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Modeling and Experimental Data for the Reverse Micellar Extraction of Proteins Using a New Surfactant

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ABSTRACT

In this study the reverse micellar extraction of α-chymotrypsin using bis(2,4,4-trimethylpentyl) sodium dithiophosphinate as the surfactant has been investigated. Experiments were performed to determine the effects of pH and salt concentration on the reverse micellar extraction and backward extraction of α-chymotrypsin in isooctane/decanol mixtures. The results showed that both pH and salt concentration influence the reverse micellar extraction of α-chymotrypsin. The pH affects the extraction of the protein through changing the sign and number of the charges carried by protein molecules and thereby affects their interactions with the charged head groups of the surfactant molecules. It was found that at high pH values the hydrogen ions compete with positively charged protein molecules and exchange with the counterions of the surfactant molecules, and as a result destroy the reverse micelles. This effect was exploited for the backward extraction of protein molecules from the reverse micellar phase. A model has been developed to correlate the experimental data of reverse micellar extraction of proteins. In the model the reverse micelles were treated as noninteractive ion-exchange sites. The activity coefficient of the protein molecules in reverse micelles was represented by the model proposed by Pitzer. It has been shown that the model can correlate the experimental data obtained in this work and those reported in other studies.

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INTRODUCTION

Separation and purification of biochemicals are important stages in their productions. Most of the biomolecules are produced in dilute media along with other chemicals that should be eventually separated and concentrated. The costs involved in these stages are usually very high and determine the cost of the final products (1). Biomolecules, on the other hand, are delicate chemicals which may be destroyed or lose their physicochemical characters during the separation process. These facts have stimulated many studies to develop new separation techniques for biomolecules.

Among the various methods proposed to separate biomolecules, reverse micellar extraction, as a surfactant-based separation process, has recently received a great deal of attention. Reverse micelles are the aggregation of surfactant molecules around minute water droplets in organic solvents. These aqueous microenvironments are also called water pools. Reverse micelles are one of the possible forms of the water-in-oil microemulsion referring to a thermodynamically stable, transparent, liquid–liquid system which contains significant amounts of water, organic, surfactant, and cosurfactant. Although not homogeneous at a molecular level, a reverse micellar phase is effectively a single-phase system. In the case of reverse micelles formed with ionic surfactants, the surfactant counterions dissociate in the water pool and form a charged envelope around the water pool. As a result of the electrostatic field produced by the charged surfactant head groups, reverse micelles favor attraction of oppositely charged molecules and entrap them in their water pools. Unlike ordinary liquid–liquid extraction, in reverse micellar extraction the extracted molecules do not leave their aqueous environment formed by the aggregates of surfactants around the water pools and yet extracted to the organic phase without direct contact with the organic solvent. This behavior reduces the possible destructive effect of the organic solvent on the biomolecules. Reverse micellar extraction of various biomolecules such as amino acids (2–4), proteins (5–8), and enzymes (9) has been the subject of numerous studies. In most of these studies sodium bis(2-ethylhexyl) sulfosuccinate (AOT) or trioctylmethylammonium chloride (TOMAC) has been used as the surfactant. There are few studies in the literature investigating the capability of other commercially available surfactants in the reverse micellar extraction of biomolecules (2, 10). Furthermore, it has been shown that the backward extraction of the biomolecules extracted using these surfactants is either not efficient or results in the presence of the surfactant in the backward extracted biomolecule solution (6). Recently, Khoshkbarchi and Vera (11) showed that a commercially available surfactant, bis(2,4,4-trimethylpentyl) sodium dithiophosphinic acid (HPSS), when converted to its sodium salt can form reverse micelles. This surfactant was then used to successfully extract amino acids

from aqueous solutions. An important advantage associated with this surfactant was its ease of backward extraction of the biomolecule to a more concentrated solution without the presence of the surfactant.

The modeling of the reverse micellar extraction of proteins has also been the subject of some studies. One class of the models developed is based on the minimization of the Gibbs free energy of the transfer of the protein molecules to the reverse micelles. This type of modeling may include the electrostatic interactions between the protein molecules and reverse micelles. For example, a simple model based on the minimization of Gibbs free energy of the extraction of the protein into the reverse micelles was developed by Caselli et al. (12). In this model the attraction of the protein molecules into the reverse micelles was attributed to the electrostatic interactions between the charged inner wall of reverse micelles and the charged protein molecules. The other class of models is based on the electrostatic interactions between charged protein molecules and reverse micelles formed by ionic surfactants. These models, which may include short-range interaction effects, mainly take advantage of the electrical double layer theory and model the electrochemical potential of the ionic species, including protein molecules, using the Poisson–Boltzmann equation and its extensions (13, 14). Within this framework the alternative to the Poisson–Boltzmann equation would be modeling the ionic species using the mean field theory (15). This method is based on a rather complex theory that in many cases is not well developed. Other statistical thermodynamic methods have also been applied to model the electrochemical potential of ionic species in reverse micelles which, due to their complexity and lack of enough information for their parameters, are not very useful for engineering applications (16).

In this study experiments are performed to determine the effects of the pH and salt concentration on the extraction of α-chymotrypsin using the reverse micelles formed by bis(2,4,4-trimethylpentyl) sodium dithiophosphinate (NaPSS). A model is developed to correlate the experimental data obtained in this work and those reported in other studies. The model considers the reverse micelles as noninteractive active sites capable of ion-exchanging with the charged protein molecules. The activity coefficients of the protein molecules in the reverse micelles, required by the model, are calculated from a model proposed by Pitzer (17). It is shown that the model can accurately correlate the experimental data.

MATERIALS AND METHODS

The surfactant used in this study, bis(2,4,4-trimethylpentyl) sodium dithiophosphinate, was prepared by first purifying a 70% solution of bis(2,4,4-trimethylpentyl) dithiophosphinic acid (HPSS) by a copper salt precipitation

method (18). The concentration of the final solution was 99.2% and was determined using a potentiometric method by titrating the solution with a 0.05 N NaOH solution with a Metrohm pH meter. The purified acid thus prepared was then reacted with a 1 N solution of sodium hydroxide to prepare its sodium salt according to the following reaction:

$$HPSS + NaOH \leftrightarrow NaPSS + H_2O \quad (1)$$

Sodium chloride (99.9% purity), isooctane, decanol, and sodium hydroxide (99.0% purity) were obtained from Merck and were used as received. α-Chymotrypsin was a product of Sigma and was used without further purification.

The reverse micellar extraction of α-chymotrypsin was performed using the phase transfer method. All experiments were performed by contacting 20 mL of an organic solution containing 200 mM decanol in isooctane with 10 mL of an aqueous solution containing 200 mM NaPSS and 10 mL of an aqueous solution containing 1.0 g/L α-chymotrypsin, NaCl, and NaOH or HCl to adjust the pH. The reason that a buffer solution is not used to adjust the pH is to ensure that the buffer ions produced from the buffer solution will not interfere in the ion-exchange process. It will be shown that this is particularly important in the backward extraction experiments. The samples were placed in a shaker and submerged in a water bath at 298.2 ± 0.1 K and shaken for 2 hours. The shaker was then turned off and the samples were left in the water bath for a week to reach equilibrium. For the backward extraction experiments, 10 mL of a sample of the organic phase containing the reverse micelles formed by NaPSS and loaded with α-chymotrypsin was contacted with 2 mL of a 2 N solution of HCl. The mixture was then shaken for 20 minutes and left to reach equilibrium in a water bath at 298.2 ± 0.1 K for 2 hours.

For both the forward and backward extraction experiments the aqueous and organic phases of each sample were separated and the aqueous phase was analyzed to measure the final concentrations of α-chymotrypsin and NaPSS and pH. The pH of the aqueous phase was measured on a Metrohm pH meter, and the NaPSS concentration was measured using the potentiometric titration method explained above. The concentration of α-chymotrypsin was measured using a Perkin-Elmer UV spectrophotometer at 280 nm. The water content of the organic phase was analyzed by Karl Fischer titration on a Brinkmann Titrator.

RESULTS AND DISCUSSION OF THE EXTRACTION EXPERIMENTAL DATA

Effect of pH

Figure 1 depicts the percent extraction of α-chymotrypsin as a function of pH of the solution. The experimental points are connected to each other by

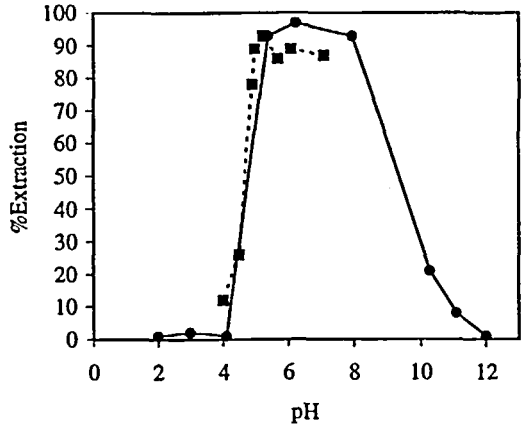

FIG. 1 Reverse micellar extraction of α-chymotrypsin as a function of pH from an aqueous solution with initial concentrations of 100 mM NaCl and 1 g/L α-chymotrypsin and an organic solution with an initial concentration of 100 mM: (——) NaPSS and (---) AOT as the surfactant. The solid and dashed lines are aids for the eyes.

a solid line to lead the eyes. As shown in this figure, the pH of the aqueous phase largely influences the reverse micellar extraction of α-chymotrypsin. As can be seen in Fig. 1, at high pH values up to the isoelectric point of the α-chymotrypsin almost no extraction takes place. At pH values around the isoelectric point of α-chymotrypsin, a sharp increase in the percent extraction of α-chymotrypsin occurs, which then becomes a plateau as the pH decreases to a pH value approximately equal to 4.0. At pH values lower than 4.0, the extraction of α-chymotrypsin decreases dramatically and almost reaches zero. Experimental data for the reverse micellar extraction of α-chymotrypsin using AOT as the surfactant as reported by Marcozzi et al. (7) are also shown in Fig. 1. The comparison of the percent extraction of α-chymotrypsin using NaPSS and AOT shows that the percent extractions of α-chymotrypsin at a certain pH value with both surfactants are very close to each other. This phenomenon suggests that the reverse micellar extraction of proteins depends more on the charged behavior of the ionic surfactants than on the chemical structure of their head groups.

Figure 2 shows the effect of pH on the water uptake of the organic phase. The water uptake is defined as the mass percent of water in the organic phase. As shown in this figure, the water uptake remains constant at moderate pH values and sharply decreases at low pH values.

In order to explain these effects it should be mentioned that protein molecules at various pH values carry different numbers of positive or negative charges. This is due to the presence of a sequence of the amino and carboxyl

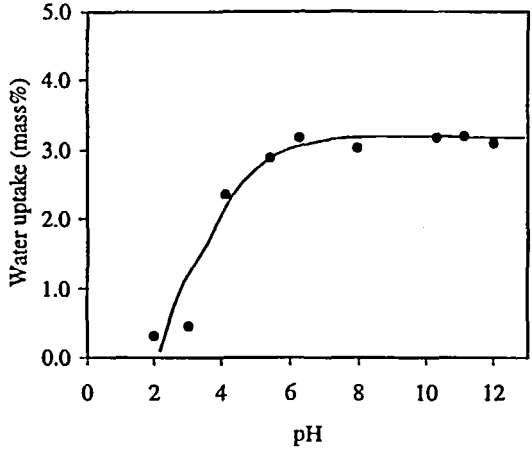

FIG. 2 Effect of the pH on the water uptake of the reverse micelles formed by NaPSS in an aqueous solution with initial concentrations of 100 mM NaCl and 1 g/L α-chymotrypsin and an organic solution with an initial concentration of 100 mM NaPSS surfactant. The solid line is an aid for the eyes.

groups on their backbones. There exists a pH value at which the numbers of negative and positive charges on a protein molecule become equal and therefore the protein molecule becomes electrically neutral. This pH value is called the isoelectric point of the protein. On the other hand, since reverse micelles formed by ionic surfactants are also charged aggregates, the driving force between the protein molecules and reverse micelles are mainly electrostatic in nature and depends on the number and sign of charges carried by the protein molecules. The α-chymotrypsin molecules at pH values higher than their isoelectric point are negatively charged and therefore repel the reverse micelles formed by NaPSS surfactant which are also dissociated and negatively charged in the water pool of the reverse micelles. Therefore, the α-chymotrypsin molecules have no tendency to be extracted to the reverse micelles formed by NaPSS. On the other hand, the α-chymotrypsin molecules at pH values lower than their isoelectric point are positively charged and are attracted to the negatively charged reverse micelles formed by NAPSS surfactant. At very low pH values, although reverse micelles formed by NaPSS and α-chymotrypsin molecules are oppositely charged, the hydrogen ions exchange with the sodium counterions of the NaPSS, converting them to HPSS according to the following reaction:

$$NaPSS + H^+ \leftrightarrow HPSS + Na^+ \quad (2)$$

The occurrence of this effect is due to the large concentration of hydrogen

ions relative to the concentration of α-chymotrypsin molecules in the aqueous solution. As a result of the mass action law, hydrogen ions win the competition and become extracted to the reverse micellar phase. It has also been shown that HPSS, unlike NaPSS, is incapable of forming reverse micelles under the conditions of these experiments (2, 11). This demolishes the structure of the reverse micelles and reduces the extraction of the α-chymotrypsin molecules to the reverse micellar phase. This explanation is further approved by observing from Fig. 2 that the water uptake also sharply decreases at low pH values. This can be the direct consequence of the destruction of the reverse micelles structure as a result of the exchange of the surfactant counterions with hydrogen ions. Later, it will be shown that this phenomenon can be exploited for the backward extraction of protein molecules.

Effect of Salt Concentration

Figures 3 and 4 show the percent extraction of α-chymotrypsin and the water uptake, respectively, as functions of NaCl molarity at a pH value of 6.08. As shown in Fig. 3, the percent extraction of α-chymotrypsin decreases as the NaCl concentration increases. A similar phenomenon happens to water uptake, and it decreases as the salt concentration increases. These phenomena can be explained by the fact that an increase in the salt concentration in the bulk aqueous phase also increases the salt concentration in the water pool of

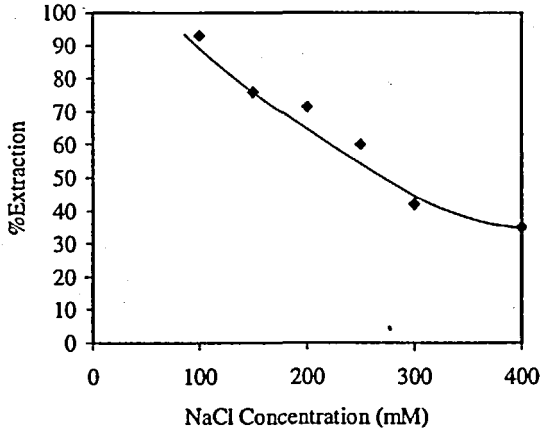

FIG. 3 Reverse micellar extraction of α-chymotrypsin as a function of NaCl concentration from an aqueous solution with initial concentrations of 1 g/L α-chymotrypsin and an organic solution with an initial concentration of 100 mM NaPSS surfactant. The solid line is the result of the correlation using the model.

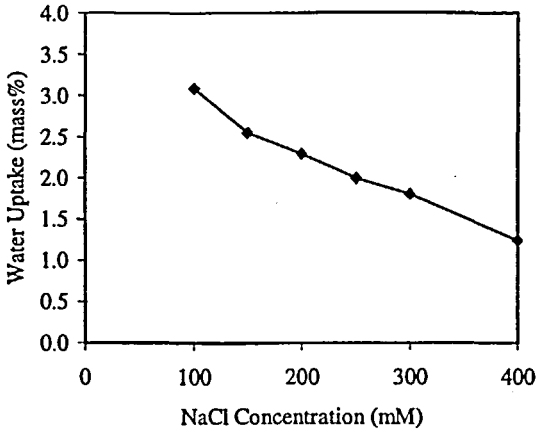

FIG. 4 Effect of the NaCl concentration on the water uptake of the reverse micelles formed by NaPSS in an aqueous solution with initial concentrations of 1 g/L α-chymotrypsin and an organic solution with an initial concentration of 100 mM NaPSS surfactant. The solid line is an aid for the eyes.

the reverse micelles. Higher ionic strength in the water pool decreases the dissociation of the surfactant molecules head groups and results in an electrostatic field inside the water pools with lower intensity. As discussed before, electrostatic interactions are the main reason for the reverse micellar extraction of proteins, and therefore an electrostatic field with lower intensity results in a lower percent of extraction of the protein molecules. Another reason for this phenomenon may be the fact that higher electrolyte concentrations lead to a decrease in the number of dissociated surfactant molecules. This leads to a weaker repulsion force between the surfactant molecules, and therefore they can form closer packed aggregates. The reverse micelles formed in this way are smaller in size and cannot accommodate the large protein molecules and also can dissolve less water in the organic phase. Therefore, the percent extraction of the protein molecules and the water uptake decrease as a result of the size exclusion effect. The reduction in the size of the reverse micelles formed by AOT surfactant with an increase in the salt concentration has been shown by the measurements performed using the light scattering. The results of these measurements are discussed by Matzke et al. (6).

Backward Extraction

It was shown that the extraction of α-chymotrypsin is possible using the reverse micelles formed by NaPSS. The next step after a successful extraction

process is the backward extraction of the protein molecules to a more concentrated solution. Experiments were performed to investigate the feasibility and efficiency of the backward extraction of α-chymotrypsin from a reverse micellar phase formed by NaPSS. As shown in Fig. 1, the percent extraction of α-chymotrypsin dramatically decreases at very low pH values. The reason for this phenomenon was attributed to the ion exchange between the hydrogen ions and sodium ions in the reverse micelles, resulting in the formation of the HPSS according to Equilibrium Reaction (2). This phenomenon was exploited for the backward extraction process. The results show that in all cases the reverse micelles formed by NaPSS were destroyed as a result of the ion exchange between the hydrogen ions and the sodium ion of the NaPSS, and therefore the α-chymotrypsin molecules were released to the new aqueous phase. The measurements performed for the water uptake indicated that no water was dissolved in the organic phase. These results also proved that high concentrations of hydrogen ions in the aqueous phase demolish the reverse micelles formed by NaPSS. As HPSS is not soluble in water, no trace of it was detected in the new aqueous phase. Experiments show that the resulting aqueous phase was several times more concentrated in α-chymotrypsin than the initial solution and the backward extraction did not introduce any contamination.

MODELING FRAMEWORK

In this section a model is developed to correlate experimental data of the effects of the pH and salt concentration on the reverse micellar extraction of proteins. In this model the reverse micellar phase is considered as a homogeneous phase containing active sites, each site representing one reverse micelle. The protein molecules are considered to be ion-exchanged with the counterions of these active sites as a result of the electrostatic forces between the charged protein molecules and charged head group of surfactant molecules. For simplicity the model is derived for reverse micelles formed by anionic surfactants similar to NaPSS used in this study. The final relations are also given for the extraction of protein with reverse micelles formed by cationic surfactants.

A protein molecule, in order to be ion exchanged with the counterions of the reverse micelles formed with anionic surfactants, should be positively charged. The number and the sign of charges carried by a protein molecule depend on the pH of the system. As mentioned before, proteins at pH values lower than their pI values are positively charged and at pH values higher than their pI values are negatively charged. The charging process of a protein in the bulk aqueous phase can be represented by a reversible equilibrium reaction between the protein molecule and hydrogen ion as:

$$P^- + H^+ \xleftrightarrow{K_P} P^+ \tag{3}$$

where P^-, P^+, and H^+ refer to the negatively and positively charged proteins and hydrogen ion, respectively. The parameter K_P is the equilibrium constant defined as

$$K_P = \frac{a_{P^+}}{a_{P^-} a_{H^+}} \tag{4}$$

where a is the molar based activity defined as

$$a_i = \gamma_i C_i \tag{5}$$

with γ being the molar-based activity coefficient. It should be noted that proteins are large molecules with large hydrocarbon backbones and very high dipole moments. Therefore, in the absence of external electrostatic fields, short-range interactions such as dipole–dipole and excluded volume effect are responsible for their interactions in aqueous solutions. Thus, the effect of the long-range interactions arising from the charged characteristic of protein molecules can be neglected. Therefore, it is reasonable to assume that the activity coefficients of the negatively and positively charged proteins in the bulk aqueous phase are equal. Using this assumption, Eq. (4) can be written as

$$C_{P^-}/C_{P^+} = 10^{(pK_P + pH)} \tag{6}$$

The ion exchange of the positively charged protein molecules on the reverse micelles can also be represented by an equilibrium reaction between the charged reverse micelles, RM, and protein molecules. As discussed before, in this model each reverse micelle acts as an active site in an ensemble of equivalent sites. Therefore, the equilibrium reaction can be written as

$$P^+ + \overline{RM} \xleftrightarrow{K_{PRM}} \overline{PRM} \tag{7}$$

The overbar symbol in Eq. (7) indicates the organic phase. In the above reaction PRM represents the protein-filled reverse micelles. The equilibrium reaction constant, K_{PRM}, for Reaction (7) can then be written as

$$K_{PRM} = \frac{\overline{a_{PRM}}}{a_{P^+} \overline{a_{RM}}} \tag{8}$$

Assuming that every protein-filled reverse micelle carries only one protein molecule, the concentration of protein molecules in the organic phase equals the concentration of protein-filled reverse micelles. Also, as the reverse micelles in this model are considered as noninteractive ion-exchange sites, their

activities are equal to their molar concentrations. Therefore, Eq. (8) simplifies to

$$K_{PRM} = \frac{\overline{C_{P^+}}}{\gamma_{P^+} C_{P^+} \overline{C_{RM}}} \tag{9}$$

The partitioning coefficient of protein molecules between the aqueous and organic phases can also be shown through an equilibrium relation as

$$P \xleftrightarrow{K_{dis}} \overline{P} \tag{10}$$

The equilibrium distribution constant, K_{dis}, for Equilibrium Reaction (10), assuming that the activity coefficients of protein molecules in the bulk aqueous phase are governed by the long-range interactions and therefore their charge signs, can be written as

$$K_{dis} = \frac{\overline{a_P}}{a_P} = \frac{\overline{a_{P^+}}}{(C_{P^+} + C_{P^-})\gamma_P} \tag{11}$$

Combining Eq. (9) with Eq. (11) gives

$$K_{dis} = \frac{\overline{\gamma_{P^+} C_{RM}} K_{PRM}}{1 + 10^{(pK_P + pH)}} \tag{12}$$

The percent of extraction, *Ext%*, can then be calculated from Eq. (12) as

$$Ext\% = \frac{K_{dis}}{1 + K_{dis}} = \frac{\overline{\gamma_{P^+} C_{RM}} K_{PRM}}{1 + 10^{(pK_P + pH)} + \overline{\gamma_{P^+} C_{RM}} K_{PRM}} \tag{13}$$

It was shown in the Experimental Section that the hydrogen ions present in the solution compete with the protein molecules to be ion exchanged with the reverse micelle sites. As a result, at low pH values the extraction of protein molecules decreases dramatically. In order to consider this phenomenon in the present model, the ion exchange of the hydrogen ions on the reverse micelle sites is mimicked by the following equilibrium reaction:

$$H^+ + \overline{RM} \xleftrightarrow{K_{HRM}} \overline{HRM} \tag{14}$$

The equilibrium reaction constant, K_{HRM}, can be written as

$$K_{HRM} = \frac{\overline{C_{RM}^{init}} - \overline{C_{RM}}}{\overline{C_{RM}} 10^{-pH}} \tag{15}$$

It should be noted that the reverse micelles after and before their reactions with hydrogen ions are regarded as noninteracting ion-exchange sites. Therefore, their activity coefficients equal unity. In Eq. (15), C_{RM}^{init} represents the

initial concentration of the reverse micelles. Substituting the concentration of reverse micelle sites, C_{RM}^{init}, from Eq. (15) to Eq. (13) leads to the following relation for the percent of the extraction:

$$Ext\% = \frac{\overline{\gamma_{P^+}} K}{[1 + 10^{(pK_P + pH)}][1 + K_{HRM} 10^{-pH}] + \overline{\gamma_{P^+}} K} \tag{16}$$

where reaction constant K for convenience is defined as

$$K = C_{RM}^{init} K_{PRM} \tag{17}$$

As mentioned before, Eq. (16) is derived specifically for the reverse micelles formed by anionic surfactants. A similar relation can be derived for the reverse micellar extraction of proteins with cationic surfactants as

$$Ext\% = \frac{\overline{\gamma_{P^-}} K}{[1 + 10^{(pK_P + pOH)}][1 + K_{HRM} 10^{-pOH}] + \overline{\gamma_{P^-}} K} \tag{18}$$

It should be noted that the Equilibrium Chemical Reaction (7) was written at a constant salt concentration and, as discussed before, salt concentration influences the extraction of reverse micellar extraction of proteins. In order to consider the effect of the salt concentration in the model presented here, the K parameter in Eq. (16) is considered to depend on the salt concentration as

$$K = \alpha + \beta C_{salt} \tag{19}$$

Therefore, in Eq. (17), in order to correlate the experimental data of reverse micellar extraction of proteins, the equilibrium reaction constants α and β, and K_{HRM}, and the activity coefficient of protein molecules in reverse micelles should be evaluated.

The activity coefficients of protein molecules in reverse micelles are represented by a model proposed by Pitzer (17). As discussed before, charged protein molecule in the water pool of the reverse micelles are surrounded by the charged head groups of ionized surfactant molecules. This gives rise to an electrostatic field in the cavity between the outer surface of the protein molecule and the inner wall of the reverse micelle. As a result, the chemical potentials of the charged species, including the protein molecule in this cavity, are highly under the influence of the long-range interaction forces. Therefore, in the calculation of the activity coefficient of the protein molecules entrapped in reverse micelles, the effect of the short-range interaction forces compared to the effect of the long-range interaction forces can be neglected. Thus the activity coefficient of the charged protein molecules using the Pitzer model can be written as

$$\ln \overline{\gamma_P} = -A\left[\frac{2z_P^2}{b}\ln(1 + b\sqrt{I_x}) + \frac{z_P^2\sqrt{I_x} - 2I_x\sqrt{I_x}}{1 + b\sqrt{I_x}}\right] \quad (20)$$

where A is the Debye–Huckel constant and b is the parameter of the Pitzer model:

$$b = 2150\left(\frac{d_{H_2O}}{DT}\right)^2 \quad (21)$$

where d_{H_2O} is the density of water, D is the dielectric constant of water, and T is the absolute temperature. In Eq. (20), I_x denotes the mole-fraction-based ionic strength, defined as

$$I_x = 0.5\sum_i x_i z_i^2 \quad (22)$$

where x is the mole fraction of the ionic species in the water pool and the sum goes over all the ionic species. The ionic species in the water pool are considered to be the charged protein molecules, surfactant head groups, surfactant counterions, and hydrogen ions. Since experimental data for the density of the water in the water pool are not available, in order to calculate the mole fractions in Eq. (22) it is assumed that the density of the solution of the water pool is the same as the density of the pure water. The mole fractions of the ionic species are calculated based on the total moles of the ionic species in the total moles of water dissolved in the reverse micellar phase obtained from the water uptake experimental data. It should be noted that the activity coefficient obtained from the Pitzer model is normalized based on the mole fraction. As in this work we approximated the density of the water pool with that of the pure water, the difference between the mole-fraction-based and molarity-based activity coefficients will be eliminated (19).

RESULT AND DISCUSSION OF THE MODEL

The model developed here was applied to correlate the experimental data of the effects of the salt concentration and pH on the reverse micellar extraction of the α-chymotrypsin obtained in this study. The number of charges carried by α-chymotrypsin at various pH values, required by the activity coefficient model, were obtained from the literature (20). The parameters K_{HRM}, α, and β were treated as adjustable parameters of the model, and their values were calculated from the experimental data of the percent extraction of the protein at various pH values and salt concentrations.

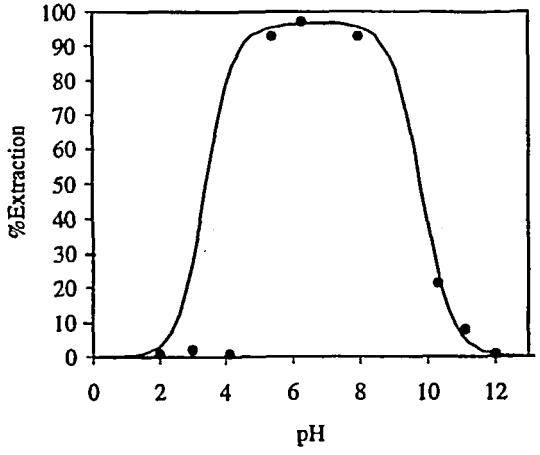

FIG. 5 Correlation of the reverse micellar extraction of α-chymotrypsin as a function of pH.

Figure 5 shows the results of the correlation of the percent extraction of α-chymotrypsin as a function of pH of the solution obtained in this study. Similarly, Fig. 6 shows the correlation of the percent extraction of Ribonuclease A and trypsin inhibitor as a function of pH of the solution with AOT as the surfactant obtained from the literature (5). As can be seen from these

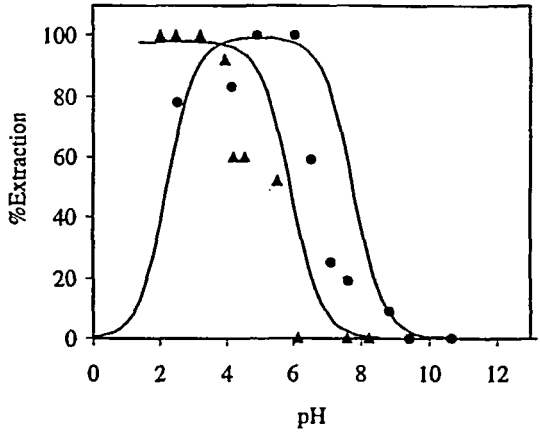

FIG. 6 Correlation of the reverse micellar extraction of two proteins as a function of pH with AOT as the surfactant. (●) Ribonuclease A and (▲) trypsin inhibitor.

figures, the model developed in this study can correlate the experimental data with good accuracy. It can also be seen that at low pH values the model slightly deviates from the experimental data. This may be due to the failure of the Pitzer model to represent the activity coefficient of protein molecules at high concentrations of hydrogen ion and the large number of charges on the protein molecules at low pH values. It is important to mention that some of the assumptions made in the derivation of the model presented here arise from the lack of experimental data. For example, more physically meaningful parameters can be obtained by measuring the aggregation number of the reverse micelles, the ratio of protein molecules to reverse micelles in the organic phase, and the ionization pattern of the charged inner wall of the reverse micelles and protein molecules at different salt concentrations.

The solid line in Fig. 3 shows the correlation of the experimental data for the reverse micellar extraction of α-chymotrypsin as a function of salt concentration. Once more it can be seen that the model represents the experimental data with good accuracy.

CONCLUSIONS

Experiments showed that the reverse micelles formed by bis(2,4,4-trimethylpentyl) sodium dithiophosphinate (NaPSS) as an anionic surfactant can extract α-chymotrypsin from aqueous solutions. It was found that both pH and salt concentration strongly influence the percent extraction. The reason for the observed phenomena was attributed to the electrostatic interactions between the charged protein molecules and charged head group of the surfactant molecules. It was shown that at low pH values the ion exchange between the hydrogen ions and surfactant counterions demolishes the structure of the reverse micelles and releases the protein molecules entrapped in them. This effect was used for the backward extraction of protein molecules from the reverse micellar phase.

A model was developed to correlate the experimental data obtained in this study and those reported in previous studies. The model considers the reverse micelles as noninteractive active sites. The activity coefficients of the protein molecules in reverse micelles are represented by the Pitzer model. It was shown that the model can accurately correlate the experimental data.

SYMBOLS

A	Debye–Huckel constant
a	activity
b	parameter of the Pitzer model
C	molar concentration

D	dielectric constant of water
d_{H_2O}	density of water
$Ext\%$	percent extraction
I_x	mole-fraction-based ionic strength
K_{dis}	protein distribution equilibrium constant
K_{HRM}	hydrogen ion-reverse micelle binding reaction constant
K_P	protein charging process reaction constant
K_{PRM}	protein-reverse micelle binding reaction constant
T	absolute temperature
x	mole fraction
z	charge number
α	adjustable parameter
β	adjustable parameter
γ	molar-based activity coefficient

Subscripts and Superscripts

init	initial concentration
H	hydrogen ion
HRM	hydrogen ion bonded reverse micelles
P	protein
PRM	protein-filled reverse micelles
RM	reverse micelles
+	positively charged
−	negatively charged

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