# Long term continuous chemoenzymatic dynamic kinetic resolution of *rac*-1-phenylethanol using ionic liquids and supercritical carbon dioxide

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The long term continuous dynamic kinetic resolution (DKR) of *rac*-phenylethanol in IL/$scCO_2$ biphasic systems was carried out by simultaneously using immobilized lipase (Novozym 435) and acidic zeolite catalysts at 50 °C and 100 bar, providing good yields (up to 98.0%) for *R*-phenylethyl propionate with excellent enantioselectivity (up to 97.3% ee) and without any activity loss during 14 days of operation.

## 1. Introduction

The preparation of chiral drugs as single enantiomers is one of the most pressing goals in pharmaceutical science because of the different types of biological activity exhibited by each enantiomer. Kinetic resolution (KR) with enzymes is the most widely used method for separating the two enantiomers of a racemic mixture. However, the main drawback of this method is that the chemical yield is limited to 50% and, it is necessary to incorporate a further separation step (*e.g.* distillation, liquid–liquid extraction, membrane process, *etc*) to isolate the desired enantiomer.[1] These limitations can be overcome by combining kinetic resolution with *in situ* racemisation of the undesired enantiomer, using so-called dynamic kinetic resolution (DKR). Classically, transition metal (*e.g.* $Ru^{2+}$) complexes[2] and, more recently, solid acids[3] (*e.g.* zeolites) have been described as suitable catalysts for the racemisation of *sec*-alcohols *via* hydrogen transfer processes. DKR based on lipases combined with metal catalysis has been assayed in anhydrous organic solvents (*e.g.* toluene) under argon atmosphere and in a discontinuous way, providing high yields (up to 99% and ee > 99.9%) of the enantiomerically pure ester product in 1–6 days. DKR approaches based on lipases combined with acidic zeolite catalysts have also been described using water/organic solvent biphasic media in a discontinuous way.[3b,3c] The potential value of H-beta zeolites as heterogeneous alcohol-racemisation catalysts for benzylic alcohols in aqueous phase has been demonstrated, while the enzymatic KR step occurred in the organic phase, leading to a corresponding *R*-ester with 78% yield and 98% ee. The use of a perfluorous acyl donor (20,20,20-trifluoroethanol 1H,1H,2H, 2H-perfluoundecanoate) in the Zr-beta zeolite/Novozym-catalyzed DKR of benzylic alcohols in organic/fluorous biphasic system lead to no clear improvement in the process (95% yield, 75% ee).[3d]

Water-immiscible ionic liquids (ILs) have recently emerged as exceptionally interesting green non-aqueous reaction media for biotransformations, because of the high level of activity,[4a] stereoselectivity[4b] and stability[5] displayed by enzymes in chemical transformations. The use of ILs as suitable reaction media for the DKR of *sec*-alcohols catalyzed by lipase/ruthenium complex has been described, leading to good yields (up to 95%) after 2–6 days of reaction.[6]

Biphasic systems based on ILs and supercritical carbon dioxide ($scCO_2$) for enzyme catalysis have been put forward as the first approach to developing integral green bioprocesses in non-aqueous media, where both the biotransformations and extraction steps are coupled in efficient reaction/separation processes.[7] In this context, DKR processes of *rac*-1-phenylethanol (**1**, see Fig. 1A) in IL/$scCO_2$ systems have been carried out combining immobilized *Candida antarctica* lipase B (CALB) with silica modified with benzenosulfonic acid groups as catalysts in a packed bed reactor at 50 °C and 100 bar.[8] However, the use of both catalysts as a simple mixture resulted in a complete loss of activity, probably due to the acid environment around enzyme particles that could produce deactivation. Consequently, it was necessary to pack catalyst particles in three different layers (immobilized enzyme-acid catalyst-immobilized enzyme) physically separated by glass wool to obtain moderate results for the *R*-ester product (70% yield, 92% ee).

This paper describes for the first time the continuous DKR of **1** in IL/$scCO_2$ systems by using a combination of immobilized CALB (Novozym 435®) and acid zeolite catalysts packed as a heterogeneous particle mixture (Fig. 1). The reactor operated as a catalytic unit able to continuously transform *rac*-**1** in *R*-**2**, combining the advantages of ionic liquids to stabilize enzymes in supercritical fluids, as a non-aqueous "green" reaction/extraction system, with the advantages of a continuous flow process for easy product separation and catalyst reuse.

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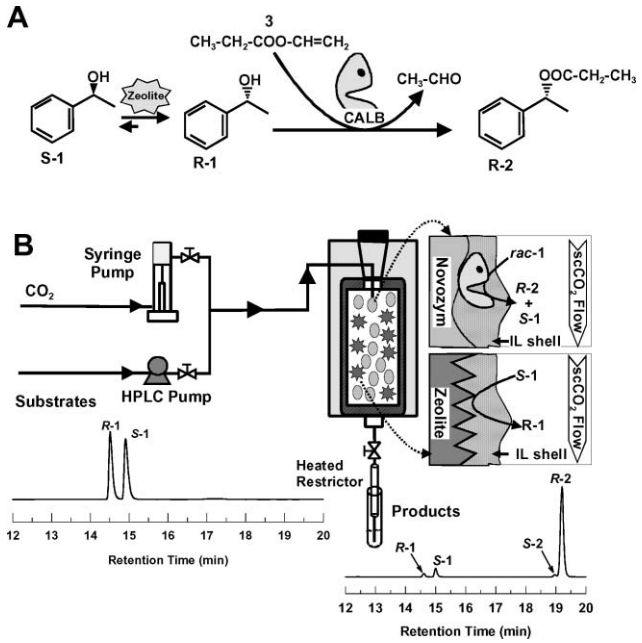


**Fig. 1** **A**. DKR of *rac*-1-phenylethanol (*rac*-**1**) catalyzed by the combined action of zeolite and immobilized *Candida antarctica* lipase B (Novozym 435). **B**. Experimental set-up of the continuous packed bed reactor containing both Novozym 435 and zeolite coated with ILs, including GC chromatograms of the substrate inlet and product exit.

## 2. Experimental

Immobilized *Candida antarctica* lipase B (Novozym 435®, EC 3.1.1.3) was a gift from Novozymes S.A. (Spain). Zeolite Beta CP811E-150 (Si:Al molar ratio = 75), and, zeolites HY CBV400 (Si:Al = 2.5) and HY CBV720 (Si:Al = 15) were obtained from Zeolysts International (PA, USA). Substrates, solvents and other chemicals were purchased from Sigma-Aldrich-Fluka (Madrid, Spain), and were of the highest purity available. The ILs 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim][$PF_6$], 99% purity) and 1-butyl-2,3-dimethylimidazolium hexafluorophosphate ([Bdmim] [$PF_6$], 99% purity) were from Solvent Innovation Inc. (Germany); 1-octadecyl-3-methylimidazolium bis((trifluoromethyl)sulfonyl)amide ([Odmim][$NTf_2$], 99% purity) was obtained from IoLiTec GmbH (Germany); trioctylmethylammonium bis((trifluoromethyl)sulfonyl)amide, [Toma][$NTf_2$], and butyltrimethylammonium bis((trifluoromethyl)sulfonyl)-amide, [Btma][$NTf_2$], were synthesized as described previously.[5]

### Adsorption of IL onto catalyst particles

In a 10-mL capacity test tube, 1 mL of IL ([Bmim][$PF_6$], [Bdmim][$PF_6$], [Odmim][$NTf_2$], [Toma][$NTf_2$], or [Btma][$NTf_2$]) were dissolved in 3 mL acetonitrile. Then, 1 g of catalyst (Novozym 435 or zeolite) was added, and the mixture was gently stirred for 30 min at room temperature. Finally, the acetonitrile was eliminated by continuous bubbling of $N_2$ for 30 min at room temperature. The resulting catalyst-IL particles were equilibrated to 0.11 water activity by over saturated LiCl solutions in closed containers at 25 °C for one week prior to use.[9]

### Racemization reactions

Standard reactions were carried out in 1-mL screw-capped vials with teflon-lined septa, containing 0.6 M *S*-**1** and 0.6 M **3** in hexane (0.5 mL overall volume). Reactions were started by adding zeolite catalyst previously coated with IL [Btma][$NTf_2$] (5 mg) and run at 50 °C in a glycerol bath for 3 h. At regular time intervals, 20 μL aliquots were taken and suspended in 480 μL hexane, and the biphasic mixture was strongly shaken for 3 min to extract all substrates and product into the hexane phase. Then, 400 μL of hexane phase were collected and mixed with 100 μL of 150 mM butyl butyrate (internal standard) solution in hexane, and finally analyzed by GC. All experiment were carried out in duplicate.

### DKR of rac-1 in $scCO_2$

Both Novozym-IL (1 g) and zeolite-IL (0.5 g) catalysts were added to a 10-mL test tube, and the resulting mixture was strongly shaken for 5 min at room temperature to obtain an homogenous distribution of particles. The final mixture was placed in the cartridge of an ISCO 220SX (Teledyne Isco, Inc, Lincoln, NE, USA) high pressure extraction apparatus of 10 mL total capacity. The apparatus is equipped with a syringe pump (ISCO model 100DX, 100 mL overall volume), needle valves and devices for pressure, temperature and flow rate control. The ISCO system was started by the continuous pumping of $scCO_2$ at 100 bar and 50 °C, which automatically opens the exit valve, bubbling continuously the $CO_2$ through a calibrated heated restrictor (1 mL/min, 70 °C). Synthetic processes were carried out for 6 h by continuously pumping a **3**:*rac*-**1** (2:1 mol:mol) mixture into the $scCO_2$ inlet flow at 6 μmol/min mass-flow rate, by using a HPLC pump (model LC-10AT, Shimadzu Europe, Duisburg, Germany) (see Fig. 1B). Substrates and products were transported by the $scCO_2$ flow through the catalytic cartridge, and then recovered by depressurizing through the calibrated heated restrictor by 30 minutes steps in a controlled amount of hexane placed on an ice-bath. Samples were analyzed by GC. In all cases, substrate and product mass-balances from the outlet were consistent with the substrate mass-flow inlet.

### GC analysis

Analysis were performed with a Shimadzu GC-2010 (Shimadzu Europe, Duisburg, Germany) equipped with FID detector. Samples were analyzed on a Beta DEX-120 column (30 m × 0.25 mm × 0.25 μm, Supelco) and a FID detector in the following conditions: carrier gas (He) at 1 MPa (105 mL/min total flow); temperature programme: 60 °C, 10 °C/min, 130 °C; split ratio, 100:1; detector, 300 °C. The retention times of compounds were as follows: **2** (3.1 min; propionic acid (PrA, 5.6 min), butyl butyrate (internal standard, 7.1 min), *R*-**1** (14.4 min), *S*-**1** (14.9 min), *S*-**2** (18.9 min) and *R*-**2** (19.3 min).

## 3. Results and discussion

To know the activity of zeolites catalyzing racemization of *S*-**1** in DKR conditions, these catalysts were tested in a hexane medium containing both *S*-**1** and **3** substrates at the same concentration. As shown in Fig. 2, the H-Beta CP811E zeolite was a suitable

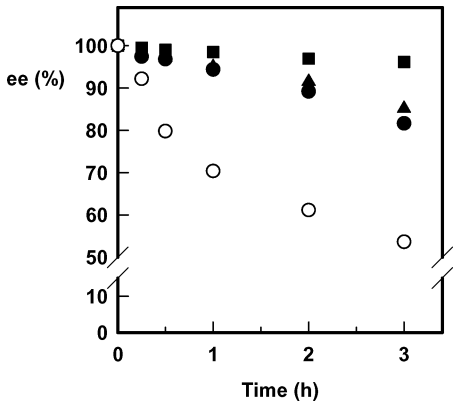


**Fig. 2** Time-course profiles of ee for the racemisation of *S*-**1** with H-Beta CP811E zeolite (○), and H-Beta CP811E (●), H-USY CBV720 (▲) H-USY CBV400 (■) zeolites coated with [Btma][$NTf_2$] in hexane at 50 °C.

catalyst for the proposed reaction in agreement with previous works, since it was able to reduce the $ee_{S\text{-}1}$ from 100 to 53.6% in 3h. Note that no synthesis of 1-phenylethypropionate was observed. However, the undesired hydrolysis of **3** catalyzed by the zeolite that produces propionic acid (PrA) was also detected (see Table 1). This fact was also observed when a silica gel modified with benzenesulfonic acid (SCX) was used as catalyst for the DKR of *rac*-1 in hexane or $scCO_2$, and is obviously a drawback for any continuous DKR process with an equilibrated mass-substrate concentration at the reactor inlet.[8]

The fact that the GC chromatograms did not show any production of acetophenone suggest that H-Beta zeolites catalyzed racemization as Brønsted acids.[3c,12] Coating of this H-Beta zeolite with [Btma][$NTf_2$] lead to a clear decrease in the undesired hydrolysis of **3**, which was accompanied by a loss in the racemisation reaction rate. This fact could be related with the enhancement of mass-transfer limitations into the hexane media resulting from the IL-coating of zeolite particles.[8] In this way, the coating of acid H-Beta zeolites by polyelectrolyte capsules to protect the pH-sensitive enzyme CALB has also been described as an interesting strategy that enables both catalysts to be used in a one-pot DKR of *sec*-alcohols.[10] In our previous studies,[8] a full enzyme deactivation was also observed when DKR processes were carried out by using a mixture of both immobilized lipase and SCX catalyst particles without IL coating, probably as a consequence of the acidification of the enzyme microenvironment.

Fajausite (H-USY) type zeolites (*i.e.* CBV720 and CBV400) coated with [Btma][$NTf_2$] were also able to catalyze the racemisation of *S*-**1**, but the observed racemisation reaction rate was clearly lower than the one obtained for the H-Beta zeolite case (see Table 1). These results agreed with previous works,[3b,3c,11,12] where de-aluminated zeolites resulted in fast racemization of **1**: the fastest reaction rates were always observed with H-Beta zeolites.

**Table 1** Zeolites coated with IL-catalyzed racemization of *S*-**1** in hexane at 50 °C

| Zeolite | Si/Al | IL | Rate (μmol $min^{-1}$ $g^{-1}$) | PrA at 3 h (%) |
|---|---|---|---|---|
| H-Beta CP811E | 75 | None | 165.6 | 8.5 |
| H-Beta CP811E | 75 | [Btma][$NTf_2$] | 37.3 | 1.4 |
| H-USY CBV720 | 15 | [Btma][$NTf_2$] | 24.7 | 1.2 |
| H-USY CBV400 | 2.5 | [Btma][$NTf_2$] | 6.9 | 0 |

The ability of Novozym coated with IL to catalyze the KR of *rac*-**1** in continuous operation with $scCO_2$ at 50°C and 100 bar has been reported to increase the yield of *R*-**2** product by only up to 50% with an excellent enantioselectivity (ee > 99.9).[8]

The suitability of acid zeolites combined with immobilized lipase catalyzing DKR of *rac*-**1** in non-aqueous monophasic systems has been studied in discontinuous way.[10,11] In spite of the different organic solvents assayed (*e.g.* toluene, hexane, 1,4-dioxane, *etc.*), as well as the reactions parameters (*e.g.* temperature, acyl donor, *etc.*), the best results were relatively poor (71% *R*-1-phenyletyl acetate yield and 87.5% ee after 6 h in toluene, improving to 82% yield and 83.1% ee after 48 h reaction time).[11] The encapsulation of H-Beta zeolite with polyelectrolytes did not improve these results (70% *R*-**1** yield and 86% ee after 72 h in toluene).[10]

In this context, a (chemo)biocatalytic packed bed reactor, based on both immobilized lipase (Novozym 435) and zeolite particles coated with ILs, was developed to carry out the DKR of *rac*-**1** in $scCO_2$ phase in continuous way (see Fig. 1B). The system operated as a biphasic reactor, the substrates being transported by the $scCO_2$ phase to each catalyst microenvironment across the IL shell, and the products returning to the supercritical phase. The stereoselective action of the enzyme permits the preferential conversion of *R*-**1** to *R*-**2**, while the acidic catalysts provide *in situ* racemisation of the *S*-**1** unreactive enantiomer. In this way, the product *R*-**2** can theoretically be obtained optically pure with a 100% yield.[13]

Five different ILs, *i.e.* [Bmim][$PF_6$], [Bdmim][$PF_6$], [Odmim][$NTf_2$], [Toma][$NTf_2$] and [Btma][$NTf_2$], were assayed, and each Novozym-zeolite-IL system was tested for 6 h. The obtained *R*-**2** yield, $ee_{R\text{-}2}$ and PrA yield in the steady state are summarised in Table 2. As can be seen in entry 1, a low $ee_{R\text{-}2}$ value (16.4%) was obtained when catalyst particles were assayed without IL coating, in spite of the reported[4–7] excellent enantioselectivity of Novozym 435 for the kinetic resolution of *rac*-**1**, suggesting that the acidic zeolite was indeed interfering with the pH-sensitive enzyme. This fact was in agreement with previous works where uncoated acid catalysts (*e.g.* zeolites) combined with immobilized lipases were assayed in DKR reactions.[8,10] Furthermore, the observed high content of PrA (58.7% of inlet acyl donor **3**) at the outlet of the reactor, corresponding to the hydrolysis of the excess of **3**, is worth noting. This fact could also be related with the loss of enzyme enantioselectivity. In this way, the use of catalyst particles coated with [Bmim][$PF_6$] (entry 2) greatly improved the efficiency of the system, because the racemizing activity of the H-Beta zeolite coupled with the enzyme catalysis was able to increase the *R*-**2** yield up to 73.8%, while the enantioselectivity of the enzyme remained unchanged (99.5% $ee_{R\text{-}2}$). In this case, the undesired hydrolysis of **3** at the reactor outlet was reduced to 23.3% of its inlet concentration, suggesting that the transport of uncontrolled amounts of water from the $CO_2$ tank towards the catalytic environment was limited by the water-immiscible IL shell coating particles. The use of other water-immiscible ILs to cover both Novozym and H-Beta zeolite particles (entries 3

 

**Table 2** Continuous DKR catalyzed by both Novozym (1 g) and zeolite catalysts coated with IL, by pumping a **3**:**1** (2:1 mol:mol) mixture at 6 μmol $min^{-1}$ mass-flow rate into the $scCO_2$ inlet flow (1 mL $min^{-1}$) of the reactor at 50 °C and 100 bar

| Entry | Zeolite | IL | *R*-2 Yield (%) | $ee_{R\text{-}2}$ (%) | PrA[i] (%) |
|---|---|---|---|---|---|
| 1 | CP811E[a] | None | 48.0 | 16.4 | 58.7 |
| 2 | CP811E[a] | [Bmim][$PF_6$][d] | 73.8 | 99.5 | 23.3 |
| 3 | CP811E[a] | [Toma][$NTf_2$][e] | 75.5 | 82.1 | 11.3 |
| 4 | CP811E[b] | [Odmim]][$NTf_2$][f] | 67.7 | 95.7 | 11.1 |
| 5 | CP811E[c] | [Bdmim][$PF_6$][g] | 59.5 | 96.2 | 10.4 |
| 6 | CP811E[c] | [Btma][$NTf_2$][h] | 72.3 | >99.0 | 11.7 |
| 7 | CBV720[c] | [Btma][$NTf_2$][h] | 72.0 | 90.4 | 11.9 |
| 8 | CBV400[c] | [Btma][$NTf_2$][h] | 98.0 | 97.3 | 10.7 |

[a] 200 mg. [b] 300 mg. [c] 500 mg. [d] Bmim: 1-butyl-3-methylimidazolium. [e] Toma: trioctylmethylammonium. [f] Odmim: 1-octadecyl-3-methylimidazolium. [g] Bdmim: 1-butyl-2,3-dimethylimidazolium. [h] Btma: butyltrimethylammonium. [i] With respect the mass inlet flow.

to 6) provided propionic acid concentrations lower than 12% of the inlet mass flow **3**, but did not achieve a clear improvement in either the *R*-**2** yield or $ee_{R\text{-}2}$, in spite of the increased amount of zeolite assayed (from 200 mg to 500 mg).

In spite of the low racemizing activity shown by fajausite (Y-USY) type zeolites (see Table 1), both CBV720 and CBV400 chemical catalysts were seen to be suitable for the DKR process of *rac*-**1** in $scCO_2$. The use of a Novozym/CBV720 mixture (2:1 w/w) coated with [Btma][$NTf_2$] allowed an interesting activity level (72% *R*-**2** yield and 90.4% $ee_{R\text{-}2}$, entry 7), which was similar to that observed for H-beta zeolite (entry 2). However, the best results (98% *R*-**2** yield and 97.3% $ee_{R\text{-}2}$, entry 8) were obtained for the Novozym/CBV400 catalysts mixture (2:1 w/w) coated with [Btma][$NTf_2$].

Fig. 3 shows the operational stability profile obtained for the continuous DKR process catalyzed by the Novozym 435/H-USY CBV400 catalyst mixture coated with [Btma][$NTf_2$] in $scCO_2$ under different conditions of pressure and temperature. As can be seen, both *R*-**2** yield and $ee_{R\text{-}2}$ profiles remained practically unchanged for 6 days of operation. At 50 °C, the increase in $scCO_2$ pressure to 120 bar involved a slight improvement in $ee_{R\text{-}2}$ (up to 98.8%), while the *R*-**2** yield was reduced to 80%. At a pressure of 120 bar, the subsequent increase of temperature to 60 °C improved the previous *R*-**2** yield (up to 90%) maintaining the same level of $ee_{R\text{-}2}$ (up to 99.5%) A further decrease in pressure to 100 bar at the same temperature (60 °C) allowed to improve the *R*-**2** yield (up to 97.5%), but slightly reduced $ee_{R\text{-}2}$ (up to 93.5). Finally, when the reactor was returned to the initial supercritical conditions (50 °C and 100 bar) after 9 days of operation, both *R*-**2** yield and $ee_{R\text{-}2}$ profiles returned to the activity levels shown at initial stages, remaining then unchanged for an additional four days of operation.

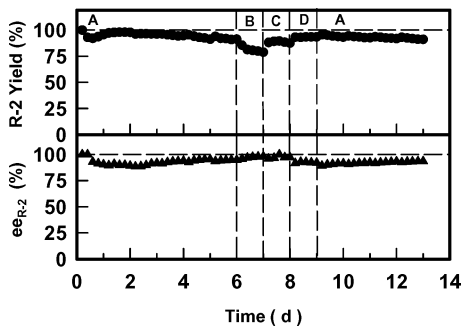


**Fig. 3** Operational stability for continuous DKR catalyzed by both Novozym 435 (1 g) and zeolite CBV400 (0.5 g) coated with [Btma][$NTf_2$] (1 mL and 0.5 mL, respectively) in $scCO_2$ at 2 μmol of **1** $min^{-1}$ mass-flow rate and (A) 50 °C and 100 bar, (B) 50 °C and 120 bar, (C) 60 °C and 120 bar and (D) 60 °C and 100 bar.

The excellences of IL-coating biocatalyst particles, as protective agents that improve both activity and stability of lipases in adverse processes (*e.g.* immobilization in sol-gel derived silica, KR in $scCO_2$ at 100 bar and 150 °C, *etc*), have been demonstrated.[14] The effects of changes in pressure and temperature on enzyme-catalyzed reactions in $scCO_2$ have recently been reviewed.[15] In the synthesis of butyl butyrate catalyzed by Novozym 435 in $scCO_2$, it was observed that, within the range 40–60 °C, an increase in temperature improves the enzyme activity at all the assayed pressures (80–150 bar). However, at a fixed temperature in the above range, an increase in pressure resulted in a decrease in the synthetic activity of the enzyme, and this effect was attributed to the increase in the density of $scCO_2$.[16] However, the effect of pressure on enantioselectivity is indeed noteworthy, although the reason for this is not clear. For example, Matsuda *et al.*[17] reported how the enantioselectivity of Novozym 435-catalyzed acetylation of *rac*-1-(p-chlorophenyl)-2,2,2-trifluoroethanol with vinyl acetate in $scCO_2$ gradually decreased when the pressure increased from 80 to 190 bar, which was also related with changes in $scCO_2$ density. Conversely, for the same immobilized lipase-catalyzed continuous kinetic resolution of *rac*-**1**, it was reported that changes in pressure did not greatly affect conversion or enantioselectivity.[18]

## Conclusions

The excellent suitability of the Novozym/H USY CBV400/[Btma][$NTf_2$] system for the continuous DKR of **1** in $scCO_2$ was clearly demonstrated. Once again, the excellent protective effect of ILs against enzyme deactivation by temperature, $CO_2$ and/or acidic pH was observed.[5,7] This work clearly demonstrates the exciting potential of multi-catalytic (enzymatic or chemo-enzymatic) systems in ILs/$scCO_2$ for synthesizing optically active pharmaceutical drugs in green and clean non-aqueous continuous processes. In this way, the use of co-solvents or surfactants may aids to overcome the limitations of $scCO_2$ to only dissolve hydrophobic compounds, allowing to extend the proposed methodology to the hydrophilic cases.[19] Fundamental studies on (bio)catalysis in IL/supercritical

fluid biphasic media should be carried out to establish clear criteria for specifically pairing the most appropriate IL and supercritical fluid with the corresponding (multi)catalytic system or bioprocess.

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