Extraction of antibiotics using aqueous two-phase systems based on ethyl lactate and thiosulphate salts

Małgorzata E. Zakrzewska[1,2], Ana V. M. Nunes[2], Aarti R. Barot[3], Alfred Fernández-Castané[3], Zoran P. Visak[3], Worapon Kiatkittipong[4] and Vesna Najdanovic-Visak[3*]

[1] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049–001 Lisbon, Portugal

[2] LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal

[3] Chemical Engineering and Applied Chemistry, Energy & Bioproducts Research Institute, Aston University, Birmingham B4 7ET, United Kingdom.

[4] Department of Chemical Engineering, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakhon Pathom 73000, Thailand.

* corresponding author: v.najdanovic@aston.ac.uk

Abstract

Ethyl lactate is a hydrophilic solvent produced from bio-renewable sources (bioethanol and lactic acid produced from corn fermentation) that is considered a “green” solvent due to its extremely low toxicity, biodegradability and negligible eco-toxicity.

This work focuses on the utilization of ethyl lactate to form aqueous two-phase systems (ATPS) in the presence of inorganic salts for the extraction of antibiotics from aqueous solutions. The performance of three thiosulfate salts ($Na_2S_2O_3$, $K_2S_2O_3$ and $(NH_4)_2S_2O_3$) as salting-out media for the extraction of chloramphenicol and tetracycline 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 atmospheric pressure (0.1 MPa) and 298.2 K. Partition coefficients of chloramphenicol and tetracycline between the two phases were determined by chemical analysis of phases in equilibrium for different initial compositions at 298.2 K. This paper is the first report to demonstrate the ability of the ATPS based on ethyl lactate to efficiently separate antibiotics. Thus, ATPS based on ethyl lactate represents a new and green platform for the extraction of antibiotics from aqueous solutions which can facilitate their detection, identification and quantification in surface waters as well as their extraction from fermentation broths.

Keywords: antibiotic extraction, environmental monitoring, liquid-liquid equilibria, ATPS

1. Introduction

Between 2000 and 2015, global antibiotic consumption increased by 65%, from 21.1 to 34.8 billion defined daily doses (DDDs), while the antibiotic consumption rate increased by 39%, from 11.3 to 15.7 DDDs per 1000 inhabitants per day, with different trends observed for high-income and low- and middle-income countries [1,2,3].

The extensive and often excessive administration of antibiotics has led to serious environmental problems. Their residues are discharged into wastewater treatment plants through urine and faeces, not to mention the improper disposal of wastes associated with pharmaceutical manufacturing in the first place [**Error! Bookmark not defined.**]. Even in trace amounts, antibiotics may cause the development, spread, and persistence of antibiotic-resistant strains, leading to a public health emergency [4]. Wastewater treatment plants (WTP) are considered as one of the main 'hotspots' of the potential evolution of antibiotic resistance into the environment [5,6]. Never designed to deal with pharmaceutical compounds, the conventional sewage treatment facilities are incapable of effective removal of antibiotics. Therefore, the development of advanced technology is of utmost importance to ensure availability and sustainable management of waters, as pointed out in the sixth goal of the United Nations 2030 Agenda for Sustainable Development [7].

The final fate of antibiotics is as relevant as their sustainable and cost-effective production process. Currently, antibiotics are in second place, just after ethanol, in the world ranking for fermentation-derived fine chemicals [8]. The last phase of the biosynthetic pathway involves the recovery of antibiotics from fermentation broths, whereby these are present at very low concentrations. The separation and purification steps combined account for 50 to 85% of the total bioprocessing cost [9]. Conventionally, after centrifugation and filtration of the microorganisms, antibiotics are separated by adsorption and solvent extraction and recrystallisation [10,11]. In practice, separation of antibiotics is a time-consuming and

multistep process that frequently uses harmful organic solvents and, still, results in low product yields.

Antibiotics are easy to denature and, in many cases, highly soluble in water, which makes their removal with organic agents even more challenging. Taking this into account, aqueous two-phase systems (ATPS) offer an alternative and biocompatible approach [12,13,14]. ATPS are composed to a large extent of water [12], and formed when at least two water-soluble components such as polymers, salts and ionic liquids, are mixed in water above certain concentrations. The separation of a compound of interest occurs through its partitioning between two aqueous liquid phases. ATPSs have already been successfully tested in the separation of antibiotics in mixtures containing, i.e., liquid polymers [15,16,17,18] and ionic liquids [19,20,21].

Recently, new ATPS based on ethyl lactate emerged due to being an environmentally friendly solvent with many attractive physicochemical features such as low volatility, non-corrosive and non-carcinogenic behaviour, broad liquid temperature range and low viscosity. Produced from ethanol and lactic acid, ethyl lactate is easily biodegradable and with very low toxicity, to the point, that the U.S. Food and Drug Administration (FDA) approved its use as a food and pharmaceutical additive [22].

Previous works have shown efficient separations of natural compounds, such as caffeine and catechin [23], amino acids [24] as well as rutin and quercetin [25], from aqueous solutions using ATPS based on ethyl lactate and various salts (citrate, tartrate, succinate, phosphate, dihydrogen phosphate and carbonate). In this paper, we further investigate using a systematic study the role of different extractants namely, thiosulphate salts. An additional novelty of this work consists in employing the ethyl lactate ATPS to extract antibiotics for the first time. The obtained results could further facilitate applications for both recovery of antibiotics from fermentation broths and environmental monitoring.

Previous work has shown efficient separations of natural compounds, such as caffeine and catechin, from aqueous solutions using ATPS based on ethyl lactate and various salts (citrate, tartrate, succinate, phosphate, dihydrogen phosphate and carbonate). In this paper, we continue with a systematic study of the role of different extractants, namely thiosulphate salts. Another novelty of this work was to employ the ethyl lactate ATPS to extract antibiotics for the first time. The obtained results could further facilitate applications for both recovery of antibiotics from fermentation broths and environmental monitoring.

Therefore, we report experimental cloud points and tie-lines of the ternary systems containing ethyl lactate, water and inorganic salt ($Na_2S_2O_3$, $K_2S_2O_3$ and $(NH_4)_2S_2O_3$) at 298 K and atmospheric pressure as well as partition coefficients of two antibiotics, tetracycline and chloramphenicol, between two phases at the same temperature.

2. Methodology

2.1 Materials

(±) Ethyl Lactate ($C_5H_{10}O_3$; CAS No. 97-64-3) with unknown stereoisomeric composition, potassium thiosulfate ($K_2S_2O_3$; CAS No. 10294-66-3), sodium thiosulphate pentahydrate ($Na_2S_2O_3$; CAS No. 10102-17-7), ammonium thiosulphate ($(NH_4)_2S_2O_3$; CAS No. 7783-18-8), chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$; CAS No. 56-75-7) and tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$, CAS No. 64-75-5) were purchased from either Sigma-Aldrich or Fisher Scientific. All chemicals were used without further purification. Their purities are presented in Table 1. Used water was distilled and deionized by a Milli-Q water filtration system from Millipore.

Mettler AT201 analytical balance with stated repeatability of $\pm 3\times10^{-2}$ mg was used to gravimetrically prepare all liquid mixtures. Though sodium thiosulphate pentahydrate was

used for preparation, the solution compositions are given in terms of anhydrous sodium thiosulphate.

2.2 Cloud points (binodal curves)

Cloud points of the ternary mixtures containing ethyl lactate, water, and salt ($K_2S_2O_3$ or $Na_2S_2O_3$ or $(NH_4)_2S_2O_3$) were determined by the titration method at a constant temperature of 298.2 K and at a pressure of 0.1 MPa as described in our previous work [26]. Binary mixtures containing various known compositions of salt and water were gravimetrically prepared in septum-sealed conical glass vials equipped with a magnetic stirrer and immersed in a temperature-controlled bath (Thermo Scientific) heated with SAHARA SC150 S19T circulator with a stability of 0.05 K. The transparent acrylic bath walls allowed full visualization of the mixture content. The binary mixtures were titrated with ethyl lactate very slowly at a constant temperature until the first turbidity was observed, which was taken as a cloud point. The final mixtures were weighted to calculate the composition, corresponding to the cloud point composition. Three replicates of each assay were performed to validate the experimental method and the average reproducibility of the composition of cloud points (in mole fraction) was ±0.003.

Solubility curves for three ternary systems were obtained by fitting the experimental cloud points using Merchuk's equation [27]:

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

where x_{EL} and x_{salt} are mole fractions of ethyl lactate and salt, respectively; while parameters *A*, *B*, and *C* are constants obtained by regression of the experimental cloud point data.

Standard deviations (*SD*) were used to compare the experimental data and data predicted by regression for x_{EL}:

$$SD(\%) = \sqrt{\frac{\sum(x_{EL}^{calc} - x_{EL}^{exp})^2}{NP}} \qquad (2)$$

where x_{EL}^{calc} and x_{EL}^{exp} are the calculated and experimental mole fractions of ethyl lactate, respectively; and *NP* is the number of experimental cloud points.

2.3 Tie-lines

The tie-lines of ternary systems containing ethyl lactate, water, and salt ($K_2S_2O_3$ or $Na_2S_2O_3$ or $(NH_4)_2S_2O_3$) were determined at 298.2 K. The ternary mixtures of known compositions were prepared in a 50 mL conical-bottom flask and stirred for at least 3 h at 298.2 K. To allow a complete phase separation, the mixtures were left still for at least 12 h at the same temperature. Ethyl lactate-rich (top) phase and salt-rich bottom phase were taken with a syringe into discrete vials and their masses were recorded by Mettler AT201 analytical balance with stated repeatability of ± 3×10^{-2} mg. The ratio between the mass of the top phase and the total mass of the mixture (α) was calculated according to the following equation:

$$\alpha = \frac{\text{mass of the top phase}}{\text{total mass of the mixture}} \qquad (3)$$

Equation (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:

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

$$x_{\mathrm{EL}}^{\mathrm{bot}} = A \cdot \exp\left[B \cdot \left(x_{salt}^{\mathrm{bot}}\right)^{0.5} - C \cdot \left(x_{salt}^{\mathrm{bot}}\right)^3\right] (5)$$

$$x_{\mathrm{EL}}^{\mathrm{top}} = \frac{1}{\alpha} x_{\mathrm{EL}}^{\mathrm{overall}} - \frac{1-\alpha}{\alpha} x_{\mathrm{EL}}^{\mathrm{salt-phase}} \qquad (6)$$

where superscripts “top”, “bot” and “overall” stand for mole fractions in ethyl lactate-rich phase (top), salt-rich phase (bottom) and overall mixture, respectively. Nonlinear set of

equations ((4), (5) and (6)) were solved using MATLAB software. Thus, the mole fraction compositions of ethyl lactate and salt in the top and bottom phases were obtained.

Three replicates of each assay were carried out to validate the experimental method. The average reproducibility of the composition of tie-lines (in mole fraction) was ±0.001.

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

$$TLL = \sqrt{\left(x_{\mathrm{EL}}^{\mathrm{top}} - x_{\mathrm{EL}}^{\mathrm{bot}}\right)^2 + \left(x_{salt}^{\mathrm{top}} - x_{salt}^{\mathrm{bot}}\right)^2} \qquad (7)$$

Another useful parameter is the slope of tie-lines (*STL*) which can be used for the construction of additional tie-lines:

$$STL = \frac{x_{\mathrm{EL}}^{\mathrm{top}} - x_{\mathrm{EL}}^{\mathrm{bot}}}{x_{salt}^{\mathrm{top}} - x_{salt}^{\mathrm{bot}}} \qquad (8)$$

2.4 Partition coefficients

Partition coefficients for chloramphenicol and tetracycline were measured by sampling the top and bottom phases followed by their analysis of antibiotic concentrations. The initial concentration of chloramphenicol and tetracycline in water (antibiotic feed solutions) was 0.15 g/L and 0.10 g/L, respectively. Known amounts of the antibiotic feed solution were mixed with known amounts of ethyl lactate and salt to form two-phase mixtures in a 15 ml sealed conical vial. These mixtures were agitated for at least 6 hours at a controlled temperature of 298.2 K. The temperature-controlled bath (Thermo Scientific) heated with SAHARA SC150 S19T circulator with a stability of 0.05 K was used to control the temperature. Subsequently, the mixtures were left still for at least 12 h at the same temperature to allow complete phase separation. Samples of both top and bottom phases were carefully taken using a syringe to avoid cross-contamination. These samples were analyzed

using UV-Vis Spectrophotometry (Evolution 220 UV-Vis Spectrophotometer, Thermo Fisher Scientific) at the wavelengths of 290 nm and 315 nm for tetracycline and chloramphenicol, respectively. The obtained spectra were analysed using ThermolINSIGHT Software.

The calibration curves for both the bottom and top phases were generated for systems containing different salts. Blank solutions used for calibration curves were prepared with the same composition in the top and bottom phases but without antibiotics. Mass balance was checked taking into account the overall quantity of antibiotics added and their quantities in each phase. The standard error between the actual mass and the sum of determined masses in the two phases was less than 2%.

Obtained concentrations of chloramphenicol (CH) and tetracycline (TC) in both phases were used to calculate the partition coefficients (K_{CH} and K_{TC}) according to the following equations:

$$K_{\mathrm{CH}} = \frac{C_{\mathrm{CH}}^{\mathrm{top}}}{C_{\mathrm{CH}}^{\mathrm{bot}}} \qquad (9)$$

$$K_{\mathrm{TC}} = \frac{C_{\mathrm{TC}}^{\mathrm{top}}}{C_{\mathrm{TC}}^{\mathrm{bot}}} \qquad (10)$$

where C is the concentration of either chloramphenicol (CH) and tetracycline (TC) in $g \cdot L^{-1}$ in ethyl lactate-rich (top) phase and salt-rich (bottom) phase. All experiments were performed in triplicate and results are given as average partition coefficients. The deviation from the average partition coefficient value was always within ±0.6.

3. Result and discussion

Experimental binodal points for three ternary mixtures composed of ethyl lactate, water and thiosulphate salt (either $Na_2S_2O_3$, $K_2S_2O_3$ or $(NH_4)_2S_2O_3$) are presented in Table 2. These

binodal points are also shown in Fig.1 along with the curves fitted with Merchuk's equation (Eq. (1)). The obtained constants *A*, *B*, and *C* for each mixture are presented in Table 3, showing standard deviations of 0.0068, 0.0128 and 0.0239 (in mole fractions) for mixtures containing $K_2S_2O_3$, $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$, respectively. Fig.1 allows comparison between binodal curves for systems containing different salts: The ability to provoke phase splitting in mixtures containing ethyl lactate and water follows the order: $Na_2S_2O_3$ > $K_2S_2O_3$ > $(NH_4)_2S_2O_3$. This phase segregation capacity can be explained by the Gibbs energy of hydration (ΔG_{hyd}) [28]: Cations with a higher salting-out ability have more negative ΔG_{hyd} value: Na^+ (-383.3 J mol^{-1}) > K^+ (-303.9 J mol^{-1}) > NH_4^+ > (-285.4 J mol^{-1}). The three different cations have the same charge but their radiuses are different and consequently, the number of water molecules in their hydration shell is 3.53, 2.61 and 2.43 for Na^+, K^+ and NH_4^+, respectively. Thus, sodium cation attracts a higher number of water molecules compared to K^+ and NH_4^+ resulting in a better ability to phase split the (ethyl lactate + water) mixture.

A similar trend can also be observed by analysing the tie-lines (Fig.2) and their length (*TLL*) and slope (*STL*) as shown in Table 4. The highest *TLL* values are observed for mixtures containing $Na_2S_2O_3$, followed by $K_2S_2O_3$ and $(NH_4)_2S_2O_3$. Higher *TLL* values correspond to a higher degree of separation and a bigger difference between compositions of the top and the bottom phases. For all mixtures, the biphasic region is composed of the top phase rich in ethyl lactate while the bottom phase is rich in salt and water.

Fig. 3 depicts a comparison of binodal curves between ethyl lactate and other phase forming solvents namely, ethanol [29] and polyethylene glycol 1500 g/mol (PEG1500) [30] using the same salt ($Na_2S_2O_3$). It is interesting to observe different trends in the degree of separation for mixtures with low and high salt concentrations. At low salt concentration, ATPS based on ethanol and ethyl lactate exhibit similar mutual solubilities which are significantly higher

than those obtained for the PEG ATPS. On the contrary, a more significant difference in mutual solubilities of ethyl lactate and ethanol solutions is observed for mixtures that are rich in salt content, where smaller amounts of salts are necessary to obtain phase splitting in the mixtures containing PEG and ethyl lactate. This might be due to the higher polarity of ethanol and ethyl lactate compared to PEG resulting in their stronger interactions with water which are more difficult to break.

Table 5 shows the calculated partition coefficients and extraction efficiencies for chloroform and tetracycline using different salts and compositions of ATPS. The highest partition coefficients of 9.1 and 61.0 in the ATPS with $Na_2S_2O_3$, were achieved for chloramphenicol and tetracycline, respectively. The highest obtained value of partition coefficient for tetracycline is comparable with literature values of 7 – 12 in ATPS composed of ethanol and $(NH_4)_2SO_4$ [31] and higher than 0.9 – 6.7 for ATPS composed of PEG and various cholinium-based salts [18]. In general, significantly higher partition coefficients for tetracycline were previously reported for ATPS based on ionic liquids ranging from 34 to 165 [32] and on glycerol formal ranging from 74 to 1522 [26].

The obtained partition coefficient of 9.1 for chloramphenicol is considerably higher than the values reported in the literature for the ATPS containing propanol and NaH_2PO_4 (2.4 – 6.7) [33]. Also, reported values for partition coefficient of chloramphenicol in the ATPS based on 1-hydroxylhexyl-3-methylimidazolium chloride ionic liquid with K_2CO_3 are lower than the ones reported in this work, ranging from 1.5 – 4.9. On the contrary, the ATPS containing the same ionic liquid and K_2HPO_4 exhibited higher partition coefficients of chloramphenicol (28 – 77)[34].

In terms of extraction efficiency, employing the system containing $Na_2S_2O_3$ showed the best results with the extraction efficiency of 100% for all studied compositions.

These results show that ATPS based on ethyl lactate can be used as a new and green platform for the extraction of antibiotics from aqueous solutions, with the potential to be implemented for their detection, identification and quantification in surface waters as well as their extraction from fermentation broths.

4. Conclusions

Binodal and tie-line data for the ternary (ethyl lactate + water + salt) mixtures, where salt stands for either $Na_2S_2O_3$ or $K_2S_2O_3$ or $(NH_4)_2S_2O_3$, were obtained at 298.2 K and atmospheric pressure. In general, the ability of salt to induce phase separation followed the order $Na_2S_2O_3$ > $K_2S_2O_3$ > $(NH_4)_2S_2O_3$, which was evident from both binodal data and tie-line-length. In comparison with other hydrophilic solvents reported in the literature, the degree of separation in the ATPS based on ethyl lactate lies between ATPS based on ethanol and polyethylene glycol.

Partition coefficients and extraction efficiencies for two antibiotics, chloramphenicol and tetracycline, both of industrial importance were determined for different compositions. The highest partition coefficients of 9.1 and 61.0 were achieved for chloramphenicol and tetracycline, respectively, both corresponding to 100% extraction efficiency.

This work demonstrates the ability of ethyl lactate based aqueous two-phase systems to extract antibiotics from their aqueous solutions with the potential to be employed as extraction media for both environmental monitoring and antibiotic production.

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Table 1. Chemicals used in this work

Chemical	Molecular formula	Purity (mass %)	CAS no.	Source
Ethyl lactate	$C_5H_{10}O_3$	> 98%	97-64-3	Sigma-Aldrich
Potassium thiosulfate	$K_2S_2O_3$	> 95%	10294-66-3	Sigma-Aldrich
Sodium thiosulphate	$Na_2S_2O_3$	99%	10102-17-7	Sigma-Aldrich
Ammonium thiosulphate	$(NH_4)_2S_2O_3$	96%	7783-18-8	Fisher Scientific
Chloramphenicol	$C_{11}H_{12}Cl_2N_2O_5$	98%	56-75-7	Fisher Scientific
Tetracycline hydrochloride	$C_{22}H_{24}N_2O_8 \cdot HCl$	≥ 99%	64-75-5	Sigma-Aldrich

Table 2. Binodal point data for the ternary mixture containing ethyl lactate (EL), water (wat), and salt (either $K_2S_2O_3$, $Na_2S_2O_3$ $(NH_4)_2S_2O_3$) at 298.2 K and at 0.1 MPa, in mole fraction.[a]

Ethyl lactate + water + $K_2S_2O_3$			**Ethyl lactate + water + $Na_2S_2O_3$**			**Ethyl lactate + water + $(NH_4)_2S_2O_3$**		
x_{salt}	x_{EL}	x_{wat}	x_{salt}	x_{EL}	x_{wat}	x_{salt}	x_{EL}	x_{wat}
0.006	0.287	0.707	0.011	0.117	0.872	0.006	0.397	0.597
0.008	0.208	0.784	0.018	0.056	0.926	0.011	0.197	0.792
0.012	0.150	0.838	0.027	0.026	0.947	0.017	0.147	0.836
0.015	0.118	0.867	0.034	0.007	0.959	0.020	0.113	0.867
0.016	0.105	0.879	0.051	0.007	0.942	0.024	0.089	0.887
0.023	0.067	0.910	0.057	0.004	0.939	0.029	0.066	0.905
0.026	0.050	0.924	0.070	0.004	0.926	0.032	0.043	0.925
0.030	0.035	0.935	0.008	0.165	0.827	0.037	0.030	0.933
0.033	0.029	0.938	0.014	0.091	0.895	0.048	0.028	0.924
0.037	0.019	0.944	0.022	0.044	0.934	0.054	0.027	0.919
0.044	0.016	0.940	0.040	0.005	0.955	0.062	0.014	0.924
0.049	0.019	0.932	0.005	0.226	0.769	0.065	0.011	0.924
0.058	0.011	0.931	0.003	0.440	0.557			

[a] Standard uncertainties, u, are $u(T) = 0.05$ K, $u(p) = 1$ kPa, and $u(x) = 0.003$

Table 3. Parameters *A*, *B*, and *C* of Merchuk equation (Eq. 1) fitted to the binodal points for three ternary mixtures at 298.2 K and at 0.1 MPa. *SD* stands for the standard deviation given by Eq. 2.

A	***B***	***C***	***SD***
Ethyl lactate + water + $K_2S_2O_3$			
1.344	-20.195	1361.26	0.0068
Ethyl lactate + water + $Na_2S_2O_3$			
2.027	-28.716	-3842.95	0.0128
Ethyl lactate + water + $(NH_4)_2S_2O_3$			
1.995	-20.271	-435.54	0.0239

Table 4. Tie-line equilibrium data for the ternary mixtures containing ethyl lactate (EL), water (wat), and salt (either $K_2S_2O_3$, $Na_2S_2O_3$ $(NH_4)_2S_2O_3$) at 298.2 K and at 0.1 MPa, in mole fraction.

Overall composition			**Top phase**			**Bottom phase**				
x_{salt}	x_{EL}	x_{wat}	x_{salt}	x_{EL}	x_{wat}	x_{salt}	x_{EL}	x_{wat}	***TLL***	***STL***
Ethyl lactate + water + $K_2S_2O_3$										
0.031	0.062	0.907	0.009	0.187	0.804	0.038	0.023	0.939	0.167	-5.66
0.034	0.080	0.886	0.007	0.244	0.749	0.044	0.014	0.942	0.233	-6.22
0.030	0.054	0.916	0.010	0.166	0.824	0.035	0.029	0.936	0.139	-5.48
Ethyl lactate + water + $Na_2S_2O_3$										
0.029	0.088	0.883	0.040	0.014	0.946	0.005	0.258	0.737	0.246	-6.97
0.047	0.051	0.902	0.054	0.004	0.942	0.003	0.356	0.641	0.356	-6.90
0.050	0.067	0.883	0.060	0.003	0.937	0.003	0.473	0.524	0.473	-8.25
Ethyl lactate + water + $(NH_4)_2S_2O_3$										
0.033	0.101	0.866	0.046	0.029	0.925	0.010	0.232	0.758	0.206	-5.64
0.034	0.115	0.851	0.049	0.024	0.927	0.009	0.275	0.716	0.254	-6.28
0.045	0.106	0.849	0.060	0.012	0.928	0.007	0.342	0.651	0.334	-6.23

[a] Standard uncertainties, *u*, are $u(T) = 0.05$ K, $u(p) = 1$ kPa, and $u(x) = 0.003$

Table 5. Partition coefficients (*K*) and extraction efficiencies (*EE*) of chloramphenicol (CP) and tetracycline (TC) in biphasic mixtures composed of ethyl lactate (EL), water (wat) and salt (either $K_2S_2O_3$, $Na_2S_2O_3$ $(NH_4)_2S_2O_3$) at 298.2 K and at 0.1 MPa. The initial concentrations of chloramphenicol and tetracycline in water (antibiotic feed solution) were 0.15 g/L and 0.10 g/L, respectively. $x_{\text{ant in water}}$ stands for mole fraction of the feed solution.

Feed			**Chloramphenicol**				**Tetracycline**			
x_{salt} (mol/mol)	x_{EL} (mol/mol)	$x_{\text{ant in water}}$ (mol/mol)	$C_{\text{CP}}^{\text{top}}$ (g/L)	$C_{\text{CP}}^{\text{bot}}$ (g/L)	K_{CP}	EE_{CP} (%)	$C_{\text{TC}}^{\text{top}}$ (g/L)	$C_{\text{TC}}^{\text{bot}}$ (g/L)	K_{TC}	EE_{TC} (%)
Ethyl lactate + water + $K_2S_2O_3$										
0.025	0.090	0.885	0.122	0.037	3.3	76.5	0.076	0.029	2.6	71.5
0.025	0.116	0.859	0.109	0.016	6.8	89.6	0.071	0.012	5.9	87.6
0.033	0.090	0.877	0.132	0.029	4.6	77.0	0.098	0.012	8.2	85.8
Ethyl lactate + water + $Na_2S_2O_3$										
0.035	0.090	0.875	0.073	0.08	0.9	40.5	0.12	0.017	7.1	99.8
0.035	0.172	0.793	0.091	0.010	9.1	99.9	0.061	0.001	61.0	100
0.048	0.090	0.862	0.101	0.051	2.0	48.4	0.139	0.003	46.3	100
Ethyl lactate + water + $(NH_4)_2S_2O_3$										
0.035	0.105	0.860	0.005	0.127	0.0	3.2	0.078	0.024	3.2	72.3
0.035	0.147	0.818	0.085	0.023	3.7	83.0	0.057	0.015	4.0	84.1
0.044	0.105	0.851	0.066	0.067	1.0	44.7	0.095	0.003	32.9	96.3

[a] Standard uncertainties, *u*, are $u(T) = 0.05$ K, $u(p) = 1$ kPa, $u(x) = 0.003$, $u(C_{CP}) = 0.002$ g/L, $u(C_{TC}) = 0.002$ g/L and $u(K) = 0.6$

Caption to Figures

Figure 1. Binodal curves of the ternary mixtures composed of ethyl lactate, water and salt ($K_2S_2O_3$ – red, $Na_2S_2O_3$ – green and $(NH_4)_2S_2O_3$ – blue) at 298.2 K in mole fraction. Symbols represent experimental binodal data while the solid lines correspond to the fittings obtained by Eq. (1) using constants given in Table 3.

Figure 2. Binodal curves (solid line) and corresponding tie-lines (dash line) for ternary mixtures containing ethyl lactate, water and salt at 298.2 K: $Na_2S_2O_3$ (a), $K_2S_2O_3$ (b) and $(NH_4)_2S_2O_3$ (c). Red, green and blue symbols represent experimental binodal data while white symbols correspond to experimental tie-line compositions. Solid and dashed lines correspond to fittings obtained by Eq. (1) and tie-lines, respectively.

Figure 3. Comparison of binodal curves for different ternary mixtures composed of $Na_2S_2O_3$, water and phase-forming solvent: ethyl lactate from this work (black circles), ethanol [29] (green circles) and PEG1500 [30] (red circles) at 298.2 K.

Figure 1

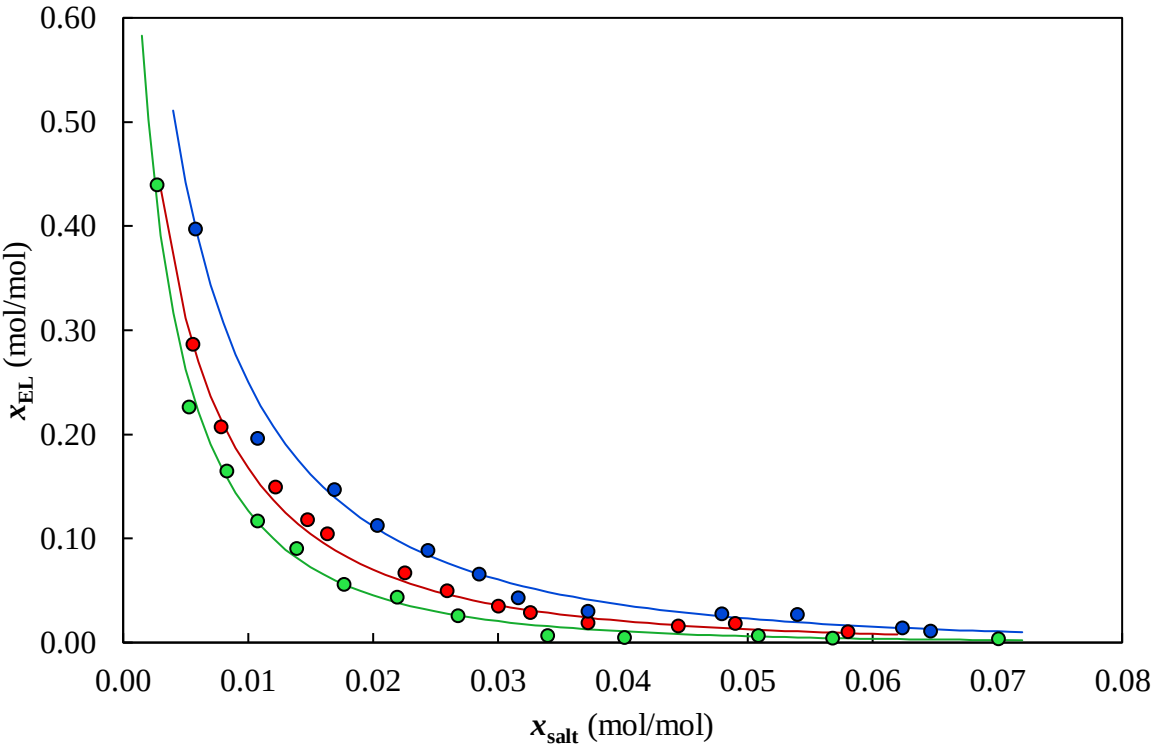

0.60
0.50
0.40
0.30
0.20
0.10
0.00
x_{EL} (mol/mol)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
x_{salt} (mol/mol)

Figure 2

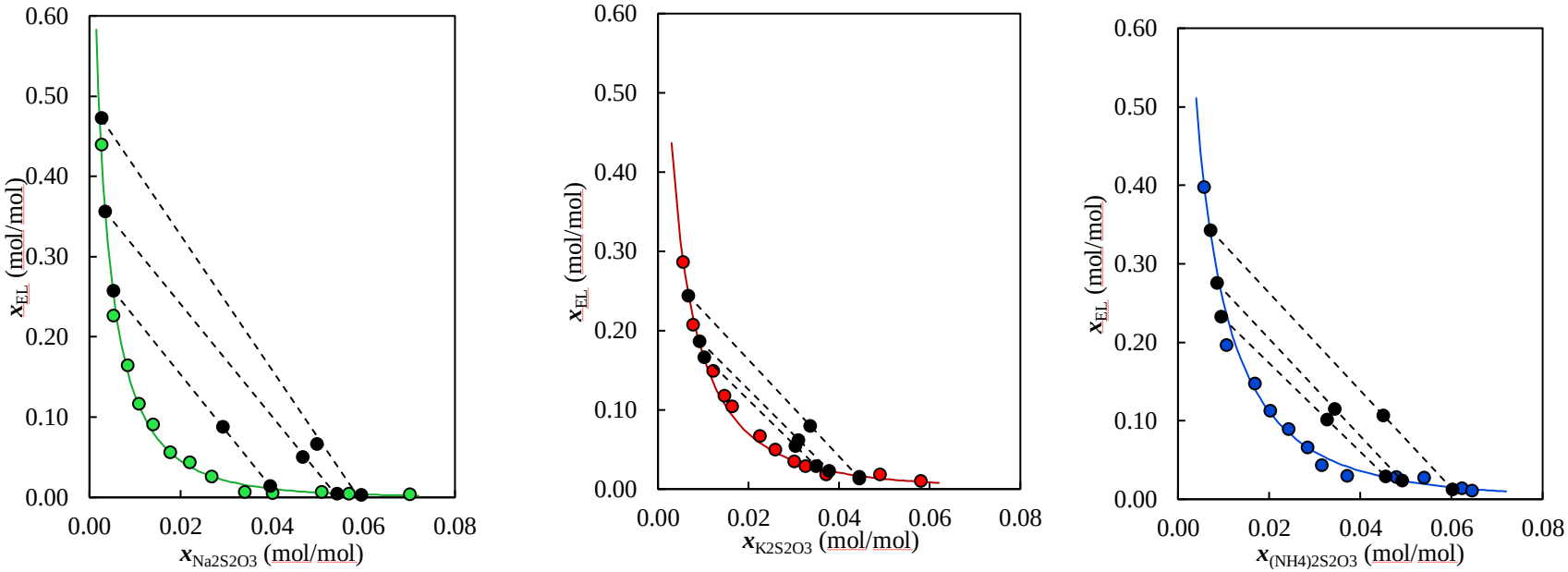
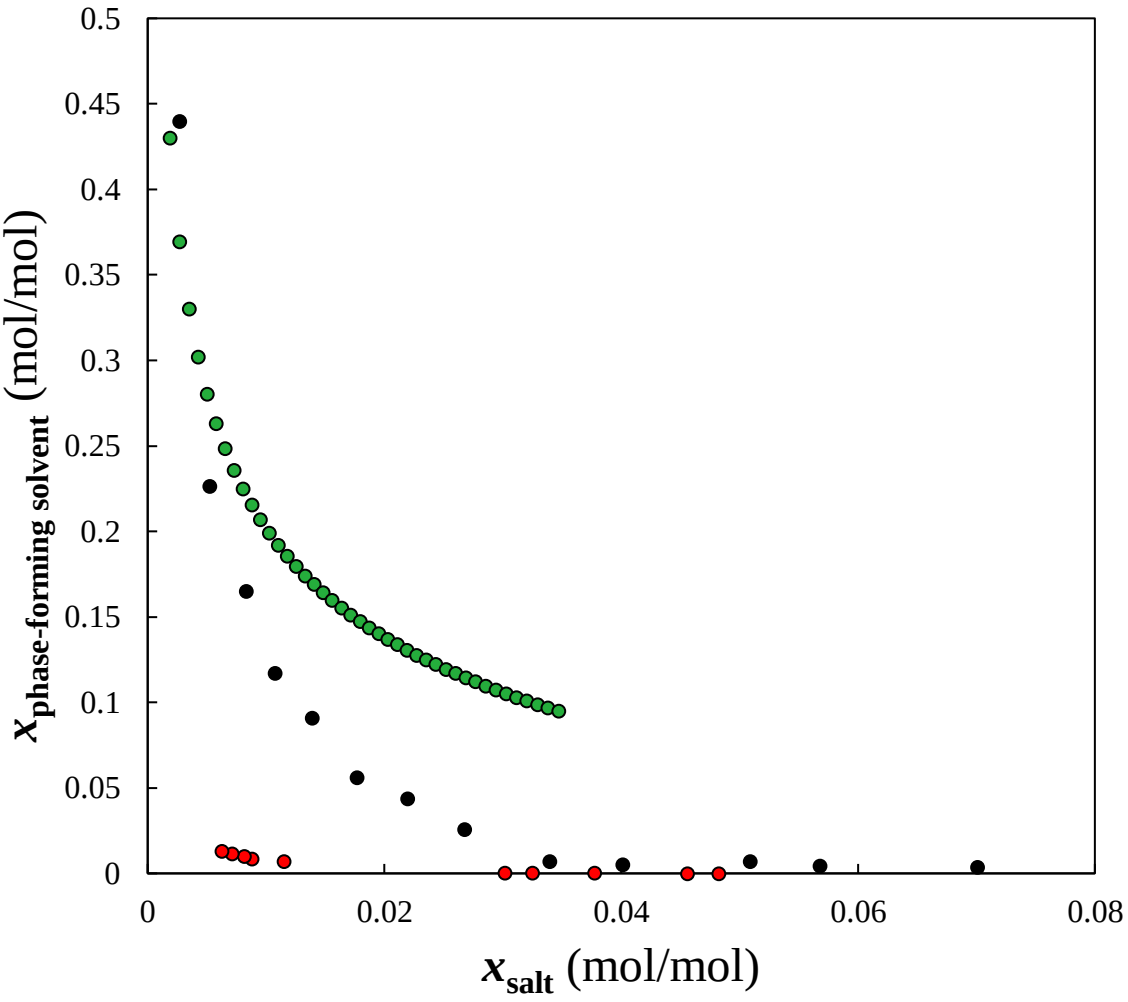

0.60
0.50
0.40
0.30
0.20
0.10
0.00
x_{EL} (mol/mol)
0.00
0.02
0.04
0.06
0.08
$x_{Na2S2O3}$ (mol/mol)
x_{K2S2O3} (mol/mol)
$x_{(NH4)2S2O3}$ (mol/mol)

Figure 3


0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.02
0.04
0.06
0.08
$x_{\text{phase-forming solvent}}$ (mol/mol)
x_{salt} (mol/mol)

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