Aqueous biphasic systems using chiral ionic liquids for the enantioseparation of mandelic acid enantiomers

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Abstract

This work aims at extending the applicability of chiral aqueous biphasic systems (ABS) to enantioseparations by using chiral ionic liquids (CILs) simultaneously as phase forming agents and chiral selectors. After determining the ternary phase diagrams of ABS composed of CILs and salts, these were used to ascertain the CIL structure and salt role on the ABS aptitude to separate mandelic acid enantiomers. Representative CIL-based ABS were further employed in optimization studies, where the mandelic acid

content, temperature, tie-line length, salt and phases weight ratio were studied. The influence of these parameters is shown to be highly dependent on the CIL-based ABS, nevertheless the results here reported suggest that the key driving the enantioseparation in these ABS is a combination of the enantiorecognition ability of a given CIL with the solubility of mandelic acid in the corresponding CIL-rich phase.

Keywords

Aqueous biphasic systems, chiral ionic liquids, enantioseparation, mandelic acid enantiomers, enantiomeric excess

Introduction

In spite of having very similar physical and chemical properties (except for their optical rotation), the optical isomers of a molecule can be discriminated by the human body. This is crucial in drug development, since the pharmacological activity usually results from only one of the enantiomers (the eutomer), while the other (the distomer) may be inert, less potent or even toxic.¹ During the past years, the pharmaceutical industry has faced pressures from the regulatory bodies regarding the commercialization of chiral drugs, so that a shift from racemic to enantiopure drugs is demanded.²

The production of enantiopure drugs remains a major challenge for the pharma industry due to the limited approaches available and their high cost.³ The preferable and most powerful pathway is the asymmetric synthesis, where the direct synthesis of the pure isomer is carried.⁴ However, it is cumbersome and it often requires the use of expensive enantiopure raw materials or specific enantioselective catalysts. The synthesis of racemates followed by their chiral resolution is a simpler, more flexible and cheaper

alternative to asymmetric synthesis.^{5, 6} Crystallization and chromatography are the most used techniques for enantioseparation, due to their simplicity of operation.⁵ Still, the former is limited by the number of racemic mixtures able to form conglomerates (estimated values of 5 to 10%), the excessive solids handling and the need for additional steps of enantiomeric enrichment; in turn, the latter suffers from high costs linked to chiral chromatographic columns, low loading capacities and poor scale-up opportunities.^{5, 7}

Enantioselective liquid-liquid extraction (ELLE) techniques are attracting much interest in enantioseparations. Beyond its simple and fast operation and low cost, ELLE affords an adequate compromise between efficiency, broad applicability and easy scale-up.⁶ By bringing together solvent extraction with chiral recognition,⁶ ELLE requires the existence of at least one chiral selector, so that chiral recognition may occur. Chiral selectors are molecules able to perform chiral recognition according to the “three-point model”, i.e. by establishing discriminative intermolecular interactions that lead to the formation of “chiral selector-enantiomer” complexes.⁸ Combinations of organic solvents/water with β -cyclodextrin-based and/or tartaric acid-inspired chiral selectors are amongst the most studied ELLE systems.⁹⁻¹⁴ Still, the large quantities of volatile organic solvents employed fail to match the recommendations of the Green Chemistry and Sustainability guidelines.^{15, 16}

Aqueous biphasic systems (ABS) are good candidates to turn enantioseparations not only into more biocompatible but also more versatile approaches.^{17, 18} The first credential is mainly afforded by the high water content, while the second is related with the wide range of phase-formers pairs (e.g. polymer-polymer, polymer-salt, salt-salt, polar organic solvents-salt),^{17, 19-35} some of them bearing chiral centers (e.g. polymer-chiral polymer, polar organic solvents-oligosaccharides),³⁶⁻³⁸ available to induce liquid-

liquid demixing in aqueous solution. The implementation of ABS to chiral separations has been achieved resorting to two distinct strategies: one that relays on the addition of an extra chiral selector to the system formulation (not essential to two-phase formation)¹⁹⁻³⁵ and another where one of the solutes has chirality, thus acting simultaneously as phase-former and chiral selector.³⁶⁻³⁸ The former has been mainly focused on the use of β -cyclodextrin derivatives, copper- β -cyclodextrin-complexes, tartaric acid derivatives, proteins and microbial cells as chiral recognition agents in polymer-salt, polymer-polymer, polar organic solvent-salt and micellar systems.¹⁹⁻³⁵ The highest enantiomeric excess obtained so far (86.7 %) was achieved in a process combining the enantioselective biotransformation and extraction of a histidine intermediate with an ABS composed of poly(ethylene) glycol, Na_2HPO_4 and microbial cells as chiral selectors.³⁰ The alternative approach, although it has lagged behind in the past years, is less complex, since less chemicals are used, thus simplifying recycling and reuse routes. Pairs of β -cyclodextrin derivatives-polar organic solvents and of two polymers, one of them chiral were successfully applied in the development of chiral ABS.³⁶⁻³⁸ So far lower enantioselectivities (maximum enantiomeric excess reported of 32.66 % for zopiclone) were achieved than those afforded by the introduction of an extra chiral selector to the ABS.³⁸ Still, there is much to explore in what regards the use of chiral phase formers in ABS and the implementation of chiral ionic liquids (CILs) is being considered.

Ionic liquids (ILs) are alternative solvents with an enormous degree of structural diversity,³⁹ allowing the design of task-specific solvents and, by their introduction in ABS, of highly performant extraction/separation approaches.⁴⁰ Being made up of ions, if one can select/develop chiral structures to function as cations, anions or both, the opportunity of creating CILs emerges. The first CIL, 1-butyl-3-methylimidazolium

lactate, was synthesized in 1996 by Seddon and co-workers.⁴¹ Ever since, although hundreds of other CILs were proposed,⁴² such as those based on carbohydrates, amino acids and alkaloids, their application in ABS for enantioseparations has seldom been addressed. Efforts to resolve racemic mixtures of amino acids, with special attention to phenylalanine, were done with ABS formed by imidazolium-⁴³ and tropine-based CILs⁴⁴ and inorganic salts. Under optimized conditions, enantiomeric excesses of 53%⁴³ and 65%⁴⁴ were attained for phenylalanine, the higher value being yielded by the precipitation of the target enantiomer at the interface.

Given the limited application of CILs in the development of ABS for chiral resolution purposes,^{43, 44} it is here intended to contribute towards the enlargement of CIL-based ABS database and to provide further insight on their enantioseparation aptitude. In an initial stage of this work, the phase diagrams of ABS composed of CILs based on quinine, L-proline and L-valine and three salts (viz. K_3PO_4 , K_2HPO_4 and K_2CO_3) were determined and characterized. The low toxicity, significant water solubility and proved chiral recognition aptitude of this set of CILs recently synthesized by some of us showcase the interest of their implementation in chiral ABS.⁴⁵ Their enantioseparation aptitude was further evaluated and optimized using mandelic acid, a key precursor in chiral pharmaceuticals manufacturing, as model chiral compound.

Experimental

Materials

Six cationic CILs were synthesized in this work: 1-methyl quininium methylsulfate, $[C_1Qui][C_1SO_4]$; *N,N*-dimethyl-L-proline methyl ester iodide, $[C_1C_1C_1Pro]I$; *N,N*-dimethyl-L-proline methyl ester methylsulfate,

[C₁C₁C₁Pro][C₁SO₄]; *N,N*-diethyl-L-proline ethyl ester bromide, [C₂C₂C₂Pro]Br; *N,N,N*-trimethyl-L-valinolium iodide, [C₁C₁C₁Val]I; and *N,N,N*-trimethyl-L-valinolium methylsulfate, [C₁C₁C₁Val][C₁SO₄]. For the synthesis, the reagents used were quinine (purity = 98%), iodomethane (purity = 99%), dimethyl sulfate (purity = 99%), dichloromethane anhydrous (purity = 99.8%), ethanol (purity = 99.8%), acetone (HPLC grade), potassium carbonate, K₂CO₃ (purity ≥ 99%), L-proline, L-Pro (purity = 99%), bromoethane (purity = 98%), acetonitrile (purity = 99.8%), chloroform (purity = 99%), L-valine, L-Val (purity = 98%), sodium borohydride (purity = 99%), sulfuric acid (purity = 99.9%), methanol (purity = 99%), ethyl acetate (purity = 99.8%), potassium hydroxide, KOH (purity = 90%), formic acid (purity = 98%), formaldehyde (37 wt % in water solution) and hydrochloric acid (37 wt% in water solution) acquired from Sigma-Aldrich. The salts used in ABS were potassium phosphate tribasic, K₃PO₄ (purity = 97%), K₂CO₃ (purity ≥ 99%) and di-potassium hydrogen phosphate trihydrate, K₂HPO₄·3H₂O (extra pure) and were respectively purchased at Alfa-Aesar, Sigma-Aldrich and Scharlau. The enantiomers used were *R*-(-)-mandelic acid, *R*-MA (purity = 99%), and *S*-(+)-mandelic acid, *S*-MA (purity = 99%), both supplied by Acros Organics. The chemical structures and abbreviations of the CILs and mandelic acid enantiomers are depicted in Figure 1.

For the HPLC-DAD analysis of the mandelic acid enantiomers, copper (II) sulphate pentahydrate, CuSO₄·5H₂O (purity > 98%), L-phenylalanine, L-Phe (purity > 98%), purchased from AnalaR and Alfa Aesar, respectively, and methanol (HPLC grade), acquired from Fisher Chemical, were used for the mobile phase. Ammonia solution at 25% was obtained from Chem-Lab. Ultra-pure water (double distilled and then treated with a Milli-Q plus 185 water purification apparatus) was used for the HPLC analysis. Syringe filters (0.45 µm) and regenerated cellulose membrane filters

(0.45 μm), acquired at Specanalitica and Sartorius, respectively, were used during filtration steps.

Figure 1. Chemical structures and abbreviations of the CILs and mandelic acid enantiomers investigated.

Synthesis of the chiral ionic liquids based on quinine, L-proline and L-valine

The six CILs here used were synthesized in our laboratory according to well-established protocols.⁴⁵ Briefly, an alkylation reaction between dimethyl sulphate and quinine yielding $[\text{C}_1\text{Qui}][\text{C}_1\text{SO}_4]$ was performed, L-valine-based CILs were obtained in a three step synthesis entailing reduction of L-valine, Eschweiler-Clark reaction and *N*-alkylation and L-proline-based CILs were synthesized by alkylation reactions between

L-proline and either iodomethane or bromoethane affording $[C_1C_1C_1Pro]I$ or $[C_2C_2C_2Pro]Br$, respectively.

Determination of the phase diagrams and tie-lines

The ternary phase diagrams of the ABS composed of CILs and K_3PO_4 , K_2CO_3 or K_2HPO_4 were determined through the cloud point titration method at $(25 \pm 1) ^\circ C$.⁴⁰ To aqueous solutions containing ca. 6-70 wt% of CILs, the alternate drop-wise addition of an aqueous solution of salt at ca. 40 wt%-50 wt% and of pure water was performed under constant stirring. The repetition of this procedure allows, by turns, entering the biphasic region (turbid solution) and reaching the monophasic region (clear solution), respectively. By weight quantification ($\pm 10^{-4}$ g) after the addition of each solution, the ternary systems compositions of the phase diagram were determined. The experimental binodal curves were fitted by equation (1)⁴⁶

$$[CIL] = Ae^{[(B[salt])^{0.5} - (C[salt]^3)]} \quad (1)$$

where $[CIL]$ and $[salt]$ are the CIL and salt weight fraction percentages, respectively, while A , B and C correspond to the fitting parameters. The tie-lines were gravimetrically determined, as originally proposed by Merchuk et al.⁴⁶ A ternary mixture composition formed by CIL + salt + water located at the biphasic region was prepared within $\pm 10^{-4}$ g, vigorously stirred and left to equilibrate at $(25 \pm 1) ^\circ C$ for at least 12 h. Both phases were then separated and weighed. The lever-arm rule by the relationship between the top CIL-rich phase and the overall system weights allowed calculating each tie-line. Detailed guidelines on the tie-lines determination can be found elsewhere.⁴⁰

Separation of mandelic acid enantiomers using ABS

Mixture points localized in the biphasic region of the phase diagrams were selected to conduct studies on racemic mandelic acid enantioseparation. The systems were gravimetrically prepared (within $\pm 10^{-4}$ g) by adding the correct amounts of CIL, salt and water along with equal amounts of two aqueous solutions of *R*-mandelic acid and *S*-mandelic acid both prepared at the same concentrations (*viz.* 10, 50 or 100 mg.mL⁻¹) to yield the desired final content in the ABS. Throughout this work, the evaluation of distinct conditions was carried out: CIL's structure, enantiomers content, temperature, TLLs, salt and mixture points along the same TL. The overall mixture compositions and conditions are detailed in Table 1. The CILs were placed in contact with the mandelic acid enantiomers in aqueous solution for at least 12 h under constant stirring (300 rpm) at the desired temperature, to promote specific interactions between the CIL and the target enantiomers, as recommended elsewhere.⁴³ The salt was added after such a period to induce liquid-liquid demixing. To this a period of equilibration of at least 12 h under the desired temperature followed, to guarantee complete separation of the phases and partition of the enantiomers among phases. The phases, the top being CIL-rich and the bottom being salt-rich, were then separated and weighed (within $\pm 10^{-4}$ g). CIL-rich phases were submitted to HPLC-DAD analysis for mandelic acid enantiomers quantification. In order to estimate the average extraction/enantioseparation parameters and the corresponding standard deviations, triplicates were performed.

The percentage extraction efficiencies of *R* and *S*-mandelic acid (EE_{R-MA} and EE_{S-MA} , %) were separately determined according to the equation 2:

$$EE_{R/S-MA}, \% = \frac{m_{R/S-MA}^{CIL}}{m_{R/S-MA}^0} \times 100 \quad (2)$$

where $m_{R/S-MA}^{CIL}$ is the mass of *R* or *S*-mandelic acid present in the CIL-rich phase and $m_{R/S-MA}^0$ is the mass of *R* or *S*-mandelic acid originally added to the ABS.

The enantiomeric excess (*e.e.*, %) present in the CIL-rich phase was calculated in accordance to equation 3:

$$e.e., \% = \frac{m_{S-MA}^{CIL} - m_{R-MA}^{CIL}}{m_{S-MA}^{CIL} + m_{R-MA}^{CIL}} \times 100 \quad (3)$$

in which m_{S-MA}^{CIL} and m_{R-MA}^{CIL} are the mass of *S* and *R*-mandelic acid present in the CIL-rich phase, respectively.

Table 1. Overall set of conditions evaluated and approximate mixture compositions used in the enantioseparation of mandelic acid studies with CIL-based ABS.

CIL	Salt	([CIL] _M , [Salt] _M) / (wt%, wt%)	([R-MA] _M , [S-MA] _M) / (wt%, wt%)	<i>T</i> (±1) / °C
<i>CIL structure</i>				
[C ₁ Qui][C ₁ SO ₄]	K ₃ PO ₄	(2.5, 18)	(0.8, 0.8)	25
[C ₁ C ₁ C ₁ Pro]I	K ₃ PO ₄	(14, 32)	(0.8, 0.8)	25
[C ₂ C ₂ C ₂ Pro]Br	K ₃ PO ₄	(10, 35)	(0.8, 0.8)	25
[C ₁ C ₁ C ₁ Val]I	K ₃ PO ₄	(10, 35)	(0.8, 0.8)	25
[C ₁ C ₁ C ₁ Val] [C ₁ SO ₄]	K ₃ PO ₄	(10, 25)	(0.8, 0.8)	25
<i>TLL</i>				
[C ₁ Qui][C ₁ SO ₄]	K ₃ PO ₄	(3, 15); (2.5, 18); (4, 20)	(0.8, 0.8)	25
[C ₂ C ₂ C ₂ Pro]Br	K ₃ PO ₄	(10, 30); (10, 35); (10, 40)	(0.8, 0.8)	25
<i>Mixture points along the same TL - Phases weight ratio</i>				
[C ₂ C ₂ C ₂ Pro]Br	K ₃ PO ₄	(30, 16); (20, 25); (10, 35); (5, 40)	(0.8, 0.8)	25
[C ₂ C ₂ C ₂ Pro]Br	K ₂ HPO ₄	(20, 25); (15, 30); (10, 35); (5, 40)	(0.8, 0.8)	25
<i>Temperature</i>				
[C ₁ Qui][C ₁ SO ₄]	K ₃ PO ₄	(2.5, 18)	(0.8, 0.8)	15, 25, 35, 45
[C ₂ C ₂ C ₂ Pro]Br	K ₃ PO ₄	(10, 35)	(0.8, 0.8)	15, 25, 35, 45
<i>MA content</i>				
[C ₁ Qui][C ₁ SO ₄]	K ₃ PO ₄	(2.5, 18)	(0.17, 0.17); (0.8, 0.8); (1.7; 1.7)	25

[C ₂ C ₂ C ₂ Pro]Br	K ₃ PO ₄	(10, 35)	(0.17, 0.17); (0.8, 0.8); (1.7, 1.7)	25
<i>Salt</i>				
[C ₂ C ₂ C ₂ Pro]Br	K ₃ PO ₄	(10; 35)	(0.8, 0.8)	25
[C ₂ C ₂ C ₂ Pro]Br	K ₂ HPO ₄	(10; 35)	(0.8, 0.8)	25
[C ₂ C ₂ C ₂ Pro]Br	K ₂ CO ₃	(10; 35)	(0.8, 0.8)	25

Mandelic acid enantiomers quantification

The liquid chromatograph HPLC Elite LaChrom (VWR Hitachi) used for this purpose was equipped with a diode array detector (DAD) 1-2455, column oven 1-2300, auto-sampler 1-2200 and pump 1-2130. A C₁₈ reversed-phase analytical column (LiChrospher 100 RP-18, 5 μ m, 250 mm $\times$ 4 mm i.d.) linked to a guard column (5 μ m, 4 mm $\times$ 4 mm) with the same stationary phase was used. Both column oven and autosampler operated at controlled temperature of 22 $^{\circ}$ C and 25 $^{\circ}$ C, respectively. The mobile phase was made up of water:methanol [85:15 (v/v)], 2 mM L-phenylalanine and 1 mM CuSO₄ at pH = 4.00 ($\pm$ 0.02), adjusted by adding an ammonia aqueous solution at 5 wt%. The separation was carried out using isocratic elution at a flow rate of 0.8 mL.min⁻¹ and the injection volume was 20 μ L. DAD was set to measure the spectrum from 200 to 600 nm, with a specific wavelength of 270 nm being used for *R*-mandelic acid and *S*-mandelic acid quantification. Calibration curves were previously determined using stock solutions prepared in water:methanol [85:15 (v/v)] at concentrations of 10 – 500 μ g.mL⁻¹ of each enantiomer. The *R* enantiomer elutes first, at a retention time of around 11.7 min, followed by *S* eluting at approximately 13.2 min. The LOD and LOQ were, respectively, 5 μ g.mL⁻¹ and 10 μ g.mL⁻¹ for both enantiomers. Intra and inter-day precisions were 0.27-3.29 % and 1.39-1.88 % for *R*-MA and 0.79-5.59 % and 4.01-6.40 % for *S*-MA, respectively. Intra and inter-day accuracies were 95.8-127 % and 96.4-118.4 % for *R*-MA, while for *S*-MA they were of 97.3-126.2 % and 93.0-124.6%, respectively. The CIL-rich phases were diluted using water:methanol [85:15 (v/v)] and filtered using syringe filters (0.45 μ m). At least two injections *per* sample were done.

Results and discussion

Ternary phase diagrams and tie-lines

The knowledge of the CIL-based ABS phase diagrams is essential for the development of enantioseparations. To accomplish this, the ternary phase diagrams composed of five CILs, [C₁C₁C₁Val]I, [C₁C₁C₁Val][C₁SO₄], [C₂C₂C₂Pro]Br, [C₁C₁C₁Pro]I and [C₁Qui][C₁SO₄], and K₃PO₄, a strong salting-out agent,⁴⁰ were measured at (25 ± 1) °C. [C₁C₁C₁Pro][C₁SO₄] was not able to form ABS with K₃PO₄. Two additional salts, K₂CO₃ and K₂HPO₄, were paired with [C₂C₂C₂Pro]Br to evaluate on the role of salt type upon ABS formation.

The ternary phase diagrams are shown in Figures 2 and 3 in weight fraction. All detailed experimental data related (ternary phase diagrams weight fraction compositions, equation 1 regression parameters and TL information) are provided as Supplemental Material (Table S1 – Table S9). The ternary phase diagrams determined in this study provide information on the CILs and salt role upon ABS formation (Figure 2 and 3). The biphasic zone is placed above the binodal curve meaning that the broader this is the more prone is the CIL to form ABS.

As observed in Figure 2, the CILs' ability to form ABS with K₃PO₄ can be ranked as follows (at fixed CIL weight fraction composition of 10 wt%): [C₁Qui][C₁SO₄] > [C₁C₁C₁Val]I > [C₁C₁C₁Val][C₁SO₄] > [C₂C₂C₂Pro]Br > [C₁C₁C₁Pro]I. Within the CILs studied, it is possible to infer on both cation ([C₁Qui][C₁SO₄] vs. [C₁C₁C₁Val][C₁SO₄] vs. [C₁C₁C₁Pro][C₁SO₄]) and anion role ([C₁C₁C₁Val]I vs. [C₁C₁C₁Val][C₁SO₄]) on the ABS formation. The cation effect is driven by the hydrophobicity-hydrophilicity of the cation, where the order [C₁Qui]⁺ > [C₁C₁C₁Val]⁺ > [C₁C₁C₁Pro]⁺ directly correlates with the octanol-water partition coefficients of their precursors (log *K*_{o/w} of 3.44, -0.08 and -0.10 for quinine, valinol and

proline methyl ester, respectively⁴⁷). In close agreement with previous studies,⁴⁰ the more hydrophobic the CIL the higher its aptitude to form ABS. It should be highlighted that, although valinol and proline methyl ester possess similar $\log K_{o/w}$, $[C_1C_1C_1Pro][C_1SO_4]$ failed to induce phase separation in presence of K_3PO_4 . This can be attributed to the higher hydrophobicity of the cation when compared to the parent compound, due to alkylation: while the addition of 3 methyl groups is done to valinol, only 2 methyl groups are added to proline methyl ester (*cf.* Figure 1)

In general, the ability of an IL anion to create ABS is related with the decrease in their hydrogen-bond accepting ability (β).^{48, 49} The aforementioned rank places I^- as a better two-phase formation inducer than $[C_1SO_4]^-$, in good agreement with their relative position in the scale of hydrogen bond basicity of ILs proposed by Cláudio *et al.*⁵⁰ Although $[C_2C_2C_2Pro]Br$ vs. $[C_1C_1C_1Pro]I$ do not allow direct comparisons, it should be noted that Br^- is a stronger hydrogen-bond acceptor than I^- ,⁵⁰ being thus expected to yield smaller biphasic regions. Since the opposite is observed, the effect of longer alkyl chains in $[C_2C_2C_2Pro]Br$ may overwhelm that of the anion nature (*cf.* Figure 1).

Figure 2. Phase diagrams of ABS composed of CILs and K_3PO_4 at $(25 \pm 1) ^\circ C$: $[C_1Qui][C_1SO_4]$ (blue dashed lined), $[C_1C_1C_1Val]I$ (red dashed-dotted line), $[C_1C_1C_1Val][C_1SO_4]$ (green dashed line), $[C_2C_2C_2Pro]Br$ (yellow solid line) and $[C_1C_1C_1Pro]I$ (orange dotted line).

Figure 3 shows the ability of three salts to promote phase separation, which can be rated as follows (at fixed CIL weight fraction composition of 10 wt%): $K_3PO_4 \approx K_2HPO_4 > K_2CO_3$. This ranking follows the Hofmeister series as previously established in the literature for ABS composed of ILs and salts.⁵¹

Figure 3. Phase diagrams of ABS composed of $[C_2C_2C_2Pro]Br$ and salts at $(25 \pm 1) ^\circ C$: K_3PO_4 (dark blue solid line), K_2HPO_4 (red dashed-dotted line) and K_2CO_3 (green dashed line).

CILs-based ABS: evaluating the impact of CILs structures in enantioseparation

An initial screening comprising the five CIL-based ABS developed (Table 1) was done in order to understand the role of the cation/anion structures on the enantioseparation of *R*- and *S*- mandelic acid structures. The extraction efficiencies (EE_{R-MA} and EE_{S-MA}) as well as enantiomeric excesses (*e.e.*) obtained are depicted in Figure 4 and detailed as Supplemental Material (Table S10). The $EE_{R/S-MA}$ values reveal a similar partition of mandelic acid between the two phases, or a preferential partition of mandelic acid for the salt-rich phase. Under the conditions adopted (initial biphasic mixture compositions, temperature and mandelic acid content – Table 1), all CILs exhibited preferable chiral recognition for the *S*-mandelic acid over the *R* enantiomer, with modest *e.e.* (1.61 ± 0.92 % to 17.37 ± 1.92 %). Moreover, valine and proline-based CILs seem to be more promising than the quinine-based CIL.

In general, electrostatic interactions between mandelic acid and the CIL cations plays an important role in the “three-point model”-based enantiorecognition process, since mandelic acid is deprotonated ($pK_{a1} = 3.75$ and $pK_{a2} = 13.57$, Figure S1 in Supplemental Material)⁴⁷ under the alkaline pH induced by K_3PO_4 . Given the chemical structures of the CILs and mandelic acid (*cf.* Figure 1) and the results found, it seems that other interactions can act in the mandelic acid enantiomeric discrimination by valine and proline-based CILs. Moreover, and contrarily to what is reported in literature when aromatic chiral recognition agents and solutes are present,⁵²⁻⁵⁴ in this specific case, π - π stacking does not seem to contribute to the enantioseparation of racemic mandelic acid, since $[C_1Qui][C_1SO_4]$ (the only CIL bearing aromatic rings) yielded the lowest *e.e.*.

It has been previously shown that factors other than the CIL structure may affect the enantioseparation ability and that such impact is dependent on the ABS phase

formers.^{43, 44} Bearing this in mind, optimization studies will be carried for two CILs, the least and the most performant ones, aiming to gain further insight on the phenomena governing enantioseparations in these CIL-based ABS.

Figure 4. Extraction efficiencies (EE_{R-MA} , yellow bars and EE_{S-MA} , green bars) and enantiomeric excesses (e.e., diamonds) obtained with five CIL-based ABS at 25 (± 1) °C.

[C₁Qui][C₁SO₄]-based ABS: evaluating the impact of mandelic acid content, temperature and TLL in enantioseparation

[C₁Qui][C₁SO₄], here identified as the weakest enantiorecognition agent, was used for further optimization to understand whether its enantioseparation ability could be improved by modifying the operational conditions. Mandelic acid content, TLL (varied by changing the mixture point) and temperature were evaluated, as presented in

Table 1. The results obtained are depicted in Figure 5 and detailed in Supplemental Material (Table S10) and suggest that, although having distinct effects on the extraction and separation of mandelic acid enantiomers, the parameters evaluated lead to better enantioseparations [from nearly 0 to a maximum *e.e.* of 7.88 ± 0.70 % obtained with [C₁Qui][C₁SO₄]-based ABS at $15 (\pm 1)$ °C].

While a decline of about 15 % on $EE_{R/S-MA}$ (a sign of mandelic acid loss of solubility in the [C₁Qui][C₁SO₄]-rich phase) is observed, an increase of ca. 3.8 times occurs by raising mandelic acid content in the system (Figure 5A). So, the enantioseparation seems to be ruled by a compromise between the solubility of mandelic acid in the CIL-rich phase and the more favorable interactions between the CIL and *S*-mandelic acid. The TLL effect is marginal under the conditions studied in this work (Figure 5B). Temperature, in turn, has a significant impact in the $EE_{R/S-MA}$, likely as a result of the increasing solubility of mandelic acid in the CIL-rich phase at 45 °C (Figure 5C). *e.e.* is higher for lower temperatures (e.g. 15 °C) where the molecular motions are slower, thus favoring the “*S*-mandelic acid-[C₁Qui][C₁SO₄]” interactions. The same behavior was previously observed in a work on the chiral separation of phenylalanine enantiomers with CIL-based ABS.⁴³

Figure 5. Impact of mandelic acid content (A), TLL (B) and temperature (C) on the extraction efficiencies (EE_{R-MA} , yellow bars and EE_{S-MA} , green bars) and enantiomeric excesses (e.e., diamonds) obtained with ABS composed of $[C_1Qui][C_1SO_4]$ and K_3PO_4 .

[C₂C₂C₂Pro]Br-based ABS: evaluating the impact of mandelic acid content, temperature, TLL, salt and phases' weight ratio in enantioseparation

Since the best enantioseparations were achieved with [C₂C₂C₂Pro]Br and [C₁C₁C₁Val][C₁SO₄], the role of the operational conditions on the performance of these ABS was further studied. The [C₂C₂C₂Pro]Br was used as a model chiral selector to evaluate the influence of mandelic acid content, temperature, TLL, salt and the phases' weight ratio, as specified in Table 1. Figure 6 overviews the results obtained with ABS composed of [C₂C₂C₂Pro]Br and K₃PO₄ and reveals a complex scenario regarding the impact of distinct operational conditions upon mandelic acid enantioseparation (detailed data provided as Supplemental Material, Table S10). Again, an indication of mandelic acid loss of solubility in the CIL-rich phase is observed. When the mandelic acid content is increased from 0.17 wt% to 1.17 wt% in the ABS, a 37% drop in $EE_{R/S-MA}$ is observed. The maximum enantioseparation is achieved at intermediate mandelic acid concentration ($e.e. = 17.37 \pm 1.92 \%$) - Figure 6A - this seeming to be the optimal mandelic acid/CIL compositions to favor "[C₂C₂C₂Pro]Br-S-mandelic acid" interactions.

The TLL influences both the extraction and enantioseparation performance of [C₂C₂C₂Pro]Br-based ABS, as shown in Figure 6B. Mixture points yielding longer TLLs, *i.e.* higher concentrations of both CIL and K₃PO₄ in both top and bottom phases, respectively, promote the extraction of mandelic acid towards the CIL-rich phase. This may be explained in the light of hydrophobic interactions occurring between the mandelic acid and the [C₂C₂C₂Pro]Br in the top phase. However, enantioseparations are less efficient under such conditions, indicating that the relative amounts of CIL/salt in the top phase and mandelic acid in the system are crucial to design efficient CIL-based ABS. Contrarily to what was observed for [C₁Qui][C₁SO₄], the temperature does not

significantly affect $EE_{R/S-MA}$ or $e.e.$ (Figure 6C). Therefore, both the solubility of mandelic acid in the $[C_2C_2C_2Pro]Br$ -rich phase and the specific interactions taking place between the CIL and the S -enantiomer seem to be unaffected on the temperature range studied.

Figure 6. Impact of mandelic acid content (A), TLL (B) and temperature (C) on the extraction efficiencies (EE_{R-MA} , yellow bars and EE_{S-MA} , green bars) and enantiomeric excesses (e.e., diamonds) obtained with ABS composed of $[C_2C_2C_2Pro]Br$ and K_3PO_4 .

The action of two additional salts (K_2HPO_4 and K_2CO_3) on the enantioseparation ability of $[\text{C}_2\text{C}_2\text{C}_2\text{Pro}]\text{Br}$ -based ABS was evaluated. It has been widely shown that the salt used has an important influence in the extraction and separation of a solute using ABS,⁴⁰ in particular if enantiomeric separations are targeted.⁴⁴ Figure 7 shows that also here the enantioseparation is dependent on the salt used. Under the conditions assessed (see Table 1), K_3PO_4 ranks first ($e.e. = 17.37 \pm 1.92 \%$), followed by K_2CO_3 ($e.e. = 5.79 \pm 0.12 \%$), while KH_2PO_4 completely failed to separate mandelic acid enantiomers ($e.e. = 0.82 \pm 0.18 \%$) (detailed data provided as Supplemental Material, Table S10). These salts create an alkaline pH to ABS ($\text{pH}_{\text{K}_2\text{HPO}_4}^{\text{CIL}} = 8.3 \pm 0.02$, $\text{pH}_{\text{K}_2\text{CO}_3}^{\text{CIL}} = 11.7 \pm 0.02$ and $\text{pH}_{\text{K}_3\text{PO}_4}^{\text{CIL}} = 13.0 \pm 0.02$), so that mandelic acid is deprotonated ($\text{pK}_{\text{a}1} = 3.75$ and $\text{pK}_{\text{a}2} = 13.57$)⁴⁷. In presence of K_3PO_4 mandelic acid deprotonates displaying a distribution of approximately 21% and 79 % for mono- and divalent ions, respectively.⁴⁷ The amount of divalent ions further decreases with decreasing pH, down to around 1.34 % (K_2CO_3) and completely vanishes at pH 9.2 (Figure S1 in Supplemental Material).⁴⁷ Better recognition ability seems to be accomplished when divalent mandelic acid ions are present in solution, in agreement with previous insights gathered in the chiral separation of mandelic acid in micellar systems containing copper- β -cyclodextrin-complexes as chiral selector.²⁰ Alongside, mandelic acid partitions majorly to the CIL-rich phase (more hydrophobic) when K_2HPO_4 is used ($EE_{R\text{-MA}} = 81.90 \pm 5.59 \%$ and $EE_{S\text{-MA}} = 81.63 \pm 5.08 \%$), while an almost equivalent distribution of mandelic acid between the two phases is observed for K_3PO_4 ($EE_{R\text{-MA}} = 44.13 \pm 0.03 \%$ and $EE_{S\text{-MA}} = 61.58 \pm 3.96 \%$) and K_2CO_3 ($EE_{R\text{-MA}} = 55.13 \pm 0.03 \%$ and $EE_{S\text{-MA}} = 62.61 \pm 2.31 \%$). Divalent mandelic acid is more polar than its monovalent congener, what may explain this extraction profile. It should be highlighted that the enantioseparation in these ABS may

be additionally influenced by specific interactions promoted by the salt ions or distinct solubility profiles exhibited by mandelic acid in the ABS phases.

Figure 7. Impact of salt on the extraction efficiencies (EE_{R-MA} , yellow bars and EE_{S-MA} , green bars) and enantiomeric excesses (e.e., diamonds) obtained with $[C_2C_2C_2Pro]Br$ -based ABS.

The body of results hitherto reported provides some evidence that the performance of CIL-based ABS in the enantioseparation of mandelic acid may be improved by manipulating the solubility of mandelic acid in the CIL-rich phase. At first glance, the preferential partition of the *S*-enantiomer to the $[C_2C_2C_2Pro]Br$ -rich phase is enhanced by constraining the mandelic acid solubility. To confirm this hypothesis, partition studies were carried out along the same TL for two distinct ABS (K_3PO_4 - and K_2HPO_4 -based), meaning that different weight ratios were used while the phases compositions were kept constant (Figure 8 and Table S10 from Supplemental Material). As shown in Figure 8A for the K_3PO_4 -based ABS, almost complete partition of

mandelic acid towards the $[C_2C_2C_2Pro]Br$ -rich phase occurs for systems possessing larger CIL-rich phases ($EE_{R-MA} = 87.17 \pm 7.70 \%$ - $94.51 \pm 5.94 \%$ and $EE_{S-MA} = 86.63 \pm 8.15 \%$ - $92.81 \pm 5.79 \%$). When the phases weight ratio is decreased, the CIL-rich phase becomes saturated, as revealed by the decreasing of mandelic acid partition ($EE_{R-MA} = 37.32 \pm 2.74 \%$ and $EE_{S-MA} = 48.10 \pm 2.62$). In previous studies distinct solubility profiles of the phenylalanine enantiomers in CILs phases were also observed.^{43, 55} A completely distinct pattern was observed by replacing K_3PO_4 by K_2HPO_4 , where neither $EE_{R/S-MA}$ nor $e.e.$ significantly vary along the tie line (Figure 8B), what must be related with the effect of the pH upon the charge of the mandelic acid as discussed above.

Figure 8. Extraction efficiencies (EE_{R-MA} , yellow bars and EE_{S-MA} , green bars) and enantiomeric excesses (e.e., diamonds) obtained with ABS composed of $[C_2C_2C_2Pro]Br + K_3PO_4$ (A) and $[C_2C_2C_2Pro]Br + K_2HPO_4$ (B) at 25 (± 1) °C and at distinct initial compositions along the same TL: binodal curve (dashed line), TL (solid line) and initial mixture composition (triangles).

Critical assessment of chiral ABS application in the enantioseparation of mandelic acid

As aforementioned, the satisfactory compromise between cost-effectiveness, broad applicability, easy operation and scale-up^{17, 18} has placed ABS in the spotlight. Four works using chiral ABS for chiral separation of mandelic acid enantiomers were previously reported. While most are based on the introduction of an extra chiral agent to the ABS,^{19, 20, 35} one proposes the use of chiral phase formers.³⁶ The phase formers are focused on polymer-salt,¹⁹ alcohol-salt³⁵ and micellar systems²⁰ incorporating β -cyclodextrin derivatives as well as on polymer-polymer³⁶ introducing a chiral compound acting as both phase former and chiral agent.³⁶ The enantioseparation ability is commonly evaluated by determining *e.e.* (Eq. 3) and/or enantioselectivity (α , the ratio between the partition coefficients of mandelic acid enantiomers). Overall, the enantioseparation abilities hitherto reported are highly dependent on the type of ABS and conditions adopted: ethanol- $(NH_4)_2SO_4$ + sulfonated- β -cyclodextrin with $\alpha = 1.69$ and *e.e.* = 16 %;³⁵ poly(ethylene) glycol- $(NH_4)_2SO_4$ + β -cyclodextrin with $\alpha = 2.46$ and *e.e.* = 42 %;¹⁹ and Triton X-114 + NaCl + copper- β -cyclodextrin complex with *e.e.* = 68 %.²⁰ The implementation of chiral phase formers yields less efficient enantioseparations ($\alpha = 1.27$).³⁶ With this strategy, employed in this work, the technological simplicity, target enantiomer polishing and ABS constituents recycling and reuse are enhanced.³⁶ Moreover with the ability to overcome technological limitations of polymeric ABS³⁶

(e.g. viscosity of the phases, limited hydrophobicity-hydrophilicity range and difficulty to find pairs of polymers able to form ABS), the CIL-based ABS here developed are somehow more efficient (maximum *e.e.* of 17.37 ± 1.92 %). However, given the limited understanding on their enantiorecognition mechanisms, the design of efficient chiral ABS platforms still relies on case-by-case studies and the broad applicability of ABS for these separations remains challenging.

Conclusions

On the search for alternative enantioseparation techniques, this work proposes the implementation of CILs as chiral phase formers in ABS to resolve racemic mandelic acid. The ternary phase diagrams of ABS constituted by five CILs bearing chirality in the cation and salts were ascertained under ambient conditions, with the hydrophobicity of the CIL cation and the salting out aptitude of the salt dictating the two-phase separation aptitude. After an initial screening where all five CILs were ranked according to their relative ability to separate mandelic acid enantiomers, a maximum *e.e.* of 17.37 ± 1.92 % was achieved with [C₂C₂C₂Pro]Br. The most and least promising CILs were object of detailed optimization, comprising the parameters mandelic acid content, temperature, TLL, salt and phases' weight ratio. With the CIL structure playing a central role, all remaining conditions were shown to influence the enantioseparation. Such impacts are highly dependent on the ABS nature: while temperature was the main factor improving the enantioseparation ability of [C₁Qui][C₁SO₄]-based ABS, [C₂C₂C₂Pro]Br-based ABS was mainly influenced by the salt used the phases weight ratio. Based on the optimization results it seems that the saturation of the CIL-rich phase rules the enantioseparation: *S*-mandelic acid (the enantiomer with higher affinity for this

set of CILs) remains in the CIL-rich phase, while *R*-mandelic acid partitions to the K₃PO₄-rich phase.

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