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Harnessing the power of natural deep eutectic solvents (choline chloride/sucrose) and polypropylene glycol in the formation of aqueous biphasic systems and the application of these systems in drug extraction†

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In recent times, there has been considerable interest in utilizing aqueous biphasic systems (ABSs) containing natural deep eutectic solvents (NADESs) for the extraction of various substances. In this study, we focused on investigating the phase behavior of ABSs composed of poly(propylene) glycol 400 and NADESs (specifically, choline chloride/sucrose with molar ratios of 2 : 1 and 1 : 1). By analyzing the compositions of tie-lines, it was observed that these ABSs, which consist of four components, exhibit characteristics similar to ternary systems. To examine the influence of molar ratios on phase separation, the binodal model was applied to the obtained binodal data. The NRTL and UNIQUAC models were employed to establish correlations for the tie-lines. Moreover, we examined the extraction capabilities of the aforementioned ABSs for three commonly used drugs: diclofenac potassium, acetaminophen, and salicylic acid. To assess the efficiency of extraction, partition coefficients and extraction efficiencies were calculated for each drug. The results revealed that the extraction efficiency of these drugs into the polymer-rich top phase is dependent on their hydrophobicity. Furthermore, we employed the Diamond–Hsu equation, along with its modified version, to establish correlations between the experimental partition coefficients of the drugs and NADES overall concentrations.

1. Introduction

Aqueous biphasic systems (ABSs) are widely used in various biotechnological applications due to their ability to separate biomolecules such as enzymes and proteins.[1,2] ABSs offer a cleaner and more sustainable alternative to traditional organic-water solvent extraction systems. Recently, deep eutectic solvents (DESs) have been employed to create ABSs for biomolecule extraction.[3–6] DESs are a type of liquid solvent that was introduced in 2003.[7] They are formed by combining and heating at least two starting chemicals, resulting in the formation of hydrogen bonds between a hydrogen-bond donor (HBD) and a hydrogen-bond acceptor (HBA). These DESs exhibit substantially lower melting points than their individual constituents. Among DESs, natural deep eutectic solvents (NADES) have gained attention as bio-based alternatives, derived from plant compounds such as organic acids, sugars, alcohols, amines, and amino acids.[3,8–10] The application of DESs as phase forming components in ABSs was pioneered by Xu and

colleagues.[4,5,11,12] Studies on these ABSs[13] have provided compelling evidence that the interactions between HBA and HBD are disrupted in the presence of water, leading to the complete breakdown of DES complexes at high water concentrations. Consequently, in DES-based ABSs, the molar ratio between HBA and HBD is disrupted in the coexisting phases, indicating that HBA and HBD function as separate phase forming components in ABSs, resulting in a quaternary mixture.[13] In the context of this research, Farias *et al.*[6] investigated the capability of natural deep eutectic solvents to form ternary-like ABSs. Their ABS system consisted of polypropylene glycol with a molar mass of 400 g mol⁻¹ (PPG400) and the NADES, with choline chloride (ChCl) serving as the HBA and glucose or urea as the HBD.

This study revealed that the combination of various factors, such as the composition of the ABS components and the hydrophobicity/hydrophilicity of the hydrogen bond donor (HBD), enables the creation of ABS systems in which the stoichiometry of the hydrogen bond acceptor (HBA) to HBD, as used in the preparation of natural deep eutectic solvents (NADES), is preserved in the equilibrium phases. As a result, these ABSs exhibit behavior similar to ternary systems.[6] Similarly, investigations have been conducted on the phase behavior of ABSs consisting of PPG400 and NADES (choline chloride: fructose)

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d3ra07066b

with different molar ratios.[14] In our previous research on the stability of natural deep eutectic solvents in aqueous solutions using vapor–liquid equilibria (VLE), we discovered that the hydrogen bonds between the initial components of the NADES were weakened when diluted with water. Ultimately, at approximately 75 wt% water content, the hydrogen bonds completely vanished.[15,16]

In this work, we investigated an aqueous biphasic system consisting of PPG400 and a natural deep eutectic solvent (NADES) composed of choline chloride as the hydrogen bond acceptor (HBA) and sucrose as the hydrogen bond donor (HBD) with molar ratios of 2 : 1 and 1 : 1. The experiments were conducted at a temperature of 298.15 K and atmospheric pressure (approximately 85 kPa). The binodal data and tie-lines were determined for these ABSs. Equations proposed by Merchuk[17] and Zafarani-Moattar et al.[18] were employed to fit binodal data. The binodal theory based on effective excluded volume (EEV)[19] was utilized to describe the influence of the two different molar ratios of NADES on the position of the binodal curves.

Tie-lines were also determined for the ABSs consisting of PPG400, ChCl : sucrose (in 2 : 1 and 1 : 1 molar ratios), and water. The purpose was to investigate whether the stoichiometry of the initial components of NADES is maintained in the top and bottom phases. Based on the obtained data, it was observed that these studied ABSs behave as either ternary or quaternary systems. The experimental tie-lines were fitted using two local composition models, namely NRTL[20] and UNIQUAC.[21,22]

In addition, our research delved into the use of these aqueous biphasic systems to separate diclofenac potassium, acetaminophen, and salicylic acid. We calculated partition coefficients (K) and their corresponding extraction efficiencies (EE%) at various tie-lines to explore how the ratio of natural deep eutectic solvents (NADES) and the characteristics of the drugs influenced their separation. To further analyze the results, we applied the Diamond–Hsu model and its modified version to fit the partition coefficient values.

2. Chemicals and methods

2.1. Chemicals

The choline chloride (>99 wt%) was provided from Daejung. Merck provided sucrose with a purity greater than 99.5 wt%, as well as PPG with a molar mass of 400 g mol^{-1}. Diclofenac potassium, acetaminophen, and salicylic acid, each with a purity exceeding 99.5 wt%, were sourced from Danna

Pharmaceutical Company in Iran. These chemicals were used without further purification. For solution preparation, double-distilled deionized water with a conductivity of 0.055 μS cm^{-1} was used. The Karl-Fischer technique was employed to determine the water content of the compounds, and additional information about the chemicals is detailed in Table 1.

2.2. Preparation of NADES

In this research, a particular type of natural deep eutectic solvent (NADES) was prepared using choline chloride as the hydrogen bond acceptor (HBA) and sucrose as the hydrogen bond donor (HBD) in different molar ratios of 2 : 1 and 1 : 1. The preparation of NADES followed the procedure outlined in previous studies.[23,24] Initially, choline chloride and sucrose were combined in a 50 mL round-bottom flask. The flask was then placed in a paraffin oil-bath heated by a hot plate stirrer. The mixture was stirred for 2 hours at a temperature of 353.15 K until a colorless and homogeneous liquid was obtained. The temperature of the NADES was continuously monitored using a thermometer with an accuracy of ±0.01 K. Subsequently, all samples were carefully stored in tightly sealed vials within a controlled moisture environment.

To assess the water content in the prepared NADES, Karl-Fischer analysis was conducted, revealing a mass fraction of water content of 0.0008.

2.3. Methods

2.3.1. Determination of phase diagrams and tie-lines. To construct the phase diagrams, the cloud point titration method was employed, following established techniques described in previous studies.[25–27] The experiments were conducted at a temperature of 298 K and atmospheric pressure (85 kPa). The method involved the gradual addition of NADES aqueous solution dropwise to a PPG400 solution until the appearance of a cloudy solution, indicating the presence of a biphasic region. Subsequently, water was added dropwise until a clear and monophasic region was observed. Aqueous solutions of PPG400 at a concentration of 60 wt% and NADES at a concentration of 60 wt% were utilized to determine the binodal curves. Throughout the experiment, continuous stirring was maintained. During the preparation of the solutions, the water contents of the chemicals were considered, and measurements were conducted using a Karl Fischer coulometer (Metrohm 751 GPD). A Shimadzu analytical balance (321-34553, Shimadzu Co.,

Table 1 Descriptions of the used chemicals

Material	Provenance	CAS number	Purity (mass fraction)	Purification method	Water content^a (mass fraction)
Choline chloride	Daejung	67-48-1	>0.99	Dried in vacuum over P_2O_5	0.0008
PPG$_{400}$	Fluka	25322-69-4	>0.99	None	0.0020
Sucrose	Merck	57-50-1	>0.995	Dried in vacuum over P_2O_5	0.0007
Diclofenac potassium	Danna. Iran	—	>0.995	None	0.0001
Salicylic acid	Danna. Iran	—	>0.995	None	0.0001
Acetaminophen	Danna. Iran	—	>0.995	None	0.0001

^{a} Determined by Karl-Fischer method.

Japan) with a precision of $\pm 1 \times 10^{-7}$ kg was used to determine the mass fraction of the components. The maximum uncertainty associated with determining the mass fraction of the polymer and NADES was found to be 0.002.

To determine the tie-lines, a total of five solutions with different compositions were prepared for each aqueous biphasic system (ABS) comprising of {PPG400 + NADES (ChCl : sucrose 2 : 1 and 1 : 1 molar ratios) + water}. For each tie-line, a precise gravimetric preparation of the biphasic region was carried out with an accuracy of $\pm 10^{-7}$ kg, involving the combination of NADES, polymer, and water. These solutions were vigorously stirred for 30 minutes, followed by centrifugation and placement in a water bath at 298 K until equilibrium was reached. The compositions of the coexisting phases were then determined through analytical methods.

The quantification of sucrose compositions was accomplished using the phenol–sulfuric acid method.[28,29] In this procedure, a 2 mL aliquot of the carbohydrate solution was mixed with 1 mL of a 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulfuric acid was rapidly added to the mixture. The test tubes were allowed to stand for 10 minutes, followed by vortexing for 30 seconds before being placed in a water bath at room temperature for 20 minutes to develop color. The light absorption at 490 nm was then recorded using a spectrophotometer (model: SPECORD 40-Series Analytik Jena AG-Germany).

For the preparation of reference solutions, the same procedure as described above was followed, with the exception that the 2 mL aliquot of carbohydrate solution was replaced with double distilled deionized water.

To ascertain the polymer concentration in both phases, the refractive index method introduced by Cheluget et al.[30] was employed. The refractive index of the solutions was measured using a refractometer (ATAGO DR-A1, Japan) with a precision of 0.0001. The uncertainty associated with the refractive index measurement was estimated to be 0.0002.

According to the refractive index method,[30] for dilute aqueous solutions of each phase of an ABS, there is a relation between the refractive index of solution, n_D, and the corresponding components mass fractions. The relation between n_D and mass fractions of polymer, w_p, choline chloride, w_c and sucrose, w_{su}, takes the following form:

$$n_D = n_0 + a_p w_p + a_c w_c + a_{su} w_{su} \quad (1)$$

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_f corresponding to polymer, choline chloride and sucrose respectively were obtained from the linear calibration plots of the corresponding refractive index of the solutions diluted in mass fraction range (C range (w/w)) as showed in Table 2. These constants together with the coefficient of determination values, R^2, are reported in Table 2. By knowing these constants, we have four unknowns in eqn (1). The n_D of diluted solutions of each phase in the mass fraction range of Table 2, was measured by a refractometer. The sucrose mass fraction w_{su} was determined by phenol–sulfuric acid procedure outlined

Table 2 The parameter values of eqn (1), a_m, for ABS containing {ChCl (c) : sucrose (f) +PPG$_{400}$ (p) + water (w)}

Material	Constant	Value	C range (w/w)	R^{2a}
PPG$_{400}$	a_p	0.1323	0 to 0.10	0.9994
ChCl	a_c	0.1452	0 to 0.08	0.9987
Sucrose	a_f	0.1486	0 to 1.80	0.9968

[a] Where the coefficient of determination, R^2, represents the respective correlation coefficient value of the linear calibration plot of the refractive index against the mass fraction of ChCl, polymer, and sucrose within the mass fraction range (C range) of each material.

above. Now by inserting the measured n_D and w_{su} in eqn (1), we obtain an equation with only two unknowns w_p, and w_c. On the other hand the sum of mass fractions in each phase is equal to unity ($w_w + w_p + w_c + w_{su} = 1$, here w_w is water mass fraction); so that, by inserting the water contents w_w value of each phase, measured by the Karl Fischer method, we will reach the second equation again with two unknowns w_p, and w_c. By solving these mentioned two equations for w_p, and w_c the mass fractions of polymer and choline chloride were determined. The uncertainty by this method was about 0.008 for each mass fraction of the ABS components.

2.3.2. Drugs partition coefficient and the corresponding extraction efficiency. To evaluate the effectiveness of the investigated ABSs in terms of drug partitioning and extraction efficiency, five mixture compositions (as presented in Table 5) were selected based on the previously determined phase diagrams for {NADES (ChCl : sucrose) + PPG400 + H$_2$O} systems.

Table 3 Experimental binodal data of mass fraction (wt%) for the systems containing {ChCl : sucrose (w_{NADES}) + PPG400 (w_p) + H$_2$O} at 298.15 K and atmospheric pressure (≈ 85 kPa)^a

w_{NADES} molar ratio 2 : 1	w_p	w_{NADES} molar ratio 1 : 1	w_p
6.33	50.97	9.44	44.77
6.48	50.36	9.62	43.98
6.61	49.75	9.99	43.21
7.04	48.04	10.33	42.21
7.49	46.30	10.83	40.99
8.14	44.04	11.37	39.40
8.72	41.63	12.03	37.18
9.57	38.63	12.64	35.57
10.30	35.88	13.43	33.91
11.21	33.33	14.09	32.01
11.94	31.10	14.92	29.98
12.74	28.61	15.71	28.98
13.48	26.79	16.82	26.53
14.25	24.74	17.66	25.53
14.90	22.86	18.68	23.88
15.79	21.23	19.74	22.59
16.77	20.17	20.68	21.44
17.33	18.49	21.79	20.05
18.47	17.73		
19.01	16.32		
20.14	15.58		

^{a} The standard uncertainties σ for temperature, pressure, and mass fraction are: $\sigma(T) = 0.05$ K; $\sigma(p) = 0.5$ kPa; and $\sigma(w_i) = 0.005$, respectively.

These mixtures were designed to have similar overall compositions to those used for obtaining the tie-lines. The prepared mixtures were subjected to vigorous stirring for a duration of 30 minutes. Subsequently, they were carefully transferred to a water bath, where they were maintained at the desired temperature. Upon the observation of phase separation, 1 mL of each phase was meticulously isolated. These separated phases were then combined and further supplemented with a 0.002 mass fraction of the drugs (diclofenac potassium, salicylic acid, and acetaminophen) under investigation.

The samples were then subjected to centrifugation at 2000 rpm for 10 minutes and kept in a water bath for 24 hours to ensure complete phase separation and reach equilibrium. After the establishment of equilibrium, the concentrations of diclofenac potassium, salicylic acid, and acetaminophen in both phases were determined using UV spectroscopy with a spectrophotometer mentioned earlier at specific wavelengths: 284 nm for diclofenac potassium, 261 nm for salicylic acid, and 303 nm for acetaminophen. In order to mitigate interference from the components present in the phases, the samples were appropriately diluted and analyzed alongside blanks containing the same phase components but without the drugs.[31]

The partitioning coefficient, K, and extraction efficiency, EE%, were calculated respectively by eqn (2) and (3) as below:

$$K = \frac{w_{\text{top}_{\text{drug}}}}{w_{\text{bot}_{\text{drug}}}} \quad (2)$$

$$\text{EE\%} = \frac{K}{K+1} \times 100 \quad (3)$$

here, $w_{\text{top}_{\text{drug}}}$ and $w_{\text{bot}_{\text{drug}}}$ are the mass fractions of drug in the top and bottom phases respectively.

3. Results and discussion

3.1. Phase diagram

The phase diagrams of studied ABS composed of PPG400, NADES (obtained at two molar ratios of ChCl : sucrose (2 : 1 and 1 : 1)), and water at 298.15 K are plotted in Fig. 1, and the experimental mass fraction data are also listed in Table 3.

The binodal data presented in Table 3 were fitted to the equations presented by Merchuk,[17] eqn (4), and Zafarani-Moattar et al.,[18] eqn (5) using a nonlinear least-square regression method.

$$w_{\text{p}} = a \exp(b w_{\text{NADES}}^{0.5} - c w_{\text{NADES}}^{3}) \quad (4)$$

$$w_{\text{p}} = \alpha + \beta \ln(w_{\text{NADES}}) + \gamma w_{\text{NADES}} \quad (5)$$

In above equations w_{p} and w_{NADES} are mass fractions of polymer and NADES, respectively. The adjustable parameters of eqn (4) and (5) {(a, b, and c) and (α, β and γ)} together with the corresponding standard deviation, sd, are presented in Table 4. The obtained sd values indicate a good performance of both equations in regeneration of binodal data.

3.1.1. The effect of molar ratio of NADES on the phase formation. The impact of the molar ratio of NADES on the biphasic region in the (PPG400 + NADES + water) system can be
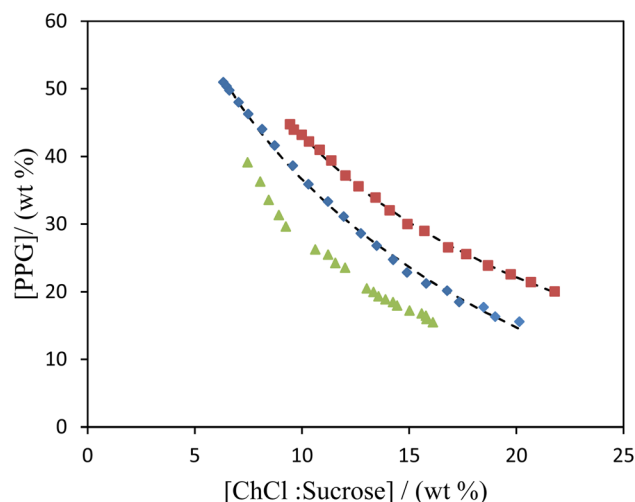

Fig. 1 Experimental binodal data of ABS composed of mixtures of ChlCl : sucrose at different molar ratio, PPG$_{400}$, and water at 298.15 K: 2 : 1 (◆); 1 : 1 (■); PPG$_{400}$ + ChCl + water (▲);[32] (—) line is calculated binodal data from eqn (4).

Table 4 The parameters values of eqn (4), (a, b, c), eqn (5), (α, β, γ), and eqn (6) ($v^{*}_{\text{p-NADES-w}}$, f), for {PPG$_{400}$ + ChCl (HBA) + sucrose (HBD) + H$_2$O} systems at 298.15 K

	Merchuk (eqn (4))			
HBA : HBD	a	b	c	100 sd[a]
1 : 1	1.9800	−4.8015	5.3315	0.27
2 : 1	1.7492	−4.8182	40.0377	0.53

	Zafarani-Moattar et al. (eqn (5))			
HBA : HBD	α	β	γ	100 sd
1 : 1	−0.5674	−0.4034	0.6976	0.21
2 : 1	−0.4509	−0.3453	0.2146	0.49

	Effective excluded volume theory, binodal model (eqn (6))		
HBA : HBD	$v^{*}_{\text{p-NADES-w}}$	f	100 sd
1 : 1	4.2672	9.9934	1.56
2 : 1	5.0020	10.7063	1.29
ChCl + PPG + water[32]	5.2765	13.6546	0.84

[a] sd = $\left(\sum_{i=1}^{N} (w_1^{\text{cal}} - w_1^{\text{exp}})^2 / N\right)^{0.5}$ where N and w_1 represented number of binodal data and mass fraction of PPG$_{400}$, respectively.

examined by referring to Fig. 1. The figure illustrates that the biphasic region for NADES expands as the molar ratio increases, suggesting that an elevated concentration of ChCl promotes phase separation. In order to assess the influence of ChCl on the phase separation, the binodal curve for the ternary (PPG400 + ChCl + water) system[32] is also depicted in Fig. 1. It is evident that the biphasic region for the ABS comprising PPG400 and ChCl is larger when compared to the ABS involving the (PPG400 +

NADES + water) system, indicating a more pronounced salting-out effect when pure ChCl is employed.

A similar trend was observed in the comparison of ABSs containing NADES (ChCl : glucose) and PPG400 with various molar ratios, as well as ABSs consisting of ChCl and PPG400, as reported by Farias *et al.*[6] It has been postulated[32,33] that in these types of ABSs, the formation of ABS is driven by a salting-out mechanism induced by ChCl, which exhibits salt-like behavior and possesses a higher capacity for forming hydration complexes. Consequently, ChCl acts as a potent salting-out agent.

In order to assess the impact of the molar ratio of NADES on the stability of the salting-out phenomenon, the binodal model developed by Guan and colleagues[19] was employed. This model enabled the correlation of the experimental binodal data presented in Table 3 with eqn (6). By utilizing this correlation, the effective excluded volume (EEV), which serves as an indicator of salting-out strength, could be determined:[34]

$$\ln\left(v^*_{\text{p-NADES-w}} \frac{w_{\text{NADES}}}{M_{\text{NADES}}} + f_{\text{NADES}}\right) + v^*_{\text{p-NADES-w}} \frac{w_{\text{p}}}{M_{\text{p}}} = 0 \quad (6)$$

$$v^*_{\text{p-NADES-w}} = \rho N_{\text{a}} v_{\text{p-NADES-w}} \quad (7)$$

Here, $v^*_{\text{p-NADES-w}}$ denotes the polymer's effective excluded volume (EEV) in an aqueous solution containing NADES. This parameter shows the spacing of the nearest polymer molecular centers. f_{NADES} is the volume fraction of the unfilled effective available volume after tight packing of NADES molecules into the network of the polymer molecules in the polymer aqueous solution. $v_{\text{p-NADES-w}}$ is the volume of polymer species at constant solution density, ρ, in presence of NADES. M_{p}, M_{NADES}, w_{p} and w_{NADES} are molar masses and mass fractions of polymer and NADES, respectively.

The EEV values, $v^*_{\text{p-NADES-w}}$, presented in Table 4 and binodal curves depicted in Fig. 1 indicate that the larger EEV values are obtained for system with greater salting-out strength. These values are increased by increasing molar ratio of HBA : HBD from (1 : 1) to (2 : 1) indicating that choline chloride plays an important role as the salting-out effect.

3.2. Analysis of tie-lines

The tie-lines and binodal curves for the ABSs composed of PPG400 and NADES (ChCl : sucrose) with two molar ratios (2 : 1 and 1 : 1) are presented in Fig. 2. The experimental tie-lines and their corresponding tie-line lengths (TLL) for the investigated systems are summarized in Table 5. The results demonstrate a consistent pattern, with the top phase consistently enriched in PPG400 (>79 wt%) across different molar ratios of NADES and tie-line lengths. The majority of the bottom phase composed of water (>50 wt%), as well as higher concentrations of ChCl and sucrose compared to the polymer, thus indicating a ChCl-rich phase. This behavior aligns with previous findings for ABSs containing NADES (ChCl : glucose or urea) and PPG400 with varying molar ratios.[6]

Fig. 2 further reveals that, among ABSs with different molar ratios, the ChCl-rich phase is more influenced by variations in
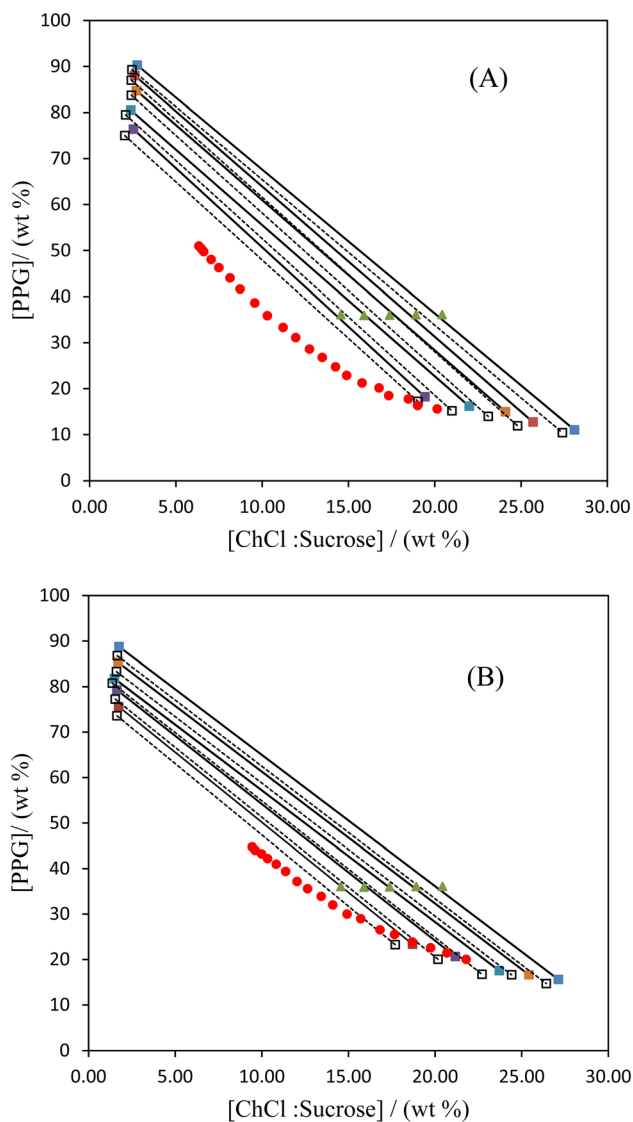

Fig. 2 Phases diagram for the ABS composed of {NADES [ChCl : sucrose] + PPG$_{400}$ + H$_2$O} at 298 K and different ChCl : sucrose molar ratios: (A) 2 : 1; (B) 1 : 1; tie-line overall composition (▲), binodal curve (●), tie-line phase composition (■). ■—■, experimental tie-lines; □....□, calculated tie-lines using the NRTL model.

the TLL compared to the PPG400-rich phase. Increasing the TLL leads to a significant reduction in the water content of the bottom phase, resulting in higher concentrations of ChCl and sucrose. However, the composition of the PPG-rich phase only undergoes slight changes in response to variations in the TLL or overall compositions. To investigate the final stoichiometry of the hydrogen bond acceptor to hydrogen bond donor (HBA : HBD) in the coexisting phases, the ChCl : sucrose molar ratios were calculated for both phases of the studied tie-lines, and the results are presented in Table 6 and Fig. 3. This comparison allows us to assess the agreement between the initially selected molar ratios (2 : 1 and 1 : 1) and the calculated final molar ratios. The fairly good agreement observed suggests that the initial molar ratios are approximately maintained, supporting the characterization of the studied ABS as a ternary-like system.

Table 5 Experimental tie-line values of {ChCl (HBA) + sucrose (HBD) + PPG$_{400}$ + H$_2$O} systems at 298.15 K and atmospheric pressure (≈ 85 kPa)^a

HBA : HBD molar ratio	Overall composition/wt%		HBA : HBD (mol : mol)		
	[HBA : HBD]	[PPG]	PPG-rich phase	ChCl-rich phase	TLL
2 : 1	14.56	36.07	2.491 ± 0.13	1.931 ± 0.09	63.10
1 : 1			1.194 ± 017	0.982 ± 0.20	61.12
2 : 1	15.9	35.92	2.351 ± 0.10	2.119 ± 0.10	66.50
1 : 1			1.231 ± 0.18	1.242 ± 0.19	65.10
2 : 1	17.38	35.98	2.799 ± 0.12	2.035 ± 0.08	66.81
1 : 1			1.090 ± 0.21	1.157 ± 0.20	65.89
2 : 1	18.91	36.07	2.489 ± 0.12	2.091 ± 0.08	67.52
1 : 1			1.225 ± 0.12	1.178 ± 0.20	68.51
2 : 1	20.43	36.1	2.598 ± 0.14	2.033 ± 0.09	68.21
1 : 1			1.295 ± 0.12	1.173 ± 0.20	71.76

^{a} The standard uncertainty of mass percent for each component is 0.8.

Table 6 The HBA (ChCl) and the HBD (sucrose) molar ratios in the ABS coexisting phases composed of mixtures of ChCl and sucrose, PPG$_{400}$, and water

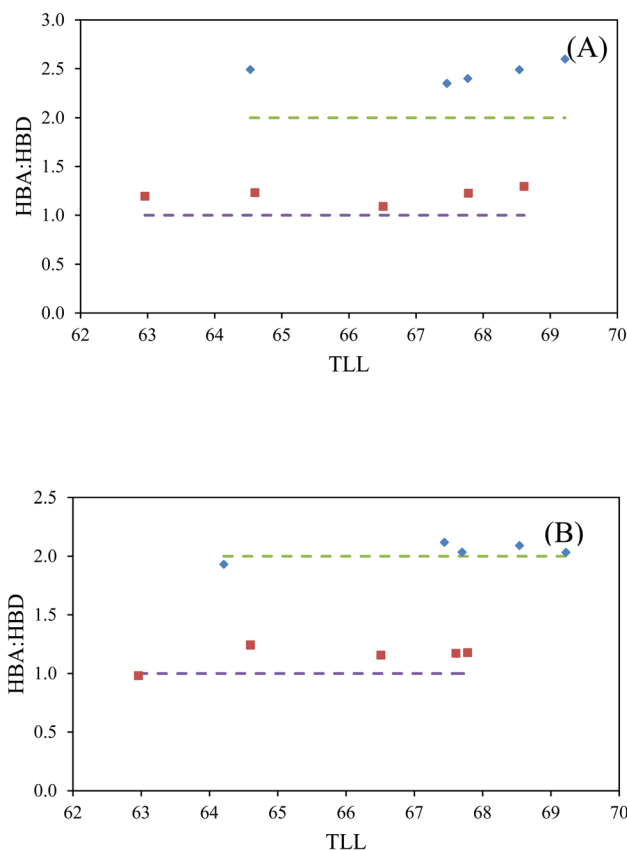| HBA : HBD molar ratio	Overall composition/wt%		HBA : HBD (mol : mol)		
	[HBA : HBD]	[PPG]	PPG-rich phase	ChCl-rich phase	TLL
2 : 1	14.56	36.07	2.491 ± 0.13	1.931 ± 0.09	63.10
1 : 1			1.194 ± 017	0.982 ± 0.20	61.12
2 : 1	15.9	35.92	2.351 ± 0.10	2.119 ± 0.10	66.50
1 : 1			1.231 ± 0.18	1.242 ± 0.19	65.10
2 : 1	17.38	35.98	2.799 ± 0.12	2.035 ± 0.08	66.81
1 : 1			1.090 ± 0.21	1.157 ± 0.20	65.89
2 : 1	18.91	36.07	2.489 ± 0.12	2.091 ± 0.08	67.52
1 : 1			1.225 ± 0.12	1.178 ± 0.20	68.51
2 : 1	20.43	36.1	2.598 ± 0.14	2.033 ± 0.09	68.21
1 : 1			1.295 ± 0.12	1.173 ± 0.20	71.76

Fig. 3 Stoichiometry of the HBA (ChCl) and the HBD (sucrose) molar ratio in the coexisting phases of ABS composed of {NADES [ChCl : sucrose] + PPG$_{400}$ + H$_2$O}: 2 : 1 (◆); 1 : 1 (■). (A) Molar ratio in the top phase; (B) molar ratio in the bottom phase. Dashed lines represent initial molar ratios.

Similar behaviors have been reported for ternary-like ABSs comprising PPG400 and deep eutectic solvents (ChCl : glucose or urea),[6] demonstrating consistency with our findings. This behavior arises when both the HBA and HBD possess highly hydrophilic properties and exhibit poor solubility in the PPG-rich phase, which serves as a hydrophobic ABS former.[6]

The findings presented in Table 6 demonstrate that the PPG-rich phase, regardless of the molar ratio (2 : 1 and 1 : 1), contains only trace amounts of NADES components. This behavior can be attributed to the highly hydrophilic nature of choline chloride and sucrose, as indicated by their log K_{ow} values of −0.325 and −3.70, respectively.[35] Due to their hydrophilicity, these components exhibit low solubility in the extremely hydrophobic polymer-rich phase, favoring their retention in the NADES-rich phase. Similar observations have been reported for ABSs consisting of PPG400 and NADES (ChCl : glucose) with various molar ratios,[6] supporting this rationale.

From vapor liquid equilibrium studies on the aqueous NADESs (ChCl : sucrose, ChCl : fructose and ChCl : urea) solutions[15,16] it was concluded that by diluting these NADESs the interaction between HBD and HBA is almost retained when the water content is less than 75 wt%. On the basis of data presented in Table 5, in the studied ABS the water contents of both phases are much lower than 75 wt% (i.e., about 6–19 wt% and 55–62 wt% in polymer-rich and NADES-rich phases, respectively), therefore NADESs are most likely stable in both phases and maintained their HBD : HBA ratio making the ABS to behave as a ternary-like mixture.

3.2.1. Local composition models for estimating the tie-line compositions. For representing the tie-line compositions two commonly used local composition models, the Nonrandom Two-Liquid (NRTL)[20] and Universal Quasi-Chemical theory (UNIQUAC)[21] which usually give satisfactory results in fitting LLE data, were applied.

3.2.1.1 NRTL model. Renon and Prausnitz[20] used the concept of local composition in the derivation of NRTL (Nonrandom Two-Liquid) equation. This equation is applicable to partially miscible as well as completely miscible systems. The activity coefficient in NRTL model is defined as follows:[20]

$$\ln \gamma_i = \frac{\sum_{j=1}^{m} X_j G_{ji} \tau_{ji}}{\sum_{i=1}^{m} X_k G_{ki}} + \sum_{j=1}^{m} \frac{X_j G_{ij}}{\sum_{k=1}^{m} X_k G_{kj}} \left(\tau_{ij} - \frac{\sum_{r=1}^{m} X_r G_{rj} \tau_{rj}}{\sum_{k=1}^{m} X_k G_{kj}} \right) \quad (8)$$

where $G_{ij} = \exp(-\alpha_{ij}\tau_{ij})$, $\alpha_{ij} = \alpha_{ji}$ and $\tau_{ii} = \tau_{jj} = 0$.

In this equation α_{ij} is a non-randomness parameter and usually set between 0.01–0.4 values. In this study, α_{ij} was fixed at 0.1. Also τ_{ij} is the binary interaction parameter which is expressed by the following equation:[20]

$$\tau_{ji} = \frac{g_{ij} - g_{ii}}{RT} \quad (9)$$

where g_{ij} is an energy parameter characteristic of the i–j interaction. The adjustable interaction parameter, Δg_{ij}, for this model is defined with $\Delta g_{ij} = g_{ij} - g_{ii}$.

3.2.1.2 UNIQUAC model. UNIQUAC (the Universal Quasi-Chemical theory) for $\ln \gamma_i$ consists of two parts: on the one hand, a combinatorial part, $\ln \gamma_i^C$, which describes the entropic contribution, and on the other hand a residual part, $\ln \gamma_i^R$,- which accounts for the intermolecular forces that are responsible for the enthalpy of mixing. The combinatorial part depends on the composition, the size and the shape of the molecules. The residual part depends on intermolecular forces. The UNIQUAC equation[21] contains adjustable interaction parameters and is defined as:

$$\ln \gamma_i = \ln \gamma_i^C + \ln \gamma_i^R \quad (10)$$

$$\ln \gamma_i^C = \ln\left(\frac{\Phi_i}{x_i}\right) + \frac{z}{2}q_i \ln\left(\frac{\theta_i}{\Phi_i}\right) + l_i - \frac{\Phi_i}{x_i}\sum_{j=1}^{m} x_j l_j \quad (11)$$

$$l_i = \frac{z}{2}(r_i - q_i) - (r_i - 1) \quad (12)$$

$$\ln \gamma_i^R = -q_i \left(1 + \ln\left(\sum_{j=1}^{m} \theta_i \tau_{ji}\right) - \sum_{j=1}^{m} \frac{\theta_i \tau_{ji}}{\sum_{k=1}^{m} \theta_k \tau_{kj}} \right) \quad (13)$$

$$\theta_i = \frac{q_i x_i}{\sum_j q_j x_j} \Phi_i = \frac{r_i x_i}{\sum_j r_j x_j} \quad (14)$$

The variables θ_i, Φ_i, and τ_{ji} are the volume fraction, area fraction, and interaction parameter between molecules i and j, respectively. The coordination number, denoted as Z, represents the count of molecules that surround the central molecule. In this context, Z is assigned a value of 10. Additionally, the parameters r and q serve as pure component molecular-structure constants, which are contingent upon the molecular size and the external surface areas. The mentioned parameters for NADES are calculated by:

$$A_i = x_1 A_1 + x_2 A_2 \quad (15)$$

Here the mole fractions of choline chloride and fructose are denoted by x_1 and x_2, respectively. The related adjustable interaction parameter to an energy parameter characteristic of the i–j interaction (Δu_{ij}) for this model is:

$$\tau_{ji} = \exp\left(-\frac{\Delta u_{ji}}{RT}\right) \quad (16)$$

The interaction parameters of the NRTL and UNIQUAC models were determined by minimizing the objective function eqn (17).

Table 7 The parameters values of NRTL and UNIQUAC models for systems containing {NADES [ChCl (HBA) : sucrose (HBD)] + PPG$_{400}$ + H$_2$O} at $T = 298.15$ K

Molar ratio of 2 : 1

NRTL

τ_{NADESw}	τ_{wNADES}	τ_{pw}	τ_{wp}	τ_{NADESp}	τ_{pNADES}	100 Dev[a]
814.8449	−136.9850	38.5273	−27.8779	−14.8109	−105.7515	0.04

UNIQUAC

Δu_{NADESw}	Δu_{wNADES}	Δu_{pw}	Δu_{wp}	Δu_{NADESp}	Δu_{pNADES}	100 Dev
−565.892	−801.410	−289.023	301.543	−1436.431	1632.871	0.41

Molar ratio of 1 : 1

NRTL

τ_{NADESw}	τ_{wNADES}	τ_{pw}	τ_{wp}	τ_{NADESp}	τ_{pNADES}	100 Dev
40.8927	−12.5615	6.3442	−2.5538	−3.0350	−10.2793	0.03

UNIQUAC

Δu_{NADESw}	Δu_{wNADES}	Δu_{pw}	Δu_{wp}	Δu_{NADESp}	Δu_{pNADES}	100 Dev
413.301	−512.325	−211.765	143.572	−1736.245	−2005.231	0.39

[a] $\mathrm{Dev} = \left(\sum_p \sum_l \sum_j \sum_T (100 w_{p,l,j,T}^{cal} - 100 w_{p,l,j,T}^{exp})^2 / 6N \right)$ where $w_{p,l,j,T}$ is the mass fraction of the component j (*i.e.* DES, polymer or water) and N is the number of tie-line data.

$$\text{OF}(X) = \left(\sum_T \sum_p \sum_n \sum_j \left(x_{p,n,j,T}^{\text{cal}} - x_{p,n,j,T}^{\text{exp}} \right)^2 \right) \quad (17)$$

In eqn (17), $x_{p,l,j,T}$ is the mol fraction of the component j in the phase p for n tie-line at working temperature. The superscripts "cal" and "exp" refer to the calculated and experimental values, respectively. LLE data of Table 5 were correlated using eqn (17) and the equilibrium condition:

$$(x_i \gamma_i)^{\text{top}} = (x_i \gamma_i)^{\text{bot}} \quad (18)$$

The tabulated outcomes of tie-line data fitting for ABS comprising (PPG400 + NADES (with two different molar ratios of HBA : HBD, namely 2 : 1 and 1 : 1) + water) employing the NRTL and UNIQUAC models have been compiled in Table 7. The negligible standard deviations exhibited in Table 7 signify that both models adequately correlate the tie-line data. Furthermore, it is observed that the NRTL model demonstrates slightly superior performance in representing tie-lines compared to the UNIQUAC model.

3.3. The performance of NADES–PPG ABS in the partition behavior of drugs

Table 8 displays the experimental partition coefficients, K, and the percentage extraction efficiencies, EE%, for the drugs under investigation, namely diclofenac potassium, salicylic acid, and acetaminophen. The variation of these coefficients concerning the Tie-Line Length (TLL) is illustrated in Fig. 4 and 5, respectively. For both molar ratios of Natural Deep Eutectic Solvents (NADES), the observed trend is consistently as follows: K (diclofenac potassium) > K (salicylic acid) > K (acetaminophen). The data in Table 8 evidently indicates that the drugs predominantly partition into the more hydrophobic phase, which is the PPG-rich phase.

3.3.1. The effect of NADES molar ratio on the drug partitioning behavior. The data presented in Table 8 at 298.15 K for each drug within the ABS system {NADES [choline chloride : sucrose] + PPG400 + H$_2$O}, employing different molar ratios of hydrogen bond acceptor (HBA) to hydrogen bond donor (HBD) (2 : 1 and 1 : 1), reveals distinct values of partition coefficients (K) and extraction efficiencies (EE%). Notably, the K values exhibit the following order: choline chloride : sucrose (HBA : HBD molar ratio of 2 : 1) > choline chloride : sucrose (HBA : HBD molar ratio of 1 : 1). This observation suggests that with an increase in choline chloride concentration, the studied drugs preferentially transfer to the PPG400 top phase. Similar results regarding drug partitioning into the top polymer-phase have been previously reported in the literature for other ABS systems, such as {NADES [choline chloride : glucose] + PPG400 + H$_2$O},[6] and {NADES [choline chloride : sucrose] + polyethylene glycol dimethyl ether + H$_2$O}.[36]

3.3.2. The effect of drug structure on the drug partitioning behavior. The observed trend of the partition coefficients (K) and extraction efficiencies (EE%) values for the drugs, as presented in Table 8, can be attributed to the chemical structures and polarities of the drugs. All of the drugs under investigation

Table 8 Partitioning coefficients, K, and extraction efficiency, EE%, values of studied drugs for the system composed of {NADES [ChCl (HBA) : sucrose (HBD)] + PPG$_{400}$ + H$_2$O} at 298.15 K and atmospheric pressure (≈ 85 kPa)[a]

HBA : HD molar ratio	Overall composition/wt%		K	EE%
	[HBA : HBD]	[PPG]		
Diclofenac potassium				
2 : 1	14.56	36.07	12.96	92.84
	17.90	36.09	10.91	91.60
	17.38	35.98	9.92	90.84
	18.91	36.07	8.67	89.66
	20.43	36.10	7.97	88.85
1 : 1	14.56	36.07	10.25	91.11
	17.90	36.09	9.79	89.78
	17.38	35.98	8.62	88.58
	18.91	36.07	7.81	86.85
	20.43	36.10	7.20	86.15
Salicylic acid				
2 : 1	14.56	36.07	11.95	92.28
	17.90	36.09	9.89	90.82
	17.38	35.98	8.81	89.81
	18.91	36.07	7.68	88.48
	20.43	36.10	6.56	86.77
1 : 1	14.56	36.07	9.85	90.78
	17.90	36.09	8.78	89.78
	17.38	35.98	7.73	88.55
	18.91	36.07	6.63	86.59
	20.43	36.10	6.22	86.15
Acetaminophen				
2 : 1	14.56	36.07	8.91	89.91
	17.90	36.09	7.16	87.75
	17.38	35.98	3.49	77.73
	18.91	36.07	2.14	68.15
	20.43	36.10	1.12	52.83
1 : 1	14.56	36.07	7.88	88.74
	17.90	36.09	4.36	81.34
	17.38	35.98	1.71	63.10
	18.91	36.07	0.92	47.92
	20.43	36.10	0.45	31.03

[a] The standard uncertainties σ for partitioning coefficient, temperature and pressure are: $\sigma(K) = 0.15$, $\sigma(T) = 0.05$ K and $\sigma(p) = 0.5$ kPa respectively.

are characterized as non-polar, leading to favorable interactions with the hydrophobic constituents in the top phase. Consequently, drug partitioning to the choline chloride (ChCl)-rich phase occurs to a lesser extent. Notably, the presence of non-polar functional groups in the drug structures enhances their affinity for the PPG-rich top phase. The high values of EE% obtained, as compiled in Table 8, indicate that a significant portion of all studied drugs is predominantly partitioned into the polymer top phase. Furthermore, the trend observed for the K values aligns well with the hydrophobicity of the drugs, which is represented by the logarithm of the octanol/water partition coefficient ($\log K_{\text{ow}}$). A higher $\log K_{\text{ow}}$ value indicates a higher degree of hydrophobicity for the solute, resulting in a lower
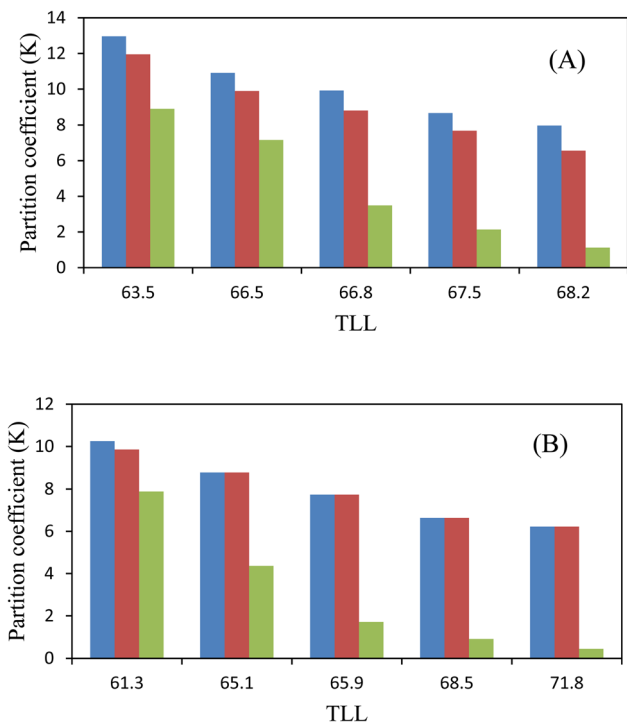
Fig. 4 Values of partition coefficient, K, in function of the TLL for each drug in ABS composed of {NADES [ChCl : sucrose] + PPG$_{400}$ + H$_2$O} at two ChCl : sucrose molar ratios: (A) 2 : 1; (B) 1 : 1. Diclofenac potassium █, salicylic acid █, acetaminophen █.
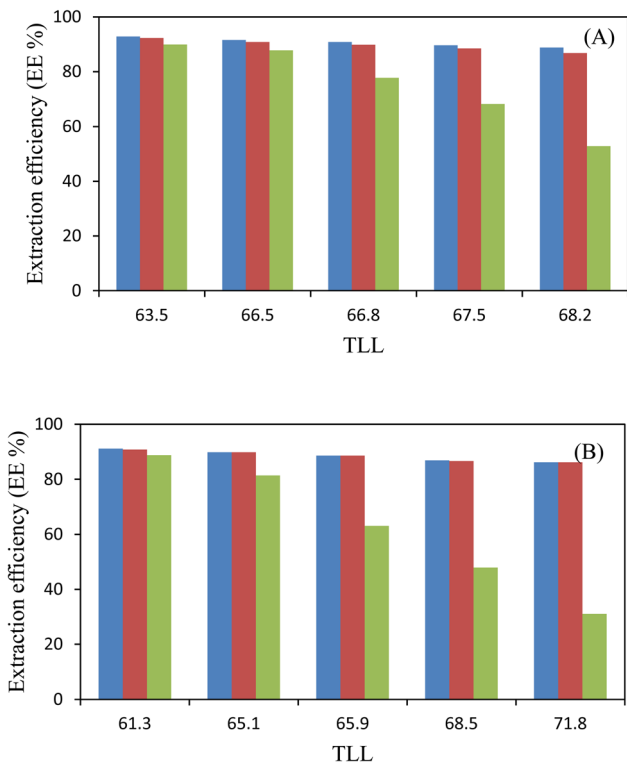

Fig. 5 Extraction efficiency, EE%, for each studied drug in ABS {NADES [ChCl : sucrose] + PPG$_{400}$ + H$_2$O} at different ChCl : sucrose molar ratios: (A) 2 : 1; (B) 1 : 1. Diclofenac potassium █, salicylic acid █, acetaminophen █.

tendency to associate with water molecules.[25] For the drugs diclofenac potassium, salicylic acid, and acetaminophen, the $\log K_{ow}$ values are reported as 4.50,[36] 2.34 (ref. 37) and 0.46,[38] respectively. Thus, the order of drug extraction efficiency is as follows: diclofenac potassium > salicylic acid > acetaminophen. This study, along with a previous investigation,[38] underscores the significance of drug hydrophobicity as a useful criterion in predicting drug partitioning between the two phases of an Aqueous Biphasic System (ABS).

3.3.3. Correlations of the partition coefficients. In order to regenerate the partition coefficient data, a model is necessary. In this study, the equation suggested by Diamond–Hsu[22] (eqn (19)) was used for correlation of experimental partition coefficients values of the studied drugs. We also attempted to improve the quality of the Diamond–Hsu model by adding one extra term to the right hand side of eqn (19) and refitting the K values to the modified polynomial equation (eqn (20)). Also, the relation between partition coefficient and NADES overall composition was established using a similar polynomial equation as eqn (21):

$$\ln K = A\Delta w(\text{PPG}_{400}) + B\Delta w(\text{PPG}_{400})^2 \qquad (19)$$

$$\ln K = A_1 + B_1\Delta w(\text{PPG}_{400}) + C_1\Delta w(\text{PPG}_{400})^2 \qquad (20)$$

$$\ln K = A_2 + B_2 w(\text{NADES}) + C_2 w(\text{NADES})^2 \qquad (21)$$

In above relations, (A and B), (A_1, B_1, and C_1) and (A_2, B_2, and C_2) are respectively the adjustable parameters of eqn (19)–(21). The symbols $\Delta w(\text{PPG}_{400})$ and $w(\text{NADES})$ are used for the mass fraction difference of PPG$_{400}$ in the top and bottom phase and the overall composition of NADES, respectively.

For each drug the results of fitting experimental partition coefficients values to eqn (19)–(21) are reported in Table S1.† To evaluate the ability of eqn (19)–(21) in representing the partition coefficient values, the variation of experimental and calculated K values by these equations are plotted in Fig. S1 to S3,† respectively. According to the sd values in Table S1 and Fig. S1–S3,† it can be concluded that the correlation with all of the eqn (19)–(21) is satisfactory; and excellent performance is obtained with the eqn (21) in which variation of K with NADES overall concentrations is considered.

4. Conclusion

Phase diagrams of an Aqueous Biphasic System (ABS) were investigated at 298.15 K and under atmospheric pressure (approximately 85 kPa). The ABS was comprised of polypropylene glycol with a molar mass of 400 g mol^{-1}, and Natural Deep Eutectic Solvents (NADES) derived from mixtures of choline chloride (ChCl) and sucrose at molar ratios of 2 : 1 and 1 : 1, acting as the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), respectively. Through a meticulous examination of tie-lines, it was determined that the original HBA : HBD stoichiometry of the NADES was approximately maintained in the equilibrium phases, suggesting that the ABS exhibits ternary-like system behavior. This finding confirms the

retention of hydrogen bonding interactions between the components of NADES in the ABS. Similar results were reported in previous studies, which investigated the stability of NADES in water *via* vapor–liquid equilibrium experiments, proposing that the hydrogen bonds between the initial components of NADES remain mostly preserved when the water content of the NADES solution is below 75 wt%. Both the NRTL and UNIQUAC local composition activity coefficient models yielded satisfactory outcomes in representing the experimental tie-lines. Additionally, the observed stronger salting-out effect of the natural deep eutectic solvent at higher molar ratios was confirmed by employing the effective excluded volume theory, which demonstrated an expansion of the biphasic region with an increase in the HBA : HBD molar ratio. Furthermore, partitioning studies of drugs within these ABSs revealed an extraction efficiency trend of diclofenac potassium > salicylic acid > acetaminophen, indicating that the drugs exhibited a preference for extraction into the polymer-rich top phase.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

References

1 P.-Å. Albertsson, Partition of proteins in liquid polymer–polymer two-phase systems, *Nature*, 1958, **182**, 709–711.

2 R. D. Rogers and M. A. Eiteman, *Aqueous Biphasic Separations*, Springer, 1995.

3 Y. Song, R. P. Chandra, X. Zhang and J. N. Saddler, Non-productive celluase binding onto deep eutectic solvent (DES) extracted lignin from willow and corn stover with inhibitory effects on enzymatic hydrolysis of cellulose, *Carbohydr. Polym.*, 2020, **250**, 116956.

4 K. Xu, Y. Wang, Y. Huang, N. Li and Q. Wen, A green deep eutectic solvent-based aqueous two-phase system for protein extracting, *Anal. Chim. Acta*, 2015, **864**, 9–20.

5 Q. Zeng, et al., Deep eutectic solvents as novel extraction media for protein partitioning, *Analyst*, 2014, **139**, 2565–2573.

6 F. O. Farias, H. Passos, A. l. S. Lima, M. R. Mafra and J. o. A. Coutinho, Is it possible to create ternary-like aqueous biphasic systems with deep eutectic solvents?, *ACS Sustain. Chem. Eng.*, 2017, **5**, 9402–9411.

7 A. P. Abbott, G. Capper, D. L. Davies, R. K. Rasheed and V. Tambyrajah, Novel solvent properties of choline chloride/urea mixtures, *Chem. Commun.*, 2003, 70–71.

8 Y. H. Choi, et al., Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology?, *Plant Physiol.*, 2011, **156**, 1701–1705.

9 M. Marchel, J. Niewisiewicz, A. S. Coroadinha and I. M. Marrucho, Purification of virus-like particles using aqueous biphasic systems composed of natural deep eutectic solvents, *Sep. Purif. Technol.*, 2020, **252**, 117480.

10 V. Selvanathan, A. D. Azzahari, A. A. A. Halim and R. Yahya, Ternary natural deep eutectic solvent (NADES) infused phthaloyl starch as cost efficient quasi-solid gel polymer electrolyte, *Carbohydr. Polym.*, 2017, **167**, 210–218.

11 N. Li, et al., Development of green betaine-based deep eutectic solvent aqueous two-phase system for the extraction of protein, *Talanta*, 2016, **152**, 23–32.

12 H. Zhang, et al., Ternary and binary deep eutectic solvents as a novel extraction medium for protein partitioning, *Anal. Methods*, 2016, **8**, 8196–8207.

13 Y. Dai, G.-J. Witkamp, R. Verpoorte and Y. H. Choi, Tailoring properties of natural deep eutectic solvents with water to facilitate their applications, *Food Chem.*, 2015, **187**, 14–19.

14 F. Ghaffari, M. T. Zafarani-Moattar and H. Shekaari, Aqueous biphasic systems created with choline chloride-fructose natural deep eutectic solvents and polypropylene glycol 400 and usage of these systems for extraction of some commonly used drugs, *Fluid Phase Equilib.*, 2021, 113348.

15 M. T. Zafarani-Moattar, H. Shekaari and F. Ghaffari, Vapor–Liquid Equilibria Study of the Aqueous Systems Containing {Choline Chloride + Glucose or Urea} and Their Deep Eutectic Solvents at 298.15 K and 85 kPa, *J. Chem. Eng. Data*, 2019, **64**, 4754–4762.

16 M. T. Zafarani-Moattar, H. Shekaari and F. Ghaffari, The study of extent of interactions between components of natural deep eutectic solvents in the presence of water through isopiestic investigations, *J. Mol. Liq.*, 2020, **311**, 113347.

17 J. C. Merchuk, B. A. Andrews and J. A. Asenjo, Aqueous two-phase systems for protein separation: studies on phase inversion, *J. Chromatogr. B: Biomed. Sci. Appl.*, 1998, **711**, 285–293.

18 M. T. Zafarani-Moattar and E. Nemati-Kande, Study of liquid–liquid and liquid–solid equilibria of the ternary aqueous system containing poly ethylene glycol dimethyl ether 2000 and tri-potassium phosphate at different temperatures: experiment and correlation, *Calphad*, 2010, **34**, 478–486.

19 Y. Guan, T. H. Lilley and T. E. Treffry, A new excluded volume theory and its application to the coexistence curves of aqueous polymer two-phase systems, *Macromolecules*, 1993, **26**, 3971–3979.

20 H. Renon and J. M. Prausnitz, Local compositions in thermodynamic excess functions for liquid mixtures, *AIChE J.*, 1968, **14**, 135–144.

21 D. S. Abrams and J. M. Prausnitz, Statistical thermodynamics of liquid mixtures: a new expression for the excess Gibbs energy of partly or completely miscible systems, *AIChE J.*, 1975, **21**, 116–128.

22 A. D. Diamond and J. T. Hsu, Fundamental studies of biomolecule partitioning in aqueous two-phase systems, *Biotechnol. Bioeng.*, 1989, **34**, 1000–1014.

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23 Y. Dai, J. van Spronsen, G.-J. Witkamp, R. Verpoorte and Y. H. Choi, Natural deep eutectic solvents as new potential media for green technology, *Anal. Chim. Acta*, 2013, **766**, 61–68.

24 A. Hayyan, et al., Fruit sugar-based deep eutectic solvents and their physical properties, *Thermochim. Acta*, 2012, **541**, 70–75.

25 H. Passos, D. J. Tavares, A. M. Ferreira, M. G. Freire and J. o. A. Coutinho, Are aqueous biphasic systems composed of deep eutectic solvents ternary or quaternary systems?, *ACS Sustain. Chem. Eng.*, 2016, **4**, 2881–2886.

26 M. T. Zafarani-Moattar, H. Shekaari and P. Jafari, Thermodynamics of acetaminophen and bovine serum albumin partitioning in ternary aqueous solutions comprising polyethylene glycol dimethyl ether 250 and choline bitartrate: Liquid-liquid equilibria, volumetric and acoustic investigations, *J. Mol. Liq.*, 2021, **323**, 115072.

27 M. T. Zafarani-Moattar, H. Shekaari and E. Pourbagherian, Study of the liquid-liquid equilibrium for aqueous ternary systems containing choline bitartrate and 1-propanol or 2-propanol at different temperatures and their performances in acetaminophen separation and alcohols recovery, *Fluid Phase Equilib.*, 2020, **514**, 112536.

28 A. A. Albalasmeh, A. A. Berhe and T. A. Ghezzehei, A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry, *Carbohydr. Polym.*, 2013, **97**, 253–261.

29 M. Dubois, K. A. Gilles, J. K. Hamilton, P. t. Rebers and F. Smith, Colorimetric method for determination of sugars and related substances, *Anal. Chem.*, 1956, **28**, 350–356.

30 E. Cheluget, S. Marx, M. Weber and J. Vera, Equilibrium in biphasic aqueous systems: a model for the excess gibbs energy and data for the system H_2O- NaCl-1-propanol at 25 °C, *J. Solution Chem.*, 1994, **23**, 275–305.

31 G. M. Ferreira, G. M. Ferreira, A. O. Maldaner, L. H. M. da Silva and M. C. Hespanhol, Partitioning of cocaine and its adulterants in aqueous two-phase systems: An alternative drug identification method, *Fluid Phase Equilib.*, 2020, **506**, 112367.

32 M. V. Quental, et al., Enhanced extraction of proteins using cholinium-based ionic liquids as phase-forming components of aqueous biphasic systems, *Biotechnol. J.*, 2015, **10**, 1457–1466.

33 C. M. Neves, et al., Aqueous biphasic systems composed of ionic liquids and polypropylene glycol: insights into their liquid–liquid demixing mechanisms, *Phys. Chem. Chem. Phys.*, 2016, **18**, 20571–20582.

34 J. G. Huddleston, H. D. Willauer and R. D. Rogers, Phase diagram data for several PEG+ salt aqueous biphasic systems at 25 C, *J. Chem. Eng. Data*, 2003, **48**, 1230–1236.

35 S. Pradhan, P. Kumar and I. Mehrotra, Characterization of aqueous organics by specific ultraviolet absorbance and octanol water partition coefficient, *J. Environ. Eng.*, 2014, **140**, 06013001.

36 P. Jafari and A. Jouyban, Partitioning behavior of caffeine, lamotrigine, clonazepam and oxcarbazepine in a biodegradable aqueous two-phase system comprising of polyethylene glycol dimethyl ether 250 and choline chloride/saccharose deep eutectic solvent, *J. Mol. Liq.*, 2021, **323**, 115055.

37 F. H. Clarke and N. M. Cahoon, Ionization constants by curve fitting: determination of partition and distribution coefficients of acids and bases and their ions, *J. Pharm. Sci.*, 1987, **76**, 611–620.

38 F. Ghaffari, M. Khorsandi, M. T. Zafarani-Moattar and H. Shekaari, Liquid–liquid equilibrium measurements and computational study of salt–polymer aqueous two phase system for extraction of analgesic drugs, *Sci. Rep.*, 2022, **12**, 13848.