Model Simulation of BSA Partitioning in Recycling Aqueous Two-Phase Systems Composed of Light-Sensitive Polymer P_{NBC}-Dextran

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Aqueous two-phase systems (ATPS) are potential industrial techniques in the separation process of biomacromolecules, antibiotics, amino acids, and organic acids, etc. Partition behavior prediction of biomolecules in ATPS is an important problem. This paper applies the empirical protein partition model and the Florry−Huggins partition model to the experimental data of light-sensitive polymer P_{NBC}-dextran ATPS developed by our laboratory. It has been found that the Flory−Huggins partition model could comply with the experimental results, with an average relative error of 1.82 % and maximum relative error of 3.03 %.

1. Introduction

Aqueous two-phase systems (ATPS) have the advantages of high biocompatibility and easy scale-up in industrial applications. Unfortunately, they have still not been applied by industry due to high cost. The key problem is that the forming phase polymer of ATPS could not be effectively recycled. The light-sensitive reversible dissolution−precipitation polymer P_{NBC} is a novel polymer forming ATPS developed by our laboratory.[1,2] This polymer is precipitated from aqueous solution under laser radiation at 488 nm or filtrated light at 450 nm. The new ATPS formed by P_{NBC} and traditional Dextran will hopefully initiate the recycle of the forming phase polymer.

Another technical problem is that the protein partition in ATPS cannot be represented by proper models very well, mainly due to the reason that most of them are derived with strict thermodynamic premises. To investigate protein partition behavior in this new P_{NBC}-dextran ATPS, the article is focused on the prediction of the partitioning coefficients of bovine serum albumin (BSA) in P_{NBC}-dextran ATPS, and this work may also provide convenience for potential applications.

2. Thermodynamic Models

Proteins and other macromolecules obey the Nernst Partitioning Rules[3] in ATPS, and that can be described as

$$K_p = \frac{m^T}{m^B} \tag{1}$$

where m^T and m^B stand for the solute concentration of the top and bottom phases, respectively (superscript B stands for the "Bottom" phase and T for the "Top" phase). According to the principle of equal chemical potential in equilibrium, partition coefficient K_p obeys the Brownstedt equation

$$\ln K_p = \frac{\Delta E}{kT} = \frac{M\lambda}{kT} \tag{2}$$

where M represents the relative weight of the molecule; λ is defined as the surface characteristic coefficient of the system; and k represents the Boltzmann constant. Since the macromolecule weight M is very large, any tiny change of λ will result in great fluctuations of K_p. λ may be influenced by temperature, pressure, phase density, and salt concentration, as well as other factors.

2.1. Empiric Protein Partition Model. Generally, the protein partition coefficient is proportional to the phase-forming polymer concentration difference of ATPS.[4] The larger the concentration difference of polymers between the top and bottom phase is, the more uneven the protein partitioning in ATPS. More proteins are biased in one of two phases.

The partition coefficient of proteins in ATPS can be expressed by the empirical formula[5,6]

$$\ln K_p = a + b \cdot \mathrm{TLL} \tag{3}$$

Here a and b are constants, and TLL represents tie-line length

$$\mathrm{TLL} = \sqrt{(m_2^T - m_2^B)^2 + (m_3^T - m_3^B)^2} \tag{4}$$

where m_2 and m_3 represent concentrations of polymer 2 and 3, respectively.

2.2. Flory−Huggins Partition Model. The Lattice theory[7] emphasizes the characteristics of macromolecule chains and can describe properties of macromolecule systems. Supposing that in an unbranched polymer each segment (for example, a polymerized component) is connected by linear sequences, then each segment occupies one site of a lattice, and the total number of lattice sites N could be described as

$$N = \sum_{i=1}^{m} M_i n_i \tag{5}$$

where n_i is the number of molecules of component i and M_i is the degree of polymerization. The volume fraction of each component Φ_i can be represented by

$$\Phi_i = \frac{M_i n_i}{N} \tag{6}$$

Flory and Huggins deduced the expression of mixed entropy, with the premise that there is no interaction effects between components and that the solution volume could not be com-

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Table 1. Partitioning Coefficient of BSA in P_{NBC}-Dextran ATPS (T = 293.15 K)

top phase (wt %)			bottom Phase (wt %)		
Dex^T	$P_{NBC}{}^T$	BSA^T	Dex^B	$P_{NBC}{}^B$	BSA^B
3.667	7.706	0.02345	6.760	0.9890	0.03847
2.999	9.347	0.02167	8.224	0.3067	0.03963
2.824	10.19	0.01773	9.379	0.2037	0.04183
2.802	10.80	0.01692	10.509	0.1743	0.04230
2.545	11.46	0.01297	11.988	0.1039	0.04367
2.503	11.86	0.01290	13.437	0.0781	0.04493

pressed. For such a system, Flory and Huggins derived the combinatorial entropy. To obtain this simple expression, Flory assumed the systems to be incompressible and thus neglected any contributions from pressure−volume work.

Flory applied solution theory to the lattice to deduce his model by using an approximate expression for the mixing energy of a polymer solution, and the effective pairwise interchange energy is defined as

$$w_{ij} = z\left[\Gamma_{ij} - \frac{1}{2}(\Gamma_{ii} + \Gamma_{jj})\right] \tag{7}$$

where the molecule interacts with z nearest neighbors, and Γ_{ij}, for instance, is the potential energy of an $i-j$ pair.

When an ATPS reaches liquid−liquid equilibrium, the repulsive interaction between two types of polymers is much greater than the mutual interaction between polymers and solvent. Through deduction we can get

$$-2w_{23} + RT\left(\frac{1}{\phi_2 M_2} + \frac{1}{\phi_3 M_3}\right) = 0 \tag{8}$$

With this formula, we can carry out the calculation of phase equilibrium. Here, subscripts 2 and 3 stand for polymer 2 and polymer 3, respectively.

When the proteins are distributed in the ATPS, a thermodynamically stable quaternary two-phase system will be formed, and we can have the following expression with regard to the infinite diluted solution free of electricity

$$\ln K_p = \ln\left(\frac{\phi_p^T}{\phi_p^B}\right) = \frac{1}{RT}[(\mu_p^{ex})^B - (\mu_p^{ex})^T] \tag{9}$$

where R is the gas constant and μ_p^{ex} is the excess chemical potential of the protein. Considering the entropic and enthalpic contribution to the partition coefficient, then the equation could be modified to

$$\ln K_p = \frac{M_p}{\rho}\left(\frac{n^T}{V^T} - \frac{n^B}{V^B}\right) - \frac{M_p}{RT}[(w_p^T - E^T) - (w_p^B - E^B)] \tag{10}$$

where n^T and n^B are the total number of molecules in the top and bottom phase, respectively. ρ is the number of lattice sites per unit volume, and V^T is the volume of the top phase. Both w and E are energies. The first term represents the entropic contribution to the partition coefficient, and the latter one the enthalpic contribution to the partition coefficient.

3. Experimental Part

3.1. Materials. The polymer P_{NBC} was synthesized and purified by our laboratory.[1] Dextran 20000 and BSA were purchased from the National Chemical Reagent Group, Shanghai, P.R. China.

3.2. Instruments and Analysis. The concentration of dextran was determined by a WZZ-2SS automatic digital polarimeter (Shanghai Accurate Scientific Instrument Co., Ltd., P.R. China).

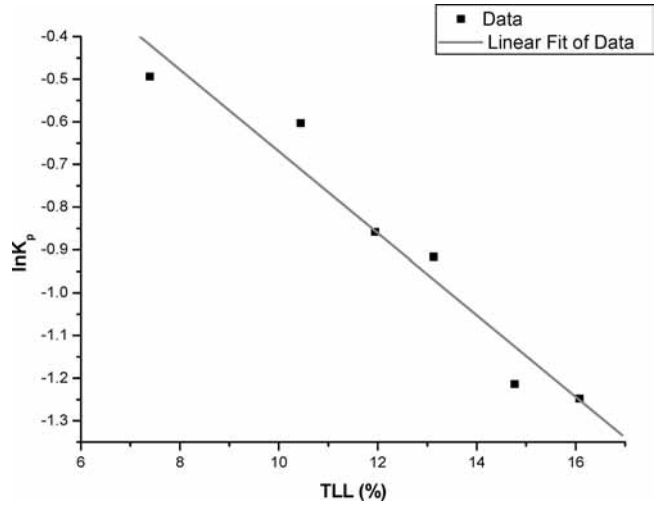

Figure 1. Fitting in empirical partitioning model (T = 293.15 K).

The concentration of the P_{NBC} polymer was determined by using a UNICO UV-2000 ultraviolet spectrophotometer (UNICO Instruments Co., Ltd., Shanghai, P.R. China) at 405 nm.[2] All of the materials were weighed by an Ohaus Adventurer AR2140 analytic balance instrument (Shanghai Jieman Industrial Measures Systematic Co., Ltd., P.R. China).

The dextran molecular weight was determined by an Agilent 1100 gel permeation chromatograph (Agilent Technologies, Inc. USA), and the chromatograph conditions were: TSK G3000Pw type soluble chromatography column (temperature: 30 °C; flow phase: 0.1 mol·L^{-1} $NaNO_3$; velocity of flow: 0.5 mL·min^{-1}). A viscosity test was used to measure P_{NBC} viscosity molecular weight.[8] Coomassie brilliant blue G250 was used to measure the standard BSA concentration at 595 nm.

3.3. Determination of the ATPS Phase Diagram. We used the node determination method[9] to measure the phase diagram and the BSA partitioning coefficient of P_{NBC}-dextran ATPS (Table 1). The experimental data were measured at (20.0 ± 0.5) °C.

4. Results and Discussion

4.1. Prediction by the Empirical Partitioning Model. By calculating the thermodynamic data in Table 1 and applying the Origin software for linear fitting, we can get the empirical formula

$$\ln K_p = 0.28827 - 0.09576 \cdot \mathrm{TLL}$$

The correlation coefficient $R^2 = 0.941$. From Figure 1, we can see that the slope of the line is negative, which implies that the value of K_p decreases and the protein is more prone to bias in the bottom phase with the increase of the polymer concentrations in the ATPS within the experimental range.

From Figure 1, we can see that $\ln K_p$ is basically linearly related to TLL. To obtain quantitative evaluation, we compared

Table 2. BSA Partitioning Coefficient Calculation by the Empirical Partitioning Model (T = 293.15 K)

$\ln K_p^{exp}$	TLL (wt %)	$\ln K_p^{cal}$	relative error of K_p (%)
−0.494	7.395	−0.420	7.70
−0.603	10.442	−0.712	10.24
−0.858	11.949	−0.856	0.23
−0.916	13.127	−0.969	5.25
−1.214	14.767	−1.125	9.43
−1.248	16.075	−1.251	0.35

the model calculation results with experimental data. The relative error of K_p is shown in Table 2.

The calculation formula of the K_p relative error is: δ % = $|(K_p^{exp} - K_p^{cal})/K_p^{exp}|\cdot 100$ %. Through calculation, we have obtained the detailed data for model evaluation: the average relative error is 5.52 %, and the maximum relative error is 10.24 %.

From linearity, the empirical protein partitioning model could be used to approximate the BSA partitioning in P_{NBC}-dextran ATPS. With a format similar to the Nernst Law, this model is obtained from our experience, so it lacks relative theoretical foundation and could not be used to indicate the relationship between molecular weight and partition coefficients. This calculation has quantified the model error, and expects that in predicting the more complicated system without salt phases. This research could provide a basic empirical model on the relationships of polymer concentration and partition coefficients.

4.2. Prediction by the Flory−Huggins Partitioning Model. Through measurement, we get: $\rho^T = 0.9977$ g·mL^{-1} and $\rho^B = 1.0256$ g·mL^{-1}.

Since the enthalpic contribution to the partition coefficient is not obvious, we could suppose it is constant. From eq 10 we can get

$$\ln K_p = \frac{M_p}{\rho}\left[\frac{n^T}{\left(\frac{m^T}{\rho^T}\right)} - \frac{n^B}{\left(\frac{m^B}{\rho^B}\right)}\right] - \text{constant}$$

$$= \frac{M_p}{\rho}[c^T\cdot\rho^T - c^B\cdot\rho^B] - \text{constant}$$

Here, the unit of concentrations, c^T and c^B, are "mol·g^{-1}".

Introducing the experimental data and applying the Origin Software, we can get Figure 2 and the linear formula as follows

$$\ln K_p = 0.25284 - 4509.3\cdot(c_p^T\cdot\rho^T - c_p^B\cdot\rho^B)$$

The correlation coefficient R^2 = 0.994. Here, the intercept stands for enthalpic contribution, and the slope stands for term of M_p/ρ. The relative error of K_p is shown in Table 3.

Through calculation of the Flory−Huggins partitioning model, we can see from Table 3 that the calculation and experimental data average relative error is 1.82 %, and the maximum average relative error is 3.03 %. The theoretical and calculated results accord with each other satisfactorily. After linear process, the linear relationships between density and partition coefficients is very obvious, with R^2 reaching as high as 0.994. We can say that the Flory−Huggins partition model can properly represent the partitioning behavior of protein in P_{NBC}-dextran ATPS.

5. Conclusions

After we solved the recycling problem of the light-sensitive P_{NBC}-dextran ATPS, a model was selected to represent the liquid−liquid equilibrium behavior for potential industrial application. In this experiment, we have focused on the thermodynamics of the ATPS and have conducted a calculation for the protein partition model. We expect this research will be interesting for the selection of a protein partition model and for the programming calculation of protein partitioning in similar ATPS in the future.

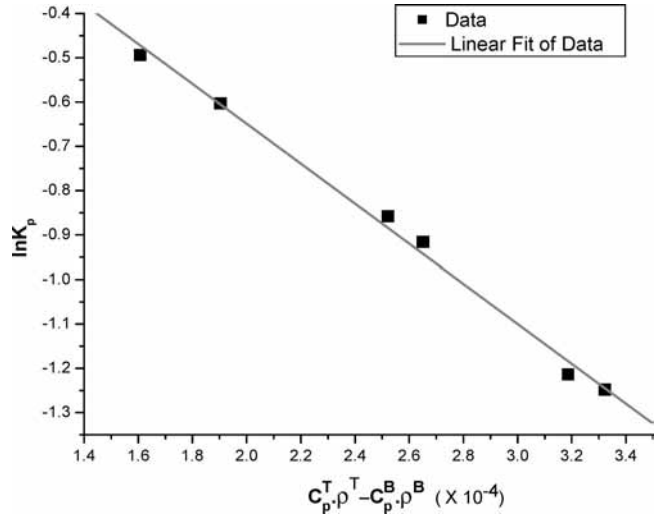

Figure 2. Fitting curve in the Flory−Huggins partitioning model (T = 293.15 K).

Table 3. BSA Partitioning Coefficient Calculation by the Flory−Huggins Partitioning Model (T = 293.15 K)

$\ln K_p^{exp}$	$\ln K_p^{cal}$	relative error of Kp (%)
−0.494	−0.472	2.29
−0.603	−0.605	0.18
−0.858	−0.884	2.59
−0.916	−0.942	2.50
−1.214	−1.184	3.03
−1.248	−1.245	0.34

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