POTENTIAL OF AQUEOUS TWO-PHASE SYSTEMS FOR THE SEPARATION OF LEVODOPA FROM SIMILAR BIOMOLECULES

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Abstract

BACKGROUND: Levodopa is a precursor of several neurotransmitters, such as dopamine, and is used in the treatment of the Parkinson’s disease. In this work, an alternative strategy was studied to separate levodopa from similar biomolecules using aqueous two-phase systems (ATPS).

RESULTS: Ternary ATPS composed of polyethylene glycol (PEG) 400 or ionic liquids (ILs), citrate buffer ($K_3C_6H_5O_7/C_6H_8O_7$) at pH 7.0 and water, and quaternary ATPS composed of PEG 400, $K_3C_6H_5O_7/C_6H_8O_7$ at pH 7.0, water and the same ILs at 5 wt%, were studied. The respective liquid-liquid phase diagrams were determined at 298 K to appraise the mixture compositions required to form two-phase systems, followed by studies of the partition of levodopa and structurally similar biomolecules (dopamine, L-phenylalanine, and L-tyrosine). Their partition coefficients and extraction efficiencies have been determined, and the selectivity of the ATPS to separate levodopa from the remaining biomolecules evaluated.

CONCLUSION: The results obtained indicated that PEG-based ATPS were the most effective to separate levodopa from L-phenylalanine while the separation from the other biomolecules was better using IL-based ATPS, in particular those based on $[P_{4444}]Cl$ and $[N_{4444}]Cl$, with extraction efficiencies of levodopa to the salt-rich phase ranging between 62.7 and 74.0%, and of the remaining biomolecules to polymer/IL-rich phase up to 91.5%.

Keywords: aqueous two-phase systems, levodopa, amino acids, extraction, separation, ionic liquids

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Introduction

Parkinson’s disease is a neurodegenerative disorder partially defined by the decrease of dopamine production.\(^1\) However, dopamine cannot be effectively used for the treatment of Parkinson’s disease because it is not able to cross the blood–brain barrier.\(^2,3\) Unlike dopamine, levodopa can cross the blood-brain barrier reaching the central nervous system, where it is converted into dopamine.\(^4,5\) Levodopa is thus a precursor of dopamine, and of other neurotransmitters, like norepinephrine and epinephrine.\(^6\)

The main treatment for the Parkinson’s disease involves the administration of synthetic levodopa.\(^7\) However, one of the common side effects of synthetic levodopa is dyskinesia (drug-induced involuntary muscle movement). Levodopa can also be obtained from a natural source and was first isolated from the seeds of *Mucuna pruriens* in 1937.\(^8\) When the value of this compound for the treatment of Parkinson’s disease became known, a large scientific interest in plants rich in levodopa was revived.\(^1\) Lieu et al.\(^9\) observed that in animal models levodopa naturally extracted from *M. pruriens* produces better results than its synthetic counterpart. Misra and Wagner\(^10\) studied the extraction of levodopa from Mucuna seeds using different solvents. A good extraction yield was obtained using a mixture of ethanol-water (1:1), using ascorbic acid as a protector. Pulikkalpura et al.\(^11\) evaluated the extraction of levodopa from the same biomass using a mixture of formic acid-alcohol (1:1). In these works, levodopa was extracted from a natural and complex feedstock (seeds of mucuna) containing different contaminants. Junnotula and Licea-Perez\(^12\) used protein precipitation methods and solid-phase extractions as extraction procedures. Overall, most of these studies addressed the use of volatile organic solvents both in the extraction and separation procedures.

Liquid-liquid extractions by aqueous two-phase systems (ATPS) have been intensively explored and used to separate and purify several biological products,\(^13\)\(^–\)\(^18\) and also to recover metal ions, radiochemicals, and synthetic drugs from complex mixtures.\(^19\) ATPS can be prepared by mixing aqueous solutions of two polymers, a polymer and a salt, or two different salts above concentrations
defined by the systems binodal curve. Polyethylene glycol (PEG) is frequently used as one of the phase forming compounds due to its low cost and facility to form a two-phase system with other neutral polymers as well as salts. Despite their simplicity and low cost, the performance of the more traditional polymer-salt systems is hampered by the limited range of polarities of the coexisting phases. To overcome this limitation, the use of ionic liquids (ILs) as phase-forming components, or in lower quantities as adjuvants or additives in polymer-salt ATPS, was proposed to enhance the extraction performance of ATPS for several biomolecules. Since the first work reporting on ATPS composed of ILs and inorganic salts, by Rogers and co-workers, the number of publications dealing with IL-based ATPS has been steadily increasing.

The main characteristics of ILs include a high solvation ability, non-flammability, high thermal and chemical stabilities, negligible vapor pressure, and a tailoring ability achieved by the large number of possible cation-anion combinations, being this last property particularly relevant and transferrable to IL-based ATPS.

Aiming at evaluating ATPS as alternative strategies for the separation of levodopa from similar biomolecules (L-phenylalanine, L-tyrosine and dopamine), two types of systems were here investigated: (i) ternary ATPS based on polyethylene glycol (PEG 400) or ILs ([C₄mim]Cl, [C₄mpyr]Cl, [C₄mpip]Cl, [N₄444]Cl, [P₄444]Cl) + (K₃C₆H₅O₇/C₆H₈O₇) at pH 7.0 + H₂O; and (ii) quaternary ATPS formed by polyethylene glycol (PEG 400) + (K₃C₆H₅O₇/C₆H₈O₇) at pH 7.0 + H₂O + ILs as additives at 5 wt%.

**Experimental**

**Materials**

Polyethylene glycol with an average molecular weight of 400 g·mol⁻¹ (PEG 400) was supplied by Sigma-Aldrich (Germany). Tri-potassium citrate monohydrate (K₃C₆H₅O₇·H₂O, 99 wt% pure) and citric acid (C₆H₈O₇, 99 wt% pure) were purchased from Fisher Scientific (USA) and Prolabo (Belgium), respectively. The ILs studied were: 1-butyl-3-methylimidazolium chloride, [C₄mim]Cl (> 99 wt% pure); 1-butyl-1-methylpyrrolidinium chloride, [C₄mpyr]Cl (> 99 wt% pure); 1-butyl-1-methylpiperidinium chloride, [C₄mpip]Cl (> 99 wt% pure); tetrabutylammonium chloride, [N₄444]Cl.
(> 97 wt% pure); and tetrabutylphosphonium chloride [P$_{4444}$]Cl (> 96 wt% pure). [C$_{4}$mim]Cl, [C$_{4}$mpyr]Cl and [C$_{4}$mpip]Cl were purchased from Iolitec (Germany), while [N$_{4444}$]Cl was acquired from Sigma-Aldrich (Germany) and [P$_{4444}$]Cl was kindly supplied by Cytec Ind. (USA). Di-sodium hydrogenphosphate (Na$_2$HPO$_4$, 99 wt% pure), sodium di-hydrogenphosphate (NaH$_2$PO$_4$, > 99 wt% pure) and ortho-phosphoric acid (85 wt% pure) were acquired at Panreac (Spain). Levodopa (> 98 wt% pure) and dopamine (> 98 wt% pure) were acquired from Sigma-Aldrich (Germany). The water employed was double distilled, passed across a reverse osmosis system and finally treated with a Milli-Q plus 185 water purification apparatus (Millipore, USA).

The partition of dopamine and levodopa was investigated in this work, whereas partition data for the amino acids L-phenylalanine and L-tyrosine were taken from the literature,$^{28}$ using the studied ATPS and at the same mixture compositions. The molecular structures and some characteristics of the biomolecules investigated are provided in Table S1 in Supporting Information.

The chemical structures and the hydrogen-bond acidity ($\alpha$) of the investigated ILs are reported in Table S2 in Supporting Information. The values of $\alpha$ were determined based on the correlations proposed by Kurnia et al.$^{29}$

**Phase diagrams**

The ATPS studied in this work were composed of water, K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ buffer at pH 7.0, PEG 400, and several chloride-based ILs. The binodal curves of the studied systems were obtained by the cloud point titration method at 298 (± 1) K and at atmospheric pressure, as previously described.$^{30}$ Aqueous solutions of K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ buffer, pH 7.0 at 50 wt% and of each IL at 75 wt% were prepared for the determination of the IL-salt phase diagrams. To determine the phase diagram corresponding to the PEG-salt ATPS, pure PEG 400 and an aqueous solution of K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ buffer, pH 7.0 at 50 wt% were used. Finally, to determine the phase diagrams for the quaternary systems using ILs as adjuvants (kept at 5 wt% in all mixture compositions), aqueous solutions of 50 wt% K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$, pH 7.0 + 5 wt% of each IL, pure PEG 400 + 5 wt% of each IL and aqueous solutions of 5 wt% of each IL were employed. In this case, ILs were assumed to be part of the solvent in the representation of the phase diagrams. Repetitive drop-wise addition of the
K₃C₆H₅O₇/C₆H₈O₇ buffer solution to the IL or PEG, or PEG/IL aqueous solutions was carried out until the detection of a cloudy solution, followed by the drop-wise addition of water (pure or aqueous solutions of each IL) until the detection of a clear solution. This procedure was carried out under constant stirring. The systems compositions were determined by the weight quantification of all components added within ±10⁻⁴ g.

The experimental binodal curves were correlated using Eq. (1):

\[ [Y] = A \exp[(BX^{0.5}) - (CX^3)] \]  

(1)

where \([Y]\) is the PEG, (PEG + IL) or IL weight percentages (wt%), \([X]\) is (K₃C₆H₅O₇/C₆H₈O₇) or (K₃C₆H₅O₇/C₆H₈O₇ + IL) wt%, and \(A\), \(B\) and \(C\) are constants obtained by the regression of the experimental data.

**Partition and separation of biomolecules using ATPS**

The partition behavior of levodopa and dopamine in the studied systems was determined in ternary ATPS composed of IL + K₃C₆H₅O₇/C₆H₈O₇ + H₂O and PEG + K₃C₆H₅O₇/C₆H₈O₇ + H₂O, and in the quaternary systems formed by PEG + K₃C₆H₅O₇/C₆H₈O₇ + H₂O + IL at 5 wt% as additive. The partition of dopamine and levodopa was investigated in this work, whereas partition data of the amino acids L-phenylalanine and L-tyrosine were taken from the literature, using the same ATPS and at the same mixture compositions. Aiming at avoiding the interference of tie-line length (TLL) in the partition coefficients, the experiments were carried out at TLL (ca. 70). The initial compositions used are reported in Table S6 in Supporting Information.

Aqueous solutions of each biomolecule (1.0 g·L⁻¹), namely levodopa and dopamine, were used as part of the water composition in the ATPS partition studies. All phase-forming components were weighted within ±10⁻⁴ g, vigorously stirred for 5 min, and allowed to equilibrate at 298 (± 1) K for at least 12 h. After this period, the top and bottom phases were carefully separated and weighted. The biomolecules concentration in each phase was measured by UV-spectroscopy, using a synergy/HT microplate reader (Biotek, USA), at a wavelength of 280 nm, using calibration curves previously established. Aiming at avoiding the interference of the salt, PEG and IL in the quantification, blank
control samples were always used. At least two individual experiments were carried out for each ATPS.

The quantification of a mixture of L-tyrosine, L-phenylalanine and levodopa (it was not possible to quantify dopamine since its peak overlaps the levodopa peak) was carried out by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD, Shimadzu, model PROMINENCE, China). HPLC analyses were performed with an analytical C18 reversed-phase column (250 × 4.60 mm), Kinetex 5 μm C18 100 Å, from Phenomenex (USA). The mobile phase consisted of 95% of a phosphate buffer solution (20 mM of NaH₂PO₄ and 10 mM Na₂HPO₄) at pH of 2.5, adjusted with ortho-phosphoric acid, and 5% of acetonitrile. The separation was conducted in isocratic mode, at a flow rate of 1.5 mL·min⁻¹ and using an injection volume of 30 μL. DAD was set at 280 nm for levodopa and 196 nm for L-tyrosine and L-phenylalanine. Each sample was analyzed at least in duplicate. The column oven and the autosampler were operated at a controlled temperature of 35 °C. Levodopa, L-tyrosine and L-phenylalanine display retention times of 2.07, 2.33 and 3.95 minutes, respectively, and their areas were used for determination of each partition coefficient.

The partition coefficients \( K \) of each biomolecule were determined according to Eq. (2),

\[
K = \frac{C_T}{C_B}
\]  

(2)

where \( C_T \) and \( C_B \) are the equilibrium concentrations (g·L⁻¹) of each biomolecule in the top and in the bottom phase, respectively. It should be remarked that the top phase corresponds to the PEG-rich phase in both the polymer-salt and polymer-salt ATPS with ILs as adjuvants, and to the IL-rich phase in the IL-salt ATPS.

The extraction efficiencies (%) \( EE \) of dopamine, L-phenylalanine and L-tyrosine are defined as the percentage ratio between the amount of each biomolecule in the top phase and that in the total mixture, according to Eq. (3),

\[
\%EE = \frac{w_T}{w_T + w_B} \times 100
\]  

(3)
where $w_T$ and $w_B$ are the total weight of each biomolecule in the top and bottom phases, respectively. Since levodopa preferentially partitions to the bottom phase, its %$EE$ is defined as the percentage ratio between the amount of levodopa in the bottom phase and that in the total mixture.

The selectivity ($S_{\text{biom/lev}}$) on the $K$ of each biomolecule (L-phenylalanine, L-tyrosine or dopamine) to the polymer-rich phase in respect to levodopa was calculated according to Eq. (4):

$$S_{\text{biom/lev}} = \frac{K}{K_{\text{lev}}}$$

(4)

where $K_{\text{lev}}$ corresponds to the partition coefficient specifically of levodopa and $K$ corresponds to the partition coefficient of the remaining biomolecules (L-phenylalanine, L-tyrosine or dopamine), as defined above.

**Results and Discussion**

**Phase diagrams**

The phase diagrams of ternary ATPS formed by different combinations of IL + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ at pH 7.0 + H$_2$O, as well as of PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ at pH 7.0 + H$_2$O, and of quaternary systems composed of PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ at pH 7.0 + H$_2$O + IL at 5 wt% as adjuvant were here measured. The binodal data (express in mass fractions) of all systems studied are presented in the Supporting Information (Tables S3 and S4). All experimental binodal curves were correlated using the Merchuk equation,$^{31}$ described by Eq. (1). The regression parameters were estimated by the least-squares regression method, and their values and corresponding standard deviations ($\sigma$) are provided in Table S5 in the Supporting Information. In general, good correlation coefficients were obtained for all systems indicating that these fittings can be used to predict the phase diagram in regions where no experimental results are available. Estimated compositions of the phases (wt%) in equilibrium for the mixtures used in the extraction experiments and respective values of TLL are presented in Table S6 in the Supporting Information.

The phase diagrams for the ternary systems studied are shown in Figure 1. All phase diagrams are presented in molality units to avoid discrepancies, which could be a result of the differences between the PEG, K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ and IL molecular weights. The experimental results show that
the ability of ILs and PEG to induce the formation of two-phase systems with the common salt follows the trend: \([P_{4444}]Cl > [N_{4444}]Cl > \text{PEG} > [C_4\text{mpip}]Cl > [C_4\text{mpyr}]Cl > [C_4\text{mim}]Cl\).

The sequence obtained in this work with a citrate-based salt is similar to that previously reported for other salts,\(^{22,32}\) meaning that the ATPS dependency on the IL cation trend is essentially independent of the salt employed. Freire et al.\(^{33}\) reported that ILs composed of the imidazolium cation, due to their aromatic character, present stronger interactions with water and are thus more difficult to be salted-out; therefore, ATPS formed by \([C_4\text{mim}]Cl\) presents the smallest biphasic region. On the other hand, quaternary ammonium and phosphonium-based ILs, being the bulkier and more hydrophobic of the ILs studied, present the largest biphasic regions. In general, the ILs ability to form ATPS correlates well with their molar volume (\(V_m\)) and \(\alpha\), described in Table S2 in Supporting Information. ILs with a lower \(\alpha\) are less able to establish hydrogen-bonds with water, and are therefore more easily salted-out.\(^{32,34}\) Their largest \(V_m\), formed essentially by alkyl chains, makes them also more hydrophobic.

In addition to the ternary systems, novel quaternary phase diagrams were determined for PEG 400 + \(K_3C_6H_5O_7/C_6H_8O_7\) at pH 7.0 + \(H_2O + \text{ILs}\) as adjuvants (5 wt%), at 298 K and at atmospheric pressure. The respective phase diagrams are shown in Fig. 2, in molality units to better evaluate the ILs impact on the phase separation.

The addition of all ILs investigated leads to an increase in the biphasic region, \(i.e.\) lower amounts of salt or PEG are required to form two-phase systems. Two quite different behaviors are here observed: the first corresponding to the cyclic ILs that have a small influence on the phase diagrams; and another when the quaternary ammonium and phosphonium salts are used with a very significant effect upon the phase diagram. Although there seems to be a similar trend on the quaternary phase diagrams to that observed for the ternary, here the presence of the IL contributes in all cases to increase the biphasic region, unlike to what is observed in the ternary phase diagrams for which some systems have biphasic regions smaller than that of the PEG system. Globally, it seems that mixtures of IL–PEG are more hydrophobic than their pure components, since the IL
preferentially migrates to the PEG-rich phase\textsuperscript{28} increasing its hydrophobicity, and are thus more easily salted-out by the citrate-based salt in aqueous media.

**Partition and separation of biomolecules using ATPS**

The $K$ of levodopa and dopamine measured in the ternary (IL + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_5$O$_7$ + H$_2$O) and quaternary (PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_5$O$_7$ + H$_2$O + IL) systems studied are shown in Figs. 3 and 4. The $K$ of L-phenylalanine and of L-tyrosine were taken from our previous work,\textsuperscript{28} and are also depicted in Figs. 3 and 4 for comparison purposes. The detailed values of $K$ are provided in the Supporting Information, Table S7.

In the systems studied all biomolecules preferentially partition to the polymer-rich phase, with exception of levodopa that significantly partitions to the salt-rich phase. The log $K_{ow}$ values of all biomolecules are given in Table S2 in Supporting Information. The log $K_{ow}$ of levodopa is -2.39, indicating that it is more hydrophilic than L-phenylalanine, L-tyrosine and dopamine (log $K_{ow}$ of -1.38, -2.26, and -0.98, respectively) and displaying thus a higher affinity to the more hydrophilic salt-rich phase. In IL-based ATPS, the Ks of the biomolecules range between 1.10 and 10.5, with L-phenylalanine and L-tyrosine being extensively extracted to the IL-rich phase when using the more hydrophobic ILs, \textit{i.e.} quaternary ammonium and phosphonium salts ($K$ values up to 10.5). On the other hand, the Ks of levodopa in these systems range between 1.10 and 1.52, meaning that the IL used seems to have a negligible effect on its partition.

In quaternary systems no significant differences are observed in the Ks obtained for the systems using ILs as adjuvants, nor among the various ILs, when compared with those obtained for the (PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_5$O$_7$ + H$_2$O) system with no IL added.

The main goal of this work was to identify the best ATPS to separate levodopa from other analogous or structurally similar compounds, such as dopamine and the amino acids L-phenylalanine and L-tyrosine to which it is associated in biologic functions or from which it can be produced.\textsuperscript{35–37} According to the results of $K$s and $S$s reported in Figs. 4 and 5, the best ternary IL-based ATPS to separate levopoda from dopamine is the one formed by [N$_{4444}$]Cl, whereas the system composed of
[P4444]Cl is the most appropriate to separate levodopa from the amino acids L-phenylalanine and L-tyrosine, with a maximum $S$ value of 8.3 and 7.7, respectively. The highest $S$ to separate levodopa from L-phenylalanine (up to 13.0) was achieved with the ternary system composed of PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$, although the quaternary systems (using ILs as adjuvants) also performs well, with $S$s ranging from 10.3 with [C$_4$mim]Cl up to 12.6 with [P4444]Cl.

The $K$s of levodopa increase in the order: PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ (0.81) < PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ + IL as adjuvants (from 0.83 to 0.86) < IL + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ (from 1.10 to 1.52), meaning that levodopa preferentially partitions to the salt-rich phase in systems composed of PEG. As the $S$s of levodopa are similar for ternary (PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ + H$_2$O, 13.0 for $S_{\text{phe/lev}}$, 6.62 for $S_{\text{tyr/lev}}$ and 1.89 for $S_{\text{dop/lev}}$) and quaternary systems (PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ + H$_2$O + IL, ranging from 10.3 to 12.6 for $S_{\text{phe/lev}}$, from 4.89 to 5.79 for $S_{\text{tyr/lev}}$ and 2.13 to 3.66 for $S_{\text{dop/lev}}$), the simpler and cheaper ternary system, with no IL added, is the preferred one for separating levodopa from similar biomolecules. This system can be seen as highly selective taking into account the chemical structure similarity (cf. Table S1 in Supporting Information) between levodopa and the remaining studied biomolecules, where levodopa is enriched in the bottom salt-rich phase and the other biomolecules are mainly present in the top polymer-rich phase.

The $\%EE$ of levodopa, dopamine, L-phenylalanine and L-tyrosine using the ternary (PEG/IL + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ + H$_2$O) and quaternary (PEG + K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$ + H$_2$O + IL) ATPS are shown in Figs. 5 and 6. The extraction efficiencies corresponding to levodopa were determined for the salt-rich phase, whereas the remaining were determined in respect to the IL- or PEG-rich phase. The detailed values of $\%EE$ are provided in the Supporting Information, Table S8.

In ternary IL-based ATPS the $\%EE$ of levodopa to the salt-rich phase range between 42.4 and 48.3%, whereas the $\%EE$ of the remaining biomolecules to the IL-rich phase range between 71.6 and 90.6%. For the PEG-based ATPS the $\%EE$ of levodopa to the salt-rich phase range between 62.7 and 74.0%, against the $\%EE$ of the remaining biomolecules to the polymer-rich phase ranging between 41.0 and 91.5%. The $\%EE$ of the studied biomolecules to the polymer-rich phase (in quaternary
systems) follow the trend: L-phenylalanine > L-tyrosine > dopamine > levodopa. With the exception of dopamine, this trend follows the biomolecules log $K_{ow}$ values given in Table S1 in Supporting Information, suggesting that more hydrophobic molecules tend to migrate preferentially to the PEG-rich phase, the most hydrophobic one. In summary, the results obtained indicate that PEG-based ATPS are the most effective to separate levodopa from L-phenylalanine while the separation from the other biomolecules is better using IL-based ATPS, in particular those based on [P4444]Cl and [N4444]Cl, with %EE of levodopa to the salt-rich phase ranging between 62.7 and 74.0%, and of the remaining biomolecules to polymer/IL-rich phase up to 91.5%. Giving the good results obtained with the PEG-salt ternary system, a mixture containing all biomolecules was used to evaluate their separation in this ternary system. For that purpose HPLC analysis was performed and was found that dopamine has the same retention time as levodopa, and for that reason only the mixture containing levodopa, L-tyrosine and L-phenylalanine was evaluated. The results obtained (levodopa $K = 0.57 \pm 0.03$; L-tyrosine $K = 3.49 \pm 0.99$ and L-phenylalanine $K = 3.05 \pm 0.06$) are in satisfactory agreement with the ones gathered with the pure biomolecules in UV. The fact that the values obtained by HPLC are lower than the ones obtained by UV are expected since the behavior of molecules in a mixture can be different when compared to the pure. Despite this fact, here it is shown that is possible to separate levodopa from the other similar amino acids.

**Conclusions**

In this work, ternary and quaternary (using ILs as adjuvants) ATPS were investigated for Levodopa purification, aiming at identifying promising systems able to separate it from similar biomolecules, such as dopamine, L-tyrosine and L-phenylalanine. The results here obtained, unlike the ones suggested in the literature, show that there is no significant advantages of using ILs as adjuvants for these separations. Here, it is shown that ternary PEG-based ATPS are the most effective to separate levodopa from other biomolecules with %EE up to 74.0%. This was reinforced by the results obtained studying the partition of each molecule in a mixture using this system. Therefore,
this study opens new routes for the development of alternative purification processes for levodopa from natural or synthetic sources.

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30. Neves CMSS, Ventura SPM, Freire MG, Marrucho IM and Coutinho JAP, Evaluation of cation influence on the formation and extraction capability of ionic-liquid-based aqueous biphasic


Fig 1. Ternary phase diagrams, at 298 K and atmospheric pressure, for the ATPS of PEG 400 / IL
+ (K$_3$C$_6$H$_5$O$_7$/C$_6$H$_8$O$_7$) pH 7.0 + H$_2$O: ◊, [C$_4$mim]Cl; o, [C$_4$mypyr]Cl; ×, [C$_4$mpip]Cl; □, [N$_4$4444]Cl;

Δ, [P$_4$4444]Cl and ▲, PEG400. Lines represent data correlations using Eq. (1).
Fig 2. Quaternary phase diagrams, at 298 K and atmospheric pressure, for the ATPS of PEG 400 + (K₃C₆H₅O₇/C₆H₈O₇) pH 7.0 + H₂O + ILs (as adjuvants): ◊, [C₄mim]Cl; o, [C₄pyr]Cl; ×, [C₄mpip]Cl; □, [N₄₄₄₄]Cl; and △, [P₄₄₄₄]Cl. ▲, ternary system corresponding to the ATPS (PEG400 + (K₃C₆H₅O₇/C₆H₈O₇)). Lines represent data correlations using Eq. (1).
Fig. 3. Partition coefficients ($K$) of L-phenylalanine, L-tyrosine, dopamine and levodopa, and Selectivities ($S_{\text{biom/lev}}$) to levodopa, using ternary ATPS.
Fig 4. Partition coefficients ($K$) of L-phenylalanine, L-tyrosine, dopamine, and levodopa, and selectivities ($S_{\text{biom/lev}}$) to levodopa, using quaternary ATPS.
Fig. 5. Extraction efficiency (%EE) of L-phenylalanine, L-tyrosine, dopamine and levodopa using PEG 400 / IL + (K$_3$C$_6$H$_5$O$_7$/C$_6$H$_5$O$_7$) pH 7.0 + H$_2$O ATPS (ternary systems).
Fig. 6. Extraction efficiency ($%EE$) of L-phenylalanine, L-tyrosine, dopamine and levodopa using PEG 400 + ($K_3C_6H_5O_7/C_6H_5O_7$) pH 7.0 + $H_2O$ + IL ATPS (quaternary systems).