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Thermodynamic Model for Partition Coefficients in the Two Protein Systems

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Abstract: The equation of state developed herein is predicated on a hard-sphere reference with perturbations introduced via a potential function to account for electrostatic forces and for attraction between protein particles. During this process, the generalized Lennard-Jones (GLJ) pair potential function is employed. The GLJ pair potential function is employed to represent the protein-protein interaction in two-protein systems. Via the use of the relation between the equation of state and the chemical potential, the phase behavior in the aqueous two-protein system can be estimated. The partition coefficients can be obtained via these processes. The calculated values of the coefficients agree fairly well with the experimental data in the given pH and ionic strength range, with no additional adjustable model parameters.

Keywords: phase equilibria, GLJ potential, partition coefficient, precipitation.

Introduction

Precipitation and crystallization are used to separate and purify proteins and to formulate protein pharmaceuticals, while crystallization is the first step in crystallographic analyses that are the cornerstone of structural biology. Both precipitation and crystallization are manifestations of protein solution and phase behavior, and these effects have been studied since the birth of protein physical chemistry.[1] Protein precipitation is widely used to recover and purify proteins from aqueous solutions.[2] In addition, protein crystallization has been extensively studied by biologists performing X-ray crystallography of protein crystals[3] (Rosenberger, 1996). However, phase diagrams of protein solutions remain less explored than those of solutions containing common polymers.

To understand the phase behavior of protein solutions, it is necessary to understand how solution conditions, such as pH, salt concentration, and salt type, influence protein-protein interactions. And it is useful for designing and optimizing protein purification and separation processes.[4,5] Protein-protein interactions are commonly measured by static light scattering, membrane osmometry, or sedimentation. But almost all experimental studies in the literature have focused on single-protein systems. However, for protein separations, we require extension to systems where two or more different proteins are present in solution. Salt-induced protein precipitation, a common early step in protein purification, can be modeled as a liquid-liquid phase equilibrium with a dilute-protein phase coexisting with a protein-rich phase.[6] With a statistical-mechanical theory for the fluid phase, such as the random phase approximation (RPA)[7] and a suitable potential of mean force, protein phase diagrams can be constructed.

Modeling of multi-protein systems requires not only information for protein-protein self-interactions, but also for cross protein-protein interactions. Calculations of the cross protein-protein interactions for a protein (2)-protein (3) pair provide useful data for determining optimum conditions to precipitate or crystallize a target protein from an aqueous protein mixture. Data for aqueous protein (2)-protein (3) interactions are scarce because it is difficult to make such measurements using static light scattering or membrane osmometry. The cross protein-protein interactions for unlike protein pairs cannot be measured directly using static light scattering or membrane osmometry.

Coen[8] and Coen *et al.*[9] have obtained limited experimental data for the precipitation of lysozyme and ovalbumin in one-protein systems with ammonium sulfate over a wide range of pH and high ionic strength. Only a few data were obtained for the phase behavior of these proteins together in an aqueous two-protein system at similar pH and ionic strength. In this work we present a two-component equation of state describing liquid/liquid equilibria in aqueous two-protein systems, then compared the obtained values of the partition coefficients for two-protein systems with experimental data.

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Model Description

Equation of State. The van der Waals-type equation of state has the general form of:

$$\frac{P}{\rho kT} = \left(\frac{P}{\rho kT}\right)_{ref} + \left(\frac{P}{\rho kT}\right)_{pert} \tag{1}$$

Here taken the reference term which is the proposed model by Kim *et al.*[10]

$$\left(\frac{P}{\rho kT}\right)_{ref} = 1 + \rho\sum_{ij}^{m} x_i x_j r_i r_j b_{ij} g_{ij}(d_{ij}^{+}) - \sum_{i}^{m} x_i(r_i - 1)\rho\frac{\partial \ln g_{ij}(d_{ij}^{+})}{\partial\rho} \tag{2}$$

where P is the pressure, $\rho = N/V$ is the number density (N is the number of molecules and V is the volume), and k is the Boltzmann constant, $x_i = N_i/N$ is the mole fraction of molecules, r_i is originally the number of segment of component i but here we suppose the number is one, and $g_{ij}(d_{ij}^{+})$ is the pair radial distribution function of hard-sphere mixtures at contact which takes the form given by Boublik-Mansoori-Carnahan-Starling (BMCS) equation[11]:

$$g_{ij}(\eta, \xi_{ij}) = \frac{1}{1 - \eta} + \frac{3}{2}\frac{\xi_{ij}}{(1 - \eta)^2} + \frac{1}{2}\frac{\xi_{ij}^2}{(1 - \eta)^3} \tag{3}$$

where η and ξ_{ij} given by:

$$\eta = \frac{\rho}{4}\sum_{i}^{m} x_i r_i b_i \tag{4}$$

$$\xi_{ij} = \left(\frac{b_i b_j}{b_{ij}}\right)^{1/3}\frac{\rho}{4}\sum_{k}^{m} x_k r_k b_k^{2/3} \tag{5}$$

For a one-component system and for mixtures of equal size, $\xi_{ij} = \eta$ and eq. (3) reduces to the Carnahan-Starling equation for hard-spheres.[12]

The additivity of hard-sphere diameters allows the expression for $b_{ij}(T)$ as follows

$$d_{ij}(T) = \frac{1}{2}[d_i(T) + d_j(T)] \tag{6}$$

$$b_{ij}(T) = \frac{2\pi}{3}d_{ij}^3(T) = \frac{1}{8}(b_i^{1/3} + b_j^{1/3})^3 \tag{7}$$

$$b_i(T) = \frac{2\pi}{3}\sigma_i^3 \tag{8}$$

And size parameter σ_{ij} can be determined by

$$\sigma_{ij} = \frac{\sigma_{ii} + \sigma_{jj}}{2} \tag{9}$$

To transform the number basis to segment basis equations, more useful variables should be introduced for polymer mixtures. These variables are segment density $\rho_r = N_r/V$, and segment fraction $\phi_i = N_i r_i/V$, where N_r is the total number of segments in the system with volume V.

$$N_r = \sum_{i}^{m} N_i r_i \tag{10}$$

And the perturbation term can be expressed by,

$$\left(\frac{P}{\rho kT}\right)_{pert} = -\frac{\rho_p U}{2kT} \tag{11}$$

where, ρ_p is the protein number density, which is expressed $\rho_p = 6\eta/\pi\sigma_p^3$ with the protein diameter σ_p, and U is the perturbation energy per unit density, which can be obtained from the following relationship.

$$U = 4\pi\int W(r) r^2 dr \tag{12}$$

We assume the potential function $W(r)$ is the sum of the GLJ potential[13] and the electrostatic potential.

$$W(r) = W_{GLJ}(r) + W_{elec}(r) \tag{13}$$

The GLJ potential can be expressed by,

$$W_{GLJ}(r) = \begin{cases} \infty & (0 < r < \sigma) \\ -\varepsilon & (\sigma < r < 2\sigma/A(\tilde{T})) \\ \dfrac{\varepsilon}{1 - A(\tilde{T})\left(\dfrac{r}{\sigma}\right)} & (2\sigma/A(\tilde{T}) < r < \lambda\sigma) \\ 0 & (r > \lambda\sigma) \end{cases} \tag{14}$$

where r is the center-to-center distance between two adjacent molecules, σ is the collision diameter, ε is the minimum potential energy, $\tilde{T} = kT/\varepsilon$ is the reduced temperature with Boltzman constant k, and $A(\tilde{T})$ is the temperature-dependent parameter,

$$A(\tilde{T}) = \frac{1.761 - 1.579\tilde{T}}{1 - \tilde{T}} \tag{15}$$

And the electrostatic potential can be expressed by,

$$W_{elec}(r) = \frac{z_1 z_2 e^2 \exp[-\kappa(r - \sigma_{23})]}{4\pi\varepsilon_0\varepsilon_r(1 + \kappa\sigma_{23}/2)^2} \quad (r > \sigma_{23}) \tag{16}$$

Where z is the valence of the protein, e is the unit of electron charge, $4\pi\varepsilon_0$ is the dielectric permittivity of free space, σ is the hard-sphere diameter, and ε_r is the relative dielectric permittivity of water. κ is the inverse of the Debye length; given by $\kappa^2 = (2e^2 N_A I)/(kT\varepsilon_0\varepsilon_r)$, then substitute these two potential functions to eq. (11), the result is

$$\left(\frac{P}{\rho kT}\right)_{pert} = -\frac{\rho_p U}{2kT} = -\frac{\rho_p}{2kT}4\pi\int W(r) r^2 dr = -\frac{\rho_p}{2kT}4\pi\int_{\sigma}^{\lambda\sigma} W_{GLJ}(r) r^2 dr - \frac{\rho_p}{2kT}4\pi\int_{\sigma}^{\infty} W_{elec}(r) r^2 dr$$

$$= -\frac{4\pi\rho_p}{2kT}\left[\int_{\sigma}^{\frac{2\sigma}{A(\tilde{T})}}(-\varepsilon r^2)dr + \int_{\frac{2\sigma}{A(\tilde{T})}}^{\lambda\sigma}\left(\frac{\varepsilon}{1 - A(\tilde{T},\eta)\left(\frac{r}{\sigma}\right)}r^2\right)dr\right]$$

$$-\frac{4\pi\rho_p}{2kT}\int_{\sigma}^{\infty}\left\{\frac{z_1z_2e^2\exp[-\kappa(r-\sigma_{23})]}{4\pi\varepsilon_0\varepsilon_r(1+\kappa\sigma_{23}/2)^2}r^2\right\}dr$$

$$-\frac{12\eta}{\tilde{T}}\left\{-\frac{4}{3A(\tilde{T})^3}+\frac{\ln[A(\tilde{T})\lambda-1]}{A(\tilde{T})^3}+\frac{\lambda^2}{2A(\tilde{T})}\right.$$

$$\left.+\frac{\lambda}{A(\tilde{T})^2}-\frac{1}{3}\right\}+\frac{3\eta z_1z_2(\kappa\sigma_{23}+1)}{2\pi\sigma_{23}^3N_AI(1+\kappa\sigma_{23}/2)^2} \quad (17)$$

and we can obtain a new equation of state,

$$\frac{P}{\rho_rkT} = 1 + \rho_r\sum_{ij}^{m}x_ix_jr_ir_jb_{ij}g_{ij}(d_{ij}^{+})$$

$$-\sum_{i}^{m}x_i(r_i-1)\rho_r\frac{\partial\ln g_{ii}(d_{ii}^{+})}{\partial\rho_r}-\frac{\rho_r}{kT}\sum_{ij}^{m}x_ix_jr_ir_ja_{ij}$$

$$-\frac{12\eta}{\tilde{T}}\left\{-\frac{4}{3A(\tilde{T})^3}+\frac{\ln[A(\tilde{T})\lambda-1]}{A(\tilde{T})^3}+\frac{\lambda^2}{2A(\tilde{T})}\right.$$

$$\left.+\frac{\lambda}{A(\tilde{T})^2}-\frac{1}{3}\right\}+\frac{3\eta z_1z_2(\kappa\sigma_{23}+1)}{2\pi\sigma_{23}^3N_AI(1+\kappa\sigma_{23}/2)^2} \quad (18)$$

Helmholtz Energy. The general equation for calculating the Helmholtz energy[14] from the given equation of state is

$$A(T,V,N_i) = \sum_{i}^{m}A_i^0(T) + \int_{V}^{\infty}\left(P - \frac{Nk_BT}{V}\right)dV$$

$$+ k_BT\sum_{i}^{m}N_i\ln\left(\frac{Nk_BT}{V}\right) \quad (19)$$

Eq. (18) can be rewritten in terms of T and ρ_r

$$\frac{A}{N_rkT} = \sum_{i}^{m}\frac{\phi_i}{r_i}\frac{A^0}{N_rkT} + \rho_r\sum_{ij}^{m}\phi_i\phi_jb_{ij}W_{ij}$$

$$-\sum_{i}^{m}\phi_i\left(1-\frac{1}{r_1}\right)\int_0^{\rho_r}\frac{\partial\ln g_{ii}(d_{ii}^{+})}{\partial\rho_r}d\rho_r$$

$$-\frac{12\eta}{\tilde{T}}\left\{-\frac{4}{3A(\tilde{T})^3}+\frac{\ln[A(\tilde{T})\lambda-1]}{A(\tilde{T})^3}+\frac{\lambda^2}{2A(\tilde{T})}\right.$$

$$\left.+\frac{\lambda}{A(\tilde{T})^2}-\frac{1}{3}\right\}+\frac{3\eta z_1z_2(\kappa\sigma_{23}+1)}{2\pi\sigma_{23}^3N_AI(1+\kappa\sigma_{23}/2)^2} \quad (20)$$

where, $\phi_i = \frac{N_ir_i}{V}$,

$$W_{ij} = \frac{1}{\rho_r}\int_0^{\rho_r}g_{ij}d\rho_r = \frac{I_1}{\eta}+\frac{3}{2}\frac{\xi_{ij}}{\eta^2}I_2+\frac{1}{2}\frac{\xi_{ij}^2}{\eta^3}I_3, \quad (21)$$

with $I_n = -I_{n-1} + \frac{1}{n-1}\frac{\eta^{n-1}}{(1-\eta)^{\eta-1}}$, $I_1 = -\ln(1-\eta)$ (22)

Chemical Potentials. The chemical potential is defined

$$\mu_k = \left(\frac{\partial A}{\partial N_k}\right)_{T,V,N_{i\neq k}} \quad (23)$$

and the Helmholt energy can be written (by substituting $r = 1$)

$$\frac{A}{N_rkT} = \sum_{i}^{m}\phi_i\frac{A^0}{N_rkT} + \rho_r\sum_{ij}^{m}\phi_i\phi_jb_{ij}W_{ij}$$

$$-\frac{12\eta}{\tilde{T}}\left\{-\frac{4}{3A(\tilde{T})^3}+\frac{\ln[A(\tilde{T})\lambda-1]}{A(\tilde{T})^3}+\frac{\lambda^2}{2A(\tilde{T})}\right.$$

$$\left.+\frac{\lambda}{A(\tilde{T})^2}-\frac{1}{3}\right\}+\frac{3\eta z_1z_2(\kappa\sigma_{23}+1)}{2\pi\sigma_{23}^3N_AI(1+\kappa\sigma_{23}/2)^2} \quad (24)$$

$$\frac{\mu_k}{kT} = \frac{\mu_k^0}{kT} + 2\rho_r\sum_{i}^{m}\phi_ib_{ik}W_{ik}$$

$$+\rho_r\sum_{ij}^{m}\phi_i\phi_jb_{ij}\left(N_r\frac{\partial W_{ij}}{\partial N_k}\right)+\ln(\phi_k\rho_rkT)+1$$

$$-\frac{24\eta}{\tilde{T}}\left\{-\frac{4}{3A(\tilde{T})^3}+\frac{\ln[A(\tilde{T})\lambda-1]}{A(\tilde{T})^3}+\frac{\lambda^2}{2A(\tilde{T})}\right.$$

$$\left.+\frac{\lambda}{A(\tilde{T})^2}-\frac{1}{3}\right\}+\frac{3\eta z_1z_2(\kappa\sigma_{23}+1)}{\pi\sigma_{23}^3N_AI(1+\kappa\sigma_{23}/2)^2} \quad (25)$$

where

$$N_r\frac{\partial W_{ij}}{\partial N_k} = \left(\frac{\partial W_{ij}}{\partial\eta}\right)\left(N_r\frac{\partial\eta}{\partial N_k}\right)+\left(\frac{\partial W_{ij}}{\partial\xi_{ij}}\right)\left(N_r\frac{\partial\xi_{ij}}{\partial N_k}\right) \quad (26)$$

with $N_r\frac{\partial\eta}{\partial N_k} = \frac{\rho_r}{4}r_kb_k = \frac{\rho_r}{4}b_k$ (27)

Partition Coefficients. Thermodynamic calculations for partition coefficients are compared with experimental data in the following way: because solvent with dissolved salt is considered to be a continuous medium, a one protein system is a pure-component system. Experimental partition coefficients reported by Coen[8] and Coen *et al.*[9] for a one-protein system are given

$$K^{one\text{-}protein} = \frac{c^{dp}}{c^{lp}} \quad (28)$$

where c^{dp} is the protein concentration in the dense phase in mg/g water, and c^{lp} is the protein concentration in the light phase. From the equation of state for a one-protein system we obtain the molar densities of the light phase and dense phase. The partition coefficient can be written

$$K^{one\text{-}protein} = \frac{\rho^{dp}}{\rho^{lp}} \quad (29)$$

where ρ^{dp} is the dense-phase protein number density and ρ^{lp} is the light-phase protein number density.

Consistent with eq. (28), in the two-protein system we have two distribution coefficients

$$K_1^{two\text{-}protein} = \frac{c_1^{dp}}{c_1^{lp}} \text{ and } K_2^{two\text{-}protein} = \frac{c_2^{dp}}{c_2^{lp}} \quad (30)$$

Because water and dissolved salt are a continuous medium, they contribute no mass to the calculated protein densities. Eq. (30) can be rewritten

$$K_1^{two\text{-}protein} = \frac{x_1' c_1^{dp}}{x_1'' c_1^{lp}} \text{ and } K_2^{two\text{-}protein} = \frac{x_2' c_2^{dp}}{x_2'' c_2^{lp}} \quad (31)$$

where x_i' is the solvent-free mole fraction of component i in the dense phase and x_i'' is the solvent-free mole fraction in the light phase.

Results and Discussion

For data reduction, we need molecular diameters for both proteins. From crystallographic data we use 50 Å for ovalbumin[15] and 34.4 Å for lysozyme.[16] For each protein, well depth ε depends on pH and on the ionic strength of the ammonium sulfate solution, as shown in Tables II and III.

Table I. Net Charge z_i of Ovalbumin, Lysozyme

pH	z_{oval}	z_{lys}	$z_{oval}z_{lys}$
3	+28	+13	+364
4	+12	+10.5	+126
5	-1	+9	-9
5.5		+8.5	
6	-8	+8	-64
7	-12	+7.5	-90
8	-14	+7	-98
9	-16	+6	-96
10	-20	+3.5	-70
11	-28	+0	0

Table II. Specific Energy Parameter (Square-Well Depth) ε/kT for Ovalbumin in an Aqueous One-Protein System Containing Ammonium Sulfate at 298 K

I/pH	3	4	5	6	7
6					2.62
7	4.82	5.41	4.77		3.46
8	5.56	6.07	5.63	4.79	4.3
9	6.33	6.94	6.22	5.29	5.05
10	6.9	7.13	7.01	6.25	6.07
11			7.49	6.8	6.74
12			8.22	6.95	7.01

Table III. Specific Energy Parameter (Square-Well Depth) ε/kT for Lysozyme in an Aqueous One-Protein System Containing Ammonium Sulfate at 298 K

I/pH	3	4	5	6	7
5		5.56	3.94	3.95	3.96
6		5.7	4.52	4.27	4.33
7	6.99	6.09	5.22	4.85	5.1
8	7.25	6.33	5.85	5.42	5.57
9	7.32	7.01	6.47	6.24	6.39

Table IV. Interaction Parameters k_{ij} for the Aqueous Two-Protein System Containing Ammonium Sulfate at 298 K

I/pH	4	5	6	7
6				-0.051
7	0.06			0.01
8	0.1			0.04
9	-0.075	-0.055	0.003	-0.02

The well depths were obtained from experimental one-protein liquid-liquid equilibrium data.[8,9]

For the two-protein system, we require binary parameter k_{12}. This binary parameter also depends on the pH and ionic strength of ammonium sulfate, as shown in Table IV. The molecular-thermodynamic framework presented here enables us to calculate phase equilibria in a manner virtually identical to that for vapor/liquid equilibria as used by chemical engineers for about fifty years. In both cases, the calculation requires an equation of state and characteristic constants that reflect intermolecular forces: for a binary mixture containing components 1 and 2, we need one set of constants for 1-1 interactions, another for 2-2 interactions, and another for 1-2 interactions. The first and second sets we obtain from experimental data for the one-component systems, but the third set requires some binary data. Thus, there is a striking similarity between conventional calculations for vapor/liquid equilibria and those for aqueous multiprotein systems. Regrettably, the latter requires more experimental information because intermolecular forces between proteins, unlike those for conventional nonelectrolyte fluids, depend on temperature, pH, ionic strength and on the nature of the salt in the aqueous medium.

Partition Coefficients. Figures 1 to 4 show some calculated results for the aqueous two-protein systems, ovalbumin-lysozyme. Figures 1 and 2 show results at pH 7, and Figures 3 and 4 at pH 4, respectively.

In Figure 1, the calculated partition coefficients in aqueous solution are compared with the corresponding experimental data.[9] This comparison is accompanied at pH 7 and ionic strength I = 9.0 mol/L ammonium sulfate. At the process of calculation, we use the value of net charge in the Table I. In the comparison of K_1, our proposed model slightly under-

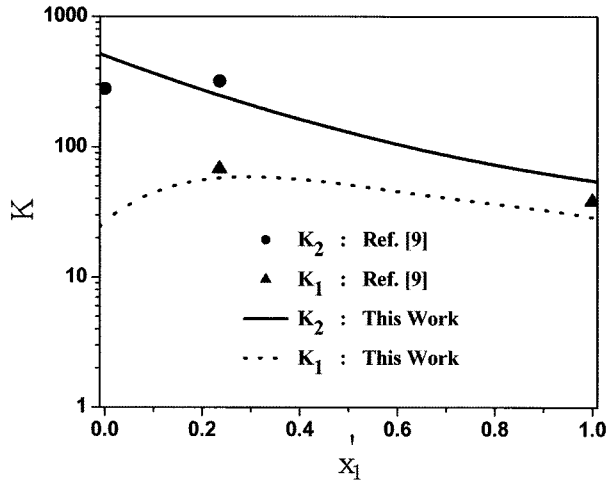

Figure 1. Phase diagrams for the system ovalbumin - lysozyme at pH 7 and ionic strength 9 of ammonium sulfate at 25 °C.

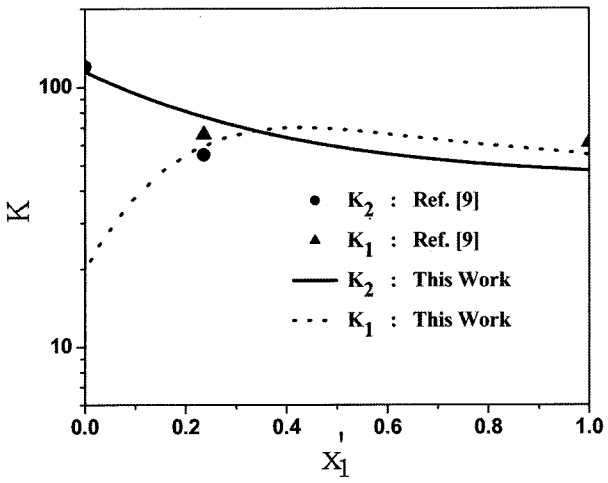

Figure 3. Phase diagrams for the system ovalbumin - lysozyme at pH 4 and ionic strength 7 of ammonium sulfate at 25 °C.

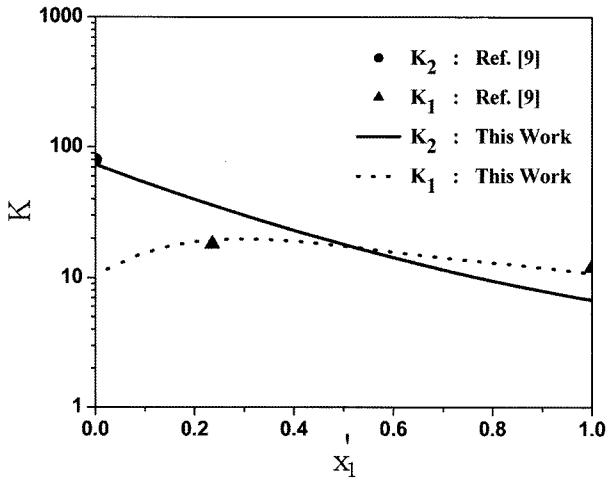

Figure 2. Phase diagrams for the system ovalbumin - lysozyme at pH 7 and ionic strength 8 of ammonium sulfate at 25 °C.

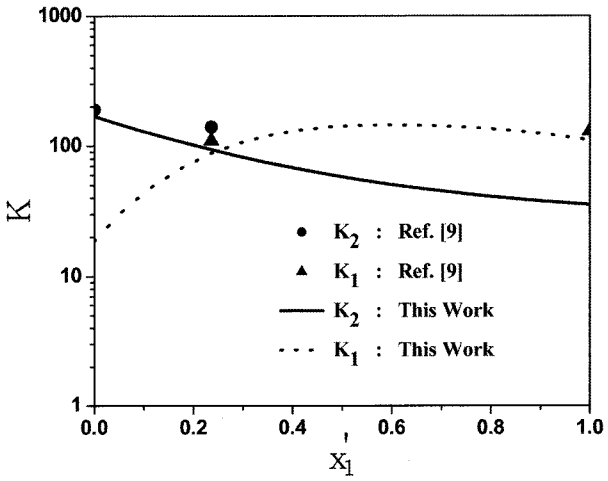

Figure 4. Phase diagrams for the system ovalbumin - lysozyme at pH 4 and ionic strength 8 of ammonium sulfate at 25 °C.

estimates the partition coefficient, but the trend of the dotted line is similar with the experimental results. In the comparison of K_2, our proposed model slightly overestimates the partition coefficient at the initial point, but the next point is reasonably agree well with calculated value.

Figure 2 shows phase behavior of ovalbumin-lysozyme at pH 7 and ionic strength $I = 8.0$ mol/L ammonium sulfate. In this figure, calculated values using our proposed model agree well with the experimental value.[9] As the mole fraction of ovalbumin increases to 0.5, the partition coefficients decrease to unity.

Figure 3 shows phase behavior at pH 4 and ionic strength $I = 7.0$ mol/L ammonium sulfate. In the comparison of K_1 and K_2, our proposed model slightly overestimates the partition coefficient, but the numerical difference is reasonable and the trend of the calculated line agrees with the experimental results.

In Figure 4, the calculated partition coefficients are compared with the corresponding experimental data of ovalbumin-lysozyme at pH 4 and ionic strength $I = 7.0$ mol/L ammonium sulfate. In the comparison of K_1 and K_2, our proposed model slightly underestimates the partition coefficient. In Figure 4, at pH 4 and ionic strength 8 molal shows similar behavior with Figure 3, except that the deviation from ideality is larger.

In these figures, dark circles and dark triangles are experimentally observed K for ovalbumin-lysozyme interactions,[9] solid line and dotted line are values calculated for corresponding pH 7 and 4 by this work, respectively. In the figures, agreement with presented experimental data is reasonable considering that no adjustable parameter for two-protein systems is introduced, the comparison of the calculated

value with the experimental data indicated that the theory is able to describe the variation of partition coefficient with pH and ionic strength reasonably.

Conclusions

We develop a new thermodynamic model taking into account a salt effect on phase behaviors for two-protein systems. The model correctly predicts liquid-liquid phase separation when high-ionic-strength ammonium sulfate is added to an aqueous mixture of ovalbumin and lysozyme. A single specific energy parameter is adjusted to model phase separation in one-protein systems. Using these parameters and one binary specific energy parameter, calculated phase behavior gives reasonable agreement with experimental data.

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