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Aqueous two-phase systems formed by biocompatible and biodegradable polysaccharides and acetonitrile

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ABSTRACT

In this work, it is shown that novel aqueous two-phase systems can be formed by the combination of acetonitrile and polysaccharides, namely dextran. Several ternary phase diagrams were determined at 25 °C for the systems composed of water + acetonitrile + dextran. The effect of the dextran molecular weight (6000, 40,000 and 100,000 g mol^{-1}) was ascertained toward their ability to undergo liquid–liquid demixing. An increase in the dextran molecular weight favors the phase separation. Furthermore, the effect of temperature (25, 35 and 45 °C) was evaluated for the system constituted by the dextran of higher molecular weight. Lower temperatures are favorable for phase separation since lower amounts of dextran and acetonitrile are required for the creation of aqueous two-phase systems. In general, acetonitrile is enriched in the top phase while dextran is majorly concentrated in the bottom phase. The applicability of this new type of two-phase systems as liquid–liquid extraction approaches was also evaluated by the study of the partition behavior of a well-known antioxidant – vanillin – and used here as a model biomolecule. The optimized conditions led to an extraction efficiency of vanillin of 95% at the acetonitrile-rich phase.

1. Introduction

Aqueous two-phase systems (ATPS) are widely applied in biotechnology for the isolation and purification of enzymes such as lipase [1–3], antioxidants, namely rutin [4] and gallic acid [5], alkaloids, such as theobromine, theophyline, nicotine and caffeine [6], antibiotics, for instance tetracycline [7,8], and antibodies [9,10]. The main advantages of ATPS rely on their scale-up possibility, on the rapid mass transfer and phase equilibrium, possibility of a continuous processing, low energy requirements, among others [11].

ATPS are usually formed by mixing two polymers in aqueous media, for instance polyethylene glycol (PEG) and dextran [12,13] or PEG and maltodextrin [14], or by one polymer and one salt, such as PEG and phosphate-based salts [15–17] or PEG and citrate-based salts [18,19]. However, some other pairs of phase-forming components can be used in the creation of alternative ATPS, such as alcohol + salt [20], ionic liquid + salt [21–23], ionic liquid + PEG [24,25] and ionic liquid + carbohydrate [26].

Previously we have also demonstrated that alternative aqueous biphasic systems can be created by combining acetonitrile and carbohydrates (monosaccharides and disaccharides) [27] as well as with polyols [28]. In this context, we attempted now the formation of novel ATPS formed by acetonitrile and polysaccharides, namely dextran.

Dextran is a water soluble biopolymer produced by a variety of lactic acid bacteria, such as *Leuconostoc* sp., and which presents two valuable properties: biodegradability and biocompatibility [29]. The chemical structure of dextran is predominantly formed by 95% of linear α-(1 → 6) linkages as the main backbone and 5% of α-(1 → 3) branch linkages [30]. This homopolymer of glucose has several targeted industrial applications, varying from food, cosmetic, and pharmaceutical to oil drilling industries [31].

Acetonitrile (CH_3CN) – ACN – is an interesting solvent due to its properties; it is aprotic and strongly polar and is obtained as a by-product from the manufacture of acrylonitrile [32]. ACN is widely used by industry in the production of perfumes, rubber products, pesticides or pharmaceuticals [33], and in chromatography processes as a mobile phase in high performance liquid chromatogra-

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phy – HPLC [34,35]. This solvent is miscible with water in all proportions [36] and its molecules are unable to strongly associate with themselves leaving a hydrogen-bond network formed by water [37,38].

The aim of the present work is to study novel aqueous two-phase systems based on acetonitrile and several dextrans of different molecular weights. The ternary phase diagrams were determined at 25 °C and the effect of the polysaccharide molecular weight was evaluated. Moreover, the effect of temperature (25, 35 and 45°) through the phase diagrams behavior was also addressed for the ATPS constituted by the dextran of higher molecular weight. These systems were also ascertained on what regards their applicability on extraction routes, and in particular on the extraction of a well-known antioxidant, vanillin (4-hydroxy-3-methoxybenzaldehyde). Vanillin is the major flavor constituent of natural vanilla – *Vanilla plantifolia*, extracted at a rate of 12,000 ton/year [39]. Vanillin is widely used as a flavoring material in confectionery, food products, beverages, perfumes and in pharmaceutical preparations [40]. Natural vanillin costs between 1200 and 4000 USD/kg, while synthetic vanillin costs around 15 USD/kg [41]. However, the chemical process usually leads to a low quality vanillin that further requires a sensitive extraction and purification procedure [42], and for which ATPS can be foreseen as an alternative approach.

2. Material and methods

2.1. Materials

The ATPS studied in this work were formed by dextran from *Leuconostoc* spp. (M_w = 100,000 g mol^{-1} – Dx-100; 40,000 g mol^{-1} – Dx-40; and 6000 g mol^{-1} – Dx-6) and acetonitrile. Dextran and acetonitrile (purity of 99.9 wt%), as well as vanillin (>99 wt% pure) were purchased from Sigma–Aldrich. The chemical structures of the phase-forming components of the ATPS studied and of the target biomolecule used in the partitioning experiments are shown in Fig. 1. Distilled and deionized water was used in all experiments.

2.2. Phase diagrams and tie-lines

The ternary phase diagrams for water, acetonitrile and the different molecular weight dextran were determined at 25, 35 and 45 (± 1) °C in atmospheric pressure by the cloud point titration method [43]. Pure acetonitrile (since it is water soluble in the whole composition range and no precipitation effects were observed during the determination of the liquid–liquid saturation curves) and a solution of dextran (Dx-100, Dx-40 and Dx-6) at 20 wt% (a concentration close and below their saturation limit in water at room temperature) was previously prepared and used for the determination of the binodal curves. Repetitive drop-wise addition of pure acetonitrile to the aqueous solution of dextran was carried out until the detection of a cloudy solution, followed by the drop-wise addition of ultra-pure water until the inspection of a monophasic region (clear and limpid solution). These additions were carried out under continuous stirring and the saturation curves were determined gravimetrically within ±10^{-4} g.

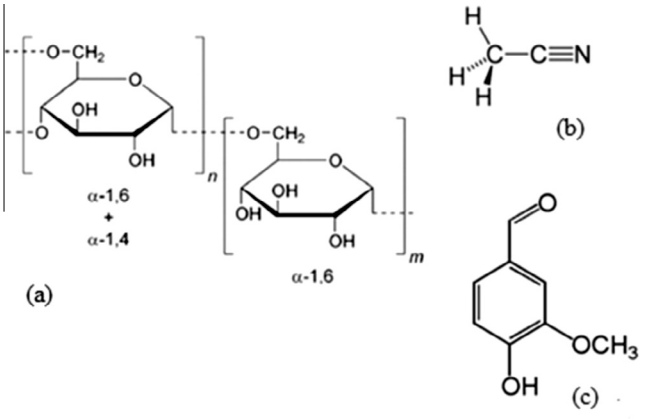

Fig. 1. Chemical structures of the phase-forming components used in the ATPS formation and of the biomolecule used as a partitioning solute: (a) dextran; (b) acetonitrile; and (c) vanillin.

The tie-lines (TLs) were obtained through a gravimetric method originally described by Merchuk et al. [44]. Several mixtures at the biphasic region of the ternary systems were prepared (total of 5 g), vigorously stirred, and allowed to reach equilibrium and phase separation, for a minimum of 18 h at 25, 35 and 45 (± 1) °C. The temperature was maintained by means of a temperature-controlled water-bath. Initially, aqueous solutions of each dextran were prepared followed by the addition of pure ACN to reach a specific mixture composition. After the equilibration step, the top and bottom phases were carefully separated and weighted within ±10^{-5} 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),

$$Y = A\exp\{(B \times X^{0.5}) - (C \times X^3)\} \quad (1)$$

where Y and X are the acetonitrile and dextran weight fraction percentages, respectively, and A, B and C are constant values obtained by the fitting of the experimental saturation curve data.

The determination of the TLs, describing the composition of Y and X at the top (T) and bottom phases (B) – Y_T, Y_B, X_T and X_B – was then accomplished by solving a system of four equations derived from Eq. (1) [43,44]. The respective tie-line lengths (TLLs) were determined through the application of Eq. (2),

$$\mathrm{TLL} = \sqrt{(X_T - X_B)^2 + (Y_T - Y_B)^2} \quad (2)$$

2.3. Partitioning of vanillin

Dextran and an aqueous solution of vanillin (0.1 g L^{-1}) were initially mixed, and after the complete homogenization, acetonitrile was added to reach the chosen mixture composition. Each ATPS (total of 5 g) was prepared in glass tubes. After the complete mixing of all components, for a given mixture composition, each tube was immersed in a thermostatic water bath from 5 to 45 (± 1) °C for at least 18 h. After equilibration, both phases were separated for the quantification of vanillin. At least three independent replicates were made and the average partition coefficients and associated standard deviations were therefore determined.

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. At the concentration of vanillin used, dilutions in the order of 1:50 (v:v) were carried out in distilled water. At these dilutions there are no interferences by the phase-forming components at 280 nm in the quantification of vanillin. Therefore, no blanks were needed for the vanillin quantification at the dilutions carried out.

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

$$K_{Van} = \frac{C_{Van,T}}{C_{Van,B}} \quad (3)$$