Phase diagram prediction of recycling aqueous two-phase systems formed by a light-sensitive copolymer and dextran

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Abstract−Our Laboratory has synthesized a novel light-sensitive and recycling copolymer P_{NBC}. The P_{NBC} copolymer could form recycling aqueous two-phase systems with Dextran. Recycling aqueous two-phase systems have important application in bioseparation engineering. Phase diagram prediction of the novel aqueous two-phase systems also is an important aspect in engineering. This article applied the Cabezas model and effective excluded volume (EEV) model to the experimental data of light-sensitive recycling aqueous two-phase systems. It has been found that the EEV model could consist with experimental data very well. The maximum absolute error and average absolute error is 0.81% (wt%) and 0.23% (wt%), respectively.

Key words: Aqueous Two-phase Systems, Light-sensitive Polymer, Liquid-liquid Equilibria, Thermodynamic Models

INTRODUCTION

The extraction technique of aqueous two-phase systems (ATPS) has advantages of high biocompatibility and of being easily scaled-up in industrial application. It can be used in the separation of bio-macromolecules, antibiotics, amino acids and organic acids etc. Unfortunately, ATPS still has not been applied to industry due to high cost. The key problem is that the forming phase polymer of ATPS cannot be effectively recycled.

The light-sensitive reversible dissolution-precipitation polymer P_{NBC} is a novel polymer forming ATPS developed by our laboratory [1,2]. It is copolymerized by N-isopropylacrylamide (NIPA), n-Butyl acrylate (BA) and chlorophyllin sodium copper salt (CHL). Under radiation by laser at 488 nm or filtrated light at 450 nm, this polymer would be precipitated from aqueous solution. Repeated experiments have proven that the P_{NBC} recovery is more than 97%. The new type of aqueous two-phase systems formed by P_{NBC} and traditional Dextran are TRY recycling aqueous two phase systems. Phase diagram prediction of the novel aqueous two-phase systems also is an important aspect in engineering. Currently, the prediction work of the new aqueous two-phase systems has not been performed. This article carries out the prediction of the phase diagram of ATPS (P_{NBC}-Dextran), and facilitates possible future applications.

THERMODYNAMIC MODELS

According to the classification of ATPS models by Cabezas [3], there are four types of thermodynamic models [4,5] to describe the liquid-liquid equilibrium of ATPS: 1. Osmotic viral extension theories (McMillan-Mayer model and Hill solution model); 2. Extensions of lattice theories (Flory-Huggins [6] model, UNIQUAC model and NRTL model [7]); 3. Incorporating integral model; 4. Other theories (group contribution model, effective excluded volume).

1. Cabezas Model

The Cabezas model [8] is attributed to osmotic viral theory; it was developed from the Hill solution theory. The model can be used for the calculation of ATPS composed of non-electrolyte polymers. By the chemical potential of the solution, Hill osmotic viral coefficients under isothermal and isotonic conditions can be obtained by relating the polymer molecular weight and the polymerization degree of the renormalization group theory.

As polymers in ATPS are polydisperse, the systems are a mixture of polymers with different polymerization degree. For simplifying deduction, we have a hypothesis that the ATPS is a ternary system composed of polymer-polymer-water, and the subscript 1 stands for water, subscripts 2, 3 stand for two kinds of polymers. According to thermodynamics, chemical potential in two phases meets with the following conditions in equilibrium:

$$\mu_1^T = \mu_1^B; \ \mu_2^T = \mu_2^B; \ \mu_3^T = \mu_3^B \tag{1}$$

The solution is different from the ideal solution condition in the following two aspects [9]:

(1) In a solution of polymers, the interaction between solvent molecules and polymer chains, the interaction between solvent molecules and solvent molecules, and interaction between polymer chains-polymer chains are quite different.

(2) The polymers have long molecule chains with pliancy. They have various conformations in water.

When studying the chemical potential of a solution, we should consider not only the property of one polymer itself, but also the influence of another kind of polymer.

Cabezas developed the Hill solution theory and introduced the second viral coefficients C_{22}, C_{33} and C_{23}. C_{23} is defined as the interaction coefficient of polymers 2, 3. He gave the chemical potential of the solution equation by using three Hill viral coefficients under isothermal and isotonic condition. After deduction, Cabezas obtained the statistical expression which is similar to the Edmond-Ogston Equation.

Neglecting high-order molality of solute, the chemical potentials

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of polymer 2, 3 are as follows:

$$\mu_2(T, P, m_2, m_3) - \mu_2^0(T, P, 0, 0) = RT[\ln m_2 + 2C_{22}m_2 + 2C_{23}m_3 + \cdots] \quad (2)$$

$$\mu_3(T, P, m_2, m_3) - \mu_3^0(T, P, 0, 0) = RT[\ln m_3 + 2C_{33}m_3 + 2C_{23}m_2 + \cdots] \quad (3)$$

Using the Gibbs-Duhem Equation, the chemical potential of water can be obtained:

$$\mu_1(T, P, m_2, m_3) - \mu_1^0(T, P, 0, 0)$$
$$= -RT[m_2 + m_3 + C_{22}m_2^2 + 2C_{23}m_2m_3 + C_{33}m_3^2 \cdots] \quad (4)$$

Where C_{ij} is a function of temperature and pressure. According to Wilson's renormalization group theory, we can obtain the expression of C_{ij} when considering polydispersity and neglecting polymerization effect.

The expanded form of viral expression is simple, and it contains clear physics meanings. The Hill viral coefficients can be obtained from the solution sediment equilibrium data, osmotic data, as well as the low-angle laser-light scattering (LALLS) measurements data, etc.

Cabezas Model has following characteristics [9]:

1. Apparently, the equation form is similar to Edmond-Ogston, and the equation need to be calculated strictly under isothermal and isotonic conditions.

2. It considers the relationship between the molecular weight and phase diagram.

3. It also considers polydispersity of the polymer, but neglects the polymer classification of two phases.

2. Tie-line Calculation in Cabezas Model

In ATPS phase diagram, polymers in top and bottom phases obey conservation of mass. The tie-line equation can be shown as follows:

$$m_2^0 = \frac{m_2^T - m_2^B}{m_3^T - m_3^B} m_3^0 + \frac{m_3^T m_2^B - m_3^B m_2^T}{m_3^T - m_3^B} \quad (5)$$

When Hill viral coefficients are available, this equation can be associated with the aforementioned Eqs. (2), (3), (4). If the initial m_2^0 and m_3^0 are known, then we can calculate other four parameters: m_2^T, m_2^B, m_3^T, m_3^B. The tie point values of the tie-line in the binodal curve could be calculated by using Matlab software programming.

3. Excluded Volume Theory

The excluded volume theory [10,11] is based on statistical geometry. Its hypothesis is that any molecular species in a solution is randomly distributed, and all of the molecules are geometrically saturated in the solution system which is represented by a binodal curve of the phase diagram.

This theory is derived from geometry. Bernal [12] thought the molecules in the binary phase system to be soft chains that are randomly distributed and coherently assembled. Based on this hypothesis, Yue Guan and other researchers [10] developed the parameter of "effective excluded volume" to describe the coexistence curves of polydisperse polymers in this system. The term, "effective excluded volume" (hereafter referred to as "EEV") refers to the acceptability of one polymer component by a network constructed of the other component.

Since the molecules are compactly packed in the solution, it is impossible for any significant size aperture to exist. As a result, the system does not accept other additional molecules unless the solution structure has been changed.

In ATPS, phase-forming polymers are treated as monodisperse. Here we adopted the root-mean-square-average molecular weight to represent that of polydisperse species.

From this study, it is shown that the molecular weight can significantly affect the binodal curve position in phase diagram

The ATPS model is derived in the following steps:

Suppose that the polymer i and polymer j are all randomly distributed in i-j binary-solution systems, and we use υ_i and υ_j to represent the number densities of two kinds of molecules. If the polymer i is considered as a centered system, the probability of no polymer j in the specified volume is given by applying the Poisson distribution (V_{ji}):

$$P_1(\underline{V} \geq V_{ji}) = \frac{(\upsilon_i V_{ji})^0}{0!} e^{-\upsilon_i V_{ji}} = e^{-\upsilon_i V_{ji}} \quad (6)$$

We call V_{ji} as the "effective excluded volume" in a binary i-j system, and it reflects the smallest spacing of species i which can accept an individual j molecule. The EEV is greater than the van der Waals Volume because of molecular size, shape and interaction between them.

In the same way, we calculate the probability of no species i in a j-centered specified system:

$$P_2 = e^{-\upsilon_j V_{ij}} \quad (7)$$

The whole solution system can be divided into effective available volume and effective unavailable volume. We can have the following expression:

$$e^{-\upsilon_i V_{ji}} = V_{ji} \upsilon_j \text{ (In the j-centered system)} \quad (8)$$

For ternary aqueous systems of i-j-0, the H_2O molecule occupies a certain proportion of the whole volume due to the hydrophilicity of the polymers. We use the EEV coefficient V_{ji0} to adjust the influence of water to the system, and have:

$$e^{-V_{ji0}\upsilon_i} = V_{ji0} \upsilon_j \text{ (In the j-centered system)} \quad (9)$$

Molecule number density is defined as:

$$\upsilon_s = \frac{\rho N_a w_s}{M_s} \text{ (s=i, j)} \quad (10)$$

Define EEV by: $V_{ji0}^* = \rho N_a V_{ji0} \quad (11)$

Considering the formulas (9-11), we can obtain a simple equation:

$$\ln\left(V_{ji0}^* \frac{w_j}{M_j}\right) + V_{ji0}^* \frac{w_i}{M_i} = 0 \quad (12)$$

It needs to be pointed out that only one phase can be used in Eq. (12). For instance, if w_i is the top-phase concentration of the polymer i, then w_j can only refer to the top-phase concentration of j, and vice versa.

Generally, polymers in the ATPS are polydisperse, and thus we could use the root-mean-square-average molecular weight to approximately replace the geometric average molecular weight <M>:

$$\langle M \rangle = \langle M_{rms} \rangle = \sqrt{M_w \cdot M_n} \quad (13)$$

4. Tie-line Calculation in Excluded Volume Theory

When polymer concentration in the solution is lower, Vainerman et al. [13] expanded and applied the Setschenow empirical equation

to ATPS to describe the experimental phase diagram for ATPS as the following relationship:

$$\ln w_j = -\ln\left(\frac{V_{ji0}^*}{M_j}\right) - \frac{V_{ji0}^*}{M_i} w_i \tag{14}$$

Applying this equation to the top-bottom of aqueous two-phase systems, we can obtain the equation of EEV tie-line:

$$\ln\frac{w_j^T}{w_j^B} = k_s(w_i^B - w_i^T) \tag{15}$$

Where: $k_s = \dfrac{V_{ji0}^*}{M_i}$ (16)

EXPERIMENTS

1. Materials and Instrument

UNICO UV-2000 ultraviolet spectrophotometer (UNICO Instruments Co., Ltd. Shanghai, P.R.China). Ohaus Adventurer AR2140 analytic balance instrument; Shanghai Jieman Industrial Measures Systematic Co., Ltd., P.R.China). WZZ-2SS automatic digital polarimeter; (Shanghai Accurate Scientific Instrument Co., Ltd. P.R. China). Agilent 1100 GPC system, 1100 detection differential instrument; Agilent Technologies, Inc., USA). The new-type polymer P_{NBC} synthesized and purified by our laboratory [1] (State Key laboratory of Bioreactor Engineering, East China University of Science & Technology. Shanghai, P.R.China). Dextran 20000, 40000, T40 (National Chemical Reagent Group, Shanghai, P.R.China).

2. Analysis

A polarimeter was employed to measure Dextran concentration. A spectrophotometer was used to measure P_{NBC} polymer concentration in 405 nm [2]. Gel Permeation Chromatography was used to determine Dextran molecular weight. Chromatogram Condition: TSK G3000Pw type soluble chromatogram column; (Temperature: 30 °C; Flow Phase: 0.1 mol/L NaNO$_3$; Velocity of Flow: 0.5 ml/min); Viscidity method was used to measure P_{NBC} viscosity molecular weight [14].

3. Determination of ATPS Phase Diagram

This paper use the Node Determination Method [15] to measure the phase diagram of P_{NBC}-Dextran ATPS. And the aforementioned experimental data is measured under 20.0±0.5 degrees Centigrade.

RESULTS AND DISCUSSION

There is no thermodynamics data on the new-type P_{NBC} polymer, and it is difficult to attain the Hill Viral osmotic coefficients C_{ij}. This polymer is photo/thermally sensitive, and its dissolution property changes in the measurement of low-angle laser-light scattering. Moreover, when GPC is used in the process of measuring molecular weight, the increasing temperature causes precipitation of the polymer in solution. So we could not obtain C_{ij} by low-angle laser-light scattering or GPC measurement method. In our study, we measured the P_{NBC}-Dextran phase diagram and related the data points to obtain the parameter C_{ij}. Matlab software was employed to the fit curve and parameter C_{ij} could be obtained. This method is effective and simple.

1. P_{NBC}-Dextran 20000 Systems

1-1. Prediction by Cabezas Model

When P_{NBC}-Dextran20000 ATPS reaches equilibrium, the chemical potential of the top phase is equal to the bottom phase:

$$\mu_i^T = \mu_i^B$$

We can obtain Hill viral osmotic coefficients C_{22} C_{33}, and C_{23} through matrix calculation.

$$\begin{bmatrix} 2m_2^T - 2m_2^B & 2m_3^T - 2m_3^B & 0 \\ 0 & 2m_2^T - 2m_2^B & 2m_3^T - 2m_3^B \\ (m_2^T)^2 - (m_2^B)^2 & 2m_2^T m_3^T - 2m_2^B m_3^B & (m_3^T)^2 - (m_3^B)^2 \end{bmatrix} \begin{bmatrix} C_{22} \\ C_{23} \\ C_{33} \end{bmatrix}$$

$$= \begin{bmatrix} \ln m_2^B - \ln m_2^T \\ \ln m_3^B - \ln m_3^T \\ m_2^B + m_3^B - m_2^T - m_3^T \end{bmatrix}$$

From seven experimental point data by Matlab (Version 7.1.0.246 Service Pack3), we can calculate the three parameters, C_{22} C_{33}, and C_{23}. The objective equation is:

$$OF = \sqrt{\frac{\sum_{i=1}^{r}(F_i^L - F_i^R)^2}{r}} \tag{17}$$

Where $F_i^L - F_i^R$ stands for the difference value of the left side of the equation minus the right side introducing experimental data, and r represents the total number of equations. By least square difference method, we can obtain the approximate solution: $C_{22}=-0.1253$; $C_{33}=-0.0839$; $C_{23}=0.0432$. The above Hill viral osmotic coefficients are all dimensionless. Fig. 1 shows the result of binodal curve fitting:

Applying the Cabezas Model to calculate 14 statistic samples of P_{NBC}-Dextran20000 ATPS, the result (Table 1) is as follows. Maximum absolute error: 3.66%, mean absolute error (wt%): $\overline{\Delta Dex^T}=$ 1.49%; $\overline{\Delta P_{NBC}^T}=2.90\%$; $\overline{\Delta P_{NBC}^B}=0.40\%$.

1-2. Prediction by EEV Model

According to the measurement results of P_{NBC} and Dextran20000, the root-mean-square molecule weights are: $<M_{rms}>_{PNBC}=375,000$ Da; $<M_{rms}>_{Dex20000}=18,264$ Da, respectively. By lsqcurvefit function we can obtain: $V_{ji0}^*=8.9748\times10^3$. Fig. 2 shows the result of binodal curve fitting:
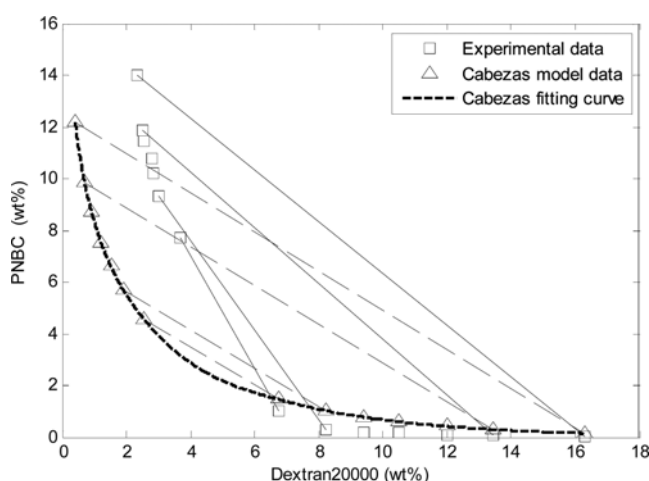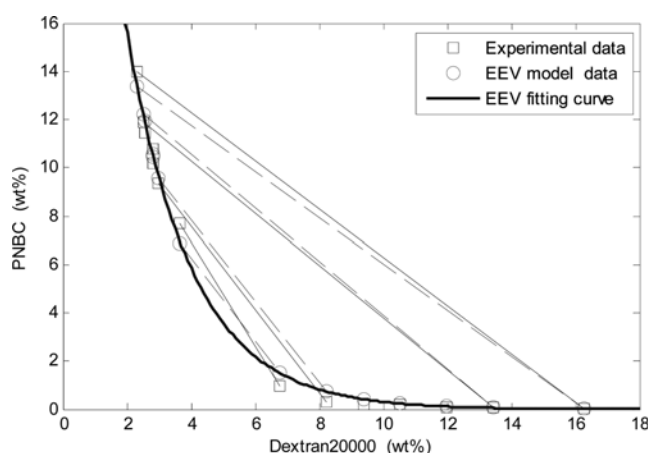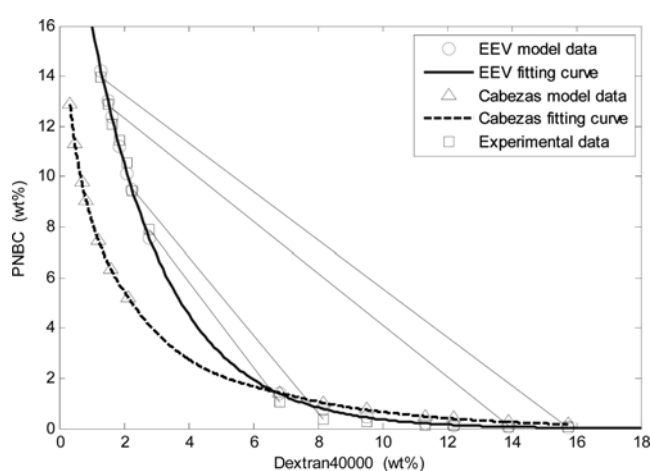

Fig. 1. Phase diagram of P_{NBC}-Dextran20000 ATPS at T=293.15 °C (predicted by cabezas model).

Table 1. Equilibrium calculation of P_{NBC}-Dextran 20000 ATPS (cabezas model) T=293.15 °C

	Top phase (wt%)					Bottom phase (wt%)					
Dex_{exp}^T	Dex_{cal}^T	ΔDex^T	P_{NBCexp}^T	P_{NBCcal}^T	ΔP_{NBC}^T	Dex_{exp}^B	Dex_{cal}^B	ΔDex^B	P_{NBCexp}^B	P_{NBCcal}^B	ΔP_{NBC}^B
2.317	0.4013	1.916	14.01	12.18	1.836	16.28	16.28	0	0.04936	0.1518	−0.1024
2.503	0.6931	1.809	11.86	9.859	2.004	13.44	13.44	0	0.07809	0.2986	−0.2205
2.545	0.9156	1.629	11.46	8.686	2.771	11.99	11.99	0	0.1039	0.4205	−0.3166
2.802	1.217	1.585	10.80	7.498	3.303	10.51	10.51	0	0.1743	0.5958	−0.4215
2.824	1.516	1.307	10.19	6.598	3.596	9.379	9.379	0	0.2037	0.7780	−0.5743
2.999	1.903	1.095	9.347	5.686	3.661	8.224	8.224	0	0.3067	1.024	−0.7171
3.667	2.557	1.109	7.706	4.547	3.159	6.760	6.760	0	0.9890	1.458	−0.4694
Δ		1.493			2.904			0			0.4031

Notes: mean absolute error $\overline{\Delta} = \dfrac{1}{n}\sum_{i=1}^{n}|c_{i,exp} - c_{i,cal}| \times 100\%$, N refers to number of calculation samples.

Fig. 2. Phase diagram of P_{NBC}-Dextran20000 ATPS at T=293.15 °C (predicted by EEV model).

Table 2. Calculation of P_{NBC}-Dextran ATPS (EEV theory) T=293.15 °C

P_{NBCexp} (wt%)	P_{NBCcal} (wt%)	ΔP_{NBC}^T (wt%)
14.011	13.3799	0.6311
11.863	12.216	−0.353
11.457	11.9641	−0.5071
10.801	10.5442	0.2568
10.194	10.4339	−0.2399
9.3472	9.5732	−0.226
7.7056	6.8948	0.8108
0.989	1.5079	−0.5189
0.3067	0.7344	−0.4277
0.20367	0.4164	−0.2127
0.17427	0.2389	−0.0647
0.10387	0.1155	−0.0117
0.07809	0.0567	0.0214
0.04936	0.0140	0.0353
Δ		0.3084

From Table 2 below, we see that the maximum absolute error of P_{NBC} is 0.81% (wt%), and mean absolute error is 0.31% (wt%).

Fig. 3. Phase diagram of P_{NBC}-Dextran40000 ATPS at T=293.15 °C (predicted by cabezas model and EEV model).

2. P_{NBC}-Dextran 40000 Systems

Applying the Cabezas Model to the calculation of 14 statistic samples of P_{NBC}-Dextran40000 ATPS, we can get Hill Viral osmotic coefficients: C_{22}=−0.1123; C_{33}=−0.0853; C_{23}=0.0478, and we can also obtain the mean absolute errors (wt%): $\overline{\Delta Dex}^T$=0.87%; $\overline{\Delta P_{NBC}^T}$= 2.32%; $\overline{\Delta P_{NBC}^B}$=0.35%. On the whole, the maximum absolute error is 3.08% (wt%), and the mean absolute error is 1.18% (wt%).

According to the measuring results, the root-mean-square molecule weight of Dextran40000 is $<M_{rms}>_{Dex40000}$=40,000 Da. By lsqcurvefit function, we can get: V_{ji0}^*=1.529×10⁴. The maximum absolute error and mean absolute error of P_{NBC} are respectively 0.45% (wt%) and 0.20% (wt%).

Fig. 3 shows the prediction results of phase diagram by Cabezas model and EEV model respectively.

3. P_{NBC}-Dextran T40 Systems

With regard to the P_{NBC}-DextranT40 Systems, we can also use the Cabezas Model to calculate the 14 statistic samples of P_{NBC}-Dextran T40 ATPS, and we have the Hill Viral osmotic coefficients as follows: C_{22}=−0.1132; C_{33}=−0.0811; C_{23}=0.0511. As for the mean absolute error (wt%), we have: $\overline{\Delta Dex}^T$=0.82%; $\overline{\Delta P_{NBC}^T}$=2.16%; $\overline{\Delta P_{NBC}^B}$=0.26%. The maximum absolute error and mean absolute error are, respectively, 2.83% (wt%) and 1.08% (wt%).
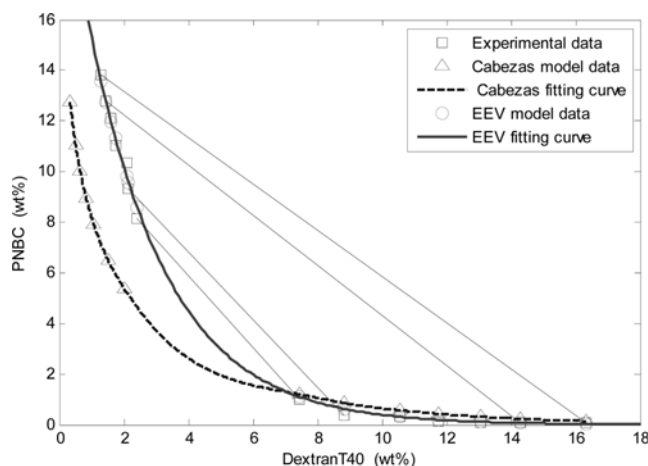
Fig. 4. Phase diagram of P$_{NBC}$-DextranT40 ATPS at T=293.15 °C (predicted by cabezas model and EEV model).

According to the measuring results, the root-mean-square mole-cule weight of DextranT40 is $<M_{rms}>_{DexT40}$=36,251 Da. Using the lsqcurvefit function, we can get the value of V_{ji0}^* as 1.6328×10^4. The maximum absolute error of P$_{NBC}$ is 0.51% (wt%), and the mean absolute error is 0.18% (wt%).

Fig. 4 shows the prediction results of phase diagram by Cabezas model and EEV model, respectively.

From the prediction result by Cabezas model, we can see that there is large deviation between the theoretical value and experiment data. One of the possible reasons is that the model is not suitable for the non-ideal solution condition. Another possible reason lies in that the minimum square method to obtain the value of Hill viral coefficients is not a good choice in this research. As a result, the C$_{ij}$ value is not accurate.

From the prediction result by EEV model, we can see the experi-mental results accord quite well with theoretical value, and the theory can be suitable for most experiments due to its wide application. P$_{NBC}$-Dextran systems complied with the principle of geometry mech-anism, and the binodal could also accord with the experimental phase diagram. Through further experiments, it can be found that the fitting effect of EEV Theory is sensitive to molecular weight of the poly-mer, which has also been reflected by its formula form.

CONCLUSIONS

The light-sensitive P$_{NBC}$-Dextran ATPS is an attempt to solve the problem of re-utilization of phase-forming polymers. It is necessary to find a model to describe the liquid-liquid equilibrium. This research applies the EEV Theory to effectively predict the phase diagram of systems. The simulation effect is satisfactory and can be tentatively applied in engineering calculations.

Besides the above-mentioned calculation of the phase diagram for the two models, we have also attained the calculation methods of tie-line for models by adding formulas (5) and (15). Up to now, our laboratory has accumulated some Matlab programs on tie-line calculation based on experimental data. With these programs, we can not only predict the polymer concentration in the top and bottom.

Recently, our laboratory synthesized another two sets of new total

recycling ATPS composed of light-sensitive [16] and pH-sensitive polymers [17]. The recovery of two formed phase polymers is over 97%. Some ideas of the study will be still applied to the new recy-cling ATPS. This study will be valuable for the future phase dia-gram prediction of ATPS.

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NOMENCLATURE

C$_{ij}$: hill osmotic viral coefficient for i and j
T : system temperature [K]
P : system pressure [Pa]
R : gas constant
m$_i$: dimensionless molality of component i [mol/mol]
V$_{ji}$: the effective excluded volume of molecular j in a real i-j binary systems
P$_1$: the probability of there being no species j in an arbitrarily located volume V$_{ji}$
P$_2$: the probability of there being no species i in an arbitrarily located volume V$_{ij}$
N$_a$: Avogadro's constant
w$_s$: weight percent concentration of component s [g/g]
V$_{ji0}^*$: the scaled effective excluded volume
$<M_{rms}>$: root-mean-square-average of the number- and weight-aver-age molecular weights [Da]
M : molecular weight of polymer [Da]
M$_w$: weight average molecular weight [Da]
M$_n$: number average molecular weight [Da]
k$_s$: setschenow coefficient

Greek Letters

μ_i : chemical potential of component i [J/mol]
μ_i^0 : standard chemical potential of component i [J/mol]
υ_s : number density of molecular s
ρ : solution density, g/ml
Δ : mean absolute deviation

Superscripts

B : bottom phase
T : top phase
L : left
R : right

Subscripts

1 : water
2 : polymer
3 : polymer

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