Formation of Reverse Micelles with Dialkyl Sodium Phosphinates and Their Use for Extraction of Amino Acids

by

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To my dear parents

Abstract

Experiments were performed to determine the ability of three anionic surfactants for the formation of reverse micelles and the reverse micellar extraction of L-lysine The surfactants studied 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) The effects of the concentration of salt, nature and concentration of surfactant and alcohol cosurfactants, on the formation of reverse micelles were studied comparatively. For the reverse micellar extraction of L-lysine, the effect of nature of the surfactant, pH and salt concentration were also comparatively studied Since all three surfactants have two identical tails and differ in their polar heads their comparative study gives an insight into the effect of the surfactant head in the formation of reverse micelles and the reverse micellar extraction of biomolecules

The results show that, under well defined conditions, these surfactants can form reverse micelles and have the ability to extract L-lysine They have a clear advantage over other surfactants for the back-extraction and concentration of amino acids into a new aqueous solution free of surfactant

Résumé

Des expériences furent réalisées afin de déterminer les performances de trois nouveaux surfactifs anioniques dans la formation de micelles inversées et dans l'extraction micéllaire inverse de L-lysine Les surfactifs étudiés furent le bis(2,4,4-trimethylpentyl) phosphinate de sodium (NaPOO), le bis(2,4,4-trimethylpentyl) monothiophosphinate de sodium (NaPSO) et le bis(2,4,4-trimethylpentyl) dithiophosphinate de sodium (NaPSS). L'effet de la concentration de sel ainsi que la nature et l'effet de la concentration de surfactif et des alcools cosurfactifs furent étudiés sur une base comparative pour la formation de micelles inversées Pour l'extraction micellaire de L-lysine, les effets de la nature du surfactif, du pH et de la concentration de sel furent également étudiés Puisque les trois surfactifs ont deux queues identiques et different dans la polarisation de leurs têtes, une étude comparative permet d'examiner l'effet de la tête du surfactif dans la formation de micelles inversées et l'extraction micéllaire inverse.

Les résultats démontrent que sous certains conditions spécifiques, ces surfactifs forment des micelles inversées et sont capables d'extraire le L-lysine Ces surfactifs ont des avantages sur certains autres dans la contre-extraction et la concentration d'acides aminés dans une nouvelle solution aqueuse en absence de surfactif.

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Chapter 1

Introduction

Reverse micellar extraction is emerging as one of the promising methods of surfactant-aided separation processes In biotechnology, recent developments have made the production of new biochemicals possible Many of these bioproducts, which are easily degradable, are produced in dilute aqueous solutions and should eventually be separated and purified. Reverse micelles provide microaqueous phases in which the biomolecule is entrapped and extracted to an organic phase without direct contact with the organic medium. Reverse micellar extraction can also be used for the extraction of metals from aqueous solutions for pollution control and wastewater treatment (Wason et al., 1988). Although the factors influencing the formation of reverse micelles, the reverse micellar extraction and the development of new surfactants have been investigated extensively (Luisi and Straub, 1984; Leodidis and Hatton, 1991; Matzke et al. 1992), there is much work to be done in the development of related technologies From an engineering point of view, the study of reverse micelles is in its infancy.

1.1 Formation of the Reverse Micelles

1.1.1 Surfactants

Surfactants are chemicals which have, in the same molecule, two distinct groups which differ greatly in their solubilities in water and in organic solvents (Winsor, 1948) Surfactants are also called amphiphiles because they are comprised of one or more lipophilic hydrocarbon tails and a hydrophilic head group. As surfactants are amphiphilic they tend to accumulate at the interfaces of polar and non-polar solvents (Eicke, 1984) Surfactants can be classified based on their charges after association in water as cationic, anionic and nonionic.

Certain amphiphiles due to their poor solubilities in water, cannot be used as primary surfactants but can be blended with other surfactant molecules to form a surfactant system These compounds are termed cosurfactants (Bourrel and Schechter, 1988).

1.1.2 Microemulsions

In polar medium, surfactant molecules above a certain surfactant concentration form aggregates called micelles. The surfactant concentration at which micelles begin forming is termed *the critical micelle concentration (CMC).* In the micelles, the polar head groups are directed outward and are in contact with the polar medium, whereas the non-polar tail groups are pointed toward the interior of the micelle to form a small region

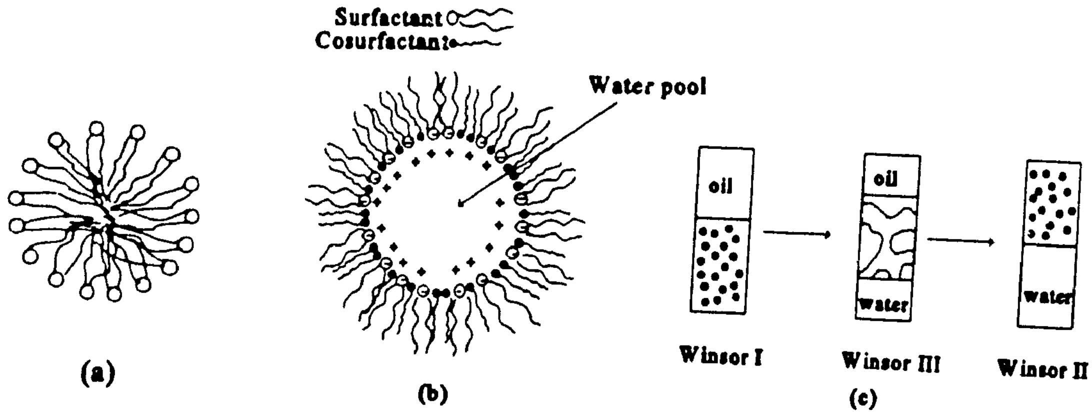


Figure 1.1 1: Schematic representation of (a): a micelle, (b): a reverse micelle and (c): transition from micellar solution to bicontinuous and reverse micellar solution.

from which the polar medium is essentially excluded. Over a narrow range of the surfactant concentration, above the CMC, the physical, colligative and spectral properties of the system undergo an abrupt change (Ruckenstein and Nagarajan, 1980).

Aggregation of surfactant molecules also occurs in a nonpolar medium and is called a reverse micelle. In reverse micelles, in contrast to micelles, the polar head groups are directed inward whereas the tail groups are directed outward and in contact with the non-polar medium shielding the inner polar core (see Figures 1.1.1 (a) & (b)). Polar compounds can be readily solubilized in this polar core, and in the case of water, the solubilized core is called *the water pool* (Luisi et al , 1988) The term microemulsion refers to isotropic, thermodynamically stable, clear, transparent, liquid-liquid colloidal systems which contain significant amounts of water, oil, surfactant and cosurfactant (Hoar and Schulman, 1943). Although not homogenous at a molecular level, a microemulsion is effectively a one phase system Winsor (1954) classified microemulsion systems into three types oil in water (O/W) in equilibrium with oil (micelles), water in oil (W/O) in equilibrium with water (reverse micelles) and bicontinuous (O+W) in equilibrium with oil and water These systems are also called Winsor microemulsion types I, II and III, respectively As shown in Figure 1.1.1 (c), the Winsor type III system consists of three phases and is a transition stage between the two extreme system types I and II Formation of each of these systems depends on the condition and composition of the phases and each type can be formed from another type by varying one or more parameters of the system

1.1.3 Phase Behaviour of Systems Containing Surfactants, Salt, an Organic Compound and Water

When oil, water, salt, surfactant and cosurfactant (if needed) are blended together and allowed to equilibrate, two or more phases may appear In many cases, almost all of the surfactant will reside in one of the phases together with various proportions of oil and water The phase containing the bulk of the surfactant is called a microemulsion phase Figures 1.1.2 (a) & (b) represent ternary diagrams of a brine, oil and surfactant system at fixed temperature, pressure and cosurfactant/surfactant ratio In Figure 1 1 2 (a) the two phase region has a plait point positioned well toward the oil side of the phase diagram

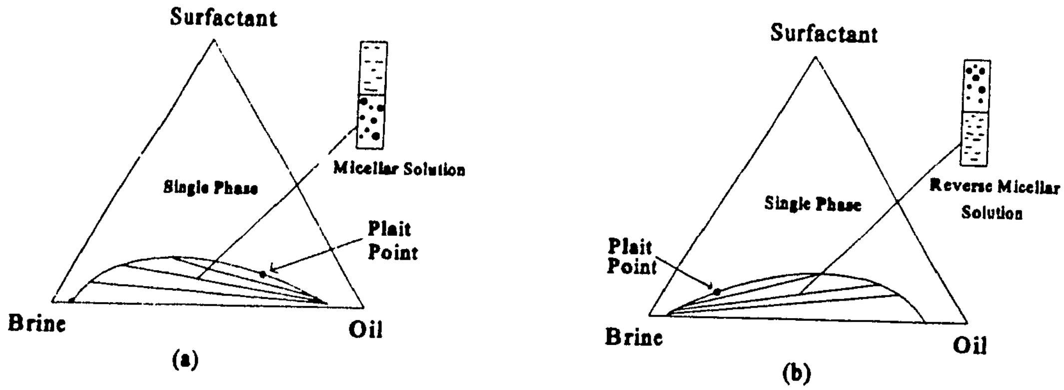


Figure 1 1.2 Schematic representation of typical phase diagrams for (a): a micellar solution and (b). a reverse micellar solution at constant temperature and constant cosurfactant/surfactant ratio.

Thus any point in this region will divide into a type I microemulsion phase (micelles) in equilibrium with an oil phase. The surfactant concentration in oil phase is very low whereas the aqueous phase contains almost all of the surfactant and some solubilized oil in the form of micelles Any point in the two phase region in Figure 1.1.2 (b), in contrast to Figure 1.1.2 (a), corresponds to an aqueous phase in equilibrium with an oil phase containing water. In this case, almost all the surfactant forms a type II microemulsion (reverse micelles) The tie lines in Figure 1.1 2 (a) are sloped in a different direction than they do in Figure 1.1.2 (b) This shows different tendencies of surfactant molecules to dissolve in the oil or in the aqueous phase. It is this distinction that determines the formation of either a type

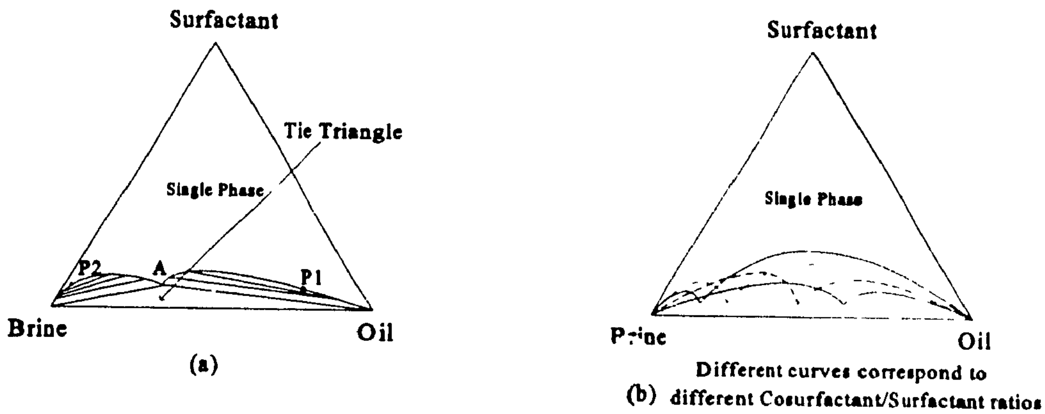


Figure 1.1.3: Schematic representation of (a). a tie triangle phase diagram (b) series of phase diagrams at different cosurfactant/surfactant ratio.

I or II microemulsions system Transformation of one phase diagram into the other (transformation from a micellar to a reverse micellar system) can be achieved by varying the temperature or the concentration of surfactant, cosurfactant or salt The behaviour of the system varies according to the structure of the surfactant lipophile or hydrophile and the oil molecules.

The transformation of a type I to type II system occurs via an intermediate three phase state, known as Winsor type III (or simply type III) system. A type I system such as the one shown in Figure 1.1 2 (a) can become a three phase system at a critical tie line, which may broaden into a tie triangle such as the one shown in Figure 1 1 3 (a) A system having an overall composition represented by a point in that area will divide into three phases One phase consists mostly of water containing small amounts of other compounds This phase is in equilibrium with a phase containing significant amounts of water, surfactant and oil, and with a phase containing mostly oil and very low amounts of the other compounds The oil phase is less dense than the other two phases and therefore it is the upper phase. The aqueous phase is the most dense and is the bottom phase and the surfactant rich phase is in the middle In the system depicted in Figure 1 1 3 (a), a change in the ratio of surfactant to cosurfactant results in, a series of phase diagrams, some of them are shown in Figure 1 1 3 (b) in a single ternary diagram For simplicity, only the boundaries separating the two phase and single phase regions are shown From the Figure 1 1 3 (b) it is evident that the intersection of the boundary curves, which corresponds to the vertex of the three phase region, shifts continuously toward the oil or the brine vertex of the phase diagram. The isotropic system near the oil vertex contains oil, surfactant and a small amount of water in terms of reverse micelles The system near the brine vertex contains water, salt, surfactant and a small amount of oil in the form of micelles In limiting conditions, the intersection point is positioned on the oil-brine axis, depicted in Figures 1 1.2 (a) and (b) There are many variables, apart from the ratio of surfactant to cosurfactant, which can transform a system composed of brine, surfactants and oil from a type I microemulsion (micellar system) to a type II (reverse micellar system) Some of these variables will be discussed in the following section

1.1.4 Factors Influencing the Phase Change

The phase transition of a system containing water, salt, surfactant, cosurfactant and oil is affected by varying one or more of the system parameters At fixed temperature and pressure the remaining variables are the salt concentration, the nature of the organic compound (the oil) and the nature and concentration of the surfactant and cosurfactant By changing any of these variables, a system may shift from a microemulsion type I to type III and eventually to type II, or the reverse All of the parameters mentioned above change the relative affinity of the surfactant for the oil and water and therefore tend to influence the system type (Bourrel et al , 1980)

- Surfactant Structure

Since surfactants are partly hydrophilic and partly lipophilic, they have in the same molecule different affinities for the aqueous and organic phases These affinities are a reflection of the strength of the cohesive energies between the surfactant tail and head with the organic and aqueous phases A balance between these forces dictates in which phase the surfactant resides

- Salt Concentration

The addition of salt to the aqueous phase promotes the migration of the surfactant to the organic phase (salting-out effect) Since the surfactant is partly hydrophilic, an increase in the salt concentration decreases the affinity of the surfactant head group with the aqueous phase, thus favouring the formation of reverse micelles Consequently, by increasing the salt concentration, the system tends to transform from type I to type III and type II. According to Leodidis and Hatton (1989), the addition of salt also increases the charge density inside the reverse micelles and screens the repulsive interactions between surfactant head groups

The salt concentration strongly influences the water uptake of reverse micellar systems The molar ratio of water to surfactant in a reverse micellar system decreases significantly with increasing the salt concentration and is also a function of the nature of the salt Aveyard et al (1986) investigated the distribution of the surfactant aerosol OT (AOT) between brine and heptane phases and found that below a certain salt concentration (70 mM), the AOT resides in the water phase and above this salt concentration, almost all of the AOT is transferred to the organic phase forming reverse micelles. Goklen (1987) found that high salt concentrations (>0 2 M) decrease the water uptake for AOT systems. Shinoda and Kunieda (1987) and Guerin and Bellocq (1988) also investigated the effect of salt concentration on the phase behaviour of the AOT in oil and water.

- Cosurfactants

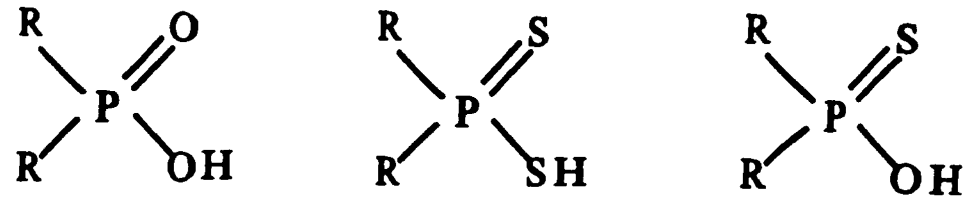
Cosurfactants participate in the interfacial region of the reverse micelles and screen the repulsive forces between the surfactant charged head groups, thus enhancing the aggregation of the surfactant molecules Since cosurfactants have polar head groups, their presence in the organic phase increases its polarity and thereby increasing the solubility of the surfactants in the organic phase The polarity of the organic phase has been shown (Shioi et al., 1991) to have a significant effect on the formation and size of the reverse micelles. Long chain alcohols are usually used as cosurfactants

- Nature of the Organic Phase

The structure of the organic phase influences the interaction forces between the surfactant tails and the organic solvent This directly affects the tendency of the surfactant to migrate to the organic phase, as well as its ability to aggregate According to Mukhejee et al (1983) shorter alkanes penetrate into the surfactant layer more effectively than do longer alkanes and thus tend to stabilize the reverse micelles Aveyard et al. (1986) showed that for AOT reverse micellar systems the water uptake drops dramatically above a certain carbon number of the hydrocarbon forming the organic phase For the same system, Ladanowski (1991) showed that water uptake increases as the length of the carbon chain of the organic solvent approaches the length of the carbon chain of the tails of the AOT and no reverse micelles will form when the chain length of the organic solvent exceeds that of the AOT tails.

1.1.5 Surfactants Studied in This Work

Few surfactants have been studied in the literature for the formation of reverse micelles. Among the surfactants which form reverse micelles, the most widely studied is Aerosol OT (AOT), the sodium salt of bis(2-ethylhexyl) sulfosuccinate (Eicke et al , 1984) and little data on the ability of the other surfactants for the formation of reverse micelles are available. In this work three commercially available dialkylphosphinic acids



CYANEX 272 CYANEX 301 CYANEX 302

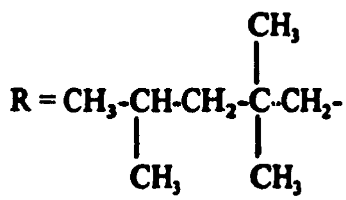
where: 

Figure 1.1.4. Structures of CYANEX 272, CYANEX 301 and CYANEX 302.

were studied. The surfactants studied were bis(2,4,4-trimethylpentyl) phosphinic acid (CYANEX 272), bis(2,4,4-trimethylpentyl) monothiophosphinic acid (CYANEX 302) and bis(2,4,4-trimethylpentyl) dithiophosphinic acid (CYANEX 301). These chemicals are used as extractants for metal ions from aqueous phases (Rickelton and Boyle, 1988). As shown in Figure 1.1.4, all these chemicals have two 2,4,4-trimethylpentyl tails and three different phosphinic acid heads. Therefore they can also play the role of a surfactant and should be able to form reverse micelles. As it was discussed in Section 1.1.4, for a surfactant, in order to form reverse micelles there should be a balance in the cohesive energies between the surfactant tail and head with the organic and aqueous phases. However, these surfactants were too hydrophobic and had very low solubility in water. These surfactants did not form reverse micelles under the conditions studied in this work. One possibility to form reverse micelles with these surfactants is to increase their cohesive energies with water so to make them more water soluble. This can be done through the change in the structure of their hydrophilic head groups Therefore, by substituting the hydrogen with sodium in their head groups and forming their sodium salt, they become more hydrophilic and could form reverse micelles (Corbridge, 1985) The procedure to substitute hydrogen by sodium in their head groups and the results of the study of the performance of the sodium salts will be described with more details in Chapter 2 and Chapter 3 Since all these surfactants have two identical tails and differ in their head groups a comparative study of them gives some insight into the effect of the surfactant head group on the formation of reverse micelles

1.2 Reverse Micellar Extraction of Amino Acids

1.2.1 Introduction

Recent developments in the field of biochemical engineering have enabled the production, by fermentation, of molecules of interest in the food, agriculture and drug industries (Jolivalt et al., 1990) These bioproducts are usually obtained in a dilute aqueous fermentation medium together with other materials such as substrates, nutrients, etc Therefore, their separation and purification is of economical importance. The need for efficient, and easily scalable separation methods for bioproducts is obvious since rate of annual production which can be as large as 500,000 tons and since the cost of purification and concentration is up to 50% of the final production cost (Eyal and Bressler, 1993).

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 beyond the laboratory size (Abbott and Hatton, 1988) Liquid-liquid extraction has been shown to be an effective separation process Since most of the bioproducts such as proteins, peptides and amino acids are hydrophilic molecules, they cannot be solubilized directly in non-polar solvents Reverse micellar extraction, due to some of its unique properties, has the potential to separate and purify these biomolecules The application of reverse micelles for the extraction of proteins and other bioproducts seems to be one of the most promising methods of the surfactant-mediated-separation processes (Hatton, 1989).

1.2.2 Extraction of Biomolecules Using Reverse Micelles

Reverse micelles provide an aqueous microenvironment in an organic medium, making it possible to solubilize proteins and other hydrophilic molecules in a bulk organic phase (Matzke et al., 1992). As discussed in section 1 1.2, reverse micelles are the aggregation of surfactant molecules around a minute water pool in an organic solvent. Reverse micelles make it possible to solubilize biomolecules in organic solvents while maintaining them in an aqueous environment. These biomolecules are trapped in water pools and are not in direct contact with the organic solvent (see Figure 1.2.1). The main

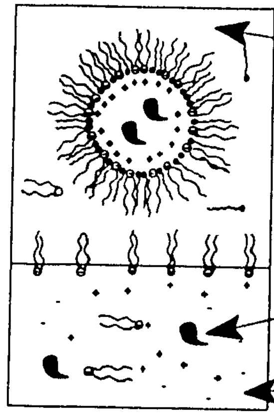


Figure 1.2.1: Schematic representation of reverse micellar extraction of biomolecules.

factor in the extraction is the electrostatic interaction between the charged guest molecules and the oppositely charged surfactant molecules (Leodidis and Hatton, 1990, Fendler et al., 1975).

Following the observation that biomolecules can be readily solubilized in reverse micelles, many investigators have studied their reverse micellar extraction. Among the biomolecules studied, proteins have received the greatest attention (Luisi et al ,1979, Fletcher et al., 1985; Abbott and Hatton, 1988; Jolivalt et al.,1990; Matzke et al., 1992; Pires and Cabral, 1993). Amino acids have been also the subject of some studies (Adachi et al., 1991; Leodidis and Hatton, 1990 a & b; 1991 c & d).

1.2.3 Amino Acids

There are 20 amino acids, each with a common backbone combined with one of 20 different side chains (also called R groups). Figures 1.2.2 a and b show the general structure of an amino acid and the structure of a particular amino acid called L-lysine. At the centre is a tetrahedral carbon atom called the α carbon (C_α) which is covalently bonded on one side to an amino group (NH_2) and on the other side to a carboxyl group (COOH) The third bond is always with hydrogen, and the forth is with a variable side chain (R) In neutral solution (pH 7), the carboxyl group loses a proton and the amino group gains one Thus an amino acid in solution is a neutral but doubly charged species called a zwitterion (Zubay, 1984) When a zwitterion amino acid is dissolved in water, it can act either as an acid (proton donor) or as a base (proton acceptor).

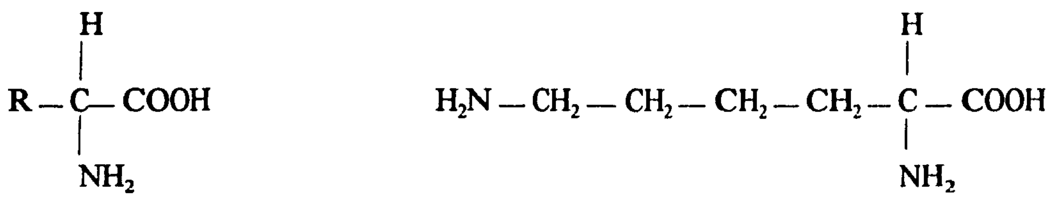
$$\text{As an acid: } H_3N^+CH(R)COO^- \rightleftharpoons H^+ + H_2NCH(R)COO^-$$

$$\text{As a base } H^+ + H_3N^+CH(R)COO^- \rightleftharpoons H_3N^+CH(R)COOH$$

In the titration curve of amino acids there is a point corresponding to a pH in which there is no net electrical charge on the molecule. This is the isoelectric pH (symbolized pI), which is the arithmetic mean of pK_1 and pK_2 (Lehninger, 1975), that is:

$$pI = (pK_1 + pK_2)/2$$

where pK_1 and pK_2 are the first and second dissociation constants. Thus, at pH higher



(a) (b)

Figure 1.2 2: Schematic of (a) general structure of amino acids (b). structure of L-lysine.

than pK_2, the amino acid has one negative charge, at pH between pK_1 and pK_2, the amino acid has one negative and one positive charge and at pH lower than pK_2, the amino acid has one positive charge. Further details are given in section 3 2.2.

1.2.4 Why Amino Acids?

Several amino acids are produced in relatively large amounts through synthesis or fermentation The cost of separation and purification of these amino acids can be up to 50% of the final production cost (Eyal and Bressler, 1993). Due to the product inhibition effect of some amino acids, such as L-lysine, upon biomass growth in fermentation their concentration must be kept low. This renders product removal difficult and expensive (Boyadzhiev and Atanassova, 1992) Therefore the search for new methods of separation is of great importance. On the other hand, there are many advantages associated with amino acids as model compounds for the investigation of solubilization in reverse micelles.

i) Amino acids are the basic units of peptides and proteins, and therefore the study of the extraction mechanism of amino acids may provide some insight into the solubilization mechanism of proteins and peptides, *ii)* The localization of guest molecules in reverse micelles is of primary importance Amino acids, because of their zwitterionic nature, are insoluble in organic solvents. Therefore, all the amino acid solubilized in an organic phase containing reverse micelles is in the water core and not dissolved in the solvent; *iii)* Amino acids are highly soluble in aqueous electrolyte solutions and easily detectable in water; *iv)* The charged state of amino acids can be controlled by varying the pH in the aqueous phase.

1.2.5 Factors Influencing the Reverse Micellar Extraction of Amino Acids

For a system formed by a particular surfactant and organic phase, 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 (Hatton, 1987; Leodidis and Hatton, 1990). The localization of amino acid molecules in the reverse micelles is closely related to their charged states (Adachi et al., 1991). As discussed in section 1.2.3, amino acids at different pH 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 salt concentration has also an important influence on the extraction of amino acids For a salt containing the same counterion of the surfactant, increasing the salt concentration favours the undissociated form of the surfactant, and thus decreases the tendency of the amino acid to be exchanged with the surfactant counter ion An increase in the salt concentration increases the charge density inside the reverse micelles, preventing the amino acid from entering the reverse micelles as a result of the salting-out effect (Leodidis and Hatton, 1991).

1.2.6 Back-Extraction of Amino Acids

Once the amino acid is extracted to the organic phase and separated from the original aqueous phase, it must be eventually recovered to a new aqueous phase This process is called back-extraction. Although without back-extraction the process for the separation of biomolecules is not complete, very little work has been done in this field The major problems with back-extraction, when possible, is the low efficiency and the presence of surfactant or other impurities in the final aqueous phase Marcozzi, et al (1991), studied the back-extraction of α-chymotrypsin from an AOT reverse micellar solution by adding silicagel to the reverse micellar solution and absorbing the extracted α-chymotrypsin Pires and Carbal (1993) also studied the back-extraction of recombinant protein from CTAB, hexadecyltrimethylammonium bromide, reverse micellar solutions by contacting them with an aqueous phase containing different amounts of KCl.

1.3 Objectives

The objectives of this project are the following:

[1]: To study the possibility of forming reverse micelles starting from dialkilphosphinc acids (CYANEX 272, CYANEX 301 and CYANEX 302).

[2]. (a) To synthesize and study three dialkyl sodium phosphinate surfactants with two identical tails and different head groups under different conditions. (b) To determine the abilities of the surfactants to form reverse micelles so as to have a comparative study to illustrate the effect of the surfactant head group on the formation of reverse micelles. The surfactants studied are 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) The independent variables to be investigated are the nature and concentration of the surfactant and cosurfactant and the concentration of NaCl. The dependent variables are the amounts of water solubilized in the organic phase and the distribution of the surfactant between the phases.

[3]· (a) To investigate comparatively the ability of the above surfactants for the reverse micellar extraction of amino acids under different conditions. L-lysine was chosen as a model amino acid The independent variables are salt concentration, initial pH and nature of the surfactant The dependent variables are the equilibrium pH, L-lysine partitioning, the water content of organic phase and the surfactant partitioning. (b) To investigate the possibility of the back-extraction of the L-lysine from the reverse micellar organic phase.

Chapter 2

Experimental Methods

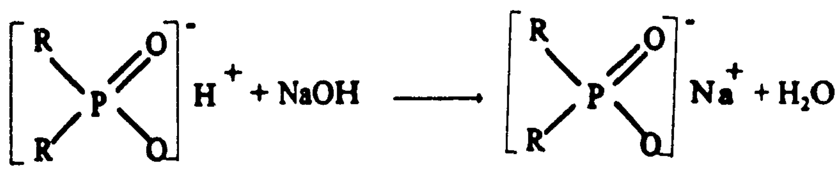
2.1 Materials

Bis (2,4,4-trimethylpentyl) phosphinic acid (CYANEX 272), 85% purity, bis (2,4,4-trimethylpentyl) monothiophospinic acid (CYANEX 302), 84% purity and bis (2,4,4-trimethylpentyl) dithiophosphinic acid (CYANEX 301), 75% purity were obtained from Cyanamid Canada Inc (Toronto, Ontario) 1-decanol , 1-octanol, 1-pentanol, 1-propanol and benzene, all 99% purity, and of reagent grade and isooctane, HPLC grade, were obtained from A & C American Chemical Ltd. (Montreal, Quebec) Sodium chloride (99 9% pure), sodium hydroxide (1 and 0.1 N solution), hydrochloric acid (1 and 0 1 N solution), cupric hydroxide (99% pure) and acetone (lab grade) were obtained from Anachemia Chemicals Ltd. (Montreal, Quebec). Karl Fischer titrant, AQUASTAR Comp 5 (pyridine free) was obtained from BDH Inc (Ville St-Laurent, Quebec). O-phthaicdicarboxaldehyde, sodium tetraborate and 2-mercaptoethanol were obtained from Pflatz and Bauer (Waterbury, CT, U.S.A) L-lysine (l-2,6-diaminohexanoic acid), 99% purity, was obtained from Sigma

Chemical Company (St Lois, MO, U S A). Deionized water with a minimum resistance of 1 megohm/cm was used in all experiments. All chemicals were used without further purification except CYANEX 272 , 301 and 302 which were purified as described below

2.2 Surfactant Preparation

In the first step the three alkyl phosphinic acids were purified through a copper salt precipitation method similar to the purification of bis(2-ethylhexyl)phosphoric acid according to the method proposed by Partridge and Jensen (1969) The purification of the surfactants is described with more details in Appendix A. The initial and final purities were determined using potentiometric titration with 0.05 N sodium hydroxide. The final purities were found to be 99 4% CYANEX 272, 99% CYANEX 301 and 99 1% CYANEX 302. In the second step the surfactants were prepared by reacting purified bis(2,4,4-trimethylpentyl) phosphinic acid (CYANEX 272), bis(2,4,4-trimethylpentyl) monothiophospinic acid (CYANEX 302), and bis(2,4,4-trimethylpentyl) dithiophosphinic acid (CYANEX 301) with stoichiometric amount sodium hydroxide The solution was placed in a volumetric flask and mixed for 30 min. Under mixing, water was added until the required volume was reached and then the solution was settled for 48 hours. The procedure was the same for all three surfactants. The reactions were the following:



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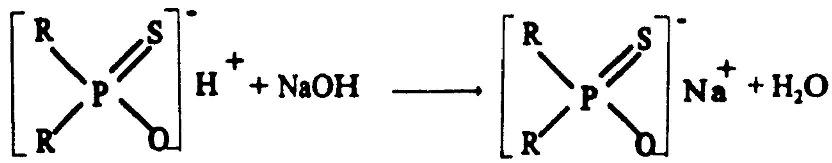
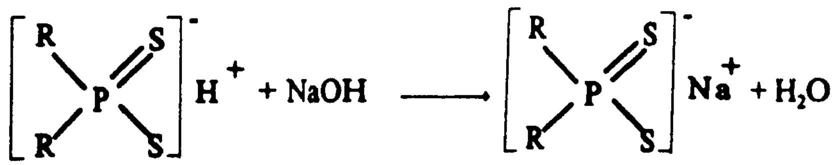
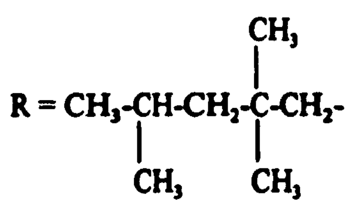


(2.2.2)



(2 2 3)

where:



The products 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)   All three surfactants were water soluble

2.3 Analytical Methods

● Surfactant Analysis

The concentration of surfactant in the aqueous and organic phase was determined by potentiometric titration with 0 05 N HCl solution. A calibration curve for the surfactant was prepared The titration experiments and the preparation of a calibration curve are described in Appendix B.

● Water Content

The water content of the organic phase was determined by Karl Fischer (KF) titration using a Metrohm-Brinkmann Model 701/1 KF Titrator The KF reaction takes place in two steps, in the first step the KF titrant forms a complex with a primary alcohol:

$$ROH + SO_2 + R'N \rightarrow [R'NH]\,SO_3R \qquad (2.3.1)$$

where R is alcohol chain and R' is a nitrogenous base In the second step this complex is titrated with I_2 and H_2O in the sample:

$$I_2 + H_2O + 2R'N + [R'NH]\,SO_3R \rightarrow [R'NH]\,SO_4R + 2\,[R'NH]I \qquad (2.3.2)$$

Usually, methanol is used as the solvent However, since the organic solvents and surfactants were long chain hydrocarbons which may not dissolve satisfactorily in methanol, propanol was employed The titration readings were calibrated for each set of experiments. Figure 2.1 shows a typical calibration curve for the system NaPOO + water + isooctane + decanol. In all experiments the KF readings were accurate to about ± 2 % of the measured values.

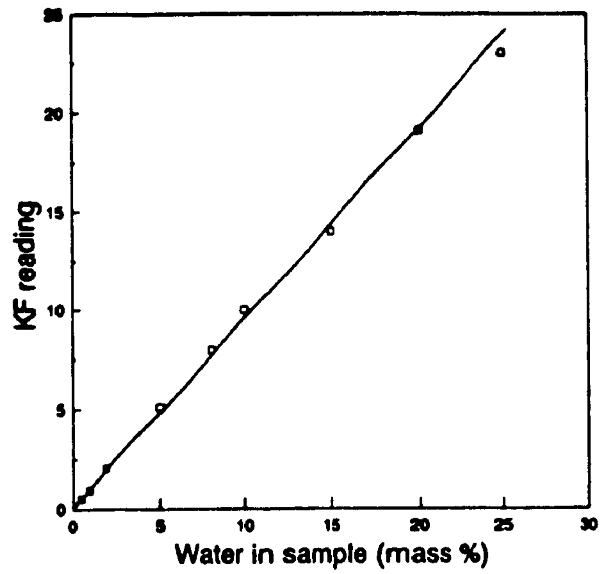


Figure 2.1: Typical Karl Fischer calibration curve for the system NaPOO + water + isooctane + decanol.

● pH Measurements

The concentration of H^+ ions in the aqueous phase was measured by a Metrohm Brinkmann 691 pH meter with a precision of ± 0.01 in the pH scale The instrument was calibrated using standard solutions for each set of experiments.

● Amino Acid Analysis

Analysis of L-lysine in the aqueous phase was performed following the method proposed by Roth (1971). The concentration of L-lysine in the aqueous phase was determined by mixing 0.6 ml of the unknown solution containing L-lysine with 20 ml labelling solution and measuring the absorbtivity at 340 nm after 5 min by a UV-visible spectroscopy on a Cary-13 Varian Spectrophotometer. The concentration of the L-lysine in the organic phase was calculated by a mass balance. The instrument was calibrated using standard solutions for each set of experiments The complete method of analysis is described in Appendix C.

2.4 Sample Preparation and Experimental Procedure

Two types of experiments were conducted One was to study the formation of reverse micelles and the other was to study the reverse micellar extraction and back-extraction of L-lysine.

- Formation of Reverse Micelles

All experiments were performed by contacting 20 ml of an aqueous solution containing the surfactant and salt with 20 ml of an organic solution containing isooctane and the cosurfactant The experiments were carried out in 125 ml glass jars with plastic caps The jars were placed on a vibrating shaker at 200 rpm and shaken for 60 min at constant temperature of 25°C The shaking and settling times were fixed through preliminary experiments (Appendix D). The samples were then left to settle for 48 hours to reach equilibrium The phases were separated using a syringe and analyzed for water and surfactant content. The aqueous phase was prepared by adding 100 mM of surfactant and 0, 50, 100, 200, 300 milimoles of NaCl in a 1 litre volumetric flask and bringing the volume to 1 litre by addition of water. The organic phase was prepared by adding 0, 50, 100,150, 200, 250, 300, 400 mM of three different cosurfactants (decanol, octanol and pentanol) in a 1 litre volumetric flask and bringing the volume to 1 litre by addition of isooctane. In all experiments before separating the phases it was verified that both phases should be transparent. The method was the same for all three surfactants.

● Reverse Micellar Extraction of L-lysine

All experiments were performed by contacting 20 ml of an aqueous solution containing surfactant , salt, hydrochloric acid and L-lysine with 20 ml of an organic solution containing isooctane and cosurfactant. The experiments were carried out in 125 ml glass jars with plastic caps. The jars were placed on a vibrating shaker at 200 rpm and shaken for 60 min at constant temperature of 25 °C. The samples were then left to settle for 72 hours to reach equilibrium. The shaking and settling times were fixed through preliminary experiments (Appendix D). The phases were separated using a syringe and the aqueous phase was analyzed for L-lysine, pH and surfactant content. The initial aqueous phase was prepared by adding 10 ml of a solution of 200 mM of surfactant and NaCl at the concentrations of 0, 80, 140, 200, 250 and 300 mM with 10 ml of a solution of 10 mM of L-lysine whose pH was adjusted by 0.1 M HCl in a glass jar In all experiments before separating of the phases it was verified that both phases should be transparent

For the back-extraction, all experiments were performed by contacting 10 ml of an aqueous solution containing HCl with 20 ml of an organic solution containing isooctane and 400 mM pentanol and reverse micellar extracted L-lysine The experiments were carried out in 25 ml glass jars with plastic caps. The jars were placed on a vibrating shaker at

200 rpm and shaken for 10 min at constant temperature of 25 °C. The samples were then left to settle for 30 min to reach equilibrium. The phases were separated using a syringe and the aqueous phase was analyzed for L-lysine and surfactant content. In all experiments before separating of the phases it was verified that both phases should be transparent.

2.5 Definitions

The experimental results in Chapter 3 are reported in terms of the parameters defined below

- Water uptake

$$Water\ uptake = \frac{mass\ of\ water\ in\ organic\ phase}{total\ mass\ of\ organic\ phase} \times 100$$

- % Surfactant in Organic Phase

$$\%\ Surfactant\ in\ organic\ phase = \frac{moles\ of\ surfactant\ in\ organic\ phase}{initial\ moles\ of\ surfactant\ in\ the\ system} \times 100$$

- % Extraction

$$\%\ Extraction = \frac{(initial - final)\ moles\ of\ amino\ acid\ in\ aqueous\ phase}{initial\ moles\ of\ amino\ acid\ in\ the\ aqueous\ phase} \times 100$$

Chapter 3

Results and Discussion

3.1 Formation of Reverse Micelles

3.1.1 Introduction

The present work is primarily oriented to the development and screening of three new surfactants and to determine their abilities to form reverse micelles under various conditions. 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). Since all these surfactants have the same two hydrocarbon tails and differ in their head groups, their comparative study can give some insight into the structure of reverse micelles. In addition if they can form reverse micelles they may be used for the reverse micellar extraction of biomolecules The variables studied for each surfactant were the nature and concentration of the cosurfactant in the organic phase and the salt concentration of the aqueous phase. In all the experiments the water and surfactant content of the organic phase was determined

Most of the data points for these experiments represent the average value of three replicate measurements from three different samples. For the water uptake experiments the maximum deviation of data from the average value was within ±5 % , while for the surfactant distribution the accuracy was ±3 %.

3.1.2 Effect of Cosurfactant

Experiments were performed to study the effect of the nature and concentration of a cosurfactant on the formation of reverse micelles, water uptake and distribution of surfactant in the system The system consisted of (isooctane + cosurfactant) + (water + surfactant + NaCl). The experiments were conducted at 25°C and at fixed salt and surfactant concentrations. In this section the results for 100 mM surfactant concentration and 100 mM salt concentration are reported Similar results for 0, 50, 200 and 300 mM salt concentrations are reported in Appendix E Pentanol, octanol and decanol at different concentrations up to 400 mM in the organic phase were used as cosurfactants and NaPOO, NaPSO and NaPSS at 100 mM concentration in the aqueous phase were used as surfactants.

Figures 3.1.1 to 3.1.3 show the effect of the cosurfactant chain length and its concentration on the water uptake and on the surfactant distribution. Some experimental observations are presented below together with a discussion of their possible causes:

[a]. For the systems studied, NaPOO, NaPSO and NaPSS, need a cosurfactant in order to form reverse micelles.

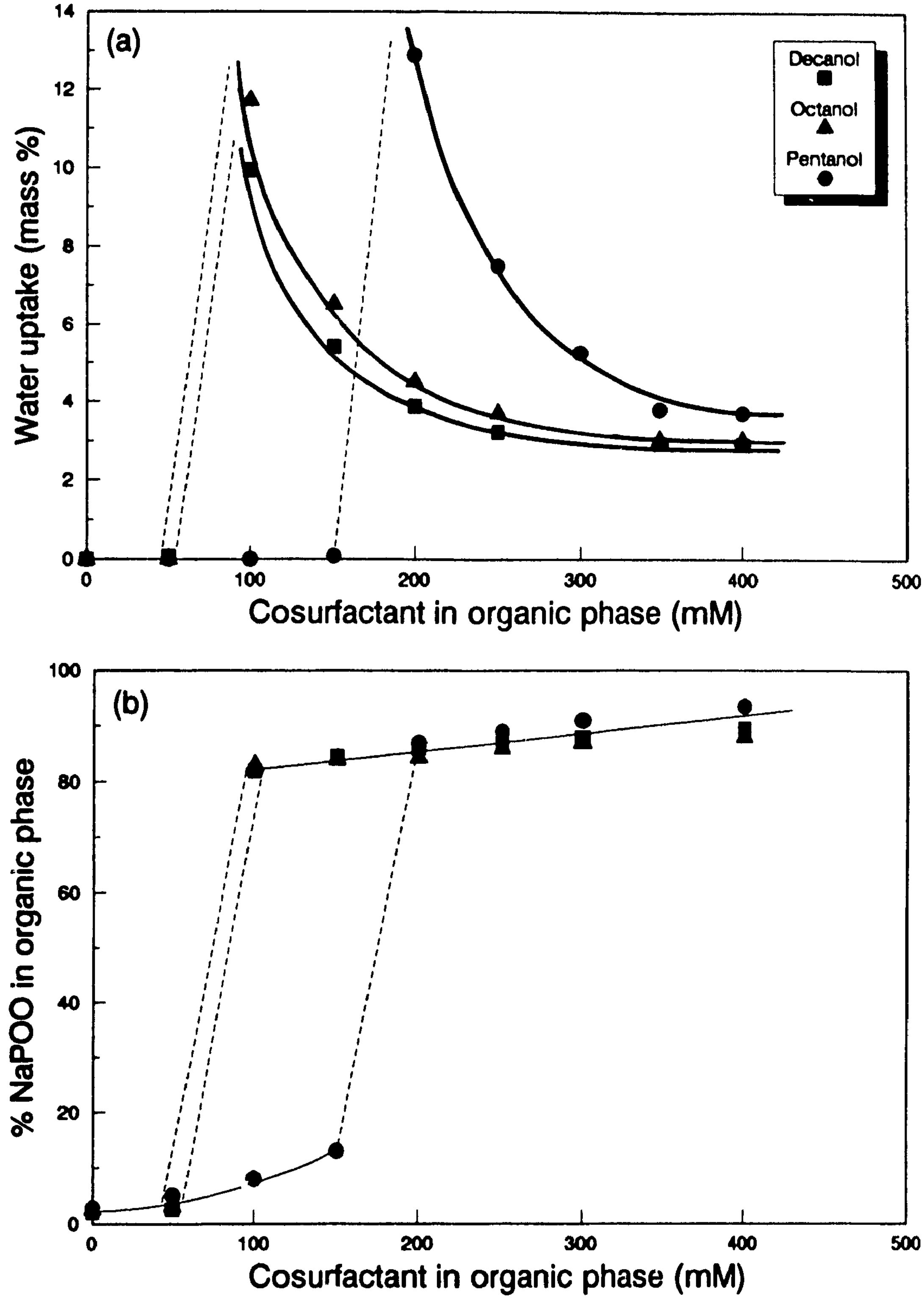


Figure 3.1.1: Effect of alcohol chain length and concentration on the (a). water uptake and (b): surfactant distribution for (■) Decanol, (●) Octanol and (▲) Pentanol at 100 mM NaPOO and 100 mM NaCl.

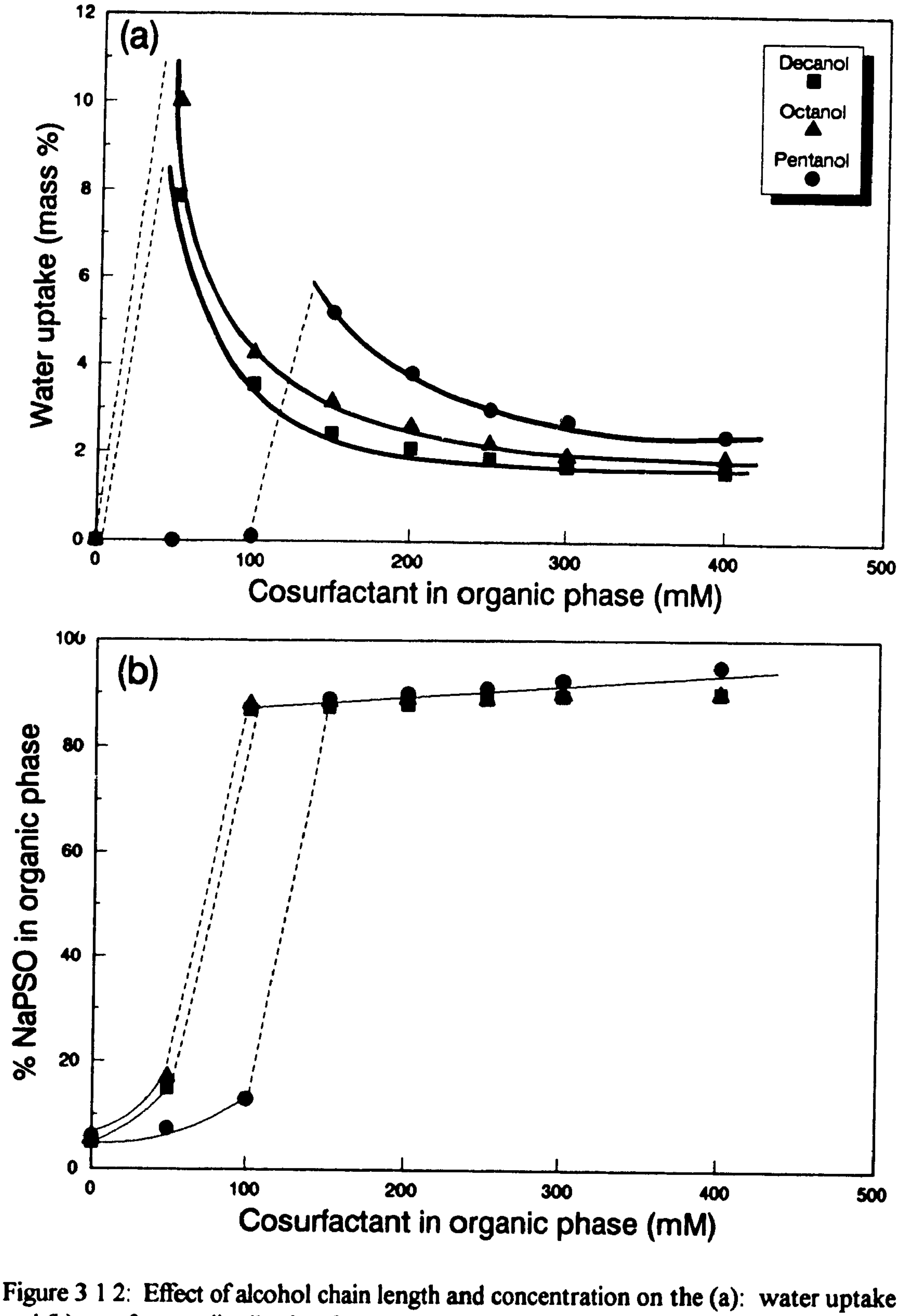


Figure 3 1 2: Effect of alcohol chain length and concentration on the (a): water uptake and (b): surfactant distribution for (■) Decanol, (●) Octanol and (▲) Pentanol at 100 mM NaPSO and 100 mM NaCl.

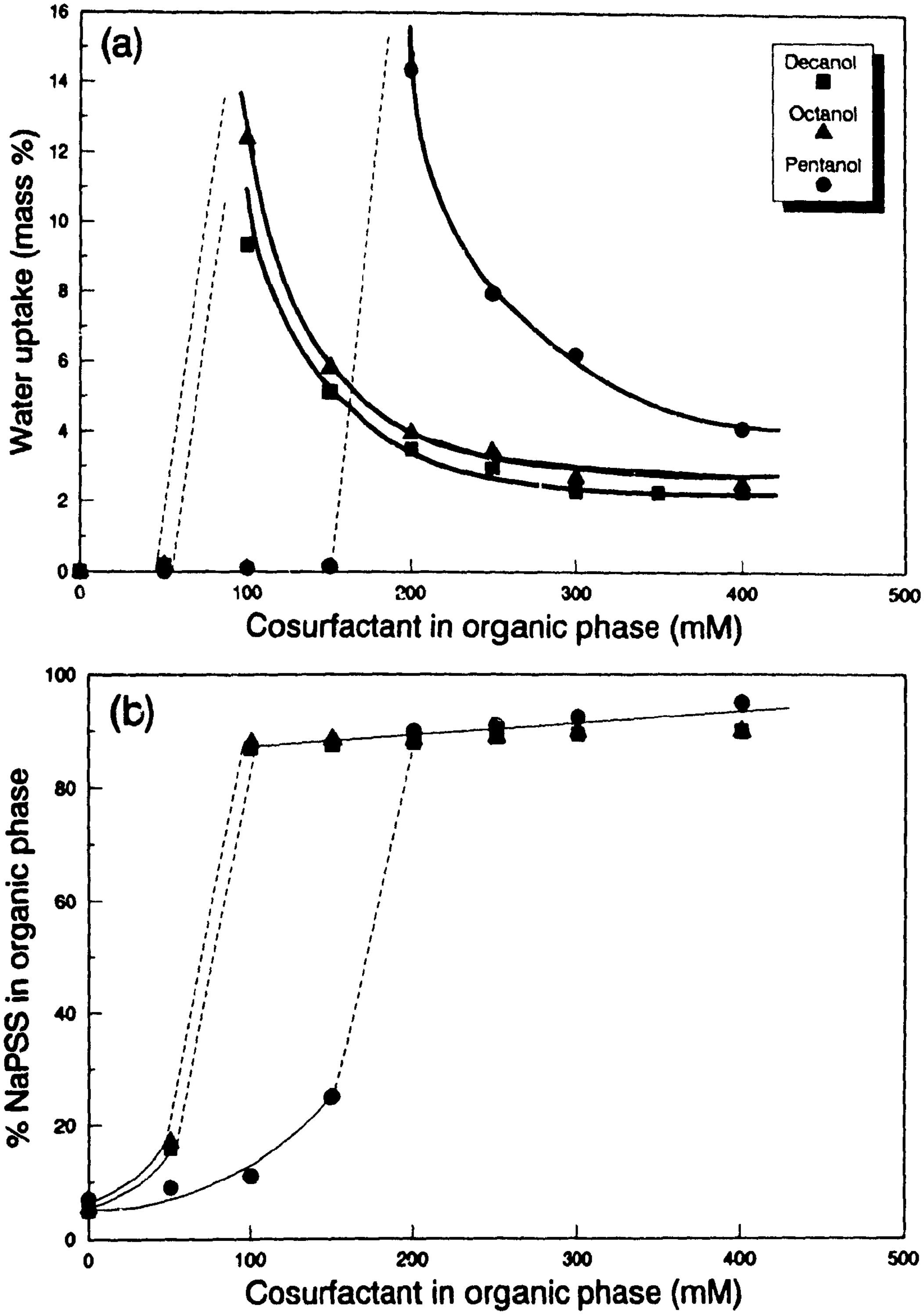


Figure 3.1.3: Effect of alcohol chain length and concentration on the (a): water uptake and (b): surfactant distribution for (■) Decanol, (●) Octanol and (▲) Pentanol at 100 mM NaPSS and 100 mM NaCl.

[b]: As shown in Figures 3.1.1 to 3.1.3, the formation of reverse micelles begins at a certain concentration of cosurfactant which we call the minimum cosurfactant concentration (MCC) The maximum water uptake occurs at the MCC At higher cosurfactant concentrations, about 90% of the total amount of the surfactant resides in the organic phase The nature of the cosurfactant does not have a strong effect on the distribution of surfactant in the phases. It is also shown that the water uptake decreases sharply as the alcohol concentration increases beyond the MCC and becomes approximately constant at higher alcohol concentrations. The trends were the same for all surfactants and alcohols tested in this study. A possible explanation for these facts is the following: The alcohol molecules, by participating at the interfacial region of the reverse micelles, screen the repulsive ion-ion interaction forces between the charged head groups of the surfactant. At a certain concentration of alcohol, migration of the surfactant molecules from the aqueous phase to the organic phase and their aggregation in the form of reverse micelles becomes possible In this role, the alcohol is acting as a cosurfactant. Since alcohols are polar molecules, adding them to a nonpolar organic solvent, increases the polarity of the organic phase and, acting as a cosolvent, favours the solubilization of surfactant molecules. Thus, addition of more alcohol decreases the number of surfactant molecules forming reverse micelles. On the other hand, addition of more alcohol, by buffering the repulsions between surfactant head groups, allows the close packing of surfactant molecules and may result in a decrease in the size and number of the reverse micelles and, consequently, the water uptake (Krei and Hustedt, 1991). In addition to the above two phenomena, it has been

shown (Leodidis and Hatton, 1989) that increasing the concentration of alcohol at the interface of the water pool of the reverse micelles results in a decrease of the dielectric constant of the water pools This decrease in dielectric constant will shift the equilibria of the surfactant from the ionic form to the undissociated form in the interfacial region of the reverse micelles The undissociated form of the surfactant will migrate to the alcohol/isooctane phase, reducing the number of surfactant molecules available for the formation of reverse micelles and further reducing the water uptake (Wang et al , 1994)

[c]: Figures 3.1.1 to 3.1 3 show that the MCC and water uptake depend on the chain length of the alcohol Reverse micelles form at a lower MCC with longer chain alcohols At the same cosurfactant concentration, once reverse micelles are formed, the water uptake is less for the longer chain alcohols. It is also apparent that the effect of the alcohol chain length becomes smaller as the alcohol chain becomes longer. To explain these experimental results it should be noted that the tendency of the surfactants (surfactant and cosurfactant) to form reverse micelles is a result of a balance between the net cohesive energy of the surfactants with the oil and water at the interface (Winsor, 1954) For two reverse micellar systems of the same surfactant, oil and brine but with a different alcohol chain length, all interactions are the same except the one due to the alcohol. The OH group is the same in both alcohols and has approximately the same cohesive energy with water The longer tail alcohol molecule has a larger cohesive energy with the organic phase than the molecule of the shorter one Thus, the number of long-tail alcohol molecules required is less than the number of short-tail alcohol molecules to produce the same cohesive effect and the

reverse micelles with longer tail alcohols can be formed at lower concentrations of alcohols.

[d] The dashed lines in Figures 3 1.1 to 3.1.3 show that the transformation from micelles to reverse micelles occurs through an intermediate three phase system Formation of a three phase system begins at a certain concentration of cosurfactant in the organic phase. Below this cosurfactant concentration, more than 95% of the total amount of the surfactant is in the aqueous phase forming micelles. Increasing the cosurfactant concentration increases the size of the third phase and at a certain cosurfactant concentration, which corresponds to the MCC, it vanishes The middle phase is a surfactant rich phase and its growth is mainly due to increases in its surfactant and water content As discussed in chapter 1.1, during the transition from micelles to reverse micelles as a result of an increase in the cosurfactant concentration, the system must pass through a three phase state in which almost all inventory of the surfactant resides in the middle phase (Winsor, 1954).

3.1.3 Effect of Salt Concentration

Experiments were performed to study the effect of salt concentration on the formation of reverse micelles, the water uptake and the distribution of the surfactant in the system. The system consisted of (isooctane + cosurfactant) + (water + surfactant + NaCl). The experiments were conducted at 25°C and at a fixed surfactant concentration. Sodium chloride at concentrations of 0, 50, 100, 200, 300 mM in the aqueous phase was used as the salt. Pentanol, at different concentrations up to 400 mM in the organic phase, was used as cosurfactant and NaPOO, NaPSO and NaPSS at 100 mM concentration in the aqueous phase were used as surfactants Similar results for decanol and octanol are reported in Appendix E

Figures 3.1.4 to 3.1 6 show the effect of salt concentration on the water uptake and on the surfactant distribution. Some experimental observations are presented below and a discussion of possible reasons for their occurrence is given

[a]: Salt concentration is an important factor which influences the phase behaviour of the NaPOO, NaPSO and NaPSS systems studied Figure 3 1 4 to 3 1 6 show that at zero salt concentration these surfactants do not form reverse micelles and that they have different behaviour at different salt concentrations A reason for this may be that, since the surfactant molecule is partly hydrophobic, the increase of the ionic strength of the aqueous phase promotes the migration of the surfactant to the organic phase and favours the formation of reverse micelles.

[b]: Figures 3.1.4 to 3.1 6 show that the MCC for each cosurfactant depends on the salt concentration such that the lower the salt concentration the higher the MCC The increase in salt concentration, by virtue of the common-ion effect, favours the undissociated form of the surfactant and increases its tendency to dissolve in the organic phase where it can form reverse micelles. Therefore, the migration of the surfactant to the organic phase can occur at lower MCC

[c]: An increase in the salt concentration reduces the equilibrium water uptake in the organic phase. This effect becomes smaller as the alcohol concentration increases

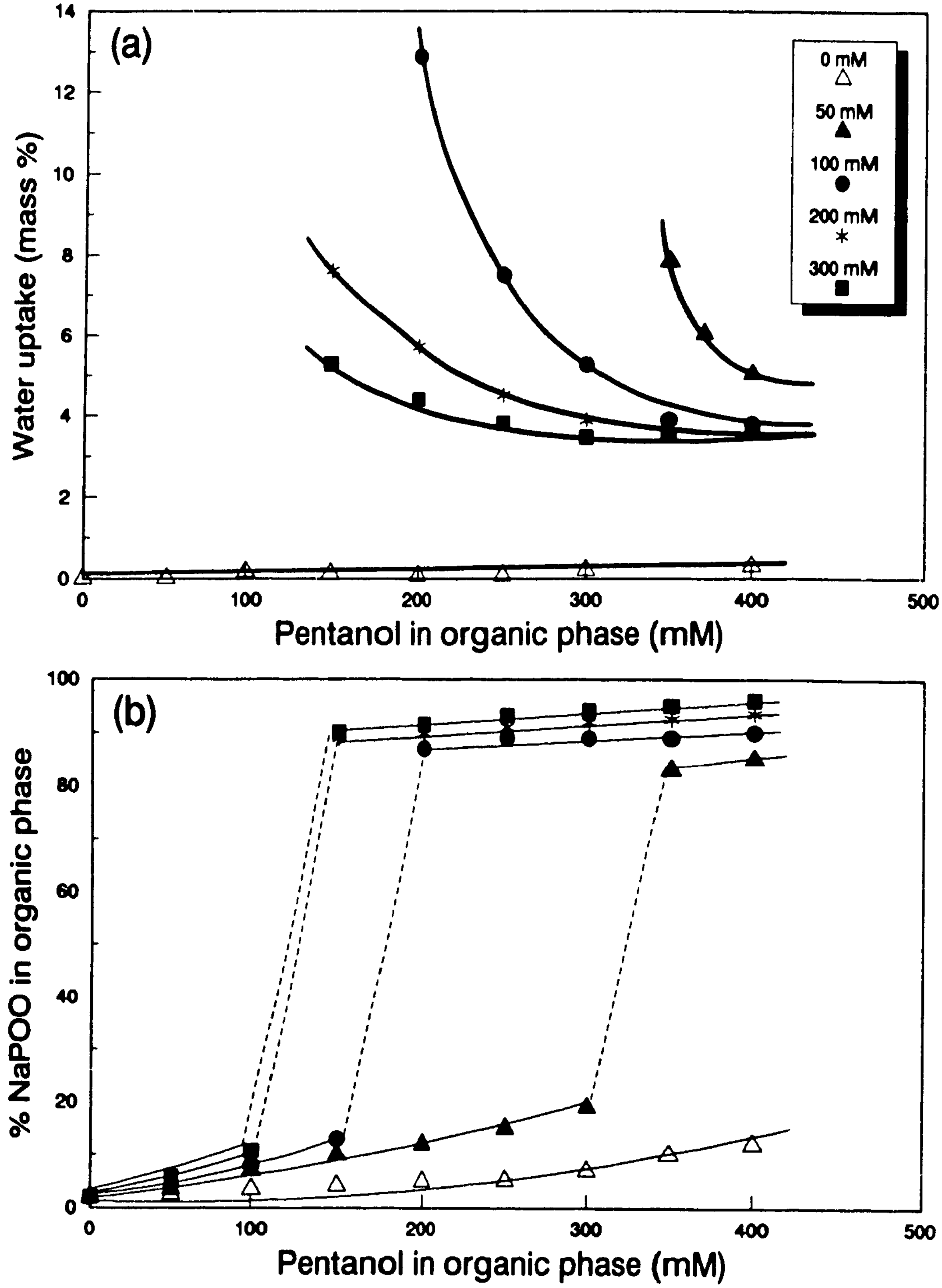


Figure 3.1.4: Effect of salt concentration on the (a): water uptake and (b): surfactant distribution for (Δ) 0 mM, (▲) 50 mM, (●) 100 mM, (★) 200 mM and (■) 300 mM NaCl at 100 mM NaPOO.

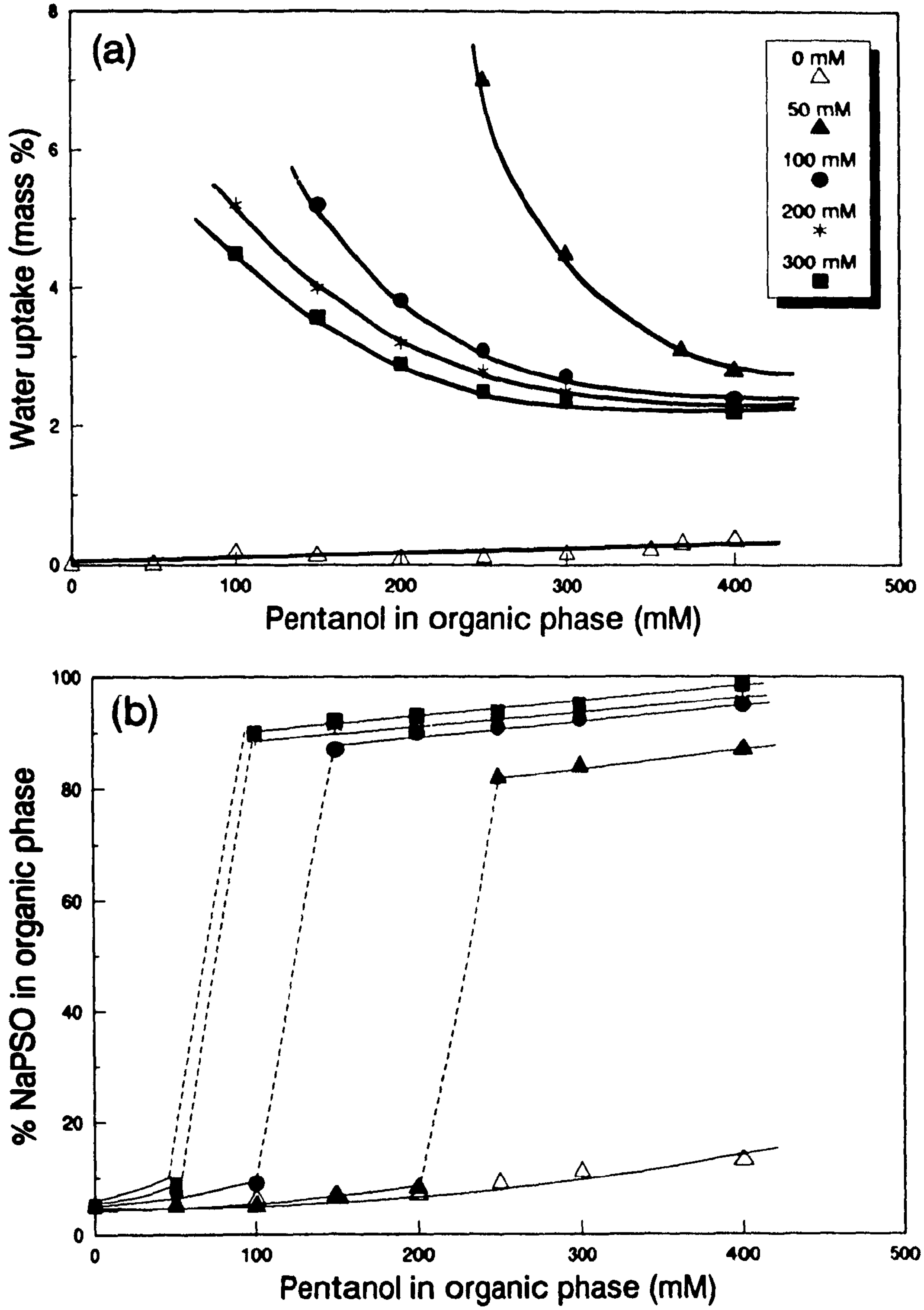


Figure 3 1 5. Effect of salt concentration on the (a) water uptake and (b) surfactant distribution for (Δ) 0 mM, (▲) 50 mM, (●) 100 mM, (★) 200 mM and (■) 300 mM NaCl at 100 mM NaPSO.

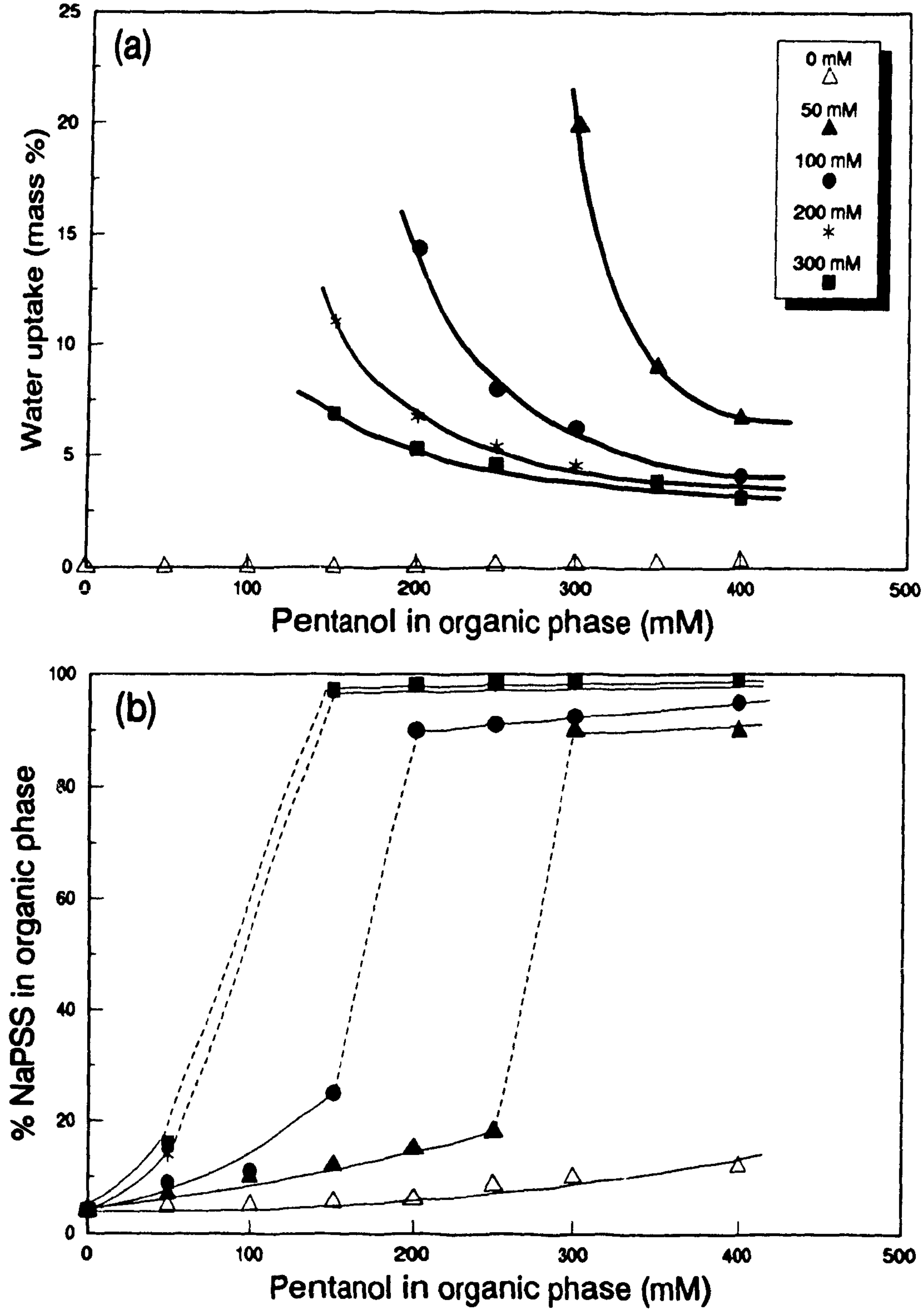


Figure 3 1.6: Effect of salt concentration on the (a): water uptake and (b): surfactant distribution for (Δ) 0 mM, (▲) 50 mM, (●) 100 mM, (★) 200 mM and (■) 300 mM NaCl at 100 mM NaPSS.

An explanation for this may be that the charge density inside the reverse micelle increases with increasing the salt concentration and therefore screens the repulsive interactions between surfactant head groups at the interfacial region of the reverse micelle. Meanwhile, the polar heads can stay closer, hence decreasing the size of reveres micelles and the water uptake (Leodidis and Hatton, 1989)

3.1.4 Effect of Surfactant Structure and Concentration

Experiments were performed to study the effect of surfactant nature and concentration on the formation of reverse micelles and the water uptake. The system consisted of (isooctane + cosurfactant) + (water + surfactant + NaCl). The experiments were conducted at 25°C and fixed salt concentration. The surfactants NaPOO, NaPSO and NaPSS were used at the concentrations of 100, 150 and 200 mM in the aqueous phase Decanol was used as a cosurfactant at different concentrations up to 400 mM in the organic phase Sodium chloride was used as salt at a concentration of 100 mM

Figures 3.1.7 to 3.1.9 show the effect of surfactant concentration and structure on the water uptake Experimental results and discussion follow.

[a]: Figures 3.1.7 to 3 1.9 show that increasing the surfactant concentration increases the equilibrium water uptake in the organic phase It is also shown that the water uptake increases in larger proportion than the concentration of the surfactant. An explanation for this can be that an increase in the number of moles of the surfactant increases the number

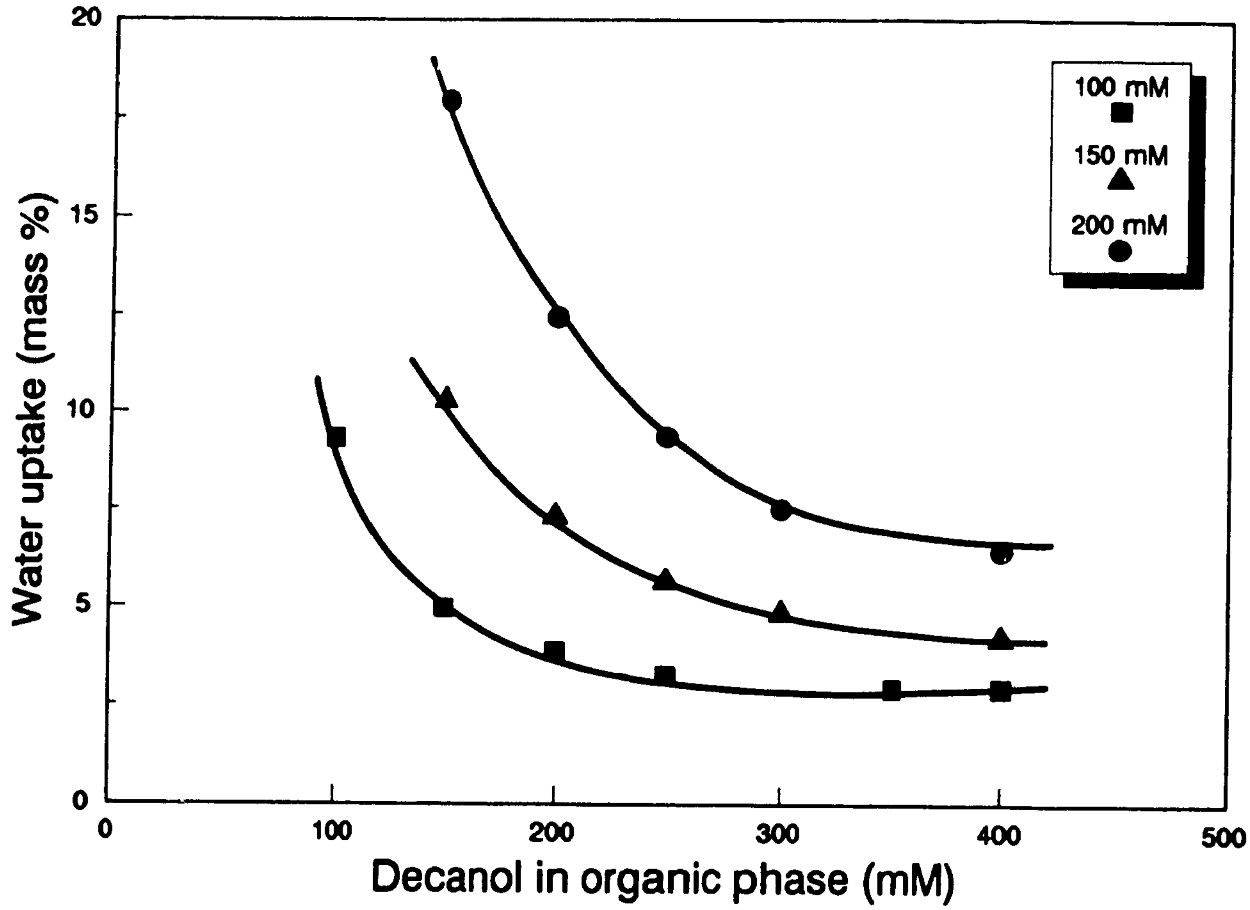


Figure 3.1.7: Effect of surfactant concentration on the water uptake for (■) 100 mM, (▲) 150 mM and (●) 200 mM NaPOO at 100 mM NaCl.

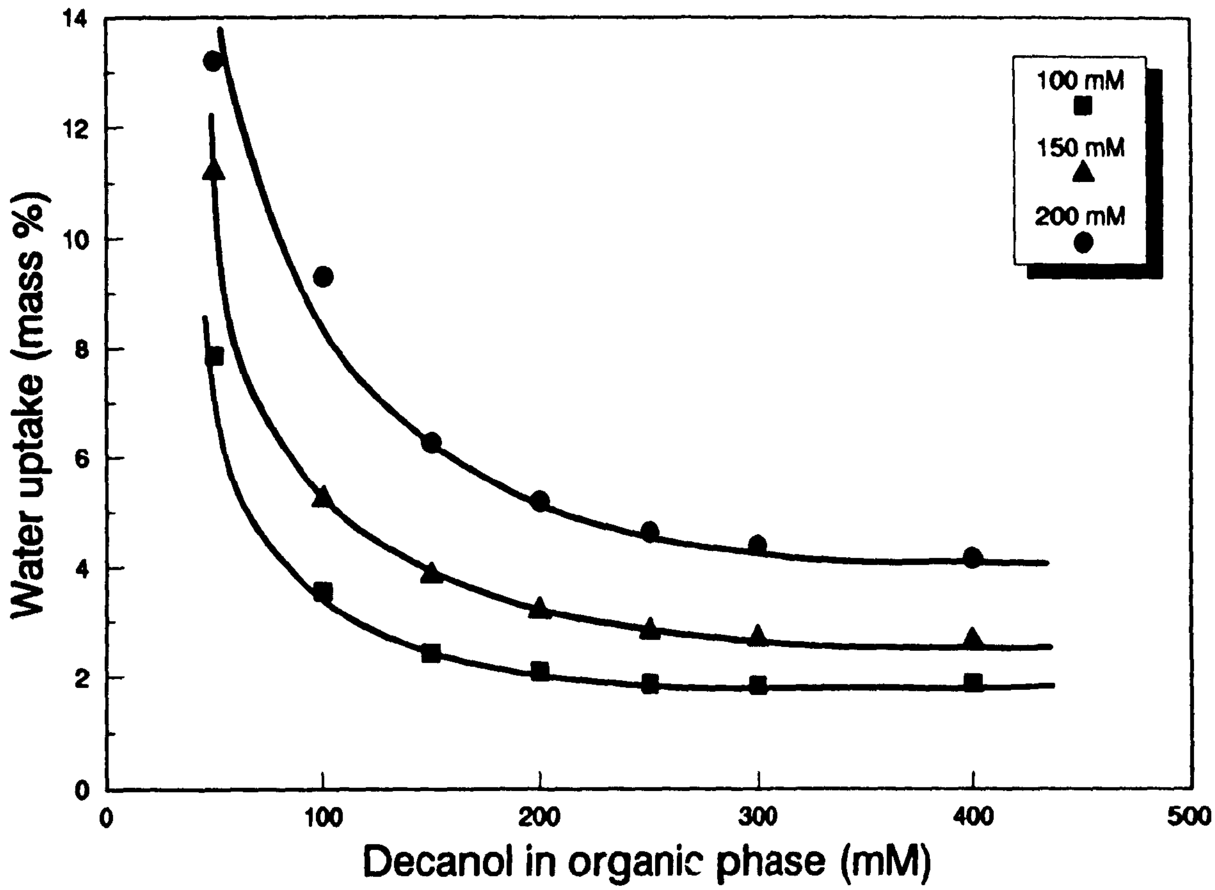


Figure 3.1.8: Effect of surfactant concentration on the water uptake for (■) 100 mM, (▲) 150 mM and (●) 200 mM NaPSO at 100 mM NaCl.

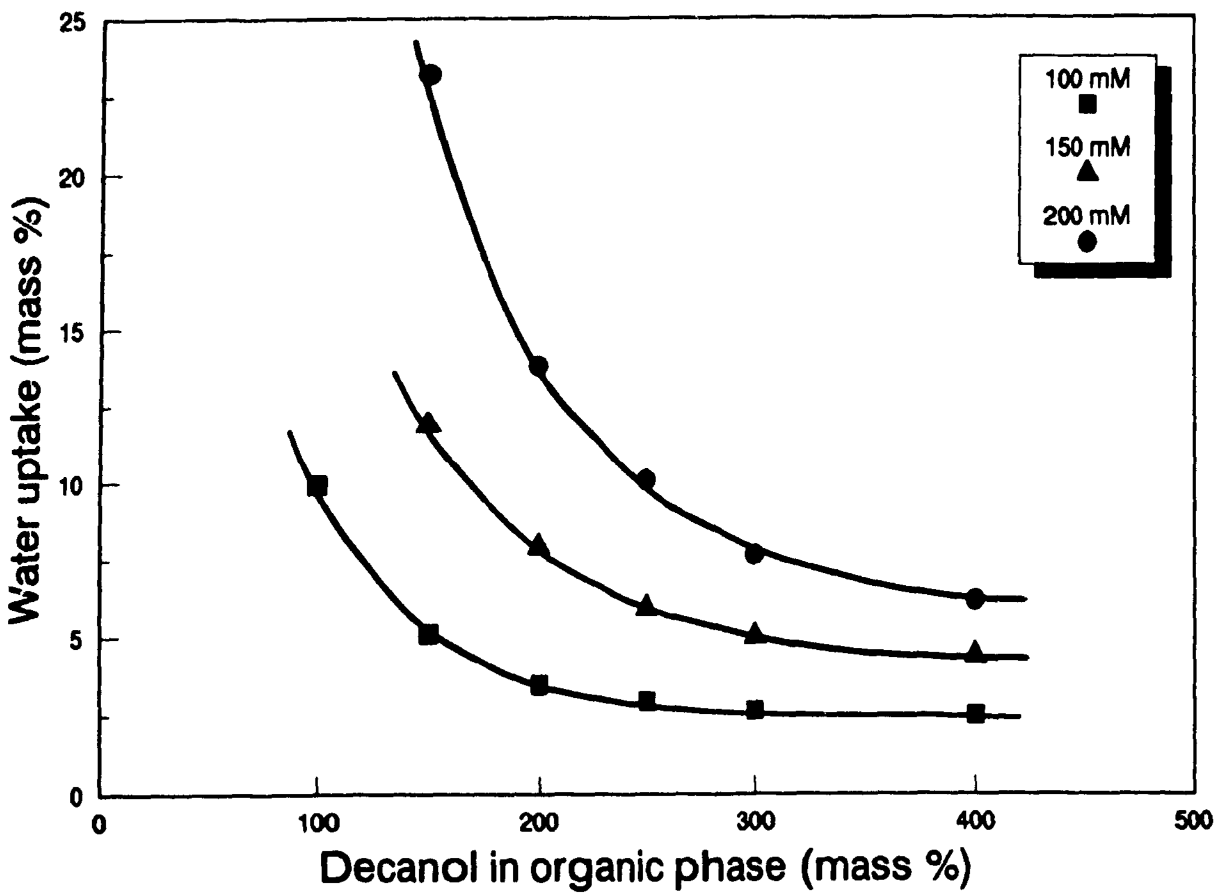


Figure 3.1.9: Effect of surfactant concentration on the water uptake for (■) 100 mM, (▲) 150 mM and (●) 200 mM NaPSS at 100 mM NaCl.

of reverse micelles through a simple mass action law. Since up to saturation of the interface, part of the surfactant molecules reside at the interfacial region, the number of surfactant molecules participating in the reverse micelles is less than the difference between the initial and the equilibrium concentration of the surfactant molecules in the aqueous phase However, as the interfacial region becomes saturated, the number of surfactant molecules at the interface of the phases does not change with the increase in the surfactant concentration Therefore all the additional molecules of surfactant would participate in the formation of reverse micelles and the increase in the water uptake is larger than the increase in the surfactant concentration

[b]: Figure 3.1.10 shows the effect of the surfactant head group on the water uptake of the system. As shown, for a fixed cosurfactant concentration, NaPOO has the largest water uptake and NaPSO has the lowest water uptake. Since the tails in all three surfactants are the same, this effect is solely due to the effect of the head groups At first sight, it is surprising that the value of the water uptake for NaPSO is not between values for NaPOO and NaPSS. However, a more detailed consideration suggests that this effect may be due to the geometry of the head groups. The surfactant molecules at the interfacial region of the reverse micelles can be divided into two smaller regions, tail and head group regions. The tail region includes the tail of both the surfactant and the cosurfactant and the penetrated oil. The head region includes the head of the surfactant and of the cosurfactant and penetrated water molecules. The tail and head regions both have a cohesive energy with oil and water Reverse micelles form when these cohesive forces are balanced This balance can be reached

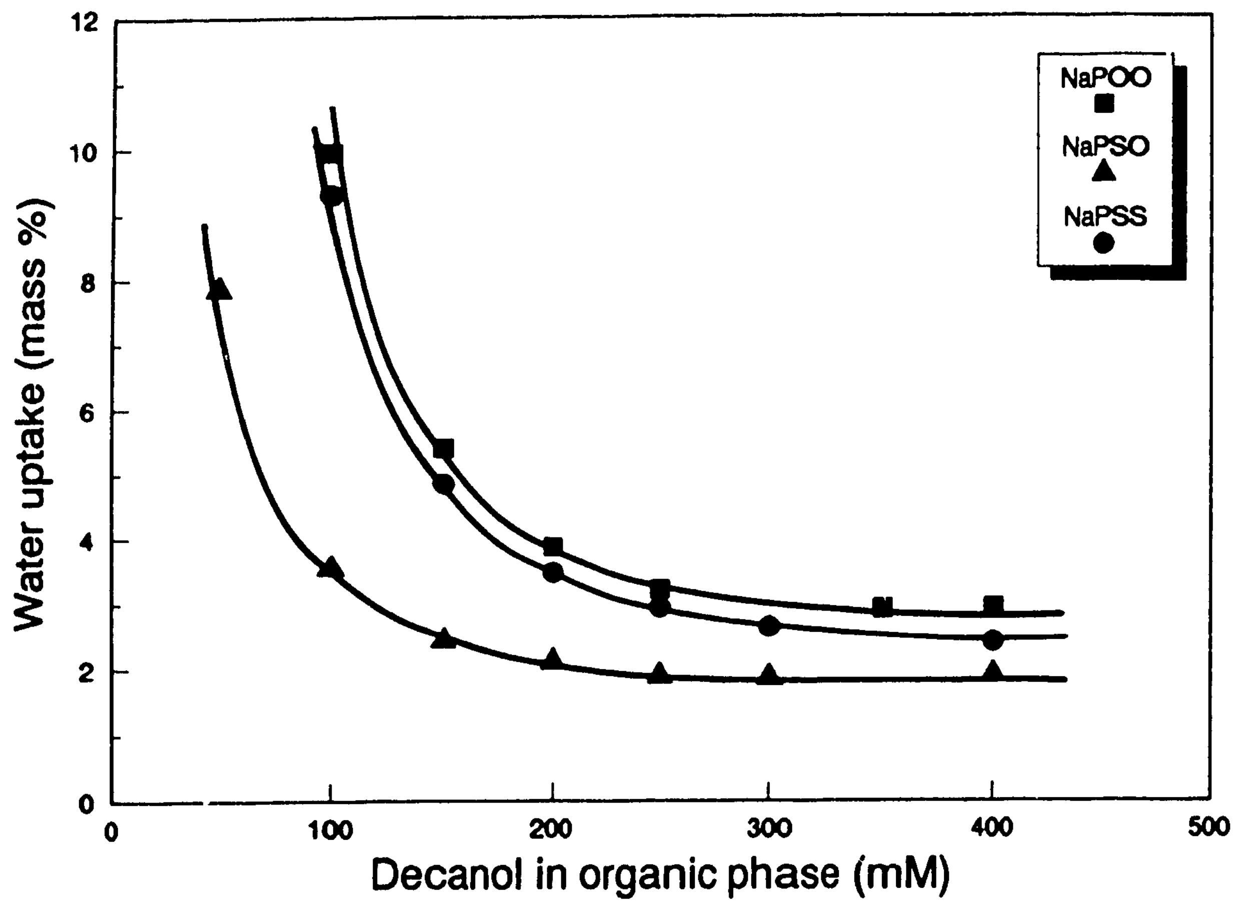


Figure 3.1.10: Effect of surfactant head group structure on the water uptake for (■) NaPOO, (▲) NaPSO and (●) NaPSS at 100 mM surfactant concentration and 100 mM NaCl.

with the help of a cosurfactant which increases the cohesive forces in the tail region and buffers the repulsion of the head groups Surfactants with different size or geometry of head groups need different amounts of cosurfactant to reach this balance They may also form reverse micelles with different geometries which would differ in water uptakes. The head groups of both NaPOO and NaPSS are symmetric. In the case of NaPSO the head is asymmetric and the head group repulsions at the interfacial region of the reverse micelles can be smaller than those for the other two (Ruckenstein and Nagarajan, 1980) Therefore the head groups of NaPSO can pack closer, forming smaller reverse micelles, and needing less cosurfactant to buffer the effect of head groups. This may be a reason for the lower water uptake and lower MCC of NaPSO in comparison to NaPSS and NaPOO which are symmetric molecules.

3.2 Reverse micellar extraction of L-lysine

3.2.1 Introduction

In section 3.1 the formation of reverse micelles with three new surfactant was described and their ability to form reverse micelles under different conditions was discussed. 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). This section presents the results obtained with these surfactants in the extraction of amino acids. Since these surfactants all have the same two hydrocarbon tails and differ only in their head groups, their comparative study is illustrative of the effect of surfactant head group in the reverse micellar extraction of amino acids. In this work L-lysine is used as a model amino acid. The variables studied were the salt concentration, pH of the aqueous phase and the nature of the surfactant. In all experiments the final water uptake, pH and surfactant and L-lysine partitioning were measured.

Most of the data points for these experiments represent the average values of three replicate samples. For water extraction experiments the maximum deviation of data from the average value was approximately ±4 % , while that for the water uptake values was ±5% of the reported values

3.2.2 Effect of pH

Experiments were performed to study the effect of pH on the reverse micellar extraction of L-lysine and on the water uptake of the system The system consisted of (isooctane + cosurfactant) + (water + surfactant + NaCl + HCl + L-lysine). The experiments were conducted at 25 °C with 100 mM surfactant, 40 mM NaCl and 5 mM L-lysine in the aqueous phase, and 400 mM pentanol in the organic phase. Similar results for salt concentrations of 70, 100 and 150 mM are reported in Appendix E. The surfactants used were NaPOO, NaPSO and NaPSS.

Figures 3.2.1 to 3.2 6 show the effect of initial pH on the partitioning of L-lysine and on the water uptake. Some experimental observations are presented below and a discussion of possible reasons for their occurrence is given:

[a]· From Figures 3.2 1 to 3 2 3 it can be seen that the pH affects the partitioning of L-lysine in the organic phase As discussed in chapter 1, amino acids are differently charged at different pH. These charged states for L-lysine are·

$H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(H_2N)(COO^-)$	$pH>pK_R=10.53$
$H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(H_3N^+)(COO^-)$	$(pK_2=8.95)<pH<pK_R$
$H_3N^+\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(H_3N^+)(COO^-)$	$(pK_1=2.18)<pH<pK_2$
$H_3N^+\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(H_3N^+)(COOH)$	$pH<pK_1$

where pK_R, pK_1 and pK_2 are the R group and the first and the second dissociation constants, respectively. Since NaPOO, NaPSO and NaPSS are anionic surfactants and, after dissociation in water are negatively charged, their tendencies for the extraction of amino acids differ with the charged state of the amino acid At pHs higher than pK_2= 8 95, L-lysine has no charge and therefore the surfactant molecules do not tend to exchange their counterions with the L-lysine and the system shows poor extraction At pHs between pK_2 = 8 95 and pK_1 = 2.18 where the L-lysine has one net positive charge (two positive and one negative charges), the surfactants can exchange their counterions with the L-lysine In the region between pH = 8.95 and pH=2.18 there is a plateau where pH does not have a major effect

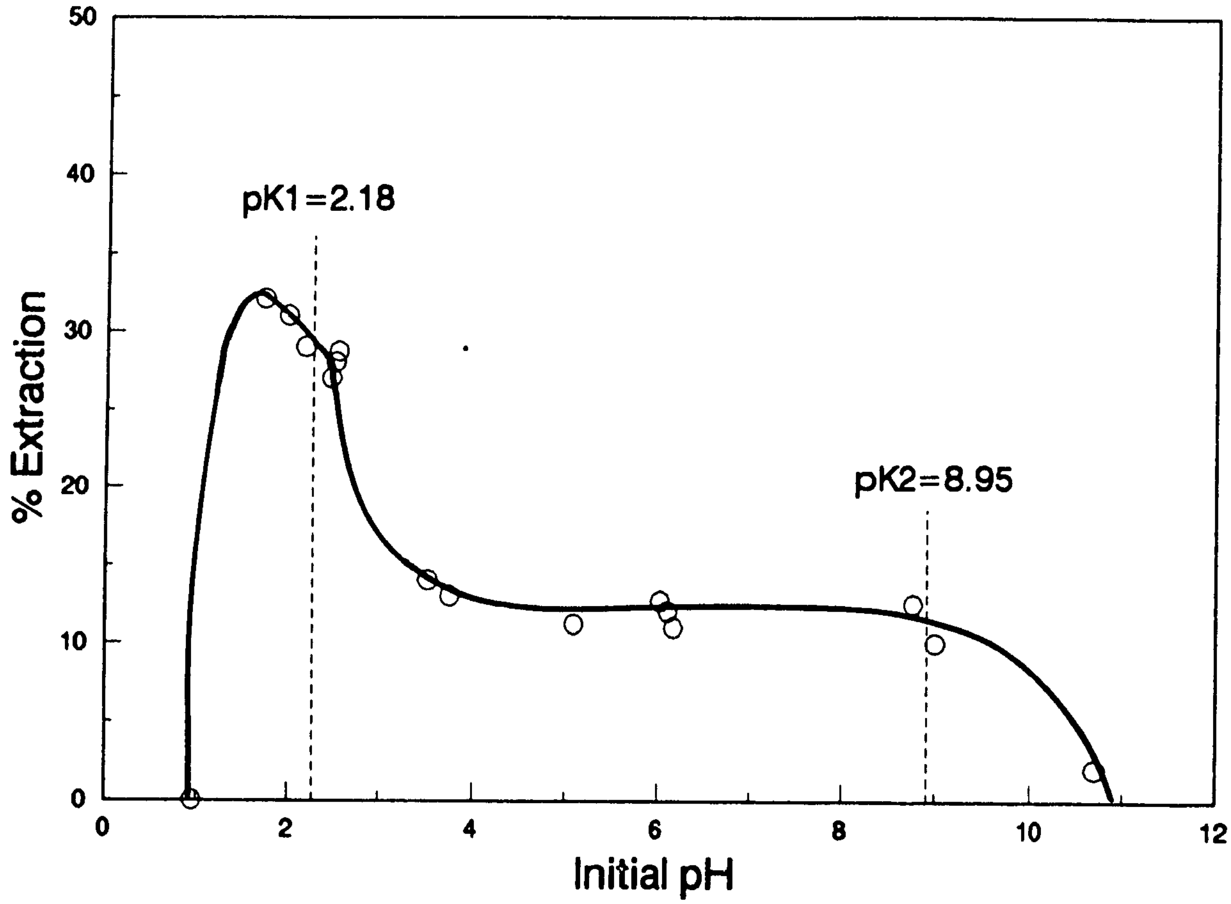


Figure 3.2.1. Effect of pH on the extraction of L-lysine at 400 mM pentanol, 100 mM NaPOO, 40 mM NaCl and 5 mM L-lysine.

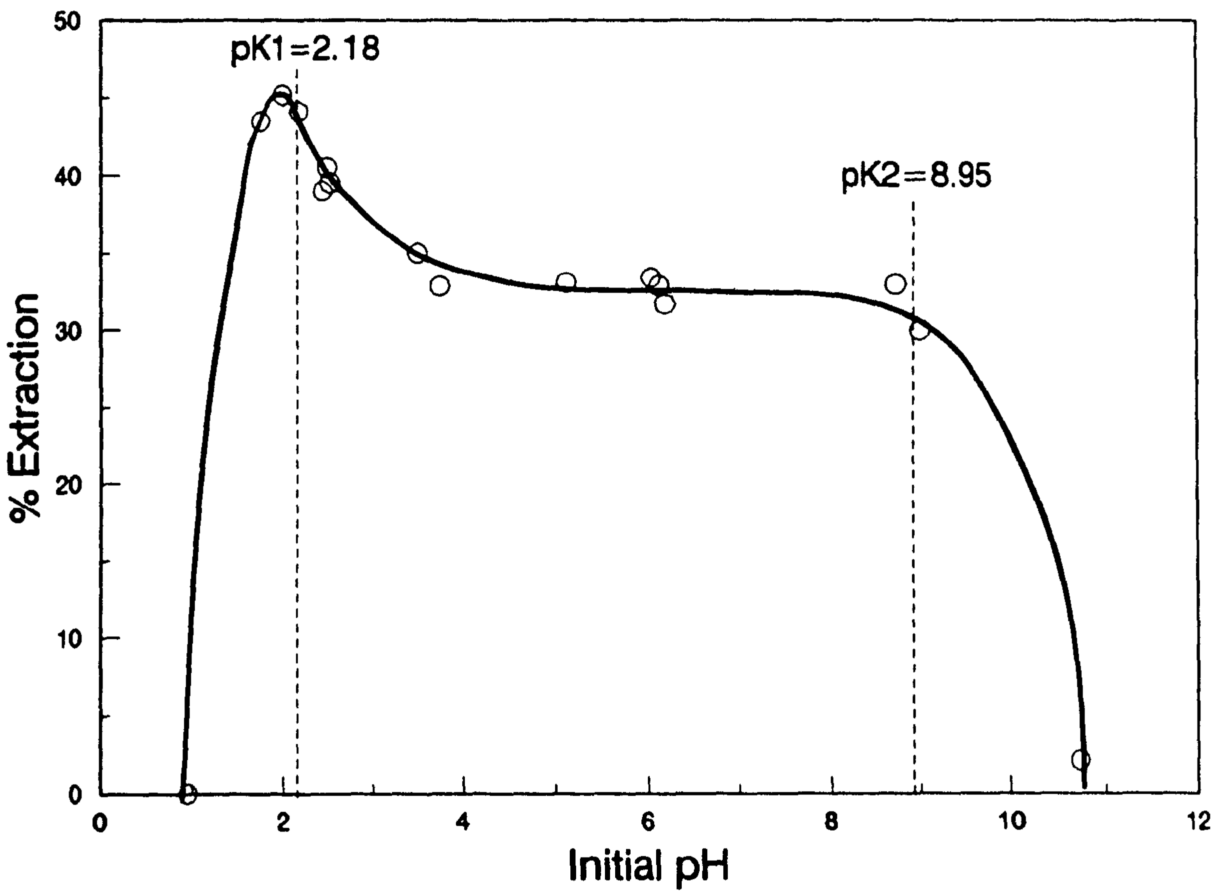


Figure 3 2 2: Effect of pH on the extraction of L-lysine at 400 mM pentanol, 100 mM NaPSO, 40 mM NaCl and 5 mM L-lysine.

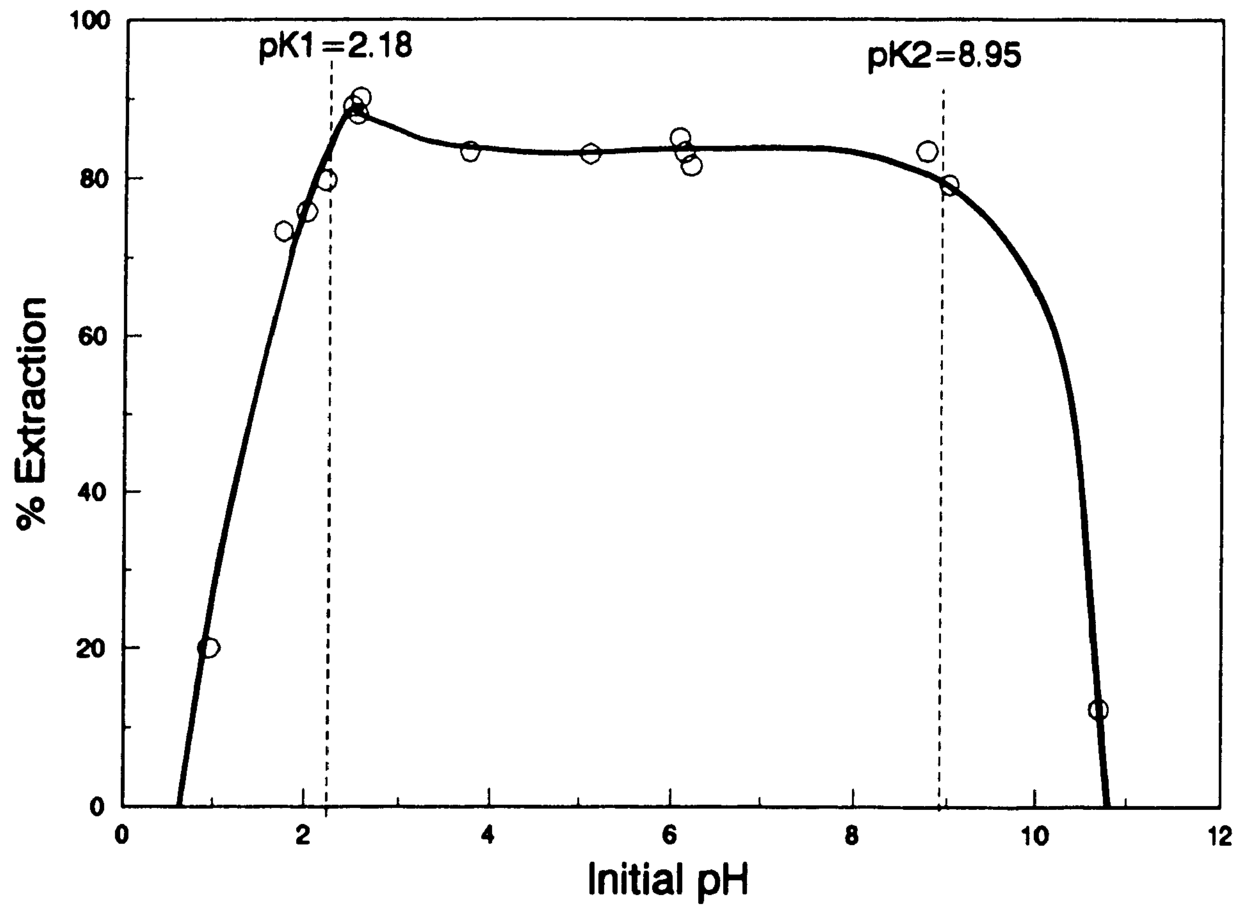


Figure 3.2 3: Effect of pH on the extraction of L-lysine at 400 mM pentanol, 100 mM NaPSS, 40 mM NaCl and 5 mM L-lysine

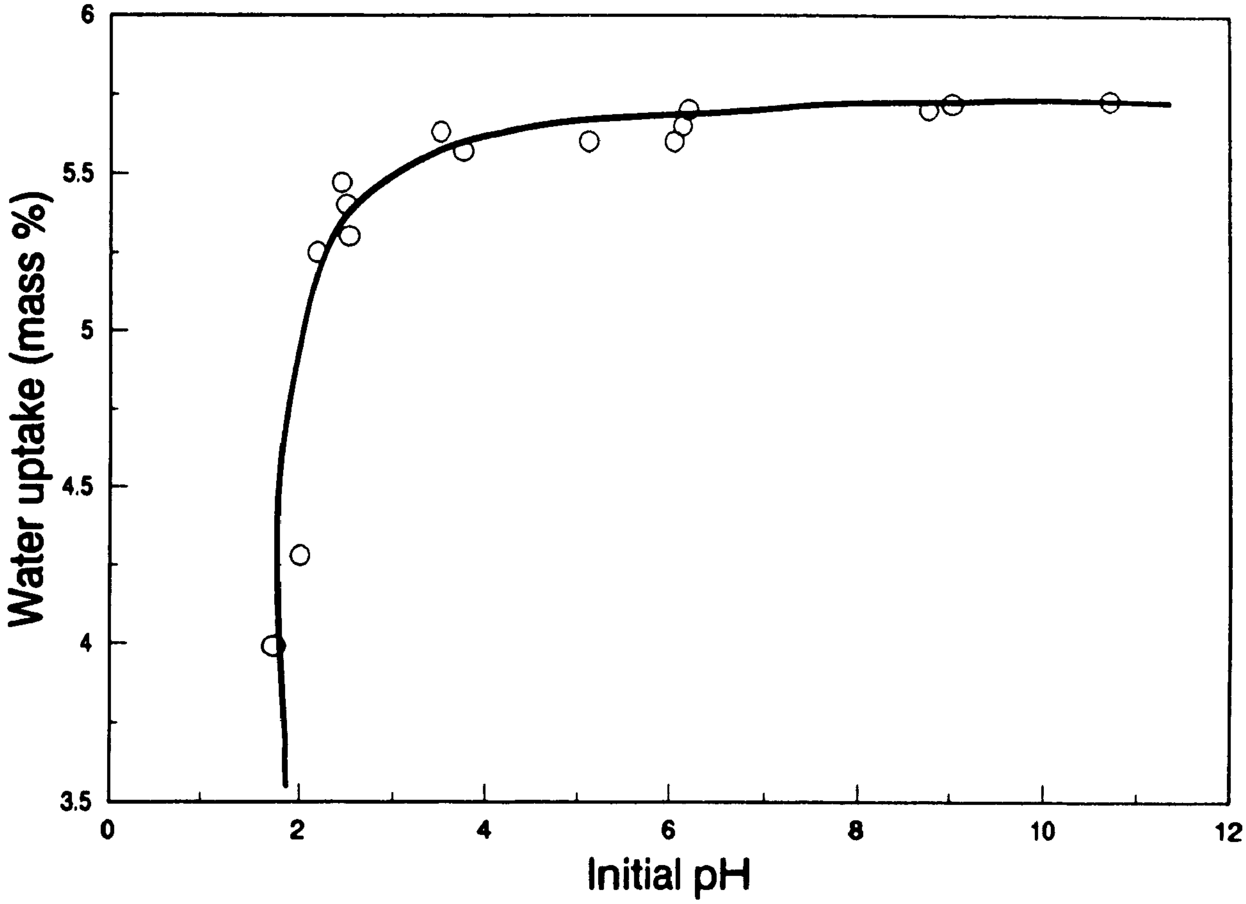


Figure 3.2.4: Effect of pH on the water uptake at 400 mM pentanol, 100 mM NaPOO, 40 mM NaCl and 5 mM L-lysine.

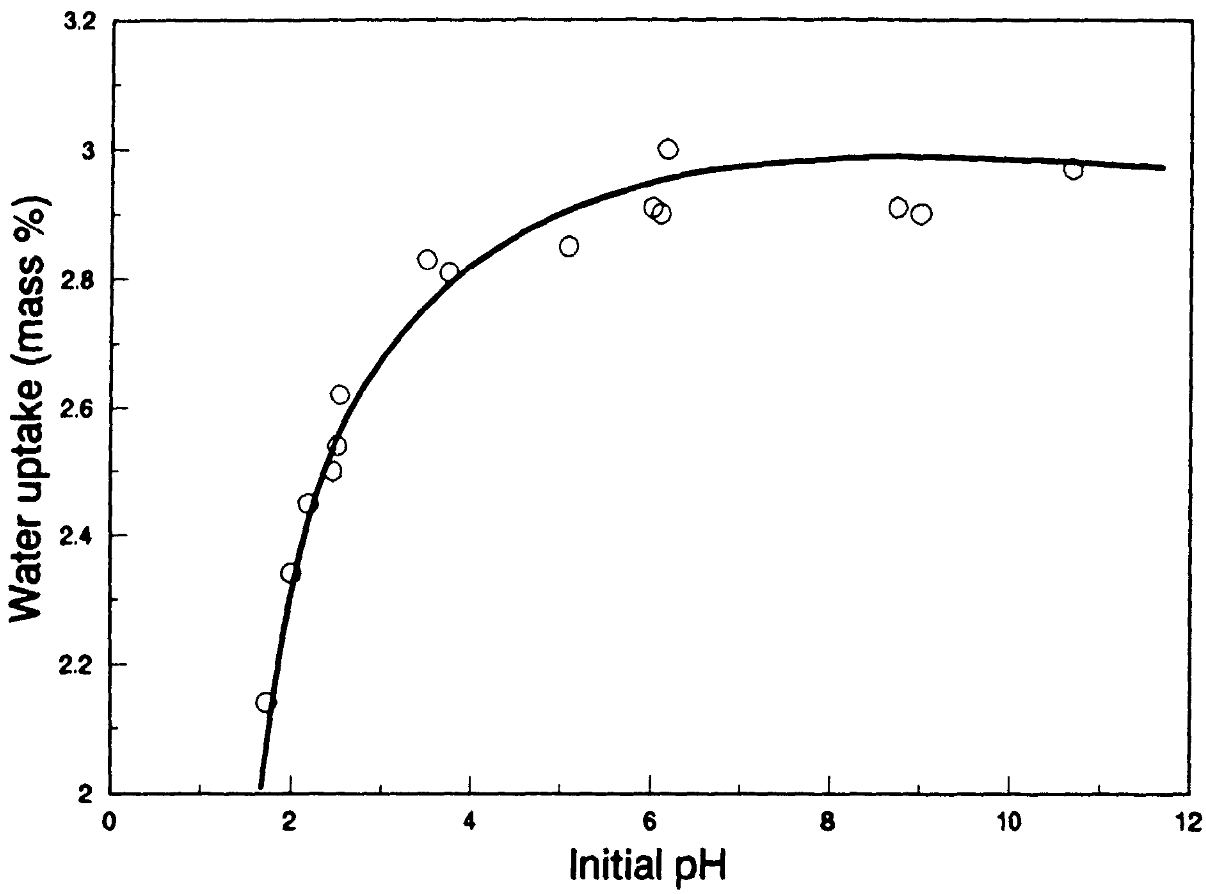


Figure 3.2.5· Effect of pH on the water uptake at 400 mM pentanol, 100 mM NaPSO, 40 mM NaCl and 5 mM L-lysine

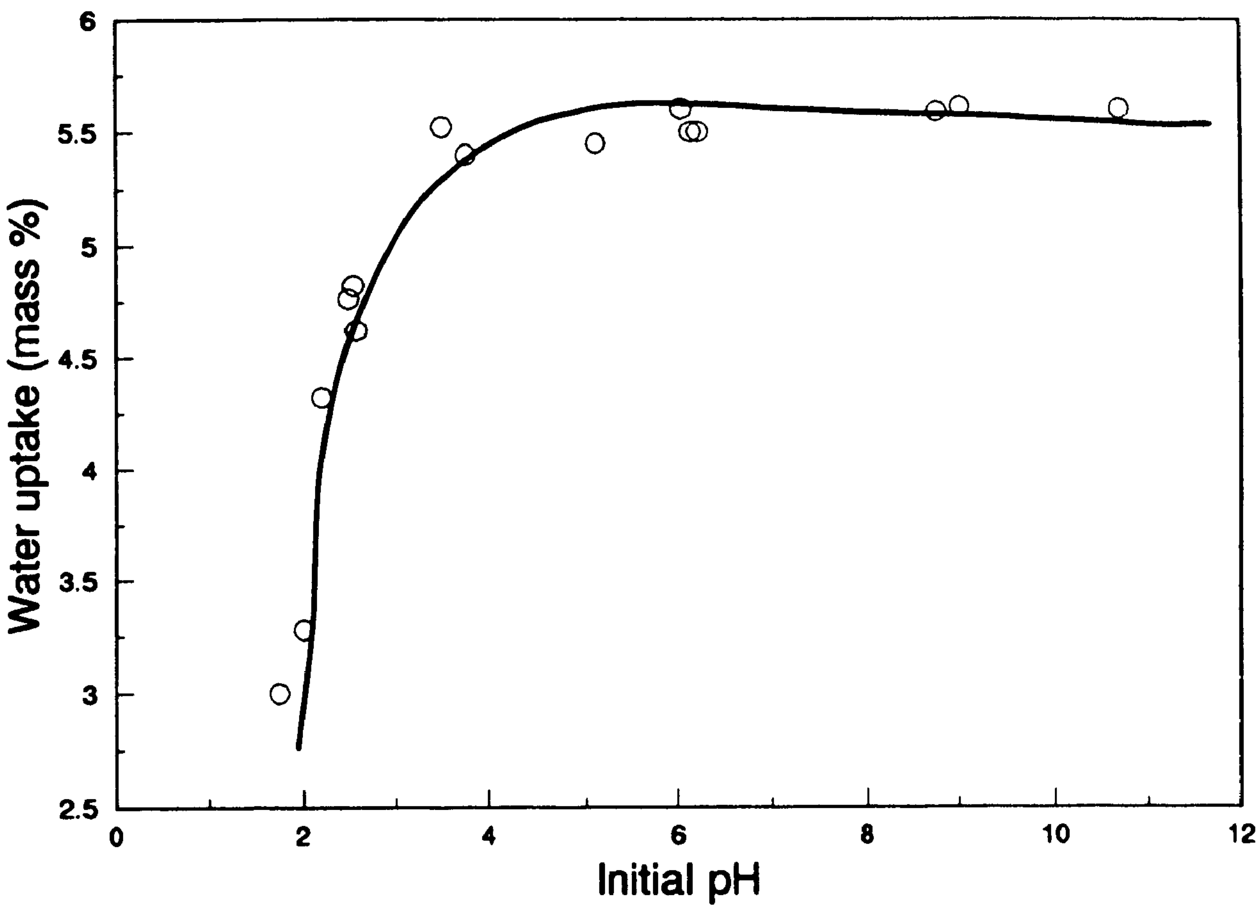


Figure 3.2.6: Effect of pH on the water uptake at 400 mM pentanol, 100 mM NaPSS, 40 mM NaCl and 5 mM L-lysine.

on the extraction of L-lysine. At a pH lower than 2.18, where the L-lysine has two positive charges, the surfactants have a higher tendency for the exchange of their counterions with L-lysine On the other hand at low pH the concentration of H^+ ions is very high and increases very fast as the pH decreases Therefore at low pH, the competition between H^+ ions and L-lysine ions is favourable for the exchange of H^+ ions and this reduces the extraction of L-lysine Therefore, at pH lower than pK_1 there is first an increase in the extraction and then a dramatic decrease in the extraction This system behaviour can be used for the back extraction of L-lysine In all extraction experiments, the final pH was from 8.8 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

[b] Figures 3 2 4 to 3.2.6 show that at low pH the water uptake decreases sharply. An explanation for this can be that, at low pH, part of the surfactant molecules exchange their counterions with H^+ ions Since these surfactants in the acid form do not form reverse micelles (see Section 1 1 5) the water uptake of the system decreases.

3.2.3 Effect of Salt Concentration

Experiments were performed to study the effect of salt concentration on the reverse micellar extraction of L-lysine, on the water uptake and on the surfactant partitioning. The system consisted of (isooctane + cosurfactant) + (water + NaCl + HCl + surfactant + L-lysine). The experiments were conducted at a constant temperature of 25°C with

100 mM surfactant and 5 mM L-lysine in the aqueous phase and with 400 mM pentanol in the organic phase. Concentrations of 25, 40, 70, 100, 125 and 150 mM of NaCl in the aqueous phase were used. The surfactants studied were NaPOO, NaPSO and NaPSS

Figures 3.2.7 to 3.2 9 show the effect of salt concentration, at three different values of the pH, on the partitioning of L-lysine and on the water uptake Some experimental observations are presented below and a discussion of the possible reasons for their occurrence is given:

[a]: Salt concentration is an important factor which influences the partitioning of L-lysine in the systems studied Figures 3 2.7 to 3 2 9 show that below 25 mM NaCl concentration, these surfactants do not form reverse micelles and stay in the aqueous phase Therefore the organic phase does not extract the L-lysine The effect of salt concentration on the formation of reverse micelles is discussed in section 3 1 3.

[b]: Figure 3.2.7 (a) to 3.2 9 (a) show that increasing the salt concentration decreases the L-lysine extraction. The extraction of L-lysine decreases linearly with salt concentration over the whole range of salt concentration and pH studied An explanation for this is that the increase in salt concentration favours the formation of the undissociated form of the surfactant by virtue of the common-ion effect. Another explanation of this effect may be that increasing the salt in the aqueous phase also increases the salt concentration in the reverse micelles and therefore expels the amino acid out of the reverse micelles (salting-out effect). A different interpretation of this phenomena is based on electrostatics (Leodidis and Hatton, 1990). According to this interpretation the amino acids have very high dipole

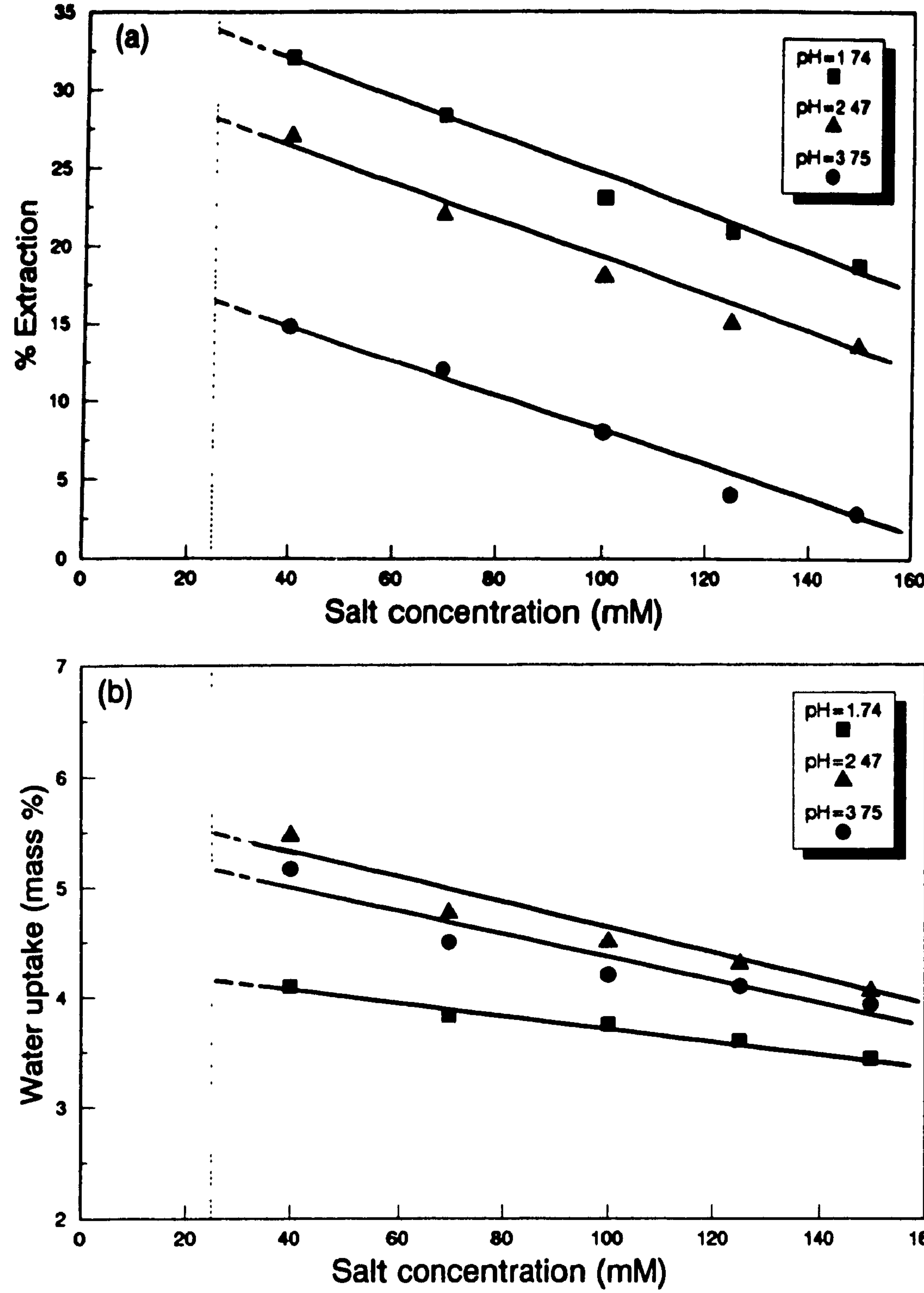


Figure 3.2.7: Effect of NaCl concentration on the (a) extraction of L-lysine and (b) water uptake at 400 mM pentanol, 100 mM NaPOO and 5 mM L-lysine.

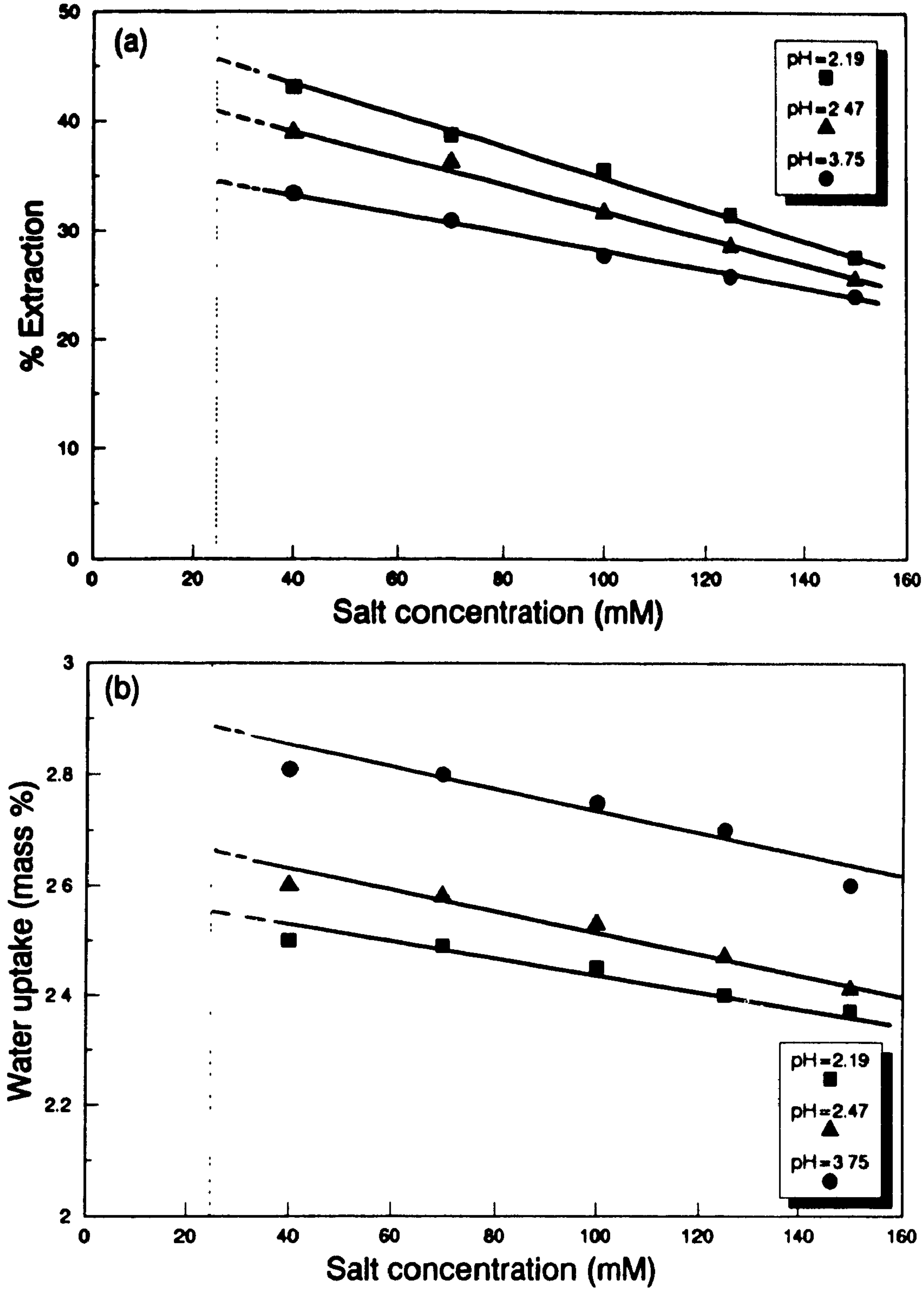


Figure 3.2.8: Effect of NaCl concentration on the (a) extraction of L-lysine and (b) water uptake at 400 mM pentanol, 100 mM NaPSO and 5 mM L-lysine

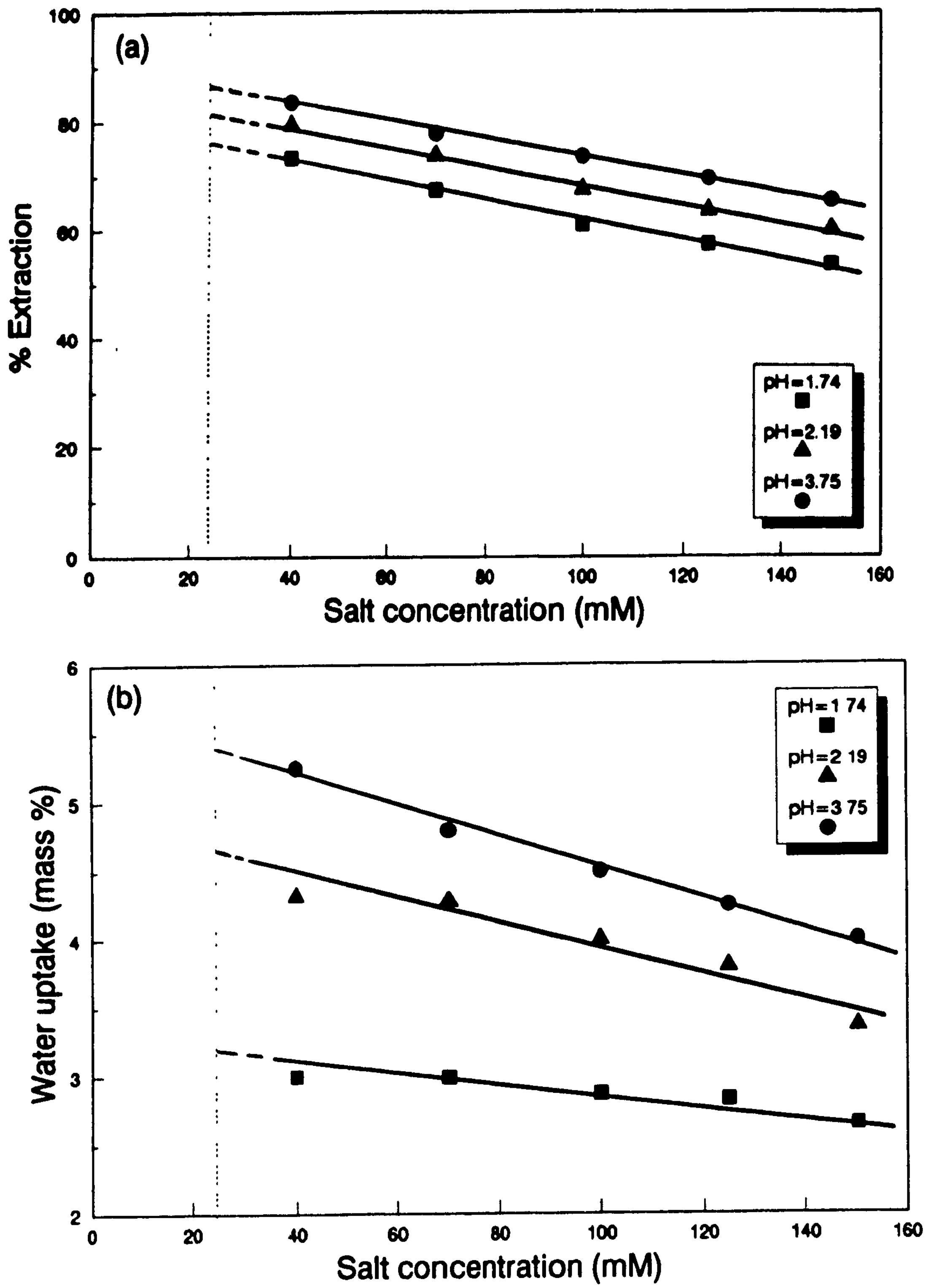


Figure 3.2 9: Effect of NaCl concentration on the (a) extraction of L-lysine and (b) water uptake at 400 mM pentanol, 100 mM NaPSS and 5 mM L-lysine.

moments in their zwitterionic form and the electric field in the water pools, close to the head groups, depends on the electrolyte concentration (Leodidis and Hatton, 1989) and the concentration of amino acid in the water pool depends on the salt concentration, reflecting the interaction of the solute with the electric field.

[c]: As shown in figures 3.2.7 (b) to 3.2.9 (b), increasing the salt concentration slightly reduces the equilibrium water uptake of the organic phase In the next section it will be seen that the water uptake for the surfactants is in the order NaPOO>NaPSS>NaPSO while the order of the extraction is NaPSS>NaPSO>NaPOO The order of the water uptake of the surfactants is the same whether or not there is extraction. Therefore, it can be concluded that the water uptake of the system, under the conditions of these experiments, can be considered to be independent of L-lysine extraction and the reason for the decrease in the water uptake with the increase in salt concentration is the same as discussed in section 3.1.3.

3.2.4 Effect of Surfactant Structure

Figures 3.2.10 and 3 2.11 show the effect of the surfactant head group on the extraction of L-lysine and the water uptake. As is evident 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 surfactants Since the

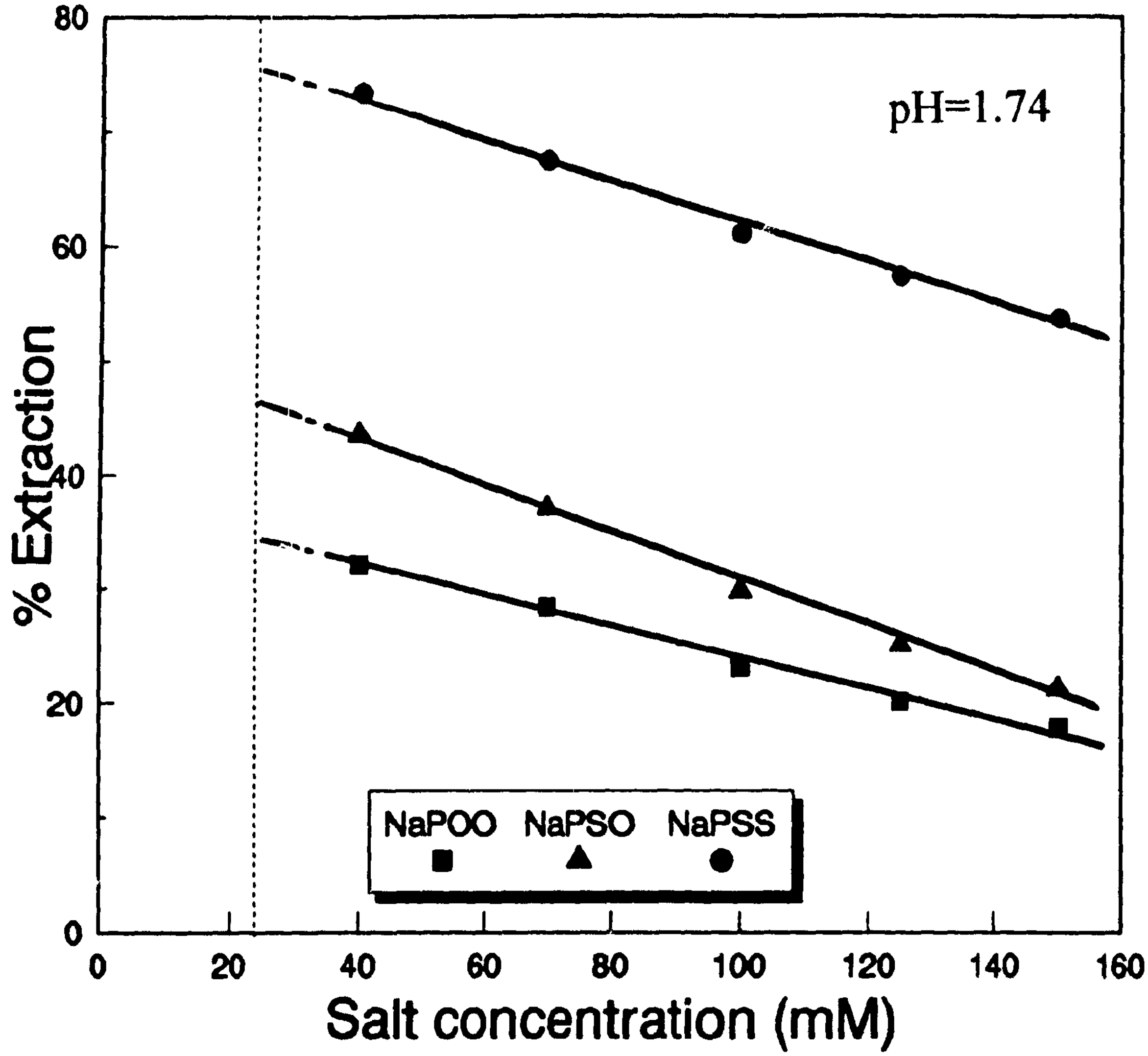


Figure 3.2.10: Effect of surfactant head group structure on the extraction of L-lysine for (■) NaPOO, (▲) NaPSO and (●) NaPSS at 400 mM pentanol, 100 mM surfactant and 5 mM L-lysine.

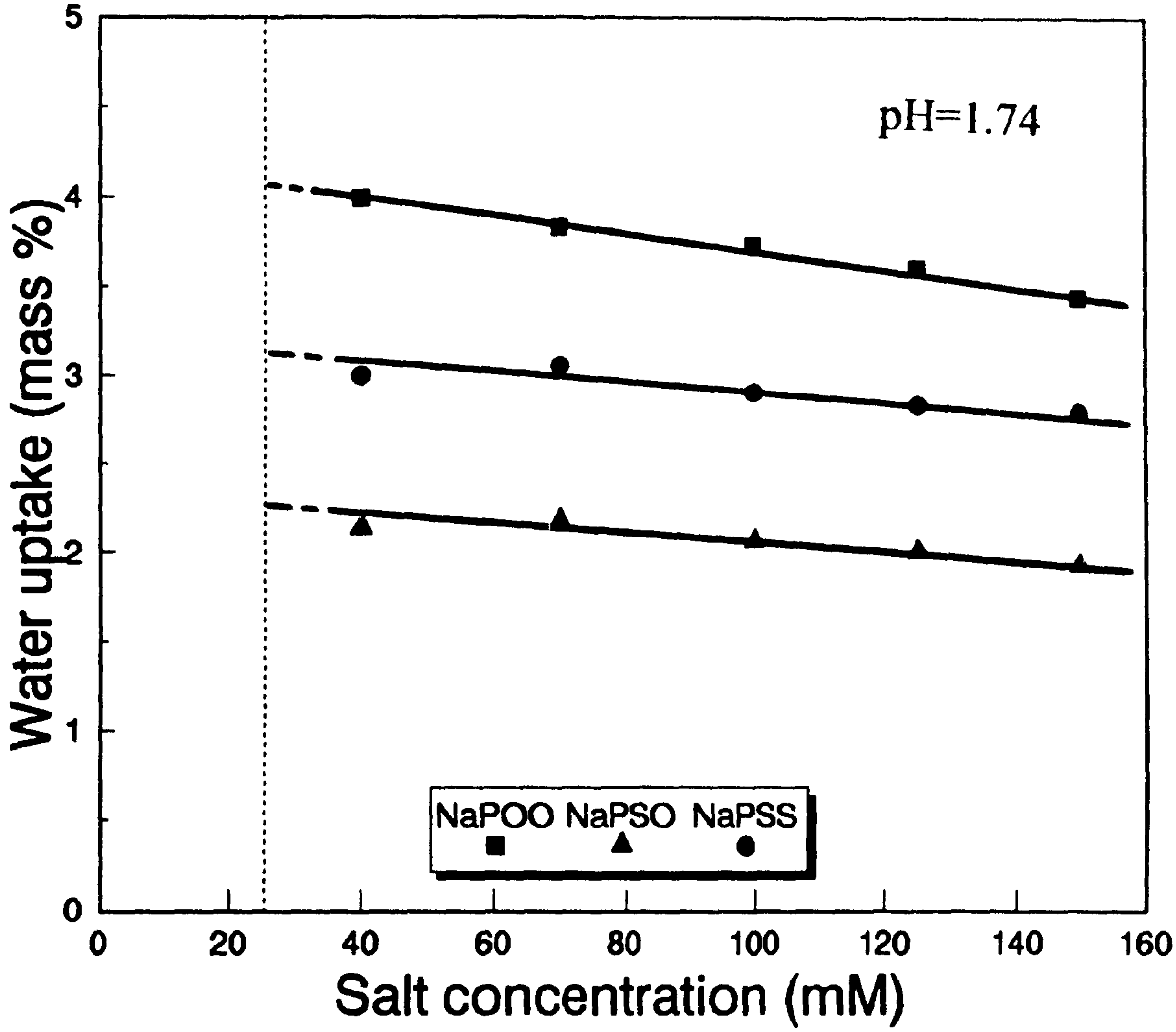


Figure 3 2.11: Effect of surfactant head group structure on the water uptake for (■) NaPOO, (▲) NaPSO and (●) NaPSS at 400 mM pentanol, 100 mM surfactant and 5 mM L-lysine electronegativity of the S atom in the organophosphosphorus compounds is higher than that of the O atom (Sole and Hiskey, 1992) then, sulphur substitution in the surfactant head group enhances the extraction Figure 3 2.11 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 as discussed in section 3.1.3 .

3.2.5 Back Extraction of L-lysine

Experiments were conducted to study the back extraction of L-lysine from the reverse micellar organic phase In order to extract L-lysine to the organic phase , reverse micellar extraction was performed at 25°C with 100 mM NaPOO, 40 mM NaCl and 5 mM L-lysine in water, and 400 mM pentanol in isooctane The pH of the aqueous phase was adjusted to 2 by adding HCl The procedure of extraction was similar to that described in section 2 4 The analysis of amino acid showed that the organic phase contained 1 33 mM L-lysine Ten ml of the loaded organic phase were contacted with 10 ml solution of 0.3 N HCl, shaken for 10 min and settled for 30 min The aqueous phase was analyzed for L-lysine The results showed that the aqueous phase contains 1 28 mM L-lysine which is equivalent to 96% back extraction The aqueous phase was analyzed for surfactant and none was detected. An explanation for this is that the H^+ ions replaced the Na^+ ions of the NaPOO and since the acid form of this surfactant cannot form reverse micelles, 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 form of the HaPOO (CYANEX 272), in contrast to NaPOO, is insoluble in water (Corbridge, 1985) no surfactant was detected in the aqueous phase

In the next step, a 10 ml solution of 4 N HCL was repeatedly contacted with 10 ml fresh loaded organic phase. The phases were shaken and settled each time and then separated The aqueous phase was again contacted with a new loaded organic phase the results are shown in Table 3.2 1

Table 3.2 1: Repeated back extraction of L-lysine

Run No.	5	10	15	20	25
L-lysine (mM)	6.52	13.2	17.5	23 9	29 3

Chapter 4

Conclusions and recommendations

4.1 Conclusions

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), can form reverse micelles in a system consisting of isooctane, alcohol, water and NaCl The concentration of salt, nature and concentration of both surfactant and alcohol cosurfactant have a major effect on the formation of reverse micelles and on the water uptake These surfactants, in order to form reverse micelles, need a minimum cosurfactant and salt concentration (at which the maximum water uptake occurs). Increasing the cosurfactant or the salt concentration, or increasing the cosurfactant chain length, decreases the water uptake Upon addition of alcohol cosurfactant to the organic phase all three surfactants passed from micelles to a three phase system and then to reverse micelles The nature of the surfactant head group was found to be a determinant factor on the water uptake For the same conditions, the water uptake decreases in the order NaPOO>NaPSS>NaPSO.

These three surfactants have the ability to extract L-lysine from dilute aqueous solutions The nature of the surfactant, the pH and the salt concentration 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 therefore at low pH the extraction decreases dramatically Increasing the salt concentration decreases the extraction through the common-ion effect The different head groups have 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 cosurfactant, 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 the water uptake with or without extraction However, the ordering was different for extraction, reflecting the differences in the factors affecting the formation of reverse micelles and reverse micellar extraction

4.2 Recommendations

Some new areas of research which can be followed as a continuation of this work would be:

[1]: To investigate the effect of different organic solvents on the formation of reverse

micelles using NaPOO, NaPSO and NaPSS

[2] To study the effect of different cosurfactants on the reverse micellar extraction of amino acids or other biochemical materials

[3] To study the possibility of reverse micellar extraction of other biochemicals and metallic ions with these surfactants

[4] To determine the effect of the surfactant head group and of the structure of the extracted molecule on the reverse micellar extraction The first systematic work can be done using different amino acids with the surfactants developed in this work

[5] To investigate the selectivity of each of these surfactants, and even of a mixture of them, on the reverse micellar extraction of different biomolecules such as proteins, peptides, etc

[6] Since at low pH these surfactants cannot form reverse micelles, they have good potential for the back extraction step Therefore, it is important to investigate the back-extraction of biomolecules from reverse micelles formed by these three surfactants In this work, the back extraction of L-lysine from reverse micelles formed by NaPOO was studied as a test case This was just a preliminary study to confirm the potential advantage of these surfactants for concentration of L-lysine However, the study of back extraction under different conditions and with the other surfactants is open for research. Obviously, studies with NaPSS should be given preference.

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Appendix A

Purification

As it was discussed in chapter 2, three surfactants studied in this work were prepared by reacting bis(2,4,4-trimethylpentyl) phosphinic acid (HPOO), bis(2,4,4-trimethylpentyl) monothiophosphinc acid (HPSO) and bis(2,4,4-trimethylpentyl) dithiophosphinic acid (HPSS) with sodium hydroxide.

Commercially available HPOO,HPSO and HPSS were not pure and their purity using potentiometric titration were found to be 85%, 84% and 75%, respectively Equivalent points for HPOO, HPSO and HPSS were found by titrating with 0 05 N NaOH in an 80% ethanol solvent.

The HPOO, HPSO and HPSS were purified through a copper salt precipitation method similar to the purification of bis(2-ethylhexyl) phosphoric acid proposed by Partridge and Jensen (1968). The procedure was as follows

A solution of 1 M of HPOO in benzene was prepared and 500 ml of it were contacted with 500 ml solution of 2 M $Cu(OH)_2$ and mixed for 2 hr until all the H^+ ions in the HPOO were replaced with Cu^{2+} according to the following reaction.

$$Cu(OH)_2 + 2HPOO \longrightarrow Cu(POO)_2 + 2H_2O \qquad (A\text{-}1)$$

For removal of the aqueous phase, the $Cu(POO)_2$ was precipitated by slow addition of acetone under stirring After complete precipitation, the $Cu(POO)_2$ was separated and washed 5 times with acetone and dried with air The $Cu(POO)_2$ was then, converted back to the acid form by reacting with a 6 M HCl solution according to the following reaction:

$$Cu(POO)_2 + 2HCl \longrightarrow 2HPOO + 2CuCl_2 \qquad (A\text{-}2)$$

The HPOO was separated from aqueous solution and was washed 5 times with 0.1 N solution of HCl and 10 times with water to remove all Cu(II) from the HPOO phase. The procedure was the same for all three surfactants The purified HPOO, HPSO and HPSS were analyzed using potentiometric titration with 0.05 N NaOH in an 80% ethanol solvent and were found to be 99 4%, 99.1% and 99%, respectively.

Appendix B

Surfactant Analysis

The concentrations of NaPOO, NaPSO and NaPSS in the aqueous phase were determined by potentiometric titration In all experiments, 10 ml of an aqueous phase containing the surfactant were titrated with 0 05 N HCl solution A pH meter was used to measure the pH of the solution At the end point of the titration a drastic change in pH was observed (see Figure B 1) In order to prepare a calibration curve, stock solutions of each of the above surfactants were prepared in the range of 0 - 0 2 M and titrated

To measure the concentrations of NaPOO, NaPSO and NaPSS in the organic phase 10 ml of the organic phase containing the surfactant were first contacted with 10 ml solution of 0 1 N HCl and shaken vigorously. The phases were then separated and the water phase was titrated with a 0 05 N NaOH solution to determine the remaining HCl The result was used to calculate the concentration of the surfactant in the organic phase Since NaPOO, NaPSO and NaPSS, in the acid form, are insoluble in water, they did not interfere the titration of the water phase A sample calculation is as given below

A sample of 20 ml aqueous solution of 0 1 M NaPOO and 0.1 N NaCl was contacted and shaken with 20 ml solution of 0 2 M decanol in isooctane After the equilibrium was reached, the phases were separated and analyzed for the surfactant. For the aqueous phase, 2 3 ml solution of 0 05 N HCl were consumed to reach the end point which were equivalent to 0.0115 M surfactant in the aqueous phase. A sample of 10 ml of the organic phase was contacted with 10 ml solution of 0 1 N HCl. To neutralize the remaining HCl in the water phase, 2 5 ml solution of 0 05 N NaOH were consumed which were equivalent to 0 087 M surfactant in the organic phase The total surfactant is then (0.0115+0 0875) = 0 099 which is in good agreement with the initial amount of 0 1M surfactant in the system

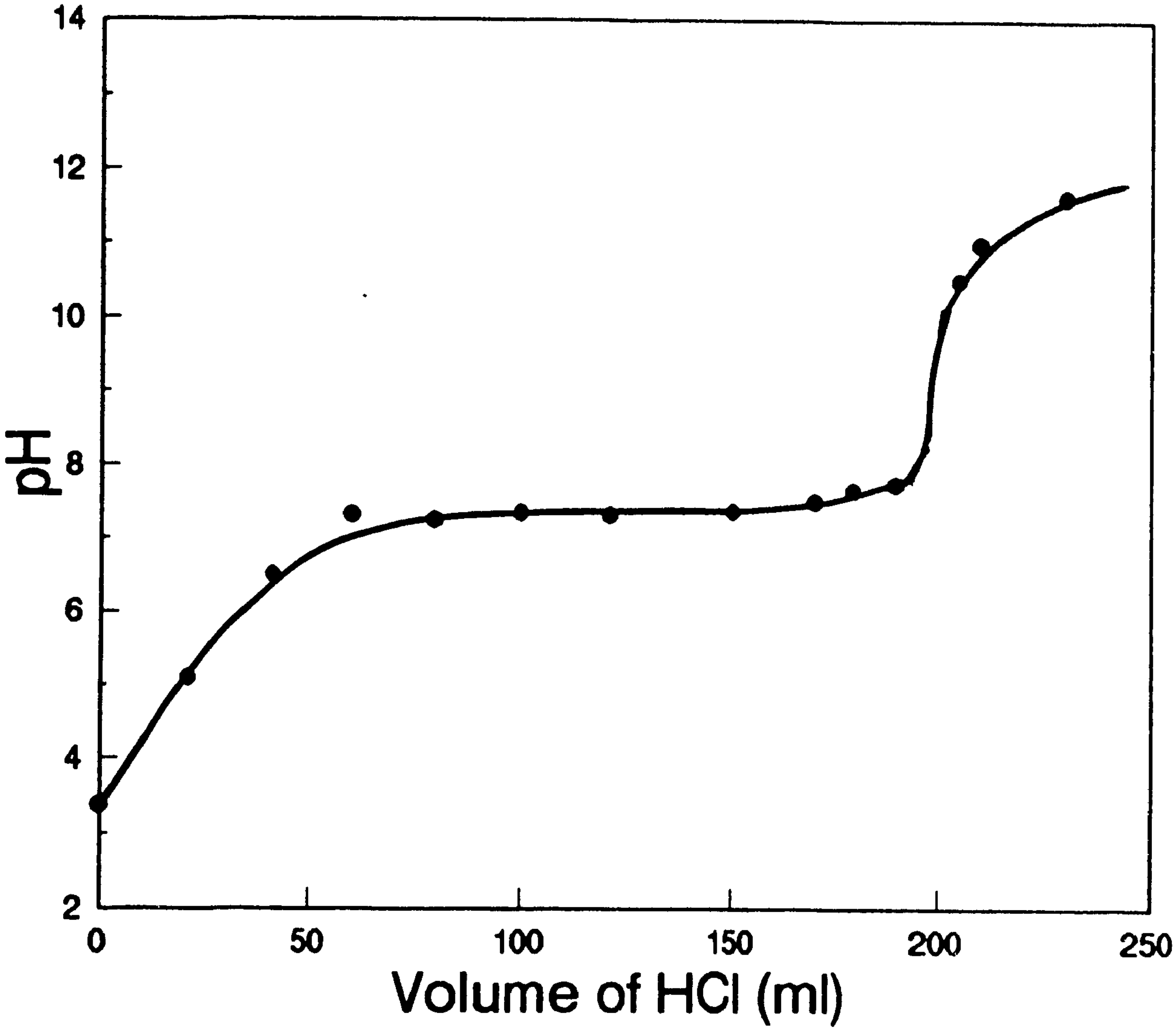

Figure B.1: Titration curve for 100 ml solution of 100 mM NaPOO with 0.05 N HCl solution.

Appendix C

Amino Acid Analysis

Analysis of the L-lysine in the aqueous phase was performed by spectrophotometry using the OPA labelling UV method proposed by Roth (1971) To prepare 1 litre of labelling solution, 0.8 gr o-phthaicdicarboxaldehyde (OPA) were dissolved in 20 ml ethyl alcohol then 2 ml 2-mercaptoethanol were added and mixed for 30 min The whole solution was added to 500 ml of a solution of 0.1 M sodium tetraborate and then the total volume was brought to 1000 ml with deionized water and mixed for 3 hr

To find the concentration of L-lysine in water, 0.6 ml of the solution containing L-lysine were added to 20 ml of labelling solution and after, 5 min, the UV absorbance spectrum at 340 nm was measured For each set of measurements a new calibration curve for L-lysine, in the concentration range of 0.5 - 5 mM, was prepared (Figure C 1)

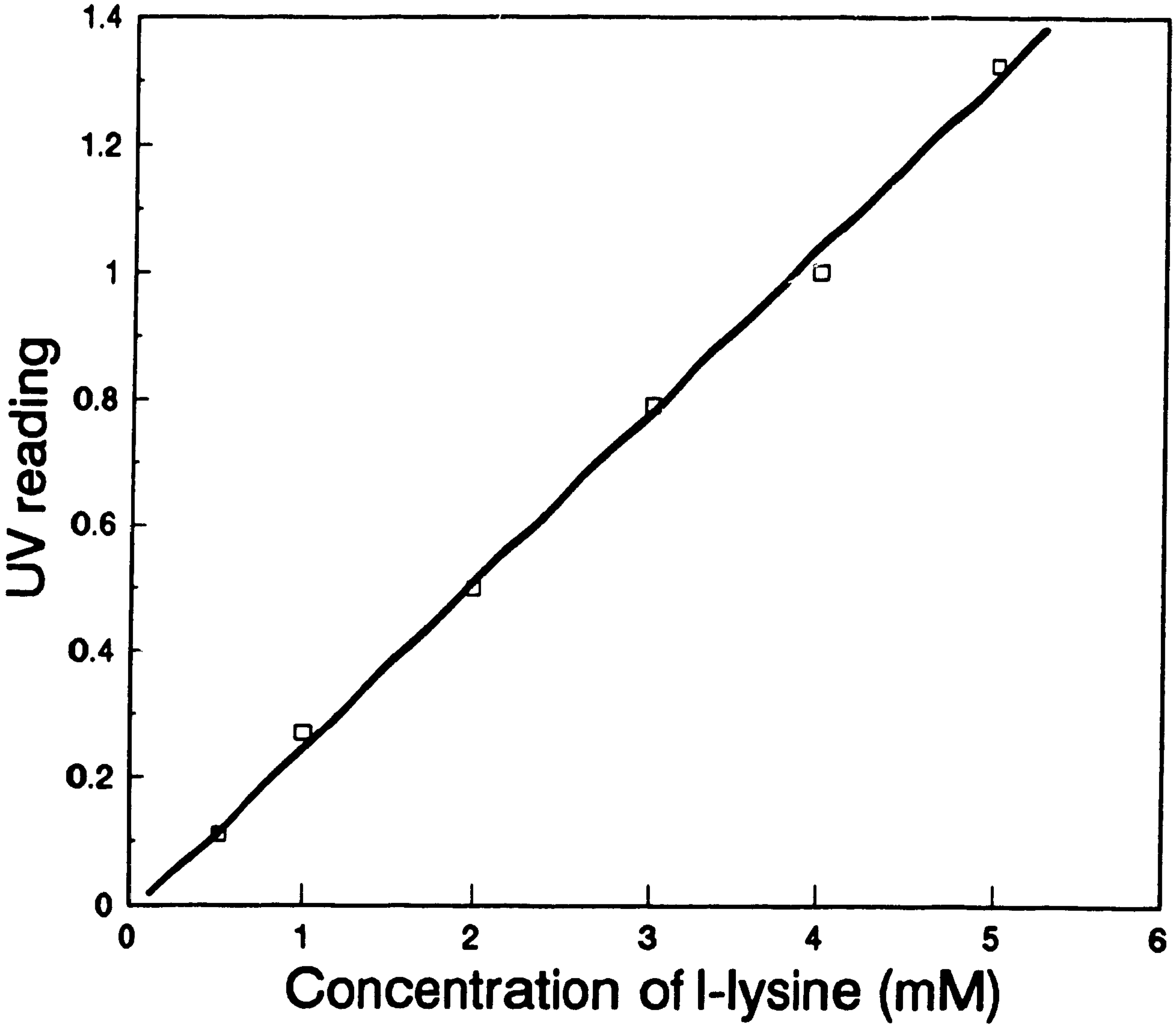

Figure C.1: Typical calibration curve for L-lysine using labelling method.

Appendix D

Preliminary experiments

Preliminary experiments were performed to find a satisfactory value for both the shaking and the settling time. Several identical samples were prepared and tested at different shaking and settling times for both the formation of reverse micelles and the reverse micellar extraction of L-lysine. Waiting time should be long enough to ensure that equilibrium is reached but not unnecessarily long. In fact, it is desirable to find the shortest possible time after which results do not change within the sensitivity of the measurements. The results did not change after 30 min shaking and 24 hr settling for more safety 1 hr shaking and 48 hr settling were fixed in all subsequent experiments A typical set of results are shown in Tables D.1 to D.2.

Table D 1· Effect of shaking and settling time on the formation of reverse micelles.

Run No	1	2	3	4	5	6	7	8	9
Shaking time (hr)	0.5	1	3	0 5	1	3	0 5	1	3
Settling time (hr)	24	24	24	48	48	48	72	72	72
Aqueous phase 0 1 M NaPOO + 0.1 M NaCl Organic phase 0 2 M decanol									
Water uptake	3 85	3.80	3 81	3 84	3 82	3.80	3.79	3.80	3.81
Aqueous phase 0 1 M NaPOO + 0.05 M NaCl Organic phase.0 2 M decanol									
Water uptake	3 06	3 08	3.13	3.05	3 12	3 13	3 02	3 09	3.08
Aqueous phase 0 1 M NaPOO + 0 1 M NaCl Organic phase 0 4 M decanol									
Water uptake	2.94	2 90	2 88	2.92	2 90	2 90	2 89	2.91	2 87

Table D.2 Effect of shaking and settling time on the reverse micellar extraction of L-lysine.

Run No.	1	2	3	4	5	6	7	8	9
Shaking time (hr)	0 5	1	3	0.5	1	3	0 5	1	3
Settling time (hr)	24	24	24	48	48	48	72	72	72
Aqueous phase 0 1 M NaPSS + 0 04 M NaCl + 0 05 M L-lysine, pH=1 74									
% Extraction	74 0	73.1	75 3	74 0	73.3	73 3	73.5	73 1	73.9
Aqueous phase 0 1 M NaPSS + 0 04 M NaCl + 0 05 M L-lysine, pH=2 19									
% Extraction	79.1	80 0	79.0	79 0	79.7	79 2	79 5	79 8	79 4
Aqueous phase 0 1 M NaPSS + 0 07 M NaCl + 0 05 M L-lysine, pH=1 74									
% Extraction	68 0	67 8	68 0	67 9	67 5	67.0	67.9	67.6	67.6

Appendix E

Experimental Data For the Formation of Reverse Micelles and Reverse Micellar Extraction of L-lysine

Table E 1 Wate- uptake and % surfactant in the organic phase for 100 mM NaPOO, NaCL free

Conc of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf. in Org Phase	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf. in Org Phase
50	0 00	2 0	0 00	2 0	0.00	2.5
100	0 00	3 0	0 00	3.2	0.16	3.5
150	0 18	7 0	0 20	5.1	0.12	4.2
200	2 50	58 2	1 90	42.0	0.07	5.0
250					0.08	5.2
300	1.75	54.5	1.85	50.0	0.20	7.0
400	1 50	60 0	1 70	57.0	0 30	12 0

Table E 2· Water uptake and % surfactant in the organic phase for 100 NaPOO at 50 mM NaCL.

Conc. of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf in Org Phase
50	0 00	8.5	0.00	10 0	0 00	3 5
100	0 57	58 0	0 19	26 2	0 00	7.0
150	4 42	80 0	5 33	78 2	0 00	10.0
200	3.11	80.0	3.48	80 0	0 00	12 0
250	2.63	83.5			0 19	15 0
300	2.51	85 0	2 71	85 1	0 99	19 0
350			2 55	87 5	7 82	83 0
400	2.35	87.1	2 44	86.1	5 03	85 0

Table E 3 Water uptake and % surfactant in the organic phase for 100 mM NaPOO at 100 mM NaCL

Conc of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf. in Org. Phase	Water Uptake (mass %)	%Surf. in Org Phase	Water Uptake (mass %)	%Surf. in Org. Phase
50	0 07	2.5	0.00	3.0	0.00	4 9
100	9 94	82 0	11.70	83 0	0.00	8.1
150	5 40	84 5	6.50	84.1	tph	13.2
200	3 87	86 0	4 50	84.3	12.87	87.1
250	3 22	87.0	3 70	86.0	7 49	89.0
300	2 92	88 0	3 00	87.0	5.25	89.0
400	2 94	90.0	3 00	89.0	3 79	90 1

tph = three phase

Table E.4 Water uptake and % surfactant in the organic phase for 100 mM NaPOO at 200 mM NaCL.

Conc. of Cosurf. in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf. in Org Phase	Water Uptake (mass %)	%Surf in Org Phase
50	tph	tph	tph	tph	0 00	5 1
100	5.56	90.0	5 88	90.0	tph	tph
150	3 79	89.5	3.98	90 1	7 60	89 0
200	3.01	91.3	3 20	91 5	5.71	90 0
250	2.82	92 0	2 92	91 0	4 50	91 0
300	2.65	92.1	2 69	92.4	3 89	92 0
400	2.60	95.0	2 48	94 3	3 69	93 5

tph = three phase

Table E 5   Water uptake and % surfactant in the organic phase for 100 NaPOO at 300 mM NaCL

Conc of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf. in Org. Phase
50	0 22	5.4	0.22	7 8	0.00	5.9
100	4 54	89.5	4 71	90 0	tph	tph
150	3 40	90 9	3 55	91 0	5.27	90.0
200	2 65	91 0	2 80	90 8	4.40	91.5
250	2 56	92 2	2 68	94 1	3.80	93 2
300	2 54	94.8	2 66	95.0	3 42	94 1
400	2 28	95 8	2 49	96.1	3.59	96.0

tph = three phase

Table E.6 Water uptake and % surfactant in the organic phase for 100 NaPSS, NaCL free.

Conc. of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf. in Org Phase	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf in Org Phase
50	0 00	2 4	0 00	3 0	0 00	5 0
100	0.00		0 00	4 2	0 00	5 0
150	0 15	9.0	0 25	5 2	0 00	5 5
200	1 50	60 4	1.00	51.0	0.00	6 0
250					0 07	8.5
300	1.50	64 5	0 94	65 0	0 14	10 0
350					0 20	
400	1.15	75 0	1 00	70 0	0 20	12 0

Table E 7 Water uptake and % surfactant in the organic phase for 100 mM NaPSS at 50 mM NaCL.

Conc of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf. in Org Phase	Water Uptake (mass %)	%Surf in Org. Phase
50	0 00	5 4	0 00	6 8	0 00	7 0
100	2 91	79 5	0 00	9 0	0 00	10.0
150	7 91	85.9	8 72	85 0	0 00	12 0
200	4 41	89 0	4 75	87.8	0.00	15.0
300	2.86	89 8	3.03	90.0	19 82	90.0
400	2.56	90 8	2 70	91.1	6 68	90.0

Table E 8: Water uptake and % surfactant in the organic phase for 100 mM NaPSS at 100 mM NaCL.

Conc of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf. in Org Phase	Water Uptake (mass %)	%Surf in Org Phase
50	0 17	16 0	0 16	17 0	0 00	9 0
100	9 35	87 0	12 36	88 1	0 10	11 1
150	5 12	87 5	5.80	88 5	tph	25 2
200	3 48	88 0	3 94	88 5	14 36	90 0
250	2 94	89 1	3.40	89.0	7 96	91 0
300	2 64	89 5	2.68	90 0	6 20	92 5
400	2 41	90 0	2.49	90 1	4 09	95 0

Table E 9 Water uptake and % surfactant in the organic phase for 100 mM NaPSS at 200 mM NaCL

Conc of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf in Org. Phase	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf in Org Phase
50	tph	tph	tph	tph	0 08	14.0
100	6.56	95.0	7 29	95 0	tph	tph
150	4 28	96. 0	4.50	95 0	11 04	97 0
200	3 33	96.2	3.57	96 1	6 72	98.0
250	2 93	97.5	2.97	98.0	5 37	98.0
300	2 62	97 5	2.57	98 0	4 52	98 5
400	2 50	98.8	2.53	99.0	3.51	99.0

tph = three phase

Table E.10: Water uptake and % surfactant in the organic phase for 100 mM NaPSS at 300 mM NaCL.

Conc. of Cosurf in Org. Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf in Org. Phase	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf in Org Phase
50	tph	tph	tph	tph	tph	tph
100	5 54	89.5	5 93	97 5	tph	tph
150	3.81	96 0	4.22	98.0	6 87	97 2
200	3 05	96 8	3 31	98 8	5 27	98 1
250	2.73	98 2	2.92	98 1	4 54	98.5
300	2.55	98 8	2 64	99 0	3 84	98 1
400	2.45	99 0	2.49	99 0	3 60	99.0

tph = three phase

Table E 11 Water uptake and % surfactant in the organic phase for 100 mM NaPSO at 100 mM NaCL.

Conc. of Cosurf in Org Phase (mM)	Decanol		Octanol		Pentanol	
	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf in Org Phase	Water Uptake (mass %)	%Surf. in Org. Phase
50	7 86	15 1	9 04	16.9.	0.00	7.5
100	3.55	87.0	4.26	88.0	thp	9.0
150	2 43	87 5	3.15	88 5	5 20	89.1
200	2 10	88.0	2 60	89.1	3.83	90.0
250	1.88	89.2	2 21	89.0	3.00	90.8
300	1.85	89 5	1.93	90.0	2 73	92.5
400	1.89	90.0	1.83	90.3	2.40	95.2

tph = three phase

Table E.12: Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPSS / 40 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0.95	4.5		
1.74	73.3	3 00	6 21
2.00	75.8	3.28	6 50
2.19	79 7	4.32	6 66
2 47	89.0	4 76	7 08
3.75	83.3	5 40	7 11
5.1	83 0	5.45	7 17
6.12	83 2	5.50	7 20
8 75	83.4	5.59	7.32
10.70	12.2	5.60	9 95

Table E 13· Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPSS / 70 mM NaCl / 5 mM L-lysine / HCl.

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0.95	5 2		5 20
1 74	67 3	3 05	6 09
2.00	68.6	3.19	6 30
2.19	74 0	4.28	6.61
2 47	76.0	4 15	6.76
3.75	77.1	4.64	6.85
5.10	73 1	4.70	6 90
6.12	74 0	4 95	7.04
8.75	74.2	5.05	7.60
10.70	3 1	5.60	9.40

Table E.14: Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPSS / 100 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0.95	1 0		
1.74	60.5	2 90	6.16
2.00	64.7	3.10	6 32
2.19	67.9	3 57	6 34
2.47	71.3	3 90	6 70
3.75	72 7	4.16	6.90
5.10	68.8	4 52	7.02
6.12	65.4	5 21	7.12
8.75	69 0	5.70	7.32
10.70	3.0	5.81	9 20

Table E.15: Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPSS / 150 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0.95	0.9		
1.74	53.6	2.79	5.90
2.00	56.9	2.97	6.02
2.19	60.0	3.36	6.32
2.47	63.5	3.64	6.60
3.75	64.2	4.01	7.25
5.10	58.2	4.92	7.42
6.12	60.1	5.32	7.70
8.75	60.0	5.20	8.01
10.70	2.0	5.40	9.54

Table E 16: Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPSO / 40 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0 95	0 0		
1.74	43.5	2 14	8.10
2.00	45.1	2.34	8.15
2.19	44.1	2.45	8 24
2 47	39 0	2 50	8 32
3.75	32.9	2.81	8 40
5.10	33.1	2 85	8 64
6.12	32.9	2.90	8.72
8.75	33.0	2 91	9 08
10.70	2.1	2 97	9 89

Table E.17 Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPSO / 70 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0.95	0.0		
1.74	37.0	2.18	8.50
2.00	39.7	2.41	8.45
2.19	36.7	2.49	8.92
2.47	37.2	2.58	8.99
3.75	30.1	2.81	8.42
5.10	28.0	2.85	8.71
6.12	26.5	2.90	8.84
8.75	27.0	2.91	9.08
10.70	0.1	2.96	10.02

Table E.18: Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPSO / 100 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0 95	0 0		
1 74	29.7	2 06	8 25
2.00	37.2	2.38	8.50
2.19	36 5	2.58	8 90
2.47	34 5	2.41	8 99
3.75	27.7	3.87	9 01
5.10	22.0	3.94	9.08
6.12	23.2	3.90	9.04
8.75	24.1	4.00	9 05
10.70	0.0	4.10	10 00

Table E.19: Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPSO / 150 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0.95	0.0		
1.74	21.1	1.93	8.32
2.00	32.5	2.34	8.56
2.19	27.6	2.40	8.72
2.47	25.3	2.41	8.85
3.75	24.6	2.57	8.92
5.10	20.4	2.63	8.99
6.12	22.1	2.74	9.08
8.75	23.2	2.80	9.04
10.70	0.0	2.85	10.20

Table E.20 Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPOO / 40 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0.95	0.0		
1.74	32.1	3.99	8.21
2.00	31.0	4.28	8.40
2.19	29.0	5.25	8.58
2.47	27.0	5.47	8.71
3.75	13.0	5.57	8.79
5.10	11.2	5.60	8.75
6.12	12.0	5.65	8.80
8.75	2.0	5.70	10.21
10.70	0.0	5.73	11.20

Table E 21 Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPOO / 70 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0 95	0 0		
1 74	28 3	3 83	8.40
2 00	28.0	4 24	8.45
2 19	25 1	4 50	8.31
2 47	22 0	4.70	8 40
3.75	12 0	4 50	8.62
5 10	6 3	4 65	8.92
6.12	0 0	4.80	9 10
8.75	0 0	5 14	10.41
10.70	0.0	5.10	11.50

Table E.22. Extraction of L-lysine for the system isooctane / 400 mM pentanol / water / 100 mM NaPOO / 100 mM NaCl / 5 mM L-lysine / HCl

Initial pH	% Extraction	Water uptake (mass %)	Final pH
0 95	0 0		
1 74	23.4	3 75	8 40
2.00	23 0	4 05	8 57
2 19	20 2	4 35	8 75
2.47	18 0	4 10	8 90
3 75	8 0	4.70	9 01
5 10	0 0	5 10	10 99
6.12	0 0	5 24	11 63
8.75	0.0	5 47	11 40
10.70	0.0	5.40	11.58