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Reverse Micellar Extraction and Backextraction of L-Lysine with Three Dialkyl Sodium Phosphinates in Pentanol/Isooctane Mixtures

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ABSTRACT

The effects of pH, salt concentration, and structure of the surfactant head on the reverse micellar extraction and backextraction of L-lysine in pentanol/isooctane mixtures have been comparatively studied. The surfactants used were bis(2,4,4-trimethylpentyl) sodium phosphinate (NaPOO), bis(2,4,4-trimethylpentyl) sodium monothiophosphinate (NaPSO), and bis(2,4,4-trimethylpentyl) sodium dithiophosphinate (NaPSS). Since all three surfactants have the same two hydrocarbon tails and differ only in their polar heads, their comparative study gives some insight into the effect of the surfactant head group on the reverse micellar extraction and backextraction of amino acids. The results show that the nature of the surfactant head, the pH, and the salt concentration have a major effect on the reverse micellar extraction of L-lysine. The percent of L-lysine extracted to the organic phase, at fixed surfactant and pentanol concentrations, decreased in the order NaPSS > NaPSO > NaPOO. The exchange with the three surfactants can be easily reversed for the backextraction of amino acids into a new surfactant-free aqueous solution.

INTRODUCTION

The production by fermentation of biomolecules of interest in the food, agriculture, and drug industries has become increasingly attractive at an industrial scale. These bioproducts are usually obtained in a dilute aqueous fermentation medium together with other materials such as substrates

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and nutrients. Therefore, their separation and purification is of economic importance. The need for efficient and easy-to-scale separation methods for bioproducts is due to their large annual production rates which can be as large as 500,000 tons and the fact that the cost of purification and concentration can be up to 50% of their final production cost (1). Various forms of chromatography (column liquid, molecular exclusion, ion-exchange affinity etc.), electrophoresis, salt and solvent precipitation, dialysis, and ultrafiltration have been applied for the purification and separation of biomolecules. However, these techniques are expensive and difficult to scale-up (2).

Liquid–liquid extraction has been shown to be an effective separation process. Since most bioproducts such as proteins, peptides, and amino acids are hydrophilic molecules, they cannot be solubilized directly in nonpolar solvents. Reverse micellar extraction, due to its unique properties, has economic potential for the separation and purification of biomolecules. Reverse micelles are the aggregation of surfactant molecules around minute water pools in an organic solvent. Some surfactants need the presence of a cosurfactant in order to form reverse micelles. Reverse micelles make it possible to solubilize biomolecules in organic solvents while maintaining them in a microaqueous environment. The biomolecules are trapped in the water pools and are not in direct contact with the organic solvent. A typical reverse micellar extraction system is depicted in Fig. 1. The main factor in extraction via reverse micelles is the electrostatic interaction between the charged guest molecules and the oppositely charged surfactant molecules (3).

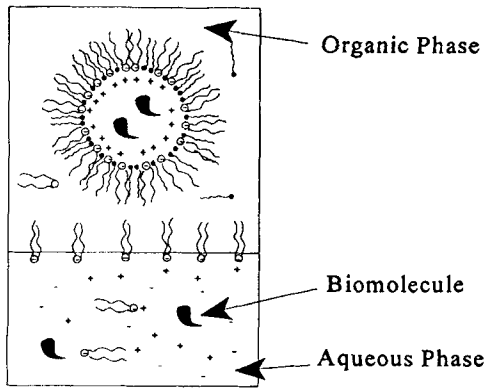

FIG. 1 Schematic representation of a reverse micellar extraction of biomolecules: surfactant molecules ~~D; cosurfactant molecules ●~~; different ions +, −.

The reverse micellar extraction of proteins has been studied extensively (2, 4–8), and the reverse micellar extraction of amino acids has also been the subject of some studies (9–13). Results have been recently reported (14) for the extraction of L-phenylalanine using 283 g/L of the ammonium salt of Cyanex 301 in decanol (undiluted). The effect of the equilibrium pH on the percent extraction and one extraction isotherm at 25°C and pH 3.0 to 3.3 were reported.

Several amino acids are produced in relatively large amounts by fermentation. Due to the product inhibition effect of some amino acids, such as L-lysine, upon biomass growth in the fermentation process, their concentration must be kept low. This renders the product removal difficult and expensive (15), and promotes the search for new methods of separation. In addition, since amino acids are the basic units of peptides and proteins, the study of their extraction may provide some insight into the solubilization of larger biomolecules. Amino acids, due to their zwitterionic nature, are insoluble in organic solvents and, therefore, all the amino acid solubilized in an organic phase containing reverse micelles dissolve in the water core and are not dissolved in the solvent.

For a system formed by a particular surfactant, cosurfactant, and organic solvent, at fixed temperature and pressure, the main factor in the extraction of amino acids is the electrostatic interaction between the charged amino acid and the surfactant molecules (10–13, 16). The localization of amino acid molecules in the reverse micelles is closely related to their charged states (9). Amino acids at different pH values have different charged states. An amino acid at a low pH is positively charged and has a tendency for ion exchange with the counterion of an anionic surfactant. The concentration of salt in the aqueous phase also has an important influence on the extraction of amino acids. For a salt containing the same counterion as the surfactant, an increase in the salt concentration favors the undissociated form of the surfactant, and thus decreases the tendency of the amino acid to be exchanged with the surfactant counterion. In general, an increase in the salt concentration in the aqueous phase increases the charge density inside the reverse micelles and prevents the amino acid from entering the reverse micelles (12).

Few surfactants have been studied in the literature for the reverse micellar extraction of amino acids. Among the anionic surfactants which have been used for the reverse micellar extraction of amino acids, the most widely studied is Aerosol OT (AOT), the sodium salt of bis(2-ethylhexyl) sulfosuccinate (17). Very limited data are available on the ability of other anionic surfactants for reverse micellar extraction and backextraction of amino acids.

One important aspect in reverse micellar extraction processes is the backextraction step. Once a biomolecule is extracted into the organic

phase and separated from the original aqueous phase, it must be eventually recovered in a new aqueous phase. This process is called backextraction. Although without backextraction the process for the separation of biomolecules is not complete, very little work has been done in this field. The major problems with backextraction, when it is possible, is its low efficiency and the presence of surfactant or other impurities in the final aqueous phase. Marcozzi et al. (18) studied the backextraction of α-chymotrypsin from an AOT reverse micellar solution by adding silica gel to the reverse micellar solution and adsorbing the extracted α-chymotrypsin. Pires and Cabral (7) studied the backextraction of recombinant protein from hexadecyltrimethylammonium bromide (CTAB) reverse micellar solutions by contacting the loaded organic phase with an aqueous phase containing different amounts of KCl.

The formation of reverse micelles with three dialkyl sodium phosphinate surfactants in isooctane/alcohol mixtures has been recently reported (19, 20). The surfactants studied were bis(2,4,4-trimethylpentyl) sodium phosphinate (NaPOO), bis(2,4,4-trimethylpentyl) sodium monothiophospinate (NaPSO), and bis(2,4,4-trimethylpentyl) sodium dithiophosphinate (NaPSS). Their structures are presented in Fig. 2. The objective of the present work was to determine the effect of pH, salt concentration, and surfactant head group structure on the reverse micellar extraction and backextraction of L-lysine. Since all these surfactants have the same two

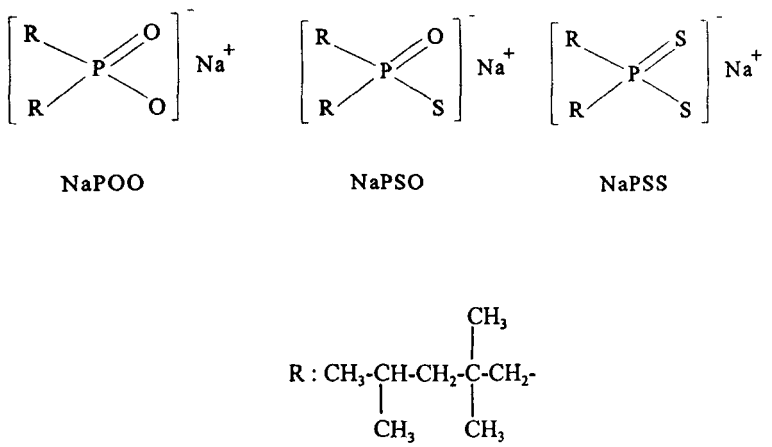

FIG. 2 Structures of bis(2,4,4-trimethylpentyl) sodium phosphinate (NaPOO), bis(2,4,4-trimethylpentyl) sodium monothiophospinate (NaPSO), and bis(2,4,4-trimethylpentyl) sodium dithiophosphinate (NaPSS).

hydrocarbon tails and differ only in their head groups, their comparative study gives some insight into the effect of the structure of the surfactant head group on the reverse micellar extraction and backextraction of amino acids.

EXPERIMENTAL METHODS

Three commercial chemicals bis(2,4,4-trimethylpentyl) phosphinic acid (CYANEX 272, 85% purity), bis(2,4,4-trimethylpentyl) monothiophosphinic acid (CYANEX 302, 84% purity), and bis(2,4,4-trimethylpentyl) dithiophosphinic acid (CYANEX 301, 75% purity), obtained from Cyanamid Canada Inc. (Toronto, Ontario), were purified by a copper salt precipitation method (21). The final purities of the acids obtained from CYANEX 272, CYANEX 301, and CYANEX 302 were 99.4, 99.0, and 99.1%, respectively, as determined by titration with a Model 93-42 Orion surfactant electrode (Orion Research Inc., Cambridge, Massachusetts) and by potentiometric titration with a 0.05 N NaOH solution using a Metrohm Brinkmann 691 pH meter. To prepare the surfactants, the purified acids were neutralized with a 1 N sodium hydroxide solution until the pH in the aqueous phase reached a value of about 8.25. The reaction was:

$$S^-H^+ + NaOH \rightleftarrows S^-Na^+ + H_2O$$

where S^- is the ionic form of the surfactant.

The three dialkyl sodium phosphinates, bis(2,4,4-trimethylpentyl) sodium phosphinate (NaPOO), bis(2,4,4-trimethylpentyl) sodium monothiophosphinate (NaPSO) and bis(2,4,4-trimethylpentyl) sodium dithiophosphinate (NaPSS), thus formed were used as surfactants in all experiments. The sodium salts of these surfactants, unlike their acid forms are completely water soluble over the whole range of surfactant concentrations used in this study.

The amino acid used was L-lysine, obtained from Sigma (St. Louis, Missouri), and used as received. *o*-Phthaldialdehyde (OPA), 2-mercaptoethanol, and sodium tetraborate were purchased from Pfaltz & Bauer Inc. (Toronto, ON). All other chemicals were obtained from A&C American Chemical Ltd. (Montreal, QC) and were used without further purification. Isooctane was HPLC grade, and all other chemicals were of reagent grade. Deionized water, with an electrical conductivity less than 0.8 μS/cm, was used for all experiments.

All extraction experiments were performed by contacting 20 mL of an organic solution containing 400 mM pentanol in isooctane with 10 mL of an aqueous solution containing 200 mM surfactant and 10 mL of an aqueous solution containing NaCl, L-lysine, and HCl to adjust the pH. All backex-

traction experiments were performed by contacting 10 mL of a loaded organic solution with 10 mL of a 1 N HCl solution. For extraction, the samples were shaken for 1 hour at 200 rpm and settled for 1 week at 23°C to reach equilibrium. For backextraction, the samples were shaken for 30 minutes at 200 rpm and settled for 1 hour at 23°C. The phases were separated and analyzed for water uptake in the organic phase and surfactant, and L-lysine content and final pH in the aqueous phase. The water content of the organic phase was determined by Karl Fischer (KF) titration using a Metrohm-Brinkmann Model 701/1 KF Titrator, and the surfactant content was determined by potentiometric titration. The pH of the aqueous phase was measured by a Model 691 pH Meter (Metrohm Ltd.). In all experiments, before separating the phases, it was verified that both phases were transparent. The concentration of L-lysine in the aqueous phase was measured with a CARY 1/3 UV spectrophotometer (Varian Techtron Pty Ltd., Victoria, Australia) using the modified OPA labeling method (22–24) at 340 nm. The OPA reagent was prepared by mixing 0.8 g OPA with 20 mL ethanol, adding 10 g sodium tetraborate and 2 mL 2-mercaptoethanol, and then bringing the volume to 1000 mL with water. New OPA reagent was prepared for each set of experiments and used immediately after preparation. In order to measure the concentration of L-lysine in an aqueous phase, 0.6 mL of a sample containing L-lysine was added, under mixing, to 20 mL of reagent solution, and then, after 5 minutes, the UV absorbance at 340 nm was measured. A calibration curve for each set of measurements was prepared.

RESULTS AND DISCUSSION

Effect of pH on the Extraction of L-Lysine and on the Water Uptake

Figures 3 and 4 show the effect of initial pH on the partitioning of L-lysine and on the water uptake. The initial organic phase was a solution of 400 mM pentanol in isooctane. The initial aqueous phase contained 100 mM NaPOO, NaPSO, or NaPSS as surfactant, 40 mM NaCl, 5 mM L-lysine, and HCl to adjust the pH. In this work, unlike in previous works with amino acids (9, 10), the pH was adjusted by addition of HCl and no buffer solution was used. Buffer solutions usually consist of weak salts which dissociate in the aqueous solution, and thus their anions compete with the amino acid in the extraction process.

From Fig. 3 it can be seen that pH has a major effect on the phase partitioning of L-lysine. This is due to the fact that amino acids have different charged states at different pH values. Since NaPOO, NaPSO, and NaPSS are anionic surfactants and, after dissociation in water are

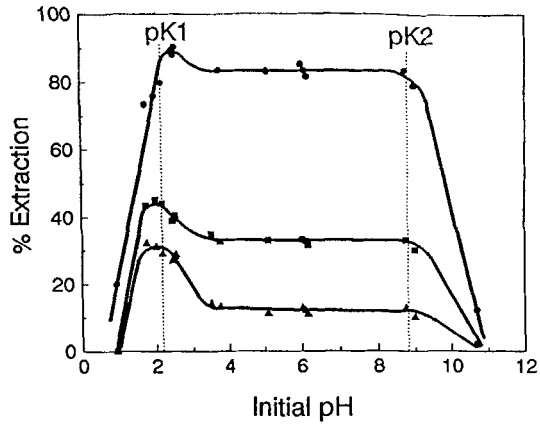

FIG. 3 Effect of surfactant structure and pH on the extraction of L-lysine for (▲) NaPOO, (■) NaPSO, and (●) NaPSS at 400 mM pentanol, 100 mM surfactant, 40 mM NaCl, and 5 mM L-lysine.

negatively charged, their tendencies for the extraction of amino acids differ with the charged state of the amino acid. L-lysine has three pK values, pK_1 = 2.18, pK_2 = 8.95, and pK_R = 10.53, which are defined as the first, second, and R group dissociation constants, respectively (25). At pH values higher than pK_2, L-lysine has no charge and therefore has no tendency to exchange with the counterion of the surfactant molecules. At

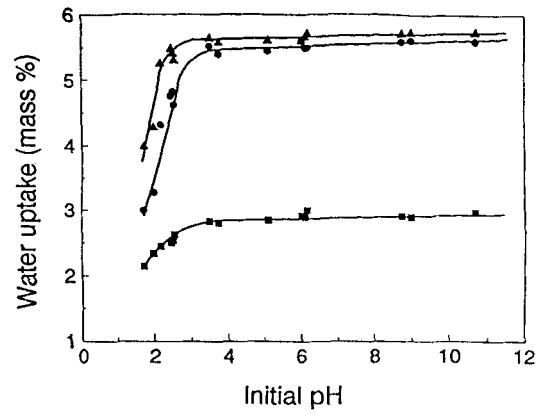

FIG. 4 Effect of pH on the water uptake for (▲) NaPOO, (■) NaPSO, and (●) NaPSS at 400 mM pentanol, 100 mM surfactant, 40 mM NaCl, and 5 mM L-lysine.

pH values between pK_1 and pK_2, the L-lysine has one net positive charge (two positive and one negative charges), and it exchanges with the counterions of the surfactant molecules. At pH values between 2.18 and 8.95, the extraction presents a plateau where pH does not have a major effect on the extraction of L-lysine. At a pH value lower than 2.18, where the L-lysine has two positive charges, it exchanges favorably with the counterion of the surfactant. On the other hand, at very low pH the concentration of H^+ ions is very high and the exchange of the H^+ ions is favored, thus reducing the extraction of L-lysine. Therefore, at pH values lower than pK_1 there is first an increase and then a dramatic decrease in the extraction. This behavior can be used for the backextraction of L-lysine. In all extraction experiments the final pH was from 8.2 to 10 for NaPOO, from 8 to 9 for NaPSO, and from 6 to 7 for NaPSS, and the final concentration of surfactant in the aqueous phase was 6.3 mM. The low equilibrium pH after extraction with NaPSS clearly shows its buffering property and explains the high percent of extraction that can be attained with it.

Figure 4 shows that at low pH the water uptake decreases sharply due to the exchange of the surfactant counterion with H^+ ions. Since these surfactants in their acid form do not form reverse micelles (19, 20), the water uptake of the system decreases.

Effect of Salt Concentration

Figures 5 to 7 show the effect of salt concentration, at three different values of the pH, on the partitioning of L-lysine and on the water uptake. Note that for Fig. 7(a) the scale for the ordinate axis is different from the scale used in Figs. 5(a) and 6(a). As shown in these figures, salt concentration is an important factor which influences the partitioning of L-lysine in the systems studied. The dashed lines in Figs. 5 to 7 show that below 25 mM NaCl concentration, these surfactants do not form reverse micelles and stay in the aqueous phase. Since there are no reverse micelles present in the organic phase, the system cannot extract the L-lysine. The effect of salt concentration on the formation of reverse micelles with dialkyl sodium phosphinates has been discussed elsewhere (19, 20).

Figures 5 to 7 show that increasing the salt concentration decreases the L-lysine extraction and also slightly decreases the water uptake. The extraction of L-lysine decreases linearly with salt concentration over the whole range of salt concentration and pH studied. This decrease is due to the fact that the increase in salt concentration favors the formation of the undissociated form of the surfactant by virtue of the common-ion effect. In addition, increasing the salt in the aqueous phase increases the salt concentration inside the reverse micelles and therefore the amino acid is kept out of the reverse micelles (salting-out effect).

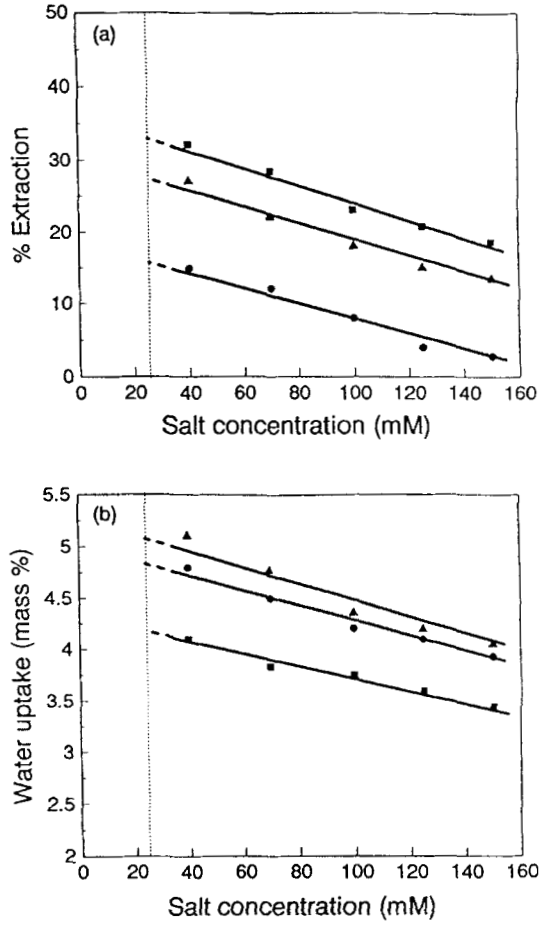

FIG. 5 Effect of NaCl concentration on: (a) extraction of L-lysine and (b) water uptake for (■) pH 1.74, (▲) pH 2.47, and (●) pH 3.75 at 400 mM pentanol, 100 mM NaPOO, and 5 mM L-lysine.

Effect of Surfactant Structure

Figures 3, 4, 8, and 9 show the effect of the surfactant head group on the extraction of L-lysine and on the water uptake. As it is evident from these figures, the structure of the head group of the surfactant has a significant effect on the extraction of L-lysine. The extraction ability of these surfactants increases in the order NaPOO < NaPSO < NaPSS. This result can be explained by the electronegativity of the head group of the surfac-

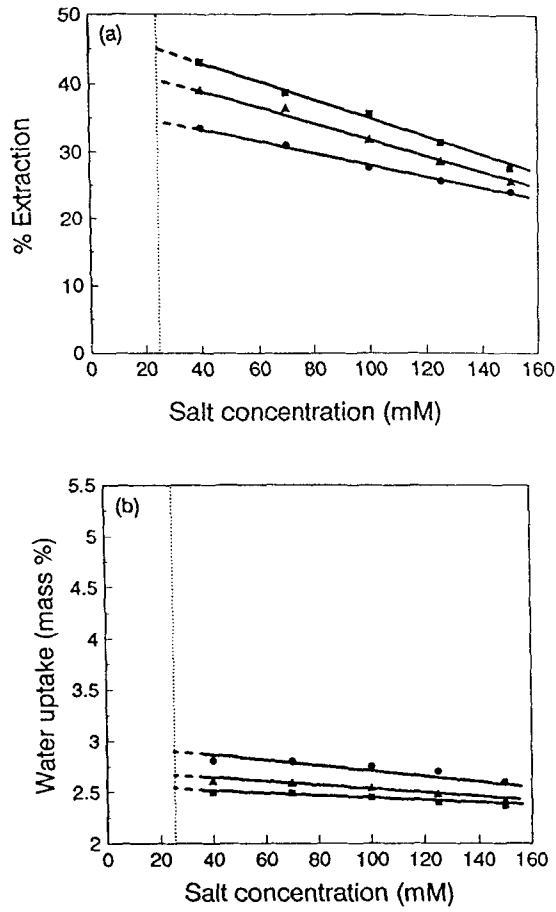

FIG. 6 Effect of NaCl concentration on: (a) extraction of L-lysine and (b) water uptake for (■) pH 2.19, (▲) pH 2.47, and (●) pH 3.75 at 400 mM pentanol, 100 mM NaPSO, and 5 mM L-lysine.

tants. Since the electronegativity of the S atom in the organophosphorus compounds is higher than that of the O atom (26), then sulfur substitution in the surfactant head group enhances the extraction. Figure 9 shows that the order of water uptake, when there is extraction, increases with surfactant in the order NaPOO > NaPSS > NaPSO. This order is different from the order of extraction but is the same as the order for the water uptake when there is no extraction (19, 20).

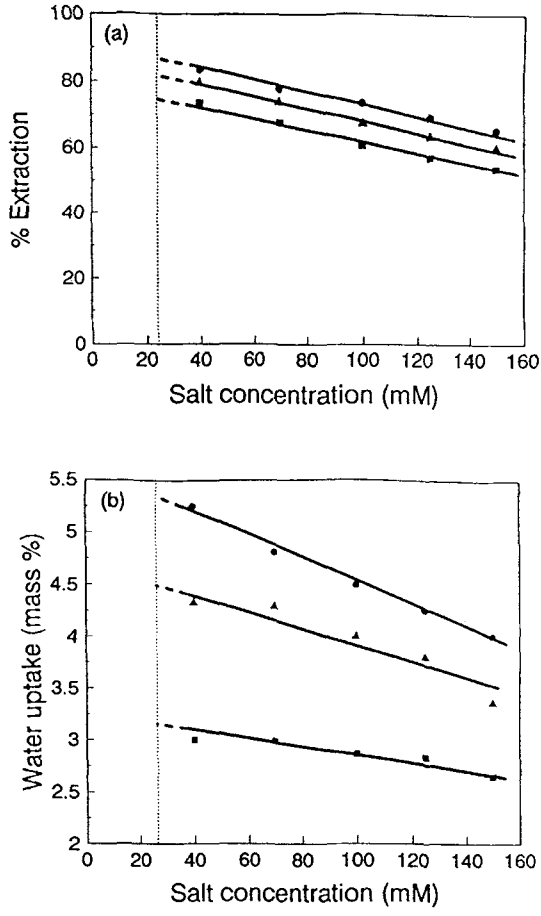

FIG. 7 Effect of NaCl concentration on: (a) extraction of L-lysine and (b) water uptake for (■) pH 1.74, (▲) pH 2.19, and (●) pH 3.75 at 400 mM pentanol, 100 mM NaPSS, and 5 mM L-lysine.

Backextraction of L-Lysine

Experiments were conducted to investigate the backextraction of the L-lysine from the organic phase reverse micellar solution. Reverse micellar extraction of L-lysine was performed under different conditions of pH and salt concentration. The samples of the loaded organic phase were then contacted with an equivalent volume of 1 N solution of HCl. The results showed a backextraction of about 90 to 96% for all samples. The efficiency

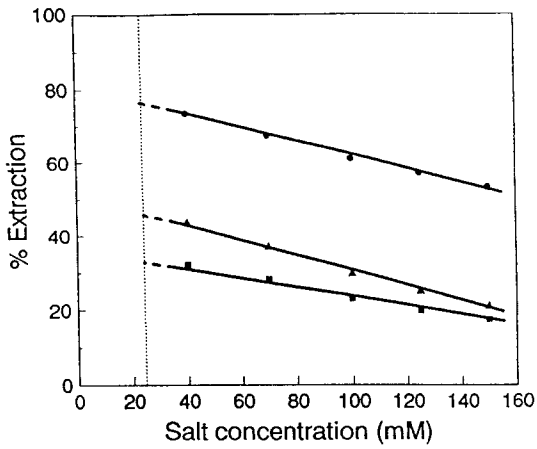

FIG. 8 Effect of surfactant structure and NaCl concentration on the extraction of L-lysine for (■) NaPOO, (▲) NaPSO, and (●) NaPSS at 400 mM pentanol, 100 mM surfactant, 5 mM L-lysine, and pH 1.74.

of the backextraction for all experiments was independent of the conditions under which extraction was performed. The aqueous phase was analyzed and no surfactant was detected. An explanation for this is that the H^+ ions replaced the Na^+ ions of the surfactants (NaPOO, NaPSO, and NaPSS) according to the following reaction:

$$S^-Na^+ + HCl \rightleftarrows S^-H^+ + NaCl$$

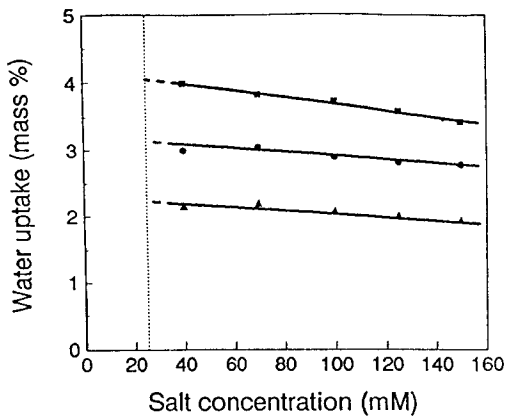

FIG. 9 Effect of surfactant structure and NaCl concentration on the water uptake for (■) NaPOO, (▲) NaPSO, and (●) NaPSS at 400 mM pentanol, 100 mM surfactant, 5 mM L-lysine, and pH 1.74.

where S^- is the ionic form of the surfactant. Since the acid form of these surfactants cannot form reverse micelles (19, 20), the reverse micelles in the organic phase were broken and the L-lysine was released to the aqueous phase. On the other hand, since the acid forms of these surfactants, in contrast to their sodium salts, are to a large extent insoluble in water (27), no surfactant was detected in the aqueous phase.

CONCLUSIONS

Reverse micellar extraction of L-lysine has been performed for the first time with the surfactants bis(2,4,4-trimethylpentyl) sodium phosphinate (NaPOO), bis(2,4,4-trimethylpentyl) sodium monothiophosphinate (NaPSO), and bis(2,4,4-trimethylpentyl) sodium dithiophosphinate (NaPSS), in a system consisting of isooctane, pentanol, water, NaCl, and HCl. The pH, concentration of salt, and structure of the surfactant head group were found to have a major effect on the reverse micellar extraction of L-lysine. The pH affects the extraction of L-lysine through changes in its charged states. At very low pH, due to the competition between L-lysine and hydrogen ions, the surfactants exchange their sodium counterions with the hydrogen ions and the extraction decreases dramatically. The nature of the head group of the surfactants has a significant effect on the reverse micellar extraction of L-lysine. The head group with higher electronegativity, NaPSS, extracts more L-lysine to the organic phase. The percent L-lysine extracted, at fixed concentration of surfactant and pentanol, decreased for the three surfactants in the order NaPSS > NaPSO > NaPOO, while the order of decreasing water uptake when there is extraction was NaPOO > NaPSS > NaPSO. The surfactants presented the same ordering for water uptake with extraction as the order previously found (19, 20) without extraction. Notably, the water uptakes of NaPOO and NaPSS are almost the same in all cosurfactant concentrations studied (19, 20). However, the capacity to extract L-lysine of these two surfactants is substantially different.

These surfactants have potential for the backextraction of the L-lysine, and other amino acids, to a new surfactant-free aqueous solution. The backextraction process is performed by contacting the loaded organic solution with a low pH aqueous solution. The efficiency of the backextraction was found to be independent of the conditions under which the extraction had been performed.

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