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Vacancy Solution Theory for Partitioning of Protein in Reverse-Micellar Systems

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ABSTRACT

Using vacancy solution theory based on a surface pressure, a thermodynamic model has been developed for prediction of the protein partitioning in aqueous solution in equilibrium with an oil phase containing reverse micelles. In the present model, one vacancy solution represents the bulk aqueous phase and the other the reverse-micellar phase. Using the concentration of protein in both reverse micelles and aqueous phase, the surface pressure was incorporated for prediction of the protein adsorption in reverse micelles and the nonideality of the system was expressed in terms of the Wilson activity-coefficient model. The present model has been applied to the extraction of bovine serum albumin with formation of reverse micelles using a cationic surfactant, cetyltrimethylammonium bromide (CTAB), in isooctane-1-hexanol. The results of prediction were in very good agreement with the experiment.

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Key Words: Vacancy solution theory; Reverse micelle; Partitioning; Bovine serum albumin; CTAB.

INTRODUCTION

Reverse micelles are aggregates of surfactant molecules containing microscopic polar cores of solubilized water, called water pools. These structures are thermodynamically stable and spontaneously form in organic solvents. The hydrophilic compounds, such as proteins, are solubilized inside the polar core of reversed micelles and stabilized in the organic solvent by a surfactant shell layer that protects the biomaterials from denaturation in the organic phase. Recently, several investigators have represented the ability of this technique in partitioning and purification of biomolecules.[1–5] In addition, reverse micelles are ideally suited for hosting enzymatic reactions, particularly those involving difficult-to-solubilize substrates, require low water content, or employ multiple biocatalysis.[6]

Since understanding of phase equilibria in reverse-micellar systems is very important for designing down-stream processing, presentation of a thermodynamic model for prediction of phase equilibria in these systems is necessary. In spite of vast researches in the application of reverse micelles for the separation and purification of biomolecules, due to the complexity and misunderstanding of the interactions among various species in the solution, researchers have not been successful in the prediction of phase equilibria in reverse-micellar systems as in ordinary vapor–liquid or liquid–liquid equilibria.

Several thermodynamic models have been implemented so far for protein partitioning from the aqueous phase using reverse micelles. One may classify the previous models into the three main groups, which are "mass-action models," "Gibbs free-energy equations," and "adsorption models."

Based on mass-action principle, three models have been presented. Woll and Hatton proposed a simple phenomenological model for protein partitioning in anionic reverse-micellar systems.[7] They assumed a pseudochemical equilibrium for complexation of the protein molecules with the hydrophobic tails of reverse micelles as empty sites. Using the mass-action principle, Rabie and Vera presented an ion-exchange model for the partitioning of protein[8] with consideration of the reaction between protein and surfactant counter ion. They assumed that at equilibrium, the concentration of protein in both aqueous and reverse-micellar phases are the same and that the activity of components in the solution should be equal to its concentration. Ashrafizadeh and Khoshkbarchi presented a different

mass-action formulation to improve the previous models.[9] They assumed that the reverse micelles act as active sites and considered a complex speciation takes place between molecules of protein and active sites to extract protein from the aqueous solution.

In the second group of the models, the researcher tried to take into account the microstructure of reverse micelles. Using shell and core model, Bratko et al. evaluated the partitioning of protein by solving the nonlinear Poisson–Boltzman equation to take into account the electrostatic contribution to the free energy of extraction.[10] Bruno et al. presented a thermodynamic model, which was based on the minimization of the Gibbs free energy of protein extraction using reverse micelles.[11] They assumed that the protein partitioning is due to the electrostatic interactions between the charged inner wall of the reverse micelles and the charged protein molecules.

Brandani et al. also proposed an adsorption model[12] based on the assumption that the reverse micelles are the active sites for adsorbing protein molecules from aqueous solution. For partitioning of the protein, they considered only those interactions, which is associated with the Langmuir adsorption isotherm.

THERMODYNAMIC FRAMEWORK

Dubinin[14] and Lucassen-Reynders[15–17] presented two concepts of "vacancy solutions" and "dividing surfaces," respectively. Based on the vacancy solution theory, in 1980, Suwanayen and Danner[13] developed a thermodynamic model for the prediction of gas adsorption. In this work, we apply this thermodynamic framework for the prediction of protein extraction in aqueous phase using reverse micelles.

Each vacancy solution was considered as an imaginary space in that it was defined as a vacuum entity with vacuum sites. The adsorbates, which are the same size as vacuum sites, occupying the remaining adsorption space. For this system, the hypothetical solvent is the vacancy, and the protein molecules are adsorbate, which can be laid into the vacancy of the reverse micelles. Based on this thermodynamic framework, liquid adsorption equilibrium can be considered as equilibrium between two vacancy solutions such as aqueous and reversed micellar phases and, thus, this equilibrium may be considered as a binary vacancy solution.

According to this model, the chemical potential of component i in the surface of the reverse micelles can be expressed as,

$$\mu_i^{(rm)} = \mu_i^{0(rm)} + RT\ln\gamma_i^{(rm)}x_i^{(rm)} + \pi\bar{a}_i \tag{1}$$

where the last term on the right hand side is the contribution of the surface potential to the chemical potential of component i in the reverse micellar phase, π is the osmotic pressure of reverse micelles, and $\bar{a}_i$ is the partial molar area of this component. Evidently this item vanishes for the aqueous phase, and thus, the chemical potential for component i in aqueous phase is:

$$\mu_i^{(aq)} = \mu_i^{0(aq)} + RT\ln\gamma_i^{(aq)}x_i^{(aq)} \tag{2}$$

Since in all cases the aqueous phase, bulk vacancy solution, is extremely dilute, the contribution of the second term on the right hand side of Equation. (2) to the chemical potential of the solvent, vacancy, is negligible. Thus, by applying the equilibrium criterion for component 2 as vacancy, we have:

$$\pi = -\frac{RT}{\bar{a}_2}\ln(\gamma_2^{(rm)}x_2^{(rm)}) \tag{3}$$

where the two terms in the bracket are the activity coefficient and surface excess-mole fraction of vacancy in the reverse-micellar phase, respectively. Based on the definition of the dividing surface, which was proposed by Lucassen-Reynders' work, the surface excess-mole fraction can be the same as the fractional coverage of component as in the following. The dividing surface, which represents the adsorbed phase, is located such that the partial molar area of components, adsorbate and vacancy, are equal to a constant. Using this definition one can show (see appendix A):

$$x_1^{(rm)} = \frac{n_1^{(rm)}}{n_1^{(rm)Max}} = \theta_1 \tag{A-9}$$

$$x_2^{(rm)} = \frac{n_2^{(rm)}}{n_1^{(rm)Max}} = 1 - \theta_1 \tag{A-10}$$

where $n_1^{(rm)}$ and $n_1^{(rm)Max}$ are number of moles and maximum moles of protein on the surface of reverse micelles, respectively, and θ_1 is the fractional coverage of protein molecules.

Using the Wilson activity-coefficient model (B-1) and Equations (A-9) and (A-10), one can show:

$$\ln\gamma_2^{(rm)} = -\ln(\theta_2^{(rm)} + \Lambda_{21}\theta_1^{(rm)}) - \theta_1^{(rm)}\left[\frac{\Lambda_{12}}{\theta_1^{(rm)} + \Lambda_{12}\theta_2^{(rm)}} - \frac{\Lambda_{21}}{\theta_2^{(rm)} + \Lambda_{21}\theta_1^{(rm)}}\right] \quad (4)$$

Finally, combining Gibbs adsorption equation (Appendix B) with the osmotic pressure Equations. (3) and (4), the concentration of protein remained in aqueous phase was obtained as:

$$[P]^{(aq)} = \left[\frac{1}{b}\frac{\theta_1}{1-\theta_1}\right]\left[\Lambda_{12}\frac{1-(1-\Lambda_{21}\theta_1)}{\Lambda_{12}+(1-\Lambda_{12})\theta_1}\right] \times \exp\left[\frac{-\Lambda_{21}(1-\Lambda_{21})\theta_1}{1-(1-\Lambda_{21})\theta_1} - \frac{(1-\Lambda_{12})\theta_1}{\Lambda_{12}+(1-\Lambda_{12})\theta_1}\right] \quad (5)$$

The first bracket of Eq. (5) is exactly the same as the Langmuir equation and the second bracket can be considered as a correction factor to the Langmuir isotherm.

EXPERIMENT

The equilibrium data of partitioning of bovine serum albumin (BSA) was obtained from aqueous phase in equilibrium with a solution of reverse micelles. At different pH, the BSA with various concentrations were extracted from an aqueous solution dispersed with reverse micelles forming in a Winsor type II by a cationic surfactant, cetyltrimethylammonium bromide (CTAB), and isooctane using 1-hexanol (4 v/v) as cosolvent at 25°C. At any value of pH, the experiments were carried out at two different values of surfactant concentration.

Bovine serum albumin (67000 Da, pI 4.7) was purchased from BDH Company and cetyltrimethylammonium bromide (CTAB) was obtained from Merck with 99% purity, and it was used without any further purification. For titration, Karl-Fischer solution and dried methanol were prepared from Fluka. The other chemicals such as solvent, cosolvent, salt, and buffer were all commercially available reagents of analytical grade.

The solution of reverse micelles was prepared by dissolving CTAB in isooctane with addition of 1-hexanol as cosolvent (4 v/v). An amount of

3.5 mL of the aqueous protein solution was brought into contact with the same volume of reverse-micellar solution into glass test tubes to solubilize the protein in the reverse micelles. The mixture was shaken for 3 min to equilibrate two aqueous and organic phases. To obtain a clear phase interface between two phases, the emulsion of reverse micelles was centrifuged at 3000 rpm for 5 min so that no precipitation appeared at the interface. Then, the test tubes were subsequently placed in a thermostat bath at 25°C for 16 hr, and finally, the two equilibrium phases were separated carefully.

The water content in the reverse micelles was measured using Karl-Fischer titrator 758 (Metrohm, Ltd., Herisau, Switzerland). The pH of the aqueous phase was monitored by a model 744-pH meter (Metrohm, Ltd). The protein concentration of aqueous solution was determined by measuring the absorbency at 280 nm using a UV/VIS spectrophotometer (Shimadzu 1201). The protein content in the reverse micelles was computed by mass-balance calculation and it was in agreement with the amount of protein, which was measured in backward extraction for all the experiments. Simultaneously, the blank experiments were performed with aqueous phase containing no protein.

RESULTS AND DISCUSSION

The three adjustable parameters in Eq. (5)—b, Λ_{12}, and Λ_{21}—were evaluated by least-squares fitting procedure utilizing the Marquardt optimization program for minimizing relative error of partition coefficient of bovine serum albumin[18] where, according to the definition, the partition coefficient of protein is defined as follows:

$$K = \frac{[P]^{(rm)}}{[P]^{(aq)}} \tag{8}$$

The results of partitioning in terms of measured and calculated partition coefficient of BSA, including the weight percent of water in reverse micelles, are presented in Tables 1 and 2. In these tables, the standard deviation was calculated based on the following equation:

$$\sigma = \left(\frac{\sum_{i=1}^{N} \left(\frac{K_i^{\exp} - K_i^{cal}}{K_i^{\exp}} \right)^2}{N} \right)^{0.5} \tag{9}$$

Table 1. The weight percent of water in reverse micelles; measured and calculated partition coefficient of BSA at pH 9.1 and pH 8.1.

pH = 9.1						pH = 8.1					
CTAB 30 mM			CTAB 20 mM			CTAB 30 mM			CTAB 20 mM		
Wt%	K^{exp}	K^{cal}	Wt%	K^{exp}	K^{cal}	Wt%	K^{exp}	K^{cal}	Wt%	K^{exp}	K^{cal}
2.477	105.2	111.0	1.673	64.5	62.9	2.240	76.9	66.6	1.656	41.8	43.2
2.553	141.2	146.8	1.700	70.6	68.8	2.313	72.8	83.7	1.633	43.5	49.1
2.358	178.9	181.0	1.718	75.5	82.2	2.309	88.4	87.3	1.505	50.5	51.7
2.449	254.6	218.8	1.724	89.4	94.4	2.302	94.1	100.9	1.565	59.9	55.2
2.229	424.6	434.6	1.660	117.7	96.2	2.100	108.0	122.8	1.559	73.8	61.3
2.229	986.6	991.9	1.694	150.9	150.1	2.200	140.8	123.6	1.601	101.5	66.7
			1.650	236.0	244.6	2.007	204.0	199.5	1.571	99.0	118.9
			1.510	501.6	495.7				1.339	186.1	190.3
									1.353	222.2	222.2
		$\sigma = 0.071$						$\sigma = 0.135$			

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Table 2. The weight percent of water in reverse micelles; measured and calculated partition coefficient of BSA at pH 7.1 and pH 6.1.

pH = 7.1						pH = 6.1					
CTAB 30 mM			CTAB 20 mM			CTAB 30 mM			CTAB 20 mM		
Wt%	K^{exp}	K^{cal}	Wt%	K^{exp}	K^{cal}	Wt%	K^{exp}	K^{cal}	Wt%	K^{exp}	K^{cal}
2.064	15.5	17.8	1.151	15.0	12.9	1.746	3.46	2.32	1.122	2.47	3.02
1.904	17.3	17.8	1.099	15.3	14.1	1.775	3.61	2.61	1.196	2.73	3.12
1.949	19.1	19.2	1.081	15.9	15.8	1.652	3.26	3.49	1.093	3.05	3.29
2.000	20.0	20.5	1.182	18.0	16.4	1.757	3.37	4.00	1.218	3.45	3.50
1.925	23.2	21.2	1.289	16.9	20.6	1.786	4.09	4.13	0.960	4.18	3.63
1.678	23.3	23.0	1.151	19.7	21.9	1.699	4.11	5.27	1.057	5.24	3.87
1.970	34.7	23.7	1.098	24.4	24.2	1.788	7.25	5.37	1.122	5.54	5.31
1.938	27.8	33.2	1.175	28.3	29.5						
1.792	44.1	35.0	1.159	43.5	36.3						
$\sigma = 0.136$						$\sigma = 0.196$					

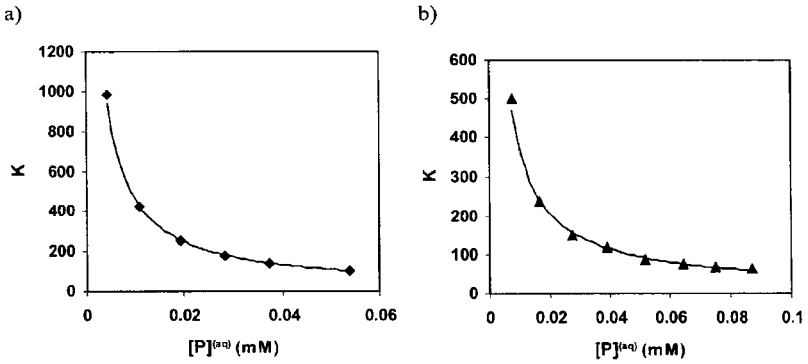

Figure 1. The partition coefficient of bovine serum albumin vs. the protein concentration in equilibrium aqueous phase at 25°C, KCl 0.1 M, pH 9.1, (a) CTAB 30 mM, and (b) CTAB 20 mM.

where N is the number of experimental data and superscripts "exp" and "cal" are indicated as the experimental and calculated values.

Figures 1–4 show the results of the vacancy solution theory based on the Wilson equation (VST-Wilson) for prediction of partition coefficient of bovine serum albumin compared to the experimental data. The solid lines represent the model and the points with labels are the experimental data.

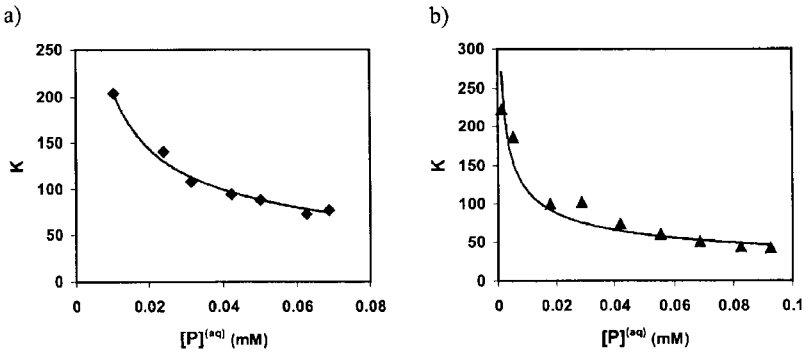

Figure 2. The partition coefficient of bovine serum albumin vs. the protein concentration in equilibrium aqueous phase at 25°C, KCl 0.1 M, pH 8.1, (a) CTAB 30 mM, and (b) CTAB 20 mM.

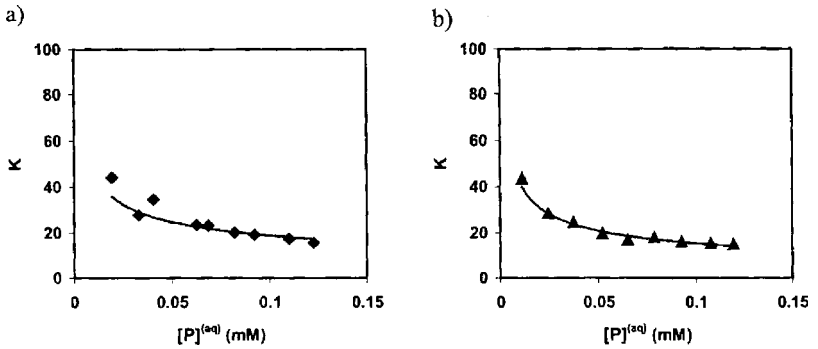

Figure 3. The partition coefficient of bovine serum albumin vs. the protein concentration in equilibrium aqueous phase at 25°C, KCl 0.1 M, pH 7.1, (a) CTAB 30 mM, and (b) CTAB 20 mM.

Each figure represents the effect of pH on partitioning of bovine serum albumin between aqueous and reverse-micellar phase.

It is clear that with increasing concentration of surfactant, the partition coefficient is enhanced, particularly at pH far from the isoelectric point (pI) of protein. In fact, the yield of the partitioning of protein depends on the quantity of reverse micelles, which is more dispersed in bulk aqueous phase with increasing surfactant. On the other hand, on adding more surfactant to the solution, it intensifies the interactions of reverse micelles with protein molecules and

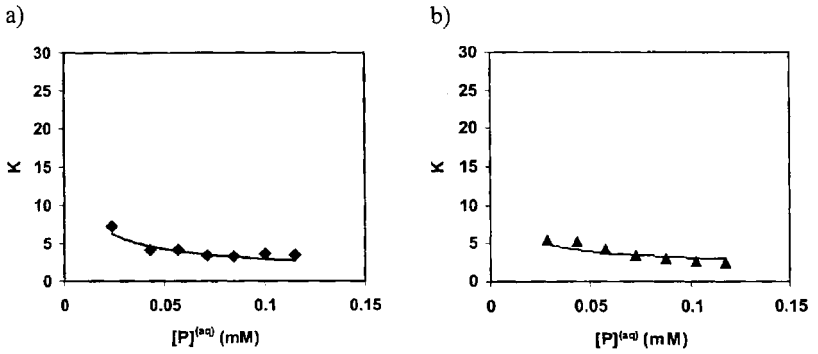

Figure 4. The partition coefficient of bovine serum albumin vs. the protein concentration in equilibrium aqueous phase at 25°C, KCl 0.1 M, pH 6.1, (a) CTAB 30 mM, and (b) CTAB 20 mM.

Table 3. The maximum number of moles of bovine serum albumin on reverse-micelle surface at different surfactant concentration and pH.

CTAB concentration (mM)	pH 6.1	pH 7.1	pH 8.1	pH 9.1
20	115.9	536.7	542.4	998.1
30	121.5	647.9	774.2	1208.2

results in the number of moles and the maximum mole number of protein on the surface of reverse micelles being changed.

The charge of the protein will be influenced strongly by the pH of the solution so that a protein at a pH value above its isoelectric point takes on a net negative charge, while below its pI, a protein has net positive charge. Thus, as the pH was increased from 6.1 to 9.1, the net negative charge on bovine serum albumin was enhanced. As shown in Figs. 1–4, the partition coefficient of bovine serum albumin increased with increasing pH because it intensifies the electrostatic interaction between the charged amino-acid residues and the charged head groups in reverse micelles.

To investigate the effect of surfactant concentration, the maximum number of moles of protein on the surface of reverse micelles in terms of pH was optimized as the fourth adjustable parameter. Table 3 shows this

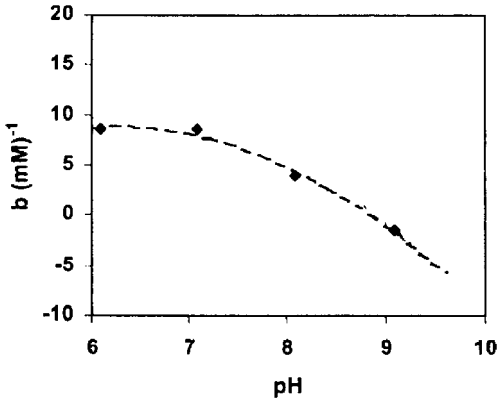

Figure 5. The adsorption parameter b against pH for partitioning of bovine serum albumin from aqueous phase in equilibrium with isooctane-1-hexanol reverse-micellar phase using CTAB.

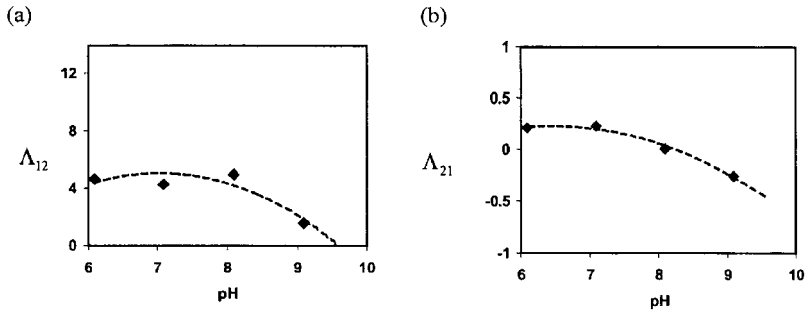

Figure 6. The Wilson parameters against pH for partitioning of bovine serum albumin from aqueous phase in equilibrium with isooctane-1-hexanol reverse-micellar phase using CTAB. (a) the parameter Λ_{12}, and (b) the parameter Λ_{21}.

parameter for each surfactant concentration at different pH. The results show that the trend of enhancing the maximum number of moles of protein on the surface, with increasing surfactant concentration and pH, follows from the actual behavior of the system.

In the second step, the dependence of the adjustable parameters on the pH was investigated. Figures 5–6 show the variation of the parameters as a function of pH. The investigation was performed for pH range below 10, because, near this value, a very thin layer of surfactant appeared and, as the pH increased from this value, the thickness of this layer (third phase) was enlarged.

CONCLUSION

The vacancy solution theory was extended as a new thermodynamic model was used for prediction of the partitioning of bovine serum albumin using reverse micelles with CTAB surfactant. The results of partitioning of the protein were compared with experimental data and it was shown that the calculated results are in very good agreement with the experimental data. Besides the accuracy of the results, the model has some advantages as in the following. Firstly, the proposed model is subjected to neither an assumption about the behavior of aqueous phase nor an assumption concerning the characteristic of the reverse micelles such as aggregation number, density of water pool, geometrical parameters of reverse micelles, etc. Moreover, unlike to the other thermodynamic model in the third group, all of the adjustable

parameters are reasonable and in the same order of magnitude. Finally, using binary adjustable parameters, the new model has a powerful potential for prediction of protein partitioning in multicomponent mixtures, particularly for solutions containing more than one protein.

NOMENCLATURE

K	partition coefficient
N	number of experimental data
[P]	protein concentration (mmol/l)
Wt%	the weight percent of water in reverse micelles
$\bar{a}_i$	partial molar surface area of i (m^2/kmol)
b	constant in Eq. (5)
$n_i^{(rm)}$	number of moles of i on reverse-micelle surface
$n_i^{(rm)_{MAX}}$	maximum number of moles of i on reverse-micelle surface
pI	isoelectric point of protein
x_i	mole fraction of i in vacancy solution

Greek Letters

γ_i	activity coefficient of i in vacancy solution
θ_i	fractional coverage of i
μ_i	chemical potential of i in vacancy solution (J/mol)
π	surface pressure (N/m)
σ	standard deviation
Λ_{ij}	Wilson parameter

Superscripts

aq	aqueous phase
cal	calculated
exp	experimental (measured)
rm	reverse micellar phase
0	standard state
max	maximum extraction limit

Subscripts

i	component i
1	protein
2	vacancy

APPENDIX

A—Dividing Surface

Similar to the Lucassen-Reynders' work, the partial molar area of both components are equal to the reciprocal of the limiting excess-adsorption concentration, so that:

$$\bar{a}_1 = \bar{a}_2 \equiv \frac{1}{\Gamma_1^{MAX}} \tag{A-1}$$

where, according to the definition;

$$\Gamma_i = \frac{n_i^{(rm)}}{A} \tag{A-2}$$

By substitution of Eq. (A-2) into (A-1):

$$\bar{a}_1 = \bar{a}_2 = \frac{A}{n_1^{(rm)_{MAX}}} \equiv a_1^{MAX} \tag{A-3}$$

By using the following thermodynamic relation, one can relate the surface excess-mole fractions $x_i^{(rm)}$, with a measurable quantity such as fractional coverage θ_i

$$\sum_i \bar{a}_i n_i^{(rm)} = A \tag{A-4}$$

or

$$\sum_i \bar{a}_i \Gamma_i = 1 \tag{A-5}$$

Then, by substituting Eq. (A-3) into (A-5) for a binary (protein and vacancy) system:

$$\Gamma_1 + \Gamma_2 = \frac{1}{a_1^{MAX}} = \Gamma_1^{MAX} \tag{A-6}$$

Since

$$x_i^{(rm)} \equiv \frac{n_i^{(rm)}}{\sum_j n_j^{(rm)}} \tag{A-7}$$

or

$$x_i^{(rm)} \equiv \frac{\Gamma_i}{\sum_j \Gamma_j} \tag{A-8}$$

With combination of Eq. (A-6) and (A-8):

$$x_1^{(rm)} = \frac{\Gamma_1}{\Gamma_1^{MAX}} = \frac{n_1^{(rm)}}{n_1^{(rm)_{MAX}}} = \theta_1 \tag{A-9}$$

$$x_2^{(rm)} = \frac{\Gamma_2}{\Gamma_1^{MAX}} = \frac{n_2^{(rm)}}{n_1^{(rm)_{MAX}}} = 1 - \theta_1 \tag{A-10}$$

B—Gibbs Adsorption Equation

For the equilibrium between aqueous the phase and the reverse-micellar phase, similar to the vapor–solid equilibrium, the Gibbs adsorption equation is as follows:

$$(RT)^2 d(\ln[P]^{aq}) = \frac{A}{n_1^{(rm)}} d\pi \tag{B-1}$$

where $[P]^{aq}$ is the protein (adsorbed component) concentration remaining in the aqueous phase and is in equilibrium with adsorbed protein in the reverse-micellar phase.

C—Wilson Equation

For a binary solution, the Wilson Equation for vacancy (component 2) in a reverse-micellar phase is as follows:

$$\ln\gamma_2^{(rm)} = -\ln(x_2^{(rm)} + \Lambda_{21} x_1^{(rm)}) - x_1^{(rm)} \left[\frac{\Lambda_{12}}{x_1^{(rm)} + \Lambda_{12} x_2^{(rm)}} - \frac{\Lambda_{21}}{x_2^{(rm)} + \Lambda_{21} x_1^{(rm)}} \right] \tag{C-1}$$

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