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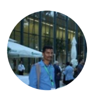
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Densities and Viscosities of Polyethylene Glycol 6000 + Triammonium Citrate + Water Systems

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The densities and viscosities of binary and ternary solutions of the aqueous two-phase systems created by the polyethylene glycol 6000 + triammonium citrate + water system were measured at different temperatures [(25, 30, 35, 40, and 45) °C] and correlated with empirical equations. The density data were compared with those available in the literature. The density data show a linear variation with the mass fraction of the polymer for all temperatures. The density and viscosity of the top and bottom phases were also measured and reported.

Introduction

Aqueous two-phase systems (ATPSs) are a relatively new and powerful separation technique, which has wide applications for the separation and purification of biomaterials, cell organs, proteins, and enzymes from their corresponding complex mixtures.[1,2] Aqueous two-phase systems are formed by soluble polymers in water by using two incompatible polymers or even an inorganic salt and a polymer. Due to its low interfacial tension, biocompatibility, and the hydrophilic nature of the polymer/salt in the system, the ATPSs provide a mild environment to preserve biological activities of labile compounds. The major advantages of ATPSs formed by polyethylene glycol (PEG) and inorganic salts are that they are nontoxic and nonflammable. The physical properties of the phase forming systems at various concentrations and temperatures are necessary for the design and scale up of such processes.[3] In the present work, an attempt is made to measure and correlate the densities and viscosities of binary (PEG 6000 + water; triammonium citrate + water) and ternary (PEG 6000 + triammonium citrate + water) systems and also the top and bottom phases of the two-phase system.

Experimental Section

Materials. Polyethylene glycol with a number average molecular mass of 6000 (cat. no. 8.07491.1000, analytical grade, Merck) and triammonium citrate ($(NH_4)_3C_6H_5O_7$) (cat. no. 21315, 'SQ' grade, Qualigens, India) were used. The polymer and salt were used without further purification. Distilled, deionized water was used for the preparation of solutions.

Apparatus and Procedures. The binary (ammonium citrate + water, PEG 6000 + water) and ternary (PEG 6000 + ammonium citrate + water) systems were prepared by adding the appropriate mass of individual solution in a 50 cm^3 centrifuge tube using an analytical balance (OHAUS-Essae-Teraoka Ltd., Japan, model AR2140) with an accuracy of 0.1 mg. The prepared solutions were brought into a Schott-Gerate CT 52 (Germany) thermostatic bath to maintain the appropriate temperature with an uncertainty of 0.05 °C. Then the densities and viscosities of the solutions were measured at different temperatures [(25, 30, 35, 40, and 45) °C]. The densities were measured by using a 5 cm^3 glass pycnometer.[4,5] The densities of pure water at different temperatures were taken from Perry's Chemical Engineers' Handbook.[6] The uncertainty of the density measurements was estimated to be ± 0.0001 $g\cdot cm^{-3}$. An Ostwald viscometer (with different capillary sizes) was used to measure the viscosities of the solutions at (25, 30, 35, 40, and 45) °C. The uncertainty of the viscosity measurements was estimated to be ± 0.002 mPa·s.

Table 1. Densities of the PEG 6000 + Water System at Various Temperatures

w_p	$\rho/g\cdot cm^{-3}$	w_p	$\rho/g\cdot cm^{-3}$
25 °C		30 °C	
0.0000	0.9970	0.0000	0.9956
0.0500	1.0051	0.0500	1.0047
0.1000	1.0137	0.1000	1.0125
0.1500	1.0217	0.1500	1.0213
0.2000	1.0311	0.2000	1.0297
0.2500	1.0397	0.2500	1.0386
0.3000	1.0487	0.3000	1.0474
0.3500	1.0584	0.3500	1.0566
0.4000	1.0679	0.4000	1.0656
0.4500	1.0766	0.4500	1.0742
0.5000	1.0856	0.5000	1.0833
35 °C		40 °C	
0.0000	0.9940	0.0000	0.9922
0.0500	1.0022	0.0500	1.0006
0.1000	1.0105	0.1000	1.0088
0.1500	1.0190	0.1500	1.0167
0.2000	1.0273	0.2000	1.0248
0.2500	1.0358	0.2500	1.0339
0.3000	1.0448	0.3000	1.0428
0.3500	1.0539	0.3500	1.0512
0.4000	1.0628	0.4000	1.0597
0.4500	1.0714	0.4500	1.0694
0.5000	1.0799	0.5000	1.0779
45 °C			
0.0000	0.9902	0.3000	1.0390
0.0500	0.9977	0.3500	1.0470
0.1000	1.0060	0.4000	1.0559
0.1500	1.0135	0.4500	1.0638
0.2000	1.0222	0.5000	1.0716
0.2500	1.0305		

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Table 2. Densities of the Aqueous Single-Phase System (PEG 6000 (p) + Triammonium Citrate (s) + Water) at Various Temperatures

		ρ /g·cm^{-3}				
w_p	w_s	T = 25 °C	T = 30 °C	T = 35 °C	T = 40 °C	T = 45 °C
0.5000	0.0200	1.0948	1.0929	1.0875	1.0835	1.0805
0.4500	0.0200	1.0862	1.0846	1.0798	1.0759	1.0718
0.4000	0.0200	1.0767	1.0750	1.0710	1.0669	1.0643
0.3500	0.0200	1.0680	1.0662	1.0624	1.0591	1.0567
0.3000	0.0200	1.0590	1.0575	1.0532	1.0497	1.0483
0.2500	0.0200	1.0498	1.0483	1.0447	1.0419	1.0398
0.2000	0.0200	1.0412	1.0386	1.0354	1.0329	1.0306
0.1500	0.0200	1.0319	1.0307	1.0278	1.0254	1.0231
0.1000	0.0200	1.0230	1.0213	1.0180	1.0165	1.0149
0.0500	0.0200	1.0148	1.0129	1.0097	1.0094	1.0064
0.4000	0.0400	1.0859	1.0838	1.0795	1.0757	1.0732
0.3500	0.0400	1.0771	1.0755	1.0710	1.0679	1.0642
0.3000	0.0400	1.0683	1.0668	1.0629	1.0585	1.0562
0.2500	0.0400	1.0588	1.0577	1.0535	1.0508	1.0472
0.2000	0.0400	1.0502	1.0478	1.0449	1.0408	1.0398
0.1500	0.0400	1.0409	1.0382	1.0351	1.0339	1.0323
0.1000	0.0400	1.0322	1.0299	1.0282	1.0252	1.0244
0.0500	0.0400	1.0238	1.0213	1.0189	1.0179	1.0160
0.2500	0.0600	1.0676	1.0666	1.0632	1.0597	1.0562
0.2000	0.0600	1.0589	1.0570	1.0540	1.0501	1.0492
0.1500	0.0600	1.0498	1.0487	1.0456	1.0432	1.0408
0.1000	0.0600	1.0412	1.0402	1.0367	1.0336	1.0326
0.0500	0.0600	1.0323	1.0320	1.0290	1.0263	1.0235
0.2000	0.0800	1.0678	1.0668	1.0632	1.0592	1.0566
0.1500	0.0800	1.0589	1.0579	1.0545	1.0515	1.0491
0.1000	0.0800	1.0502	1.0491	1.0461	1.0420	1.0409
0.0500	0.0800	1.0410	1.0402	1.0370	1.0347	1.0333
0.1000	0.1000	1.0592	1.0584	1.0550	1.0514	1.0491
0.0500	0.1000	1.0502	1.0492	1.0467	1.0436	1.0412

Table 3. Coefficients of Equation 3

temperature/°C	ρ_o	A	AARD/%[a]
25	0.9970	0.1752	0.0741
30	0.9956	0.1745	0.0367
35	0.9940	0.1710	0.0479
40	0.9922	0.1686	0.0487
45	0.9902	0.1627	0.0377

[a]
$$\mathrm{AARD} = \frac{1}{N}\sum_{i=1}^{N}\left(\left(\frac{100(\rho^{\exp} - \rho^{\mathrm{cal}})}{\rho^{\exp}}\right)^2\right)^{0.5}$$

Similarly, the solutions for the formation of aqueous two-phase systems were prepared by mass in a 50 cm^3 centrifuge tube, and the appropriate temperature of the solution was maintained in the thermostatic bath with an uncertainty of 0.05 °C. At the desired temperature, the systems were stirred and well mixed and allowed to settle for 12 h to ensure the proper phase separation, which was indicated by the absence of turbidity in each phase. Samples of the top phase and bottom phase were taken using a pipet.[4] Then the densities and viscosities of both the top and bottom phases of the aqueous two-phase systems were measured at different temperature of (25, 30, 35, and 40) °C. The uncertainties of the measurement of density and viscosity were ± 0.0001 g·cm^{-3} and ± 0.002 mPa·s, respectively. All the viscosity measurements were conducted in triplicate, and the average values are reported.

The concentration of PEG 6000 was measured by the refractive index method (Abbe Refractometer (Advance Research Instruments Co., New Delhi, model R-4)), whereas the concentration of the triammonium citrate was determined by the conductivity method[7,8] (Digital Conductivity Meter, model EC-2000, Line Seiki-Japan). Both the refractive index and conductivity of the phases were measured at 30 °C through the calibration method. Initially, the calibration charts of refractive index and conductivity were prepared through the known phase composition. The relation between the refractive index, n, and the mass fraction of polymer, w_p, and that of salt, w_s, is given by[9]

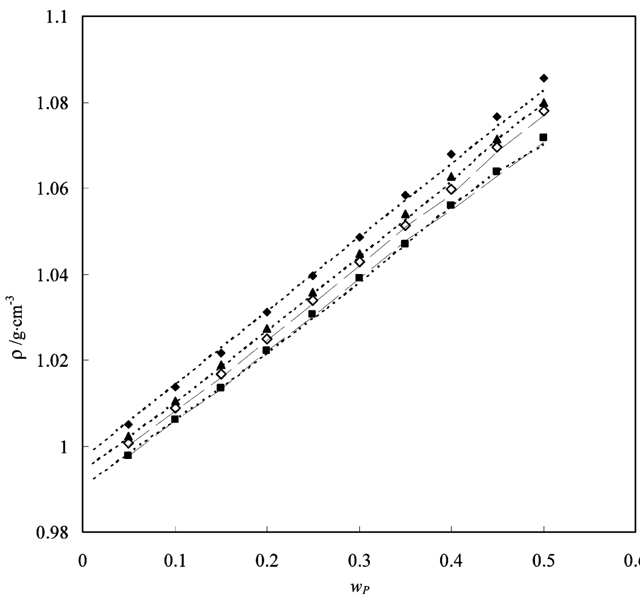

Figure 1. Comparison of present experimental data with literature data on aqueous phase polymer density. ◆, experimental at 25 °C; ▲, experimental at 35 °C; ◇, experimental at 40 °C; ■, experimental at 45 °C; - - -, Zafarani-Moattar et al.[10] data (at (25, 35, and 45) °C); — — —, Ali Eliassi et al.[11] data (at (40 and 45) °C).

$$n = a_o + a_1 w_p + a_2 w_s \tag{1}$$

The values of coefficients a_0, a_1, and a_2 for the present systems were obtained as 1.3285, 0.1522, and 0.2150, respectively. The uncertainty of the mass fraction of PEG was ± 0.0002. Similarly, the relation between the conductivity and the salt concentrations is represented by

$$\kappa = b_o + b_1 w_s \tag{2}$$

where κ is the conductivity (μS·cm^{-1}); the values of b_o and b_1 for triammonium citrate solution are 2.1786 and 799.4, respec-

Table 4. Coefficients of Equation 4

T/°C	A	B	ρ_o/g·cm^{-3}	AARD/%
25	0.1781	0.4513	0.9970	0.0626
30	0.1762	0.4479	0.9956	0.0423
35	0.1697	0.4373	0.9940	0.0519
40	0.1654	0.4282	0.9922	0.0446
45	0.1633	0.4221	0.9902	0.0468

Table 5. Viscosities of the PEG 6000 (p) + Water and Triammonium Citrate (s) + Water Systems at Various Temperatures

PEG 6000 (p) + water		triammonium citrate (s) + water	
w_p	η/mPa·s	w_s	η/mPa·s
		25 °C	
0.0000	0.894	0.0000	0.894
0.0500	1.675	0.0200	0.924
0.1000	2.545	0.0400	0.931
0.1500	4.314	0.0600	0.949
0.2000	7.520	0.0800	0.949
0.2500	13.077	0.1000	1.010
0.3000	21.262		
0.3500	33.657		
0.4000	49.978		
0.4500	71.306		
0.5000	95.933		
		30 °C	
0.0000	0.801	0.0000	0.801
0.0500	1.512	0.0200	0.827
0.1000	2.254	0.0400	0.837
0.1500	3.624	0.0600	0.875
0.2000	6.186	0.0800	0.950
0.2500	10.354	0.1000	1.161
0.3000	16.723		
0.3500	26.032		
0.4000	38.524		
0.4500	55.905		
0.5000	76.646		
		35 °C	
0.0000	0.723	0.0000	0.723
0.0500	1.238	0.0200	0.748
0.1000	1.835	0.0400	0.764
0.1500	2.976	0.0600	0.795
0.2000	5.142	0.0800	0.889
0.2500	8.694	0.1000	1.102
0.3000	14.052		
0.3500	21.966		
0.4000	32.898		
0.4500	47.144		
0.5000	65.060		
		40 °C	
0.0000	0.656	0.0000	0.656
0.0500	1.061	0.0200	0.690
0.1000	1.560	0.0400	0.695
0.1500	2.534	0.0600	0.737
0.2000	4.308	0.0800	0.831
0.2500	7.296	0.1000	1.038
0.3000	11.892		
0.3500	18.187		
0.4000	26.896		
0.4500	38.567		
0.5000	53.183		
		45 °C	
0.0000	0.599	0.0000	0.599
0.0500	0.882	0.0200	0.631
0.1000	1.270	0.0400	0.645
0.1500	2.024	0.0600	0.671
0.2000	3.447	0.0800	0.770
0.2500	5.636	0.1000	0.975
0.3000	9.200		
0.3500	13.783		
0.4000	20.235		
0.4500	28.795		
0.5000	39.201		

Table 6. Coefficients of Equations 5 and 6

T/°C	A	B	C	AARD/%
		PEG 6000 + Water (Equation 5)		
25	947.3957	−120.1252	19.2589	0.4438
30	741.2727	−102.5078	17.4922	0.4033
35	615.3113	−76.5962	12.6279	0.3633
40	484.1000	−52.9103	9.5296	0.3210
45	341.7897	−28.4292	6.1337	0.5224
		Triammonium Citrate + Water (Equation 6)		
25	976.825	−102.67	3.4689	0.3322
30	958.1699	−88.3504	2.8533	0.5380
35	919.764	−82.4125	2.8336	0.2730
40	893.238	−80.6228	2.9302	0.3076
45	879.461	−78.9878	2.8280	0.2645

tively; and the estimated errors are within ± 1.0 %. The uncertainty of the mass fraction of triammonium citrate was ± 0.0001.

Results and Discussion

The densities and viscosities of aqueous solutions of PEG 6000 and the densities of PEG 6000 + triammonium citrate + water solutions at various temperatures are given in Tables 1 and 2. The densities are found to decrease with an increase in temperature and increase with an increase in PEG 6000 concentration. The densities of aqueous PEG 6000 solutions could be correlated by using the following equation[9]

$$\rho/\mathrm{g{\cdot}cm^{-3}} = Aw_p + \rho_o/\mathrm{g{\cdot}cm^{-3}} \tag{3}$$

where ρ is the density of the solution; ρ_o is the density of pure water at the corresponding temperature; and w_p is the mass fraction of PEG 6000. The constant A and the average absolute relative deviation (AARD) were given in Table 3 for different temperatures [(25, 30, 35, 40, and 45) °C]. The present experimental data at (25, 35, and 45) °C were compared with the literature data of Zafarani-Moattar et al.,[10] and for (40 and 45) °C the experimental data were compared with the data of Ali Eliassi et al.[11] (Figure 1). Further, the experimental density data for the aqueous PEG 6000 system available in the literature at different temperature were analyzed using eq 3. The AARDs of the data with respect to their temperature are: 1.45 % for 25 °C (Zafarani-Moattar et al.[10]); 1.975 % for 35 °C (Rahbari-Sisakht et al.,[12] Zafarani-Moattar et al.[10]); 2.81 % for 40 °C (Ali Eliassi et al.[11]), and 1.98 % for 45 °C (Zafarani-Moattar et al.,[10] Rahbari-Sisakht et al.,[12] Ali Eliassi et al.[11]).

The densities of the PEG 6000 + triammonium citrate + water systems could be correlated as follows

$$\rho/\mathrm{g{\cdot}cm^{-3}} = Aw_p + Bw_s + \rho_o/\mathrm{g{\cdot}cm^{-3}} \tag{4}$$

where w_p and w_s are the mass fractions of polymer and salt, respectively. Constants A and B and the pure water densities at different temperatures with corresponding AARD were given in Table 4. It can be observed that the constants of the equation are dependent on the temperature.

In the present work, a polynomial of the following form[13] is used to represent the present viscosity data (Table 5) on the PEG 6000 + water and triammonium citrate + water systems at (25, 30, 35, 40, and 45) °C.

$$\eta/\mathrm{mPa{\cdot}s} = Aw_p^3 + Bw_p^2 + Cw_p + \eta_o/\mathrm{mPa{\cdot}s} \tag{5}$$

$$\eta/\mathrm{mPa{\cdot}s} = Aw_s^3 + Bw_s^2 + Cw_s + \eta_o/\mathrm{mPa{\cdot}s} \tag{6}$$

where η is the absolute viscosity of the solution and η_o is the viscosity of water at the corresponding temperature. The

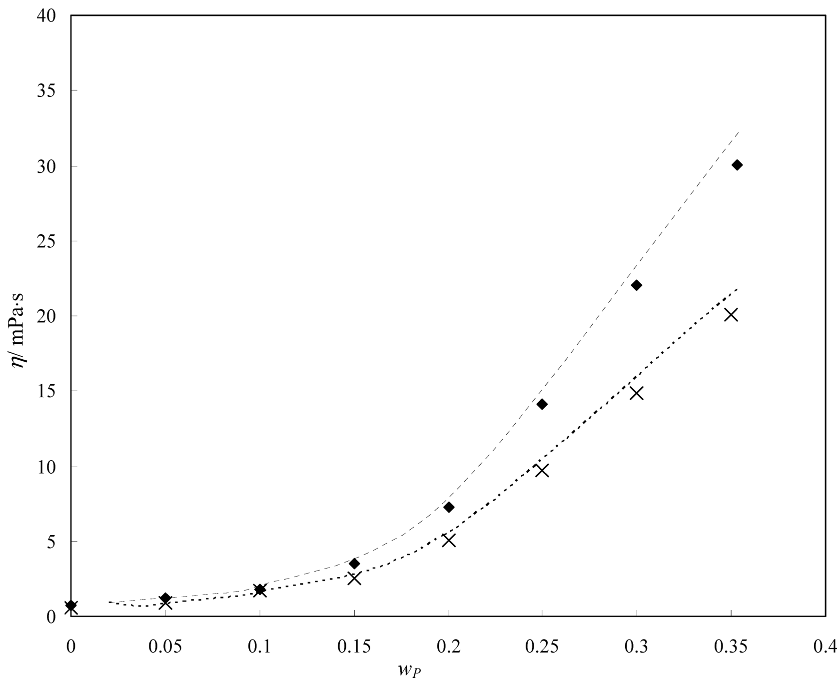

Figure 2. Comparison of present experimental data with literature data on aqueous phase polymer viscosity. ◆, experimental at 35 °C; ×, experimental at 45 °C; - - -, Rahbari-Sisakht et al.[12] data (at (35 and 45) °C).

Table 7. Viscosities of the Aqueous Single-Phase System (PEG 6000 (p) + Triammonium Citrate (s) + Water) at Various Temperatures

		η/mPa·s				
w_p	w_s	T = 25 °C	T = 30 °C	T = 35 °C	T = 40 °C	T = 45 °C
0.5000	0.0200	85.659	68.551	57.741	46.719	35.037
0.4500	0.0200	64.527	50.126	41.483	34.020	25.508
0.4000	0.0200	45.231	34.740	29.356	23.440	18.064
0.3500	0.0200	30.883	23.565	19.634	16.140	12.384
0.3000	0.0200	19.557	15.378	12.730	10.612	8.290
0.2500	0.0200	12.260	9.640	8.004	6.594	5.146
0.2000	0.0200	7.225	5.951	4.849	3.977	3.209
0.1500	0.0200	4.395	3.635	2.928	2.400	1.963
0.1000	0.0200	2.801	2.414	1.920	1.565	1.309
0.0500	0.0200	2.091	1.797	1.427	1.161	0.993
0.4000	0.0400	40.810	31.301	26.280	21.094	16.179
0.3500	0.0400	28.011	21.571	17.799	14.341	11.099
0.3000	0.0400	18.121	14.142	11.539	9.455	7.511
0.2500	0.0400	11.441	8.917	7.345	5.933	4.766
0.2000	0.0400	7.005	5.651	4.555	3.654	3.027
0.1500	0.0400	4.305	3.506	2.837	2.293	1.899
0.1000	0.0400	2.844	2.376	1.886	1.526	1.302
0.0500	0.0400	2.068	1.817	1.407	1.131	0.993
0.2500	0.0600	10.701	8.339	6.838	5.516	4.406
0.2000	0.0600	6.601	5.270	4.277	3.407	2.845
0.1500	0.0600	4.184	3.410	2.708	2.196	1.836
0.1000	0.0600	2.765	2.345	1.857	1.496	1.283
0.0500	0.0600	1.982	1.693	1.370	1.119	0.984
0.2000	0.0800	6.336	5.132	4.158	3.317	2.785
0.1500	0.0800	4.040	3.345	2.686	2.165	1.840
0.1000	0.0800	2.697	2.330	1.856	1.510	1.319
0.0500	0.0800	1.941	1.697	1.410	1.166	1.038
0.1000	0.1000	2.821	2.471	2.015	1.652	1.442
0.0500	0.1000	2.032	1.850	1.559	1.318	1.189
		a = 1.9290, AARD = 0.2693 %	a = 1.6938, AARD = 0.4056 %	a = 1.3891, AARD = 0.3196 %	a = 1.0206, AARD = 0.5336 %	a = 1.0435, AARD = 0.4093 %

coefficients of the polynomial equation A, B, and C are determined by regression analysis. These values along with the average absolute relative deviation (AARD) are given in Table 6. The viscosities increase with an increase in PEG 6000 concentration and decrease with an increase in temperature. The present experimental data at (35 and 45) °C are compared with the literature data of Rahbari-Sisakht et al.[12] (Figure 2). Further, the literature data were fitted using the present model, and the AARD values are found to be 4.2536 and 3.8738 for (35 and 45) °C, respectively.

The viscosities of the PEG 6000 + triammonium citrate + water systems are given in Table 7. The viscosities of the PEG 6000 + triammonium citrate + water systems could be correlated by using the Grunberg-like equation[14] as follows

$$\ln(\eta_m/\text{mPa·s}) = c_1 \ln(\eta_p/\text{mPa·s}) + c_2 \ln(\eta_s/\text{mPa·s}) + c_1 c_2 a \quad (7)$$

where

Table 8. Densities and Viscosities of the PEG 6000 (p) + Triammonium Citrate (s) + Water Aqueous Two-Phase System at Various Temperatures

w_p	w_s	w_p^T	w_s^T	w_p^B	w_s^B	η^T mPa·s	η^B mPa·s	ρ^T g·cm^{-3}	ρ^B g·cm^{-3}
						T = 25 °C			
0.1200	0.1400	0.2041	0.0973	0.0090	0.2049	6.276	1.735	1.0750	1.0858
0.1500	0.1400	0.2547	0.0848	0.0038	0.2236	10.358	1.895	1.0795	1.0946
0.1800	0.1400	0.3001	0.0736	0.0032	0.2421	16.021	2.154	1.0828	1.1037
0.2100	0.1400	0.3489	0.0623	0.0035	0.2566	25.321	2.401	1.0870	1.1123
0.2400	0.1400	0.3959	0.0523	0.0069	0.2768	38.013	2.827	1.0899	1.1215
						AARD = 0.885 %	AARD = 1.37 %	AARD = 0.11 %	AARD = 0.278 %
						T = 30 °C			
0.1200	0.1400	0.2299	0.0861	0.0031	0.2053	6.482	1.495	1.0744	1.0830
0.1500	0.1400	0.2804	0.0736	0.0042	0.2207	10.511	1.667	1.0778	1.0912
0.1800	0.1400	0.3239	0.0661	0.0033	0.2354	15.652	1.784	1.0810	1.0998
0.2100	0.1400	0.3632	0.0573	0.0029	0.2542	22.245	2.042	1.0847	1.1081
0.2400	0.1400	0.4042	0.0511	0.0057	0.2733	31.643	2.346	1.0889	1.1179
						AARD = 0.923 %	AARD = 0.917 %	AARD = 0.063 %	AARD = 0.25 %
						T = 35 °C			
0.1200	0.1400	0.2607	0.0736	0.0049	0.1980	7.135	1.302	1.0716	1.0794
0.1500	0.1400	0.3095	0.0623	0.0069	0.2116	12.054	1.426	1.0751	1.0883
0.1800	0.1400	0.3494	0.0573	0.0058	0.2299	16.352	1.589	1.0790	1.0966
0.2100	0.1400	0.3913	0.0486	0.0055	0.2463	24.123	1.746	1.0819	1.1047
0.2400	0.1400	0.4315	0.0411	0.0025	0.2632	33.641	1.948	1.0855	1.1138
						AARD = 2.151 %	AARD = 0.803 %	AARD = 0.065 %	AARD = 0.179 %
						T = 40 °C			
0.1200	0.1400	0.2845	0.0661	0.0026	0.1977	7.265	1.145	1.0686	1.0734
0.1500	0.1400	0.3229	0.0598	0.0021	0.2119	10.542	1.225	1.0726	1.0825
0.1800	0.1400	0.3729	0.0523	0.0070	0.2295	16.532	1.358	1.0757	1.0915
0.2100	0.1400	0.4095	0.0473	0.0048	0.2452	22.381	1.495	1.0793	1.1003
0.2400	0.1400	0.4411	0.0436	0.0027	0.2625	28.137	1.676	1.0827	1.1089
						AARD = 1.998 %	AARD = 0.646 %	AARD = 0.091 %	AARD = 0.2 %

$$c_1 = w_p/(w_p + w_s)$$
$$c_2 = w_s/(w_p + w_s)$$

η_p and η_s are the viscosities of aqueous solutions of PEG 6000 and aqueous solutions of triammonium citrate, respectively. The values of constant a along with the AARD are given in Table 7.

The liquid−liquid equilibrium and the tie line composition of the mixtures of polyethylene glycol 6000 + triammonium citrate + water at different temperatures [(25, 30, 35, and 40) °C] were already studied and reported.[8] The densities and viscosities of the top and bottom phases of the PEG 6000 + triammonium citrate + water aqueous two-phase systems are estimated for the same mixture compositions reported for tie line estimation, at different temperatures [(25, 30, 35, and 40) °C] and are reported in Table 8. Further, the density and viscosity data for the phases were analyzed using eqs 4 and 7, respectively, and the relative deviations were also presented in Table 8.

Conclusions

In the present study, the densities, refractive index, and viscosities of the binary and ternary mixtures of PEG 6000 + triammonium citrate + water based aqueous two-phase systems were measured and correlated for five different temperatures. The density and viscosity of the top and bottom phases were measured and reported for different temperatures.

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