Phase Separation Conditions for Sugaring-Out in Acetonitrile−Water Systems

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This paper reports the phase separation conditions for "sugaring out", a new phase partition method. It was observed in our laboratory that the addition of a monomeric sugar or a disaccharide in an acetonitrile (ACN)−water mixture created two phases with the upper phase rich in acetonitrile and a lower aqueous phase. Potential applications of this method have been explored for the extraction of high value products such as biomolecules (proteins and antibiotics), hydroxymethyl furfural, furfural, syringic acid, ferulic acid, and *p*-coumaric acid from the aqueous phase. However, the detailed phase separation conditions are unknown for this method. Hence, this work was undertaken to study the phase separation conditions for sugaring-out. The effect of temperature and glucose concentration on sugaring-out was investigated. Tie line data of ACN−water−glucose system were measured and satisfactorily correlated with the Othmer−Tobias and Bancroft equations. It was observed that high glucose concentration and low temperatures favor sugaring-out. The advantages of sugaring-out make this method suitable for a large number of applications in the chemical and biotechnology industry.

Introduction

In recent years, phase separation from a homogeneous solution by creating a second immiscible phase has been investigated as an alternative to the traditional liquid−liquid extraction for the separation of chemical species and recovery of biological products.[1−7] The techniques that have been exploited include salting-out[1−4] and aqueous two-phase systems[6,7] (ATPS's), each with its advantages and shortcomings. The use of polar solvents in salting-out results in high extraction efficiencies. However, the use of a high concentration of salt for phase separation may lead to unwanted reactions.[8] Further, some salts such as K_2HPO_4 change the environment pH and thus may damage the product, if the product is sensitive to the change in pH.[2] In addition, the design of equipment is a critical problem in salting-out as salts corrode the vessels easily.[9,10] ATPS's apply a combination of polymer−polymer or a polymer and salt which creates the second immiscible phase. It has been used for separation of biological materials.[6] The limitations of ATPS's are the high cost of polymer and the difficulties in recovery of polymers.

Acetonitrile (ACN), because of its physicochemical properties, is widely used as a solvent in the extraction of high value products as well as in reverse-phase high-pressure liquid chromatography applications.[11] The recovery of ACN is essential for cost reduction. Traditional approaches used for separating ACN from the aqueous phase include cooling below subzero temperatures (253 K)[12,13] and salting-out.[1,2,14] Gu et al.[13] observed a phase separation at a subzero temperature of 256 K where the top phase was rich in ACN (16.87 mol·kg^{-1}). In another study by Pence and Gu, [15] the liquid−liquid equilibrium of the ACN−water system was studied. They obtained an ACN-rich phase containing 0.738 mass fraction of ACN at 255 K. There are few reports of ACN separation using salting-out. Le et al.[1] used different salts such as NaCl, Na_2SO_4, or NH_4Cl to separate ACN and extract erythromycin from an aqueous phase at room temperature. The maximum ACN concentration of 17.25 mol·kg^{-1} was observed in the upper phase at 100 g·L^{-1} NaCl. Gu and Shih[2] tested different salts (K_2HPO_4, KH_2PO_4, NaCl, NH_4HCO_3, and KCl) for salting-out and for the separation of proteins at lower temperatures (277 K) and obtained the best separation with K_2HPO_4.

Sugaring-out is a new phase partition method in which a monomeric carbohydrate or a disaccharide is used to trigger phase separation in an ACN−water mixture.[16,17] The addition of sugar (monomers or disaccharides) above a critical concentration in the mixture induces two-phase formation, with the upper phase rich in ACN and the lower phase rich in water. Sugaring-out may provide an entirely new platform for the extraction of organic compounds and other products from the aqueous phase. This method has been successful in the separation of ACN.[16,17]

Sugaring-out uses sugars which do not react with the components of the system. Further, it does not change the environment conditions (e.g., pH) and can occur at higher temperature (> 277 K). Thus, the application of sugar and relatively high temperature (> 277 K) for phase separation makes this method better than existing methods. However, as a new phase separation method, the conditions for sugaring-out in the form of a phase diagram have not been reported. The current work was undertaken to investigate the effect of temperature ((279 to 288) K) and sugar concentration ((105 to 180) g·L^{-1}) on two-phase formation and phase composition.

Materials and Methods

Chemicals. The high-performance liquid chromatography (HPLC) grade ACN (CH_3CN) with a purity of 0.999 (mass

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fraction) and D-glucose (0.999 mass fraction) ($C_6H_{12}O_6$, certified ACS grade) were purchased from Fisher Scientific Co. (Pittsburgh, PA). *n*-Butanol ($C_4H_{10}O$) (≥ 0.994 mass fraction) was purchased from Sigma Aldrich Chemicals (St. Louis, MO). Deionized (DI) water from an in-house facility was used.

Sugaring-Out Phase Separation. Glucose solutions of different concentrations ((105 to 180) $g \cdot L^{-1}$ with 15 $g \cdot L^{-1}$ increments) were prepared in DI water. Five milliliters of the glucose solution were mixed with 5 mL of ACN in capped 15 mL scale test tubes. The sugar concentration is given in the aqueous phase. The mixture was then shaken thoroughly at room temperature using a vortex (Fisher Vortex Genie, Pittsburgh, PA) and placed in a refrigerator circulator bath (± 0.1 K) (Poly Science digital temperature controller, Niles, IL) filled with DI water. The temperature was measured with an Ertco (272 to 324) K (0.1 K subdivisions) thermometer from Fisher Scientific Co. (Pittsburgh, PA). All of the tubes were equilibrated overnight at each temperature ranging from (279 to 288) K (± 0.2) with 3 ± 0.2 K increments to form a two-phase system. After phase separation, the volumes (accuracy 0.1 mL) of the upper phase (ACN-rich) and the lower phase (water-rich) were recorded. The density of each phase was measured using a weighing balance (OHAUS analytical plus accuracy −0.1 mg). Samples were collected by disposable syringe for the estimation of glucose and ACN.

Analytical Methods. The concentration of glucose in the samples was estimated by a HPLC system (± 0.04 $g \cdot L^{-1}$). The HPLC system consist of a Waters (Milford, MA) e2695 separation module and a Waters 2414 refractive index detector monitored by an Empower pro software version 6.2 (Waters Corporation Milford, MA). The column used was Aminex HPX-87P (300 mm × 7.8 mm) equipped with Microguard Carbo-P cartridge (30 mm × 4.6 mm) from Biorad (Hercules, CA). The column temperature and the temperature of the refractive index detector were kept at 358 K and 323 K, respectively. The mobile phase was ultra pure grade water (0.45 μm filtered and 18.2 $\Omega \cdot$cm conductivity, 0.6 $mL \cdot min^{-1}$). All samples were filtered through 0.22 μm filters into auto sampler vials. The coefficient of determination, R^2, of all standard curves was greater than 0.9998. Whenever it was required, samples were diluted to fit in the concentration ranges of the standard curves by DI water before analysis.

The concentration of ACN was determined by gas chromatograph (± 0.03 $g \cdot L^{-1}$) (GC Hewlett Packard 5890 Series II, Avondale, PA) equipped with an auto sample injector (Hewlett Packard 7673A automatic injector) and a flame ionization detector (FID). A DB-WAX 30 m × 0.250 mm × 0.25 μm fused silica capillary column (J & W Scientific) was installed into the system. The oven temperature was programmed from (313 to 463) K at a 20 $K \cdot min^{-1}$ rate. The injector and detector temperature was set to 493 K and 523 K, respectively. The carrier gas was He at a 0.72 $mL \cdot min^{-1}$ flow rate. Butanol was used as an internal standard. Peaks, areas, and percentages were calculated using Agilent Technologies GC Chemstation software (Agilent Technologies, Germany). The amount of water in each phase was calculated by the material balance (± 0.03 $g \cdot L^{-1}$).

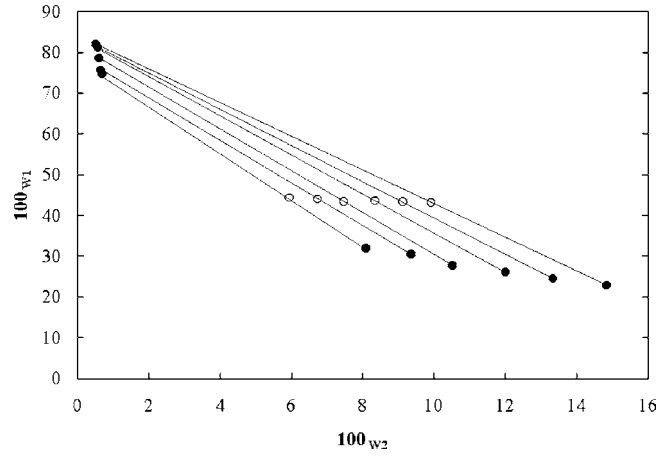

Figure 1. Phase diagram in mass fraction of ACN (1) + glucose (2) + water (3) showing the binodal curve, ●, and the overall and node compositions, ○, of several fitted tie lines at 279 K.

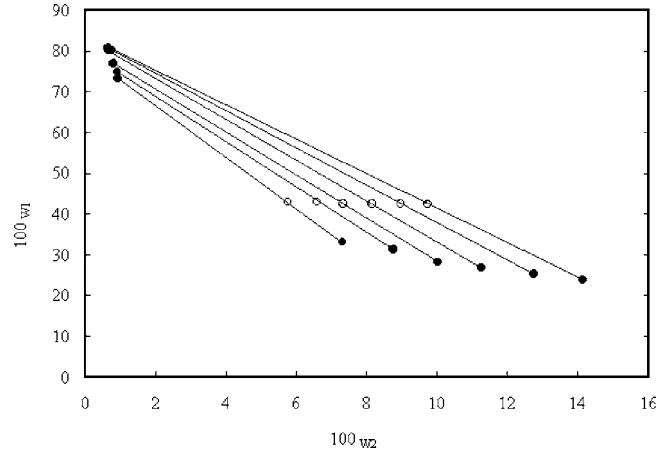

Figure 2. Phase diagram in mass fraction of ACN (1) + glucose (2) + water (3) showing the binodal curve, ●, and the overall and node compositions, ○, of several fitted tie lines at 282 K.

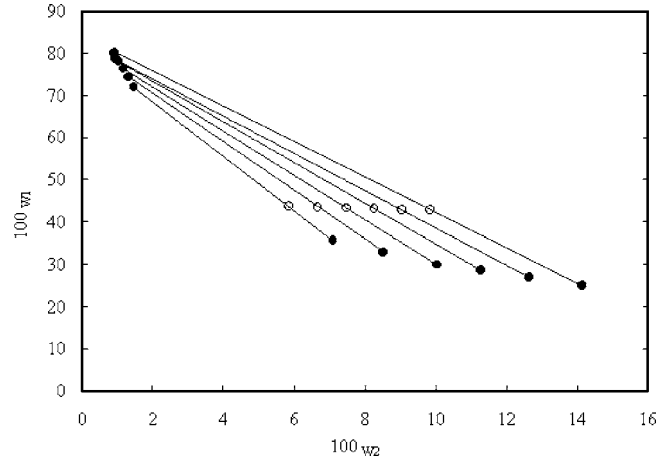

Figure 3. Phase diagram in mass fraction of ACN (1) + glucose (2) + water (3) showing the binodal curve, ●, and the overall and node compositions, ○, of several fitted tie lines at 285 K.

Results and Discussion

Phase diagrams for the ACN−glucose system examined at four temperatures are shown in Figures 1 to 4, and the tie-line composition is included in Table 1. It can be seen from Figures 1 to 4 that, at a fixed temperature, an increase in glucose concentration increases the ACN concentration in the upper phase. Further, at a fixed glucose concentration, an increase in temperature results in the poor separation of ACN (Table 1). For the present study, the highest ACN mass fraction (0.82) was achieved at a lower temperature (279 K) and high glucose concentration (glucose mass fraction in the feed = 0.0991). No phase separation was observed at 288 K for the lowest glucose mass fraction in the mixture (0.584).

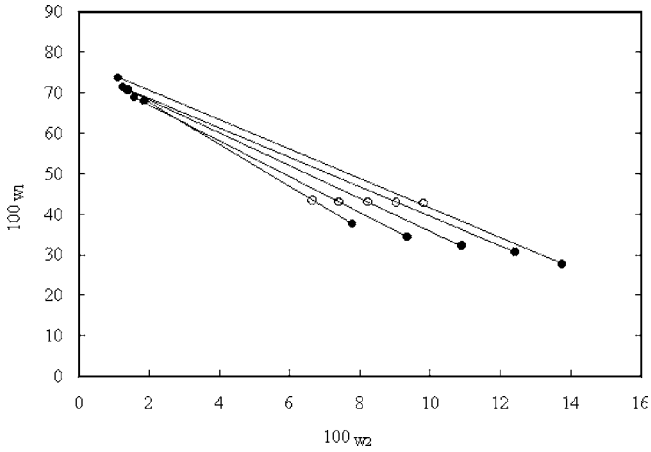

Figure 4. Phase diagram in mass fraction of ACN (1) + glucose (2) + water (3) showing the binodal curve, ●, and the overall and node compositions, ○, of several fitted tie lines at 288 K.

Table 1. Tie-Line Compositions of ACN (1) + Glucose (2) + Water (3) at Different Temperatures[a]

	tie 1	tie 2	tie 3	tie 4	tie 5	tie 6
			279 K			
w_1 (mixture)	0.4444	0.4409	0.4343	0.4373	0.4344	0.4327
w_2 (mixture)	0.0594	0.0673	0.0746	0.0834	0.0912	0.0991
w_1 (top)	0.7476	0.7567	0.7861	0.8124	0.8185	0.8202
w_1 (bottom)	0.3201	0.3051	0.2779	0.2609	0.246	0.2294
w_2 (top)	0.0068	0.0064	0.006	0.0057	0.0053	0.0051
w_2 (bottom)	0.0809	0.0935	0.1051	0.12	0.1333	0.1484
			282 K			
w_1 (mixture)	0.4301	0.4307	0.4257	0.4266	0.4265	0.4251
w_2 (mixture)	0.0575	0.0658	0.0731	0.0814	0.0895	0.0974
w_1 (top)	0.7337	0.75	0.7695	0.8028	0.8037	0.8087
w_1 (bottom)	0.3323	0.3152	0.2833	0.2692	0.2537	0.2396
w_2 (top)	0.0091	0.009	0.0079	0.0072	0.0067	0.0063
w_2 (bottom)	0.073	0.0874	0.1001	0.1125	0.1274	0.1414
			285 K			
w_1 (mixture)	0.4373	0.4357	0.4341	0.4320	0.4303	0.4294
w_2 (mixture)	0.0584	0.0665	0.0746	0.0825	0.0903	0.0983
w_1 (top)	0.7206	0.7443	0.7635	0.7814	0.7878	0.802
w_1 (bottom)	0.3572	0.3298	0.3003	0.2866	0.2713	0.2503
w_2 (top)	0.0145	0.0131	0.0115	0.0102	0.0095	0.009
w_2 (bottom)	0.0708	0.0849	0.1003	0.1125	0.1263	0.1413
			288 K			
w_1 (mixture)	0.4346	0.4307	0.4312	0.4303	0.4278	
w_2 (mixture)	0.0664	0.0740	0.0823	0.0903	0.0980	
w_1 (top)	0.6808	0.6888	0.7085	0.7153	0.7378	
w_1 (bottom)	0.3761	0.3447	0.3233	0.307	0.2764	
w_2 (top)	0.0184	0.0156	0.0138	0.0123	0.011	
w_2 (bottom)	0.0777	0.0934	0.1089	0.1241	0.1375	

[a] Data presented as mass fraction; uncertainty ± 0.0002.

The reliability of the tie-line composition was checked by the empirical correlation equations of Othmer−Tobias eq 1 and Bancroft eq 2[18−21]

$$\left(\frac{1 - w_1^{\mathrm{T}}}{w_1^{\mathrm{T}}}\right) = K\left(\frac{1 - w_1^{\mathrm{B}}}{w_1^{\mathrm{B}}}\right)^n \quad (1)$$

$$\left(\frac{w_3^{\mathrm{B}}}{w_2^{\mathrm{B}}}\right) = K_1\left(\frac{w_3^{\mathrm{T}}}{w_2^{\mathrm{T}}}\right)^r \quad (2)$$

where w_1^{T} is the mass fraction of ACN in the top phase; w_1^{B} is the mass fraction of ACN in the bottom phase; w_2^{T} is the mass fraction of sugar in the top phase; w_2^{B} is the mass fraction of sugar in the bottom phase; w_3^{T} is the mass fraction of water in the top phase; w_3^{B} is the mass fraction of water in the bottom phase, and K, n, K_1, and r are the fit parameters. Equations 1 and 2 are linearized by taking the logarithm on both sides of the equations to determine the fit parameters. The values of the above parameters (K, n, K_1, and r) are included in Table 2. It can be seen that the eqs 1 and 2 satisfactorily correlate the tie-line data of ACN−water−glucose systems. Recently, eqs 1 and 2 have been successfully used for correlating the tie-line compositions of various systems containing liquid + salt or sucrose + water.[18−21]

Table 2. Values of the Parameters of Equations 1 and 2

T/K	K	n	R^2	K_1	R	R^2
279.15	0.8146	−0.8906	0.9455	0.0572	0.8771	0.9976
282.15	0.7855	−0.9354	0.9381	0.0706	0.766	0.9971
285.15	0.6264	−1.1078	0.9911	0.085	0.6973	0.9972
288.15	0.5324	−1.5505	0.97	0.1751	0.4863	0.9983

The tie-line length was calculated using eq 3.

$$\mathrm{TLL} = \sqrt{(w_2^{\mathrm{T}} - w_2^{\mathrm{B}})^2 + (w_1^{\mathrm{T}} - w_1^{\mathrm{B}})^2} \quad (3)$$

and the slope of the tie line is defined as

$$\mathrm{slope} = \left|\frac{w_1^{\mathrm{B}} - w_1^{\mathrm{T}}}{w_2^{\mathrm{B}} - w_2^{\mathrm{T}}}\right| \quad (4)$$

The tie-line length and slope were plotted against the glucose concentration (Figures 5 and 6). It was observed that the tie-line length increases with the increase in glucose concentration at a fixed temperature (Figure 5). Further, the tie-line length does not increase significantly with an increase in glucose concentration after a critical glucose concentration. At 279 K there is a trivial increase in the tie-line length above 150 g·L^{-1}

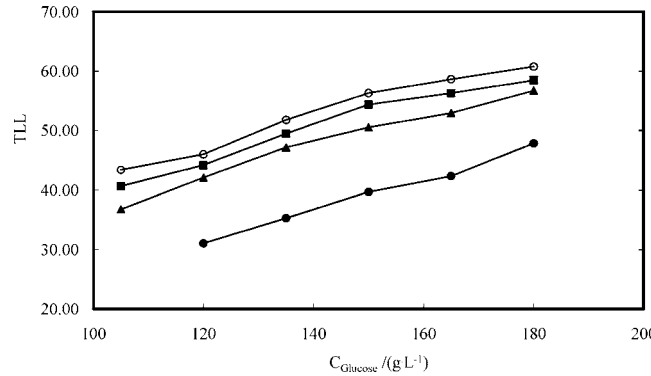

Figure 5. Effect of glucose concentration [C_{Glucose}/(g·L^{-1})] on tie-line length (TLL) at different temperatures (○, 279 K; ■, 282 K; ▲, 285 K; ●, 288 K). (TLL is defined by eq 3.)

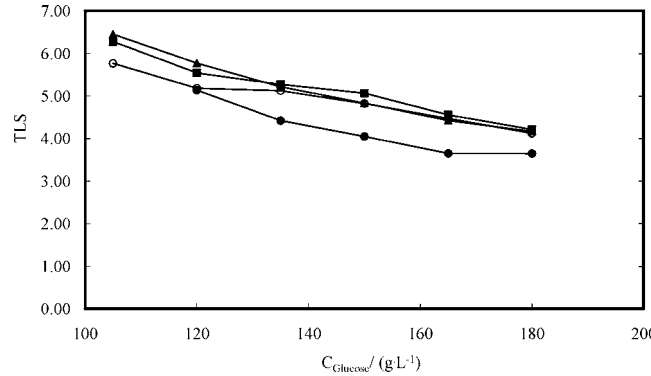

Figure 6. Effect of glucose concentration [C_{Glucose}/(g·L^{-1})] on tie-line slope (TLS) at different temperatures (○, 279 K; ■, 282 K; ▲, 285 K; ●, 288 K). (TLS is defined by eq 4.)

glucose. Similarly, at (282 and 285) K, there is not much of an increase in the tie-line length beyond a glucose concentration of 150 $g \cdot L^{-1}$. Thus, at a fixed temperature, above the critical glucose concentration, the increase in phase separation is insignificant. The effect of temperature on phase separation can be clearly seen from Figure 5. For a fixed glucose concentration, the tie-line length increases with a decrease in temperature which shows that better separation is achieved at low temperature. These observations were confirmed by the tie-line slope (Figure 6). The tie-line slope decreases with the increase in glucose concentration. Further, the change in slope was insignificant after a critical glucose concentration (165 $g \cdot L^{-1}$). Thus, the tie-line length and its slope confirm that the best phase separation conditions for sugaring-out are low temperature (279 K) and high glucose concentration (180 $g \cdot L^{-1}$). This can be explained based on the interaction among ACN, water, and glucose at the molecular level. ACN molecules have a strong dipole−dipole interaction which is expected to be stronger than the dispersion ones. In aqueous solution, ACN dimers are mostly destroyed and partially replaced by water−ACN complexes (N−H−O hydrogen bonds).[22] Takamuku et al.[23] reported that ACN molecules form three-dimensional clusters and these clusters are surrounded by water molecules through hydrogen bonding and dipole−dipole interactions. Further, the network between ACN and water molecules was enhanced when the ACN mass fraction in the feed was less than 0.6.[23] In the present study, ACN mass fraction in the mixture was always less than 0.6. Thus, it is assumed that the existing hydrogen bonds between ACN and water molecules may have been replaced by glucose molecules. Hence, a better separation was observed at high glucose concentrations. Further, the effect of temperature on phase separation may be justified by the following theory. The degree of hydrogen bonding depends on temperature. At low temperatures, the distance between nearest water molecules decreases.[24] Thus, ACN molecules will find fewer places at a lower temperature to replace water molecules. As a result, at lower temperatures, more ACN molecules are forced out, resulting in a better separation.

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