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Thermophysical Properties of Polymers in Aqueous Two-Phase Systems[1]

V. Cesi,[2,3] B. Katzbauer,[2] M. Narodoslawsky,[4] and A. Moser[2]

This work concentrated on very precise density measurements of aqueous two-phase systems (ATPS). The relations between density and polymer concentration of solutions of binary systems were determined experimentally at 293.15 K at concentrations ranging from 0 to 20% (w/w) of polymers mostly used for ATPS: PEG, dextrans, and Reppal PES. The effect of temperature on density in the temperature range from 293.15 to 323.15 K was determined for binary dextran 250,000 and PEG 20,000 systems. Equations that describe the relation between density and polymer concentration in polymer–water binary systems were calculated and combined for predicting the density of mixtures of ternary systems. When these values were compared with measured densities of ternary polymer systems, no significant difference was found (less than 0.2% of dextran). A combination of density and total dry matter analysis was therefore taken as a new method for determining the composition of upper and lower phases after phase equilibria of ternary systems. Perfect agreement from tie line plotting, mass balance, and volume ratio was observed.

KEY WORDS: aqueous two-phase system; density; polymer concentration.

1. INTRODUCTION

When two water-soluble but incompatible polymers, such as dextran and polyethylene glycol (PEG), are added to water, two liquid phases may form. In the case of dextran and PEG, the upper phase is always PEG-rich, whereas the lower phase is dextran-rich [1]. As discovered by Albertsson [1], these phases offer different physical and chemical environments.

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[2] Institut für Biotechnologie, Technische Universität Graz, Petersgasse 12, A-8010 Graz, Austria.

[3] To whom correspondence should be addressed.

[4] Institut für Verfahrenstechnik, Technische Universität Graz, Inffeldgasse 25, A-8010 Graz, Austria.

Materials such as proteins, nucleic acids, viruses, and smaller molecules, such as alcohols and organic acids, may partition differently between them. ATPS are therefore of great utility for separation and purification purposes in biological processes. In recent years, great interest has been focused on the measurement, correlation and prediction of thermodynamic properties of polymers and their mixtures. The absence of consistent and reliable data has hindered developments in the latter fields [2].

2. MEASUREMENTS

2.1. Materials and Equipment

Density measurements were carried out using an Anton Paar density meter (Model DMA 48, Graz, Austria). This instrument determines the density (mass specific to volume) of liquids and gases by electronically measuring the period of oscillation of a swinging U-shaped tube containing the sample, since the natural frequency of the tube is influenced by the sample's mass. The precision of the instrument is $\pm 0.1\ \mathrm{kg \cdot m^{-3}}$ for a measurement range of 500 to 1500 $\mathrm{kg \cdot m^{-3}}$, and the uncertainty of the temperature control system is less than ± 0.05 K. The density meter was calibrated every 100 days, as recommended by the manufacturer, and controlled daily by means of doubly distilled water. An Hitachi U-3210 spectrophotometer, equipped with sample temperature control, was used to measure the absorbance of ternary system samples.

A Sartorius laboratory balance (L 420 P) was used for preparing polymer–water samples. The precision of this balance is $\pm 10^{-6}$ kg. For total dry matter (TDM) determination, a Sartorius analytic balance (A 210 P) with a precision of 10^{-7} was used.

PEG of a molecular weight of 20,000 (Art. 818897) was purchased from Merck-Schuchard (Schuchardt, 8011 Hohenbrunn bei München). All other chemicals, namely, PEG of average molecular weight 8000 (lot 73H0278), D-(+)-glucose (lot 53H063125), and dextrans of average molecular weights 9300 (lot 61H0368), 19,200 (lot 92H0688), 250,000 (lot 92H0572), 500,000 (lot 31H0298), and 200,000 (lot 63H0372) were obtained from Sigma Chemical Co. (P.O. Box 14508, St. Louis, MO 63178, USA). Reppal PES 100 and Reppal PES 200 are products from REPPE AB, S-352 50 Växjö, Sweden. Reppal PES are potato starches modified for use in aqueous two-phase systems by hydrolsis and hydroxypropylation with propylene oxide [3].

2.2. Procedures

All indicated conentrations are expressed as the weight percentage. Polymer stock solutions (10, 20, and in some cases 25%) were prepared by dissolving appropriate quantities of the solid in doubly distilled water. For dextrans and Reppal PES powders, the water content [determined by total dry matter (TDM) measurement] of the solid was taken into account. TDM was measured by drying the samples for 3 h at 378.15 K. Binary system samples and samples for the determination of binodal curves of ternary system were prepared by mixing in test tubes the appropriate quantities of PEG and dextran solutions with water. Ten grams of a system was usually prepared. For very accurate binodal line determations, a new strategy for sample preparation was substituted for the classical turbidity titration [1]. Every sample was prepared separately, and the onset of turbidity was determined by spectrometric measurement of absorption at 600 nm. Absorption values higher than 0.050 were considered turbid and as marking the beginning of phase separation. For partitioning experiments, 100-g solutions were prepared in glass beakers, magnetically stirred, and poured into 90-ml separation funnels. The phases were allowed to equilibrate for 20 h at 293.15 K. A syringe was used to harvest the top phase, while the bottom phase was collected through the funnel's valve. The composition of each phase was determined with a combination of density and dry matter measurements, which have never been reported for ATPS.

3. RESULTS

Table 1 presents the relationship between density and solution concentration for different types of polymers in binary systems. The temperature at measurement was 293.15 K and the concentrations ranged from 0 to 20%. The results show that the type of polymer, but not its molecular weight, determines the solution's density. For the two polyethylene glycols of different molecular weights, 8000 and 20,000, no difference in solution density at a given concentration is observed. The glucose monohydrate and the Reppal PES 100 and Reppal PES 200 solutions (polymer molecular weights of 100,000 and 200,000, respectively) also have the same density at any concentration within the investigated range. And for dextran solutions of fixed concentration, large increases in the polymer's molecular weight (from 9300 to 2,000,000) result in only slight increases in the solution's density. The effect of temperature on solution density in the temperature range of 293.15 to 323.15 K is reported in Table II for the dextran 250,000–water binary system and in Table III for the PEG 20,000–water system. For the dextran 250,000–water system, the relationship between

Table I. Relationship Between Density (kg · m^{-3}) and Solution Concentration for Different Types of Polymers in Binary Systems at 293.15 K[a]

Polymer	0.0%	1.0%	2.0%	5.0%	10.0%	15.0%	20.0%
PEG 8000	998.2	999.9	1001.6	1006.7	1015.2	1024.1	1033.1
PEG 20,000	998.2	999.9	1001.6	1006.7	1015.2	1024.1	1033.1
Glucose	998.2	1002.0	1005.9	1017.6	1037.6	1058.4	1080.0
R							
100,000	998.2	1002.0	1005.9	1017.5	1037.7	1058.5	1080.1
200,000	998.2	1002.0	1005.8	1017.5	1037.6	1058.3	1080.0
D							
9300	998.2	1002.1	1006.0	1008.0	1038.5	1059.8	1081.9
19,200	998.2	1002.1	1006.1	1008.3	1039.5	1061.3	1083.8
250,000	998.2	1002.2	1006.2	1018.4	1039.4	1061.4	1084.3
500,000	998.2	1002.2	1006.2	1008.5	1039.6	1061.6	1084.9
2,000,000	998.2	1002.2	1006.3	1018.9	1040.5	1063.0	1087.1

[a] R, marks for Reppal PES 100, and Reppal PES 200, respectively; D, marks for dextrans of different molecular weights.

dextran concentration and solution density can be expressed by the binomial equation

$$Y_1 = a_1 X_1^2 + b_1 X_1 + c_1 \qquad (1)$$

where Y_1 is the solution's density minus that of pure water, at the same temperature and pressure, and X_1 is the dextran concentration (%). The coefficients a_1, b_1, and c_1 are temperature dependent. For the PEG

Table II. Effect of Temperature on Solution Density in the Temperature Range 293.15 to 323.15 K for the Dextran 250,000–Water Binary System

	T (K)						
% dextran	293.15	298.15	303.15	308.15	313.15	318.15	323.15
0.0	998.2	997.0	995.7	994.1	992.3	990.3	988.0
1.0	1002.2	1001.0	999.6	998.0	996.2	994.2	992.0
2.0	1006.2	1005.0	1003.6	1001.9	1000.1	998.1	995.9
5.0	1018.4	1017.2	1015.7	1014.0	1012.2	1010.1	1007.9
10.0	1039.4	1038.1	1036.5	1034.8	1032.9	1030.8	1028.5
15.0	1061.2	1059.7	1058.1	1056.2	1054.2	1052.0	1049.8
20.0	1083.9	1082.3	1080.4	1078.5	1076.6	1074.4	1072.1
25.0	1107.6	1105.8	1103.9	1101.8	1099.6	1097.4	1094.9

Table III. Effect of Temperature on Solution Density in the Temperature Range 293.15 to 323.15 K for the PEG 20,000–Water Binary System

	T (K)						
% PEG	293.15	298.15	303.15	308.15	313.15	318.15	323.15
0.0	998.2	997.0	995.7	994.0	992.2	990.2	988.0
1.0	999.9	998.8	997.4	995.8	993.9	991.9	989.5
2.0	1001.6	1000.5	999.0	997.3	995.5	993.4	991.0
5.0	1006.7	1005.4	1003.9	1002.1	1000.1	998.0	995.6
10.0	1015.2	1013.8	1012.1	1010.2	1008.1	1005.8	1003.3
15.0	1024.1	1022.6	1020.6	1018.5	1016.2	1013.7	1011.2
20.0	1033.1	1031.6	1029.4	1027.0	1024.6	1021.9	1019.2

20,000–water system, a linear relationship between PEG concentration and solution density was found. It can be expressed with Eq. (2):

$$Y_2 = a_2 X_2 + b_2 \tag{2}$$

where Y_2 is the density of the solution minus that of pure water, X_2 is the PEG concentration (%), and coefficients a_2 and b_2 are again temperature dependent. At any given temperature, the density of water is constant, so calculations were simplified by subtracting its value from a solution's density.

Ternary systems were also studied. First, Eqs. (1) and (2) were algebraically combined to predict empirically the densities of ternary system solutions. To simplify the resulting expression, the following substitution was made: X_2, the percentage of PEG in a solution, was replaced by the term $\mathrm{TDM} - X_1$. Since total dry matter is a measurable quantity, this reduced the number of unknown variables in the equation to one, X_1. The resulting equation, Eq. (3), has the form

$$Y_3 = a_1 X_1^2 + b X_1 + c + a_2 (\mathrm{TDM}) \tag{3}$$

where $b = b_1 - a_2$, $c = c_1 + b_2$, and TDM is expressed as a percentage of the solution. Y_3 is a ternary solution's density minus that of water. The coefficients of Eq. (3) are presented in Table IV.

Three mixtures of the dextran 250,000–PEG 20,000–water system were then prepared: Mix A, 9.0% PEG + 0.46% dextran; mix B, 1.0% PEG + 14.5% dextran; and mix C, 1.25% PEG + 8.0% dextran. The densities of these ternary mixtures were measured at temperatures ranging from 293.15 to 323.15 K. The difference between two measuring temperatures was 5 K, as it had been for binary systems.

Table IV. Temperature-Dependent Coefficients of Eq. (3) for Dextran 250,000–PEG 20,000–Water Systems in the Temperature Range 293.15 to 323.15 K

Temp. (K)	Coeff. a_1	Coeff. b	Coeff. c	Coeff. a_2
293.15	0.016053	2.2583	0.013028	1.7000
298.15	0.014045	2.2970	0.156968	1.6718
303.15	0.015893	2.2855	0.011783	1.6371
308.15	0.012248	2.3526	0.013093	1.6153
313.15	0.013201	2.3536	0.154768	1.5816
318.15	0.013223	2.3652	0.052385	1.5564
323.15	0.014205	2.3729	0.000920	1.5316

Using the coefficients of Eq. (3), the densities of mixtures A, B, and C were calculated and compared with the measured values. All results are shown in Table IV. All differences between the measured and the calculated densities are, over the whole temperature range, very small. None exceed 0.4 $\text{kg} \cdot \text{m}^{-3}$, which is insignificant. This can be verified by expressing the discrepancies in terms of dextran percentage, as shown in the last column of Table V. From these data it was concluded that for binary and ternary systems, the same relationships can be used, since Eq. (3) is reduced to the corresponding binary equation [Eq. (1) or (2)] when applied to a binary system. Equation (3) can be used either to predict a solution's density from the concentrations of its components or to determine a solution's polymer content from its density and TDM. The latter approach was used for the determination of dextran and PEG contents in the upper and lower phases of ternary systems after phase equilibrium and separation. The phase diagrams and phase composition of three prepared ternary systems at 293.15 K are given below. The dextran 250,000–PEG 20,000–water system is shown in Fig. 1 (central points represent starting compositions), while Fig 2. shows the Reppal PES 100–PEG 20,000–water and Reppal PES 200–PEG 20,000–water systems (central points for starting compositions). After the compositions of both phases (upper and lower) were calculated from their densities and TDM contents, the results were plotted in Figs. 1 and 2, tie lines were drawn between them, and in every case they passed through the points that represent the starting mixtures. The fact that these three compositions (the total system, the top phase, and the bottom phase) can be represented by three points on a straight line in the phase diagram is, according to the literature [1], a confirmation of the accuracy of the calculation method. All tie lines in Figs. 1 and 2 are parallel, and polymer mass balances (based on measured phase volume ratios) also verified the results. For this work, binodal lines were determined very precisely at the onset of turbidity. The tie line ends do not lie on the binodal lines (especially in the case of the Reppal polymers). This is probably due to the polydispersity of the polymers, the difference in the size of their particles as described in the literature [1, 3, 4], if, in a system of polydisperse polymers, we construct a binodal from the onset of turbidity, or from the compositions of the phases, the two loci may not be identical. Polydispersity was not the subject of investigation of this work, but according to the data in Table I, differences in molecular weight have no significant influence on density and, accordingly, no effect on the final results of calculations such as those shown above (concentration of polymers in mixtures).

Table V. Comparison Between Calculated and Measured Density of Mixtures for Dextran 250,000–PEG 20,000–Water Systems in the Temperature Range 293.15 to 323.15 K

Mix.[a]/T (K)	Real density $(\mathrm{kg \cdot m^{-3}})$	Measured Y_3 $(\mathrm{kg \cdot m^{-3}})$	Calculated Y_3 $(\mathrm{kg \cdot m^{-3}})$	Difference $(\mathrm{kg \cdot m^{-3}})$	Difference (% dextran)
A/293.15	1015.4	17.2	17.1	0.1	0.05
B/293.15	1061.0	62.8	62.5	0.3	0.15
C/293.15	1033.4	35.2	34.8	0.4	0.20
A/298.15	1013.9	16.9	17.0	−0.1	0.05
B/298.15	1059.5	62.5	62.3	0.2	0.10
C/298.15	1032.0	35.0	34.9	0.1	0.05
A/303.15	1012.2	16.5	16.5	0.0	0.00
B/303.15	1057.8	62.1	61.9	0.2	0.10
C/303.15	1030.4	34.7	34.5	0.2	0.10
A/308.15	1010.3	16.3	16.3	0.0	0.00
B/308.15	1055.9	61.9	61.7	0.2	0.10
C/308.15	1028.7	34.7	34.6	0.1	0.05
A/313.15	1008.2	16.0	16.2	−0.2	0.10
B/313.15	1054.0	61.8	61.6	0.2	0.10
C/313.15	1026.8	34.6	34.5	0.1	0.05
A/318.15	1006.0	15.8	15.8	0.0	0.00
B/318.15	1051.8	61.6	61.3	0.3	0.15
C/318.15	1024.6	34.4	34.2	0.2	0.10
A/323.15	1003.6	15.6	15.5	0.1	0.05
B/323.15	1049.4	61.4	61.1	0.3	0.15
C/323.15	1022.3	34.3	34.1	0.2	0.10

[a] Mixture A, 9.0% PEG + 0.46% dextran; mixture B, 1.0% PEG + 14.5% dextran; mixture C, 1.25% PEG + 8.0% dextran.

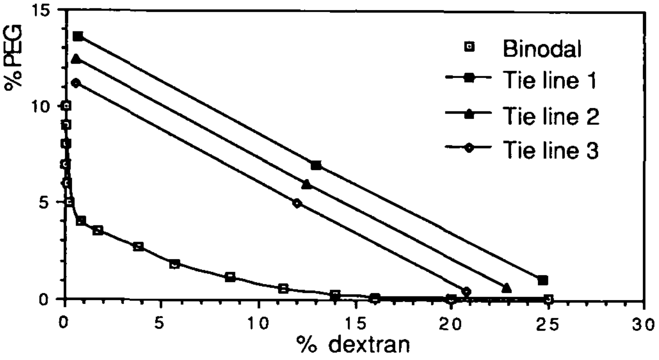

Fig. 1. Phase diagram and phase compositions of the dextran 250,000–PEG 20,000 system at 293.15 K.

The combination of density measurement and TDM assay is a new, succesful method for the determination of the composition of phases in ATPS. This method can be applied to ternary systems of two polymers in aqueous solution based on data obtained from the corresponding binary systems, as long as the polymers give solutions of different densities at equal concentrations. Examples of use of this method for other types of ATPS that are widely used in the industry (solutions of PEG-salts) will be published in the future. Since density measurements can be done rapidly (between 2 and 3 min), results are easy to verify, so the method can successfully replace the polarimetric analysis [1] classically used in ATPS for PEG–starch systems.

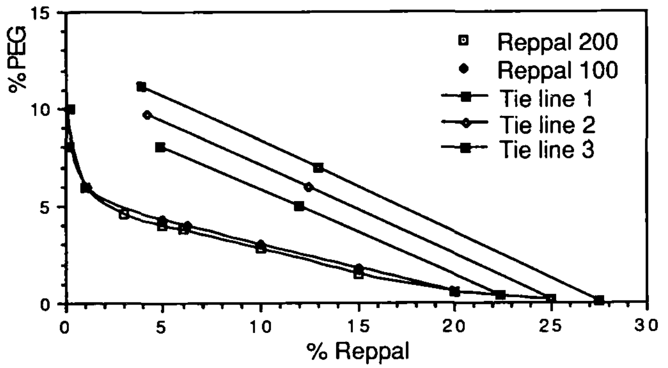

Fig. 2. Phase diagram and phase compositions of the Reppal PES 100–PEG 20,000 and Reppal PES 200–PEG 20,000 systems at 293.15 K.

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