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Extraction of some essential amino acids using aqueous two-phase systems made by sugar-based deep eutectic solvents

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Aqueous two-phase systems (ATPSs) have long been recognized as versatile and efficient tools for the extraction of biomolecules, including amino acids. Recent advancements in the field have introduced a novel approach by utilizing deep eutectic solvents (DES) to form ATPs. This study aimed to determine the phase diagrams for an ATPS made of polyethylene glycol dimethyl ether 250 and two types of NADESs, namely choline chloride as a hydrogen bond acceptor (HBA), and either sucrose or fructose as a hydrogen bond donor (HBD) with a molar ratio of 1 : 2. The measured tie-line results revealed that the hydrogen bonds of NADES may not be entirely disrupted in aqueous solutions, and thus, these ATPSs act as ternary-like systems. Additionally, the binodal data were fitted using two semi-empirical equations, namely Merchuk and Zafarani-Moattar *et al.* equations. Furthermore, the ATPSs mentioned above were applied to extract three amino acids, namely L-arginine, L-phenylalanine, and L-tyrosine, and demonstrated good extraction levels. Finally, the Diamond–Hsu equation and its modified version were utilized to correlate the experimental partition coefficients of the amino acids. These advancements pave the way for the development of improved extraction methodologies and the exploration of new applications in the field of biotechnology, pharmaceuticals, and beyond.

1. Introduction

With the advent of green chemistry, many investigators have been paying considerable attention to the discovery and application of green solvents.[1] Aqueous two-phase systems (ATPSs) are environmentally friendly alternatives for traditional organic water solvent extraction systems. An ATPS is mostly composed of water and non-volatile components. These systems have been used for many years in biotechnological applications such as the separation of amino acids, enzymes, proteins, *etc.*[2–4]

Recently, ATPSs based on deep eutectic solvents (DESs)[5–8] have been created to extraction of biomolecules. This innovation brings forth several distinctive features that enhance the partitioning process of amino acids. First, DES is composed of a hydrogen bond donor and acceptor, which facilitates the formation of ATPS with water. This unique property allows for

the creation of DES-based ATPS with tailored physicochemical properties, such as tunable polarity and viscosity, offering a wide range of design options for specific amino acid partitioning. Furthermore, DES-based ATPS exhibit excellent biocompatibility, making them highly suitable for biomolecule separation applications. The inherent low toxicity and biodegradability of DES ensure minimal interference with the integrity and functionality of the partitioned amino acids. Moreover, DES possesses a wide liquid range, enabling their application over a broad temperature range, expanding the operational flexibility of the partitioning process. Overall, the integration of deep eutectic solvents into aqueous two-phase systems for amino acid partitioning introduces a novel and promising approach that combines the unique properties of DES with the well-established advantages of ATPS, offering a platform for efficient and environmentally friendly separation and purification of amino acids.[9] However, ATPs made by natural deep eutectic solvents (NADES) have some advantages like low toxicity, and more stability in comparison of ATPs based on DESs. Natural deep eutectic solvents are bio-based deep eutectic solvents made up of two or more compounds derived from plants, such as organic acids, sugars, alcohols, amines, and amino acids.[5,10–12] Xu and co-workers[6,7,13,14] were used DESs for first time as a phase forming component of ATPS. Investigation on these kinds of ATPSs[15] provided convincing evidence about impairing interactions of the HBA and HBD in presence of water

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and that DES complexes can be entirely broken at high water concentrations. In other words, in DES-based aqueous two-phase systems, the HBA and HBD molar ratio is destroyed in the coexisting phases, demonstrating that the HBA and HBD act as phase forming ATPS components separately and ATPS is a quaternary mixture.[15] In this line of research, Farias et al.[8] have made a study about the ability of natural deep eutectic solvents to create ternary-like aqueous biphasic systems. Their ATPS was consisted of PPG$_{400}$ and the NADES (choline chloride as HBA and glucose or urea as HBD). This investigation indicated that a combination of factors, including the nature of the ATPS components and the hydrophobicity/hydrophilicity of the HBD, enables the formation of systems in which the HBA : HBD stoichiometry used in creation of NADES is retained in the co-existing phases, thus these ATPSs behave as ternary-like systems.[9] Similar studies on phase behavior and stability of natural deep eutectic solvents in aqueous solutions have been carried out.[16–18] Also, to investigation of DESs applications for extraction of macromolecules and amino acids the DES-based ATPSs have been applied for partitioning of proteins,[6,7,13,14] dyes,[8] phenolic compounds, amino acids and alkaloids.[19–22]

In this work, aqueous two-phase systems composed of polyethylene glycol dimethyl ether with a molar mass of the 250 g mol^{-1} (PEGDME$_{250}$), and a natural deep eutectic solvent (ChCl:sucrose or ChCl:fructose with 1 : 2 molar ratio), were studied at 298.15 K and under pressure of 85 kPa. For these aqueous two-phase systems, the binodal and tie-line values were determined. Merchuk[23] and Zafarani-Moattar et al.[24] equations were used to fit the binodal data. Tie-lines for { PEGDME$_{250}$ + ChCl:sucrose + water} and { PEGDME$_{250}$ + ChCl:fructose + water} ATPSs were also determined to see whether stoichiometry of initial components of NADES is maintained in the top and bottom phases or not, and from these data it can be considered that studied ATPSs act as ternary or quaternary systems.

Also, the application of these aqueous two-phase systems was studied for partitioning of some amino acids namely L-arginine, L-phenylalanine, and L-tyrosine. The partitioning coefficients, K, and the corresponding extraction efficiency, EE%, at each tie-line were calculated to the investigation of effect of amino acid nature and the kind of HBD in structure of NADES on the separation of mentioned amino acids. Finally, the Diamond–Hsu[25] equation and its modified form were used to fit the partitioning coefficient values.

2. Chemicals and methods

2.1. Chemicals

The choline chloride (>99.0% w/w) was provided from Daejung. The sucrose (>99.5% w/w), fructose (>99.5% w/w), PEGDME with a molar mass of 250 g mol^{-1} (>99.0% w/w), L-arginine (>99.0% w/w), L-phenylalanine (>99.0% w/w), and L-tyrosine (>99.0% w/w) were purchased from Merck. All of the materials were utilized without additional purification and also the solutions were prepared with double distilled deionized water. To determine the water content of compounds, the Karl-Fischer technique was utilized.

2.2. Preparation of NADES

In this study, two kinds of NADES (ChCl:sucrose and ChCl:fructose) with 1 : 2 molar ratio were prepared. According the procedure described previously,[26,27] for preparing NADES, first choline chloride as HBA and sucrose or fructose as HBD were mixed in a 50 mL round-bottom flask. After that, the flask was immersed in a tiny paraffin oil bath heated by a hot plate stirrer. Flask contents were stirred for 2 h at 353.15 K until a colorless, homogeneous liquid formed. A thermometer (0.01 K) was used to continually check the temperature of the NADES. After processing, all samples were stored in well-sealed vials in a moisture-controlled environment.

The temperature of the NADES was continuously monitored with a thermometer ($\pm$0.01 K). The samples were provided in a moisture controlled environment and then after preparation they kept in well-sealed vials. The Karl-Fischer analysis indicated the mass fraction water content of the prepared NADES to be 0.0008.

2.3. Methods

2.3.1. Determination of phase diagrams and tie-lines. The phase diagrams were created using the cloud point titration technique.[28–30] In this method NADES aqueous solution was added dropwise to PEGDME$_{250}$ solution until a cloudy solution which is biphasic region was observed, then water was added until a clear and monophasic region was detected. To determine the binodal curves, 60 wt% of PEGDME$_{250}$ aqueous solutions and 60 wt% of each NADES aqueous solutions (with molar ratio 1 : 2) were used. Throughout the experiment, the solution was constantly stirred. During the preparation of the solutions water contents of chemicals, which were measured by Karl Fischer coulometer (Metrohm 751 GPD) were taken into account. An analytical balance (Shimadzu, 321-34553, Shimadzu Co., Japan) with a precision of $\pm 1 \times 10^{-7}$ kg was used to determine the mass fraction of the components. The maximum uncertainty in determining the mass fraction of polymer and NADES was found to be 0.002.

Five different solutions of each ATPS consist of {PEGDME$_{250}$ + NADES (ChCl:sucrose or ChCl:fructose with 1 : 2 molar ratio) + water} were prepared to determination of tie-lines. A mixture of NADES, PEGDME$_{250}$ and water for each tie-line was prepared gravimetrically within $\pm 10^{-7}$ kg and at the biphasic region. The prepared solutions to reach equilibrium for 30 min were vigorously stirred, then centrifuged and placed in water bath with temperature of 298.15 K. The compositions of coexisting phases were analytically determined. The phenol–sulfuric acid method[31,32] was used to quantify the sucrose and fructose compositions. For this purpose, first in a test tube a 2 mL aliquot of a carbohydrate solution was mixed with 1 mL of 5% aqueous solution of phenol. Then, 5 mL of concentrated sulfuric acid was added rapidly to the mixture; the test tubes allowed standing for 10 min, and they were vortexed for 30 seconds before being placed in a room temperature water bath for 20 minutes for color development. Finally, a spectrophotometer (Model: SPECORD 40-Series Analytik Jena Germany) was used to record light absorption at 490 nm. For preparation

of reference solutions the same method as explained above was used, except that the double distilled deionized water was used instead of the 2 mL aliquot of carbohydrate.

Mohr method,[33] known as argentometric method, is one of the significant methods for determination of chloride in water. Choline chloride is a quaternary ammonium salt with choline cation and chloride anion. This method determines the chloride ion concentration of a solution by titration with silver nitrate using potassium chromate as indicator.

There are some common chemical indicators that are utilized with argentometric titrations: (I) the chromate ion, CrO_4^{2-} (the Mohr method); (II) adsorption indicators such as fluorescein (the Fajans method); (III) the ferric ion, Fe^{3+} (the Volhard method).[34,35]

Mohr indicator reaction is based on the following reactions:

$$Cl^- + AgNO_3 \rightarrow AgCl + NO_3^-$$

$$2Ag^+ + CrO_4^{2-} \rightarrow Ag_2CrO_4$$
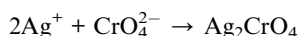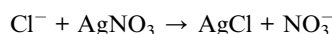
The concentration of titrant rises sharply near the equivalence point, and the solubility of Ag_2CrO_4 is exceeded. The appearance of red precipitate marks the endpoint.

$$ppm\left(\frac{mg}{1} \text{ as } Cl^-\right) = \frac{(A - B) \times N \times 35460}{V} \quad (1)$$

where A and B are the volumes of $AgNO_3$ for sample and blank, respectively. The normality of $AgNO_3$ is N, and V is the volume of water sample taken.

To determination of the polymer concentration in both phases the refractive index method was used which proposed for first time by Cheluget et al.[36] In our work a refractometer (ATAGO DR-A1, Japan) with a precision of 0.0001 was applied to measure the prepared solutions. The refractive index uncertainty was estimated to be 0.0002.

According to the refractive index method,[36] there is a relation between the refractive index of solution, n_D, and the corresponding components mass fractions in dilute aqueous solutions of each phase of an ATPS. The relation between n_D and mass fractions of polymer, w_p, choline chloride, w_c and sucrose, w_{su}, takes the following form:[16]

$$n_D = n_0 + a_p w_p + a_c w_c + a_{su} w_{su} \quad (2)$$

here, n_0 is the refractive index of pure water for which the value 1.3325 at $T = 298.15$ K was obtained. The values of constants a_p, a_c and a_{su} corresponding to polymer, choline chloride and sugar respectively were obtained from the linear calibration plots of the corresponding refractive index for the diluted solutions in range of mass fraction (C range(w/w)), which are reported in Table 1: values of the parameters of eqn (1), a_m, for ATPs containing {choline chloride (c): sucrose (su) + PEGDME$_{250}$ (p) + water (w)} and {choline chloride (c): fructose (su) + PEGDME$_{250}$ (p) + water (w)}system.

2.3.2. Determination of partition coefficient and the extraction efficiency for amino acids. To assess the performance of the investigated ATPSs in terms of amino acid partitioning

Table 1 Values of the parameters of eqn (1), a_m, for ATPs containing {choline chloride (c): sucrose (su) + PEGDME 250 (p) + water (w)} and {choline chloride (c): fructose (su) + PEGDME 250 (p) + water (w)} system

Material	Constant	Value	C Range (w/w)	$^a R^2$
ChCl	a_c	0.1452	0 to 0.08	0.9987
PEGDME$_{250}$	a_p	0.1323	0 to 0.10	0.9994
Sucrose	a_{su}	0.1486	0 to 1.80	0.9968
Fructose	a_{su}	0.1492	0 to 1.70	0.9908

^{a} Where, R^2, represented respective correlation coefficient value of the linear calibration plot of the refractive index against mass fraction of choline chloride, polymer and sugars at the mass fraction range (C range) of each material.

and extraction efficiency, the five mixture compositions based on the phase diagrams for {NADES (ChCl:sucrose or ChCl:fructose) + PEGDME$_{250}$ + H$_2$O} systems which determined before, were chosen. These mixtures had similar overall compositions used to obtain tie-lines. The prepared mixtures were vigorously stirred for 30 minutes before being put in a temperature-controlled water bath. After observation of phase separation 1 mL of each phase was carefully separated, mixed again and then 0.002 mass fractions of amino acids (L-arginine, L-phenylalanine, and L-tyrosine) was added to each sample. The samples were centrifuged at 2000 rpm for 10 minutes and kept in a water bath for 24 hours to ensure complete phase separation and equilibrium. Finally, after careful separation of phases in equilibrium, the concentrations of L-arginine, L-phenylalanine, and L-tyrosine in both phases were determined by UV spectroscopy using a spectrophotometer mentioned. To avoid interference from the phase components, the samples were diluted and analyzed against the blanks containing the same phase components but without amino acid.[37]

The partitioning coefficient, K, and extraction efficiency, EE%, were calculated respectively by eqn. (3) and (4) as below:

$$K = \frac{w\text{bot}_{amin}}{w\text{top}_{amin}} \quad (3)$$

$$EE\% = \frac{K}{K + 1} \times 100 \quad (4)$$

here, $w\text{top}_{amin}$ and $w\text{bot}_{amin}$ are the mass fractions of amino acid in the top and bottom phases respectively.

3. Results and discussion

3.1. Phase diagrams

For each studied ATPS made by PEGDME$_{250}$, NADES (ChCl:sucrose and ChCl:fructose (with molar ratio of 1 : 2)), and water phase diagrams at 298.15 K are plotted in Fig. 1, and the experimental mass fractions are also presented in Table 2.

The binodal data in Table 2 were fitted by Merchuk,[23] eqn (5), and Zafarani-Moattar et al.,[24] eqn (6) using a nonlinear least-square regression method.
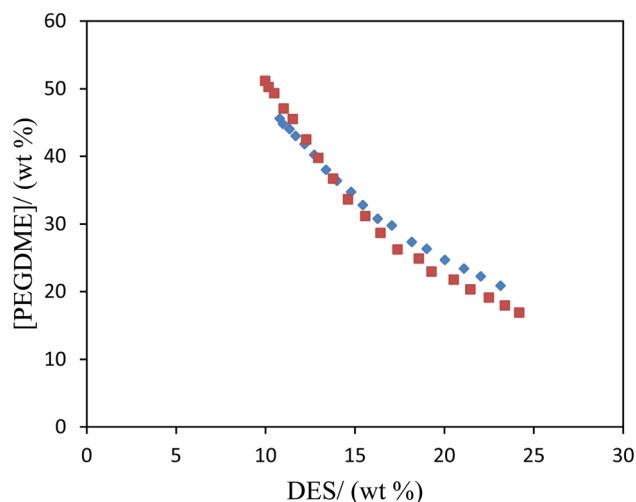
Fig. 1 Experimental binodal data of ATPSs composed of mixtures of ChlCl:sucrose and ChCl:fructose at 1 : 2 molar ratio, PEGDME$_{250}$, and water at 298.15 K: ChlCl:sucrose (◆); ChCl:fructose (■).

$$w_p = a \exp(bw_{NADES}^{0.5} - cw_{NADES}^3) \qquad (5)$$

$$w_p = \alpha + \beta \ln(w_{NADES}) + \gamma w_{NADES} \qquad (6)$$

In above equations w_p and w_{NADES} are mass fractions of polymer and NADES, respectively. The adjustable parameters of eqn. (5)

Table 2 Experimental binodal data of mass fraction (wt%) for the systems containing {ChCl:sucrose (w_{NADES}) + PEGDME$_{250}$ (w_p) + H$_2$O} and {ChCl:fructose (w_{NADES}) + PEGDME$_{250}$ (w_p) + H$_2$O} at 298.15 K and atmospheric pressure ($\approx$ 85 kPa).[a]

w_{NADES}		w_{NADES}	
ChCl:sucrose 1 : 2	w_p	ChCl:fructose 1 : 2	w_p
10.79	45.59	9.98	51.16
10.97	44.80	10.17	50.27
11.34	44.03	10.49	49.35
11.68	43.03	11.01	47.09
12.18	41.81	11.52	45.53
12.72	40.22	12.28	42.49
13.38	38.00	12.95	39.76
13.99	36.39	13.78	36.69
14.78	34.73	14.61	33.64
15.44	32.83	15.58	31.18
16.27	30.80	16.43	28.68
17.06	29.80	17.38	26.25
18.17	27.35	18.57	24.9
19.01	26.35	19.28	22.97
20.03	24.70	20.52	21.77
21.09	23.41	21.45	20.32
22.03	22.26	22.49	19.12
23.14	20.87	23.38	17.94
		24.19	16.9

[a] The standard uncertainties σ for temperature, pressure, and mass fraction are: σ (T) = 0.05 K; σ (p) = 0.5 kPa; and σ (w_i) = 0.005, respectively.

Table 3 The parameters values of eqn (5), (a, b, c), eqn (6), and (α, β, γ) for { PEGDME$_{250}$ + ChCl (HBA) + sucrose or fructose (HBD) + H$_2$O} systems at 298.15 K

	Merchuk (eqn (5))			
HBA : HBD (1 : 2)	a	b	c	100. sd[a]
ChCl:sucrose	2.3922	−5.0170	2.5096	0.26
ChCl:fructose	3.9377	−6.4102	0.0189	0.48

	Zafarani-Moattar *et al.* (eqn (6))			
	α	β	γ	100.sd
ChCl:sucrose	−0.7178	−0.4824	0.9492	0.24
ChCl:fructose	−1.3033	−0.7065	1.9455	0.45

[a] $sd = \left(\sum_{i=1}^{N} (w_1^{cal} - w_1^{exp})^2 / N \right)^{0.5}$ where N and w_1 represented number of binodal data and mass fraction of PEGDME$_{250}$, respectively.

and (6) {(a, b, and c) and (α, β and γ)} together with the corresponding standard deviation, sd, are presented in Table 3. The obtained values of sd indicate a good performance of both equations in regeneration of binodal data.

3.1.1. The effect of HBD on the phase formation. Fig. 1 shows the effect of sugar as HBD in the structure of NADES on the biphasic region of (PEGDME$_{250}$ + NADES + water) systems. This can be seen in Fig. 1, the biphasic region for NADES mixed by choline chloride and sucrose or fructose have a similar behavior and the biphasic region for both of {PEGDME$_{250}$ + ChCl:sucrose + water} and {PEGDME$_{250}$ + ChCl:fructose + water} are very close. This behavior indicates that the ATPS formation is dominated by the choline chloride with the HBD having a small effect on phase-forming ability.

3.2. Analysis of tie-lines

The tie-lines together with binodal curves are presented in Fig. 2 for ATPSs composed of NADES (ChCl:sucrose and ChCl:fructose) with 1 : 2 molar ratio, PEGDME$_{250}$, and water. For mentioned systems five tie-lines and their corresponding length (TLL) were characterized. The related data are listed in Table 4. It can be seen from this table, the top phase is always rich in PEGDME$_{250}$, and this behavior happens for both of studied ATPS and different tie-line length. The majority of bottom phase is composed of higher concentration of ChCl and sucrose or fructose than polymer and water. Therefore, bottom phase denoted as ChCl-rich phase.

Fig. 2 shows that, for the studied ATPSs, the ChCl-rich phase is more affected than the polymer-rich phase by variation of the TLL; so that by increasing the TLL the amount of water in bottom phase decreases remarkably leading to increase of the ChCl:sucrose or ChCl:fructose concentrations. But, the polymer-rich phase composition is only slightly changed by variations of the TLL or overall compositions. To investigate the final stoichiometry of HBA: HBD in the coexisting phases, the molar ratios for the NADESs (ChCl:sucrose and ChCl:fructose)
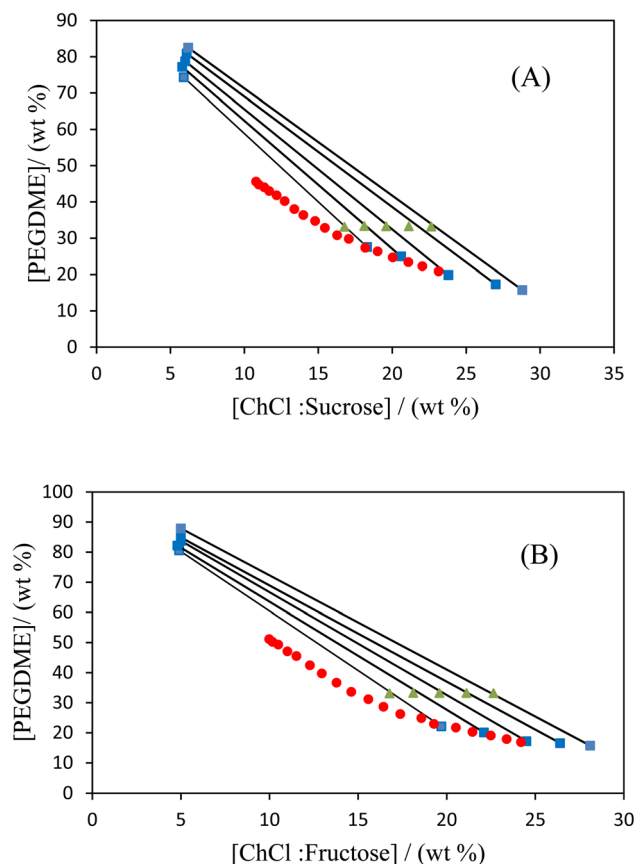
Fig. 2 Phases diagram for the ATPSs composed of {NADES + PEGDME$_{250}$ + H$_2$O} at 298 K: (A) ChCl:sucrose; (B) ChCl:fructose; tie-line overall composition (▲), binodal curve (●), tie-line phase composition (■).

were computed in each phases, and the results are listed in Table 5. This table enables us to compare the final HBA : HBD molar ratios in both phases with those selected initially as 1 : 2; the fairly good agreement between initial and calculated final molar ratios indicates that the initial molar ratios are

approximately retained and both studied ATPSs may be acted as a ternary-like system. In fact, this behavior can happen when the HBA and HBD both show extremely hydrophilic properties and poor solubility in the polymer-rich phase.[8]

Also, the data reported in Table 4 indicate that in polymer-rich phase (in both studied ATPSs) there are only very small amounts of NADES components. This behavior can be related to sucrose, fructose, and choline chloride high hydrophilic properties (log K_{ow} values are −3.70,-1.55, and −5.16, respectively);[38] so that these components have a low solubility in the hydrophobic polymer-rich phase and therefore prefer to stay in the bottom phase.[8]

3.3. The performance of NADES-PEGDME ATPSs in the partition behavior of amino acids

The experimental partition coefficients, K, and the percentage extraction efficiencies, EE%, of investigated amino acids (L-arginine, L-tyrosine, and L-phenylalanine) are presented in.

Table 6 and their variation with TLL can be seen in Fig. 3 and 4, respectively. For both the studied ATPSs the observed trend is as follows: K (L-arginine) > K (L-tyrosine) > K (L-phenylalanine). It is observed from Table 6 that amino acids effectively partitioned to the ChCl-rich phase (more hydrophilic phase).

3.3.1. The effect of each studied NADES on the amino acid partitioning behavior. The reported values of the experimental partition coefficients, K, and the extraction efficiencies, EE%, in Table 6 for each studied amino acid at 298.15 K in the ATPSs composed of (NADES (ChCl:sucrose and ChCl:fructose) + PEGDME$_{250}$ + water) with 1 : 2 molar ratio of HBA : HBD indicate that, the K values for system containing (NADES (ChCl:fructose) + PEGDME$_{250}$ + water) are slightly higher than the K values for system containing (NADES (ChCl:sucrose) + PEGDME$_{250}$ + water) and these values are very close to each other, this behavior maybe is because of the biphasic region of studied systems. Also, the studied amino acids in both mentioned ATPSs transferred preferentially to the bottom ChCl-rich phase.

Table 4 Experimental tie-line values of {ChCl (HBA) + sucrose or fructose (HBD) + PEGDME$_{250}$ + H$_2$O} systems at 298.15 K and atmospheric pressure (≈85 kPa).[a]

HBA : HBD molar ratio 1 : 2	Overall composition/wt%		Polymer-rich phase composition/wt%			ChCl-rich phase composition/wt%			
	[HBA : HBD]	[PEGDME]	[HBA]	[HBD]	[PEGDME]	[HBA]	[HBD]	[PEGDME]	TLL
ChCl:sucrose	16.77	33.11	1.09	4.8	74.29	3.59	14.71	27.56	47.84
ChCl:fructose			1.51	3.4	80.6	5.61	14.11	22.16	60.55
ChCl:sucrose	18.11	33.3	1.02	4.85	77.18	4.43	16.2	24.95	53.57
ChCl:fructose			1.47	3.39	82.18	7.34	14.92	20.15	63.36
ChCl:sucrose	19.59	33.25	1.12	4.89	78.75	5.03	18.81	19.83	60.66
ChCl:fructose			1.52	3.39	83.75	7.93	16.62	17.23	68.12
ChCl:sucrose	21.12	33.24	1.12	5.03	80.89	5.33	21.65	17.29	65.89
ChCl:fructose			1.52	3.5	84.89	9.15	17.31	16.59	70.07
ChCl:sucrose	22.64	33.19	1.09	5.12	82.49	6.14	22.69	15.78	69.21
ChCl:fructose			1.48	3.58	87.89	9.58	18.69	15.75	74.14

[a] The standard uncertainty of mass percent for each component is 0.8.

Table 5 The HBA (ChCl) and the HBD (sucrose or fructose) molar ratios in the ATPS coexisting phases composed of mixtures of ChCl and sucrose or fructose, PEGDME$_{250}$, and water

HBA : HBD molar ratio 1 : 2	Overall composition/wt%		HBA : HBD (mol : mol)		
	[HBA : HBD]	[PEGDME]	Polymer-rich phase	ChCl-rich phase	TLL
ChCl:sucrose	16.77	33.11	0.562	0.602	47.84
ChCl:fructose			0.569	0.512	60.55
ChCl:sucrose	18.11	33.3	0.511	0.666	53.57
ChCl:fructose			0.531	0.632	63.36
ChCl:sucrose	19.59	33.25	0.550	0.652	60.66
ChCl:fructose			0.569	0.614	68.12
ChCl:sucrose	21.12	33.24	0.539	0.599	65.89
ChCl:fructose			0.553	0.679	70.07
ChCl:sucrose	22.64	33.19	0.529	0.659	69.21
ChCl:fructose			0.538	0.656	74.14

Table 6 Partitioning coefficients, K, and extraction efficiency, EE%, values of studied amino acids for the system composed of {NADES [ChCl (HBA): sucrose or fructose (HBD)] + PEGDME$_{250}$ + H$_2$O} at 298.15 K and atmospheric pressure ($\approx$ 85 kPa)^{a,b}

HBA : HD molar ratio 1 : 2	Overall composition/wt%		K	EE%
	[HBA : HBD]	[PEGDME]		
L-Arginine				
ChCl:sucrose	16.77	33.11	2.82	73.82
	18.11	33.30	2.95	74.68
	19.59	33.25	3.09	75.55
	21.12	33.24	3.23	76.36
	22.64	33.19	3.45	77.53
ChCl:fructose	16.77	33.11	2.96	74.75
	18.11	33.30	3.09	75.55
	19.59	33.25	3.23	76.36
	21.12	33.24	3.37	77.12
	22.64	33.19	3.59	78.21
L-Tyrosine				
ChCl:sucrose	16.77	33.11	2.50	71.43
	18.11	33.30	2.63	72.45
	19.59	33.25	2.77	73.47
	21.12	33.24	2.91	74.42
	22.64	33.19	3.13	75.79
ChCl:fructose	16.77	33.11	2.66	72.68
	18.11	33.30	2.78	73.54
	19.59	33.25	2.95	74.68
	21.12	33.24	3.08	75.49
	22.64	33.19	3.28	76.64
L-Phenylalanine				
ChCl:sucrose	16.77	33.11	2.15	68.25
	18.11	33.30	2.28	69.51
	19.59	33.25	2.42	70.76
	21.12	33.24	2.56	71.91
	22.64	33.19	2.78	73.54
ChCl:fructose	16.77	33.11	2.31	69.79
	18.11	33.30	2.44	70.93
	19.59	33.25	2.56	71.91
	21.12	33.24	2.74	73.26
	22.64	33.19	2.94	74.62

^{a} The standard uncertainties σ for partitioning coefficient, temperature and pressure are: σ (K) = 0.15, σ (T) = 0.05 K and σ (p) = 0.5 kPa respectively. ^{b} The standard uncertainty σ for partitioning coefficient is: σ (K) = 0.1.

3.3.2. The effect of amino acids structure on their partitioning behavior. The chemical structures and polarities of amino acids are responsible for the observed trend of the K and EE% values (Table 6). All of the studied amino acids because of their polar properties have favorable interactions with hydrophilic phase (ChCl-rich phase), and therefore, partitioning of these amino acids to the polymer-rich phase occurs significantly less frequently. In fact, the presence of polar groups in amino acids structure increases its tendency to ChCh-rich bottom phase. The obtained high values of EE% collected in Table 6 imply that all of the investigated amino acids
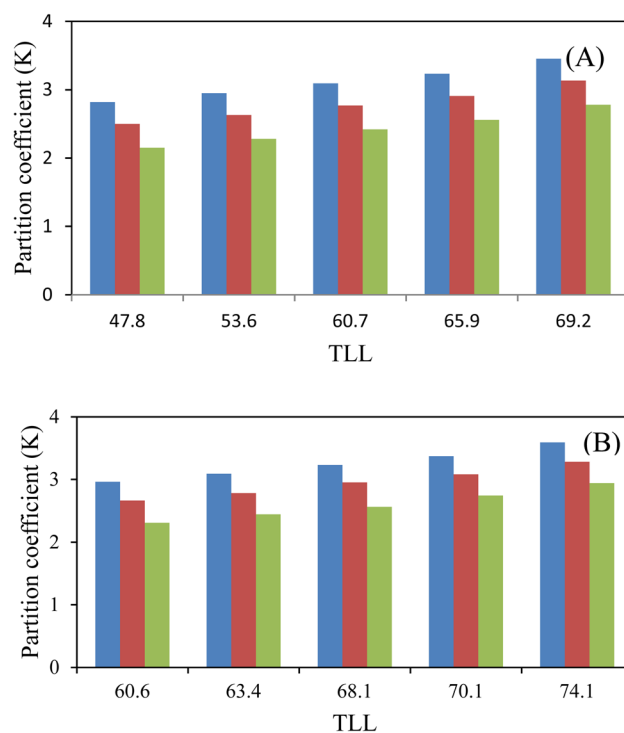
Fig. 3 Values of partition coefficient, K, in function of the TLL for each amino acid in studied ATPSs composed of {NADES + PEGDME$_{250}$ + H$_2$O}: (A) ChCl:sucrose; (B) ChCl:fructose. L–arginine ▮ L–tyrosine ▮ L–phenylalanine ▮ .
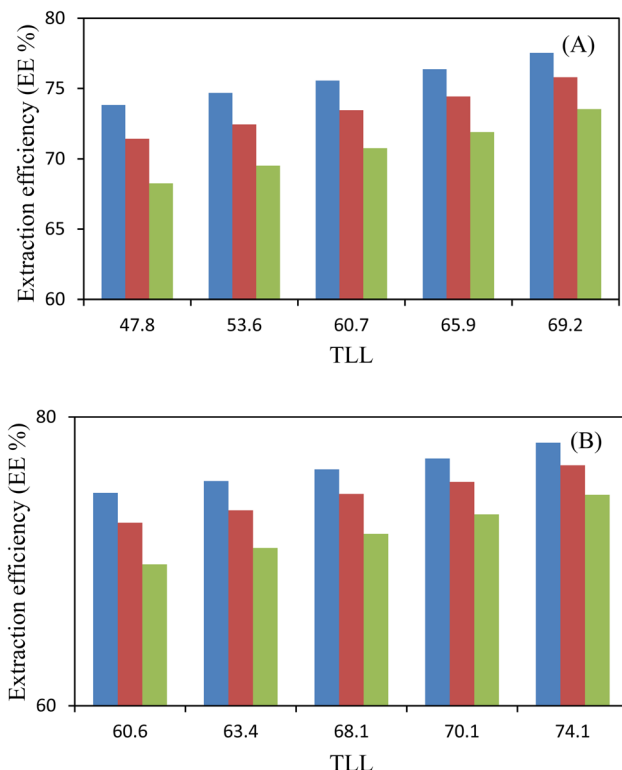
Fig. 4 Extraction efficiency, EE%, in function of the TLL for each amino acid in studied ATPSs composed of {NADES + PEGDME$_{250}$ + H$_2$O}: (A) ChCl:sucrose; (B) ChCl:fructose. L-arginine ▬ L-tyrosine ▬ L-phenylalanine ▬.

partitioned mostly in the ChCh-rich bottom phase. The observed trend for the amino acids K values is in good agreement with their hydrophobicity values defined by the logarithm of octanol/water partition coefficient (log K_{ow}); so that lower log K_{ow} values show that the solute have more hydrophilicity and higher tendency to water molecules.[28] The log K_{ow} values of amino acids are −4.20, −1.49 and −1.18 (ref. 39) for L-arginine, L-tyrosine, and L-phenylalanine, respectively. Therefore the amino acid extraction efficiency is in the order: L-arginine > L-tyrosine > L-phenylalanine. This study and previous one[39] indicate that the hydrophobicity of amino acids can be an important criteria to predict of solute partitioning between two-phases of an ATPS.

3.3.3. Correlations of the partition coefficients. For regeneration of the partition coefficient data a model is required; and here, the equation proposed by Diamond–Hsu[25] (eqn (7)) and its modified form were used for correlation of experimental partition coefficients values of the studied amino acids:

$$\ln K = A\Delta w(\text{PEGDME}_{250}) + B\Delta w(\text{PEGDME}_{250})^2 \quad (7)$$

$$\ln K = A_1 + B_1\Delta w(\text{PEGDME}_{250}) + C_1\Delta w(\text{PEGDME}_{250})^2 \quad (8)$$

In above relations, (A and B), and (A_1, B_1, and C_1) are respectively the adjustable parameters of eqn (7) and (8). The symbol $\Delta w(\text{PEGDME}_{250})$ is used for the mass fraction difference of PEGDME$_{250}$ in the top and bottom phase.

Table 7 The values of parameters of eqn (7) and (8) along with the standard deviation of the models, sd, from the experimental values of partition coefficients for the {PEGDME$_{250}$ + ChCl (HBA) + sucrose or fructose (HBD) + H$_2$O} at 298.15 K and atmospheric pressure (≈85 kPa)

HBA : HBD molar ratio 1 : 2	Diamond–Hsu (eqn (7))		
	A	$10^4 \times B$	sd
L-Arginine			
ChCl:sucrose	0.0303	−1.8232	0.58
ChCl:fructose	0.0220	−0.6173	0.42
L-Tyrosine			
ChCl:sucrose	0.0253	−1.2838	0.50
ChCl:fructose	0.0176	−0.1635	0.10
L-Phenylalanine			
ChCl:sucrose	0.0188	−0.5853	0.71
ChCl:fructose	0.0114	−0.4697	0.68

Modified Diamond–Hsu (eqn (8))				
	A_1	B_1	$10^4 \times C_1$	sd
L-Arginine				
ChCl:sucrose	1.4621	−0.0219	2.7535	0.09
ChCl:fructose	1.9717	−0.0387	4.0318	0.05
L-Tyrosine				
ChCl:sucrose	1.3694	−0.0236	3.0029	0.10
ChCl:fructose	1.4306	−0.0265	3.2097	0.04
L-Phenylalanine				
ChCl:sucrose	1.2501	−0.0258	3.3279	0.31
ChCl:fructose	1.9534	−0.0487	5.0755	0.52

For each amino acid the results of fitting experimental partition coefficients values to eqn (7) and (8) together with the corresponding standard deviations, sd are reported in Table 7. According to the sd values in Table 7, we conclude that correlation with all of the eqn (7) and (8) is satisfactory; and excellent performance is obtained with the eqn (8).

4. Conclusion

This study focused on the applications and composition of aqueous two-phase systems (ATPS) utilizing natural eutectic solvents as environmentally friendly alternatives. Specifically, phase diagrams were constructed for two ATPSs consisting of polyethylene glycol dimethyl ether (with a molar mass of 250 g mol^{-1}) and two types of natural deep eutectic solvents (NADESs). The NADESs were composed of choline chloride as the hydrogen bond acceptor (HBA), and either sucrose or fructose as the hydrogen bond donor (HBD), in a 1 : 2 molar ratio. The phase diagrams were studied at a temperature of 298.15 K and atmospheric pressure (approximately 85 kPa). Thorough analysis of the tie-lines revealed that the initial stoichiometry of HBA : HBD in each NADES was approximately maintained in the coexisting phases. Consequently, the ATPSs exhibited

properties resembling a ternary system, indicating that the hydrogen bonding interactions between the components of the NADESs were largely preserved within the ATPSs. Furthermore, the performance of these ATPSs in partitioning certain amino acids, namely L-arginine, L-tyrosine, and L-phenylalanine, was investigated. The experimental partition coefficients (K) and percentage extraction efficiencies (EE%) for the amino acids exhibited the following trend: L-arginine > L-tyrosine > L-phenylalanine. These results indicate that the chemical structures and polarities of the amino acids influence the observed variations in K and EE% values, with amino acids showing a preference for extraction into the ChCl-rich bottom phase.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare that they have no conflict of interest.

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