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Application of deep eutectic solvent-based aqueous two phase systems for extraction of analgesic drugs

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The ability of biphasic-aqueous systems to efficiently and simultaneously purify active pharmaceutical compounds has led to extensive study of these systems. As a new environmentally friendly separation technology, deep eutectic solvent (DES)-based aqueous two-phase systems (ATPSs) are extensively applied for the extraction and separation of various bioactive compounds. In this study, two DES-based ATPSs consisting of choline chloride/fructose and choline chloride/glucose as DESs with a molar ratio of 2 : 1 and tripotassium phosphate (K_3PO_4) were prepared. The measured binodal data correlated with Merchuk and Zafarani-Moattar *et al.* equations. Moreover, the ATPSs were employed to investigate the separation of pharmaceuticals. The partition coefficient and the effect of factors such as the concentration of the deep eutectic solvent on drug partitioning were investigated as novel discoveries. Drugs are likely to be removed in the top DES-rich phase, according to the current data. Finally, the compositions of five tie-lines for each ATPS were meticulously determined. Othmer–Tobias, Bancraft, and Setschenow equations were used for correlation of tie-line data.

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1. Introduction

The biotechnology industry has made great strides in the last thirty years in the production of biological products through the application of traditional techniques, including precipitation, membrane-based, and chromatography technologies, for the separation, recovery, and purification of biomolecules. Cost-effective and biocompatible methods are crucial to ensuring successful scalability and satisfying customer needs for biotechnology products.[1] In separation operations, aqueous two phase systems (ATPSs) are widely used.[2,3] These systems offer a sustainable and environmentally friendly alternative to conventional organic-water solvent extraction methods. Recent advancements in the field have focused on the use of deep eutectic solvents (DESs) to create ATPSs for a wide range of separation and analytical purposes.[4–8]

The novelty of DES-based ATPSs lies in their unique composition and tunable properties, which distinguish them from traditional ATPSs. Deep eutectic solvents are formed by mixing two or more components, typically a hydrogen bond donor and acceptor that combine to create a liquid at room temperature with enhanced solubility and selectivity for various compounds.[9] These systems are recognized for their biodegradability, low toxicity, and sustainable nature, making them ideal candidates for green extraction methods.[10–12] In the context of drug extraction, DES-based ATPSs offer several advantages, including higher efficiency in selectively partitioning bioactive compounds, improving the solubility of poorly water-soluble drugs, and facilitating the recovery of delicate pharmaceutical agents without compromising their stability.[13,14] This innovative approach opens new possibilities for the extraction, separation, and purification of therapeutic drugs, presenting a more sustainable and effective alternative to conventional extraction techniques. The adaptability of DES-based ATPSs to a wide range of compounds highlights their potential for broader applications in the pharmaceutical industry, particularly in drug development and formulation. Their adaptability is based on their distinct molecular structure and chemical properties, which means that to develop their applications, it is necessary to comprehend their thermodynamics and phase behavior. Xu and colleagues have played a pioneering role in demonstrating the use of DESs as phase-forming components in ATPSs.[5,6,15,16] They extracted proteins using sugar-based DESs. They created DESs for this reason, which are composed of mixes of D-glucose and D-sorbitol as HBD and ChCl as HBA. Their findings demonstrated the high protein extraction capacity of these sugar-based DESs. Subsequently, the utilization of Deep Eutectic Solvent/Aqueous Two-Phase Systems (DES/ATPS) was employed for the extraction of numerous natural active compounds, including organic raw materials,[17] amino acids,[18] pharmaceuticals,[19] and enzymes such as pepsin.[20] This system is characterized by a straightforward operational process and has demonstrated excellent performance in the large-scale recovery of biological products.[21,22]

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However, investigations conducted by Coutinho and colleagues[23] revealed that the hydrogen bond between the HBA and HBD of DES might break at higher water content in ATPS. Consequently, the initial molar ratios of HBA and HBD cannot be sustained, leading to their distribution in either the upper or lower phase. In such instances, the authentic DES ceases to exist, compromising the fusion-enhancing property of DES. Farias *et al.*[23] proposed that a pseudo-ternary system can be established when both HBA and HBD exhibit high hydrophilicity and poor solubility in the other phase-forming components. This configuration enables the maintenance of the stoichiometry of the initial DES to a certain extent. This pseudo-ternary DES-based ATPS is categorized as a true quaternary system, preserving the initial molar ratios of HBD and HBA. This is crucial for ensuring the stability of DES properties under specific conditions.[24,25] According to the results obtained from the sources, it has been determined that DESs have a high ability to form aqueous two-phase systems and separate pharmaceutical substances. Despite the challenges identified, DES-based ATPSs still hold considerable potential for applications in extraction procedures.[23,26]

Therefore, in this study, K_3PO_4 and two deep eutectic solvents made of fructose or glucose as the hydrogen bond donor (HBD) and choline chloride as the hydrogen bond acceptor (HBA) with a molar ratio of 2 : 1 were created as aqueous two-phase systems. The experiments were carried out under atmospheric pressure and 298.15 K in temperature. For these ATPSs, the binodal and tie-line values were found. Equations from Zafarani-Moattar *et al.*[27] and Merchuk[28] were applied to fit the binodal data. Additionally, tie-lines were established for the ATPSs made up of water, K_3PO_4, and DESs. Tie-line compositions were modeled using the Othmer–Tobias and Bancraft equations[29,30] and Setschenow-type equation.[31] Our research delved into using these ATPSs to separate ibuprofen, acetaminophen, and aspirin. For this purpose, partition coefficients (K) and extraction efficiencies (EE%) of the studied drugs were calculated at various tie-lines to describe how the concentration of DESs and the characteristics of the drugs influenced their separation.

2. Experimental measurements

2.1. Chemicals

The specific information about the utilized chemicals, including details on purity, CAS number, and origin, is presented in Table 1. To prepare the solutions, double-distilled deionized water with a conductivity of 0.055 μS cm^{-1} was employed, and all chemicals were utilized without undergoing additional purification processes. The determination of water contents in the chemicals was conducted using the Karl-Fischer method.

2.2. Preparation of DES

In this study, two forms of DES were created in molar ratios of 2 : 1 using fructose or glucose as the hydrogen bond donor (HBD) and choline chloride as the hydrogen bond acceptor (HBA). The process described in earlier investigations[22,32] was followed in the fabrication of DES. First, sugars and choline chloride were mixed together in a 50 mL round-bottom flask. Next, using a hot plate stirrer, the flask was submerged in a paraffin oil bath that was heated. At 353.15 K, the mixture was agitated for two hours to produce a colorless, uniform liquid. A thermometer with an accuracy of ±0.01 K was applied to measure the DES temperature continuously. All samples were then properly preserved in airtight vials in a temperature-controlled environment. All the materials were dried before making the DES. Karl-Fischer analysis (Metrohm 751 GPD) was used to obtain the prepared DES water content, and the results showed a 0.0008 mass fraction of water content.

2.3. Methods

2.3.1. Preparation of binodal curves and tie-lines. The cloud point titration technique was applied to determine the binodal curves, adhering to accepted procedures that have been previously reported in investigations.[26,33,34] The temperature and pressure used for the trials were 298.15 K and 85 kPa, respectively. In this method, amounts of DES solution were added dropwise to a K_3PO_4 solution gradually until a hazy solution appeared, signifying the presence of a biphasic zone. Water was then added drop by drop until a zone that was clear and monophasic was observed. The binodal curves were found using 60% (w/w) K_3PO_4 and 60% (w/w) DES. Stirring was done constantly during the experiment. Using an analytical balance (Shimadzu, 321-34553, Shimadzu Co., Japan) with a precision of $\pm 10^{-7}$ kg, the mass fraction of the components was determined. It was found that there was a maximum uncertainty of 0.002 in the mass fraction determination of both salt and DES.

For each ATPS, a total of five distinct solutions consisting of {K_3PO_4 + DES (ChCl/fructose or ChCl/glucose with a 2 : 1 molar

Table 1 Information on used chemicals[a,b]

Chemicals	Source	CAS no.	Mass percent (purity)
Ibuprofen	Zahravi (Iran)	—	≥990
Acetaminophen	Zahravi (Iran)	—	≥990
Aspirin	Zahravi (Iran)	—	≥990
Choline chloride	Daejung	67-48-1	>0.990
Fructose	Merck	57-48-7	>0.995
Glucose	Merck	50-99-7	>0.995
Tri-potassium phosphate (K_3PO_4)	Merck	7778-53-2	>0.980

[a] The suppliers provided the purities of the used components. [b] Water contents were determined using the Karl-Fischer method.

ratio) + water} were created to identify the tie-lines. Using a mixture of DES, K_3PO_4, and water, a precise gravimetric preparation of the biphasic zone was performed for each tie-line, with an accuracy of $\pm 10^{-7}$ kg. These solutions were vigorously stirred for 30 minutes; then, they were centrifuged and heated to 298 K in a water bath to achieve equilibrium. Then, analytical techniques were used to identify the compositions of each phase. After separation of the two phases, a flame photometer (JENVEY model PFP7, England) was used to determine the concentrations of potassium phosphate (K_3PO_4) in the top and bottom phases.

The concentration of sugars was obtained using the phenol–sulfuric acid method.[35,36] In this process, a test tube containing 1 mL of a 5% aqueous phenol solution was combined with a 2 mL aliquot of the carbohydrate solution. After that, 5 milliliters of sulfuric acid were quickly added to the mixture. To produce color, the sample tubes were immersed in a water bath at 298.15 K for 20 minutes after standing for 10 minutes and vortexing for 30 seconds. Light absorption was measured using a spectrophotometer (model: SPECORD 40-Series Analytik Jena AG-Germany).

The identical steps as previously described were used to prepare the reference solutions, with the exception that the 2 mL aliquot of sugar solution was substituted with double-distilled deionized water.

The refractive index method[37] was used to calculate the concentration of choline chloride in both phases. A refractometer (ATAGO DR-A1, Japan) was used to test the refractive index of the solutions with 0.0001 precision. An estimated 0.0002 was the related uncertainty of the refractive index measurement.

The refractive index technique[37] states that there is a relationship between the mass fractions of the relevant components and the refractive index of the solution, n_D, for diluted aqueous solutions containing a choline chloride, sugar and salt for each phase of ATP. The relation between n_D and mass fractions of salt, w_s, choline chloride, w_c and sugars, w_{su}, takes the following form:

$$n_D = n_0 + a_s w_s + a_c w_c + a_{su} w_{su}, \quad (1)$$

where n_0 represents the refractive index of pure water, determined to be 1.3325 at a temperature (T) of 298.15 K. The constants a_s, a_c and a_{su}, associated with salt, ChCl, and sugars, respectively, were derived from calibration plots of the refractive index corresponding to diluted solutions within the range of mass fraction, as presented in Table 2. The method yielded an uncertainty of approximately 0.008 for the mass fraction determination of each component of the ATPs.

2.3.2. Separation of drugs. According to the previously determined phase diagrams of {DES (ChCl/fructose or ChCl/glucose) + K_3PO_4 + H_2O} systems, five mixture compositions (presented in Table 3) were chosen to assess the efficacy of the investigated ATPs with respect to ibuprofen, acetaminophen, and aspirin partitioning and extraction efficiency. The general makeup of these combinations was intended to resemble that of the ones that were utilized to create the tie-lines. After vigorously swirling for half an hour, the prepared combinations were placed in a water bath and heated to the appropriate temperature. Following the observation of phase separation, 1 mL of each phase was carefully separated, combined, and supplemented by 0.002 mass fractions of ibuprofen, acetaminophen, and aspirin. To guarantee total phase separation and equilibrium, the samples were then centrifuged for 10 minutes at 2000 rpm and then placed in a water bath for 24 hours. UV spectroscopy (model: SPECORD 40-Series Analytik Jena AG-Germany) was used to measure the amounts of ibuprofen, acetaminophen, and aspirin in both phases following the establishment of equilibrium. The samples were suitably diluted and tested beside blanks that had the same phase composition without drug to reduce interference from the components present in the phases.[38] In the UV spectrophotometric method for measuring drug concentration, the solvent's effect on UV absorption is first eliminated. This is typically done by using a blank cell containing only the solvent. After adjusting and zeroing the device against the blank, the sample containing the drug is placed into the UV cell, and its light absorption at a specific wavelength is measured.

Table 2 The parameters of eqn (1), a_m, for ATPs containing the{ChCl (c) : sugars (su) + K_3PO_4 (s) + water (w)} system

Material	Constant	Value	C range (w/w)	R^{2a}
ChCl	a_c	0.1452	0–0.08	0.9975
K_3PO_4	a_s	0.1308	0–0.10	0.9992
Fructose	a_{su}	0.1473	0–1.20	0.9973
Glucose	a_{su}	0.1474	0–1.20	0.9978

[a] For the mass fraction range (C range) of each material, R^2 denotes the corresponding correlation coefficient value of the linear calibration plot of the refractive index against the mass fraction of ChCl, salt, and sugars.

Table 3 Mass fractions (wt%) of experimental binodal data for {DES (w_{DES}) + K_3PO_4 (w_s) + H_2O} systems at 298.15 K[a]

w_{DES} ChCl/fructose	w_s	w_{DES} ChCl/glucose	w_s
41.56	26.70	43.12	24.64
41.10	26.91	42.56	24.99
40.39	27.32	42.06	25.14
39.76	27.68	41.36	25.58
38.8	28.15	38.98	26.70
37.91	28.58	37.18	27.67
36.81	29.06	35.57	28.48
35.68	29.65	33.79	29.39
34.45	30.3	32.13	30.42
33.19	30.99	30.5	31.35
31.92	31.58	28.89	32.33
30.89	32.26	27.48	33.27
29.7	32.98	25.97	34.34
28.49	33.57	24.61	35.24
27.42	34.24	23.31	36.04
26.12	34.87	22.08	36.89

[a] Standard uncertainties (u) for mass fraction, pressure, and temperature are $u(w_i) = 0.005$; $u(p) = 0.5$ kPa; and $u(T) = 0.05$ K, respectively (0.68 level of confidence).

Eqn (2) and (3) were used to determine the extraction efficiency (EE%) and partitioning coefficient (K), respectively. The results are as follows:

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

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

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

3. Results and discussion

In this study, K_3PO_4 and two deep eutectic solvents made of fructose or glucose as the hydrogen bond donor (HBD) and choline chloride as the hydrogen bond acceptor (HBA) with a molar ratio of 2 : 1 were created as aqueous two-phase systems.

3.1. Phase diagram

The binodal curves of obtained ATPs containing K_3PO_4, DES (with a 2 : 1 molar ratio of ChCl/fructose and ChCl/glucose), and water at 298.15 K are plotted in Fig. 1, and the experimental mass fraction data are listed in Table 3.

The binodal data presented in Table 3 were fitted to the equations presented by Merchuk[28] (eqn (4)) and Zafarani-Moattar *et al.*[27] (eqn (5)) using a nonlinear least-square regression method:

$$w_s = a\exp(bw_{\text{DES}}^{0.5} - cw_{\text{DES}}^{3}), \quad (4)$$

$$w_s = \alpha + \beta\ln(w_{\text{DES}}) + \gamma w_{\text{DES}}, \quad (5)$$

where w_s and w_{DES} are mass fractions of salt and DES, 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 the good performance of both equations in the regeneration of the binodal data.

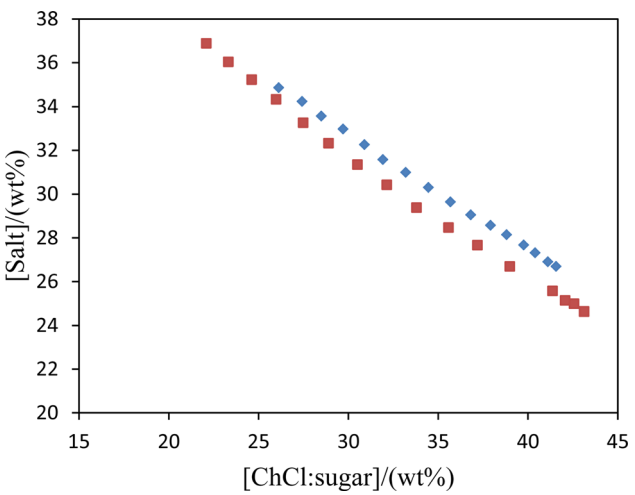

Fig. 1 Phase diagram for the systems {DES (2 : 1 molar ratio) + K_3PO_4 + water} at 298 K: ChlCl/fructose (◆); ChCl/glucose (■).

Table 4 Parameters of eqn (4) and (5) for {K_3PO_4 + ChCl/sugars + H_2O} systems at 298.15 K

	Merchuk (eqn (4))			
DES	a	b	c	100sd[a]
ChCl/fructose	1.7885	−2.3702	12.2545	0.09
ChCl/glucose	2.7008	−3.4492	8.0556	0.09
	Zafarani-Moattar *et al.* (eqn (5))			
DES	α	β	γ	100sd
ChCl/fructose	0.0670	−0.3889	−0.6151	0.09
ChCl/glucose	−0.4069	−0.5707	0.1608	0.08

[a] $\text{sd} = (\sum_{i=1}^{N}(w_1^{\text{cal}} - w_1^{\text{exp}})^2/N)^{0.5}$, where w_1 and N represent the mass fraction of salt and the number of binodal data, respectively.

3.2. Analysis of tie-lines

Fig. 2 shows the tie-lines and binodal curves for the ATPs made of K_3PO_4 and NADES (ChCl/glucose or ChCl/fructose) at a molar ratio of 2 : 1. Table 5 provides an overview of the experimental tie-lines and the tie-line lengths (TLL) for the studied systems. The top phase is constantly enriched in DES with varying tie-line lengths according to the data, which follow a consistent pattern. Higher K_3PO_4 concentrations than DES made up the majority of the bottom phase, suggesting a salt-rich phase.

3.2.1. Othmer–Tobias and Bancraft equations. The reliability of the obtained tie-lines was ascertained by the correlation of the Othmer–Tobias (eqn (6))[29] and Bancroft (eqn (7)) equations:[30]

$$\left(\frac{1 - w_{\text{DES}}^{\text{top}}}{w_{\text{DES}}^{\text{top}}}\right) = K\left(\frac{1 - w_s^{\text{bot}}}{w_s^{\text{bot}}}\right)^n, \quad (6)$$

$$\left(\frac{w_w^{\text{bot}}}{w_s^{\text{bot}}}\right) = K_1\left(\frac{w_w^{\text{top}}}{w_{\text{DES}}^{\text{top}}}\right)^r, \quad (7)$$

where K, n, K_1 and r are fitted parameters. The parameters fitted by Othmer–Tobias and the model along with the associated (R^2) and standard deviations are listed in Table 6.

The presence of a linear relationship between the two sides of the above equations indicates the acceptability of the obtained results. In the present work, we investigate this issue to clarify the results and witness the linearity of the relationship between the two sides and the acceptability and consistency of the obtained results. Using the deviations (Dev.) achieved, we conclude that eqn (6) and (7) can be applied to correlate the tie-lines of the mentioned systems.

3.2.2. Setschenow-type equation. To correlate tie-line data and investigate the salting-out ability in our studied ATPS systems composed of K_3PO_4, DES (obtained at one molar ratio of ChCl/fructose and ChCl/glucose (2 : 1)), and the water Setschenow equation[31] at $T = 298.15$ K are used, which is a simple two-parameter relation proposed by Hey and coworkers:[31]

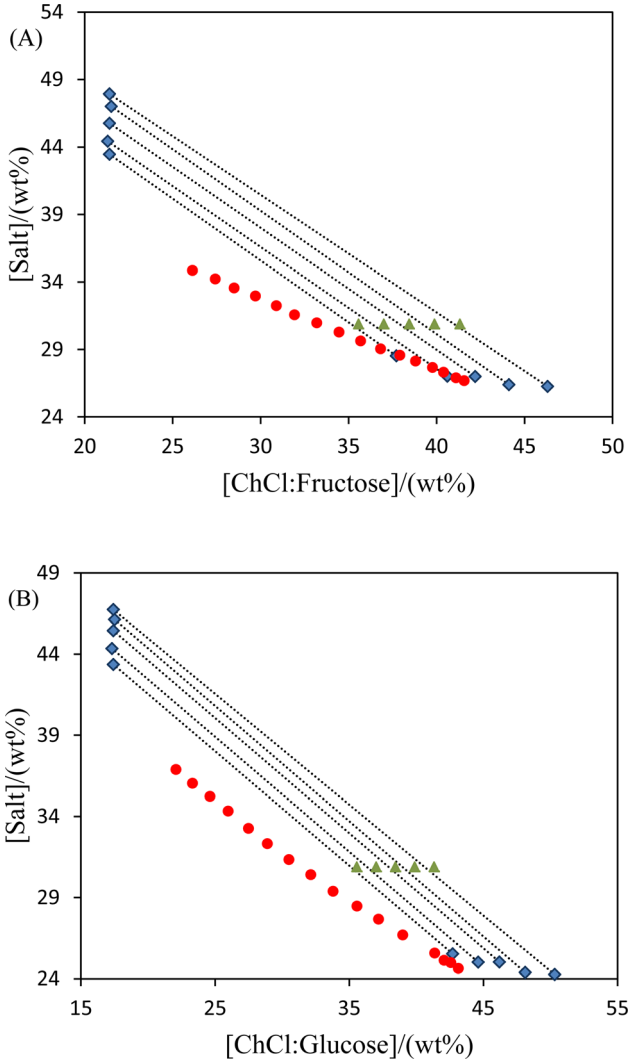

Fig. 2 Phase diagram and tie-line data for the systems {DES (2 : 1 molar ratio) + K_3PO_4 + water} at 298 K: (A) ChCl/fructose; (B) ChCl/glucose; binodal curve (●), overall composition of tie-line (▲), and phase composition (■).

Table 6 Values of parameters of Othmer–Tobias and Bancroft for {K_3PO_4 + ChCl/sugars + H_2O} at $T = 298.15$ K

Othmer–Tabias and Bancroft equations (eqn (6) and (7))				
	K	n	K_1	r
ChCl/glucose	2.727	2.037	0.636	0.545
ChCl/fructose	2.792	2.068	0.649	0.553

$$\ln\left(\frac{m_{DES}^{top}}{m_{DES}^{top}}\right) = k_{DES} + k_s\left(m_{salt}^{bot} - m_{salt}^{top}\right), \quad (8)$$

where k_s is the salting-out coefficient, k_{NADES} is a constant and m_{NADES} and m_{salt} are the molality of DES and K_3PO_4, respectively. To evaluate the reliability of eqn (8), in describing the soluting-out ability of DESs in these biocompatible ATPSs containing K_3PO_4 by determining the soluting-out coefficient, k_s, from fitting experimental tie-lines the following objective function (eqn (9)) was used at studied temperature. The values of k_{NADES} and k_s are illustrated in Table 7 along with the correlation coefficient, R^2, and sd values at different temperatures.

$$Of = \sum_T \sum_P \sum_l \sum_j \left(w_{T,P,l,j}^{cal} - w_{T,P,l,j}^{exp}\right), \quad (9)$$

where $w_{T,P,l,j}$ represents the mass percent of the component j in the phase (DES, K_3PO_4 and water) for lth tie-line at working temperature. The superscripts "*cal*" and "*exp*" indicate the calculated and experimental values, respectively. The corresponding correlation coefficient values, R^2, together with the tie-line experimental data, values of the fitted parameters (k_s, k_{NADES}) and deviations are listed in Table 7. This table shows that the k_s values for the two studied systems are very similar. This value for the system countering ChCl/glucose is slightly higher than that of another system, which shows that the soluting-out effect of the DES composed of ChCl/glucose is a little more than that of the DES composed of ChCl/fructose.

Table 5 Tie-line experimental data at 298.15 K for the {ChCl (HBA) + sugars (HBD) + K_3PO_4 + H_2O} system[a]

HBA : HBD	Overall composition/wt%		ChCl-rich phase/wt%		Salt-rich phase/wt%		
Molar ratio 2 : 1	[HBA : HBD]	[K_3PO_4]	[HBA : HBD]	[K_3PO_4]	[HBA : HBD]	[K_3PO_4]	TLL
ChCl/fructose	35.56	30.90	43.47	28.53	21.41	37.71	23.89
ChCl/glucose			42.71	25.53	17.41	43.37	30.96
ChCl/fructose	37.00	30.90	44.44	27.02	21.31	40.61	26.82
ChCl/glucose			44.61	25.02	17.31	44.35	33.45
ChCl/fructose	38.43	30.90	45.78	27.00	21.41	42.19	28.72
ChCl/glucose			46.19	25.03	17.41	45.45	35.29
ChCl/fructose	39.91	30.90	47.03	26.39	21.53	44.11	31.05
ChCl/glucose			48.11	24.39	17.51	46.13	37.54
ChCl/fructose	41.35	30.90	47.94	26.26	21.41	46.31	33.25
ChCl/glucose			50.31	24.26	17.41	46.74	39.85

[a] Standard uncertainties (u) for mass fraction, pressure, and temperature are $u(w_i) = 0.005$; $u(p) = 0.5$ kPa; and $u(T) = 0.05$ K, respectively (0.68 level of confidence).

Table 7 Values of parameters Setschenow-type, (k_{NADES}, k_s), for {K_3PO_4 + ChCl/sugars + H_2O} at $T = 298.15$ K[a]

Setschenow type equation (eqn (8))			
System	k_{NADES}/kg K mol^{-1}	k_s/kg K mol^{-1}	Dev.
ChCl/glucose	1.7431	0.1181	0.03
ChCl/fructose	1.7341	0.1119	0.04

[a] sd $= \left(\sum_{i=1}^{N} (w_1^{cal} - w_1^{exp})^2/N\right)^{0.5}$, where N and w_1 denote the mass percent of salt and the number of binodal data, respectively.

3.3. Partitioning behavior of drugs in the prepared ATPs

Table 8 shows the values of the extraction efficiency (EE%) and partition coefficients (K), which are determined using eqn (2) and (3) for the chosen drugs. As can be observed, we used five combinations with different weight percentages of DES (35.56, 37.00, 38.43, 39.91, and 41.35 wt%) and a constant concentration of salt (30.90 wt%) to investigate how changing DES concentration influences drug separation. The partition coefficients for ibuprofen, acetaminophen, and aspirin are shown in Fig. 3, and they increase as DES concentrations increase. Operating points are in a wider biphasic zone (longer tie-line) as the DES concentration increases. Additionally, these drugs are partitioned into the DES-rich phase, as shown in Fig. 4. More hydrogen bonds between DES molecules and drug molecules develop when the weight fraction of the DES is larger. Higher partition coefficients and greater extraction efficiency are the results of this improved interaction, which encourages greater drug partitioning into the DES phase. For both DESs, the observed trend due to the similar structure of sugars is very close and very slightly different as follows: K (ChCl/glucose) > K (ChCl/fructose).

Table 8 Partition coefficients (K) and extraction efficiency (EE%) for the {DES [ChCl : sugar] + K_3PO_4 + H_2O} system at 298.15 K[a]

	Overall composition/ wt%			
Molar ratio 2 : 1	[DES]	[K_3PO_4]	K	EE%
Ibuprofen				
ChCl/fructose	41.35	30.90	2.50	71.43
	39.91	30.90	2.33	69.97
	38.43	30.90	2.18	68.55
	37.00	30.90	1.99	66.56
	35.56	30.90	1.72	63.24
ChCl/glucose	41.35	30.90	2.62	72.38
	39.91	30.90	2.45	71.01
	38.43	30.90	2.30	69.70
	37.00	30.90	2.11	67.85
	35.56	30.90	1.84	64.79
Acetaminophen				
ChCl/fructose	41.35	30.90	3.51	77.83
	39.91	30.90	3.34	76.96
	38.43	30.90	3.19	76.13
	37.00	30.90	3.01	75.06
	35.56	30.90	2.73	73.19
ChCl/glucose	41.35	30.90	3.62	78.35
	39.91	30.90	3.45	77.53
	38.43	30.90	3.30	76.74
	37.00	30.90	3.12	75.73
	35.56	30.90	2.84	73.96
Aspirin				
ChCl/fructose	41.35	30.90	5.69	85.05
	39.91	30.90	5.52	84.66
	38.43	30.90	5.37	84.30
	37.00	30.90	5.19	83.84
	35.56	30.90	4.91	83.08
ChCl/glucose	41.35	30.90	5.87	85.44
	39.91	30.90	5.70	85.07
	38.43	30.90	5.55	84.73
	37.00	30.90	5.37	84.30
	35.56	30.90	5.09	83.58

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

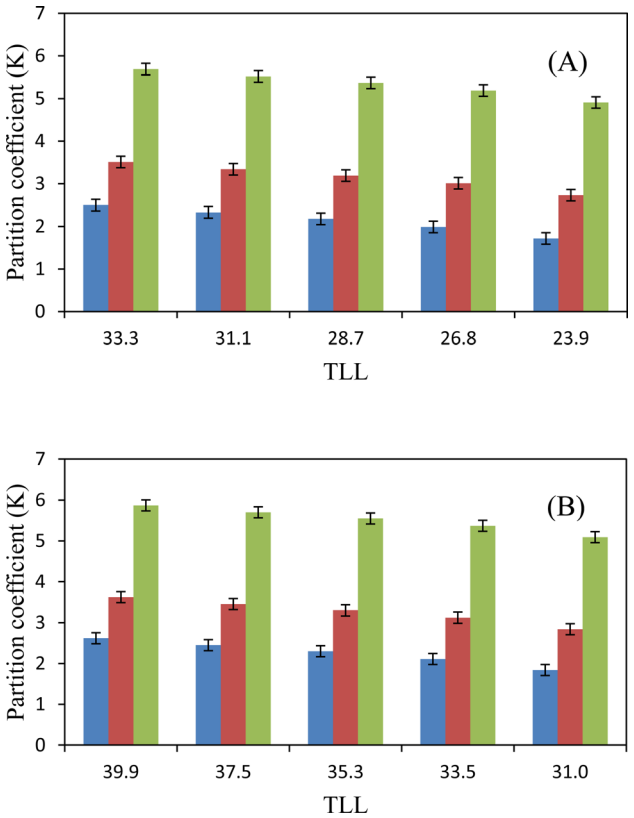

Fig. 3 Partition coefficient, K, as a function of the TLL in ATPSs composed of {DES (2 : 1 molar ratios) + K_3PO_4 + H_2O}: (A) ChCl/fructose; (B) ChCl/glucose. Ibuprofen acetaminophen aspirin.

3.3.1. Effect of drug structure.

The chemical structures and polarity of the medications can be used to explain the observed trend of the partition coefficients (K) and extraction efficiency (EE%) values for ibuprofen, acetaminophen, and aspirin, as shown in Table 8. The non-polar nature of the studied medicines results in advantageous interactions with the hydrophobic components in the top phase. Consequently, there is less medication partitioning in the salt-rich phase. Interestingly, the ibuprofen, acetaminophen, and aspirin structures have non-

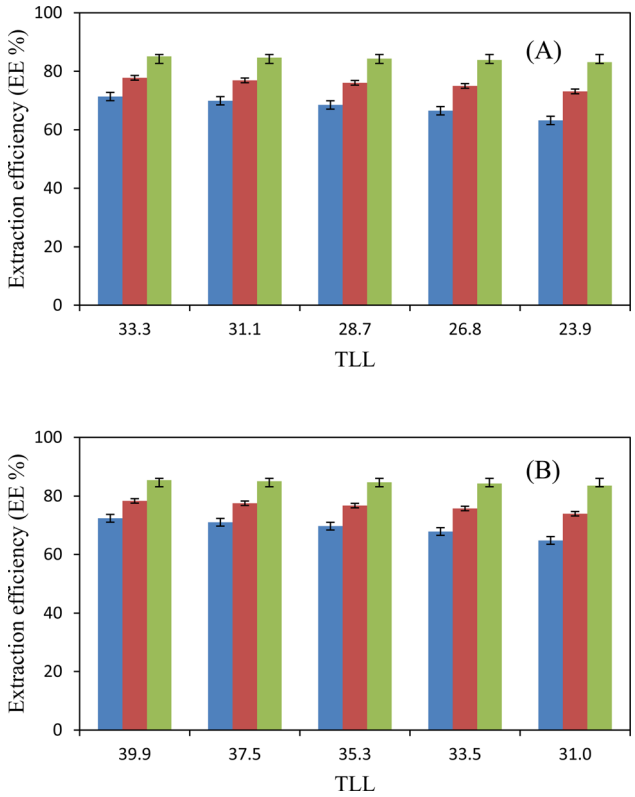

Fig. 4 Extraction efficiency, EE%, as a function of the TLL in ATPSs composed of {DES (2 : 1 molar ratios) + K_3PO_4 + H_2O}: (A) ChCl/fructose; (B) ChCl/glucose. Ibuprofen acetaminophen aspirin.

polar functional groups that increase their affinity for the DES-rich top phase. Table 8 shows that a considerable proportion of pharmaceuticals are mostly partitioned into the DES top phase based on the high values of EE% found.

Moreover, the K value trend is consistent with ibuprofen, acetaminophen, and aspirin hydrophobicity, as indicated by the logarithm of the octanol/water partition coefficient ($\log K_{ow}$). An increase in $\log K_{ow}$ indicates an increase in the hydrophobicity of a solute, which reduces its tendency to bind with water molecules.[39] The $\log K_{ow}$ values for ibuprofen, acetaminophen, and aspirin are 3.97,[3] 2.34,[40] and 1.40,[41] respectively. This work emphasizes the importance of drug hydrophobicity as a helpful factor in forecasting drug partitioning between the two phases of an aqueous two-phase system (ATPs), as in a previous study.[26]

3.3.2. Effect of pH on the partitioning of drugs. Potassium phosphate can affect the pH of water when dissolved. K_3PO_3 is a salt of phosphorous acid (H_3PO_3), but in its dissociated form, potassium phosphate acts more like a base than an acid. When it dissolves in water, it dissociates into potassium (K^+) ions and phosphate ions (PO_3^{3-}). Phosphate ions (PO_3^{3-}) can react with water molecules to form hydroxide ions (OH^-) through hydrolysis. This reaction increases the concentration of OH^- in the solution, making the water more basic. As a result of the increased OH^- concentration, the pH of the water increases. This means that K_3PO_3 increases the pH of water, making it more alkaline.[42,43] As can be understood from Table 8, all three drugs have acidic groups and are slightly soluble in the salt-rich phase.

4. Conclusion

In this work, two deep eutectic solvents were synthesized and employed in conjunction with K_3PO_4 to create ATPSs. These solvents included ChCl as HBA and fructose or glucose as HBD at a molar ratio of 2 : 1. Two correlation equations, Merchuk and Zafarani-Moattar, were satisfactorily used to represent the experimental binodal data. The compositions of the five tie-lines for each ATPS were carefully determined. Three Othmer–Tobias, Bancraft, and Setschenow equations were used for the correlation of the tie-line data. With precision in the standard deviation values obtained from each model, we can observe that the performance of the models used in fitting the tie-line data is good.

In addition, the capability of the studied systems in drug separation was used to partition ibuprofen, acetaminophen, and aspirin. The results showed that higher concentrations of DESs had a positive effect on the partition coefficient for the studied pharmaceuticals, and they had a tendency to move to the top DES-rich phase. This phenomenon is primarily influenced by the hydrophobic nature and structural characteristics of the drug.

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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