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Partitioning of DNP-amino acids in ionic liquid/citrate salt based Aqueous Two-Phase System

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B S T R C T

In this work, the partitioning of four N-(2,4-dinitrophenyl)-amino acids (DNP-amino acids) in novel Aqueous Two-Phase Systems (ATPS) based on 1-hexyl-3-methylimidazolium dicyanamide (C_6Mim DCA) and biodegradable citrate organic salt, is evaluated.

Firstly, the tie-lines and respective tie-line lengths for ve ATPS (ionic liquid: C*n*Mim DCA with $n = 2,3,4$ and 6 and C_4pyr DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)) were determined at $T = 298.15$ K and atmospheric pressure. The tie-lines were correlated using Othmer-Tobias and Bancroft equations. The partition coef cients of N-(2,4-Dinitrophenyl)-glycine; N-(2,4-Dinitrophenyl)-L-alanine; N-(2,4-Dinitrophenyl)-L-valine and N-(2,4-Dinitrophenyl)-L-leucine in the {C_6Mim DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)} ATPS were measured at $T = 298.15$ K and atmospheric pressure, aiming to evaluate the influence of the structure of the DNP-amino acids on the partition coef cients.

1 Introduction

Aqueous Two-Phase Systems (ATPS) is an extraction technique that presents several advantages such as: environmentally friendly media, low cost and easy of scaling-up. In the mid-1950s, Albertsson [1] proposed the use of ATPS as an alternative to traditional liquid-liquid equilibria (LLE) separation. The ATPS consist of two immiscible aqueous-rich phases based on polymer-polymer, polymer-salt or salt-salt combinations and are a clean replacement to the conventional LLE systems since they are mostly composed of water (up to 70 in the overall systems).

The ATPS have been widely and mostly studied in the eld of separation, puri cation and recovery of different molecules such as proteins, enzymes, nucleic acids, antibodies and antibiotics [2]. But the use of ATPS has also other applications such as preconcentration, separation and recovery of metal ions [3].

In the biotechnological industry, the puri cation or recovery of biomolecules requires the use of the liquid-liquid extraction technique, which generally uses organic solvents that can affect the biological activity or chemical structure of the biomolecule, for this reason the use of ATPS with compounds as ionic liquids and organic salts is a promising option for this type of processes. The aim of this study is to evaluate the viability of the use of ATPS based on ionic liquids and organic salts as an extraction method for amino acids.

The main advantage of the use of ionic liquids in the ATPS is the high versatility of these compounds, the possibility of synthesizing different ionic liquids thanks to the possible multiple combinations of cations and anions, and therefore designs an ionic liquid for a speci c application."

The use of inorganic salts in ATPS can lead to an environmental problem such as its discharge into effluent streams. For this reason, in this work we propose the use of biodegradable and non-toxic salts, such as citrate organic salts.

The rst work about the formation of ATPS with an ionic liquid was reported in 2003 by Rogers et al. [4], in this study the ionic liquid was the butylmethylimidazolium chloride ionic liquid (IL) which forms an ATPS with the addition of the inorganic salt K_3PO_4.

The research on ATPS based ionic liquids and inorganic salts have gained interest in the last years [5–22], but the ATPS formed by ionic liquids and other compounds as organic salts have seldomly been investigated [23–30].

Although the study of the partitioning of amino acids in ATPS has been studied [31–35], there are few publications about the extraction of amino acids in ATPS containing ionic liquids and these works report partitioning of other amino acids such as: L-tryptophan and L-tyrosine, in ATPS-based on ionic liquids with Cl and Br

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as anion [23,24,36].

In a previous publication [37] the binodal curves of the systems (ionic liquid: C*n*Mim DCA with $n = 2,3,4$ and 6 and C_4pyr DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)) studied here were presented. It was shown that the system containing the C_6Mim DCA ionic liquid leads to the larger heterogeneous region, which is an advantage for separation processes. Therefore, partition coef cients of different N-(2,4-Dinitrophenyl)-amino acids (DNP-amino acids) in only {C_6Mim DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)} ATPS were measured at $T = 298.15$ K and atmospheric pressure.

Besides, with the aim of studying the influence of alkyl chain length of cation of the ionic liquid to form two-phase systems, the ATPS composed by different ionic liquids based on imidazolium and pyrrolidinium cation and dicyanamide anion together biodegradable organic salt were studied with the determination of the tie-lines and tie-line length (TLL) at $T = 298.15$K and atmospheric pressure.

2 Experimental

2.1. Materials

All ionic liquids (ILs) were acquired at IoLiTec with mass fraction purity higher than 0.98, and water and halide contents lower than 100 ppm. In order to reduce the water content and volatile compounds to negligible values, ILs were dried at constant stirring at moderate temperatures ($T = 343$ K) and under moderate vacuum ($p = 0.2$ Pa) for at least 48h prior to their use.

The sodium citrate tribasic dihydrate salt was purchased from Sigma-Aldrich with mass fraction purity higher than 0.99 and was dried by lyophilization (Scan vac. Cool-safe 55-4) before its use. N-(2,4-Dinitrophenyl)-amino acids were purchased from TCI (Tokio Chemical Industry) and were used as received, mass fraction purity higher than 0.98 for all DNP-amino acids.

The stock solutions (of salt and DNP-amino acids) were prepared using double distilled deionized water. The concentration of stock solution of salt was of 37 (percentage weight) and 0.2 for the stock solution of the four DNP-amino acids, and their concentrations were obtained gravimetrically on an Mettler AX-205 Delta Range balance with an uncertainty of 3 10^{-4} g.

In Table 1 the purities and the suppliers of the all components are presented.

2.2. Apparatus and procedure

2.2.1. Tie-lines determination

To obtain the tie-lines (TL), ternary mixtures with different compositions of ionic liquid + salt + water (total mass equal to 6 g) within the biphasic region were gravimetrically prepared within an uncertainty of the measurement of $\pm 10^{-4}$ g. The mixtures were vigorously stirred in a vortex (VWR, model VV3) and kept for at least 24 h at $T = 298.15$ K in a thermostatic bath (Techne, Tempette TE-8D) within an uncertainty of the measurement of the temperature of 0.2 K, to ensure complete separation of the phases. After equilibration, both phases (top and bottom phases) were carefully separated and analysed.

The binodal curves of all ternary systems are presented in a previous paper [37].

In all cases, the top phase corresponds to the ionic liquid rich phase and the bottom phase to the salt rich phase.

The ionic liquid concentration in both phases was measured using UV–Vis spectrophotometry (Thermo Scienti c Varioskan flash) at 298 nm, the water content was determined gravimetrically after freezedrying and the salt amount was obtained by mass balance.

The tie-line length (TLL) is a numerical indicator of the composition difference between the two phases and is generally used to correlate trends in the partitioning of solutes between both phases. For the calculation of each tie-line length (TLL) the following equation was used [7,11]:

$$TLL = [([IL]_{top} - [IL]_{bot})^2 + ([salt]_{top} - [salt]_{bot})^2]^{1/2} \quad (1)$$

The reliability of the tie-lines was checked by Othmer-Tobias [38] and Bancrof [39] (eqs (2) and (3)):

$$\log\left[\frac{1 - IL]_{top}}{IL]_{top}}\right] = K_1 + n \log\left[\frac{1 - salt]_{bot}}{salt]_{bot}}\right] \quad (2)$$

$$\log\left[\frac{1 - water]_{bot}}{salt]_{bot}}\right] = K_2 + r \log\left[\frac{1 - water]_{top}}{IL]_{top}}\right] \quad (3)$$

where $[water]_{top}$ and $[water]_{bot}$ are the weight fraction percentage fractions of water in the top and bottom phases in equilibrium, respectively. The tted parameters, *K*, *n* and *r* and the correlation coef cients (R^2) are given in Table 2.

2.2.2. DNP-amino acids partitioning

The determination of the partition coef cients of the four DNP-amino acids (N-(2,4-Dinitrophenyl)-glycine; N-(2,4-Dinitrophenyl)-L-alanine; N-(2,4-Dinitrophenyl)-L-valine and N-(2,4-Dinitrophenyl)-L-leucine) in {C_6Mim DCA (1) + sodium citrate salt (2) + water (3)} ATPS was performed according to following procedure: appropriate amounts of ionic liquid C_6Mim DCA, sodium citrate salt and water to achieve the same feed compositions of the tie-lines determined on the LLE, were weighted (these feed samples are presented in Table 3).

Table 1
Names, suppliers purities and water content of the ionic liquids, salt and DNP-amino acids used in this work.[a]

Chemical	Name	Supplier	Purity (weight fraction)	Water contet (ppm)[b]
C_2Mim DCA	1-ethyl-3-methylimidazolium dicyanamide	Iolitec	≥0.99	80
C_3Mim DCA	1-propyl-3-methylimidazolium dicyanamide	Iolitec	≥0.99	75
C_4Mim DCA	1-butyl-3-methylimidazolium dicyanamide	Iolitec	≥0.98	95
C_6Mim DCA	1-hexyl-3-methylimidazolium dicyanamide	Iolitec	≥0.99	90
C_4Mpyr DCA	1-butyl-1-methylpyrrolidinium dicyanamide	Iolitec	≥0.98	85
$C_6H_5Na_3O_7$	Sodium citrate tribasic dihydrate	Sigma-Aldrich	≥0.99	
$C_8H_7N_3O_6$	N-(2,4-Dinitrophenyl)-glycine	TCI	≥0.98	
$C_9H_9N_3O_6$	N-(2,4-Dinitrophenyl)-L-alanine	TCI	≥0.98	
$C_{11}H_{13}N_3O_6$	N-(2,4-Dinitrophenyl)-L-valine	TCI	≥0.98	
$C_{12}H_{15}N_3O_6$	N-(2,4-Dinitrophenyl)-L-leucine	TCI	≥0.99	

[a] The structure of ionic liquids was checked by ^{1}H NMR.
[b] The water content was measured using a Mettler Toledo C20 Coulometric KF Titrator.

Table 2
Parameters obtained using Othmer-Tobias equation (eq (2)) and Bancroft equation (eq (3)) and correlation coef cients.

ATPS	Othmer-Tobias			Bancroft		
	n	K_1	R^2	r	K_2	R^2
C_2MimDCA (1)+ $C_6H_5Na_3O_7$ (2)+water (3)	2.0261	−0.5959	0.960	0.3834	0.2906	0.920
C_3MimDCA (1)+ $C_6H_5Na_3O_7$ (2)+water (3)	0.9570	−0.3401	0.996	1.3901	0.4077	0.988
C_4MimDCA (1)+ $C_6H_5Na_3O_7$ (2)+water (3)	1.4725	−0.6950	0.992	0.732	0.496	0.983
C_6MimDCA (1)+ $C_6H_5Na_3O_7$ (2)+water (3)	0.6321	−0.5496	0.995	1.9104	−0.9888	0.9928
C_4MpyrDCA (1)+ $C_6H_5Na_3O_7$ (2)+water (3)	2.3825	−1.2375	0.978	0.4097	0.5281	0.970

For each tie-line, six replicates were prepared, different amounts of the solute stock solution of DNP-amino acids (0, 20, 40, 60, 80 and 100 mg) were added and water was added in the corresponding amount (100, 80, 60, 40, 20 and 0 mg) with the aim of keeping the composition constant in the system. The samples were prepared in Eppendorf of 1.5 mL, the total mass was 1.1 g. The Eppendorf were vigorously shaken on vortex mixer for 15 min, and then centrifuged (Minispin, Eppendorf, at 13.4 10^3 rpm for 15 min) and after they were left overnight at $T = 298.15$K to achieve complete phase separation.

Solute quanti cation in top and bottom phases was assessed by absorbance measurements at 362 nm using UV–Vis spectrophotometer (Thermo Scienti c Varioskan flash).

To minimize the possible interferences from other components (salt and ionic liquid) the top and the bottom phases were diluted with water, the dilution factor (DF) was 40 for the top phase and 1.33 for the bottom phase. In addition, the samples were analyzed against blanks which contained the same phase components but without amino acid. It is important to note that no interferences were found. The maximum peak of absorbance of C_6mimDCA ionic liquid is at 298 nm and this ionic liquid does not present value of absorbance at 362 nm, wavelength in which the absorbances of the amino acids studied in this work were determined.

From the representation of the absorbance in the top phase ($Abs_{(top)}$) versus the absorbance in the bottom phase ($Abs_{(bottom)}$) (see an example in Fig. S2, supporting information) the partition coef cients (K) for the four amino acids were calculated using equation (4) [32,33], where DFtop and DFbottom are the dilution factor of the top and of the bottom, respectively.

$$K = \frac{Abs\ (top)}{Abs\ (bottom)} \frac{DFtop}{DFbottom} \quad (4)$$

Table 3
Feed compositions ($[IL]_{feed}$, $[salt]_{feed}$) and compositions (weight fraction percentage) of the salt and ionic liquid on the top-phase and bottom phase ($[salt]_{top}$, $[IL]_{top}$,-$salt]_{bot}$ and $[IL]_{bot}$) and respective values of tie-line lengths (TLL) for the systems {ionic liquid (1) + citrate salt (2) + water (3)} at $T = 298.15$ K and atmospheric pressure.[a]

Number of TL	$[salt]_{feed}$	$[IL]_{feed}$	$[salt]_{top}$	$[IL]_{top}$	$[salt]_{bot}$	$[IL]_{bot}$	TLL
{C_2Mim DCA (1) + $C_6H_5Na_3O_7$ (2) + water (3)}							
TL 1	26.295	17.608	6.308	50.679	33.916	3.990	0.535
TL 2	22.821	21.223	7.407	46.163	32.467	5.407	0.473
TL 3	19.672	24.249	9.263	42.313	30.283	6.882	0.405
TL 4	16.603	30.907	7.201	48.503	32.729	4.633	0.500
{C_3Mim DCA (1) + $C_6H_5Na_3O_7$ (2) + water (3)}							
TL 1	26.641	13.328	4.658	51.872	32.003	2.804	0.555
TL 2	23.349	16.694	6.088	48.892	29.906	3.443	0.503
TL 3	16.757	23.721	10.630	42.875	24.743	5.073	0.378
TL 4	13.213	28.545	12.351	40.670	23.110	5.802	0.332
TL 5	9.980	33.483	13.482	39.967	22.098	6.051	0.310
{C_4Mim DCA (1) + $C_6H_5Na_3O_7$ (2) + water (3)}							
TL 1	23.285	13.504	3.708	55.542	28.271	1.889	0.582
TL 2	19.965	16.862	5.375	51.261	25.801	2.489	0.515
TL 3	16.679	20.041	7.742	45.543	23.027	3.516	0.425
TL 4	13.287	25.014	9.413	42.839	21.320	4.108	0.376
TL 5	9.994	30.164	10.165	39.774	20.629	4.884	0.335
{C_6Mim DCA (1) + $C_6H_5Na_3O_7$ (2) + water (3)}							
TL 1	26.598	10.190	1.280	67.481	30.424	1.997	0.720
TL 2	23.254	13.525	1.550	65.756	28.111	2.071	0.693
TL 3	19.921	17.026	1.945	64.889	25.533	2.110	0.672
TL 4	13.262	23.226	3.642	58.769	19.241	2.411	0.576
TL 5	10.032	26.719	5.553	54.891	15.593	2.630	0.510
{C_4Mpyr DCA (1) + $C_6H_5Na_3O_7$ (2) + water (3)}							
TL 1	26.605	10.013	1.937	70.401	30.108	0.413	0.746
TL 2	23.183	13.502	2.950	64.523	27.827	0.726	0.673
TL 3	20.008	16.605	4.049	56.148	25.915	1.407	0.576
TL 4	16.821	20.268	5.723	52.584	23.580	1.806	0.517
TL 5	13.217	25.191	6.854	46.657	22.239	2.656	0.444

[a] Standard uncertainties, u, are $u(T) = 0.2$ K, $u(p) = 10$ kPa, $u(w$, weight fraction percentage$) = 10^{-3}$.

3 Results and discussion

3.1. Tie-lines

The tie-lines were obtained for systems {ionic liquids (1) + citrate salt (2) + water (3)} where the ionic liquids are: CnMim DCA (with $n = 2,3,4$ and 6) and C_4pyr DCA combined with sodium citrate salt, at $T = 298.15$ K and atmospheric pressure. The feed compositions, experimental tie-lines, along with the respective lengths are reported in Table 3. As example of the tie-lines obtained for this type of systems, Fig. 1 displays the tie-lines for the system: {C_6Mim DCA (1) + sodium citrate salt (2) + water (3)}. In addition, in Fig. S1 the other systems are shown. In these gures it can be observed that the compositions of the tie-lines match very well with the binodal curves determined in a previous work [37].

It was observed that an increase in the TLL leads to an increase in

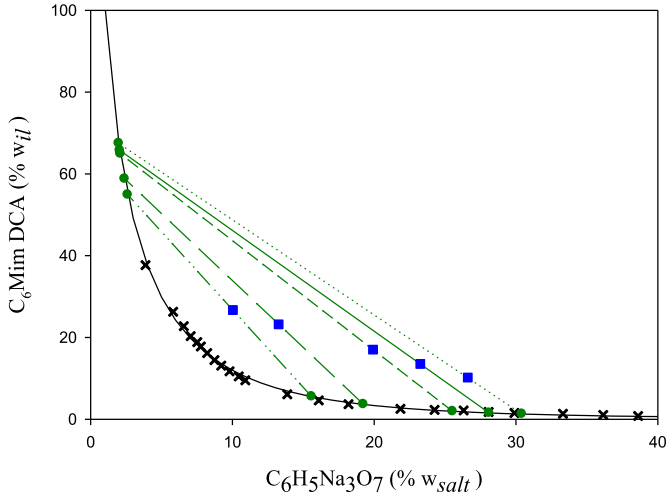

Fig 1 Tie-lines for ternary system composed of {C_6Mim DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)} at $T = 298.15$K and atmospheric pressure. (X) binodal curve (ref. 37), (●) tie-line data, (.) TL1, (–) TL2, (—) TL3, (– –) TL4, (–..–) TL5 and (■) feed samples.

the size of bottom phase, while the size on the top phase decreases. In all cases, the top phase was the ionic liquid rich phase and the bottom phase was rich in salt. In addition, Table S1 shows the values of the masses obtained from both phases (mass of the top-phase and mass of the bottom-phase) for the all ternary systems.

An increase in the value of the TLL (this means increase in the salt concentration and decrease in ionic liquid concentration on the feed) gives higher values of ionic liquid in top phase and lower values of ionic liquid in the bottom phase. This behavior is represented in Fig. 1.

In systems such as those studied in this work (containing two ionic species), the possibility of ion exchange in the equilibrium of phases is an important issue that must be addressed. To con rm that there is no ion exchange in the systems presented here, nuclear magnetic resonance (NMR H1) of the two liquid equilibrium phases (top phase and bottom phase) for the 5 systems studied and for the different tie-lines have been performed.

Figure S3 a) and b) (Supplementary information) present NMR H1 for top and bottom phases of the system {C6Mim DCA (1) + sodium citrate salt (2) + water (3)} for TL5 (see composition of this TL in Table 3), as an example. In these gures the NMR H1 for the pure ionic liquid (C6Mim DCA) and the pure salt (sodium citrate) are also displayed. In Fig. S3a) the top phase is presented and in Fig. S3b) the bottom phase. These gures clearly show that there is not ion exchange.

3.2. Partitioning

In Table 4 the partition coef cients (K) calculated with equation (4), for the four DNP-amino acids together with the feed composition and the tie-line length of the tie-lines for the system {C_6Mim DCA (1) + sodium citrate salt (2) + water (3)} are presented. In this Table it can be observed that an increase in the value of the TLL leads to an increase on the partition coef cient value. This is in agreement with the obtained values for the absorbance on the top and on the bottom phase (an increase in the TLL gives higher values of the absorbance in the top and lower values for the absorbance in the bottom phase), and it agrees with the expected results, since the top phase is the rich ionic liquid phase and the bottom phase is the rich salt phase.

Fig. 2 represents the logarithm of the partition coef cients for four DNP-amino acids as function of TLL. These results are in accordance with the commonly used expression:

$$\mathrm{LnK} = \alpha \quad \mathrm{TLL} \tag{5}$$

where α is a constant value which depends of the ATPS composition.

Fig. 2 shows that the highest distribution coef cients are obtained for the DNP-leucine, the order found was: Kleucine Kvaline Kalanine Kglycine, which is in close agreement with previous works [31–35] for the partitioning of DNP-amino acids on

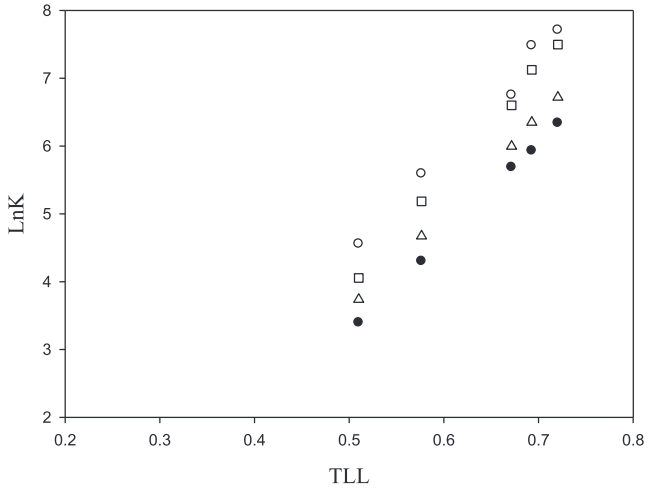

Fig 2 Logarithm of the partition coef cients of the DNP-amino acids versus tie-line length for the {C_6Mim DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)} system at $T = 298.15$K and atmospheric pressure. DNP-amino acids: (○) DNP-leucine, ( ) DNP-valine, ( ) DNP-alanine and (●) DNP-glycine.

APTS composed by PEG and salts. Although these systems do not contain ILs it is interesting to observe the same behaviour.

The values of the partition coef cients for the four DNP-amino acids, which are high, clearly indicate that this system is an ef - cient alternative for the puri cation of the amino acids.

The relative hydrophobicity of the amino acids residues was estimated by Zaslavsky et al. [40], as an average equivalent number of methylene units, n(CH2), and is different from real alkyl chain length. The positive value of n(CH2) means that a solute is hydrophobic and its relative hydrophobicity is equal to the one with n units of CH2 groups. In Fig. 3 the values of n(CH2) are plotted versus logarithms of partition coef cients, lnK. Linearity observed in Fig. 3 can be explained using equation (6).

$$\mathrm{LnK_{DNP\text{-}aa}} = \mathrm{C} + \mathrm{E} \quad \mathrm{n(CH2)} \tag{6}$$

where n(CH2) is the equivalent number of methylene units of a solute and C and E are constants, which represent the total contribution of the non-alkyl part of the DNP-amino acid structure and the contribution of CH2 to the partition coef cient, respectively.

4 Conclusions

In this work, 5 novel ILs-based ATPS were studied. The ILs selected for this work were the following: CnMim DCA (with n = 2,3,4 and 6) and C_4pyr DCA, and were combined with the biodegradable sodium citrate tribasic dihydrate organic salt. The tie-lines and tie-line lengths were experimentally determined at

Table 4
Partition coef cients obtained for four DNP-amino acids for the system {C_6Mim DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)} at $T = 298.15$ K and atmospheric pressure.[a]

Number of TL	$[salt]_{feed}$	$[IL]_{feed}$	TLL	*K*			
				DNP-leucine	DNP-valine	DNP-alanine	DNP-glycine
TL 1	26.598	10.190	0.720	2232	1800	828.4	567.0
TL 2	23.254	13.525	0.693	1776	1242	572.0	376.5
TL 3	19.921	17.026	0.672	856.1	734.9	401.8	295.0
TL 4	13.262	23.226	0.576	268.5	178.4	107.1	73.84
TL 5	10.032	26.719	0.510	95.41	57.71	42.00	29.85

[a] Standard uncertainties, *u*, are $u(T) = 0.2$ K, $u(p) = 10$ kPa, $u(K) = 10^{-2}$.

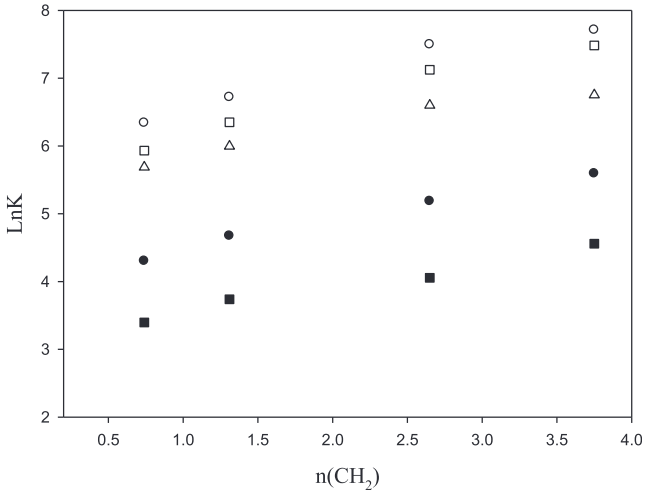

Fig 3 Logarithm of partition coef cients of the DNP-amino acids versus of the average number of methylene groups for the ve tie-lines of the {C_6Mim DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)} system at $T = 298.15$K and atmospheric pressure. Number of tie-lines: (○) TL1, ( ) TL2, ( ) TL3, (●) TL4 and (■) TL5.

$T = 298.15$ K and atmospheric pressure. It can be observed that an increase in the value of the TLL gives higher values of ionic liquid in top phase and lower values of ionic liquid in the bottom phase.

The partition coef cients of N-(2,4-Dinitrophenyl)-glycine; N-(2,4-Dinitrophenyl)-L-alanine; N-(2,4-Dinitrophenyl)-L-valine and N-(2,4-Dinitrophenyl)-L-leucine in the {C_6Mim DCA (1) + sodium citrate tribasic dihydrate (2) + water (3)} ATPS were determined at $T = 298.15$ K and atmospheric pressure.

The obtained results show that an increase in the value of the TLL leads to an increase on the partition coef cient value and that the highest distribution coef cients were obtained for the DNP-leucine. The order found for the partition coef cients was as follows: Kleucine Kvaline Kalanine Kglycine.

The high values of the partition coef cients obtained in this work show that this ATPS is very adequate for future biotechnology applications.

Acknowledgments

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Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fluid.2018.11.021.

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