Phase Equilibrium Compositions, Densities, and Viscosities of Aqueous Two-Phase Poly(ethylene glycol) + Poly(acrylic acid) System at Various Temperatures

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The effect of temperature on the densities and viscosities of aqueous solution of poly(acrylic acid) (PAA) of different mass fraction (from 0.05 to 0.50) and liquid−liquid equilibrium, densities, and viscosities for the aqueous two-phase PEG-6000 + PAA + water system at equilibrium have been measured. The densities and viscosities of the PAA + water system at different temperatures were correlated as a function of mass fraction. The binodal curves for the PEG + PAA + water system have been correlated using a four-parameter equation. The increase in temperature results in an increase in the two-phase region. The tie line compositions have been fitted to both the Othmer−Tobias and the Bancroft equations. A linear dependency of both the plots indicates the consistency of the data; however, the Bancroft equation gives the best fit.

Introduction

Aqueous two-phase systems (ATPS) find extensive usage in industrial biotechnology for the separation and purification of biomolecules such as proteins, enzymes, viruses, chloroplasts, and nucleic acids.[1,2] Adding either two polymers or a polymer and a salt to water can form ATPS. Only some pairs of compounds induce this phase separation and do so for compositions within a specific range.[3] These systems comprise mass fraction of water of (80 to 90) % in equilibrium phases, each phase containing mainly one of the compounds and small amounts of the other.[4]

The most common polymer−polymer-based ATPS are those formed with poly(ethylene glycol) and dextran. This pair of polymers is usually chosen as a separating medium because the systems thus formed constitute a facile environment for biomolecules, where the polymers employed do not have any denaturing properties. The industrial usage of the PEG + dextran system is limited due to higher cost of dextran.[1,3] Poly(acrylic acid) (PAA), which is hydrophilic in nature and lower in cost forms a suitable substitute for dextran in the formation of two phase system with PEG for the separation of biomolecules.

For the preparation and theoretical modeling of such a separation system, knowledge of the liquid−liquid equilibria (LLE) and physicochemical properties of aqueous two-phase systems is needed. Phase diagram data are also necessary for the development of models that predict phase partitioning.[5−8] In this present work, densities and viscosities of aqueous PAA solution with mass fraction of PAA from 0.05 to 0.50 and a comprehensive set of phase compositions, densities, and viscosities of PEG + PAA + water-based ATPS were also measured at different temperatures.

Experimental Section

***Materials*.** Poly(ethylene glycol) with a molecular weight of 6000 (Catalog No. 8.07491.1000) was purchased from Merck Chemicals, Germany. Poly(acrylic acid, sodium salt) of average molecular weight of 2100 was procured from Aldrich Chemicals Company (Milwaukee, WI). For the present work, the polymers were used without further purification. Milli-Q water was used throughout the experiments.

Apparatus and Procedure for the Preparation of ATPS. A glass jacketed vessel of 50 cm^3 volume was used for the experimental determination of LLE.[9] The glass vessel was provided with an external jacket in which water at constant temperature was circulated using a thermostat. The temperature was controlled to within ± 0.05 K. The binodal curves were determined using the turbidity method.[10] In this method, a known amount of aqueous poly(acrylic acid) was added to poly(ethylene glycol) dropwise till the appearance of turbity. The first appearance of turbidity indicates that the system is about to enter the two-phase area. For the determination of the tie lines, feed samples were prepared by mixing appropriate amounts of polymers and water by mass basis in the vessel. The thermostat was set at desired temperatures of (293.15, 303.15, and 313.15) K and the samples were stirred for 1.5 h. Then the mixture was allowed to settle for 24 h. After separation of the two phases, the densities and viscosities of each phase were determined.

Density Measurement. The density of PEG solutions has been reported by other workers,[11−17] but the density variation of PAA with different temperatures has not been reported. In this study, the densities of aqueous solutions of various mass fractions of PAA were measured in the temperature range from (293.15 to 323.15) K. The results of measurements were fitted to a third-order polynomial.

The solutions were prepared by mass, using an analytical balance with 0.0001 mg accuracy (Sartorius, model Bp 211D). The density measurements were carried out using a 10 cm^3 glass pycnometer. The volume of the pycnometer was calibrated as a function of temperature using Millipore Milli-Q water. The density of water was taken from Perry's *Chemical Engineering Handbook*.[18] The density measurements were carried out at different temperatures of (293.15 to 323.15) K. The pycnometer

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Table 1. Densities and Viscosities of the PAA (1) + Water (2) System at Different Temperatures (293.15 to 323.15) K

w_1	ρ/g·cm^{-3}	η/Pa·s	w_1	ρ/g·cm^{-3}	η/Pa·s
		T = 293.15 K			
0.00	0.9982	0.0101	0.30	1.1020	0.0436
0.05	1.0148	0.0125	0.35	1.1201	0.0582
0.10	1.0309	0.0165	0.40	1.1412	0.0773
0.15	1.0492	0.0206	0.45	1.1572	0.0857
0.20	1.0669	0.0264	0.50	1.1751	0.1090
0.25	1.0856	0.0362			
		T = 298.15 K			
0.00	0.9970	0.0089	0.30	1.0984	0.0371
0.05	1.0136	0.0109	0.35	1.1178	0.0502
0.10	1.0297	0.0140	0.40	1.1382	0.0643
0.15	1.0478	0.0176	0.45	1.1545	0.0703
0.20	1.0659	0.0215	0.50	1.1717	0.0889
0.25	1.0827	0.0296			
		T = 303.15 K			
0.00	0.9956	0.0080	0.30	1.0987	0.0338
0.05	1.0113	0.0097	0.35	1.1168	0.0412
0.10	1.0289	0.0122	0.40	1.1362	0.0482
0.15	1.0464	0.0154	0.45	1.1526	0.0580
0.20	1.0642	0.0189	0.50	1.1690	0.0755
0.25	1.0802	0.0254			
		T = 308.15 K			
0.00	0.9940	0.0072	0.30	1.0981	0.0267
0.05	1.0099	0.0086	0.35	1.1140	0.0366
0.10	1.0273	0.0110	0.40	1.1319	0.0426
0.15	1.0453	0.0138	0.45	1.1492	0.0506
0.20	1.0619	0.0166	0.50	1.1667	0.0622
0.25	1.0784	0.0217			
		T = 313.15 K			
0.00	0.9921	0.0066	0.30	1.0966	0.0262
0.05	1.0104	0.0081	0.35	1.1116	0.0337
0.10	1.0268	0.0099	0.40	1.1293	0.0372
0.15	1.0450	0.0127	0.45	1.1468	0.0443
0.20	1.0607	0.0150	0.50	1.1632	0.0537
0.25	1.0774	0.0195			
		T = 318.15 K			
0.00	0.9901	0.0060	0.30	1.0915	0.0230
0.05	1.0084	0.0073	0.35	1.1082	0.0287
0.10	1.0249	0.0089	0.40	1.1265	0.0328
0.15	1.0422	0.0115	0.45	1.1447	0.0390
0.20	1.0580	0.0140	0.50	1.1611	0.0476
0.25	1.0755	0.0166			
		T = 323.15 K			
0.00	0.9880	0.0055	0.30	1.0844	0.0221
0.05	1.0056	0.0070	0.35	1.1035	0.0293
0.10	1.0209	0.0088	0.40	1.1199	0.0307
0.15	1.0386	0.0112	0.45	1.1359	0.0366
0.20	1.0548	0.0131	0.50	1.1524	0.0442
0.25	1.0702	0.0170			

was filled with the solution and immersed in the water bath to attain thermal equilibration. A constant temperature water bath was used to control the temperature with an uncertainty of ± 0.1 K (Julabo, Type F12). After thermal equilibrium was achieved, the pycnometer was removed from the bath and then cleaned and dried. Densities were determined from the measurements of the weight of the samples and the pycnometer volume. The measurements were carried out three times, and the uncertainty of the analysis was estimated to be ± 2 %.

Viscosity Measurements. Many researchers have reported the viscosity variation of aqueous PEG solutions at different temperatures and compositions.[19−23] Viscosities of the aqueous PAA solutions and that of the top and bottom phases of the aqueous PEG + PAA + water-based two-phase system were determined using an Ostwald viscometer at (293.15 to 323.15) K.[15,23] The measurements were performed in triplicate, and the uncertainty of the analysis was estimated to be ± 2 %.

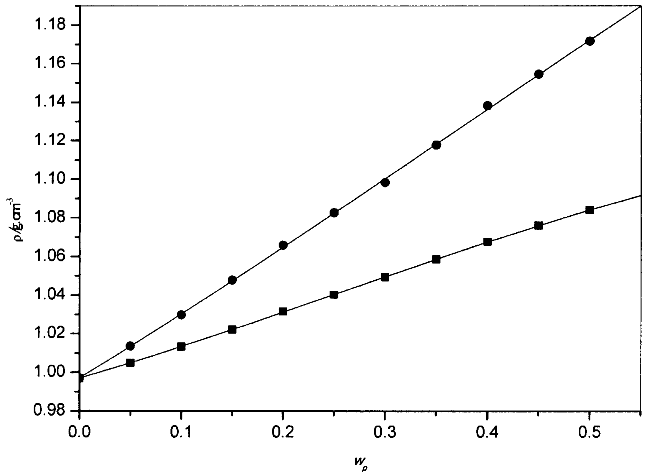

Figure 1. Densities (g·cm^{-3}) of PAA (1) + water (2) solutions: (■, ref 15, PEG-2000, 298.15 K; ●, this work, PAA, 298.15 K).

Results and Discussion

Density and viscosity values of aqueous solutions of the PAA + water system at different temperatures and mass fraction are presented in Table 1. It is observed from Table 1 that an increase in PAA composition increases the density of the polymer solution, whereas the densities of the polymer solution decrease with an increase in the temperature for the same concentration of the polymer. It is usual to express the density in terms of mass fraction according to the following equation:[12,17]

$$\rho/\mathrm{g\cdot cm^{-3}} = A + B_1 w + B_2 w^2 + B_3 w^3 \tag{1}$$

where ρ is the density of the solution at the measured temperature; A, B_1, B_2, and B_3 are coefficients of the polynomial; and w is mass fraction of PAA in the solution. Values of coefficients A, B_1, B_2, and B_3 are obtained by regression. These values along with the average percentage of relative deviation (ARD) are given in Table 2. The density variations of PAA have been compared with the densities variations of PEG[15] at similar concentration and temperature (298.15 K) of the polymer, which is shown in Figure 1. A similar trend was observed and reported by Murugesan and Perumalsamy[15] for PEG solutions.

The densities of the PEG-6000 + PAA + water systems are given in Table 3. It is observed from the Table 3 that the density of the top phase varies from (1.0797 to 1.0476) g·cm^{-3}, while the density of the bottom phase is (1.1261 to 1.1258) g·cm^{-3}. The increase in temperature from (298.15 to 313.15) K decreases the densities of both the phases at any particular concentration of PEG and PAA. There is only a marginal variation in the density of the top phase whereas significant variation is observed for the bottom phase for the system at a particular temperature. The Othmer−Tobias correlation (eq 2) and the Bancroft potential equation (eq 3) reproduce satisfactorily the equilibrium concentrations for both the phases:

$$\left(\frac{1 - w_{3t}}{w_{3t}}\right) = k\left(\frac{1 - w_{1b}}{w_{1b}}\right)^n \tag{2}$$

$$\left(\frac{w_{2b}}{w_{1b}}\right) = k_1\left(\frac{w_{2t}}{w_{3t}}\right)^r \tag{3}$$

w_{3t} is the mass fraction of PEG in the top phase; w_{1b} is the mass fraction of PAA in the bottom fraction; w_{2b} and w_{2t} are the fractions of the water mass in the bottom and top phases, respectively; and k, n, k_1, and r are the parameters to be

Table 2. Density and Viscosity Coefficients of Polynomial Equation 1 for Aqueous Solutions of PAA (1) +Water (2)

	ρ						η					
T/K	A	B_1	B_2	B_3	SD	100 × ARD	A	B_1	B_2	B_3	SD	100 × ARD
293.15	0.9982	0.3196	0.1435	−0.1493	0.0010	0.0036	0.0105	0.0214	0.2823	0.1315	0.0025	0.0032
298.15	0.9970	0.3225	0.0993	−0.0877	0.00117	0.0084	0.0096	0.0006	0.3374	−0.0485	0.0022	0.2504
303.15	0.9956	0.3173	0.1452	−0.1688	0.0008	0.0018	0.0077	0.0360	0.0827	0.2194	0.0016	0.0623
308.15	0.9937	0.3313	0.0622	−0.0667	0.0007	0.0023	0.0074	0.0151	0.1590	0.0575	0.0010	0.0989
313.15	0.9924	0.3499	−0.0353	0.0392	0.0008	0.0035	0.0069	0.0074	0.2047	−0.0693	0.0012	0.2286
318.15	0.9904	0.3525	−0.0898	0.1399	0.0007	0.0072	0.0061	0.0142	0.1277	0.0162	0.0009	0.0579
323.15	0.9882	0.3401	−0.0727	0.1012	0.0010	0.0036	0.0057	0.0124	0.1583	−0.0633	0.0013	0.0162

Table 3. Liquid−Liquid Equilibrium Composition and Physical Properties of Aqueous Two-Phase PEG6000 (3) + PAA (1) + Water (2) System at Various Temperatures

			T = 298.15 K				T = 303.15 K				T = 313.15 K			
overall composition			ρ/g·cm^{-3}		η/Pa·s		ρ/g·cm^{-3}		η/Pa·s		ρ/g·cm^{-3}		η/Pa·s	
w_3	w_1	w_2	top phase	bottom phase	top phase	bottom phase	top phase	bottom phase	top phase	bottom phase	top phase	bottom phase	top phase	bottom phase
0.2128	0.1403	0.6469	1.0797	1.1261	0.1742	0.0450	1.0640	1.0963	0.1698	0.0408	1.0629	1.0924	0.1679	0.0413
0.2125	0.1405	0.6470	1.0773	1.1295	0.2040	0.0493	1.0616	1.0994	0.1708	0.0416	1.0617	1.1043	0.1713	0.0420
0.2110	0.1417	0.6473	1.0725	1.1370	0.2062	0.0494	1.0592	1.1167	0.1767	0.0430	1.0572	1.1066	0.1740	0.0426
0.2108	0.1418	0.6474	1.0682	1.1401	0.2068	0.0504	1.0569	1.1190	0.1793	0.0433	1.0527	1.1090	0.1777	0.0430
0.2097	0.1426	0.6477	1.0646	1.1449	0.2088	0.0509	1.0521	1.1214	0.1829	0.0438	1.0526	1.1185	0.1792	0.0436
0.2080	0.1431	0.6489	1.0624	1.1498	0.2094	0.0520	1.0498	1.1262	0.1833	0.0442	1.0476	1.1258	0.1826	0.0438

Table 4. Values of the Parameters of Equations 2 and 3

k	n	SD	K_1	r	SD
1.2601	0.8332	0.0009	1.3636	1.3276	0.0014

Table 5. Binodal Data for PEG-6000 (3) + Poly(acrylic acid) (1) + Water (2) Systems at (293.15, 303.15, and 313.15) K

T = 293.15 K		T = 303.15 K		T = 313.15 K	
w_3	w_1	w_3	w_1	w_3	w_1
0.3183	0.0499	0.3162	0.0434	0.3195	0.0375
0.2820	0.0664	0.284	0.0552	0.2755	0.054
0.2644	0.0738	0.2631	0.0635	0.2453	0.0634
0.2435	0.0838	0.211	0.0885	0.2161	0.0762
0.2300	0.0891	0.197	0.0967	0.1911	0.0885
0.2193	0.0927	0.1835	0.1049	0.1651	0.0983
0.2097	0.0983	0.158	0.1215	0.1585	0.1026
0.2018	0.1035	0.1457	0.1277	0.1503	0.1077
0.1834	0.1134	0.1337	0.134	0.1324	0.1187
0.1501	0.1333	0.1246	0.1397	0.1031	0.1406
0.1296	0.1478	0.1143	0.1456	0.0939	0.1479
0.1180	0.1531	0.1009	0.1553	0.0904	0.1538
0.1052	0.1653	0.0958	0.1591	0.0833	0.1585
0.0960	0.1736	0.0921	0.1634	0.0818	0.1625
0.0843	0.1828	0.0902	0.1654	0.0739	0.1734
0.0804	0.1883	0.0866	0.1693		
0.0732	0.1932	0.0819	0.1741		
0.0677	0.1973	0.0802	0.1734		

Table 6. Binodal Curve Coefficients of Polynomial Equation 1 for Aqueous Two-Phase PEG6000 (3) + PAA (1) + Water (2) System

T/K	A	B_1	B_2	B_3	SD	100 × ARD
293.15	0.4637	−3.2203	7.1335	−4.7553	0.00187	0.0534
303.15	0.4384	−3.2069	8.3073	−9.9497	0.00226	0.0498
313.15	0.4478	−3.7205	8.7162	1.8797	0.00192	0.0389

determined. A linear dependency of the log $\{(1 - w_{3t})/w_{3t}\}$ versus log $\{(1 - w_{1b})/w_{1b}\}$ and log(w_{2b}/w_{1b}) versus log(w_{2t}/w_{3t}) plots indicate acceptable consistency of the results. Values for these parameters are given in Table 4. The best fit was obtained using the Bancroft potential equation.

The experimental data for the effect of temperature on the binodal curve are presented in Table 5, and the data are plotted in Figure 2. It is observed from the figure that with an increase in temperature, the binodal curve tends to move toward the origin. The binodal curve was fitted using polynomial eq 1.[24] Values of polynomial coefficients A, B_1, B_2, and B_3 obtained by regression are given in Table 6. The corresponding percentage average relative deviations (ARD) for different temperatures are also presented. The relative deviations for all the system is very low, indicating better agreement with the experimental data.

The viscosities and mass fraction of PAA solution at various temperatures are given in Table 1. The variation of mass fraction and viscosity of PAA with different temperatures are plotted in Figure 3. From the figure, it is observed that the viscosities increase with an increase in PAA concentration and decrease with an increase in temperature. Kirincic and Klofutar[25] have successfully used a third-order polynomial to represent their viscosity data with respect to the mass fraction of PEG-2000 and the nonelectrolyte system. Hence, in the present work a polynomial fit is used to represent the viscosity data on the PAA + water system. The values along with the average relative deviation (ARD) are given in Table 2, whereas the viscosity data of the top and bottom phases for the PEG + PAA + water system are given in Table 3. The viscosity of the top phase is more than the viscosity of the bottom phase. An increase in temperature results in a decrease in the viscosity values. The viscosity of both the phases increases with an increase in polymer concentrations for a given temperature.

The binodal curve, densities, and viscosities of aqueous PAA solution and PEG + PAA + water system have been deter-

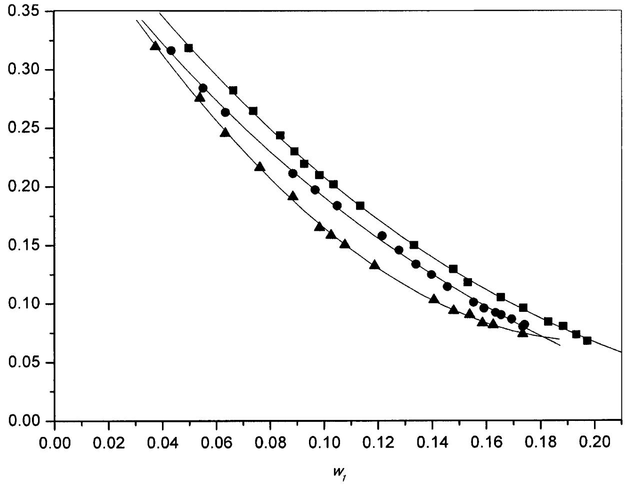

Figure 2. Binodal curves for PEG-6000 (3) + PAA (1) + water (2) systems at ■, 293.15 K; ●, 303.15 K; ▲, 313.15 K.

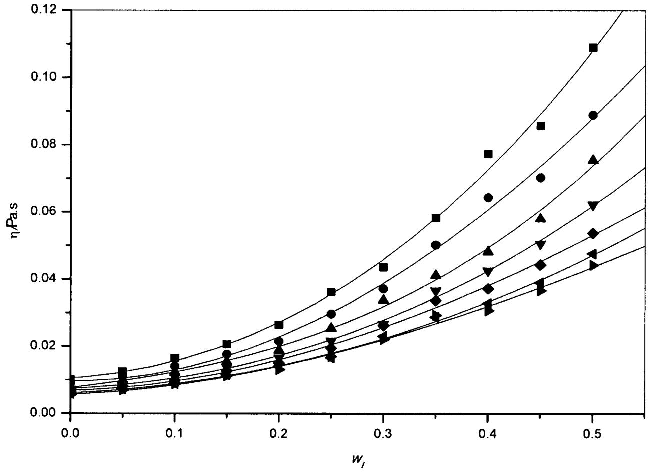

Figure 3. Relationship between the viscosities and mass fraction for PAA (1) + water (2): ■, 293.15 K; ●, 298.15 K; ▲, 303.15 K; ▼, 308.15 K; ◆, 313.15 K; left-facing solid triangle, 318.15 K; right-facing solid triangle, 323.15 K.

mined. The tie lines compositions were fitted to both the Othmer−Tobias and the Bancroft equations. A linear dependency of both the plots indicates acceptable consistency of the results. The best fit was obtained using the Bancroft potential equation. The present investigation indicates that PAA is a good alternative to dextran to be used as one of the phase-forming polymer in aqueous two-phase systems.

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