



Salting-out and salting-in effects of organic compounds and applications of the salting-out effect of Pentasodium phytate in different extraction processes



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ARTICLE INFO

Article history:

Received 24 October 2016

Accepted 17 March 2017

Available online 30 March 2017

Keywords:

Salting-out

Salting-in

Organic compounds

Pentasodium phytate

Separation

ABSTRACT

The influence of different uncharged and charged organic compounds on the Lowest Solution Temperature (LST) of a water/dipropylene glycol propyl ether (DPnP) mixture is investigated. Depending on the nature of the additive, a salting-out or salting-in behavior could be observed. For the binary mixture, a salting-out effect is associated with a decrease of the LST with increasing additive concentrations. The reverse effects are observed with increasing salting-in additive concentrations. Typical sugars, short carboxylate sodium salts, ammonium organic salts and amino-acids were found to be salting-out, whereas all studied sweeteners and organic acids showed a salting-in behavior. The Pentasodium phytate ($(\text{Phy}^{5-}, 5\text{Na}^+)$) was found being the most efficient organic salting-out compound. Three possible applications were investigated in order to compare the salting-out effect of $(\text{Phy}^{5-}, 5\text{Na}^+)$ to classical inorganic salts. First, the liquid-liquid extraction of 5-hydroxymethylfurfural (HMF) was investigated and compared to former results obtained using lithium, sodium and aluminium sulfate salts. Secondly, we considered a liquid-liquid, ethanol-water separation and compared the results to the one obtained using ammonium sulfate and potassium pyrophosphate. Finally, the salting-out effect of $(\text{Phy}^{5-}, 5\text{Na}^+)$ on glycerol was investigated and also compared to the inorganic salts, sodium chloride and lithium sulfate as well as potassium and sodium phosphates.

Due to higher water solubility, $(\text{Phy}^{5-}, 5\text{Na}^+)$ allowed a more pronounced separation of HMF than the tested sulfate salts. This high water solubility drives also to a more pronounced separation of ethanol in comparison to ammonium sulfate; potassium pyrophosphate being the most water soluble and most efficient salt to separate ethanol and water. The use of $(\text{Phy}^{5-}, 5\text{Na}^+)$ and potassium pyrophosphate showed a salting-out effect on glycerol in contrast to the inorganic salts sodium chloride and lithium sulfate for which a salting-in effect on glycerol was observed. The salting-out effect of sodium triphosphate was limited by its water solubility.

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1. Introduction

Since many decades, a huge number of authors deal with the topic 'specific ion effects'. The pharmacologist Franz Hofmeister established the first concept concerning ionic effects. It dates back to the year 1888 and is generally based on the ability of electrolytes to precipitate proteins from solution. According to Hofmeister, salts can be classified with regard to their ability to increase (salting-in) or decrease (salting-out) the

solubility of proteins in water. The resulting Hofmeister series gives a typical ordering of cations and anions, as shown in Fig. 1 [1–4].

Roughly speaking, the specific ion effect correlates with the charge density of the ions. Anions on the left of the series exhibit a high charge density and remain highly hydrated in the bulk, they are also called kosmotropes. In contrast, anions on the right side of the series have a low charge density and a less tightly bonded hydration shell, hence they tend to associate with nonpolar surfaces [3,5] and are called chaotropes. However, these expressions are only of historical importance and somewhat misleading, because no long-range structure-making or structure-breaking effect can be noted neither for single-charged kosmotropes nor for single-charged chaotropes [6–11].

In comparison to the ones dealing with specific inorganic ion effects, the number of scientific papers concerning the salting-out or salting-in effects of organic salts or uncharged organic molecules is small. In the following, we have decided to focus our investigation on the specific

Abbreviations: LST, Lowest solution temperature; DPnP, Dipropylene glycol propyl ether; HMF, 5-Hydroxymethylfurfural; V_{UP} , Volume of the upper phase; $V_{rel,UP}$, relative volume of the upper phase; V_i , initial volume; w_{UP} (EtOH), ethanol mass fraction of the upper phase; m_{UP} (EtOH), ethanol mass in the upper phase; d_{UP} , density of the upper phase; R_{UP} , ethanol ratio in the upper phase; m_i (EtOH), initial mass of ethanol in the sample; P (EtOH), ethanol purification coefficient.

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Cations									
NH(CH ₃) ₄ ⁺	NH ₄ ⁺	Cs ⁺	Rb ⁺	K ⁺	Na ⁺	Li ⁺	Mg ⁺	Ca ⁺	
salting-out					salting-in				
SO ₄ ²⁻	HPO ₄ ²⁻	OAc ⁻	F ⁻	OH ⁻	Cl ⁻	Br ⁻	NO ₃ ⁻	I ⁻	ClO ₄ ⁻ SCN ⁻
Anions									

Fig. 1. Typical Hofmeister series of some anions and cations.

effects of some of these often “neglected” molecules on the Lowest Solution Temperature (LST).

First, the present article reports the evolution of the LST of the binary mixture of water and an organic solvent: dipropylene glycol propyl ether (DPnP) upon the addition of different chemical compounds. Similar experiments using the same model system DPnP/water, adding inorganic salts were already performed and published by some of us [12]. The temperature dependent phase diagram of DPnP/water is given in Fig. 2a.

The mixtures are clear and monophasic below the LST and become biphasic with increasing temperature above the LST. In presence of a high quantity of water, the DPnP molecules are difficult to be dehydrated. At low water content, the last water molecules are too strongly bound to the alcoholic function and perhaps to the dioxypropylene (OP) function of the DPnP molecules to be removed even at high temperature.

The evolution of the LST in the water/DPnP system with increasing concentration of sodium thiocyanate (NaSCN) and sodium chloride (NaCl) is shown in Fig. 2b. In one of our previous studies, NaCl was reported as salting-out and NaSCN as salting-in. According to our explanations, the salting-out effect came from a competition for water between the salts, the OH and the OP groups of the DPnP molecule. The salting-in effect must be related with a lowering of the chemical potential of DPnP. The origin might be an association of the DPnP with the SCN⁻ anion or at least a weak interaction or simply the presence of different conformations of the DPnP molecules [12,13]. The star (★) in the monophasic area, near to phase boundary, corresponds to the binary mixture used for the present investigation and the one already published [12].

The most efficient organic salting-out agent Pentasodium phytate ((Phy⁵⁻, 5Na⁺)) can be extracted from cereal and is considered as a waste and a potential “green” salting-out agent. Phytic acid has found some applications mainly in food industry, medicine and dental care as well as in corrosion inhibition. It was already used for separation of rare earth metals not using its salting-out capacity but its property to

chelate metal cations [14]. More recently, some more applications have been published, e.g. its use as a flame retardant [15], and in the coating or in the production of new materials [16,17].

In the second part of the paper we focus on some applications of the salting-out properties of (Phy⁵⁻, 5Na⁺). For these applications the solvents concerned are not DPnP but 1-butanol, ethanol and glycerol. In the case of applications discussed below, 1-butanol is used as extractive solvent for 5-hydroxymethylfurfural (HMF) and glycerol. Concerning the ethanol, a liquid separation was performed from aqueous solutions. It can be noted that the binary phase diagram DPnP/water presents a LST, the binary phase diagram 1-butanol/water presents a highest solution temperature (HST) and the aqueous ethanol solutions have neither a LST nor a HST in the considered temperature range.

First, HMF/water separation performance using the (Phy⁵⁻, 5Na⁺) is compared to former results obtained with lithium sulfate (Li₂SO₄), sodium sulfate (Na₂SO₄), aluminium sulfate (Al₂(SO₄)₃) as salting-out agents in a liquid-liquid extraction of HMF from water with 1-butanol as extractive solvent. These salts were reported to be the most efficient in former studies [18,19].

Secondly, (Phy⁵⁻, 5Na⁺) and potassium pyrophosphate (K₄P₂O₇) were used to separate ethanol from water. Ethanol purification is performed generally in industry using distillation processes. A comparison with the performance of K₄P₂O₇, an inorganic phosphate salt with very high water solubility (1870 g/L at 25 °C) [20] was also conducted. Addition of a salting-out salt can improve the separation process [21,22]. The salting-out effect of (Phy⁵⁻, 5Na⁺) for ethanol is then compared to the one of ammonium sulfate ((NH₄)₂SO₄) which is well-known for its ethanol-water phase separation power. A comparison to K₄P₂O₇ is done as an example of phosphate inorganic salts, these latter being recently proposed in different liquid-liquid extraction processes implicating bio-fermentation products.

Finally, for the separation of glycerol from water with 1-butanol as extractive solvent, the salting-out power of (Phy⁵⁻, 5Na⁺), K₄P₂O₇, sodium triphosphate (Na₅P₃O₁₀), NaCl and Li₂SO₄ are compared.

2. Experimental

2.1. Chemicals

Dipropylene glycol propyl ether (≥98.5%), ethanol (≥99.8%), Choline chloride (≥99%), L-Arginine, Disodium malate (≥95%), Sodium nicotinate (≥98%), Oxalic acid (≥99%), Pentasodium phytate (≥99%), Sodium saccharinate (≥98%), Sodium acetate (≥99%), Sodium chloride (≥99%), Potassium pyrophosphate (97%), Sodium N-cyclohexylsulfamate (≥99%) and Monosodium L-glutamate monohydrate (>99%) were purchased from Sigma Aldrich. 1-Butanol was obtained from VWR (>99.8%).

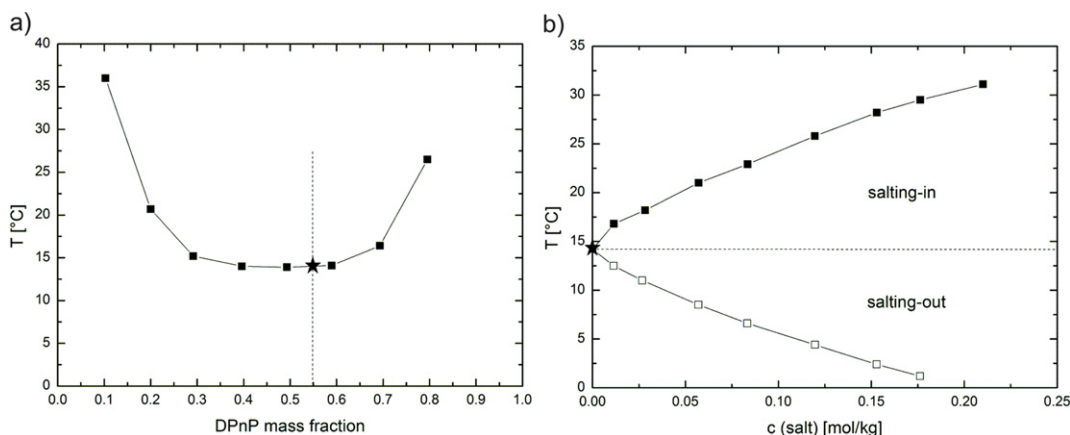


Fig. 2. - a) Phase diagram of the binary mixture water/DPnP as a function of temperature. The star (★) in the monophasic area corresponds to the binary mixture used for the present investigation. - b) Effect of (□) NaCl and (■) NaSCN on the binary mixture DPnP/water (★). Above the lines (—) mixtures turned turbid and are biphasic.

Acetylacetone (99%), Trisodium citrate ($\geq 99\%$), Citric acid monohydrate ($\geq 99\%$), Fructose ($\geq 99\%$), Glucose ($\geq 99\%$), Glycolic acid ($\geq 99\%$), Glycine ($\geq 99\%$), L-Proline ($\geq 99\%$), L-Cystein ($\geq 99\%$), L-Alanine ($\geq 99\%$), Malonic acid ($\geq 99\%$), Sodium triphosphate ($\geq 98\%$) and Sorbitol ($\geq 99\%$) were purchased from Merck. Potassium acesulfamate ($\geq 99\%$), Betaine hydrochloride ($\geq 99\%$), L-Serine ($\geq 99\%$), Sodium thiocyanate ($\geq 98\%$), Tartaric acid ($\geq 99\%$) and Trimethylamine N-oxide dihydrate ($\geq 99\%$) were received from Fluka. DL-Carnitine hydrochloride ($\geq 98\%$) and Xylitol ($\geq 99\%$) were obtained from Alfa Aesar. Isomalt was received from Beneo-Palatinit. Triethylphenylammonium chloride ($\geq 99\%$) was purchased from TCI. Ectoine ($\geq 96\%$) was obtained from bitop. Aspartame ($\geq 99\%$) was received from Ajinomoto. 5-Hydroxymethylfurfural was purchased from Molekula (99.1%). Water was taken from a Millipore purification system. The molecular structures of all the organic compounds tested are reported in the supporting information according to their chronological order in the different figures presented in the text. The content of sodium in the phytate was checked using atomic emission spectroscopy and was found to be equal to 5 mol/mol of phytate. The water content of Pentasodium phytate was determined by Karl-Fischer titration and was found to be $< 1\%$.

2.2. LST determination

The phase transition from clear to turbid was determined as a function of temperature. To this purpose, samples were cooled to approximately 0°C using an ice-water bath. The bath and the samples were stirred using a magnetic stir bar. A digital thermometer was used to measure the temperature of the bath. While slowly heating, the phase behavior was determined by visual observation. The temperature at which samples turned turbid was referred to as the lowest solution temperature (LST). The LST was found for a DPnP mass fraction of 0.55 [23]. All determined cloud points were reproducible on cooling and heating.

2.3. Determination of the HMF partition coefficient

The sample preparation as well as quantitative analysis of HMF in the aqueous and the organic phase in the biphasic area of the systems water/1-butanol/HMF and water/1-butanol/HMF/salt are performed according to [18]. The partition coefficients K_{HMF}^w were calculated using the equilibrium weight fractions of HMF in the 1-butanol and in the aqueous phases according to the following equation:

$$K_{HMF}^w = \frac{w_{HMF}^{1-butanol}}{w_{HMF}^{water}}$$

where $w_{HMF}^{1-butanol}$ and w_{HMF}^{water} denote the weight fractions of HMF in the 1-butanol and aqueous phase, respectively.

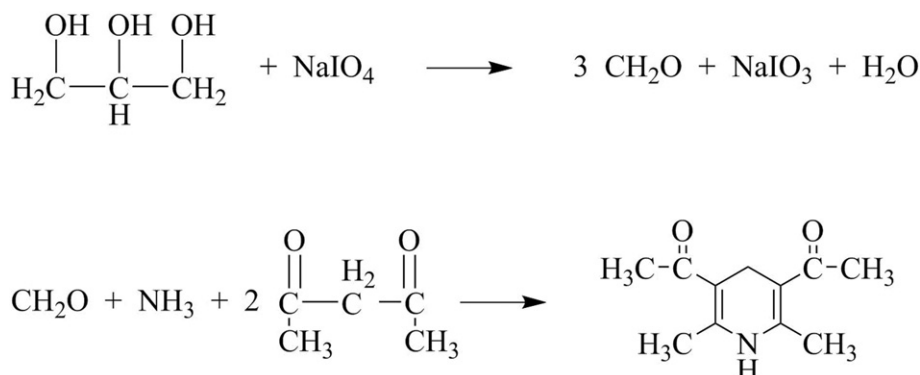


Fig. 3. Chemical reaction of the colorimetric assay for the quantification of glycerol in the biphasic systems water/1-butanol/glycerol as well as water/1-butanol/glycerol/salt.

2.4. Determination of the relative volume of the ethanol rich phase and of the ethanol purification coefficient

2.4.1. Determination of the relative volume of the ethanol rich phase

Samples were prepared by first weighing the desired salt masses into graduated test tubes of 20 ml, followed by the addition of equal amounts of water and ethanol (w/w). Complete dissolution of the salt in the mixture was carried out by mechanical stirring. The volume of the upper phase (V_{UP}) is determined after a complete phase separation detected by visual observation. The relative volume of the upper phase ($V_{rel,UP}$) is calculated by dividing the volume of the upper phase by the volume of the corresponding binary system water/ethanol without salt (V_i). $V_{rel,UP}$ is equal to 1 if no phase separation occurs and cannot be inferior to 0.5; the density of ethanol being lower than the one of water. All the measurements were performed at room temperature ($25^\circ\text{C} \pm 1$).

$$V_{rel,UP} = V_{UP} / V_i$$

2.4.2. Determination of the ethanol purification coefficient

The ethanol mass fraction ($w_{UP}(EtOH)$) of the upper phases was determined by gas chromatography (HP-6890). Samples from the ethanol rich phases were carefully taken by using syringes and were diluted with water by factor 1:100. An aqueous 1-propanol (1 wt%) solution was used as internal standard. The diluted samples were combined gravimetrically 1:1 with the internal standard solution. Peak separation of ethanol (3.7 min) and 1-propanol (4.1 min) was realized via a column temperature gradient of $10^\circ\text{C}/\text{min}$ ranging from 60 to 120°C using a polyethylene glycol packed column (DB-WAXetr, Agilent). The dimension of the capillary was 30 m in length and 0.320 mm in diameter with $0.50 \mu\text{m}$ of film thickness. Three replicate measurements of each sample were carried out and average values were considered. The ethanol mass in the upper phase ($m_{UP}(EtOH)$) was determined by dividing V_{UP} with the density of the upper phase (d_{UP}) and multiplying the obtained mass of the upper phase with $w_{UP}(EtOH)$.

$$m_{UP}(EtOH) = \frac{V_{UP}}{d_{UP}} w_{UP}(EtOH)$$

Densities (d_{UP}) were measured using an automated density meter DMA 5000 M (Anton Paar). Here, the period of oscillation of a U-shaped borosilicate glass tube filled with the sample is measured.

The ethanol ratio in the upper phase ($R_{UP}(EtOH)$) is calculated by dividing $m_{UP}(EtOH)$ by the value of the total mass of ethanol in the

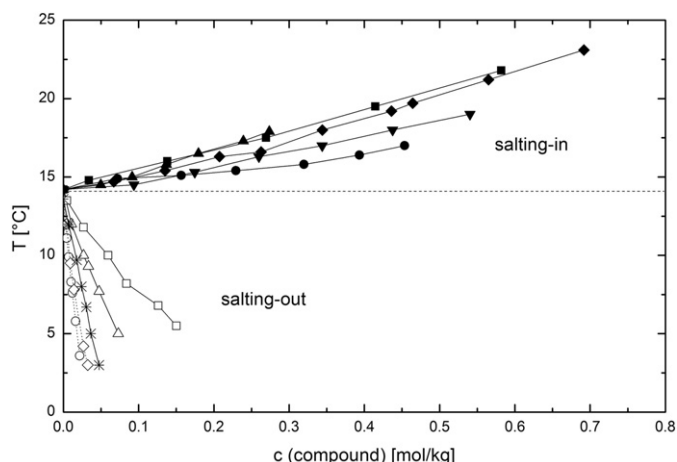


Fig. 4. Shift in the LST of the DPnP/water mixture upon the addition of short organic acids or short organic sodium carboxylate salts. Symbols represent experimental data: (■) Citric acid, (▲) Tartaric acid, (◆) Malonic acid, (▼) Glycolic acid, (●) Oxalic acid, (□) Sodium nicotinate, (△) Sodium acetate, (*) Monosodium L-glutamate, (◇) Disodium malate, (○) Trisodium citrate.

original prepared samples ($m_i(\text{EtOH})$).

$$R_{UP}(\text{EtOH}) = \frac{m_{UP}(\text{EtOH})}{m_i(\text{EtOH})}$$

Finally, the purification coefficient ($P(\text{EtOH})$) is calculated using the following equation:

$$P(\text{EtOH}) = \frac{R_{UP}(\text{EtOH})}{V_{UP}} V_i$$

2.5. Determination of the glycerol partition coefficient

Glycerol quantification in the aqueous and organic phases of the biphasic systems water/1-butanol/glycerol as well as water/1-butanol/glycerol/salt was performed using a modified colorimetric assay reported in [24]. To do this, aqueous and organic samples were diluted with water using dilution factors of 1/1000 up to 1/3000 (w/w) and subsequently 0.5 ml of each dilution were combined with 1.5 ml of working

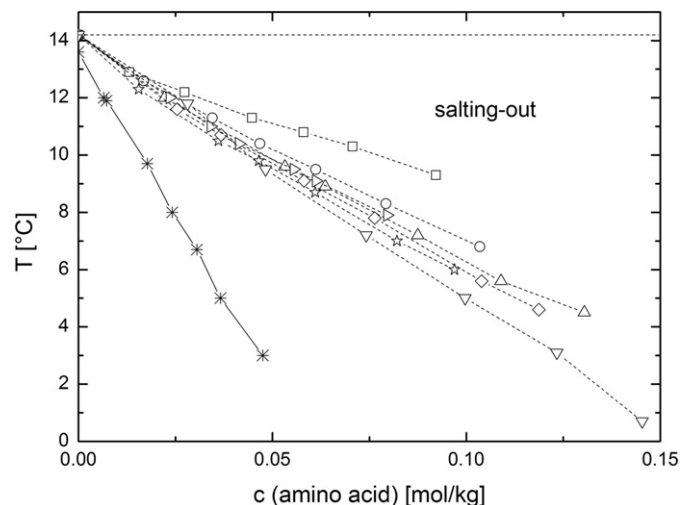


Fig. 5. Shift in the LST upon the addition of amino acids. Symbols represent experimental data: (□) L-Cysteine, (○) L-Proline, (△) Glycine, (▷) L-Arginine, (◇) L-Alanine, (☆) L-Serine, (▽) Ectoine, (*) Monosodium L-glutamate.

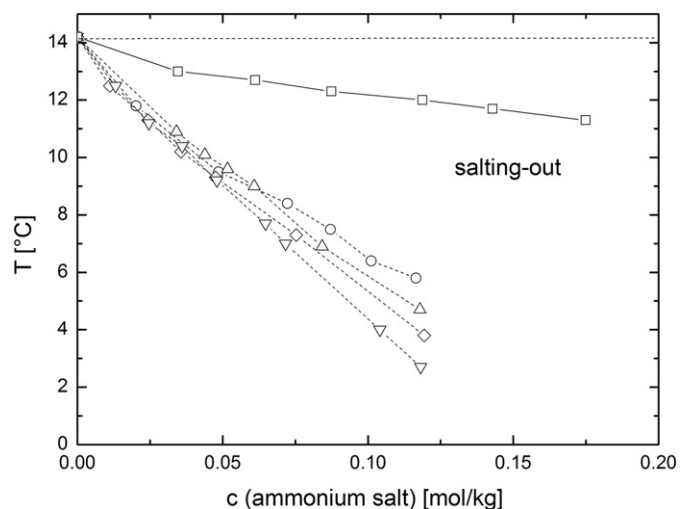


Fig. 6. Shift in the LST upon the addition of quaternary ammonium salts. Symbols represent experimental data: (□) Triethylphenylammonium chloride, (○) Betaine hydrochloride, (△) DL-Carnitine hydrochloride, (◇) Choline chloride, (▽) Trimethylamine-N-oxide.

solvent (ethanol-water mixture 1/1 (v/v)). After the addition of 1.2 ml of ammonium acetate buffered NaIO_4 solution and 1.2 ml of an ammonium acetate buffered acetylacetone solution, all samples were thermostated at 70 °C for 5 min to ensure the quantitative chemical reaction which is illustrated in Fig. 3. Glycerol gets splitted into three molecules of formaldehyde by use of NaIO_4 (glycol cleavage, Malaprade reaction). In a next step, formaldehyde reacts with two molecules of acetylacetone leading to the formation of 3,5-diacetyl-1,4-dihydropyridine (Hantzsch's reaction). The pyridine derivative has an absorption maximum at 410 nm allowing the detection of very small amounts of glycerol. The samples were cooled to room temperature with tap water and extinction values were collected by applying UV/VIS spectrophotometry. Weight fractions of glycerol were calculated using a corresponding calibration curve.

The partition coefficients K_{Glycerol}^w were calculated using the equilibrium weight fractions of glycerol in the 1-butanol and in the aqueous phases according to the following equation:

$$K_{\text{Glycerol}}^w = \frac{w_{\text{Glycerol}}^{1\text{-butanol}}}{w_{\text{Glycerol}}^{\text{water}}}$$

where $w_{\text{Glycerol}}^{1\text{-butanol}}$ and $w_{\text{Glycerol}}^{\text{water}}$ denote the weight fractions of glycerol in the 1-butanol and aqueous phase, respectively.

3. Results and discussions

3.1. Evolution of the LST of the DPnP/water mixture upon the additive concentration

Results concerning the evolution of the LST of the DPnP/water mixture upon the addition of either short organic acid, short organic sodium carboxylate salt, natural sugars, sweeteners, amino acid and ammonium salt are presented in Fig. 4–7.

Fig. 3 reports the evolution of the LST values by adding various concentrations of short organic acids and short sodium carboxylate salts. All the added short organic acids are salting-in and all the short organic sodium carboxylate salts show a salting-out behavior. The salting-in of DPnP by organic acids is much smaller than the salting-out by carboxylates. The short organic acids are very soluble in water and can form some molecular associations with the DPnP molecule in favor of its hydration, similarly to NaSCN . The energy cost for DPnP dehydration is then higher and the LST increases with increasing concentration of the short organic acids. The nearly linear LST increases are strongly specific

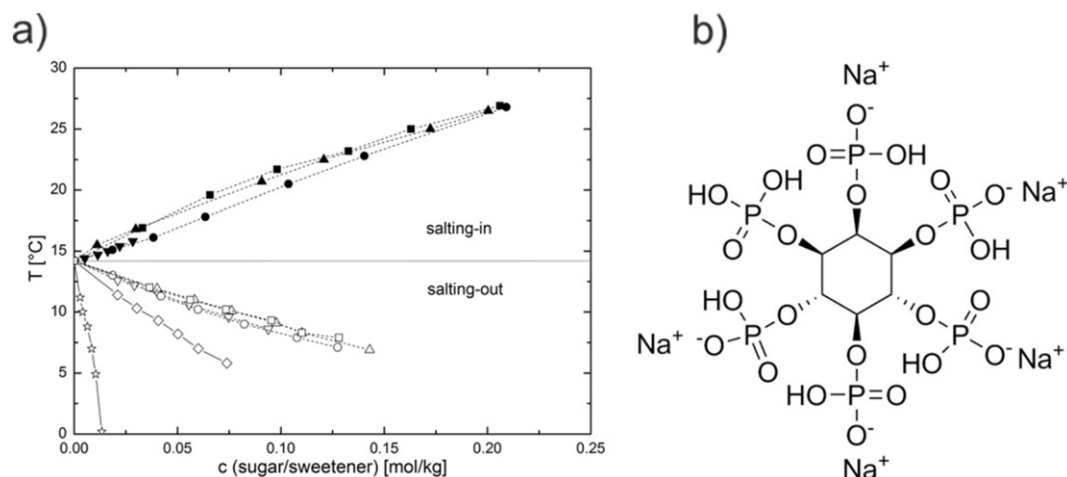


Fig. 7. a) Shift in the LST upon the addition of sugar or sweetener. Symbols represent experimental data: (■) Potassium acesulfamate, (▲) Sodium saccharinate, (●) Sodium N-cyclohexylsulfamate, (▼) Aspartame, (□) Glucose, (△) Fructose, (▽) Sorbitol, (○) Xylitol, (◇) Isomalt, (☆) $(\text{Phy}^{5-}, 5\text{Na}^+)$. - b) Possible molecular structure of $(\text{Phy}^{5-}, 5\text{Na}^+)$.

to the short organic acids and follow the order: Citric acid > Tartaric acid > Malonic acid > Glycolic acid > Oxalic acid. Citric acid shows the highest salting-in effect which can be explained by the formation of pronounced intermolecular hydrogen bonds giving a stronger hydrophobicity to the molecules in solution. The short organic carboxylate salts are more soluble in water than the short organic acids. The pronounced hydration of carboxylate salts leads to less water molecules being available as solvent for DPnP molecules. Their salting-out power is reflected by the nearly linear decrease of the LST values with increasing salt concentration. The decrease of the LST value is also specific and shows the following order: trisodium citrate > disodium malate > sodium acetate > sodium nicotinate. It can be noted that the most salting-in acid corresponds to the most salting-out salt.

The salting-out effect of Monosodium L-glutamate appears comparable to the one of the carboxylate sodium salts. This effect is more pronounced than in presence of the other tested amino acids (see Fig. 4).

The evolutions of the LST values adding various concentrations of amino acids are depicted in Fig. 5. All the tested amino-acids show a salting-out behavior, weakly specific of the added molecule. Like in presence of increasing concentrations of short organic carboxylate salts, a nearly linear decrease of LST values is obtained with increasing amino acids concentrations.

The following order concerning the salting-out power is observed: monosodium L-glutamate > ectoine > L-alanine > L-arginine > L-glycine > L-proline > L-cysteine.

The well-known food additive monosodium L-glutamate shows the highest salting-out power due to two carboxylate functions in the structure and show a behavior comparable to the one of disodium malate, see Fig. 4. Monosodium L-glutamate is responsible of the Umami taste [25]. The fact that it shows a salting-out effect in comparison to the salting-in effect observed for the sweeteners (see Fig. 7), may reflect a different mode of action on the G proteins which are present at the surface of the tongue. It can be also noted that a higher concentration of monosodium L-glutamate than of other sweeteners is necessary to get the desired taste. At high concentrations of L-cysteine, the decrease of the LST values is significantly lower compared to the other investigated amino-acids. L-cysteine is the only amino-acid having sulfur in its molecular structure.

The change of the LST upon addition of quaternary ammonium salts is reported in Fig. 6. As in presence of increasing concentrations of short organic carboxylate salts or amino acids, the curves obtained are decreasing nearly linearly, with a slight specificity. The salting-out power follows the order: Trimethylamine N-oxide > choline chloride > DL-carnitine hydrochlorine > betaine hydrochlorine > triethylphenylammonium

Table 1

α coefficients for different additives.

Chemical compound	α coefficient (°C/mmol of additive in 1 mol of DPnP/water mixture)	Related figure
Sodium thiocyanate	2.20	2
Sodium chloride	-2.01	2
Lithium sulfate	-8.65	
Sodium sulfate	-10.48	
Aluminium sulfate	-29.06	
Ammonium sulfate	-7.19	
Potassium pyrophosphate	-16.53	
Sodium triphosphate	-25.65	
Tetrasodium pyrophosphate	-30.03	
Citric acid	0.66	4
Tartaric acid	0.40	4
Malonic acid	0.37	4
Glycolic acid	0.26	4
Oxalic acid	0.16	4
Sodium nicotinate	-1.58	4
Sodium acetate	-3.34	4
Disodium malate	-9.46	4
Trisodium citrate	-13.33	4
L-Cysteine	-1.41	5
L-Proline	-1.98	5
Glycine	-2.08	5
L-Arginine	-2.21	5
L-Alanine	-2.22	5
L-Serine	-2.32	5
Ectoine	-2.58	5
Monosodium L-glutamate	-6.36	5
Triethylphenylammonium chloride	-0.42	6
Betaine hydrochloride	-1.96	6
DL-Carnitine hydrochloride	-2.25	6
Choline chloride	-2.35	6
Trimethylamine N-oxide	-2.73	6
Potassium acesulfamate	1.72	7
Sodium saccharinate	1.69	7
Sodium N-cyclohexylsulfamate	1.73	7
Aspartame	1.61	7
Glucose	-1.39	7
Fructose	-1.43	7
Xylitol	-1.58	7
Sorbitol	-1.65	7
Isomalt	-3.19	7
$(\text{Phy}^{5-}, 5\text{Na}^+)$	-27.41	7

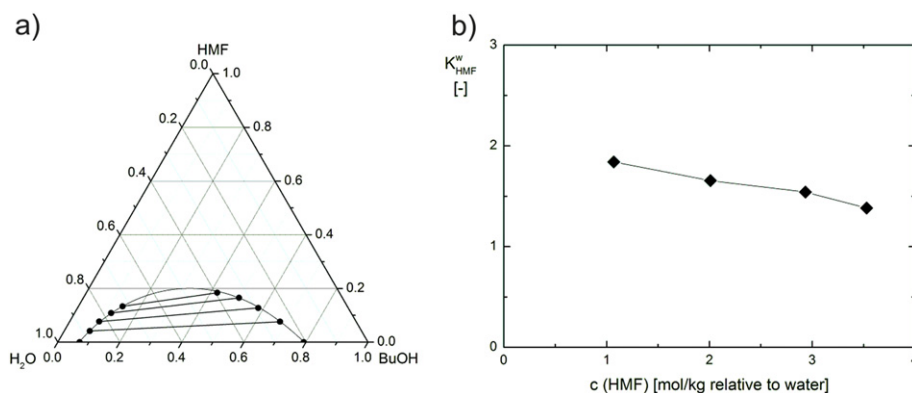


Fig. 8. - a) Ternary phase diagram of the system water/1-butanol/HMF at 25 °C and 1 bar presented in weight fractions. - b) Partition coefficients of HMF in the corresponding ternary system water/1-butanol/HMF/ at 25 °C and 1 bar.

chloride. Note that trimethylamine-N-oxide is a zwitterionic salt, in contrast to the cationic character of the others.

Fig. 7a) shows the evolutions of the LST depending on sugar and sweetener concentrations. It appears that all the investigated sugars have a salting-out power and all the sweeteners show a salting-in behavior.

Again, the salting-in behavior can result from direct weak interactions or a higher degree of hydration of DPnP. Both cases would lead to a lowering of the chemical potential of DPnP molecules in solution. The curves are all decreasing nearly linearly and no pronounced specific effect can be observed. This salting-in behavior is in agreement with the fact that these sweeteners have to salt-in the G-proteins of the tongue to be active and to give a strong sugar taste.

Compared to the four tested sugar molecules (glucose, fructose, xylitol, sorbitol), a significantly stronger salting-out effect is observed in the presence of isomalt and (Phy⁵⁻, 5Na⁺). The molecular structure of (Phy⁵⁻, 5Na⁺) is depicted in Fig. 7b. The increased salting-out power of isomalt can be explained by the presence of nine alcoholic functions in the molecular structure, compared to five or six in the case of glucose, fructose, xylitol, and sorbitol. Phytic acid contains 6 phosphate functions and these phosphate functions are even more water-binding than the nine alcoholic functions of isomalt.

In all cases, a linear shift in the LST was found over a wide concentration range. Referring to earlier works by Bauduin and al [12], curves are presented as:

$$LST(c) = LST(c=0) + \alpha c$$

where $LST(c)$ is the minimum demixing temperature in °C, $LST(c=0)$ the corresponding temperature of the binary mixture DPnP/water, c is the concentration of additive in mmol per 1 mol of DPnP/water mixture. The α values obtained with additive are given in Table 1. Positive values

indicate a salting-in behavior whereas negative values indicate a salting-out behavior. In the scope of the studied organic salts, (Phy⁵⁻, 5Na⁺) shows the most pronounced salting-out effect on DPnP from water with an α value of -27.41 .

Based on this observation, we decided to compare the salting-out effect of this organic salt to the one of inorganic salts for three different industrial processes, which are the separation of HMF, ethanol, and glycerol from aqueous phases. These three processes are very important in the framework of bio-fermentations. As it is well-known, HMF and bio-ethanol are two prominent compounds for the production of bio-fuel.

3.2. Salting-out properties of (Phy⁵⁻, 5Na⁺) and liquid-liquid extractions of HMF, ethanol and glycerol

3.2.1. HMF partition coefficient

The experimentally determined liquid-liquid equilibrium (LLE) of the ternary system water/1-butanol/HMF is illustrated in Fig. 8. The LLE data and the corresponding partition coefficients of HMF depending on the HMF-feed concentration were taken from [18].

In Fig. 9, experimentally determined partition coefficients of HMF between the aqueous and the organic phase are shown. They are obtained after liquid-liquid extraction in the system water/1-butanol at 25 °C and 1 bar as a function of the salt concentration relative to the water content (Fig. 9a in weight percent and Fig. 9b in molality relative to water). It can be seen that (Phy⁵⁻, 5Na⁺) has a lower efficiency at low salt concentrations compared to the inorganic salts (Li₂SO₄, Na₂SO₄, Al₂(SO₄)₃). Partition coefficients obtained with Li₂SO₄ and Al₂(SO₄)₃ are comparable and superior to those obtained with Na₂SO₄. Due to the high water solubility of (Phy⁵⁻, 5Na⁺) (≥ 50 mg/ml), at high salt concentrations, a higher value of the partition coefficient can

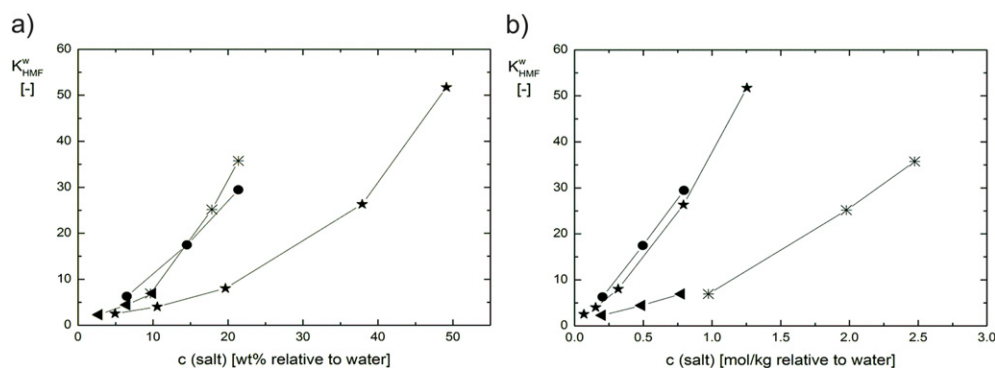


Fig. 9. Partition coefficients of HMF in quaternary systems water/1-butanol/HMF/salt at 25 °C, 1 bar and a constant HMF molality of 4 mol/kg relative to water. - a) Partition coefficients vs. wt% of salt relative to water. - b) Partition coefficients vs. molality of salt relative to water. Symbols represent experimental data: (*) Li₂SO₄, (◄) Na₂SO₄, (●) Al₂(SO₄)₃, (★) (Phy⁵⁻, 5Na⁺).

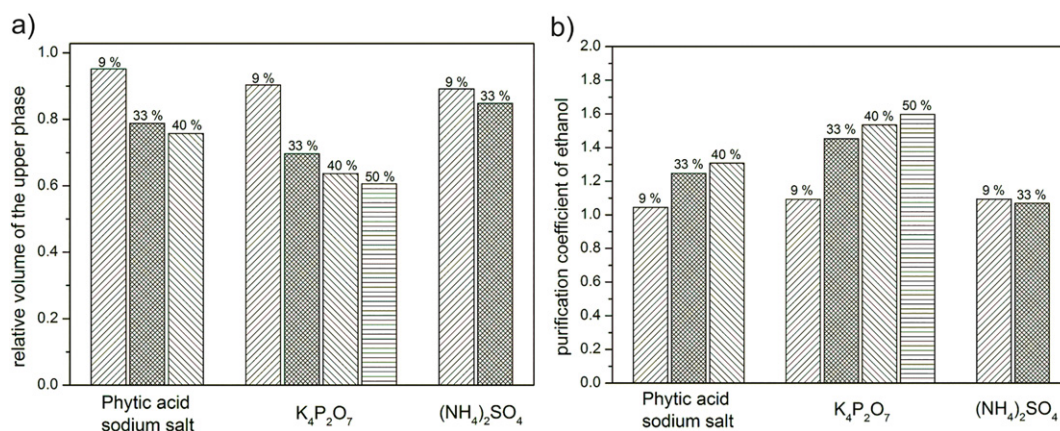


Fig. 10. - a) Relative volume of the upper phase to a comparable monophasic binary solution of ethanol and water (1:1; wt%) without salt obtained at 25 °C and 1 bar, after the addition of (■) 9%; (■) 33%; (■) 40%; (■) 50% of $(Phy^{5-}, 5Na^+)$, $K_4P_2O_7$ and $(NH_4)_2SO_4$. - b) Purification coefficient of ethanol obtained at 25 °C and 1 bar, after the addition of (■) 9%; (■) 33%; (■) 40%; (■) 50% of $(Phy^{5-}, 5Na^+)$, $K_4P_2O_7$ and $(NH_4)_2SO_4$.

be reached. Fig. 9b illustrates the HMF partition coefficients depending on the salt molality relative to water. Due to the high molecular weight of $(Phy^{5-}, 5Na^+)$, the efficiency is comparable to the one obtained with the most efficient inorganic salt $Al_2(SO_4)_3$. But as the solubility of $(Phy^{5-}, 5Na^+)$ in water is higher than that of $Al_2(SO_4)_3$, a higher value of the HMF partition coefficient can be achieved.

3.2.2. Relative volume of the ethanol rich phase and ethanol purification coefficient

In Fig. 10a, the volume of the upper phase after ethanol separation from water at 25 °C and 1 bar, relative to the volume of a comparable monophasic binary solution of ethanol and water (1:1, wt%) without salt is presented depending on the weight percentages (wt%) of different added salts, $(Phy^{5-}, 5Na^+)$, $K_4P_2O_7$ and $(NH_4)_2SO_4$. At a total amount of 9 wt% of salt, $(NH_4)_2SO_4$ is the most efficient additive for ethanol separation from water, $V_{rel,UP} = 0.89$ and in addition the phase separation occurs very fast compared to the other salts. At a salt content of 33 wt%, the ammonium salt is precipitated and shows a less effective ethanol separation from water compared to the other salts. $K_4P_2O_7$ is the best salting-out agent followed by $(Phy^{5-}, 5Na^+)$.

In Fig. 10b, the purification coefficients of ethanol at 25 °C and 1 bar are presented depending on the used salt and its total concentration in the samples. It appears that the purification coefficients obtained using $(NH_4)_2SO_4$ and $K_4P_2O_7$ are the best ($P(EtOH) = 1.09$) at 9 wt% of salt in

comparison to the one of $(Phy^{5-}, 5Na^+)$ ($P(EtOH) = 1.04$). The purification coefficients reported using $(Phy^{5-}, 5Na^+)$ ($P(EtOH) = 1.25$) and $K_4P_2O_7$ ($P(EtOH) = 1.45$) are superior to the one of $(NH_4)_2SO_4$ ($P(EtOH) = 1.07$) in presence of 33 wt% of salt. The purification coefficient found at this concentration using $(NH_4)_2SO_4$ is limited by the solubility of this salt in water. In presence of 40 wt% of salt, a higher purification coefficient is observed in case of $K_4P_2O_7$ ($P(EtOH) = 1.53$) compared to the one obtained using $(Phy^{5-}, 5Na^+)$ ($P(EtOH) = 1.31$). The purification coefficient in the presence of 50 wt% of added $K_4P_2O_7$ is the best one of all tested mixtures ($P(EtOH) = 1.60$).

3.2.3. Glycerol partition coefficient

Partition coefficients of glycerol between the aqueous and the organic 1-butanol phase in the quaternary systems water/1-butanol/glycerol/salt are represented in Fig. 11, depending on the salt concentration relative to water at 25 °C and 1 bar (Fig. 11a in weight percent and Fig. 11b in molality relative to water). In the ternary system water/1-butanol/glycerol without added salt, the partition coefficient $K_{Glycerol}^w$ has a value of 0.16 at a glycerol-feed concentration of 4 mol/kg water. In case of NaCl and Li_2SO_4 , the partition coefficients of glycerol decrease with increasing salt concentration. That is, the salts have a salting-in effect on glycerol which is more pronounced for NaCl. All used phosphate salts induce a salting-out effect on glycerol with $(Phy^{5-}, 5Na^+)$ being the most effective one. Due to a higher water solubility of $K_4P_2O_7$, higher

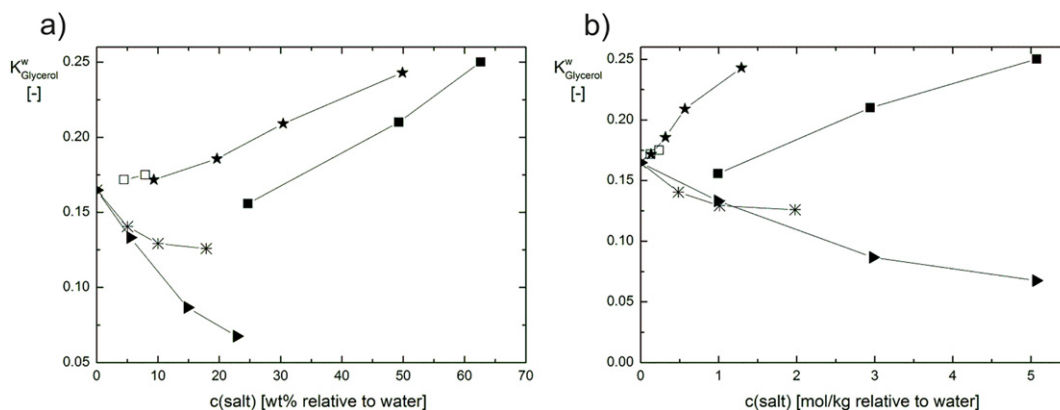


Fig. 11. Partition coefficients of glycerol in quaternary systems water/1-butanol/glycerol/salt at 25 °C, 1 bar and a constant glycerol molality of 4 mol/kg relative to water. - a) Partition coefficients vs. wt% of salt relative to water. - b) Partition coefficients vs. molality of salt relative to water. Symbols represent experimental data: (*) Li_2SO_4 , (▶) NaCl, (□) $Na_3P_3O_{10}$, (■) $K_4P_2O_7$, (★) $(Phy^{5-}, 5Na^+)$.

partition coefficients of glycerol can be achieved compared to (Phy^{5-} , 5Na^+). The salt effect of $\text{Na}_5\text{P}_3\text{O}_{10}$ is limited due to its low solubility in water.

4. Conclusion

Salting-in and salting-out behaviors have been reported for different organic compounds. The salting-in and -out properties (we keep this expression also for the uncharged molecules) seem to be associated to the molecular structure and therefore to the molecular family and perhaps to a particular biological property. In the scope of the tested compounds, a salting-out effect was observed for typical sugars, short carboxylate sodium salts, ammonium organic salts and amino-acids. In contrast, a salting-in effect appears using sweeteners and short organic acids. Obviously, similar effects are typical of a certain class of molecules with common biological functions.

Among the tested organic molecules, the (Phy^{5-} , 5Na^+) showed the most pronounced salting-out effect. Note that phytic acid is a cheap and food-agreed ingredient.

In tested applications, the use of (Phy^{5-} , 5Na^+) leads to a more pronounced separation of HMF than the tested sulfate salts and a more pronounced separation of ethanol from water in comparison to the ethanol separation from water induced by $(\text{NH}_4)_2\text{SO}_4$. In contrast to the common inorganic salts NaCl and Li_2SO_4 , (Phy^{5-} , 5Na^+) shows even a salting-out effect on glycerol from water using 1-butanol as extracting solvent.

Acknowledgement

The authors gratefully acknowledge the financial support of the IGF-project 17114N/1 of the DECHEMA e.V. that was funded by the Federal Ministry of Economics and Technology (grant no. 005-1009-0053) based on an enactment of the German Federal Parliament (BMWi). The project was supported within the program "Promoting the Industrial Collective Research (IGF)" with the help of the German Federation of Industrial Research Associations (AiF) (IGF-project 17114N/1).

We further express our gratitude to Florian Mertes and Max Hohenschutz for their excellent technical support.

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