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

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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.

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)

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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]

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 ±3·10−2 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:

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

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:

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

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.

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