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Aqueous two-phase systems based on acetonitrile and carbohydrates and their application to the extraction of vanillin

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ABSTRACT

Aqueous two-phase systems (ATPS) are an important technique for the extraction and purification of biomolecules. In aqueous media, many pairs of solutes can be used to prepare ATPS and, in spite of their interest, scarce attention has been given to the use of mono- and disaccharides. In this context, this work addresses the use of acetonitrile and carbohydrates to prepare aqueous two-phase systems and their application in the partition of vanillin. The phase diagrams were determined at 298 K and the impact of the carbohydrate structure on the liquid–liquid demixing was evaluated. Besides high purity carbohydrates, commercial food grade sugars were also tested and are shown to be able to form ATPS. Their impurities affect, however, the phase separation and tend to reduce the two-phase region. The studied ATPS were investigated for the extraction of vanillin that favorably partitions towards the acetonitrile-rich phase with partition coefficients higher than 3.0 and recoveries up to 91% attained in a single step.

1. Introduction

Aqueous two-phase systems (ATPS) are formed when a pair of solutes leads to the formation of two macroscopic liquid phases when dissolved in water above certain concentrations. This phenomenon was first observed by Beijerinck in 1896; however, it was not until 1956 that the potential use of these systems as a separation technique in biotechnology was realized [1]. Nowadays, the scientific literature is prodigal in studies concerning ATPS for the extraction and purification of biomolecules such as antibiotics (e.g. ciprofloxacin [2]), anthocyanins [3], amino acids (e.g. L-methionine [4]), proteins (e.g. lectin [5]) and enzymes (e.g. lipase [6,7]).

Their versatility, high efficiency, high yield, improved purification factor, selectivity, low-cost and fast mass transfer rates are the main advantages of ATPS [8,9]. Originally, these systems were based on aqueous mixtures of two incompatible polymers such as polyethylene glycol (PEG), dextran and maltodextrin [10–12]. Nevertheless, the high viscosity of the coexisting phases led to the development of systems formed by polymers and inorganic salts such as potassium phosphate, sodium citrate and calcium chloride [13–15]. Other components like organic solvents have also been used, e.g. alcohols [16,17], but the application of this type of systems is limited due to the interference of alcohols in the biological activity of several biomolecules. Recently, a new kind of ATPS was reported using ionic liquids (ILs) and inorganic salts [18–20] or saccharides [21,22]. ILs have also been proposed as potential adjuvants in conventional polymer-salt-based ATPS aiming at tailoring their extraction efficiency for particular added-value compounds [23].

Scarce attention has been devoted to the use of carbohydrates as potential substitutes of inorganic salts and polymers for the formation of ATPS. The use of sugars in ATPS was already addressed by Wang and co-workers [24] in combination with acetonitrile, as well as by Wu and co-workers [21] and Freire and co-workers [22] in combination with ionic liquids.

Acetonitrile, CH_3CN, also known as cyanomethane or methyl cyanide, is a colorless aprotic solvent which is fully miscible in water at temperatures close to room temperature. The acetonitrile molecules do not strongly interact with themselves and tend to form a hydrogen bond network with water molecules [25]. Acetonitrile is an important chemical widely used in industry in the production of perfumes, rubber products, pesticides or pharmaceuticals. It is also usually applied as a mobile phase in high-performance liquid chromatographic or as solvent to extract fatty acids from animal and vegetable oils [26]. The extraction of biomolecules using acetonitrile–water systems was attempted at

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temperatures below 0 °C [27] whereas extractions of Pt(IV), Pd(II) and Rh(III) at room temperature were performed using ATPS composed of acetonitrile and carbohydrates [28]. No reports were found regarding the extraction of vanillin in these systems.

Carbohydrates, with the general formula $(CH_2O)_x$, are a large and diverse group of organic compounds including sugars, cellulose and starch. These molecules are non-charged, biodegradable, nontoxic and a renewable feedstock. They are classified in monosaccharides, oligosaccharides (2–10 linked monosaccharides) and polysaccharides (>10 linked monosaccharides) [29]. Carbohydrates are polyhydroxy aldehydes or ketones with high affinity for water since several –OH groups, with a dual donor/acceptor character, can be involved in hydrogen bonding, and thus, present an inherent salting-out character (also known as *sugaring-out* effect). Therefore, carbohydrates are potential substitutes to conventional salts used in the formation of ATPS with the advantage of creating more friendly environments to the biomolecules.

This work is focused on the development of novel carbohydrate-acetonitrile-based ATPS and on the evaluation of the carbohydrate structure through the phase separation ability. For that purpose, various carbohydrates were investigated, namely monosaccharides (glucose, mannose, galactose, xylose, arabinose, and fructose) and disaccharides (sucrose and mannose). Moreover, commercial sucrose, fructose and glucose commonly used in the food industry were also tested. The ternary phase diagrams were determined at 298 K, and the respective binodal curves, tie-lines and tie-line lengths are reported. Finally, to appraise on the extractive potential of the proposed ATPS, the partitioning of a common antioxidant, vanillin, was additionally investigated.

2. Materials and methods

2.1. Materials

The ATPS studied in this work were formed by several carbohydrates and acetonitrile. The carbohydrates used were sucrose (>99.5 wt.% pure from Himedia), D-(+)-maltose (⩾98.0 wt.% pure from Sigma), D-(+)-glucose (>99.5 wt.% pure from Scharlau), D-(+)-mannose (>99.0 wt.% pure from Aldrich), D-(+)-galactose (>98.0 wt.% pure from GPR Rectapur), D-(+)-xylose (⩾99.0 wt.% pure from Carlo Erba), L-(+)-arabinose (>99.0 wt.% pure from BHD Biochemicals), and D-(−)-fructose (>98.0 wt.% pure from Panreac). The acetonitrile, HPLC grade with a purity of 99.9 wt.%, was purchased from Sigma. The vanillin (>99 wt.% pure) was supplied by Aldrich. Commercial fructose, sucrose and glucose are of food grade and were obtained in a local supermarket at Aracaju, Sergipe, Brazil. Distilled and deionized water was used in all experiments.

2.2. Phase diagrams and tie-lines

The studied systems comprise acetonitrile and different carbohydrates, and which can be divided into monosaccharides (D-(+)-glucose, D-(+)-mannose, D-(+)-galactose, D-(+)-xylose, L-(+)-arabinose, and D-(−)-fructose) and disaccharides (sucrose and D-(+)-maltose). The ternary phase diagrams were determined at 298 (±1) K and at atmospheric pressure by the cloud point titration method. Stock solutions of the carbohydrates (≈40–70 wt.%, depending on the carbohydrate solubility saturation in water) and acetonitrile (≈80 wt.%) were previously prepared and used for the determination of the phase diagrams. Repetitive drop-wise addition of the carbohydrate 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). All these additions were carried out under continuous stirring.

The tie-lines (TLs) were obtained through a gravimetric method originally described by Merchuck and co-workers [30]. For the calculation of TLs, 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, both top and bottom phases were separated and weighted using a Metller Toledo AL-204 balance (±0.0001 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 the following equation:

$$Y = A \times \exp(BX^{0.5} - CX^3) \quad (1)$$

where Y and X are the acetonitrile and carbohydrate weight percentages, respectively, and A, B and C are constants parameters obtained by the regression.

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 Y_T, Y_B, X_T and X_B,

$$Y_T = A \ \exp\left(BX_T^{0.5} - CX_T^3\right) \quad (2)$$

$$Y_B = A \ \exp\left(BX_B^{0.5} - CX_B^3\right) \quad (3)$$

$$Y_T = (Y_M/\alpha) - ((1-\alpha)/\alpha)Y_B \quad (4)$$

$$X_T = (X_M/\alpha) - ((1-\alpha)/\alpha)X_B \quad (5)$$

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 mass of the mixture. The system solution results in the acetonitrile and carbohydrate concentration in the top and bottom phases, and thus, TLs can be simply represented.

The tie-line length (TLL) was determined through the application of following equation:

$$\mathrm{TLL} = \sqrt{(X_T - X_B)^2 - (Y_T - Y_B)^2} \quad (6)$$

2.3. Partitioning of vanillin

The partitioning systems for vanillin were prepared in graduated glass centrifuge tubes weighing the appropriate amounts of carbohydrate, acetonitrile and an aqueous solution containing vanillin. Vanillin was at a concentration of 0.4 g cm^{-3} in the initial aqueous solution. After the complete mixing of all components, each system was centrifuged at 3000*g* for 10 min to favor the phase separation, and then each tube was placed in a thermostatic bath at 298.15 (±0.01) K for at least 18 h. The volume of each phase was further measured. After, both phases were carefully separated for the quantification of vanillin and for the determination of their density, viscosity and pH values.

The density and viscosity of the bottom phase (carbohydrate-rich) were determined in the temperature range from (298.15 to 323.15) K, and at atmospheric pressure, using an automated SVM 300 Anton Paar rotational Stabinger viscosimeter–densimeter. The pH values (±0.02) of the top and bottom phases were measured at 298 K using a HI 9321 Microprocessor pH meter (HANNA Instruments).

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 making use of a calibration curve previously established.

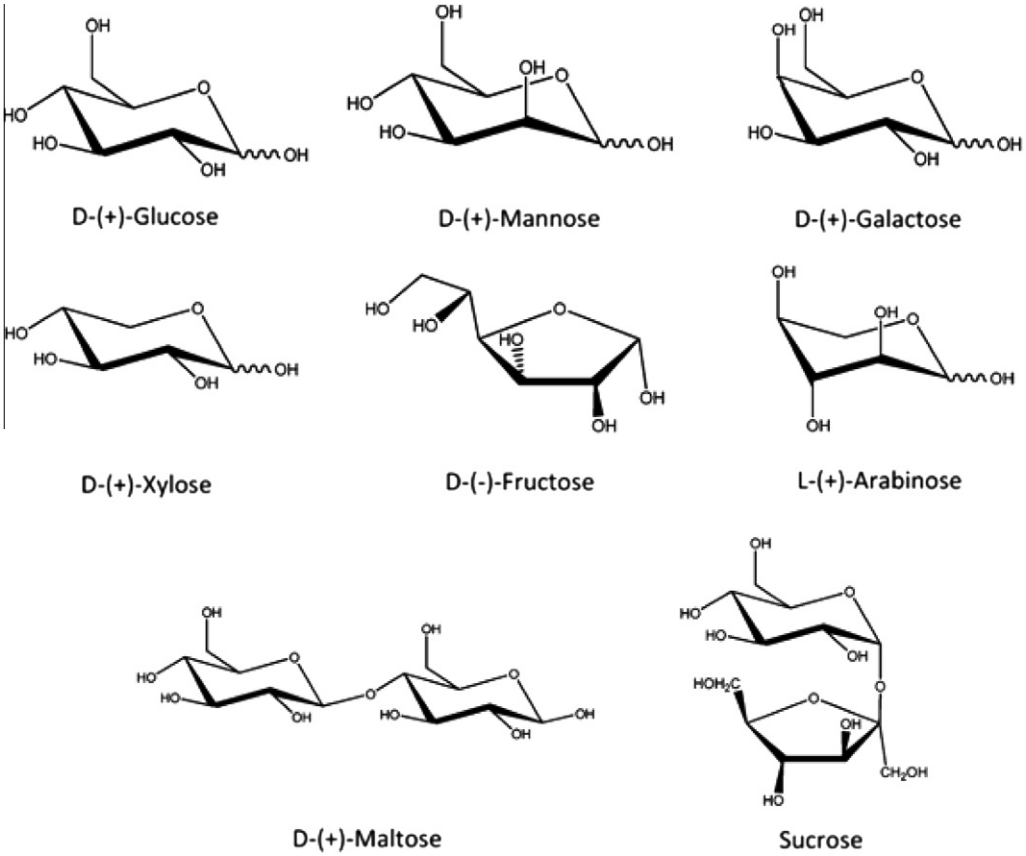

Fig. 1. Chemical structure of the monosaccharides and disaccharides studied.

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

$$K_{van} = \frac{C_T}{C_B} \tag{7}$$

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 (carbohydrate-rich) phases, respectively. The recoveries of vanillin (R_T) for the top phase was evaluated using the following equation:

$$R_T = \frac{C_T}{(C_T + C_B)} \times 100 \tag{8}$$

where C and the subscripts T and B are described above.

3. Results and discussion

3.1. Phase diagrams and tie-lines

The systems investigated in this work are formed by acetonitrile and a large array of carbohydrates. The molecular structures of the studied carbohydrates are depicted in Fig. 1. The experimental phase diagrams for each monosaccharide (D-(+)-glucose, D-(+)-mannose, D-(+)-galactose, D-(+)-xylose, L-(+)-arabinose, and D-(−)-fructose), disaccharide (sucrose and D-(+)-maltose) and commercial carbohydrates (glucose, fructose and sucrose), were determined at 298 K and atmospheric pressure. The corresponding phase diagrams are presented in Figs. 2–4 and allow the analysis of the carbohydrate potential to induce an ATPS. All binodal curves are represented in molality units to avoid disparities in the evaluation of the carbohydrate potential in inducing the liquid–liquid demixing and which could simple result from their distinct molecular weights. The experimental weight fraction data are provided in the Supplementary information (Tables S1–S4).

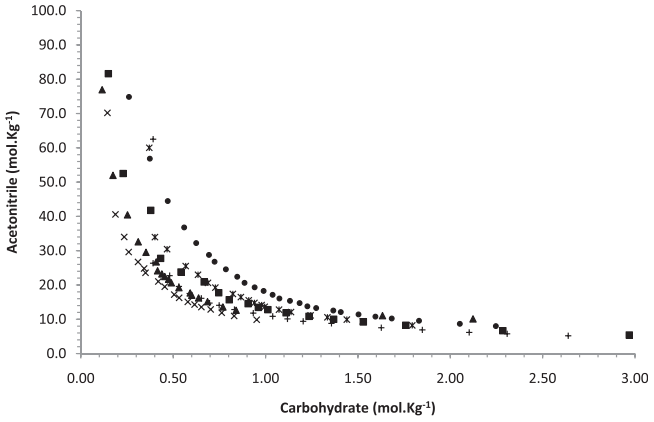

Fig. 2. Phase diagrams for the ternary systems composed of acetonitrile + carbohydrate + water at 298 K. ■ – D-(−)-Fructose, ▲ – D-(+)-Glucose, ● – D-(+)-Xylose, × – D-(+)-Galactose, ∗ – L-(+)-Arabinose, + – D-(+)-Mannose.

The addition of a concentrated carbohydrate aqueous solution to acetonitrile leads to phase separation: a top acetonitrile-rich phase and a bottom carbohydrate-rich phase. According to Galema and co-workers [31] the hydration of carbohydrates depends on the ratio between the axial and equatorial hydroxyl groups. Thus, the carbohydrates can be classified into three groups of decreasing hydration: (a) both OH(2) and OH(4) are axial (D-(+)-talose); (b) OH(4) is equatorial and OH(2) is either axial (D-(+)-mannose) or

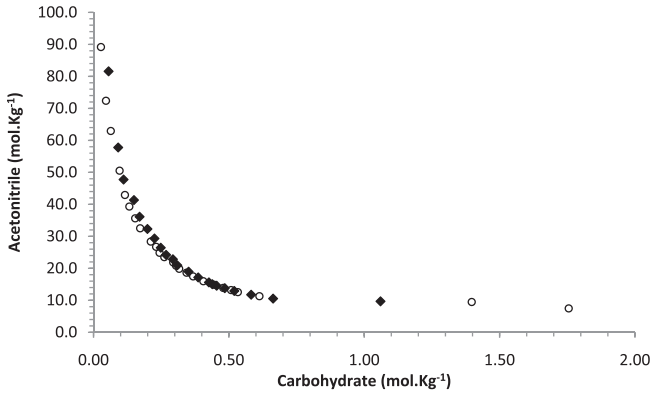

Fig. 3. Phase diagrams for the ternary systems composed of acetonitrile + carbohydrate + water at 298 K. ♦ – Sucrose; ○ – D-(+)-Maltose.

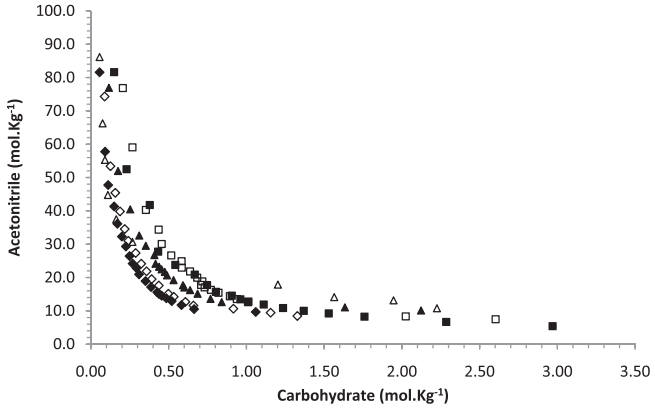

Fig. 4. Phase diagrams for the ternary systems composed of acetonitrile + carbohydrate + water at 298 K. ♦ – Sucrose; ◇ – Commercial sucrose, ■ – D-(−)-Fructose; □ – Commercial fructose, ▲ – D-(+)-Glucose, △ – Commercial glucose.

equatorial (D-(+)-glucose); (c) OH(4) is axial and OH(2) is equatorial (D-(+)-galactose). The binodal curves for the systems with acetonitrile and the various monosaccharides, and depicted in Fig. 2 shows indeed an increasing tendency of phase separation proportional to their hydration ability: D-(+)-xylose < L-(+)-arabinose ≈ D-(−)-fructose < D-(+)-glucose < D-(+)-mannose < D-(+)-galactose.

Aldoses with five carbon atoms such as L-(+)-arabinose and D-(+)-xylose are less effective in promoting the ATPS formation due to a lower number of hydroxyl groups and, consequently, a lower hydration ability and less favorable conformation for hydrogen bounding with water.

The comparison between the isomers D-(+)-glucose (an aldose with a 6-sided ring) and D-(−)-fructose (a ketose with a 5-sided ring) suggests that ketoses are less effective in inducing the formation of two aqueous phases. Epimers of aldoses with six carbon atoms, which are distinguished by the position of the hydroxyl group at carbon 2, epimers D-(+)-glucose and D-(+)-mannose, have similar abilities to induce ATPS formation. However, the position of the hydroxyl group at carbon 4, as for the epimers D-(+)-glucose and D-(+)-galactose, is relevant and facilitates the phase formation with D-(+)-galactose. Therefore, the orientation of the hydroxyl at carbon 4 plays an important role in the ATPS formation ability.

The phase diagrams shown in Fig. 3 show the effect of the two disaccharides on the formation of ATPS. Sucrose consists of glucose and fructose linked by a glycosidic bond while maltose is formed by two glucose units. These disaccharides have similar capabilities for ATPS formation in systems formed with acetonitrile at 298 K.

Fig. 4 shows the comparison between the high purity and commercial forms of sucrose, glucose and fructose towards the ATPS formation. The binodal curves show a decreasing order in inducing ATPS according to: sucrose > commercial sucrose > glucose > fructose ≈ commercial fructose > commercial glucose. The use of commercial carbohydrates leads to a decrease of the biphasic region envelope which may be a result of a low purity level and to the presence of impurities. The difference was more pronounced when using commercial glucose (corn syrup) and glucose due to the presence of other sugars such as isomaltose, maltose and maltotriose and as already pointed out by Pontoh and Low [32].

All the binodal curves were fitted using Eq. (1). The regression coefficients (R^2) and the fitted parameters *A*, *B* and *C*, estimated by least-squares regression, are reported in Table 1. Fig. 5 presents four examples of the correlation of the data for the systems composed of acetonitrile + carbohydrate (D-(−)-fructose, sucrose, D-(+)-glucose or L-(+)-arabinose) + water. The results for the remaining systems are presented in Supplementary information (Figs. S1–S4). To complete the phase diagrams, several TLs and respective TLLs were further calculated and their values are reported in Table 2. Some examples of the TLs representation are shown in Fig. 5.

The application of ATPS in industrial processes for biomolecules extraction and purification also depends on their physical properties. Particularly, large differences in the densities of both phases favor the phase separation whereas low viscosities increase the mass transfer coefficients. Hence, the characterization of the densities and viscosities of the phases are important issues when envisaging the process scale-up. In this sense, the densities and viscosities for the sugar-rich phase were here determined. It should be remarked that acetonitrile, at 298.15 K, presents a density of 0.7766 g cm^{-3} and a viscosity of 0.3369 mPa s [33]. These values are below the values of pure water at the same temperature (0.9991 g cm^{-3} and 1.0 mPa s) [34] and thus the properties of the acetonitrile-rich phase were not determined due to a lack of a proper equipment to measure densities and viscosities within this range. Furthermore, the sugar composition (the more dense and viscous compound) in the acetonitrile-rich phase is always below 7 wt.% (Supplementary information – Tables S1–S4). For the

Table 1
Adjusted parameters obtained from the regression of Eq. (1) for the ternary systems composed of acetonitrile + carbohydrate + water at 298 K and atmospheric pressure.

Carbohydrate	Regression parameters			
	A	*B*	*C*	R^2
Sucrose	114.5 ± 2.2	−0.280 ± 0.008	$2.8 \times 10^{-5} \pm 0.5 \times 10^{-5}$	0.9964
D-(+)-Maltose	102.0 ± 1.3	−0.245 ± 0.006	$3.8 \times 10^{-5} \pm 0.6 \times 10^{-5}$	0.9962
D-(+)-Glucose	122.6 ± 2.7	−0.332 ± 0.011	$4.4 \times 10^{-5} \pm 1.3 \times 10^{-5}$	0.9962
D-(+)-Mannose	127.6 ± 5.8	−0.356 ± 0.014	$2.8 \times 10^{-16} \pm 1.7 \times 10^{-6}$	0.9954
D-(+)-Galactose	123.3 ± 3.0	−0.375 ± 0.011	$1.1 \times 10^{-5} \pm 9.0 \times 10^{-6}$	0.9978
D-(−)-Fructose	134.6 ± 2.2	−0.342 ± 0.006	$7.1 \times 10^{-16} \pm 1.1 \times 10^{-6}$	0.9978
D-(+)-Xylose	177.7 ± 6.2	−0.394 ± 0.012	$3.4 \times 10^{-6} \pm 3.2 \times 10^{-6}$	0.9960
L-(+)-Arabinose	151.6 ± 5.6	−0.393 ± 0.006	$4.1 \times 10^{-7} \pm 4.1 \times 10^{-6}$	0.9965

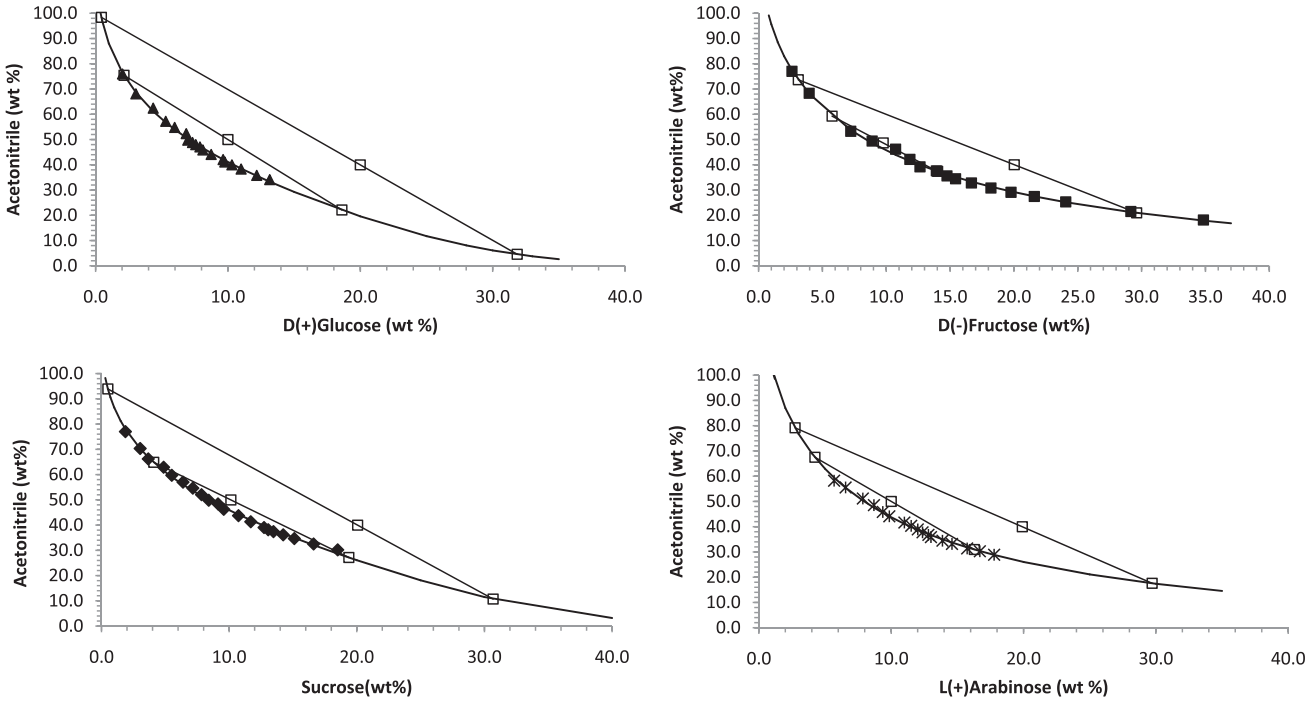

Fig. 5. Phase diagrams for the ternary systems composed of acetonitrile + carbohydrate at 298 K (■ – D-(+)-Fructose, ▲ – D-(+)-Glucose, ∗ – D-(+)-Arabinose, and ♦ – Sucrose), □ – TL data, (—) binodal adjusted data through Eq. (1).

Table 2
Weight fraction compositions (TLs) at the top (T) and bottom (B) phases, initial mixture composition (M), and respective TLLs for the several systems composed of acetonitrile (Y) and carbohydrate (X) at 298 K and atmospheric pressure.

Carbohydrate	100 × Weight fraction (wt.%)						
	Y_M	X_M	Y_T	X_T	Y_B	X_B	TLL
Sucrose	39.97 ± 0.03	20.07 ± 0.05	93.91 ± 0.01	0.50 ± 1.99	10.71 ± 0.09	30.68 ± 0.03	88.51
	49.94 ± 0.02	10.14 ± 0.10	64.86 ± 0.02	4.09 ± 0.24	27.15 ± 0.04	19.38 ± 0.05	40.69
D-(+)-Maltose	40.05 ± 0.03	19.96 ± 0.05	92.15 ± 0.01	0.17 ± 5.84	6.59 ± 0.15	32.66 ± 0.03	91.52
	49.95 ± 0.02	10.02 ± 0.10	65.24 ± 0.02	3.31 ± 0.30	18.13 ± 0.06	23.97 ± 0.04	51.44
D-(+)-Glucose	39.98 ± 0.03	19.99 ± 0.05	98.37 ± 0.01	0.44 ± 2.26	4.56 ± 0.03	31.86 ± 0.22	58.98
	49.98 ± 0.02	10.00 ± 0.10	75.42 ± 0.02	2.14 ± 0.47	22.09 ± 0.05	18.61 ± 0.05	55.81
D-(+)-Mannose	40.04 ± 0.03	20.00 ± 0.05	77.14 ± 0.02	1.99 ± 0.50	17.62 ± 0.06	30.88 ± 0.03	66.15
	49.92 ± 0.02	9.98 ± 0.10	69.29 ± 0.02	2.93 ± 0.34	28.34 ± 0.04	17.84 ± 0.06	43.58
D-(+)-Galactose	49.99 ± 0.02	10.01 ± 0.10	73.92 ± 0.02	1.87 ± 0.54	21.46 ± 0.01	19.73 ± 0.05	55.42
	45.00 ± 0.02	8.01 ± 0.12	32.02 ± 0.03	12.54 ± 0.08	54.21 ± 0.02	4.80 ± 0.21	23.50
D-(−)-Fructose	40.03 ± 0.03	20.03 ± 0.05	73.66 ± 0.02	3.11 ± 0.32	20.96 ± 0.03	29.62 ± 0.05	58.98
	48.65 ± 0.02	9.76 ± 0.10	59.28 ± 0.02	5.76 ± 0.17	37.61 ± 0.03	13.92 ± 0.07	23.15
D-(+)-Xylose	39.95 ± 0.03	20.05 ± 0.05	79.31 ± 0.01	4.20 ± 0.24	20.77 ± 0.01	27.78 ± 0.04	63.11
	49.99 ± 0.02	10.02 ± 0.10	50.06 ± 0.02	10.26 ± 0.10	50.06 ± 0.02	10.26 ± 0.10	–
L-(+)-Arabinose	39.97 ± 0.03	19.91 ± 0.05	79.15 ± 0.02	2.73 ± 0.37	17.59 ± 0.01	29.72 ± 0.03	67.21
	50.01 ± 0.02	10.00 ± 0.02	67.51 ± 0.02	4.24 ± 0.24	30.97 ± 0.03	16.28 ± 0.06	38.47

carbohydrate-rich phase the densities range from 1.0004 g cm^{-3} (galactose) to 1.0991 g cm^{-3} (glucose) whereas the viscosities are between 1.3995 mPa s (galactose) and 3.5977 mPa s (maltose). The densities and viscosities at 298.15 K and 323.15 K for the carbohydrate-rich phase of different systems are presented in Table 3. These values are significantly lower than the viscosities obtained for ATPS constituted by polymers such as polypropylene glycol (polymer-rich phase: 18.1–64.7 mPa s and Na_2SO_4-rich phase: 1.91–3.73 mPa s [35]) or ionic liquids (ionic-liquid-rich phase: 8.0–1.03 mPa s [8]). The low viscosity of acetonitrile–carbohydrate ATPS favor the mass transfer on extraction processes as well as the phases handling at an industrial scale.

3.2. Partitioning of vanillin

The application of the investigated systems as alternative extractive techniques was studied using the partitioning of vanillin, a widely used flavoring agent [36] as model system. For each system, two different compositions were investigated: 20 wt.% carbohydrate + 40 wt.% acetonitrile and 10 wt.% carbohydrate + 50 wt.% acetonitrile. The pH values of both phases of each ATPS are presented in Table 4. These values range between 5.48 and 7.06. Vanillin is present as a neutral molecule at these conditions [37]. The influence of the pH in the chemical structure of vanillin is shown in Fig. S5 in Supplementary information.

Table 3
Experimental value of densities (ρ) and viscosities (η) of the carbohydrate-rich phase at 298.15 K and 323.15 K.

Carbohydrate	System	ρ (g cm^{-3})		η (mPa s^{-1})	
		298.15 K	323.15 K	298.15 K	323.15 K
Sucrose	*A*	1.0984	1.0808	3.5606	1.8145
	B	1.0535	1.0343	2.2117	1.1956
D-(+)-Maltose	*A*	1.0968	1.0793	3.5977	1.8345
	B	1.0678	1.0495	2.5711	1.3809
D-(+)-Glucose	*A*	1.0991	1.0825	3.2582	1.6831
	B	1.0358	1.0173	1.8355	1.0510
D-(+)-Mannose	*A*	1.0990	1.0813	3.0513	1.6009
	B	1.0314	1.0121	1.7401	0.9794
D-(+)-Galactose	*B*	1.0429	1.0243	1.8812	1.0643
	C	1.0004	0,9797	1.3995	0.8233
D-(+)-Xylose	*A*	1.0738	1.0550	2.5039	1.3760
	B	1.0091	0.9872	1.4637	0.8606
L-(+)-Arabinose	*A*	1.0919	1.0729	2.6091	1.4102
	B	1.0288	1.0083	1.6412	0.9372
D-(−)-Frutose	*A*	1.0977	1.0782	2.8406	1.4917
	B	1.0228	1.0021	1.6096	0,9216

A: 40 wt.% acetonitrile + 20 wt.% carbohydrate; *B*: 50 wt.% acetonitrile + 10 wt.% carbohydrate); and *C* (45 wt.% acetonitrile + 8 wt.% carbohydrate).

Table 4
pH values of the acetonitrile(top)- and carbohydrate(bottom)-rich phases at 298 K.

Carbohydrate	System *A*		System *B*	
	Top phase	Bottom phase	Top phase	Bottom phase
Sucrose	7.06	6.35	6.97	6.76
D-(+)-Maltose	6.55	5.97	6.92	6.64
D-(+)-Glucose	6.84	5.69	6.98	6.28
D-(+)-Mannose	7.00	6.28	6.89	6.41
D-(+)-Galactose	–	–	6.81	6.09
D-(+)-Xylose	6.64	5.83	5.96	5.95
L-(+)-Arabinose	6.78	5.73	6.34	6.14
D-(−)-Fructose	6.36	5.80	6.34	5.48

A: 40 wt.% acetonitrile + 20 wt.% carbohydrate; *B*: 50 wt.% acetonitrile + 10 wt.% carbohydrate.

For all systems the partition coefficients of vanillin are higher than 1 and demonstrate the preferential affinity of vanillin towards the acetonitrile-rich phase (Fig. 6). This preferential migration is in good agreement with the octanol–water partition coefficient of vanillin ($\log K_{ow}$ = 1.19 [38]) which indicates the preferential affinity of vanillin for more hydrophobic phases. Acetonitrile ($\log K_{ow}$ = −0.17) is indeed more hydrophobic than carbohydrates ($-2.30 < \log K_{ow} < -4.70$) and support the trend observed (http://chemspider.com).

The effect of system composition, namely the TLL, on the extraction ability was studied by changing the point of the initial mixture, acetonitrile–carbohydrate, from 40–20 wt.% to 50–10 wt.%. The composition of each phase is described in Table 2. A large decrease in the partition coefficient was observed with the system composed of mannose (K_{van} = (9.67 ± 0.04) and (3.66 ± 0.01)) with the decrease of the TLL. An opposite pattern was verified with the system constituted by xylose and for which the partition coefficient increases from (6.95 ± 0.01) to (8.74 ± 0.03) with a decrease in the TLL. It should be remarked that Gu and Zhang [27] studied the partitioning of various biomolecules in the system composed of acetonitrile and water at sub-zero temperatures (−10 °C). Most compounds preferentially partitioned for the water-rich phase [27] contrarily to what was observed here.

The K_{van} rank at different mixtures compositions is similar to the order of formation of ATPS previously noted. For instance, for the mixture composition constituted by 20 wt.% of carbohydrate and 40 wt.% of acetonitrile, the order of partition coefficients is,

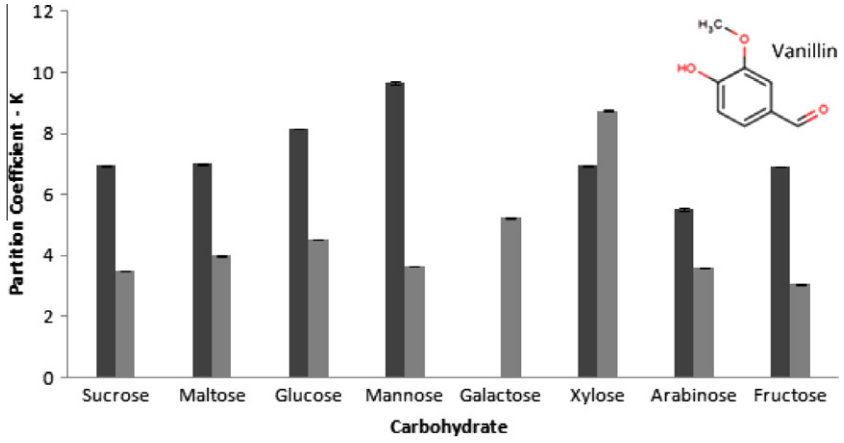

Fig. 6. Partition coefficient of vanillin between the acetonitrile- and the carbohydrate-rich phase at 298 K. ■ – 40–20 wt.% acetonitrile–carbohydrate and ■ 50–10 wt.% acetonitrile–carbohydrate.

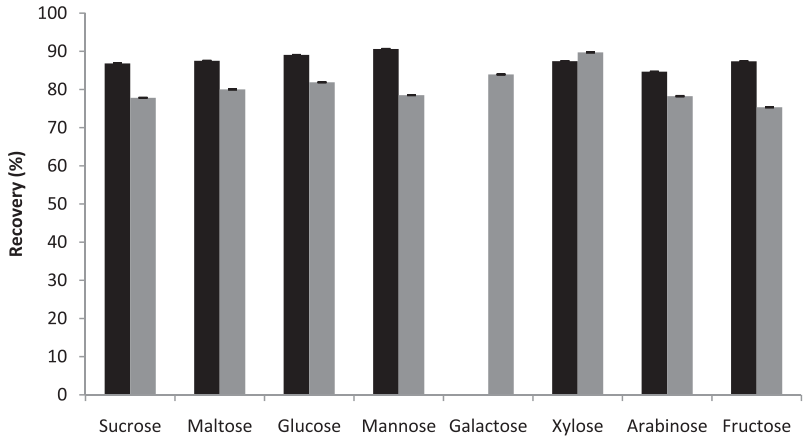

Fig. 7. Recovery of vanillin in the top phase (acetonitrile-rich phase) at 298 K. ■ – 40–20 wt.% acetonitrile–carbohydrate and ■ 50–10 wt.% acetonitrile–carbohydrate.

Aldoses with 6C:	D-(+)-glucose < D-(+)-mannose
Aldoses with 5C:	L-(+)-arabinose < D-(+)-xylose
Monossacharides:	Aldoses with 5C ≈ D-(−)-fructose (Ketose) < Aldose with 6C
Dissacharides:	Sucrose ≈ D-(+)-maltose

In addition, for the mixture point composed of 10 wt.% of carbohydrate and 50 wt.% of acetonitrile, the partition coefficient values increase according to,

Aldoses with 6C:	D-(+)-mannose < D-(+)-glucose < D-(+)-galactose
Aldoses with 5C:	L-(+)-arabinose < D-(+)-xylose
Monossacharides:	Aldoses with 5C ≈ D-(−)-fructose (Ketose) < Aldose with 6C
Dissacharides:	Sucrose < D-(+)-maltose

All the results indicate that the hydration capacity of the carbohydrate leads to an exclusion effect of the biomolecule towards the acetonitrile-rich phase and confirms the *sugaring-out* effect reported by other authors [24,39]. In addition, for aldoses with six carbon atoms, the order is inversely proportional to the dielectric constant of each carbohydrate: D-(+)-mannose (4.25) ≈ D-(+)-glucose (4.27) > D-(+)-galactose (3.28) [40].

Based on the quantification of vanillin in each phase, the recoveries of vanillin at the acetonitrile-rich phase were also determined and are presented in Fig. 7. As observed with the partition coefficients, the recoveries indicate a preferential migration of vanillin to the acetonitrile-rich phase. The recovery of vanillin ranges between 75%, with the system formed by acetonitrile and galactose, and 91%, with the system constituted by acetonitrile and glucose. In general, high recovery efficiencies are attained in a single step.

4. Conclusions

This study reports novel ATPS formed by acetonitrile and a large array of carbohydrates (monosaccharides and disaccharides). The ternary phase diagrams, tie-lines and tie-line lengths were determined at 298 K and at atmospheric pressure. Based on the phase diagrams behavior it was shown that the ATPS formation is a result of the hydration capacity of each sugar. Besides high purity carbohydrates, commercial food grade sugars were also investigated and shown to be less able to form ATPS.

To explore the applicability of the investigated systems, the partitioning of vanillin was studied in several ATPS at two different mixture compositions. In all the extraction essays vanillin preferentially migrates for the acetonitrile-rich phase. The trend on the partition coefficients is dependent on the hydration capacity of each carbohydrate. The recovery of vanillin in the acetonitrile-rich phase ranged between 75% and 91% in a single step.

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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.2012.11.001.

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