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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.

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/$(NH_4)_2SO_4$, $MgSO_4$, KCl or KCH_3CO_2 [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, CH_3CN, 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 foodstuffs [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),

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

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 $[\mathrm{ACN}]_T$, $[\mathrm{ACN}]_B$, $[\mathrm{Polyol}]_T$ and $[\mathrm{Polyol}]_B$,

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

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

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

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

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),

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

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,

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

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),

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