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Novel aqueous two-phase systems composed of acetonitrile and polyols: Phase diagrams and extractive performance



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ABSTRACT

A large number of works has been devoted to the study of alternative constituents to form aqueous two-phase systems (ATPS); however, scarce attention has been given to polyols as two-phase forming components. This work addresses the potential use of polyols (glycerol, erythritol, xylitol, sorbitol and maltitol) to create ATPS in presence of acetonitrile. Novel ternary phase diagrams were determined at 298 K and the impact of the polyol chemical structure through the liquid–liquid demixing was evaluated. It is shown that the ability for phase separation largely depends on the number of hydroxyl groups present in each polyol. Polyols with a higher number of hydroxyl groups are better phase separating agents increasing thus the ability for two-phase formation. The partitioning of a model biomolecule, vanillin, was also assessed to ascertain on these systems applicability as alternative extractive techniques. In all systems, vanillin preferentially migrates to the acetonitrile-rich phase (more hydrophobic layer) with recoveries higher than 89%, except to glycerol. This pattern was confirmed by solid–liquid solubility studies of vanillin in aqueous solutions containing diverse polyols supporting thus their phase separating ability. These novel systems can be used as alternative ATPS for the extraction and recovery of added-value biomolecules.

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1. Introduction

The extraction of biomolecules usually requires the use of several and combined processes, such as solvent and ultrasound assisted extraction [1], microwave assisted extraction [2] and supercritical fluid extraction [3], followed by purification steps involving precipitation, centrifugation, filtration, dialysis or chromatography [4]. This two-step process makes the downstream processing responsible for 50–80% of the final cost of biotechnological-based products [5]. In this sense, aqueous two-phase systems (ATPS) can be foreseen as a possible alternative that is easy to scale up, presents low cost and leads to a high product purity as well as to a high yield, while maintaining the biological activity of the molecules due to their water-rich environment [6,7].

ATPS have been studied in the recovery and purification of diverse biomolecules, namely proteins [8], enzymes [9,10], nucleic acids [11], flavor compounds (vanillin [12]; 6-pentyl- α -pyrone

[7]), antioxidants (ascorbic acid [13]), alkaloids [14], and antibiotics (tetracycline [15–17]).

Since the first observation (by Beijerinck in 1886) demonstrating that ATPS can be formed by mixtures of agar and starch or gelatin in aqueous media many other pairs of phase-forming constituents have been explored [18]. In the past decades, ATPS have shown capable to be created by two polymers (dextran/polyethylene glycol [19]) or by a polymer-salt combination (polypropylene glycol/(NH₄)₂SO₄, MgSO₄, KCl or KCH₃CO₂ [20]), and which can be labeled as “traditional systems”. In recent times, other compounds have been successfully used in the replacement of the traditional constituents, such as the pairs alcohol–salt [13], ionic liquid–salt [21–23], ionic liquid–polymer [24,25], and ionic liquid–carbohydrate [26]. Recently, pioneering ATPS based on acetonitrile and sugars have also been reported [27–29].

Acetonitrile (ACN) is an organic solvent widely used by industry in the production of perfumes, rubber products, pesticides or pharmaceuticals [30] or as a mobile phase in reverse phase high performance liquid chromatography (HPLC) in separation and purification processes [31,32]. Acetonitrile is also a by-product from the manufacture of acrylonitrile [33]. Acetonitrile, CH₃CN, also known as cyanomethane or methyl cyanide, is one aprotic

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solvent miscible with water in the whole composition range, similar to the dimethyl sulfoxide or acetone behavior, and its molecules do not strongly interact with themselves leaving a hydrogen bond network formed by water [34].

Polyols, usually known as sugar alcohols, are a hydrogenated form of carbohydrates and whose carbonyl group has been reduced to a primary or secondary hydroxyl group [35]. Polyols may mimic the structure of water and maintain an artificial sphere of hydration around macromolecules [36]. Due to their properties, polyols are widely used in pharmaceuticals, confectionery products, chewing gums, mixed juice [37] and as substituent of sucrose in food-stuffs [38].

Taking into account the continuous investigation on novel phase-forming components to create ATPS, this work addresses innovative ATPS formed by polyols of different chemical structure and acetonitrile. The corresponding phase diagrams, tie-lines and tie-line lengths were determined at 298 K. Moreover, to investigate the extractive performance of these novel systems, they were used in the partitioning of vanillin (used here as a standard biomolecule). Vanillin (3-methoxy-4-hydroxybenzaldehyde) is the major component of natural vanilla and it is widely used as a flavoring material in confectionery, food products, beverages, perfumes and in pharmaceutical preparations [39]. Currently, vanillin is naturally produced via a multistep curing process of the green vanilla pods of the orchid plant (10%). However, the majority of vanillin (90%) is actually synthetically produced [40].

2. Material and methods

2.1. Materials

The ATPS studied in this work were formed by polyols and acetonitrile. All compounds were purchased from Sigma-Aldrich: glycerol (>99.5 wt% pure), erythritol (≥ 99 wt% pure), xylitol (>99 wt% pure), sorbitol (>98 wt% pure), maltitol (>98 wt% pure), acetonitrile (HPLC grade with a purity of 99.9 wt%) and vanillin (>99 wt% pure). Distilled and deionized water was used in all experiments.

2.2. Phase diagrams and tie-lines

The ternary phase diagrams were determined for each polyol and acetonitrile at 298 (± 1) K and atmospheric pressure by the cloud point titration method. Stock solutions of each polyol (≈ 30 –80 wt%, depending on the polyol solubility saturation in water) and acetonitrile (≈ 80 –100 wt%) were previously prepared and used for the determination of the phase diagrams. Repetitive drop-wise addition of the polyols solution to the aqueous solution of acetonitrile was carried out until the detection of a cloudy solution, followed by the drop-wise addition of ultra-pure water until the detection of a monophasic region (clear and limpid solution). These additions were carried out under continuous stirring and the saturation curves were determined gravimetrically within $\pm 10^{-4}$ g.

The tie-lines (TLs) were obtained through a gravimetric method originally described by Merchuck and co-workers [41]. A mixture at the biphasic region of each ternary system was prepared, vigorously stirred, and allowed to reach equilibrium and phase separation, for a minimum of 18 h at 298 (± 1) K. After the equilibration step, the top and bottom phases were carefully separated and weighted within $\pm 10^{-4}$ g. Each individual TL was determined by the application of the lever-arm rule, which describes the relationship between the weight of the top phase and the overall system weight and composition. For that purpose, the binodal curves were correlated using Eq. (1),

$$[\text{ACN}] = A \exp \left\{ \left(B \times [\text{Polyol}]^{0.5} \right) - \left(C \times [\text{Polyol}]^3 \right) \right\} \quad (1)$$

where [ACN] and [Polyol] are the acetonitrile and polyol weight fraction percentages, respectively, and A , B and C are constants parameters obtained by the regression of the experimental binodal data.

The determination of the TLs was then accomplished by solving the following system of four equations (Eqs. (2)–(5)) for the four unknown values of $[\text{ACN}]_T$, $[\text{ACN}]_B$, $[\text{Polyol}]_T$ and $[\text{Polyol}]_B$,

$$[\text{ACN}]_T = A \exp \left\{ \left(B \times [\text{Polyol}]_T^{0.5} \right) - \left(C \times [\text{Polyol}]_T^3 \right) \right\} \quad (2)$$

$$[\text{ACN}]_B = A \exp \left\{ \left(B \times [\text{Polyol}]_B^{0.5} \right) - \left(C \times [\text{Polyol}]_B^3 \right) \right\} \quad (3)$$

$$[\text{ACN}]_T = ([\text{ACN}]_M/\alpha) - ((1-\alpha)/\alpha)[\text{ACN}]_B \quad (4)$$

$$[\text{Polyol}]_T = ([\text{Polyol}]_M/\alpha) - ((1-\alpha)/\alpha)[\text{Polyol}]_B \quad (5)$$

where the subscripts M, T and B denote, respectively, the initial mixture, and the top and bottom phases. The value of α is the ratio between the mass of the top phase and the total weight of the mixture. The system solution results in the acetonitrile and polyol concentration in the top and bottom phases, and thus, TLs can be simply represented.

The respective tie-line lengths (TLLs) were determined through the application of Eq. (6),

$$\text{TLL} = \sqrt{([\text{Polyol}]_T - [\text{Polyol}]_B)^2 + ([\text{ACN}]_T - [\text{ACN}]_B)^2} \quad (6)$$

2.3. Partitioning of vanillin

The partitioning liquid–liquid systems for vanillin were prepared in graduated glass centrifuge tubes weighing the appropriate amounts of each polyol, acetonitrile and an aqueous solution containing vanillin. Vanillin was at 0.4 g dm^{-3} in the initial aqueous solution. After the complete mixing of all components for a given mixture composition, each system was centrifuged at 2000g for 10 min, and then each tube was placed in a thermostatic bath at 298.15 (± 0.01) K for at least 18 h. After the two phases become clear and transparent, and the interface was well defined, the bottom phase was carefully withdrawn using a long needle syringe and a pipette for removing the top phase [42]. The volume of each phase was initially measured and both phases were further separated for the quantification of vanillin and for the determination of their pH values. At least three independent replicates were made and the average partition coefficients and associated standard deviations were therefore determined.

The pH values (± 0.02) of the top and bottom phases were measured at 298 K using a DIGIMED DM-20 pH meter.

The concentration of vanillin at each aqueous phase was quantified through UV-spectroscopy, using a Varian Cary 50 Bio UV–Vis spectrophotometer, and at a wavelength of 280 nm using a calibration curve previously established [12].

The partition coefficient of vanillin was determined taking into account the concentration of the antioxidant in each phase and according to,

$$K_{\text{van}} = \frac{C_T}{C_B} \quad (7)$$

where K_{van} is the partition coefficient of vanillin, C represents the vanillin concentration, and the subscripts T and B denote the top (acetonitrile-rich) and bottom (polyol-rich) phases, respectively.

The recovery of vanillin (R_T) in the top phase was evaluated using Eq. (8),

$$R_T = \frac{100}{1 + \frac{1}{K_{\text{van}} \times R_v}} \quad (8)$$

where R_v is the ratio between the volumes of the top (V_T) and bottom (V_B) phase.

2.4. Determination of vanillin solubility

Vanillin was added in excess amounts to aqueous solutions of xylitol and sorbitol (20, 15, 10 and 5 wt%) and equilibrated in an air oven under constant agitation using an Eppendorf Thermomixer Comfort equipment. The equilibrium temperature was 303 (± 0.5) K. Previously optimized equilibration conditions were established: stirring velocity of 750 rpm and at least for 72 h. After the saturation conditions all samples were centrifuged in a Hettich Mikro 120 centrifuge to properly separate the macroscopic phases during 20 min at 4500 rpm. After centrifugation, samples of the liquid phase were carefully collected and the amount of vanillin was quantified through UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at a wavelength of 280 nm. A proper calibration curve was previously established. At least three individual samples of each aqueous solution, and at each concentration of polyol, were quantified in order to determine the average solubility of vanillin and the respective standard deviation.

3. Results and discussion

3.1. Phase diagrams and tie-lines

Although there are many reports in literature describing ATPS, this work is the first evidence that systems based on acetonitrile and polyols also undergo phase separation in aqueous media. The molecular structures of the constituents of these novel systems are depicted in Fig. 1.

The solubility of a given solute in water is affected by the presence of other species that can act as phase separating agents. Polyols are non-ionic compounds with an enhanced ability to be hydrated due to their large number of –OH groups [43]. Therefore, polyols tend to act as phase separating agents [26,44,45].

The experimental phase diagrams for each polyol (glycerol, erythritol, xylitol, sorbitol and maltitol) and acetonitrile were determined at 298 K and atmospheric pressure. The experimental weight fraction data are provided in the Supporting Information (Tables S1 and S2). The corresponding phase diagrams are depicted in Fig. 2 and allow the analysis of the polyol potential to induce the liquid–liquid demixing. All solubility curves are represented in molality units to avoid disparities in the evaluation of the polyol capability to form ATPS and which could result from their different molecular weights. It should be remarked that for the studied systems based on acetonitrile and each polyol, the bottom phase cor-

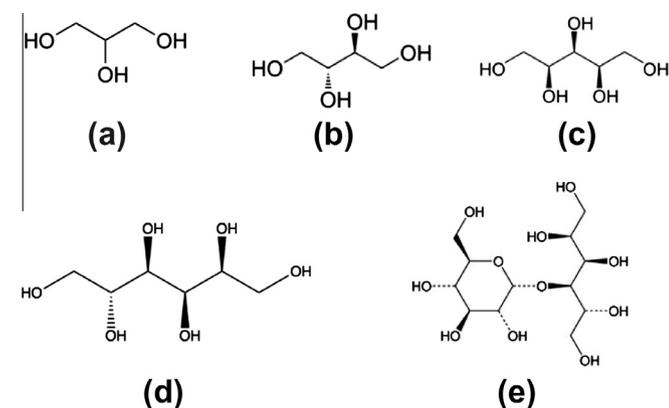


Fig. 1. Chemical structure of the studied polyols: (a) glycerol; (b) erythritol, (c) xylitol, (d) sorbitol and (e) maltitol.

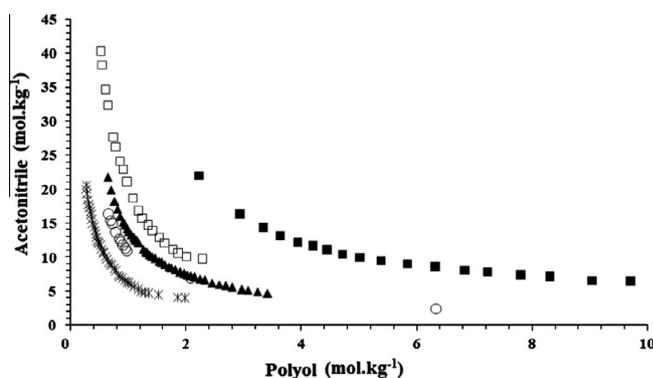


Fig. 2. Binodal curves for ternary systems composed of acetonitrile + polyol + water at 298 K and atmospheric pressure. ■, glycerol; □, erythritol; ▲, xylitol; ○, sorbitol; *, maltitol.

responds to the polyol-rich phase whereas the top phase is the acetonitrile-rich phase.

According to Fig. 2, the formation of ATPS is favoured in the following order: glycerol < erythritol < xylitol < sorbitol < maltitol. In general, the capability of alditols for ATPS formation is directly proportional to the increasing carbon number, and thus, of hydroxyl groups from 3 (glycerol) to 9 (maltitol) – cf. Fig. 1. According to Freire and co-workers [26] the number of hydroxyl groups present in each polyol is directly associated with its capability to hydrogen-bond with water and, therefore, to act as a phase separating species. In this context, polyols with more hydroxyl groups are those that are more able to form ATPS with acetonitrile that is excluded towards a second and aqueous liquid phase.

All experimental binodal data were fitted using the empirical relationship described by Eq. (1). The fitted parameters A , B and C (estimated by least-squares regression), and the corresponding standard deviations (σ) and regression coefficients (R^2) are reported in Table 1. As can be seen by the correlation coefficients obtained, Eq. (1) provides an accurate description of the experimental binodal saturation curves. Fig. 3 presents the correlation of the data by Eq. (1) for the several systems investigated and composed of acetonitrile + polyol + water. To complete the phase diagrams, several TLs and respective TLLs were further calculated and their values are reported in Table 2. The TLs are also represented in Fig. 3.

3.2. Partitioning of vanillin

In order to evaluate the polyol structure and respective concentration in the extraction of vanillin, two different mixtures compositions (30 wt% polyol + 30 wt% acetonitrile and 35 wt% polyol + 30 wt% acetonitrile) were investigated.

In all systems, the top phase (acetonitrile-rich) is more acid (pH ranging between 4.7 and 5.7) than the bottom (pH varying between 6.5 and 6.8). The pH values of the coexisting phases used

Table 1
Parameters A , B and C (and corresponding standard deviation, σ), obtained from the regression of the experimental binodal data by Eq. (1), and correlation coefficients (R^2), for the ternary systems composed of acetonitrile + polyol + water at 298 K and atmospheric pressure.

Polyol	$A \pm \sigma$	$B \pm \sigma$	$C \pm \sigma$	R^2
Glycerol	155.3 ± 6.2	-0.295 ± 0.008	$4.0 \times 10^{-17} \pm 1.4 \times 10^{-7}$	0.997
Erythritol	160.9 ± 5.4	-0.384 ± 0.012	$3.6 \times 10^{-14} \pm 3.5 \times 10^{-6}$	0.999
Xylitol	142.2 ± 2.1	-0.375 ± 0.004	$1.1 \times 10^{-14} \pm 4.0 \times 10^{-7}$	0.999
Sorbitol	115.1 ± 14.3	-0.329 ± 0.035	$7.4 \times 10^{-7} \pm 1.2 \times 10^{-6}$	0.989
Maltitol	132.3 ± 2.1	-0.371 ± 0.005	$6.3 \times 10^{-15} \pm 4.3 \times 10^{-7}$	0.999

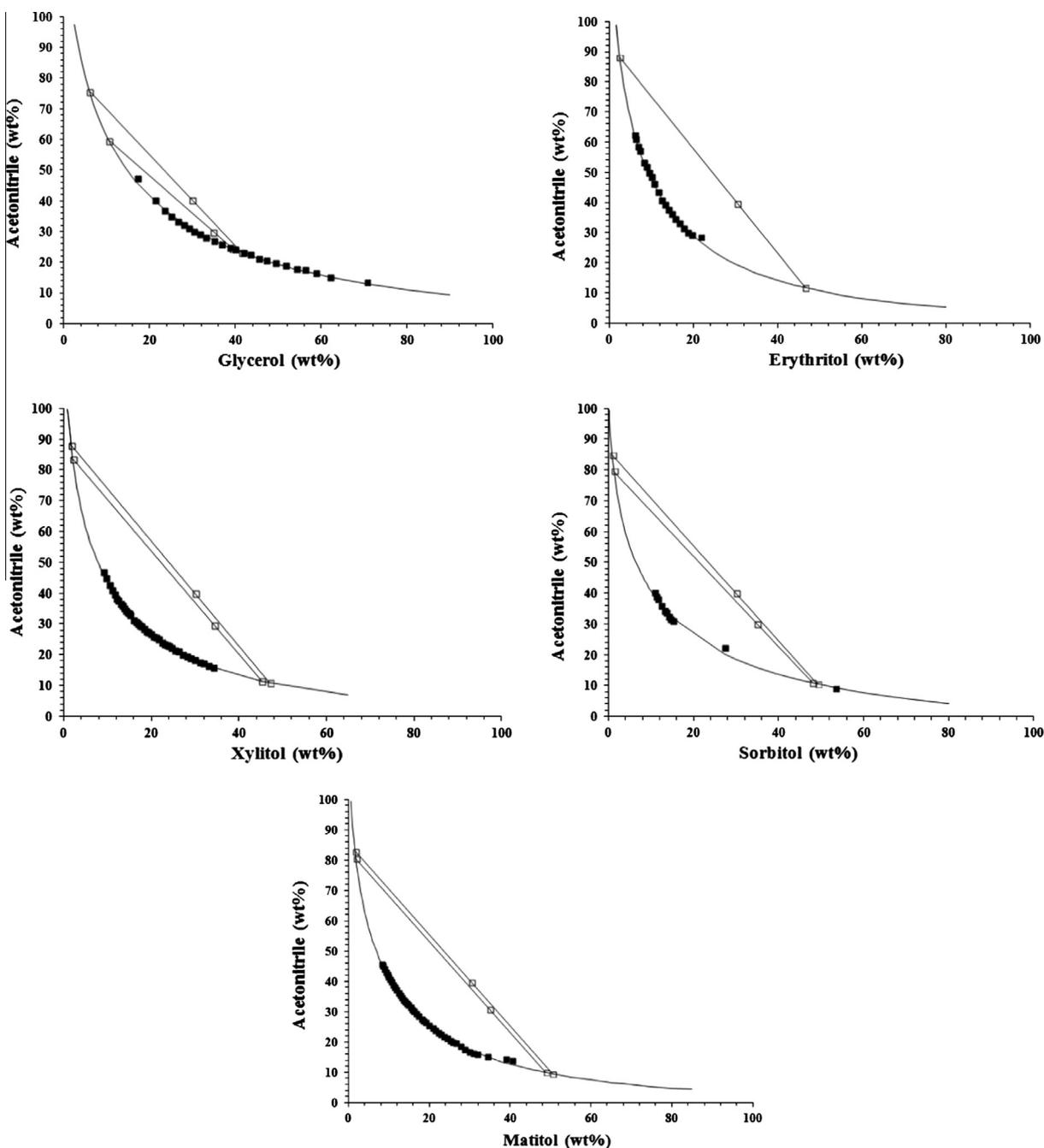


Fig. 3. Phase diagrams for ternary systems composed of acetoneitrile + polyol + water at 298 K and atmospheric pressure. ■, experimental solubility data; □, TL data; —, fitting by Eq. (1).

in the partitioning experiments are reported in Table 3. In all systems, vanillin is mainly present as a neutral molecule ($pK_a = 8.2$) [46,47]. The influence of the pH in the chemical structure of vanillin is shown in Supporting Information (Fig. S1).

The partition coefficient of a given biomolecule depends on the main interactions and solute/solvent properties which favour an one-side migration. These can include dispersive-type interactions, hydrogen-bonding and electrostatic forces, as well as the biomolecule size, solubility and affinity for a given phase. Moreover, the associated magnitude further depends on the composition of the system and biomolecules nature [5].

The partition coefficients of vanillin at two different mixture compositions, at 298.15 K, are shown in Fig. 4 and Table S.3 of

the Supporting Information. The compositions of the coexisting phases, i.e., the respective TLs are reported in Table 2. In all systems, vanillin preferentially migrates for the acetoneitrile-rich phase with partition coefficient values higher than 7.16 ± 0.43 . Vanillin has preference for more hydrophobic and organic phases as reflected by its octanol–water partition (K_{ow}) coefficient value: $\log(K_{ow}) = 1.19$ [48]. In fact, acetoneitrile is more hydrophobic ($\log(K_{ow}) = -0.17$) than all the studied polyols, namely glycerol ($\log(K_{ow}) = -1.84$), erythritol ($\log(K_{ow}) = -2.47$), xylitol ($\log(K_{ow}) = -3.10$), sorbitol ($\log(K_{ow}) = -3.73$) and maltitol ($\log(K_{ow}) = -5.50$) [49]. Moreover, the partition coefficient of vanillin for the acetoneitrile-rich increases with the hydrophilicity of the polyol, with the exception of maltitol. This trend also follows the ability for

Table 2
Mass fraction composition for the TLs and respective TLLs, at the top (T) and bottom (B) phase, and initial biphasic composition of the mixture (M), composed of acetonitrile ([ACN]) and polyol ([Polyol]) at 298 K and atmospheric pressure.

Polyol	Weight fraction/(wt%)						TLL
	[ACN] _M	[Polyol] _M	[ACN] _T	[Polyol] _T	[ACN] _B	[Polyol] _B	
Glycerol	40.15 ± 0.03	30.04 ± 0.03	15.62 ± 0.02	5.94 ± 0.17	23.14 ± 0.04	41.59 ± 0.02	63.18
	29.92 ± 0.03	34.99 ± 0.03	59.69 ± 0.02	10.49 ± 0.10	24.34 ± 0.04	39.41 ± 0.03	45.66
Erythritol	39.71 ± 0.03	30.42 ± 0.03	88.12 ± 0.01	2.45 ± 0.41	11.62 ± 0.09	46.64 ± 0.02	88.34
Xylitol	39.94 ± 0.03	29.98 ± 0.03	88.13 ± 0.01	1.63 ± 0.61	10.87 ± 0.01	47.08 ± 0.02	89.64
	29.52 ± 0.03	34.40 ± 0.03	83.70 ± 0.01	2.00 ± 0.50	11.44 ± 0.09	45.21 ± 0.02	84.19
Sorbitol	40.00 ± 0.03	30.05 ± 0.03	84.84 ± 0.01	0.86 ± 1.16	10.47 ± 0.01	49.26 ± 0.02	88.73
	29.99 ± 0.03	35.04 ± 0.03	79.58 ± 0.01	1.26 ± 0.79	10.85 ± 0.09	48.04 ± 0.02	83.15
Maltitol	39.78 ± 0.03	30.34 ± 0.03	82.99 ± 0.01	1.58 ± 0.63	9.49 ± 0.11	50.50 ± 0.02	88.29
	30.59 ± 0.03	34.68 ± 0.03	80.54 ± 0.01	1.79 ± 0.56	9.92 ± 0.10	48.81 ± 0.02	84.84

Table 3
pH values of the top (acetonitrile-rich) and bottom (polyol-rich) phases at 298 K and atmospheric pressure.

Polyol	System A		System B	
	Top	Bottom	Top	Bottom
Glycerol	4.74	5.62	5.40	6.80
Erythritol	4.96	5.93	5.05	6.52
Xylitol	5.34	6.10	5.46	6.76
Sorbitol	4.70	5.83	5.54	6.79
Maltitol	5.68	6.57	5.46	6.82

System A: 40 wt% acetonitrile + 30 wt% polyol; System B: 30 wt% acetonitrile + 35 wt% polyol.

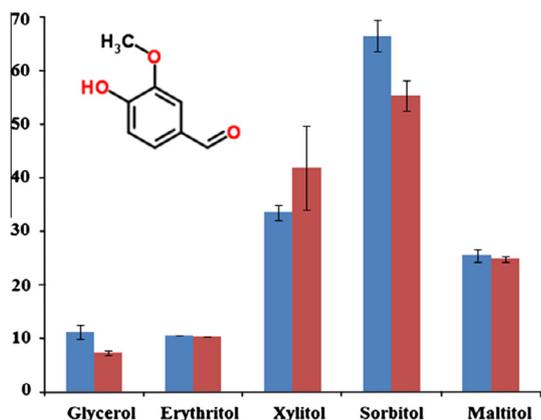


Fig. 4. Partition coefficients of vanillin (K_{van}) in ATPS composed of: ■ – 30 wt% polyol + 40 wt% acetonitrile and 30 wt% water; ■ – 35 wt% polyol + 30 wt% acetonitrile and 35 wt% water. The chemical structure of vanillin is also shown as an insert.

two-phase formation previously described indicating that stronger phase separating compounds improve the migration of the biomolecule for the opposite phase. This pattern of the phase separating capacity of the different polyols was further confirmed by the determination of the solubility of vanillin in aqueous solutions containing polyols at different concentrations. The results obtained are depicted in Fig. 5 and Table S.4 of the Supporting Information, and it shows a decrease on the vanillin solubility with the increase on the polyols concentration supporting thus the partition coefficients observed.

The maximum partition coefficient was observed with sorbitol whereas the lowest value was observed with maltitol. With an increase in the hydroxyl groups number in the polyol structure there is an increase in interactions by hydrogen-bonding (glycerol – 3 and sorbitol – 6) with vanillin, which did not overcome the affinity of vanillin with the top phase and the increase of K_{van} . However,

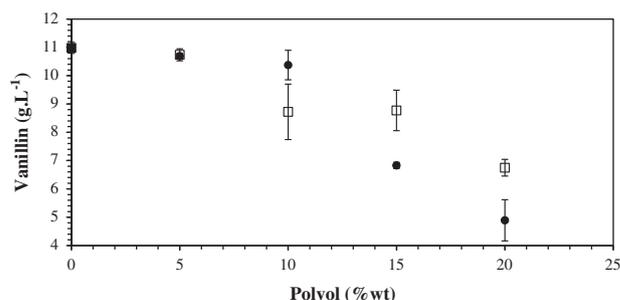


Fig. 5. Solubility of vanillin at 303 K in ■, H₂O; and aqueous solutions of ● sorbitol and □ xylitol.

maltitol has almost the double of the –OH groups (11) compared to sorbitol, and probably it occurs a stronger interaction of this polyol with vanillin leading to a subsequent decrease of the partition coefficient of vanillin when compared with other polyols.

The partition coefficients of vanillin observed in this work in acetonitrile-polyol ATPS are higher than those observed in other systems composed of acetonitrile + mannose ($K_{\text{van}} = 9.67$) [28] or ionic liquid + K₃PO₄ ($K_{\text{van}} = 49.5$) [12], although lower than those observed in ethanol + K₂HPO₄ ($K_{\text{van}} = 430$) [13] systems. The gathered results support the idea that non-ionic ATPS formed by acetonitrile and polyols are therefore an alternative extractive route for added-value biomolecules, such as vanillin.

The influence of the polyol chemical structure was also evaluated through the vanillin recovery parameter – Fig. 6 and Table S.3 of the Supporting Information. In general, the recovery

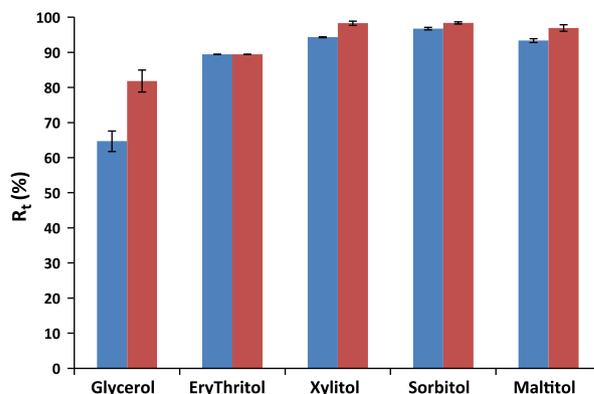


Fig. 6. Recovery of vanillin in the acetonitrile-rich phase (R_t) in ATPS composed of: ■ – 30 wt% polyol + 40 wt% acetonitrile and 30 wt% water; ■ – 35 wt% polyol + 30 wt% acetonitrile and 35 wt% water.

values are above 89%, except for glycerol, and reflect the high preference of vanillin for the acetonitrile-rich phase. In terms of recovery, the values tend to increase with the increase in the number of hydroxyl groups at the polyol and from glycerol to maltitol. Polyols with less than 4 hydroxyl groups present an average of R_T below 82% (glycerol), and similar to the value reported by Cardoso and co-workers [28] using acetonitrile + sugar ATPS. Polyols with more than 4 hydroxyl groups lead to an average R_T above 89% (xylitol, sorbitol and maltitol), and similar to the value described by Reis and co-workers [13] using systems constituted by alcohols + potassium phosphate salts. The enhanced recovery of vanillin to the acetonitrile-rich phase suggests that the systems investigated in this work represent a viable alternative for extractive purposes while avoiding the use of charged species or high-charge density salts. Dhamole and co-workers reported recovery of proteins in ACN + glucose aqueous two-phase system above 97.16% at 18 °C [50].

4. Conclusion

This work shows, for the first time, that ATPS can be formed by acetonitrile and a wide variety of polyols at specific concentrations in aqueous media. The ternary phase diagrams, tie-lines and tie-line lengths were determined at 298 K and atmospheric pressure. The results obtained indicate that polyols act as phase separating species leading to the exclusion of a second acetonitrile-rich phase. Moreover, the higher the number of hydroxyl groups at the polyol the higher it is the separation ability observed. The two-phase separation is favored in the following order: glycerol < erythritol < xylitol < sorbitol < maltitol. A proof of principle showing that these novel ATPS can be used to extract biomolecules was also demonstrated using vanillin as a model compound. Vanillin preferentially partitions for the acetonitrile-rich phase with partition coefficients ranging from 7 to 67 and which are dependent on the phase separating ability of each polyol. Moreover, the recovery of vanillin at the acetonitrile-rich phase showed to be higher than 89%, except to glycerol, supporting the huge potential of these novel systems to be explored in the extraction of the most diverse added-value compounds.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.seppur.2014.01.004>.

References

- [1] D. Jadhav, B.N. Rekha, P.R. Gogatre, V.K. Rathod, Extraction of vanillin from vanilla pods: a comparison study of conventional soxhlet and ultrasound assisted extraction, *J. Food Eng.* 93 (2009) 421–426.
- [2] U.K. Sharma, K. Sharma, N. Sharma, A. Sharma, S.P. Singh, A.K. Sinha, Microwave-assisted efficient extraction of different parts of *Hippophae rhamnoides* for the comparative evaluation of antioxidant activity and quantification of its phenolics constituents by reverse-phase high-performance liquid chromatography (RP-HPLC), *J. Agric. Food Chem.* 56 (2008) 374–379.
- [3] R. Hartono, G.A. Mansoori, A. Suwono, Prediction of solubility of biomolecules in supercritical solvents, *Chem. Eng. Sci.* 56 (2001) 6949–6958.
- [4] A. Pessoa Filho, B.V. Kilikian, Purificação de produtos biotecnológicos, Editora Manole Ltda, São Paulo, 2005.
- [5] P.A. Albertson, Partition of Cell Particles and Macromolecules, Wiley Interscience, New York, 1986.
- [6] X. Wu, L. Liang, Y. Zou, T. Zhao, J. Zhao, F. Li, L. Yang, Aqueous two-phase extraction, identification and antioxidant activity of anthocyanins from mulberry (*Morus atropurpurea* Roxb.), *Food Chem.* 129 (2011) 443–453.
- [7] M. Rito-Palomares, A. Negrete, E. Galindo, L. Serrano-Carreón, Aroma compounds recovery from mycelial cultures in aqueous two-phase processes, *J. Chromatogr. B* 743 (2000) 403–408.
- [8] J.A. Asenjo, B.A. Andrews, Aqueous two-phase systems for protein separation: a perspective, *J. Chromatogr. A* 1218 (2011) 8826–8835.
- [9] J.M.P. Barbosa, R.L. Souza, A.T. Fricks, G.M. Zanin, C.M.F. Soares, A.S. Lima, Purification of lipase produced by a new source of *Bacillus* in submerged fermentation using an aqueous two-phase system, *J. Chromatogr. B* 879 (2011) 3853–3858.
- [10] R.L. Souza, J.M.P. Barbosa, G.M. Zanin, M.W.N. Lobão, C.M.F. Soares, A.S. Lima, Partitioning of porcine pancreatic lipase in a two-phase systems of polyethylene glycol/potassium phosphate aqueous, *Appl. Biochem. Biotechnol.* 16 (2010) 288–300.
- [11] F. Luechau, T.C. Ling, A. Lyddiatt, Primary capture of high molecular weight nucleic acids using aqueous two-phase systems, *Sep. Purif. Technol.* 66 (2009) 202–207.
- [12] A.F.M. Cláudio, M.G. Freire, C.S.R. Freire, A.J.D. Silvestre, J.A.P. Coutinho, Extraction of vanillin using ionic-liquid-based aqueous two-phase systems, *Sep. Purif. Technol.* 75 (2010) 39–47.
- [13] I.A.O. Reis, S.B. Santos, L.A. Santos, N. Oliveira, M.G. Freire, J.F.B. Pereira, S.P.M. Ventura, J.A.P. Coutinho, C.M.F. Soares, A.S. Lima, Increased significance of food wastes: selective recovery of added-value compounds, *Food Chem.* 135 (2012) 2453–2461.
- [14] H. Passos, M.P. Trindade, T.S.M. Vaz, L.P. Costa, M.G. Freire, J.A.P. Coutinho, The impact of self-aggregation on the extraction of biomolecules in ionic-liquid-based aqueous two-phase systems, *Sep. Purif. Technol.* 108 (2013) 174–180.
- [15] J.F.B. Pereira, F. Vicente, V.C. Santos-Ebinuma, J.M. Araújo, A. Pessoa, M.G. Freire, J.A.P. Coutinho, Extraction of tetracycline from fermentation broth using aqueous two-phase systems composed of polyethylene glycol and cholinium-based salts, *Process Biochem.* 48 (2013) 716–722.
- [16] S. Shahriari, L.C. Tomé, J.M.M. Araújo, L.P.N. Rebelo, J.A.P. Coutinho, I.M. Marrucho, M.G. Freire, Aqueous biphasic systems: a benign route using cholinium-based ionic liquids, *RSC Adv.* 3 (2013) 1835–1843.
- [17] C.F.C. Marques, T. Mourão, C.M.S.S. Neves, A.S. Lima, I. Boal-Palheiros, J.A.P. Coutinho, M.G. Freire, Aqueous biphasic systems composed of ionic liquids and sodium carbonate as enhanced routes for the extraction of tetracycline, *Biotechnol. Progr.* 29 (2013) 645–654.
- [18] L.A. Ferreira, J.A. Teixeira, L.M. Mikheeva, A. Chait, B.Y. Zaslavsky, Effect of salt additives on partition of nonionic solutes in aqueous PEG–sodium sulfate two-phase system, *J. Chromatogr. A* 1218 (2011) 5031–5039.
- [19] C. Grolßmann, R. Tintinger, J. Zhu, G. Maurer, Aqueous two-phase systems of poly(ethylene glycol) and dextran— experimental results and modeling of thermodynamic properties, *Fluid Phase Equilib.* 106 (1995) 111–138.
- [20] X. Zhao, X. Xie, Y. Yan, Liquid–liquid equilibrium of aqueous two-phase systems containing poly(propylene glycol) and salt ((NH₄)₂SO₄, MgSO₄, KCl, and KAc): experiment and correlation, *Thermochim. Acta.* 516 (2011) 46–51.
- [21] K.E. Gutowski, G.A. Broker, H.D. Willauer, J.G. Huddleston, R.P. Swatloski, J.D. Holbrey, R.D. Rogers, Controlling the aqueous miscibility of ionic liquids: aqueous biphasic systems of water-miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations, *J. Am. Chem. Soc.* 125 (2003) 6632–6633.
- [22] C.M.S.S. Neves, S.P.M. Ventura, M.G. Freire, I.M. Marrucho, J.A.P. Coutinho, Evaluation of cation influence on the formation and extraction capacity of ionic-liquid-based aqueous biphasic systems, *J. Phys. Chem. B* 113 (2009) 5194–5199.
- [23] S.P.M. Ventura, C.M.S.S. Neves, M.G. Freire, I.M. Marrucho, J. Oliveira, J.A.P. Coutinho, Evaluation of anion influence on the formation and extraction capacity of ionic-liquid-based aqueous biphasic systems, *J. Phys. Chem. B* 113 (2009) 9304–9310.
- [24] M.G. Freire, J.F.B. Pereira, M. Francisco, H. Rodríguez, L.P.N. Rebelo, R.D. Rogers, J.A.P. Coutinho, Insight into the interactions that control the phase behaviour of new aqueous biphasic systems composed of polyethylene glycol polymers and ionic liquids, *Chem. Eur. J.* 18 (2012) 1831–1839.
- [25] J.F.B. Pereira, S.P.M. Ventura, F.A. Silva, S. Shahriari, M.G. Freire, J.A.P. Coutinho, Aqueous biphasic systems composed of ionic liquids and polymers: a platform for the purification of biomolecules, *Sep. Purif. Technol.* 113 (2013) 83–89.
- [26] M.G. Freire, C.L.S. Louros, L.P.N. Rebelo, J.A.P. Coutinho, Aqueous biphasic systems composed of a water-stable ionic liquid + carbohydrates and their applications, *Green Chem.* 13 (2011) 1536–1545.
- [27] B. Wang, T. Ezejias, H. Feng, H. Blaschek, Sugaring-out: a novel phase separation on extraction system, *Chem. Eng. Sci.* 63 (2008) 25958–26000.
- [28] G.B. Cardoso, T. Mourão, F.M. Pereira, A.T. Fricks, M.G. Freire, C.M.F. Soares, A.S. Lima, Aqueous two-phase systems based on acetonitrile and carbohydrates and their application to the extraction of vanillin, *Sep. Purif. Technol.* 104 (2013) 106–113.
- [29] P.B. Dhamole, P. Mahajan, H. Feng, Phase separation conditions for sugaring-out in acetonitrile– water systems, *J. Chem. Eng. Data* 55 (2010) 3803–3806.
- [30] D. Zhang, Y. Zhang, Y. Wen, K. Hou, J. Zhao, Intrinsic kinetics for the synthesis of acetonitrile from ethanol and ammonia over Co–Ni/c–Al₂O₃ catalyst, *Chem. Eng. Res. Des.* 89 (2011) 2147–2152.

- [31] M. Taha, H.L. Teng, M.J. Lee, Phase diagrams of acetonitrile or (acetone + water + EPPS) buffer phase separation systems at 298.15 K and quantum chemical modeling, *J. Chem. Thermodyn.* 54 (2012) 134–141.
- [32] Y. Gu, P.H. Shih, Salt-induced phase separation can effectively remove the acetonitrile from the protein sample after the preparative RP-HPLC, *Enzyme Microb. Technol.* 35 (2004) 592–597.
- [33] P. Pollak, G. Romeder, F. Hagedorn, H.P. Gelbke, *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley Online Library, 2000.
- [34] T. Takamuku, M. Tabata, A. Yamaguchi, J. Nishimoto, M. Kumamoto, H. Wakita, T. Yamaguchi, Liquid structure of acetonitrile-water by X-ray diffraction and infrared spectroscopy, *J. Phys. Chem. B* 102 (1998) 8880–8888.
- [35] T. Ichikawa, Y. Yano, F. Fujita, T. Kashiwabara, K. Nagao, The enhancement effect of three sugar alcohol on the fungicidal effect of benzethonium chloride toward *Candida albicans*, *J. Dent.* 36 (2008) 965–968.
- [36] B. Steinitz, Sugar alcohol display nonosmotic roles in regulating morphogenesis and metabolism in plants that do not produce polyols as primary photosynthetic products, *J. Plant Physiol.* 155 (1999) 1–8.
- [37] S. Nojiri, N. Taguchi, M. Oishi, S. Suzuki, Determination of sugar alcohols in confectioneries by high-performance liquid chromatography after nitrobenzoylation, *J. Chromatogr. A* 893 (2000) 195–200.
- [38] R.K. Talja, Y.H. Roos, Phase and state transition effects on dielectric, mechanical, and thermal properties of polyols, *Thermochim. Acta* 380 (2001) 109–121.
- [39] N.J. Walton, M.J. Mayer, A. Narbad, Vanillin, *Phytochemistry* 63 (2003) 505–515.
- [40] D.J. Fitzgerald, M. Stratford, M.J. Gasson, A. Narbad, Structure-function analysis of the vanillin molecule and its antifungal properties, *J. Agric. Food Chem.* 53 (2005) 1769–1775.
- [41] J.C. Merchuck, B.A. Andrews, J.A. Asenjo, Aqueous two-phase systems for protein separation: studies on phase inversion, *J. Chromatogr. B* 711 (1998) 285–293.
- [42] A.S. Lima, R.M. Alegre, A.J.A. Meirelles, Partitioning of pectinolytic enzyme in polyethylene glycol/potassium phosphate aqueous two-phase systems, *Cabohydr. Polym.* 50 (2002) 63–68.
- [43] Y.R. Gokarn, A. Kosky, E. Kras, A. McAuley, R.L. Remele Jr., Excipients for protein drugs, in: A. Katdare, M.V. Chaubal (Eds.), *Excipient Development for Pharmaceutical, Biotechnology, and Drug Delivery Systems*, Informa Healthcare USA Inc., New York, 2006, pp. 291–332.
- [44] M.G. Freire, P.J. Carvalho, A.M.S. Silva, L.M.N.B.F. Santos, L.P.N. Rebelo, I.M. Marrucho, J.A.P. Coutinho, Ion specific effects on the mutual solubilities of water and hydrophobic ionic liquids, *J. Phys. Chem. B* 113 (2009) 202–211.
- [45] J.F.B. Pereira, A.S. Lima, M.G. Freire, J.A.P. Coutinho, Ionic liquids as adjuvants for the tailored extraction of biomolecules in aqueous biphasic systems, *Green Chem.* 12 (2010) 1661–1669.
- [46] R. Li, Z. Jiang, L. Mao, H. Shen, Adsorbed resin phase spectrophotometric determination of vanillin or/and its derivatives, *Talanta* 47 (1998) 1121–1127.
- [47] V.E. Tarabanko, Y.V. Chelbina, V.A. Sokolenko, N.V.A. Tarabanko, Study of vanillin extraction by octylamine, *Solvent. Extr. Ion Exc.* 25 (2007) 99–107.
- [48] A. Noubigh, A. Mgaidi, M. Abderrabba, Temperature effect on the distribution of some phenolic compounds: an experimental measurement of 1-octanol/water partition coefficients, *J. Chem. Eng. Data* 55 (2010) 488–491.
- [49] Chemspider, *The Free Chemical Database*, <<http://www.chemspider.com/>>.
- [50] P.B. Dhamole, P. Mahajan, H. Feng, Sugaring out: a new method for removal of acetonitrile from preparative RP-HPLC eluent for protein purification, *Process Biochem.* 45 (2010) 1672–1676.