The aim of this work is to investigate the quaternary systems which contain (polyethylene glycol/isopropyl alcohol) + ethanol + salt (Na₂SO₄/Na₂CO₃) + water to understand their liquid-liquid equilibrium (LLE) and salting-out ability and apply them to definite uses. For this purpose, phase diagrams and liquid-liquid equilibrium (LLE) data of the investigated quaternary systems were experimentally determined at 308.15, 318.15, and 328.15 K. Binodal curves were fitted to three empirical nonlinear equations, whereas tie-lines were fitted to the Setschenow-type equation and another two-parameter equation. All the models were successfully correlated with the experimental data. What is more, factors such as temperature, different organic solvent, and type of salt are studied. It was demonstrated that temperature has no influence in binodal curves and the salting-out ability follows the order Na₂CO₃ > Na₂SO₄. Finally, we chose the best system (isopropyl alcohol + ethanol + Na₂CO₃ + H₂O) to find out the extraction efficiency when dealing with vitamin B₆. The results show that when the temperature is 308.15 K and pH is 5.02, the extraction efficient can reach a top level. It serves as a theoretical basis for the extraction, separation, and purification.

1. Introduction

Vitamin B₆, as an indispensable part of human body, can regulate body fluids, prevent hair loss, and has a central role in amino acid metabolism and modulating the activity of steroid and other hormones [1]. In recent years, events concerning food safety have increased public attention. Vitamin B₆ exists in all kinds of food, and it is important to know how much it is contained in food. Therefore, a rapid, accurate, and safe measurement analysis must be established [2].

Since aqueous biphasic systems were proposed by Beijernick in 1896 [3], the aqueous two-phase system (ATPS) as a new technology that is safe and easy to control and has a simple process is now gradually taking the place of traditional organic-water solvent extraction systems. The ATPS is usually composed of two or more polymers, a polymer, and a salt [4, 5]. They have mild separation conditions, rapid balance processes, and high rate of product recovery. As a “green chemistry,” ATPS now is widely used in the separation of protein [6, 7], DNA [8], drugs [9], antibiotic [10, 11], and metal ion [12]. So, we pay more attention to find a new ATPS to extract vitamin B₆.

With the development of science and technology, a new system is born. Small-molecule organic solvent ATPSs are led to our increased use in searching ATPSs. For example, binodal data for ethanol, 2-propanol, and 1-propanol + MgSO₄/ ZnSO₄ + water systems experimentally determined at 303.15 K and 313.15 K [13, 14] and ethanol + salt [(NH₄)₂SO₄ [15], NaOH [16], NaF [17], and K₂HPO₄ [18] ATPSs have been reported. Comparing traditional ATPSs with the small-molecule organic solvent ATPS, the small-molecule organic solvent
ATPS has some advantages. For example, it is easy to control, has a low cost, and is environmentally friendly. So, it has been successfully used in the separation and purification of hesperidin [19] and pigments [20]. However, it also has shortcomings, such as large amount of phase-forming materials used and having a higher volatility of alcohol. In order to reduce these situations, we use quaternary systems to replace ternary systems. So, the ATPS containing polymer and small-molecule, such as polymer/alcohol and binary alcohol to form an ATPS, is studied in the following passage.

In this work, phase diagrams and liquid-liquid equilibrium (LLE) data for the quaternary system polyethylene glycol + ethanol + salt (Na$_2$SO$_4$) + water were investigated at $T = 308.15$ K, 318.15 K, and 328.15 K, and those for the system isopropyl alcohol + ethanol + salt (Na$_2$SO$_4$/Na$_2$CO$_3$) + water were examined at $T = 308.15$ K and those at atmospheric pressure have been studied. In the above, system polyethylene glycol/isopropyl alcohol and ethanol were mixed in different proportions and formed homogeneous and stabilized solutions. From what we discussed, the bimodal curves were fitted to three nonlinear equations. What is more, we have discussed what the temperature, salts, and different composition of organic solution can do on the phase separation abilities of these systems. In addition, some factors such as salt, pH, and temperature were discussed in the system of vitamin B$_6$ extraction.

2. Materials and Methods

2.1. Materials. Analytical-grade Na$_2$CO$_3$, Na$_2$SO$_4$, ethanol, polyethylene glycol, and isopropyl alcohol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), Tianjin Baishi Chemical Co., Ltd. (Tianjin, China), and Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China), respectively. All the above materials have a minimum mass fraction of 99.5%. Vitamin B$_6$ was purchased from a local supermarket and was ultrasonically degassed in advance. Double-distilled deionized water was also used in the experiments. The sources and purities of chemicals used in the study are shown in Table 1.

2.2. Apparatus and Procedure

2.2.1. Determination Method of Binodal Curves Data. Before the experiment, we added polyethylene glycol and isopropyl alcohol into ethanol until the mass fraction of ethanol reached 0.200 and 0.333, respectively. In the following text, “MA” denotes the mass fraction of ethanol in the mixture of polyethylene glycol and ethanol or isopropyl alcohol and ethanol. So, the values of MA$_1$ and MA$_2$ were 0.200 and 0.333, respectively.

The binodal curves were determined by the cloud point method at 308.15, 318.15, and 328.15 K. First, known mass fractions of polyethylene glycol alcohol and ethanol were added drop-wise using a small-sized pipette to create heterogeneous systems until the mixture became cloudy. Then, known amounts of double-distilled deionized water were subsequently added to the tube to create a transparent one-phase system. After recording the data, the mass of the tube was measured again until there was some precipitation at the bottom of the vessel for obtaining sufficient data for the construction of a phase diagram. The data of isopropyl alcohol and ethanol at $T = 308.15$ K was obtained by the same method. Throughout this preparation procedure, the tubes were incubated in an external jacket through which water at a constant temperature was circulated using a DC-2008 water thermostat (Tianjin Taite Instrument Factory, China). The temperature was controlled to within ±0.05 K. The masses of the starting and added solutions were measured using an analytical balance (BC 124S, Beijing Sartorius Instrument Co., Ltd., China) with a precision of ±0.0001 g.

2.2.2. Determination Method of Tie-Line Data. After the collecting of the binodal data, five tie-lines were determined for each of the studied systems. Systems with a known polyethylene glycol/isopropyl alcohol and ethanol composition (total weight of approximately 10 g) were vigorously shaken in separate tubes and then placed in a thermostat bath for at least 48 h until the solution separated into two clear phases to reach a phase equilibration. Then the upper phase was carefully removed by pipette. The mass of the upper phase was determined by an analytical balance, and the mass of the lower phase was obtained by subtraction. Both the top phase and bottom mixture solutions with concentrations in the range of 1%–50% w/w were prepared, and their refractive indexes were determined at the experimental temperatures using an Abbe refract meter (modal WAY [2WAJ], Shanghai Physical Optics Instrument Co., Ltd.). After that, the salt solutions with concentration in the range of 0.1%–25% w/w were added to the above alcohol mixture solutions, and the corresponding reactive indexes were determined at the experimental temperatures. A comparison of the refractive indexes for the two groups of solutions revealed that there was some influence of salt on the refractive index when solved in the alcohol solutions. A comparison of the refractive indexes revealed that the salt content influence on the refractive index is not obvious. Hence, the salt concentrations in the two phases were determined directly by atomic absorption spectrophotometer. Since the refractive index of mixture depends on the mass fractions of alcohol and salt, the alcohol concentrations were determined by Abbe refractometer and in accordance with the following equation:

$$n_{D} = n_0 + a_1 w_1 + a_2 w_2,$$

(1)

where $n_0$ is the refractive index of deionized water; $a_1$ and $a_2$ are experimental fitting parameters; $w_1$ and $w_2$ are mass fractions of alcohol and salt, respectively. The values of parameters were given in Table S1 (Supplementary Materials). For the measurement of alcohol, we used an Abbe refractometer with an uncertainty of 0.0002 to determine the refractive indexes. The refraction indexes were put into the corresponding equation to obtain the concentration of the alcohol mixture in the phases. The mass fraction of water in both the top phase and bottom phase was obtained from the law of mass conservation.

When ion exchanging exists in both the top phase and bottom phase, it may affect the validity of the concentration
of both the top phase and bottom phase. Aiming to check it, we used the method above and corrected it with mathematical model. Firstly, the concentration of both the top phase and bottom phase was accurately determined; then MATLAB software was used to deal with these dates and the dates met with the “lever principle” [21]. The method was used in the literature in recent years [22, 23].

The tie-line length (TLL) and the slope of the tie-line (S) at different compositions were calculated using equations (2) and (3), respectively [24]:

$$TLL = \left[ (w_1^t - w_1^b)^2 + (w_2^t - w_2^b)^2 \right]^{0.5},$$

$$S = \frac{(w_1^t - w_1^b)}{(w_2^t - w_2^b)},$$

where \(w_1^t\), \(w_1^b\), \(w_2^t\), and \(w_2^b\) represent the equilibrium mass fractions of the polyethylene glycol/isopropyl alcohol and salt (\(\text{Na}_2\text{SO}_4/\text{Na}_2\text{CO}_3\)) in the top and bottom phases, respectively.

### 2.2.3. Vitamin B6 Analysis

A certain portion of sample solution containing isopropyl alcohol, ethanol, \(\text{Na}_2\text{CO}_3\), and \(\text{H}_2\text{O}\) was transferred into a 10 mL centrifuge tube. Then the centrifuge tube with ATPS was put on the analytical balance, and the 2 g of the standard working condition B6 prepared in advance was added drop-wise to the tube using a syringe. The mixture was stirred thoroughly at room temperature (25°C). Still, an 802 centrifuge was used at 2000 rpm for 25 min to ensure that phase separation operated completely. To finish these, the centrifuge tubes were then placed in water thermostat DC-2008 (Tianjin Taisite Instrument Co., China) with an uncertainty of 0.05°C at the setpoint temperature of 35°C for 12 h to reach phase equilibrium. After that, B6 and alcohols were enriched in top phase and salt was enriched in bottom phase. The volume of top phase was read accurately and part of them was aspirated into a 50 \(\mu\text{L}\) microsyringe and directly injected into the HPLC system for analysis. The pH value was adjusted by hydrochloric acid and ammonia water.

The extraction efficiency of B6 was defined as the ratio between the amount of the B6 in the top phase and the initial amount of the B6.

$$E(\%) = \frac{V t \times C_t}{m_i} \times 100\%,$$

where \(C_t\) is the concentration of B6 in the top phase after the phase equilibrium, \(V_t\) is the volume of the top phase after the phase equilibrium, and \(m_i\) is the amount of B6 added initially.

### 2.2.4. HPLC-UV Analysis

A 1200 HPLC system (Agilent, USA) essentially comprised of a quaternary pump and a variable wavelength UV-VIS detector (Agilent, USA) was employed for the determination of DBP. All data processing and facility control were conducted via Agilent Chem Station software. Acetonitrile and water (88:12 by volume) were used as the mobile phase with gradient elution on the reversed phase chromatography column (Shim-pack VP-ODS column, 150 × 4.6 mm, 5 \(\mu\text{m}\)), and the flow rate was 1.0 mL/min. The injected volume was 5 \(\mu\text{L}\). The detection wavelength was 228 nm using the UV-VIS detector.

### 3. Results and Discussion

#### 3.1. Phase Behaviors of the IL-Salt ILATPS

The binodal data of the quaternary system polyethylene glycol + ethanol + \(\text{Na}_2\text{SO}_4\) + water determined at \(T = 308.15, 318.15\), and 328.15 K, system isopropyl alcohol + ethanol + \(\text{Na}_2\text{SO}_4\) + water at \(T = 308.15\) K, and system isopropyl alcohol + ethanol + \(\text{Na}_2\text{CO}_3\) + water at \(T = 308.15\) K are presented in Table 2.

The binodal data were fit to the following system of equations using the empirical nonlinear expression developed by Merchuk et al. [25]:

$$w_1 = a \exp\left(bw_2^{0.5} - cw_2^3\right),$$

where \(w_1\) is the mass fraction of the mixture of the alcohol, \(w_2\) is the mass fraction of the salt, and \(a\), \(b\), and \(c\) are fitting parameters. The fitting parameters obtained from fitting the experimental binodal data to equation (5) are presented in Table 3, along with the correlation coefficients \(R^2\) and the corresponding standard deviations (sd).
Table 2: Binodal data for the mixture of polyethylene glycol/isopropyl alcohol + ethanol + salt (Na$_2$CO$_3$/Na$_2$SO$_4$) + water ATPS at different temperatures$^a$.

| Temperature ($K$) | Polyethylene glycol + ethanol + Na$_2$SO$_4$ + H$_2$O system at $T = 308.15K$ | Polyethylene glycol + ethanol + Na$_2$SO$_4$ + H$_2$O system at $T = 318.15K$ | Polyethylene glycol + ethanol + Na$_2$SO$_4$ + H$_2$O system at $T = 328.15K$ |
|------------------|--------------------------------|--------------------------------|--------------------------------|
| $T$              | $W_2$ | $W_1$ | $W_2$ | $W_1$ | $W_2$ | $W_1$ | $W_2$ | $W_1$ | $W_2$ | $W_1$ |
| 308.15K          | 0.200 | 46.51 | 2.20  | 30.59 | 5.47  | 27.23 | 6.67  | 20.13 | 9.97  | 8.57  | 16.79 |
| 318.15K          | 0.200 | 43.94 | 2.92  | 24.72 | 8.09  | 19.61 | 10.50 | 14.07 | 13.24 | 7.93  | 16.99 |
| 328.15K          | 0.200 | 47.94 | 2.12  | 26.00 | 7.69  | 14.22 | 13.32 | 10.15 | 15.42 | 7.64  | 16.92 |

| Temperature ($K$) | Isopropyl alcohol + ethanol + Na$_2$SO$_4$ + H$_2$O system at $T = 308.15K$ | Isopropyl alcohol + ethanol + Na$_2$SO$_4$ + H$_2$O system at $T = 318.15K$ | Isopropyl alcohol + ethanol + Na$_2$SO$_4$ + H$_2$O system at $T = 328.15K$ |
|------------------|--------------------------------|--------------------------------|--------------------------------|
| $T$              | $W_2$ | $W_1$ | $W_2$ | $W_1$ | $W_2$ | $W_1$ | $W_2$ | $W_1$ | $W_2$ | $W_1$ |
| 308.15K          | 0.333 | 48.25 | 0.96  | 30.49 | 5.06  | 26.14 | 7.04  | 21.16 | 9.91  | 16.05 | 13.62 |
| 318.15K          | 0.333 | 43.37 | 1.65  | 30.36 | 5.10  | 25.98 | 7.11  | 21.09 | 10.01 | 15.97 | 13.78 |
| 328.15K          | 0.333 | 47.94 | 2.12  | 26.00 | 7.69  | 14.22 | 13.32 | 10.15 | 15.42 | 7.64  | 16.92 |
In order to obtain a more accurate fitting result, we use another two nonlinear empirical equations to correlate the experimental binodal data [26, 27]:

\[
\begin{align*}
\text{Equation (4)} & : \\
& w_1 = a_1 \exp\left(\frac{w_1}{b_1}\right) + a_2 \exp\left(\frac{w_1}{b_2}\right) + c, \\
& \text{Equation (5)} & : \\
& w_1 = a \exp\left(\frac{w_1}{b}\right) + bw_0^{0.5} + cw + dw_2^2, \\
& \text{Equation (6)} & : \\
& w_1 = \exp\left(\frac{w_1}{b}\right) + \exp\left(\frac{w_2}{b}\right) + c,
\end{align*}
\]

where \(w_1\) and \(w_2\) are the mass fractions of the alcohol and the salt, respectively. The fitting parameters \(a, b, c, d\) along with the corresponding standard deviations (sd\(^a\)) and correlation coefficient \(R^2\) are listed in Tables 4 and 5.

In order to obtain a more accurate fitting result, we use another two nonlinear empirical equations to correlate the experimental binodal data [26, 27]:

\[
\begin{align*}
\text{Equation (4)} & : \\
& w_1 = a_1 \exp\left(\frac{w_1}{b_1}\right) + a_2 \exp\left(\frac{w_1}{b_2}\right) + c, \\
& \text{Equation (5)} & : \\
& w_1 = a \exp\left(\frac{w_1}{b}\right) + bw_0^{0.5} + cw + dw_2^2, \\
& \text{Equation (6)} & : \\
& w_1 = \exp\left(\frac{w_1}{b}\right) + \exp\left(\frac{w_2}{b}\right) + c,
\end{align*}
\]

where \(w_1\) and \(w_2\) are the mass fractions of the alcohol and the salt, respectively. The fitting parameters \(a, b, c, d\) along with the corresponding standard deviations (sd\(^a\)) and correlation coefficient \(R^2\) are listed in Tables 4 and 5.

Equations (4)–(6) have been successfully used for the correlation of binodal data of IL-based ATPSs [28–30], polymer-based ATPSs [31, 32], and hydrophilic alcohol-based ATPSs [33, 34]. Based on the obtained \(R^2\) and sd\(^a\) values in Tables 3–5, it can be concluded that equations (5)–(7) are...
perfectly suitable for correlating the binodal data of the investigated systems. Furthermore, equation (6) shows the most satisfactory accuracy among the three expressions due to its $R^2$.

### 3.1.1 Tie-Line Data and Correlation

The tie-line data of the ATPSs composed of polyethylene glycol + ethanol + Na$_2$SO$_4$ and water determined at three different temperatures and isopropyl alcohol + ethanol + Na$_2$SO$_4$/Na$_2$CO$_3$/H$_2$O system at $T = 308.15$ K are listed in Table 6.

The Setschenow-type equation [35] has been successfully used to correlate the tie-line data for polymer + salt ATPSs [36]. This equation has the following form:

$$\ln \left( \frac{c_i}{c_j} \right) = k_{MA} (c_i - c_j) + k_S (c_i^b - c_j^b).$$  \hfill (8)

In this equation, $k_S$ represents the salting-out coefficient, $c_i$ and $c_j$ are the molarities of the alcohol mixture and salts, respectively, and $k_{MA}$ is a parameter relating the activity coefficient of the alcohol mixture to its concentration. The superscripts “$i$” and “$j$” represent the top phase and the bottom phase, respectively. The fitting parameters of the equation, the corresponding correlation coefficient values ($R^2$), and the standard deviations (sda) are provided in Table 6.

As we can see from Table 6, the Setschenow-type equation can be satisfactorily applied to the correlation of tie-line data for these systems.

A relatively simple equation was also used to correlate the tie-line data:

$$\ln \left( \frac{w_i}{w_j} \right) = \beta + k (w_i^b - w_i^t),$$  \hfill (9)

where $k$ signifies the salting-out coefficient and $\beta$ represents a constant related to the activity coefficient. The superscripts “$t$” and “$b$” represent the top phase and the bottom phase, respectively. The fitting parameters of the equation, the corresponding correlation coefficient values ($R^2$), and the standard deviations (sda) are provided in Table 8.

This equation was successfully used to correlate the tie-line data for polymer-salt ATPSs [37]. This expression shows strong agreement when correlated with the experiment tie-line data. It can be seen from Tables 7 and 8 that equation (9) has a better accuracy for the investigated system. Moreover, the results show good reliability of the experimental tie-line data and calculation methods used.

### 3.1.2 Effect of Temperature on Binodal Curves

It has been reported that temperature plays a positive role in forming the ATPS consisting of 2-propanol, NaOH, and water [16]. Figure 1 shows the binodal curves for the systems consisting of polyethylene glycol + ethanol + Na$_2$SO$_4$ + water at $T = 308.15$, 318.15, and 328.15 K to discuss the influence of temperature on the binodal curves. It shows that temperature has little influence on the phase equilibrium compositions, so there is no significant change in the slope of the binodal curves with increased temperature. This situation mainly occurs because when the salt concentration in the system with the alcohol mixture of (MA$_1$ = 0.200), Na$_2$SO$_4$, and the water is less than 25% by mass fraction, the system
will be less sensitive to changes in temperature, and the liquid-liquid equilibrium condition will be maintained.

### Table 6: Tie-line data for the mixture of polyethylene glycol/isopropyl alcohol + ethanol + salt (Na₂CO₃/Na₂SO₄) + water ATPSs at different temperatures.

| T (K) | Total system | Top phase | Bottom phase | TLL | S |
|-------|--------------|-----------|--------------|-----|---|
|       | 100W₁ | 100W₂ | 100W₁ | 100W₂ | 100W₁ | 100W₂ | 100W₁ | 100W₂ | 100W₁ | 100W₂ |
| Polyethylene glycol + ethanol + Na₂SO₄ + H₂O system | | | | | | | | | | |
| 308.15 | 19.53 | 11.57 | 27.35 | 6.65 | 4.01 | 21.33 | 18.33 | −1.59 |
|       | 14.00 | 14.18 | 23.54 | 8.18 | 5.78 | 19.35 | 19.71 | −1.91 |
|       | 15.00 | 13.85 | 24.99 | 7.57 | 5.06 | 20.11 | 11.75 | −1.59 |
|       | 16.49 | 12.79 | 24.41 | 7.81 | 5.34 | 19.80 | 13.17 | −1.59 |
|       | 17.98 | 12.16 | 25.78 | 7.25 | 4.69 | 20.52 | 15.70 | −1.59 |
| 318.15 | 20.89 | 11.94 | 31.17 | 5.79 | 2.27 | 23.07 | 21.70 | −1.67 |
|       | 17.20 | 12.76 | 25.32 | 7.90 | 4.04 | 20.64 | 15.34 | −1.67 |
|       | 18.79 | 12.22 | 27.26 | 7.15 | 3.36 | 21.46 | 17.99 | −1.67 |
|       | 16.54 | 12.91 | 26.04 | 8.42 | 4.53 | 21.80 | 16.43 | −1.67 |
|       | 18.00 | 12.36 | 25.70 | 7.75 | 3.90 | 20.80 | 16.43 | −1.67 |
| 328.15 | 27.84 | 7.34 | 31.83 | 5.73 | 3.84 | 20.35 | 28.09 | −1.91 |
|       | 26.25 | 8.39 | 30.50 | 6.16 | 4.27 | 19.89 | 24.81 | −1.91 |
|       | 27.34 | 12.44 | 26.72 | 7.53 | 5.80 | 18.48 | 13.03 | −1.91 |
|       | 22.11 | 10.17 | 27.84 | 6.22 | 6.11 | 19.05 | 15.34 | −1.91 |
|       | 16.50 | 13.60 | 28.51 | 6.90 | 5.07 | 19.12 | 12.12 | −1.91 |

### Table 7: Parameters of equation (8) for the mixture of polyethylene glycol/isopropyl alcohol + ethanol + salt (Na₂CO₃/Na₂SO₄) + water ATPSs at different temperatures.

| T (K) | kᵣ | Intercept | R² | δ (kᵣ)ᵇ | δ (intercept)ᶜ | sdᵇ |
|-------|-----|-----------|----|---------|---------------|-----|
| Polyethylene glycol + ethanol + Na₂SO₄ | | | | | | |
| 308.15 | 1568.3615 | 0.1233 | 0.9860 | 93.3915 | 0.0660 | 0.0032 |
| 318.15 | 3330.5112 | −0.4158 | 0.9908 | 159.8895 | 0.1000 | 0.0033 |
| 328.15 | 1312.4646 | 0.3960 | 0.9918 | 59.5733 | 0.0514 | 0.0523 |
| Isopropyl alcohol + ethanol + Na₂SO₄ | | | | | | |
| 308.15 | 697.6802 | 0.0941 | 0.9976 | 23.9236 | 0.0242 | 0.0510 |

### 3.1.3. Effect of the Mass Composition of the Alcohol on Binodal Curves

Figure 2 shows the binodal curves for the systems consisting of polyethylene glycol + ethanol + Na₂SO₄ + water at T = 308.15 K and the systems consisting of isopropyl alcohol + ethanol + Na₂SO₄ + water at T = 308.15 K to discuss the influence of the mass composition of the alcohol on binodal curves. It shows that mass composition of the alcohol can affect the phase equilibrium composition, so there are some changes in the slope of the binodal curves with the change of the mass composition of the alcohol. The change can be described as follows: when the salt concentration is greater than 7.3% by mass fraction, the biphasic region of the polyethylene glycol + ethanol + Na₂SO₄ + water system is larger than that of
isopropyl alcohol + ethanol + Na$_2$SO$_4$ + water system. Hence, the salting-out ability of the former system is greater than the latter. When the salt composition is lower than 7.3% by mass fraction, the biphasic region of the isopropyl alcohol + ethanol + Na$_2$SO$_4$ + water system is larger than that of polyethylene glycol + ethanol + Na$_2$SO$_4$ + water system. Hence, the salting-out ability of the latter is greater than the former.

Furthermore, the binodal curves of (polyethylene glycol + ethanol)/polyethylene glycol 1500 [38]/polyethylene glycol 4000/polyethylene glycol 8000 [39] + Na$_2$SO$_4$ + water systems were shown in Figure S1 (in Supplementary Materials). It can be seen from the figure that the ATPSs containing polyethylene glycol 1500, polyethylene glycol 4000, and polyethylene glycol 8000 have larger two-phase ranges, which show that the ATPS containing polyethylene glycol can more easily form two-phase system than the combination of polyethylene glycol and ethanol.

3.1.4. Effect of Salt on Binodal Curves. System concerning isopropyl alcohol + ethanol + Na$_2$SO$_4$/Na$_2$CO$_3$ + water ATPSs at $T = 308.15, 318.15$, and $328.15$ K is also discussed in this paper. As we can see from Figure 3 the salting-out ability of system containing Na$_2$CO$_3$ is better than that containing Na$_2$SO$_4$. And the salting-out ability increased when salt composition increased. This may be because Na$_2$CO$_3$ has a smaller molecular weight than that of Na$_2$SO$_4$ in the same salt composition. Also, we can see from Table 6 that the absolute value of entropy ($S$) of Na$_2$SO$_4$ is larger than that of Na$_2$CO$_3$. The larger the absolute value of entropy ($S$), the easier to form a salting-out ability.

3.1.5. Effect of Temperature on Tie-Line. Figure 4 shows the tie-line for the systems consisting of polyethylene glycol + ethanol + Na$_2$SO$_4$ + water at $T = 308.15, 318.15$, and $328.15$ K to discuss the influence of temperature on the tie-line. It can be seen from the figure that the tie-lines at the three temperatures are almost parallel and it demonstrates that temperature has no effect on tie-line. And this result is similar to the effect of temperature on binodal curves.

3.1.6. Effect of Salt on Tie-Line. System concerning isopropyl alcohol + ethanol + Na$_2$SO$_4$/Na$_2$CO$_3$ + water ATPSs at $T = 318.15$ K is also discussed in this paper. As we can see from Figure 5, the salting-out ability of system containing Na$_2$CO$_3$ is better than that containing Na$_2$SO$_4$. The salting-out ability increased when salt composition increased. Also, the trend is similar to the effect of salt on binodal curves.

3.2. Separation Behaviors of B$_6$ in ILATPS. According to what has been discussed above, ATPS (isopropyl alcohol + ethanol + Na$_2$CO$_3$ + H$_2$O), with a better ability of
3.2.1. Effect of the Salts on Extraction Efficiency. The discussions above show that Na₂CO₃ has a better salting-out ability than Na₂SO₄. Experiments have been done to find out what salt can do to affect the extraction efficiency. Different salts (Na₂CO₃, Na₂SO₄, and NaH₂PO₃) with the same weight were added into three centrifuge tubes containing the same system (isopropyl alcohol + ethanol + H₂O) individually. Then 2 g of the standard working B₆ was added into the centrifuge tubes. The mixtures were then stirred thoroughly at T = 308.15 K. An 802 centrifuge was used at 2000 rpm for 25 min to ensure phase separation. To finish these, the centrifuge tubes were then placed in a thermostatic water bath at the setpoint temperature of T = 308.15 K for 12 h to reach phase equilibrium. And the HPLC system was used for analysis of these systems. The result is shown in Table 9; we can read from the table that the extraction efficiency of Na₂CO₃ is 84.24% and it is the largest among the three ATPSs. It is perfectly similar to the study we have discussed earlier.

3.2.2. Effect of Temperature on the Extraction Efficiency. Temperature is always a factor that affects chemical reactions, so it can affect the extraction efficiency. Effect of temperature on the ATPS (isopropyl alcohol + ethanol + Na₂SO₄ + Na₂CO₃ + H₂O) was also investigated in our study. In the beginning, several ATPSs (isopropyl alcohol + ethanol + Na₂CO₃ + H₂O) of identical component contents were formed by mixing four components in centrifuge tubes; then 2 g of the standard working B₆ was added into the centrifuge tubes. The mixtures were then stirred thoroughly at T = 308.15 K. An 802 centrifuge was used at 2000 rpm for 25 min to ensure phase separation. To finish these, the centrifuge tubes were then placed in a thermostatic water bath at six different setpoint temperatures (303.15, 308.15, 313.15, 318.15, 323.15, and 328.15 K) for 12 h to reach phase equilibrium. The top phase was analyzed using the HPLC method finally. The results are shown in Figure 7; we can see that temperature did affect the extraction efficiency: the extraction efficiency initially increased and then decreased during the increase in temperature. The maximum value of extraction efficiency reached 83.01% when temperature is 308.15 K.

3.2.3. Effect of pH on the Extraction Efficiency. pH can not only affect the phase separation but also be a factor that acts on extraction efficiency. So, the extraction of B₆ was studied.
in different pH range from 3.25 to 7.47. As shown in Figure 8, the extraction efficiency initially increased and then decreased during the increase of pH. The maximum value of extraction efficiency reached 89.32% when pH was 5.02.

4. Conclusions

Liquid-liquid equilibrium data have been determined for polyethylene glycol + ethanol + Na₂SO₄ + water ATPSs at \( T = 308.15 \), 318.15, and 328.15 K and isopropyl alcohol + ethanol + Na₂SO₄/Na₂CO₃ + water ATPSs at \( T = 308.15 \) K. The Merchuk equation and other equations were successfully used to correlate binodal data, and the tie-lines were described by the Setschenow-type equation and a two-parameter equation. The effect of temperature on the binodal curves of the investigated systems is that the temperature did not affect the separated two-phase area. Salting-out ability can be ranked as Na₂SO₄ < Na₂CO₃ at the same temperature, and the phase separation ability of the alcohol mixture was that when the salt concentration is higher than 7.3% by mass fraction, the salting-out ability of the polyethylene glycol + ethanol + Na₂SO₄ + water ATPSs system at \( T = 308.15 \) K is greater than isopropyl alcohol + ethanol + Na₂SO₄ + water ATPSs at \( T = 308.15 \) K, and when the salt composition is lower than 7.3% by mass fraction, the salting-out ability of isopropyl alcohol + ethanol + Na₂SO₄ + water ATPSs system at

### Table 9: The date of extraction efficiency on salts.

| Salt       | Na₂CO₃ | Na₂SO₄ | NaH₂PO₃ |
|------------|--------|--------|--------|
| Extraction efficiency (%) | 84.24  | 81.77  | 75.98  |

![Figure 6: HPLC by UV detection of real samples: left, deionized water sample after ATPS extraction; right, deionized water sample added with 50 mg/mL vitamin B₆ after ATPS extraction.](image)

![Figure 7: Effect of temperature on the extraction efficiency.](image)

![Figure 8: Effect of pH on the extraction efficiency.](image)
$T = 318.15 \text{ K}$ is greater than the other. As a viable pre-treatment technique for complicated samples, ATPS, combined with a HPLC method, has been successfully used to quantitatively determine vitamin $B_6$. The optimum working conditions were considered for an aqueous two-phase system composed of isopropyl alcohol + ethanol + $\text{Na}_2\text{CO}_3$ operating at $T = 308.15 \text{ K}$ and at a pH of 5.02.

**Data Availability**

The data supporting the conclusions of this work are included in this manuscript. Other datasets generated and analyzed during the current work are available from the corresponding author on reasonable request.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (21906039), Natural Science Foundation of Shaanxi Province (2019Q-382), Funding Project for Introduced Overseas Scholars of Hebei Province (C20190321), Fundamental Research Funds for the Central Universities of Chang’an University (300102299505 and 300102290501), Program for Water Resources Research and Promotion of Hebei Province (2019-55), and Doctoral Research Fund of Hebei GEO University (BQ2019041).

**Supplementary Materials**

Table S1: values of parameters of equation (1): the mixture of polyethylene glycol/isopropyl alcohol + ethanol + salt ($\text{Na}_2\text{CO}_3/\text{Na}_2\text{SO}_4$) + water ATPSs at different temperatures. Figure S1: the binodal curves of alcohol + $\text{Na}_2\text{SO}_4$ + water systems: polyethylene glycol 1000 + ethanol + $\text{Na}_2\text{SO}_4$ + water at $T = 308.15 \text{ K}$; polyethylene glycol 1500 + $\text{Na}_2\text{SO}_4$ + water at $T = 313.15 \text{ K}$ (ref. 39); polyethylene glycol 4000 + $\text{Na}_2\text{SO}_4$ + water at $T = 308.15 \text{ K}$ (ref. 40); polyethylene glycol 8000 + $\text{Na}_2\text{SO}_4$ + water at $T = 308.15 \text{ K}$ (ref. 39). (Supplementary Materials)

**References**

[1] D. A. Bender, *Vitamin $B_6$ Physiology, Encyclopedia of Human Nutrition* (Third Edition), pp. 340–350, Elsevier, Amsterdam, Netherlands, 2013.

[2] Y. Qi, J. Ma, X. Chen, F.-R. Xiu, Y. Chen, and Y. Lu, “Practical aptamer-based assay of heavy metal mercury ion in contaminated environmental samples: convenience and sensitivity,” *Analytical and Bioanalytical Chemistry*, vol. 412, no. 2, pp. 439–448, 2019.

[3] M. W. Beiernick, “Original mitteilung über eine eigentümlichkeit der loslichen starke,” Zentralbl. Bakteriol. Parasitenk. Infektionskrankh, vol. 122, pp. 699–701, 1896.

[4] S. Ketnawa, N. Rungraeng, and S. Rawdkuen, “Phase partitioning for enzyme separation: an overview and recent applications,” *International Food Research Journal*, vol. 24, no. 1, p. 24, 2017.

[5] H. Li, Y. Pang, X. Wang et al., “Phospholipase D encapsulated into metal-surfactant nanocapsules for enhancing biocatalysis in a two-phase system,” *RSC Advances*, vol. 9, no. 12, pp. 6548–6555, 2019.

[6] E. Lladosa, S. C. Silverio, O. Rodriguez, J. A. Teixeira, and E. A. Macedo, “(Liquid + liquid) equilibria of polymer-salt aqueous two-phase systems for laccase partitioning: UCON 50-HB-5100 with potassium citrate and (sodium or potassium) formate at 23°C,” *The Journal of Chemical Thermodynamics*, vol. 55, pp. 166–171, 2012.

[7] C. He, S. Li, H. Liu, K. Li, and F. Liu, “Extraction of testosterone and epistosterone in human urine using aqueous two-phase systems of ionic liquid and salt,” *Journal of Chromatography A*, vol. 1082, no. 2, pp. 143–149, 2005.

[8] C. Celeste, O. Aguilar, and M. Rito-Palomares, “Application of an aqueous two-phase systems strategy for the potential recovery of a recombinant protein from alfalfa (Medicago sativa),” *Separation and Purification Technology*, vol. 77, no. 1, pp. 94–98, 2011.

[9] S. Xu, Q. Zhu, Q. Luo, and Y. Li, “Influence of ions and temperature on aqueous biphasic systems containing ionic liquid and ammonium sulfate,” *Journal of Chemical & Engineering Data*, vol. 64, no. 7, pp. 3319–3417, 2019.

[10] Y. Li, N. Zhang, S. Xu, Q. Zhu, and J. Hu, “The influence of temperature on the phase behavior of ionic liquid aqueous two-phase systems,” *Journal of Dispersion Science and Technology*, vol. 40, no. 6, pp. 874–883, 2019.

[11] Q. Wu, D.-Q. Lin, Q.-L. Zhang, D. Gao, and S.-J. Yao, “Evaluation of a PEG/hydroxypropyl starch aqueous two-phase system for the separation of monoclonal antibodies from cell culture supernatant,” *Journal of Separation Science*, vol. 37, no. 4, pp. 447–453, 2014.

[12] X. J. Xie and Z. X. Zhang, “Determination of vitamin $B_6$ by fluorimetry after aqueous two-phase extraction based on ionic liquid,” *Journal of Analytical Science*, vol. 27, pp. 513–515, 2011.

[13] R. Lertlapwasin, N. Bhawawet, A. Imyim, and S. Fuangwasi, “Ionic liquid extraction of heavy metal ions by 2-amino-thiophenol in 1-butyl-3-methylimidazolium hexafluorophosphate and their association constants,” *Separation and Purification Technology*, vol. 72, no. 1, pp. 70–76, 2010.

[14] W. Guo, J. Ma, Y. Wang, J. Han, Y. Li, and S. Song, “Liquid-liquid equilibrium of aqueous two-phase systems composed of hydrophilic alcohols ( ethanol/2-propanol/1-propanol) and MgSO$_4$/ZnSO$_4$ at (303.15 and 313.15) K and correlation,” *Thermochimica Acta*, vol. 546, pp. 8–15, 2012.

[15] Y. Li, S. Yang, W. Zhang, X. Lu, H. Yang, and J. Hu, “Phase equilibria and salt effect on the aqueous two-phase system of ethanol + 2-propanol + salt [ (NH$_4$)$_2$SO$_4$/Na$_2$SO$_4$] + water,” *Journal of Thermal Analysis and Calorimetry*, vol. 127, no. 3, pp. 2473–2487, 2017.

[16] J. Han, Y. Wu, Y. Xiang, Y. Wang, J. Ma, and Y. Hu, “Liquid-liquid equilibrium of hydrophilic alcohol + sodium hydroxide+water systems: experimental and correlation,” *Thermochimica Acta*, vol. 566, pp. 261–267, 2013.

[17] E. L Nan, G. R. Williams, H.-H. Song, J. Quan, H.-L. Nie, and L.-M. Zhu, “Liquid-liquid Solid triple-phase data for aqueous two-phase systems comprising ethanol-1-propanol-2-propanol-acetone and salts,” *Journal of Chemical & Engineering Data*, vol. 58, no. 12, pp. 3314–3319, 2013.

[18] Z. W. Bai, Y. H. Chao, M. L. Zhang et al., “Partitioning behavior of papain in ionic liquids-based aqueous two-phase systems.”
systems,” Journal of Chemistry, vol. 2013, Article ID 938154, 6 pages, 2013.

[19] H. J. Zhu, Y. Ding, and Y. W. Jia, "Hesperidin extraction from orange peel with new aqueous two-phase system,” Journal of Food Science and Biotechnology, vol. 32, pp. 995–1001, 2013.

[20] X. Liu, T. Mu, H. Sun, M. Zhang, and J. Chen, "Optimisation of aqueous two-phase extraction of anthocyanins from purple sweet potatoes by response surface methodology,” Food Chemistry, vol. 141, no. 3, pp. 3034–3041, 2013.

[21] Y. Lu, Z. J. Tan, and Y. S. Yan, "The design and implementation of the data processing system for liquid-liquid equilibrium experiments of ATPS based on VB and MATLAB,” Computer and Applied Chemistry, vol. 29, pp. 1195–1198, 2012.

[22] E. L. Nan, “Exploring the liquid/liquid/solid boundary and constructing the phase ratio database in hydrophilic organic solvent/salt aqueous two-phase systems,” MS thesis, A thesis submitted to Donghua University for the Master’s degree, Shanghai, China, 2014.

[23] J. J. Ma, "Construction of small molecular water-miscible alcohol-salt aqueous two phase systems and application on the extraction of plant pigment,” MS thesis, A Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Engineering, Bailey, TX, USA, 2013.

[24] J. Chen, S. K. Spear, J. G. Huddleston, J. D. Holbrey, R. P. Swatloski, and R. D. Rogers, "Application of poly(ethylene glycol)-based aqueous biphasic systems as reaction and reactive extraction media,” Industrial & Engineering Chemistry Research, vol. 43, no. 17, pp. 5358–5364, 2004.

[25] J. C. Merchuk, B. A. Andrews, and J. A. Asenjo, "Aqueous two-phase systems for protein separation: studies on phase inversion,” Journal of Chromatography B: Biomedical Sciences and Applications, vol. 711, no. 1–2, pp. 285–293, 1998.

[26] G. H. Jonathan, D. W. Heather, and D. R. Robin, "Phase diagram data for several PEG + salt aqueous biphasic systems at 25°C,” Journal of Chemical & Engineering Data, vol. 48, no. 5, pp. 1230–1236, 2003.

[27] M. Hu, Q. Zhai, Z. Liu, and S. Xia, "Liquid–Liquid and Solid–Liquid equilibrium of the ternary system ethanol + cesium sulfate + water at (10, 30, and 50) °C,” Journal of Chemical & Engineering Data, vol. 48, no. 6, pp. 1561–1564, 2003.

[28] F. J. Deive, M. A. Rivas, and A. Rodríguez, "Sodium carbonate as phase promoter in aqueous solutions of imidazolium and pyridinium ionic liquids,” The Journal of Chemical Thermodynamics, vol. 43, no. 8, pp. 1153–1158, 2011.

[29] Y. Deng, T. Long, D. Zhang, J. Chen, and S. Gan, "Phase diagram of [amim] Cl + salt aqueous biphasic systems and its application for [amim] Cl recovery,” Journal of Chemical & Engineering Data, vol. 54, no. 9, pp. 2470–2473, 2009.

[30] Y. Pei, J. Wang, L. Liu, K. Wu, and Y. Zhao, "Liquid–Liquid equilibrium of aqueous biphasic systems containing selected imidazolium ionic liquids and salts,” Journal of Chemical & Engineering Data, vol. 52, no. 5, pp. 2026–2031, 2007.

[31] L. A. Ferreira and J. A. Teixeira, "Salt effect on the aqueous two-phase system PEG 8000–Sodium sulfate,” Journal of Chemical & Engineering Data, vol. 56, no. 1, pp. 133–137, 2011.

[32] M. Forouatan, "Liquid–Liquid equilibria of aqueous two-phase poly(vinylpyrrolidone) and K2HPO4/KH2PO4 Buffer: effects of pH and temperature," Journal of Chemical & Engineering Data, vol. 52, no. 3, pp. 859–862, 2007.

[33] H. Shekaari, R. Sadeghi, and S. A. Jafari, "Liquid–Liquid equilibria for aliphatic alcohols + dipotassium oxalate + water,” Journal of Chemical & Engineering Data, vol. 55, no. 11, pp. 4586–4591, 2010.

[34] Y. Wang, S. Hu, J. Han, and Y. Yan, “Measurement and correlation of phase diagram data for several hydrophilic alcohol + citrate aqueous two-phase systems at 298.15 K,” Journal of Chemical & Engineering Data, vol. 55, no. 11, pp. 4574–4579, 2010.

[35] Y. L. Li, X. J. Lu, J. Hao, and C. Q. Chen, “Liquid-liquid equilibrium data for the ionic liquid N-ethyl-pyridinium bromide with several sodium salts and potassium salts,” Journal of Chemistry, vol. 2013, Article ID 857272, 11 pages, 2013.

[36] M. T. Zafarani-Moattar and S. Hamzehzadeh, "Liquid-liquid equilibria of aqueous two-phase systems containing polyethylene glycol and sodium succinate or sodium formate,” Calphad, vol. 29, no. 1, pp. 1–6, 2005.

[37] M. T. Zafarani-Moattar and P. Seifi-Aghjekohal, "Liquid-liquid equilibria of aqueous two-phase systems containing polyvinylpyrrolidone and tripotassium phosphate or dipotassium hydrogen phosphate: experiment and correlation,” Calphad, vol. 31, no. 4, pp. 553–559, 2007.

[38] Barreto and C. Rodrigues, "Liquid–liquid equilibrium data and thermodynamic modeling for aqueous two-phase system peg 1500 + sodium sulfate + water at different temperatures,” Journal of Chemical & Engineering Data, vol. 64, no. 2, pp. 810–816, 2019.

[39] M. González-Amado, E. Rodil, A. Arce, A. Soto, and O. Rodriguez, "The effect of temperature on polyethylene glycol (4000 or 8000)-(sodium or ammonium) sulfate aqueous two phase systems,” Fluid Phase Equilibria, vol. 428, pp. 95–101, 2016.