The Analysis of Surfactants and Their Determination in Surface Water by Liquid Chromatography and Liquid Chromatography - Mass Spectrometry.

By

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Abstract.

Linear alkylbenzene sulphonates (LAS) and alkylphenol polyethoxylates (APEO), the surfactants most used in this country, are complex mixtures of oligomers and homologues. Due to their excellent surface active properties they are widely used as ingredients in many detergent formulations both in the home and more importantly in industry.

For several years research has been carried out in order to understand the environmental impact of these widely used groups of compounds. However, some of the analytical techniques developed are not able to give reliable information concerning individual oligomer / homologue levels. As the toxicological profile of these compounds is dependent on the individual levels present this data is of great importance.

Surfactants are most often introduced into the environment through wastewaters. In order to try and actively contribute to this area, laboratory investigations were undertaken to develop chromatographic techniques which would be able to determine individual oligomer / homologue levels in environmental surface water samples. Even if extensive sample clean up is used these samples are by nature, very 'complex'. Mass spectrometry lends itself to the analysis of environmental samples as it is able to give detailed structural data which aids in eliminating signal contributions from interfering compounds. To this end, work was carried out in order to develop liquid chromatography methods which are compatible with conventional mass spectrometers and could be used for the determination of the environmental levels of both LAS and APEO.

CONTENTS

Glossary of terms used in this thesis.

ABS = alkylbenzene sulphonates.

ACN = acetonitrile.

AE = alcohol ethoxylate.

AP_9EO = an alkylphenol polyethoxylate which contains nine $-(CH_2CH_2O)-$ units in the hydrophilic chain of the molecule (the subscripted number indicates the number of $-(CH_2CH_2O)-$ units in the hydrophilic chain of the molecule).

APcI = atmospheric pressure chemical ionisation.

APEC = alkylphenoxy carboxylic acid.

APEO = alkylphenol polyethoxylate.

API = atmospheric pressure ionisation.

C18 = octadecyl silyl - a stationary phase used in HPLC columns.

CI = chemical ionisation.

CTAS = Cobalt Thiocyanate Active Substances Test.

EI = electron impact ionisation.

EM = emission wavelength.

EX = excitation wavelength.

GC = gas chromatography.

GC/MS = gas chromatography/ mass spectrometry.

GCB = Graphitized Carbon Black - modified powdered carbon.

HPLC = high performance liquid chromatography.

Igepal CA-630 = a trade name of a commercial octylphenol ethoxylate formulation.

IPA = iso-propyl alcohol.

LAEC = linear alkylphenoxy carboxylic acid.

LAS = linear alkylbenzene sulphonates.

LC/MS = liquid chromatography/ mass spectrometry.

LC/TSP/MS = liquid chromatography/ thermospray/ mass spectrometry.

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

LOD = limit of detection.

m/z = mass to charge ratio.

mbar = millibar.

MBAS = Methylene Blue Active Substance Test.

MS = mass spectrometry.

MTBE = methyl-tertiary-butyl ether.

Nansa SS = a trade name for a commercial alkylbenzene sulphonate formulation.

NOEC = no observed effect concentrations.

NP = nonylphenol.

NP_9EO = a nonylphenol polyethoxylate which contains nine $-(CH_2CH_2O)-$ units in the hydrophilic chain of the molecule (the subscripted number indicates the number of $-(CH_2CH_2O)-$ units in the hydrophilic chain of the molecule).

NPEC = nonylphenoxy carboxylic acid.

NPEO = nonylphenol polyethoxylate.

OBSA = octylbenzene sulphonic acid.

OPEO = octylphenol polyethoxylate.

PB = Particle Beam interface.

PB/CI/MS = Particle Beam /chemical ionisation/Mass Spectrometry

RMM = relative molecular mass.

r.s.d. = relative standard deviation.

SAX = strong anion exchange.

s.d. = standard deviation

SDS = sodium dodecyl sulphate.

SPC = sulphophenyl carboxylate.

SPE = Solid Phase Extraction.

STPs = sewage treatment plants.

Synperonic = a trade name for a commercial nonylphenol ethoxylate formulation.

THF = tetrahydrofuran.

TIC = total ion chromatograph

TMS = trimethyl silyl - a stationary phase used in HPLC columns.

Triton X 100 = a trade name of a commercial octylphenol ethoxylate formulation.

TSK = The brand name of a stationary phase for HPLC supplied by TosoHaas.

TSP = thermospray interface.

UV = ultra-violet light.

Y_{LG} = Liquid - Gas surface energy.

Y_{SG} = Solid - Gas surface energy.

Y_{SL} = Solid - liquid surface energy.

Chapter 1

Surfactant structure and formulation

1.0) Common Surfactants

Surface active agents, or surfactants are important in all areas of our daily life, not only are they used as active constituents of cleaning agents (Soaps, Detergents, etc.) but they are also vital in the stabilisation of emulsions (e.g., Foods, Cosmetics, pharmaceuticals), as mould release agents in the plastics industry, in fabric softening, in contraceptive pastes, in flotation, and in oil well drilling as well as in a host of other applications. They have the effect of lowering surface or interfacial tension, which increases solubility, along with other related properties such as wetting, (Fig. 1). In this diagram, no equilibrium is possible and so the drop spreads out until it is uni-molecular. This is a result of the increase in solid-gas surface energy (Y_{SG}), i.e., when surfactant is added $Y_{SG} - [Y_{SL} + Y_{LG} \cos\theta] \rangle 0$. Where Y_{LG} = liquid-gas surface energy, Y_{SL}= solid- liquid surface energy and Y_{SG} = solid-gas surface energy .

There are four basic classes of amphiphilic molecules which make up the diverse range of chemicals commonly known as surfactants. Each consist of a hydrophilic (water compatible) head group, known as the hydrophile and a hydrophobic (water repellent) hydrocarbon tail, the hydrophobe. The hydrophile can be:

(a) Anionic: e.g. Sodium dodecyl sulphate in which the hydrophobic portion of the molecule carries a residual negative charge, $C_{12}H_{25}SO_3^-Na^+$.

(b) Cationic: e.g. Hexadecyltrimethyl ammonium bromide, in which the hydrophobic portion carries a residual positive charge, $C_{16}H_{33}N^+(CH_3)_3Br^-$.

(c) Amphoteric: e.g. Alkyldimethyl betaine in which both positive and negative centres are to be found on the molecule, $RCH(CH_3)(CH_3)_2N^+CH_2COO^-$.

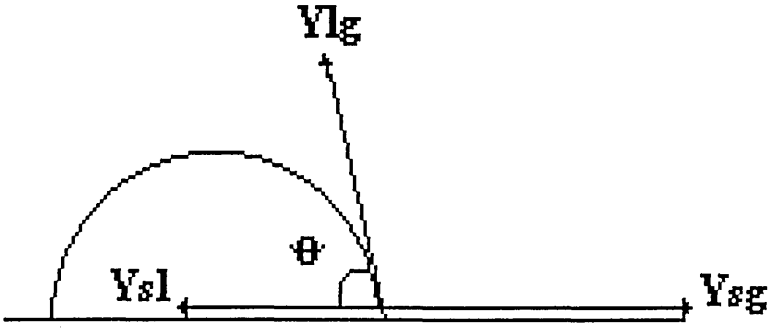
(d) Non-ionic: e.g. Dodecyl alcohol ethoxylate in which there is no residual charge, $CH_3(CH_2)_{10}CH_2(OCH_2CH_2)_nOH$.

When dissolved in water or another solvent, surfactants orientate themselves at the interface between the liquid and solid, or liquid, or gaseous phase and modify the properties of the interface. The modification may be accompanied by frothing or foaming and by the formation of colloids, emulsions, suspensions, dispersions, aerosols, or foams.

When a drop of water is on a solid surface :

Y_{LG} = liquid - gas surface energy Y_{SL} = solid- liquid surface energy

Y_{SG} = solid-gas surface energy



If the horizontal forces balance, the drop is at equilibrium.

$$Y_{SG} - [Y_{SL} + Y_{LG} \cos\theta] = 0$$

When surfactant is added $Y_{SG} - [Y_{SL} + Y_{LG} \cos\theta] > 0$

Figure 1.0) The spreading of a liquid drop.

1.1) Surfactant classes:

1.1.1) Cationic Surfactants :

A cationic surfactant is an amphiphatic species in which the hydrophobic head group carries a positive charge. Cationic surfactants account for about 5-10% of total surfactant production. The positively charged end of the surfactant may be associated with various chemical functional groups, i.e. Sulphonium, Phosphonium, Iodonium compounds etc. These have been seen to exhibit bacterial or insecticidal properties. However, due to their high expense and toxicity, only compounds in which the positive charge resides on a nitrogen atom, contained in either a protonated amino or a quaternary ammonium group, are of commercial significance. Also heterocyclic compounds, such as morphine, imidazoline, and pyridine have some commercial usage as surfactants, although it is the simple alkyl amines ($R\text{-}NH_2$) in which R is a linear hydrocarbon derivative which have 80% of the market share.

1.1.2) Chemical reactions of the amphiphilic amines.

Due to the presence of a lone pair of electrons, the nitrogen atom of an amine is strongly nucleophilic and basic. The basicity range is , secondary > primary > tertiary. The amino group reacts strongly with inorganic and organic acids to yield the corresponding amine salt.

$$RNH_2 + HA \rightarrow RNH_3^+A^- \rightarrow RNH_3^+ + A^-.$$

Amines also undergo typical nucleophilic reactions such as alkylation addition to activated double bonds and ring cleaving. These reactions are of commercial importance since they lead to the formation of speciality cationic surfactants as follows:

Carboxymethylation can be used to convert amines into amphoterics i.e., alkyl amino acids.

$$RNH_2 + CH_2{=}CHCOOH \rightarrow RNHCH_2CH_2CO_2H.$$

At low pH = CATIONIC. At high pH = ANIONIC.

They give enormous scope for the preparation of speciality chemicals and prove to be the most commercially significant chemical modification procedures. At this time cationic surfactants only play a small role when considering the total world usage of surfactants.

1.1.3) Non-ionic Surfactants.

The term non-ionic surfactant refers chiefly to polyethylene oxide and polypropylene oxide derivatives, but other surfactants are also included in this category, such as hydroxyhexitol derivatives and fatty amine oxides. Non-ionic surfactants are usually prepared by the addition of ethylene oxide to compounds containing one or more active hydrogen atoms, such as alkyl phenols, fatty acids, fatty alcohols, fatty mercaptans, fatty amines and polyols. The alkylphenol polyethoxylates (APEOs) are manufactured by a base catalysed reaction of ethylene oxide with alkylphenols (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 is almost 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 complicated mixture of a variety of compounds. The most common classification of non-ionic surfactants is into the following types :

i) Polyoxyethylene alcohols and polyoxyethylene esters of fatty acids

These are surfactants with the general structures :

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

Ethoxylates are by far the most important group of alkoxylated non-ionic surfactants.

Polyoxyethylene surfactants (ethoxylates) were introduced in the US as a textile chemical shortly before the 1940's. The water solubility of these compounds arises from the recurring ether linkage in the polyethoxyethylene chain. A single oxyethylene group contributes slightly more to hydrophilicity than a 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 activity of the ethoxylates is not adversely influenced by water hardness.

ii) Alcohol Ethoxylates (AE).

Alcohol ethoxylates have emerged as the principal non-ionic surfactant in the consumer detergent product market. They are cheap to manufacture and are highly biodegradable. They vary in physical form from liquids 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 oxide units actually contains significant amounts of other ethoxylates ranging from 0-20 ethylene oxide units.

iii) Alkylphenol ethoxylates (APEO).

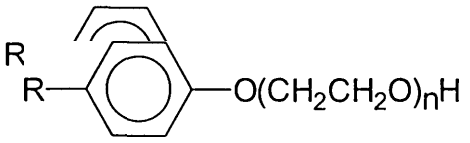
The physical and performance properties of these surfactants are similar to those of alcohol ethoxylates. They are derived mostly from alkyl phenols containing branched alkyl side chains, typically nonyl and octyl groups. This branching leads to a decrease in biodegradability in comparison to the alcohol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

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

The most important non-ionic surfactants in terms of environmental impact are the

Alkylphenol ethoxylates, (APEOs). An estimated 350,000 tonnes per year of APEOs are currently being used in the US, W. Europe and Japan[1].

The general formula for an Alkylphenol Ethoxylate (APEO) is :



Nonylphenol ethoxylates are the most commonly used APEOs. This molecule has an alkyl chain containing nine carbon units and an ethoxylate chain which can vary from 1 to 40 units.

Concern has increased about the world-wide usage of APEOs because of their relatively stable and toxic biodegradation intermediates[2]. Many European countries are now looking at alternative formulations and in the UK a voluntary ban has been introduced on their domestic use. It is hoped that the use of these products in both domestic and industrial detergents will be phased out in EC member countries by the year 2000.

1.1.4) Anionic Surfactants.

Carboxylate, sulphonate, sulphate and phosphate are the polar, solubilizing groups found in anionic surfactants. In dilute solutions of soft water, these groups are combined with a 12 carbon-chain hydrophobe for the best surfactant properties. In neutral or acidic media or in the presence of heavy metal salts the carboxylate group loses most of its solubilizing power. Of the cations (counter ions) sodium and potassium impart water solubility whereas calcium, barium and magnesium promote oil solubility. Anionic surfactants are divided into the following types :

i) Carboxylates.

Most of the commercial carboxylates are soaps. The general structure of a soap is $RCOO^-M^+$, where R is a straight chain hydrocarbon (C_9-C_{21}), and M^+ a metal or

ammonium ion. In soft water soaps show excellent detergency; however, bivalent metal ions (Ca^{2+} etc.) cause unsightly "curds". For this reason and from an economic point of view syndets, a combination of synthetic surfactants notably alkylbenzene sulphonates, and builders, notably 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 leads to surfactant molecules of unusually low surface tension.

ii) Alkylbenzene sulphonates (ABS).

Alkylbenzene sulphonates are effective surfactants which respond well to builders and foam boosters in detergents 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 thus :

$$RR'CHCl + C_6H_6 \xrightarrow{HF} RR'CHC_6H_5 + HCl$$

or

$$R'CH{=}CHR + C_6H_6 \xrightarrow{HF} R'CH(CH_2R)C_6H_5$$

followed by

$$RR'CHC_6H_5 \xrightarrow{SO_3/Air\ or\ oleum} RR'CHC_6H_4SO_3H$$

$$RR'CHC_6H_4SO_3H \xrightarrow{NaOH} RR'CHC_6H_4SO_3Na$$

Chapter 1 References

1) Greek BF, Layman PL, Chemical Engineering News, 63, 29,1989.

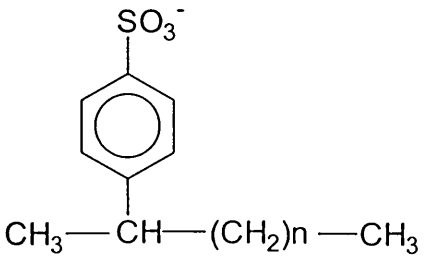
2) Mcleese DW, Sergeant DB, Metcalfe CD, Zitko V, Burridge LE, Bulletin of Environmental Contamination and Toxicology, 24, 575,24, 1980.

2.0) Introduction.

Normally, surfactants are discharged with waste water after their use. As a result it can be assumed that they and / or their degradation products will ultimately be found in the environment. Due to their huge usage their ecotoxicological potential must not be ignored. It is necessary therefore to measure the concentrations of the most commonly used surfactants, i.e. the anionic and non-ionic classifications in environmental samples. As these are present as mixtures of a particular generic classification (each having a slightly different toxic potential), it must be concluded that methods which can assess individual oligomer or homologue levels are more valuable as analytical tools than the conventional calorimetric techniques commonly used (Chapter 3).

2.1) Anionic Surfactants.

The present world-wide surfactant consumption is approximately 15 million tonnes per year[1]. More than 50% of the estimated figure is accounted for by soaps. The most common anionic surfactants are the Linear Alkylbenzene Sulphonates, (LAS), (Figure 2.0), which have a total production estimated at 1.8 million tonnes per year. This represents 25% of the total consumption of synthetic surfactants. In the industrial world i.e., the US, W. Europe and Japan the figure for the total LAS consumption is approximately one million tonnes per year. LAS consist of a long non-polar hydrocarbon side chain linked to a sulphonated benzene group. This gives rise to the generic classification for this type of molecule, "the alkylbenzene sulphonates". Commonly, the alkyl chain varies in length from 11 to 14 carbon units.



n = 11-14.

Figure 2.0) The Structure of Linear Alkylbenzene Sulphonate (LAS).

2.2) Toxicity

There have been several studies on the toxicity of LAS[2-6]. The general consensus of opinion is that toxicity to aquatic life increases with increasing chain length of the alkyl chain. The acute toxicity of commercial LAS is typically in the range of 1-10 mg L^{-1}. Biodegradation intermediates are 100 to 10,000 times less toxic than the parent molecule. They are normally present in river waters, at concentrations well below threshold levels which equate to chronic effects.

In mammals LAS is normally excreted before ultimate degradation via urine and faeces. LAS is absorbed and passed into the blood in the intestine. Eventually it 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 in urea from the kidneys. Any LAS which was not absorbed in the kidneys will be found in excreted faeces[7]. In experiments using radio-labelling techniques LAS metabolites were detected in rat urine (40-58% of original LAS concentration) and in faeces between (39-56% of original LAS concentration) over a 4 day period. A total of 19% of the original LAS concentration ingested remained intact[7].

De Henau[8] tested several commercial seedlings for LAS toxicity. He concluded that foliage showed visible signs of toxic effects when the soil containing the seedlings was sprayed with a solution of LAS at concentrations of 1000 mg L^{-1} and above. No reduction in growth occurred in any species at concentrations between 0.4 mg Kg^{-1}-2.2 mg Kg^{-1}. These data were collected under stress conditions and when compared to the measured concentrations of 0.9-2.2 mg Kg^{-1} in the environment, it would seem that LAS in sludge applied to soil does not represent a hazard to terrestrial plant life. Kimberle[9] has produced a paper which presents LAS toxicity values as No Observed Effect Concentrations (NOEC) . These values were used to a produce a hazard data assessment model. He states that shorter chain LAS, i.e., (C_{10}) is less toxic to fish and invertebrates than LAS with longer chain lengths i.e., (C_{14}). For C_{10}, the NOEC was shown to equal 10 mg L^{-1}, however, for C_{14}, the NOEC equalled 0.1 mg L^{-1}. For commercial formulations with an average chain length of $C_{11.8}$, the NOEC equalled

1.2 mg L^{-1} whereas those with an average chain length of $C_{13.3}$, the NOEC equalled 0.6 mg L^{-1}.

2.2.1) Toxicity in waterways.

It is thought that laboratory tests often over estimate the toxic potential of chemicals. In the environment there is usually a lower exposure level than in test tanks due to the greater expanse of water under consideration and the complex makeup of the water system. It is interesting to note that many types of fish are able to treat small amounts of LAS as naturally occurring bile salts and hence thus their digestion and excretion becomes fairly easy. Kimberle[9] studied the world wide data for LAS and has concluded that it is possible to find LAS in concentrations ranging from 0.01 mg L^{-1} to 10 mg L^{-1} in the environment. Using these data for toxicological effects he concludes that algae, invertebrate and fish have a 100 fold tolerance level to LAS exposure and effects. Sivak[10] states that acute toxic effects to aquatic life forms generally occur in adult vertebrates and invertebrates at surfactant concentrations ranging from 1 to 2 mg L^{-1}, juvenile and developmental stages show effects at somewhat lower concentrations. Table 2.0, displays a summary of the LC_{50} concentrations of LAS for various aquatic species.

Table 2.0) A summary of the LC_{50} concentrations of LAS for various aquatic species.

LC Exposure.	Concentration (mg L^{-1})	Species.	Ref.
24 - 96 Hr	1-10	Fish	11
96 Hr	0.4	Marine Shrimp	12
96 Hr	0.2	Lobster	12
144 Hr	5	Fresh water Clam (Anodonta Cataractae)	12
360	1	Soft Shelled Clam (Mya Arenaria)	12
96 Hr	3	Common Mussel (Mytilus Edulis L)	13
360 Hr	0.5	Common Mussel (Mytilus Edulis L)	13
850 Hr	0.14	Common Mussel (Mytilus Edulis L)	13

2.2.2) Toxicity of Sewage Sludges:

Under anaerobic conditions LAS is totally resistant to biodegradation, in contrast to NP_1EO and NP_2EO which are transformed to NP[14]. Early studies carried out using MBAS showed little or no LAS removal during anaerobic digestion[15]. Marcomini et al.[16] have studied fresh sludge and sludge obtained from landfill sites. Under aerobic conditions > 90% of LAS, NP and NP_1EO had disappeared over a 15 year period. However, over the same time under anaerobic conditions, samples showed little or no biodegradation. The degradation products of LAS, i.e. the sulphophenyl carboxylates show a characteristic homologue distribution in sewage effluent and groundwater[17]. Intermediate chain lengths of between 5 and 8 are found to persist over those with less than 5 and greater than 9. Similar results have been reported by Taylor and Nickless[18], in laboratory experiments using river water spiked with LAS. Table 2.1, displays environmental concentrations of LAS and the methods used to determine those concentrations.

2.2.3) Human toxicity.

The present day use of LAS does not appear to represent a hazard to human health. This is due to the large amount of LAS required to produce chronic effects in mammalian systems and also from the low order of acute mammalian toxicity. Acute oral LD_{50} values in rodents range from 650 mg Kg^{-1} to 2480 mg Kg^{-1} [19]. Simultaneous oral and subcutaneous administration of LAS (300 mg Kg^{-1} and 1 mg Kg^{-1}, respectively), to rhesus monkeys for 28 days produced no effects other than reduced weight gain[19].

2.3) Biodegradation of organic compounds.

Biodegradation can be split into two stages[20]:

(i) Primary degradation of a compound, such that the compound is metabolised into intermediates which are persistent. Their persistence means there is a delay or 'lag' in the biodegradation process before ultimate degradation is complete. Presumably this lag period for LAS and APEOs occurs until microbes capable of ring desulphonation and / or ring oxidation in most biodegradation media are formed.

(ii) Ultimate degradation of a compound occurs when the compound of interest is totally transformed into its basic elements, i.e., H_2O and CO_2 in the case of carbon containing molecules.

2.3.1) Biodegradation of LAS.

The effect of surfactant structure on primary biodegradation can be summarised as follows.

(a) The structure of the hydrophobe has a pronounced effect on the biodegradability; any branching of the hydrophobe will deter biodegradation.

(b) In general with the exception of polyethoxylate non-ionics[21,22] the nature of the hydrophile has little effect on the rate of surfactant biodegradation.

(c) Increased distance between sulphonate group and the far end of the hydrophobe group increases the speed of primary degradation of LAS.

The bacteria responsible for surfactant biodegradation use oxidation as a means of chemical breakdown. The three general oxidative mechanisms and pathways which appear to be particularly important in the bacterial attack on surfactants are:

(i) Terminal or ω oxidation (often the initial oxidation step) which is responsible for the initial attack on the terminus of the hydrophobe.

(ii) β oxidation, the process whereby the aliphatic portion of the hydrophobe is degraded.

(iii) Aromatic oxidation where a molecule containing a benzene ring is broken down.

It is thought that biodegradation attack begins at the end of the alkyl chain via mechanisms also used in the degradation of aliphatic hydrocarbons[23-27]. Evidence for these ω oxidation mechanisms is conflicting. Several differing pathways exist depending on the particular micro-organisms involved, the structural features of the hydrocarbon and the conditions of the media within which it is placed. Degradation can start via the dehydrogenation of the alkyl chain to an alkene followed by hydration to a primary alcohol, (Fig 2.1, step A) and then through to a carboxylic acid. After the alcohol has been formed the next step is an oxidation reaction to form the aldehyde[27]. This is carried out

by alcohol dehydrogenase enzymes. The ultimate product of this process is a carboxylic acid, (Fig 2.2)

The next step in the pathway is the shortening of the alkyl chain. Generally this step is carried out via a β-oxidation process; however, occasionally it can be carried out via a α–oxidation process which is less well understood. Alternatively, a more direct route can be followed, where ultimately the ring is shortened by one carbon unit after the formation of a diol, (Fig 2.1, step B). In both routes a carboxylic acid moiety is eventually formed with one less carbon unit in the chain by the loss of carbon dioxide[9,17,20,28], Figure 2.2.

Oxidation is used by cellular organisms to convert two carbon units at a time into acetyl groups which are used for energy or synthesis reactions by the cell. Coenzyme A (HSCoA) is used to catalyse the reaction. The basic principles are given in detail in many biochemistry text books[29].

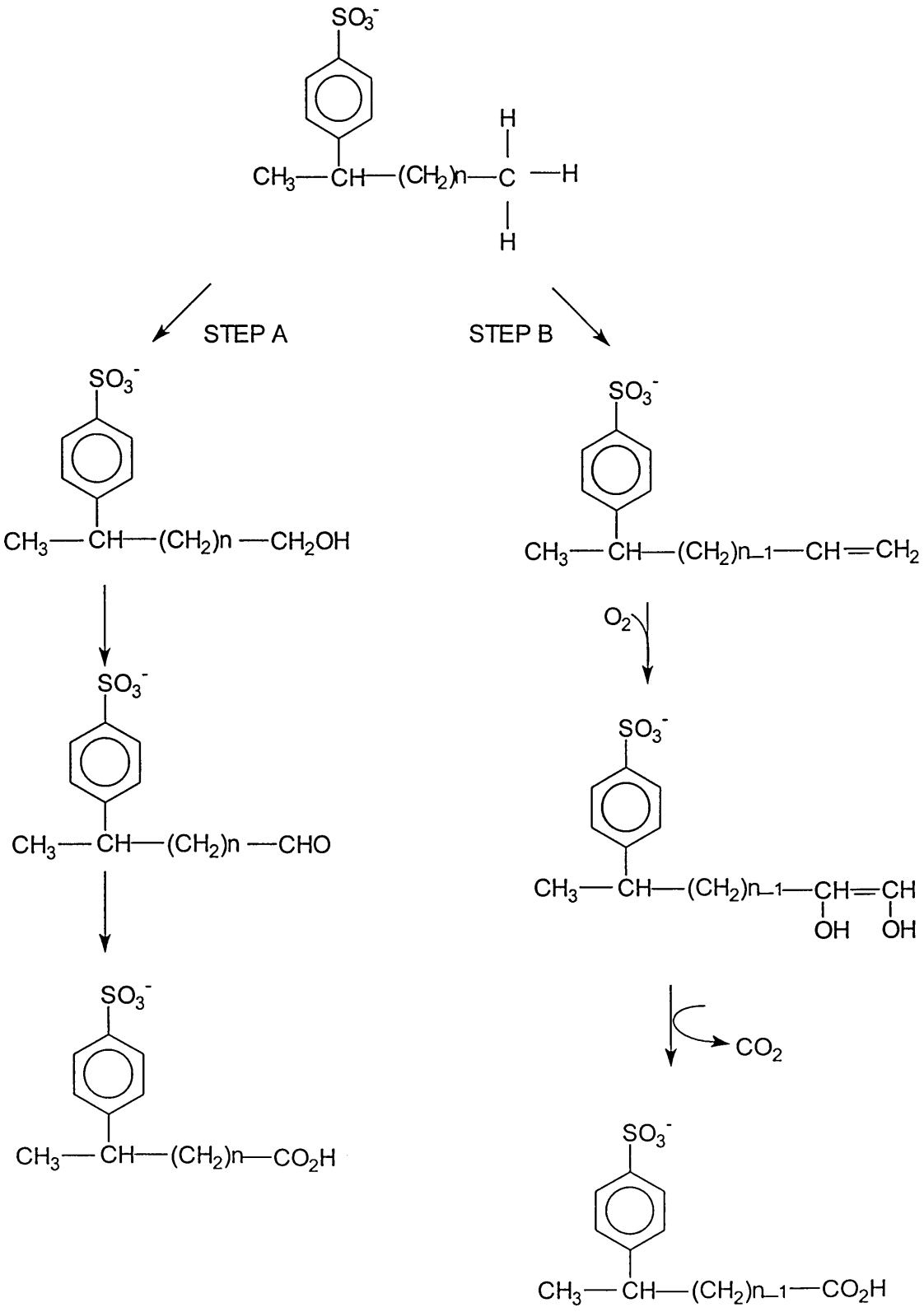


Figure 2.1) The possible oxidation steps for the oxidation of the alkyl chain of LAS.

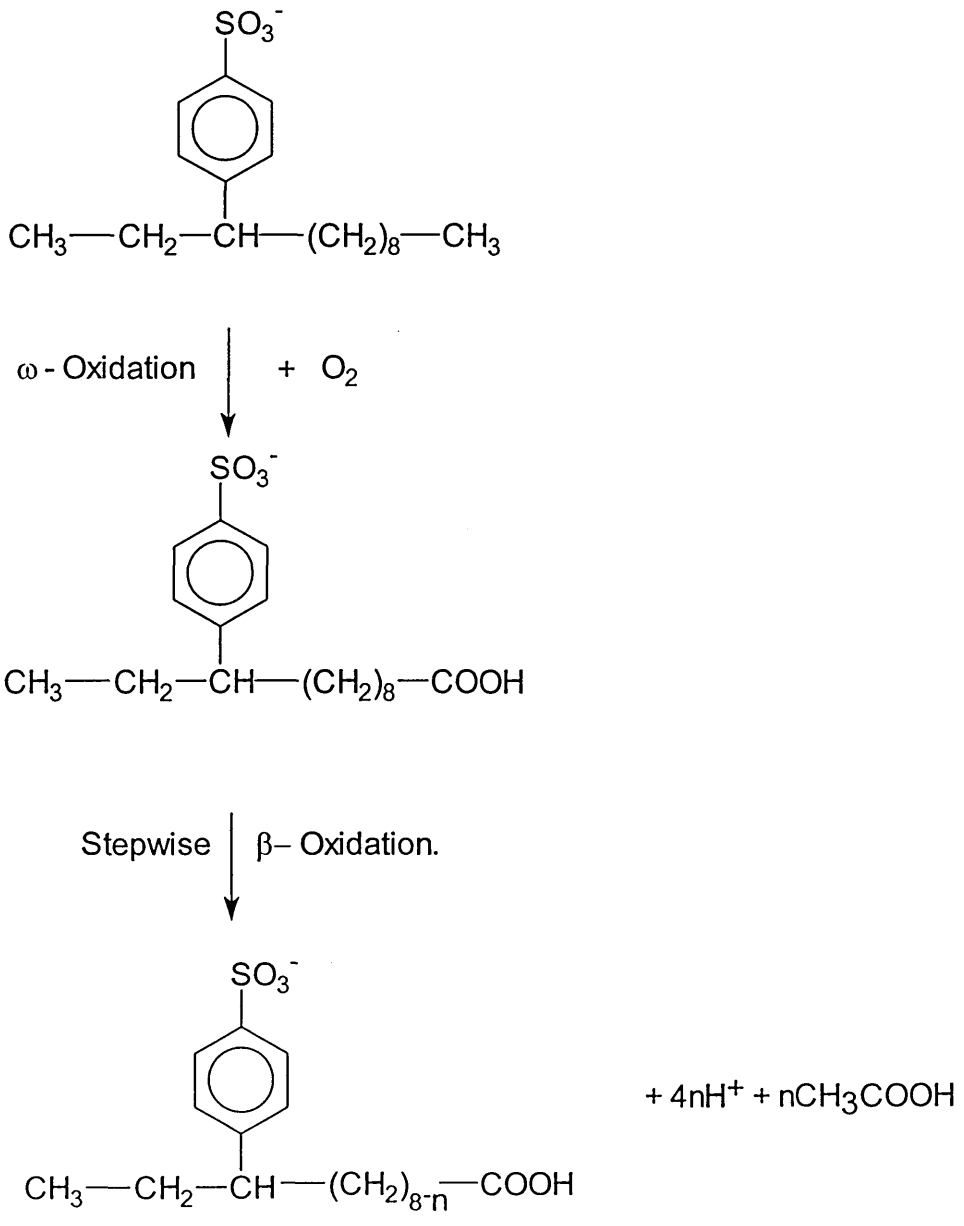


Figure 2.2) The primary degradation steps of LAS.(see reference 20).

Table 2.1) Environmental concentrations of LAS.

Sample Conc / mg L^{-1}	Extraction Method.	Analysis Method.	Ref.
Germany River Water = 0.04 R. Sediment = 16.2 μg g^{-1} Sewage Influent = 4.0 Effluent 0.07	River waters : extraction using solid phase extraction cartridge (SPE) followed by separation using a C_8 column. Sediments etc., extraction using methanol followed by anion exchange to isolate then C_8 column.	Reverse phase gradient HPLC using a C_{18} column Mobile phase = $NaClO_4$ (aq) / $NaClO_4$ in acetonitrile. UV detection at 230 nm.	30
Japan River = 0.097 μg ml^{-1} - 0.377 μg ml^{-1}		Reverse Phase HPLC with C8 column. acetonitrile / $NaClO_4$ (aq). Fluorescence detection Ex = 225 nm, Em = 295 nm.	31
Japan River = 0.086μg ml^{-1} - 0.091 μg ml^{-1}	TSK 10 mm x 3 mm pre-column used to extract analyte from sample.	TSK (polymer based anion exchange column) mobile phase Acetonitrile / 10 mM $NaClO_4$ (aq) UV detection at 220 nm.	32
Japan River = 0.100 μg ml^{-1}	TSK 10 mm x 3 mm pre column used to extract analyte from sample.	C_4 HPLC column with Acetonitrile / 0.1M $NaClO_4$ (aq). UV detection at 220 nm.	33
Japan. Rivers = 0.001 μg ml^{-1}- 0.84 μg ml^{-1}	Sep-pak C_{18} SPE cartridge	Reverse Phase HPLC. Isocratic run using C_8 column. Acetonitrile/ $NaClO_4$ (aq) mobile phase. UV detection at 225 nm.	34
Spain LAS in sewage 5.46 g Kg^{-1}	Soxhlet extraction : solid NaOH / methanol. Followed by C_{18} SPE clean up.	Reverse Phase HPLC Acetonitrile/ $NaClO_4$ (aq) Fluorescence detection Excitation = 225 nm Emission = 295 nm.	35

Table 2.1) continued

Sample Conc. / mg L^{-1}	Extraction Method.	Analysis Method.	Ref.
USA. Sewage Influent = 6.2 Sewage effluent = 0.066 Water = 0.025	C_2 SPE cartridge followed by an SAX SPE cartridge. 200 ml of river water used.	Isocratic TMS HPLC column THF/ 0.1M $NaClO_4$ (aq). Fluorescence Em = 225 nm EX = 290 nm.	36
Italy LAS influent = 6.7 LAS effluent = 0.08 SPC influent = 4.5 SPC effluent = 1.4	C_{18} SPE cartridge	Reverse phase gradient elution using C_{18} column. Fluorescence detection EX = 225 EM = 295 nm.	37
Iran Rivers = 0.61 μg L^{-1} - 0.78 μg L^{-1}	C18 boned silica membrane disks.	Methylene blue active test	38
Italy Fresh Sludge < 5 g Kg^{-1}	Soxhlet extraction with Methanol / NaOH.	HPLC C_{18} column. Acetonitrile / $NaClO_4$ (aq). Fluorescence detection Ex = 225 nm Em = 295 nm.	39
Switzerland Digested Sludge = 7.3 g Kg^{-1} (dry)	Soxhlet extraction.	Reverse Phase gradient HPLC with C_8 column.	40
Italy LAS raw sewage < 0.3 Treated sewage LAS < 208 μg L^{-1}	SPE using Graphitized carbon black (GCB) which allows preferential elution of NP, NPEO, LAS and SPC.	Reverse phase HPLC using C_8 with Acetonitrile (aq) / TFA 0.02 %.	41

Table 2.1) continued

Sample Conc. / mg L^{-1}	Extraction Method.	Analysis Method.	Ref.
Switzerland Raw waste water = 4.8 Mechanical treated waste water = 3.8 Biologically treated = 0.45	Extracted using a Sep-pak C_{18} SPE cartridge	Reverse phase gradient HPLC using C_8 gradient elution with IPA + Acetonitrile / $NaClO_4$ (aq). Fluorescence Ex = 225 nm Em = 295 nm.	42
USA SPC Sewage effluent = 51 μ L^{-1} Ground water = 8 μ L^{-1}	Ion exchange followed by solvent extraction and absorption chromatography.	GC/MS. Derivatization by reaction with PCl_5 followed by condensation with $F_3C\text{-}CH_2\text{-}OH$ to form trifluoroethyl esters.	43

2.4) Biodegradation of Non-ionic Surfactants.

The biodegradation of non-ionic surfactants is complex in nature. Bacterial inocula can attack non-ionic surfactants at three different points depending on the structure of the surfactant in question. Three different initial points of attack in aerobic conditions have been suggested[44]. The mechanisms can be summarised as shown below:

(1) A central fission mechanism in which the hydrophobe is cleaved from the hydrophile. β–oxidation is then responsible for the further conversion of the linear chains to CO_2 and H_2O.

(2) ω–Hydrophobe attack in which the far end of the hydrophobe is first oxidised to a carboxylic acid. Biodegradation then proceeds via the β–oxidation of the alkyl chain.

(3) ω–Hydrophile attack which occurs via the oxidation of the polyoxyethylene chain initially to a carboxylic acid. The mechanism of this process is not fully understood.

Under aerobic conditions the biodegradation rate and pathway which are followed are dependent on the type of bacterial inocula present and the structure of the intact surfactant. Linear alcohol ethoxylates (LAE) are known to biodegrade to CO_2 and H_2O via the central fission mechanism in which the hydrophobe is cleaved from the hydrophile[44], (Figure 2.3).

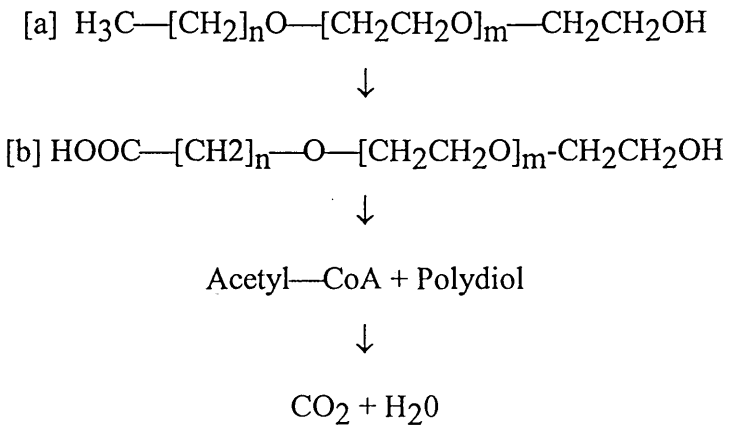


Figure 2.3) The biodegradation pathway of LAE.

Where structure [a] is Linear Alcohol Ethoxylate, LAE.

Where structure [b] is Linear Alcohol Ethoxycarboxylic acid LA_nEC where n = 0,1.

Schoberl et al.[45] postulated that the biodegradation of APEO takes place via the hydrolytic removal of an ethylene oxide group. This occurs via the oxidation of a cleaved ethylene oxide unit to glycolic acid and finally to glycoxylic acid.

The first step in the process is the oxidation of the ethylene oxide chain to a carbonyl group, followed by the hydrolytic removal of glycolic (hydroacetic) acid. Kravetz et al.[46-48] however, have suggested a substantial transformation of the aromatic ring together with simultaneous oxidation of hydrophile and hydrophobe. Swisher[44] and Ball et al.[50] believe the major mechanism involved in the bio-transformation involves the hydrolytic cleavage and oxidation of the terminal -OH group, (Figure 2.4).

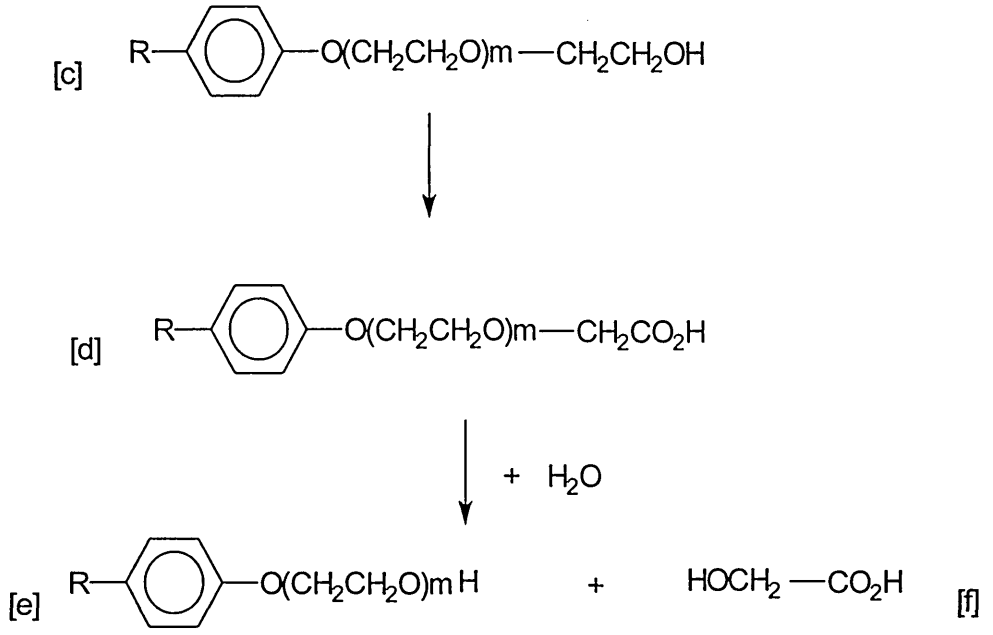


Figure 2.4) Biodegradation of APEO.

Where structure [c] intact alkylphenol ethoxylate (APEO).

Where structure [d] alkylphenoxy carboxylic acid (APEC).

Where structure [e] alkylphenol ethoxylate with an ethoxylate chain length reduced by one unit.

Where structure [f] 2-hydroxyethanoic acid.

The work by Schoberl[45] cannot account for the presence of APEC found in the environment[49,50,55]. Experimental evidence seems to suggest that highly branched alkylphenol ethoxylates and alcohol ethoxylates biodegrade via the ω-hydrophile

oxidation pathway. During waste water treatment four main nonylphenolic compounds are produced due to ω–hydrophile oxidation[55] . The primary degradation products of alkylphenol polyethoxylates (APEO) are commonly: (1) Short chain alkylphenol ethoxylates (n = 1,2), (2) alkylphenoxy carboxylic acids (n = 0,1) and (3) alkylphenols. In aerobic conditions the biodegradation products can consist of a mixture of the short chain alkylphenol ethoxylates, alkylphenoxy carboxylic acids and a residual amount of longer chain alkylphenol ethoxylates (n = 3-20), (Figure 2.5).

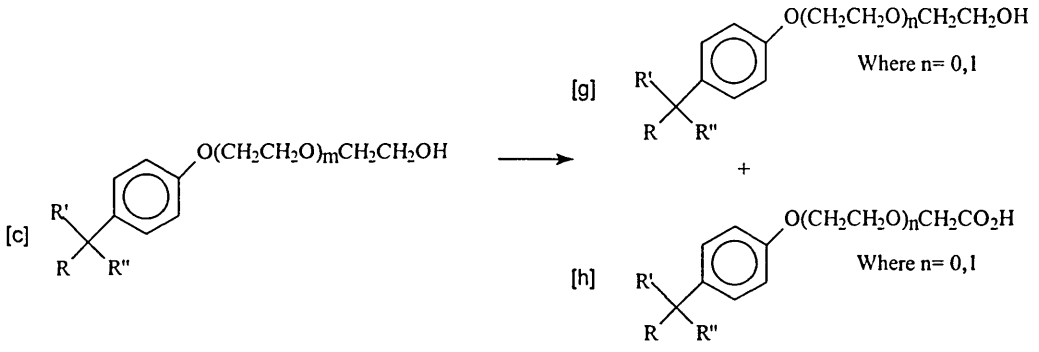


Figure 2.5) Aerobic degradation products of the alkylphenol ethoxylates.

Where Structure [c] is the intact alkylphenol polyethoxylate. (m=1-20 ethoxylate units).

Structure [g] short chain alkylphenol polyethoxylate (APEO). Typically n= 0,1

Structure [h] short chain alkylphenoxy carboxylic acids (APEC). Typically n= 0,1

In anaerobic conditions the degradation products consist of the shorter chain alkylphenol ethoxylates and alkylated phenols, (Figure 2.6).

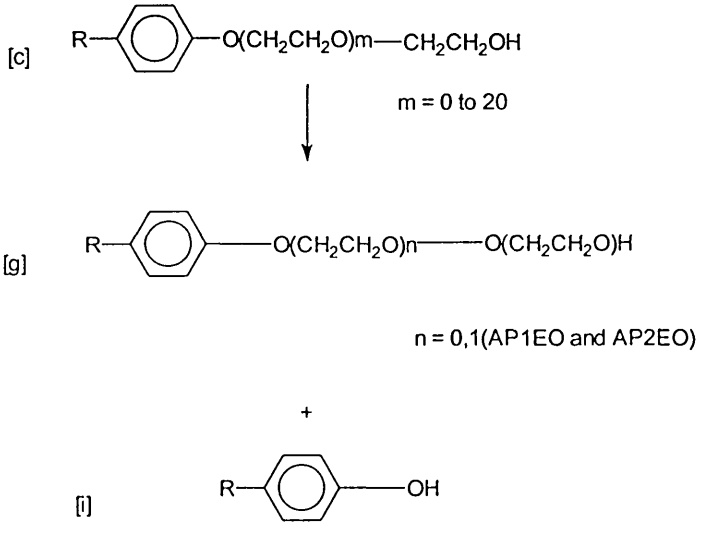


Figure 2.6) Anaerobic degradation products of Alkylphenol ethoxylates.

Where [c] Intact Alkylphenol Ethoxylate.

Where [g] Short chain Alkylphenol Ethoxylate, (n = 0,1).

Where [i] Alkylphenol.

Several authors[49,50] have carried out studies which have detected only short chain carboxylated intermediates. However, Ahel et al.[49] have shown that the measured concentrations of AP_nEC oligomer correspond to the major part of AP_nEO initially added. They also noted that the relative abundance of APEC oligomers closely resembles the original composition of individual AP_nEO oligomers and suggested that the alternative transformation mechanism involving the hydrolytic ether cleavage of either AP_nEO or AP_nEC, or substantial transformation of the alkyl ring[48] is of minor importance.

However, in earlier work Ahel et al.[52] detected no higher APEC than AP_3EC. There is therefore confusion as to whether biodegradation via carboxylation only becomes important after shortening of the polyethylene oxide chain to 1 to 3 units. Ahel et al.[52] suggested that the loss of glucolic acid after carboxylation of the higher AP_nEO could be so fast that none of the longer AP_nEC were found as a result.

This group[49] have also used various aerobic mixed bacterial cultures, isolated from waste water, river water and forest soil to investigate the biodegradation of short chain alkylphenol ethoxylates (n=1 to 3). Almost complete transformation of samples occurred in 6 to 23 days when added to synthetic sewage at total concentrations in the range of 0.5 mg L^{-1}. The analysis of shake cultures at the end of the exponential phase revealed a significant presence of alkylphenoxy carboxylic acids. These accounted for 90% of the APEO originally added to the growth media. This suggests that the most important transformation process is the carboxylation of the terminal alcohol group. Similar biotransformation rates were observed using bacterial cultures isolated from river water and waste water from a detergent manufacturing plant. However, significantly lower rates were obtained for the bacterial culture isolated from forest soil. It is probable that this sample did not contain bacterial cultures which were adapted to biodegrade surfactant samples, unlike the polluted water. Ahel et al[49] go on to argue that APEO

cannot be regarded as truly persistent under aerobic conditions since biotransformation was shown to occur even in a mineral medium where APEO were the only source of organic carbon.

Experimental evidence[6] suggests that the distance of the terminal alcohol group from the branched alkylbenzene moiety known as the hydrophilic / lipophilic balance (HLB) is probably more important than lipophilicity in determining the biodegradation pathway of APEOs. Kravetz[46] found that the structure of the hydrophobe of an alcohol ethoxylate has a significant influence on its biodegradation pathway and biodegradation rate, in experiments carried out in a medium containing those bacterial inocula present in waste treatment plants.

A direct comparison of AE and APE biodegradation[45,47] indicated that AE's degrade more quickly than APE's. In other work[53] it was demonstrated that linear AE underwent complete primary degradation within 3 days whereas branched chain APE having an EO content which were essentially the same as AE showed only 20% primary biodegradation in 30 days. Temperature[53,55] also has an important role to play in the biodegradation of non-ionic surfactants. A reduction in temperature results in a reduction in the rate of biodegradation for APE surfactants, however, there is little or no effect observed for branched or linear AE surfactants. The change in rate of biodegradation with changes in temperature has a marked effect on the concentration of APEO and their degradation products in the environment[54].

It is clear from the conflicting evidence found in the literature that the biodegradation of APEO is a very complex process which is strongly influenced by the condition of the media within which the surfactant is present.

2.4.1) Half Lives

Ahel et al.[49] report the biotransformation half lives of short chain APEO as 2-3 days. Values reported in the literature range from a few hours to a few weeks[50]. Field studies on APEO in sewage treatment and natural waters[51] seem to imply that AP_1EO and AP_2EO which are rapidly produced from parent APEO can be readily biotransformed under aerobic conditions such as secondary sewage treatment plants and in natural waters

into APECs. Half lives of approximately 1-2 days have been reported by Ahel and Giger[54].

2.5) The environmental fate of APEOs

Studies have been carried out to look into the environmental fate of APEO. These were carried out by introducing NPEOs into Sewage Treatment Plants (STPs)[55] and then by monitoring the STPs[54] output into river water. It was found that the raw waste water contained NPEO and its degradation products. The distribution of $NP_{3-18}EO$ oligomers in non-treated and mechanically treated waste water was seen to be very similar to a commercial sample formulation. However, NP and $NP_{1-2}EO$ had a much higher population. The effect of sewage treatment on the four main phenolic classes is very pronounced.

This is summarised below (Table 2.2) :

Table 2.2) The influence of sewage treatment on non-ionic surfactants and their degradation products.

i) Primary effluents were found to contain :			
$NP_{3-20}EO$	$NP_{1-2}EO$	NP	$NP_{1-2}EC$
82.4 %	11.5 %	3 %	3.1 %
ii) Secondary effluents were found to contain :			
$NP_{3-20}EO$	$NP_{1-2}EO$	NP	$NP_{1-2}EC$
28 %	21.8 %	3.9 %	46.1 %

Hence, clearly biological treatment results in the formation of $NP_{1-2}EC$.

In order to judge the efficiency of sewage treatment plants, it is necessary to compare the amount of NPEO in raw sewage with the levels of all alkylphenolic compounds found in secondary effluents. It has been observed that approximately 60-65% of all alkylphenol ethoxylate and related compounds that enter sewage treatment are released back into the environment. Approximately 19% of nonylphenol polyethoxylates and their degradation products introduced into STPs are released into the environment in

the form of NPEC, 11% as lipophilic $NP_{1-2}EO$, 25% as NP and 8% as untransformed NPEO. Presumably total biodegradation accounts for the shortfall in nonylphenol ethoxylate type compounds. It should be noted that almost all of the released NPEO and NPEC are discharged into natural waters in secondary effluents whereas 90% of NP is disposed of to the environment as sludge, due to the lipophilic nature of NP.

Monitoring[54] of the Glatt river (which receives eluents from several STPs) showed that nonylphenoxy carboxylic acids were the most abundant alkylphenol ethoxylate type compounds at concentrations between 2-71 $\mu g\ L^{-1}$ and intact NPEO the least at 1-7.7 $\mu g\ L^{-1}$. Ratios of all alkylphenol ethoxylates and their degradation products were similar to those found in secondary effluents. The rate of elimination of NP_nEO(where n = 3-20), $NP_{1-2}EO$ and NP was found to be very similar to those of STPs. As a consequence of the transformation processes those compounds underwent significant compositional changes. NP_nEO decreased from 21 to 3.5% of the total whereas NPEC increased from 51 to 85%. Giger et al.[59] measured NP_nEO surfactants at concentrations of between 400-2200 $mg\ L^{-1}$ in raw and mechanically treated sewage. It was shown that these surfactants were efficiently removed by the activated sludge treatment process. It was thought that both biotransformation and physico-chemical processes determine the behaviour and fate of nonylphenol ethoxylate compounds during the sewage treatment process. Primary treatment caused a reduction in the lipophilic NP, NP_1EO and NP_2EO. The activated sludge process reduced the concentration of NP, NP_1EO, NP_2EO and most significantly $NP_{3-20}EO$. The concentrations of carboxylated compounds (NP_1EC, NP_2EC) however, were much higher in secondary effluent than in primary effluent. Tertiary treatment caused a further decrease in NP, NP_1EO and NP_2EO but had no effect on the NPECs. Analysis of sludges indicated that a strong accumulation of NP during sludge digestion occurred. Table 2.4 (page 33), shows typical environmental concentrations of non-ionic surfactants.

2.6) Metabolites of APEO in the marine environment.

The environmental behaviour of the nonylphenol ethoxylates in sewage released into the marine environment has been studied[56]. Waters and sediments from the Venice

lagoon were selected as it receives treated and untreated domestic and industrial effluents. A portable re-suspending device allowed the analysis of the first 0.01 to 0.15 mm of the sediment layer in the lagoon. NP, NP_1EO and NP_2EO were detected in the range 0.15-13.7 $\mu g\ g^{-1}$ (dry weight basis). 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-suspended sediment showed a seasonal dependence. Twice as much material was found in April and July compared with February. However, the total concentration of biodegradation product per unit of sediment surface was one order of magnitude higher in February. The growth of micro algae within the bay was thought to be a related factor. The micro algae on average contained NP, NP_1EO and NP_2EO at concentrations of 0.25 $\mu g\ g^{-1}$ +/- 0.15 $\mu g\ g^{-1}$ (dry weight). In water APEO oligomers with up to 13 ethoxy units were found in an average concentration of 0.6 - 4.5 $\mu g\ L^{-1}$.

If the toxic metabolites of APEO can be found in sludge it is possible that bottom feeding animals such as mussels may ingest these compounds. The uptake and excretion of aminocarb, which contains nonylphenol formulations, by mussels has been monitored by Mcleese et al.[57]. They concluded that significant contamination of bivalves will not occur if the concentration of aminocarb (4-dimethylamino 3-methylphenyl methylcarbamate) and nonylphenol in water are less than 0.01 mg L^{-1}, due to their low accumulation coefficients. Wahlberg et al.[58] found concentrations of nonylphenol in blue mussels (Mytilus Edulis) between 0.2 to 0.4 $\mu g\ g^{-1}$. NP_1EO at roughly 0.075 to 0.275 $\mu g\ g^{-1}$, NP_2EO at roughly 0.04 to 0.125 $\mu g\ g^{-1}$ and NP_3EO at 0.03 to 0.04 $\mu g\ g^{-1}$. Estimated biological concentration factors in fish tissues range from 13 to 410 for NP, 3 to 300 for NP_1EO and 3 to 330 for NP_2EO. Factors of less than 10,000 are considered to be too low for bioaccumulation. Conversely, algae are though to have a relatively high bioaccumulation factor, estimated at 10,000. As the levels of alkylphenol ethoxylate and their degradation products were found to be less than those estimated for algae, biomagnification did not occur through the food chain. Levels in fish were recorded at 5.8 mg Kg^{-1}. It is always prudent however, to note bioaccumulation effects when considering environmental assessments[63].

2.7) Toxicity.

Table 2.3 below displays a summary of the toxicity data available for APEOs.

Table 2.3) Toxicity data for APEOs and for NP.

Lethal Conc. (LC) Exposure.	NP Conc. (mg L^{-1})	APEO Conc. (mg L^{-1})	Species.	Ref.
LC_{50}	0.13-1.4	1.3-1000	All Fish (acute)	66
LC_{50} 96 Hr	0.3	4.6	fat headed minnow (acute)	66
LC_{50}	10	/	All Invertebrates (acute)	66
LC_{50} 48 Hr	0.190	14	Daphnia magna (acute)	66
EC_{50}	0.025-0.750	0.210-5000	All Algae (acute range)	66
48 Hr		1.5	Invertebrate (D. Magna)	62

Looking at Table 2.3 it can be seen that the toxicity of APEOs and their degradation products varies widely from species to species. Generally alkylphenol ethoxylates are less toxic than the corresponding Alkylphenol. However, the toxicological effect of alkylphenols upon bottom feeding species decreases upon adsorption onto sediments.

The sub lethal effects of NP cause a decrease in byssus strength (the byssus is used by mussels to grip onto surfaces)[13]. Also a change of scope for growth was obtained at a concentration as low as 0.056 mg L^{-1}.

For NP_1EC no toxicity data seems to be available other than the SMP tests carried out by Argeese and co-workers[63]. They monitored an in vitro response of sub-mitochondrial particles (SMPs) obtained from a bovine heart tissue. Toxicity is estimated by determining the effects of any particular compound on the energy coupled reverse electron transfer (RET), which was induced by ATP and succinate at the first site level of the respiratory chain and reduces exogenous NAD^+ to NADH. The results obtained indicated that NP_1EC had shown toxic characteristics at a concentration = 8.2 mg L^{-1},

NP = 1.8 mg L^{-1} and NPEO = 1.3 mg L^{-1}. In comparison LAS = 0.6 mg L^{-1}. The toxicity of degradation products of LAS, i.e., SPC, ranged from 470 to 18000 mg L^{-1}. Hence the biodegradation of the ethoxy chain of APEOs results in huge changes in the toxic properties of these molecule. Although NP and NP_1EC have differing hydrophobicities they exhibit LC_{50} values within the same order of magnitude. Therefore, the length of the hydrophobic chain present on the molecule has a significant influence on toxicity.

2.8) Oestrogenic properties of Non-ionic Surfactants.

It is known that many chemicals present in the environment as pollutants are oestrogenic in nature[72], many, such as the APEO and their degradation products are environmentally persistent, (Section 2.5). This property multiplies any toxicological properties which the compounds may have.

Natural oestrogens play a major role in the reproductive processes of both male and female species. Their presence at elevated levels in males has been thought to account for the growth of some forms of cancer[73]. It has also been concluded that exposure to oestrogenic compounds might account for the increase in infertility and sexual organ abnormalities in human males[74]. Purdom et al.[75] observed that sewage effluent treatment plants could change the sexual characteristics of fish. It was thought that this was linked to the exposure of the fish to an oestrogenic substances, namely the biodegradation products of APEOs. Previous work[76] (which was inspired by research conducted by Soto[77] and Mueller[78]) showed that many of the phenolic compounds produced via sewage treatment of long chain alkylphenol polyethoxylates promote a response from fish cells which was normally only caused by oestrogenic compounds. The in-vitro bioassay (based on the oestrogen dependent synthesis of vitellogenin by hepatocytes) showed that 4-nonylphenol, 4 tert-octylphenol, 4 tert-butylphenol, 4-nonylphenol diethoxylate, NP9 and 4-nonylphenoxy carboxylic acids are all weakly oestrogenic. Their potencies varied between 10^{-4} to 10^{-6} of the activity of 17-β oestradiol. In general, the order of oestrogenicity was OP >NP_1EC >NP = NP_2EO. The oestrogenic activity observed was confined to the para substituted compounds. The potency of polyethoxylated compounds (NP_2EO, NP_9EO and $NP_{40}EO$) decreased with

increasing ethoxymer chain length. However, as longer chain ethoxymers are more water soluble than alkylphenol they are more bio-available to organisms. This could result in the multiplication of their toxicological potency.

The significance of the ability of AP's to mimic natural oestrogens in mammalian species has been demonstrated by White et al.[79]. Oestrogen responsive breast cancer cells were exposed to alkylphenolic compounds which resulted in increased cell growth. However, it seems that the oestrogenicity of APEOs depends on their chain length, APEOs with more than three ethoxylates showed very little activity in the assay used. This is directly comparable to the toxicological trends of the alkylphenolic compounds[60,61]. There is some confusion as to whether or not the weak oestrogenic nature of the short chain alkylphenols (i.e., NP_2EO) is due to the activity of the hydrophilic group. White et al.[79] have indicated that the short chain alkylphenol ethoxylates are inactive when exposed to mammalian or fish oestrogen receptors. It may be that biotransformation in the cell causes a change in structure of APEOs which then produces a compound which has oestrogenic properties. Further work is needed, however, to clarify this situation.

There is no clear data available in the literature on the lowest concentration of alkylphenolic compounds required to cause a harmful oestrogenic response in mammals. Jobling and Sumpter[76], have shown that in vivo levels of nonylphenol (at $> 20\ \mu g\ L^{-1}$) induce high plasma levels of vitellogenin, a protein found normally in female fish. It is possible to find the more active alkylphenols in the environment at these concentrations. Also, because of the bioaccumulative potential of the alkylphenolic compounds[57], it is likely that they will be more potent at much lower concentrations in vivo than in vitro. Since fish use lipids, rather than carbohydrates, as an energy source, it is possible that alkylphenols stored within adipose tissue may be transported around the animals body, when fat is burnt during energy production. The action of environmental pollutants which have the ability to simulate the action of naturally occurring hormones such as 17-β-oestradiol is still not fully understood. It will be interesting to see what this line of research unveils in the future. Generally, due

to the complexity of environmental matrices any trends observed in the laboratory cannot be used as an effective predictive model without modification.

Table 2.4) continued.

Sample Concentration/ mg L^{-1}	Method of Extraction	Method of Analysis	Reference
England Raw Sewage NP = 0.0002 - 0.330 Rivers Aire NP = 0.0016 - 0.180 Thames = 0.0008 - 0.0022 Lea = <0.0009 - 0.012 Wye = 0.0002 - 0.0027 Ouse = 0.2 - 0.0053 Estuaries Wear = 0.00008 Tees = 0.00009 - 0.0032 Tyne = <0.00008 Blyth = <0.00008 Mersey = <0.00008 - 0.00032 Poole Harbour = <0.00008 Southampton water = 0.00008	Rivers : 100-200 ml sample taken NaCl added before extraction to prevent adsorption onto filter paper used to remove particulate matter. Estuaries : 300 ml of sample taken.	GC/MS used to analyse sample. Nonylphenol peaks integrated using the cluster of ions corresponding to masses 107 and 135. These cluster ions are seen because the manufacturing process of nonylphenols produces a variety of isomers. butylphenol used as internal standard (m/z = 150). LOD = 50 ng L^{-1}.	71

Table 2.4) continued.

Sample Concentration/ mg L^{-1}	Method of Extraction	Method of Analysis	Reference
Australia Raw sewage = 107 - 0.11 effluent = 0.061 - 0.05	Gas stripping (ethyl acetate sublation)	HPLC : cyano column 95:5 ACN/H_20	64
Italy Sewage Influent NPEO = 0.157 - 0.352 Effluent = 0.00074 - 0.012	10 and 100 ml of raw sewage extracted by passing sample through 1g of Graphitized Carbon Black (GCB).	HPLC using C_8 Column with Electrospray MS detection.	69

Continued over the page.

Table 2.4) continued.

Sample Concentration/ mg L^{-1}	Method of Extraction	Method of Analysis	Reference
Italy Raw Sewage NPEO = 0.06-0.35 NP = 0.002 - 0.040 (LAS = 3.4 - 10.3) Treated Sewage NPEO = 0.002 - 0.208 NP = 0.0007 - 0.004 (LAS = 0.021 - 0.208) NPEC = 0.010 - 0.145 (SPC = 0.22 - 1.15)	10 and 100 ml of Influent and effluent samples extracted by passing sample through 1g of GCB.	HPLC using C8 column with Fluorescence detection.	70

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Table 2.4) continued.

Sample Concentration/ mg L^{-1}	Method of Extraction	Method of Analysis	Reference
Sewage Sludge NP = 1000 -<100 mg Kg^{-1}	Solvent extraction	GC/ECD or GC/MS (pentafluorobenzoyl chloride derivatives).	58
Swiss River Water NP<0.004, NP_1EO<0.008 NP_2EO<8, NP_1EC<0.030 NP_2EC<0.060. Swiss River Sediments NP = 0.19 - 2.83 μg g^{-1}* NP_1EO = 0.1 - 8.85 μg g^{-1}* NP_2EO = 0.08 - 2.72 μg g^{-1}* * Dry weight.	River Water samples Extraction carried out via the Wickbold procedure. River Sediments Steam distillation/ Solvent Extraction. NP_1EC and NP_2EC : Chloroform extraction / Silica column clean up.	Normal phase HPLC -NH_2 column.	54

Table continued over the page.

Sample Concentration/ mg L^{-1}	Method of Extraction	Method of Analysis	Reference
Swiss Municipal Waters Total conc.' $NP_{1-18}EO$ = 0.8-2.0	Gaseous Stripping into ethyl acetate. Recovery for Total APEO =87%	HPLC with UV detection 277 nm. For resolution of oligomers -NH_2 column with Hexane/PI gradient. Total APEO -C_8 column used.	67
Switzerland Primary Effluent NPEC = 0.001 - 0.017 Secondary Effluents NPEC = 0.071 - 0.330 Swiss River = 0.002 - 0.116	Liquid/Liquid Extraction using Chloroform (83% efficient) or alternatively Gaseous Stripping into ethyl acetate. (65% efficient).	Normal Phase HPLC -NH_2 column with UV detection at 277 nm or GC/MS (samples methylation carried out using BF_3 or HCl/MeOH)	68
Italy Sewage Influent NPEO = 0.157 - 0.352 Effluent = 0.00074 - 0.012	10 and 100 ml of raw sewage extracted by passing sample through 1g of Graphitized Carbon Black (GCB).	HPLC using C_8 Column with Electrospray MS detection.	69

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Table 2.4) The Environmental profile of APEOs and their degradation products.

Sample Concentration/ mg L^{-1}	Method of Extraction	Method of Analysis	Reference
<u>Australia</u> Raw Sewage = 0.11-107 Secondary Effluent = 0-0.06 Primary Treated effluent = N/D.	Gaseous Stripping into ethyl acetate. 800 ml sample extracted cleaned up using ion-exchange. Recovery 60-92 % efficient.	Total NPEOs = HPLC C_{18} column $NP_{>4}EO$ = cyano column $NP_{<4}EO$ = C_{18} with higher carbon loading All Gradient systems. UV detection at 229 nm	64
<u>Southern England</u> Total APEO in influents = 0.13-0.41 Sewage effluents = 0.04-0.228	250 ml of influent and 1L of effluent extracted using sublimation / ion-exchange and alumina chromatography.	Gradient HPLC using -NH_2 column. 0.1% CH_3CO_2H in MTBE/0.1% CH_3CO_2H in ACN/MeOH (95/5) at 2 ml/min. Fluorescence detection Ex =230 , Em =302 nm.	65

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Chapter 3

Methods for the determination of Surfactants.

3.0) Introduction.

Determination of surfactants in surface waters is required owing to their toxicity to aquatic micro-organisms and potential oestrogenic effects, (as discussed in chapter 2). The complexity of surfactants, which are often mixtures of related compounds differentiated by variation in carbon chain length, has meant that analytical methodology has concentrated on determination by class. Simple, relatively fast and inexpensive quantitative or semi-quantitative methods based on titrimetric or spectrophotometric methods[1,2], (Section 3.2 and Section 3.3), have been commonly used. Generally, methods which depend on a spectrophotometric reaction are no longer acceptable since they do not reliably reflect the concentration of either the individual surfactant types within the general class of non-ionic, anionic or cationic or the concentration of individual homologues/oligomers (i.e. chain length distribution) within a class. As a result of the non-specific and insensitive nature of spectrophotometric techniques, several other techniques have been devised[1-8]. Currently, most work published concerning the detection of surfactants in the environment utilises some form of chromatographic separation step followed by UV, Fluorescence or Mass Spectrometric detection. Liquid chromatography is by far the most popular chromatographic technique. Due to the low sample volatility of both intact APEO, LAS and their degradation products (with the exception of the short chain APEO homologues[9,10]), GC can only be used after lengthy derivatization procedures. Due to the complexity of the matrices and the low concentration at which surfactants are found in the environment, means that when analysing samples it is often necessary to extract and pre-concentrate the analyte of interest prior to analysis.

3.1) Sample preparation.

Extraction of the analyte of interest using solid phase extraction (SPE) has three purposes, firstly, isolation of the sample followed by subsequent elution allows the selection of a solvent which is compatible with the HPLC system in use, secondly, the sample can be concentrated if it is present in small quantities. Finally, the sample of interest can be cleaned up in order to remove any impurities which may degrade the

quality of the resulting chromatographic run. Soxhlet extraction is used extensively for solid sample matrices[11], whilst liquid/liquid extraction has been used for the analysis of surface waters[12]. However, it is solid phase extraction (SPE) which offers the most convenient method of sample manipulation in the analysis of surface active chemicals. Extraction using SPE is achieved after, (1) initially conditioning the cartridge with de-ionised water then with an organic solvent such as methanol. (2) Before the stationary phase dries the aqueous sample is passed through. Finally, the cartridge is allowed to dry (3) and a small quantity of solvent (usually approx. 3 ml) is used to elute the analyte of interest (4), (Figure 3.0).

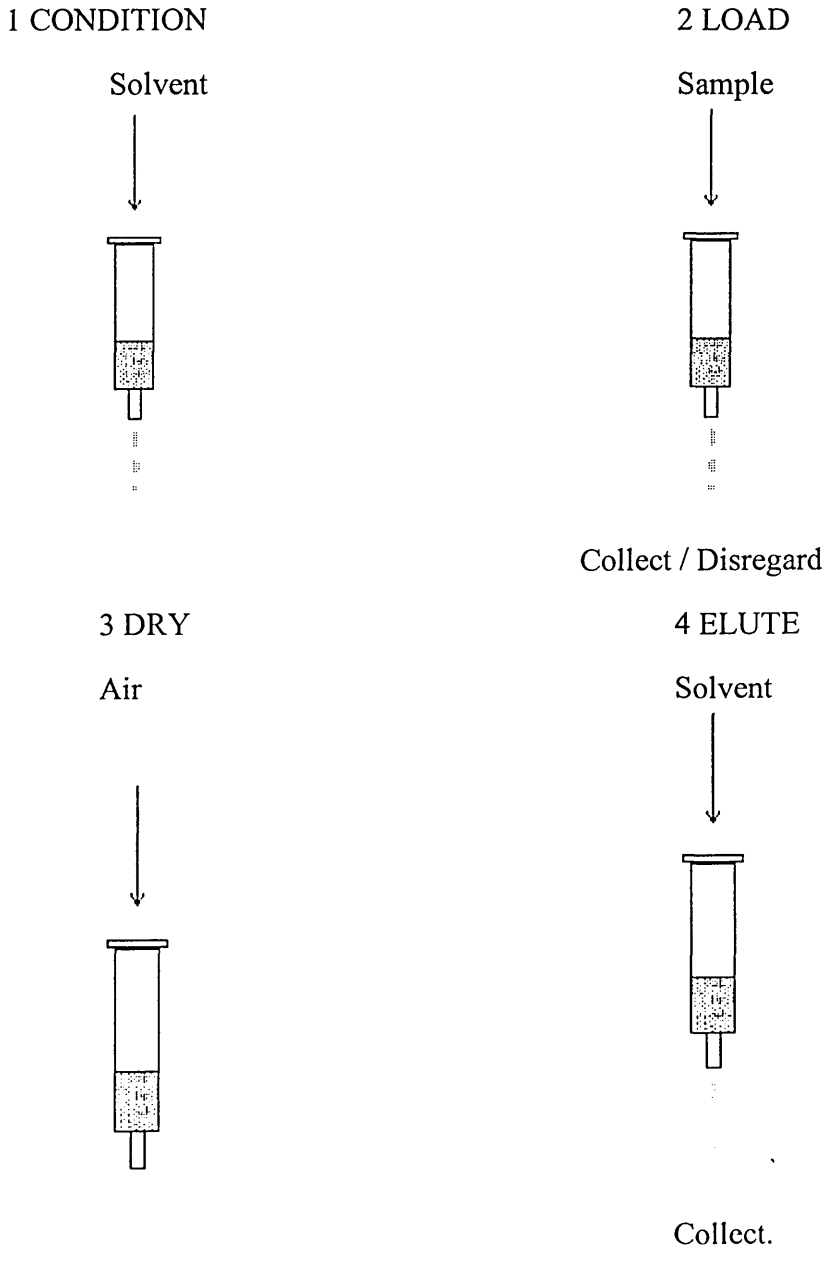


Figure 3.0) The Principle of Solid Phase Extraction.

Solid phase extraction cartridges are now commercially available with several different stationary phases i.e., Reverse phase C_{18}, C_8, C_1, Diol, Normal phase Si, NH_2, and ion exchange, i.e., SAX and SCX. As a result it is theoretically possible to tailor the type of stationary phase used in order to (1) retain the compound of interest on the stationary phase whilst allowing interfering compounds to pass through. Informed use of solvent washes can be used to further 'clean up' the sample. After drying the cartridge the analyte of interest can then be eluted in a small volume of solvent so as to concentrate the sample or, (2) retain interfering compounds thus leaving a 'cleaner' sample to pass through the cartridge. By using a combination of these two techniques it is possible to produce a purer concentrated sample.

Many workers have used SPE in order to pre-concentrate and clean-up surfactants, (Chapter 2, tables 2.2 and 2.4, respectively). Marcomini et al.[12] used an enrichment process involving percolation through an octadecyl silica cartridge for the extraction of LASs, APEOs and NPs and achieved recoveries of 67% and higher. Duplicate solutions were used for the determination, 35 μg of LAS and 15μg of NPEO were added to 100 ml of secondary sewage effluent which already contained 22μg L^{-1} of LAS and 93μg L^{-1} of NPEO. Cartridges were rinsed with 3 ml of acetonitrile, 3 ml of methanol and 5 ml of doubly distilled water. Next, samples were then passed through the cartridges. Samples were then passed through the cartridges at a flow rate of 10-15 ml/min. Elution was achieved using 3 ml of acetone. Three commercially available cartridges showed no great variation in their ability to pre-concentrate the analytes. Matthijs and De Henau[13] have used a more complex system in order to remove interfering NPEO during the analysis of LAS. Firstly, an octyl silica column is used, followed by an anion exchange column which will not retain any non-ionics. Di Corcia et al.[14] have proven the worth of graphitized carbon black (GCB) as a stationary phase for SPE cartridges. By heating carbon to 2700-3000 ^oC in an inert atmosphere it is possible to produce "graphitized carbon black", (GCB). It is thought that the benzpyrylium salts at the GCB surface are able to act as both an anion-exchanger and a non-specific sorbent. Non-ionic and anionic surfactants and their degradation products have been simultaneously isolated from influent and effluent

water treatment samples[15]. By exploiting the presence of positively charged active centres on the surface of the GCB surface, it was possible to fractionate the surfactant samples by differential elution from the surface of the GCB.

3.2) Determination of Non-ionic Surfactants.

Historically, the spectrophotometric technique involving the use of cobalt thiocyanate (CTAS) to form a blue colour complex with non-ionic surfactants was widely used[1]. The major shortcoming of the CTAS method was insufficient specificity in the presence of anionic and cationic surfactants. A reaction based on the complexation of surfactant molecules with Dragendorff reagent was successfully used for the analysis of non-ionic surfactant biodegradation. This method was modified from the Wickbold procedure[2]. Unfortunately all complexing reactions had insufficient sensitivity to the lowest oligomers (nEO <5) and were unspecific. Environmental samples contain high levels of the more toxic short chain APEOs. It is therefore undesirable to use the CTAS method to monitor complex degradation samples.

3.3) Determination of Anionic Surfactants.

The colorimetric methods used for the measurement of anionic surfactants in aqueous samples[3] are based on the formation of an ion-pair between the anionic surfactant and a cationic dye. The complex is extractable into a non polar solvent, where both the free surfactant and the dye remain in the aqueous phase. Methylene blue is generally used for this purpose. Termed the methylene blue active substances test (MBAS) this method has drawbacks similar to those of the CTAS method used for non-ionic surfactant determination. These are that; (a) any substance that would form an extractable complex with the dye yields a positive bias and (b) compounds of opposite charge to the surfactant (positive in this case, e.g., quaternary amines) will compete with the dye to ion-pair with the surfactant. This results in a negative bias. Sullivan and Swisher[4] determined that only 10 - 20 % of the MBAS concentration in rivers was actually due to the presence of LAS.

3.4) UV Spectroscopy as a means of Surfactant determination.

The absorption of electromagnetic radiation in the UV region of the spectrum is useful as a tool in the determination of surfactants as it is sensitive and relatively selective. Essentially, the conjugated ring present in the structure of LAS and APEOs means that UV spectroscopy offers a reliable and convenient means of detection. When coupled to chromatographic techniques reliable and reproducible methods can be developed which are able to determine the makeup of a complex mixture which represents a commercial surfactant formulation.

Absorption of UV or visible light occurs via a two step process. Firstly, the molecular species is excited :

$$\mathbf{M + h\nu \rightarrow M^*}$$

The excited species can then relax via several routes :

(1) Production of heat. This process is normally not detectable.

(2) Photo decomposition of the sample.

(3) Re-emission of radiation.

The most useful in terms of analysis is process (3), as it can be related to a particular functional group which may be present in the sample. The lifetime of the excited state is usually between 10^{-8} - 10^{-9} seconds.

The absorbance of energy in the UV or visible region of the electromagnetic spectrum results in an excited species in which bonding electrons have been 'excited' to an orbital of higher energy. The wavelength of the radiation which will produce a maximum in absorbance is governed by the particular type of bond in which the electrons are held. It is possible to place absorbance due to the type of functional group present in a molecule into three separate categories.

(1) π,σ, and n electrons.

(2) d and f electrons.

(3) Charge transfer electrons.

All organic compounds are capable of absorbing electromagnetic radiation; however, absorption of energy by electrons in single bonds, i.e., $\sigma \rightarrow \sigma^*$ occurs at low

energies. Due to instrumental problems it is impractical to measure these absorbances at the wavelengths (<185 nm) at which they occur.

Saturated compounds containing atoms with unshared electrons pairs (non-bonding (n) electrons) can undergo $n \rightarrow \sigma^*$ transitions. As they require less energy than $\sigma \rightarrow \sigma^*$ transitions λ_{MAX} can be seen at a longer wavelength, i.e., 200 nm. The ε_{MAX} associated with this type of absorption range from ~ 100 to 3000 L cm^{-1} mol^{-1} (low to intermediate). The most useful transition for organic analysis is the $\pi \rightarrow \pi^*$. Absorption maxima fall into the experimentally convenient 200 to 700 nm range. Molar extinction coefficients range from 1000 to 10,000. Molar extinction coefficients for the other type of π transition, i.e., the $n \rightarrow \pi^*$ are usually between 10 and 100 L $cm^{-1}mol^{-1}$.

Change in solvent polarity can have an affect on the absorption maxima of analytes in solution. For this reason it is advisable to use HPLC mobile phase solvents to dissolve sample in order to avoid solvent fronts which could interfere with analyte peaks. Generally an increase in solvent polarity will cause a decrease in the λ_{MAX} involving $n \rightarrow \pi^*$ transitions but increase the λ_{MAX} of a $\pi \rightarrow \pi^*$ transition. Hydrogen bonding will affect $n \rightarrow \pi^*$ transitions causing hypsochromic shift. (blue shift). The energy of the n orbital is lowered by the approximate energy of the hydrogen bonds. When the $n \rightarrow \pi^*$ transition occurs the remaining n electron cannot remain in the H-bond so as a result energy is retained in the π^* excited energy level.

$\pi \rightarrow \pi^*$ transitions are affected to a much greater degree than $n \rightarrow \pi^*$ transitions by solvent effects. A bathochromic shift (red shift) occurs as attractive polarisation forces between the solvent absorber present lower the energy of both the excited and un-excited states. However, as the effect on the excited state is greater the energy difference is reduced so an increase in solvent polarity results in a red shift.

Functional groups which contain valence electrons are called chromophores. A rough identification of the absorber can be made depending on the λ_{MAX}. This process can be misleading due to differences seen if solvents are varied and also if more than one chromophore is present within the molecule. It should be noted that the presence of conjugation within a system will cause a shift of λ_{MAX} to longer wavelength as π^* orbital energies are lowered.

The UV spectra of aromatic hydrocarbons are complex. Benzene has 3 absorption peaks, the longer wavelength band (256 nm) contains a series of sharp peaks due to vibrational transitions acting in combination with the basic electronic transitions. Ring substitution with a group which contains n electrons enables these electrons to interact with the π electrons of the ring. This interaction has the effect of stabilising the π^* state, so lowering its energy., i.e. a red shift occurs.

3.5) UV Detectors for HPLC.

The general principle of UV absorbance detectors is that mobile phase from the column is passed through a small flow cell held in a radiation beam of the UV/visible photometer or spectrophotometer, (Figure 3.1). The mobile phases used in reverse phase mode generally do not absorb above 210 nm so generally they do not interfere with the λ_{MAX} (200-700 nm) of most organic molecules.

Absorption of radiation by solutes as a function of concentration is given by the Beer - Lambert law.

$$A = \varepsilon.b.c$$

Where A = absorbance

b = path length of the cell (cm)

c = concentration of the solution to be measured (mol dm^{-3})

ε = Molar extinction coefficient L $mol^{-1}cm^{-1}$.

Beer's law is generally linear up to concentration of ~ 0.01M. However, chemical dissociations, i.e., acid / base reactions affect the linearity. Also, non-monochromatic light from the source will have the effect of lowering signal integrity.

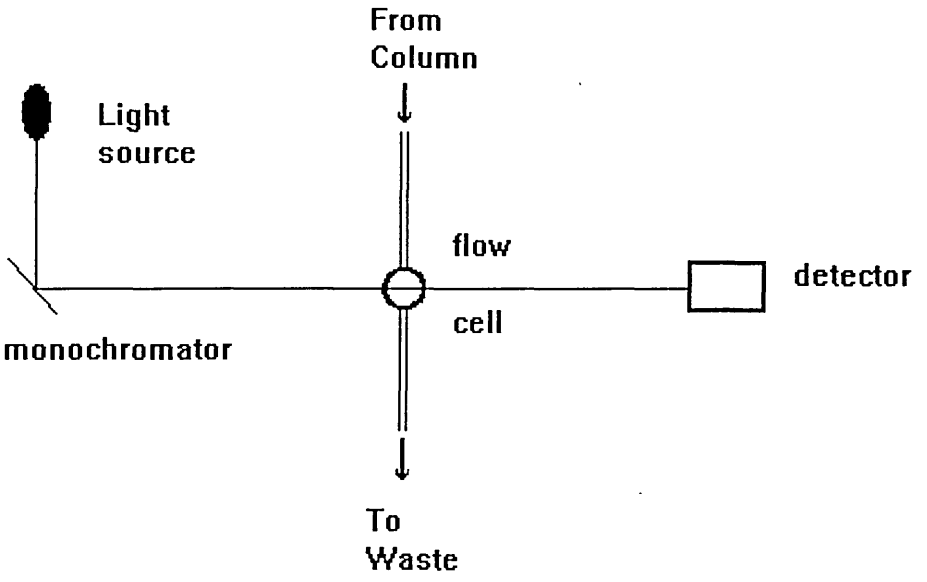


Figure 3.1) A single wavelength detector for HPLC.

3.6) Fluorescence.

A Fluorescence detector for HPLC has two distinct advantages over a conventional UV HPLC detector. Firstly, the fluorescence detector works by measuring the radiation emitted by the analyte. The energy is at a longer wavelength than that used to excite the analyte. As a result these measurements are made against a black background. This cannot be said of UV detection which relies on measuring the amount of light which is absorbed by the analyte. Thus as a result fluorescence can be an inherently more sensitive technique than UV spectroscopy. Secondly, it is possible to distinguish between analytes due to their fluorescent wavelength characteristics, i.e. the excitation wavelength, λ_{ex} and the emission wavelength λ_{em}. As two individual wavelengths are used fluorescence can be said to be more specific than UV detection.

Photoluminescence occurs when molecules of an analyte absorb photons. There are two types of luminescence, fluorescence and phosphorescence. Fluorescence differs from phosphorescence in the respect that electronic energy transitions responsible for photoluminescence do not involve a change in electronic spin. As a result fluorescent radiation is short lived, 10^{-9}-10^{-6} seconds, whereas, phosphorescence requires at least 10^{-4} seconds and can take as long as 10^{2} seconds.

When defining the process of absorption and emission of light it is necessary to assume that radiant energy can only be absorbed in definite units, or quanta. The energy carried by one quantum is proportional to its frequency of oscillation.

$E = h\nu = hc/\lambda$ where ν = The frequency of oscillation.

λ = The related wavelength.

h = Plank's constant.

c = The velocity of light.

Since the energy of a single quantum is too small for convenience, it is usual to talk about the energy associated with N quanta, where $N = 6.023 \times 10^{23}$, i.e., the number of single molecules in a gram of analyte. The absorption light quanta promote electrons in the lowest vibrational level of the ground (singlet) electronic state, S_0 to higher electronic states S_1, S_2.....S_n. This process can take as little as 10^{-15} seconds. The probability of the absorption is governed by the selection rules of quantum mechanics. Energy absorbed in this way can be dissipated in a number of photo-chemical or photo-physical processes. If the amount of energy absorbed is great enough photo dissociation may occur. Energy transfer may occur if the appropriate overlapping energy levels are present.

Following absorption of energy the molecule relaxes to the lowest vibrational level of the first excited single electronic state by radiationless routes. This can occur within 10^{-12} seconds. The transition from an excited state S_1 to the ground state S_0 results in the emission of radiation. This emission of radiation is known as fluorescence.

Fluorescence and phosphorescence spectroscopy are very sensitive techniques since in contrast to UV absorption spectroscopy, the emitted radiation is usually detected at right angles to the incident beam. This is advantageous since as light is emitted against a dark background, longer wavelength luminescent energy can be detected whilst scattered light from the source can be limited by the use of monochromators or filters.

Emitted light I_f can be linked to incident radiation I_0 by the equation :

$$I_f = I_0\Phi_f(1-e^{-\varepsilon bc}).$$

ε = Molar extinction coefficient

b = Path length of cell.

c = Molar concentration.

The constant Φ_f is the quantum yield of fluorescence and is defined by:

$$\Phi_f = \text{number of photons emitted}/\text{number of photons observed.}$$

For very dilute solutions the observed fluorescence intensity is directly proportional to concentration and I_f is given by:

$$I_f = I_0\Phi_f(2.3\varepsilon bc).$$

This relationship is valid at absorbance values less than 0.05A. Above this value inner effects may occur, i.e., re-absorption of emitted light by the fluorophore or by other compounds present.

It can be seen that the intensity of fluorescence emissions is directly proportional to the intensity of the incident radiation. Sensitivity in absorption measurements is limited by the ability of the instrument to discriminate between two nearly equal signals due to I and I_0. As a result the detection limit for even the most favourable cases is rarely lower than 10^{-8}moles. However, fluorimetric instruments are limited only by the intensity of the excitation source and the ability to detect low light levels. Amounts of analyte as low as 10^{-12} moles can be measured.

3.7) Factors affecting Fluorescence

When a molecule is promoted to an electronically excited state it loses the excess energy in a number of ways; the principal decay routes are fluorescence, non-radiative decay and photochemical reactions. Fluorescence can only happen if the rate constant for radiative transition is large compared with that of each of the other routes. Molecules should also have the following characteristics:

i) The electron which is promoted to a higher level in the absorption transition should not be located in an orbital involved in bonding. Otherwise bond dissociation may accompany excitation.

ii) The energy of the lowest spin allowed absorption should be reasonably low since the greater the energy of excitation the more probable the occurrence of photo decomposition.

iii) The intensity of absorption is directly proportional to the rate constant for the radiative transition and the spin allowed electronic absorption of the lowest energy is very large, (i.e. high molar absorptivity). Molecules possessing π electrons have these characteristics. Since the π electrons are less strongly held than σ electrons, they can be promoted to π^* anti-bonding orbitals by the absorption of fairly low energy without the disruption of molecular bonding. $\pi \rightarrow \pi^*$ is strongly allowed ($\varepsilon_{max} \sim 10^4$) and as a result aromatic compounds possessing $\pi \rightarrow \pi^*$ singlet state are strongly fluorescent. The 'n' bonding electrons of heterocyclics and molecules containing carbonyl groups undergo transitions to π^* orbitals. However, these transitions are much less intense, ($\varepsilon_{MAX} \sim 10^2$). Transitions involving σ-bonding electrons occur at very high energies so bond disruption is likely to occur. Some non-aromatic but highly conjugated compounds such as β-carotene and vitamin A are fluorescent due to the occurrence of $\pi \rightarrow \pi^*$ transitions. In general, however, the vast majority of fluorescent compounds are aromatic. Compounds containing rigid planar aromatic rings are particularly fluorescent. The size of the aromatic system also directly influences the excitation and emission wavelengths and the fluorescence intensity. An increase in conjugation i.e., in the series benzene, naphthalene, anthracene results in increases in fluorescent wavelengths. The type and position of a substituent group on an aromatic compound can affect fluorescence. When substituent groups, i.e. auxochromes are in the ortho/para position fluorescence is suppressed. Electron withdrawing groups such as halogens and alkoxy groups, decrease fluorescence quantum yields. Substitution in aromatic systems by hetero atoms greatly affects fluorescence characteristics. Compounds such as pyrrole, pyrazine etc., do not fluoresce or fluoresce weakly since the molecules posses at least one pair of 'lone' electrons. Absorption of energy results in an $n \rightarrow \pi^*$ transition, with a

high probability of a radiationless transition to a lower triple state resulting in phosphorescence. If a benzene ring is added to a heterocyclic molecule an increased absorption intensity can be observed with an shift of the absorption maximum to a longer wavelength.

3.8) Instrumentation for the measurement of fluorescence

The basic components for the measurement of fluorescence are similar to those used for UV absorption spectroscopy, (Figure 3.2). The only major differences are that the detector is placed at right angles to the sample cell and that radiation of a different wavelength to that shone through the sample is detected. A discrete emission wavelength is detected by using an emission monochromator or filter between the sample cell and the detector.

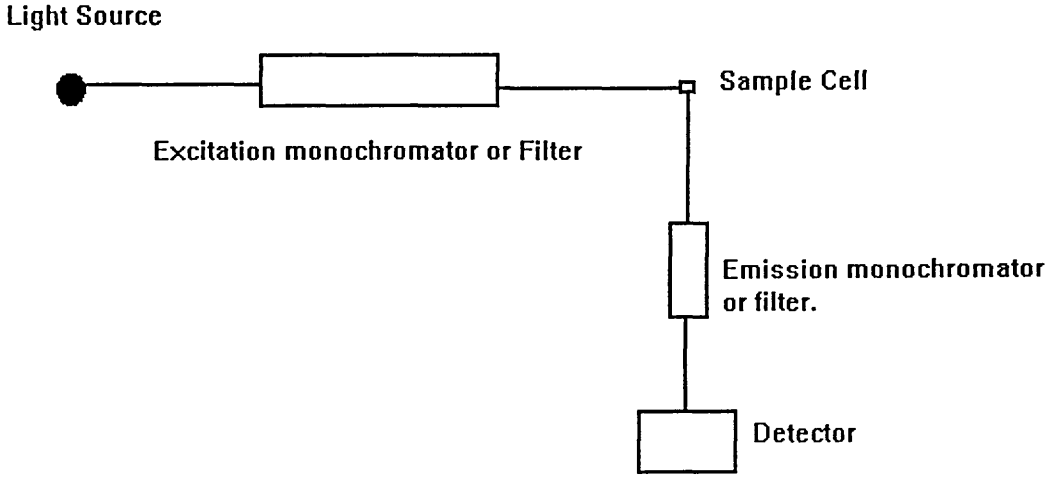


Figure 3.2) The basic components of a typical fluorimeter.

(a) The light Source:

Mercury or xenon arc lamps are generally used for the light source in commercial fluorimeters. Tungsten and hydrogen lamps are generally considered to be of too low intensity, since the magnitude of the output signal and thus the sensitivity is directly proportional to the source power P_0. Xenon arc lamps produce intense radiation by the passage of current through an atmosphere of xenon. The spectrum is continuous over the range 250-600 nm.

(b) Filters and monochromators:

Most modern spectrofluorimeters are equipped with grating monochromators, although absorption filters have been employed.

(c) Detectors:

A fluorescent signal is of low intensity. Therefore, large amplification factors are required for measurement. Generally commercial fluorescence instruments use a photomultiplier tube for detection. The limit of sensitivity of a photomultiplier is usually governed by the level of dark current (the current produced by the tube with no incident radiation falling on it). Dark current is caused by thermal activation and can be reduced by cooling.

(d) Flow cell:

Glass or silica flow cells are employed which are either round or rectangular in shape.

3.9) Gas Chromatography (GC) and Gas Chromatography / Mass Spectrometry (GC/MS).

It is possible to separate mixtures of surfactants using gas chromatography; however due to lack of volatility LAS samples must first be desulphonated[16]. The detection of APEO is possible if the sample contains APEOs with ethylene oxide chains less than 5 units. However, APEOs with chain length greater than 5 EO units generally require derivatization in order to achieve an acceptable suitable degree of sensitivity. Wahlberg et al.[17] used pentafluorobenzoate derivatives for the analysis of APEO in mussel tissue. Although generally derivatization is required for the analysis of surfactants using GC this form of analysis is theoretically capable of offering excellent peak resolution. The use of GC/MS adds to this technique by offering further structural information. The simplest interface for the combination of GC and MS is a direct line from the end of the chromatographic column to the mass spectrometer. Gas chromatography/ Mass spectrometry (GC/MS) has been applied to the analysis of non-ionic surfactants. However, due to the lack of volatility it is only applicable to nonylphenol and the short chain phenolic compounds.

GC/MS using electron impact ionisation (EI) has been used to determine surfactant concentrations[18]. Stephanou used chemical ionisation (CI) mass spectrometry using methane gas to detect tertiary-octylphenol in effluent samples[19]. Generally, however, derivatization is used to improve sensitivity. Hing Bing Lee and Peart[20] determined levels of NP in sewage after converting NP into its acetyl derivative with acetic anhydride. Ahel et al.[21] determined levels of NPEC in sewage effluents after a methylation reaction. Chalaux et al.[22] used pentafluorobenzyl bromide derivatives to determine NPEO and NP levels using GC/MS in sewage samples. The determination of NP and NPEO in water, sludge and mussel samples has been achieved by using pentafluorobenzoates by Wahlberg et al.[17]. GC/MS has also been successfully used for the determination of LAS. Trehy et al.[11] determined LAS concentrations in water and sediment samples by conversion of the sulphonate group into the trifluoro methyl sulphonate derivatives via a sulphonate chloride intermediate.

3.10) Liquid Chromatography / Mass Spectrometry (LC/MS).

A mass spectrometer is probably the ideal detector for liquid chromatography, as it is capable of providing both structural information and quantitative analysis of separated compounds. The limitations of combining liquid chromatography with mass spectrometry have made the development of suitable interfaces a major challenge. A number of on-line interfaces have been developed for high performance LC/MS interfacing, based on several operating principles. The subject has also been discussed in many reviews [23-28].

The ideal LC interface should allow a wide range of ionisation modes and be able to transport efficiently solute into the ion source using normal HPLC flow rates. Willoughby and Browner designed the "MAGIC" interface[29] principally because this aerosol based interface allows solvent to evaporate quickly. It does not increase band spreading, but most importantly thermal desorption is not required (which could cause sample degradation). Ligon and Dorm[30] described a modified version of this particle beam type LC/MS interface which is compatible with the vacuum requirements of a high resolution double focusing MS. This interface is similar in design to the one used during this work. Its design is shown in Figure 3.3. In this interface a nebuliser is used to convert the LC effluent into an aerosol. After the addition of a dispersion gas, the aerosol is allowed to drift through the heated desolvation chamber in which the particles tend to lose volatile solvents by evaporation. At this stage the measured pressure is approximately 500 mbar. "Dry" particles then pass through a capillary nozzle forming a high velocity stream.

The central stream is then directed into the mass spectrometer using two skimmers. Vacuum pumps between the skimmers divert most of the solvent vapours away from the mass spectrometer and transfer a large fraction of the sample molecules into the MS. 90% of helium and solvent are removed after the first rotary vacuum pump reducing the pressure to 10 mbar and 90% of the remaining solvent and helium after the second rotary pump, reducing the pressure to 1 mbar. It has been shown that[31-33] low concentrations of ammonium acetate (i.e. 0.01M) beneficially increase the ion abundance and so the sensitivity of the interface. The same workers also recognise the

inability of the interface to operate efficiently with eluants containing more than 70% H_2O.

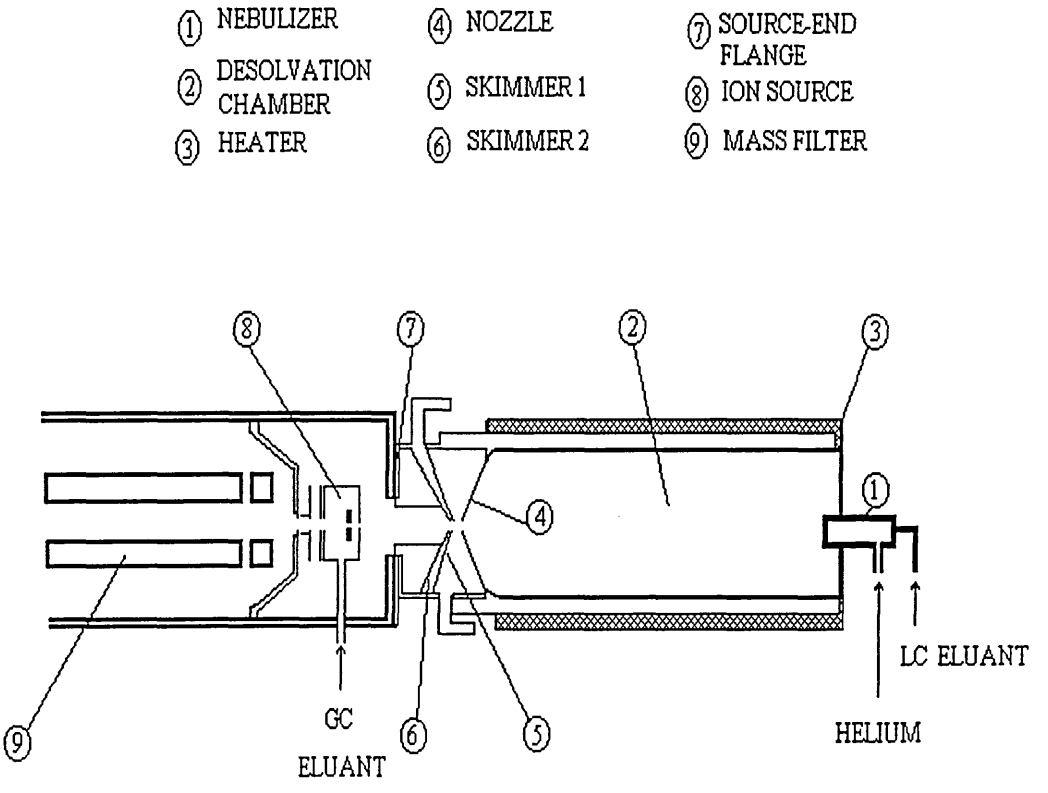


Figure 3.3) Schematic of a Particle beam (PB) interface.

Apffel and Perry[33] have produced a mathematical model which shows the non-linear behaviour of the interface. This model is in close agreement with experimental data. The effect of 10 HPLC mobile phases and 24 analytical probes was investigated. It was found that although certain combinations of probes and additives showed improved linear response, no single additive appeared to completely alleviate the non-linear behaviour. Also, Voyksner et al.[34] have produced a paper dealing with the optimisation of a HPLC/PB/MS system for several compounds. It was shown that the sensitivity of the interface was greatest for solvents of low heat capacities (methanol > acetonitrile > isopropanol > water). Furthermore, optimal sensitivity was obtained at low solvent flow rates (about 0.4-0.6 ml min^{-1}). Parameters such as desolvation temperature, helium flow rate to the nebuliser and nebuliser position resulted in a minimum change in sensitivity.

For the same compounds, HPLC/MS with a thermospray interface often resulted in less structural information than obtained by particle beam. A review of particle beam liquid chromatography-mass spectrometry has been published which compares different commercial designs[28].

3.10.1) Particle Beam LC/MS analysis of Surfactants.

Solka and Curtis[35] have described the ability of Particle Beam (PB) LC/MS to provide useful analytical information for non-volatile cationic surfactants. Using a HPLC mobile phase of a 50/50 methanol/water (flow rate = 0.6 ml/min) and an Extrel ELQ mass spectrometer with a Thermabeam particle beam interface it was possible to show that the particle beam volatilisation processes generated species which were directly related to the structure of the original sample. The same workers[36] have also produced spectra of LAS and ethoxylated alkyl sulphates by direct flow injection of a 1 μg sample into a post HPLC column flow (mobile phase 50/50 methanol/water flow rate 0.6 ml/min). An extrel ELQ 400 mass spectrometer and an Extrel thermabeam particle beam interface was used to gather mass spectral data. PB/CI/MS spectra of these anionics reveal competitive flash desorption of intact ionic species and pyrolytic formation of aliphatic alcohols. The influence of ion source temperature on the competition of these volatilisation processes is opposite in nature to that observed for quaternary ammonium compounds[35].

Alexander and Quinn[37] used LC/MS to detect and identify surfactants in commercial laundry agents and shampoos. Analyses were performed using both electron impact (EI) and chemical (CI) ionisation. Methane CI readily produced protonated molecular ions on some surfactants, and combined with structural information obtained from EI analyses, identification of alkylbenzene sulphonates and alkylphenol polyethoxylates is possible. The presence of alkylated sulphonates in detergents and shampoos was also investigated. Experiments were carried out on a HP 5988 quadrupole mass spectrometer, with a Hewlett Packard particle beam (PB) interface. A Polymer Industries C_{18} HPLC column (15 cm × 4.6 mm i.d.) was used to separate alkylated sulphonates.

The gradient system used in these experiments was as follows :

Time	0	15	20	25
%A	85	30	0	85

Where solvent A = 5% acetonitrile + 10 mM ammonium hydroxide

solvent B = 95% acetonitrile + 10 mM ammonium hydroxide.

The source temperature of mass spectrometer was held at 275 ^{0}C, whilst methane pressure within the source was held at 1.1 mbar.

Langon and Schilling[38] used LC/PB/MS to analysis non-ionic surfactants of the alkylphenol polyethoxylate type. Via chemical ionisation (CI), it was observed that the presence of ammonia in the reagent gas produced the $(M+18)^+$ ammonium adduct ion. Fragmentation at 44 atomic mass intervals corresponds to ethoxy polymeric units in the molecule was observed. A HP 5988A mass spectrometer was used with a 1090 liquid chromatograph coupled by a thermabeam interface. For positive CI a 1% mixture ammonia in methane was used as the reagent gas while the source temperature was held at 250 ^{0}C.

A Hypersil aminopropyl (5µm 25 cm × 2.1 mm i.d.) column was used to provide separation via HPLC, with a gradient system which was as follows :

Time	% A
0	95
10	72.5
20	40

Where solvent A= Hexane : IPA (98:2) and solvent B= IPA : Water (50:50)

Reasonable resolution of octyl and nonyl oligomers can be seen.

Clarke et al.[39] used particle beam liquid chromatography / mass spectrometry to analyse finished drinking water for alkylphenol ethoxylates and their acetic acid derivatives. Five hundred litres of finished water were extracted with an on-line continuous liquid/liquid extractor with dichloromethane and concentrations of alkylphenol polyethoxylates (n=3-8), at 7.4 ng L^{-1} were detected.

Aromatic sulphonic acids have been determined in aqueous wastewater samples using anion exchange-liquid chromatography. Separations employing anion exchange columns using acetonitrile and ammonium acetate or sodium hydroxide required a membrane suppresser for desalting.

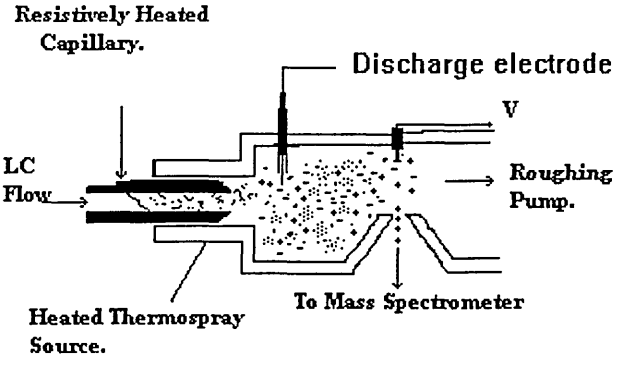
Mass spectra obtained from injecting 2.0 μg of aromatic sulphonic acids on-column show a molecular ion and major diagnostic fragmentation ions corresponding to the loss of SO_2, HSO_2, SO_3 and HSO_3. It is interesting to note that memory effects were observed. This has been a recurrent problem during the analysis of linear alkyl benzene sulphonates (LAS)[31].

3.10.2) The analysis of Surfactants using Thermospray.

A typical modern thermospray (TSP) ion source is shown schematically, (Figure 3.4). LC eluant is passed through a heated capillary tube into a heated ion chamber. The internal diameter of the capillary is about 0.1 mm. The temperature of the capillary is adjusted to a level where the solvent is partially vaporised. As a result a supersonic vapour is formed which contains small electrically charged droplets when aqueous solvents are used. This charging does not arise as a result of the application of external fields. As the chamber is heated and partially evacuated the droplets continue to vaporise and shrink. Eventually free ions are expelled as the droplets decrease in size. These ions leave the TSP source through a small orifice in the sampling cone and then travel onwards towards the mass spectrometer. A repeller electrode can be used to aid this transfer process. The production of ions via evaporation can be enhanced by the addition of an electrolyte to the mobile phase; normally ammonium acetate is used at a concentration of 0.1M. Positive and negative ions may be generated, depending on the acidity and basicity of the gaseous sample. If more structural information is required an electrical discharge electrode can be used. This produces a plasma which ionises the sample.

The TSP interface will accept HPLC solvent flow rates in the approximate range of 0.5 - 2.5 ml min^{-1} but the source will not tolerate involatile buffer solutions. Intact protonated molecules ($[M+H]^+$) are often seen. Sensitivity tends to be very dependent

on the compound type, solvent composition and ionisation mode and can be as good as 1 ng or as poor as 10 μg for a full scan spectra. The lowest sensitivities are usually found for highly labile analytes. Reproducibility of TSP spectra is not very good. TSP spectra appearance can be influenced by solvent composition and probe / source temperature.



3.4) Diagram to Show a Thermospray interface for a Mass Spectrometer.

Evans[40] has described the use of thermospray LC/MS in the analysis of surfactants. He showed that the thermospray mass spectra of linear primary alcohol ethoxylates are characterised by intense $(M+NH_4)^+$ ions, with little or no structural information. Limits of detection for this analysis were quoted in the low nanogram region for each species analysed. The thermospray method was applied to the analysis of surface water and sewage effluent samples. The method was validated for concentrations of individual alcohol ethoxylates in the range 0.06 to 2.17 μg L^{-1} by spiking 1 litre samples.

Schroder[41] examined the concentrations of a range of surfactants in sewage treatment plants. TSP LC/MS incorporating flow injection as a means of sample introduction was used to generate mass spectra. The resulting mass spectrum can be regarded as a survey of the components present. Using this technique it was possible to identify both anionic and non-ionic surfactants in waste water plant effluent.

3.10.3) Atmospheric Pressure Ionisation (API).

In this method ions are formed when LC eluant enters a source region maintained at atmospheric pressure. As a result the ion source of the instrument contains sample ions together with solvent vapour and a nebulizer gas (usually nitrogen). The advantages of eluant introduction at atmospheric pressure are two fold; Firstly, problems concerning liquid / vacuum compatibility are avoided and secondly, capillary chromatography can be used without the need for modification.

The three forms of ionisation used with this particular sample introduction technique are, (a) heated nebulizer used in conjunction with a discharge electrode (b) electrospray and (c) ion spray. During this work type (a) was utilised. This process works by producing pneumatic nebulization via the heated nebulizer to convert the liquid flow into droplets which are then swept by means of a sheath gas through a tubular heated to vaporise the analyte/solvent mixture. The mixture then enters the ion formation region where a discharge electrode initiates chemical ionisation at atmospheric pressure using the vaporised solvent as reagent gas. These CI processes take place within a so called wall-less reaction region which is defined by gas flow in the source, (Figure 3.5). It is common to operate with flows from the HPLC column of between 0.5 and 2 ml min^{-1}. The sampling orifice (approximately 200 to 300μm) leads to an intermediate vacuum stage. Successive vacuum stages may be used to improve the ion-to-neutral ratio in the stream which eventually enters the mass analyser by pumping away neutral molecules while ions are directed towards the next orifice by means of electric fields. Pattanaargson et al.[42] have used atmospheric pressure chemical ionisation/mass spectrometry (APcI/MS) as a means of determination of APEO oligomer distribution. Liquid chromatography/electrospray mass spectrometry has been investigated by Crescenzi and Samperi[43]. This method can only separate non-ionic surfactants through differences in alkyl chain length, it does not allow the determination of ethoxymer chain length distribution. The distribution of ethoxymer chain length is important as it gives an indication of biological degradation (degradation leads to a shortening of the ethylene oxide chain length) and a rough guide as to how toxic a

particular sample may be (shortening of the ethylene oxide chain length results in an increase in sample toxicity).

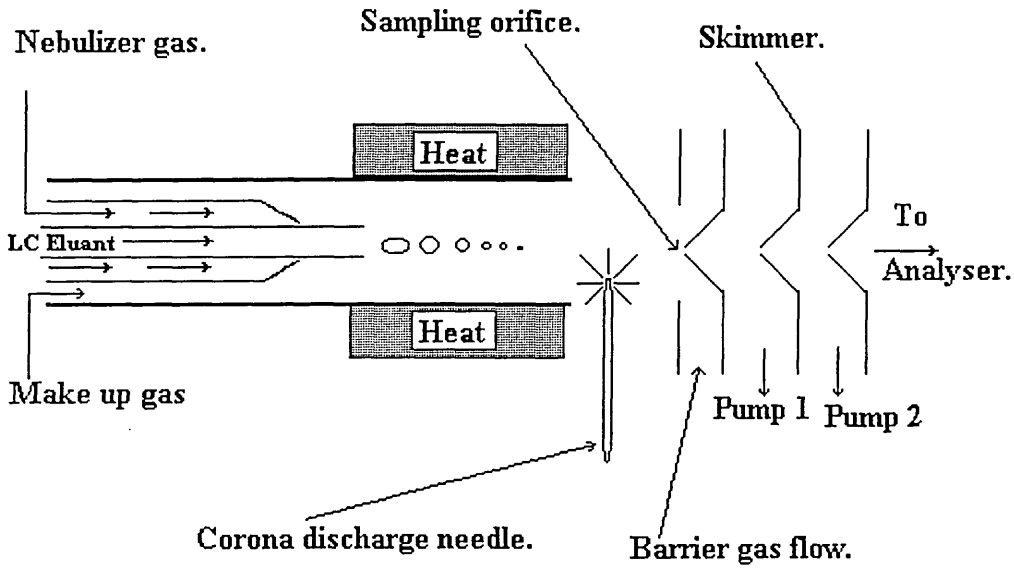


Figure 3.5) Schematic of a typical API interface.

In all of this previous work, with the exception of the experiments carried out by Di Crescenzi and Samperi[43], some compromise in either practical sample volumes or chromatographic integrity or both has been required in order to use mass spectrometry for sample detection. In the GC work lengthy derivatization reactions are necessary. Particle beam requires large initial sample volumes to achieve the desired sensitivity. In the thermospray work optimum HPLC conditions for the separation of individual oligomers were not used as they are not compatible with this type of interface. The APcI work used only direct introduction of non-ionic surfactant samples. No chromatographic separation of oligomers was attempted before introduction. Therefore work was carried out during this project to investigate the marriage of mass spectrometry with a liquid chromatography method. This method is able to give more data concerning the structural diversity of APEO and LAS type surfactants.

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Chapter 4.

An investigation of HPLC methods for the analysis of Surfactant formulations.

4.0) Introduction.

The main body of work carried during this part of the project concerned the development of HPLC methods which are compatible with mass spectrometric detection. Mass spectrometry offers unambiguous detection when coupled to liquid chromatography. It does not rely principally on peak retention times as its means of identification unlike conventional detection such as UV or fluorescence. It is therefore desirable to use this technique when looking at analytes in complex sample matrices such as surface waters.

Four HPLC methods have been developed in order to analyse surfactant samples. These methods are summarised below:

a) A normal phase system which employs a cyano column to separate non-ionic surfactants. This method is explained fully in section 4.3.

b) A normal phase system which is capable of determinations involving non-ionic surfactants, (Section 4.4).

c) A reverse phase system which employs a C_{18} chromatographic column which can be used for the determination of anionic LAS, (Section 4.5). This method employs a linear gradient in order to separate individual LAS homologues. Although this method gives excellent base-line/base-line separation of LAS homologues it is time consuming due to the linear gradient applied. Thus it is of limited use for routine analysis.

d) A reverse phase system which employs a C_1 analytical column. This system can be used for the simultaneous determination of a mixture of both anionic (LAS) and non-ionic (alkylphenol ethoxylates) surfactants, (Section 4.6). This method has been used to obtain LC/PB/MS and LC/APcI/MS chromatograms of commercial surfactant formulations. Initial experiments have shown that the resolution of nonylphenol ethoxylates is not as good as that obtained for octylphenol ethoxylates.

All of the HPLC methods developed separate the homologue / oligomer distributions present in typical standard surfactant mixtures so that low molecular mass homologue / oligomers units are eluted first. Each following peak then represents an increase in homologue / oligomer chain length. For APEOs this represents an ethoxymer unit increase of one -(CH_2CH_2O)- group. For LAS this represents an increase of one -CH_2- unit for each homologue.

4.1) Experimental

Standard solutions of Triton X100 ((an octylphenol ethoxylate) Sigma-Aldrich, Poole, Dorset, UK.) or Igepal CA-630 (an octylphenol ethoxylate with an average ethylene oxide chain length of nine units) (a gift from Rhone Poulenc, Leeds, UK), Synperonic 9 (a nonylphenol ethoxylate with an ethylene oxide chain length of nine units) (a gift from ICI Materials Research Centre, Wilton, Middlesborough, UK) or Igepal C0-630 (a nonylphenol ethoxylate with an average ethylene oxide chain length of nine units) (a gift from Rhone Poulenc, Leeds, UK) Synperonic 5 (a nonylphenol ethoxylate with an ethylene oxide chain length of five units) (a gift from ICI Materials Research Centre, Wilton, Middlesborough, UK) and Nansa SS (a commercial dodecyl alkylbenzene sulphonate formulation with an alkyl chain distribution of 10 to 13 carbon units, Albright and Wilson, Castleford, UK) were prepared by weighing and then dissolving in relevant HPLC mobile phase for each particular experiment (Section 4.3 - 4.6). Octylphenol and n-nonylphenol were purchased from Sigma-Aldrich, Poole, Dorset, UK. Octylbenzene sulphonic acid was purchased from Sigma-Aldrich, Poole, Dorset, UK. All solvents used were HPLC grade, (Sigma-Aldrich, Poole, Dorset, UK).

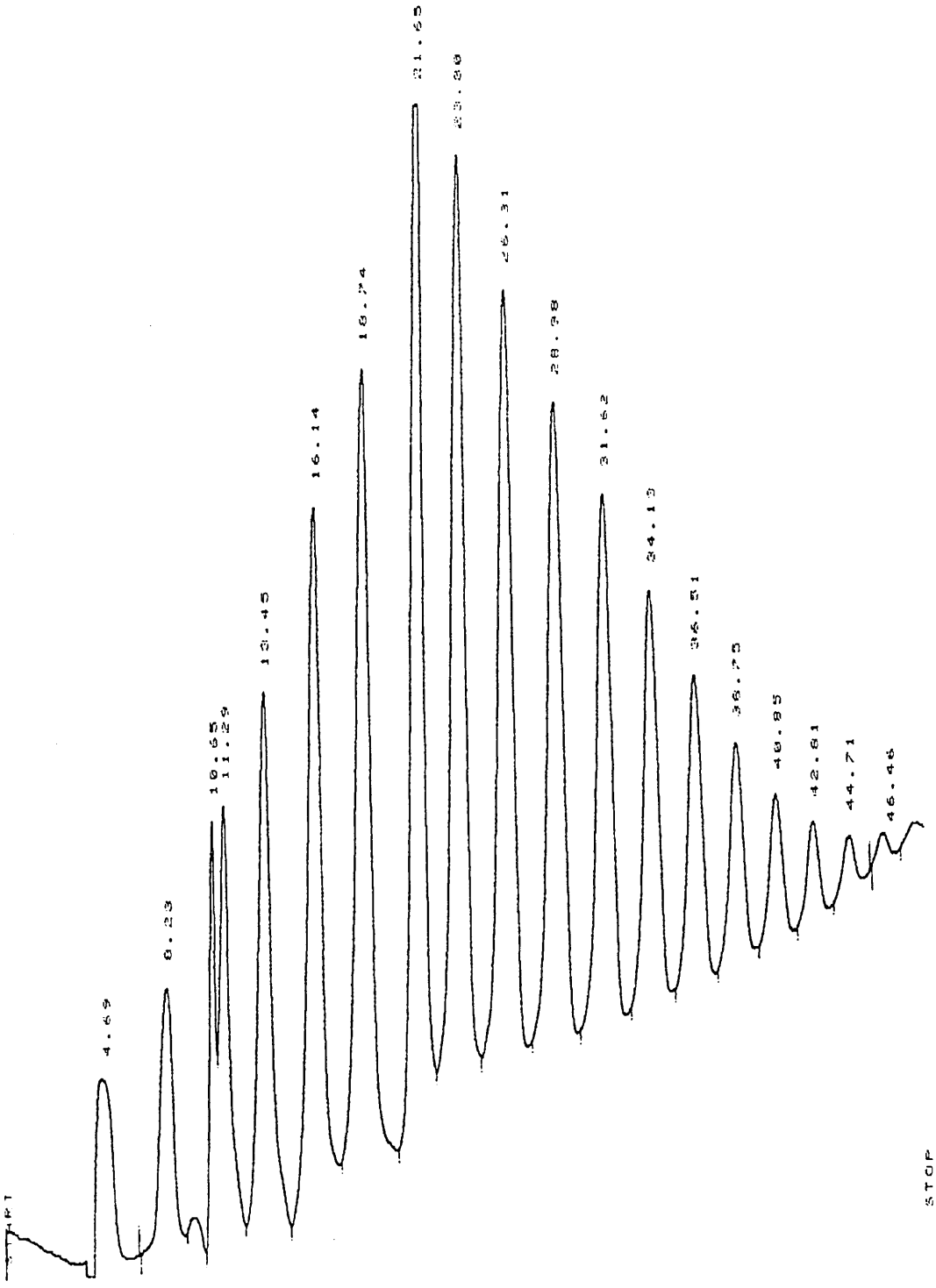
4.2) High Performance Liquid Chromatography

All analyses were performed on a Gilson 302 gradient pumping system. Column and mobile phase were as described in each experimental section, 4.3-4.6. UV detection at 260 nm (Unicam UV HPLC detector) was used for the experiments in section 4.3. In this case data was processed by a Hewlett Packard 3394 (Palo Alto, CA, USA) integrator. Injections were made manually using a Rheodyne injection valve fitted with

a 50 µl sample loop. A Hewlett Packard 1046A (Palo Alto, CA, USA) fluorescence detector λ_{ex} = 220 nm and λ_{em} = 302 nm for alkylphenol ethoxylate and λ_{ex} = 220 nm and λ_{ex} = 290 nm for linear alkylbenzene sulphonates was used for experiment in sections 4.4-4.6. Data was processed by a Shimazdu integrator. Injections of 50 µl were made using a Waters Wisp 712 auto sampler.

4.3) Analysis of Non-ionic Surfactants using normal phase Chromatography.

Using a 25 cm × 4.6 mm i.d. Cyano column (Anachem Ltd, Luton, Bedfordshire) alkylphenol polyethoxylates have been separated according to oligomer distribution. Figure 4.0 displays a representative chromatogram of a nonylphenol polyethoxylate, (NP_9EO). This NPEO has an average of nine ethylene oxide units in the hydrophilic chain.



Retention time (Min.)

Figure 4.0) 200 ppm injection of a NP9 standard solution (UV detection at 280 nm).

This method is based on the work by Pilc[1]. However, modifications to the linear gradient and flow rate have been made in order to make the system compatible with LC/PB/MS.

A constant flow rate of 0.7 ml min^{-1} was maintained while a linear gradient was applied :

Time (min).	%A	%B
0	95	5
50	50	50

Where A = n-Hexane and B = 2- methoxyethanol / Propan-2-ol (75/25).

All reagents used were HPLC grade.

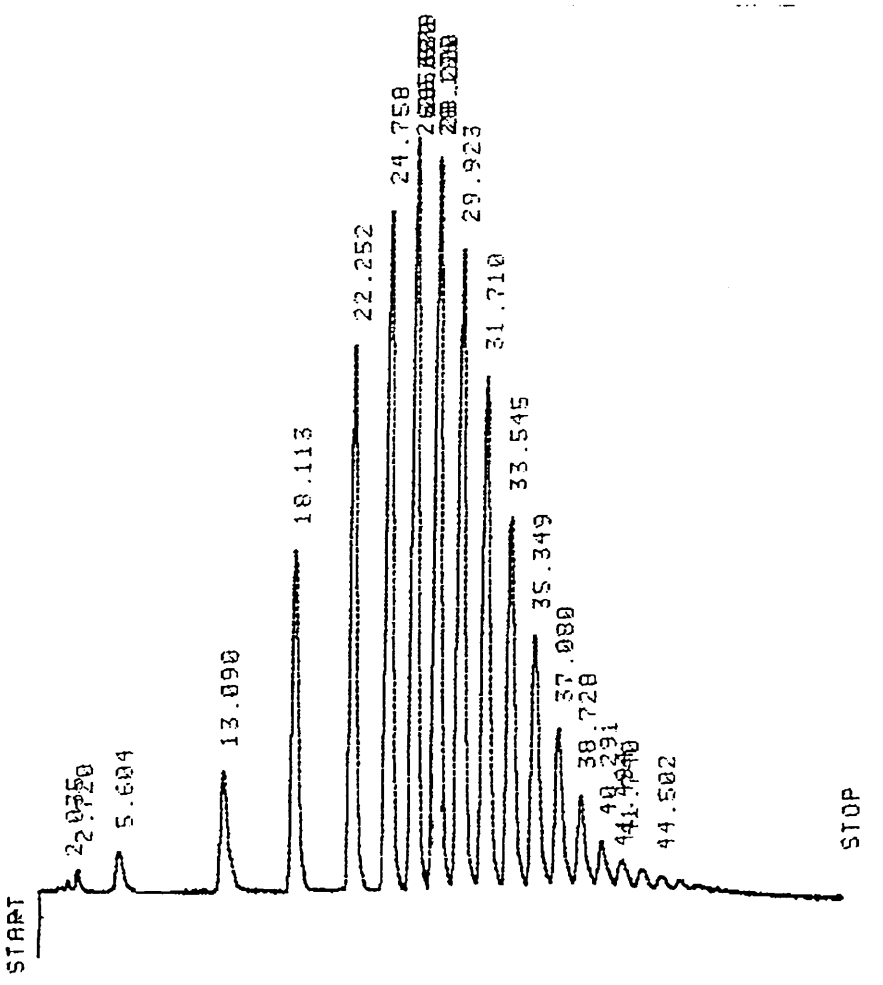
Although this method gives "base line / base line" separation of individual non-ionic oligomers it is not possible to separate anionic surfactants using this system. Generally this classification of surfactants appear as an unresolved peak. It is probable that phase switching could be utilised during these experiments in order to characterise different surfactant classification simultaneously. However, in order to achieve this the mobile phases used to achieve satisfactory elution would need to be miscible with conventional polar reverse phase solvents, which were used in the separation of LAS via a C_{18} column (Section 4.5). Unfortunately, the solvents used in this experiment limit the eluant range which can be used in experiments to separate anionic surfactants using reverse phase conditions. As a result it was necessary to try more polar elution solvents for the separation of non-ionic species which are more compatible, (Section 4.4).

Experiments have been carried in order ascertain whether this method is suitable for use with LC/PB/MS systems, (section 7.2.1).

Work carried out using the APcI interface using the cyano column and the corresponding mobile phase constituents produced poor results which suggest that a more polar mobile phase would be necessary in order to use this interface. (Section 7.2.5).

4.4) Non-ionic Surfactant oligomer separation using an amino HPLC column.

Using an 3μm amino 15 cm × 4.6 mm i.d Spherisorb HPLC column (Phase Sep, Clwyd, Wales) separation of alkylphenol polyethoxylates has been achieved based on the conditions first devised by Holt[2]. A typical standard chromatogram for 200 ppm Triton X100 is shown, (Figure 4.1).



Retention Time (Min.)

Figure 4.1) Separation of a standard mixture of OPEO using an amino column.

This method clearly separates OPEO ethoxymers. The peak at 5.6 min is OP_3EO, (using the nomenclature OP_xEO where x represents the number of ethylene oxides units in the hydrophilic chain) and the peak at retention time (Rt) = 44.5 min is $OP_{19}EO$. The advantage of this system is that it utilises solvents which are compatible with reverse phase conditions. As a result it would be possible to carry out mixed polarity phase switching. Flow rate and gradient conditions have been adjusted in order to make the method compatible with LC/PB/MS systems.

The method uses a constant flow rate of 0.3 ml min^{-1} and a linear gradient as follows:

Time / (min)	%A	%B
0	90	10
30	10	90

All samples were made up in solvent A, where A = tert-butyl methyl ether (TBME) + 0.1% acetic acid. Solvent B = Acetonitrile : methanol (95:5) + 0.1% acetic acid. Using this method it is also possible to separate a NPEO standard mixture. A typical chromatogram of a 500 ppm NP_9EO standard is shown below, (Figure 4.2).

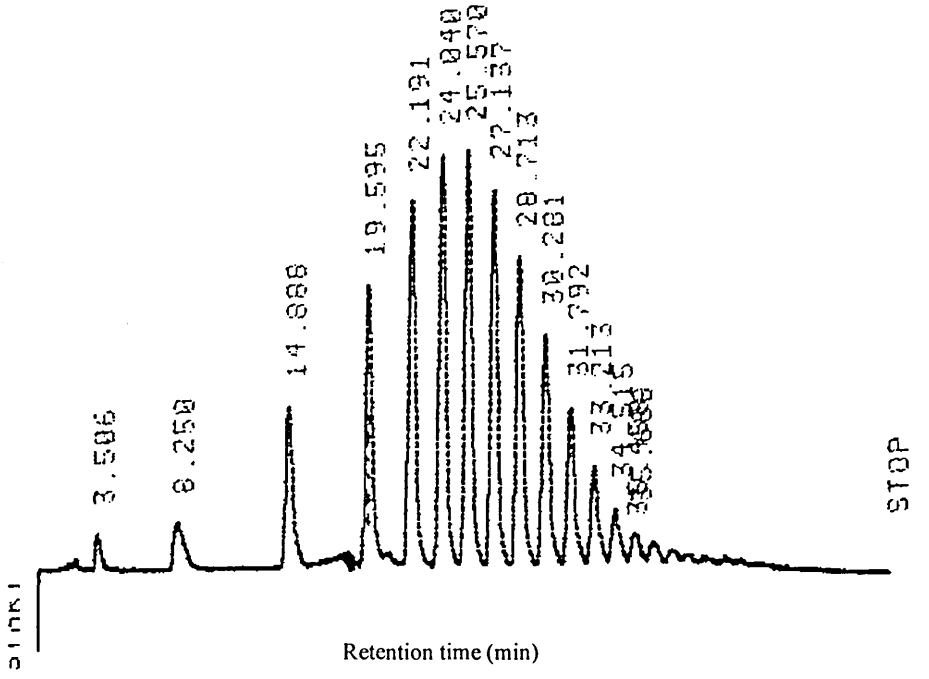
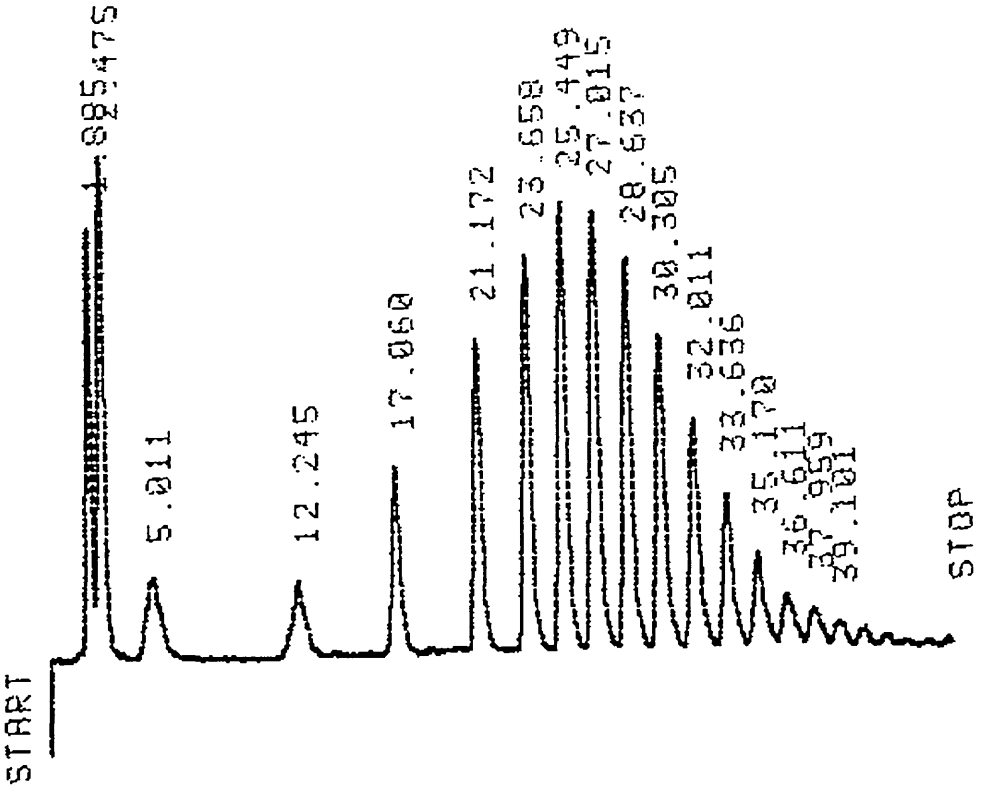


Figure 4.2) Separation of a standard mixture of NP_9EO using an amino column.

The first NPEO (NP_3EO) peak has a retention time (Rt) = 3.5 min The final ethoxymer detectable at this concentration ($NP_{16}EO$) has a retention time of roughly 38 min.

It can be seen that this method gives "base line" separation of nonyl and octylphenol ethoxylates and could also separate nonyl and octylphenols in an alkylphenol ethoxylate mixture (NP_9EO 200 ppm, 20 ppm NP_1EO and NP_2EO mixture in made up TBME (Figure 4.3).



Retention Time (min)

Figure 4.3) HPLC separation of a NP_9EO standard spiked with a NP_1EO and NP_2EO mixture.

Using these conditions NP_1EO and NP_2EO appear to elute at Rt's = 1.8 and 2.5 min, respectively.

This was confirmed by running a standard solution of the NP_1EO and NP_2EO mixture, (Figure 4.4).

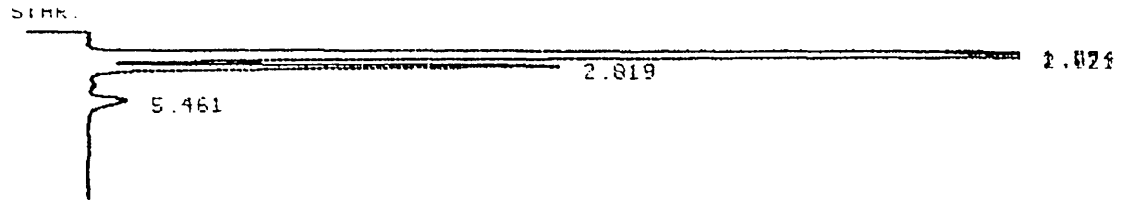


Figure 4.4) HPLC separation of a mixture of NP_1EO and NP_2EO.

Figure 4.3 displays a chromatogram of a NP_9EO spiked with NP_1EO and NP_2EO. In Figure 4.3 it can be that NP_1EO (Rt = 1.89) and NP_2EO (Rt = 2.4), have very similar retention times (Rt) to those in Figure 4.4. It is interesting to note the presence of a small amount of NP_3EO Rt = 5.4 min in Figure 4.4. Chromatograms of nonylphenol (NP) (Figure 4.5) and octylphenol (OP) (Figure 4.6) are shown below.

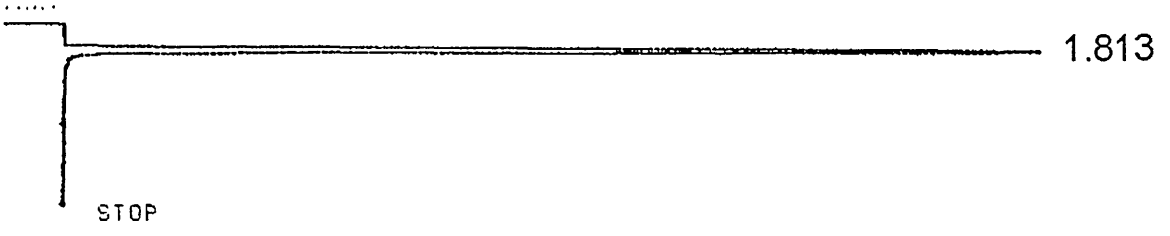


Figure 4.5) HPLC analysis of n-nonylphenol (10 ppm) standard solution.

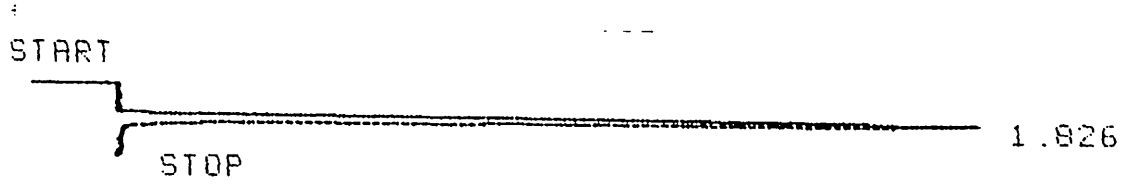


Figure 4.6) HPLC analysis of octylphenol (10 ppm) standard solution.

Clearly both compounds have very similar retention times to each other and also to NP_1EO. Therefore, if this method was to be used to measure these compounds a gradient in which the increase in solvent B (acetonitrile/methanol (95/5)) was less rapid should be used.

These methods (Section 4.3 and 4.4) are ideally compatible with the optimum conditions for a particle beam mass spectrometer interface. This particular type of interface is unable to cope with solvents with a high polarity. However, experiments using these methods resulted in poor chromatograms.

The conditions used in this experiment are not, however, compatible with the thermospray mass spectrometer interface. Due to the method of ion production here buffers need to be present in order to ionise the analyte of interest. It is possible to add a solution of buffer after the column (known as "Post Column Addition"). However, due to the limited solubility of buffer solutions with the mobile phase used in these experiments this was not practically possible. Due to time constraints it was not possible to investigate the compatibility of the conditions used in these experiments with mass spectrometric detection via an API interface.

4.5) HPLC separation of Anionic Linear Alkylbenzene Sulphonate (LAS).

The separation of a commercial surfactant formulation (Nansa SS, Albright & Wilson, UK) of LAS has been achieved using a linear gradient system with a C18 5μm Spherisorb ODS 1 25 cm x 4.6 mm i.d HPLC column, (HPLC Technology, Macclesfield, Cheshire, UK). The chromatogram shown (Figure 4.7) displays a 200 ppm solution of Nansa SS in solvent B. A linear gradient was applied with a flow rate of 0.5 ml min^{-1} :

Time/ (min)	%A	%B
0	100	0
50	50	50

Where solvent A = 0.1M NH_4OAc (aq) and solvent B = Acetonitrile + 0.1M NH_4OAc. Fluorescence detection was used, λ_{ex} = 230 nm and λ_{em} = 290 nm.

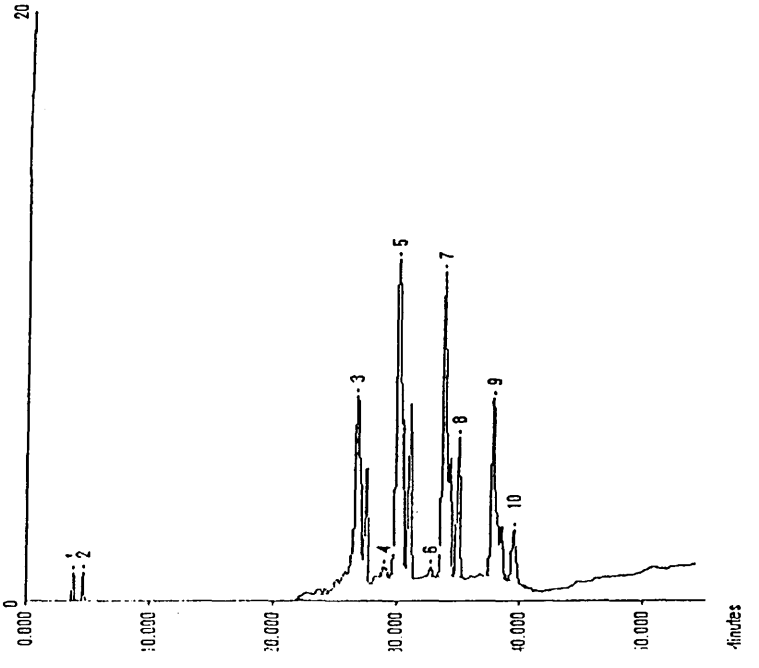


Figure 4.7) Typical HPLC chromatogram of a 200 ppm Nansa SS standard solution in 0.1M NH_4OAc.

Although this method gives excellent separation of individual homologues the time taken for a complete run (including re-equilibration time) is in excess of 1 hr, so if multiple sample analysis is required this method does not seem viable. This factor is

also true of the methods discussed in section 4.3 and 4.4, developed for non-ionic surfactant determination. It was therefore desirable to develop a method which can cut down analysis time whilst still giving the desired separation of individual homologues. This problem has been overcome by the development of an isocratic method for the simultaneous determination of a mixture of both APEO and LAS.

4.6) Reverse phase simultaneous detection of Anionic and Non-ionic Surfactants

To the best of the authors knowledge this is the first time an isocratic HPLC method has been developed for the simultaneous determination of anionic and non-ionic surfactants. It uses a trimethylsilyl column (C_1) with simple isocratic HPLC elution which is compatible with mass spectrometric detection. The analysis of anionic surfactants using a trimethylsilyl column has been reported by Castles and co-workers[3] . Using a THF / 0.1 M $NaClO_4$ eluant they were able to separate the various LAS oligomers during a 12 min run time, but were unable to resolve the various positional isomers in the mixture at the same time. Wang and Fingus[4] perfected the rapid separation of octylphenol ethoxylates using a trimethylsilyl column and a methanol / 0.01M ammonium acetate mixture. This method gives separation of oligomers over an average of 35 min. Elution of individual ethoxymers begins at roughly 8 min.

Initially experiments were carried out for each type of surfactant (i.e., APEOs and LAS) using the C_1 column and a mobile phase containing various organic solvents and phase modifiers, (i.e. methanol, THF, acetonitrile with sodium perchlorate or ammonium acetate). It was found that an aqueous solution of acetonitrile or methanol gave the best resolution. As sodium perchlorate solutions can potentially cause explosions when used in conjunction with particle beam interfaces, ammonium acetate was preferred as a phase modifier. The addition of this resulted in a sharpening of LAS peaks.

Seeing the potential of the trimethylsilyl (C1) column to allow rapid separation of both classes of surfactant using a single chromatographic phase, experiments were carried out in order to investigate the possibility of dual elution using an isocratic

system which would be compatible with mass spectrometer interfaces, allowing mass spectrometric detection. Eventually, after extended experimentation two isocratic methods were developed.

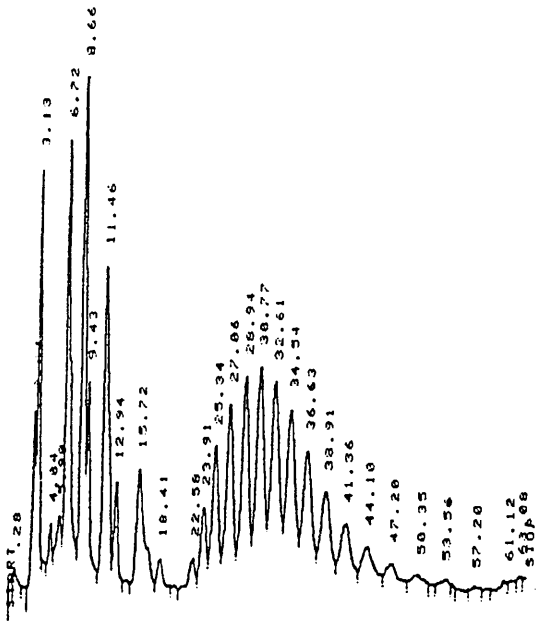
These methods are described below:

System A : 65% water / 35% Acetonitrile with an overall buffer concentration of 0.065M ammonium acetate. Flow rate : 0.7 ml min^{-1}.

System B : 58 % methanol / 42 % 0.008M ammonium acetate(aq).

Flow rate : 0.7 ml min^{-1}.

System A allows the resolution of positional isomers of LAS, which can be seen as side peaks on the main peaks for each LAS homologue. Figure 4.8 shows a chromatogram of a standard mixture of NP_9E0, LAS and octylbenzene sulphonic acid (OBSA) (40 ppm, 10 ppm and 2 ppm respectively). It can be seen that the octylbenzene sulphonic acid eluted at retention time (Rt) = 3.13. LAS oligomer distribution was between Rt = 6.72 min and 15.72 min and the NP_9EO ethoxymer distribution was between Rt = 22.58 min and 50.35 min. The partially resolved side peaks indicate the presence of the positional isomers of individual LAS homologues (representing LAS with an alkyl chain containing 11 to 14 carbon units, respectively).



Retention Time (Min).

Figure 4.8) Standard chromatogram of a standard mixture OBSA, LAS and NP9EO.

System B does not resolve the positional isomers of LAS. This leads to a more simplified chromatographic trace, (Figure 4.9). LAS distribution begins with the peak at 5 min and ends with the fourth peak at 10 min. The non-ionic distribution begins with the small peak at 11 min and ends with the twentieth peak at 58 min. This could be of benefit for trace analysis of LAS concentrations in surface waters.

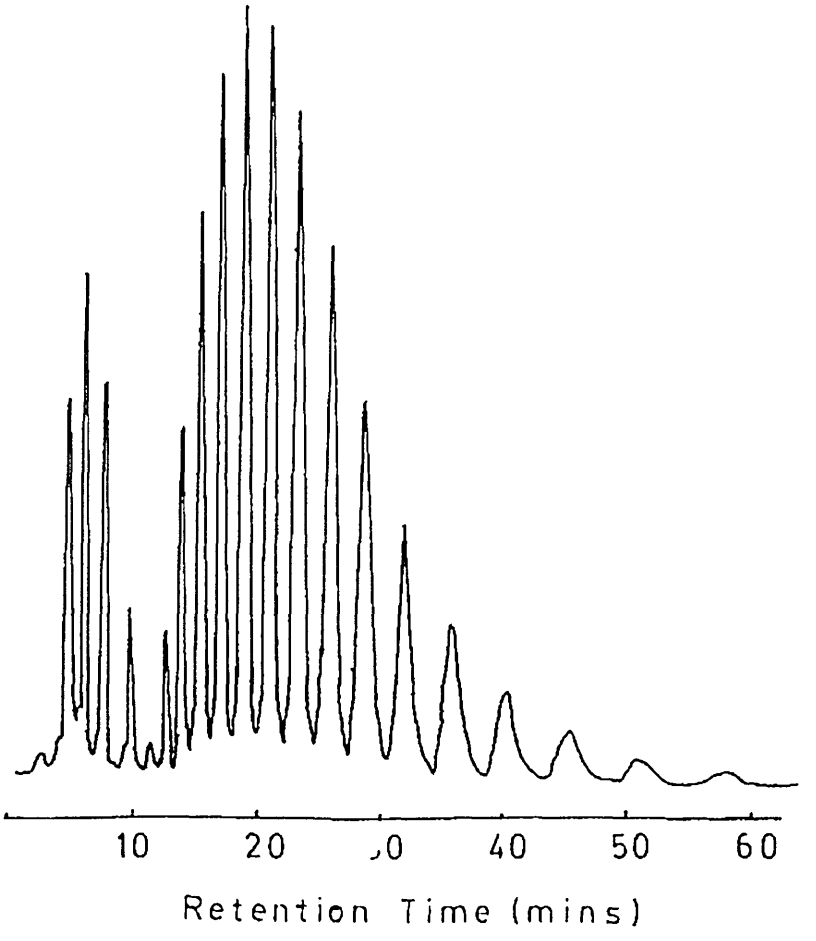
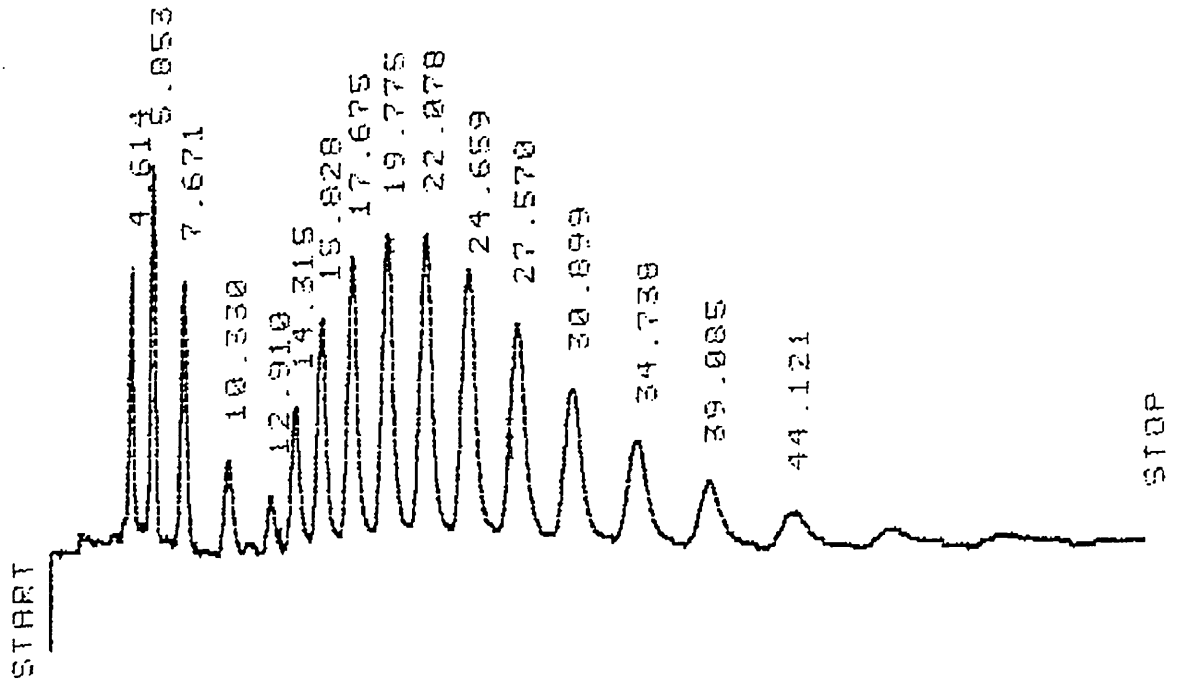


Figure 4.9) A typical HPLC chromatogram of a standard mixture of LAS and NP_9EO.

It can be seen (Figures 4.8 and 4.9) that the resolution of individual ethoxymers is not as sharp as that of the octylphenol ethoxylates, (Figure 4.10). This phenomenon has been reported by Wang and Fingus[4] also. Unfortunately as their publication does not include a representative chromatogram of an NP9 sample it is impossible to compare each method's resolving power. During this research it was noticed that some C1 columns were able to resolve NPEO oligomers to a greater degree than others. This led to a 15 cm x 4.6 mm i.d S5 Spherisorb 5μm C1 column manufactured by Hichrom (Reading, UK) being the column of preference.



Retention Time (min)

Figure 4.10) Typical HPLC chromatogram of a standard solution of Triton X100 and Nansa SS (40 ppm and 15 ppm, respectively).

Figure 4.10 shows the typical chromatographic separation obtained for a mixture containing Nansa SS (LAS) and Triton X100 (APEO) using a C1 column (chromatographic conditions as in System B Section 4.6). LAS distribution begins with the peak at 4.6 min and ends with the peak at 10.3 min. Non-ionic surfactant distribution begins with the peak at 12.9 min and ends with the peak at roughly 50 min.

4.7) Conclusions.

The aim of this work was to produce high performance liquid chromatography methods which would separate commercial surfactant formulations. Commercial LAS consists of a complex mixture of homologues which vary according to alkyl chain length. Generally this varies between 11 and 14 carbon units. In addition, further variation is introduced due to the variable position of the alkyl chain. The alkyl chain is normally in the para position; however, small quantities can be meta substituted. APEO formulations are even more complex. They contain a mixture of oligomers where the ethoxy chain length can vary between 1 and 30 units. The alkyl chain position on the

aromatic ring of the molecule can also vary. Generally, the alkyl chain of OPEO is straight, whereas it can be branched in APEO formulations. To compound these problems, manufacturers do not characterise these products fully. There are two reason for this (a) industrially it is cheaper to produce surfactants this way, (b) commercially the properties of a particular surfactant can be altered by blending with another type to produce a product of the desired qualities. As a result it is not necessary to strictly control the structure of a particular surfactant classification within a formulation, as the formulation itself can be monitored to produce a product with desirable properties.

The HPLC methods described in sections 4.3-4.6 have been used to characterise surfactants. Care was taken to either develop or modify the methods so that they would be compatible with current interfacing techniques used with mass spectrometers.

Three types of interface were used during this project. Table 4.0 summarises the characteristics of each interface used. Each required slightly different mobile phase characteristics, so as a result several HPLC methods were tailored to optimise the performance of the chosen interface. A full description of each interface and the results on combining the two techniques can be found in Chapter 7.

Table 4.0) An approximate assessment of the characteristic of the LC/MS interfaces used in this project.

LC/MS Type	Limit of detection	LC flow range	Solvent types	semi-volatile samples	Involatile /thermally labile samples	mol. mass range
Thermospray	✓✓(✓✓)	✓✓✓✓✓	✓✓✓(✓)	✓✓✓✓✓	✓✓✓✓	✓✓✓✓
Particle beam	✓✓	✓✓✓(✓)	✓✓✓✓	✓✓✓✓✓	✓(✓)	✓(✓)
API	✓✓✓✓(✓)	✓✓✓✓✓	✓✓✓(✓)	✓✓✓✓	✓✓✓✓✓	✓✓✓✓

✓ indicates performance. ( ) indicate varying characteristics.

It can be seen from table 4.0 that the particle beam interface for example, can accommodate a wide range of solvent types. It was therefore possible to use all of the methods developed in this work using the particle beam interface.

However, due to low sensitivity it was also necessary to investigate other methods. It is advantageous to use mobile phases containing a degree of inorganic salts when using the thermospray interface so as to aid ionisation. Therefore, the methods in section 4.5 and 4.6 were developed. Both use solvents which are compatible with the interface and have buffer added as part of the mobile phase. Commonly, buffers are added at values approximately 0.1M (Section 7.1.3). It was not, however, possible to add inorganic buffer at a concentration of 0.1M to the mobile phase of the methods described in section 4.6 as this resulted in retention times which were too long practically in duration. Ammonium acetate was one of the buffers added. It was used to increase the level of inorganic buffer prior to entry of the mobile phase into the thermospray mass spectrometer interface. It was added at a concentration of approximately 0.1M post-column in the hope of increasing ionisation efficiencies when using this interface. If ammonium acetate was added at this level to the mobile phase reservoir (so it was at a concentration of 0.1M before entering the column) the chromatographic separation of the sample would of been seriously affected. Due to its high sensitivity the LC/MS interface of choice would appear to be the atmospheric pressure ionisation (API) interface. Theoretically it can be used with polar and to some

degree relatively non-polar solvents. Therefore the methods described in section 4.3 and 4.6 were used with this interface.

Methods mentioned (Section 4.3-4.6) were developed by trial and error. It was important, however, to produce final methods with solvent ratios and flow rate which are compatible with UV, Fluorescence and Mass Spectrometric techniques. Due to the complex nature of the sample it was not generally possible to predict the result of a change in mobile phase composition, column packing size or flow rates.

The work carried out in order to develop separation of LAS on reverse phase systems the (C1(trimethylsilyl) and C18 column) is a good example of this, (section 4.5-4.6). This was particularly true of LAS separation using the C1 column. Several organic solvent / buffer ratios were tried and their affect on peak resolution monitored. The addition of inorganic buffer can have a dramatic affect on resolution. This can be tentatively explained by the fact that, in reverse phase systems LAS anions are retained on the stationary phase surface through a hydrophobic interaction between the alkyl chain of the anionic surfactant and the stationary phase. This would create a negatively charged layer at the surface of the stationary phase. As mobile phase ionic strength increases with the addition of buffer it is probable that there will be an interaction between a cationic (i.e., NH_4^+) species and the retained LAS. This would reduce the ionic character of the LAS surfactant anionic group, thus increasing the interaction between the LAS surfactant and the stationary phase. It has been shown (section 4.6) that it is possible to separate APEO type surfactants by using the C1 column. The addition of buffer here did not have a great affect on the retention profile of these surfactants. However, changes in the levels of organic solvents did affect the retention profile of this compound. When acetonitrile was used in the mobile phase a solvent ratio of around 35% acetonitrile / 65% buffer solution provided the optimum separation of oligomers and homologues whereas when the mobile phase contained methanol, which is more polar, a solvent ratio of 58% methanol / 42% buffer solution was needed to produce optimum conditions. It should also be noted that less ammonium acetate was needed. The separation of LAS could be achieved satisfactorily with the use of 0.008M

NH_4OAc instead of 0.065M which was used in the case of acetonitrile containing mobile phases.

Chapter 4 References.

1) Pilc JA, Sermon P, J. Chromatogr., 398, 375, 1987.

2) Holt MS, McKerrell EH, Perry J, Watkinson RJ, J. Chromatogr., 362,1986.

3) Castles MA, Moore BL and Ward SR, Analytical Chemistry, 61, 2534,1989.

4) Wang Z, Fingus M., J. Chromatogr., 641, 125, 1993.

Chapter 5

Extraction of Surfactants from aqueous samples by Solid Phase Extraction (SPE).

5.0) Introduction.

As surfactants are present in surface waters at low concentration it is necessary to pre-concentrate before analysis. Solid phase extraction has been used in order to do this during this project. This technique has the added advantage of allowing sample clean-up prior to analysis. This process can therefore result in improved peak shape and resolution. However, as with any analytical technique optimisation is required. Therefore, a series of experiments were carried out in order to optimise the extraction process. A study of three different organic solvents, (a) methanol, (b) acetonitrile and (c) acetone, was carried out in order to determine which would maximise anionic and non-ionic surfactant recovery. Experiments were also carried out in order to determine the efficiency of extraction of anionic surfactant (Nansa SS) and non-ionic surfactant (Synperonic 9) individually and as a mixture. Initially these experiments used surfactant concentrations (Nansa SS = 2.5 mg L^{-1}, Synperonic 9 = 5.6 mg L^{-1}) which are greater than the environmental concentration of the surfactant in many waterways. Later, after problems with adsorption of surfactant onto laboratory glassware had been resolved it was possible to study the extraction profile of surfactants at environmentally realistic concentrations (Synperonic 9 = 14 $\mu g\ L^{-1}$ and Nansa SS = 2 $\mu g\ L^{-1}$).

During this work adsorption of the surfactant formulations onto the surfaces of the glassware used in the extraction experiments was noted. Surfactant concentrations in surface waters are very low, usually in the range of 0.01 to 1 ppm. Therefore with a 500 fold pre-concentration step (as has been used in this work), it is important to be able to produce linear calibration curves when standard concentrations are less than 100 ppm. If adsorption of any surfactant onto glassware occurs it would obviously change the actual concentration of a surfactant solution held within that flask. If the number of 'sites' that surfactant molecules could attach to is presumed to be constant for a particular type of flask then as the concentration of a surfactant solution decreases loss of fluorescent signal due to adsorption would become more prominent. As a result 'Flask Transfer' experiments were carried out (Section 5.1.1) in order to investigate the significance of surfactant adsorption onto the laboratory glassware used with a view to correcting the problem.

5.1) Experimental.

Standard solutions of Synperonic 9 ((a nonylphenol ethoxylate) a gift from ICI Materials Research Centre, Wilton, Middlesborough, Cleveland, UK.) and Nansa SS (a commercial dodecyl alkylbenzene sulphonate formulation with an alkyl chain length distribution of 10 to 13 units, (Albright and Wilson, Castleford, UK.)) were prepared by (a) dissolving in HPLC mobile phase for standard surfactant solutions and (b) Millipore grade water for samples to be extracted by SPE.

All analyses were performed on a Gilson 302 gradient pumping system. Column and mobile phase were as described in section 4.3 and 4.5 for non-ionic and anionic surfactants respectively. Injections were made manually using a Rheodyne injection value fitted with a 50 µl sample loop. A Hewlett Packard 1046A (Palo Alto, CA, USA.) fluorescence detector λ_{ex} = 220 nm and λ_{em} = 302 nm for alkylphenol ethoxylate and λ_{ex} = 220 nm and λ_{em} = 290 nm for linear alkylbenzene sulphonates was used. Data was relayed to a Shimazdu integrator.

5.1.1) Flask transfer experiments.

Solutions of LAS (2.0 mg L^{-1}) were made up from 25 mg L^{-1} stock solutions in silanized and unsilanized glass. The 25 mg L^{-1} stock solutions themselves in silanized and unsilanized glassware were prepared by accurate weighing of LAS and octylbenzene sulphonic acid (OSA) (for use as an internal standard) into volumetric flasks and dissolution into mobile phase.

The 2.0 mg L^{-1} solutions were allowed to stand for 5 minutes at room temperature after vigorous shaking and then four aliquots of 50 µl were analysed by HPLC using the method described in section 4.6 method B of this thesis. In order to minimise surface effects the aliquots were taken by inserting the HPLC syringe 2 cm below the sample surface.

The 2.0 mg L^{-1} solutions were then transferred to a subsequent silanized and unsilanized glass volumetric flask (as appropriate) and the procedure repeated. Six such transfers were carried out for each solution. The flasks used were silanized by treatment with trimethylchlorosilane (Sigma-Aldrich, Poole, Dorset) overnight. They were then

washed with hexane and then methanol, then air dried. A similar set of experiments were carried out using a standard solution (2.5 mg L^{-1}) of an alkylphenol ethoxylate with an average chain length of nine ethoxylate units (Synperonic 9).

5.1.2) Experiments to investigate the linearity of calibration curves produced from Surfactant standards.

The linearity of calibration curves produced via the analysis of non-ionic surfactants was investigated by use of standard solutions contained in a) glass volumetric flasks b) silanized glass volumetric flasks of the same grade, or c) grade 'B' polypropylene plastic volumetric flasks (BDH, Dorset, England). A standard stock solution of 56 ppm of Synperonic 9 was made up in a solution of MeOH / 0.008M ammonium acetate (58 / 42), mobile phase conditions for HPLC method (Section 4.6b). Dilutions were performed using Gilson (Villiers le Bel, France) plastic tipped automatic pipettes with grade 'A' glass volumetric flasks. Standards of concentrations, 2.24, 5.6, 8.4, 11.2 ppm were analysed by HPLC using the conditions described in section 4.6 method B of this thesis and the peak areas of individual ethoxymers used to construct calibration curves. Injections were carried out in duplicate. This procedure was repeated using silanized volumetric flasks of the same grade, then by using grade 'B' poylpropylene plastic volumetric flasks (BDH, Dorset, England).

5.1.3) Comparison of the effect of organic solvents on Surfactant pre-concentration using C_{18} Solid Phase Extraction cartridges (SPE).

In order to determine which organic solvent should be used to both condition the SPE cartridges and elute a mixture of LAS and APEO type surfactants from the cartridge a rapid experiment was carried out using 3 different organic solvents.

Hypersil C_{18} (Shandon, Warrington, Cheshire, UK) solid phase extraction cartridges (500 mg packing material) were conditioned with either (a) 10 ml of acetonitrile (b) 10 ml of acetone or (c) 10 ml of methanol, then 10 ml of Millipore grade distilled water. A standard surfactant mixture (600 mg L^{-1} of both Nansa SS and Synperonic 9 in 100 ml of distilled Millipore water) was passed through each cartridge.

The cartridges were then rinsed with 10 ml of distilled Millipore grade water. After they were thoroughly dry, 10 ml of one organic solvent (methanol, acetonitrile and acetone) was passed through one of the three different cartridges, individually. The eluant was collected, blown to dryness using a stream of nitrogen then reconstituted using HPLC mobile phase.

(i) Extraction efficiency of LAS (Nansa SS) was measured by using a linear gradient system with an octadecyl 5μm Spherisorb ODS 1 25 cm x 4.6 mm i.d. HPLC column (HPLC Technology, Macclesfield, Cheshire). A flow rate of 0.5 ml min^{-1} was applied :

Time / (min)	%A	%B
0	100	0
50	50	50

Where solvent A = 0.1M $NaClO_4$ (aq) and Solvent B = 0.1M $NaClO_4$ in acetonitrile. Peak areas obtained were compared against a standard chromatogram of the same initial concentration.

(ii) Non-ionic surfactant (Synperonic 9) extraction efficiency was determined using a 25 cm x 4.6 mm i.d. cyano column (Anachem Ltd, Luton, Bedfordshire), (Section 4.3). Alkylphenol ethoxylates were separated according to oligomer distribution. A constant flow rate of 0.7 ml min^{-1} was applied :

Time/(min)	%A	%B
0	95	5
50	50	50

Where A = n-hexane and B = 2-methoxyethanol / propan-2-ol (75/25). Here UV detection (280 nm) was employed. The peak areas obtained were compared against a standard chromatogram of the same initial concentration.

5.1.4) Extraction of Surfactant from aqueous samples using SPE cartridges.

Extended experiments were carried out in order to determine extraction efficiency. Initial experiments were carried out at concentrations in excess of normal environmental surfactant concentrations. This was done so as to minimise error introduced into the experiment as a result of surfactant adsorption on the glassware used.

a) Non-ionic surfactant extraction.

Three Synperonic 9 samples in Millipore grade water (4.8 mg L^{-1}) were extracted in order to calculate % recoveries of non-ionic surfactants. Standards were used to construct a calibration graph. SPE conditions used were as follows :

i) Conditioning step : 7 ml methanol, 7 ml Millipore grade water.

ii) Deposition step : Before the cartridge dried 50 ml of sample was passed through at a flow rate of 5 ml min^{-1}.

iii) Rinsing step : 20 ml of Millipore grade water was used to rinse the flask (which was then passed through the cartridge) then 30 ml of Millipore grade water was passed through the SPE cartridge.

iv) Elution Step : After the cartridge had air dried, 3 ml of methanol was passed through the cartridge, the eluant being collected in a grade "A" 10 ml volumetric flask. This solvent was then blown off under a steady stream of N_2. The flask were then made up to volume using hexane. This solution represents the analytical mixture used. Chromatographic conditions were as described in section 5.1 of this thesis. A central peak in the ethoxymer distribution was chosen to calculate peak area response to simplify calculations of the recovery data.

b) At low surfactant concentrations

Further experiments were carried out at environmental concentrations after the linearity of the method has been assessed, (Section 5.2.5). Conditions were as described in section 5.1.4a, except that polypropylene volumetric flasks were used in place of glass volumetric flasks. Also, an initial 500 ml sample size was used instead of 100 ml,

after which the eluted analyte was reconstituted with MeOH / 0.008M (aq) ammonium acetate (58 / 42) to a volume of 1 ml. The HPLC method mentioned in Section 4.6 method B was used to analyse each sample. Samples were spiked at 11.2 $\mu g\ L^{-1}$ with NP9 and recoveries calculated from calibration data generated from peak areas of the HPLC chromatograms of standards.

c) Anionic Surfactants.

Three extracts of Nansa SS in Millipore grade water (2.5 mg L^{-1}) were analysed in order to calculate % recoveries of the analyte. Standards were used to construct a calibration graph for LAS.

The SPE process was carried out as described in section 5.1.3, except that samples were eluted with 3 ml of methanol into a 1 ml volumetric flask. Following this, a steady stream of N_2 was applied in order to evaporate the excess methanol in the flask. Samples were then reconstituted in 1 ml of 0.1 M $NaClO_4$.

5.1.5) Simultaneous recovery of Nansa SS and Synperonic 9 using SPE.

Experiments were carried out in order to investigate the extraction profile of a surfactants mixture solution containing both anionic and non-ionic surfactant.

a) high surfactant concentration

Standards were used to construct a calibration graph and 3 extracts (Millipore grade water spiked with Nansa SS = 2.48 mg L^{-1}, Synperonic 9 = 5.6 mg L^{-1}) were analysed in order to calculate % recoveries of the analytes of interest. A reverse phase system which employs a C1 HPLC column, (Section 4.6 method A) was used.
Samples were pre-concentrated using SPE Hypersep C18 cartridges using the following conditions :

(i) Conditioning	(i) 7 ml methanol
	(ii) 10 ml Millipore grade water.
(ii) Deposition	100 ml of sample solution was passed though SPE cartridge.

(ii) Elution

20 ml of Millipore grade water was used to wash the volumetric flask used (which was then passed through the SPE cartridge). Next the cartridge was washed with 20 ml of Millipore grade water.
The cartridge was then allowed to air dry.
3 ml of methanol was then passed through the cartridge and collected in a 1 ml volumetric flask.
The methanol was blown to dryness using a steady stream of N_2. The extract then dissolved in acetonitrile/ 0.05 M ammonium acetate (35/65).

b) at low Surfactant concentration.

Conditions were as described in section 5.1.4a, except that polypropylene volumetric flasks were used in place of glass volumetric flasks. Dilutions were performed using Gilson (Villiers le Bel, France) plastic tipped automatic pipettes. Also a 500 ml sample size was used. The eluted analyte was reconstituted with MeOH / 0.008M ammonium acetate (58 / 42). The HPLC method mentioned in Section 4.6 method B was used to analyse each sample. Each sample was spiked at 14 $\mu g\ L^{-1}$ Synperonic 9 and 2 $\mu g\ L^{-1}$ Nansa SS. Recoveries were calculated using calibration data generated from peak areas of HPLC chromatograms of standards.

5.2) Results

5.2.1) The transfer of Surfactant solutions between volumetric flasks.

In order to investigate whether or not a surfactant solution could be retained on the surface of laboratory glassware, surfactant solutions were transferred to six identical volumetric flasks. Any change in peak areas recorded would give an indication of whether or not the surfactant sample was being retained on the surface of the volumetric

flasks used. Table 5.0 and Figure 5.0 shows the results of transferring a solution of Nansa SS between six 100 ml volumetric flasks.

Table 5.0) Flask transfer of a standard Nansa SS solution.

Ratio Values				
LAS Peak	1	2	3	4
flask 1	0.491	0.65	0.59	0.315
flask 2	0.474	0.65	0.56	0.291
flask 3	0.461	0.623	0.55	0.294
flask 4	0.446	0.617	0.52	0.28
flask 5	0.448	0.584	0.508	0.268
flask 6	0.468	0.596	0.513	0.256
Mean	0.465	0.620	0.540	0.284
s.d.	0.017	0.027	0.032	0.021
r.s.d.	3.66	4.35	5.93	7.39
% Loss	-5	-8	-8	-6

The experiment was repeated with glassware using 6 silanized 100 ml volumetric flasks. Refrigerated stock solution was allowed to reach room temperature then a 10 fold dilution was taken as in the previous experiment. Contact times were equivalent to the previous experiment. Table 5.1 and Figure 5.1 show the result of transferring a Nansa SS solution between six 100 ml silanized volumetric flasks.

Table 5.1) Flask transfer of Nansa SS between silanized flasks.

Ratio Values				
LAS Peak	1	2	3	4
flask 1	0.52	0.73	0.65	0.36
flask 2	0.40	0.54	0.49	0.28
flask 3	0.39	0.52	0.45	0.25
flask 4	0.43	0.50	0.44	0.24
flask 5	0.41	0.52	0.45	0.24
flask 6	0.40	0.52	0.45	0.24
Mean	0.43	0.55	0.49	0.27
s.d.	0.05	0.08	0.07	0.04
r.s.d.	11.62	14.54	14.28	14.81
% Loss	-23	-29	-31	-33

Figure 5.3) Comparison of signal variation after transfer of NP9 solution into 6 different silanized volumetric flasks.

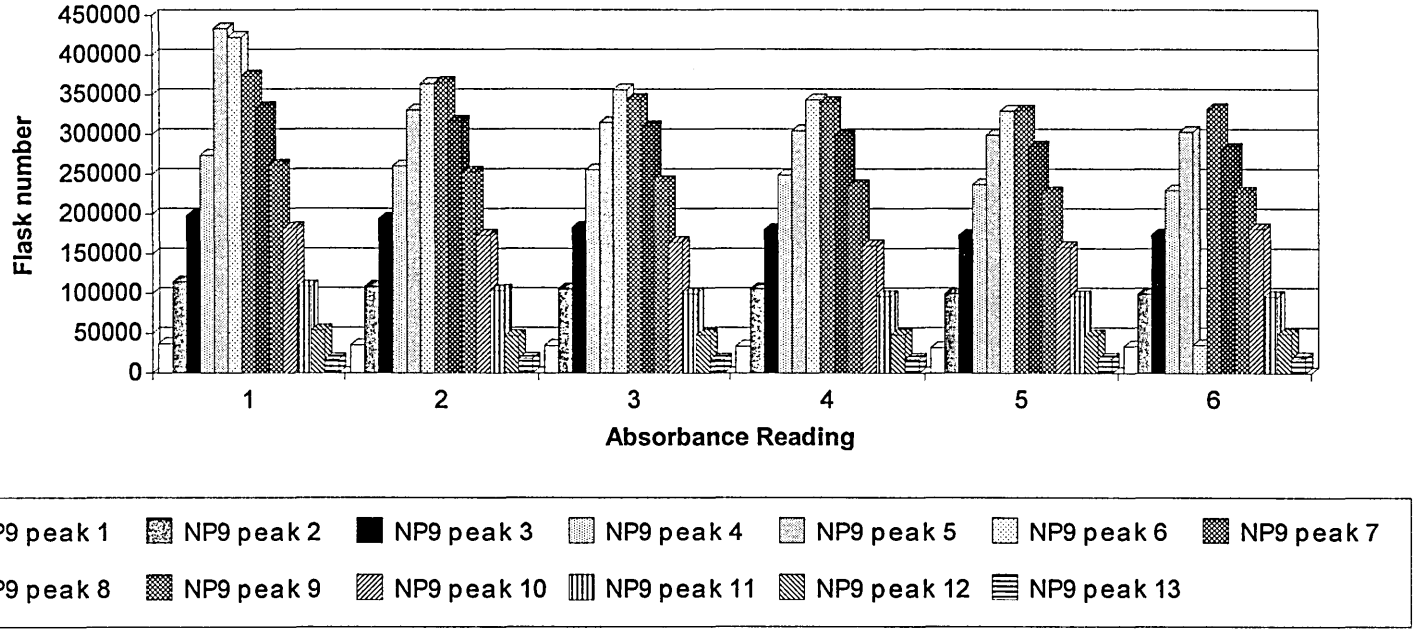


Figure 5.2) Comparison of signal variation after transfer of NP9 solution into 6 different 100 ml volumetric flasks.

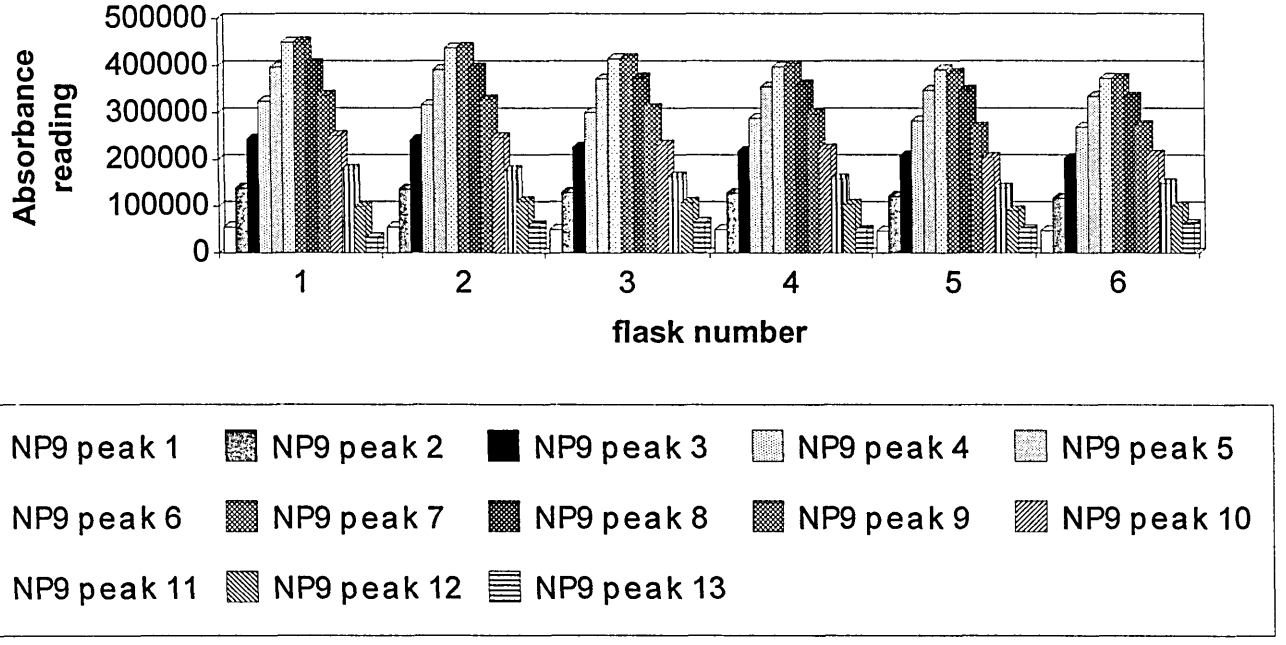


Table 5.3) NP9 oligomer variation after silanized flask transfer.

Mean absorbance value													
Flask no.	NP9 Peak Number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	37077	114739	197931	274027	433639	422461	373732	334102	261809	182517	109023	53815	17299
2	35180	108794	194301	260436	331053	364041	365450	316861	252886	173633	103634	46766	18271
3	34632	106203	183537	256256	315350	356433	344651	310367	241180	164768	99617	48068	17101
4	33947	105995	180579	249355	304921	343692	340385	299594	236144	160675	96386	48440	17393
5	32012	100200	173655	237560	299754	329788	330039	284946	227555	158617	96396	47315	17836
6	33641	99440	174279	230261	303558	35222	332397	282358	227411	181814	96086	49282	19012
% Loss	-9	-13	-12	-16	-30	-23	-11	-15	-13	-0.3	-12	-8	10
s.d.	1692	5671	10135	15873	51358	35391	17897	19799	13879	10513	5214	2540	720
r.s.d.	5	5	6	6	15	10	5	6	6	6	5	5	2

Table 5.2) NP9 oligomer variation after unsilanized flask transfer.

Mean absorbance value													
Flask no.	NP9 Peak Number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	55957	139237	243789	324946	397224	449454	449934	404325	336639	250661	178144	99155	32375
2	56205	136333	241627	316421	391496	437718	438876	395058	327799	247371	176736	110519	58929
3	50677	129798	226841	300698	372155	414757	414775	374696	309605	231511	163417	108114	65045
4	50341	127892	217815	288154	355083	397570	399773	359643	297959	222896	158907	104789	48980
5	47039	121566	208240	283277	348090	391610	383984	347778	269420	204145	138726	90397	49869
6	48050	117731	202980	270092	335399	373004	373014	333050	273505	209550	149324	99928	61838
% Loss	-8	-15	-17	-17	-16	-17	-17	-18	-19	-16	-16	1	91
s.d.	3892	8275	16957	20794	24647	29004	30334	27530	27610	19163	15389	7275	11913
r.s.d.	8	6	8	7	7	7	7	7	9	8	10	7	23

Figure 5.1) Comparison of signal ratio variation after the tranfser of a Nansa SS solution into six different 100ml silanized volumetric flasks

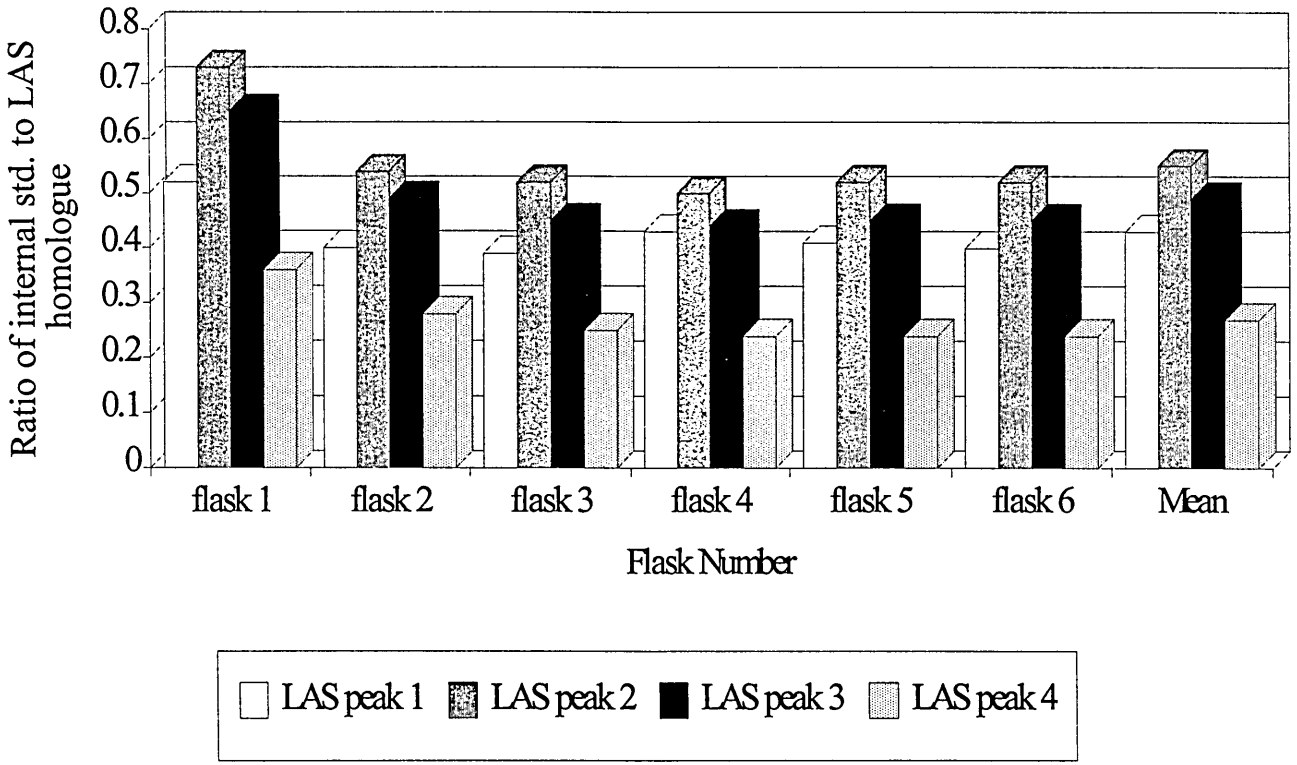
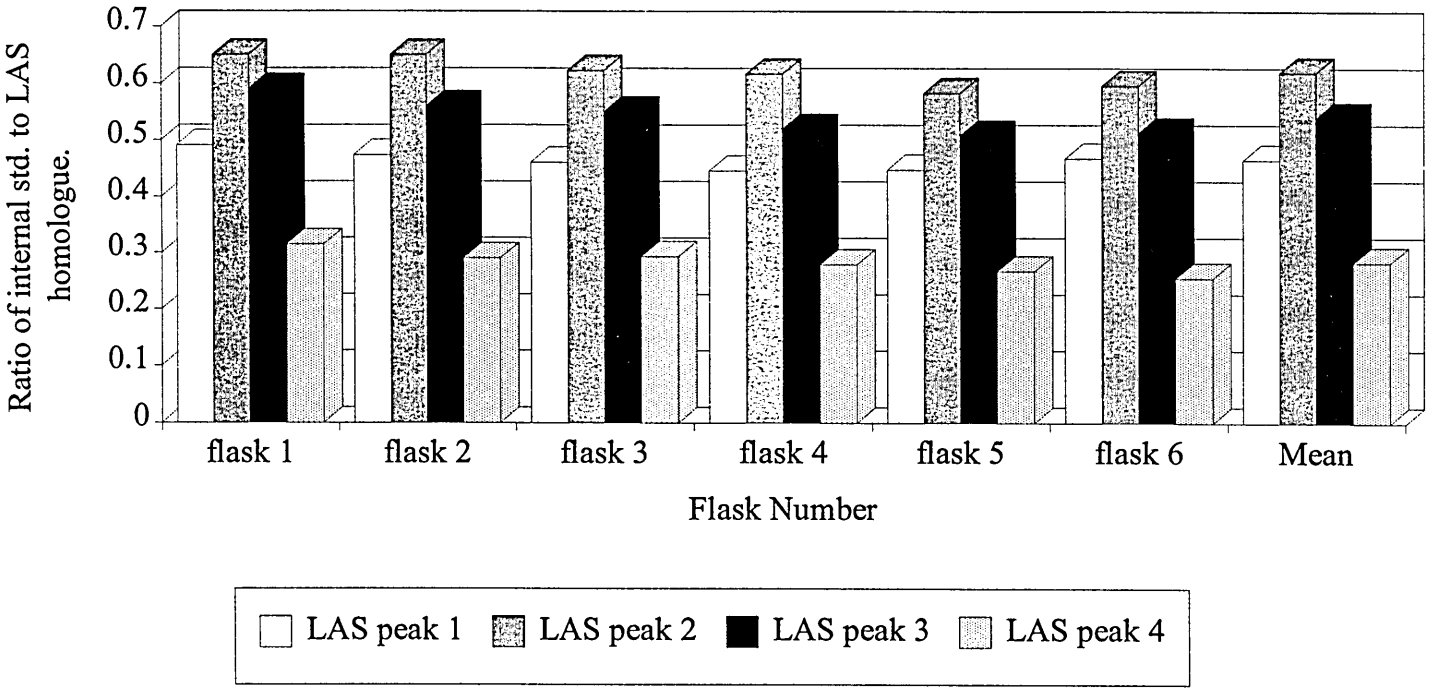


Figure 5.0) Comparison of signal variation after transfer of a Nansa SS solution between six different 100ml volumetric flasks.



Comparing Tables 5.0 and 5.1 it can be seen that silanization results in an increase in LAS deposition onto flask walls. This presumably is as a result of the introduced trimethyl groups interacting with LAS.

Table 5.2 and Figure 5.2 show the variation in signal for the transfer of a standard 2.5 ppm Synperonic 9 solution between six unsilanized glass volumetric flasks. Both Table 5.3 and Figure 5.3 show the variation in signal for the transfer of a standard 2.5 ppm Synperonic 9 solution after transfer between six silanized glass volumetric flasks. Broadly speaking, adsorption onto glassware seems to decrease after silanization of the volumetric flasks used in these experiments. Tables 5.2 and 5.3 show that the relative standard deviation (r.s.d.) of the surfactant signal for the silanized glassware is lower than for the unsilanized which suggests that silanization of glassware may improve signal reproducibility. However, the r.s.d. of the data in both cases ranges from 5 to 15. Quantification of samples containing low surfactant concentrations could be influenced by this apparent high variation in r.s.d. As a measure of method precision experiments were undertaken to look into the linearity of standard curves produced via experiments using silanized and unsilanized glassware.

5.2.2) Standard curves of Non-ionic Surfactants.

Typically the concentration of NP9 in the environment is in the region of 0.01 ppm. Assuming an extraction method will pre-concentrate 500 fold, calibration graphs used need to be linear over a concentration range of 1 to 10 ppm at least. Table 5.4 shows the results of regression values calculated for several oligomers of standards Synperonic 9 solutions at concentrations of 2.24, 5.6, 8.4, 11.2 ppm. Dilutions were performed using Gilson (Villiers le Bel, France) plastic tipped automatic pipettes. Chromatographic conditions were as in Section 4.6 method B. Injections were carried out in duplicate. This procedure was repeated using silanized volumetric flasks of the same grade, (Table 5.5).

Table 5.4) Regression values for Synperonic 9 standards contained in unsilanized glassware.

Ethoxymer number	Regression value
3	0.9967
4	0.9979
5	0.9982
6	0.9979
7	0.9978
8	0.9982
9	0.9977
10	0.9981
11	0.9970

Table 5.5) Regression values for Synperonic 9 standards contained in silanized glassware.

Ethoxymer number	Regression value
3	0.9970
4	0.9983
5	0.9980
6	0.9981
7	0.9984
8	0.9984
9	0.9980
10	0.9981
11	0.9983

Comparing Tables 5.5 and 5.6 it can be seen that silanization of glassware results in slightly better regression values. However, it would be desirable to produce calibration

curves which are linear in nature. Further experiments were carried out in order to investigate whether plastic volumetric flasks would be more effective for use with surfactant solutions. Results are shown in Tables 5.7 and 5.8. Work carried out by Marcomini et al.[1] showed the benefit of adding sodium dodecyl sulphate (SDS) to standard surfactant solutions to improve linearity. As a result it was decided to adopt this procedure to investigate whether or not this addition would improve standard linearity. Sodium dodecyl sulphate was added at a concentration of 250 mg L^{-1}.

Table 5.7) Regression values for Synperonic 9 standards containing 250 mg L^{-1} of SDS held within silanized glassware.

Ethoxymer number	Regression value
3	0.9993
4	0.9978
5	0.9993
6	0.9997
7	0.9978
8	0.9997
9	0.9998
10	0.9998
11	0.9998

Table 5.8) Regression values for Synperonic 9 standards containing 250 mg L^{-1} of SDS held within Plastic volumetric flasks.

Ethoxymer number	Regression value
3	0.998
4	0.998
5	0.9995
6	1.0
7	0.9996
8	1.0
9	0.9999
10	0.9999
11	0.9999

Tables 5.7 and 5.8 show that the use of plastic polypropylene flasks leads to an improvement in calibration curve linearity. The use of SDS to aid linearity was investigated further by repeating the experiment using polypropylene flasks without the addition of SDS.

Table 5.9) Regression values for NP9 oligomers obtain from standards stored in plastic volumetric flasks without the addition of SDS.

Ethoxymer number	Regression value.
3	0.9992
4	0.9999
5	0.9999
6	0.9999
7	0.9999
8	0.9999
9	0.9999
10	0.9990
11	0.9999

Comparison of Tables 5.8 and 5.9 shows the variation in linearity when using plastic volumetric flasks with and without added SDS to standard solutions. The addition of SDS did not produce a marked improvement in linearity. Therefore, in order to reduce potential variation during sample preparation it was decided that it would be prudent not to add SDS to standards or samples when monitoring the environmental concentration of APEOs.

5.2.3) Experiment to determine the effect of different organic solvents on Surfactant pre-concentration using SPE.

Figures 5.4 and 5.5 show recovery data from Tables 5.10 and 5.11, respectively. Clearly, they show that methanol is the best solvent to use to elute a mixture of APEO and LAS surfactants.

Methanol gave a total Nansa SS (LAS) recovery of roughly 78% and a total Synperonic 9 (NP9) recovery of 118%. No peaks greater than peak no. 5 (equivalent to NP_8EO) were detected (i.e. eluted) using acetone. Similarly no peaks greater than peak

no. 12 (equivalent to $NP_{15}EO$) were detected (from the SPE cartridge) using acetonitrile. Although methanol gave 'high' recovery values for APEO, i.e., greater than 100 % it was observed that recovery of LAS was only practically possible using methanol (use of acetonitrile and acetone resulted in poor LAS recoveries). These results were obtained via comparison of peaks areas between a standard and a spiked reagent water sample of the sample concentration (600 mg L^{-1} for Nansa SS and NP_9EO), prior to extraction. Although standard calibration graphs should be used for accurate determination of recovery data, (Section 5.2.4-5.2.5) it was felt that this experiment indicated a general trend which showed that methanol should be used to elute surfactant solutions from SPE cartridges.

5.2.4) Extraction of reagent water spiked with Surfactants using SPE.

a) Non-ionic Surfactants.

Table 5.12 shows recovery data obtained for the extraction of alkylphenol ethoxylates. A central peak in the ethoxymer distribution was chosen in order to calculate peak area response in order to simplify percentage recovery calculations. This experiment was repeated and results are shown in Table 5.13.

Table 5.10) Variation in the recovery of Nansa SS from surfactant solution containing Nansa SS and Synperonic 9 using 3 different organic solvents.

Solvent Used For Extraction	**Recovery (%)**			
Peak Number	1	2	3	4
Acetonitrile	44	39	26	22
Acetone	17	12	8	12
Methanol	81	75	77	79

Table 5.11) Variation in the recovery of Synperonic 9 from a surfactant solution containing Nansa SS and Synperonic 9 using 3 different organic solvents.

Solvent Used For Extraction	**Recovery (%)**														
Peak Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acetonitrile	79	73	77	73	85	87	90	86	88	83	96	97	86	0	0
Acetone	118	58	44	23	13	0	0	0	0	0	0	0	0	0	0
Methanol	149	110	112	106	105	107	108	108	114	115	134	146	184	67	108

Figure 5.4) Graph to show the variation in recovery from a C18 SPE cartridge of Nansa SS from a surfactant solution containing Nansa SS and Synperonic NP9 using 3 different orgainc eluants.

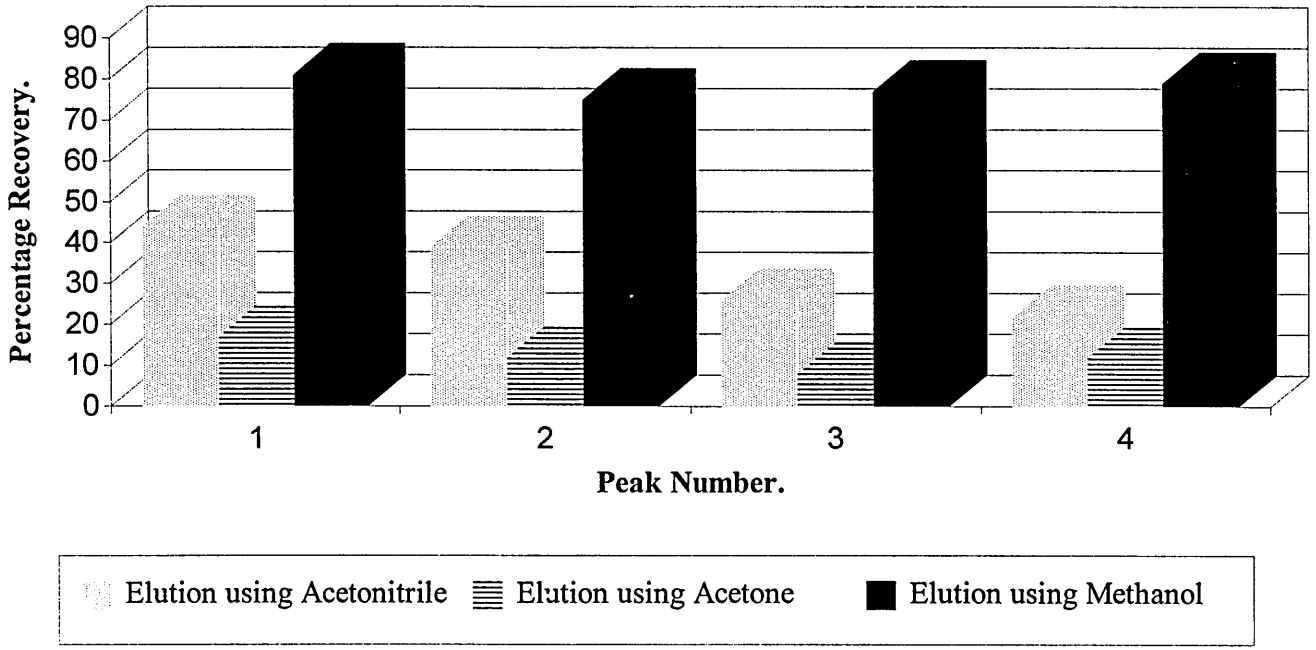


Figure 5.5) Graph to show the variation in recovery from a C18 SPE cartridge of Synperonic 9 from a surfactant solution containing Nansa SS and Synperonic 9 using 3 different organic eluants.

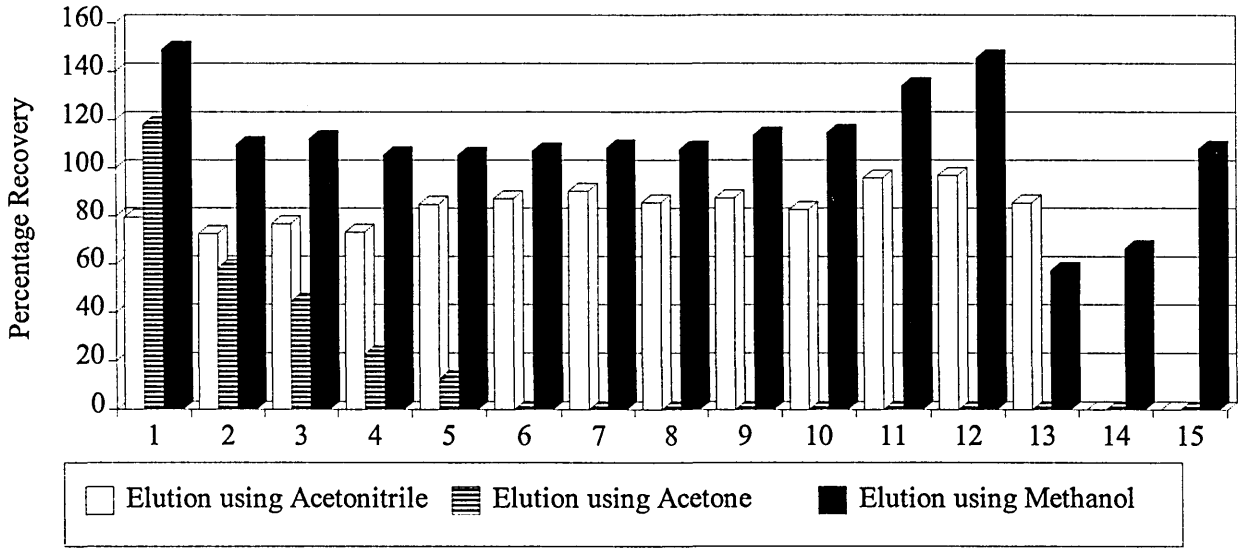


Table 5.12) Recovery Data for a single peak within the Synperonic 9 oligomer distribution.

Sample	Initial sample conc/(ppm).	sample conc/(ppm) after extraction	Recovery /(%)
1	4.72	4.17	88
2	4.80	4.17	87
3	4.62	6.05	131
			Average = **102**

Table 5.13) Repeat experiment of recovery for a single peak within the Synperonic 9 oligomer distribution.

Sample	Initial sample conc/(ppm).	sample conc/(ppm) after extraction	Recovery /(%)
1	4.82	4.56	95
2	6.30	5.76	91
3	6.35	5.23	82
			Average = **89**

Clearly if data for sample (3) in Table 5.12 is disregarded both experiments give very similar recovery values with an average of between 88 and 89%.

b) Anionic surfactant

Tables 5.14 and 5.15 show the corresponding data obtained for SPE extraction of Nansa SS samples in Millipore grade water.

Table 5.14) Recovery data for a single peak within the LAS homologue distribution.

Sample	Initial sample conc/(ppm).	sample conc/(ppm) after extraction	Recovery /(%)
1	2.00	1.53	77
2	2.12	2.07	98
3	2.89	3.13	108
			Average = **94**

Table 5.15) Repeat experiment of the recovery data for a single peak within the LAS homologue distribution.

Sample	Initial sample conc/(ppm).	sample conc/(ppm) after extraction	Recovery /(%)
1	2.01	1.82	91
2	2.16	2.08	96
3	2.52	2.70	107
			Average = **98**

The second homologue within the LAS distribution was chosen to base the recovery data on as it elutes in a fairly reasonable time (retention time = 30 min) and is also in the centre of the elution distribution. Again it can be seen using these conditions it is possible to efficiently extract surfactant samples from aqueous media.

c) mixture of anionic and non-ionic surfactants.

Tables 5.16 to 5.17 show recovery data for a mixture containing LAS and Synperonic 9 (Millipore water spiked at 2.48 mg L^{-1} and 5.6 mg L^{-1}, respectively). Table 5.15 shows data calculated by using the first LAS peak (retention time = 8 min) and Table 5.16 shows data calculated by using peak 3 of Synperonic 9 oligomer distribution (retention time = 28 min). In each case a calibration graph was constructed

for the relevant peak by using standard solutions. Recoveries were then calculated for each sample by using the calibration graph.

Table 5.16) Recovery data for 1 peak of the LAS homologue distribution in a mixture of LAS and Synperonic 9.

Sample	Initial sample conc/(ppm).	sample conc/(ppm) after extraction	Recovery /(%)
1	2.48	2.21	89
2	2.48	2.34	94
3	2.48	2.01	83
			Average = **89**

Table 5.17) Recovery data for 1 peak of the Synperonic 9 distribution in a mixture of LAS and Synperonic 9.

Sample	Initial sample conc/(ppm).	sample conc/(ppm) after extraction	Recovery /(%)
1	5.61	5.81	104
2	5.61	5.47	98
3	5.61	5.33	95
			Average = **99**

5.2.5) Extraction of Surfactants from the reagent water spiked at environmental concentrations using SPE.

Table 5.18 and Figure 5.6 show the recoveries achieved of 4 Millipore grade water (500 ml) samples spiked at 11.2 μg L^{-1} with Synperonic 9. Polypropylene volumetric flasks were used instead of glass volumetric flasks. C18 SPE extraction cartridges were used. Recoveries were calculated from calibration data generated from peak areas of chromatographed standards.

Table 5.19 and Figures 5.7 to 5.8 show the results obtained for the simultaneous extraction of both LAS and NP9. Reagent water samples (500 cm^3) were spiked at 14 $\mu g\ L^{-1}$ Synperonic 9 and 2 $\mu g\ L^{-1}$ Nansa SS. The recoveries of each oligomer of NP9 ranged from 54 - 95 %. The recoveries of each individual homologue of Nansa SS ranged from 69 - 149%.

Table 5.18) Recoveries of individual oligomers from spiked reagent water.

	Recovery (%)								
Ethoxymer No.	3	4	5	6	7	8	9	10	11
Extract 1	81	66	86	106	110	118	114	87	71
Extract 2	94	69	88	107	108	127	115	103	81
Extract 3	60	63	84	103	111	121	121	95	72
Extract 4	76	55	88	107	113	123	116	90	70
Mean	78	63	87	106	111	122	117	94	74
s.d.	14.0	6.0	1.9	1.9	2.1	3.77	3.12	6.7	5.1
r.s.d.	18.0	9.5	2.2	1.8	1.9	3.1	2.67	7.1	6.9

Table 5.19) Recoveries of individual oligomers and homologues from reagent waters spiked with NP9 and LAS.

	Recovery (%)									Recovery (%)			
Ethoxymer No.	3	4	5	6	7	8	9	10	Homologue No.	1	2	3	4
Extract 1	54	46	96	67	66	76	90	116		82	78	69	110
Extract 2	54	67	76	71	91	90	88	89		77	71	86	143
Extract 3	68	92	95	91	90	89	91	92		110	97	99	149
Extract 4	66	80	85	81	85	86	84	82		78	87	84	96
Extract 5	54	74	76	78	81	77	76	73		77	75	63	69
Mean	59	72	86	78	83	84	86	90		85	80	80	113
s.d.	7.2	17.1	9.8	9.9	9.6	8.6	8.2	9.9		10.3	10.3	10.7	16.5
r.s.d.	12.2	23.8	11.4	12.7	11.7	10.2	9.5	8.1		12.1	12.9	13.4	14.6

Figure 5.6) Graph to show the variation in recovery of individual oligomers of Synperonic 9 from spiked reagent water.

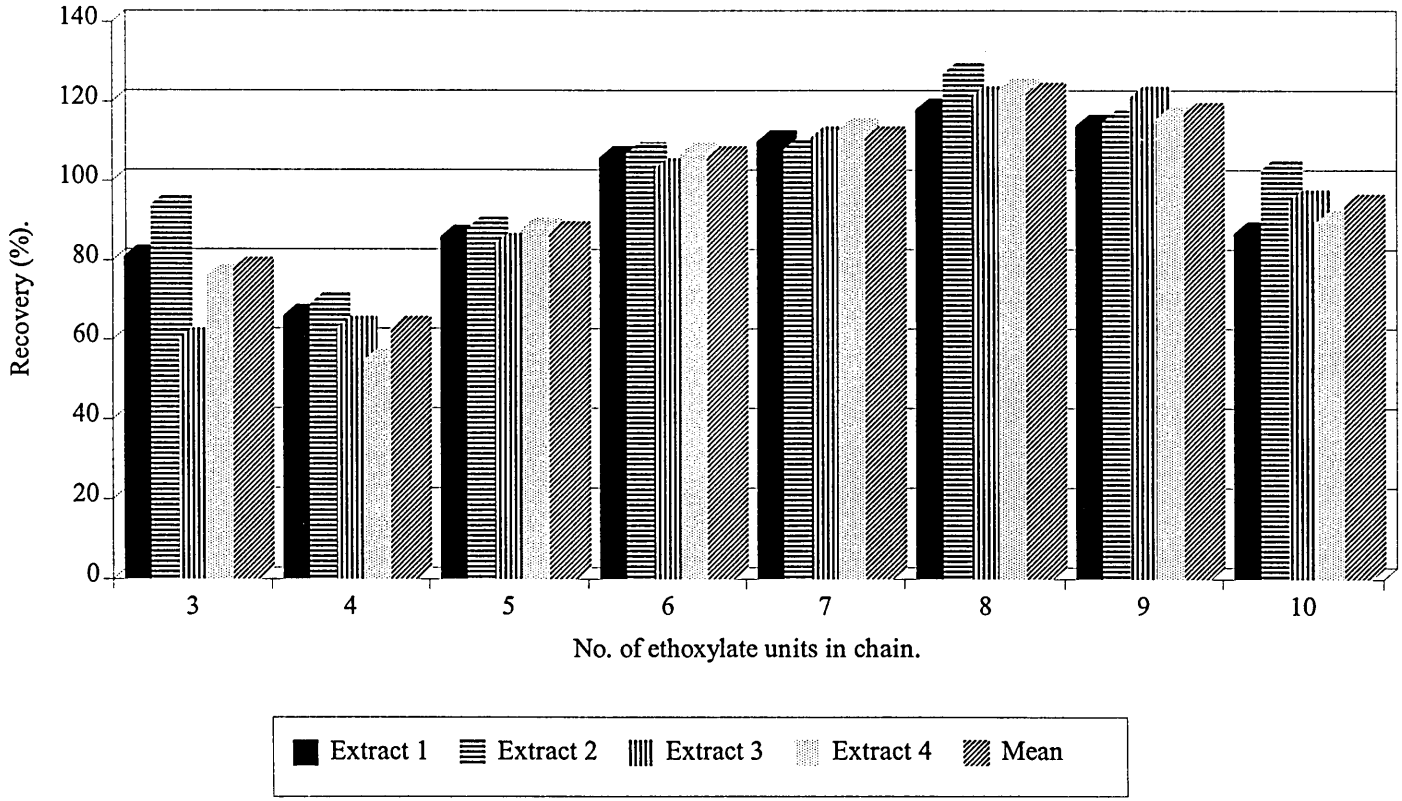


Figure 5.7) Graph to show the variation in Nansa SS homologue recovery from a mixture of Nansa SS and NP9.

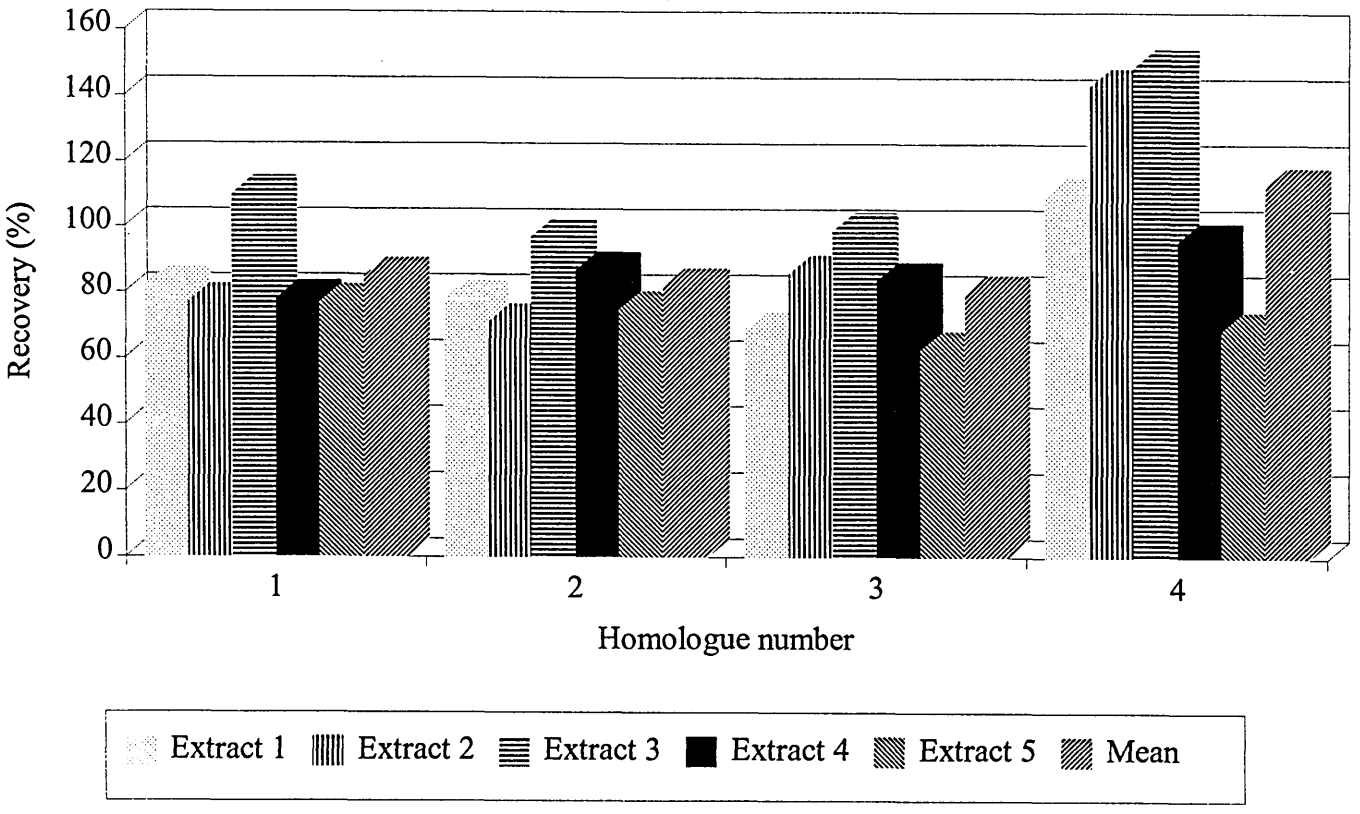
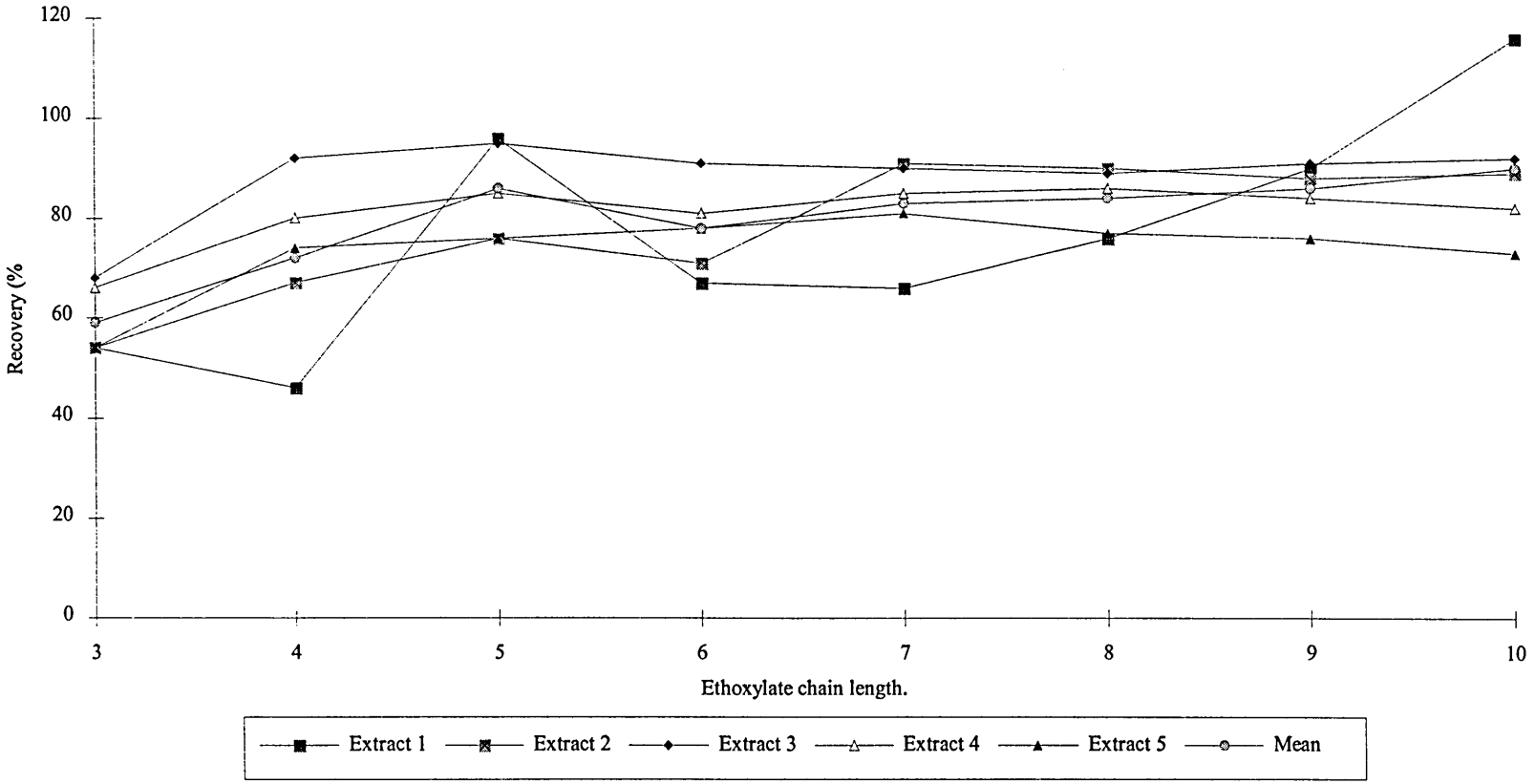


Figure 5.8) Graph to show the variation in Synperonic ethoxymer recovery from a mixture of Synperonic 9 and Nansa SS.



5.3) Conclusions.

In order to quantify surfactant concentrations in aqueous solutions calibration graphs are produced using standards. The peak area of a surfactant homologue/oligomer from an extracted aqueous sample can then be compared with the graph produced from the standards and as a result the concentration of the surfactant formulation can be estimated. In this work HPLC has been used to separate the complex mixtures which are surfactant formulations. This makes it possible to determine individual homologue/oligomer concentrations. Prior to analysis surfactant standards and samples are dissolved in the same 'mobile phase'. The HPLC method mentioned in section 4.6 method B was used in these experiments. Thus standards and samples were dissolved in the mobile phase solvent which was composed of MeOH/ 0.008M ammonium acetate (58%/42%). It was thought that adsorption of surfactant molecules onto the walls of the glassware used in these experiment could potentially reduce standard curve linearity. Therefore a series of experiments were conducted with silanized and unsilanized glassware and plastic volumetric flasks. Experiments which transferred the same solution between identical glass volumetric flasks, 'flask transfer' (Section 5.2.1), showed a loss in signal for each surfactant component in the standard solution used. It was therefore concluded that surfactant molecules were being adsorbed onto the surface of the volumetric flasks. These experiments were repeated with silanized glassware. These results were inconclusive. The adsorption of non-ionic Synperonic 9 (NP9) decreased after silanization whereas that of anionic Nansa SS (LAS) increased after treatment. The linearity of calibration graphs was then studied to give a more practical indication of which type of flask should be used. From these experiments it was concluded that plastic volumetric flasks were the most suitable for use during this project.

Glass is manufactured from silica. However, only a small amount of glass is made from pure silica. It is generally considered to be too expensive for general use due to the input of energy which is required to produce molten silica. The very high temperatures needed to produce silica glass can be lowered via the addition of various oxides to the melt, thus obtaining silicate glass. It, like other forms of glass, consists of

randomly orientated chains, sheets and three-dimensional networks of SiO_4. Na_2CO_3 can be added in small amounts to produce soda glass which is used for cheap laboratory glassware. If B_2O_3 is used, B^{3+} replaces some Si^{4+} in the tetrahedral skeleton. After the replacement of Si^{4+} with B^{3+} there are areas of overall negative charge which are balanced by incorporation of cations such as Na^+ into the structure. Borosilicate glass containing B^{3+} or sometimes Al^{3+} are important as they have a low coefficient of expansion, can withstand heat changes without cracking and are less prone to chemical attack than soda glass. Their structure can be considered to be an amorphorous undercooled liquid of extremely high viscosity. It is probable that the surfactant molecules within the surfactant solutions used, interact with any charge present at the surface of the borosilicate glass vessels. It is however, not clear what the exact mechanism for these interactions are. The plastic volumetric flasks were made of polypropylene polymer. This substance is a tough plastic, which is considered to be chemically inert and is not wet by water. Therefore, it is not surprising that flasks made from polypropylene should not be prone to surfactant adsorption.

As surfactants are present in surface waters at low concentration, it is necessary to pre-concentrate before analysis. Several workers have used a variety of sample preparation techniques (i.e., liquid/liquid or solid phase extraction (SPE)) prior to the analysis of surfactants (Section 2, Tables 2.1 and 2.4). Solid phase extraction (SPE) has become one of the most popular for a number of reasons. This technique is (a) easy to use (b) quick and lastly, (c) easily tailored to be relatively specific for a group of compounds. Specificity can be realised by informed choice of conditioning/elution solvent and of SPE cartridge stationary phase. It should be noted, however, that stationary phase chemistry can change between batches. It is frustrating that manufacturers generally, do not issue detailed information regarding the chemistry of specific stationary phases, experiments with several 'C18' cartridges from different batches and different manufacturers showed varying ability to retain anionic and non-ionic surfactant, eventually, these problems were overcome and solid phase extraction has been used in the pre-concentration and clean up of aqueous surfactant solutions during this project. Initial experiments to determine the best elution solvent showed that

methanol was the most desirable eluant, (Section 5.2.3). Table 5.18 and Figure 5.6 show the recoveries of individual oligomers from reagent water spiked with Synperonic 9 (NP9) (11.2 μg L^{-1}). Recoveries of individual oligomers range from 63 to 122%, (r.s.d. values ranged from 2.2 to 18.0). Table 5.19 and Figure 5.7 show the results obtained for the simultaneous extraction of both LAS and NP9 (Nansa SS and Synperonic 9, respectively). Reagent water samples were spiked with 14.0 μg L^{-1} of nonylphenol ethoxylate and 2 μg L^{-1} of Nansa SS. Mean recoveries for individual oligomers from reagent water spiked with Synperonic 9 ranged from 59 to 90% (r.s.d. values ranged from 8.1 to 23.8) and for Nansa SS mean homologue recoveries ranged from 80 to 113%, (r.s.d values ranged from 12.1 to 14.6). Using these data the limit of detection using fluorescence detection based on 2x signal-to-noise ratio definition for the most intense oligomer/homologue distribution is estimated to be equivalent to 0.05 μg L^{-1} for alkylphenol ethoxylates and 0.005 μg L^{-1} for alkylbenzene sulphonate in the original sample with a concentration factor of 500 generated by the extraction procedure.

It was noticed that throughout the experiments with spiked reagent water samples recoveries greater than 100 % were common. This theoretically could have been caused by injector carry over. However, a blank injection of HPLC mobile phase was carried out at the beginning and end of each experiment which never showed any fluorescent response. A sample blank was also processed during each run and this never showed any fluorescent response. This blank (500 ml of reagent water) extracted in the same way as a spiked reagent water sample (Section 5.1.4b) was blown to dryness using nitrogen gas. The extract was then made to volume using mobile phase. If an incorrect volume of solvent was added to reconstitute the extract (after the extract was blown to dryness), the result would of been an incorrect dissolution concentration for all oligomers/homologues. This could of resulted in recoveries which were greater than 100% for all oligomers/homologues. This was not the case during this work, a response greater than 100 % could be seen for some oligomers/ homologues during analysis of an extracted sample but not for all present. However, correlation coefficients obtained were satisfactory and as sample recovery is based on calibration graphs produced for

each individual oligomer /homologue (i.e. a 'like for like' comparison) this would not present a problem. Although this problem could not be solved this methodology was used to quantify environmental samples, (Section 6 of this thesis).

Chapter 5 References

1) Marcomini A, Capri S and Giger W, J. Chromatogr., 403, 243-252, 1987.

Chapter 6

Determination of Surfactants in surface waters by Liquid Chromatography and Solid Phase Extraction.

6.0) Introduction.

Using the methodology described in section 4.6 method B experiments were carried out to analysis surface waters for APEO type non-ionic surfactants. Due to its close proximity to Sheffield Hallam University, grab samples were taken from the River Rother in South Yorkshire (UK). As high levels of sediment were present in the surface water samples it was necessary to use a strong anion exchange (SAX) SPE in conjunction with reverse phase C18 SPE in order to clean up the extracted sample prior to analysis via HPLC with fluorescence detection. Further extraction experiments were also conducted on grab samples taken from Langstone harbour entrance, Portsmouth (UK). As a result it has been be possible to determine whether the different sample matrix in the marine sample affects the recovered oligomer distribution and therefore, to make comparisons between the APEOs found in both the freshwater and the marine environment.

6.1) Experimental

a) River water samples

Grab samples were taken from the river bank of the River Rother in South Yorkshire (UK), transferred to plastic bottles then stored at 4 °C overnight. The samples were taken at an approximate sampling depth of 0.25 M from the surface of the river. All samples were processed the next day.

The methodology described in section 5.1.3b was used to extract and purify surface water samples. Sample pH was, however, increased to 11 via the addition of sodium hydroxide to ensure ionisation of the anionic species within the sample. A SAX SPE cartridge (500 mg, Shandon, Warrington, Cheshire, UK), was placed in series with the C18 cartridge to remove interfering anionic species. The C18 cartridge was conditioned prior to use (Section 5.1.3b). Stock standard Synperonic 9 (a gift from ICI Materials Research Centre, Wilton, Middlesborough, Cleveland, UK) was, made up in HPLC mobile phase (Section 4.6 method B) and diluted to produce standards of similar concentration to the extracted analyte. A calibration graph was constructed from the standards and used to quantify APEO concentration.

b) marine water samples.

Using the methodology described in section 4.6 method B experiments were carried out in order the analyse marine waters for APEO type non-ionic surfactants. Grab samples were taken from Langstone harbour entrance, Portsmouth (UK).

After sampling, samples were transferred to plastic bottles then frozen prior to analysis. The samples were allowed to fully thaw then were stirred to ensure uniformity.

The methodology described in section 5.1.3b was used to extract and purify surface water samples, although a sample size of 100 ml was used. Thus after extraction and elution to 1 ml, a 100 fold pre-concentration was achieved. A stock solution of NP9 was made up by dissolving an appropriate amount of Synperonic 9 in HPLC mobile phase (Section 4.6 method B). A calibration graph was constructed using stock solution diluted to produce standards of similar concentration to the extracted analyte and used to quantify APEO concentration. In this case it was not deemed necessary to use the SAX cartridge clean-up procedure.

6.2) Results.

a) Surface waters

In chromatograms obtained after the extraction of the high sediment containing surface water samples using a C18 SPE cartridge only (Figure 6.0a) several large interfering peaks eluted early in the chromatogram. It was assumed that these might be acidic compounds originating from sediment material. Hence it was decided to remove all anionic compounds from the sample extract by employing a SAX solid-phase extraction cartridge in series with the C18 cartridge. This resulted in the cleaner chromatogram shown in Figure 6.0c, where the characteristic distribution pattern for alkylphenol ethoxylate oligomers can be clearly seen and compared with the trace produced by a standard sample (Figure 6.0b).

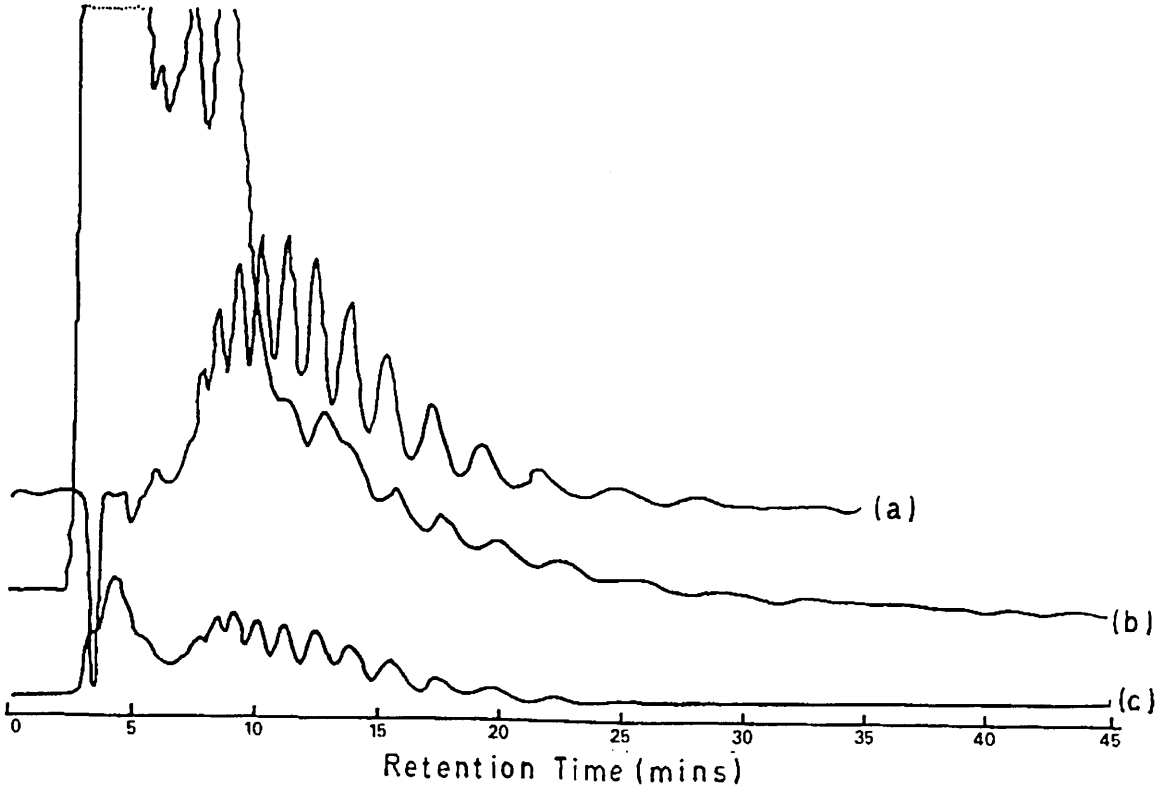


Figure 6.0) HPLC chromatograms for the analysis of extracts from the River Rother (South Yorkshire, UK). Trace (a), extraction using C_{18} SPE cartridge only. Trace (b), standard NP9. Trace (c), extraction using SAX and C18 SPE cartridges together.

Table 6.0 and Figure 6.1 show the APEO oligomer concentration for the extracted waste water sample.

Table 6.0 Concentration of APEO oligomer concentration for the extracted waste water sample.

APEO Peak Number	3	4	5	6	7	8	9	10	Mean	Total
Conc.[a]/ mg L^{-1}	2.3	4.1	4.0	4.1	3.7	3.1	3.1	1.7	3.3	26.1
% recovery of APEO from Spiked water[b]	78	63	87	106	111	122	117	94		
Conc./mg L^{-1} of APEO allowing for SPE error[c]	2.7	5.6	4.5	3.8	3.4	2.4	2.6	1.8	3.4	27.2

[a]Limit of detection was taken to be x3 signal to noise. No oligomers greater than $AP_{10}EO$ were detected in this case. The concentration quoted refers to oligomer concentration after pre-concentration.

[b]Figures quoted are for mean recovery data of 4 separate 500 ml Millipore grade water samples spiked with Synperonic 9 at 14 μg L^{-1}, (Section 5.7 table 5.6)

[c]Obtained by using the following equation A = [(100-B/100)xC] + C.

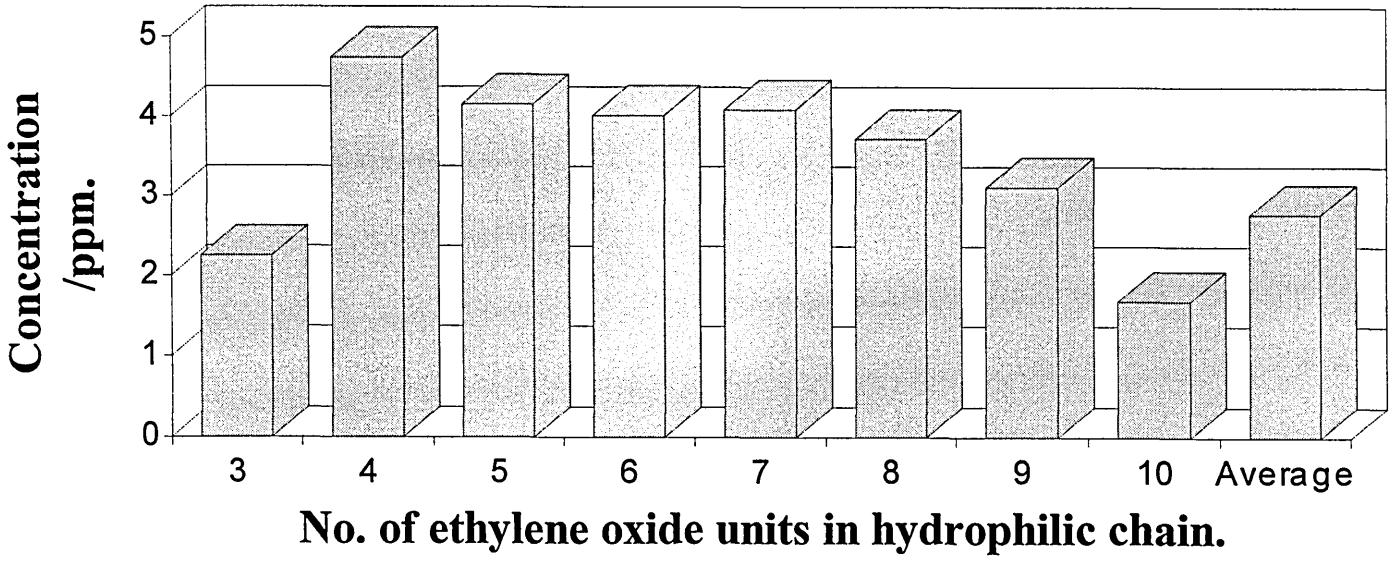
Where A = concentration of surfactant oligomer in mg L^{-1} allowing for SPE error.

B = % APEO recovery from spiked water.

C = concentration of APEO in mg L^{-1}.

Table 6.0 shows the average oligomer concentration = 3.3 mg L^{-1}. Thus allowing for a 500 fold pre-concentration step the actual average oligomer concentration was calculated at 6.6 μg L^{-1} and the total oligomer concentration was 26.1 mg L^{-1}.Thus allowing for a 500 fold pre-concentration step the actual total oligomer concentration was calculated at 52.2 μg L^{-1}. If the individual loss of oligomer distribution is taken into account by multiplying by its average recovery from APEO spiked water the total APEO recovery was 27.2 mg L^{-1} and the overall mean was 3.4 mg L^{-1}. Allowing for a 500 fold pre-concentration in the purification step the actual total oligomer concentration was calculated at 54.5 μg L^{-1}.

Figure 6.1) Graph to show the variation in ethylene oxide chain length concentration in an APEO sample recovered from the River Rother, South Yorkshire, UK.



Allowing for a 500 fold pre-concentration step the actual average oligomer concentration was calculated at 6.8 $\mu g\ L^{-1}$.This is higher than the mean value calculated via a comparison of sample peaks with the standard calibration graph only.

b) Marine water

Figure 6.2 shows a typical chromatogram obtained for a stock Synperonic 9 sample. Figure 6.3 shows a typical chromatogram obtained for a sample blank (500 ml of reagent grade water) and Figure 6.4 shows a chromatogram of an extracted marine sample. Clearly, it can be seen that the sediment levels in this sample are not as great as those found in the surface water sample. Figure 6.4 does, however, show that there is some degree of extracted material which elutes at an earlier stage of the chromatographic run, before APEO. It was not apparent whether or not the peaks observed at the early stage of the chromatographic run (< 8 min.) were due to LAS. The blank (Figure 6.3) contains peaks which co-elute and therefore interfere with eluting LAS homologues using this chromatographic procedure. Therefore unambiguous LAS identification due to peak retention time and fluorescence detection cannot be assumed.

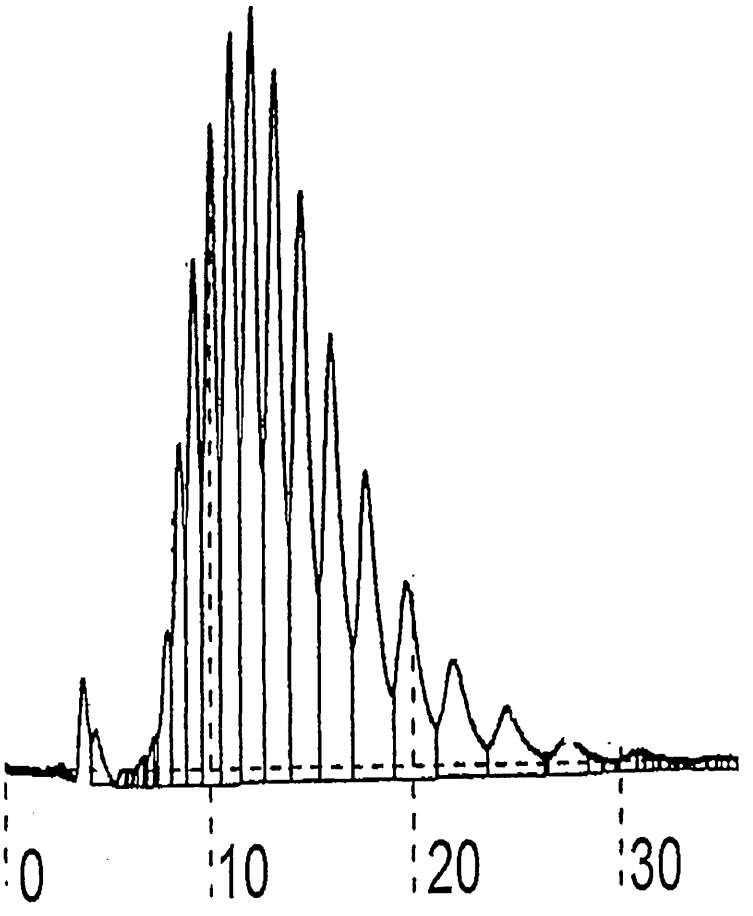


Figure 6.2) Typical HPLC chromatogram of an NP9 standard used to construct a calibration graph to determine APEO concentration in waters from Langstone harbour entrance (Portsmouth).

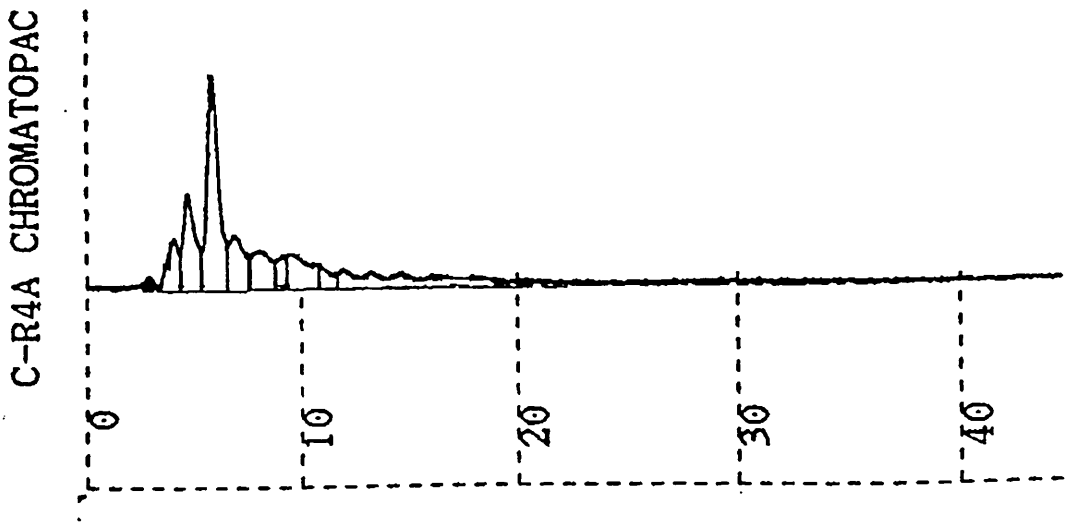


Figure 6.3) Chromatogram of a blank extract, obtained using the same methodology as the extracted sample.

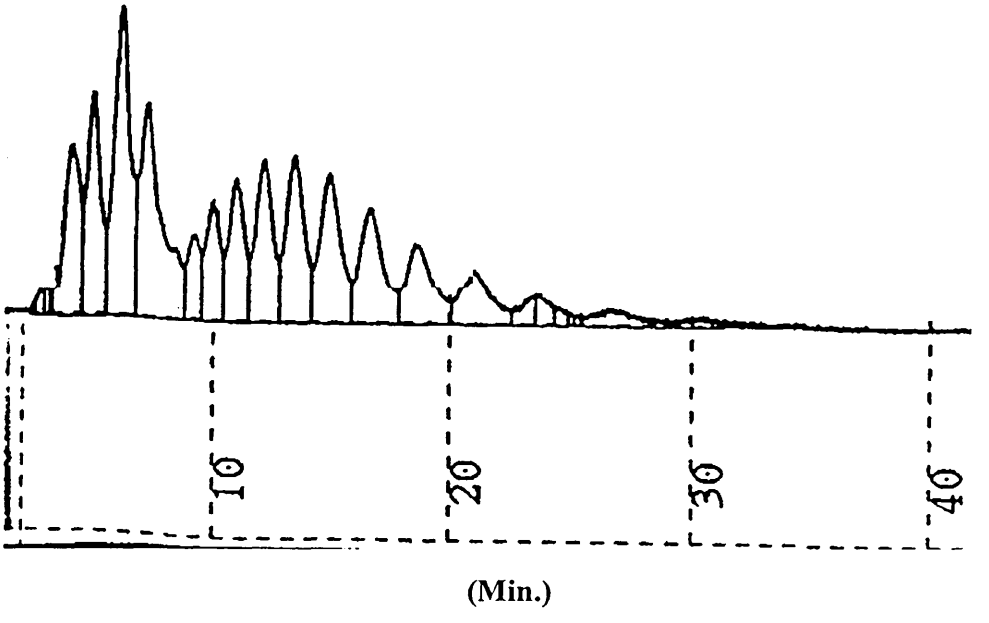


Figure 6.4) Typical chromatogram of a sample extracted from the entrance of Langstone Harbour, Portsmouth, UK. For chromatographic conditions see text.

Table 6.1 and Figure 6.5 show the oligomer distribution obtained from the extracted marine water sample.

Table 6.1) Concentration APEO oligomers extracted from marine sample.

APEO Peak Number	3	4	5	6	7	8	9	10	11	Mean[b]	Total
Conc.[a]/ mg l^{-1}	2.6	3.1	2.9	3.1	3.0	2.8	2.7	1.8	0.36	2.5	22.5
% APEO of recovery from Spiked water[c]	78	63	87	106	111	122	117	94	74		
Conc./mg l^{-1} of APEO allowing for SPE error[d]	3.2	4.3	3.3	2.9	2.7	2.2	2.2	1.9	0.45	2.6	23.4

[a]Limit of detection was taken to be x3 signal to noise. No oligomers greater than $AP_{11}EO$ were detected in this case. The concentration quoted refers to oligomer concentration after pre-concentration obtained by a comparison of sample peaks with a standard calibration graph.

[b] Mean quoted is of all 11 oligomers.

[c]Figures quoted are for mean recovery data of 4 separate 500 ml Millipore grade water samples spiked with Synperonic 9 at 14 μg l^{-1}, (Section 5.7 table 5.6)

[d]Obtained by using the following equation A = [(100-B/100)xC] + C.

Where A = concentration of surfactant oligomer in mg L^{-1} allowing for SPE error.

B = % APEO recovery from spiked water.

C = concentration of APEO in mg L^{-1}.

Table 6.1 shows the average oligomer concentration = 2.5 mg L^{-1}. Thus allowing for a 100 fold pre-concentration step the actual average oligomer concentration was calculated at 25 μg L^{-1}. It has been noted that the concentration of oligomer number 11 is very low. If this value is omitted the mean of the remaining oligomers = 2.8 mg L^{-1}. The total oligomer concentration = 22.5 mg L^{-1}. Thus allowing for a 100 fold pre-concentration step the actual total oligomer concentration was calculated at 225 μg L^{-1}.

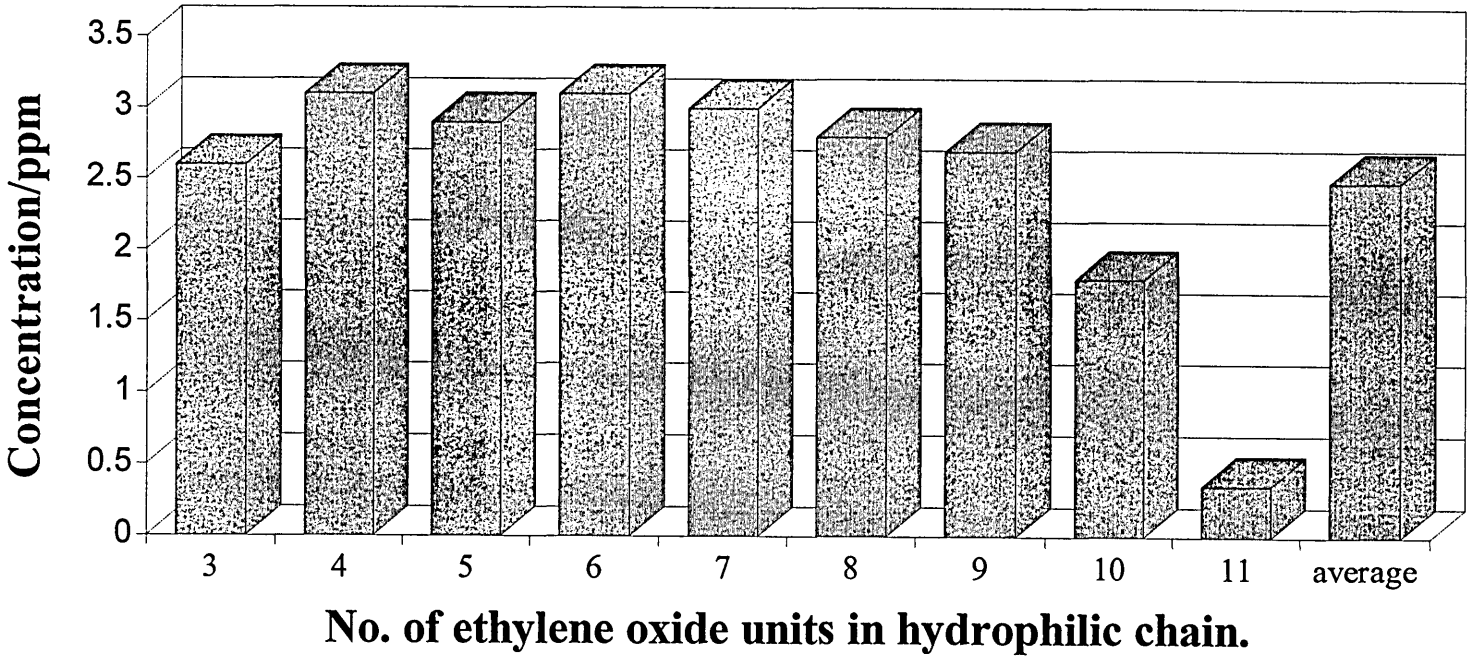
It can be seen that if the individual loss of oligomer distribution is taken into account by multiplying by its average recovery from APEO spiked water the overall mean (2.6 mg L^{-1}) is in good accordance with the mean value calculated via a comparison of sample peaks with a standard calibration graph. The total oligomer

concentration = 23.4 mg L^{-1}. Thus allowing for a 100 fold pre-concentration step the actual total oligomer concentration was calculated at 234 μg L^{-1}.

6.3) Conclusions

It can be seen that oligomer distributions of the river water extract and the marine extract are similar. The marine sample obtained from the entrance to Langstone Harbour, Portsmouth, contained a total APEO concentration of 225 μg L^{-1}. The surface water sample collected from the River Rother contained a total APEO concentration of 52.2 μg L^{-1}. The concentration of APEO in the river water sample is typical of values quoted in the literature, (Section 2, Table 2.4). Nonylphenol (NP) a degradation product of NPEO has been recorded at concentrations ranging from <0.2 to 12 μg L^{-1} in UK rivers, to < 0.08 μg L^{-1} in UK estuaries[1]. There is at present very little data available in the literature concerning the concentration of APEO in the UK marine environment. However, a concentration of 25 μg L^{-1} does also fall into the recorded aquatic APEO concentration range, (Section 2, Table 2.4). Marcomini et al.[2] have reported NPEO oligomers of up to 13 ethoxy units at an overall concentration of range of 0.6-4.5 μg L^{-1} in marine waters from the Venice lagoon. Soxhlet extraction was used by Marcomini et al.[2] to determine the concentration of the polar degradation products of APEO, i.e., NP, NP_1EO and NP_2EO in lagoon waters. A portable re-suspending device allowed the analysis of the first 0.01 to 0.15 mm of the sediment layer, where the sum of NP, NP_1EO and NP_2EO was in the range 0.15 to 13.7 μg L^{-1}. Therefore, it is possible that the high level of sedimentation may have reduced the apparent concentration of APEO in the River Rother sample due to the association with the particulate matter.

Figure 6.5) Graph to show the variation in ethylene oxide chain length concentration of an APEO sample recovered from Langstone harbour entrance.



The concentration of APEO found in Langstone Harbour (total APEO concentration of 225 μg L^{-1}) is above the nonylphenol threshold for sub-lethal effects[3] for NP which is reported to be 56 μg L^{-1}. Unfortunately no data was presented on APEO toxicity in this paper. Therefore it is not possible to assess whether or not APEO represents a toxicological threat to mussels at this concentration.

It was not possible to report the levels of LAS during these experiments. The method described in section 6.1a obviously makes it impossible to detect any LAS as it will be retained by the SAX cartridge and not observed, while the marine sample contained interfering peaks which could co-elute with LAS. Further work is required to investigate the possibility of selectively fractionating the anionic components trapped onto the SAX cartridge such that simultaneous analysis of LAS and APEOs would be possible in this situation. Work carried out using graphitized carbon black (GCB) SPE cartridges by Di Corcia et al.[4,5] and Crescenzi et al.[6] is applicable in this situation. Using GCB it is possible to fractionate a mixture of APEO and LAS type surfactants and their degradation products (short chain alkylphenol ethoxylates (containing 1 to 2 ethoxy units), alkylphenol carboxylates and the sulphophenyl carboxylates, respectively) by exploiting active sites within the GCB cartridge sorbent bed. GCB has on its surface positively charged oxygen complexes which are able to bind anions. These active centres allow GCB to act as both an anion exchanger and a non-specific adsorbent. Therefore, a number of experiments could be carried out to characterise both LAS and APEO in samples containing a high level of interfering species. If GCB cartridges were used with an isocratic reverse HPLC method (Section 4.6 method B), (i.e., retain either LAS on the cartridge), it would be possible to chromatograph the APEO fraction as usual then (a) characterise LAS by eluting from the cartridge (leaving the interfering species retained), or (b) elute LAS and interfering species together and use a modified mobile phase or column to separate. It would also be possible to fractionate within a classification of compound, i.e. the anionic compounds found in surface waters, by using varying strengths of acidic eluants. It may then be possible to fractionate LAS from the other anionic species found in surface waters. If these species could be removed while retaining LAS and APEOs it would then be possible to elute

both surfactant samples together, which obviously would be more practical and efficient. Obviously, many more samples need to be processed in order to accurately assess APEO concentrations in river and marine samples. It would be prudent to measure APEO concentration in both surface water and sediment in order to gather a fuller picture of the environmental profile of APEO in the waterways.

In the future, in order to obtain a full picture of the environment effects of the two most industrially used surfactant molecules (LAS and APEO) it would seem necessary to monitor both the intact surfactant molecules and their degradation products. It is known (Section 2.4) that the more environmentally persistent products of APEO degradation are (a) the short chain ethoxylates, AP_1EO and AP_2EO, (b) alkylphenol carboxylates (AP_1EC and AP_2EC) and (c) alkylphenol. The degradation products of APEO are more toxic than the intact parent molecule[6]. LAS degrades to sulphophenyl carboxylates in the environment, (section 2.3.1) although this material is considerably less toxic than its parent compound (section 2.2).

Alkylphenol carboxylates and sulphophenyl carboxylates standards are not available commercially. Therefore, it would be necessary to synthesis them. Alkylphenol carboxylates can be prepared via the oxidation of short chain alkylphenol ethoxylates with Jones reagent. The oxidation products can then be extracted using diethyl ether and purified using silica gel chromatography. Sulphophenyl carboxylates can be prepared via the sulphonation of phenylcarboxylic acid, the product of which can be purified using liquid extraction. Both methods have been described by Marcomini et al.[7]. It is likely that the chromatographic method developed (Section 4.6 method B) to analyse surfactant mixtures simultaneously would need to be modified in order to do this.

Although high performance liquid chromatography coupled with fluorescence chromatography is a powerful analytical technique it is not infallible. Indeed an example of this was seen during the analysis of marine samples during this work. Co-elution of compounds with a similar fluorescent profile as the analyte of interest is possible during the analysis of environmental samples. Therefore experiments were carried out during this project to couple the HPLC methods developed with mass spectrometric detection, (Chapter 7).

Chapter 6 References.

1) Blackburn MA, Waldock MJ, Wat. Res., 29, 1623, 1995.

2) Marcomini A, Pavoni B, Sfriso A and Orio AA, Marine Chemistry, 29, 307, 1990.

3) Granmo A, Ekelund R, Magnusson K and Berggren M, Environmental Pollution, 59, 115,1989.

4) Di Corcia A, Samperi R, Marcomini A, Environmental Science Technology, 28, 850, 1994.

5) Di Corca A, Marchese S, Samperi R, J. Chromatogr., 642, 163, 1993.

6) Crescenzi C, Di Corcia A, Samperi R, Marcomini A, Analytical Chemistry, 67, 1797, 1995.

7) Marcomini A, Di Corcia A, Samperi R, Capri S, J. Chromatogr., 644, 59, 1993.

Chapter 7

A comparison of LC/MS methods for the analysis of Surfactant formulations.

7.0) Liquid Chromatography-Mass Spectrometry (LC/MS).

Experimental work employing three different LC/MS interfaces for the determination of surfactants is described in this section. The interfaces used were (a) Particle Beam (PB) (b) Thermospray (TSP) and (c) Atmospheric Pressure Chemical Ionisation (APcI). A description of these interfaces is given in section 3. Initially it was hoped that particle beam interfacing would allow LC/MS identification of surfactants in surface waters. Satisfactory peak resolution was obtained for the HPLC method employing a cyano column which uses non-polar organic solvents. Using the C1 column method, (Section 4.6 method B), would have allowed the simultaneous determination of both LAS and APEO. However it was not possible to resolve surfactant peaks using this method in conjunction with the PB interface. Compromises in peak resolution were required in order to allow the simultaneous determination of the different surfactant classes in a reasonable time scale. This meant that the resolution of the C1 column method even when using conventional detection was not as good as that obtained for the cyano column method, (Section 4.3). However, even taking this into consideration the C1 column method lost more in terms of resolution than did the cyano method when transferring the method to MS detection. The increase polarity of the HPLC mobile phase no doubt was largely responsible for this. A brief investigation of thermospray interfacing showed it was possible to produce good mass chromatographic data. However, due to instrumental failure it was not possible within the time constraints imposed to ascertain how well individual surfactant peaks could be resolved. The most successful analyses were obtained when the C1 column HPLC method described in section 4.6 method B of this thesis was used in conjunction with the Atmospheric Pressure Chemical Ionisation (APcI) interface.

7.1) Experimental

7.1.1) The use of Liquid Chromatography-Particle Beam-Mass Spectrometry (LC/PB/MS) for the characterisation of Surfactant samples.

Experiments were carried out using a Trio-1 mass spectrometer (Micromass, Altrincham, Cheshire, UK, (formerly VG Organic)) and a Linc particle beam interface (Micromass, Altrincham, Cheshire, UK, (formerly VG Organic)). EI^+ ionisation was used for all experiments. The instrumental conditions used are shown below :

Helium pressure = 30 psi.

Particle beam desolvation temperature = 50°C

Source Temperature = 250°C

Surfactant samples were separated into individual oligomers using the cyano column HPLC method, (Section 4.3). A flow rate of 0.6 ml min^{-1} was employed. Eluant from the HPLC column was allowed to enter the particle beam interface directly.

7.1.2) Experiments to investigate the effects of post column addition on the sensitivity of LC/PB/MS for Surfactant detection .

Due to the poor sensitivity obtained when using the particle beam interface for mass spectrometric detection of surfactants, experiments were carried out in order to try and improve the apparent limit of detection (LOD), (0.5 mg on column) which obviously would require very large volumes of surface water to be extracted to enable this technique to be utilised as an environmental monitoring technique. Currently something in the region of 10 to 100 L would have to be pre-concentrated in order to facilitate LC/PB/MS quantification. Undoubtedly, this would be an interference prone and time consuming procedure.

Experiments were carried out to try and improve the limit of detection for this method. In order to increase speed of analysis the HPLC column usually used in conjunction with these solvents was replaced with a 25 cm length of stainless steel tubing identical to that used in the conventional HPLC set up. As a result no oligomer separation

was seen and the NP9 standard was detected as an unresolved peak. Hexane, one of the solvents used in this method was used as an 'eluant' to carry the NP9 sample into the desolvation chamber of the mass spectrometer. A standard loop injection of NP9 (0.5 %) in hexane was carried out. Differing solvents and / or buffers were added 'post column' in an attempt to improve the LOD.

Particle beam interface conditions were as follows :

Helium Pressure = 30 psi.

Particle beam desolvation chamber temperature = 50°C.

Particle beam desolvation chamber temperature = 50°C.

Figure 7.0 shows a schematic diagram of the instrumentation used in the experiment.

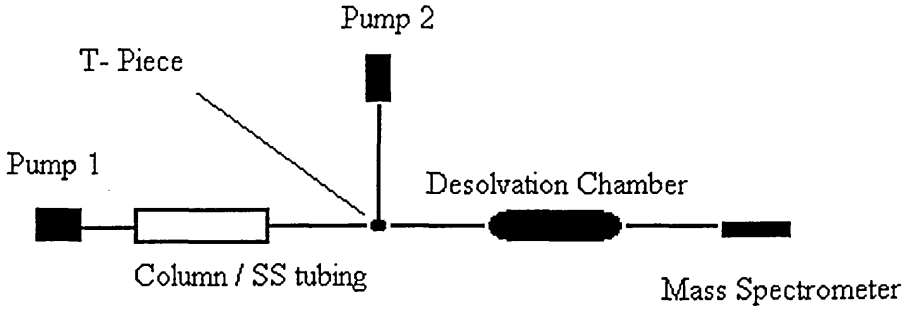


Figure 7.0) Schematic diagram of the instrumentation used in post column addition of various solvents and buffers (see text for details).

Pump 1 was used to pump the mobile phase eluant in to the mixing tee. Pump 2 was used to pump the various solvents and solvent / buffer solutions into the tee piece which allowed mixing of the two solutions. NP9 was monitored in selected ion monitoring (SIM) mode using EI^+ ionisation. The ions monitored were m/z = 311, 205, 161 and

135. A total flow rate of 0.8 ml min^{-1} into the particle beam interface was maintained by altering pump flow rates of the two pumps. This provided a convenient way of altering the ratio of eluant to post column addition solvent, enabling the effects of a change of ratio to be examined. A flow rate of 0.8 ml min^{-1} was chosen as it is within the limits of the interface and also allowed the addition of solvents from two separate pumps at reasonable flow rates. Obviously if an analytical column was used changes in flow rate would affect oligomer separation, this is another reason why it was necessary to kept the solvent flows constant.

7.1.3) The use of Liquid Chromatography/Thermospray/Mass Spectrometry (LC/TSP/MS) for the characterisation of Surfactants.

It was hoped that LC/TSP/MS would lead to a more sensitive LC/MS method than had been seen using LC/PB/MS. Therefore experiments were carried out to determine the suitability of the thermospray interface for LC/MS detection of surfactants. All experiments were carried out on a Micromass, (formerly VG Organic), Trio-3 triple quadrupole mass spectrometer (Altrincham, Cheshire, UK). Both positive and negative ionisation modes were utilised. Throughout the experiments the instrument was used in single quadrupole mode. The instrumental conditions used are shown below:

Capillary Temp = 70°C

Source Temp = 250 °C

Electrode voltage = 70 V

As this interface is compatible with polar solvents it was hoped that surfactants mixtures (both anionic and non-ionic) could be separated using the C_1 column method (Section 4.6 method B) before introduction into the mass spectrometer. The mobile phase used in this method was Methanol / 0.08 M ammonium acetate 58/42. Mobile phase was pumped at a rate of 0.5 ml min^{-1}. A 0.15 M aqueous ammonium acetate

solution was added post column (Section 7.1a) at a rate of 0.5 ml min^{-1} which made the total concentration of ammonium acetate entering the interface roughly equal 0.1 M.

7.1.4) The use of Liquid Chromatography-Atmospheric Pressure Chemical Ionisation (APcI)- Mass Spectrometry for the characterisation of Surfactants.

All analyses were performed on a Micromass (Altrincham, Cheshire, UK, (formerly VG Organic)) Quattro triple quadrupole mass spectrometer, equipped with a Micromass Atmospheric Pressure Chemical Ionisation (APcI) liquid chromatography mass spectrometry interface. A Hewlett Packard 1050 (Palo Alto, Ca., USA) HPLC system was used in this case (HPLC conditions, section 4.6). Data was acquired in full scan mode range 50-1000 at 3 sec. / scan. Alternative positive ion / negative ion switching was used to acquire spectra both in positive and negative ion modes.

The API discharge Voltage	:	3.5 KV
High Voltage Lens	:	500V
Source Temperature	:	120 ^{o}C
API Probe Temperature	:	500 ^{o}C

All solvents used in the experiment are described in Section 7.1 were HPLC grade (Sigma-Aldrich, Poole, UK). All HPLC mobile buffers used in the experiment described in Section 7.1 were HPLC grade (Sigma-Aldrich, Poole, UK)

7.2.1) Liquid Chromatography-Particle Beam-Mass Spectrometry (LC/PB/MS).

A typical EI^+ total ion chromatogram for a 50 µl injection (100 µg on column) of a 2000 ppm NP9 standard solution is shown below, (Figure 7.1). (HPLC conditions, Section 4. 3). Flow rate was set at 0.6 ml min^{-1}.

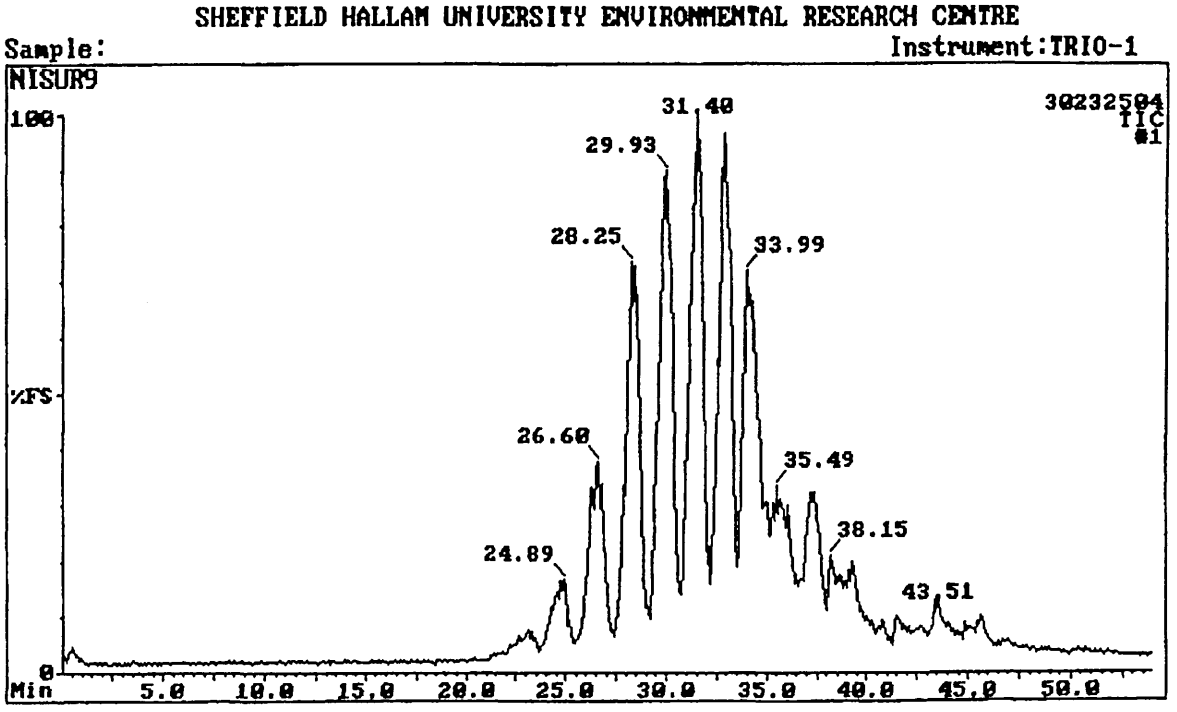


Figure 7.1) EI^+ TIC of a 2000 ppm standard solution of NP9 obtained via LC/PB/MS.

It can be seen that the resolution of each individual ethoxymer is not as good as that obtained using UV detection. Presumably this band broadening was caused by memory affects associated with sample transportation across the desolvation chamber of the interface. Mass chromatograms of individual ethoxymer peaks (NP_7EO, NP_8EO, NP_9EO, $NP_{10}EO$) show characteristic fragmentation patterns, (Figure 7.2, Figure 7.3, Figure 7.4 and Figure 7.5, respectively). The EI^+ mass spectra obtained showed both a molecular ion and structurally significant fragmentation allowing the peak at 28.2 min in Figure 7.1 to be assigned to NP_7EO (molecular ion $(M)^{\cdot+}$ (m/z 528)), i.e., the molecular mass of a NPEO with an ethylene oxide chain length of 7 units, i.e.

NP_7EO. The peaks at 29.3 min, 31.4 min and 33.9 min produced mass chromatograms (Figure 7.3, Figure 7.4 and Figure 7.5, respectively) which have molecular ions (m/z 572, 616 and 660), corresponding to NP_8EO, NP_9EO and $NP_{10}EO$, respectively.

The analysis for alkylbenzene surfactants by LC/PB/MS using EI^+ ionisation leads to production of several structurally characteristic fragmentation ions[1]. The mass spectra corresponding to NP_7EO to $NP_{10}EO$ all show an homologous ion series characteristic of a alkylbenzene, (Figures 7.2 to 7.5), i.e., $C_6H_5(CH_2)^+_n$: n =1 (m/z 91), n = 2 (m/z 105), n =3 (m/z 119), n = 4 (m/z 133), n = 5 (m/z 147), n = 6 (m/z 161), n = 7 (m/z 175) and n = 8 (m/z 189).

Compounds containing an alkylphenol group will also produce a $(M\text{-}C_nH_{2n+1})^+$ ion series. This can be seen in the mass spectra of NP_7EO to NP_9EO (Figures 7.2, 7.3, 7.4 and 7.5). Tentatively, fragmentation may occur via the loss an ethyl group ($-C_2H_5$) from the alkyl chain attached to the substituted benzene ring. For example, Figure 7.2 displays the mass spectrum of NP_7EO (RMM 528), the loss of 29 Da to m/z 499 can be assigned to the loss of an ethyl group ($-C_2H_5$). Clearly, Figure 7.2 shows the presence of a series of ions separated by 14 Da, i.e., m/z 485, m/z 471, m/z 457 and m/z 443 These ions arise via the fragmentation of the alkyl chain and correspond to an ion series, $(M\text{-}C_nH_{2n+1})^+$.

A similar series of fragmentation ions is observed in the mass spectra of NP_8EO, (Figure 7.3), i.e., m/z 543, m/z 529, m/z 515, m/z 501, m/z 487; of NP_9EO, (Figure 7.4), i.e., m/z 587, m/z 573, m/z 559, m/z 545, m/z 531, and of $NP_{10}EO$, (Figure 7.5), i.e., m/z 631, m/z 617, m/z 603, m/z 589, m/z 575. All of these fragmentation ions corresponding to an ion series, $(M\text{-}C_nH_{2n+1})^+$.

The peak at m/z 89 in Figures 7.2-7.5 is likely to be part of the series :

$$-[CH_2CH_2O]_n\text{-}H^+$$

Here the ethoxylate chain of the of NP_9EO oligomers is cleaved. Cleavage at the point furthest from the benzene ring of the molecule will produce a fragment with m/z 45 Da, i.e., $[CH_2CH_2O]_n\text{-}H^+$. Fragmentation ions in the series will then show a decrease

of 44 Da, as the point of fragmentation moves closer to the benzene phenyl group of the molecule[2] :

i.e., $[CH_2CH_2O]_n\text{-}H^+$ n = 2 (m/z 89), n = 3 (m/z 133), n = 4 (m/z 177).

This series can be seen in all the mass spectra produced, Figures 7.2-7.5.

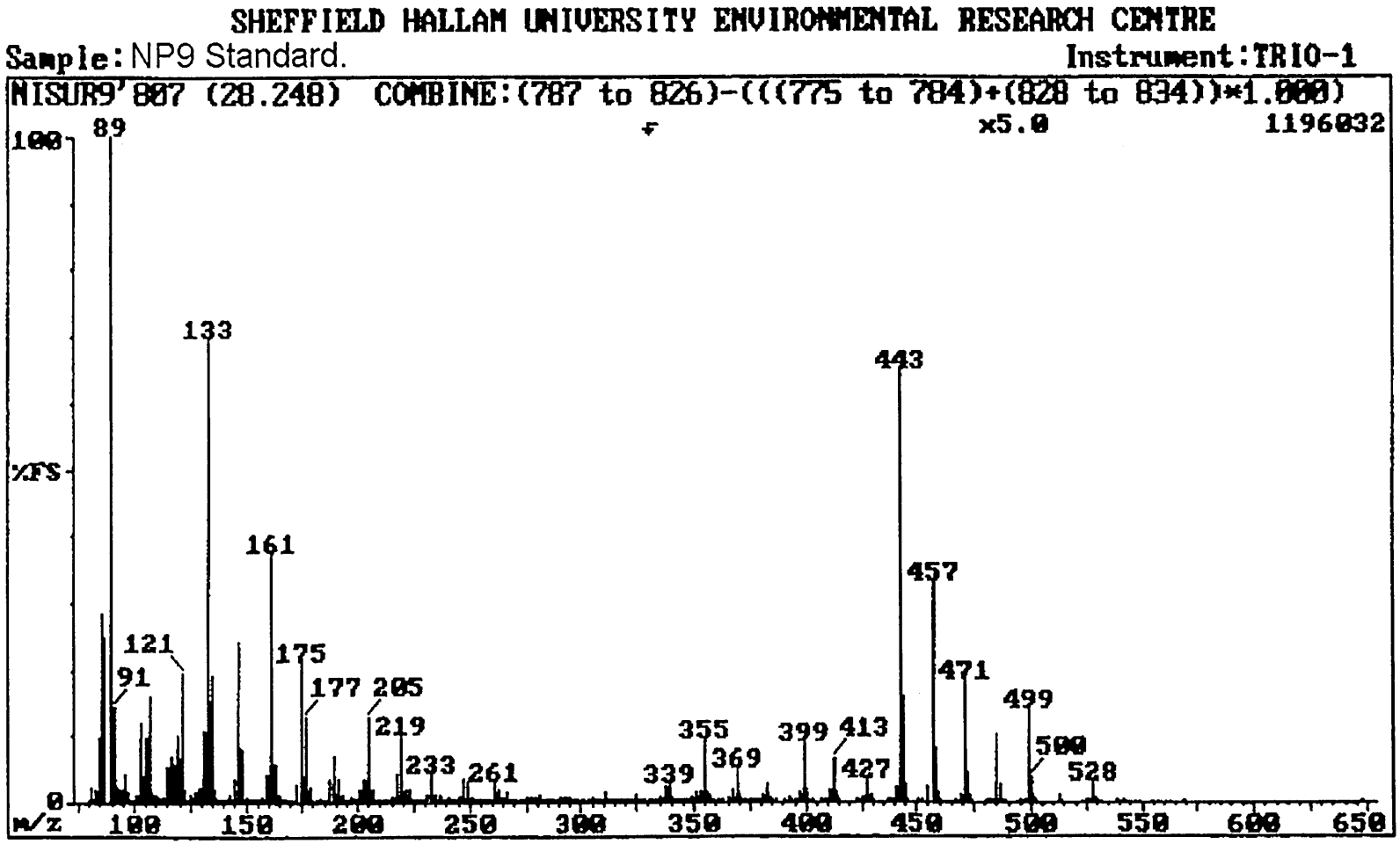


Figure 7.2) Mass Spectrum of peak at 28.2 min (NP_7EO ethoxymer peak).

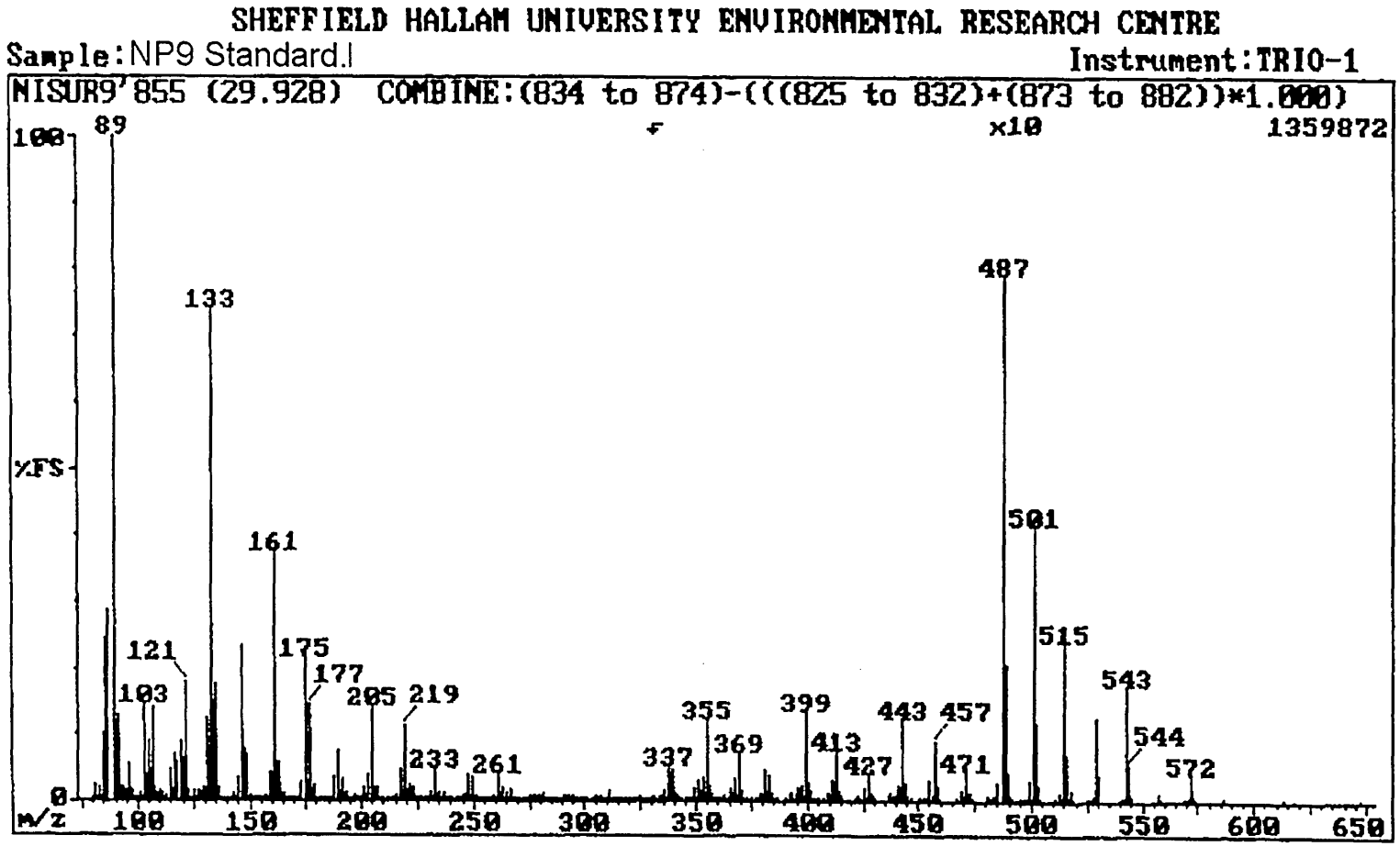


Figure 7.3) Mass Spectrum of peak at 29.3 min (NP_8EO ethoxymer peak).

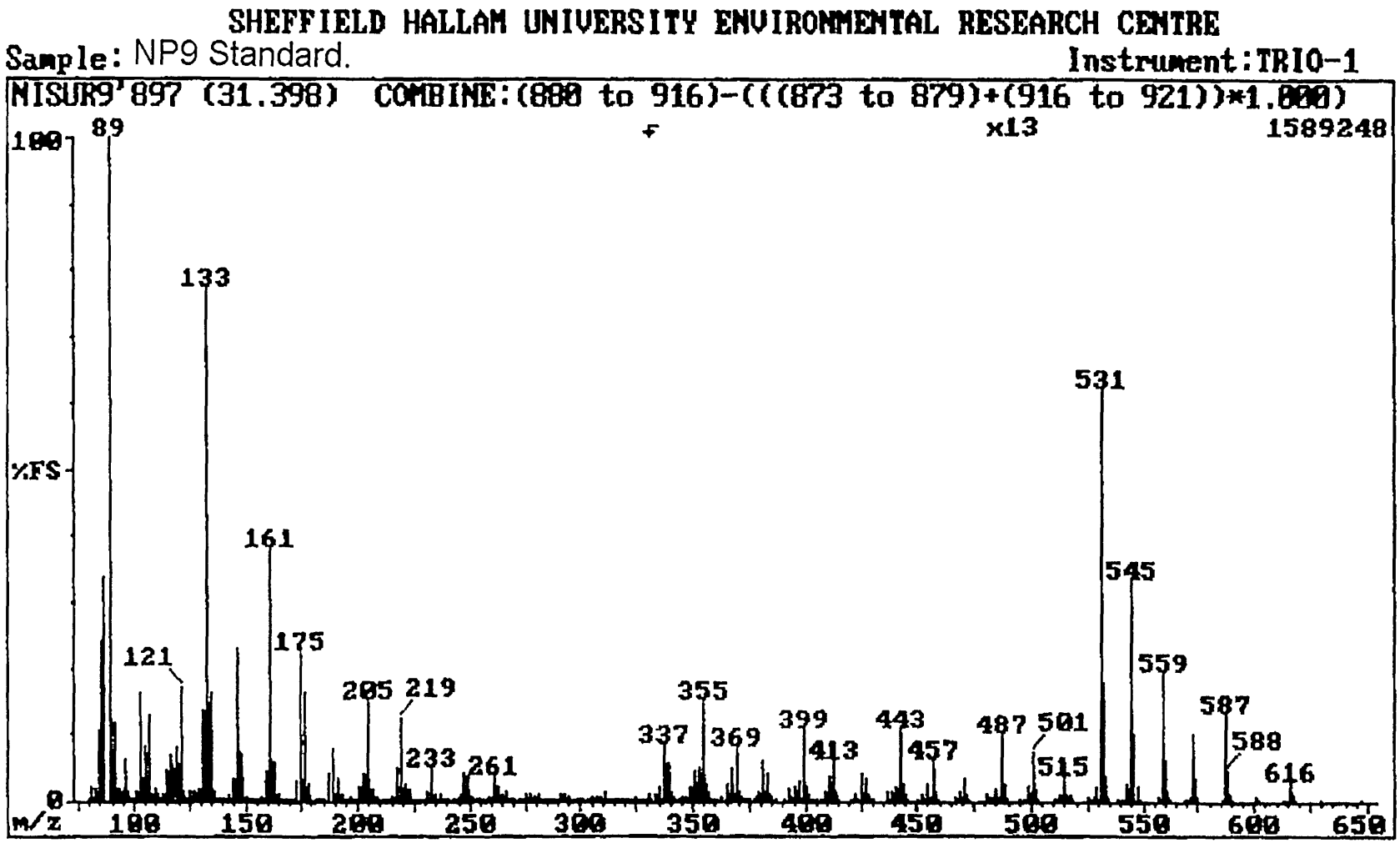


Figure 7.4) Mass Spectrum of peak at 31.4 min (NP_9EO ethoxymer peak).

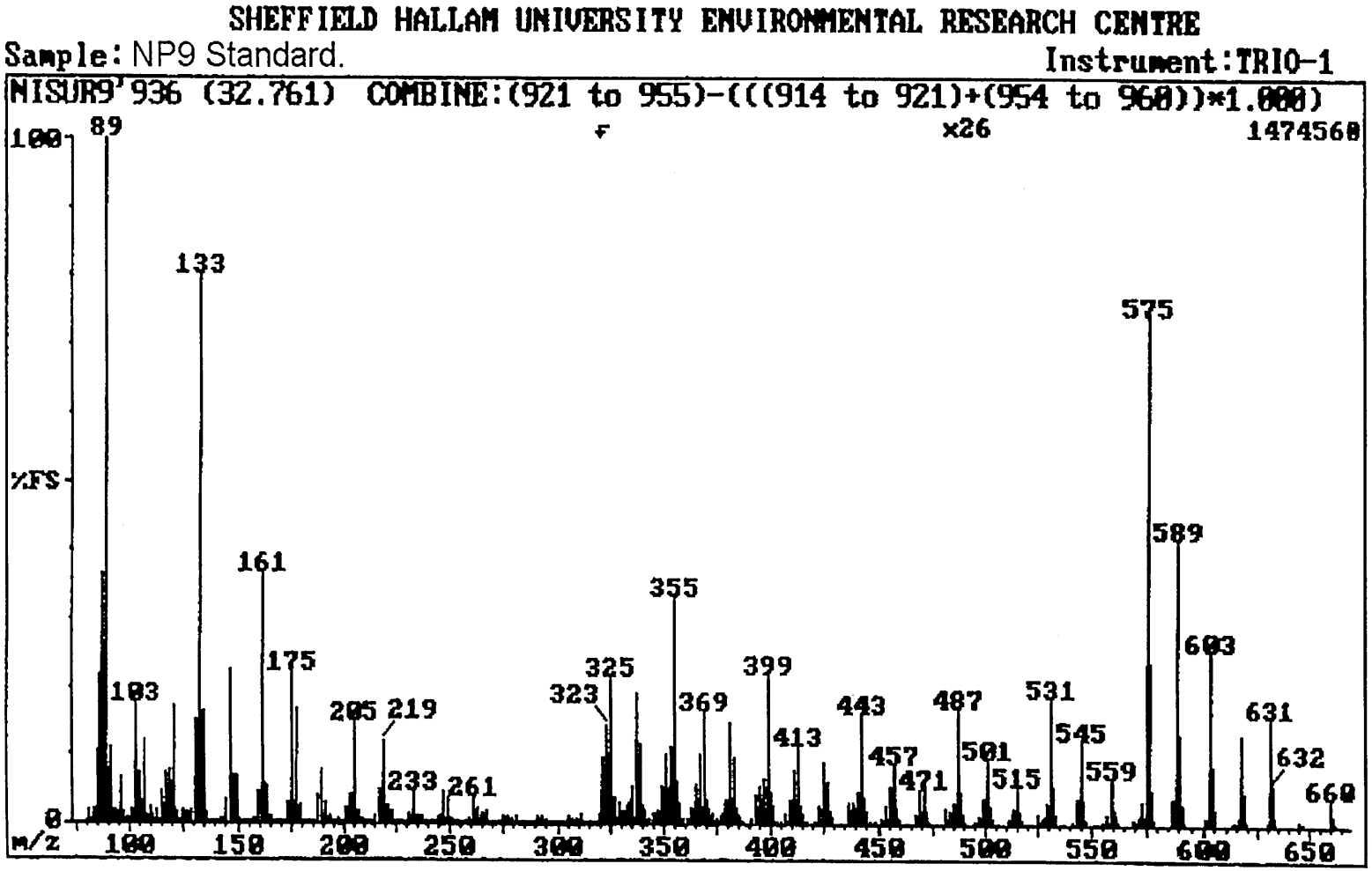


Figure 7.5) Mass Spectrum of peak at 33.9 min ($NP_{10}EO$ ethoxymer peak).

7.2.2) Post column addition experiments.

Tables 7.1-7.5 display the results obtained after the post column addition (added using pump 2, (Figure 7.0)) of various salts and solvents. Pump 1 was used to pump the two mobile phase eluants usually used. This was done either singularly or as a mixture, (conditions are discussed in the relevant table). Injections were carried out in duplicate, mean peak areas for various characteristic fragmentation ions as shown, (Tables 7.1-7.5).

Table 7.0) Post column addition of butanol.

SIM Peak	Pump conditions / %[a]	Mean Peak area
311	Pump 1 = hexane 100 Pump 2 = 0	140847200
205	as above	268460064
161	as above	446446080
135	as above	355144384
311	Pump 1 = hexane 75 Pump 2 = butanol 25	47853692
205	as above	94494024
161	as above	144055712
135	as above	123484568
SIM Peak	Pump conditions / %[a]	Mean Peak Area.
311	Pump 1 = hexane 50 Pump 2 = butanol 50	54520772
205	as above	145962528
161	as above	222507408
135	as above	131763768
311	Pump 1 = hexane 25 Pump 2 = butanol 75	25458210
205	as above	75719352
161	as above	111985192
135	as above	74690144
311	Pump 1 = Solvent B[b] 75 Pump 2 = butanol 25	44996164
205	as above	122779616
161	as above	172397280
135	as above	88442768

Table 7.1 Post column addition of 0.05M ammonium oxalate[c] in butanol

SIM Peak	Pump conditions / %[a]	Mean Peak area
311	Pump 1 = hexane 100 Pump 2 = 0	46483648
205		110971648
161		86108160
135		123810816
311	Pump 1 = hexane 75 Pump 2 = ammonium oxalate[c] 25	94325184
205		215100944
161		397861120
135		281628672
SIM	Pump Conditions / %[a]	Mean Peak Area
311	Pump 1 = hexane 50 Pump 2 = ammonium oxalate[c] 50	61108488
205		125344972
161		203530624
133		190113632

Table 7.2) post column addition of 0.05M ammonium acetate in butanol

SIM Peak	Pump conditions / %[a]	Mean Peak area
311	Pump 1 = Hexane = 100 Pump 2 = 0	57428712
205		132652240
161		263203888
135		185683312
311	Pump 1 = hexane 75 Pump 2 = 0.05 M ammonium acetate 25[d]	4390576
205		124339496
161		263203888
135		126277736
311	Pump 1 = hexane 50 Pump 2 = ammonium acetate 50[d]	51823436
205		153370240
161		286247296
135		159631616

Table 7.3) Post column addition of propanol.

SIM Peak	Pump conditions / %[a]	Mean Peak area
311	Pump 1 = hexane 100 Pump 2 = 0	42635304
205		59131756
161		104179456
135		96545520
311	Pump 1 = hexane 50 Pump 2 = propanol 50	30667961
205		55923650
161		80695390

Table 7.4) Post column addition of ethyl acetate.

SIM Peak	Pump conditions / %[a]	Mean Peak area
311	Pump 1 = hexane 100 Pump 2 = 0	42635304
205		59131756
161		104179456
135		96545520
311	Pump 1 = hexane 75 Pump 2 = ethyl acetate 25	47946696
205		83820720
161		126141248
135		72978044
SIM	Pump conditions / %[a]	Peak area
311	Pump 1 = hexane 50 Pump 2 = ethyl acetate[e] 50	39561274
205		72884742
161		89540540
135		60481428

Table 7.5) Addition of 0.05M ammonium acetate in ethyl acetate[e]

SIM Peak	Pump conditions / %[a]	Mean Peak area
311	Pump 1 = hexane 100 Pump 2 = 0	71194016
205		241226304
161		409945952
135		186144432
311	Pump 1 = hexane 75 Pump 2 = ethyl acetate[e] 25	64288240
205		119067808
161		175155408
135		158486352
311	Pump 1 = hexane 50 Pump 2 = ethyl acetate[e] 50	72703696
205		140111296
161		211880720
135		166556816

[a] indicates the percentage each pump contributes to total flow of 0.8 ml min^{-1}.

[b] solvent B = 2- methoxyethanol / propan-2-ol

[c] solution of 0.05M ammonium oxalate in butanol. Ammonium oxalate found to be only sparingly soluble.

[d] solution of 0.05M ammonium acetate in butanol.

[e] solution of 0.05M ammonium acetate in ethyl acetate solution.

It can be seen from the results displayed in Figures 7.6 to 7.11, that the addition of solvents and /or solvents and buffers did not significantly increase the sensitivity of this method. Generally peak areas obtained after the 'post column addition' were less than for hexane alone indicating that the non-polar character of hexane plays a more important role in the transportation of sample into the ionisation chamber of the mass spectrometer than the presence of salts. The addition of salts to the HPLC mobile has been described as being of some use in increasing analyte signal[3] when utilising PB interfacing.

Figure 7.7) Graph to show the variation in signal of monitored selected ions of NP9 with the addition of various solvents.

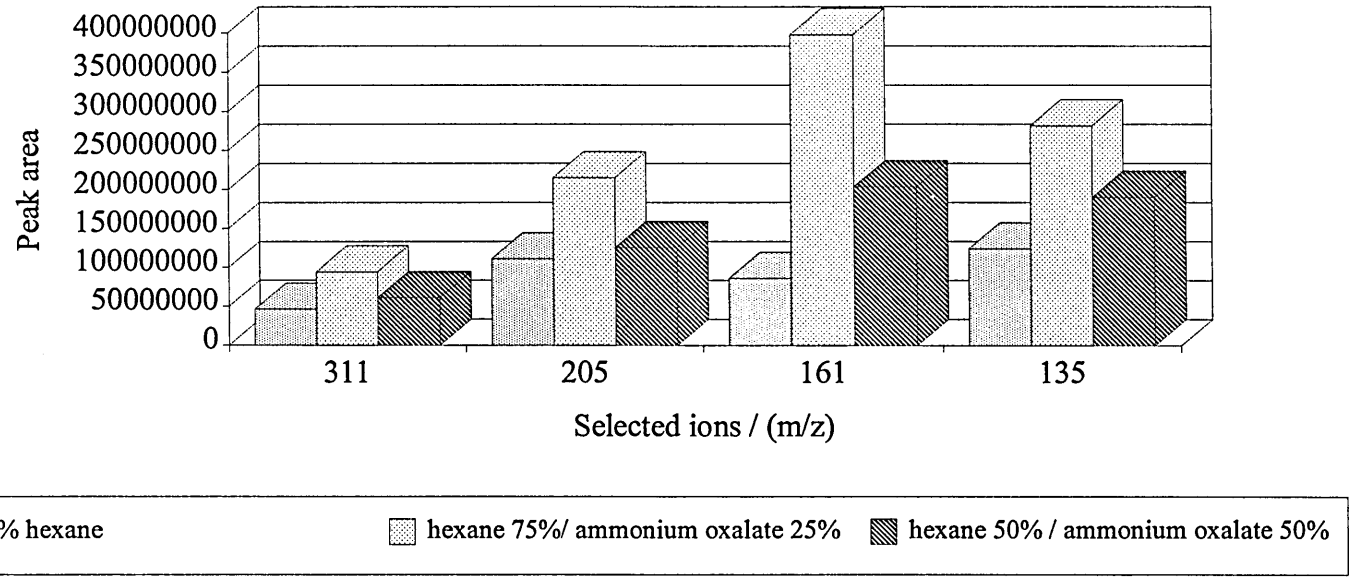


Figure7.8) Graph to show the variation in signal of monitored selected ions of NP9 with the addition of various solvents.

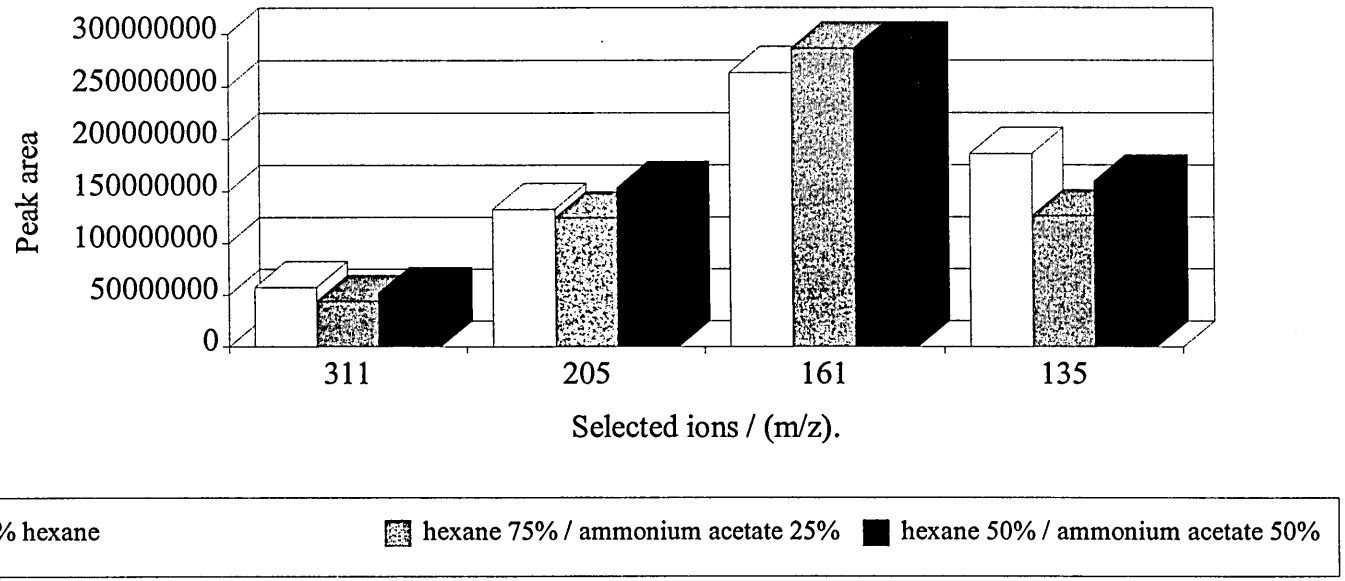


Figure 7.10) Graph to show the variation in signal of monitored selected ions of NP9 with the addition of various solvents.

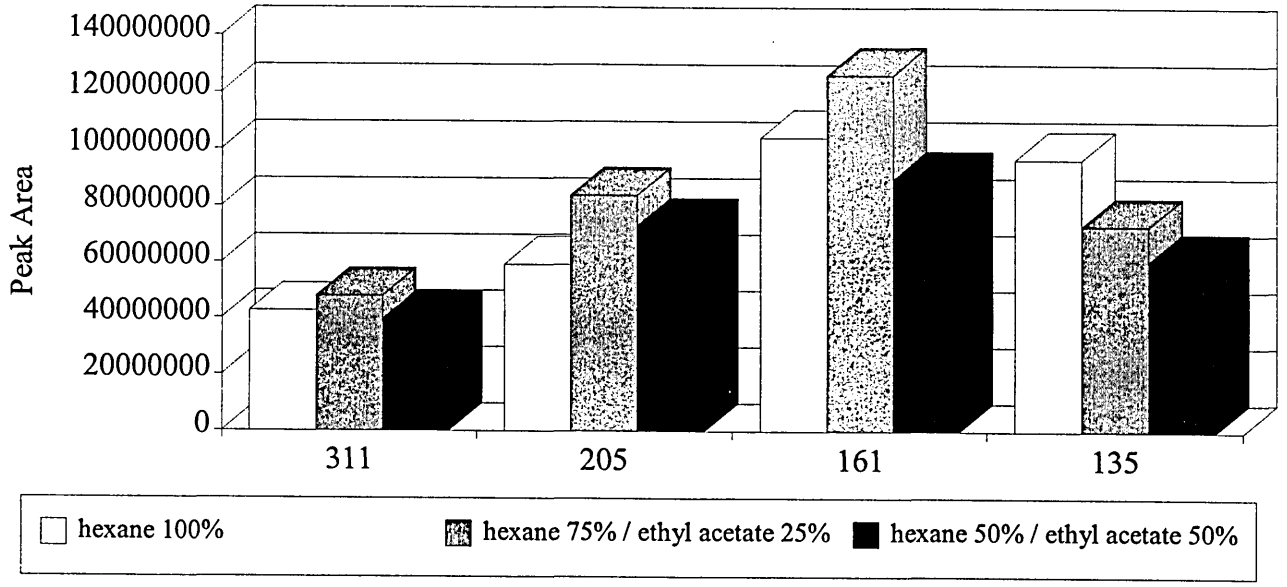
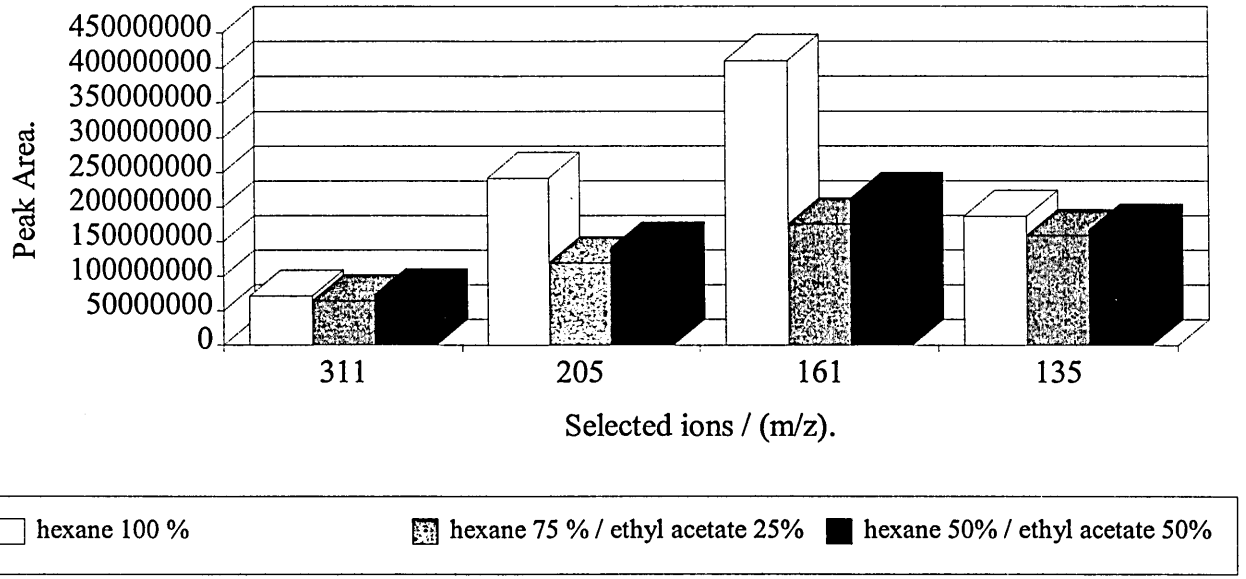


Figure 7.11) Graph to show variation in signal of monitored selected ions of NP9 with the addition of various solvents.



7.2.3) Liquid Chromatography-Thermospray-Mass Spectrometry (LC/TSP/MS) for the determination of Surfactants.

Figure 7.12 shows the results of a preliminary investigation into the compatibility of the mobile phase used in this method (Methanol / 0.08 M ammonium acetate 58/42). Mobile phase was pumped at a rate of 0.5 ml min^{-1}. A 0.15 M aqueous ammonium acetate solution was added post column (as in section 4.2) at a rate of 0.5 ml min^{-1} which made the total concentration of ammonium acetate entering the interface roughly equal 0.1 M. Figure A shows a 50 µl loop injection of a 500 ppm LAS solution in mobile phase using TSP^{+} ionisation.

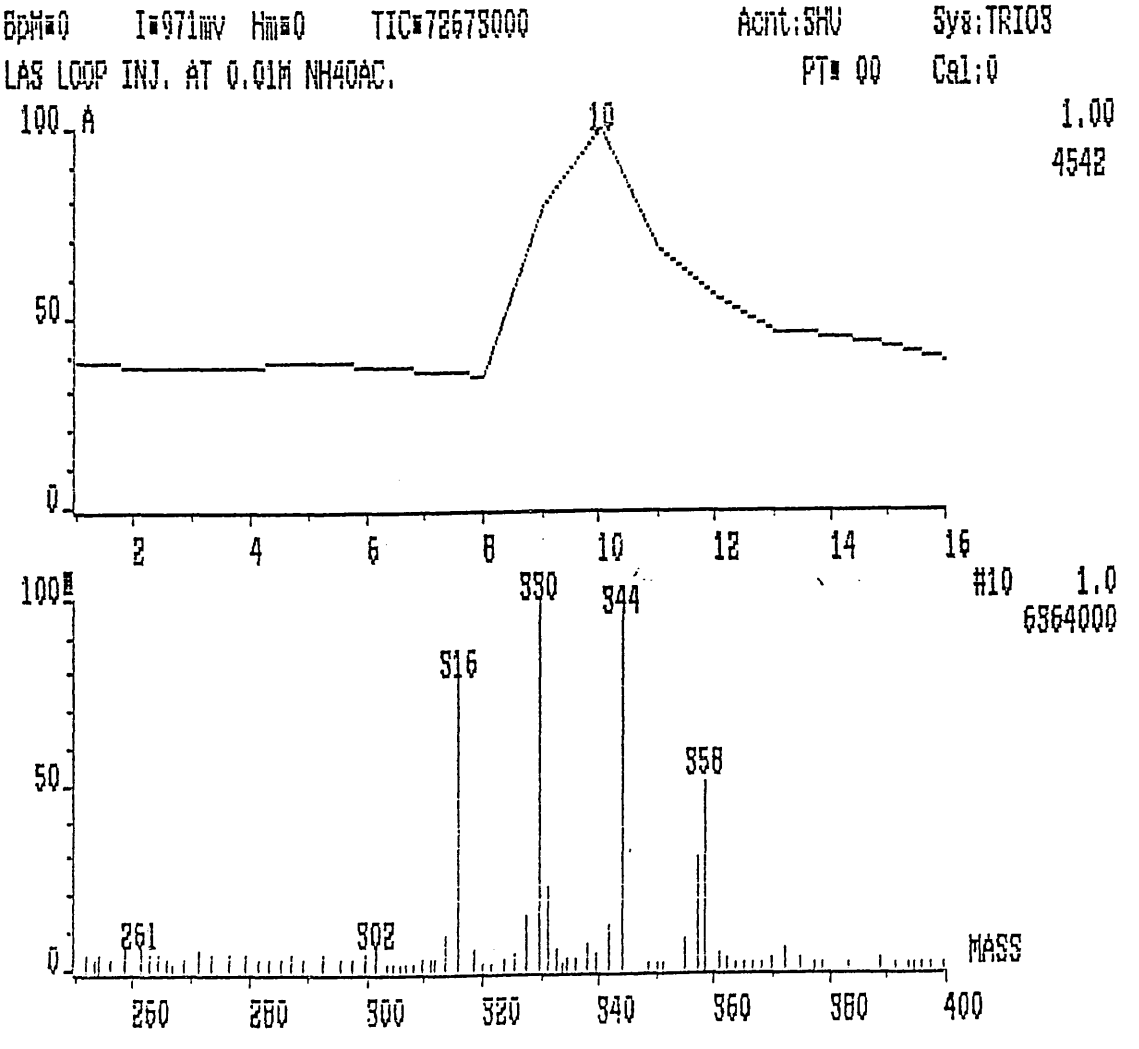


Figure 7.12) Mass spectrum produced from a Loop injection of a 500 ppm standard of LAS. Produced in positive ionisation mode.

As can be seen the method gives limited structural mass spectral information. The peaks at m/z 302, 316, 330, 344, 358 and 376 are (Figure 7.12), derived from LAS homologues arising from a $(M+NH_4)^+$ ion species, i.e. 301, 315, 329, 343, 357 and 375. This corresponds to an LAS mixture with alkyl chain lengths of 9, 10, 11, 12, 13 and 14. If the ionisation efficiency of LAS homologue with an alkyl chain length of C9 and C14 is taken to be roughly the same as the rest of the homologues present, it can be seen that these two homologues are present at very low levels. This would explain why only four peaks are commonly observed using UV or fluorescence detection. Indeed the peaks corresponding to C9 and C14 are hardly distinguishable from background noise. Figure 7.13 shows a 50µl injection of the same solution using the mass spectrometer in TSP^- mode. The peaks seen in the spectrum show that $(M-H)^-$ molecular ions are obtained for the LAS homologues present i.e. ions at m/z 281, 296, 311, 325, 339 and 355 which equate to homologues with alkyl chain lengths of 9, 10, 11, 12, 13 and 14 carbon units. The peak at m/z 355 in the mass spectrum is effectively 2 mass units greater than the mass of the molecular ion of the homologue of LAS containing 14 carbon atoms in the alkyl chain. This suggests that the instrument used was not calibrated properly. The total ion chromatogram (TIC) in positive ion mode is approximately 16 times more intense than the total ion chromatogram produced by detection of the standard solution using TSP^- ionisation. This suggests that positive ion mode should be used for trace detection.

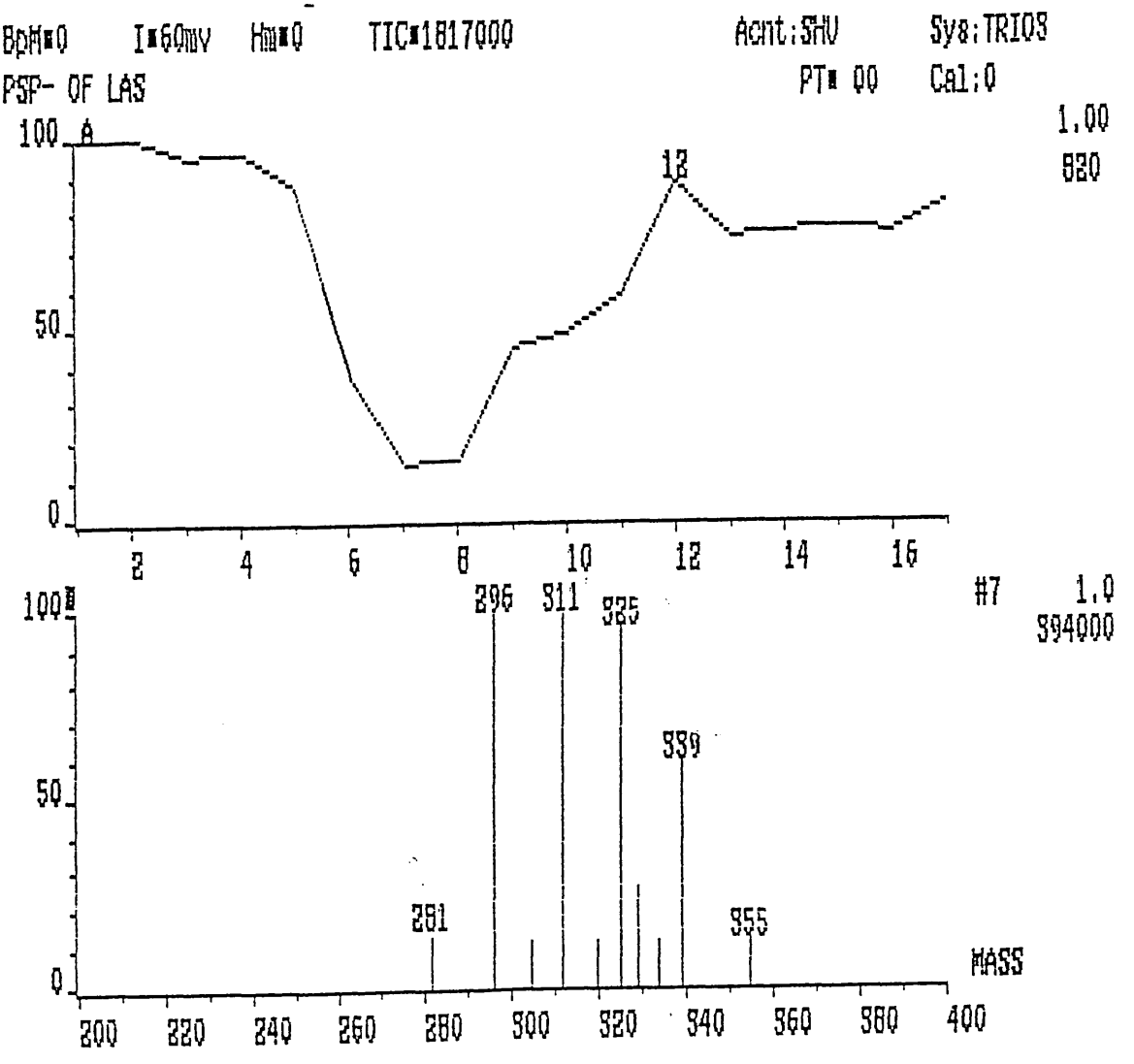


Figure 7.13) Mass spectrum of a 50µl Loop injection of a 500 ppm LAS standard obtained in EI- mode.

Figure 7.14a shows the TIC obtained in positive ionisation mode by injecting 50µl of the standard LAS mixture and using the chromatographic procedure described in section 4.6. Post column addition of ammonium acetate was used as described earlier. Figure 7.14b shows ion chromatograms corresponding to the molecular ion of each LAS homologue (from top to bottom : C10LAS to C13LAS). Each trace shows a sum over two masses to allow for variation in quadrupole calibration. It can be seen that each component within the LAS mixture is resolved under these conditions.

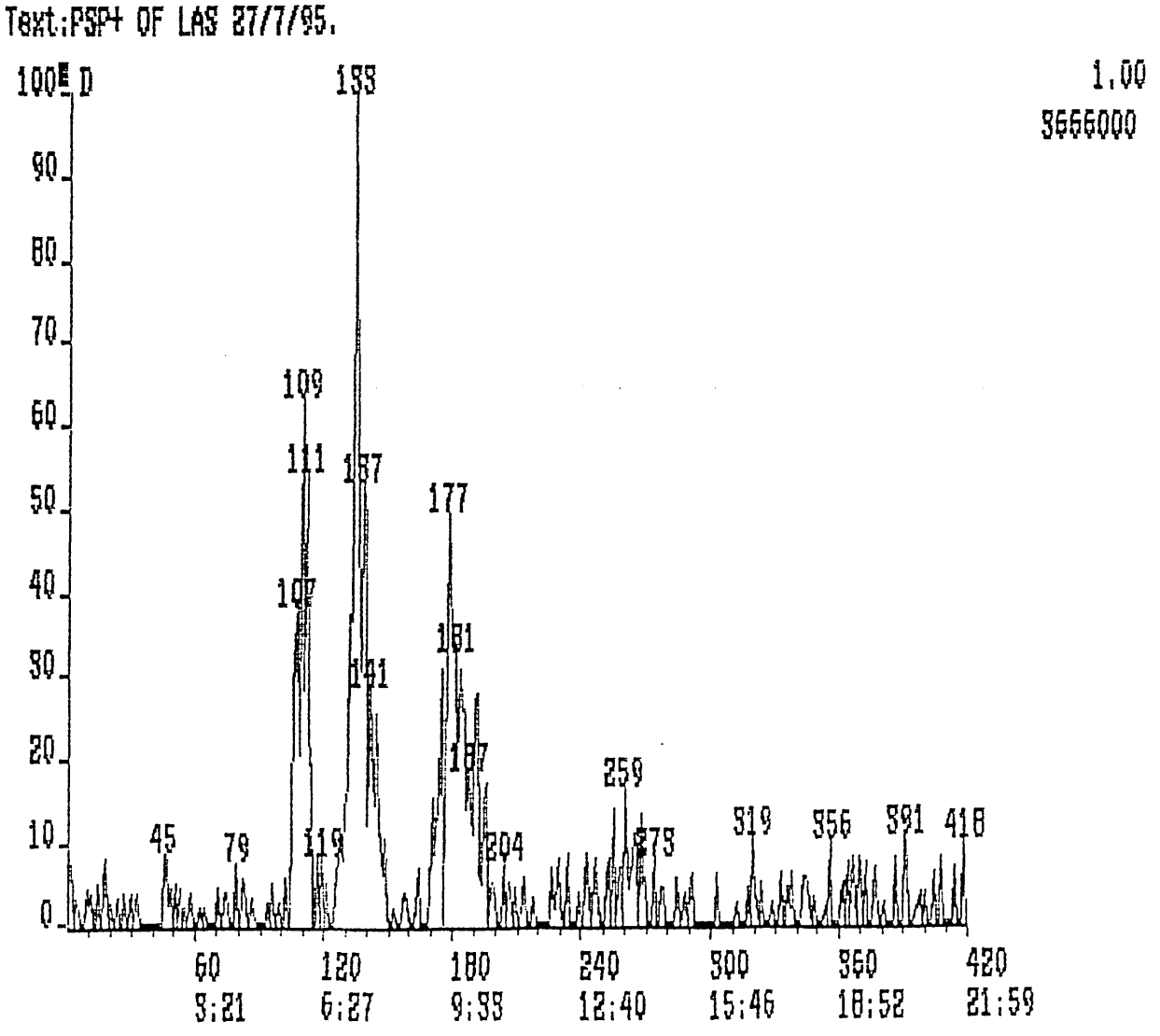


Figure 7.14a) Total ion chromatogram (TIC) of a 50µl injection of a LAS standard mixture obtained using chromatographic conditions described in section 4.6.

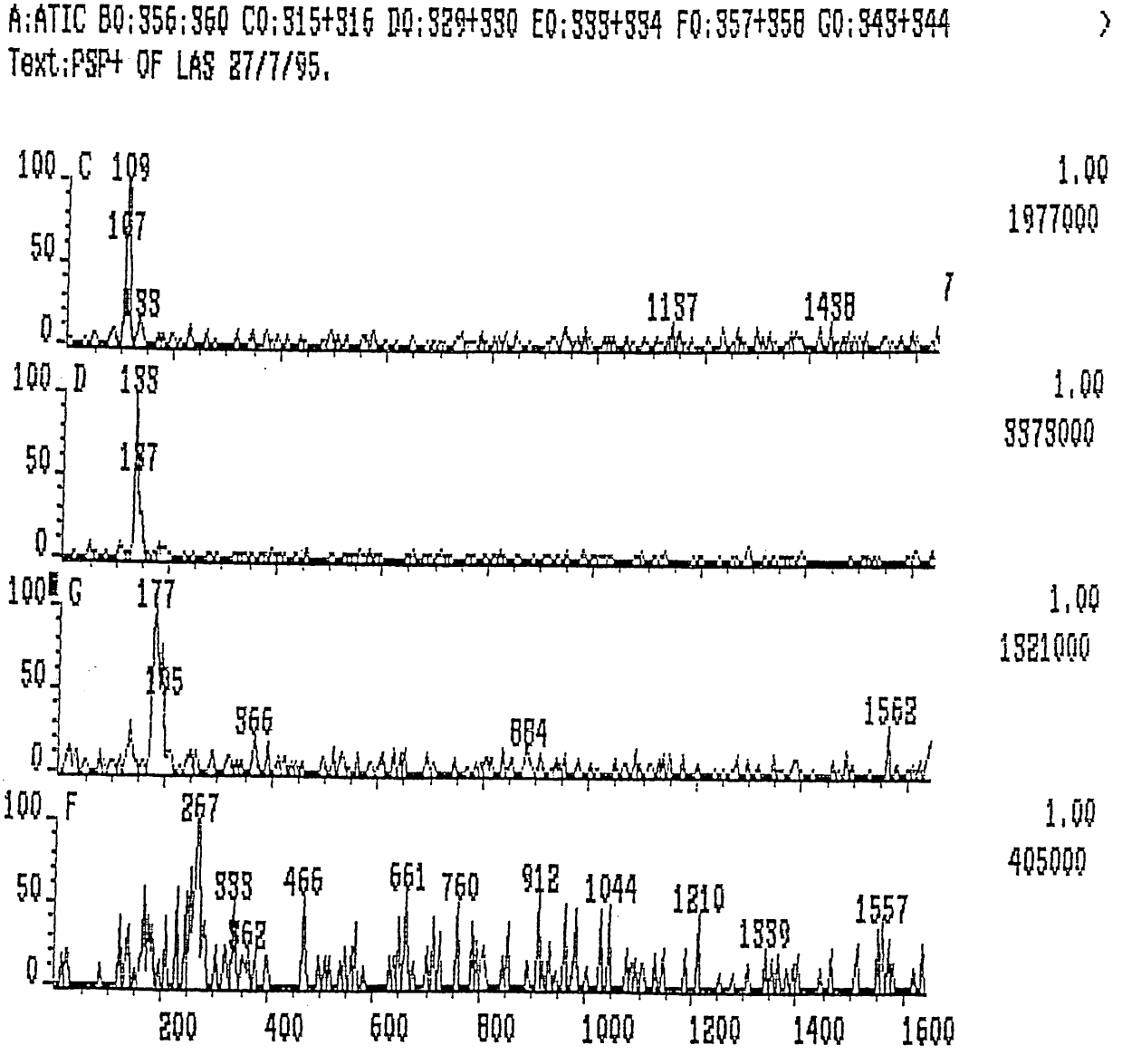


Figure 7.14b) Ion chromatograms of C10LAS to C13LAS resulting from a 50µl injection of LAS standard mixture obtained using chromatographic conditions (section 4.5).

Non-ionic surfactants were also tested for compatibility with the thermospray interface. Ammonium acetate was added post column as described in the previous section. Figure 7.15 shows the mass spectrum obtained from a loop injection of 50 µl of a 500 ppm Triton X100 sample in MeOH / 0.08M ammonium acetate (aq) 58/42 solution (representing the HPLC mobile phase used in these experiments). This

spectrum was produced with the instrument in positive ion mode. It was not possible to produce negative ion spectra of the non-ionic surfactants.

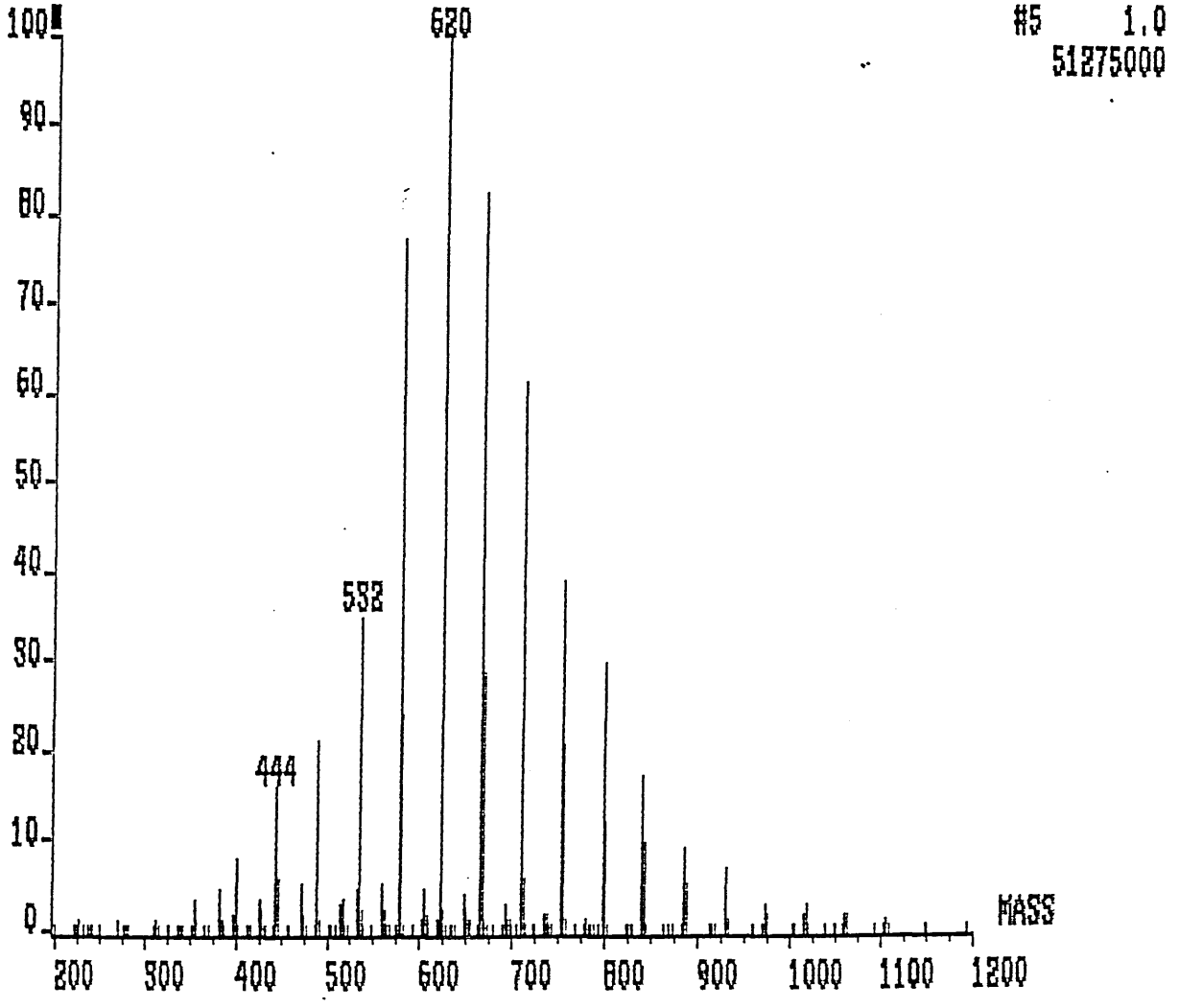


Figure 7.15) Mass spectrum of the standard Triton X100 solution loop injection.

The spectrum shown in Figure 7.15 displays a characteristic series of $(M+NH_4)^+$ ions for the individual ethoxymers within the Triton X100 octylphenol ethoxylate formulation. Each individual ethoxymer is separated by 44 mass units. This

results from a stepwise increase in ethylene oxide chain length (each additional unit has a mass of 44 Daltons). It can be seen that each ion represents detectable levels of individual ethoxymers beginning at m/z 356 i.e., OP_3EO to $OP_{16}EO$ (m/z 928).

Figures 7.16 and 7.17 show the TSP+ mass spectra obtained from loop injections of different NPEO formulations (NP5 and NP9, respectively). Using the same methodology 50 µl of a 500 ppm sample was injected. Like OPEO, the peaks seen in a mass spectrum of NPEO are in fact molecular adduct ions of the individual ethoxylates within the sample mixture. Again they can be assigned to a $(M+NH_4)^+$ species. As a result of this characteristic ionisation pattern it can been seen (Figure 7.16) that NP_5EO has a detectable ethoxylate chain distribution ranging from 2 to 10 ethylene oxide units. As would be expected, the peak of maximum intensity corresponds to m/z 459, i.e., $(M+NH_4)^+$ of NP_5EO.

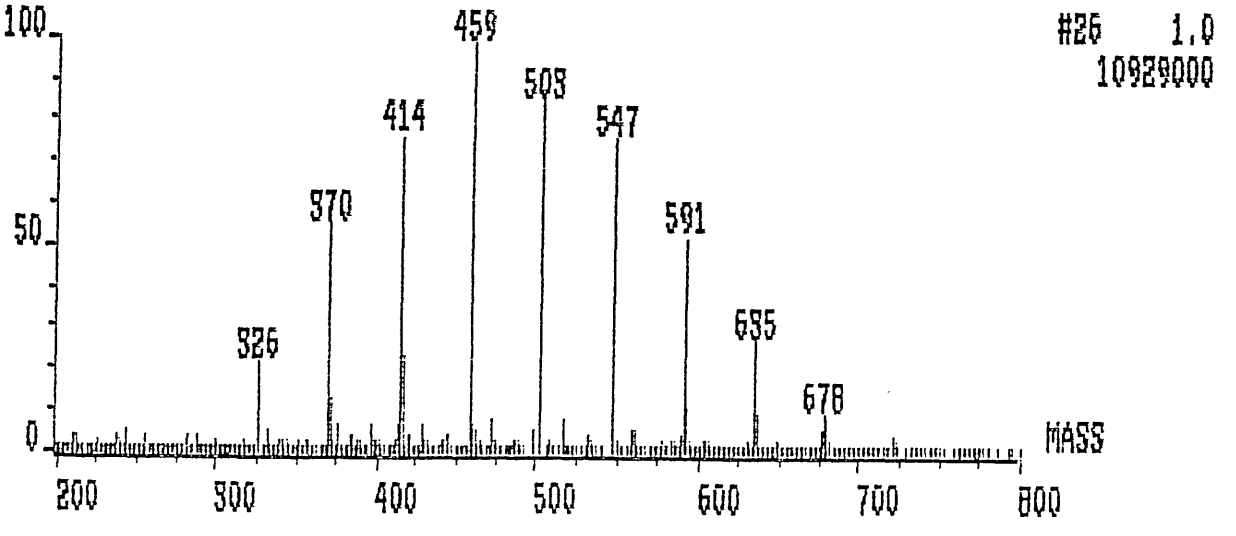


Figure 7.16) Loop injection of a NP5 surfactant formulation in HPLC mobile phase.

Figure 7.17 shows the mass spectrum of a NP9 formulation. Here the ethoxylate distribution ranges from NP_3EO to $NP_{14}EO$. The peak of maximum intensity results from a ammonium adduct ion m/z 634, i.e., NP_9EO.

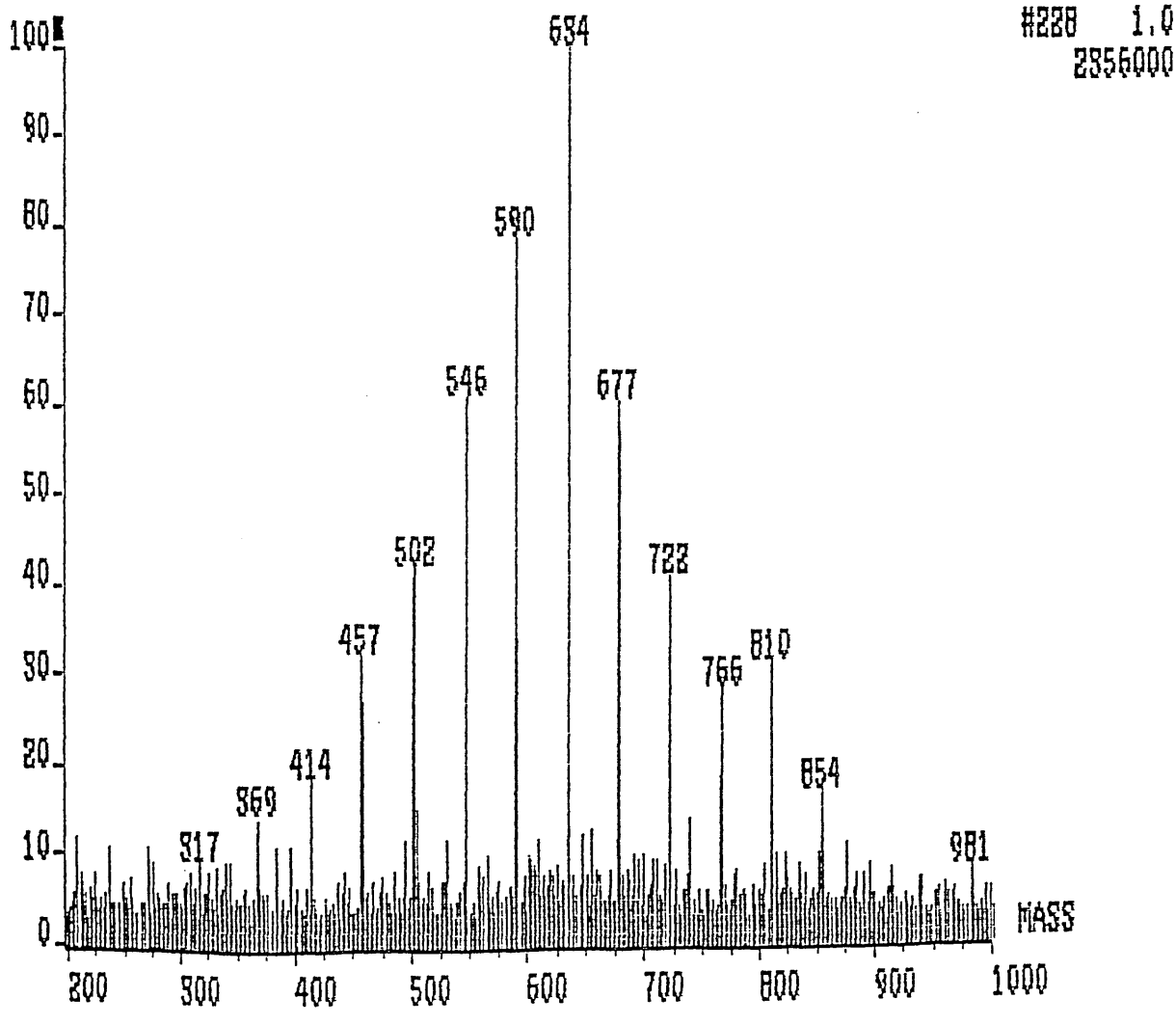


Figure 7.17) Loop injection of a NP9 standard mixture in HPLC mobile phase.

Figure 17.8 show the TIC of a Triton X100 standard resolved using the HPLC conditions described in section 4.6.

The peaks resulting from OP_3EO ($(M+NH_4^+)$ m/z 356) to $OP_{15}EO$ ($(M+NH_4^+)$ m/z 888) are displayed simultaneously. Each peak displayed is a member

of the series 356, 400, 444, ... to 888, i.e. shown from the loop injection of Triton X100 to represent adduct ions of the individual ethoxylate within the Triton sample. It can be seen that using this technique it is possible the resolve individual ethoxylates within a mixture.

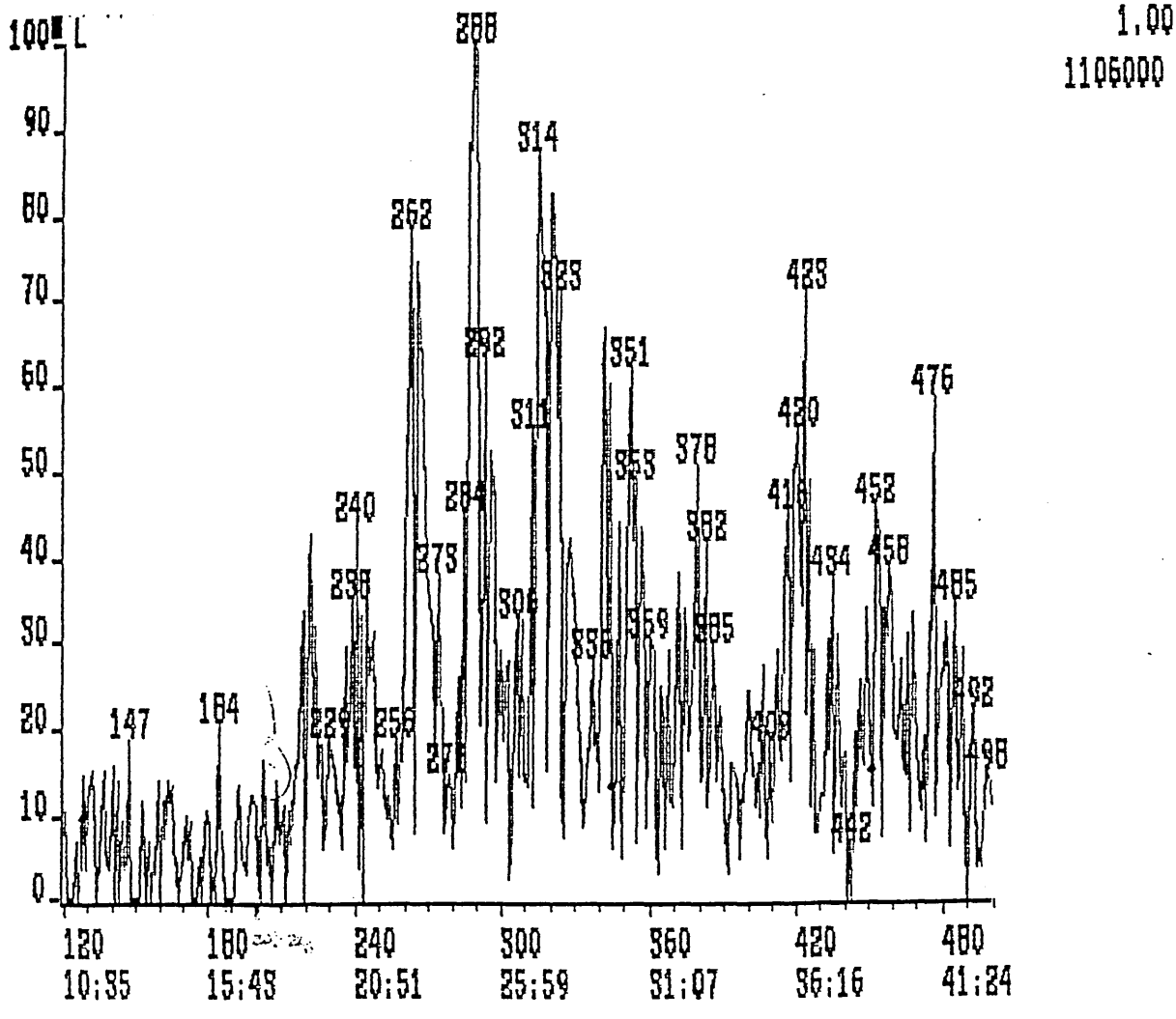


Figure 7.18) TIC of a Triton X100 standard sample resolved using the HPLC conditions described in section 4.6.

Figure 7.19 shows ion chromatograms for OP_4EO to $OP_{14}EO$. Clearly, there is little or no overlap of individual OPEOs.

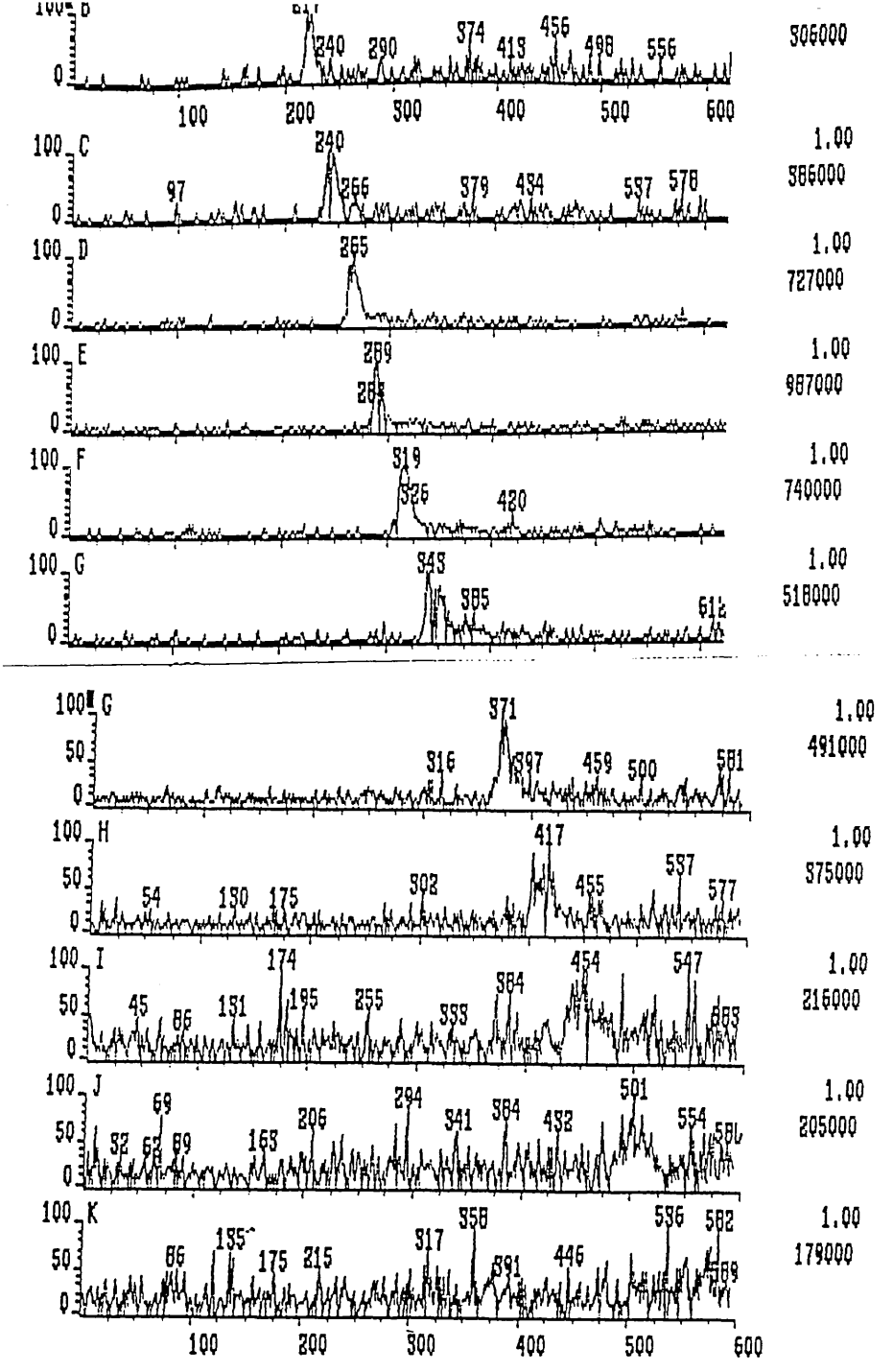


Figure 7.19) LC/TSP/MS ion chromatograms of individual Triton X100 oligomers (top trace OP_4EO to bottom trace $OP_{14}EO$).

7.2.4) Simultaneous determination of Non-ionic and Anionic Surfactants (using LC/TSP/MS).

This technique has been achieved via the use of the 'C1 column' HPLC method mentioned in section 4.6. Figure 7.20 displays total ion chromatogram together with ion chromatograms for the various NPEO homologues NP_3EO to $NP_{15}EO$.

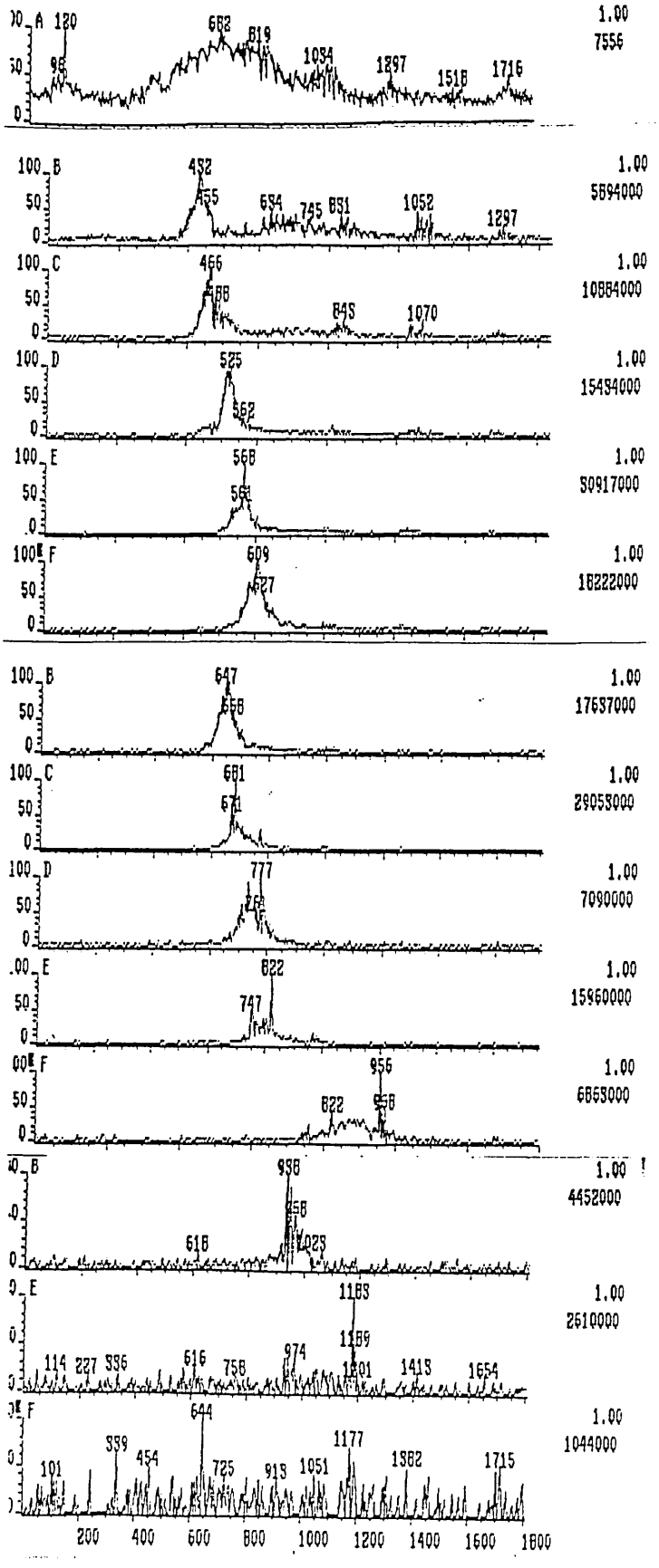


Figure 7.20) Total ion chromatogram and ion chromatograms for the various NPEO homologues (Top trace NP_3EO to $NP_{15}EO$ bottom trace) produced in positive ion mode.

Using this technique it is possible to show that the chromatographic conditions used do in fact resolve anionic LAS from an APEO type of non-ionic surfactant. Figure 7.21 shows ion chromatograms for the final eluting LAS fraction m/z 358 (C13 LAS, top trace) and the first NPEOs to elute, NP_2EO m/z 326 (bottom trace) and NP_3EO m/z 370 (middle trace). It can seen that no NP_2EO is present in the sample and the NP_3EO is clearly resolved from LAS with thirteen carbon atoms in its alkyl chain.

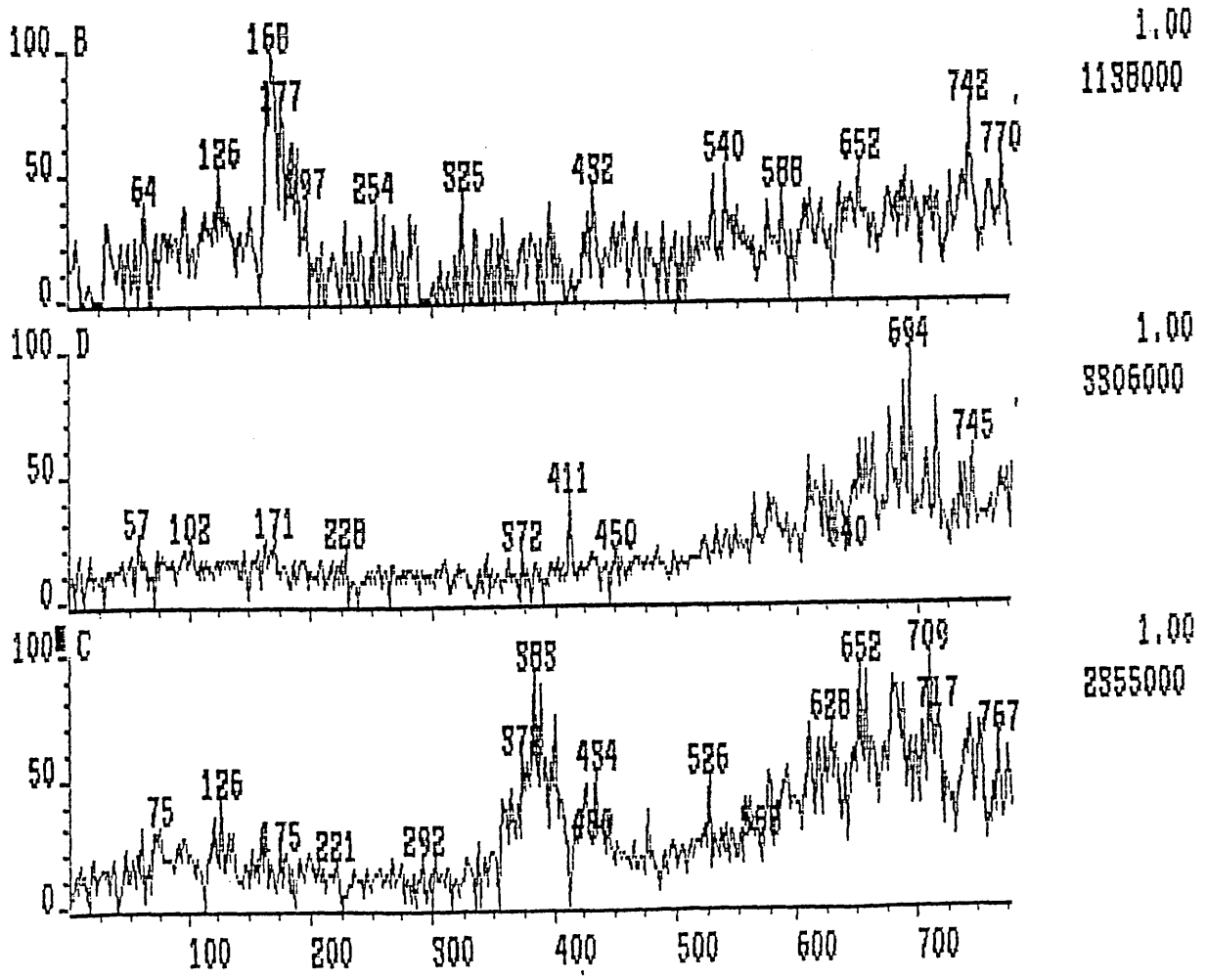


Figure 7.21) Ion chromatograms of C13 LAS (top trace), NP_3EO (middle trace) and .NP_2EO and NP_3EO combined (bottom trace). Resolved using the HPLC method described in Section 4.6 method B via LC/TSP^+/MS detection.

The limit of detection for non-ionic surfactants using this method in full scan mode was equal to 5 μg on column, which allowed the detection of the major peak of that particular formulation (at three times signal to noise). Therefore for a NP9 formulation, NP_9EO could be monitored at 5 μg on column and above. However, using this principle it would not be possible to monitor the individual APEOs with ethoxylate chain lengths above and below the mean ethoxylate value, as these molecules are present in smaller concentrations within the formulation. For LAS (with an alkyl chain containing eleven carbon units) the limit of detection (three times signal to noise) was approximately 2 μg on column. Clearly although these values represent an improvement with respect to particle beam interfacing several litres of surface water would have to be processed via SPE to pre-concentrate the analyte to a satisfactory level.

As this technique has been shown to give very little structural information it would be desirable to use MS/MS experiments in order to obtain structurally significant mass spectral information. This is especially important as in the environment, surfactant molecules are normally present in very complex matrices. Unfortunately due to instrumental problems this was not possible during the project. However, Schroder[2] has examined surfactants in sewage treatment plants via Thermospray mass spectrometry using both FIA and conventional HPLC. In positive ion mode ions corresponding to $(M+NH_4)^+$ adducts of LAS were observed at m/z 316, 330 and 344. In negative ion mode $(M-H)^-$ ions were detected at m/z 297, 311 and 325. It was also possible to monitor the presence of a series of ions at 44 Daltons apart beginning at m/z 468. This series was assigned to $(M+NH_4)^+$ ions from oligomers of a polyethylene glycol ether type non-ionic surfactant. Using a product ion scan MS/MS studies were used in order to confirm this assignment. This resulted in sufficient structural information to allow interpretation. Samples were spiked to a level of 300 ppm and above followed by an injection on column of 100 μl which suggest limits of detection as good as, if not better than, those that were achieved during this work.

7.2.5) Liquid Chromatography / Atmospheric Chemical Ionisation (APcI) / Mass Spectrometry.

An investigation of the compatibility of the mobile phase with the atmospheric pressure liquid chromatography mass spectrometry is shown in Figures 7.22, 7.23a, 7.23b and 7.24. The HPLC method discussed in section 4.6 method B was used to separate a mixture of anionic and non-ionic surfactants. This method utilises a trimethylsilyl (TMS) column with an aqueous methanol mobile phase. Figure 7.22 shows the total ion chromatogram for the analysis of a 10 ppm standard mixture of both alkylbenzene sulphonate (LAS) and octylphenol ethoxylates (OPEO) by HPLC/APcI/MS in positive ion mode.

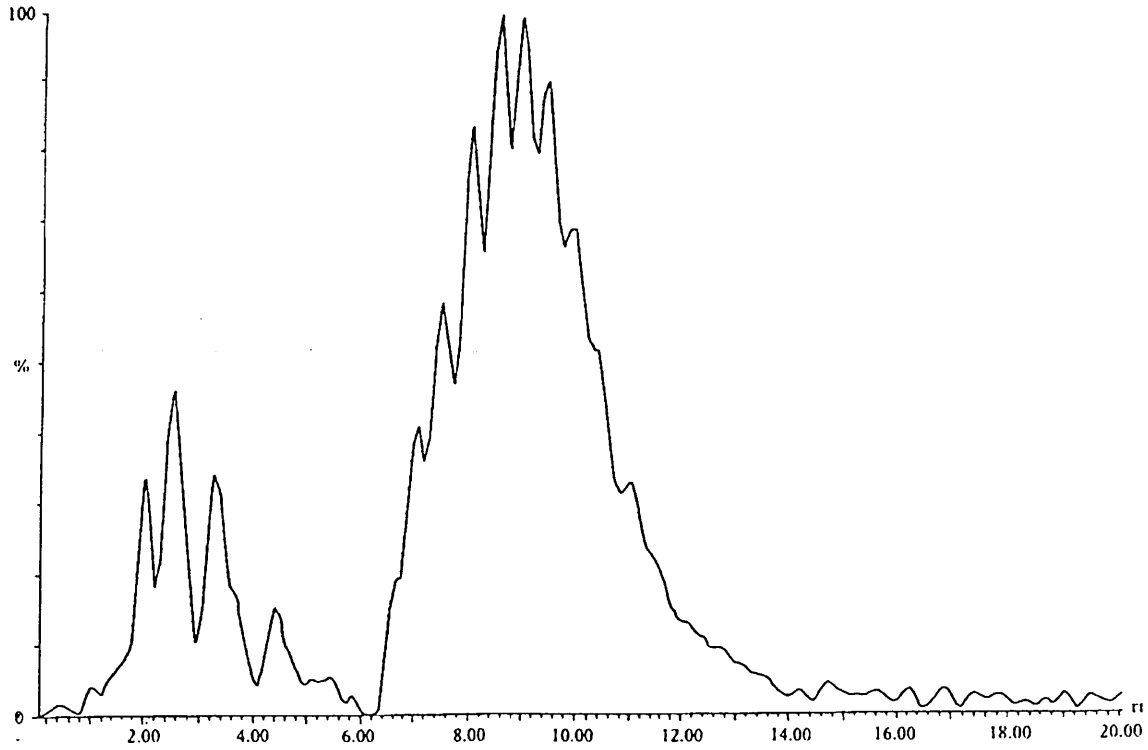


Figure 7.22) APcI positive total ion chromatogram for the analysis of a standard solution of octylphenol ethoxylate and linear alkylbenzene standards. Using HPLC conditions mentioned in section 4.6b.

It can be seen that peaks arising from both anionic LAS and non-ionic OPEO can be seen in this ionisation mode. However, in negative ion mode only the anionic surfactants are observed. The relative sensitivity for the detection of anionic surfactant

is approximately 5:1 in favour of the positive ion mode, (peak area comparison). It would therefore seem desirable to use this ionisation mode for quantitative work.

Investigating the mass spectra generated it is possible to assign structures to the peaks shown in the total ion chromatogram. A comparison of mass spectra from one dodecylbenzene sulphonate component of this standard mixture is shown in Figures 7.23a and 7.23b. In Figure 7.23a the APcI positive ion spectrum from the eluting peak at retention time 3.2 min is shown. The major ion in this spectrum at m/z 385 can be assigned to a $(M + 2Na)^+$ ion.

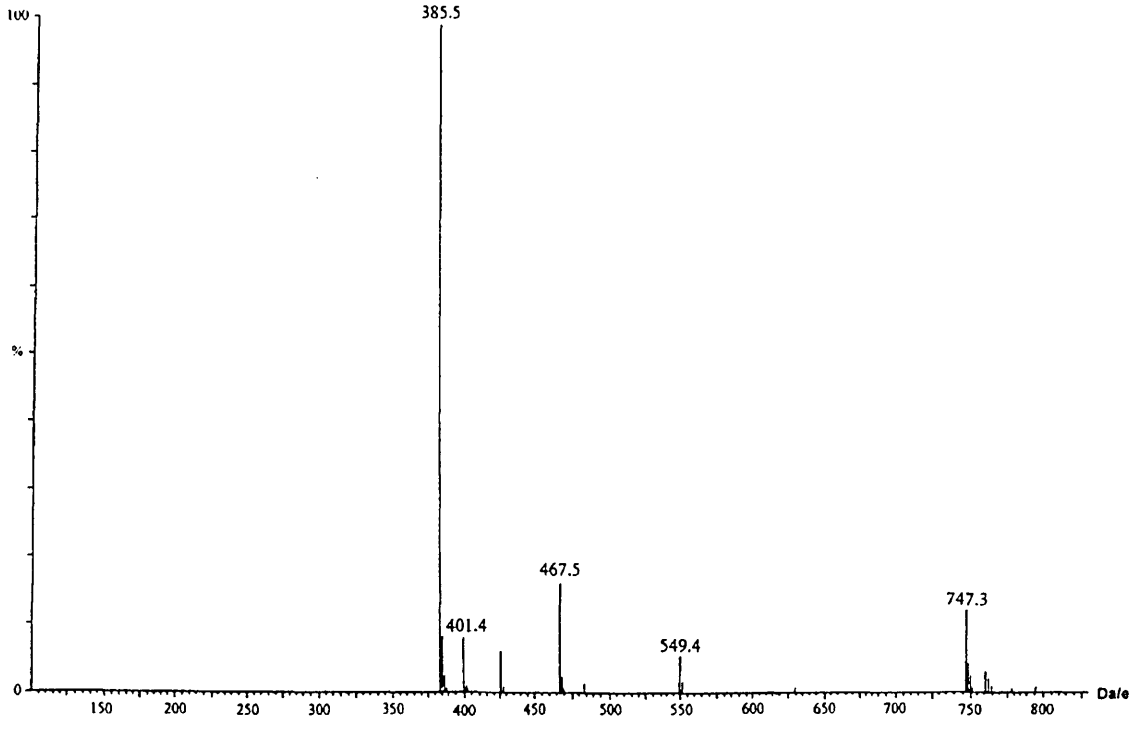


Figure 7.23a) Positive ion APcI mass spectrum for the peak eluting at retention time = 3.2 min in figure 7.22.

The corresponding negative ion spectrum is shown in Figure 7.23b. In this spectrum the $(M-H)^-$ species at m/z 339 dominates.

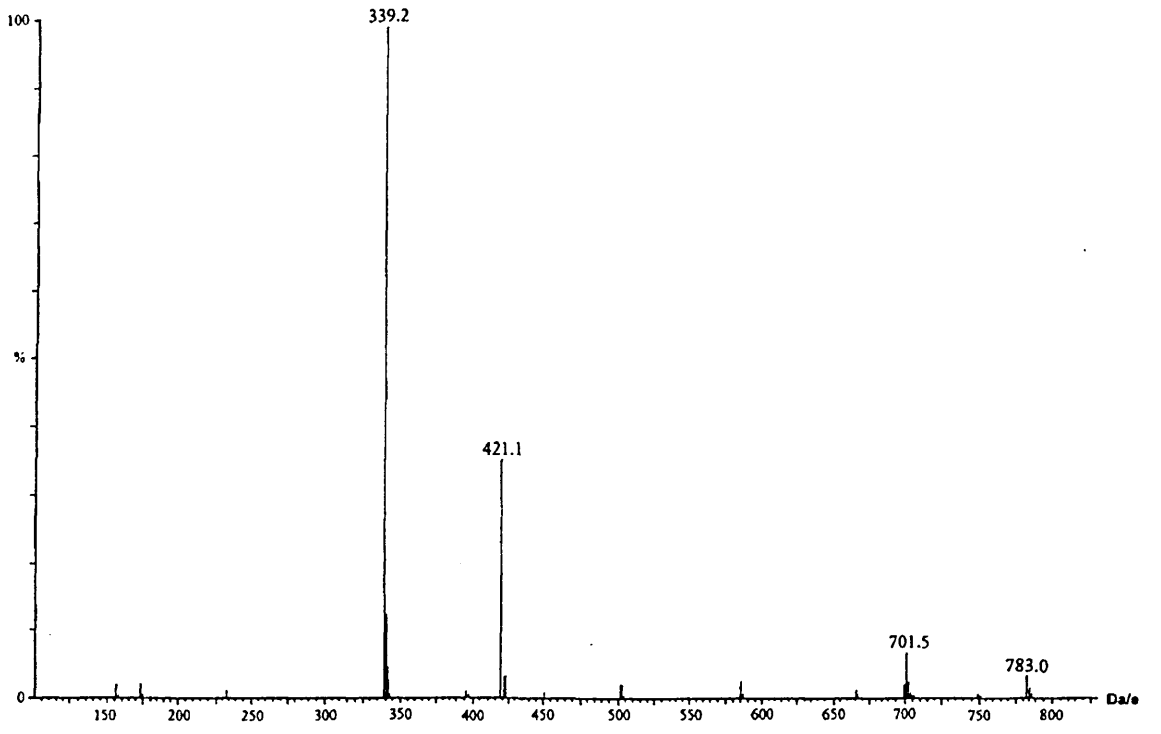


Figure 7.23b) Negative ion APcI mass spectrum for the peak eluting at retention time = 3.2 min in figure 7.22.

These two spectra allow the assignment of this peak to a LAS homologue with a side chain of thirteen carbon units.

Figure 7.24 shows the APcI positive ion spectrum for the peak eluting at retention time = 8.92 min.

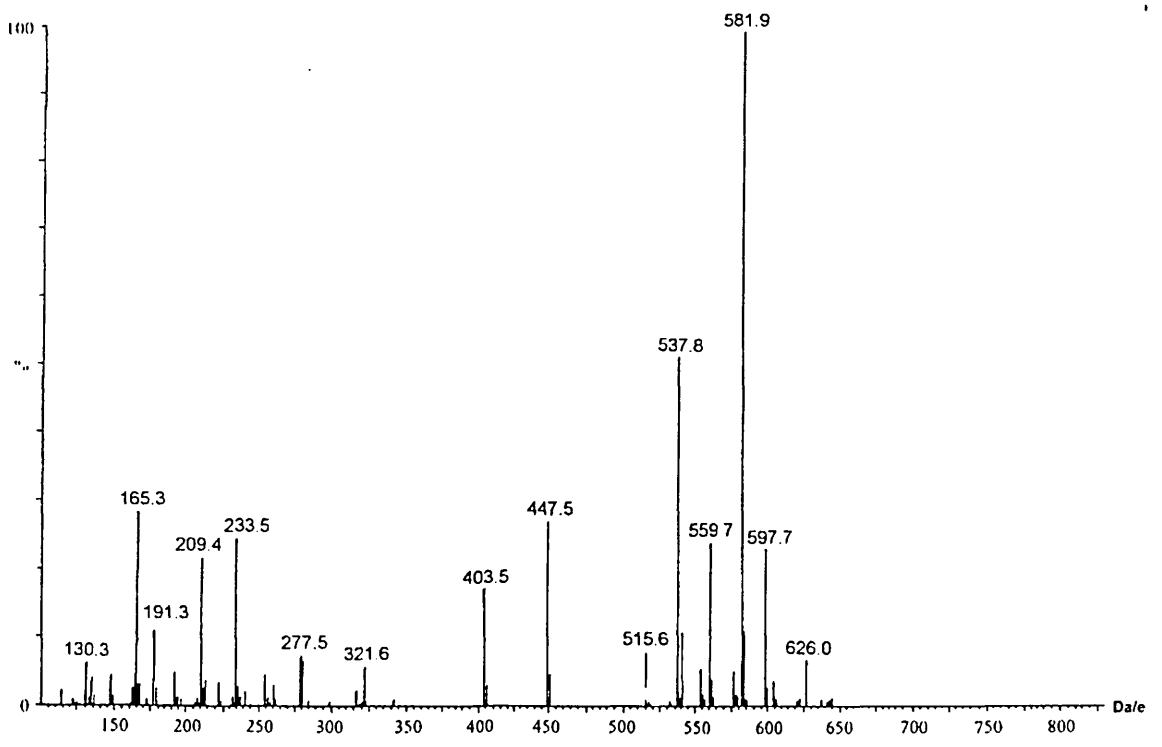


Figure 7.24) Positive ion APcI mass spectrum for the peak eluting at retention time = 8.2 min in figure 7.22.

This mass spectrum exhibits a small $(M+H)^+$ ion at m/z 559. However, the dominant pseudo-molecular species are sodium adduct ions. These can be observed and assigned to $(M+Na)^+$ at m/z 581 $((M-1)+2Na)^+$ at m/z 604 and $((M-2)+3Na)^+$ at m/z 626. Therefore each ethylene oxide unit has the ability to gain sodium atoms from the sodium rich sample. This data indicates that the peak is due to an eight ethylene oxide containing oligomer of Triton X100. No negative ion APcI mass spectra could be produced for Triton X100. It should be noted that in this non background subtracted data a $(M+H)^+$ ion at m/z 515, $(M+2Na)^+$ ion at m/z 537, $(M-2+3Na)^+$ ion at m/z 583 can be seen representing a seven unit ethylene oxide containing oligomer.

7.3) Conclusions.

Experiments using three different LC/MS interfaces for the determination of surfactants were carried out during this project. The interfaces used were (a) Particle Beam (PB) (b) Thermospray (TSP) and (c) Atmospheric Pressure Ionisation (API). Initially it was hoped that particle beam interfacing would allow LC/MS identification of surfactants in waste waters. Satisfactory peak resolution was obtained for the HPLC method employing a cyano column which used non-polar organic solvents. Ideally, during this research it was desirable to use the TMS column method, (Section 4.6 method B). This would allow the simultaneous determination of both LAS and APEO. However, it was not possible to resolve surfactant peaks using this method and the PB interface. Due to instrumental failure before a LC/TSP/MS method was optimised it was not possible to characterise surfactant solutions using the TMS column method, (Section 4.6b). However, it was possible to achieve satisfactory resolution of a standard mixture of Nansa SS and OP_9EO using LC/APcI/MS instrumentation.

Unfortunately it was not possible to determine levels of surfactants in waste waters using LC/MS techniques for several reasons. LC-PB-MS showed excellent compatibility with a chromatographic method based on elution of APEO type molecules from a cyano column with non polar solvents (Section 4.3). However, the limit of detection of this method (0.5 mg on column for individual APEOs) was not practical in terms of environmental analysis where total surfactant concentrations are typically 0.1 mg L^{-1} and below. $M^{\cdot +}$ type molecular ions were produced in EI^+ mode. However, with the instrument in EI^- mode no response was observed. Although it is possible to separate APEO type molecules using polar solvents (Section 4.6) it was not possible to produce mass chromatograms with satisfactory separation. Thus presumably the particle beam interface reduced the resolution of individual APEO oligomers prior to mass spectrometric detection. On a practical level it seemed as if surfactant was retained in the desolvation chamber and did not pass through it unhindered to the ion source of the mass spectrometer. During cleaning of the interface large deposits of surfactant could be seen on the nozzle cone and skimmers (Section 3, Figure 3.3). Nebulizer gas pressure, distance of nebulizer probe into desolvation chamber and desolvation chamber

temperature conditions were all experimented with but peak resolution and indeed sensitivity could not be altered to any great degree. Although band broadening effects when using the particle beam interface have been observed before[4], surfactant formulations seem to be particularly prone to this phenomenon. During this work it was observed that the broadening of peaks became worse after each analysis until a point was reached where no peak resolution at all was achieved. This undoubtedly was due to the deposition of surfactant onto the walls of the desolvation chamber which decreased the size of the nozzle aperture. This would increase residence time within the desolvation chamber and therefore increase the probability of adsorption onto the surface of its walls.

Post column addition experiments of solvent and salts were carried out to try and increase the sensitivity of the LC-PB-MS (Section 7.1.2). It can be seen from the results displayed in Figures 7.6 to 7.11 that the addition of solvents and /or solvents and buffers did not significantly increase the sensitivity of this method. Generally peaks obtained after the 'post column addition' were smaller than for hexane (one of the column eluants used in the HPLC method, section 4.3) alone, indicating that the non-polar character of hexane plays a more important role in the transportation of sample into the ionisation chamber of the mass spectrometer than the presence of salts. The presence of salts has been described as being of some use in increasing analyte signal[3].

The sensitivity of the thermospray interface used in this project for APEO in TSP^+ mode was in the region of 5μg on column for individual oligomers and 2μg on column for individual homologues of LAS in full scan mode. For LAS TSP^+ ionisation was approximately 16 more sensitive than for TSP^- ionisation. In TSP^+ mode $(M+NH_4)^+$ type molecular adduct ions were produced for both LAS and APEO. In TSP^- mode it was not possible to produce negative ion spectra of non-ionic surfactants. However, anionic LAS produced $(M-H)^-$ type molecular ions. Unfortunately these values are not low enough to promote the use of LC-TSP-MS for the environmental determination of surfactants. It was not possible to determine the interface's ability to produce resolved mass chromatograms of individual APEO and LAS molecules due to instrumentational failure before this method was optimised.

LC/APcI/MS was compatible with a reverse phase chromatographic method, (Section 4.6). In positive ion mode the API interface produced sodium adduct molecular ions, $(M-(x-1)+xNa)^+$ (where x = number of sodium ions), and in negative ion mode anionic LAS produced molecular ions of the $(M-H)^-$ type. It was therefore possible to produce total ion chromatograms of a mixture of APEO and LAS type surfactants in a single chromatographic run. In positive ion mode it was possible to detect both anionic LAS and non-ionic APEO molecules. However, in negative ion mode only the anionic species are observed. The relative sensitivity for the detection of anionic surfactant is approximately 5:1 in favour of the positive ion mode, (via peak area comparison). In full scan positive ion mode the LOD for individual anionic LAS homologues and individual APEO oligomers was at least 50 ng on column. Therefore it would seem possible to use this method to detect surfactants in environmental waste waters. However, due to the complexity of environmental samples and the limited structural information obtained using this type of interface it may be necessary to use MS/MS experiments to obtain a definite positive identification. Obviously further experiments are required if this technique is to be used to obtain environmental information concerning LAS and APEO type surfactants. MS/MS studies need to be carried out to ascertain whether or not it would be possible to obtain further structural information on LAS and APEO type surfactants.

Also, instrumental performance with regard to linear range and limit of detection needs to be investigated. If the environmental 'profile' of the surfactants in question is to be monitored it will be necessary to investigate the mass spectra of the degradation products of these molecules (Section 2.3.1 and 2.4), in particular nonylphenol, short chain APEOs and alkylphenoxycarboxylic acids.

Chapter 7 References

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8.0) Conclusions

The present world-wide surfactant consumption is approximately 15 million metric tonnes per year[1]. More than 50% of the estimated figure is accounted for by soaps. The most common anionic surfactants, the linear alkylbenzene sulphonates (LAS), have a total production estimated at 1.8 million tonnes per year. This can be regarded as 25% of the total consumption of synthetic surfactants. In the industrialised world, i.e., the US, Europe and Japan, the figure for the total LAS consumption is approximately 1 million tonnes per year. LAS consists of a long chain non-polar hydrocarbon chain linked to a sulphonated benzene group which gives rise to the generic classification for this type of molecule as alkylbenzene sulphonates. Commonly, the alkyl chain varies in length from 11 to 14 carbon units. Non-ionic surfactants do not contain discrete charges. They do, however, contain highly polar, hydrophilic, ethoxylate groups. Most non-ionic surfactants are not single compounds but are rather the products of a reaction between ethylene oxide and organic compounds such as alkyl alcohols, alkylphenols and fatty acids. These reactions produce mixtures which have a range of ethoxymer chain lengths. The trivial nomenclature for these compounds is generally based on their average ethoxymer chain length with for example 'NP9' being used to describe a nonylphenol ethoxylate formulation with an average chain length of nine. The parent compound of this type of surfactant can be described as the alkylphenol polyethoxylate (APEO). An estimated 350 000 tonnes per year of APEOs are currently used in the US, Japan and Europe[2].

Obviously, due the widespread usage of these compounds it is necessary to monitor their environmental levels, particularly as they can be widely found in waterways. However, the work of Soto[3] demonstrated a weak oestrogenic response for nonylphenol has raised the profile of interest in the determination of surface active agents. Following this work, Jobling and Sumpter[4] have shown that alkylphenol ethoxylates and their associated degradation products are weakly oestrogenic in nature. Sharpe and Skakkebaek[5] have linked these compounds, along with other environmental pollutants, to the apparent decrease in sperm production by mammals and increase in sexual reproductive problems observed throughout the Western Hemisphere.

The chemical complexity of surfactants, which are often mixtures of related compounds differentiated by variation in carbon chain length, has meant that analytical methodology has concentrated on determination by class. Simple, relatively fast and inexpensive quantitative or semi-quantitative methods based on titrimetric or spectrophotometric methods, have been commonly used. Generally, however, methods which depend on a spectrophotometric reaction are no longer acceptable since they do not reliably reflect the concentration of either the individual surfactant types within the general classes of non-ionic, anionic or cationic, or the concentration of individual compounds (i.e. chain length distribution) within a class.

For these reasons work carried during this project has been centred around the use of liquid chromatographic methods which have allowed the separation of individual homologues/oligomers within a surfactant formulation. Initially, these methods were coupled to conventional ultra violet detectors which were used for identification and quantification purposes. It quickly became apparent that fluorescence detection would have to be used for detection at environmental concentrations. Although fluorescence detection offers a degree of selectivity it was felt that mass spectrometric detection would have to be included in any methodology produced to guarantee unambiguous quantification. To this end methods incorporating LC/PB/MS, LC/TSP/MS and LC/API/MS have been investigated. It was found that only LC/API/MS has the sensitivity necessary to detect surfactants at environmental levels unless extreme concentration regimes are adopted.

Due to the low levels of surfactants in surface water, typically 0.1 mg L^{-1} or below, pre-concentration was necessary. To this end solid phase extraction was utilised. Commercially available chemically bonded octadecyl reverse phase solid phase extraction cartridges allowed the clean-up and concentration of surfactants in (0.5 L) surface water. Experiments employing spiked reagent water (14 μg L^{-1} nonylphenol ethoxylate and 2 μg L^{-1} Nansa SS) indicated recoveries for each nonylphenol ethoxylate oligomer ranging from 54 to 95% and for each homologue of Nansa SS ranging from 69 to 149%. The methodology devised was used to measure the concentration of APEOs in river and harbour waters. Total APEO oligomer

concentration in a grab sample taken from the River Rother, near Sheffield, South Yorkshire, was found to be 52.2 $\mu g\ L^{-1}$. A grab sample taken from the entrance of Langstone Harbour was found to contain a total APEO oligomer concentration of 225 $\mu g\ L^{-1}$.

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Symposia and Meetings attended.

Restek capillary GC training course. November 1995.

RSC Analytical Division R&D topics meeting.

University of Bradford, 13-14 July 1994.

Poster Presentation.

BMSS 20 th annual meeting

12-15th September, 1994

Poster Presentation.

RSC Analytical Division R&D topics meeting.

University of Hertfordshire, 18-19 July 1995.

Oral presentation given.

43 rd ASMS annual meeting

Atlanta, Georgia

21st-26st May, 1995,

Poster Presentation.

Environmental Analysis meeting

Sheffield Hallam University, 1995

Oral Presentation given.

In addition the author has attended a series of departmental seminars on a variety of topics.

Published Papers

During the course of this study two papers have been published. The references for these papers are given below, followed by copies of each.

MR Clench, SD Scullion, R Brown and J White, Spectroscopy Europe, 6, 16, 1994.

SD Scullion, MR Clench, M Cooke and A Ashcroft, Journal of Chromatography A, 733, 207, 1996

Liquid chromatography/mass spectrometry in environmental analysis

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Introduction

The selectivity afforded by the use of mass spectrometry as a detection method for liquid chromatography has proved attractive in many areas of analysis. Its widespread adoption in environmental applications has, however, been hampered by the plethora of interface types and the deserved reputation of earlier interfaces for unreliability and poor sensitivity. However, the advent of more robust interfaces in the 1980s and 90s has led to several groups applying LC/MS techniques in the environmental area.

In this article rather than attempt an exhaustive review of LC/MS techniques, brief descriptions of the current "state of the art" will be offered and specific application areas examined. The techniques to be covered are Thermospray (TSP), Particle Beam (PB), Atmospheric Pressure Chemical Ionisation (APCI) and Electrospray/IonSpray (ES/ISP). Applications in the analysis of polycyclic aromatic hydrocarbons, surfactants, dyestuffs and pesticides will be described. For a complete description of the various interface types the interested reader is referred to books by Ardrey[1] and Yergey *et al.*[2]

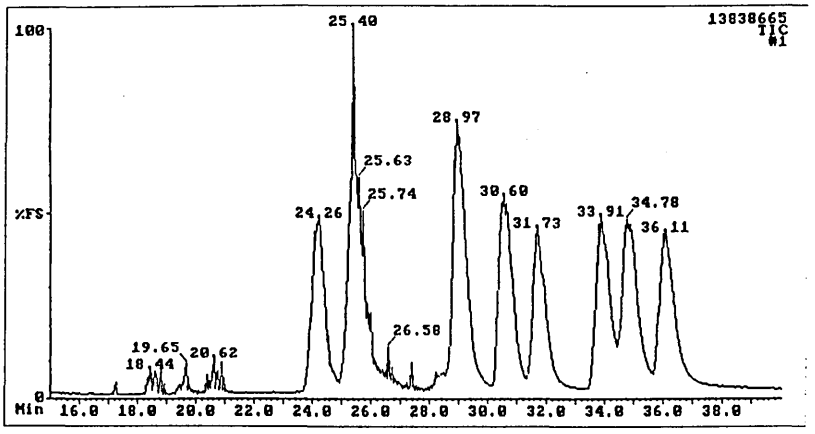


Figure 2. Determination of 16 PAHs by particle beam LC/MS (selected ion monitoring mode) (100 ng per component on column).

Polycyclic aromatic hydrocarbons

Determination of polycyclic aromatic hydrocarbons (PAHs) in a range of matrices is required owing to their carcinogenic properties. Several groups have applied the particle beam technique (Figure 1) to the analysis of (PAHs).[3,4,5]

The particle beam technique is intrinsically the simplest of the available interfaces. Eluant from the HPLC column is nebulised and sprayed into a heated desolvation chamber. The mixed nebulising gas, mobile phase and sample stream enters the dual stage momentum separator where the majority of the mobile phase and nebulising gas is removed. This results in a stream of "snowballs" of sample clustered with a few solvent molecules entering a

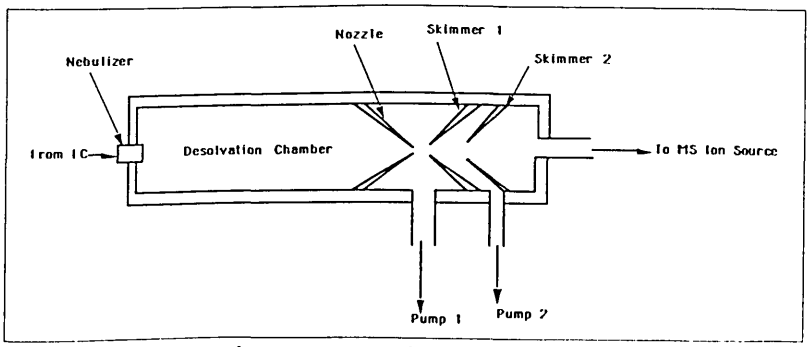
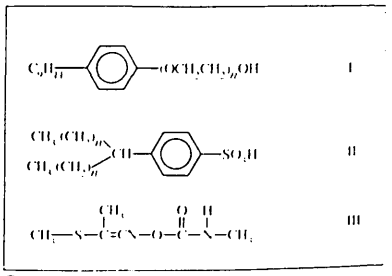


Figure 1. Schematic representation of a particle beam LC/MS interface.



Structures.

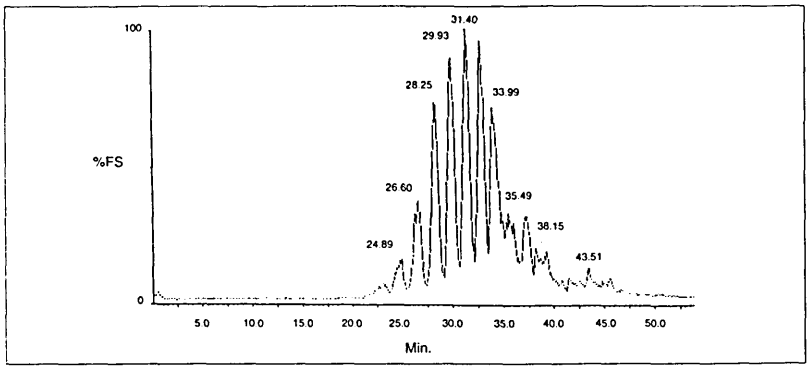


Figure 3(a). Analysis of a commercial nonyl phenol ethoxylate type non-ionic surfactant by particle beam LC/MS.

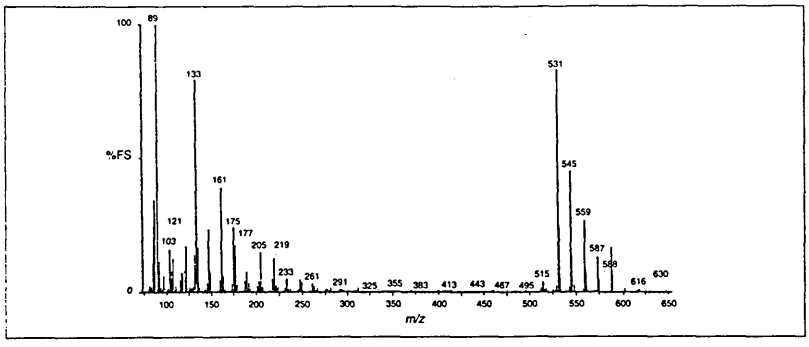


Figure 3(b). Mass spectrum of peak at t_r = 31.4 min. from the above chromatogram (identified as the nine ethylene oxide unit containing oligomer).

conventional electron impact (EI) or chemical ionisation (CI) ion source.

The data shown in Figure 2 come from the analysis of the standard US EPA mixture of 16 PAHs using PB/LC/MS in the selected ion monitoring (SIM) mode. In this analysis we have attempted to minimise the reduction in sensitivity of the PB/LC/MS technique with increasing aqueous content in the mobile phase, by adding 0.2 ml min^{-1} of acetonitrile post column. In this data where each component was injected at the 100 ng level on column, the sensitivity of, for example, benz(α)anthracene (t_r = 24.3 min.) which normally elutes at 25% aqueous content is increased by a factor of three. However, as can be clearly seen the relative sensitivity of PB/LC/MS for PAHs still dramatically increases with increasing RMM. The earlier eluting compounds, e.g. acenapthene (RMM 154), are not detectable at these levels and the relative sensitivity for chrysene (RMM 202, t_r = 20.62 min.) is only around 10% of that of benz(β)fluoranthene (t_r = 28.97 min., RMM 252). In general the limits of detection achievable with this technique do not approach those of the normally used fluorescence detection methods for the smaller compounds although they are comparable above RMM 252.

Surfactants

The determination of surfactants in drinking, surface and groundwater is currently a very "live" topic of analysis. It has been shown[6] that non-ionic surfactants of the alkylphenol ethoxylate type, e.g. Structure I, and their degradation products exhibit weak oesterogenic properties and may be implicated in the increase in male infertility in the western world.

The analysis of non-ionic surfactants of this type is made complex by the fact that they are formulated as structures such as Structure I with a distribution of ethoxylate chain length. The length of the alkyl chain may also vary. Analysis may be carried out by spectrophotometric or LC methods employing conventional detectors. However, neither of these techniques offers unambiguous identification of surfactant type and chain length. Hence LC/MS has been investigated as a possible method of analysis.

Particle beam LC/MS has been used in the determination of non-ionic surfactants in surface[3] and drinking water.[7] Clark *et al.*[7] determined alkylphenol ethoxylates in drinking water after continuous liquid/liquid extraction of 500 litres of finished water. The resulting extract was then analysed by PB/LC/MS and detection limits of ng l^{-1} were obtained. Figure 3(a) shows the total ion chromatogram obtained from the analysis of a standard NP9 nonylphenol ethoxylate carried out in our own laboratory, by PB/LC/MS. Figure 3(b) shows the mass spectrum obtained from the peak eluting at t_r = 31.4 min. The EI mass spectrum obtained shows both a molecular ion and structurally significant fragmentation allowing this peak to be assigned to be a nine ethylene oxide unit containing nonylphenol ethoxylate.

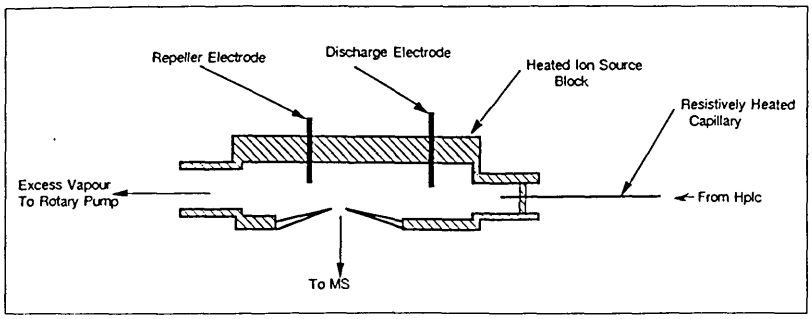
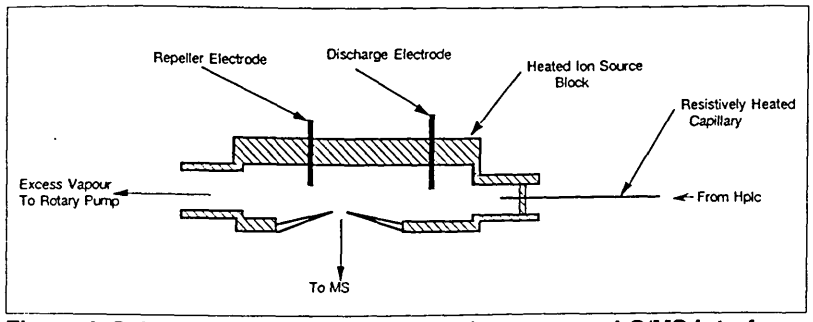


Figure 4. Schematic representation of a thermospray LC/MS interface.

The thermospray interface (Figure 4) has also been successfully applied to the analysis of surfactants. In thermospray LC/MS the HPLC eluant is sprayed through a resistively heated capillary into the heated thermospray ion source. The droplets so formed then shrink under the action of heat from the ion source block. When the droplets have shrunk such that the coloumbic repulsion between charged species in the droplet exceeds the surface tension of the droplet, ions are ejected into the gas phase. This process is known as ion evaporation and is facilitated by the addition of a volatile electrolyte (often ammonium acetate) to the mobile phase or post column. The ammonium actetate may also serve as a source of NH_4^+ ions for conventional chemical ionisation processes to occur in the gas phase. Thermospray spectra generally show reduced fragmentation compared to EI spectra and are "CI like" showing either protonated molecular species $(M + H)^+$ or adduct ions $(M + NH_4)^+$ in positive ion mode.

Papers describing the use of thermospray in the analysis of surfactants have appeared from Evans *et al.*[8] and Schroder.[9] Evans showed that the thermospray mass spectra of linear primary alcohol ethoxylates are characterised by intense $(M + NH_4)^+$ ions, with little structural information. They established limits of detection in the low nanogram region for each species analysed. The thermospray method was applied to the analysis of surface water and sewage effluent samples by using solid phase extraction as a method of sample preparation. The method was validated for concentrations of individual alcohol ethoxylates in the range 0.06 to 2.17 ppb by spiking 1 litre samples.

Schroder[9] examined the concentrations of a range of surfactants in sewage treatment plants by thermospray LC/MS using flow injection mode and a tandem mass spectrometer. In flow injection mode the sample is injected into the mobile phase which is pumped into the interface without passing through a column. The resulting mass spectrum can be regarded as a "survey" of components present. This technique is obviously best suited to "soft ionisation" techniques such as thermospray which yield molecular species in abundance. Figure 5 shows the mass spectrum obtained from a sample of waste water treatment plant inffluent by this technique. In this positive ion spectrum ions corresponding



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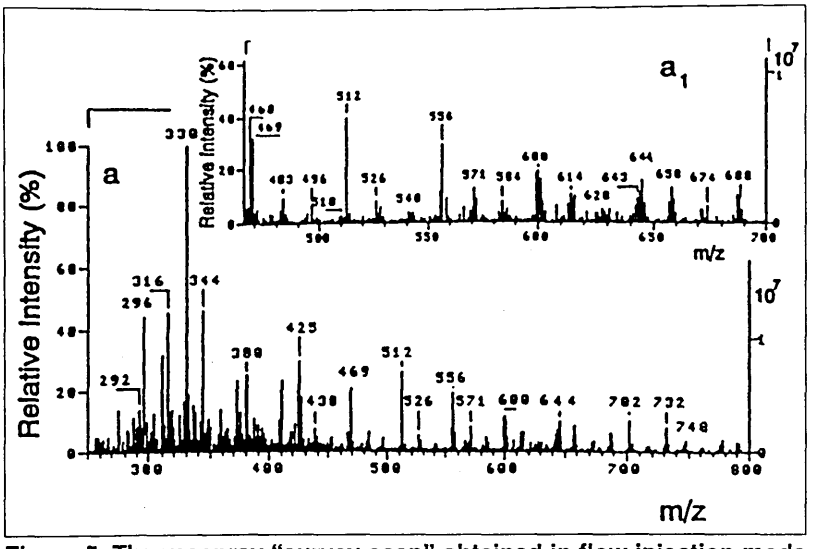

Figure 5. Thermospray "survey scan" obtained in flow injection mode from a waste water extract. Note the series of ions at *m/z* 316, 330, 344 corresponding to anionic surfactants and the series 44 daltons apart beginning at *m/z* 468 corresponding to non-ionics. (Reproduced from Reference 9 by Permission of Elsevier Science BV).

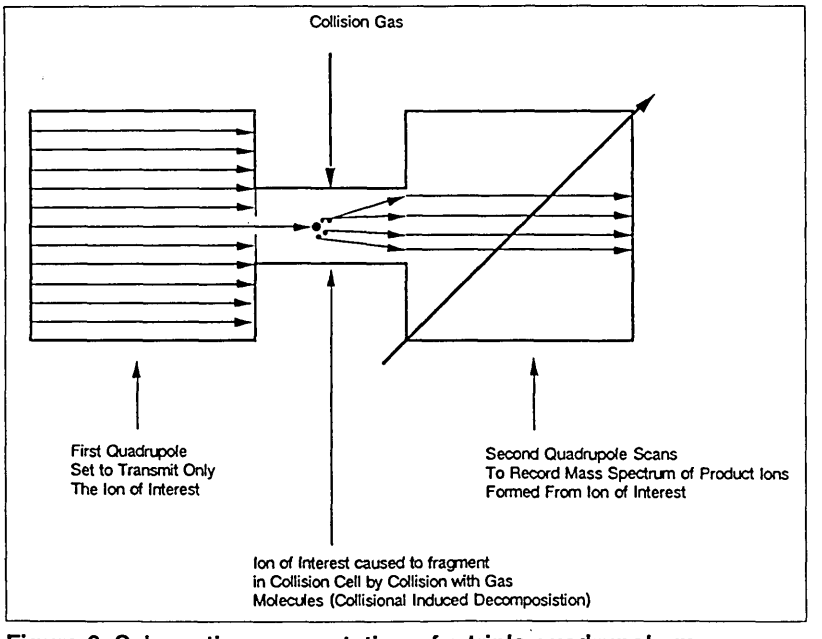

Figure 6. Schematic representation of a triple quadrupole mass spectrometer, showing the experimental set up for obtaining product ion scans.

to the presence of a series of linear alkyl benzene sulphonates (Structure II) (an anionic surfactant formulation) can be observed at *m/z* 316, 330 and 344. Also the series of ions 44 daltons apart beginning at *m/z* 468 may be assigned to surfactant and represent $(M + NH_4)^+$ ions from the oligomers of a polyethylene glycol ether type non-ionic surfactant.

Confirmation of this assignment was obtained by acquiring product ion spectra from some of these ions using the tandem mass spectrometer. This technique is shown diagrammatically in Figure 6, as it is performed on a triple quadrupole mass spectrometer, the type of tandem instrument used in this work. The first quadrupole mass filter is set to transmit the ion of interest (in this case the $(M + NH_4)^+$ ion occurring at *m/z* 468) into the collision cell, where collisions with a gas cause it to fragment. The "product ions" thus formed are then separated by mass to charge ratio in the second quadrupole mass filter and a mass spectrum recorded. Figure 7 shows the product ion spectrum record from the *m/z* 468 ion. As can be seen sufficient structural information is now obtained in order to allow interpretation. This technique along with the other experiments available on tandem instruments is often used to generate additional structural information.

Dyestuffs

Straub *et al.*[10] have produced an interesting paper comparing the performance of three different LC/MS techniques for the analysis of azo dyes. They compared results obtained from the use of thermospray and particle beam from those obtained from electrospray. In the electrospray technique the (Figure 8) eluant from the LC column is passed through a capillary held at around 5 kV. This high potential creates a fine spray of charged droplets. These droplets are broken up either by collisions with a "curtain gas" or by heat or a combination of the two. Ionisation then proceeds from the smaller droplets via ion evaporation as previously described for thermospray. This technique is more often applied in the analysis of high molecular weight biopolymers although Henion *et al.*[11] have also used electrospray MS, interfaced to capillary electrophoresis and in a later paper[12] with liquid chromatography in conjunction with a high flow rate thermal nebuliser system, to analyse dyestuffs.

A comparison of the type of spectra obtained is shown in Figures 9(a, b and c). The expected differences in fragmentation between thermospray and particle beam in electron impact mode, are observed. Figure 9(c) also exhibits the phenomenon of the generation of multiply charged ions commonly seen in electrospray mass spectra. In this case the dye Acid Orange 10 which contains two sodium sulphonate groups exhibits dominant peaks arising from doubly negatively charged ions, when analysed by electrospray ionisation mass spectrometry in negative ion mode.

Pesticides

Each of the techniques described above has been applied to the analysis of pesticides. Two US EPA methods have been published describing the use of LC/MS for this application, Method 8321A describes the use of thermospray LC/MS for the analysis of chlorinated phenoxy acid herbicides and Method 553 the use of particle beam in the analysis of nitrogen containing compounds.

A paper by Pleasance *et al.*[13] provides an interesting comparison between the interfaces already described and the

more recently developed technique of Atmospheric Pressure Chemical Ionisation (APCI). In this paper a comparison between the techniques for the analysis of N-methyl carbamate pesticides is presented.

In the APCI interface (Figure 10), pneumatic nebulisation is employed to convert the mobile phase into droplets. These are then carried by a sheath gas through a heated tube where vaporisation of the solvent and analyte takes place. The solvent and sample vapour now flows towards the ion formation region where a corona discharge initiates chemical ionisation at atmospheric pressure. In this APCI technique the vaporised mobile phase is used as the reagent gas, unlike conventional CI where reagent gas is introduced into the ion source.

A comparison of spectra obtained for methomyl (Structure III) is shown in Figure 11, and is indicative of the type of spectra that are obtained from each of the techniques. The "ion spray" (electrospray with a nebuliser) spectrum Figure 11(a) is dominated by the $(M + H)^+$ ion at m/z 163. In order to increase the amount of structurally significant fragmentation it was necessary to use either collisionally induced decomposition in the source [Figure 11(b)] or an MS/MS product ion scan as previously described [Figure 11(c)]. The APCI spectrum [Figure 11(d)] again shows an $(M + H)^+$ ion but in this case appreciable fragmentation is observed. The particle beam spectra are conventional EI and CI spectra. In Figure 11(g) the thermospray spectrum is dominated by intense $(M + NH_4)^+$ and $(M + H)^+$ ions with little fragmentation. This data is very typical of the type of spectra generated by each of the techniques.

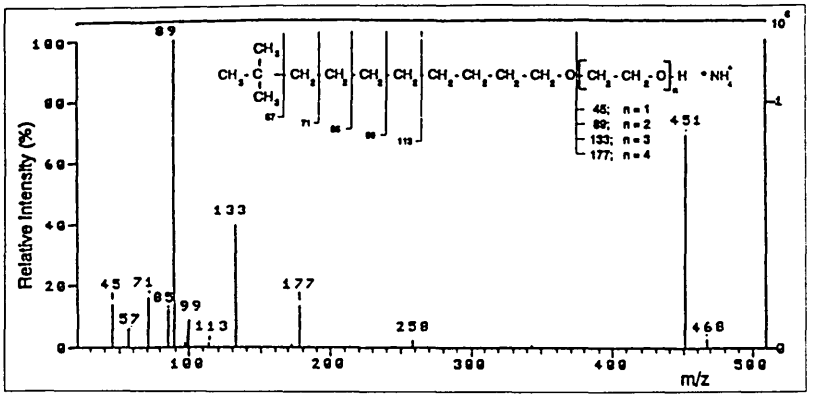

Figure 7. Product ion spectrum from the peak at *m/z* 468 in Figure 5. The structurally significant fragmentation induced in this type of experiment allows unambiguous identification of this ion as arising from a polyethylene glycol type non-ionic surfactant. (Reproduced from Reference 9 by Permission of Elsevier Science Publishers BV).

Table 1 (adapted from Reference 13) shows the absolute limits of detection for a methyl carbamate pesticides obtained by Pleasance *et al.* The trends shown again provide typical relative values. Taking, as an example, methomyl and scaling the sensitivity obtained to the particle beam EI results, we arrive at approximate values for the relative sensitivities of APCI (4250) > ISP (635) > TSP (90) > PB(EI) (1). These figures amply illustrate the reasons for the current interest in the APCI technique, the sensitivity obtainable with this technique is often a couple of orders of magnitude greater than that for either thermospray or particle beam.

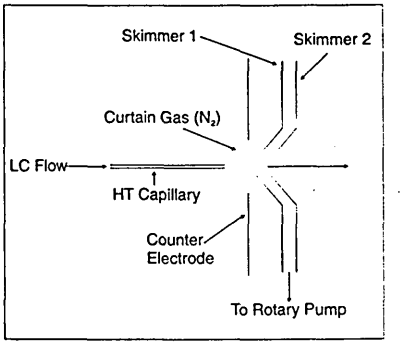

Figure 8. Schematic representation of an electrospray LC/MS interface. (Note in the "ion spray" version of this technique the creation of a spray of droplets from the LC eluant is assisted by the use of a pneumatic nebuliser.)

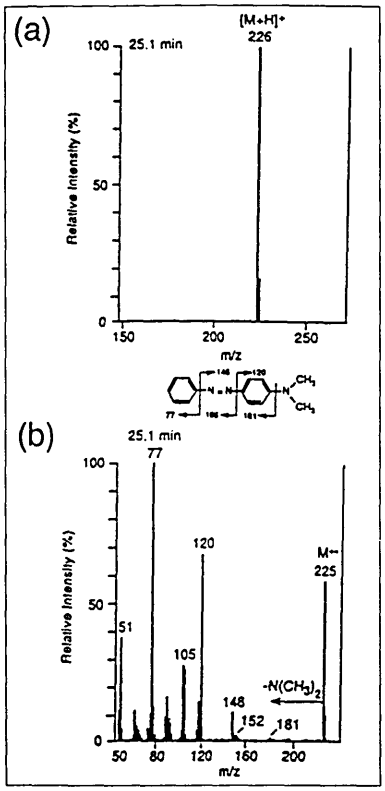

Table 1. Comparison of limits of detection obtained for methomyl by different LC/MS techniques. Adapted from Reference 13 by Permission of Elsevier Science Inc., copyright 1992 The American Society for Mass Spectrometry.

Compound name	Ion Spray	APCI	Particle Beam (EI)	Thermospray
Methomyl	0.40	0.06	254	2.8
Aldicarb	1.5	0.07	>500	0.9
Aldicarb sulphoxide	1.0	0.18	152	2.5
Aldicarb sulphone	1.8	0.1	79	1.1
Carbaryl	1.0	0.05	10	0.8
Methiocarb	0.6	0.07	19	0.8
Carbofuran	0.3	0.05	55	0.8
3-hydroxy-carbofuran	1.0	0.07	7	1.1

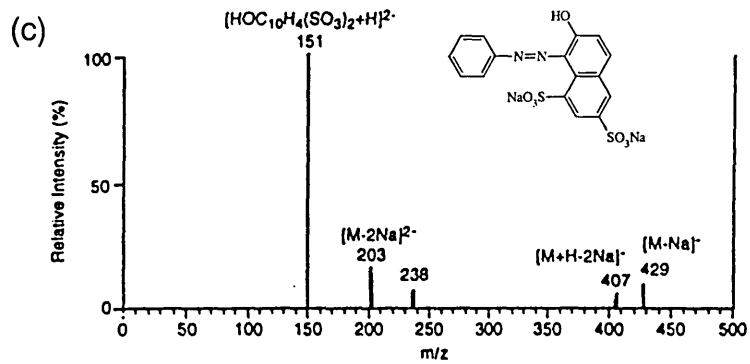

Figure 9. Comparison of mass spectra obtained from dyestuffs. (a) Thermospray positive ion mass spectrum of Solvent Yellow 2. (b) Particle Beam (EI) mass spectrum of Solvent Yellow 2. (c) Electrospray negative ion mass spectrum of Acid Orange 10. Adatpted from Reference 10 with permission of Elsevier Science Publishers BV.

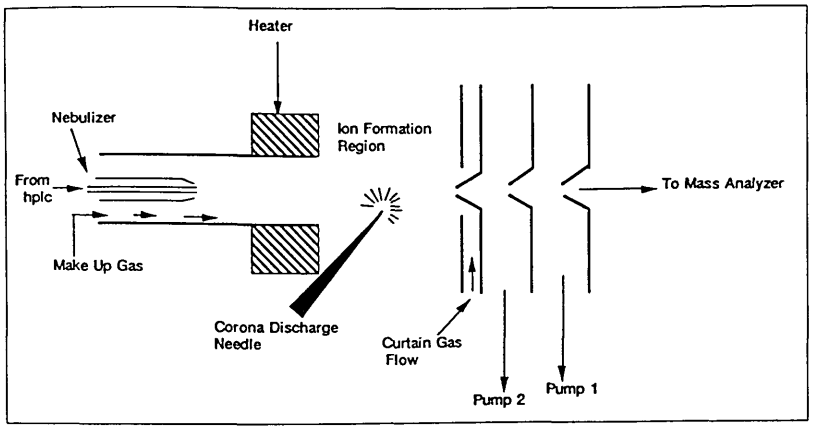

Figure 10. Schematic representation of an atmospheric pressure chemical ionisation (APCI) LC/MS interface.

Conclusions

Liquid chromatography mass spectrometry is a technique that has offered a great deal of promise in environmental analysis for a number of years. It is now starting to deliver. Developments in interface technology have led to the current situation where a choice of reliable techniques is on offer. Each has found useful areas of application. The analytical scientist entering this area for the first time still has to make choices, e.g. the trade off between sensitivity and structural information, when choosing to use particle beam or APCI. However, there is now enough information in the literature for these to be informed decisions rather than "inject and hope".

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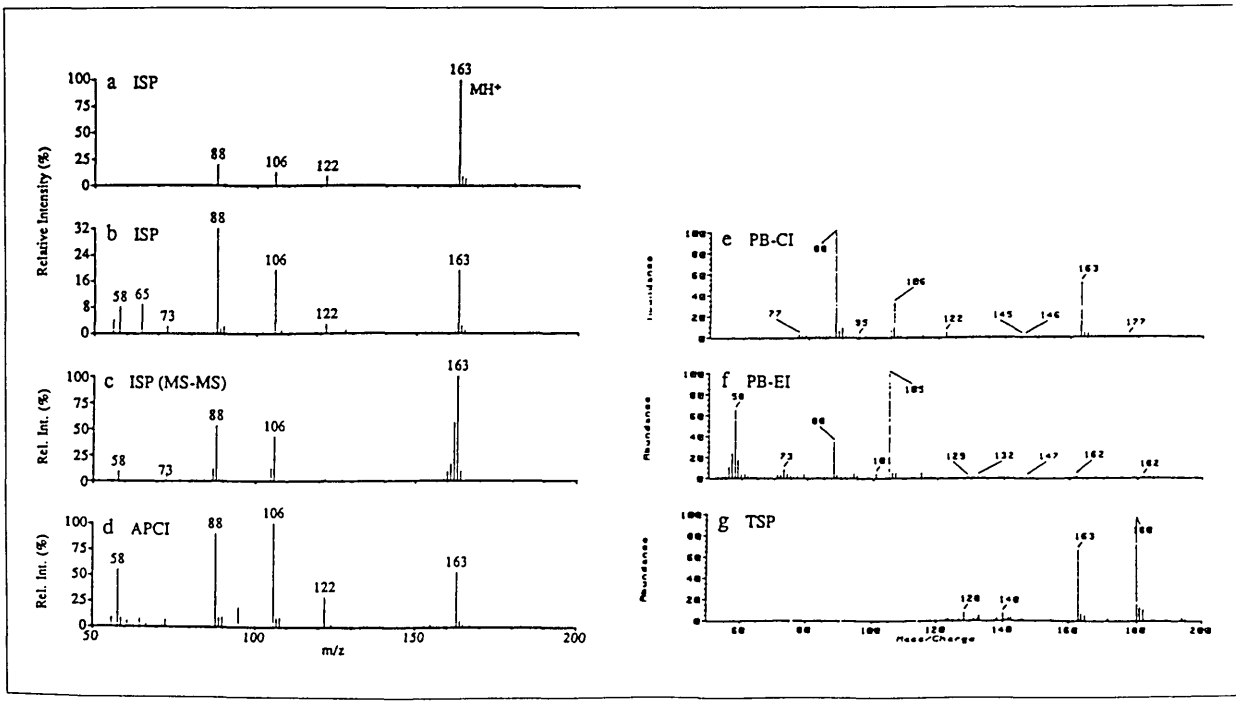

Figure 11. Comparison of mass spectra obtained from methomyl (RMM = 162). (a) Ion spray, (b) ion spray with "in source" collisional induced decomposition, (c) ion spray MS/MS (product ion scan), (d) APCI, (e) particle beam (CI), (f) particle beam (EI), (g) thermospray. Reproduced by Permission of Elsevier Science Inc. from Reference 13, copyright 1992 The American Society for Mass Spectrometry.

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Determination of surfactants in surface water by solid-phase extraction, liquid chromatography and liquid chromatography–mass spectrometry

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Abstract

Determination of surfactants in surface waters is required owing to their toxicity to aquatic micro-organisms and potential oestrogenic effects. We have investigated methods for the determination of two types of surfactants by solid-phase extraction with C_{18} and SAX cartridges. HPLC separation of anionic and non-ionic surfactants on a C_1 (TMS) column has been achieved and detection via both fluorescence and liquid chromatography–mass spectrometry (atmospheric pressure chemical ionisation interface) is reported. Recoveries are approximately 100% using the method developed with detection limits of 50 ng on column for detection by fluorescence. Alkylphenol ethoxylates have been detected in samples taken from the River Rother in South Yorkshire, UK, at levels of 5.6 $\mu g\,l^{-1}$ using the described methodology.

Keywords: Water analysis; Liquid chromatography–mass spectrometry; Environmental analysis; Surfactants; Alkylphenol ethoxylate surfactants; Ethoxylates

1. Introduction

Surfactants are a group of chemicals which are widely used both industrially and domestically, hence they have become ubiquitous in the environment. Surfactants interact with other molecules to confer either hydrophilicity or hydrophobicity according to type of surfactant. Surfactant molecules may be divided into three individual main classes. These are anionic surfactants, e.g. alkylphenol sulphonates, cationic surfactants, e.g. quaternary ammonium salts, and non-ionic surfactants such as those produced by reacting alkylphenols with ethylene oxide.

The present world-wide surfactant consumption is approximately 15 million metric tonnes per year [1]. More than 50% of the estimated figure is accounted for by soaps. The most common anionic surfactants, the linear alkylbenzene sulphonates (LAS), have a total production estimated at 1.8 million tonnes per year. This can be regarded as 25% of the total consumption of synthetic surfactants. In the industrialised world, i.e., the US, W. Europe and Japan, the figure for the total LAS consumption is approximately 1 million tonnes per year. LAS consists of a long non-polar hydrocarbon side chain linked to a sulphonated benzene group which gives rise to the generic classification for this type of molecule as alkylbenzene sulphonates. Commonly, the alkyl chain varies in length from 11 to 14 carbon units.

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Non-ionic surfactants do not contain discrete charges; they do, however, contain highly polar, hydrophilic, hydroxyl groups. Most non-ionic surfactants are not single compounds but rather are the products of a reaction between ethylene oxide and organic compounds such as alkyl alcohols, alkylphenols and fatty acids. These reactions produce mixtures which have a range of ethoxymer chain lengths. The trivial nomenclature for these compounds is generally based on their average ethoxymer chain length with for example 'NP9' being used to describe a nonylphenol ethoxylate formulation with an average ethoxymer chain length of nine. The parent compound of this type of surfactant is described as the alkylphenol polyethoxylate (APEO). An estimated 350 000 tonnes per year of APEOs are currently used in the US, W. Europe and Japan [2].

Currently widespread interest in the determination of surface active agents in water has been generated by the work of Jobling and Sumpter [3], who have shown that alkylphenol polyethoxylates and their associated degradation products are weakly oestrogenic in nature. This work developed research originally carried out by Soto [4], who demonstrated a weakly oestrogenic response for nonylphenol. Sharpe and Skakkebaek [5] have linked these compounds, along with other environmental pollutants, to the apparent decrease in sperm production and increase in sexual reproductive problems observed throughout the Western Hemisphere.

The chemical complexity of surfactants, which are often mixtures of related compounds differentiated by variation in carbon chain length, has meant that analytical methodology has concentrated on determination by class. Simple, relatively fast and inexpensive quantitative or semi-quantitative methods based on titrimetric or spectrophotometric methods, have been commonly used. Generally however, methods which depend on a spectrophotometric reaction are no longer acceptable since they do not reliably reflect the concentration of either the individual surfactant types within the general classes of non-ionic, anionic or cationic, or the concentration of individual compounds (i.e. chain length distribution) within a class.

Major developments have been made in the identification and quantification of surfactants by gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC). The GC–MS work is typified by the work of Stephanou et al. [6] where capillary GC–MS in conjunction with both electron impact and chemical ionisation was described. Many papers describing the use of HPLC for the analysis of surfactants have appeared [7–11]. Both normal- and reversed-phase separations have been used in conjunction with UV and fluorescence detection.

Marcomini and co-workers [12,13] have described the determination of alkylphenol ethoxylates and linear alkylbenzene sulphonates (LAS) in water. Isolation of surfactants from aqueous solution was achieved by solid-phase extraction [13,14]. Chromatographic analysis was by a combination of normal- and reversed-phase HPLC with either fluorescence or UV detection. The described methodology, whilst allowing determination of the homologue distribution for LAS, does not effect chain length separation for alkylphenol ethoxylates.

Castles et al. [15] have described a method for the determination of LAS. This method utilises a trimethylsilyl (TMS) column and an isocratic HPLC method using THF with added sodium perchlorate. Wang and Fingus [16] have described a method for the determination of alkylphenol ethoxylate using an isocratic HPLC method employing a TMS column with a methanol–ammonium acetate mobile phase. We have developed this method in order to allow the separation of two classifications of surfactants simultaneously including individual oligomer separation for the anionic alkylbenzene sulphonates and the non-ionic alkylphenol ethoxylates.

Growing attention is being paid to the analysis of the degradation products of commonly used surfactants. The biotransformation of LAS leads to the formation of mono- and dicarboxylic sulphophenyl acids (SPC). These are formed via the ω-oxidation of the molecules alkyl chain. β-Oxidation results in a shortening of this chain by two carbon units at a time [17]. APEOs are degraded to shorter-chain ethoxylates, alkylphenoxy acids and nonylphenol under aerobic conditions via ω-oxidation [18]. In this case, however, the biotransformation process is not fully understood. Data concerning the detection of surfactants and their degradation products have been published [18–21].

The advent of suitable interface technology has also led ourselves and other groups to investigate liquid chromatography–mass spectrometry for the determination of surfactants. Particle-beam liquid

chromatography–mass spectrometry (PB-LC–MS) has been used in the determination of surfactants in drinking water. Clark et al. [22] determined alkylphenol ethoxylates in drinking water after continuous liquid–liquid extraction of 500 l^{-3} of finished water. The resulting extract was analysed by PB-LC–MS and low detection limits of parts per trillion (10^{-12}) were obtained. Although this method produces excellent sensitivity the initial 500 l^{-3} sample size taken would appear to make it impractical for routine use. A second limitation of the described methodology arises from the use of a chromatographic system which does not achieve individual oligomer separation.

Papers describing the use of thermospray LC–MS in the analysis of surfactants have appeared from Evans et al. [23] and Schroder [24]. Evans et al. showed that the thermospray mass spectra of linear primary alcohol ethoxylates are characterised by intense $[M+NH_4]^+$ ions, with little or no structural information. They established limits of detection in the low nanogram region for each species analysed. The thermospray method was applied to the analysis of surface water and sewage effluent samples by using solid-phase extraction as a method of sample preparation. The method was validated for concentrations of individual alcohol ethoxylates in the range 0.06 to 2.17 $\mu g\, l^{-1}$ by spiking 1-l samples.

Schroder [24] examined the concentrations of a range of surfactants in sewage treatment plants by thermospray LC–MS using flow injection as a means of sample introduction. The resulting mass spectrum can be regarded as a 'survey' of components present. Using this technique it was possible to identify both anionic and non-ionic surfactants in waste water plant influent. Liquid chromatography–electrospray mass spectrometry of surfactants has been investigated by Crescenzi et al. [25] after their extraction from raw sewage, treated water, river water and drinking water. The analysis of municipal water revealed the presence of analytes at ng l^{-1} levels. Pattanaargson et al. [26] have used APCI-MS as a means of determination of oligomer distribution, but have not applied this technique to environmental samples.

In all of this previous work, with the exception of the experiments carried out by Crescenzi et al. [25], some compromise in either practical sample volumes or chromatographic integrity or both has been required in order to use mass spectrometry for detection. In the particle beam work large initial sample volumes were required to achieve the desired sensitivity. In the thermospray work optimum HPLC conditions for the separation of individual oligomers were not used as they were not compatible with this type of interface. The APCI work used only direct introduction of non-ionic surfactant samples. No chromatographic separation of oligomers was attempted before introduction.

We now report a method for the determination of linear alkyl benzene sulphonates and alkylphenol ethoxylates in water using a simple solid-phase extraction procedure followed by isocratic HPLC separation on a C_1 column. Data from a preliminary investigation of the compatibility of the mobile phases used with the C1 column and liquid chromatography–mass spectrometry with an atmospheric pressure chemical ionisation (APCI) interface is reported. The analysis of the degradation products of anionic and non-ionic surfactants has not been addressed using this method.

2. Experimental

2.1. Samples

Standard solutions of Triton X100 (an octylphenol ethoxylate) (Sigma Chemicals, Poole, Dorset, UK), Synperonic 9 (a nonylphenol ethoxylate) (a gift from ICI Materials Research Centre, Wilton, Middlesborough, UK) and Nansa SS (a commercial dodecyl alkylbenzene sulphonate formulation, Albright and Wilson, Castleford, UK) were prepared by weighing and dissolution in HPLC mobile phase (details below)

Grab samples of surface water (2 l) were taken from the River Rother in South Yorkshire, UK (map reference OSS 111 435 877). The samples were stored in amber glass bottles at 4°C prior to analysis. At the time of sampling water pH was found to be 6.9

2.2. Extraction and clean-up of samples

Water samples were extracted using either a Shandon (Warrington, Cheshire, UK) Hypersep C_{18} solid-phase extraction cartridge (500 mg packing

material) alone or placed in series with a SAX solid-phase extraction cartridge (Whatman, NJ, USA). The cartridges were first conditioned with 7 ml of methanol and then 7 ml of reagent water. Samples were then taken through under vacuum and the cartridges air-dried. The cartridges were washed with 12 ml of water–methanol (70:30). Elution of surfactants was achieved using 3 cm^3 of methanol. Finally, the sample extract was blown down to dryness using a steady flow of nitrogen. The samples were dissolved in the appropriate HPLC mobile phase prior to analysis.

2.3. High-performance liquid chromatography

All HPLC analyses were performed on a Gilson 302 gradient pumping system. The column employed was a Hichrom (Reading, UK) 15 cm×4.6 mm I.D. TMS HPLC column. Fluorescence detection was carried out using a Hewlett-Packard 1046A (Palo Alto, CA, USA) fluorescence detector, λ_{ex}=220 nm and λ_{em}=302 nm for alkylphenol ethoxylates and λ_{ex}=220 nm and λ_{em}=290 nm for the linear alkyl benzene sulphonates. Data were output to a Shimazdu integrator. Injections (50 μl) were made using a Waters Wisp 712 autosampler.

Two isocratic mobile-phase systems were employed. System A: 65% water–35% acetonitrile with an overall buffer concentration of 0.065 *M* ammonium acetate; flow-rate, 0.7 ml min^{-1}. System B: 58% methanol–42% 0.008 *M* ammonium acetate (aq); flow-rate, 0.7 ml min^{-1}.

2.4. Liquid chromatography–mass spectrometry

All analyses were performed on a VG Organic (Altrincham, Cheshire, UK) Quattro triple quadrupole mass spectrometer, equipped with a VG Organic atmospheric pressure chemical ionisation (APCI) liquid chromatography–mass spectrometry interface. A Hewlett-Packard 1050 (Palo Alto, CA, USA) HPLC system was used in this case (see above for HPLC conditions). Data were acquired in full scan mode, range 50–1000 at 3 s/scan. Alternate positive ion/negative ion switching was used to acquire spectra of both positive and negative ion modes.

The APCI source conditions were as follows: corona discharge voltage, 3.5 kV; high voltage lens, 500 V; source temperature, 120°C; APCI probe temperature, 500°C.

3. Results and discussion

Fig. 1 shows a typical HPLC–fluorescence chromatogram from a standard mixture of commercial surfactants, Nansa SS and Triton X100. These data were obtained using mobile-phase system A. Via this simple isocratic experiment it was possible to separate the anionic and non-ionic surfactants and each of their individual homologues (LAS) and ethoxymers (OPEO). Peaks from the Nansa SS eluting at t_R 3.1–15.7 min and those for Triton at t_R 22.6 min onwards. Of interest are the side peaks on the alkylbenzene sulphonate homologue peaks. These may be observed at t_R 2.6, 9.4 and 12.9 min and we attribute them to positional isomers of each LAS homologue.

Fig. 2 displays a typical HPLC-fluorescence chromatogram from the same mixture of standards obtained using HPLC mobile-phase system B. Using this mobile-phase system it was also possible separate the anionic and non-ionic surfactants so that each individual homologue (LAS) and ethoxymer (OPEO) was observed. Peaks from the LAS eluting at t_R 4.5–10 min and those for OPEO at t_R 12 min onwards. However, this method does not show the

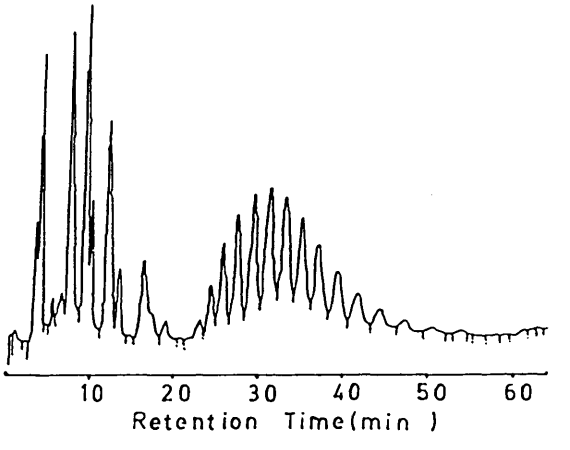

Fig. 1. HPLC chromatogram for the separation of linear alkyl benzene sulphonate and octylphenol ethoxylate surfactant standards on a C_1 (TMS) column using mobile-phase system A (see text for further details).

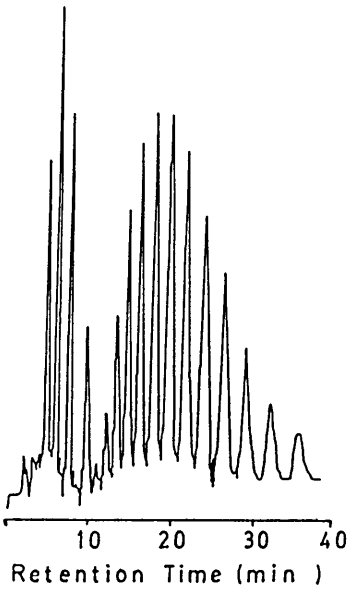

Fig. 2. HPLC chromatogram for the separation of linear alkylbenzene sulphonate and octylphenol ethoxylate surfactant standards on a C_1 (TMS) column using mobile-phase system B (see text for further details).

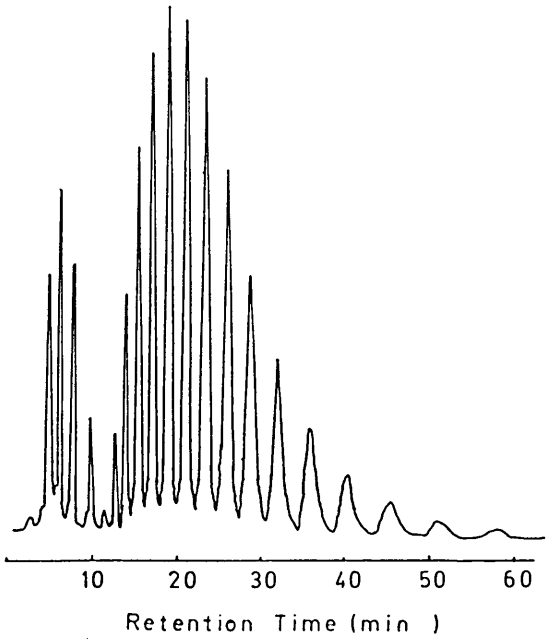

Fig. 3. HPLC chromatogram for the separation of linear alkylbenzene sulphonate and nonylphenol ethoxylate surfactant standards on a C_1 (TMS) column using mobile-phase system B (see text for further details).

positional isomers of the individual LAS homologues, but does allow more rapid analyses. Increasing chain length, be it alkyl in the case of LAS and ethoxylate in the case of OPEO, results in an increased retention time with respect to each component of the mixture. This statement is valid in both HPLC methods reported.

The chromatographic methodology developed is also applicable to mixtures of LAS and nonylphenol ethoxylates (Fig. 3). Peaks for Nansa SS elute from 5.5 min to 10 min and those for NP9 from 11.5 min onwards. The chromatographic resolution of the nonylphenol ethoxylates is not as good as that for the octylphenol ethoxylates but is still adequate for sample analyses. This phenomenon has also been observed by Wang and Fingus [27]. During our research it was noticed that some C_1 columns were able to resolve NPEO oligomers to a greater degree than others. This led to the selection of the column manufactured by Hichrom (Reading, UK). It was also noted during this work that a particular column's ability to resolve NPEO oligomers degrades more quickly that its ability to resolve OPEO oligomers. In situations where LAS, NPEO and OPEO were all present in the sample, the OPEO and NPEO would co-elute. It is in this situation that the specificity introduced by liquid chromatography–mass spectrometry would be useful and some preliminary data from analysis by this method are reported below.

During recovery experiments adsorption of analyte onto the surface of the glassware used was a recurrent theme. In order to minimise this problem acid washes were carried out followed by silanization; however, this was shown to be unsuccessful. Subsequently, use of PTFE volumetric flasks for the storage of standard and extracted samples improved calibration curve linearity. Soto [4] has described the leakage of nonylphenol from plastic vessels. Obviously environmental analysis looking at non-ionic surfactants and their degradation products (which include alkylphenols) could be affected by this problem. However, no such occurrence has been observed during this work. This may be due to the use of mobile phase for the preparation of solutions or to the type of plastic vessel used.

Table 1 shows the recoveries achieved using the

Table 1
Recovery data for a NP9 alkylphenol exthoxylate from reagent water using C_{18} solid-phase extraction (for experimental conditions see text)

	Ethoxymer number									
	3	4	5	6	7	8	9	10	11	12
Extract 1	81	66	86	106	110	118	118	114	87	71
Extract 2	94	69	88	107	108	127	127	115	103	81
Extract 4	76	55	88	107	113	123	123	116	90	70

single stage C_{18} solid-phase extraction method from samples of reagent water. Samples were spiked at 11.2 $\mu g\,l^{-1}$ with nonylphenol ethoxylate and recoveries calculated from calibration data generated from peak areas in the HPLC chromatograms of standards. The recoveries of each individual ethoxymer from 3–12 chain length of ethylene oxide units are shown in Table 1. The recoveries for each oligomer ranged from 63 to 122%. Using these data the limit of detection using fluorescence detection based on 2× signal-to-noise ratio definition for the most intense peak in the oligomer distribution is estimated to be equivalent to 0.05 $\mu g\ l^{-1}$ for alkylphenol ethoxylates and 0.005 $\mu g\ l^{-1}$ for alkylbenzene sulphonate in the original sample with a concentration factor of 500 generated by the extraction procedure. Table 2 shows results obtained from the simultaneous extraction of both LAS and NP9. Reagent water samples (500 cm^{-3}) were spiked with 14 $\mu g\ l^{-1}$ of nonylphenol ethoxylate and 2 μl^{-1} of Nansa SS. The recoveries for each oligomer of NP9 ranged from 54 to 95%. The recoveries for each individual homologue of Nansa SS ranged from 69 to 149%.

This methodology was applied to the analysis of samples of surface water taken from the River Rother in South Yorkshire, UK. In chromatograms obtained after extraction of this high-sediment-containing sample using a C_{18} SPE cartridge only (Fig. 4a) several large interfering peaks eluted early in the chromatogram. It was assumed that these might be acidic components originating from the sediment. Hence it was decided to remove all anionic components from the sample extract by employing a SAX solid-phase extraction cartridge in series with the C_{18} cartridge. This methodology results in the cleaner chromatogram shown in Fig. 4c where the characteristic distribution pattern for alkylphenol ethoxylate oligomers can be clearly seen and compared with the chromatogram obtained from a standard sample (Fig. 4b). Any LAS present in the sample is now obviously retained by the SAX cartridge and not observed. Further work is required to investigate the possibility of selectively fractionating the anionic components trapped onto the SAX cartridge such that simultaneous analysis of LAS and APEOs would still be possible in such a situation. Using external calibration the level of alkylphenol ethoxylate found in these samples was calculated at 5.6 $\mu g\ l^{-1}$ taking an average of the individual oligomers.

Data from a preliminary investigation of the compatibility of the mobile-phase system employed with liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry (LC–APCI-MS) is shown in Figs. 5, 6 and 7. Fig. 5 shows the

Table 2
Recovery data for a NP9 alkylphenol exthoxylate and Nansa SS linear alkylbenzene sulphonate from reagent water using C_{18} solid-phase extraction (for experimental conditions see text)

	Ethoxymer number								Homologue number			
	3	4	5	6	7	8	9	10	1	2	3	4
Extract 1	54	46	96	67	66	76	90	116	82	78	69	110
Extract 2	54	67	76	71	91	90	88	89	77	71	86	143
Extract 3	68	92	95	91	90	89	91	92	110	97	99	149
Extract 4	66	80	85	81	85	86	84	82	78	87	84	96
Extract 5	54	74	76	78	81	77	76	73	77	75	63	69

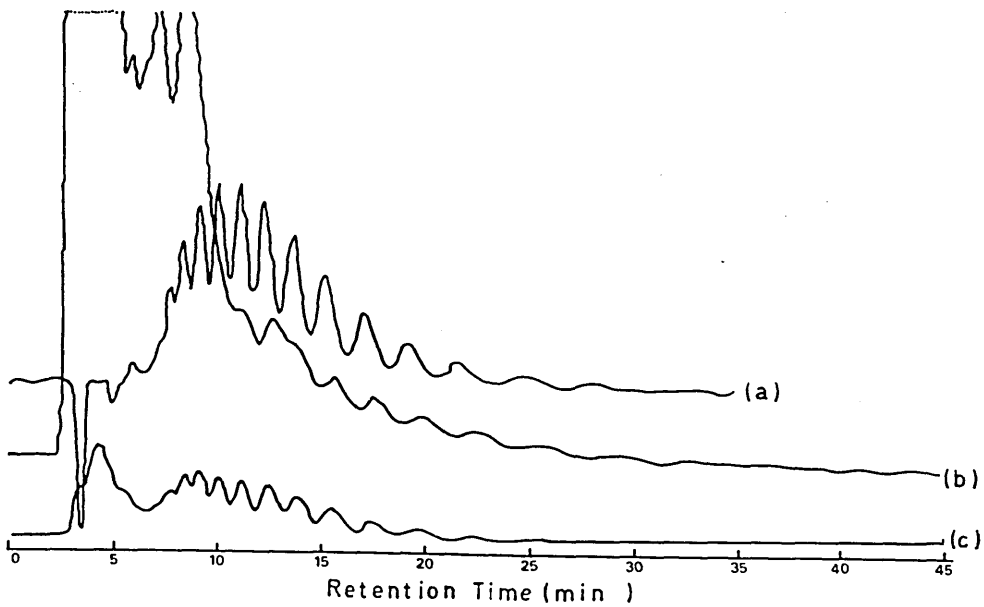

Fig. 4. HPLC chromatograms for the analysis of extracts from the River Rother (South Yorkshire, UK). Trace (a), extraction using C_{18} SPE cartridge only. Trace (b), standard NP9. Trace (c), extraction using C_{18} and SAX SPE extraction cartridges. Mobile-phase conditions A used throughout.

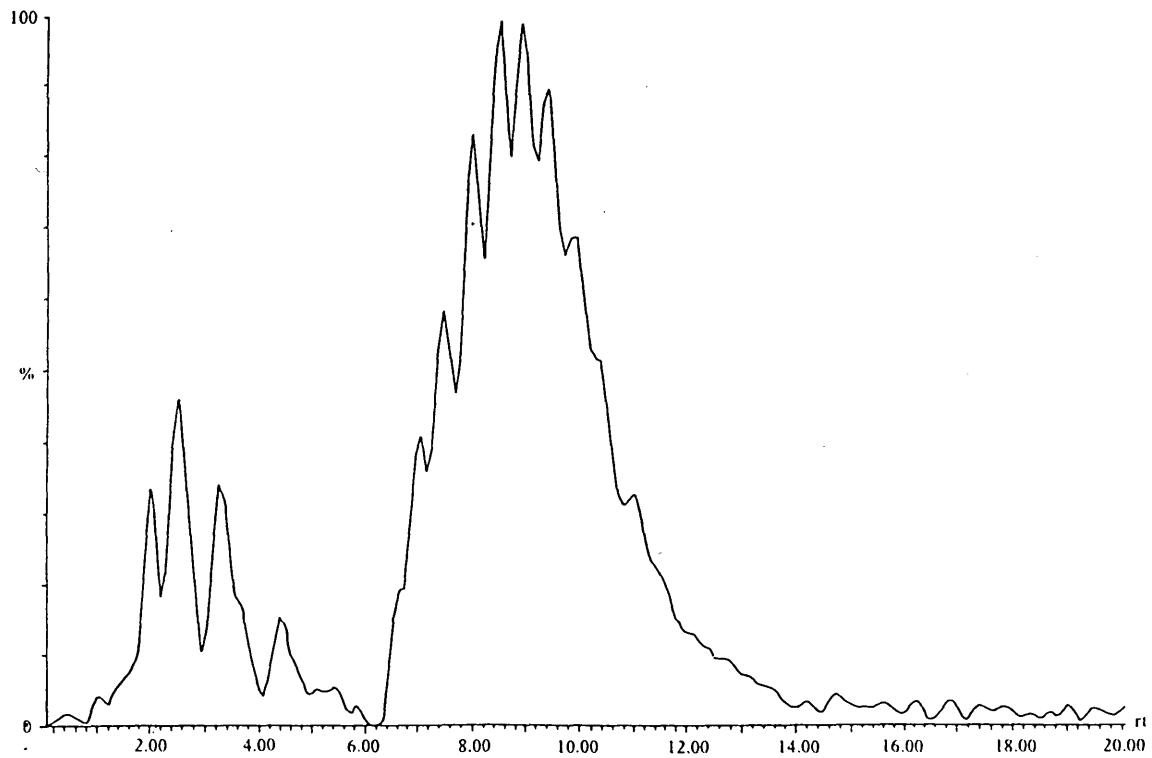

Fig. 5. APCI positive total ion chromatogram for the analysis of a standard solution of octylphenol ethoxylate and linear alkylbenzene standards (using HPLC conditions A).

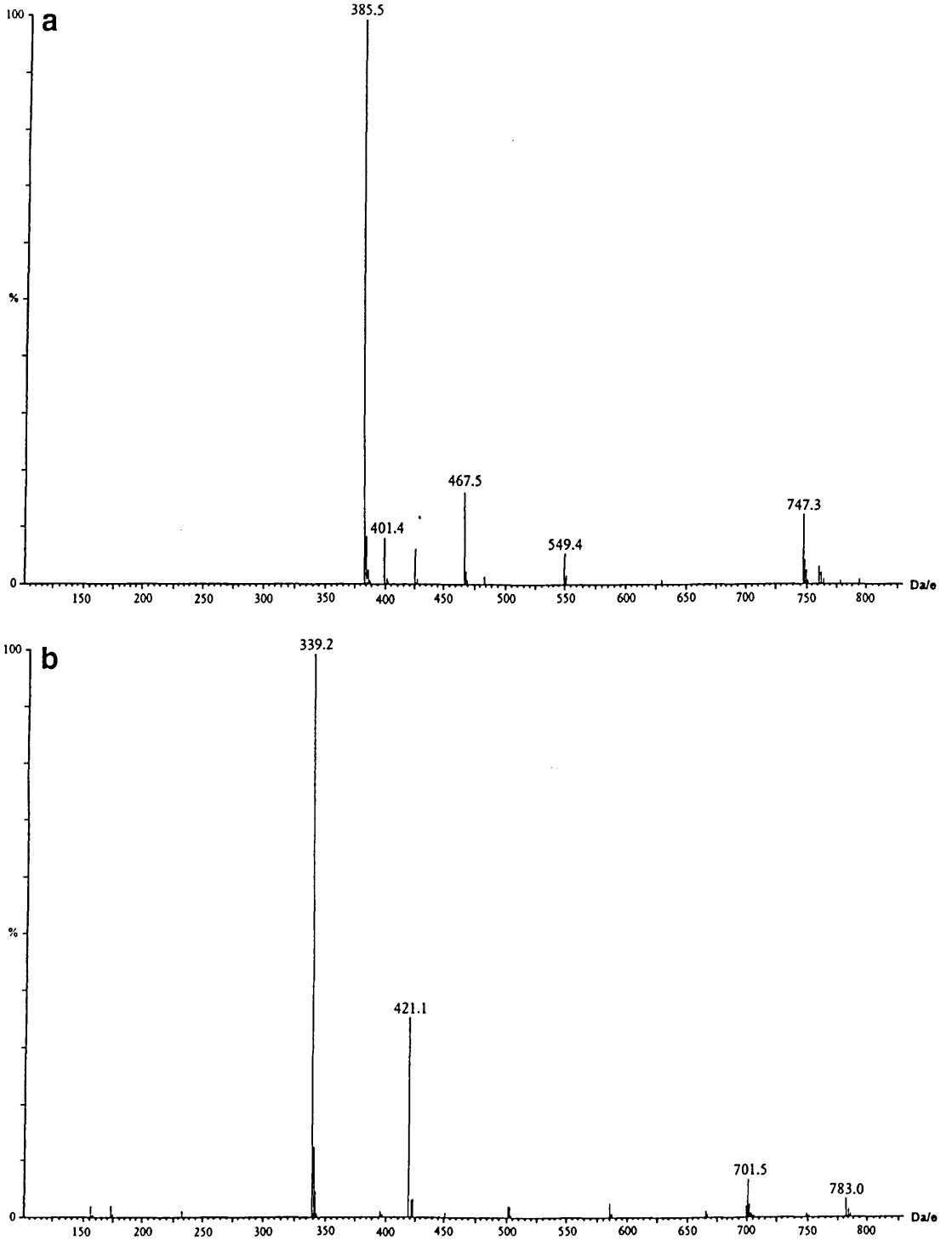

Fig. 6. (a) Positive ion APCI mass spectrum for the peak eluting at $t_R = 3.2$ min in Fig. 5. (b) Negative ion APCI mass spectrum for the peak eluting at $t_R = 3.2$ min in Fig. 5. Taken together these data can be assigned as arising from the LAS homologue with a side chain of thirteen $-CH_2-$ units.

total ion chromatogram for the analysis of a 0.001% standard solution of LAS and octylphenol ethoxylates by LC–APCI-MS in positive ion mode. As can be seen, peaks arising from both the anionic alkylbenzene sulphonate and non-ionic octylphenol ethoxylate can be seen in this ionisation mode. However, in negative ionisation mode only the anionic surfactants are observed. The relative sensitivity for detection of anionic surfactant is approximately 5:1 in favour of the positive ion mode (based on a comparison of peak areas). It is therefore desirable to use this ionisation mode for quantitative work.

A comparison of mass spectra from one dodecyl benzene sulphonate component of this standard mixture is shown in Fig. 6. In Fig. 6a the APCI positive ion spectrum from the peak eluting at t_R 3.2 min is shown. The major ion in this spectrum at m/z 385 can be assigned to a $[M+2Na]^+$ ion. The corresponding negative ion spectrum is shown in Fig. 6b. In this spectrum as might be expected, the $[M-H]^-$ peak at m/z 339 dominates. These two spectra together allow an assignment of this peak as arising from the LAS homologue with a side chain of thirteen $-CH_2-$ units.

Fig. 7 shows the APCI positive ion mass spectrum from the peak eluting at $t_R=8.92$. This mass spectrum exhibits a small $[M+H]^+$ ion at m/z 559. However, the dominant pseudo-molecular species are sodium adduct ions. These can be observed and assigned to $[M+Na]^+$ at m/z 581 $[M+2Na]^+$ at m/z 604 and $[M+3Na]^+$ at m/z 626. This multiple adduction can be explained if it is assumed that each ethylene oxide unit has the potential to gain a sodium atom from this obviously sodium-rich sample. Taken together these data can be used to identify this peak as the eight ethylene oxide unit containing oligomer of Triton X100. This non-ionic surfactant did not yield any negative ion APCI mass spectra. Also observable in this non background subtracted data

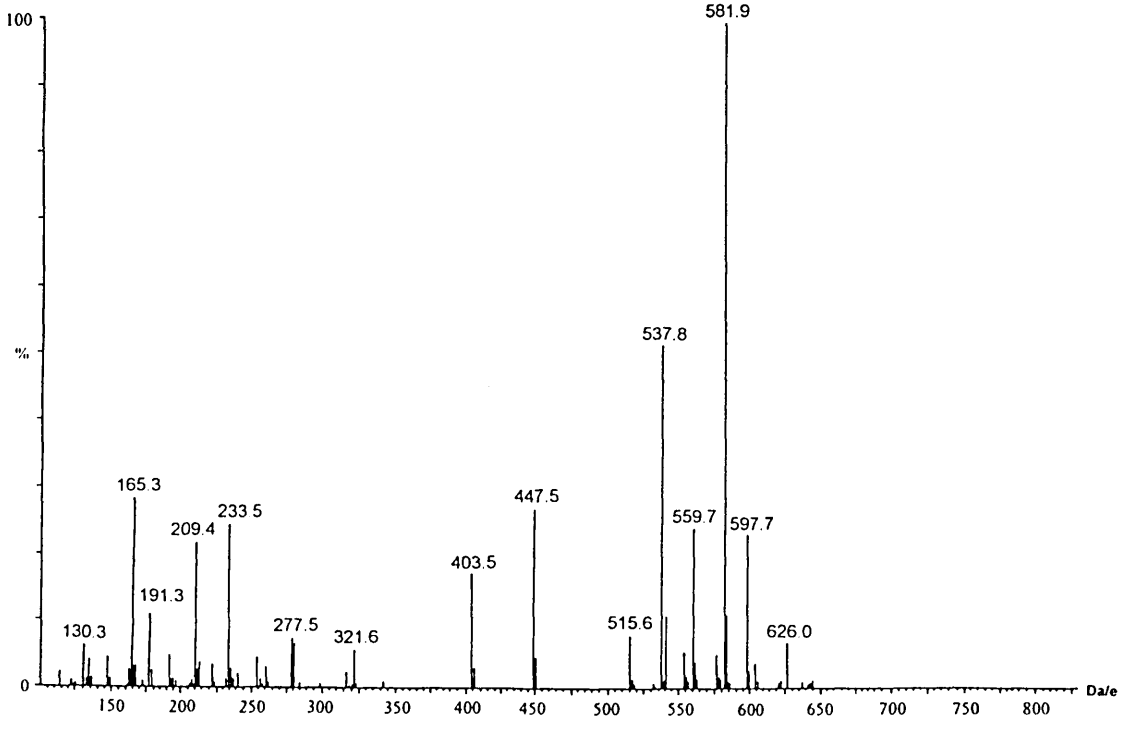

Fig. 7. Positive ion APCI mass spectrum for the peak eluting at $t_R=8.2$ min in Fig. 5. This can be assigned to the eight ethoxylates containing oligomer of the octylphenol ethoxylate standard. (For discussion see text.)

are the equivalent $[M+H]^+$ ion at m/z 515 $[M+Na]^+$ at m/z 537 $[M+2Na]^+$ at m/z 560 and $[M+3Na]^+$ at m/z 583 for the seven ethylene oxide units containing oligomer.

4. Conclusions

A method allowing the determination of common anionic and non-ionic surfactants has been developed. The method allows both total surfactant and individual homologue (LAS) or oligomer (APEO) distribution to be determined. Simultaneous extraction of LAS and alkylphenol ethoxylates is possible using a C_{18} solid-phase extraction cartridge only, and simultaneous analysis, i.e. extraction and chromatographic separation has been demonstrated for these analytes in reagent water. However, for the surface water samples analysed which contained a high level of sediment, it was found necessary to add a further SPE clean-up stage which removed all anionic components, including the LAS. Further work is required to investigate the possibility of selectively fractionating the anionic components trapped on the SAX cartridge such that simultaneous analysis would still be possible in this situation.

Fluorescence detection provides a sensitive and specific method of detection. Where additional confirmation of peak assignments is required, a preliminary investigation has indicated that LC–APCI-MS is compatible both with the mobile-phase systems used for chromatographic analysis and offers adequate sensitivity.

In future work we will investigate methods for the selective elution of LAS from the SAX cartridge and the application of the developed methodology to the determination of surfactants in the marine environment.

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