

Article

Density, refractive index and liquid–liquid equilibrium data of polyethylene glycol 3000 + potassium formate + water at different pH values



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ARTICLE INFO

Article history:

Received 26 February 2017

Received in revised form 21 June 2017

Accepted 3 July 2017

Available online 21 July 2017

Keywords:

Liquid–liquid equilibrium data

Density

Polyethylene glycol

Potassium formate

ABSTRACT

New liquid–liquid equilibrium data for polyethylene glycol (PEG) 3000 + CHO_2K + H_2O systems were measured at 298.15 K and pH values of 7.95, 8.40 and 9.98. It was found that an increase in pH caused the binodal curve to be displaced downward and the two-phase region to expand. Accordingly, the binodal curve was adjusted to the Pirdashti equation and the tie-line compositions were correlated using the Othmer–Tobias, Bancroft and Hand equations. The study measured the refractive index and densities of several homogeneous binary and ternary solutions. The solutions were used for calibration within a range of 0% to 30% of the mass of the PEG and potassium formate. The density and refractive index data show a linear variation with the mass fraction of the polymer and the salt. The effect of pH on the binodal, tie-line lengths (TLL) and slope of the tie-line (STL) in the systems was examined. It was found that an increase in pH increased the TLL and decreased the STL. It was observed that the density of the aqueous two-phase system was influenced by the TLL. The difference in density between phases ($\Delta\rho$) increased as the TLL and pH increased. It was found that the TLL and $\Delta\rho$ showed a linear relationship. The effective excluded volume (EEV) of the PEG was obtained and it was found that EEV also increased as the pH increased.

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1. Introduction

One method of preparing an aqueous two-phase system (ATPS) is to use an aqueous solution which is immiscible at specific concentrations of polymer–polymer, salt–salt and polymer–salt molecular compounds [1]. ATPS was first investigated by Beijerinck [2]. Albertsson later investigated other aspects of ATPS, including its use in biomolecule purification (such as proteins) and cell particles [3]. Studies have reported on the merits of employing the conventional method of ATPS. These include faster processing and shorter time [4], economical affordability and low material costs [5,6], low energy consumption, non-toxicity and being environmentally friendly [7,8].

ATPS is a more efficient, powerful and flexible method to decontaminate and isolate biomolecules than alternative methods using different compounds [9–11]. Vernau and Kula [12] found that ATPS effects the separation of biomolecules, including the mass of the molecules, physical properties of the polymer (hydrophobicity), type of salt and its selection, polymer system composition, pH level, temperature and distance from the stationary point. Polyethylene glycol (PEG) has been used in studies because it is affordable, is non-poisonous, burns quickly and is straightforward to control.

Inorganic salts can be used to create ATPS with PEG phosphates. Commonly-used ones are sulfates of sodium, potassium, and ammonium

salts [13]. The use of these salts on the large scale can cause environmental problems [14,15]. Potassium formate ($\text{K}^+ \text{CHOO}^-$) is the potassium salt of formic acid (CHOOH). It rapidly dissolves in water and is present as potassium cations (K^+) and formate anions (CHOO^-), which are very mobile in an aquatic environment. The negative effects of formate on the environment include oxygen consumption from microbial degradation, subsurface denitrification, manganese, iron and sulfate reduction and methanogenesis as well its effect on perennial plants [16]. It is widely used in the oil-drilling industry and indirect refrigeration systems as a secondary refrigerant [17], as an ice-removal agent in airport runways and road maintenance [16], as an additive to modified triethylene glycol [18], in air-cooled absorption chillers [19], as a sustainable green hydrogen source in an organic transformation [20], as a palladium catalyst [21] and as a catalyst of ruthenium encapsulated in an aluminum oxyhydroxide-support [22].

Formates are organic anions that can be used to produce an ATPS showing better performance with regard to environmental and toxicity issues. Some researchers have found formates as a different substance for creating an ATPS with PEG because they can prevent pollution and virulent disease and can be successfully used in biological wastewater treatment plants [23–26]. Little experimental data (PEG + water + formate) is available, however, and there are many research opportunities when working on this system. Lladosa *et al.* [27] reported on UCON 50-HB-5100 + potassium formate + water systems as measured at 298.15 K. Silverio *et al.* [26,14] characterized PEGs with different molecular mass (1500 and 8000) when combined with potassium phosphate.

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Our previous study [28] reported on data derived from the binodal curves and tie-lines of PEG 3000 + trisodium citrate + water systems at 298.15 K and different pH values. In the current study, phase equilibrium data for PEG 3000 + potassium formate ATPS was determined at 298.15 K and pH levels of 7.95, 8.40 and 9.98. The binodal curves and tie-line lengths (TLLs) satisfactorily correlated. The PEG and salt compositions were then analyzed using calibration curves and the effects of pH on the binodal curve and TLL and slope of the tie-line (STL) were examined. The experiment was processed at low temperatures as an important condition for protein stability.

2. Experimental

2.1. Materials

PEG with a mass average of 3000 g·mol⁻¹ and sodium hydroxide (NaOH; mass purity > 0.99%) were obtained from Merck (Germany) and used without further purification. Potassium formate (KCHO₂; purity pa > 99.0 wt%) was supplied by Sigma-Aldrich (Darmstadt, Germany). Distilled deionized water (conductivity = 17.8 μS·cm⁻¹) was used to prepare the solutions. All chemicals were dried for at least 24 h (433.15 K for salts and 313.15 K for polymers) to eliminate the adsorbed water.

2.2. Apparatus and procedure

2.2.1. Analytical method

The compositions in both phases were obtained by measuring the physical properties of refractive index (n_D) and density (ρ) of binary (PEG 3000 + water, potassium formate + water) and ternary (PEG 3000 + potassium formate + water) systems at 298.15 K using a CETI refractometer (Belgium) with an accuracy of 0.0001 n_D and using an Anton Paar oscillation U-tube densimeter (model: DMA 500, Austria) with a precision of $\pm 10^{-4}$ g·cm⁻³. The calibration equations were calculated beforehand. Duplicate data measurement was employed and the average values of the parameters were reported. This data was correlated using Eq. (1) as:

$$Z = a_0 + a_1w_p + a_2w_s + a_3w_p^2 + a_4w_s^2 + a_5w_pw_s \quad (1)$$

where Z denotes the physical properties (density or refractive indices), w_p denotes the mass fraction of the polymer, w_s denotes the mass fraction of the salt and the fitting parameters are denoted by a_0 to a_5 . The density and refractive indices of the PEG 3000 + potassium formate + water systems are shown in Table S1. A practical approach employed for determination of the phase composition of PEG–salt ATPS is shown in Fig. 1.

2.2.2. Binodal curve

The titration method was selected to ascertain the binodal curves [29]. To achieve the cloud point, titration was conducted by adding the polymer (titrant) to salt solutions of specific concentrations. A binary solution of potassium formate (50% w/w) and PEG 3000 (50% w/w) was prepared. The salt solution was prepared and titrated against the polymer. Special care was taken when adding the salt solution dropwise continuously to reach turbidity so as to determine the end point. To remove the turbidity, water was added over a period of time. The same method was used to obtain the other binodal. An appropriate ratio of potassium formate to sodium hydroxide was mixed so that the pH values of the salt solutions could be accurately adjusted using a pH meter (827 pH; Metrohm; Switzerland).

2.2.3. TLL and STL

The tie lines were obtained using the equilibrium set designed based on previously described procedures [30]. Four samples were selected at each pH. Adequate amounts of PEG, salt stock and water were mixed in 15 ml graduated cylinders at 298.15 K to prepare 10 g of feed samples using an analytical balance (A&D; model GF300; Japan) with an accuracy of $\pm 10^{-4}$ g. Appropriate ratios of potassium formate and sodium hydroxide were mixed so that the pH values of the salt stock could be accurately adjusted using a pH meter.

The samples were stirred for 10 min and settled in a thermostatic bath (Mettmert; model INE400; Germany) to keep the temperature constant at ± 1 K and allow the mixtures to settle for 24 h. The tubes were spun in a Hermle Z206A centrifuge (Germany) at 6000g for 10 min to separate the phases, which showed no turbidity or difficulty discerning the top and bottom of the samples. After the achievement of equilibrium, syringes were used to withdraw the phases. Accordingly, the top phase was sampled first, with care being taken to leave a layer of

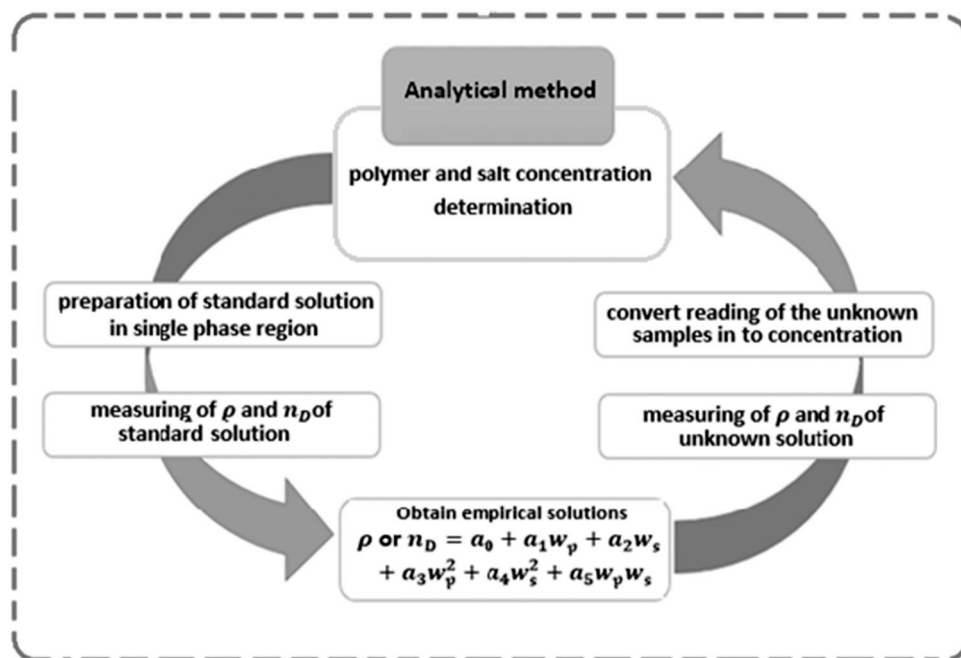


Fig. 1. Practical approach for determination of phase composition and binodal curve of PEG–salt ATPS.

material at least 0.5 mm thick above the interface. The bottom phase remained in the glass vessel and was removed with a long needle. To determine the concentration of polymer and salt, the physical properties (density and refractive index) of the top and bottom phases were measured. The TLL was obtained using Eq. (2), which presents an observational measurement of the compositions in the two phases.

$$\text{TLL} = \sqrt{(w_p^{\text{top}} - w_p^{\text{bottom}})^2 + (w_s^{\text{bottom}} - w_s^{\text{top}})^2} \quad (2)$$

The STL was obtained using Eq. (3) as the ratio of the difference in the mass fraction of polymer and salt in both the top and bottom phases. w_i is the mass fraction of the PEG and salt and top and bot denote the top and bottom phases, respectively.

$$\text{STL} = \frac{w_p^{\text{top}} - w_p^{\text{bottom}}}{w_s^{\text{top}} - w_s^{\text{bottom}}} \quad (3)$$

2.2.4. Binodal curve and TLL correlation

The Pirdashti equation [28] is appropriate for recreation of the binodal curves of the systems investigated for binodal data correlation as:

$$w_p = (a + bw_p)^{-1} \quad (4)$$

where a , b , and c denote the fitting parameters and w_p and w_s denote the mass fraction of the polymer and salt, respectively. The binodal data of this expression was correlated using least-squares regression.

The Othmer–Tobias [31] (Eq. (5)), Bancroft [32] (Eq. (6)) and Hand [33] (Eq. (7)) correlations were used to control and ensure the reliability and consistency of the data derived from the tie-line.

$$\left(\frac{1 - w_p^{\text{top}}}{w_p^{\text{top}}}\right) = k \left(\frac{1 - w_s^{\text{bottom}}}{w_s^{\text{bottom}}}\right)^n \quad (5)$$

$$\left(\frac{w_w^{\text{bottom}}}{w_s^{\text{bottom}}}\right) = k_1 \left(\frac{w_w^{\text{top}}}{w_p^{\text{top}}}\right)^r \quad (6)$$

$$\ln \frac{w_w^{\text{bottom}}}{w_s^{\text{bottom}}} = a + k_2 \ln \frac{w_w^{\text{top}}}{w_p^{\text{top}}} \quad (7)$$

Table 1
Values of coefficients obtained from Eq. (1)

	a_0	a_1	a_2	a_3	a_4	a_5	R^2
n_D	1.332353	0.091962	0.135431	0.021186	0.021476	1.000000	0.9998
$\rho/\text{g} \cdot \text{cm}^{-3}$	0.996283	0.552640	0.164441	0.178023	0.017750	1.000000	0.9996

where k , n , k_1 , and r denote the adjusted parameters. This experimental data can also be adapted to the equation provided by Guan *et al.* [34] as:

$$\ln(V^* \cdot w_p/M_p) + V^* \cdot w_s/M_s = 0 \quad (8)$$

where M denotes the representative molecular mass ($\text{g} \cdot \text{mol}^{-1}$) and V^* denotes the effective excluded volume (EEV) of the PEG [35,36].

Next, a simple two parameter equation was used to correlate the LLE data [34]:

$$\ln\left(\frac{w_s^{\text{top}}}{w_s^{\text{bot}}}\right) = b + k \cdot (w_p^{\text{bot}} - w_p^{\text{top}}) \quad (9)$$

where b and k are fitting parameters that can be regarded as effective virial (or active) coefficients and the salting-out coefficient of the salt, w_i is the mass fraction of the PEG and salt and top and bot denote the top and bottom phases, respectively.

3. Results and Discussion

3.1. Fitting parameters of calibration equation

Table 1 shows the coefficients having the nominal values of a_0, a_1, a_2, a_3, a_4 , and a_5 from Eq. (1). The refractive index and density of pure water at 298.15 K were 1.3323 and 0.9962, respectively. The average absolute relative deviation in this concentration range was 0.0058% for the refractive index and 0.03% for the density.

3.2. Binodal curve

The binodal experimental data plotted in Fig. 2 are shown in Table 2.

Fig. 2 shows the effect of pH on the binodal curve of the PEG 3000 + potassium formate ATPS at pH values of 7.95, 8.40 and 9.98 at 298.15 K. The two-phase area broadened as the pH increased and the binodal displacement descended as the moderate pH increased. Such displacement indicates the need for lower concentrations of phase polymers for ATPS formation. Previous studies have reported similar results [37–39].

The functions of pH are solute charging, charging the species ratio and binodal location [40]. Sadeghi and Jamehbozorg [41] increased the pH and observed a behavior known as “salting-out” after studies of salt in water. One outcome of this phenomenon is a decrease in the interaction hydrogen-bond. Reasons for this could be the decrease in hydration and the solubility of the polymer because of the greater attraction of salt ions to water over the polymer [42]. The polymer can be eliminated from the solution as an outcome of this process [39].

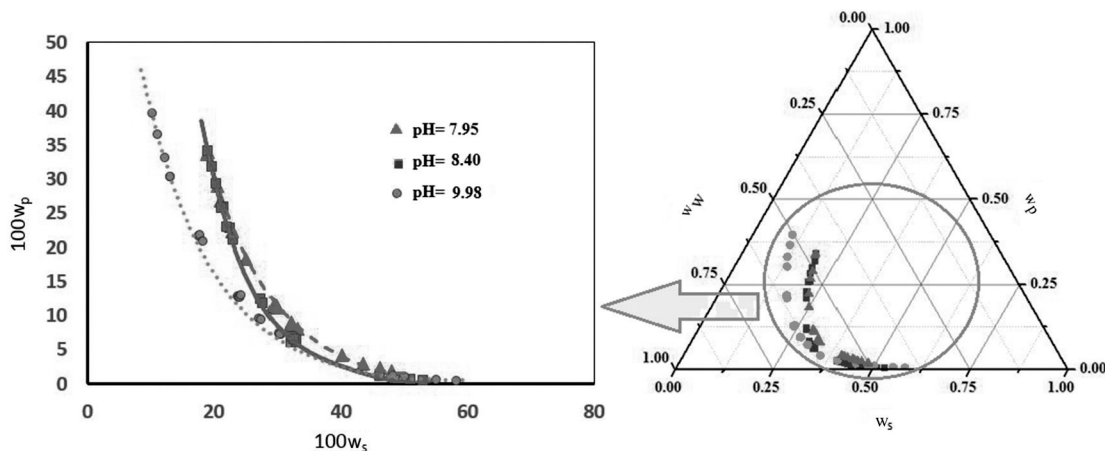


Fig. 2. Phase diagram of PEG (3000) + potassium formate (KCHO_2) + H_2O (3) two-phase system at $T = 298.15$ K and pH values of 7.95 ($\blacktriangle$), 8.40 ($\blacksquare$) and 9.98 ($\bullet$) experimental binodal. The solid curves represent the empirical model.

Table 2

Binodal curve data of PEG 3000 + potassium formate water system at 298.15 K for different pH values

pH = 7.95		pH = 8.40		pH = 9.98	
100w _p	100w _s	100w _p	100w _s	100w _p	100w _s
34.27 ± 0.02	18.89 ± 0.01	30.90 ± 0.03	19.76 ± 0.02	20.19 ± 0.03	17.54 ± 0.02
27.53 ± 0.01	21.10 ± 0.01	28.43 ± 0.01	20.42 ± 0.01	19.98 ± 0.02	17.66 ± 0.01
22.99 ± 0.01	22.91 ± 0.02	24.42 ± 0.02	21.61 ± 0.02	19.09 ± 0.02	18.16 ± 0.02
18.64 ± 0.02	25.03 ± 0.00	21.85 ± 0.02	22.48 ± 0.01	11.45 ± 0.02	23.79 ± 0.01
12.12 ± 0.03	29.37 ± 0.01	20.34 ± 0.01	23.05 ± 0.00	11.34 ± 0.01	23.89 ± 0.02
11.83 ± 0.00	29.62 ± 0.03	11.70 ± 0.02	27.39 ± 0.02	11.25 ± 0.02	23.98 ± 0.03
11.66 ± 0.01	29.76 ± 0.00	11.23 ± 0.02	27.71 ± 0.01	11.06 ± 0.04	24.16 ± 0.02
11.47 ± 0.01	29.93 ± 0.01	6.39 ± 0.01	32.15 ± 0.02	8.46 ± 0.02	27.11 ± 0.02
9.32 ± 0.02	32.03 ± 0.01	6.31 ± 0.01	32.24 ± 0.01	8.24 ± 0.00	27.40 ± 0.01
9.23 ± 0.01	32.12 ± 0.02	5.85 ± 0.02	32.83 ± 0.02	6.46 ± 0.02	30.07 ± 0.01
9.15 ± 0.02	32.21 ± 0.03	5.81 ± 0.02	32.89 ± 0.02	6.32 ± 0.03	30.33 ± 0.02

The data in Table 3 shows that the PEG concentration of the top phase increased as the salt composition increased at a constant PEG molecular mass. The ability of salt to increase the water structure can explain this. A decrease occurs in hydration and PEG solubility because of the stronger attraction of salt ions to water than PEG. Eventually hydrophilic PEG is salted separately and is removed from the remnant of the solution at a specific concentration. This means that the absorption of PEG and water decreased and in the two-phase system of PEG and salt, water was driven from the PEG-rich phase to the salt-rich phase. This increased the PEG concentration of the PEG-rich phase and somewhat diluted the salt-rich phase (the salt concentration decreased) and the volume of the salt-rich phase increased at the expense of the PEG-rich phase [41–43].

Fig. 3 shows this binodal as compared with previous studies [14,15,26]. When the molecular mass of the PEG increased, biphasic formation occurred at lower concentrations of polymer and salt. Larger heterogeneous regions were observed for PEG 8000–salt than for PEG 600–salt ATPs. This was to be expected because as the polymer molecular mass increased, hydrophobicity increased and water solubility decreased, leading to polymer salting-out. Because the density also increased and the differences in the binodals were not significant, systems with PEG 600 and 8000 are potentially valuable for extraction purposes, mainly for the separation of target solutes. A lower density can have a positive effect on which PEG should be used.

3.3. TLL and STL

Table 3 shows the composition of the tie-line in the form of plots in Figs. 4 and 5. The binodal curve for the PEG + potassium formate + water system at 298.15 K is also provided.

The tie-lines were obtained through correlation with each resulting set of phase compositions (final, top and bottom). Moreover, there are simultaneous phases in the composition. A bulk balancing test

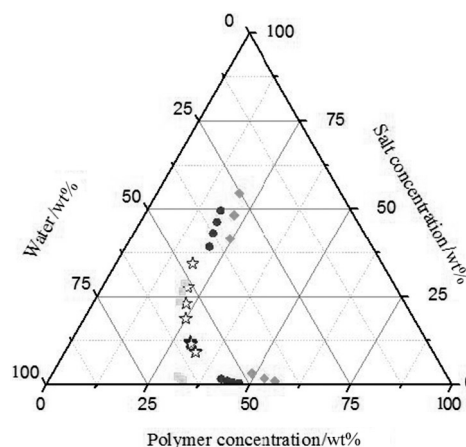


Fig. 3. Experimental binodal curves for PEG 3000 + potassium formate + water (☆) compared to PEG 600 + potassium formate + water (●) [26], PEG 1500 + potassium formate + water (◆) and PEG 8000 + potassium formate + water (■) [14] at 298.15 K.

was conducted by initially considering the amount of the mass per phase for the top and bottom on the basis of equilibrium compounds. Volume and density measurements were used to calculate the mass of each phase. The error of the mass balance was less than <2%. Table 3 shows that an increase in pH increased the TLL and decreased the STL.

The effects of pH on the equilibrium phase composition, TLL and STL are shown for the PEG 3000 + potassium formate + water system in Table 3 and Figs. 4 and 5. The STL values were not constant and slight variations can be observed for the different tie-lines of a given ATPS. It was found that the absolute value of the STL decreased as the TLL increased in this system when the pH level increased, which may be

Table 3

Phase composition, tie-line data and physical properties of PEG 3000 + potassium formate + water ATPs at 298.15 K and 0.1 MPa

pH	Total system/wt%		Top phase				Bottom phase				TLL	STL
	100w _p	100w _s	100w _p	100w _s	$\rho(\pm 0.0001)/\text{g} \cdot \text{cm}^{-3}$	n_D	100w _p	100w _s	$\rho(\pm 0.0001)/\text{g} \cdot \text{cm}^{-3}$	n_D		
9.98	25.00	25.00	36.57	11.01	1.1621	1.4354	0.67	55.03	1.3591	1.3940	56.80	0.82
	25.00	27.00	39.57	10.14	1.1621	1.4390	0.50	58.11	1.3813	1.3965	61.86	0.81
	28.00	18.00	30.38	13.05	1.1627	1.4275	1.25	48.15	1.3117	1.3893	45.61	0.83
	26.00	22.00	33.09	12.11	1.1622	1.4310	1.05	50.01	1.3242	1.3903	49.62	0.84
8.40	25.00	25.00	30.90	19.76	1.2260	1.4563	0.58	51.02	1.3285	1.3885	43.55	0.97
	25.00	27.00	34.02	19.01	1.2304	1.4638	0.45	53.00	1.3423	1.3900	47.77	0.99
	28.00	18.00	22.93	22.11	1.2165	1.4366	1.07	46.15	1.2959	1.3857	32.49	0.91
	26.00	22.00	25.63	21.23	1.2194	1.4434	0.80	48.45	1.3110	1.3868	36.84	0.91
7.95	25.00	25.00	20.57	29.02	1.2264	1.4530	46.14	2.30	1.3036	1.3930	36.98	1.04
	25.00	27.00	19.01	33.89	1.2300	1.4634	48.01	1.91	1.3149	1.3931	43.17	1.10
	28.00	18.00	22.91	23.18	1.2244	1.4402	40.15	4.16	1.2704	1.3951	25.67	1.10
	26.00	22.00	21.10	27.06	1.2236	1.4479	43.5	2.99	1.2883	1.3934	32.88	1.07

Standard uncertainties: $u(w_i) = 0.002$; $u(P) = 5 \text{ kPa}$; $u(T) = 0.05 \text{ K}$.

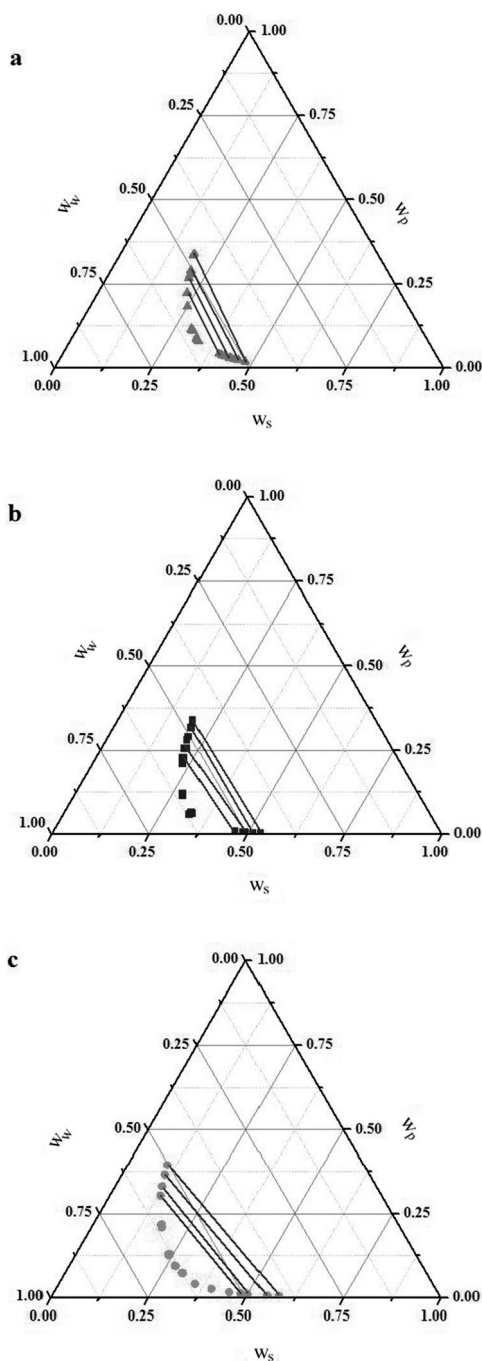


Fig. 4. Phase diagram and tie-line of PEG + potassium formate + water ATPS at $T = 298.15$ K and: (a) pH 7.95; (b) pH 8.40; (c) pH 9.98.

because of the reduction in the solution hydrodynamic volume. Comparable conduct has been shown in previous studies [14,15,26,37,44]. Waziri *et al.* [45] and Shahbazinasab and Rahimpour [37] reported a decrease in the intrinsic viscosity of the polymer solution with a decrease in pH.

Note that a decrease in pH modified the polymer chains, making their structure more compact, because the intrinsic viscosity of a polymer solution was proportional to its hydrodynamic volume. Studies have shown that the concentrations of the PEG and salt-rich phases increase and decrease, respectively, following an increase in the pH of the aqueous two-phase PEG–salt system. Once more, the polydispersity of the PEG could be the main reason for this [14]. The current study also examined the effects of TLL on the difference in density between the

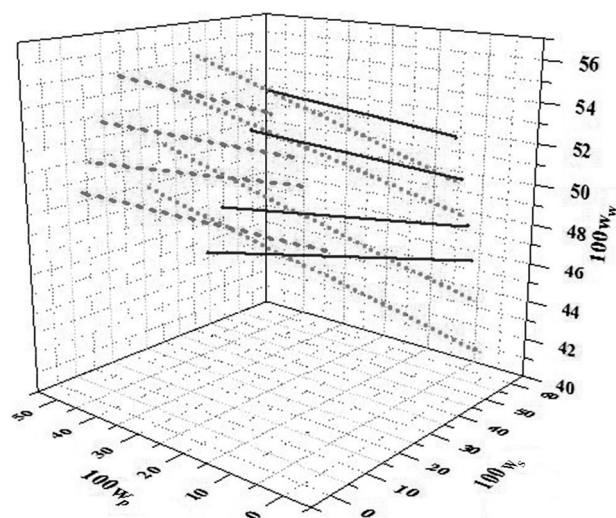


Fig. 5. Tie-line of PEG (3000) + potassium formate + H_2O ATPS at $T = 298.15$ K and pH values of 7.95, 8.40 and 9.98. Experimental tie line: (...) pH 7.95; (—) pH 8.40; (...) pH 9.98.

phases ($\Delta\rho$) of the ATPSs. Table 3 and Fig. 6 show that the $\Delta\rho$ increased as the TLL and pH increased. The $\Delta\rho$ between the phases shows a linear relationship with TLL. Similar results have been reported in previous studies [38,44,46–49].

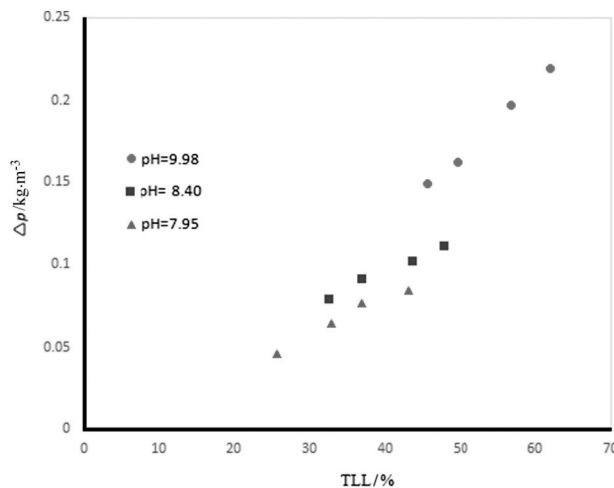


Fig. 6. Relationship between $\Delta\rho$ and TLL for PEG 3000 + potassium formate + water at different pH values.

3.4. Binodal curve and tie-line data correlation

Table 4 provides the coefficients of Eq. (4) with the resulting standard deviations of the systems.

Table 4

Values of parameters in Eq. (3) for PEG 3000 + potassium formate + water at different pH values

pH	<i>a</i>	<i>b</i>	<i>c</i>	R^2	<i>S</i>
7.95	0.999747	0.000005	0.000055	0.9987	0.0022
8.40	0.999346	0.000014	0.000110	0.9991	0.0011
9.98	0.999600	0.000007	0.000070	0.9998	0.0007

Standard deviation; Q_{cal} = calculated value, Q_{exp} = experimental value; *N* = number of values.

Table 5

Values of parameters in Eqs. (5), (6) and (7) for PEG 3000 + potassium formate + water at different pH values

pH	Eq. (5)				Eq. (6)				Eq. (7)			
	<i>k</i>	<i>n</i>	<i>R</i> ²	<i>s</i>	<i>k</i> ₁	<i>r</i>	<i>R</i> ²	<i>s</i>	<i>k</i> ₂	<i>a</i>	<i>R</i> ²	<i>s</i>
7.95	40.3465	−1.2254	0.9435	0.07	1.8293	1.5017	0.9668	0.03	0.5643	−0.1633	0.9565	0.05
8.40	0.7578	0.4622	0.9916	0.01	2.4146	1.9656	0.9988	0.01	0.4611	−0.2760	0.9922	0.01
9.98	131.3005	−1.9551	0.9899	0.02	2.0740	1.0015	0.9999	0.1	1.0609	−0.5899	0.9835	0.03

The standard deviations indicate that Eq. (4) could be used to correlate the binodal curves of the studied systems. The binodal curves indicate model reliability. The tie-line compositions for PEG 3000 + potassium formate + water were correlated using the Othmer–Tobias (Eq. (5)), Bancroft (Eq. (6)) and Hand (Eq. (7)) equations, which were perfectly practiced in previous systems containing polymer–salt molecular compounds. Table 5 shows the parameter values obtained from the results.

V^{*} denotes the EEV of PEG. Nonlinear regression was used to calculate the EEV (Table 6). Although the performance of this model was worse than for the Pirdashti equation, it has a more powerful theoretical background (statistical geometry), enabling better measurement of the salting-out effect of the different salts. Apart from this contradictory feature, both models have been adopted for correlation of the binodal data from the polymer/polymer and polymer/salt ATPSs [14,34,36].

Table 6
EEV parameter correlation

pH	EEV/g · mol ^{−1}	LSE [ⓐ]
7.95	941.05	0.0034
8.40	997.11	0.0020
9.98	1107.00	0.0329

[ⓐ] Least squares error $\left(\min \frac{1}{n} \sum_{i=1}^n (\text{error})^2 \right)$.

Linear regression was used to find the values for *b* and *k* in Eq. (9). The fitted parameters are shown in Table 7.

Table 7
Fitting parameter for Eq. (9) and statistics of regression

pH	<i>k</i>	<i>b</i>	<i>R</i> ²
7.95	2.9687	−42.9370	0.9907
8.40	3.2110	−30.0622	0.8995
9.98	4.4638	−24.0876	0.9978

4. Conclusions

The current study provides new findings on the use of liquid–liquid equilibrium data for PEG 3000 + potassium formate + water systems at pH values of 7.95, 8.40, and 9.98 at 298.15 K. Although reliable and valid data on the composition and system properties are necessary to design the extraction process, such data is inaccessible. A direct relationship was found between two-phase area expansion and an increase in pH, and is likely caused by the effect of salting-out. An increase in pH decreased the slope of the equilibrium tie-lines, while the lengths of tie-lines increased in the biphasic system. This may be because of a decrease in solution hydrodynamic volume. The calibration method was applied to measure the refractive index and density of the phases. The experimental binodal data for all investigated systems satisfactorily correlated with the Pirdashti equation and the tie-line compositions were fitted to the Othmer–Tobias, Hand and Bancroft equations. The difference in density between the phases increased as the tie-line length and pH increased. It was found that tie line length and difference in density

between phases vary linearly. Moreover, EEV increased following an increase in pH. PEG 3000 + potassium formate at a pH of 9.98 was the ATPS with the largest EEV and heterogeneous region.

Supplementary Material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cjche.2017.07.003>.

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