Partitioning of amino acids in the novel biphasic systems based on environmentally friendly ethyl lactate

Ishara Kamalanathan, Luca Canal, Joe Hegarty, Vesna Najdanovic-Visak*

Engineering Department, Faculty of Science and Technology, Lancaster University, United Kingdom.

*corresponding author: v.najdanovic@lancaster.ac.uk, Tel: +44 (0)1524 593586

Abstract

For the first time, we report on the performance of biphasic system composed of ethyl lactate, water and inorganic salt (K_3PO_4, K_2HPO_4 and K_2CO_3) for the separation of amino acids (L-phenylalanine, L-tryptophan and L-tyrosine) from their aqueous solutions. Cloud points (solubility curve) and tie-lines for three ternary (ethyl la ctate + water + inorganic salt) systems at 298.2 K and 313.2 K at atmospheric pressure were determined. For certain composition range, these mixture exhibit biphasic systems – top and bottom phases rich in ethyl lactate and salt, respectively. Partition coefficients of amino acids and their extraction efficiencies, as essential parameters for design of any separation process, were measured at two temperatures – 298.2 K and 313.2 K. The maximum values of partition coefficients were observed for the system containing K_3PO_4: 3.5, 3.7 and 11.9 for L-phenylalanine at 313.2 K, L-tyrosine at 298.2 K and L-tryptophan at 313.2 K, respectively. The obtained results clearly showed that the biphasic systems based on ethyl lactate are suitable for the efficient and sustainable recovery of amino acids from solutions with water.

Keywords: extraction, ATS, liquid-liquid equilibria, solubility, salting-out

1. Introduction

Amino acids are building blocks that play central roles in both human and animal nutrition and health. In the world market for fermented products excluding ethanol, amino acids are the second most important category after antibiotics [1]. In particular, aromatic amino acids (L-phenylalanine, L-tryptophan and L-tyrosine) have various applications as dietary supplements, animal feed and valuable precursors for the production of high value compounds such as anti-Parkinson's drug L-dopa and p-hydroxycinnamic acid [2],[3].

Amino acids can be produced by three methods: chemical synthesis, fermentation, and enzymatic catalysis. Extraction of L-amino acids from protein hydrolysate is limited due to scalability and raw material availability issues. Fermentation is by far the most popular for being cost competitive, sustainable and for ease of large scale production. Over the years the production of amino acid by fermentation has been optimised with the use of different strains, mutants and carbon sources [1-2].

Conventional recovery methods of amino acids from their aqueous solutions include ion exchange chromatography [4], flotation [5], liquid membrane [6] and solvent extraction [7]. However, these methods have low yield, high maintenance cost and use hazardous organic solvents that are flammable and toxic to humans, micro-organisms and the environment. Furthermore, organic solvent-aqueous systems are not suitable for amino acid recovery due to the low solubility of amino acids in these systems [8].

Aqueous biphasic systems are clean alternatives that have gained growing interest in recent years as a simple and benign separation technique with benefits of ease of process integration, possibility of continuous operation and easy scale up. It is a liquid-liquid extraction method composed of two aqueous phases, generally a polymer-aqueous salt solution [9] or ionic liquid-aqueous [10] salt systems.

Aqueous biphasic systems have been applied for the recovery of aromatic amino acids using a range of hydrophilic compounds, including polymers [11-12], polymer mixtures [13], non-ionic surfactants [14], ionic liquids [15] as shown in Table 1. In addition, partitioning of amino acids in the biphasic (water + alcohol) [16-17] and in the (water + acetonitrile) [18] systems were reported. Key factors that influence the partition coefficient of amino acid are their hydrophobicity, hydrophobic and electrostatic interactions and salting out effects [15]. Larger the amino acid hydrophobicity, the greater affinity it has for the more hydrophobic region of the biphasic system (generally top phase rich in polyethylene glycol) leading to higher partition coefficients. The hydrophobicity of aromatic amino acids is in the following order: l-tryptophan > L-phenylalanine> L-tyrosine. Hence, the partition coefficients for L-tryptophan for all systems are greater than the other two amino acids [19], [12] (see Table 1).

Ethyl lactate, a monobasic ester is one of the most promising alternate green solvents to emerge in recent years [20]. It has favourable characteristics such as low toxicity, high biodegradability and very low eco-toxicity. Furthermore it has been approved by the FDA (USA Food and Drug Administration) for use in food products. From an economic perspective, the production cost of ethyl lactate is competitive due to manufacture from renewable carbohydrate feed stocks [21-22]. Ethyl lactate has been demonstrated to be a good solvent for a range of compounds such as caffeine [23], hydrocarbons [24] and phenolic compounds and flavonoids [25-26] as well as a suitable sustainable solvent for organic synthesis [27].

In this work, for the first time ethyl lactate based biphasic system was successfully demonstrated to recover and enrich three aromatic amino acids (L-tryptophan, L-phenylalanine and L-tyrosine). Cloud points and tie-lines of the ternary systems containing ethyl lactate, water and salt (potassium phosphate, potassium hydrogen phosphate and potassium carbonate) are determined at 298.2 K and 313.2 K. In addition, partition coefficients of L-tryptophan, L-phenylalanine and L-tyrosine between ethyl lactate – rich and aqueous phases at two temperatures of 298.2 K and 313.2 K are presented.

2. Experimental section

2.1 Materials

Table 2 summarizes details of all chemicals used in this work source. Ethyl lactate (CAS 687-47-8, purity 98 mass%) , L-phenylalanine (CAS 63-91-2, purity > 98 mass%), L-tryptophan (CAS 73-22-3 , purity > 98 mass%), L-tyrosine (CAS 60-18-4, purity > 98 mass%), potassium phosphate tribasic K_3PO_4 (CAS 7778-53-2 , purity > 98 mass%), potassium phosphate dibasic K_2HPO_4 (CAS 7758-11-4 , purity > 98 mass%) and potassium carbonate K_2CO_3 (CAS 584-08-7, purity > 99 mass%) were supplied by Sigma–Aldrich. All chemicals were used without further purification. Water was distilled and deionized using a Milli-Q water filtration system from Millipore. All liquid mixtures were gravimetrically prepared using Mettler AT201 analytical balance with stated repeatability of $\pm 3\cdot10-2$ mg.

2.2 Methods

2.2.1 Cloud points (solubility curve)

Cloud points of the ternary systems containing ethyl lactate, water and salt (either K_3PO_4 or K_2HPO_4 or K_2CO_3) were determined by the cloud point titration at the constant temperatures of 298.2 K and 313.2 K, according to the procedure already described in the literature [28-29],[15]. Binary mixtures containing salt and water of known compositions (approximately in the region from 0.02 to 0.40 in mass fraction of salt) were placed in septum-sealed conical glass vials immersed in the temperature-controlled bath at 298.2 K or 313.2 K (±0.1 K). These contents were titrated with ethyl lactate at a constant temperature. Cloud points were taken as the appearance of turbidity in the sample. After the turbidity was observed, final mixtures were weighted by Mettler AT201 analytical balance with stated repeatability of $\pm 3 \cdot 10^{-2}$ mg in order to calculate the composition corresponding to the cloud point composition. Three replicates of each assay were carried out in order to validate the experimental method. The average reproducibility of composition (in mass fraction) was ±0.001.

Obtained cloud points were fitted using the equation given by Merchuk et al. [30] to obtain solubility curves:

$$w_{EL} = A \cdot \exp\left[B \cdot x_{salt}^{0.5} - C \cdot x_{salt}^{3}\right] \qquad (1)$$

where w_{EL} and w_{salt} are the ethyl lactate and salt mass fraction, respectively, while parameters A, B, and C are constants obtained by the regression of the experimental cloud point data. The experimental and fitted data were compared in terms of the absolute average deviations (AAD) of the ethyl lactate mass fraction according to:

$$AAD(\%) = \frac{1}{NP}\sum_{i}\frac{\left|w_{EL,i}^{calc} - w_{EL,i}^{\exp}\right|}{w_{EL,i}^{\exp}}\times 100 \qquad (2)$$

where $w_{EL,i}^{calc}$ and $w_{EL,i}^{\exp}$ are the calculated and experimental mass fractions, respectively, and *NP* is the number of available cloud points.

2.2.2 Tie-lines

The biphasic ternary (ethyl lactate + water + salt (either K_3PO_4 or K_2HPO_4 or K_2CO_3)) mixtures of known composition were agitated in a conical-bottomed flask for at least three hours at constant controlled temperature of 298.2 K or 313.2 K. Next, the mixtures were left still for at least 12 hours at the given temperature, to allow a complete phase separation into top phase (ethyl lactate rich phase) and bottom phase (salt-rich phase). Using a Hamilton syringe, the samples of both phases were taken into separate vials and their masses were recorded by Mettler AT201 analytical balance with stated repeatability of $\pm 3\cdot 10^{-2}$ mg. The overall mass of the biphasic ternary system was significantly high (approximately 50 g) in order to decrease the error associated with the sampling by Hamilton syringe. Ratio between the mass of the top phase and the total mass of the mixture (α) was calculated according to:

$$\alpha = \frac{\text{mass of the top phase}}{\text{total mass of the mixture}} \qquad (3)$$

Using lever-arm rule and constants *A*, *B*, and *C* obtained by the regression of the experimental cloud points (eq. (1)) the following equations are obtained:

$$w_{EL}^{EL-phase} = A \cdot \exp\left[B \cdot \left(w_{salt}^{EL-phase}\right)^{0.5} - C \cdot \left(w_{salt}^{EL-phase}\right)^{3}\right] \qquad (4)$$

$$w_{EL}^{salt-phase} = A \cdot \exp\left[B \cdot \left(w_{salt}^{salt-phase}\right)^{0.5} - C \cdot \left(w_{salt}^{salt-phase}\right)^{3}\right] \qquad (5)$$

$$w_{EL}^{EL-phase} = \frac{1}{\alpha} w_{EL}^{overall} - \frac{1-\alpha}{\alpha} w_{EL}^{salt-phase} \qquad (6)$$

where superscripts “EL-phase”, “salt-phase”, and “overall” stand for mass fractions in ethyl lactate–rich phase, salt–rich phase and overall mixture, respectively. Equations (4), (5) and (6) were solved using MATLAB software and the solution results in the mass fraction composition of ethyl lactate and salt in the top and bottom phases.

2.2.3 Partition of amino acids

Firstly, solutions of amino acids in water were prepared gravimetrically. Their initial concentrations were 2.200 $g \cdot L^{-1}$ for L-phenylalanine and L-tryptophan while the initial concentration of L-tyrosine was 0.404 $g \cdot L^{-1}$. This difference in concentrations is due to much lower solubility of L-tyrosine in water compared to L-phenylalanine and L-tryptophan. Next, biphasic systems containing 12 mass% salt, 39 mass% ethyl lactate and 49 mass% of the (water + amino acid) solution were prepared in the case of systems containing K_3PO_4 and K_2HPO_4. The overall composition for the (ethyl lactate + water + K_2CO_3) system was 14 mass% salt, 40 mass% ethyl lactate and 46 mass% of the (water + amino acid). These were agitated for at least six hours, at constant and controlled temperatures of 298.2 K or 313.2 K (± 0.1 K).

Subsequently, the mixtures were left still for at least 12 h to allow complete phase separation. Samples from both phases were taken into the vials using a Hamilton syringe and prepared for further analysis. Concentrations of L-phenylalanine, L-tryptophan and L-tyrosine in both phases (top phase rich in ethyl lactate and bottom phase rich in salt) were determined using UV-Vis spectroscopy (Evolution 220 UV-Visible Spectrophotometer, Thermo Fisher Scientific), at wavelengths of 257 nm, 280 nm and 276 nm, respectively. Interferences of both ethyl lactate and salts on the concentration were found to be significant. Therefore, the respective calibration curves were established for each individual compound using blank solutions with the same composition in the top and bottom phases but without amino acids.

Obtained compositions of amino acids and masses of in both phases allowed checking material balance. Standard error between the actual mass and sum of determined masses in two phases was always below 3%.

Partition coefficients (K_i) and extraction efficiency (EE_i) were calculated according to:

$$K_i = \frac{C_i^{EL\ \ phase}}{C_i^{salt\ \ phase}} \qquad (7)$$

$$\%EE_i = 100 \cdot \frac{m_{EL-phase} \cdot C_i^{EL\text{-}phase}}{m_{\mathrm{salt}\text{-}phase} \quad C_i^{salt\text{-}phase}} \qquad (8)$$

where $C_i^{EL-phase}$ and $C_i^{salt-phase}$ are concentration of amino acid (either L-phenylalanine or L-tryptophan or L-tyrosine) in the ethyl lactate-rich (top) phase and salt-rich (bottom) phase, respectively. $m_{EL-phase}$ and $m_{salt-phase}$ are masses of ethyl lactate-rich (top) and salt-rich (bottom) phase, respectively.

pH values of samples of both phases were measured by HI 2211 Microprocessor pH meter (HANNA instruments) with precision to within ± 0.01. Associated pH standard solutions (pH 4, 7 and 10) were used for calibration.

All experiments were repeated three times. The deviation from the average K_i value was always within ±0.1.

2.3 Results and Discussion

Obtained experimental cloud points for three ternary systems composed of ethyl lactate, water and inorganic salt (either K_3PO_4 or K_2HPO_4 or K_2CO_3) at two temperatures (298.2 K and 313.2 K) are shown in Figure 1 and in Table 3. Cloud point data were correlated with equation (1) and obtained parameters *A*, *B* and *C* for each mixture are presented in Table 4, showing reasonable fitting with the average absolute deviations ranging from 4.0% to 21%. Figure 1 shows that, at both temperatures, K_3PO_4 and K_2HPO_4 salts have similar abilities to provoke phase splitting in ethyl lactate - water mixtures despite their difference in ionic force. On the other hand more K_2CO_3 has to be added to the (ethyl lactate + water) system to obtain phase splitting. This trend can be related to the ions' Gibbs free energy of hydration (ΔG_{hyd}) [31], associated with electron density. Ions with higher electron density interact more with water molecule resulting in the better salting-out. It can be noted that the anion with a higher salting-out ability has a more negative ΔG_{hyd} value, PO_4^{3-} (-2765 $Jmol^{-1}K^{-1}$) > HPO_4^{2-} (-1789 $Jmol^{-1}K^{-1}$) > CO_3^{2-} > (−1476 $Jmol^{-1}K^{-1}$).

It is interesting to compare the obtained cloud point data for the biphasic systems based on ethyl lactate with similar systems based on polyethylene glycols and ionic liquids as presented in Figure 2 for K_3PO_4 (Fig.2a), K_2HPO_4 (Fig.2b) and K_2CO_3 (Fig.2c). For all studied systems, smaller amounts of salts are necessary to obtain phase splitting in the (PEG + water) [32-33] compared to the (ethyl lactate + water) systems which is probably due to the higher polarity of ethyl lactate resulting in the stronger interaction with water which are more difficult to break. Aqueous system containing phosphonium ionic liquid [34] exhibits similar solubility curve as PEG (Fig.2a) while systems containing immidazolim ionic liquids [35-36] showed similar solubility curve as ethyl lactate systems (Fig.2a), b) and c)).

Tie-lines data for the ternary system containing ethyl lactate, water and salt (K_3PO_4 or K_2HPO_4 or K_2CO_3) are also included in Fig.2. Higher tie-line length means bigger difference between compositions of the top and bottom phases or, in other words, higher degree of separations. As expected, the tie-line length is enhanced by increase of the overall salt concentration.

Table 5 shows measured partition coefficients (*K*) of three amino acids (L-phenylalanine, L-tyrosine and L-tryptophan) in the biphasic systems containing ethyl lactate, water and inorganic salt (K_3PO_4 or K_2HPO_4 or K_2CO_3), along with extraction efficiencies and values of pH in the top and bottom phases at 298.2 K. The partition coefficients were in the range of 2.6 to 3.3, 0.4 to 2.6 and 2.6 to 5.3, for L-phenylalanine, L-tyrosine and L-tryptophan, respectively. The most of obtained values for partition coefficients were favourable (higher than unity), meaning that the amino acids were more concentrated in the ethyl lactate rich phase. Only exception is the value of 0.4 for L-tyrosine in the system containing K_2CO_3, suggesting that the concentration of L-tyrosine is higher in the salt rich phase. Considering partition coefficients values for other biphasic system from Table 1, obtained values in the biphasic systems based on ethyl lactate are much higher than ones observed when employing organic solvents as extraction agents and comparable or higher than values obtained for biphasic systems based on polymers, ionic liquids or surfactants. Obtained partition coefficients for L-phenylalanine of 2.6 to 3.3 are higher than for any reported biphasic systems. For L-tyrosine, the obtained partition coefficients in biphasic system based on ethyl lactate (0.4 – 2.6) are comparable with other biphasic systems based on ionic liquids and polyethylene glycol but higher than ones reported for systems based on triton. Similar values of partition coefficients were observed for L-tryptophan as for other biphasic systems.

Results from Table 5 also show that there is no clear correlation between pH and partition coefficients.

Figure 3 shows the comparison of partition coefficients at two different temperatures, 298 K and 313 K. Partition coefficient of L-phenylalanine was slightly enhanced with temperature rise, reaching the maximum value of 3.5 at 313 K for system containing K_3PO_4 salt. Similar trend was observed for partition coefficient of L-tyrosine in the K_2CO_3 system, while increasing the temperature resulted in decrease of the partition coefficient in the K_3PO_4 and K_2HPO_4 systems. Significantly higher partition coefficients for L-tryptophan were observed at higher temperature in the systems containing K_3PO_4 and K_2CO_3, reaching value of 11.9. On the contrary, only slight enhancement of the partition coefficient with increasing temperature was observed for L-tryptophan in the K_2HPO_4 system.

Conclusions

In this work, the solubility curves for ternary biphasic system composed of ethyl lactate, water and inorganic salt (K_3PO_4, K_2HPO_4 and K_2CO_3) along with tie-lines at two temperature (298.2 K and 313.2) are reported. Partition coefficients of amino acids (L-phenylalanine, L-tyrosine and L-tryptophan) and their extraction efficiencies were measured at two temperatures – 298 K and 313 K.

The obtained values of partitioning coefficients are higher than ones observed when employing organic solvents as extraction agents and comparable or higher than values obtained for biphasic systems based on polymers, ionic liquids or surfactants.

The maximum values of observed partition coefficients were observed for the system containing K_3PO_4: 3.5, 3.7 and 11.9 for L-phenylalanine at 313 K, L-tyrosine at 298 K and L-tryptophan at 313 K, respectively.

The obtained results clearly showed that the biphasic systems based on ethyl lactate are suitable for the efficient and sustainable recovery of amino acids from solutions with water.

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Table 1. Comparison of obtained partition coefficients (*K*) of amino acids (L-phenylalanine-PA, L-tyrosine-TYR and L-tryptophan-TRY) in aqueous biphasic system based on ethyl lactate with other systems from literature.

Amino acid	System	*T* / K	*K*	Ref.
PA	1-butanol + water	298	0.3 – 0.4	[16]
	acetonitrile + water	263	0.1	[18]
	dextran + (Ficoll or PEG or Ucon) + NaCl	298	0.8 – 0.9	[13]
	PEG6000 + water + Na_2SO_4	298	1.1	[11]
	PEG6000 + water + $MgSO_4$	298	0.8 – 1.0	[12]
	PEG6000 + water + Na_2SO_4	298	2.0 – 2.5	[12]
	PEG6000 + water + $(NH_4)_2SO_4$	298	1.0 – 1.3	[12]
	triton + water + Na3citrate	298	0.6 – 0.9	[14]
	triton + water + $MgSO_4$	298	0.9 – 1.1	[14]
	C_4mimBr + water + K_3citrate/citric acid	298	1.8 – 2.2	[15]
TYR	1-butanol + water	298	0.1 – 0.2	[16]
	PEG6000 + water + Na_2SO_4	298	1.3	[11]
	PEG6000 + water + $MgSO_4$	298	1.3 – 1.5	[12]
	PEG6000 + water + Na_2SO_4	298	1.4 – 2.0	[12]
	PEG6000 + water + $(NH_4)_2SO_4$	298	1.2 – 1.8	[12]
	triton + water + Na3citrate	298	0.3 – 0.4	[14]
	triton + water + $MgSO_4$	298	0.5 – 0.9	[14]
	C_4mimBr + water + K_3citrate/citric acid	298	1.0 – 3.0	[15]
TRY	1-butanol + water	298	0.6 – 0.7	[16]
	acetonitrile + water	263	0.2	[18]
	octanol + water	298	1.1	[17]
	dextran + (Ficoll or PEG or Ucon) + NaCl	298	0.9 – 1.2	[13]
	PEG6000 + water + Na_2SO_4	298	4.4	[11]
	PEG6000 + water + $MgSO_4$	298	2.4 – 3.9	[12]
	PEG6000 + water + Na_2SO_4	298	2.9 – 6.9	[12]
	PEG6000 + water + $(NH_4)_2SO_4$	298	2.5 – 4.9	[12]
	triton + water + Na3citrate	298	1.6 – 2.7	[14]
	triton + water + $MgSO_4$	298	1.0 – 1.4	[14]
	C_4mimBr + water + K_3citrate/citric acid	298	2.0 – 15	[15]

Table 2. Chemicals used in this work. All chemicals were used without further purification.

Name	Source	Stated Purity /mass%
ethyl lactate	Sigma Aldrich	> 98
L-phenylalanine	Sigma Aldrich	> 98
L-tryptophan	Sigma Aldrich	> 98
potassium phosphate tribasic	Sigma Aldrich	> 98
potassium phosphate dibasic	Sigma Aldrich	≥ 98
potassium carbonate	Sigma Aldrich	≥ 99
distilled and deionized water	In house	n/a

Table 3. Cloud point data for the ternary systems containing ethyl lactate (EL), water and salt (K_3PO_4, K_2HPO_4, and K_2CO_3) at 298.2 K and 313.2 K at pressure of 0.1 MPa in mass fractions.[a]

w_{salt}	w_{EL}	w_{salt}	w_{EL}	w_{salt}	w_{EL}	w_{salt}	w_{EL}	w_{salt}	w_{EL}	w_{salt}	w_{EL}
Ethyl lactate + water + K_3PO_4				Ethyl lactate + water + K_2HPO_4				Ethyl lactate + water + K_2CO_3			
T = 298.2 K		T = 313.2 K		T = 298.2 K		T = 313.2 K		T = 298.2 K		T = 313.2 K	
0.005	0.837	0.005	0.915	0.006	0.793	0.005	0.921	0.003	0.786	0.012	0.82
0.008	0.764	0.008	0.851	0.014	0.644	0.016	0.750	0.006	0.735	0.022	0.73
0.014	0.655	0.012	0.800	0.020	0.612	0.030	0.683	0.015	0.655	0.058	0.601
0.024	0.572	0.016	0.754	0.035	0.500	0.042	0.647	0.043	0.564	0.098	0.482
0.041	0.499	0.022	0.710	0.062	0.392	0.052	0.551	0.06	0.509	0.119	0.411
0.058	0.423	0.032	0.655	0.082	0.343	0.079	0.421	0.092	0.426	0.148	0.335
0.074	0.377	0.044	0.582	0.113	0.257	0.120	0.325	0.117	0.356	0.192	0.23
0.089	0.323	0.055	0.523	0.141	0.172	0.153	0.281	0.145	0.276	0.211	0.1921
0.098	0.310	0.088	0.436	0.186	0.098	0.172	0.193	0.16	0.234	0.235	0.147
0.114	0.275	0.105	0.368	0.236	0.050	0.208	0.131	0.202	0.122	0.271	0.092
0.124	0.251	0.133	0.310	0.287	0.035	0.291	0.072	0.229	0.086	0.287	0.063
0.135	0.235	0.167	0.245	0.345	0.020	0.375	0.055	0.292	0.018	0.309	0.0282
0.144	0.207	0.189	0.185	0.395	0.014			0.334	0.029	0.35	0.02
0.158	0.185	0.221	0.147								
0.170	0.162	0.244	0.111								
0.192	0.115	0.259	0.102								
0.199	0.095	0.280	0.082								
0.221	0.058	0.298	0.080								
0.241	0.041										
0.250	0.043										
0.260	0.041										
0.289	0.038										

[a] Maximal standard uncertainties u are $u(T) = \pm 0.1$ K, $u(x) = 0.001$, $u(p) = 10$ kPa

Table 4. Parameters *A*, *B* and *C* of equation (1) fitted to the experimental cloud points at 298.2 K and 313.2 K. r^2 and *AAD* stand for correlation coefficient and absolute average deviations (equation (2)), respectively.

Mixture	*A*	*B*	*C*	r^2	*AAD* %
			T = 298.2 K		
ethyl lactate + water + K_3PO_4	1.057 ± 0.018	-3.710 ± 0.102	81.5 ± 7.7	0.9979	6.4
ethyl lactate + water + K_2HPO_4	1.060 ± 0.028	-3.935 ± 0.172	89.3 ±16.3	0.9974	21.4
ethyl lactate + water + K_2CO_3	0.868 ± 0.012	-2.085 ± 0.090	114.9 ± 7.9	0.9974	9.3
			T = 313.2 K		
ethyl lactate + water + K_3PO_4	1.152 ± 0.013	-3.287 ± 0.071	44.7 ± 3.4	0.9989	4.0
ethyl lactate + water + K_2HPO_4	1.171 ± 0.053	-3.281 ± 0.273	62.3 ± 19.2	0.9896	15.9
ethyl lactate + water + K_2CO_3	1.055 ± 0.017	-2.361 ± 0.080	66.3 ± 3.3	0.9991	7.2

Table 5. Partition coefficients (*K*) of amino acids (L-phenylalanine-PA, L-tyrosine-TYR and L-tryptophan-TRY) in aqueous biphasic systems containing ethyl lactate (EL), water and inorganic salt (K_3PO_4 or K_2HPO_4 or K_2CO_3) at 298.2 K and pressure of 0.1 MPa. The initial concentration of amino acids in water were 2.200 g·L^{-1} for L-phenylalanine and L-tryptophan while the initial concentration of L-tyrosine was 0.404 g·L^{-1}. Values of *pH* in the top and bottom phases as well as obtained extraction efficiencies (*EE*) are included.[a]

Amino acid	w_{salt} (w/w)	w_{EL} (w/w)	$C^{top\ phase}_{amino\ acid}$ (g L^{-1})	$C^{bottom\ phase}_{amino\ acid}$ (g L^{-1})	*K*	$pH_{top\ phase}$	$pH_{bottom\ phase}$	*EE* (%)
				ethyl lactate + water + K_3PO_4				
PA	0.111	0.379	1.570	0.482	3.26	6.6	8.0	82.1
TYR	0.112	0.383	0.412	0.113	3.65	6.0	7.8	99.9
TRY	0.111	0.381	1.929	0.740	2.61	6.0	7.7	87.5
				ethyl lactate + water + K_2HPO_4				
PA	0.111	0.379	1.889	0.764	2.47	5.8	6.8	95.7
TYR	0.114	0.380	0.377	0.178	2.12	5.8	7.0	99.2
TRY	0.111	0.381	2.034	0.381	5.34	5.6	6.8	99.7
				ethyl lactate + water + K_2CO_3				
PA	0.139	0.396	1.617	0.615	2.63	6.5	10.6	73.9
TYR	0.139	0.399	0.107	0.255	0.42	6.8	10.6	27.2
TRY	0.140	0.399	1.970	0.401	4.91	6.5	10.5	91.6

[a] Maximal standard uncertainties *u* are *u*(*K*) = ± 0.1, *u*(*C*) = 0.002 g L^{-1}, *u*(*C*) = 0.002 g L^{-1}, *u*(*pH*) = 0.1, *u*(*p*) = 10 kPa

Captions to Figures

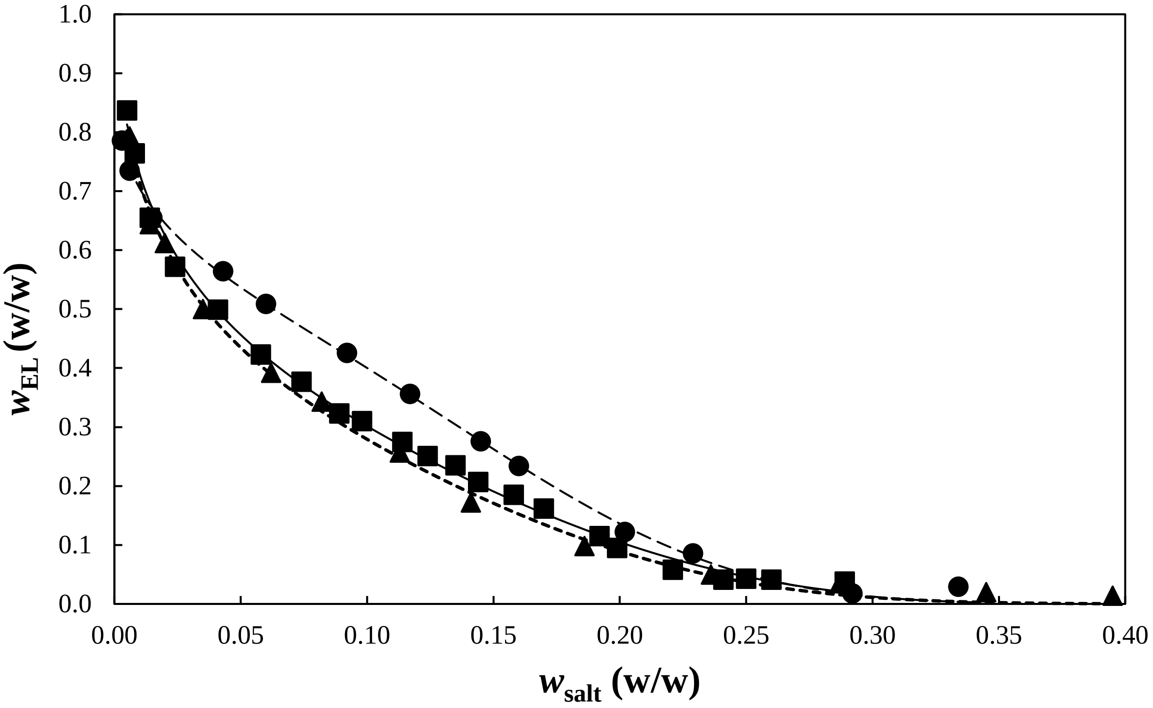
Figure 1. Cloud points for the ternary mixture composed of ethyl lactate (EL), water and inorganic salt (salt) under atmospheric pressure, where salt stands for either K_3PO_4 (filled squares) or K_2HPO_4 (filled triangles) or K_2CO_3 (filled circles) at: 298.2 K (a) and 313.2 K (b) Curves present fittings obtained by equation (1) using parameters given in Table 3 for systems containing K_3PO_4 (solid line), K_2HPO_4 (dotted line) and K_2CO_3 (dashed line).

Figure 2. Comparisons of cloud point data for different systems composed of: a) K_3PO_4 + water + either ethyl lactate (this work where filled and empty squares are cloud points and tie-line points, respectively) or polyethylene glycol 2000 g/mol [32] (solid line) or tetrabutylphosphonium bromide [34] (dotted line) or tributylmethylphosphonium methylsulphate [34] (dashed line) or 1-butyl-3-methylimidazolium bromide [35] (dashed-dotted line) [35]; b) K_2HPO_4 + water + either ethyl lactate (this work where filled and empty triangles are cloud points and tie-line points, respectively) or polyethylene glycol 2000 g/mol [33] (solid line) or tetrabutylphosphonium bromide [35] (dotted line) or 1-ethyl-3-methylimidazolium dimethyl phosphate [36] (dashed line); c) K_2CO_3 + water + either ethyl lactate (this work where filled and empty circles are cloud points and tie-line points, respectively) or polyethylene glycol 2000 g/mol [32] (solid line) or 1-butyl-3-methylimidazolium bromide [35] (dotted line) or 1-ethyl-3-methylimidazolium dimethyl phosphate [36] (dashed line).

Figure 3. Partition coefficients of L-phenylalanine (a), L-Tyrosine (b) and L-Tryptophan (c) in different aqueous biphasic systems at 298.2 K (black columns) and 313.2 K (white columns). The overall mass fractions were $w_{\text{salt}} = 0.11$ and $w_{\text{EL}} = 0.380$ for systems containing K_3PO_4 and K_2HPO_4 while the overall mass fractions were $w_{\text{salt}} = 0.14$ and $w_{\text{EL}} = 0.40$ for system containing K_2CO_3.

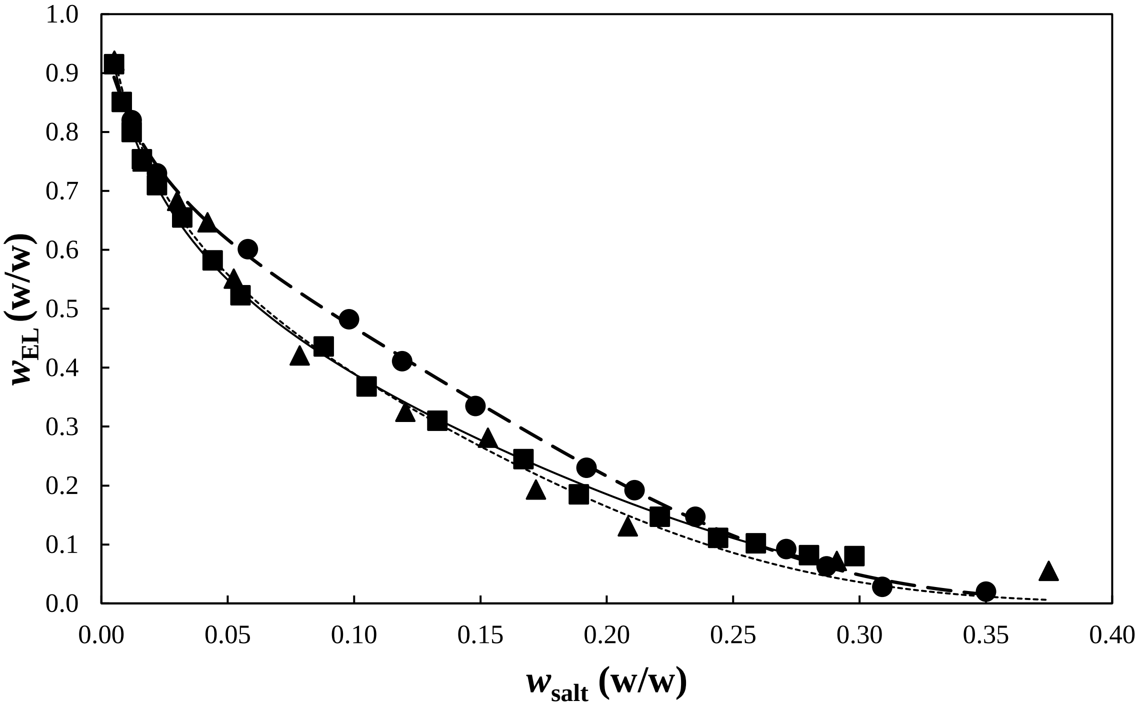
Figure 1

a)



1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
w_{EL} (w/w)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
w_{salt} (w/w)

b)



1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
w_{EL} (w/w)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
w_{salt} (w/w)

Figure 2 a)

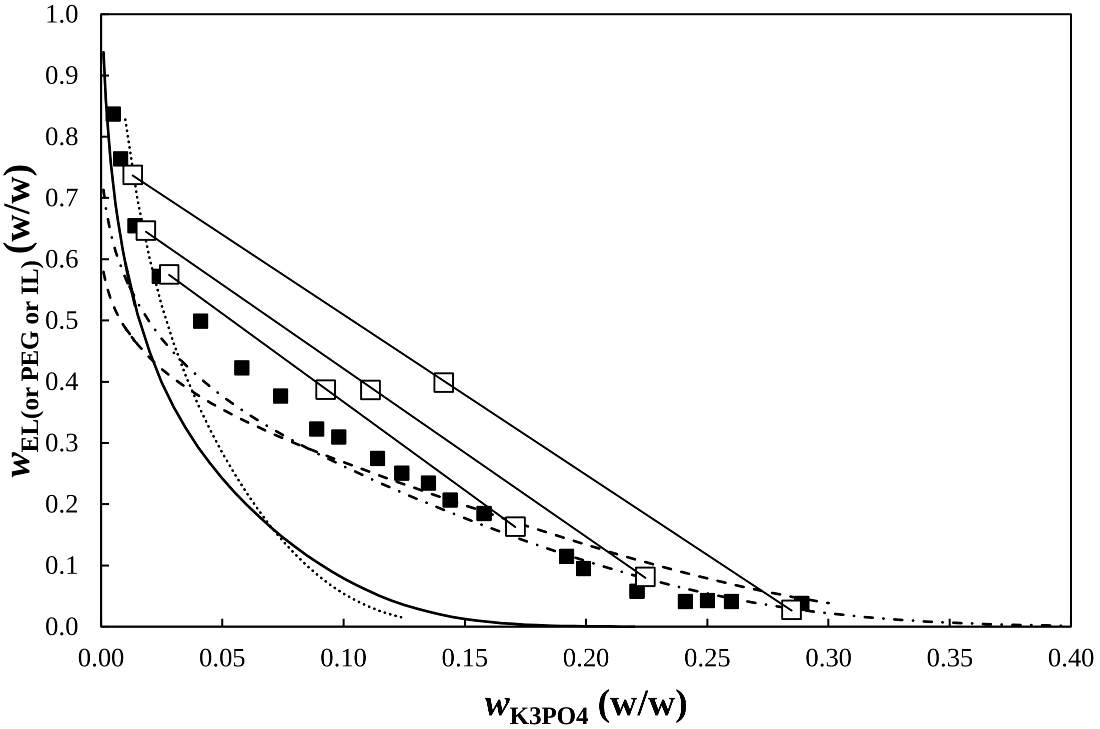


Figure 2 b)

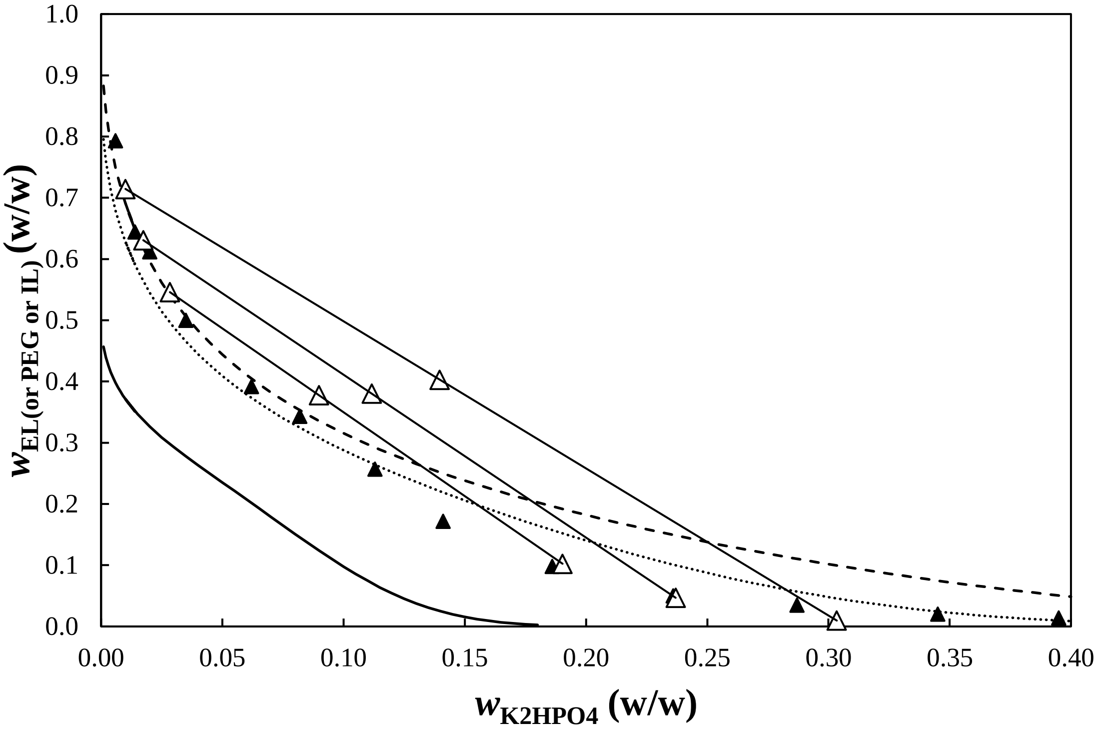


Figure 2c)

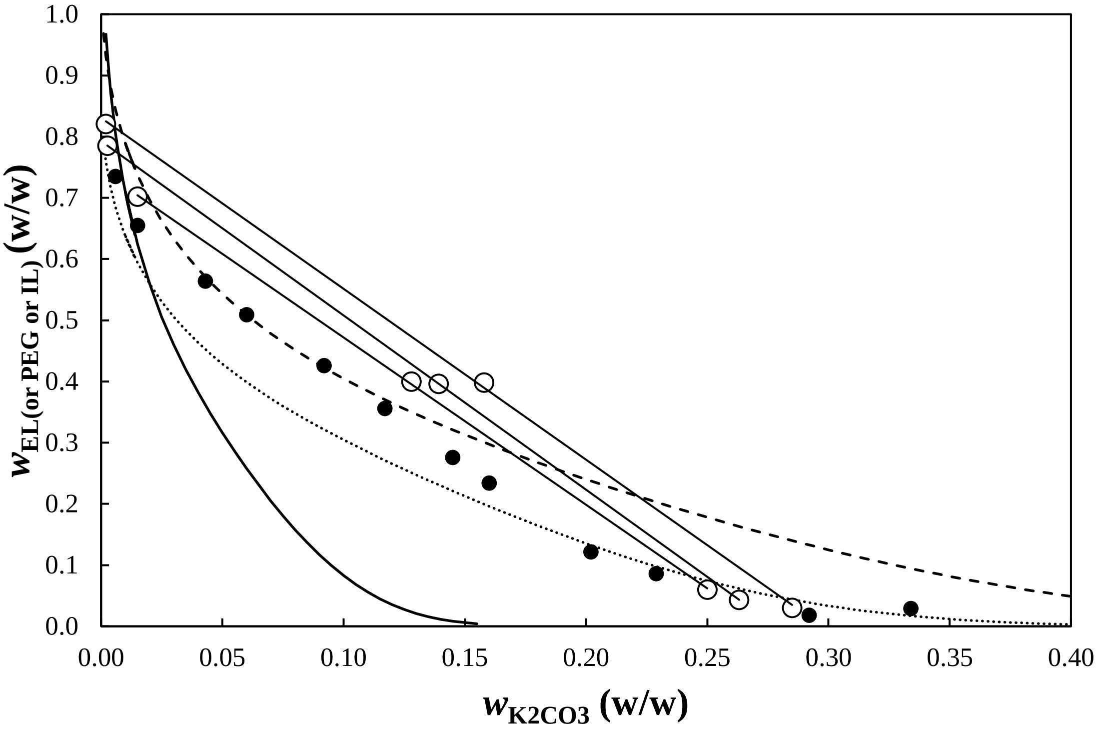
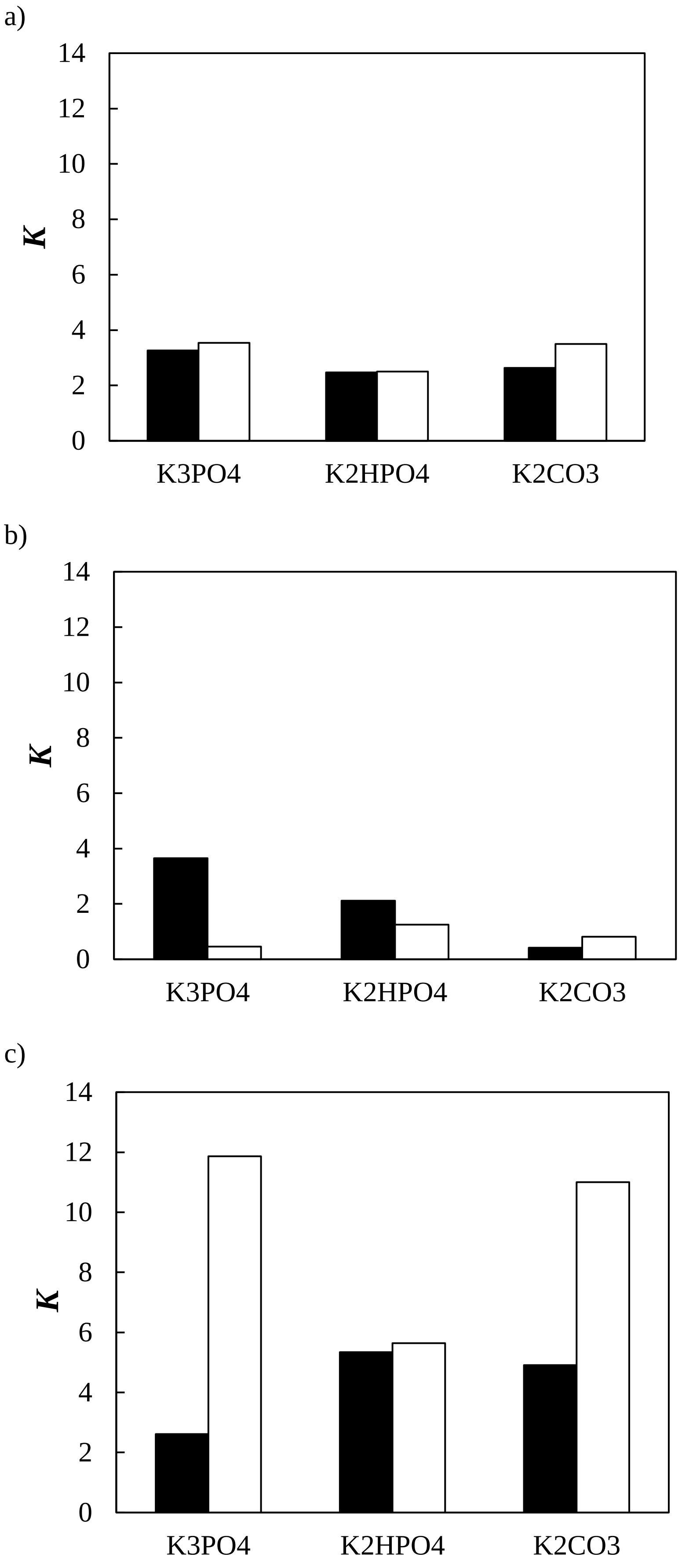

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
$w_{EL(or\ PEG\ or\ IL)}$ (w/w)
w_{K2CO3} (w/w)

Figure 3


a)
14
12
10
8
6
4
2
0
K
K3PO4
K2HPO4
K2CO3
b)
14
12
10
8
6
4
2
0
K
K3PO4
K2HPO4
K2CO3
c)
14
12
10
8
6
4
2
0
K
K3PO4
K2HPO4
K2CO3

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