Solvent Extraction and Ion Exchange

ISSN: 0736-6299 (Print) 1532-2262 (Online) Journal homepage: https://www.tandfonline.com/loi/lsei20

Aqueous Biphasic Systems Using Chiral Ionic Liquids for the Enantioseparation of Mandelic Acid Enantiomers

Francisca A. e Silva, Mariam Kholany, Tânia E. Sintra, Magda Caban, Piotr Stepnowski, Sónia P. M. Ventura & João A. P. Coutinho

To cite this article: Francisca A. e Silva, Mariam Kholany, Tânia E. Sintra, Magda Caban, Piotr Stepnowski, Sónia P. M. Ventura & João A. P. Coutinho (2018) Aqueous Biphasic Systems Using Chiral Ionic Liquids for the Enantioseparation of Mandelic Acid Enantiomers, Solvent Extraction and Ion Exchange, 36:6, 617-631, DOI: 10.1080/07366299.2018.1545344

To link to this article: https://doi.org/10.1080/07366299.2018.1545344

SOLVENT EXTRACTION AND ION EXCHANGE
2018, VOL. 36, NO. 6, 617–631
https://doi.org/10.1080/07366299.2018.1545344

Taylor & Francis
Taylor & Francis Group

Aqueous Biphasic Systems Using Chiral Ionic Liquids for the Enantioseparation of Mandelic Acid Enantiomers

Francisca A. e Silva [a], Mariam Kholany [a], Tânia E. Sintra [a], Magda Caban [b], Piotr Stepnowski [b], Sónia P. M. Ventura [a], and João A. P. Coutinho [a]

[a]CICECO, Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal; [b]Department of Environmental Analysis, Institute for Environmental and Human Health Protection, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland

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 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 (TLL), salt and phases weight ratio were studied. The influence of these parameters is shown to be highly dependent on the CIL-based ABS, however 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] Asymmetric synthesis usually entails laborious and prolonged development processes, with uncertain outcome. Moreover, it often requires the use of expensive enantiopure raw materials or specific enantioselective catalysts.[3,5] The synthesis of racemates followed by their chiral resolution is a simpler, more flexible and cheaper alternative to asymmetric synthesis.[6,7] Crystallization and chromatography are the most used techniques for enantioseparation, due to their simplicity of operation.[6] Still, the former is limited by the number of racemic mixtures able to form conglomerates (estimated values from 5 to 10%), the excessive solids handling and the need for additional steps of enantiomeric

CONTACT João A. P. Coutinho jcoutinho@ua.pt CICECO, Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro 3810-193, Portugal

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lsei.

Supplemental data for this article can be accessed here.

enrichment; in turn, the latter suffers from high costs linked to chiral chromatographic columns, low loading capacities, and poor scale-up opportunities.[6,8]

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.[7] By bringing together solvent extraction with chiral recognition,[7] 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," that is, by establishing discriminative intermolecular interactions that lead to the formation of "chiral selector-enantiomer" complexes.[9] Combinations of organic solvents/water with β-cyclodextrin-based and/or tartaric acid-inspired chiral selectors are amongst the most studied ELLE systems.[10–15] Still, the large quantities of volatile organic solvents employed fail to match the recommendations of the Green Chemistry and Sustainability guidelines.[16,17]

Aqueous biphasic systems (ABS) are good candidates to turn enantioseparations not only into more biocompatible but also more versatile approaches.[18,19] 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),[18,20–36] some of them bearing chiral centers (e.g. polymer-chiral polymer, polar organic solvents-oligosaccharides),[37–39] 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 relies on the addition of an extra chiral selector to the system formulation (not essential to two-phase formation)[20–36] and another where one of the solutes has chirality, thus acting simultaneously as phase-former and chiral selector.[37–39] 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.[20–36] 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.[31] 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.[37–39] 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.[37–39] 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.[39] 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,[40] allowing the design of task-specific solvents and, by their introduction in ABS, of highly performant extraction/separation approaches.[41] 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 1999 by Seddon and co-workers.[42] Ever since, although hundreds of other CILs were proposed,[43] 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-[44] and tropine-based CILs[45] and inorganic salts. Under optimized conditions, enantiomeric excesses of 53%[44] and 65%[45] 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,[44,45] 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. 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.[46] 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, $[C_1C_1C_1Pro][C_1SO_4]$; *N,N*-diethyl-L-proline ethyl ester bromide, $[C_2C_2C_2Pro]Br$; *N,N,N*-trimethyl-L-valinolium iodide, $[C_1C_1C_1Val]I$; and *N,N,N*-trimethyl-L-valinolium methylsulfate, $[C_1C_1C_1Val][C_1SO_4]$. For the synthesis, the reagents used were quinine, Qui (purity = 98%), iodomethane, CH_3I (purity = 99%), dimethyl sulfate, $(CH_3)_2SO_4$ (purity = 99%), dichloromethane anhydrous, CH_2Cl_2 (purity = 99.8%), ethanol, C_2H_5OH (purity = 99.8%), acetone, C_3H_6O (HPLC grade), potassium carbonate, K_2CO_3 (purity ≥ 99%), L-proline, L-Pro (purity = 99%), bromoethane, CH_3CH_2Br (purity = 98%), acetonitrile, CH_3CN (purity = 99.8%), chloroform, $CHCl_3$ (purity = 99%), L-valine, L-Val (purity = 98%), tetrahydrofuran anhydrous, C_4H_8O (purity = 99.9%), sodium borohydride, $NaBH_4$ (purity = 99%), sulfuric acid, H_2SO_4 (purity = 99.9%), methanol, CH_3OH (purity = 99%), ethyl acetate, $C_4H_8O_2$ (purity = 99.8%), potassium hydroxide, KOH (purity = 90%), formic acid, HCOOH (purity = 98%), formaldehyde, CH_2O (37 wt % in water solution) and hydrochloric acid, HCl (37 wt% in water solution) acquired from Sigma-Aldrich. The salts used in ABS were potassium phosphate tribasic, K_3PO_4 (purity = 97%), K_2CO_3 (purity ≥ 99%), and dipotassium hydrogen phosphate trihydrate, $K_2HPO_4{\cdot}3H_2O$ (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 S1 from Supplemental Material.

For the HPLC-DAD analysis of the mandelic acid enantiomers, copper (II) sulphate pentahydrate, $CuSO_4 \cdot 5H_2O$ (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.

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.[46] Briefly, an alkylation reaction between dimethyl sulfate and quinine yielding $[C_1Qui][C_1SO_4]$ 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 iodomethane, bromoethane, or dimethyl sulfate affording $[C_1C_1C_1Pro]I$, $[C_2C_2C_2Pro]Br$ or $[C_1C_1C_1Pro][C_1SO_4]$, respectively. Relevant features of CILs, namely melting temperature (T_m, °C), decomposition temperature (T_d, °C) and specific rotations ($[\alpha]_D^{25}$) can be consulted in Supplemental Material, Table S1.[46]

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 (±1)°C.[41] To aqueous solutions

containing ca. 6–70 wt% of CILs, the alternate drop-wise addition of an aqueous solution of salt at ca. 40–50 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[47]

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

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.[47] 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 (±1)°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.[41]

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 Supplemental Material, Table S2. 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.[44] 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 ($EE_{\text{R-MA}}$ and $EE_{\text{S-MA}}$, %) were separately determined according to the Equation 2:

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

where $\mathrm{m}^{\mathrm{CIL}}_{R/S-\mathrm{MA}}$ is the mass of *R* or *S*-mandelic acid present in the CIL-rich phase and $\mathrm{m}^{0}_{R/S-\mathrm{MA}}$ 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:

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

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

Mandelic acid enantiomers quantification

Mandelic acid enantiomers were quantified by HPLC-DAD using an analytical method adapted from Yue et al. with modifications.[48] The liquid chromatograph HPLC Elite LaChrom (VWR Hitachi) used for this purpose was equipped with a diode array detector (DAD) l-2455, column oven l-2300, auto-sampler l-2200 and pump l-2130. A C_{18} reversed-phase analytical column (LiChrospher 100 RP-18, 5 µm, 250 mm × 4 mm i.d.) linked to a guard column (5 µm, 4 mm × 4 mm) with the same stationary phase was used. The column oven and autosampler were operated at controlled temperature of 22°C and 25°C, respectively. The mobile phase was made up of water:methanol [85:15 (v/v)], 2 mM L-phenylalanine and 1 mM $CuSO_4$ at pH = 4.00 (±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 µL. 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 $\mu g.mL^{-1}$ 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 $\mu g.mL^{-1}$ and 10 $\mu g.mL^{-1}$ 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 µm). 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, $[C_1C_1C_1Val]I$, $[C_1C_1C_1Val][C_1SO_4]$, $[C_2C_2C_2Pro]Br$, $[C_1C_1C_1Pro]I$, and $[C_1Qui][C_1SO_4]$, and K_3PO_4, a strong salting-out agent,[41] were measured at 25 (±1)°C. $[C_1C_1C_1Pro][C_1SO_4]$ was not able to form ABS with K_3PO_4. Two additional salts, K_2CO_3 and K_2HPO_4, were paired with $[C_2C_2C_2Pro]Br$ to evaluate the role of salt type upon ABS formation.

The ternary phase diagrams are shown in Figures 1 and 2 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 (Tables S3–S11). The ternary phase diagrams determined in this study provide information on the CILs and salt role upon ABS formation (Figures 1 and 2). 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 1, the CILs' ability to form ABS with K_3PO_4 can be ranked as follows (at fixed CIL weight fraction composition of 10 wt%): $[C_1Qui][C_1SO_4]$ > $[C_1C_1C_1Val]I$ > $[C_1C_1C_1Val][C_1SO_4]$ > $[C_2C_2C_2Pro]Br$ > $[C_1C_1C_1Pro]I$. Within the CILs studied, it is possible to infer on both cation ($[C_1Qui][C_1SO_4]$ *vs.* $[C_1C_1C_1Val][C_1SO_4]$ *vs.* $[C_1C_1C_1Pro][C_1SO_4]$) and anion role ($[C_1C_1C_1Val]I$ *vs.* $[C_1C_1C_1Val][C_1SO_4]$) on the ABS formation. The cation effect is driven by the hydrophobicity-hydrophilicity of the cation, where the order $[C_1Qui]^+$ > $[C_1C_1C_1Val]^+$ > $[C_1C_1C_1Pro]^+$ 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, respectively[49]). In close agreement with previous studies,[41] the more hydrophobic the CIL the higher is 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

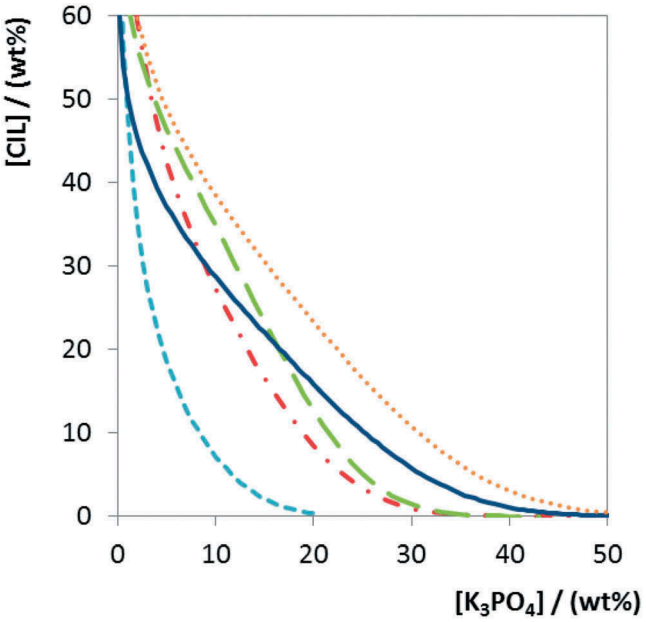

Figure 1. Phase diagrams of ABS composed of CILs and K_3PO_4 at (±1)°C: $[C_1Qui][C_1SO_4]$ (blue dashed line), $[C_1C_1C_1Val]I$ (red dashed-dotted line), $[C_1C_1C_1Val][C_1SO_4]$ (green dashed line), $[C_2C_2C_2Pro]Br$ (dark blue solid line), and $[C_1C_1C_1Pro]I$ (orange dotted line).

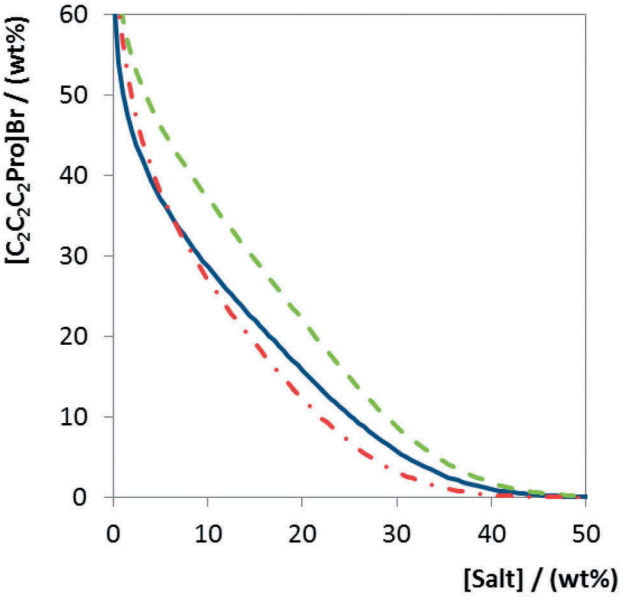

Figure 2. Phase diagrams of ABS composed of $[C_2C_2C_2Pro]Br$ and salts at (±1)°C: K_3PO_4 (dark blue solid line), K_2HPO_4 (red dashed-dotted line), and K_2CO_3 (green dashed line).

hydrophobicity of the cation when compared to the parent compound, due to alkylation: while the addition of three methyl groups is done to valinol, only two methyl groups are added to proline methyl ester (*cf.* Figure S1 in Supplemental Material).

In general, the ability of an IL anion to create ABS is related with the decrease in their hydrogen-bond accepting ability (β).[50,51] 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.*[52] 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^-,[52] 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 S1 in Supplemental Material).

Figure 2 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.[53,54]

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

An initial screening comprising the five CIL-based ABS developed (Supplemental Material, Table S2) 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 ($EE_{R\text{-}MA}$ and $EE_{S\text{-}MA}$) as well as enantiomeric excesses (*e.e.*) obtained are depicted in Figure 3 and detailed in Supplemental Material (Table S12). The $EE_{R/S\text{-}MA}$ 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 S2 in Supplemental Material), 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 play an important role in the "three-point model"-based enantiorecognition process, since mandelic acid is deprotonated (pKa_1 = 3.75 and pKa_2 = 13.57, Figure S2 in Supplemental Material)[49] under the alkaline pH induced by K_3PO_4. Given the chemical structures of the CILs and mandelic acid (*cf.* Figure S1 in Supplemental Material) and the results found, it seems that other interactions can act in the

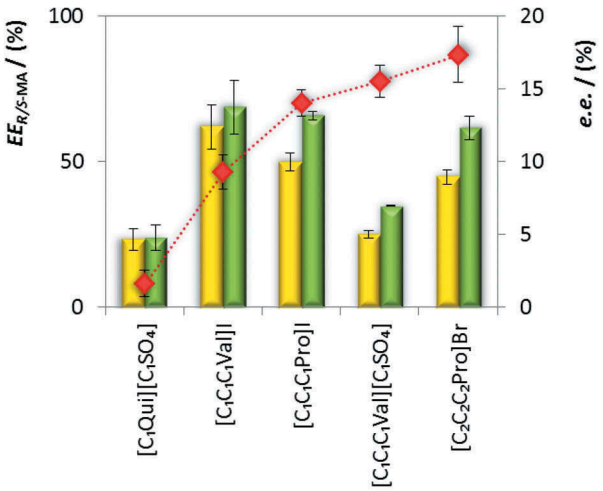

Figure 3. Extraction efficiencies ($EE_{R\text{-}MA}$, yellow bars and $EE_{S\text{-}MA}$, green bars) and enantiomeric excesses (*e.e.*, diamonds) obtained with five CIL-based ABS at 25 (±1)°C.

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,[55–57] 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.[44,45] 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.

$[C_1Qui][C_1SO_4]$-based ABS: evaluating the impact of mandelic acid content, temperature and TLL in enantioseparation

$[C_1Qui][C_1SO_4]$, 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 Supplemental Material (Table S2). The results obtained are depicted in Figure 4 and detailed in Supplemental Material (Table S12) 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 $[C_1Qui][C_1SO_4]$-based ABS at 15 (±1)°C].

While a decline of about 15 % on $EE_{R/S\text{-}MA}$ is observed, an increase of ca. 3.8 times in *e.e.* occurs by raising mandelic acid content in the system (Figure 4a). 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 4b). Temperature, in turn, has a significant impact in the $EE_{R/S\text{-}MA}$, likely as a result of the increasing solubility of mandelic acid in the CIL-rich phase at 45°C (Figure 4c). Furthermore, Figure 4c provides support of a trade-off between $EE_{R/S\text{-}MA}$ and *e.e.* parameters. *e.e.* is higher for lower temperatures (e.g. 15°C) where the molecular motions are slower, thus favoring the "*S*-mandelic acid-$[C_1Qui][C_1SO_4]$" interactions. The same behavior was previously observed in a work on the chiral separation of phenylalanine enantiomers with CIL-based ABS.[44]

$[C_2C_2C_2Pro]Br$-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 $[C_2C_2C_2Pro]Br$ and $[C_1C_1C_1Val][C_1SO_4]$, the role of the operational conditions on the performance of these ABS was further studied. The $[C_2C_2$

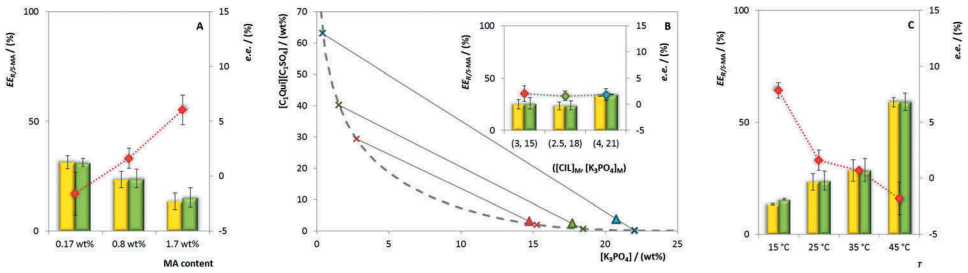

Figure 4. Impact of mandelic acid content (a), TLL (b), and temperature (c) on the extraction efficiencies ($EE_{R\text{-}MA}$, yellow bars and $EE_{S\text{-}MA}$, green bars) and enantiomeric excesses (*e.e.*, diamonds) obtained with ABS composed of $[C_1Qui][C_1SO_4]$ and K_3PO_4.

C_2Pro]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 S2 in Supplemental Material. Figure 5 overviews the results obtained with ABS composed of [$C_2C_2C_2$Pro]Br and K_3PO_4 and reveals a complex scenario regarding the impact of distinct operational conditions upon mandelic acid enantioseparation (detailed data provided as Supplemental Material, Table S12). When mandelic acid content is increased from 0.17 wt% to 1.7 wt% in the ABS, a 37% drop in $EE_{R/S\text{-}MA}$ is observed. The maximum enantioseparation is achieved at intermediate mandelic acid concentration (*e.e.* = 17.37 ± 1.92 %) - Figure 5a - this seeming to be the optimal mandelic acid/CIL compositions to favor "[$C_2C_2C_2$Pro]Br-*S*-mandelic acid" interactions. A shift towards *R*-enantiomer higher partitions to the CIL-rich phase seems to occur (negative *e.e.* values are merely indicative of a *R*-mandelic acid enriched mixture) at cost of *e.e.* at the lowest (*e.e.* of 4.91 ± 1.34 %) and highest (*e.e.* of 6.40 ± 2.92 %) concentrations ascertained. Although this behavior may seem odd, similar results are found in literature, showing that low to intermediate concentrations are enantioseparation boosters.[20,24,37,39,58] It should however be emphasized that the type of system, chiral selector, operational conditions and racemic compound may lead to different dependencies.[26,45,48,59] The lowest concentration of mandelic acid investigated seems to restrict the occurrence of "[$C_2C_2C_2$Pro]Br-*S*-mandelic acid" interactions, thus limiting enantioseparation.[45]

The TLL influences both the extraction and enantioseparation performance of [$C_2C_2C_2$Pro]Br-based ABS, as shown in Figure 5b. Mixture points yielding longer TLLs, *i.e.* higher concentrations of both CIL and K_3PO_4 in both top and bottom phases, respectively, promote the extraction of mandelic acid toward the CIL-rich phase. This may be explained in the light of hydrophobic interactions occurring between the mandelic acid and the [$C_2C_2C_2$Pro]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 [C_1Qui][C_1SO_4], the temperature does not significantly affect $EE_{R/S\text{-}MA}$ or *e.e.* (Figure 5c). Therefore, both the solubility of mandelic acid in the [$C_2C_2C_2$Pro]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.

The action of two additional salts (K_2HPO_4 and K_2CO_3) on the enantioseparation ability of [$C_2C_2C_2$Pro]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,[41] in particular if enantiomeric separations are targeted.[45] Figure 6 shows that also here the enantioseparation is dependent on the salt used. Under the conditions assessed (see Table S2 in Supplemental Material), K_3PO_4 ranks first (*e.e.* = 17.37 ± 1.92 %), followed by K_2CO_3 (*e.e.* = 5.79 ± 0.12 %), while K_2HPO_4 completely failed to separate mandelic acid enantiomers (*e.e.* = 0.82 ± 0.18 %) (detailed data provided as Supplemental Material, Table S12). These salts create an alkaline pH in ABS ($pH^{CIL}_{K_2HPO_4}$= 8.32 ± 0.02, $pH^{CIL}_{K_2CO_3}$= 11.68 ± 0.02 and $pH^{CIL}_{K_3PO_4}$= 12.99 ± 0.02), so that mandelic acid is deprotonated (pKa_1

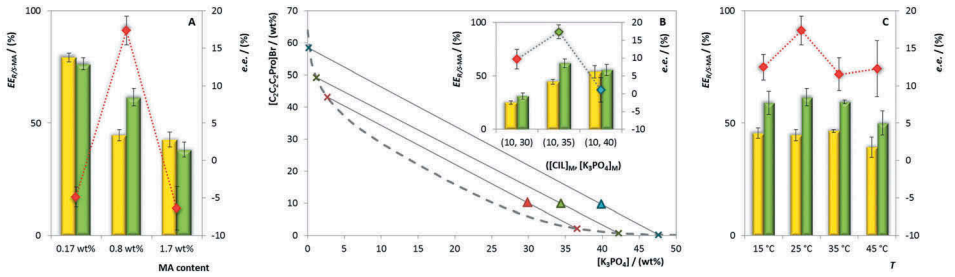

Figure 5. Impact of mandelic acid content (a), TLL (b), and temperature (c) on the extraction efficiencies ($EE_{R\text{-}MA}$, yellow bars and $EE_{S\text{-}MA}$, green bars) and enantiomeric excesses (*e.e.*, diamonds) obtained with ABS composed of [$C_2C_2C_2$Pro]Br and K_3PO_4.

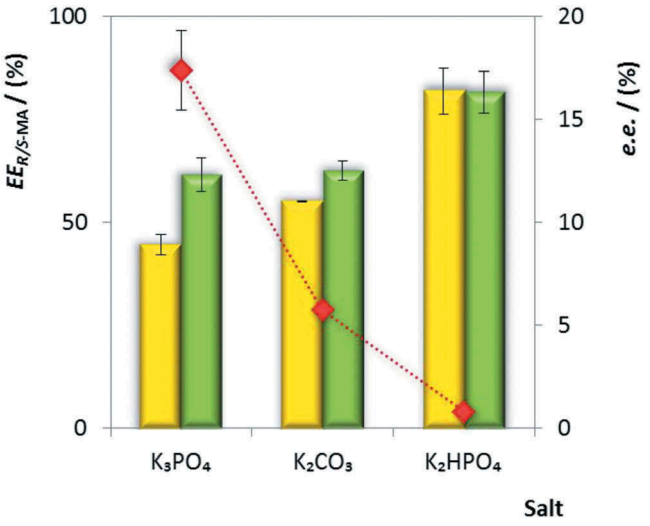

Figure 6. Impact of salt on the extraction efficiencies ($EE_{R\text{-}MA}$, yellow bars and $EE_{S\text{-}MA}$, green bars) and enantiomeric excesses (*e.e.*, diamonds) obtained with $[C_2C_2C_2Pro]Br$-based ABS.

= 3.75 and pKa_2 = 13.57).[49] In presence of K_3PO_4 mandelic acid deprotonates displaying a distribution of approximately 79% and 21% for mono- and divalent ions, respectively.[49] 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 S2 in Supplemental Material).[49] 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.[21] Alongside, mandelic acid partitions majorly to the CIL-rich phase (more hydrophobic) when K_2HPO_4 is used (EE_{R-MA} = 81.90 ± 5.59 % and EE_{S-MA} = 81.63 ± 5.08 %), while an almost equivalent distribution of mandelic acid between the two phases is observed for K_3PO_4 (EE_{R-MA} = 44.62 ± 2.50 % and EE_{S-MA} = 61.58 ± 3.96 %) and K_2CO_3 (EE_{R-MA} = 55.13 ± 0.03 % and EE_{S-MA} = 62.61 ± 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 corresponding ABS phases.

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 7 and Table S12 from Supplemental Material). As shown in Figure 7a 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 ($EE_{R\text{-}MA}$ = 87.17 ± 7.70% – 94.51 ± 5.94% and $EE_{S\text{-}MA}$ = 86.63 ± 8.15% – 92.81 ± 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 ± 2.74% and EE_{S-MA} = 48.10 ± 2.62). In previous studies, distinct solubility profiles of the phenylalanine enantiomers in CILs phases were also observed.[44,60] A completely distinct pattern was observed by replacing K_3PO_4 by K_2HPO_4, where neither $EE_{R/S\text{-}MA}$ nor *e.e.* significantly vary along the tie line (Figure 7b), what must be related with the effect of the pH upon the charge of the mandelic acid as discussed above.

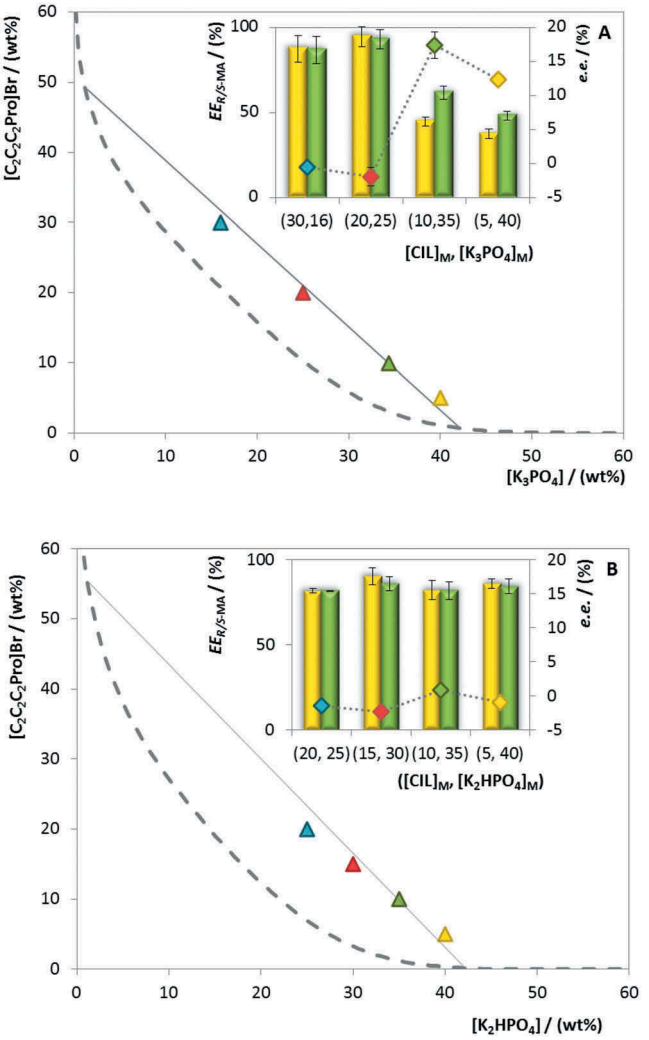

Figure 7. Extraction efficiencies ($EE_{R\text{-}MA}$, yellow bars and $EE_{S\text{-}MA}$, green bars) and enantiomeric excesses (*e.e.*, diamonds) obtained with ABS composed of $[C_2C_2C_2Pro]Br + K_3PO_4$ (a) and $[C_2C_2C_2Pro]Br + K_2HPO_4$ (b) at 25 (±1)°C and at distinct initial compositions along 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[18,19] 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,[20,21,36] one proposes the use of chiral phase formers.[37] The phase formers are focused on polymer-salt,[20] alcohol-salt,[36] and micellar systems[21] incorporating β-cyclodextrin derivatives as well as on polymer-polymer introducing a chiral compound acting as both phase former and chiral agent.[37] 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 α = 1.69 and *e.e.* = 16 %[36]; poly(ethylene) glycol-$(NH_4)_2SO_4$ + β-cyclodextrin with α = 2.46 and *e.e.* = 42 %[20]; and Triton X-114 + copper-β-cyclodextrin complex with *e.e.* = 68 %.[21] Yet, the implementation of chiral phase formers yields less efficient enantioseparations (α = 1.27).[37] With this strategy, also employed in this work, the technological simplicity, target enantiomer polishing and ABS constituents recycling and reuse are enhanced.[37] Moreover, with the ability to overcome technological limitations of polymeric ABS[37] (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 %). In addition, the possibility of using chiral cationic and/or anionic groups[43] affords the opportunity of designing specific CILs structures able to interact more specifically with some particular enantiomeric structures, avoiding the use of complex extraction systems, since these can act simultaneously as chiral selectors and solvents. Most purification systems reported in literature lack specificity towards the enantiomers structures, which leads to low specificity on their separations. Moreover, ILs can be combined with a large range of phase formers to generate ABS,[41] providing an extra degree of tailoring the enantioseparations. Such features offer unique opportunities to plan ABS to match specific enantioseparations.[41] Finally, some CILs show great promise in the field as they can be synthesized by simple, practical and benign routes,[43] using natural precursors[43] and available in large scale.[5] 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 $[C_2C_2C_2Pro]Br$. 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 $[C_1Qui][C_1SO_4]$-based ABS, $[C_2C_2C_2Pro]Br$-based ABS was mainly influenced by the salt used and 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 K_3PO_4-rich phase.

Acknowledgments

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, financed by national funds through the FCT/MEC and when appropriate cofinanced by FEDER under the PT2020 Partnership Agreement. ChiResus - Chiral resolution of drugs in centrifugal partition chromatography using enantioselective aqueous biphasic systems (POCI-01-0145-FEDER-030750), supported by the Operational Program Competitiveness and Internationalization, in its FEDER/FNR component, and the Foundation for Science and Technology, in its national State Budget component (OE). M. Caban acknowledges COST Action CM1206 (EXIL – Exchange on Ionic Liquids) for the STSM grant (Ref. COST-STSM-ECOST-STSM-CM1206-011115-066588).

Funding

This work was supported by the Fundação para a Ciência e a Tecnologia [UID/CTM/50011/2013]; Fundação para a Ciência e a Tecnologia [IF/00402/2015]; Fundação para a Ciência e a Tecnologia [SFRH/BD/94901/2013]; Fundação para a Ciência e a Tecnologia [SAICTPAC/0040/2015].

ORCID

Francisca A. e Silva http://orcid.org/0000-0001-6735-5437
Mariam Kholany http://orcid.org/0000-0002-6279-2053
Tânia E. Sintra http://orcid.org/0000-0002-7686-5970
Magda Caban http://orcid.org/0000-0002-8040-5862
Piotr Stepnowski http://orcid.org/0000-0001-5022-6962
Sónia P. M. Ventura http://orcid.org/0000-0001-9049-4267
João A. P. Coutinho http://orcid.org/0000-0002-3841-743X

References

[1] Nguyen, L. A.; He, H.; Pham-Huy, C. Chiral Drugs: An Overview. *Int. J. Biomed. Sci.* 2006, *2*(2), 85–100.
[2] FDA's Policy Statement for the Development of New Stereoisomeric Drugs. *Chirality*. 1992, *4*(5), 338–340. DOI: 10.1002/chir.530040513.
[3] Mane, S. Racemic Drug Resolution: A Comprehensive Guide. *Anal. Methods*. 2016, *8*(42), 7567–7586. DOI: 10.1039/C6AY02015A.
[4] Farina, V.; Reeves, J. T.; Senanayake, C. H.; Song, J. J. Asymmetric Synthesis of Active Pharmaceutical Ingredients. *Chem. Rev*. 2006, *106*(7), 2734–2793. DOI: 10.1021/cr040700c.
[5] Blaser, H.-U. Chirality and Its Implications for the Pharmaceutical Industry. *Rend. Fis. Acc. Lincei*. 2013, *24*(3), 213–216. DOI: 10.1007/s12210-012-0220-2.
[6] Lorenz, H.; Seidel-Morgenstern, A. Processes To Separate Enantiomers. *Angew. Chem. Int. Ed*. 2014, *53*(5), 1218–1250. DOI: 10.1002/anie.v53.5.
[7] Schuur, B.; Verkuijl, B. J. V.; Minnaard, A. J.; de Vries, J. G.; Heeres, H. J.; Feringa, B. L. Chiral Separation by Enantioselective Liquid-Liquid Extraction. *Org. Biomol. Chem*. 2011, *9*(1), 36–51. DOI: 10.1039/c0ob00610f.
[8] Maier, N. M.; Franco, P.; Lindner, W. Separation of Enantiomers: Needs, Challenges, Perspectives. *J. Chromatogr. A*. 2001, *906*(1), 3–33.
[9] Davankov, V. A. The Nature of Chiral Recognition: Is It a Three-Point Interaction? *Chirality*. 1997, *9*(2), 99–102. DOI: 10.1002/(ISSN)1520-636X.
[10] Tang, K.; Song, L.; Liu, Y.; Pan, Y.; Jiang, X. Separation of Flurbiprofen Enantiomers by Biphasic Recognition Chiral Extraction. *Chem. Eng. J*. 2010, *158*(3), 411–417. DOI: 10.1016/j.cej.2010.01.009.
[11] Tang, K.; Zhang, P.; Pan, C.; Li, H. Equilibrium Studies on Enantioselective Extraction of Oxybutynin Enantiomers by Hydrophilic β-cyclodextrin Derivatives. *AIChE J*. 2011, *57*(11), 3027–3036. DOI: 10.1002/aic.v57.11.
[12] Tang, K.; Yi, J.; Liu, Y.; Jiang, X.; Pan, Y. Enantioselective Separation of R,S-phenylsuccinic Acid by Biphasic Recognition Chiral Extraction. *Chem. Eng. Sci*. 2009, *64*(18), 4081–4088. DOI: 10.1016/j.ces.2009.06.029.
[13] Tang, K.; Chen, Y.; Liu, J. Resolution of Zopiclone Enantiomers by Biphasic Recognition Chiral Extraction. *Sep. Purif. Technol*. 2008, *62*(3), 681–686. DOI: 10.1016/j.seppur.2008.03.029.
[14] Ren, Z.; Zeng, Y.; Hua, Y.; Cheng, Y.; Guo, Z. Enantioselective Liquid–Liquid Extraction of Racemic Ibuprofen by l-Tartaric Acid Derivatives. *J. Chem. Eng. Data*. 2014, *59*(8), 2517–2522. DOI: 10.1021/je500292c.
[15] Tang, K.; Li, H.; Liu, Y. Kinetic Study for Biphasic Recognition Chiral Extraction of Naproxen Enantiomers with Hydrophobic L-Iso-Butyl Tartrate and Hydrophilic Hydroxypropyl-β-Cyclodextrin. *Solv. Ext. Ion Exc*. 2012, *30*(3), 291–305. DOI: 10.1080/07366299.2011.639252.
[16] Glavič, P.; Lukman, R. Review of Sustainability Terms and Their Definitions. *J. Clean. Prod*. 2007, *15*(18), 1875–1885. DOI: 10.1016/j.jclepro.2006.12.006.
[17] Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford university press, 2000.
[18] Iqbal, M.; Tao, Y.; Xie, S.; Zhu, Y.; Chen, D.; Wang, X.; Huang, L.; Peng, D.; Sattar, A.; Shabbir, M. A. B.; et al. Aqueous Two-Phase System (ATPS): An Overview and Advances in Its Applications. *Biol. Proced. Online*. 2016, *18*, 18. DOI: 10.1186/s12575-016-0048-8.
[19] Soares, R. R. G.; Azevedo, A. M.; Van Alstine, J. M.; Aires-Barros, M. R. Partitioning in Aqueous Two-Phase Systems: Analysis of Strengths, Weaknesses, Opportunities and Threats. *Biotechnol. J*. 2015, *10*(8), 1158–1169. DOI: 10.1002/biot.201400532.

[20] Tan, L.; Long, Y.; Jiao, F.; Chen, X. Enantioselective Extraction of Mandelic Acid Enantiomers by Aqueous Two-Phase Systems of Polyethylene Glycol and Ammonium Sulfate Containing β-cyclodextrin as Chiral Selector. *J. Iran. Chem. Soc.* 2011, *8*(4), 889–896. DOI: 10.1007/BF03246544.
[21] Xing, J.-M.; Li, -F.-F. Chiral Separation of Mandelic Acid by Temperature-Induced Aqueous Two-Phase System. *J. Chem. Technol. Biotechnol.* 2012, *87*(3), 346–350. DOI: 10.1002/jctb.v87.3.
[22] Li, L.-H.; Li, -F.-F. Chiral Separation of α-cyclohexyl-mandelic-acid by Aqueous Two Phase System Combined with Cu2-β-cyclodextrin Complex. *Chem. Eng. J.* 2012, *211–212*, 240–245. DOI: 10.1016/j.cej.2012.09.058.
[23] Chen, -L.-L.; Li, -F.-F.; Tan, Z.-J. Chiral Separation of α-cyclohexylmandelic Acid Enantiomers Using Ionic Liquid/Salt Aqueous Two-Phase System. *Chem. Pap.* 2015, *69*(11), 1465–1472. DOI: 10.1515/chempap-2015-0162.
[24] Chen, X.; Wang, J.; Jiao, F. Efficient Enantioseparation of Phenylsuccinic Acid Enantiomers by Aqueous Two-Phase System-Based Biphasic Recognition Chiral Extraction: Phase Behaviors and Distribution Experiments. *Process. Biochem.* 2015, *50*(9), 1468–1478. DOI: 10.1016/j.procbio.2015.05.014.
[25] Zhuang, J.; Yang, W.; Chen, X.; Jiao, F. Enantioseparation of Phenylsuccinic Acid Enantiomers Using Aqueous Two-Phase Flotation and Their Determination by HPLC and UV Detection. *Chromatographia.* 2014, *77*(9), 679–685. DOI: 10.1007/s10337-014-2668-y.
[26] Wang, J.; Chen, X.; Jiao, F. Enantioseparation of Phenylsuccinic Acid Enantiomers Based on Aqueous Two-Phase System with Ethanol/Ammonium Sulfate: Phase Diagrams Optimization and Partitioning Experiments. *J. Incl. Phenom. Macro. Chem.* 2015, *81*(3), 475–484. DOI: 10.1007/s10847-015-0477-z.
[27] Jiao, F.; Wang, J.; Jiang, X.; Yang, H.; Shi, S.; Chen, X.; Yu, J. Biphasic Recognition Enantioseparation of Ofloxacin Enantiomers by an Aqueous Two-Phase System. *J. Chem. Technol. Biotechnol.* 2015, *90*(12), 2234–2239. DOI: 10.1002/jctb.2015.90.issue-12.
[28] Arai, T.; Kuroda, H. Distribution Behavior of Some Drug Enantiomers in an Aqueous Two-Phase System Using Counter-Current Extraction with Protein. *Chromatographia.* 1991, *32*(1), 56–60. DOI: 10.1007/BF02262467.
[29] Chen, Z.; Zhang, W.; Wang, L.; Fan, H.; Wan, Q.; Wu, X.; Tang, X.; Tang, J. Z. Enantioseparation of Racemic Flurbiprofen by Aqueous Two-Phase Extraction with Binary Chiral Selectors of L-Dioctyl Tartrate and L-Tryptophan. *Chirality.* 2015, *27*(9), 650–657. DOI: 10.1002/chir.22481.
[30] Shinomiya, K.; Kabasawa, Y.; Ito, Y. Enantiomeric Separation of Commercial D,L-Kynurenine with an Aqueous Two-Phase Solvent System by Cross-Axis Coil Planet Centrifuge. *J. Liq. Chromatogr. Relat. Technol.* 1998, *21*(1–2), 135–141. DOI: 10.1080/10826079808001942.
[31] Ni, Y.; Zhou, J.; Sun, Z. Production of a Key Chiral Intermediate of Betahistine with a Newly Isolated Kluyveromyces Sp. In an Aqueous Two-Phase System. *Process. Biochem.* 2012, *47*(7), 1042–1048.
[32] Chen, X.; Liu, L.; Jiao, F.; Wang, Z. Extraction of Phenylalanine Enantiomers by Aqueous Two Phase Systems Containing Combinatorial Chiral Selector. *Chin. J. Chem.* 2012, *30*(4), 965–969. DOI: 10.1002/cjoc.201100224.
[33] Ekberg, B.; Sellergren, B.; Albertsson, P.-Å. Direct Chiral Resolution in an Aqueous Two-Phase System Using the Counter-Current Distribution Principle. *J. Chromatogr. A.* 1985, *333*, 211–214. DOI: 10.1016/S0021-9673(01)87344-1.
[34] Chen, X.-Q.; Dong, Q.-L.; Yu, J.-G.; Jiao, F.-P. Extraction of Tryptophan Enantiomers by Aqueous Two-Phase Systems of Ethanol and (NH4)2SO4. *J. Chem. Technol. Biotechnol.* 2013, *88*(8), 1545–1550. DOI: 10.1002/jctb.4001.
[35] Tong, S.; Ito, Y.; Ma, Y. Enantioseparation of Dl-Tryptophan by Spiral Tube Assembly Counter-Current Chromatography and Evaluation of Mass Transfer Rate for Enantiomers. *J. Chromatogr. A.* 2014, *1374*, 77–84. DOI: 10.1016/j.chroma.2014.11.013.
[36] Li, -F.-F.; Tan, Z.-J.; Guo, Z.-F. Enantioseparation of Mandelic Acid and α-cyclohexylmandelic Acid Using an Alcohol/Salt-Based Aqueous Two-Phase System. *Chem. Pap.* 2014, *68*(11), 1539–1545. DOI: 10.2478/s11696-014-0594-y.
[37] Tan, Z.; Li, F.; Zhao, C.; Teng, Y.; Liu, Y. Chiral Separation of Mandelic Acid Enantiomers Using an Aqueous Two-Phase System Based on a Thermo-Sensitive Polymer and Dextran. *Sep. Purif. Technol.* 2017, *172*, 382–387. DOI: 10.1016/j.seppur.2016.08.039.
[38] Wang, J.; Liu, Q.; Rong, L.; Yang, H.; Jiao, F.; Chen, X. Enantioselective Extraction of Phenylsuccinic Acid in Aqueous Two-Phase Systems Based on Acetone and β-cyclodextrin Derivative: Modeling and Optimization through Response Surface Methodology. *J. Chromatogr. A.* 2016, *1467*, 490–496. DOI: 10.1016/j.chroma.2016.06.054.
[39] Wang, J.; Yang, H.; Yu, J.; Chen, X.; Jiao, F. Macrocyclic β-cyclodextrin Derivative-Based Aqueous-Two Phase Systems: Phase Behaviors and Applications in Enantioseparation. *Chem. Eng. Sci.* 2016, *143*, 1–11. DOI: 10.1016/j.ces.2015.12.019.
[40] Freemantle, M. Designer Solvents: Ionic Liquids May Boost Clean Technology Development. *Chem. Eng. News.* 1998, *76*(13), 32–37. DOI: 10.1021/cen-v076n013.p032.
[41] Freire, M. G.; Cláudio, A. F. M.; Araújo, J. M. M.; Coutinho, J. A. P.; Marrucho, I. M.; Lopes, J. N. C.; Rebelo, L. P. N. Aqueous Biphasic Systems: A Boost Brought about by Using Ionic Liquids. *Chem. Soc. Rev.* 2012, *41*(14), 4966–4995. DOI: 10.1039/c2cs35151j.

[42] Earle, J. M.; McCormac, B. P.; Seddon, R. K. Diels-Alder Reactions in Ionic Liquids. A Safe Recyclable Alternative to Lithium Perchlorate-Diethyl Ether Mixtures. *Green Chem*. 1999, *1*(1), 23–25. DOI: 10.1039/a808052f.
[43] Payagala, T.; Armstrong, D. W. Chiral Ionic Liquids: A Compendium of Syntheses and Applications (2005–2012). *Chirality*. 2012, *24*(1), 17–53. DOI: 10.1002/chir.21975.
[44] Wu, D.; Zhou, Y.; Cai, P.; Shen, S.; Pan, Y. Specific Cooperative Effect for the Enantiomeric Separation of Amino Acids Using Aqueous Two-Phase Systems with Task-Specific Ionic Liquids. *J. Chromatogr. A*. 2015, *1395*, 65–72. DOI: 10.1016/j.chroma.2015.03.047.
[45] Wu, H.; Yao, S.; Qian, G.; Yao, T.; Song, H. A Resolution Approach of Racemic Phenylalanine with Aqueous Two-Phase Systems of Chiral Tropine Ionic Liquids. *J. Chromatogr. A*. 2015, *1418*, 150–157. DOI: 10.1016/j.chroma.2015.09.058.
[46] Sintra, T. E.; Synthesis of More Benign Ionic Liquids for Specific Applications. PhD Thesis, University of Aveiro, 2017.
[47] Merchuk, J. C.; Andrews, B. A.; Asenjo, J. A. Aqueous Two-Phase Systems for Protein Separation: Studies on Phase Inversion. *J. Chromatogr. B Biomed. Sci. Appl*. 1998, *711*(1), 285–293.
[48] Yue, Y.; Jiang, X.-Y.; Yu, J.-G.; Tang, K.-W. Enantioseparation of Mandelic Acid Enantiomers in Ionic Liquid Aqueous Two-Phase Extraction Systems. *Chem. Pap*. 2014, *68*(4), 465–471. DOI: 10.2478/s11696-013-0467-9.
[49] Chemspider. The Free Chemical Database at. http://www.chemspider.com (accessed Feb 10, 2018).
[50] Cláudio, A. F. M.; Ferreira, A. M.; Shahriari, S.; Freire, M. G.; Coutinho, J. A. P. Critical Assessment of the Formation of Ionic-Liquid-Based Aqueous Two-Phase Systems in Acidic Media. *J. Phys. Chem. B*. 2011, *115* (38), 11145–11153. DOI: 10.1021/jp204865a.
[51] Passos, H.; Dinis, T. B. V.; Cláudio, A. F. M.; Freire, M. G.; Coutinho, J. A. P. Hydrogen Bond Basicity of Ionic Liquids and Molar Entropy of Hydration of Salts as Major Descriptors in the Formation of Aqueous Biphasic Systems. *Phys. Chem. Chem. Phys*. 2018. DOI: 10.1039/C8CP01401A.
[52] Cláudio, A. F. M.; Swift, L.; Hallett, J. P.; Welton, T.; Coutinho, J. A. P.; Freire, M. G. Extended Scale for the Hydrogen-Bond Basicity of Ionic Liquids. *Phys. Chem. Chem. Phys*. 2014, *16*(14), 6593–6601. DOI: 10.1039/c3cp55285c.
[53] Shahriari, S.; Neves, C. M. S. S.; Freire, M. G.; Coutinho, J. A. P. Role of the Hofmeister Series in the Formation of Ionic-Liquid-Based Aqueous Biphasic Systems. *J. Phys. Chem. B*. 2012, *116*(24), 7252–7258. DOI: 10.1021/jp300874u.
[54] Li, S.; He, C.; Liu, H.; Li, K.; Liu, F. Ionic Liquid-Based Aqueous Two-Phase System, a Sample Pretreatment Procedure Prior to High-Performance Liquid Chromatography of Opium Alkaloids. *J. Chromatogr. B*. 2005, *826*(1), 58–62. DOI: 10.1016/j.jchromb.2005.08.005.
[55] Kacprzak, K.; Maier, N.; Lindner, W. Unexpected Enantioseparation of Mandelic Acids and Their Derivatives on 1,2,3-Triazolo-Linked Quinine Tert-Butyl Carbamate Anion Exchange-Type Chiral Stationary Phase. *J. Sep. Sci*. 2010, *33*(17–18), 2590–2598. DOI: 10.1002/jssc.201000393.
[56] Chen, S. The Enantioseparation of Amino Acids on a Teicoplanin Chiral Stationary Phase Using Non-Aqueous Mobile Phases after Pre-Column Derivatization with Sulfur-Containing Reagents: The Considerations of Mobile Phase Composition and Analyte Structure Variation on Resolution Enhancement. *Biomed. Chromatogr*. 2006, *20*(8), 718–728. DOI: 10.1002/bmc.587.
[57] Jitsukawa, K.; Katoh, A.; Funato, K.; Ohata, N.; Funahashi, Y.; Ozawa, T.; Masuda, H. Kinetic Resolution of rac-Phenylalanine by Stereoselective Complexation to a Chiral Cobalt Complex through π–π Stacking Interaction. *Inorg. Chem*. 2003, *42*(20), 6163–6165. DOI: 10.1021/ic030135g.
[58] Meng, H.; Yan, T.; Jiao, F.; Wang, S. Enantioseparation of Phenylsuccinic Acid Enantiomers by Solvent Sublation with Collaborative Selectors. *J. Solution Chem*. 2017, *46*(12), 2159–2170. DOI: 10.1007/s10953-017-0689-5.
[59] Wang, Z.; Hou, Z.; Yao, S.; Lin, M.; Song, H. A New and Recyclable System Based on Tropin Ionic Liquids for Resolution of Several Racemic Amino Acids. *Anal. Chim. Acta*. 2017, *960*, 81–89. DOI: 10.1016/j.aca.2017.01.050.
[60] Tang, F.; Zhang, Q.; Ren, D.; Nie, Z.; Liu, Q.; Yao, S. Functional Amino Acid Ionic Liquids as Solvent and Selector in Chiral Extraction. *J. Chromatogr. A*. 2010, *1217*(28), 4669–4674. DOI: 10.1016/j.chroma.2010.05.013.