

Influence of the Molecular Weight of Polymer on the Poly Vinyl Pyrrolidone and Zinc Sulfate Phase Diagram of Aqueous Two-Phase Systems

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ABSTRACT: The study obtained the Liquid – Liquid Equilibrium (LLE) for Aqueous Two-Phase Systems (ATPSs) containing poly (vinyl pyrrolidone) (PVP) (10000, 24000, and 40000) and zinc sulfate at 298.15 K. The phase area is expanded according to the increased molecular weight of the polymer. Density and refractive indices were employed as the physical properties for obtaining phase composition and end of tie-line evaluations. The effect of molecular weight is discussed in relation to binodal curve, Tie-Line Length (TLL), and Slope of Tie Line (STL) determinations. Following the increase in polymer molecular weight value, the TLL increased and STL slightly decreased. Accordingly, Pirdashti's equation was used to correlate binodal curve evaluations of these systems. Furthermore, tie-line data points were correlated by Othmer-Tobias, Hand, and Bancroft equations. Finally, the study determined the Effective Excluded Volume (EEV) of salt into the PVP aqueous solution.

KEYWORDS: ATPS; Phase diagram; Refractive index; Poly (vinyl pyrrolidone); Zinc sulfate; Density.

INTRODUCTION

Aqueous Two-Phase Systems (ATPSs), also known as aqueous biphasic systems have been utilized very extensively in many different industries to separate proteins[1, 2], recombinant proteins[3], enzymes[4], nucleic acids[5], cells[6], drug molecules[7], metal ions[8], dyes[9], and small organic species[10]. ATPSs are generally made from an aqueous solution of two water-soluble polymers or a polymer and a salt [10,11]. Polyethylene glycol (PEG) is a polymer that is often used in this technique. Polyvinylpyrrolidone (PVP) is a synthetic hydrophilic polymer, its aqueous solutions with a suitable polymer or salt form a two-phase system [11,12].

In regard to PVP–salt systems, very few reports on the Liquid–Liquid Equilibrium (LLE) of aqueous PVP–organic salt are provided. Zafarani-Moattar and Sadeghi (2002) [13] have been determined LLE data for the PVP (K15) - sodium dihydrogen phosphate ATPS at 293.15, 298.15, and 303.15 K. It was found that for the studied system in the PVP-rich region, an increase in temperature caused the expansion of the one-phase area; while, for the salt-rich region, expansion of the two-phase area was observed with increasing the temperature. It was also observed that the slope of all equilibrium tie-lines increased with increasing temperature. Sadeghi (2006) have been determined

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1021-9986/2021/2/627-637

11/\$/6.01

the phase diagrams and compositions of coexisting phases for ATPSs containing PVP (K15) – sodium citrate[14], and PVP (K15) – potassium citrate at various temperatures of 298.15, 308.15, 318.15 and 328.15 K. The effect of temperature on the salting-out effect of PVP by citrate salt has been studied. It was found that, an increasing in temperature caused the expansion of two-phase region. Increasing temperature also increases the concentration of PVP in the PVP-rich phase, while the salt-rich phase will be somewhat mole diluted. In our previous papers, we have reported LLE data for PVP (K30)-potassium phosphate [16], and PVP (K30)-tri sodium citrate[17] ATPSs at various pH values at 298.15K. The two-phase area was expanded with increasing pH. Also, with pH enhancement, the slope and length of the equilibrium tie-lines were augmented for the mentioned biphasic system.

However, all attempts have included investigating the effects of temperature and pH on LLE data and according to our knowledge, only rarely has the effect of PVP molecular weight on LLE data been considered.

ATPS based on polymer and sulfate salt have applied for separation of metal ions [18,19], drugs [20], dyes[21] and biological materials [22]. Between salts of sulfate, the ability of the cation to promote the formation of ATPS and as a result of better separation followed the order zinc > ammonium > potassium > sodium. As far as we know, there is no report on the LLE data for PVP and zinc sulfate aqueous system. Consequently, this paper aimed at obtaining phase equilibrium data of PVP at different molecular weights (K17, K25 and K30) and zinc sulfate and water at 298.15 K.

After the phase-forming species have been selected, the next step is to select a particular tie-line. Coordinates for all “potential” systems will lie on a tie-line; the tie-line connects two nodes on the binodal, which represent the final concentration of phase components in the top and bottom phases. Moving along the tie-line coordinates denote systems with differing total compositions and volume ratios, but with the same final concentration of phase components in the top and bottom phases. Therefore, systems will differ in stability and the result of an individual change, or a combination of changes, will give a corresponding deviation in the Tie-Line Length (TLL) and, thus, in the composition and volume of the phases. Tie-lines are commonly parallel and hence the Slope

of the Tie-Line (STL) can also be calculated, $STL = \Delta Y1/\Delta X2$ thus, facilitating the construction of further tie-lines [23]. Also, an attempt was made to correlate the binodal curve, Tie Line Length (TLL) and slope of Tie Line (STL).

Finally, the binodal curves were successfully fitted by *Pirdashti* equations [24]. The tie-lines were correlated by using *Othmer–Tobias* [25], *Bancroft* [26] and *Hand* [27] equations.

EXPERIMENTAL SECTION

Materials

To prepare the materials, PVP [HO (C₂H₄O)_n H] with different molecular weights (M_w) of (40000, 24000 and 10000) g/mol and zinc sulfate with minimum purity of 99.5% by mass were obtained from Merck. Distilled deionized water (conductivity=17.8μS.cm⁻¹) was used to prepare the solutions. All chemicals were dried for at least 24 h (433.15 K for salts and 313.15K for polymers) in order to eliminate adsorbed water.

Apparatus and Procedure

Analytical Method

The study obtained compositions in both phases by evaluations of density and refractive indices as two physical properties at 298.15 K, while calibration equations were calculated beforehand. The next step was preparation of the homogeneous ternary mixtures with compositions from 0 to 30% (total solute composition) based on weight. Then, each of the two physical properties was measured at 298.15 K, as cited in *González-Amado et al.* (2016) [28]. Equation 1 was used to calculate PVP and salt concentration connecting the refractive index and density index to the concentration of salt and PVP at 298.15 K for each ATPS,

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

In Equation 1, Z stands for the physical properties (density or refractive index), w_p denotes the weight fraction of polymer, w_s indicates the weight fraction of salt, and the fittings of parameters are indicated by a_0 to a_5 . Accordingly, the refractive index was conducted at 298.15 K to obtain the refractive index using a refractometer (CETI Belgium model) with accuracy of 0.0001 n_D . Then, an Anton Paar oscillation U-tube densitometer (model: DMA 500) was used for density measurements with precision of $\pm 10^{-4}$ g.cm⁻³.

Binodal Curve

The study employed apparatus similar to that cited in *Pirdashti et al.* (2015) [29]; the equilibrium was obtained using a glass vessel with volume of 25 cm³. Moreover, an external jacket containing water at a constant temperature was used beside the glass vessel and the temperature was controlled within ± 0.05 K. The cloud-point method [23] was adopted to obtain the binodal curve and investigated by the titration method. Accordingly, exact amounts of polymer (titrant) were added to an aqueous solution salt with a known concentration (or vice versa), this was done step by step, through stirring until the solution became cloudy.

The TLL and STL

The tie lines were obtained by using the equilibrium set designed by this study and based on a previously described Procedure [30]. For this purpose, four samples were selected for each M_w ; prepared by mixing adequate amounts of PVP, salt, and water in the vessels. Each sample was then stirred for 5 min and then left to settle for 24 h under controlled temperature to ensure equilibrium. The study employed Hermle Z206A (made in Germany) to centrifuge the tubes at 6000 rpm for 5 min to separate the obtained phases, which showed no turbidity and difficulty in the separation of top and bottom samples. After equilibrium was reached, syringes were used to withdraw the phases. Accordingly, the top phase was sampled first, care was taken to leave a layer of material at least 0.5 mm thick above the interface. The bottom phase remained in the glass vessel with a long needle. The TLL provided an empirical measurement of the composition for each of the two phases, which can be calculated by the following equation:

$$TLL = \sqrt{(w_{pt} - w_{pb})^2 + (w_{sb} - w_{st})^2} \quad (2)$$

Where w_p and w_s indicate the mass fraction of PEG and salt. Moreover, the superscripts of t and b indicate the top and bottom phases, respectively.

STL is obtained from Equation 3. In other words, STL is the ratio of the difference in the weight fraction of polymer (w_p) and salt (w_s) in both top and bottom phases.

$$STL = \frac{w_{pt} - w_{pb}}{w_{st} - w_{pb}} \quad (3)$$

Binodal Curve and TLL Correlation

For the binodal data correlation, the Pirdashti's equation is appropriate to reproduce the binodal curves of the investigated systems

$$w_p = (a + bw_s)^{\frac{-1}{c}} \quad (4)$$

Where a , b , and c represent the fitting parameters, and w_p and w_s demonstrate polymer and salt weight fractions, respectively. Binodal data of the above expression were correlated by least-square regression.

Othmer-Tobias (Eq. (5)) and Bancroft (Eq. (6)) and Hand (Eq. (7)) correlation equations were used to ensure reliability of the measured tie-line compositions [25-27].

$$\left(\frac{1-w_{pt}}{w_{pt}}\right) = k \left(\frac{1-w_{sb}}{w_{sb}}\right)^n \quad (5)$$

$$\left(\frac{w_{wb}}{w_{sb}}\right) = k_1 \left(\frac{w_{wt}}{w_{pt}}\right)^r \quad (6)$$

$$\ln \frac{w_{wb}}{w_{sb}} = a + k_2 \ln \frac{w_{wt}}{w_{pt}} \quad (7)$$

Where w_{pt} is the mass fraction of polymer in the top phase, w_{sb} is the mass fraction of salt in the bottom phase, w_{wb} and w_{wt} are mass fractions of water in the bottom and top phases, respectively, and k , n , k_1 , and r are the adjusted parameters.

To correlate the liquid-liquid equilibrium data, a simple two parameter equation was employed based on the the binodal theory (*Guan et al.* [31]):

$$\ln(w_{st}/w_{sb}) = b + k \cdot (w_{pb} - w_{pb}) \quad (8)$$

In equation 8, b and k are fitting parameters that can be regarded as "effective" virial (or activity) coefficients and the salting-out coefficient of the salt. w_i is the mass fractions of polymer and salt, and the superscripts in the equation indicate the top and bottom (bot) phases. Besides, the obtained experimental data can also be adapted to the equation provided by *Guan et al.* (1993) [31].

$$\ln(v^* \cdot W_{PVP} / M_{PVP}) + v^* \cdot W_{salt} / M_{salt} = 0 \quad (9)$$

Where M_{PVP} and M_{salt} stand for the polymer and salt molecular weight, respectively. Moreover, V^* denotes the Effective Excluded Volume (EEV) of the target salt in the PEG aqueous solution [28,32].

Table 1: The value of the coefficients observed from eq. 1.

ATPS type	Physical property	a_0	a_1	a_2	a_3	a_4	a_5	R^2
PVP(K30) and zinc sulfate and water	n_D	1.3325	0.016	0.0091	0.0005	0.0004	1.0000	0.9996
	$\rho/\text{g.cm}^3$	0.9962	0.0202	0.0636	0.0005	-0.0032	1.0000	0.9995
PVP(K25) and zinc sulfate and water	n_D	1.3327	0.0157	0.0008	0.0005	0.0005	1.0000	0.9998
	$\rho/\text{g.cm}^3$	0.9961	0.0202	0.0638	0.0005	-0.0032	1.0000	0.9997
PVP(K17) and zinc sulfate and water	n_D	1.3328	0.0159	0.0016	0.0004	0.0006	1.0000	0.9999
	$\rho/\text{g.cm}^3$	0.9966	0.0184	0.0630	-0.0008	-0.0030	1.0000	0.9998

RESULT AND DISCUSSION

Fitting parameters of calibration equation

The values of the coefficients equation (1), a_0 , a_1 , a_2 , a_3 , a_4 and a_5 for the studied system are shown in Table 1.

From the coefficient of determination (R^2), it is concluded that equation 1 could be adequately applied to calculate the polymer and salt concentration by measuring refractive index and density index of unknown solution. a_0 , a_1 , a_2 , a_3 , a_4 and a_5 are dimensionless. According to the coefficients given in Table 1, the effect of polymer concentration on the refractive index of solution is greater than the effect of salt concentration ($a_1 > a_2$ for n_D). Vice versa, the effect of salt concentration on the density of solution is greater than the effect of the concentration of polymer on it ($a_1 < a_2$ for ρ). As can be seen from this table, the importance of the coefficients (a_1 and a_2) is greater than the other coefficients (a_3 and a_4), i.e. $a_1 \gg a_3$ and $a_2 \gg a_4$.

Binodal Curve

The binodal curve data of the PVP and zinc sulfate and water system are presented in Table 2.

Fig. 1 shows the binodal curves obtained by experimental and correlated by Pirdashti's equation. The effect of polymer molecular weight is clear; the phase area is expanded according to increased molecular weight of the polymer [33]. In other word, when the molecular weight of the PVP was increased, biphasic formation occurred at lower concentrations of polymer and salt. Larger heterogeneous regions were observed for PVP (K30) and salt than other mention ATPS's. This means that the system formed by PVP K30 has better phase forming ability and can be considered as a more efficient ATPS for separation. This was to be expected, because when

the polymer's molecular weight increases, hydrophobicity is higher and water solubility is lower, leading to the polymer salting-out. Similar conclusions were previously found by other authors [27-29].

From Table 2 and Fig. 1, it is also observed that, the difference between binodal curves is related to weight fractions of polymer and salt. For weight fractions of PVP > 0.08 the binodal curves tend to join the binodal curves obtained with PEG 24000 and 40 000. A dissimilar effect occurs for concentrations of $\text{ZnSO}_4 > 0.18$ for the binodal curves obtained with PVP 10000, 24000, and 40 000 and at lower molecular weights of PVP, the difference between binodal curves is less.

TLL and STL

Tie line compositions are given in Table 3 and Figs. 2 and 3 presents the tie lines and the binodal curve together for the PVP and zinc sulfate and water system at 298.15 K.

The tie lines were determined by connecting each corresponding set of phase compositions; total, top and bottom. The coexisting phases were closed in the composition evaluations. The mass balance between the beginning mass of each component and amounts in the bottom and top phases were ascertained according to the equilibrium compositions. Then, mass evaluations were calculated for each phase based on volume and density measurements. The relative error in the mass balance was less than $< 2\%$. The effects of polymer molecular weight on the equilibrium phase compositions, the TLL and STL have been indicated for the PVP and zinc sulfate and water system in Table 3 and Figs. 2 and 3. Accordingly, the STL values are not constant and slight variations are observed for the different tie-lines of a given ATPS. Furthermore, it was found that the absolute value of the STL decreases as the TLL increases in this system if the PVP molecular

Table 2: Binodal curve data of the PVP and zinc sulfate and water system at 298.15 K and 0.1 MPa at different MW values

$M_W = 10000$		$M_W = 24000$		$M_W = 40000$	
$100w_p$	$100w_s$	$100w_p$	$100w_s$	$100w_p$	$100w_s$
46.72±0.02	14.32±0.01	35.01±0.01	10.35±0.02	46.62±0.01	7.72±0.01
41.75±0.01	14.64±0.02	25.84±0.02	11.32±0.03	30.55±0.01	9.43±0.03
33.88±0.01	15.23±0.03	22.34±0.02	12.01±0.00	26.09±0.01	10.11±0.02
30.17±0.00	15.56±0.04	16.84±0.01	13.43±0.01	14.85±0.03	12.85±0.02
22.84±0.01	16.35±0.01	14.96±0.01	14.22±0.01	12.77±0.02	13.51±0.02
19.88±0.02	16.74±0.02	14.13±0.03	14.47±0.01	13.45±0.02	13.16±0.02
16.32±0.02	17.50±0.01	11.99±0.01	15.69±0.00	13.08±0.03	13.33±0.01
14.90±0.01	17.73±0.03	9.68±0.03	16.84±0.02	12.72±0.00	13.47±0.00
13.17±0.01	18.20±0.01	8.88±0.02	17.89±0.04	10.65±0.01	14.61±0.00
12.05±0.03	18.54±0.01	8.43±0.01	18.52±0.03	7.52±0.02	16.43±0.03
9.84±0.02	19.40±0.01	7.50±0.01	19.15±0.02	7.04±0.02	16.62±0.02
8.87±0.02	20.14±0.02	5.46±0.02	21.06±0.01	5.45±0.02	18.60±0.01
7.27±0.00	21.10±0.01	3.89±0.02	23.32±0.01	5.10±0.03	18.77±0.01
5.06±0.01	22.56±0.02	2.73±0.03	25.23±0.01	4.96±0.02	18.87±0.02
3.39±0.01	24.78±0.01			4.55±0.01	19.48±0.00
2.49±0.02	26.71±0.03			4.27±0.04	19.69±0.00
1.82±0.02	28.91±0.02			2.99±0.01	22.32±0.01
1.10±0.03	32.88±0.01			2.21±0.02	24.56±0.04
0.81±0.03	35.61±0.01				

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

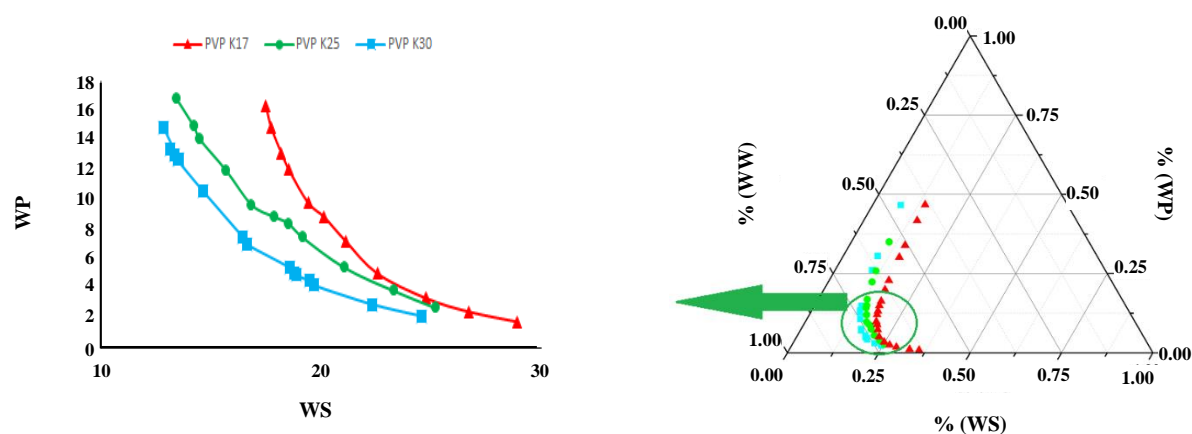


Fig. 1: Phase diagram of the PVP and zinc sulfate and H_2O (3) two-phase system at $T = 298.15 \text{ K}$ and various MW (10000, 24000, 40000): experimental binodal (10000 (\blacktriangle), 24000 (\bullet), 40000 (\blacksquare); (3) the solid curves represented correlated binodal.

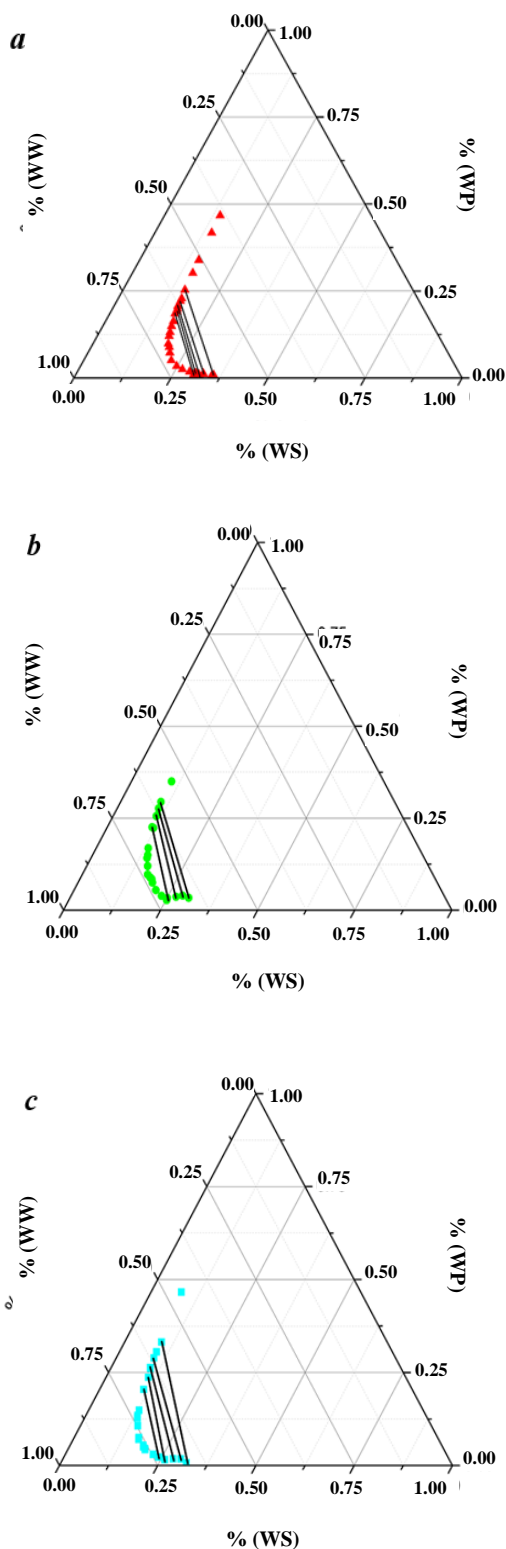


Figure 2. the tie lines of the PVP and zinc sulfate and water two-phase system at $T = 298.15\text{ K}$ and MW 10000 (a), 24000 (b) and 40000 (c)

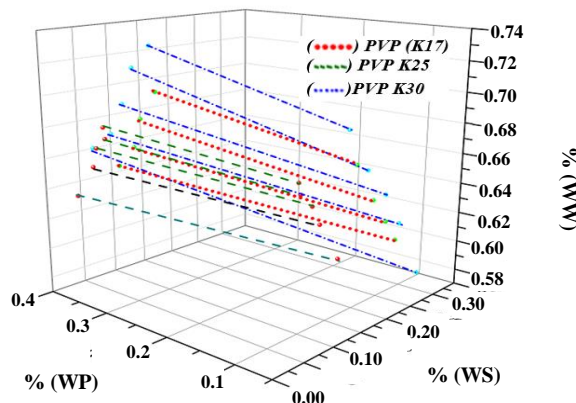


Fig. 3: The tie line of the PVP (K17,K25,K30) and zinc sulfate and water at different MW (10000,24000,40000) two-phase system at $T = 298.15\text{ K}$; experimental tie line; (•••••) PVP (K17); (•••••) PVP K25; (•••••)PVP K30.

weight value is augmented, which may be because of the reduction in the solution hydrodynamic volume. Furthermore, a comparable conduct for PEG-salt ATPS has been shown in other research [27-29]. Once more, the polydispersity of the polymer may be the main reason for this coincidence [27].

Moreover, according to Table 3 and Fig. 4, density evaluations of the bottom phase were close to 1.2 g/cm^3 , while those of the top phase ranged from 1.08 to 1.2 g/cm^3 . The study considered the effects of TLL on the the density difference between the phases ($\Delta\rho$) of the aqueous two-phase systems. $\Delta\rho$ increased with an increment in the TLL and PVP molecular weight. Besides, the density differences between the phases show a linear relationship with TLL. Other studies have shown comparable experiment results [19,30-32].

Although ATPS with a larger area is more suitable for the separation process and this phenomenon has been observed with increasing molecular weight of the polymer. However, as density also increases, and the differences in the binodals are not significant, systems with PVP (K17) can be envisaged as interesting and useful for extraction purposes, mainly in the separation of target solutes. Density being lower can have a positive effect on the decision of which PVP should be used. There is a paradox here. Increasing the molecular weight and lower density are opposed to each other. Optimized system for separation process depending on the environmental conditions and the type of system the solute is chosen [29].

Table 3: Phase composition, tie-line data and physical properties of PVP(K30,25,17) and zinc sulfate and water aqueous two-phase system at 298.15 K and 0.1 MPa.

M_w	Total System (% mass)		Top phase				Bottom phase				TLL	-STL
	$100w_p$	$100w_s$	$100w_p$	$100w_s$	$\frac{\rho(\pm 0.0001)}{gr. cm^{-3}}$	n_D (± 0.0001)	$100w_p$	$100w_s$	$\frac{\rho(\pm 0.0001)}{gr. cm^{-3}}$	n_D (± 0.0001)		
40000	15.00	15.00	20.44	11.22	1.1014	1.3796	2.26	24.03	1.1347	1.3613	26.89	1.45
	16.00	16.00	23.72	10.78	1.1056	1.3849	1.56	26.02	1.1441	1.3622	30.48	1.35
	17.00	17.00	26.35	9.95	1.1063	1.3887	1.80	28.03	1.1556	1.3648	33.95	1.32
	18.00	18.00	28.92	9.57	1.1094	1.3928	1.78	29.98	1.1661	1.3669	39.50	1.43
	19.00	19.00	33.18	9.37	1.1168	1.4001	0.75	31.93	1.1747	1.3672	22.99	1.37
24000	16.00	16.00	22.56	11.55	1.1069	1.3835	3.98	25.10	1.1438	1.3655	27.18	1.36
	17.00	17.00	25.56	11.15	1.1106	1.3883	3.61	27.18	1.1544	1.3671	29.78	1.31
	18.00	18.00	27.66	10.69	1.1123	1.3915	3.95	28.71	1.1634	1.3693	32.98	1.29
	19.00	19.00	29.42	10.39	1.1141	1.3943	3.35	30.59	1.1724	1.3703	22.39	1.30
10000	18.00	18.00	19.19	17.02	1.1202	1.3831	1.43	30.66	1.1676	1.3668	24.85	1.28
	15.00	21.00	20.65	16.45	1.1193	1.3850	1.02	31.69	1.1725	1.3672	31.11	1.27
	20.00	20.00	25.35	15.94	1.1233	1.3926	0.88	35.16	1.1910	1.3706	22.12	1.29
	12.00	22.00	18.50	16.83	1.1181	1.3817	0.98	30.34	1.1652	1.3657	26.46	1.27
	19.00	19.00	22.12	16.39	1.1211	1.3875	1.29	32.71	1.1784	1.3688		

Standard uncertainties: $u(w_i) = 0.01$; $u(P) = 5$ kPa; $u(T) = 0.05$ K.

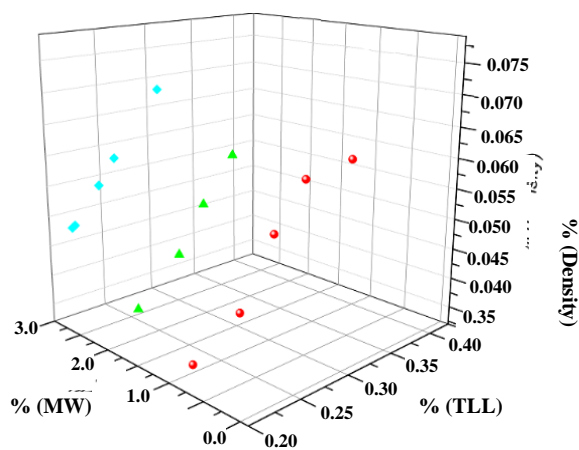


Fig. 4: Relationship between density difference ($\Delta\rho$) and tie line length (TLL) for the PVP and zinc sulfate and water at different molecular weight values.

Binodal curve and tie-line data correlation

The coefficients of Equation 4, along with the corresponding standard deviations of the investigated systems are provided in Table 4.

According to the acquired standard deviation, it was concluded that equation 4 was almost suitable to make correlations of binodal curves of the studied systems. Figures determined by the binodal curves are used to indicate reliability of the model.

Also, linear dependence of plots of $\log((1 - w_{t1})/w_{t1})$ against $\log((1 - w_{b2})/w_{b2})$ and $\log(w_{b3}/w_{b2})$ against $\log(w_{t3}/w_{t1})$ indicates acceptable consistency of the results. The corresponding correlation coefficient values, R_1 and R_2 , are also given in Table 5.

Also, linear regression was used to find values for β and k values for equation 8. The fitting parameter for equation 8 are also given in Table 6.

Moreover, V^* is the EEV of the salt into the PVP aqueous solution. As indicated in Table 7, a nonlinear regression was used to obtain EEV values. Although this model performed less well than Pirdashti's equation, it had a stronger theoretical background (statistical geometry), which enabled evaluation of the salting-out effect in the different salts that were tested. However, studies have shown that the correlation of binodal data from polymer/polymer and polymer/salt ATPs has been conducted through successful

Table 4: Values of parameters of equation 4 for PVP (K17, K25, K30) and zinc sulfate and water at different molecular weight values.

M_w	a	b	c	R^2
10000	-0.2498	0.0370	0.3300	0.9992
24000	-0.0792	0.0227	0.5251	0.9959
40000	0.2350	0.0245	0.2230	0.9998

Table 5: Values of the parameters of Eqs.5, 6 and for PVP and zinc sulfate and water at different molecular weight (M_w) values.

M_w	k	n	R^2	k_1	r	R^2	k_2	a	R^2
10000	0.9454	1.8363	0.9948	0.7429	1.8859	0.9975	0.5351	0.1521	0.9978
24000	0.7795	1.3483	0.9907	0.7406	1.3024	0.9951	0.7754	0.2236	0.9945
40000	0.6166	1.5965	0.9948	0.5357	1.6271	0.9875	0.5971	0.3994	0.9836

Table 6: Fitting parameter for equation(8) and statistic of regression.

M_w	k	b	R^2
10000	0.0297	-0.0665	0.9964
24000	0.0456	0.1061	0.9965
40000	0.0363	-0.1042	0.9460

Table 7: Effective excluded volume parameter correlation.

M_w	EEV(g/mol)	LSE*
10000	1585.23	0.3776
24000	2242.02	0.0093
40000	2826.84	0.0072

adoption of both models [18, 31, 32]. In total, taking into account the EEV values from Table 7, it is also observed that, at higher molecular weights of PVP, the difference between binodal curves is less.

CONCLUSIONS

In this study, experiments presented new results for the liquid-liquid equilibrium data of PVP (K17, K25 and K30) and zinc sulfate and water system at 298.15 K. Results demonstrated that the two-phase area expanded according to increased PVP molecular weight. On the other side, the polymer molecular weight enhancement had an opposite relationship with the slope of the equilibrium tie-lines as it decreased, while the length of tie-lines increased in the biphasic system. It may be because of the reduction in the solution hydrodynamic volume. Furthermore, the experimental binodal data for all of the investigated

systems satisfactorily correlated with the *Pirdashti* equation and the tie-line compositions were fitted to both the *Othmer-Tobias*, *Hand* and *Bancroft* equations. Moreover, evaluations of density, and refractive indices of the binary and ternary mixtures of the aqueous two-phase PVP (K17, K25 and K30) and zinc sulfate and water systems were measured and correlated at 298.15 K. Furthermore, empirical models were developed to describe some physical properties of the binary and ternary systems. The models reproduced the data determined by experiments precisely. The calibration method was applied to measure both the refractive index and density of the phases. The density difference between the phases increased with an increment in the tie line length and polymer molecular weight. It was found that the tie line length and density difference between the phases change linearly with each other. Moreover, the EEV was increased

following the polymer molecular weight increment. Thus, PVP (K30) and zinc sulfate are the ATPS with the largest EEV and heterogeneous region.

Received : Aug. 7, 2019 ; Accepted : Nov. 18, 2019

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