THE INFLUENCE OF SURFACTANTS ON THE SOLUBILIZATION, EXTRACTION AND BIODEGRADATION OF MODEL POLYCYCLIC AROMATIC HYDROCARBONS

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2004

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A THESIS SUBMITTED
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
DEPARTMENT OF CHEMICAL & BIOMOLECULAR
ENGINEERING
NATIONAL UNIVERSITY OF SINGAPORE
2004

ACKNOWLEDGEMENT

I would like to express my gratitude to my supervisors, Dr. Chen Bing-Hung for his instructive and patient supervision throughout this project, and A/P Bai Renbi for his great help at the late stage of my work.

I also would like to take this chance to thank my colleagues in the research group and the laboratory officers at the department for their help in the experimental work.

Finally, thank the National University of Singapore for providing the financial support to this research project and the scholarship during my study in NUS.

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SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) are highly toxic chemicals. Their high hydrophobicity contributes to their low aqueous solubility and persistence in the environment. Consequently, effective techniques are needed to increase their bioavailability and to monitor their existence in the environment. In this work, the potential use of linear alcohol ethoxylate nonionic surfactants Tergitol 15-S-X (X=5, 7, 9 and 12) in the solubilization, preconcentration and biodegradation of model PAHs was explored.

The solubilization capacities of Tergitol 15-S-X (X=7, 9 and 12) for model PAHs were measured. The effects of various factors including the HLB values of surfactants, hydrophobicity of PAH, temperature and salinity on solubilization capacity were examined. The results showed that this type of surfactant has comparable solubilization capacity for PAH with the traditionally used surfactants. For surfactants of the homolog, those with lower HLB numbers have greater solubilization capacity. The logarithms of the micelle-water partition coefficients of selected PAHs could be correlated linearly to the logarithms of their octanol-water partition coefficients, which means that hydrophobicity data of PAH can be used to predict the solubilization capacity of a surfactant. It was also observed that increasing temperature or increasing sodium chloride concentration could improve the solubilization capacity of the surfactants. This is attributable to the increase in aggregation number and micelle size.

Simple preconcentration processes using Tergitol 15-S-5 and Tergitol 15-S-7 were developed to preconcentrate model PAHs from aqueous solutions. The preconcentration was enhanced with the addition of suitable salts. Various factors including salt concentration, surfactant concentration and hydrophobicity of PAH on the preconcentration factors and recoveries of model PAHs were examined. Preconcentration factors and recoveries higher than 90% were obtained. The partition coefficients of PAHs between the surfactant-rich phase and the aqueous phase were also measured when Tergitol 15-S-7 was used as extractant. The results showed that the partition coefficient was independent of surfactant concentration and increased with salt concentration. The partition of PAHs into the surfactant-rich phase is also driven by the hydrophobic affinity of PAH to surfactant aggregates.

The biodegradability of Tergitol 15-S-X (X=7, 9 and 12) was tested. The effects of them on the biodegradation of phenanthrene were investigated. The results showed that these surfactants were not toxic and could be readily biodegraded by the marine bacteria *Neptunomonas naphthovorans* (ATCC 700638) used in the experiments. A first-order kinetics was observed for their biodegradation. It was also observed that solubilization by these surfactants enhanced the biodegradation of phenanthrene. This is attributable to the increased solubility of phenanthrene. However, at the same phenanthrene concentration, the bioavailability of phenanthrene decreases with increase in surfactant concentration. This may be due to the fact that relatively larger fractions of phenanthrene were solubilized into the micellar phase with an increase in surfactant concentration. Or, in another word, the fraction of phenanthrene in aqueous phase that can be directly utilized by the bacteria becomes smaller. The slower mass-transfer from the micellar to the aqueous phase at higher surfactant concentrations may also contribute to the reduced bioavailability.

NOMENCLATURE

Symbol	**Description**
a	constant
A_2	second viral coefficient, cm^3mol/g^2
b	constant
	first-order endogenous respiration coefficient, h^{-1}
c	solution concentration for Zimm plot measurement, mg/mL
C	apparent solubility of PAH in micellar solution, mg/L
C_a	aqueous phase concentration of PAH in micellar solution, mg/L
C_{cmc}	PAH solubility at CMC, mg/L
C_m	concentration of PAH in micellar phase, mg PAH/mg micellized surfactant
C_{mic}	concentration of PAH in micellar phase, mg/L (bulk solution)
C_{surf}	surfactant concentration, mg/L
C_S	PAH concentration in surfactant-rich phase, mg/L
C_W	PAH concentration in aqueous phase after cloud point separation, mg/L
CMC	critical micelle concentration, mg/L
D_0	diffusion coefficient of surfactant molecules
f_C	preconcentration factor
ΔG_{mic}	free energy change of micellization, kJ/mol

ΔH_{mic}	enthalphy change of micellization, kJ/mol
HLB	hydrophilic-lipophilic balance number of surfactant
K	optical constant for vertical polarized incident light
k	first-order rate constant, hr^{-1}
	Mass-transfer coefficient of phenanthrene, cm/min
k_B	Boltzmann's constant
k_l	lumped mass-transfer coefficient, min^{-1}
K_m	micelle-water partition coefficient
K_{mw}	micelle-water partition coefficient, L/mg
K_p	partition coefficient in cloud point extraction
K_{ow}	octanol-water partition coefficient
MSR	molar solubilization ratio
M_w	molecular weight, g/mol
m_w	mass of water in surfactant-rich phase, mg
m_t	total mass of surfactant-rich phase, mg
n	number of EO groups in surfactant molecules
	refractive index of solvent
N_A	Avogadro's number, mol^{-1}
N_{ag}	aggregation number of micelle
N_c	number of carbons in the hydrophobic group of surfactant molecules
N_h	number of hydrophilic group in surfactant molecules
P_L	Laplace pressure, N/cm^{-2}
q	magnitude of scattering wave vector
R	recovery of PAH by cloud point extraction, %

R_m	recovery of PAH by micellar solubilization, %
R_T	total recovery of phenanthrene in extraction from spiked sand, %
R_g	radius of gyration, nm
R_h	hydrodynamic radius of micelle, nm
R_V	phase volume ratio
ΔR_θ	excess Rayleigh ratio
S	total surfactant concentration, mg/L or mg carbon/L
S_{mic}	surfactant concentration in micellar phase, mg/L
S^*	asymptotic surfactant concentration, mg carbon/L
ΔS_{mic}	entrophy change of micellization, kJ/(mol·K)
T	absolute temperature, ^{o}K
T_D	bacteria doubling time, hr
X_m	mole fraction of PAH in micellar phase
X_a	mole fraction of PAH in aqueous phase
V_c	core volume of micelle, Å^3
V_m	micellar volume, nm^3
V_s	molecular volume of surfactant, mL/mol; volume of surfactant-rich phase, mL
V_t	total volume of surfactant solution, mL
V_w	volume of aqueous phase, mL
$V_{w,mol}$	molar volume of water at experimental temperature, mL/mol

Greek Letters

α	solubilization extent of phenanthrene
β	bioavailable factor of phenanthrene in micellar phase
η_0	solvent viscosity, $gcm^{-1}sec^{-1}$
γ	water content of surfactant-rich phase
	interfacial tension across the micelle-water interface, mN/m
μ	specific growth rate, hr^{-1}
λ_0	vacuum wavelength of incident light, nm
π	pi
ω	molar concentration of water, mol/L

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Chapter 1 Introduction

1.1 Research Background

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment and have been of great environmental concern, as they are either known or suspected carcinogens or mutagens (Brown et al., 1999; Gerde et al. 2001; Mizesko et al. 2001; Tsai et al., 2001; Oanh et al., 2002; Tang et al., 2003). PAHs constitute a class of hazardous organic chemicals consisting of two or more fused benzene rings in linear, angular or cluster arrangements. They are released to the environment either from natural origins, such as forest fires, or from anthropogenic activities. PAHs form, for example, during the incineration of solid waste, as a result of the incomplete combustion of fossil fuels and during the disposal of coal tar. The emission of PAHs from various sources has been a serious environmental problem (Liu et al., 2001; Chao et al., 2002; Kuusimaki et al., 2002; Zhu and Wang, 2003).

Due to their carcinogenic and mutagenic characteristics, strict legal controls are now imposed to regulate their production, usage and emission, in which the determination of trace of PAHs has to be addressed. Their low aqueous solubility often necessitates some preconcentration procedures prior to their analytical determination. Cloud-point extraction (CPE) using readily biodegradable nonionic surfactant is a promising technique (Bai et al., 2001).

PAHs are very hydrophobic and have very low aqueous solubility. For example, their solubility ranges from 32.5 ppm for naphthalene down to 0.14 ppm for pyrene at 25 °C (Edwards et al., 1991). As a result, PAHs often show a strong tendency to be sorbed onto soil and incorporated into soil micropores. The biodegradation of soil-bound PAHs is, consequently, a two-step process that involves mobilization of PAHs from solid to aqueous phase and subsequent catabolism by microorganisms (Van der Meeren and Verstraete, 1996; Zheng and Obbard, 2000, 2002a, Dong and Lei, 2003) The mass-transfer rate of PAHs into the aqueous phase was shown to be the rate-limiting step in their biodegradation (Grimberg et al., 1995; Boonchan et al., 1998; Kose et al., 2003; Shor et al., 2003). It is, thus, very essential to enhance their mass-transfer for a successful remediation process. To solve this problem, a surfactant-enhanced bioremediation technique has been a research focus in recent years.

In general, a surfactant molecule consists of a hydrophilic headgroup and one or two hydrophobic parts. The hydrophobic tail, usually a long hydrocarbon or fluorocarbon chain, acts to reduce solubility in water while the polar head, often ionizable, has the opposite effect. These unique amphiphilic structures and properties contribute to the versatility of surfactants for numerous applications. Surfactant molecules can accumulate along the air-liquid and liquid-liquid interfaces and thus reduce both surface tensions and interfacial tensions at the same time. In addition, if the surfactant concentration exceeds a certain threshold, called the critical micelle concentration (CMC), at temperature higher than its Krafft temperature, surfactant monomers in aqueous solution will aggregate to form micelles of colloidal-size. Under such a condition, the hydrophobic solubilizates are incorporated into the hydrophobic cores of the micelles, which is often called solubilization. More explicitly, solubilization may be defined as the spontaneous dissolving of a substance by reversible interaction with the micelles of a surfactant in a solvent to form a thermodynamically stable isotropic solution with reduced thermodynamic activity of the solubilized material (Rosen, 1989).

At ambient temperature, surfactants with proper structures are soluble in water. With an increase in temperature, the solubility of surfactant molecules diminishes. Above a certain threshold, they will completely lose their water solubility and separate out of the solution. This threshold temperature is termed as cloud point (CP). Clouding is a normal phenomenon of nonionic surfactants. Certain zwitterionic surfactants and some cationic surfactants also exhibit phase separation in the presence of high concentrations of electrolytes (Lang and Morgan, 1980; Saitoh and Hinze, 1991). At the cloud point, a homogenous surfactant solution separates into two phases, one of which contains much of the surfactant, which is normally named as surfactant-rich phase, while the other phase contains largely of water with surfactant concentration at or slightly above its CMC. This phase change is reversible. When the system is cooled to a temperature below the cloud point, the two phases merge into a clear solution again. Upon phase separation, the compounds that are originally present in the micellar solution and bound to the micelles can be extracted into the surfactant-rich phase. The small volume of the surfactant-rich phase has the effect of concentrating the compounds. The process is referred as cloud point extraction (CPE) or cloud point preconcentration. CPE offers several advantages over conventional liquid-liquid extraction, including experimental convenience, low cost, and the possibility of using nontoxic and less dangerous reagents (Quina et al., 1999; Huddleston et al., 1999; Szymanowski, 2000; Materna and Szymanowski, 2002; Kulichenko et al., 2003).

Despite the many advantages of cloud point extraction, there exist some difficulties in sample analysis due to the large UV absorbance or fluorometric signals of surfactants containing double-bond structures, such as phenyl groups in the surfactants of polyoxyethylene alkylphenyl ether. The signals of these surfactants always overlap those of PAHs of low molecular wights. The typical surfactants of this type commonly used in cloud point extraction include the Triton series, the Igepal series and the PONPE series. To alleviate this problem, researchers have attempted the use of alternative extractants including polymers, anionic surfactants such as sodium dodecyl sulfate and primary ethoxylated alcohols such as the Brij series and the Genapol series. However, little success has been achieved in using polymer to extract PAHs. The use of anionic surfactants often requires the addition of salt and the *pH* adjustment, usually to very low values (Sicilia et al., 2002). Moreover, the Brij and the Genapol surfactants would not undergo phase separation unless at higher temperatures, for example, 70 ^{o}C for Polyoxyethylene (10) Oleyl Ether (Brij97) and 90 ^{o}C for Polyoxyethylene(4) lauryl ether (Brij 30) (Fernández et al., 1998). Use of the surfactants with phenyl groups often poses other problems because of their aromatic groups. Due to their resistance to biodegradation, these surfactants have been restricted in many major applications. The search for environmentally friendly surfactants and the development of a simple extraction process are two key issues for the widespread application of CPE.

As an alternative to traditional solvent extraction, CPE, especially the extraction of environmental pollutants, is still at its initial stage. To date, not many reports can be found on the extraction of PAH by this technique (Ferrer et al., 1996; Sirimanne et al., 1996; Pino et al., 2001, 2002; Merino et al., 2002). The high extraction efficiency of PAH by CPE has been reported. However, no work has been reported on the partition of these chemicals between the surfactant-rich phase and the aqueous phase. The partition equilibrium is of critical importance because it determines the extraction efficiency of a surfactant for the solute of interest. There are many factors that can affect the partition of solutes. Several authors have studied the effect of various experimental factors on the partition mainly of polar aromatic hydrocarbons between surfactant-rich phase and aqueous phase, including surfactant concentration, time, acidity, *pH* and additives, as well as on their recovery in the CPE (Frankewich and Hinze, 1994; Akita and Takeuchi, 1995, 1996; Materna et al., 2001).

In the previous studies, CPE has been limited to preconcentration and separation of analytes in aqueous solutions. It has also a promising application in the collection of environmental pollutants in the washing solutions for remediation applications. Even in bioremediation applications, the level of PAHs remaining in the solution after biodegradation is normally still far excessive of their permitted levels in water system. Therefore, it is a good idea to apply CPE to collect the residues of toxic pollutants in the washing solution. It provides an effective way to reduce the volume of the washing solution, which makes it easy to handle the pollutants (Hinze and Promauro, 1993; Komaromy and von Wandruszka, 1995; Pramauro and Prevot, 1995). The surfactant-rich phase, which contains the pollutants, is combustible. This makes disposal by incineration possible. The aqueous solution left can then be reused.

In addition, most of the surfactants used in the extraction process were hydrophilic. However, it has been long known that some mesophases of the surfactants, such as the lamellar liquid crystalline phase, have even better solubilization capacity and higher hydrophobic affinity than the micellar phases. They can provide even more hydrophobic space for the hydrophobic solutes (Rosen, 1989). However, there is hardly work found in the literature, in which the surfactants that form mesophases were ever used in the surfactant-based extraction technique. It may be because the mesophases are usually too viscous to separate them from the other phases and to inject into the HPLC directly. The other reason is that the phase volume of the surfactant-rich phase, i.e. the mesophase, is very large and consequently, it is not easy to achieve a high preconcentration factor.

Surfactant-enhanced bioremediation has been a research focus in recent years. Surfactants can increase the solubility of slightly water-soluble compounds through the process of solublilization. The effect of solubilization is to increase the apparent solubility of the compounds. When a hydrophobic solute is present in excess, its apparent solubility increases linearly with surfactant concentrations above CMC.

In recent years, the solubilization of PAH from pure PAH solids or other matrices by micellar solutions of surfactants has been studied by some authors (Edwards et al., 1991; Guha et al., 1998a; Huang and Lee, 2001; Grasso et al., 2001; An et al., 2002). Most of the examined surfactants are nonionic because this type of surfactant is less capable of forming foam, low toxicity and a high solubilization capacity for PAH, as well as low sorption to soil compared with cationic and anionic surfactants (Auger et al., 1995).

The role of surfactant in the biodegradation of PAH was also extensively studied in the past decades. Although there is no doubt that surfactant can increase the solubility of hydrocarbons, conflicting results have been reported on its ability to enhance the biodegradation process. Inhibition of biodegradation was normally observed at surfactant concentrations approaching or higher than CMC (Cort and Bielefeldt, 2002). Various inhibition mechanisms have been proposed. Searching for the right surfactants, which are both efficient in enhancing the solubilization and biodegradation of PAHs is of critical importance for a successful bioremediation process.

Another important aspect in surfactant-mediated bioremediation is the biodegradability of surfactants, which is becoming a critical criterion for surfactant selection in field applications. The toxicity and potential biodegradation products of a surfactant are the most important criteria for the selection of surfactant in soil remediation applications (Volkering et al., 1997). A suitable surfactant must not cause environmental concerns and must be readily biodegradable to levels consistent with regulations (Abdul et al., 1992). The common aromatic-based surfactants are generally resistant to biodegradation and always leave highly toxic intermediates (Patoczka and Pulliam, 1990). Therefore, readily biodegradable and hence environmentally benign surfactants are preferred choices. However, the biodegradation of surfactants has either positive or negative effects on the biodegradation of primary substrates. In the case that a surfactant is consumed as a preferable substrate, its biodegradation may retard or even inhibit the biodegradation of a primary substrate. The biodegradation of surfactants may also serve to support bacterial growth and the biodegradation of primary substrates. In addition, the biodegradation of a surfactant may affect the bioavailability of a primary substrate due to the decrease in surfactant concentration and hence a reduction in solubilization extent. However, in literature, the biodegradability of surfactants was often not considered.

1.2 Objectives

Based on the research progress of the two techniques, that is, cloud point extraction and surfactant-mediated bioremediation, the objectives of this project are to improve these two techniques in the following aspects.

First, develop a simple and more environmentally friendly cloud point extraction process for the trace determination of model PAHs in aqueous systems. To achieve this, surfactants with low cloud points and better environmental fitness will be employed. Ideally, the selected surfactants will not cause disturbance in sample analysis.

Second, examine whether the introduction of surfactants forming mesophases, i.e. the slightly hydrophobic surfactant, could really effectively enhance the performance of the surfactant-based extraction process on the polycyclic aromatic hydrocarbons (PAHs).

Third, explore the extraction mechanisms. For this objective, the partition of model PAHs will be studied together with their recovery. The effects of various factors on the partition and recovery of PAHs will be studied. Information obtained here will also be helpful to find ways to improve the extraction efficiency.

Forth, study the biodegradability of the surfactants to be selected, the role of solubilization on the biodegradation of a model PAH, phenanthrene. Examine the effects of solubilization on biodegradation and bioavailability of phenanthrene.

Information obtained here will be helpful to the understanding of the mechanisms of the effects of solubilization on biodegradation.

Suitable surfactants from the series, Tergitol 15-S-X will be selected in this study, because of their ready biodegradability and high solubilization capacity for PAHs. These surfactants are mixtures of secondary alcohol ethoxylates on the hydrocarbon chains having 11 to 15 carbons. These ethoxylated alcohols are a new generation of surfactants, which were developed as an alternative to the conventional surfactants such as nonyl phenol ethoxylates due to their readily biodegradable nature. The linear molecular structure of this type of surfactant also offers an advantage of low UV absorbance and non-fluorometric signal. Some of these surfactants have low cloud points, which make them good choices for cloud point preconcentration process.

1.3 Organisations of This Thesis

This thesis is composed of eight chapters. The contents of the following chapters are given below.

Chapter 2 gives a detailed review on the research works reported in recent years.

Detailed descriptions on the materials and experimental procedures used in this study are given in Chapter 3.

The experimental results are summarized in Chapter 4 to 7. Chapter 4 gives the solubilization results of model PAHs by Tergitol 15-S-X (X=7, 9 and 12) surfactants. First, the solubilization capacity of Tergitol 15-S-7 was compared with a few commonly used surfactants. The effects of various factors including HLB number of surfactant, hydrophobicity of PAHs, temperature and salinity on solubilization were studied. Changes in micellar properties including micelle size, molar weight, and aggregation number were examined by light scattering technique. The synergistic solubilization of PAH mixtures was also investigated.

In Chapter 5, the aqueous phase behavior of two surfactants, Tergitol 15-S-5 and Tergitol 15-S-7 are described. The temperatures needed for the phase separation of these two surfactants were measured and factors affecting the phase separation, especially electrolytes were investigated. Based on the observations, suitable salts were selected to enhance the extraction and preconcentration of model PAHs.

Results on the preconcentration and extraction of model PAHs are presented in Chapter 6. To examine the effectiveness of the cloud point extraction process, PAHs at trace concentrations in aqueous solutions are extracted. The partition coefficients of the PAHs were measured and correlated to their octanol-water partition coefficients. The effects of various factors on the water content of surfactant-rich phase, phase volume ratio, partition and recovery of the PAHs were studied. The effectiveness of a process, which combines the micellar solubilization and cloud point extraction from spiked sand samples is also reported in this chapter.

Chapter 7 summarizes the results on the biodegradation of a model PAH, phenanthrene in the micellar solution of three surfactants, Tergitol 15-S-X (X=7, 9 and 12). The biodegradability of these surfactants was examined and the effects of solubilization on the biodegradation of phenanthrene were investigated.

Results reported in the preceding chapters are summarized in chapter 8. Major conclusions are drawn and recommendations on future work are also given in this chapter.

Chapter 2 Literature Review

2.1 Solubilization of PAH by Nonionic Surfactants

Solubilization has been widely investigated either experimentally or theoretically. Various techniques including the dynamic and static light scattering, small-angle neutron scattering, fluorescence quenching, cryo-TEM, NMR, ESR and so on, have been employed (Adamson and Gast, 1997). Studies on the solubilization kinetics show that solubilization process can be controlled either by diffusion (Grimberg et al., 1995; Johnson et al., 1999; Prak et al., 2000) or by some interfacial factors (Carroll, 1981; Chen et al., 1997, 1998; Kabalnov, 1998).

2.1.1 Factors Affecting Solubilization

Solubilization is a partition process of organic substances between an aqueous and a micellar phase. This process can be affected by many factors, including the properties of surfactants and solubilizates, as well as some environmental factors.

The most important properties of a surfactant determining its solubilization power are its hydrophilic-lipophilic balance number (HLB) and molecular structure (Kile et al., 1989; Edwards et al., 1991; Diallo et al., 1994). Surfactants with lower HLB can provide a more hydrophobic environment for solubilization. That is the one that contributes to the difference in the solubilization capacity of surfactants in the homolog. Another mechanism reported by some authors is that surfactants with lower HLB have greater micelle core volumes compared with surfactants of the homolog, but with higher HLB. This mechanism was used to explain the greater solubilization capacity of dodecyl alcohol ethoxylates for nonpolar hydrophobic organic compounds including dodecane, decane, hexane and cyclohexane (Diallo et al., 1994). Lower HLB number favours solubilization of hydrocarbons. However, with the decrease in HLB number, the aqueous solubility of surfactants diminishes. Surfactants with HLB number higher than 10 are normally considered for the field applications. Molecular structure is also an important factor that affects the micellar solubilization capacity of a surfactant. Pennell et al. (1997) reported that polyoxyethylene (20) sorbitan monooleate (Witconol 2722 or Tween 80) could solubilize 2-3 times more dodecane and PCE (tetrachloroethylene) than the nonylphenol ethoxylate Tergitol NP-15 and the linear lauryl alcohol ethoxylate Witconol SN-120, although these surfactants have the similar HLB values and aggregation numbers. The longer hydrophobic chain of Witconol 2722 results in a substantially larger micelle core volume.

Hydrophobicity of a solubilizate is an important factor that governs its partition into the micellar phase. For nonpolar hydrophobic compounds, the micellar core is the primary site for solubilization. The solubilization is driven by the hydrophobic affinity of solute molecules to the micellar core. Valsaraj and Thibodeaux (1989) reported a linear relationship between the logarithm of micelle-water partition coefficient, K_m and octanol-water partition coefficient, K_{ow} for eleven organic compounds solubilized by sodium dodecyl sulfate. Edwards et al (1991) also reported a linear relationship between $\log K_m$ and $\log K_{ow}$ for the solubilization of several PAHs by a few nonionic surfactants. Diallo et al. (1994) studied the solubilization of several alkanes and aromatic hydrocarbons by dodecyl alcohol ethoxylates. It was observed that at the same HLB number of surfactant, the micelle-water partition coefficient, K_m of alkanes increased with their octanol-water partition coefficients K_{ow}. A linear relationship between $log\ K_m$ and $log\ K_{ow}$ was also reported when three representative PAHs, naphthalene, phenanthrene and pyrene were solubilized by perfluorinated surfactant micelles (An et al., 2002). The polarity of solutes is also an important factor affecting the solubilization. For polar compounds, unlike non-polar ones, solubilization preferably occurs at the polar interfacial area between the micelles and water. For slightly polar solubilizates such as benzene, partition both in the interior of micelles and at the interface of micelles and water has been reported (Nagarajan et al., 1984).

Temperature is one of the very important environmental factors that affect the solubilization behaviors of surfactants. Shinoda and Takeda (1970) reported a 60% enhancement in *WSR* (weight solubilization ratio) of decane in methoxydecaoxyethylene decyl ether, corresponding to an increase of temperature from 10 to 30^{o}C. Pennell et al. (1997) reported an increase of more than 20% in *WSR* of dodecane, PCE and 1,2-dichlorobenzene (DCB) by three nonionic surfactants when the experimental temperature was increased from 10 to 25 ^{o}C. The influence of temperature on solubilization capacity of surfactants can be attributed to the changes in micellar properties, such as aggregation number and micelle size. The effects of temperature on micellar properties have been studied by some authors (Streletzky and Phillies, 1995; Charlton and Doherty, 2001). Increasing temperature can also enhance the thermal agitation of surfactant molecules, which may lead to enhanced solubilization (Rosen, 1989).

Inorganic salts present in the water system can influence the performance of nonionic surfactants because their presence affects the formation of micelles. Pennell et al. (1997) studied the effect of calcium chloride on the solubilization capacity of Witconol 2722 for dodecane, PCE and DCB. Enhanced solubilization was observed at salt concentrations above 60g/L. The presence of salts can alter both structural and dynamic properties of micelle, which in turn affect the solubilization capacity. Phillies and Yambert (1996) investigated the effect of NaCl concentration (from 0 to 0.6M) on the structure of polyoxyethylene(10) isooctylphenyl ether (Triton X-100) micelles, and observed that both micelle size and hydration increased with salt concentration. Using rotating disk voltammeter in conjunction with ferrocene acting as an electroactive probe, Charlton and Doherty (2000) studied the structure evolution of Triton X-100 micelles in the presence of KCl. Micellar hydrodynamic radius was observed to vary linearly from 4.22 to 6.21nm with respect to KCl concentration (ranging from 0.01 to 0.8M), indicating the progressive evolutionary growth of the micelles due to the increasing aggregation number and hydration. Correspondingly, the average molecular weight of the micelles increased from 77,700 to 326,000 Da, which is predominately due to water entrapment at the periphery of the self-assembled structures. Kim et al. (2000) reported the effect of electrolytes on the solubilization capacity of pyrene by the dodecyl sulfate micelles. It was observed that increasing concentration of the electrolyte increased the aggregation number, which consequently increased the solubilization capacity of the surfactant. Using static and dynamic light scattering, together with the fluorescence probe technique, Molina-Bolívar (2001) investigated the effect of KCl on the micelle formation and structure of Triton X-100. It was found that the addition of this salt induced a decrease in the CMC and an increase in both aggregation number and hydration. The increased aggregation number accounted for the micelle growth.

The solubilization efficiency of a nonionic surfactant for a hydrocarbon can also be affected by the presence of other organic compounds. A synergistic effect was observed for the solubilization of hexane in the presence of small amounts of benzene (Nagarajan et al., 1994). The effect could be attributable to the distribution of benzene at both the interfacial and interior sites of the micelles. It was also discovered that the fractional distribution of hexane was affected by the polar groups of the surfactant and the amount of benzene present in the micelles. Solubilization of PAH mixtures by a nonionic surfactant, Triton X-100, was studied (Guha et al., 1998a). A synergistic effect was also observed in the solubilization of binary and ternary mixture of naphthalene, phenanthrene and pyrene. The results indicated that the presence of the less hydrophobic compounds could increase the solubility of the more hydrophobic ones. The proposed mechanism is that the less hydrophobic ones can partition at the interface of water and the micelle core, which reduces the interfacial energy and hence gives a larger core volume. When the solutes are all nonpolar, the solubility of one compound will be reduced by the presence of the others due to competition for the solubilization site in the micelle cores. Solubilization of PAH mixtures in micellar nonionic surfactant solutions was also reported by some other authors (Prak and Pritchard, 2002b). The observation is that both the PAH-PAH and micelle-PAH interactions could affect the concentrations of individual PAH in the micellar solutions. The interactions between individual PAH can influence their aqueous solubility, while the micelle-PAH interaction can affect the distribution of PAHs in the micellar phase. Besides, the solubilization in mixed surfactant solutions was also reported (Zhu and Chiou, 2001).

2.1.2 Quantitative Study on Solubilization

Quantitative studies on the solubilization capacities of surfactants could be found elsewhere in the literatures (Edwards et al., 1991; Diallo et al., 1994; Pennell et al., 1997). There are a few common expressions to quantify the solubilization capacity of surfactants, one of which is the micelle-water partition coefficient (K_m). Two others are the mass solubilization ratio (*WSR*) and the molar solubilization ratio (*MSR*). The mass solubilization ratio (*WSR*) is defined as the weight of the PAH solubilized by the unit mass of surfactant above its *CMC*. Similarly, the molar solubilization ratio (*MSR*) is described as the moles of PAH solubilized by each mole of surfactant above its *CMC*, which is shown as the follows (Edwards et al., 1991):

$$MSR=(C-C_{cmc})\cdot M_{surf}/[(C_{surf}-CMC)\cdot M_{PAH}] \qquad (2.1)$$

where C is the total apparent solubility of PAH in micellar solution at the particular surfactant concentration equal to C_{surf}; and C_{cmc} is the saturation concentration of PAH at CMC. M_{surf} and M_{PAH} are the molar weight of surfactant and PAH. The *MSR* as well as *WSR* can be obtained from the slope of solubilization curve. Here, the term "apparent solubility" is used, since the solubility of PAH contains contribution made by solubilization.

The micelle-water partition coefficient K_m is a parameter that indicates the distribution of organic molecules between the "micellar phase" and the "aqueous phase".

$$K_m = X_m / X_a \qquad (2.2)$$

where X_m is the mole fraction of PAH in the micellar phase and X_a is the mole fraction of PAH in the "micelle-free aqueous phase". The mole fraction of PAH in the "micellar phase", X_m, can be calculated in terms of *MSR* (Edwards et al., 1991),

$$X_m = MSR / (1 + MSR) \qquad (2.3)$$

The mole fraction of PAH in the aqueous phase is approximated for dilute solutions by

$$X_a = C_{cmc} \cdot V_{a,\,mol} / M_{PAH} \qquad (2.4)$$

where $V_{a,\,mol}$ is the molar volume of water at the experimental temperature.

The effects of surfactants on the dissolution of hydrocarbons from various matrices have also been reported. Surfactants are able to improve the mass-transfer of hydrophobic pollutants from a solid or non-aqueous liquid phase into the aqueous phase by decreasing the interfacial tension and by accumulating the hydrophobic compounds in the micelles (Tiehm, 1994; Volkering et al., 1995).

The solubilization power of surfactants is very crucial in selecting suitable surfactants for the successful applications of the surfactant washing and biodegradation processes, as well as the cloud-point extraction technique. With the increasing environmental awareness and stricter regulations on the use of environmentally acceptable surfactants, development and selection of new types of surfactants have been the focus of much research (Van der Meeren and Verstraete, 1996).

2.2 Aqueous Phase Behaviour of Nonionic Surfactants

2.2.1 Micelle Formation in Aqueous Solution

A surfactant molecule contains both a hydrophilic and one or two hydrophobic tail. When a surfactant is dissolved in water, its hydrophobic groups distort the structure of water and consequently increase the free energy of the system (Rosen, 1989). To reduce the free energy, surfactant molecules will orient at the air-water interface with the hydrophobic groups directed away from water. With increase in surfactant concentration to a critical value (CMC), the adsorption of surfactant at the interface becomes saturated and the surfactant molecules will reduce the free energy by forming aggregates, which are named micelles. The CMC values of some selected nonionic surfactants are given in Table 2.1. The values of some other surfactants used in this study will be given in Table 2.4 and Table 3.1.

Table 2.1 CMC Values of Some Nonionic Surfactants

Surfactant	CMC (mM)	Reference
Type:Polyoxyethylene alkyl phenylether		
Igepal CA-720 (C_8PE_{12})[a]	0.23	Edwards, et al., 1991
Tergitol NP-10 ($C_9PE_{10.5}$)[a]	0.054	
PONPE-7.5 ($C_9PE_{7.5}$)	0.085	Quina and Hinze, 1999
Polyoxyethylene alkyl ether		
C_8E_5	7.1	Frankewich et al., 1994
$C_{10}E_5$	0.84	
$C_{12}E_5$	0.062	
$C_{12}E_6$	0.067	
$C_{12}E_8$	0.081	
$C_{16}E_6$	0.001	Berthod et al., 2001
$C_{16}E_{10}$	0.0006	
$C_{16}E_{30}$	0.0001	

[a]: P denotes phenyl group

Depending on the surfactant molecular structure, temperature and solution conditions, micelle can assume different shapes. The major types of micelles appear to be spherical, elongated cylindrical (rod-like), lamellar (disk-like), and vesicles. Figure 2.1 gives the schematic description of a few common micelle structures.

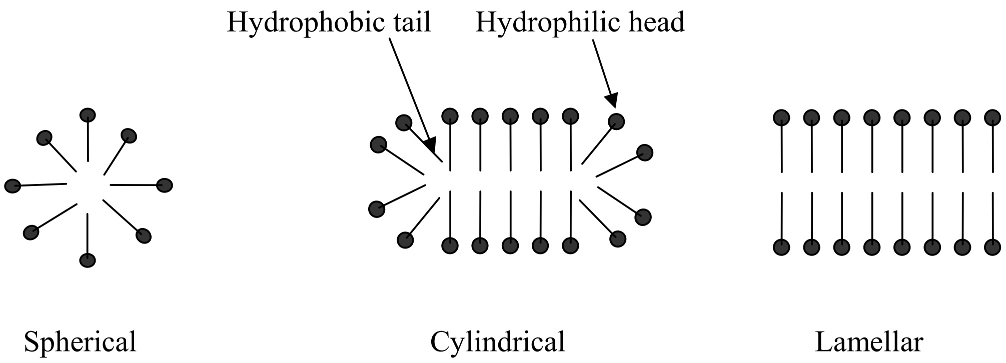


Figure 2.1 A Schematic Description of a few Common Structures of Micelles in Aqueous Solution

For a surfactant, the micellar structure depends on its molecular structure. A theory to predict the micellar structure has been developed based on the geometry of various micellar shapes and the space occupied by the hydrophilic and hydrophobic groups of the surfactant molecules (Israelachvili et al., 1977). A parameter, $V_H/l_c a_0$ was proposed, in which V_H and l_c are the volume occupied by the hydrophobic groups and length of those groups in the micellar core, a_0 is the cross-sectional area occupied by the hydrophilic group at the micelle-solution interface. The following relationship between the value of the parameter and micellar structure in aqueous solution was given:

Value of $V_H/l_c a_0$	structure of micelle
0-1/3	spherical
1/3-1/2	cylindrical
1/2-1	lamellar

In aqueous solution, surfactant with bulky or loosely packed hydrophilic groups and long, thin hydrophobic groups tend to form spherical micelles, while those with short, bulky hydrophobic groups and small, close-packed hydrophilic groups tend to form cylindrical or lamellar micelles. Beside surfactant molecular structure, some other factors including temperature and presence of additives also affects the micellar structure.

Thermodynamic Parameter for Micelle Formation

As mentioned above, in a nonionic surfactant solution, the formation of micelle is related to the free energy change by the aggregation of surfactant monomers.The free energy change ΔG_{mic} can be expressed as (Shinoda, 1953; Molyneux, 1965):

$$\Delta G_{mic} = 2.303RT \log x_{CMC} \tag{2.5}$$

where x_{CMC} is the molar fraction of the surfactant in the liquid phase at the CMC. In aqueous solutions, the CMC is generally low, so x_{CMC} can be approximated by CMC/ω, without significant error. Therefore,

$$\Delta G_{mic} = 2.303RT \log x_{CMC} = 2.303RT(\log CMC - \log \omega) \tag{2.6}$$

where ω is molar concentration of water. From Eq. (2.6)

$$\log CMC = \Delta G_{mic} / (2.303RT) + \log(\omega) \tag{2.7}$$

$$\Delta G_{mic} = \Delta H_{mic} - T\Delta S_{mic} \tag{2.8}$$

Substitute Eq. (2.8) into Eq. (2.7), it arrives that:

$$\log CMC = (\Delta H_{mic} - T\Delta S_{mic}) / (2.303RT) + \log(\omega) \tag{2.9}$$

It can be concluded that any factor that changes the entropy and enthalpy of the micellization process can affect the CMC. From Eq. (2.8), ΔH_{mic} and ΔS_{mic} have opposite effects on the free energy change ΔG_{mic}. For aqueous systems, ΔG_{mic} is mainly contributed by the entrophy change. The ΔH_{mic} is often positive and even negative, is much smaller than the value of $T\Delta S_{mic}$. Therefore, the micellization process is governed primarily by the entrophy gain, and the driving force for the process is the tendency of the hydrophobic group to transfer from water to the interior of the micelle (Rosen, 1989). On the contrary, an increase in the hydrophilic group increases the CMC of nonionic surfactant. However, normally the change in CMC per oxyethylene unit is much smaller than that per methylene unit in the hydrophobic chain (refer to Table 2.1).

2.2.2 Factors Affecting CMC of Nonionic Surfactants

There are many factors that affect the micelle formation and CMC of a nonionic surfactant. These mainly include the surfactant chemical structure, temperature and the presence of salts and solvents.

2.2.2.1 Surfactant Chemical Structure

With increase in the alkyl chain length of the hydrophobic group, the CMC of a non-ionic surfactant decreases, which can be attributed to the reduced free energy of micelle formation. For example, for polyoxyethylene *n*-alkyl alcohols, the following relationship was obtained between the CMC and carbon numbers in the alkyl chain (Schick, 1987):

$$logCMC = -0.5 \cdot C\# + 1.89 \qquad (2.10)$$

where $C\#$ is the carbon number.

Eq. (2.10) does not incorporate the effect of the hydrophilic part on the micelle formation. As a matter of fact, the length of the hydrophilic head also plays an important role. For linear polyoxyethylene alkyl ether surfactants, the CMC value was found to correlate well with the number of oxyethylene units (EO#) and methylene groups (or number of carbon C#) (Berthod et al., 2001).

$$logCMC = 0.011C\#\cdot EO\#+0.135C\#-0.433EO\#+1.117 \quad (2.11)$$

The correlation was found to be well when 4<EO#<16 and 3<C#<30.

2.2.2.2 Temperature

For a nonionic surfactant, an increase in temperature normally causes changes both in ΔH_{mic} and ΔS_{mic}. Therefore, the net effect of temperature will depends on the relative magnitude of the changes in these two parameters. For most nonionic surfactants, at temperature below 50 ^{o}C, ΔG_{mic} appears to become more negative with increase in temperature, but to become more positive with further increase in temperature. Consequently, for many nonionic surfactants, the CMC decreases to a minimum when the temperature is 50 ^{o}C and then rises with further increase in temperature (Rosen, 1987).

2.2.2.3 Electrolytes

The presence of salts can influence the micelle formation. Due to the water-ion interactions (hydration of ion), the work needed to creat the volume in water to accommodate the hydrophobic parts of a surfactant is affected. This results in a change in the activity coefficient of the surfactant. If the required work is increased by the presence of the electrolyte, then the activity coefficient of the surfactant is increased and it is “salted out”. On the other hand, if the required work is decreased, the surfactant is “salted in” (Rosen, 1989). When the monomeric form of a surfactant is salted out, micellization is favored and the CMC is decrease. “Salting in” electrolytes have the opposite effects. The magnitude of the effects of the anions and cations appears to depend on the radius of the hydrated ions; the smaller the radius, the greater the effects.

2.2.2.4 Solvents

The presence of a small amount of organic compounds can greatly influence the micelle formation of non-ionic surfactants. The effects of solvent on the micelle formation will be discussed in the section of effects of alcohol on the cloud point of Tergitol 15-S-7 in the Chapt. 5.

2.2.3 Clouding Phenomenon

2.2.3.1 Lower Consolute Behavior

When a micellar solution of a nonionic surfactant is heated above its cloud point temperature (T_c), the micellar solution separates into surfactant-rich and surfactant-poor (dilute) phases. The phase separation is attributable to the absence of long-range electrostatic interactions between the nonionic aggregates and the decreasing hydration of the nonionic hydrophilic groups with increasing temperature (Smith et al., 1995). For polyoxyethylene surfactants, a plot of the cloud point temperature versus the surfactant concentration is U-shaped. This means that a minimum cloud point exists at a certain surfactant concentration and the phase envelop is a lower consolute boundary. A schematic representation of the clouding phenomenon for nonionic surfactant solutions is given in Figure 2.2.

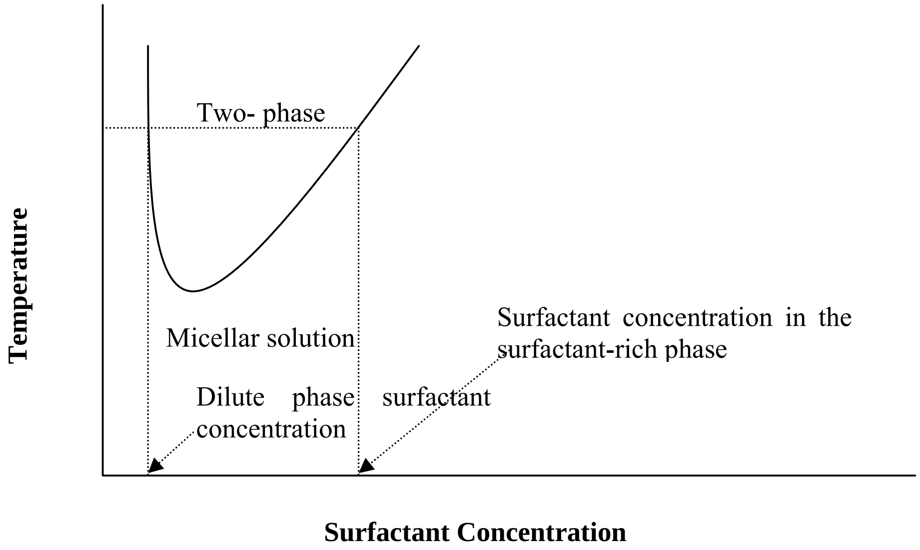


Figure 2.2 A Schematic Representation of the Lower Consolute Phase Behaviour of Nonionic Surfactants.

The lower consolute behaviour has also been observed with certain zwitterionic surfactants and ionic surfactants when electrostatic interactions are screened at high electrolyte concentrations (Herrmann et al., 1966; Laughlin, 1978; Pospichil, 1986).

2.2.3.2 Mechanisms of Clouding Phenomenon

The mechanism of the clouding phenomenon has been discussed by some authors. Some researchers suggested that the clouding phenomenon was caused by the rapid micellar growth and the large increase in aggregation number of the micellar solution in response to heat (Osipow et al., 1957; Schick, 1967). However, it was also attributed to the attractive intermicellar interactions, which become more important in the vicinity of cloud point (Triolo et al., 1982; Hayter and Zulauf, 1982; Zulauf and Rosenusch, 1983; Penfold et al., 1991). In nonionic micellar solutions in the absence of electrolytes, no electrostatic interaction exists between the micelles. The intermicellar interactions can be neglected at dilute micellar concentrations. However, excluded volume effects can be expected to become noticeable for volume fractions greater than 0.01. The deviations from the ideal behavior arise in the form of second virial interactions (Hall, 1987).

The interaction mechanism was also supported by Valaulikar and Manohar (1985). They observed that the cloud point of Triton X-100 was very sensitive to the addition of small amounts (10^{-5} M) of ionic surfactants. The cloud point increased linearly with the ionic surfactants concentrations up to a maximum of 1 molecule per micelle of Triton X-100. At such low concentrations, the ionic surfactants exist in the solutions either in the form of monomer or mixed micelle with the nonionic surfactant. The charging of the micellar surface made it difficult for the micelles to overcome the potential barrier and correspondingly the cloud point was raised. Kumar et al. (2002) investigated the salt-induced cloud point in anionic surfactant solutions. They observed that the addition of tetra-*n*-butylammonium bromide (Bu_4NBr) could reduce the cloud points of two ionic surfactants, sodium dodecylsulfate (SDS) and sodium dodecylbenzenesulfonate (SDBS), while it raised the cloud point of a nonionic surfactant, Triton X-100. The observations are also in favor of the attraction mechanism. However, some authors argued that the two mechanisms, micellar growth and attractive interaction were not mutually exclusive (Cebula and Ottewill, 1982). A recent study using small angle neutron scattering technique also suggested that both micellar growth and attractive interaction contribute to the clouding of *n*-alkyl polyglycol ether type surfactants (Glatter et al., 2000). Some authors gave a thermodynamic interpretation for the clouding phenomena in surfactant and polymer solutions. The clouding seems to be the result of the change in balance of hydrophilic and hydrophobic interactions (Blankschtein et al., 1986). Hence, factors that can break this balance will influence the cloud point. As temperature increases, nonionic surfactants will change from water-soluble to oil-soluble. As the temperature reaches a certain level, the balance will be broken and the surfactant molecules separate out of the solution. Consequently the homogenous surfactant solution separates into two distinct phases.

2.2.4 Factors Affecting Cloud Point

2.2.4.1 Surfactant Molecular Structure

Nonionic surfactants owe their solubility in water to the hydration of polyethylene oxide (PEO) chains. Therefore, the solubility increases as the PEO chain length increases (Pandit and Caronia, 1988). For a particular class of nonionic surfactant, the number of ethylene oxide (EO) units in the molecules has a dramatic effect on the cloud point. As the number of EO group increases, the cloud point becomes higher. For example, the cloud point of 1wt% Triton X-100 aqueous solution, with an average of 9.5 EO units, is about 67 ^{o}C. In comparison, that of polyoxyethylene (8) isooctylphenyl ether (Triton X-114), with an average of 7.5 EO units, at the same aqueous concentration falls to 25 ^{o}C (Koshy et al., 1996).

Efforts have been made to predict the cloud point from the surfactant molecular structures. Gu and Sjöblom (1992) observed a linear relationship between the cloud point and the logarithm of the ethylene oxide group number (EO#) for alkyl ethoxylates, alkylphenyl ethoxylates and methyl capped alkyl ethoxylate ester, as well as a linear relationship between the cloud point and alkyl carbon number (C#) for linear alkyl ethoxylates. The empirical relationship derived by these authors is as follows,

$$CP = A \cdot \log(EO\#) - 5.5 \cdot C\# - B \tag{2.12}$$

where A and B are empirical constants depending on the surfactant class. Eq. (2.12) shows that CP is a balance between the hydrophilic and hydrophobic properties of the nonionic surfactant. A more general empirical relationship, Eq. (2.13) was developed for the estimation of the cloud point of pure nonionic surfactants of the alkyl ethoxylate class with a varity of hydrophobic tail structures using the logarithm of the ethylene oxide number and three topological descriptors (Huibers et al., 1997).

$$\begin{aligned} CP = {} & (264 \pm 17) + (86.1 \pm 3) \cdot \log(EO\#) + (8.02 \pm 0.78) \cdot {}^{3}k - (1284 \pm 86) \cdot {}^{0}ABIC \\ & - (14.26 \pm 0.73) \cdot {}^{1}SIC \end{aligned} \tag{2.13}$$

where ${}^{3}k$ is the third order kier shape index for the hydrophobic tail, ${}^{0}ABIC$ is the zeroth order average binding information content of the tail, and ${}^{1}SIC$ is first order structural information content of the tail. This equation successfully correlated the cloud point of alkyl ethoxylate nonionic surfactants with a set of 62 structures composed of linear alkyl, branched alkyl, cyclic alkyl and alkylphenyl ethoxylates. However, it seems that the correlation fails for surfactants with short ethylene oxide chains.

For linear polyoxyethylene alkyl ether nonionic surfactant, a more simple correlation function was obtained as Eq. (2.14) (Berthod et al., 2001).

$$CP = 11\sqrt{(24 - C\#)EO\# + 0.014C\#^2} - 48 \tag{2.14}$$

Same as Eq.(2.11), Eq. (2.14) is also valid within the ranges of 4<EO#<16 and 3<C#<30. Despite of its simple form, this correlation fails at high alky chain length.

The cloud points of some nonionc surfactants that have been widely used in cloud point extraction are tabulated in Table 2.2. The values of another few nonionic surfactants will be given in Table 3.1

Table 2.2 Cloud Points of Some Nonionic Surfactants

Surfactant	Cloud Point[a] (^oC)	Reference
Type: Polyoxyethylene alkyl phenylether		
POE-7.5-nonylphenyl ether (PONPE-7.5)	1	Martínez et al., 2000
POE-7.5-octylphenyl ether (Trion X-114)	25	Gu and Galera-Gómez, 1995
Type: Polyoxyethylene alkyl ether		
$C_{10}E_5$	44	Frankewich et al., 1994
$C_{12}E_5$	31.7	
$C_{12}E_7$	63.4	
$C_{12}E_4$ (Brij 30)	4	Böckelen and Niessner 1993
$C_{12}E_{23}$ (Brij 35)	100	
$C_{18}E_{10}$(Brij 76)	71	
$C_{18}E_{10}$ (Brij 97)[b]	85	Fernández et al., 1998
$C_{12}E_{10}$	90	Berthod et al., 2002
$C_{13}EO_8$ (Genapol X-80)	42	Böckelen and Niessner 1993

[a]: 1 wt% surfactant solution; [b]: contains a double bond in the hydrophobic part

2.2.4.2 Effects of Additives

The solubility and cloud point of a nonionic surfactant can be altered by the presence of many additives. It is imperative to understand the magnitude and nature of the influence of these additives, so that a surfactant system can be suitably tailored to exhibit clouding behavior at the desired temperature (Goel, 1999). The effects of various additives on the cloud point of nonionic surfactants have been investigated. The most extensively studied additives are electrolytes and a second surfactant (Kenkare et al., 1996; Schott, 1995,1997, 2001; Kim and Shah, 2002). Although the cloud points of nonionic surfactants are normally independent of surfactant concentration at concentrations below 0.5% or above 5%, the cloud points are dependent on their own concentration and are strongly dependent on additives at concentrations between these two values (Schott, 1997).

Effects of Salts

It has been known since the 1950's that salts have significant effects on the cloud points of nonionic surfactant solutions (Kenkare et al., 1996). Electrolytes at low concentrations (<0.01 M) do not influence obviously, whereas, at high concentrations, they can increase or decrease the cloud point significantly. Generally, the effects of electrolytes have been explained in two ways. One explanation is that the influence of electrolytes arises from their effects on the water structure. In liquid water, water molecules exist as two forms, either as free molecules or in aggregated form, which can be described by the following equilibriums,

$$nH_2O \Leftrightarrow (H_2O)_n \qquad (2.15)$$

$$R_1OR_2+2H_2O \Leftrightarrow R_1OR_2\cdot 2H_2O \qquad (2.16)$$

$$M^{p+} + mH_2O \Leftrightarrow [M(H_2O)_m]^{p+} \qquad (2.17)$$

$$X^{q-} + lH_2O \Leftrightarrow [X(H_2O)_l]^{q-} \qquad (2.18)$$

$$M^{p+} + k(R_1OR_2) \Leftrightarrow [M(R_1OR_2)_k]^{p+} \qquad (2.19)$$

where R_1OR_2 denotes a nonionic surfactant molecule; M^{p+} and X^{q-} are the cation and anion of a salt. The addition of electrolytes can break the equilibrium of (2.15) and thus promote or inhibit the hydration of the EO group. If the cation can complex the ether oxygen, as indicated in Eq.(2.19), it will also dehydrate the surfactant. Ions that have the effect of enhancing the formation of water aggregates are referred as water "structure-making" ions, and those with the opposite effect are called "structure-breaking" ions (Zaslavsky, 1995). Addition of "structure-making" ions leads to a reduced number of free water molecules, which, in turn, leads to a reduced extent of hydrogen bond formation between water molecules and the ether group in nonionic surfactants. As a result, the solubility of surfactant molecules will diminish and the cloud point is lowered. Therefore, the "structure-making" ions have a "salting-out" effect on surfactant solubility. Structure-breaking salts have the opposite effect on the solubility and cloud point of surfactant.

However, a unanimous conclusion has not been reached for the electrolyte effects on micelle formation and clouding phenomenon. Another explanation is the competition for water of hydration between the ether oxygen of the EO group and the anions and cations of the inorganic salts. This mechanism is also described by Eq. (2.16) to Eq. (2.18). Generally, the order of the effects of the anions is that of the hofmeister or lyotropic series. In other words, the lower the lytropic number of the anions, the greater is their ability to reduce the cloud point (Schick, 1987). Despite of the different explanations, the two ways of interpretation on the salt effects agree on the point that the clouding phenomenon of nonionic surfactants is caused by the dehydration of the EO groups. Ions that have "salting-in" effects enhance the hydration of surfactant and hence increase the cloud point. Wherease, "salting-out" ions have the opposite effects. In this thesis, "salting-in" and "salting-out" will be used to describe the effects of the electrolytes.

Koshy et al. (1996) studied the effects of various inorganic salts on the cloud point of Triton X-100 and Triton X-114. It was observed that the presence of alkali metal chlorides decreased the cloud point of these two surfactants, with sodium chloride having the strongest effect. The decrease in cloud point was attributed to the dehydration of the EO groups of the surfactant molecules because of the competition for water between EO groups and alkali metal ions. Examination of the effects of various sodium halides in this study showed that, like sodium chloride, sodium bromide also lowered the cloud point of the surfactants. However, sodium iodide increased the cloud points, which was attributed to the water structure breaking effect of iodide ion. Schott (1997) studied the effect of chaotropic anions on the cloud point of Triton X-100. It was observed that the change in cloud point versus salt molality rose to a maximum in a parabolic fashion followed by steep decreases. As was pointed out by the author, the ascending branches were caused by the disruption of the water structure due to the chaotropic effect of the anions. When most of the self-association of water molecules has been disrupted, further increase in chaotropic salt concentration cannot induce additional increase in cloud point, the salt-out effect of the alkali metal cations became dominant, and the cloud point dropped upon further increase in salt concentration.

Effects of Ionic Surfactants

Mixed ionic-nonionic surfactants are of considerable importance in field applications (Huang and Gu, 1990). In the area of enhanced oil recovery, for example, such systems might have advantageous solubilization behaviors: exhibiting cloud point higher than those of pure nonionic surfactants and may also having Krafft points lower than those of the pure anionic surfactant. Mixed surfactants may be employed over a wider range of temperature, salinity, and hardness conditions than the individual surfactants (Sadaghiania and Khan, 1991). Many researchers reported that the cloud points of nonionic surfactants could be dramatically increased in the presence of small amounts of ionic surfactants, either anionic or cationic (Nilsson and Lindman, 1984; Valaulikar and Manohar, 1985; Gu, et al., 1989). The mechanism is that the addition of ionic monomer can increase the electrostatic repulsion between nonionic micelles, thus making it more difficult for the micelles to aggregate together. This can also explain why the cloud points of nonionic surfactant solutions are not affected by the addition of uncharged surfactants (Huang and Gu, 1990; Sadaghiania and Khan, 1991). Studies were also carried out on the change of cloud point in the presence of both ionic surfactants and electrolytes. It was found that inorganic electrolyte at concentrations that only had a slight effect on the cloud point of Triton X-114 could decrease the cloud point drastically in the presence of sodium dodecyl sulfate (SDS) or cetyltrimethylammonium (CTAB) (Gu and Galera-Gómez, 1995). The added electrolytes screened the micellar charge and so the cloud point is lowered. For two nonionic surfactants, the cloud points of their mixtures normally lie between the cloud points of individual surfactant.

Effects of Organic Compounds

The influences of other organic compounds on the cloud point of nonionic surfactants have been studied. Gu and Galera-Gómez (1999) investigated the effect of different alcohols and other polar organic additives on the cloud point of Triton X-100. The results showed that the addition of polar organic compounds infinitely miscible with water increased the cloud point of this surfactant, whereas the addition of the polar compounds partially soluble in water decreased its cloud point. The difference was attributed to the modifications of the surfactant-solvent interactions. In the former case, the addition of the polar compounds may modify the structure of the solvent water, leading to the formation of a less polar medium, which, in addition to the adsorption of the additives at the micelle-water interface, could restrict the micellization and hence lead to increase in cloud point. In the later case, the molecules of the additives may be solubilized in the micelles and adsorbed at the micelle-water interface, which could justify the micellar growth and a depression in the cloud point.

Al-Ghamdi and Nasr-El-Din (1997) also studied the effect of various oilfield chemicals and additives on the cloud point of the Triton-X series. Their results indicate that the effect of the chemicals depends on the number of ethylene oxide groups in the surfactant.

2.2.5 Applications of Clouding Phenomenon

The most popular application of the clouding phenomenon is the cloud point extraction technique. As an alternative to conventional solvent extraction, this technique has a promising future in many areas. These include the extraction of metal ions (Okada, 1992; Paleologos et al., 2001) and the separation and purification of biomolecules (Garg et al., 1994; Schwarz et al., 1997; Sirimanne et al., 1998; Tani et al., 1998; Carabias et al., 1999; Collén et al., 2002). Recently, increasing interest has been shown on the extraction of environmental pollutants (Froschl et al., 1997; Wu and Huang, 1998; Seronero et al., 2000; Pino et al., 2001, 2002).

2.3 Cloud Point Extraction and Its Limitations in Previous Studies

Cloud point extraction was initially developed by Watanabe (Watanabe, 1978) for the extraction of metal ions (Ni(II)) from aqueous solutions with the nonionic surfactant, Triton X-100 as the extractant. 1-(2-thiazolylazo)-2-naphthol (TAN) was used as a chelating agent to form a complex with the ions. In recent years, this technique has been used by other researchers to preconcentrate metals (Saitoh et al., 1989; Buhai and Rigan, 1990; Silva et al., 1997, 1998; Wuilloud et al., 2002; Paleologos et al., 2002; Manzoori and Karim, 2003; Pereira and Arruda, 2003). Normally, the process was facilitated with the addition of chelating agents to form chelates in the surfactant aggregates. However, extraction without the addition of chelating agents has also been reported (Luconi et al., 2000; Sombra et al., 2003).

Bordier (1981) extended the application of CPE into biochemical area. In the study, Triton X-114 was used to separate hydrophobic membrane proteins. Because of the simple extraction procedure, this technique has been employed to purify/separate a variety of membrane proteins, enzymes and receptors (Clemetson et al., 1984; Tauber et al., 1985; Tiruppathi et al., 1986; Zhang and Hager, 1987; Saitoh et al., 1995; Minuth et al., 1995, 1996; Selber et al., 2001a,b; Choi et al., 2003). Most of the applications to date used CPE to separate proteins with different hydrophobicity. More hydrophobic ones can be extracted preferably into the surfactant-rich phase. Recently, Choi et al. (2003) combined the pressurized liquid extraction and cloud point extraction for the rapid sample preconcentration of herbal materials prior to analysis by high-performance liquid chromatography.

One of the most obvious advantages using surfactant in CPE is that the commercial surfactants suitable for the extraction process are generally more biocompatible than the organic solvents used in the traditional solvent-solvent extraction techniques. However, like traditional solvent extraction, additional proteins/biomaterials other than the targeted ones could also be co-extracted into the respective phases. Thus, subsequent steps such as column chromatography and electrophoretic procedures are needed to achieve final separations. However, the use of CPE in such systems has proven to be very effective at eliminating many of the tedious, time-consuming steps previously required in the conventional purification schemes, such as ammonium sulfate precipitation, for bioanalytes (Quina and Hinze, 1999).

Increasing interest has been shown recently on the cloud point extraction of environmental pollutants (Pramauro, 1990; Pinto et al., 1995; Revia and Makharadze, 1999; Santana et al., 2002; Sanz et al., 2002). Cloud point extraction was utilized generally in these studies to preconcentrate environmental compounds prior to the liquid or gas chromatographic analysis. The growing interest in this aspect is attributable to the quest for a simple and efficient preconcentration technique for the analytical determination of toxic chemicals with low aqueous solubility. The extraction efficiency of a variety of environmental pollutants, such as PAHs and PCBs (polychlorinated biphenyls) from water systems by various surfactants has been reported.

Pinto et al. (1994) used the CPE to preconcentrate PAH from both bottled and river water samples prior to the determination by HPLC with fluorescence detection. Triton X-114 was used as the extracting agent and the extraction was carried out at 40 ^{o}C. In this study that negligible sorption of PAH in the presence of surfactant onto the container was reported and almost complete recoveries, even in the presence of considerable levels of humic acids, were observed. The effective prevention of loss of analytes is another advantage of the CPE. The enrichment factors (the ratio of the peak intensities with and without preconcentration) ranging from 14.1 to 30.2 were obtained. However, Triton X-114 disadvantageously rendered a strong signal, which disturbed the analyses of six out of the ten PAHs used in their study. To alleviate this problem, the detection parameters were optimized for minimum background signal and maximum sensitivity for the PAHs, at the expense of losing the detection sensitivity for some compounds. In addition, optimization of analyzing conditions may require a lot of work and is analytes specific.

The preconcentration of PAHs in water sample from both bottled and network supply was studied by Ferrer et al. (1996). Triton X-114 was again used as extracting agent and the PAHs were determined by HPLC with fluorescence detection and wavelength programming. In order to avoid the disturbance of this surfactant in the sample analysis, a clean-up step using a silica-gel column was utilized to separate the surfactant from the PAHs prior to sample analysis. The disadvantage of using the clean-up is that it increased the loss of analytes and hence resulted in low recoveries. In addition, it made the process more complicated.

To alleviate the disturbance from surfactants, efforts have been put in the selection of suitable surfactant. For example, use of anionic surfactants, such as sodium dodecyl sulfate (SDS), sodium dodecylbenzenesulfonic acid (SDB-SA), sodium dodecanesulfonic acid (SDSA) and sodium dioctylsulfonic acid (Aerosol OT) to preconcentrate PAHs, was attempted (Casero et al., 1999; Sicilia et al., 1999; Merino et al., 2002). These surfactants have low fluorescence and UV absorbance signals at the PAH detection wavelengths because of the absence of aromatic moieties in the surfactant molecules. Their low retention time, owing to their polar character, allowed the sensitive determination of more polar PAHs with low molecular weight without the need for a clean-up step to remove the surfactants. The presence of these surfactants also leads to negligible sorption of PAHs onto the container. However, use of this type of surfactant requires the addition of acid to induce the phase separation and relatively longer induction time for phase separation to occur. The most significant drawback of anionic surfactant-mediated extraction is reflected on the low recovery achieved for weakly basic polar compounds. Because in such acidic medium, which is required for phase separation to occur in anionic surfactant solutions, these compounds are in their ionic form (Sicilia et al., 2002)

Other types of surfactant in attempts include primary ethoxylated alcohols such as Brij series (Fernández et al., 1998). Compared with surfactants such as Triton X-114, this type of surfactant has low UV absorbance and gives no fluorescence signal because their molecules contain no aromatic moiety. However, the cloud point of this kind of surfactant is usually high.

Polymers were also used as an alternative in CPE. Saitoh et al. (1999) used a water soluble polymer, poly(*N*-isopropylacrylamide) to extract hydrophobic organic compounds including PAHs. HPLC with UV detection was utilized for the determination of the compounds. In contrast to the widely used poly(oxyethylene alkylphenyl ether) type surfactant, e.g. Triton series or PONPE series, this polymer has very small ultraviolet absorption. However, for polar compounds, the effectiveness of the polymer-water systems is limited. Unlike the polymer-water system, surfactant-water systems can offer both a hydrophilic and a hydrophobic environment to solute species simultaneously, which gives rise to a partitioning selectivity based on the hydrophobicity of solutes. In addition, compared to surfactants, the number of polymers suitable to extraction applications is restricted due to their more complicated phase behavior. Unlike the simple binary surfactant-water system, more complex ternary polymer1-polymer2-water systems are required in many cases.

Another alternative to eliminate the disturbance from surfactants is to use detectors other than UV or fluorescence, such as electrochemical detectors (Pinto et al., 1992, 1995). However, it has limited applications since it is only feasible for electroactive analytes.

CPE has also been employed to extract environmental pollutants from soil/sediment samples. Bockelen and Niessner (1993) studied the micellar extraction and CPE of PAH from soil suspensions by different nonionic-ionic surfactants. Recoveries up to 100% were obtained. Stangl and Niessner (1994) reported the decontamination of napropamide from soil. Micellar solutions of a nonionic surfactant Polyethylene glycol monoalkyl ether (Genapol X-80) were used to extract napropamide spiked on the soil. Cloud point extraction was then applied to concentrate napropamide solubilized in the solutions. The recoveries by this process were reported from 54% to 102%, depending on the initial sample concentration. The effects of different soils on the extraction extent were also studied. The drawback of this study is that the cloud point of this surfactant is very high. Even with the addition of sodium sulfate, the CPE had to be carried out at 75 °C. Komaromy and Wandruszka (1995) studied the decontamination of oil-polluted soil by CPE. Triton X-114 was utilized. The oil was first recovered by micellar extraction and then CPE was applied. Recoveries ranging from 42% to 105% were achieved. The possible causes for the recoveries of more than 100% were not given in the studies. It may be caused by experimental errors, which were unfortunately not provided. As is realized in this thesis work, a major cause of errors in the recovery of the cloud point extraction process is the precise determination of the volume of the surfactant-rich phase. The small volume and high solute concentration of that phase can leads to significant errors if the volume can not be precisely determined. To reduce the volume of the surfactant-rich phase, the extraction was carried out at 50 ^{o}C. The requirement of high temperature is also another disadvantage to this process. Pinto et al. (1994) also reported the effectiveness of an extraction process for PAH from solid samples by micellar solutions of Triton X-114 and a following preconcentration step at 40 ^{o}C. High recoveries from 94% to 100% were obtained. Merino et al. (2002) evaluated the effectiveness of the acid-induced cloud point extraction (ACPE) to extract polycyclic aromatic hydrocarbons (PAHs) from different environmental samples (soils, sediments and sludges), prior to chromatographic analysis. Variables affecting the ACPE efficiency were optimized. Recoveries obtained for four to five aromatic ring PAHs ranged from 71 to 98%. Although the method does not require additional clean-up or preconcentration steps, it needs the addition of high concentration of acid (4.2 M HCl) and high temperature (60 °C) and equilibrating time (one hour) are needed for optimal extraction.

As has been discussed above, though much significant successes of CPE have been reported, the selection of suitable surfactants still remains a very important issue. A suitable surfactant should have high extraction efficiency for the targeted compounds and low cloud point, but cause no disturbance in sample analysis.

Compared with the study on recovery and preconcentration efficiency of solutes by CPE, little work has been reported on the partition or distribution characteristics of solutes between the aqueous and surfactant-rich phase. Since the extraction of solutes into the surfactant-rich phase is governed by the solubilization process, generally, the factors favored by the solubilization process will help the partition in CPE.

Akita and Takeuchi (1996) studied the equilibrium distribution of aromatic compounds between the aqueous solution and a coacervate (surfactant-rich phase) of a nonionic surfactant, polyoxyethylene nonyl phenyl ether having an average ethylene oxide number of 10 (PONPE10). The effects of various factors including surfactant concentration, sodium chloride concentration and *pH* on the partition coefficient were investigated. It was observed that the partition coefficient was independent of surfactant concentration and could be improved in the presence of sodium chloride. The effect of sodium chloride is attributable to its effect in lowering the cloud point of the surfactant, which consequently leads to a decrease in the volume of the surfactant-rich phase. The effect of *pH* on partition coefficient was interpreted in the term of the acid dissociation constant of the solutes. It was also observed that solutes with a stronger effect to lower the cloud point of the surfactant had greater partition coefficient.

Sakulwongyai et al. (2000) studied the distribution of chlorinated ethanes between the surfactant-rich phase and the aqueous phase in the cloud point extraction with two surfactants, Octylphenyl-polyethylene glycols with molar ratio of ethylene oxide group to octylphenyl 7 (Igepal CA-620) and 9 (Igepal CA-630) as extractants. It was found that high hydrophobicity of the solutes favored the partition into the surfactant-rich phase. It was also observed that increasing temperature could improve the partition coefficient, which was attributed to the larger solubilization capacity at higher temperatures, smaller volume of the surfactant-rich phase and the decrease in the amounts of micellized surfactant in the aqueous phase. Despite of the detailed study on the various factors, there are some aspects that need to be improved if the extraction process is to be applied efficiently. First, in this study, it seems that the extraction took a too long time (two days) to arrive equilibrium. To accelerate the process, external forces such as centrifugal forces could be used. Second, if the preconcentration factors (a solute concentration in the coacervate phase/its initial concentration) are to be calculated, it was found that the values are less than 10 in most of cases. The preconcentration factor is a direct measure of a preconcentration process. The low preconcentration factors mean that the preconcentration efficiency is not high enough. The process needs to be optimized.

Prediction on parameters affecting the distribution of organic toxic pollutants in cloud point extraction was proposed as well (Szymanowski and Apostoluk, 2000). The estimation method was based on the Abraham model, which describes the solute-solvent interactions for various compounds in the same solvent system (Abraham, 1993). The model takes into account a number of solute properties such as hydrophobicity and dipolarity. Twelve aromatic compounds were used. It was observed that the distribution ratio always increases with the solute hydrophobicity, and also depends on the hydrophobicity of the nonionic surfactant. It was also found that the solubilization data by the nonionic micellar solutions could be used to determine the usefulness of the cloud point extraction technique.

Also with the aid of Abraham model, Materna et al. (2001) studied the distribution characteristics of phenols in CPE. In this study, new oxyethylated methyl dodecanoates with average degrees of oxyethylation ranging between 5 and 14 were used. The effects of factors including the characteristic of phenols (e.g. hydrophobicity, hydrogen-bound acidity, hydrogen-bound basicity, etc.), the average degree of oxyethylation of surfactant, the electrolyte concentration, and the overheating temperature on the distribution coefficients were studied. Distribution coefficients ranging from 20 to 100 were obtained. It was discovered that the kinetics of the separation process was limited by the slow coalescence of the fine droplets of surfactant-rich phase.

Despite the numerous reports on CPE in the literature, the surfactants used in CPE were limited to the hydrophilic surfactants with higher cloud points. In contrast, utilization of hydrophobic or slightly hydrophobic surfactants was rarely reported. Unlike the hydrophilic surfactants, hydrophobic nonionic surfactants do not bear the clouding phenomenon. Instead, they usually exist as dispersions in water and mainly used as emulsifier. However, these surfactants do separate into distinct phases upon changes in temperature or presence of additives, such as inorganic salts. For example, the bicontinous L_3 phase, rich in surfactant but having very small phase volume, coexists with the excess water phase as a result of heating up the lamellar dispersions (L_α+W). The advantage of using such hydrophobic surfactants is based on their higher solubilization power but lower phase separation temperature.

2.4 Effect of Surfactant on Biodegradation

Bioremediation technique has been attracting researchers in recent years. However, this technique is limited in the case of sparingly soluble hydrocarbons, such as PAH. These substances have great affinity to soil, which contributes to their low bioavailability. Stimulated by the dramatic solubilization capacity of surfactants for this family of compounds, a new technique, surfactant-enhanced bioremediation has been a research focus in recent years (Mulligan et al., 2001; Cort et al., 2002; Ruiz et al., 2002; Zheng and Obbard, 2002a,b). It's generally assumed that the surfactant micellar phase serves as a source of substrate. As the microorganism depletes the hydrocarbons in the aqueous phase, the micelle-solubilized hydrocarbons diffuse into the aqueous phase (Liu et al., 1995). Compared with biodegradation process, the exchange of hydrocarbons between aqueous phase and micellar phase is very fast.

Although it has been agreed unanimously that surfactants can enhance the apparent solubility and dissolution of hydrocarbons from contaminated soil (Pennell et al., 1993; Yeom et al., 1996; Johnson et al., 1999; Billingsley et al., 2002; Garon et al., 2002), conflicting results still have been reported on the ability of surfactant to enhance biodegradation process. The focus of contest is whether solubilization is conducive or inhibitory to the microbial uptake of hydrocarbons. The enhanced biodegradation in the micellar solution can be attributable to the increased solubility and bioavailability of substrate to bacteria (Guerin and Jones, 1988; Bury and Miller, 1993; Liu et al., 1995; Volkering et al., 1995; Jahan et al., 1997; Madsen and Kristensen, 1997; Bardi et al., 2000; Schippers et al., 2000; Prak and Pritchard, 2002a), surfactant-enhanced substrate transport through the microbial cell wall (Miller and Bartha, 1989; Noordman et al., 2002), increased interfacial area in the presence of surfactant (Robichaux and Myrick, 1972; Liu, 1980; Rittman and Johnson, 1989), and the enhanced contact of bacteria to the hydrocarbon-water interface (Holden et al., 2002). The inhibitory effect was always observed at surfactant concentrations approaching and exceeding the CMC. Potential mechanisms of inhibition include toxicity of surfactant to the microorganism (Aronstein et al., 1991; Tsomides et al., 1995; Mosche and Meyer, 2002), that surfactant is used as preferable substrate (Mulkins and Steward, 1974; Laha and Luthy, 1991, 1992), and that surfactant inhibits direct contact between cells and hydrocarbon surface (Foght et al., 1989; Efroymson and Alexander, 1991). It was also observed that the effect of surfactant was also related to the specific bacteria involved (Boonchan et al., 1998; Allen et al., 1999), which means that the specific interactions between bacteria and surfactant also plays an important role.

Since the micellar phase contains most of the dissolved substrate, the bioavailability of solubilized hydrocarbons decides the overall effect of solubilization on their biodegradation. The bioavailability of hydrocarbons in the micellar phase has been studied as well. Bury and Miller (1993) examined the effect of micellar solubilization on biodegradation rates of *n*-decane and *n*-tetradecane. A model with Monod equation (Eq.2.20) to describe the cell growth and substrate consumption was used to study the biodegradation of these two hydrocarbons in micellar solutions of liner primary alchol ethoxylate Neodol 25-7 and a surfactant mixture of 90 wt% Neodol 25-9 and 10 wt% Neodol 25-3.

$$\frac{dS}{dt} = -\frac{dX/dt}{Y} = -\frac{\mu_{\max} S}{Y(K_s + S)} X \qquad (2.20)$$

where S is substrate concentration; X is biomass concentration; $\mu_{\max}$ is the maximum specific growth rate; Y (mg dry cell produced/mg carbon oxided) is yield coefficient and K_s is half saturation constant. Results showed that when hydrocarbon is solubilized in small micelles of surfactant, its rate of biodegradation can be substantially enhanced. This was attributable to the increase in the aqueous solubility of hydrocarbon. The solubilization of *n*-decane and *n*-tetradecane by surfactant micelles reduced the doubling time of microorganisms and greatly increases the rate of hydrocarbon consumption. It was also observed that the specific growth rate, μ was a function of surfactant concentration and the length of surfactant hydrophilic head, which was attributed to the degradation of surfactant. However, the biodegradation of the surfactants were not measured.

Tiehm (1994) compared the biodegradation of surfactant presolubilized phenanthrene and fluoranthene with their biodegradation in crystal form. The degradation of PAHs was inhibited by sodium dodecyl sulfate because this surfactant was preferred as a growth substrate. However, it was found that growth of mixed cultures on phenanthrene and fluoranthrene solubilized by a nonionic surfactant was exponential, which indicated the high bioavailability of the solubilized hydrocarbons. It was also observed that nonionic surfactants of the alkylethoxylate type and the alkylphenolethoxylate type with an average EO number of 9 to 12 monomers were toxic to several PAH-degrading cultures. Toxicity decreased with increasing hydrophilicity of the surfactants.

Liu, et al. (1995) quantified the bioavailability of micelle-solubilized naphthalene to naphthalene-degrading microorganisms. Two nonionic surfactants, Brij 30 and Triton

X-100 were used. Results showed that naphthalene solubilized by surfactant micelles was bioavailable and degradable by the mixed bacterium cultures.

Tiehm and Frizsche (1995) studied the biodegradation of both single and mixture of PAHs presolubilized by surfactant. Accelerated biodegradation rates were found for both single and mixed PAHs presolubilized compared with the rate of PAHs in crystal form. This indicated that the solubilization increased the bioavailability of PAHs.

Guha and Jaffé (1996a) studied the biodegradation kinetics of phenanthrene partitioned into the micellar phase of nonionic surfactants. Based on the assumption that phenanthrene partitioned into micellar micelles is directly available to microorganisms, they incorporated a bioavailability fraction coefficient, f, into the mathematical model. The model also took into account a number of processes, such as partition onto apparatus, volatilization, and partition onto biomass of phenanthrene. Results showed that, the bioavailability coefficient of Brij 35 is zero. It means that the microoganism used in the experiments could not attack the phenanthrene in the micelles, whereas, the f values of Polyethylene (9.5) glycol nonylphenyl ether (Triton N101), Triton X-100, and Brij 30 were between 0 and 1. With an increase in surfactant concentrations, f approached zero, indicating that phenanthrene cannot be transferred from the micelles to the cells. They also suggested that f is not only a function of the surfactants, but most likely also of the bacterial culture. Mechanism of bioavailability of hydrophobic compounds partitioned into the micellar phase of nonionic surfactant was suggested as a result from their further research work (Guha and Jaffe, 1996b). They also studied the bioavailability of PAH mixtures partitioned into the micellar phase of another nonionic surfactant (Guha et al., 1998b).

Zhang et al. (1997) studied the effects of biosurfactants on the dissolution, bioavailability, and biodegradation of phenanthrene. A mathematical model that describes the combined effects of solubilization and biodegradation, including description of bioavailability within surfactant micelles, was suggested. Various bioavailability of substrate within micelles of different surfactants was found. Experimental results indicated that the effect of a surfactant on biodegradation was a combination of the solubilizing power of the surfactant and the bioavailability of the substrate within the surfactant micelles.

In a kinetics study of biodegradation on the surfactant-solubilized fluoranthene, Willumsen and Arvin (1999) found that surfactant-solubilized fluoranthene could promote the rate of fluoranthene degradation, but, to a lesser extent than expected based on the observed surfactant-enhanced fluoranthene solubilization. They suggested that the bioavailability of micelle-solubilized fluoranthene might be one factor controlling mineralization in such system.

The effects of surfactant on PAH biodegradation and vice versa were also studied by some others (Chen et al., 2001). It was observed that naphthalene and phenanthrene biodegradation varied in the presence of different surfactants. The surfactant biodegradation is also responding differently to the presence of naphthalene and phenanthrene. The effects could be attributed to the inhibition of surfactants on the biodegradation of PAHs and the competitiveness between surfactants and PAHs.

Garcia et al. (2001) studied the effect of nonionic surfactant Brij 35 on the bioavailability of solid and Teflon-sorbed dibenzofuran for *Sphingomonas sp.* strain

HH19K. It was observed that the presence of this surfactant accelerated the dissolution and biodegradation of solid dibenzofuran by a factor of 2. However, it slowed down the initial biodegradation of desorbing dibenzofuran. They proposed that two processes might reduce the bioavailability of sorbed dibenzofuran. First, desorbing dibenzofuran rapidly accumulated in the surfactant micelles, leading to reduced truly water-dissolved dibenzofuran concentration, which controls the biodegradation rate. Second, Brij 35 suppressed the contact between bacteria and Teflon. This increased the average diffusion distance of dibenzofuran to the bacteria, which in turn flattened the gradient of the dissolved dibenzofuran concentration between the sorbent and the cells.

As aforementioned, the effect of a surfactant on the biodegradation of hydrocarbons is contributed by many factors. The bioavailability of substrates in the micellar phase decides the overall performance of surfactants in the bioremediation process. Consequently, it is also the reason that much research work has been carried out in this aspect. For a successful field application, the selection of surfactants is the most important step. Such a surfactant should be nontoxic to the microorganism and pose no environmental problems, also should have a good solubilization capacity for the targeted contaminants. All these factors together with the bioavailability of the compounds solubilized in the micelles of the surfactant should be examined before field applications. In addition, the surfactant selection must also consider operation factors, such as turbidity and foam generation (Deshpande et al., 1999).

The importance of solubilization on biodegradation cannot be over emphasised. A successful surfactant-enhanced bioremediation process not only comes from the efficient solubilization, but also depends on the effectiveness of bacteria to degrade the solubilized contaminants. Searching for surfactant that is conducive to both of the process is what future research should be directed to.

It is thus one purpose of this study is to get a better understanding on the effect of solubilization on biodegradation and to study the bioavailability of PAH solubilized in surfactant micellar phase.

2.5 Biodegradation of Surfactant

The biodegradation of surfactants can influence, enhancing or inhibiting, the biodegradation of primary substrate of interest. Consequently, it is necessary to examine the biodegradability of surfactants to the bacteria in the selection of suitable surfactant.

Biodegradation of surfactant has been the subject of substantial researches since the 1950s when synthetic detergents became popular in daily life (Zhang et al., 1999; Staples et al., 2001; Eichhorn et al., 2002; Řezníčková et al., 2002). However, the surfactant concentrations of primary interest in biodegradations were in the neighbourhood of 10 mg/L, which is usually 10-100 times lower than their critical micelle concentrations (CMCs). To enhance the soil remediation process, surfactants should be applied at concentrations much higher than their respective CMCs. However, information about the biodegradation of surfactant at such concentrations is scarce. It has not been till recent years that such studies have been carried out. Salanitro and Diaz (1995) investigated the anaerobic biodegradability of anionic and nonionic surfactants. Results showed that linear primary alcohol sulfates and a linear alcohol ethoxylate (LAE-8) could be readily biodegraded after a 15-30 day lag phase. The extent of degradation of a branched alkyl phenol ethoxylate was lower.

Lajoie et al. (1997) studied the simultaneous biodegradation of nonionic surfactants and polychlorinated biphenyls (PCBs). The surfactants were used both to increase the solubility of PCBs and to support the growth of surfactant-degrading strains engineered for PCB degradation.

Jahan et al. (1999) modelled the influence of nonionic surfactants, polyoxyethylene sorbitanmonopalmitate (Tween 40), Brij 35, Triton X-114 and Corexit 0600 (blend of surfactant esters) on the biodegradation kinetics of phenanthrene. A mathematical model was presented based on the rates of solid dissolution, substrate biodegradation and oxygen uptake. The model accounted for the concurrent utilization of surfactants for cell growth. The result showed that the presence of surfactants enhanced the biodegradation rate of phenanthrene, which is mainly by increasing the dissolution rate of phenanthrene from solid phase to aqueous phase.

Zhang et al. (1999) studied the aerobic biodegradation kinetics of four anionic and nonionic surfactants at sub- and supra- critical micelle concentrations. Both primary and ultimate biodegradation of the surfactants were investigated. For the two anionic surfactants, sodium dodecyl sulphate (SDS) and sodium dodecylbenzene sulfonate (SDBS), their primary biodegradation were determined by MBAS (methylene blue active substances) assays, and for the two alchol ethoxylate nonionic surfactants, Tergitol 15-S-12 and Witconol SN-90, CTAS (cobalt thiocyanate active substances) assays was used. The ultimate biodegradation of the four surfactants was determined by TOC (total organic carbons) measurement. It was observed that SDBS showed no biodegradation. Increasing surfactant concentrations from sub- to supra-CMC significantly decreased both primary and ultimate biodegradation of the three other surfactants. As was proposed by the authors, the inhibition may be attributed to the limited bioavailability of surfactants in the micellar phase as compared to the monomeric surfactants.

2.6 Properties and Applications of Tergitol Surfactants

2.6.1 Applications of Tergitol 15-S-X Surfactants

Tergitol 15-S-X surfactants are mixtures of linear secondary alcohols reacted with ethylene oxide. This type of surfactant has the following general structural formula,

$C_{12-14}H_{25-29}[OCH_2CH_2]_XOH$

This type of surfactant contains no double or π bonds in their molecules, therefore they do not cause disturbance in sample analysis using HPLC with fluorescence detection, which is an efficient method for the analysis of aromatic hydrocarbons. Therefore, usage of these surfactants can simplify the cloud point extraction process by eliminating the washing step for the separation of surfactants from analytes or saves time on the optimization of HPLC operating conditions in order to minimize the disturbance from surfactants. This type of surfactant is also readily biodegradable in the environment. Therefore, use of these surfactants will not pose dangers to the environment. These surfactants are proven performers in numerous diverse industries, which are listed below (from the supplier).

- Household and industrial laundry detergents
- Hard-surface cleaners and degreasers

- Industrial and institutional cleaners
- Hydrocarbon and water-based laundry prepotters
- Power car washes and care products
- Paper deinking, rewetting, pulping and deresinating
- Oil-in-water emulsions
- Textile wet processing
- Dye assist and levelling agents for carpets and textiles
- Wetting agent, coupling agent, and emulsifier for fiber lubricants
- Emulsifier for polyethylene textile softeners
- Dispersant, stabilizer, wetting agent for paints
- Agricultural dispersant and wetting agent
- Metal cleaners and acid-cleaning compounds
- Low-temperature soak-tank cleaning systems
- Oil field chemicals
- Circuit board cleaners
- Leather hide soaking, tanning and dyeing operations

The increasing popularity of this type of surfactant is attributable to their better features compared with other types of surfactant. A comparison is given in Table 2.3. Though Tergitol 15-S-X surfactants have been used in so many areas, their application in the extraction and bioremediation has been rarely reported. Due to the readily biodegradability nature of these surfactants, they are ideal candidates for environmental applications. The purpose of this work is to select suitable surfactants from this series and to explore their potential in extraction and biodegradation applications.

Table 2.3 Comparison of Tergitol 15-S-X to Other Types of Surfactants (from supplier)

Characteristic	Tergitol 15-S-X	PAE*	NPE*	OPE*
Initial Foam Height	Moderate	Moderate	Moderate	Moderate
Foam Collapse Rate	Rapid	Slow	Moderate	Slow
Wetting	Excellent	Good	Good	Excellent
Hard Surface Cleaning	Excellent	Good	Excellent	Excellent
Handling (Pour Point)	Excellent	Poor	Good	Good
Dissolution Time	Excellent	Fair	Fair	Fair
Solvent Solubility	Excellent	Fair	Good	Good

*PAE=Primary Alcohol Ethoxylate, NPE=Nonylphenol Ethoxylate, OPE=Octylphenol Ethoxylate

2.6.2 Selection of Surfactants

Table 2.4 Properties of Tergitol 15-S-X surfactants Reported by the Supplier

Tergitol 15-S-X X=	Form	Cloud Point (°C)	HLB	EO#	CMC (mg/L)
3	liquid	insoluble	8.0	3	insoluble
5	liquid	dispersible	10.5	5	dispersible
7	liquid	37	12.1	7.3	39
9	liquid	60	13.3	8.9	56
12	Semi-solid	88	14.7	12.3	110
15	solid	>100	15.4	15	180
20	solid	>100	16.3	20	280
30	solid	>100	17.4	31	710
40	solid	>100	18.0	41	2200

Cloud Point: 1 wt% aqueous solution, ^{o}C; CMC: 25°C

The selection of surfactants should be based on their physical properties. Table 2.4 summarizes the selected properties from the supplier of this type of surfactant.

For preconcentration process, surfactants with lower cloud points are preferred. As can be seen from Table 2.4, Tergitol 15-S-7 has a low cloud point temperature, which is just above ambient temperature. Therefore, it is a good candidate for this process. With the addition of some suitable additives, the extraction may be carried out at ambient temperature. In addition, this surfactant has been accepted by the U.S. Department of Agriculture for general-purpose cleaning or as an ingredient in general-purpose cleaner for use in federally inspected meat and poultry processing plants.

Due to its slight hydrophobicity, Tergitol 15-S-5 forms dispersion in water. This surfactant undergoes complicated phase changes upon increase in temperature. Above a certain temperature, which is 23.5 °C for 1 wt% solution (from preliminary experiments), two phases form in the solution (L_3+W), which resembles the cloud point separation (L_1+W) for most nonionic surfactants. Both the L_1 and L_3 phase are rich in surfactants. Therefore, this phase behavior of Tergitol 15-S-5 may be used for the preconcentration of PAHs. For Tergitol 15-S-X, when X is above 7, their cloud points are much higher and hence are not good choices. For an example, 1wt% Tergitol 15-S-9 has a cloud point of 60 °C, which is well above the ambient temperature.

For surfactant washing in soil remediation, normally surfactants with HLB numbers greater than 10 are suitable choices. In addition, surfactants with low CMC values and high solubilization capacity are preferred. According to Table 2.4, Tergitol 15-S-7, Tergitol 15-S-9 and Tergitol 15-S-12 are good choices.

According to the information provided above, in this thesis, the solubilization capacity of Tergitol 15-S-7, Tergitol 15-S-9 and Tergtiol 15-S-12 for model PAH was examined. The preconcentration efficiency of Tergitol 15-S-5 and Tergitol 15-S-7 was tested. The effects of Tergitol 15-S-7, Tergitol 15-S-9 and Tergitol 15-S-12 on the biodegradation of model PAH, as well as the biodegradability of these surfactants were also examined.

Chapter 3 Materials and Methods

3.1 Reagents

3.1.1 Surfactants

Nonionic surfactant, Tergitol 15-S-X (X= 5, 7, 9 and 12) were obtained from Union Carbide (see Section 2.6). Triton X-100, Tween 20 and Tween 80 were obtained from Sigma. Neodol 25-7 was obtained from Shell Chemicals. It is a linear primary alcohol ethoxylates, with an average EO number of 7.0. Sodium dodecyl sulfate (SDS) was purchased from Sigma. The selected properties of the nonionic surfactants are given in Table 3.1. The properties of Tergitol 15-S-X surfactants has been given in Table 2.2

Table 3.1. Selected Properties of Nonionic Surfactants Studied in This Work

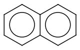
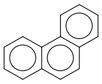
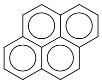
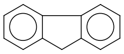
Surfactant	Molecular Weight (Da)	HLB[a]	CMC (mg/L)	Cloud Point[b], (°C)
Tween 20	1228	16.7	60[d]	98.5[d]
Tween 80	1308	15.0	13 - 15[c]	75[d]
Triton X-100	624	13.5	43[e], 140[c]	65[c]
Neodol 25-7	515	12.3	9[c]	49[c]

[a] Calculated values using HLB= degree of ethoxylation expressed in wt% / 5
[b] 1 wt% solution
[c] Provided by supplier
[d] Shiau et al., 1995
[e] Guha and Jaffé, 1996a

3.1.2 PAHs

Naphthalene, fluorene, phenanthrene, and pyrene were obtained from Aldrich. The information about the PAHs is given in Table 3.2

Table 3.2 Physical Data of the Model PAHs Used in This Work

Name	Molecular Formula	Molecular Structure	Molecular Weight	M.P.* °C	Purity (≥%)
Naphthalene	$C_{10}H_8$		128.17	81	98
Phenanthrene	$C_{14}H_{10}$		178.23	101	98
Pyrene	$C_{16}H_{10}$		202.26	150	98
Fluorene	$C_{13}H_{10}$		166.22	116	98

* melting point

3.1.3 Salts and Alcohols

Five reagent grade salts NaCl, Na_2SO_4, $CaCl_2{\cdot}2H_2O$, Na_2SO_4, $Na_3PO_4{\cdot}12H_2O$ and NaI were purchased from Merck. The effects of these salts on the cloud point of Tergitol 15-S-7 were examined. The effect of NaCl on the phase separation of Tergitol 15-S-5 was also studied. Five alcohols, methanol, ethanol, *n*-propanol, *n*-butanol and *n*-pentanol are also obtained from Merck. All the alcohols have a purity of over 98%. Their effects on the cloud point of Tergitol 15-S-7 were measured as well.

3.1.4 Medium for Bacterial Cultivation

Marine Broth 2216, obtained from Becton Dickinson was used for the cultivation of the bacteria. Marine Agar 2216 (Becton Dickinson) was used as solid media for the bacterial cultivation and maintenance.

An artificial seawater mineral salt medium based on the ionic compositions of seawater was used (Dyksterhouse et al., 1995) in the biodegradation experiments. It contains (per litter of deionized water) 22.79 g of NaCl, 11.18 g of $MgCl_2{\cdot}6H_2O$, 3.98

g of Na_2SO_4, 1.46 g of $CaCl_2 \cdot 2H_2O$, 1.3 g of TAPSO {3-[N-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid}, 0.72 g of KCl, 0.27 g of NH_4Cl, 89 mg of $Na_2HPO_4 \cdot 7H_2O$, 83 mg of NaBr, 31 mg of $NaHCO_3$, 27 mg of H_3BO_3, 24 mg of $SrCl_2 \cdot 6H_2O$, 2.6 mg of NaF, and 2.0 mg of $FeCl_2 \cdot 4H_2O$. The pH value of this solution was adjusted with 2N hydrochloric acid to 7.5.

3.2 Sand

Sand used in the extraction experiments was obtained from High Performance Concrete Laboratory of The National University of Singapore. It was sieved and particles with a size range from 500~800 μm was used. The porosity is 0.35. Before being spiked, the sand was washed with acetone to get ride of the organic contents and then dried at 500 ^{o}C for 48 hours.

3.3 Bacteria

The bacteria, *Neptunomonas naphthovorans* (ATCC 700638) was used in the biodegradation experiments. The bacteria arrived in freeze-dried form. It can grow on phenanthrene as a sole carbon source (Hedlund et al., 1999). Incubation conditions are shown as the followings:

Temperature: 20 °C
Atmosphere: aerobic

The purity of the culture was checked once in two weeks by plate stripping method. 10 μL cultures maintained on the slant marine agar were taken and diluted 100 times in 10 mL sterilized DI water. Then, aliquots of 200 μL were spread on solidified marine agar plate. After the plates were dried, they were inverted and wrapped in parafilm, and then incubated at 20 °C for two weeks. The resulting colonies are white, shiny, moist and entire, which suggests the culture is pure. TEM images showed that the bacteria are rod-shaped (2.0~3.0 μm in length and 0.7~0.9 μm in diameter) and are motile with a single polar flagellum (Hedlund et al., 1999).

3.4 Apparatus

3.4.1 HPLC

Figure 3.1 is a photograph of the HPLC system used in the experiments.

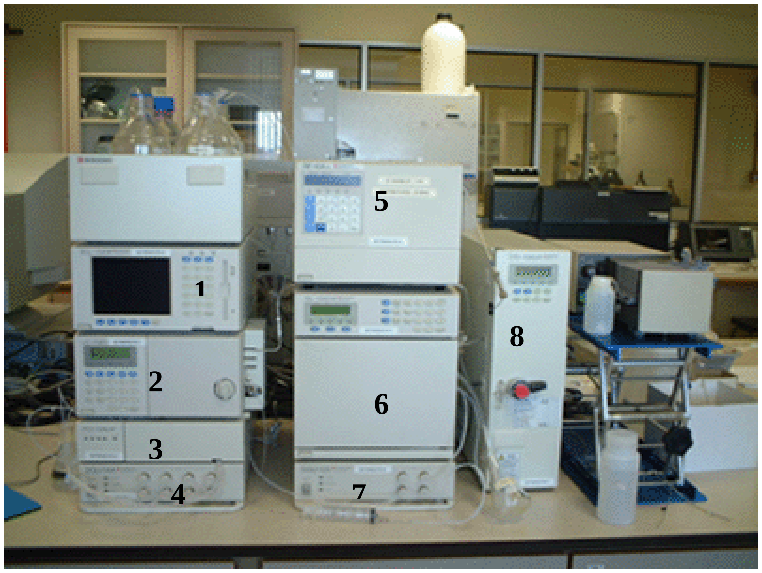


Figure 3.1 A Photo of the HPLC System Used in the Study.
1-system controller; 2-liquid chromatograph; 3-pump; 4,7- degaser; 5-fluorenscence detector; 6- auto injector; 8-column oven

It was equipped with a LC-10ATVP pump, two DGU-14A degassers, an SIL-10ADVP auto injector, a CTO-10ASVP column oven, an SCL-10AVP system controller, and an RF-10AXL fluorescence detector. PAH concentrations were obtained using a Shimadzu software Class-VP5.03. The column is an Agilent PAH C_{18} column

(250×4.6 mm) packed with 5 μm particles and connected with the guard cartridge holder (Agilent 79918PH-100). Mobile phase is a mixture of methanol (80%) and ultra pure water (20%), and the flow rate is 1 mL/min.

3.4.2 Light Scattering

Dynamic and static laser light scattering experiments was carried out with a light scattering apparatus from the Brookhaven Instrument Corporation (NY, USA). This system consists of a BI-200SM motor-driven Goniometer, an advanced BI-9000AT digital autocorrelator and a model 95 argon ion laser. Figure 3.2 is a photograph of the light scattering system.

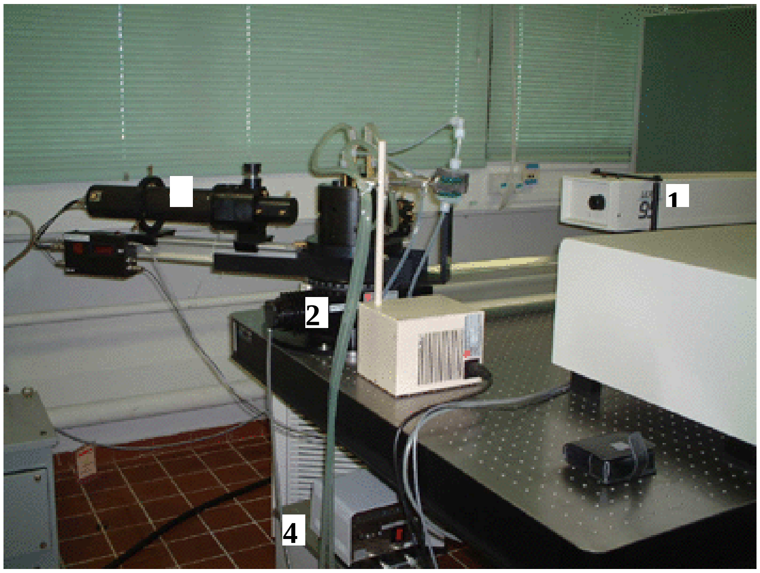


Figure 3.2 A Photo of the Laser Light Scattering System Used in the Study.
1-laser; 2- Goniometer; 3- autocorrelator; 4-water bath

3.4.3 Drop Shape Surface Tensiometer

A surface tensiometer (Krüss Drop Shape Analysis System, DSA-10) was used to determine CMCs of surfactants. In this work, the pendant-drop mode was chosen.

3.4.4 TOC

A Shimadzu TOC-5000A total organic carbon analyzer was used for the determination of surfactant concentration and biomass. The machine is equipped with an ASI-5000A auto sampler. The furnace temperature is set at 680 °C.

3.5 Experimental Procedures

3.5.1 CMC Determination

To determine the CMC of surfactants in deionized water (Chapter 4) and in mineral medium (Chapter 7), concentrated surfactant solutions and series of dilutions were prepared. The surface tensions were measured with the surface tensiometer DSA 10. The CMC values were estimated by plotting the surface tension data against the logrithm of surfactant concentration as the abscissa. The transition between the descending line for surfactant concentration less than CMC and the line obtained at surfactant concentrations greater than CMC was taken as the CMC values.

3.5.2 Solubilization Equilibrium

All the solubilization experiments were carried out using 15 mL screw-capped culture tubes. The vials were first coated with the PAH of interest and then filled with 10 mL surfactant solutions at concentrations above its CMC. To coat the tube wall with PAH (either pure PAH or PAH mixtures), the PAH was first dissolved in methanol (HPLC grade) and then let the methanol to evaporate at about 70 ^{o}C. The amount of the PAH in each vial was all in excess.The vials were then agitated on an orbital shaker in an air-conditioned room of 22 °C over a period up to two weeks before HPLC analysis. Solubilization experiments at 30 °C were conducted in a water bath shaker with good temperature control. Experiments at 15 °C were carried out in an incubator. The equilibrium solubility of PAH was taken as the average value of triplicate analysis. The standard errors are less than 2%. The samples were filtered through the cellulous membrane syringe filters having pore size of 0.2 μm to remove the fine particles. The filters were pre-saturated by filtering corresponding solutions of 2 mL. The excitation wavelengths for naphthalene, fluorene, phenanthrene, and pyrene are 215, 215, 248 and 230 nm, and the emission wavelengths are 345, 350, 395, and 385 nm respectively. The detection limit of the fluorescence detector for all the PAHs is 1 ppb.

3.5.3 Micelle Size and Aggregation Number Measurement

The dynamic and static laser light scattering experiments were performed on the laser light scattering instrument. The BIC Zimm plot software was used to obtain the molecular weights of micelles, from which the aggregation number could be estimated, and the hydrodynamic radii of micelles were calculated with the BI-DLSW software.

The molecular weight and the second virial coefficient can be obtained from the following equation on the Zimm plot:

$$\frac{Kc}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{q^2 {R_g}^2}{3}\right) + 2A_2c \qquad (3.1)$$

where R_θ is the Rayleigh ratio; q is the magnitude of the scattering wave vector; R_g is the radius of gyration; M_w is the weight-averaged molecular weight; c is the surfactant concentration; A_2 is the second virial coefficient; and K is the optical constant, which is given as the following equation for vertical polarized incident light:

$$K = \frac{4\pi^2 n_o^2}{N_A \lambda_0{}^4} \cdot \left(\frac{dn}{dc} \right)^2 \qquad (3.2)$$

where n_o is the refractive index of the solvent, the continuous media; λ_o is the wavelength of incident light in vacuum; N_A is Avogadro's number; and dn/dc is the specific refractive index increment of the micellar solutions.

The hydrodynamic radius, R_h of the micelle was obtained from the dynamic light scattering (DLS), commonly referred as quasi-elastic light scattering (QELS) as well. In contrast to the static light scattering experiments that focus on the time-averaged intensities at any given scattered angle, the dynamic light scattering experiments use the information on the variation of intensity with time, which contains the information on the random motion of the particles and therefore can be used to measure the diffusion coefficient of the particles. The fluctuating signal in the time-dependence intensity of the scattered light due to the random motion of the particle can be processed by forming the autocorrelation function, $g(t_d)$, t_d being the delay time. For a monodisperse suspension of rigid and globular particles, the autocorrelation function is given by

$$g(t_d) = A \exp(-2q^2 D\, t_d) + B \qquad (3.3)$$

where D is the translational diffusion coefficient, principle quantity measured by DLS; A is the optical constant determined by the instrument design; and B is a constant background term. The measured diffusion coefficient D can then be used to determine the particle sizes using the following Stokes-Einstein equation:

$$R_h = \frac{k_B T}{6\pi\eta_0 D} \tag{3.4}$$

where k_B is the Boltzmann constant; T is the absolute temperature of the solution; and η_o is the viscosity of the fluid. It has to mention that the DLS experiments in this study were all carried out at the scattered angle equal to 90°.

3.5.4 Measurement of Cloud Point

The cloud point determination was carried out in a water bath for Polyscience with good temperature control of ±0.1 °C. Cloud point is taken as the temperature at which a surfactant solution turns into turbid upon being heated up. The determination was repeated three times for each solution and the errors on the average values of CP are within 0.2 °C.

3.5.5 Cloud Point Extraction from Aqueous Solutions

Stock solution of PAHs was prepared by dissolving weighed amounts of PAHs into 50 mL HPLC grade methanol. The CPE is conducted in 30 mL centrifuge tubes. In a typical experiment, calculated amount of surfactant was added into about 20 mL deionized water along with various volumes of PAH stock solution, and then suitable amounts of sodium sulfate or sodium chloride was added into the tube. The final solution was 25 mL. The solution was then put into an Eppendorf centrifuge 5810R at

22 °C. After being equilibrated statically for 15 minutes, the solutions were centrifuged at 3500 rpm for 20 minutes to achieve complete separation of the two phases. Clear interface between the two phases, surfactant rich phase and water phase, can be seen.

An aliquot from the surfactant rich phase was withdrawn and transferred into HPLC auto sampling vials for analysis. Averages were taken of triplicate analysis to determine the concentrations of the PAHs in the surfactant-rich phase. To determine the PAH concentrations in the aqueous phase, the surfactant-rich phase was carefully removed from above the aqueous phase, and then triplicate samples from the aqueous phase were withdrawn for direct HPLC analysis. The recovery and partition coefficient are determined using the following definitions.

Recovery by CPE: the percentage of PAH extracted from the bulk solution into the surfactant-rich phase:

$$R = \frac{C_s V_s}{C_0 V_t} \times 100\% = \frac{C_0 V_t - C_w (V_t - V_s)}{C_0 V_t} \times 100\% = \left[1 - \frac{C_w}{C_0}\left(\frac{1}{1+R_V}\right)\right] \times 100\% \tag{3.5}$$

where C_s and C_w (mg/L) are the concentration of PAHs in the surfactant-rich phase and aqueous phase respectively; C_0 (mg/L) is the initial PAHs concentration in the bulk solution before phase separation; V_s and V_w (mL) are the volume of the phases correspondingly. V_t (mL) is the total volume of the solutions and R_V is the phase volume ratio of the surfactant-rich phase to the aqueous phase.

**Partition coefficient K_p: ** defined by

$$K_p = C_s / C_w \tag{3.6}$$

The schematic description of phase equilibrium in CPE is shown in Figure 3.3.

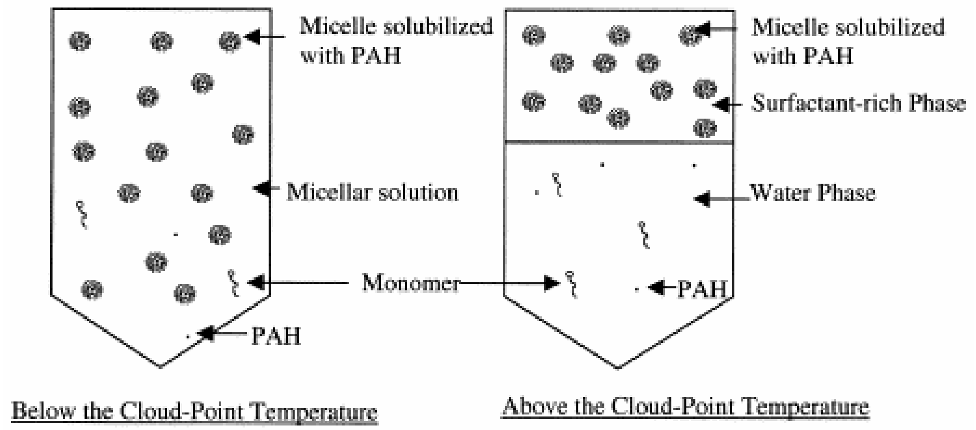


Figure 3.3 A Schematic Description of Phase Equilibrium in CPE.

Water content of the surfactant-rich phase, preconcentration factor of PAHs and phase volume ratio are defined as follows,

Water content, γ of the surfactant-rich phase: defined as the mass percentage of water in that phase,

$$\gamma = \frac{m_w}{m_t} \times 100\% \tag{3.7}$$

where m_w (mg) is the mass of water in surfactant-rich phase; and m_t (mg) is the total mass of that phase.

Preconcentration Factor f_C: defined as the ratio of the PAH concentration in the surfactant-rich phase to the initial PAH concentration in the bulk solution.

$$f_C = C_s / C_0 \tag{3.8}$$

The preconcentration factor defined here is a direct measure of the effectiveness of a preconcentration method.

Phase Volume Ratio R_V:

$$R_V = V_s / V_w \tag{3.9}$$

3.5.6 Procedure for the Decontamination of Spiked Sand

Extraction Procedure: the extraction procedure is described in Figure 3.4.

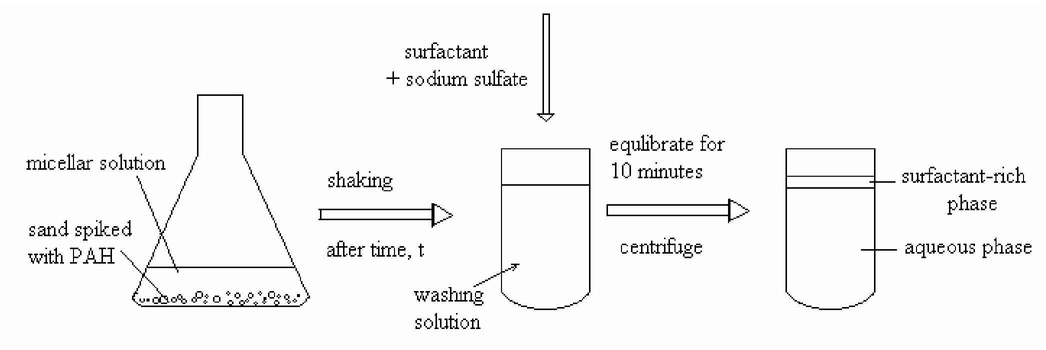


Figure 3.4 Description of the Procedure of Phenanthrene Extraction from Spiked Sand.

The process is composed of the following three steps

Step 1. Preparation of sample sand: Firstly, the sample sand was spiked with phenanthrene dissolved in acetone. To make the spiking as homogeneous as possible, the mixture of the sand and the phenanthrene solution in acetone was agitated on an orbital shaker at 100rpm in an air-conditioned room of 22 ^{o}C. Acetone was then removed by evaporation. The phenanthrene content of spiked sand was determined by TOC measurement. The measured value is 0.6 mg phenanthrene/g sand.

Step 2. Micellar solubilization: 10 g spiked sand was added to each 500 mL Erlenmeyer flask. Then, 200 mL micellar solution of Tergitol 15-S-7 at various concentrations was added into the Erlenmeyer flasks, which were placed on the shaker operated at 150 rpm. To measure the dissolution kinetics, aliquots of 0.5 mL solution was withdrawn at certain time intervals. The samples were then centrifuged for HPLC analysis. For a constant interfacial area, the dissolution rate of phenanthrene can be described by a first-order mass-transfer model (Grimberg et al., 1994).

$$\frac{dC}{dt} = ka\left(C^{*} - C\right) = k_{l}\left(C^{*} - C\right) \tag{3.10}$$

where C (mg/L) is the phenanthrene concentration in solution at time t; C^{*} (mg/L) is the saturation concentration; k (cm/min) is mass-transfer coefficient; a (cm^{-1}) is the specific surface area of sand sample; and k_l (min^{-1}) is the lumped mass-transfer coefficient. Since the specific surface area of the sand sample is difficult to be determined, the lumped mass-transfer coefficients at different surfactant concentrations were obtained using this model.

Step 3. Cloud point extraction: after micellar solubilization has reached equilibrium, the washing solutions in the flasks were transferred into 25 mL centrifuging tubes and surfactant was added to the tubes to achieve desired concentration, and then a weighed amount of sodium sulfate was added into the solution. After sodium sulfate was completely dissolved, the tubes were centrifuged for 20 minutes at 4000 rpm and then equilibrated for 15 minutes. Samples from the surfactant-rich phase were withdrawn for HPLC analysis.

The recovery of phenanthrene by this process is described below,

The recovery of Phenanthrene by Micellar Solubilization from Spiked Sand, R_m

$$R_m = \left(CV_w / \left(C_{soil}{}^0 M_{soil} \right) \right) \times 100\% \tag{3.11}$$

where C is the concentration of PAH in washing solution (mg/L); V_w is the volume of washing solution; $C_{soil}{}^0$ is the initial concentration of PAH sorbed on sand (mg/g); and M_{soil} is sand mass (g).

Total Recovery from Spiked Sand, R_T:

$$R_T = \left(C_s V_s / \left(C_{soil}{}^0 M_{soil} \right) \right) \times 100\% = R_m \times R \tag{3.12}$$

where R is the recovery of cloud-point extraction

3.4.7 Biodegradability Test of the Nonionic Surfactants

Biodegradability of the surfactants at concentrations up to 1.0 wt% by the bacteria was tested in 250 mL glass Erlenmeyer flasks. Each flask contains 100 mL artificial seawater mineral solution with surfactant at various concentrations. Cultures growing in marine broth were harvested at the late exponential growth stage and centrifuged. The bacteria pellets were washed, centrifuged again and resuspended in 50 mL mineral medium. Aliquots of 5 mL were added in to each flask stoppered with cotton plugs. The solutions were then incubated at 20 ^{o}C. The bacteria growth was observed visually.

3.4.8 Biodegradation Experiments

3.4.8.1 Biodegradation of Surfactants

The biodegradation of surfactants was carried out in 500 mL glass Erlenmeyer flasks. Bacteria, grown on each individual surfactant, Tergitol 15-S-X (X= 7, 9 and 12), were harvested at the late exponential growth stage. Aliquots of the cultures were centrifuged and the bacteria was washed once with and resuspended in mineral water. Aliquots of the suspension were used as inocula. Biodegradation was carried out in a water bath shaker operated at 150 rpm and 22 °C. Uninoculated surfactant solutions at the same concentrations were used as control. At time intervals, samples were withdrawn and centrifuged at 10,000 rpm at room temperature (22 °C) for 15 minutes to remove the bacteria.

3.4.8.2 Effect of Solubilization on Biodegradation of Phenanthrene

Biodegradation of Solubilized Phenanthrene: The experiments were also carried out in 500 mL Erlenmeyer flasks. To study the effect of solubilization on biodegradation of phenanthrene, the biodegradation of phenanthrene at the same initial concentration and different surfactant concentrations was carried out. To acclimatise the bacteria to surfactants, the inocula used were taken from an exponential growth culture grown in mineral medium supplemented with 1 mg/L of phenanthrene and 200 mg/L of individual surfactant. To prepare the micellar solutions solubilized with phenanthrene, 500 mL Erlenmeyer glass flasks were welded with cylinder glass wells (3.8 cm in id; 1 cm in height) at the center of the flask bottom. Phenanthrene powders were melted in the wells to form phenanthrene solid with certain surface area. 200 mL micellar solution with surfactant concentration lower than the experimental concentrations was added into each flask. After certain time, the solutions were filtered through Whatman #50 filter paper (dia 15.0 cm, hardened) five times to remove the fine phenanthrene particles in the micellar solutions. To prevent loss of surfactant due to adsorption on the filter paper, the filter paper was presaturated by filtering 50 mL micellar solution solubilized with phenanthrene. All the filtered solutions were mixed and distributed again into individual flasks to ensure the same initial phenanthrene concentration. Weighted surfactant was added to other flasks to get the desired surfactant concentrations. Samples of 1.0 mL were taken at certain time intervals and centrifuged to remove bacteria. The clear supernatant from each sample was used for the determination of phenanthrene concentration. Sterilized uninoculated solutions were used to control the abiotic losses of phenanthrene. The biomass and phenanthrene concentration were measured at certain time intervals.

To study the effect of initial phenanthrene on biodegradation, different concentrations of phenanthrene were applied at a same surfactant concentration. The following procedure is used to prepare the solutions: 200 mL surfactant solution at a certain concentration was added into each flask with phenanthrene solid, which was then put into a water bath shaker operating at 150 rpm at 22 ^{o}C. Different phenanthrene concentration can be acquired by controlling the dissolution time. The micellar solutions were filtered for five times to remove fine phenanthrene particles. The inoculums were taken from an exponential growth culture supplemented with 1 mg/L of phenanthrene and 200 mg/L of surfactant as carbon source. Abiotic losses of phenanthrene were monitored using sterilized uninoculated controls.

Chapter 4 Solubilization of PAH by Nonionic Surfactants

4.1 Introduction

In this chapter, the nonionic surfactants, Tergitol 15-S-X (X = 7, 9 and 12), comprising mixtures of secondary alcohol ethoxylates, were examined on their solubilization efficiency for model PAHs. These Tergitol surfactants are environmentally benign and easily biodegradable, in contrast to the conventional alkylphenolpolyethoxylate surfactants like Triton X-100, which have been shown to leave relatively stable metabolites octylphenol or nonylphenol compounds that are toxic to both marine and fresh species (Van der Meeren and Verstraete, 1996).

Additionally, the choice of the Tergitol surfactants, especially Tergitol 15-S-7, is also based on the known high solubilization power for large triglyceride oils and fatty alcohols (Chen et al., 1997, 1998), and its high extraction efficiency for PAHs (Bai et al., 2001). Despite the fact that the solubilization of PAHs by other surfactants could be found elsewhere in the literature, use of this surfactant has not been reported yet. It is, thus, the purpose of this chapter to attempt to report the solubilization power of these Tergitol surfactants on the model PAHs and their selected properties in the micellar solutions. The information obtained in this chapter will provide some solubilization information of the model PAHs by the Tergitol surfactants for the aforementioned successful applications in the biodegradation processes and the cloud-point extraction technique.

The CMC value of the Tergitol 15-S-7 in DI water was measured by a pendant drop tensiometer. Its solubilization capacity for a model PAH, phenanthrene, was compared with those of three frequently reported surfactants, namely, Tween 20, Tween 80 and Triton X-100. Solubilization capacity was expressed as weight solubilization ratio, molar solubilization ratio and micelle-water partition coefficient. To understand the effect of HLB number on solubilization, its solubilization capacity was compared with those of two other surfactants of the homolog, namely Tergitol 15-S-9 and Tergitol 15-S-12. The micelle-water partition coefficients of four model PAHs, namely naphthalene, fluorene, phenanthrene and pyrene, were correlated to their octanol-water partition coefficients, which is a measure of hydrophobicity. This information is helpful to the understanding of the solubilization mechanism and is practically useful in predicting the solubilization efficiency of a surfactant. Effects of temperature and salinity on solubilization capacity of Tergitol 15-S-7 surfactant for phenanthrene were also investigated along with the micellar properties. The change in hydrodynamic radius and aggregation number of micelles with temperature was measured by the dynamic and static laser light scattering techniques. Effect of salinity on the enhancement of phenanthrene solubility was also discussed in terms of conformation changes in the micelles due to the possible coordination of sodium cations and oxygen atoms on the ethylene oxide groups of the surfactant. Finally, the synergistic effect of naphthalene and pyrene on the solubilization of phenanthrene was also studied. Results of this chapter have been published (Li and Chen, 2002a).

4.2 Results and Discussion

4.2.1 Determination of CMC

The surface tensions of solutions of Tergitol 15-S-7 in DI water at 22 °C were measured at different surfactant concentration and the results are given in Figure 4.1. The surface tension initially decreases with increase in surfactant concentration and then approaches a constant value. Two tangent lines were drawn for the two sections and the CMC is taken as the axial value of the cross point. From the figures, the CMC of Tergitol 15-S-7 is 39.5 mg/L, which corresponds to the value from the provider (0.0039 wt%)

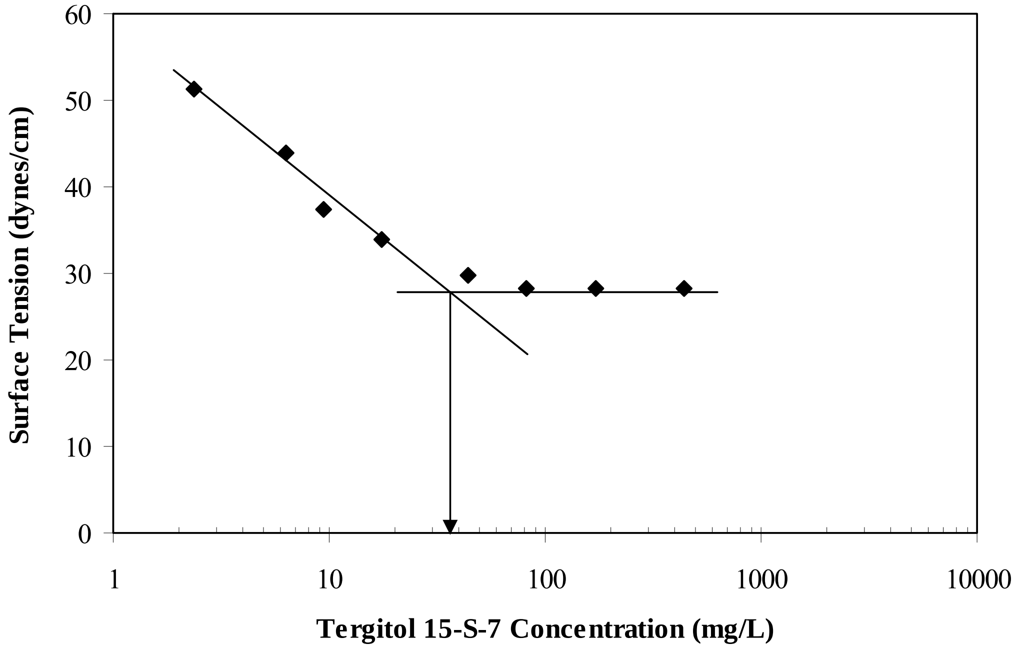


Figure 4.1 Determination of CMC of Tergitol 15-S-7 in Deionized Water at 22 °C.

4.2.2 Solubilization Capacity of Tergitol 15-S-7 for Model PAHs

The solubilization of phenanthrene at 22 °C by solutions at surfactant concentrations below and above the CMC of Tergitol 15-S-7 and three other "conventional" surfactants, Tween 20, Tween 80 and Triton X-100 is shown in Figure 4.2.

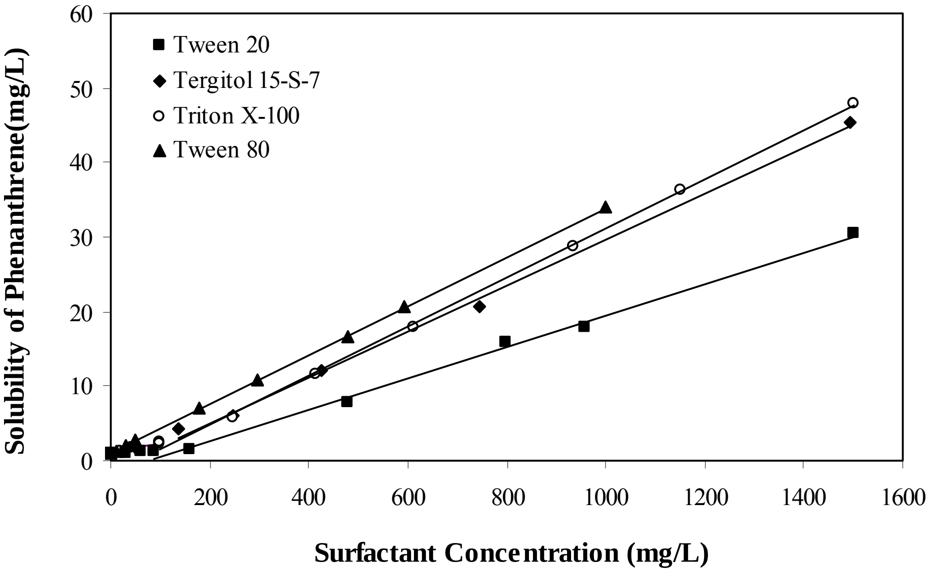


Figure 4.2 Solubilization of Phenanthrene by Nonionic Surfactants.

The equilibrium solubility of phenanthrene increases linearly with increasing concentration of surfactant above the CMC. It is easily understood that increasing the surfactant concentration will increase the number density of the micelles, where phenanthrene molecules are solubilized in the hydrophobic cores (Rosen, 1989). Increasing the surfactant concentration will also increase the solubilization rates of hydrophobic solubilizates (Carroll 1981; Chen et al., 1997, 1998). The slope of the solubilization curve is the mass solubilization ratio (*WSR*), which is a dimensionless quantity equal to the mass ratio of the solubilized phenanthrene to that of the surfactant. The corresponding *MSR* values could then be easily garnered from the aforementioned *WSR* values with the information on the molecular weights of the surfactants and the solubilizates. The values of *WSR* and *MSR* as well as $log\ K_m$ are listed in Table 4.1.

Table 4.1 Solubilization Data for Phenanthrene by Different Surfactants

Surfactant	WSR (ppm/ppm)	MSR (mol/mol)	*log K_m*
Tergitiol 15-S-7	0.031	0.089	5.73
Tergitol 15-S-9	0.020	0.066	5.61
Tergitol 15-S-12	0.017	0.071	5.63
Tween 20	0.021	0.140	5.78
Tween 80	0.033	0.241	5.98
Triton X-100	0.034	0.118	5.72

It is found that Tergitol 15-S-7 has relatively comparable solubilization capacity for phenanthrene, on the *WSR* basis, to those of three "conventional" surfactants. Nevertheless, the low CMC and molecular weight as well as readily biodegradable nature that Tergitol 15-S-7 possesses may contribute to widespread applications in the future. Moreover, Tergitol 15-S-7 has been successfully demonstrated in the cloud-point extraction process to concentrate the polycyclic aromatic hydrocarbons (PAHs) in aqueous samples prior to the analytic analysis by using HPLC equipped with fluorescence detector (Bai et al., 2001). Furthermore, use of the Tergitol 15-S-7, in contrast to the Triton X-100, will never render the unwanted interference on the fluorometric signals of the PAHs on the HPLC system in this application.

The solubilities of phenanthrene in various surfactant solutions below the corresponding CMCs remained almost unchanged from about 1 mg/L in water to a value near 2 mg/L at the CMCs. Increasing surfactant concentration further, the solubility of phenanthrene increase dramatically. Therefore, the value of 2 mg/L is appropriately taken as the value of C_{cmc} for phenanthrene, i.e., the phenanthrene concentration at the CMC of the surfactant.

4.2.3 Factors Affecting Solubilization

4.2.3.1 HLB number of Surfactant

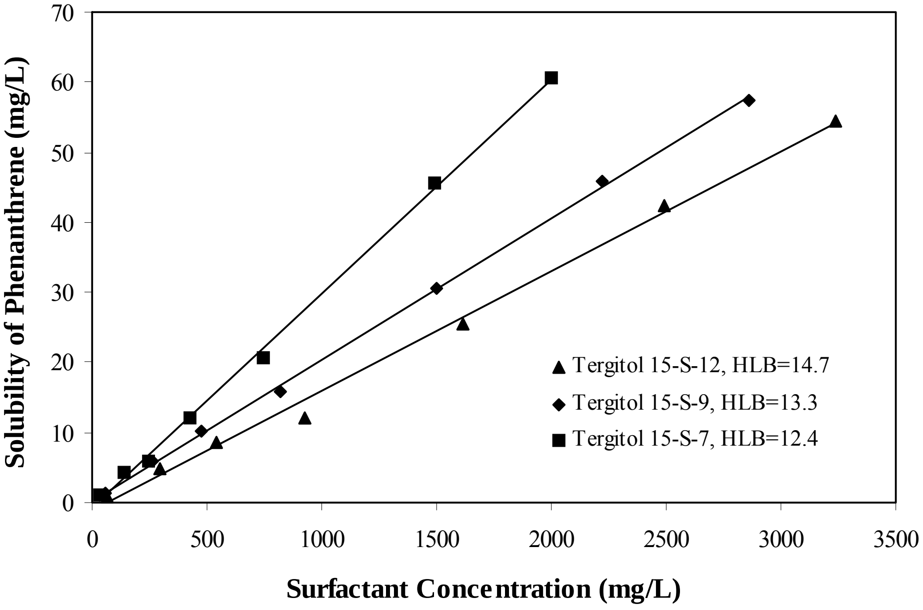


Figure 4.3 Solubilization of Phenanthrene by Surfactant of Tergitol Series.

Figure 4.3 exhibits the solubilization curves of phenanthrene by the micellar solutions of Tergitol 15-S-X (X = 7, 9 and 12), respectively, at 22 ºC. The effect of hydrophile-lipophile-balance (HLB) value of the surfactant on the solubilization capacity is clearly demonstrated in this figure. Among these three Tergitol surfactants used in this study, Tergitol 15-S-7 has the lowest HLB value but the greatest solubilization capacity expressed in *WSR*. The hydrophobicity of a surfactant can be roughly estimated from its HLB value. The lower the HLB value that the surfactant has, the more hydrophobic it is. In aqueous solutions, surfactants with lower but not too low HLB values will tend to form micelles that have more hydrophobic environment in their cores or palisade shells, where hydrocarbons tend to reside. That is, the solubility of the hydrocarbon solubilizates will increase accordingly (Rosen, 1989). The factor of HLB value is a good indicator to judge the solubilization capacity of surfactant of the same homolog.

Another explanation is that the surfactants with lower HLB values, if able to form micelles, will form larger micelles. That is, the core or the shell of the micelles has larger volume, compared to that formed from the surfactant of the homologous series but having larger HLB value, which could house more hydrocarbon molecules. This effect can also be observed from the cloud point temperature of the surfactant. In general, the surfactants with lower HLB values have lower cloud points. As approaching the cloud point, the surfactant will tend to dehydrate and the micelles will aggregate and grow. This will be discussed later in this session.

Diallo et al. (1994) successfully elucidated the effect of HLB value in explaining the solubilization capacity of nonionic surfactant series, containing the mixtures of the primary dodecyl alcohol ethoxylates (Witco), for 11 nonpolar hydrophobic organic compounds including alkanes and aromatic hydrocarbons. It is noteworthy to mention that the HLB value cannot be used as the sole factor to account for the solubilization capacity of the surfactants having different structures. For example, Tween 80 has a higher HLB number (15.0) than Tergitol 15-5-7 (12.4), but it has a greater solubilization capacity. The discussion on the effect of HLB values on the solubilization will be more meaningful only when the surfactants are of the same homologous series.

4.2.3.2 PAH Hydrophobicity

Figure 4.4 shows the different solubilization behavior of model PAHs by the micellar solutions of Tergitol 15-S-7 at 22 ºC. The surfactant concentrations range from 50 to 1000 mg/L. Similarly, it is found that the solubility of PAH increases linearly with the surfactant concentration above its CMC. The *WSR* values for naphthalene, fluorene, phenanthrene and pyrene are found at 0.065, 0.046, 0.031 and 0.015, respectively. Correspondingly, the *MSR* values are 0.264, 0.144, 0.091 and 0.039.

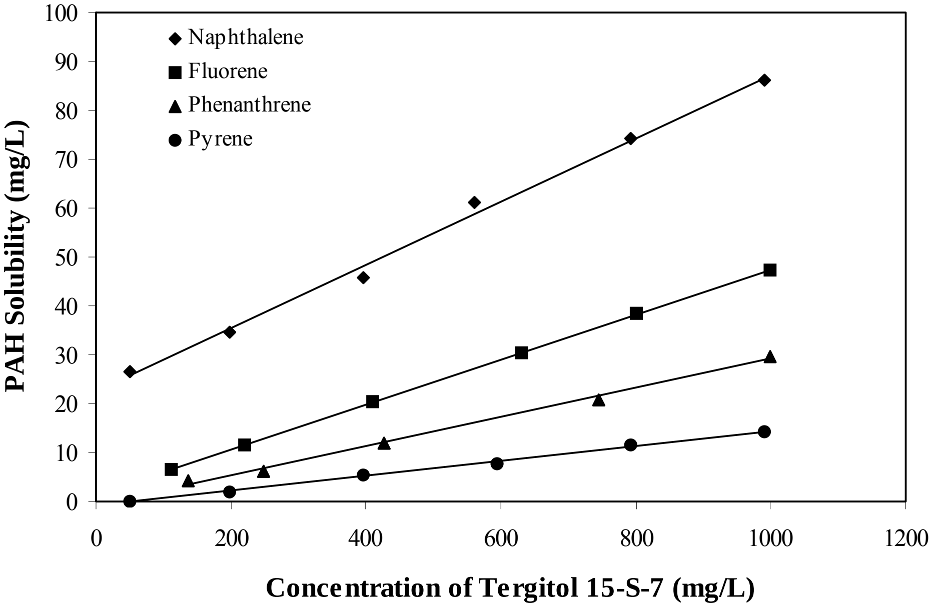


Figure 4.4 Solubilization of PAHs by Tergitol 15-S-7 micellar solutions at 22 °C.

The micelle-water partition coefficients of these PAHs by Tergitol 15-S-7 are calculated and plotted in Figure 4.5 with respect to their octanol-water partition coefficients. The values of logK_{ow} are 3.34, 4.47, 4.57 and 5.52 for naphthalene, fluorene, phenanthrene and pyrene respectively (Yalkowsky, 1999). Likewise, the concentrations of naphthalene and fluorene as well as pyrene at the CMC of Tergitol 15-S-7 are found at 27, 3 and 0.2 mg/L, respectively. These values are slightly different but very close to those values found by Edwards et al. (1991) at 25 ºC at the CMCs of the surfactants employed in their study. They are 40–50 mg/L for naphthalene, 2–3 mg/L for phenanthrene, as well as 0.2–0.4 mg/L for pyrene. In general, the larger K_{ow} of the particular hydrophobic solubilizate implies the more hydrophobic nature it has and the greater tendency it possesses in partitioning into the micellar phase. The logarithms of K_m and K_{ow} are found to have a good linear relationship (Figure 4.5). The correlation can be expressed as

$$log\ K_m = 0.85\ log\ K_{ow} + 1.87 \tag{4.1}$$

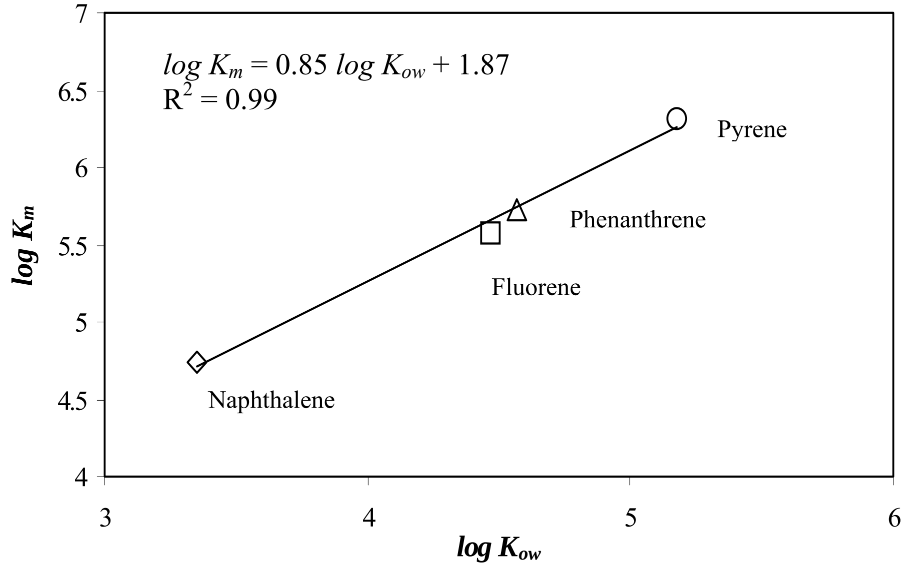


Figure 4.5 Correlation of *log* K_m and *log* K_{ow} for PAHs in Tergitol 15-S-7 micellar solution.

The findings are in accord with the results of Valsaraj and Thibodeaux (1989), who investigated the solubilization of eleven hydrophobic organic compounds by an anionic surfactant, sodium dodecyl sulfate, and reported a good linear relationship between *log* K_m and *log* K_{ow}. They obtained a slope of 0.847 and an intercept of 1.09 on the *log* K_m–*log* K_{ow} curve. Indeed, Valsaraj and Thibodeaux (1989) derived the following relationship for *log* K_m and *log* K_{ow}:

$$\log K_m = \left(1.00 - 18.0\frac{P_L}{RT}\right)\cdot \log K_{ow} + B \tag{4.2}$$

where P_L is the Laplace pressure existing across the curved micelle-water interface; T is the temperature; and R is the universal gas constant. For spherical micelles, P_L can be estimated by $P_L = 2\gamma / r$, where γ is the interfacial tension across the micelle-water interface and r is the radius of the micelle. If the micelle-water interfacial tension can be approximated with the surface tension between the air and the Tergitol 15-S-7 micellar solution, which is about 30 mN/m for 0.1 wt% Tergitol 15-S-7 solution as reported by the manufacturer, and the radius of micelle could be regarded as about 10 nm from the DLS results, the slope between *log* K_m–*log* K_{ow} curve is near 0.94, which is slightly larger than the experimental value of 0.85 for Tergitol 15-S-7 solution. Precise determination of the micellar size always presents some difficulty by using the light scattering technique. If the micelles are spherical, the average radii of these micelles could be found near 5.5 nm using the aggregation numbers obtained from the static light scattering experiments (to be discussed later in details and also see Table 4.2). Furthermore, with the experimental data available for the polycyclic aromatic hydrocarbons (Miller et al., 1985), the theoretically predicted slope in the Eq. (4.2) is, instead, given as (1 – 20.4 P_L/RT). Thus, the theoretically predicted slope of the Eq. (4.2) is near 0.89, which is closer from but still higher than the experimental value at 0.85.

Furthermore, Edwards, Luthy and Liu (1991) studied the solubilization of five hydrophobic compounds, including three PAHs, in Triton X-100 solutions and reported the linear relationship between the logarithms of K_m and K_{ow} as well, which indicated a slope of about 0.81 and an intercept of 1.85 on the $log\ K_m$–$log\ K_{ow}$ curve. Similarly, a surface tension of 30 mN/m is reported by the manufacturer for 0.1 wt% Triton X-100 and the micellar size is in the order of magnitude near 10 nm, the theoretical value for the slope is estimated at 0.94 using Eq. (4.2).

In addition to the difficulty in the precise determination of the micellar size, one possibility attributed to the discrepancy may be arisen from the specific interaction between the surfactant molecules and the hydrophobic solubilizates in the micelles. Eq. (4.2) was derived based on the assumptions, for simplicity, that the driving force for water to octanol transfer of a hydrophobic molecule is solely the entropic change, and that only the Laplace pressure contributes to the chemical potentials of the hydrophobic solutes in the bulk and micellar phases, respectively (Valsaraj and Thibodeaux, 1989). It is well known that the solubility of the hydrophobic solubilizates in the micelles is lower than that of bulk-phase hydrophobic solutes arisen from the counteraction by the Laplace pressure acting on the convex micellar surface (Rosen, 1989). Indeed, the free energy and the chemical potential may be attributed by different factors (Rosen, 1989). For instance, the surfactant molecules may interact with the solubilizates in the micelles through different routes, such as the coordination among the surfactant and water molecules as well as the solubilizates. This will be discussed later in this section to account for the higher-than-expected solubility of phenanthrene in the micellar solutions with presence of NaCl. Another possibility that contributes to the discrepancy is to approximate the micelle-water interfacial tension with the surface tension. In fact, the interfacial tension should be much lower than the surface tension. In addition, considering that the a fraction of phenanthrene is solubilized on the micellar shell, the micelle radii should be a value between the length of alkyl chains (*ca.* 2 nm) and calculated micelle radii, 5.5 nm. Upon the correction of these two parameters, the predicted slope will be closer to the experimentally obtained values.

Nevertheless, Jafvert et al. (1994) proposed the following approximate equation to relate the octanol-water partition coefficient, K_{ow}, to the micellar-water partition coefficient, K_m:

$$K_m = K_{ow}\left[aN_c - bN_h\right] \tag{4.3}$$

where a and b are fitted parameters; N_c is the number of carbons in the hydrophobic group, and N_h is the number of hydrophilic groups.

4.2.3.3 Temperature

The solubilization of the five PAHs in Tergitol 15-S-7 micellar solutions at different temperatures, such as 15 °C and 30 °C, were measured as well. The results are given in Fig. 4.6.

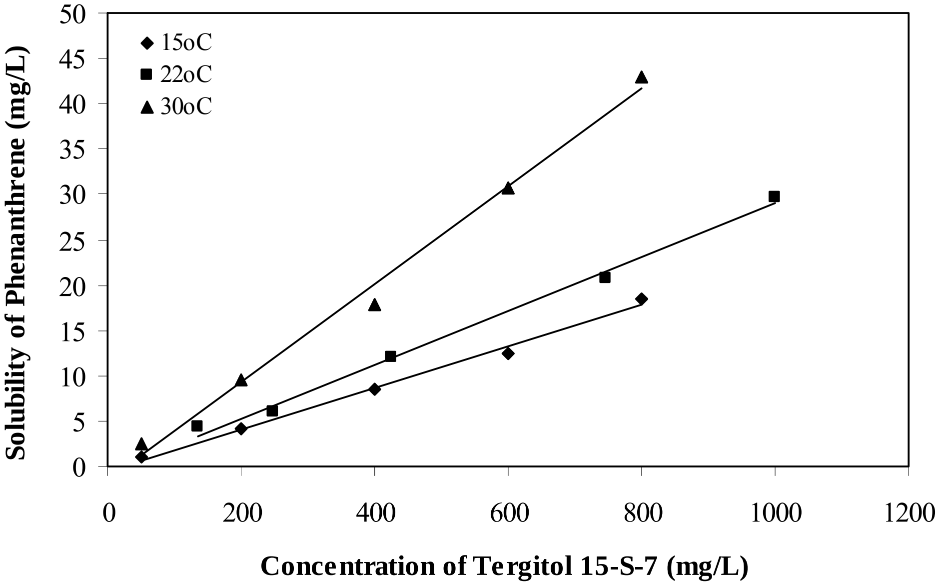


Figure 4.6 Effect of temperature on solubilization of phenanthrene by Tergitol 15-S-7 micellar solutions.

Figure 4.6 clearly exhibits the difference in the solubilization of phenanthrene due to the temperature effect. It indicates that, below cloud point temperature, an increase in temperature can really enhance the solubility of phenanthrene. Generally, an increase in temperature will result in an increase in the extent of solubilization for both polar and nonpolar solubilizates (Rosens, 1989). However, the main mechanism due to the effect of temperature may differ by surfactants of different types. For example, in ionic surfactant solutions, increasing temperature will enhance the thermal agitation of surfactant molecules so as to increase the space available for solubilization in the micelle (Rosen, 1989; Pennell et al., 1997). In contrast, the increase in temperature in the nonionic surfactant solutions tends to increase the aggregation numbers and/or the sizes of the micelles (Rosens, 1989). This is also shown here in Figure 4.7 and reflected in Table 4.2 for Tergitol 15-S-7.

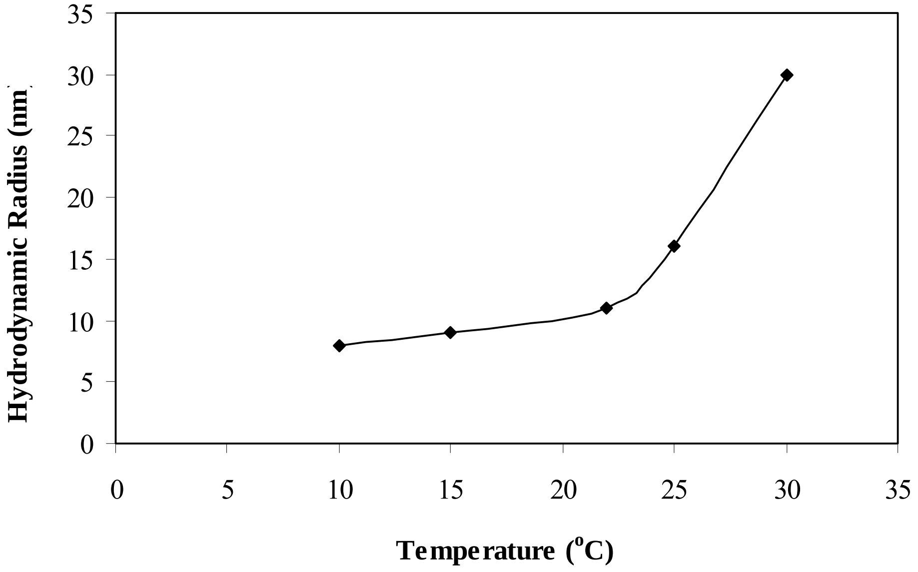


Figure 4.7 Influence of Temperature on the Micellar Size of Tergitol 15-S-7.

Table 4.2 Properties of Tergitol 15-S-7 Micelles Obtained from Light Scattering

Temp	Molecular Weight, Da	Aggregation Number	Hydrodynamic Radius, nm	Core Vol. of micelle[a], nm^3	Micellar Vol.[b], nm^3
15 °C	1.42×10^5	276	9.0	93	477
22 °C	2.13×10^5	414	11.0	139	716
30 °C	4.00×10^5	777	31.0	286	1344

[a] Calculated values using Eq. (4.4) [b] Calculated values using Eq.(4.5)

It is worthy of mentioning that, with light scattering technique, the direct information regarding the micellar volume or the core volume of the micelles is not readily obtainable. However, information on the hydrodynamic radius of micelle can usually be used as an indicator of the micellar size. The aggregation number of micelles at different temperatures was determined by measuring the weight-averaged molecular weight of the micelles using the static light scattering and the Zimm plot. A typical Zimm plot is given in Appendix A. The growth of micelle is more rapid when the temperature increases from 22 ºC to 30 ºC. This is very common for the nonionic surfactants near the cloud points (Rosen, 1989). The cloud point temperature of this surfactant at 1 wt% is about 37.8 ºC (Bai et al., 2001). Increasing the temperature from 15 ºC to 30 ºC, the aggregation numbers in Tergitol 15-S-7 micelles raise from 276 to 777. Similarly, the hydrodynamic radii of the micelles grow from 9 to 31 nm. The increase is also reflected in the core volume of the micelle.

Because of the difficulties present in the direct measurement, attempts have been made to estimate the core volume of the micelle, V_c. Tanford (1980) estimated the core volume of a micelle, V_c in Å^3, by the following equation:

$$V_c = N_{ag} \left[27.4 + 26.9 \left(N_c - 1 \right) \right] \quad (4.4)$$

where N_{ag} is the aggregation number and N_c is the number of carbon atoms of the surfactant lipophile. Subsequently, Diallo et al. (1994) modified Eq. (4.4) and proposed the following approximate equation to quantify the micellar volume, V_m, of dodecyl alcohol ethoxylates (Witco) that were employed in their study to solubilize the benzene, toluene and xylene:

$$V_m = N_{ag} \left(V_s + 4\, N_{EO} \cdot V_w \right) \quad (4.5)$$

where V_s is the surfactant molecular volume; N_{EO} is the number of ethylene oxide groups; and V_w is the molecular volume of water. Eq. (4.5) was derived based on the assumptions that (i) the total volume of a micelle is equal to the volume of its core and hydrated polyoxyethylene shell volumes; (ii) in average 4 water molecules are bound to each ethylene oxide monomer; and (iii) the micellar aggregation number is not affected by solubilization. As aforementioned, the first assumption is generally true for small nonpolar but polarizable hydrocarbons, such as benzene, which can either be solubilized either in the palisade layer or in the inner hydrophobic core of the micelle (Rosen, 1989). For the large nonpolar but slightly polarizable molecules like phenanthrene, they will be mainly solubilized in the inner core of the micelle and slightly, if possible, in the deep palisade layer near the core. The second assumption is expected to hold in most cases because the ratio of water/EO ranging from 2 to 6 was often reported for ethoxylated nonionic surfactants (Foster et al., 1982; Nilsson and Lindman, 1983). Therefore, the core volume of the Tergitol 15-S-7 micelle estimated by Eq. (4.4) may render useful information on qualitatively estimating the solubilization capacity. Alternatively, based on the molecular similarity between Tergitol 15-S-7 and the Witco surfactants employed by Diallo et al. (1994), the micellar volume of Tergitol 15-S-7 at 15, 22 and 30 ºC can be calculated approximately using Eq. (4.5). Consequently, the effective space to house the phenanthrene molecules in the Tergitol 15-S-7 micelles will be some value between those obtained from Eq. (4.4) and (4.5), respectively.

The calculated values of core volume V_c and micellar volume V_m are given in Table 4.2, using V_s and V_w obtained from the density data and the average molecular weight of surfactant and water at experimental temperature. It clearly demonstrated that the effective volume for the phenanthrene molecules to be solubilized increases with increasing temperature. However, care should be taken in estimating the micellar volume when the Eq. (4.4) and (4.5) are used. The assumption that the aggregation number is not affected by solubilization is generally valid for hydrocarbons which are solubilized predominantly in the POE shell of ethoxylated nonionic surfactant micelles. For compounds such as phenanthrene, which can only be partially solubilized on the shell, the aggregation number may be changed. More accurate estimation of micellar volume can be done by measuring the aggregation number in the presence of such solutes.

Diallo et al. (1994) pointed out that the capacity of ethoxylated nonionic surfactants to solubilize alkanes is governed primarily by the volume of the micelles. As aforementioned, the large nonpolar organic compounds tend to be solubilized in the hydrophobic cores of the micelles, an increase in the core volume will, therefore, contribute to the enhancement in the solubility. Equivalently but in different way of explanation, Pennell et al. (1997) reported that a decrease in micellar core volume could reduce the solubilization capacity.

As temperature is increased and the cloud point is approached, the solubilization of nonpolar solubilizates increases very rapidly, probably because of an increase in the aggregation number of the micelles and/or the larger micellar size arisen from the dehydration and the resulted aggregation of the micelles. In addition, for secondary effect, temperature-rise can enhance the thermal agitation of the nonionic surfactant molecules in the micelles, which can also lead to greater solubilization capacity (Rosen, 1989; Pennell et al., 1997). The aggregation numbers were obtained by measuring the molecular weight (MW) of the micelles. The measured micelle MWs of Tergitol 15-S-7 at 15, 22 and 30 ^{o}C are given in Table 4.2.

4.2.3.4 Salinity

The effect of salinity, e.g. the NaCl concentration, in Tergitol 15-S-7 micellar solution on the solubilization of phenanthrene and the selected properties of 1 wt % Tergitol 15-S-7 solution was investigated and is shown in Figures 4.8 and 4.9.

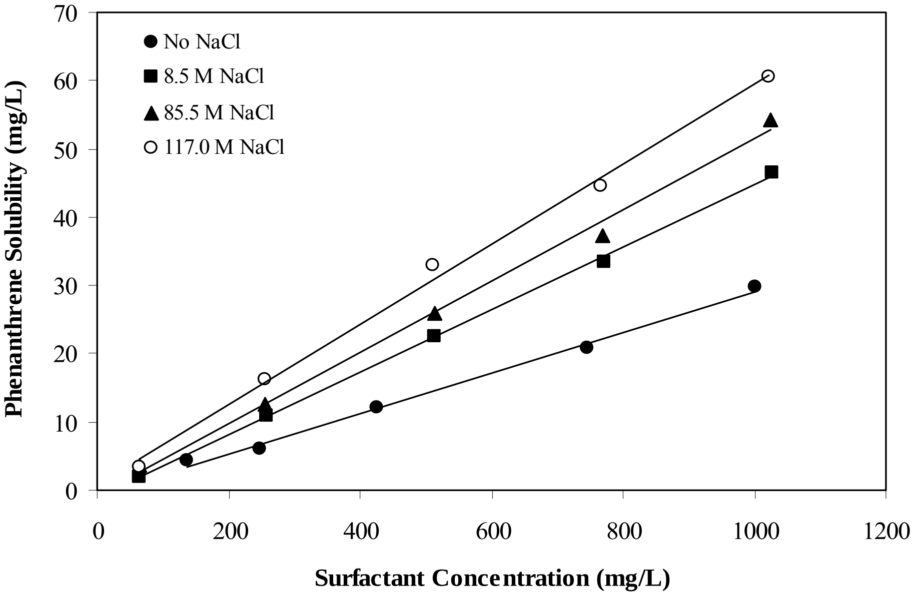


Figure 4.8 Effect of salinity on solubilization of phenanthrene by Tergitol 15-S-7 solution.

The solubility of phenanthrene in the micellar solutions of Tergitol 15-S-7 is enhanced significantly with increasing concentration of sodium chloride. The *WSR* increases almost double from 0.031 in NaCl-free surfactant solution to 0.052 and 0.059 in those with addition of 85.5 and 171.0 M NaCl, respectively (see Figure 4.8).

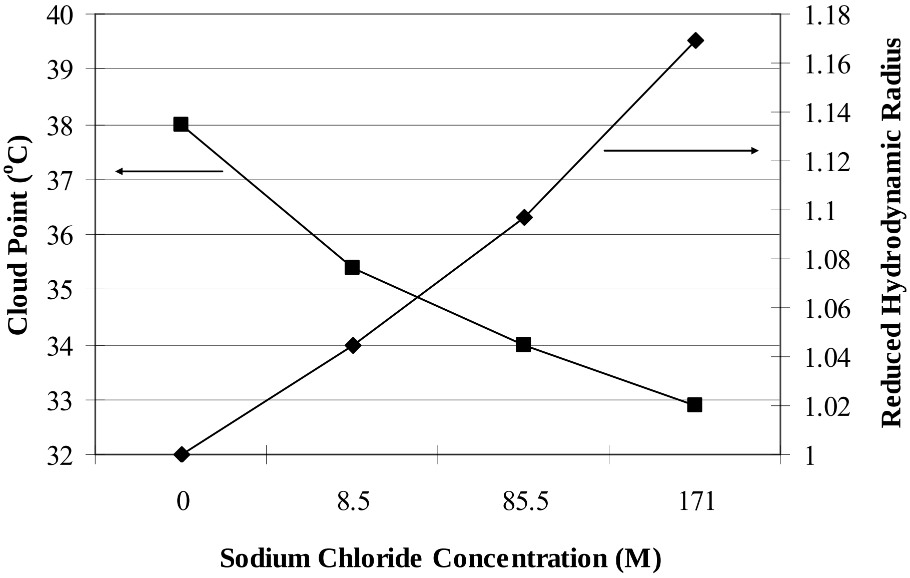


Figure 4.9 Effect of salinity on the micellar size and the cloud point in 1 wt% Tergitol 15-S-7 solution.

The cloud points of 1 wt% Tergitol 15-S-7 solutions decrease from 37.8 ºC in the NaCl-free solution to 34 °C and 32.9 ºC in those with the presence of 85.5 and 171.0 M NaCl, respectively. Nevertheless, the hydrodynamic radius increases only by about 10 and 17%, correspondingly. It is consistent with the measurements by the static light scattering, which reveals that the aggregation number in the Tergitol 15-S-7 micelles with NaCl concentration at 85.5 M increases slightly to 443, compared to 414 obtained in salt-free micelles at the same temperature. It is noteworthy to mention that the aggregation number could not be obtained in the Tergitol 15-S-7 micellar solution with addition of 171.0 M owing to the very high *dn/dc* value, which exceeds the maximum value allowed by this instrument. The increase in the solubility of phenanthrene due to the increasing concentration of NaCl is somewhat consistent with the increasing aggregation number and the decreasing cloud point. Rosen (1989) has pointed out that the addition of neutral electrolyte to solutions of nonionic polyoxyethylenated surfactant increases the extent of solubilization of hydrocarbons at a given temperature in those cases where electrolyte addition can cause an increase in the aggregation number of the micelles.

However, it is obvious that the increase in the solubility of phenanthrene in Tergitol 15-S-7 solutions resulted from addition of NaCl might exceed what one would expect merely from the slim increase of aggregation number and micellar size. One of the conjectures is that the adsorption of the cations, Na^+ in this study, to the Tergitol 15-S-7 molecules, especially at the ether oxygen in the ethylene oxide groups, changes the conformation through the coordination of 6 ether oxygen atoms to the sodium cation in a tetragonal-pyramidal manner and, thus, form some ordered structure like microcages which would interact preferably with the planar molecules like phenanthrene. This effect of coordination has been found in some other systems containing polymers having longer chains of ethylene oxide monomers (Rester et al., 2000; Zhu et al., 2001). This competition between Na^+ and water molecules to the ether oxygen atoms is indeed manifested as well in the lowering of cloud point and the increase in aggregation number of the micelles. As a result, the phenanthrene molecules can be solubilized in the more shallow position of the palisade layer near the hydrophilic portion of micelles and the micelle-water interface. That is, the effective volume increases significantly even though the micellar size changes very slightly.

Another possible reason is the desorption/hydration of sodium ions around the EO group of the micelle. The hydration of sodium ions leads to the reduced hydration of the hydrophilic group due to the reduced water thermodynamic activity. This gives a smaller head group of the surfactant molecules in the micelle (Attard et al., 2000). As the EO size reduces, the micelle aggregation number increases and the cloud point is reduced.

4.2.3.5 Synergistic Solubilization

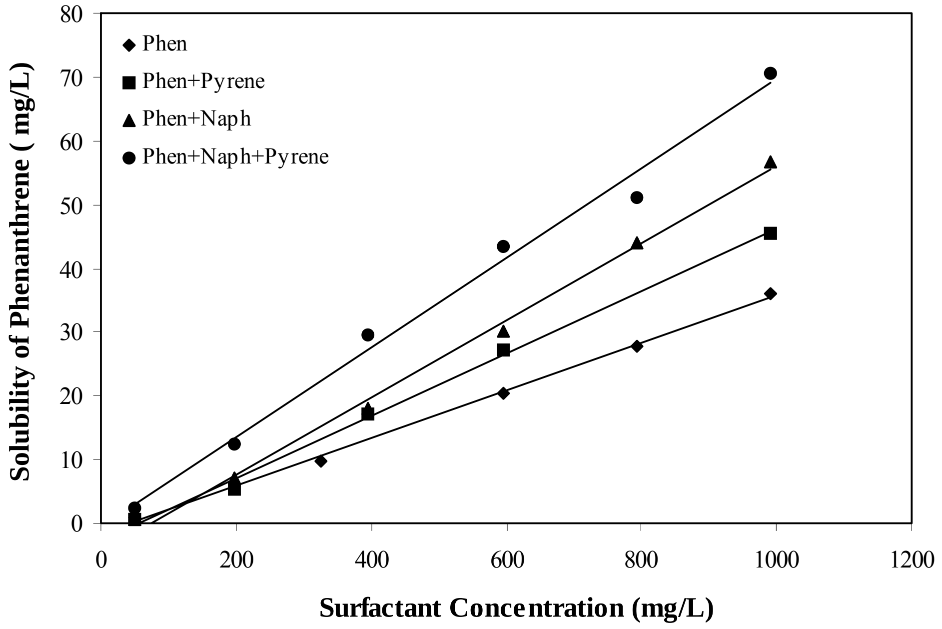


Figure 4.10 Solubilization of Phenanthrene in the Presence of Naphthalene and Pyrene. (Naph: naphthalene; Phen: phenanthrene)

Synergistic effect was observed in Figure 4.10 when phenanthrene was solubilized by Tergitol 15-S-7 at 22 °C in the presence of other two PAHs, naphthalene and pyrene. The amounts of all the three PAHs are far excess their saturation solubility in the micellar solutions. It is clearly shown that the presence of naphthalene and pyrene enhanced the solubilization of phenanthrene by this surfactant. Naphthalene has a greater effect than pyrene and in the ternary mixture phenanthrene shows the greatest solubility. The mass solubilization ratios of phenanthrene are 0.038, 0.049, 0.060 and 0.070 respectively for systems with phenanthrene, binary mixture of phenanthrene and pyrene, binary mixture of phenanthrene and naphthalene, and the ternary mixture.

Similar results were reported by Guha et al. (1998a) who used Triton X-100 as surfactant. The phenomena was attributed to the characteristics of PAH solubilization. PAH can be solubilized not only in the hydrophobic micellar core, but also at the core-water interface, the result is reduced surface tension and larger core volume. The increase in micelle volume is evidenced by the decrease in cloud point of surfactant solutions. Normally, in a micellar solution, increase in micellar size can result in lower cloud point of the solution. The cloud points of the surfactant solution at 1 wt% saturated with single phenanthrene, binary mixture of phenanthrene and pyrene, binary mixture of phenanthrene and naphthalene, and ternary mixture of these three PAHs are 35.4, 35.2, 34.2 and 33.8 °C respectively. Theoretically, the smaller the PAH molecule, the greater its tendency to be solubilized at the interface because of its higher polarity. Therefore, compared with pyrene, naphthalene has a greater effect to lower the core-water interfacial tension and leads to larger increase in the effective volume for phenanthrene solubilization. The difference is also reflected in their effects on the cloud point. In the presence of pyrene the cloud point is 35.2 ^{o}C, which is higher than 34.2 ^{o}C, the value in the presence of naphthalene. It is worthy of mentioning that the presence of pyrene has another effect of competing with phenanthrene for the hydrophobic interior core, which will lead to reduced solubilization of phenanthrene. The overall effect of the presence of pyrene is a compromise between the two effects. Finally, in the ternary system, phenanthrene has the greatest solubility. This is because the presence of both pyrene and naphthalene has a greater effect to lower the interfacial tension than individual compound, which results in the lowest cloud point, 33.8 ^{o}C .

The solubilization of pyrene can also be enhanced by the presence of naphthalene and phenanthrene. The results are given in Figure 4.11.

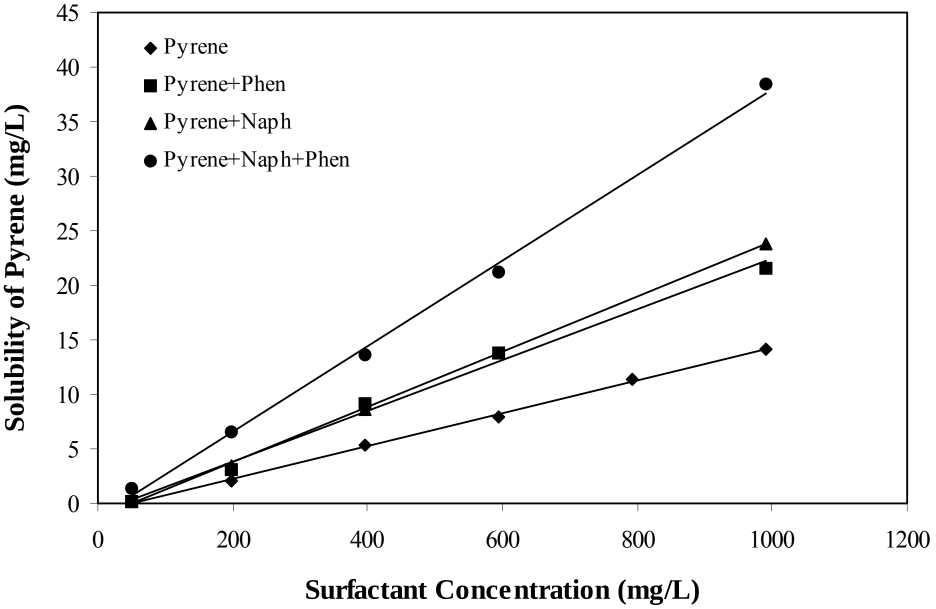


Figure 4.11 Solubilization of Pyrene in the Presence of Naphthalene and Phenanthrene.

At the same surfactant concentration, the greatest solubility of pyrene was also achieved in the solutions solubilized with these three PAHs. Finally, it was also observed in the experiments that the solubility of naphthalene was reduced in the presence of phenanthrene and pyrene. The reduced solubility of naphthalene is due to the competition of phenanthrene and pyrene for the solubilization site in the micellar core, because these two PAHs are more hydrophobic than naphthalene, and hence have greater affinity to the hydrophobic micellar core.

4.3 Conclusions

Based on the experimental results, the following conclusions can be drawn:

(1) Tergitol 15-S-X (X= 7, 9 and 12) are good candidates for the solubilization of PAHs, due to their comparable solubilization capacity with traditionally used surfactants and readily biodegradability nature.

(2) For surfactants of the homologous series, HLB number could be used as one criterion in the selection of surfactants for solubilization process. Normally, surfactants with lower HLB number have greater solubilization capacity.

(3) The solubilization of PAHs into the micellar phase is driven by the hydrophobic affinity of the PAHs molecules for the surfactant micelles. The partition of PAH between the micellar and aqueous phase can be predicted by their octanol-water partition coefficients.

(4) Increasing temperature can improve the solubilization capacity of the nonionic surfactants. This is attributable to the increased aggregation number and micelle size.

(5) Increasing salinity can also increase the solubilization capacity. This is due to the increased aggregation number and micelle size. Conformation change might also be one contribution.

Chapter 5 Aqueous Phase Behavior of Nonionic Surfactants

5.1 Introduction

The aqueous phase behavior of surfactants is the basis for the selection of suitable surfactants for a certain process. For the extraction process, the phase separation temperature is a primary criterion. The separation is desired to occur at ambient temperature so that a simple extraction process can be established. Hence, it is imperative to acquire knowledge of the phase-separation characteristics of a suitable surfactant before it is to be applied in an extraction process.

In this chapter, the phase behaviors in aqueous solutions, especially the phase separation of two surfactants, namely Tergitol 15-S-5 and Tergitol 15-S-7 were studied. The phase separation temperatures of Tergitol 15-S-7 (cloud point) and Tergitol 15-S-5 were firstly measured in deionized water as a function of their concentrations. The phase changes in the Tergitol 15-S-5 were observed with the aid of cross polarizers and an optical microscope, as well as with visual observations. The phase separation temperatures of this surfactant at concentration of 2 wt% in the presence of 1 to 5 M sodium chloride were measured. The cloud point of Tergitol 15-S-7 was observed visually. The effects of some additives including inorganic salts, ionic and nonionic surfactants, as well as alcohols on the cloud point of 1 wt% Tergitol 15-S-7 solution were investigated. Information about the effects of some inorganic salts and pentanol on the cloud point of Tergitol 15-S-9 was also otained. Based on the results obtained in this chapter, suitable salts and salt concentrations were selected to facilitate the extraction and preconcentration of model PAHs in the next chapter.

5.2 Results and Discussion

5.2.1 Aqueous Phase Behavior of Tergitol 15-S-5

As a slightly hydrophobic surfactant, the solutions of the nonionic surfactant Tergitol 15-S-5 usually form a lamellar phase, in contrast to the micellar phase shown in the solutions containing the hydrophilic surfactants. In order to predict the extraction performance, a series of investigations on the phase behavior is, thus, in need. The phase change of the 2 wt% Tergitol 15-S-5 with respect to the temperature was observed visually with the crossed polarizers or by the optical microscopy with the aid of the crossed polarizers. Its phase behavior is quite complicated.

At the temperature between 18.5 and 20.5 °C, the sample containing 2 wt% Tergitol 15-S-5 only appears as a homogeneous and milky solution, which also exhibits strong birefringence under the crossed polarizers. Coincidently, islands of birefringence were observed as well on a very tiny drop of such solution by the optical microscope with the crossed polarizers. That is, the 2 wt% surfactant solution exists as a dispersion of the lamellar phases (L_α). A microscopy of the lamellar liquid crystalline phase of Tergitol 15-S-5 in deionized water is given in Figure 5.1. Similar textures of lamellar crystalline phase of polyoxyethylene surfactant have been reported (Walsh and Tiddy, 2003).

Increasing the temperature further, the entire solution separates into two distinct isotropic phases at about 26.3 °C. Compared with the well-known phase sequence of the linear ethoxylated nonionic surfactants, these two distinct phases are the excess water W phase and the bicontinuous L_3 sponge phase, respectively, in which the streaming birefringence could be frequently seen under shear. The former is sometimes referred as the surfactant-lean phase, while the latter is called as the surfactant-rich phase. This is also consistent with the previous observations by Tungsubutra and Miller (1994). It is noted that the surfactant concentration in the *W* phase is approximately equal to its critical micelle concentration. Consequently, the hydrophobic extractants, if present in the solution, will go to the L_3 phase, as most of the surfactant moiety will exist in that phase. Preconcentration and extraction takes place, accordingly.

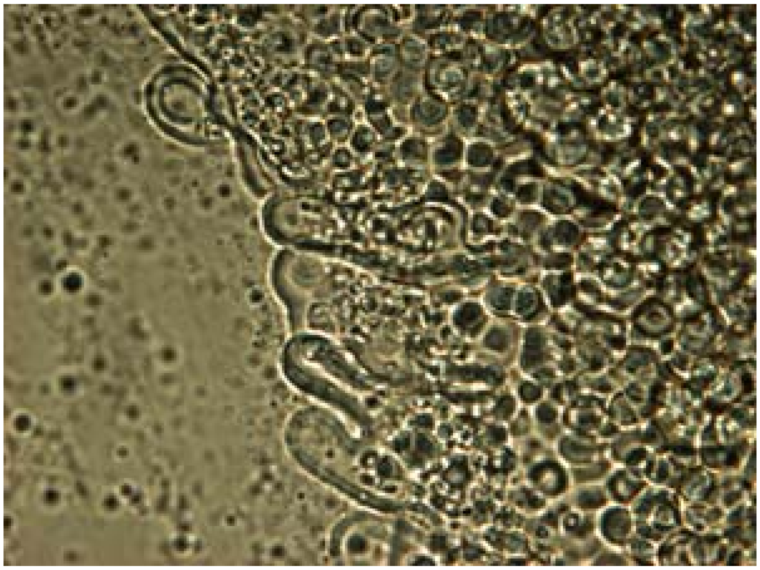


Figure 5.1 Texture of Lamellar Crystalline Phase of Tergitol 15-S-5 in Deionized water at 20 ^{o}C. x500

5.2.1.1 Phase Separation Temperature at Different Surfactant Concentrations

To acquire more information about the phase separation of this surfactant, the phase separation temperature of this surfactant at concentrations between 1wt% and 10 wt% is given in Figure 5.2. Above the curve, the two phases, L_3 and water phase coexist in the solutions. The phases below the curve are not labled. It was observed that the phase behavior is very complex. The intermediate phases in the transition from the W+La phases to the W +L_3 phases was not identified, since the detailed phase diagram is not the main theme of this work.. The figure below shows that the phase separation temperature of Tergitol 15-S-5 increases with surfactant concentration above 1 wt%. In 1 wt% surfactant solution, the temperature is 23.5 °C. It increases to 33.0 °C when the surfactant concentration increases to 10 wt%. This is due to that, at higher surfactant concentrations, more structured water-surfactant systems are present. Therefore, higher temperatures are required to remove the water molecules, which are barriers for the interactions between surfactant aggregates (Koshy et al., 1996). The reproducibility of the temperatures acquired in this chapter is very high. The errors are normally within ±0.2°C.

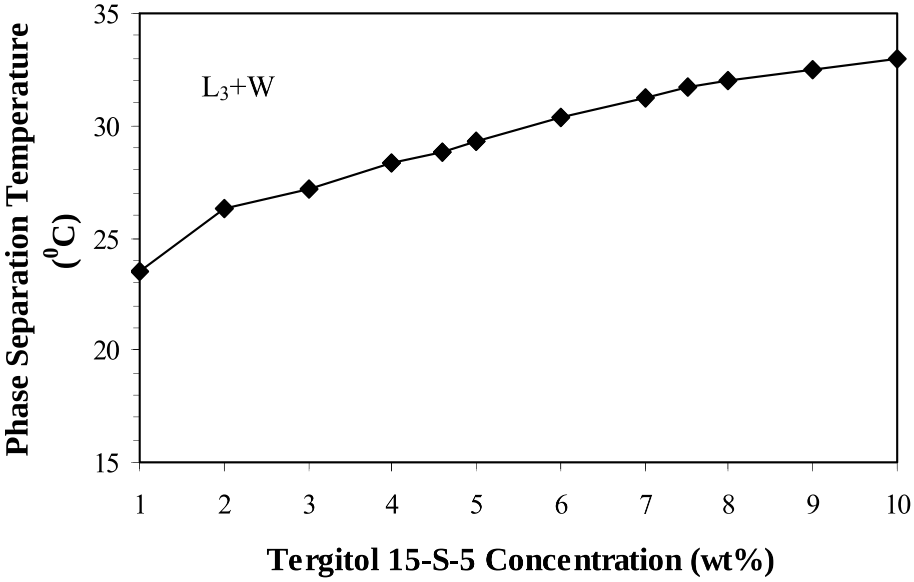


Figure 5.2 Phase Separation Temperature of Tergitol 15-S-5 at Different Concentrations.

5.2.1.2 Effect of Sodium Chloride on Phase Separation Temperature

Addition of sodium chloride leads to a significant drop in the phase-separation temperature (Figure 5.3). The phase separation temperature of this surfactant decreases almost linearly with the sodium chloride concentration. At a salt concentration of 4M, phase separation occurs below 2 °C. It is desired to bring down the phase separation temperature below that of the surroundings, so that the analysis could be facilitated at the ambient conditions.

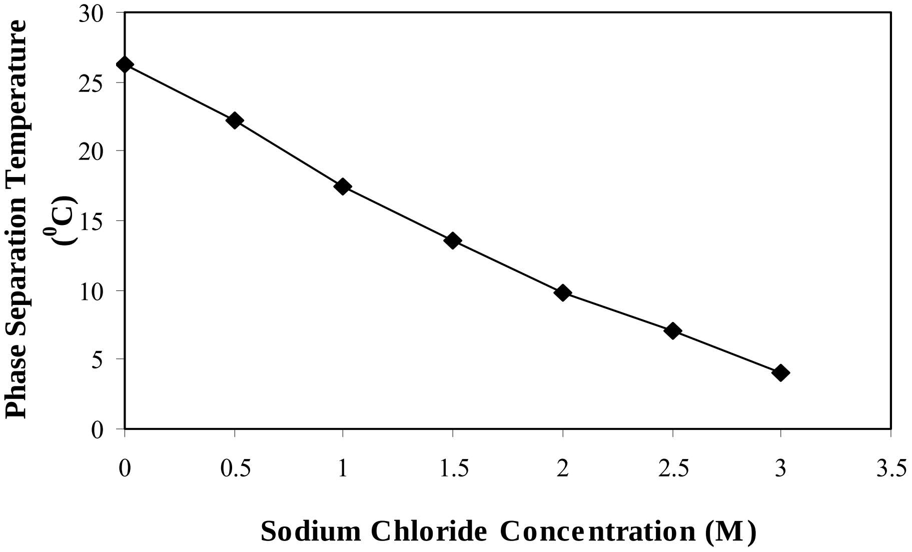


Figure 5.3 Effect of Sodium Chloride on the Phase Separation Temperature of 2wt% Tergitol 15-S-5.

The presence of ions such as chloride ions could dehydrate the surfactant aggregates by competing with the EO group for water to form hydrated ions. Ions with this "salting-out" effect lowers the surfactant solubility. Increasing salt concentration to a certain level, the surfactant molecules separate out of the solution. Moreover, addition of NaCl will enlarge the density difference between the L_3 and *W* phases so as to ease the phase separation and sample handling.

5.2.2 Aqueous Phase Behavior of Tergitol 15-S-7

Compared with Tergitol 15-S-5, the phase behavior of Tergitol 15-S-7 is relatively simple. This is due to the longer polyoxyethylene chain of its molecule, which contributes to its better miscibility with water.

5.2.2.1 Effect of Surfactant Concentration

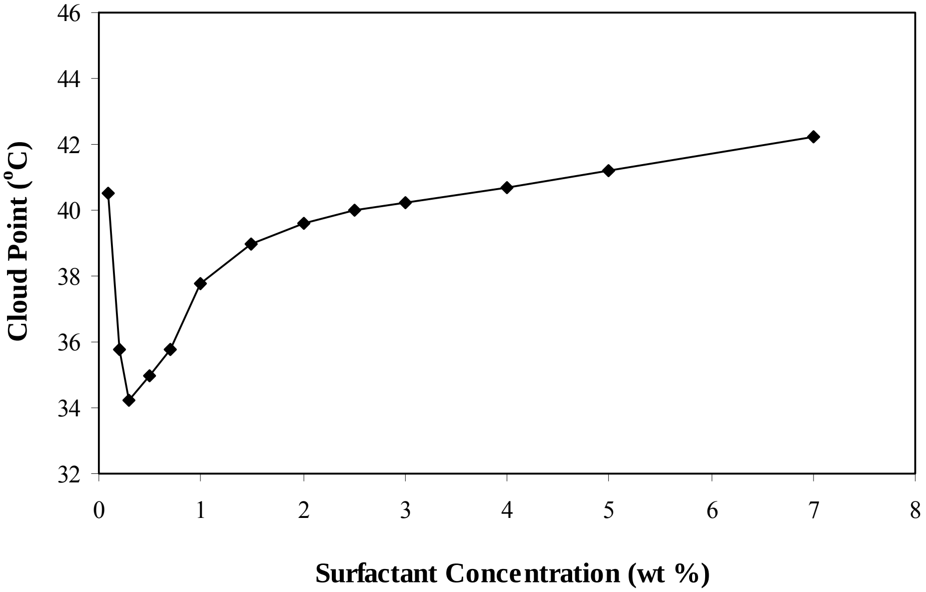


Figure 5.4 Cloud Point of Tergitol 15-S-7 as a function of surfactant concentration.

The cloud point of Tergitol 15-S-7 was measured as a function of its concentration. The curve is given in Figure 5.4. The cloud point of Tergitol 15-S-7 increases monotonically with its own concentration above 0.3 wt%. A lower consolute phase boundary was also obtained for this surfactant. A minimum of cloud point exists at 0.3 wt%. As has been mentioned, the phase separation in nonionic surfactant solutions is attributable to the absence of long-range electrostatic interactions between the aggregates and the decreasing hydration of the non-ionic hydrophilic groups with increasing temperature. The phase separation is attributable to the absence of long-range electrostatic interactions between the aggregates and the decreasing hydration of the non-ionic hydrophilic groups with increasing temperature. The decrease in cloud point at surfactant concentrations less than 0.3 wt% is attributable to the increase in micelle concentration. With increase in surfactant concentration, the interaction becomes stronger, and hence, the cloud point is decreased. After reaching a minimum value, further increase in surfactant concentration leads to more structured water-surfactant system and dehydration can not occur as easily as in dilute micellar solutions. As a result, the cloud point is increased. The minimum point is the net effect of the increased inter-micellar interaction which favors clouding, and the increasing difficulties in dehydration that unfavors phase separation, with increase in surfactant concentration. It was also observed that when the surfactant concentration was reduced to below 0.1 wt% (1000 mg/L), though the surfactant concentrations were still far above its CMC, clouding was hard to happen even at very high temperatures. Similar trend of change in cloud point temperature with surfactant concentration has been reported on some other surfactants (Sadaghiania and Khan, 1991).

5.2.2.2 Effect of Inorganic Salts

The cloud points of 1 wt% Tergitol 15-S-7 in the presence of a few inorganic salts were measured and the results are shown in Figure 5.5. The figure indicates that the addition of inorganic electrolytes can either decrease or increase the cloud points of Tergitol 15-S-7. The change is almost linearly proportional to the concentrations of the electrolytes in the solution. The inorganic salts, except NaI, have a depressing effect on the cloud point of this surfactant. Na_3PO_4 has the most dramatic effect, which is followed by Na_2SO_4. Addition of small amount of these two salts can decrease the cloud points of Tergitol 15-S-7 to ambient temperature. Compared to Na_3PO_4 and Na_2SO_4, NaCl and $CaCl_2$ are much less effective

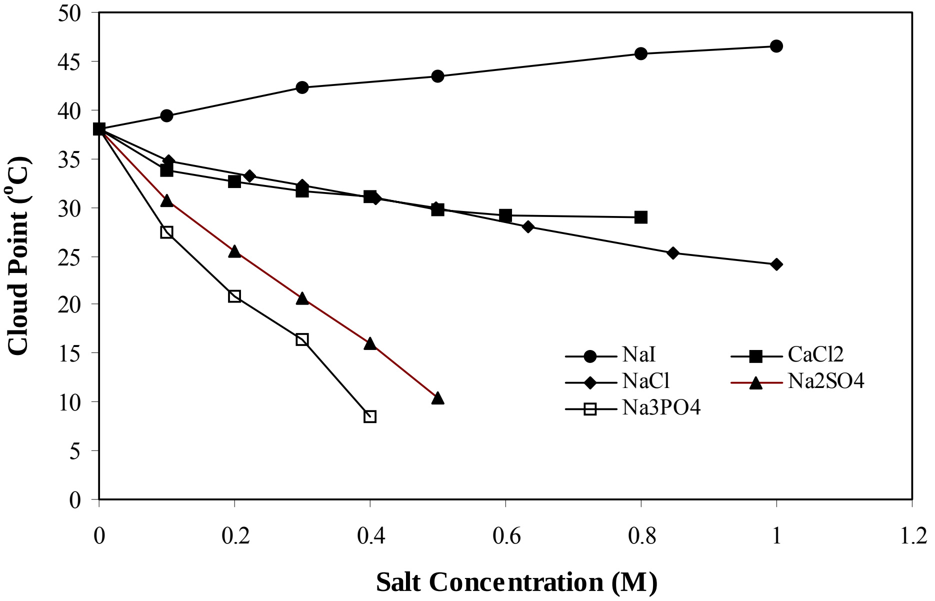


Figure 5.5 Effect of Inorganic Salts on Cloud Point of 1 wt%Tergitol 15-S-7.

Among the five salts examined, four of them have the same cation, Na^+, but their cloud point depressing capacity is quite different from one another. This difference is attributed to their different anions. Some ions, e. g., OH^-, F^-, Cl^-, SO_4^{2-} and PO_4^{3-} with lyotropic numbers less than 8 can produce large increases in the viscosity and surface tension of water. They have high electronegativity and generate strong electrostatic fields that not only polarize, immobilize, and electrostrict the adjacent water molecules but also induce additional order beyond the first water layer. The result is reduced solvent property and thermodynamic activity of water, as a result of which, the surfactant is dehydrated. The presence of these ions "salts out" the surfactant. The magnitude of effects of the anion and cataion are dependent on the radius of the hydrated ion, that is, the lytropic number; the smaller the radius, the greater the effect. Therefore, the order of the effectiveness in decreasing the cloud point is 1/3 PO_4^{3-}> 1/2 SO_4^{2-} > F^- >Cl^-, and $NH4^+$ > K^+ > Na^+ > Li^+ > 1/2 Ca^{2+}. It has been reported that I^- and SCN^- increase the cloud point because their presence ehances the hydration of the EO group (Schott et al., 1984). The anions of this type are generally monovalent and relatively large in size, with lyotropic numbers greater than 11 and most of them are lewis bases with low electronegativity and high polarizability. Compared to the "salting-out" ions, these ions have a "salting-in" effect on surfactant molecules. It is worth mentioning that the effects of cations are relatively small in comparison with those of anions, especially with the large, polyatomic ones.

Rapid micellar growth and the large increase in aggregation number have also been reported as a contributor to the clouding phenomenon. Increase in micellar size and aggregation number of this surfactant in the presence of sodium chloride has been reported in Chapter 4. Dramatic increase in the aggregation number was also observed in the presence of sodium sulfate. The measured aggregation numbers in the presence of 0.1 and 0.2 M sodium sulfate are 711 and 2660, compared with 414 in deionized water. The micellar growth in the presence of the slats could be attributed to the dehydration of surfactant micelle, which leads to smaller headgroup and hence the micellar growth.

Based on the cloud point investigation, we can conclude that Na_3PO_4 and Na_2SO_4 are efficient cloud point depressors. A small amount of them can make it possible for the surfactant to cloud at ambient temperature, which not only saves much heating energy, but also simplifies the cloud point extraction process. Na_2SO_4 will be used in the cloud point extraction when Tergitol 15-S-7 is used as extractant in Chapter 6. Its neutral nature may extend its use to a wide range of compounds, especially polar compounds. The extraction efficiency of this type of compounds can be affected by the pH value of the solution. The extraction efficiency of Tergitol 15-S-7 for a model PAH, phenanthrene in the presence of Na_3PO_4 has been reported elsewhere (Li and Chen, 2002b).

To get more information about the effect of salt, the cloud point of Tergitol 15-S-9 in the presence of sodium sulfate and sodium phosphate was also measured and the results are shown in Figure 5.6. The figure indicates that the two salts also have great effects on the cloud point of this surfactant. With the addition of 0.6 M sodium sulfate or 0.4 M sodium phosphate, the cloud point of 1 wt% Tergitol 15-S-9 can be reduced to ambient temperature (about 22 °C). However, to achieve effective preconcentration, small volume of the surfactant-rich phase is preferred. Correspondingly, the concentration of sodium sulfate and sodium phosphate should be at least 0.7 and 0.5 M respectively.

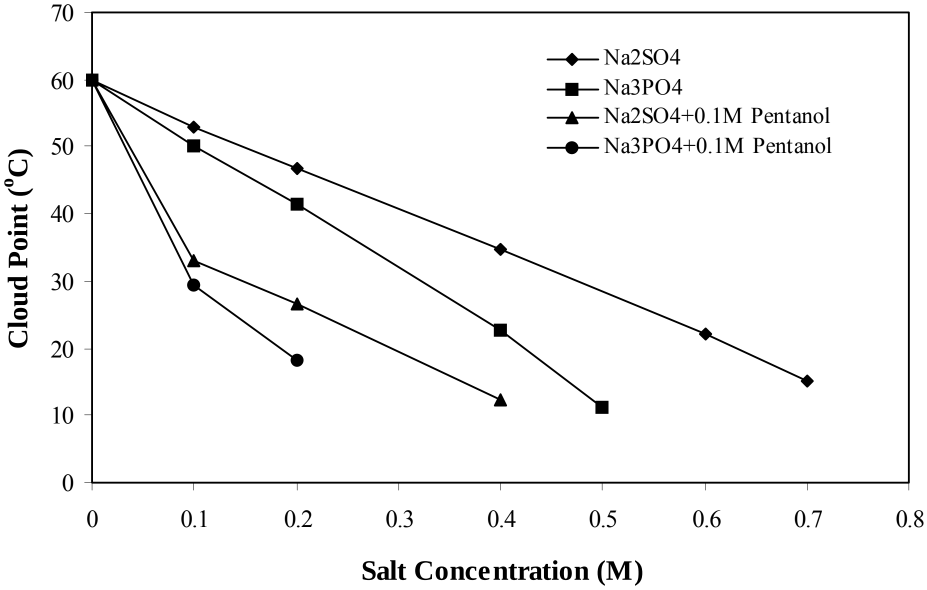


Figure 5.6 Effect of Inorganic Salts and Pentanol on Cloud Point of 1 wt%Tergitol 15-S-9.

The extraction by Tergitol 15-S-9 was not carried out in this study. It is worth mentioning that the value of investigation into the effects of inorganic salts is not only in providing information for the CPE applications. The reduction in the cloud-point temperature owing to the presence of electrolytes is sometimes utilized to increase the low-temperature surface activity of surfactants. It is also useful when an application requires a surfactant to be above its cloud point such as in some textile scouring operations.

5.2.2.3 Effect of Ionic Surfactants

The cloud points of Tergitol 15-S-7 at concentration of 1 wt% in the presence of an ionic surfactant, sodium dodecyl sulphate (SDS) were measured (Figure 5.7).

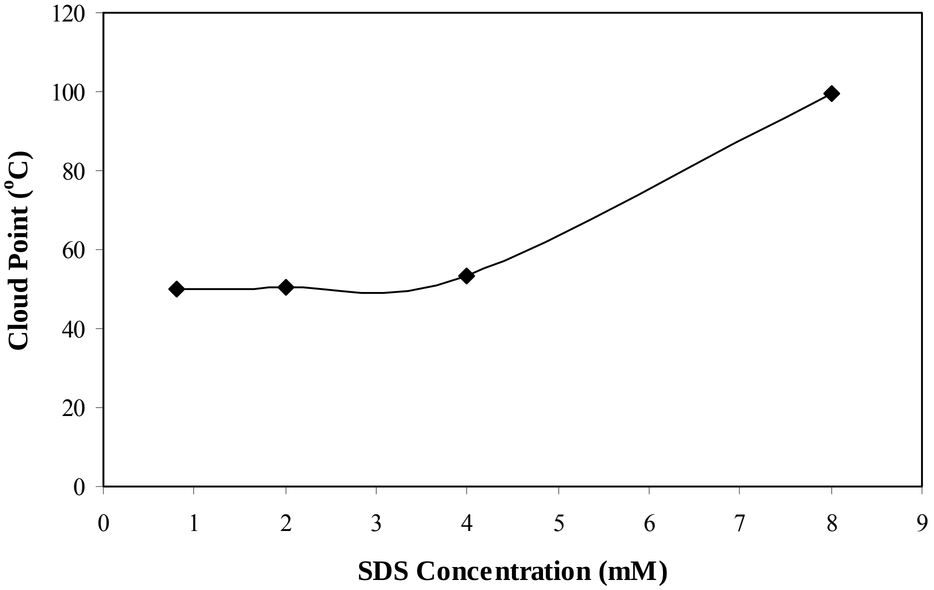


Figure 5.7 Cloud Point of 1 wt% Tergitol 15-S-7 in the Presence of SDS.

The results show that the presence of SDS increased the cloud point of Tergitol 15-S-7. The charged SDS molecules can be adsorbed on the nonionic surfactant micelles or form mixed micelles with nonionic surfactant molecules. This results in increased electrostatic repulsion between nonionic micelles, thus making it more difficult for the micelles to aggregate together, which leads to increase in the cloud point. This effect becomes more dramatic when the concentration of the ionic surfactants approaches its CMC (8 mM for SDS).

5.2.2.4 Effect of Nonionic Surfactants

The cloud points of Tergitol 15-S-7 mixtures with two other nonionic surfactants, Tergitol 15-S-9 and Neodol 25-7 were measured. The results are given in Figure 5.8. The total surfactant concentration in surfactant mixtures is fixed at 1 wt%. It was observed that the cloud point of a binary surfactant solution falls between the cloud points of the two constituent surfactants. The measured cloud points of Tergitol 15-5-7, Tergitol 15-S-9 and Neodol 25-7 at concentration of 1 wt% are 37.8, 62.5 and 46.2 °C respectively. It indicates that mixed nonionic surfactants might be used to achieve desired cloud point. Another advantage of using mixed surfactants is that the performance can be adjusted by simply changing its composition.

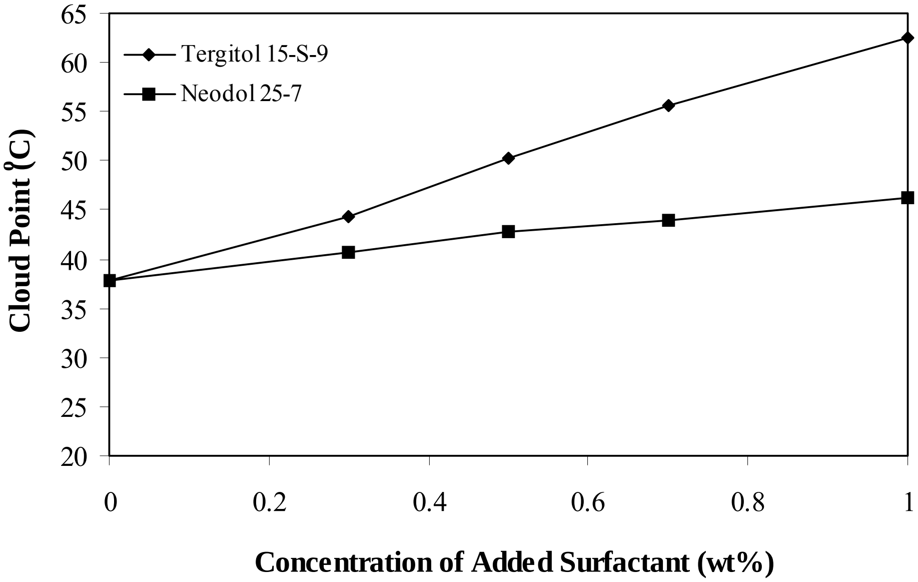


Figure 5.8 Cloud Point of Binary Surfactant Mixtures of Tergitol 15-S-7/Tergitol 15-S-9 or Tergitol 15-S-7/Neodol 25-7(Total surfactant concentration: 1 wt%).

5.2.2.5 Effect of Alcohols

The effects of a few alcohols on the cloud points of 1 wt% Tergitol 15-S-7 solutions are shown in Figure 5.9. As is indicated in the figure, addition of short-chained alcohols including methanol, ethanol and propanol increases the cloud point. On the contrary, the presence of long-chain alcohols, *n*-butanol and *n*-pentanol, has the effect of decreasing the cloud point. Similar results were reported by Gu and Galera-Gómez (1999), who examined the effect of alcohols on the cloud point of the nonionic surfactant Triton X-100. The results also agree with those reported by other authors who studied the phase behaviors of nonionic surfactant/water/alcohol systems (Kahlweit et al., 1991, 1995).

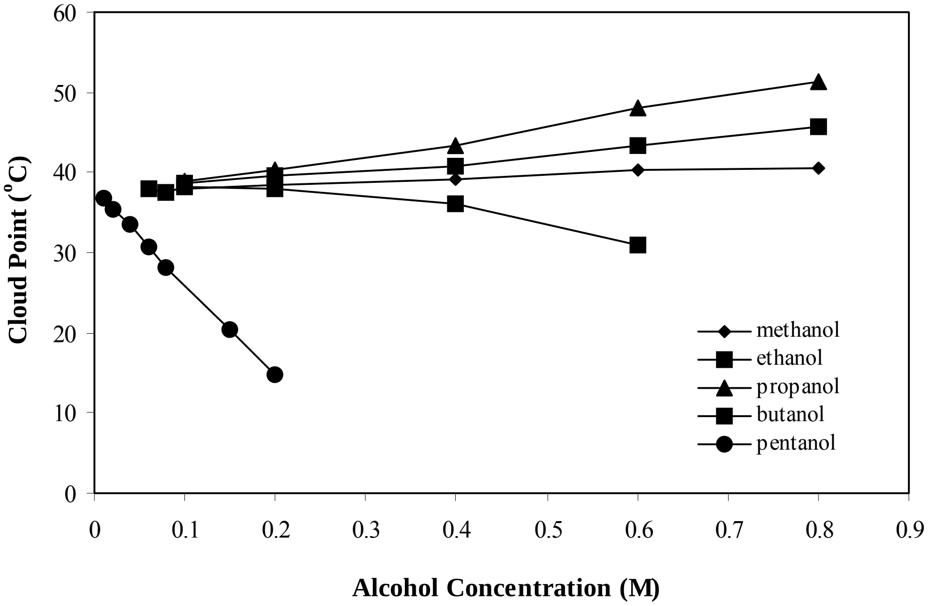


Figure 5.9 Effects of Alcohols on the Cloud Point of 1 wt% Tergitol 15-S-7.

Short-chain alcohols are miscible with water. The presence of such alcohols can modify the solvent, water, leading to the formation of a less polar medium. This factor combining with the adsorption of these alcohols at the micelle-water interface originates a restriction to the micellization of surfactant molecules, which leads to an increase in cloud point (Gu and Galera-Gómez, 1999). It was observed that, replacing water with 1-propanol or propylene glycol, the amphiphilicity of the surfactant, octaethylene glycol dodecyl ether ($C_{12}E_8$), decreases. Eventually the surfactant does not from micelles in the alcohol-dominant solvent (Aramaki et al., 1999). On the contrary, alcohols with longer chain length are partially soluble in water. Their molecules may be solubilized in the micelles and adsorbed at the micelle-water interface leading to the growth of micelles and the depression in cloud point (Gu and Galera-Gómez, 1999). Hydration of $C_{12}E_8$ molecules and hence increase in the cloud point was observed when water was replaced by glycerol. The "salting-out" effect by glycerol is similar to that by inorganic salts. In particular, it was suggested that multi-connected micelles was formed at high glycerol and surfactant concentration (Aramaki et al., 1999).

The significance of this observation is that combinations of suitable additives, such as pentanol and inorganic salts might be used to enhance cloud point separation of surfactants with higher cloud points, such as Tergitol 15-S-9. It was observed that, for this surfactant, addition of inorganic salts alone could not achieve effective phase separation. In a cloud point extraction process, the cloud point of a solution should be much lower than the experimental temperature. Otherwise, the volume of the surfactant-rich phase will be too large to achieve the efficient preconcentration. Unfortunately, at very high salt concentrations, unexpected viscous phases usually form in the surfactant-rich phase. This makes the sampling more difficult. The cloud point of 1 wt% Tergitol 15-S-9 in the presence of 0.1 M pentanol, sodium sulfate and sodium phosphate are given in Figure 5.6. The figure shows that the addition of 0.1 M pentanol with these two salts can reduce the cloud point of Tergitol 15-S-9 significantly. In the presence of pentanol, the cloud point is 12.4 °C at sodium sulfate concentration of 0.4 M, compared to 34.8 °C at the same sodium sulfate concentration but without addition of any *n*-pentanol. At 0.1 M *n*-pentanol and 0.6 M sodium sulfate, the solution remains clouding when the temperature is constantly reduced to below 2 °C. That is, the cloud point is lower than 2 °C. It was also observed that in the presence of 0.1 M pentanol, the cloudy solutions at Na_3PO_4 concentration of 0.4 and 0.5 M turned into solid when the temperature was reduced to about 8 and 4 °C respectively. This means that structured phase might form in Tergitol 15-S-9 solutions at lower temperatures in the presence of both pentanol and sodium phosphate.

5.3 Conclusions

The following conclusions can be made based on the observations in this chapter.

(1) The phase separation temperature of Tergitol 15-S-5 increase monotonically with surfactant concentration above 1 wt%. A minimum cloud point of Tergitol 15-S-7 exists at surfactant concentration of 0.3 wt%. Above this concentration, the cloud point increases.

(2) The presence of inorganic salts can either increase or decrease the cloud point of Tergitol 15-S-7. Sodium sulfate and sodium phosphate are very effective in lowering the cloud point of this surfactant and Tergitol 15-S-9. With the addition of these two salts, clouding can be induced at room temperature. The phase separation of Tergitol 15-S-5 can be greatly enhanced with the addition of sodium chloride.

(3) The presence of an ionic surfactant, SDS above a certain concentration increases the cloud point of Tergitol 15-S-7. This might be due to the adsorption of SDS molecules to the Tergitol 15-S-7 micelle, which increased the electrostatic repulsion between nonionic micelles.

(4) The cloud points of binary mixtures of Tergitol 15-S-7/Tergitol 15-S-9 or Tergitol 15-S-7/Neodol 25-7 are between the cloud points of individual surfactants. Mixtures of surfactants with suitable compositions can be prepared to get desired cloud point.

(5) Alcohols of short chain length elevate the cloud point, while longer chain length alcohols have depressing effect. The presence of both pentanol and sodium sulfate/sodium phosphate is more effective in lowering the cloud point.

(6) Finally in this chapter, the cloud points of Tergitol 15-S-X (X = 7, 9 and 12) could be correlated linearly to the logarithm of their oxide number.

Chapter 6 Extraction of PAH by Nonionic Surfactants

6.1 Introduction

In this chapter, a simple and effective extraction process based on the aqueous phase behavior of two surfactants, Tergitol 15-S-5 and Tergitol 15-S-7 was developed to extract model PAHs. Since they do not render any fluorometric signal, their presence does not disturb the analysis of PAHs. The selection of these two surfactants was also based on the following two facts. The first is the low temperatures for their phase separation. As was observed in Chapter 5, Tergitol 15-S-7 has a low cloud point temperature of around 38°C at 1 wt% concentration and the phase separation can be facilitated with the addition of some additives, such as sodium sulfate. Complete phase separation can also occur easily in Tergitol 15-S-5 solutions with the aid of sodium chloride. Another criterion is the high solubilization capacity of Tergitol 15-S-X surfactants for PAH. The solubilization of PAHs by Tergitol 15-S-7 has been reported in Chapter 4. Tergitol 15-S-5 is more hydrophobic than Tergitol 15-S-7; therefore, theoretically this surfactant should have a greater solubilization capacity.

To facilitate phase separation, Na_2SO_4 and NaCl were introduced to lower the phase separation temperatures of Tergitol 15-S-7 and Tergitol 15-S-5, respectively. With the addition of these two salts, complete phase separation in the aqueous solutions of these two surfactants could occur within a few minutes. The water contents of the surfactant-rich phases were measured as a function of both surfactant and salt concentration. The proficiency of the cloud point extraction process was evaluated. The effects of salt and surfactant concentration on phase volume ratios, preconcentration factors, partition coefficients and recoveries of model PAHs were studied. The effects of temperature on the water content and preconcentration factor with Tergitol 15-S-5 as extractant was also investigated. To have a better understanding on the extraction mechanism, the partition coefficients of selected PAHs were correlated to their octanol-water partition coefficients.

The extraction of phenanthrene from spiked sand sample was also examined with Tergitol 15-S-7 as extractant. The dissolution kinetics of phenanthrene from the sand matrices by micellar solutions was studied first. The effect of surfactant on mass transfer of phenanthrene into aqueous phase was observed by fitting the experimental data to a first-order dissolution model. The effect of surfactant and salt concentration on recovery of the extraction process was observed. The total recoveries of phenanthrene by the process, which combines micellar solubilization and cloud point extraction were also given.

6.2 Results and Discussion

6.2.1 Extraction by Tergitol 15-S-7

6.2.1.1 Effect of Sodium Sulfate on Phase Separation Temperature

Since Na_2SO_4 will be used as the cloud point depressor in the following extraction experiments, it is important to first obtain some information about the phase behavior of Tergitol 15-S-7 solutions in the presence of this salt. The cloud points of Tergitol 15-S-7 at various concentrations in the presence and absence of Na_2SO_4 were measured against its concentration. Figure 6.1 gives the coexistence curves of the solutions. The area above each curve is a two-phase region.

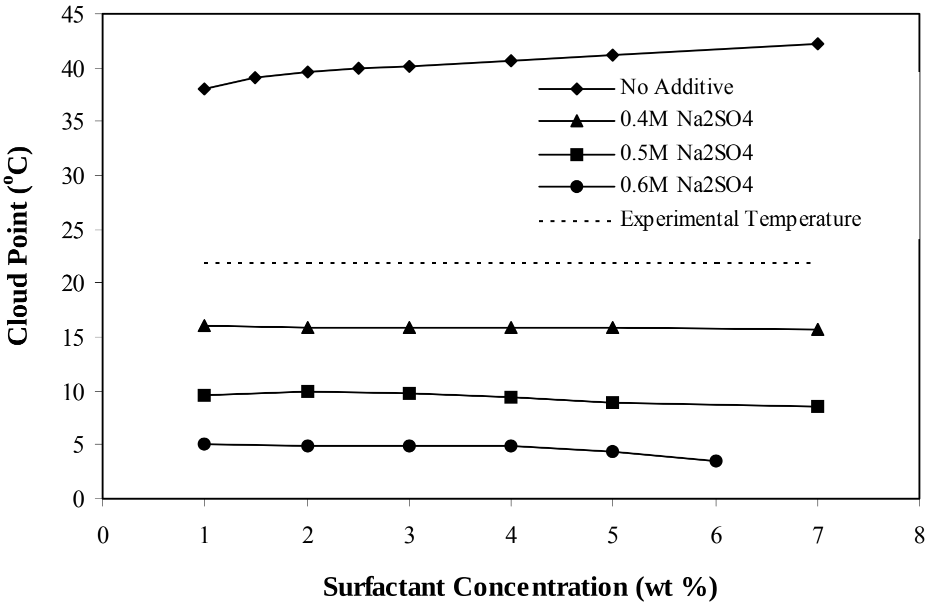


Figure 6.1 Cloud Points of Aqueous Solution of Tergitol 15-S-7 at Different Surfactant and Sodium Sulfate Concentrations.

6.2.1.2 Water Content of Surfactant-rich Phase

The water content of the surfactant-rich phase at different Tergitol 15-S-7 and sodium sulfate concentrations is given in Figure 6.2. The results show that the surfactant-rich phase of Tergitol 15-S-7 contains a large fraction of water. At the same salt concentration, water content is almost independent of surfactant concentration. In contrast, the salt concentration has a profound effect on it. For example, at sodium sulfate concentration of 0.4 M, the water content in the surfactant-rich phase of 1 wt% Tergitol 15-S-7 solution is around 74 wt%. It is diminished to about 57 wt% when the salt concentration is increased to 0.6 M. The reduced water content is attributable to the more dehydrated nature of the surfactant-rich phase at higher salt concentrations. Presence of sodium sulfate can dehydrate the surfactant molecules by enhancing the self-association of water molecules. The higher the salt concentration, the greater is this effect. When most of the free water molecules have associated into aggregated form, further increase in salt concentration will have little effect.

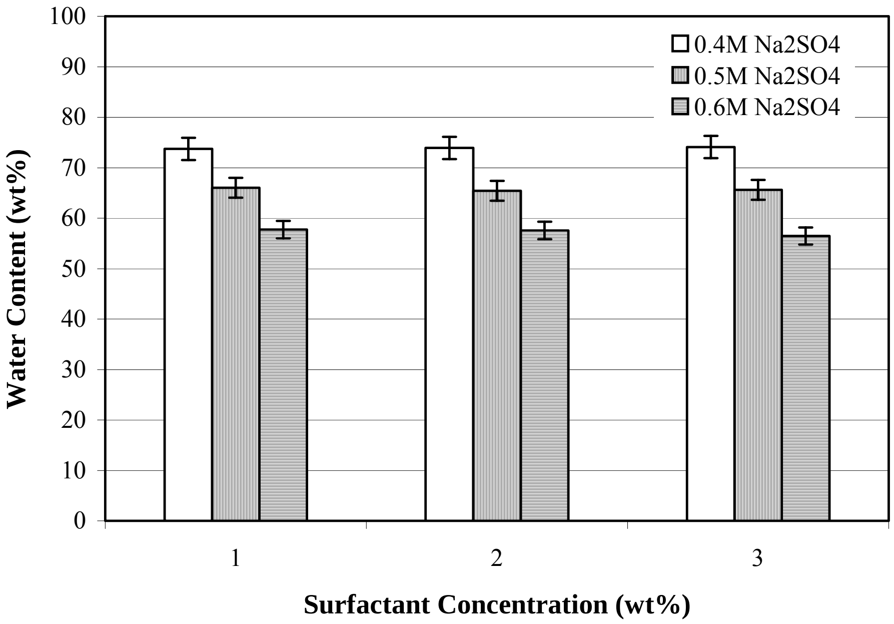


Figure 6.2 Water Content of Tergitol 15-S-7 as a Function of Surfactant and Sodium Sulfate Concentrations.

6.2.1.3 Phase Volume Ratio

The ratios of the volume of the surfactant-rich phase to that of the aqueous phase were measured. The results are given in Figure 6.3. The results show that, at the same surfactant concentration, increasing sodium sulfate concentration decreases the volume ratio and, in another word, decreases the volume of the surfactant-rich phase. This is attributable to the smaller water contents at higher salt concentrations. Lower phase volume ratios were achieved at lower surfactant concentrations due to the smaller amount of surfactant present in the solution. The effects of sodium sulfate concentration on the water content and phase volume ratio can be expected from the tie-line between the coexisting phases described in Fig. 2.2.

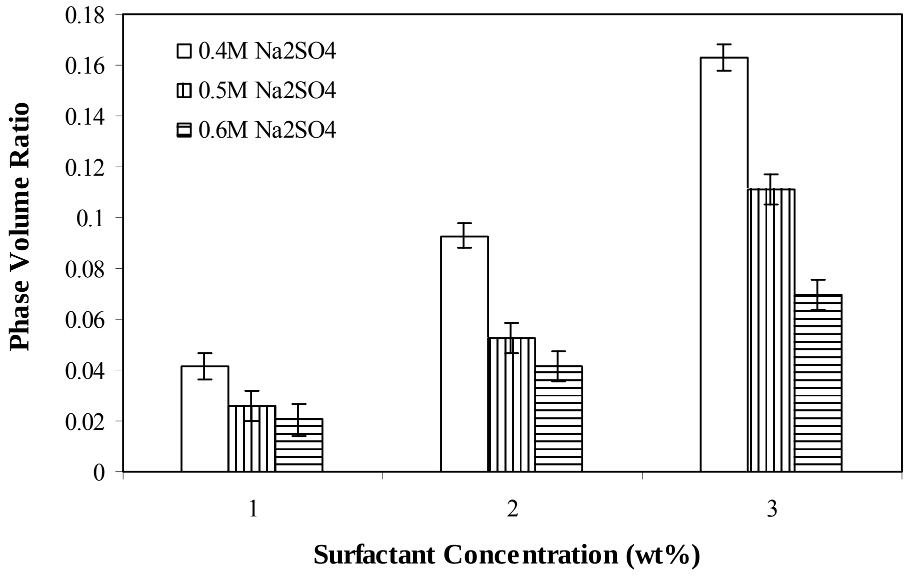


Figure 6.3 Phase Volume Ratio of Tergitol 15-S-7 as a Function of Surfactant and Sodium Sulfate Concentrations.

6.2.1.4 Preconcentration Factor

As aforementioned, the most popular surfactants found in literature, such as Triton X-100 and Triton X-114, cause problems in sample analysis using HPLC equipped with a UV or a fluorescence detector due to their large UV absorbance and fluorometric signals. Indeed, it was observed that Triton X-100 and Triton X-114 could give out detectable signals over a very large range of wavelengths. That is, the excitation wavelength covers from 200 to 260 nm and the emission wavelength from 300 to 400 nm. These ranges coincide with those of typical PAHs. Moreover, the low retention time of these two surfactants contributes to their disturbance to the analysis of low molecular weight PAHs. Figure 6.4 is a chromatogram of four PAHs in the presence of Triton X-100. It is obvious that the signal of the aromatic ring contained in this surfactant overlaps with those of naphthalene and fluorene. On the contrary, Tergitol 15-S-7 does not render any nuisance (Figure 6.5).

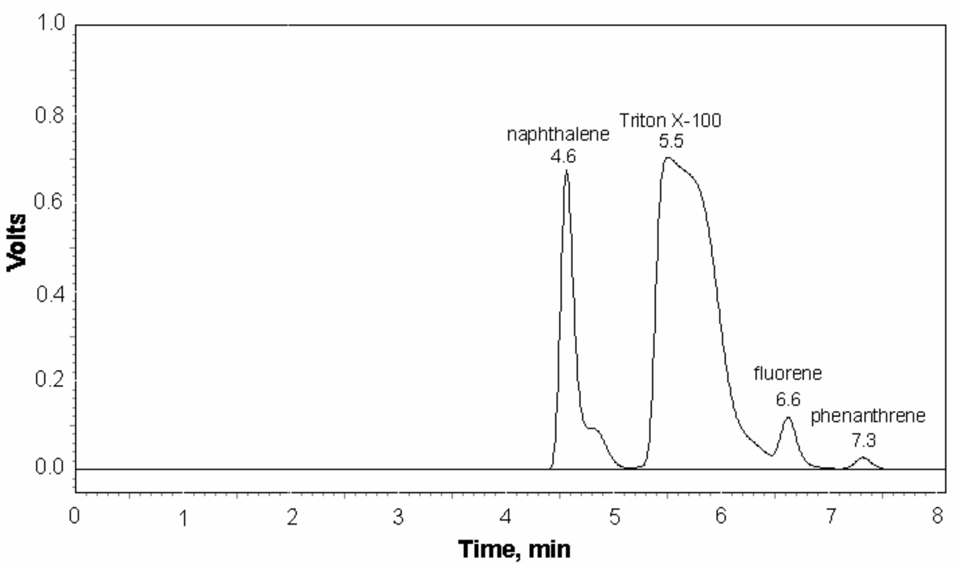


Figure 6.4 A Chromatogram of PAH Mixture Solubilized in Micellar Solution of Triton X-100.

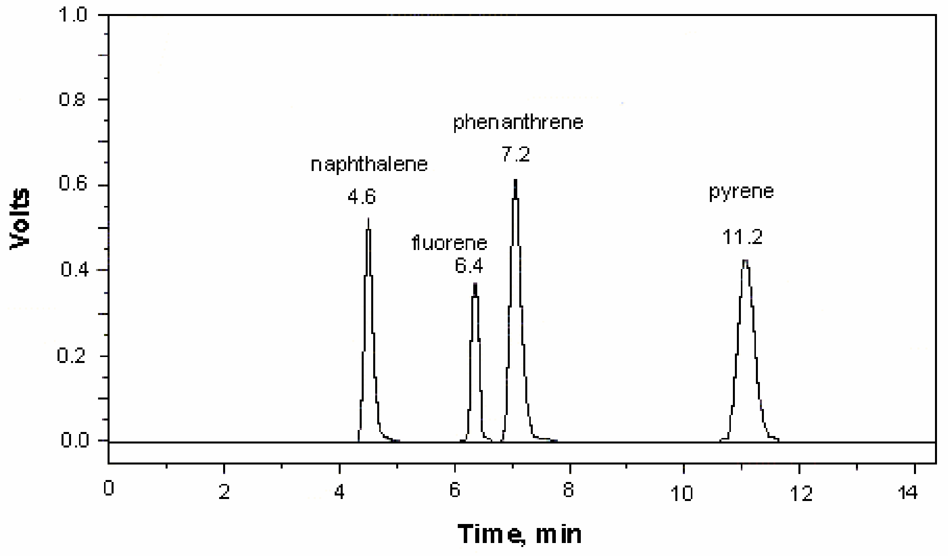


Figure 6.5 A Chromatogram of PAH Mixture Solubilized in Micellar Solution of Tergitol 15-S-7.

The effect of sodium sulfate and surfactant concentration on the preconcentration factor, as is defined in Eq. (3.8), of phenanthrene is shown in Figure 6.6.

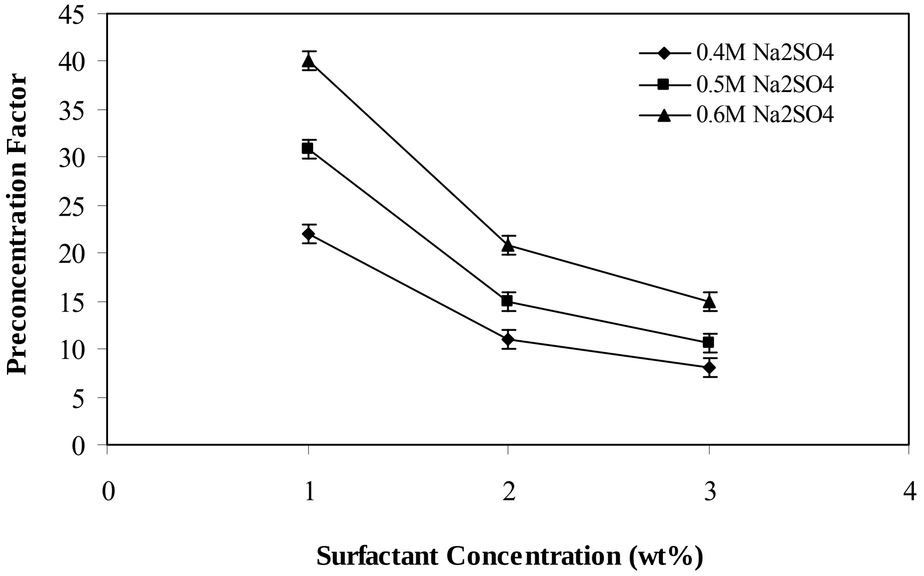


Figure 6.6 Preconcentration Factor of Phenanthrene at Different Concentrations of Tergitol 15-S-7 and Sodium Sulfate.

The results indicate that the higher preconcentration factor can be obtained at the lower surfactant concentrations with more salt added. At the surfactant concentration of 1 wt% with added sodium sulfate at 0.6 M, the preconcentration factor is close to 40. That is, the phenanthrene initially present in the bulk solutions can be concentrated by about 40 times prior to be analyzed. It is especially helpful for the analysis of trace amount of compounds in the aqueous systems. However, the preconcentration factor is found to be independent of the initial concentration of phenanthrene, ranging from 2 to 20 mg/L.

In spite of not being shown here, no significant difference among the preconcentration factors for all the four PAHs is observed in this study regarding their initial concentrations. That is, the preconcentration factor is a combined function of the surfactant concentration and the added amount of Na_2SO_4 only, rather than the initial concentration of the PAHs. It is known that the presence of the surfactant can significantly increase the solubility of the PAHs (Li and Chen, 2002a). For example, the solubility of the phenanthrene in 1 wt% Tergitol 15-S-7 solution at 25°C is reported near 307 ppm, a remarkable increase from *ca.* 1 – 2 ppm for that in the micelle-free aqueous solution at the same temperature.

In this study, the surfactant-rich phases are far undersaturated with the PAHs. That is, the PAHs initially present in the bulk solutions are almost completely recovered into the surfactant-rich phase. Therefore, the mass of the PAHs in the cloud-point extraction process can be described by the following equation:

$$V_t C_0 = V_s C_s + V_w C_w \approx V_s C_s \tag{6.1}$$

where V_t, V_w and V_s denote the volume of the initial bulk solution as well as that of the water phase and surfactant-rich phase after phase separation; C_0, C_w and C_s stand for the initial PAH concentration in the bulk solution, and that in the water phase and surfactant-rich phase. It is of note that, in Eq. (6.1), V_tC_0 can be appropriately approximated by V_sC_s. Though V_s is smaller than V_w, the PAH concentration in the surfactant-rich phase C_s is a few orders of magnitude larger than that in the water phase C_w.

The preconcentration factors, f_c, defined as the ratios of the PAH concentration in the surfactant-rich phase to that in the bulk phase initially, will remain constant in the same surfactant solutions with the same amount of added Na_2SO_4, regardless of the initial PAH concentration. The preconcentration factor could be simplified as the volume ratio of the bulk phase before phase separation to the surfactant-rich phase after phase separation.

$$f_c = C_s / C_0 = V_t / V_s \qquad (6.2)$$

The larger preconcentration factor at the lower surfactant concentration and at higher salt concentration is mainly attributable to the smaller phase volume of the surfactant-rich phase. Interestingly, more Na_2SO_4 added to the surfactant solutions will lead to smaller water contents in the surfactant-rich phase with a smaller phase volume. As a result, a larger preconcentration factor can be achieved.

6.2.1.5 Partition of PAH

The partition of model PAHs between the surfactant-rich phase and the aqueous phase was investigated. The effects of a few factors including surfactant concentration, hydrophobicity of PAH and concentration of sodium sulfate on the partition were studied.

Effect of Surfactant Concentration on Partition. Figure 6.7 displays the equilibrium partition of selected PAHs between the surfactant-rich and water phase after cloud-point separation from the micellar solution initially containing 2 wt% Tergitol 15-S-7 and 0.5 M Na_2SO_4. Since the curves shown in the figure are linear, it implies that the concentrations of the PAHs in the surfactant-rich are still undersaturated. The equilibrium partitions of these PAHs are also examined for those contained in the micellar solutions having 1 and 3 wt% Tergitol 15-S-7 with 0.5 M Na_2SO_4 added. The results are tabulated in the Table 6.1.

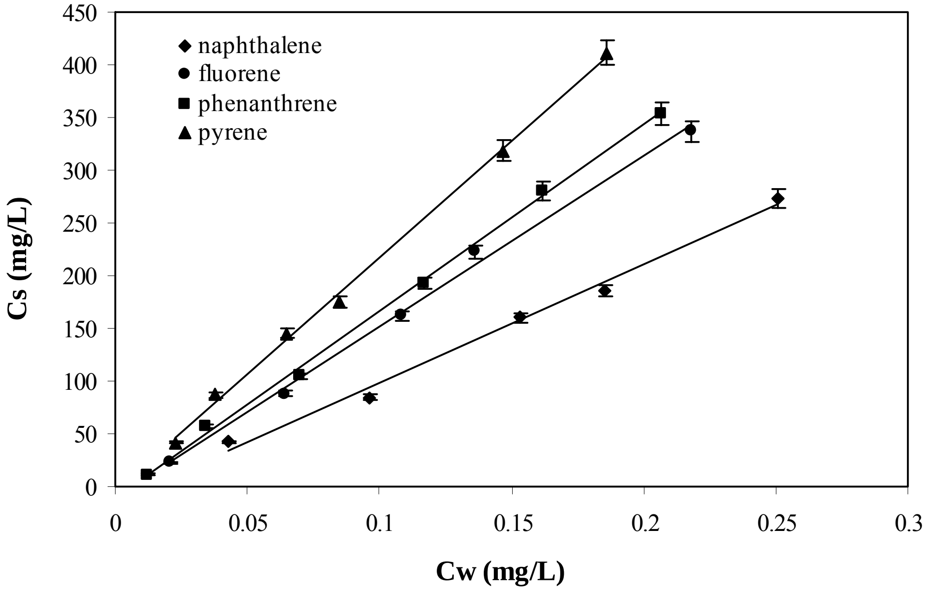


Figure 6.7 Partition of PAH at 2 wt% Tergitol 15-S-7. The cloud-point extraction process was initiated by using 0.5 M Na_2SO_4.

Table 6.1: Effect of Surfactant Concentration on the Equilibrium Partition Coefficients, K_p, of PAHs in the Micellar Solutions with 0.5 M Na_2SO_4 Added.

Tergitol 15-S-7	*log K_p*			
(wt%)	Naphthalene	Fluorene	Phenanthrene	Pyrene
1	3.05	3.23	3.26	3.36
2	3.05	3.22	3.25	3.36
3	3.06	3.23	3.25	3.36

The results in Table 6.1 obviously demonstrate that the equilibrium partition of PAH is almost independent of the surfactant concentrations within the experimental precision. It indicates that the equilibrium partition holds in CPE in the same manner as that observed in conventional solvent extraction.

The slopes of the curves shown in the Figure 6.6 bear the same definition as the equilibrium partition coefficients, K_p. The values of the K_p corresponding to the four PAHs used in this study increase in the order as pyrene > phenanthrene >fluorene> naphthalene. Such an order coincides with the order of the octanol-water partition coefficients (K_{ow}) of these compounds (Figure 6.8). The logarithmatic values of octanol-water partition coefficient of the four PAHs have been given in Chapter 4. The same trend was found as well in the micellar solutions containing Tergitol 15-S-7 at 1 and 3 wt%, with addition of 0.5 M Na_2SO_4. The logarithms of K_p and K_{ow} are found to have a good linear relationship (Figure 6.8). The correlation can be expressed as the following equation:

$$\log K_p = 0.165 \log K_{ow} + 2.50 \quad (6.3)$$

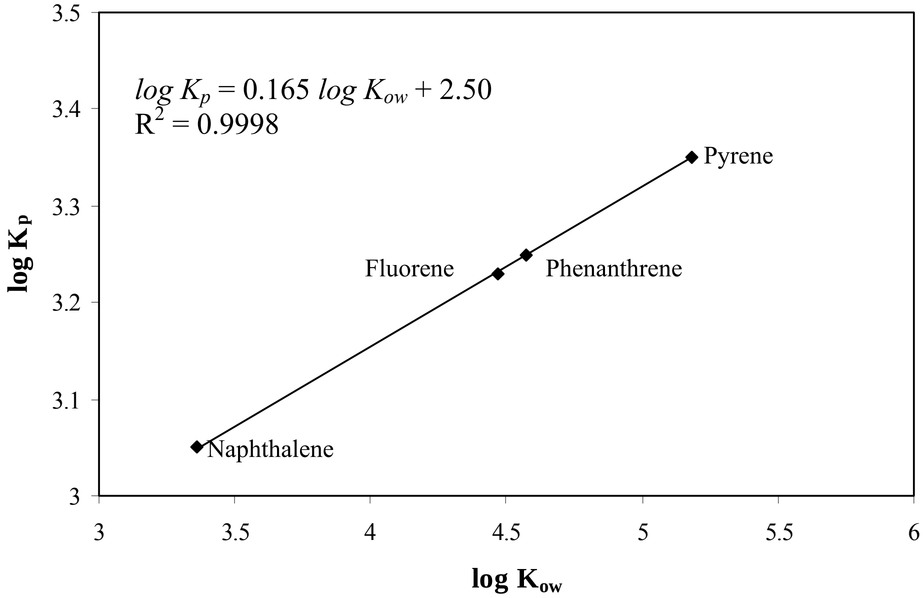


Figure 6.8 Correlations of the CPE Partition Coefficients to the Octanol-water Partition Coefficients. The CPE process was initiated by addition of 0.5 M Na_2SO_4.

Eq. (6.3) implies that the extraction of the PAH molecules into the surfactant-rich phase is driven by the hydrophobic affinity between the PAH molecules and the surfactant aggregates. In the cloud-point extraction process, any component originally binding to the surfactant aggregates in the bulk solution will preferentially be extracted to the surfactant-rich phase, usually in much smaller volume.

In the extraction process, the partition of solutes depends on the specific solute-solvent interaction, which can be determined by various factors. A five-parameter Abraham model, expressed as Eq. (6.4) has been used successfully to describe the equilibrium partition of solute between the coexisting phases (Abraham, 1993).

$$log\ SP = c + r\,R_2 + s\,\pi_2^H + a\,\Sigma\,\alpha_2^H + b\,\Sigma\,\beta_2^H + v\,(V_x/100) \tag{6.4}$$

Five parameters are used in this model to account for the physicochemical properties of solutes of interests. In this model, *SP* refers to some property of a series of solutes in fixed phases. It can be the water-micelle partition coefficient or the gas-liquid partition coefficient in a given liquid. R_2 is the excess molar refraction of the solute and π_2^H represents the dipolarity/polarity of the solute. $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ stand for the hydrogen-bond acidity and basicity of the solute, respectively. V_x is the McGowan characteristic molar volume of the solute, which is also an indicator for the hydrophobicity of the solute (Szymanowski, 2000). *c*, *r*, *s*, *a*, *b* and *v* are regression constants.

Recently, Szymanowski and Apostoluk (2000) demonstrated that $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$ and V_x are the most important parameters affecting the equilibrium partition of aromatic solutes in the cloud-point extraction containing the nonionic surfactant, PONPE-10, a polyoxyethylene nonyl phenyl ether as the extractant.

As the four PAHs used in this study have similar structures, the hydrogen-bond acidity $\Sigma\alpha_2^H$ and basicity $\Sigma\beta_2^H$ of the solutes will make little difference, if not negligible, to the partition coefficient. Consequently, V_x, the hydrophobicity of the compound, will be the most important parameter affecting the equilibrium partition of the PAHs. Likewise, a good linear relationship is found between the $logK_p$ and $logV_x$, shown in Figure 6.9.

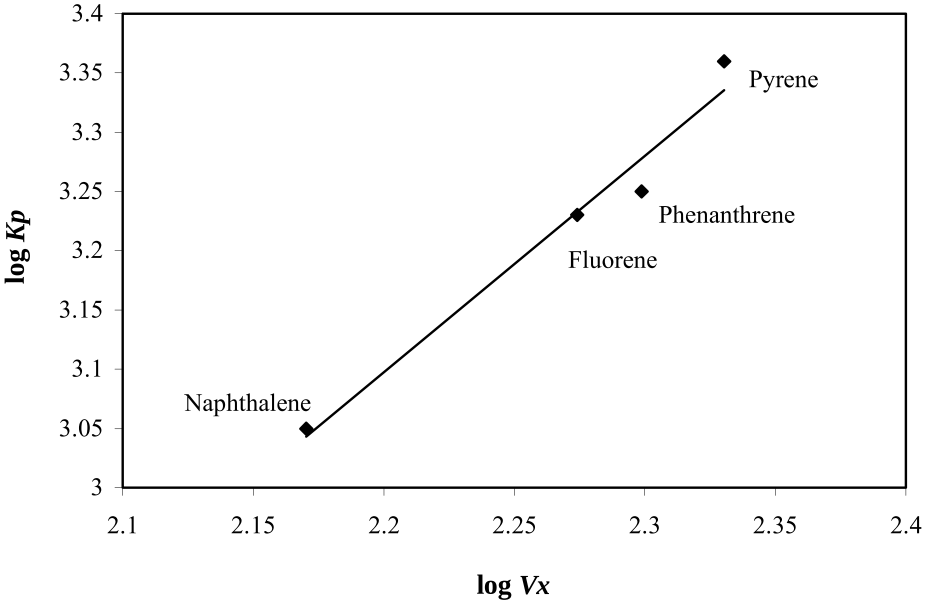


Figure 6.9 Correlations of the CPE Partition Coefficients to the Molar Volume, V_x. The cloud-point extraction process was initiated by addition of 0.5 M Na_2SO_4.

The values of V_x for the four PAH, naphthalene, fluorene, phenanthrene and pyrene are 148, 188, 199 and 214 cm^3/mol (Karcher, 1985). It has been reported that for hydrophobic compounds such as PAHs, a linear relationship could be established between their octanol-water partition coefficient and molar volume (Miller et al., 1985). This indicates that the molar volume of a hydrophobic compound can be a measure of its relative hydrophobicity. The linear relationship between the logarithm of K_{ow} and V_x also indicates that the equilibrium partition of the solute in the cloud-point extraction process can be positively correlated to its hydrophobicity.

Effect of Sodium Sulfate Concentration on Partition Coefficient. The concentration effect of the added Na_2SO_4 on the equilibrium partition of naphthalene, phenanthrene and pyrene in 2 wt% Tergitol 15-S-7 micellar solution was investigated as well. The partition coefficients are given in the Table 6.2. As aforementioned, addition of excessive Na_2SO_4, for example, 0.7 M to the 2 wt% Tergitol 15-S-7 solution, depresses the cloud-point too much and leads to the formation of very viscous surfactant-rich phase, which gives great difficulty to the further sample analysis. In contrast, addition of insufficient Na_2SO_4 will not lower the cloud-point temperature enough below the ambient temperature and cannot initiate the cloud-point phase separation. Table 6.2 shows obviously that the partition coefficient of the PAH increases with the increasing concentration of added Na_2SO_4. That is, more PAH will tend to partition to the surfactant-rich phase with more added Na_2SO_4. The enhanced partition is mainly due to the smaller water content and the lesser volume of the surfactant-rich phase at higher salt concentration.

Table 6.2 Effect of the Na_2SO_4 Concentration in 2 wt% Tergitol 15-S-7 Solution on the Partition Coefficient, K_p, of the PAHs

Na_2SO_4 Conc. (M)	$logK_p$		
	Naphthalene	Phenanthrene	Pyrene
0.4	2.35	2.77	3.03
0.5	3.05	3.25	3.36
0.6	3.22	3.69	3.77

6.2.1.6 Recovery of PAH

Effects of Surfactant Concentration and PAH Initial Concentration. Figure 6.10 shows the recovery of phenanthrene present at various concentrations by Tergitol 15-S-7 at concentrations of 1, 2 and 3 wt%. The sodium sulfate concentration used is 0.5 M.

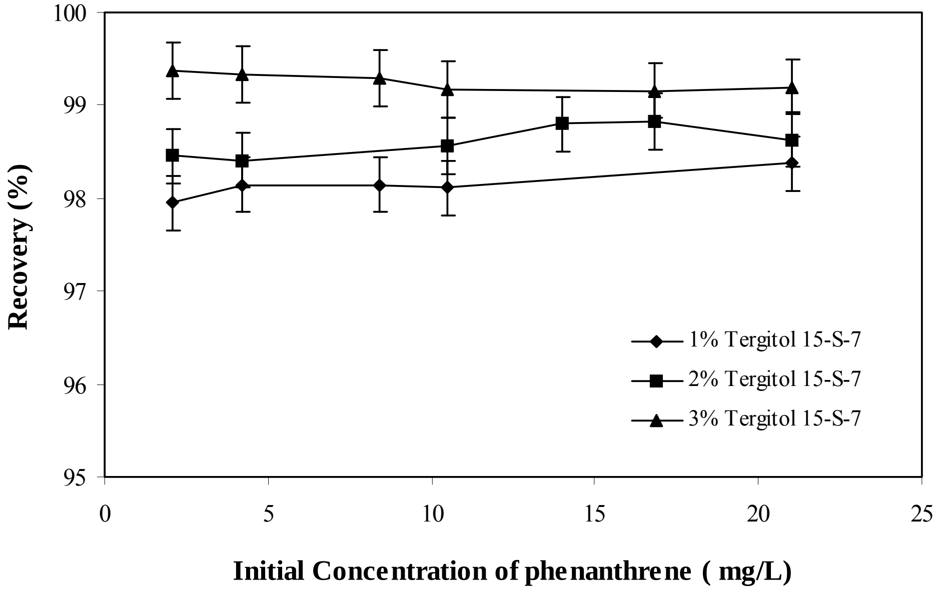


Figure 6.10 Effects of Surfactant Concentration and Initial Phenanthrene Concentration on Recovery of Phenanthrene.

From Figure 6.10, it is seen that more than 98% of the phenanthrene originally present in the aqueous solution was extracted into the surfactant-rich phase. The recovery of phenanthrene is independent of its own initial concentrations. It was also observed that the recovery increased with surfactant concentration.

Effect of PAH Hydrophobicity on Recovery. To observe the effect of PAH hydrophobicity on the recovery, the recovery of naphthalene and pyrene at different surfactant and initial concentrations are give in Figures 6.11 and 6.12.

Figure 6.10 to Figure 6.12 show that the recovery of pyrene is slightly higher than that of phenanthrene, which is slightly higher than that of naphthalene at the same surfactant concentration. In the study on partition, it was observed that more hydrophobic PAHs have larger partition coefficients. That is, they can be extracted more favourably into the surfactant-rich phase. Combining Eq. (3.5), (3.6) and (3.9), the following relationship between recovery and partition coefficient can be derived,

$$R = \frac{C_s V_s}{C_0 V_0} = \frac{C_s V_s}{C_s V_s + C_w V_w} = \frac{1}{1 + \frac{1}{K_p} \frac{1}{R_V}} \tag{6.5}$$

or,

$$\frac{1}{R} = 1 + \frac{1}{R_V} \frac{1}{K_p} \tag{6.6}$$

Eq. (6.5) is based on the assumption that loss of PAH is negligible. This is reasonable in CPE. It was observed that R_v was not affected by the presence of different PAHs. Hence, $1/R_v$ is a linear function of $1/K_p$. Eq. (6.6) indicates that solutes with greater partition coefficient also can be recovered more effectively.

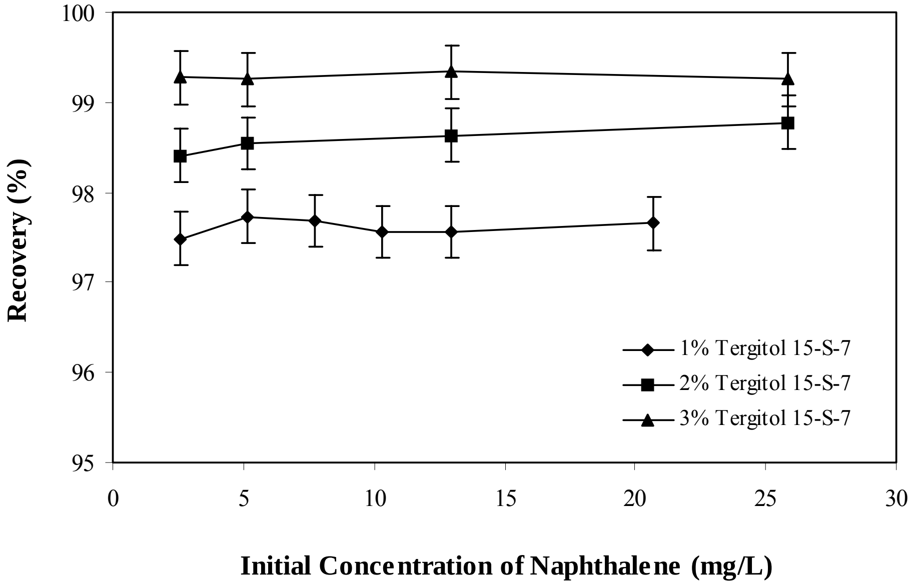


Figure 6.11 Recovery of Naphthalene at Different Surfactant Concentrations and Initial Naphthalene Concentrations.

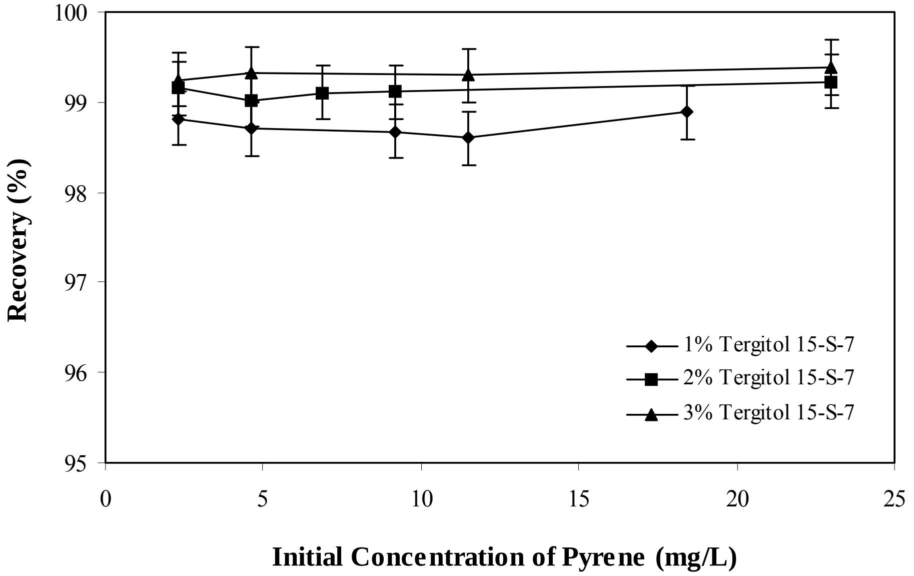


Figure 6.12 Recovery of Pyrene at Different Surfactant Concentrations and Initial Pyrene Concentrations.

Effect of Salt Concentration on Recovery. Figure 6.13 shows the recovery of phenanthrene at different concentrations of sodium sulfate. The surfactant concentration used is 2 wt%. From the results it can be concluded that an increase in the salt concentration leads to increased recovery. This change is about 2-3% from 0.4 to 0.6 M Na_2SO_4. Increased recovery was also observed for naphthalene and pyrene at higher salt concentrations.

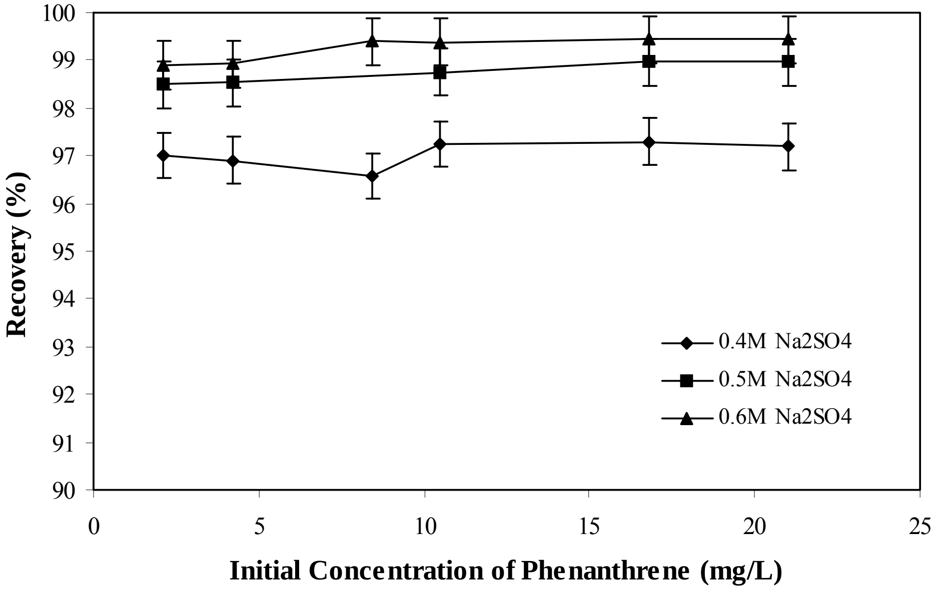


Figure 6.13 Effects of Na_2SO_4 Concentration on Recovery of Phenanthrene at Various Initial Phenanthrene Concentrations.

6.2.1.6 Estimation of Loss of PAHs

The presence of surfactant can minimize the loss of analytes caused by adsorption. Negligible sorption of PAHs onto the containers in the presence of surfactant Triton X-114 at 0.5 wt% was reported (Pinto et al., 1994). The loss of these three PAHs due to their adsorption on the centrifuge tubes and the glass sampling vials for HPLC analysis were estimated by measuring the change in the concentration after equilibrating the samples in the containers for 2, 5 and 24 hours. The surfactant concentration used is 1 wt%. The initial PAH concentrations were 2.23, 2.54 and 2.01 ppm for naphthalene, phenanthrene and pyrene respectively. The results are given in Table 6.3. The losses of phenanthrene due to its adsorption onto containers are negligible within the uncertainty of the HPLC analyses. Therefore, it can be concluded that Eq. (3.5), which is based on the mass balance, is thus accurate enough for the calculation of PAH recovery.

Table 6.3 Sorption of PAHs onto Centrifuge Tubes and Sampling Vials

PAH	Recovery, %					
	Centrifuge Tube			Sampling Vial		
	2 hr	5hr	24 hr	2 hr	5 hr	24 hr
naphthalene	99.3	98.5	100.2	99.1	100.9	98.8
phenanthrene	97.3	100.8	98.0	96.7	101.6	100.1
pyrene	98.5	97.0	99.4	99.2	100.3	100.0

6.2.2 Extraction by Tergitol 15-S-5

6.2.2.1 Sodium Chloride Enhanced Phase Separation of Tergitol 15-S-5

The phase separation of this surfactant in the presence of sodium chloride has been discussed in Chapter 5. It was observed that when the salt concentration was above 1M, complete phase separation could be achieved easily. The addition of salt not only increased the difference between the density of water and surfactant phase, but also enhanced the phase separation by accelerating the formation of fine droplets of surfactant. In the extraction experiments, to accelerate phase separation, samples were centrifuged at 3000 rpm. It was observed that complete phase separation could be achieved in a few minutes.

6.2.2.2 Water Content of Surfactant-rich Phase

The water contents of Tergitol 15-S-5 surfactant-rich phase in the presence of sodium chloride are given in Figure 6.14.

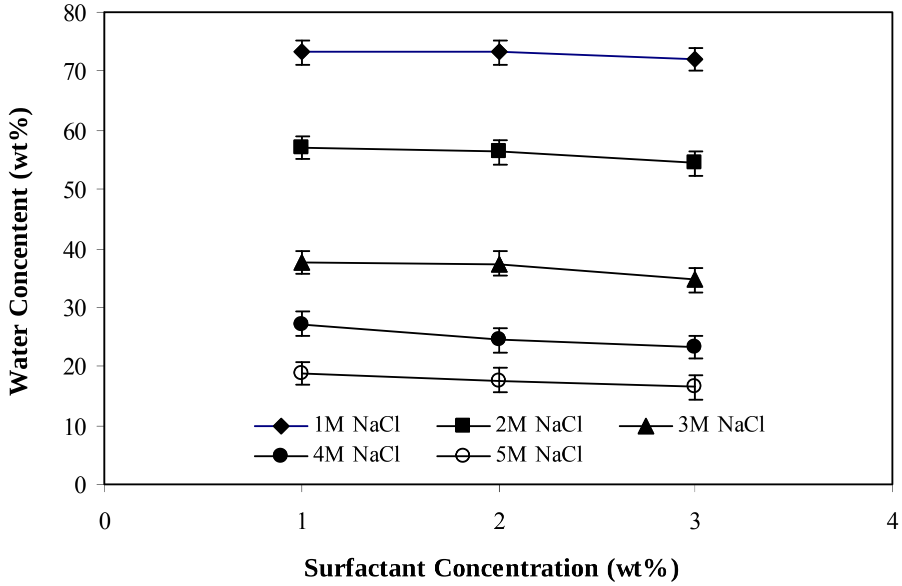


Figure 6.14 Water Content of Tergitol 15-S-5 as a Function of Surfactant and Sodium Chloride Concentrations.

At sodium chloride concentration of 1 M, the water content of Tergitol 15-S-5 is over 80 wt%. Increasing salt concentration to 5 M, the water content is reduced to less than 20 wt%. This is also due to the dehydrating effect of salt on the surfactant molecules.

6.2.2.2 Phase Volume Ratio

The effect of sodium chloride on the phase volume ratio of Tergitol 15-S-5 is shown in Figure 6.15. The result is like that of sodium sulfate on Tergitol 15-S-7. That is, increasing salt concentration and decreasing surfactant concentration leads to smaller volumes of the surfactant-rich phase, and enhances the preconcentration of solutes.

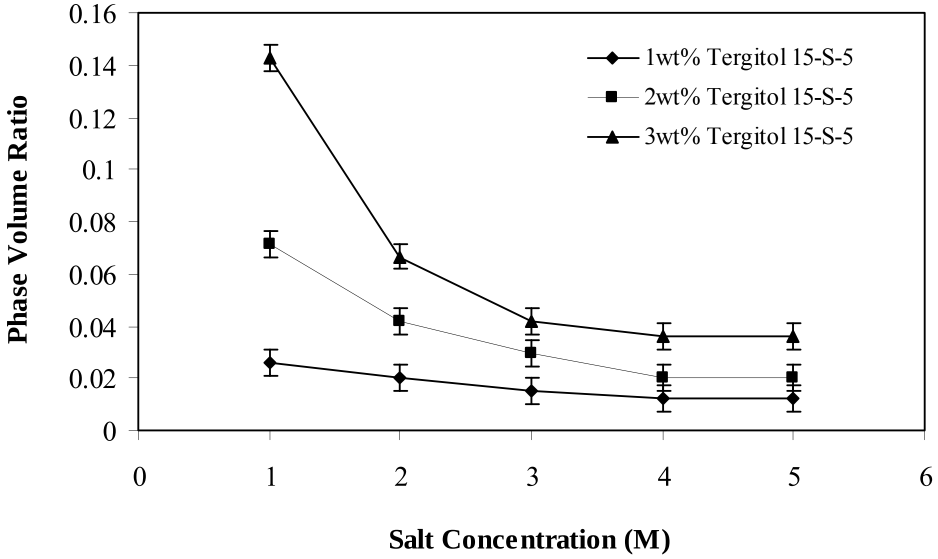


Figure 6.15 Phase Volume Ratio of Tergitol 15-S-5 as a Function of Surfactant and Sodium Chloride Concentrations.

6.2.2.3 Preconcentration Factor

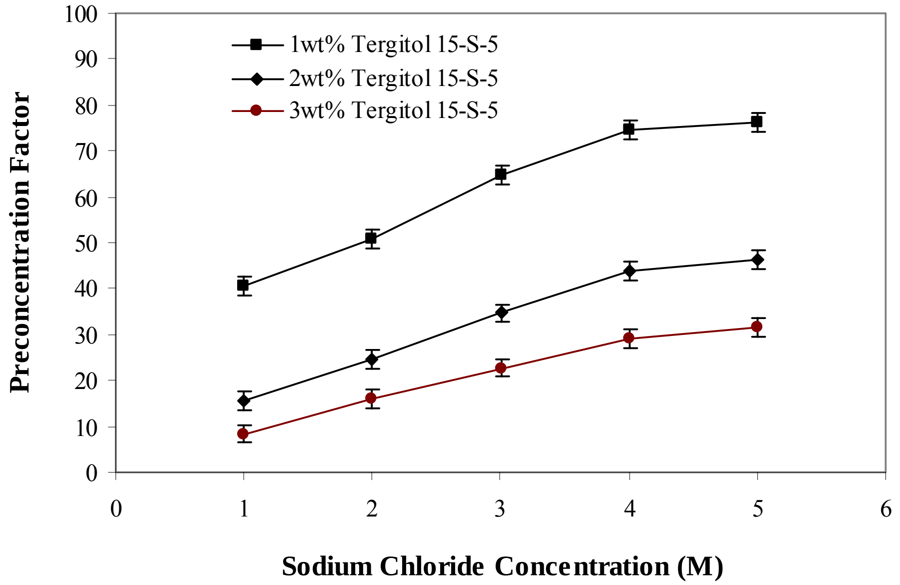


Figure 6.16 Preconcentration Factor of Phenanthrene at Different Concentrations of Tergitol 15-S-5.

The preconcentration factor of phenanthrene by Tergitol 15-S-5 was measured. Figure 6.16 gives the preconcentration factors at different surfactant concentrations and sodium chloride concentrations. The effect of sodium chloride on the preconcentration factor is also similar to that of sodium sulfate when Tergitol 15-S-7 is used as extractant. At sodium chloride concentration of 5 M and surfactant concentration of 1 wt%, a high preconcentration factor of about 75 can be obtained.

The effectiveness of sodium sulfate on the preconcentration factor was also examined and compared to that of sodium chloride. The result is given in Figure 6.17.

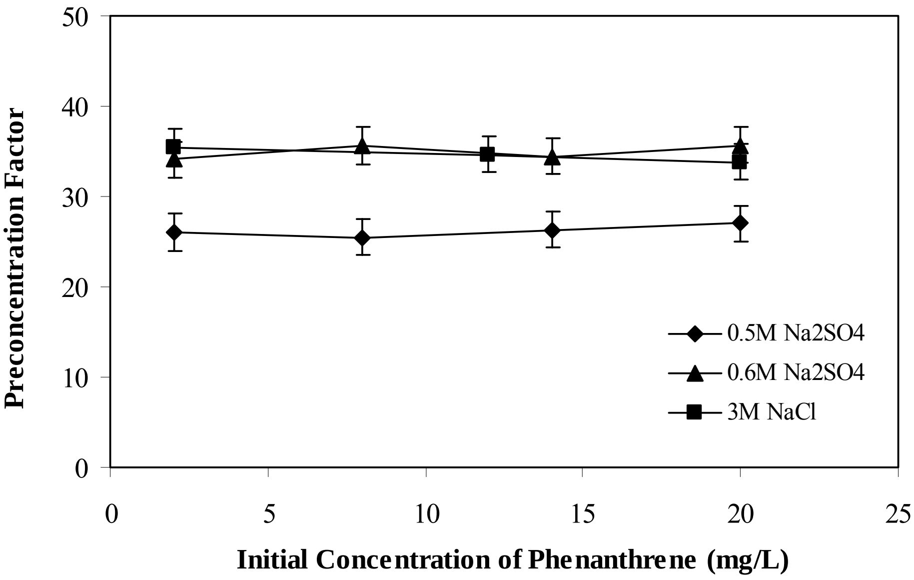


Figure 6.17 Preconcentration Factor of Phenanthrene in the Presence of Different Salts with Tergitol 15-S-5 as Surfactant.

It can be concluded that sodium sulfate is much more efficient in the preconcentration of phenanthrene. That is, effect of adding 0.6 M Na_2SO_4 is as same effectiveness as that of NaCl at 3 M. Their different strength in enhancing the preconcentration factor arrives from the difference in their effect on water structure. SO_4^{2-} are stronger water structure-making ions. In another word, it is more efficient in enhancing the association of water molecules. Unfortunately, when the concentration of Na_2SO_4 is above 0.6 M, unexpected phase change occurred. The oil-like surfactant-rich phase disappears and the surfactant molecules separated out from the solution and aggregated at the top of the solution like solid powders. This may be due to the strong dehydrating effect of this salt.

The temperature effect on the preconcentration efficiency of Tergitol 15-S-5 is shown in Figure 6.18. The surfactant and salt concentrations are 2 wt% and 3 M respectively. The initial phenanthrene concentration used here is 2 ppm. The results show that higher preconcentration efficiency can be obtained by increasing the experimental temperature. Increasing the temperature can dehydrate the surfactant molecules further more (Materna et al., 2001), leading to smaller water contents and smaller volumes of the surfactant-rich phase, which in turn contributes to higher preconcentration factors. At 30 ^{o}C, the preconcentration factor is about 50. Combining information provided in Figure 6.16, it can be seen that at this temperature, the preconcentration factor of 50 is larger than that obtained at 22 ^{o}C with the same surfactant concentration but a higher salt concentration of 5 M. This observation indicates that increasing temperature and salt concentration has the same effect on the preconcentration process. They both can dehydrate the surfactant and hence decrease the water content of the surfactant-rich phase. The water contents at different temperatures were also measured and given in Figure 6.18. It was also observed that with increase in temperature, the change in water content became slower. This is because that when most of the water contained in the surfactant-rich phase has been removed, further increase in temperature will have little effect.

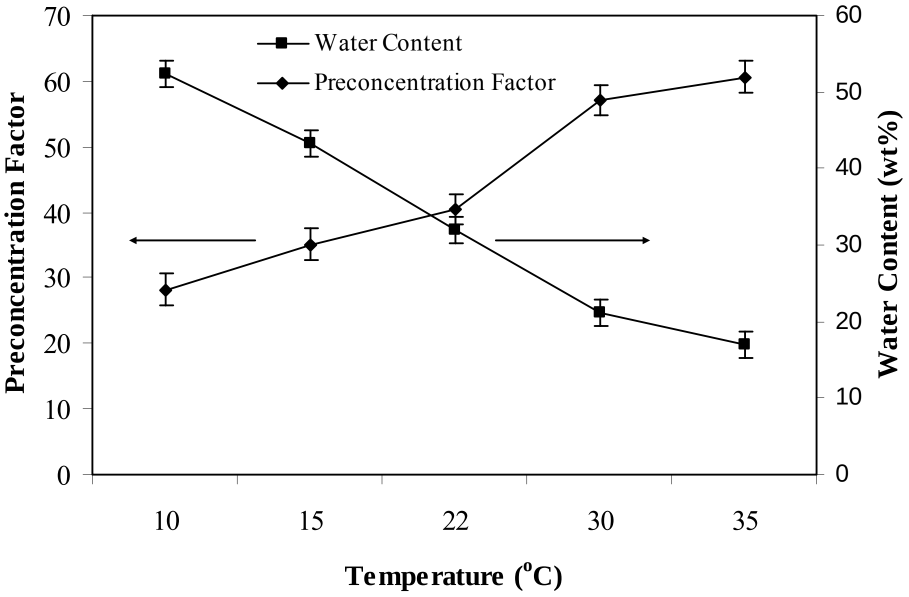


Figure 6.18 Effect of Temperature on Water Content and Preconcentration Factor of 2 wt% Tergitol 15-S-5.

6.2.2.4 Recovery and Partition Coefficient

It was observed that the concentration of phenanthrene left in the water phase after phase separation is very low and that in the surfactant-rich phase is a few orders of magnitude higher. For example, at surfactant concentration of 2 wt% and initial phenanthrene concentration of 2 ppm, its concentration in water phase is around 1 ppb. However, the phenanthrene concentrations in the surfactant-rich phase are about 30 ppm and 94 ppm at sodium chloride concentration of 1 and 5 M respectively. At initial phenanthrene concentrations below 20 ppm, the phenanthrene concentrations in the aqueous phases are below 50 ppb at the surfactant and sodium chloride concentrations used in the study. If the loss of phenanthrene is assumably negligible, which is quite reasonable in surfactant extraction process, more than 99% of phenanthrene can be recovered. Due to the low aqueous concentration of phenanthrene in the aqueous phase, precise determination of phenanthrene in that phase presents some difficulties.

Successive extraction may be necessarily applied. Therefore, the partition coefficient of phenanthrene in the extraction by this surfactant was not measured. However, the lower limit of the partition coefficient can be estimated by Eq.(6.5). Rearrange this equation, it arrives

$$Kp = \frac{1}{R_V}\frac{R}{1-R} \tag{6.6}$$

Since the recovey R was observed to be higher than 99%, K_p should be higher than $99/R_V$. In the study, the maximum R_V (0.14) was obtained at 3w% of Tergitol 15-S-5 and 1M of sodium chloride. Therefore, the lowest partition coefficient is 99/0.14=707. This is the lower limit of the partition coefficient of phenanthrene.

6.2.3 Comparison between Tergitol 15-S-5 and Tergitol 15-S-7

As is indicated by the results, both of these two surfactants are good candidates for the preconcentration of PAHs from aqueous solutions. The effect of surfactant concentration, additives and initial PAH concentration on the preconcentration factor and recovery of PAH follows the same trend. That is, increasing surfactant concentration decreases preconcentration factor, but improves the recovery. Increasing salt concentration can improve both the preconcentration factor and recovery. The preconcentration factor and recovery is independent of the initial PAH concentrations. However, higher recoveries were achieved when Tergitol 15-S-5 was used, which is attributable to its higher hydrophobicity. Higher preconcentration factors can also be achieved with this surfactant with the addition of sodium chloride. For example, at a same sodium sulfate concentration of 0.5 M and a same surfactant concentration of 2 wt%, the recovery for 5 mg/L phenanthrene is 99.5% and 98.5% for Tergitol 15-S-5 and Tergitol 15-S-7 respectively. Under the same conditions, the preconcentation factor of phenanthrene is 25.5 and 15 accordingly for the two surfactants.

6.3 Decontamination of Spiked Sand

6.3.1 Dissolution of Phenanthrene from Spiked Sand Sample

The dissolution kinetics of phenanthrene from spiked sand into water and into solutions of various surfactant concentrations are described in Figures 6.19 and 6.20 respectively. The saturation concentration and the lumped mass-transfer coefficients obtained by fitting experimental data to Eq. (3.10) is given in Table 6.4. The figures indicate that a first-order model is good enough to describe the dissolution of phenanthrene in this study. For contaminated soil, the dissolution in the presence of surfactant normally involves three steps (Yeom et al., 1996): the first step is the diffusion of phenanthrene within matrix; the second step is the mass-transfer at the interface (film diffusion); and the third step is partitioning into the micellar phase. The last step is much faster than the other two steps (Geheln and DeSchryver, 1993). The dissolution kinetics of a pollutant from contaminated soil also depends on many factors. The most important one is the contamination history of the soil. For weathered soil with a long history of contamination, the matrix diffusion normally dominates the process and the dissolution cannot be simply described by a first-order model. Whereas, in the case of artificially contaminated soil or soil with a short contamination history, the film diffusion is more important.

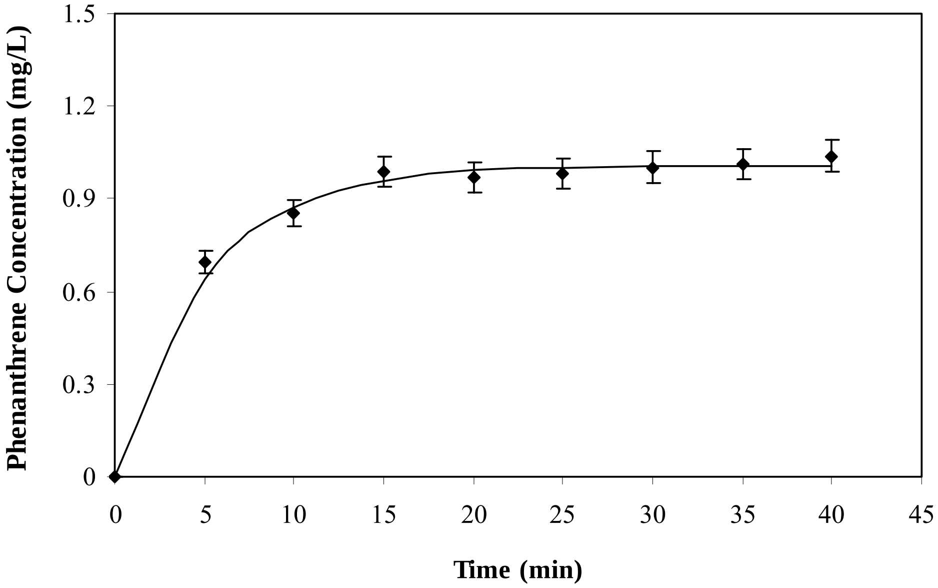


Figure 6.19 Dissolution of Phenanthrene from Spiked Sand Sample into Water.

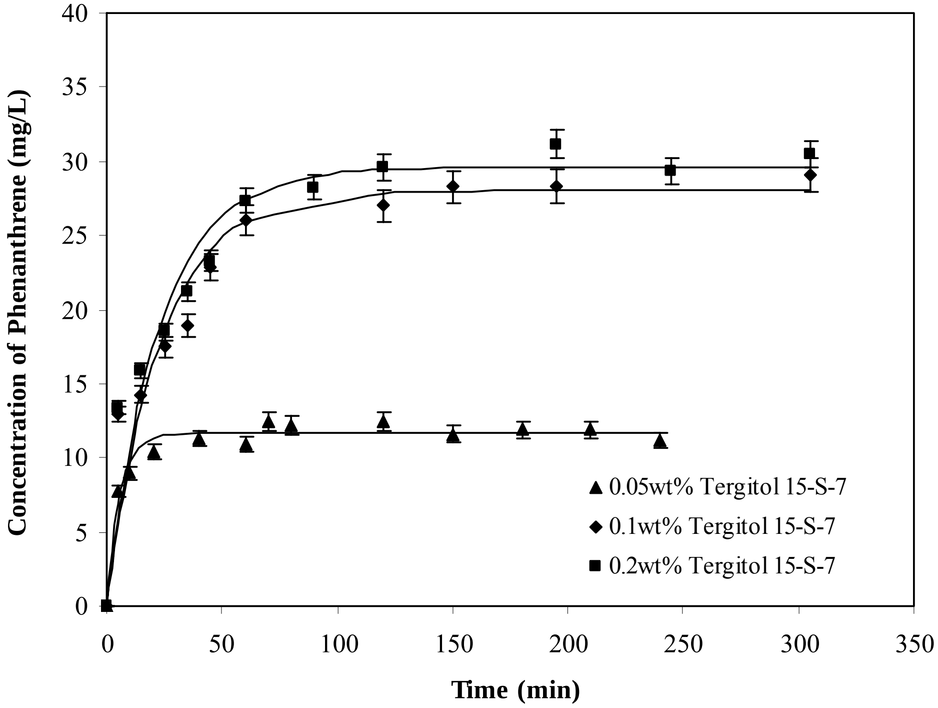


Figure 6.20 Dissolution of Phenanthrene from Spiked Sand Sample into Surfactant Solutions.

Table 6.4 Fitted Dissolution Parameters and Recoveries by Micellar Solubilization

Surfactant Concentration, wt%	C^*, mg/L		k_l, min^{-1}	Recovery R_m, %
	Measured value	Fitted value		
0	1.02	1.01	0.201	3.4
0.05	11.80	11.65	0.179	39.3
0.10	28.56	28.01	0.043	95.2
0.20	29.81	29.55	0.044	99.4

The results show that with increase in surfactant concentration, the lumped mass-transfer coefficient decreases. If the surface areas of the sand samples added into each solution are assumed to be the same, then the mass-transfer coefficient *k* decreases with the increase in surfactant concentration. Dissolution of phenanthrene includes the mass transport of the phenanthrene in the aqueous phase and the solublilized phenanthrene from the interface to the bulk solution. The decrease in mass-transfer coefficient can be attributed to the slower mass-transfer of phenanthrene in the micellar phase. Individual phenanthrene molecules in the aqueous phase are much smaller than the surfactant micelles, so their mass transport is faster than that in the micellar phase. Similar results were reported for the dissolution of phenanthrene from solid phenanthrene surface into surfactant solutions (Grimberg et al., 1995). However, the enhancement of solubilization capacity with increasing surfactant concentration compensates the loss in the mass-transfer coefficient and results in an overall increase in dissolution rates.

6.3.2 Cloud Point Extraction for Preconcentration of Phenanthrene

Phenanthrene in the surfactant washing solutions was preconcentrated by adding surfactant to achieve final surfactant concentrations of 1, 2 and 3 wt%. Sodium sulfate was applied at concentrations of 0.4, 0.5 and 0.6 M. The recoveries by cloud point extraction *R* and the total recoveries, R_T of the process were calculated and given in Table 6.5.

Same with the results obtained in the extraction in aqueous system, the recovery of phenanthrene by CPE from the washing solutions also increase with the surfactant and sodium sulfate concentrations. It was observed that the total recoveries of phenanthrene from the spiked sand sample are over 90%.

Table 6.5 Recovery of Phenanthrene by Cloud Point Extraction

C_{Surf} in Micellar Solubilization, wt%	C_{surf} in Cloud Point Extraction, wt%	C_{Na2SO4}, M	*R*, %	R_T, %
0.1	1	0.5	98.1±0.2	93.4
	2	0.4	97.0±0.4	92.3
	2	0.5	98.7±0.2	94.0
	2	0.6	99.2±0.3	94.4
	3	0.5	99.3±0.2	94.5
0.2	1	0.5	98.0±0.3	97.4
	2	0.4	97.2±0.3	96.6
	2	0.5	98.5±0.2	97.9
	2	0.6	99.0±0.2	98.4
	3	0.5	99.4±0.3	98.8

6.4 Conclusions

Based on the observations in this chapter, the following conclusions can be drawn:

(1) Tergitol 15-S-7 and Tergitol 15-S-5 are both very effective in the preconcentration and extraction of PAH from spiked water samples. The extraction

process is very simple compared with those reported in the literature, which is attributable to their low phase separation temperatures and non-fluorometric absorbance.

(2) Addition of suitable salts can not only facilitate the phase separation, but also improve the preconcentration efficiency. Increasing salt concentration, higher preconcentration factors and recoveries were obtained. This is due to the more dehydrated nature of the surfactant molecules, which is also reflected in the lower water content and smaller volume of the surfactant-rich phase. The recovery of PAH increases with surfactant concentration and hydrophobicity of PAH.

(3) When Tergitol 15-S-7 is used as extractant, the partition of PAH is observed to be independent of surfactant concentration and increase with sodium sulfate concentration. Extraction of PAHs into the surfactant-rich phase is driven by the hydrophobic affinity of PAH molecules to the surfactant aggregates.

(4) Increasing temperature can also improve the extraction efficiency. This is also due to the more dehydrated nature of the surfactant molecules. Smaller volume and water content of the surfactant-rich phase was observed at elevated temperature.

(5) The process, which combines micellar solubilization and cloud point extraction, is a good way for the decontamination of pollutant soil/sand matrix. Cloud point extraction could be used for the collection of pollutants from the washing solution for further treatment.

Chapter 7 Surfactant-mediated Biodegradation of Phenanthrene

7.1 Introduction

Biodegradation of poorly soluble polycyclic aromatic hydrocarbons (PAHs) has been a challenge in bioremediation, and surfactant-enhanced bioremediation of PAH contaminants has attracted great attention in recent years. Though many types of surfactants have been attempted, special interest is being shifted to those that are environmentally benign. Moreover, despite that surfactant can increase the solubility of these compounds, conflicting results still have been reported on the effects of surfactants on their biodegradation. The bioavailability of solubilized hydrocarbons is the focus of contest. Thus, it is the attempt of this chapter to investigate the bioavailability of a model PAH solubilized in the micellar solution of surfactants Tergitol 15-S-X. Selection of these surfactants is primarily based on their readily biodegradable nature. Attempts on this type of surfactants in bioremediation process have not been reported elsewhere.

In this chapter, the investigation of the biodegradation of phenanthrene as a model PAH solubilized in micellar solutions of three non-ionic surfactants, Tergitol 15-S-X (X=7, 9 and 12), by a type of marine bacteria, *Neptunomonas naphthovorans* (ATCC 700638), is presented. The solubilization capacities, CMC, and cloud points of these surfactants in artificial seawater were examined. The biodegradation of the surfactants was performed at different surfactant concentrations. The biodegradation data of the surfactants was fitted to a first-order kinetics model. The biodegradability of the surfactants was determined in terms of asymptotic concentration. The effects of surfactant concentrations, initial phenanthrene and biomass concentrations on the bioavailability of phenanthrene solubilized in the micellar solutions of the three surfactants were evaluated. It was observed that the biodegradability of phenanthrene decreased with the increase of surfactant concentrations, and increased with the increase of initial phenanthrene concentrations. The results were analyzed in terms of solubilization extents and aqueous concentrations of phenanthrene, which are two factors that determine the fraction of phenanthrene directly available to the bacteria.

7.2 Results and Discussion

7.2.1 CMC and Solubilization Capacity in Mineral Solution

The CMC of the three surfactants, Tergitol 15-S-X (X=7, 9 and 12) in the artificial seawater mineral medium were measured and the results are tabulated in Table 7.1. As is indicated in the table, the CMCs of the surfactants are lower than the corresponding values in deionized water provided by the supplier (refer to Table 2.4). The difference is attributable to the presence of various salts in the mineral solutions. The presence of salts can modify the micellar properties. Normally, salts with "salting-out" effect on a nonionic surfactant favor the micellization of the surfactant and hence the CMC is reduced. The effects of additives on the micellization of surfactant molecules have been reported (Schott, 1995). The overall "salting-out" effect of the mineral medium is reflected in the decrease in the cloud points of the surfactants, as is given in Table 7.1. For example, the cloud point of Tergitol 15-S-7 at a concentration of 1 wt% is 37.8 °C in DI water and it drops to 30.9 °C in the mineral medium. It needs to mention that such a low cloud point will not pose a significant disadvantage over the *in-situ* application of this surfactant. Practically, the applicable surfactant concentrations adopted in field applications are normally below 0.5 wt% (about 5000 mg/L). At lower surfactant concentrations, especially at concentrations less that 0.1 wt% (1000 mg/L), clouding is difficult to happen in the micellar solutions, which may due to the weak attractive interactions between the micelles.

The measured solubilization capacities of these three surfactants for phenanthrene are given in Figure 7.1. The respective mass solubilization ratios (*WSR*) are given in Table 7.1. The values of *WSR* in deionized water are 0.031, 0.020 and 0.017 for Tergitol 15-S-7, Tergitol 15-S-9 and Tergitol 15-S-12, respectively, as were measured in Chapter 4. The enhancement of the solubilization capacity is also due to the presence of the salts in the mineral medium. The effects of salts on solubilization have been discussed in Chapter 4.

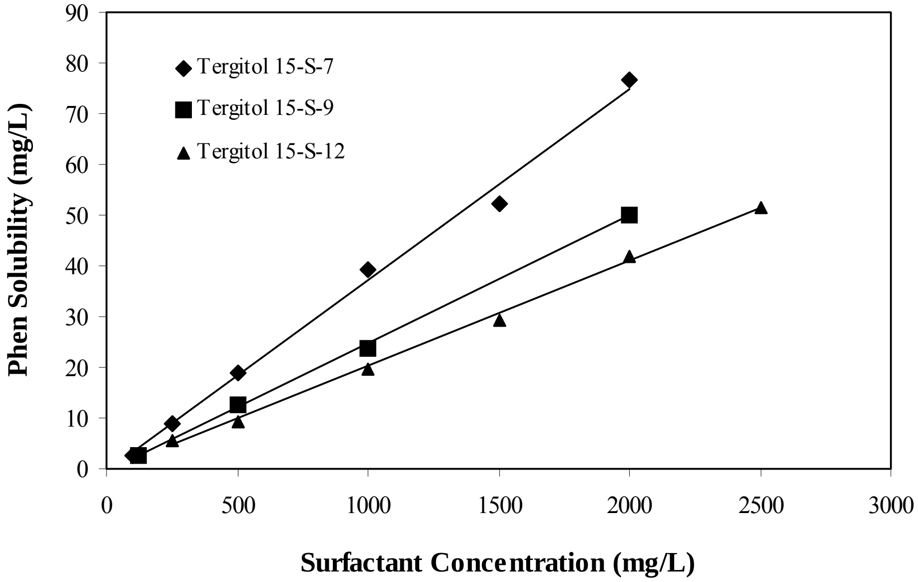


Figure 7.1 Solubilization Capacity of Surfactant for Phenanthrene in Mineral Medium.

Table 7.1 CMCs and Solubilization Data of Surfactants for Phenanthrene in Mineral Medium

Surfactant	CMC (mg/L)	WSR	K_{mw}	C_a (mg/L)	Cloud Point* (°C)
Tergitol 15-S-7	32	0.038	0.033	1.15	30.9
Tergitol 15-S-9	45	0.025	0.025	0.98	51.7
Tergitol 15-S-12	75	0.021	0.022	0.95	76.5

*Surfactant concentration: 1 wt%

7.2.2 Determination of Applicable Surfactant Concentrations

The surfactant concentration used in the *in-situ* applications could be up to 0.5 wt%. However, at such high concentrations, some surfactants are toxic to or inhibit the growth of bacteria. Hence, it is necessary to determine the ranges of surfactant concentrations that are suitable to the bacterial growth. The surfactants were supplied as the sole carbon source at a series of concentrations from 200 to 10,000 mg/L (1 wt%). Bacterial growth was observed at all the concentrations, although a longer lag period was observed with the increase in surfactant concentrations. Therefore, the Tergitol surfactants are biodegradable and not toxic to the bacteria, *Neptunomonas naphthovorans*. One of the surfactants, Tergitol 15-S-7, has been used in the biodegradation of palmitic acid (Lim, 2002). Surfactant concentrations up to 0.5 wt% were used in that study. Although inhibition of palmitic acid degradation was observed at such high surfactant concentration, which may be due to limited bioavailability of palmitic acid in the micellar phase, toxicity of this surfactant to the mixed bacteria (ATCC 55644) was not observed.

7.2.3 Biodegradation of Surfactant

The ultimate biodegradation of the surfactants was monitored by TOC measurement. A correlation curve of TOC to surfactant concentration is shown in Figure 7.2, which displays that the carbon contents of these three surfactants are around 60%. The carbon contents in decreasing order are Tergitol 15-S-7 > Tergitol 15-S-9 > Tergitol 15-S-12. However, the difference is not significant. The TOC values calculated from the chemical formulae are 63.5%, 62.5% and 60.9% respectively, which are close to the measured values.

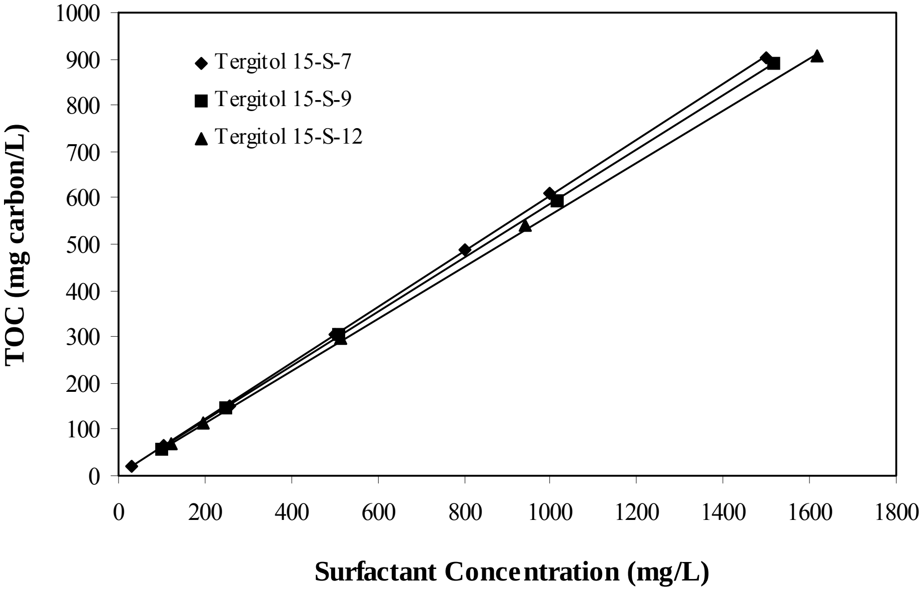
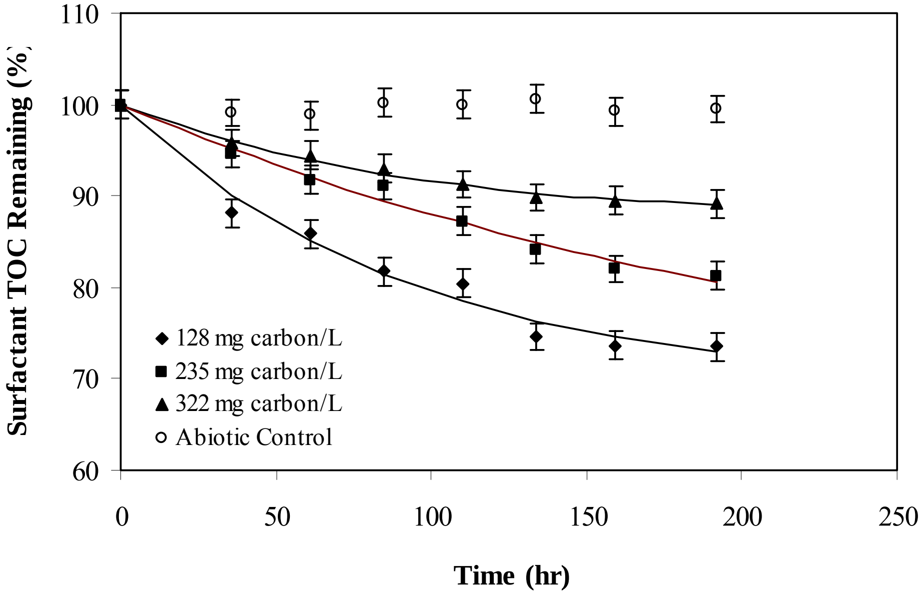


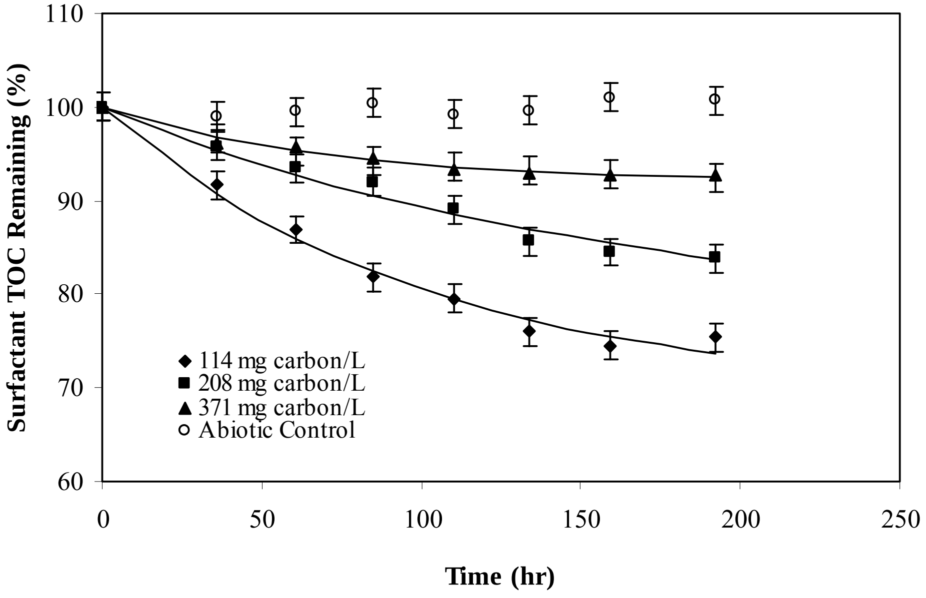
Figure 7.2 TOC VS. Surfactant Concentration.

7.2.3.1 Biodegradation of Surfactants at Different Concentrations

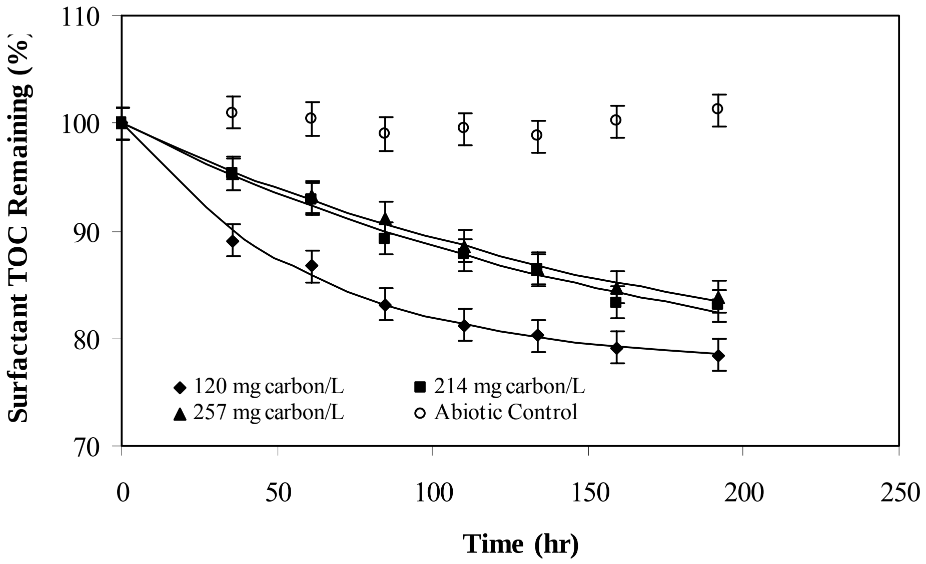
The biodegradation of these three surfactants at concentrations above their CMCs was carried out. The biodegradation data of these three surfactants are given in Figures 7.3 (a-c). For each surfactant, three concentrations were tested. Good biodegradability of these surfactants was confirmed. For example, at the initial TOC contents of Tergitol 15-S-7 at 128, 235, and 322 mg carbon/L, respectively, which are corresponding to the initial concentration at *ca.* 200, 400 and 600 mg/L, near 26, 19 and 11% were mineralized after 192 hours, respectively.



(7.3 a) Tergitol 15-S-7



(7.3 b) Tergitol 15-S-9



(7.3 c) Tergitol 15-S-12

Figure 7.3 Biodegradation of Surfactants at Different Concentrations (Biomass: 0.97 mg carbon/L).

The experimental data were fitted very well to a first-order kinetic model.

$$\frac{dS}{dt} = -k \cdot (S - S^*) \tag{7.1}$$

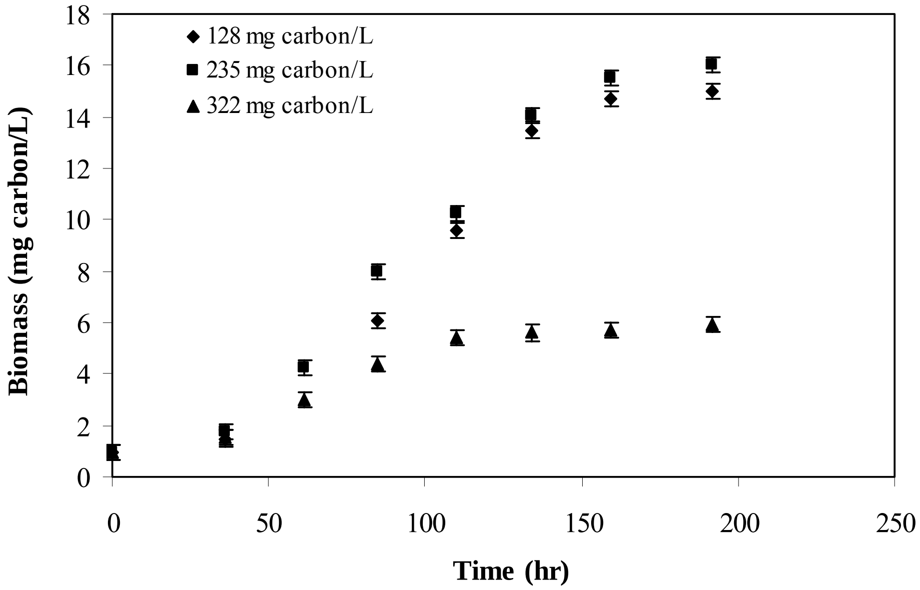
Integrate the above equation and one can get

$$S = S^* + (S_0 - S^*) \cdot e^{-kt} \tag{7.2}$$

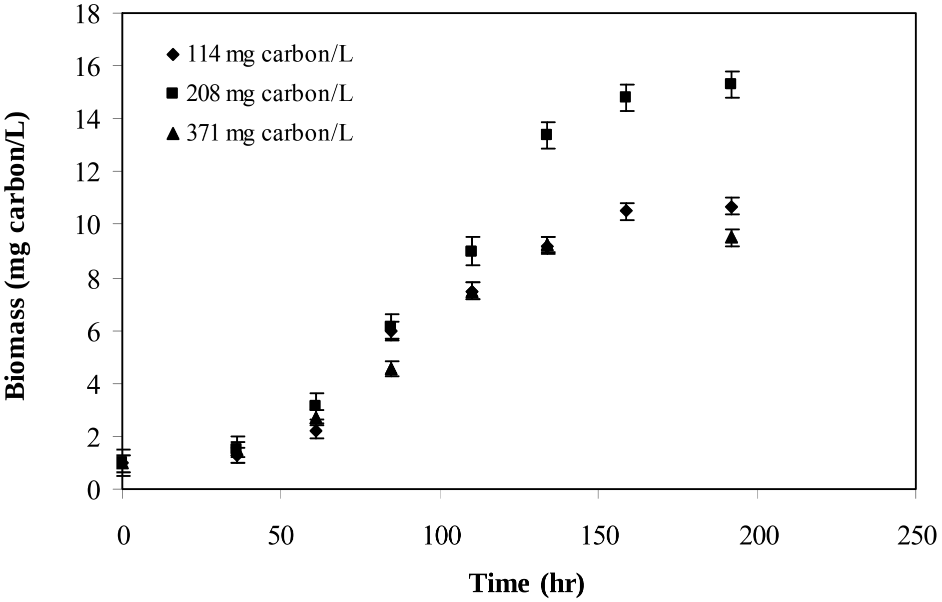
where S is the surfactant concentration in TOC (mg carbon/L) at time t; S^* is the asymptotic concentration (mg carbon/L) and k is the first-order rate constant (hr^{-1}).

Eq. (7.1) is based on the assumption that the biodegradation is not linked closely to bacterial growth. This is reasonable in the case of ultimate biodegradation of compounds with long chain molecules, such as surfactants. Before being mineralized, large surfactant molecules need to be broken down into smaller ones. This is generally called primary biodegradation. While primary biodegradation may contribute mainly to the increase in biomass, ultimate biodegradation may mainly serve to maintain the bacterial growth. A first-order kinetics was also reported by some authors in the biodegradation of a few nonionic surfactants, including Tergitol 15-S-12 (Zhang et al., 1999).

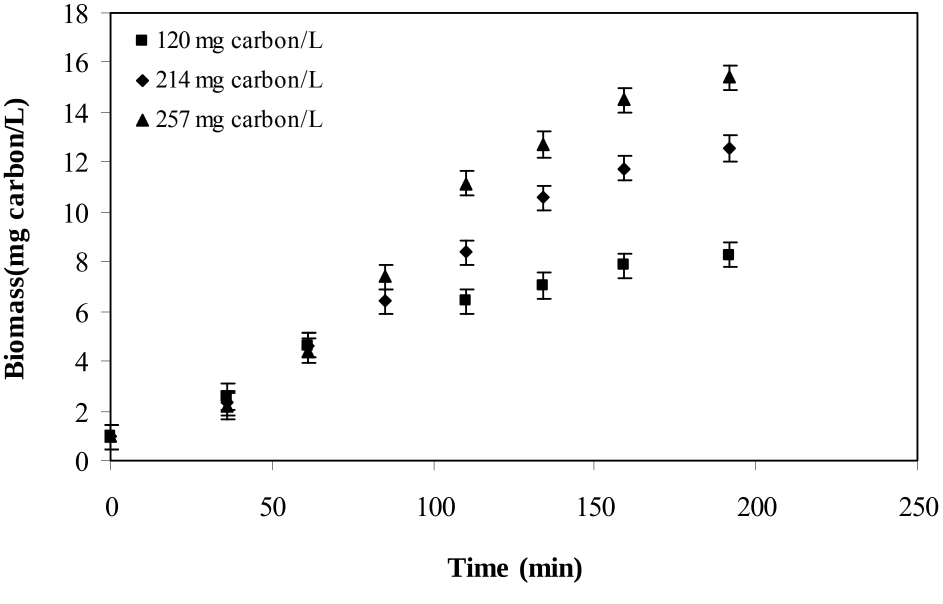
The fitted parameters, S^* and k in the first-order model are given in Table 7.2. The values of $(1–S^*/S_0)$, which can be taken as an index of biodegradability, are also tabulated in the table. The results showed that there is a slight increase in the biodegradability of Tergitol 15-S-7 and Tergitol 15-S-9 initially with increase in their concentrations. Then, their biodegradability decreased when the concentration is further increased. This may indicate a slight inhibition of biodegradation at higher surfactant concentrations. The biodegradability of Tergitol 15-S-12 was not observed to decrease within the concentration range of this study. The mechanism of inhibition may be due to the change of micelle structure at higher surfactant concentrations. At higher surfactant concentrations, more structured and packed micelles can be formed, which may hinder the close contact between the micellized surfactant and bacteria. The decreased biodegradability at high surfactant concentrations has been reported (Zhang et al., 1999). The reduced biodegradability was also reflected in the bacterial growth. Figure 7.4 (a) shows the curves of the bacterial growth with Tergitol 15-S-7 as the carbon source. Bacterial growth was enhanced when the surfactant concentration was increased from 128 to 235 mg/L. However, reduced bacterial growth was observed at 322 mg/L of surfactant. Similar results were obtained for Tergitol 15-S-9 and the results are given in Figure 7.4 (b). The bacterial growth on Tergitol 15-S-12 is given in Figure 7.4 (c), from which it can be concluded that the microbial growth is not inhibited within the concentration range. The non-inhibitory effects of Tergitol 15-S-12 could be due to the smaller concentration range used for this surfactant and its high CMC values (75 mg/L). The maxium concentration of this surfactant is 257 mg carbon/L, which is much lower than the corresponding values of the other two surfactants. The concentration increase from 214 to 257 mg/L is less than one CMC. Inhibitory effect could be expected at higher surfactant concentrations.



(7.4 a) Tergitol 15-S-7.



(7.4 b) Tergitol 15-S-9.



(7.4 c)Tergitol 15-S-12.

Figure 7.4 Bacterial Growths on Surfactants

Table 7.2 Parameters of First-order Biodegradation Kinetics of Surfactant

Surfactant	Concentration, S_0 (mg carbon/L)	k (hr^{-1})	S^* (mg/L)	$(1-S^*/S_0)$
Tergitol 15-S-7	128	0.0109	88.09	0.312
	235	0.0041	150.98	0.358
	322	0.0110	281.42	0.126
Tergitol 15-S-9	114	0.0099	78.83	0.309
	208	0.0035	135.25	0.350
	371	0.0147	341.61	0.079
Tergitol 15-S-12	120	0.0164	93.14	0.224
	214	0.0056	157.10	0.266
	257	0.0050	187.66	0.270

Initial biomass: 0.97 mg carbon/L

It is noteworthy to point out that the inhibition observed in this study could not be induced by the toxicity of surfactants because it has been demonstrated that the bacteria can survive at surfactant concentrations up to 1 wt%. Results in Table 7.2 also indicate that Tergitol 15-S-12 has a much lower biodegradability than the other two surfactants, while the biodegradability of Tergitol 15-S-7 is slightly higher than that of Tergitol 15-S-9. Since these three surfactants have the same hydrophobic chain in their molecules, the difference might be attributable to the longer hydrophilic moiety of the Tergitol 15-S-12 molecule, which makes the micelle surface more hydrophilic and hinders the contact of hydrophobic cell surface to the micelles. For secondary linear alcohol ethoxylates, a significant resistance to degradation was demonstrated on increasing the ethoxylate chain length (Karsa and Porter, 1995). Additionally, the hydrophilic part of a surfactant molecule is the dominant proportion of degradative scission (Hoffmann et al., 1999; Řezníćková et al., 2002). The effects of the molecular structure on the biodegradability of surfactants have been reported by some other authors (Terzić et al.,1992)

Specific Growth Rate and Doubling Time

The growth rate μ of bacteria was obtained from the following equation,

$$\frac{dX}{dt} = \mu X - bX \tag{7.3}$$

where b is the endogenous respiration coefficient of the bacteria. Further integrating the Eq. (7.3), the following equation is achieved:

$$\ln\left(\frac{X}{X_0}\right) = (\mu - b) \cdot t \tag{7.4}$$

With the Eq. (7.4), the growth rate μ can be easily obtained by plotting $ln(X/X_0)$ versus t. Furthermore, according to Eq. (7.4), the doubling time, t_D can be acquired:

$$t_D = \ln(2)/(\mu - b) \approx 0.693/(\mu - b) \tag{7.5}$$

The endogenous respiration of bacteria was monitored. No carbon source was assuredly provided. The first-order endogenous respiration coefficient b was obtained by fitting experimental data to the following equation.

$$ln(X/X_0) = -b \cdot t \tag{7.6}$$

The change of biomass with time is plotted in Figure 7.5. The slope of the curve is equal to $-b$. That is, the endogenous respiration coefficient of the bacteria is found at 0.0026 hr^{-1}. It needs to mention that the respiration coefficient obtained here should be different from the one during the biodegradation process. However, due to the difficulties in obtaining the real value, normally it is approximated by the one when no carbon source is supplied. Fortunately, because of the small value of this parameter, the approximation does not cause significant errors.

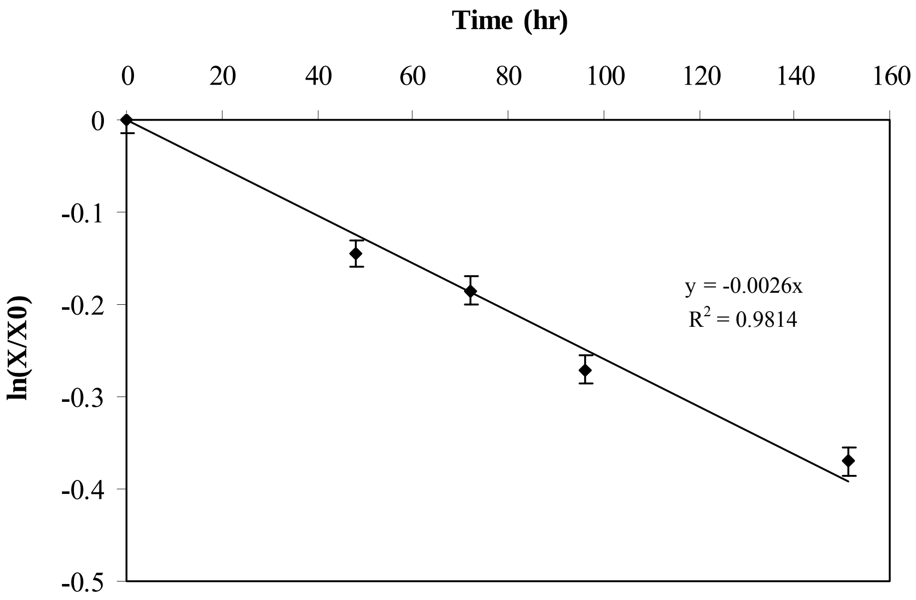


Figure 7.5 Endogenous Respiration of Bacteria.

The specific growth rate and doubling time of the bacteria on the surfactants are tabulated in Table 7.3. Within the surfactant concentration ranges used in the study, the specific growth rate is almost the same at the different surfactant concentrations.

Table 7.3 Specific Growth Rate on Surfactant and Doubling Time

Surfactant	Concentration (mg carbon/L)	μ (hr^{-1})	t_D (hr)
Tergitol 15-S-7	128	0.021	37. 66
	235	0.022	35.72
	322	0.021	37.66
Tergitol 15-S-9	114	0.025	30.94
	208	0.029	26.25
	371	0.023	33.97
Tergitol 15-S-12	120	0.027	28.40
	214	0.029	26.25
	257	0.029	26.25

7.2.4 Effect of Solubilization on Biodegradation

7.2.4.1 Phenanthrene Biosorption

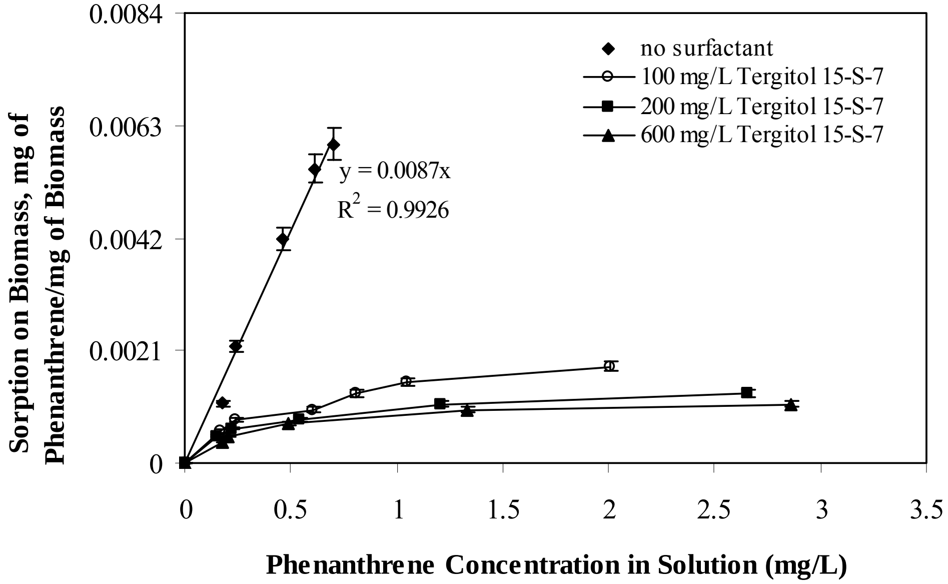


Figure 7.6 Effect of Tergitol 15-S-7 on the Biosorption of Phenanthrene

Figure 7.6 gives the sorption of phenanthrene on biomass in the absence and presence of Tergitol 15-S-7. It demonstrates that in the absence of this surfactant, the biosorption of phenanthrene is a linear function of phenanthrene concentration within its solubility (0.7 mg/L) in the mineral medium. The slope of the curve is 0.0087 equals to its partition coefficient between biomass and the medium. It can be concluded that in the absence of surfactant, the biosorption may contribute to significant abiotic loss. Thus, its biosorption should be analyzed. However, in the presence of surfactant, the biosorption is very low. At a certain surfactant concentration, the biosorption first increases with phenanthrene concentration and then approaches a constant value, which means that the sorption of phenanthrene on the biomass is staturated. The results in this figure could also explain why the addition of surfactant can effectively reduce the sorption of hydrophobic contaminants on the polluted matrices such as soil and other organic matters. The sorption of these contaminates in many cases is a factor that limits their bioavailability and controls the remediation process (Salloum et al., 2002).

7.2.4.2 Effect of Surfactant Concentration on Phenanthrene Biodegradation

To examine the solubilization extent on the biodegradation of phenanthrene, experiments were conducted at constant initial phenanthrene concentrations and different surfactant concentrations. Figure 7.7 shows the biodegradation of phenanthrene in the absence and presence of Tergitol 15-S-7 at 100, 200 and 400 mg/L.

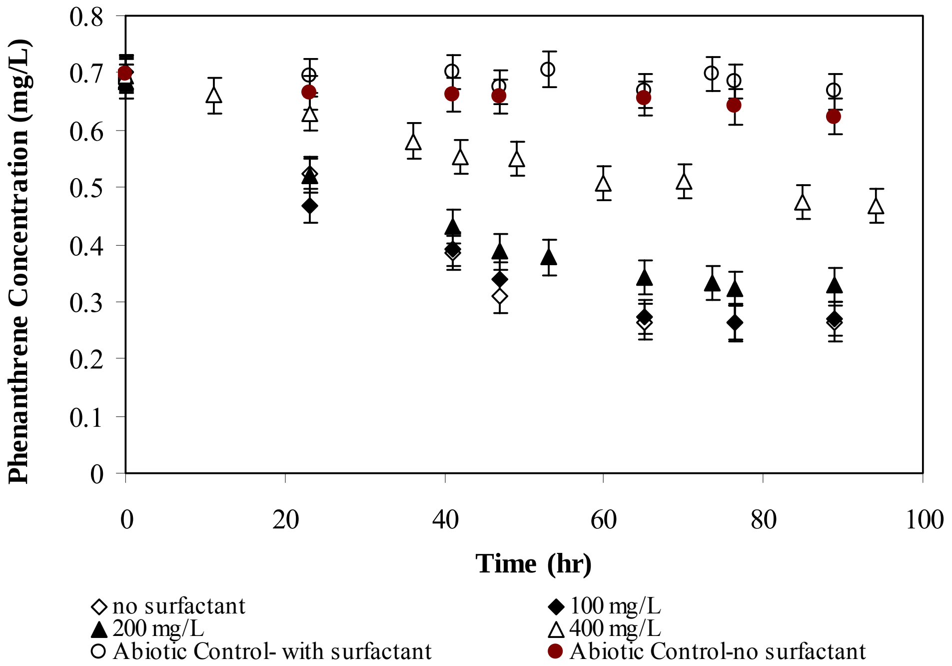


Figure 7.7 Effect of Tergitol 15-S-7 Concentration on the Biodegradation of Phenanthrene (Initial phenanthrene concentration: 0.70 mg/L; initial biomass: 0.22 mg carbon/L).

The concentration of phenanthrene in these experiments was 0.70 mg/L. Therefore, the addition of surfactant in this experiment was not for increasing the solubility of phenanthrene in the solution but for examining how the surfactant concentration may affect biodegradation of phenanthrene in the medium. It appears that a surfactant concentration up to 100 mg/L did not influence phenanthrene biodegradation. With further increases of the surfactant concentrations to above 100 mg/L, however, the results show that the extent of biodegradation of phenanthrene in the medium was decreased with the increase of the surfactant concentrations. Hence, the presence of Tergitol 15-S-7 inhibits the degradation of phenanthrene. The inhibitory effect is greater at higher surfactant concentration. Inhibitory effect was also observed at Tergitol 15-S-7 concentrations above 0.1 wt% (1000 mg/L) when palmitic acid was used as substrate (Lim, 2002). Despite of this adverse effect of Tergitol 15-S-7, the biodegradation of phenanthrene could indeed be enhanced by this surfactant, because of the enhancement in the aqueous solubility of phenanthrene by this surfactant. The results will be given in Figure 7.9 and Figure 7.14.

Since it has been demonstrated that adsorption of phenanthrene on biomass in the absence of surfactant may contribute to its significant abiotic loss, it is necessary to estimate the loss of phenanthrene caused by biosorption. However, the biomass concentration during the biodegradation of phenanthrene in the absence of surfactant is very low, with the maximum of 5 mg carbon/L. Based on the partition coefficient in Figure 7.6, the calculated loss of phenanthrene due to sorption onto biomass is within 0.001 to 0.01 mg/L (Figure 7.8). Therefore, the biosorption here only contribute to negligible abiotic loss.

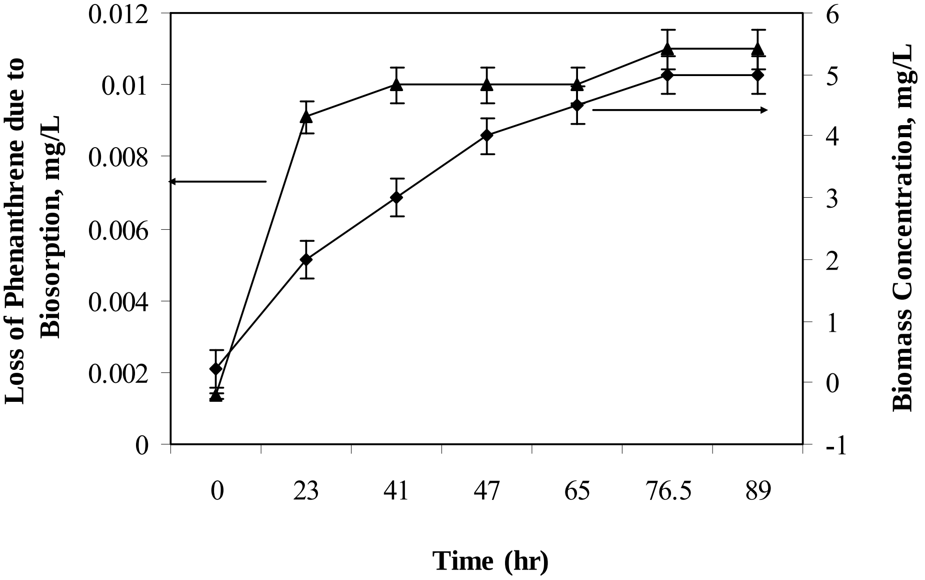


Figure 7.8 Biosoprtion of Phenanthrene in the Absence of Tergitol 15-S-7 (initial phenanthrene concentration: 0.7 mg/L; initial biomass: 0.22 mg/L)

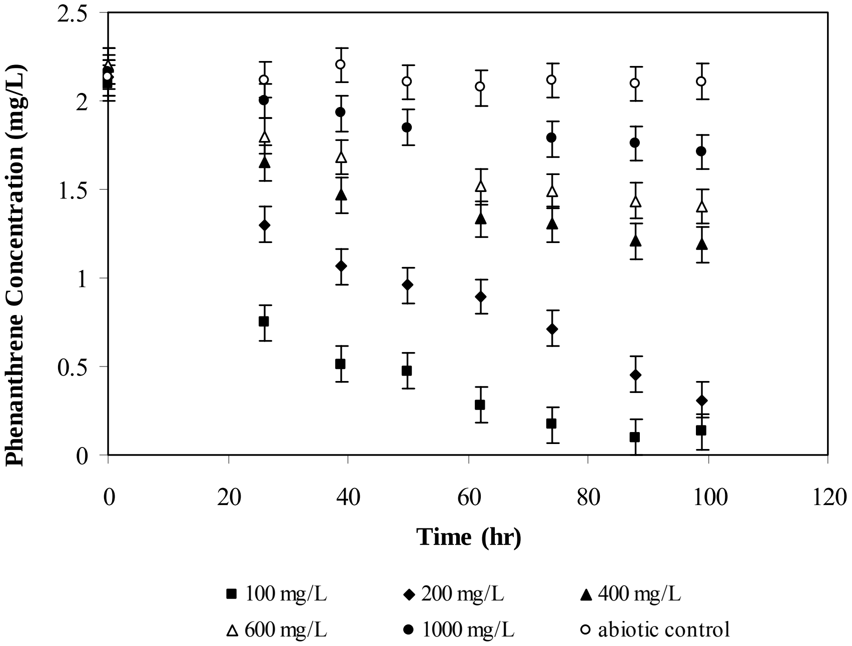


Figure 7.9 Effect of Tergitol 15-S-7 Concentration on Biodegradation of Phenanthrene (Initial phenanthrene concentration: 2.13 mg/L, Initial Biomass: 0.22 mg carbon/L).

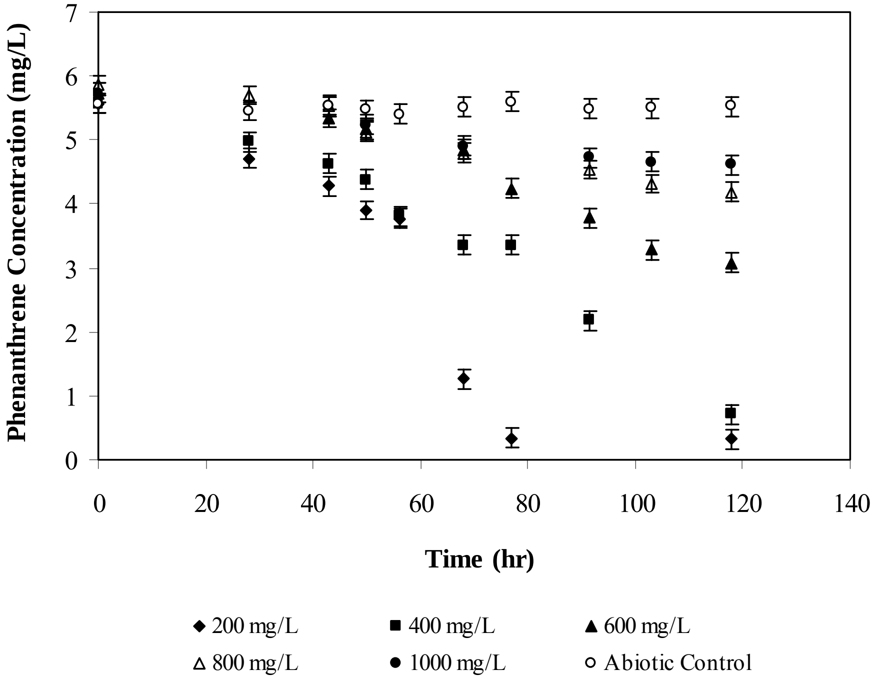


Figure 7.10 Effect of Tergitol 15-S-7 Concentration on Biodegradation of Phenanthrene (Initial phenanthrene concentration: 5.66 mg/L, Initial Biomass: 0.15 mg carbon/L).

The results in Figures 7.9 and 7.10 exhibit the effects of Tergitol 15-S-7 concentrations on the biodegradation of phenanthrene at different initial phenanthrene concentrations much greater than its saturated solubility in the artificial seawater medium without the addition of surfactant. Results similar to that in Figure 7.7 were obtained. That is, increasing surfactant concentration the biodegradability of phenanthrene is reduced. The reduced microbial growths at higher surfactants were also observed and given in Figure 11. In Figure 7.9, at the surfactant concentration of 100 and 200 mg/L only about 0.1 mg/L of phenanthrene remains at the end, that is, about 2 mg/L of phenanthrene has been degraded, which is about three times of the solubility (0.7 mg/L) of phenanthrene in the mineral medium in the absence of surfactant. However, when the surfactant concentration is 1000 mg/L, the amount of phenanthrene degraded is only about 0.4 mg/L.

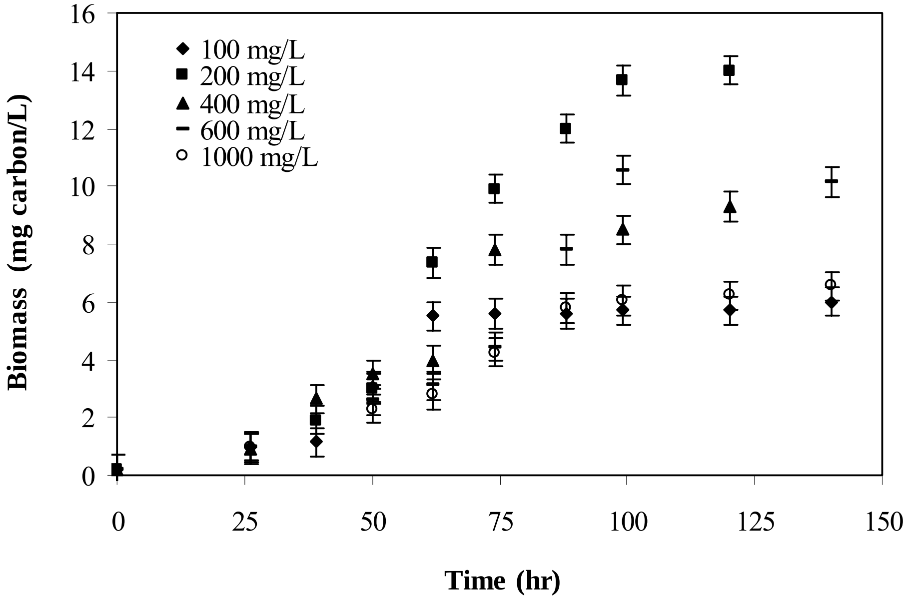


Figure 7.11 Effects of Tergitol 15-S-7 on Microbial Growth in the Presence of Phenanthrene (initial phenanthrene concentration: 2.13 mg/L; initial biomass: 0.22 mg carbon/L).

Combining the results in Figure 7.7 and 7.9, the use of surfactant seems to have two competing effects on the biodegradation of phenanthrene: first, at a constant initial phenanthrene concentration, with increase in surfactant concentration, the bioavailability of phenanthrene in the solution is reduced, that is, the presence of surfactant Tergitol 15-S-7 is not beneficial to the phenanthrene biodegradation; second, the presence of this surfactant can, on the other hand, increase the bioavailability of phenanthrene by increasing its solubility through the solubilization process. The second conclusion will be more clearly demonstrated when the surfactant concentration is fixed, while the initial phenanthrene concentration is varied (to be shown in Figure 7.14). To conclude, the surfactant has two opposite effects on the biodegradation of phenanthrene. As a consequence, pre-determination of suitable surfactant concentrations should be carried out prior to an *in-situ* bioremediation application. In fact, for a certain substrate concentration, the reduced bioavailability of the substrate with increase in surfactant concentration has been reported by many authors for other surfactant/bacteria combinations (Laha and Luthy, 1992; Grimberg & Aitken, 1995; Volking et al., 1995; Grimberg et al., 1996; Guha and Jaffe, 1996; Cort et al., 2002; Cort and Bielefeldt, 2002; Doong and Lei, 2003).

In Figure 7.10, at surfactant concentration of 200 and 400mg/L, a sharp drop in phenanthrene concentration was observed after 60 and 80 hours respectively. The drop in phenanthrene concentration is due to the loss of solubilization capacity caused by both of its ultimate and primary biodegradation. Coincidently, precipitation of phenanthrene crystals was observed. The solubilization capacities of the surfactant solutions after the biodegradation experiments were tested and compared with the results in Figure 7.1. The results showed that at the same TOC concentration of surfactant the solubilization capacities of the surfactant solutions used for biodegradation were significantly reduced. This means the primary biodegradation, which leads to the breakdown of surfactant molecules, could invoke a significant loss in solubilization capacity. Theoretically, the loss of surfactant due to its consumption by the bacteria may improve the bioavailability of phenanthrene. The reduction of surfactant concentration can reduce the fraction of total phenanthrene in the micellar phase. As a consequence, the fraction of phenanthrene in the aqueous phase increases, which contributes to a higher bioavailability. A reduction of surfactant may also contribute to the formation of a less compact micellar structure, which can also be conducive to uptake of phenanthrene from the solution by the bacteria. Reduced bioavailability of phenanthrene in the micellar solutions of Tergitol 15-S-9 and Tergitol 15-S-12 at higher surfactant concentrations were also observed (see Figures 7.12 and 7.13).

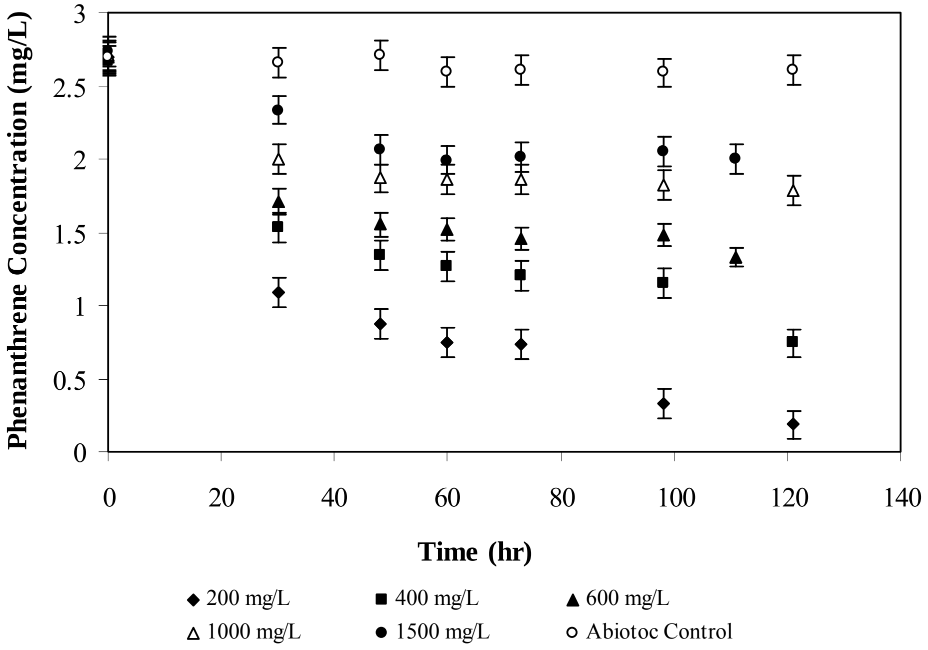


Figure 7.12 Biodegradation of Phenanthrene Solubilized by Tergitol 15-S-9 (initial phenanthrene concentration: 2.64 mg/L; initial biomass: 0.27 mg carbon/L).

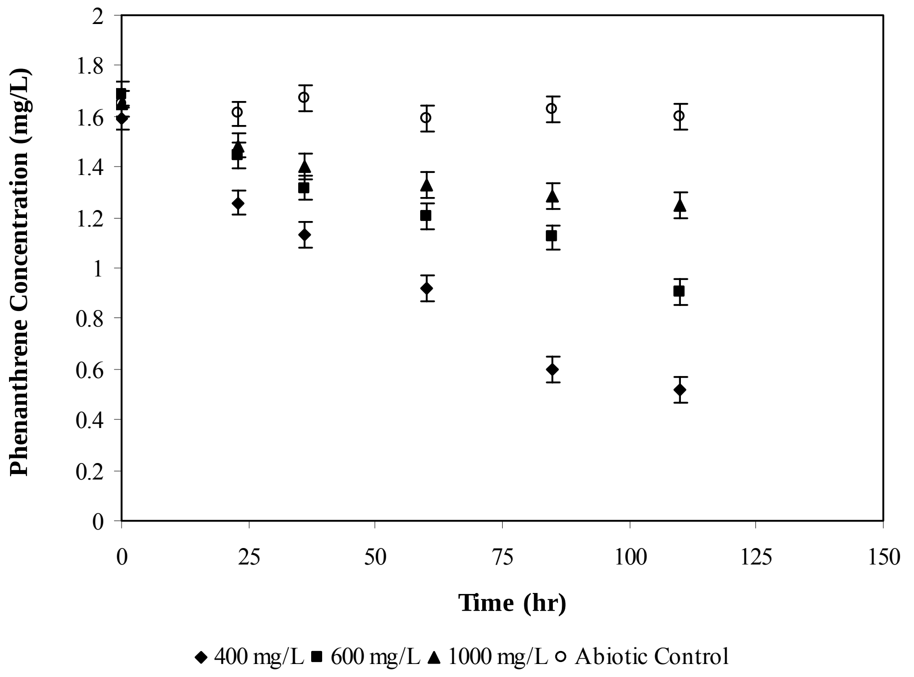


Figure 7.13 Biodegradation of Phenanthrene Solubilized by Tergitol 15-S-12 (initial phenanthrene concentration: 1.64 mg/L; initial biomass: 0.97 mg/L).

7.2.4.3 Effect of Initial Phenanthrene Concentration

With the change of initial phenanthrene concentrations, the concentrations of phenanthrene in the aqueous and, especially, in the micellar phases will vary accordingly. The increase of phenanthrene in the aqueous phase is beneficial to the biodegradation since phenanthrene in this phase is directly accessible to the bacteria and hence has a higher bioavailability. As aforementioned, the phenanthrene in the micellar phase is generally less biologically available for biodegradation. The increase of the phenanthrene concentrations in the micellar phase however makes the micellar phase be more “saturated” with phenanthrene. The difference between the saturation concentration and the actual concentration of phenanthrene in the micellar phase at a given surfactant concentration indicates a tendency of the phenanthrene molecules in the micelles to remain in the micelles or to diffuse from the micellar phase into the aqueous phase. A greater difference means that the phenanthrene molecules in the micellar phase have a greater tendency to stay in the micelles than to diffuse into the aqueous phase, and the phenanthrene molecules in the micelles in this case will be less biologically available during the biodegradation of phenanthrene than in the case of a smaller difference. An increase in phenanthrene concentration in the micellar phase also leads to an enhanced mass-transfer between micellar and aqueous phase, as well as the mass transfer between micelles and cells.

Figure 7.14 shows the results of biodegradation of phenanthrene at different initial concentrations in the medium with 550 mg/L of Tergitol 15-S-7.

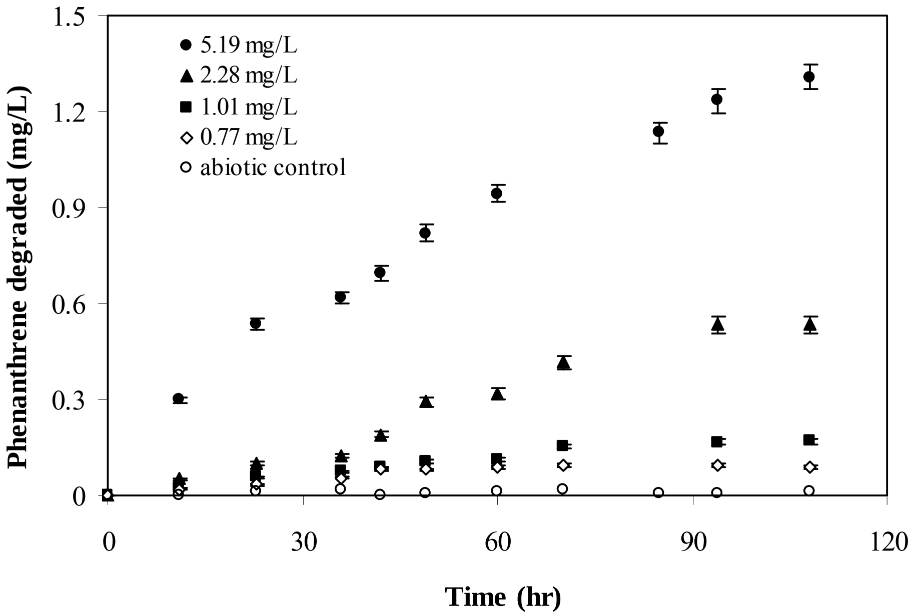


Figure 7.14 Effect of Initial Phenanthrene Concentration on its Biodegradation (Tergitol 15-S-7 Concentration: 550 mg/L; Initial Biomass: 0.22 mg carbon/L).

To get a clearer picture of the effects, the amount of phenanthrene biodegraded is given as a function of time. It is clearly observed that, for the concentrations studied, the increase of the initial phenanthrene concentrations resulted in a greater amount of phenanthrene being biodegraded. It shows that at low intial phenanthrene concentrations such as 0.77 mg/L, only about 0.1 mg/L phenanthrene is biodegraded. However, when the phenanthrene concentration is increased to 5.18 mg/L, about 1.3 mg/L of phenanthrene can be degraded.

7.2.4.4 Effect of Biomass

Figure 7.15 shows the effect of initial biomass on the biodegradation of phenanthrene at a surfactant concentration of 100 mg/L and an initial phenanthrene concentration of 1.05 mg/L.

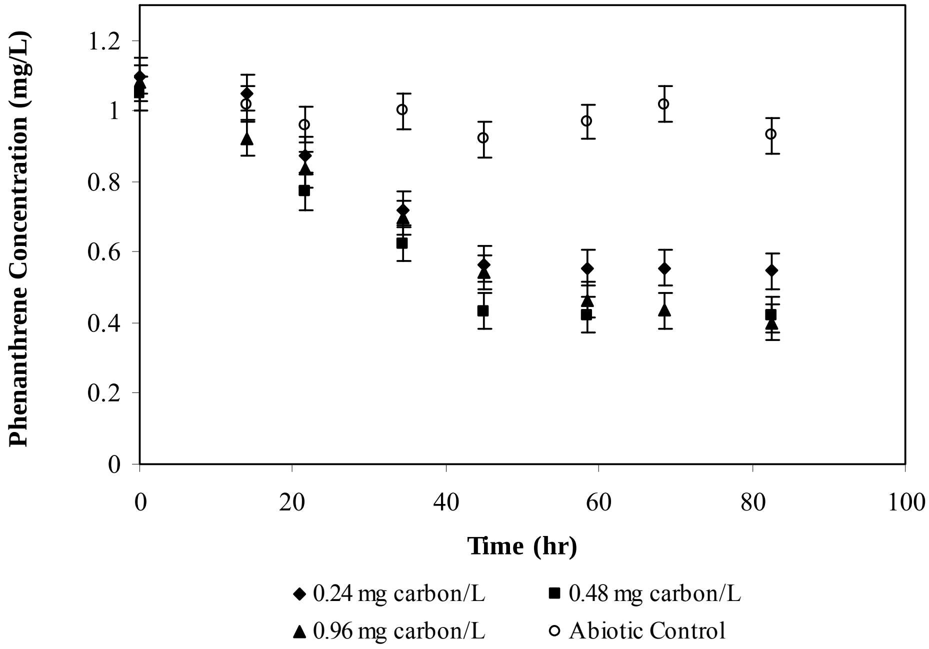


Figure 7.15 Effect of Initial Biomass Concentration on Biodegradation of Phenanthrene (Tergitol 15-S-7 Concentration: 100 mg/L; initial phenanthrene concentration:1.05 mg/L).

It is found that the increase of the initial biomass concentration from 0.24 mg carbon/L to 0.96 mg carbon/L slightly increased the biodegradation of phenanthrene. After 80 h, the biodegradation of phenanthrene was about 0.53 mg/L at 0.24-0.48 mg carbon/L biomass, as compared to 0.68 mg/L at 0.96 mg carbon/L biomass. More bacteria in the medium can consume the phenanthrene in the aqueous phase at a faster rate, which would create a greater concentration difference of phenantherene between the aqueous and the micellar phases and thus enhance more phenanthrene molecules to diffuse from the micellar phase into the aqueous phase. As a result, more phenanthrene molecules were biologically available and hence an increased biodegradation of phenanthrene was obtained.

7.2.4.5 Mechanisms of the Surfactant Effects on Biodegradation

The biodegradation of phenanthrene includes the degradation of free phenanthrene molecules in the aqueous phase and degradation of phenanthrene in the micellar phase. The biodegradation of free phenanthrene molecules is controlled by the diffusion of the molecules to the cell surface or enzyme sites. The phenanthrene in the micellar phase is degraded either by first diffusing into the aqueous phase and then utilized by the bacteria or by directing microbial uptake from the micelles. The first process is controlled by the kinetics of micellar aggregation. The relaxation time of the micelle is typically on the order of milliseconds to microseconds. Therefore, the first process is normally not a rate limiting step. The second process is discribed in Figure 7.16.

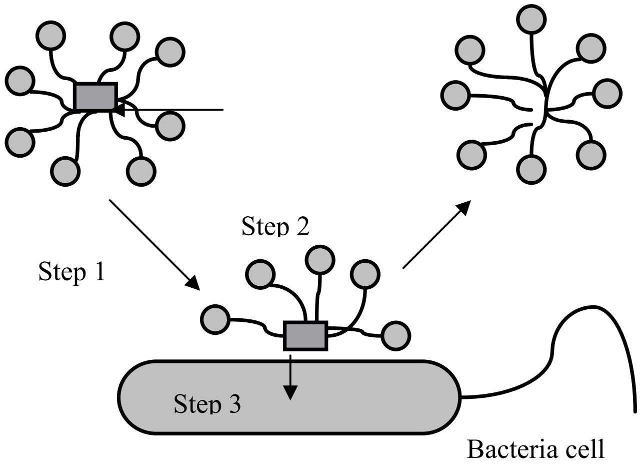


Figure 7.16 Bioavailability of a Substrate in the Micellar Phase

As shown in Figure 16, the mass transfer from micelle into cell is composed of three steps. The first step is the transport of the micelle solubilized with a sustrate to the vicinity of the cells or enzymes by mixing. The second step is the exchange of the filled micelles with the hemimicellar layer of surfactant molecules formed around the cells. The formation of hemi-micelle layer around the cell or other substrates has been proposed and used successfully by many authors to describe the biodegradation (Guha and Jaffé, 1996) and dissolution of PAH (Grimberg et al., 1995; Volking et al., 1995). And the third step is the transfer of the substrate from the hemi-micelle to the cell. In a well-stirred system, the first step is also not a rate limiting step. The second and the third step normally controlls the bodegradation of a substrate in the micellar phase. The process is affected by the specific interactions between the micelle surface and the cell surface. It has been reported that the specific interaction between the micelle surface and the cell surface, such as the affinity of the two surfaces, is a limiting factor in controlling the transport of the substrate from the micelle to the cell (Guha and Jaffé, 1996; Boonchan et al., 1998; Allen et al., 1999).

Based on the experimental results presented above, three mechanisms may be conjectured on the effects of surfactant:

Mechanism 1: Lower bioavailability of phenanthrene at higher surfactant concentrations. For a certain amount of phenanthrene, increasing surfactant concentration, more phenanthrene will partition into the surfactant micelles. Compared to the phenanthrene in the aqueous phase, the phenanthrene in the micellar phase has a lower bioavilability, since phenanthrene in this phase largely can not be accessed by the bacteria directly. It has to be transferred into the aqueous phase before being utilized by the bacteria. Although it is possible that the bacteria can take the substrate in the micelle directly, the fraction of the directly available substrate normally decreases with increase in surfactant concentration.

Mechanism 2: Slower mass transfer at high surfactant concentrations. It has been shown that the biodegradability of the surfactants decreases with increase in their concentration. Therefore, when phenanthrene is being consumed, a smaller amount of phenanthrene will diffuse into the aqueous phase at a higher surfactant concentration. In addition, at higher surfactant concentrations, the phenanthrene was dispersed in a large number of micelles, which leaves a smaller fraction of phenanthrene in the aqueous phase. This induces a lower concentration gradient between the individual micelle and the aqueous phase, as well as a reduced concentration gradient between individual micelle and cell. It will result in a reduced mass-transfer of phenanthrene from the micellar phase to the aqueous phase and to the cells (Doong and Lei, 2003). Therefore, the lower surfactant concentrations are more appropriate in the viewpoint of achieving higher bioavailability. To improve the bioavailability, selection of suitable bacteria is very important, because the specific interaction between bacteria and surfactant may influence significantly the bacterial uptake of solubilized substrates (Allen et al., 1999).

Mechanism 3: Reduced microbial activity at higher surfactant concentrations may also contribute to the low biodegradability of phenanthrene.

7.2.4.6 Biodegradability and Bioavailability

Likewise, the biodegradability of phenanthrene, B_d, may be expressed as:

$$B_d = 1 - \frac{C_f}{C_0} \tag{7.7}$$

where C_f is the phenanthrene concentration at the end of the biodegradation process or the concentration before the total loss of solubilization power at low surfactant concentrations (refer to Figures 7.10); and C_0 is the initial phenanthrene concentration.

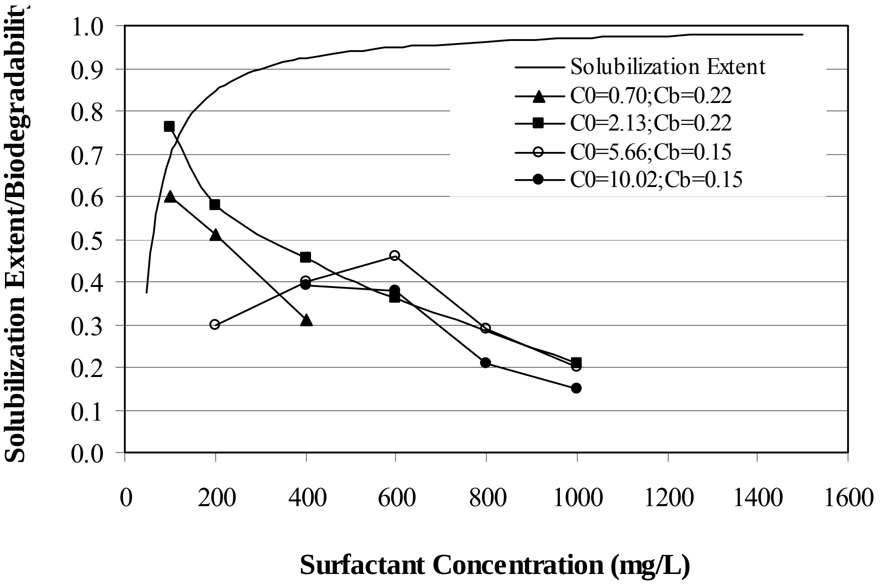


Figure 7.17 Solubilization Extent and Biodegradability of Phenanthrene as a Function of Tergitol 15-S-7 Concentration.

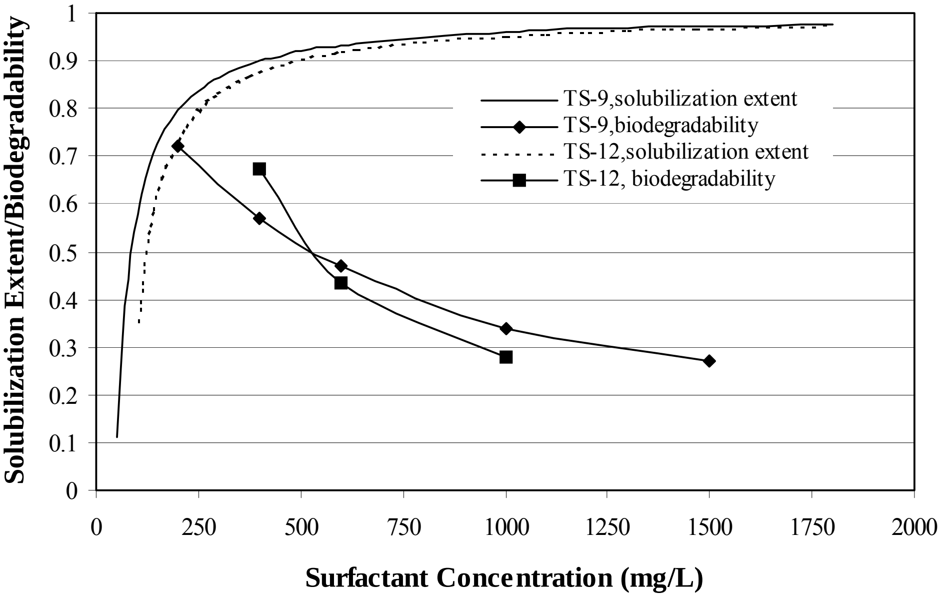


Figure 7.18 Solubilization Extent and Biodegradability of Phenanthrene as a Function of Tergitol 15-S-9 and Tergitol 15-S-12 Concentration.

Experimental results have shown that at the same initial phenanthrene concentration, the biodegradability of phenanthrene decreases with increase in surfactant concentration, or the availability of phenanthrene to the bacteria is reduced (Figures 7.17 and 7.18). For examples, at an initial phenanthrene concentration of 2.13 mg/L, the biodegradability decreased from 0.76 at 100 mg/L of Tergitol 15-S-7 to 0.21 when the surfactant concentration was increased to 1000 mg/L.

The decrease in biodegradability of phenanthrene may be attributed the reduced bioavailability of phenanthrene in the micellar solution at higher surfactant concentrations. Since the solubility of phenanthrene in the micellar solution increases with the increase of surfactant concentrations in the medium, greater percentages of the total phenanthrene in the medium will be solubilized into the micellar phase and become less biologically available for biodegradation.

If one defines α as the solubilization extent of phenanthrene in the micellar solution, there are:

$$\alpha = C_{mic} / C \tag{7.8}$$

$$C = C_{mic} + C_a \tag{7.9}$$

where C_{mic} (mg/L) is the concentration of phenanthrene in the micellar phase; C_a (mg/L) is the concentration of phenanthrene in the aqueous phase; and C (mg/L) is the apparent concentration of phenanthrene solubilized in the micellar solution (including both in the aqueous phase and in the micellar phase). Furthermore, C_{mic} can be calculated using the following equations,

$$C_{mic}=S_{mic}\cdot K_{mw}\cdot C_a \tag{7.10}$$

$$S_{mic} = S - CMC \tag{7.11}$$

where S_{mic} (mg/L) is the surfactant concentration in micellar phase; and K_{mw} (L/mg) is the micelle-water partition coefficient of phenanthrene. The partition coefficient K_{mw} is defined as the ratio of the phenanthrene concentration in micellar phase, C_m (mg phenanthrene/ mg micellized surfactant) to its aqueous concentration C_a, (mg phenanthrene/L bulk solution), i. e.

$$K_{mw} = \frac{C_m}{C_{aqu}} = \frac{C_{mic}}{S_{mic}C_{aqu}} = \frac{\alpha C}{S_{mic}(1-\alpha)C} = \frac{\alpha}{S_{mic}(1-\alpha)} \tag{7.12}$$

The rearrangement of Eq.(7.12) gives

$$\frac{1}{\alpha} = 1 + \frac{1}{K_{mw}S_{mic}} \tag{7.13}$$

Eq.(7.13) indicates that the solubilization extent, α, increases with the surfactant concentration, S_{mic}, and is independent of the phenanthrene concentration, C. This is understandable since the distribution of phenanthrene between the two phases, i.e. the aqueous and micellar phases, is determined by the partition equilibrium and the amount of micelles in the micellar solution. The increase of α with these three surfactant concentrations, as calculated from Eq.(7.13), is also shown in Figures 7.17 and 7.18.

Bioavailability of phenanthrene may be depicted by an effective concentration of phenanthrene in the micellar solution available for biodegradation. The effective concentration, C_e, can conceptually be assumed to include the phenanthrene dissolved in the aqueous phase and a fraction of the phenanthrene in the micellar phase (Guha and Jaffé, 1996), i.e.

$$C_e = C_a + \beta \cdot C_{mic} \tag{7.14}$$

where β is a coefficient, ranging from 0 to 1, dependent on surfactant concentration. From Eqs.(7.10) and (7.12), one can have:

$$C_a = \frac{C}{1 + S_{mic} K_{mw}} \tag{7.15}$$

Introduction of Eqs.(7.10) and (7.15) into Eq.(7.14) leads to:

$$C_e = \left(\frac{1 + \beta K_{mw} S_{mic}}{1 + K_{mw} S_{mic}} \right) \cdot C \tag{7.16}$$

with $\beta = 1$, $C_e = C$ and $\beta = 0$, $C_e = C_a$.

With further substitution of Eq.(7.12) into Eq.(7.16), it gives

$$C_e = \left[1 - (1 - \beta) \cdot \alpha \right] \cdot C \tag{7.17}$$

At a constant initial phenanthrene concentration C and with an increase in the surfactant concentration, β decreases but α increases. It can be seen from Eq.(7.17) that, the effective concentration or the bioavailability of phenanthrene in the micellar solution decreases with the increase of surfactant concentration. Eq.(7.17) also indicates that, at a constant surfactant concentration (i.e. constant α and β), the effective concentration or the bioavailability of phenanthrene in the micellar solution increase with the initial phenanthrene concentration, C. Since phenanthrene is biodegradable, a greater effective concentration or the bioavailability of phenanthrene in the micellar solution would be converted to a higher biodegradability of the phenanthrene. Hence, Eq.(7.17) implies that biodegradation of phenanthrene in the micellar solution will be affected inversely by the concentration of the surfactant, and be enhanced by the initial phenanthrene concentration dissolved in the micellar solution. This conclusion is in fact in agreement with the experimental results.

The effect of surfactant concentration and initial phenanthrene concentration on phenanthrene biodegradation may be further explained as in the following. From Eq.(7.15), it can be seen that the increase of surfactant concentration (i.e. S_{mic}) reduces C_a, the phenanthrene concentration in the aqueous phase (more phenanthrene distributed into the micellar phase). This makes phenanthrene in the micellar solution be less biologically available because phenanthrene in the aqueous phase is more accessible than phenanthrene in the micellar phase by bacteria.

Apparently, the increase of initial phenanthrene concentration, C, in the micellar solution will increase C_a; see Eq.(7.15). On the other hand, the micellar phase will be more saturated with phenanthrene at a higher C because more phenanthrene is dissolved into the micellar phase. As a consequence, the difference between the actual concentration and the saturation concentration (determined by the solubility; see Fig.7.1) in the micellar phase will be reduced. In this case, phenanthrene in the micellar phase will then have a greater tendency to diffuse out from the micellar phase in the aqueous phase and become accessible to bacteria if the phenanthrene in the aqueous phase is consumed by bacteria.

7.2.4.7 Biodegradation of Surfactant in the Presence of Phenanthrene

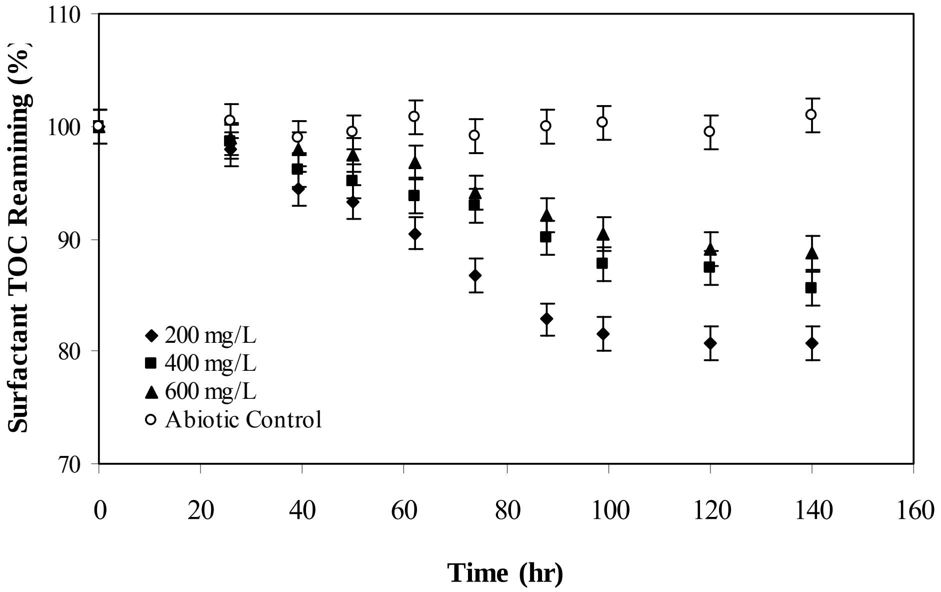


Figure 7.19 Biodegradation of Tergitol 15-S-7 in the Presence of Phenanthrene (Initial phenanthrene Concentration: 2.13 mg/L; Initial Biomass: 0.22 mg carbon/L).

Figure 7.19 shows the biodegradation of Tergitol 15-S-7 solubilized with 2.13 mg/L of phenanthrene. Compared with its biodegradation in the absence of phenanthrene, which is given in Figure 7.20, a slower degradation rate is evident at the early stage when phenanthrene is present. That is, phenanthrene is preferably degraded by the bacteria. However, the ultimate biodegradability of Tergitol 15-S-7 at 400 and 600 mg/L is slightly higher than the corresponding values in the absence of phenanthrene. This means that the presence of phenanthrene is conducive to the biodegradation of this surfactant at higher concentrations. The presence of phenanthrene could support the bacterial growth, which in turn enhanced the degradation of surfactants. Similar results were obtained for the other two surfactants. Figure 7.21 displays the biodegradation of Tergitol 15-S-12 in the presence and absence of phenanthrene.

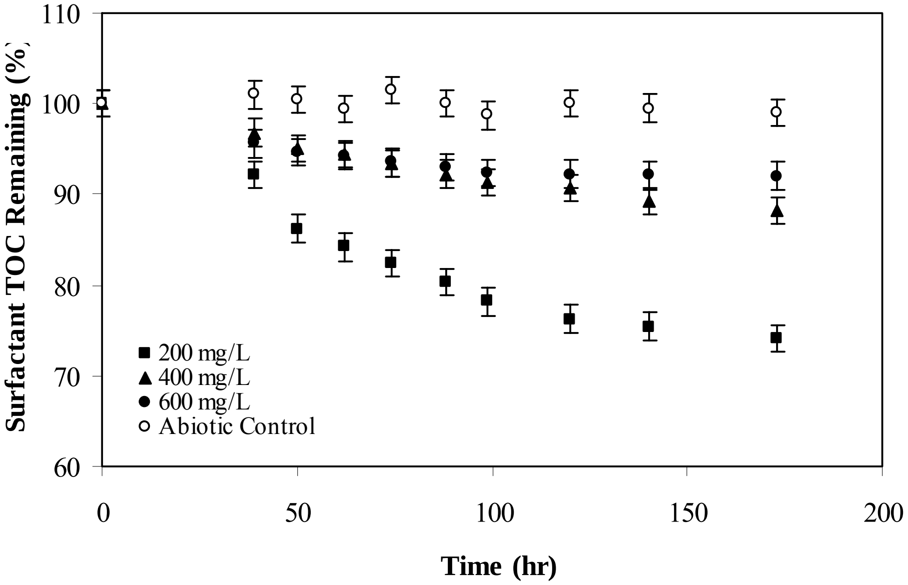


Figure 7.20 Biodegradation of Tergitol 15-S-7 in the Absence of Phenanthrene (Initial Biomass: 0.22 mg carbon/L).

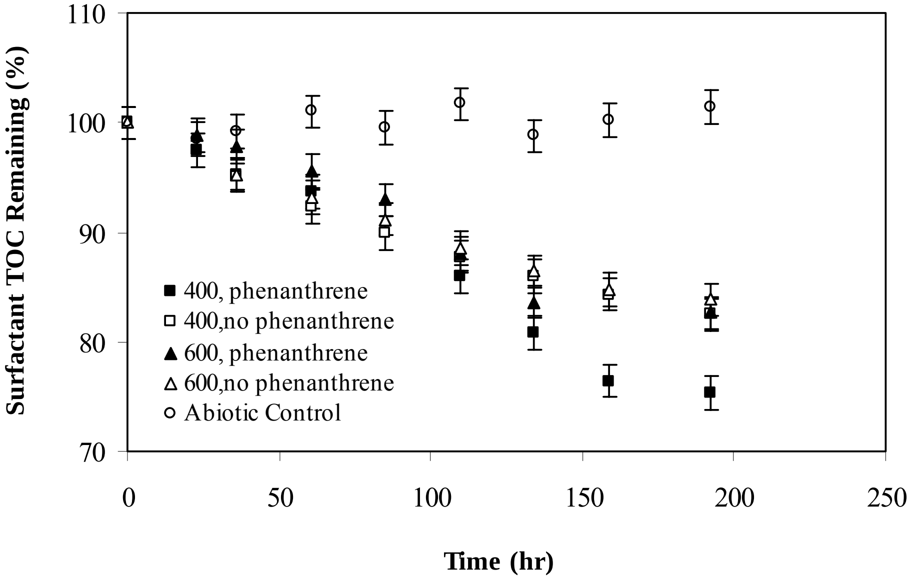


Figure 7.21 Effect of Phenanthrene on the Biodegradation of Tergitol 15-S-12 (Surfactant concentration: 400 and 600 mg/L; initial phenanthrene concentration: 1.64 mg/L; initial biomass: 0.97 mg/L).

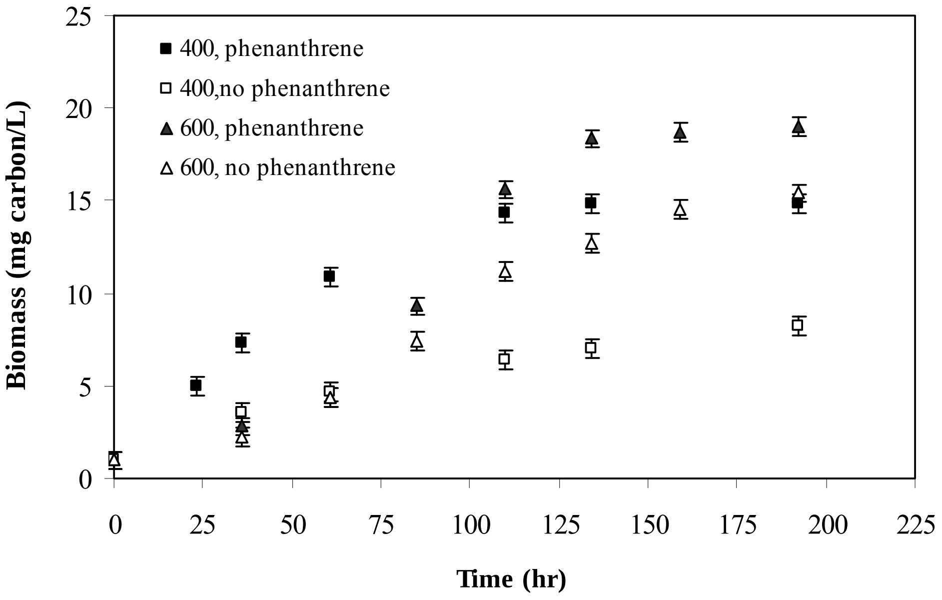


Figure 7.22 Effect of Phenanthrene on Bacterial Growth on Tergitol 15-S-12 (Surfactant concentration: 400 and 600 mg/L; initial phenanthrene concentration: 1.64 mg/L; initial biomass: 0.97 mg/L).

The enhanced biodegradation of surfactant in the presence of phenanthrene is also indicated in the biomass growth. Figure 7.22 gives the bacterial growth on Tergitol 15-S-12 in the presence and absence of phenanthrene. The results show that the presence of phenanthrene supports the bacterial growth. Hence, the biodegradability of the surfactant is higher.

7.3 Implications for Surfactant-mediated Bioremediation

The observations in this study are very meaningful to the application of surfactant in *in-situ* bioremediation process since readily biodegradable surfactants will be the preferences in the future field applications. The advantage of using readily biodegradable surfactants is their low toxicity and non-accumulation in the environment. However, their readily biodegradable nature also poses a big disadvantage. That is, loss of solubilization power. To compensate this, lots of preliminary work is needed to do for the selection of suitable surfactant and suitable surfactant concentrations. Economic consideration requires low usage of surfactants. Experimental results of this chapter indicate that lower surfactant concentration may contribute to higher bioavailability. However, for effective solubilization, it should not be reduced without bound. In addition, loss of surfactant during the in-situ application is sometimes significant. Therefore, to estimate the loss of surfactants due to the biodegradation and other processes such as sorption by soil are of crucial importance. There are also some other important operating factors to be considered in the screening of suitable surfactant system, such as turbidity and foam generation (Deshpande et al., 1999). For a selected surfactant, efforts also need to make on the screening of suitable microorganisms. Firstly, such a microorganism should be tolerant of the surfactant (or the surfactant is not toxic to it) over a wide range of concentration. Secondly, it does not preferably use the surfactant as carbon source. Thirdly, it should be compatible with the surfactant, or in other words, the surfactant does not inhibit the attachment of the cells to the micelle-water interface, so that direct microbial uptake of hydrocarbons from the micellar phase is possible. This feature could improve bioavailability of the solubilized hydrocarbons.

7.4 Conclusions

Based on the results, the following major conclusions can be drawn:

(1) Tergitol 15-S-X (X=7, 9, 12) are not toxic and readily biodegradable to the marine bacteria *Neptunomonas naphthovorans* (ATCC 700638). Bacterial growth was observed at surfactant concentrations up to 1 wt%. The biodegradation can be described by a first-order kinetics model. The biodegradability of the surfactants decreased when the surfactant concentrations are increased to 600 mg/L. Tergitol 15-S-

12 has a much lower biodegradability than the other two surfactants, while the biodegradability of Tergitol 15-S-7 and Tergitol 15-S-9 are close to each other. The difference might be attributable to the longer hydrophilic moiety of the Tergitol 15-S-12 molecule, which makes the micelle surface more hydrophilic and hinders the contact of hydrophobic cell surface to the micelles and to the hydrophobic moiety.

(2) There are two competing effects of the surfactant on the biodegradation of phenanthrene. On the one hand, at the same apparent phenanthrene concentration, the biodegradability of phenanthrene decreases with increase in surfactant concentration. That is, the presence of the surfactant reduced the bioavailability of phenanthrene in the micellar solution. In other words, the surfactant is not beneficial to the biodegradation of phenanthrene. On the other hand, the use of surfactant can enhance the biodegradation or increase the bioavailability due to the increased solubility of phenanthrene, which is a result of solubilization. Therefore, suitable surfactant concentrations need to be pre-determined for an in-situ bioremediation application. The reduced bioavailability of phenanthrene in the micellar phase at higher surfactant concentrations could be attributed to the larger solubilization extent. That is the relatively larger fraction of total phenanthrene was in the micellar phase. Or, in another word, the fraction of phenanthrene in aqueous phase that can be directly utilized by the bacteria becomes smaller. The reduced mass-transfer from the surfactant micelles into aqueous phase may make a greater contribution to the reduced biodegradability at higher surfactant concentrations.

(3) At the same surfactant concentration but different initial phenanthrene concentrations, the biodegradability of phenanthrene is larger when more phenanthrene is solubilized in the micellar solution. This is attributable to the increase of phenanthrene concentration in the aqueous phase, which serves as a direct food source for the bacteria, and a greater mass transfer rate of phenanthrene from the micellar phase to the aqueous phase when the micelles are more “saturated”.

(4) Suitable surfactant concentrations need to be optimized for the successful *in-situ* bioremediation applications.

Chapter 8 Conclusions

8.1 Conclusions

In this work, nonionic surfactants of Tergitol 15-S-X (X = 5, 7, 9 and 12) were examined on their effectiveness in solubilizing, extracting and enhancing the biodegradation of polycyclic aromatic hydrocarbons. Choice of these surfactants is based on their readily biodegradability. They are linear secondary alcohol ethoxylates and do not possess any aromatic group in their molecules.

In Chapter 4, the solubilization capacity of Tergitol 15-S-X (X = 7, 9 and 12) was examined. It was observed that Tergitol 15-S-7 had comparable solubilization capacity as conventional surfactants, such as Triton X-100. For the surfactants from the same series, the solubilization capacity increases with decreasing HLB numbers. The solubilization capacity of these three surfactants are in the order of Tergitol 15-S-7 > Tergitol 15-S-9 >Tergitol 15-S-12. With Tergitol 15-S-7 as a model, various factors that may affect the solubilization capacity of the surfactants were tested. The hydrophobicity of solubilizate is a crucial factor. A good linear relationship was obtained between $logK_m$ and $logK_{ow}$. Temperature is also a very important factor. The solubilization capacity was observed to increase with increase in temperature. This is attributable to the increase in the micelle aggregation number and the size of the micelles. The salinity effect was also examined. Increasing salinity has a positive effect on the solubilization capacity. Increasing aggregation number and micelle size was also observed with the aid of light scattering analysis. Change in conformation of the micelles in the presence of Na^+ may also increase the solubilization capacity. Synergic effect was observed when mixtures of PAH were used. For these three PAHs, naphthalene, phenanthrene and pyrene used in the study, the solubility of phenanthrene was increased by the presence of naphthalene and pyrene. The highest solubility of phenanthrene was obtained in the ternary mixtures. Similar results were obtained for pyrene in the presence of naphthalene and phenanthrene.

The phase behavior of Tergitol 15-S-5 and Tergitol 15-S-7 was observed in Chapter 5. The phase separation temperatures of these two surfactants at concentrations above 1 wt% were measured. The results showed that the temperature increased with increase in surfactant concentration. At 1 wt%, the value is 23.5 and 37.8 ^{o}C for Tergitol 15-S-5 (W+L_3) and Tergitol 15-S-7 (W+L_1), respectively. To facilitate phase separation, the effects of sodium chloride on Tergitol 15-S-5 solutions were examined. It was observed that the presence of this salt could significantly reduce the phase separation temperature. The increase in salt concentration could also facilitate the phase separation by increasing the density of water, dehydrating the surfactant-rich phase, and hence reducing the volume of the surfactant-rich phase. The effects of some factors on the phase separation temperature or cloud point of Tergitol 15-S-7 were studied. Among the salts examined, sodium sulfate and sodium phosphate could decrease the cloud point of this surfactant dramatically. The difference in the effects of different salts can be attributed to the different effects of salts on the solvent property of water and consequently, the solubility of surfactant molecules. Salts that can break the association of water molecules, which enhances the solvent property of water, have the effect of elevating the cloud point. The presence of an ionic surfactant, SDS above a certain concentration was observed to increase the cloud point of Tergitol 15-S-7. This might be due to the adsorption of charged SDS molecules on the micelles of the nonionic surfactant. The result, showing increased electrostatic repulsion between nonionic micelles, makes it more difficult for the micelles to aggregate together. The cloud points of binary mixtures of Tergitol 15-S-7/Tergitol 15-S-9, Tergitol 15-S-9/Neodol 25-7 was observed to be between the cloud points of the constituent surfactants. The effects of alcohols on the cloud point of Tergitol 15-S-7 were also investigated. The observation indicates that alcohols of short chain length elevate the cloud point, while alcohols with longer chain length have depressing effect. Finally in this chapter, the cloud points of Tergitol 15-S-X (X=7, 9 and 12) could be correlated linearly to the logarithm of their oxide number.

In Chapter 6, a simple but effective extraction process was developed for the extraction of model PAHs. The results showed that the slightly hydrophobic surfactant Tergitol 15-S-5, which forms mesophases, (L_3) rather than the L_2 phase in cloud point extraction by hydrophilic surfactants could indeed be used for the surfactant-based extraction process on the polycyclic aromatic hydrocarbons (PAHs). Due to its higher hydrophobicity, this surfactant has higher extraction efficiency than Tergitol 15-S-7. NaCl and Na_2SO_4 were used to enhance the phase separation of Tergitol 15-S-5 and Tergitol 15-S-7 solutions respectively. It was observed that increasing salt concentration could improve the preconcentration factor. This is attributable to the smaller water content of the surfactant-rich phase and hence the smaller volume of that phase. Higher preconcentration factor could also be obtained at lower surfactant concentrations and higher temperature. This is also due to the smaller volume of the surfactant-rich phase. The partition of model PAHs was measured when Tergitol 15-S-7 was used. It was observed that the partition coefficient was independent of surfactant concentration, and could be improved by increasing Na_2SO_4 concentration. A good linear relationship was obtained between $logK_p$ and $logK_{ow}$, which indicates that the partition of PAH into the surfactant-rich phase is driven by the hydrophobic affinity of PAH molecules to the surfactant molecules. The partition coefficients of PAHs were also correlated linearly to their molar volumes. Results of recovery showed that increasing surfactant and salt concentration could improve the recovery of PAH. Recoveries higher than 90% were obtained. This indicates that extraction using the two nonionic surfactants is efficient for the recover of PAHs, from aqueous solutions. A process, which combines micellar solubilization and cloud point extraction, was used to recover phenanthrene from spiked sand samples. The dissolution kinetics of phenanthrene from sand into water and micellar solution was measured. A first-order kinetics was observed. The results showed that the lumped mass-transfer coefficient, k_l decreased with increase in surfactant concentration, while recovery of the micellar solubilization process increased. The total recovery of the process for phenanthrene is over 90%. The results obtained in this chapter showed that Tergitol 15-S-5 and Tergitol 15-S-7 are good choice for the preconcentration and extraction of PAH. Use of these surfactants can provide both a simple process and high preconcentration factor and recovery.

Biodegradation experiments showed that Tergitol 15-S-X (X= 7, 9 and 12) are readily biodegradable to the marine bacteria (ATCC 700638). A first-order kinetics model can be used to describe the biodegradation process. Solubilization by these surfactants enhanced the biodegradation of phenanthrene. The enhancement can be attributed to the increased solubility and hence larger bioavailability. At the same apparent phenanthrene concentration, the bioavailability of phenanthrene decreases with increase in surfactant concentration. This may be due to the relatively larger fraction of totally phenanthrene in the micellar phase. Or, the fraction of phenanthrene in aqueous phase that can be directly utilized by the bacteria becomes smaller. At the same surfactant concentration, the biodegradability of phenanthrene is larger at higher phenanthrene concentration. The above results indicate that surfactant concentration is an important factor that determines the efficiency of the biodegradation process. Lower surfactant concentrations are beneficial to the process. However, biodegradation and other factors such as adsorption to the soil matrix could contribute to a significant loss of surfactant. Therefore, suitable surfactant concentrations need to be predetermined for *in-situ* bioremediation applications.

8.2 Recommendations for Further Research

Tergitol 15-S-X surfactants were used for the first time in this study for the solubilization, extraction and biodegradation of PAHs in aqueous solutions. The results are very encouraging. However, this project is only a primary study. Further research is recommended to carry out in the following aspects.

(1) To examine the effectiveness of theses surfactants in the solubilization and extraction of PAHs from naturally contaminated matrix.

(2) The partition of PAH in PAHs mixtures between the surfactant-rich phase and the aqueous phase needs to be studied, as they normally coexist rather than exist individually in the environment. The partition of one PAH may be affected by the presence of other PAHs, since synergistic solubilization has been observed.

(3) Further studies may need to be carried out to improve the bioavailability of PAH solubilized in the micellar solutions of Tergitol 15-S-X surfactants. These may

include the investigation into the inhibition mechanism of these surfactants to the marine bacteria used in the study. For example, whether the formation of micelles inhibits the microbial uptake of PAH, or equivalently, whether the PAH molecules in the micelles can be easily accessed by the bacteria? As has been reported that the affinity of the cell surface to the micelle-water interface is an important factor that affects the bioavailability of a substrate in the micelles, characterization of cell surface and the affinity examination may have to be performed. On the other hand, selection of suitable microorganisms, which have a great affinity to the micelles and are less likely to use the surfactants as carbon sources, may also need to be accomplished. To maintain sufficient solubilization capacity during the biodegradation of PAHs, the surfactants should not be too easily biodegraded by the microorganism. Nevertheless, the selected microorganism should be versatile in decomposing a great number of PAH components.

References

Abdul, A. S., T. L. Gibson, C. C. Ang, J. C. Smith and R. E. Sobczynski. In-situ Surfactant Washing of Polychlorinated Biphenyls and Oils from a Contaminated Site, Ground Wat., 30(2), pp. 219-231.1992.

Abraham, M. H. Scales of Solute Hydrogen-bonding- Their Construction and Application to Physicochemical and Biochemical Processes, Chem. Soc. Rev., 22, pp.73-83. 1993.

Adamson, A. W. and A. P. Gast. Physical Chemistry of Surfaces. 6th ed., New York: Wiley. 1997.

Al-Ghamdi, A. M. and H. A. Nasr-El-Din. Effects of Oilfield Chemicals on the Cloud Point of Nonionic Surfactants, Colloid Surface A, 125, pp. 5-18. 1997.

Akita, S. and H. Takeuchi. Cloud Point Extraction of Organic Compounds from Aqueous Solutions with Nonionic Surfactant, Separ. Sci. Technol., 30, pp. 833-846. 1995.

Akita, S. and H. Takeuchi. Equilibrium Distribution of Aromatic Compounds between Aqueous Solutions and Coacervate of Nonionic Surfactants, Separ. Sci. Technol., 31, pp. 401-412. 1996.

Allen, C. C. R., D. R. Boyd, F. Hempenstall, M. J. Larkin and N. D. Sharma. Contrasting Effects of a Nonionic Surfactant on the Biotransformation of Polycyclic Aromatic Hydrocarbons to cis-Dihydrodiols by Soil Bacteria. Appl. Environ. Microbiol. 65 (3). Pp. 1335-1339. 1999.

An, YJ, E. R. Carraway and M. A. Schlautman. Solubilization of Polycyclic Aromatic Hydrocarbons by Perfluorinated Surfactant Micelles, Wat. Res., 36, pp. 300-308. 2002.

Aramaki, K., U. Olsson, Y. Yamaguchi and H. Kunieda. Effect of Water-Soluble Alcohols on Surfactant Aggregation in the $C_{12}EO_8$ Systems, Langmuir, 15, pp. 6226-6232. 1999.

Aronstein, B. N., Y. M. Calvillo and M. Alexander. Effect of Surfactant at Low Concentrations on the Desorption and Biodegradation of Sorbed Aromatic Compounds in Soil, Environ. Sci. Technol., 25, pp. 1728-1731. 1991.

Attard, G. S., S. Fuller and G. J. T. Tiddy. Influence of Added Electrolytes on the Lytropic Phase Behavior of Triethylammoniodecyloxycyanobiphenyl Bromide (OCB-C10NEt3Br), J. Phys. Chem. B., 104, pp. 10426-10436.

Auger, R. L., A. M. Jacobson and M. M. Domach. Effect of Nonionic Surfactant Addition on Bacterial Metabolism of Naphthalene: Assessment of toxicity and overflow metabolism potential, J. Hazard. Mater., 43, pp. 263-272. 1995.

Bai, D. S., J. L. Li, S. B. Chen and B.-H. Chen. A Novel Cloud-Point Extraction Process for Preconcentrating Selected Polycyclic Aromatic Hydrocarbons in Aqueous Solution, Environ. Sci. Technol., 35, pp. 3936-3940. 2001.

Bardi, L., A. Mattei, S. Steffan and M. Marzona. Hydrocarbon Degradation by a Soil Microbial Population with ß-Cyclodextrin as Surfactant to Enhance Bioavailability. Enzyme Microb. Tech., 27, pp. 709-713. 2000.

Berthod, A., S. Tomer and J. G. Dorsey. Polyoxyethylene Alkyl Ether Nonionic Surfactants: physicochemical properties and use for cholesteroldetermination in food, Talanta, 55, pp. 69-83. 2001.

Billingsley, K. A., S. M. Backus, S. Wilson, A. Singh and O. P. Ward. Remediation of PCBs in Soil by Surfactant Washing and Biodegradation in the Wash by *Pseudomonas sp LB400*. Biotechnol. Lett., 24(21), pp. 1827-1832. 2002

Blankschtein, D., G. M. Thurston and G. B. Benedek. Phenomenological Theory of Equilibrium Thermodynamic Properties and Phase Separation of Micellar Solutions, J. Chem. Phys., 85, pp. 7268-7288. 1986.

Böckelen, A. and R. Niessner. Combination of Micellar Extraction of Polycyclic Aromatic Hydrocarbons from Aqueous Media with Detection by Synchronous Fluorescene, Fresenius J. Anal. Chem., 346, pp. 435-440. 1993.

Boonchan, S., M. L. Britz and G. A. Stanley. Surfactant-enhanced Biodegradation of High Molecular Weight Polycyclic Aromatic Hydrocarbons by Stenotrophomonas maltopholia, Biotechnol. Bioeng., 59, pp. 482-494.1998.

Bordier, C. Phase Separation of Integral Membrane Proteins in Triton X-114 Solutions, J. Biol. Chem. 256, pp. 1604-1611. 1981.

Brown, D. G., C. D. Knightes and C. A. Petter. Risk Assessment for Polycyclic Aromatic Hydrocarbon NAPLs Using Component Fractions. Environ. Sci. Technol. 33 (24). Pp. 4357-4363. 1999.

Buhai, L. and M. Rigan. Liquid Solid Extraction System Based on Tween 40-Salt-H_2O without Organic Solvent, Talanta, 37, pp. 885-888. 1990.

Bury, S. J. and C. A. Miller. Effect of Micellar Solubilization on Biodegradation Rates of Hydrocarbons, Environ. Sci. Technol., 27, pp.104-110. 1993.

Carabias, M. R., G. E. Rodriguez, A. J. Dominguez and M. J. Hernandez. Cloud Point Extraction as a Preconcentration Step Prior to Capillary Electrophoresis, Anal. Chem. 71(13), pp. 2468-2474. 1999.

Carrol, B. J. The Kinetics of Solubilization of Nonpolar Oils by Nonionic Surfactant Solutions, J. Colloid Interf. Sci., 79, pp. 126-135. 1981.

Casero, I., D. Sicilia, S. Rubio and D. Pérez-Bendito. An Acid-induced Phase Cloud Point Separation Approaching Using Anionic Surfactants for the Extraction and Preconcentration of Organic Compounds. Anal Chem., 71, pp. 4519-4526. 1999.

Cebula, D. J. and R. H. Ottewill. Neutron Scattering Studies on Micelles of Dodecylhexaoxyethylene Glycol Monoether, Colloid Polym. Sci. 260, pp. 1118-1120. 1982.

Charlton, I. D. and A. P. Doherty. Electrolyte-Induced Structural Evolution of Triton X-100 Micelles., J. Phys. Chem. B., 104, pp. 8327-8332. 2000.

Charlton, I. D. and A. P. Doherty. Combined Effect of Temperature and Electrolyte on Triton X-100 Micellar Diffusion, Colloid Surface A, 182, pp. 305-310. 2001.

Chao, C. Y. H., L. L. Yeung and P. S. H. Choi. Quantification of Polycyclic Aromatic Hydrocarbons and Aliphatic Hydrocarbons in Air Particulate Samples in Homes. Indoor Built Environ., 11(3), pp. 123-133. 2002.

Chen, B.-H., C. A. Miller and P. R. Garrett. Rates of Solubilization of Triolein into Nonionic Surfactant Solutions. Colloid Surface A, 128, pp.129-143.1997.

Chen, B.-H., C. A. Miller and P. R. Garrett. Rates of Solubilization of Triolein/fatty Acid Mixtures by Nonionic Surfactant Solutions, Langmuir, 14, pp. 31-41.1998.

Chen, G., K. A. Strevett and B. A. Vanegas. Naphthalene, Phenanthrene and Surfactant Biodegradation. Biodegradation. 12. pp. 433-442. 2001.

Choi, M. P. K., K. K. C. Chan, H. W. Leung and C. W. Huie. Pressurized Liquid Extraction of Active Ingredients (Ginsenosides) from Medicinal Plants Using Nonionic Surfactant Solutions. J. Chromatogr. A. 98. pp. 153-162. 2003

Clemetson, K. J., D. Bienz, M.-L. Zahno and E. F. Lüscher. Distribution of Platelet Glycoproteins and Phosphoproteins in Hydrophobic and Hydrophilic Phases in Triton X-114 Phase Partition, Biochem. Biophys. Acta, 778, pp. 463-469. 1984.

Collén, A., K. Selber, T. Hyytiä, J. Persson, T. N. Setlä, M. Bailey, R. Fagerström, M. R. Kula, M. Penttilä, H. Stålbrand and F. Tjerneld. Primary Recovery of a Genetically Engineered *Trichoderma reesei* Endoglucanase I (Cel 7B) Fusion Protein in Cloud Point Extraction Systems. Biotechnol. Bioeng., 78(4), pp. 385-394. 2002.

Cort, T. L. and A. R. Bielefeldt. A kinetic Model for Surfactant Inhibition of Pentachlorophenol Biodegradation. Biotechnol. Bioeng. 78 (6). pp. 606-616. 2002.

Cort, T. L., M. S. Song and A. R. Bielefeldt. Nonionic Surfactant Effects on Pentachlorophenol Biodegradation. Wat. Res., 36, pp. 1253-1261. 2002.

Deshpande, S., B. J. Shiau, D. Wade, D.A. Sabatini and J. H. Harwell. Surfactant Selection for Enhancing Ex Situ Soil Washing, Wat. Res., 33, pp. 351-360. 1999.

Diallo, M. S., L. M. Abriola and W. J. Weber. Solubilization of Nonaqueous Phase Liquid Hydrocarbons in Micellar Solution of Dodecyl Alcohol Ethoxylates, Environ. Sci. Technol., 28, pp. 1829-1837. 1994.

Doong, R. A. and W. G. Lei. Solubilization and Mineralization of Polycyclic Aromatic Hydrocarbons by *Pseudomonas putida* in the Presence of Surfactant J. Hazar. Mater., 96 (1), pp. 15-27. 2003.

Dyksterhouse, S. E., J. P. Gray, R. P. Herwig, J. C. Lara and J. T. Staley. Cycloclasticus pugetti gen. nov., sp. nov., an Aromatic Hydrocarbon-Degrading Bacterium from Marine Sediments. Int. J. Syst. Bacteriol., 45, pp. 116-132. 1995.

Edwards, D. A., R. G. Luthy and Z. Liu. Solubilization of Polycyclic Aromatic Hydrocarbons in Micellar Nonionic Surfactant Solution, Environ. Sci. Technol., 25, pp. 127-133. 1991.

Efroymson, R. A. and M. Alexander. Biodegradation by an Arthrobacter Species of Hydrocarbons Partitioned into an Organic Solvent, Appl. Environ. Microbiol., 57 (5), pp. 1441-1447. 1991.

Eichhorn, P., S. V. Rodrigues, W. Baumann and T. P. Knepper. Incomplete Degradation of Linear Alkybenzene Sulfonate Surfactant in Brazilian Surface Waters and Pursuit of Their Polar Metabolites in Drinking Waters. Sci. Total. Environ., 284, pp. 123-134. 2002.

Fernandez, A. E., Z. S. Ferrera and J. J. S. Rodriguez. Determination of polychlorinated biphenyls by liquid chromatography following cloud-point extraction, Anal. Chim. Acta, 358, pp. 145-155. 1998.

Ferrer, R, J. L. Beltrán and J. Guiteras. Use of Cloud Point Extraction Methodology for the Determination of PAHs Priority Pollutants in Water Samples by High-Performance Liquid Chromatography with Fluorescence Detection and Wavelength Programming, Anal. Chim. Acta, 330, pp. 199-206. 1996.

Foght, J. M., D. L. Gutnick and D. W. S. Westlake. Effect of Emulsam on Biodegradation of Crude Oil in Pure and Mixed Cultures, Appl. Environ. Microbiol., 55, pp. 36-42, 1989.

Foster, K. R., B. R. Epstein, P. C. Jenin and R. A. Mackay. Dielectric Studies on Nonionic Microemulsions, J. Colloid Interf. Sci., 88, pp. 233-246. 1982.

Frankewich, R. P. and W. L. Hinze. Evaluation and Optimization of the Factors Affecting Nonionic Surfactant-Mediated Phase Separations, Anal. Chem., 66 (7), pp. 944-954. 1994.

Froschl, B., G. Stangl and R. Niessner. Combination of Micellar Extraction and GC-ECD for the Determination of Polychlorinated Biphenyls (PCBs) in Water, Fresenius J. Anal. Chem., 357, pp. 743-746. 1997.

Garcia, J. M., L. Y. Wick and H. Harms. Influence of the Nonionic Surfactant Brij35 on the Bioavailability of Solid and Sorbed Bidenzofuran, Environ. Sci. Technol., 35, pp. 2033-2039. 2001.

Garg, N., I. Y. Galaev and B. Mattiasson. Use of a Temperature-Induced Phase-Forming Detergent (Triton X-114) as Ligand Carrier for Affinity Partitioning in an Aqueous Three-Phase System, Biotechnol. Appl. Biochem., 20, pp. 199-215. 1994.

Garon, G., S. Krivobok, D. Wouessidjewe and F. Seigle-Murandi. Influence of Surfactant on Solubilization and Fungal Degradation of Fluorene. Chemosphere, 47, pp. 303-309. 2002.

Gehlen, M. H. and F. C. De Schryver. Time-resolved Fluorescence Quenching in Micellar Assemblies, Chem. Rev., 93, pp. 199-221. 1993.

Gerde, P., B. A. Muggenburg, M. Lundborg and A. R. Dahl. The Rapid Alveolar Absorption of Diesel Soot-adsorbed Benzo[a]pyrene: Bioavailability, Metabolism and Dosimetry of an Inhaled Particle-borne Carcinogen, Carcinogenesis, 22, pp. 741-749. 2001.

Glatter, O., G. Fritz, H. Lindner, J. B. Popela, R. Mittelbach, R. Strey and S. U. Egelhaaf. Nonionic Micelles Near the Critical Point: Micellar Growth and Attractive Interaction, Langmuir, 16, pp. 8692-8701. 2000.

Goel, S. K. Critical Phenomena in the Clouding Behavior of Nonionic Surfactants Induced by Additives, J. Colloid Interf. Sci., 212, pp. 604-606. 1999.

Grasso, D, K. Subramaniam, J. J. Pignatello, Y. Yang and D. Ratté. Micellar Desoprtion of Polynuclear Aromatic Hydrocarbons from Contaminated Soil, Colloids Surf., 194, pp. 65-74. 2001

Grimberg, S. J., M. D. Aitken and W. T. Stringfellow. The Influence of a Surfactant on the Rate of Phenanthrene Mass Transfer into Water, Wat. Sci. Technol., 30, pp. 23-30. 1994.

Grimberg S. J. and M. D. Aitken. Biodegradation Kinetics of Phenanthrene Solubilized in Surfactant Micelles. In: Hinchee R. E., Brockman F. J. and Vogel C. M. (Eds.), Microbial Processes for Bioremediation, pp. 59-66. Battelle Press, Columbus, OH.

Grimberg, S. J., J. Nagel and M. D. Aitken. Kinetics of Phenanthrene Dissolution into Water in the Presence of Nonionic Surfactants, Environ. Sci. Technol., 29, pp. 1480-1487. 1995.

Grimberg, S. J., W. T. Stringfellow and M. D. Aitken. Quantifying the Biodegradatio of Phenanthrene by *Pseudomonas Stutzeri P16* in the Presence of a Nonionic Surfactant, Appl. Environ. Microbiol., 62, pp. 2387-2392. 1996.

Gu, T.R. and J. Sjöblom. Surfactant Structure and Its Relation to the Krafft Point, Cloud Point and Micellization-some Empirical Relationships. Colloid Surface, 64, pp. 39-46. 1992.

Gu, T.R., S. F. Quin and C. M. Ma. The Effect of Electrolytes on the Cloud Point of Mixed-Solutions of Ionic and Nonionic Surfactants, J. Colloid Interf. Sci., 127, pp. 586-588. 1989.

Gu, T. R. and P. A. Galera-Gómez. Clouding of Triton X-114: The effect of Added Electrolytes on the Cloud Point of Triton X-114 in the Presence of Ionic Surfactants, Colloid Surface A., 104, pp. 307-312. 1995.

Gu, T. R and P. A. Galera-Gómez. The Effect of Different Alcohols and Other Polar Organic Additives on the Cloud Point of Triton X-100 in Water, Colloid Surface A, 147, pp. 365-370. 1999.

Guerin, W. F. and G. E. Jones. Mineralization of Phenanthrene by a Mycobacterium sp, Appl. Environ. Microbiol., 54, pp. 937-944. 1988.

Guha, S. and P. R. Jaffé. Biodegradation Kinetics of Phenanthrene Partitioned into the Micellar Phase of Nonionic Surfactant, Environ. Sci. Technol., 30, pp. 605-611. 1996 (a).

Guha, S. and P. R. Jaffé. Bioavailability of Hydrophobic Compounds Partitioned into Micellar Phase of Nonionic Surfactants, Environ. Sci. Technol., 30, pp. 1382-1391. 1996 (b).

Guha, S., P. R. Jaffe and A. P. Catherine. Solubilization of PAH Mixtures by a Nonionic Surfactant, Environ. Sci. Technol., 32, pp. 930-935. 1998 (a).

Guha, S., P. R. Jaffé and C. A. Peters. Bioavilability of Mixtures of PAHs Partitioned in to the Micellar Phase of a Nonionic Surfactant, Environ. Sci. Technol., 32, pp. 2317-2324. 1998 (b).

Hall, D. G. Thermodynamics of Micelle Formation, In Nonionc Surfacatants: physical chemistry, Schick M. J. (ed.) pp. 233-296. New York: Dekker. 1987.

Hayter, J. B. and M. Zulauf. Attractive Interactions in Critical Scattering from Nonionic Micelles, Colloid Polym. Sci. 260, pp. 1023-1028. 1982

Hedlund, B. P., A. D. Geiselbrecht, T. J. Bair and J. T. Staley. Polycyclic Aromatic Hydrocarbon Degradation by a New Marine Bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov., Appl. Environ. Microbiol. 65. pp. 251-259. 1999.

Herrmann, K. W., J. G. Brushmiller and W. L. Courchene. Micellar Properties and Critical Opalescence of Dimethyalkylphosphine Oxide Solutions, J. Phys. Chem. 70, pp. 2909-2918. 1966.

Hinze, W. L. and E. A. Pramauro. A Critical Review of Surfactant-Mediated Phase Separations (Cloud Point Extractions): Theory and Applications, Crit. Rev. Anal. Chem., 24, pp. 133-177. 1993.

Hoffmann, J., Komárek, K., Řezníćková, I., Richter, P. Biodegradation Course of Oxyethylenated Medium Alcohols in Aqueous System. Toxicol. Environ. Chem. 72, pp.145-158. 1999.

Holden, P.A., M. G. La Montagne, A. K. Bruce, W. G. Miller and S. E. Lindow. Assessing the Role of Pseudomonas Aeruginosa Surface-active Gene Expression in Hexadecane Biodegradation in Sand. Appl. Environ. Microbiol., 68(5), pp. 2509-2518. 2002.

Huang, H.-L. and W.-M. G. Lee. Enhanced Naphthalene Solubility in the Presence of Sodium Dodecyl Sulfate: Effect of Critical Micelle Concentration, Chemosphere, 44, pp. 963-972. 2001.

Huang, Z. and T. R. Gu. The Effect of Mixed Cationic-Anionic Surfactants on The Cloud Point of Nonionic Surfactant, J. Colloid Interf. Sci, 138, pp. 580-582.1990.

Huddleston, J. G., H. D. Willauer, S. T. Griffin and R. D. Rogers. Aqueous Polymer Solutions As Environmentally Benign Liquid/Liquid Extraction Media, Ind. Eng. Chem. Res.,38, pp. 2523-2539. 1999.

Huibers, P. D. T., D. O. Shah and A. R. Katritzky. Predicting Surfactant Cloud Point from Molecular Structure. J. Colloid Interf. Sci., 193, pp. 132-136. 1997.

Israelachvili, J. N., F. J. Mitchell and B. W. Ninham, Theory of Self-assembly of Lipid Bilayers and Vesicles. Biochim. Biophys. Acta, 470 (2), pp. 185-201. 1977.

Jafvert, C. T., P. L. Van Hoof and J. C. Heath. Solubilization of Nonpolar Compounds by Nonionic Surfactant Micelles, Wat. Res., 28, pp. 1009-1017. 1994.

Jahan, K., T. Ahmed and W. J. Maier. Factors Affecting the Nonionic Surfactant-enhanced Biodegradation of Phenanthrene. Wat. Environ. Res., 69(3), pp. 317-325. 1997.

Jahan K, T. Ahmed and W. J. Maier. Modeling the Influence of Nonionic Surfactants on Biodegradation of Phenanthrene. Wat. Res., 33(9), pp. 2181-2193. 1999.

Johnson, J. C., S. B. Sun and P. R. Jaffé. Surfactant Enhanced Perchloroethylene Dissolution in Porous Media: The Effect on Mass Transfer Rate Coefficients, Environ. Sci. Technol., 33, pp. 1286-1292. 1999.

Kabalnov, A. Thermodynamic and Theoretical Aspects of Emulsions and Their Stability. Curr. Opin. Colloid In., 3(3), pp. 270-275. 1998.

Kahlweit, M, R. Strey and G. Busse. Effect of Alcohols on the Phase-Behavior of Microemulsions. J Phys. Chem., 95(13), pp. 5344-5352. 1991.

Kahlweit, M., G. Busse and B. Faulhaber. Preparing Microemulsions with Alkyl Monoglucosides and the Role of n-Alkanols. Langmuir, 11(9), pp. 3382-3387. 1995.

Karcher, W. Spectral Atlas of Polycyclic Aromatic Compounds. pp. 21, Dordrecht; Boston: Kluwer Academic Publisher, 1985.

Karsa, D. R. and M. R. Porter. Biodegradability of Surfactants. pp. 204-230. London: Blackie Academic and Professional. 1995.

Kenkare, P. U., C. K. Hall and P. K. Kilpatrick. The effect of Salts on the Lower Consolute Boundary of a Nonionic Micellar Solution, J. Colloid Interf. Sci., 184, pp. 456-468. 1996.

Kile, D. E. and C. T. Chiou. Water Solubility Enhancement of DDT and Trichlorobenzene by Some Surfactants Below and Above the Critical Micelle Concentration, Environ. Sci. Technol., 23, pp. 832-838. 1989.

Kim, J-H., M. M. Domach and R. D. Tilton. Effect of Electrolytes on the Pyrene Solubilization Capacity of Dodecyl Sulfate Micelles, Langmuir, 16, pp. 10037-10043. 2000.

Kim, E. J. and D. O. Shah. Cloud Point Phenomenon in Amphiphilic Drug Solutions Langmuir. 18 (26), pp. 10105-10108. 2002.

Komaromy-Hiller, G. and R. von Wandruszka. Decontamination of Oil-polluted Soil by Cloud Point Extraction, Talanta, 42, pp. 83-88. 1995.

Kose, T., T. Mukai, K.Takimoto and M. Okada. Effect of Non-aqueous Phase Liquid on Biodegradation of PAHs in Spilled Oil on Tidal Flat. Water Res., 37 (8), pp.1729-1736. 2003.

Koshy, L., A. H. Saiyad and A. K. Rakshit. The Effect of Various Foreign Substances on the Cloud Point of Triton X-100 and Triton X-114, Colloid Polym. Sci., 274, pp. 582-587. 1996.

Kulichenko, S.A., V.O. Doroschuk and S.O. Lelyushok. The Cloud Point Extraction of Copper(II) with Monocarboxylic Acids into Non-ionic Surfactant Phase. Talanta, 59 (4), pp.767-773. 2003.

Kumar, S., D. Sharma, Z. A. Khan and K.U. Din. Salt-induced Cloud point in Anionic Surfactant Solutions: Role of the Heasgroup and Additives. Langmuir, 18, pp. 4205-4209. 2002

Kuusimaki L, Y. Peltonen, E. Kyyro, P. Mutanen, K. Peltonen and K. Savela. Exposure of Garbage Truck Drivers and Maintenance Personnel at a Waste Handling Centre to Polycyclic Aromatic Hydrocarbons Derived from Diesel Exhaust. J. Environ. Monitor, 4(5), pp. 722-727. 2002.

Laha, S. and R. G. Luthy. Inhibition of Phenanthrene Mineralization by Nonionic Surfactants in Soil-water Systems, Environ. Sci. Technol., 25, pp.1920-1930. 1991.

Laha, S. and R. G. Luthy. Effect of Nonionic Surfactants on the Solubilization and Mineralization of Phenanthrene in Soil-water Systems, Biotechnol. Bioeng., 40, pp. 1367-1380. 1992.

Lajoie C. A, A. C. Layton, J. P. Easter, F. M. Menn and C. S. Sayler. Degradation of nonionic surfactants and polychlorinated biphenyls by recombinant field application vectors. J Ind. Microbiol. Biotechnol., 19(4): 252-262.1997.

Lang, J. C. and R. C. Morgan. Nonionic Surfactant Mixtures. I. Phase Equilibria in $C_{10}E_4$–H_2O and Closed-loop Coexistence, J. Chem. Phys. 73 (11), pp. 5849-5858. 1980.

Laughlin, R. G. The Aqueous Phase Behavior of Surfactants. pp.148-154. London: Academic Press.1994.

Laughlin, R. G. Advances in Liquid Crystals. New York: Academic Press. 1978

Li, J.- L., Chen, B-H. Solubilization of Model Polycyclic Aromatic Hydrocarbons by Nonionic Surfactants. Chem. Eng. Sci. 57: 2825-2835. 2002 (a).

Li, J.-L. and B.-H. Chen. Cloud-point Extraction of Phenanthrene by Nonionic Surfactants, J. Chin Inst. Chem. Eng, 33(6), pp. 581-589, 2002 (b).

Lim, T.Y. Effect of Surfactants on Solubilization and Biodegradation of Selected Free Fatty Acids in Palm Oil Wastes. Master Thesis. The National University of Singapore. 2002.

Liu, D. Enhancement of PCBs Biodegradation by Sodium Ligninsulfonate, Wat. Res., 14, pp. 1467-1475. 1980.

Liu, Y. J., L. Z. Zhu and X. Y. Shen. Polycyclic Aromatic Hydrocarbons (PAHs) in Indoor and Outdoor Air of Hangzhou, China. Environ. Sci. Technol. 35(5), pp. 840-844. 2001

Liu, Z.B., A. M. Jacobson and R. G. Luthy. Biodegradation of Naphthalene in Aqueous Nonionic Surfactant Systems, Appl. Environ. Microbiol., 61, pp.145-151. 1995.

Luconi, M. O., M. F. Silva, R. A. Olsina and L. P. Fernandez. Cloud Point Extraction of Lead in Saliva via Use of Nonionic PONPE 7.5 Without Added Chelating Agent, Talanta, 51, pp. 123-129. 2000.

Madsen, T and P. Kristensen. Effects of bacterial inoculation and nonionic surfactants on degradation of polycyclic aromatic hydrocarbons in soil. Environ. Toxicol. Chem. 16(4), pp. 631-637. 1997.

Manzoori, J.L. and N.G. Karim. Selective Cloud Point Extraction and Preconcentration of Trace Amounts of Silver as a Dithizone Complex Prior to Fame Atomic Absorption Spectrometric Determination. Anal. Chim. Acta, 484 (2), pp.155-161.2003.

Materna, K., I. Milosz, I. Miesiac, G. Cote and J. Szymanowski. Removal of Phenol from Aqueous Stream by the Cloud Point Extraction Technique with Oxyethylated Methyl Dodecanoates As Surfactant, Environ. Sci. Technol., 35, pp. 2341-2346. 2001.

Materna, K. and J. Szymanowski. Separation of Phenols from Aqueous Micellar Solutions by Cloud Point Extraction. J. Colloid Interf. Sci., 255 (1), pp.195-201. 2002

Merino, F., S. Rubio and D. Pérez-Bendito. Acid-induced Cloud Point Extraction and Preconcentration of Polycylic Aromatic Hydrocarbons from Environmental Solid Samples, J. Chromatogr. A, 962, pp. 1-8. 2002.

Miller, R. M. and R. Bartha. Evidence for Liposome Encapsulation for Transport-limited Microbial Metabolism of Solid Alkanes, Appl. Environ. Microbiol, 55(2), pp. 269-274. 1989.

Miller, M. M., S. P. Waslk, G. L. Huang, W. Y. Shiu and D. Mackay. Relationships between Octanol-water Partition Coefficient and Aqueous Solubility, Environ. Sci. Technol., 19, pp. 522-529. 1985.

Minuth, T., J. Thommes and M. R. Kula. Extraction of Cholesterol Oxidase from Nocardia-Rhodochrous using a Nonionic Surfactant-Based Aqueous 2-Phase System, J. Biotechnol., 38, pp.151-164. 1995.

Minuth, T., J. Thommes and M. R. Kula. A Closed Concept for Purification of the Membrane-Bound Cholesterol Oxidase from *Nocardia rhodochrous* by Surfactant-Based Cloud Point Extraction, Organic-Solvent Extraction and Anion-Exchange Chromatography, Biotechnol. Appl. Biochem., 23, pp. 107-116. 1996.

Mizesko, M. C., C. Grewe, A. Grabner and M. S. Miller. Alteration at the *Ink4a* Locus in Transplacentally Induced Murine Lung Tumors, Cancer Letters, 172, pp. 59-66. 2001.

Molina-Bolívar, J. A., J. Aguiar and C. R. Carnero. Light Scattering and Fluorescence Probe Studies on Micellar Properties of Triton X-100 in KCl Solutions, Mol. Phys., 99(20), pp.1729-1741. 2001.

Mosche, M. and U. Meyer. Toxicity of Alkylbenzene Sulfonate in Anaerobic Digestion: influence of exposure time, Wat. Res. 36. pp. 3253-3260. 2002.

Mulkins-Phillips, G. J. and J. E. Steward. Effect of Four dispersants on Biodegradation and Growth of Bacteria on Crude Oil, Appl. Environ. Microbiol., 28, pp.547-552. 1974.

Mulligan, C. N., R. N. Yong and B. F. Gibbs. Surfactant-enhanced Remediation of Contaminated Soil: a review. Eng. Geol., 60, pp. 371-380. 2001.

Nagarajan, R., M. A. Chaiko and E. Ruchenstein. Locus of Solubilization of Benzene in Surfactant Micelles, J. Phys. Chem., 88, pp.1916-1922. 1984.

Nilsson, P. G. and B. Lindman. Water Self-diffusion in Nonionic Surfactant Solutions: Hydration and Obstruction Effects, J Phys. Chem., 87, pp. 4756-4761. 1983.

Nilsson, P. G. and B. Lindman. Mixed Micelles of Nonionic and Ionic Surfactants. A Nuclear Magnetic Resonance Self-Diffusion and Proton Relaxation Study. J. Phys. Chem., 88, pp. 5391-5397. 1984.

Noordman, W. H., J. H. J. Wachter, G. J. de. Boer and D. B. Janssen. The Enhancement by Surfactants of Hexadecane Degradation by *Pseudomonas aeruginosa* Varies with Substrate Availability. J. Biotechnol., 94, pp. 195-212, 2002.

Oanh, N.T.K., L. Nghiem and Y.L. Phyu. Emission of Polycyclic Aromatic Hydrocarbons, Toxicity, and Mutagenicity from Domestic Cooking Using Sawdust Briquettes, Wood, and Kerosene, Environ. Sci. Technol. 36 (5), pp.833-839. 2002.

Okada, T. Temperature-induced Phase Separation of Nonionic Polyoxyethylated Surfactant and Application to Extraction of Metal Thiocyanates, Anal. Chem., 64(18), pp. 2138-2142. 1992.

Osipow, L., F. D. Snell and J. Nichson. Proceedings of 2nd International Conference on Surface Activity. pp.50. New York: Academic Press. 1957.

Paleologos, E. K., M. A. Koupparis, M. I. Karayannis and P. G. Veltsistas. Nonaqueous Catalytic Fluorometric Trace Determination of Vanadium Based on the Pyronine B-hydrogen Peroxide Reaction and Flow Injection after Cloud Point Extraction, Anal. Chem., 73(18), pp. 4428-4433. 2001.

Paleologos, E.K., M. I. Prodromidis, D. L. Giokas, A. C. Pappas and M. I. Karayannis. Highly Selective Spectrophotometric Determination of Trace Cobalt and Development of a Reagentless Fiber-optic Sensor, Anal. Chim. Acta, 467 (1-2), pp.205-215. 2002.

Pandit, N. K. and J. Caronia. Comparison of the Cloud Point Behavior of Triton X-100 in H_2O and D_2O, J. Colloid Interf. Sci., 122, pp.100-103. 1988.

Patoczka, J. and G. W. Pulliam. Biodegradation and Secondary Effluent Toxicity of Ethoxylated Surfactants. Wat. Res. 24. pp. 965-972. 1990.

Penfold, J., E. Staples and P. G. Cummins. Small Angle Neutron Scattering Investigation of Rodlike Micelles Aligned by Shear Flow. Adv. Colloid. Interf. Sci. 34, pp. 451-476.1991.

Pennell, K. D., L. M. Abriola and W. J. Weber Jr. Surfactant-Enhanced Solubilization of Residual Dodecane in Soil Columns. 1. Experimental Investigation, Environ. Sci. Technol., 27, pp. 2332-2340. 1993.

Pennell, K. D., M. A. Andrew, L. M. Abriola and M. S. Diallo. Solubilization of Dodecane, Tetrachloroethylene, and 1,2-Dichlorobenzene in Micellar Solution of Ethoxylated Nonionic Surfactants, Environ. Sci. Technol., 31, pp. 1382-1389. 1997.

Pereira, M.D. and M.A.Z. Arruda. Trends in Preconcentration Procedures for Metal Determination Using atomic Spectrometry Techniques. Microchim Acta, 141 (3-4), pp. 115-131. 2003.

Phillies, G. D. J. and J. E. Yambert. Solvent and Solute Effects on Hydration and Aggregation Number of Triton X-100 Micelles, Langmuir, 12, pp. 3431-3436. 1996.

Pino, V. J. H. Ayala, A. M. Afonso and V. González. Cloud-point Preconcentration and HPLC Determination of Polycyclic Aromatic Hydrocarbons in Marine Sediments. Fresenius J. Anal. Chem., 371, pp. 526-531. 2001.

Pino, V., J. H. Ayala, A. M. Afonso and V. González. Determination of Polycyclic Aromatic Hydrocarbons in Seawater by High-performance Liquid Chromatography with Fluorescence Detection Following Micelle-mediated Preconcentration. J. Chromatogr. A. 949, pp. 291-299, 2002.

Pinto, C. G., J. L. P. Pavón and B. M. Cordero. Cloud Point Preconcentration and High-Performance Liquid-Chromatographic Analysis with Electrochemical Detection. Anal. Chem., 64(20), pp. 2334-2338. 1992.

Pinto, C. G., J. L. P. Pavón and B. M. Cordero. Cloud Point Preconcentration and High-Performance Liquid Chromatographic Determination of Polycyclic Aromatic Hydrocarbons with Fluorescence Detection, Anal. Chem., 66, pp. 874-881. 1994.

Pinto, C. G., J. L. P. Pavón and B. M. Cordero. Cloud Point Preconcentration and High Performance Liquid Chromatographic Determination of Organophosphorus Pesticides with Dual Electrochemical Detection, Anal. Chem., 67(15). pp. 2606-2612. 1995.

Pospichil, K. H. Phase Behavior of Ternary Systems: H_2O-Oil-Nonionic Amphiphile with n-Alkyldimethylphosphine Oxide, Langmuir, 2, pp. 170-173. 1986.

Prak, D. J. L., L. M. Abriola, W. J. Weber Jr., K. A. Bocskay and K. D. Pennell. Solubilization Rates of n-Alkanes in Micellar Solutions of Nonionic Surfactants, Environ. Sci. Technol., 34, pp. 476-482. 2000.

Prak, D. J. L., and P. H. Pritchard. Degradation of Polycyclic Aromatic Hydrocarbons Dissolved in Tween 80 Surfactant Solutions by Sphingomonas paucimobilis EPA 505. Can. J. Microbiol., 48(2), pp. 151-158. 2002 (a).

Prak, D. J. L., and P. H. Pritchard. Solubilization of Polycyclic Aromatic Hydrocarbon Mixtures in Micellar Nonionic Surfactant Solutions. Wat. Res., 36(14), pp. 3463-3472. 2002 (b).

Pramauro, E. Concentration and Removal of Chloroaromatic Pollutants using Micelle-Mediated Methods, Ann. Chim., 80, pp. 101-109. 1990.

Pramauro, E. and A. B. Prevot. Solubilization in Micellar Systems-Analytical and Environmental Applications, Pure Appl. Chem., 67, pp. 551-559. 1995.

Quina, F. H. and W. L. Hinze. Surfactant-Mediated Cloud Point Extractions: An Environmentally Benign Alternative Separation Approach, Ind Eng. Chem. Res., 38, pp. 4150-4168. 1999.

Rester, U., M. Moser, R. Huber and W. Bode. L-Isoaspartate 115 of Porcine β-Trypsin Promotes Crystallization of Its Complex with Bdellastasin, Acta Crystallog., Sect. D: Biol. Crystallogr., D56(5), pp. 581–588. 2000.

Revia, R. L. and G. A. Makharadze. Cloud-Point Preconcentration of Fulvic and Humic Acids, Talanta, 48, pp. 409-413. 1999.

Řezníćková, I., Hoffmann, J. and Komárek. Biodegradation of Technical Mixtures of Oxyethylenated Aliphatic Alcohols in an Aqueous Environment, Chemosphere, 48, pp. 83-87. 2002.

Rittman, B. E. and N. M. Johnson. Rapid Biological Clean-up of Soils Contaminated with Lubricating Oil, Wat. Sci. Technol., 21, pp. 209-219. 1989.

Robichaux, T. J. and H. N. Myrick. Chemical Enhancement of the Biodegradation of Crude-oil Pollutants, J. Petr. Technol., 24, pp. 16-20. 1972.

Rosen, M. J. Surfactants and Interfacial Phenomena. pp.108-206. New York: Wiley. 1989.

Ruiz-Aguilar, G. M. L., J. M. Fernandez-Sanchez, R. Rodriguez-Vazquez and H. Poggi-Varaldo. Degradation by White-rot Fungi of High Concentrations of PCB Extracted from a Contaminated Soil, Adv. Environ. Res., 6(4), pp. 559-568. 2002.

Sadaghiania, A. S. and A. Khan. Clouding of a Nonionic Surfactant: The Effect of Added Surfactants on the Cloud Point, J. Colloid Interf. Sci., 144, pp.191-200. 1991.

Saitoh, T., Y. Kimura, H. Watanabe and K. Haraguchi. Distribution Equilibria of Metal-chelates with Thiazolylazo Dyes between Two Phases Formed from an Aqueous Micellar Solution of a Nonionic Surfactant, Anal. Sci., 5(5), pp. 577-581. 1989.

Saitoh, T. and W. L. Hinze. Concentration of Hydrophobic Organic Compounds and Extraction of Protein Using Alkylammoniosulfate Zwitterionic Surfactant Mediated Phase Separation (Cloud Point Extraction), Anal. Chem., 63(21), pp. 2520-2525. 1991.

Saitoh, T., H. Tani, T. Kamidate and H. Watanabe. Phase-Separation in Aqueous Micellar Solutions of Nonionic Surfactants for Protein Separation, Trends Anal Chem., 14, pp. 213-217. 1995.

Saitoh T., Y. Yoshida, T. Matsudo, S. Fujiwara, A. Dobashi, K. Iwaki, Y. Suzuki and C. Matsubara. Concentration of Hydrophobic Organic Compounds by Polymer Mediated Extraction, Anal. Chem., 71(20). pp. 4506-4512. 1999.

Sakulwongyai, S., P. Trakultamupatam, J. F. Scamehorn, S. Osuwan and S. D. Christian. Use of a Surfactant Coacervate Phase to Extract Chlorinated Aliphatic Compounds from Water: Extraction of Chlorinated Ethanes and Quantitative Comparison to Solubilization in Micelles, Langmuir, 16, pp. 8226-8230. 2000.

Salanitro, J. P. and L. A. Diaz. Anaerobic Biodegradability Testing of Surfactants, Chemosphere, 30(5), pp. 813-830. 1995.

Salloum, M. J., B. Chefetz and P. G. Hatcher. Phenanthrene Sorption by Aliphatic-rich Natural Organic Matter, Environ. Sci. Technol., 36, pp. 1953-1958. 2002.

Santana, C. M., Z.S. Ferrera and J.J.S. Rodriguez. Use of Non-ionic Surfactant Solutions for the Extraction and Preconcentration of Phenolic Compounds in Water Prior to Their HPLC-UV Detection, Analyst, 127 (8), pp.1031-1037. 2002.

Sanz, C.P., Z.S. Ferrera and J.J.S. Rodriguez. Extraction and Preconcentration of Polychlorinated Dibenzo-p-dioxins Using the Cloud-point Methodology - Application to Their Determination in Water Samples by High-performance Liquid Chromatography, Anal. Chim. Acta, 470 (2), pp.205-214. 2002.

Schick, M. J. Nonionic Surfactants. pp. 571-580. New York: Dekker. 1967.

Schick, M. J. Nonionic Surfactants. pp. 109-183. New York: Dekker. 1987.

Schippers, C., K. Geßner, T. Müller and T. Scheper. Microbial Degradation of Phenanthrene by Addition of a Sophorolipid Mixture, J. Biotechnol., 83, pp. 189-198. 2000.

Schott, H. Effect of Inorganic Additives on Solutions of Nonionic Surfactants. X. Micellar Properties, J. Colloid Interf. Sci. 173, pp. 265-277. 1995.

Schott, H. Effect of Inorganic Additives on Solutions of Nonionic Surfactant: XIV. Effect of Chaotropic Anions on the Cloud Point of Octoxynol 9 (Triton X-100), J. Colloid Interf. Sci., 189, pp. 117-122. 1997.

Schott, H. Effect of Inorganic Additives on Solutions of Nonionic Surfactants: XVI. Limiting Cloud Points of Highly Polyoxyethylated Surfactants. Colloids Surf. 186, pp. 129-136. 2001.

Schwarz, A., G. C. Terstappen and A. H. Futerman. Isolation of Gangliosides by Cloud-Point Extraction with a Nonionic Detergent, Anal. Biochem., 254, pp. 221-225. 1997.

Selber, K., A. Collen, T. Hyytia, M. Penttila, F. Tjerneld and M. R. Kula. Parameters Influencing Protein Extraction for Whole Broths in Detergent Based Aqueous Two-phase Systems. Bioseparation, 10 (4-5), pp.229-236. 2001 (a).

Selber, K., S. Muller, H. Gieren, J.Thommes, T. Sottmann, R. Strey and M. R. Kula. Physical Properties of Detergent-based Aqueous Two-phase Systems. Bioseparation. 10 (4-5), pp.243-253. 2001 (b).

Seronero, L. C., M. E. F. Laespada, J. L. P. Pavón and B. M. Cordero. Cloud Point Preconcentration of Rather Polar Compounds: Application to the High-performance Liquid Chromatographic Determination of Priority Pollutant Chlorophenol. J. Chromatogr. A., 897, pp. 171-176. 2000.

Shiau B. J., D. A. Sabatini and J. H. Harwell. Properties of Food Grade Surfactants Affecting Subsurface Remediation of Chlorinated Solvents, Environ. Sci. Technol., 29(12), pp. 2929-2935. 1995.

Shinoda, K. and H. Takeda. The Effects of Added Salts in Water on the Hydrophile-lipophile Balance of Nonionic Surfactants: The Effects of Added Salts on the Phase Inversion Temperature of Emulsions, J. Colloid Interf. Sci., 32, pp. 642-646. 1970.

Shor, L. M.; W.S. Liang, K.J. Rockne, L.Y. Young, G.L.Taghon and D.S. Kosson. Intra-aggregate Mass Transport-limited Bioavailability of Polycyclic Aromatic Hydrocarbons to Mycobacterium Strain PCO1, Environ. Sci. Technol., 37 (8), pp. 1545-1552. 2003.

Sicilia, D., S. Rubio, D. Pérez-Bendito, N. Maniasso and E. A. G. Zagatto. Anionic Surfactants in Acid Media: A New Cloud Point Extraction Approach for the

Determination of Polycyclic Aromatic Hydrocarbons in Environmental Samples, Anal. Chim. Acta., 392, pp. 29-38. 1999.

Sicilia, D., S. Rubio and D. P. Bendito. Evaluation of the Factors Affecting Extraction of Organic Compounds Based on the Acid-induced Phase Cloud Point Approach. Anal. Chim. Acta, 460, pp. 13-22. 2002

Silva, M. F., L. P. Fernández, R. A. Olsina and D. Stacchiola. Cloud Point Extraction, Preconcentration and Spectrophotometric Determination of Erbium(III)-2-(3,5-dichloro-2-pyridylazo)-5-dimethylaminophenol, Anal. Chim. Acta, 342, pp. 229-238. 1997.

Silva, M. F., L. P. Fernández and R. A. Olsina. Monitoring the Elimination of Gadolinium-based Pharmaceuticals: Cloud Point Preconcentration and Spectrophotometric Determination of Gd(III)-2-(3,5-dichloro-2-pyridylazo)-5-dimethylaminophenol in urine, Analyst, 123, pp. 1803-1807. 1998.

Sirimanne, S. R., J. R. Barr, D. G. Patterson and L. Ma. Quantification of Polycyclic Aromatic Hydrocarbons and Polychlorinated Dibenzo-p-dioxins in Human Serum by Combined Micelle-Mediated Extraction (Cloud-Point Extraction) and HPLC, Anal. Chem., 68, pp. 1556-1560. 1996.

Sirimanne, S. R., D. G. Patterson, L. Ma and J. B. Justice. Application of Cloud-point Extraction/Reversed-phase High Performance Liquid Chromatography: A Preliminary

Study of the Extraction and Quantification of Vitamins A and E in Human Serum and Whole Blood. J. Chromatogr. 716, pp. 129-137. 1998.

Smith, A. M., M. C. Holmes, A. Pitt, W. Harrison and G. J. T. Tiddy. Lamellar Phases in Contact with the Lower Consolute Loop of Ionic Surfactant-water Systems, Langmuir, 11, pp. 4202-4204. 1995.

Sombra, L. L., M. O. Luconi, L. P. Fernandez, R. A. Olsina, M. F. Silva and L. D. Martinez. Assessment of Trace Aluminium Content in Parenteral Solutions by Combined Cloud Point Preconcentration - Flow Injection Inductively Coupled Plasma Optical Emission Spectrometry, Pharmaceut. Biomed Anal., 30 (5), pp. 1451-1458. 2003

Stangl, G. and R. Niessner. Cloud Point Extraction of Napropamide and Thiabendazole from Water and Soil, Mikrochim. Acta., 113, pp. 1-8. 1994.

Staples C. A., C. G. Naylor, J. B. Williams and W. E. Gledhill. Ultimate Biodegradation of Alkylphenol Ethoxylate Surfactant and Their Biodegradation Intermediates. Environ. Toxicol. Chem., 20 (11), pp. 2450-2455. 2001.

Streletzky, K. and D. C. J. Phillies. Temperature-Dependence of Triton X-100 Micelle Size and Hydration, Langmuir, 11, pp. 42-47. 1995.

Szymanowski, J. Surfactant Enhanced Non-classical Extraction J. Radioanal Nucl Chem., 246, pp. 635-642. 2000.

Szymanowski, J. and W. Apostoluk. Estimation of Distribution Parameters of Organic Solutes in Cloud Point Extraction, J. Colloid Interf. Sci., 228, pp. 178-181. 2000.

Tani, H., T. Kamidate and H. Watanabe. Aqueous Micellar Two-Phase Systems for Protein Separation, Anal. Sci., 14, pp. 875-888. 1998.

Tanford, C. The Hydrophobic Effect: Formation of Micelles and Biological Membranes. New York: Wiley. 1980.

Tang, Y., K.C. Donnelly, E. C.Tiffany and M.M. Mumtaz. Neurotoxicity of polycyclic aromatic hydrocarbons and simple chemical mixtures, J Toxicol. Env. Heal A 66 (10), pp. 919-940. 2003

Tauber, A. I., J. Wright, F. K. Higson, S. A. Edelman and D. J. Waxman. Purification and Characterization of the Human Neutrophil NADH-cytochrome b5 Reductase. Blood, 66, pp. 683-678. 1985.

Terzić, S., D. Hršak, M. Ahel. Primary Biodegradation Kinetics of Linear Alkylbenzene Sulphonates in Estuarine Waters. Wat. Res., 26(5), pp. 585-591. 1992.

Thomposn, L., J. M. Walsh and G. J. T. Tiddy. A Comparison of the Physical Chemistry and Oily Soil Detergency Properties of Primary and Secondary Alcohol Ethoxylates, Colloids Surf. A, 106, pp. 223-235. 1996.

Tiehm, A. Degradation of Polycyclic Aromatic Hydrocarbons in the Presence of Synthetic Surfactants, Appl. Environ. Microbiol., 1, pp. 258-263. 1994.

Tiehm, A. and C. Frizsche. Utilization of Solubilized and Crystalline Mixtures of Polycyclic Aromatic Hydrocarbons by a *Mycobacterium sp*., Appl. Microbiol. Biotechnol., 42, pp. 964-968. 1995.

Tiruppathi, C., D. H. Alpers and B. Seetharam. Phase Separation of Rat Intestinal Brush Border Membrane Proteins Using Triton X-114, Anal. Biochem., 153, pp. 330-335. 1986.

Triolo, R., L. J. Magid, J. S. Johnson, H. R. Child. Small-Angle Neutron Scatterig from Aqueous Micellar Solutions of a Nonionic Surfactant as a Function of Temperature, J. Phys. Chem., 86, pp. 3689-3695. 1982.

Tsai, P. J., H. Y. Shieh, W. J. Lee and S. O. Lai. Health-risk Assessment for Workers Exposed to Polycyclic Aromatic Hydrocarbons (PAH) in a Carbon Black Manufacturing Industry, Sci. Total Environ., 278, pp. 137-150. 2001.

Tsomides, H. J., J. B. Hughes, J. M. Tomas and C. H. Ward. Effect of Surfactant Addition on Phenanthrene Biodegradation in Sediment. Environ. Toxicol. Chem. 14(6). pp. 953-959. 1995.

Tungsubutra, T., C. A. Miller. Effect of Secondary Alcohol Ethoxylates on Behavior of Triolein-Water-Surfactant Systems, J. Am. Oil Chem Soc, 71, pp. 65-73. 1994.

Valaulikar, B. S. and C. Manohar. The Mechanism of Clouding in Triton X-100: The Effect of Additives, J. Colloid Interf. Sci., 108, pp. 403-406. 1985.

Valsaraj, K. T. and L. J. Thibodeaux. Relationships Between Micelle-Water and Octanol-Water Partition Constants for Hydrophobic Organics of Environmental Interest, Wat. Res., 23, pp. 183-189. 1989.

Van der Meeren, P. and W. Verstraete. Surfactants in Relation to Bioremediation and Wastewater Treatment. Current Opin. in Colloid Interf. Sci., 1, pp. 624 – 634. 1996.

Volkering, F., A. M. Breure, J. G. Vanandel and W. H. Rulkens. Influence of Nonionic Surfactants on Bioavailability and Biodegradation of Polycyclic Aromatic Hydrocarbons. Appl. Environ. Microbiol., 61(5), pp. 1699-1705. 1995.

Volkering, F., A. M. Breure and W. H. Rulkens. Microbiological Aspects of Surfactant Use for Biological Soil Remediation, Biodegradation, 8, pp. 401-417. 1997.

Walsh, J. M. and G. J. T. Tiddy. Liquid Crystal Phase Behaviour of Branched Poly(oxyethylene) Surfactants, Langmuir, 19, pp. 5586-5594. 2003.

Watanabe, H. and H. Tanaka. A Nonionic Surfactant as a New Solvent for Liquid-Liquid Extraction of Zinc (II) with 1-(2-Pyridylazo)-2-Naphthol, Talanta, 25, pp. 585-589. 1978.

Willumsen, P. A. and E. Arvin. Kinetics of Degradation of Surfactant-Solubilized Fluoranthrene by a Sphingomonas paucimobilis, Environ. Sci. Technol., 33, pp. 2571-2578. 1999.

Wu, Y. C. and S. D. Huang. Trace Determination of Hydroxyaromatic Compounds in Dyestuffs Using Cloud Point Preconcentration, Analyst, 123, pp. 1535-1539. 1998.

Wuilloud, J. C. A. de, R. G. Wuilloud, M. F. Silva, R. A. Olsina and L. D. Martinez. Sensitive Determination of Mercury in Tap Water by Cloud point Extraction Pre-concentration and Flow Injection-cold Vapor Inductively coupled Plasma Optical Emission Spectrometry. Spectrochimica Acta Part B, 57, pp. 365-374. 2002.

Yalkowsky, S. H. Solubility and Solubilization in Aqueous Media, Am. Chem. Soc. Washington DC, 1999, chapt. 7.

Yeom, I. T., M. M. Ghosh and C. D. Cox. Kinetic Aspects of Surfactant Solubilization of Soil-Bound Polycyclic Aromatic Hydrocarbons, Environ. Sci. Technol., 30, pp. 1589-1595. 1996.

Zaslavsky, B. Y. Aqueous Two-phase Partitioning. pp. 28-36, New York: Marcel Dekker, INC. 1995.

Zhang, T.-F. and L. P. Hager. A Single-Step Large-Scale Purification of Pyruvate Oxidase. Arch. Biochem. Biophys., 257, pp. 485-487. 1987.

Zhang, Y. M., W. J. Maier and R. M. Miller. Effect of Rhamnolipids on the Dissolution, Bioavailability, and Biodegradation of Phenanthrene, Environ. Sci. Technol., 31, pp. 2211-2217. 1997.

Zhang, C. L., K. T. Valsaraj, W. D. Constant and D. Roy. Aerobic Biodegradation Kinetics of Four Anionic and Nonionic Surfactants at Sub- and Supra- Critical Micelle Concentrations (CMCs), Wat. Res., 33(1), pp. 115-124, 1999.

Zheng, Z. and J. P. Obbard. Removal of Polycyclic Aromatic Hydrocarbons from Soil Using Surfactant and the White Rot Fungus *Phanerochaete chrysosporium*, J. Chem. Technol. Biotechnol., 75, pp. 1183-1189. 2000.

Zheng, Z. M. and J. P. Obbard. Polycyclic Aromatic Hydrocarbon Removal from Soil by Surfactant Solubilization and Phanerochaete Chrysosporium Oxidation. J Environ Qual., 31 (6), pp.1842-1847. 2002(a).

Zheng, Z. M. and J. P. Obbard. Removal of Surfactant Solubilized Polycyclic Aromatic Hydrocarbons by *Phanerochaete chrysosporium* in a Rotating Biological Contactor Reactor. J. Biotechnol., 96, pp. 241-249. 2002 (b).

Zhu, L. Z. and C. T. Chiou. Water Solubility Enhancement of Pyrene by Single and Mixed Surfactant Solutions. J. Environ. Sci.-CHINA., 13(4), pp. 491-496. 2001.

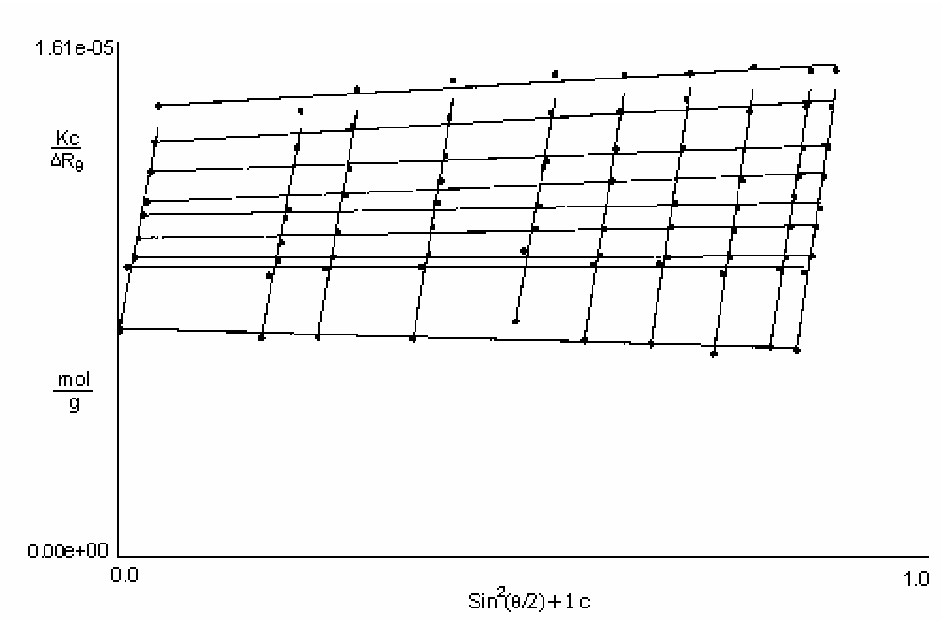
Zhu, L. Z. and J. Wang. Sources and Patterns of Polycyclic Aromatic Hydrocarbons Pollution in Kitchen Air, China. Chemosphere, 50(5), pp. 611-618. 2003.

Zhu, W., X. Wang, B. Yang and X. Tang. A Novel Ionic-conduction Mechanism Based on Polyurethane Electrolyte, J. Polym. Sci., Part B: Polym. Phys., 39(11), pp. 1246–1254. 2001

Zulauf, M. and J. P. Rosenbusch. Micelle Clusters of Octylhydroxyoligo (oxyethylenes), J. Phys. Chem. 87, pp. 856-862. 1983.

Appendix A

A typical Zimm Plot Obtained for Tergitol 15-S-7 at 22 °C in Deionized Water.



Appendix B

List of Publications

PAPERS IN PRINT

Li, J. L., R. B. Bai, and B. H. Chen. Preconcentration of Phenanthrene from Aqueous Solution by a Slightly Hydrophobic Nonionic Surfactant, Langmuir, 20, pp. 6068-6070, 2004.

Li, J. L. and R. B. Bai. Effect of a Commercial Alcohol Ethoxylate Surfactant on Biodegradation of Phenanthrene in a Saline Water Medium by *Neptunomonas Naphthovorans*. Biodegradation, 2004 (In press)

Li, J. L. and B.-H. Chen. Equilibrium Partition of Polycyclic Aromatic Hydrocarbons in a Cloud Point Extraction Process, J. Colloid Interf. Sci., 263(2), pp.625-632, 2003.

Li, J. L. and B.-H. Chen. Solubilization of Model Polycyclic Aromatic Hydrocarbons by Nonionic Surfactants, Chem. Eng. Sci., 57, pp. 2825-2835, 2002.

Li, J. L. and B.-H. Chen. Cloud-point Extraction of Phenanthrene by Nonionic Surfactants, J. Chin Inst. Chem. Eng., 33(6), pp. 581-589, 2002.

Bai, D. S., J. L. Li, S. B. Chen and B.-H. Chen. A Novel Cloud-Point Extraction Process for Preconcentrating Selected Polycyclic Aromatic Hydrocarbons in Aqueous Solution, Environ. Sci. Technol., 35, pp. 3936-3940. 2001.

PAPERS SUBMITTED

Li, J. L., R. B. Bai, and B.-H. Chen. Biodegradation of Alcohol Ethoxylates Non-ionic Surfactants and Its Effect on the Bioavailability of Phenanthrene in Micellar Solutions.

CONFERENCES

Li, J. L., D. S. Bai and B.-H. Chen. Solubilization of Polycyclic Aromatic Hydrocarbons by Nonionic surfactants. AIChE, Los Angeles, 2000. Poster Presentation.

Li, J. L., D. S. Bai and B.-H. Chen. Enhanced Solubilization of Polycyclic Aromatic Hydrocarbons By Nonionic Surfactants. IWA Sludge Management Conference, Taipei, Mar. pp. 25-28, 2001.

Li, J. L. and B.-H. Chen. Preconcentration of Phenanthrene by Nonionic Ethoxylated Alcohols. APCChE 2002, New Zealand, Poster Presentation.