was previously inaccessible. The kinetic resolution of (*R,S*)-1-phenylethanol by transesterification with vinyl acetate, catalyzed by a immobilized *Candida antarctica* lipase B, was successfully carried out in all assayed ionic liquids. Ionic liquid [bmim][BF<sub>4</sub>] has been shown to act as an excellent non-aqueous media, since the conversion of 99.3 % was achieved after 5 hours of reaction performance.

Bubble points of binary mixtures of (*R,S*)-1-phenylethanol/CO<sub>2</sub> and vinyl acetate/CO<sub>2</sub> were determined, as well. From the experimental results it can be seen that by increasing the temperature from 40 °C to 120 °C, phase transition pressures for vinyl acetate/CO<sub>2</sub> system ( $x_{VA} = 0.297 - 0.316$ ) are increased. On the contrary, for (*R,S*)-1-phenyl-ethanol/CO<sub>2</sub> system ( $x_{1-PE} = 0.410 - 0.483$ ), at temperatures above the 60 °C, the phase transition pressures were slowly decreasing. However, more detailed investigations and further experiments will be employed to gain the sufficient knowledge about the phase equilibrium of (*R,S*)-1-phenylethanol/CO<sub>2</sub> and vinyl acetate/CO<sub>2</sub> systems. Based on obtained phase equilibrium measurements, enzyme-catalyzed transesterification of (*R,S*)-1-phenylethanol in SC CO<sub>2</sub> will be design.

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