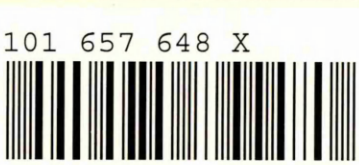

The Analysis of Salt Resistant Surfactants Used in Enhanced Oil Recovery

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A thesis submitted in partial fulfilment of the Requirements of Sheffield Hallam University for the degree of Doctor of Philosophy

June 2001

Abstract.

Commercial nonylphenol ethoxysulphonate (NPEOS), octylphenol ethoxysulphonate (OPEOS) surfactant formulations and mixtures of alkyl aryl sulphonate and NPEOS surfactant formulations, are used for enhanced oil recovery (EOR). The surfactants have been analysed by liquid chromatography (LC), liquid chromatography-mass spectrometry (LC-MS) and matrix assisted laser desorption ionization mass spectrometry. Mixed-mode C18/SAX and C8/SAX columns were used for both liquid chromatography and liquid chromatography-mass spectrometry analyses of NPEOS and OPEOS. NPEOS and alkyl aryl sulphonate surfactant mixtures were separated using a mixed-mode C4/SAX column.. Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) spectra were obtained using either alpha-cyano-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid as matrix with the addition of lithium chloride to simplify the mass spectra obtained. Data obtained from each method indicate that the NPEOS formulation has an ethoxymer chain length ranging from 2-13 units with average of 6.26. This is in broad agreement with earlier studies, although the range was reported as 2-15. However, the data obtained suggest that the OPEOS formulation has an ethoxymer chain length ranging from 1-8 ethoxymer units with an average chain length of 3.67. This is in contrast to earlier studies carried out by LC only, which suggested that the chain length ranged from 2 to 6 ethoxymer units with an average of 3.6. A method for the extraction of NPEOS and OPEOS from sea-water and reagent water, and alkyl aryl sulphonate from sea-water only, using graphitised carbon black (GCB) solid phase extraction (SPE) cartridges has also been developed.

In the last section of this thesis the chemical oxidation of NPEOS used Fenton's reagent and biological oxidation of NPEOS using a microorganism (*Paracoccus halodenitrificans*) is reported. The intermediate products formed in the chemical oxidation have been identified and characterized by LC, LC-ES-MS and MALDI/MS techniques. The major products formed are dicarboxylic acids and single carboxylic acids. The aerobic biodegradation of NPEOS was carried out over five days. The

oxygen uptake was measured each day. The biodegradation intermediate products were analysed by LC and data indicate that the same products were formed as those from the chemical oxidation of NPEOS.

In the Name of God, Most Merciful, Most Compassionate

The author wishes to express his sincere gratitude to Dr. Malcolm Clench for his supervision, constant advice, encouragement and support throughout this project. A special thanks go to Professor David Allen for his scientific advice and encouragement. I would also like to thank the technical staff within the Divisions of Chemistry and Biomedical Science at Sheffield Hallam University. A special thank you to Matthew, Maria, Peter, Edward, Marinela, Anna and Michael for their friendship and valuable support.

I would like also to gratefully acknowledge to the Libyan Petroleum Research Centre (PRC) for offering the sponsorship which has made this work possible.

My gratitude also goes out to my family and my friends. Special thanks go to my parents who have supported me, with words of encouragement, throughout my education and I would not have made it this far without them.

To : my parents,

my wife,

my daughters Noha , Nihad and

my son Mohammed.

Table of Contents

Glossary of terms used in this thesis

ABS = alkylbenzene sulphonates.

ACN = acetonitrile.

AE = alcohol ethoxylate.

APCI = atmospheric pressure chemical ionisation.

APEC = alkylphenol carboxylic acid.

APEO = alkylphenol ethoxylate.

APEOS = alkylphenol ethoxylatesulphonate.

API = atmospheric pressure ionisation.

BiAS = bismuth active substance.

BOD = biochemical oxygen demand.

C_{18} = octadecyl silyl- a stationary phase used in HPLC columns.

CO_2 = carbon dioxide.

CI = chemical ionisation.

CMC = critical micelle concentration.

CTAS = Cobalto Thiocyantate Active Substance.

ECI = electron capture detector.

EI = electron impact ionisation.

EM = emission wavelength.

EOR = enhanced oil recovery.

EX = excitation wavelength.

FAB = fast atom bombardment.

GC = gas chromatography.

GC/MS = gas chromatography/ mass spectrometry.

GCB = graphitised carbon black.

HFBA = heptafluorobutyric anhydride.

HPLC = high performance liquid chromatography.

IFT = interfacial tension.

LAEC = linear alkylphenoxy carboxylic acid.

LAS = linear alkylbenzene sulphonates.

LC/MS = liquid chromatography/ mass spectrometry.

LC_{50} = the "Lethal Concentration" of a substance at which 50% of species die.

LD_{50} = lethal dose.

m/z = mass to charge ratio.

MBAS = Methylene Blue Active Substance.

MS = mass spectrometry.

MTBE = methyl-tertiary-butyl ether.

NCI = negative chemical inoisation.

NOEC = no observed effect concentration.

NP = nonylphenol.

NPEC = nonylphenol carboxylic acid.

NPEO = nonylphenol polyethoxylate.

ODE = oil displacement efficiency.

OECD = organisation for economic cooperation and development.

OPEO = octylphenol ethoxylate.

PB = Particle Beam interface.

PFBCI = pentafluorobenzyl chloride.

ppb = parts-per-billion.

PPAS = potassium picrate Active Substance.

ppm = parts-per-million.

RMM = relative molecular mass.

RSD = relative standard deviation.

SAX = strong anion exchange.

SDS = sodium dodecyl sulphate.

SPC = sulphophenyl carbxylate.

SPE = solid phase extraction.

STPs = sewage treatment plants.

TIC = total ion chromatography.

TMS = trimethyl sily-a stationary phase for HPLC.

TSP = thermospray interface.

UV = ultra-violet light.

VSD = volumetric-sweep efficiency.

CHAPTER ONE

Enhanced Oil Recovery

1.0 Petroleum History

All over the world, at various depths beneath land and sea, there are accumulations of crude oil formed long ago by the decomposition of animal and vegetable remains. Crude oil is an extremely complex and variable mixture of compounds containing thousands or tens of thousands of different chemical species. Many are hydrocarbons varying from simple aliphatics and aromatics to complex, multi-ring structures of high molecular weight. However, within crude oil these are also compounds which contain sulphur, nitrogen, oxygen and other elements. The proportion of these many components varies widely in different deposits: generally, the higher the content of short chain compounds the lighter and more mobile the crude. Viscous heavy oils, and even more so bitumens, are deficient in the light fractions, possibly reflecting the extent of *in-situ* maturation or the result of their loss over geological time by evaporation or microbial action. Some components of crude oil are known to be susceptible to microbiological attack: they tend to be the lower molecular weight materials and an attack on pure hydrocarbon always seems to require the involvement of molecular oxygen. A few bacterial species may be capable of anaerobic metabolism on compounds containing elements additional to carbon and hydrogen but not, it seems, on pure hydrocarbons. Viscosity is of course, highly dependent on temperature so that the effective mobility of the crude oil in the reservoir is the result both of its composition and of the local temperature. The physical properties of the oil in any particular location thus reflect its history as well as its environment.

In its widest sense, the term "petroleum" embraces all hydrocarbons occurring naturally in the earth. However, in its narrower, commercial sense, "petroleum" is usually restricted to the liquid deposit i.e. crude oil, gaseous products being termed "natural gas" and the solid ones "bitumen" "asphalt" or "wax" according to their composition. Another type of deposit "oil sands" is unconsolidated sandstone deposit containing very heavy crude oil termed bitumen. Bitumen is chemically similar to conventional crude oil but has a greater density and a much greater viscosity.

Accumulations of crude oil are found throughout the world in the interstices of porous sandstone and within fine fractures and pores of limestone and chalk (Figure1.1). Reservoirs occur at all depths, from the surface to the limit of contemporary drilling technology at several kilometers and presumably beyond: the environmental conditions within each reservoir will naturally reflect its location. Temperature is directly related to depth although the thermal gradient does vary in different regions. Before the extraction of oil begins, reservoirs are commonly under considerable pressure, largely from dissolved gas but often with a contribution from an underlying aquifer which communicates ultimately with surface water and is thus subject to a hydrostatic head of pressure corresponding to depth.

Most crude oils, in fact, contain gaseous and solid hydrocarbon in solution. The gases come either out of solution on the release of pressure as the crude oil is produced or during the first stage of refining. This contributes to total natural gas production. Some of the solids are recovered during refining as bitumen and wax, some stay in solution in the

liquid oil products. Natural gas is also found associated with crude oil as a gas cap above the oil or on its own, unassociated with oil.

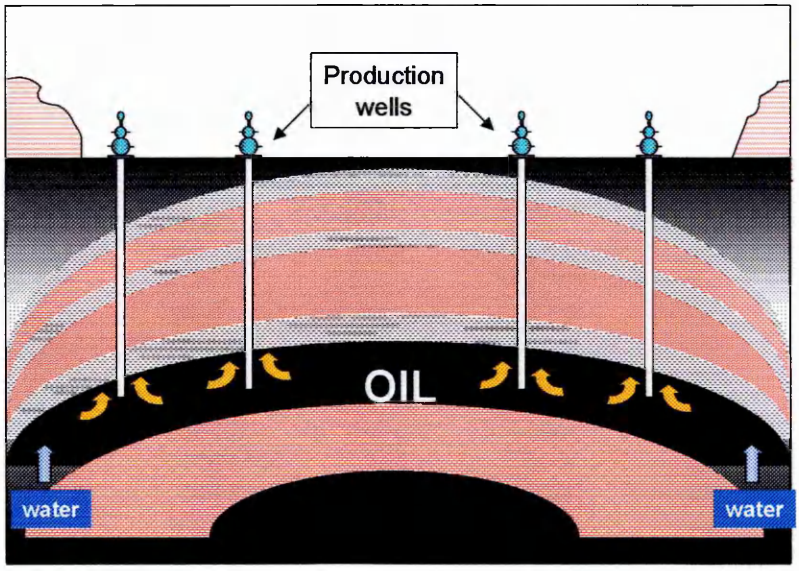

Figure 1.1 Water-driver reservoir

Crude oil and natural gas are the raw materials of the petroleum industry. It is the business of the industry to find them, to win them from the ground, to manufacture technically useful products from them and to sell the products in the markets of the world.

The origins of the modern petroleum industry lie in the rapid growth in the demand for artificial light that occurred early in the nineteenth century. Buildings were more spacious and were used more for leisure activities. Factories with their new expensive machinery had to work night shifts. Railroads and steamboats needed light to run after dark. An

important part of the rapidly increasing demand for light was supplied by gas made from coal. The fact that a combustible gas can be produced by heating coal was known as early as 1700, but the first practical use of coal gas was by William Murdoch in Birmingham, England, about 1800. In 1816 the use of coal gas was common in London and by 1825 it was used for lighting streets in most of the large cities of the U.S. However, it had been noticed early that the manufacture of gas from coal resulted in the formation of some condensable liquids, which were oily and could be used for illumination. The first practical manufacture of illuminating oil from mineral source was by James Young of Manchester who patented his process in England in 1850. His process was originally the fractional distillation of petroleum, although he later made oil from a type of oil shale.

However, the modern era of oil production is generally considered to have begun on Aug. 27,1859 in Titusville (Pennsylvania) when Edwin L. Drake discovered crude oil and drilled the first well to be sunk specifically for oil. He struck oil at 69.50 ft and production ($1.6m^3/d$) began. Within the year, a further 175 oil-wells had been drilled in Pennsylvania, and within two years other wells were drilled that produced thousands of barrels per day [1] The search for petroleum spread rapidly to other parts of North America. Although this is generally taken as the start of the modern petroleum industry, small quantities of oil were being produced in Russia by 1856 and in Romania by 1857. Developments followed in other countries and by the year 1900, the world annual output of the crude oil was 141x 10^6 barrels, from 11 countries [2], and production was averaging just over 40, 000 barrels a day (as shown in Table 1.1).

Country	Barrels/day
Russia	206310
USA	173830
East indies	9090
Poland	6410
Romania	4920
India and Burma	2950
Japan	2380
Canada	1940
Germany	980
Peru	830
Italy	30
Total	409670

Table 1.1 World production of petroleum in 1900 [2].

By 1920, in spite of the devastation caused by World War I, world output had increased to 695×10^6 barrels. By 1946, just after the end of World War II, world output had increased to $2{,}750 \times 10^6$ barrels. In the years that have elapsed since then, the output of crude oil has continued to increase at an average annual rate of growth of more then 7%, equivalent to the doubling of production every ten years. By 1973, commercial oil production was being obtained from 62 countries and totaled 19.9×10^9 barrels, roughly 140 times the output in 1900. However, it is interesting to note that, since man-made frontiers have little relationship to the geological factors which control the size and productivity of individual oil-fields, 85% of the total output comes from only 12 countries [2]. World production capacity by region and country in 1999 was as shown in Table 1.2.

Country	MM Brl	% of total
Indonesia	1,207.1	1.9
United Arab Emirates	2,048.8	3.2
Saudi Arabia	7,564.7	11.8
Iran	3,439.0	5.4
Venezuela	2,800.4	4.4
Kuwait	1,872.7	2.9
Libya	1,287.2	2.0
Nigeria	1,939.8	3.0
Qatar	608.5	0.9
Iraq	2,719.8	4.3
Algeria	749.6	1.2
OPEC	26,237.7	41.0
North America	7,268.6	11.4
Latin America	9,213.0	14.4
Eastern Europe	7,606.1	11.9
Western Europe	6,141.2	9.6
Middle East	20,289.0	31.7
Africa	6,517.9	10.2
Asia and Far east	6,286.0	9.8
Oceania	634.9	0.9
World Total	**63,956.8**	**100**

Table 1.2 Principal Oil-Producing Countries and Regions, 1999 [3].

1.1. Oil Recovery

Oil recovery, traditionally, has been subdivided into three stages (Figure1.2): primary, secondary, and tertiary. These stages describe the production from a reservoir in a chronological sense.

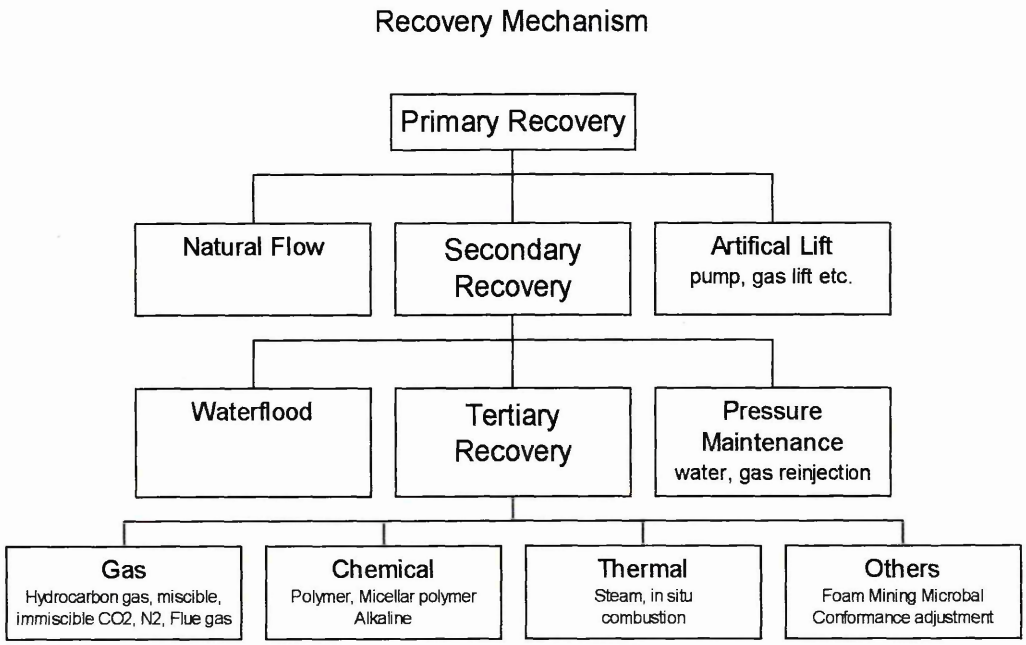

Figure 1.2. Recovery Mechanism

1.1.1 Primary Oil Recovery

Oil is produced using the natural reservoir energy to drive the oil through the complex pore network to the producing well by three main mechanisms :-

- Expansion of the oil, gas (if present), connate water and the compaction of rock as the pressure falls.

- As the reservoir pressure falls to below the bubble point during production, some of the more volatile components are released and come out of solution as small gas bubbles. The bubbles are initially trapped in the pores and displace oil as they expand (solution gas drive). As the pressure falls further the individual bubble increase in size, join together and the gas can begin to flow. Depending on the vertical permeability some of the gas may segregate to the gas cap at the top of the reservoir, or if no gas cap is present, it forms a secondary gas cap. This gas cap can expand, and in doing so displace more oil (gas cap drive).
- If the oil reservoir is part of a much larger aquifer system, substantial quantities of water may flow into the oil zone, so displacing oil

The pressure is usually high enough initially to lift the oil up the producing wells to the surface, but as oil is produced, the reservoir pressure declines and the rate of oil production falls. Production can be maintained for a time by these primary mechanisms by pumping the production wells, but the recovery factor is small unless there is good aquifer drive, and most of the oil remains in the reservoir.

1.1.2. Secondary Oil Recovery

When the natural pressure decreases, energy must be supplied to the reservoir in order to recover some of this residual oil. This extra energy can be introduced by injection of water or gas or by supplying heat. This stage of production is termed secondary oil recovery, or more specifically water flooding, gas injection or thermal methods. Thermal

methods would be in the context of secondary recovery for viscous heavy oils since increasing the oil's temperature lowers its viscosity.

Powered water flooding is very common nowadays. For example, the majority of North Sea fields have such facilities on their platforms, and also around 50% of the current US annual production is aided by water flooding. It moves oil to the production wells by keeping the reservoir pressure usually to about that of bubble point. This ensures that no gas blocks the pores. Also at the bubble point, the hydrocarbon thermodynamics dictate that the oil will have its lowest viscosity and largest formation volume factor, so that the most oil will be displaced under the smallest pressure gradients

1.1.3. Tertiary Oil Recovery

Primary and secondary crude oil recovery processes frequently leave substantial quantities remaining in place in the reservoir. Estimates range from 50-70% [4] of the original reservoirs. During secondary recovery, continuous water injection results in ever increasing production of water and eventually only injected water is produced, leaving behind "residual oil". The oil remaining after water flooding is retained in the pore space of reservoir rock at lower concentration than original and exists as ganglia trapped in individual pore clusters. It may also remain as films partly coating the pore walls or as a continuous phase in the pores which were not invaded during water flooding. Capillary and viscous forces are mainly responsible for the retention of residual oil. Capillary forces dominate in water-wet reservoirs. The residual oil becomes discontinuous and forms ganglia because of high interfacial tension between oil and water. Since the oil droplet diameter is larger than the pore throat, oil does not move towards the producing well.

Viscous forces may prevent oil movement and injection fluid with low viscosity overtakes. The relation between capillary and viscous forces is given by capillary number which varies directly as the viscosity of the injected fluid and inversely as the interfacial tension between the injected fluid and the reservoir fluid.

The extent of oil recovery by injection fluid is also dependent upon (a) the fraction of oil in the reservoir that is contacted by the fluid, called volumetric-sweep efficiency (VSE), (b) the fraction of oil that is displaced from pores invaded by the fluid, called oil displacement efficiency (ODE). Total oil recovery efficiency is controlled by the product VSE x ODE.

1.2. Enhanced Oil Recovery

Enhanced oil recovery (EOR) refers to any method used to recover more oil from a reservoir than would be produced by primary recovery [5]. The development of enhanced oil recovery (EOR) processes has been ongoing since the end of world war II, when operators who owned reservoirs with declining reserves recognized that significant quantities of oil remained in their reservoirs after primary and secondary recovery (primary water flooding). Research and field activity increased as production from major reservoirs declined, worldwide consumption of oil increased, and discoveries of major new reservoirs became infrequent. Intense interest in EOR processes was stimulated in response to the oil embargo of 1973 and the following energy "crisis". The collapse of oil prices in 1981 has resulted in significant rapid changes in EOR field technology, development and field testing.

Worldwide production from EOR projects at the start of 1994 remained about 1.9 million barrels per day ($1.9x10^6$ brl/d) or about the same as at the beginning of 1992. The $1.6x10^6$ brl/d represents about 3.2% of the worlds oil production. In 1994, US, EOR production of 709,000bbl/d represented about 10% of the total production [6].

1.2.1 Enhanced Oil Recovery (EOR) Methods

The intent of enhanced oil recovery methods [7] is to:-

- Improve sweep efficiency by reducing the mobility ratio between injected and in-place fluid.
- Eliminate or reduce the capillary and interfacial forces and thus improve displacement efficiency.
- Act on both phenomena simultaneously.

Overall, these three effects lead to an increase in the efficiency of oil recovery.

Oil recovery mechanisms are dependent on two principles: increasing volumetric sweep efficiency of the injected fluid and increasing oil displacement efficiency by injected fluid. In both, chemicals are used to modify the properties of an injected fluid whether water, steam, a miscible gas such as CO_2 or natural gas, or an immiscible gas, usually nitrogen. Poor reservoir volumetric sweep efficiency is the greatest obstacle to increasing oil recovery [8].

Wettability is defined as the tendency of one fluid to spread on or adhere to a solid surface (rock) in the presence of another immiscible fluid [9]. As many as 50% of all sandstone reservoirs and 80% of all carbonate reservoirs are oil-wet [10]. Strongly water-wet

reservoirs are quite rare [11]. Rock wettability can affect fluid injection rates, flow patterns of fluid within the reservoir, and oil displacement efficiency [11]. Rock wettability can strongly affect its relative permeability to water and oil [9,12]. When a rock is water-wet, water occupies most of the small flow channels and is in contact with most of the rock surfaces as a film. Crude oil does the same in oil-wet rock wettability, hence adsorption of polar material, such as surfactants and corrosion inhibitors, or adsorption of polar oil crude oil components [13], can strongly alter the behavior of the rock [12].

When water is injected into a water-wet reservoir, oil is displaced ahead of the injected fluid. Injection water preferentially invades the small-and medium-sized flow channels or pores. As the water front passes, uncovered oil is left in the form of spherical, unconnected droplets in the center of pores or globules of oil extending through interconnected rock pores. In both cases, the oil is completely surrounded by water and is immobile. There is little oil production after injection water breakthrough at the production well [9]. In an oil-wet rock, water resides in the larger pores, oil exists in the smaller pores or as a film on flow channel surface. Injected water preferentially flows through the large pores and only slowly invades the smaller flow channels, resulting in a higher produced water/oil ratio and a lower oil production rate than in the water-wet case.

Chemical methods of enhanced oil recovery are characterized by the addition of chemicals to water in order to generate fluid properties or interfacial conditions that are more favorable for oil displacement. Polymer flooding, using polyacrylamides or

polysaccarides, is conceptually simple and inexpensive, and its commercial use is increasing despite the fact that it raises potential production by only small amounts. Surfactant flooding is complex, requiring detailed laboratory testing to support field project design. Addition of surfactants to the injection water [14,15] can displace the oil remaining near the well (Figure 1.3). The concentration of surfactant in the injection water is relatively high (1–3%). However, the total amount of surfactant used is not great because it is necessary only to displace the oil from a 2–3 m radius around the injection well. It is also expensive and is used in few large-scale projects. Alkaline flooding has been used only in those reservoirs containing specific types of high-acid-number crude oils.

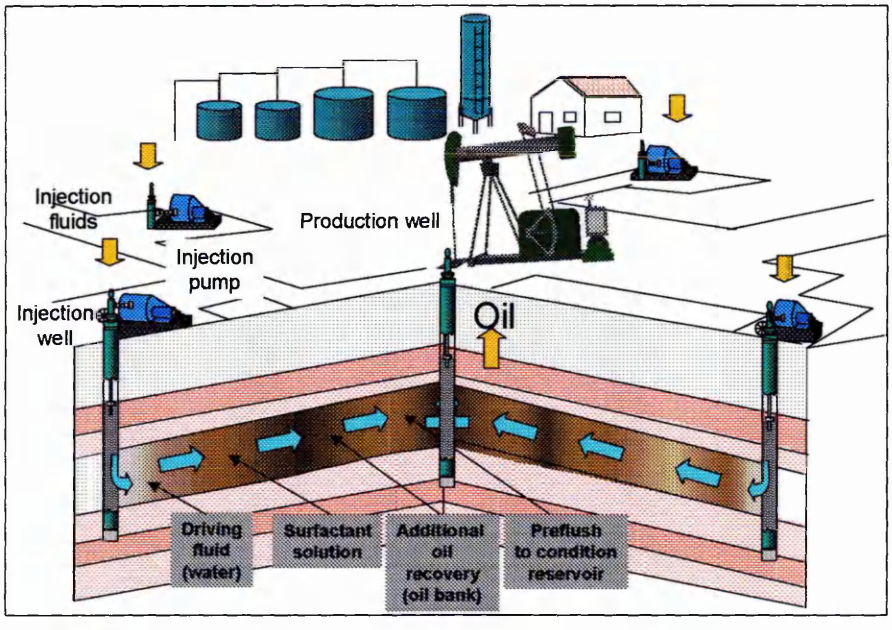

Figure 1.3. Surfactant process

Miscible methods have the greatest potential for enhanced oil recovery of low-viscosity oils. Among these methods, injecting gases miscible with reservoir crude oil can result in low interfacial tension promoting a high oil displacement efficiency [16]. The process of miscible gas flooding using carbon dioxide is depicted in Figure 1.4. Other suitable gases include natural gas and flue gas. Carbon dioxide is of most interest in the United States; hydrocarbon miscible projects represent 80% of Canadian EOR production [5]. Supercritical CO_2 [16] and various hydrocarbon injectants [16,17] undergo physical interactions with crude oil that result in stripping out of the low molecular weight components, which increases oil production. The rapid or gradual development of miscibility with remaining crude oil constituents results in oil mobilization. Either partial or complete miscibility with the oil may be developed, depending on the nature of the injectant, crude oil properties, and reservoir conditions, particularly temperature. However, interaction of the injectant with the crude oil can alter rock wettability and thus reduce injection rates and decrease oil recovery.

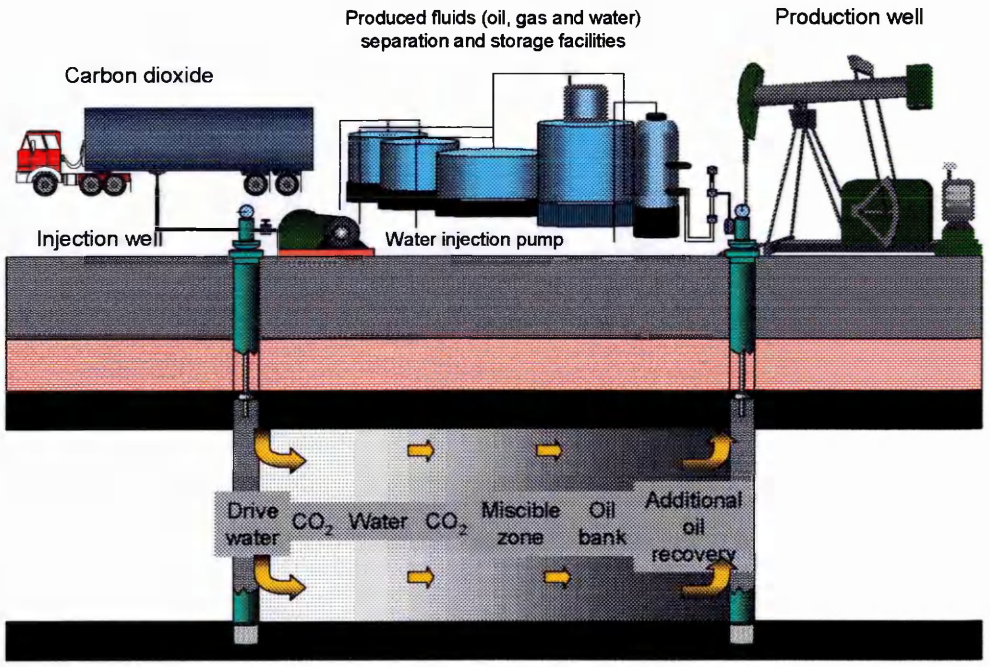

Figure 1.4. Carbon dioxide miscible process

Another method of using CO_2 is called cyclic CO_2 stimulation or "huff' n' puff" [18]. A limited amount of CO_2 is injected into a reservoir over hours or days. The well is then shut in for a soak period of day to weeks to allow the CO_2 to interact with the crude oil, swelling the oil and reducing its viscosity. The well is then opened, the CO_2 provides a solution gas drive, and oil mobilization by the CO_2 soak is produced. Nonmiscible gases such as nitrogen have also been used as EOR injection fluids.

Thermal methods provide a driving force and add heat to the reservoir to reduce oil viscosity and / or vaporize the oil. This makes the oil more mobile, so that it can be more

efficiently driven to producing wells. In heavy oil fields, water flooding is often omitted and steam injection is begun immediately after primary production (Figure 1.5). Steam injection temperatures of typically 175-230 °C have been used in California oil fields since the early 1960s, whilst injection temperature can reach 300 °C in Canadian and Venezuelan EOR projects. The performance of steam injection EOR can be estimated with less uncertainty than other methods. *In-situ* combustion is normally applied to reservoirs containing low-gravity oil, but has been field tested under a wide variety of reservoir conditions. Only a few projects have proven economical enough to advance to a commercial scale. To date, in situ, combustion has been most effective for the recovery of viscous oils in moderately thick reservoirs. The basic principles of the most promising EOR methods used are given in Table 1.3.

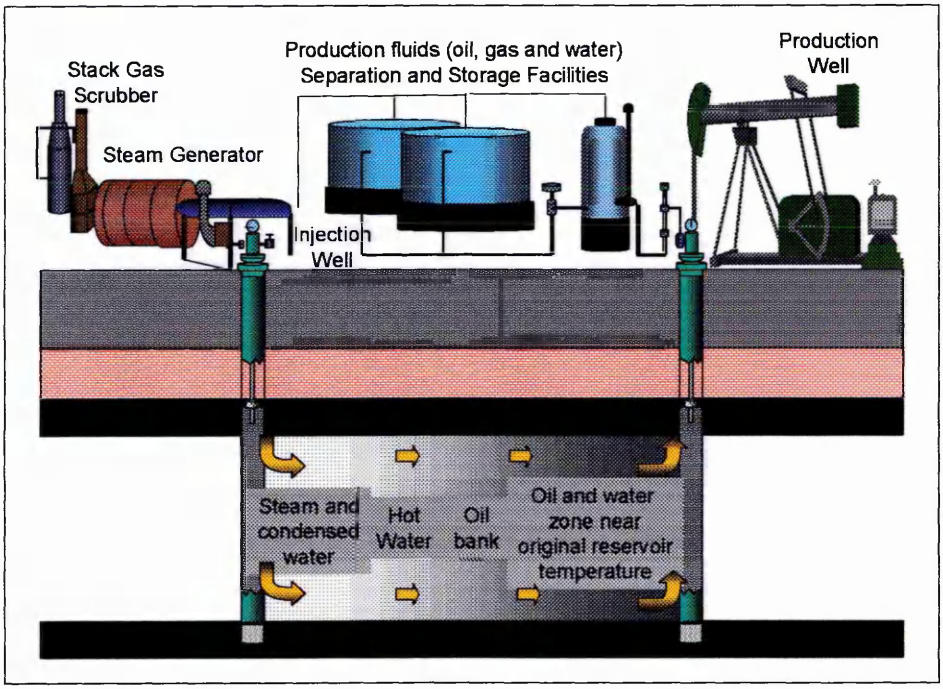

Figure 1.5. Steam flooding process

	Method Used	Basic Principle
Chemical methods	Polymer-augmented water flooding, surfactant flooding; alkaline flooding; immiscible CO_2 displacement	Improvement of sweep efficiency, improvement of displacement.
Miscible methods	Miscible fluid displacement using CO_2, nitrogen, alcohol, LPG or rich gas dry gas.	Improvement of displacement efficiency.
Thermal methods	Cyclic steam injection; steam drive, in situ combustion.	Improvement of both sweep efficiency and displacement efficiency.

Table 1.3. Methods of enhanced Oil Recovery [7].

Other technologies for EOR also exist. Microbial-enhanced oil recovery (MEOR) involves injection of carefully chosen microbes. Subsequent injection nutrient is sometimes employed to promote bacterial growth. The main nutrient source for the microbes is often the crude oil in the reservoir. A rapidly growing microbe population can reduce the permeability of thief zones improving volumetric efficiency. Microbes, particularly species of Clostridium and Bacillus, have also been used to produce surfactant, alcohol, solvents, and gas in *situ* [19]. These chemicals improve water flooding oil displacement efficiency. Microbes adsorb and grow on reservoir rock surfaces fed injected nutrient [20] and may have application in plugging thief zones near injection well-bores. Controlling the rate and location of bacterial growth and chemical production can be difficult. Bacterial growth near well-bores has been a common problem causing reduced injection rates and productivity.

1.1 Surfactants in Enhanced Oil Recovery

Our dependence on oil has increased tremendously. Oil has no longer remained a dirty business but has become a business of billion of dollars. New fields are being explored and technologies are being developed to exploit the areas which were earlier inaccessible. At the same time, attempts are being made to economize the existing processes and to recover maximal crude oil from established and producing reservoirs. Chemicals are used in various operations of the oil industry. However, a particular class of chemical, the surface-active agents (surfactants), has found wide ranging application in petroleum industry. Drilling, work-over, primary, secondary and tertiary recovery and environmental production, all use surfactants in one or other form.

As previously noted surfactants are used in enhanced oil recovery. In other areas of petroleum production, they are used in;-

- Drilling fluids: emulsification of oils, to disperse solids, and to modify the rheological properties of drilling and completion fluids.
- Mist drilling: convert intrusion water to foam in air drilling.
- Work-over of producing wells: emulsify, and disperse sediment in clean-out of well, modify wetting of formation at producing zone.
- Producing wells: demulsify crude petroleum and inhibit corrosion of well tubing storage tanks, and pipeline.
- Secondary recovery in flooding operation, release crude oil from the formation surface, i.e. preferential wetting.
- Refined petroleum products-detergent, sludge dispersant, and corrosion inhibitor in fuel oil, crankcase oil and turbine oil.

In the primary recovery stage, when crude oil is produced, it is often accompanied by gas and water (brine). The three components i.e. gas, oil and water are separated before despatching crude oil to refineries. The gas can form a foam with crude oil in the presence of carboxylic acids and phenols of low molecular weight [21]. This can cause loss of valuable crude oil in gas. Defoamers are used to break foams and thus prevent loss of oil. Lower alcohols like propanol and butanol, organic polar molecules, sorbitan monolaurate, sorbitan trioleate, PLURONIC type non-ionic surfactants, polypropylene glycols and their derivatives, mineral oils blended with surfactants and silicones alone or blended with surfactants are used as defoamers [22]. Callaghan *et al* [21], in their laboratory studies,

observed that two defoamers, one a silicone glycol and another unsubstituted polydimethyl siloxane worked well with aged crude oil while with fresh oil, only the silicone glycol worked.

Water is generally produced along with crude oil in the form of a water-in-oil emulsion and this has to be broken down for various commercial and operation reasons. Asphaltenes, resins, wax, solid and metalloporphyrins stabilize such emulsions. Water has been encountered with oil since the beginning of the petroleum industry and since then all sorts of available chemicals such as soda, soap, phenols, fatty acids and their salts have been used to demulsify crude oil emulsion.

Water flooding is by far the most economical and widely practised of secondary recovery processes. Water injected through the injection wells supplements the depleted energy of the reservoir and drives oil towards the production well/wells. The interfacial tension (IFT) between crude oil and injected water is high and the viscosity of the injected water is low. Therefore, a major portion of oil is still retained in the reservoir. Much of this uncovered oil is in the form of discontinuous globules trapped in pore spaces. These discrete droplets cannot deform themselves due to high interfacial tension and are unable to pass through the pore throat. Surfactant addition to water can lower interfacial tension between oil and water and permit droplets to deform so that they can pass through pore channels and reach producing wells. Interfacial tension of the order of 10^{-2} mN/m is generally accepted to be desirable for good recovery by surfactant-aided water flooding.

Surfactant use for oil recovery was thought of even as early as 1920s when DeGroot [24] described water-soluble surfactants such as a polycyclic sulphonic compound and wood sulphite liquor in concentrations of 25 to 1000 ppm for use during water flooding. Laboratory tests have indicated that dilute surfactants solutions remove more oil than water alone [25,26]. Surfactants which have been used with some success in water flooding, either in the laboratory or in the field, include Igepal CA (as octyl phenyl ether, non-ionic) [27], sodium sulphoresinoleate, oleoglcerol sulphates, Gardinal WA (sodium lauryl sulphate), Igepon T (sodium N-methyl-N-oleoyl taurate, an anionic), Ninol 55 (an amide, non-ionic), a cationic condensation product of fatty acids and ethanolamines, and OT (dioctyl sodium sulphosuccinate, an anionic). Cox and Hancock [27] have patented a formulation of sulphated polyoxyethylene alcohol which can be used to enhance the efficiency of water flooding.

Amphoteric surfactants also find application in water flooding [28]. For example, Hydrocarbyl dimethyl ammonium propane sulphonate, and Hydrocarbyl dihydroxyethyl ammonium propane sulphonate

Downs and Hoover [29] used alkoxylated substituted phenol formaldehyde resin of relatively high molecular weight to recover additional oil during water flooding. This surfactant acted by wettability alteration. The loss of surfactant by adsorption and by interaction with reservoir rock had been considered a serious impediment to the process since the beginning. The surfactants also lose their effectiveness under reservoir

temperatures and salinity. Anionic surfactants may be precipitated by interaction with divalent cations such as, Ca^{2+}, Mg^{2+}.

In tertiary oil recovery, additives such as polymers and surfactants are also used. These help in a number of ways: (i) by lowering the oil/water interfacial tension: (ii) by the spontaneous emulsification of the oil; and (iii) by increasing the wettability of the oil-bearing rock. Overall, these three effects lead to an increase in the efficiency of oil recovery. The modification of the wetting characteristics of the oil-bearing rock by the addition of surfactants is a major area of research into enhanced oil recovery[30].

Much of the success of surfactant flooding depends on the ability of the surfactant to reduce oil-water interfacial tension (IFT) to very low values (10^{-2}- 10^{-3} mN/m). Whereas in laboratory experiments this can be accomplished quite easily with relatively small surfactant concentrations (as low as 0.1% by weight), in field applications, the efficiency of the surfactant is drastically reduced by interaction with reservoir rock (adsorption) and by interaction with reservoir fluids affecting oil-brine surfactant phase behavior[31]. The latter effect is especially important when the brine salinity is high and, particularly, when divalent ions (Ca^{2+}, Mg^{2+}) are present.

As a consequence of the above considerations, the following main properties must be taken into account when considering a surfactant for potential application in enhanced oil recovery:-

- Solubility in hard water

- Long-term chemical stability and resistance to bacterial attack.
- Sensitivity of phase behavior to the variation in salinity that may arise from interactions with rock surface and, in particular, clay-type minerals
- Sensitivity of phase behavior to the variation in surfactant concentrations.
- Adsorption onto reservoir rock.
- Cost.

Surfactant flooding can lower interfacial tension between oil and injected fluid to 10^{-3} mN/m or even lower. Thus they increase capillary number and oil displacement efficiency. Terms like micellar-microemulsion-detergent-surfactant-soluble oil-ultra low tension and chemical-flooding have been used to describe enhanced oil recovery by surfactants. The process involves injection of a micellar solution of oil, water, surfactant, co-surfactant (to modify solvency of surfactant) and salts into the formation and it is in turn replaced by a mobility control buffer solution, which in turn is displaced by injection water. Preflooding may or may not be needed. Oil and water are displaced ahead of the micellar slug and a stabilized oil and water bank develops. Micellar flooding can be low concentration or high concentration. In low concentration surfactant flooding, low surfactant concentration micellar solution is injected at large pore volumes (15-60%) and in high concentration surfactant flooding, surfactant concentration is high and pore volume is low (3-20%). The latter process is known to give better efficiency than the former.

Displacement by micellar solutions is in fact one of the most important tertiary recovery processes. This process is known in the petroleum industry by several names. Hill *et al*

[32], Larson *et al* [33], Shah *et al* [34], and Van Poollen [35], name the process as "surfactant flooding" . The term "micellar flooding" was used by Davis *et al* [36], Gogarty *et al* [37-40], Farouq ali *et al* [41], Gupta *et al* [42]. Sayyouh *et al* [43] and Trusenaki *et al* [44]. Based on interfacial tension criteria, Foster [45], and Bleakly [46], used the term "low tension water flooding". The term "microemulsion" was introduced by Healy *et al* [47-50]. Holm [51] used the term 'soluble oil flooding. Bleakly [46] and Danielson [52] named the process "Maraflooding". The "Maraflooding" process was first introduced by Gogarty and Tosch [53].

Oil displacement tests in water wet Berea sandstone cores containing residual crude oil flooded with water have shown that high tertiary recoveries can be obtained using the sodium salts of readily available carboxylic acids. Using a 10% pore volume surfactant slug containing 3.0% sodium isostearate and 3.0% isopentyl alcohol, followed by a polyacrylamide mobility buffer, resulted in a 92% tertiary oil recovery, which compares well with recoveries using petroleum sulphonates. Oil recoveries were highly dependent on pH and added base. Aliphatic C_{18} carboxylates give higher recoveries at lower pH using sodium bicarbonate as the added base (pH 8.5) rather than sodium hydroxide, sodium carbonate or sodium orthiosilicate (pH 11-13). In contrast, aromatic carboxylate e.g. sodium P(pentylnonyl) benzoate, gave higher recoveries at higher pH using sodium carbonate rather than sodium bicarbonate. Carboxylates with branched alkyl groups, e.g., isostearate, gave higher tertiary oil recoveries than unbranched carboxylates, e.g., oleate or stearate. Carboxylates were found to give good oil recoveries even when significant amounts of calcium ion were present [54].

Since the 1970s, when the oil supply shortage was felt all over the world, there have been many attempts at micellar flooding. Petroleum sulphonates, being cheap, were the first to be used in this process [55,56]. Surfactants considered practical for EOR applications have some water-solubility. Petroleum sulphonates are soluble because of the ionic sulphonate group SO^{-}_{3}. Petroleum sulphonate solutions have been found to produce low interfacial tension (IFT) under appropriate condition of surfactant and salt concentration [57-59]. However, the limitation of petroleum sulphonate is that the surfactant solutions become unstable and phase separation occurs when salt concentration is increased beyond 2 or 2.5% NaCl [60]. In general, petroleum sulphonates with high polysulphonate content are not good EOR candidates. Also, as a rule of thumb relative to solubility, Gale and Sandvik [61] state that petroleum sulphonates with equivalent weights above 450 are normally oil-soluble and not water-soluble. Lower-equivalent-weight sulphonates tend to be water-soluble. Later on, the emphasis shifted to more effective surfactants and since then various types of anionic and non-ionic surfactants have been used to improve oil displacement efficiency of the slug. A few such formulations are: alcohol ethoxylates, alkylphenols [62], ethoxylated sulphates, sulphonates or carboxylates [63-65], alpha-olefin sulphonates of general formula $R\text{-}CH{=}CH\text{-}(CH_2)_n\text{-}SO_3^{-}Na^{+}$ [66,67], alpha-sulphonated fatty acid esters [68], alkyl (or alkylaryl) ethoxylated sulphonates [69], alkylaryl alkoxy sulphate [70], alkylaryl ether sulphates [71], carboxymethylated ethoxylates [72], and branched carboxymethylated alcohol ethoxylates [73], ethylene oxide condensates with p-diisobutylene phenol, p-tripropylene phenol and p-n dodecyl phenol with mean degree of ethoxylation 3,5 and 8 [74], ethoxylated $C_{12\text{-}15}$ alcohols with 75% linearity, $C_{13\text{-}14}$ alcohols with 40% linearity and degree of ethoxylation 3,5 and 8 [74]. Increasing the length of an

ethoxy chain reduces critical micellar concentration (CMC) [75]. Cosurfactant requirement can be minimized by using a surfactant having a short-branched hydrophobe or branched (vs linear) alkyl substituent on an aromatic group [76]. Blends of surfactants optimized for sea-water or reservoir brine include linear alkyl xylene sulphonate/ alcohol ether sulphate mixtures [77]. Alpha-olefin sulphonates have been found to possess good salt tolerance and chemical stability at elevated temperature, and appear to exhibit good oil solubilization and low interfacial tension over a wide range of temperatures [78]. While being less salt tolerant, alkyl aromatic sulphonates exhibit excellent chemical stability. The nature of the alkyl group, the aryl group and ring isomer distribution produced in the Friedel-Crafts alkylation of the aromatic compounds can be adjusted to optimize surfactant performance under a given set of reservoir conditions. Ethoxylated alcohols have also been added to some anionic surfactant formulations to improve interfacial properties [79].

Neuman et al [80] have studied the following salt tolerant surfactants and found mixtures of then to be useful:-

1. Fatty alcohol polyethoxylated propylene sulphonates with formulae

   $C_{13}H_{27}/\ C_{15}H_{31}O\ (CH_2CH_2O)_xCH_2CH_2CH_2SO^-_3K^+$

   $i\text{-}C_{13}H_{27}O(CH_2CH_2O)_xCH_2CH_2CH_2SO^-_3K^+$

2. Nonylphenol polyethoxylated propylene sulphonates with formulae

   $C_9H_{19}\text{-}C_6H_4O(CH_2CH_2O)_xSO_3^-K^+$

   X=2,3,4,6,9.5

3. Petroleum sulphonates with mean molar masses in the range 416-496, active

substance 60% weight by weight and oil content 30%.

4 Nonylphenol ethoxymethylene carboxylates with EO = 6.

5. Fatty alcohol ethoxymethylene carboxylates with EO = 6.

6. Tributylphenol ether sulphonate, EO = 8.

Schmidt et al [81] used mixture of surfactants containing (a) ether sulphonates, (b) polyglycol ethers (c) primary or secondary C_{8-12} alkane sulphonates, petroleum sulphonates, olefin sulphonates, alkylbenzene sulphonates or a mixture of these for tertiary recovery of petroleum. Kalpakci and Yvonne [82] used a mixture of two sulphonates

(a) $C_6H_{13}C_8H_{17}CHCH_2(OCH_2CH_2)_2SO^-_3Na^+$

(b) $C_{20}H_{41}(OCH_2CH_2)_3SO^-_3Na^+$

in a range of concentrations. The method is claimed to be useful in achieving of high temperature (more than 120°C), high pressure, high divalent ion concentration and high salinity. High concentrations (1-10%) of lignosulphonates have sufficient interfacial activity to increase oil recovery from unconsolidated sands [83] and have been shown to interact synergistically with petroleum sulphonates to produce an ultra low interfacial tension [84]. Low molecular weight ethoxylates, sulphated or sulphonated lignin phenols have been used alone in surfactant floods [85] and were found to recover more than 75% of the oil remaining after waterflooding. Naae and Debons [86] prepared an alkylphenol lignin surfactant by the reaction of alkylphenol with Kraft lignin and used it for oil recovery.

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CHAPTER TWO

Surfactants and Methods for Their Analysis

2.0 Chemical Structures of Surfactants

Introduction

The term "surfactant" is a convenient shortening of the terms "surface-active agent" and "interface active agents". Surfactants have great impact on all aspects of our daily life, either directly, as components of household detergents and personal care products or indirectly, in the production and processing of the materials which surround us.
Surfactants have been brought to public attention increasingly since World War II, and have become a universally accepted term to describe organic substances having certain characteristics of structure and properties.

The term "detergent" is often used interchangeably with surfactant as a designation for a substance capable of cleaning. Detergents can also encompass inorganic substances when these do in fact perform a cleaning function. More often, however, the term detergent refers to a combination of surfactants and other substances, formulated to enhance functional performance (specifically cleaning) over that of the surfactant alone.

The older detergents, soaps are salts of higher fatty acids, such as sodium stearate, $C_{17}H_{35}COO^{-}Na^{+}$, which have been around for many years and have played an important role in health and hygiene. However, the last forty years have seen the rise of the synthetic surfactants. These more versatile "surface active agents" now make up a massive market that encompasses the entire world. The global usage of surfactants (excluding soap) currently stand at over 10 million tonnes with a value of over $14 billion.

Although the surfactants market is now growing more slowly in the developed countries of Western Europe, North America and Japan, there is now a trend of significant growth in south Asia and Latin America (Table2.1) [1].

Area	1995/ 10^3 tonnes	2005/ 10^3 tonnes	% increase/ a
Western Europe	2100	2165	0.3
North America	1800	1960	1.0
Japan	565	655	1.5
Latin America	1575	1785	2.6
Asia-Pacific	2690	4340	6.1
Rest of World	1645	2765	6.8
Total	**10,220**	**13870**	**3.6**

Table 2.1 Projected global surfactant usage [1].

The growth of the surfactants market in developing countries is mainly due to expansion in the household cleaning and laundry products sectors. Global usage is predicted to increase to 18 million tonnes by 2050, as the hygiene standards and cleaning practices of Europe, the US and Japan are gradually adopted by the developing world. Despite this growth in the surfactant industry, soap usage is expected to remain constant at 8 million tonnes; this is mainly because of its relatively poor performance (for example, it generates scum in water).

The surfactants market can be subdivided into main categories:-

- Cleaning products e.g. household detergents, consumer products, personal care products and industrial and institutional cleaning.
- Processing aids products that make use of the surfactant's surface active properties e.g. emulsifiers for producing water-based polymer latex used in paint and inks; formulation aids for crop protection chemicals, textile auxiliaries and fiber lubricants, defoamers; and oil field chemicals.

Surfactants can be characterized by the following features:-

- Surfactant molecules are composed of groups of opposing solubility tendencies, typically an oil-soluble hydrocarbon chain and water-soluble ionic group.
- A surfactant is soluble in at least one phase of a liquid system.
- At equilibrium, the concentration of surfactant solute at a phase interface is greater than its concentration in the bulk of the solution.
- Surfactant molecules form oriented monolayers at phase interfaces.
- Surfactants form aggregates of molecules called micelles when the concentration of the surfactant solute in the bulk of the solution exceeds a limiting value, the so-called "critical micelle concentration" (CMC), the value of which is a fundamental characteristic of each solute-solvent system.

In general, it is the presence of two structurally dissimilar groups within a single molecule that is the most fundamental characteristic of surfactants. The surface behavior

i. e. the surface activity of the surfactant molecule, is determined by the make-up of the individual groups, their relative size and their location with the surfactant molecule. These interfacial and solution properties give rise to the following, surface-active properties:-

Emulsification/ demulsification, wetting/ re-wetting, foaming, dispersing, defoaming , detergency and solubilising. These properties or combinations of them have use in many diverse areas such as the food industry, textiles, mineral extraction and processing, agriculture, and the paper industry.

Surfactants can be produced from both petrochemical and / or oleochemical (natural) feedstocks. It interesting to note that while 80% of Western European surfactants are petroleum-based, 55- 65% of all surfactants in the Asia-region are oleochemical-based. It was predicted that in 2000 three quarters of all surfactants would be derived from petrochemical-based intermediates [1].

2.1. Types of Surfactant.

Surfactants are surface active because they concentrate at interfacial regions: air-water, oil-water, and solid-liquid interfaces, for example. The surface activity of surfactants derives from their amphiphilic structure, meaning that their molecules contain one water-soluble (hydrophilic) and one insoluble (hydrophobic) moiety. Surfactants are classified into four different groups of amphiphilic molecule as shown in Figure 2.1.

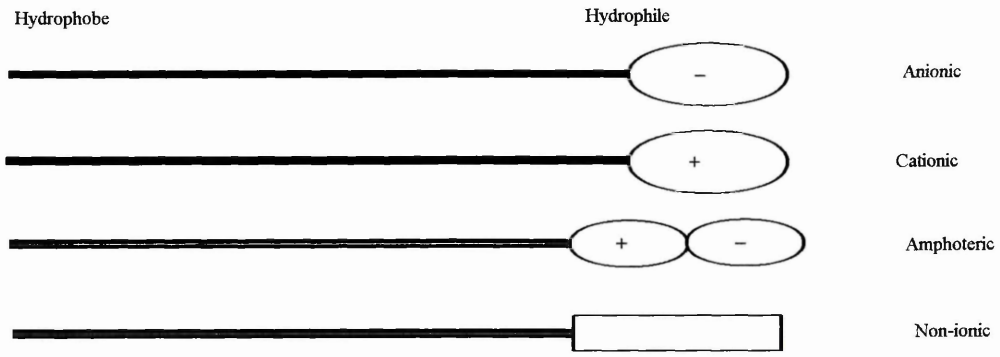


Figure 2.1. The four classes of surfactant.

General formulae and acronyms of the most widespread surfactants are shown in Table2.2.

Common name (acronym)	**Hydrophobic group**	**Hydrophilic group**
Linear alkylbenzenesulphonates (LAS)	C_6H_4-$C_nH_{2n+1}^-$ n =10– 14	– SO^-_3
Alkylethoxylate sulphates (AES)	$C_nH_{2n+1}^-$ n =12–15	–(OCH_2CH_2)n-OSO^-_3 n =1–8
Alkylsulphate (AS)	$C_nH_{2n+1}^-$ n =12–15	– OSO^-_3
Nonylphenol ethoxylates (NPEO)	C_9H_{19}–C_6H_4–	– (OCH_2CH_2)nOH n =1–40
Octylphenol ethoxylates (OPEO)	C_8H_{17}–C_6H_4–	– (OCH_2CH_2)nOH n =1–23
Linear alcohol ethoxylates (AEO)	$C_nH_{2n+1}^-$ n =12–18	– (OCH_2CH_2)nOH n = 1–23

Table 2.2. General formulae and acronyms of the most widely used surfactants.

2.1.1 Anionic Surfactants

Anionic surfactants have been used since so long ago that most of the history of surfactants has to be devoted to them. Event at present, they command the greater part of surfactant consumption, indicating their reasonable cost and performance. In anionic surfactants, the hydrophilic moiety carries a negative charge. Most anionic surfactants (sulphonates, sulphates, carboxylates and phosphates) are polar, and water-soluble. In dilute solutions in soft water, these groups are combined with a 12- 15 carbon chain hydrophobe for the best surfactant properties. In neutral or acidic/ basic media, or in the presence of metal salts, e.g. Ca^{2+}, the carboxylate group loses most of its solubilizing power.

Of the cations (counter ions) associated with polar groups, sodium and potassium impart water solubility, whereas calcium, barium and magnesium promote oil solubility. Ammonium and substituted ammonium ions give rise to both water and oil solubility. Triethanolammonium is a commercially important example. Salts (anionic surfactants) of these ions are often used in emulsification. Anionic surfactants are divided into the following groups:-

2.1.1.1 Carboxylates

Most of the commercial carboxylates are soaps. The general structure of a carboxylate soap is $RCOO^{-}M^{+}$, where R is a straight chain hydrocarbon ($C_{9\text{-}21}$) and M^{+} is a metal or ammonium ion. Soaps show excellent detergency in soft water; however, bivalent metal ions (Ca^{2+} etc.) cause unsightly "scum". For this reason, and from an economic point of

view, "syndets", a combination of synthetic surfactants i.e. alkylbenzene sulphonates (ABS) and builders i.e. pentasodium tripolyphosphate, have replaced soaps in soap powders. Carboxylates with a fluorinated chain have also been developed. Replacement of hydrogens on the hydrophobe by fluorine atoms has led to surfactant molecules of unusually low surface tension.

2.1.1.2 Sulphonates

The sulphonate group, $-SO_3M$, attached to an alkyl, aryl, or alkylaryl hydrophobe, is a highly effective solubilizing group. Sulphonic acid surfactants are chemically stable in nature and their salts are relatively unaffected by pH, they are stable to oxidation, and because of the strength of the C- S bond, are also stable to hydrolysis. Sulphonates interact moderately with the hardness cations, Ca^{2+} and Mg^{2+}, but significantly less so than do the carboxylates.

Alkylbenzene sulphonates (ABS) have been the most commercially used anionic surfactants since their introduction in the 1940s. Alkylbenzene sulphonates are effective surfactants which respond well to builders and foam boosters in detergent formulations. These properties, together with the low cost and availability at consistent quality, account for their dominant position in household laundry products. Alkylbenzene sulphonates are manufactured as is shown in Figure 2.2.

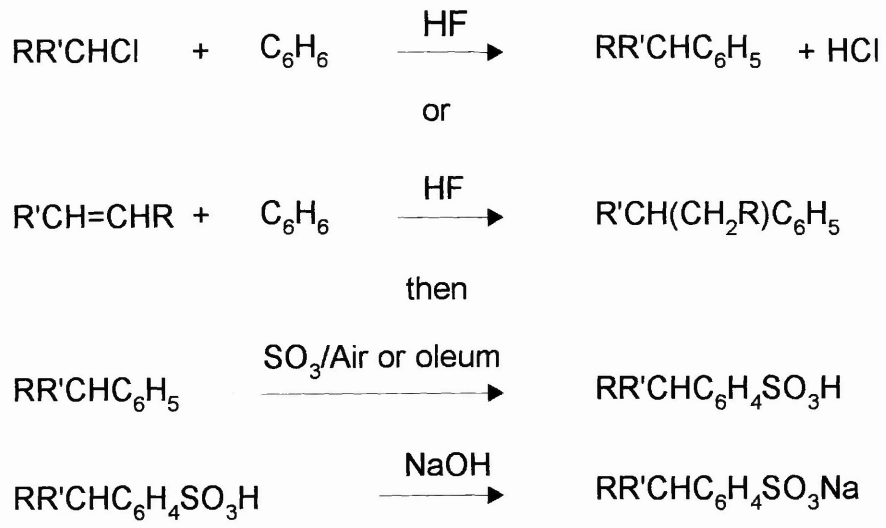


Figure 2.2. The manufacture of ABS.

Alkylbenzene sulphonates (ABS) are regarded as "hard" detergents because of their relatively slow biodegradation rate as compared to the "soft" linear alkylbenzene sulphonates (LAS) detergents which are more quickly degraded by common microorganisms. The presence of significant levels of these detergents in sewage and surface water has been known to cause foaming problems in sewage and water treatment plant operations. The persistence of these detergents in river water can also cause adverse effects to aquatic life. The foaming problem has led the industrialised countries to switch in the 1960s from ABS to LAS, which has since been found to be a satisfactory solution [2].

The common anionic surfactants are the linear alkylbenzene sulphonates (LAS), with a structure shown in Figure 2.3, and possess both hydrophilic and hydrophobic characteristics, due to the sulphonate ion and the long-chain alkyl group of the molecule.

Their ability to reduce the surface tension and to form micelles is the reason for the widespread use of surfactants not only in the detergent industry but also in many other industries like the textile, cosmetic, food and leather industries and in the flotation of ore minerals [3]. LAS which are present in commercial formulations consist of complex mixture of C_{10}-C_{14} homologues and of positional isomers resulting from attachment of the phenyl ring to the carbon atoms (from the second to the central one) of the linear alkyl chain. LAS have a total production estimated at 290,000 tonnes per year in Western Europe [1]. This represents 25% of the total consumption of synthetic surfactants. In the industrial world (i.e. United State, Western Europe and Japan) the figure for the total LAS consumption is approximately one million tonnes per year.

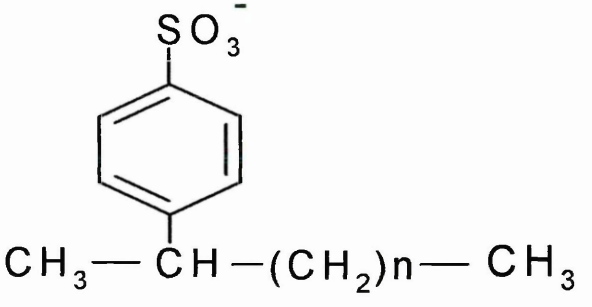


Figure 2.3 Structure of LAS

After use, most linear alkylbenzene sulphonates (LASs) are disposed of via sewage treatment facilities. It can be largely removed (95% for activated sludge process) during the sewage treatment process as result of biodegradation and adsorption to solids. A small quantity of LAS enters surface water, as part of the treated effluent, and soil, as part of the sludge when the latter is applied in agriculture as fertilizer. The presence of surfactants in the environment causes disturbances in the ecological equilibrium. These

surfactants have a toxic impact on aquatic organisms, due to interactions with biomembranes [3].

2.1.1.3 Sulphates

The sulphate group, $-OSO_3M$, where M is a cation, represents the sulphuric acid half-ester of an alcohol and is more hydrophilic than the sulphonate group because of the presence of an additional oxygen atom. Attachment of the sulphate group to the hydrophobe through the C-O-S linkage limits hydrolytic stability, particularly under acidic conditions. Usage of alcohol sulphate (AE) and alcohol ethoxylate sulphate (AES) systems has expanded dramatically since the 1970s as the detergent industry has reformulated consumer products to improve biodegradability, lower phosphate content, and to move from powered to liquid products.

Commercial production of alcohol sulphates (AS) started in Europe in the 1930's and these products have become well established in specialty markets. The hydrophobes are predominantly of petrochemical origin, although in the 1990's some shift is evident to oleochemical hydrophobes obtained by reduction of fatty acids and esters. Alcohol ethoxylate sulphate products have experienced dramatic growth since the 1960's. Most of the increase has come from expanded usage in consumer detergent products, resulting from :- expanding availability of relatively low cost primary straight-chain alcohols from petrochemical sources; superior biodegradability of alcohol ethoxylate sulphates compared to corresponding alkylphenol ethoxylate sulphates; the necessity of hardness-insensitive surfactants in heavy-duty laundry detergents brought about by phosphate

content reduction; expansion of reliable, inexpensive sulphation technology; and use of the less expensive ethylene oxide, which lowers overall cost. Alcohol ethoxylate sulphates have several advantages over alcohol sulphates including lower sensitivity to hardness with respect to foaming and detersive effectiveness, less irritation to skin and eyes, and high water solubility. Alcohol ethoxylate sulphates generally contain from 10 to 40% ethylene oxide calculated on the weight of the starting alcohol. These are offered commercially as light-colored, odorless liquids containing 30-70% of the active ingredients.

2.1.2 Non-ionic Surfactants

Non-ionic surfactants carry no discrete charge when dissolved in aqueous media. Hydrophilicity in non-ionic surfactants is provided by hydrogen bonding with water molecules [4]. Oxygen atoms and hydroxyl groups readily form strong hydrogen bonds, whereas ether and amide groups form hydrogen bonds less readily. Hydrogen bonding provides solubilization in neutral and alkaline media. In a strongly acid environment, oxygen atoms are protonated, providing a quasi-cationic character. Each oxygen atom makes only a small contribution to water solubility and therefore more than a single oxygen atom is needed to solubilize a non-ionic surfactant in water. Non-ionic surfactants are compatible with ionic and amphoteric surfactants, because the polyoxyethylene group can easily be introduced by the reaction of ethylene oxide with any organic molecule containing an active hydrogen atom, and so a wide variety of hydrophobic structures can be solubilized by ethoxylation. Non-ionic surfactants are usually prepared by the addition of ethylene oxide to compounds containing one or more

active hydrogen atoms, such as alkylphenols, fatty acids, fatty alcohols, fatty mercaptans, fatty amines and polyols. The alkylphenol polyethoxylates (APEO) are manufactured by a base-catalysed reaction of ethylene oxide with alkylphenol (AP). Nonylphenol polyethoxylates (NPEOs) are therefore made by the reaction of nonylphenol (NP) with ethylene oxide. NP is produced from phenol and nonene using acid catalysis. The NP produced almost is completely substituted in the para position. As nonene is a branched nine carbon containing olefin made by trimerizing propylene, many isomers of APEO are obtained during manufacture. Also as the manufacturing process produces a mixture of ethylene oxide oligomers which follow a Gaussian distribution (generally from 1 to 30), APEO commercial formulations are very complex in nature.

Many of these reactions used for the manufacture of non-ionic surfactants produce a complex mixture of compounds. The most common classification of non-ionic surfactants is into the following types:-

2.1.2.1 Polyoxyethylene Alcohols and Polyoxyethylene Esters of Fatty Acids

These are surfactants with the general structure:-

$$RO\text{-}(CH_2CH_2O)_xH \quad \text{or} \quad RCOO\text{-}(CH_2CH_2O)_xH$$

Poly(ethylene oxide) surfactants (ethoxylates) are by far the most important group of alkoxylated non-ionic surfactants. Poly(ethylene oxide) surfactants (ethoxylates) were introduced into the U.S. as a textile chemical shortly before the 1940's. The water solubility of these compounds arises from recurring ether linkages in the polyoxyethylene chain. A single oxyethylene group contributes slightly more to hydrophilicity than single

methylene CH_2 contributes to hydrophobicity, so that complete miscibility with water occurs when 65-70% of the molecule by weight is polyoxyethylene. The surface active properties of the ethoxylates are not adversely influenced by water hardness.

2.1.2.2 Alcohol Ethoxylates(AE)

Alcohol ethoxylates have emerged as the principal non-ionic surfactants in the consumer detergent product market. They are cheap to manufacture and highly biodegradable. They vary in physical from (liquid to waxes) depending on their ethylene oxide content. Alcohol ethoxylates are polydisperse with respect to ethylene oxide chain length. An AE containing nominally eight ethylene units actually contains significant amounts of other ethoxylates ranging from 0-20 oxyethylene units.

2.1.2.3 Alkylphenol ethoxylates (APEOs)

The physical and performance properties of these surfactants are similar to those of AE. They are derived mostly from alkylphenols (APs) containing branched alkyl side chains, typically nonyl and octyl groups. This branching leads to a decrease in biodegradability in comparison to the alcohol ethoxylates. However, water solubility and dispersive properties are increased as a result of branching.

Commercial alkylphenol ethoxylates are usually produced by the base-catalysed ethoxylation of alkylated phenol. As phenols are slightly more acidic than alcohols, their reaction with ethylene oxide to form the mono-adduct is faster. The product therefore does not contain unreacted phenols, and thus the distribution of individual ethoxylates in

the commercial mixture is narrower and alkylphenol ethoxylates are more soluble in water. The general formula for APEO is shown in Figure 2.4.

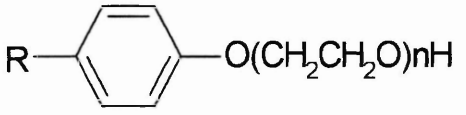
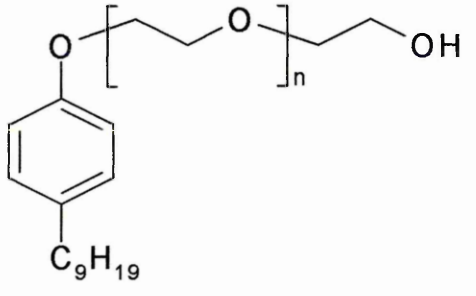


Figure2.4. The general formula for APEO.

Nonylphenol ethoxylates (NPEOs) are the most commonly used APEO. The molecule has an alkyl chain (R= C_9H_{19}) containing nine carbon units and an ethoxylate chain that can vary from one to forty units (Figure2.5)



Figuer2.5. Structure of nonyl phenol ethoxylates..

There is increasing concern with regard to the world-wide usage of APEO, because of their relatively stable and toxic biodegradation intermediates [5]. Many European countries are now looking at alternative formulations, and in the UK, a voluntary ban has been introduced on their domestic usage.

2.1.3 Cationic Surfactants

The hydrophobic moiety of cationic surfactants carries a positive charge which resides on an amino or quaternary nitrogen. The positive charged cationic surfactants are more strongly adsorbed than anionic or non-ionic surfactants on a variety of substances including textiles, metal, glass, plastic, minerals, and human tissue, which can often carry a negative surface charge. The only cationic surfactant found in any quantity in the environment is ditallow dimethylammonium chloride (DTDMAC), which is a group of quaternary ammonium salts of distearyldimethylammonium chloride (DSDMAC) [6]. Cationic surfactants have antibacterial properties and are frequently used in disinfectants. Their antistatic and substantive properties make them ideal for use in fabric conditioners, and their ability to "hydrophobe" a surface makes them ideal emulsifiers, such as in bitumen emulsion for road repair.

2.1.4 Amphoteric Surfactants

Amphoteric surfactants contain both an acidic and basic hydrophilic group. Ether or hydroxyl groups may also be present to aid the hydrophilicity of the surfactant molecule. Examples of amphoteric surfactants include amino acids and their derivatives in which the nitrogen atom tends to become protonated with decreasing pH of the solution. Amino acid salts, under these conditions, contain both a positive and a negative charge on the same molecule. In alkyldimethyl betaine $RCH(CH_3)(CH_3)_2N^+CH_2COO^-$, discrete opposing charges are present in the molecule at all pH values. Because of their mildness and high foaming properties, amphoteric surfactants are generally found in toiletries and

cosmetic formulations. They are also used in industrial applications where a high stability foam profile is needed.

The relatively low usage of cationic and amphoteric surfactants (Table2.3) makes them much less environmentally significant.

Class	Sales 1996 / 10^3 tonnes	Sales 2005/ 10^3 tonnes
Anionic	760	765
Non-ionic	1015	1140
Cationic	190	200
Amphoteric	50	60
Total	**2070**	**2165**

Table 2.3. Current and projected sales of surfactants by type[1].

2.2. Introduction-The Analysis of Surfactants

There are many problems which can be encountered by the analytical chemist when determining surfactants in the different matrices in which they are found, i.e. river, water, sludge, raw sewage, sea, etc. The complete analysis of surfactants of all types is tedious and time-consuming. Problems are encountered due to differences between the four classes of surfactants, and, particularly because many commercial surfactants are not specific structures but homologous series with similar repeating structures, or complex mixtures consisting of homologues, oligomers and positional isomers. For example, the anionic surfactant LAS may contain as many as thirty-five different homologues and phenyl positional isomers. The alkyl chain can vary from ten to fourteen methylene units, and the phenyl group is distributed quite evenly along all of the positions on the chain (with the exception of the two end groups). This last phenomenon is due to the manufacturing processes used in industry.

The various biodegradation products of these surfactants further complicate analysis. Recent concern over the effects of surfactants and their biodegradation intermediates on the environment means that any analytical method must be capable of providing information about the different isomers.

Not only is it necessary to classify the type of surfactants present in a sample, it is also necessary to determine its structure and provide quantitative information down to the

parts-per-billion (ppb) level or less. Many investigators have attempted to find suitable tools of analysis [7-14].

2.2.1 Non-Specific Methods

The need to determine routinely low concentrations (0.2 mg/L) of surfactants in laboratory test liquors and environmental samples has led in the first place to the development of analytical methods capable of assessing the important members of the main surfactant classes (e.g. the colorimetric methylene blue procedure for anionics [15,16] and the Wickbold [17] potentiometric titration method for alkoxylated non-ionic surfactants). The chemistry of these summary methods is such that the determinations are not specific for surfactants alone. These methods give fairly accurate results for clean samples and some of the methods are now standard for legislative biodegradation test protocols. However, the lack of specificity and sensitivity of these non-specific methods means that their application to the determination of surfactants in environmental samples, where concentrations are generally lower and many more interferences are encountered, is less satisfactory. At best they only provide conservative estimates of surfactants. In addition, these methods are usually unable to cope with the complete range of environmental matrices, e.g. sludge, sediment and soil samples. The need to demonstrate the distribution and fate of major chemicals in the environment is increasingly important for assessments of their environmental safety.

2.2.1.1 Anionic Surfactants

In the determination of trace amounts of anionic surfactants, and in particular LAS, the methylene blue analytical method is by far the most widely used. Methylene blue (Figure2.1) is a cationic dye, which in the form of a salt with an inorganic anion such as chloride or sulfate is insoluble in many organic solvents.

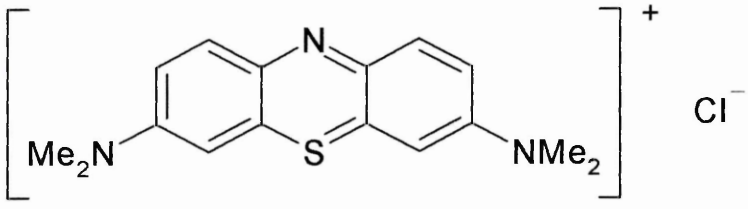


Figure 2.1 Methylene blue chloride

In the presence of an anionic surfactant, an ion-pair is formed between the methylene blue cation and the surfactant that is less soluble in water than the individual components and can therefore be separated into an organic solvent. The complex forms a blue colour that is readily determined by colorimetry, the intensity of the colour is relative to the amount of surfactant in the system. This analytical method depends on two factors. The first is the ability of the anionic hydrophilic group to combine with the methylene blue dyestuff and the second that the overall complex is then sufficiently hydrophobic to be extracted into chloroform from an aqueous medium.

As the name MBAS (methylene blue active substance) suggests, the procedure is not specific to anionic surfactants. The method was originally published by Longwell and Maniece in 1955 [16] and subsequently modified by Abbott in 1962 [18]. Other developments for the analysis of environmental samples by the MBAS techniques

involve the isolation of the surfactant by the foam sublation technique of Wickbold [17]. The methylene blue method is responsive not only of anionic surfactants, but generally of any material containing a single strong anionic centre, strong enough to form a stable ion-pair with the methylene blue cation, and at the same time containing a hydrophobic group sufficiently lipophilic to be more soluble in the organic layer. Other commonly occurring compounds can give partial responses with methylene blue, thus causing positive interference. These are 17,900 ppm NaCl at pH. 1.8 which gives the same colour as 10ppm alkyl sulphate surfactants, as does 1040 ppm of nitrate or 40 ppm thiocyanate [19]. Whilst careful choice of method can reduce the interference, such species can still cause falsely high results. Limits of detection using this method are around 0.01-0.02mg/L in favorable conditions.

2.2.1.2 Non-ionic Surfactants

The historical methods for the analysis of non-ionic surfactants in waters and sewage have been reviewed by Longman in 1975 [20] and by Heinerth in 1966 [21] and all involve essentially two stages. The first stage is the extraction procedure to separate the non-ionic surfactant into an organic solvent and the second stage is the estimation of the extracted surfactant using chemical methods which, in general, involve reaction with the hydrophilic portion of the surfactant. The extraction procedures used fall into two broad classes, the first standard solvent extraction procedure and the second, which is now much more widely used, the solvent sublation procedure of Wickbold [17].

The development of analytical methodology has been very much focused on the two major alkoxylated non-ionics, i.e. alcohol ethoxylates (AEO) and alkylphenol ethoxylates (APEO) to determine them in laboratory tests of biodegradability and toxicity and their environmental monitoring. The three methods detailed below all depend upon the complex formation between the ethoxylated chain of the non-ionic surfactant and either inorganic metal or organic salts. As with the anionic MBAS methods, the chemistry of these complex formation reactions is such that they cannot be specific for non-ionic surfactant determinations without suitable clean-up of samples.

2.2.1.2.1 Cobaltothiocyanate Active Substances (CTAS) [22,23]

The basis of the CTAS method is similar to that for MBAS; a complex is formed between ammonium cobaltothiocyanate and the ethoxylate chain which can be extracted from an aqueous phase into a solvent and this is followed by colorimetric estimation. In common with all of the non-specific methods for non-ionic surfactants described here, little or no reaction is obtained for surfactants containing on an average of less than three to four ethoxy units.

The CTAS method and Wickbold [17] solvent sublation technique are used to concentrate and separate intact surfactant from non-surfactant materials that can interfere in their subsequent material estimation. With this technique surface-active materials including the non-ionics are removed form dilute aqueous samples (containing 10% sodium chloride and 0.5% sodium bicarbonate) into an overlying layer of ethyl acetate by bubbling a gas (air or nitrogen) through it. Separation is achieved by adsorption of the

surfactant on the surface of the bubbles and its subsequent transfer to the liquid-liquid interface where it is allowed to partition into the organic solvent. Any anionic surface-active materials in the resulting extract are removed by non-aqueous ion-exchange. The limit of detection for the CATS method is approximately 0.1mg/L.

2.2.1.2.2 Potassium Picrate Active Substance (PPAS) [24]

The basis of this method is the interaction of the ethoxymer chain with a large excess of potassium ions in aqueous solution to form positively charged complexes which are readily extractable into 1,2-dichloroethane as picrate ion-association compounds The concentration of non-ionic surfactant is determined by the absorbance of the picrate ion at 378nm. The technique is well suitable for the determination of low concentration of non-ionic surfactants in marine and surface water, with a limit of detection of 2-200µg/L.

2.2.1.2.3 Bismuth Active Substance (BiAS)

The BiAS procedure, reported by Wickbold [17,25] is the European standard method for determining alcohol ethoxylate in biodegradation test liquors. This method is based on two solvent sublation steps with ethyl acetate, cation-exchange chromatography, and precipitation of the non-ionic surfactant with modified Dragendorff reagent (barium chloride-potassium tetraiodobismuthate (III)). The resulting non-ionic surfactant complex precipitate is then dissolved and the liberated bismuth ion is titrated potentiometrically with pyrrolidine dithiocarbamate complexone as measure of non-ionic surfactant. This method provides a good estimate for the concentration of total non-ionic surfactants (AEO and APEO) present in raw sewage, but still leads to a significant

overestimation of the concentration of non-ionics in the final effluent of sewage treatment plants (STP) and river water samples [26,27]. The liberated bismuth can also be determined by atomic absorption (A.A) or Ultraviolet (UV) spectroscopy. The BiAS method has gained the same status for non-ionic surfactants as the MBAS method has for anionic surfactant. The limit of detection of the method is 0.05-0.1 mg/L.

2.2.2 Specific Analytical Methods

2.2.2.1 Gas Chromatography

2.2.2.1.1 Anionic Surfactants

In recent years good progress has been made towards developing analytical methodologies for the specific determination of individual anionic surfactants in support of environmental testing. This is particularly well illustrated for LAS, the major anionic surfactant in use, which can now be determined at low concentration (as well as its many homologues and phenyl positional isomers) in almost any testing laboratory for any environmental matrix.

Gas chromatography (GC) is a technique based upon the principle that each component of a mixture undergoes its own characteristic partition between the gas phase and stationary (liquid or solid) phase packed in a column. The degree of separation of the sample components is determined mainly by the partition coefficients of the substance (solutes). These in turn are functions of the solute interaction with stationary phase and of the vapor pressure of the solute at the column temperature. There are some other

factors which affect the separation of the solutes and the appearance of the subsequent chromatogram, such as solute diffusion, carrier-gas, velocity, mass transfer effect, etc. It will be readily appreciated that the sample components require full vaporization prior to the partition so that the carrier-gas flow can drive them into the column; this is the first step of gas chromatography. Then one may suppose that the higher the volatility of the sample, the easier the gas chromatography and in general this is true . In this connection, it is logical that gas chromatography was first applied to the analysis of volatile materials such as petroleum products and only later to that of organic substances of little or no volatility such as drugs and polymers, and of some inorganic compounds. In the case of substances of little or no volatility (which includes surfactants), the samples require pretreatment by chemical reaction or thermal decomposition in order to permit GC analysis.

Gas chromatography has played an important role in the analysis of surfactants; it is an important application of GC to separate complex mixtures, especially of alkyl homologues and their isomers. However, there is a difficulty in the practical application. As mentioned above, samples need to be vaporized before separation in the column, and in order to achieve this surfactants have to be converted to characteristic non-surfactive hydrophobic oils by derivatisation, which can then be subjected to GC analysis.

The gas chromatographic procedure is useful as it allows the determination of the individual LAS homologues and all phenyl positional isomers. It is very sensitive and specific and can accurately determine μg amounts of LAS in a broad range of

environmental matrices. It requires, however, complicated and tedious sample pretreatment and is therefore less appropriate for routine work. Due to the presence of the sulphonate group, an LAS salt is involatile, and therefore some form of derivatisation procedure must be performed prior to analysis by gas chromatography (GC). Derivatisation techniques offer an alternative approach to desulphonation for increasing the volatility of LAS for GC analysis. There have been several successful derivatisation methods for LAS reported in the literature. These include desulphonation in boiling phosphoric acid [28] and conversion to the sulphonyl chloride [29] or methyl sulphonate [30]. Modern GC methods tend to utilise mass spectrometric detection (GC-MS) rather than flame ionization detectors (FID) or electron capture detector (ECD); this is mainly because of the enhanced selectively and sensitivity available from mass spectrometry.

Hon-nomi and Hanya [30] have, however, described improvement of the sulphonate derivatisation techniques for the analysis of low concentration of LAS in river water by GC and GC/ MS. The LAS was concentrated from river water by extraction into chloroform as its methylene blue complex. The methylene blue cation was removed by a non-aqueous cation-exchange step. The resulting LAS material was treated with phosphorus pentachloride and methanol to give the corresponding methyl sulphonate derivative. As a final clean-up step, the methylene sulphonate derivative was then purified by passage through a silica gel column, to remove interfering organic material, prior to GC or GC/MS analysis. Calibration was performed by external standardisation with dodecylbenzene sulphonate. Analysis of the river Tama in Japan showed the level of LAS to be greater then 3 ug/L.

Trehy *et al* [31] have reported the results of a study on a method for the determination of LAS and Dialkyltetralin sulphonate (DATS) in environmental water and sediment samples at low microgram per liter concentration by GC/MS. LAS and DATS (minor components present in LAS) were extracted using C_8 solid phase extraction (SPE) cartridge. Following extraction, the resulting analytes were allowed to react with phosphorus pentachloride and then trifluoroethanol added to form their trifluoroethyl sulphonate derivatives, which they claim is an improvement on the methyl sulphonate method developed by Hon-nami *et al* [30]. Trifluoroethyl sulphonates are prepared instead of methyl esters in order to enhance sensitivity and selectivity for electron capture negative chemical ionization GC/MS. The limit of detection for the method was found to be *ca*. 0.001mg/L for both LAS and DATS. LAS concentrations in the influent and effluent of a trickling filter wastewater treatment facility in Utah, USA were found to be 2.7mg/L and 0.14mg/L receptively. DATS concentration were 0.22 and 0.052mg/L.

Further studies on the concentration of LAS and DATS, in influents and effluents of 10 US domestic wastewater treatment plants were conducted by Trehy *et al* [32]. The derivatisation electron capture/ gas chromatograph /mass spectrometry (GC/MS) method was employed. Two types of sewage treatment plant (STP) were studied; activated sludge and trickling filter. The activated sludge process removed > 99% LAS and around 95% of DATS. The trickling filter was shown to be less efficient, with 85% removal of LAS and 65% DATS. Concentrations of LAS in receiving water downstream of the ten sites ranged from < 0.001 to 0.094mg/L and < 0.001 to 0.023 mg/L for DATS.

2.2.2.1.2 Non-ionic Surfactants

The determination of the environmentally significant non-ionic surfactant alkylphenol ethoxylate (APEO) suffers from a similar volatility problem to LAS. APEO with a short polyethoxylate chain are amenable to direct determination using GC. Quantitative GC analysis of non-ionic surfactants of a higher degree of ethoxylation, therefore, requires derivatisation reactions to transform the APEO into more volatile compounds. APEO analysis by GC without derivatization has mainly been used on the volatile biodegradation products of APEO, namely NP2EO, NP1EO, and NP.

GC coupled to MS becomes more and more an important method for the determination of APEO/ AP in environmental matrices because of its sensitivity and selectivity . GC/MS provides the basis for the reliable identification of these analytes by their fragmentation pattern apart from quantitation of these compounds. Giger *et al* [33,34] have successfully applied GC/MS in the electron ionisation (EI) mode for the analysis of NP2EO, NP1EO and NP in wastewaters and river waters. Quantitation was performed by addition of tribromophenol [33] or n-nonylbenzene [34] as internal standards to the extracts just before the GC determination. Detection limits for both methods ranged between 1.0 and 10.0 µg/L.

Jobst and Fresenius [35] have performed determinations of NP from soil samples by GC/MS in the selected ion monitoring (SIM) mode for sensitive detection (0.05- 0.1mg/ kg soil) and selective identification. The internal standard 4-n- nonylphenol not included

in technical NP was added to the original sample to correct for non-quantitive recoveries during isolation.

Blackburn and Waldock [36] have determined the concentrations of nonylphenols (NP) and octylphenol (OP) in rivers and estuaries of England and Wales. Extraction was performed by C_8 SPE cartridge, the resulting extracts were analysed directly by GC/ MS without any derivatisation procedure. The survey included six rivers, the final effluent from twelve sewage treatment works, six estuaries and one harbour-mouth. The highest concentration of nonylphenol was found in the effluent from a sewage works and was found to be 330μg/L. The works in question serves an area with a large amount of textile industry nearby. The concentration of NP in the river Aire into which the treated effluent was discharged was 180μg/L, which is approaching the LC_{50} for Daphnia (300μg/L). The majority of the river sampled contained < 0.2- 5μg/L NP,the highest recorded being 10μg/L. Estuarine concentrations were lower as a result of dilution and dispersion processes caused by tidal flow. The highest concentrations were recorded in the outer Tees estuary (5.2μg/L nonylphenol, and 13μg/L octylphenol) and in the Mersey. Over 80% of the estuarine samples contained < 0.1μg/L NP. Concentrations of octylphenol (OP) were less than 1μg/L at all sites, reflecting the low use of octylphenolpolyethoxylates in the U.K. Detection limits using this method were 30-200μg/L for nonylphenol and 50- 250μg/L for octylphenol.

GC/EI-MS is used for mass spectral characterization of individual nonyl chain isomers of NP, NP1EO and NP2EO. By use of high-capillary GC/EI-MS, Wheeler *et al* [37] were

able to separate 22 isomers of technical NP. Analysis of the corresponding mass spectra indicated the presence of five distinct groups of octylphenol ethoxylate (OPEO) and OPEC and give very reliable information on the molecular weight of the analytes due to the presence of the adduct ions $[M+H]^+$and $[M+C_2H_5]^+$ [38,39]. Thus, GC/CI-MS is a suitable method for the selective identification of these compounds in environmental matrices.

Derivatisation of NP and NPEO with pentafluorobenzyl chloride (PFBCl) or heptafluorobutyric anhydride (HFBA) provides derivatives with high electron affinities which can be specifically and sensitivity-analyzed by GC coupled to an electron capture detector (ECI), EI- MS, or negative chemical ionisation (NCI) MS.

Walhlberg *et al* [40] have used a GC/MS method for the determination of nonylphenol (NP) and nonylphenol ethoxylate (NPEO) in sewage sludge as their pentafluorobenzoates. The detection limits were 0.1mg/kg for nonylphenol and 0.4, 1 and 2mg/kg respectively for NP1-3EOs. Following extraction from the various matrices by solvent extraction, levels of nonylphenol in sludge samples were found to range from *ca*. 25- 1100mg/kg. Level of NPEOs were much lower, ranging from less than the limit of detection to 125mg/kg.

The acidic biodegradation products of APEO, namely APEC, have been also identified by GC/MS after derivatization. Methylation of APEC with diazomethane [41], a 10% solution of BF_3 in methanol [34] or 1 M solution of HCl in methanol [34] or silylation of

APEC with BSTFA [42] yields derivatives which can be easily chromatographed on fused silica capillary columns.

Field *et al* [43] have developed methods for quantitative determination of nonylphenol ethoxycarboxylate biodegradation intermediate products from nonylphenol ethoxylate (NPEO) by GC/MS in paper mill effluents, municipal sewage treatment plant effluents, and river water samples. The analytes were extracted from samples by a strong anion-exchange (SAX) extraction disk and following derivatization with methyl iodide, samples were spiked with 2-chloroepidine as internal standard. The detection limit for this method was found to be 0.2, 0.4, 2.0 and 2.0μg/L respectively for (NP1EC, NP2EC, NP3EC and NP4EC). The total concentration of NPEC in paper mill effluents ranged from below the limit of detection to 1300μg/L, effluents typically containing less than 100μg/L NPECs. Over half of the paper mill effluents contained only NP1EC and NP2EO, and in all cases, NP2EC was the dominant oligomer. The average proportions of NPECs in paper mill effluents were NP1EC (16%), NP2EC(72%), NP3EC (10%), and NP4EC (2%). All NPEC oligomers were detected in all the municipal sewage treatment plants effluents tested with the average proportion of NPECs as follows: NP1EC (7%), NP2EC (54%), NP3EC(31%) and NP4EC (8%), indicating that municipal STPs on average have a higher percentage of NP3EC and NP4EC relative to NP1EC. Of the eight US river samples tested, five gave concentrations for NP1EC and NP2EC above the limit of detection for the method, and again, NP2EC was the dominant oligomer.

2.2.2.2 High Performance Liquid Chromatography

High performance chromatography (HPLC) is a widely used technique in surfactant analysis as it is able to distinguish these charged compounds on the basis of their hydrophobicity. It is therefore used to analyse complex mixtures of non-volatile anionic or neutral surfactants (which may not need prior derivatization). The major advantage of HPLC is the ability to separate and quantitate the various homologues and oligomers by the length of the alkyl and ethoxylate chains.

2.2.2.2.1 Anionic Surfactants

High performance liquid chromatography (HPLC) has been reported [44-46] as a simple and efficient analytical method for determining linear alkylbenzene sulphonate (LAS) in environmental samples. HPLC does not have the superior resolution of GC but it allows the separation of the main LAS homologues without preliminary derivatization. It offers a practical tool for environmental monitoring by combining selectivity and speed of analysis. The method presented by Matthijs and De Henan [44] has been widely applied to determine LAS in samples of sewage treatment plants[44,47,48], river waters [44,49], sediments [44] and sludge-amended soils [50,51]. LAS is isolated from environmental matrices by methanol extraction. Sample clean-up is achieved by anionic-exchange chromatography followed by solid phase extraction (SPE). The sensitivity for total LAS is 10μg/kg in aqueous samples and 100μg/kg in solid samples [52].

Kikuch *et al* [52] developed the necessary concentration and clean-up procedures to allow the determination of LAS by HPLC in a range of environmental samples including

river and sea water, sediment and fish tissue samples. They combined trace enrichment and clean-up of LAS on C_8 reversed-phase mini-columns with an HPLC fluorescence detection determination (λ_{ex}=231nm, λ_{em} =288nm). To recover LAS from aqueous samples, samples were filtered and methanol added to the filtrate. Sediment samples required repeated methanol extraction and fish tissue was homogenised with methanol. All filtrates and extracts were, as required, diluted with distilled water to reduce their methanol contents to below 10% prior to passage through C_{18} reversed-phase mini-columns. The samples were then analysed using HPLC with an ODS column at 40°C using a mobile-phase of 0.1 M sodium chlorate in acetonitrile/water (60:40). The detection limits for this method, were approximately 0.1µg/L, 0.03µg/g and 0.3µg/g for sea water, sediment and fish samples respectively.

LAS in environmental matrices can be extracted by solid phase extraction (SPE) and then analysed by HPLC on a C_{18} column using an acetonitrile /0.33M sodium perchlorate mobile phase. When this method was applied to sewage treatment plant (STP) the amounts in influent and effluent detected were 0.4 and 0.14mg/L LAS, respectively [54]. Other HPLC phases used for the separation of LAS include C_4 (55), polystyrenedivinylbenzene [56] and C_1[57].

Matthijs *et al* [44] also developed method for determination of LAS from aqueous samples, sediment, sludge and soil using a C_{18} solid phase extraction column. The extract was then further purified over a strong anion-exchange column and analysed by HPLC

using fluorescence detection. The method allowed the determination of the individual alkyl chain lengths.

2.2.2.2.2 Non-ionic Surfactants

The need to determine and differentiate between the two largest groups of non-ionic surfactants, (i.e. APEO and AEO), for the purposes of environmental testing has led to the development of specific methods for these materials. To date, particular emphasis has been given to the determination of APEO, used almost exclusively in industrial cleaning applications, which are generally considered as less environmentally acceptable.

Surfactants are complex mixtures consisting of various homologues and oligomers by variations in the length of the alkyl and ethoxylate chain. Consequently, HPLC separation of APEO into individual molecules is a two-dimensional problem best solved by using different HPLC stationary phases. Polar normal-phase columns separate non-ionic surfactants by their interaction with the hydrophilic polyethoxylate chain without resolving the hydrophobes, while nonpolar reversed-phase columns separate them by their interaction with the hydrophobic chain only, eluting the ethoxymers as a single peak. The ring chromophore in APEO molecules enables direct and sensitive UV or fluorescence detection. Therefore, normal and reversed-phase HPLC provide a quite simple and suitable technique for the environmental analysis of APEO and their metabolites. Recently three reviews [5,58,59] have described the enormous amount of data published on the determination of non-ionic surfactants (particularly of the APEO type) by HPLC over the last fifteen years or so.

Normal-phase HPLC is mostly applied to obtain information about the ethoxylate chain distribution of APEO. Therefore, quantitative determinations of APEO in samples from waste water, sewage treatment, and the aquatic environment are often performed by normal-phase HPLC to reveal the changes in the APEO composition due to biodegradation. Ethoxylate distribution has also been determined by several different normal-phase systems. Giger *et al* [60,61] used an aminopropyl column for the determination of NPEO and their biodegradation intermediates NP, NP1EO and NP2EO in waste water and river water. The authors used a hexane/ isopropanol gradient system coupled to UV detection (277nm). Samples were extracted by steam distillation and solvent extraction; the resulting analysis showed river water from the Glatt river in Switzerland to contain 3.9µg/L NP, 23.4µg/L NP1EO, 9.4µg/L NP2EO and 0.8-2.3µg/L NPEO. A digested municipal sewage sludge was found to contain 1.6g/kg NP. Rothman [62] also used an aminopropyl column for the determination of OPEO and NPEO with UV detection. The method used a gradient mobile-phase system isooctane, dichloromethane and methanol. The same method was used by for the determination of NPEO around the Krka river estuary, Croatia [63]. Following extraction by C_{18} SPE, untreated municipal waste water was found to contain 70-2960µg/L NPEO, with estuarine concentrations ranging between 0.7 and 17µg/L.

Boyd-Boland and Pawliszyn [64] have been developing a new method for the determination of APEO in environmental samples by coupled solid phase microextraction (SPME) with HPLC. The authors found that the best SPME phase for the extraction of

APEO was Carbowax/ divinylbenzene. This method was used simply to quantitatively determine the presence of alkylphenol in the sewage sludge to level of 10μg/L.

In order to increase sensitivity and selectivity, another method for the determination of APEO in water samples using an aminopropyl column has been developed by Holt *et al* [65]. Extraction and purification of APEO in sewage-influent and sewage- effluent samples was carried out by sublation, ion-exchange and alumina chromatography. The resulting extracts were analysed by HPLC using a gradient mobile phase system of methyl tetra-butyl ether (MTBE) and acetonitrile/ methanol (containing 0.1% acetic acid) with fluorescence detection (λ_{ex}=230nm, λ_{em} =302nm). A detection limit of 0.2ng for each individual homologue of APEO was achieved. The total APEO in influents from two sewage treatment plants in South East England varied from 126 to 410μg/L and in effluent levels from 40 to 228μg/L.

Lee *et al* [66] have reported a method of analysis for APEO in river water and sediment samples. They also used an amino-propyl column but with isocratic elution and fluorescence detection. The detection limits of APEO were , 0.02μg/L and 0.015μg/g d.w. respectively. Ethoxylate distributions have also been determined on silica [67-69], alumina [70], cyano [71], 10 pac [72], porous graphitic carbon (PGC) [73] and C_1 (TMS) [73] columns to good effect.

Reversed-phase HPLC can be used as a complementary method to normal-phase HPLC. It is able to separate the homologous compounds OPEO and NPEO but not the coeluting

ethoxylate oligomers. Thus, the quantitative analysis of NP and NPEO from waste water, river water or sewage sludge can be performed on C_8 column with isocratic methanol/ water elution and UV detection at wavelength 277nm. Reversed-phase HPLC has been used to provide information on the identity of the alkyl chain and also to determine the overall concentrations of APEO and as a rapid screening method [74,68,60,61].

2.2.2.2.3 Simultaneous Determination of Anionic and Non-ionic Surfactants

The determination of two classes of surfactant in one chromatographic run has been described by a number of groups. Marcomini and co-workers [75] were the first to describe a simultaneous technique to determine both anionic LAS and non-ionic APEO surfactants by reversed-phase HPLC on C_8 and C_{18} columns with acetonitrile and water gradient elution containing 0.02 M sodium perchlorate ($NaClO_4$) and 5% isopropanol for the determination of LAS and NPEO in laundry detergent. As would be expected from a reversed-phase method, the LAS and APEO surfactants were separated according to their alkyl chain length. The C_{18} column was also able to resolve the LAS positional isomers as well. However, for information on ethoxylate distribution, a separate normal-phase experiment was required.

Another method was developed by Marcomini and Giger [76] for the determination of both LAS and APEO, (i.e. 4-octylphenolpolyethoxylates [OPEO], and nonylphenolpolyethoxylates [NPEO]), and 4-nonylphenol (NP) in sewage sludges and river sediments by reversed-phase HPLC on C_8 columns using water/ acetonitrile gradient elution. LAS are separated according to their alkyl chain length, and coelution of NPEO

and NP is observed in contrast to the separation of NPEO and OPEO. Samples were extracted by soxhlet extraction. Recoveries of 85 to 100% were found for LAS, APEO, and NP. Results showed sewage sludge to contain 7.3g/kg LAS, 1.2g/kg NP, 0.22g/kg NP1EO and 0.03g/kg NP2EO. River water sediment contained 5.6g/kg LAS, 0.9g/kg NP, 0.80g/kg NP1EO and 0.70g/kg NP2EO. The NP, NP1EO and NP2EO concentrations were determined by normal-phase HPLC. No higher NPEO ethoxylates were found in any of the samples.

The method developed by Marcomini and co-workers has also been used for the determination of LAS and APEO in the marine environment [77], and applied to the analysis of the carboxylic biodegradation intermediates of LAS and APEO as well [78].
Di Corcia *et al* [79] have been developing methods which allow the simultaneous extraction of LAS and its sulphophenyl carboxylate (SPC) biodegradation intermediates, and NPEO and their corresponding NPEC and NP biodegradation intermediates, using three different elution systems. The samples were extracted using a SPE cartridge filled with graphitised carbon black (GCB). Recoveries of all compounds were in the range 89-99%. The method was used for the determination of all the above compounds in raw and treated sewage of mechanical-biological treated plant. The analysis method used was that developed by Marcomini and co-workers [78].

Graphitised carbon black (GCB) is an adsorbing medium which is produced by heating carbon blacks at 2700- 3000 °C in an inert atmosphere. GCB is an essentially non-specific, non-porous sorbent. A range of GCB is available with surface areas ranging

about between 8 and $100m^2/g$, depending on the type of starting material submitted to the graphitisation process. GCBs are capable of acting as both reverse-phase and anion exchange sorbents. The anion exchange sites are relatively few in number. They are thought to have a chromene-like structure, that is a burnt-off residue left over from the heating of carbon blacks [80] in producing graphitic carbon. In the presence of water and particularly of acid, this surface group is rearranged to form benzpyrylium salts (see Figure 2.2). The presence of these positively charged chemical impurities on the GCB surface enable it to act as both an anion-exchanger and a non-specific sorbent.

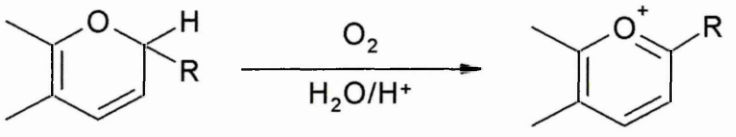


Figure 2.2 Rearrangement of chromene-like structure to benzpyrylium salt in graphitised carbon black.

Scullion *et al* [81] have published a method for the simultaneous determination of LAS and APEO using C_1 HPLC column. The method was an improvement on that developed by Marcomini and co-works [75] as it enables the resolution of the LAS positional isomers (if needed) and also the APEO ethoxymers in the same run. The issue of carboxylic biodegradation intermediates of these surfactants was not addressed.

2.2.2.3 Capillary Electrophoresis

Capillary electrophoresis (CE) is a relatively new separation technique [82], which has increasingly been applied for the analysis of ionic surfactants during the past few years [3,83-86]. The analytes are separated according to their electrophoretic mobilities

(determined by charge and size of the ion). Several buffer parameters, such as pH, ionic strength, and organic solvent content, can be varied to influence the migration behavior, leading to an improved peak resolution. Neutral surfactants do not possess an electrophoretic mobility. Therefore, additional interactions with buffer constituents are necessary for the CE separation of non-ionic compounds. In micellar electrokinetic capillary chromatography (MECC), micelle-forming agents, usually sodium dodecylsulfate (SDS), supply the possibility for such an interaction [87]. Hydrophobic substances with similar structure are difficult to resolve in MECC owing to their low solubility in water and high partition coefficients into the micellar phase. For that reason, another separation mode has been developed [88]. With the addition of organic modifiers in concentrations higher than 20% to electrophoretic buffers containing charged micelles, micelle formation will be inhibited, but the surfactants and nonionic analytes can still interact. The interaction between nonionic analytes and surfactants is called solvophobic association. Separation is based on differences in the strength of analyte-surfactant association complexes, which results in differences in effective electrophoretic mobilities. Non-ionic surfactants of the alkylphenol type have been analyzed using solvophobic association with sodium dodecyl sulfate (SDS) in buffers with high contents of acetonitrile and other organic solvent [89-91]

Another possibility is the derivatization to anionic compounds with phthalic anhydride, which has been carried out for alkylphenol ethoxylates before separation by capillary electrophoresis [92]. This method does not require any further interactions in the electrolyte system for electrophoretic migration of analytes and could be very useful for

the analysis of fatty alcohol ethoxylates (FAE). The nonabsorbing FAEs have to be transformed into chromophoric components to carry out UV detection. The reaction with phthalic anhydride leads to both absorbing and anionic analytes, allowing a less problematical electrophoretic separation.

Capillary electrophoresis (CE) analyses of technical nonylphenol ethoxylate (NPEO) and octylphenol ethoxylate (OPEOs) using fused-silica capillary(57cm x75μm i.d) and UV detection were performed to separate the surfactants into individual EO oligomers [90,91]. In systematic investigations, the separation efficiency could be considerably improved by addition of sodium dodecyl sulfate (SDS) and high amounts of acetonitrile (20-40%) to the electrophoretic buffer. Despite these results, the applicability of CE to the determination of non-ionic surfactants is limited because of the insufficient peak resolution of low and higher ethoxylates and relatively low detection sensitivity.

Heinig *et al* [93] have shown CE to be useful for the determination of the fatty alcohol ethoxylate (FAEs) surfactants in laundry detergent. Before separation, surfactants were derivatised with phthalic anhydride to render them suitable for UV detection at 200 nm. The authors found the method to be an excellent tool for the rapid finger printing of technical products and household formulations, and the results were comparable to those obtained by a standard HPLC method.

Bullock [89] has reported a CE method for the analysis of Triton X-100 (an octylphenol ethoxylate surfactant) standard. Excellent resolution of the X-100, which indicated about

20 oligomers, was achieved using 25mM boric acid buffer pH 8.6 in a (35:65) acetonitrile/water solution containing 50 mM sodium dodecyl sulphate (SDS) solution. The analytes were determined by UV detection at 200nm. The authors suggested a solvophobic mechanism for the separation which involves the association of the hydrophobic portions of the Triton ethoxymers with the hydrophobic portions of the SDS molecule.

Shamsi and Danielson [94] have used CE with indirect photometric detection for the simultaneous determination of mixtures of tetralkylammonium cationic surfactants and alkanesulphonate anionic surfactants. Salimi-Moosav and Cassidy [85] have also reported the separation of alkanesulphonates, but this time the method utilised non-aqueous EC with direct UV detection at 214nm.

2.2.2.4 Liquid Chromatography-Mass Spectrometry

Chromatographic analysis of surfactants and their biodegradation products is an analytical problem which is typically solved by different methods of liquid chromatography (LC). However, these methods lack sufficient specificity to identify without doubt the presence of traces of surfactants in complex matrices. Even when analyzing commercial mixtures of surfactants, MS data can be of support for assigning definitive identity to chromatographic peaks. Thus, coupling liquid chromatography to mass spectrometry is the key to the future of many LC methods. Research in methodologies in MS, notably LC-MS, has greatly benefited from such an international need and now can serve to fulfil the goals initially sought by such a technique, that is the

monitoring of non-volatile and polar target compounds with a specificity and sensitivity similar to GC-MS.

In the past 20 years, a large variety of interfaces have been developed to make the high vacuum of the mass analyzer compatible with the large amounts of liquids exiting from the LC column. LC-MS offers an excellent combination technique to achieve on-line separation as well as molecular weight and structural information on separated compounds. LC-MS has been extensively reviewed in the past years. Several books and review papers [95-101] which illustrate the principles, instrumentation and applications of LC-MS have been published. LC-MS is widely used in environmental sample analysis and has recently been applied to analysis of trace organic compounds in water [102-105] and specifically to surfactants in water [106-110].

The electrospray (ES) interface is the most important device introduced for LC-MS coupling. ES has opened new and exciting perspectives to the LC-MS technique. It is sufficient to say that the ES interface enables LC-MS analysis of compounds having molecular masses up to 400 000u (unified atomic mass units) [111] as the ES process is able to form multiply charged ions depending on the acid-base chemistry and hydration energy of the molecules. The ability to increase charge (z) permits the analysis of large molecular masses on a conventional quadrupole which have a limited m/z range of about 4000. The versatility of this interface is making it extremely popular among both analytical chemists and biochemists.

In the ES process, gas-phase ions are softly generated leading to formation of $[M+H]^+$ (positive-ion mode) or $[M-H]^-$ (negative-ion mode) even for the most thermally labile and non-volatile compounds. Spectra from non-basic analytes may also display intense signals for Na^+,K^+, NH_4^+ adduct ions. These cations are always present as impurities in organic solvents used as organic modifiers of the LC mobile-phase.

A very interesting option offered by the ES-MS system, which is not often practised, is that, by raising the electrical field in the desolvation chamber, the quasi-molecular ion can be accelerated to such a point that multiple collisions with residual molecules from the drying gas generate characteristic fragment ions. Provided the target compound is not coeluted, "in source" collision-induced dissociation (CID) spectra closely resemble those obtained by the more costly tandem MS technique [112]

.

Various interfaces have been used for the determination of surfactants by LC-MS. Escott and Chandler [113] proposed a LC-TS-MS procedure for analyzing simultaneously LAS and polyethoxylate-based non-ionic surfactants. They pointed out that the use of ammonium acetate had a dual role as a chromatographic ion-pair-forming agent for anionic species, such as LAS, and as a volatile electrolyte for TS-MS detection.

Popenoe *et al* [114] have developed a method using LC-ES-MS for monitoring for alkyl sulphates (AS) and thirty six alkylethoxylate sulphates (AES) in STP influents and effluents as well as in receiving water. Detection was performed in the ion-negative mode. Chromatographic separation was attained by C_8 column with gradient elution. Although the mobile-phase contained 0.3 mM ammonium acetate, sufficiently well

shaped peaks for the negative charged analytes were obtained. The surfactants were extracted from the aqueous matrices by SPE, C_2 cartridge. The effectiveness of using the ES-MS arrangement as a LC detector was fully evidenced by the fact that, although coeluted with AS, quantitation of AS could be achieved by extracting ion chromatograms at m/z relative to their $[M-H]^-$ ions from the TIC chromatogram. Limits of detection for AES in river waters were reported to be about 500 and 10 ng/L in the full-scan and SIM modes, respectively.

A very sensitive analytical procedure for determining AEO and NPEO in influent and effluents of STP, river water and drinking waters samples by SPE with a GCB cartridge followed by LC-ES-MS, has been developed by Marcomini *et al* [115]. Although the mobile-phase was acidified with 0.1 mM of TFA, analytes were detected as $[M+Na]^+$ adduct ions. The behavior can be explained by considering that polyethoxylate-containing compounds have a great tendency to form stable complexes with inorganic cations. Chromatography was adjusted in order to elute all the oligomers of NPEO and AEO homologues as single peaks. Analyte quantification was performed by the internal standard method, using $A_{10}EO_6$ as internal standard. This compound is found in negligible amounts in commercial AEO mixtures. Under these conditions, the limit of detection (S/N =3) was estimated to be 20pg/ component injected into the column or 0.6, 0.02, 0.02 and 0.0002 μg/L of each analyte in the influents and effluents of STP, river water and drinking water, respectively.

2.2.2.5 Other Mass Spectrometry Techniques

Organic MS has been a tool of analytical chemistry for more than 30 years; but has been applied to the analysis of surfactants only over past few years, in conjunction with GC but mainly with LC [116]. MS relies on creating charged ions for detection and consists of many variations, including the following: (i) electron ionisation [117], (ii) chemical ionisation [118], (iii) field ionisation, (iv) field desorption [119], (v) collisionally activated dissociation [120], (vi) fast-atom bombardment [121-125], (vii) photoionisation, and (viii) surface emission. MS is also used in combination with other methods such as GC-MS [126,127] LC-MS, and MS-MS with two different units in tandem. Electron ionisation (EI) is by far the most common method used for sample ionization in MS

.

MS is an unambiguous method for the determination of the molecular structure of the parent ions, and can give directly the weight distribution of surfactant oligomers. Fragmentation patterns yield information about the kind of the isomers, such as the location of side chains and degree of branching.

MS and, in particular, tandem mass spectrometry (MS-MS) allows determination of complex mixtures without prior derivatisation and chromatographic separation. However, the benefits that mass spectrometry provides such as simple preparation and short analysis times have to be balanced against the high cost of the instrumentation and cost of day to day running. However, some new ionisation techniques have been applied

to the direct identification of APEO and their degradation products in environmental samples

Fast atom bombardment (FAB) is useful for the identification of APEO, especially the higher oligomers, and its metabolites in environmental matrices. Ventura *et al* [128] produced a method for the identification of surfactants and their acidic metabolites in raw and drinking water by fast bombardment mass spectrometry (FAB-MS) and FAB-MS/MS. The surfactants were extracted from samples using an XAD-2 resin, followed by fractionation into acids, bases and neutrals. The acidic components were then derivatised to form the methyl ester by reaction with BF_3/MeOH. Analysis of the fractions by FAB-MS showed that non-ionic surfactants of the alkylphenol and alcohol ethoxylate types were the most abundant type of surfactant in both the raw and drinking water of the Barcelona area in Spain. The presence of their acidic biodegradation products NPEC in raw water, and bromononylphenol ethoxylates and brominated NPEC in drinking water, was also identified. The presence of brominated compounds in both raw and river water was thought to be due to the high concentration of bromide ions in discharges from salt mines located in the upper course of the river. The anionic surfactant LAS was also identified along with small amounts of cationic surfactants. The authors also used accurate mass measurements and tandem MS to aid identification where needed.

Borgerding and Hiles [129] also have developed a quantitative method for the analysis of LAS in a wastewater treatment plant by FAB-MS. The surfactants were extracted by C_{18}

disks. The method allowed the simultaneous analysis of all LAS homologues by examining the parent ions of m/z 183, which is a product ion common to all LAS homologues when subjected to collisionally induced dissociation (CID). It was also possible to determine branched alkylbenzenesulphonate (ABS) by examining the parent ions of m/z 197. Analysis time was approximately four minutes, because no chromatographic separation was required. The limit of detection was determined to be 0.5ug/L based in a 1L sample.

Evans *et al* [130] have developed a method to determine AEO in river water and effluents from STP, based on LC-TS-MS. Samples were extracted by a C_8, SPE cartridge and aliquots of the final extracts were then injected into a C_{18} HPLC column. This was operated isocratically with a mobile-phase composed of a water-tetrahydrofuran (55:45,v/v) solution. By using this mobile-phase, the column was able to separate AEO according to the lengths of both the alkyl and ethoxy chains as well as the highly branched AEO compounds from essentially linear ones. A continuous post-column addition of aqueous ammonium acetate was done before TS-MS detection. Individual AEO were identified by both $[M+H]^+$ and $[M+NH_4]^+$adduct ions. To compensate for variations in the instrumental response due to ionization efficiency, quantification was performed by an internal standard procedure. An $A_{11}EO$ mixture with an average of 9 ethoxy units, which is virtually absent in commercial detergent formulations, was used as internal standard. Although not applied to the analysis of unspiked real water samples, this method has the potential to quantify concentrations of AEOs at levels of 25-100ppb and less than 3ppb for individual homologues

Levsen *et al* [131] analyzed OPEO, NPEO and AEO by LC-MS with electron ionisation (EI). As an interface, a moving-belt device was used. While EI spectra from aromatic surfactants showed distinct peaks for the molecular ion, these were absent in spectra from the nonaromatic AEO surfactants. For these species, the EI ionization process produced abundant amounts of fragment ions with the general formula $[(CH_2CH_2O)_nH]^+$. Branched NPEO could be distinguished from linear ones, as decomposition of the former compounds produced abundant amounts of a fragment at m/z 131 due to the loss of the hexyl radical. Abundant production of quasi-molecular ions from the surfactants considered was achieved by a much softer ionization technique, i. e. chemical ionisation (CI) with isobutane as reagent gas. For NPEO and OPEO homologues with up to six ethoxy units, CI spectra displayed intense signals relative to daughter ions formed as a result of the loss of nonene (NPEO) or octane (OPEO). The CI spectra of higher homologues showed fragment ions at m/z 291 (NPEO) and 277 (OPEO) formed by the cleavage of the C-O bond at the level of the second oxyethylene unit. Conceivably, the moving-belt interface, which involves a thermal desorption step from the belt to the ion source, discriminated analytes on the basis of their volatilities, this resulting in distortion of the apparent relative abundances of the various oligomers. The moving-belt interface is no longer used today.

In any case, the same MS data could be achieved by using a commercially available interface, e.g. PB, since even this interface serves to link LC with an EI ion source. Probably, however, the same negative effect cited above could again occur with the PB interface.

Pattanaargsorn *et al* [132] have used atmospheric pressure chemical ionization (APCI) mass spectrometry for the determination of the oligomer distribution of APEO and fatty alcohol ethoxylate (FAEO). The instrument was operated in positive ion mode, and protonated molecular ions were seen for both types of surfactants. This method again showed the ability of mass spectrometry for the determination of mixtures of surfactants without the need for prior chromatographic separation. In this case the method was not used for the analysis of environmental samples.

Strife *et al* [133] used ion trap mass spectrometry for the analysis of the surfactant components of a shampoo. The authors operated the mass spectrometer in MS mode to provide a greater insight into the molecular structure of complex mixture than would be available by MS/MS alone.

In the last years, matrix-assisted laser-desorption/ ionization mass spectrometry (MALDI/ MS) was established as a powerful technique for the determination of the mass of large biomolecules and synthetic polymers [134-138]. MALDI is still a relatively new method of ionization in mass spectrometry. However, it is an extremely powerful tool when combined with time-of-flight (MALDI-TOF) detection, for the analysis of high molecular weight biopolymers with high sensitivity. MALDI-TOF mass spectrometry is a recently introduced [134,139] soft ionization technique that allows desorption and ionization of very large molecules even if in complex mixtures, such as the wide molecular weight distribution (MWD) present in synthetic and natural macromolecules. MALDI has evolved from the older technique of laser desorption/ ionisation (LDI) which involved the

irradiation of a solid sample with short, intense pulses from a laser in the IR or UV region, to produce quasi-molecular ions from thermally labile molecules. LDI however, has a number of important limitations, as it tends to be most efficient when the laser wavelength matches an intense absorbance band in the analyte molecule and then tends to lead to fragmentation with the loss of important molecular weight information [140].

The process of using a matrix to aid laser desorption of intact protein molecular ion was reported almost simultaneously and independently by Tanaka and co-workers [138] and Karas and Hillenkamp [134]. Tanaka's group used a liquid matrix of a fine powder of cobalt (300 Å) suspended in glycerol to produce LDI spectra of proteins and polymers with molecular weights up to 25 kDa, and multiply charged lysozyme quasi-molecular ions of 100 kDa. Karas and Hillenkamp [134] produced spectra using a solid UV absorbing organic matrix. Equal volumes of a very dilute solution of analyte (10^{-5} M) and nicotinic acid (10^{-3} M) were mixed, and a drop of the mixture applied to a metallic substrate. After air drying, spectra of various proteins such as β-lactoglobulin (MW 18277 Da) and albumin (MW 67000 Da) were recorded.

Since then, considerable attention has been paid to the various factors involved in obtaining good quality MALDI spectra, which include the laser ion source, choice of matrix, the solvent evaporation technique and method of detection.

MALDI-TOF has now become the method of choice (along with electrospray mass spectrometry) for molecular weight analysis of large biopolymers, a significant

achievement being the analysis of a singly charged human immunoglobulin species (MW= 1 Mda) [141].

There have been only a few papers published on the MALDI-MS of surfactants. Just and co-workers [142] have compared the use of MALDI with supercritical fluid chromatography (SFC) for molar mass determination of some APEO surfactants. They produced good MALDI spectra of these surfactants using 2,5-dihydroxybenzoic acid (DHB) as the matrix. Spectra of the APEO showed a mixture of $[M+Na]^+$ and $[M+K]^+$ adducts. Direct comparison between SFC and MALDI-MS proved that MALDI-MS provided a much better differentiation in the higher mass range but seemed to cause some discrimination in the lower mass region, with lower intensities than expected observed for lower molecular weight ethoxymers.

Thomson *et al* [143] produced MALDI-MS spectra for three classes of surfactant - non-ionic, anionic and cationic. The anionic surfactants, sodium dodecylsulphate (SDS) and sodium dodecylbenzene sulphonate, were successfully analysed to produce negative ion spectra, either simply as an aqueous solution allowed to dry on the metal probe, as in the case of SDS, or dispersed in ethylene bis [3-(2-naphthyl)acylate] as in the case of the latter anionic surfactant. Cationic surfactants, cetyldimethylethylammonium bromide, cetylpyridinium chloride and benzalkonium chloride produced good quality, intense spectra either neat or dispersed in DHB. Good spectra of the NPEO surfactants IGEPAL CO-850, CO-880 and CO-890 were produced as their sodium adducts by dispersal in DHB with NaCl added as a source of cations.

Parees *et al* [144] have briefly compared electrospray, FAB and MALDI ionisation techniques for the analysis of some commercial nonylphenol ethoxylate (NPEO) surfactants. Their findings seemed to indicate that the data obtained from the two methods are comparable for lower average molecular weight surfactant mixtures. However at higher average molecular weights, although the electrospray and MALDI results were similar, the FAB data exhibited distinctly lower molecular weight distributions than electrospray and MALDI, possibly due to fragmentation.

Another comparison has been made between MALDI, reversed phase HPLC and thin-layer chromatography (TLC) for the analysis of non-ionic surfactants. In this work Cumme and co-workers [145] compared the molecular weight information given by MALDI, TLC (using mass spectrometry to analyse the fractions) and HPLC. The MALDI spectra were obtained using DHB as the matrix, and the HPLC analysis was performed using a C_{18} column with an isocratic iospropanol : water (45:55) mobile phase containing 0.1% TFA. The results from these experiments did not show any major discrepancy between the average molecular weight found by HPLC and TLC with that found by MALDI. However the authors [145] state that for MALDI, ethoxymers with masses below 405 Da were not included in the results because of interferences from matrix ions.

Recently Willetts *et al* [146] have reported a method for the determination of nonylphenol ethoxylates in surface water by MALDI/MS [146]. Following extraction/preconcentration by C_{18} solid phase extraction, the sample was mixed with a

standard MALDI matrix (2,5-dihydroxybenzoic acid) with the addition of LiCl. The limit of detection was 40 μg/L based on a 250 ml sample.

Of the methods described for determination of surfactants in reagent-water and sea-water, HPLC and mass spectrometry have the advantage of being the most versatile and efficient and here were chosen as the methods for use in the initial part of the investigation described in this thesis.

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CHAPTER THREE

Determination of Alkylphenol Ethoxysulphonates and Related Surfactant Mixtures in Reagent Water and Sea-Water by Solid Phase Extraction and HPLC.

3.0 Introduction

Anionic and non-ionic surfactants have been widely used for Enhanced oil recovery (EOR) processes. Non-ionics have been used primarily as co-surfactants to improve the behavior of surfactant systems. Non-ionics are much more tolerant of high-salinity brine, but their surface-active properties (in particular reduction of interfacial tension (ITF)) are not generally as good as those of anionics. Anionic surfactants have been the most extensively used type of surfactant in EOR since they have good surfactant properties, are relatively stable, exhibit relatively low adsorption on reservoir rock, and can be manufactured economically. However, the very low cost anionic surfactants such as petroleum sulphonates cannot be used for off-shore reservoirs where sea-water is the only source of injection water, since they are not sufficiently salt tolerant [1].

Many papers have shown that it is possible to synthesize anionic surfactants that tolerate high concentrations of multivalent cations [2-5]. Anionic surfactants containing multiple units of ethylene oxide (EO) and/ or propylene oxide (PO) in their mid-section were found to satisfy many of the desired conditions. Commercial anionic surfactants of the propoxy and ethoxy type are polydisperse in the PO- and EO-groups. Ethoxylated anionic sulphonates are also fairly stable with respect to desulphonation by breaking of the C-S bond, under ordinary reservoir conditions [6]. Water solvolysis, H^+ catalyzed hydrolysis, and nucleophilic (HS^- and Cl^-) displacement reactions have , however, been observed. Each and every one of these reactions can dominate the decomposition rate under different conditions.

Although EO-sulphates are cheaper than the corresponding EO-sulphonates, they are hydrolysed at high temperatures and low pH. The pH of injected seawater is normally changed from about 8 to 4-6 due to the solubilization of CO_2 and ion-exchange between water and the reservoir rock. At 60 °C, the half life time for EO-sulphates is estimated to be about 7 and 30 years at pH=5 and pH =8, respectively [7]. The rate of hydrolysis increases exponentially with increasing temperature.

Alkylphenol ethoxylatesulphonates (APEOS) (Figure 3.1a and b) are a class of anionic surfactants. When the ethoxymer chain length of an APEOS is greater than three it is completely soluble in sea-water [8]. The development of APEOS and also alkylaryl sulphonates (Figure3.1c) that are able to tolerate both hard water and high salinities is therefore of great interest to the oil industry. Mixtures of these surfactants can be used for the chemical flooding of off-shore oil reservoirs with the use of sea-water as the injection fluid. Mixing the expensive APEOS with the less expensive alkyl aryl sulphonates leads to a reduction in the cost of chemical flooding in comparison to the use of APEOS alone [9]. This is, according to Rosen [10], due to synergism in mixed micelle formation. Usually, oilfield connate waters as well as the injection water for off-shore situations , have a relatively high content of divalent cations.

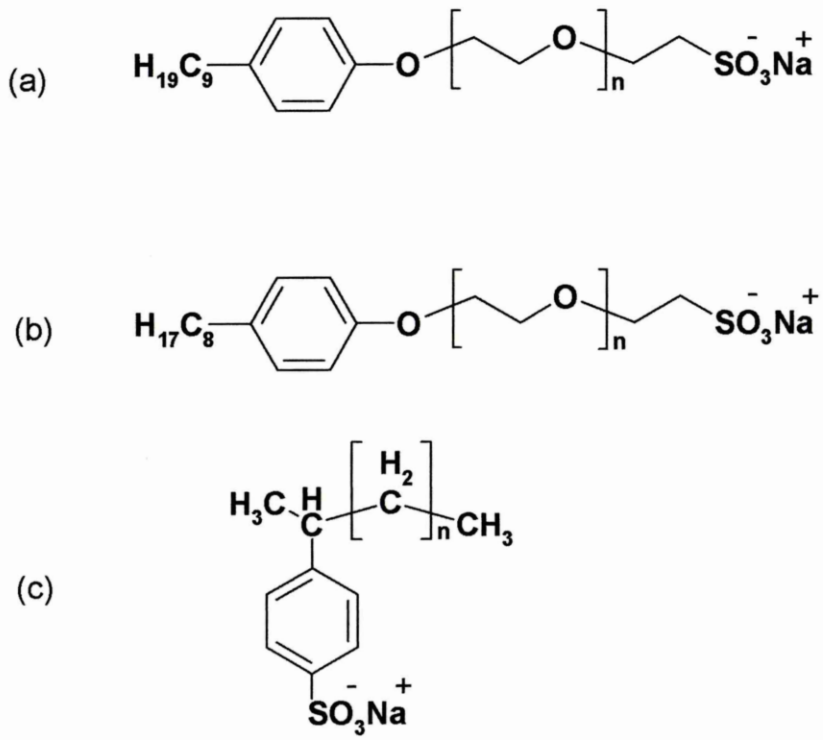


Figure 3.1 Structure of (a) Nonylphenol Ethoxylatesulphonate. (b) Octylphenol Ethoxylatesulphonate. (c) A typical alkyl aryl sulphonate.

The use of alkyl aryl sulphonates for chemical flooding has been suggested by Puerto and Reed [11]. For off-shore reservoirs, where sea-water is the only source of injection water, low cost surfactants such as petroleum sulphonates cannot be used. These materials are not sufficiently salt-tolerant. However, ethoxylated sulphonates have proved to be more resistant toward multivalent cations, and hence there is current interest in these surfactant systems [12,13].

The traditional method for the determination of anionic surfactants is based on a color forming reaction with methylene blue [14,15]. The method is, however, non-specific and

incapable of differentiating among individual compounds within the homologous series of anionic surfactants.

High performance liquid chromatography (HPLC) is the most widely used technique in surfactant analysis. Shamsi and Danielson [16] have compared the separation of long chain-alkyl sulphate, alkanesulphonate and alkyl phosphate anionic surfactants on three mixed-mode (RP8, RP4 and RP phenyl / anion-exchange) stationary phases. They suggested that the RP phenyl /anion mixed-mode column is the most suitable for the separation of a mixture of alkyl sulphate, alkane- sulphonate and alkyl phosphate surfactants due to short analysis times and high peak efficiencies [17].

Fjeld and Austed [8] have reported a HPLC method for the separation of nonylphenol ethoxylatesulphonate (NPEOS) and octylphenol ethoxylatesulphonate (OPEOS) surfactants at sea-water salinity using two different mixed-mode reversed-phase/ion-exchange columns (C_8/ SAX and C_{18} /SAX columns). They were able to separate the different APEOS oligomers up to degree of ethoxylation of at least 15. Furthermore, the oligomers of unconverted ethoxylated alcohols could be qualitatively detected and impurities of the type R-(EO)-R could be analysed in one chromatographic determination. They concluded that HPLC was an excellent quality test for commercial APEOS.

3.1 Experimental

3.1.1 Reagents and Materials

A commercial NPEOS formulation manufactured by Hoescht and an OPEOS formulation manufactured by PPG industries were gifts from Dr Tor Austad (Rogaland University centre, Norway). Synthetic sea-water was purchased from BDH (Poole, Dorset, U.K.). Graphitised carbon black (GCB) cartridges (500mg) were purchased from Supelco (Poole, Dorset, U.K.). Strong Anion-Exchange (SAX) cartridges (200mg) were purchased from Varian (Frampton Avenue, Harbor City, USA).

All solvents used were HPLC grade and were purchased from Fisher Scientific (Loughborough, U.K.). All water used was Milli-Q grade. Ammonium acetate was HPLC grade (Fisher Scientific). Acetic acid used was HPLC grade (Fisher Scientific).

3.1.2 Sample Pretreatment

In order to simplify the chromatograms obtained from the surfactant formulations, residual non-ionic starting material was selectively removed by the use of SAX solid phase extraction cartridges. The surfactant solutions were acidified to pH 3-4 with glacial acetic acid. The cartridge was washed with methanol (5ml) and distilled water (10ml). The acidified surfactant solution was passed through the cartridge which was subsequently washed with 2% acetic acid in methanol (5ml) to selectively elute the non-ionic surfactants. The cartridge was then washed with methanol (5ml) before elution of the anionic fraction in 2 M HCl in methanol:water (50:50) (15ml). This was evaporated

to dryness and the now "pure" NPEOS and OPEOS were dissolved in the appropriate solvent for further analysis.

3.1.3 Sample Preparation

All surfactants NPEOS, OPEOS and DBS were dissolved in synthetic sea-water/ distilled water.

3.1.4 Solid Phase Extraction Methodology

NPEOS and OPEOS were dissolved in synthetic sea-water and distilled water. The surfactant solutions were acidified to pH=3 (with conc. HCl) prior to extraction/ preconcentration by solid phase extraction (SPE). SPE was performed using a GCB cartridge method based that developed by Di Corcia *et al* [18]. The method uses three eluents:-

Eluent A. Dichloromethane/ methanol (70:30)

Eluent B. 25 mmol/L formic acid in dichloromethane/ methanol (90:10)

Eluent C. 5 mmol/L tetramethylammonium hydroxide (TMAOH) in dichloromethane/methanol (90:10).

The extraction procedure was as follows:-

1. The cartridge were first washed with dichloromethane (DCM) (50ml), methanol (MeOH) (50ml) and acetonitrile (ACN) (50ml) and left to stand in ACN overnight.
2. Condition cartridge with:-

7ml eluent C.

7 ml methanol

30 ml acidified water (pH 2, with conc. HCl)

3. Sample added to cartridge
4. Cartridge washed with:-

   7 ml water

   Air dried for 1 min

   2 ml methanol

   Air dried

5. Analytes extracted with:-

   7 ml eluent A

   7 ml eluent B

   7 ml eluent C

The fractions obtained with eluent C were evaporated to dryness under steady steam of N_2 gas and redissolved in 1ml HPLC mobile phase (eluents A and B were discarded).

3.1.5 HPLC instrumentation and conditions

All HPLC analyses were performed on a Waters 600-MS gradient HPLC system. The columns used were Alltech (Carnforth, Lancashire, U.K.) mixed-mode reversed-phase/ion-exchange columns, mixed-mode RP8/ anion 100Å, 7µm (150x4.6mm) and mixed-mode RP18/ anion 100Å, 5µm (150x4.6mm) and mixed-mode RP4/ anion 100Å, 5µm (150x4.6mm). Fluorescence detection was carried out using a Jasco FP-920 (Cheltenham, U.K.) fluorescence detector, $\lambda_{ex.}$=225nm and $\lambda_{em.}$=295nm. Data were

output to a Hewlett Packard HP 3396A integrator. Injections (100µl) were made using a Perkin-Elmer ISS-101 autosampler.

The mobile phase gradient used is shown in Table 3.1. All analyses of NPEOS were completed using the C8/SAX column described above and all analyses of OPEOS were completed using the C18/ SAX column.

Time (min.)	Flow Rate (ml/min.)	Solvent A Vol. %	Solvent B Vol. %	Gradient Curve
0	1	60	40	6
20	1	70	30	6
30	1	80	20	6
40	1	60	40	6

Table 3.1 Solvent A: Acetonitrile (ACN). Solvent B: Distilled water containing 0.1M Ammonium acetate (pH=4.7).

3.2 Results and Discussion

Austad and Fjelde [8,9] describe three HPLC methods based on mixed-mode reversed-phase / ion-exchange columns of C_4, C_8 and C_{18} types for the analysis of alkylphenol ethoxylatesulphonates and alkyl aryl sulphonates. These were used to quantify each of the three surfactant groups at sea-water salinities. The HPLC gradient program they employed is shown as Table 3.2.

Time (min.)	Flow Rate (ml/ min.)	Solvent A Vol. %	Solvent B Vol. %	Gradient Curve
0	2	60	40	6
10	2	70	30	6
20	2	80	20	6
80	2	60	40	6

Figure 3.2 Solvent A: Acetonitrile. Solvent B: Distilled water containing 0.02 M Potassium dihydrogen Phosphate (KH_2PO_4 (pH=4.7)).

3.2.1 Alkylphenol ethoxylatesulphonates

High performance liquid chromatography (HPLC) is an ideal technique for the analysis of small molecules (mol. wt. <1000). This technique offers the speed and resolution of gas chromatography without the need for the analytes to be volatile. Reversed-phase high performance liquid chromatography (RP-HPLC) with a buffered mobile phase is a powerful technique for separation of both neutral and ionic compounds. In the present work, in order to extend the life time of all columns used and to make separation

compatible with the electrospray interface of the Quattro I mass spectrometer (which is incompatible with the use of phosphate buffers), potassium dihydrogen phosphate (KH_2PO_4), was replaced by ammonium acetate buffer solution. Ammonium acetate is a good choice as a buffer system for LC-MS since it is:-

(i) Chemically stable, non-toxic, inexpensive and readily available; (ii) a good buffering medium in the pH range most useful for RP-HPLC; (iii) highly soluble in methanol and acetonitrile (ACN); (iv) an excellent masking agent for residual silanol groups on chromatographic media leading to greatly improved separation; (v) fully ionized and almost neutral in water; (vi) compatible with all commonly used HPLC detectors (UV-Vis, fluorescence, electrochemical); (vii) able to accelerate rates of proton equilibrium, important for ionic compounds; and (viii) relatively volatile and easily removed when used in conjunction with a mass spectrometer [19].

A secondary (and unexpected) benefit of the change of buffer system was a reduction in the retention times for the individual ethoxylate oligomers, in comparison to the published separations [8,9], without any adverse effect on their chromatographic resolution. Thus the analyses of nonylphenol ethoxylatesulphonate and octylphenol ethoxylatesulphonate were achieved with the simple ammonium acetate buffer system and this was employed for all subsequent HPLC and LC-MS analyses.

Figure 3.2 shows a typical HPLC fluorescence chromatogram for NPEOS obtained using the mobile phase conditions given in table 3.1 and a C_8/SAX mixed-mode column. The chromatogram shows excellent resolution of NPEOS ethoxymers. The peak at

retention time (t_r) = 25.46 min is NP_2EO, (using the nomenclature NP_xEO where x represents the number of ethylene oxide units in the hydrophilic chain) and the peak at 6.61min is $NP_{13}EO$. The advantage of this system is that it utilises solvents which are compatible with reversed-phase conditions.

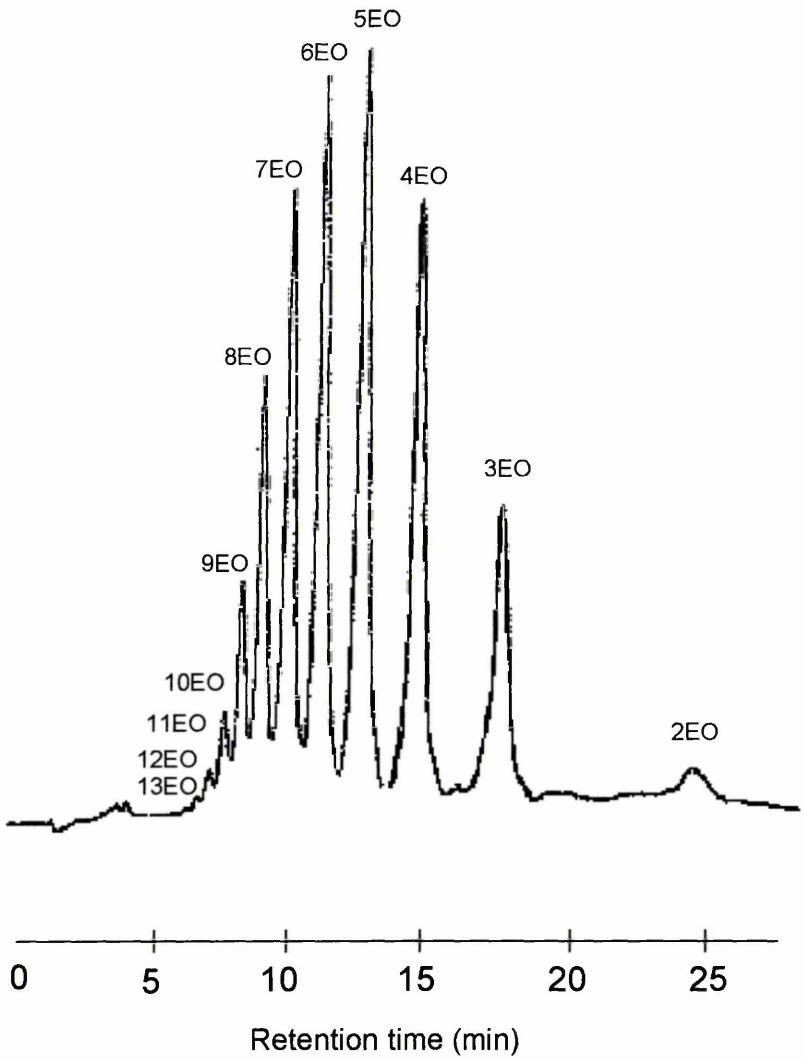


Figure 3.2 HPLC chromatogram of NPEOS on C8/SAX column.

Figure 3.3 shows the typical chromatogram obtained for OPEOS using the gradient program given in table 3.1 with the C_{18}/SAX column. The OP_1EOS peak had a retention time (r_t) =27.69 minutes and the largest ethoxymer (OP_8EOS) had a retention time of (r_t) =7.27min.

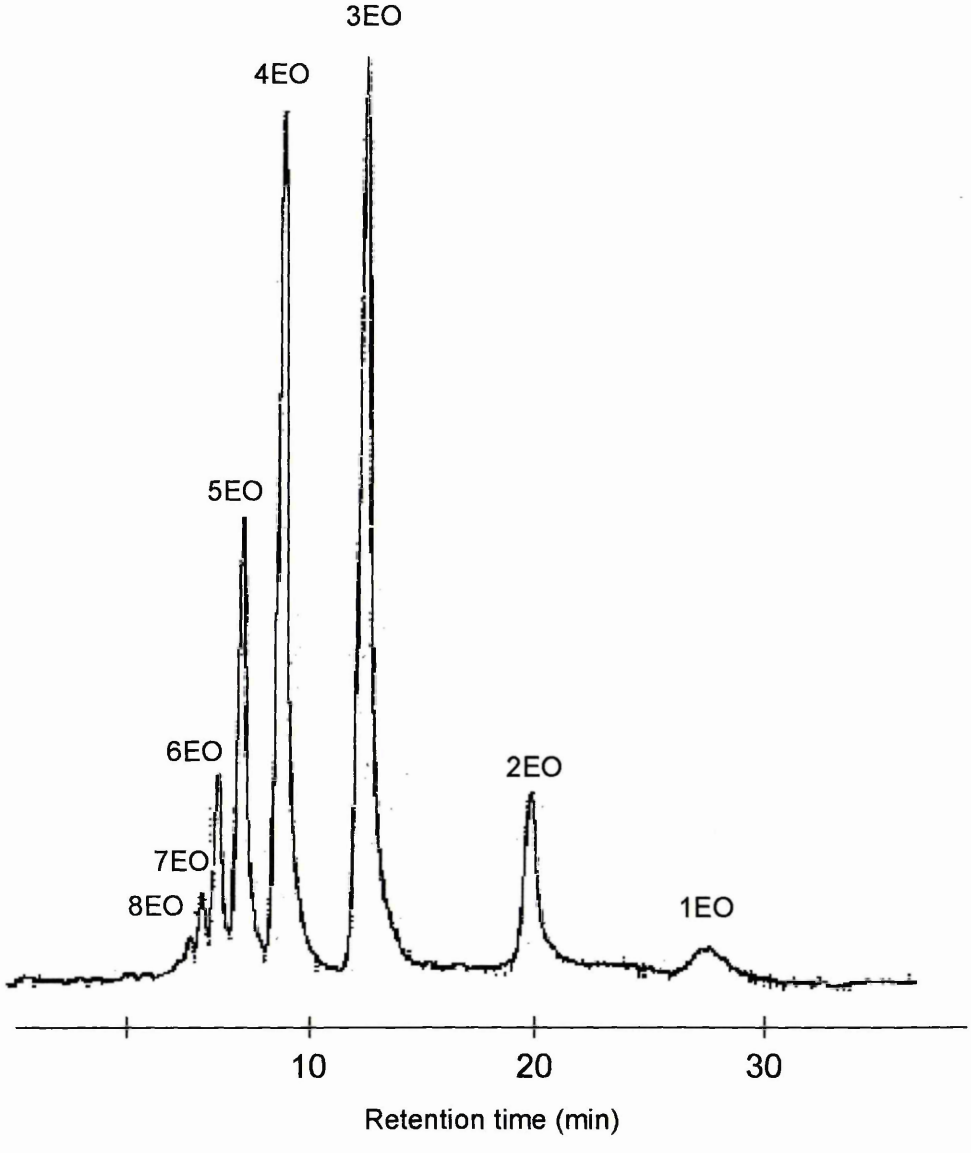


Figure 3.3 HPLC chromatogram of OPEOS on C_{18}/SAX

Figure 3.4 indicates that calculating an average of the values obtained from the HPLC chromatogram of the NPEOS formulation gives an ethoxymer chain length ranging from

2-13 units with an average of approximately 6.00. An approximation of the mole fraction of each ethoxymer indicated by the HPLC technique was calculated measuring the peak height of individual ethoxymers in the HPLC data and quoting it as a percentage of the total height (these calculations were also carried out using peak areas and no differences were observed). The average number of ethoxymers was then calculated as a weighted average based on these data. This is obviously a very approximate way of handling the data in terms of calculating accurate mole ratios of individual ethoxymers but does enable a simple comparison between the data obtained from HPLC and other techniques. This is in broad agreement with earlier studies although the range of ethoxymer units was reported in these to be from 2-15.

Figure 3.5 indicates that the average number of EO units calculated from the HPLC chromatogram of OPEOS, approximately 3.87, is in broad agreement with the earlier studies carried out HPLC only which gave an average EO number of 3.6 [8]. The calculated average number of EO units for OPEO was determined as above. However in our studies, the range of EO units for the OPEOS was found to be from 1-8, and this is not in agreement with the earlier work carried out by HPLC only, where the range was reported to be from 2-6 units. The discrepancy does appear to arise from the assignment of peaks in the HPLC chromatogram, with Austad and Fjelde [8] appearing to have misidentified the peaks in their tentative assignment. The misidentification results from the lack of pure ethoxymer standards (Austad and Fjelde are, in fact, cautious in their peak assignments in the paper) and this result is a clear demonstration of the advantage of the use of mass spectrometry either as MALDI or LC-MS in the analysis of polymers.

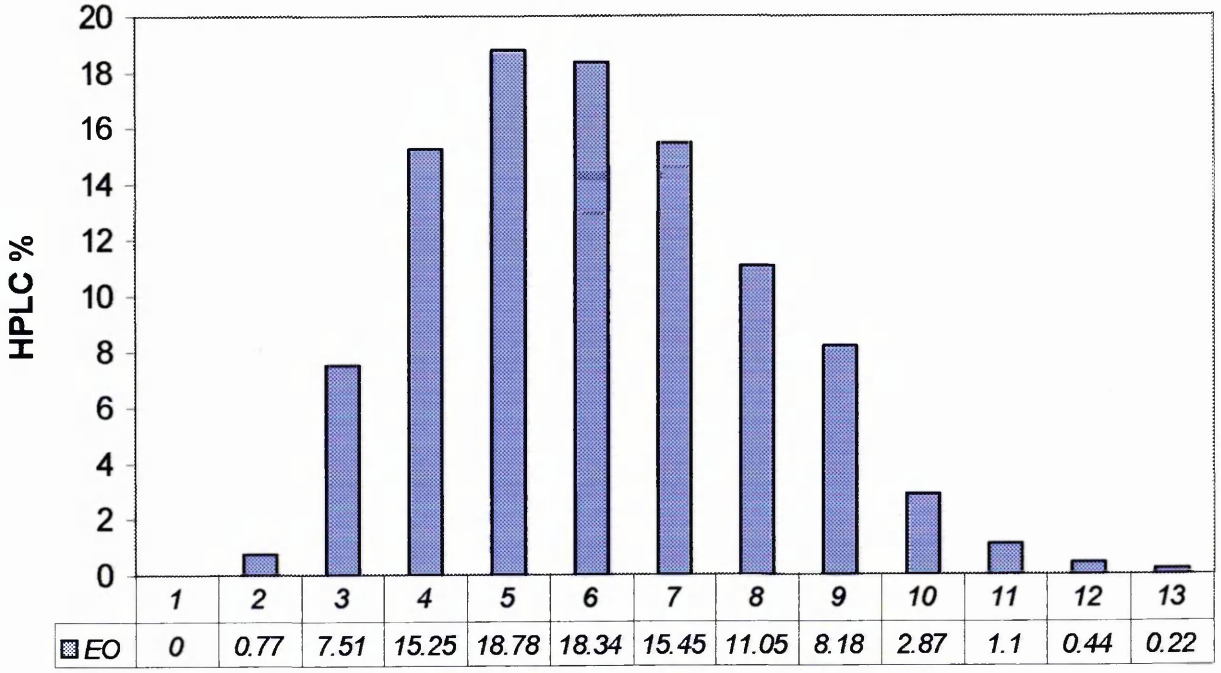


Figure 3.4 Graph showing the average number of ethoxymer units calculated from the HPLC chromatogram for NPEOS.

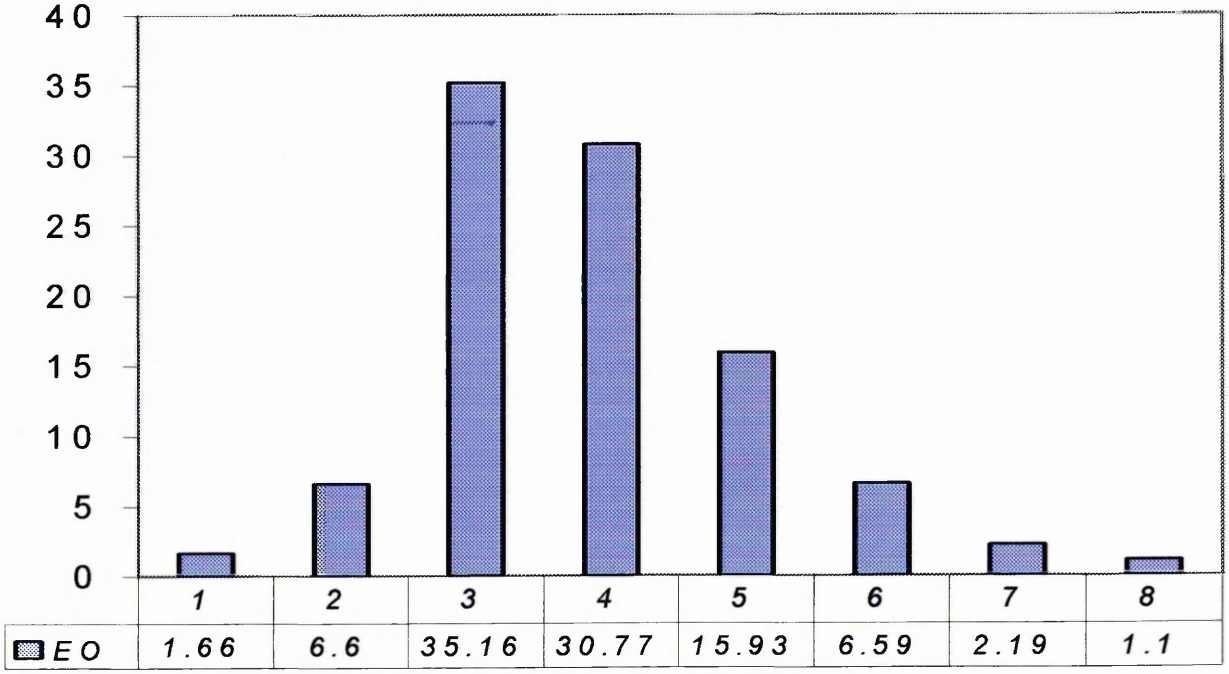


Figure 3.5 Graph showing the average number of ethoxymer units calculated from the HPLC chromatogram for OPEOS.

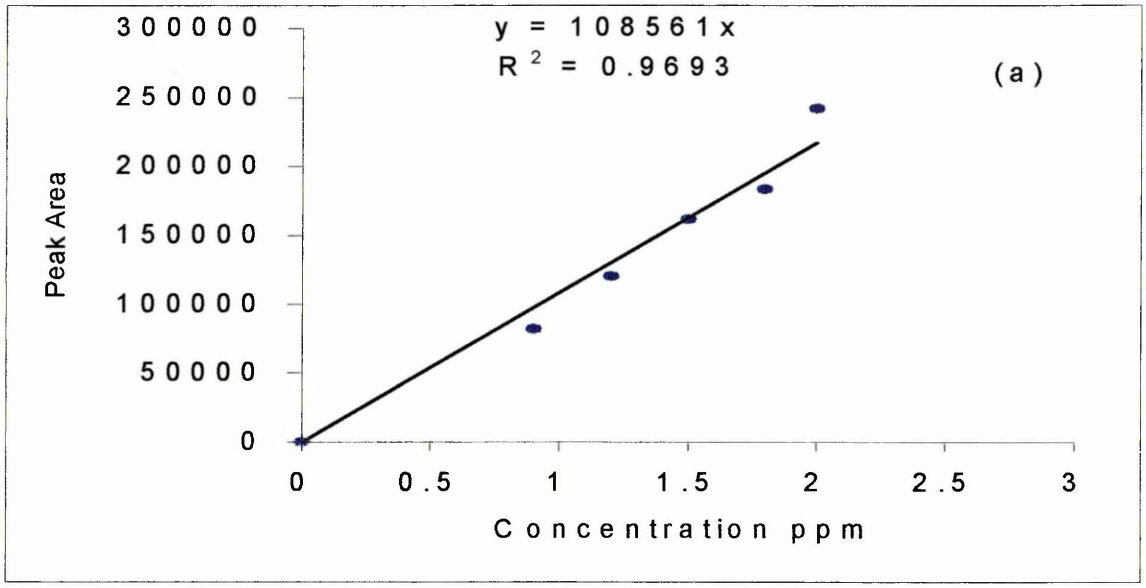
The method developed by Di Corcia *et al* [18] for the extraction of aromatic surfactants and their biodegradation intermediates from raw and treated sewage worked successfully, for APEOS on decreasing the concentration of the ion pair reagent, tetramethylammonium hydroxide (TMAOH), to 5mmol/L. Excellent recoveries were obtained for the extraction of APEOS from both reagent water and sea-water. The recovery data for extraction of NPEOS and OPEOS from each of these matrices are shown in Tables 3.3 and 3.4. As can be seen, excellent and reproducible recoveries were obtained for both surfactants from distilled water and seawater. These are the first data reported showing the extraction of these surfactants from sea-water by SPE and the good sensitivity and recoveries do suggest that SPE followed by HPLC with fluorescence detection is the method of choice for the determination of these compounds in surface water samples.

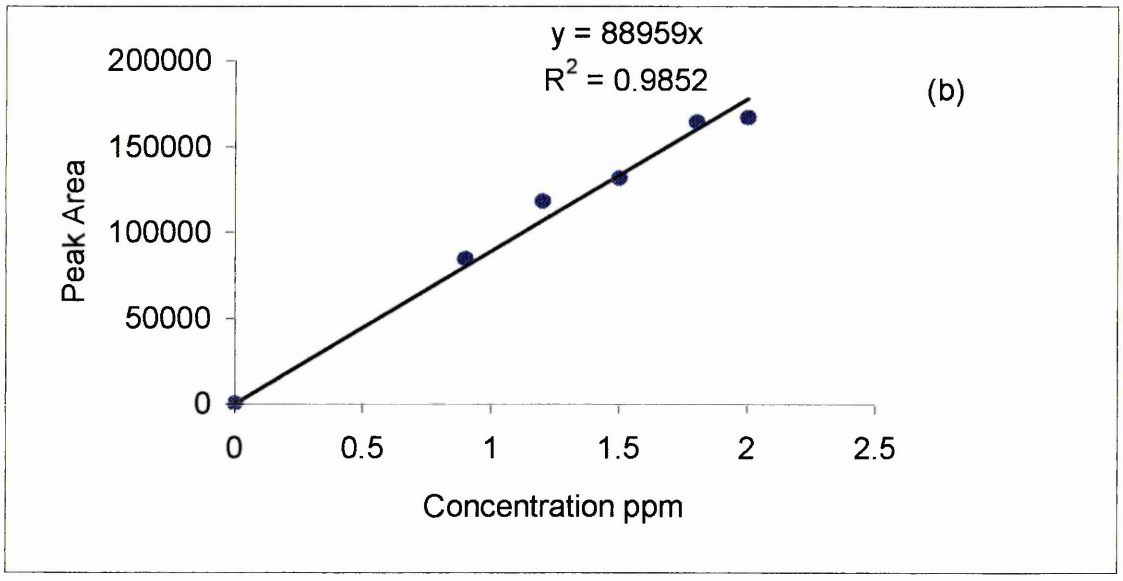
Type of water	Added in ppm	Found in ppm	Recovery %	RSD %
Distilled water	1.50	1.52	101	6.9
(3 replicates)	0.61	0.60	98	1.25
Sea-water	1.51	1.62	106	0.96
(3 replicates)	0.61	0.63	103	0.08

Table 3.3. Recoveries of NPEOS from sea-water and reagent water using GCB, SPE cartridges and extraction method of Di Corcia [18].

Figures 3.6 (a,b) show calibration curves obtained for the analysis of standard solutions of NPEOS, containing 0.90,1.20, 1.50, 1.80 and 2.00ppm dissolved in reagent water and sea-water. A plot of NPEOS concentration against peak area gives a linear relationship

(R^2=0.9693 and R^2=0.9852). These data are based on five different concentrations of NPEOS and the recovery is an average of three replicates.



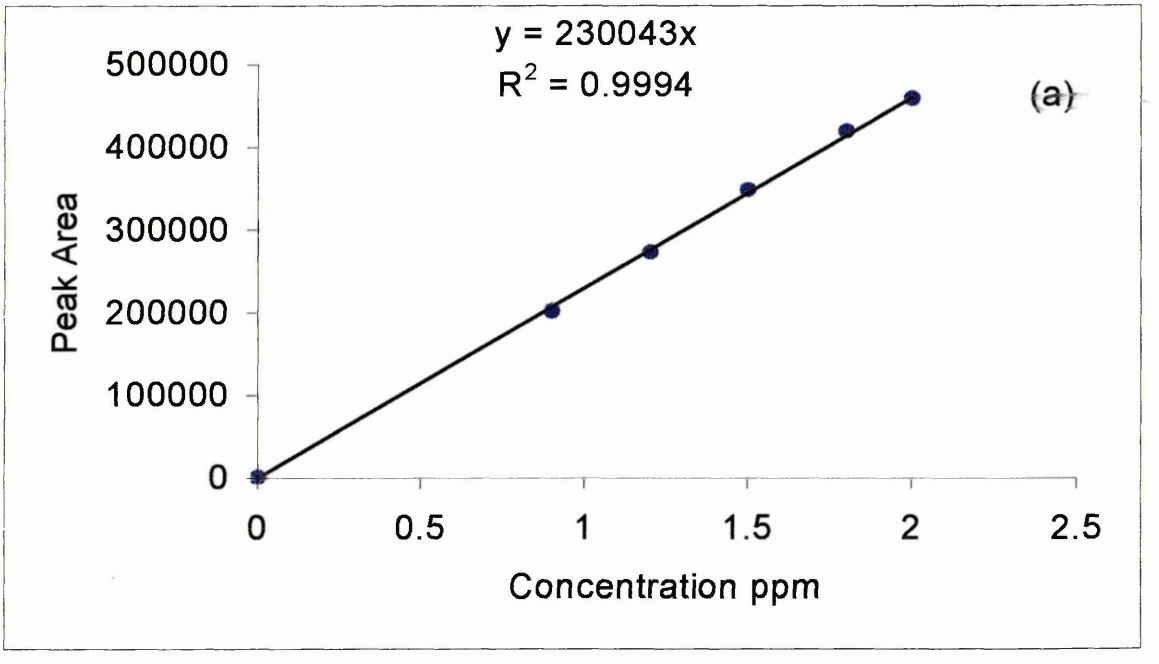


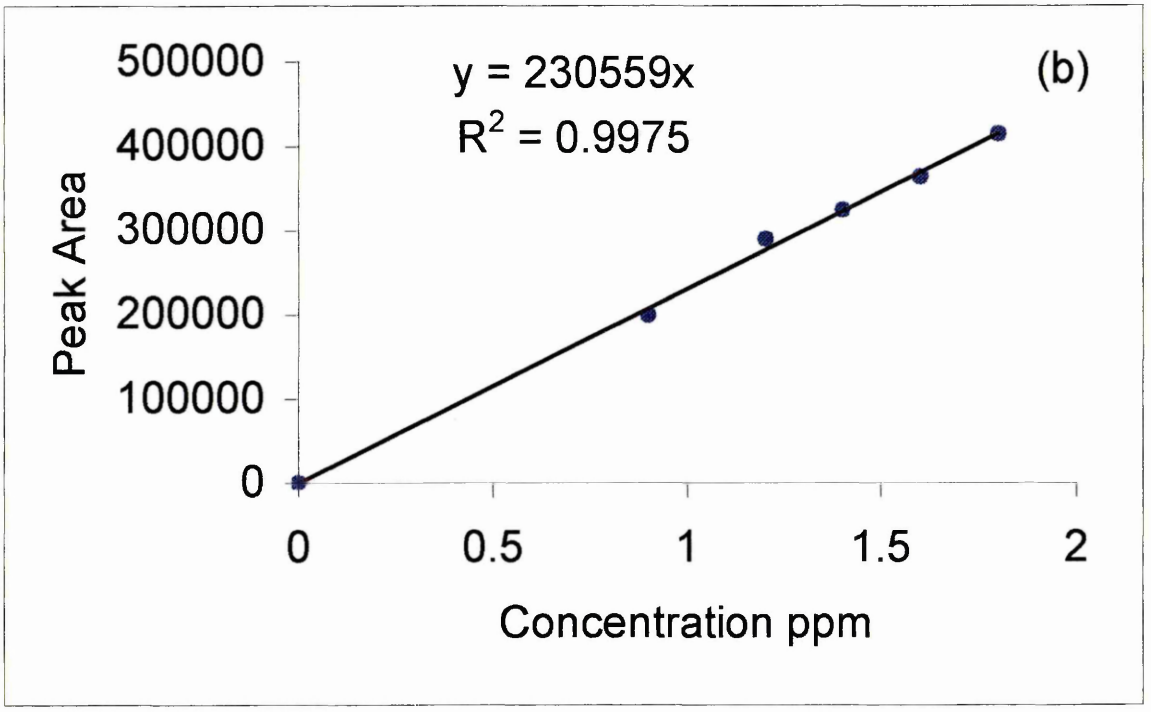
Figures 3.6 Calibration curves and unknown concentrations of NPEOS in (a) sea-water; (b) distilled water.

Type of water	Added in ppm	Found in ppm	Recovery %	RSD %
Distilled water (3 replicates)	1.56	1.55	99	8.55
Sea-water (3 replicates)	1.51	1.56	103	1.86

Table 3.4. Recoveries of OPEOS from sea-water and reagent water using GCB, SPE cartridges and the extraction method of Di Corcia [18].

Figures 3.7 (a,b) show calibration curves from five different concentrations of OPEOS in (a) sea-water , and (b) distilled water. A plot of OPEOS concentration against peak area in each of these matrices gives a good linear relationship (R^2=0.9994 and R^2=0.9975). The average of three replicates when measuring from these calibration curves indicates good results for OPEOS. As can be seen from Table 3.4, excellent recoveries of OPEOS were obtained for reagent and sea-water samples at the 1.5 ppm level.





Figures 3.7 (a and b)Calibration curves and unknown concentrations of OPEOS in (a) sea-water (b) distilled water.

3.2.2 Mixtures of ethoxylated and non-ethoxylated surfactants

The retention order of the NPEOS oligomers suggests that an electrostatic ion-pair interaction is the main retention mechanism involved [9]. The retention order was also in line with the expected salt tolerance of the anionic oligomers, i.e. the retention time increases with decreasing EO number. It was observed that the low EO number oligomers were retained more strongly on the C_{18} column relative to the C_8 column, and furthermore, dodecyl benzene sulphonate DBS^-, did not elute from any of these columns, even at very high acetonitrile fractions. These observations suggest that hydrophobic interactions between the surfactant tail and the stationary phase are also playing an important role in the separation of anionic surfactants on these mixed-mode columns.

In order to lower the hydrophobic interaction, Fjelde and Austad [9] used a C_4/SAX mixed-mode column for the separation of a mixture NPEOS and DBS^-. The gradient system used is shown in Table 3.5. The mobile phase contained 0.02 M potassium dihydrogen phosphate (KH_2PO_4) and phosphoric acid (pH 4.7) as buffer, in order to protonate the bonded amino-group, $-NR_2H^+$.

Time (min.)	Flow Rate (ml/min)	Solvent A Vol. %	Solvent B Vol. %
0	2	20	80
20	2	20	80
45	2	70	30
80	2	70	30

Table 3.5 Solvent A: Acetonitrile. Solvent B: distilled water containing KH_2PO_4 as buffer (pH4.7).

In the present work we also replaced KH_2PO_4 by ammonium acetate as buffer to increase the life-time and efficiency of the column. The solvent gradient used is shown in table 3.6.

Time (min.)	Flow Rate (ml/min)	Solvent A. Vol. %	Solvent B Vol. %	Gradient Curve
0	1	60	40	6
35	1	80	20	6
40	1	60	40	6

Table 3.6 Solvent A: acetonitrile ; Solvent B: distilled water containing ammonium acetate as buffer (pH4.7).

Figure 3.8 shows the typical chromatographic separation obtained for a mixture containing NPEOS and DBS^- using the C_4/SAX column. The resolution of individual homologues of DBS^- is not as sharp as that of the ethoxymers of NPEOS. The resolution of the NPEOS oligomers is good and comparable to the previous results obtained using the C_8 mixed-mode column. The NPEOS distribution begins with the peak at 4.00 min ($NP_{13}EOS$) and ends with the peak at 20.51 min. DBS distribution begins with the peak at 24.14 min and ends with the peak at 31.47 min.

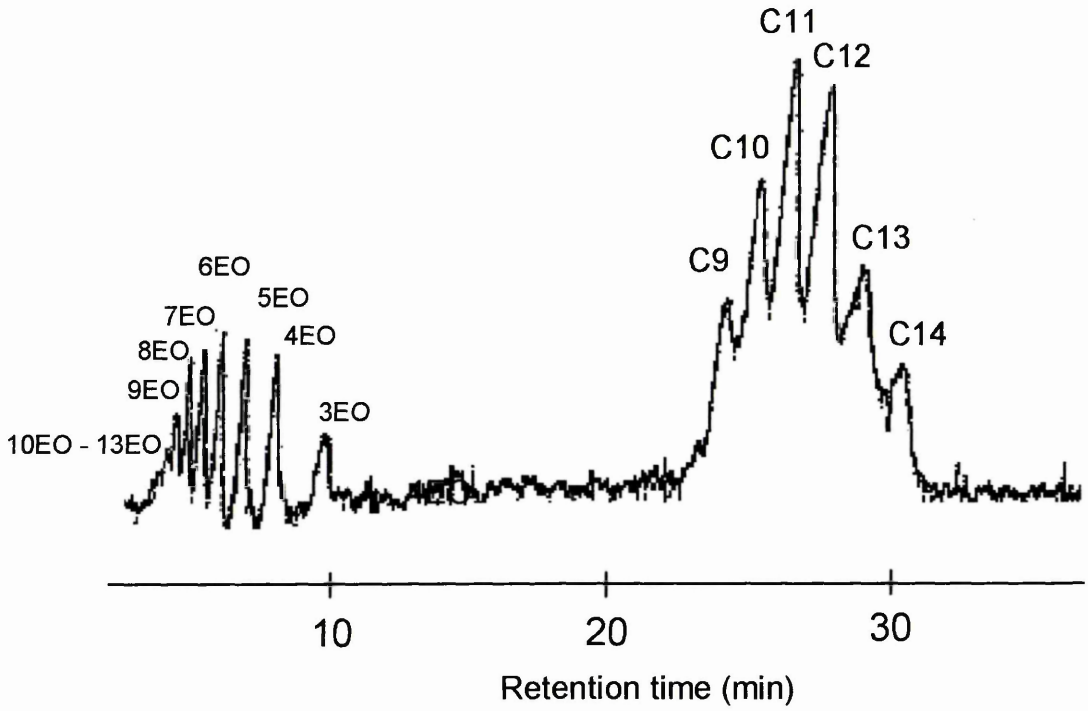


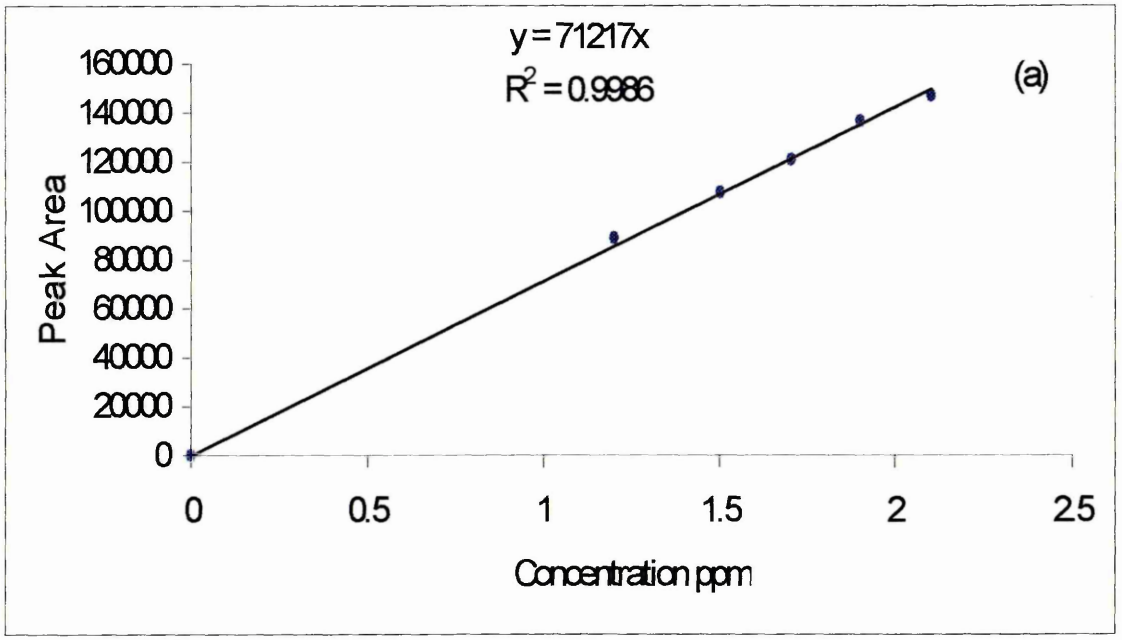
Figure 3.8 HPLC chromatogram of mixture of DBS and NPEOS using C_4/SAX

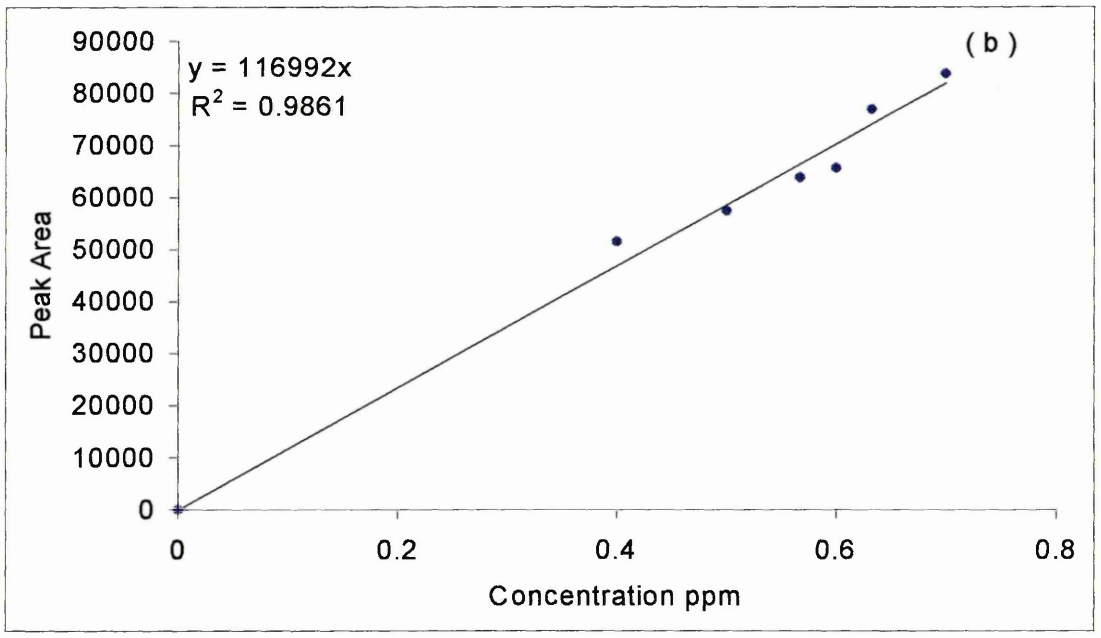
The method previously described (Section 3.1.4) was used for the extraction of a mixture of NPEOS and DBS^- at a mole ratio of 1:3 from sea-water. The recovery data for the extraction of these mixtures are shown in Table 3.7. These data are the mean of three recoveries.

Surfactants (3 replicates)	Added ppm	Found ppm	Recovery %	RSD %
DBS	1.83	1.60	87	5
NPEOS	0.61	0.63	103	0.08

Table 3.7 Recoveries of mixture of the DBS and NPEOS from sea-water using GCB, SPE cartridges and extraction method of Di Corcia [18].

Figures 3.9 (a and b) show calibration curves made by injecting standard solutions of DBS^- containing different concentrations of 1.20, 1.502, 1.703, 1.9 and 2.102 ppm and of NPEOS containing 0.40, 0.50, 0.56, , 0.60, 0.63 and 0.70 ppm, dissolved in sea-water. Plots of DBS^- and NPEOS concentrations against peak area give linear relationships (R^2=0.9986 and R^2=0.9861). These results were obtained from five different concentrations of DBS^- and six different concentrations of NPEOS.





Figures 3.9 (a and b). Calibration curves of standard solutions and unknowns of (a) DBS^- and (b) NPEOS dissolved in sea-water.

3.3 Conclusion

The aim of this work was to produce high performance liquid chromatographic and solid phase extraction methods which would separate and extract certain commercial surfactant formulations from sea-water. Commercial NPEOS and OPEOS consist of complex mixtures of oligomers which vary according to ethoxymer chain length. Data obtained from the HPLC method indicate that the NPEOS formulation has an ethoxymer chain length ranging from 2-13 units with an average of approximately 6.00 (calculating an average of the values obtained from HPLC techniques). This is in broad agreement with earlier studies, although the range of ethoxymer units was reported in these to be from 2-15. The HPLC data obtained for the OPEOS formulation suggests that it has an ethoxymer chain length ranging from 1-8 ethoxymer units with an average chain length of 3.87. This is in contrast to earlier studies carried out by HPLC only, which suggested that the chain length ranged from 2 to 6 ethoxymer units, with an average of 3.6.

Commercial mixtures of NPEOS and alkyl aryl sulphonates can be separated and quantitatively analyzed by use of a mixed-mode reversed-phase/ ion-exchange HPLC method using a the C_4/SAX column.. The individual oligomers of the NPEOS are separated well in the range 2-13. The alkyl aryl sulphonates of DBS are separated well enough to enable quantitative determination of the relative amounts.

In order to quantify surfactant concentrations in surface water and sea-water, calibration graphs were produced using standards for each surfactant. The peak area of surfactant oligomers from extracted each of these matrices can then be compared to the graph

produced from the standards, and as a result, the unknown concentration of the surfactant formulation can be estimated. A method for the extraction of NPEOS and OPEOS surfactants from reagent water and sea-water using graphitised carbon black (GCB) solid phase extraction cartridges has also been developed which gives excellent recoveries from both reagent water and sea-water at ppm levels. Since HPLC has been used to separate the complex mixtures which are surfactant formulations, this makes it possible to determine individual oligomer concentrations.

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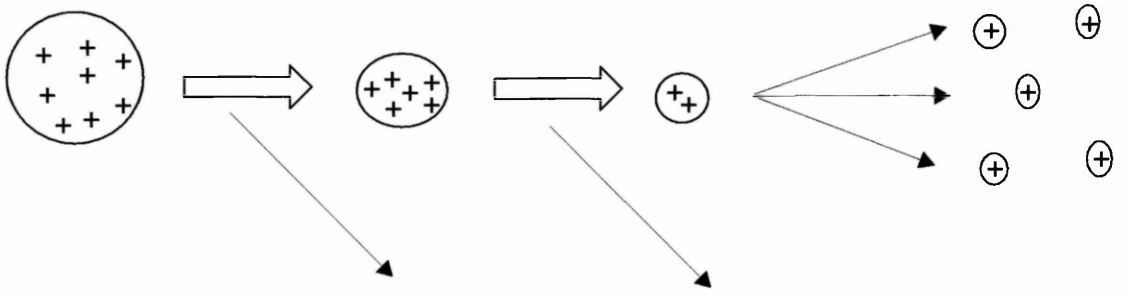
CHAPTER FOUR

A comparison of LC-MS and MALDI/MS Methods for Analysis of NPEOS and OPEOS Surfactant Formulations

4.0 Introduction

Electrospray ionisation (ESI) is an atmospheric pressure ionsation (API) technique. Thus, ionisation takes place at atmospheric pressure and ions are then transferred into the vacuum system of the mass spectrometer. Ionization is achieved during the electrostatic nebulisation of a solution of analyte ions by a large electrostatic field gradient (3kV/cm) between a spray needle and counter electrode. Highly charged droplets are formed in a dry "bath gas" of nitrogen. These charged droplets shrink as neutral solvent evaporates until the charge density exceeds the Rayleigh limit and coulombic repulsion causes the droplet to divide (Figure 4.1). The Iribane-Thomson [1,2] model suggests that the smaller droplets continue to evaporate and the process repeats until evaporation of charged solutes occurs.



Solvent evaporates from the droplets, causing the droplet to reduce in size, until coulombic repulsion occurs, causing ions to be ejected.

Figure 4.1 Mechanism of Ion Evaporation Stage of Electrospray

The evaporation of solvent is aided through the use of nebulising and "bath gases" (usually heated nitrogen, although the use of electron scavenging gases such as SF_6, has been suggested for negative ion electrospray, in order to prevent electrical discharges).

The nozzle skimmer arrangement allows the ions to be sampled from atmospheric pressure into the high vacuum of the instrument by passing through differentially pumped (rotary pumps) chambers. A typical commercial electrospray source is represented in Figure 4.2.

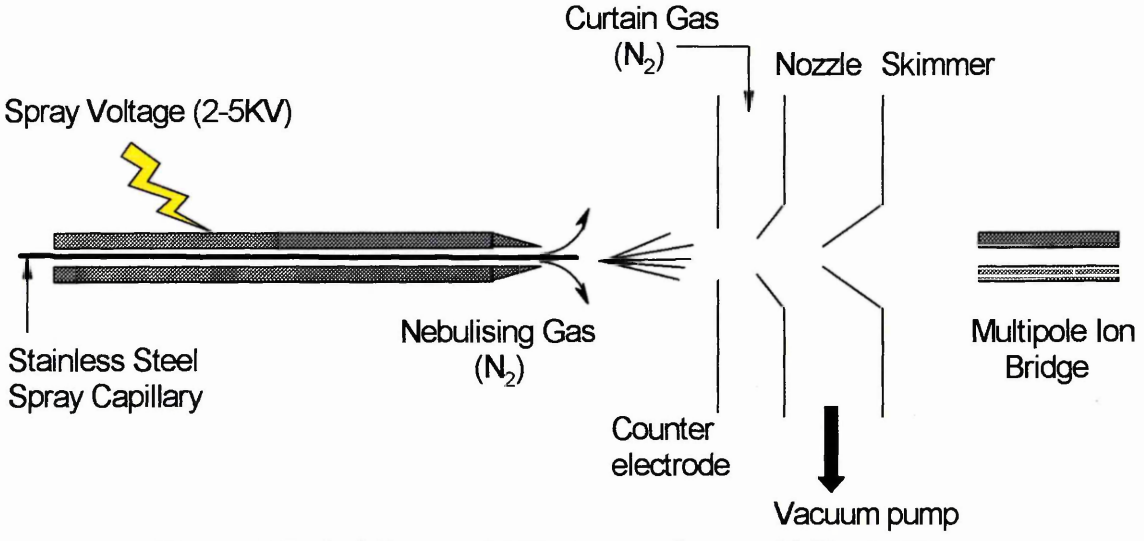


Figure 4.2 Typical Commercial Electrospray Source with Nozzle/ Skimmer Arrangement and Drying Gas.

Electrospray ionisation was originally described by Dole *et al* [3,4], who examined the intact ions of synthetic polymers in excess of 100 kDa RMM, with detection by Faraday-cage. No other data were published in the following ten years until Fenn *et al* [5,6] used ESI with a quadrupole mass analyser. Almost simultaneously, the work was independently reproduced by a Russian group using a magnetic sector instrument. They demonstrated the fundamental aspects of ESI, the analysis of modest molecular weight biomolecules and its ability as an interface for combining liquid chromatography and mass spectrometry [7].

Although the electrospray process is a "soft-ionization" method, structural information on analytes can also be easily achieved by collision-induced fragmentation with a suitable

adjustment of the electrical field existing in the intermediate-pressure desolvation chamber located between the ionization source and the mass analyzer region, so called "cone-voltage" fragmentation.

A number of liquid chromatographic methods have been proposed for determining those analytes of environmental interest that are not amenable to analysis by the gas chromatography/mass spectrometry technique. However, because of the legal implication of many environmental data, coupling liquid chromatography (LC) with mass spectrometry (MS) is key for the future of LC procedures. Electrospray ionisation (ESI) has rapidly emerged as a very promising technique for interfacing LC to MS. The LC/ES/MS system has proven to be a sensitive technique for analyzing compounds of environmental interest that exist as ions in solution [8,9].

Matrix-assisted laser desorption/ionisation-mass spectrometry (MALDI-MS) permits the analysis of high molecular weight compounds with high sensitivity. MALDI is a method that allows for the ionisation and transfer of a sample from a condensed phase to the gas phase in a fashion similar to fast atom bombardment (FAB). The primary difference between MALDI and FAB is that while FAB uses an atom or ion beam and a liquid matrix, MALDI uses a solid matrix, and the ionising beam is laser light. Ion formation in MALDI is accomplished by directing a pulsed laser beam onto a sample suspended or dissolved in a matrix. The matrix plays a key role in this technique by absorbing the laser light energy and causing the matrix material to vaporize. (The vaporized matrix will carry some of the sample with it). Once in the gas phase, the matrix may play a role in

the ionisation of the analyte molecules. The charged molecules will then be directed by electrostatic lenses from the ionisation source into the mass analyzer (Figure 4.3 shows ionisation in a typical MALDI/ TOF instrument). Uncharged molecules will often react with the matrix or other molecules to produce charged species, transferred electrostatically into the mass analyzer. Once the molecules in the sample are vaporized, time-of-flight mass analysis is often used to separate the ions according to their mass-to-charge ratio (m/z).

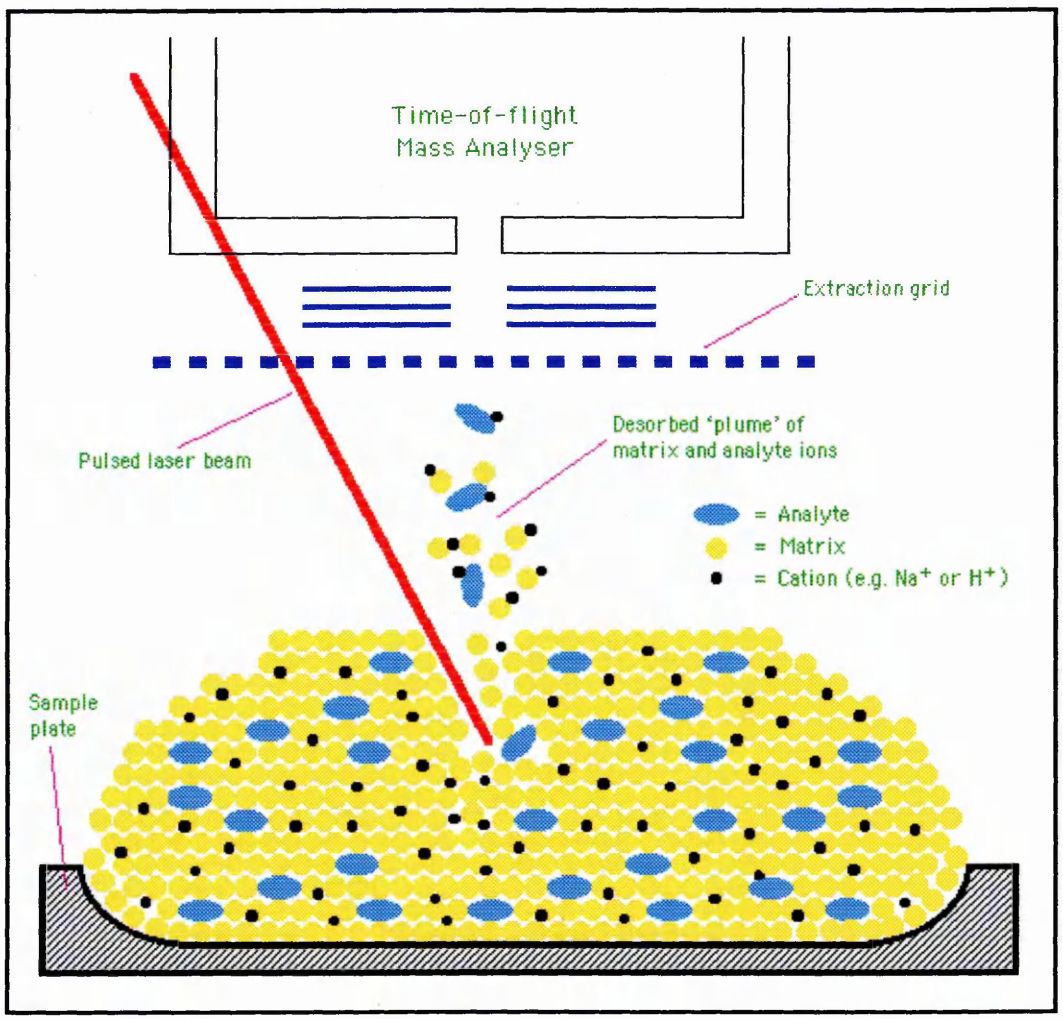


Figure 4.3 A typical commercial MALDI-TOF system.

4.1 Experimental

4.1.1 Reagents and Materials

A commercial NPEOS formulation manufactured by Hoescht and an OPEOS formulation manufactured by PPG industries were gifts from Dr Tor Austad (Rogaland University centre, Norway). Strong Anion-Exchange (SAX) cartridges(200mg) were purchased from Varian (Frampton Avenue, Harbor City, USA). Alpha-cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid were purchased from Aldrich (Poole, Dorest, U.K.).

All solvents used were HPLC grade and were purchased from Fisher Scientific (Loughborough, U.K.). All water used was Milli-Q grade. Ammonium acetate was HPLC grade (Fisher Scientific). Acetic acid used was HPLC grade (Fisher Scientific).

4.1.2 Sample Pretreatment

In order to simplify the chromatograms and mass spectra obtained from the surfactant formulations, residual non-ionic starting material was selectively removed by the use of SAX solid phase extraction cartridges. The surfactant solutions were acidified to pH 3-4 with glacial acetic acid. The cartridge was washed with methanol (5ml) and distilled water (10ml). The acidified surfactant solution was passed through the cartridge which was subsequently washed with 2% acetic acid in methanol (5ml) to selectively elute the non-ionic surfactants. The cartridge was then washed with methanol (5ml) before elution of the anionic fraction in 2 M HCl in methanol:water (50:50) (15ml). This was evaporated to dryness and the now "pure" NPEOS and OPEOS were dissolved in the appropriate solvent for further analysis.

4.1.3 HPLC instrumentation and conditions

All HPLC analyses were performed on a Waters 600-MS gradient HPLC system. The columns used were Alltech (Carnforth, Lancashire, U.K.) mixed-mode reversed-phase/ion-exchange columns, mixed-mode RP8/ anion 100Å, 7µm (150x4.6mm) and mixed-mode RP18/ anion 100Å, 5µm (150x4.6mm).

4.1.4 Liquid chromatography- Mass Spectrometry

All analyses were performed on a Quattro I (Micromass Manchester U.K.), mass spectrometer, equipped with an electrospray ionisation (ESI) source. A Jasco Pu-980 intelligent HPLC pump system was used in this case. All other HPLC conditions were as described above (see Table 3.1in Chapter Three of this thesis for the HPLC gradient systems employed). The mass spectrometer was operated in positive ion mode. A capillary voltage of 3.5 kv, and a skimmer cone voltage of 30V were used. Mass spectra were collected in full- scan mode, over the range 100- 1200 m/z at 3s/scan. The source temperature was maintained at 80°C. Nitrogen was used as the drying and nebulising gas.

4.1.5 MALDI-MS

All MALDI analyses were performed on a Finnigan Vision 2000 reflectron-based time-of-flight (MALDI/TOF/MS) instrument. Nonylphenol ethoxylatesulphonates (NPEOS) and octylphenol ethoxylatesulphonates (OPEOS) were dissolved in methanol : water(50:50). Alpha–cyano-4-hydroxycinnamic acid (90mg/ml) and 2,5-dihydroxybenzoic acid (90mg/ml) were dissolved in 0.1% TFA in methanol. Lithium chloride (10mg/ml) was dissolved in methanol. 200µl of the NPEOS or OPEOS

solutions , 40µl of matrix and 10µl of LiCl were mixed and 1µl of the resulting solution was placed on the stainless steel target of the instrument for analysis.

4.2 Results and Discussion

A study of both NPEOS and OPEOS surfactant formulations in aqueous samples has been carried out by the combination of ion-exchange RP-HPLC and mass spectrometry.

Figure 4.4 shows the electrospray positive ion LC-MS base peak intensity chromatogram of NPEOS, and selected mass chromatograms are given in Figure 4.5 (a)-(k). Using the chromatographic conditions employing the ammonium acetate buffer system, the NPEOS ethoxymers eluted between 18.32 to 31.12 minutes. Figure 4.6 (a)-(j) shows the mass spectra obtained for each of the ethoxymers. Interestingly, the spectra for the NPEOS oligomers in Figure 4.6 show that the peaks produced have m/z values corresponding to NH_4^+ adducts, the sodium ion presumably being completely displaced by the use of the ammonium acetate in the mobile-phase. Ions corresponding to $[M\text{-}Na+H+NH_4]^+$ adducts for NPEOS ethoxymers were observed at m/z 478, 522, 566, 610, 654, 698, 742, 786, 830 and 874.

Figure 4.7 shows the corresponding base peak intensity chromatogram for the analysis of chromatograms are shown in Figure 4.8 (a)-(j). The mass spectra (Figure 4.9(a)-(h)) obtained differ by 44 Da relative to the various ethoxymers of OPEOS and the peaks produced have m/z values corresponding to NH_4^+adducts. In electrospray positive ion mode, the ions corresponded to $[M\text{-}Na+H+NH_4]^+$adducts and are observable at m/z 377, 421, 465, 509, 553, 597, 641 and 685.

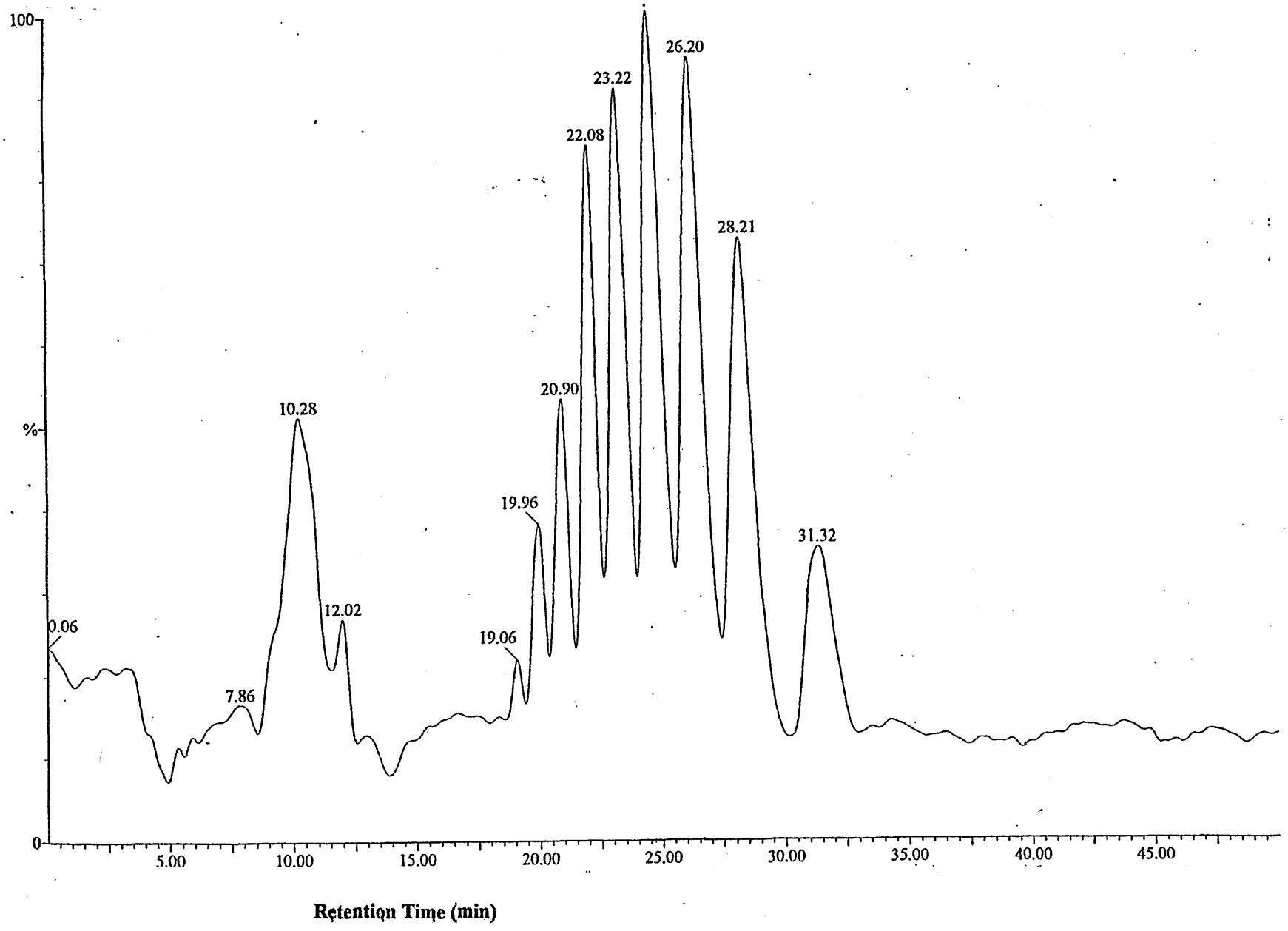


Figure 4.4 LC-ES-MS (positive ion) base peak intensity chromatogram of NPEOS surfactant.

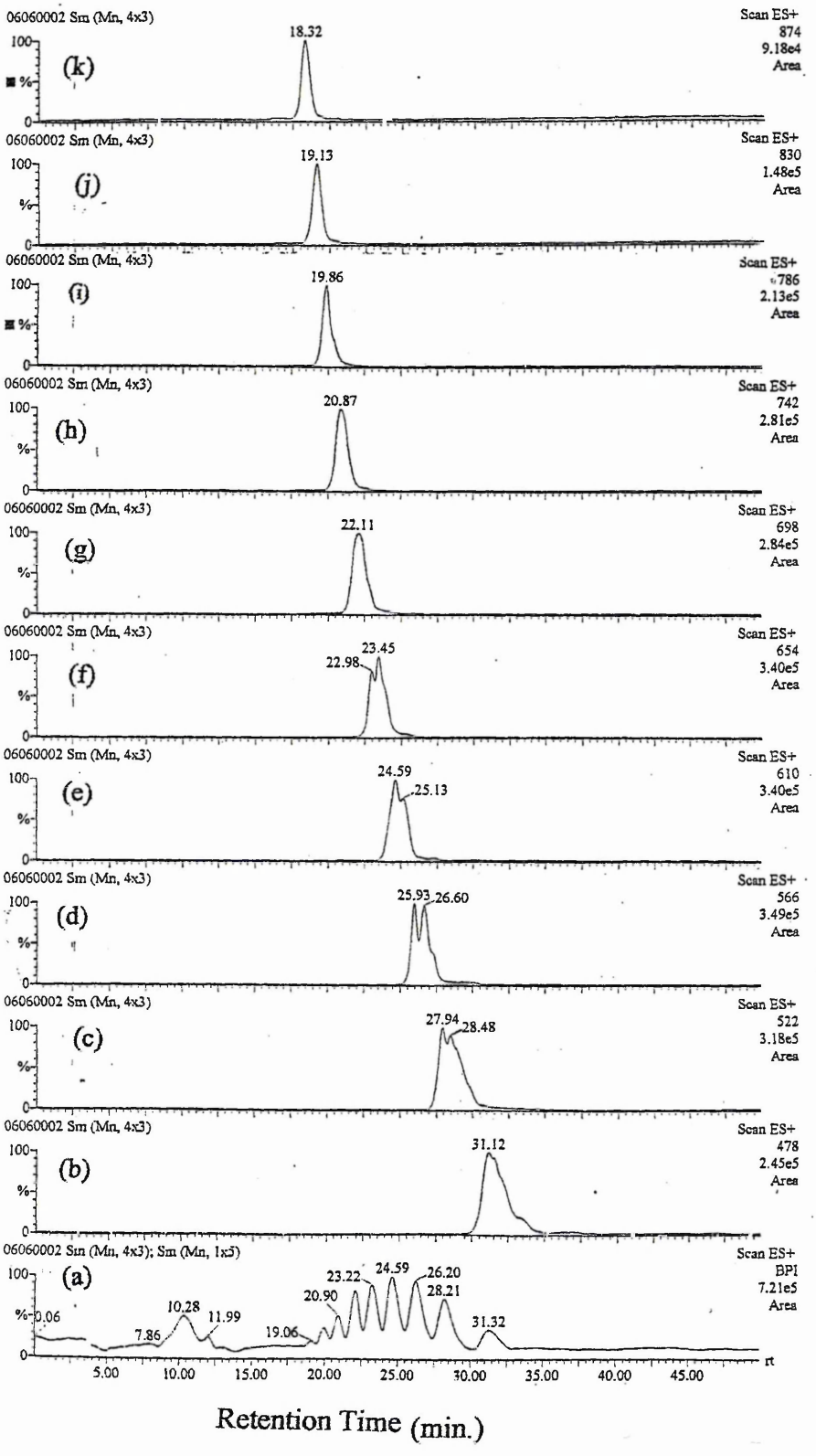


Figure 4.5.Individual mass chromatograms (a)-(j)and (k), the base peak intensity mass chromatogram, for the positive ion LC-ES-Ms analysis of a commercial NPEOS surfactant.

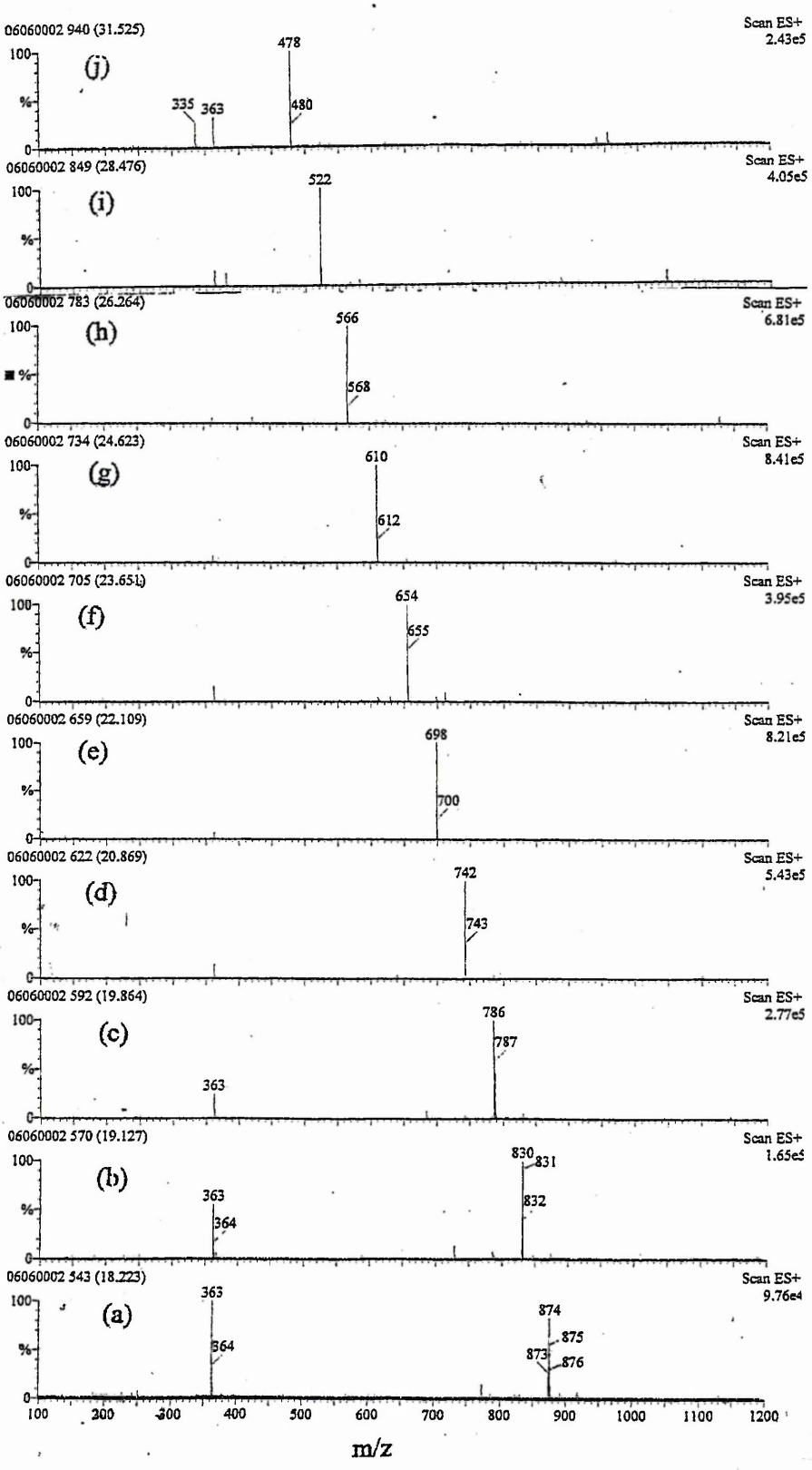


Figure 4.6. Electrospray positive ion mass spectra for the individual oligomers (a)-(j) of a commercial NPEOS surfactant formulation.

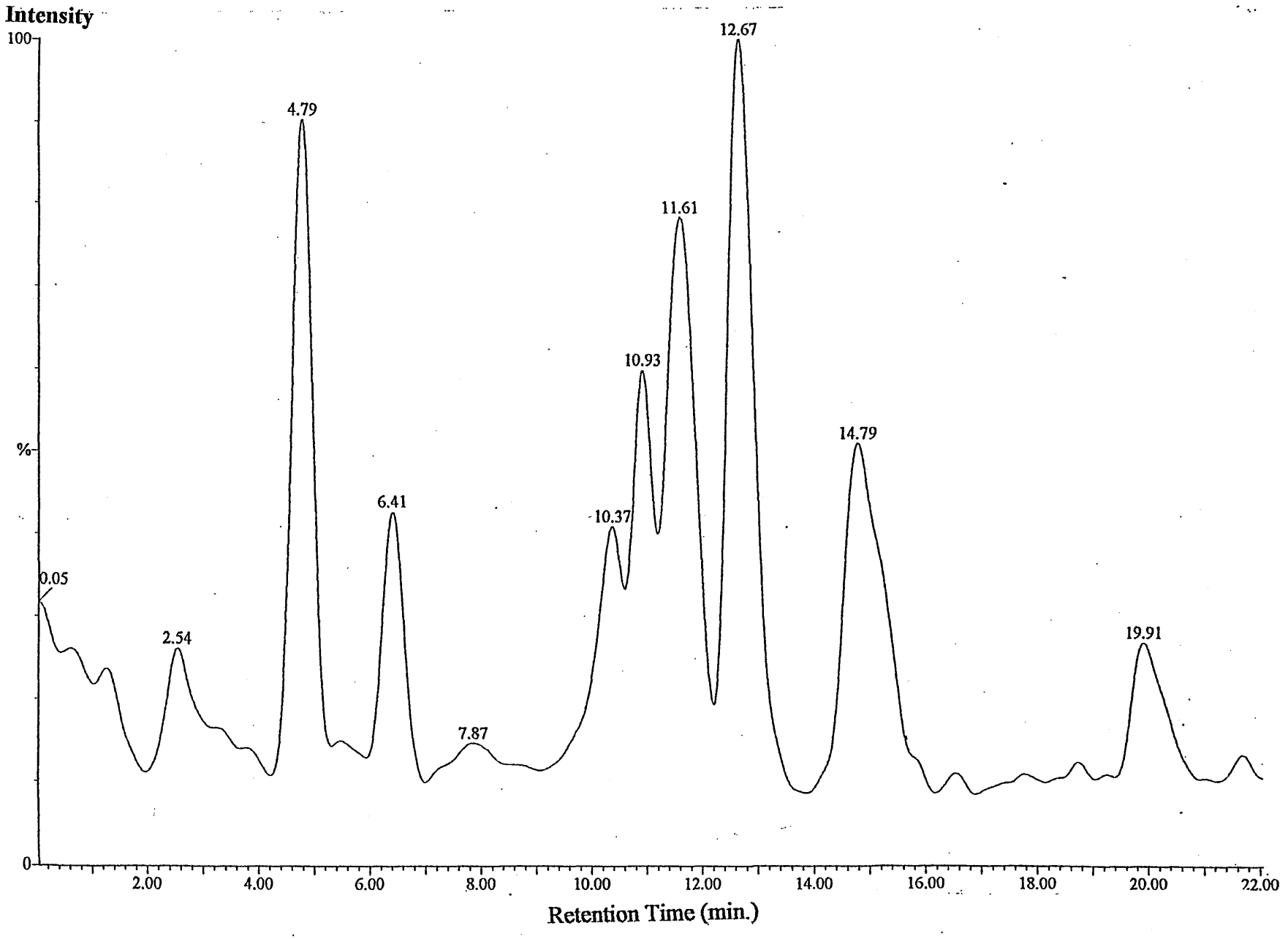


Figure 4.7. LC-ES-MS (positive ion) base peak intensity chromatogram of commercial OPEOS surfactant.

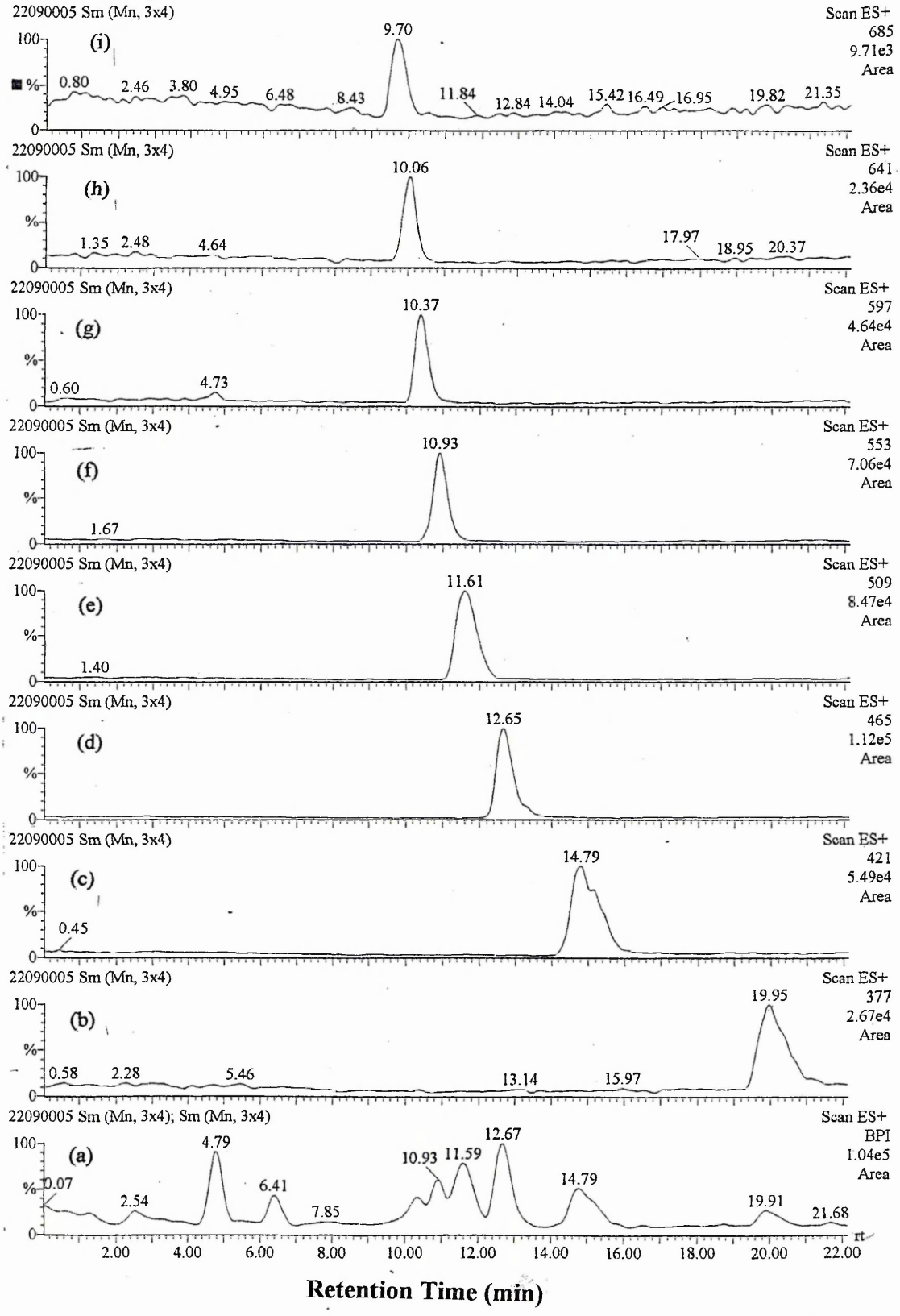


Figure 4.8. Individual mass chromatograms (a)-(h)and (i), the base peak intensity mass chromatogram, for the positive ion LC-ES-Ms analysis of a commercial OPEOS surfactant.

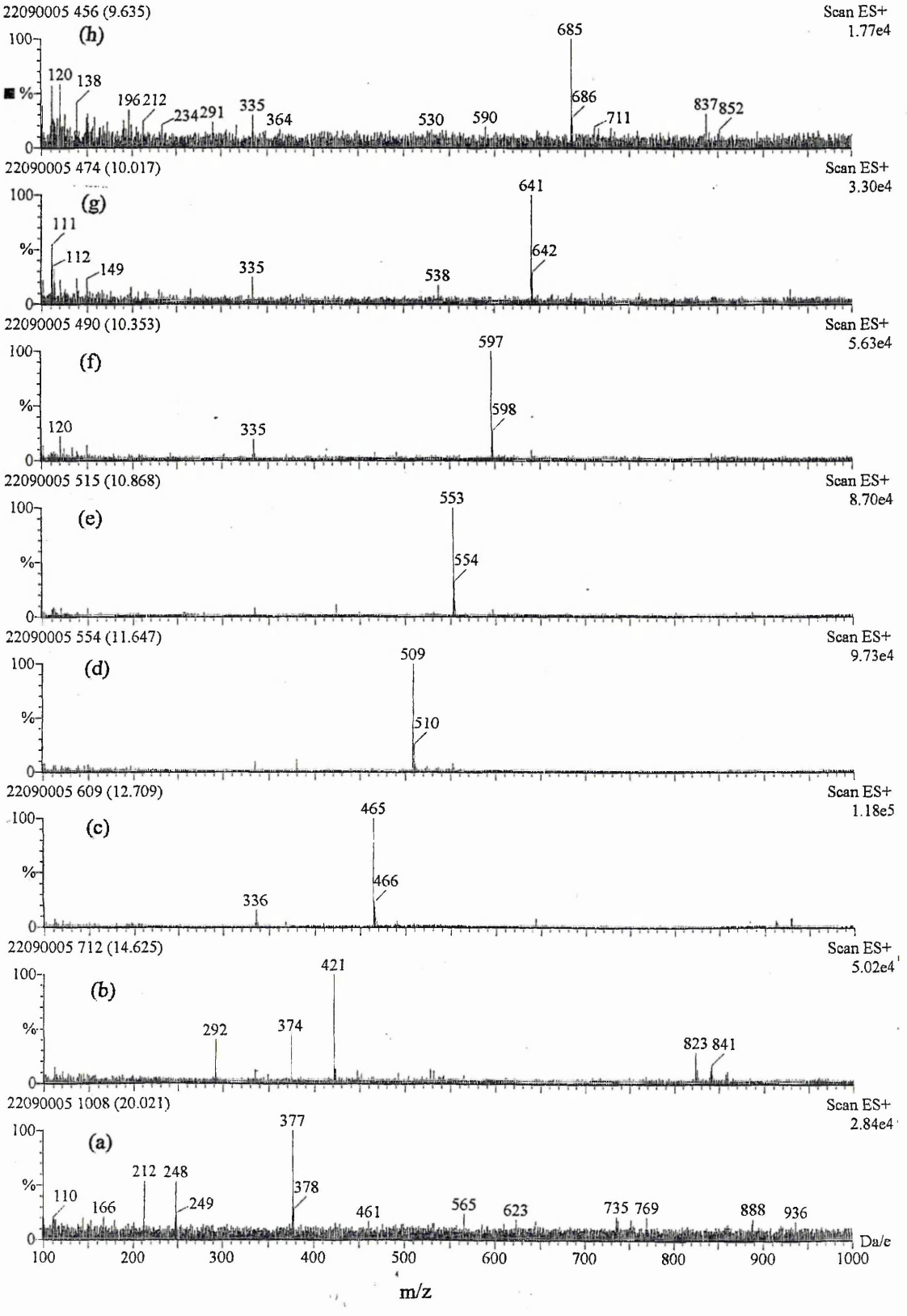


Figure 4.9. Electrospray positive ion mass spectra for the individual oligomers (a)-(h) of a commercial OPEOS surfactant formulation.

MALDI-TOF mass spectrometry has been used widely in the analysis of synthetic polymers by addition of an excess of a suitable metal cation during sample preparation [10-15]. Addition of a metal cation leads to the production of solely $[M+cation]^+$species. Just and co-workers [16] added an excess of lithium chloride to alkylphenol ethoxylates before analysis by MALDI-TOF. This led to the production of almost solely lithium adducts instead of the mixture of potassium and sodium adducts which they observed before the addition of lithium chloride. Their reason for adding lithium to the samples was to counteract the low-mass discrimination shown in the comparison between MALDI-TOF and SFC.

The addition of lithium chloride had the effect of suppressing the discrimination, enabling them to discern the lower ethoxymers. The most likely explanation of this effect is probably due to the fact that in producing solely $[M+Li]^+$ adducts, the signal due to each ethoxymer is represented by one peak, whereas without the addition of lithium chloride, the signal for each ethoxymer is split between two peaks (the sodium and potassium adducts) which are much smaller in intensity than if the signal were represented by one peak. Therefore, the addition of lithium chloride to the samples before crystallisation should counteract this phenomenon and lead to much less complicated spectra.

Willetts *et al* [17] recently reported a MALDI-TOF/ MS method for the analysis of nonyl phenol ethoxylate non-ionic surfactants by the addition of lithium chloride to produce solely $[M+Li]^+$ adducts in surface water samples.

In the present work, analysis of both NPEOS and OPEOS surfactants using MALDI-MS has been demonstrated. The addition of 10μl of a 10mg/ml solution of lithium chloride results in a relatively simple mass spectrum for NPEOS (Figure 4.10). The spectrum exhibits an envelope of $[M-Na+2Li]^+$ adduct ions at m/z (429.2, 473.3, 517.7, 561.4, 605.9, 649.3, 692.5, 737.3, 781.6, 825.1, 869.0 and 912.7). Hence these data suggest that the surfactant formulation contained molecules with between 2-13 ethylene oxide (EO) units.

The MALDI-MS spectrum of OPEOS (Figure 4.11) obtained with addition of lithium chloride, shows excellent resolution of individual ethoxymers in this type of surfactant. A typical envelope of intense peaks representing $[M-Na+ 2Li]^+$ adducts for 1-8 ethylene oxide-containing species was produced (m/z 370.9, 415.1, 459.5, 503.8, 547.9, 591.9, 636.2, and 680.5) with an average chain length of 3.14 units.

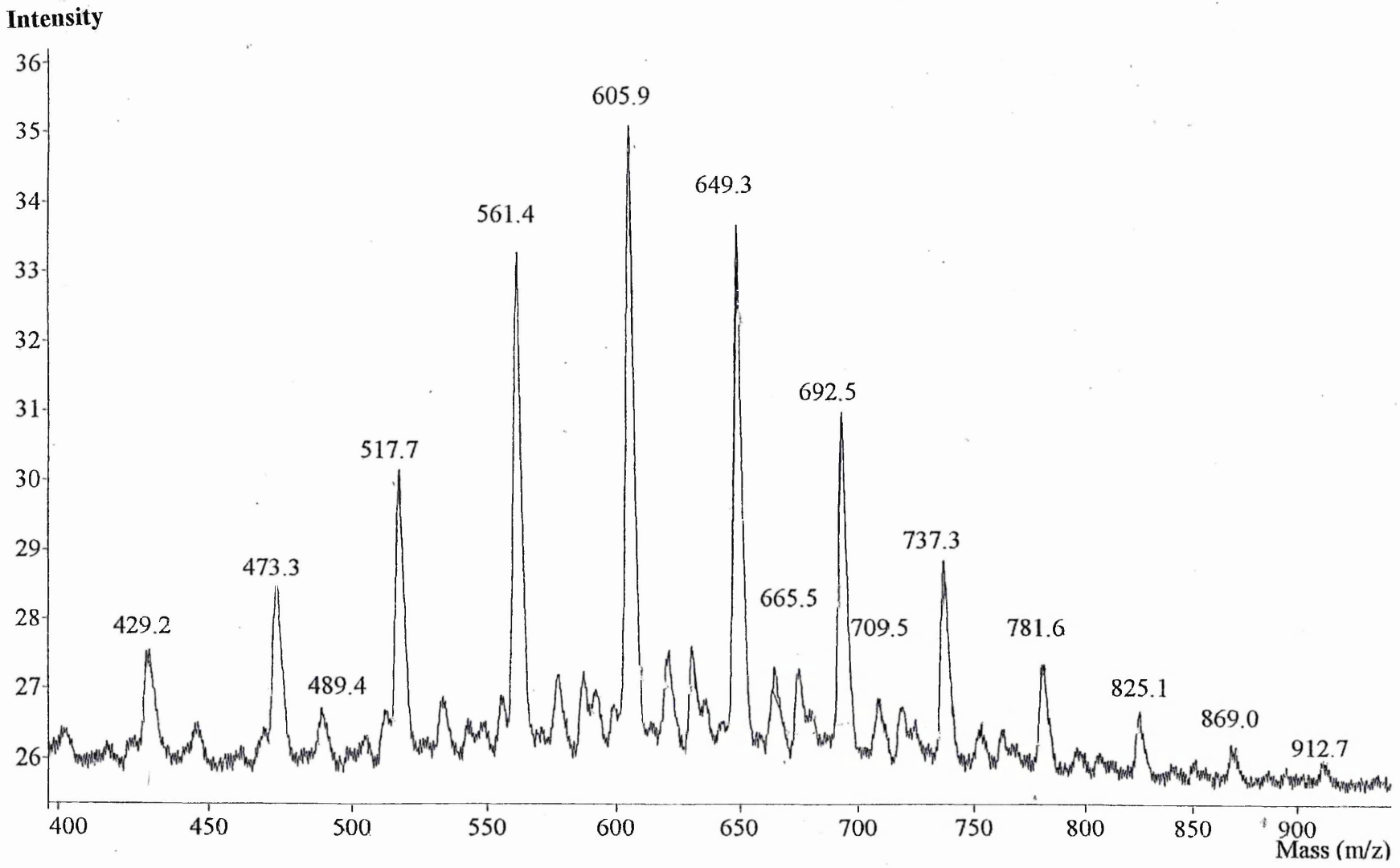


Figure 4.10. MALDI/ MS spectrum of a NPEOS surfactant.

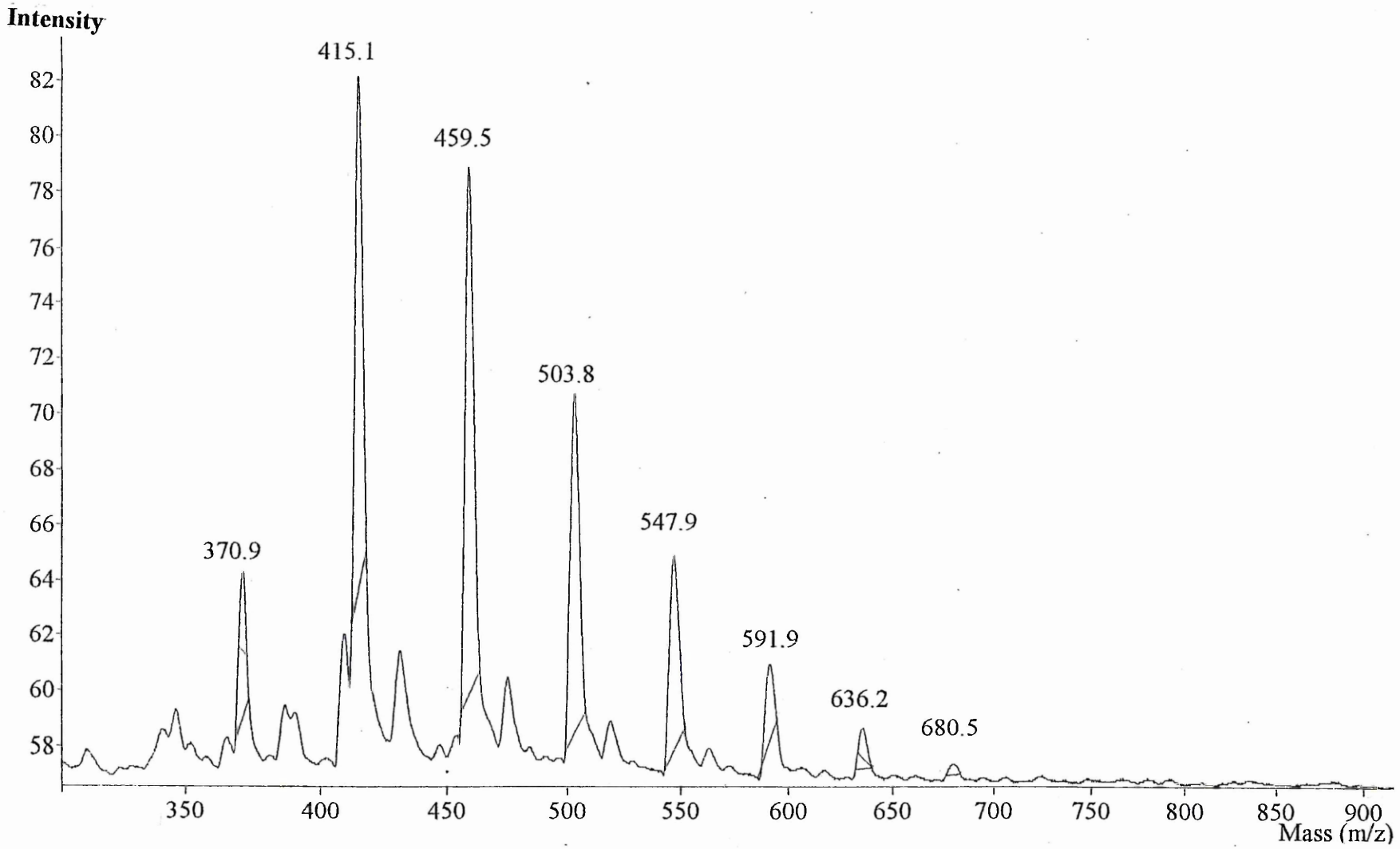
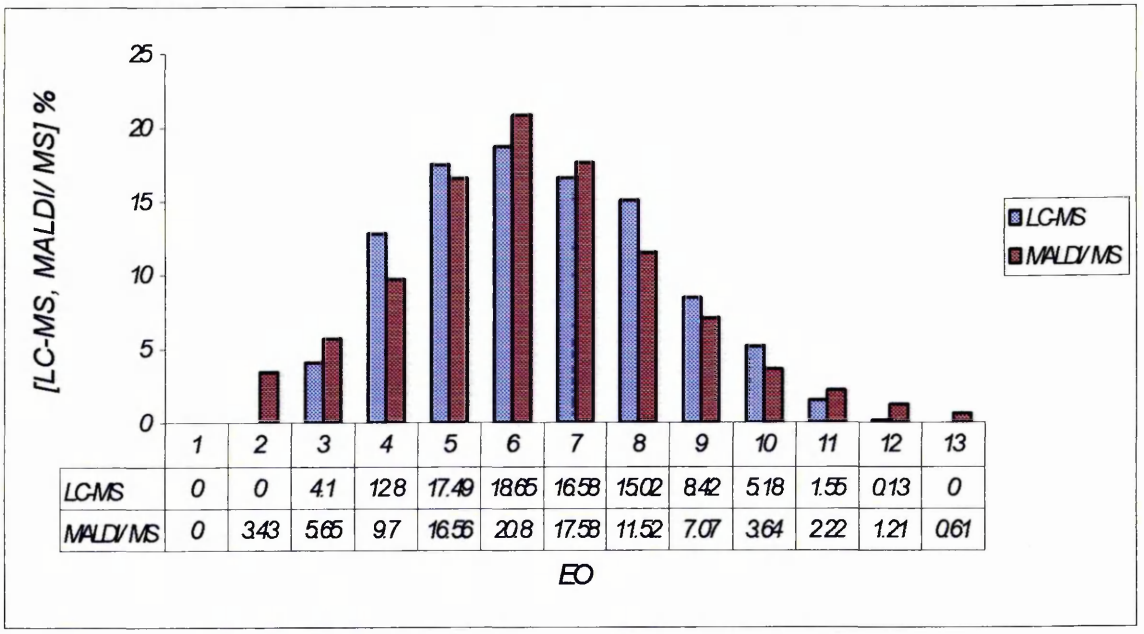


Figure 4.11 MALDI/ MS spectrum of a OPEOS surfactant.

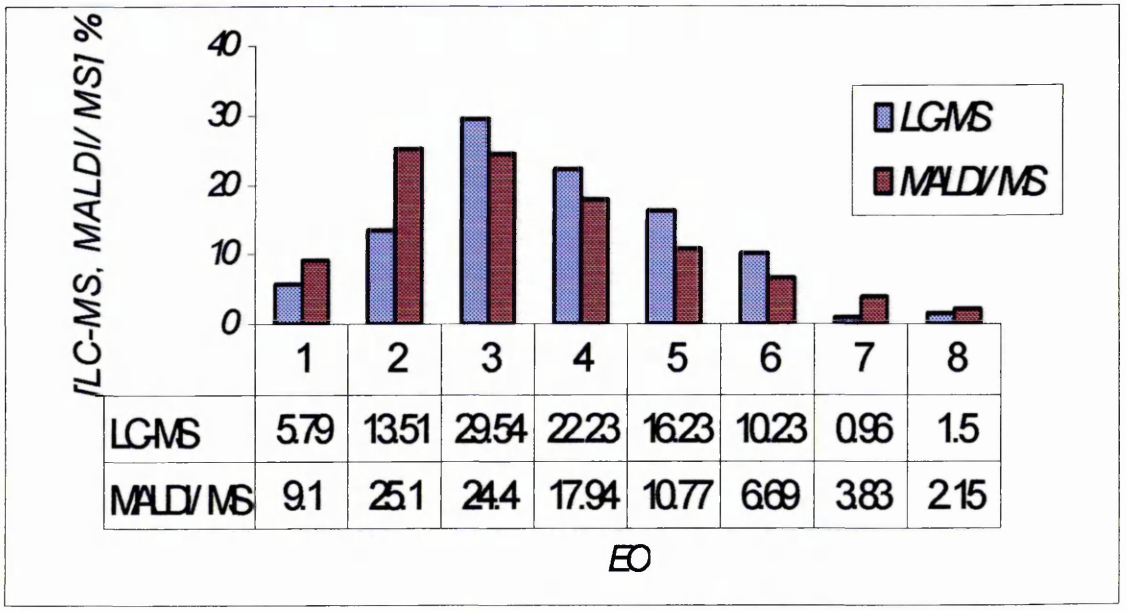
A comparison of the results obtained from two different techniques (MALDI-MS and LC-MS) for NPEOS and OPEOS surfactants is given in Figures 4.12 and 4.13. An approximation of the mole fraction of each ethoxymer indicated by each technique was calculated measuring the peak height of individual ethoxymers in the LC/MS and MALDI/MS data and quoting it as a percentage of the total height. The average number of ethoxymers was then calculated as a weighted average based on these data. This is obviously a very approximate way of handling the data in terms of calculating accurate mole ratios of individual ethoxymers but it does enable a simple comparison between the data sets to be made. As can be seen from Figure 4.12 for NPEOS, the data obtained for average length of the ethylene oxide chain (EO number) are in reasonable agreement, with values of 6.46 from LC/MS and 6.32 from MALDI/MS. In earlier studies conducted by HPLC, only the average EO number was determined as 6.19 [18]. However, in the present study, the ranges of EO units found by LC/MS (3-12) and MALDI/MS (2-13) are narrower than that reported in the earlier HPLC work (2-15).

Figure 4.13 indicates that the average number of EO units calculated for OPEOS by LC/MS and MALDI/MS techniques is in broad agreement (3.72 and 3.14 respectively) and these data are also in agreement with the earlier studies carried out by HPLC which gave an average EO number of 3.6 [18]. However in our studies the range of EO units for the OPEOS was found to be from 1-8 and this is not in agreement with the earlier work carried out by HPLC only, in which the range was reported to be from 2-6 units.



	1	2	3	4	5	6	7	8	9	10	11	12	13
LC-MS	0	0	4.1	12.8	17.49	18.65	16.58	15.02	8.42	5.18	1.55	0.13	0
MALDI/ MS	0	3.43	5.65	9.7	16.56	20.8	17.58	11.52	7.07	3.64	2.22	1.21	0.61

Figure 4.12 Graph showing the average number of ethoxymer units calculated from LC-MS and MALDI/ MS chromatogram for NPEOS.



	1	2	3	4	5	6	7	8
LC-MS	5.79	13.51	29.54	22.23	16.23	10.23	0.96	1.5
MALDI/ MS	9.1	25.1	24.4	17.94	10.77	6.69	3.83	2.15

Figure 4.13 Graph showing the average number of the ethoxymer units calculated From LC-MS and MALDI/ MS chromatogram for OPEOS.

4.3 Conclusion

The LC-ESI-MS and MALDI/MS techniques have been shown to be very useful for the analysis of commercial NPEOS and OPEOS surfactant formulations. LC-ES-MS spectra have shown a range of ethoxymers units for NPEOS surfactants from 3-12 and ethoxymer units for OPEOS surfactants from 1-8.

MALDI/MS has been shown to be the most useful method for the analysis of NPEOS and OPEOS surfactants. The addition of lithium chloride as a source of lithium ions during sample preparation was shown to produce solely $[M+Li]^+$ adducts, suppressing the formation of sodium and potassium adducts and producing a much simpler spectrum. MALDI/ MS spectra have shown a range of ethoxymer units for NPEOS from 2-13 and average ethoxymer units for OPEOS from 1-8.

Both of the mass spectral techniques provide sufficiently precise measurements of the molecular weight of each surfactant species. Data obtained show the average number of ethoxymers for NPEOS to be 6.46 by LC-MS and 6.32 by MALDI/ MS. The average for OPEOS was 3.72 by LC-MS and 3.14 by MALDI/ MS. Hence the MALDI/ MS results agreed well with LC-MS results for NPEOS and OPEOS surfactants.

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18. Austad T. and Fjelde I.; Anal. Lett. 25 (1992) 957.

CHAPTER FIVE

A Study of the Degradation of Nonylphenol Ethoxysulphonate Surfactants by Chemical and Biological Oxidation

5.1 Chemical Oxidation of Nonylphenol Ethoxylatesulphonate (NPEOS) Surfactants.

Introduction

In these studies, Fenton's reagent has been used for the oxidation of commercial NPEOS surfactant. The oxidation products have been identified using different techniques (HPLC, MALDI/MS and LC-MS). The main aim of this investigation was to assist in the interpretation of data that were obtained from the biodegradation of NPEOS by using microorganisms. These data are also of interest, however, for considerations of the potential of chemical oxidation for the treatment of waste streams containing these surfactants.

Industrial and municipal waste waters contain a wide variety of pollutants, both organic (phenols, aromatics, amines, chlorinated compounds) and inorganics (sulphides, heavy metals). Technologies for the destruction or detoxification of hazardous organic wastes are in urgent demand, particularly since growing restrictions are placed on land disposal and the need to clean up existing waste sites. Optimal wastewater treatment is today's challenge. On the one hand there are very different kinds of wastewater composition, and on the other there are many different kinds of possible treatments. Depending on final water quality requirements and economic aspects, some processes are better suited than others. Physical separation of suspended solids, oils and greases and biological treatments have been shown to be very economic and reliable systems for most cases (municipal wastewater, food and farm processing water, etc.). There are, however, cases in which the effectiveness of these treatments drops (soluble substances for physical

separation, non-biodegradable and/or toxic substances for biological treatments). For this last case, which is common in chemical processing wastewater, different chemical processes, most of them based on oxidation-reduction reactions, are being applied. Processes such as chlorination, ozonation, UV irradiation, electrochemical treatments and processes based on $OH^{\bullet}$radical attack have been investigated. Although these treatments have proved effective, they are expensive and commercially unattractive (in comparison to physical and biological methods).

Chemical oxidation of dissolved organic compounds and micropollutants is increasingly being considered in water and wastewater treatment because the technology has the potential of converting harmful organic compounds to innocuous inorganic compounds such as carbon dioxide and water. In chemical oxidation processes, structural changes and changes in chemical properties arise as a result of oxidative degradation reactions, and higher percentages of oxygen appear in the products, in the form of alcohols, carboxylic acids etc. Oxidation of organic compounds with oxidants such as ozone or $OH^{\bullet}$radicals usually yields more oxidized ones which are more easily biodegradable [1-4]. This is the general idea that makes some investigators think of a combination of a chemical oxidation process followed by a biological one [5,6].

Sawyer [7] has investigated in detail the chemistry associated with molecular oxygen, particularly the reactivity of oxygen-containing reagents such as hydrogen peroxide, oxygen radicals e.g. hydroxyl radicals $OH^{\bullet}$ and the metal-induced activation of dioxygen and its subsequent behavior.

The use of Fenton's reagent (a common method to produce hydroxyl radicals) in advanced oxidation processes (AOPs), is considered highly promising for treating refractory compounds. In 1894 Fenton [8] first developed the chemistry of a convenient and widely used method for the elimination of organic pollutants from aqueous solutions. However, its application as an oxidising process for destroying toxic organic was not practiced until the late 1960s [9]. Since then, it has been used in direct oxidation of phenolic wastes, of refractory organics in municipal waste-water, and of nonbiodegradable industrial wastes to render them more biodegradable for aerobic and anaerobic processes. Fenton's reagent is one of the best known metal-catalyzed oxidation reactions of water-miscible organic compounds. It consists of a ferrous salt such as $Fe(II)SO_4$ (or any other ferrous complex), and H_2O_2. The mixture, at low pH (i.e. pH 3.5), results in Fe^{2+} catalytic decomposition of H_2O_2 and proceeds via a free radical chain process which produces hydroxyl radicals [10,11]. The use of Fenton's reagent is convenient [12] because it does not load the system with harmful or toxic substances, it does not lead to a salinity increment (ferric ions are precipitated as hydroxide), and it does not require complicated equipment. The so-called Fenton sludge, produced by neutralization after the reaction, does create subsequent disposal problems but constitutes the only limitation of the process.

There are mechanisms proposed for the reaction of hydrogen peroxide with $Fe^{2+}$and Fe^{3+}. The "classical" mechanisms for these reactions involve hydroxyl radical intermediates that can attack organic compounds. The classical reaction of Fe^{2+} with H_2O_2, known as the Fenton reaction [13,14], generates $OH^{\bullet}$ in the rate-limiting step (eq1); the $OH^{\bullet}$may

subsequently be scavenged by reaction with another Fe^{2+} (eq2) or they may react with an organic compound.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot} + HO^{-} \quad (1)$$

$$OH^{\cdot} + Fe^{2+} \longrightarrow HO^{-} + Fe^{3+} \quad (2)$$

Fe^{3+} catalytically decomposes H_2O_2 to O_2 and H_2O. The classical "radical chain" mechanism proposed for the simple Fe^{3+}(aq) system (i.e., no complexing ligands other than water) involves $OH^{\bullet}$ and hydroperoxyl radicals ($HO_2^{\bullet}$) and is shown in equations 3-7 [13,15,16]:

$$Fe^{3+} + H_2O_2 \rightleftharpoons Fe{-}OOH^{2+} + H^{+} \quad (3)$$

$$Fe{-}OOH^{2+} \longrightarrow HO_2^{\cdot} + Fe^{2+} \quad (4)$$

$$Fe^{2+} + HO_2^{\cdot} \longrightarrow Fe^{3+} + HO_2^{-} \quad (5)$$

$$Fe^{3+} + HO_2^{\cdot} \longrightarrow Fe^{2+} + H^{+} + O_2 \quad (6)$$

$$OH^{\cdot} + H_2O_2 \longrightarrow H_2O + HO_2^{\cdot} \quad (7)$$

In the presence of excess peroxide, $[Fe^{2+}]$ is small relative to $[Fe^{3+}]$, since reaction 4 is generally much slower than reaction 1 [16]. As can be seen in equation 7, H_2O_2 can act as an $OH^{\bullet}$ scavenger as well as an initiator. Due to the formation of Fe^{3+} during the reaction, it is normally accompanied by the precipitation of $Fe(OH)_3$. The reaction can

also lead to the cleavage of C-C bonds and in some cases complete mineralization, giving rise to CO_2 and H_2O. For this reason, Fenton's reagent has been extensively applied in the waste water treatment of resistant pollutants.

Molecular oxygen is generally not a strong enough oxidant to oxidize most organic compounds. However, wet air oxidation (WAO) and advanced oxidation reactions may be successfully used for the treatment of wastewater. These techniques use dissolved oxygen at elevated temperature and pressure to enhance the oxidation process and Fenton chemistry can be used to catalyse these reactions [17].

Surfactants belong to a class of organic pollutants resistant to biological degradation [18] and consequently they have received increasing attention [19] in the last few years because of their large-scale domestic and industrial use, resulting in significant environmental problems [20]. Hence there is interest in methods for their disposal and catalysed WAO offers one such route.

5.1.1 Experimental

5.1.1.1 Reagents and Materials

A commercial NPEOS formulation manufactured by Hoescht was gifted from Dr Tor Austad (Rogaland University centre, Norway). Hydrogen peroxide solution (30% v/v) was purchased from BDH (Poole, Dorset, U.K.). Iron(II)sulfate heptahydrate (99 %) was purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Strong Anion-Exchange (SAX) cartridges (500mg) were purchased from Varian (Frampton Avenue, Harbor City, USA). All solvents used were HPLC grade and were purchased from Fisher Scientific (Loughborough, U.K.). All water used was Milli-Q grade. Ammonium acetate and acetic acid used were HPLC grade (Fisher Scientific)

51.1.2 Sample Pretreatment

In order to simplify the chromatograms and mass spectra obtained from the oxidation products from the various formulations, residual non-ionic starting material was selectively removed by the use of SAX solid phase extraction cartridges. The surfactant solutions were acidified to pH 3-4 with glacial acetic acid. The cartridge was washed with methanol (5ml) and distilled water (10ml). The acidified surfactant solution was passed through the cartridge which was subsequently washed with 2% acetic acid in methanol (5ml) to selectively elute the non-ionic surfactants. The cartridge was then washed with methanol (5ml) before elution of the anionic fraction in 2 M HCl in methanol:water (50:50) (15ml). This was evaporated to dryness and the now "pure" NPEOS was dissolved in distilled water.

5.1.1.3 HPLC instrumentation and conditions

All HPLC analyses were performed on a Waters 600-MS gradient HPLC system. The column used was an Alltech (Carnforth, Lancashire, U.K.) mixed-mode reversed-phase/ion-exchange columns, mixed-mode RP8/ anion 100Å, 7µm (150x4.6mm). Fluorescence detection was carried out using a Jasco FP-920 (Cheltenham, U.K.) fluorescence detector, $\lambda_{ex.}$=225nm and $\lambda_{em.}$=295nm. Data were outputted on a Hewlett Packard HP 3396A integrator. Injections (100µl) were made using a Perkin-Elmer ISS-101 autosampler. The mobile phase gradient used is given in Table 3.1 (Chapter Three of this thesis).

5.1.1.4 Liquid chromatography- Mass Spectrometry

All analyses were performed on a Quattro I (Micromass Manchester U.K.), mass spectrometer, equipped with an electrospray ionisation (ESI) source. A Jasco Pu-980 intelligent HPLC pump system was used in this case and all other HPLC conditions are as previously described. The mass spectrometer was operated in positive ion mode. A capillary voltage of 3.5 kv, and a skimmer cone voltage of 30V were used. Mass spectra were collected in full- scan mode, scanning over the range 100- 1200 m/z at 3s/scan. The source temperature was maintained at 80°C. Nitrogen was used as the drying and nebulising gas.

5.1.1.5 MALDI-MS

MALDI analyses were performed on a Finnigan Vision 2000 reflectron based time-of-flight (MALDI/TOF/MS) instrument. The oxidation products were dissolved in methanol : water (50:50). 2,5-Dihydroxybenzoic acid (90mg/ml) was dissolved in 0.1% TFA in methanol. Lithium chloride (10mg/ml) was dissolved in methanol. 200µl of the oxidation product solutions , 40µl of matrix and 10µl of LiCl were mixed and 1µl of the resulting solution was placed on the stainless steel target of the instrument for analysis.

5.1.1.6 Reaction Conditions

The optimized Fenton oxidation procedures were used under conditions proposed by Ito *et al.*[21]. The reaction was carried out in a three necked-flask fitted with a pressure equalising dropping funnel, a nitrogen inlet and an outlet. A solution of $FeSO_4 .7H_2O$ (0.0175g, 3.6 mmol) in 25 ml of acidic water (pH 2.8) (5% sulphuric acid) was added to stirred solution of NPEOS (0.1g, 0.35mmol) in 50 ml of acidic water (pH 2.8) (5% sulphuric acid) followed by the dropwise addition of hydrogen peroxide solution 27% w/v (0.25g, 2.2mmol) in 25 ml of water while the reaction flask was cooled in ice. The reactions were repeated under a nitrogen sealed system and in an open system respectively, but both systems showed the same oxidation products. After the addition of hydrogen peroxide solution, the oxidation reaction mixture was stirred at room temperature for a period of time which ranged from 1 to 5 hours.

5.1.1.7 Sample Preparation

Once the reaction was completed, 5% ammonia solution was added to adjust the pH to 9 in order to precipitate ferric ions as hydroxide, and then the suspension was filtered. The aqueous reaction mixture was extracted with 3x100 cm^3 of ethyl acetate. The organic layers were combined and dried using magnesium sulphate, and the extract was evaporated on a rotary evaporator to give a greasy residue and dissolved in 5ml (50:50) methanol and water.

5.1.2 Results and Discussion

An NPEOS surfactant possesses two principal units, which can be oxidatively attacked by $OH^{\bullet}$radicals, namely, the ethylene oxide chain , and the alkyl chain. Figure 5.1 shows some of the possible oxidation products for a NPEOS surfactant.

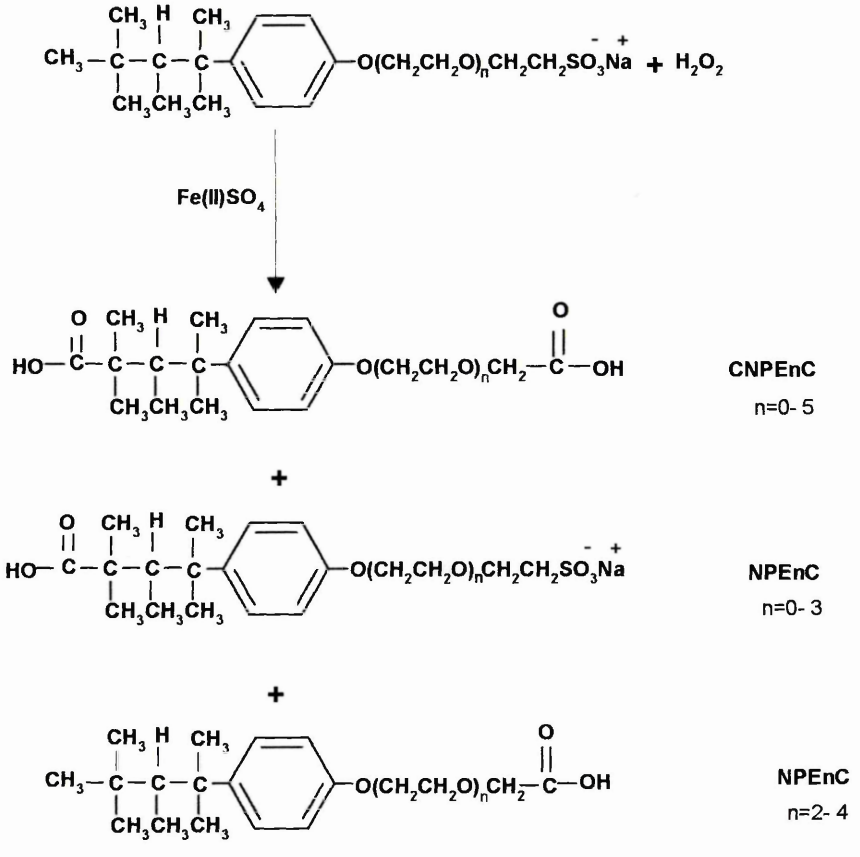

Figure 5.1.Oxidation of NPEOS by Fenton Reagent.

This study involved the oxidation of NPEOS using Fenton's reagent under different experimental condition and for different times. The oxidation reactions were carried out over time periods (after addition of hydrogen peroxide to the reaction mixture) of 1 hour,

2 hours, 3 hours, 4 hours and 5 hours respectively and the oxidation products were observed as soon as was practicable after addition of hydrogen peroxide.

Initial identification of oxidation products formed in the oxidation reactions of NPEOS was made by the use of HPLC techniques. Figure 5.2 (a-f) shows the variation in the HPLC fluorescence chromatograms of the oxidation products of NPEOS obtained. The peaks between (t_r) 5.104 min and (t_r) 6.840 min were tentatively identified as a mixture of dicarboxylic acids ($CNPE_nC$) and monocarboxylic acids (NPE_nC). The oxidation reaction of NPEOS using the Fenton reagent was monitored over five hours. A 1ml sample was taken out every 15 minutes in the first 1 hour and dissolved in 100 ml distilled water and analysed by HPLC, and then sampling was carried on at hourly intervals. Complete conversion to intermediate oxidation products was observed at the end of five hours (Figure 5.1(f)) to give what appeared to be one major set of products, probably (based on the regular pattern of peaks) members of the same homologous series.

The tentative identification was based on the work of DeVoogt et al [22] who have proposed a new biodegradation pathway for nonylphenol ethoxylate (NPEO) surfactants. Analyses of spiked river water samples using LC-ES-MS after extraction using C_{18} cartridge SPE revealed relatively fast primary degradation of NPEO, with > 99% degradation observed after 4 days. The generally proposed degradation pathway of EO is via chain shortening. It was shown that the initiating step of the degradation is ω−carboxylation of the individual ethoxylate chains, leading to the formation of metabolites with long carboxylated EO chains (NPEC). Further degradation was found to

proceed gradually into short-chain carboxylated EO with the most abundant species being NPE_2C. The oxidation of the nonyl chain proceeded concomitantly with this degradation, leading to metabolites which contained both a carboxylated ethoxylate and a carboxylated alkyl chain, of varying length (CAPEC). The identity of the CAPEC metabolites was confirmed by the fragmentation pattern obtained with LC-ES-MS/MS.

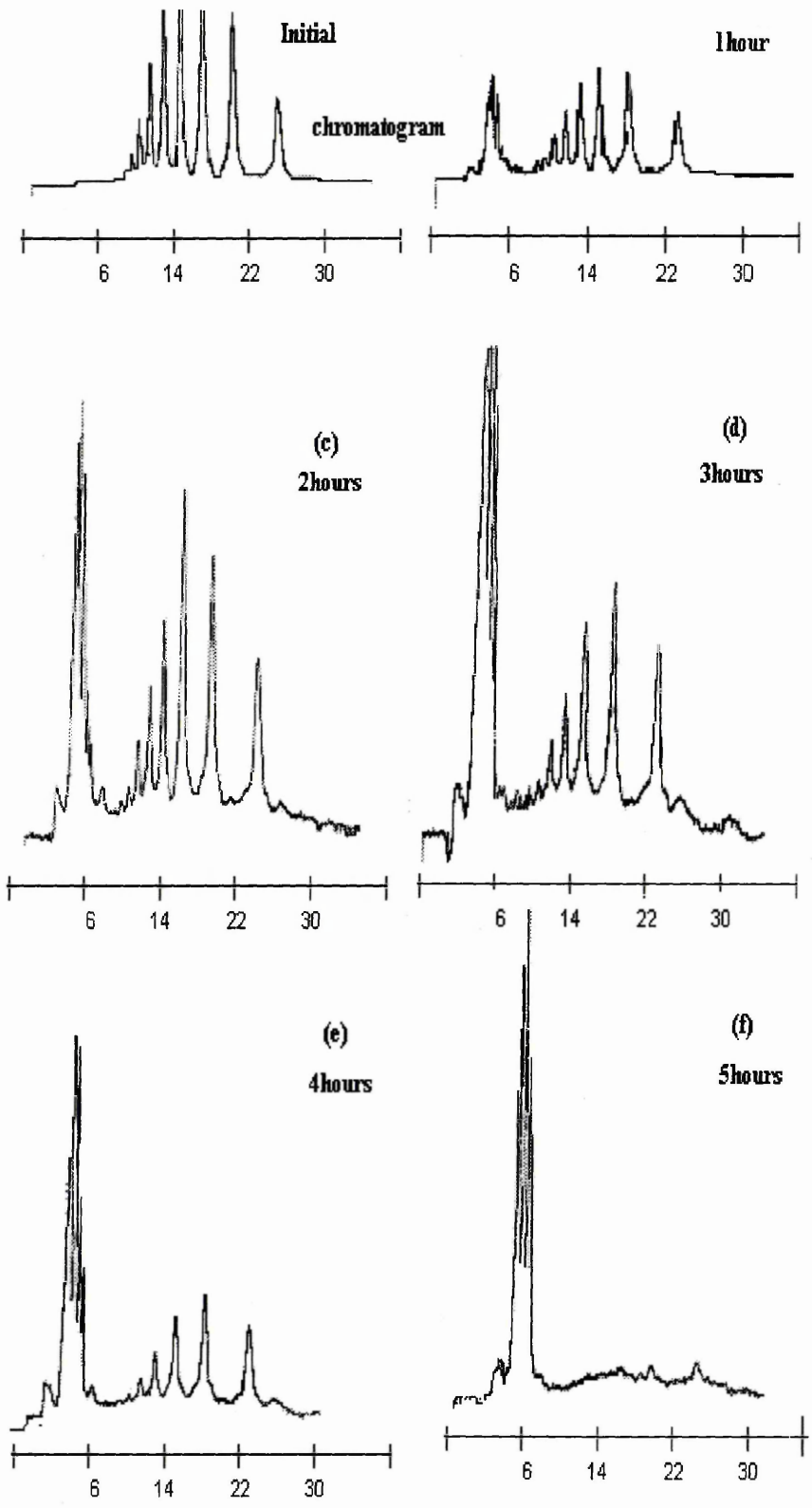

Figure 5.2 HPLC chromatograms for separation of oxidation products of NPEOS by mixed-mode column (RP/8) using mobile phase in Table 3.1.

The oxidation products of NPEOS were further studied by MALDI/ MS and electrospray mass spectrometry to confirm their identities.

The MALDI/MS spectrum of oxidation intermediate products of NPEOS (Figure 5.3) obtained with addition of lithium chloride shows excellent resolution of individual ethoxymers in this type of oxidation product. An envelope of intense peaks representing $[M+Li]^+$ adducts for 0-4 ethylene oxide units containing dicarboxylic acid species was typically produced (m/z 315.0, 359.0, 403.2, 447.0 and 491.1). Ions corresponding to the monocarboxylic acid can be m/z 329.0, 372.9, 416.9, 461.0 and 504.9. Hence these data suggest that the intermediate oxidation products formulation contained molecules with between 0 –5 ethylene oxide (EO) units and were a mixture of products as suggested by HPLC data.

The electrospray positive ion mass spectra of the oxidation products were obtained by direct infusion of the diluted reaction mixtures. The spectra obtained (e.g. Figure 5.4) exhibit ions corresponding to both single and dicarboxylic acids species. Ions corresponding to $[M+NH_4]^+$ species for the dicarboxylic acids can be seen at m/z 326, 370, 414, 458, 502 and 546. The corresponding series of $[M+H]^+$ ions can be seen at m/z 309, 353, 397 and 441.

Ions corresponding to $[M+NH_4]^+$ species for the monocarboxylic acids produced by oxidation of the ethoxylate chain can be seen at m/z, 384, 428, 472 and 516. Ions corresponding to $[M+NH_4]^+$ species for the monocarboxylic acids produced by oxidation of the hydrocarbon chain can be seen at m/z, 375, 419, 463 and 507.

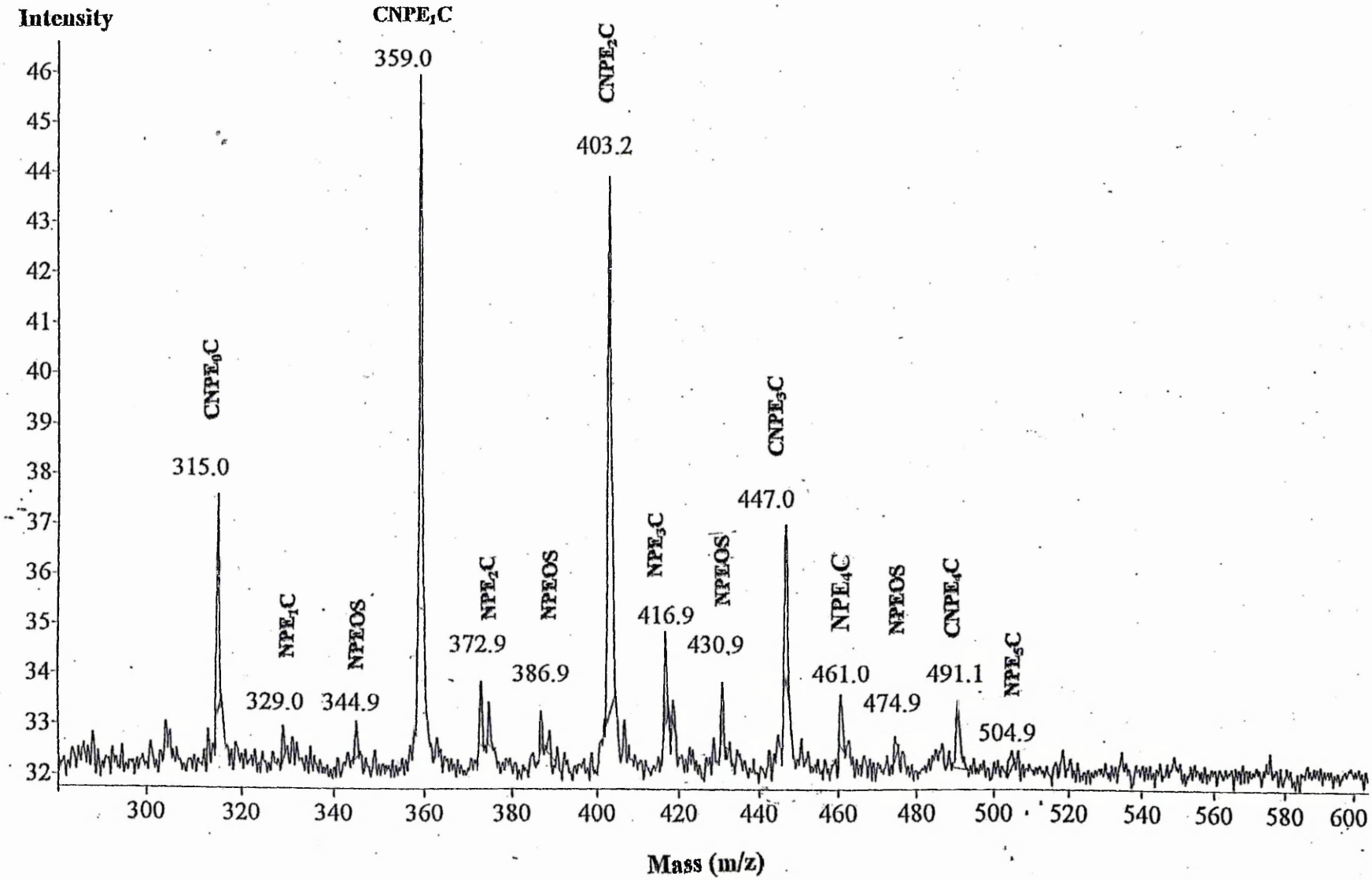

Figure 5.3 MALDI/MS of oxidation intermediate products from oxdation of NPEOS

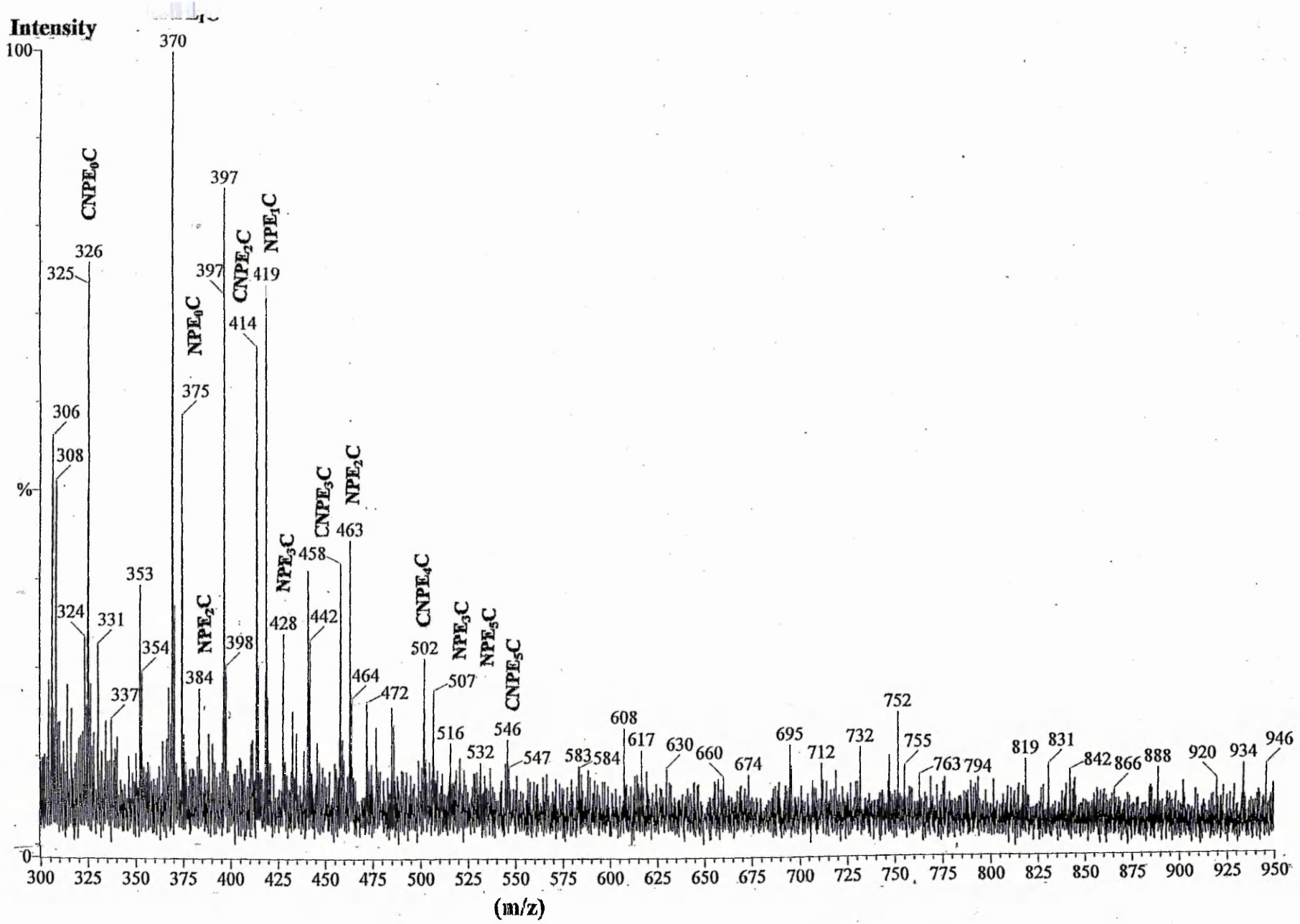

Figure 5.4 LC-MS of oxidation intermediate products from oxidation of NPEOS.

The data obtained from the three different techniques indicate that there is mixture of three different types of product formed from the oxidation of NPEOS. The MALDI/MS data appears to suggest that the formation of NPEOS intermediates bearing a carboxylic group on opposite side chains (dicarboxylic acid) was the major product type. From these data, the oxidation reactions of NPEOS can be divided into three stages. The first process is a rapid ω−oxidation of the ethoxylate chain that occurs without any observable time-lag. The next stage occurs more slowly, in which the long-chain NPE_nC are degraded to short-chain, mainly, $CNPE_nC$ (n = 0-5). The third stage is the oxidation of the alkyl chain that starts as soon as NPE_nC is being formed.

The m/z values correspond to $[M+Li]^+$ ions expected from dicarboxylic acids. The peak of m/z 315 corresponds to the $[M+Li]^+$ ion formed for a dicarboxylic acid species where both the alkyl chain and the first ethoxylate group have been carboxylated i.e. the compound shown Figure 5.5 has been formed.

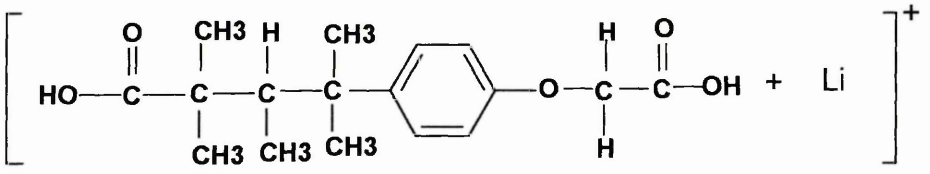

Figure 5.5. $CNPE_0C$ MALDI/MS ion for peak m/z 314.8.

The formation of this product is also confirmed by the ES-MS data where the peak of m/z 326 corresponds to the $[M+NH_4]^+$ ion formed for the dicarboxylic acid species where

both the alkyl chain and first ethoxylate group have been carboxylated i.e. as shown in Figure 5.5.

The data obtained from the chemical oxidation of NPEOS anionic surfactants shows both a carboxylated ethoxylate and a carboxylated alkyl chain. This leads to the formation of dicarboxylic acids ($CNPE_nC$ (n=0-5)) and two types of monocarboxylic acids (ethoxylate chains or nonyl chain carboxylated) to give NPE_nC (n=0-3 and n=2-5). Thus, the oxidation of NPEOS appear to follow the same pathways as NPEO.

5.2 Biological Oxidation of Nonylphenol Ethoxylatesulphonate (NPEOS) Surfactants

Introduction

In the atmosphere currently prevailing at the Earth's surface, the most stable form of carbon is its fully oxidised state, CO_2. The atmosphere contains 2.6x 10^{12} tonnes of carbon as CO_2, which is in equilibrium with even larger amounts (1.3 x 10^{14} tonnes) in solution in the rivers, lakes and oceans [23]. From this pool of oxidised carbon begins a series of complex biological processes driven primarily by solar energy, which lead to the reduction of CO_2 to C-C, C-H and other bonds in an enormous variety of organic compounds that collectively constitute the biosphere. In effect, photosynthetic organisms, predominantly the green plants and marine algae, are the primary producers which transduce solar energy into chemical energy by converting CO_2 into complex assemblies of reduced organic compounds. These materials constitute the base of numerous interrelated food-chains involving herbivores, carnivores and omnivores. During the flux of carbon through these routes, some is oxidised by the consuming organisms to drive energy, while the resulting CO_2 is returned to the atmosphere. However, much organic material enters the soils, water and sediments in the environment either during senescence of plants or the excretions from, or death of, higher organisms. The deposition of organic compounds in the environment provides another pool, this time of reduced carbon compounds which, in the presence of CO_2, are thermodynamically unstable with respect to CO_2 under aerobic conditions.

An integral part of the evolutionary process that has produced this plethora of organic compounds is the evolution of microorganisms that are able to utilise these materials as nutrients. Microorganisms use organic compounds (and other elements such as N and S) in the environment not only as sources of the carbon from which to build cell components, but also for the energy they need to achieve that biosynthesis in order to maintain the low entropy state embodied in the highly organised structures characteristic of biological systems. This energy is also used to accomplish other functions such as motility and bioluminescence. Thus, environmental deposits of organic compounds serve as nutrients and as an energy source for microorganisms which ultimately complete the re-oxidation of carbon to CO_2 in the atmosphere and surface waters, thereby closing the cycle.

Most of the surfactants reaching the environment (soil and natural waters) do so from consumer products via the use of sewage sludge on land, effluents from wastewater treatment plants (WWTP) and industrial discharges into freshwater and marine sites. Other sources of surfactant contamination are from the use of surfactant dispersants for fuel oil spillages and surfactant-enhanced remediation of subsoil after spillage and contamination with non-aqueous liquids.

Historically, potential surfactant contamination of the environment followed the shift from the use of soap-based detergents to synthetic surfactants. Over the past 45 years, a number of factors caused significant change in the detergent industry. The first of these took place in 1965, when the industry voluntarily switched its anionic workhorse

surfactants, branched alkylbenzenesulphonates (ABS), to linear alkylbenzenesulphonates (LAS) upon the discovery that the less biodegradable ABS was largely responsible for excessive foaming in receiving water. During the 1960s, phosphorus, present as phosphate builders in household laundry and some institutional detergents, was found to be a limiting nutrient in the eutrophication of lakes and streams. This finding has resulted in a number of states and municipalities enacting legislation limiting the use of phosphates in detergent products. Phosphates, in the form of sodium tripolyphosphate or potassium pyrophosphate, were the only low-cost builder capable of reducing water hardness concentrations to levels where hardness-sensitive LAS would perform a good cleaning job.

The fate of a substance in an environmental compartment is related to its susceptibility to degradation. This degradation may occur by abiotic physical chemical processes such as hydrolysis or photolysis or by the action of living organisms, in the process known as biodegradation. In practice, this is by far the most important process by which the majority of substances are degraded in the environment. Biodegradation may take place in the presence of oxygen (aerobic) or in the absence of oxygen (anaerobic). The aquatic and terrestrial environments are generally aerobic although anaerobic conditions may exist in aquatic sediments and sub-soil environments. Biodegradation of surfactants in wastewater, in treatment plants, and in the ultimate receiving bodies is primarily the result of bacterial action, just as is the case with the other organic components of the waste. The biochemical metabolic reactions involved appear to be much the same whether surfactant or not, although there are certain characteristic features exhibited in

surfactant biodegradation which arise from the nature of surfactants themselves i.e. strongly hydrophilic and strongly hydrophobic groups joined together in the same molecule.

Biodegradation is the molecular breakdown of an organic substance by the enzymatic action of living microorganisms which use the substance for food. Conversion of the substance occurs stepwise with the formation of metabolites and biodegradation intermediates. These may degrade further at faster or slower rates compared to their precursors [24]. The processes involved are described as :-

(i) Primary biodegradation which means biodegradation of a substance to an extent sufficient to remove a characteristic property of the original intact molecule. For surfactants, this has been measured by loss of foaming capacity or ability to reduce surface tension. Primary biodegradation can leave high levels of organic residues altered in form from the original material.

(ii) Ultimate biodegradation is biodegradation which proceeds through a sequence of enzymatic attacks to ultimately produce the simplest structures possible in the biodegradation media. In aerobic biodegradation, such as that which consumes oxygen in the aeration sections of sewage treatment plants, CO_2, H_2O, methane and mineral salts of other elements are formed. In practice, ultimate biodegradability is generally assessed in an aerobic system by measuring carbon dioxide, the oxygen consumed during the biodegradation process ("biochemical oxygen demand" or BOD) or by measuring the level of organic carbon remaining in solution during the course of the biodegradation study. Each such method has its own intrinsic problem leaving aside any experimental

problems associated with the measurements themselves. The measurement of carbon dioxide production or the expression of BOD is a positive indication that some measure of ultimate degradation has occurred. However, microorganisms, like higher organisms, use biodegradable organic materials not only as an energy source but also as a food source to build up their cellular mass. Depending on the nature of the substance in question and the food supply available to the microorganisms (the more food, the more they 'put on weight'), only approximately 60-70% of the theoretical carbon dioxide production or BOD will be found for even a very easily biodegradable substance such as sodium acetate.

The measurement of dissolved organic carbon (DOC) in solution as an indication of ultimate degradation can both in principle and in practice indicate high levels of biodegradation since any of the substance converted into cellular biomass will be removed by filtration or centrifugation. However, it is not applicable to substances which are sparingly soluble in water or strongly sorptive, and can also be misleading where sparingly soluble or sorptive metabolites are formed.

To the best of this author's knowledge, no data on the biodegradation of APEOS type surfactants has been published. It was felt important to examine their biodegradation since it appeared that they have the potential to biodegrade to toxic/ endocrine-disrupting intermediates in a similar manner to APEO. This could have important consequences for their use in EOR. The data from the chemical oxidation of NPEOS reported earlier in this chapter support this hypothesis. Although the biodegradation of APEOS has not

been studied, the biodegradation of APEO and LAS has been extensively examined. Since each of these classes of surfactants have structural features of APEOS it was felt important to examine the literature.

5.2.1 Biodegradation of Non-ionic Surfactants

Non-ionic surfactants biodegrade in a very complex manner. They can be attacked at three different points by microorganisms, the position of which is dependent upon the structure of the surfactant. In aerobic conditions, the three different points of attack are summarised below [25].

- **Central Fission Mechanism**

The hydrophobic unit is cleaved from the hydrophilic unit. β-Oxidation is then responsible for the further conversion of the linear chain to carbon dioxide and water.

- **ω–Hydrophobe Attack**

The far end of the hydrophobe is firstly oxidised to an acid. Biodegradation then proceeds via β–oxidation of the alkyl chain.

- **ω–Hydrophile Attack**

This occurs via the oxidation of the polyoxyethylene chain initially to carboxylic acid. However, the mechanism is not fully understood.

The pathway and rate of biodegradation is dependent upon the type of microorganism present and the structure of the intact surfactant. Linear alcohol ethoxylates (LAE) biodegrade to water and carbon dioxide by the central fission mechanism (Figure 5.6) [25].

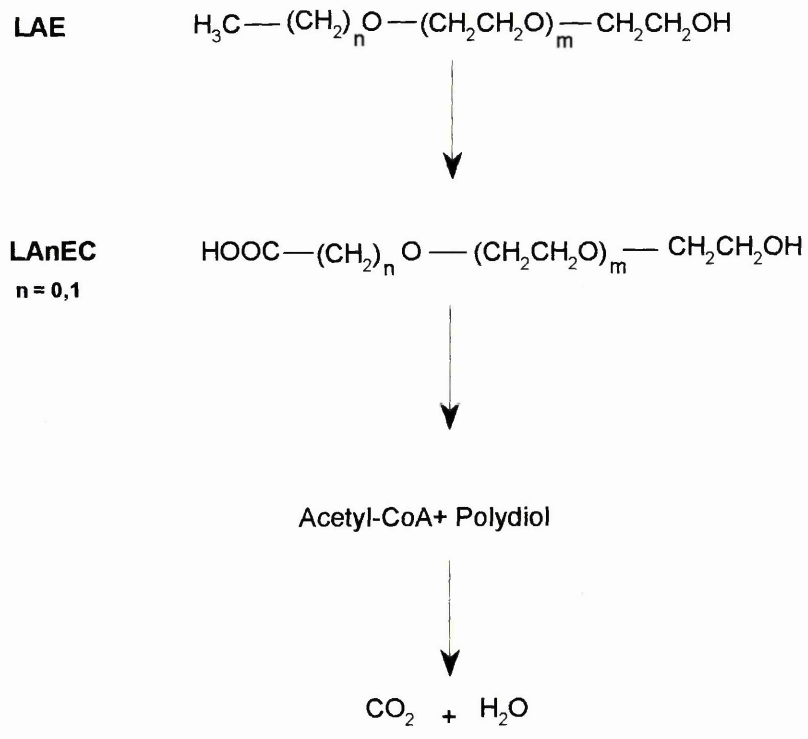

Figure 5.6. The biodegradation pathway of LAE [25].

5.2.1.1 Environmental Effects

The major problems of surfactants in the environment arise from the two classes of materials LAS and APEO but in the case of the APEO the environmental problems relate more to the biodegradation products rather than to the APEO themselves. Primary biodegradation of APEO is by stepwise removal of ethylene oxide monomers and is understood to be facilitated by bacterial metabolism of the hydrophilic ethoxylate chain [25]. Numerous studies have investigated the biodegradation of APEO and reported a wide range of degradation rates depending on the kind of test system and conditions employed [26]. While ultimate biodegradation results in the complete mineralisation of the aromatic structure of APEO, most studies have indicated that only primary

biodegradation is particularly rapid, resulting in an accumulation of persistent metabolites such as nonylphenol (NP) or octylphenol (OP), short chain mono-, di- and tri-ethoxylates (i.e. NP1EO, NP2EO, NP3EO) and phenoxy carboxylates (i.e. NP1EC, NP2EC) [27-29], which have greater aquatic toxicity than the original, fully ethoxylated NPEO [30].

The biodegradation of APEO by bacteria in sea water polluted with urban sewage is brought about by bacteria of the *pseudomonas genus* of marine origin. Few other species of Gram-negative bacteria are able to degrade APEO with nine or ten ethoxymer groups. *Pseudomomas strains* degrade only down to four or five ethoxymer groups, although other species of bacteria which are unable to degrade the long chain APEO are able to degrade the APEO with four or five ethoxymer units down to the two ethoxy group compounds [31].

A number of internationally standardized test methods have been established for assessment of the biodegradability of surfactants, and some studies have been conducted to investigate the environment fate of APEO, by the introduction of NPEO into sewage treatment plants, and subsequently monitoring the plant output into river water.

In a study of the Glatt River [32] Ahel *et al* found that while STPs were indeed reducing the amount of long ethoxylate chain species, the elimination rate of all nonylphenolic compounds (70 ± 15 %) was significantly lower than the elimination of BOD (biochemical oxygen demand) (86 ± 9 %), indicating that nonylphenolic compounds are not the most biodegradable fraction of sewage. No net elimination of NP1EO and

NP2EO was seen in the results, indicating that their formation during activated sludge treatment was faster than their degradation. In addition, the concentrations of NP1EC and NP2EC in secondary effluents were 2.1–7.6 time higher than in primary effluents, indicating significant formation in this class of compound during aerobic biological treatment (Table 5.1). Thus, while sewage treatment effectively degrades the long ethoxylate chains, the resulting shorter chains and carboxylic degradation products (which are much more resistant to biodegradation) remain and are present in the final effluent from the treatment plants.

	NP13 –20 EO	**NP1–2 EO**	**NP**	**NP – 2EC**
Primary Effluent	82.4 %	11.5 %	3 %	3.1 %
Secondary Effluent	28 %	21.8 %	3.9 %	46.1 %

Table 5.1 The influence of sewage treatment on non-ionic surfactants and their degradation products [data from ref. 32].

Ahel *et al* [33] have reported the results of another study of the Glatt River in Switzerland which receives effluents from several sewage treatment plants. Results indicated that the biodegradation products NPEC were the most abundant APEO type compound (2 –7.1 μg/L) and, as would be expected, and intact NPEO were the least abundant (1– 7.7 μg/L). Ratios of APEO and their biodegradation products were found to resemble those of secondary effluents. These types of compound were seen to undergo

significant changes due to the biodegradation process. Contribution from intact NPEO decreased from 21 % to 3.5 % of the total; in contrast, NPEC increased from 51 to 85 %.

Mann *et al* [34] have evaluated the biodegradability of OPEO by field trials with a trickling filter sewage treatment plants. Biodegradability rose from 26 % in March to a level of about 80 % in late August and September. During November and December biodegradation fell until by the end of January it was only 20-25 %. The decrease in biodegradation was attributed to low biological activity during the cold winter months.

Stiff *et al* [35] have studied the effect of temperature on the removal of OPEO on a laboratory scale. Tests were carried out at 8,11 and 15 °C using the "porous pot" activated sludge technique over a period of 122 days. At 20 mg/L the percentage removal was high during the period at 15 °C but at 11– 12 °C and at 8 °C it varied between 40– 95% and 20– 80%, respectively, indicating that at low temperatures, stable populations of microorganisms adapted to OPEO could not be maintained.

Rudling *et al* [36] reported a study of the biodegradation of NPEO, using the Organisation for Economic Cooperation and Development (OECD) screening test. NPnEO (n = 8, 10, 14) were degraded to an extent of more than 90% within 12 days. Fifty percent of the predominantly formed degradation product NP2EO was eliminated at 20 °C after 28 days, while at 15 °C, no degradation was observed.

Schoberl *et al* [37] have investigated NPEO in pond water and sea water at different temperatures over a period of 50 days. The extent of primary biodegradation of NPEO at 20 – 23 °C was 33 – 36% in pond water and 95% in sea water. At 3 – 4 °C they were degraded to a maximum of 37% in pond water and to only 15% sea water.

Trocme *et al* [38,39] have studied the biodegradability of NP in sludge-amended soil and in a compost-sand stone mixture spiked with 100 and 1000 mg/kg technical NP. In both cases, biodegradation of NP was approximately 90% after 100 mg/kg treatment but only approximately 60% after 1000 mg/kg treatment within 40 days. A rapid decrease in the respiration rates of the soils in 1000 mg/kg samples by the fourth day indicated a toxic effect of NP on the microorganisms. Kirchmann *et al* [40] have spiked soil samples with 10 and 500 mg/kg NP and determined the soil respiration by measurement of the CO_2 evolution. At high concentrations of NP respiration was significantly higher compared with the untreated control, whereas no effect was measured at the lower concentration of 10 mg/kg. In both cases the concentration of NP reached the detection limit of 0.02 mg/kg after 20 days. These results are apparently contrary to those of Trocme *et al* [38,39], but a reason for that may be the different concentration levels and experimental conditions.

5.2.1.2 Metabolites of APEO in the Marine Environment

Studies have been conducted on the environmental behavior of sewage released into the marine environment. Waters and sediments from the Venice Lagoon were analysed as it receives treated and untreated domestic and industrial effluents [41].

NP, NP1EO and NP2EO were detected in the range 0.15– 13.7 μg/g (dry weight basis) in the first 0.01 to 13.7 mm of the sediment layer in the lagoon (using a portable re-suspending device). This was equivalent to a five-fold increase in concentration compared with the underlying 5 cm of sediment. The amount of NP and short chain NPEO bound to re-suspending sediment showed a seasonal dependence. In April and July, twice as much material was found compared to that in February. However, in February, the total concentration of biodegradation product per unit of sediment surface was one order of magnitude higher, possible due to the growth of micro algae. On average, the micro algae contained NP, NP1EO and NP2EO at concentration of 0.25 +/- 0.15 μg/g (dry weight). In water APEO oligomers (up to thirteen ethoxylate units) were found in an average concentration of 0.6 – 4.5 μg/L.

If toxic metabolites of APEO can be found in sludge, it is a possibility that bottom-feeding animals, such as mussels, may consume these compounds. McLeese and co-workers [42] studied the uptake and excretion of aminocarb (containing nonylphenyl formulations) by mussels. They suggested that significant contamination of bivalves does not occur if the concentrations of the aminocarb and nonylphenol in water are less than 0.01 mg/L.

Wahlberg *et al* [43] reported concentrations of between 0.2 and 0.4 μg/L nonylphenol, 0.075-0.275 μg/L NP1EO, 0.04-0.125 μg/L and 0.03-0.04 μg/L NP3EO in blue mussels (*Mytilus Edulis*). The study was carried out by putting mussels in cages at different depths and distances down the coast from a manufacturer of surfactants in Sweden.

5.2.1.3 Toxicity

The biodegradation products of NPEO such as NP, NP1EO and NP2EO have a high degree of lipophilic character, and therefore, bioaccumulate in aquatic organism. Hence, the effect of APEO in the environment is a very important issue, especially because of their persistence and the toxicity of their biodegradation products, in particular, alkylphenols.

A survey of the literature by Thiele *et al* [44] showed that most studies of APEO toxicity have concentrated on nonylphenol. McLeese *et al* [45] determined the LC_{50} (the concentration that kills 50% of a population) of NP for various species living in the sea (Table 5.2).

Species	Test Duration (h)	LC_{50} (mg/L)
Freshwater clam	144	5.0
Shrimp	96	0.4
Soft-shelled clam	144	>1.0
Lobster	96	0.2
Salmon	96	0.9

Table 5.2 NP LC_{50} data for various marine organisms [45].

Terrestrial animals are much less sensitive than aquatic species to surfactants. For example the median lethal dose (LD_{50}) for oral ingestion by mammals generally lies in the range 500-5000 mg of surfactant per kg of body weight, which is comparable to sodium chloride or sodium bicarbonate [25].

5.2.1.4 Oestrogenic Properties of Alkylphenol Ethoxylates

The majority of APEO are used in aqueous solutions; therefore, they are discharged into municipal and industrial waste waters which enter sewage treatment plants. During the different steps of sewage treatment, a complex biodegradation process of APEO takes place, leading to the formation of several biorefractory metabolites [36,46-50]. The degradation products 4-alkylphenol diethoxylate (AP2EO), 4-alkylphenol monoethoxylate (AP1EO), [(4-alkylphenoxy)ethoxy] acetic acid (AP2EC), (4-alkylphenoxy) acetic acid (AP1EC), and 4-alkylphenols. 4-Alkylphenols, formed by shortening of the hydrophilic ethoxylate chains are persistent, strongly lipophilic, and more toxic than the parent compounds. Acute toxicity data of NP to aquatic organisms range from 0.18 to 5.0 mg/L, dependent on species and experimental conditions [45,51]. In comparison, the LC_{50} for NP9/10EO to fish are 5.0-11.0 mg/L [52]. The release of these highly toxic compounds via secondary effluents or sewage sludge could be harmful to the aquatic or terrestrial environment.

Recently, toxicity results have shown that the nonylphenolic compounds are endocrine disruptors that can pose a health risk to humans and to wildlife. According to Soto *et al* [51], 10 μM NP displays similar oestrogenic effects to 30ppm 17β-oestradiol in inducing

the proliferation of MCF_7 human breast cancer cells; in addition, NP can cause feminization of male fish [53] and affect adversely testicular size and spermatogenesis in rats [55,56].

5.2.2 Biodegradation of Anionic Surfactants

LAS are generally regarded as biodegradable surfactants. Very high levels of biodegradation (97-99%) have been found in some waste water treatment plants (WWTP), under aerobic conditions. The biodegradation of LAS is affected by a number of factors amongst which are the concentration of dissolved oxygen [57], complexing with cationic surfactants [58,59], the formation of insoluble calcium and magnesium salts [60], the presence of other organic contaminants [61,62] and the effect of LAS on the pH during aerobic degradation [63]. In sewage-contaminated groundwater, the rates of LAS biodegradation increase with dissolved oxygen concentration and the longer alkyl chain homologues (C_{12} and C_{13}) are preferentially biodegraded. However, the removal of LAS was found to be 2 – 3 times greater under laboratory conditions than in field tracer studies [57].

The rate and pathway of LAS biodegradation have been the object of many studies. These have been reviewed by Swisher [25] and Schöberl [64]. The pathway of LAS biodegradation is shown in Figure 5.7 . Initial attack is ω–oxidation of the terminal methyl groups of the alkyl chain. The methyl group that is further from the phenyl group is attacked first, and the resulting oxidation products, a series of alkanoic acid that are shortened by two carbons at a time via β–oxidation. The resulting short-chain

intermediate is often called sulphenyl carboxylate (SPC). The ring structure is hydroxylated next by oxygenases in preparation for ring cleavage. Ring cleavage is the proposed rate-limiting step in LAS biodegradation. Dioxygenase-catalyzed ring cleavage is proposed to occur at the 1–2 position of the ring followed by desulphonation. Desulphonation prior to ring cleavage is another possible mechanism. Once ring opening and desulphonation have occurred, the resulting aliphatic intermediates can enter common pathways for further oxidation or assimilation into biomass.

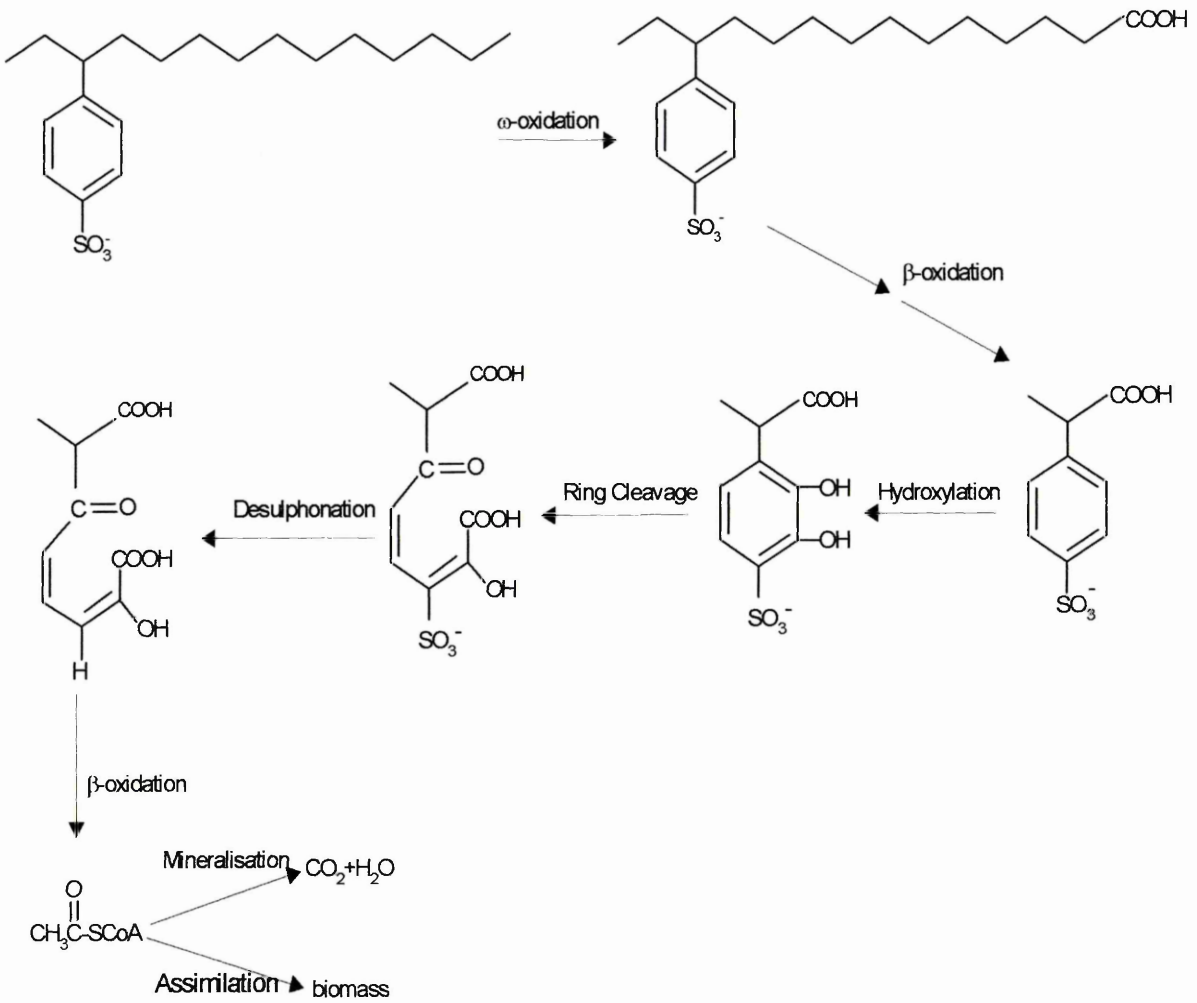

Figure 5.7. Biodegradation pathway of LAS.

All alkyl aryl sulphonates and petroleum sulphonates would be expected to follow the same basic catabolic scheme: (a) terminal oxidation and shortening of the alkyl portion; (b) ring (phenyl or naphthyl) hydroxylation ; (c) ring opening (cleavage) and desulphonation ; (d) further breakdown via common intermediary metabolic pathway, ultimately to CO_2 or assimilation of intermediates into biomass. The structure of the parent molecule has great importance for the extent of biodegradation and its rate. Other effects of structure on biodegradability are as follows:-

- For a given homologue, the greater the distance between the sulphonate group and the more distant terminal methyl group on the alkyl chain, the faster the degradation. That is, internal phenyl isomers degrade slower than external (e.g. 2-,3-phenyl) isomers.
- The effect of alkyl chain length size is uncertain. Differences in biodegradation rates between surfactants with longer or shorter alkyl chains may actually reflect solubility (microbial uptake) and inhibitory effects.

5.2.2.1 Environmental Effects/ Toxicity

There have been several studies on the toxicity of LAS [65-67], which show that toxicity to aquatic life increases with increasing chain length of the alkyl chain. For commercial LAS to be acutely toxic, it must be in the environment at a concentration of between 1 – 10mg/L. LAS biodegradation intermediates are 100 to 1000 times less toxic than the parent compound, and are normally present in river waters at concentrations well below threshold levels which equate to chronic effects.

In mammals LAS is usually excreted, before ultimate degradation, via urine and faeces. It is absorbed and passed into the blood in the intestine and eventually arrives in the liver where it is broken down via ω– and β– Oxidation. The hydrophilic compounds i.e. short chain carboxylates, are passed to the blood while LAS and longer chain sulphophenyl carboxylates (SPC) move into bile for the whole process to continue. The short chain SPC are finally excreted as urea from the kidneys. Any LAS which was not absorbed in the kidneys will be found in excreted faeces [68].

In experiments using radio-labeling techniques, LAS metabolites were detected in rat urine (40-58 % of original LAS concentration) and in the faeces (39-56 % of original LAS concentration) over a four day period. A total of 19 % of the original LAS concentration ingested remained intact [68].

De Henau *et al* [69] tested several commercial seedlings for LAS toxicity, and concluded that foliage showed visible signs of toxic effects when the soil containing the seedlings was sprayed with a solution of LAS at concentration of 100 mg/L and above. In all species, no reduction in growth occurred between 0.4 and 2.2 mg/kg. These data were collected under stress conditions and when compared to the measured concentration of 0.9 to 2.2 mg/kg in the environment, it would seem that LAS in sludge applied to soil does not represent a hazard to terrestrial plant life.

Kimberle [70] reported LAS toxicity values as *No Observed Effect Concentrations (NOEC)*, and the values were used to produce a hazard data assessment model. He

reported that the shorter chain LAS (i.e.C_{10}) is less toxic to fish and invertebrates than LAS with longer chain lengths (i.e.C_{14}). The NOEC for C_{10} was 10 mg/L compared to 0.1 mg/L for C_{14} . For commercial applications with average chain length of $C_{11.8}$ the NOEC was 1.2 mg/L; double the amount of those with an average chain length of $C_{13.3}$.

A summary of the LC_{50} concentrations of LAS for various aquatic species is shown in Table5.3.

Species	Test Duration (h)	LC_{50} (mg/L)
Marine shrimp [45]	96	0.40
Lobster [45]	96	0.20
Common mussel [71]	96	3.00
Fresh water clam [45]	144	5.00
Soft shelled clam [71]	360	1.00
Common mussel [71]	360	0.50
Common mussel [71]	850	0.14

Table 5.3 LC_{50} concentrations of LAS for a variety of aquatic species

In study of various animals from marine and river environments, Bressan *et al* [72] found that the concentration range in which LAS displayed acute effects range from 0.25 to 200 mg/kg. The tolerances observed were very dependent on the LAS concentration and the organism in question. Among the more sensitive organisms examined were copepods

and embryos of the sea urchin, *Paracentrotus lividus*, while among the more resistant were the fresh water molluscs *A. cygnea* and *U. elongatulus*.

5.2.2.2 Biodegradation of NPEOS in Sea-water

Although there are many different kinds of micro-organism in sea-water (algae, bacteria, protozoa), the most important of these are bacteria, since they have greater and faster growth capacity than others, and can survive longer in a wider variety of substrates.

Since it is proposed that NPEOS are useful for EOR application in high salinity environments, e.g., off-shore oilfields, their biodegradation by salt-resistant bacteria is of interest. In this work the biodegradation of NPEOS by two different strains of salt-resistant bacteria has been studied and the data obtained are compared with those from chemical oxidation and from the biodegradation of NPEO. As previously discussed this is particularly important since NPEOS would appear to have the potential to biodegrade to endocrine disrupting metabolites.

5.2.3 Experimental

5.2.3.1 Reagents and Materials

A commercial NPEOS formulation manufactured by Hoescht was gifted by Dr Tor Austad (Rogaland University centre, Norway). Synthetic sea-water was purchased from BDH (Poole, Dorset, U.K.). *Paracoccuss halodentitrificans* was purchased from the National Collections of Industrial and Marine Bacteria (NCIMB) Ltd (Aberdeen, Scotland, U.K.). *Bacterium T-52* was purchased from American Type Culture Collection as ATCC 27042 (Manassas, Virginia, USA). Strong Anion-Exchange (SAX) cartridges (500mg) were purchased from Varian (Frampton Avenue, Harbor City, USA). All solvents used were HPLC grade and were purchased from Fisher Scientific (Loughborough, U.K.). All water used was Milli-Q grade. Ammonium acetate and acetic acid used were HPLC grade (Fisher Scientific)

5.2.3.2. Culture conditions for *Paracoccus halodenitrificans and Bacterium T-52.*

5.2.3.2.1 *Paracoccus halodenitrificans*

The medium used for the growth of *Paracoccus species* contained 3.0gm yeast extract, 5.0gm peptone, 750ml filtered, aged sea-water, 250ml distilled water The temperature was maintained at 25-27 °C and the pH to 7.3 ± 2.

5.2.3.2.2 *Bacterium T-52*

Medium (A)

The basal medium composition contained 7.5g K_2HPO_4, 0.8g $(NH_4)_2SO_4$, 1.0g KH_2PO_4, 0.1g $MgSO_4.7H_2O$, 10.0mg $FeSO_4.\ 7H_2O$, 1.0ml salts solution (4.4g $ZnSO_4.7H_2O$, 3.0g $MnSO_4.H_2O$, 6.0g $CaCl_2.H_2O$, 0.2g $CuCl_2 2H_2O$ and 1.82g $(NH_4)_6\ Mo_7O_{24}.\ 4H_2O$ in 1000ml distilled water) and 70g NaCl in 1000ml distilled water and adjust pH to 6.9.

Medium (B)

The basal medium composition contained 2.44g Na_2HPO_4, 1.52g KH_2PO_4, 0.20g $MgSO_4.\ 7H_2O$, 0.50g $(NH_4)_2SO_4$, 0.05g $CaCl_2.2H_2O$, 10ml trace element solution (0.50g EDTA, 0.20g $FeSO_4.7H_2O$), and 100ml trace element solution (0.10g $ZnSO_4.7H_2O$, 0.03g $MnCl_2.4H_2O$, 0.30g H_3BO_3, 0.20g $CoCl_2.6H_2O$, 0.01g $CuCl_2.2H_2O$, 0.02g $NiCl_2.\ 6H_2O$, 0.03g $Na_2\ MoO_4\ 2H_2O$) and 34g NaCl in 1000ml distilled water and adjust pH to 6.9.

5.2.3.3. Sample Pretreatment

In order to simplify the chromatograms of the biodegradation products residual non-ionic starting material was selectively removed from the formulation by the use of SAX solid phase extraction cartridges. The surfactant solutions were acidified to pH 3-4 with glacial acetic acid. The cartridge was washed with methanol (5ml) and distilled water (10ml). The acidified surfactant solution was passed through the cartridge which was subsequently washed with 2% acetic acid in methanol (5ml) to selectively elute the non-ionic surfactants. The cartridge was then washed with methanol (5ml) before elution of the anionic fraction in 2 M HCl in methanol:water (50:50) (15ml). This was evaporated

to dryness and the now "pure" NPEOS was dissolved in the synthetic sea-water/ distilled water.

5.2.3.4. HPLC instrumentation and conditions

All HPLC analyses were performed on a Waters 600-MS gradient HPLC system. The columns used were Alltech (Carnforth, Lancashire, U.K.) mixed-mode reversed-phase/ion-exchange columns, mixed-mode RP8/ anion 100Å, 7μm (150x4.6mm). Fluorescence detection was carried out using a Jasco FP-920 (Cheltenham, U.K.) fluorescence detector, $\lambda_{ex.}$=225nm and $\lambda_{em.}$=295nm. Data were output to Hewlett Packard HP 3396A integrator. Injections (100μl) were made using a Perkin-Elmer ISS-101 autosampler. The mobile phase gradient used is as shown in Table 3.1 in Chapter three. All analyses of the biodegradation products of NPEOS using both microorganisms (*Paracoccus halodenitrificans and Bacterium T-52)* were completed using the C8/SAX column described above.

5.2.3.5. Biodegradation of NPEOS using Paracoccus species.

The biodegradation of high purity of NPEOS was carried out in fifteen volumetric flasks (ca. 250 ml) containing NPEOS (1500 μg/L) dissolved in synthetic sea-water. To ten of the flasks was added 1ml of *Paracoccus halodenitrificants* (250,000 cells) from the culture samples. The other five flasks contained NPEOS dissolved in sea-water only and were used as blanks in the measurement of biochemical oxygen demand (BOD). All samples were kept in a water bath at constant-temperature (20± 0.5 °C) and in the dark for 5- days. Each day, three flasks were taken. The BOD was measured, for one blank

and for one sample containing both microorganism and NPEOS. The second sample was extracted and analysed by HPLC.

5.2.3.6 Biodegradation of NPEOS using Bacterium T-52 species.

The surfactant, NPEOS, (0.094 g/L) was added to 100 ml of medium A and medium B. These media were held in six (*ca*. 250cm^3) Erlenmeyer flasks (three contained medium A and three medium B). These experiments were carried out for sterilized basal media mixtures. The media were inoculated with adapted *Bacterium T-52* and the flasks were shaken on a reciprocal shaker at room temperature, which ranged from 27 to 29 °C. Growth of *Bacterium T-52* was followed by reading the optical density (OD) at λ= 640 nm.

5.2.3.7 Sample Preparation

Before extraction, the aqueous samples were vigorously shaken to ensure adequate mixing and suspension of particulate material. Analytes were extracted from the bioassay by 3x25 ml of ethyl acetate. The organic layers were combined and dried using magnesium sulphate, and the extract was evaporated on a rotary evaporator to give a greasy residue which was dissolved in (50:50) methanol and water.

5.2.3.8 Method for the determination of Dissolved Oxygen (DO)

Dissolved oxygen was determined using a Russell CD400 dissolved oxygen meter. For this instrument, standard tables give values for solubility of oxygen in pure water at various temperatures. These values are given in mg/L (ppm) and relate to pure water in

equilibrium with water vapour-saturated normal air at the standard atmospheric pressure of 760 mmHg. In this work, the table of salinity correction values, which can be used to determine oxygen solubility in sea-water over the temperature range 0-35 °C, were used. The value given is subtracted from the corresponding value in the solubility column for each degree of salinity and expressed in parts per thousand (° / 00).

Thus the oxygen solubility = solubility in pure water(mg/L) –salinity of water (salinity correction).

5.2. 4 Results and Discussion.

The biodegradability of the surfactants is mostly governed by the molecular structure of the hydrophobe. The surfactants with branched hydrophobes are generally more resistant to biodegradation than those with linear ones .

Basically, biodegradation is measured by exposing the test compounds to microorganisms and analyzing the system at intervals to determine such things as the disappearance of the test compound, the formation of degradation products or the uptake of oxygen. Many combinations of microbiological environments with analytical methods have been used depending on the exact objectives of the work.

Biochemical oxygen demand (BOD) is one of the oldest methods used to measure oxygen uptake. Substrate, bacterial inoculum and oxygen are generally placed in a glass vessel and O_2 uptake determined by chemical analysis, manometrically or by an oxygen electrode. The NPEOS surfactant biodegradation by *Paracoccus halodeintrificans* was studied as a batch experiment, where the NPEOS served as the sole carbon source. NPEOS degradation was measured in terms of oxygen uptake by the microorganism. This is shown in Figure 5.8, which shows the gradually decreasing value of oxygen from the first day to the fifth day for sample mixtures containing NPEOS surfactant and microorganism in synthetic sea-water. This clearly indicates that the bacteria were consuming the oxygen in the solution in order to break down NPEOS to biodegradation intermediate products.

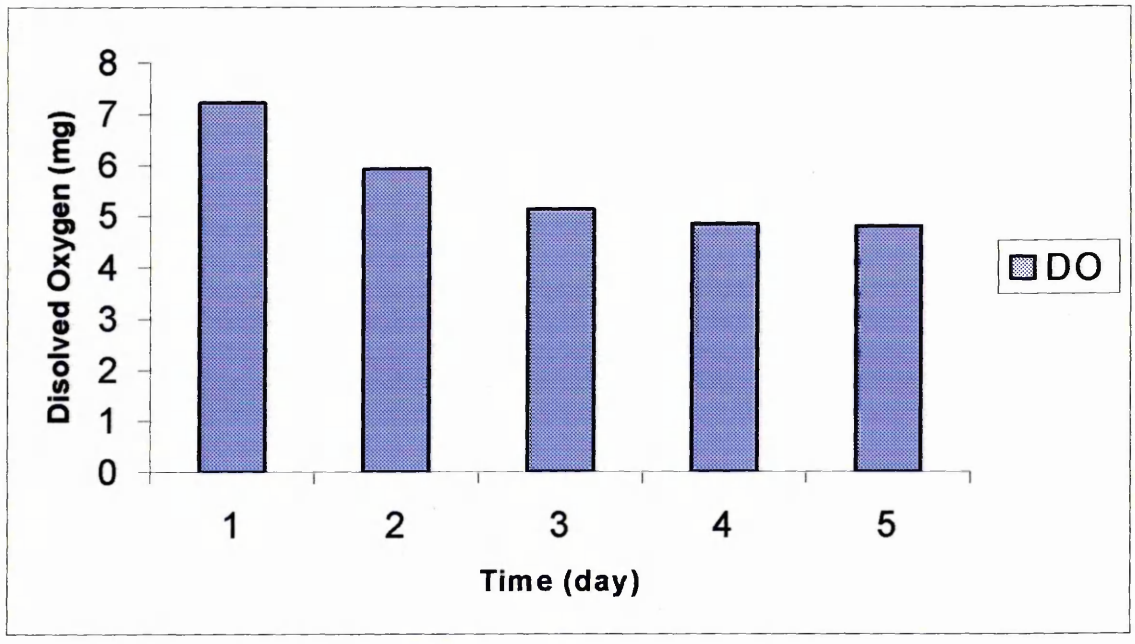

Figure 5.8: Decrease in Dissolved Oxygen During the Biodegradation of NPEOS By Paracoccus halodenitrificans.

The data for the HPLC analysis of the second set of samples are shown in Figure 5.9 (a-f). As can be seen, some new peaks occur at the retention times between (t_r) 4.20 and (t_r) 6.00 and these are initially observed in the 6 hours sample (Fig. 5.9) and increase in intensity over the five day study.

The retention time data obtained from chemical oxidation and biodegradation using bacteria species for NPEOS are slightly different, because the test solutions were analysed at different times. However, as can be seen, the emergence of a similar pattern of peaks to that seen in the chemical oxidation experiments was observed between t_r= 5.104 mins and t_r = 6.840 mins.

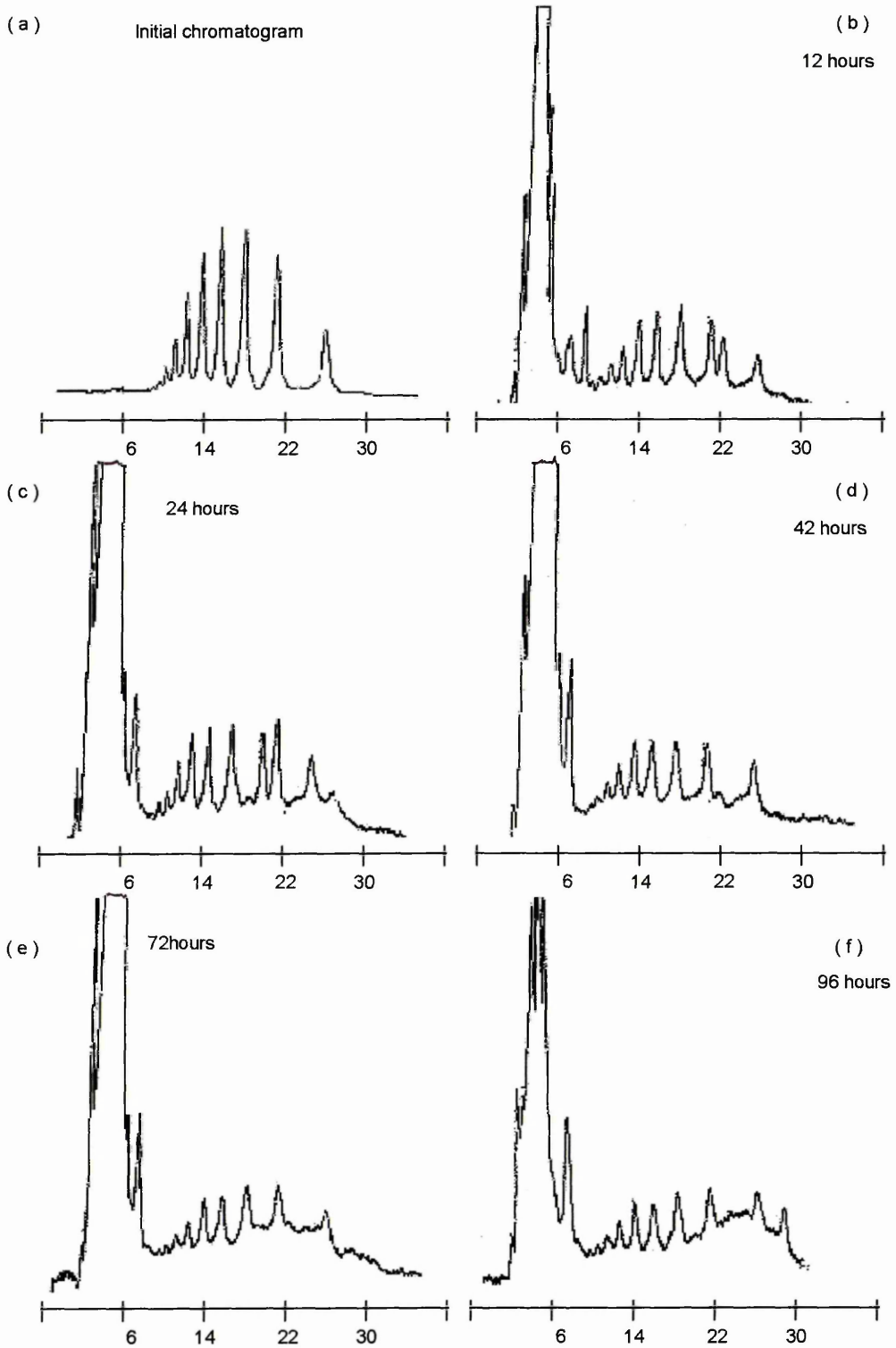

Figure 5.9. HPLC chromatograms for the separation of biochemical oxidation products from biodegradation of NPEOS for 5-days.by Paracoccus halodenitrificans.

Ornston et al [73] have proposed pathway for metabolism of two-carbon molecules that could be available for ethylene glycol metabolism, provided that ethylene glycol could be oxidised to glycolate. Carlos et al [74] have reported that *Bacterium T-52* can oxidise ethylene glycol to glycolate and glyoxylate and utilize both as sole carbon sources for growth. In this work *Bacterium T-52* was found to be unable to oxidize NPEOS.

The data from the *Paracoccus halodenitrificans* experiments Fig. 5.9 (a)-(f), however, show significant biodegradation of the NPEOS surfactant. Based on retention time data and the pattern of peaks observed, it is possible to tentatively identify these peaks as a similar mixture of $CNPE_nC$ compounds to that observed in the chemical oxidation experiments. Hence it has been demonstrated that the biodegradation of NPEOS surfactant does appear to follow the same pathway as NPEO, proposed by De Voogt [22]. Therefore it can be predicted that these surfactants will eventually degrade to the endocrine disrupting substance nonylphenol. This has important implications for their use in marine environments and further experiments should be carried out in order to prove this.

Conclusion

The experiment carried out on the oxidation of the NPEOS surfactant using Fenton's reagent, indicated a very fast oxidation reaction to give new products, and in a few hours NPEOS was completely converted to intermediate oxidation products. The presently available experimental data obtained from three different techniques (HPLC, MALDI/MS and LC-MS) for the oxidation products showed that the major intermediate oxidation products were dicarboxylic acids, $CNPE_nC$ (n=0-5), with some traces of mono-carboxylic acids (in two positions). For the oxidation of NPEOS, the mixture of $Fe(II)/H_2O_2$ was an effective oxidation system. The proposed degradation mechanism initially is via a hydroxyl radical attack on the ethoxylated chain (ω-oxidation) and then the second step, in which the long-chain NP_xEC are degraded to mainly short-chain, NP1EC occurs more slowly. The third steps is the attack on the terminal methylene group of the alkyl chain. These data suggest a similar degradation pathway for NPEOS as that for NPEO.

In the second part of this study, experiments on the biodegradation of NPEOS surfactant were carried out by using two different type of microorganisms (*Paracoccus and Bacterium T-52*). In the experiment using the *Paracoccus species* with NPEOS over a 5-day period, degradation to dicarboxylic acids ($CNPE_n$ C) and monocarboxylic acids was observed. However, HPLC chromatograms obtained from the experiment using *Bacterium T-52 species* over 45 days showed that this type of microorganism was unable to degrade NPEOS surfactant.

The overall aim of the work reported in this Chapter was to identify the products obtained from the biodegradation of NPEOS in sea-water. It is felt that strong evidence has been obtained that the primary biodegradation of NPEOS leads to the formation of the same intermediates as those formed from NPEO and hence further degradation to NP would be as expected pathway. This should be investigated further.

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CHAPTER SIX

General Conclusions and Future Work

6.0 Conclusion

Alkylphenol ethoxysulphonate (NPEOS and OPEOS) and alkyl aryl sulphonate (sodium dodecyl benzene sulphonate (DBS^-Na^+)) anionic surfactants have been found to tolerate high salinity water containing divalent cations. Thus, these chemicals are important candidates for use in enhanced oil recovery (EOR) processes in off-shore reservoirs where sea water is the injection fluid. In the work described in this thesis, three different analytical methods (HPLC, LC-MS and MALDI/MS) for NPEOS, OPEOS and DBS^-Na^+ surfactants analysis have been developed.

HPLC separation methods for NPEOS, OPEO and a mixture of 3:1 DBS^-Na^+ and NPEOS using C8/SAX, C18/SAX and C4/SAX mixed-mode columns and a fluorescence detector have been developed. Each method shows excellent resolution for all surfactants.

A range of extraction methods was investigated for the extraction of NPEOS and OPEOS surfactants from distilled water and sea-water, and for the extraction DBS^-Na^+ surfactants from sea-water only. A method reported in the literature was successfully adapted for the extraction of these surfactants. It used graphitised carbon black (GCB) SPE cartridges, and it was applied by decreasing the concentration of the ion pair reagent, tetramethylammonium hydroxide (TMAOH), from that used in the published method. The recovery data for the extraction of NPEOS (at two different concentrations) and OPEOS from each of the matrices were excellent and reproducible. Recovery data for the extraction of DBS^-Na^+ from sea-water were also excellent and reproducible. Slightly poorer reproducibility for the extraction of NPEOS and OPEOS surfactants from distilled

water was observed and the reason for this is unknown. It may, however be due to the surfactants existing in differing ionic forms in distilled water. These are the first data reported showing the extraction of these three surfactants from sea-water by SPE and the good sensitivity and recoveries obtained do suggest that SPE followed by LC with fluorescence detection would be the method of choice for the determination of these compounds in surface water samples.

A method was developed for qualitative analysis of NPEOS and OPEOS surfactants by MALDI mass spectrometry. Solutions were mixed with a concentrated solution of either α-cyano-4-hydroxycinamic acid (HCCA) for NPEOS or 2,5-dihydroxybenzonic acid (DHB) as a matrix and a concentrated solution of lithium chloride was also added as a source of lithium ions before crystallisation. The addition of lithium ions formed solely $[M+Li]^+$adducts, and created much cleaner, less complicated spectra.

The LC methods developed were compatible with LC-MS. In electrospray positive ion mode, ions observed corresponded to $[M-Na+H+NH_4]^+$ adducts for NPEOS and OPEOS surfactants, the original sodium presumably being completely displaced by the use of the ammonium acetate in the mobile phase.

Data obtained from each method indicate that the NPEOS formulation has an ethoxymer chain length ranging from 2-13 units with average of approximately 6.26 (calculating an average of the values obtained from each technique). The NPEOS data obtained by LC-MS indicates about one oligomer unit less than LC and MALDI/MS techniques. This is

in broad agreement with earlier studies, although the range of ethoxymer units was reported in these to be from 2-15. The data for the OPEOS formulation suggests that it has an ethoxymer chain length ranging from 1-8 ethoxymer units with an average chain length of 3.58.

In order to identify the intermediate products that were obtained from the biodegradation of NPEOS using bacteria, chemical oxidation studies using Fenton's reagent were carried out and the intermediate oxidation products identified.

The experiment carried out on the oxidation of NPEOS surfactants using Fenton's reagent, indicated a very fast oxidation reaction, and in few hours NPEOS completely converted to intermediate oxidation products. The presently available experimental data obtained from three different techniques (HPLC, MALDI/MS and LC-MS) for the oxidation products indicates that the major products are dicarboxylic acids $CNPE_nC$ (n = 0 - 4) along with some traces of single carboxylic acids (NPE_nC), with carboxylation occurring at both the ethoxymer chain and alkyl chain

The biodegradation of NPEOS surfactants was investigated by using two different type of microorganisms (*Paracoccus* and *Bacterium* T-52). Initial data appear to suggest that the biodegradation of NPEOS by *Paracoccus halodenitirificans* also leads to the formation of $CNPE_nC$ and NPE_nC, in themselves potentially toxic substances. If this is confirmed by further work it would prove that NPEOS has the potential to be harmful to a marine environment.

6.1 Future work

It would be interesting to repeat the experiments on the biodegradation of NPEOS using real sea-water and surface-water samples, collected from the Mediterranean around off-shore and in-shore oil fields. The extraction method developed in this work and instruments such as LC, LC-MS and MALDI/MS could then be used to investigate and obtain information about the effect of microorganisms living in Mediterranean sea-water on the NPEOS surfactants, to the identification of the biodegradation products, and potential effects on human and marine life considered.

It also would be of interest to carry out biodegradation experiment using different EOR surfactants i.e OPEOS and alkyl aryl sulphonates. The possible effects of biodegradation products from these types of surfactant on humans and marine organisms could then be discussed.

The chemical oxidation work could be extended by trying to carry out further experiments on the oxidation of OPEOS and alky aryl sulphonates by using Fenton's reagent. Analysis of the intermediate products by different techniques might confirm the structure of in the biodegradation work. The complete analysis of alkyl aryl sulphonates by LC-MS/MS and MALDI/MS could also be carried out.

Meetings and Symposia Attended

September 1998 BMSS—Annual Conference, University of Warwick