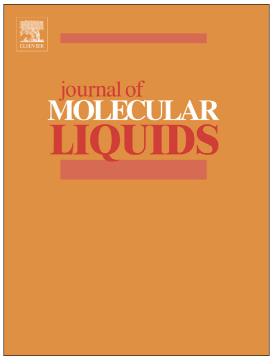

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Novel Aqueous Biphasic System Based on Ethyl Lactate for Sustainable Separations: Phase Splitting Mechanism

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Abstract

Ethyl lactate is a hydrophilic green solvent which is bio-renewable and biodegradable with low toxicity towards humans and animals. For the first time, we report that aqueous solutions of ethyl lactate separate into two aqueous phases upon addition of salts. The performance of trisodium citrate, disodium tartrate and disodium succinate as salting-out media for the separation of natural organic compounds, such as caffeine and catechin, from their aqueous solutions was examined. In this respect, cloud points for the ternary solutions composed of ethyl lactate, water and salt were determined at ambient pressure (0.1 MPa) at 298.2 K. Partition coefficients of caffeine and catechin between two phases were determined by chemical analysis of phases in equilibrium for different initial compositions at 298.2 K. The obtained results clearly demonstrate the ability of the ternary mixture to phase separate, providing good salting-out media for the efficient and sustainable separation from aqueous solution. ^{1}H-NMR spectroscopy was employed to elucidate the mechanisms of phase splitting in the ternary (ethyl lactate + water + salt) systems at molecular level. The discovery of aqueous biphasic system (ABS) containing ethyl lactate as hydrophilic solvent opens a new and green platform for extraction of various compounds from aqueous solutions.

Keywords: aqueous two-phase system, green extraction, natural organic compounds

1. Introduction

The separation and purification costs to recover compounds from aqueous solutions can vary from 20 to 50% of their total production cost [1]. Solvent extraction using hazardous organic solvents is the most used method for recovery. Many biologically active compounds are hydrophilic, meaning that their extraction from aqueous solutions using organic solvent is not a promising option. For these two reasons, aqueous biphasic systems (ABS), formed by mixing a hydrophilic polymer (*e.g.* polyethylene glycols – PEGs or dextrin), water and inorganic salt have been attracting interest of academic community for many years [2]. Adding a hydrophilic solvent and salt triggers the formation of two phases where a biologically active compound is more concentrated in the solvent-rich phase. In the contrast to phases involving organic solvent extraction, both phases in an aqueous two-phase system are rich in water offering a biocompatible environment for various biologically active substances, such as proteins, viruses, enzymes, nucleic acids and other biomolecules both in industry and academia [3]. Beside biocompatibility, the ABS are characterized by very low interfacial tension [4], between 0.0001 and 0.1 dyne/cm compared to 1–20 dyne/cm for water-organic solvent systems. This low interfacial tension assures efficient mass transfer because of a high interfacial contact area of the dispersed phases. Another benefit of ABS based on polymers is their stabilizing influence on structures and activity of biomolecules.

Another alternative for salting out is to use of an ionic liquid as a hydrophilic substance in combination with inorganic salt to induce aqueous biphasic system. This extraction method has been the focus of a significant amount of research during the past decade [5]. Similar to molten salts, ionic liquids are compounds solely composed of ions but with melting points close to room temperature. Due to their physicochemical properties, ionic liquids have been implemented in aqueous two-phase systems for extraction of various biologically active substances, including proteins [6], amino acids [7], antioxidants [8], antibiotics [9], pesticide

[10] and mycotoxins [11]. However, most ionic liquids have low biodegradability and low biocompatibility.

In the last few years, another hydrophilic green solvent – ethyl lactate, produced from bio-renewable sources (bioethanol and lactic acid produced from corn fermentation) has attracted attention from both industry and academia. Due to its extremely low toxicity towards humans and animals [12], biodegradability and negligible eco-toxicity for several life forms [13], ethyl lactate is approved by the USA Food and Drug Administration as additive for food and pharmaceutical formulations [14].

Chemical structure of ethyl lactate has the ability to form intra- and intermolecular association via hydrogen bonds, either as proton donor or proton acceptor [15],[16]. On the other hand, ethyl lactate dissolves non-polar compounds such as paraffin oils, suggesting the presence of the van der Waals interactions [17]. Because of this dual nature, ethyl lactate is a good solvent for a large number of different solutes including caffeine [18], phenolic compounds and flavonoids [19],[20] and hydrocarbons [17].

This work reveals, for the first time, that ethyl lactate forms aqueous two-phase system with water and salts, opening a new way for recovery of various compounds from their water solutions. We have selected two model compounds to study their partitioning between ethyl lactate and salt rich phases: caffeine and catechin.

Caffeine is the most widely consumed and studied natural products in history. Caffeine is an important raw material for pharmaceutical and food industries that might be produce synthetically, but naturally isolated caffeine from coffee beans is preferred. Two most used processes for caffeine extraction are Swiss water® and supercritical process. In the Swiss process®, water is used as a solvent to extract caffeine from coffee beans followed by recovery of caffeine from water by adsorption on activated carbon. The recovery of caffeine from this adsorbent is very poor and uneconomical. On the other hand, in the supercritical process, high-pressure carbon dioxide is used to extract caffeine from previously soaked coffee beans followed by water extraction of caffeine from carbon dioxide. Furthermore, caffeine is recognized as a track anthropogenic input into rural freshwater, urban marine systems, ground water, rivers, lakes and wells [21]. Thus, recovery of caffeine from its aqueous solutions is an issue in both processes.

Catechin was chosen as another model component due to its extensive utilization as nutraceutical for human health, pharmaceutical and as ingredient for increasing the product shelf life. It belongs to a group of polyphenolic compounds that widely occur in the plants. Extraction and purification of catechins is a challenging task [22].

In this work, cloud points and tie-lines of the ternary systems containing ethyl lactate, water and salt (trisodium citrate, disodium tartrate or disodium succinate) are determined at 298.2 K. Also, partition coefficients of caffeine and catechin between two aqueous phases are presented, showing efficient extraction. Selected salts are cheap, environmentally friendly and contain divalent or trivalent sodium cation and multiply charged anionic species, promising good separation ability. Furthermore, ^{1}H-NMR spectroscopy was employed to understand mechanisms of phase splitting in the ternary (ethyl lactate + water + salt) systems at molecular level allowing interpretation of macroscopic solution property at the level of molecular interactions.

2. Experimental section

2.1 Chemicals and preparation of solutions

Ethyl lactate (CAS 687-47-8, purity 98%), trisodium citrate (CAS 6132-04-3, purity ≥ 99.0%), disodium tartrate (CAS 6106-24-7, purity ≥ 99.0%), disodium succinate (CAS150-

90-3, purity ≥ 98.0%), caffeine (CAS58-08-2, purity 99.0 %) and catechin (CAS154-23-4, purity ≥ 96.0%) were supplied by Sigma–Aldrich.

Deuterated solvents, sodium 3-trimethylsilylpropionate (TSP, CAS 24493-21-8, purity 98%) and water (D_2O, purity 98%) were purchased from Cambridge Isotope Laboratories Inc. All chemicals were used without further purification.

All liquid solutions were gravimetrically prepared using Mettler AT201 analytical balance with stated repeatability of ± $3 \cdot 10^{-2}$ mg.

2.2 Equipment and methodology

2.2.1 Cloud points

Cloud points of the ternary systems containing ethyl lactate, water and salt (trisodium citrate or disodium tartrate or disodium succinate) were determined by the cloud point titration method at the constant temperature. Binary mixture of known composition was titrated with the third component at a constant temperature (± 0.1 K) under stirring until the turbidity in the sample was observed which was taken as the cloud point. After the turbidity was observed, final mixtures were weighed by Mettler AT201 analytical balance with stated repeatability of ± $3 \cdot 10^{-2}$ mg, allowing calculation of the composition corresponding to the cloud-point. The experimental procedure was validated by comparison with literature data for the cloud points obtained for the system containing PEG400, water and trisodium citrate [23] which showed a good agreement of less than 1% deviation between measured and literature mass fraction data. The validity of the experimental method for the cloud points was further confirmed by carrying out replicas of the assays at random conditions. The average deviation of mass fraction data was less than 2%.

2.2.2 Cloud points fitting

Obtained cloud points were fitted using the equation given by Merchuk et al. [24]:

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

where x_{EL} and x_{salt} are the ethyl lactate and salt mass fraction, respectively, while parameters *A*, *B*, and *C* are constants obtained by the regression of the experimental cloud point data. The experimental and fitted data were compared in terms of the absolute average deviations (AAD) of the ethyl lactate mass fraction according to:

$$AAD(\%) = \frac{1}{NP}\sum_i \frac{\left|x_{EL,i}^{calc} - x_{EL,i}^{exp}\right|}{x_{EL,i}^{exp}} \cdot 100 \qquad (2)$$

Where $x_{EL,i}^{calc}$ and $x_{EL,i}^{calc}$ are the calculated and experimental mass fractions, respectively, and *NP* is the number of available cloud points.

2.2.3 Tie-lines

The tie-lines of the ternary systems were determined according to the procedure previously described in literature [9]. Ternary mixtures of desired compositions containing of ethyl lactate, salt (trisodium citrate or disodium tartrate or disodium succinate) and water, total mass of approximately 40 g, at the biphasic region were prepared, vigorously agitated, and left to equilibrate for at least 12 h at 298 ± 1 K. After that period, both phases were carefully separated and individually weighed within $\pm 3 \cdot 10^{-2}$ mg. Each experiment was repeated three times and reported results are average values.

Each tie line was determined using lever-arm rule according to the following equations:

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

$$x_{EL}^{salt-phase} = A \cdot exp\left[B \cdot \left(x_{salt}^{salt-phase}\right)^{0.5} - C \cdot \left(x_{salt}^{salt-phase}\right)^{3}\right] \qquad (4)$$

$$x_{EL}^{EL-phase} = \frac{1}{\alpha} x_{EL}^{overall} - \frac{1-\alpha}{\alpha} x_{EL}^{salt-phase} \qquad (5)$$

where superscripts "*EL-phase*", "*salt-phase*", and "*overall*" stand for mass fractions in ethyl lactate–rich phase, salt–rich phase and overall mixture, respectively. Parameter α is the ratio between the mass of the top phase and the total mass of the mixture. Equations 3, 4 and 5 were solved using MATLAB software and the solution results in the mass fraction composition of ethyl lactate and salt in the top and bottom phases.

The tie-line lengths (*TLL*) at different compositions were calculated by the following equation:

$$TLL = \sqrt{\left(x_{EL}^{EL-phase} - x_{EL}^{salt-phase}\right)^2 + \left(x_{salt}^{EL-phase} - x_{salt}^{salt-phase}\right)^2} \qquad (6)$$

2.2.4 Partition coefficients

Partition coefficients for caffeine and catechin were obtained by sampling of phases. The initial concentration of both solutes (caffeine or catechin) in water was 0.195 mass% (feed solution) in all partitioning experiments. Known amount of the feed solution (m_{feed}) was mixed with a known amount of ethyl lactate and salt. These samples were agitated for at least 6 hours, at constant and controlled temperatures of 298.2 K. Next, the mixtures were left still for at least 12 hours at 298.2 K to allow complete phase separation. Using a syringe

(Hamilton, Bonaduz, Switzerland), samples from both phases were taken and prepared for further analysis to determine their composition.

Concentrations of caffeine and catechin in both phases were determined using UV-Vis spectroscopy (Evolution 220 UV-Visible Spectrophotometer, Thermo Fisher Scientific), at wavelengths of 272 nm and 278 nm for caffeine and catechin, respectively. Respective calibration curves were established for each individual compound. Interferences of both ethyl lactate and salts on the concentration of solutes were found not to be significant. It was also possible to check material balance taking into account overall quantity of caffeine or catechin and its concentration in each phase. Standard error between the actual mass and sum of determined masses in two phases was always below 3%.

The concentrations of sampled phases were used to calculate the partition coefficients for caffeine and catechin ($K_{caffeine}$ and $K_{catechin}$) according to the relationship:

$$K_{caffeine} = \frac{x_{caffeine}^{EL-phase}}{x_{caffeine}^{salt-phase}} \qquad K_{catechin} = \frac{x_{catechin}^{EL-phase}}{x_{caffeine}^{salt-phase}} \qquad (7)$$

Where $x_{caffeine}^{EL-phase}$ and $x_{caffeine}^{salt-phase}$ are mass fractions of caffeine in EL-rich phase and salt-rich phase, respectively; $x_{catechine}^{EL-phase}$ and $x_{catechine}^{salt-phase}$ are mass fractions of catechin in EL-rich phase and salt-rich phase, respectively. All the results presented are the average of at least two replicate analyses. The maximum observed standard error for obtained partition coefficients for different replicas of the same assays is less than 5%.

The extraction efficiencies (%*EE*) of each component *i* were determined according to Eq. (8):

$$\%EE_i = 100 \cdot \frac{m_{EL-phase} \cdot x_i^{EL-phase}}{m_{feed} \cdot x_i^{feed}} \qquad (8)$$

where $m_{EL-phase}$ and m_{feed} are masses of the top (EL-rich) phase and initial mass of feed (solute in water solution) while $x_i^{EL-phase}$ and x_i^{feed} are mass fraction of solutes (caffeine or catechin) in the top phase and in the initial feed solution ($1.95 \cdot 10^{-3}$ mass/mass), respectively.

In addition, the biphasic systems containing ethyl lactate, water and salt of the same compositions (without partitioning component) were prepared. These were agitated for at least 6 hours, at constant and controlled temperatures of 298 K. Next, the mixtures were left still for at least 12 h to allow complete phase separation. Samples of both phases were carefully taken and their pH value was measured by was measured by HI 2211 Microprocessor pH meter (HANNA instruments) with precision to within ± 0.01. Respective pH standard solutions (pH 4 and 7) were used for calibrations.

2.2.5 NMR Spectroscopy

^{1}H NMR spectroscopy (Bruker AMX400 spectrometer) was employed to study molecular interactions existing in the mixtures containing ethyl lactate, salt (trisodium citrate or disodium tartrate or disodium succinate) and water. The NMR spectrometer was locked and spectra were calibrated using TSP solution in D_2O (c = 1.72 mg/ml) as an external standard in a small capillary inside NMR tube to avoid direct contact and interference of TSP and D_2O on analysed solutions. The following sets of solutions were made gravimetrically: 1) Four binary solutions containing 1mol/kg of salt(trisodium citrate, disodium tartrate, disodium succinate) and ethyl lactate in water; 2) Two ternary solutions with 0.3 mol/kg and 1.3 mol/kg of salt in solvent containing 1mol/kg of ethyl lactate in water; and, 3) Two ternary solutions with 0.3 mol/kg and 1.3 mol/kg of ethyl lactate in solvent containing 1mol/kg of salt in water. The labels (a to h) were used to designate hydrogen atoms of observed chemical shifts as shown in Figure 1. The obtained ^{1}H NMR chemical shifts ($\Delta\delta_H$) for each solution are listed in the Electronic Supplementary Information (Table S1). These allowed calculation of chemical shift deviations ($\Delta\delta_H$) according to the following equations:

$$\Delta\delta_{H,1} = \delta_H(ethyl\ lactate + water + salt) - \delta_H(ethyl\ lactate + water) \quad (9)$$

$$\Delta\delta_{H,2} = \delta_H(ethyl\ lactate + water + salt) - \delta_H(salt + water) \quad (10)$$

where $\Delta\delta_{H,1}$ is a chemical shift deviation when salt is added to the (ethyl lactate + water) system and $\Delta\delta_{H,2}$ is a chemical shift deviation when ethyl lactate is added to the (salt + water) system.

3. Results

Experimental cloud points and tie-lines for the ternary mixtures containing ethyl lactate, water and salt (trisodium citrate, disodium tartrate ordisodium succinate) at 298.2K are shown in Table 1 which were used to construct Fig. 2 and Fig. 3. According to Figure 2, it seems that the ability of all three studied salts to provoke two-phase splitting in (ethyl lactate + water) mixture is very similar. However, it is necessary to consider mole fraction instead of mass fractions in order to examine a real influence due to difference in molecular masses. Thus, Figure S1 (Supplementary Information) shows the cloud point curves for three mixtures in mole fraction, demonstrating that the ability of salt to provoke phase splitting is in the order citrate > tartrate >succinate. Citrate and tartrate salts have very similar anions, but citrate salt possess higher ionic force (trivalent vs. divalent) leading to the highest ability for phase splitting. On the contrary, tartrate and succinate salts have the similar ionic forces (both are divalent), but their anions have a significant difference: tartrate has two -OH groups making it more polar then succinate and more soluble in aqueous solutions. Table 1 also contains tie-line lengths (*TLL*) calculated according to Eq.(6) which is an important parameter showing the degree of separation. Higher TLL values correspond to bigger difference between compositions of the top and bottom phases - higher degree of separations. As expected, increase of the overall salt concentration enlarged the tie-line length for three studied systems.

Cloud points for both studied solutions were correlated using equation (1) and obtained parameters *A*, *B* and *C* of each system are shown in Table 3. Correlations are reasonable good with the average standard deviations of 7.0%, 8.1% and 7.4% for solutions with trisodium citrate, disodium tartrate anddisodium succinate, respectively.

Few biphasic ternary mixtures composed of ethyl lactate, salt and water with different compositionwere selected to study partitioning coefficient of caffeine and catechin. Partition coefficient of a solute between two phases of ternary mixture depends on initial compositions (feed). Thus, for comparison, all partition studies were performed using identical initial compositions as it can be observed in Table 3 (systems 1, 2 and 3). In addition, Table 3 includes measured pH values of phases in equilibrium. It can also be observed that the values of pH for salt-rich (bottom) and ethyl lactate-rich phases are relatively similar for all studied salts. Also, the pH of the ternary systems involving trisodium citrate and disodium succinate salts are very similar (approximately 7.2-7.3) while the pH of system with disodium tartrate is somewhat more acidic (approximately 6.4-6.5).

For all studied systems, partition coefficients for catechin and caffeine are higher than unity (Fig. 4 and Table 3) – ranging from 1.6 to 6.4 for caffeine and 3.0 to 28.6 for catechin. In general, $K_{\text{catechin}} > K_{\text{caffeine}}$ which is likely due to difference in their structure. Catechin has five –OH groups which are capable to form hydrogen bonds with both water and ethyl lactate, and one ether group which can interact with non-polar part of ethyl lactate molecule. In contrast, caffeine has four nitrogen atoms but none of the hydrogens are attached to them, making them unavailable for hydrogen bonding. Obtained values of partition coefficient for caffeine are comparable with their literature values of 0.4 in octanol-water system [25], 8.0 in methylene chloride-water [26] and 4.8 in aqueous biphasic system composed of tributyl(methyl)-phosphonium methylsulphate, K_3PO_4 and water [27]. On the other hand, obtained partition coefficient for catechin is considerably higher than values reported in literature: 0.8 in octanol-water [28] and from 8 to 21 in aqueous biphasic system composed of ethylene oxide-propylene oxide polymer and different salts.

^{1}H NMR (Nuclear Magnetic Resonance) was employed to reveal the molecular-level mechanisms which rule the phase splitting in mixtures containing water, ethyl lactate and salt. Table S1 in Electronic Supplementary Information (ESI) document contains chemical shifts and deviations for various mixture compositions. H-signals belonging to -OH groups from citrate anion, tartrate anion and ethyl lactate seem not to appear as separate probably due to interaction with water. Also, two aqueous signals appear in spectra (designated as a and b in Figure 1). The larger signal shift (b in Fig.1) changes its position greatly (~0.10 ppm) while smaller signal (a in Fig.1) is much smaller and present at 4.79 ppm which prompt us to conclude that principal signal (b) is associated to water in our investigated system and quite sensitive to solution composition and pH while the smaller one (a) is most probably related to HOD from capillary solution and therefore irrelevant [29].

Using obtained chemical shifts and their deviations presented in Table S1 (SI), it was possible to construct Figures 5, 6 and 7 showing dislocation of all ^{1}H-NMR signals with addition of salt to the (EL+water) and addition of ethyl lactate to the (salt+water) mixture. Mixtures containing different salts showed similar trends. Addition of a salt to the (ethyl lactate + water) mixture resulted in negative chemical shifts for all hydrogens of ethyl lactate and water (Figures 5a), 6a) and 7a)), indicating that interactions between ethyl lactate and water molecules become less attractive with addition of salt.

It can be also observed that all hydrogen signals belonging to ethyl lactate (designated as f, h, h and i in Fig.1) dislocate equally toward lower negative chemical shift values, suggesting that salt-anion and ethyl lactate do not interact directly, but through mediation of their hydrated cores. The consequence of this interaction is transfer of some electron density uniformly to ethyl lactate resulting also in uniform dislocation of chemical shifts of its H-atoms.

For systems containing trisodium citrate and disodium succinate, further addition of ethyl lactate at higher concentration (1.2 mol/kg) does not contribute to further increase of dislocation of water and salt shifts in respect to shifts of ethyl lactate at lower concentration (0.3 mol/kg) (see Figures 5b) and 7b)). Different trend was observed for tartrate salt (Fig.6b)) – further increase of ethyl lactate concentration resulted in further increase of positive shifts. This might be because, on the contrary to citrate and succinate anions, tartrate anion possess two –OH groups capable to form stronger hydrogen bonds with water.

4. Conclusions

Formation of aqueous biphasic system based on environmentally friendly solvent (ethyl lactate) was reported in this work for the first time. Could points of the ternary systems composed of water, ethyl lactate and salt (trisodium citrate or disodium tartrate or disodium succinate) along with corresponding tie-lines were experimentally determined at 298K. In addition, partition coefficients of biologically active compounds, namely caffeine and catechin were reported, showing favourable separation potential. Observations of phase behaviour and results of ^{1}H-NMR spectroscopy revealed the molecular mechanism of phase splitting.

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Table 1.Cloud points and tie-line equilibrium data for the ternary system containing ethyl lactate (EL), water (wat) and salt (trisodium citrate, disodium tartrate or disodium succinate) at 298.2 K and at 0.1 MPa. x_{EL}, x_{wat} and x_{salt} stand for mass fractions of ethyl lactate, water and salt, respectively, while TLL is tie-line length calculated according to Eq.(6).[a]

	x_{EL}	x_{wat}	x_{salt}	*TLL*	x_{EL}	x_{wat}	x_{salt}	*TLL*	x_{EL}	x_{wat}	x_{salt}	*TLL*
	ethyl lactate + water + trisodium citrate				ethyl lactate + water + disodium tartrate				ethyl lactate + water + disodium succinate			
Cloud points	0.727	0.268	0.005		0.664	0.323	0.013		0.806	0.186	0.008	
	0.610	0.373	0.017		0.516	0.442	0.042		0.635	0.337	0.028	
	0.478	0.481	0.041		0.431	0.510	0.059		0.531	0.423	0.046	
	0.385	0.549	0.066		0.333	0.581	0.086		0.409	0.518	0.073	
	0.310	0.593	0.097		0.299	0.598	0.103		0.301	0.596	0.103	
	0.254	0.631	0.115		0.248	0.629	0.123		0.220	0.649	0.131	
	0.169	0.679	0.152		0.153	0.682	0.165		0.214	0.654	0.132	
	0.111	0.693	0.196		0.160	0.678	0.162		0.143	0.686	0.171	
	0.086	0.705	0.209		0.114	0.682	0.204		0.124	0.677	0.199	
	0.059	0.688	0.253		0.08	0.692	0.228		0.105	0.686	0.209	
	0.034	0.690	0.276		0.069	0.657	0.274					
Tie- lines	0.608	0.375	0.017		0.597	0.38	0.023		0.602	0.368	0.030	
	0.300	0.569	0.131	0.553	0.300	0.57	0.130	0.501	0.280	0.594	0.126	0.431
	0.090	0.700	0.210		0.126	0.681	0.193		0.189	0.658	0.153	
	0.740	0.255	0.005		0.673	0.313	0.014		0.687	0.294	0.019	
	0.350	0.501	0.149	0.755	0.35	0.51	0.14	0.669	0.300	0.57	0.130	0.564
	0.032	0.701	0.267		0.05	0.693	0.257		0.145	0.681	0.174	
	0.676	0.315	0.009		0.648	0.335	0.017		0.742	0.244	0.014	
	0.320	0.540	0.140	0.657	0.33	0.534	0.136	0.613	0.32	0.545	0.135	0.662
	0.059	0.705	0.236		0.074	0.693	0.233		0.106	0.698	0.196	

[a] Standard uncertainties *u* are: $u(T) = \pm 0.1$ K, $u(x) = \pm 0.003$, $u(p) = \pm 1$ kPa.

Table 2. Parameters A, B and C of equation (1) fitted to the experimental cloud points at 298.2 K and at 0.1 MPa. Parameters σ, r^2 and AAD stand for standard error, correlation coefficient and absolute average deviations (Eq.(2)), respectively.[a]

$A \pm \sigma$	$B \pm \sigma$	$C \pm \sigma$	r^2	AAD / %
		ethyl lactate + water + trisodium citrate		
0.940 ± 0.018	-3.389 ± 0.114	85.87 ± 8.47	0.9984	7.0
		ethyl lactate + water + disodium tartrate		
1.037 ± 0.054	-3.655 ± 0.259	69.86 ± 14.94	0.9921	8.1
		ethyl lactate + water + disodium succinate		
1.141 ± 0.048	-3.700 ± 0.245	97.22 ± 20.39	0.9938	7.6

[a] Standard uncertainties u are: $u(T) = \pm 0.1$ K, $u(p) = \pm 1$ kPa.

Table 3. Partition coefficients of caffeine (K_{caffeine}) and catechin (K_{catechin}) in biphasic mixtures composed of ethyl lactate, water and salt, where salt is either trisodium citrate (Na_3citrate) or disodium tartrate (Na_2tartrate) or disodium succinate (Na_2succinate) at 298 K and at 0.1 MPa. The initial concentration of both solutes (caffeine or catechin) in water was 0.195 mass% in all partitioning experiments. x_{EL} and x_{salt} stand for mass fractions of ethyl lactate and salt in initial ternary mixture (feed), respectively.[a]

Salt	System	mass fraction in the feed		K_{caffeine}	$EE_{caffeine}$ %	K_{catechin}	$EE_{catechin}$ %	pH	
		x_{EL}	x_{salt}					top phase	bottom phase
Na_3citrate	1	0.40	0.09	6.4	91.3	10.6	94.4	7.07	7.13
	2	0.50	0.09	4.4	91.1	16.1	95.8	7.05	7.09
	3	0.40	0.13	3.4	79.7	3.0	75.2	7.37	7.38
Na_2tartrate	1	0.40	0.09	2.8	83.6	6.0	91.6	6.40	6.78
	2	0.50	0.09	3.6	88.2	6.4	95.7	6.47	6.58
	3	0.40	0.13	3.6	82.1	28.6	96.5	6.40	6.45
Na_2succinate	1	0.40	0.09	2.9	70.4	2.6	71.1	7.25	7.25
	2	0.50	0.09	1.6	78.6	8.5	93.2	7.45	7.37
	3	0.40	0.13	3.5	79.9	6.5	87.7	7.37	7.34

[a] Standard uncertainties u are: $u(T) = \pm 0.1$ K, $u(K) = \pm 0.1$, $u(EE\%) = \pm 0.7$, $u(p) = \pm 1$ kPa.

Caption to Figures

Figure 1. Designation of hydrogen atoms of compounds whose chemical shift was analysed by ^{1}H-NMR.

Figure 2. Cloud-point data for the ternary solutions containing ethyl lactate (EL), water and salt at 298.2 K and at 0.1 MPa, in mass fractions: trisodium citrate (filled triangles), disodium tartrate (filled circles) and disodium succinate (filled squares). Solid curvespresent fittings obtained by equation (1) using parameters given in Table 1.

Figure 3. Cloud point curves (solid line) and corresponding tie-lines (dash line) for ternary solutions containing ethyl lactate (EL), water and salt at 298.2 K and at 0.1 MPa: trisodium citrate (a), disodium tartrate (b), and disodium succinate (c). Solid curves present fittings obtained by equation (1) using parameters given in Table 1.

Figure 4. Partition coefficients of caffeine K_{caffeine} (a), and catechin K_{catechin} (b), in biphasic systems composed of ethyl lactate, water and salt (trisodium citrate, disodium tartrate and disodium succinate) at 298 K and at 0.1 MPa, for different initial compositions (in mass fraction): 1) $x_{\text{EL}} = 0.40$, $x_{\text{salt}} = 0.09$ (black); 2) $x_{\text{EL}} = 0.50$, $x_{\text{salt}} = 0.09$ (grey); 3) $x_{\text{EL}} = 0.40$, $x_{\text{salt}} = 0.13$ (white). The initial concentration of both solutes (caffeine or catechin) in water was 0.195 mass% in all partitioning experiments.

Figure 5. ^{1}H-NMR chemical shift deviations for ternary system composed of ethyl lactate, water and trisodium citrate (Na_3citrate): a) Effect of addition of Na_3citrate to the (ethyl lactate + water) mixture ($\Delta\delta_{H,1}$); and b) Effect of addition of ethyl lactate to the (Na_3citrate + water) mixture ($\Delta\delta_{H,2}$). Shifts b (grey circles) and c (minus) belong to water and Na_3citrate ($-C\underline{H_2}COO^-$), respectively, while shifts f (empty squares), g (empty triangle), h (plus) and i (empty diamonds) belong to H atoms of ethyl lactate as designated in Figure 1.

Figure 6. ^{1}H-NMR chemical shift deviations for ternary system composed of ethyl lactate, water and disodium tartrate (Na_2tartrate): a) Effect of addition of Na_2tartrate to the (ethyl lactate + water) mixture ($\Delta\delta_{H,1}$); and b) Effect of addition of ethyl lactate to the (Na_2tartrate + water) mixture ($\Delta\delta_{H,2}$). Shifts b (grey circles) and c (minus) belong to water and Na_3citrate (-$C\underline{H_2}COO^-$), respectively, while shifts f (empty squares), g (empty triangle), h (plus) and i (empty diamonds) belong to H atoms of ethyl lactate as designated in Figure 1.

Figure 7. ^{1}H-NMR chemical shift deviations for ternary system composed of ethyl lactate, water and disodium succinate (Na_2succinate): a) Effect of addition of Na_2succinate to the (ethyl lactate + water) mixture ($\Delta\delta_{H,1}$); and b) Effect of addition of ethyl lactate to the (Na_2succinate + water) mixture ($\Delta\delta_{H,2}$). Shifts b (grey circles) and c (minus) belong to water and Na_3citrate (- $C\underline{H_2}COO^-$), respectively, while shifts f (empty squares), g (empty triangle), h (plus) and i (empty diamonds) belong to H atoms of ethyl lactate as designated in Figure 1.

Figure 1

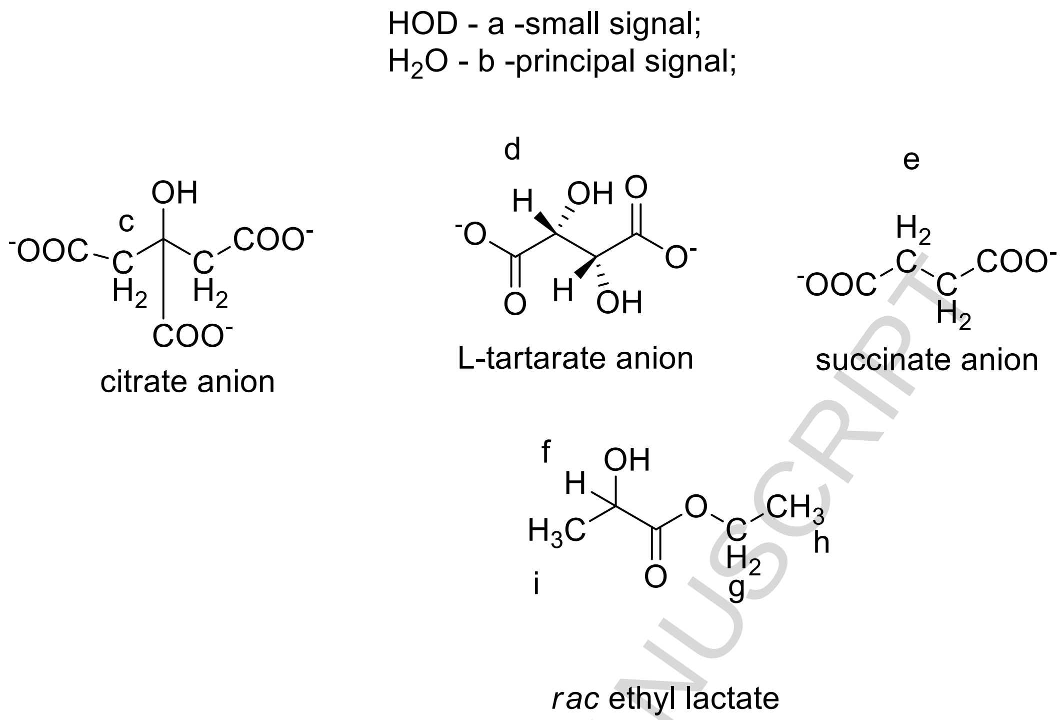
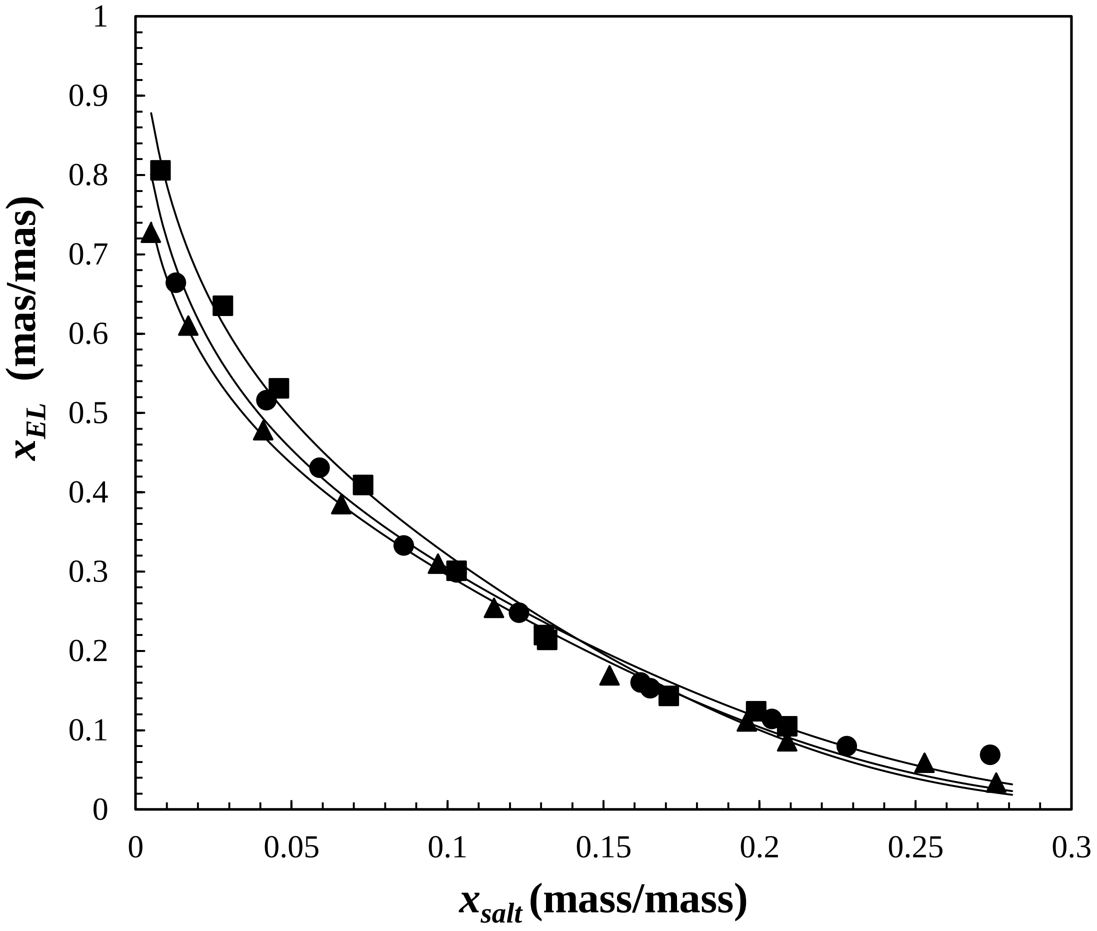

HOD - a -small signal;
H$_2$O - b -principal signal;
c
citrate anion
d
L-tartarate anion
e
succinate anion
f
g
h
i
rac ethyl lactate

Figure 2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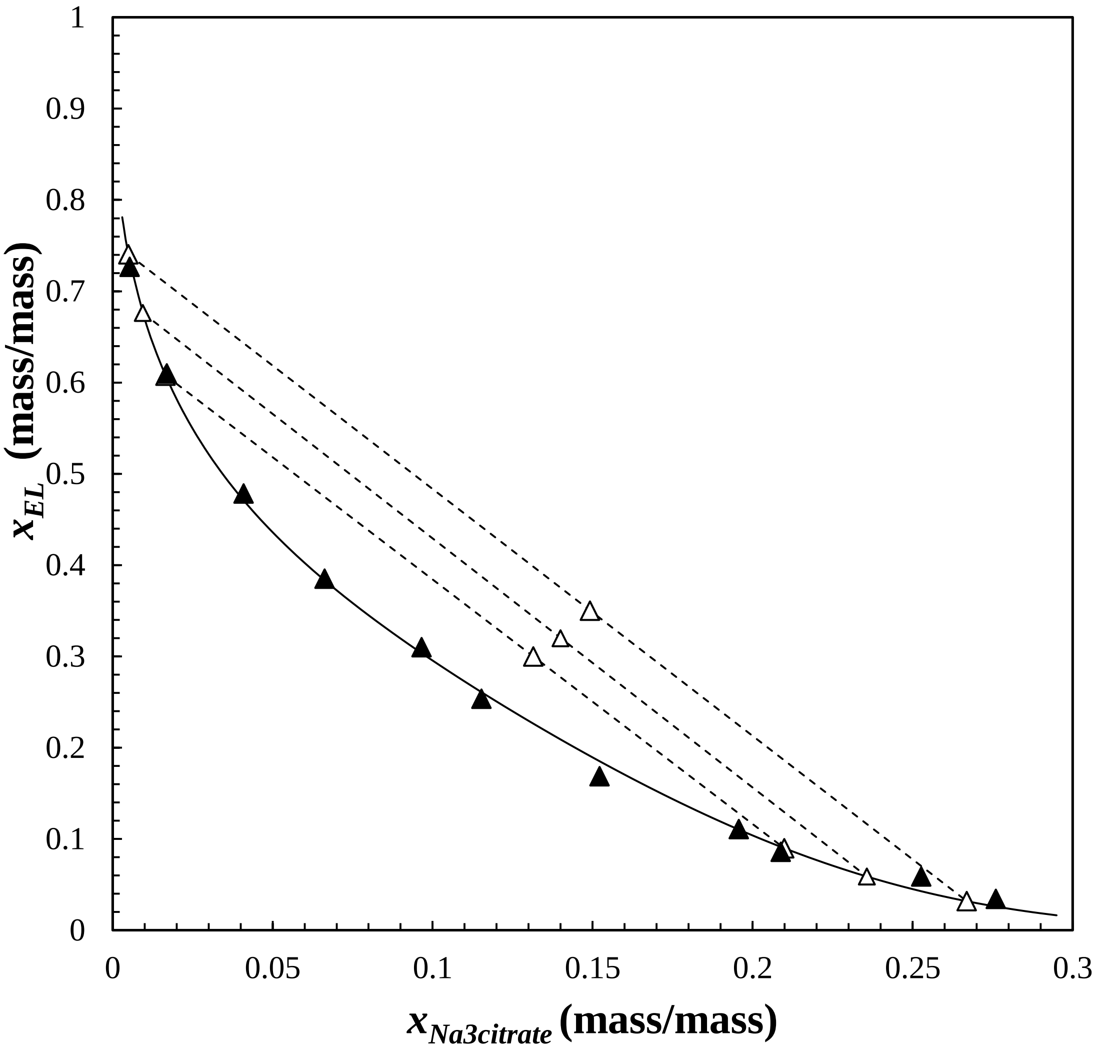
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.05
0.1
0.15
0.2
0.25
0.3
x_{EL} (mas/mas)
x_{salt} (mass/mass)

Figure 3 a)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0.7
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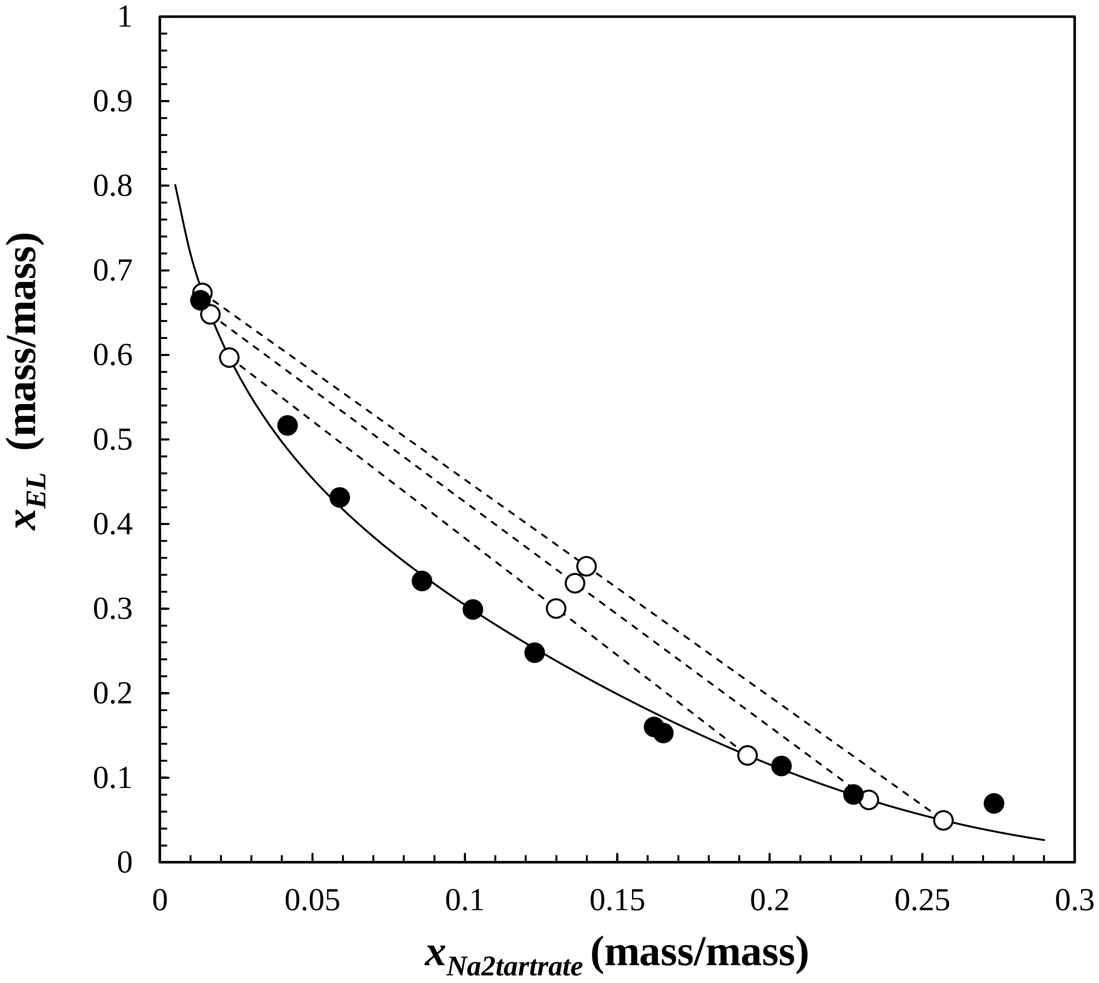
0.5
0.4
0.3
0.2
0.1
0
0
0.05
0.1
0.15
0.2
0.25
0.3
x_{EL} (mass/mass)
$x_{Na3citrate}$ (mass/mass)

Figure 3 b)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0.7
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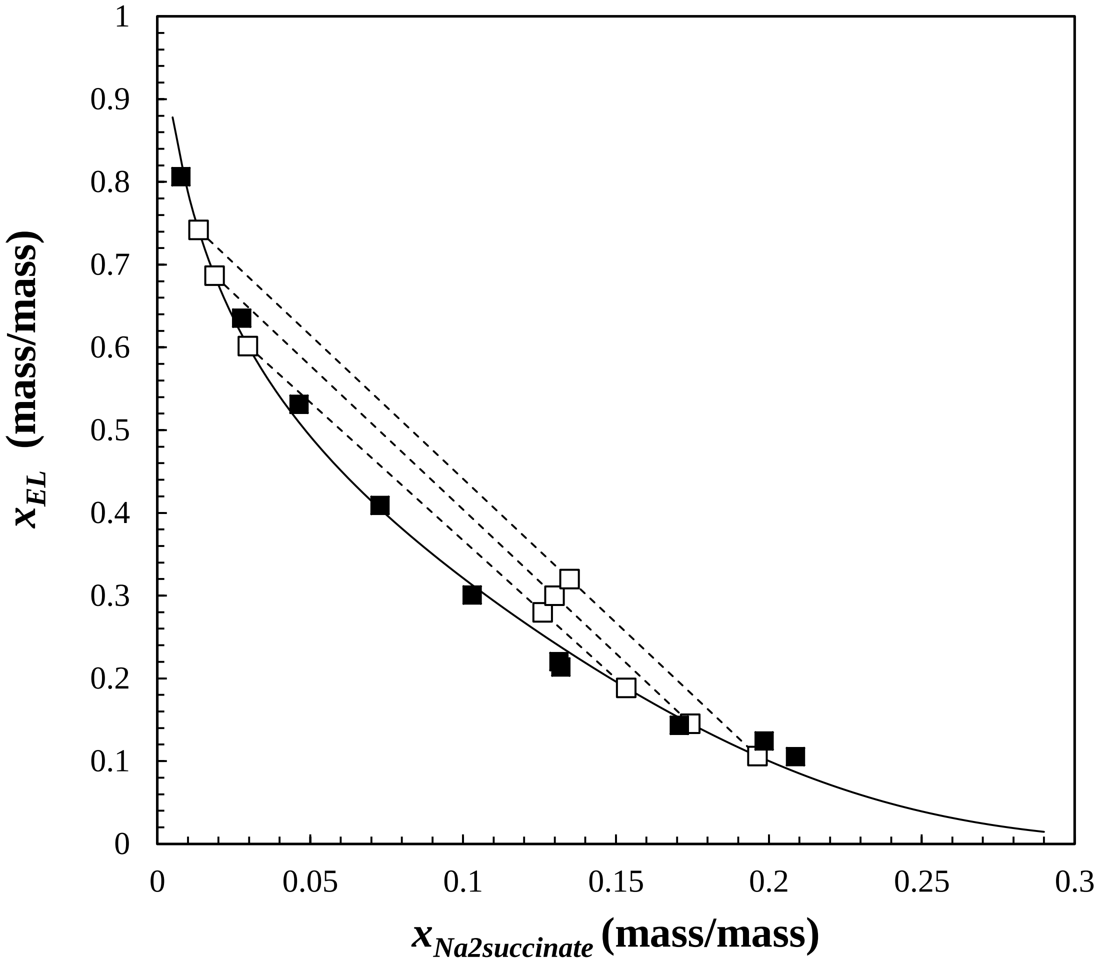
0.5
0.4
0.3
0.2
0.1
0
0
0.05
0.1
0.15
0.2
0.25
0.3
x_{EL} (mass/mass)
$x_{Na2tartrate}$ (mass/mass)

Figure 3c)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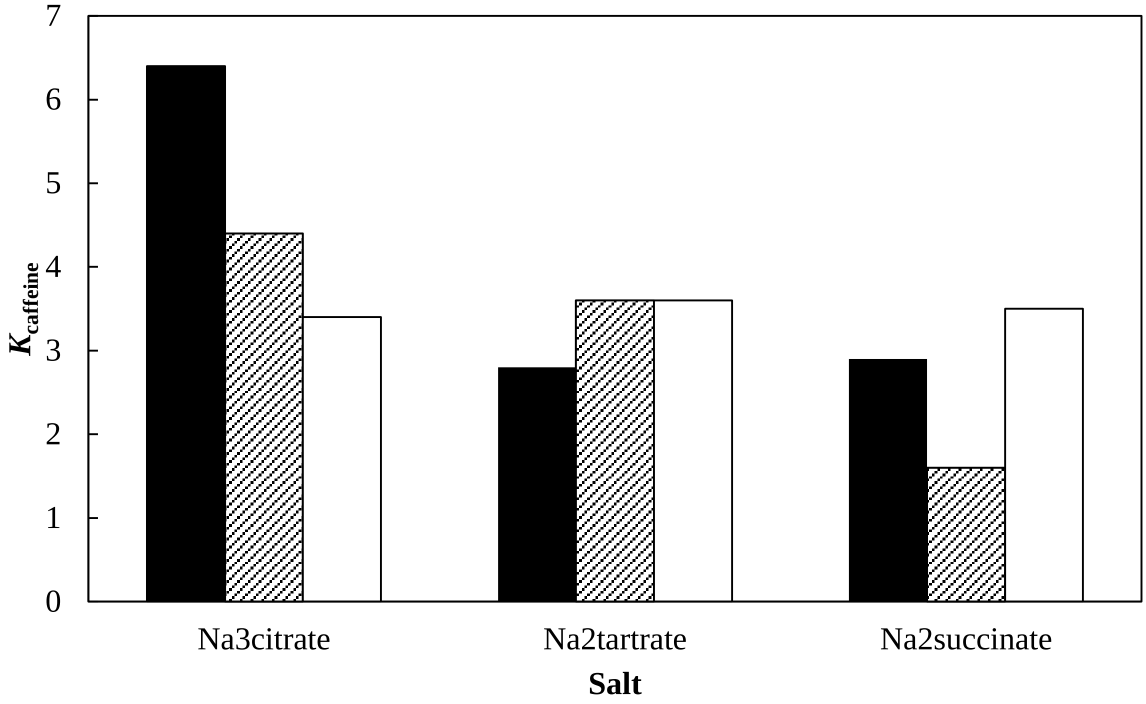
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.05
0.1
0.15
0.2
0.25
0.3
x_{EL} (mass/mass)
$x_{Na2succinate}$ (mass/mass)

Figure 4

a)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6
5
4
3
2
1
0
Kcaffeine
Na3citrate
Na2tartrate
Na2succinate
Salt

b)

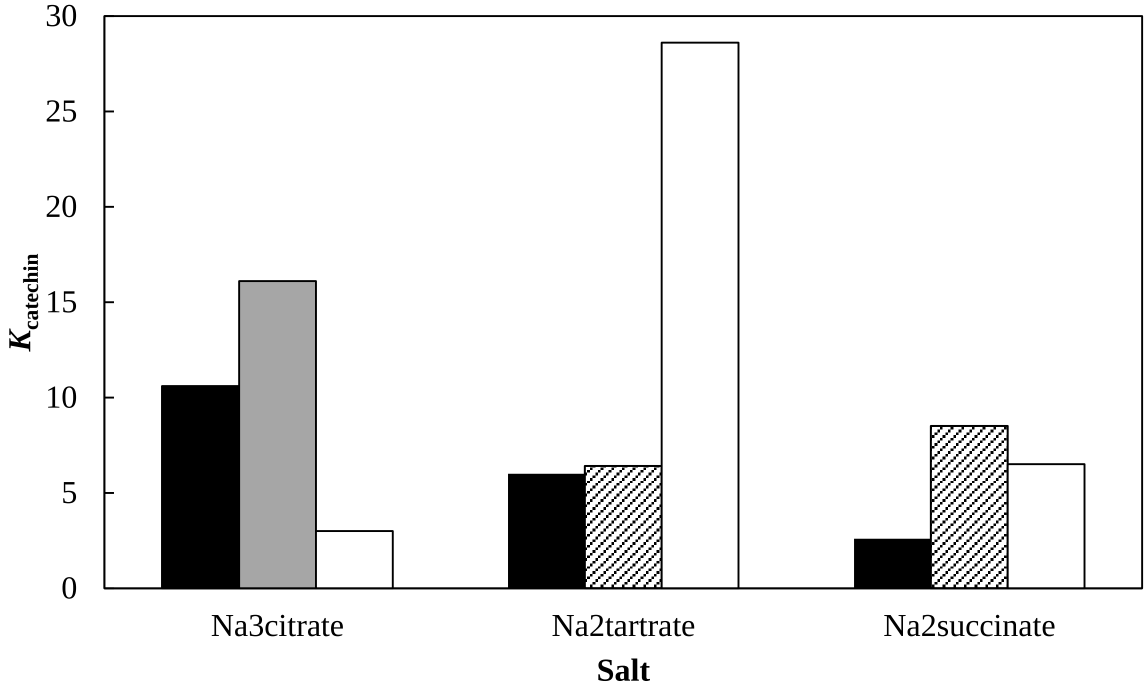
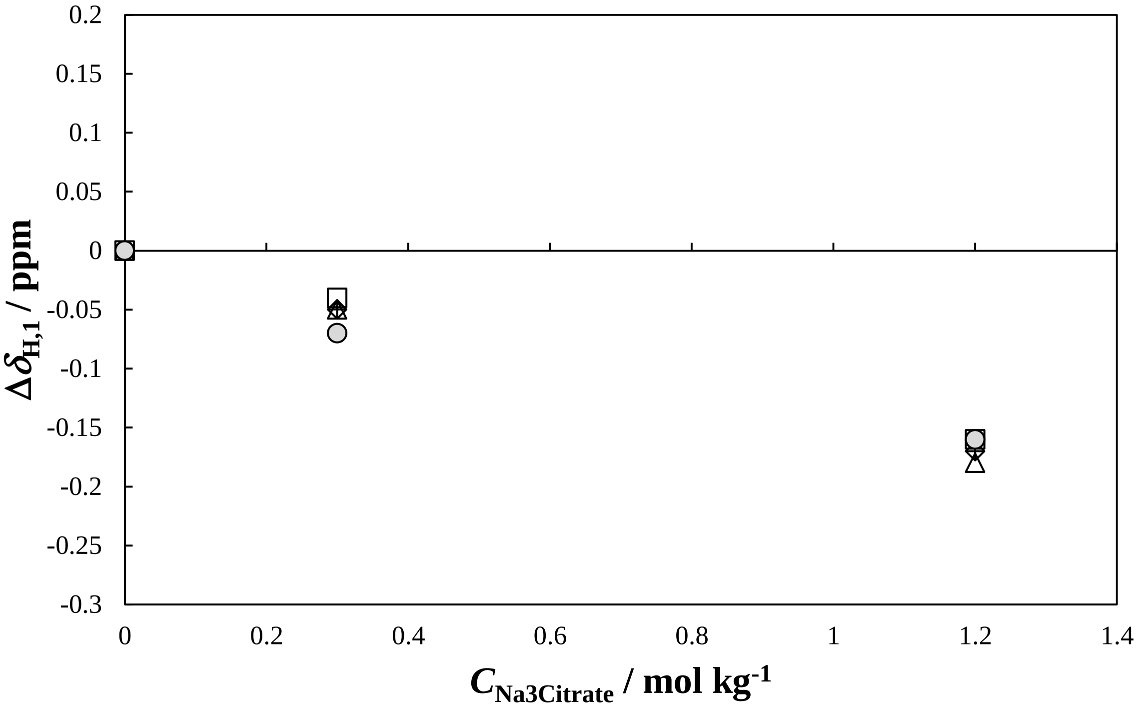

30
25
20
15
10
5
0
Kcatechin
Na3citrate
Na2tartrate
Na2succinate
Salt

Figure 5

a)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0.15
0.1
0.05
0
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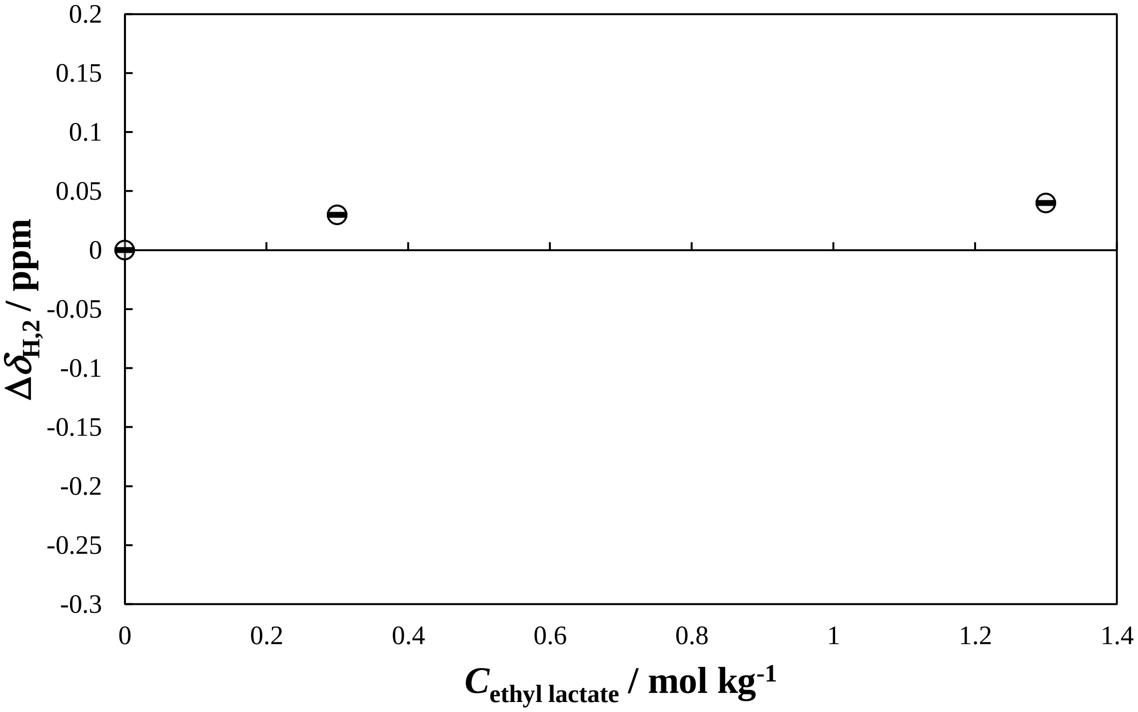
-0.15
-0.2
-0.25
-0.3
0
0.2
0.4
0.6
0.8
1
1.2
1.4
$\Delta\delta_{H,1}$ / ppm
$C_{Na3Citrate}$ / mol kg^{-1}

b)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0.1
0.05
0
-0.05
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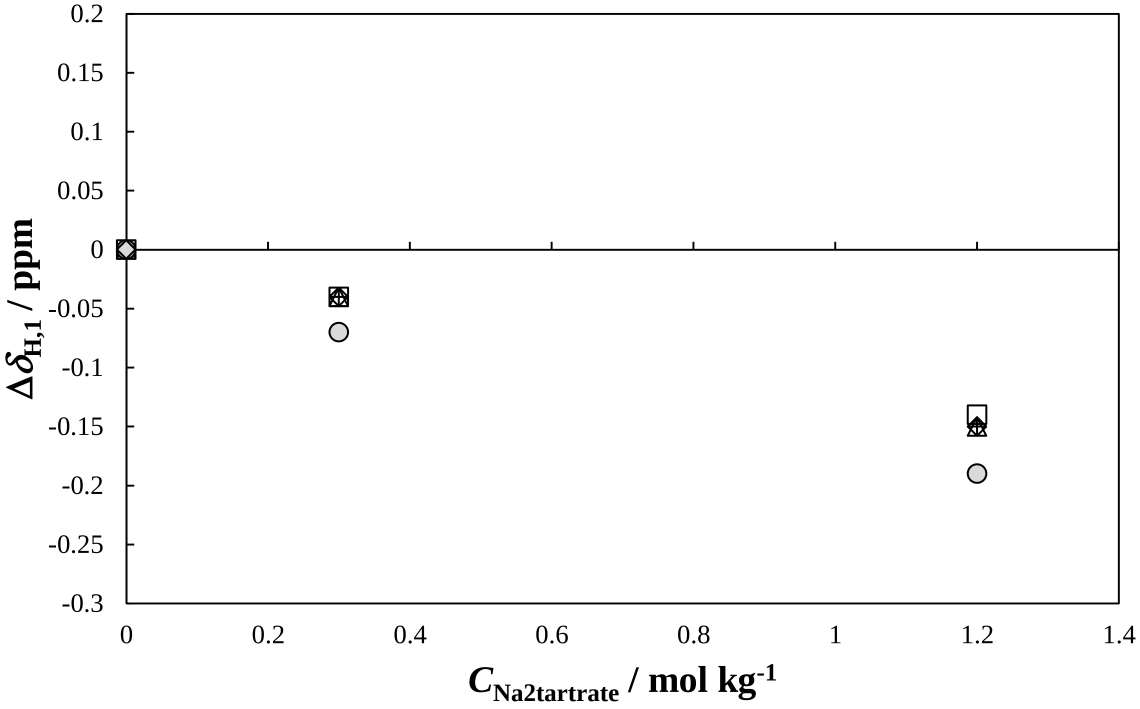
-0.15
-0.2
-0.25
-0.3
0
0.2
0.4
0.6
0.8
1
1.2
1.4
$\Delta\delta_{H,2}$ / ppm
$C_{ethyl\ lactate}$ / mol kg^{-1}

Figure 6

a)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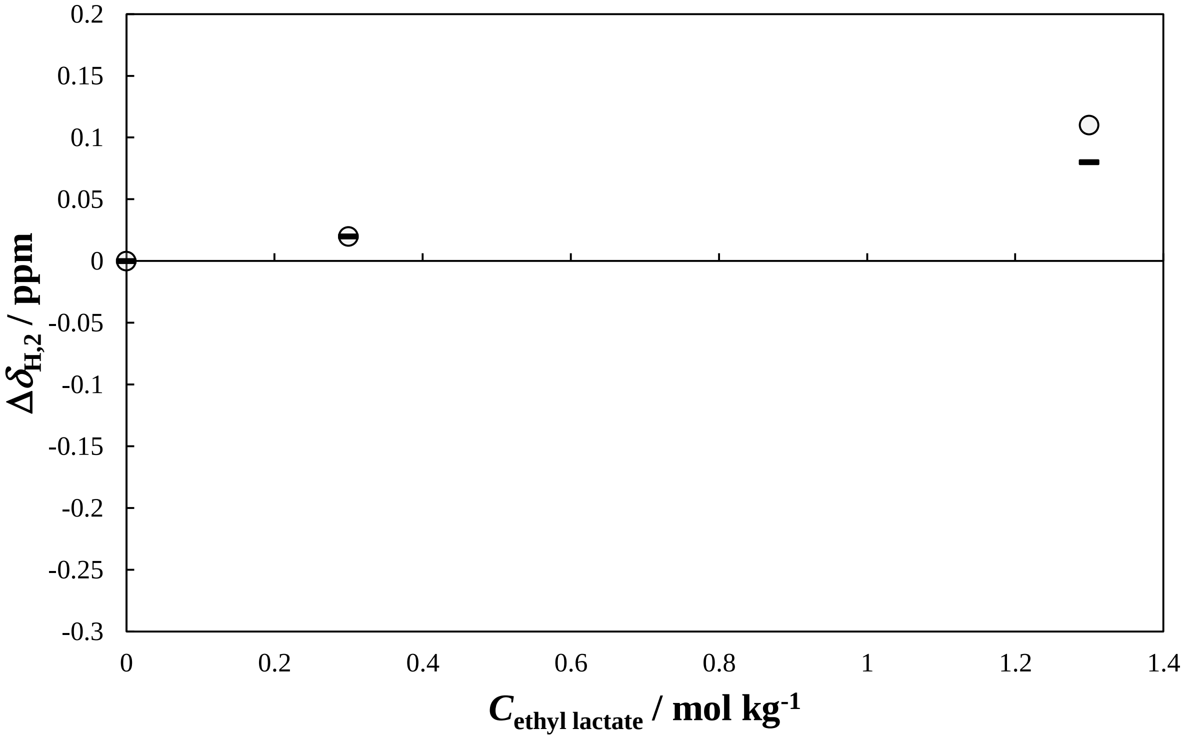
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
Δδ_H,1 / ppm
0
0.2
0.4
0.6
0.8
1
1.2
1.4
C_Na2tartrate / mol kg-1

b)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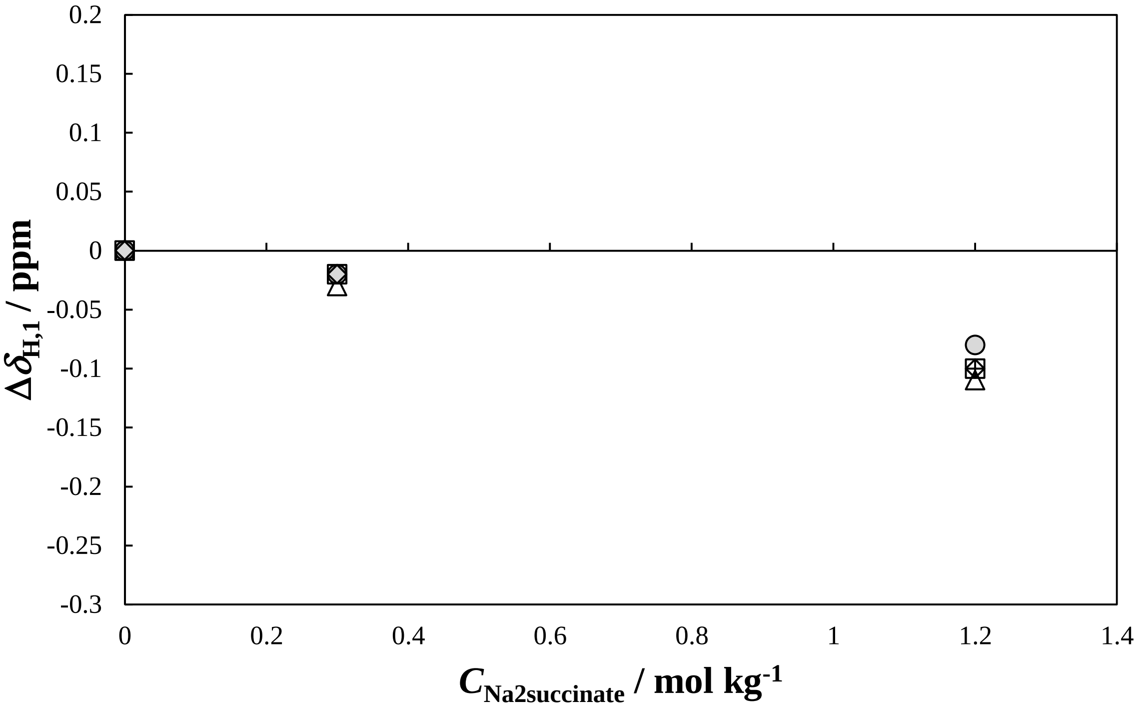
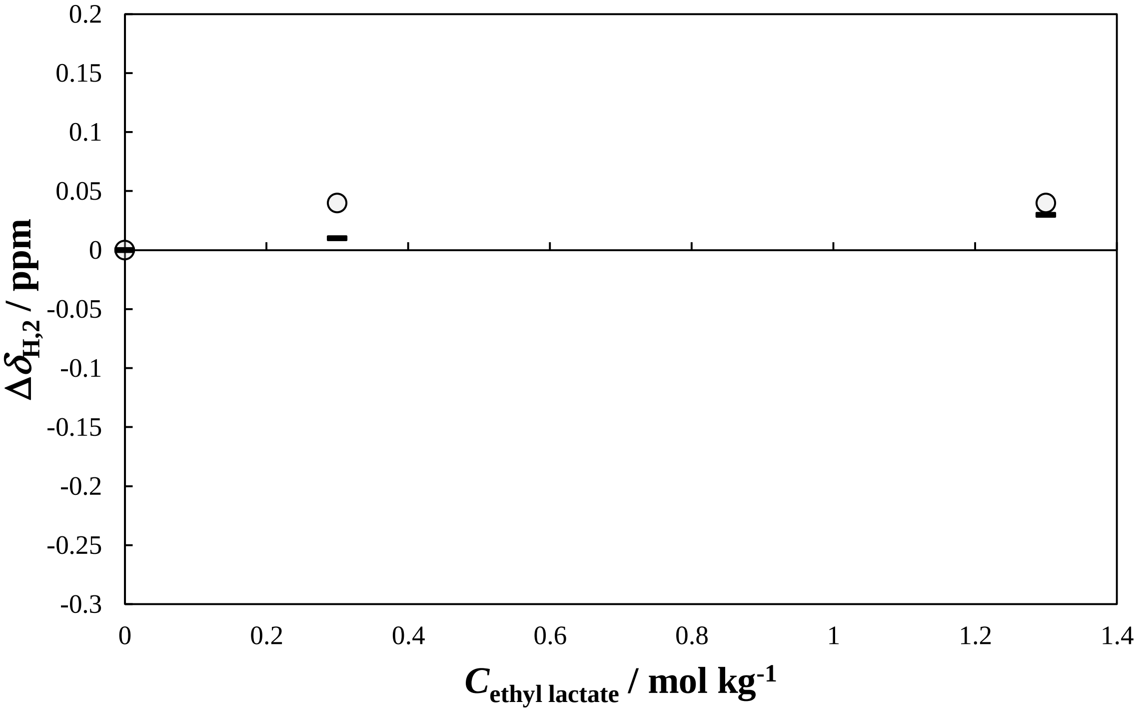
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
Δδ_H,2 / ppm
0
0.2
0.4
0.6
0.8
1
1.2
1.4
C_ethyl lactate / mol kg-1

Figure 7

a)


0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
$\Delta\delta_{H,1}$ / ppm
0
0.2
0.4
0.6
0.8
1
1.2
1.4
$C_{Na2succinate}$ / mol kg^{-1}

b)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0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
$\Delta\delta_{H,2}$ / ppm
0
0.2
0.4
0.6
0.8
1
1.2
1.4
$C_{ethyl\ lactate}$ / mol kg^{-1}

Graphical abstracts

Aqueous biphasic mixtures based on ethyl lactate are promising systems for sustainable separations.

Highlights

- Novel green platform for extraction of various compounds from aqueous solutions.
- Ethyl lactate aqueous solutions form aqueous biphasic systems upon salt addition.
- ^{1}HNMR analysis has reveal molecular interactions.
- Measured partition coefficients show favourable separation potential.

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