The Influence of Surfactants on the Solubility of Acenaphthene and Phenanthrene and their Extraction from Spiked Soils

by

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Submitted in partial fulfilment of the requirements for the degree of Master of Science in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, 2005

DECLARATION

I hereby certify that this dissertation is my own work, except when specifically acknowledged in the text. Neither the present dissertation, nor any part thereof has been submitted to any other University for a degree.

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ACKNOWLEDGEMENTS

The author wishes to express her gratitude to the following persons for their assistance in her research:

Prof. D. Jaganyi, especially for arranging the manufacture of the specialised laboratory equipment and for his supervision during the thesis compilation

Dr. F. Khan, for proofreading the initial drafts and her recommendations

Mr. V. Dorasamy, for the analyses of the soil samples and his assistance regarding the soil science aspect of the thesis

Mr. E. Abib and the other users of the Postgraduate Soil Science Laboratory, for their assistance in the usage of the centrifuge and centrifuge tubes and for the usage of bench space

Mr M. Somaru and all the technical staff and Mr D. Reddy and all other postgraduates at the Chemistry department for their assistance

Sasol for the funding of this research and Mr Martin Ginster and team at Sasol for their assistance and advice during the research

My parents for supporting me throughout this work, I would have not made it without you

Sadhna, for being a friend when I really needed one

Finally to God Almighty for seeing me through it all, Hare Krishna!

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LIST OF ABBREVIATIONS USED

API	American Petroleum Institute
CEC	cation exchange capacity
CMC	critical micelle concentration
DPE	dual phase extraction
EO	ethylene oxide
HLB	hydrophile-lipophile balance
HOC	hydrophobic organic compound
LTTD	low- temperature thermal desorption
MSR	molar solubilisation ratio
NOM	natural organic matter
PAH	polyaromatic hydrocarbon
PCB	polychlorinated biphenyl
PCP	pentachlorophenol
POE	polyoxyethylene
SDS	sodium dodecylsulfate
SEAR	surfactant enhanced aquifer remediation
SOM	soil organic matter
SVE	soil vapour extraction
VOC	volatile organic compound
VRU	volume reduction unit

ABSTRACT

In the first phase of the study, the effect of five Safol surfactants on the aqueous solubility of phenanthrene and acenaphthene was determined. The fixed variables were temperature and ionic strength, while surfactant concentration and pH were varied. Quantification of the polyaromatic hydrocarbons (PAHs) was conducted by UV-Visible spectrophotometry.

The surfactants had little or no effect on analyte solubilisation below the critical micelle concentration (CMC) while a linear relationship between surfactant concentration and amount of solubilised phenanthrene was observed above CMC concentrations. Safol 45E5 had the highest phenanthrene molar solubilisation ratio (0.83) of the five surfactants tested. The solubilisation of phenanthrene increased marginally (4.1 % for Safol 45E12 and 15.2 % for Safol 45E7) by decreasing the pH from 8 to 5. The concentration of solubilised acenaphthene was 8.4 % higher than phenanthrene in a 1 mM solution of Safol 45E7. The aqueous solubility of phenanthrene was enhanced 11.0, 21.2, 19.6, 15.9 and 14.7 times in 1 mM solutions of Safol 45E3, 45E5, 45E7, 45E9 and 45E12 respectively.

Seasand, Longlands sand, Longlands soil and a standard soil sample were spiked with the two PAHs and aged for two weeks. API sludge provided by Sasol and unspiked samples of the above mentioned sorbents were subjected to determinations of organic matter content, particle size distribution and moisture content. The spiked soils and sands and the sludge samples were then washed in various concentrations of Safol 45E7 (0.5, 1.0 and 2.0 mM) at the same temperature used in the solubility studies. A soil mass to solution volume of 1g to10 mL was used. Analyses of the soil and sand samples were conducted by High Pressure Liquid Chromatography (HPLC).

Using a 2 mM Safol 45E7 surfactant solution, 100 % and 90 % of phenanthrene and acenaphthene were respectively extracted from Longlands sand and 88 % and 100 % of phenanthrene and acenaphthene were removed from seasand. 8.4 % phenanthrene

and 8.17 % of acenaphthene was removed from Longlands soil, while 7.03 % phenanthrene and 6.64 % acenaphthene was removed from the standard soil sample. In the sand desorption studies, the amount of desorbed contaminants initially increased rapidly with increasing surfactant concentration, before levelling off at equilibrium. The amount of desorbed acenaphthene and phenanthrene increased exponentially with increasing surfactant concentration while contaminant concentrations decreased with increasing time in the Longlands soil and standard soil desorption experiments.

Dry API sludge samples were also subjected to soil washing studies. The washed samples were Soxhlet extracted and analysed by gas chromatography. The 0.5 mM and 1 mM Safol 45E7 washed sludge samples showed respective phenanthrene peak area percent reductions representing a 44 % and 47 % extraction of phenanthrene from the API sludge.

CHAPTER ONE

INTRODUCTION

1.1. Polyaromatic Hydrocarbons (PAHs)

1.1.1 Introduction

The products of heating coal to high temperatures in the absence of air are coke, coal gas and coal tar. Coal tar consists mainly of hydrocarbons, including benzene and other aromatic hydrocarbons.[1] Polyaromatic hydrocarbons (PAHs) are characterised by two or more fused benzene rings. In order for a compound to be classified as aromatic, it has to obey Hückel's Rule, which states that for any compound to be aromatic, it must have $(4n + 2)$ π electrons (where n is any whole number). [2] PAHs are planar molecules with delocalised electrons in the benzene ring.[3]

PAHs with three or more benzene rings are poorly soluble in water and have a high K_{ow} (octanol-water) partition coefficient.[4] The octanol-water partition coefficient, K_{ow}, is defined in Equation 1.1:

$$K_{ow} = \frac{C_{octanol}}{C_{water}} \qquad (1.1)$$

Where $C_{octanol}$ is a compound's solubility in octanol and C_{water} is the compound's aqueous solubility. Compounds with low K_{ow} values (< 10) are considered hydrophilic and those with high K_{ow} values (> 10^4) are considered hydrophobic

The two analytes studied were acenaphthene and phenanthrene.

1.1.2. Physical Properties of Acenaphthene and Phenanthrene

Acenaphthene is found naturally in crude oil. It is also emitted from petroleum refineries, coal tar distillation, coal combustion and diesel-fuelled engines.[5] Acenaphthene is used as a dyestuff intermediate; in insecticides and fungicides and in the manufacture of plastics.[6]

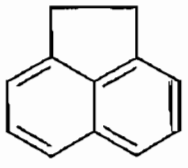
Acenaphthene is toxic to fish at minimum concentrations of 600 – 1700 μg L^{-1}. It has been reported to be moderately toxic to humans via the intraperitoneal (area that contains the

abdominal organs) route.[7] It also acts as an irritant to the eyes, skin and mucous membranes[5] and is reported to be carcinogenic. [6]

Table 1.1.a. displays the physical properties of acenaphthene, while Figure 1.1.a. is the structural representation of an acenaphthene molecule.

Table 1.1.a. Physical Properties of Acenaphthene

Property[8]	Value
Molecular weight	154.211 g mol^{-1}
Melting Point	95 °C
Boiling Point	279 °C
Water Solubility (20 °C)	3.47 mg L^{-1}
Log K_{ow}[9]	3.96



$C_{12}H_{10}$

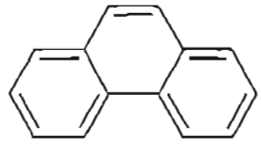
Figure 1.1.a: Molecular Structure of Acenaphthene

Phenanthrene is a component of crude oil and is used in the manufacture of dyestuffs and explosives; in biochemical research and in drug synthesis.[10] The compound has been reported to be toxic to fish at 3.2 mg L^{-1} and invertebrates at 0.6 mg L^{-1}.[11] In humans, experimental tumorigenic data via skin contact has also been reported.[12]

Table 1.1.b. displays the physical properties of phenanthrene, while Figure 1.1.b. is the structural representation of a phenanthrene molecule.

Table 1.1.b. Physical Properties of Phenanthrene

Property[8]	Value
Molecular weight	178.233 g mol^{-1}
Melting Point	99.5 °C
Boiling Point	340 °C
Water Solubility (20 °C)	1.18 mg L^{-1}
Log K_{ow}[9]	4.52



$C_{14}H_{10}$

Figure 1.1.b: Molecular Structure of Phenanthrene

1.2. Soils

1.2.1. Introduction

Soils are porous, heterogeneous mixtures of inorganic and organic matter, water and air.[13] Due to increased concern about inorganic and organic contaminants in soil and water and their impact on plant, animal and human health, the emphasis of soil chemistry now includes environmental soil chemistry, along with the traditional studies on plant and growth nutrition. The basis of environmental soil chemistry is the study of chemical reactions between soils and environmentally important plant nutrients, radionuclides, metals and organic chemicals.[14]

Biological, geological and hydrological weathering processes lead to the formation of soil at the land surface. Soils exhibit an approximately vertical stratification (the soil horizons) produced by the continual influence of percolating water and living organisms and therefore differ from weathered rock. Soils are open systems because they exchange matter with the surrounding atmosphere, biosphere and hydrosphere and undergo continual biological and chemical transformations that link them physically with the atmosphere and hydrosphere.[15]

Knowledge of environmental soil chemistry enables one to predict the fate of contaminants in the surface and subsurface environments. An understanding of the chemistry and mineralogy of inorganic and organic soil components is necessary to comprehend the multitude of chemical and physical transformations that contaminants may undergo in the soil environment, *viz.* equilibrium and kinetic processes such as *dissolution, precipitation, polymerisation, adsorption/desorption* and *oxidation-reduction.*

Dissolution is the separating of a soil solid into its component parts into the soil solution. *Precipitation* occurs when supersaturated conditions exist in the soil solution resulting in the deposition of a substance from the soil solution.[14] *Polymerisation* occurs when chemical species involving molecular units in a repetitive structure form polymers. Examples of polymers are $Al_2(OH)_2^{4+}$, $Fe_2(OH)_2^{4+}$ and biopolymers such as proteins and polysaccharides.[16] *Adsorption* occurs at the solid-liquid interface. In *physisorption,* one

material will sorb onto another material by weak Van der Waals forces of attraction. *Chemisorption* involves adsorbed molecules held to the surface by stronger covalent forces similar to those occurring between atoms and molecules.[17] *Oxidation-reduction* reactions involve the transfer of electrons from one substance to another. H, C, N, O, S, Mn, Fe and Cu are elements that commonly undergo redox reactions.[16] These processes affect the solubility, mobility, speciation and toxicity of contaminants in soils, surface waters and groundwaters.[14]

1.2.2. Composition, Structure and Classification of Soils

About 50 - 67% of the soil volume is made of solid matter. Of this material, typically more than 90% is represented by inorganic compounds, except for peat and muck soils wherein organic material accounts for more than 50% of the solid matter.[15]

The different soil size fractions generally used in the mechanical analysis of fine earth samples are the coarse sand, fine sand, silt and clay size fractions. Table 1.2.a. lists these soil fractions and particle size diameters.[18,19]

Table 1.2.a: Classification of Soils by Particle Size

Fraction	Particle Size Diameter
Coarse Sand	2.0 - 0.2 mm
Fine Sand	0.2 - 0.02 mm
Silt	0.02 – 0.002 mm
Clay	<0.002 mm

Figure 1.2.a.[20] shows the Sand Grade Chart and Texture Chart which enables the classification of soils according to texture and particle size.

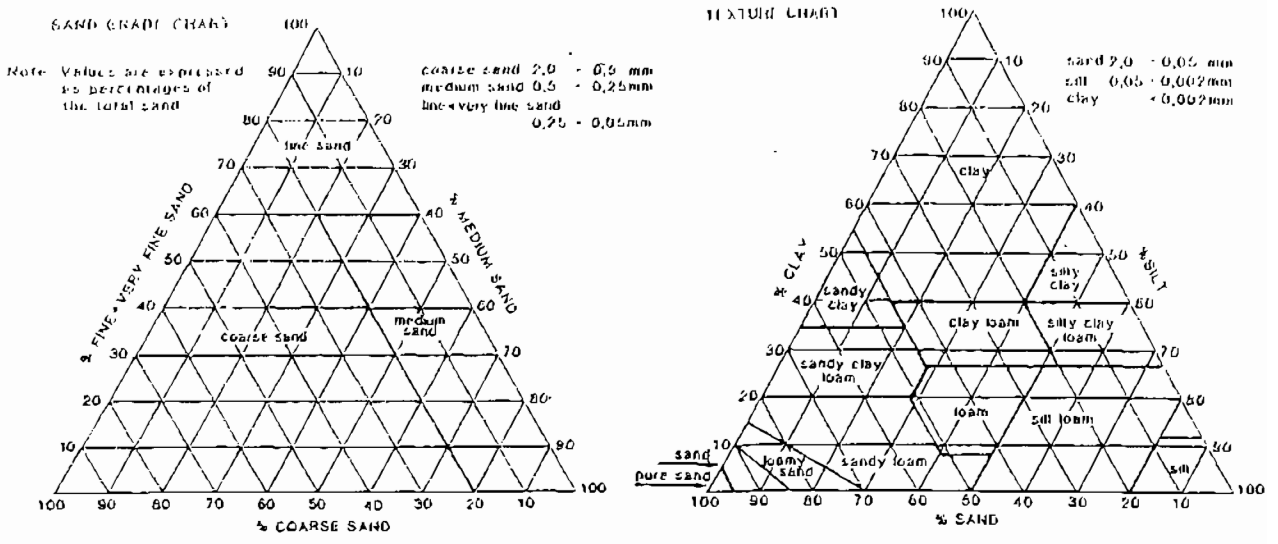


Figure 1.2.a: Classification of Soil According to Texture and Particle Size[21]

1.2.3. Inorganic Soil Matter

Inorganic or mineral soils are generally very low in organic matter (1-10% m/m), and occupy a much greater proportion of the total land area compared to organic soils.[13] Although soils have a variable nature, a number of solid phases of relatively uniform mineral composition have been identified in soils. Oxygen and silicon are the most abundant elements in soil that combine with other elements to form the fifteen common minerals listed in Table 1.2.b.[15]

Table 1.2.b: List of Typical Soil Minerals[15]

Name	Chemical Formula	Importance
Quartz	SiO_2	Abundant in sand and silt
Feldspar	$(Na,K)AlO_2[SiO_2]_3$, $CaAl_2O_4[SiO_2]_2$	Abundant in soil that is not leached extensively
Mica	$K_2Al_2O_5[Si_2O_5]_3Al_4(OH)_4$, $K_2Al_2O_5[Si_2O_5]_3(Mg,Fe)_6(OH)_4$	Source of K in most temperate-zone soils
Amphibole	$(Ca,NA,K)_{2,3}(Mg,Fe,Al)_5(OH)_2[(Si,Al)_4O_{11}]_2$	Easily weathered to clay minerals and oxides
Pyroxene	$(Ca,Mg,Fe,Ti,Al)(Si,Al)O_3$	Easily weathered
Olivine	$(Mg,Fe)_2SiO_4$	Easily weathered
Epidote	$Ca_2(Al,Fe)_3(OH)Si_3O_{12}$	Highly resistant to chemical weathering, used as index mineral in pedologic studies
Tourmaline	$NaMg_3Al_6B_3Si_6O_{27}(OH,F)_4$	
Zircon	$ZrSiO_4$	
Rutile	TiO_2	
Kaolinite	$Si_4Al_4O_{10}(OH)_8$	Abundant in clay as products of weathering, source of exchangeable cations in soils
Smectite	$M_x(Si,Al)_8(Al,Fe,Mg)_4O_{20}(OH)_4$, where M= interlayer cation	
Vermiculite		
Chlorite		
Allophane	$Si_3Al_4O_{12}.nH_2O$	Abundant in soils derived from volcanic ash
Imogolite	$Si_2Al_4O_{10}.5H_2O$	
Gibbsite	$Al(OH)_3$	Abundant in leached soils
Goethite	$FeO(OH)$	Most abundant Fe oxide
Hematite	Fe_2O_3	Abundant in warm regions
Ferrihydrite	$Fe_{10}O_{15}.9H_2O$	Abundant in organic horizons
Birnessite	$(Na,Ca)Mn_7O_{14}.2.8H_2O$	Most abundant Mn oxide
Calcite	$CaCO_3$	Most abundant carbonate
Gypsum	$CaSO_4.2H_2O$	Abundant in arid regions

Quartz, feldspar, mica, amphibole, pyroxene and olivine are primary minerals because they are inherited from parent material as opposed to being precipitated through weathering processes. The key feature in these minerals is the Si-O bond, which is more covalent and stronger than most metal oxygen bonds. These minerals show greater resistance to both chemical and physical weathering. The minerals listed from kaolinite to gypsum are classified as secondary minerals because they almost always result from the weathering of primary silicates. These secondary minerals are often less than 0.002 mm in diameter (c.f. clay) and have a relatively poorly ordered atomic structure.[15]

1.2.4. Soil Organic Matter (SOM)

Plant tissue is the primary source of soil organic matter and becomes part of the soil horizon upon decomposition and digestion by soil microorganisms. Animals are secondary sources of soil organic matter (SOM) because they feed on the primary plant tissue, thereby creating waste products and also contribute their own bodies to SOM at the end of their life cycles.[13]

SOM forms stable complexes with micronutrient elements such as zinc, iron and copper. It exhibits low bulk density and low particle density. SOM possesses extensive surface area for adsorption and many other reactions while exhibiting low specific heat and low conductivity which mean its surface warms up easily. It has the greatest affinity for hydrophobic contaminants compared to other soil solids, and thus plays a crucial role in contaminant sorption kinetics. SOM resists compaction and improves water filtration, and improves soil structure. Organic matter decomposes and thus elements are recycled.[22]

1.2.5. Factors Affecting Contaminant Sorption and Desorption

The extent of the sorption of chemicals onto soil particles affects their mobility, bioavailability and toxicity. The physical availability of organic contaminants is affected by their rates of sorption and desorption onto solids. Properties affecting the sorption of contaminants onto soil are solute concentration and residence time and soil physical properties and chemical properties.

In a study to determine the effect of concentration on the sorption of pentachlorophenol (PCP) on soil, the rate of sorption was observed to increase with increasing PCP concentration, but only up to a concentration of 13 mg L^{-1}.[23] Weber *et al*[24], Braida *et al*[25] and Schlebaum *et al*[26] have reported that the sample with a higher initial PAH content always reached equilibrium faster than the sample with the lower PAH concentration.

Sorption of hydrophobic organic compounds (HOCs) can be divided into two phases, a rapid initial sorption phase followed by a slow sorption stage. Increased residence time results in slow increases in sorption.[22] As the contact time between the soil and contaminant increases, the contaminant could become more difficult to remove from the matrix.

The physical properties of soil include *particle size, soil temperature, pore volume* and *soil moisture content*. These properties can affect sorption and desorption of contaminants.

Generally, adsorption increases with decreasing particle size because of the increase in surface area per unit volume. Particle size therefore becomes an important property in silt (2 μm < particle diameter < 75 μm) and clay (particle diameter < 2 μm).[27] Finer particles also tend to have higher organic matter content, which generally leads to a higher adsorption of organic contaminants.[28]

The rate of chemical reactions in soils is enhanced at higher temperatures.[29] Under dry conditions, high temperatures lead to the upward movement of capillary water, bringing dissolved salts to the surface, which may lead to increased precipitation. Very low temperatures inhibit microbial activity and the movement of organic matter into the soil.[30] In a study to determine the effect of temperature on organic compound adsorption to soil, Sleep *et al*[31] reported a decrease in sorption coefficients for toluene, perchloroethylene and naphthalene by 35, 40 and 60 % respectively when the temperature was changed from 25 °C to 90 °C. This suggests that thermal remediation techniques such as hot-water and steam flushing could assist in the removal of sorbed organic soil contamination.

The pore volume is the volume of soil occupied by air and water and is determined by the packing of the soil solids. If the particles are closely packed as in sands or compact subsoils, then the total porosity is low. Porous, aggregated soils have high pore volumes.

There are two types of pores, micropores and macropores. Micropores are defined as having a diameter of 0.06 mm or less and are filled with water that inhibits the movement of air within the soil. [13] Macropores, in contrast, allow for the movement of air and water. Thus, larger pores can be emptied out quicker than smaller pores. The movement of a molecule within a channel of soil pores is dependant on its size relative to the soil pore size.[32]

As soil moisture content increases, the amount of air filled pore space is reduced. However, as water content increases, water molecules displace contaminant molecules and sorption decreases, thus increasing the volatilisation of contaminants.[33] Water also mobilizes contaminants through the soil. Upward mobility of contaminants may occur through capillary action.

Chemical properties of soil include *cation exchange capacity (CEC), pH* and *ionic strength.* These properties can also have a profound effect on contaminant sorption and desorption.

Soil colloids that are made of clay and humus particles tend to possess nett negative surface charges, causing the particles to behave like giant anions. Cations such as Ca^{2+}, Mg^{2+}, K^{+} and NH_4^{+} that are associated with the soil minerals are attracted to these charged surfaces. The strength of this attraction may lead to some cations being exchanged with others. The total negative charge on the surface is known as the cation exchange capacity, CEC. Soil organic matter contributes significantly to the soil's cation exchange capacity. As discussed in Section 1.2.4, soil organic matter (SOM) has the highest affinity for hydrophobic organic contaminants compared to other soil solids, and thus an increase in SOM results in an increase in HOC sorption. As the pH increases, the CEC increases and the preference for polyvalent cations increases. An increase in fractional organic carbon content of the soil leads to an increase in the CEC and the soil surface becomes more negative[34]; the CEC also increases with an increase in the clay mineral content, especially montmorillonite.[29]

Materials between structural layers of minerals, including cations, hydrated cations, organic molecules, and hydroxide octahedral groups and sheets are defined as the interlayer. The sorption capacities of clays are affected by the type of interlayer cation

present. Hundal *et al*[35] demonstrated that the sorption capacities of K-saturated smectites were greater than those of Na-saturated smectites. The hydrophobicity of a smectite was affected by the amount of layer charge, the location of the charge i.e. tetrahedral or octahedral site and the hydration energy of the cation that hydrated the charge.

Sorption has been shown to decrease with increasing soil pH.[36] This behaviour has been related to the surface charge of humic materials. As surface charge increases, humic materials are thought to become less hydrophobic by charge repulsion. This makes the humic materials more hydrophilic and reduces their sorption affinity for hydrophobic solutes.[37]

Lee *et al*[38] demonstrated that at a higher ionic strength the sorption affinity between soil and naphthalene was increased. Stauffer *et al*[39] also observed a marginal increase in hydrophobic organic compound (HOC) sorption with increase in ionic strength. This was attributed to a 'salting out' effect, i.e., a decrease in aqueous solubility of HOCs at an increased ionic strength.

1.3. Surfactants

1.3.1. Introduction

The word surfactant is derived from the phrase *surface-active agent*.[40] The surface activity of a surfactant is due to its amphiphilic nature.[41,42] Amphiphiles contain both a hydrophobic hydrocarbon tail group and a hydrophilic head group,[39] thus making them semi-soluble in both aqueous and organic solvents. Figure 1.3.a. shows different representations of a common surfactant, sodium dodecylsulfate.[43]

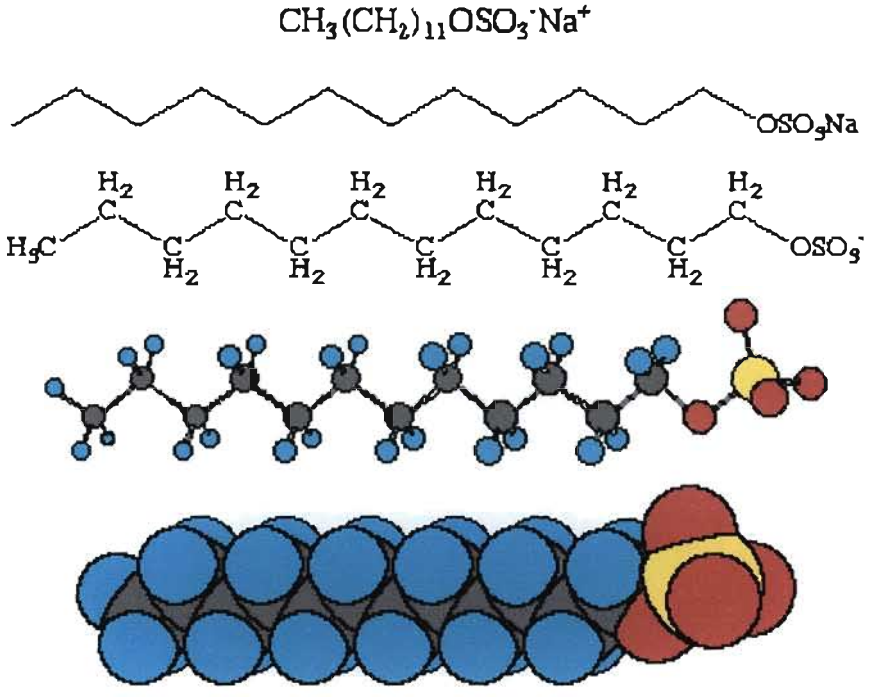


Figure 1.3.a: Sodium Dodecylsulfate Surfactant Molecule

As can be seen in Figure 1.3.a, the surfactant tail group consists of a long lipophilic hydrocarbon tail. Surfactants are also amphipathic compounds because they avoid the organic and aqueous phases, congregating instead at the organic/aqueous interface, or forming micelles.[44] Micelles are dynamic clusters that form once a critical concentration of surfactant monomers is exceeded. This concentration is called the critical micelle concentration or CMC. Figure 1.3.b is a two dimensional representation of a sodium dodecylsulfate micelle.[42]

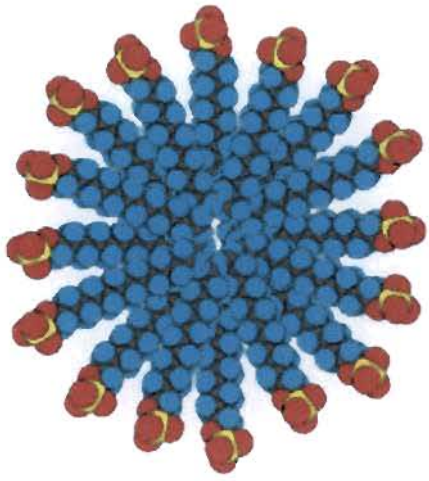


Figure 1.3.b. : Sodium DodecylSulfate Micelle

1.3.2. Surfactant Behaviour in Water

To understand micelle formation and surfactant behaviour in water, one has to understand the concept of surface tension. Water molecules are very strongly associated in the aqueous phase due to hydrogen bonding. This is illustrated in Figure 1.3.c.

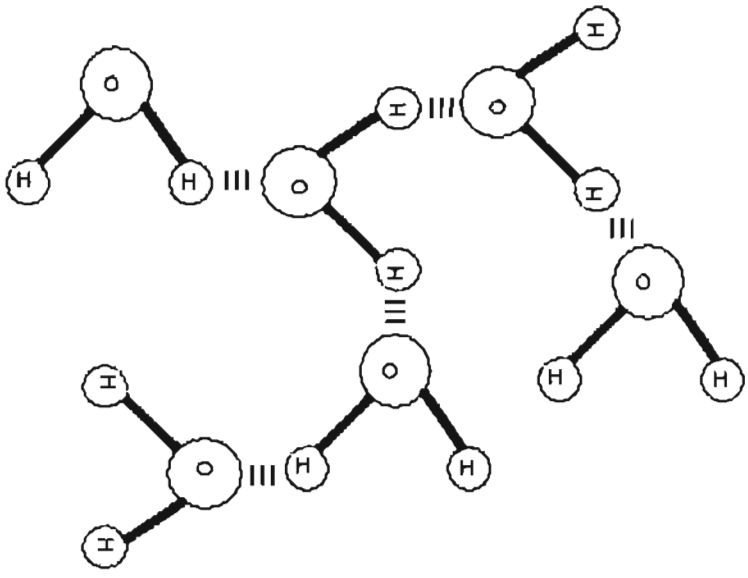


Figure 1.3.c: Hydrogen Bonding in Water Molecules

Water molecules at the surface behave differently to the water molecules below the surface. If one considers the surface water molecule S in Figure 1.3.d[45], the net force acting on it is directly inward. This net inward force acting on all surface water molecules causes water to seek the minimum surface area per unit volume, thus water has the tendency to form droplets or spheres. This is known as surface tension. Surface tension thus slows the wetting of a surface, thereby inhibiting the cleaning process.

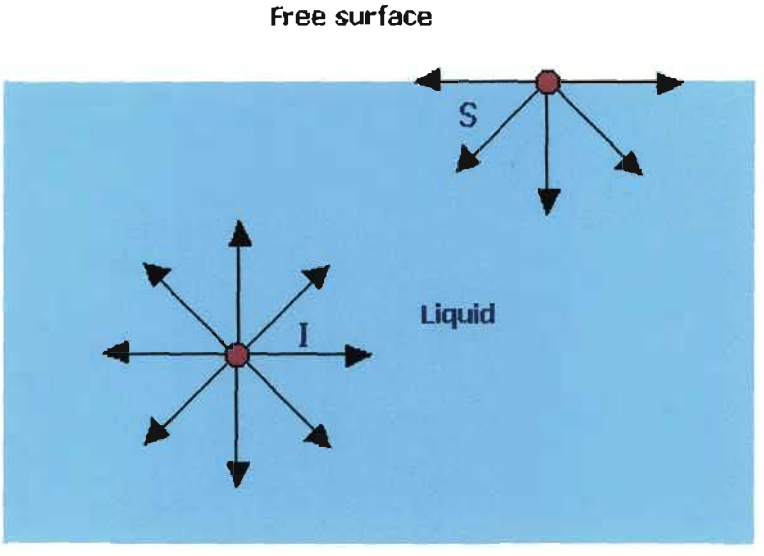


Figure 1.3.d: Forces Acting on Water Molecules

Surfactants aid the cleaning process because they reduce the surface tension of a liquid by reducing the contact angle (angle formed between two substances in contact, as determined by their surface tensions) thus making wettability easier. Figure 1.3.e shows the alignment of monomers at the water's surface and the formation of a spherical micelle in water.[46] The hydrophilic heads of the surface surfactant monomers are attracted to the water molecules while the hydrophobic tails are oriented away from water. In a spherical micelle, the hydrophilic head points to the water, while the hydrophobic tail is directed inward to the core of the micelle.

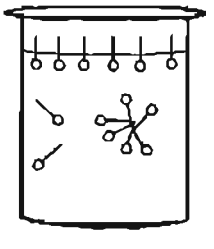


Figure 1.3.e: Micelle formation in water

In addition to spherical micelles, other types of micelle include reversed micelles, admicelles and hemimicelles. A reversed micelle has the surfactant heads pointing inward toward the core and the tails pointing outward. This micelle would form in a nonpolar liquid. Admicelles (Figure 1.3.f) and hemimicelles (Figure 1.3.g) form at the liquid-solid interface.[39] Hemimicelles are a single layer of monomers sorbed onto a solid surface whilst admicelles are aggregated layers of surfactant monomers.

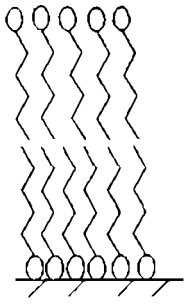


Figure 1.3.f: admicelle

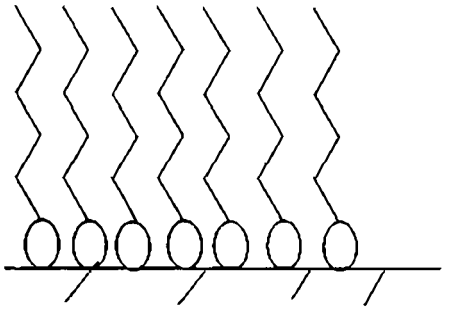


Figure 1.3.g: hemimicelle

1.3.3. Properties of Surfactants and their Applications

Surface-active properties of surfactants include *foaming, emulsification, dispersion, detergency* and *solubilisation.*[47]

Foaming, emulsification and *dispersion* involve surfactants suspending, respectively, a gas, immiscible liquid or a solid in liquid.[45] A *foam* is a gas dispersed in a liquid. It is formed when gas introduced beneath the surface of the liquid is enclosed by the expansion of the liquid around the gas in a thin film.[48] *Foaming* is desirable in shaving foams, bubble baths and shampoos, but is undesirable in applications where agitation or high pressure spraying is used. In the latter, foaming will reduce the contact between the liquid reactants, thus reducing the contact time. In high pressure spraying application foaming reduces the pressure from the jet stream.

An *emulsion* is a reasonably stable suspension of two immiscible liquids. Emulsion stability could range from minutes to years.[46] Two types of emulsions exist, *macroemulsions* and *microemulsions*. Macroemulsions (particle size range 0.2 to 50 μm) are physical dispersions of one fluid in the other and are relatively unstable. Microemulsions (particle size range 0.01 to 0.20 μm) on the other hand are composed of

submicroscopic particles that are suspended by Brownian motion and hence are thermodynamically stable. Macroemulsions are frequently highly viscous dispersions, whereas microemulsions are extremely fluid with relatively low viscosities.

Dispersion is the suspension of a solid in liquid. Two types of *dispersions* exist, *lyophobic* and *lyophilic*. In lyophobic dispersions, the dispersed solids are not well solvated; in lyophilic dispersions, the dispersed solids are well solvated by layers of solvent molecules, thus making them less susceptible to aggregation and thus more stable.

Detergency involves three steps. The soiled substrate is first wetted; then the soil is removed from the substrate and finally soil is suspended and prevented from redepositing on the substrate. *Solubilisation* refers to the micellar uptake of an insoluble compound to form a clear and stable solution.[49]

As a result of these properties, surfactants are used in the chemical manufacturing industry and are important constituents of toiletries and detergents. Surfactants are also used in the stabilisation of emulsions (*e.g.* convenience foods, paints) and oil-well drilling.[50]

1.3.4. Surfactant Classification

Surfactants are classified according to the nature of their head group in aqueous media. [40,51] Table 1.3.a lists the surfactants according to their head group.

Table 1.3.a: Classification of Surfactants

Charge on head group	Classification of surfactant
Positive (+)	Cationic (ammonium chlorides, amine acetates)
Negative (-)	Anionic (fatty acids, sodium dodecyl sulfate)
No charge (0)	Nonionic (polysorbates, cholesterol)
Positive and negative	Zwitterionic/amphoteric (ammonium sulfates, amine oxides)

Anionic surfactants are the most widely manufactured surfactants because of their low manufacturing cost and their detergent action. They have higher critical micelle

concentration (CMC) values than with nonionic surfactants. This is advantageous because monomers diffuse rapidly compared to large micelles. Also, the soil molecule is electrostatically attracted to the anionic head, thus making soil removal easier.[40,48]

Cationic surfactants are expensive to manufacture and have a poor detergent action; applications include softeners, anticaking agent in fertilisers, dispersing agents in bitumen and corrosion inhibitors in oilfields. [49] They are readily adsorbed onto solids from aqueous solution.

Nonionic surfactants are most widely used in the food and animal feed industries. Other areas of use include mining, flotation, oil recovery, textiles and fibres, cosmetics, paints and polymers because of their ability to form extremely stable emulsions.[46]

In acidic solutions, amphoteric surfactants form cations and in alkaline solutions they form anions. These surfactants are used with anionic surfactants in shampoos, foam baths, shower gels and washing powders. Amphoteric surfactants are milder on the skin than anionic surfactants.[48]

1.3.5. Surfactant Parameters

An in-depth understanding of surfactant properties viz. hydrophile-lipophile balance (HLB number), cloud point, aggregation number and turbidity are required to optimise surfactant selection for any given application.

HLB Number

The *HLB number* predicts the emulsifying properties of a nonionic surfactant and is applicable to nonionic surfactants only and a proposed formula for charged surfactants has proved to be unsuccessful. Equation 1.2 can be used to estimate the HLB number.[49]

$$HLB = \frac{molar\ \%\ of\ the\ hydrophilic\ group}{5} \qquad (1.2)$$

Surfactants with low HLB numbers have a weak hydrophilic end and a strong lipophilic end and are therefore oil soluble. Conversely, surfactants with high HLB numbers have a strong hydrophilic end and a weak lipophilic end and are therefore water-soluble.[40] The

HLB number effectively compares the polyaromatic (PAH) solubilisation powers of surfactants. The lower the HLB number, the greater the solubilising power of the surfactant.

Cloud Point

The cloud point temperature is an indication of the maximum temperature to which a surfactant solution should be heated and is only applicable to non-ionic surfactants Ethylene oxide derivatives are soluble in water due to the hydrogen bond between water and the ethylene oxide group. When an ethylene oxide derivative solution is heated, dehydration takes place and the product precipitates out of solution. The temperature at which this occurs is known as the *cloud point*, as the solution becomes cloudy. Above this temperature, solubility decreases rapidly. The product dissolves again on cooling.

Aggregation Number

The *aggregation number* is the average number of monomers making up a micelle.[52] This number is dependant on the nature of the surfactant and the temperature of the aqueous solution and is important because it determines the size of the core of the micelle, which in turn, determines how much solute it can accommodate. *Turbidity* is the cloudiness caused by suspended particles in solution.[53]

1.3.6. Surfactants in Soil and Aquifer Remediation

Due to environmental concerns, surfactants have assumed an important role in the remediation of PAH-contaminated soil and aquifer material.

Surfactants have been found to be useful in soil washing and bioremediation studies[54] and can be used in various ways to enhance the remediation of hydrophobic organic compound (HOC) contaminated soils or sediments. Cationic surfactants can be irreversibly sorbed onto soils to enhance HOC sorption and immobilisation; nonionic surfactants can reduce the interfacial tension between water, sediment and non-aqueous phase liquid (NAPL) to induce two-phase flow;[40] surfactants can increase the bioavailability of a substrate in bioremediation and the HOCs can be desorbed from soil by micellar surfactant solutions.[55]

1.3.7. Micellar Solubilisation of Organic Compounds by Non-ionic Surfactants

Micelles are dynamic aggregates, with sizes ranging typically in the nanometre range. The residence time of a monomer within a micelle at a given coordination number is of the order of 0.2 – 10 μs, and the mean lifetime of a micelle is in the millisecond time scale. The rate of transfer of hydrophobic organic hydrocarbons (HOCs) between the aqueous and micellar phases is rapid. A first order rate coefficient of 10 μs^{-1} was measured for the transfer of pyrene into a sodium dodecylsulfate micelle.[56]

Micellar solubilisation involves the transfer of the soluble compound from the aqueous phase to the micellar phase. Solubilisation can occur at different sites in a micelle as shown in Figure 1.3.h. These sites have been identified as: a) the micelle-water interface, b) between the hydrophilic head groups, c) in the micelle palisade layer and d) the inner core of the micelle.[57]

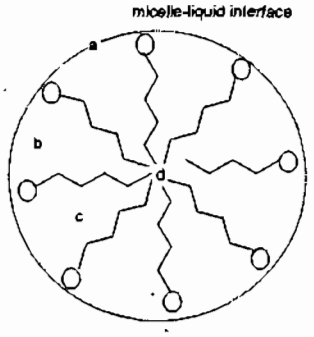


Figure 1.3.h: Solubilisation Sites in a Micelle

The site at which solubilisation occurs depends both on the surfactant and the solute, e.g. in an ethoxylated non-ionic surfactant, nonpolar HOCs will be solubilised into the micellar core, while polar HOCs will be solubilised in the polyoxyethylene (POE) shell. [58]

1.3.8. Factors Affecting the Micellar Solubilisation of PAHs

The amount of solute that can be accommodated by a micelle depends on the surfactant structure, aggregation number, micelle geometry, ionic strength, temperature, solute polarity, solute hydrophobicity and solute size.[51]

The aggregation number increases with increasing temperature below the surfactant cloud point. Thus, the hydrodynamic radius and the core volume of the micelle also increase.[59] Increasing the temperature also increases the aqueous solubility of the organic compound and the thermal agitation of the surfactant molecule. These factors serve to enhance PAH solubilisation.

Pennell *et al* [60] demonstrated that surfactants with a larger alkyl chain length are able to solubilise more hydrocarbons than shorter chain surfactants with similar HLB values. The longer hydrophobic chain creates a larger micelle core volume. In the study of the solubilisation of compounds of differing polarity in non-ionic surfactant solution, the authors concluded that larger quantities of the less hydrophobic compound were solubilised. This is explained by the fact that the more hydrophobic compound will only be solubilised into the core of the micelle, while the less hydrophobic compound can be solubilised into the core of the micelle and the polyoxyethylene (POE) shell.

The solubilisation rate of an organic molecule into a micelle is dependant on the size of the molecule because larger molecules move slowly. Fewer large molecules can be accommodated by the micelle than a smaller molecule.[61]

1.3.9. Adsorption of Surfactants onto Surfaces

Surfactants may be lost during soil and aquifer remediation processes due to mechanisms such as precipitation with natural hardness in groundwater, sorption onto soil and biodegradation by soil microorganisms.[62,63] A micelle, being an aggregate, will break up on contact with soil because of monomer adsorption at the soil-water interface.

Factors affecting surfactant sorption onto surfaces include *surfactant chain length, pH, ionic strength, surfactant concentration, soil organic matter* and in the case of nonionic surfactants, the *number of ethoxylate groups.*

In a study of cationic surfactant adsorption onto silica, Goloub and Koopal[64] found that increasing the *aliphatic chain length* resulted in an increase in the hydrophobic attraction with the surface as well as an increase in the lateral attraction with the hydrocarbon chains

of surfactant. As the *pH* increased, the amount of surfactant adsorbed onto the surface also increased. There was a steeper increase in adsorption at higher *ionic* strengths. In another study of factors affecting surfactant sorption onto surfaces, at low *ionic strengths*, anionic surfactant adsorption decreased with an increase in pH.[65] For high sorption capacity soils (e.g. clays), surfactant adsorption increased with increasing *ionic strength*. However, adsorption remained independent of *ionic strength* for low sorption capacity soils. Changes in nonionic surfactant sorption were inversely proportional to changes in *pH*, since changes in pH can change the surface properties of a soil.

The sorption of nonionic surfactants was shown to reach a maximum at a *surfactant concentration just below or at the CMC*.[67] At low *surfactant concentrations*, sorption of surfactant as monomers may occur, while at *higher surfactant concentrations*, surfactants sorb as bilayer aggregates or admicelles.[66] At *concentrations greater than the CMC*, the amount of sorbed surfactant may reach a constant.

John *et al*[67] concluded that nonionic surfactant adsorption is inversely proportional to the *number of surfactant ethoxylate groups*, especially in soils with a high sorption capacity. The nonionic surfactant bonds to the soil via hydrogen bonding. Nonionic surfactant adsorption is also inversely proportional to the *ethylene oxide chain length*. This is because long chains hinder the formation of admicelles due to chain-chain interactions. *Soil organic matter* enhances the sorption process. This could be due to hydrophobic interaction with the hydrocarbon chains of the nonionic surfactant.

1.3.10. Surfactants and The Environment

In order for surfactants to be environmentally acceptable, they should ideally be biodegradable, non-toxic to human, animal and plant life and be recyclable. If biodegradable, the biodegradation products should also be non-toxic. Some surfactants have the ability to interact with cell membranes[68] by adsorbing at interfaces and binding through hydrophobic interactions with proteins. This can cause disruption in normal cell function and also affects enzymic activity.[69]

While some surfactants could be environmentally agreeable, others may prove to be otherwise. Surfactants can be moderately toxic to fish and aquatic invertebrates.[70, 71]

Nonylphenol ethoxylates have been banned in Western Europe because they have been found to have endocrine disrupting effects in freshwater organisms at concentrations of 20 $\mu g\ l^{-1}$.[69,72]

Two primary alcohol ethoxylate non-ionic surfactants, Dobanol 45-7 and Dobanol 45-11, were tested for biodegradability and 96 – 98% degradation was achieved in the 5 to 10 °C temperature range. The toxicity of the degradation products was tested on rainbow trout over a period of 7 days. No adverse effects were noted on the fish.[69]

Warisnoicharoen *et al*[73] investigated the toxicity of the nonionic surfactants, polyoxyethylene-10-oleyl ether (C18:1E10), polyoxyethylene-10-dodecyl ether (C12E10), and *N,N*-dimethyl-dodecylamine-N-oxide (C12AO) to human bronchial cells. Systems containing C12E10 and C12AO were toxic at concentrations around or below their critical micelle concentrations. Surfactant toxicity was suggested to be due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

1.4. Soil Washing

1.4.1. Introduction

Soil washing incorporates a combination of physical and chemical techniques to clean contaminated soils.[74] Soil washing may be done ex-situ or in-situ. Ex-situ soil washing involves excavating contaminated soil, which is then fed into a soil-washing unit. Contaminants are removed by contacting the soil with a washing solution.

In-situ soil flushing is applied to unexcavated soils using a groundwater extraction and reinjection system. A solvent and/or a surfactant solution is added to the soil and the contaminants in the leachate or groundwater are then recovered.[75]

Chu *et al*[76] identified two distinct stages in which soil washing could occur.

- In the first stage, hydrocarbons can be extracted from the soil by surfactant monomers. It was noted that the washing performance was directly proportional to the available monomer content.
- In the second stage, the soil is saturated with surfactant so micellization can occur. The HOCs can then be solubilised by the micelles. These two stages are analogous to the soil rollup and solubilisation mechanisms discussed later in Section 1.4.2.d..

1.4.2. The Soil Washing Process

Although different soil washing processes vary slightly from each other, the basic unit processes and principles are standard. These unit processes are particle size separation, washing of sand size fraction, treatment of fines and effluent treatment.

1.4.2.a. Description of the Equipment Used in Soil Washing

A *trommel* (Figure 1.4.a)[77] is a large mechanically operated screen that consists of a rotating perforated cylinder with its axis at a slight angle to the horizontal. A *grizzly* (Figure 1.4.b)[78] is a large hand operated screen. It has a plane screening surface composed of longitudinal bars (up to 3 metres long) fixed in a rectangular framework. In a

hydrocyclone (Figure 1.4.c)[79], feed is introduced at a high tangential velocity. Separation of particles is effected in the centrifugal field. A *belt filter* (Figure 1.4.d)[80] is an endless belt arranged in the horizontal plane and running over pulleys. Rubber or wiper blades drag against the cake surface and can be used to isolate the filtrate and washing zone from each other. The abovementioned equipment is used to screen oversized soil fractions.

Figure 1.4.a: Trommel

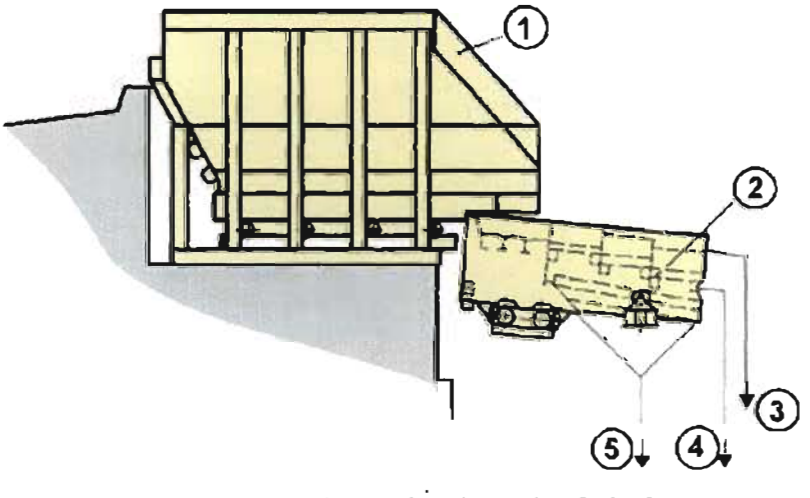


Figure 1.4.b: Cross-Section of a Grizzly

1. Plate Feeder
2. Vibrating Grizzly Screen
3. Oversize to the crusher
4. By-pass of the crusher
5. Waste

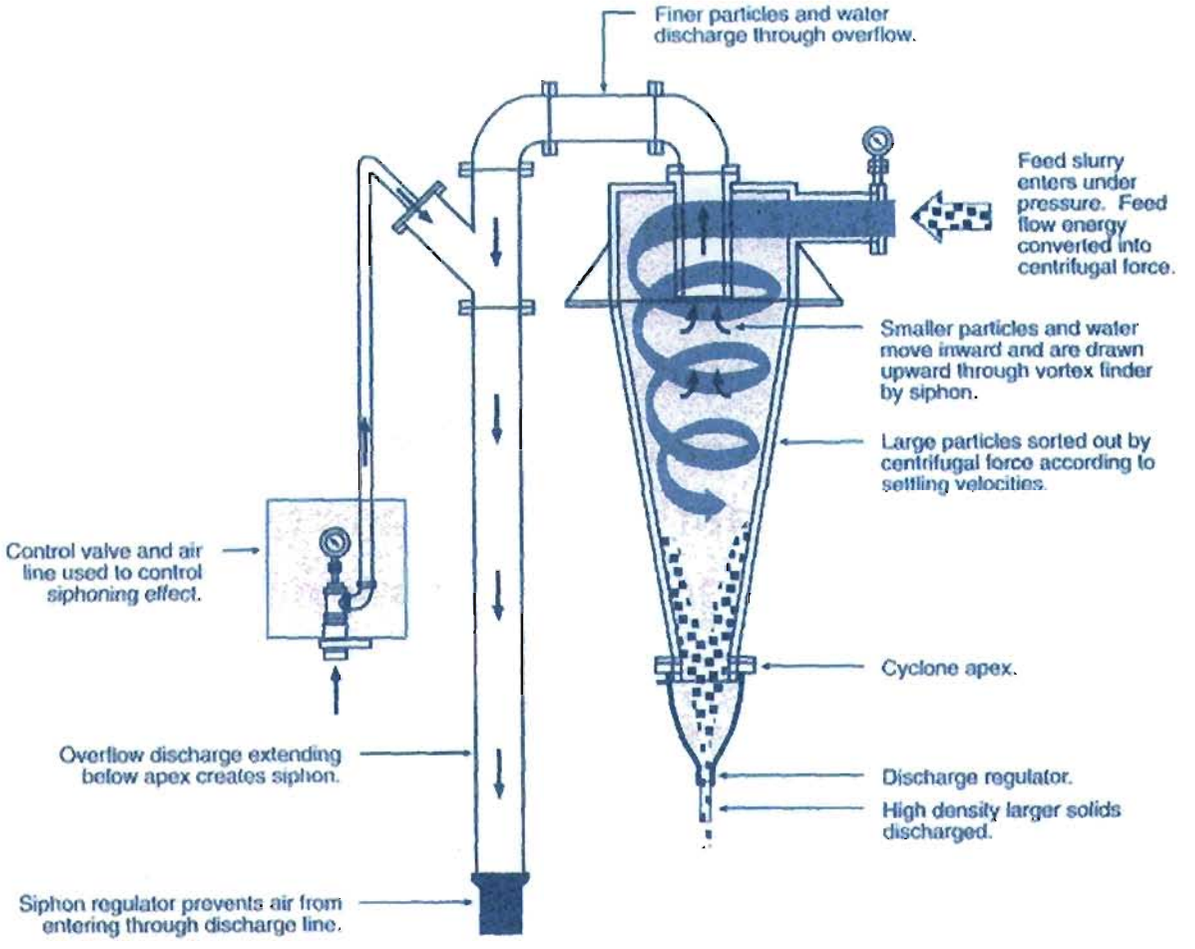


Figure 1.4.c: Schematic Diagram of a Hydrocyclone

Figure 1.4.d: Belt Filter

1.4.2.b. Particle Size Separation

The concentration of contaminants in the soil matrix increases with decreasing particle size as demonstrated for soil samples L, A and H in Figure 1.4.e.[81] This is due to an increased surface area per unit volume (Section 2.4). The three soil fractions significant to soil washing are the oversize fraction (particle diameter > 5mm), the sand fraction (0.063 μm < particle diameter < 5mm) and the fine particle fraction (particle diameter < 0.063 μm).[74] If fines constitute a small fraction of the bulk soil, the fines could be washed from the soil particles, leaving behind a clean, coarse fraction.[82]

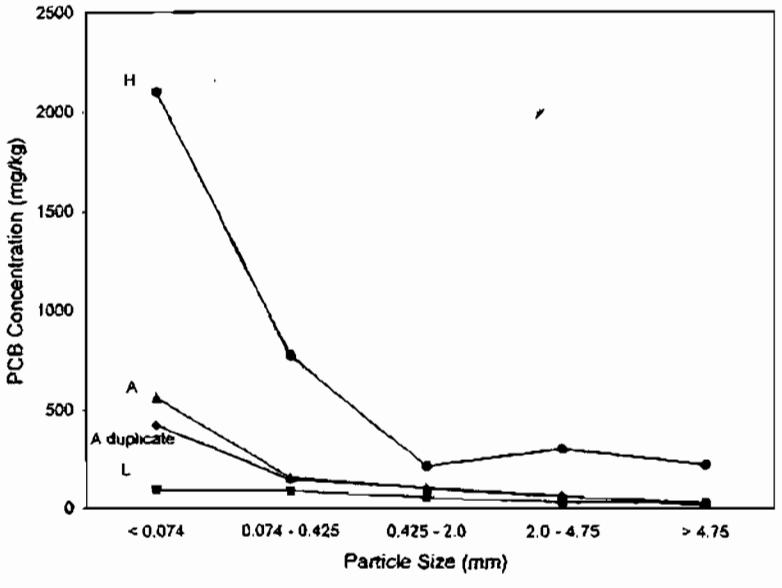


Figure 1.4.e: Concentration of Polychlorinated Biphenyls in Different Soil Size Fractions

Noyes[83] related the effectiveness of soil washing to particle size distribution (Table 3).

Table 1.4.a: Effectiveness of Soil Washing in Relation to Particle Size Distribution

Particle size distribution (mm)	Effectiveness
> 2.00	Oversize pretreatment requirements
0.25-2.00	Effective soil washing
0.063-0.25	Limited soil washing
<0.063	Difficult soil washing (Clay fraction)

Only contaminated soil is fed to the soil washing plant, therefore, the gross oversize debris must be removed *in-situ*. The pre-screening can be achieved using a hopper mounted with a vibrating grizzly, and a trommel screen to screen out particles of diameter greater than 50 mm.[74]

Since smaller particles adhere to larger particles, the soil can be broken up in an attrition scrubber.[84] Attrition forces arise from the friction of particles against one another or against a rigid surface.[85] In a volume reduction unit (VRU) shown in Figure 1.4.f,[86] high attrition is achieved by blending soil with a small volume of water and washing additive in a trough bottom hopper fitted with a ribbon blender. The washed mixture then moves on to a trommel that is sprayed with additional wash water. Particles of diameter greater than 2 mm are screened out. Thus, the clean coarse fraction is removed from the fines.[82] Sandscrews combine countercurrent washing of the soil with hydrosizing and flotation to remove fines from soil slurry.[84] Hydrocyclones have also been employed in particle size separation. In the cyclone, the sand is able to exit from the bottom and the fines from the top.[74,82] Froth flotation may also be used for particle size separation. A flotation surfactant reduces the surface tension binding the sand and contaminant. The froth is generally passed onto the fines management stream. If there is a significant density difference between the sand and contaminant, spiral concentrators may be employed.

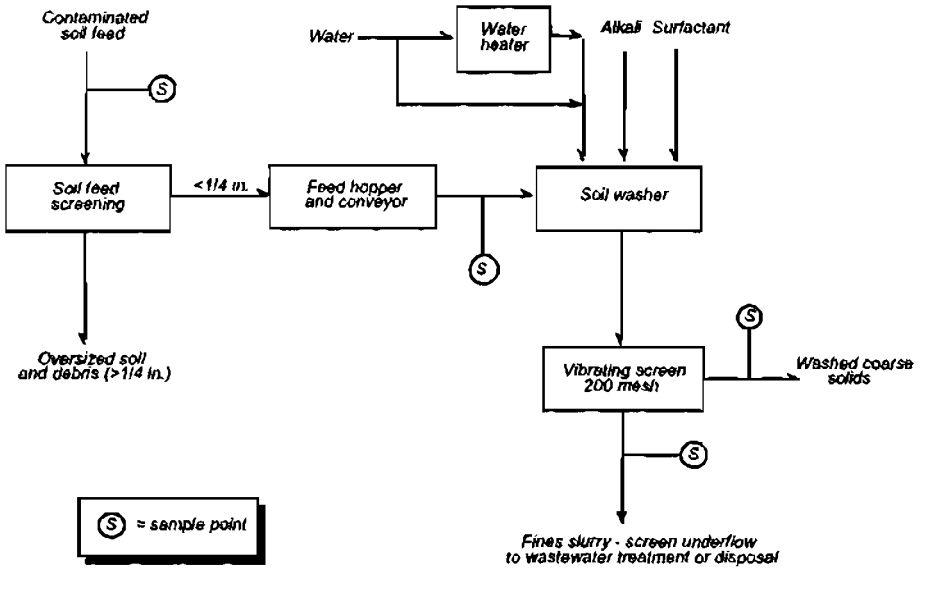


Figure 1.4.f: Schematic Diagram of a Volume Reduction Unit (VRU)

1.4.2.c. Treatment of Fines

The fine particles are managed in a separate system. This is a difficult fraction to treat because of the high contaminant concentration and complex binding and speciation of contaminants. Soil particles finer than 20 μm can only be washed if the contaminants can be solubilized by the wash water or separated by flotation.[84]

Alternatively, fines are treated like wastewater sludge by polymer addition, sedimentation, thickening and dewatering. The use of biological additives to bioremediate the fines 'sludge' can also be considered. The sludge is thickened to approximately 15 % dry solids and the water is recycled to the screening area. The thickened solids are then filtered under pressure using a belt filter press. The influent is then converted to an approximately 50 % dry solids filter cake, within which the contaminants are contained. The cake can be treated *ex-situ*.[74]

1.4.2.d. Wash Water Additives

Additives should be avoided if plain water can be used to achieve appreciable volume reduction of contaminants. Additives complicate recycling of wash water, however, it is necessary to use additives if the contaminants are greatly hydrophobic. Commonly used additives include acids, bases, surfactants, dispersants and chelating agents. (Acids and chelating agents are used in metal contaminated soil washing.) [82]

Two mechanisms are involved in surfactant enhanced soil washing. Below the critical micelle concentration (CMC), washing occurs with the soil rollup mechanism and above the CMC, washing occurs via solubilization. In the soil rollup mechanism, the first step involves surfactant monomers aligning at the soil-contaminant and soil-water interfaces thus increasing the contact angle between the soil and the contaminant. Monomers adsorbed on the contaminant's surface cause repulsion between the monomer's head group and the soil particles, thereby loosening the hygroscopic bond between the contaminant and the soil. In the second step, convective currents displace the contaminant from the soil. In solubilisation, contaminants are partitioned into the hydrophobic core of the surfactant micelles.[87]

Surfactant choice is governed by numerous factors, eg. soil particle size fraction, cost, recyclability, toxicity, the nature of contaminant and surfactant recovery, which are assessed by bench-scale testing.[88] In addition, the predominant mechanism (soil rollup or solubilization) has to be determined. Nonionic surfactants are preferred for soil rollup mechanisms because of their lower CMC. However anionic surfactants are more resistant to sorption and precipitation losses and could be more economically viable. Soil-surfactant-contaminant interactions that impact on the efficacy of the surfactant to solubilize the contaminant are also important. Surfactant properties such as foaming, phase separation and precipitation affect the soil washing process.[87]

1.4.2.e. Effluent Treatment

To make the soil washing process economically viable, the wash water may be recycled. Firstly, any residual fines in the effluent have to be removed. This can be achieved by flocculation. Reducing the pH to the 4 – 5 range reverses the soil washing process of dispersion by reversing the charge on the fines, and promotes flocculation and settling. Centrifuges and filters can also be employed to remove fines from the effluent.[84]

Subsequently, the contaminants are removed from the effluent. This can be achieved by precipitation[84] or solvent extraction.[89]

1.4.3. Factors Affecting Soil Washing

It is difficult to formulate a single wash water solution for soils contaminated with both metals and organic compounds, as they require different approaches. Sequential washing, which involves removing only one group of contaminant at a time, may be required.[90]

Soils with a very high organic content often require pre-treatment. A high humus content binds to the soil, thereby decreasing the mobility of the organics.[91] Soils with a high volatile organic compound (VOC) content may require an emission control unit.[84,90] The cleaned soil fractions may be backfilled or dumped. Soil washing is generally carried out at elevated temperatures because the rate of the chemical reaction is enhanced. The viscosity of the solution is reduced, thus reducing the surface tension that reduces bubbles formation. Surface attractive forces are also reduced at higher temperatures, thus making

soil dispersion easier.[92] Increasing the pH of the system can also enhance the solubilization of organic compounds.[88]

1.4.4. Soil Washing Studies

Abdul *et al*[93] washed polychlorinated biphenyl (PCB) contaminated sandy aquifer material with a non-ionic ethoxylated surfactant solution. The sand was washed intermittently in a column type experiment. After 20 washings, 66, 86 and 56 % of the contaminants were removed by 5 000, 10 000 and 20 000 ppm solutions of surfactant respectively.

Pennell[94] *et al* investigated the remediation of tetrachloroethylene contaminated soils using a 4 % solution of polyoxyethylene (POE) sorbitan monoleate. 90 % of the contaminant was removed in the 20-30 mesh soil column, compared to 97 % for the 40-120 mesh soil column. In an earlier study, Pennell[95] *et al* conducted a similar investigation with dodecane as the analyte. Column flushing with a 43 000 mg L^{-1} solution of the non-ionic POE sorbitan monoleate surfactant was shown to enhance the solubilisation of the dodecane by up to five orders of magnitude when compared with flushing with plain water.

Dwarakanath[96] *et al* improved surfactant performance in soil column washing using a surfactant and co-solvent formulation. Their results demonstrated that up to 99.9% of contaminants could be removed from the soil column with only 1 to 2 pore volumes of surfactant flooding. It was also noted that mobilization experiments required only 2 to 4 pore volumes of surfactant flooding compared to 11 to 20 pore volumes for solubilisation experiments.

Abdul[97] *et al* conducted laboratory and field scale *in-situ* washing studies of PCB contaminated soils. The laboratory and field results compared well with each other for washings of 5.7 pore volumes and 8 pore volumes. Under laboratory conditions, 85 % of the contaminant was removed after 105 pore volume washings. In the field study, 25 % of the contaminant was recovered from the leachate after two phases of flushing with surfactant.

There are many variables to consider in soil washing. These include the nature of the soil, the nature and binding of the contaminant, the type of surfactant to be used, in-situ or ex-situ soil washing, the cost of the application and compatibility with other techniques like bioremediation. Thus laboratory and pilot scale soil washing trials have to be undertaken before the field scale investigation because of the multitude of factors affecting the process

1.5. Bioremediation

1.5.1. Introduction

Bioremediation is a biological method of treatment involving the controlled use of microorganisms to break down xenobiotic chemical substances into less hazardous or benign components. Many microbial species occurring naturally in soil and water have been found to be capable of remediating hydrocarbon contaminated soil.[98] Aerobic degradation of these substances results in the formation of carbon dioxide and water, while anaerobic degradation results in the formation of carbon dioxide and methane.[99]

Bioremediation is considered an attractive alternative to chemical and mechanical means of remediation because it can be cost effective and can also result in the destruction of the contaminants.[100,101] However, natural biodegradation processes tend to be very slow.[102] Studies have shown that surfactant addition enhances bioremediation processes.[103] To understand how surfactants can enhance microbial processes, one has to understand the factors affecting microbial processes.

1.5.2. Factors Affecting Microbial Processes

Biodegradation occurs most rapidly or exclusively in the aqueous phase,[104] which is limited by oil phase partitioning, adsorption and diffusion processes.[105] This in turn influences the bioavailability, i.e. the fraction of substrate available for microbial attack, as intimate contact is needed between the microorganisms and the contaminant to increase biodegradation rates.[99] The mechanisms which influence polyaromatic hydrocarbon bioavailability have been outlined as;

- PAH soil-water partitioning equilibria,
- PAH mass transfer (desorption) rates, and
- microbial processes for PAH uptake and degradation.[106]

Aerobic degradation is generally faster than anaerobic degradation and is therefore the preferred method of treatment.[99] The oxygen levels of soil can be improved by tilling,

composting with bulking agents to increase porosity or by venting. In slurries, this can be achieved by sparging or the addition of hydrogen peroxide.[107]

Moisture is necessary for microbial metabolism and adequate moisture is needed for the microbes, nutrients and contaminants to be transported through the soil.[99,107] Nutrients such as nitrogen and phosphorus are essential for microorganisms to be able to degrade contaminants. Manilal[104] *et al.* demonstrated that phenanthrene mineralisation was enhanced by the addition of phosphates.

The optimum pH range for microbial activity is 6 - 8.[99] It may be necessary to determine the optimum pH for a microbe, because changes in pH affect the structure and activity of the microbe.[108] Soil temperature also plays a crucial role in the metabolic activity of microbes and the decomposition of organic matter. Optimum metabolic activity occurs between 15 °C and 35 °C. Most bacteria are unable to thrive at temperatures less than 4 °C or temperatures greater than 40 °C.[108]

The nature and concentration of the contaminant affects the rate of biodegradation. A higher initial contaminant concentration means a faster uptake rate of the substrate into the cells, thus enhancing the rate of biodegradation.[109] Volkering *et al*[110] observed different biodegradation mechanisms for crystalline and sorbed PAHs. Crystalline PAH degradation occurred only at supra-CMC (concentrations much higher than the CMC) levels, while sorbed PAH degradation was stimulated at sub-CMC and supra-CMC levels (concentrations above or below the CMC). In highly weathered soils, chemical and biological weathering may transform the contaminant into a recalcitrant compound, making bioremediation difficult.

Artificially grown bacteria may outperform natural bacteria in degradation because the native microbes may need to achieve a critical population in order to yield demonstrable contaminant degradation; the metabolic range of the native bacteria may not be able to degrade certain compounds.[99,108] However, artificially grown bacteria must first be fully acclimatised to the field conditions.

Surfactants can increase the aqueous solubility of hydrophobic organic compounds (HOCs), thus increasing their bioavailability. Polyaromatic hydrocarbon (PAH)

mineralisation can be inhibited at concentrations above the critical micelle concentration (CMC) and no degradation enhancement effect may be observed by sub-CMC surfactant concentrations on PAH mineralisation.[111] Diluting the surfactant to sub-CMC levels could reverse the inhibitory effect. It has been suggested that the inhibitory effect is due to a reversible physiological surfactant micelle-bacteria interaction[100] and may also be due to the total destruction of the cell membrane.[106] Factors to be considered when selecting a surfactant for bioremediation studies are its PAH solubilising ability, extent of sorption onto sediment materials, toxicity to bacteria and fate in the environment.

1.5.3. Methods of Bioremediation

Ex-situ methods of bioremediation include *composting* or *biopiling* and *landfarming*. In *composting* or *biopiling*, excavated contaminated soils are heaped into piles and microbial bioremediation activity is stimulated through aeration and/or addition of minerals, nutrients and moisture. Aeration is done by forcing air through slotted or perforated piping placed throughout the pile.[112] In *landfarming* the soil is not heaped into piles, rather, it is applied in a thin layer to a ground surface and aeration is achieved by tilling or ploughing.[112]

Bioventing, soil vapour extraction (SVE) and *low-temperature thermal desorption (LTTD)* are in-situ bioremediation techniques. *Bioventing* is used in the bioremediation of compounds with a low volatility. It combines the physical process of soil venting with the microbial process of bioremediation. [113] Creating a vacuum in the vadose zone creates airflow through the soil.[99, 112] The negative pressure increases the volatilisation of hydrocarbon compounds sorbed to the soils in the vadose zone. *Soil vapour extraction (SVE),* is a technique used for reducing concentrations of volatile substances in the unsaturated zone. By applying a vacuum in the unsaturated zone, contaminated air is removed from the soil and replaced by clean atmospheric air. This causes the continuous transfer of contaminant from the aqueous and solid phases into the gaseous phase.[114] In *low-temperature thermal desorption (LTTD),* soils are heated to temperatures that result in the volatilisation and desorption of contaminants from the soil. A gas or steam injection system is used to heat the soil.[113] The vaporised contaminants are then treated in an afterburner, catalytic oxidation chamber, condenser or carbon adsorption unit before being released into the atmosphere.[112]

Solidification aims to remove the free liquid from a contaminated material. This not only decreases the surface area of the material, but also produces a solid product of high structural integrity. Solidification can involve the encapsulation of fine waste particles (microencapsulation) or large blocks of wastes (macroencapsulation). The waste material becomes mechanically bound to the solidified matrix so that the release rate of the contaminants in the environment is significantly decreased. *Stabilisation* refers to converting contaminants into their least soluble, mobile or toxic form.[108]

1.6. Aims and Objectives of Study

Sasol is the South African leader in oil-from-coal technology and is a global player in the international chemical and fuel industry. One of the issues facing Sasol is the disposal of hazardous hydrocarbon waste. Waste streams are sent to Enviroserv for disposal in its Holfontein landfill site at an approximate cost of R 1500/ton. Sasol has, however, conducted research showing that biopiling and composting are cheaper methods of API sludge disposal as opposed to disposal by Enviroserv.

Soil washing using surfactants may be considered as an alternative to biopiling and composting. Literature has revealed that surfactant can increase the solubilization of polyaromatic hydrocarbons (PAHs), making it easier to remove contaminants from the soil. Because Sasol is a manufacturer of the nonionic Safol 45 series of surfactant, successful soil washing studies would imply that the surfactant could be marketed internationally for soil and aquifer remediation purposes.

The objective of this project was to investigate the aqueous solubility of PAHs in a series of Safol 45 surfactants (Safol 45E3, 45E5, 45E7, 45E9 and 45E12), under conditions of varying pH and concentration, with temperature and ionic strength as fixed variables. The surfactant with the highest solubilisation capacity was then used to wash API sludge, PAH spiked soils and sand to determine if the surfactant was able to solubilise the sorbed contaminants.

1.7. References for Chapter One

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CHAPTER TWO

EXPERIMENTAL METHODS AND ANALYSIS

2.1. Experimental Procedures

The solubilities of two PAH analytes, phenanthrene and acenaphthene, were tested in water and aqueous surfactant solutions of varying concentrations. The temperature was maintained at 40 °C, but the pH values of the solutions were varied between 5 and 8.

2.1.1. Solubility Studies in Aqueous Media

The aims of the solubility studies were to identify the surfactant with the best solubilization capacity and to determine the optimum experimental pH. The surfactant and pH were then to be used in the analyte desorption studies.

A series of nonionic Safol surfactants provided by Sasol Pty were used in the study. Phenanthrene (98 %) and acenaphthene (97 %) were purchased from Sigma-Aldrich Chemicals. To vary the pH of the solutions, MES (2-(*N*-morpholino) ethanesulphonic acid) and Tris (*N-[tris*(hydroxymethyl) methyl] glycine) buffers having a purity of greater than 99 % were purchased from Fluka and Aldrich respectively. Anhydrous sodium acetate (99 %) was obtained from Saarchem. Potassium chloride (99 %, Saarchem) was used to maintain a constant ionic strength of the buffered solutions. The organic solvents, which were all obtained from Merck, were of HPLC grade and included ethanol, hexane, methanol, cyclohexane, and glacial acetic acid. Only Ultrapure water (Modulab Water Purification Systems) was used.

Water or surfactant solution (300 mL) (buffered or unbuffered), and a magnetic stirrer bar were placed in a glass flat-bottomed one-necked flask. The neck was sealed with a latex septum and the flask immersed in a water bath set at 40 °C. The water bath and the flask with solution were equilibrated overnight using a Thermomix 1441 heater stirrer. Insulating spheres (Gallenkamp) were used to maintain a constant temperature and prevent water evaporation in the insulated water bath. This setup is illustrated in Figure 2.1.a.

At the start of the reaction, the septum was removed, 0.05 g of analyte weighed in a plastic weighing boat was added to the flask through a funnel and the septum replaced. The flask was stirred by means of a variable speed control immersion stirrer. Sampling was done

through the septum via a 0.25 μm syringe filter (Micro Filtration Systems), using a plastic syringe (5 mL, Set Inject) attached to a needle (Sigma Aldrich, 20 gauge, 100 mm). Drawing out the first samples two or three times and pushing it back into the flask preconditioned the filter. A 1 mL of sample was pipetted from the syringe using an automatic Eppendorf pipette, and the sample placed into a No.1 glass polytop vial with an aluminium foil covered lid (to reduce evaporation). The sample was then diluted using 1 mL of ethanol. This procedure was used for the solubility studies. The samples were analyzed within two hours of sampling using a Cary 100 Bio UV-Visible spectrophotometer.

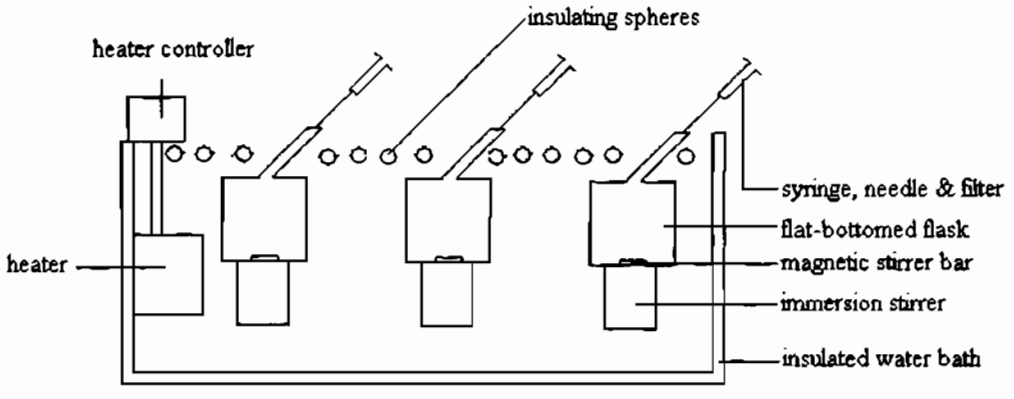

Figure 2.1.a : Experimental Setup for Solubility Studies

The solubility of phenanthrene was tested in water and in solutions of varying concentrations of each of the five surfactants. Since micellization occurs at the critical micelle concentration, the surfactant concentrations were selected by using the CMC value and two concentrations below and above the CMC. This was done in order to investigate surfactant behaviour below and above the CMC. Since the CMC for the surfactants were different, solutions of different concentrations were prepared (Table 2.1.a).

Table 2.1.a: Concentrations† of Surfactant Solution Prepared

Surfactant	Safol 45E3	Safol 45E5	Safol 45E7	Safol 45E9	Safol 45E12
CMC	0.00294	0.00447	0.00391	0.00504	0.00782
Solutions Prepared	0.0025 0.003 0.010 0.10 0.500 1.00	0.003 0.004 0.005 0.010 0.500 1.00	0.00035 0.004 0.010 0.500 1.00	0.004 0.005 0.010 0.500 1.00	0.005 0.008 0.010 0.500 1.00

†All concentrations are expressed in units of mmol L^{-1}

The Safol surfactants differ in the number of ethoxylate groups they contain. For example, Safol 45E3 has 3 ethoxylate groups, while Safol 45E12 has 12 ethoxylate groups. Thus, these surfactants have varying physical properties such as CMC, cloud point, pour point and hydrophile-lipophile (HLB) number. Lists showing the physical properties of these surfactants and the ethoxylate distribution provided in Appendix A.

Because Safol 45E5 had the lowest cloud point (34 °C), the experimental temperature was fixed at 40 °C for all the solubility studies conducted. It was assumed that at 40 °C all the surfactants will be soluble and any chance of precipitation from Safol 45E5 will be minimal.

The solubility studies involving Safol 45E7 and Safol 45E12 were subjected to pH variation. Since the intentions of using surfactants were to solubilise PAHs to promote bacterial activity, the pH range that was investigated was between pH 5 to 8. The specific pHs that were investigated were 5, 6, 7 and 8, while the concentration of the surfactants were 0.1, 0.5 and 1 mM. At surfactant concentrations of 1 mM, sampling became difficult due to the clogging of the filter, thus higher surfactant concentrations were not considered.

The buffered pH 5 solutions were prepared by using sodium acetate and acetic acid. A solution of 0.2 M sodium acetate solution was prepared by dissolving 57.435 g of sodium acetate in 3.5 L of Ultrapure water, while 0.2 M acetic acid solution was prepared by

adding 18.015 g of glacial acetic acid to 1.5 L of Ultrapure water. The two solutions were then mixed together to maintain a volume ratio of 70:30 for sodium acetate to acetic acid. To maintain an ionic strength of 0.1 M, 33.842 g of potassium chloride was added to the pH 5 solution. Buffered solutions at pH 6 and 7 were prepared by using MES with sodium hydroxide and Tris with hydrochloric acid respectively. For pH 6, a mass of 4.2953 g of MES was made up into 4 L of Ultrapure water to which 28.4987 g of potassium chloride was added to maintain a constant ionic strength of 0.1 M. The pH of this solution was adjusted to pH 6 by the addition of approximately 100 mL of 0.1 M sodium hydroxide solution. For the pH 7 solution, the 0.2 M Tris solution was made by adding 30.29 g of Tris to 1.25 L of Ultrapure water. To this, 0.1 M hydrochloric acid solution prepared by adding 25.94 g of 32% hydrochloric acid to 2.275 L of Ultrapure water was added, after which 33.8613 g of potassium chloride was added to maintain a constant ionic strength of 0.1 M before filling the volumetric flask to a 5 L mark using Ultrapure water. The solubility of acenaphthene was only investigated at pH 8 in 0.10, 0.50 and 1.0 mmol L^{-1} solutions of Safol 45E5, 45E7 and 45E12.

It was important to develop a simple method for quantifying the amount of analyte dissolved in the solution. Therefore, several experimental routes were investigated before a reproducible procedure was obtained. The first attempt involved using hexane to extract the analyte from the aqueous solution sampled. Three millilitres of hexane was added to a 1 mL aqueous sample. This mixture was shaken in a separating funnel. The aqueous layer, which was at the bottom, was then collected. The organic layer that remained behind was then dried using anhydrous sodium sulfate. The sample was then analysed using UV-Visible spectrophotometry. The results obtained were not reproducible possibly because the analyte was sorbing onto the drying agent, thus causing the variation in the results. The second method involved extracting the analyte with cyclohexane. No drying agent was used. The results were not reproducible. A possible cause of this could have been the minute percentage of water entering the organic phase. The third method involved using hexane as the extracting solvent and filtering the solution through a Whatman phase separator filter paper. This proved to be cumbersome and time consuming, resulting in loss of solvent and irreproducible results, even though the results were better than the first two attempts. The method that was finally adopted was one where no extraction or filtering process was involved. Samples were diluted using ethanol and their absorbance determined using UV-Visible spectrophotometry.

2.2. Desorption Studies

The moisture content of the soil samples was determined since the experimental soil moisture content had to be fixed at 16.67 % to represent field wet conditions. The soil and sludge particle size fractions and organic matter contents were also determined because these have an effect on the sorption properties of soils.

A standard soil sample was obtained from the Soil Fertility Laboratory at Cedara College of Agriculture. Seasand was obtained from the Durban beachfront while Longlands soil sample was provided by the Soil Science Laboratories of the University of KwaZulu-Natal (Pietermaritzburg). Sasol provided an American Petroleum Institute (API) separator waste hydrocarbon sludge sample from a waste stream at Secunda. The purpose of this sample was to test if surfactant can extract the analytes of interest from it.

2.2.1. Determination of Soil Moisture Content

Three samples (5 g) of standard soil (Cedara), Longlands soil and sludge were weighed accurately in porcelain crucibles. The crucibles were placed in a drying oven set at 105 °C for 3 days or until constant mass. The samples were then cooled in a dessiccator (using silica gel with blue moisture indicator as a drying agent) and reweighed. The moisture content was then determined by difference.

2.2.2. Determination of Soil Clay, Silt and Sand Content

Soil particle size analysis was conducted at the University of KwaZulu-Natal Soil Science Laboratories using the Pipette Method[1]. This method involves the direct sampling of a dispersed sub sample, at a given time and depth, based on Stokes Law at a specific temperature. Particle size fractions that were measured in this method were clay (particle size diameter less than 0.002 mm), silt (particle size diameter between 0.002 and 0.05 mm), fine sand (particle size diameter between 0.10 and 0.25 mm), medium sand (particle size diameter between 0.25 and 0.50 mm) and coarse sand (particle size diameter between 0.50 and 2.0 mm).

The soil sample was air-dried and ground to pass a 2 mm sieve. The air-dry moisture content of a sub-sample was determined by the procedure described in Section 2.2.1. 20.00 g of air-dried soil was accurately measured into a 100 mL beaker. Ten millilitres of Calgon solution (35.7 g of sodium hexametaphosphate and 7.9 g sodium carbonate was dissolved in deionised water and made up to 1 L) and 15 mL of deionised water was added to the beaker. The soil:solution ratio should be restricted to about 1:1.5 in order to minimise energy loss during ultrasound treatment. The samples were treated for 3 minutes with the ultrasonic probe (Labsonic 2000 with an output of 350 – 400 W, complete with soundproof box.) at maximum output. Considerable heat was generated in the suspension as well as in the instrument itself, and adequate time had to be allowed for cooling of the instrument during treatment of a series of samples. The dispersed sample was washed through the 0.053 mm sieve into a 1 L measuring cylinder with distilled water. A squirt bottle was used for this operation, and care was taken to ensure that all the fine material passed through the sieve. The liquid volume in the measuring cylinder was made up to the 1 L mark with distilled water. When the suspension had reached ambient temperature the soil was brought into suspension by at least 40 firm (up and down) strokes of the plunger using a metal rod 600 mm in length with a perforated disc 50 mm in diameter attached at one end during a 20 second period.

Determination of Silt Content

The plunger was removed and immediately a 20 mL sample suspension was pipetted (soil pipette of approximately 20 mL capacity designed according to Black, 1965). This represented the content of coarse silt plus fine silt plus clay according to Stokes' Law (See Table B1 in Appendix B). The sample was discharged from the pipette into a pre-weighed 50 mL beaker. The beaker was placed in an oven at 105 °C for drying overnight and then the mass of the sample was accurately determined.

Determination of Clay Content

After the required settling time for clay, a pipette sample was taken 75 mm below the surface to represent the clay content. In the same way as before, the sample was dried

at 105 °C. The following day, the beakers were removed from the oven, and allowed to cool in a desiccator and then re-weighed.

Determination of Sand Content

The soil fraction that did not pass through the 0.053 mm sieve was transferred from the top of the sieve into a 250 mL beaker using a water squirt bottle and was then dried in the oven. This dried sample was sieved through 0.500 mm, 0.250 mm and 0.106 mm sieves using a sieve shaker. The mass of each sieve and the empty pan was then recorded. The coarse sand fraction was represented by the material that did not pass through the 0.500 mm sieve, the material that did not pass between the 0.250 mm sieve represented the medium sand fraction and the fine sand fraction was represented by the material that passed through the 0.250 mm sieve. The percentage of each particle size fraction was determined by dividing the weight of each oven-dried fraction by the oven-dried weight of the total treated sample.

2.2.3. Quantification of Organic Matter

Organic carbon content was quantified using the Walkley-Black Procedure[2] at the University of KwaZulu-Natal Soil Science Department. This procedure involves the reduction of chromate ($Cr_2O_7^{2-}$) by soil organic carbon compounds and the subsequent determination of unreduced chromate by oxidation reduction titration with Fe^{2+}. Air-dried soil sample was ground using a porcelain mortar and pestle and sieved to pass a 0.5 mm sieve; a sample of 0.5 g (weighed accurately to 0.01 grams) was transferred into a 500 mL Erlenmeyer flask. Ten millilitres of the potassium dichromate solution (49.04 g L^{-1} made up with deionised water) was then added to the flask and mixed by swirling. Twenty millilitres of conc. H_2SO_4 (not less than 96 %) was then added in a fumehood and mixed gently for 1 minute by slowly rotating the flask. The mixture was allowed to stand for 30 minutes. A blank was also prepared, omitting the soil, and was treated in exactly the same way.

After 30 minutes, the flasks containing the sample mixture and the blank were each diluted by the addition of 170 mL of deionised water, 10 mL of 85 % H_3PO_4, 0.2 g NaF and 5

drops of ferroin indicator (14.85 g o-phenanthroline plus 6.95 g ferrous ammonium sulfate made up to I L) followed by a thorough mixing of the contents. Not all the soil organic matter is oxidised by the potassium dichromate. The residual amount in the sample flask was determined by titrating with iron (II) ammonium sulfate, (FAS, $(Fe(NH_4)_2(SO_4)_2.6H_2O$, approximately 0.5M (196.1g in 800 mL deionised water to which 20 mL conc. H_2SO_4 was added and made up to 1 L with H_2O). However, the blank titration was run first so as to standardise the FAS and to make the recognition of the sample solution endpoint easier.

The amount of FAS used was noted and used to determine the amount of Fe^{2+} used. 6 moles of Fe^{2+} reduces one mole of $Cr_2O_7^{2-}$ as shown in the redox equation below, thus the amount of unreduced chromate could be calculated. The amount of unreduced chromate was subtracted from the total initial amount of chromate to determine the amount of organic carbon oxidised as three moles of carbon will reduce two moles of chromate.

$$Cr_2O_7^{2-} + 14\,H^+ + 6\,e^- \rightarrow 2\,Cr^{3+} + 7\,H_2O$$
$$6\,Fe^{2-} \rightarrow 6\,Fe^{3+} + 6\,e^-$$
$$Cr_2O_7^{2-} + 14\,H^+ + 6\,Fe^{2+} \rightarrow 2\,Cr^{3+} + 6\,Fe^{3+} + 7\,H_2O$$

2.2.4. Identification of Organic Compounds in API Sludge

The API sludge sample was analysed for organic compounds to find out if phenanthrene and acenaphthene were in the sludge (Appendix D). USEPA Method 3540C[3] involving soxhlet extraction was used to extract the organic compounds. Sludge samples, both wet and dry, that had not been subjected to the washing process and sludge samples that were washed in 0.5 mM Safol 45E7 solution and 0.1 mM Safol 45E7 solution were soxhlet extracted. The dry mass equivalent of the samples (taking into account the moisture content (Section 2.2.1)) that was used was 5 g.

Each sludge sample was mixed with an equal mass of drying agent (anhydrous sodium sulphate which had previously been dried in an oven and stored in a dessicator). The

mixture was placed in an extraction thimble (Whatman single thickness cellulose extraction thimble, 28 mm internal diameter × 80 mm external length) that was later placed in an extraction chamber suspended above a round-bottomed flask containing the solvent. The refluxing process was achieved using a condenser connected to a water tap. The extraction solvent was a mixture of 150 mL each of dichloromethane and hexane. A few boiling chips were added to the flask which was heated using a heating mantle. The samples was extracted for 24 hours. The solvent in the flask was then evaporated to 2 mL using a Kuderna-Danish concentrator. The sample was then sent to Umgeni Water (PLC) Laboratory for GC-MS analysis. Figure 2.2.a shows the experimental setup used for soxhlet extraction.

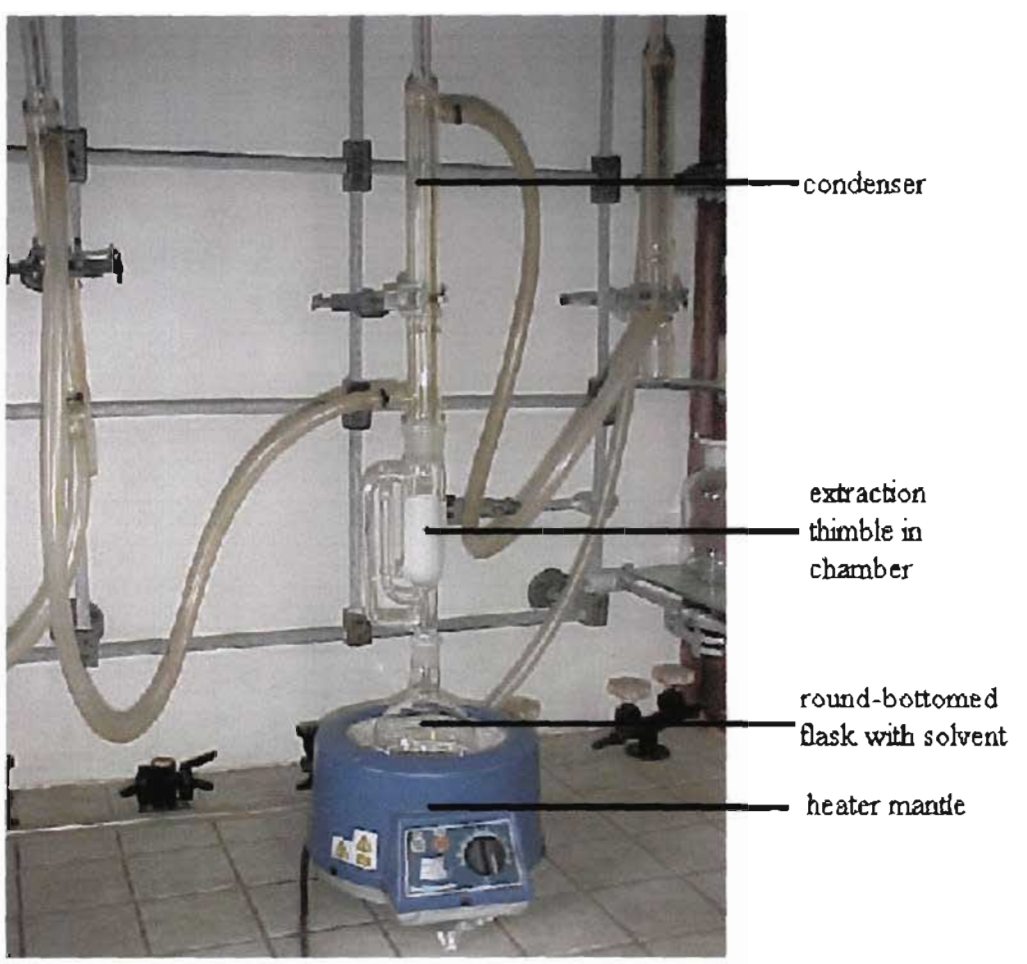

Figure 2.2.a: Experimental Setup used for Soxhlet Extraction

2.2.5. Soil Washing

The air-dried standard soil sample from Cedara was used as received since it was made up of uniform particle size. The supplied sample had already been crushed and sieved. The air-dried Longlands soil was divided into two batches. The first batch was ground coarsely with a porcelain pestle and mortar to break up agglomerates. The second batch was washed to remove all fine particles until the water ran clear and only the sand fraction remained. The Longlands sand fraction and the sea sand were oven-dried at 105°C for 24 hours before use.

The spiking solution was prepared by dissolving 1.5 g of phenanthrene and acenaphthene in 100 mL of acetone. The amount of soil sample used for each wash was 30 g. This was weighed into an amber screw top glass bottle having an aluminium foil lined lid. This was necessary to prevent possible degradation enhancement caused by light. To represent field moisture levels at 16.67 %, 5 mL of water was added to the soils and thoroughly mixed. To this, 1 mL of spiking solution was added dropwise using an automatic pipette, while mixing the soil. The bottles were left open with occasional turning for 3 hours to effect acetone evaporation. The samples were then sealed and refrigerated at 4 °C[4] for a minimum of 14 days to allow the aging process to take place. A total of 13 bottles were prepared for each soil sample since the washing process involved different concentrations of surfactant and the experiments had to be done in duplicate for reproducibility purposes.

The spiked and aged samples were then subjected to desorption studies. An optimum soil mass to water volume ratio of 1 g to 10 mL for soil washing was used.[5] Thirty grams of spiked sand (Longlands sand and seasand) and 300 mL of surfactant solution (0.5 mM, 1 mM and 2 mM) or water (as a reference solution) was placed in a flat-bottomed one-necked flask that was rotated through 360° as a way of stirring the mixture. The flask, having the neck sealed with a latex septum, rotated in a water bath set at 40°C. Samples were drawn through the septum via a 0.25 μm cellulose acetate syringe filter (Micro Filtration Systems) into a 5 mL plastic syringe (Set Inject) attached to a needle (Sigma Aldrich, 20 gauge, 100 mm). Drawing the first 2 mL of sample and pushing it back into the reaction vessel preconditioned the filter. To ensure reproducible sampling, an Eppendorf

pipette was used to sample 1 mL from the syringe into a 2 mL Whatman autosampler glass vial containing 1 mL of acetonitrile. The remaining sample in the syringe was pushed back into the flask.

The abovementioned procedure was modified for washing of the soils containing particle size fractions smaller than sand, i.e. the sludge, standard soil (Cedara) and unwashed Longlands soil. This is because the fine particles clogged the cellulose acetate filters and made the sampling process difficult. Therefore the experimental procedure was kept the same, except, that the amount of sludge (or spiked and aged soil) was reduced to 5 g and the surfactant solution to 50 mL. This was placed into a 50 mL centrifuge tube (Beckman) that replaced the flat-bottomed flask. At the specified time intervals, the tubes with their contents were removed and centrifuged using a Sorvall RC *26* PLUS (SA 600 rotor) apparatus set at 40°C and rotating at 15 000 rpm for 10 minutes. The temperature was maintained at 40°C so that the solubility of the analyte did not change. At the end of this process, the sampling procedure was repeated as explained above and the tubes were placed back into the water bath. All the samples were then analysed using HPLC.

2.3. Analysis of Polyaromatic Hydrocarbon Compounds from Solubility Studies

2.3.1. Introduction

UV-Visible spectrophotometry was considered a suitable technique for the solubility studies because of the sensitivity of the technique and the relative speed of sample analysis. It was logical to use UV-Visible spectrophotometry instead of HPLC or GC because only one analyte needed to be analyzed at a time therefore requiring no separation. The instrument used was a Varian Cary 100 Bio UV-Vis spectrophotometer.

2.3.2. The Principles of UV-Vis Spectophotometry

UV-Visible spectrophotometry is a sensitive technique that can detect samples of low concentrations in the range of 10^{-4} to 10^{-6} M. Figure 2.3.a shows a schematic diagram of a UV-Vis spectrophotometer.[6] The principle components of spectroscopic equipment include

the radiation source, the sample container, the monochromator, the detector and the detector output-measuring instrument. Monochromators are usually prisms or gratings. The region of the electronic spectrum determines the type of prism used. Glass prisms are generally used for the UV-Visible region. The eye, photographic plate and photoelectric cells are used as detectors for the visible region, while the latter are employed for the ultraviolet region.[7]

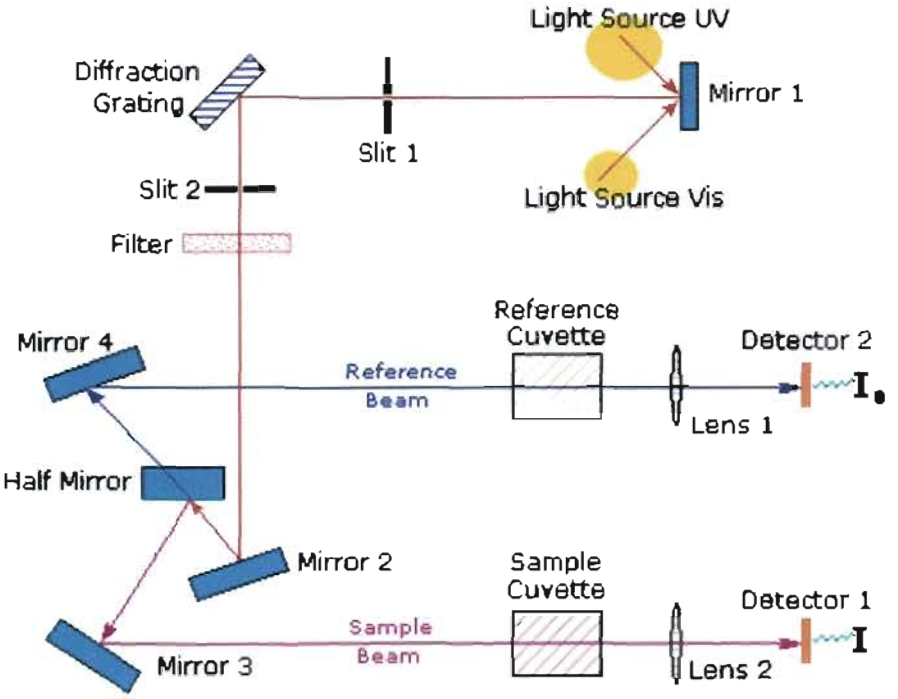

Figure 2.3.a : Schematic Diagram of a Double Beam UV-Vis Spectrophotometer

A spectrophotometer measures the transmittance of light by a sample. The transmittance, T, can be expressed as absorbance, A, by use of Equation 2.1. :

$$A = -\log_{10} T \tag{2.1}$$

but

$$T = \frac{I}{I_o} \tag{2.2}$$

where I_o is the intensity of the incident light

I is the intensity of the transmitted light

Therefore $$A = \log \frac{I_o}{I} \qquad (2.3)$$

The absorption spectrum is directly dependent on the Beer-Lambert-Bouguer expression shown as equation 2.4., commonly referred to as Beer's Law.

$$A = \varepsilon CL \qquad (2.4)$$

where A is the absorbance of the sample

ε is the molar absorptivity ($m^2 mol^{-1}$)

C is the concentration of the absorbing substance in a transparent solvent (M)

L is the path length through the absorbing sample in cm

Thus the concentration of a substance can be determined by using Equation 2.4.

2.3.3. Calibration Curves for Phenanthrene and Acenaphthene

In order to quantify the amount of phenanthrene and acenaphthene, it was important to scan the pure samples so that a representative spectrum could be obtained. As a result, UV-spectra were determined by scanning solutions of phenanthrene and acenaphthene dissolved in a 50:50 v/v water-ethanol mixture from 190 nm to 800 nm. The peak having the maximum absorbance was selected for quantification purposes. The UV-spectra for phenanthrene and acenaphthene are shown in Figures 2.3.b and 2.3.c respectively. From UV-spectra, maximum absorbance occurred at 251 nm and 227 nm for phenanthrene and acenaphthene respectively. These values compare well with literature values of 251 nm and 230 nm for the two PAHs. [8,9] The absorbances at these two wavelengths were therefore used to prepare the calibration graphs and quantification of the PAHs in the solubility studies.

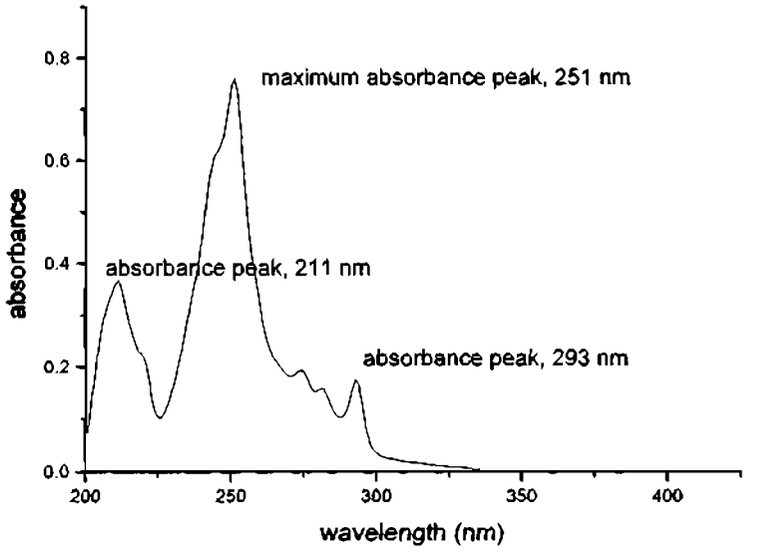

Figure 2.3.b: The UV-Spectrum for Phenanthrene

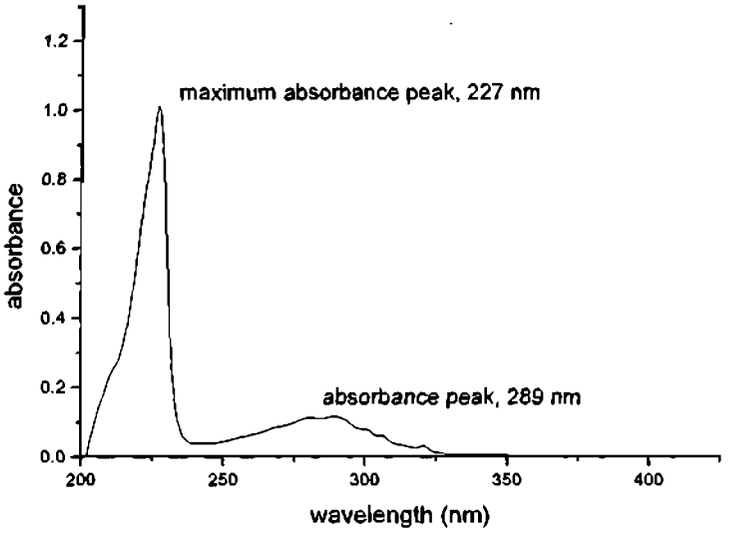

Figure 2.3.c: The UV-Spectrum for Acenaphthene

Calibration graphs were constructed for each of the analytes dissolved in a 50:50 v/v solution of ethanol-water. A 10 mg L^{-1} analyte stock solution was prepared by dissolving 0.0010 g of each analyte in 50 mL of HPLC grade ethanol from Merck Laboratory Supplies, to which 50 mL of Ultrapure Water (Modulab Water Purification Systems) was

added. This was made up to the mark in a 100 mL volumetric flask using a 50:50 v/v solution of ethanol-water. Six standard solutions were prepared by diluting the stock solutions with 50:50 v/v solution of ethanol-water. Table 2.2 shows the concentrations of the standards prepared and their corresponding absorbances. Figures 2.3.d and 2.3.e show the corresponding graphs of concentration versus absorbance plotted using Origin 5.0 data analysis and technical graphics software. A linear response was obtained over the chosen concentration range. The straight-line equations and correlation coefficients for each PAH are displayed in Table 2.3.a.

Table 2.3.a: Calibration Concentrations used to Generate Calibration Graph and the Corresponding Absorbance Values

Standard Conc ($mg\ L^{-1}$)	Phenanthrene Absorbance	Acenaphthene Absorbance
0.5	0.202	0.276
1	0.385	0.516
2	0.761	1.011
3	1.067	1.473
4	1.516	1.879
5	1.837	-
Equation	$y = 0.37x$	$y = 0.47x$
R^2	0.9993	0.9991

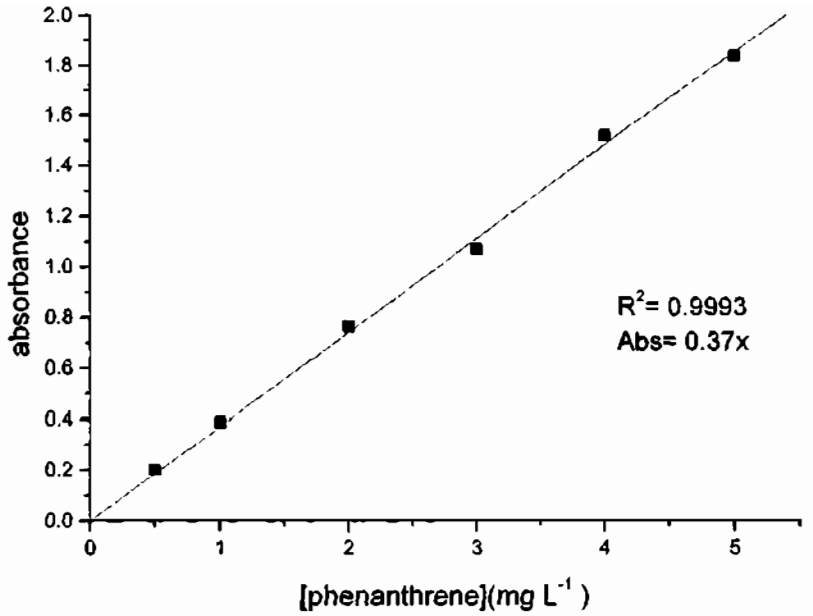

Figure 2.3.d: Calibration Graph of Phenanthrene in a Water-Ethanol Solvent System

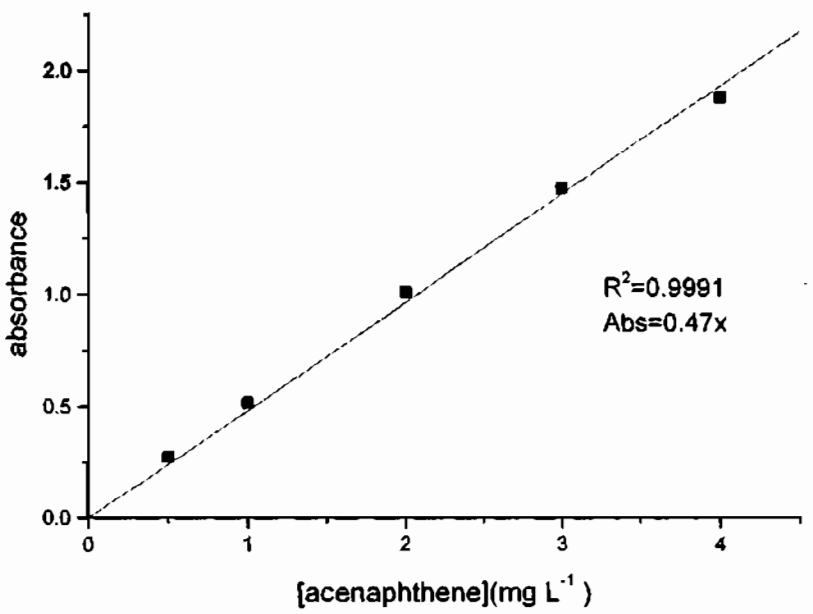

Figure 2.3.e: Calibration Graph of Acenaphthene in a Water-Ethanol Solvent System

2.4. Analysis of PAH Compounds from Soil Washing

2.4.1. Introduction

A High Pressure Liquid Chromatography (HPLC) instrument is generally used to monitor non-volatile organic compounds. HPLC is advantageous over many other techniques because it can effect the separation, identification (through comparison with known standards) and quantification of components and thus was chosen as the method of analysis for soil washing studies. Gas Chromatography (GC) can also effect component identification and separation but is not applicable to non-volatile compounds. Techniques such as nuclear magnetic resonance spectroscopy (NMR) and UV-Vis require the samples to be pure, but HPLC can separate and identify impure samples. The main components of an HPLC system are the solvent reservoirs, solvent degasser, vacuum pump, high-pressure pump, injection port, analytical column and detector. Figure 2.4.a shows a schematic diagram of an HPLC unit.

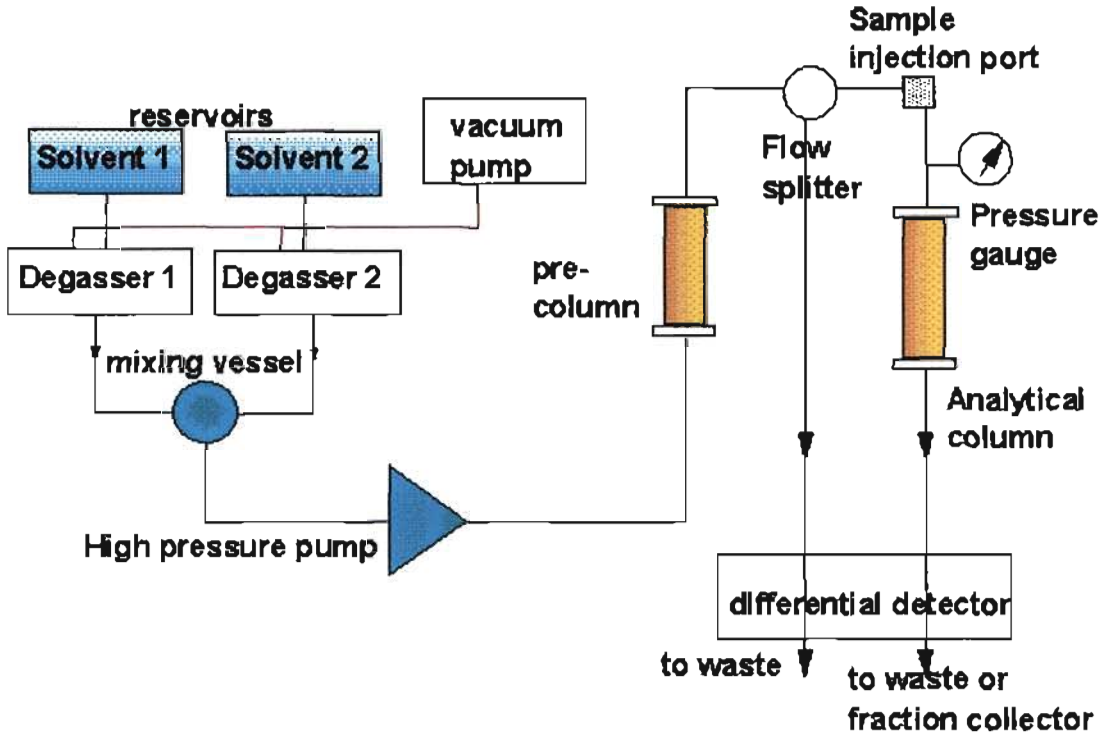

Figure 2.4.a : Schematic Diagram of an HPLC Unit

2.4.2. The Principles of the HPLC

Chromatographic separation involves the distribution of a sample mixture between two phases. One phase is stationary (analytical column) while the other is mobile (solvents). The *stationary phase* (or adsorbent) is either a solid, porous or a surface-active material in small particle form (typically the μm range). In HPLC, stationary phases are typically bonded covalently onto micro porous silica (SiO_2) particles. The *mobile phase* (or eluent) is a solvent or a mixture of solvents able to carry the sample through the system. In *normal-phase* liquid chromatography, the stationary phase is polar and the mobile phase is non-polar, e.g. hexane or isopropyl ether. In *reverse-phase* liquid chromatography, the stationary phase is non-polar and the mobile phase is moderately polar, e.g. acetonitrile:water or methanol. The choice of phase depends on the polarity of the analyte. In normal phase, the least polar analyte is eluted first and increasing the solvent polarity decreases elution time. Conversely, the most polar analyte is eluted first in reverse phase decreasing the solvent polarity decreases elution time of the analyte. For applications of isocratic elution, only one solvent system is required, but HPLC systems can handle up to four solvents for gradient elutions. The solvents are sparged with an inert gas, generally helium, to remove any dissolved gases before use. The mobile phase enables analytes to move through the column. After the column has been equilibrated with mobile phase, the sample is injected manually or via autosamplers. Most injection ports work with a fixed loop injection system to reduce error caused by variable injection volumes.

To understand the separation of species injected into the column, the equilibrium distribution ratio, K, of the components between the mobile phase, C_m, and stationary phase, C_s, must be considered. K is defined in Equation 2.5.[10]

$$K = \frac{C_s}{C_m} \tag{2.5}$$

Molecules that are more strongly attracted by the stationary column and are less soluble in the mobile phase will move through the column at a slower rate than those that behave otherwise, hence the separation. The detector shows separated components as peaks on a chromatogram. A common problem in chromatography is the broadening of the peaks. Broadening is a function of thermodynamic and kinetic processes in the column with the

bandwidth being affected by three factors namely: eddy diffusion, longitudinal diffusion and resistance to mass transfer.[11] Eddy diffusion involves the movement of solute particles at different velocities and along different path lengths around the stationary phase particles within the column. Using packing of the smallest possible diameter and ensuring uniform column packing can minimize eddy diffusion. Longitudinal diffusion describes the axial random molecular motion of solute particles within the mobile phase and becomes significant at low mobile phase velocities. At low velocities high diffusion rates of a solute in the mobile phase causes the solute molecules to disperse axially while moving through the column. This results in the broadening of the solute band. Resistance to mass transfer is the uneven rate at which solute is adsorbed and desorbed by the stationary phase. This results in some of the molecules at the front of the band being swept ahead before equilibration occurs as with the bulk of the molecules, while the molecules at the back of the band being left behind by the moving mobile phase. This also results in band broadening. The faster the mobile phase moves, the less time there is for equilibrium to be approached and the greater the resistance to mass transfer contribution to band broadening.[12] Narrow-shaped peaks are achieved when the three above-mentioned processes are minimized.

Separated sample components enter the detector after passing through the column. Most HPLC detectors are based on the absorption of ultra-violet and visible radiation. Deuterium and tungsten lamps are used to cover this wavelength range in some instruments, while others may use photodiodarray detectors. The samples are identified from their retention times at a specific wavelength for a fixed set of variables, i.e. mobile phase, stationary phase and sample matrix.

2.4.3. Experimental Conditions for HPLC in the Current Work

Varian CP Scanview Software (Version 5.0, 1999, Application 335 - HPLC)[13] was used to determine a suitable mobile phase for the HPLC analysis. Keywords used in the program search were separation of PAHs using a reverse phase column. The software database search presented a mobile phase of 70:30 v/v acetonitrile-water as the best mobile phase for the separation of 16 PAHs using a reverse phase column. Park *et al*[14] also used a 70:30 v/v acetonitrile-water mobile phase for the identification of surfactant extracted pentachlorophenol using HPLC.

HPLC grade acetonitrile (Riedel de Haen) and Ultrapure water (Modulab Water Purification Systems) were used to prepare the mobile phase. The Ultrapure water and acetonitrile were individually filtered through a 0.45 µm pore diameter nylon membrane filter (Whatman) to remove any possible impurities before mixing.

Pure samples of phenanthrene and acenaphthene were dissolved in a 50:50 acetonitrile-water mixture. In addition, a composite sample containing the two PAHs was also prepared. These samples were then subjected to HPLC analysis using the chosen mobile phase. The detector was set at 227 nm and 251 nm. These wavelengths are where the maximum absorbance was observed in UV-Vis spectrophotometry for acenapththene and phenanthrene respectively. Although 227 nm was the wavelength at which acenaphthene absorbed at a maximum, phenanthrene absorption intensity was strong enough to be monitored at this wavelength as well. Thus all sample analysis and calibration was conducted at 227 nm. The single solution samples were therefore injected into the HPLC to determine the individual retention times of each PAH as shown in Figures 2.4.b and 2.4.c. The composite sample having both analytes in solution, was analysed so as to see how good the separation of the peaks were as shown in Figure 2.4.d. The individual retention times were found to be 10.67 minutes for acenaphthene and 11.95 minutes for phenanthrene. The instrument specification and conditions that were used in the current study are summarised in Table 2.4.a. All injections were passed through a guard column as a prevention measure.

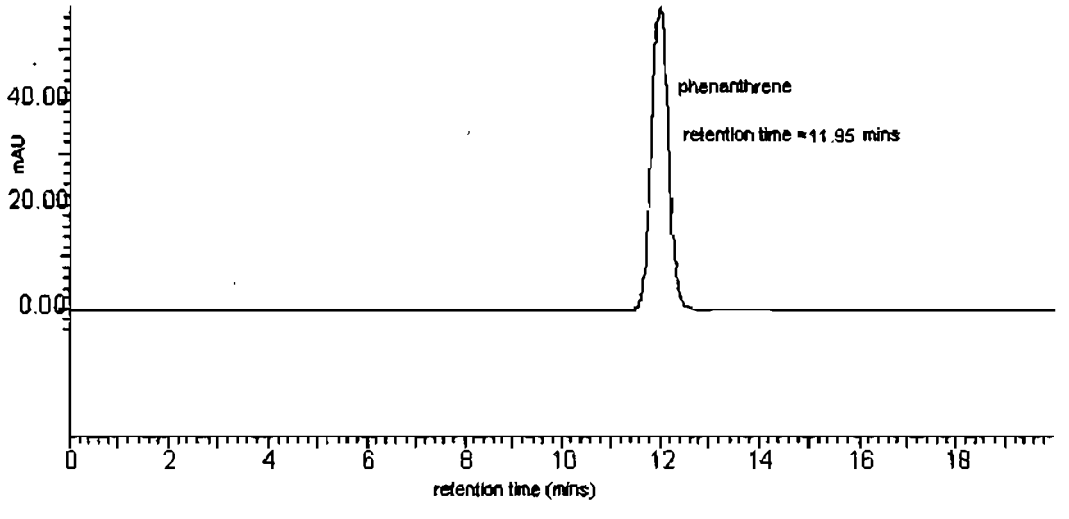

Figure 2.4.b: HPLC Chromatogram of Phenanthrene

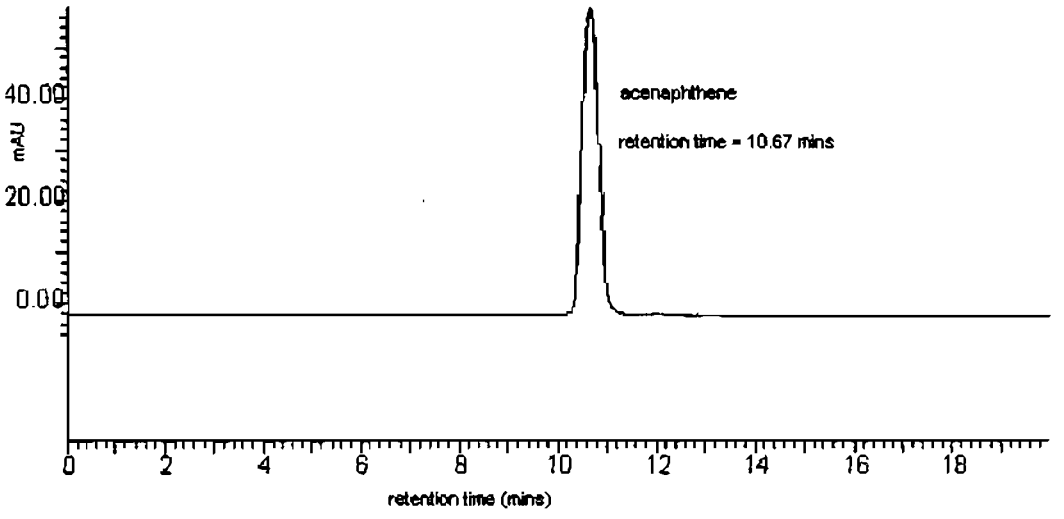

Figure 2.4.c: HPLC Chromatogram of Acenaphthene

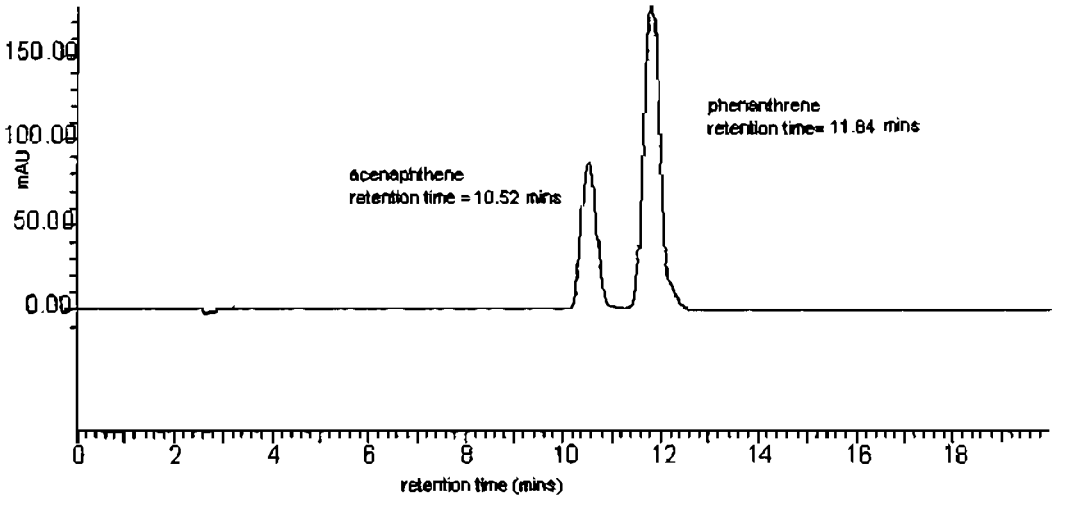

Figure 2.4.d: HPLC Chromatogram of Acenaphthene and Phenanthrene

At the end of analysis, a standard shutdown procedure was performed. The mobile phase was changed from 70:30 v/v acetonitrile-water (100 %) to methanol (100 %) over a period of 20 minutes. The latter was then run for 30 minutes. The column was always stored in methanol (100 %) to prevent corrosion in the column and instrument. The column was also routinely regenerated with a standard cleanup procedure. This was done by first running methanol-water (10 %) through the column for 1 hour and was followed by switching to methanol (100 %) over a 10 minute period. Methanol was then pumped through the column for 30 minutes and then switched to the 70:30 v/v acetonitrile-water mobile phase over a period of 10 minutes. The mobile phase was then run for a further 20 minutes. This was done to remove any possible PAH and surfactant build-up in the column.

Table 2.4.a: The HPLC component specifications and conditions used during routine analysis

Instrument	Perkin Elmer 200 Series
Detector	Perkin Elmer Series 200 Diode Array
Lamp (190 nm to 400 nm)	Deuterium
Pump	Perkin Elmer Series 200
Injection valve	Perkin Elmer Series 200 Autosampler
Fixed loop volume	20 µL
Software	Turbochrom Workstation
Guard column	Supelco Nucleosil C18 (10 mm)
Column	Waters Spherisorb C18
Column dimensions	4.6 x 250 mm
Stationary phase	Silica bonded C_{18} alkyl chains
Particle Size	5µm
Mobile Phase flow rate	1 mL/min

2.4.4. Calibration Curves for the PAH Compounds

A stock solution containing 45 mg L^{-1} acenaphthene and 58 mg L^{-1} phenanthrene was prepared by dissolving 0.0045 g of acenapthene and 0.0058 g of in 50 mL of acetonitrile, followed by the addition of 50 mL of Ultrapure Water and then making the solution up to the mark in a 100 mL volumetric flask with 50:50 v/v acetonitrile-water. A total of five

standard solutions were prepared by dilution from the stock solution. These were injected into the HPLC and their corresponding peak heights determined. These results including the concentrations are tabulated in Table 2.4.b. The calibration plots of analyte concentration versus peak height for phenanthrene and acenaphthene were contructed and are shown in Figures 2.4.e and 2.4.f respectively. These graphs were plotted using Origin 5.0 data analysis and technical graphics software. The data points fitted a linear curve. The straight line equations and correlation coefficients are shown as inserts in the respective figures.

Table 2.4.b: Results of Analyte Calibration

Standard	Phenanthrene Conc. (mg L^{-1})	Peak Height (μV)	Acenaphthene Conc. (mg L^{-1})	Peak Height (μV)
1	1.16	6987	0.9	5944
2	2.9	15698	2.25	12957
3	5.8	35222	4.5	28219
4	11.6	66417	9.0	49867
5	14.5	82630	11.25	59855

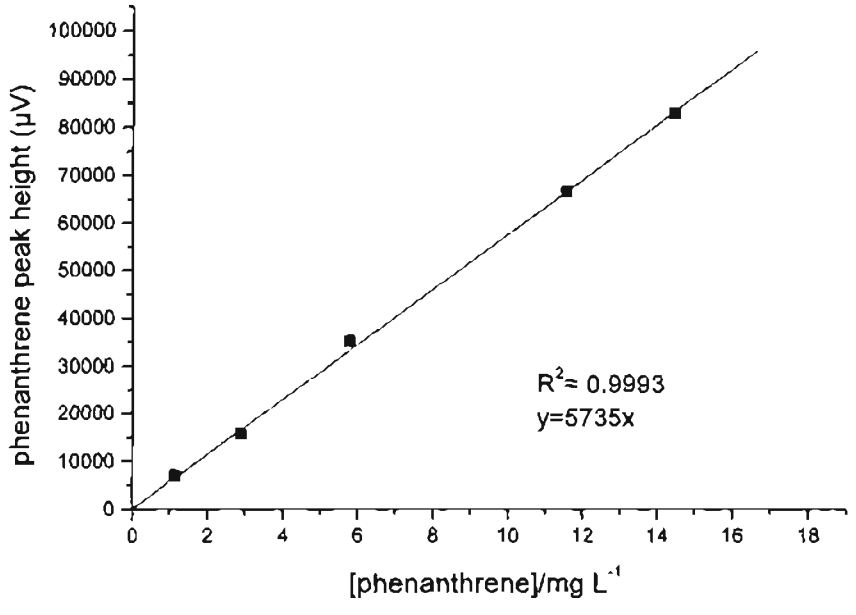

Figure 2.4.e: Calibration Graph for Phenanthrene

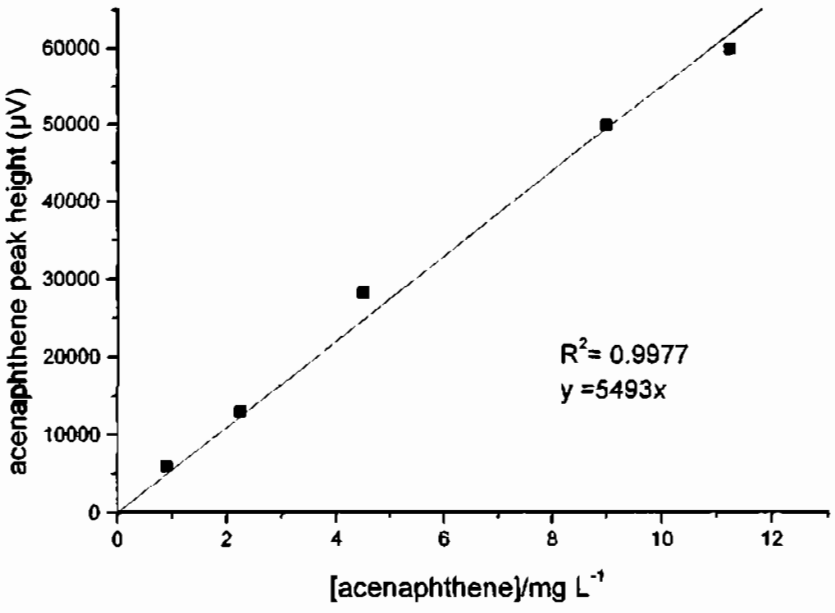

Figure 2.4.f: Calibration Graph for Acenaphthene

Figure 2.4.g shows an HPLC trace of a sample obtained from washing a spiked standard soil with surfactant solution.

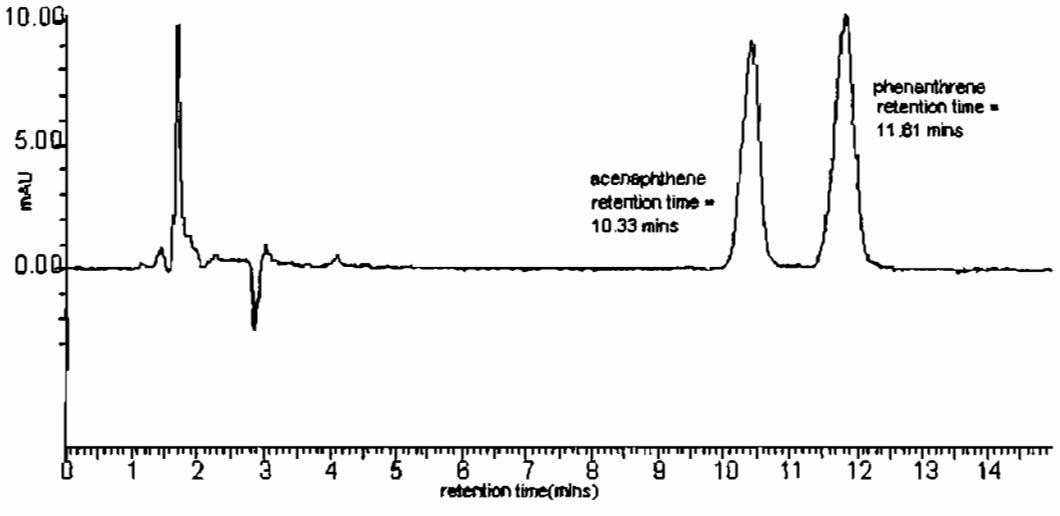

Figure 2.4.g: HPLC Trace of Spiked Standard Soil Extracted with 1 mM Surfactant Solution

2.5. Analysis of PAH Compounds from Sludge Washing

2.5.1. Introduction

The GC-MS system comprises a gas chromatograph, a mass spectrometer and a computer system. The main components of the gas chromatograph (GC) are the carrier gas supply, injector, column, column oven, detector and recorder as displayed in Figure 2.5.a.

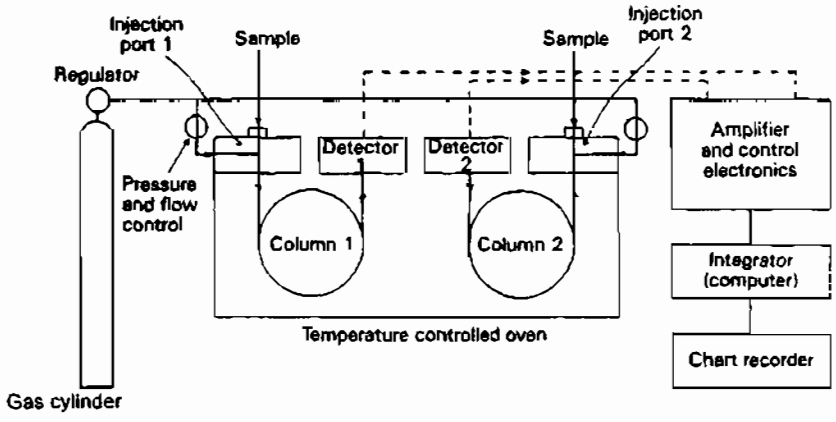

Figure 2.5.a: Schematic Diagram of a GC System[15]

The components of a mass spectrometer (MS) are the sample inlet system and ion source, mass analyser, detector, and control and signal processing electronics as shown in Figure 2.5.b.[15]

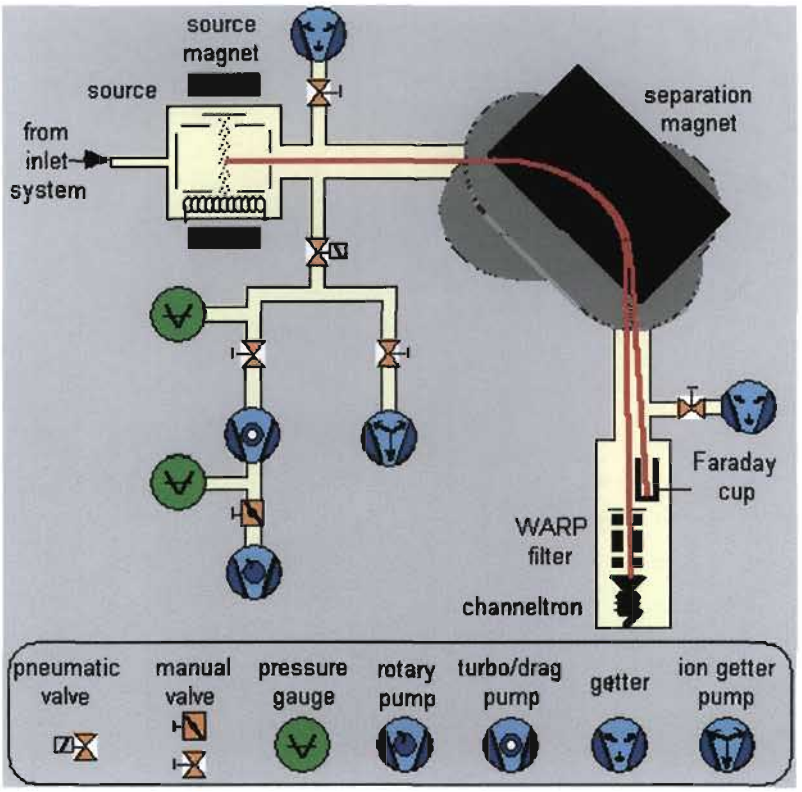

Figure 2.5.b: Schematic Diagram of an MS System

The extract from washing the API sludge with surfactant was initially analysed using HPLC. However, no peaks were detected. This was attributed to the possibility of the concentration of the analytes being too low to be detected by HPLC or the anlaytes being absent. The washed and unwashed API samples were then Soxhlet-extracted as described in section 2.2.4. Since the Soxhlet-extracted samples were dissolved in dichloromethane/hexane mixture, reverse phase HPLC could not be used for analysis. Therefore GC-MS was chosen as the method of analysis for the soxhlet-extracted samples because it is more sensitive and useful in separating and identifying compounds in a complex mixture. GC is able to separate, quantify and identify a wide range of substances, ranging from permanent gases and hydrogen isotopes to fatty acids and waxes. In mass spectrometry, sample quantities as low as 1 μg are required to obtain a spectrum, thus this technique can provide information about samples too small to be identified by other techniques.[16]

2.5.2. The Principle of the GC-MS

In GC-MS, the sample is separated using the gas chromatograph, and then the column effluent is directly passed to the mass spectrometer for identification and quantification.[17]

In gas chromatography, the stationary phase is a solid packed into a column, or lining a capillary column. An inert, non-flammable gas like helium is used as the mobile phase. The sample components are distributed between the two phases to effect separation. The principles of GC are similar to HPLC, except the mobile phase in GC is a gas. The distribution coefficient between the two phases has been described by Equation 2.5.

Liquid samples are introduced into the column by injection via a syringe through a self-sealing rubber septum. The injection port is generally a flash-vaporisation injector device, set on a heated block maintained at 10 to 50 °C above the column temperature. The injector ports may be used split or splitless modes. Part of the sample entering the column is flushed away when the carrier gas is purged through the injector port in the split mode. This is to prevent column overload and to reduce the amount of solvent loaded onto the column. In splitless mode, the entire injected sample enters the column, creating a better-defined separation. Splitless injector ports are designed to handle samples with low analyte concentrations.

For good GC-MS results, the GC procedure must be optimised. A uniform and reproducible chromatogram with good resolution is required. This can be achieved by the optimisation of several parameters including the choice of carrier gas, column type, and stationary phase. The carrier gas must be inert so as not to interfere with the separation process. Two types of open tubular columns exist; narrow-bore or capillary columns and wide-bore columns. Capillary columns have a greater resolution than do wide-bore columns. The selected stationary phase should have minimal volatility to prevent column bleeding. After injection into the GC, the sample components are swept down the column by the carrier gas which is normally helium.

Mass spectrometry involves the bombarding of molecules with electrons. This results in the fragmentation of the molecules into their respective ions. The parent molecular ion

undergoes further fragmentation, giving rise to new ions, which in turn can fragment further as shown in Equation 2.6.[17]

$$M + e^- \rightarrow M^{+\cdot} + 2e^- \rightarrow A^+ + B^+ + \ldots\ldots\ldots \quad (2.6)$$

where M is the molecule bombarded, $M^{+\cdot}$ is the molecular ion and A^+, B^+ are the primary fragmentation products.

These ions are then accelerated and focused by using electrical and magnetic fields. The ions are then electrically or electromagnetically separated by mass. The mass to charge ratio, *m/z* is observed. The parent molecular ion appears as the highest *m/z* value and gives the molecular weight of the component analysed. The structure of the compound is confirmed by the fragmentation pattern.

Methods used to produce ions in a mass spectrometer include electron impact ionisation (EI), chemical ionisation (CI) and field ionisation (FI). However, field ionisation techniques are generally used in GC-MS. Ions in the FI source are produced by a high positive electric field around 10^8 V cm^{-1}. The 12 to 13 eV of energy usually available from this electric field is sufficient to ionise most organic molecules which have an ionisation potential between 7 and 13 eV. Less excess energy is available in FI techniques compared to CI and EI, this results in less fragmentation and the parent ion can usually be observed.

2.5.3. Summary of Experimental Conditions for GC-MS in the Current Work

One microlitre of the sample was injected into the HP 6890 series Gas Chromatograph interfaced to an HP 5973 Mass Selective Detector (MSD) and controlled by HP Chemstation software (version b.02.05, 1989-1997). The chromatographic separation was achieved using a DB-5 capillary column (30.0 m x 250 μm x 0.25 μm). The column stationary phase comprised of 5%-diphenyl-95% dimethylpolysiloxane. The specific instrument conditions are listed in Table 2.5.a.

Table 2.5.a: Instrument Operating Conditions

Parameter	Value	Parameter	Value
Oven Temperature Programme		Front Inlet	
Initial Temp	50°C	Initial Temp	250 °C
Final Temp	300°C	Pressure	48.6 kPa
Initial Time	2 minutes	Purge Flow	50.0 ml/min
		Purge time	1.0 min
Equilibration Time	0.50 min	Total Flow	53.8ml/min
Ramp		Gas Saver	On
Rate	10°C/min	Saver Flow	20.0 ml/min
Initial Temp	270°C	Saver Time	2.0 min
Final Time	3 minutes	Gas type	Helium
MS Detector		Column 1	
Solvent Delay	5.0 min	Maximum Temp	325°C
EM absolute	false	Initial Flow	1.0 ml/min
EM offset	494	Nominal Initial Pressure	48.7 kPa
Resulting EM voltage	2658.8	Average Velocity	36 cm/sec
Low mass scan parameters	35m/z	Post Run	
High mass scan parameters	500 m/z	Post Time	0.0 min
Injector Conditions		Front injector	7673 Injector
Injection mode	Splitless	Sample washes	**6**
Injector Temp	250°C	Sample pump	6
Injector volume	1 µL	Post Injection	
Thermal Aux		Solvent A washes	6
Initial time	0.0 min	Solvent B washes	6
Initial Temp	280°C	Viscosity Delay	0 secs
		Plunger Speed	fast

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CHAPTER THREE

THEORY

3.1. Partitioning of PAHs Between Micellar and Aqueous Pseudophase

The solubility of slightly water soluble hydrophobic organic compounds can be enhanced dramatically in solutions of surfactants at concentrations greater than the CMC.[1] Surfactant solubilisation results in an isotropic colloidal solution, which is stable in the sense that it has the lowest possible sum of free energies of its components.[2] Polycyclic aromatic hydrocarbons are examples of compounds that may be solubilised by surfactants.[3,4]

The hydrocarbon solubilisation capacity of a surfactant can be measured by the molar solubilisation ratio (*MSR*) or the micelle-water partition coefficient, K_m. The *MSR* is defined as the number of moles of organic compound solubilised per mole of surfactant micellised in solution (Equation 3.1).[5]

$$MSR = \frac{S_{PAH,mic} - S_{PAH,cmc}}{C_{surf} - CMC} \qquad (3.1)$$

where $S_{PAH,mic}$ is the apparent solubility of the polyaromatic hydrocarbon (PAH) at a surfactant concentration C_{surf};
$S_{PAH,cmc}$ is the apparent solubility of the PAH at the critical micelle concentration (CMC).
All units in Equation 3.1 are quoted in moles per litre.

The increase in solubilisate concentration per unit increase in micellar surfactant concentration is equivalent to the *MSR*. In the presence of excess hydrophobic compound, the *MSR* may be obtained from the slope of the curve that results when the solubilisate concentration is plotted against surfactant concentration.

An alternative approach in quantifying surfactant solubilisation is to determine the partitioning of the organic compound between the micelles and the monomeric surfactant solution with a mole fraction micelle-phase/aqueous-phase partition

coefficient. The micelle-water partition coefficient, K_m, is an indication of the distribution of the PAH between the micellar and aqueous phases and is defined in Equation 3.2 as:

$$K_m = \frac{X_m}{X_a} \tag{3.2}$$

where X_m is the mole fraction of the hydrocarbon in the micellar phase and
X_a is the mole fraction of the hydrocarbon in the aqueous phase.[6]

The value of X_m may be calculated as given in Equation 3.3:

$$X_m = \frac{S_{PAH,mic} - S_{PAH,cmc}}{C_{surf} - CMC + S_{PAH,mic} - S_{PAH,cmc}} \tag{3.3}$$

For saturated systems (those containing excess organic phase in equilibrium with a micellar solution), the relationship between X_m and MSR is defined in Equation 3.4:

$$X_m = \frac{MSR}{1 + MSR} \tag{3.4}$$

For dilute solution solutions, X_a is defined by Equation 3.5 as:

$$X_a = S_{PAH,cmc} V_m \tag{3.5}$$

where C_{sat} is the hydrocarbon aqueous solubility;
V_m is the molar volume of water.[7]

Therefore, K_m can be expressed as:

$$K_m = \frac{S_{PAH,mic} - S_{PAH,cmc}}{[(C_{surf} - CMC + S_{PAH,mic} - S_{PAH,cmc})(S_{PAH,cmc}V_m)]}$$

(3.6)

In terms of the *MSR*, K_m can be expressed as:

$$K_m = \frac{MSR}{(1 + MSR)(S_{PAH,cmc}V_m)} \tag{3.7}$$

3.2. Theory of Soil Washing

The distribution coefficient, K_d, of a hydrophobic organic carbon (HOC) between the soil and surfactant solution phase has been described by Chu *et al*[8] as shown in Equation 3.8:

$$K_d = \frac{[P]_{soil}}{[P]_w + [P]_{mic}} \tag{3.8}$$

Where $[P]_{soil}$ is the pollutant concentration (mol L^{-1}) in the soil phase, $[P]_w$ is the pollutant concentration (mol L^{-1}) in water and $[P]_{mic}$ is the pollutant concentration (mol L^{-1}) in the surfactant micelles.

Equation 3.8 can be re-arranged by taking the reciprocal, resulting in Equation 3.9:

$$\frac{1}{K_d} = \frac{[P]_w}{[P]_{soil}} + \frac{[P]_{mic}}{[P]_{soil}} \tag{3.9}$$

$1/K_d$ is the performance indicator of soil washing. $[P]_w$ and $[P]_{mic}$ is the pollutant concentration in the liquid phase. For HOCs, $[P]_w/[P]_{soil}$ can usually be neglected, since $[P]_w$ represents the HOC's aqueous solubility, and is usually very small compared to $[P]_{mic}$. Equation 3.10 can be rewritten:

$$\frac{1}{K_d} = \frac{[P]_{liq}}{[P]_{soil}} \tag{3.10}$$

Where $[P]_{liq}$ is the total aqueous phase concentration of the pollutant.

Chu *et al*[9] identified two distinct stages in which soil washing could occur. In the first stage HOCs can be extracted from the soil by surfactant monomers. It was noted that the washing performance was 1:1 proportional to the available monomer content. In the second stage, the soil is saturated with surfactant so micellisation can occur. The HOCs can then be solubilised by the micelles. These two stages are analogous to the soil rollup and solubilisation mechanisms discussed in Section 1.3.

3.3. References for Chapter Three

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CHAPTER FOUR

RESULTS, DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1. Solubility Studies of Phenanthrene and Acenaphtene in Aqueous Media

The aim of the solubility studies was to investigate the influence of Safol surfactants on the aqueous solubility of phenanthrene and acenaphthene. The solubility of phenanthrene was tested in five different Safol surfactants. The experiments were carried out in an insulated water bath with the temperature fixed at 40°C. The surfactant concentration was varied to test solubilisation trends below and above the critical micelle concentration (CMC) as described in Section 2.1. Initially, the solution pH was unaltered and this was at pH 8. After testing the solubility of phenanthrene in the five surfactants, Safol 45E7 and Safol 45E12 were chosen for pH variation studies. Buffered surfactant solutions with a fixed ionic strength of 0.1 M were prepared as described in Section 2.1, the pH studies were conducted at pH 5, 6 and 7 (Refer to Chapter two, pg 50, par. 3). In addition to phenanthrene, the solubility of acenaphthene was also tested in Safol 45E7 and Safol 45E12. Analysis for the solubility studies was by UV-Vis spectrophotometry.

The solubility curves of phenanthrene concentration versus time for all five surfactants are shown in Figures 4.1.a to 4.5.a; Figures 4.1.b to 4.5.b show the correlation between the phenanthrene concentration and the apparent solubility of the surfactant. The graphs were plotted using Origin 5.0 software and the curves were fitted using the first order exponential decay function and linear regression.

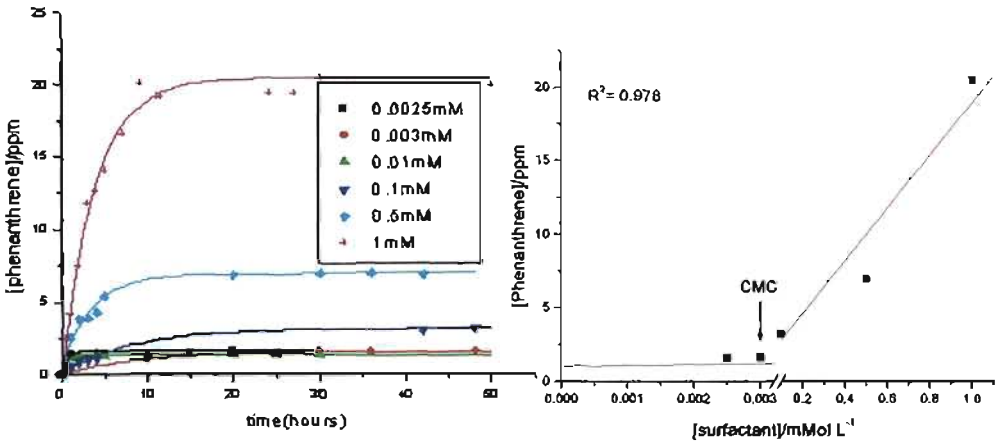


Figure 4.1.a **Figure 4.1.b**

Figure 4.1.a: Phenanthrene Solubilisation Curve For Safol 45E3

Figure 4.1.b: Graph of Phenanthrene Concentration vs Surfactant Concentration for Safol 45 E3

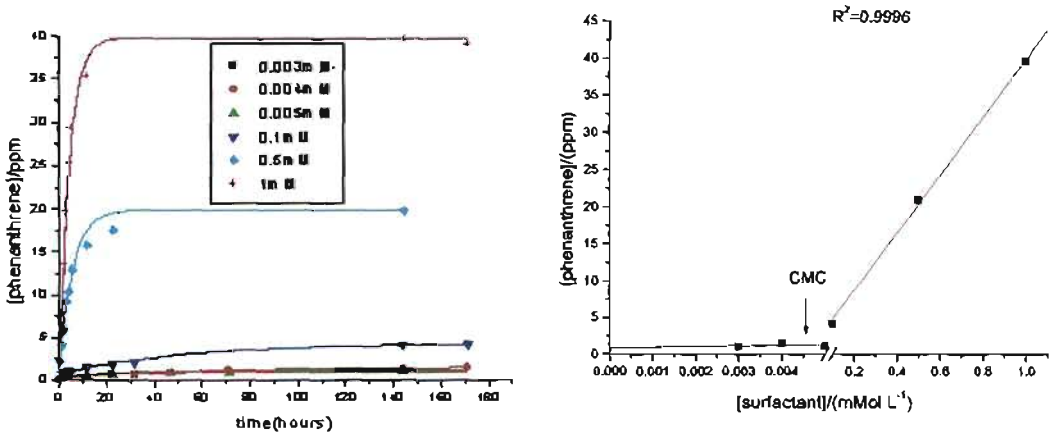
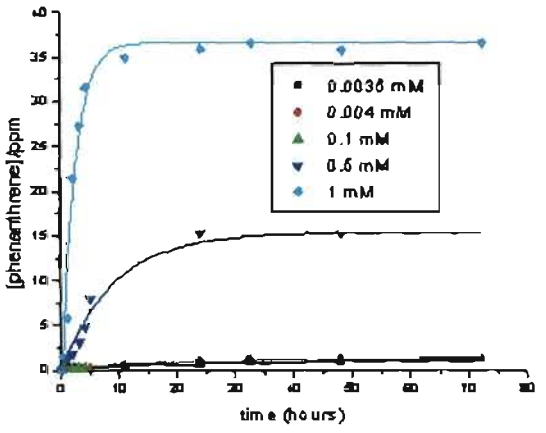


Figure 4.2.a **Figure 4.2.b**

Figure 4.2.a: Phenanthrene Solubilisation Curve For Safol 45E5

Figure 4.2.b: Graph of Phenanthrene Concentration vs Surfactant Concentration for Safol 45E5



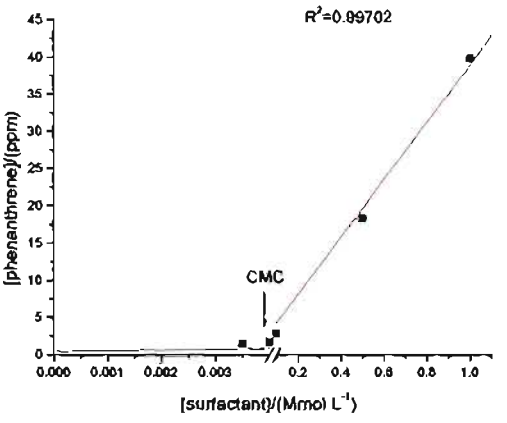
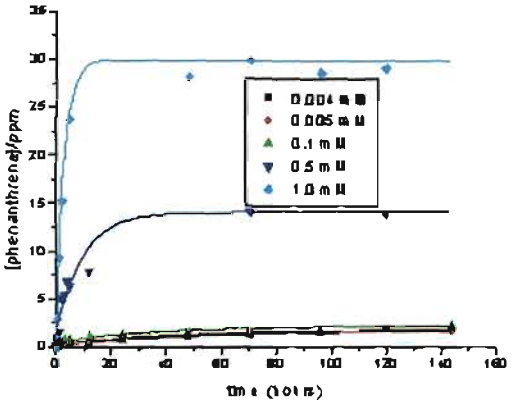


Figure 4.3.a **Figure 4.3.b**

Figure 4.3.a: Phenanthrene Solubilisation Curve For Safol 45E7

Figure 4.3.b: Graph of Phenanthrene Concentration vs Surfactant Concentration for Safol 45E7



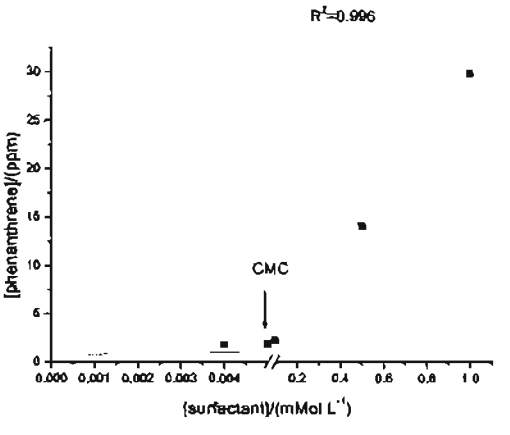
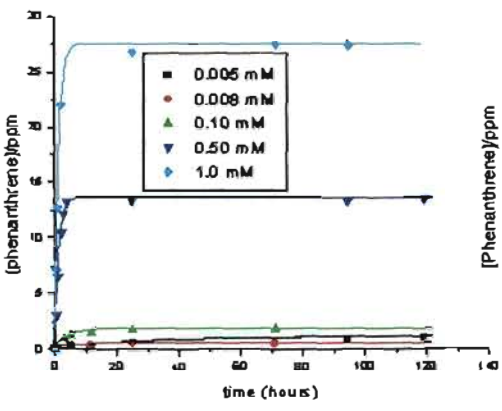


Figure 4.4.a **Figure 4.4.b**

Figure 4.4.a: Phenanthrene Solubilisation Curve For Safol 45E9

Figure 4.4.b: Graph of Phenanthrene Concentration vs Surfactant Concentration for Safol 45E9



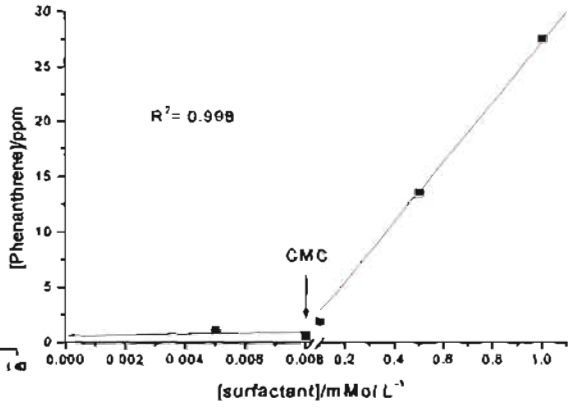


Figure 4.5.a **Figure 4.5.b**

Figure 4.5.a: Phenanthrene Solubilisation Curve For Safol 45E12

Figure 4.5.b: Graph of Phenanthrene Concentration vs Surfactant Concentration for Safol 45E12

The linear responses in Figures 4.1.b to 4.5.b indicate that the amount of phenanthrene solubilised was directly proportional to the surfactant concentration, for the surfactant concentration ranges studied. This trend is consistent with literature reports.[1,2] Different trends are noted for surfactant concentrations below and above the CMC. Upon closer inspection of these figures, it can be seen that below the CMC, the graph is relatively flat, while the linear response can only be seen at and above the CMC. This trend is consistent with theory[3], as micellisation only starts at the CMC. Below the CMC, the surfactant exists as monomers, and the hydrocarbon solubility should be similar to that in pure water.

4.1.1. Calculation of Molar Solubilisation Ratios and Partition Coefficients

A measure of the effectiveness of a particular surfactant in solubilising a given solubilisate is known as the molar solubilisation ratio (MSR). Surfactant solubilisation can also be quantified by the micelle-phase/aqueous-phase partition coefficient, K_m, which is the ratio of the mole fraction of the compound in the micellar pseudophase, X_m, to the mole fraction of the compound in the aqueous pseudophase, X_a. The abovementioned parameters are defined in Chapter 3. The molar solubilisation ratios and K_m for each surfactant at each concentration at 40 °C at pH 8 were calculated from Equations 3.1. and 3.7. respectively and are shown in Tables 4.1 to 4.5.

Table 4.1: Molar Solubilisation Ratios of Phenanthrene in Safol 45E3

C_{surf} (mMol L^{-1})	$S_{PAH,mic}$ (mMol L^{-1})	MSR	K_m	Log K_m
0.003	4.661×10^{-6}			
0.01	4.041×10^{-6}			
0.1	9.0×10^{-6}	0.0447	166	2.22
0.5	1.97×10^{-5}	0.0396	148	2.17
1.0	5.81×10^{-5}	0.0540	200	2.30

CMC = 0.00294 mmol L^{-1} $S_{PAH,cmc}$ = 4.661 E-6 mol L^{-1} V_m = 55.51 mol L^{-1}

Table 4.2: Molar Solubilisation Ratios of Phenanthrene in Safol 45E5

C_{surf} (mMol L^{-1})	$S_{PAH,mic}$ (mMol L^{-1})	MSR	K_m	Log K_m
0.005	2.201×10^{-6}			
0.1	9.1×10^{-6}	0.0670	417	2.62
0.5	4.267×10^{-5}	0.0861	525	2.72
1.0	8.548×10^{-5}	0.0832	513	2.71

CMC = 0.00447 mMol L^{-1} $S_{PAH,cmc}$ = 2.699 E-6 mol L^{-1} V_m = 55.51 mol L^{-1}

Table 4.3: Molar Solubilisation Ratios of Phenanthrene in Safol 45E7

C_{surf} (mMol L^{-1})	$S_{PAH,mic}$ (mMol L^{-1})	MSR	K_m	Log K_m
0.004	3.153×10^{-6}			
0.1	4.432×10^{-6}	0.0133	74	1.87
0.5	2.820×10^{-5}	0.0505	275	2.44
1.0	6.720×10^{-5}	0.0643	347	2.54

CMC = 0.00391 mMol L^{-1} $S_{PAH,cmc}$ = 3.153 E-6 mol L^{-1} V_m = 55.51 mol L^{-1}

Table 4.4: Molar Solubilisation Ratios of Phenanthrene in Safol 45E9

C_{surf}(mMol L^{-1})	$S_{PAH,mic}$(mMol L^{-1})	MSR	K_m	Log K_m
0.005	2.891×10^{-6}			
0.1	3.508×10^{-6}	0.00650	40	1.60
0.5	2.229×10^{-5}	0.0392	234	2.37
1.0	4.727×10^{-5}	0.0446	263	2.42

CMC = 0.00504 mMol L^{-1} $S_{PAH,cmc}$ = 2.891 E-6 mol L^{-1} V_m = 55.51 mol L^{-1}

Table 4.5: Molar Solubilisation Ratios of Phenanthrene in Safol 45E12

C_{surf} (mMol L^{-1})	$S_{PAH,mic}$ (mMol L^{-1})	MSR	K_m	Log K_m
0.008	7.63×10^{-7}			
0.1	1.986×10^{-6}	0.0132	309	2.49
0.5	1.934×10^{-6}	0.0377	851	2.93
1.0	3.928×10^{-5}	0.0388	891	2.95

CMC = 0.00782 mMol L^{-1} $S_{PAH,cmc}$ = 7.63 E-7 mol L^{-1} V_m = 55.51 mol L^{-1}

The same trends for the MSR and K_m values are observed from Tables 4.1 to 4.5. The MSR and K_m values increased as surfactant concentration increased from 0.1 through to 1mM. There was a steeper rise from 0.1 to 0.5 mM than from 0.5 to 1 mM. The increase in MSR and K_m values is expected because the micelle quantity increases with increasing surfactant concentration, thereby increasing the solubilisation of phenanthrene.

Based on the equilibrium phenanthrene concentrations for a 1 mM surfactant solution at 40 °C, the order of solubilisation capacity was E5>E7>E3>E9>E12. Safol 45E5 had the highest molar solubilisation ratio of 0.083 at 40 °C, which can be seen from the MSRs of various surfactants in Table 4.6.

Table 4.6: Molar Solubilisation Ratios of Phenanthrene in Various Surfactant Solutions

Surfactant	MSR	Log K_m	Source
Safol 45E3	0.054	2.30	Current Work
Safol 45E5	0.083	2.71	Current Work
Safol 45E7	0.064	2.54	Current Work
Safol 45E9	0.045	2.42	Current Work
Safol 45E12	0.038	2.95	Current Work
Ammonium Perflurooctonate (APFO)	2.31×10^{-4}	-	An *et al*[4]
Lithium perfluorooctanesulfonate (LiFOS)	7.97×10^{-4}	-	An *et al*[5]
Sodium dodecyl diphenyloxide disulfonate (C12-DPDS)	0.0406	5.58	Hasegawa *et al*[6]
Sodium hexadecyl diphenyloxide disulfonate (C16-DPDS)	0.0592	5.74	Hasegawa *et al*[6]
Monoalkyl monosulfonate (C10-MAMS)	0.065	5.79	Deshpande *et al*[7]
Monoalkyl disulfonate (C10-MADS)	0.016	5.18	Deshpande *et al*[7]
Monoalkyl disulfonate (C12-MADS)	0.035	5.53	Deshpande *et al*[7]
Monoalkyl disulfonate (C16-MADS)	0.067	5.80	Deshpande *et al*[7]
Dialkyl disulfonate (C10-DADS)	0.17	6.17	Deshpande *et al*[7]

The calculated MSRs are of the same order of magnitude as those reported by Hasegawa *et al*[6] and Deshpande *et al*[7] and two orders of magnitude greater than the values reported by An *et al.*[4,5] Hasegawa *et al* and An *et al* conducted their studies at 22 °C and Deshpande *et al* conducted their studies at 23 °C while the current work done at 40 °C, thus negating a direct comparison between the values quoted in literature and the current work because the temperature dependence of the MSR was not evaluated.

The hydrophobicity of a surfactant decreases with increasing ethylene oxide chain length.[8] The solubility of a neutral organic molecule decreases with increasing ethylene oxide chain length.[9] The hydrophile-lipophile balance (HLB number) of the Safol surfactants increases

as the number of ethoxylate chains increase (Appendix A, Table A1). Surfactants with low HLB numbers have a weak hydrophilic end and a strong lipophilic end and are therefore oil soluble. The lower the HLB value, the more hydrophobic the surfactant molecule will be, thus on a mass basis, the surfactant with the lowest HLB value should solubilise the most phenanthrene and acenaphthene. This trend was evident in the surfactant series from Safol 45E5 to Safol 45E12 at 40 °C and was also observed by Grimberg *et al.*[10] The only exception was Safol 45E3. Void and Void[11] reported that the aggregation numbers of surfactant micelles increase at lower CMCs and HLB numbers, and other reports state that the micelle shape shifts from spherical to asymmetrical as the cloud point is approached[12,13]. These factors result in the formation of a larger micelle, which can therefore solubilise a greater amount of hydrocarbon. This correlates with the observed results, where the phenanthrene solubilisation increased with decreasing CMC and HLB number, with the exception of Safol 45E3.

The solubility of acenaphthene was also determined at pH 8 in solutions of 0.1, 0.5 and 1 mM Safol 45E7 and Safol 45E12 at surfactant concentrations of 0.1mM, 0.5 mM and 1mM. The equilibrium concentrations of each concentration of the surfactants were obtained by fitting first order exponential decay function using Origin 5.0 software and the plot is shown in Figure 4.6. These values have been compared with those for phenanthrene in Table 4.7.

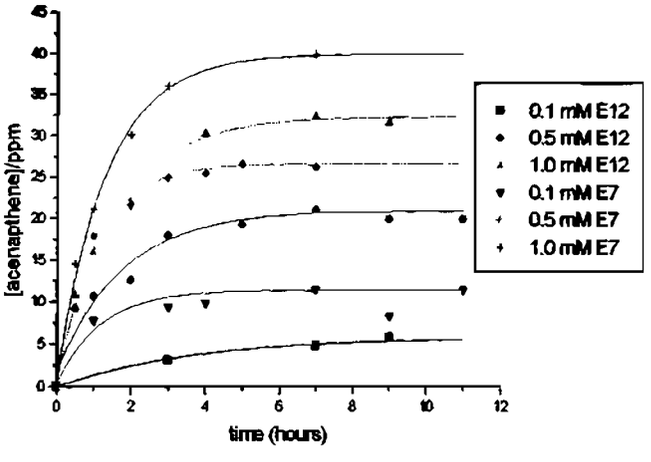


Figure 4.6: Solubility of Acenapthene in Safol 45E7 and Safol 45E12

Table 4.7: Equilibrium Concentrations of Phenanthrene and Acenaphthene in SaFol 45E7 and Safol 45E12

Concentration (mM)	Acenaphthene (ppm)		Phenanthrene (ppm)	
	Safol 45E7	Safol 45E12	Safol 45E7	Safol 45E12
0.1	11.38	5.83	1.09	1.84
0.5	26.62	20.99	15.36	13.55
1	39.95	32.35	36.60	27.51

The equilibrium concentrations of acenaphthene were higher than phenanthrene for the concentrations of Safol 45E7 and Safol 45E12 tested. This result could be due to the fact that acenaphthene is a smaller molecule than phenanthrene, and solubilisation capacity has been observed to increase with decreasing molecular size.[14]

4.1.2. Effect of pH on Phenanthrene Solubility

The pH of 1mM solutions of surfactant were measured at 20.5 °C (Table 4.8). Included in the table are the cloud points of each surfactant.

Table 4.8: pH of 1 mM solutions at 20.5 °C

Surfactant	pH	Cloud Point (°C)
Safol 45E3	7.77	46
Safol 45E5	8.10	34
Safol 45E7	8.00	44
Safol 45E9	7.89	75
Safol 45E12	7.90	95

Although Safol 45E5 showed the highest phenanthrene solubilisation capacity, Safol 45E7 was chosen for the pH studies because the experimental temperature (40 °C), was higher than the cloud point of Safol 45E5 (34°C). A surfactant reaches its maximum aqueous solubility at its cloud point; beyond this temperature surfactant will precipitate out of solution. Safol 45E12 was chosen as a second surfactant to investigate the effect of pH at the two extreme ends of solubilisation. The pH values investigated were pH 5, 6 and 7. The sub-CMC surfactant concentrations were not used in any further solubility studies, as

no significant difference was observed in the solubilisation of phenanthrene in water or sub-CMC concentrations of surfactant. The results of the pH studies are shown in Figures 4.7, 4.8 and 4.9. The equilibrium phenanthrene concentrations in different concentrations of Safol 45E7 and Safol 45E12 were determined by fitting first order exponential decay function through the points and are shown in Table 4.9. Also included is the percentage enhancement of solubilisation which was obtained by comparison to the values at pH 8.

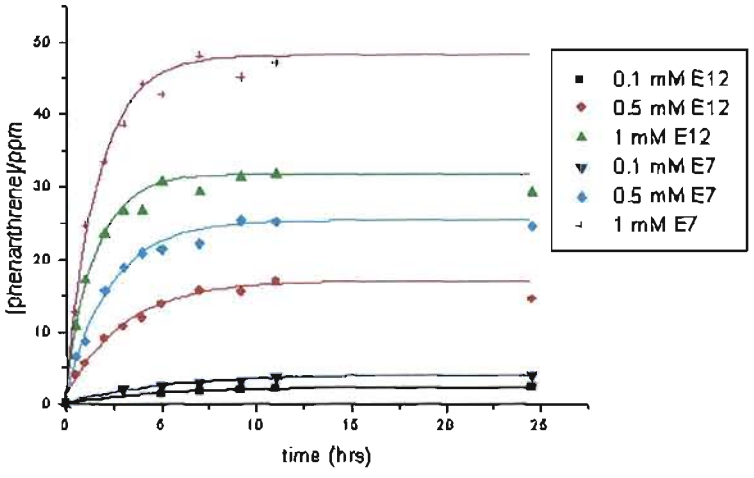


Figure 4.7: Solubility of Phenanthrene in Safol 45 E7 and Safol 45 E12 at pH 5

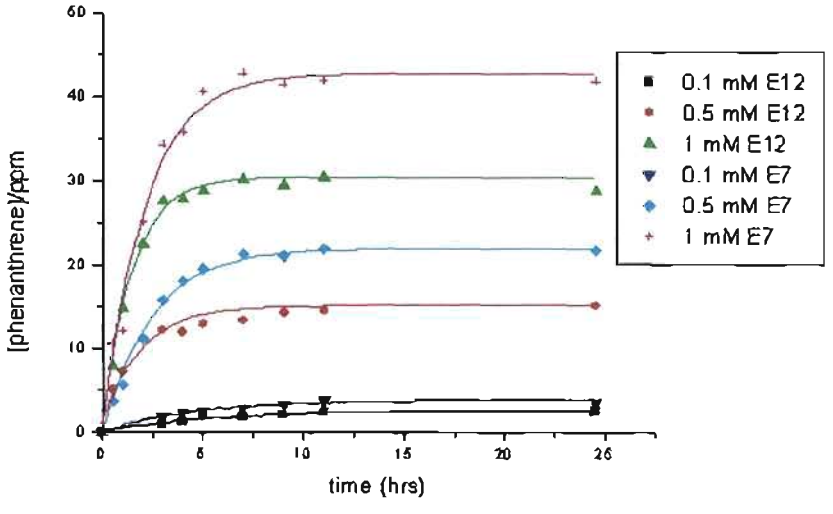


Figure 4.8: Solubility of Phenanthrene in Safol 45 E7 and Safol 45 E12 at pH 6

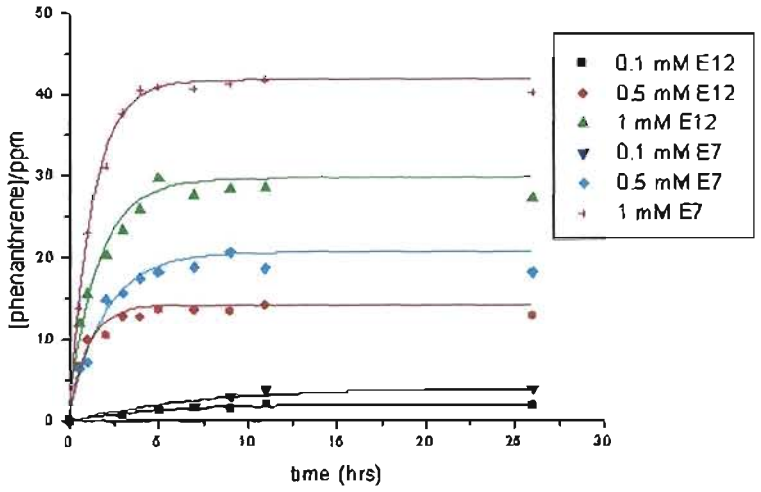


Figure 4.9: Solubility of Phenanthrene in Safol 45 E7 and Safol 45 E12 at pH 7

The enhancement in phenanthrene solubility from pH 8 to pH 5, 6 and 7 is shown in Table 4.9. The formula of the enhancement factor (EF) calculated for a 1mM solution is:

$$EF = \frac{[PAH]_{pHx} - [PAH]_{pH8}}{[PAH]_{pH8}} \times 100 \qquad \text{(Eq 4.1)}$$

Where $[PAH]_{pHx}$ is the concentration of the PAH at pH 5, 6 or 7 and $[PAH]_{pH8}$ is the PAH concentration at pH 8.

Table 4.9: Comparison of Equilibirum Phenanthrene Concentrations at pH 5, 6, 7 and 8

pH	[Safol 45E7]/mM				[Safol 45E12]/mM			
	0.1	0.5	1	EF (%)	0.1	0.5	1	EF (%)
5	4.25	24.82	46.54	27.2	2.42	15.99	30.51	10.9
6	3.67	22.11	42.75	16.8	2.50	14.32	29.72	8.03
7	4.07	19.27	41.40	13.1	1.83	13.57	28.53	3.71
8	1.09	15.36	36.60	-	1.84	13.55	27.51	-

The equilibrium concentrations of 1 mM Safol 45 E7 and Safol 45E12 at pH 5, 6 and 7 were compared to the equilibrium concentrations of 1 mM Safol 45 E7 and Safol 45E12 at pH 8 to obtain the enhancement factor.

The pH studies indicated an increase in phenanthrene solubilisation when lowering the pH from 8 to 5. The phenanthrene solubilisation capacity of Safol 45E7 and Safol 45E12

increased with decreasing pH. As pH decreases, the base protonates, creating a positively charged ionic species, and solubility increases. The ionic form of a neutral organic molecule does not interact with a surfactant micelle as strongly as the neutral molecule. As the pH increases, the organic molecule becomes ionized and thus the solubility decreases.[15] Safol 45E7 showed the highest increase (15.2 %) in phenanthrene solubilisation at pH 5.

4.1.3. Comparing the Solubility of Phenanthrene in Water and Safol Surfactant

The solubility of phenanthrene was tested in water (40 °C) to determine the enhancement (if any) of phenanthrene solubilisation by using Safol surfactants. Figure 4.10 shows the solubility of phenanthrene in water at 40 °C. Figure 4.10 was obtained by following the experimental procedures described in Section 2.1.1, while Figure 4.11 was plotted using data from literature.[16]

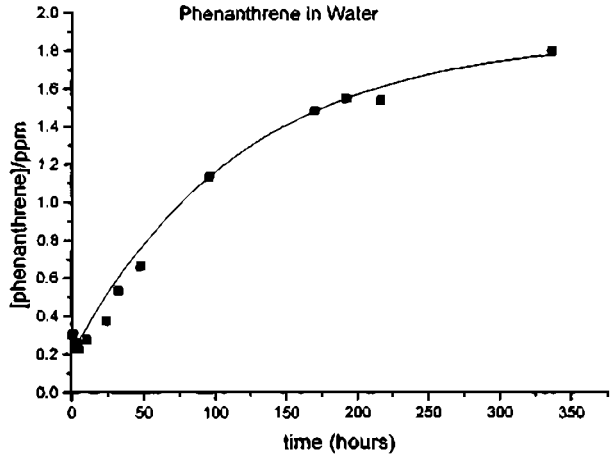


Figure 4.10: Solubility of Phenanthrene in Water

Chen *et al*[17] reported that the solubilisation rates of hydrophobic compounds increase with the addition of surfactant. This observation was consistent with the current work as the time taken to reach equilibrium in the surfactant solutions was approximately 12 hours, compared to the 350 hours in water.

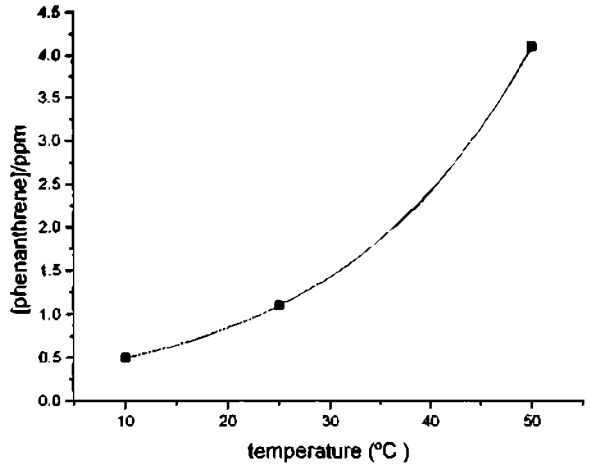


Figure 4.11: Phenanthrene Aqueous Solubility as a Function of Temperature

The experimentally determined aqueous solubility of phenanthrene at 40 °C was 1.87 ppm. Using the equation for the curve in Figure 4.11, the calculated aqueous solubility of phenanthrene at 40 °C was 2.44 ppm. This difference maybe accounted for by experimental errors.

Table 4.10: Comparison between Phenanthrene Solubilisation in Water and Safol Surfactants

Medium	$[Phenanthrene]_{eqm}$/ppm	Enhancement Factor
Water	1.87	1
Safol 45E3 (1 mM)	20.50	10.96
Safol 45E5 (1 mM)	39.63	21.20
Safol 45E7 (1 mM)	36.60	19.57
Safol 45E9 (1 mM)	29.80	15.94
Safol 45E12 (1mM)	27.51	14.71

Several conclusions can be made from the solubility studies. The aqueous solubility of phenanthrene was enhanced between 10.96 to 21.20 times in 1 mM solutions of five Safol surfactants (Table 4.10). Phenanthrene solubilisation increased linearly with increasing surfactant concentration above the critical micelle concentration (CMC) (Figures 4.1b – 4.5b). The aqueous solubility of phenanthrene below the CMC was similar to that of

water. Lowering the pH from 8 to 5 showed an increase in phenanthrene solubilisation (Table 4.9). At 20 °C, phenanthrene had an aqueous solubility of 1.18 g mL^{-1} while acenaphthene had an aqueous solubility of 3.47 mg L^{-1}. In 1 mM surfactant solution at 40 °C, the phenanthrene and acenaphthene had average solubilities of 40.41 ppm and 39.1 ppm respectively.

At a fixed experimental temperature of 40 °C and pH 8, Safol 45E5 had the highest solubilisation capacity for phenanthrene and acenaphthene. Lowering the pH from pH 8 to pH 5, resulted in an increase in phenanthrene solubilisation.

4.2. Analyte Desorption Studies

4.2.1. Determination of Moisture Content

The moisture content (Section 2.2.1) of the soil and sludge samples are displayed in terms of weight percent in Table 4.11. The moisture content of the samples were determined to standardise the mass and the moisture content in the soil spiking procedure so as to represent field-wet conditions.[18]

Table 4.11: Moisture Content of Different Soil Samples in Weight Percent

Sample	Mean ± S.D. (Weight %)
Sludge (Wet)	59.92 ± 0.43
Standard Soil	5.67 ± 0.032
Longlands (Unwashed)	0.66 ± 0.05

From the results, the Longlands and standard soil samples had moisture contents lower than usual field wet conditions of 16.67 %. Because of this these samples were not oven dried but their moisture content was adjusted to 16.67 % before spiking. However, the moisture content of the sludge sample was more than the field conditions. Thus the sludge had to be oven-dried before being used in desorption studies.

4.2.2. Determination of Clay, Silt and Sand Content

The particle size fractions of the soil, sand and sludge samples were determined by the Pipette Method[19] (Section 2.2.2). Particle size affects the sorption characteristics of soils and is thus vital in interpreting desorption studies.[20] As particle size decreases, sorption increases because of the increase of surface area per unit volume. The results of particle size analysis for the various soil and sludge samples are displayed in Table 4.12.

Table 4.12: Particle Size Fractions of Soil and Sludge Samples

Sample	% Clay	% Silt	% Sand		
			Fine	Medium	Coarse
Sludge (Dry)	11.73	39.93	15.17	5.17	28
Sludge (Wet)	25.30	65.35	7.29	1.26	0.80
Seasand	0.94	0.96	73.35	22.00	2.75
Standard Soil	16.21	63.49	19.30	0.80	0.20
Longlands Soil	9.86	12.59	44.80	27.80	4.95
Longlands Sand	0.23	0.87	58.35	37.40	3.15

Sludge samples set hard when dried and it was therefore difficult to grind them to their individual particle sizes. It is thus more appropriate to use wet sludge results for particle size analysis. The higher the clay and silt content, the greater the surface area because these particles are the finest soil fractions (< 0.02 mm in particle size diameter).[21,22] From Table 4.12, it can be concluded that the order of decreasing surface area of the soils investigated was wet sludge, standard soil, dry sludge, Longlands soil, seasand and Longlands sand.

4.2.3. Quantification of Organic Matter

The organic matter contents of the sorbents were determined by the Walkley Black procedure (Section 2.2.3) and the results of the analysis are shown in Table 4.13. Soil organic matter content affects sorption properties of the soil as discussed in Section 1.2.

An increase in soil organic matter generally leads to an increase in sorption because of the high surface area of organic matter.[23] The sludge was found to have an organic matter content of 27.76 %. Organic matter was not detected in the washed Longlands sand and seasand had a negligible organic matter content of 0.06 %. Longlands soil had a relatively low organic matter content of 0.33 %, while standard soil had an organic matter content of 3.72 %.

Table 4.13: Organic Matter Content of Soils and Sludge

Sample	% Organic Matter
Sludge (Dry)	27.76
Sludge (Wet)	27.76
Seasand	0.06
Standard Soil	3.72
Longlands (Unwashed)	0.33
Longlands (Washed)	0.00

4.2.4. Determination of Organic Compounds and Quantification of Phenanthrene in the Sludge

API sludge samples, both unwashed and washed using Safol 45E7 were subjected to soxhlet extraction as described in Section 2.2.4. The soxhlet-extracted samples were analysed using GC-MS. Figure 4.12 (arrow indicate the position of the phenanthrene peak) shows a typical GC-MS chromatogram of the soxhlet extracted sample while Table 4.14 shows the results of the analysis.

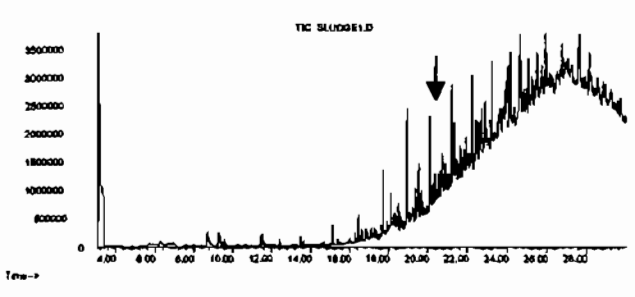


Figure 4.12. GC-MS Analysis of Soxhlet Extracted Unwashed Dry Sludge

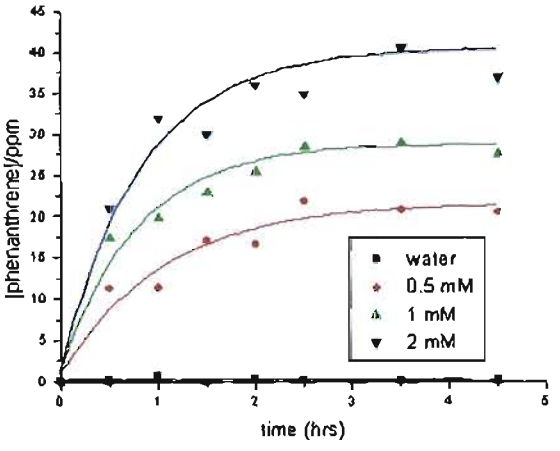
Table 4.14: Phenanthrene Area Percent as Determined from Screening of Sludge

Sample	Phenanthrene Area %
Unwashed Dry Sludge	0.68
Unwashed Wet Sludge	0.60
Washed Sludge (0.5mM solution)	0.38
Washed Sludge (1mM solution)	0.36

Dry sludge and wet sludge had phenanthrene peak area percentages of 0.68 and 0.60 % respectively. Only the dry sludge samples were subjected to surfactant washing as wet samples could possibly have contained volatile organic compounds. The 0.5 mM and 1 mM Safol 45E7 washed sludge samples showed phenanthrene peak area percents to have decreased to 0.38 and 0.36 % respectively, which represent a 44 % and 47 % extraction of phenanthrene from the dry API sludge. The results also showed that doubling the surfactant concentration from 0.5 mM to 1 mM only enhances the extraction by 3 %. It is worth mentioning that these numbers are relative to each other since the exact concentration was not determined by GC-MS analysis.

4.2.5. Soil and Sludge Washing

Spiked seasand, Longlands sand, standard soil, Longlands soil were subjected to desorption studies (Section 2.2.5). The sand samples were washed in rotating glass flat-bottomed flasks, while the soil samples were washed in rotating centrifuge tubes. Both the flasks and tubes were immersed in an insulated water bath set at 40°C and the surfactant extracts analysed using HPLC. The plots of extract analyte concentration versus time are shown in Figures 4.16 to 4.23. The desorption curves were fitted using Origin 5.0 software.



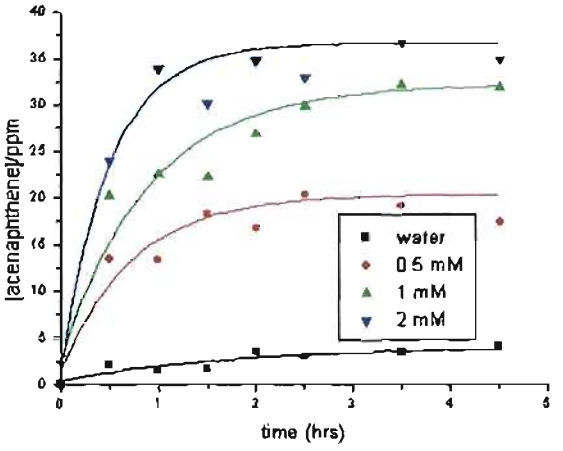
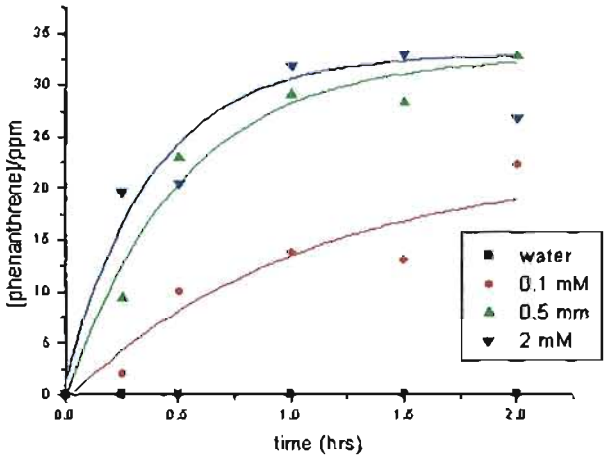


Figure 4.16 **Figure 4.17**

Figures 4.16 and 4.17: Phenanthrene and Acenaphthene Desorption Curves For Washed Longlands Sand



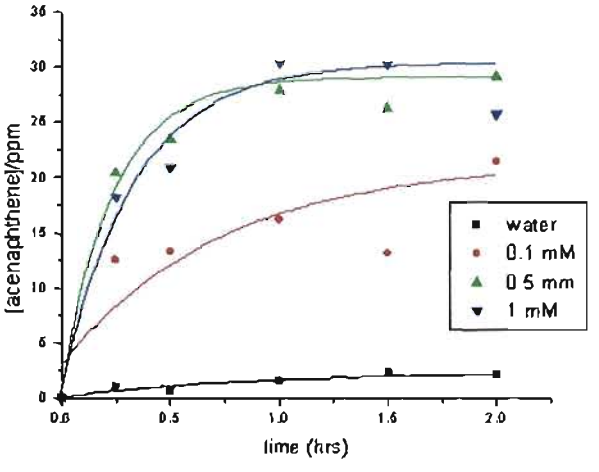
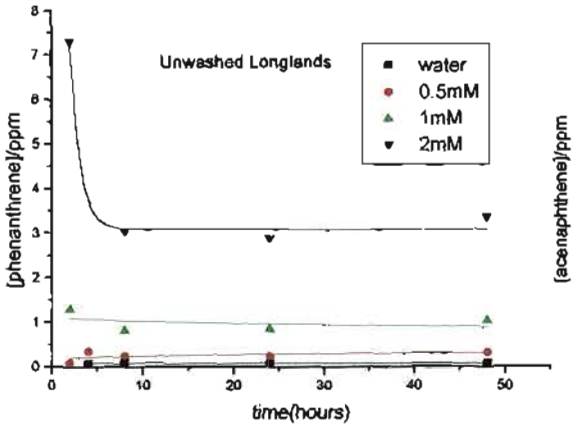


Figure 4.18 **Figure 4.19**

Figures 4.18 and 4.19: Phenanthrene and Acenaphthene Desorption Curves For Seasand



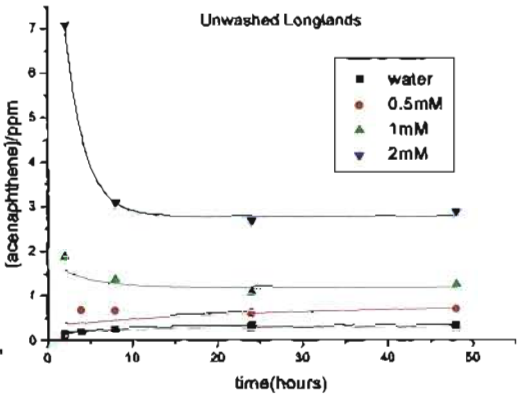
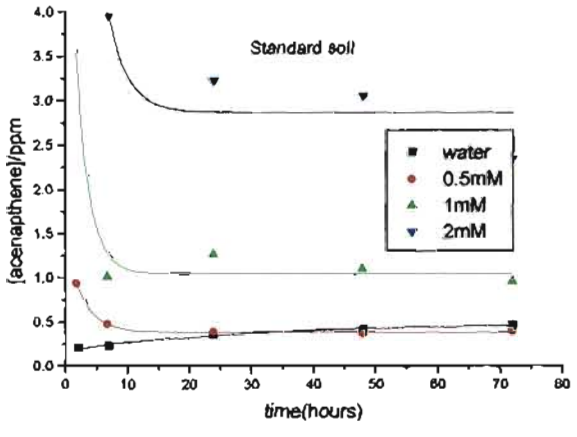


Figure 4.20 **Figure 4.21**

Figures 4.20 and 4.21: Phenanthrene and Acenaphthene Desorption Curves For Unwashed Longlands Soil



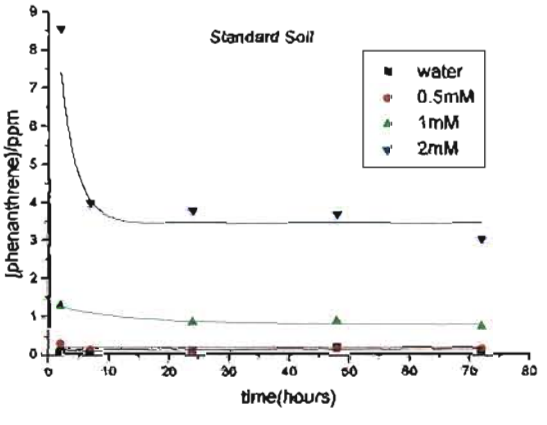


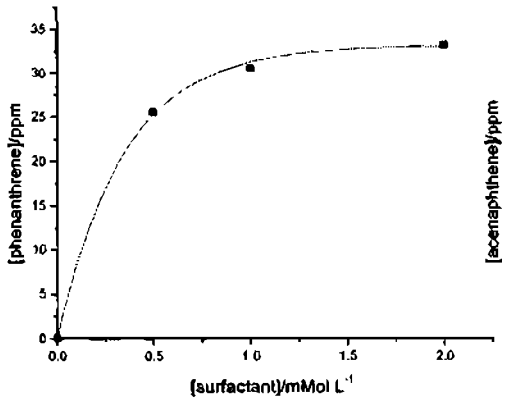
Figure 4.22 **Figure 4.23**

Figures 4.22 and 4.23: Phenanthrene and Acenaphthene Desorption Curves For Standard Soil

Two very different desorption trends are observed for the sands (Figures 4.16 to 4.19) and the soils (Figures 4.20 to 4.23). The sands exhibited an initial rapid desorption phase, followed by levelling off at equilibrium. Similar desorption trends were observed by Roy *et al*[24] and Yeom *et al*.[25] In the soil desorption studies, the analyte concentration decreased with increasing time before levelling off. This could be due to sorbed surfactants on the solid surface, which form admicelles or hemimicelles. These aggregates behave similarly to micelles in aqueous solution.[26] Thus the sorbed surfactant acted as a sink for phenanthrene in aqueous solution. Urano *et al* reported that surfactant sorption increased with increasing organic content of the sorbent.[27] However, Brownawell *et al* found that sorption of non-ionic surfactants onto soils is mostly determined by the fraction of swelling clays in the soil and not the organic content.[28] Thus, the possibility of surfactant sorbing onto the soils was greater because of their higher organic matter and clay content than the sands.

Figures 4.24 to 4.31 show the plot of phenanthrene and acenaphthene concentration versus Safol 45E7 concentration for the desorption studies. The data points were fitted using Origin 5.0 software.

In the solubility studies a linear relationship was observed between surfactant concentrations (of up to 1 mM) and the concentration of phenanthrene solubilised. Figures 4.24 to 4.27 show an initial rapid linear increase in the desorption process from sand with increasing surfactant concentration, which gradually levels off to an equilibrium at a critical concentration. Similar experimental observations were made by Chang *et al*[29] and Zheng *et al*.[30]



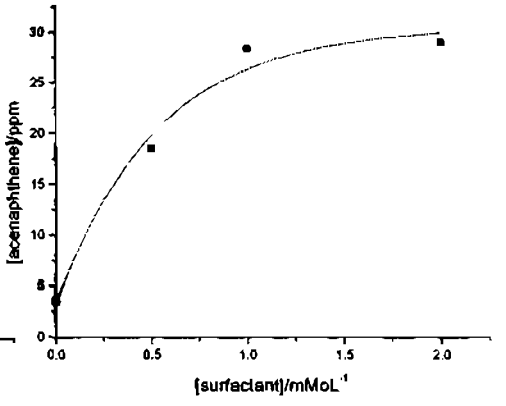
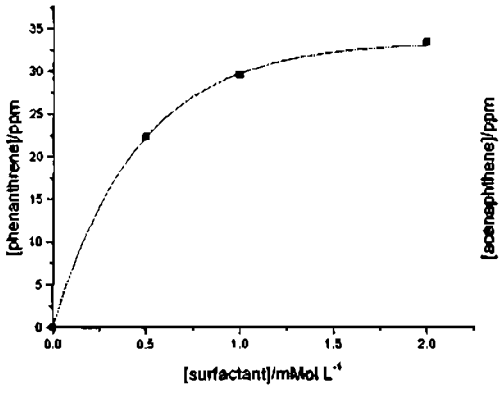


Figure 4.24 **Figure 4.25**

Figures 4.24 and 4.25: Graph of Phenanthrene and Acenaphthene Concentration vs Safol 45 E7 Surfactant Concentration for Seasand



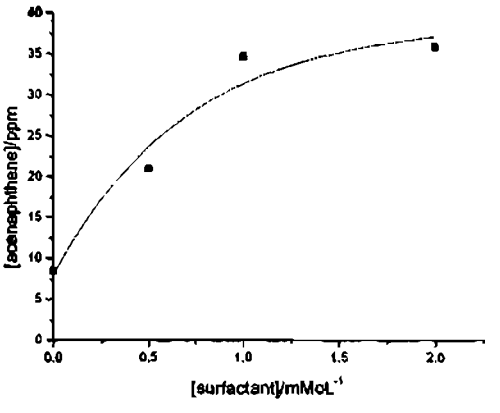
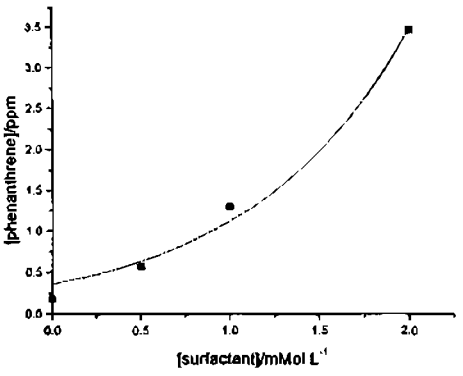


Figure 4.26 **Figure 4.27**

Figures 4.26 and 4.27: Graph of Phenanthrene and Acenaphthene Concentration vs Safol 45 E7 Surfactant Concentration for Longlands Sand

In the case of the soils, Figures 4.28 and 4.31 show an exponential increase in desorbed phenanthrene and acenaphthene concentration with increased surfactant concentration.



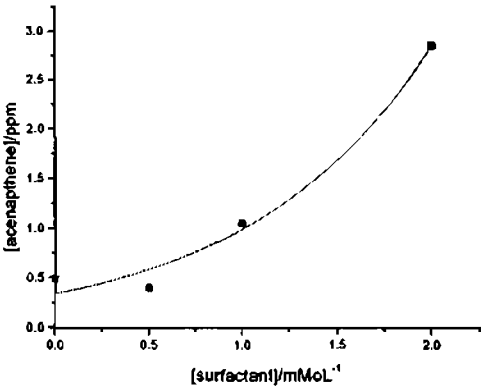
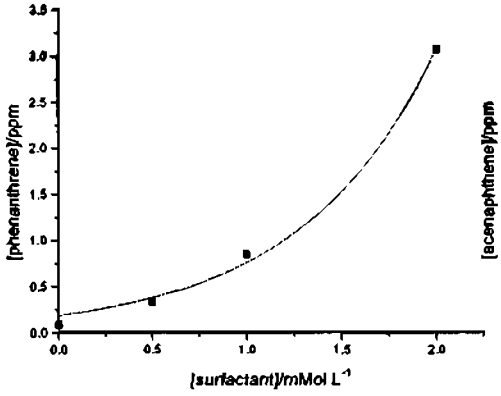


Figure 4.28 **Figure 4.29**

Figures 4.28 and 4.29: Graph of Phenanthrene and Acenaphthene Concentration vs Safol 45 E7 Surfactant Concentration for Standard Soil



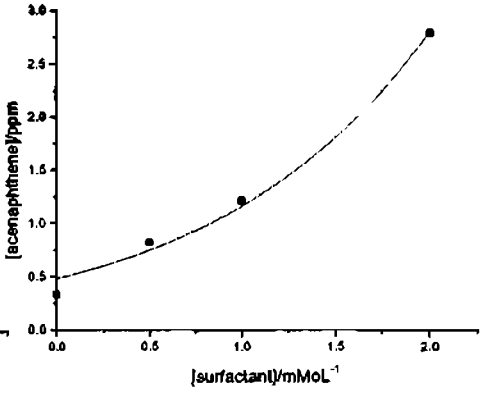


Figure 4.30 **Figure 4.31**

Figures 4.30 and 4.31: Graph of Phenanthrene Concentration vs Safol 45 E7 Surfactant Concentration for Unwashed Longlands Soil

Table 4.15 shows the equilibrium concentrations of acenaphthene and phenanthrene as determined from the plots of hydrocarbon concentration versus time for the analyte in the desorption studies.

Table 4.15: Equilibrium Concentrations of Acenaphthene and Phenanthrene in Sorbent Washing Studies

Soil	Water	0.5 mM	1 mM	2 mM
Acenaphthene (ppm)				
Seasand	3.5	18.5 (5.3)	28.4 (8.1)	29.1 (8.3)
Longland Sand	8.5	20.9 (2.5)	34.7 (4.1)	35.8 (4.2)
Standard Soil	0.49	0.40 (0.82)	1.1 (2.2)	2.9 (5.9)
Longlands Soil	0.33	0.82 (2.5)	1.2 (3.6)	2.8 (8.5)
Phenanthrene (ppm)				
Seasand	0.081	25.5 (315)	30.5 (377)	33.2 (410)
Longland Sand	0.33	22.4 (67.9)	29.6 (89.7)	38.4 (116.4)
Standard Soil	0.18	0.57 (3.2)	1.3 (7.2)	3.5 (19.4)
Longlands Soil	0.087	0.35 (4.0)	1.2 (13.8)	3.1 (35.6)

Shown in parentheses is the ratio of surfactant solubilisation to the solubilisation values for pure water. The results indicate a significant difference in equilibrium analyte concentrations of the sands and the soils. These differences may be attributed to the physical differences between the sands and soils i.e., the particle size fractions and the organic matter content. Longlands sand and seasand had combined clay and silt fractions of 1.1 and 1.9 % respectively compared with 22.5 and 79.7 % for Longlands soil and standard soil respectively. The results in the current work can be compared to the investigations of Sheets *et al*[31], who reported higher contaminant content in the finer size soil fractions, which he attributed to increased surface area that increases the number of sites available for contaminant sorption. No organic matter was detected in Longlands sand while seasand had an organic matter content of 0.06 %. Longlands soil and standard soil had organic matter contents of 0.33 and 3.72 % respectively. Finer grained soils generally have a higher organic matter content which also contributes to greater adsorption of contaminants.

For Longlands soil and standard soil, Figures 4.20 to 4.23 show that the amount of contaminant being extracted decreased exponentially with increasing time before reaching a plateau phase. From studies reported in literature, this is possibly due to surfactant sorption onto the soil surface. Ko *et al* [32] reported the sorption of sub-CMC concentrations of sodium dodecyl sulfate onto kaolinite reaching equilibrium in approximately 6 hours. The study also reported Tween sorption at concentrations above the CMC. The sorption of the two surfactants at different concentrations was attributed to the natural organic matter content of the soil samples and solids with low fractional organic carbon contents displaying nonionic surfactant sorption above the critical micelle concentration while solids with a higher organic content showed nonionic surfactant sorption below the CMC. Another reason for the decrease in hydrocarbon solubilisation with increasing time could be attributed to the solubilisation of humic material by surfactant micelles[33] as both soils had appreciable organic matter contents. Humic material contains aromatic rings with carboxylic and phenolic groups, sugars and peptides.[34] Polyaromatic hydrocarbon molecules can thus partition into the hydrophobic part of humic substances or bind to the aromatic moiety of the humic matter.[35]

It is important to note that although the standard soil sample had organic matter content approximately 10 times greater than the Longlands soil, both soils showed the same desorption trends and similar solubilised contaminant values (Table 4.15). This could be possibly due to differences in the heterogeneity of the organic fractions in both samples. Karapanagioti *et al*[36] observed that sediment organic matter heterogeneity affects the sorption behaviour of contaminants. Their study showed that even a small percentage of coal containing particles (< 3%) increased the soil sorption capacity. Coaly organic matter also displays slow irregular sorption kinetics compared to soil with organic coating around quartz crystals, which displayed fast linear sorption kinetics. The opaque organic matter fraction dominates the sorption process and thus determining this fraction allows one to predict the sorption properties of soils.[37,38]

The soil washing extraction indicator value, $\frac{1}{K_d}$ was calculated from Equation 3.10 (Chapter 3), and the results are displayed in Table 4.16. $[P]_{liq}$ was calculated from the equilibrium analyte concentration, while $[P]_{soil}$ was determined by extracting the total amount of analyte left in the soil after spiking with acetonitrile and analysing using HPLC.

Table 4.16: Soil Washing Performance Indicators

Soil	Safol 45E7 Conc. (mM)	$\frac{1}{K_d}$ (Phenanthrene)	$\frac{1}{K_d}$ (Acenaphthene)
Seasand	0.5	0.68	0.55
	1	0.81	0.73
	2	0.88	0.86
Longlands Sand	0.5	0.61	0.56
	1	0.80	0.93
	2	1.04	0.96
Standard Soil	0.5	0.092	0.023
	1	0.038	0.035
	2	0.084	0.082
Longlands Soil	0.5	0.012	0.0092
	1	0.026	0.025
	2	0.070	0.066

The seasand and Longlands sand had indicator values ranging between 0.68 and 1.04 for phenanthrene and between 0.55 and 0.96 for acenaphthene across the surfactant concentration range for both phenanthrene and acenaphthene. This means that at a surfactant concentration of 2 mM, most if not all of the contaminant had been removed from the sands. The indicator values for standard soil and Longlands soil were between 0.012 and 0.092 for phenanthrene and between 0.0092 and 0.082 for acenaphthene over the same range. This indicates that negligible amount of contaminant was removed from the soil.

Another important point to note is that even though the sludge had the highest organic matter content and smallest particle size amongst the soils and sands subjected to the desorption studies, almost half of the phenanthrene in the sludge was removed by washing with surfactant. This is attributed to the fact that the washed sludge sample was rinsed with clean water prior to soxhlet extraction to prevent interference by the surfactant in the GC-MS analysis. The clean water may have removed some adsorbed surfactant (admicelles) containing hydrocarbons.

A comparison of equilibrium analyte concentrations comparing the solubility studies to the Longlands sand desorption studies is made in Table 4.17.

Table 4.17: Equilibrium Concentrations of Phenanthrene and Acenaphthene in Safol 45E7

Concentration (mM)	Solubility Studies		Longlands Sand Desorption	
	Acenaphthene (ppm)	Phenanthrene (ppm)	Acenaphthene (ppm)	Phenanthrene (ppm)
0.5	26.62	15.36	20.9	22.4
1	39.95	36.60	34.7	29.6
2	-	-	35.8	38.4

With the exception of the phenanthrene in aqueous 0.5 mM Safol 45E7 solution, all equilibrium analyte concentrations were higher in the solubility studies compared to the desorption studies. This phenomenon could be due to the fact that only one analyte was studied at a time in the solubility studies, compared with two analytes for the desorption studies. When more than one analyte is present, the PAHs tend not to reach their single solution solubility and their individual solubilities are thus lowered.[39] Another reason for lowered PAH solubility in the desorption studies is that the addition of soil to a surfactant solution increases the CMC compared to aqueous solutions.[40] Thus more nonionic surfactant is required to reach the CMC in soil-surfactant systems probably due to the sorption of surfactant onto soil.[41]

4.3. Conclusions

Under conditions of 40 °C, pH 8 and varying surfactant concentrations, Safol 45E5 showed the highest solubilisation capacity of a 1 mM solution with a molar solubilisation ratio of 0.083, compared with MSRs of 0.054, 0.064, 0.045 and 0.038 for Safol 45E3, 45E7, 45E9 and 45E12 respectively. Below the CMC, the amount of phenanthrene solubilised was similar to that of pure water. At surfactant concentrations higher than the CMC, there was a linear relationship between phenanthrene solubilisation and surfactant concentration. The aqueous of solubility of phenanthrene was enhanced 10.96, 21.20, 19.57, 15.94 and 14.71

times in 1 mM solutions of Safol 45E3, 45E5, 45E7, 45E9 and 45E12 respectively. A 15.2 % and 4.1 % enhancement in phenanthrene solubilisation was noted for respectively Safol 45E7 and Safol 45E12 on decreasing the pH from 8 to 5. Equilibrium concentrations of acenaphthene were higher than phenanthrene.

In the desorption studies at 40 °C and using a 2 mM Safol 45E7 surfactant solution, 104 % and 90 % of phenanthrene and acenaphthene respectively were removed from Longlands sand and 88 % and 116 % of phenanthrene and acenaphthene were removed from seasand. The values in excess of 100 % are probably due the experimental error in spiking each sample separately. In the soil desorption studies, 8.4 % phenanthrene and 8.17 % of acenaphthene was removed from Longlands soil, while 7.03 % phenanthrene and 6.64 % acenaphthene was removed from the standard soil sample. Thus, it was concluded that removal of acenaphthene and phenanthrene was markedly easier for sands compared to soils. Different solubilisation trends were observed for the sands and soils. In the case of the sands, the amount of contaminant solubilised was linearly dependent on surfactant concentration before levelling off at equilibrium. The amount of contaminant solubilised in the soils showed an exponential increase with increasing surfactant concentration.

The sludge samples showed 44% and 47% reductions in phenanthrene content for samples washed in 0.5 mM and 1 mM Safol 45E7 solutions respectivley.

4.4. Recommendations

The effect of temperature on surfactant solubilisation could be conducted using at least one surfactant. Since surfactant solubility reaches a maximum at the cloud point, it is recommended to test the solubility of contaminants in each surfactant at their individual cloud points. Safol 45E9 and 45E12 have cloud points of 75 °C and 95 °C respectively. While these temperatures may be impractical in daily laboratory practice, it is important to know how the solubilisation capacity of each surfactant compares at their individual cloud points, thus the cloud point could be used a guideline to determine what surfactant to use for a given application depending on the temperature at which the application is to be conducted.

Phenanthrene and acenapthene solubility was only tested up to a surfactant concentration of 1 mM; higher concentrations of surfactant could be tested to determine the exact range of the linear dependence of aqueous contaminant solubilisation on surfactant concentration. The pH range studied was 5, 6, 7 and 8. Contaminant solubilisation increased with a decrease in pH. The pH range could be further investigated at lower and higher pHs as trends sometimes change at critical values. The effect of ionic strength was not investigated; this variable could be manipulated to determine if it has any significant effect on contaminant solubilisation.

On an industrial scale, the cost of adding pH and ionic strength buffers to surfactant solution and increasing the operating temperature will have to be weighed against the increase in PAH solubilisation.

Soil washing studies were conducted in batch experiments. Continuous soil column washing experiments could be conducted, and the effect of rinsing the washed soil with clean water should be investigated. The effect of pH, ionic strength and temperature also need to be investigated. Since most of the contaminant tends to sorb in the finer, high clay and organic matter soil fractions, soil washing as a volume reduction process should be investigated.

Due to the complex nature of API sludge and its high content of fine soil fractions, alternative methods of surfactant-enhanced polyaromatic hydrocarbon solubilisation like bioremediation and bioreactors should be investigated in order to compare the different techniques in terms of expense, efficacy and time. On an industrial scale, the recycling of used surfactant solutions should be considered and the environmental impact of the surfactants must be assessed.

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APPENDIX

APPENDIX A

Surfactant Properties (Referred to in Section 2.1.1, p 50)

Table A1: Physical Properties of Safol Surfactants and Method of Determination

Property	Method	Unit	Safol45E3	Safol45E5	Safol45E7	Safol45E9	Safol45E12
Water Content	ASTM D1744-92	%	0.1	0.09	0.04	0.07	0.1
Mean Mol wt	Calculated	g/mol	352.86	463.7	544.7	630.36	700.4370
Density@20°C	ASTM 1298	kg/k	0.9315	0.9645	0.9876	1.0099	1.0335
Density@50°C	ASTM 1298	kg/l	0.9126	0.9456	0.9687	0.991	1.052
HLB	Calculated	Griffen	7.034	9.82	11.7	13.06	15.2
Polyethylene Glycols	ASTM D4252	%	0.5	0.7	0.9	0.8	1.38
PH(5% in H_2O)	ASTM 1172-89	-	6.4	7.9	6.7	7.3	6.6
EO Content	ASTM 2959-29	%	35.17	49.1	58.5	65.3	76
Pour Point	ASTM D97	°C	0	8	10	18	24
Cloud Point	ASTM D2024	°C	46	34	44	75	95
Acid Number	ASTM D3242-98	mgKOH/g	0.08	0.03	<0.01	<0.01	0.07
Hydroxyl no.	IB-AL-01	mgKOH/g	159	121	103	89	80.1
Free Alcohol	HT-GC	Area %	19.47	8.91	12.81	2.76	4.14
Viscosity@40°C	ASTM D445	cst	21.08	27.6	35.0	45.7	53.4
CMC	Calculated	mMol/l	0.00294	0.00447	0.00391	0.00504	0.00782

Table A2: Ethoxylate Distribution in Safol Surfactants (Referred to in Section 2.1.1, p 50)

	3 EO	SAFOL 45E3	5 EO	SAFOL 45E5	7 EO	SAFOL 45E7	9 EO	SAFOL 45E9	12 EO	SAFOL 45E12
	EO-groups	Area %	EO-groups	Area %	EO-groups	Area %	EO-groups	Area %	EO-groups	Area %
alcohol	0	19.47	0	8.91	0	7.94	0	2.76	0	4.14
1EO	1	13.47	1	6.64	1	5.41	1	1.99	1	2.50
2EO	2	13.13	2	8.35	2	6.16	2	2.65	2	2.33
3EO	3	12.31	3	10.11	3	7.78	3	3.53	3	2.90
4EO	4	10.58	4	10.85	4	9.18	4	4.61	4	3.65
5EO	5	8.21	5	10.44	5	9.16	5	5.36	5	3.76
6EO	6	6.28	6	9.96	6	9.65	6	6.46	6	4.50
7EO	7	4.84	7	9.07	7	10.33	7	7.58	7	5.24
8EO	8	3.38	8	7.65	8	9.80	8	8.58	8	6.63
9EO	9	2.11	9	6.20	9	8.31	9	9.29	9	7.81
10EO	10	1.25	10	4.63	10	6.08	10	9.58	10	8.40
11EO	11	0.68	11	3.25	11	3.38	11	9.32	11	9.01
12EO	12	0.30	12	2.05	12		12	8.41	12	9.09
13EO	13	0.11	13	1.13	13		13	7.04	13	8.48
14EO	14	0.19	14	0.50	14		14	5.54	14	7.18
15EO	15		15	0.15	15		15	3.97	15	5.64
16EO	>15		16		16		16	2.25	16	3.30
17EO					17		17	0.81	17	1.85
18EO							>17	0.14	18	0.95

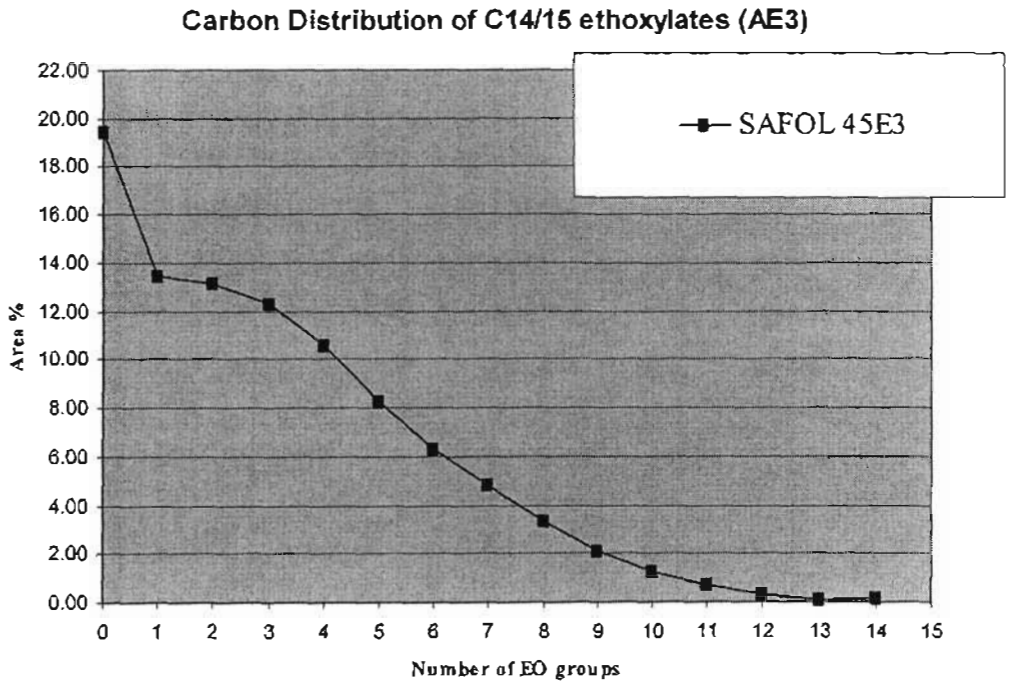

Figure A1: Carbon Distribution Curve for Safol 45E3

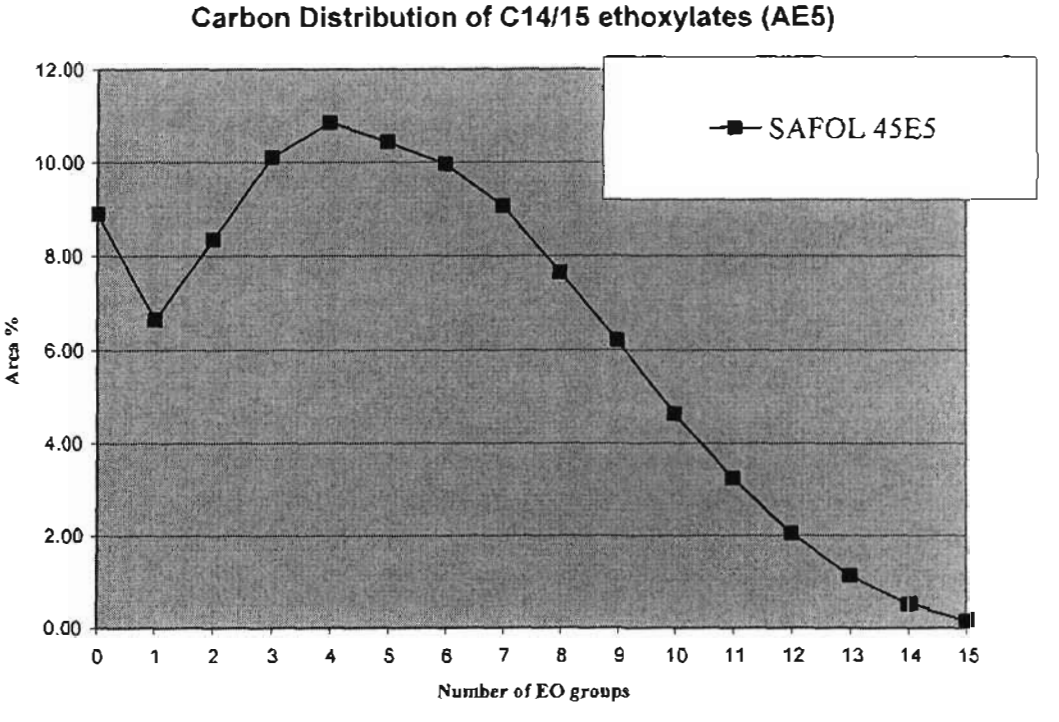

Figure A2: Carbon Distribution Curve for Safol 45E5

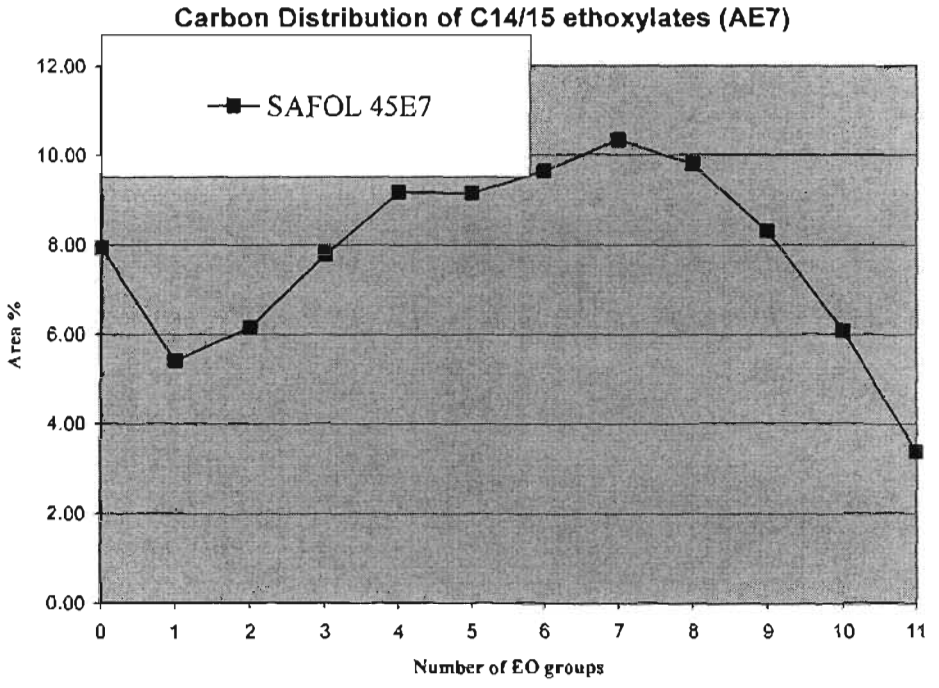

Figure A3: Carbon Distribution Curve for Safol 45E7

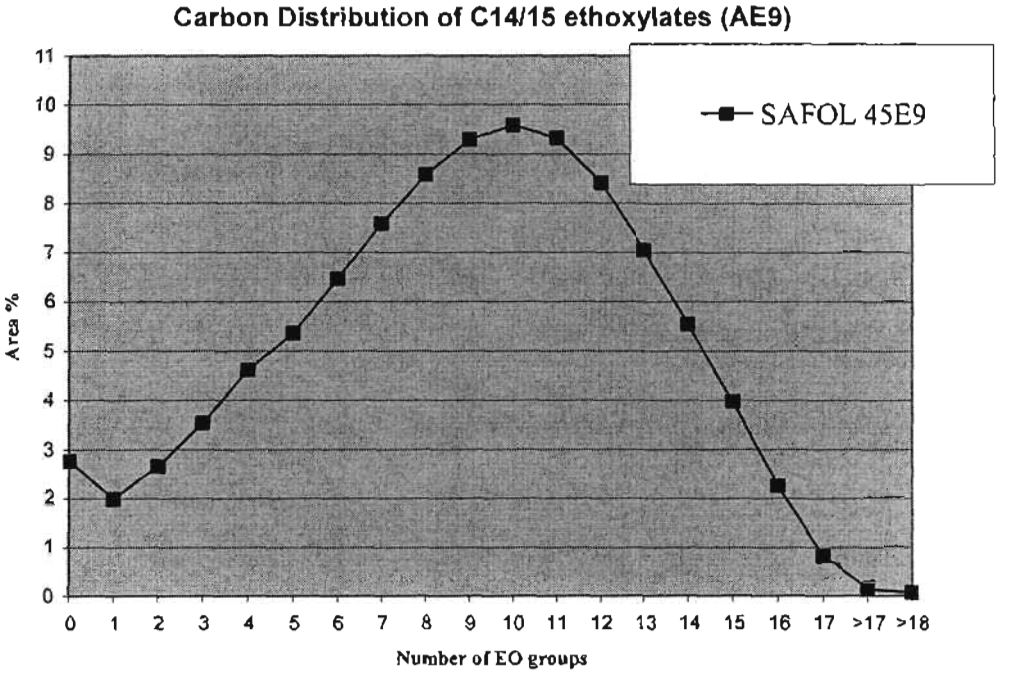

Figure A4: Carbon Distribution Curve for Safol 45E9

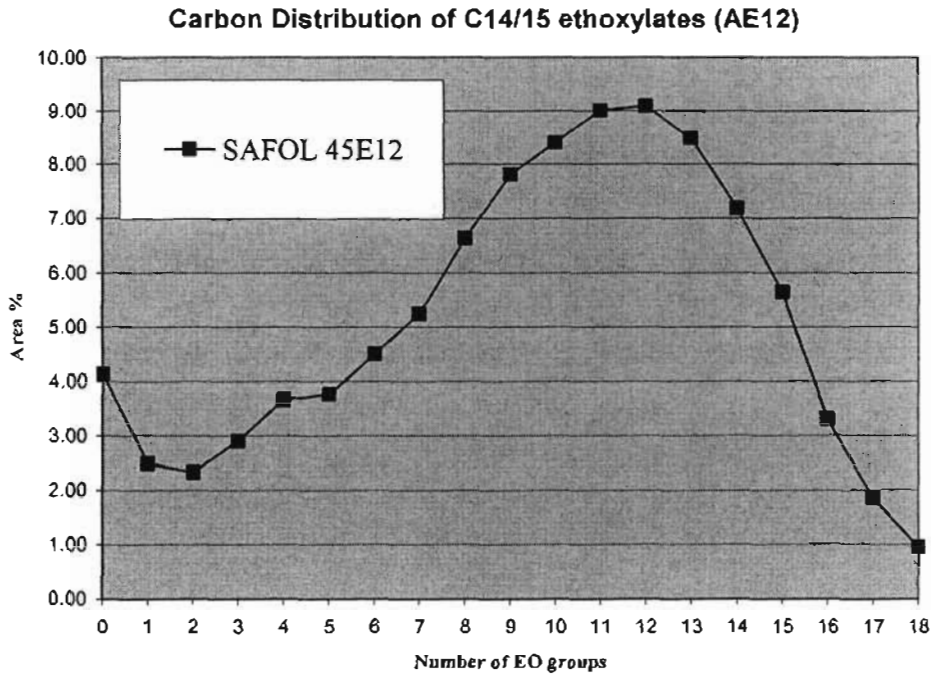

Figure A5: Carbon Distribution Curve for Safol 45E12

APPENDIX B (Referred to in Section 2.2.2, p 53)

TABLE B1: Sedimentation times for particles of silt (0.05 mm), fine silt (0.02 mm) and clay (0.002 mm) size particles in water, according to Stokes' Law.

Temperature oC	Settling time for indicated particle diameter				
	0.05mm at 125mm depth (i.e.for Si+C1 reading - only used in measurement of "unbound"Si+CI)	0.02 mm at 100 mm depth (i.e. for fiSi+Cl reading)		0.002 mm at 75 mm depth (i.e. for CI reading)	
	sec	min	sec	hrs	min
16	-	5	14	6	32
17	-	5	7	6	24
18	60	5	1	6	16
19	59	4	54	6	8
20	57	4	48	6	0
21	56	4	41	5	52
22	54	4	35	5	43
23	53	4	28	5	35
24	51	4	22	5	28
25	50	4	16	5	20
26	49	4	10	5	13
27	48	4	4	5	6
28	46	4	0	4	59
29	45	3	55	4	52
30	44	3	49	4	46

APPENDIX C1: RAW DATA FOR SOLUBILITY STUDIES: ABSORBANCE VALUES CONVERTED TO CONCENTRATION UNITS OF PPM

pH 8
Safol 45E3:

Time(hours)	0.0025 mM	0.003 mM	0.01 mM	0.1 mM	0.5 mM	1 mM
0	0	0	0	0	0	0
0.5	--	--	--	--	--	2.4468
1	1.42595	--	1.2153	0.55093	2.51971	4.19682
2	1.27201	--	1.31252	0.93983	3.79173	7.47812
3	--	--	--	1.02895	3.87274	11.85319
4	--	--	1.37734	1.15048	4.22113	12.63908
5	--	--	1.26391	--	5.323	14.04883
7	--	--	--	--	--	16.64146
9.25	--	--	--	--	--	20.15772
10	--	0.99654	1.28821	--	--	--
11	--	--	--	--	--	19.20169
11.5	--	--	--	--	--	19.20169
15	1.54748	1.41785	1.40164	--	--	--
20	1.57989	--	1.42595	--	6.90288	--
24	--	--	--	--	--	19.37993
25	1.49887	1.45025	1.39354	--	--	--
27	--	--	--	--	--	19.39613
30	1.51507	1.57178	1.40974	--	6.92719	20.498
36	--	1.57989	--	--	6.9515	--
42	--	--	--	3.09496	6.87048	--
48	--	1.6447	--	3.17597	--	--
50	--	--	--	--	--	19.99568

Safol 45 E5

Time(hours)	0.003 mM	0.004 mM	0.005 mM	0.1 mM	0.5 mM	1 mM
0	0	0	0	0	0	0
0.5	--	--	--	--	2.27666	4.15631
1	--	--	--	0.59144	4.0915	6.39246
2	0.36459	--	0.32408	0.86691	5.88204	13.71665
3	0.4213	0.32408	--	0.8183	9.13903	19.80123
4	--	0.31679	--	1.02895	10.40294	25.424
5	--	0.32408	--	0.93173	12.96316	29.39397
11.5	--	--	0.56714	1.40164	15.73404	35.30842
22.5	0.51042	0.53473	0.67246	1.83105	17.53268	--
31.5	0.59144	0.59955	--	2.01739	--	--
46.5	0.84261	--	0.8102	--	--	--
71	--	1.09377	0.95603	--	--	--
144	0.98034	1.42595	1.02085	4.0915	19.78503	39.63487
171	--	1.48266	--	4.22113	--	39.08394

Safol 45E7

Time(hours)	0.0035 mM	0.004 mM	0.1 mM	0.5 mM	1 mM
0	0	0	0	0	0
0.5	0.32406	0.32649	0.29571	0.34351	1.46646
1	0.24305	0.25358	0.26006	1.37726	5.81722
2	0.26249	0.24224	0.33216	1.82285	21.46214
3	0.23575	0.25358	0.32406	3.1515	27.33607
4	0.22684	0.2714	0.33216	4.80421	31.63012
5	0.22279	0.21874	--	8.05293	--
11	0.2795	0.45369	0.65622	--	35.09776
24	0.5185	0.82636	0.81015	15.36052	35.99708
32.5	0.68863	1.02079	1.08561	--	36.60473
48	0.81015	1.23954	--	15.2633	35.81884
72	1.10991	--	--	--	36.6
96	--	1.45828	1.69322	--	
170	1.46638	1.71753	2.18742	--	
192	1.38536	1.69322	2.41426	--	

Safol 45E9

Time(hours)	0.005 mM	0.008 mM	0.1 mM	0.5 mM	1 mM
0	0	0	0	0	0
0.5	--	--	--	0.55904	3.02204
1	0.29572	0.2544	0.56714	1.57178	9.31727
2	--	--	--	5.10425	15.1831
3	0.28924	0.24549	0.78589	5.52555	--
4	--	--	--	6.82186	--
5	0.33056	0.52663	0.76159	6.38436	23.74689
12	0.38079	0.4294	1.05326	7.8022	--
24.33	0.76159	0.72108	1.26391	--	--
48	1.11807	1.26391	1.55558	--	28.14627
70.5	1.25581	1.68521	--	14.04883	29.79907
96.5	1.44215	1.56368	--	--	28.55947
120	--	1.82294	--	13.85438	28.99698
144	1.74193	1.73382	2.21184	--	--

Safol 45E12

Time(hours)	0.005 mM	0.008 mM	0.1 mM	0.5 mM	1 mM
0	0	0	0	0	0
0.5	--	--	--	3.03014	7.16215
1	--	--	--	6.5707	12.76061
2	--	--	--	10.48396	22.06979
3	--	--	0.87501	12.15297	--
4	--	--	--	13.20622	--
5	0.35406	0.28924	1.2234	--	--
7	--	--	--	--	--
11.5	0.34028	0.397	1.49887	--	--
25	0.62385	0.46181	1.77433	13.35206	26.78514
71	--	0.53473	1.83915	--	27.49001
94.5	0.91552	--	--	13.27104	27.41709
119	1.06946	--	--	13.54651	27.51431
122.5	--	--	--	--	--

pH 5

Safol 45E7 and 45E12

Time(hours)	Safol 45E12 Concentration (mM)			Safol 45E7 Concentration (mM)		
	0.1	0.5	1	0.1	0.5	1
0.5		3.95376	10.83234		6.59501	12.65529
1	--	5.56606	17.06276	--	8.66101	24.5814
2	--	9.04181	23.54434	--	15.71784	33.51788
3	--	10.69461	26.66361	2.12272	18.82899	38.62212
4	--	11.86129	26.73652	--	20.83828	44.17198
5	1.45025	13.78956	30.77131	2.62504	21.3244	42.74603
7	1.76623	15.66922	29.31295	2.8762	22.1427	48.15815
9.17	1.98498	15.52339	31.40326	3.19218	25.30247	45.0794
11	2.30906	16.96554	31.71924	3.74311	25.22956	47.15351
24.5	2.38198	14.50254	29.24003	4.0915	24.5733	--

pH 6

Safol 45E7 and 45E12

Time(hours)	Safol 45E12 Concentration (mM)			Safol 45E7 Concentration (mM)		
	0.1	0.5	0.1	0.5	0.1	0.5
0.5		4.926	7.87512		3.6945	10.6541
1		7.16215	14.84282		5.59847	12.03954
2		11.0835	22.49109		11.22124	25.14854
3	0.94793	12.18537	27.61154	1.83915	15.79885	34.27136
4	1.28011	11.94231	27.90321	2.25235	18.08361	35.75402
5	1.83915	12.86594	28.80253	2.52782	19.50956	40.54229
7	1.75813	13.36826	30.23658	2.84379	21.3244	42.75413
9	2.0498	14.25948	29.48309	3.32991	21.09755	41.41731
11	2.37388	14.50254	30.40672	3.84034	21.94015	41.92773
24.5	2.50351	15.11829	28.72961	3.48385	21.77811	41.77379

pH 7

Safol 45E7 and 45E12

Time(hours)	Safol 45E12 Concentration (mM)			Safol 45E7 Concentration (mM)		
	0.1	0.5		0.1	0.5	
0.5	--	6.78946	11.99903	--	6.5464	13.89489
1	--	9.99784	15.5882	--	7.26747	23.01772
2	--	10.58118	20.30355	--	14.86713	30.99816
3	0.68867	12.88214	23.45522	--	15.75024	37.7228
4	--	12.78492	25.95873	--	17.58129	40.5909
5	1.29632	13.59512	29.72615	--	18.34288	40.93119
7	1.70142	13.64373	27.73307	1.80674	18.92622	40.73674
9	1.48266	13.41687	28.41363	2.94102	20.70865	41.32008
11	1.96878	14.25948	28.58377	3.79173	18.78038	41.83861
26	1.79864	12.88214	27.22264	3.91325	18.19704	40.18581

APPENDIX C2: Desorption Studies: Tables showing amount of analyte desorbed (in ppm) for given time interval

Seasand

Acenaphthene

Time (hours)	Water	0.5 mM	1 mM	2 mM
0.25	0.99	12.49	20.43	18.26
0.5	0.56	13.28	23.42	20.92
1	1.55	16.15	27.91	30.38
1.5	2.3	13.1	26.29	30.24
2	2.1	21.4	29.07	25.69

Seasand

Phenanthrene

Time (hours)	Water	0.5 mM	1 mM	2 mM
0.25	0.051	2.038	9.39	19.6
0.5	0.05	10	22.88	20.44
1	0.058	13.8	29.04	31.84
1.5	0.068	13.04	28.2	32.88
2	0.066	22.24	32.68	26.76

Longlands Sand

Acenaphthene

Time (hours)	Water	0.5 mM	1 mM	2 mM
0.5	2.13	13.56	20.35	24.03
1	1.5	13.42	22.55	33.88
1.5	1.61	18.28	22.24	30.1
2	3.44	16.79	26.86	34.8
2.5	2.978	20.37	29.91	32.97
3.5	3.4	19.13	32.14	36.66
4.5	3.98	17.34	31.85	34.82

Longlands Sand

Phenanthrene

Time (hours)	Water	0.5 mM	1 mM	2 mM
0.5	0.162	11.34	17.44	21
1	0.7	11.44	19.8	31.95
1.5	0.149	17.16	22.96	30
2	0.331	16.7	25.48	36.06
2.5	0.22	21.9	28.5	35.06
3.5	0.149	20.9	29	40.7
4.5	0.162	20.65	27.7	37

Longlands Soil

Acenaphthene

Time (hours)	Water	0.5 mM	1 mM	2 mM
2	0.14	0.11	1.89	7.08
4	0.2	0.71	1.12	2.65
8	0.27	0.7	1.4	3.09
24	0.33	0.63	1.1	2.69
48	0.33	0.74	1.29	2.89

Longlands Soil

Phenanthrene

Time (hours)	Water	0.5 mM	1 mM	2 mM
2	-	0.089	1.28	7.3
4	0.065	0.35	0.68	2.09
8	0.093	0.25	0.81	3.04
24	0.068	0.24	0.84	2.87
48	0.082	0.33	1.03	3.35

Standard Soil

Acenaphthene

Time (hours)	Water	0.5 mM	1 mM	2 mM
2	0.21	0.95	4.22	8.15
7	0.23	0.49	1.02	3.95
24	0.37	0.4	1.27	3.22
48	0.42	0.38	1.11	3.05
72	0.47	0.41	0.97	2.34

Standard Soil

Phenanthrene

Time (hours)	Water	0.5 mM	1 mM	2 mM
2	0.101	0.32	1.31	8.55
7	0.093	0.17	0.6	3.99
24	0.144	0.15	0.89	3.79
48	0.22	0.19	0.93	3.7
72	0.14	0.18	0.77	3.03

Appendix C3: Statistical Analysis for Results Obtained

Surfactant Variation: Unaltered pH

Analyte: Phenanthrene

Conc(mM)	Safol45E3	Safol45E5	Safol45E7	Safol45E9	Safol45E12
	1.90 ±0.61	0.028 ±0.010			
	0.10 ±0.0087	0.017 ±0.0024	0.016 ±0.0019	0.019 ±0.0017	0.030 ±0.0080
	1.79 ±0.35	0.044 ±0.010	0.023 ±0.0020	0.023 ±0.0024	0.12 ±0.0080
0.1	0.12 ±0.016	0.020 ±0.0028	0.014 ±0.0013	0.027 ±0.0075	0.20 ±0.020
0.5	0.28 ±0.030	0.18 ±0.017	0.12 ±0.017	0.10 ±0.024	0.72 ±0.041
1.0	0.26 ±0.0069	0.24 ±0.013	0.54 ±0.042	0.34 ±0.029	0.71 ±0.046

pH Variation

Analyte: Phenanthrene

surfactant	Conc(ppm)	Unfixed pH	pH5	pH6	pH7
Safol45E7	0.1	0.014 ±0.0013	0.14 ±0.020	0.28 ±0.056	0.36 ±0.088
	0.5	0.12 ±0.017	0.42 ±0.026	0.42 ±0.017	0.59 ±0.062
	1.0	0.54 ±0.042	0.64 ±0.043	0.49 ±0.035	0.75 ±0.032
Safol45E12	0.1	0.20 ±0.020	0.24 ±0.043	0.24 ±0.045	0.39 ±0.12
	0.5	0.72 ±0.041	0.36 ±0.031	0.52 ±0.047	0.73 ±0.094
	1.0	0.71 ±0.046	0.65 ±0.049	0.79 ±0.031	0.54 ±0.046

Analyte Variation: Acenaphthene

Unfixed pH

surfactant	Conc(ppm)	Unfixed pH
Safol45E7	0.1	0.48 ±0.25
	0.5	0.95 ±0.14
	1.0	0.76 ±0.079
Safol45E12	0.1	0.28 ±0.15
	0.5	0.57 ±0.044
	1.0	0.55 ±0.034

APPENDIX D: Umgeni Sludge Analysis (Referred to in Section 2.2.4, p 55)

The given information shows the compound likely to be present for a given retention time using a database. The compounds of importance to the analysis were highlighted. Acenaphthene was not detected in the sludge, but phenanthrene was. The analogues of phenanthrene were also highlighted because they could indicate the degradation of phenanthrene by natural organisms.

Search Libraries: C:\DATABASE\wiley275.L Minimum Quality: 80

Retention Time	Library/ID
3.16	Pentane, 3-ethyl-2,2-dimethyl
3.59	2-Hexene
3.65	1-Buten-3-yne, 2-methyl-
3.68	exo-2-Bromonorbornane
3.78	3-formylpyrrole
3.81	2-Pentene, 3-methyl-, (Z)-
3.85	2-Butyn-1-ol, 4-methoxy-
3.89	2,4-Pentadieneoic Acid
3.93	6-Oxabicyclo[3.2.1]octane-7-one
3.99	3-ethynyl-2H-azirine
4.02	Cyclobutanone, 2,2,3,3-tetramethyl
4.10	5-Methoxy-1-tetralone
4.12	N-Nitroso-2,2,4,4-tetradeuteroazet
4.15	Furan, 2-methyl- 2-Methyl
4.21	Azetidine, 1-nitroso-
4.24	Decane, 2-methyl-
4.29	Triclofos
4.33	O-d-3-Cyclohexen-1-ol 3-Cyclohe
4.35	3-Methyl-2-(2-methyl-2-buten
4.42	2-n-Butylacrolein Hexanal, 2-me
4.46	Acetic acid, chloro-, ethyl ester
4.52	Disulfide, butyl (1,1-dimethylethy
4.56	hloroiodomethane Methane, chlo
4.63	7-(1,2-butadienyl)bicyclo[2.2.1]he
4.68	1,4-Cyclohexadiene, 1-methyl-
4.70	Acetonitrile, (dimethylamino)-
4.82	1H-Pyrazole, 4,5-dihydro-5-methyl-
4.84	2-Buten-1-ol, 3-methyl-
4.86	1-(hydroxymethyl)-2-vinylcyclopent
4.88	3-Buten-2-ol, 2-methyl-
4.91	Benzenamine Aniline
4.95	3-Penten-1-yne Propenylac
4.98	Bicyclo[2.2.1]heptan-2-ol, exo-
5.03	Phenol Izal ENT 1814
5.05	1-Penten-3-yne, 2-methyl-
5.09	Benzenamine Aniline
5.20	1,3,5-Cycloheptatriene
5.22	1,3-dithiethane
5.23	Hydrazinecarbothioamide
5.38	Ethane, chloro-
5.43	Methane, chloro-
5.46	2-fluoropyridine Pyridine,
5.47	3-Cyclohexen-1-ol
5.64	2-Hydroxy-2-methyl-but-3-enyl 2-me
5.73	2-Hexanone
5.90	3-Hexanol
5.97	Methane, nitroso-
6.28	6,6-Dimethylcyclohexa-2-en-1-ol
6.63	2-Hexene, 3,5,5-trimethyl-
6.80	2-Hexene, 2,5,5-trimethyl-
6.93	1-Cyclopenten-3-ol
7.20	3(5)-Di-1,2,4-triazole
7.30	1,3-Cyclopentadiene
7.47	2-Pentanethiol, 4-methyl-
7.49	Propanoic acid, 3-mercapto-, ethyl
7.61	Benzene, 1,2-dimethyl-

7.63	Pyrazine, ethenyl- 2-Viny
7.71	1,2-dithiacyclopentane
7.95	2,3,3-Trimethyl-1-hexene
8.19	Cyclooctane, (1-methylpropyl)-
8.37	Cyclohexane
8.53	1H-Azepine, hexahydro
8.59	methyl 2-hydroxy-4-methylpentanoat
8.70	2-Butenal, 3-methyl-
8.80	Thiophene, 2-butyl-
8.96	1-Silacyclo-3-pentene
9.16	Cyclohexane, 1-methyl-3-propyl-
9.27	Cyclohexane, (1,2,2-trimethylbutyl
9.32	2-fluoropyridine
9.41	Carbonic acid, dipentyl ester
9.59	Benzenethiol Thiophenol
9.72	1-Docosene
9.85	Norborneol 2
10.20	Benzene, 1-ethenyl-3-methyl-
10.31	Decane
10.43	2-Hexadecanol
10.57	Methyl 1,2-Dimethyl-2-propenyl Eth
10.68	Heptane, 2,4-dimethyl-
10.78	(2-Furyl)isopentyl ether
10.90	Benzenemethanol
10.98	E-1-phenylpropene Benzene, 1-
11.07	Benzaldehyde, 2-hydroxy-
11.14	1H-Indene
11.28	Phenol, 2-methyl-
11.34	Benzene, (2-methylpropyl)-
11.41	Hexyl octyl ether
11.50	n-Propyl cis-1-propenyl sulfide
11.58	Benzenemethanethiol
11.63	Phenol, 4-methyl- p-Creso
11.79	heptanoic acid
11.84	Benzene, 1,2,3,4-tetramethyl-
11.91	2-Nonanone Methyl heptyl
12.00	Phosphoramidous difluoride
12.06	Undecane
12.11	Nonanal
12.15	2H-1-Benzopyran 3-Chromen
12.20	Disulfide, bis(1-methylethyl)
12.32	Ethanone, 1-(5-methyl-1,2,3-thiadi
12.43	2-ethylthiolane
12.50	di-(3-methylbutyl) ether
12.58	Hexanoic acid, 2-methylpropyl este
12.63	trans-3-Chloro-4-fluoro-3-hexene
12.67	Phenol, 4-ethyl- p-Ethylp
12.76	Benzene, 2-butenyl- 1-Phe
12.84	Phenol, 2,6-dimethyl- 1-H
12.94	Benzene, 2-ethenyl-1,4-dimethyl-
13.02	Benzene, (2-methylbutyl)-
13.09	alpha.-Terpinene $$ 1,3-Cyclohexa
13.15	Phenol, 3,5-dimethyl-
13.19	Cyclopropane, 1-hexyl-2-methyl-
13.29	Octanoic Acid
13.37	2,4-Dimethylthiophenol Benzenet
13.49	Azulene Cyclopentacyclohe
13.64	Dodecane
13.68	Hexathiepane 1,2,3,4,5,6-
13.72	Decanal
13.76	Benzene, (methylsulfinyl)-
13.81	Naphthalene, 1,2,3,4-tetrahydro-6-
13.86	2,5-Cyclohexadiene-1,4-dione, 2-hy
13.94	Benzofuran, 4,7-dimethyl-
13.97	Propanoic acid, 2-methyl-, 2-pheny
14.05	Phenol, 3-(1-methylethyl)-
14.08	Benzoic acid, 4-methyl-, methyl es
14.11	2-Oxazolidinethione, 4,4-dimethyl-
14.15	2-Chloro-2-oxo-1,3,2.lambda.(5)-di
14.23	Benzene, 1,4-dimethyl-2-(2-methylp
14.30	1-pyrrolizidinone

14.35	Methyl(1-phenylcyclobutyl)ether
14.42	Benzoic acid, 3-methyl-
14.45	2-(p-Tolyl)ethylamine Benzeneet
14.49	Bicyclo [3.3.1]Non-1-ol-3-one
14.52	Phenol, 2-ethyl-5-methyl-
14.58	4-methylpentylbenzene
14.63	Toluene-D5
14.69	Nonanoic Acid
14.78	Ethanone, 1-(4-methoxyphenyl)-
14.83	Benzene, 1,2,4-trimethyl(1-methyle
14.90	1H-Inden-1-one, 2,3-dihydro-
15.01	2-Methylthio-4-methoxypyrimidine
15.09	Tridecane
15.15	Naphthalene, 1-methyl-
15.20	6,8-Dioxabicyclo[3.2.1]octane, 7-e
15.23	Naphthalene, 6-ethyl-1,2,3,4-tetra
15.26	Benzaldehyde, 2,4,6-trimethyl-
15.30	1,1'-Bicyclohexyl
15.39	Naphthalene, 1-methyl-
15.47	2,4,6-Trimethylindane
15.58	Phenol, 2-(methylthio)-
15.64	Benzaldehyde, 2-methyl-
15.68	Undecane, 4,4-dimethyl-
15.71	3-Hydroxy-2-methylglutaric acid di
15.75	Ethane, 2,2-dichloro-1,1,1-trifluo
15.80	Butanethioic acid, 3-oxo-, S-(1,1-
15.84	2-Pentanone, 4-methyl-4-phenyl-
15.90	Tridecane, 4-methyl-
15.97	Undecane, 4-ethyl-
16.02	Benzene, heptyl-
16.07	Tridecane, 3-methyl-
16.10	1-Methyl-2-N-hexylbenzene
16.15	Dodecane
16.20	3-Pyridinecarbonitrile, 1,4-dihydr
16.23	5-n-Propyltetralin
16.30	1,1'-Biphenyl
16.36	2,4-Dimethyl-6-tert-butyl phenol
16.41	2-Ethyl-4,6-Dimethylindane
16.46	Tetradecane
16.51	2-Ethylnaphthalene
16.59	2-Butanone, 4-(ethenylphenyl
16.66	Naphthalene, 2,6-dimethyl-
16.73	6-N-Butyl-1,2,3,4-Tetrahydronaphth
16.78	Benzene, 4-(2-butenyl)-1,2-dimethy
16.86	Naphthalene, 1,2-dimethyl-
16.91	Naphthalene, 1,4-dimethyl-
16.97	Benzaldehyde, 4-methyl-
17.03	2-Tert-Butyl-4,5-Dimethylphenol
17.10	Fluorene, 1,2,3,4,4a,9a-hexahydro-
17.13	Tetradecane, 5-methyl-
17.18	Naphthalene, 2,7-dimethyl-
17.21	Tetradecane, 4-methyl-
17.28	Biphenylene
17.34	Naphthalene, 2,7-dimethyl-
17.37	Benzene, octyl- 1-Phenylo
17.45	Ethyl P-Methyl-Cinnamate
17.51	5-methyl-2-oxatricyclo[6.5.0.0(4,8
17.58	Phenol, 2-methyl-5-(1-methylethyl)
17.63	1-Hexadecene
17.68	1,1'-Biphenyl, 4-methyl-
17.74	Pentadecane
17.80	2,3-dihydro-1H-cyclopent[e]azulene
17.84	Nonadecane
17.95	Naphthalene, 2-(1-methylethyl)-
18.02	3-Isopropyl-2-(1-Pyrrolidinyl)-1-C
18.06	Bicyclo[2.2.1]heptane-2exo,3endo-d
18.12	Dibenzofuran Dibenzo[b,d]
18.21	Naphthalene, 2,3,6-trimethyl-
18.28	Naphthalene, 1,4,6-trimethyl-
18.31	Decane, 5-propyl-
18.37	Pentadecane, 5-methyl-

18.44	Pentadecane, 4-methyl-
18.51	1,4,6-Trimethylnaphthalene
18.61	Pentadecane, 3-methyl-
18.67	Benzene, dodecyl- 1-Pheny
18.69	Naphthalene, 2,3,6-trimethyl-
18.72	Benzene, (1,3-dimethylbutyl)-
18.78	1,1'-Biphenyl, 2,2'-dimethyl-
18.86	1-Nonadecene
18.95	Hexadecane
19.00	Naphthalene, 1,4,6-trimethyl-
19.09	1,1'-Biphenyl, 4,4'-dimethyl-
19.18	Benzene, 1,1'-methylenebis-
19.22	1,5,5-Trimethyl-4-phenyl-cyclopent
19.26	2,3-dihydro-1H-cyclopent[e]azulene
19.39	3,4-Dihydropyrrolo[1',2':3,4]pyrim
19.47	Hexadecane, 7-methyl-
19.51	Docosane, 6-methyl-
19.55	1,1-Dicyano-2-Methyl-3-Phenylprope
19.62	Hexadecane, 4-methyl-
19.68	Dibenzofuran, 4-methyl-
19.77	Hexadecane, 3-methyl
19.82	6-Azaspiro[2.5]octa-4,7-diene, 6-a
19.85	Oxirane, hexadecyl- 1,2-Epoxyoc
19.88	Benzene, decyl-
19.95	Naphthalene, 1-methyl-7-(1-methyle
20.00	4-tert-butyl-1,2-benzenedithiol
20.10	N-Nonadecane
20.17	Heptadecane, 2,6-dimethyl-
20.27	9H-Fluorene, 1-methyl- 1-
20.30	9H-Fluorene, 2-methyl-2-
20.38	Methylfluorene
20.47	2-N-Decyl-2,3-Dihydroindene 1H-
20.50	Methylfluorene
20.57	Dodecane, 2-methyl-6-propyl-
20.62	3-Chloro-2H-1-benzopyran-2-ol
20.67	1,1-Dicyano-2-Methyl-4-(P-Methyphe
20.73	3-(1-methylpyrrol-2-yl)indole
20.79	Heptadecane, 2-methyl-
20.83	1,1-Dicyano-2-Methyl-4-(P-Methyphe
20.89	Benz[f]indan-1,2,3-trione 2-oxime
20.96	2,5,6,7-Tetrahydrophenaleno[1,9-bc
21.03	Benzene, undecyl-
21.10	Benzene, chlorotriethyl-
21.20	Octadecane
21.23	**Phenanthrene**
21.30	triisopropylvinylsilane
21.34	Anthracene
21.45	Pentadecanoic acid
21.56	9H-Fluorene, 2,3-dimethyl-
21.63	Pentadecane
21.67	Methyl (E)-3-(4-chorophenyl)acryla
21.72	Pentadecane, 5-methyl-
21.80	Octadecane, 4-methyl-
21.84	Octadecane, 2-methyl-
21.94	Methyl 2-N-Pentyl-1-Cycloheptene
22.05	(E)-1-(4-methylphenyl)-2-phenyleth
22.12	Benzene, dodecyl- 1-Pheny
22.18	9(10H)-Anthracenone
22.23	Nonadecane
22.36	9(10H)-Anthracenone
22.43	Anthracene, 2-methyl-
22.59	**Methyl-Phenanthrene**
22.64	Nonadecane, 9-methyl-
22.70	Anthracene, 9-methyl-
22.81	Heptadecane
22.88	Hexadecanoic acid
22.95	Nonadecane, 3-methyl-
23.01	1,1'-dimethoxyferrocene
23.08	4-(2-Phenylvinyl)pyridine, trans
23.11	Benzeneacetonitrile, .alpha.-pheny
23.19	[2,2](9,9)Anthraceneophane

23.23	Eicosane
23.34	1-Methylamino-2-Azafluoren-9-One
23.40	Benzene, 1,1'-(fluorocyclopropylid
23.43	**Phenanthrene, 4-methoxy-**
23.48	2-tert-butyl-4,5-diphenyl-1H-imida
23.60	Heptadecane
23.66	**Phenanthrene, 2,5-dimethyl-**
23.71	**Phenanthrene, 2,7-dimethyl-**
23.78	Syn-1(6),8(13)-Dimethano-[14]Annul
23.85	**Phenanthrene, 4,5-dimethyl-**
23.91	(+)-.Beta.-Selinene
23.97	Sulfur
24.04	1H-Pyrazole, 1-(4-chlorophenyl)-4
24.12	Fluoranthene
24.17	N-Nonadecane
24.23	11H-indolo[3,2-c]quinoline 3,4-
24.39	5.beta.-androstane-3.alpha.,5,17.b
24.53	Docosane
24.64	Pyrene
24.75	Octadecanoic acid
24.83	Benzo[b]naphtho[2,3-d]furan
24.96	Nickel, Cyclopentadienyl-(4,4-dime
25.15	4-Bromo-3,5-dimethylbenzylidene di
25.19	Nickel, Cyclopentadienyl-(4,4-dime
25.25	11H-Benzo[b]fluorene 2,3-
25.31	**Phenanthrene, 2,3,5-trimethyl-**
25.35	trans-phenyl(2-phenylcyclopropyl)m
25.40	Heneicosane
25.50	11H-Benzo[b]fluorene 2,3-
25.56	5,6-dihydro-2-butyl-4,6-dimethyl-4
25.65	11H-Benzo[b]fluorene 2,3-
25.74	Pyrene, 1-methyl-
25.81	7-Exo-Phenyl-2,3-Benzonorcaradiene
25.89	6-chloro-11,11-dimethyl-10,11-dihy
25.95	Tricosane
26.03	Pyrene, 1-methyl-
26.11	Loganin aglycone
26.18	5H-phenanthro[4,5-bcd]pyran-5-one
26.24	Nonadecane
26.33	1,1':2',1"-Terphenyl o-T
26.36	Chrysene, 5-methyl-
26.43	7-methyl-2,4-diphenylquinoline
26.48	2,3-dimethoxy-5-phenyl-1,4-benzene
26.54	(z,z)-3-methyl-3h-cyclonona[def]bi
26.59	Benzaldehyde, 2-methyl-, (2,4-dini
26.63	Ferrocene, (3-hydroxypropyl)-
26.78	Tetracosane
26.89	1,1':2',1"-Terphenyl o-T
26.94	Pyrene, 1,3-dimethyl-
27.00	1,1':2',1"-Terphenyl o-T
27.07	1-Chloro-2-Dimethylamino-2-Methylt
27.15	Dihydrolinderalactone 4,7-Metha
27.20	3-Cyano-2-methyl-4-(methylthio)-6-
27.24	7H-Benz[de]anthracen-7-one
27.29	Ethyl 4,7-diphenyltriazolo[4,5-c]p
27.35	4-(1,1'-bibicyclo(2.2.2)octyl)yl m
27.38	N-Eicosane
27.42	2,2'-Spirobi-(s-hydrindacen)-1-one
27.45	3-(4-Methoxy-3-tert-butyl-5-methyl
27.47	trans-3,4-Dihydro-2,2-dimethyl-6-n
27.51	Benzene, (2,4-cyclopentadien-1-yli
27.61	Hexadecane
27.69	Triphenylene
27.77	Benzene, tridecyl-
27.79	Naphtho[2,1-b]furan-4-carboxylic a
27.84	Benzo[1,2-b:3,4-b']bisbenzofuran
27.92	Heneicosane
27.99	Methyl (3',6'-dioxocyclohexa-1',4'
28.02	Propane, 2-chloro-1,1,1,3,3,3-hexa
28.13	1,2-Benzenedicarboxylic acid, bis
28.20	6-Chloro-4-phenyl-1,4-dihydroquino

28.22	2-(5-hydroxybenzofuran-3-yl)-4-hyd
28.31	Benzopyrido (2,1-A)Isoindole
28.34	2,-dimethyl-1,1':3,1"-tetrphenyl
28.37	**1-Phenanthrenol, 1,4,4a,4b,5,6,7,8**
28.41	1a,12b-Dihydrobenzo[c]phenanthren
28.43	(+-)Heritol
28.45	3-Formyl-18(19)-norabieta-2,8,11,1
28.57	Tetracosane
28.63	Triphenylene, 2-methyl-
28.73	2-[2'-oxo-4'-(3,4-dimethoxyphenyl)
28.84	Triphenylene, 2-methyl-
28.95	Chrysene, 1-methyl-
29.06	4-(N-(4-chlorophenyl)amino)-5,6-di
29.09	Benz[a]anthracene, 11-methyl- (
29.12	Benzo[b]naphtho[2,3-d]thiophene, 6
29.21	Heptadecane
29.23	**Benzo[c]phenanthrene, 5,8-dimethyl**
29.25	Chrysene, 5-methyl-
29.28	5.alpha.-Cholest-22-en-16.beta.-ol
29.33	Cholestan-6-one
29.35	benz[e]acephenanthrylen-3a-(1h)-ol
29.38	benzylidenetriphenylphosphorane-bo
29.40	[1,2'-Binaphthalene]-5,5',8,8'-tet
29.44	2-(4-Methoxy-2-(trimethylsilyl)phe
29.48	5.beta.-Cholestane
29.58	2,2'-Binaphthalene
29.64	Debromoisoaplysin-20
29.70	Nonadecane
29.74	2-formyl-8-isopropyl-peri-xantheno
29.82	2,6,10-Trimethylundecan-(5E)-2,5,9
29.86	Piperazine, 1-methyl-4-(1,2,3,4-te
29.92	4-Hexen-2-yn-1-one, 1-phenyl-5-(1-