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Equilibrium Data for Poly(propylene glycol) + Sucrose + Water and Poly(propylene Glycol) + Fructose + Water Systems from (15 to 45) °C

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Liquid−liquid equilibrium data for aqueous two-phase systems formed by poly(propylene glycol) + sucrose and poly(propylene glycol) + fructose were measured from (15 to 45) °C and (15 to 25) °C, respectively. The influence of temperature on the behavior of these systems was also analyzed. A rise in temperature normally led to an increase of the slope of the tie line, decreasing the quantity of carbohydrate necessary for phase separation and increasing the biphasic area.

Introduction

In 1896, Beijerinck[1] observed that when aqueous solutions of starch and jelly (or agar−agar and jelly) were mixed a system composed of two liquid phases formed, presenting an inferior phase rich in starch (or agar−agar) and a superior phase rich in jelly. Further studies performed by Ostwald and Hertel[2] showed that various types of starch, at different concentrations, can be mixed for phase separation to occur. In 1947, Dobry and Boyer-Kawenoki[3] tested a large number of polymer pairs, observing phase splitting in most of them.

Albertsson,[4] in 1958, proposed the application of aqueous two-phase systems (ATPS) for bioseparation purposes. He used aqueous systems constituted by poly(ethylene glycol) (PEG) and dextran for separating various biocompounds.[5,6]

Ever since, many studies have been performed, expanding the use of ATPS for the partition of animal and vegetable cells, chloroplasts, enzymes, metals, mitochondrias, mushrooms, and their spores, nucleic acids, and proteins. ATPS are suitable for the separation of biomaterials, due to their low interfacial tension and high water content, which preserve the biological activity of labile constituents.[7,8]

Measurements of equilibrium data are very necessary for the development of extraction processes using ATPS. Experimental data for aqueous systems formed by PEG + salt and PEG + dextran are largely available.[9−20]

Aqueous systems containing PEG and salt are particularly useful because they present relatively low cost and low viscosity, providing an efficient phase separation.[7,8] However, dextran is a high-cost polymer, which makes difficult the scale-up of bioseparation processes that apply this constituent.[21,22]

Maltodextrin (MD) may be used as a lower-cost substitute for dextran.[22−24] Low molar mass saccharides, such as fructose, glucose, maltose, and sucrose, may also be utilized for dextran replacement, with the additional advantage that such compounds are of common occurrence in the biochemical, biological, chemical, and food industries. Monteiro Filho et al.[21] studied the liquid−liquid equilibrium of aqueous systems formed by synthetic polymers, glucose, and maltose

Various studies also analyzed the applicability of poly(propylene glycol) (PPG), a synthetic polymer structurally analogous to PEG, for the partition of biomolecules. PPGs with low molar masses present high solubility in water, whereas higher molar mass polymers are partially soluble.[21,24−26]

In view of that, this work aims to expand and facilitate the application of ATPS in bioseparation processes by measuring equilibrium data for aqueous systems composed of PPG ($M = 400\ \mathrm{g{\cdot}mol^{-1}}$) + sucrose from (15 to 45) °C and of PPG ($M = 400\ \mathrm{g{\cdot}mol^{-1}}$) + fructose from (15 to 25) °C, as well as by analyzing the effect of the temperature on the behavior of these systems.

Experimental Section

Materials. The analytical grade reagents used were PPG ($M = 400\ \mathrm{g{\cdot}mol^{-1}}$) (Sigma, USA), sucrose (Merck, USA), and fructose (VETEC, BR), with purities higher than 99 %.

Experimental Procedure. Each system was prepared in triplicate by adding appropriate quantities of water, PPG, and a stock solution of the saccharide (0.50 mass fraction) into a centrifuge tube, obtaining 10 g of mixture. The water used in making the systems was previously distilled and deionized with a Milli-Q device (MILLIPORE, USA).

Each mixture was previously stirred for about 2 min, centrifuged at 3500g for 20 min using an EPPENDORF (GmbH) centrifuge, and subsequently transferred to a thermostatic bath (TECNAL, BR) with a given uncertainty of ± 0.1 °C, where the system was allowed to settle for approximately 24 h at the operational temperature, from (15 to 45) °C, to obtain clear phase separation and to reach equilibrium. Each resulting phase was carefully collected with a syringe, leaving a 5 mm thick layer above the interface. The amount of saccharide was experimentally determined by polarimetry, utilizing a BELLINGHAM + STANLEY (U.K.) instrument. The quantity of water was measured by freeze drying, using a FREEZONE (USA) apparatus. Each sample was previously weighed and subsequently frozen for around 24 h. The amount of water was obtained from the variation of the sample weight before and

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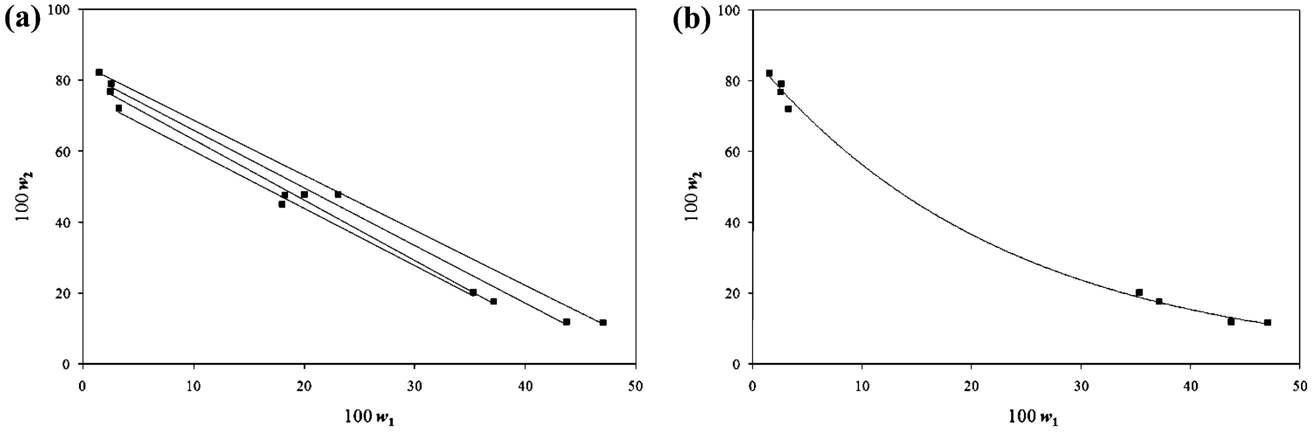

Figure 1. Equilibrium data for aqueous systems formed by ■, PPG 400 (1) + sucrose (2) at 15 °C ((a) —, tie line; (b) —, binodal curve).

Table 1. Equilibrium Data for Aqueous Systems Formed by PPG 400 (1) + Sucrose (2) from (15 to 45) °C

	overall		top phase		bottom phase	
tie line	$100 w_1$	$100 w_2$	$100 w_1$	$100 w_2$	$100 w_1$	$100 w_2$
			15 °C			
1	47.70 ± 0.20	18.26 ± 0.20	76.94 ± 0.50	2.50 ± 0.50	17.74 ± 0.22	37.12 ± 0.22
2	45.09 ± 0.01	17.99 ± 0.01	72.21 ± 0.90	3.24 ± 0.90	20.35 ± 0.75	35.32 ± 0.75
3	47.88 ± 0.01	20.02 ± 0.01	79.21 ± 0.36	2.56 ± 0.36	12.01 ± 0.44	43.72 ± 0.44
4	47.90 ± 0.77	23.10 ± 0.77	82.37 ± 0.35	1.48 ± 0.35	11.70 ± 0.92	47.02 ± 0.92
			25 °C			
1	47.99 ± 0.01	17.98 ± 0.01	72.96 ± 0.86	2.95 ± 0.86	26.62 ± 0.19	32.66 ± 0.19
2	44.94 ± 0.07	18.10 ± 0.07	73.99 ± 0.53	2.63 ± 0.53	31.16 ± 0.38	25.10 ± 0.38
3	43.00 ± 0.00	16.81 ± 0.00	70.75 ± 0.20	2.72 ± 0.20	30.88 ± 0.64	23.33 ± 0.64
4	47.68 ± 0.22	20.60 ± 0.22	79.55 ± 0.25	1.82 ± 0.25	20.16 ± 0.08	37.79 ± 0.08
			45 °C			
1	30.71 ± 0.04	15.87 ± 0.04	77.45 ± 0.00	3.41 ± 0.00	17.40 ± 0.97	20.65 ± 0.97
2	35.15 ± 0.02	19.94 ± 0.02	79.90 ± 0.00	2.36 ± 0.00	12.37 ± 0.19	31.25 ± 0.19
3	45.04 ± 0.04	17.92 ± 0.04	82.87 ± 0.08	1.68 ± 0.08	13.54 ± 0.89	37.50 ± 0.89
4	36.03 ± 0.00	21.96 ± 0.00	77.08 ± 0.05	2.05 ± 0.05	14.94 ± 0.80	34.98 ± 0.80

Table 2. Equilibrium Data for Aqueous Systems Formed by PPG 400 (1) + Fructose (2) from (15 to 25) °C

	overall		top phase		bottom phase	
tie line	$100 w_1$	$100 w_2$	$100 w_1$	$100 w_2$	$100 w_1$	$100 w_2$
			15 °C			
1	35.00 ± 0.00	21.00 ± 0.00	68.97 ± 0.74	4.44 ± 0.74	22.03 ± 0.69	29.91 ± 0.69
2	39.79 ± 0.06	26.04 ± 0.06	78.63 ± 0.71	3.08 ± 0.71	4.15 ± 0.18	50.36 ± 0.18
3	47.92 ± 0.01	18.02 ± 0.01	83.79 ± 0.18	1.93 ± 0.18	12.43 ± 0.97	40.85 ± 0.97
4	41.99 ± 0.02	21.47 ± 0.02	78.72 ± 0.74	3.35 ± 0.74	12.33 ± 0.67	39.60 ± 0.67
			25 °C			
1	34.93 ± 0.00	22.00 ± 0.00	67.14 ± 0.97	5.04 ± 0.97	18.72 ± 0.88	31.80 ± 0.88
2	39.83 ± 0.01	25.94 ± 0.01	80.04 ± 0.62	2.21 ± 0.62	1.14 ± 0.50	51.24 ± 0.50
3	48.00 ± 0.03	18.04 ± 0.03	83.02 ± 0.45	2.57 ± 0.45	3.20 ± 0.23	46.82 ± 0.23
4	42.01 ± 0.00	21.50 ± 0.00	79.41 ± 0.00	2.36 ± 0.00	3.09 ± 0.15	45.36 ± 0.15

after the drying process, corresponding to the mass of water evaporated. The quantity of PPG was determined from the difference between the mass of the dried sample and the amount of the saccharide, previously determined by polarimetry. Each measurement was made in triplicate.

Results and Discussion

Tables 1 and 2 present equilibrium data for ATPS formed by PPG 400 + sucrose, from (15 to 45) °C, and by PPG 400 + fructose, from (15 to 25) °C. Figures 1 and 2 present examples of equilibrium diagrams for ATPS containing sucrose and fructose at 15 °C. Four tie lines were achieved for each temperature studied. Tables 1 and 2 also show each measurement uncertainty, which was statistically analyzed by the estimation of the resultant standard deviation. The obtained values demonstrate the fine reproducibility of the experimental data.

The influence of the temperature on phase equilibrium was analyzed by applying the slope of the tie line (STL) concept. The STL temperature dependence reflects the kinetic and potential energy balance (ΔU/kT) existent in each pair potential formed due to the interactions between all ATPS forming components, which promote the partitioning of each of them between the phases to minimize the free energy of the systems.[27] Such a value is computed as the ratio between the variation of the polymer and saccharide concentration in each phase of the system. Tables 3 and 4 present the values of the STL found for PPG 400 + sucrose + water systems, from (15 to 45) °C, and for PPG 400 + fructose + water systems from (15 to 25) °C.

Figures 1 and 2 and Tables 3 and 4 show that the STL normally exhibited an increase with a rise of temperature for both the PPG 400 + sucrose and PPG 400 + fructose aqueous systems. A pronounced increase of the biphasic area also occurred with the rise of temperature.

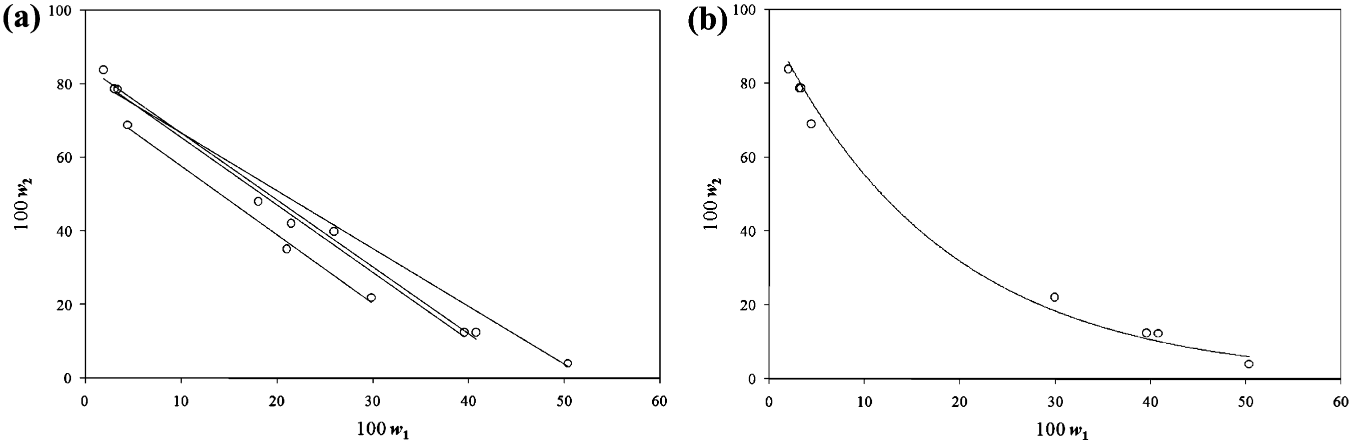

Figure 2. Equilibrium data for aqueous systems formed by ○, PPG 400 (1) + fructose (2) at 15 °C ((a) —, tie line; (b) —, binodal curve).

Table 3. STL Values for Aqueous Systems Formed by PPG 400 and Sucrose

tie line	T/°C 15	25	45
1	1.72	1.60	3.48
2	1.62	1.90	2.34
3	1.64	1.94	1.94
4	1.55	1.65	1.90

Table 4. STL Values for Aqueous Systems Formed by PPG 400 and Fructose

tie line	T/°C 15	25
1	1.84	1.92
2	1.58	1.62
3	1.82	1.80
4	1.82	1.80

It should be mentioned that the interaction between the polymer and saccharide is an endothermic event and that the rise of temperature would have to be favorable to such an interaction, increasing the constant of binding. As a result, less saccharide would be necessary for polymer chain saturation and, consequently, for phase separation.[19] However, the values of saccharide−PEO enthalpic interaction are very small leading a weak enthalpic contribution. Therefore, the entropy change appears to prevail over the enthalpy as the driving force for phase separation in such systems.

Conclusions

Equilibrium data for PPG 400 + sucrose + water systems, from (15 to 45) °C, and PPG 400 + fructose + water systems, from (15 to 25) °C, were measured. The obtained experimental data showed fine reproducibility. The influence of the temperature on the behavior of the systems was analyzed as well. The rise of temperature normally led to an increase of the STL, which decreased the amount of carbohydrate necessary for phase separation and increased the biphasic area.

Literature Cited

(1) Beijerinck, M. W. Über eine Eigentümlichkeit der löslichen Stärke. *Centralblatt Bakt.* **1896**, *2*, 697−699.
(2) Dobry, A.; Boyer-Kawenoki, F. Phase separation in polymer solution. *J. Polym. Sci.* **1947**, *02*, 90−100.
(3) Ostwald, W.; Hertel, R. H. *Kolloid Z. Z. Polym.* **1929**, *47*, 258.
(4) Albertsson, P. A. Particle fractionation in liquid two-phase systems. The composition of some phase systems and the behavior of some model particles in them. Application to the isolation of cell walls from microorganisms. *Biochem. Biophys. Acta* **1958**, *27*, 378−395.
(5) Albertsson, P. A. *Partition of Cells Particles and Macromolecules*, 3rd ed.; Wiley & Sons, Inc.: New York, 1986.
(6) Ananthapadmanabhan, K. P.; Goddard, E. D. Aqueous Biphase Formation in Poly (ethylene oxide) + Inorganic Salt Systems. *Langmuir* **1987**, *03*, 25−31.
(7) Asenjo, J. A. *Separation Processes in Biotechnology*; Marcel Dekker, Inc.: New York, 1990.
(8) Diamond, A. D.; Hsu, J. T. Aqueous Two-Phase Systems for Biomolecule Separation. *Ad. Biochem. Eng.* **1992**, *47*, 89−135.
(9) King, R. S.; Blanch, H. W.; Prausnitz, J. M. Molecular thermodynamics of aqueous two-phase systems for bioseparations. *AIChE J.* **1998**, *34*, 1585−1594.
(10) Sawant, S. B.; Joshi, J. B.; Sikdar, S. K. Partition coefficient of bovine serum albumin in two-phase aqueous systems. *J. Biotechnol. Technol.* **1988**, *02*, 41−46.
(11) Hespanhol Da Silva, M. D.; da Silva, L. H. M.; Paggioli, F. J.; Coimbra, J. S. R.; Minim, L. A. Aqueous biphasic systems: an efficient alternative for extraction of ions. *Quim. Nova* **2006**, *29*, 1332−1339.
(12) Zaslavsky, B. Y. *Aqueous Two-Phase Partitioning*: *Physical Chemistry* and *Bioanalytical Applications*; Marcel Deckker, Inc.: New York, 1995.
(13) Carvalho, C. P.; Coimbra, J. S. R; Maffia, M. C.; Costa, I. A. F.; Minim, L. A.; Silva, L. H. M. Equilibrium data for PEG 4000 + salt + water systems from (278.15 to 318.15) K. *J. Chem. Eng. Data* **2007**, *52*, 351−356.
(14) Coimbra, J. S. R.; Thoemmes, J.; Kula, M. R.; Silva, L. H. M.; Meirelles, A. J. A. Separation of β-lactoglobulin from cheese whey using an aqueous two-phase system. *Arch. Biol. Technol.* **1997**, *40*, 189−196.
(15) Hammer, S.; Pfennig, A.; Stumpf, M. Liquid−Liquid and Vapor−Liquid Equilibrium in Water + Poly (ethylene glycol) + Sodium Sulphate. *J. Chem. Eng. Data* **1994**, *39*, 409−413.
(16) Hatti-Kaul, R. Aqueous two-phase systems: a general overview. *Mol. Biotechnol.* **2001**, *19*, 269−277.
(17) Sé, R. A. G.; Aznar, M. Liquid−liquid equilibrium of the aqueous two-phase system water + PEG 4000 + potassium phosphate at four temperatures: experimental determination and thermodynamic modeling. *J. Chem. Eng. Data* **2002**, *47*, 1401−1405.
(18) da Silva, L. H. M.; Hespanhol da Silva, L. H. M.; Mesquita, A. F.; Nascimento, K. S.; Coimbra, J. S. R.; Minim, L. A. Equilibrium Phase Behavior of Triblock Copolymer + Salt + Water Two-Phase Systems at Different Temperatures and pH. *J. Chem. Eng. Data* **2005**, *50*, 1457−1461.
(19) Silva, L. H. M; Loh, W. Calorimetric investigation of the formation of aqueous two-phase systems in ternary mixtures of water, poly (ethylene) oxide and electrolytes (or dextran). *J. Phys. Chem. B* **2000**, *104*, 10069−10073.
(20) Zafarani-Moattar, M. T.; Gasemi, J. Liquid−Liquid Equilibrium of Aqueous Two-Phase Systems Containing Poly (ethylene glycol) and Ammonium Dihydrogen Phosphate or Diammonium Hydrogen Phosphate: Experiment and Correlation. *Fluid Phase Equilib.* **2002**, *198*, 281−291.
(21) Monteiro Filho, E. S.; Coimbra, J. S. R.; Minim, L. A.; Silva, L. H. M.; Meirelles, A. J. A. Liquid−liquid equilibrium for ternary systems containing a sugar + a synthetic polymer + water. *J. Chem. Eng. Data* **2002**, *47*, 1346−1350.

(22) Silva, L. H. M.; Meirelles, A. J. A. Phase equilibrium in poly (ethylene glycol) + maltodextrin aqueous two-phase systems. *Carbohydr. Polym.* **2000**, *42*, 273−278.

(23) Silva, L. H. M.; Meirelles, A. J. A. Bovine serum albumin, α-lactoalbumin and *β*-lactoglobulin partitioning in poly (ethylene) glycol + maltodextrin aqueous two-phase systems. *Carbohydr. Polym.* **2000**, *42*, 279−282.

(24) Silva, L. H. M.; Meirelles, A. J. A. Phase equilibrium and protein partitioning in aqueous mixtures of maltodextrin with poly (propylene glycol). *Carbohydr. Polym.* **2001**, *46*, 267−274.

(25) Molyneaux, P. *Water Soluble Synthetic Polymers*: *Properties and Behavior*; CRC Pr., Inc.: Boca Raton, FL, 1983.

(26) Johansson, H. O.; Karlström, C.; Tjerneld, F. Temperature induced phase partitioning of peptides in water solutions of ethylene oxide and propylene oxide random copolymers. *Biochem. Biophys. Acta* **1997**, *1335*, 315−325.

(27) da Silva, L. H. M.; da Silva, M. C. H.; de Aquino, R. A. N.; Francisco, K. R.; Cardoso, M. V. C.; Minim, L. A.; Coimbra, J. S. R. Nitroprusside−PEO enthalpic interaction as a driving force for partitioning of the [Fe(CN)(5)NO](2-) anion in aqueous two-phase systems formed by poly(ethylene oxide) and sulfate salts. *J. Phys. Chem. B* **2006**, *110*, 23540−23546.

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