

Liquid-liquid phase equilibria of aqueous biphasic systems based on glycerol formal: application on tetracycline recovery from water

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Abstract

Biopharmaceuticals are commonly present in relatively low concentrations in aqueous solutions, making their detection and purification detrimental. In this work, we used novel aqueous biphasic systems based on glycerol formal to extract an important antibiotic – tetracycline. We report cloud points (solubility curve) and tie-lines for three ternary systems, containing glycerol formal (GF), water and inorganic salt (either K_3PO_4 , K_2HPO_4 or K_2CO_3) at constant temperature of 298 K and at 0.1 MPa. The tie-line data of these ternary systems were correlated using the non-random two-liquid (NRTL) model and binary interaction parameters of activity coefficients were estimated. The experimental and correlated tie-line data were compared in terms of average root mean square deviation and showed satisfactory agreements. The partition coefficients of tetracycline between two phases were measured and corresponding extraction efficiencies were calculated. The maximum value of partition coefficient was 1551 for the system containing K_3PO_4 , followed by values of 1145 and 927 for systems containing K_2CO_3 , and K_2HPO_4 , respectively. The calculated extraction efficiencies were very high – greater than 98.8%, demonstrating high potential for using aqueous biphasic systems, which are based on glycerol formal, for separation and purification process.

Keywords: antibiotics, downstream recovery, purification technique, biopharmaceuticals, salting-out.

1. Introduction

Aqueous two-phase system (ATPS) or aqueous biphasic system (ABS) is a liquid-liquid extraction technique which is an alternative method for separation and purification of biological active substances from aqueous solutions. In these systems, the addition of a hydrophilic compound and salt to aqueous solution forms two-phase systems, where both phases are rich in water, offering polar and low toxicity medium for extraction of various biologically active compounds. This is of particular interest for lipophilic biopharmaceutical such as tetracycline antibiotics which have to be protonated to obtain salt derivatives with higher bioavailability and associated with their higher solubility in water. For example, the solubility of tetracycline hydrochloride (tetracycline·HCl) in water is approximately three orders of magnitude greater than that in less polar organic solvents¹. This means that the extraction of tetracycline·HCl from aqueous solutions using organic solvent is not a promising option.

Aqueous two-phase systems (ATS), formed by mixing a hydrophilic polymer (*e.g.* polyethylene glycols – PEGs or dextrin), water and inorganic salt have attracted interest of both industry and academia for many years^{2,3}, offering an efficient extraction of various biologically active substances, such as proteins, viruses, enzymes, nucleic acids and other biomolecules^{4,5}. Another alternative is to use an ionic liquid as a hydrophilic substance to form a two-phase system⁶. Due to their physicochemical properties and tunability, ionic liquids have been implemented in aqueous two-phase systems for extraction of various biologically active substances, such as proteins⁷, antioxidants⁸ and antibiotics⁹. However, commonly used ionic liquids based on imidazolium and pyridinium cations show considerable toxicity to biomolecules, microorganisms, plants and animals¹⁰ as well as low biodegradability¹¹.

In the last few years, glycerol formal, commercially available as a mixture of two 5- and 6-membered ring isomers, has attracted attention as hydrophilic solvent due to its very low toxicity¹². Glycerol formal is an industrially important compound derived from glycerol which has a massive surplus globally due to increased biodiesel demand. It is used as a solvent for various injectable preparations, for binders, in insecticide preparations as well as a solvent for inks and paints¹³. However, its utilization as a solvent for separation and reaction processes remains scarcely reported.

To the best of our knowledge, this is the first report on using glycerol formal to form aqueous biphasic systems. The performance of three inorganic salts (K_3PO_4 , K_2HPO_3 and K_2CO_3) as salting-out media for the separation of tetracycline was investigated. Tetracycline is an essential antibiotic used for veterinary purposes, human therapy and pesticide applications. Tetracycline is produced by fermentation using *Streptomyces* microorganisms, yielding an aqueous solution with low concentration of antibiotic, typically 0.2 – 2 g/L¹⁴. This low concentration requires an extensive separation and purification steps: liquid–liquid extraction, ion-exchange, reverse micellar extraction, chromatography, crystallization, etc. Thus, the focus of this work is the extraction of tetracycline antibiotic from their aqueous solutions using aqueous biphasic system based on glycerol formal and inorganic salts (K_3PO_4 , K_2HPO_4 and K_2CO_3).

2. Experimental

2.1 Materials

Glycerol formal (C₄H₈O₃; CAS No. 99569-11-6), potassium diphosphate (K₂HPO₄; CAS No.7758-11-4), potassium triphosphate (K₃PO₄; CAS No.7778-53-2), potassium carbonate (K₂CO₃; CAS No.584-08-7) and tetracycline-HCL (C₂₂H₂₄N₂O₈·HCl; CAS No.64-75-5) were purchased from Sigma Aldrich and used without further purification. Their purities are presented in Figure 1. Water was distilled and deionized using a Milli-Q water filtration system from Millipore.

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

2.2 Cloud points (solubility curve)

Solubility curves of the ternary mixtures containing glycerol formal, water and salt (either K₃PO₄ or K₂HPO₄ or K₂CO₃) were determined by the cloud point titration method at a constant temperature of 298 K at pressure of 0.1 MPa as described in our previous papers^{15,16}. Gravimetrically prepared binary mixtures, containing salt and water of known compositions, were placed in a septum-sealed conical glass vials equipped with magnetic stirrer and immersed in the temperature-controlled bath (Thermo Scientific™) heated with SAHARA SC150 S19T circulator with a stability of 0.02 K. The transparent acrylic bath walls allowed full visualization of the mixture content. The binary mixtures were titrated with glycerol formal very slowly at a constant temperature until the first turbidity was observed which was taken as a cloud point. The final mixtures were weighted in order to calculate the composition, corresponding to the cloud point composition. Three replicates of each assay were carried out in order to validate the experimental method. The average reproducibility of composition of cloud points (in mass fraction) was ± 0.001 .

Cloud points were fitted to Merchuk's equation,¹⁷ permitting construction of solubility curves:

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

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

$$AAD(\%) = \frac{1}{NP} \sum_i \frac{|x_{GF}^{calc} - x_{GF}^{exp}|}{x_{GF}^{exp}} \cdot 100 \quad (2)$$

where x_{GF}^{calc} and x_{GF}^{exp} are the calculated and experimental mass fractions of ethyl lactate, respectively, and NP is the number of available cloud points.

2.3 Determination of tie-lines

Known compositions of biphasic mixtures containing glycerol formal, water and inorganic salt (either K_3PO_4 or K_2HPO_4 or K_2CO_3) were made in a 50 mL conical-bottomed flask. The mixtures were stirred for at least three hours at 298 K. Next, the mixtures were left still for at least 12 hours at 298 K to allow a complete phase separation into top phase (glycerol formal - rich phase) and bottom phase (salt-rich phase). Using a precise syringe, the two phases were separated into discrete vials and their masses were recorded by Mettler AT201 analytical balance with stated repeatability of $\pm 3 \cdot 10^{-2}$ mg. Ratio between the mass of the top phase and the total mass of the mixture (α) was calculated according to:

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

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

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

$$x_{GF}^{\text{salt-phase}} = A \cdot \exp \left[B \cdot \left(x_{\text{salt}}^{\text{salt-phase}} \right)^{0.5} - C \cdot \left(x_{\text{salt}}^{\text{salt-phase}} \right)^3 \right] \quad (5)$$

$$x_{GF}^{\text{GF-phase}} = \frac{1}{\alpha} x_{GF}^{\text{overall}} - \frac{1-\alpha}{\alpha} x_{GF}^{\text{salt-phase}} \quad (6)$$

where superscripts “GF-phase”, “salt-phase” and “overall” stand for mass fractions in glycerol formal-rich phase, salt-rich phase and overall mixture, respectively. MATLAB software was used to solve nonlinear set of equations ((4), (5) and (6)), resulting in the mass fraction compositions of ethyl lactate and salt in the top and bottom phases.

Three replicates of each assay were carried out in order to validate the experimental method.

The average reproducibility of composition of tie-lines (in mass fraction) was ± 0.003 .

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

$$TLL = \sqrt{\left(x_{GF}^{\text{GF-phase}} - x_{GF}^{\text{salt-phase}} \right)^2 + \left(x_{\text{salt}}^{\text{GF-phase}} - x_{\text{salt}}^{\text{salt-phase}} \right)^2} \quad (7)$$

2.4 Partition coefficients

Feed solution of tetracycline in water ($1.124 \text{ g}\cdot\text{L}^{-1}$) was prepared gravimetrically. Known amounts of the feed solution, glycerol formal and inorganic salt (either K_3PO_4 or K_2HPO_4 or K_2CO_3) were mixed to obtain biphasic systems. These were stirred for at least 6 hours at 298 K and followed by a 12-hours rest period to allow a complete phase separation. Using a syringe (Hamilton, Bonaduz, Switzerland), samples from both phases were taken and prepared for further analysis to determine the composition of tetracycline by Spectrophotometry (Evolution 220 UV-Visible Spectrophotometer, Thermo Fisher Scientific). The calibration curves for both bottom and top phases were generated for systems containing different salts. Blank solutions used for calibration curves were with the same composition in the top and bottom phases but without tetracycline. Mass balance was checked taking into account overall quantity of tetracycline and its quantities in in each

phase. Standard error between the actual mass and sum of determined masses in two phases was always below 3%.

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

$$K_{TC} = \frac{C_{TC}^{GF-phase}}{C_{TC}^{salt-phase}} \quad (8)$$

where $C_{TC}^{GF-phase}$ and $C_{TC}^{salt-phase}$ are concentrations of tetracycline ($\text{g}\cdot\text{L}^{-1}$) in glycerol formal-rich (top) phase and salt-rich phase (bottom). All experiments were performed three times and results are given as average partition coefficients (observed deviations was always within ± 1).

pH of each liquid phase in equilibrium was measured at 298 K using HI 2211

Microprocessor pH meter (HANNA instruments) with precision of ± 0.01 . To this end, blank biphasic mixtures (without tetracycline) containing glycerol formal, water and salt with the same compositions as partitioning coefficient experiments were prepared and agitated, for at least 6 hours, at constant and controlled temperature of 298 K. The complete phase separation of the two liquids phases was achieved by leaving mixtures still for at least 12 hours. Samples of both phases were carefully taken and their pH was measured. The estimated uncertainty in pH was ± 0.1 .

3. Results and discussion

3.1 Experimental solubility and tie-line data

Table 2 shows experimental cloud points and tie-line compositions for mixtures containing glycerol formal, water and inorganic salt (i.e. K_3PO_4 , K_2HPO_4 or K_2CO_3) at 298 K. Cloud point data were correlated with equation (1) and the obtained constants A , B and C for each mixture are presented in Table 3, showing very good correlation coefficients (r^2) and the average absolute deviations calculated by equation (2) ranging from 3.1 to 4.2%. A

comparison between cloud point (binodal) curves for systems containing different salts is presented in Figure 1 in both mass and mole fractions. The three salts were able to form aqueous biphasic systems with glycerol formal and water. Areas above and below the binodal curves represent two-phase and single-phase regions, respectively. At their low concentrations, K_3PO_4 and K_2HPO_4 salts have similar abilities to provoke phase splitting of glycerol formal - water mixtures despite their difference in ionic forces. On the other hand, K_3PO_4 has a stronger salting-out ability than K_2HPO_4 at higher concentration. Also, K_2CO_3 is a weaker promotor of two-phase region than K_3PO_4 for the whole concentration region. It is interesting to compare performances of salts with the same number of potassium cations, K_2HPO_4 and K_2CO_3 : at low salt concentrations, much smaller quantities of K_2HPO_4 are needed to trigger phase-splitting while two salts appear to have very similar abilities at high concentrations. This clear influence of salt concentration, on how different salts performed, cannot be explained solely by the Gibbs free energy of hydration (ΔG_{hyd}), where ions with a higher salting-out ability have more negative ΔG_{hyd} value: PO_4^{3-} ($-2765 \text{ Jmol}^{-1}\text{K}^{-1}$) > HPO_4^{2-} ($-1789 \text{ Jmol}^{-1}\text{K}^{-1}$) > CO_3^{2-} ($-1476 \text{ Jmol}^{-1}\text{K}^{-1}$). In fact, other effects might play a major role, namely ion speciation as well as interactions involving glycerol formal. Obtained tie-lines data for the ternary systems containing glycerol formal, water and salt (K_3PO_4 or K_2HPO_4 or K_2CO_3) are also included in Table 4. Higher tie-line lengths (*TLL*) are observed for mixtures with higher salt contents in the bottom phase, corresponding to a bigger difference between compositions of the top and bottom phases, and by inference, a higher degree of separation.

3.2 Partition coefficients

Table 5 shows obtained partition coefficients (*K*) of tetracycline between two phases in the mixtures containing glycerol formal, water and inorganic salt (either K_3PO_4 , K_2HPO_4 or K_2CO_3), along with extraction efficiencies and values of pH in both phases at 298 K. All

values for partition coefficients were high (74 – 1552), evidencing a higher affinity of tetracycline for the glycerol formal-rich phase and resulting in high extraction efficiencies. Table 6 shows comparison of tetracycline's partition coefficients for various aqueous biphasic systems, which were reported in literature, based on a range of hydrophilic solvents, including polymers, alcohols and ionic liquids. The partition coefficients obtained in this work for glycerol formal based systems are higher than in aqueous biphasic systems based on a polymer (polyethylene glycol) and short-chain alcohols (propanol and ethanol). They are also comparable or higher than those obtained in the ionic liquid-based systems. The highest values for pH in both phases are observed for the system with K_3PO_4 (13.3 – 13.8), followed by pH for the system containing K_2CO_3 (12.5 – 12.9) and for the system containing K_2HPO_4 (9.7 – 10.3). The values for salt-rich (bottom) and glycerol formal-rich (top) phases are relatively similar for all studied mixtures, assuring that the partition is not greatly influenced by the differences in the charge of tetracycline molecule but dominated by its interaction with both phases and ions of salt.

3.3 NRTL model correlation

Along with ΔG_{hyd} , ion speciation and interactions, involving glycerol formal and water, play a role on salt performance. The experimentally obtained tie-line data at 298 K, for each investigated ternary system, have been correlated with the non-random two liquid (NRTL) model¹⁸:

$$\ln(\gamma_i) = \frac{\sum_{j=1}^3 \tau_{ji} G_{ji} x_j}{\sum_{k=1}^3 G_{ki} x_k} + \sum_{j=1}^3 \frac{x_j G_{ij}}{\sum_{k=1}^3 G_{kj} x_k} \left(\tau_{ij} - \frac{\sum_{k=1}^3 \tau_{ki} G_{ki} x_k}{\sum_{k=1}^3 G_{kj} x_k} \right) \quad (9)$$

where x_i is the mole fraction of component i while the parameters τ_{ij} , τ_{ji} , τ_{kj} , G_{ij} , G_{ki} and G_{kj} are the adjustable parameters for each binary pair in the NRTL model and i , j and k are indices representing all components in ternary mixture. Adjustable parameters G are given as:

$$G_{ij} = \exp(-\alpha_{ij} \tau_{ij}) \quad (10)$$

$$\tau_{ij} = a_{ij} + \frac{b_{ij}}{T} \quad (11)$$

where T is temperature and α_{ij} is a non-randomized parameter which in this work was taken to be 0.3 (i.e. mixture of nonpolar with polar non-associated species). The binary parameters for each coefficient, in terms of relevant coefficients a_{ij} and b_{ij} , were regressed by minimizing the sum of the squares of the differences between the experimental and calculated mole fractions of each component of both liquid phases for each ternary system using the MAXIMUM-LIKELIHOOD method as the objective function and the Britt–Luecke algorithm of the properties module of Aspen plus software.

The comparison of experimental and calculated data was performed by the average root mean square deviation (RMSD)¹⁹ according to:

$$\text{RMSD} = \sqrt{\frac{\sum_m \sum_l \sum_k (x_{klm,\text{exp}} - x_{klm,\text{calc}})^2}{\sigma_w}} \quad (12)$$

where x_{exp} and x_{calc} stand for the experimental and calculated mole fractions, respectively.

Indices k , l , and m denote the component, phase, and tie-line, respectively, and σ_w is the standard deviation of the experimental data.

Table 7 illustrates the binary interaction parameters and corresponding RMSD values. The values of binary interaction parameters allowed predictions of tie-lines which were in good agreement with experimental data as shown by the values of the RMSD values. The NRTL model can therefore be used to reproduce the tie-lines of the ternary mixture.

4. Conclusions

Liquid-liquid equilibrium data for the ternary (glycerol formal + K_3PO_4 + water), (glycerol formal + K_2HPO_4 + water) and (glycerol formal + K_2CO_3 + water) mixtures were obtained at 298 K and at atmospheric pressure. In general, the ability of salt to induce phase separation followed the order $K_3PO_4 > K_2HPO_4 > K_2CO_3$. Experimental tie-lines were efficiently correlated by the NRTL model and its interaction parameters were predicted.

Furthermore, this work demonstrates possibility of using these novel solvent systems for extraction of tetracycline, an industrially important antibiotic, with extraction efficiencies greater than 98.8%.

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Table 1. Chemical compounds in this work

Compound	Molecular Formula	Purity (wt %)	CAS no.	Source
Glycerol formal	C ₄ H ₈ O ₃	≥ 98.0	99569-11-6	Sigma Aldrich
Potassium diphosphate	K ₂ HPO ₄	≥ 99.0	7758-11-4	Sigma Aldrich
Potassium triphosphate	K ₃ PO ₄	≥ 99.0	7778-53-2	Sigma Aldrich
Potassium carbonate	K ₂ CO ₃	≥ 99.0	584-08-7	Sigma Aldrich
Tetracycline-HCL	C ₂₂ H ₂₄ N ₂ O ₈ · HCl	≥ 99.0	64-75-5	Sigma Aldrich

Table 2. Cloud points equilibrium data for the ternary system containing glycerol formal (GF), water (wat) and salt (either K_3PO_4 or K_2HPO_4 or K_2CO_3) at 298 K and at 0.1 MPa, in mass fraction.^a

glycerol formal + K_3PO_4 + water			glycerol formal + K_2HPO_4 + water			glycerol formal + K_2CO_3 + water		
x_{GF}	x_{salt}	x_{wat}	x_{GF}	x_{salt}	x_{wat}	x_{GF}	x_{salt}	x_{wat}
0.767	0.030	0.203	0.844	0.023	0.133	0.850	0.038	0.112
0.675	0.053	0.272	0.695	0.059	0.246	0.755	0.066	0.179
0.583	0.083	0.334	0.559	0.088	0.353	0.615	0.106	0.279
0.528	0.101	0.371	0.455	0.134	0.411	0.501	0.151	0.348
0.445	0.139	0.416	0.387	0.167	0.446	0.422	0.185	0.393
0.362	0.194	0.444	0.355	0.193	0.452	0.381	0.203	0.416
0.296	0.226	0.478	0.312	0.225	0.463	0.329	0.232	0.439
0.211	0.261	0.528	0.243	0.260	0.497	0.276	0.265	0.459
0.189	0.282	0.529	0.225	0.288	0.487	0.216	0.300	0.484
0.145	0.316	0.539	0.196	0.322	0.482	0.127	0.350	0.523
0.097	0.362	0.541	0.171	0.377	0.452	0.096	0.388	0.516
0.029	0.437	0.534	0.131	0.433	0.436	0.078	0.415	0.507

^a Standard uncertainties u are: $u(T) = \pm 0.1$ K, $u(x) = \pm 0.001$, $u(p) = \pm 1$ kPa.

Table 3. Constants A , B and C of equation (1) fitted to the cloud points for three ternary mixtures at 298 K with associated Standard Deviations (σ). r^2 and AAD stand are the correlation coefficient and absolute average deviations calculated by equation (2), respectively.

	$A \pm \sigma$	$B \pm \sigma$	$C \pm \sigma$
glycerol formal + K_3PO_4 + water	1.149 ± 0.030	-2.321 ± 0.100	24.446 ± 1.685
glycerol formal + K_2HPO_4 + water	1.356 ± 0.050	-2.970 ± 0.138	6.076 ± 1.752
glycerol formal + K_2CO_3 + water	1.392 ± 0.035	-2.452 ± 0.089	20.099 ± 1.269

Table 4. Tie-line equilibrium data for the ternary system containing glycerol formal (GF), water (wat) and salt (either K_3PO_4 or K_2HPO_4 or K_2CO_3) at 298 K and at 0.1 MPa, in mass fractions. Tie-line length (*TLL*) were calculated according to Eq. (7).^a

Overall composition			GF-rich phase (top)			salt-rich phase (bottom)			<i>TLL</i>
x_{GF}	x_{salt}	x_{water}	x_{GF}	x_{salt}	x_{water}	x_{GF}	x_{salt}	x_{water}	
<u>glycerol formal + K_3PO_4 + water</u>									
0.193	0.292	0.515	0.418	0.155	0.427	0.112	0.341	0.547	0.358
0.250	0.301	0.449	0.649	0.060	0.291	0.036	0.430	0.534	0.716
0.300	0.247	0.453	0.620	0.068	0.313	0.081	0.370	0.549	0.618
<u>glycerol formal + K_2HPO_4 + water</u>									
0.297	0.250	0.453	0.397	0.161	0.442	0.211	0.325	0.463	0.248
0.407	0.200	0.393	0.572	0.075	0.352	0.161	0.386	0.453	0.515
0.403	0.247	0.350	0.672	0.050	0.278	0.106	0.465	0.430	0.702
<u>glycerol formal + K_2CO_3 + water</u>									
0.200	0.351	0.449	0.725	0.066	0.209	0.068	0.422	0.510	0.747
0.419	0.201	0.380	0.581	0.114	0.305	0.144	0.349	0.506	0.496
0.459	0.250	0.291	0.806	0.049	0.146	0.022	0.504	0.474	0.906

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

Table 5. Partition coefficients of tetracycline (K_{TC}) in biphasic mixtures composed of glycerol formal, water and salt (either K_3PO_4 or K_2HPO_4 or K_2CO_3) at 298 K and at 0.1 MPa. The initial concentration of tetracycline in water was $1.124 \text{ g}\cdot\text{L}^{-1}$. x_{GF} , x_{salt} and $x_{TC,sol}$ are mass fractions of glycerol formal, salt and the tetracycline in water solution, respectively.

x_{GF}	x_{salt} g/g	$x_{TC,sol}$	$pH^{GF-phase}$	$pH^{salt-phase}$	$C_{TC}^{GF-phase}$ g·L ⁻¹	$C_{TC}^{salt-phase}$ g·L ⁻¹	K_{TC}	EE %
K₃PO₄								
0.193	0.292	0.515	13.8	13.6	2.302	0.019	121	86
0.250	0.301	0.449	13.8	13.6	1.522	0.001	1522	81
0.300	0.247	0.453	13.6	13.3	1.179	0.005	236	97
K₂HPO₄								
0.297	0.250	0.453	10.0	9.7	1.289	0.011	117	98
0.400	0.201	0.399	10.1	9.8	0.859	0.002	430	98
0.400	0.247	0.353	10.3	10.0	0.821	0.001	821	97
K₂CO₃								
0.200	0.351	0.449	12.5	12.9	2.237	0.006	373	97
0.419	0.201	0.38	12.2	12.8	0.817	0.011	74	99
0.459	0.25	0.291	12.5	12.9	0.654	0.001	654	92

^a Standard uncertainties u are: $u(T) = \pm 0.1 \text{ K}$, $u(x) = \pm 0.001$, $u(pH) = \pm 0.1$, $u(C_{\text{tetracycline}}) = \pm 0.003 \text{ g}\cdot\text{L}^{-1}$

Table 6. Partition coefficients of tetracycline (K_{TC}) in different aqueous biphasic systems at 298 K.

Solvent ^a	Salt	K	Lit.
PEG600	various cholinium-based salts	0.9 – 6.7	20
PEG600	Na ₂ SO ₄	13 – 18	20
[Ch][Cl]	K ₃ PO ₄	23 – 30	20
[bmim][Br]	K ₂ HPO ₄	60 – 180	21
[bmim][Cl]	K ₂ HPO ₄	130 – 275	21
2-propanol	(NH ₄) ₂ SO ₄	6 – 30	22
ethanol	(NH ₄) ₂ SO ₄	7 – 12	22
[bmim][Cl]	Na ₂ CO ₃	73	14
[bmimPyr][Cl]	Na ₂ CO ₃	82	14
[P ₄₄₄₄][Cl]	Na ₂ CO ₃	82	14
Glycerol formal	K ₃ PO ₄	121 – 1552	this work
Glycerol formal	K ₂ HPO ₄	117 – 821	this work
Glycerol formal	K ₂ CO ₃	74 – 645	this work

^a PEG600-polyethylene glycol with molecular mass of 600 g/mol; [Ch][Cl]- choline chloride; [bmim]-1-butyl-3-methylimidazolium; [bmimPyr]-1-butyl-1-methylpyrrolidinium; [P₄₄₄₄]-tetrabutylphosphonium.

Table 7. Binary interaction parameters of NRTL model for the system glycerol formal (1) + salt (2) + water (3) at 298 K.

<i>ij</i>	τ_{ij}		<i>RMSD</i>
	<i>a_{ij}</i>	<i>b_{ij}</i>	
Glycerol formal (1) + K ₃ PO ₄ (2) + water (3)			
12	1.80903	0	
21	10.3418	0	
32	8.03682	-538.763	0.1159
23	1.66666	-1192.05	
31	4.75867	0	
13	-1.05965	0	
Glycerol formal (1) + K ₂ HPO ₄ (2) + water (3)			
12	-0.031883	764.57	
21	0.229893	0.229893	
32	0.0922911	1602.7	0.7194
23	-0.066058	-598.075	
31	0.0922911	1602.7	
13	-0.066058	-598.075	
Glycerol formal (1) + K ₂ CO ₃ (2) + water (3)			
12	0.208555	352.875	
21	1.12504	2305.2	
32	-1.13382	3391.13	0.1036
23	-0.066058	4122.2	
31	-0.041522	1252.57	
13	-3.02198	3601.87	

Caption to Figures

Figure 1. Phase diagram for the ternary mixture composed of glycerol formal (GF), water and inorganic salt (K_3PO_4 – filled circles, K_2HPO_4 (filled triangles) and K_2CO_3 (filled squares) at 298 K: a) in mass fraction; and b) in mole fraction. Curves present fittings obtained by equation (1) using constants given in Table 3.

Figure 1a)

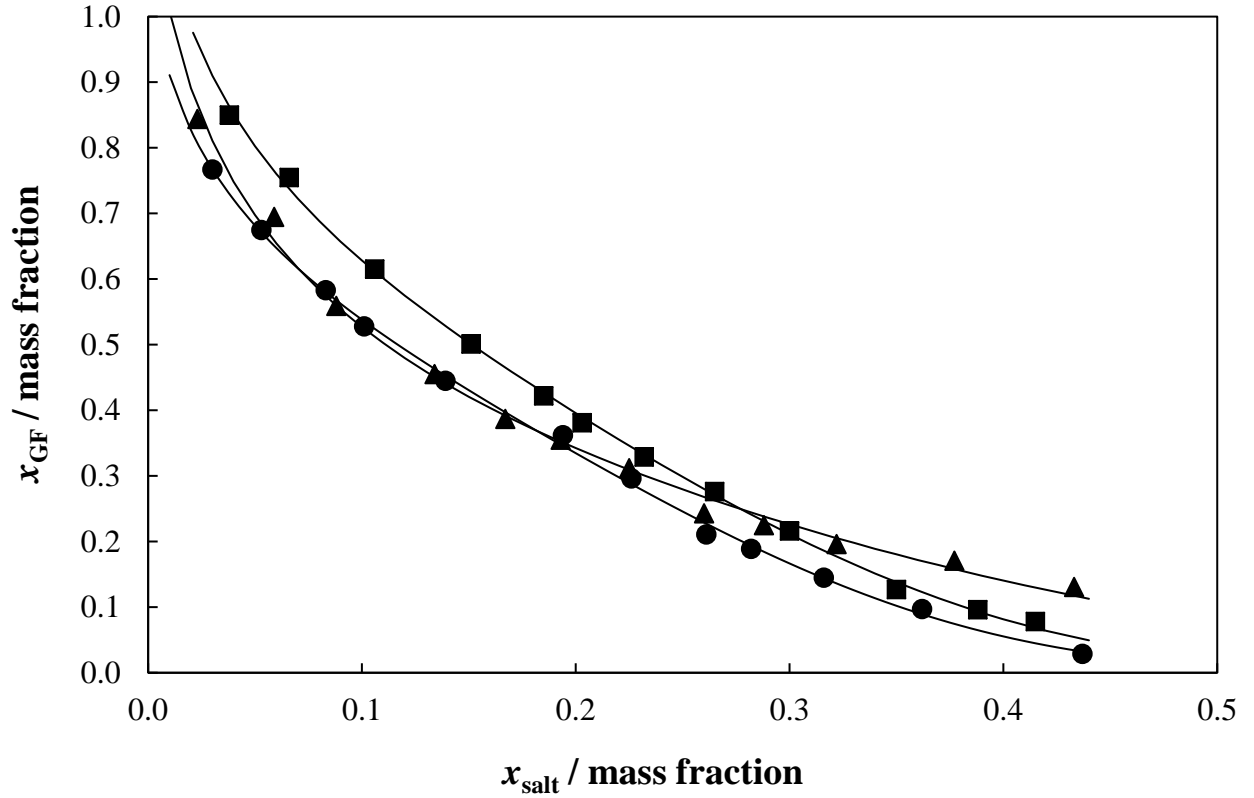
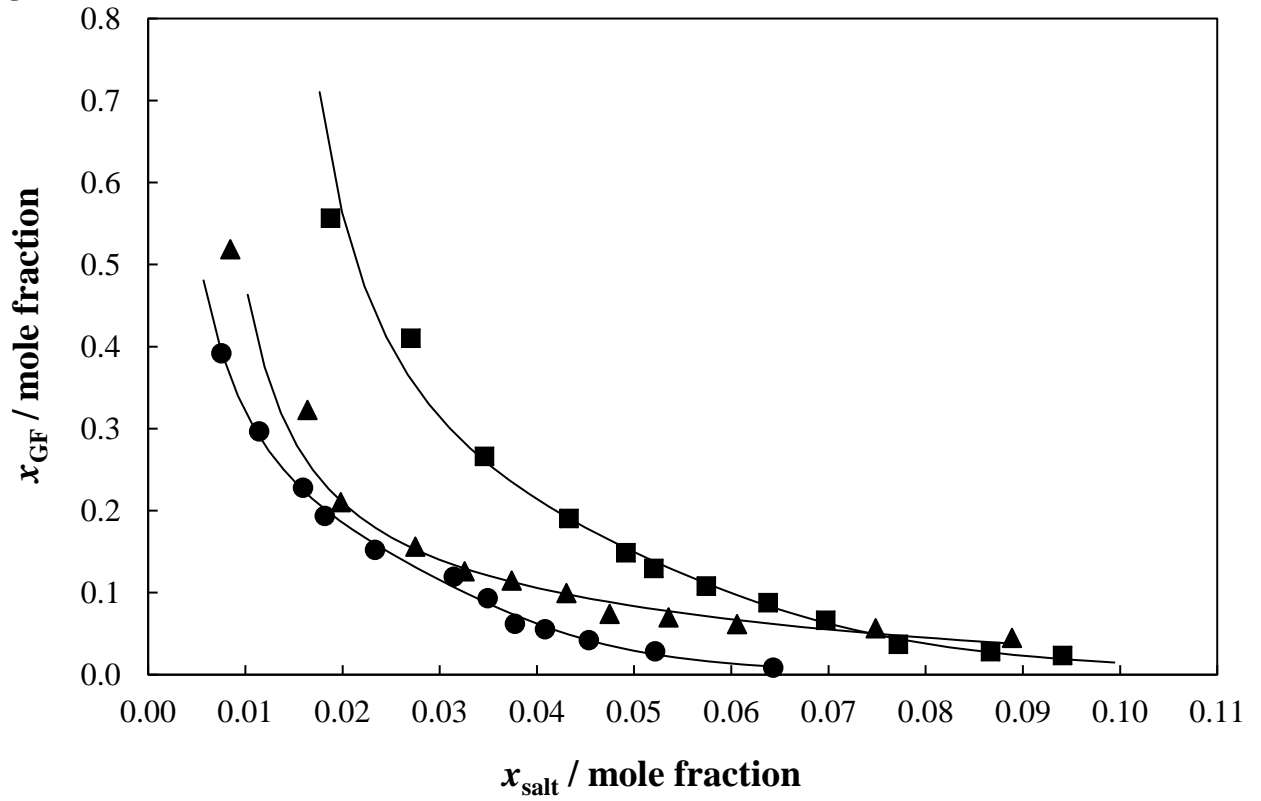


Figure 1b)



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