

Phase Behavior of Green Aqueous Biphasic Systems Based on Ethyl Lactate and Choline Salts

Published as part of Journal of Chemical & Engineering Data special issue "Aqueous Two-Phase Systems for Separation Processes: From Fundamental Data to Applications".

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Cite This: <https://doi.org/10.1021/acs.jced.5c00447>



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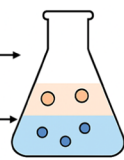


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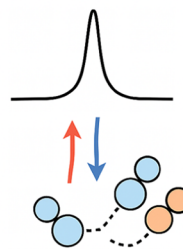
Liquid-liquid Equilibria of Green Aqueous Biphasic Systems

Ethyl lactate - rich phase

Choline salt - rich phase



Molecular Interactions using NMR Analysis



ABSTRACT: Aqueous biphasic systems (ABS) have recently emerged as an economic and sustainable solution for the separation and isolation of biomolecules. Ethyl lactate (EL) is an attractive phase-forming component, as it is a biorenewable, biodegradable, and nontoxic solvent. In this study, cloud points and tie-line data for ethyl lactate (EL)-based aqueous biphasic systems (ABS) with four choline salts—choline bicarbonate (ChHCO_3), choline chloride (ChCl), choline bitartrate (ChBitar), and choline dihydrogen citrate (ChH_2Cit)—were experimentally determined at 298.2 and 328.2 K. For both temperatures, three models were used to fit the data: the three-parameter Merchuk's equation, a two-parameter correlation, and the effective excluded volume. The molecular-level interactions and dynamic behavior within the ABS systems were investigated using diffusion nuclear magnetic resonance. EL– ChH_2Cit showed the most significant changes in diffusion coefficients and water shifts, indicating increased viscosity and altered water structuring. In contrast, ChCl effects were primarily viscosity-driven, ChBitar exhibited complex, nonlinear trends suggestive of solvation or aggregation phenomena, whereas ChHCO_3 uniquely displayed peak splitting, pointing to multiple EL environments. This work provides novel insights into the design of green solvent systems and contributes to the development of alternatives to hazardous organic solvents, with potential applications across biotechnology, pharmaceuticals, and the green chemistry industries.

1. INTRODUCTION

Aqueous biphasic systems (ABS) are a type of liquid–liquid extraction technique widely used to separate, concentrate, purify, and enrich biomolecules.^{1–4} They have emerged as a promising, economic, and sustainable separation technology.³ ABS are formed by mixing different phase-forming components, resulting in various types of biphasic systems. The different phase-forming components are usually selected with the extraction goal in mind and can include polymer–polymer, polymer–salt, ionic liquid–polymer, ionic liquid–salt, or alcohol (short chain)–salt.^{1,5} When properly chosen, these phase-forming components form two immiscible phases that coexist at equilibrium, with each component dominant in either the top or bottom phase depending on its properties and

the prevailing conditions. ABS have gained attention for their mild extraction conditions that preserve the stability of sensitive biomolecules and minimize the reliance on harmful organic solvents.³

The growing awareness of the environmental impact of industrial effluents has sparked interest in developing and using cleaner and more efficient solvents. Among the alternatives,

Received: June 30, 2025

Revised: September 29, 2025

Accepted: September 30, 2025

Table 1. Chemicals Used in This Work

Chemical	Chemical formula	Abbreviation	Purity (mass %)	CAS no.	Source
(−)-Ethyl L-lactate	C ₅ H ₁₀ O ₃	EL	>98%	687-47-8	Sigma-Aldrich
Choline bicarbonate	C ₆ H ₁₅ NO ₄	ChHCO ₃	80% (aqueous solution)	78-73-9	Sigma-Aldrich
Choline chloride	C ₅ H ₁₄ ClNO	ChCl	≥98%	67-48-1	Sigma-Aldrich
Choline hydrogen L-(+)-bitartrate	C ₉ H ₁₉ NO ₇	ChBitar	>98%	87-67-2	Thermo Fisher Scientific
Choline dihydrogen citrate	C ₁₁ H ₂₁ NO ₈	ChH ₂ Cit	≥98%	77-91-8	Sigma-Aldrich

ethyl lactate (EL), derived from biorenewable sources, stands out for its low toxicity and biodegradability.⁵ Thus, EL is generally recognized as safe (GRAS) food additive by the United States Food and Drug Administration (FDA).⁶ ABS based on EL have recently gained footing as an effective method for recovering biomolecules, namely antibiotics,⁷ amino acids,⁸ antioxidants, and flavonoids^{9,10} from aqueous solutions.

Alongside solvents such as EL, there is increasing interest in identifying sustainable and benign phase-forming salts. EL being water-soluble and hydrophilic makes it a suitable precursor since it easily mixes for ABS formation and facilitates easy phase separation.¹¹ Choline-based salts are particularly attractive because they are derived from choline, a vitamin-like, water-soluble nutrient. Choline salts are promising as salts in ABS because they are low in toxicity, easy to handle, and derived from low-cost natural resources, which aligns with green chemistry principles.¹² In addition, by varying the type of choline salt (anion choice), researchers can precisely modulate pH, ionic strength, viscosity, and phase composition, providing a powerful toolbox for designing application-specific ABS for a wide range of solutes.¹³ The combination of EL and choline salts aligns with green chemistry principles, as both are biodegradable, have tunable phase separation, and are derived from natural low-cost precursors. Gaining deeper insight into their phase-forming ability and equilibrium behavior will pave the way for the effective application in environmentally friendly biomolecule extraction and purification techniques. The use of these systems has been explored for biomolecule recovery⁷ and biowaste processing,¹¹ thereby facilitating the recovery of bioactive substances from waste and advancing circular economy. EL–choline salt systems have also been purposeful in food¹⁴ and pharmaceutical processing,⁷ where they are considered effective for the extraction and purification of proteins, vitamins, polyphenols, enzymes, antibiotics, and other pharmaceuticals from complex mixtures. They have also found application in the formulation and functionalization of drug delivery systems.¹¹

Previous work has explored ABS prepared with EL and inorganic salts.^{7–10} Barroca et al.¹⁵ reported the use of either polyethylene glycol (PEG) or EL with choline salts (choline chloride, choline bicarbonate, choline (2*R*,3*R*)-bitartrate, and choline dihydrogen citrate) for the extraction of pharmaceutical compounds. However, their study was limited to a single temperature (298.15 K), with tie-line data reported only for the bitartrate system and no biphasic region identified for the choline chloride system.

Considering the potential of these ABS systems for green applications, comprehensive research is required on their phase formation ability, molecular interactions, and equilibrium phase behavior to further advance their application. To the best of our knowledge, there are no known studies examining the temperature-dependent phase behavior of ABS based on

EL and choline salts, highlighting an important gap in the literature.

In this study, we investigate the equilibrium phase behavior of aqueous biphasic systems (ABS) composed of ethyl lactate (EL) and four choline-based salts, including choline chloride (ChCl), choline bicarbonate (ChHCO₃), choline bitartrate (ChBitar) and choline dihydrogen citrate (ChH₂Cit), at two temperatures: 298.2 and 328.2 K. This temperature range was selected to be representative of those typically employed in industrial and biotechnological separation processes, where mild to moderate temperatures are preferred to minimize energy consumption and avoid biomolecule degradation. To complement the macroscopic phase equilibria data, we also employed diffusion-ordered NMR spectroscopy (DOSY) to probe the molecular-level interactions in monophasic aqueous solutions of EL and choline salts.

2. EXPERIMENTAL METHODS

2.1. Materials. Ethyl lactate (C₅H₁₀O₃; CAS No. 687-47-8), choline chloride (C₅H₁₄ClNO; CAS No. 67-48-1), choline bicarbonate (C₆H₁₅NO₄; CAS No. 78-73-9), choline bitartrate (C₉H₁₉NO₇; CAS No. 87-67-2), and choline dihydrogen citrate (C₁₁H₂₁NO₈; CAS No. 77-91-8) were purchased from either Sigma-Aldrich or Thermo Fisher Scientific. All chemicals were used without further purification. Their purities are listed in Table 1. The water used for sample preparation was distilled and deionized by a Milli-Q water filtration system from Millipore. A Mettler AT201 analytical balance with a stated repeatability of 3×10^{-2} mg was used to gravimetrically prepare all liquid mixtures.

2.2. Cloud Points and Correlations. The cloud point titration method was used to determine the cloud points of ternary mixtures, including ethyl lactate, water, and choline salts (ChHCO₃, ChCl, ChBitar, or ChH₂Cit) at 298 K, 328.2 K, and 0.1 MPa, as described in the literature.⁷ Binary mixtures containing different known salts and water compositions were prepared in septum-sealed conical glass vials with magnetic stirrers placed in a temperature-controlled bath (Thermo Fisher Scientific) heated with a SAHARA SC150 S19T circulator with a stability of 0.05 K. The contents of the mixture could be fully visualized owing to the transparent acrylic bath walls. Until the first turbidity was noticed, which is considered a cloud point, the binary mixtures were titrated with ethyl lactate very carefully and at a constant temperature. To determine the composition that corresponds to the composition of the cloud point, the final mixtures were weighed. The experimental procedure and the average repeatability of the composition of cloud points (in mass fraction) were validated using three replicates of each assay. The average deviation of mass fraction data was <2%. Experimental cloud points were fitted using Merchuk's equation:¹⁶

$$w_{\text{EL}} = A \times \exp[B \times w_{\text{choline salt}}^{0.5} - C \times w_{\text{choline salt}}^3] \quad (1)$$

where x_{EL} and $x_{choline\ salt}$ are mass fractions of EL and choline salt, respectively, while parameters A , B , and C are constants obtained by regression of the experimental cloud point data. Other exponents of Merchuk's equation (0.05 and 1.5), previously shown to present better fittings for ethyl lactate-based ABS,¹⁷ were also tested in this work. The following two equations were also used for the correlation of cloud data:

$$w_{sol} = A \times \exp(B \times w_{choline\ salt}) \quad (2)$$

$$\ln\left(V_{213}^* \frac{w_{choline\ salt}}{M_{choline\ salt}} + f_{213}\right) + V_{213}^* \frac{w_{EL}}{M_{EL}} = 0 \quad (3)$$

Eq 2 is another version of Merchuk's equation that uses two adjustable parameters (A and B).

Eq 3 is a cloud model based on the statistical geometry methods developed by Guan et al.,¹⁸ which was used to calculate the effective excluded volume (EEV) of salt in different ABS systems. In eq 3, V_{213}^* , f_{213} , $M_{choline\ salt}$, and M_{EL} are the scaled EEV of the choline salt, the volume fraction of unfilled available volume after tight packing of the salt molecules into the network of an aqueous solution, and the molar masses of choline salts and EL, respectively. For comparison of the experimental data and data calculated by models, the root-mean-square deviation (RMSD) and coefficient of determination (r^2) were used according to eqs 4 and 5.

$$RMSD = \sqrt{\frac{\sum_i (w_{EL}^{calc} - w_{EL}^{exp})^2}{N}} \quad (4)$$

$$r^2 = 1 - \frac{\sum_i (w_{EL}^{exp} - w_{EL}^{calc})^2}{\sum_i (w_{EL}^{exp} - w_{EL}^{mean})^2} \quad (5)$$

where w_{EL}^{exp} , w_{EL}^{calc} , and w_{EL}^{mean} are the experimental, calculated, and mean values of the mass fraction of EL, respectively, while N is the number of data points.

2.3. Tie Lines. The method used for determining the tie lines has been thoroughly detailed in previous publications.⁷ The solvent, salt, and water solutions were combined in 15 mL glass vials, in accordance with the desired final composition, to create the two-phase systems. The tubes were manually agitated for 10 min and then placed in a thermostatic bath (SAHARA) for 12 h at either 298.2 or 328.2 K to attain the thermodynamic equilibrium of the phases.

After this period, full phase separation occurred with the top phase being rich in EL and the bottom phase rich in salt. Both phases were carefully separated and weighed individually using a Mettler AT201 analytical balance, with an accuracy of 3×10^{-2} mg. The experiments were repeated with varying equilibration times, and it was determined that 2 h were sufficient to reach equilibrium. However, for convenience, the samples were allowed to sit for at least 12 h.

Eq 1 and the obtained constants for A , B , and C were used in combination with the Lever-arm rule to obtain the following set of equations:

$$w_{EL}^{top} = A \times \exp[B \times (w_{choline\ salt}^{top})^{0.5} - C \times (w_{choline\ salt}^{top})^3] \quad (6)$$

$$w_{EL}^{bot} = A \times \exp[B \times (w_{choline\ salt}^{bot})^{0.5} - C \times (w_{choline\ salt}^{bot})^3] \quad (7)$$

$$w_{EL}^{overall} \times m_{overall} = w_{EL}^{bot} \times m_{bot} + w_{EL}^{top} \times m_{top} \quad (8)$$

$$w_{salt}^{overall} \times m_{overall} = w_{salt}^{bot} \times m_{bot} + w_{salt}^{top} \times m_{top} \quad (9)$$

where w_{EL}^{bot} and $w_{choline\ salt}^{bot}$ denote the fractions of ethyl lactate and salt in the bottom phase, respectively, while w_{EL}^{top} and w_{salt}^{top} denote the corresponding mass fractions of ethyl lactate and salt in the top phase. $m_{overall}$, m_{top} , and m_{bottom} are the masses of the overall mixture, the top phase, and the bottom phase, respectively.

With the aid of the solver tool in Microsoft Excel, unknown variables w_{EL}^{top} , w_{salt}^{EL} , w_{salt}^{top} , and w_{salt}^{bot} in the nonlinear set of eqs 1, 7, 8, 9 were solved. As a result, the top and bottom phases' mass fraction compositions for salt and ethyl lactate were determined. Each test was run three times to validate the experimental design. Another useful parameter, the slope of tie lines (STL) was determined using eq 10. The composition of tie lines' average repeatability (in mass fraction) was taken into consideration. The tie-line length (TLL) at different compositions was calculated by using eq 11.

$$STL = \frac{w_{EL}^{top} - w_{EL}^{bot}}{w_{choline\ salt}^{top} - w_{choline\ salt}^{bot}} \quad (10)$$

$$TLL = \sqrt{(w_{EL}^{top} - w_{EL}^{bot})^2 + (w_{choline\ salt}^{top} - w_{choline\ salt}^{bot})^2} \quad (11)$$

2.4. NMR Spectroscopy. For each ethyl lactate–choline salt system, six samples were investigated by NMR spectroscopy. These included compositions corresponding to both the top (ethyl lactate-rich) and bottom phases (salt-rich), aqueous samples of ethyl lactate and choline salts only, and intermediate samples for both the ethyl lactate-rich and choline salt-rich systems. The compositions of all samples used for the NMR study are listed in Table S1.

All NMR measurements were carried out on a nonspinning 300 MHz Bruker Avance spectrometer, using a 5 mm BBO probe equipped with a z-gradient coil producing a maximum gradient of 55 G cm⁻¹. All NMR measurements were performed at 298.15 K. No deuterated solvents were added to the samples. Experiments were acquired without the use of the lock, and shimming was achieved based on the area of the acquired FID.

Proton NMR experiments were recorded with a single increment. For each sample, two spectra were recorded: one from a neat sample and the second using an insert containing TSP in 99% D₂O for locking and referencing.

Diffusion NMR experiments used the Oneshot sequence.¹⁹ The high viscosity of the samples effectively suppressed convection effects in the bulk solution.^{19,20} Ten magnetic field gradient amplitudes, ranging from 7.1 to 28.4 G cm⁻¹, were applied in equal increments of the gradient squared. The gradient encoding time for all experiments was 1 ms, and all gradients were half-sine in shape. The diffusion delay time, Δ , was adjusted for each species studied to achieve approximately 80% signal attenuation. A summary of these delay times can be found alongside the sample compositions in Table S1. For each gradient amplitude, 16 transients of 16384 complex data points were acquired for a total experimental time of ca. 15 min. DOSY spectra and associated diffusion coefficients were subsequently processed using the DOSY Toolbox software package.²¹

Table 2. Cloud Point Data for the Ternary Mixture Containing Ethyl Lactate (EL), Water (Wat), and Choline Salt (Salt) (Either ChCl, ChHCO₃, ChBitar, or ChH₂Cit) at 298.2 K, 328.2 K, and 0.1 MPa in Mass Fraction^a

EL + water + ChCl			EL + water + ChHCO ₃			EL + water + ChBitar			EL + water + ChH ₂ Cit		
298.2 K											
<i>w</i> _{salt}	<i>w</i> _{EL}	<i>w</i> _{wat}	<i>w</i> _{salt}	<i>w</i> _{EL}	<i>w</i> _{wat}	<i>w</i> _{salt}	<i>w</i> _{EL}	<i>w</i> _{wat}	<i>w</i> _{salt}	<i>w</i> _{EL}	<i>w</i> _{wat}
0.106	0.851	0.044	0.116	0.764	0.120	0.007	0.835	0.158	0.115	0.747	0.139
0.160	0.703	0.137	0.137	0.659	0.204	0.018	0.746	0.235	0.148	0.671	0.181
0.192	0.593	0.215	0.164	0.558	0.279	0.033	0.669	0.299	0.210	0.589	0.201
0.270	0.449	0.281	0.220	0.437	0.343	0.064	0.558	0.378	0.261	0.515	0.225
0.341	0.331	0.328	0.292	0.321	0.388	0.117	0.427	0.456	0.329	0.427	0.244
0.411	0.246	0.343	0.366	0.221	0.413	0.117	0.426	0.456	0.406	0.353	0.241
0.503	0.184	0.313	0.418	0.178	0.404	0.168	0.338	0.494	0.424	0.331	0.245
0.581	0.153	0.266	0.498	0.131	0.371	0.177	0.321	0.502	0.471	0.284	0.246
0.665	0.115	0.220	0.590	0.104	0.306	0.239	0.241	0.520	0.512	0.245	0.243
			0.668	0.085	0.247	0.285	0.202	0.513	0.547	0.219	0.233
			0.736	0.079	0.185	0.406	0.094	0.500	0.634	0.158	0.208
						0.531	0.015	0.453	0.667	0.134	0.199
328.2 K											
<i>w</i> _{salt}	<i>w</i> _{EL}	<i>w</i> _{wat}	<i>w</i> _{salt}	<i>w</i> _{EL}	<i>w</i> _{wat}	<i>w</i> _{salt}	<i>w</i> _{EL}	<i>w</i> _{wat}	<i>w</i> _{salt}	<i>w</i> _{EL}	<i>w</i> _{wat}
0.099	0.828	0.072	0.083	0.800	0.117	0.009	0.935	0.057	0.096	0.799	0.106
0.126	0.722	0.152	0.098	0.694	0.207	0.020	0.866	0.113	0.128	0.722	0.151
0.171	0.604	0.225	0.135	0.578	0.286	0.047	0.777	0.176	0.174	0.641	0.185
0.234	0.480	0.286	0.181	0.461	0.357	0.079	0.684	0.236	0.174	0.641	0.185
0.290	0.383	0.327	0.239	0.349	0.412	0.084	0.664	0.252	0.272	0.495	0.233
0.310	0.342	0.349	0.307	0.247	0.446	0.122	0.584	0.294	0.348	0.391	0.261
0.428	0.215	0.357	0.342	0.211	0.447	0.136	0.548	0.316	0.398	0.331	0.271
0.450	0.202	0.349	0.457	0.141	0.403	0.169	0.484	0.346	0.486	0.251	0.263
0.529	0.164	0.308	0.551	0.117	0.332	0.198	0.434	0.368	0.564	0.196	0.240
0.619	0.120	0.262	0.646	0.094	0.260	0.226	0.382	0.391	0.644	0.145	0.211
0.668	0.114	0.218	0.742	0.071	0.186	0.278	0.305	0.417			
						0.292	0.282	0.427			
						0.356	0.208	0.436			
						0.389	0.157	0.453			
						0.429	0.103	0.468			
						0.469	0.048	0.482			
						0.510	0.045	0.445			
						0.550	0.043	0.406			

^aStandard uncertainties *u* are *u*(*T*) = 0.1 K, *u*(*x*) = 0.001, and *u*(*p*) = 1 kPa.

3. RESULTS AND DISCUSSION

3.1. Cloud Point Data and Correlations. The experimental cloud points for four ternary mixtures containing EL + water + choline salts (either ChHCO₃, ChCl, ChBitar, or ChH₂Cit) at 298.2 K, 328.2 K, and 0.1 MPa are presented in Table 2. All studied systems presented a top phase enriched with EL and a salt-rich bottom phase, which is consistent with previously published work on ABS systems based on EL.^{7–10} The data were used to build the cloud point curves presented in Figures 1 and 2.

According to Figure 1, ChBitar exhibits the largest biphasic region, and the ability for phase splitting to occur follows the order ChBitar > ChHCO₃ > ChCl > ChH₂Cit. Both the type and concentration of the salt contribute to this behavior, influencing the size of the phase regions formed. These results align with previous findings,⁹ which report that ChBitar exhibits a larger biphasic region compared to ChCl. Because the anions have different molar masses, the mass fraction plots can be misleading. In mole fraction units as shown in Figure S1, the cloud points remain separate at low salt, showing ion-specific effects, but the curves come together at high salt where ionic strength dominates. In this framework, bitartrate forms many hydrogen bonds with water and EL and ties up water, so

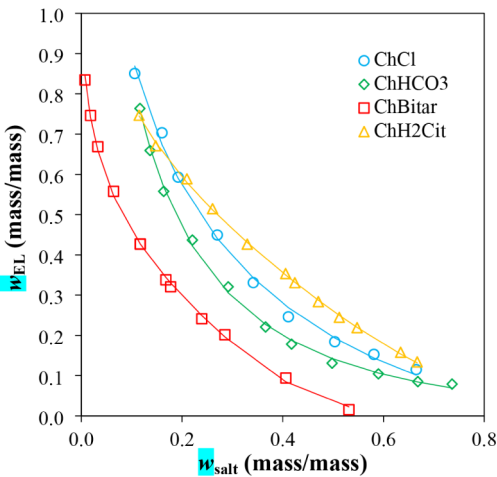


Figure 1. Cloud point curves of the ternary mixtures composed of ethyl lactate, water, and choline salts (ChCl—blue, ChHCO₃—green, ChBitar—red, and ChH₂Cit—yellow) at 298.2 K in mass fraction. Symbols represent experimental cloud point data, while the solid lines correspond to the fittings obtained by eq 1 with constants presented in Table S2.

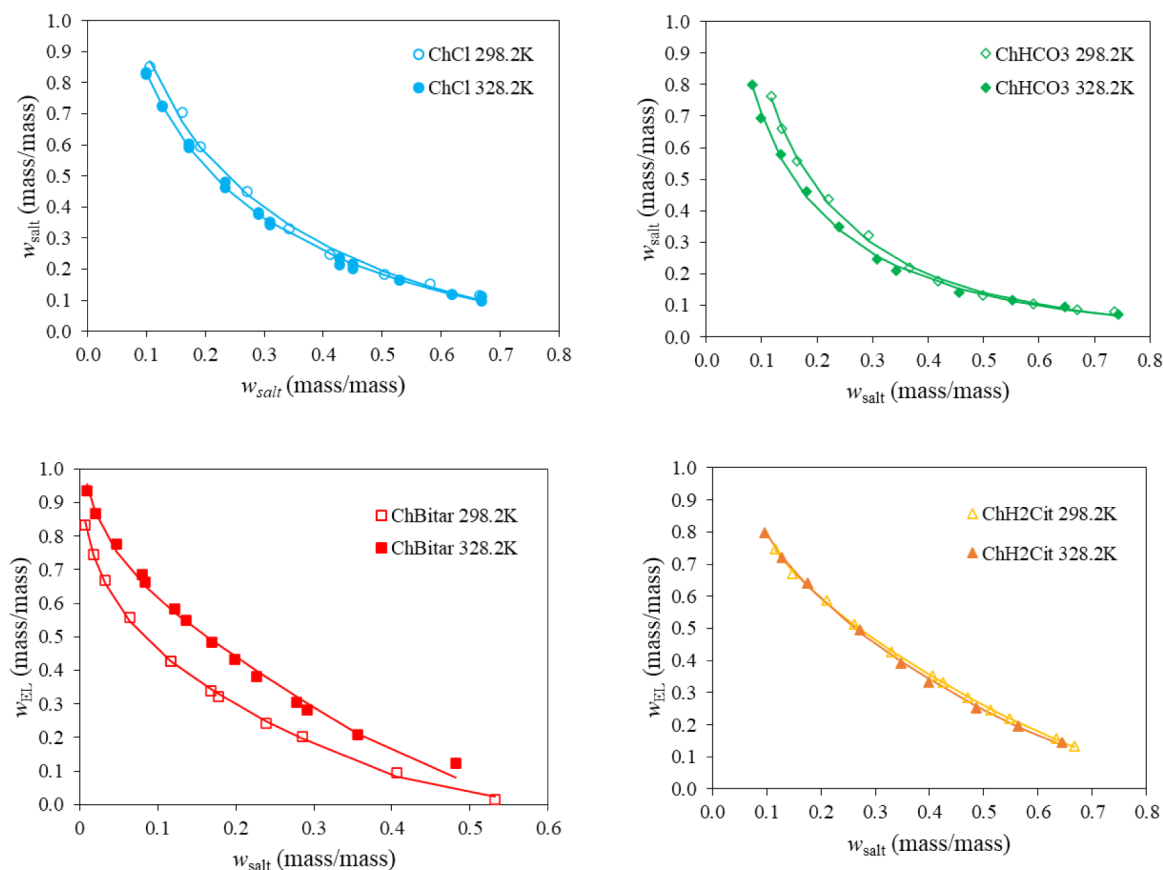


Figure 2. Comparison of cloud point curves of the ternary mixtures composed of ethyl lactate, water, and choline salts (ChCl—blue, ChHCO₃—green, ChBitar—red, and ChH₂Cit—yellow) at 298.2 and 328.2 K in mass fraction. Empty symbols represent experimental cloud point data at 298.2 K, filled symbols represent cloud point data at 328.2 K, while the solid lines correspond to the fittings obtained by eq 1 with constants presented in Table S2.

it salts out EL most strongly. Bicarbonate structures water more than chloride, so it salts out EL more effectively than chloride. Dihydrogen citrate is highly hydrated and can bind at several sites. It strengthens the water network and interacts with EL and choline cation, which stabilizes mixing and shifts cloud points to higher salt. This is consistent with reports on choline citrate versus choline bitartrate systems²² and with our NMR results (section 3.4).

A comparison of cloud point curves at 298.2 and 328.2 K can be seen in Figure 2. No significant temperature-dependent shifts were observed for systems with ChCl, ChHCO₃, and ChH₂Cit, whereas the ChBitar system exhibited a decrease in the biphasic region with increased temperature. The weak temperature dependence observed for ChCl, ChHCO₃, and ChH₂Cit suggests that phase separation in these systems is primarily entropy-driven. In contrast, the distinct behavior of ChBitar indicates a stronger contribution of enthalpic interactions, namely hydrogen bonding between the bitartrate anion, water, and ethyl lactate. Unlike chloride, bicarbonate, or citrate, the bitartrate anion is bulky and asymmetric, with both charged and neutral functional groups. This allows it to form extensive hydrogen bond networks not only with water but also directly with ethyl lactate. As the temperature increases, these hydrogen bonds weaken, reducing the enthalpic driving force for phase separation. At the same time, the entropy gain from water structuring is insufficient to offset this loss of enthalpic stabilization, leading to contraction of the biphasic region.

Two sets of exponents in Merchuk's equation were tested: the widely used (0.5 and 3.0) and those optimized for EL systems (0.05 and 1.5).¹⁷ Results of the fittings with all parameters are presented in Table S2. At 298.2 K, the exponent pair (0.50, 3.00) gave excellent fits ($r^2 \geq 0.998$, very low RMSD) for every salt except ChCl, where the set (0.05, 1.50) gave only a marginally better fit. At 328.2 K, the trend is reversed: (0.05, 1.50) yielded the best fit for ChCl, ChBitar, and ChH₂Cit, whereas the (0.50, 3.00) set performed better for ChCO₃. It should be noted that the empirical models based on Merchuk's equation were used only to correlate the experimental cloud point data, serving as a convenient mathematical description rather than a rigorous thermodynamic consistency test of the LLE data.

Table 3 shows the values for EEV obtained from the correlation of cloud point data using eq 3. The r^2 ranged from 0.9219 to 0.9993, and the RMSD ranged from 0.0002 to 0.0233, giving satisfactory correlation but lower than the values obtained with the two previous models.

In contrast to Merchuk's empirical correlation, the EEV model is based on excluded-volume (packing) theory¹⁸ and thus provides mechanistic insight into salting out: a larger V_{213}^* means less room for EL and water to stay mixed, so phase separation happens at lower salt content, while a smaller f_{213} means the system has less space to accommodate mixing, which promotes phase separation and results in a larger biphasic region. At 298.2 K, V_{213}^* decreases in the order

Table 3. Summary of Root-Mean-Square Deviations (RMSD from eq 4) and Coefficient of Determination (r^2 from eq 5) Obtained Using the EEV Model (eq 3) at 298.2 and 328.2 K, 0.1 MPa

System	NP ^a	V_{213}^* / g mol ⁻¹	f_{213}	r^2	RMSD
298.2 K					
EL + ChCl + wat	9	159	0.2103	0.9910	0.0104
EL + ChHCO ₃ + wat	11	213	0.1170	0.9354	0.0178
EL + ChBitar + wat	12	408	0.0449	0.9993	0.0002
EL + ChH ₂ Cit + wat	12	282	0.0548	0.9958	0.0008
Average				0.9804	0.0073
328.2 K					
EL + ChCl + wat	11	155	0.2512	0.9559	0.0125
EL + ChHCO ₃ + wat	11	231	0.1195	0.9219	0.0233
EL + ChBitar + wat	9	318	0.0612	0.9953	0.0015
EL + ChH ₂ Cit + wat	12	282	0.0548	0.9958	0.0008
Average				0.9672	0.0095

^aNP, Number of points.

ChBitar > ChH₂Cit > ChHCO₃ > ChCl, while f_{213} is lowest for ChBitar and ChH₂Cit. The high V_{213}^*/f_{213} of ChBitar agrees with its large biphasic region, whereas ChCl, with the smallest V_{213}^* , is the weakest salting out salt. ChH₂Cit is an exception: despite values suggesting strong phase separation, it forms a smaller biphasic region due to extensive hydrogen bonding and complexation that stabilize mixing. Regarding the temperature effect, V_{213}^* remains nearly constant for ChCl and ChH₂Cit and shows only a slight increase for ChHCO₃, whereas ChBitar exhibits a marked decrease, consistent with its reduced biphasic region at higher temperatures. Overall, EEV captures the packing-driven contribution to demixing, while deviations highlight the role of specific molecular interactions.

3.2. Comparison with Different Cloud Point Curves Based on EL from the Literature. To validate the measurements, cloud point data for the EL + water + ChH₂Cit, ChHCO₃, and ChBitar systems at 298.2 K were compared with literature data from Barroca et al.¹⁵ As shown in Figure S2 (), close agreement was obtained, with the root-mean-square deviations in EL mass fraction of 0.018, 0.038, and 0.054, respectively.

Figure 3 shows a comparison between the cloud point curves in this work and other systems based on EL with other salts reported in the literature, including organic salts such as sodium tartrate and potassium sodium tartrate⁹ as well as trisodium citrate and disodium.¹⁰

Sodium and potassium tartrate or citrate salts form biphasic systems at lower salt content compared with the corresponding choline salts, indicating that alkali cations promote phase separation more efficiently. The only exception is choline bitartrate, which exhibits cloud points close to those of potassium sodium tartrate salt, consistent with its strong salting out behavior. This behavior is consistent with the well-known link between salting out strength and the ions' Gibbs free energies of hydration (ΔG_{hyd}): the more negative the ΔG_{hyd} of a salt's cation or anion, the more strongly it binds water, and the more effectively it drives phase splitting. The Gibbs free energy for choline cation was calculated by the model developed by Marcus²³ using radius of the choline cation being 2.44 Å. The resulting value, -124 kJ mol^{-1} (detailed calculations in the Supporting Information) is significantly less

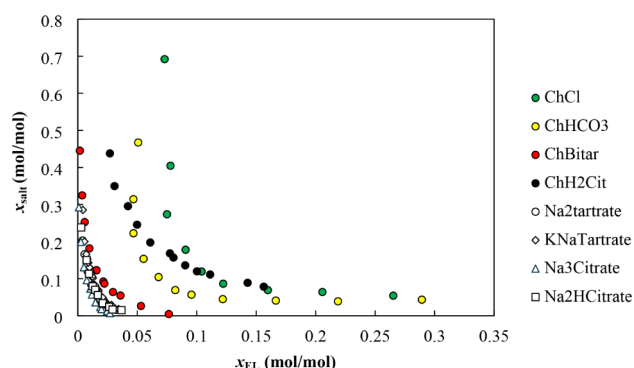


Figure 3. Comparison of cloud point curves in mole fractions for different ternary mixtures composed of ethyl lactate, water, and choline salts from this work at 298.2 K (ChCl—green circles, ChHCO₃—yellow circles, ChBitar—red circles, and ChH₂Cit—black circles) with other salts reported previously in the literature (empty symbols).^{8–10}

negative than that of Na⁺ (-383 kJ mol^{-1}).²³ Consequently, choline salts must reach higher concentrations before a biphasic mixture forms, whereas salts containing more strongly hydrated inorganic cations achieve salting out at lower loadings.

3.3. Tie-Line Data. Tie lines play a crucial role in enabling our understanding of the partitioning behavior of the solute in the biphasic region before extraction can begin. The behavior of the biphasic region, the initial concentration, and the composition of the individual phases at equilibrium facilitate our understanding of how a solute is distributed between the two immiscible aqueous phases. The cloud point curves with matching tie lines for ternary mixtures containing ethyl lactate, water, and salt (ChCl, ChHCO₃, ChBitar, or ChH₂Cit) at 298.2 K and at 328.2 K are shown in Figures 4 and 5, respectively. A minimum of three tie lines were determined for each of the four systems. All tie-line data are summarized in Table 4, along with the slope of the tie line (STL) and tie-line length (TLL) calculated using eq 10 and eq 11, respectively.

In all four systems, an increase in the overall EL or salt composition resulted in a higher TLL, depicting a higher degree of phase separation. All four systems gave high TLL values, suggesting a good phase separation ability.

3.4. Molecular Interactions from NMR Analysis. Examples of the DOSY spectra obtained in this study for the ethyl lactate–choline citrate system are presented in Figure 6.

The ethyl lactate–choline citrate system exhibited the most significant changes in NMR data in response to variations in the sample composition. The left-hand spectrum depicts an overlaid set of three spectra, beginning with a sample of ethyl lactate in water only (S3) and then progressively replacing part of the water with choline citrate. The final sample composition corresponds to the ethyl lactate-rich top layer (T0) in a biphasic aqueous solution. The right-hand spectrum depicts a second set of three overlaid spectra, starting with a sample of choline citrate in water only and then progressively replacing part of the water with ethyl lactate. The final sample composition corresponds to the choline salt-rich bottom layer (B0) in a biphasic aqueous solution. A set of annotated ¹D ¹H spectra acting as a guide to identify which peak belongs to which species can be found in Figure S3.

In the series of samples in which choline citrate was increasingly added to aqueous ethyl lactate samples, the

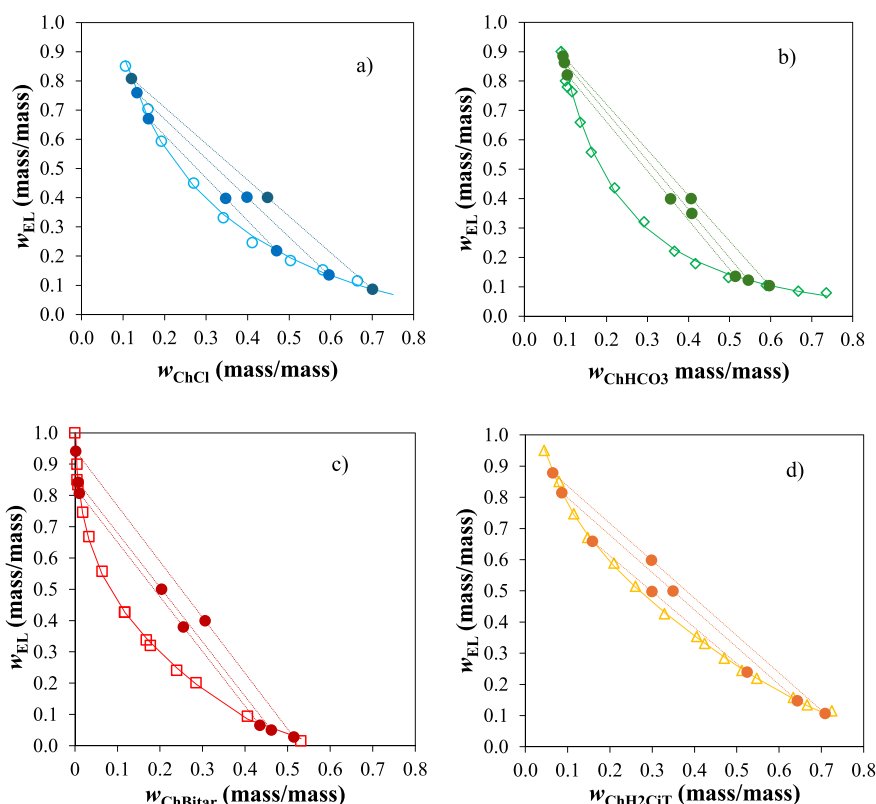


Figure 4. Cloud point curves (solid lines) and corresponding tie lines (dash lines) for ternary mixtures containing ethyl lactate, water, and salts at 298.2 K: ChCl—blue, ChHCO₃—green, ChBitar—red, and ChH₂Cit—yellow. Empty symbols represent experimental cloud point data, while filled symbols correspond to experimental tie-line compositions. Solid and dashed lines correspond to fittings obtained by eq 1 and tie lines, respectively.

diffusion coefficients of all species present decreased. Larger decreases in diffusion coefficient, ca. 80% over the set of three samples, were observed for water, choline ions, and citrate ions. However, these changes in diffusion coefficients were not accompanied by changes in the chemical shifts, except for the water peak, where a $\Delta\delta$ of over 0.40 ppm was observed. This strong water response indicates extensive hydration and hydrogen bonding by citrate, which helps explain why the biphasic region of ChH₂Cit is smaller than predicted from excluded-volume arguments, as strong enthalpic interactions offset the entropic packing drive.

Similar behavior was observed in the series of samples where ethyl lactate was increasingly added to aqueous choline citrate samples. The diffusion coefficients of all species were lower for these samples, indicating a higher viscosity of the choline citrate samples. These all decreased by up to 80% as ethyl lactate was added. Only the water peak exhibited any significant change in its chemical shift, moving 0.40 ppm to a higher chemical shift.

Choline chloride exhibited the smallest responses to the changes in sample composition (Figure S4). In the series of samples where choline chloride was increasingly added to aqueous ethyl lactate samples, the diffusion coefficients of all species present again decreased. The percentage decreases in choline and water diffusion coefficients were the same for both species, ca. 33%. There were only small changes observed in the chemical shifts for all species, with the largest $\Delta\delta$ observed, 0.05 ppm, for water. This indicates that the bulk viscosity of the system is changing, but there is only a small change to the water structure upon adding the salt. These weak NMR

responses are consistent with the relatively small biphasic region of ChCl, which behaves as the weakest salting out agent in this study.

Adding ethyl lactate to choline chloride samples reveals much the same behavior. The diffusion coefficients of all species decrease to a similar degree, indicating the increasing viscosity of the system. Only small changes in chemical shift are observed, with the solvent composition having practically no effect on the chemical shifts of the ethyl lactate peaks. The largest change in chemical shift is again observed in the water peak, with a $\Delta\delta$ of 0.05 ppm observed.

The ethyl lactate–choline bitartrate exhibited a wider range of behavior, broadly intermediate between the previous two examples (Figure S5). Again, the changes in chemical shift upon changing the sample composition are small. When adding the salt to ethyl lactate, shifts of no more than 0.025 ppm are observed. In contrast to the citrate and chloride salts, the ethyl lactate peaks move first to a higher chemical shift, then to a lower one. Upon initially adding salt to ethyl lactate, diffusion coefficients actually increase, contrary to all other experimental observations, but then fall again as the concentration of salt further increases. In the other series of samples, when adding ethyl lactate to salt solutions, the initial addition causes a large decrease in diffusion coefficient, a drop of ca. 40%, while there is only a smaller decrease when more ethyl lactate is added. Similar behavior is observed in the spectral data, with chemical shifts initially moving about 0.5 ppm but showing little change upon further modifications to the sample composition. The nonlinear behavior suggests competing effects between bitartrate's hydrogen bonding with

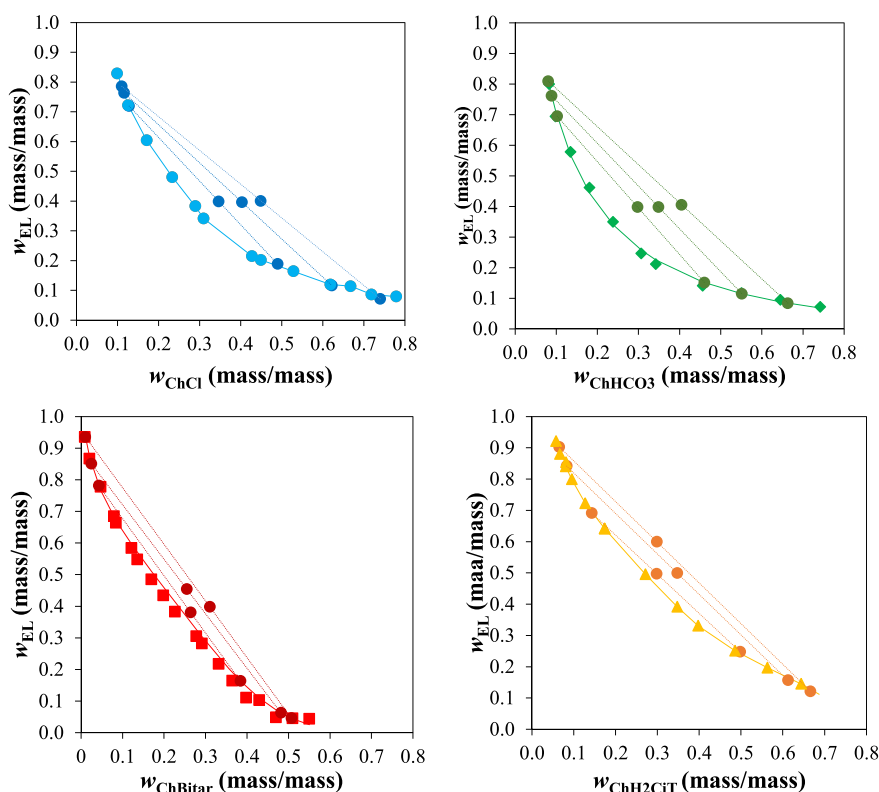


Figure 5. Cloud point curves (solid line) and corresponding tie lines (dash line) for ternary mixtures containing ethyl lactate, water, and salts at 328.2 K: ChCl—blue, ChHCO₃—green, ChBitar—red, and ChH₂Cit—yellow. Empty symbols represent experimental cloud point data, while filled symbols correspond to experimental tie-line compositions. Solid and dashed lines correspond to fittings obtained by eq 1 and tie lines, respectively.

EL/water and its disruption of water structure. Such competition is consistent with its large excluded volume, strong salting out behavior, and marked temperature sensitivity observed in the LLE data, although further study would be needed to resolve the molecular details.

The ethyl lactate–choline carbonate system exhibited markedly different behavior compared to the other three samples (Figure S6). The most striking behavior is the splitting of peaks, where the characteristic doublet and triplet of the two methyl groups are transformed into a triplet and quartet. This implies two different ethyl lactate environments in solution. It was not possible to resolve the two species using regular NMR experiments, but recent advances in NMR spectroscopy such as pure shift or GEMSTONE experiments may be able to better resolve the different species present. These results demonstrate that the choice of choline salt anion strongly influences the microstructural and dynamic properties of ethyl lactate-based mixtures, with implications for their design and application in biphasic and tunable solvent systems. Given their low toxicity and biodegradability, these systems are compatible with biomolecule processing and offer opportunities for integration into industrial bioprocesses where sustainable and selective separation is required.

4. CONCLUSIONS

Cloud point and tie-line data for the four ternary mixtures composed of ethyl lactate, water, and choline salt (either ChCl, ChBitar, ChHCO₃, or ChH₂Cit) at 298.2 and 328.2 K were investigated. The ability of the salt to induce phase separation at 298.2 K followed the order ChBitar > ChHCO₃ > ChCl > ChH₂Cit. Three models—three-parameter Merchuk's equa-

tion, a two-parameter correlation, and the effective excluded volume—gave good fittings. Tie-line length (TLL) and tie-line slope (STL) decreased with increasing temperature for all systems, while an increase in the overall EL or salt composition resulted in higher TLL.

The NMR spectroscopy study of ethyl lactate with various choline salts revealed distinct molecular interactions and dynamic behaviors dependent on salt composition. The EL–ChH₂Cit system showed the most pronounced changes, with diffusion coefficients decreasing by ~80% as water was replaced by ChH₂Cit or EL, indicating significant viscosity increases. This was accompanied by a 0.40 ppm chemical shift in water but minimal shifts for other species, suggesting that solvent restructuring primarily affects water molecules rather than ionic or organic components. The EL–ChBitar system exhibited anomalous behavior, with initial diffusion coefficient increases upon salt addition, followed by decreases, implying potential transient aggregation or competing solvation effects. In contrast, the ChHCO₃ system showed peak splitting of the distinctive ethyl lactate signals (triplet → quartet), suggesting two distinct ethyl lactate environments unresolved by conventional NMR.

By elucidating the temperature-dependent phase behavior and molecular dynamics of ethyl lactate–choline salt ABS, this study lays the groundwork for their application in water purification, pharmaceutical recovery, and bioprocessing, areas where sustainable separation technologies are increasingly in demand.

Table 4. Tie-Line Equilibrium Data for the Ternary Mixtures Containing Ethyl Lactate (EL), Water (Wat), and Choline Salt (Either ChCl, ChHCO₃, ChBitar, or ChH₂Cit) at 298.2 K, 328.2 K, and 0.1 MPa in Mass Fraction^a

Overall composition			Top phase			Bottom phase			TLL	STL
w_{salt}	w_{EL}	w_{wat}	w_{alt}	w_{EL}	w_{wat}	w_{salt}	w_{EL}	w_{wat}		
298.2 K										
Ethyl lactate + water + ChCl										
0.348	0.398	0.255	0.162	0.670	0.168	0.470	0.218	0.312	0.78	−1.46
0.399	0.401	0.200	0.134	0.759	0.107	0.596	0.135	0.269	0.96	
0.448	0.400	0.151	0.120	0.808	0.072	0.701	0.086	0.213	1.09	−1.24
Ethyl lactate + water + ChHCO ₃										
0.357	0.399	0.244	0.105	0.820	0.075	0.514	0.135	0.351	0.92	−1.67
0.407	0.400	0.193	0.094	0.885	0.021	0.597	0.104	0.299	1.04	−1.55
0.409	0.349	0.242	0.098	0.862	0.040	0.546	0.122	0.332	0.98	−1.65
Ethyl lactate + water + ChBitar										
0.255	0.379	0.365	0.011	0.807	0.183	0.435	0.065	0.500	0.87	−1.75
0.204	0.500	0.296	0.008	0.842	0.151	0.462	0.050	0.488	0.92	−1.74
0.306	0.399	0.295	0.002	0.941	0.057	0.515	0.028	0.458	1.05	−1.78
Ethyl lactate + water + ChH ₂ Cit										
0.300	0.497	0.203	0.159	0.659	0.182	0.525	0.239	0.236	0.80	−1.15
0.350	0.499	0.151	0.086	0.814	0.099	0.644	0.147	0.209	0.99	−1.20
0.299	0.598	0.103	0.065	0.878	0.057	0.709	0.106	0.185	1.09	−1.20
328.2 K										
Ethyl lactate + water + ChCl										
0.347	0.399	0.254	0.128	0.719	0.153	0.490	0.188	0.322	0.98	−0.68
0.404	0.396	0.201	0.116	0.763	0.120	0.622	0.116	0.262	1.01	−0.78
0.449	0.400	0.151	0.111	0.786	0.104	0.740	0.071	0.189	1.06	−0.88
Ethyl lactate + water + ChHCO ₃										
0.298	0.398	0.304	0.103	0.695	0.203	0.460	0.151	0.389	0.92	−0.66
0.349	0.398	0.253	0.089	0.762	0.149	0.552	0.114	0.334	0.99	−0.71
0.405	0.405	0.190	0.081	0.809	0.110	0.663	0.084	0.253	1.07	−0.80
Ethyl lactate + water + ChBitar										
0.384	0.163	0.452	0.043	0.781	0.176	0.265	0.380	0.355	1.18	−0.55
0.507	0.046	0.447	0.011	0.935	0.054	0.311	0.398	0.291	1.37	−0.56
0.482	0.063	0.455	0.025	0.850	0.125	0.255	0.454	0.291	1.32	−0.58
Ethyl lactate + water + ChH ₂ Cit										
0.30	0.497	0.204	0.144	0.691	0.165	0.498	0.247	0.254	1.00	−0.80
0.35	0.499	0.153	0.084	0.841	0.075	0.613	0.156	0.231	1.13	−0.77
0.30	0.599	0.102	0.066	0.902	0.032	0.666	0.121	0.212	1.19	−0.77

^aStandard uncertainties u are $u(T) = 0.1$ K, $u(x) = 0.001$, and $u(p) = 1$ kPa.

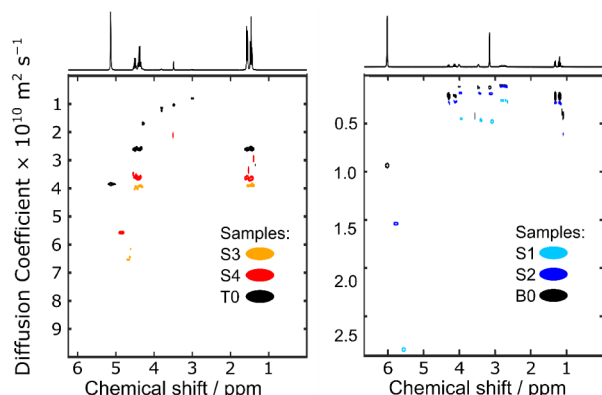


Figure 6. Overlaid DOSY NMR spectra of aqueous ethyl lactate/choline citrate mixtures. The left-hand spectrum depicts S3, a pure ethyl lactate solution; T0, a mixture corresponding to the top layer in a biphasic solution; and S4, an intermediate ethyl lactate–choline citrate mixture. The right-hand spectrum depicts S1, a pure choline citrate solution; B0, a mixture corresponding to the bottom layer in a biphasic solution; and S2, an intermediate ethyl lactate–choline citrate mixture.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jced.5c00447>.

Compositions of all samples used for NMR study; Obtained parameters of the Merchuk's equations; Proton NMR spectra of aqueous ethyl lactate samples; Overlaid DOSY NMR spectra of aqueous ethyl lactate containing a choline salt (chloride, bitartrate, or carbonate); and calculation of the molar Gibbs free energy of hydration of the salt ions (PDF)

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Author Contributions

The manuscript was written with contributions from all authors. V.N.-V.: Conceptualization; resources; data curation; writing—original draft, review, and editing; supervision. M.E.: Methodology; formal analysis; investigation; writing—original draft; visualization. K.A.: Methodology; formal analysis; investigation. P.T.: Conceptualization; writing—review and editing; supervision. W.K. and P.R.: Writing—review and editing; supervision. R.E.: Methodology; formal analysis; investigation; visualization; writing—review and editing.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

M.E. gratefully acknowledges the Commonwealth Scholarship Commission for her PhD scholarship, and K.A. thanks the Thailand Science Research and Innovation under the Research and Researchers for Industries (RRI) Ph.D. Program (Grant No. PHDS9I0016) for his visiting scholarship at Aston University.

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