Aqueous two-phase systems formed by maltodextrin and acetonitrile: Phase diagrams and partitioning studies

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**Abstract**

Novel aqueous two-phase systems (ATPS) formed by water, acetonitrile and maltodextrin with different dextrose equivalent values (DE) are here described and characterized. The respective ternary phase diagrams were determined at 298.15 K. It was found that ATPS formed by maltodextrin and acetonitrile require lower amounts of phase forming-components to undergo liquid-liquid demixing than similar systems formed by mono- and disaccharides. To evaluate the ability of these systems as extraction strategies, experiments with vanillin were carried out. Conditions such as DE and concentration of maltodextrin, concentration of acetonitrile, and temperature were evaluated. In all the investigated systems, vanillin partitions preferentially to the top phase - acetonitrile-rich phase - with a partition coefficient ($K_{van}$) of 1. The best conditions for vanillin partitioning to this phase were obtained using maltodextrin DE 16.5–19.5 at 35 wt% + acetonitrile at 35 wt% + water at 30 wt%, at 308.15 K, for which $K_{van}$ and recovery in top phase ($R_T$) was 12.4 and 91.8%, respectively. The temperature has no statistical influence in the vanillin partitioning. According to the data gathered at different temperatures, it was found that the vanillin partitioning process is spontaneous, endothermic and governed by entropic forces. Finally, the density and viscosity of the studied aqueous two-phase system (ATPS) coexisting phases were determined, showing significant differences in viscosity and density between the phases, which is highly beneficial for phase separation and scaled-up applications.

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**1. Introduction**

Maltodextrins (Mx) are a class of biopolymers (molecular weight between 900 and 18,000 Da) highly soluble in water, and obtained from thermic, enzymatic and/or acid hydrolysis of starch. These compounds are formed by $\alpha$-glucose units linked by 1-4-glucosidic bonds, with the number of reducing sugar content described as the dextrose equivalent value – DE, which may range from 3 to 20. For instance, the starch DE is zero, while glucose DE is 100 [1,2]. These biopolymers are employed in cosmetic, pharmaceutical and food industries, mainly due to their film forming ability, to enhance the rheological properties of pastes and gels, and to retard the crystallization and decrease the hygroscopy of a mixture. As a result, maltodextrins are able to improve the shelf-life stability of food matrices. Furthermore, maltodextrins are odor-, color- and tasteless, and for this reason are also employed as encapsulating agents and as additives for the elaboration of bio-based materials [3–5].

Maltodextrins can be additionally used as a constituent of aqueous two-phase systems (ATPS), mainly to replace the more expensive dextran [6,7]. ATPS are formed when two water-soluble compounds are mixed in water over certain concentrations, resulting in two liquid phases, a top and a bottom aqueous-rich phase [8]. This technique is widely investigated in the purification of proteins [9], enzymes [10], anthocyanins [11], alkaloids [12], flavors [13,14], antimicrobial agents [15] and antibodies [16]. Furthermore, it was also investigated as a simultaneous extraction-purification platform for pesticides [17] and endocrine disruptors [18].
The main advantages of ATPS over other purification and concentration protocols are attributed to their high water content, low interfacial tension, scaling up feasibility, and recycling capability [8,19,20]. Several compounds combinations in water are able to form ATPS, such as polymers [21,22], inorganic salts [23,24], carbohydrates [25], ionic liquids [26], amino acids [27], organic solvents such as alcohols [13], tetrahydrofuran [28] and acetonitrile [29,30].

Acetonitrile (ACN) is an aprotic, colorless, and water-soluble polar solvent [31]. This two-carbon aliphatic nitrile is also known as cyanoethane or methyl-cyanamide, whose molecular structure is CH₃CN. ACN is a widely employed solvent in chromatography [32], and in the cosmetic and pharmaceutical industries [33]. Based on its properties, this work addresses the identification and characterization of ATPS based on acetonitrile and maltodextrin combinations. The respective ternary phase diagrams were determined at 298.15 K. To explore the feasibility of these systems to act as extraction strategies, the vanillin partitioning was investigated. The effects of DE of maltodextrin, concentration of phase-forming components and temperature were investigated. Vanillin (4-hydroxy-3-methoxybenzaldehyde) was chosen because it is widely used as flavoring in foods, beverages, cosmetics and drugs [34]. Moreover, this natural compound is extracted together with other biomolecules from vanilla (Vanilla planifolia, Vanilla tahitiensis, and Vanilla pompon), for which alternative cost-effective purification platforms need to be explored.

2. Material and methods

2.1. Materials

The maltodextrins (Mx) with different dextrose equivalent (DE) values (Mx 13–17 (product number 419,680) and Mx 16.5–19.5 (product number 419,699)) were purchased from Sigma-Aldrich, and used without prior purification. Vanillin (purity > 99 wt%) and acetonitrile (HPLC grade 99.9 wt%) were also acquired from Sigma-Aldrich. The chemical structures of the ATPS phase-forming components and vanillin are shown in Fig. 1 (Table DB1 in Data in Brief). Distilled and deionized water was used in all experiments.

2.2. Phase diagrams

Aqueous solutions of maltodextrin (40 wt%) and acetonitrile (80 wt%) were initially prepared. The experimental binodal curves data were determined gravimetrically (±10⁻⁴ g) by the cloud point method at (298.15 ± 1.00) K and (0.10 ± 0.01) MPa as previously established in our works [11,12,28]. The drop-wise addition of acetonitrile was carried out to the maltodextrin solution until turbidity detection (biphasic area). Then, water was drop-wise added to the system to perform it clear (monophasic system). The procedure was accomplished under constant magnetic stirring and was repeated until no two-phase formation was detected. The experimental binodal curves were correlated using Eq. (1) [35].

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

(1)

where [Mx] and [ACN] are the maltodextrin and acetonitrile mass fraction percentage, and A, B and C are constants obtained by the regression of experimental data.

2.3. Vanillin partitioning

The partition behavior of vanillin in the studied systems was determined in ternary ATPS composed of Mx, ACN and water, at mixture compositions selected at the biphasic region, and optimized by changing the dextrose equivalent of maltodextrin (13–17 and 16.5–19.5), maltodextrin concentration (15–35 wt%), ACN concentration (20–35 wt%) and temperature (278.15–308.15 K). Appropriate amounts of the system constituents were weighted in graduated centrifuge tubes to form 10 g of total ATPS, each containing 0.75 mg of vanillin. The mixture was vortexed and the phase separation promoted by centrifugation (3000 rpm for 10 min at each temperature of interest) using a Hettich Universal 320 R centrifuge. The systems were then left in a thermostatic bath (Marconi MA-127, Piracicaba-SP, Brazil) for at least 12 h to reach the equilibrium at the desired temperature. All tubes were sealed to avoid ACN vaporization. The two phases were carefully withdrawn using a pipette for the top phase and a syringe with a long needle for the bottom phase. Their weights and volumes were determined.

The concentration of vanillin at each aqueous phase was quantified through UV-spectroscopy, using a Varian Cary 50 Bio UV–Vis spectrophotometer, at a wavelength of 280 nm in triplicate to obtain in the final average concentration, partition coefficient and recovery, with the associated standard deviation, 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 deionized 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 [36].

The partition coefficients (Kᵥₐₜ) were determined according to Eq. (2):

\[ K_{v_{an}} = \frac{C_T}{C_B} \]  

(2)

where C is the concentration of vanillin (mg.L⁻¹), and the subscripts T and B correspond to the top and bottom phase, respectively.

The vanillin recovery in top phase (Rᵥ) was also determined, according to Eq. (3):

\[ R_{v_{an}} = \frac{100}{1 + \frac{1}{(K_{v_{an}} \times R_{v_{B}})}} \]  

(3)

where \( R_{v_{B}} \) is the volume ratio between the volume of the top phase (Vᵥ) and the volume of the bottom phase (V_B).

Each ATPS was prepared at least in triplicate, allowing to establish the average and the associated standard deviation of the vanillin partition coefficient and recovery at the top phase. It should be remarked that the top phase corresponds to the ACN-rich phase, whereas the Mx-enriched phase corresponds to the bottom phase.

The standard molar Gibbs free energy of transfer (\( \Delta_{g} G^{m} \)), the standard molar enthalpy of transfer (\( \Delta_{g} H^{m} \)) and standard molar entropy of transfer (\( \Delta_{g} S^{m} \)) associated to the vanillin partition process, from the Mx to the ACN-rich phase, were determined by the van’t Hoff approach at different temperatures (from 278.15 to 308.15 K). The following isochors were used to determine the molar
thermodynamic functions of transfer (Eq. (4))
\[
\Delta_{T} C_{m}^{\text{e}} = \Delta_{T} H_{m}^{\text{e}} - T \times \Delta_{T} S_{m}^{\text{e}} = -RT \ln(K_{\text{van}})
\]
where \( T \) is the temperature (K) and \( R \) is the ideal gas constant. The enthalpy and entropy contributions can be directly deduced from the linear approximation of \( \ln(K_{\text{van}}) \) versus \( T^{-1} \).

2.4. Density and viscosity of the ATPS coexisting phases

The density and viscosity of the top and bottom phases were determined at different temperatures (283.15–343.15 K), with an uncertainty of ±0.02 K, using an automated SVM 3000 Anton Paar rotational Stabinger viscometer-densimeter, which was previously calibrated using standards solutions. The kinematic viscosity had a relative uncertainty of 0.35%, while the absolute uncertainty of density is \( 5 \times 10^{-6} \) g cm\(^{-3} \).

3. Results and discussion

For the suitable application of ATPS in purification processes, it is necessary the prior knowledge of the respective ternary phase diagrams, mainly to address the mixtures compositions required to form two-phase systems, and equilibrium data on the target biomolecule partitioning between the phases. In this work, the phase diagrams of novel ATPS based on acetonitrile and maltodextrin with different values of dextrose equivalent (13–16 and 16.5–19.5) were determined at (298.15 ± 1.0) K. These systems were then evaluated in terms of their extraction performance for vanillin, for which the effects of the dextrose equivalent value, the carbohydrates, and temperature were appraised.

3.1. Phase diagrams

The experimental solubility data (weight fraction) of the ternary systems formed by water, maltodextrin and acetonitrile are given in the Data in Brief (Table DB2), whereas their representation is shown in Fig. 2. This figure also has solubility/binodal curves data for ATPS formed by ACN and other carbohydrates, such as a monosaccharide (\( \alpha \)-glucose), a disaccharide (sucrose), and a polysaccharide (dextran, molecular weight of 6000 and 100,000 Da) for comparison purposes. In Fig. 2 the respective binodal curves are presented, for which compositions above each binodal curve lead to the formation of two-phase systems, whereas mixture compositions below result in the formation of monophasic mixtures. In the orthogonal representation given, the amount of water is omitted, corresponding to the content required to reach 100 wt% of a given mixture composition.

Carbohydrates with low molecular weight (\( M_w \)), namely monosaccharides like glucose (\( M_w = 180 \) g mol\(^{-1} \)) and disaccharides like sucrose (\( M_w = 342 \) g mol\(^{-1} \)), have a low two-phase formation ability (small biphasic regime in the phase diagram) when compared to heavier carbohydrates. On the other hand, dextrins appear as the more efficient species to lead to ATP formation. ATPS formed by maltodextrins display a biphasic area size between those display by sucrose and dextran, meaning that they display an intermediate ability to create two-phase systems with ACN. It was already demonstrated that the increase in the molecular weight of carbohydrates, e.g. mono- and disaccharides, increases the ATPS formation ability [37]. This finding is also true for polysaccharides, such as observed with dextrans (e.g., \( M_w = 6000 \) versus 100,000 Da) [38]. Furthermore, an increase in the \( M_w \) of saccharides usually represents an increase in the number of hydroxyls group and in the ability of these species to establish hydrogen-bonds with water [39]. Overall, in all of these ATPS, the carbohydrates act as the salting-out species, leading to the exclusion of acetonitrile towards the opposite phase. The maltodextrins molecular weight is inversely proportional to the dextrose equivalent value. According to Gregson and coworkers [40], maltodextrins with \( M_w \) values between 5 and 18 ranged the \( M_w \) between 2446 and 1031 Da (maize), 2895-968 Da (topioca – a Brazilian starch product) and 2261-1017 Da (maize from Cargil). Assuming the correlation between \( M_w \) and DE values, (Eq.(1))

All the binodal curves experimental weight fraction data were fitted using Eq. (1) – correlations shown in Figure DB2 in the Data in Brief. The respective parameters were estimated by the least-squares regression method. These parameters (\( A, B \) and \( C \)), and the corresponding standard deviations (\( \sigma \)) and regression coefficients (\( R^2 \)) are reported in Table 1. Good correlation coefficients (0.994–0.997) were obtained for both systems containing maltodextrins.

3.2. Partitioning of vanillin

The application of the novel ATPS in the separation of vanillin was studied by evaluating the effect of dextrose equivalent of maltodextrin, concentration of maltodextrin and acetonitrile, and

![Fig. 2. Binodal curves of ternary systems constituted by ACN + water + carbohydrates at 298.15 K and 0.10 MPa.](image)

Table 1

<table>
<thead>
<tr>
<th>DE of Maltodextrin</th>
<th>Parameters and Regression Coefficient</th>
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<tbody>
<tr>
<td></td>
<td>( A \pm \sigma )</td>
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<tr>
<td>13–17</td>
<td>35.5 ± 0.2</td>
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<tr>
<td>16.5–19.5</td>
<td>34.5 ± 0.3</td>
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* Standard uncertainties: u(T) = 1 K, u(P) = 10 kPa.
temperature on the partition coefficient and recovery of vanillin in the top (ACN-rich) phase. The top phase or acetonitrile-rich phase \( (\log K_{\text{ow}} \text{ of ACN} = -0.17) \) (www.chemspider.com) is more hydrophobic than the bottom phase or maltodextrin-rich phase in \( (\log K_{\text{ow}} \text{ of Mx} = -3.24) \) (www.guidechem.com), thus favoring the preferential migration of vanillin \( (K_{\text{ow}} = 1.19) \) [41] to the acetonitrile-rich phase, and as observed in all investigations discussed below.

The results showing the effect of DE of maltodextrin in the vanillin partitioning behavior are given in Fig. 3 (detailed data given in Table DB3 in the Data in Brief), both in \( K_{\text{van}} \) and \( R_T \) at 298.15 K. These results correspond to an ATPS formed by 25 wt% of Mx and 35 wt% of ACN at 298.15 K. The \( K_{\text{van}} \) obtained range from 5.9 ± 0.7 (with DE 13–17) to 7.6 ± 0.1 (with DE 16.5–19.0), while the recovery of vanillin in the top phase only undergoes a slight increase from 85 ± 1% to 87.8 ± 0.8% with the DE increase. Therefore, vanillin preferentially migrates to the ACN-rich phase, which may be also a result of the salting-out effect exerted by the saccharide. Given the results obtained, in the following experiments it was used maltodextrin with DE 16.5–19.5.

Fig. 4 (detailed data given in Table DB4 in the Data in Brief) depicts the influence of maltodextrin concentration (from 15 to 35 wt%) with Mx with a DE 16.5–19.5 in the partition coefficient and recovery of vanillin in the ATPS top phase at 298.15 K. In this study, the ACN concentration was kept at 35 wt%. In general, an increase in the maltodextrin concentration favors the partition of vanillin to the opposite phase, which again justifies the saccharide salting-out aptitude. The \( K_{\text{van}} \) increases from 2.58 ± 0.06 (Mx at 15 wt%) to 12.2 ± 0.5 (Mx at 35 wt%), while the vanillin recovery in top phase reached 91.6 ± 0.1% at a concentration of maltodextrin of 35 wt%.

In order to evaluate the influence of the acetonitrile concentration in the vanillin partitioning, experiments were carried out using ATPS formed by Mx 16.5–19.5 at 35 wt% + ACN at concentrations ranging from 20 to 30 wt%, at 298.15 K and 0.10 MPa. The results obtained are depicted in Fig. 5, and given in Table DB5 in the Data in Brief. As observed with Mx, an increase in the ACN concentration leads to an increase in the partition coefficient and recovery of vanillin in the top phase, reaching maximum values of 12.2 ± 0.5 and 91.6 ± 0.5%, respectively. This trend is associated with the vanillin hydrophobic character and increased phase hydrophobicity as the ACN content increases.

Amongst the several conditions evaluated, the ATPS constituted by Mx 16.5–19.5 (at 35 wt%) + acetonitrile (at 35 wt%) + water (30 wt%) was identified as the best system to enrich vanillin at the acetonitrile-rich phase. Therefore, this ATPS composition was used to appraise the effect of temperature (from 278.15 to 308.15 K) in the vanillin partitioning and related thermodynamic functions of transfer. The results obtained are presented in Fig. 6 (detailed data given in Table DB6 in the Data in Brief). The temperature has a neglected effect on the partitioning of vanillin, which ranged from 11.8 ± 0.5 (278.15 K) to 12.4 ± 0.8 (308.15 K). Furthermore, the recovery of vanillin in the top phase is also not sensitive to temperature variations, at least at the temperatures investigated, ranging between 91.7 ± 0.2% (278.15 K) and 91.8 ± 0.6% (308.15 K). Similar results were observed by Lima and co-workers [38] for ATPS based on dextran 100,000 (18 wt%) and acetonitrile (30 wt%).
The thermodynamic parameters of transfer, such as the standard molar Gibbs energy ($\Delta_r G_m^0$), enthalpy ($\Delta_r H_m^0$) and entropy ($\Delta_r S_m^0$) of transfer, were additionally determined to better understand the partition process of vanillin (results given in Figure DB3 in the Data in Brief). The vanillin partitioning to the acetonitrile-rich phase using the studies ATPS is favorable ($\Delta_r G_m^0 < 0$, namely $-6.20 \text{ kJ mol}^{-1}$), and the transfer of vanillin is an endothermic process ($\Delta_r H_m^0 > 0$, namely $11.11 \text{ kJ mol}^{-1}$). The $\Delta_r S_m^0$ of vanillin is $24.52 \text{ J mol}^{-1}$ and $T \times \Delta_r S_m^0$ is higher than $\Delta_r H_m^0$, meaning that entropic contributions have an important role in the partitioning process.

The vanillin partitioning in systems based on monosaccharides (glucose), disaccharides (sucrose) or polysaccharides (dextran and maltodextrin) and ACN at 298.15 K and 0.10 MPa are compared in Fig. 7 (detailed data given in Table DB7 in the Data in Brief). In all cases, vanillin preferentially migrates to the top phase (ACN-rich phase), and the highest values of partition coefficient (12.2) and recovery of vanillin (91.6%) were found using ATPS formed by maltodextrin, proving that the systems disclosed in this work display a better extraction performance for vanillin than those previously studied [37,38].

Other ATPS were investigated in the vanillin partitioning. Coutinho and co-workers [36] employed ionic liquids + $\text{K}_3\text{PO}_4$ and observed that the vanillin concentration influences its partitioning, and for the conditions closest to this work (vanillin concentration of 1 g dm$^{-1}$) the $K_{\text{van}}$ was 44.98 using the IL 1-butyl-3-methylimidazolium chloride [C$_\text{4}$mim]Cl. Mozafari and co-workers [42] studied the vanillin partitioning in systems formed by cholinium chloride and $\text{K}_3\text{PO}_4$, with the vanillin again migrating to the ionic-liquid-rich phase ($K_{\text{van}} = 11.4 \pm 0.5$; recovery = 93.5 $\pm$ 0.2%). ATPS formed by alcohols and potassium phosphate were also investigated in the vanillin partitioning, with the best $K_{\text{van}}$ ($430 \pm 46$) and $R_T$ (99.93 $\pm$ 0.01%) found for ethanol + $\text{K}_2\text{HPO}_4$ systems [13]. Still, in all these works, strong salting-out inorganic salts were used, favoring thus the vanillin partitioning to the opposite phase.

### 3.3. Density and viscosity of the ATPS coexisting phases

In the previous section, it was demonstrated that the best conditions to enhance the vanillin partitioning to the top phase occurs with the ATPS composed of maltodextrin 16.5–19.5 (35 wt %) + acetonitrile (35 wt %) + water (30 wt%). Therefore, this mixture composition was used to additionally determine the density and viscosity of the coexisting phases at different temperatures to better characterize these systems when their scale-up application is envisaged. When the difference of densities between the phases is high, the phase separation is improved; furthermore, low viscosities favor mass transfer and reduce energetic inputs [43].

According to Puig and co-workers [44] density and viscosity are important characteristics of fluids, moreover the authors report that density decrease linearly with increasing temperature:

$$\rho = a + bT$$

where $\rho$ is the density, $T$ is the temperature absolute, $a$ and $b$ are the linear parameters.

While the viscosity represent the resistance offered by a fluid to flow and it can be described by means of the Arrhenius equation:

$$\eta = A_1 \times \exp\left(\frac{E_a}{RT}\right)$$

where $\eta$ is the kinematic viscosity, $A_1$ is a constant, $E_a$ is the activation energy for flow, $R$ is the ideal universal gas constant and $T$ is the temperature absolute.

Dokic and co-workers also observed that the viscosity of aqueous solution of maltodextrin changed with the temperature according to Arrehnius equation [45].

Fig. 8 (detailed given in Table DB8 in the Data in Brief) depicts the density and viscosity profile of the coexisting phases (top and bottom) for the studied ATPS at different temperatures. The parameters $a$ and $b$ (density), and $A_1$ and $E_a$ (viscosity) are shown in Table 2.

The exponential and linear trends of viscosity and density, respectively, along temperature are consistent with the traditional behavior of these properties in ATPS. The viscosity and density of...
the top phase range between 2.72 × 10⁻⁷ — 6.70 × 10⁻⁷ m² s⁻¹ (0.213–0.566 m/s) and 0.783–0.844 g cm⁻³, respectively, and in the bottom phase range between 1.71 × 10⁻⁵ — 3.51 × 10⁻⁴ m² s⁻¹ (20.41–439.20 m/s) and 1.196–1.236 g cm⁻³. Compared to other studied ATPS, for instance formed by polymers and salts [46] or ionic liquids and salts [43], and although the Mx-rich phase displays a high viscosity, the ACN-rich phase displays a significantly lower viscosity, being this the phase in which the target vanillin molecule is enriched. Furthermore, there is a larger difference in the densities of the coexisting phases of the ATPS based on ACN + Mx when compared to ATPS formed by polymers and salts [46] or ionic liquids and salts [43], which is highly for phase separation [47].

4. Conclusions

Novel ATPS constituted by acetonitrile and maltodextrin of different value of dextrose equivalents are here shown to occur, and were characterized in terms of phase diagrams and extraction performance. Maltodextrins with a DE 13–17 and 16.5–19.5 form ATPS with acetonitrile, and are of better performance than mono- and disaccharides in what concerns the phase-forming components contents required to form ATPS. Vanillin was investigated as a model biomolecule, for which the ATPS extraction ability was studied by the evaluation of the effect of the DE value, maltodextrin and acetonitrile concentration, and temperature. The best system and operational conditions studied lead to a θcrit of 12.4 ± 0.8 and θt of 9.18 ± 0.6%. These values are higher than those observed in previous works reporting on the use of ATPS formed by ACN and monosaccharides, disaccharides or a different polysaccharide (dextran). By addressing the thermodynamic functions of transfer of vanillin, it was found that its partition is spontaneous, endothermic and governed by entropic forces. Finally, the density and viscosity of the studied ATPS coexisting phases were determined showing that the differences in viscosity and density between the phases are significant, which is highly beneficial for phase separation and scaled-up applications.

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