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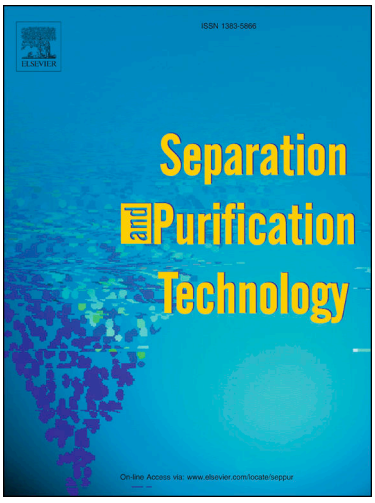

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Sugaring-out Assisted Organic-Aqueous Biphasic Systems: Characteristics, Mechanisms and Applications

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Highlights

- Organic solvent-sugar ABSs were reviewed until 2023
- A database is provided for all sugars and organic solvents studied so far
- A database was prepared for the methods used to determine the phase diagrams
- A database was presented for various target materials extracted by these type of ABSs
- The effect of different parameters on the phase diagram of these systems was examined

Contents

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Abstract

During the past decade, sugaring-out assisted aqueous biphasic systems (ABSs) have been the focus of extensive research due to their widespread applications in the green extraction, separation and purification of various biomolecules and other value-added substances. Due to the biodegradability, electrical neutrality and non-toxicity of carbohydrates, sugaring-out extraction and sugaring-out effect have shown advantages over salting-out extraction methods, such as being simple, stable, cost-effective, fast detachable, and able to avoid equipment corrosion. There are three types of sugar-based ABSs including organic solvent-sugar, polymer-sugar, and ionic liquid (IL)-sugar ABSs, which have been formed from the sugaring-out of aqueous organic solvent, polymer, and IL solutions, respectively. In this work, we review all the ABSs composed of sugars and organic solvents that have been reported in the literature up to 2023, and databases will be provided for: (1) all the sugars and organic solvents studied so far, (2) the methods used for determination of phase diagrams, (3) the models used for the correlation of the phase diagram data and (4) the different target materials extracted, separated, purified and analyzed using these type of ABSs, along with the composition used to form the ABS. We examine the proposed molecular causes for the formation of these ABSs, as well as the effect of different parameters on the phase diagram and the phase formation capability of these systems. Finally, we also examine four of the main applications of organic solvent-sugar ABSs along with the parameters affecting them: (i) partitioning behavior of biomolecules and other added-value compounds, (ii) extraction of different target molecules from real matrixes, (iii) recovery and removal of organic solvents from aquatic environments and (iv) their use in analytical chemistry. In addition, based on the analysis of the data reported so far, this critical review provides a judicious assessment of the existing literature, and the challenges in the sugaring-out effects previously investigated. The scope of further research is also discussed.

Keywords: Aqueous biphasic systems; Sugar; Sugaring-out; Organic solvent; Extraction; Biomaterials.

1. Introduction

An aqueous biphasic system (ABS) was first, and accidently, observed by Beijerinck in 1896 [1], when he mixed an aqueous solution of agar with soluble starch or gelatin. This was also the first example of an ABS where sugaring-out played a relevant role. Later Albertsson applied these systems for extraction purposes [2]. Initially, ABSs consisted of two incompatible polymers such as polyethylene glycol (PEG), dextran (DEX), and/or maltodextrin [2–4]. One of the types of ABSs that have been studied most extensively is PEG-DEX ABSs. In these systems, PEG, the more hydrophobic solute, is sugared-out by DEX to form a two-phase system in which dextran, which is more hydrophilic, dominates the denser lower phase and PEG the upper phase. While on these systems formed by polysaccharides the sugaring out mechanism is present it may not be dominant since entropic factors also significantly influence the phase separation. Since then many ABSs were introduced based, mainly, on water soluble polymers. In the last two decades, with the aim of more effective and optimal extraction of various materials, other types of ABSs have been formed by mixing a variety of two incompatible additives in water such as: polymer-polymer, polymer-salt, polymer-ionic liquid (IL), polymer-surfactant, polymer-amino acid, polymer-sugar, polymer-deep eutectic solvent (DES), IL-salt, IL-amino acid, IL-sugar, IL-surfactant, salt-organic solvent, sugar-organic solvent, IL-organic solvent, amino acid-surfactant, salt-surfactant. In addition, ABSs containing micelle and reverse micelle can also be created using the solutions of two ionic and/or non-ionic surfactants [5,6]. By addition of a soluting-out agent as a mass-separating agent (MSA) into an aqueous solution of another solute with more hydrophobicity than the soluting-out agent, a second immiscible phase is created which leads to the formation of an ABS. The term soluting-out effect refers to the decrease in the solubility of a solute in an aqueous solution due to the addition of

another solute. If the added solute is salt, this phenomenon is called salting-out (decreasing the solubility of a solute in an aqueous solution due to the addition of a salt).

Vapor-liquid equilibria behavior of ternary aqueous solutions of two incompatible solutes with different hydrophobicity capable of inducing phase separation show that the constant water activity lines in the monophasic and biphasic regions respectively show negative and positive deviations from the semi ideal behavior [7–19]. The negative deviation from the semi ideal behavior indicates that the interaction of each solute with water molecules is strengthened by the presence of another solute. In these ternary systems, because of the unfavorable interactions between two incompatible solutes, the solutes exclude themselves from the vicinity of each other by strengthening their interactions with water molecules which decreases the entropy of the system (the hydration water has less entropy than bulk water). With increasing the concentration of the solutes, this exclusion will increase and, ultimately, ABS formation would become favorable entropically. The formation of ABS causes the partial dehydration of the solutes and the increase in the entropy of the aqueous system. Since, both the equilibrium phases in ABSs are aqueous, with high water contents, the interface tension between the two-phases is low (in the range of 10^{-4}-10^{-1} $mN{\cdot}m^{-1}$, orders of magnitude smaller than those of oil-water (o/w) systems) and therefore these systems provide a mild environment for cells, cell organelles and biologically active substances.

This feature of ABSs, along with their non-toxicity, low operational cost and ease in scale up, has made these systems good candidates for the recovery and purification of various biomolecules such as nucleic acids and proteins [2–4,20,21]. Furthermore, the possibility of creative design and manipulation of ABS by choosing the right components, easy design of multi-stage treatment, recovery of constituents of phases [22–24], the possibility of modifying the ABSs with affinity ligands that significantly increase recovery yields and purification performance and introduction of aqueous biphasic flotation (ABF) [25,26] method have made ABS a more attractive separation method. In addition to the extraction, separation, purification and enrichment of proteins [27] and cell organelles [28,29], ABSs have also been applied for nano particles [22,30–33], metal ions [34–36], antibiotics [37,38], antibodies [39], small organic species [40], biopharmaceutical products [41] from the complex mixtures in which they are produced and as green reaction media [42]. The ability of ABSs to separate and purify different substances depends on the different selective partitioning of substances in two equilibrium phases. Transient factors have an effect on the partitioning of substances in ABS, including: type and concentration of the system constituents, temperature, pH and volume ratio of the ABS [5,43,44]. Additionally, partitioning behavior in ABSs also depends on the characteristics of the solutes being partitioned such as: molar mass, hydrophobicity, and superficial electrochemical charge, among others [43]. In order to significantly increase the recovery efficiency and purification of the molecule of interest, the aqueous two phase affinity partitioning (ATPAP) has been used. In these strategies, the affinity partitioning can be achieved by the modification of at least one phase constituent attaching an affinity ligand, [45] or using the free ligand, with specificity for the target compounds, as additive on the system (without any modification of phase constituents) [46]. Recently, the unique ability of ABSs to create aqueous–aqueous interfaces through phase separation and the characteristics of these interfaces have created new opportunities in biomedical applications such as developing interface-assisted design of artificial cells and cyto-mimetic materials, fabrication of cyto- and bio-compatible microparticles, cell micropatterning, 3D bioprinting, and microfluidic separation of cells and biomolecules [47].

Carbohydrates (a.k.a saccharides or sugars), with the general formula $C_x(H_2O)_y$ are polyhydroxy aldehydes or ketones and form a large and diverse group of organic compounds including sugars, cellulose and starch. Carbohydrates, classified as monosaccharides, oligosaccharides (2–10 linked monosaccharides such as disaccharides, trisaccharides, …) and polysaccharides (those with more than

10 linked monosaccharides), are usually highly hydrophilic in nature due to the presence of multiple –OH groups. They have a dual donor/acceptor character and can participate in hydrogen bonding and thus have inherent soluting-out effect (in this case known as sugaring-out effect). Due to their biodegradability, environmentally friendly, electrical neutrality, FDA-approved and non-toxicity, carbohydrates are prone to be used instead of other hydrophilic solutes such as salts as a green soluting-out agent to form ABSs. Furthermore, carbohydrates can act simultaneously as both phase former and chiral selector to form chiral ABSs that are useful for chiral resolution purposes such as obtaining enantiopure drugs and amino acids [48–55]. A review of published articles so far shows that carbohydrates have been used as sugaring-out agents to produce three types of ABSs: organic solvent-sugar, polymer-sugar, and IL-sugar ABSs that have been formed from the sugaring-out of aqueous organic solvent, polymer, and IL solutions, respectively. Recently, DESs-sugars ABSs have also been introduced, which can be classified as IL-sugar ABS due to the similarity of the DESs with ILs [56,57]. Compared to salting-out, sugaring-out has some advantages for the formation of ABSs and also for the extraction of biomolecules including mild extraction conditions, low corrosion and fouling to extraction equipment and service as the substrate in fermentation [58]. Besides, the use of organic solvents, instead of ILs or polymers, to produce sugar-organic solvent ABSs presents a lower cost, and faster and easier separation processes. Organic solvents are cheap materials with low viscosity, allowing fast mass transfer during extraction. Meanwhile, the recovery of an organic solvent is also easily achieved by evaporation. In this work, all the organic solvent-sugar ABSs reported in the literature up to 2023 (61 articles) have been reviewed and, the databases will be provided for: (1) all the sugars and organic solvents studied so far, (2) the methods used for determination of phase diagrams and (3) the different target materials extracted, separated, and purified by these type of ABSs, along with the composition used to form the ABS. We also examine the proposed mechanisms for the formation of these ABSs, correlations of their phase diagrams as well as the effect of different parameters on the phase diagram and the phase formation capability of these systems. Finally, we also examine four of the main applications of organic solvent-sugar ABSs along with the parameters affecting them: (i) partitioning behavior of biomolecules and other added-value compounds in them, (ii) extraction of different target molecules from real matrixes, (iii) recovery and removal of organic solvents from aquatic environments and (iv) their use in analytical chemistry.

2. Organic solvent-sugar aqueous biphasic systems

When a sugar is added to an organic solvent + water mixture, due to the greater hydrophobicity of the organic solvent than the sugar, the organic solvent is separated from the organic solvent-water mixture, so that an organic solvent + sugar ABS is formed, in which the upper phase is rich in the organic solvent and the lower phase is rich in the sugar. This phenomenon as well as the partitioning of a hydrophobic target is illustrated in Figure 1.

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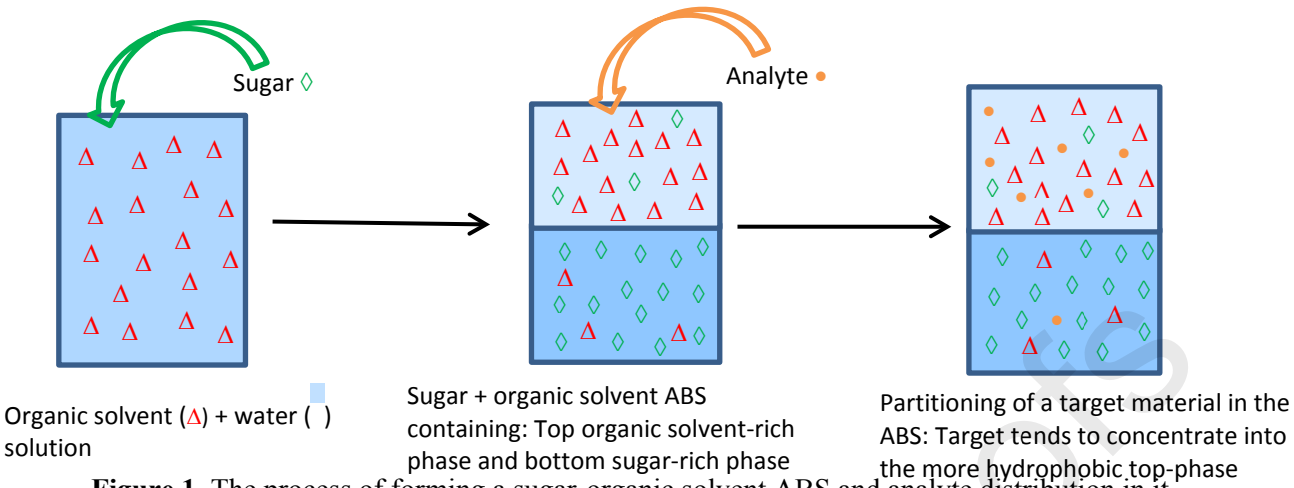

Figure 1. The process of forming a sugar-organic solvent ABS and analyte distribution in it

As can be seen, in this case, after a certain amount of sugar is dissolved in an organic solvent + water mixture, the organic solvent is expelled into the upper phase while some of the water moves to the more hydrophilic lower phase. Furthermore, the hydrophobic target tends to concentrate in the more hydrophobic organic solvent-rich phase. Figure 1 also shows that the water content (identified by the intensity of the blue background color) decreases in the order: bottom phase > initial overall mixture > top phase. Given the potential features of the sugar-organic solvent ABSs (described before), several studies have focused on determining their phase diagrams as well as their use for the extraction, separation, purification and determination of various materials [59–119]. A thorough review of the literature from 2008 to December 2023 shows that 61 articles have been published so far on the sugar-organic solvent ABSs that have either had their phase diagrams determined or used for different purposes. Figure 2 shows the number of articles per year for all three types of sugar-based ABSs including polymer-sugar (20 articles), IL-sugar (23 articles) and organic solvent-sugar (61 articles) ABSs until 2023. The methodology used for literature searching and selecting the articles was a web of science search of the phrases "sugaring-out + acetonitrile (alcohol or organic solvent)", and "aqueous two-phase (biphasic) system + sugar (carbohydrate) + acetonitrile (alcohol or organic solvent)" as well as using the references of the found articles. As can be seen, the organic solvent-sugar ABSs have been studied more extensively than the other two, especially in recent years. In fact, no article was reported in the literature about the organic solvent-sugar ABSs until 2007. In 2008, Wang et al. [119] showed for the first time that by adding a sugar to the acetonitrile-water mixture, phase separation occurs if the sugar concentration exceeds a threshold.

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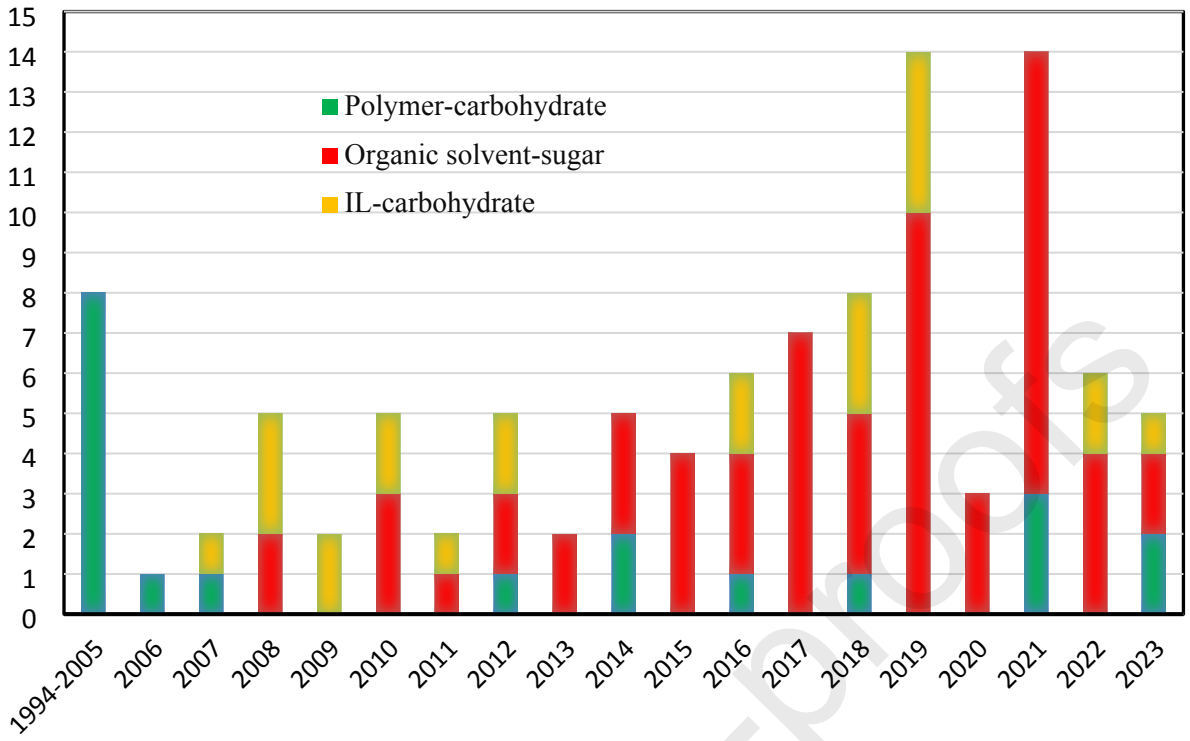

Figure 2. Number of articles per year for sugar-based ABSs until 2023

Other authors showed that the addition of a concentrated aqueous solution of sugars to other organic solvents such as propanol, butanol and THF also results in phase separation. As mentioned above, this type of ABSs, in which the organic solvent is sugared-out by carbohydrates, was first observed in acetonitrile-water mixture, where acetonitrile was separated from the acetonitrile-water mixture by the addition of sugars [115,119]. In this case, acetonitrile was repelled into the upper phase after a certain amount of sugar was dissolved in an acetonitrile−water mixture, and the water was attracted to the lower phase. Many other organic solvent-sugar ABSs have been studied so far in order to determine their phase diagram [59–64,68,69,71,82,85,87,88,91,92,104,115,117–119] or use them for the extraction, separation, purification or determination of different materials [59,61–69,71,77,82–84,86–93,95–101,103–111,113,115–117]. Among organic solvents, acetonitrile has been the most extensively studied. Acetonitrile (cyanomethane or methyl cyanide) is a colorless aprotic solvent that is completely miscible in water at room temperature. It is used in electrochemistry, liquid chromatography, increasing the catalytic efficiency of enzymes as well as a solvent or starting material for the synthesis of organic and inorganic chemicals. Instead of having strong interactions with themselves, acetonitrile molecules tend to form hydrogen bond networks with water molecules [120]. Acetonitrile forms an azeotrope with water at 86% acetonitrile and 14% water, which has a boiling point of 76 °C. Due to the presence of significant amounts of water in the upper organic (acetonitrile) phase, high salt concentration, as well as corrosion of equipment and deposits caused by salt, the salting-out method has not been used to separate acetonitrile from water [119]. However, Wang et al. [119] showed that the sugaring-out effect can be used to separate acetonitrile from its water mixture by adding a monosaccharide or a disaccharide to the acetonitrile-water mixture. Acetonitrile has been extensively studied with different carbohydrates including glucose, mannose, galactose, xylose, arabinose, fructose, sucrose, maltose, maltodextrin, sorbitol, glycerol, erythritol, xylitol, maltitol, sorbose, rhamnose, and xylopyranose to form the organic

solvent-carbohydrate ABSs (see tables 1 and 3). In addition to acetonitrile, other organic solvents such as tetrahydrofuran [64,68,87], 1-propanol [69,71,77,92,104], 2-propanol [71,87,91,92,104], 1-butanol [60], t-butanol [87,92] and propylene glycol [76] have been used, although very limited, to form the organic solvent-sugar ABSs. Xie et al. [85] introduced sucrose and glucose into the concentrated (acetone + 1-butanol + ethanol) (ABE) solution in order to investigate the separation of ABE from the concentrated ABE solution and it was found that more than 89.5 wt. % water from the concentrated ABE solution could be removed by the sugaring-out method. In Figure 3, the structure of various carbohydrates and polyols used in the literature to form solvent-sugar ABSs is shown.

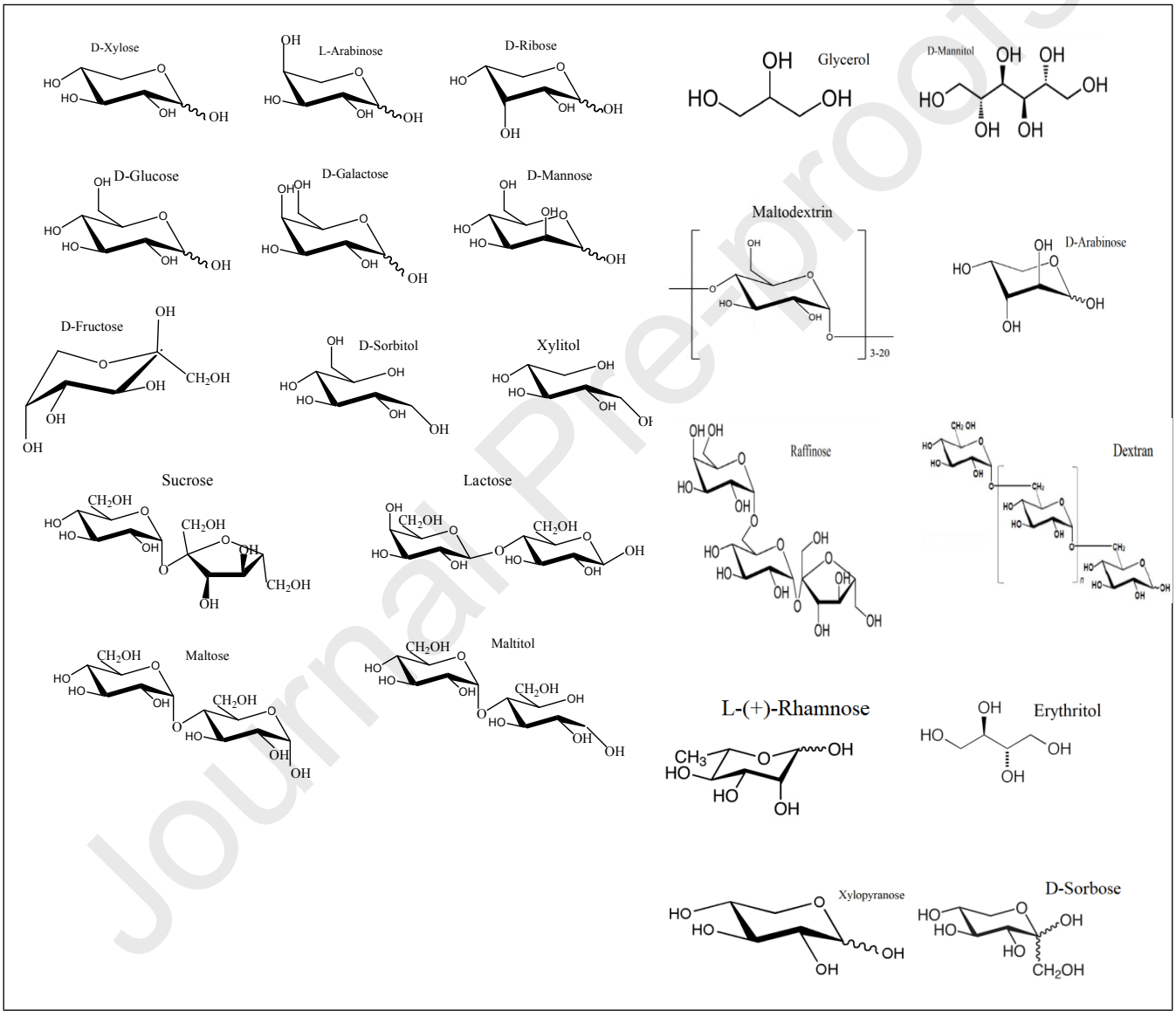

Figure 3. The structure of various carbohydrates and polyols used in the literature to form sugar-based ABSs

2.1. Phase diagrams of organic solvent-sugar aqueous biphasic systems

The phase diagram is one of the important characteristics of aqueous two-phase system, which acts like a fingerprint and it is unique for each system under certain conditions (such as temperature, pH, etc.).

Usually, two types of phase diagrams are used to describe the phase equilibrium in aqueous two-phase systems: (a) Rectangular (or orthogonal) phase diagram and (b) Triangular phase diagram. Figure 4 shows the rectangular and triangular phase diagrams for a hypothetical system consisting of *solute 1* + *solute 2* + water.

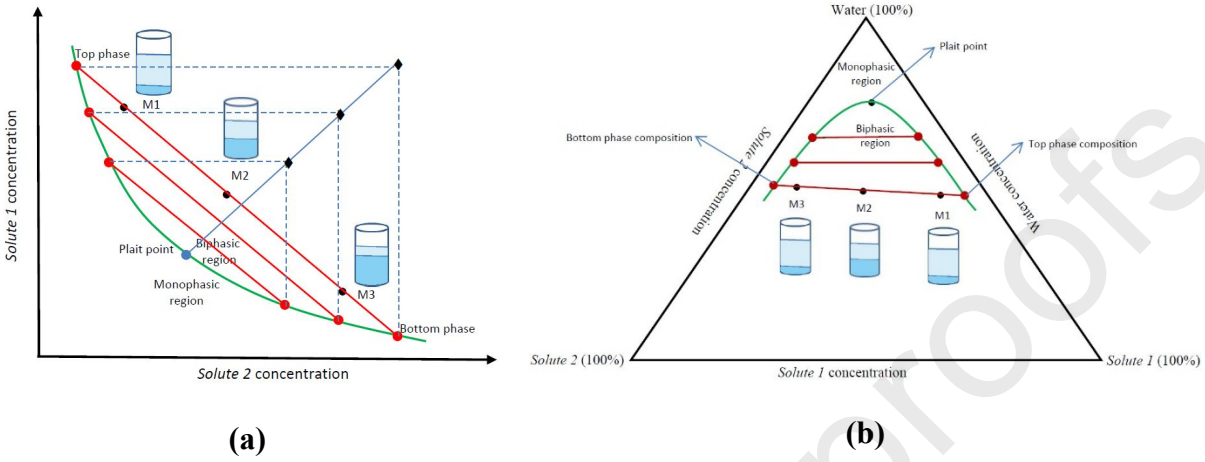

Figure 4. Rectangular **(a)** and triangular **(b)** phase diagrams for a hypothetical system composed of *solute 1* + *solute 2* + water system. Green curve, binodal; Red lines, tie-line; Blue line, auxiliary curve. M1, M2 and M3 = total composition of ABS

Each phase diagram consists of a curve called binodal curve and a series of straight lines called tie-lines. The binodal curve is the boundary between the one-phase and two-phase regions. A tie line on a phase diagram is a line that connects two end points on the binodal curve, each of which representing the final composition of one of the equilibrium phases after the complete phase separation. All the potential systems (e.g., M1, M2 and M3) have the same top and bottom phases with similar equilibrium compositions because of being on the same tie-line. Plait (or critical) point is the point at which the compositions and volumes of the two phases become equal. Binodal curves are usually determined using the visual cloud point titration and turbidimetric titration methods. In the cloud point titration method, repeated drop-wise addition of a solution of the sugar as concentrated as possible (with known concentration) to an aqueous solution as concentrated as possible (with known concentration) of the organic solvent is carried out until a cloudy (biphasic) mixture is detected. After that, a defined amount of distilled water is carefully dripped onto the cloudy ternary mixture to reveal a clear single-phase system. In this way, a point of the binodal curve is obtained. These two successive steps are repeated until complete binodal curve covering a wide concentration range is obtained. The measurements are carried out on a transparent double-walled glass binodal cell under constant stirring, where water (thermostatic fluid) at a certain temperature circulates in the outer shell of the binodal cell. In the visual turbidimetric titration method, various mixtures of sugar, organic solvent and water were gravimetrically prepared within the biphasic region, in glass tubes and thoroughly mixed. Water was added until a clear solution was identified. With the knowledge of the initial mixture composition and of the added water amount, the system composition was determined and a new point on the binodal curve identified. Tie-lines in the phase diagram of aqueous two-phase systems are obtained by two analytical and gravimetric methods. In the analytical method, the composition of each component at the coexisting phases is determined by experimental methods. For this purpose, ternary mixtures in the two-phase region are

gravimetrically prepared and allowed to equilibrate at a given temperature. After the equilibrium was achieved and the two phases became clear and transparent, and the interface was well defined, the samples from the top and bottom phases were meticulously separated for analysis. The gravimetric method was initially proposed by Merchuk et al. [121]. Here the tie-lines are determined on the basis of the mass balance coupled to experimental binodal data fitted with the Merchuk equation [121]:

$$w_1 = Aexp\left[Bw_2^{0.5} - Cw_2^3\right] \quad (1)$$

where w_1 and w_2 are the mass fraction percentages of organic solvent and sugar, respectively. A, B and C are fitting parameters obtained by least squares regression. In this method, the mass fraction of each component at the coexisting phases is determined by the lever-arm rule. In fact, for each tie-line we need four mass fractions w_1^t, w_1^b, w_2^t and w_2^b. The equations 2–5 for each tie-line are solved and the unknown values of mass fractions of organic solvent and sugar in the top and bottom phases are calculated:

$$w_1^t = Aexp\left[B\left(w_2^t\right)^{0.5} - C\left(w_2^t\right)^3\right] \quad (2)$$

$$w_1^b = Aexp\left[B\left(w_2^b\right)^{0.5} - C\left(w_2^b\right)^3\right] \quad (3)$$

$$w_1^t = \left(\frac{w_2^m}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right)w_2^b \quad (4)$$

$$w_2^t = \left(\frac{w_2^m}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right)w_2^b \quad (5)$$

In the above equations, b, t, and m are the bottom phase, the top phase, and the overall mixture, respectively. α is the ratio between the mass of the bottom phase and the mass of the total mixture for each sample.

The slope (STL) and length (TLL) of the tie-lines are two useful parameters to describe the liquid-liquid equilibrium behavior and also to verify trends in the partition or recovery coefficients in ABS. These parameters are calculated using the following equations:

$$TLL = \sqrt{\left(w_1^t - w_1^b\right)^2 + \left(w_2^t - w_2^b\right)^2} \quad (6)$$

$$STL = \frac{w_1^t - w_1^b}{w_2^t - w_2^b} \quad (7)$$

Table 1 reports the composition of organic solvents and sugars to form ABS, whose phase diagrams have been reported in the literature. As can be seen, in the most cases the visual cloud point titration method has been used to determine the binodal curves of the investigated organic solvent-sugar ABSs. Chow et al. [104] used the visual turbidimetric titration method to determine the binodal curves of the 1/2-propanol-sugar ABSs. Both the empirical and semi-empirical (gravimetric) methods have been used to determine the tie-lines of the investigated organic solvent-sugar ABSs. In the empirical method, in most cases, the sugar and organic solvent concentrations have been determined by HPLC and GC methods [85,115,118,119]. Ebrahimi and Sadeghi [71] used polarimetry to ascertain the sugar concentration and then the refractometry method to determine the concentration of propanol in both phases.

Table 1. ABSs composed of organic solvents and carbohydrates reported in the literature

ABS		Phase diagram			
Organic solvent	**Carbohydrate**	**Binodal**	**Tie-lines**	***T*** **/ °C**	**Ref.**
Acetonitrile (ACN)	D-(+)-Glucose, D-(+)-Mannose, D-(+)-Galactose, D-(+)-Xylose, L-(+)-Arabinose, D-(-)-Fructose, Sucrose, D-(+)-Maltose, commercial carbohydrates (Glucose, Fructose and Sucrose)	Visual cloud point titration method	Gravimetric method	25	[59]
ACN	Glucose, Xylose, Arabinose, Fructose, Sucrose, Maltose	Not determined	Analytical method, Carbohydrate: HPLC ACN: GC	1, 23	[119]
ACN	Glucose, Fructose, Sucrose, Maltose	Visual cloud point titration method	Gravimetric method	25	[61]
ACN	MD with a DE 13-17 and 16.5-19.5	Visual cloud point titration method	Not determined	25	[62]
ACN	Sorbitol, Maltose monohydrate, Fructose	Visual cloud point titration method	Gravimetric method	25	[63]
Tetrahydrofuran (THF)	D-(+)-Xylose, D-(+)-Fructose, L-(+)-Arabinose, D-(-)-Arabinose, D-(+)-Mannose, D-(+)-Glucose, D-(+)-Sucrose, D-(+)-Maltose, commercial sugars (Sucrose, Glucose and Fructose)	Visual cloud point titration method	Gravimetric method	25	[64]

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THF	Glycerol, Erythritol, Xylitol, Sorbitol, Maltitol	Visual cloud point titration method	Gravimetric method	25	[68]
1-Propanol	Glucose, Maltose	Visual cloud point titration method	Gravimetric method	25	[69]
1-Butanol	Maltitol, Maltose, Sucrose, D-(+)-Glucose, D-(−)-Fructose, Xylitol, D-(+)-Xylose	Visual cloud point titration method	Not determined	10, 20, 30, 40, 50, 60, 70, 80	[60]
1-Propanol , 2-Propanol	L-(+)-Arabinose, D-(+)-Xylose, D-(+)-Glucose, D-(+)-Galactose, D-(-)-Fructose, Sucrose, Maltose, Xylitol, Maltitol	Visual cloud point titration method	Analytical method, Carbohydrate: Polarimetery Alcohol: Refractive index	25, 45	[71]
Acetone +1-butanol + ethanol	Sucrose, Glucose	Not determined	Analytical method, Carbohydrate: HPLC Organic solvents: GC	27, 39.4, 49.4,	[85]
ACN	Dextran	Visual cloud point titration method	Gravimetric method	25, 35, 45	[82]
1-Propanol, 2-Propanol	Glucose, Maltose, Sucrose	Visual turbidimetric titration method	Not determined	Not reported	[104]
ACN	Glucose, Xylose	Not determined	Analytical method,	1	[115]

			Carbohydrate: HPLC ACN: GC		
ACN	Glycerol, Erythritol, Xylitol, Sorbitol, Maltitol	Visual cloud point titration method	Gravimetric method	25	[117]
ACN	Glucose	Not determined	Analytical method, Glucose: HPLC ACN: GC	6, 9, 12, 15	[118]
ACN	Maltodextrin	Visual cloud point titration method	Gravimetric method	25	[88]
t-Butanol, THF, 2-Propanol	Glucose, Fructose	Visual cloud point titration method	Not determined	30	[87]
t-Butanol, 1-Propanol, 2-Propanol	Galactose, Glucose, Fructose, Mannose, Sucrose, Xylose	Visual cloud point titration method	Analytical method, Glucose: HPLC Organic solvent: GC	25	[92]
2-Propanol	Glucose	Visual cloud point titration method	Not determined	25	[91]

2.2. Correlation of phase diagram of organic solvent-sugar aqueous biphasic systems

The Merchuk equation (eq. 1) [121] has extensively been used for the correlation of the binodal data of different organic solvent-carbohydrate ABSs [59,61–64,68,69,82,117]. Shoushtari [88] applied the following four parameter equation for fitting their experimental binodal data of acetonitrile (1) + maltodextrin (2) ABS:

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$$w_1 = exp\left(A + Bw_2^{0.5} + Cw_2 + Dw_2^2\right) \quad (8)$$

Ebrahimi et al. [60] used the Setschenow's equation [122], as fallows, for the correlation of binodal data of 1-butanol (1) + sugar (2) + water (3) system at the water-rich region:

$$lnm_1 = lnm_1^{\circ} - k_s m_2 \quad (9)$$

where m_1° and m_1 refer, respectively, to the solubility of 1-butanol in pure water and in aqueous carbohydrate solutions with molality of m_2 and k_s is the sugaring-out coefficient. In fact, in the low concentration of 1-butanol, the plot of lnm_1 against m_2 was linear and therefore to quantify the sugaring-out effect of the carbohydrates on the aqueous 1-butanol solutions, the binodal data at the water-rich region were linearly fitted to the above equation. They found that the sugaring-out coefficient of the investigated carbohydrates increased with the increase of their hydrophilic nature and followed the order: disaccharides > hexose monosaccharides > pentose monosaccharides [60].

Shoushtari et al. [63] used the effective excluded volume (EEV) model [123] to correlate the binodal curves of acetonitrile (1) + sorbitol, maltose and fructose (2) ABSs. The EEV model is based on the statistical geometry and has the following form:

$$ln\left(v_{123}^* \frac{w_1}{M_1}\right) + v_{123}^* \frac{w_2}{M_2} = 0 \quad (10)$$

where M_1 and M_2 are molar mass of acetonitrile and carbohydrate, respectively. v_{123}^* represents the EEV of the carbohydrate in solution, and a higher value indicates a lower carbohydrate concentration required for phase separation. v_{123}^* is taken as an adjustable parameter.

The Othmer−Tobias equation [124], as follows, has been used by several authors [61,63,69,118] to correlate tie-line composition of organic solvent + carbohydrate ABSs:

$$\frac{1-w_1^t}{w_1^t} = k_1 \cdot \left(\frac{1-w_2^b}{w_2^b}\right)^{n_1} \quad (11)$$

The Bancroft equation, as follows, has also been used by several authors [61,63,69,85,118] to correlate the tie-line compositions:

$$\frac{1-w_1^b-w_2^b}{w_2^b} = k_2 \cdot \left(\frac{1-w_1^t-w_2^t}{w_1^t}\right)^{n_2} \quad (12)$$

where w_i^t and w_i^b refer to the weight fraction of component i in the top and the bottom phases, respectively and k_1, k_2, n_1, and n_2 are adjustable parameters.

Xie et al. [85] checked the reliability of the tie-line data of ABE (1) + sugar (2) (sucrose and glucose) ABSs by the Bancroft correlation in which “acetone + 1-butanol + ethanol” was taken as one component 1. They also used the following extended Bancroft equation for the correlation of their tie-line data:

$$\frac{1-w_1^b-w_2^b}{w_2^b} = k_3 \cdot \left(\frac{1-w_1^t-w_2^t}{w_1^t}\right) + n_3 \quad (13)$$

where k_3 and n_3 are adjustable parameters. Ebrahimi and Sadeghi [71] ascertained the reliability of the Setschenow-type equation modified by Hey [125], in describing different 1-propanol + glucose, fructose, sucrose, maltose and maltitol ABSs via correlating the tie-lines data by the following equation:

$$ln\frac{m_1^t}{m_1^b} = k_s\left(m_2^b - m_2^t\right) + k_a\left(m_1^b - m_1^t\right) \quad (14)$$

where m_1^t, m_1^b, m_2^t and m_2^b are the coexisting phases' composition in molality scale. k_s is the sugaring-out coefficient and k_a is related to alcohol-alcohol interactions. k_s and k_a are adjustable parameters obtained from the correlation of the experimental tie-line data with the above equation. They found that the obtained values of the sugaring-out coefficient (k_s) increase with a decrease in temperature and for the investigated carbohydrates follow the order: maltose > maltitol > sucrose > D-glucose > D-fructose, which is in agreement with the extent of the biphasic region of the systems [71].

In summary, in most of the works done, Merchuk equation (eq. 1) [121] has been used to correlate the binodal curve data. Other equations (eqs. (8), (9) and (10)) used to correlate the binodal curve data had almost the same performance as Merchuk equation. Regarding to the tie-line data of organic solvent-sugar ABSs, in most cases, The Othmer−Tobias and Bancroft equations have been used to correlate them. In fact, apart from Setschenow-type equation, which has a thermodynamic basis to some extent, until now all the equations that have been used to correlate the binodal curve and tie-line data of these systems are simple mathematical equations that do not have a thermodynamic basis. The molecular thermodynamics models such as regular solution [126], Wilson [127], NRTL [128], and UNIQUAC [129] models have not yet been used to correlate the experimental liquid-liquid equilibrium data of these types of ABSs.

2.3. Mechanism of organic solvent-sugar aqueous biphasic system formation

As mentioned above, Wang et al. [119] showed for the first time that by adding a monosaccharide (glucose, xylose, arabinose or fructose) or a disaccharide (sucrose or maltose) to the acetonitrile-water mixture, phase separation occurs if the sugar concentration exceeds a threshold. They suggested that by adding sugars into an aqueous acetonitrile mixture, some of the original acetonitrile-water hydrogen bonds are replaced by new sugar-water hydrogen bonds, which may force the acetonitrile molecules to separate from the water molecules and form a new phase [119]. Due to the strong dipole-dipole interaction, acetonitrile molecules are dimers in the pure state. In the presence of water, acetonitrile dimers are partially replaced by acetonitrile–water interactions [130]. Takamuku et al. [120] investigated the structure of acetonitrile + water mixtures by X-ray diffraction and IR spectroscopy and found that the acetonitrile molecules form 3D clusters surrounded by water molecules through hydrogen bonding and dipole-dipole interactions. Saielli and Bagno [112] by molecular dynamics simulation showed that acetonitrile-water interactions are weakened by sugars, because they preferentially hydrogen bond with water. Marshall et al. [102] investigated the mechanisms of organic solvent (tetrahydrofuran and acetonitrile) separation from water using sugars (dextrose, sucrose, ribose, fructose and mannose, maltitol, erythritol, sorbitol, xylitol). By bottle tests and nuclear magnetic resonance (NMR), they showed that above 0.25 M, all investigated sugars effectively separate organic solvents from water [102]. Interfacial tension measurements at 0.5 M, also showed that while all sugars had similar phase separation ability above a critical concentration, maltitol and sucrose dimers had a stronger effect on the phase diagrams compared to the other monomeric sugars tested. Furthermore, the authors [102] used the Attenuated Total Reflectance – Fourier Transform Infrared spectroscopy (ATR-FTIR) to investigate the

effect of sugars on the hydrogen bonding and on the nitrile band. It was found that, all the investigated sugars induced a blue shift of the H-bonding of absorbance peaks for double H-bond donor (DD) and single H-bond donors (SD), in either water or in aqueous solutions of organic solvents. Indeed, both THF and ACN increase the effect of sugars on water H-bonding by increasing the ratio between species that are most effective at structuring water (SD) and those that contribute to water structure the least (DD). This indicates that H-bonding in either deionised (DI) water, or mixtures of THF-DI water or ACN-DI water is strengthened by sugars. However, the magnitude of the effect of sugars on the ratio SD/DD H-bonding was solvent-dependent and for a certain sugar concentration followed the order: DI water < THF-DI water mixtures < ACN-DI water mixtures. This is likely because sugars are not soluble in either THF or ACN, which therefore excludes them and promotes their interactions with water. Moreover, in all cases sugars increased the relative amount of free nitrile, indicating a weaker interaction between ACN and water in the presence of sugar. Therefore, water-sugar H-bonding weakens/replaces interactions between AN-water or THF-water, leading to phase separation.

Ebrahimi and Sadeghi [71] obtained the binodal curves for 1-propanol + glucose, xylose, galactose, arabinose, fructose, sucrose, maltitol, maltose and xylitol and 2-propanol + glucose, xylitol, fructose, maltose, sucrose and maltitol ABSs at 298.15 and 318.15 K and concluded that a lower number of -OH groups leads to a lower affinity of carbohydrates for water and thus a weaker sugaring-out power of the carbohydrates. In general, the higher the number of -OH groups in the sugar, the higher the sugar hydration capacity and thus its phase separation ability. The more hydrophilic nature of 2-propanol compared to 1-propanol makes the hydrogen bonds of 1-propanol with water more easily replaced by the sugar-water hydrogen bonds than 2-propanol. Thus the aqueous solutions of 1-propanol are more easily sugared-out by carbohydrates than 2-propanol, and then the biphasic region of 1-propanol-sugar ABS is wider than that of 2-propanol-sugar ABS [71]. Likewise, Xie et al. [85] by investigating the sugar + ABE ABS stated that due to the strong hydrogen bonding between water and sugar molecules, water molecules have priority being attracted to the lower sugar-rich phase and the organic molecules are held together due to their lower polarity indexes and form a new top phase. Due to more hydroxyl groups, sucrose showed a greater sugaring-out effect than glucose at the same sugar concentration [85]. Due to the less hydrophilic nature of 1-butanol compared to ethanol and acetone, 1-butanol is more easily sugared-out and therefore the decrease in concentration of ethanol and acetone in the lower phase were smaller than that of 1-butanol [85]. Ebrahimi et al. [60] investigated the clouding thermodynamics of aqueous 1-butanol solutions in the absence and presence of different carbohydrates by the following equations:

$$\Delta G_m = RT_{cp} ln x_i \tag{15}$$

$$\Delta S_m = -R\left(ln x_i + T_{cp}\left(\frac{d ln x_i}{dT}\right)\right) \tag{16}$$

$$\Delta H_m = \Delta G_m + T_{cp}\Delta S_m \tag{17}$$

where ΔG_m, ΔS_m and ΔH_m are molar gibbs free energy, entropy and enthalpy changes of clouding (phase separation) process, respectively. R is the universal gas constant and x_i is the solute (1-butanol at the water-rich region, and water at the 1-butanol-rich region) mole fraction in a solution with cloud point temperature T_{cp}. The obtained results showed that the phase separation in the butanol-rich and water-rich regions were enthalpy and entropy-driven, respectively. It was also found that all the investigated carbohydrates (maltitol, maltose, sucrose, glucose, fructose, xylitol, xylose) decreased the mutual solubility of butanol and water (sugaring-out effect) and increased the spontaneity of phase separation of aqueous butanol solutions (ΔG_m became more negative in the presence of carbohydrate). The

magnitude of ΔG_m increased with increasing the sugaring-out power of the carbohydrates. Furthermore, the more negative values of ΔG_m in the water-rich mixtures compared to the alcohol-rich region confirmed the more thermodynamically favorable phase separation in the water-rich region.

In summary, the mechanism of organic solvent-sugar ABS formation can be described as follows. In a ternary aqueous organic solvent-sugar system capable of ABS formation, because of the unfavorable interactions between the organic solvent and sugar molecules, the sugars exclude themselves from the vicinity of organic solvents by strengthening their interactions with water molecules, which reduces the entropy of the system (the hydration water has less entropy than bulk water) and thus makes it unstable. As the concentration of sugar and organic solvent increases, this exclusion, and also the instability of the system, increase and finally two phases are separated and the formation of ABS becomes entropically favorable. In addition, the weakening of hydrogen bonds between water molecules and the organic solvent in the presence of sugar facilitates the separation of the organic solvent from the aqueous phase. With the formation of ABS, sugar and organic solvent are separated, and therefore the interactions of sugar with water, which were strengthened in the presence of organic solvent, are weakened and partial dehydration occurs, and as a result, the entropy (and also the stability) of the system increases. By adding sugar to the organic solvent + water mixture, the existing organic solvent-water hydrogen bonds are replaced by sugar molecules. In fact, sugars have a greater ability to form hydrogen bonds with water molecules than the organic solvents that can form ABS with sugars due to their polyhydroxy structure and therefore they act as phase separating species leading to the exclusion of a second organic solvent-rich phase. Table 2 compares the values of the octanol–water partition coefficient, log K_{ow}, which can be considered as a measure of the degree of hydrophobicity, for different organic solvents and sugars. The higher the K_{ow} value, the higher the hydrophobicity and the lower the solubility in water and polar substances. The fact that the more hydrophilic sugars with more hydrophobic organic solvents have a greater tendency to form ABS supports the aforementioned mechanism for the formation of organic solvent-sugar ABSs.

Table 2. The octanol–water partition coefficient (log K_{ow}) of carbohydrates, organic solvents and some target materials [131].

	Organic solvents								
Compound	Propylene glycol	Acetonitrile	Ethanol	Acetone	2-Propanol	1-Propanol	t-Butanol	THF	1-Butanol
log K_{ow}	-0.92	-0.34	-0.31	-0.24	0.05	0.25	0.35	0.46	0.88

	Targeted components								
Compound	Caffeine	Codeine	Vanilin	Diuran	Erythromycin	Curcumin	Cefixime	Cefazolin	Cephalexin
log K_{ow}	-0.07	1.19	1.21	2.60 [132]	3.06 [63]	3.29	-0.40 [61]	-0.58 [61]	0.65 [61]

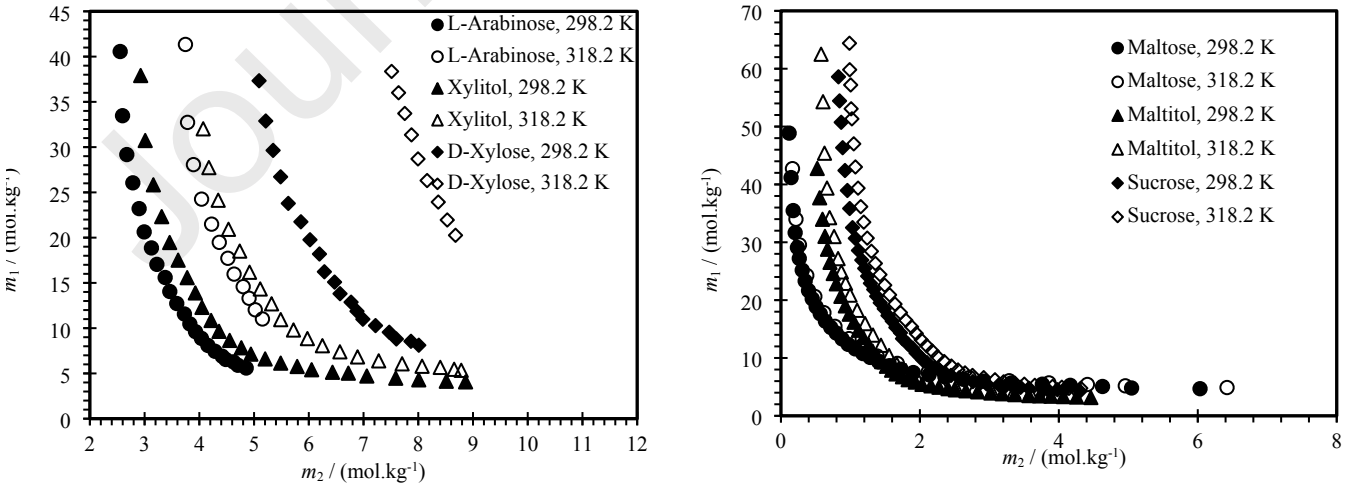
	Carbohydrates
log K_{ow}	-1.08 to -5.61

2.4. Effect of different parameters on the phase diagram of organic solvent-sugar ABSs

2.4.1. Effect of temperature

The results gathered from the literature show that the biphasic region of organic solvent-sugar ABSs expands with decreasing temperature, such that lower temperatures are favorable for phase separation because smaller amounts of organic solvent and carbohydrate are required [71,82,85,115,118,119]. As example, Figure 5 shows the binodal curves of 1-propanol - pentose monosaccharides and 1-propanol - disaccharides ABSs at different temperatures. As can be seen, the ability to form ABS decreases with increasing temperature because the two-phase region decreases with increasing temperature.

In order to justify the effect of temperature on the sugaring-out effects in acetonitrile-water system, Dhamole et al. [118] stated that at low temperatures, the distance between the nearest water molecules decreases and therefore, acetonitrile molecules find fewer places to replace water molecules. Consequently, at lower temperatures, more acetonitrile molecules are released in the presence of sugar, resulting in better phase separation. Xie et al. [85] showed that at the same sugar concentration, the water content in the top (ABE-rich) phase as well as in the bottom (water-rich) phase increases with increasing temperature and stated that the effect of temperature is favorable and unfavorable for the top and bottom phases, respectively. Therefore, the total weight fraction of ABE and consequently its recovery decreases with increasing temperature. Ebrahimi and Sadeghi [71] found that the sensitivity of the propanol-carbohydrate ABS to temperature decreases with increasing the sugaring-out ability of the carbohydrates (Figure 5).

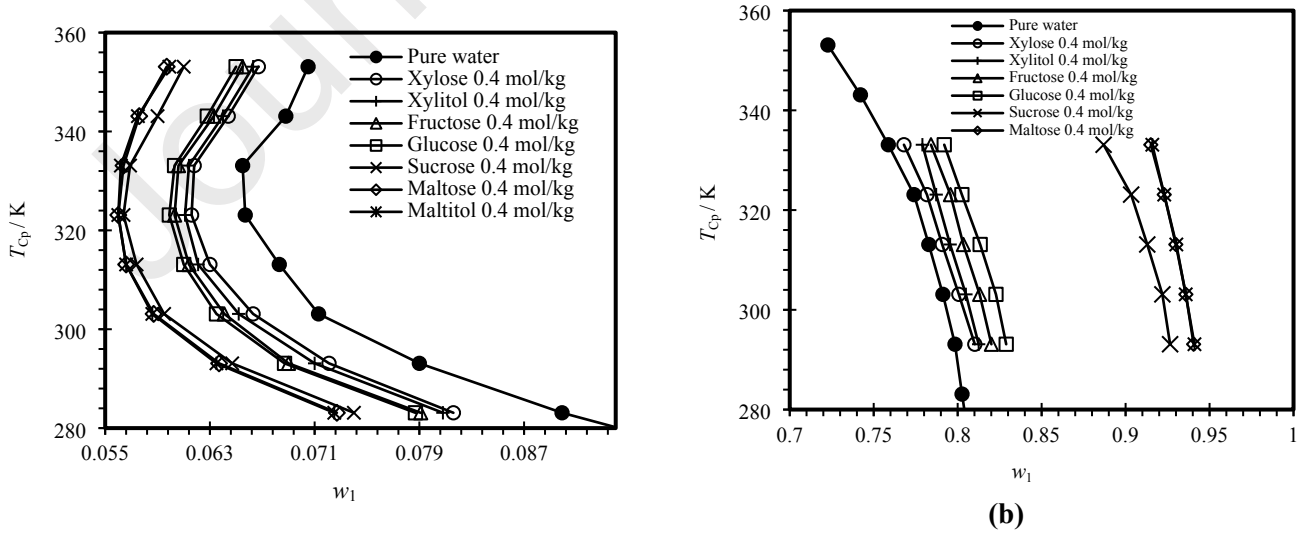


(a) **(b)**

Figure 5. Binodal curves as molality of alcohol, m_1, against molality of carbohydrate, m_2, for ABS composed of **(a)** {water + 1-propanol + pentose monosaccharides}, and **(b)** {water + 1-propanol + disaccharides}, at T = (298.2 and 318.2) K [71].

Regarding to the effect of temperature on the tie-lines, Dhamole et al. [118] found that for a given concentration of glucose, the tie-line length of acetonitrile + glucose ABSs decreases with temperature, indicating that better separation is achieved at low temperatures. This is the only report in this respect but from the behavior of the binodal curves it is safe to assume that this is an universal behavior.

In the organic solvent-sugar ABSs, the organic solvent is sugared-out by the sugar and therefore the more hydrophilic sugars combined with more hydrophobic organic solvents have a greater tendency to form ABS. The effect of temperature on the phase formation ability of these systems can be investigated and justified in terms of the effect of temperature on the hydrophilicity and hydrophobicity of the constituents of the two-phase system. Isopiestic studies of vapor-liquid equilibria behavior of different sugar + water solutions at different temperatures showed that water activities of aqueous sugar solutions do not change with temperature [16] and the temperature does not have any significant effect on the degree of hydrophilicity of sugars. Regarding the effect of temperature on the alcohol-water interactions, it has been found [60] that the solubility of 1-butanol in water in the absence and presence of sugars first decreases with increasing temperature (1-butanol becomes more hydrophobic) and passes through a minimum at about 57 °C, and then increases at higher temperatures (1-butanol becomes less hydrophobic) (Figure 6). On the other hand the solubility of water in 1-butanol increases continuously by increasing temperature. This supports the idea that, the degree of hydrophobicity of alcohols decreases with increasing temperature. Therefore increasing the immiscibility region of organic solvent + carbohydrate systems by decreasing temperature is due to a small increase in the hydrophobicity of organic solvents by decreasing temperature.



(b)

(a)

Figure 6. Cloud point temperature, T_{Cp}, versus 1-butanol mass fraction, w_1, for ternary systems of {1-butanol + aqueous solutions of 0.4 mol.kg^{-1} of sugars}: **(a)** water-rich branch; **(b)** butanol-rich branch [60].

Therefore, because the hydrophilic character of sugars does not significantly change with temperature, while the hydrophobicity of organic solvents decreases with increasing temperature, the ability of carbohydrates to sugaring-out organic solvents in aqueous solutions decreases with temperature, and higher concentrations of sugar and solvent is required for phase separation. Although the effect of temperature on the binodal curve of the organic solvent-sugar ABSs is well-established and clearly understood, the effect of temperature on the tie-lines and composition of equilibrium phases has not yet been adequately studied and not enough information in this field has been reported in the literature.

2.4.2. Effect of the carbohydrates

Regarding the ability of carbohydrates to induce the formation of acetonitrile-based ABS, Cardoso et al. [59] obtained the order maltose > sucrose > galactose > mannose > glucose > fructose > arabinose > xylose, Cardoso et al. [82] obtained the order dextran 100000 > dextran 40000 > dextran 6000 >> maltitol > maltose, Shoushtari et al. [63] obtained the order sorbitol > maltose monohydrate > fructose, Xie et al. [85] obtained the order sucrose > glucose, Cardoso et al. [117] obtained the order maltitol > sorbitol > xylitol > erythritol > glycerol, Moradi and Shahrouzi [61] obtained the order maltose > sucrose > glucose > fructose, and Souza et al. [62] obtained the order dextran 100000 > dextran 6000 > maltodextrin DE 13-17 ≈ maltodextrin DE 16.5-19.5 > sucrose > glucose

Xie et al. [85] showed that by adding sucrose and glucose to a homogeneous {ABE (40 wt%) + water (60 wt%)} mixture, a biphasic system was formed in which the water content in the upper phase under sugar-free condition decreased to 14.31 wt % and 16.72 wt %, respectively. This indicates that sucrose showed greater sugaring-out effect on the liquid−liquid equilibria of the water + ABE system than glucose at the same initial sugar concentration.

Regarding the ability of carbohydrates to induce the formation of 1-propanol and 2-propanol-based ABSs, Ebrahimi and Sadeghi [71] obtained the order of maltose (m_1 > 9 mol·kg-1) > maltitol > maltose (m_1 < 9 mol·kg^{-1}) > sucrose > galactose > glucose > fructose ≥ arabinose > xylitol > xylose (Figure 7) and Chow et al. [104] obtained the order maltose > sucrose > glucose. Chong et al. [69] showed that maltose forms an aqueous biphasic system with 1-propanol more easily than glucose because less sugar is required.

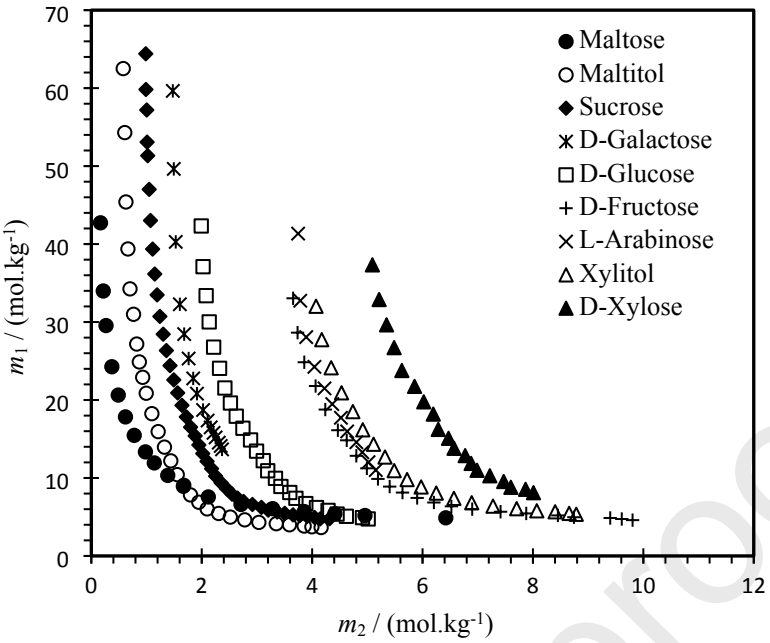


Figure 7. Binodal curves as molality of alcohol, m_1, against molality of carbohydrate, m_2, for 1-propanol-sugar ABS produced at 318.2 K [71].

The ability of the sugars to facilitate the clouding process in 1-butanol + water mixture, follows the order: maltitol ≈ maltose > sucrose > glucose > fructose > xylitol > xylose [60]. Sun et al. [92] obtained the order galactose > glucose > fructose > mannose ≈ sucrose > xylose for the phase separation abilities of t-butanol with sugars.

Regarding the ability of polyols to induce the formation of THF-based ABS, Sousa et al. [68] obtained the order maltitol > sorbitol > xylitol > erythritol > glycerol in which, the ability to form a biphasic system increased from glycerol (3 OH groups) to maltitol (9 OH groups). The ability of different carbohydrates to induce the formation of THF-based ABS was obtained as D-(+)-maltose > D-(+)-sucrose > D-(+)-glucose ≈ D-(+)-mannose > D-(-)-fructose ≈ L-(+)-arabinose ≈ D-(-)-arabinose > D-(+)-Xylose [64].

Cardoso et al. [82] found that the biphasic region of dextran-acetonitrile ABS increased by increasing the dextran (a polysaccharide) molecular weight due to more hydroxyl groups. Cardoso et al. [59] showed that the commercial carbohydrates form a smaller biphasic region than high purity carbohydrates, which may be a result of lower purity levels and the presence of impurities.

The results gathered from the literature show that the ability of carbohydrates to sugaring-out organic solvents in aqueous solutions is influenced by the number of carbohydrate hydroxyl groups as well as their stereochemistry. In general, it was found that the higher the number of OH groups in carbohydrates (or polyols), the greater the ability to form a biphasic system with acetonitrile [59,61,62,82,117], 1-propanol [69,71,104], 2-propanol [71,104], 1-butanol [60] and THF [64,68]. The ability of different carbohydrates to sugaring-out the organic solvents decreases in the order of polysaccharides > disaccharide > hexose monosaccharides > pentose monosaccharides. Pentose monosaccharides (arabinose and xylose) were less effective in promoting the ABS formation with organic solvents than

hexose monosaccharides (galactose, mannose, glucose and fructose) due to the lower number of OH groups. As the molecular weight of saccharides (mono, di and polysaccharide) increases, the number of OH groups and thus their ability to form hydrogen bonds with water increases, which lead to an increase in their ability to form a biphasic system with organic solvents in aqueous solutions. In general, the lower the number of OH groups, the lower the affinity of the carbohydrate for water, the less favorable conformation for hydrogen bounding with water, the weaker the sugaring-out ability of the carbohydrate and thus the lower the phase separation ability.

In addition to the total number of OH groups, their relative position on the pyranose ring also plays a decisive role in the degree of hydration of sugars and then their sugaring-out ability. Since the hydration of carbohydrates depends on the ratio between the axial and equatorial OH groups [133], the monosaccharides can be classified into three groups of decreasing hydration (see Figure 3):

(a) Pentose: OH(2) is equatorial and OH(4) is either axial (L-arabinose) or equatorial (D-xylose and D-ribose);

(b) Hexose: OH(4) is equatorial and OH(2) is either axial (D-mannose) or equatorial (D-glucose);

(c) Hexose: OH(4) is axial and OH(2) is equatorial (D-galactose).

Different studies on the sugaring out abilities of the pentose monosaccharides show the higher sugaring-out power of arabinose compared to xylose. Regarding to hexose monosaccharides, the trends galactose > mannose > glucose > fructose [59], galactose > glucose > fructose > mannose [92] and glucose ≈ mannose > fructose [64] have been observed for their sugaring-out ability on organic solvents in aqueous solutions. As can be seen, although there is a consensus on the order of galactose > glucose > fructose there is no agreement for the position of mannose. Fructose (a ketohexose with a 5-sided ring) was less effective than its isomer (glucose, an aldohexose with a 6-sided ring) in inducing the ABS formation which indicates that the aldohexoses are more effective in promoting phase separation than the ketohexoses. Glucose and mannose epimers distinguished by the OH(2) position had almost similar abilities to induce ABS formation with organic solvents. However, the comparison between the epimers glucose and galactose (and also xylose and arabinose), which are distinguished by the OH(4) position, suggested that galactose is more effective in inducing the aqueous biphasic formation (arabinose is more effective than xylose). Thus, the orientation of OH(4) plays an important role in the ABS formation ability, so that OH(4) in the axial position has a greater sugaring-out effect than OH(4) in the equatorial position. Since glucose has a greater sugaring-out power than fructose, disaccharide maltose (glucose-glucose) has higher capabilities for ABS formation than sucrose (glucose-fructose). In other words, the trend of maltose (glucose-glucose) and sucrose (glucose-fructose) conforms the sugaring-out ability of their constituent subunits.

As discussed, in these ABSs, the sugar acts as soluting-out agent and therefore the more hydrophilic sugars are more prone to form ABS with organic solvents. The number and relative position of –OH groups can affect the hydrophilicity of sugars, and therefore the phase behavior of organic solvent-sugar ABSs. The effect of the number of sugar OH groups on the phase diagram has been widely studied and there is a good agreement in the obtained results between various authors. However, the results gathered from different studies show that the influence of the relative position of –OH groups (relevant stereochemistry) on the phase diagram of this type of ABSs is still unclear and there is no complete agreement between the results obtained in different studies. Therefore more extensive systematic research is needed to evaluate the overall role of stereoisomeric effects on ABS formation. Moreover, the effect of chirality on the phase diagram of these systems is still unknown, which seems to have little

effect on the phase behavior and thus requires very precise measurements. In addition, there is no information in the literature about the effect of sugars on the composition of phases in equilibrium and liquid-liquid equilibrium data of organic solvent-sugar ABSs and this also requires further investigation.

2.4.3. Effect of organic solvent

Organic solvents such as acetonitrile [59,61,62,82,117], tetrahydrofuran [64,68,87], 1-propanol [69,71,77,92,104], 2-propanol [71,87,91,92,104], 1-butanol [60], t-butanol [87,92] and propylene glycol [76] in aqueous solutions can be sugared-out by various carbohydrates including monosaccharides (such as galactose, mannose, glucose, fructose, arabinose and xylose), disaccharides (such as maltose and sucrose), polysaccharides (such as dextran and maltodextrin) and polyols (such as maltitol, sorbitol, xylitol, erythritol, glycerol) to form an ABS with the upper organic solvent-rich and lower sugar-rich phases. Ternary aqueous ethanol + carbohydrate mixtures cannot form stable ABS. Since the hydrophilicity of ethanol is not very different from that of carbohydrates, both ethanol and carbohydrate can act as soluting-out agent, which is known as alcoholing-out and sugaring-out phenomenon. In these systems, the alcoholing-out phenomenon appears as carbohydrate precipitation, while the sugaring-out phenomenon leads to ABS formation and a very slight change in the concentration ratio of the solutes leads to the replacement of the soluting-out agents [71]. Figure 8 compares the binodal curves of acetonitrile + glucose, THF + glucose, 1-propanol + glucose, 2-propanol + glucose and 1-butanol + glucose ABSs at 298.15 K. As can be seen the ability of glucose to sugaring-out and form ABS with different organic solvents follows the order 1-butanol > THF > acetonitrile > 1-propanol > 2-propanol. In fact, the potential of these organic solvents to undergo liquid-liquid demixing with glucose in aqueous solutions, except for acetonitrile, corresponds to the order of their log K_{ow} value (see Table 2).

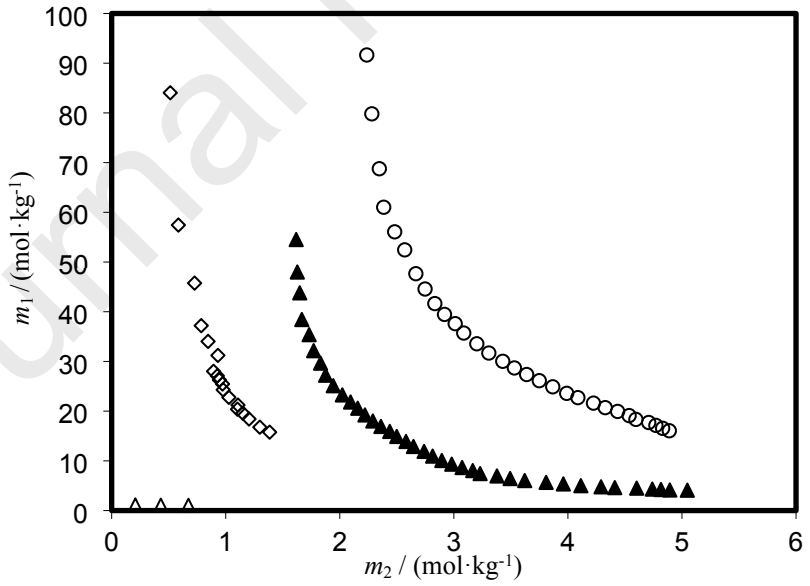


Figure 8. Binodal curves as molality of organic solvent, m_1, against molality of glucose, m_2, for different organic solvent-glucose ABSs produced at 298.2 K. Δ, 1-butanol [60]; ■, THF [64]; ◇, Acetonitrile [59]; ▲, 1-propanol [71]; ○, 2-propanol [71].

Despite the wide range of organic solvents, only a limited number of organic solvents have been used to form an aqueous two-phase system with sugars, and there are still many unknown issues in this field that require more work and research. The possibility to use log K_{ow} as guide to identify organic solvents for ABS formation is promising and would deserve a more extensive study.

2.5. Application of organic solvent-sugar aqueous biphasic systems

In most of the ABSs that have been introduced so far, one of the constituents of the phases is inorganic salts as salting-out agents to increase the extraction efficiency. Among the disadvantages of using high concentrations of inorganic salts in the formation of salt-based ABSs, we can mention the recovery of salts, unwanted reactions, corrosion of equipment and destructive effects on some biological products.

The major advantages of using sugar (sugaring-out) instead of salt (salting-out) include eco-friendly and straightforward process, fast phase separation, cost-effectiveness, low energy requirement, the role of the substrate in fermentation, as the sugar acts as the agent of cell pre-treatment, and the fact that it is able to preserve the pH of the solution, the properties of the extractants and avoid equipment corrosion. Furthermore, many real samples (such as honey, jam, fruit molasses, etc.) used for the extraction and recovery of target substances contain sugar, which can simultaneously act as a phase-separating agent in addition to the target substance substrate.

The results gathered from the literature show that this type of ABS has been used for partitioning, purification, extraction, separation and determination of the different target analytes including food coloring, herbal and flavoring supplements, antioxidant and extracts [59,62,69–71,77–79,82,86,99,111,115,117], amino acids [87,88], herbicide [64,68], pesticides [100,101,103,107], proteins [65,73–75,104], enzymes [65], alkaloids [71], antiseptic [106], antibiotics [61,63], metal ions [66,96], drugs [67,78,80,81,84,88,95,97,106,110,111,116], phenolic compounds [76,86,89,93,98], lipid acids [72,83], dyes [119], oligosaccharides [108], organic acids [91,92] and organic solvents [90,96,105,109,113] which have been collected in Table 3. By carefully examining the various articles published about the applications of the organic solvent-sugar ABSs, the application of these systems can be divided into four main purposes, all of which are based on the unequal distribution of target molecules between equilibrium phases: (i) studying the partitioning behavior of biomolecules and other value-added compounds between phases, (ii) extraction of target molecules from real matrixes, (iii) recovery and removal of organic solvents from aquatic environments and (iv) quatification of target analytes in different samples. As can be seen from Table 3, acetonitrile-sugar-water is the most used extraction system due to the high efficiency extraction of compounds with wide polarity range, and the excellent compatibility with the chromatography technology. In order to investigate the performance of the organic solvent-sugar ABSs in the extraction, purification and separation of a target material, *i*, the effectiveness indicators are the partition coefficient, K_i, extraction efficiency (extractability), EE_i, recovery, R_i, and extraction yield, EY_i, which are defined by the following equations:

$$K_i = \frac{C_i^t}{C_i^b} \tag{18}$$

$$R_i = EY_i = EE_i = \frac{amount\ of\ analyte\ i\ in\ the\ top\ phase}{amount\ of\ analyte\ i\ in\ the\ orginal\ sample} \tag{19}$$

Usually ABSs are applied in two ways to investigate their extraction performance: (i) extraction of a specific target material from a real sample, and (ii) distribution of a certain amount of a target substance

i (manually added to the ABS) between the two phases. In the second case, since the total amount of the target is equal to the sum of the amount of target in the two phases, the above equations can be written as:

$$R_i = EY_i = EE_i = \frac{C_i^t V^t}{C_i^t V^t + C_i^b V^b} = \left(\frac{K_i R_V}{1 + K_i R_V}\right) = \left(\frac{w_i^t}{w_i^t + w_i^b}\right) \quad (20)$$

where C_i^t and C_i^b represent the target *i* concentration in the top and bottom phases, respectively, $w_i^t$and w_i^b represent the weight of target component in the top and bottom phases, respectively. R_V is volume ratio or phase ratio, $R_V = V^t/V^b$, and V^t and V^b are the top and bottom phase volumes at equilibrium, respectively. Selectivity, *S*, which is a measure to express the relationship between the partition coefficient of the target molecule and other contaminants, is obtained from the following equation:

$$S = \frac{K_{target\ molecule}}{K_{contaminant}} \quad (21)$$

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Table 3. Carbohydrate-organic solvent ABSs reported in the literature for the extraction and separation of different materials

Target compound	ABS	Description	*T* / °C	Ref.
		(i) Partitioning behavior		
Curcumin (Cur)	1-Propanol + Glucose and Sucrose	Curcumin spontaneously partitions to the more hydrophobic alcohol-rich top phase. For both systems, the magnitudes of K_{Cur} and EE_{Cur}% increase by decreasing the water content ratio (ratio of the weight fraction of water in the upper phase to the lower phase, H_2O_{ratio}) and increasing the TLL or R_V. The maximum value of K_{Cur} and recovery percentages increase by increasing the sugaring-out ability of the carbohydrates (sucrose > glucose). From the linear relation found between the logK_{Cur} values and H_2O_{ratio}, it can be inferred that the water affinity/non-affinity of curcumin has a fundamental designation on their partitioning trend.	25	[77]
- Caffeine (Caf) - Codeine (Cod) - Vanillin (Van)	1-Propanol and 2-Propanol + Glucose, Sucrose and Maltitol	Caf and Van tend to concentrate in the more hydrophobic propanol-rich top phase, whereas Cod tends to concentrate in the more hydrophilic sugar-rich bottom phase. The values of K increase by increasing TLL and decreasing H_2O_{ratio}. The selectivity index (K_{Caf}/K_{Cod}) increase by T, sugar hydrophobicity and TLL.	25, 45	[71]
Van	Acetonitrile + Glucose, Mannose, Galactose, Xylose, Arabinose, Fructose, Sucrose, and Maltose	Van tends to partition for the acetonitrile-rich phase. The K_{Van} rank is dependent on the hydration capacity of each carbohydrate and is similar to the order of formation of ABS previously noted. The EE_{Van}% in the acetonitrile-rich phase ranged between 75% (galactose) and 91% (glucose) in a single step.	25	[59]

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Van	Acetonitrile + Glycerol, Erythritol, Xylitol, Sorbitol, and Maltitol	Van tends to partition into the acetonitrile-rich phase with K_{Van} between 7 and 67. With the exception of maltitol, the K_{Van} values are proportional to the degree of polyol hydrophilicity, so that the highest K_{Van} was observed with sorbitol and the lowest value with maltitol. Furthermore, for all polyols except glycerol, the recovery of Van in the acetonitrile-rich phase was higher than 89% and increased as the number of OH groups in the polyol increased from glycerol to maltitol.	25	[117]
Van	Acetonitrile + Maltodextrin (with a DE 13-17 and 16.5-19.5)	Vanillin partitions preferentially to the ACN-rich phase. K_{van} and EE_{van}% increase with an increase in the ACN and MD concentration. The highest value of K_{van} (12.4) and EE_{van}% (91.8 %) was found using 35 wt.% acetonitrile + 35 wt.% maltodextrin DE 16.5-19.5 ABS at 308.15 K. These values are higher than those observed in ABS formed by ACN and monosaccharides, disaccharides or a different polysaccharide (dextran). From the values of K_{van} at different temperatures, the thermodynamic functions of vanillin partition process were obtained. Vanillin partitioning process was spontaneous, endothermic and governed by entropic forces. Temperature has a neglected effect on K_{van} (and also EE_{van}%) which ranged from 11.8 (91.7%) at 278.15 K to 12.4 (91.8%) at 308.15 K.	5, 15, 25,35	[62]
Van	Acetonitrile + Dextran (Dx-6, Dx-40 and Dx100)	Van tends to concentrate in the acetonitrile-rich phase.	5, 15, 20, 25, 30, 35, 45	[82]

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		The highest value of K_{van} (11.3) and EE_{van}% (95%) was found using an ABS composed of 18 wt.% of Dx-100 (dextran with the higher molecular weight) and 30 wt.% of acetonitrile at 25 °C.		
-Aspartic Acid -Tyrosine	2-Methyl-2-propanol, Tetrahydrofuran, and 2-Propanol + Glucose and Fructose	In order to find specific extraction of amino acid, the extraction characteristics of twenty amino acids have been investigated. In term of the hydrophobic effect, THF-glucose ABS was a better media for amino acid separation and partitioning of the amino acids in this ABS increased by increasing the amino acids hydrophobicity, except aspartic acid and tyrosine. The specific extraction of aspartic acid and tyrosine was caused by the aggregation between these amino acids and sugars.	30	[87]
Sudan I	Acetonitrile + Glucose	Sudan I tends to concentrate in the more hydrophobic CAN-rich top phase. The extraction rate of Sudan I increases with increasing glucose concentration so that after the glucose concentration exceeds 25 g/L, the extraction rate becomes more than 80%.	1	[119]
- Cephalexin - Cefazolin - Cefixime	Acetonitrile + Glucose, Fructose, Sucrose, and Maltose	All three cephalosporins have negative surface charges and due to the repulsion between cephalosporins and acetonitrile they tend to concentrate in the 28arbohydrate-rich bottom phase. As the pH value increases, the surface net charge of cephalosporins becomes more negative and the repulsion becomes stronger and consequently partition coefficient increases. The values of *K* increase with increasing the sugar concentration (increasing TLL). The values of *K* for cephalexin and cefazolin follow the order: glucose > fructose > maltose > sucrose and the highest partition coefficients (recovery rates) for cephalexin and cefazolin were 19.2	27	[61]

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		(93%) and 16.7 (93%), respectively, corresponding to the 50 wt. % acetonitrile + 16 wt. % glucose ABS. The partition coefficient of cefixime follows the ascending order: glucose < fructose < maltose < sucrose and the maximum amount of $K_{cefixime}$ was 6.4 with a recovery percentage of 81% in 50 wt. % acetonitrile + 16 wt. % sucrose ABS.		
-Antivirals -Antidiabetics -β-blockers	Acetonitrile + Sucrose	Four different modes of liquid-liquid microextraction (LLM) including dispersive liquid-liquid microextraction (DLLM), binary and ternary salting-out and sugaring-out induced liquid-liquid microextraction (SALLM and SULLME) were applied and compared for pre-concentration of 14 drugs from three pharmacological classes in aqueous samples. Drugs were Antivirals (Sofosbuvir, Daclatasvir, Velpatasvir and Ledipasvir), Antidiabetics (Sitagliptin, Dapagliflozin, Repaglinide, Gliclazide and Nateglinide) and β-blockers (Atenolol, Aceubtolol, Bisoprolol and Beaxolol). In SULLME, acetonitrile is added to a glass test tube containing the aqueous sample, and then sucrose (as a phase separating agent) is added to it, and then the obtained solution is centrifuged. Then the upper layer containing the drug is removed and transferred to HPLC vials for analysis. Compared with the other modes, SULLM found to be the most efficient and thus it was applied for sample preparation of the antivirals in human plasma.		[84]
Erythromycin A (EA)	ACN + Sorbitol, Maltose and Fructose	EA partitions predominantly to the ACN-rich phase because of its hydrophobic nature. K_{EA} decreases with increasing the ACN concentration and temperature. The highest values of K_{EA} were obtained to be 4.73	25	[63]

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		and 1.6 for (Sorb. 15 wt.% + ACN 35 wt.%) and (Fruc. 15 wt.% + CAN 40 wt.%) systems, respectively.		
Glatiramer acetate (GA)	ACN + Maltodextrin	The separation of GA from a mixture containing its constituent amino acids (tyrosine, glutamic acid, alanine, and lysine) was investigated using the ACN + Maltodextrin ABS. In order to determine optimum conditions with the best performance, the primary studies were done so that amino acids and GA were fed into ABS individually and the effect of pH and phase composition on the partitioning of GA and amino acids was investigated. In this case, glutamic acid and alanine partition predominantly to the ACN-rich top phase while GA, tyrosine, and lysin partition toward the maltodextrin-rich bottom phase. In the next step, all of them (GA and the amino acids) were fed into the optimum ABS (15 wt. % maltodextrin, 35 wt. % ACN, volume ratio = 1.61, and pH = 6), and the concentration of GA in the top and bottom phases of the optimum broth feed was analyzed. It was found that under the optimal feed and in the presence of the amino acids, GA partitions toward the top phase and its partition coefficient and selectivity were 1.17 and 62% (the summation of the amino acids partition coefficients was taken as Kcontaminant), respectively. Also, alanine and glutamic acid partition toward the bottom phase in the presence of the other amino acids. Finally, to recover phase components, ACN was evaporated and maltodextrin was precipitated by adding methanol.	25	[88]
Diuran	THF + Xylose, Fructose, L-(+)-Arabinose, D-(-)-Arabinose, Mannose, Glucose, Sucrose, Maltose, and commercial sugars (Sucrose, Glucose and Fructose)	Diuran partitions predominantly to the THF-rich phase with partition coefficient ranging from 1.23 (commercial glucose) to 16.19 (pure glucose) and recovery between 49.45 % (commercial glucose) and 92.70 % (pure glucose).	25	[64]

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Diuran	THF + Glycerol, Erythritol, Xylitol, Sorbitol, and Maltitol	Diuran preferably partitions to the top THF-rich phase. Similar to the phase forming ability, the diuran partitioning ability of the investigated polyols is proportional to the hydration capacity of the polyols (number of hydroxyl groups) and follows the order: glycerol < erythritol < xylitol < sorbitol < maltitol. The highest value of recovery of herbicide diuran (93%) was obtained using THF + maltitol ABS.	25	[68]
-Syringic acid -Furfural -Para-coumaric acid -Ferulic acid -5-Hydroxymethyl furfural	Acetonitrile + Glucose and Xylose	The distribution of syringic acid, furfural, para-coumaric acid, ferulic acid and 5-hydroxymethyl furfural in the CAN + Glucose/Xylose ABSs was examined and it was found that all of these compounds tend to concentrate in the CAN-rich top phase. With the increase of sugar concentration, the volume of the upper phase (as well as the phase ratio) and as a result the distribution coefficient of the above organic compounds increases. In addition, glucose as a mass separating agent is more effective than xylose in extracting organic compounds from their sugar solutions (with the exception of furfural).	1	[115]
BSA	1-Propanol, 2-Propanol + Glucose, Maltose, and Sucrose	BSA tends to concentrate in the more hydrophobic alcohol-rich phase. Due to the relatively higher hydrophobicity of 1-propanol than 2-propanol, 1-propanol + carbohydrate ABS showed better BSA partition coefficient than 2-propanol + carbohydrate ABS. Among the investigated carbohydrates, maltose had the best protein partition efficiency due to its higher sugaring-out effect. Therefore, the protein partition efficiency of the studied ABSs is similar to their phase-forming ability and the 1-propanol + maltose ABS exhibited an overall better partition efciency of BSA to the alcohol-rich top phase. A maximum partition coefficient of 20.01 and recovery yield of 95.42% of BSA were achieved with 35 wt. % 1-propanol + 22 wt.% maltose ABS at pH 5.0 for 10 wt.% BSA load. The FTIR analysis	25	[104]

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		showed that the protein structure remained unchanged during the separation process.		
Pd (II), Pt (IV) and Rh (III)	Diisopentyl sulfide (DIPS) + CAN + Glucose	A sugaring-out assisted "DIPS + CAN + glucose" aqueous three phase system was used to extract and separate Pd, Pt and Rh from their hydrochloric acid aqueous solution. Pd, Pt and Rh respectively concentrate into the DIPS-rich top phase, CAN-rich middle phase and sugar-rich bottom phase. The effects of different phase-forming agents, concentrations of glucose and HCl, and initial volume ratio of added acetonitrile to aqueous glucose solution (V_{ACN}:V_{aq}) on the partition behavior of the investigated metals have been investigated. The increase in volumes of middle phase by increasing glucose concentration led to a corresponding increase in w_{Pt} in the middle phase. However, the increase in V_{ACN}:V_{aq} and HCl concentration in initial solution hampered the three-liquid-phase selective separation of Pt, Pd and Rh. Finally the recovery of Pd(II) and Pt(IV) from the loaded phases after three-phase separation were also examined.	6, 10, 15, 20, 25	[66]
(ii) Extraction or recovery from real matrix				
(ii-1)Sugaring-out assisted liquid-liquid extraction				
- Chlorogenic acid (CGA) - Flavonoids	1-Propanol + Glucose and Maltose	These ABSs were used to extract the bioactive compounds CGA, flavonoids, and TPC from haskap leaves (*Lonicera caerulea*). CGA, flavonoids, and TPC partition to the alcohol-rich top phase.	25	[69]

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- Total phenolic content (TPC)		The values of K_i (EE_i%) for CGA, flavonoids, and TPC in the 1-propanol + glucose ABS were obtained as 1.84 (74.0%), 2.88 (82.1%) and 2.41 (79.4%), respectively. The values of K_i (EE_i%) for CGA, flavonoids, and TPC in the 1-propanol + maltose ABS were obtained as 4.76 (73.8%), 5.52 (76.0%) and 4.14 (69.5%), respectively. The salt (NaH_2PO_4 and $(NH_4)_2SO_4$) + ethanol ABSs had higher EE than sugar + propanol ABSs. HPLC analysis showed fewer components in the extracts obtained from ATP extraction than the conventional extract, which together with the *K* values supports the concept that ABS integrates biomolecule concentration and partial purification.		
- Chlorogenic acid - Flavonoids - Total phenolic content	1-Propanol + Glucose and Maltose	In this work, the effects of recycling on the aqueous biphasic extraction of bioactive compounds CGA, flavonoids, and TPC from haskap leaves (*Lonicera caerulea*) have been investigated. This work uses ATPE parameters optimized in the previous study [69] and it was found that with two recycling stages, the extraction performance was maintained.	25	[86]
- Para-coumaric acid (pCA) - Ferulic acid (FA)	Acetonitrile + Glucose, Xylose, and Sucrose	Sugaring-out assisted liquid–liquid extraction (SULLE) was used to extract p-CA and FA from different agriculture residues obtained after alkaline hydrolysis. pCA tends to concentrate in the organic phase. 91.6% (wt.%) separation of pCA was achieved (in the top phase) with sugaring out. HPLC analysis of the ACN-rich top phase (obtained after phase separation) showed pCA as main component rather than FA.	4	[70]
10-Hydroxy-2-decenoic acid (10-HAD)	Acetonitrile + Glucose	SALLE (with NaCl and $MgSO_4$) and SULLE (with glucose) were used for the partition of 10-HDA and the co-extraction of protein from royal jelly. 10-HAD and co-extracted protein tend to concentrate in the organic top phase.	Not reported	[72]

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		Extraction efficiency (EY) of 10-HDA in the ACN-rich phase depends on the phase ratio (R_V) and to obtain EY higher than 80%, the R_V was suggested to be higher than 0.8. NaCl SALLE and SULLE are less efficient than MgSO4 SALLE in the 50% (v/v) ACN-water mixture. In SALLE with NaCl and SULLE, phase ratio and subsequently also the partition efficiency increase by increasing the initial concentration of ACN in ACN-water mixture so that EYs in NaCl SALLE and SULLE improve to be similar with that in MgSO4 SALLE by increasing the initial concentration of ACN in the ACN-water mixture. In SULLE, the co-extracted protein slightly increased with the raise of sugar concentration, and significantly increased at high ACN concentration.		
Protein	Acetonitrile + Glucose, Sucrose, Maltose, Fructose, and Xylose	Sugaring-out assisted Liquid Biphasic Electric Flotation system (LBEF) was used for the extraction of protein from microalgae specie of *Chlorella vulgaris* FSP-E to top organic phase. The external electric force provided to the ABS is used to disrupt the rigid microalgae cell wall and releases the intracellular contents. The separation efficiency and protein recovery of the LBEF system were 74% and 69.7%, respectively, and in the absence of electric field supply were 61.6% and 48.8%.	25	[73]
Protein	Acetonitrile + Glucose, Fructose, Sucrose, and Maltose	Sugaring-out assisted Liquid Biphasic Flotation (LBF) combined with ultrasonication was applied to extract protein from *Chlorella vulgaris* FSP-E. The ultrasonication is used for cell disruption and releases the intracellular contents. The Effects of different factors such as microalgae biomass concentration, sonication probe location, sonication time, ultrasonic pulse mode, glucose concentration, sugar types, acetonitrile concentration and the flow rate in the flotation system on the separation efficiency and protein recovery were evaluated	ambient	[74]

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		and a maximum efficiency (86.38%) and yield (93.33%) were attained at the optimized conditions.		
Protein	Acetonitrile + Glucose, Sucrose, Maltose, and Fructose	Sugaring-out assisted LBF combined with sonication was used for the isolation of protein from green microalga, *Chlorella sorokiniana* CY1. Sonication aids in breaking down the cell wall during extraction. Different factors influencing the performance of extraction were investigated and the highest protein yield of 80% and the corresponding separation efficiency of 49%, were achieved after optimization through various parameters, suggesting that the high content of protein within microalgae could be extracted on an industrial scale, as LBF is easy to be scaled up with lower cost. Among the investigated sugars, glucose was the most suitable sugar in forming the phases with acetonitrile to enhance the production of protein (52% of protein).	room	[75]
- Gallic acid (GA) - Ellagic acid (EA)	Ethanol (EtOH) and Propylene glycol (PG) + Glucose	The feasibility of employing sugaring-out concentration for the extraction of total phenolics, GA and EA from *Terminalia chebula* Retz was investigated. Two EtOH + glucose and PG + glucose ABSs were employed. It was found that by adding glucose to the aqueous solutions of EtOH + extracts of T. chebula and PG + extracts of *T. chebula*, the mixtures separate into two phases: (1) the EtOH or PG-rich top phase, into which phenolic compounds were concentrated and (2) the glucose-rich bottom phase. The distribution coefficients of total phenolics, GA and EA in the ethanolic system were 1.98, 4.89 and 3.52, respectively, while those for PG system were 1.98, 4.34 and 3.22, respectively.	29, 40, 55, 70, 81	[76]

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-Podophyllotoxins -Flavones	Hexane-Ethyl acetate-Methanol (HEM) + Sucrose, Glucose, Fructose, Maltose, D-Galactose, D-Sorbose, Mannose, Rhamnose, and Xylopyranose	In this work, a sugaring-out strategy for the counter-current chromatographic (CCC) separation of podophyllotoxins and flavones in the extract of the *Dysosma versipellis* (traditional chinese medicine) by adding a dose of sugars as modifiers into the two-phase hexane-ethyl acetate-methanol-water (HEMWat) solvent systems has been presented. Although different sugars have different sugaring-out effects on the resolution and selective separation of some components of *Dysosma versipellis*, all of the investigated sugars increase the partition coefficients of almost all of targets in the two-phase HEMWat solvent systems. In this case, the sugaring-out effects may be due to sugars competing for hydrogen bonding with the analytes. By increasing the sugar content and decreasing the probability of forming analyte-water hydrogen bonds, the analytes are pushed into the organic phase. As the partition coefficients of targets increase, the CCC separation improves.	room	[81]
Lipids	Acetonitrile + Glucose, Sucrose, Fructose, and Maltose	The potential of sugaring-out assisted LBF method to recover lipids from the microalgae *Chlorella sorokiniana* CY1 for biofuel production was investigated. Various factors affecting the extraction performance such as type and concentration of mass separating agent (sugar), liquid biphasic volume ratio and flotation time were investigated and optimized to achieve the highest lipid recovery yield (74%) which was obtained using fructose as the mass separating agent with a solution concentration of 150 g/L, in a flotation time of 15 min with a mass separating agent-ACN volume ratio of 1:2. In addition, the effectiveness of sugar as a cell disruption agent was also observed whereby the amount of lipid extracted was comparable to the amount extracted from microalgae cells pretreated with microwaves.		[83]

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-Caffeic acid -*p*-Coumaric acid -Isoferulic acid -Dimethoxycinnamic acid -Cinnamic acid -Kaempferol -Caffeic acid phenethyl ester	ACN + Glucose	SULLE method was used for the extraction of multiple phenolic compounds from propolis. Extraction yields (EYs) of phenolic compounds in the upper ACN-rich phase increase by increasing the sugar concentration and decreasing the polarity of phenolic compounds. Less polar phenolic compounds (kaempferol and caffeic acid phenethyl ester) have a greater affinity for the upper ACN phase and therefore have a higher extraction yields. For more-polar phenolic compounds (caffeic acid, p-coumaric acid, isoferulic acid, dimethoxycinnamic acid, and cinnamic acid), increasing the initial concentration of ACN in the ACN–H_2O mixture is suggested for archiving higher EYs.	Not reported	[98]
Succinic acid (SA)	t-Butanol + Glucose	The feasibility of employing SULLE combined crystallization to separate succinic acid from synthetic fermentation broths was investigated. Glucose was dissolved in a simulated fermentation broth, the pH which had been adjusted by adding NaOH. By adding organic solvents to the mixture, an ABS was formed in which SA was extracted and concentrated in the solvent phase. The systems consisting of different organic solvents and glucose were screened and it was found that t-butanol showed remarkable extraction efficiency of SA. Various factors affecting the extraction performance of t-butanol + glucose system such as tie line length, t-butanol concentration, glucose concentration, system pH, succinic acid concentration, temperature, and inorganic salt addition were evaluated. By a system consisting of 27 wt.% glucose and 40 wt.% t-butanol at pH 3 and 25 °C, the partition coefficient and extractability of SA were obtained as 5.14 and 88.15 %, respectively. Finally, the top phase was treated using vacuum distillation to recover t-butanol for reuse. Subsequently, in order to purify and obtain SA crystals, gradient crystallization was conducted and then water-washing was carried out to remove glucose and obtain the	5, 15, 25, 35, 45	[92]

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		high purity of SA. Finally, the SA crystals were dried. An identical total yield (73%) and a higher purity (98%) of succinic acid were obtained.		
Lactic acid	Isopropanol + Glucose	Sugaring-out extraction was applied to extract lactic acid from the fermentation broth. Sugar was dissolved in a simulated fermentation broth, the pH which had been adjusted by adding NaOH. By adding organic solvents to the mixture, an ABS was formed in which lactic acid was extracted and concentrated in the solvent-rich top phase. The systems consisting of different organic solvents and different sugars were screened and it was found that the isopropanol+glucose system is more favorable in partitioning and recovering lactic acid and therefore this system was selected and optimized. By a system consisting of 12 wt.% glucose and 40 wt.% isopropanol, the partition coefficient and recovery of lactic acid were obtained as 1.39 and 84.27 %, respectively. Finally, sugar as a phase separating agent was considered to be fermented as carbon source for lactic acid production. Therefore, the feasibility of the reutilization of glucose-rich bottom phase was investigated for lactic acid fermentation. The yield of lactic acid from glucose was 91.1% in the bottom phase-based fermentation vs. 94.0% in the normal fermentation. It seems that sugaring-out extraction can couple the fermentation and separation for lactic acid production.	25	[91]
2,3-Butanediol (2,3-BD)	t-Butanol + Glucose	The feasibility of employing SULLE to separate 2,3-BD from fermentation broths was investigated. Sugar was dissolved in 2,3-BD fermentation broth to obtain sugar mixture and then the organic solvents was added into the sugar mixture and a biphasic system was appeared. 2,3-BD was extracted and concentrated in the solvent-rich top phase. The systems consisting of different organic solvents and different sugars were screened and the effects of glucose addition, 2,3-BD concentration and inorganic salt addition were investigated. It was found that the t-butanol+glucose system is more favorable in partitioning and recovering 2,3-BD and	25	[105]

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		therefore this system was selected and optimized. A system consisting of 1.2 g glucose, 4 ml t-butanol and 4 ml fermentation broth containing 60.3 g/L 2,3-BD, separates into two phases in which 75.6% 2,3-BD was distributed into the top phase, and 80.4% glucose into the bottom phase, and 78.0% soluble proteins and 86.8% lactic acid were separated from 2,3-BD. Finally the glucose-rich bottom phase after removing part of t-butanol was reused for 2,3-BD fermentation.		
Acetoin	Ethyl acetate + Glucose	Sugaring-out extraction coupled with fermentation (SOECF) was applied to produce acetoin by Bacillus subtilis DL01. By mixing 1000 mL ethyl acetate with the sugar mixture, obtained by adding 500 g glucose to 500 ml fermentation broth (60.5 g/L acetoin, 14.0 g/L glucose, 2.7 g/L formic acid, 3.8 g/L acetic acid, and 2.3 g/L lactic acid), a two phase system was formed in which 61.2% acetoin was extracted into the solvent-rich top phase and most of the sugar (nearly 100%) as well as by-products (coloring matters and organic acids) remained in the bottom phase. Pure acetoin could be obtained by distillation of concentrated solution of top phase, while the bottom phase could be used as carbon source for next batch of fermentation.	25	[90]
Ethanol	t-Butanol + Glucose	In this work, a preliminary study of sugaring-out extraction coupled with ethanol fermentation using raffinate phase has been done to separate ethanol from fermentation broth. Using the system consisting of 10 wt.% glucose, 30 wt.% butanol and 2 wt.% ammonium sulfate, ethanol recovery in both simulated ethanol fermentation broth and real broth reached 70% using a single sugaring-out extraction.	25	[113]
Erythromycin	ACN + Glucose	Sugaring-out extraction of erythromycin from fermentation broth has been carried out using acetonitrile as solvent and glucose as a mass separating agent. Erythromycin tends to concentrate in the organic phase.	4	[80]

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		Various factors affecting the extraction performance such as glucose concentration, temperature, acetonitrile/water ratio and pH were investigated and optimized to achieve the maximum extraction of erythromycin which was 88% (wt.) with the following optimal conditions: glucose 156.3 g/L; temperature 4 °C; acetonitrile /water ratio 1 and pH 8.3. Furthermore, the antimicrobial activity of erythromycin was maintained during sugaring-out extraction.		
Erythromycin	ACN + Glucose	In this work, the ultrasound-mediated sugaring-out extraction (UMSOE) of erythromycin from real fermentation broth was investigated. Ultrasound was combined with the ABS to improve the extraction time. Different parameters affecting the extraction (temperature, glucose concentration, time, pulse, probe position and ACN/water ratio) were optimized for a model system and then applied for UMSOE of erythromycin from real fermentation broth. Under the optimized process conditions, the maximum extraction efficiencies of 91.6% and 87% were obtained for UMSOE of erythromycin from the model system and real fermentation broth respectively.	4, 12, 20, 28, 36	[97]
Erythromycin	ACN + Glucose	Sugaring-out extraction method was used to extract erythromycin from the model system (only ACN and erythromycin) and simulated fermentation broth (composition (g/L): starch 5, soybean flour 2.7, CaCO3 0.5, NaCl 0.2 and (NH4)2SO4 0.557). The optimized conditions for sugaring-out extraction of erythromycin were (glucose 15.6% w/w, temperature 4 ◦C, pH 8.3, and solvent/broth ratio 1:1). Then the ACN-rich phase obtained at the end of sugaring-out extraction, which contained 80% erythromycin, was further processed to separate erythromycin using combined antisolvent-evaporative crystallization method.	4	[95]
		(ii-2)Matrix-induced sugaring-out liquid-liquid extraction		

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β-(2-1)-Fructooligosaccharides (FOS)	ACN + Yacon water extract	Since dried yacon roots contain 40%-80% sugar (mainly fructooligosaccharides, sucrose, glucose and fructose), an ABS is formed by adding ACN to the aqueous extract of yacon roots with the ACN-rich upper phase and the water-rich lower phase. This ABS was used to extract and purify FOS from water extract of yacon. Most of FOS was extracted into the ACN-rich phase. The effects of sugar concentration in yacon water extract, extraction temperature and the volume ratio of ACN to yacon/water slurry (V_{ACN}:V_{AQ}) on the extraction efficiency of FOS from yacon roots were investigated. It was found that the FOS mass fraction in the top phase increases by increasing sugar concentration, decreasing temperature and increasing VACN:VAQ ratio.	6, 10, 15, 20, 25	[108]
(iii) recovery and removal of organic solvents				
- BSA - Trypsin - Pepsin	Acetonitrile + Glucose	Separation of ACN from an aqueous solution containing protein induced by glucose (sugaring-out) was studied. The effect of glucose addition on protein recovery from an ACN-water system was investigated at 6 and 18 °C. More than 95% of the proteins were retained in aqueous phase after phase separation due to their hydrophilic nature and also majority of the ACN was separated from the aqueous phase. Protein recovery (and also ACN concentration) was unaffected by change in temperature, glucose concentration and initial concentration of ACN. High recovery of proteins, removal majority of ACN and no alteration in pH are the advantages of this method.	6 and 18	[65]
Acetonitrile	Acetonitrile + Sucrose	Three procedures containing different combinations of fractional distillation, cooling, salting-out (by $CaCl_2$), and sugaring-out (by	76.5	[109]

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		sucrose) methods were used to ACN recovery from a residual aqueous solution. The protocols using $CaCl_2$ as phase separating agent guaranteed the highest total ACN recovery yields. But considering only the first distillation followed by the addition of sucrose without any subsequent steps, the recovery efficiency of ACN was better than when $CaCl_2$ was used as the phase separation agent.		
-Acetonitrile -Tetrahydrofuran -Cr(VI)	ACN + Fructose	In this work, the purification of water contaminated with chromium (VI) and THF or ACN using reducing sugar fructose along with HCl has been investigated. Without HCl, fructose separates ACN and THF from water, but does not partition Cr(VI) into the organic solvent phase. When HCl is added to solutions of Cr(VI) and fructose in ACN-water or THF-water, it causes migration of Cr(VI) from water to the separated phase of THF or ACN. Therefore, when fructose and HCl are used together, they enable simultaneous removal of Cr(VI) and organic solvents from water. Other acids (such as H2SO4) allow Cr(VI) migration into ACN or THF only in the presence of chloride salts, indicating that both pH and chloride ions are responsible for Cr(VI) migration in ACN or THF.	Not reported	[96]
		(iv) Analytical purpose:		
		(iv-1)Sugaring-out assisted liquid-liquid extraction		
Fucoxanthin	Acetonitrile + Glucose	Sugaring-out assisted liquid-liquid extraction method was used to determine fucoxanthin contents in Icelandic edible seaweeds. Glucose solution with different concentration was mixed with acetonitrile extracts to induce phase separation and fucoxanthin partition to the organic upper layer. It was found that the plot of fucoxanthin partition coefficient against glucose concentration had a maximum at glucose concentration 70 $mg{\cdot}mL^{-1}$ and in fact the	6	[79]

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		highest yield of fucoxanthin was found when fucoxanthin solution (fucoxanthin + ACN) is mixed with 70 mg·mL^{-1} glucose.		
-Bisphenol A (BPA) -Bisphenol B (BPB)	Acetonitrile + Glucose	SULLE method combined with HPLC with fluorescence detection was used to determine BPA and BPB in royal jelly. SULLE was employed for the sample preparation of BPA and BPB from royal jelly sample. Various factors affecting the extraction performance such as amount of mass separating agent (glucose), initial ACN concentration and sample size were investigated and optimized to achieve the optimal SULLE procedure (0.3 g sample extracted by 2 mL ACN-water mixture (50%, v/v), followed by phase separation with the addition of 0.4 g glucose). The obtained extract was injected into the reversed-phase HPLC system without clean-up step.	Not reported	[93]
-Lopinavir (LPV) -Ritonavir (RTV))	ACN + Glucose	SULLE and liquid chromatography (LC) with mass spectrometric (MS/MS) detection were used to determine two antiviral drugs LPV and RTV in human plasma. Under the ACN + glucose ABS, ACN can be used as an acceptor phase to extract antiviral drugs. Extraction recovery for SULLE was approximately 55% for LPV and 50% for RTV. An aliquot of the upper layer containing the extracted sample is injected into an LC-MS/MS system after dilution with water. The differences between measured concentrations with SULLE and SALLE (Salting-out LLE) were comparable or equivalent.	10	[67]
-Pseudoephedrine -Lidocaine -Propranolol	2-Ethyl hexanol, 1-Heptanol and 1-Octanol + Glucose, Fructose, Galactose and Sucrose	Sugaring-out assisted electromembrane extraction (EME) was combined with HPLC to enhance the extraction of four basic model drugs (pseudoephedrine, lidocaine, propranolol, and ketoconazole). After optimizing the extraction conditions, the method was evaluated in real biological samples including human plasma and urine in order to prove the efficiency of sugaring-out	Not reported	[116]

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-Ketoconazole		effect in EME systems. In this type of EME, in addition to improving the transfer of analytes through the supported liquid membrane, the whole extraction system also became more stable than the conventional system.		
-Evodiamine (EVO) -Rutaecarpine (RUT) -Limonin (LIM) -Rutaevin (RVN) -Obacunone (OBA) -Liquiritigenin (LIQN) -Dehydroevodiamine (DHE) -Liquiritin (LIQ) -Isoliquiritin (ILIQ)	ACN + Glucose	In this work, six sample extraction methods include (1) PPT with methanol, (2) PPT with acetonitrile, (3) LLE with tertiary-butyl ether-dichloromethane, (4) LLE with ethyl acetate-n-butanol, (5) SALLE with ammonium acetate-ACN, and (6) SULLE with glucose-ACN were investigated with the aim of finding the best method for simultaneous determination of alkaloids (EVO, RUT, DHE), terpenoids (LIM, RVN, OBA), and flavonoids (LIQ, ILIQ, LIQN) in rat plasma using a UHPLC-MS/MS system. In order to determine optimum conditions with the best performance for the nine compounds, first the primary studies were done with a mixture of target component standards isolated from Tetradium ruticarpum and Glycyrrhiza uralensis. Among the 6 extraction methods evaluated, the SALLE method was chosen (due to high recovery, satisfactory matrix interference and wide coverage of analytes). Next, the SALLE method was used to determine the pharmacokinetic behavior of the nine compounds in rat plasma after intragastric administration with an extract from Tetradium ruticarpum and Glycyrrhiza uralensis (Wuzhuyu-Gancao pair). The extraction recoveries of DHE, EVO, RUT, LIM, RVN, OBA, LIQN, LIQ and ILIQ by the SULLE method were obtained as 79.96, 90.55, 97.11, 97.23, 84.89, 84.78, 76.90, 21.33 and 54.66%, respectively.	4	[94]
-2,4-dichlorobenzyl alcohol -Amylmetacresol	ACN + Glucose, Fructose and Sucrose	A procedure based on the solid sample dissolution combined with sugaring-out liquid-liquid extraction (partitioning of target analytes between the aqueous and acetonitrile phases) followed by subsequent analysis (by UV-Visible spectrophotometry) was used for separation and determination of antiseptics (2,4- dichlorobenzyl alcohol and amylmetacresol) from artificial azo dyes (Yellow	Not reported	[106]

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-Yellow Orange S, -Azo Rubine -Ponceau 4R		Orange S, Azo Rubine, Ponceau 4R) in the throat lozenge pharmaceuticals. In fact, due to overlapping absorption spectra of dyes, 2,4-dichlorobenzyl alcohol and amylmetacresol, their simultaneous spectrophotometric determination in the same phase based on the individual calibration curves is impossible. When a sugar is added to a ACN + water solution of the throat lozenges samples, an aqueous biphasic system is formed, in which the artificial water-soluble dyes are remained in the lower sugar-rich aqueous phase and 2,4-dichlorobenzyl alcohol and amylmetacresol are extracted into the upper ACN-rich phase. The analytical results agreed fairly well with the HPLC-UV method. The effects of water-acetonitrile ratio and volume of mixture, type and concentration of sugar, sample weight, mixing time and pH were investigated.		
Procainamide (PA)	ACN + Glucose, Fructose and Sucrose	Automated SULLE method combined with HPLC-UV system was used to determine procainamide in human urine samples. When a sugar is added to a homogeneous ACN + water solution of the urine sample, an aqueous biphasic system is formed, in which PA is extracted into the upper ACN-rich phase. The analytical results agreed fairly well with the capillary electrophoresis method. The effects of phase ratio, type and concentration of sugar, mixing time, pH and PA distribution in the sugaring-out system were investigated.	ambient	[110]
-Malathion -Diazinon -Imidacloprid -Triadimefon	ACN + Glucose	Automated in-syringe sugaring-out liquid-liquid extraction (IS-SULLE) method coupled with HPLC-MS/MS system was used to extract and determine pesticides (malathion, diazinon, imidacloprid and triadimefon) in fruit (apple, cherry, raspberry, orange and pineapple) juices matrix. When glucose is added to a homogeneous ACN solution of a fruit juice sample, an aqueous biphasic system is formed, in which pesticides are extracted into the upper ACN-rich phase. After phase separation, the ACN-rich	20-50	[103]

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		phase containing pesticides was used for the HPLC-MS/MS analysis with no further sample treatment. Before testing with real examples, for model systems, the various factors affecting the pesticides extraction such as phase ratio, concentration of glucose, stirring time, temperature, pH and salt addition were studied and optimized for each pesticide. The partition coefficients (at 25 °C) in water/acetonitrile system in the presence of glucose were equal to 11.8, 68.8, 7.2 and 8.9 for malathion, diazinon, imidacloprid and triadimefon, respectively.		
-Honokiol -Magnolol	ACN + Glucose, Fructose and Sucrose	SULLE method coupled with HPLC was used to determine honokiol and magnolol in traditional Chinese herbal formula Huoxiang-Zhengqi oral liquid. By adding sugar to aqueous mixture of ACN + Huoxiang-Zhengqi oral liquid, an ABS is formed. The upper organic layer containing honokiol and magnolol was removed and analyzed by HPLC method. The various factors affecting the extraction including the type and dosage of sugar, the volume of ACN, pH, temperature, and vortex time were investigated. RSD values for repeatability and reproducibility were less than 1.62% and 1.80%, respectively. The recoveries were in the range of 85.8–103.7%.	-10, 0, 10, 20, 30 and 40	[111]
		(iv-2)Matrix-induced sugaring-out liquid-liquid extraction		
Sulfonamides (SAs)	Acetonitrile + honey (sugar)	SULLE method combined with HPLC with fluorescence detection was used to determine SAs in honey. Considering the average of 80% sugars in honey, phase separation is easily done by adding ACN aqueous solution to honey and the SAs are extracted from honey into the upper organic layer. Then, an aliquot of the upper organic layer containing the extracted SAs is injected directly or after dilution into the HPLC system.	room	[78]

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Phenolic compounds (PCs)	ACN + Honey	A matrix-induced SULLE method combined with HPLC-ECD technique was used for the extraction and determination of phenolic compounds (eight phenolic acids and nine flavonoids) in honey. By adding acidified honey aqueous solution to ACN, an ABS is formed and the PCs are extracted from honey into the upper organic layer. The upper organic layer containing PCs was collected, dried, dissolved with methanol/water solution (v/v, 1:1) and injected into the HPLC system. To achieve the maximum efficiency, the various factors affecting the recovery, such as pH, ionic strength, extraction times and the volume of extracting solvent, were investigated.	Not reported	[89]
-Neonicotinoid Pesticides	ACN + Honey	A matrix-induced sugaring-out liquid-liquid extraction method was used for the determination of three neonicotinoid pesticides in honey samples. The honey sample, which contains a high concentration of sugars (70 wt.% monosaccharides), itself was mixed directly with acetonitrile + water mixture to trigger the phase separation and the neonicotinoid pesticides were extracted into the upper ACN-rich phase and the sugars remained in the lower aqueous phase. Then, the ACN-rich phase containing the neonicotinoid pesticides were injected into the HPLC system for the analysis. The various factors affecting the recoveries and response signal such as the sample amount of honey and the initial ACN concentration were investigated.	Not reported	[100]
5-Hydroxymethylfurfural (HMF)	ACN + Honey	A Subzero-temperature assisted liquid-liquid extraction (STLLE) was used for the determination of HMF in honey. Honey sample were mixed with ACN-water solution and after vortex, the mixed solution was cooled at -20 ℃ to trigger phase separation. The upper phase was collected, diluted with ACN aqueous solution and analyzed by HPLC. In STLLE, freezing performance is used to separate the ACN from the ACN-water mixture.	-20	[99]

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-Cyprodinil -Trifloxystrobin -Prometryn -Propachlor -Fenitrothion -Chlorpyrifos -Profenofos -Phosalone	ACN + Molasses	In this study, some pesticides (cyprodinil, trifloxystrobin, prometryn, propachlor, fenitrothion, chlorpyrifos, profenofos, and phosalone) were extracted from molasses samples (grape molasses, carob molasses, and mulberry molasses) by SULLE combined with dispersive liquid-liquid microextraction (DLLME) method and then determined by GC-Mass spectrometry analysis. Here, molasses, which contains 84%–88% sugar, itself is used as the sugar-rich phase. In the first SULLE step, molasses is added to a homogeneous ACN + water mixture and then an aqueous biphasic system is formed, where the pesticides are extracted into the upper ACN-rich phase and the sugars remain in the lower aqueous phase. Then, in the second DLLME step, the ACN-rich phase containing the pesticides and toluene were used as the dispersing and extraction solvents, respectively. Finally, the analysis of the toluene phase containing pesticides after extraction was performed by GC–MS.	4	[107]
-Fenitrothion -Phosalone -Profenofos -Chlorpyrifos	ACN + Fruit jam	An analytical method for the determination of organophosphorus pesticides (fenitrothion, phosalone, profenofos, and chlorpyrifos) in fruit (cherry, strawberry, blackberry, apricot, blueberry) jams using sugaring-out liquid-liquid microextraction method followed by HPLC-UV analysis was developed where the sugars in the sample matrix triggered phase separation. Pre-homogenized jam sample (1 g) was added to 600 µl of ACN/water mixture (50/50%, v/v) and the mixture was shaken and centrifuged. Finally, the upper organic layer containing the organophosphorus pesticides was withdrawn with a microsyringe and transferred to a glass vial for the HPLC/UV analysis. The various factors affecting the extraction efficiency, including the type of organic solvent, the composition, and volume of the organic solvent/water mixture, shaking time, and pH were optimized.		[101]

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2.5.1. Studying the partitioning behavior of biomolecules and other value-added compounds between phases

Like other aqueous two-phase systems, one of the basic studies conducted on the organic solvent-sugar two-phase systems is the study of the distribution behavior of different target substances between the equilibrium phases. The purpose of this type of studies is to determine the factors affecting the distribution behavior so that, based on the characteristics of the target materials, the distribution of these materials can be predicted among the phases, and based on this information, a special two-phase system can be designed for a specific purpose. Usually, the distribution behavior study is used as a preliminary study to design the optimal conditions for the main application (one of the 2.5.2, 2.5.3 and 2.5.4 cases). In order to determine the optimal conditions with the best performance for the main application, first as preliminary studies, the distribution behavior of pure materials is investigated individually among the phases and the optimal conditions are determined by studying the effect of various factors including the type and concentration of phase components, temperature, pH, TLL, etc. A literature review showed the partition behavior of various compounds between the phases of organic solvent-sugar ABSs that has been investigated, and these are listed in Table 3 along with a summary of the obtained results. When a sugar is added to an aqueous organic solvent mixture, the sugar at a high concentration may induce the mixture to form a biphasic system in which the organic solvent and low polarity (more hydrophobic) solutes tend to go to the top phase while part of the water and high polarity (more hydrophilic) components tend to remain at the lower (sugar-rich) phase. According to the values of H_2O_{ratio} (the ratio of the weight fraction of water in the upper phase to the weight fraction of water in the lower phase), the organic solvent-rich phase has a higher hydrophobic character than the sugar-rich phase. In most cases, the target molecules partition predominantly to the more hydrophobic, organic solvent-rich, phase because of their hydrophobic nature. However, some target components such as cephalexin, cfazolin, cefixime and codeine tended to concentrate in the lower carbohydrate-rich phase. In Table 2, the values of the octanol–water partition coefficient, log K_{ow}, for some investigated target molecules have also been given. As can be seen, all the target materials that tend to concentrate in the organic solvent-rich phase have much larger log K_{ow} than carbohydrates.

2.5.2. Extraction of different target molecules from real matrixes

The sugaring-out assisted liquid-liquid extraction (SULLE), as a novel technique, has been successfully applied to extract different materials from real matrixes. The results gathered from the literature show that this type of ABS has been used for the extraction of chlorogenic acid, flavonoids and total phenolic content from haskap leaves (*Lonicera caerulea*) [69,86], *p*-coumaric acid and ferulic acid from different agriculture residues [70], protein from royal jelly [72] and microalgae [73–75], total phenolics, gallic acid and ellagic acid from *Terminalia chebula* Retz [76], podophyllotoxins and flavones from *Dysosma versipellis* (traditional chinese medicine) [81], lipids from the microalgae *Chlorella sorokiniana* CY1 for biofuel production [83], multiple phenolic compounds (caffeic acid, *p*-coumaric acid, isoferulic acid and dimethoxycinnamic acid) from propolis [98], succinic acid [92], lactic acid [91], 2,3-butanediol [105], ethanol [113], acetoin [90] and erythromycin [80,95,97] from fermentation broths and β-(2-1)-fructooligosaccharides from yacon roots [108]. All of them are reported in Table 3 along with a summary of the obtained results.

In order to improve the extraction efficiency or extraction time, novel intensification methods such as flotation [26], microwave [134], fermentation [90], crystallization [92] and ultrasonication [135] have been combined with ABSs. Moharkar et al. [97] investigated ultrasound-mediated sugaring-out

extraction (UMSOE) for rapid extraction of erythromycin from real fermentation broth. Ultrasound-assisted ABS appears as a promising technology due to the cavitation effect produced by the interaction of ultrasound waves (frequency above 20 kHz). The physical effects of cavitation such as micro-turbulence, micro-streaming, microjets, and shock waves are highly useful to overcome the mass transfer limitation in the extraction process. Sugaring-out assisted liquid biphasic flotation technique combined with ultrasonication [74,75] and external electric force [73] have been introduced for the extraction of proteins from microalgae. The sonication [74,75] or external electric force [73] provided to the ABS has been used to disrupt cells and release intracellular contents. One of the most important advantages of the SULLE method compared to other methods is that in the liquid-liquid extraction process, besides being a phase separating agent, sugar can simultaneously play other roles such as cell disruption agent and substrate in fermentation. For example, the suitability of sugaring-out assisted liquid biphasic flotation technique for lipid extraction from microalgae has been proven, where sugar acts as a cell disruption agent in addition to the role of phase separating agent [83]. Another examples are the sugaring-out extraction of lactic acid [91], 2,3-butanediol [105] and acetoin [90] from the fermentation broth in which, sugar as a phase separating agent is considered to be fermented as carbon source for lactic acid, 2,3-butanediol and acetoin production. Because of the sustainable and renewable properties and wide industrial applications of biofuels and bio-based chemicals, the application of sugaring-out extraction to recovery of bio-based chemicals from fermentation broths/aqueous solutions is of particular importance. The advantages of using sugars as the sugaring-out agents include its low corrosion to extraction equipment, mild extraction condition, and service as the substrate in fermentation [58]. So far, this method has been used for the extraction of succinic acid [92], lactic acid [91], 2,3-butanediol [105], acetoin [90], ethanol [113], and 1,3-propanediol [114] from the fermentation broth. In these cases, the sugaring-out extraction can couple the fermentation and separation for biochemical production. By adding organic solvents to the sugar mixture (mixture of sugar and fermentation broth), an ABS is formed in which the target bio-based chemical is extracted into the organic solvent-rich top phase and most of the sugar as well as by-products (coloring matters and organic acids) remain in the bottom phase. Pure target biochemical can be obtained by the evaporation of the concentrated solution of top phase, while the bottom phase can be used as carbon source for next batch of fermentation. In fact, the sugar-rich bottom phase after removing part of organic solvent is reused for target bio-based chemical fermentation.

Natural sweeteners such as honey, dates, fruit molasses, fruit jams, dried fruits, etc. contain a high concentration of sugar and can be used as a phase formation agent. By adding a sample of natural sweeteners to the organic solvent + water mixture, a two-phase system is created in which the various target molecules present in the sample are extracted into the upper organic phase and sugar remains in the lower aqueous phase. This method, in which the matrix is simultaneously the phase forming agent and the source of the target molecules, is called the matrix-induced sugaring-out liquid-liquid extraction method and may find applications in the pretreatment of food samples with a high concentration of sugars. As an example, Zhang and Liu [108] showed that an ABS is formed by adding ACN to the aqueous extract of yacon roots with the ACN-rich upper phase and the water-rich lower phase. This ABS was used to extract and purify β-(2-1)-fructooligosaccharides from water extract of yacon. Most of FOS was extracted into the ACN-rich phase.

2.5.3. Recovery and removal of organic solvents from aquatic environments

Water-miscible organic solvents such as acetonitrile, ethanol, propanol, isopropanol, form an azeotrope with water, which prevents its recovery as a pure compound by conventional distillation process. Among

organic solvents, acetonitrile are widely used as a solvent in several fields of chemistry. Aqueous acetonitrile solutions at different concentrations are also used in analytical chemistry, especially in separation methods such as liquid chromatography. On the other hand, since the decomposition of acetonitrile produces highly toxic hydrogen cyanide gas, the development of experimental protocols for the recovery of this compound, is of particular importance. Several unconventional methods for the recovery of acetonitrile from a solution have been reported in the literature, of which the use of salts as phase separating agents for liquid-liquid extraction is one of the most widely used. However, salting-out presents problems due to the high salt concentration required, including undesired chemical reactions, equipment corrosion, and difficulties in separating unstable biological components. As an alternative to the salting-out liquid-liquid extraction, the sugaring-out technique has emerged, which can compensate for the above-mentioned limitations of conventional salting-out effects and have advantages over them.

Dhamole et al. [65] explored the use of sugaring-out as a new phase separation method for the separation of acetonitrile from a simulated preparative RP-HPLC effluent. da Silva et al. [109] used the combination of fractional distillation and sugaring-out (by sucrose) method to acetonitrile recovery from a liquid chromatography residue. Telepanich et al. [96] investigated the purification of water contaminated with Cr(VI) and tetrahydrofuran or acetonitrile using reducing sugar fructose along with HCl. Without HCl, fructose separates tetrahydrofuran or acetonitrile from water, but does not partition Cr(VI) into the organic solvent phase. However, when fructose and HCl are used together, they enable simultaneous removal of Cr(VI) and organic solvents from water.

2.5.4. Determination and analysis of a number of target analytes in different samples

The procedure for the determination of a target analyte is based on the separation of the organic solvent phase, containing the target analyte from the homogeneous sample solution after injection of sugar (as a phase separating agent) into the mixing chamber of the flow system. For this purpose, first the real sample containing the target analyte is dissolved in the mixture of organic solvent + water to form a homogenous solution for the extraction. Then, the organic solvent phase together with the target analyte is separated from the aqueous solution by adding a phase separating agent such sugars. After phase separation, the organic solvent-rich phase containing the analyte is used for the HPLC analysis with no further sample treatment. In this respect, acetonitrile is the most used organic solvents, because of its smaller volume to produce phase separation, better partition of both hydrophilic and hydrophobic compounds, and the compatibility with HPLC instruments. Furthermore, the acetonitrile phase can be separated from aqueous phase by various methods such as significantly lowering the temperature of the mixture, adding salts, sugars or hydrophobic solvents to the mixture. As can be seen from Table 3, the sugaring-out assisted liquid-liquid extraction method combined with a analyzing method such as HPLC has been used to determine fucoxanthin contents in Icelandic edible seaweeds [79], bisphenol A and bisphenol B in royal jelly [93], antiviral drugs Lopinavir and Ritonavir in human plasma [67], the drugs (pseudoephedrine, lidocaine, propranolol, and ketoconazole) in human plasma and urine [116], alkaloids (Evodiamine, Rutaecarpine, Dehydroevodiamine), terpenoids (Limonin, Rutaevin, Obacunone), and flavonoids (Liquiritin, Isoliquiritin, Liquiritigenin) in rat plasma [94], antiseptics (2,4- dichlorobenzyl alcohol and amylmetacresol) in the throat lozenge pharmaceuticals [106], procainamide in human urine samples [110], pesticides (malathion, diazinon, imidacloprid and triadimefon) in fruit (apple, cherry, raspberry, orange and pineapple) juices matrix [103], and honokiol and magnolol in traditional Chinese herbal formula Huoxiang-Zhengqi oral liquid [111].

The matrix-induced sugaring-out liquid-liquid extraction method in the honey sample coupled with HPLC has been reported by different authors for the determination of sulfonamides [78], phenolic compounds [89], neonicotinoid pesticides [100] and 5-Hydroxymethylfurfural [99] in honey. Similarly, the matrix-induced sugaring-out in the molasses samples coupled with HPLC was reported to determine some pesticides in grape molasses, carob molasses, and mulberry molasses [107]. Alkan and Cabuk [101] developed an analytical method for the determination of organophosphorus pesticides (fenitrothion, phosalone, profenofos, and chlorpyrifos) in fruit jams using sugaring-out liquid-liquid microextraction method followed by HPLC-UV where the sugars in the sample matrix triggered phase separation.

2.5.5. Effect of different parameters on distribution behavior in organic solvent-sugar ABSs

The results collected from the literature show that various factors affect the distribution behavior and consequently the extraction and recovery efficiency of the target molecules among the phases of these ABSs, which include: type and concentration of organic solvent and sugar, volume of the organic solvent/water mixture, shaking time, vortex time, mixing time, pH, the sample amount, ionic strength, extraction times, temperature, the volume of extracting solvent, phase ratio, salt addition, volume of mixture, tie line length, liquid biphasic volume ratio and flotation time. In order to determine optimum conditions with the best performance, usually the primary studies are done so that pure standard target materials are fed into the ABS individually and the effect of different parameters on the partitioning behavior is investigated. In Table 3, various factors affecting the extraction performance are discussed along with the obtained results.

In general, the values of partition coefficient increase by increasing TLL and decreasing H_2O_{ratio}. Moreover, the partition efficiency of ABSs is similar to their phase-forming ability, so that the more hydrophilic the carbohydrate and the more hydrophobic the organic solvent, the higher partition coefficient of the target molecules. One of the factors affecting the extraction performance is sugar or organic solvent concentration, which is associated with changing the phase ratio or volume ratio. It has been found that the extraction efficiency usually increases with increasing phase ratio (increasing the volume of the upper phase in which the relatively non-polar analyte is concentrated). Zhang et al. [79] investigated the acetonitrile + glucose ABS for the liquid-liquid extraction of fucoxanthin from Icelandic edible seaweeds. As can be seen from Figure 9, as glucose concentration increases, the phase ratio also increases, which was visualized as the accumulation of organic phase (Figure 9a and b). Figure 9c shows that the plot of fucoxanthin partition coefficient against glucose concentration had a maximum at glucose concentration 70 $mg{\cdot}mL^{-1}$ and in fact the highest yield of fucoxanthin was found when fucoxanthin solution (fucoxanthin + acetonitrile) is mixed with 70 $mg{\cdot}mL^{-1}$ glucose. This figure shows that as the concentration of glucose increases, the distribution coefficient of fucoxanthin first increases, reaches a maximum, and then begins to decrease.

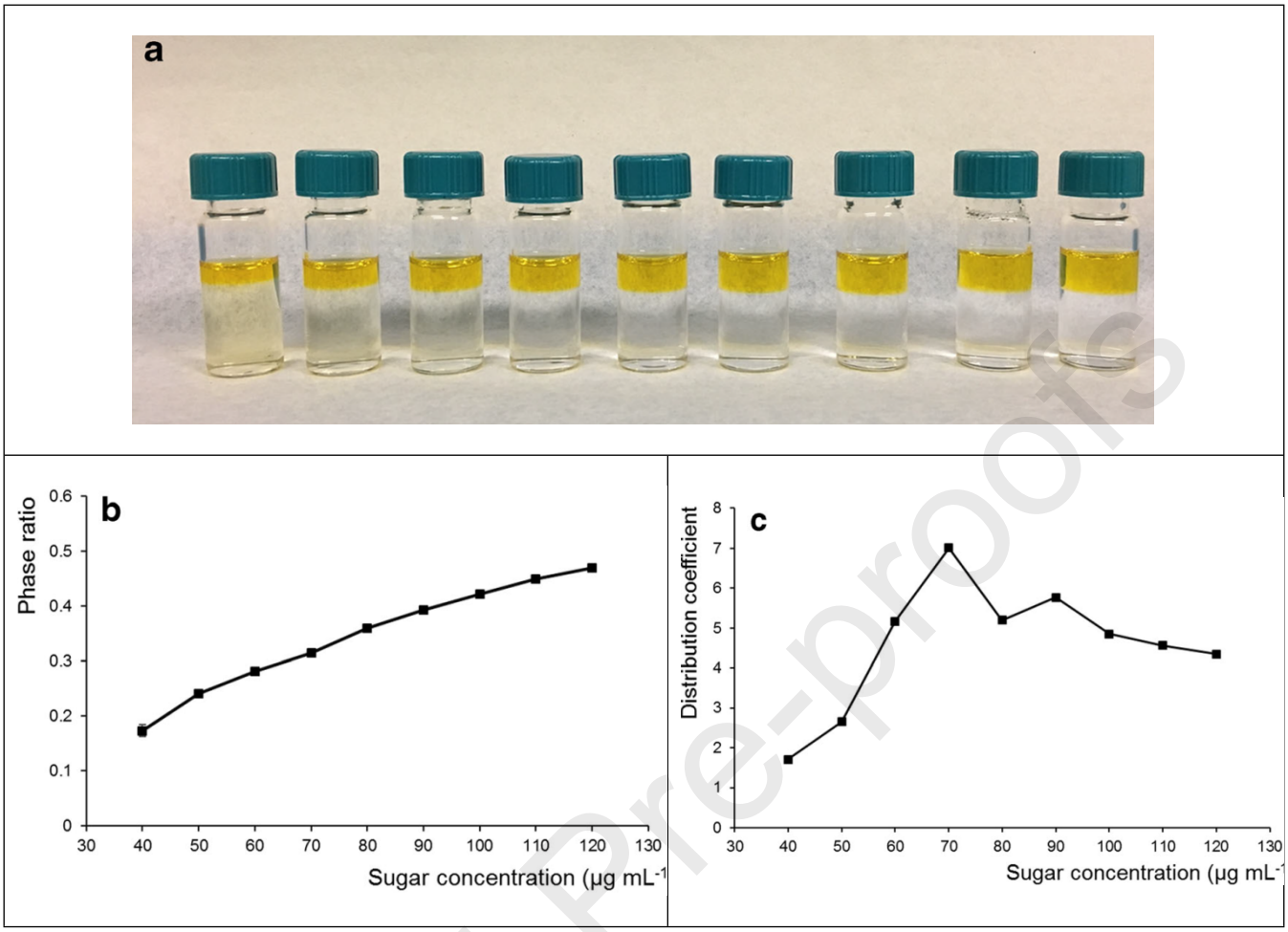

Figure 9. Characterization of sugaring-out-induced acetonitrile-water phase separation at different glucose concentrations. (**a**) Fucoxanthin (seen as a yellow-colored layer) distribution in the two-phase system. Tubes varied in glucose concentration of sugar solution 40, 50, 60, 70, 80, 90, 100, 110, and 120 mg·mL^{-1} (left to right). (**b**) Phase ratio in the two-phase system. (**c**) Distribution coefficient in the two-phase system [79] .

Tu et al. [72] applied the SULLE with glucose-acetonitrile ABS, for the partition of 10-hydroxy-2-decenoic acid (10-HDA) from royal jelly, and for the co-extraction of proteins and it was found that, phase ratio and subsequently the partition efficiency increase by increasing the initial concentration of acetonitrile in acetonitrile-water mixture. They also found that, the phase ratio increases with glucose amount, and reached a plateau over 0.4 g. Similarly, the extraction yields of 10-HDA linearly increased from 32.6% to 59.3% as the amount of glucose increased from 0.2 g to 0.4 g, and reached the plateau over 0.4 g. It appears that high phase ratio leads to the high extraction yields of 10-HDA from royal jelly. A similar behavior was also obtained when SULLE method combined with HPLC-fluorescence detection was used to determine bisphenol A (BPA) and bisphenol B (BPB) in royal jelly in which SULLE method (with acetonitrile as organic solvent and glucose as phase separating agent) was employed for the sample preparation of BPA and BPB from royal jelly sample [93]. Dhamole et al. [70] investigated the extraction of para-coumaric acid (pCA) from different agriculture residues and separation of pCA using sugaring-out by glucose + acetonitrile ABS. It was found that the separation efficiency of pCA increased with the increase in both sugar concentration and acetonitrile volume. By adding sugar to acetonitrile aqueous solutions, the acetonitrile-water hydrogen bonds are replaced by

sugar (the phase forming agent), which leads to remove water molecules from acetonitrile allowing the self-association of acetonitrile molecules and the formation of a separate phase. Therefore, the higher the concentration of sugar, the greater the disruption of acetonitrile-water hydrogen bonds and therefore more acetonitrile molecules go to the upper phase, which increases the volume of the upper phase and its extraction ability. On the other hand, increasing the amount of acetonitrile leads to an increase in the volume of the upper and hence it is expected to increase the separation efficiency. Similar behaviors were observed by Moharkar and Dhamole [80] during the sugaring-out extraction of erythromycin from fermentation broth using acetonitrile as solvent and glucose as a phase forming agent. Similarly, Wang et al. [115] found that for acetonitrile + Glucose/Xylose ABSs, with increasing sugar concentration, the volume of the upper phase (as well as the phase ratio) and thus the distribution coefficients of the organic compounds syringic acid, furfural, para-coumaric acid, ferulic acid and 5-hydroxymethyl furfural increase. Zhang et al. [66] proposed a novel sugaring-out assisted aqueous three liquid phase system composed of diisopentyl sulfide + acetonitrile + glucose to extract and separate Pt(IV), Pd(II) and Rh(III) from their hydrochloric acid solution. They found that [66], Pd, Pt and Rh tend to concentrate into the diisopentyl sulfide-rich top phase, acetonitrile-rich middle phase and sugar-rich bottom phase, respectively and therefore, this three-phase extraction system leads to the selective separation of Pt, Pd and Rh in three different liquid phases.

2.6. Challenges and future scope

2.6.1. Difficulties in predicting the partitioning behavior

The mechanism of analyte distribution in these systems is still unknown and few studies have been conducted in this field, therefore, predicting the distribution of a specific analyte in an aqueous two-phase system and the role of various factors in order to design optimal conditions is one of the challenges of these systems. On the other hand, due to the complexity of these systems, theoretical studies, especially molecular dynamic simulation, have not been used for this purpose. Therefore, studying the mechanism of analyte distribution and using computational methods to predict analyte distribution is one of the open topics in this field. For example, COSMO-RS [136], which is a quantum chemical calculation tool that only requires the chemical structure of compounds for calculations, will be a useful tool for predicting partition coefficients in these systems. Therefore, investigating the predictability of this technique for the aqueous two-phase systems discussed in this work is a suggested topic for future research, which, if confirmed, can be used to screen and select the most suitable system for the separation process.

2.6.2. Lack of a logical relationship between partition coefficient and factors affecting it

Due to the limited knowledge base compared to other established methods and the lack of basic understanding of the partition behavior on this type of ABS, there is a fundamental limitation in predicting the behavior of these systems that usually requires time-consuming and expensive experimental studies. In general, although specific interactions between the target substance and one of the phase components can play an effective role in the distribution mode, in most of the cases studied so far, the degree of hydrophobicity of the analyte is decisive. Considering that the difference in the degree of hydrophobicity of sugar and organic solvent plays a decisive role in phase separation, therefore, the separation efficiency of ABSs is also similar to their phase forming ability, so that whenever a parameter

increases the two-phase area, the distribution coefficient of the analyte will also increase. However, in the case of organic solvent-sugar ABSs, the various factors affecting the extraction performance have not been systematically investigated. More effort in this field is necessary to establish a logical relationship between the partition coefficient and these factors, and based on this, it can be used to design a special system for the extraction and separation of a specific target substance.

2.6.3. Selectivity

Selectivity is another challenge of these systems. As mentioned, in most cases, the degree of hydrophobicity of the analyte determines its distribution. Therefore, although this method has a high selectivity for analytes whose hydrophobicity difference is high, it is not selective for the target substances whose hydrophobicity difference is low and they are all concentrated in one phase. However, as can be seen from Table 3, most studies have been conducted on the ability of these systems to extract a single target compound, and studies on extraction from multicomponent mixtures to evaluate their selectivity are rare. Therefore, the experimental and theoretical study of the selectivity of these systems is another field of future research. In other words, the lack of information about the selectivity of ABS is an area of opportunity in the study of ABS in terms of selectivity of the target product from a mixture. Furthermore, in most cases, the extraction of hydrophobic analytes from the sample to the organic solvent-rich phase has been investigated, and the extraction of hydrophilic analytes into the aqueous phase has been poorly investigated. Therefore, the use of these systems to extract target materials from the hydrophobic substrates should be object of future research. Also, chirality is one of the advantages of sugar-based ABSs compared to other types of aqueous two-phase systems, which has not yet been exploited for the organic solvent-sugar ABSs, and more research is needed in this field. In fact, the issue of chiral selectivity can be one of the unique capabilities of sugar-based aqueous two phase systems, and success in this field requires more extensive theoretical and experimental research.

2.6.4. Phase-recycling processes

The lack of effective phase-recycling processes, and also, the need for additional steps, possibly time-consuming, costly and reducing efficiency, to recover the target molecule from ABS phases (which has made this technique to be considered as the primary recovery step (downstream processing)) are the limitations and weaknesses that have hindered the widespread industrial application of ABSs. Although compared to other types of aqueous two-phase systems, effective measures have been taken on sugar-organic solvent systems, and it is easier to recover organic solvent and sugar than materials such as salt, polymer, surfactant, and ionic liquids, recovery of sugar and organic solvent from the upper and lower phases after the extraction operation is still another challenge of these systems. A possible method to recover sugars from the lower phase is to evaporate the organic solvent and water from the mixture followed by crystallization of the sugar. Another method commonly used when using sugaring-out for the separation of bio-based chemicals is to reuse the sugar-rich bottom phase after removing part of the organic solvent for target bio-based chemical fermentation. In this case, sugar as a phase separating agent was considered to be fermented as carbon source for bio-based target production. Organic solvent from the top phase can be removed by vacuum distillation.

2.6.5. The negative role of organic solvents in the adoption of these ABSs for medical, pharmaceutical and food applications

Considering the biotoxicity, flammability, and volatility of organic solvents, their use may have a negative role in the acceptance of the organic solvent-sugar ABSs for medical, pharmaceutical and food applications. Due to the nascent nature of sugar-organic solvent ABSs, established techniques such as chromatography have a developed infrastructure, a broad user base, and a proven track record, which poses a significant challenge to the wider adoption of sugar-based ABS. Furthermore, stringent regulatory requirements for new purification processes, especially in sensitive industries (e.g., pharmaceuticals) and the lack of standardized protocols may delay or prevent the wider application of sugar-organic solvent ABS.

2.6.6. Others

Limited purification capacity (requiring larger volumes for processing large quantities), phase instability (changes in temperature, pH, and sample addition may affect phase separation and stability), and limited automation (currently, ABS technology is less automated compared to other established purification techniques) are the most important limitations of ABSs for the extraction purposes which may hinder their competitiveness in large-scale applications. The less tunable performance of organic solvents compared to ionic liquids and polymers is another of the limitations of organic solvent-based aqueous two-phase systems compared to other ABSs. In addition, due to its newness compared to other two-phase aqueous systems such as polymer-polymer, polymer-salt and organic solvent-salt ABSs, it has been less investigated and needs more studies. For example, in order to improve the separation efficiency, the old ABSs have been applied using various counter-current chromatography (CCC) methods to achieve multi-step separations, while these investigations have not been performed on the organic solvent-sugar ABSs and can the subject of future research in this field.

One of the important advantages of these systems is that by only adding an organic solvent (as the only chemical used) to the natural sweeteners such as honey, dates, fruit molasses, fruit jam, dried fruits, an organic solvent-sugar ABS can be created in which the matrix simultaneously acts as both a phase separation agent and the substrate containing the target sample. Considering the wide range of natural substances containing sugar and the abundance of extracts and added value substances in them, the matrix-induced sugaring-out liquid-liquid extraction method can be used in the pretreatment of many food samples as well as the extraction of various substances from them. Furthermore, the matrix-induced SULLE, if combined with other instrumental methods (spectrofluorimetry, gas-chromatography, etc), may have future applications for screening multiple contaminants in some sugar-rich foods. Moreover, its sensitivity can be improved by its combination with different preconcentration methods (for example, solid-phase extraction). The sugaring-out extraction coupling fermentation of bio-based chemicals and biofuels gives a highlighted example for separation of sugar-based chemicals from fermentation broths, which provides a novel approach to integrate sugaring-out extraction into fermentation for production of sugar-based chemicals. Research on the matrix-induced SULLE as well as sugaring-out combined with fermentation is still in early stages. Further research is needed to investigate the relationship between selective extraction, phase separation and phase structure in these systems.

Another advantage of these types of ABSs is economic efficiency. Due to the low price of organic solvents and sugar and the abundance of natural sources of sugar, the organic solvent-sugar ABSs are cheaper than other aqueous two-phase systems and their use has economic benefits.

3. Conclusions

The organic solvent-sugar ABSs as the novel sugaring-out assisted aqueous two-phase systems show enormous potential for a wide range of applications, and the additional advantages shared by carbohydrates and ABS (e.g. high water contents aqueous media, biocompatibility, biodegradability, low viscosity, low cost, fast and easy phase separation) contribute to their great potential as novel approaches in extraction and separation processes.

Understanding the thermodynamics of this emerging type of ABS gives them great potential, and therefore, in this review article, the organic solvent-sugar ABS were considered and their phase formation mechanism, the effect of various factors on the phase diagram, and their applications in the extraction of different biomaterials and other added-value materials have been addressed. All articles published in the literature from 2008 to December 2023 were reviewed, and based on them, databases were presented for the following: (1) all the sugars and organic solvents which have been studied so far, (2) the methods used for determination of phase diagrams and (3) the different target materials extracted, separated, and purified by these type of ABSs, along with the composition used to form the ABS. Therefore, this review provides the possibility in cases such as (1) guidance for future research studies, (2) distinguishing high-quality data from doubtful data, (3) setting the desired phase diagram by controlling the conditions and (4) designing an ABS for extracting a specific species and a specific application.

The results gathered from the literature show that this type of ABS has been used for partitioning, purification, concentration, extraction and separation of wide range of target materials including food coloring, herbal and flavoring supplements, antioxidant and extracts, herbicide, proteins, enzymes, alkaloids, antibiotics, metal ions, drugs, phenolic compounds, lipid acids and dyes which have been tabulated in this paper and the various factors affecting the extraction performance have also been investigated. Although considerable progress has been made in this emerging field, more work still needs to be done to overcome the current challenges and to expand sugar-based ABSs applications towards a much wider range of practical applications.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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