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Analytical Methods for the Determination of Surfactants in Surface Water

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Abstract

The determination of surfactants in environmental surface water is required due to recent concern over possible adverse health effects that have been associated with them. This thesis is concerned with two aspects of the analysis of non-ionic and anionic surfactants in surface water.

An HPLC phase-switching method has been developed in an attempt to overcome the problem of an interfering anionic species (thought to be humic acids) that masks the presence of any linear alkylbenzene sulphonate surfactants in river water samples. This problem has arisen following the development of an HPLC method for the determination of linear alkylbenzene sulphonates and alkylphenol ethoxylate surfactants in surface water in a previous research project. The phase-switching method allows the mobile phase to be diverted to either a C_1 or C_{18} column or both. The linear alkylbenzene / humic acid portion was diverted to the C_{18} column after elution from the C_1 column; the alkylphenol ethoxylate portion of the sample was then allowed to separate on the C_1 column as usual. Then the linear alkylbenzene / humic acid portion was separated on the C_{18} column using a different mobile phase. The method works well with standards; however, with real samples it was not clear as to the identity of the peaks that may or not be linear alkylbenzene sulphonates. In addition, recent batches of the Spherisorb C_1 column were unable to adequately resolve the nonylphenol ethoxylate ethoxymers.

The reason for this loss of resolution was investigated by elemental analysis and x-ray photoelectron spectroscopy. Bulk percentage carbon and surface carbon coverage both showed a similar trend. The earlier batch of Spherisorb column that produced the best resolution of nonylphenol ethoxylate ethoxymers had the lowest surface carbon coverage and the lowest percentage bulk carbon. Recent batches of the Spherisorb column along with columns from Supelco and Hypersil contained higher levels of carbon. These results suggest that resolution of the ethoxymers is due to the unreacted hydroxyl groups on the silica surface, and that the presence of the alkyl moiety actually hinders the process. In order to account for this a "pseudo reverse phase" mechanism has been invoked for this separation.

The second section of this thesis involves the development of a new qualitative and quantitative method for the determination of nonylphenol ethoxylate surfactants in surface water by matrix-assisted laser desorption / ionisation time-of-flight mass spectrometry. The sample was mixed with a concentrated solution of 2,5-dihydroxybenzoic acid or α-cyano-4-hydroxycinnamic acid as a matrix. Approximately 1 μL of the resulting solution was added to a stainless steel target and, after evaporation of the solvent, the target was placed into the mass spectrometer. The resulting spectra showed intense $[M+Na]^+$ and $[M+K]^+$ adducts for each ethoxymer group. Extracted samples from the River Don analysed by this method showed a similar characteristic envelope of peaks, corresponding to sodium and potassium adducts for nonylphenol ethoxylates. For quantitative determinations Triton X-100, an octylphenol ethoxylate surfactant, was added as an internal standard. A concentrated solution of lithium chloride was also added to produce much less complicated spectra consisting of solely $[M+Li]^+$ adducts. Good linear relationships were seen for each individual ethoxymer over the entire distribution. The method showed excellent results for spiked surface water samples, but the concentrations of nonylphenol ethoxylates in recent samples were below the current limit of detection for this method of 100 μg/L.

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Chapter 1

The Structure and Formulation of Surfactants and their Effects on the Environment

1.0 Introduction

Surfactants have a major impact on all aspects of our daily life, either directly in household detergents or in personal care products, or indirectly in the production and processing of materials which surround us. The term surfactant is a convenient shortening of the term *surface-active agent*.

Before the 1950s soaps such as sodium stearate were the major surfactants and played an important role in improvements in health and hygiene. However, the last forty years have seen the rise of the synthetic surfactant. These more versatile agents now make up a massive market that encompasses the entire world; global surfactant usage (excluding soap) currently stands at over 10 million tonnes with a value of over $14 billion.

While growth in the developed countries of Western Europe, Japan and North America is now very slow, there now a trend of significant growth in South Asia and Latin America (Table 1.1) [1].

Area	1995 / 10^3 tonnes	2005 / 10^3 tonnes	%increase / a
Western Europe	2100	2165	0.3
North America	1800	1960	1.0
Japan	565	655	1.5
Latin America	1575	1785	2.6
Asia-Pacific	2690	4340	6.1
Rest of World	1645	2765	6.8
Total	**10220**	**13870**	**3.6**

Table 1.1 Projected global surfactant usage [1]

The large growth seen in developing countries is mainly due to expansion in the laundry and household cleaning products sectors. Global usage is predicted to increase to 18 million tonnes by 2050. Despite this growth in the surfactant industry, soap usage is

expected to remain constant at 8 million tonnes; this is mainly because of its relatively poor performance (for example, it generates scum in water).

The surfactants market can be subdivided into two areas: -

- **Cleaning products** e.g. household detergents, consumer products, personal care products and industrial and institutional cleaning.
- **Process Aids** – products that make use of the surfactant's surface active properties e.g. emulsifiers, formulation aids for crop protection chemicals, fibre lubricants, defoamers and oil field chemicals.

Surfactants can be characterised by the following features: -

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

Generally, it is the presence of two structurally dissimilar groups within a single molecule that is the fundamental characteristic of surfactants. The surface behaviour, i.e. the surface activity, of the surfactant molecule is dependent on the make-up of the individual groups, their relative size and their location within the surfactant molecule.

Surfactants can be divided into four different classes of amphiphilic molecule (Figure 1.1). Each consists of a hydrophobic (water-hating) hydrocarbon tail - *the hydrophobe* and a hydrophilic (water-loving) head group – *the hydrophile.*

The *hydrophile* can be: -

- **Non-ionic**

  E.g. dodecyl alcohol ethoxylate $CH_3(CH_2)_{10}CH_2(OCH_2CH_2)_nOH$.

  Non-ionics are a very widely used class of surfactant. They have uncharged polar head groups, usually based on poly(oxyethylene). Non-ionic surfactants offer a broad range of properties such as wetting, dispersion, detergency, lubrication, emulsification and anti-static properties.

- **Anionic**

  E.g. sodium dodecyl sulphate $C_{12}H_{25}SO_3^-Na^+$.

  Anionic surfactants carry a negatively charged surface-active portion. As a class they represent the most widely used group of surfactant, especially in detergent applications where they show excellent cleaning properties and are usually good foaming agents.

- **Cationic**

  E.g. hexadecyltrimethylammonium bromide $C_{16}H_{33}N^+(CH_3)_3Br$.

  Cationics have a positively charged surface-active portion. Their antibacterial properties mean they are excellent for use in disinfectants. Cationic surfactants also have excellent anti-static properties that make them ideal for use in fabric conditioners.

- **Amphoteric**

E.g. alkyldimethyl betaine $RCH(CH_3)(CH_3)_2N^+CH_2COO^-$.

Amphoteric surfactants contain both anionic and cationic groups, enabling them to behave as an anionic, non-ionic or cationic surfactant depending on the pH. Their mildness and high foaming properties make them ideal for use in toiletries and cosmetic formulations. They are also used in industrial applications where a high stability foam profile is needed.

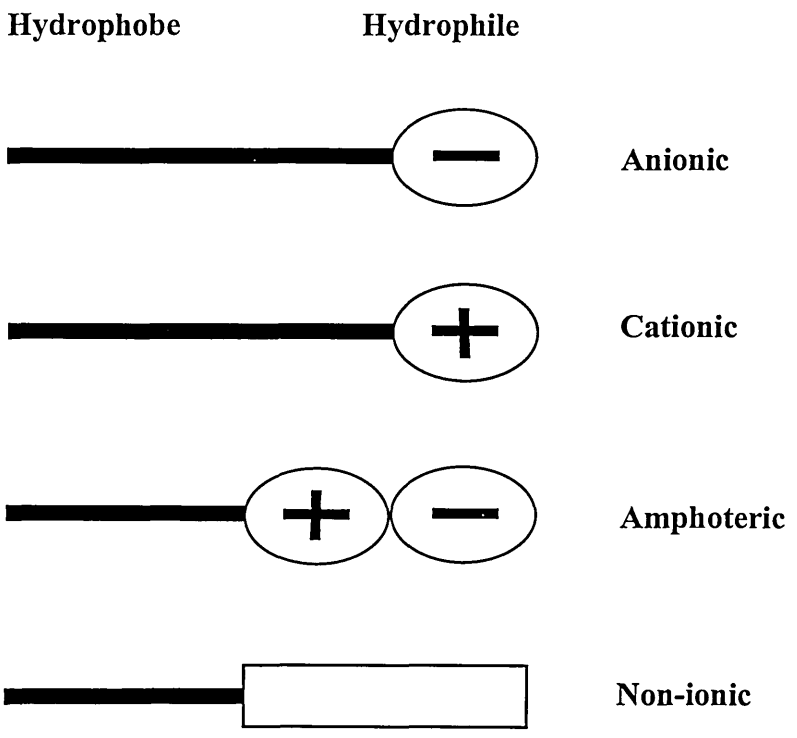

Figure 1.1 The four different classes of surfactant

The cationic and amphoteric classes of surfactants will not be discussed in the proceeding sections as their relatively low usage (Table 1.2) makes them much less environmentally significant.

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

Table 1.2 Current and projected sales of surfactants by type [1]

Over the last decade, environmental pressures and a growing consumer awareness of green issues have driven detergent formulation. Once organic compounds find their way into the environment they are subject to attack from various micro-organisms. Biodegradation of organic compounds can be split into two processes: -

1. Primary Degradation

The compound is metabolised into intermediates, which are persistent. This means that there is a delay in the biodegradation process before ultimate degradation is complete. This delay occurs until microbes are capable of ring oxidation/sulphonation.

2. Ultimate Degradation

The compound is totally transformed into its basic elements, i.e. water and carbon dioxide, in the case of carbon containing molecules.

Usually surfactants are discharged with wastewater after their use. Consequently, it is assumed that they and their degradation products will be found in the environment. Due to their enormous usage, their ecotoxicological potential must not be ignored, and it is therefore necessary to measure the concentrations of the most commonly used surfactants, i.e. the non-ionic and anionic classifications in environmental samples. As these surfactants are present in the environment as mixtures of a particular generic classification (each having a slightly different toxic potential), methods that assess

individual oligomer or homologue levels are more valuable as analytical tools than the conventional colorimetric techniques commonly used (discussed in chapter 2).

1.1 Non-ionic Surfactants

1.1.1 Structure

The term non-ionic surfactant refers chiefly to polyethylene oxide and polypropylene oxide derivatives; however, other surfactants are included in this category, such as hydroxyhexitol derivatives and fatty amine oxides.

Non-ionic surfactants are 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 most common classifications of non-ionic surfactants are shown below: -

- **Polyoxyethylene alcohols and polyoxyethylene esters of fatty acids**

These surfactants have the general structure: -

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

Polyethylene surfactants (ethoxylates) are by far the most important group of alkoxylated non-ionic surfactants, and were first introduced in the United States as a chemical in the textile industry in the late 1930s. The compounds are water soluble due to 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 ethoxylates is not adversely influenced by water hardness.

- **Alcohol Ethoxylates (AE)**

As they are cheap to manufacture, these have emerged as the principal non-ionic surfactant in the consumer detergent product market. They are highly biodegradable and their physical form (liquids to waxes) is dependent on their ethylene oxide content. AE are polydisperse with respect to ethylene oxide chain length. An alcohol ethoxylate that generally contains eight ethylene oxide units actually contains significant amounts of other ethoxylates ranging from zero to twenty ethylene oxide units.

- **Alkylphenol Ethoxylates (APEO)**

These surfactants are similar to AE physically and in performance. They are derived mostly from alkyl phenols (APs) containing branched alkyl side chains, typically nonyl and octyl groups. This increase in branching leads to a decrease in biodegradability in comparison with the AE. On the other hand, solubility and dispersive properties are increased as a result of branching.

Commercial APEO are usually produced by the base catalysed ethoxylation of alkylated phenols. As phenols are more acidic than alcohols, their reaction with ethylene oxide to form the mono adduct is faster, and the product does not contain any unreacted phenol; thus the distribution of individual ethoxylates in the commercial mixture is narrower and APEO are more soluble in water. The process produces a mixture of ethylene oxide oligomers, which follows a Gaussian distribution (generally from one to thirty); thus APEO commercial formulations are very complex in nature. The general formula for APEO is shown in Figure 1.2.

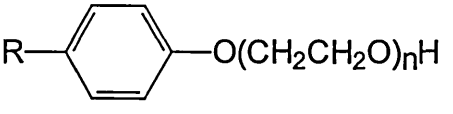

Figure 1.2 The general formula for APEO

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

There is increasing concern with regard to the world-wide usage of APEO, 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 by the year 2000 the use of these products in both domestic and industrial detergents will be phased out in EC member countries.

1.1.2 Biodegradation

Non-ionic surfactants biodegrade in a very complex manner. They can be attacked at three different points by micro-organisms, the position of which is dependent upon the structure of the surfactant. In aerobic conditions, the three different points of attack are summarised below [3].

- **Central Fission Mechanism**

The hydrophobe is cleaved from the hydrophile. β–oxidation is then responsible for the further conversion of the linear chains to carbon dioxide and water.

- **ω-Hydrophobe Attack**

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

- **ω-Hydrophile Attack**

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

The pathway and rate of biodegradation are dependent upon the type of micro-organism present and the structure of the intact surfactant. Linear alcohol ethoxylates (LAE) biodegrade to water and carbon monoxide by the central fission mechanism (Figure 1.3) [3].

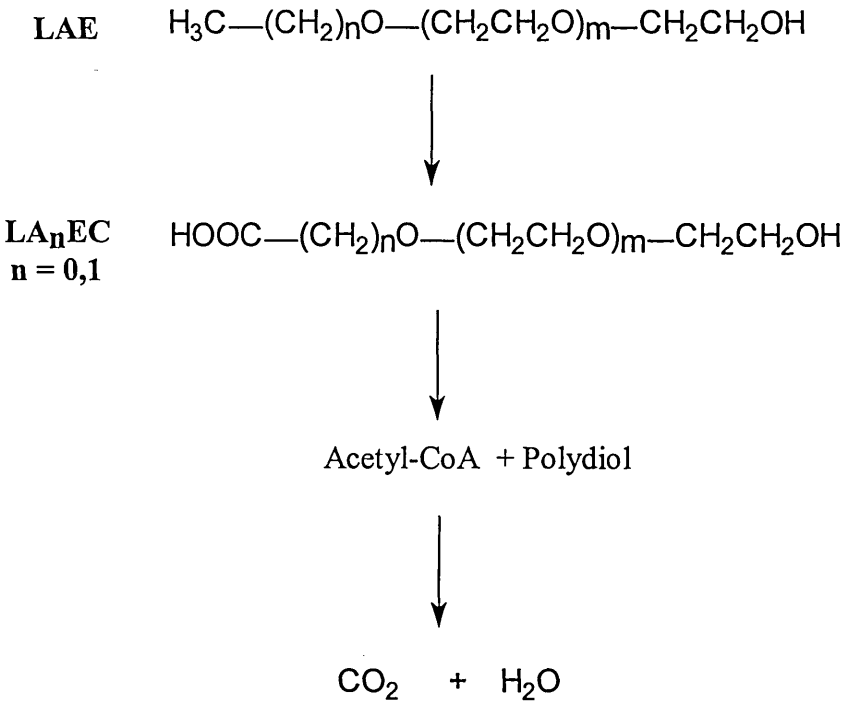

Figure 1.3 The biodegradation pathway of LAE

It has been suggested that the biodegradation of APEO takes place via the hydrolytic removal of an ethylene oxide group [4], which occurs via the cleavage of an ethylene oxide unit to glycolic acid and then to glycoxylic acid.

In this process the first step is the oxidation of the ethylene oxide chain to a carbonyl group, followed by the hydrolytic removal of glycolic (hydroxyacetic acid). However, Ball *et al.* [5] and Swisher [3] believe the major mechanism involved in the biotransformation involves the hydrolytic cleavage and oxidation of the terminal –OH

group (Figure 1.4). Conversely, Kravetz *et al.* [6-8] have suggested a substantial transformation of the aromatic ring together with simultaneous oxidation of the hydrophile and hydrophobe.

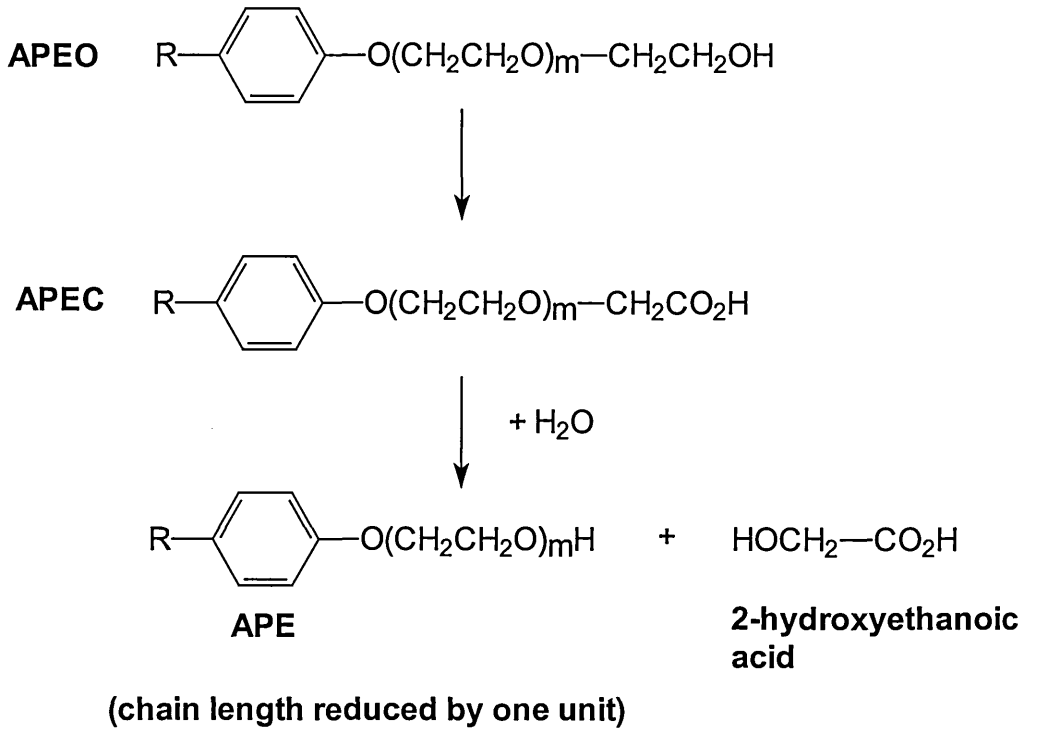

Figure 1.4 Biodegradation of APEO

Schoberl [4] could not account for the presence of APEC found in the environment, and experimental evidence suggests that the highly branched alcohol and alkylphenol ethoxylates biodegrade via the ω-hydrophile oxidation pathway.

Four main nonylphenolic compounds are produced due to ω-hydrophile oxidation [9]. These primary oxidation products of APEO are normally: -

- short chain APEO (m = 1,2)
- APECs (m = 0,1)
- Alkylphenols

A mixture of these biodegradation products is present in aerobic conditions, with a residual amount of longer chain APEO (m = 3-20) (Figure 1.5). In anaerobic conditions

the degradation products consist of the shorter chain APEO and alkylated phenols (Figure 1.6).

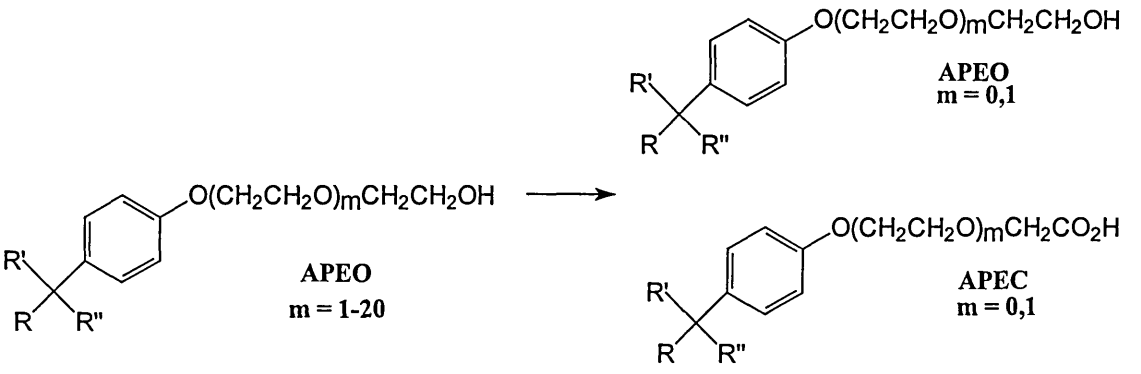

Figure 1.5 Aerobic degradation products of the APEO

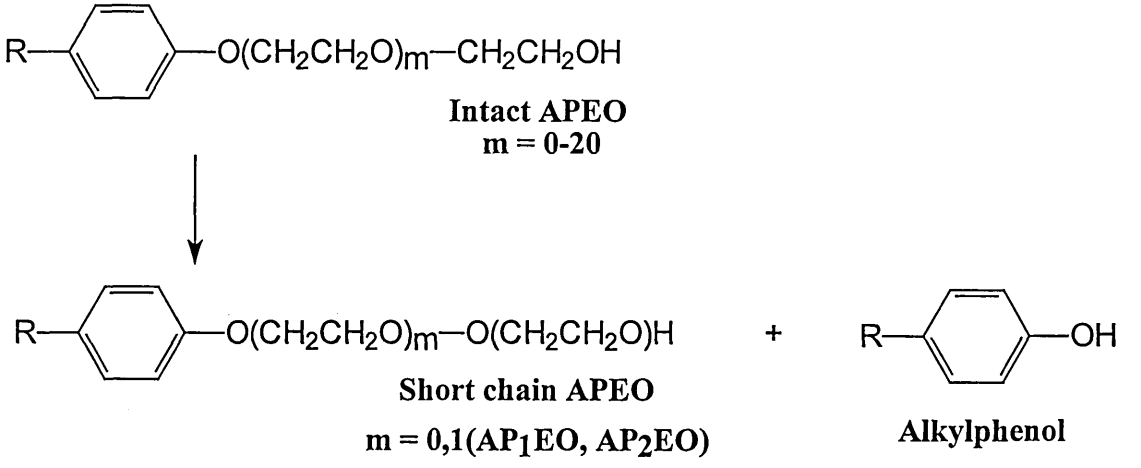

Figure 1.6 Anaerobic degradation products of APEO

1.1.3 Environmental Effects

Studies have been conducted to investigate the environmental fate of APEO, by the introduction of NPEO into sewage treatment plants (STP), and subsequently monitoring the plant output into river water.

In a study of the Glatt River [9] Ahel *et al.* found that while STPs were indeed reducing the amount of long ethoxylate chain species, the elimination rate of all nonylphenolic compounds (70 ± 15 %) was significantly lower than the elimination of BOD (biochemical oxygen demand) (86 ± 9 %), indicating that nonylphenolic compounds are

not the most biodegradable fraction of sewage. No net elimination of NP1EO and NP2EO was seen in the results, indicating that their formation during activated sludge treatment was faster than their degradation. In addition, concentrations of NP1EC and NP2EC in secondary effluents were 2.1–7.6 times higher than in primary effluents, indicating significant formation in this class of compound during aerobic biological treatment (Table 1.3). Therefore while sewage treatment effectively degrades the long ethoxylate chains, the resulting shorter chains and carboxylic degradation products (which are much more resistant to biodegradation) remain and are present in the final effluent from the treatment plants.

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

Table 1.3 The influence of sewage treatment on non-ionic surfactants and their degradation products [data from ref 9]

Ahel *et al.* [10] have reported the results of another study of the Glatt River in Switzerland which receives effluents from several sewage treatment plants. Results indicated that the biodegradation products NPECs were the most abundant APEO type compound (2-7.1 μg/L) and, as would be expected, intact NPEO were the least abundant (1-7.7 μg/L). Ratios of APEO and their biodegradation products were found to resemble those of secondary effluents. These types of compound were seen to undergo significant changes due to the biodegradation process. Contribution from intact NPEO decreased from 21 % to 3.5 % of the total; in contrast, NPECs increased from 51 to 85 %.

1.1.4 Metabolites of APEO in the Marine Environment

Studies have been conducted on the environmental behaviour of sewage released into the marine environment. Waters and sediments from the Venice Lagoon were analysed as it receives treated and untreated domestic and industrial effluents [11].

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

If toxic metabolites of APEO can be found in sludge, it is a possibility that bottom-feeding animals, such as mussels, may consume these compounds. McLeese and co-workers [2] studied the uptake and excretion of aminocarb (containing nonylphenol formulations) by mussels. They suggested that significant contamination of bivalves does not occur if the concentrations of the aminocarb and nonylphenol in water are less than 0.01 mg/L.

Wahlberg *et al.* [12] reported concentrations of between 0.2 and 0.4 μg/L nonylphenol, 0.075-0.275 μg/L NP1EO, 0.04-0.125 μg/L NP2EO and 0.03-0.04 μg/L NP3EO in blue mussels (*Mytilus Edulis*). The study was carried out by putting mussels in cages at

different depths and distances down the coast from a manufacturer of surfactants in Sweden.

1.1.5 Toxicity

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

A survey of the literature by Thiele *et al.* [13] showed that the majority of studies of APEO toxicity have concentrated on nonylphenol. McLeese *et al.* [14] determined the LC_{50} (the concentration that kills 50% of a population) of NP for various species living in the sea (Table 1.4).

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

Table 1.4 NP LC_{50} data for various marine organisms [14]

Terrestrial animals are much less sensitive than aquatic species to surfactants. For example the median lethal dose (LD_{50}) for oral ingestion by mammals generally lies in the range 500-5000 mg of surfactant per kg of body weight, which is comparable with sodium chloride or sodium bicarbonate [3].

1.1.6 Oestrogenic Properties of Alkylphenol Ethoxylates

In recent years, much evidence has been found to link several man-made chemicals to adverse reproductive health effects in both man and the environment. An excellent review on this subject has been published by Toppari *et al.* [15]. These chemicals are said to be endocrine-disrupting. The endocrine system is critical to the functioning of both animals and plants, controlling growth, maintenance and reproduction. Certain types of substance are able to interact with receptors and thereby interfere with the synthesis and actions of hormones in the body, causing disruption of the physiological processes under the control of the endocrine system. Some chemicals mimic or block the feminising effects in animals of the natural female sex hormones called oestrogens; these are referred to, respectively, as oestrogenic or antioestrogenic substances. Others mimic or block the masculinising effects of male sex hormones called androgens; these are referred to, respectively, as androgenic or antiandrogenic substances. Table 1.5 shows the different categories of these chemicals, some examples of their uses and their mode of action.

Category	Example(s)	Uses	Mode of Action
Organotins	Tributyltin chloride	Anti-fouling agents	Oestrogenic
Organochlorine pesticides	DDT, dieldrin, lindane	Insecticides	Oestrogenic and antiandrogenic
Polychlorinated organic compounds	Dioxins	By-products of incineration and industrial chemical processes	Antioestrogenic
	Polychlorinated biphenyls (PCBs)	Formerly used in some electrical equipment	
Alkylphenols	Nonylphenol	Used in production of nonylphenol ethoxylate surfactants and other polymers	Oestrogenic
Alkylphenol ethoxylates	Nonylphenol ethoxylates	Surfactants, spermicides	Oestrogenic
Biphenolic compounds	Bisphenol A	Used in production of polycarbonates and epoxy resins	Oestrogenic
Phthalates	Dibutylphthalate, butylbenzenephth -alate	Plasticisers	Oestrogenic
Synthetic steroids	Ethinyl oestradiol	Contraceptive pill	Oestrogenic

Table 1.5 Oestrogenic chemicals, their uses and mode of action [15]

The idea that alkylphenols of APEO surfactants exhibit oestrogenic effects stems from the work by Soto and co-workers [16]. They found that NP (an important biodegradation product of NPEO surfactants and a plastic additive) produced an oestrogenic response when tested in the human breast tumour MCF_7 cell line and in castrated rat models. Furthermore, Jobling and Sumpter [17] have shown that 4-nonylphenol, 4-octylphenol, 4-nonylphenol carboxylic acid, Tergitol NP9 (a

nonylphenol ethoxylate surfactant) and 4-nonylphenol diethoxylate are all weakly oestrogenic to rainbow trout. Oestrogenic potency was found to decrease with increasing ethoxy chain length. Work by Jobling *et al.* [18] has also shown that exposure to these alkylphenolic compounds causes male rainbow trout to excrete vitellogenin (a substance normally excreted from the liver of female rainbow trout in response to oestrogen production) and concomitant inhibition of testicular growth. Studies on rats [19] have shown that 4-octylphenol, 4-octylphenol ethoxylates and butylbenzyl phthalate cause a small but significant reduction in mean testicular weight, and that 4-octylphenol and butylbenzyl phthalate cause reductions of 10-21 % in daily sperm production. Recent work by Routledge and Sumpter [20] on the development of a recombinant yeast screen for assessing oestrogenic activity has shown that the biodegradation intermediates of alkylphenol ethoxylate surfactants i.e. 4-octylphenol, 4-nonylphenol, 4-nonylphenoxy carboxylic acid and 4-nonylphenol diethoxylate, show a positive result to the screen. However, the parent surfactants themselves produced a negative result for oestrogenic activity.

Whether or not these chemicals are having any effect on humans is very hard to tell; however, there have recently been some worrying indications of possible endocrine disruption. A recent study [15] suggests that over the last fifty years sperm production has dropped from 113 million/mL to 66 million/mL. However, this still remains a very contentious issue, with many reports suggesting that the differences may be due to variations in the method of counting sperm and different population groups. Also recent evidence suggests that the number of incidents of testicular cancer has increased sharply over the last decade [21] and that the number of cases of the reproductive abnormalities cryptorchidism (undescended testes) and hypospadias (urethra opening on the underside

of the penis) is also on the increase, although again, data is limited and not yet conclusive.

1.2 Anionic Surfactants

1.2.1 Structure

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

Anionic surfactants are divided into the following groups: -

- **Carboxylates**

Most of the commercial carboxylates are soaps, the general structure being $RCOO^- M^+$, where R is a straight hydrocarbon chain (C_9-C_{21}) and M^+ is a metal or ammonium ion. Soaps show excellent detergency in soft water; however, bivalent metal ions (Ca^{2+} etc.) cause unsightly *'curds'*. For this reason, and from an economic point of view, *'syndets'*, a combination of synthetic surfactants (alkylbenzene sulphonates, ABS) and builders (pentasodium triphosphate), have replaced soaps in soap powders. Carboxylates with a fluorinated chain have also been developed. Replacement of hydrogens on the hydrophobe by fluorine atoms has led to surfactant molecules of unusually low surface tension.

- **Alkylbenzene sulphonates (ABS)**

Alkylbenzene sulphonates respond well to builders and foam boosters in detergent formulations. These properties, together with the low cost and availability at consistent quality, account for their dominant position in household laundry products. Their synthesis is shown in Figure 1.7.

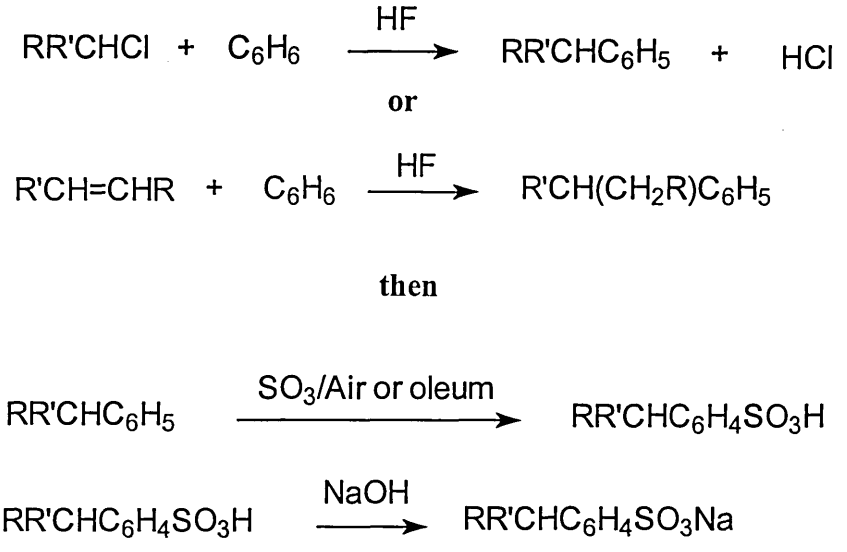

Figure 1.7 The synthesis of ABS

The most common anionic surfactants are the linear alkylbenzene sulphonates (LAS) (Figure 1.8) which have a total production estimated at 290,000 tonnes per year in Western Europe [1]. This represents 25 % of the total consumption of synthetic surfactants. In the industrial world (i.e. United States, Western Europe and Japan) the figure for the total LAS consumption is approximately one million tonnes per year.

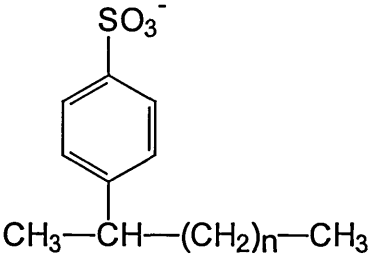

Figure 1.8 The structure of LAS

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 eleven to fourteen carbon units.

1.2.2 Biodegradation

Biodegradation of LAS has been thoroughly reviewed by Schöberl [22] and Swisher [3]. Microbial biodegradation seems to take place in four main steps: -

- Oxidative conversion of one (or both) of the methyl groups of the alkyl chain into a carbonyl group (ω-oxidation).
- Oxidative shortening of the alkyl chain by steps of two carbon units (β-oxidation) until only 4-5 carbon atoms remain.
- Oxidative ring splitting.
- Cleavage of the carbon-sulphur bond. The remaining products then enter the central metabolic pathways (Krebs cycle and glyoxylate cycle).

The actual structure of the surfactant can have an important effect on the speed and efficiency of biodegradation: -

- The structure of the hydrophobe has a pronounced effect on the biodegradability; any branching of the hydrophobe will deter biodegradability.
- The greater the distance between the sulphonate group and the far end of the hydrophobe, the faster the rate of primary biodegradation.
- While biodegradation begins at the end of the hydrophobic chain via ω-oxidation, the exact method seems to depend on the particular micro-organism involved, the media in which biodegradation occurs and the structural features of the chain itself. The ultimate product following ω-oxidation is always the

carboxylic acid, but this can be achieved in two ways; either oxidation to the alcohol, then further oxidation to the acid via the aldehyde, or dehydrogenation to the alkene followed by oxidation to the diol and then finally to the acid.

Figure 1.9 shows the overall sequence of the biodegradation pathway for LAS.

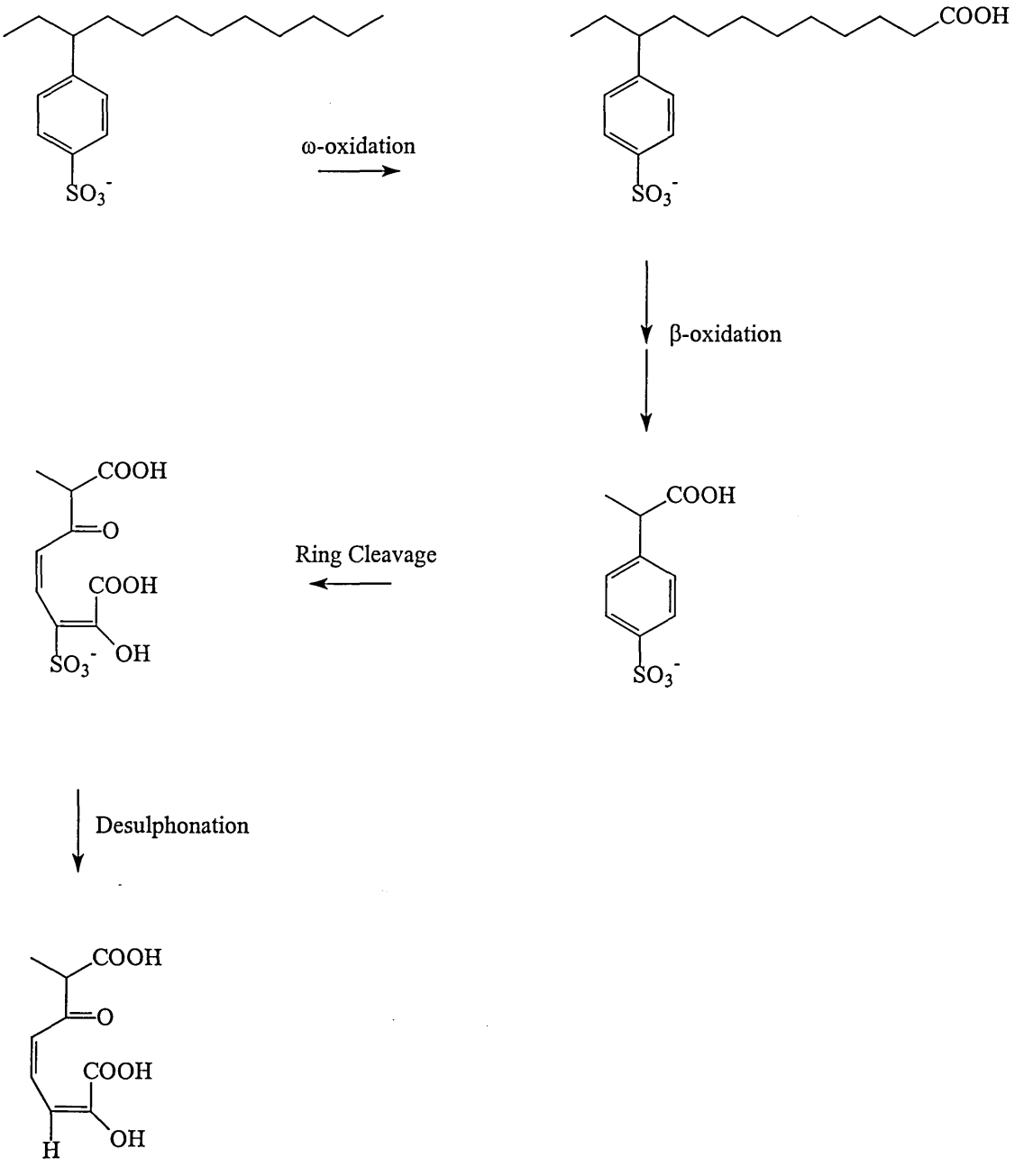

Figure 1.9 Biodegradation pathway of LAS

1.2.3 Environmental Effects / Toxicity

There have been several studies on the toxicity of LAS [23-25], which generally agree that toxicity to aquatic life increases with increasing chain length of the alkyl chain. For commercial LAS to be acutely toxic, it must be in the environment at a concentration between 1-10 mg/L. LAS biodegradation intermediates are 100 to 1000 times less toxic than the parent compound, and are normally present in river waters at concentrations well below threshold levels which equate to chronic effects.

In mammals LAS is usually excreted, before ultimate degradation, via urine and faeces. It is absorbed and passed into the blood in the intestine and eventually arrives in the liver where it is broken down via ω and β oxidation. The hydrophilic compounds i.e. short chain carboxylates are passed to the blood while LAS and longer chain sulphophenyl carboxylates (SPC) move into bile for the whole process to continue. The short chain SPC are finally excreted in urea from the kidneys. Any LAS which were not absorbed in the kidneys will be found in excreted faeces [26].

In experiments using radio-labelling techniques LAS metabolites were detected in rat urine (40-58 % of original LAS concentration) and in the faeces (39-56 % of original LAS concentration) over a four day period. A total of 19 % of the original LAS concentration ingested remained intact [26].

De Henau *et al.* [27] tested several commercial seedlings for LAS toxicity, and concluded that foliage showed visible signs of toxic effects when the soil containing the seedlings was sprayed with a solution of LAS at concentrations of 1000 mg/L and above. In all species, no reduction in growth occurred between 0.4 and 2.2 mg/kg. These data were collected under stress conditions and when compared with the

measured concentrations of 0.9 to 2.2 mg/kg in the environment, it would seem that LAS in sludge applied to soil does not represent a hazard to terrestrial plant life.

Kimberle [28] reported LAS toxicity values as *No Observed Effect Concentrations* (NOEC), and the values were used to produce a hazard data assessment model. He reported that the shorter chain LAS (i.e. C_{10}) is less toxic to fish and invertebrates than LAS with longer chain lengths (i.e.C_{14}). The NOEC for C_{10} was 10 mg/L compared to 0.1 mg/L for C_{14}. For commercial applications with an average chain length of $C_{11.8}$ the NOEC was 1.2 mg/L; double the amount of those with an average chain length of $C_{13.3}$.

There is controversy over whether there is an overestimation of the toxicity of chemicals from the results of laboratory tests. This is because there is usually a lower exposure level in the environment compared with that in test tanks due to the greater expanse of water under consideration and the complex makeup of the water system. Additionally many types of fish are able to treat small amounts of LAS as naturally occurring bile salts, and hence easily digest and excrete them.

A study by Kimberle [28] on the worldwide data for LAS concluded that it is possible to find LAS in concentrations ranging from 0.01 to 10 mg/L in the environment. From this he suggested that there is a one hundred fold difference in the tolerance levels of invertebrates, algae and fish to LAS exposure.

A summary of the LC_{50} concentrations of LAS for various aquatic species is shown in Table 1.6.

Species	Test Duration (h)	LC_{50} (mg/L)
Marine shrimp [14]	96	0.4
Lobster [14]	96	0.2
Common mussel [29]	96	3
Fresh water clam [14]	144	5
Soft shelled clam [14]	360	1
Common mussel [29]	360	0.5
Common mussel [29]	850	0.14

Table 1.6 LC_{50} concentrations of LAS for a variety of aquatic species

In a study of various animals from marine and river environments, Bressan *et al.* [30] found that the concentration range in which LAS display acute effects was ranged from 0.25 to 200 mg/kg. The tolerances observed were very dependent on the LAS concentration and the organism in question. Among the more sensitive organisms examined were copepods and embryos of the sea urchin, *Paracentrotus lividus*, while among the more resistant were the fresh water molluscs *A. cygnea* and *U. elongatulus*.

- **Sewage Sludges**

The degradation products of LAS, i.e. sulphophenyl carboxylates show a characteristic homologue distribution in sewage effluent and groundwater [31]. Intermediate chain lengths (between five and eight) are found to persist more than shorter or longer chain lengths. Taylor and Nickless [32] reported similar results in laboratory experiments using river water spiked with LAS.

- **To Humans**

At the moment the levels of LAS used do not appear to present a hazard to human health, as a large amount of LAS are required to produce chronic effects in mammalian systems. Acute oral LD_{50} values in rodents range from 650 to 2480 mg/kg. No effects apart from reduced weight gain were observed when rhesus monkeys were

simultaneously subscribed oral and subcutaneous administration of LAS (300 and 1 mg/kg, respectively) [33].

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Chapter 2

Methods for the Determination of Surfactants in the Environment

2.0 Introduction

Most commercial surfactants in use today are complex mixtures comprising of homologues, oligomers and positional isomers. For example, the anionic surfactant LAS may contain as many as thirty-five different homologues and phenyl positional isomers. The alkyl chain can vary from ten to fourteen methylene units, and the phenyl group is distributed quite evenly along all of the positions on the chain (with the exception of the two end groups). Analysis is further complicated by the various biodegradation products of these surfactants. As described in Chapter 1, recent concern over the effects of surfactants and their biodegradation intermediates on the environment means that any analytical method must be capable of providing information about the different isomers etc. present at concentrations down to the parts-per-billion level or less.

2.1 Non-Specific Methods

The need for routine low-concentration determinations of surfactants in laboratory and environmental samples led to the development of analytical methods capable of assessing the important members of the main surfactant classes. The chemistry of these methods is such that the determinations are not specific for the surfactants alone. These methods give fairly accurate results for clean samples and some of the methods are now standard for legislative biodegradation test protocols. However, the lack of specificity and sensitivity of these non-specific methods makes them less satisfactory for environmental samples where concentrations are lower and there are large amounts of interfering compounds.

2.1.1 Anionic Surfactants

For determinations of anionic surfactants, and in particular LAS, the methylene blue analytical method is the most widely used. Methylene blue (Figure 2.1) is a cationic dye, which in its normal form as the chloride or sulphate salt is insoluble in organic solvents. In the presence of an anionic surfactant, an ion-pair is formed between the methylene blue cation and the surfactant that is less soluble in water than the individual components and can therefore be separated into an organic solvent.

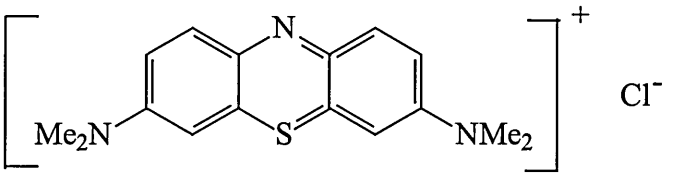

Figure 2.1 Methylene blue chloride

The complex forms a blue colour that is readily determined by colorimetry; the intensity of the colour is relative to the amount of surfactant in the system.

As the name MBAS (methylene blue active substances) suggests, the procedure is not specific to anionic surfactants. The MBAS procedure was originally developed by Longwell and Maniece [1]. It is responsive to any compound containing a single strong anionic centre, strong enough to form a stable ion-pair with the methylene blue cation, and at the same time containing a hydrophobic group sufficiently lipophilic to be more soluble in the organic layer. Other commonly occurring molecules can give partial responses with methylene blue, thus causing a positive interference. These are 17900 ppm NaCl at pH 1.8 which gives the same colour as 10 ppm alkyl sulphate surfactant, as do 1040 ppm of nitrate or 40 ppm thiocyanate [2]. Whilst careful choice of method can reduce the interference, such species can still cause falsely high results. Limits of detection using this method are around 0.01-0.02 mg/L in favourable conditions.

2.1.2 Non-ionic Surfactants

As almost all non-ionic surfactants are of the polyethoxylate type, only these will be discussed in this section.

Several non-specific methods are available for the determination of non-ionic surfactants. The three methods detailed below all depend upon the complex formation between the ethoxylated chain of the non-ionic surfactant and either inorganic metal or organic salts. As with the anionic MBAS method, the chemistry of these reactions is not specific to non-ionic surfactants without suitable cleanup of samples.

2.1.2.1 Cobaltothiocyanate Active Substances (CTAS) [3,4]

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

To concentrate and separate intact surfactant from non-surfactant materials that may interfere with the determination, the CTAS method uses a solvent sublation technique developed by Wickbold [5]. In the Wickbold method surface active materials including the non-ionics are removed from dilute aqueous samples (containing 10 % sodium chloride and 0.5 % sodium bicarbonate) into an overlying layer of ethyl acetate by bubbling a gas such as air or nitrogen through it. Separation is achieved by adsorption of the surfactant on the surface of the bubbles and subsequent transfer to the liquid-liquid interface where it is allowed to partition into the organic solvent. Any anionic

surface-active materials in the resulting extract are removed by non-aqueous ion-exchange. The limit of detection for the CTAS method is approximately 0.1 mg/L.

2.1.2.2 Bismuth Active Substances (BiAS) [6]

In this method non-ionic surfactants are extracted using a solvent sublation technique such as the Wickbold method described earlier. The non-ionic surfactants are precipitated with a modified Dragendorff reagent {barium chloride – potassium tetraiodobismuthate(III)}; the resulting non-ionic surfactant complex precipitate is dissolved and the liberated bismuth ion is titrated potentiometrically with pyrrolidine dithiocarbamate complexone as a measure of the non-ionic surfactant. The liberated bismuth can also be determined by atomic absorption or Ultraviolet (UV) colorimetry.

The BiAS method has gained the same status for non-ionic surfactants as the MBAS method has for anionic surfactants. The limit of detection of the method is 0.05-0.1 mg/L.

2.1.2.3 Potassium Picrate Active Substances (PPAS) [7]

The PPAS method involves the interaction of ethoxy chain with a large excess of potassium ions in aqueous solution to form a positively charged complex which is readily extractable into 1,2-dichloroethane as its picrate ion-association compound. The concentration of non-ionic surfactant is determined by the absorbance of the picrate ion at 378 nm.

The method is particularly suited to the determination of low concentrations of non-ionic surfactants in marine and surface waters, as it has a limit of detection of 2-200 μg/L.

2.2 Gas Chromatography

2.2.1 Anionic Surfactants

The presence of the sulphonate group on most anionic surfactants means that some form of derivatisation procedure must be performed prior to analysis by gas chromatography (GC). There have been several successful derivatisation methods for LAS in the literature. These include desulphonation in boiling phosphoric acid [8] and conversion to the sulphonyl chloride [9] or methyl sulphonate [10]. Modern GC methods tend to utilise mass spectrometric detection (GC-MS) rather than flame ionisation detectors (FID) or electron capture detectors (ECD); this is mainly because of the enhanced selectivity and sensitivity available from mass spectrometry.

Hon-nami and Hanya [10] used GC-MS for the determination of LAS in river water. Extraction was performed using the MBAS method described above, LAS was then removed from the methylene blue and converted to the methyl ester by treatment with phosphorus pentachloride and then methanol. As a final cleanup step, the resulting extract was then passed through a silica column. Calibration was performed by external standardisation with dodecylbenzene sulphonate. Extracts of the river Tama in Japan showed levels of LAS to be greater than 3 μg/L.

Trehy *et al.* have reported the results of a study involving the determination of LAS and dialklytetralin sulphonates (DATS) in environmental water and sediments [11]. LAS and DATS (by-products of LAS manufacture) were extracted using C_8 solid phase extraction (SPE) cartridges. Following extraction, the resulting analytes were allowed to react with phosphorus pentachloride and then trifluoroethanol to form their trifluoroethyl sulphonate derivative – an improvement on the procedure developed by Hon-nami *et al.* [10]. The formation of the trifluoroethyl sulphonate derivative enhances

the sensitivity and selectivity for electron capture negative chemical ionisation GC-MS. The limit of detection for the method was found to be *c.a.* 0.001 mg/L for both LAS and DATS. LAS concentrations of the influent and effluent of a trickling filter wastewater treatment facility in Utah, USA were found to be 2.7 mg/L and 0.14 mg/L, respectively. DATS concentrations were 0.22 and 0.052 mg/L.

A further study of ten US domestic wastewater treatment plants by Trehy *et al.* [12] employed the same GC-MS method and the same derivatisation procedure. Two types of sewage treatment plant were studied; activated sludge and trickling filter. The activated sludge process removed >99 % LAS and around 95 % for DATS. Trickling filter was shown to be less efficient with 85 % removal of LAS and 65 % DATS. Concentrations of LAS in receiving water down stream of the ten sites ranged from <0.001 to 0.094 mg/L and <0.001 to 0.023 mg/L for DATS.

2.2.2 Non-ionic Surfactants

The determination of the environmentally significant non-ionic surfactants alkylphenol ethoxylates (APEO) suffers from a similar volatility problem to LAS. While it is possible to determine the alkylphenol biodegradation products directly by GC, the APEO themselves require derivatisation and, even then, it is only possible to determine the first four oligomers AP1-4EO.

Blackburn and Waldock [13] have determined the concentration of alkylphenols in rivers and estuaries of England and Wales. Extraction was performed by C_{18} SPE; the resulting extracts were analysed directly by GC-MS without any derivatisation procedure. The survey included six rivers, the final effluent from twelve sewage treatment works, six estuaries and one harbour mouth. The highest concentration of nonylphenol found in effluent from sewage works was found to be 330 µg/L.

The works in question serves an area with a large amount of textile industry nearby. Actual concentration of nonylphenol in the river Aire into which the treated effluent was discharged was 180 μg/L which is approaching the LC_{50} for *Daphnia* (300 μg/L). The majority of the other rivers sampled contained <0.2 – 5 μg/L nonylphenol, the highest being 10 μg/L. Estuarine concentrations were lower as a result of dilution and dispersion processes caused by tidal flow.

The highest concentrations were recorded for the Tees estuary – 5.2 μg/L nonylphenol and 13 μg/L octylphenol; the other estuaries sampled contained less than 0.1 μg/L nonylphenol. No octylphenol was found anywhere else, reflecting the low use of octylphenol ethoxylates in the UK. The limit of detection for the method was 30 – 200 ng/L nonylphenol and 50 – 250 ng/L octylphenol. In conclusion, the authors suggested that apart from areas where there are high levels of industrial usage of alkylphenols, UK concentrations were below levels that would cause concern.

GC-MS has been used by Wahlberg *et al.* [14] for the determination of nonylphenol and nonylphenol ethoxylates (NPEO) in sewage sludge as their pentafluorobenzoates. Limits of detection were 0.1 mg/kg for nonylphenol and 0.4, 1 and 2 mg/kg, respectively, for NP1-3EOs. Following removal from the various matrices by solvent extraction, levels of nonylphenol in sludge samples were found to range from *ca.* 25 to 1100 mg/kg. Levels of NPEOs were much lower, ranging from less than the limit of detection to 125 mg/kg.

Field *et al.* [15] have reported the determination of nonylphenol ethoxycarboxylate biodegradation intermediates of NPEO by GC-MS following derivatisation with methyl iodide. The analytes were extracted from paper mill effluents, municipal sewage treatment plants and river water samples by strong anion exchange (SAX) extraction

disks after being spiked with 2-chlorolepidine as an internal standard. Limits of detection for the method were found to be 0.2, 0.4, 2.0 and 2.0 μg/L, respectively, for NP1-4EC. Total concentrations of NPEC in paper mill effluents ranged from below the limit of detection to 1300 μg/L, although all but two were less than 100 μg/L. Over half the effluents sampled contained only NP1EC and NP2EC, and in all cases, NP2EC was dominant. All four oligomers of NPEC were found in the sewage treatment plants; the percentages of each were NP1EC (7 %), NP2EC (54 %), NP3EC (31 %) and NP4EC (8 %). Of the eight US rivers tested, five gave concentrations for NP1EC and NP2EC above the limit of detection for the method; again, NP2EC was the dominant oligomer.

2.3 Capillary Electrophoresis

To the author's knowledge no results have been published on the use of capillary electrophoresis (CE) for the determination of surfactants in the environment.

Bullock [16] has reported a CE method for the analysis of Triton X-100 (an octylphenol ethoxylate surfactant) standards. Good resolution of the Triton X-100 ethoxymers was achieved using 25 mM boric acid buffer at pH 8.6 in a 35:65 acetonitrile / water solution containing 50 mM sodium dodecylsulphate (SDS) solution. The analytes were determined by UV detection at 200 nm. The author suggested a solvophobic mechanism for the separation, which involves the association of the hydrophobic portions of the Triton ethoxymers with the hydrophobic portions of the SDS molecule.

Shamsi and Danielson [17] have used CE with indirect photometric detection for the simultaneous determination mixtures of tetralkylammonium cationic surfactants and alkanesulphonate anionic surfactants. Salimi-Moosavi and Cassidy [18] have also reported the separation of alkanesulphonates; this time the method utilised non-aqueous CE with direct UV detection at 214 nm.

Heinig *et al.* [19] have shown CE to be useful for the determination of fatty alcohol ethoxylate surfactants in laundry detergents. Before separation, surfactants were derivatised with phthalic anhydride to render then suitable for UV detection at 200 nm. The authors found the method to be an excellent tool for the rapid "fingerprinting" of technical products and household formulations, and that results were comparable with those obtained by a standard HPLC method.

2.4 High-performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is ideally suited to the determination of complex mixtures of non-volatile, anionic or neutral surfactants – in most cases without the need for prior derivatisation.

2.4.1 Anionic Surfactants

The method by Tong and Tan [20] typifies the HPLC methodology for the determination of LAS in environmental matrices. Following extraction by C_8 SPE, samples were analysed on a C_{18} column using an acetonitrile / 0.33 M sodium perchlorate mobile phase. Application of the method to a sewage treatment plant showed influent and effluent to contain 0.40 and 0.14 mg/L LAS, respectively.

Other HPLC phases used for the separation of LAS include C_4 [21], polystyrene-divinylbenzene [22] and C_1 [23].

2.4.2 Non-ionic Surfactants

Three recent reviews [24,25,26] describe the enormous amount of data published on the determination of non-ionic surfactants (particularly of the APEO type) by HPLC over the last fifteen years or so.

Ethoxylate distribution has been determined by several different normal phase systems. Rothman [27] used an aminopropyl column for the determination of OPEO and NPEO with UV detection. The method used a gradient mobile phase system of isooctane, dichloromethane and methanol. Ahel and Giger [28,29] also used an aminopropyl column for the determination of NPEO and their biodegradation intermediates NP, NP1EO and NP2EO in waste water and river water. The authors used a hexane / isopropanol gradient system with UV detection. Samples were extracted by steam distillation and solvent extraction; the resulting analysis showed river water from the Glatt river in Switzerland to contain 3.9 μg/L NP, 23.4 μg/L NP1EO, 9.4 μg/L NP2EO and 0.8 – 2.3 μg/L NPEO. Digested municipal sewage sludge was found to contain 1.6 g/kg NP.

The same method was used by for the determination of NPEO around the Krka River estuary, Croatia [30]. Following extraction by C_{18} SPE, untreated municipal wastewater was found to contain 70 – 2960 μg/L NPEO with estuarine concentrations ranging between 0.7 and 17 μg/L.

The method developed by Ahel and Giger was also used by Boyd-Boland and Pawliszyn [31] who coupled solid phase microextraction (SPME) to HPLC for the determination of APEO in the environment. The authors found that the best SPME phase for the extraction of APEO was Carbowax / divinylbenzene. The method was used to determine OPEO in a sewage sludge to a level of 10 μg/g.

Another method for the determination of APEO in raw sewage and sewage effluent using an amino propyl column has been developed by Holt *et al.* [32]. Samples were extracted by sublation and ion exchange chromatography and the resulting extracts analysed by HPLC using a gradient mobile phase system of tert. butyl methyl ether and

acetonitrile / methanol (containing 0.1 % acetic acid). Total APEO concentration in two sewage treatment plants in South East England varied from 126 to 410 μg/L, with effluent levels varying between 40 and 228 μg/L.

Ethoxylate distributions have also been determined on silica [33,34,35], alumina [36], cyano [37], 10 pac [38], porous graphitic carbon (PGC) [39] and C_1(TMS) [39] columns to good effect.

Reverse phase HPLC using C_{18} columns provides information on the identity of the alkyl chain and can also be used to determine the overall concentration of APEO and as a rapid screening method [41,34,28,29].

2.4.3 Simultaneous Determination of Anionic and Non-ionic Surfactants

The determination of the two classes of surfactant in one chromatographic run has been described by a number of groups.

The simultaneous determination of LAS and APEOs was first reported by Marcomini and co-workers [42]. In this method the authors used C_8 and C_{18} reversed phase HPLC columns with water / acetonitrile gradient elution in the presence of 0.02 M sodium perchlorate and 5 % isopropanol for the determination of LAS and NPEO in laundry detergents. As would be expected from a reverse phase method, the LAS and APEO surfactants were separated by alkyl chain length. The C_{18} column was also able to resolve LAS positional isomers as well. However, for information on ethoxylate distribution a separate normal phase method was required.

The method was also used by Marcomini and Giger [43] for the determination of LAS and NPEO in sewage sludges and river sediments. Samples were extracted by soxhlet extraction. Results showed sewage sludges to contain 7.3 g/kg LAS, 1.2 g/kg NP, 0.22

g/kg NP1EO and 0.03 g/kg NP2EO. River water sediment contained 5.6 g/kg LAS, 0.9 g/kg NP, 0.80 g/kg NP1EO and 0.70 g/kg NP2EO. The NP, NP1EO and NP2EO concentrations were determined by normal phase HPLC. No higher NPEO ethoxymers were found in any of the samples.

The method developed by Marcomini and co-workers has also been used for the determination of LAS and APEO in the marine environment [44], and extended to the analysis of the carboxylic biodegradation intermediates of LAS and APEO as well [45].

Di Corcia *et al.* [46] developed a method that allowed the simultaneous extraction of LAS and its sulphophenyl carboxylate biodegradation intermediates, NPEO and their corresponding NPEC and NP using three different elution systems. The method was used for the determination of all of the above in raw and treated sewage of a mechanical-biological treatment plant. Samples were extracted using a SPE cartridge filled with graphitised carbon black (GCB) and analysed by the method developed by Marcomini and co-workers [45].

Graphitised carbon blacks are produced by heating carbon blacks at 2700-3000 °C in an inert atmosphere. GCBs are essentially non-specific, non-porous sorbents with surface areas ranging from between 8 and 100 m^2/g, depending on the type of starting material used. GCBs are capable of acting as both reverse phase and anion exchange sorbents. The anion exchange sites are relatively few in number. They are thought to have a chromene-like structure, that is a burnt-off residue left over from the heating of the blacks [47] in producing graphitic carbons. In the presence of water and particularly acid this surface group is rearranged to form benzpyrylium salts (see Figure 2.2). The presence of these positively charged chemical impurities on the GCB surface enables it to act as both an anion exchanger and a non-specific sorbent.

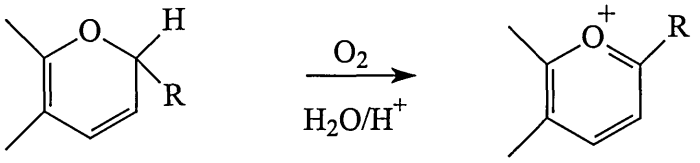

Figure 2.2 Rearrangment of chromene-like structure to benzpyrylium salt in graphitised carbon black

Recently Scullion *et al.* [48] published a method for the simultaneous determination LAS and APEO using a C_1 HPLC column. The method was an improvement on that developed by Marcomini and co-workers [42] as it enabled the resolution of the LAS positional isomers (if needed) and also the APEO ethoxymers in the same run. The carboxylic biodegradation intermediates of these surfactants was not addressed.

2.4.4 Liquid Chromatography - Mass Spectrometry

The recent development of modern, robust interfaces for the coupling of HPLC to mass spectrometry enables mass spectrometry to be used as a highly specific and sensitive detector for low level non-volatile, polar molecules.

An excellent review on the subject of the determination of surfactants by liquid chromatography – mass spectrometry (LC-MS) has been published by Di Corcia [49].

Various interfaces have been used for the determination of surfactants by LC-MS. These include a mechanical transport interface [50], particle beam [51], thermospray [52,53], electrospray [54] and atmospheric pressure chemical ionisation (APCI) [55,48].

2.5 Mass Spectrometry

Mass spectrometry and in particular tandem mass spectrometry (MS/MS) allows the determination of complex mixtures without prior derivatisation and chromatographic separation. However, the benefits that mass spectrometry provide such as simple sample

preparation and short analysis times have to be balanced against the high cost of the instrumentation and cost of day to day running.

Ventura *et al.* [56] have produced a method for the identification of surfactants and their acidic metabolites in raw and drinking water by fast atom bombardment mass spectrometry (FAB-MS) and FAB-MS/MS. The surfactants were extracted from the samples using an XAD-2 resin, followed by fractionation into acids, bases and neutrals. The acidic components were then derivatised to form the methyl ester by reaction with BF_3/MeOH. Analysis of the fractions by FAB-MS showed that non-ionic surfactants of the alkylphenol and alcohol ethoxylate types were the most abundant type of surfactant in both the raw and drinking water of the Barcelona area of Spain. The presence of their acidic biodegradation products NPECs in raw water, and bromononylphenol ethoxylates and brominated NPECs in drinking water was also identified. The presence of brominated compounds in both raw and river water was thought to be due to the high concentration of bromide ions in discharges from salt mines located in the upper course of the river. The anionic surfactant LAS was also identified along with small amounts cationic surfactants. The authors also used accurate mass measurements and tandem MS to aid identification where needed. No attempt was made to quantify any of the surfactants identified.

Borgerding and Hites [57] have also used FAB-MS for the quantification of LAS surfactants in a wastewater treatment plant. The surfactants were extracted by C_{18} solid phase extraction disks. The method allowed the simultaneous analysis of all LAS homologues by scanning the parent ion of m/z 183 which is a product ion common to all LAS homologues when subjected to collision induced dissociation (CID). It was also possible to determine branched alkylbenzene sulphonates (ABS) by scanning the parent ions of m/z 197. Analysis time was approximately four minutes because no

chromatographic separation was required; the limit of detection was determined to be 0.5 μg/L based on a 1 L sample.

Analysis of influent and effluent from the wastewater treatment plant showed that greater than 99 % of LAS was removed by the plant. However, detailed analysis showed that LAS was removed from wastewater through adsorption onto the sludge as well as by biodegradation. In fact, concentrations of the longer chain homologues of LAS on primary sludge were higher than the concentrations in the influent to the plant. This is because the longer chain LAS homologues accumulate on the sludge due to their increased hydrophobicity.

Atmospheric pressure chemical ionisation (APCI) mass spectrometry has been used for the determination of the oligomer distribution of APEO and fatty alcohol ethoxylates [58]. The instrument was operated in positive ion mode, and protonated molecule ion were seen for both types of surfactants. This method again showed the value of mass spectrometry for the determination of mixtures of surfactants without the need for prior chromatographic separation In this case the method was not used for the determination of environmental samples.

Strife *et al.* [59] used ion trap mass spectrometry for the analysis of the surfactant components of a shampoo. The authors operated the mass spectrometer in MS^n mode to provide a greater insight into the molecular structure of complex mixture than would be available by MS/MS alone.

The application of matrix-assisted laser desorption / ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) for the determination of surfactants is described in Chapter 5 of this thesis.

Of the methods described for the determination of surfactants in environmental samples, HPLC and mass spectrometry have the advantage of being the most versatile and efficient.

The initial aims of this project were to extend the earlier work carried out on surfactants at Sheffield Hallam University by Scullion *et al.* [48]. This involved the separation of LAS from co-eluting anionic interferences in surface water samples using a phase-switching method.

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Chapter 3

Separation of Linear Alkylbenzene Sulphonates from Humic Acids in Environmental Extracts by HPLC Phase-Switching

Scullion *et al.* [1] have reported the simultaneous separation of linear alkylbenzene sulphonate (LAS) and nonylphenol ethoxylate (NPEO) surfactants by HPLC. To the author's knowledge this was the first HPLC method for the simultaneous separation of both the LAS homologues and NPEO ethoxymers in the same run on the same column. The method utilises a Spherisorb S5 C_1(TMS) column supplied by HiChrom Ltd (Reading, UK) with an aqueous, isocratic mobile phase system.

Surfactants are extracted from river water samples using a C_{18} solid phase extraction (SPE) method. Resulting extracts showed several large interfering peaks eluting early in the chromatogram which masked any LAS peaks that may have been present in the sample. It was assumed that the early eluting peaks were acidic components originating from the sample. In order to remove all anionic components from the sample a strong anion exchange (SAX) SPE cartridge was used in series with the C_{18} cartridge. This methodology resulted in a much cleaner chromatogram, although any LAS present in the sample was removed by the SAX cartridge along with the anionic interference. The resulting chromatograms showed the typical envelope of ethoxymers characteristic of alkylphenol ethoxylates. Using this method a concentration of 5.6 μg/L for NPEO was determined by external standard calibration for a surface water sample from the River Don, South Yorkshire, UK.

The most likely cause of the anionic interference reported by Scullion *et al.* is humic acids. These are allomelanins found in soils, coals and peat, resulting from the decomposition of organic matter, and in particular dead plants. They consist of a mixture of complex macromolecules with polymeric phenolic structures and are slightly

water samples and extracts used.

The occurrence of humic acids in environmental extracts has been noted in the literature by Di Corcia *et al.* [2] and also by Castles and co-workers [3] who attributed a high fluorescent background, when analysing extracts from C_{18} and C_8 SPE systems, to contamination by humic acids.

In order to overcome this problem of interference from humic acids in surface water samples it was felt that a phase-switching approach might enable the humic acid / LAS fraction of the chromatogram to be switched onto a C_{18} column. The greater resolution obtainable on the C_{18} column may allow the humic acids to be separated from any LAS that may be present in the sample.

3.1.1 Reagents and Materials

All solvents used were HPLC grade and were obtained from Fisher Scientific (Loughborough, UK). All water used was Milli-Q grade. Ammonium acetate was HPLC grade (Fisher Scientific) and sodium perchlorate was obtained from Aldrich (Poole, Dorset, UK).

Triton X-100 (an octylphenol ethoxylate surfactant) was purchased from Aldrich, Synperonic NP9 (a nonylphenol ethoxylate surfactant) was a gift from ICI Materials Research Centre (Wilton, Middlesborough, UK). Nansa SS (a commercial dodecylbenzene sulphonate formulation) was obtained from Albright and Wilson (Castleford, UK).

Graphitised carbon black (GCB) SPE cartridges (500 mg) were purchased from Supelco (Poole, Dorset, UK).

3.1.2 Samples

Grab samples of surface water (2 L) were taken from the River Aire in West Yorkshire. Samples were preserved by the addition of 1 % formalin (an aqueous solution of 37 % formaldehyde) and were stored in the dark at +4 °C.

3.1.3 Solid Phase Extraction Methodology

Extraction / preconcentration was performed following the method developed by Di Corcia *et al.* [2]. The method uses three different eluents: -

Eluent A. Dichloromethane / methanol (70:30)

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

Eluent C: 10 mM tetramethylammonium hydroxide in dichloromethane / methanol (90:10)

The extraction procedure was as follows: -

1. Sample acidified to ~pH 3 with conc. HCl.
2. Cartridge conditioned with:-

   7 mL eluent C

   3 mL methanol

   30 mL acidified water (pH 2 with conc. HCl)
3. Sample added to cartridge
4. Cartridge washed with:-

   7 mL water

   Air dried for 1 min

   2 mL methanol

   Air dried
5. Analytes extracted with:-

   7 mL eluent A

   7 mL eluent B

   7 mL eluent C

Eluents A and C were combined, evaporated to dryness under a steady stream of N_2 and redissolved in 1 mL HPLC mobile phase (eluent B was discarded).

3.1.4 HPLC Instrumentation and Conditions

All HPLC analyses were performed on a Gilson 302 gradient pumping system. Fluorescent detection was carried out using a Jasco FP-920 (Cheltenham, UK)

sulphonates, and 220 nm excitation and 290 nm emission for alkylphenol ethoxylates. All data were output to a Schimadzu integrator. Injections of 50 μL were made using a Waters Wisp 712 autosampler.

Two isocratic mobile systems were used with the C_1 column; 65 % water / 35 % acetonitrile with an overall buffer concentration of 0.065 M ammonium acetate, and 58 % methanol / 42 % 0.008 M aqueous ammonium acetate solution.

An isocratic mobile phase system of acetonitrile / 0.33 M sodium perchlorate was used with the C_{18} column.

3.1.5 Phase-Switching Methodology

Phase-switching experiments were performed on a MUST Multiport Streamswitch (Spark Holland, Emmen, Netherlands). This basically consisted of two Rheodyne injection valves and a set of switches to control the flow through and between the two valves.

3.2 Results and Discussion

3.2.1 Extraction with GCB SPE Cartridges

The extraction of both anionic and non-ionic surfactants using graphitised carbon black (GCB) solid phase extraction cartridges has been employed by Di Corcia and co-workers [2] to good effect. The procedure uses three different eluents to individually fractionate non-ionic and anionic surfactants and their respective biodegradation products. It was hoped that this method would be able to fractionate the humic acid interference from any linear alkylbenzene sulphonates in surface water samples.

3.2.1.1 Standards

Previous data suggested that the Spherisorb C_1 columns obtained from HiChrom had an average lifetime of about nine months; after this time the ability of the column to resolve the NPEO ethoxymers reduced dramatically. At the start of this project the Spherisorb column available was about a year old and showed poor resolution of the NPEO ethoxymers. Therefore, a Hypersil SAS C_1 column was purchased from Shandon (Cheshire, UK) as this particular brand of column had not been tried before and it was hoped that the extra length of the column would improve resolution further.

Figure 3.1 shows the chromatogram of a mixture of Nansa SS and Triton X-100 standards obtained using the methanol version of the mobile phase on the Hypersil column. The chromatogram shows excellent resolution of both of the LAS homologues and the Triton X-100 ethoxymers. Note that the acetonitrile version of the mobile phase was unable to effect any resolution of the Triton X-100 ethoxymers; the reason for this selectivity seen only with the methanol version of the mobile phase was unclear at the time of writing. However, the column produced very poor resolution of the Synperonic

(Figure 3.2). Again, the reason for this observation was unclear, although it is worth noting that it has previously been observed [1] that the Spherisorb column's ability to resolve NPEO ethoxymers degraded more quickly than it's ability to resolve OPEO ethoxymers.

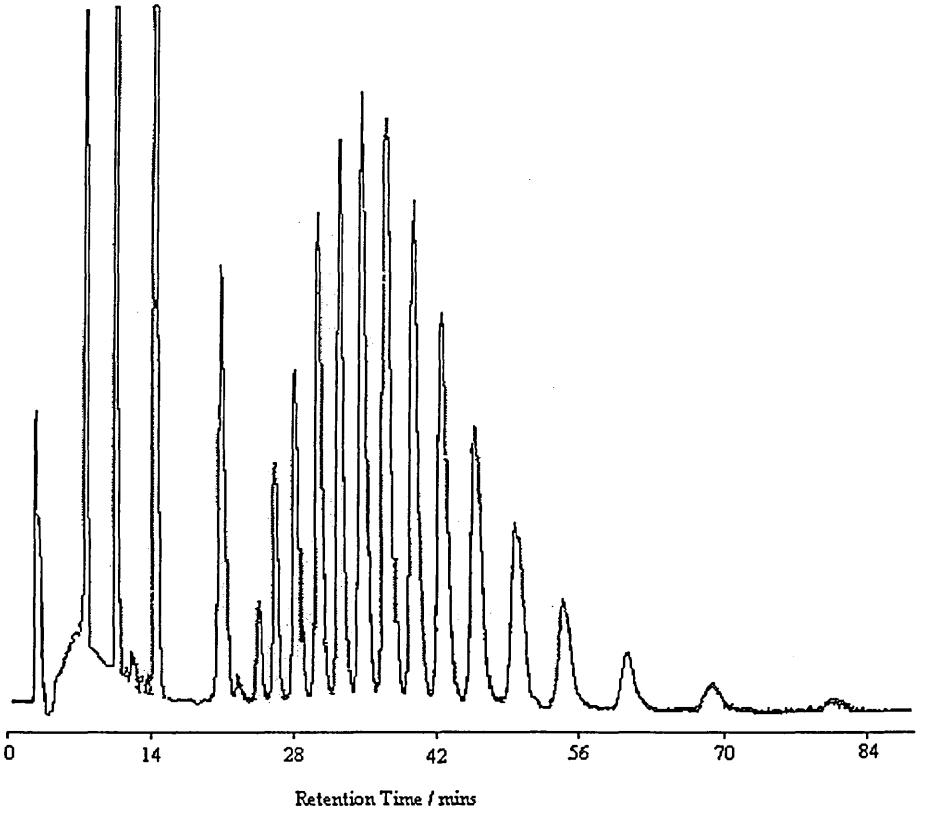

Figure 3.1 HPLC chromatogram of a mixture of Nansa SS and Triton X-100 standards on a Hypersil SAS C_1 column

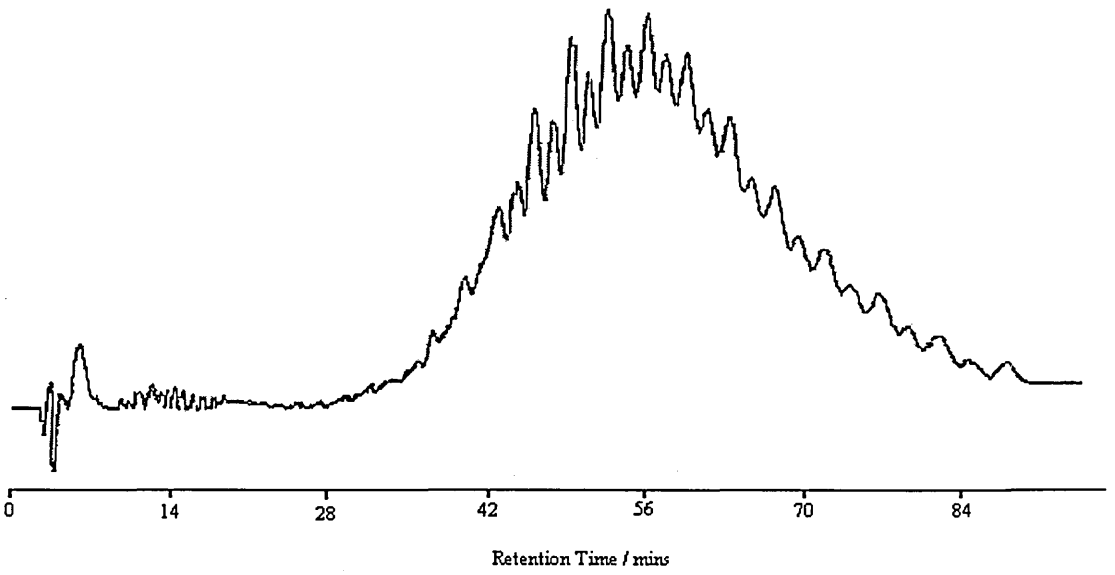

Figure 3.2 HPLC chromatogram of a Synperonic NP9 standard on a Hypersil SAS C_1 column

Extraction of Milli-Q water spiked with Nansa SS and Triton X-100 produced excellent recoveries of around 100% for both classes of surfactant; the results are shown in Tables 3.1 and 3.2.

3.2.1.2 Samples

The resulting chromatogram produced from an extract of water from the River Aire is shown in Figure 3.3. The chromatogram, produced on the Hypersil column, contains a large peak, or series of peaks, eluting over the first twenty minutes of the run. This would appear to be the same interfering anionic species seen in previous work, the identity of which is thought to be humic acids occurring naturally in the river water. This is supported by the fact that the water samples were brown in colour, a good indication of the presence of humic substances. As mentioned above, the appearance of these interfering compounds in the sample totally masks the presence of any LAS that may be present in the sample. The samples do, however, show the presence of an envelope of peaks characteristic of alkylphenol ethoxylate surfactants. The identity of

the envelope resembles the results obtained with the standard Synperonic NP9. In addition, the samples from the River Aire originate downstream from an important woollen industry site, which uses NPEO in washing processes. These results would suggest that while the GCB extraction method is able to extract these surfactants from the environment, it is not able to fractionate the humic acids from any LAS present in the sample. However, quantification by external standard calibration showed these samples to contain a total NPEO concentration of 214 μg/L.

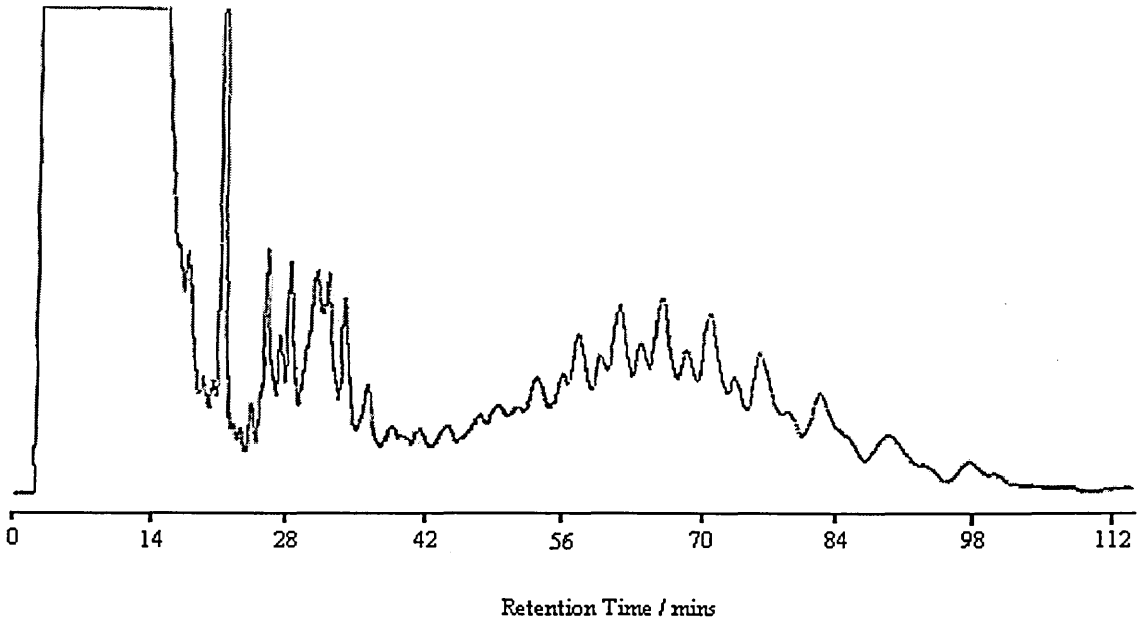

Figure 3.3 HPLC chromatogram of an extract from the River Aire

Ethoxymer	1	2	3	4	5	6	7	8	9	10	11
% Recovery	75	120	104	101	100	99	99	96	96	94	107

Table 3.1 Triton X-100 ethoxymer recoveries

Homologue	1	2	3	4
% Recovery	87	91	92	96

Table 3.2 Nansa SS homologue recoveries

3.2.2 Phase-Switching

In an attempt to overcome the problem of the humic acid interference a phase-switching method was developed.

From previous work and the literature it was known the LAS would separate on a C_{18} column using an acetonitrile / perchlorate mobile phase [4] and therefore it might be possible to separate LAS from the humic acids on this column. This could be achieved by running the C_1 method as usual but "chopping" the LAS / humic acid fraction onto the C_{18} column as it eluted from the C_1 column. The NPEO separation could then be carried out as usual and the acetonitrile / perchlorate mobile phase could then be directed to the C_{18} column to separate the LAS / perchlorate fraction left on the column.

A diagram of the arrangement used for this procedure is shown in Figure 3.4. The system basically consists of two Rheodyne high pressure injectors connected together to allow the solvent from the pump to pass through either or both of the columns depending on the position of the switches on the valve.

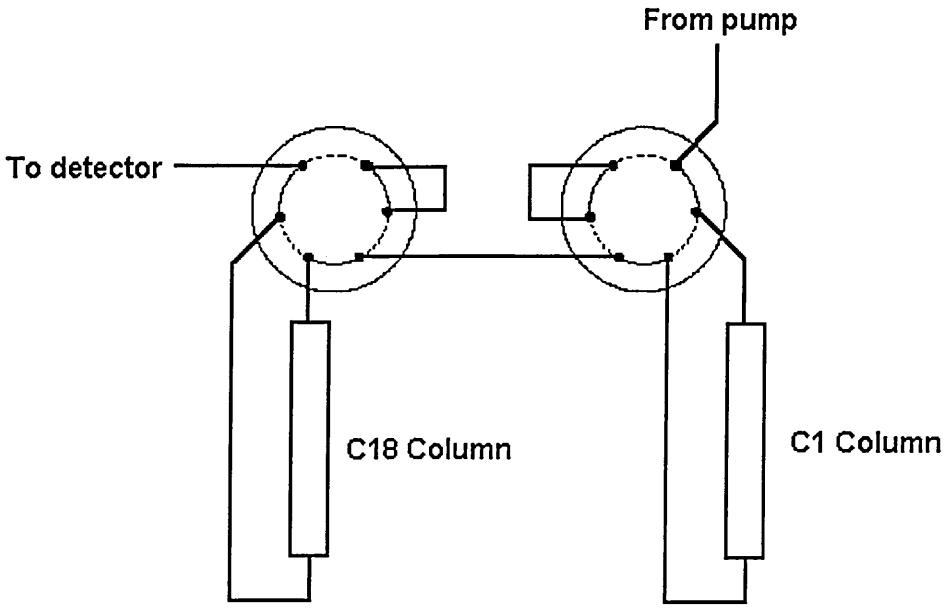

Figure 3.4 Diagram of the phase-switching arrangement

Figure 3.5 shows a phase-switched chromatogram of a Nansa SS / Synperonic NP9 standard mixture through a Supelcosil LC-1 C_1 column and a Spherisorb C_{18} column. Two different mobile phases were used in the phase-switching method; these were acetonitrile / ammonium acetate (35:65) – mobile phase A, and mobile phase B – acetonitrile / sodium perchlorate (55:45). For the first minute mobile phase A was allowed to flow through the C_1 column only. Then the C18 column was added in series for nine minutes. The C18 column was then removed from the flow leaving just the C_1 column in line until the NPEO ethoxymers had separated (about 30 mins). Finally the C_1 column was removed from the flow and the C_{18} column was reintroduced using mobile phase B to separate the LAS homologues. As can be seen from the chromatogram the LAS peaks now elute after the NPEO ethoxymers. The Supelco C_1

Although the resolution of the NPEO ethoxymers is very poor, the principle is illustrated quite nicely.

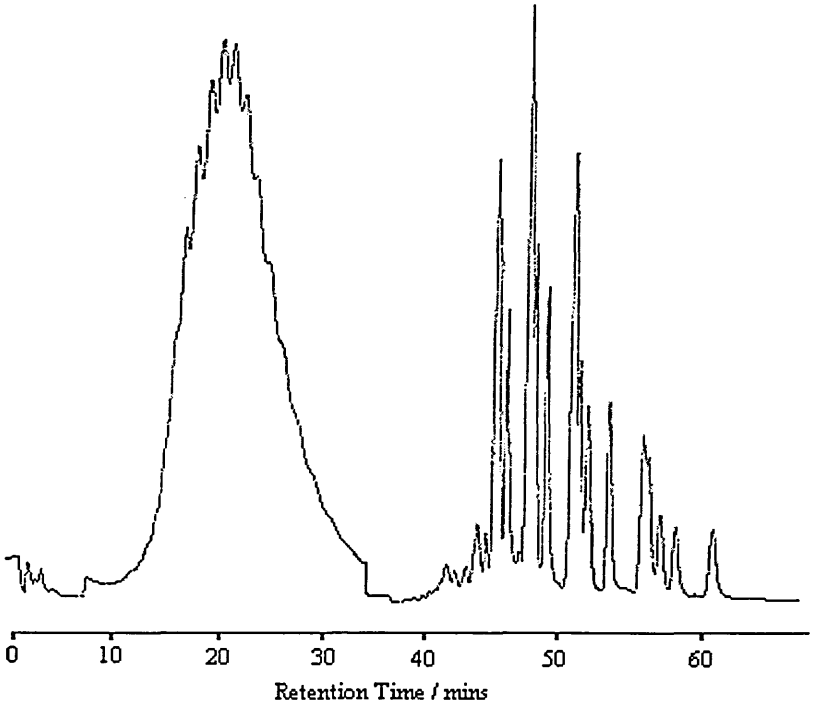

Figure 3.5 HPLC phase-switching chromatogram of a mixture of Nansa SS and Triton X-100 standards

Figure 3.6 shows an extracted sample run through the phase-switching procedure described above. There are no peaks typical of NPEO ethoxymers at the time expected (around 20 mins), instead there is just one peak at 19.46 mins. This single peak could possibly be a completely unresolved envelope of NPEOs. The identity of the large peak at 4.4 mins is not certain at the time of writing; however, it would appear to have peaks that resemble the last part of an ethoxymer distribution in its tail.

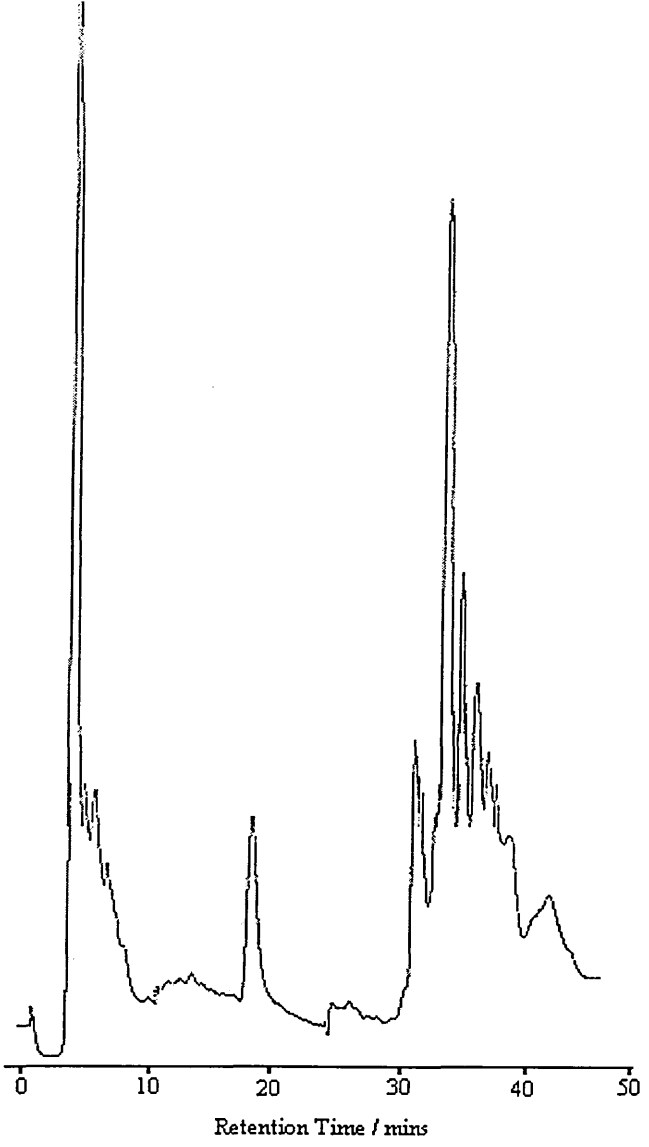

Figure 3.6 HPLC phase-switching chromatogram of an extracted sample from the River Aire

The peaks between 30 and 47 mins are possibly due to LAS but they could just as easily be humic acids or some other component in the sample. To positively identify these peaks would involve interfacing the method to a mass spectrometer using an electrospray or APCI interface.

3.2.3 New Spherisorb Column

As the Hypersil column was unable to achieve the resolution of NPEO ethoxymers required a new Spherisorb S5 C_1 column was purchased from HiChrom. However, as seen in Figure 3.7, the chromatogram of Synperonic NP9 using the new column did not produce the same resolution of NPEO ethoxymers achieved by previous batches of the column. This was true for both the methanol and acetonitrile mobile phase systems. Contact with the column manufacturer's found that there were no known changes to the Spherisorb stationary phase packed in the column; a replacement column was no better.

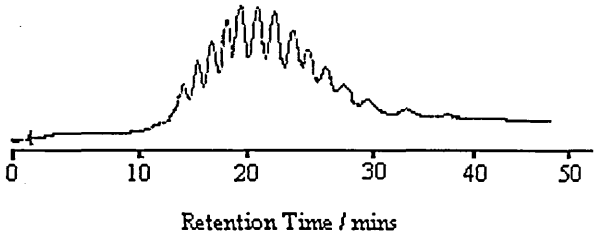

Figure 3.7 HPLC chromatogram of a Synperonic NP9 standard on a new Spherisorb S5 C_1 column

3.3 Conclusion

Two methods have been investigated for the separation of humic acids from any LAS surfactants that may be present in surface water samples.

A solid phase extraction procedure using graphitised carbon black cartridges has been followed with a view to separating the humic acids from the LAS by fractionation in the cartridge. While recoveries of the two classes of surfactants were excellent, the anionic fraction from the column still contained humic acids.

A phase-switching method has also been developed in an attempt to combat the problem of the masking of any LAS in river water samples by what are suspected to be humic

acids encountered in previous work and also in work shown here. The method employs C_1 and C_{18} columns coupled to Rheodyne injection valves in such a way that the mobile phase could be diverted through either or both columns. This allowed the combined LAS / humic acid peak to be "cut" from the C_1 column to the C_{18} column. The NPEO ethoxymers were then allowed to separate on the C_1 column as usual, followed by the attempted separation of any LAS present in the sample from the humic acids on the C_{18} column using an acetonitrile / perchlorate mobile phase.

The method was found to work for standard mixtures of LAS and NPEO but it is not clear whether the technique worked for real samples. The portion of the chromatogram that should have been the LAS / humic acids had a resemblance to the standard mixture of LAS but peak identification by retention time was not possible because retention times depend on the time at which "phase-switching" occurs. The only certain way to identify these peaks would be to use LC-MS with a sensitive interface such as electrospray or APCI; such techniques were not available in our laboratory at that time.

Another problem that appeared during this work was the inability of recent batches of Spherisorb S5 C_1 column to adequately resolve NPEO ethoxymers. Work described here, and work by a previous PhD student has shown that the Spherisorb column was the only column capable of adequately resolving both OPEO and NPEO ethoxymers. The Supelcosil LC-1 column from Supelco was able to separate both classes of ethoxylate surfactant but never as well as the Spherisorb column. The Hypersil SAS column produced excellent resolution of OPEO ethoxymers but very poor resolution of NPEO ethoxymers. The reason for this wide range of performance and the sudden lack of resolution achieved with Spherisorb columns will be investigated further in the next chapter.

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Chapter 4

The Characterisation of C1(TMS) HPLC Stationary Phases by X-ray Photoelectron Spectroscopy and Elemental Analysis

4.0 Introduction

In Chapter 3 of this thesis, problems were described with the separation of nonylphenol ethoxylates on recent batches of Spherisorb C1(TMS) stationary phase from HiChrom Ltd. along with the inability of C1(TMS) columns available from other suppliers to achieve the same separation. The carbon content of the individual phases was measured in order to ascertain the reason for this comparative lack of resolution as this is most likely to be the main factor in the separation. This was performed by x-ray photoelectron spectroscopy and bulk carbon analysis of the silicas. It was anticipated that differences in the concentration of the trimethylsilyl group bonded to the silanols on the surface of the silica would be observed.

4.1 The Use of Silica in HPLC Stationary Phases

4.1.1 The Silica Gel Surface

Silica gel particles consist of a core of silicon atoms joined by siloxane bonds (silicon-oxygen-silicon bonds). The surfaces of the particles contain some residual, uncondensed hydroxyl groups from the original polymeric silicic acid. These surface hydroxyl groups confer the polar properties of the silica gel and enable the formation of alkyl-bonded phases. However, the silica gel surface does not consist solely of uncondensed hydroxyl groups. The surface can contain more than one type of hydroxyl group, strongly bound or "chemically" adsorbed water and loosely bound or "physically adsorbed" water depending on previous treatment of the gel.

Surface hydroxyl groups can be one of three types: a single hydroxyl group that is attached to a silicon atom that has three siloxane bonds joining it to the gel matrix; geminal hydroxyl groups that are two hydroxyl groups attached to the same silicon atom that is joined to the matrix by two siloxane bonds; one of three hydroxyl groups

attached to the same silicon atom attached to the matrix by only one siloxane bond. Examples of type of hydroxyl bond are shown in Figure 4.1.

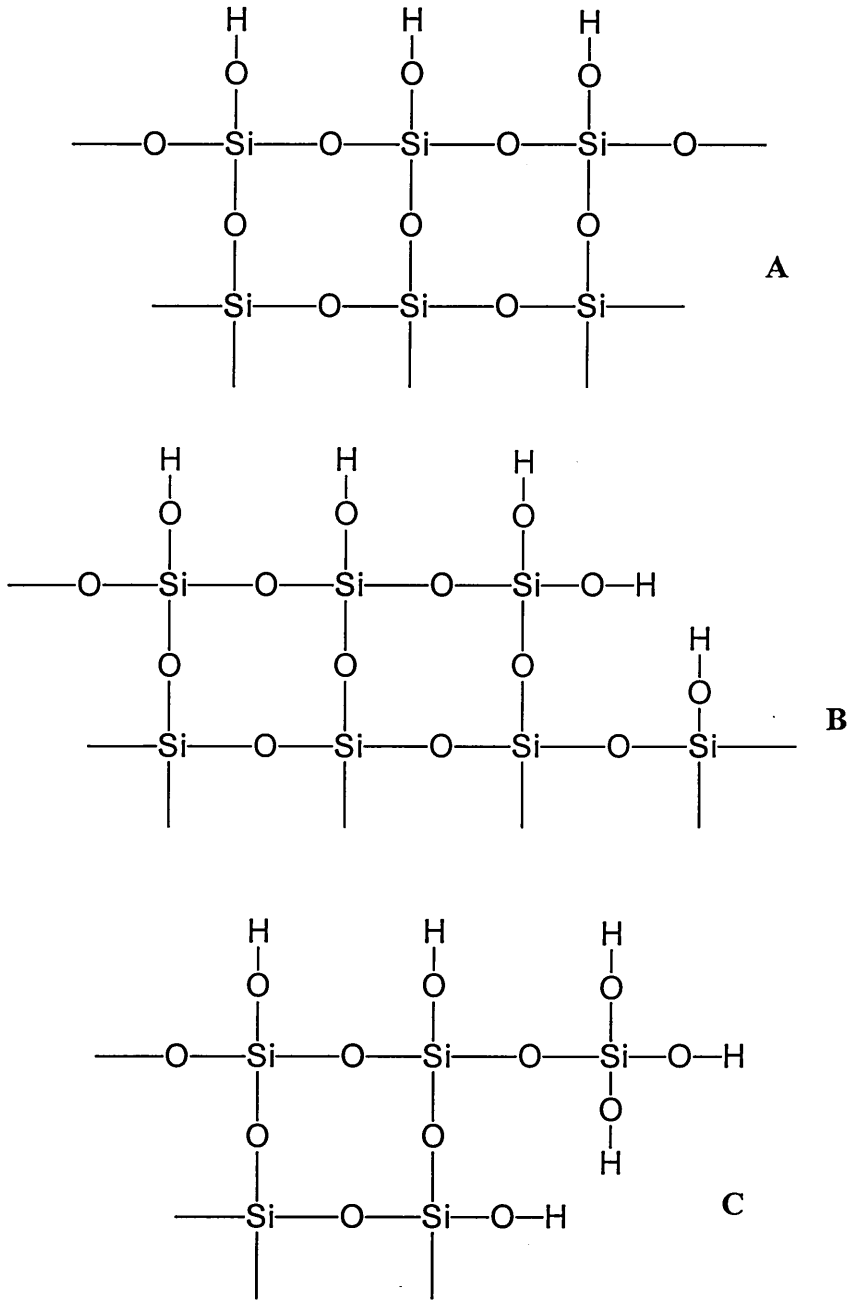


Figure 4.1. Different forms of hydroxyl groups possible on the surface of the silica substrate. A) Single hydroxyl group attached to silicon atoms. B) Double or geminal hydroxyl groups attached to silicon atoms. C) Triple hydroxyl groups attached to silicon atoms

As mentioned above, the surface of the silica is more complex than the examples represented in Figure 4.1. It is possible for water to be hydrogen bonded to the surface hydroxyl groups, and multiple layers of water to be physically adsorbed on top of these.

However, silica is more commonly used as a stationary phase for HPLC in the form of a bonded phase, involving the reaction of the surface hydroxyl groups with organosilane molecules. Unbonded silica is a less popular choice because of its highly polar nature resulting in extremely long retention times for many important classes of solutes such as molecules of biological interest. In addition, the range of solvents needed for the efficient use of silica gel as a stationary phase is complex compared with the simple aqueous solvent mixtures used with alkyl-bonded phases. However, the highly polar nature of silica gel can also be an advantage; silica gel is very useful in the separation of polarisable substances such as polynuclear aromatic hydrocarbons and mixtures of compounds of weak polarity such as phenols and esters.

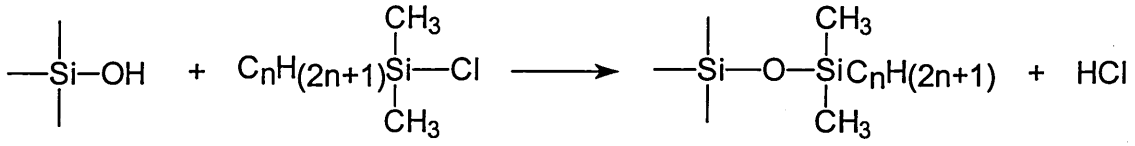
4.1.2 Alkyl-Bonded Silica Stationary Phases

Alkyl-bonded stationary phases are the most popular choice of stationary phase used today. While the number of different bonded phases available is enormous, most are based on C_{18} and C_8 alkyl groups, the other common types being C_1, phenyl, cyano propyl and aminopropyl.

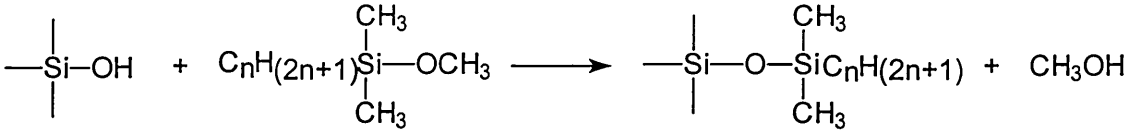
The most efficient bonded phase is one that has the minimum number of hydroxyl groups unreacted, and consequently, the maximum surface coverage. Steric hindrance from the alkyl moiety itself restricts the number of silanol groups that can be bonded and there is little that can be done to avoid this problem. Another reason for incomplete silanisation is the exclusion of the reagent molecule from the smaller pores on the silica surface. This is particularly true for relatively large reagent molecules such as dimethyloctadecylchlorosilane that is used to make C_{18} phases.

4.1.3 The Synthesis of Bonded Phases

Bonded phases are synthesised by reaction of the silica with either a chlorosilane or an alkoxysilane. These reactions are shown in Figure 4.2 below.



Chlorosilane



Alkoxysilane

Figure 4.2 Chlorosilane and alkoxysilane reactions for the preparation of bonded phases

These reactions are carried out in solvents such as toluene and xylene using pyridine as a scavenger for any hydrochloric acid released by the reaction. The same conditions are used in both laboratory and large scale synthesis.

4.2 Retention Mechanisms

When the silica surface is in contact with a solvent, the surface is covered with a layer of the solvent molecules. If the mobile phase consists of a mixture of two solvents, the surface is partially covered by one solvent and partially with the other. Thus, any solute interacting with the stationary phase may be presented with two, quite different types of surface with which to interact.

4.2.1 Normal Phase

Retention in normal phase, or adsorption chromatography, is due to interaction of polar functional groups on the analyte with discrete sites on the surface of the stationary phase. Two different mechanisms have been proposed to account for the nature of the adsorption process.

The competition model deals with the case of non-polar and moderately polar mobile phases, which interact with the surface of the stationary phase largely by dispersive and weak dipole interactions. The model assumes monolayer coverage of the surface with solvent molecules. Analyte retention then occurs by a competitive displacement of a mobile phase molecule from the surface of the adsorbant. This process is depicted in Figure 4.3a.

When polar solvents are used, the solvent interaction model proposes the formation of mobile phase layers adsorbed onto the stationary phase surface. The composition of the bilayer formed depends on the concentration of polar solvent in the mobile phase. Analyte retention occurs by interaction (either association or displacement) of the solute with the secondary layer of adsorbed mobile phase molecules (Figure 4.3b).

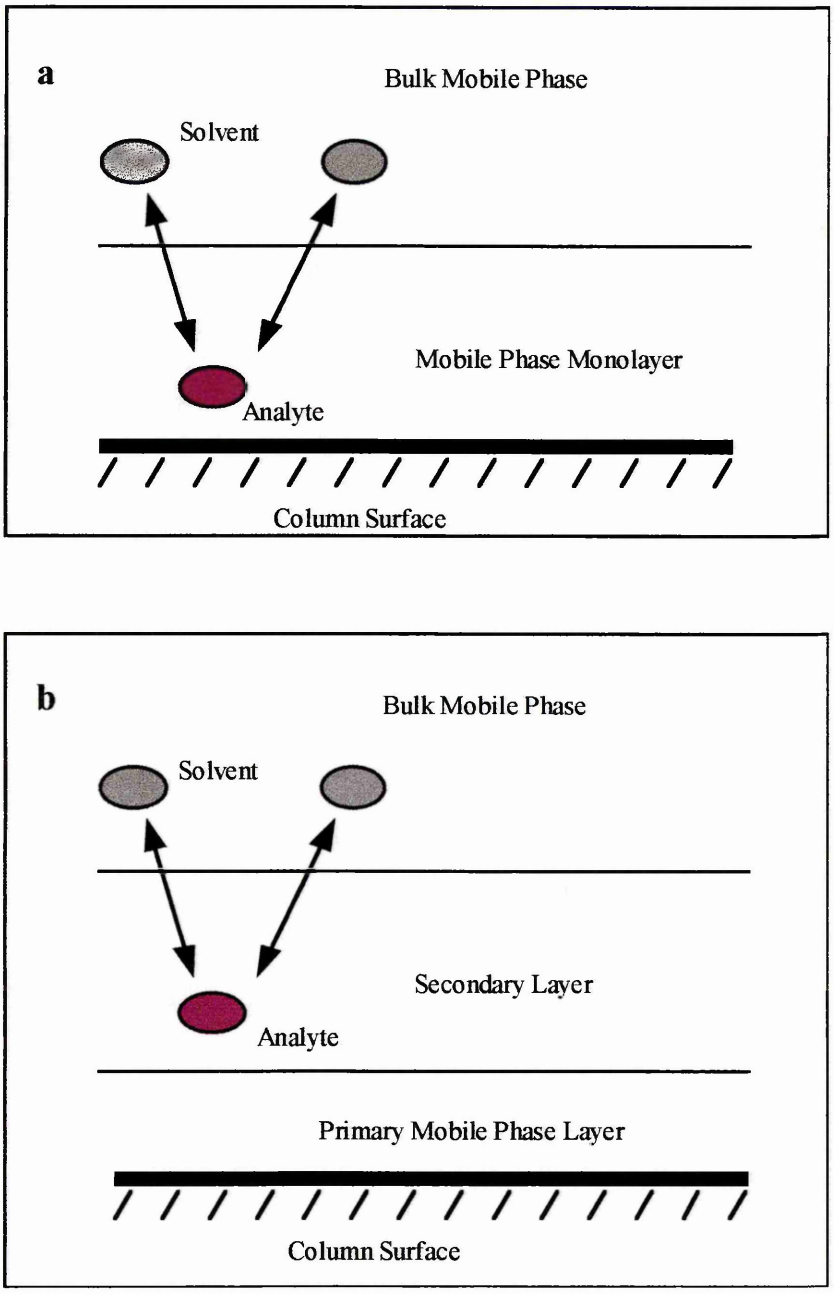


Figure 4.3 Two possible models of the mechanism of retention in normal phase chromatography. a) the Competition model, and b) the Solvent Interaction model

4.2.2 Reverse Phase

The mechanism of separation in reverse phase chromatography is not as straightforward as the normal phase mechanism described above. The dispersive forces between analyte and the non-polar stationary phase are too weak to account for the degree of separation seen in reverse phase chromatography.

Perhaps the best description of retention in reverse phase chromatography is the solvophobic theory. This theory is based on the assumption that the stationary phase is a uniform layer of a non-polar ligand. If the analyte binds to the stationary phase then the amount of surface area of the analyte exposed to the mobile phase is reduced. The analyte is sorbed due to this solvent effect; that is the analyte is sorbed because it is solvophobic. Therefore, analytes are retained more as a result of interactions (solvophobic) with the mobile phase rather than through specific interactions with the stationary phase. This phenomenon means that mobile phase composition has more influence on separation than the stationary phase (Figure 4.4).

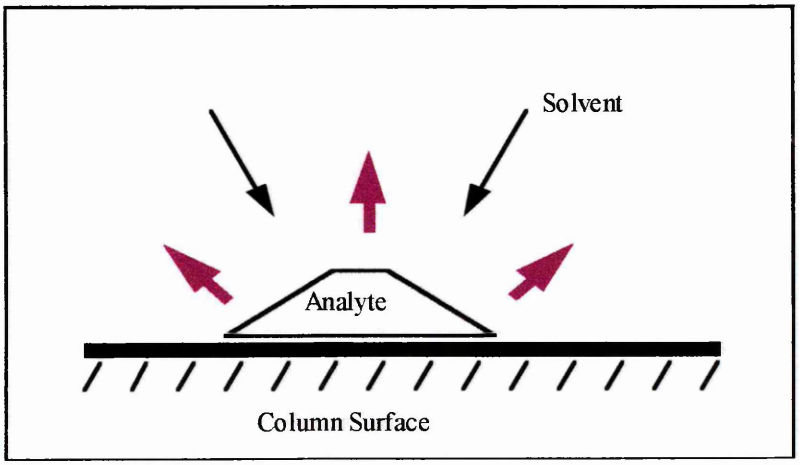


Figure 4.4 Representation of interaction between analyte, mobile phase and stationary phase in reverse phase chromatography

4.3 Methods for the Characterisation of Silica HPLC Stationary Phases

4.3.1 Chromatographic Characterisation

Traditional methods for the characterisation of silica based HPLC stationary phases tended to rely on chromatographic properties such as retention time, capacity factor and efficiency.

Kimata and co-workers [1] used numerous solutes to test various properties of C_{18} alkyl-bonded silica stationary phases. Alkylbenzenes with various alkyl groups were used to test hydrophobic properties. Triphenylene and *ortho*-terphenyl, having similar hydrophobic property but a difference in planarity, were used to test the shape selectivity of the stationary phase. Fluorene and diphenylmethane, and perylene and 1,1'-binaphthyl were also used for this purpose. Caffeine and theophylline were used for testing the hydrogen-bonding ability of the stationary phase. Alkyl amines with pK_a values above nine such as procainamide, N-acetylprocainamide, and benzylamine were used in buffered mobile phases to assess the contribution of ion exchange sites on silica surfaces. Phenol and benzyl alcohol were used to normalise the retentions of these polar compounds in order to compare the differences in hydrophobic properties of various packing materials.

Welsch *et al.* [2] used the crown ethers 18-crown-6 and 12-crown-4 along with other solutes of varying polarity such as aniline, pyridine, benzene, phenol, benzyl alcohol, benzonitrile, nitrobenzene and butyrophenone to study silanol effects in reversed phase columns.

Homologues of alkanoate and perfluoroalkanoate esters have been used by Gilpin *et al.* [3] as solute probes for the characterisation of the surface composition of alkyl bonded phases under reversed phase conditions. The authors used the results from the study to

determine a simple equation that describes the influence of the surface phase composition on a solute's retention.

Engelhardt and Jungheim [4] studied methods for the comparison and characterisation of C_8 and C_{18} reverse phases. Using a standard water/methanol eluent they were able to differentiate a C_8 from a C_{18} column by the elution order of ethyl benzoate and toluene; with the C_8 phase ethyl benzoate eluted together with or after toluene, whereas with the C_{18} phase ethylbenzoate always eluted before toluene. The same eluent composition was used to study the suitability of stationary phases for the separation of basic solutes. Good phases (those that produced symmetrical peak shapes for basic solutes) eluted aniline before phenol.

Cruz and co-workers [5] have used a variety of chromatographic data in order to classify the ever-increasing range of commercially available C_{18} reverse phase stationary phases. The similarities and differences observed between the different columns tested were used to form a rational column selection protocol by either matching the column characteristics to the analyte's physio-chemical properties or by systematic evaluation of columns from various categories.

4.3.2 Instrumental Methods

Nuclear magnetic resonance (NMR) spectroscopy has been used quite extensively for the characterisation of alkylbonded silica stationary phases, as described in an excellent review by Albert and Bayer [6]. In the review the authors describe the use of ^{29}Si cross-polarisation-magic angle spinning (CP-MAS) NMR spectroscopy to yield information on the variety and quantity of surface species of both pure and modified silica gel. In addition, ^{13}C CP-MAS NMR spectroscopy can be used to study the dynamic properties of the attached alkyl chains, in particular the differences between monofunctionalised

silica and cross-linked polymers. ^{13}C and ^{29}Si CP-MAS NMR spectroscopy have also been used to study the endcapping of different phases with hexamethyldisilazane and trimethylchlorosilane.

Infrared (IR) spectroscopy has been used for the characterisation of alkyl-bonded silica stationary phases by for example Tripp and Hair [7]. They used an in situ liquid IR cell to monitor the reaction between octadecyltrichlorosilane and silica.

The percentage bulk carbon of stationary phases has been used for their characterisation by a number of groups [8,9,10]. Determinations are carried out by elemental analysis, normally in conjunction with nitrogen and hydrogen analysis. The results give an overall percentage of carbon in the stationary phase both on the outer surface of the silica and any that may be contained in the pores of the silica.

Recently, highly surface-specific techniques such as x-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS) have been employed in the study of silica HPLC stationary phases. Such techniques are particularly important, as it is at the surface where the most important chromatographic interactions are thought to occur.

Miller and Linton [11] used XPS to characterise thermally treated SiO_2 surfaces to good effect. Linton and co-workers [12] then went on to use XPS along with CP-MAS NMR, Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) and gravimetric analysis to study the reactivity of hydroxyl groups on silica with trimethylchlorosilane. All of the instrumental techniques gave responses that correlated well with increasing TMS surface coverages on Lichrosorb Si 60. Results of the study showed geminal silanols to be much more reactive than single silanols. A comparison of these

techniques showed them to be complementary since their surface and molecular surface specificity vary widely.

XPS and time of flight (TOF)-SIMS have been utilised by Brown *et al.* [9] in the characterisation of a range of alkyl-bonded silica HPLC stationary phases. A variety of alkyl-bonded silica stationary phases were prepared with alkyl chain lengths ranging from C_1 to C_{18}, and including cyanopropyl and phenyl groups. TOF-SIMS identified dimethylalkylsilyl molecular ions, along with ions attributable to the dimethylalkylsilyl chains attached to fragments of the silica surface, showing that the dimethylalkylsilyl chains are covalently bonded to the silica surface. Comparison between percentage carbon by XPS and bulk carbon analysis indicated that the dimethylalkylsilyl groups are found preferentially at the surface of the silica particles. A comparison of the ratios of the intensities of predominantly silicon-based ions to predominantly hydrocarbon-based ions from TOF-SIMS data and % Si / % C from XPS analyses showed that data obtained from the two techniques correlated well.

The same group has also correlated surface analysis data from XPS and TOF-SIMS with retention behaviour of neutral, acidic and basic solutes [10]. Significant correlations were observed between the capacity factor (k) and the XPS C:Si atomic ratio, which was similar to that obtained between k and the bulk % C or k and the bonded alkyl chain length. Similar correlations were also achieved between k and the SIMS alkyl:Si ion peak area ratios. The overall result of the study confirmed that both XPS and SIMS could generate surface chemical data that has a direct relevance to the prediction of chromatographic behaviour.

4.4 X-ray Photoelectron Spectroscopy

4.4.1 Introduction

X-ray photoelectron spectroscopy (XPS) is based on the photoelectric effect described by Hertz over one hundred years ago; however, the first commercial spectrometers did not appear until about thirty years ago. Today XPS is regarded as a sophisticated surface science technique, finding use in biology, chemistry, physics and materials science.

4.4.2 The Photoelectric Effect

Each atom present at the surface of a material (except hydrogen) possesses (in addition to valence electrons) core electrons that are not directly involved in bonding. The "binding energy" (E_b) of each core electron is characteristic of the individual atom to which it is bound. In the XPS experiment, a sample surface is irradiated by a source of low-energy x-rays under ultrahigh vacuum. The interaction of an x-ray photon with the sample leads to the ejection of photoelectrons having a kinetic energy (E_k) related to the x-ray energy ($h\nu$) and E_b by equation 1: -

$$E_k = h\nu - E_b \text{.....Eqn (1).}$$

If the photoelectrons have sufficient kinetic energy to escape from the surface, photoemission is said to occur, the entire process being called the photoelectric effect.

In practice, other instrument related parameters have to be incorporated into equation 1. These are the recoil effect (E_R), which is a minor component compared with the others and is generally ignored, and the instrument work function ϕ. As XPS can be applied to insulators as well as conductors, the equation can contain a δE term which reflects the static charging of the sample; this factor reduces the kinetic energy of the outgoing electrons. Hence equation 2 can be written: -

$$E_k = h\nu - E_B - E_R - \phi - \delta E \quad \text{.....Eqn (2).}$$

As the energy levels are quantised, the photoelectrons have a kinetic energy distribution, N(E), consisting of a series of discrete bands that essentially reflects the "shells" of the electronic structure of the atoms in the sample. The experimental determination of N(E) by a kinetic energy analysis of the photoelectrons produced by exposure to x-rays is termed x-ray photoelectron spectroscopy (XPS).

4.4.3 Sampling Depth

After photoionisation the next step for the photoelectron is to travel through the solid to escape into the vacuum, without energy loss, before it can be analysed and detected as a characteristic photoelectron. In XPS the incident x-rays penetrate up to a depth of several microns, but the stopping power of solids for electrons is several orders of magnitude higher than it is for x-rays. Therefore electrons of the energy range 50-1000eV will typically travel between only two and ten atomic layers before they lose energy through inelastic scattering events with other electrons and hence cannot contribute to the characteristic photoelectron energy peak at energy E_k. This very small sampling depth (2-5 nm) is what gives XPS its inherent surface specificity.

4.4.4 Instrumentation

Instrumentation for XPS consists of the following: -

- An ultrahigh vacuum environment.
- A sample manipulation system.
- A x-ray source.

- An electron analyser and detection system.
- A computer control and data manipulation system.

A schematic representation of a typical XPS system is shown in Figure 4.5.

4.4.4.1 Ultrahigh Vacuum Environment

The XPS experiment must be carried out under ultrahigh vacuum conditions for three reasons. First, the emitted photoelectrons must be able to travel from the sample through the analyser to the detector without colliding with gas phase particles. Secondly, some components such as the x-ray source require vacuum conditions to remain operational. Finally, the surface composition of the sample under investigation must not change during the experiment.

XPS spectrometers consist of two vacuum chambers; the main chamber (also called the analytical chamber) houses the main instrumental components of the spectrometer and is where analysis takes place; there is also a smaller load-lock or sample introduction/preparation chamber. In its simplest form, the load-lock chamber is a small volume chamber that can be isolated from the analytical chamber and then back-filled to atmospheric pressure. Samples are placed in the load-lock chamber, which is then evacuated, typically with a turbomolecular pump. After the chamber has been pumped down, the samples are then transferred to the analytical chamber for analysis. The load-lock chamber is held at a modest vacuum of 10^{-6} torr, while the analytical chamber is held under ultrahigh vacuum of 10^{-9}-10^{-10} torr.

4.4.4.2 Sample Manipulation System

Once samples have been placed in the analytical chamber, they need to be positioned for analysis. This is usually accomplished with a holder/manipulator. Modern manipulators have the ability to translate a sample in three directions and to rotate it in one or two directions. In spectrometers capable of multisample analysis these translation and rotation motors are controlled by computer.

4.4.4.3 X-ray Source

Most commercial x-ray sources consist of a magnesium and/or aluminium anode that is bombarded by high-energy (15 keV) electrons to produce characteristic AlK_α or MgK_α emission lines. Magnesium and aluminium are chosen because; they are the only elements that produce sufficiently high x-ray energy to excite core-level electrons of all elements; their x-ray spectra are relatively clean with very few satellites or other peaks; they have a narrow line width and are suitably conductive and have high enough melting points. Many sources also include a monochromator to remove interferences from satellite peaks and Bremsstrahlung background which can cause assignment and interpretation problems. Monochromators generally use back-diffraction from the (1010) face of a quartz crystal, following the Bragg equation: -

$$n\lambda = 2d\sin\theta \quad \text{.....Eqn (3).}$$

where n=diffraction order, λ=x-ray wavelength, d=crystal spacing and θ=Bragg angle.

However, as well as the many advantages, sources containing monochromators suffer from the problem of very low x-ray flux at the sample when compared with sources without monochromators.

4.4.4.4 Electron Analyser and Detection System

In XPS, spectral resolution is of great importance if accurate determination of electron binding energies is to be achieved.

The most common form of electron analyser in XPS is the concentric hemispherical sector analyser (CHA). To achieve the desired spectral resolution the CHA is operated in constant analyser energy (CAE) mode. This is achieved by applying a constant voltage across the hemispheres, allowing electrons of a particular energy to pass between them; thus resolution is constant across the entire energy range.

In practice, an electrostatic transfer lens assembly transfers photoelectrons from the sample to the focal point of the analyser. When they reach this point they are electrostatically retarded before actually entering the analyser itself and those electrons whose energy now matches the pass energy of the analyser are transmitted between the analyser hemispheres and, subsequently, detected by the electron detector.

Electron detection is performed by a channel electron multiplier array. In this system, each incident photoelectron causes a secondary electron cascade resulting in an output pulse of up to 10^8 electrons.

4.4.4.5 Computer Control and Data Manipulation

Modern XPS systems come with advanced data systems that allow acquisition, storage and processing of data. Control of analyser, detector, x-ray gun and sample position is easily managed from the computer control system. The computer will also allow complex data manipulation such as quantification, peak fitting and quantification of the relative contribution from different chemical states.

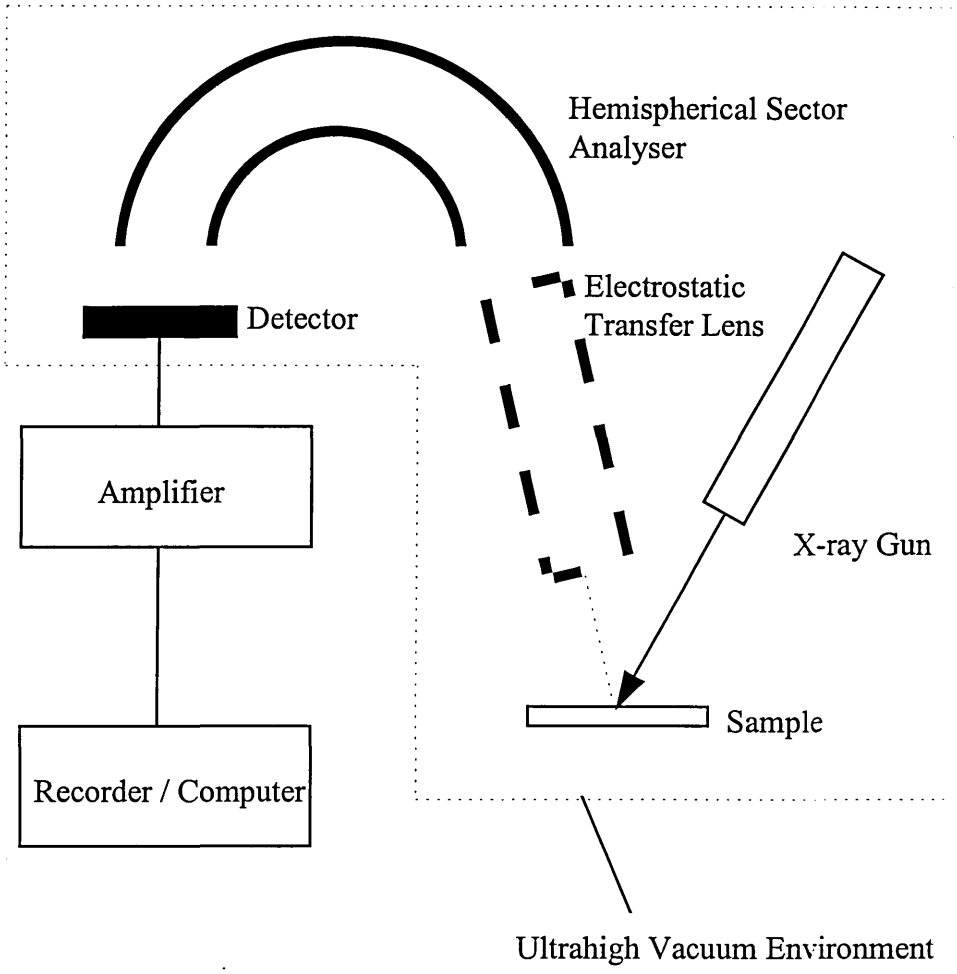


Figure 4.5 Schematic diagram of XPS system

4.5 Experimental

4.5.1 Materials

Spherisorb S5 C1 150 mm x 4.6 mm I.D. HPLC columns were obtained from HiChrom (Reading, UK). A Supelcosil LC-1 C1(TMS) 150 mm x 4.6 mm I.D. HPLC column was purchased from Supelco (Poole, Dorset, UK). A Hypersil C1 SAS 250 mm x 4.6 mm I.D. HPLC column was obtained from Hypersil (Runcorn, Cheshire, UK).

4.5.2 XPS Instrumental Parameters

All x-ray photoelectron spectra were produced on a VG ESCALAB Mk II using Al Kα x-rays ($h\nu$ = 1486.6 eV). The x-ray gun was operated at 14 kV, 20 mA. A wide scan spectrum (0-1000 eV) and high-resolution spectra of the Si 2p (95-120 eV), C 1s (275-300 eV), In $3p^1$ (435-460) and O 1s (520-545 eV) regions were recorded for each sample. The analyser was operated in fixed transmission mode with a pass energy of 20 eV for survey scans and 20 eV for narrow scan data. With this instrument all parameters such as the x-ray gun, analyser etc. were computer controlled with the exception of the sample position, which was controlled manually. VGS 5250 software on a PDP 11/53 data system was used for data acquisition and analysis. Quantification was performed using the software supplied with the data system. Note that In $3p^1$ scans were not included in quantification.

4.5.3 XPS Sample Preparation

Samples for XPS analysis were prepared using an "indium mirror" technique. A small square (*c.a.* 2 x 2 mm) of indium foil (Aldrich, Poole, Dorset, UK) was applied to a heated XPS sample stub, mounted on a hot plate. Upon melting the indium was spread over the stub, using a scalpel blade, until a mirrored surface was achieved. This surface

was then pressed into the silica sample. After cooling, the stub was shaken to remove any excess silica.

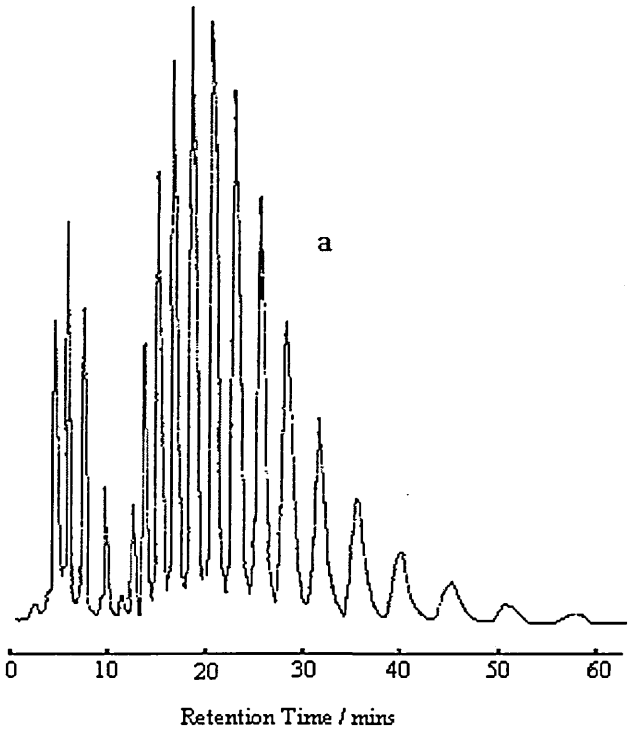
4.5.4 Elemental Analysis

Elemental analysis was carried out by Medac Ltd (Brunel Science Centre, Surrey, UK).

4.6 Results and Discussion

The separation developed by Scullion *et al.* [13] was, to the author's knowledge, the first HPLC method for the simultaneous separation of anionic and non-ionic surfactants that featured resolution of both the LAS homologues and NPEO ethoxymers (Figure 4.6a). The method was derived from two previous methods for the separation of these classes of surfactant. The resolution of LAS homologues on a C_1 column by Castles *et al.* [14], and the method by Wang and Fingas [15] for the separation of NPEO ethoxymers using a C_1 column. The new, simultaneous method was produced on a Spherisorb S5 C1 column (batch no. 1317) supplied by HiChrom Ltd. Repeating the method on a Supelcosil LC-1 C1(TMS) column purchased from Supelco demonstrated inferior resolution of the NPEO ethoxymers (Figure 4.6b). A similar situation was described by Wang and Fingas [15] for the separation of OPEO ethoxymers on Spherisorb and Supelco C_1 stationary phases.

As described in Chapter 3, later batches of the Spherisorb column (batch no. 1334) from HiChrom were unable to achieve the same resolution of NPEO ethoxymers as that obtained on previous batches (Figure 4.6c). In addition, a Hypersil SAS C1 column obtained from Hypersil was also unable to achieve adequate resolution of NPEO (Figure 4.6d).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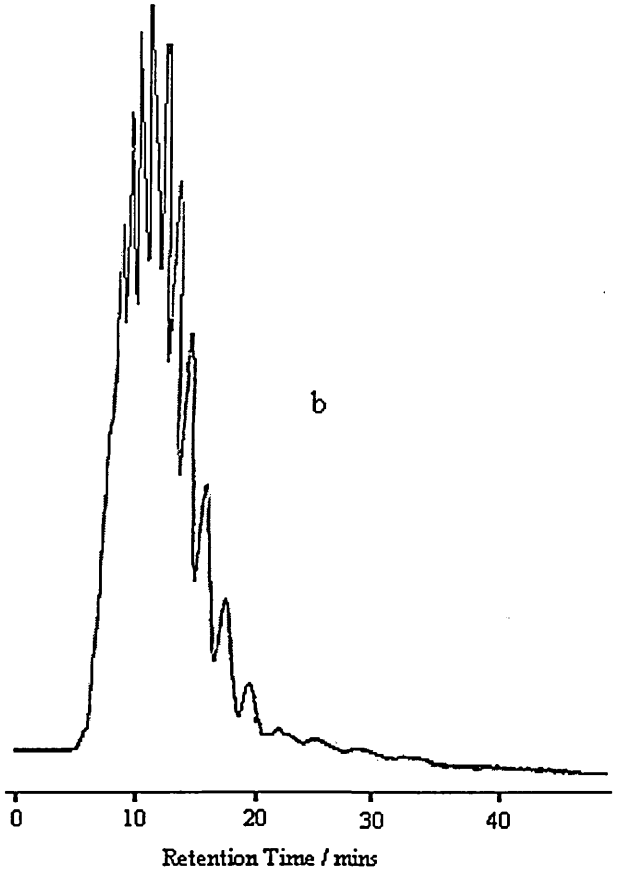
Retention Time / mins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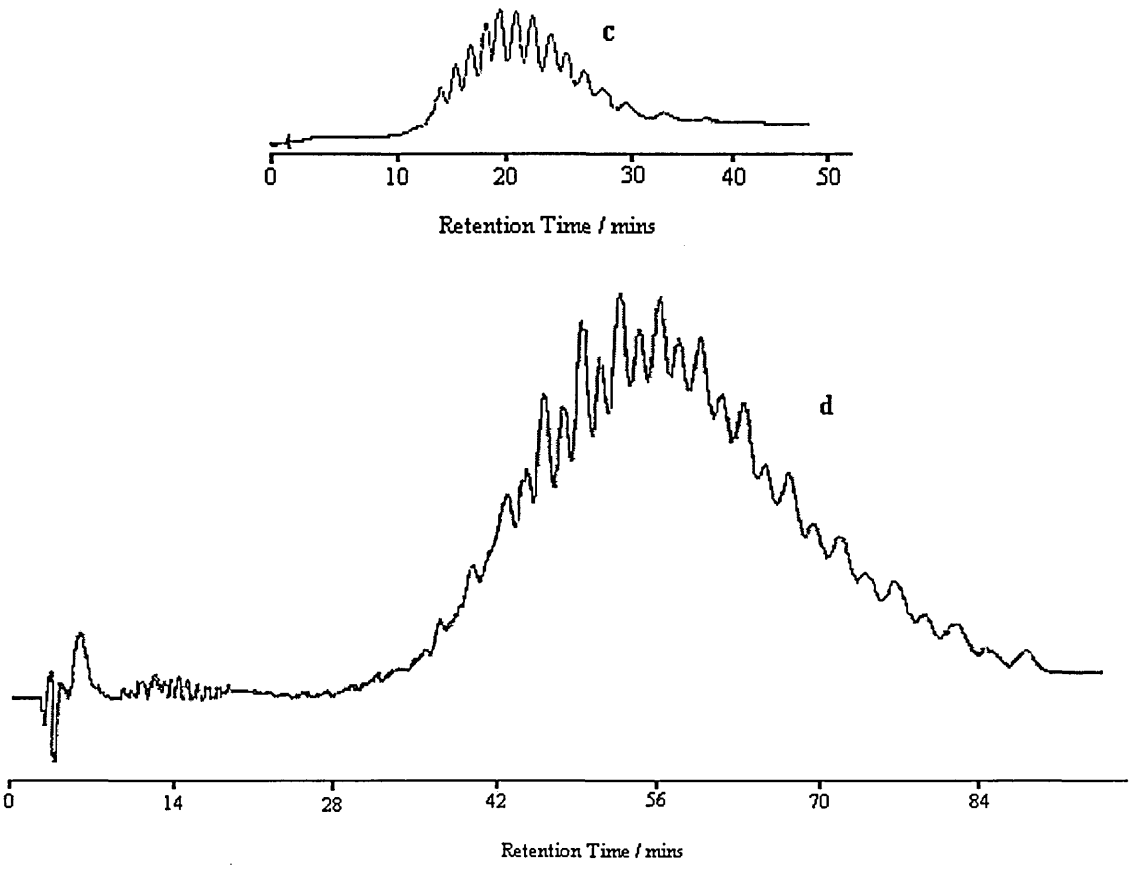


Figure 4.6 HPLC chromatograms of Synperonic NP9 produced on a) older batch (1317) of Spherisorb column [13], b) Supelco column, c) later batch (1334) of Spherisorb column and d) Hypersil column

The percentage bulk carbon loading data obtained by elemental analysis are shown in Table 4.1. These data are in good agreement with a previous study by Brown and co-workers [9] who quoted a figure of 2.14 percent carbon for bulk carbon analysis of a C1 phase from Hypersil. This equates well with the figure of 2.03 obtained in this work for the Hypersil column.

Sample	% Bulk Carbon
Spherisorb S5 C1 – 1317	0.33
Spherisorb S5 C1 – 1334	1.45
Supelco LC-1	0.93
Hypersil SAS	2.03

Table 4.1 Percentage bulk carbon by elemental analysis of various C_1 stationary phases

The pattern of results produced by elemental analysis suggests an obvious trend that equates well with the ability of the different alkyl-bonded phases to resolve the NPEO ethoxymers. Spherisorb S5 C1-1317 which produced the best resolution of all of the phases examined, had the least carbon by bulk analysis. Next, in order of increasing percentage carbon, was the phase from Supelco that was also able to separate the ethoxymers, but not with the resolution of the Spherisorb S5 C1-1317. The other two phases, both of which were unable to resolve the ethoxymers of NPEO, showed the highest carbon content. Therefore, it would seem from these results that the phases with the least amount of carbon (by elemental analysis) produced the superior separation.

The wide and narrow scan spectra obtained by XPS analysis are shown in Figures 4.7 – 4.12. As can be seen for each of the stationary phases examined, the oxygen and carbon narrow scans show two peaks for each element. For the oxygen scans the peak with a binding energy of *ca.* 537 eV is due to oxygen on the surface of the silica. In the case of the carbon scans, the peak with a binding energy of *ca.* 287 eV is due to carbon on the silica surface. The other peak in these scans is due to oxygen and carbon, respectively, on the surface of the indium used in sample preparation. This was identified by running scans on a blank indium mirror, without the addition of silica.

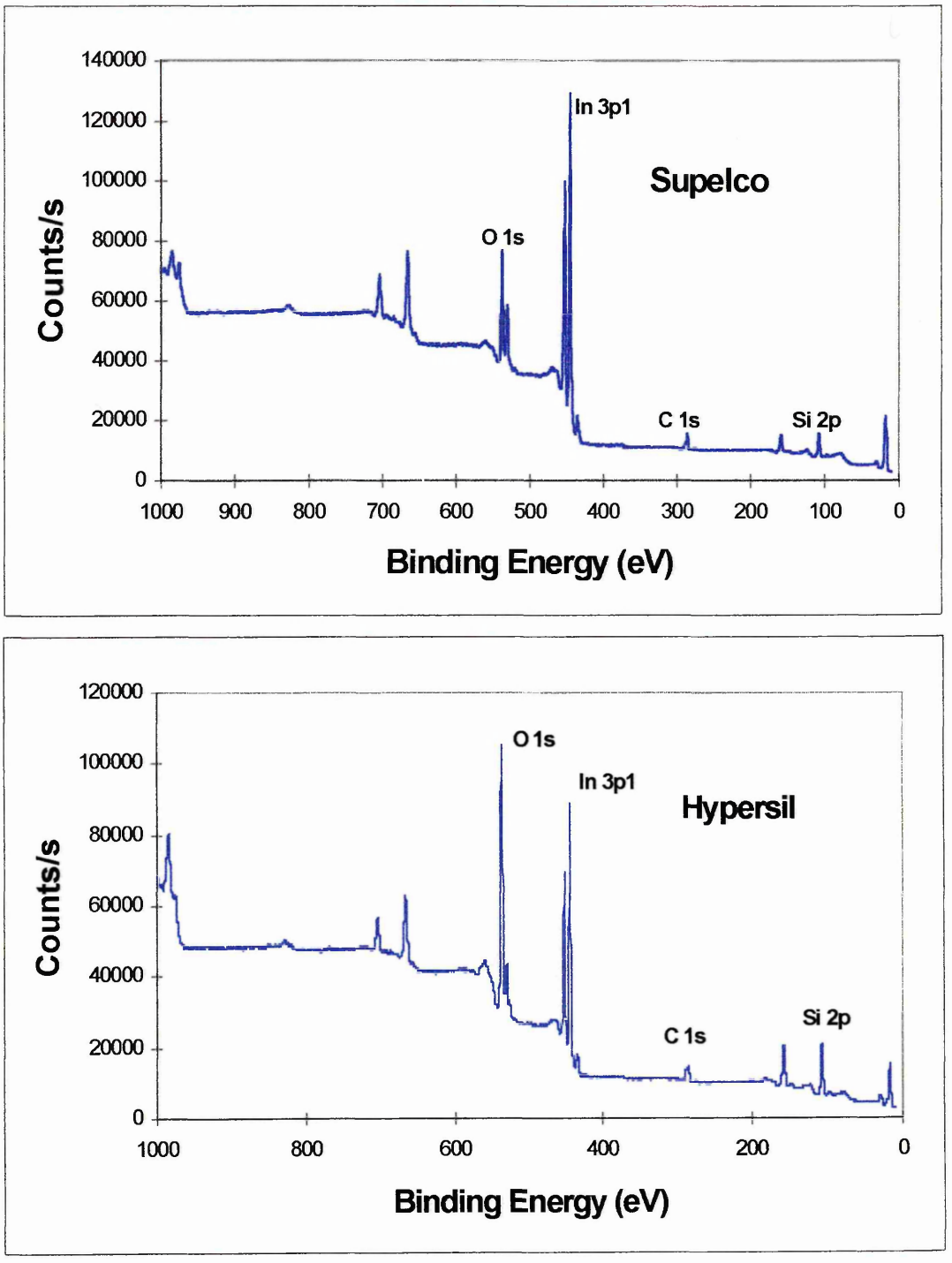


Figure 4.7 XPS wide scan data for Supelcosil LC-1 and Hypersil SAS stationary phases

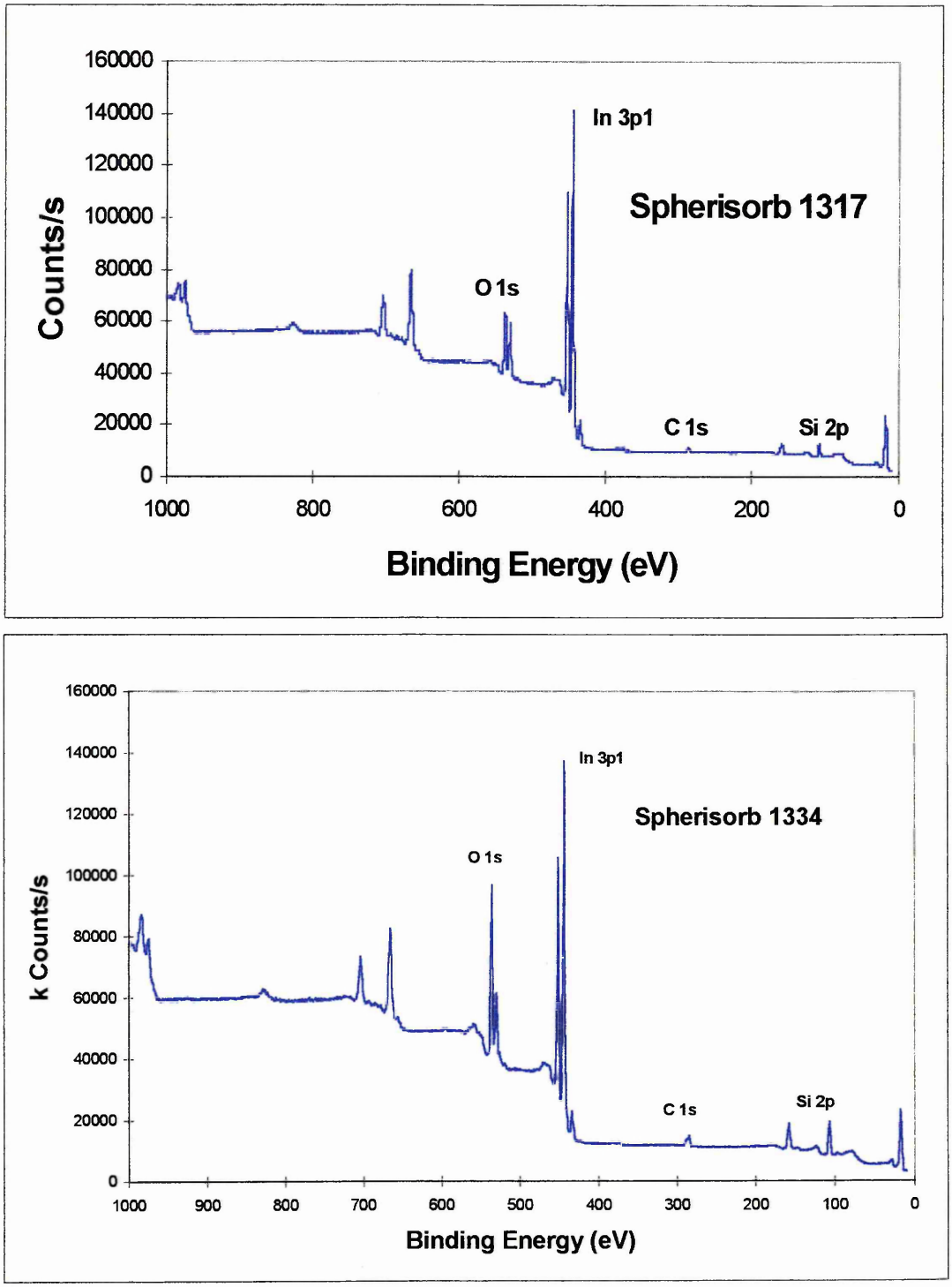


Figure 4.8 XPS wide scan data for Spherisorb S5 C1-1317 and 1334 stationary phases

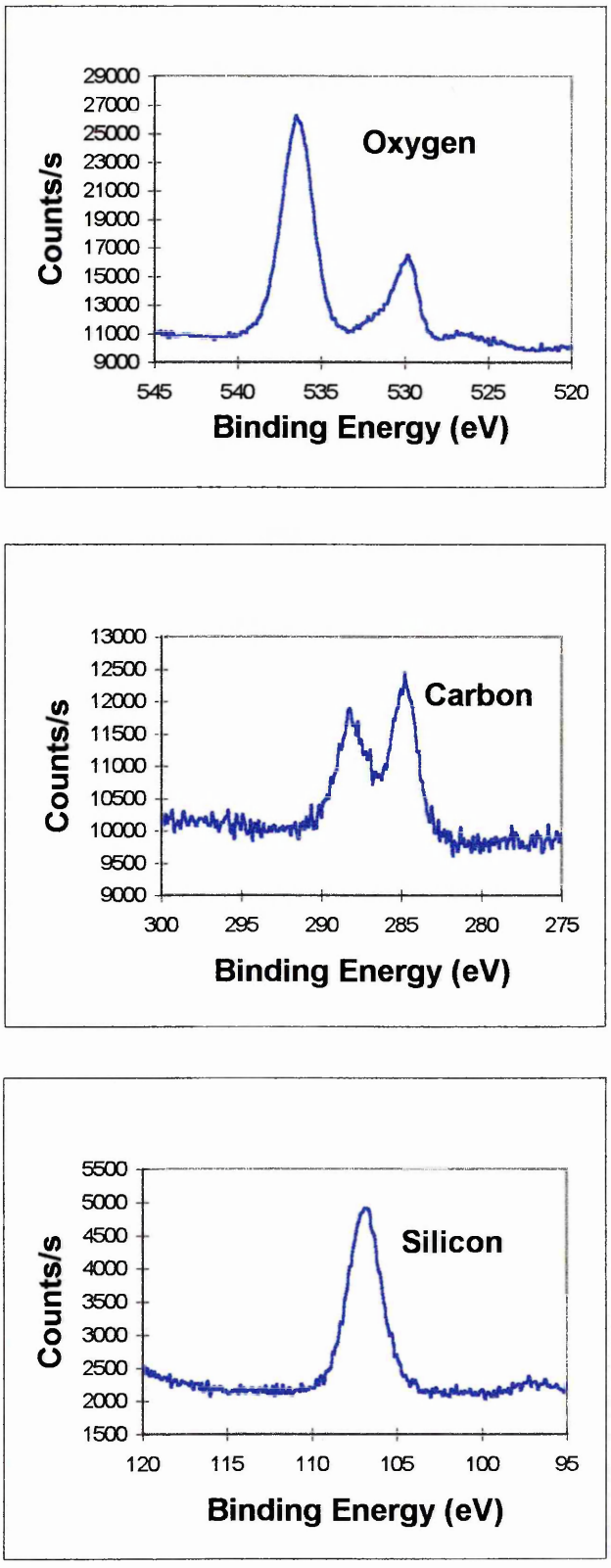
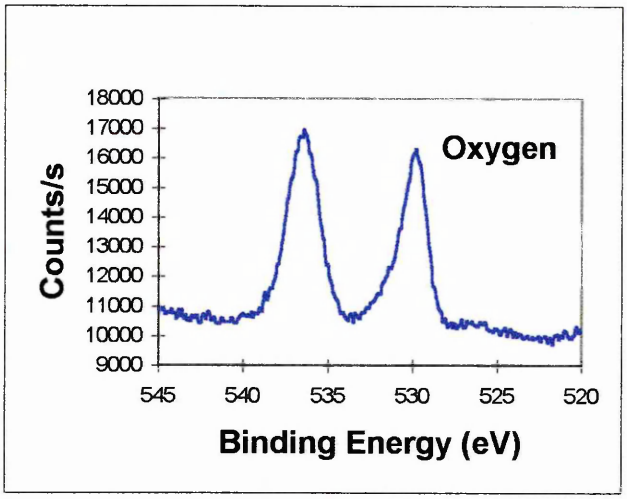
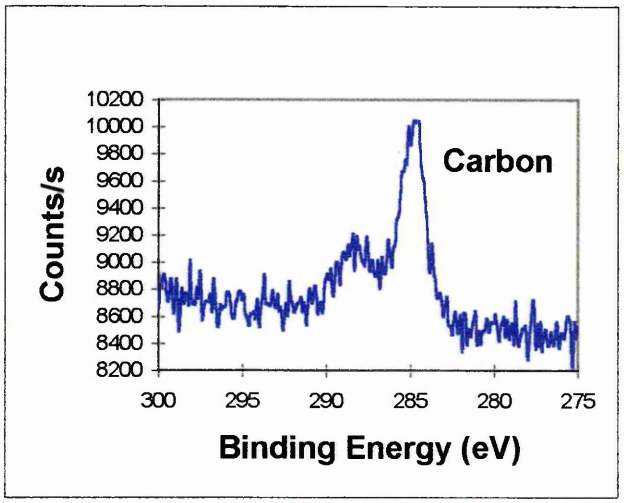


Figure 4.9 Oxygen, carbon and silicon XPS narrow scan data for Spherisorb S5 C1-1334 stationary phase





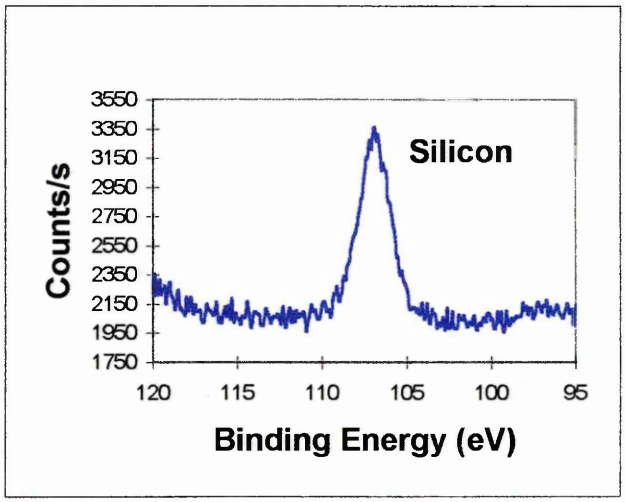
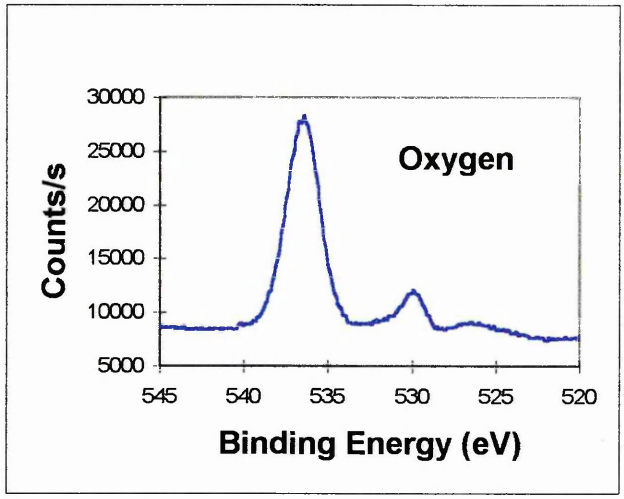
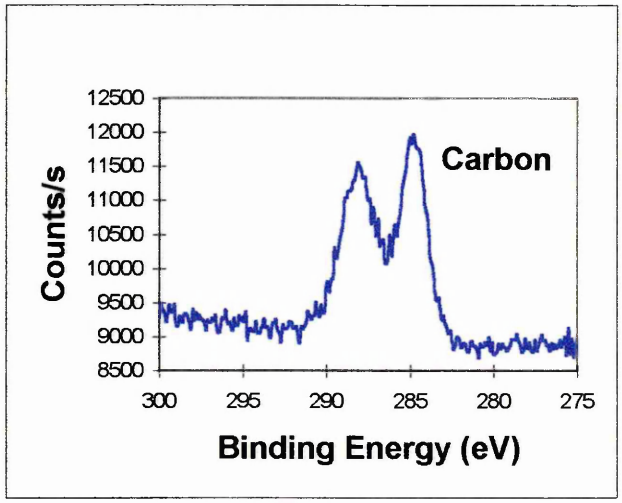


Figure 4.10 Oxygen, carbon and silicon XPS narrow scan data for Spherisorb S5 C1-1317 stationary phase





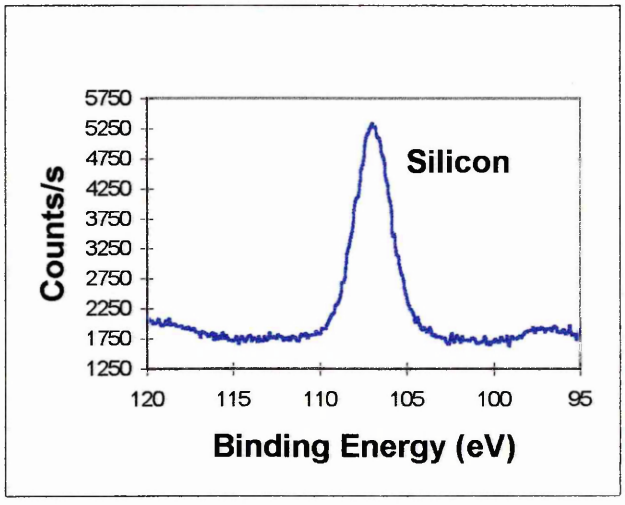
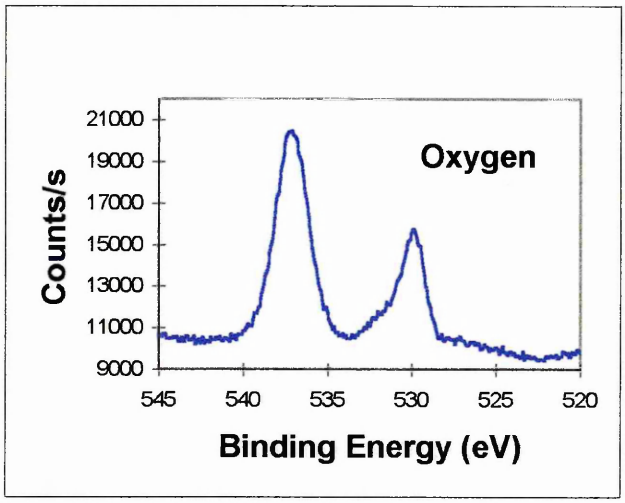
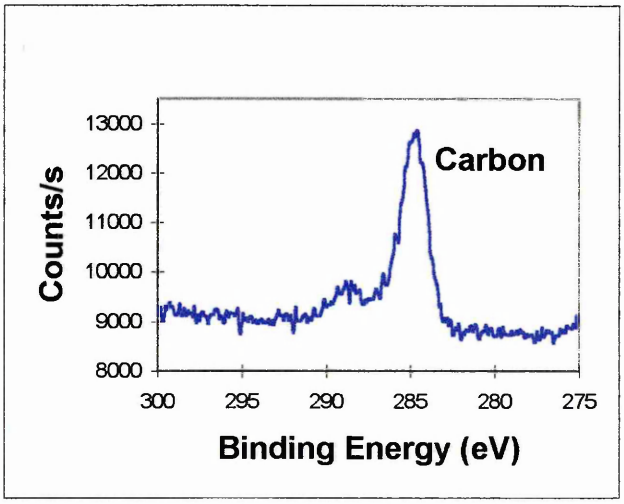


Figure 4.11 Oxygen, carbon and silicon XPS narrow scan data for Hypersil SAS stationary phase





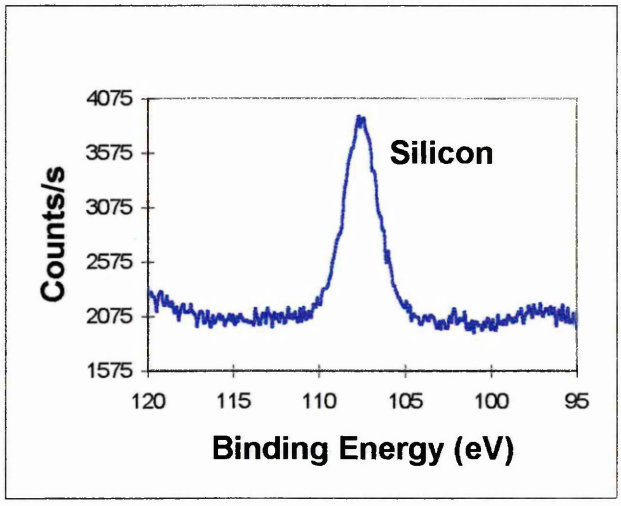


Figure 4.12 Oxygen, carbon and silicon XPS narrow scan data for Supelcosil LC-1 stationary phase

The atomic percentages determined for each of the three elements investigated are shown in Table 4.2. The carbon / silicon ratios of each of the stationary phases examined are between 0.1 and 0.2; figures which show good agreement with those published by Linton *et al.* [12] in their study of TMS silica stationary phases by XPS.

Sample	%O	%Si	% C
Spherisorb S5 C1 - 1317	55.33	40.46	4.21
Spherisorb S5 C1 - 1334	56.16	37.45	6.39
Supelco LC-1	59.08	37.18	3.75
Hypersil SAS	54.77	38.49	6.75

Table 4.2 XPS data

Surface carbon determined by XPS is a better representation than bulk carbon of the alkyl-bonded phase available for chromatographic interaction with an analyte species. A comparison of the percentage carbon determined by XPS with the percentage bulk carbon as determined by elemental analysis show a different pattern for the four phases studied. It is not surprising that the results are different because the bulk carbon results include all the carbon present in the sample, not just that on the surface. Nevertheless, the XPS results do not follow any sensible pattern in relation to the chromatographic performance of the phases. However, since these values do not take into account the surface areas of the base silicas they may be misleading.

The best approximation of surface coverage by the alkyl-bonded phase is achieved by incorporating the surface area (supplied by the manufacturers) of the non-bonded silica with the percentage carbon measured by XPS. Surface coverage of the alkyl-bonded phase, as specified by the manufacturers, is listed in Table 4.3. These values are normally based on the average percentage carbon for a number of batches of stationary phase, with the results being rounded to the nearest whole number (also quoted in Table

4.3) (the method used to determine percent carbon was unknown). By using percentage carbon values from XPS determinations the alkyl coverage at the surface of the bonded silica is measured. It is expected that this should represent the alkyl moieties available for chromatographic interactions. The data shown in Table 4.4 is calculated using equation 4 [16]:

$$N(\mu mol/m^2) = \frac{10^6 P_c}{1200 n_c - P_c (M-1)} \frac{1}{S} \quad \text{.....Eqn (4).}$$

Where N is the surface coverage (μmol/m^2); Pc is the percent carbon of the bonded phase; n_c is the number of carbons in the bonded silane molecule; M is the molecular weight of the bonded silane molecule; and S is the specific surface area of the non-bonded silica in m^2/g.

Sample	%C	Surface Area, S (m^2/g)	Surface Coverage, N (μmol/m^2)
Spherisorb S5 C1 - 1317	4.0	220	1.08
Spherisorb S5 C1 - 1334	4.0	220	1.08
Supelco LC-1	3.0	170	5.29
Hypersil SAS	2.0	170	3.4

Table 4.3 Column manufacturers data

Sample	Surface Coverage, N (μmol/m^2)
Spherisorb S5 C1 - 1317	6.2
Spherisorb S5 C1 - 1334	10.3
Supelco LC-1	7.1
Hypersil SAS	14.4

Table 4.4 Surface carbon coverage calculated using equation 4

The values calculated using equation 4 give a completely different picture of the situation. These values follow the same pattern as that seen in the bulk carbon results; the phase that produced the best resolution had the lowest coverage of trimethylsilyl groups. Although the surface carbon values are much higher than the bulk figures, this is expected as the alkyl moiety is preferentially bound to the surface of the silica.

Hence it appears that for the separation of NPEO on a C_1(TMS) column low surface coverage of the alkyl moiety gives the superior separation. This observation is difficult to account for by invoking a purely reversed phase mechanism for this separation. The separation would appear to involve both an adsorption and partition mechanism. Evidence for the adsorption mechanism lies in the fact that the most strongly retained species are the most hydrophilic (longest ethoxylate chain), and by the data presented here that shows the phase with the lowest surface coverage of alkyl material produces the best separation. The fact that increasing the concentration of the organic component of the mobile phase decreased the retention time would suggest a partition mechanism. Ibrahim and Wheals have reported the separation of nonylphenol ethoxylates on a silica column [17]. Their separation showed very similar characteristics to the separation achieved on earlier batches of the Spherisorb column. The authors described the separation as "pseudo-reverse-phase", justifying the statement by the fact that, as in this work, it uses typical reverse phase eluents, but the most strongly retained oligomers were the most hydrophilic. Interestingly, Ibrahim and Wheals also found their separation worked best on Spherisorb silica, compared with Hypersil and LiChrosorb silicas. The mobile phase used was an acetonitrile / phosphate buffer (pH 3) gradient, which again is very similar to that used here.

In reverse phase or partition chromatography, the most strongly retained solutes are the most non-polar. In the separations described here, the most strongly retained ethoxymer

has the longest ethoxylate chain. If polarity is based on hydrophilicity, then the most strongly retained ethoxymer, that is, the most hydrophilic, is also the most polar. This theory is suggested by Ibrahim and Wheals [17] and by Wang and Fingas [15]. On this basis, the order of elution achieved here is not characteristic of a reversed phase mechanism.

Ibrahim and Wheals also found that trimethylsilylation of the surface of the Spherisorb silica dramatically reduced resolution of the NPEO ethoxymers. This suggests the major retention mechanism to be hydrogen bonding between surface silanols and the ethoxylate groups on the analyte molecule. This makes sense, as separation is seen to be on the basis of ethoxylate chain length and not alkyl chain length (separation of alkylphenol ethoxylate surfactants by alkyl chain length has been shown on C_{18} and C_8 columns by several groups e.g. Marcomini *et al.* [18]).

The results presented here and that of other groups [15,17] would seem to indicate that the separation of NPEO on a C_1 column is not a function of the trimethylsilyl groups, but in fact, separation is achieved on the remaining unreacted silanol groups. This conclusion is supported by the fact that previous batches of Spherisorb C_1 stationary phase from HiChrom have a lower surface carbon coverage, and therefore a lower coverage of the trimethylsilyl group, than more recent batches of the same material which were unable to exhibit the same degree of resolution. Further evidence supporting this conclusion is seen in the work of Ibrahim and Wheals [17] who found that forming the trimethylsilyl derivative of Spherisorb silica led to a dramatic loss in resolution of NPEO ethoxymers. The incorporation of trimethylsilyl groups, above a certain concentration, has a negative effect on the resolution of NPEO ethoxymers.

However, the original separation also included separation of LAS homologues. LAS homologues were separated by alkyl chain length, a separation which is very typical of

a reverse phase mechanism, the homologue with the shortest alkyl chain eluting first. A reverse phase separation such as this could not take place on a purely silica stationary phase; the trimethylsilyl moiety bonded to the silica surface on the C_1 phase must be the factor that effects the separation of the LAS homologues. This is supported by the fact that all of the phases used in this study were capable of adequate resolution of the LAS homologues. It would seem therefore, that for the simultaneous separation of the LAS homologues and the ethoxymers of NPEO a C_1 stationary phase must be used. The phase must have trimethylsilyl groups bound to the surface of the silica in order to achieve separation of the LAS homologues, but the degree of coverage by the trimethylsilyl moiety must not be greater than *c.a.* 6.5 $\mu mol/m^2$ in order to achieve adequate resolution of the NPEO ethoxymers. Results from other studies mentioned above would also suggest that only Spherisorb silica is able to provide this resolution of ethoxymers. The reason for this is unknown at present.

4.7 Conclusion

Four C_1(TMS) HPLC stationary phases have been analysed by elemental analysis and x-ray photoelectron spectroscopy in an attempt to determine the reason for the inability of recent batches of Spherisorb S5 C1 material to resolve NPEO ethoxymers.

The elemental analysis data for bulk carbon showed good agreement with the data of other workers [9]. These data indicated that the Spherisorb S5 C1-1317 material obtained from HiChrom Ltd. (the only phase shown to be capable of resolving the NPEO ethoxymers) contained the lowest percentage of carbon. Recent batches of the Spherisorb material (1334) exhibited a much higher percentage of carbon. While Supelcosil LC-1 stationary phase purchased from Supelco showed a degree of separation, the resolution achieved was never as good as that by the Spherisorb S5 C1-

1317 material; a higher value for percentage carbon was determined by elemental analysis for this phase.

The percentage carbon determined by XPS provides a better representation of carbon available for chromatographic interaction than bulk methods as its surface specificity means that only surface carbon is examined, i.e. carbon which is chromatographically important. Data from the XPS analyses did not produce the same pattern as that seen by elemental analysis. However, when taking the surface area of the base silicas into account, the results this time showed a similar trend to the elemental results. XPS results showed that the stationary phase that exhibited the best resolution of NPEO ethoxymers also had the lowest surface coverage of the alkyl moiety.

Ibrahim and Wheals [17] have shown a similar separation of NPEO ethoxymers on a silica column using a similar mobile phase system. This, together with the results obtained here, suggests that perhaps the trimethylsilyl groups are not needed for the separation, and that perhaps they are even a hindrance to the separation mechanism.

The actual separation mechanism in operation would appear to have characteristics of both normal and reverse phase mechanisms. The fact that the method separates the analyte by length of the ethoxymer chain is indicative of a normal phase mechanism. A reverse phase mechanism is suggested by evidence that increasing the concentration of the organic component of the mobile phase decreases retention. Although the fact that the most strongly retained ethoxymer is the most hydrophilic, and by inference the most polar, is not typical of a reverse phase mechanism. This type of separation has been termed a "pseudo-reversed-phase" mechanism.

The results suggest that this separation actually takes place on the unreacted silica sites, and that the presence of the trimethylsilyl groups has a negative effect on the separation

of the NPEO ethoxymers. However, it must be remembered that the method also included the separation of LAS homologues. Since this part of the method separates these homologues on the basis of alkyl chain length, it has to be assumed that it is the trimethylsilyl groups that effect the separation in this case, and are therefore essential if the separation is to remain simultaneous.

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Chapter 6

Conclusions and Future Work

6.0 Conclusions

The vast quantity of surfactants that are used today make them major environmental pollutants. Of the four classes of surfactant used, anionic and non-ionic are the most environmentally significant, as these are more widely used than the cationic and amphoteric type.

The non-ionic surfactant nonylphenol ethoxylate (NPEO) and its biodegradation products have been shown in the literature to be weakly oestrogenic in nature. NPEOs along with other pollutants such as DDT, bisphenol A and tributyltin chloride are thought to disrupt the endocrine systems of a wide variety of animals and fish, and may be related to recent problems in human reproductive health. While NPEOs have been largely replaced by alcohol ethoxylates, which are thought to be less damaging to the environment, NPEOs still find use in some industrial cleaning processes, such as in the woollen industry, and hence still find their way into the environment.

Work by a previous Ph.D. student involved the development of a HPLC method for the simultaneous determination of linear alkylbenzene sulphonates (LAS) and alkylphenol ethoxylate surfactants in surface water [1]. However, following clean up / preconcentration using C_{18} solid phase extraction cartridges, the resulting chromatograms showed a large anionic interference that co-eluted with, and masked any LAS that may have been present in the sample.

In the work described in this thesis, an attempt was made to remove this anionic species that is now thought to be humic acids. A different extraction procedure was investigated to determine whether the humic acids could be fractionated from the LAS. The method from the literature which was employed [2] used graphitised carbon black (GCB) SPE

cartridges. While recovery results showed the method to be slightly better than the C_{18} method [1], the resulting chromatograms still showed a large peak eluting at the same time as LAS.

Therefore, a phase-switching method was developed to try to separate the LAS and humic acids chromatographically. The phase-switching set up allowed the LAS / humic acid portion to be directed to a C_{18} column following elution from the C_1 column. The alkylphenol ethoxylates were allowed to separate as usual, and then the C_1 column was removed from the flow and the humic acids were separated from the LAS on the C_{18} column. While this method worked well with standards, the results from an extracted river water sample were very inconclusive. The identities of the peaks observed in this chromatogram were not obvious and it was decided that LC-MS would be needed to characterise the peaks properly; the instrumentation for this was not available at the time.

During this work, another problem occurred involving the ability of new C_1 columns to adequately resolve the NPEO ethoxymers. The original method was developed on a Spherisorb S5C1 column obtained from Hichrom Ltd. During the development of the method, and in subsequent work, it was shown that C_1 columns from Supelco and Hypersil were unable to attain the same resolution of ethoxymers as produced with the Spherisorb column. However, later batches of the Spherisorb column were also unable to produce the same resolution as that observed in earlier batches.

This problem was investigated using elemental analysis and x-ray photoelectron spectroscopy to determine the percentage bulk and surface carbon, respectively, of the column. Bulk and surface carbon results revealed that the column which provided the

best resolution of NPEO ethoxymers had the lowest surface carbon coverage. This suggested that the resolution of the ethoxymers was effected by the remaining surface hydroxyls on the surface of the silica, whereas the presence of the trimethylsilyl group actually hindered the separation. This conclusion was supported by the fact that a method has been published that described the resolution of NPEO ethoxymers on a Spherisorb silica column [3]. The authors of this work also found that only Spherisorb silica was able to achieve adequate resolution.

Separation is based on increasing ethylene oxide chain length; the species with the shortest ethoxy chain elutes first from the column. If increasing hydrophilicity is taken to be equivalent to increasing polarity, then the fact that the most hydrophilic species eluted last, along with the evidence that the separation worked best on the column with the least carbon, showed that the separation might follow an adsorption or normal phase mechanism. However, increasing the organic component of the mobile phase decreased the retention time, which is typical of a reverse phase or partition mechanism. Therefore, the resolution of NPEOs on a C_1 column showed evidence of both normal and reverse phase mechanisms. It is important to note that the trimethylsilyl moiety is essential for the separation of the LAS homologues and therefore essential to the simultaneous nature of the method.

A new method was developed for the qualitative and quantitative determination of NPEO surfactants in surface waters by matrix-assisted laser desorption / ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Samples were mixed with a concentrated solution of either 2,5-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxycinamic acid (HCCA) as a matrix. Approximately 1 μL of this solution was

applied to a stainless steel target and the solvent was allowed to evaporate, leaving matrix / analyte crystals. Analysis of the resulting crystals showed spectra of intense $[M+Na]^+$ and $[M+K]^+$ adducts for NPEOs. Analysis of surface water samples from the River Don in South Yorkshire also produced characteristic spectra of NPEOs, with peaks corresponding to sodium and potassium adducts. While these were excellent results, showing the method to be very capable of the qualitative determination of alkylphenol ethoxylate surfactants in surface water, there was no quantitative aspect to the results.

The lack of shot-to-shot reproducibility inherent to MALDI-TOF MS makes quantitative determination difficult. As the surfactant octylphenol ethoxylate differs from NPEO by a single methylene unit (14 Da) it was decided that this would be an ideal candidate for an internal standard, as it would provide a reference peak for each analyte peak. The internal standard was added either before extraction or with the matrix; and a concentrated solution of lithium chloride was also added before crystallisation as a source of lithium ions. The addition of lithium ions led solely to the formation of $[M+Li]^+$ adducts, and created much cleaner, less complicated spectra essential for inclusion of the internal standard. Excellent linear relationships were achieved down to 10 mg/L NPEO (equivalent 200 μg/L before extraction). However, spectra of extracted samples from Langstone Harbour in Portsmouth and the River Don did not show any signals corresponding to NPEOs. The levels of NPEOs in these new samples must have been below the limit of detection for this method (100 μg/L). The extraction of a large quantity of water from the River Don produced very noisy spectra that contained many peaks, some of which could possibly, but not definitely, be attributed to NPEOs. Peaks for NPEOs were observed in spiked surface water samples

indicating that the method was capable of measuring these surfactants in surface water at higher concentrations. This method could prove useful for the rapid screening of more polluted environments such as sewage treatment plants as its analysis time of approximately ten minutes per sample compares well with the established HPLC method which has analysis times of more than thirty minutes.

6.1 Future Work

It would be interesting to repeat the phase-switching method but instead coupling the system to a mass spectrometer via an electrospray or APCI interface. It might then be possible to determine whether or not the peaks in the latter half of the chromatogram are due to LAS in the sample. However, the use of a mass spectrometer for detection and identification of unknown components of the chromatogram would add a large amount of complexity and cost to the method.

In order to investigate the retention of NPEO on C_1 stationary phases further it would be interesting to pack some columns with Spherisorb silica containing different surface concentrations of the trimethylsilyl moiety. Using XPS it would then be possible to determine the optimum concentration needed for effective resolution of NPEO ethoxymers and LAS homologues.

The MALDI method could be extended by trying to lower the limit of detection, possibly by further investigation of the optimum matrix / analyte ratio. It would also be interesting to analyse some samples that are known to be more polluted with NPEOs such the influent and effluent from sewage treatment plants. The method could also be extended to the determination of linear alkylbenzene sulphonates and possibly even

biodegradation intermediates of both non-ionic and anionic surfactants. However, the low molecular weight of these molecules may mean they are subject to interference from matrix ions.

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Chapter 5

The Determination of Nonylphenol Ethoxylate Surfactants in Surface Water by Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry

5.0 Introduction

Matrix-assisted laser desorption/ionisation (MALDI) is still a relatively new method of ionisation in mass spectrometry. However, it is an extremely powerful tool when combined with time-of-flight detection, for the analysis of high molecular weight biopolymers. MALDI has evolved from the older technique of laser desorption/ionisation (LDI) which involved the irradiation of a solid sample with short, intense pulses from a laser in the IR or UV region, to produce quasimolecular ions from thermally labile molecules. LDI, however, has a number of important limitations, as it tends to be most efficient when the laser wavelength matches an intense absorbance band in the analyte molecule. In addition, direct absorption of the radiation by the molecule tends to lead to fragmentation with the loss of important molecular weight information [1].

The process of using a matrix to aid laser desorption of intact protein molecular ions was reported almost simultaneously and independently by Tanaka and co-workers [2] and Karas and Hillenkamp [3]. Tanaka's group used a liquid matrix of a fine powder of cobalt (300 Å) suspended in glycerol to produce LDI spectra of proteins and polymers with molecular weights up to 25 kDa, and multiply charged lysozyme quasimolecular ions of 100 kDa. Karas and Hillenkamp [3] produced spectra using a solid UV absorbing organic matrix. Equal volumes of a very dilute solution of analyte (10^{-5} M) and nicotinic acid (10^{-3} M) were mixed, and a drop of the mixture applied to a metallic substrate. Following evaporation of the solvent to form crystals containing both matrix and analyte, spectra of various proteins such as β-lactoglobulin (MW 18277 Da) and albumin (MW 67000 Da) were recorded.

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

MALDI-TOF has now become the method of choice (along with electrospray mass spectrometry) for molecular weight analysis of large biopolymers, a significant achievement being the analysis of a singly charged human immunoglobulin species (MW ~1 MDa) [4].

5.1 MALDI-TOF Instrumentation

In MALDI-TOF, the laser beam hits the sample, producing ions that are then accelerated by a grid in front of the target that is held at +/- 10-20 kV. After acceleration the ions enter a field-free region and fly towards the detector at the end of the tube (Figure 5.1).

5.1.1 The Time-Of-Flight Mass Analyser

The time-of-flight (TOF) mass analyser is the ideal complement to MALDI, given that MALDI characteristically generates short pulses of ions at a well-defined point in space and time. In addition, the TOF analyser, in theory, does not have an upper mass limit, making it ideal for analysing very high molecular weight biopolymers.

Time-of-flight analysers separate ions of different mass by exploiting their different velocities when accelerated through a potential (V).

Since: -

$$zeV = \frac{mv^2}{2} \quad \text{.....Eqn (1)}$$

the velocity (v) of an ion of mass (m) is:-

$$v = \left(\frac{2zeV}{m}\right)^{1/2} \quad \text{.....Eqn (2)}$$

Therefore the velocity of any particular ion is dependent on its mass so that, if a bunch of ions (m_1, m_2, m_3.....m_n) are accelerated and allowed to pass into a field-free region, the ions will arrive at the detector (see Figure 5.1) at different times depending on their velocities. Now, supposing the field-free region has length l, then, for a velocity v: -

$$t = \frac{l}{v} \quad \text{.....Eqn (3)}$$

Combining equations (2) and (3): -

$$t = \frac{\left(\frac{m}{z}\right)^{1/2} l}{(2eV)^{1/2}} \quad \text{.....Eqn (4)}$$

Therefore, the time for an ion to reach the detector is proportional to the square root of its m/z value. Consequently the larger the mass of an ion (assuming it is singly charged), the longer it takes to traverse the field-free region.

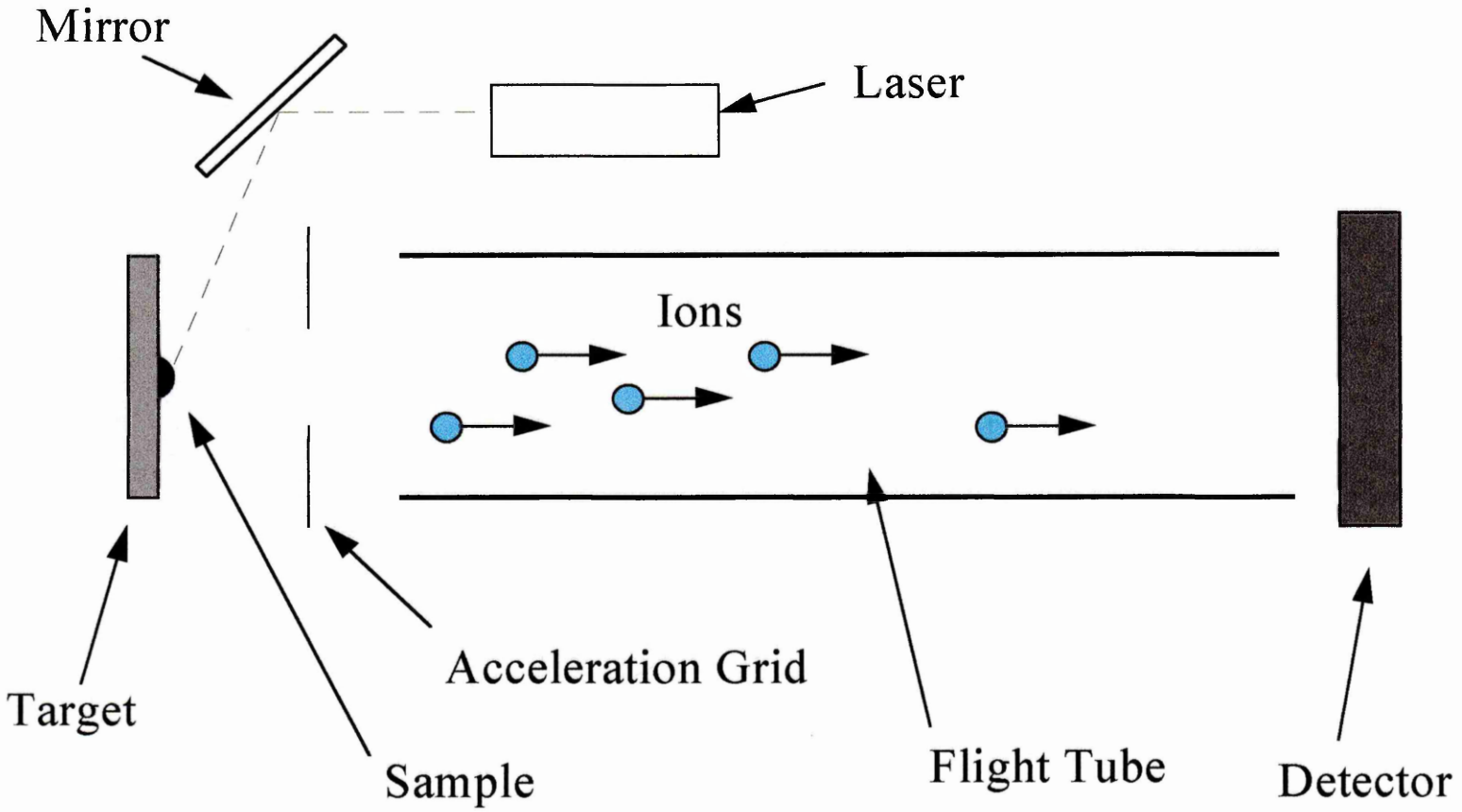
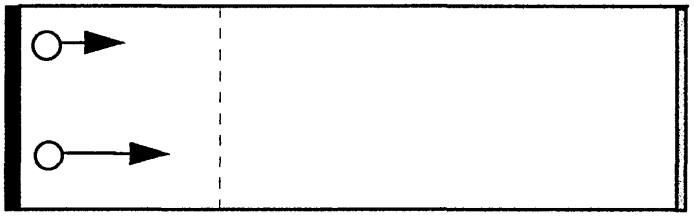


Figure 5.1 A basic schematic of MALDI-TOF instrumentation.

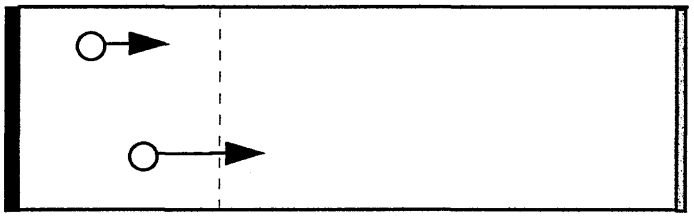
5.1.2 Delayed Extraction and Reflections

The resolving power of this (linear) time-of-flight analyser is quite poor. This lack of resolution results from the spread of energies imparted to the ions at the desorption/ionisation stage.

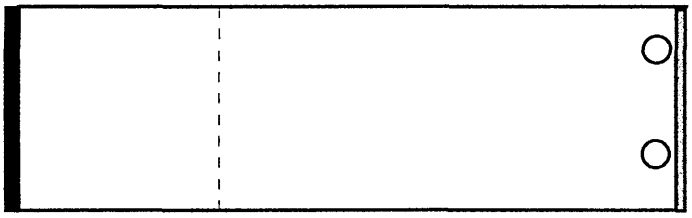
Following ionisation, the initial velocity imparted on desorbed analyte ions is nearly independent of mass; thus the initial kinetic energy is proportional to mass of the analyte. In delayed extraction a time delay in the range of a few hundred *ns* between the laser pulse and the acceleration of the ions into the field-free region is used to focus the ions at the detector. During this period the energy spread of the ions is converted into an additional spatial spread (although the kinetic energy distribution is not lost). Thus ions with greater kinetic energy will experience the most acceleration from the grid and will be travelling towards the detector faster than those ions with greater kinetic energy. At a defined point in time the faster moving ions will have caught up with their slower moving counterparts causing a bunching of ions at a particular point. In delayed extraction either the time delay or the accelerating potential are set so that this point of bunching is focussed on the detector. This situation is represented schematically in Figure 5.2.



Immediately following laser pulse ions formed with differnt KEs



Between laser pulse and extraction energies converted into spatial spread



Following extraction ions reach detector at the same time

Figure 5.2 Schematic representation of delayed extraction.

The problem of low resolution has also been overcome in some instruments by utilising a device known as a reflectron that acts as an "ion mirror". This replaces the detector at the end of the flight tube and consists of several plates. A homogeneous electric field is applied between the entrance and end plates. This field retards and reflects ions based on an electrostatic principle. Ions of the same mass but slightly higher kinetic energy penetrate the field to a greater extent than their less energetic counterparts. Therefore, the faster moving ions have further to travel, spending more time in the mirror. This results in the bunching of ions of the same mass in space so they arrive at the detector (set at an angle to the mirror) in a much shorter span of time. The addition of a reflectron

can significantly increase the resolution of the instrument but also has the drawback of reducing the sensitivity by a factor of ten.

5.2 The MALDI Mechanism

In the MALDI process the matrix absorbs the UV laser radiation and rapidly breaks down. It then expands into the gas phase and at the same time carries with it undamaged analyte molecules. The high matrix to analyte ratio serves to reduce associations between analyte molecules and provides protonated and free radical species that ionise the sample molecules [5]. The detailed mechanism of ionisation in MALDI is still not properly understood, although a number of theories have been described in the literature.

The *homogeneous bottleneck* mechanism proposed by Vertes and co-workers [6] attempts to describe the energy transfer processes during and after the laser pulse. The authors propose that the fragile guest molecules survive in the high-energy-density-lattice by a "cooling down" of the lattice by sublimation and by poor energy transfer to the guest molecules. The rate of evaporation increases exponentially as a function of the lattice energy density. The faster the energy transfer into the lattice, the greater the rate of cooling by sublimation, which is a favourable situation due to slower energy transfer to the guest molecules. Thus, there are two competing rates of energy transfer, from the matrix to the guest molecules and their desorption by sublimation. A bottleneck is formed in energy transfer to the embedded guest molecules: this makes their energy content lag behind that of the matrix. Therefore when a sufficiently high rate of sublimation can be achieved, the guest molecules will desorb internally cold and will not fragment. From these principles, the authors give four methods for controlling rates; two by reducing the energy transfer: -

- Keeping the matrix to analyte ratio as high as practicably possible,
- Using a matrix with as poor a frequency overlap with the frequencies of the analyte as possible,

And two by enhancing the volatilisation rate: -

- Using a matrix with a low sublimation temperature.
- Using a laser pulse short enough to promote volatilisation instead of degradation.

Vertes, Irinyi and Gijbels [7] then went on to suggest a hydrodynamic model which focused on the actual expansion into the gas phase. It has been determined experimentally that the average forward velocities and velocity distributions of analyte ions were the same as those of the matrix ions. This suggests that analyte ions are entrained in the expanding matrix plume and that kinetic energies of analyte ions increase with increasing mass [8]. These results are represented schematically in Figure 5.3.

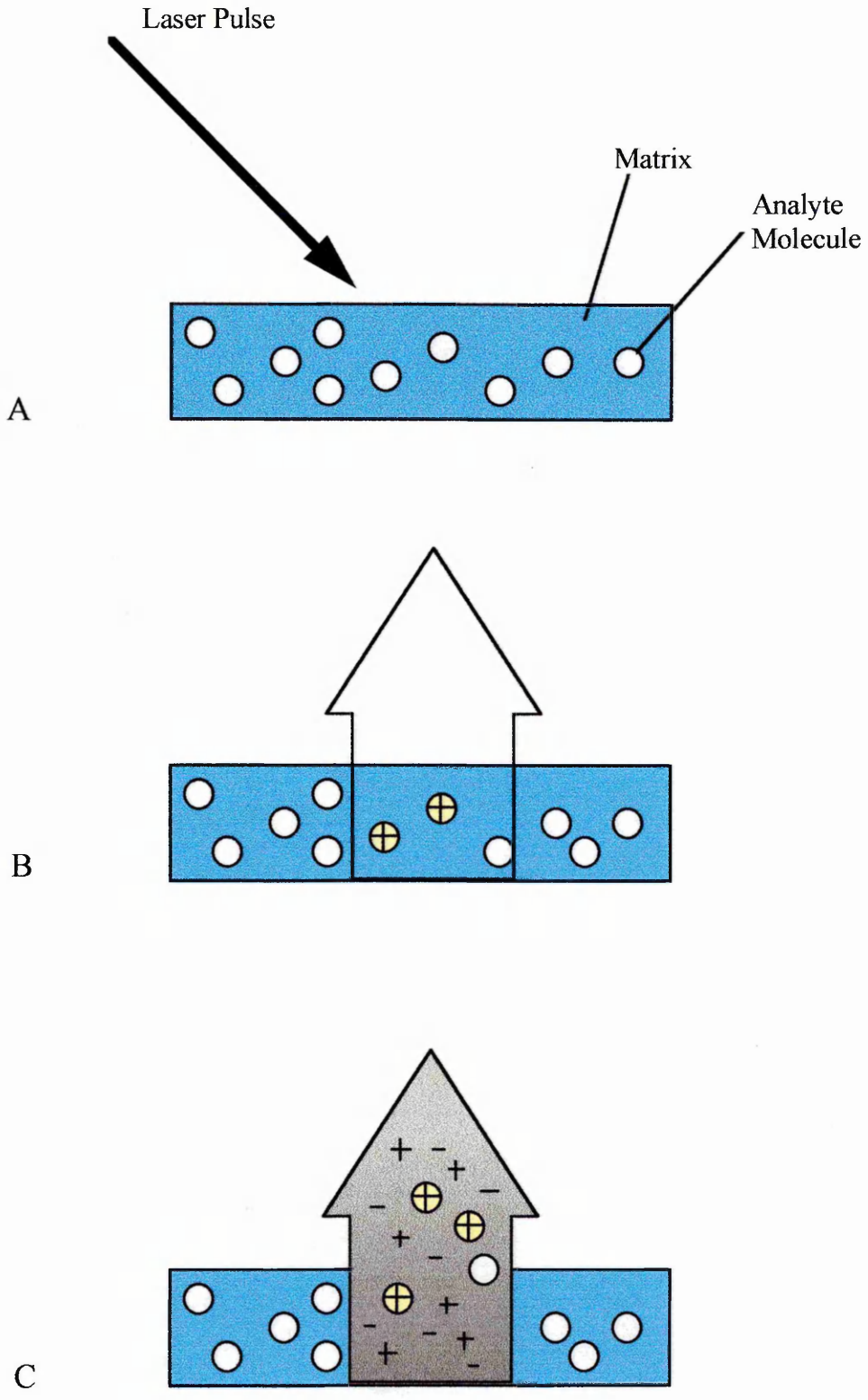
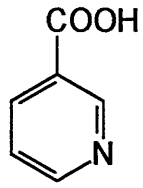
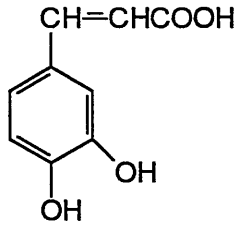
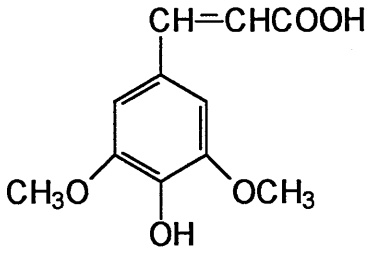
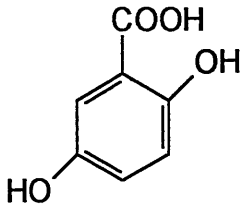
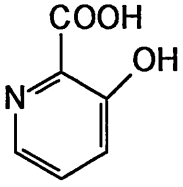


Figure 5.3 Schematic representation of the MALDI process. A) Absorption of UV radiation by the matrix causing ionisation of the matrix; B) dissociation of the matrix, phase change to supercompressed gas and transfer of charges to analyte molecules; C) expansion of the matrix at supersonic velocity, entrainment of analyte in the matrix plume and transfer of charges to analyte molecules

5.3 The Matrix

The lack of a definitive model of the MALDI mechanism has made the selection of suitable molecules for use as matrices somewhat of a 'hit and miss' process. Numerous small, UV absorbing organic molecules have been shown to be useful matrices for MALDI-TOF MS. The most common of these are shown in table 5.1 below.

Matrix	Structure
Nicotinic Acid	
Caffeic acid	
Sinapinic Acid	
2,5-Dihydroxybenzoic Acid	
3-Hydroxypicolinic Acid	

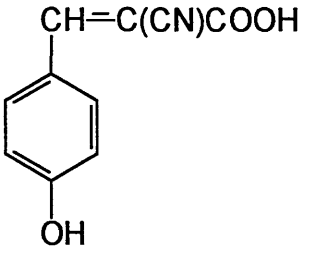
α-Cyano-4-hydroxycinnamic Acid	

Table 5.1. Common matrices used in MALDI mass spectrometry.

5.4 MALDI of Low Molecular Weight Compounds

While considerable attention has been paid to the MALDI of high molecular weight molecules, relatively little work has focussed on MALDI of small molecules. This perhaps has been due to the low resolving power of the time-of-flight mass analyser and the interference of low-mass ions introduced by the matrix. Lidgard and Duncan [9] have investigated the MALDI of a range of small molecules, including carbohydrates, sterols, amino acids and phthalocyanins using α-cyano-4-hydroxycinnamic acid (HCCA) and 2,5-dihydroxybenzoic acid (DHB) matrices. They found good correlation between calculated and experimental masses using matrix to analyte ratios of 1000:1. Also, Goheen *et al.* [10] have shown low molecular weight MALDI spectra of organic acids, oxyanions and amine-based chelating agents with a view to using MALDI as a tool for analysing the contents of hazardous waste tanks containing heavy metals such as ^{60}Co and Pu. DHB was used as the matrix in a ratio of 100:1 with these samples. Duncan and co-workers [11] also found this matrix to be effective for the analysis of 3,4-dihydroxyphenylanaline (DOPA) and the peptide H-Ser-Ala-Leu-Arg-His-Tyr-NH_2. Good signal responses for $[M+H]^+$ ions were produced in the range 100-1000:1 matrix to analyte ratio (above this level the higher signal response was accompanied by a marked increase in peak width).

5.5 MALDI of Synthetic Polymers

The use of MALDI for the characterisation of synthetic polymers was not properly addressed until around 1992 [12]. However it is interesting to note that the analysis of synthetic polymers was described by Tanaka *et al.* [2] in 1988. In their pioneering work describing the use of a cobalt / glycerol matrix, they successfully obtained spectra of polyethylene glycols (PEG) and polypropylene glycols (PPG).

As previously mentioned this apparent lack of enthusiasm was mainly because few important synthetic polymers were compatible with the sample preparation techniques developed for biomolecules using small organic acids as the matrix. In addition to this, by their nature, synthetic polymers are polydisperse and hence the signal is spread among many peaks, and not just one or two as in the case of biomolecules. This has the effect of increasing the signal to noise ratio.

The first real work on synthetic polymers was by Bahr and co-workers [12]. They produced MALDI spectra of PEG and polymethyl methacrylate (PMMA) using the conventional matrix DHB, sometimes with the addition of alkali metal salts, sodium chloride, potassium chloride or lithium chloride as a source of cations or for more homogeneous cationization. An attempt to analyse polystyrene in the same manner was unsuccessful. Microscopic observation of the sample preparation showed separation of the polystyrene (PS) from the DHB crystals. Therefore 2-nitrophenyloctylether, a highly viscous liquid was used as a matrix for PS. Although this method produced results for PS the spectra were not of good quality and showed poor resolution.

A good overview of methods for the determination of synthetic polymers by MALDI, has been published by Räder and Schrepp [13]. A number of new matrices, as well as

DHB [14], have been described, including some for the analysis of non-polar hydrocarbon polymers. These include 1,8,9-trihydroxyanthracene (dithranol) [15], trihydroxyacetophenone (THAP) [16], trans-3-indoleacrylic acid (IAA), 1,4-di-(2-(5-phenyloxazolyl))benzene [17] and 2-(4-hydroxyphenylazo) benzoic acid (HABA). The source of cations is also an important consideration in the analysis of synthetic polymers. While alkali metal salts have been used (as mentioned above), the addition of silver ions has been widely used, normally in the form of silver (I) acetylacetonate or silver (I) trifluoroacetate.

Traditional methods for the characterisation of synthetic polymers are gel permeation chromatography (GPC), supercritical fluid chromatography (SFC), vapour pressure and membrane osmometry, viscometry, light scattering and analytical ultracentrifugation. Trathnigg *et al.* [16] have compared MALDI with SFC and GPC for the characterisation of PEG. In general, the results from each technique were in good agreement; however, in some cases differences were observed in the average molecular distribution by MALDI. This was attributed to inhomogeneity of the analyte in the matrix due to different solubility of the oligomers leading to "fractionated" precipitation during evaporation, and possible partial evaporation of lower oligomers in the vacuum of the mass spectrometer. Another comparison between MALDI and GPC for the analysis of PEG and MeO-PEG [18] showed MALDI to give better agreement with the theoretical values than GPC data. In conclusion, the authors decided that MALDI offers a more convenient method for the characterisation of polymers of low molecular weight and narrow molecular weight distribution than GPC. The main advantages of MALDI were stated as being, the speed of analysis with no need for chromatographic separation or simultaneous calibration and better accuracy in molecular weight determinations.

5.6 MALDI of Surfactants

To date there have been few papers published on the MALDI-MS of surfactants. Just and co-workers [19] compared the use of MALDI with supercritical fluid chromatography (SFC) for molar mass determination of some alkylphenol ethoxylate surfactants. Good quality MALDI spectra of these surfactants were produced using DHB as the matrix (ratio not reported). Spectra of the alkylphenol ethoxylates showed a mixture of $[M+Na]^+$ and $[M+K]^+$ adducts. Direct comparison between SFC and MALDI-MS proved that MALDI-MS provided better differentiation in the higher mass range but seemed to cause some discrimination in the lower mass region, with lower intensities than expected seen for the low molecular weight oligomers.

Thompson *et al.* [20] produced MALDI-MS spectra for three classes of surfactant; non-ionic, anionic and cationic. The anionic surfactants sodium dodecylsulphate (SDS) and sodium dodecylbenzenesulphonate were successfully analysed to produce negative ion spectra, either simply as an aqueous solution allowed to dry on the metal probe, as in the case of SDS, or dispersed in ethylene bis[3-(2-naphthyl)acylate] as in the latter case. The authors note that spectra were obtained for SDS when dispersed in DHB, but interfering matrix ions made interpretation of the resulting spectra difficult. Also the sodium dodecylbenzenesulphonate spectra were similarly cluttered with matrix ions. Cationic surfactants, cetyldimethylethyl ammonium bromide, cetylpyridinium chloride and benzalkonium chloride produced good, intense spectra, either neat or dispersed in DHB. Good spectra of the nonylphenol ethoxylate surfactants IGEPAL CO-850, CO-880 and CO-890 were produced as their sodium adducts by dispersal in DHB with sodium chloride added as a source of cations.

Parees *et al.* [21] have briefly compared electrospray, FAB and MALDI ionisation techniques for the analysis of some commercial nonylphenol ethoxylate surfactants. Their findings seemed to indicate that the data obtained from the two methods are comparable for lower average molecular weight surfactant mixtures. However at higher average molecular weights although the electrospray and MALDI results were similar, the FAB data exhibited distinctly lower molecular weight distributions than electrospray and MALDI, possibly due to fragmentation. The authors also found that for the mixture with the highest average molecular weight the MALDI results were significantly higher than the other techniques. The authors gave no reason for this phenomenon.

The low molecular weight discrimination that has been observed in the MALDI-MS of some polymers [22,23,24] has been addressed by attempting to derivatise the polymer prior to analysis [22]. Samples of octylphenol ethoxylates (OPEO) were derivatised with phthalic anhydride using imidazole as the catalyst, and the resulting derivatives were analysed with DHB as the matrix with good effect. Spectra reported show less discrimination of low molecular weight ethoxymers and a better correlation with results from ^{13}C NMR and the wet chemical 'hydroxyl test'. An added bonus of the derivative method is the increased molecular weight imparted on the surfactants, which has the effect of moving them away from any interfering matrix ions. Another comparison has been made between MALDI, reversed phase HPLC and thin-layer chromatography (TLC) for the analysis of non-ionic surfactants [25]. In this work Cumme and co-workers compared the molecular weight information given by MALDI, TLC (using mass spectrometry to analyse the fractions) and HPLC. The MALDI spectra were obtained using DHB as the matrix, and the HPLC analysis was performed using a C_{18} column with an isocratic iospropanol / water (45:55) mobile containing 0.1 % TFA. The results from

these experiments did not show any major discrepancy between the average molecular weight found by HPLC and TLC and those found by MALDI. However, the authors state that for MALDI, ethoxymers with masses below m/z 405 were not included in the results due to interference from matrix ions.

5.7 Experimental

5.7.1 Instrumentation

All MALDI-TOF-MS measurements were carried out using a LaserTof 1500 instrument (Scientific Analysis Instruments, Manchester, UK). This is a linear time-of-flight instrument equipped with a N_2 UV laser operating at a wavelength of 337 nm. The instrument uses a stainless steel target with either eight or twenty-five sample positions, each position being a well of 2.5 mm in diameter. Unless otherwise stated all analyses were carried out using an accelerating voltage of 10 kV and spectra were the sum of thirty-two shots.

5.7.2 Materials

The matrix materials 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid were obtained from Aldrich (Poole, Dorset, UK). Synperonic NP5, NP9, NP12 and NP14 (a nonylphenol ethoxylate surfactant with average ethylene oxide chain lengths of 5, 9, 12 and 14 respectively) were a gift from ICI Materials Research Centre, Wilton, Middlesbrough, UK. Triton X-100 (an octylphenol ethoxylate with an average of 9 ethylene oxide units in the hydrophilic chain) was obtained from Aldrich (as above). All solvents were HPLC grade, and obtained from Fisher Scientific (Loughborough, UK). Water used was Milli-Q grade.

5.7.3 Sample Preparation

All samples, standards and matrices were dissolved in methanol and then differing volumes of matrix and analyte solutions were mixed in appropriate ratios. Approximately 1 μL of the resulting mixture was applied to the target and allowed to air dry.

5.7.4 Sample Extraction / Preconcentration

Grab samples of 2 L were obtained from Langstone Harbour, Portsmouth, UK or the River Don, South Yorkshire, UK. Prior to extraction all samples were stored in polyethylene bottles at +4 °C using 1 % formaldehyde as a preservative.

Extraction/preconcentration was then carried out by solid phase extraction using C_{18} cartridges based on the method described by Scullion *et al.* [26]. The cartridges were first conditioned with methanol (7 mL) followed by water (7mL). The sample was then passed slowly through the cartridge. After the sample stage the cartridge was washed with water/methanol (70:30)(12 mL). Elution was carried out with methanol (5 mL). Extracts were then evaporated to dryness under a steady stream of N_2 and redissolved in 1 mL methanol.

5.8 Results and Discussion

5.8.1 Analysis of Standard Formulations of nonylphenol ethoxylates

The masses used in the discussion of this chapter can be referred to in Appendix 1 at the end of this thesis.

Initial studies were conducted to determine the matrix to analyte ratio that produced the best spectra. The resulting spectra suggested that the optimum matrix to analyte ratio was between 10 and 5:1. Ratios below 5:1 did not produce good crystals, probably due to over-dilution of the matrix. Matrix to analyte ratios of 50:1 and above showed interfering matrix peaks, especially at the lower end of the spectrum. Figure 5.4 shows the spectrum obtained from the 5:1 ratio. The spectrum shows the typical envelope of peaks associated the ethoxymer distribution within the formulation. The spectrum would appear to show two peaks for each ethoxymer over the mass range m/z 400 – 1000. These ions (m/z 419, 463, 507, 551, 595, 639, 683, 728, 772, 816, 860, 903 and 947) and (m/z 435, 479, 523, 567, 611, 655, 699, 744, 788, 833, 877, 921, 965 and 1009) represent the sodium and potassium adducts, respectively, of each ethoxymer over the range of 4 to 17 ethylene oxide units. The source of these cations is most likely to be sodium and potassium contamination of the standard and/or the matrix. Peaks representing the 1-3 ethylene oxide unit containing species were too small to be discerned. The same was true of the 18+ ethylene oxide containing units.

Further experiments were carried out to assess the viability of the method for the analysis of other alkylphenol ethoxylate surfactant formulations. Figure 5.5 shows the spectrum of Synperonic NP12 (10 mg/mL). The spectrum shows $[M+Na]^+$ adducts for the 7-21 ethylene oxide containing species (m/z 552.1, 596.1, 639.8, 684.0, 728.2, 772.5, 817.1,

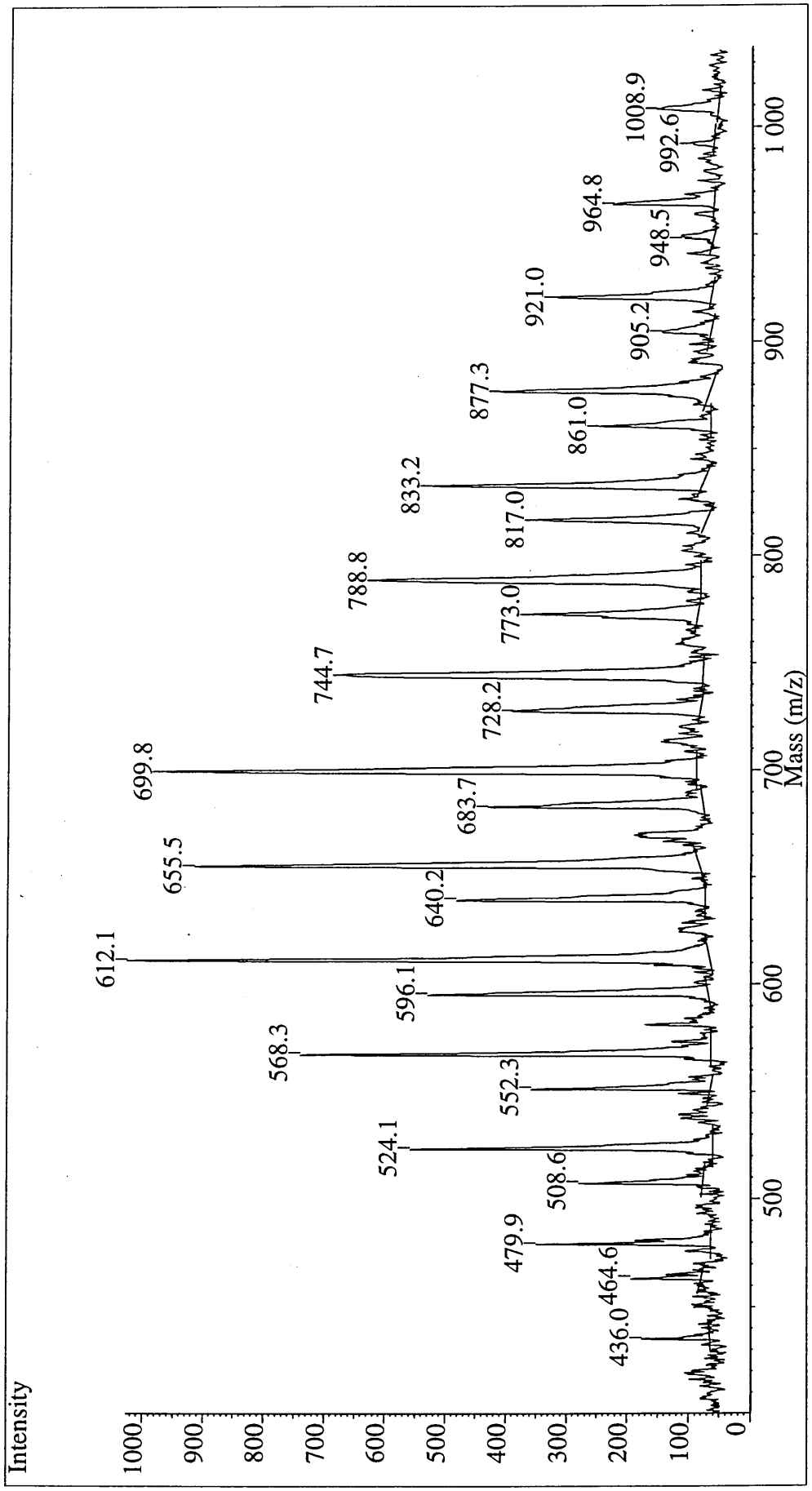


Figure 5.4 MALDI-TOF spectrum of Synperonic NP9

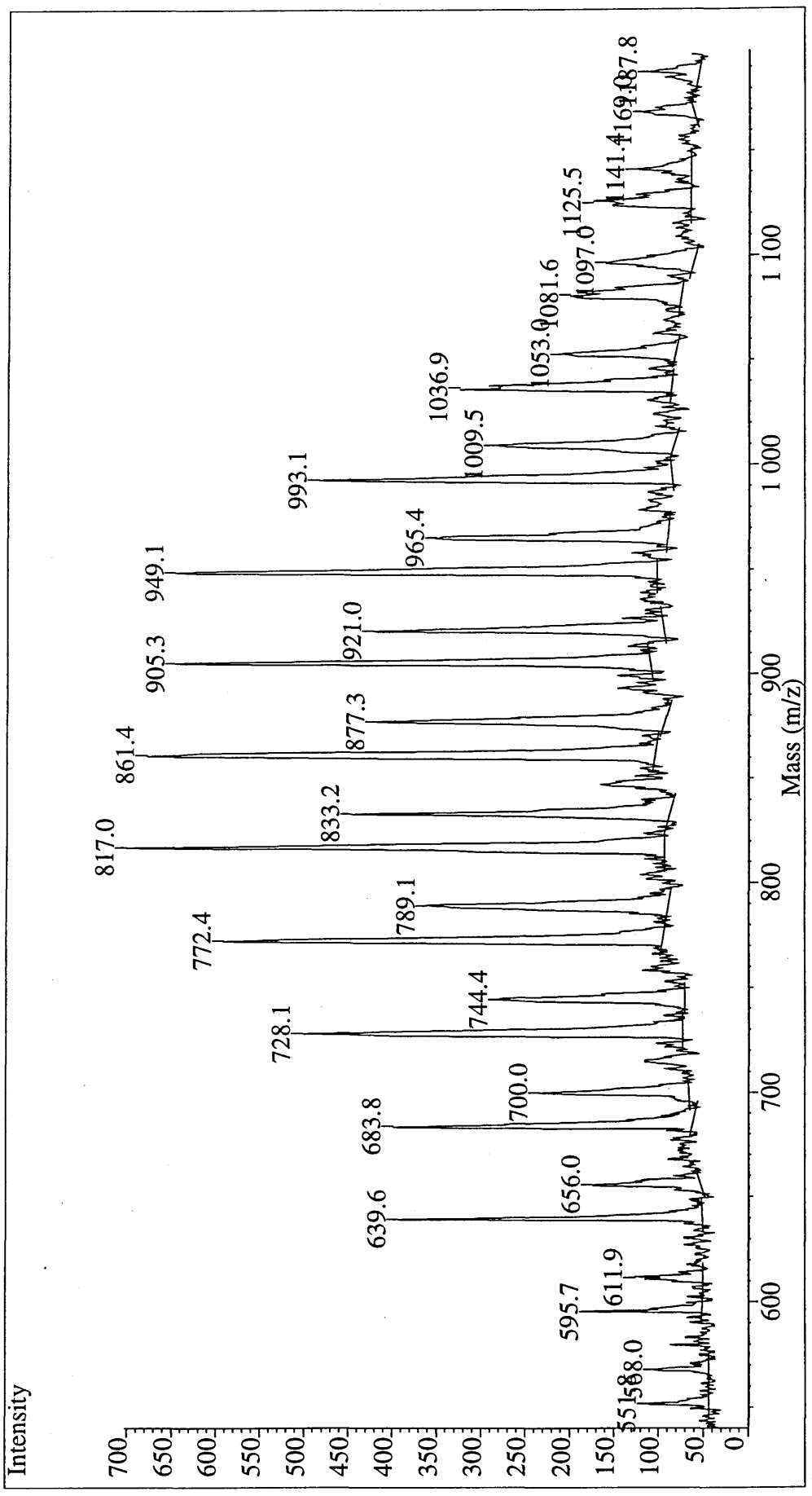


Figure 5.5 MALDI-TOF spectrum of Synperonic NP12

861.4, 905.4, 949.3, 993.4, 1037.2, 1082.0, 1126.1 and 1169.2). Also present are the corresponding potassium adducts (m/z 568.2, 611.8, 656.4, 700.3, 744.6, 789.1, 833.4, 877.5, 921.3, 965.6, 1009.4, 1053.2, 1097.1, 1141.4 and 1187.6).

Figure 5.6 shows the spectrum of Synperonic NP14 (10 mg/mL). The spectrum shows $[M+Na]^+$ adducts for the 6-22 ethylene oxide containing species (m/z 508.1, 551.9, 595.7, 639.7, 683.6, 727.5, 771.8, 816.3, 860.6, 904.9, 948.7, 992.6, 1036.5, 1080.4, 1124.5, 1168.4 and 1212.9). Once again the potassium adduct are also present (m/z 524.7, 567.6, 611.6, 655.6, 699.4, 743.7, 788.2, 832.3, 876.8, 920.8, 964.9, 1008.5, 1052.6, 1096.6, 1140.3, 1185.1 and 1228.5).

Figure 5.7 shows the spectrum of Triton X-100 (10 mg/mL). This spectrum shows $[M+Na]^+$ adducts for the 5-17 ethylene oxide containing species (m/z 450.2, 494.1, 538.1, 582.0, 626.1, 670.0, 714.0, 758.3, 802.7, 847.1, 891.3, 935.9 and 979.6). The spectrum of Triton X-100 also shows $[M+K]^+$ adducts (m/z 466.6, 510.1, 554.9, 598.1, 642.1, 686.2, 730.4, 774.9, 819.0, 863.6, 907.8, 952.0 and 951.4).

5.8.2 Analysis of River Water Extracts by MALDI-TOF

Following the success of the analysis of standard nonylphenol ethoxylate (NPEO) formulations by MALDI-TOF, it was decided to analyse extracts from the River Don used in earlier work. Samples from the River Don were previously found to have particularly high concentrations of what was thought to be NPEO due to the characteristic envelope of peaks observed by HPLC.

The River Don extracts were mixed with DHB in the same ratio as that used for the standards.

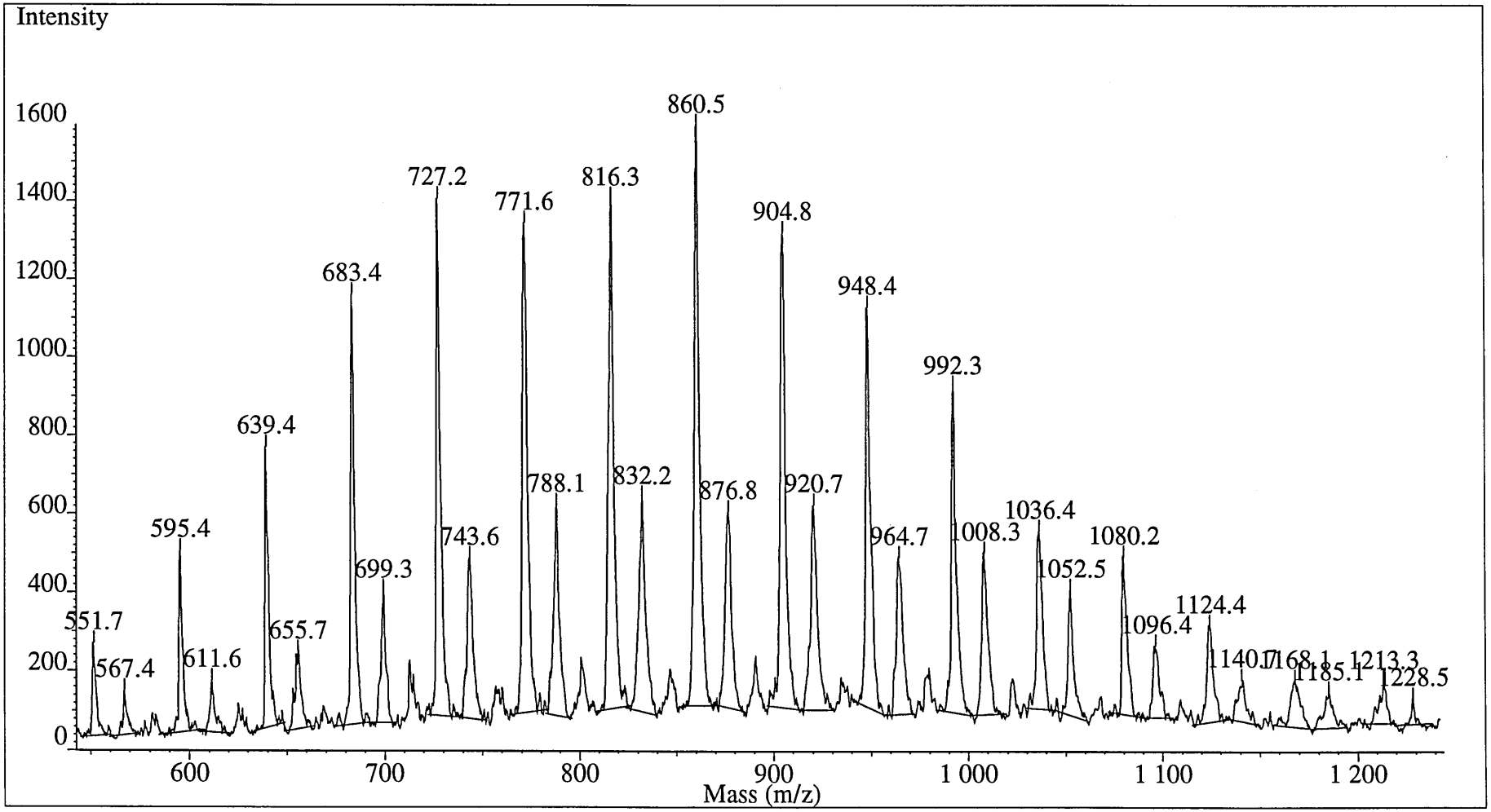


Figure 5.6 MALDI-TOF spectrum of Synperonic NP14

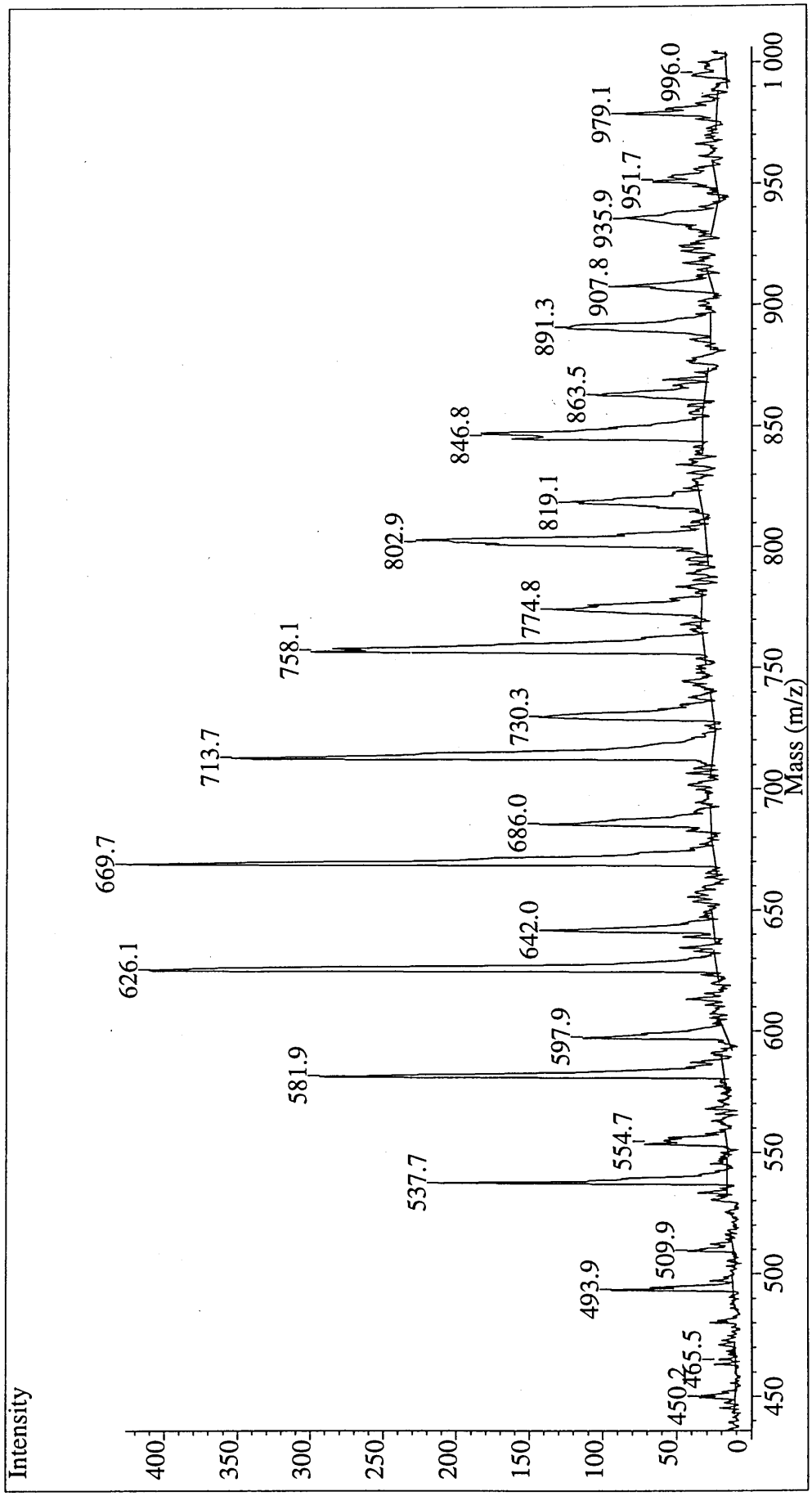


Figure 5.7 MALDI-TOF spectrum of Triton X-100

Excellent results were obtained when analysing the river water extracts by MALDI-TOF. Two separate river water extracts were analysed and different results were obtained for each sample.

Extract one (Figure 5.8) produced the simpler spectra of the two extracts. A typical envelope of ethoxymers were seen over the mass range m/z 500-1000 which correspond to $[M+Na]^+$ adducts for NPEOs species containing between 6 and 15 ethylene oxide units. It is possible that peaks representing the species containing 1 and 2 ethylene oxide units are present in the spectrum (m/z 287.1 and 333.6). However, this region is complicated by peaks due to the matrix and other contaminants, so their identification is uncertain at the time of writing.

Extract two (Figure 5.9), produced a similar envelope of peaks to that seen in extract one. In this case however, the resulting spectrum is complicated by both $[M+Na]^+$ and $[M+K]^+$ adducts both being present, and also by what would appear to be a third series of peaks (m/z 566, 611, 655, 700,745,803,847,892,935,980); once more the identity of which is unknown at the time of writing. The envelope of peaks covers the mass range m/z 400-1200 and represent NPEO containing 4 to 20 ethylene oxide units. Again, it is possible to make out ethoxymers lower in the spectrum but, as with extract one, it is difficult to identify them conclusively as NPEO due to interference from matrix and other contaminants.

The results from the analysis of environmental extracts are very interesting; the spectra produced are remarkably simple considering the complex nature of the matrix from which they were taken. Both spectra are composed of adducts of alkali metal cations which is not surprising as any environmental sample is likely to contain sodium,

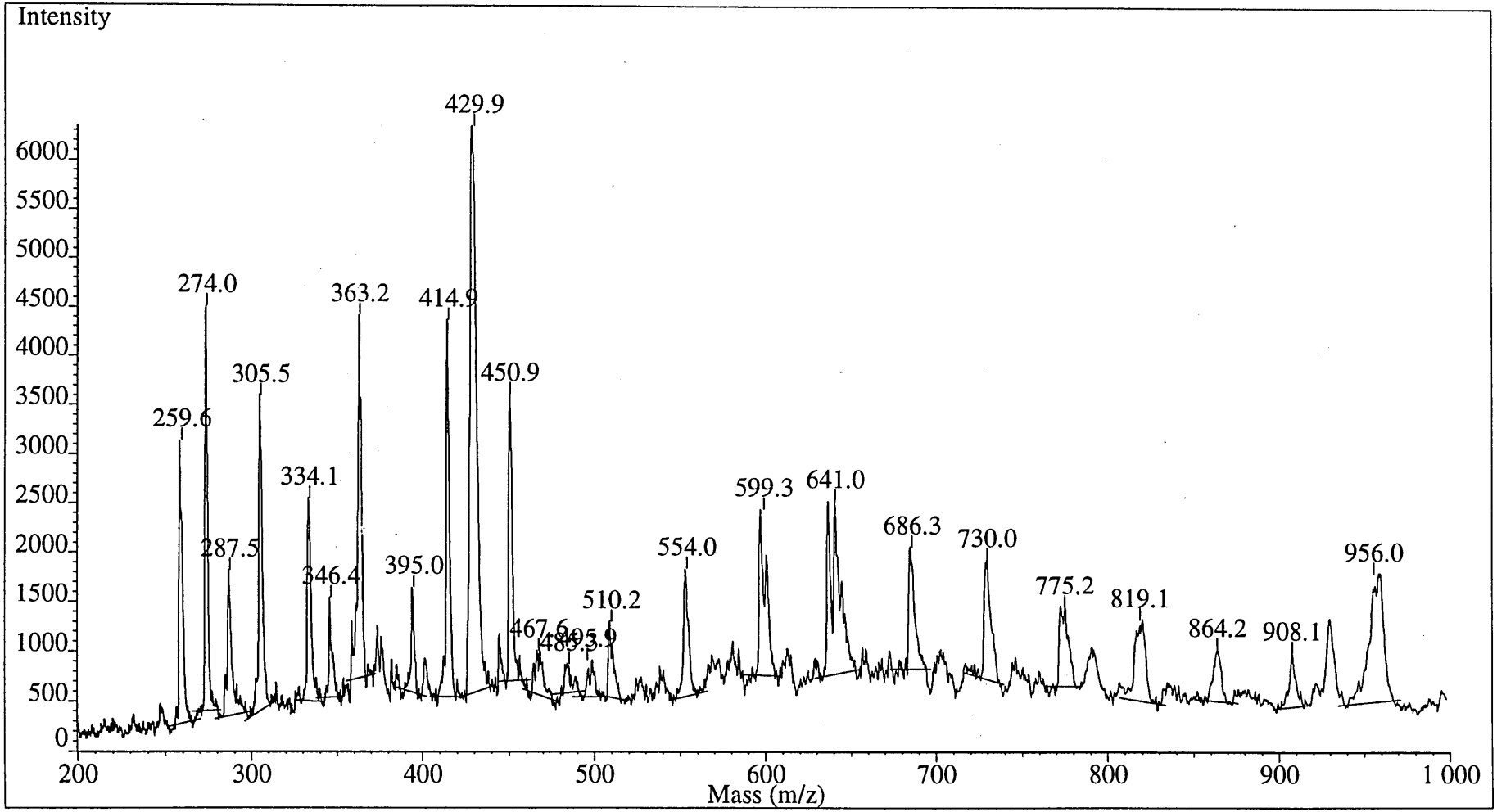


Figure 5.8 MALDI-TOF spectrum of extract 1 from the River Don

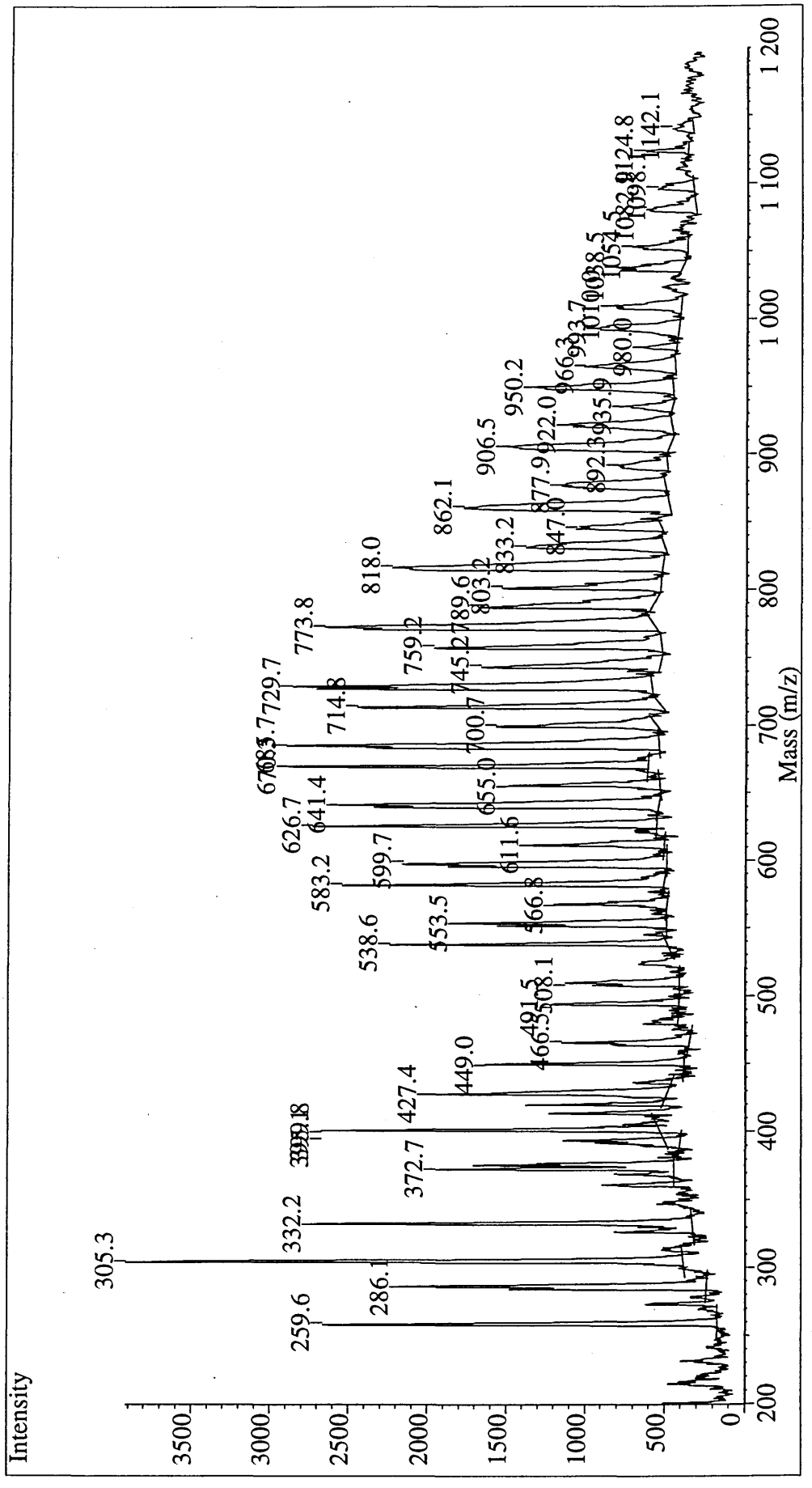


Figure 5.9 MALDI-TOF spectrum of extract 2 from the River Don

potassium and other metal species from both natural and industrial/domestic sources. However, there is no way of determining the concentration of NPEO in the samples using the current methodology. In order to gain quantitative information a new experimental procedure would have to be developed.

5.8.3 Quantitative Analysis by MALDI-TOF Mass Spectrometry

Although a large amount of work has been conducted on the qualitative aspects of MALDI, such as improving resolution and mass accuracy, comparatively little has been carried out on quantitative MALDI.

Quantification in MALDI is complicated by its inherent lack of shot-to-shot reproducibility. This lack of reproducibility means that for a series of analyte/matrix sample targets prepared under identical conditions, the response is highly variable. This is largely due to the non-uniform (and the poorly understood) nature of the crystallisation of the matrix/analyte complex, and hence the extremely variable nature of the fine structure of the analyte on the target surface. The MALDI process is very sensitive to laser power. When the power is below the desorption/ionisation threshold, no signals are observed. As the laser power is increased above this threshold the analyte signal increases dramatically; therefore, small changes in laser power can lead to very large fluctuations in analyte signal. As sample preparation for MALDI tends to lead to quite wide distributions of both crystals on the target and analyte in the matrix crystals, desorption/ionisation thresholds can vary from spot-to-spot and shot-to-shot. Therefore, the signal from a particular analyte is not necessarily linearly proportional to the quantity of analyte.

Tang and co-workers [27] used cytochrome C as an internal standard for the quantification of lysozyme and myoglobin. The addition of a known quantity of material as an internal standard having similar chemical properties should produce a signal level more or less independent of the above experimental variables. Thus, inhomogeneous sample distribution on the target and the strong dependence of desorption on laser power should equally affect both the analyte and the material put into the sample for calibration. Results from this work showed that a good linear relationship could be achieved using a chemically similar internal standard. The authors then went on to show that using a chemically dissimilar molecule (a poly-T oligomer) as an internal standard did not produce a linear relationship.

Therefore, an internal standard used for quantification in MALDI should have the following *ideal* properties [28]: -

- It must be completely resolved from the analyte.
- It must be chemically stable during the analysis.
- It should be chemically similar to the analyte (including ionisation and extraction efficiencies) and should not react with the analyte.
- It should be close to the analyte in mass and concentration to avoid instrumental errors.

The ideal candidate is an isotope-labelled version of the analyte as this would have virtually identical crystallisation properties to that of the analyte. This theory has been investigated by Duncan *et al.* [11] in the quantitative analysis of peptides. In this work three different strategies were employed; use of deuterium labelled acetylcholine as an internal standard for the analysis of acetylcholine, use of ^{13}C labelled DOPA for the quantification of DOPA and also the use of the peptide Ac-Ser-Ile-Arg-His-Tyr-NH_2 for

the analysis of H-Ser-Ala-Leu-Arg-His-Tyr-NH_2. The last one being a structural analogue of the analyte instead of an isotopically labelled version; good linear relationships ($R^2 > 0.95$) were obtained in all three cases. The authors noted that although in theory the isotopically labelled approach was the best, in reality the structural analogue was the most practical. This was justified for two reasons, the first being the fact that common linear MALDI instruments lack the resolving power to reliably separate species whose masses may only differ by one mass unit. The second is that the cost and complexity of isotope labelling (particularly at high mass) becomes prohibitive.

Gusev *et al.* [28,29] have achieved good linearity using the structural analogue approach for the analysis of cyclosporin A using cyclosporin D as an internal standard in methanol and whole blood matrices. They have also shown excellent results using human arg-insulin, insulin Chain B, cytochrome C, des-octapeptide and des-pentapeptide as internal calibrants for the quantification of bovine insulin.

Internal standards have also been used to good effect for quantification by MALDI of proteins [30], carbohydrates [31], oligonucleotides [32] and sulphonamide antibiotics [33].

5.8.4 Quantitative MALDI of Nonylphenol Ethoxylates

The quantitative analysis of alkylphenol ethoxylate surfactants by MALDI represents a more complicated situation than the typical single component analyte mentioned previously. Due to the large spread of species seen in the spectra of alkylphenol ethoxylates, a suitable (conventional) internal standard would have to have a mass which would allow it to 'insert into one of the spaces' between the individual ethoxymer peaks. As mentioned previously, the ideal solution is to use an isotope-labelled version of the

analyte, as this would effectively produce an internal standard for each ethoxymer in the sample just a few mass units higher in the spectrum. However, the resolution produced by the LaserTof 1500 in the analysis of alkylphenol ethoxylates is not good enough to reliably resolve peaks of such a small mass difference. In addition, because of the complexity and cost of isotopically labelling alkylphenol ethoxylates, it was deemed that this approach was not appropriate.

There is, however, another candidate for use as an internal standard. OPEO differ from NPEO by only a single methylene unit in the hydrophobic chain, and the LaserTof 1500 should be able to adequately resolve the mass difference of 14 Da. Using OPEO as an internal standard for NPEO has the added advantage of effectively being an internal standard for each individual ethoxymer, as in the case of using an isotopically labelled internal standard. As OPEO are not generally found in the environment there should not be any problems with interference from environmental samples.

5.8.5 Addition of Lithium Chloride

Spectra of various formulations of NPEO and OPEO surfactants show both sodium and potassium adducts. The presence of these adducts would make a spectrum of nonyl and OPEO very complicated indeed.

The addition of an excess of a suitable metal cation during sample preparation has been used widely in the analysis of synthetic polymers by MALDI-TOF mass spectrometry [17,12,16,23,34,15]. Addition of a metal cation leads to the production of solely $[M+cation]^+$ species. Just and co-workers [19] added an excess of lithium chloride to alkylphenol ethoxylates before analysis by MALDI-TOF. This led to the production of almost solely lithium adducts instead of the mixture of potassium and sodium adducts

which they saw before the addition of lithium chloride. Their reason for adding lithium to the samples was to counteract the low-mass discrimination observed in the comparison between MALDI-TOF and SFC. Mass discrimination of polydisperse polymers has been observed by other groups [22,23,24].

The addition of lithium chloride had the effect of suppressing the discrimination, enabling them to discern the lower ethoxymers. The most likely explanation of this effect is probably that in producing solely $[M+Li]^+$ adducts, the signal due to each ethoxymer is represented by one peak. Whereas without the addition of lithium chloride the signal for each ethoxymer is split between two peaks (the sodium and potassium adducts) which are much smaller than if the signal were represented by only one peak.

Discrimination at low-mass is also seen to a small degree in spectra described above. Therefore, the addition of lithium to the samples before crystallisation should counteract this phenomenon and lead to much less complicated spectra that would enable quantification using OPEO.

The addition of 10 μL of a 10 mg/mL solution of lithium chloride resulted in a much less complicated looking spectrum (Figure 5.10). Again, a typical envelope of peaks is seen (m/z 403, 447, 491, 535, 579, 623, 667, 711, 755, 799, 843, 887, 931 and 975). These masses correspond to the $[M+Li]^+$ adducts of the 4-17 ethylene oxide containing species of NPEOs. Although the addition of lithium chloride produced a visually simpler spectrum, it does not seem to have helped with the low-mass discrimination. The reason for this is not clear; however, the addition of lithium chloride should increase the sensitivity of the technique in the analysis of lower concentrations of NPEO.

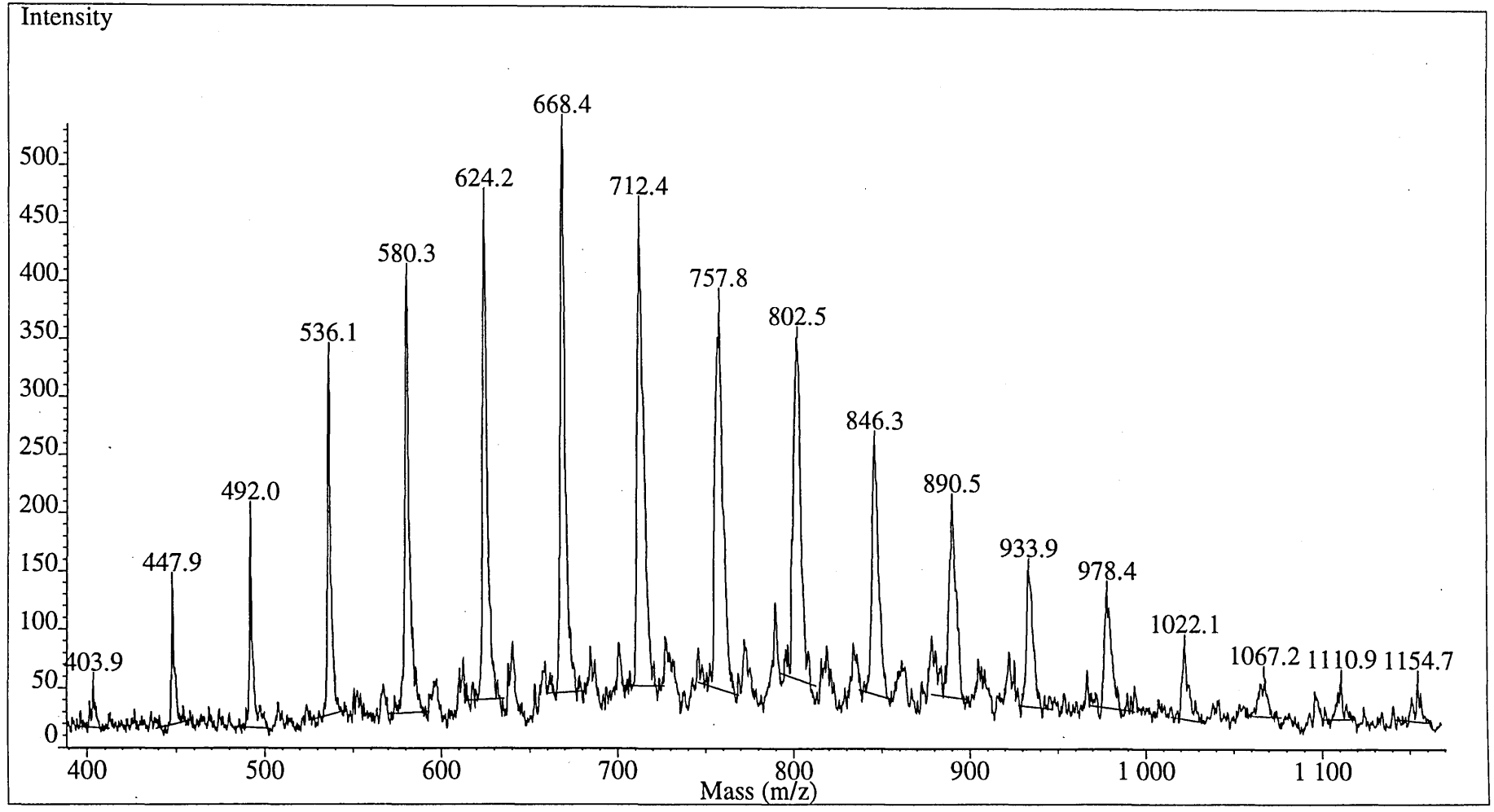


Figure 5.10 MALDI-TOF spectrum of Synperonic NP9 with the addition of LiCl

5.8.6 The Analysis of Other Alkylphenol Ethoxylate Formulations Using Lithium Chloride

Further experiments were carried out to assess the viability of adding lithium chloride to other alkylphenol ethoxylate surfactant formulations.

Figure 5.11 shows the spectrum of Synperonic NP5 (10 mg/mL). Again, only $[M+Li]^+$ adducts were produced (m/z 272, 315, 359, 403, 447, 491, 535, 579, 623, 711, 755, and 800) for the 1-12 ethylene oxide containing species.

Figure 5.12 shows the spectrum of Synperonic NP12 (10 mg/mL). The spectrum shows $[M+Li]^+$ adducts for the 4-21 ethylene oxide containing species (m/z 403, 447, 491, 535, 579, 623, 667, 711, 755, 799, 843, 886, 930, 974, 1018, 1062, 1106 and 1150).

The spectrum of Synperonic NP14 (10 mg/mL) is shown in Figure 5.13. The spectrum shows $[M+Li]^+$ adducts for the 3-21 ethylene oxide containing species (m/z 360, 403, 491, 535, 579, 623, 667, 711, 755, 799, 843, 887, 930, 974, 1018, 1062, 1106 and 1151).

Figure 5.14 shows the spectrum of Triton X-100 (10 mg/mL), and shows $[M+Li]^+$ adducts for the 3-19 ethylene oxide containing species (m/z 345, 389, 433, 477, 521, 564, 608, 652, 696, 740, 785, 828, 872, 916, 959, 1001 and 1048).

5.8.7 Use of α-Cyano-4-hydroxycinnamic Acid as a Matrix

α-Cyano-4-hydroxycinnamic acid is another useful matrix for MALDI-TOF MS. Therefore this was investigated as an alternative to DHB for the analysis of NPEO.

The resulting spectrum of a NP12 standard (Figure 5.15) shows α-cyano-4-hydroxycinnamic acid to be as good a matrix as 2,5-dihydroxybenzoic acid for the

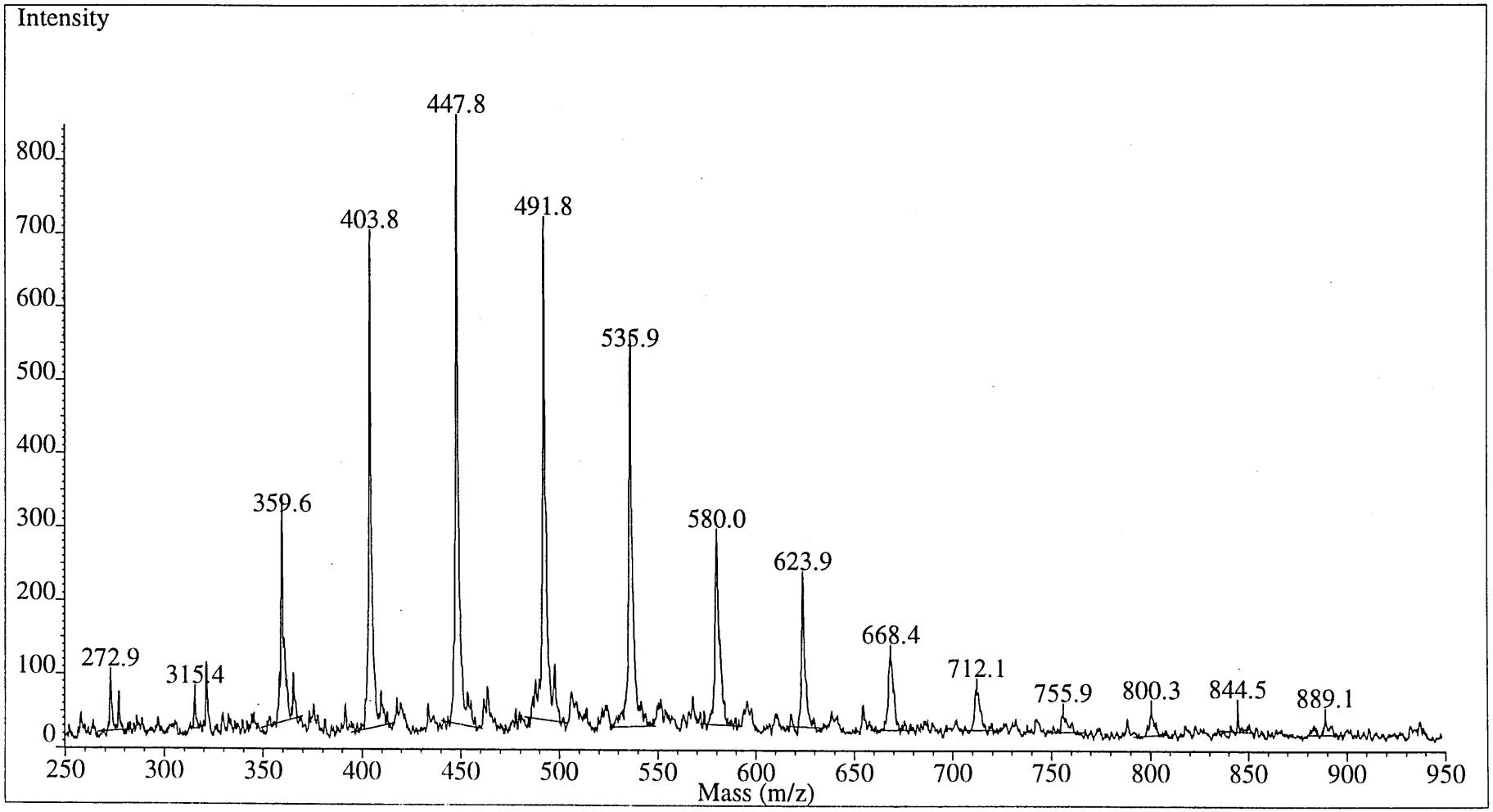


Figure 5.11 MALDI-TOF spectrum of Synperonic NP5 with the addition of LiCl

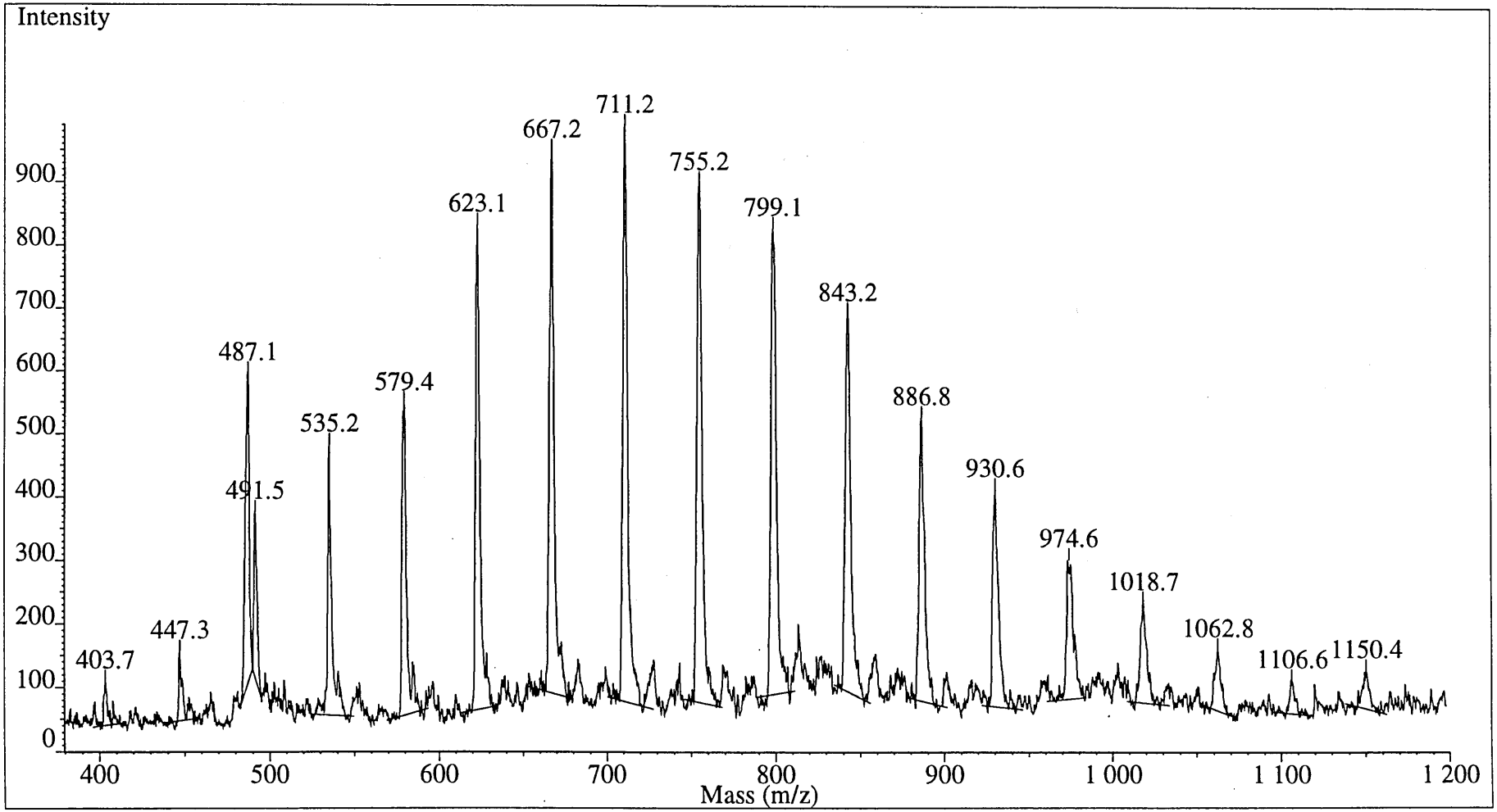


Figure 5.12 MALDI-TOF spectrum of Synperonic NP 12 with the addition of LiCl

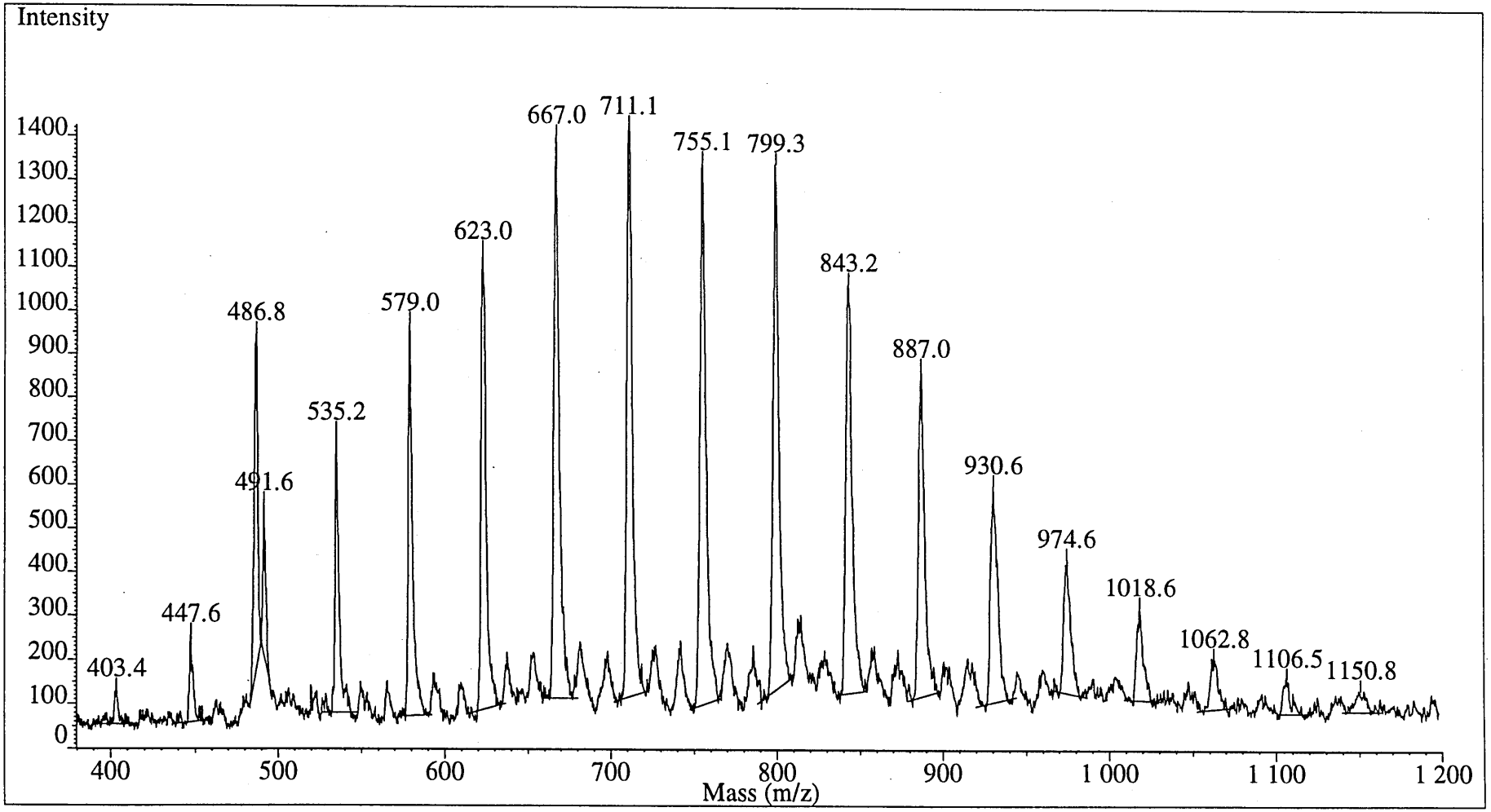


Figure 5.13 MALDI-TOF spectrum of Synperonic NP14 with the addition of LiCl

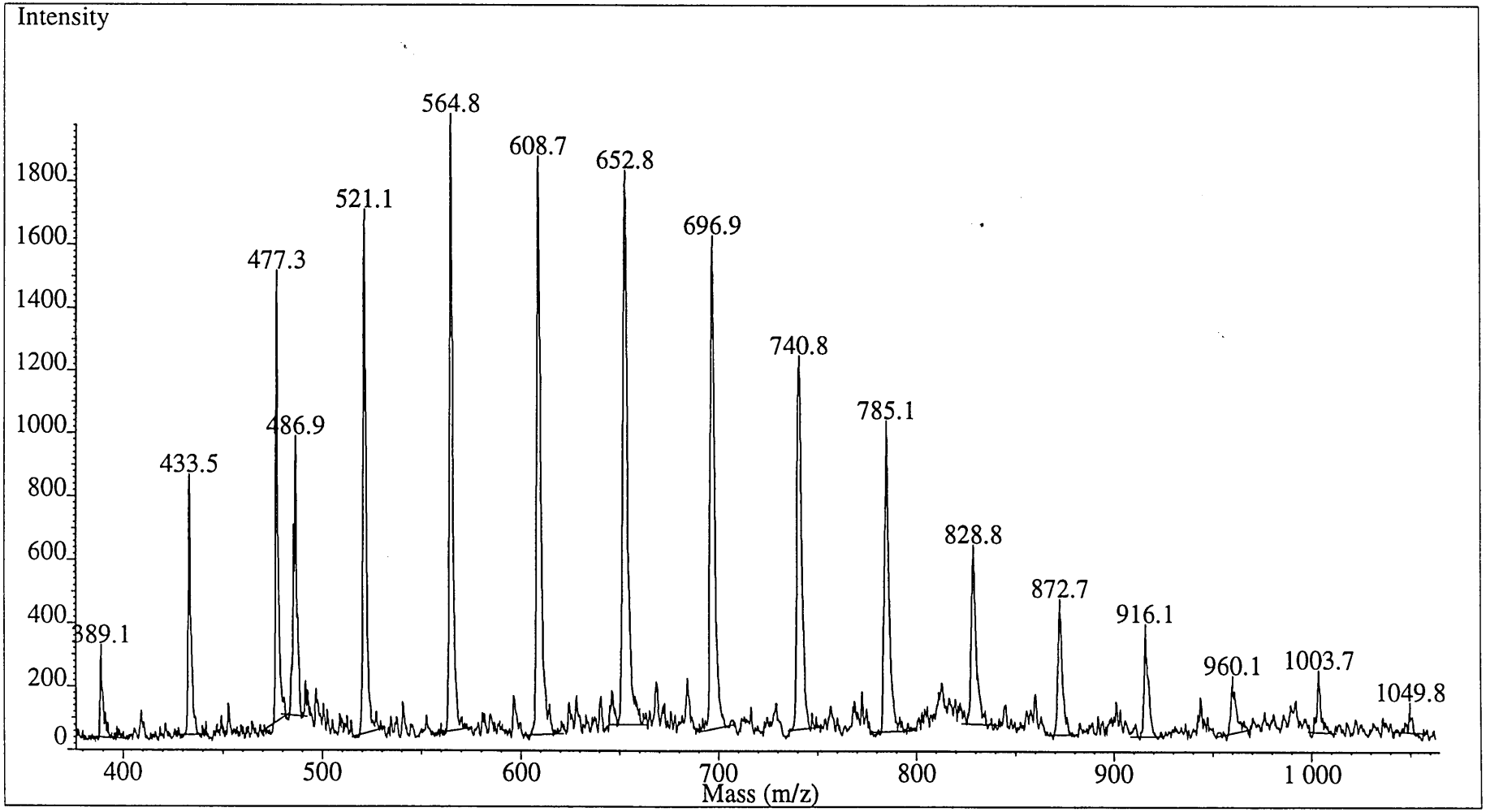


Figure 5.14 MALDI-TOF spectrum of Triton X-100 with the addition of LiCl

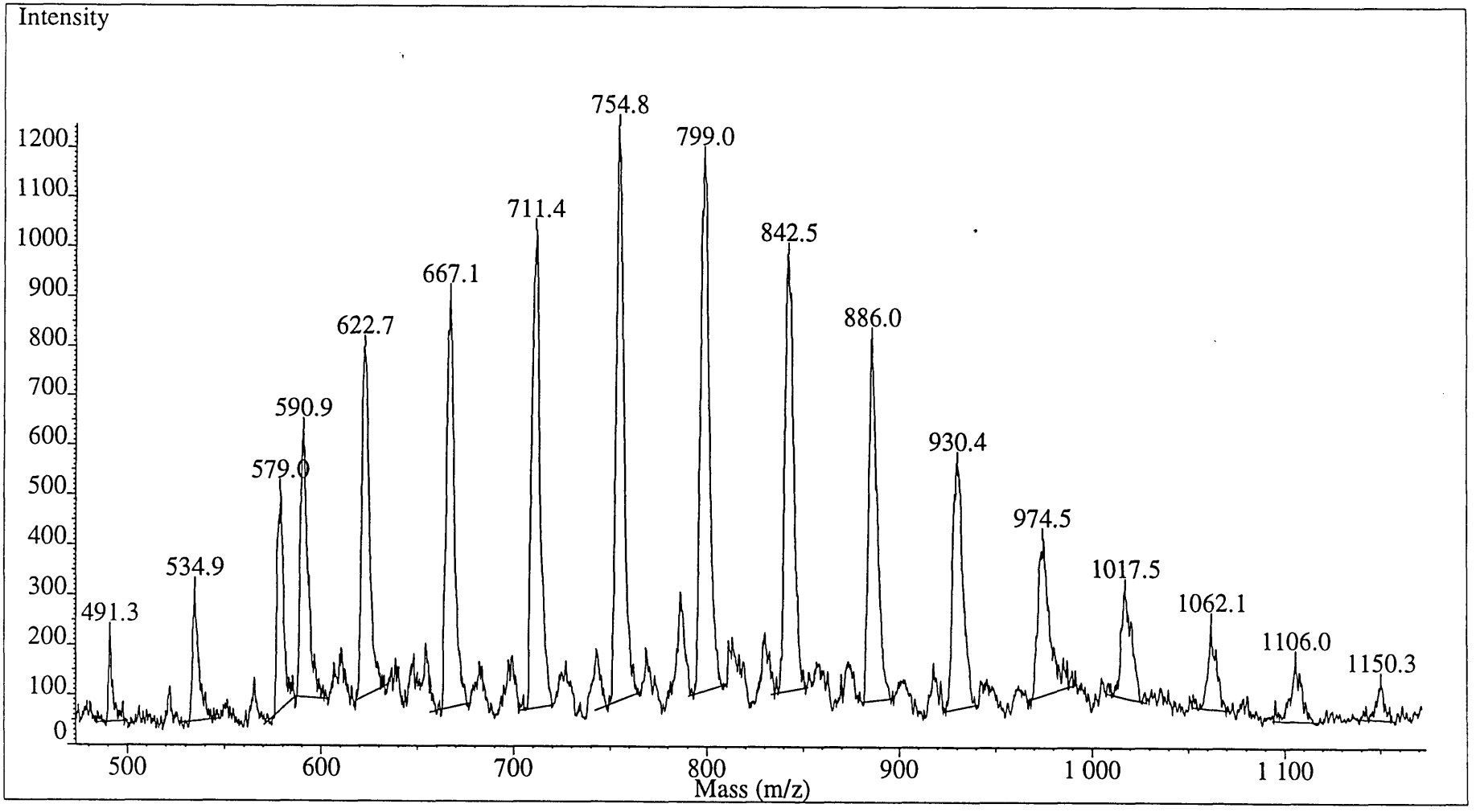


Figure 5.15 MALDI-TOF spectrum of Synperonic NP12 using HCCA as the matrix

analysis of NPEO surfactants. A typical envelope of intense peaks representing $[M+Li]^+$ adducts for 6-21 ethylene oxide containing species was produced (m/z 490.9, 534.6, 578.4, 590.6, 622.5, 666.6, 710.6, 754.5, 798.5, 842.2, 885.9, 929.9, 974.0, 1017.9, 1061.6, 1105.9 and 1150.1).

5.8.8 Effect of Mixing Synperonic NP9 and Triton X-100

In order for OPEO to be used as an internal standard for NPEO there must be adequate resolution between ethoxymers of the two types of surfactant.

Figure 5.16 shows a spectrum of Synperonic NP9 (10 mg/L) and Triton X-100 (10 mg/L) using DHB as the matrix. Lithium chloride (10 mg/mL) was added to the sample before crystallisation. Excellent resolution between each ethoxymer was achieved over the mass range m/z 330-1020 (3-18 ethylene oxide units). A similar situation was achieved using HCCA as the matrix (Figure 5.17).

5.8.9 Calibration Using Triton X-100 Internal Standard

The concentrations of standards used in the initial development of this work are very high and do not represent the concentrations of surfactants that would be found in the environment. Therefore, it was decided to conduct calibration studies on more realistic concentrations, starting with the range 100-500 mg/L Synperonic NP9 and then progress to lower concentrations if successful.

Using a Synperonic NP9 concentration of 500 mg/L in a 1:5 (v/v) ratio with the DHB (10 mg/L) solution used previously produced spectra with high levels of matrix interference. Increasing the analyte component of the matrix / analyte ratio reduced the spectral quality further; observation of the targets after evaporation of the solvent

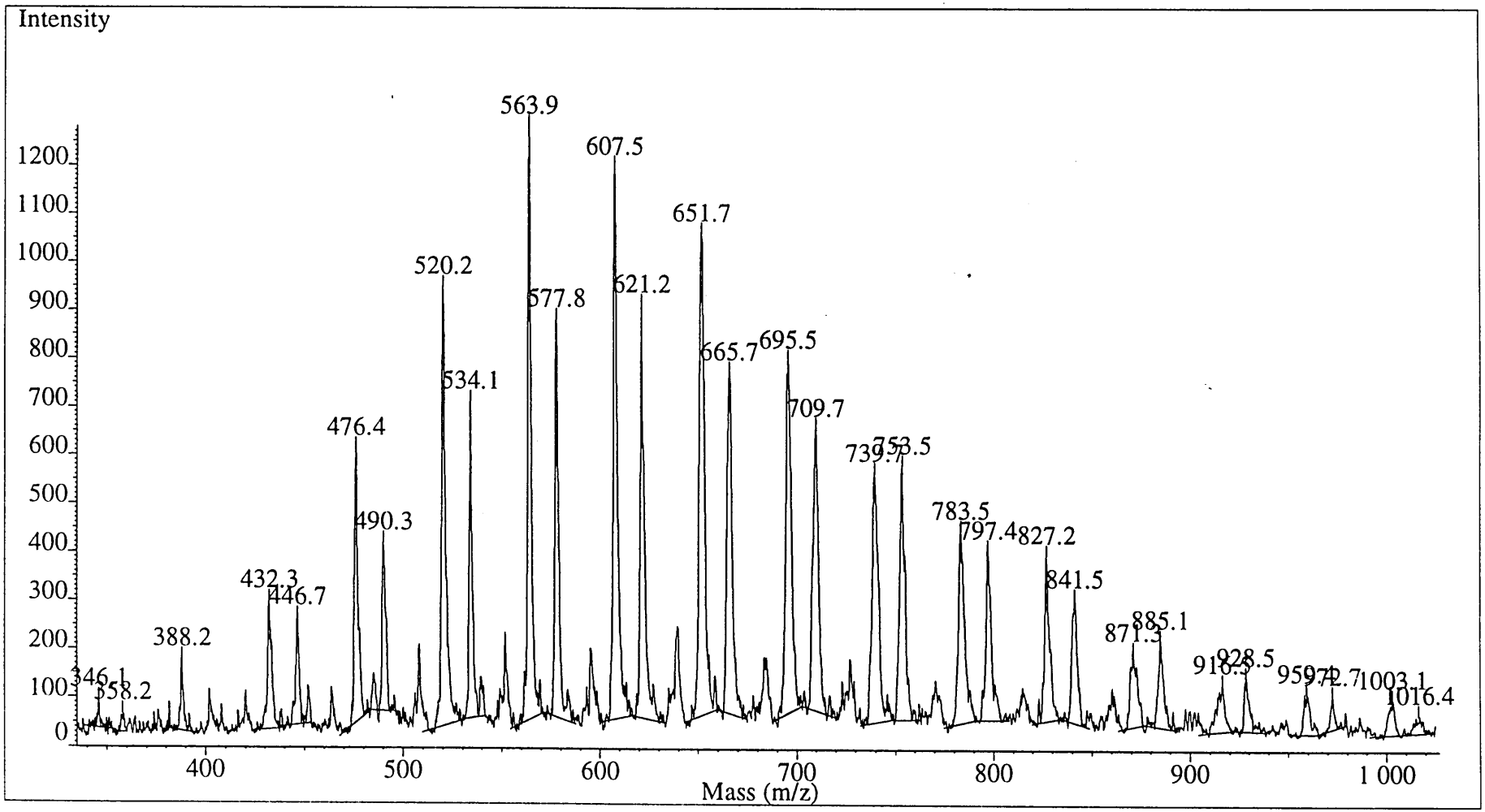


Figure 5.16 MALDI-TOF spectrum of Synperonic NP9 and Triton X-100 using DHB as the matrix

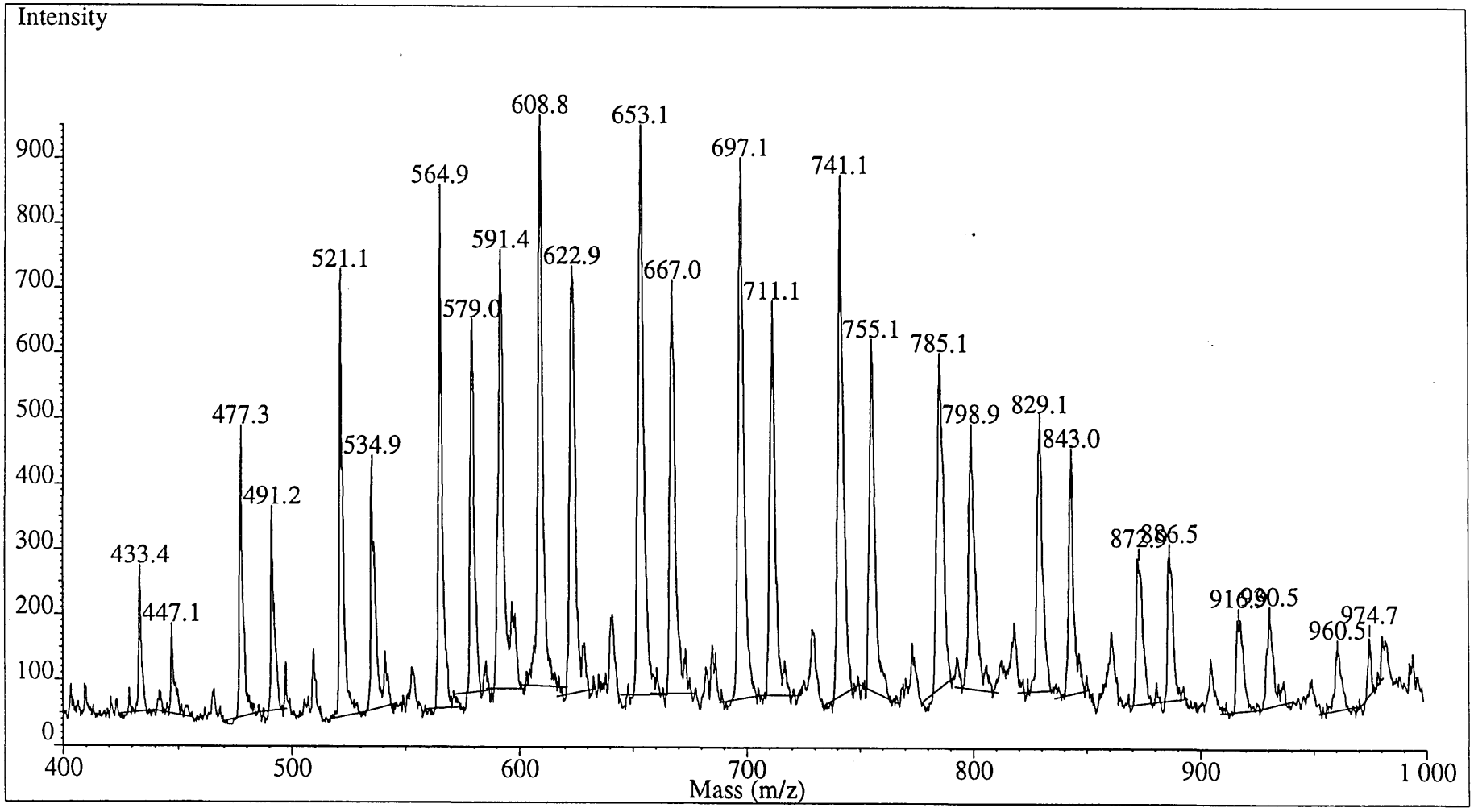


Figure 5.17 MALDI-TOF spectrum of Synperonic NP9 and Triton X-100 using HCCA as the matrix

showed that very few crystals were being formed. This was thought to be due to over dilution of the matrix. The only way around this was to increase the concentration of the matrix solution used to make up the matrix / analyte solution. DHB concentrations of 100 mg/mL and above were prepared without excessive heating and stirring (which can lead to supersaturation and the formation of crystals upon cooling) by the addition of 0.1% trifluoroacetic acid to the solution. It was then found that good quality spectra could be produced using a matrix / analyte ratio of 1:5 (v/v) using the 100 mg/ml DHB and 500 mg/L Synperonic NP9 solutions, respectively.

A plot of NPEO concentration against the NPEO / OPEO ratio (Figure 5.18) shows a good linear relationship (R^2 = 0.9926). These results are based on the average of four spectra; the result used for each spectrum is the average of the peak areas of ethoxymers 4-17. This relationship is true for both DHB and HCCA matrices.

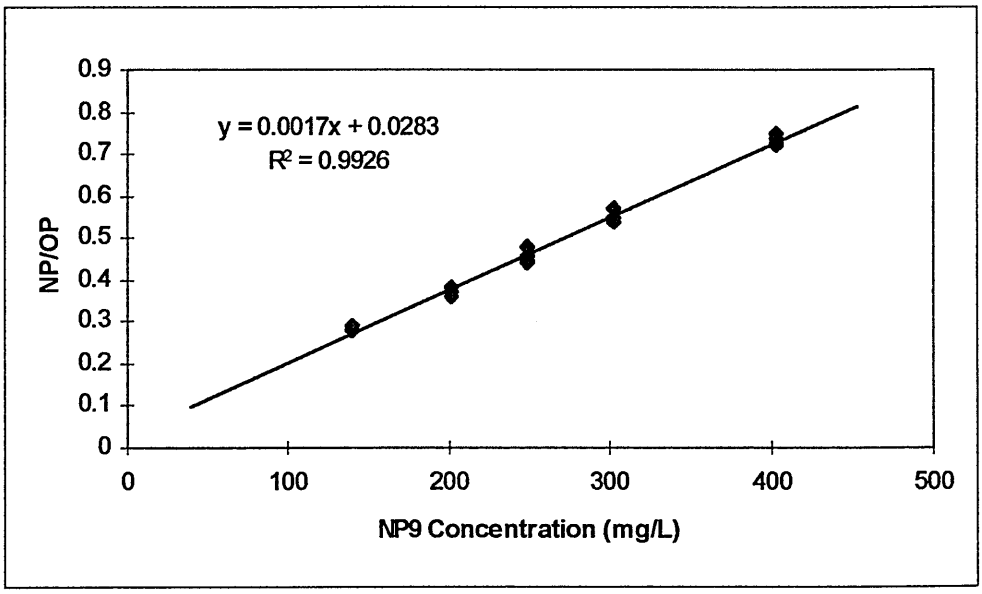


Figure 5.18. NPEO/OPEO ratio over the range 139.4-604 mg/L Synperonic NP9 (using DHB as matrix).

The concentration of NPEO used to obtain the data described above is relatively high. Assuming environmental concentrations of ~200 µg/L, after extraction / concentration of

250 mL sample to a final volume of 1 mL, this would equate to a final concentration of 50 mg/L. Therefore, the procedure detailed above was repeated using Synperonic NP9 concentrations of 10-50 mg/L. The results from this experiment are plotted in Figure 5.19. Again, a good linear relationship was achieved ($R^2 = 0.9734$) for ethoxymers 4-17.

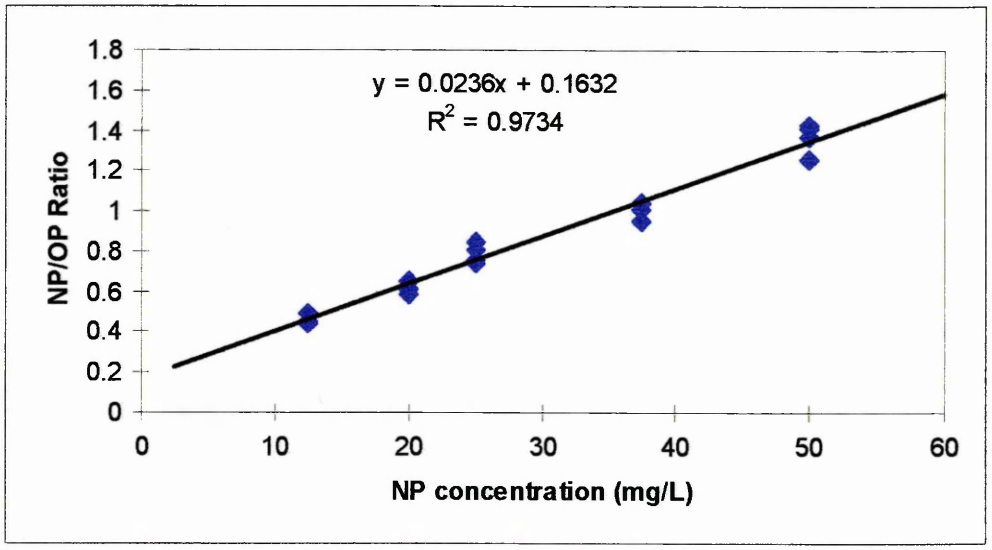


Figure 5.19. NPEO/OPEO ratio over the range 12.5-50 mg/L Synperonic NP9 (using DHB as matrix).

The environmental distributions of NPEO surfactants do not necessarily follow the same Gaussian distribution that is seen in standard surfactant formulations such as Triton X-100 and Synperonic NP9. As described in chapter one, the molecular weight distribution of NPEO can vary greatly, depending on the particular application. In addition, biodegradation in the environment proceeds, initially, via ω-oxidation of the ethoxylate chain; therefore environments where such biodegradation is prevalent will show a bias towards shorter ethoxylate chain lengths. Therefore, the approach of taking the average of the areas all of the ethoxymers for calibration, described previously, had to be altered in order to allow for the variance of ethoxymer distribution that may be seen in extracts of environmental samples. To account for this variation calibration must take place on an ethoxymer by ethoxymer basis.

Individual calibration curves were constructed for each ethoxymer. The percentage of each ethoxymer in the standard was calculated by dividing each individual area of the standard spectrum produced in section **5.8.5** by the sum of all of the areas. This figure can then be used to calculate the concentration of each ethoxymer in a standard solution of Synperonic NP9. The results of these calculations are shown in table 5.2.

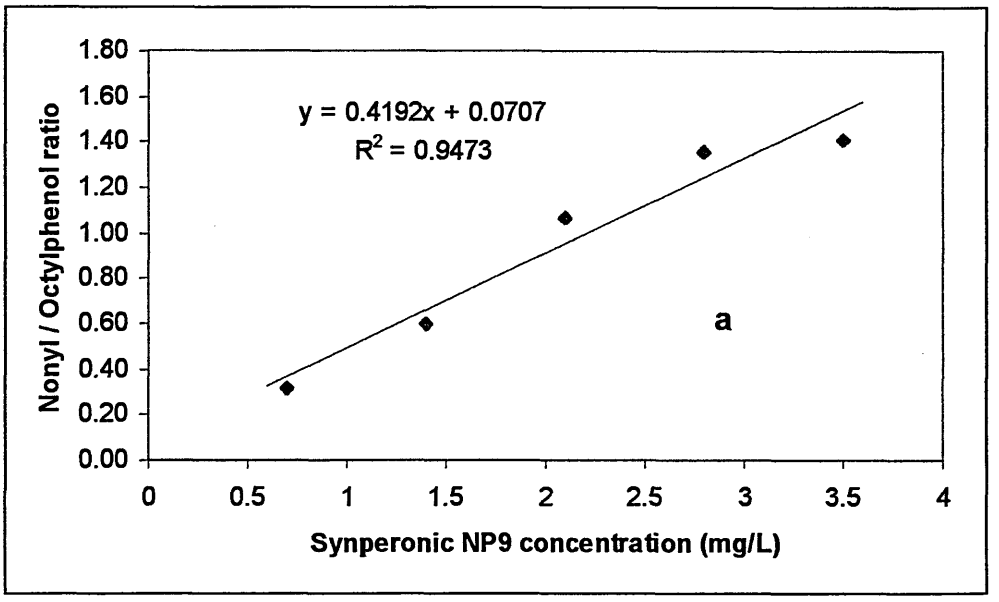
Individual calibration graphs using the data presented above gave R^2 values of >0.79 for each ethoxymer (table 5.3). Although the linearities of the curves for individual ethoxymers are not as good as the results obtained using all of the ethoxymers in the calculation, a linear relationship can still be seen in each case. Examples of three of these calibration curves obtained are displayed in Figure 5.20.

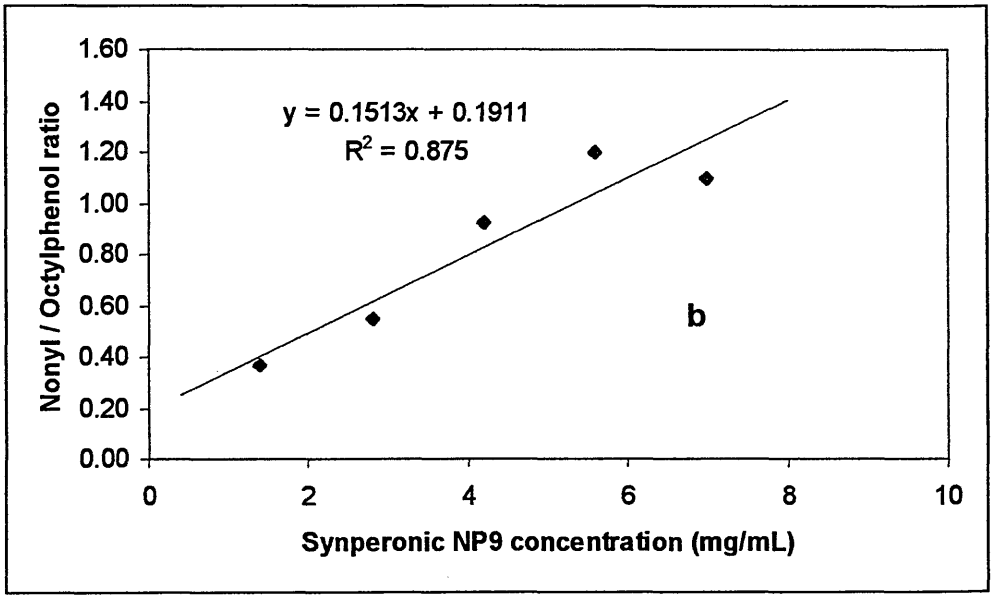
Ethoxymer	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Proportion of signal due to ethoxymer	0.003	0.010	0.030	0.070	0.100	0.120	0.140	0.130	0.120	0.090	0.070	0.050	0.040	0.020

Table 5.2. Proportional of signal due to each ethoxymer of Synperonic NP9.

Ethoxymer	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Linearity (R^2)	-	0.7888	0.8789	0.9283	0.9473	0.9676	0.8470	0.8750	0.8903	0.8662	0.8653	0.9276	0.8077	-

Table 5.3. Linearity (R^2) values for ethoxymers 4-17.





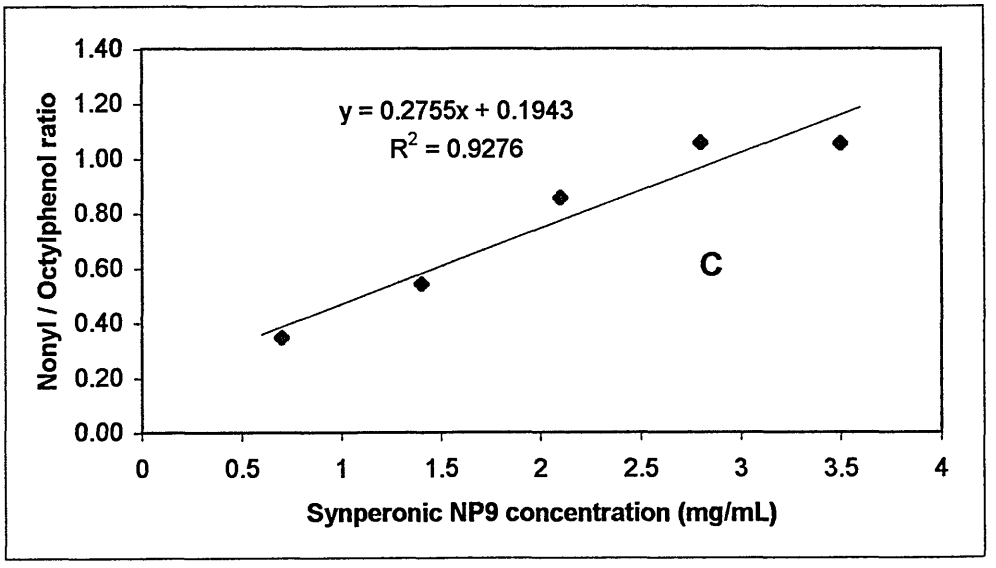


Figure 5.20. Examples of individual ethoxymer calibration curves. a) species containing eight ethylene oxide units, b) species containing eleven ethylene oxide units and c) species containing fifteen ethylene oxide units.

5.8.10 Recovery of Synperonic NP9 from Spiked Milli-Q Water

In order to test the calibration achieved above, the method was applied to the extraction of a spiked sample of Milli-Q water.

The sample volume was 250 mL H_2O spiked with Synperonic NP9 (200 μg/L) and Triton X-100 (188 μg/L) which would give final concentrations of 50 mg/L Synperonic NP9 and 47.1 mg/L Triton X-100 following extraction.

The extraction method developed by Scullion *et al.* [26] was shown to give recoveries of between 63% and 122% for the individual ethoxymers of Synperonic NP9. Analysis by MALDI-TOF produced similar results.

The data in table 5.4 shows the recovery data based on four extractions. As can be seen the percentage recoveries for each ethoxymer lie between 84% and 115 %. Percentage RSD's for the recoveries are at least as good as those generated from results obtained by HPLC, and are good enough to allow the method to be used for environmental samples.

Ethoxymer	4	5	6	7	8	9	10	11	12	13	14	15	16	17
% Recovery	84.6	95.2	102.4	101.9	96.5	96.0	93.3	114.8	99.7	102.5	114.9	91.5	95.6	111.2
% RSD	6.7	2.4	5.3	6.4	1.4	2.0	2.9	4.8	8.8	10.4	9.4	6.2	4.1	1.0

Table 5.4. Individual ethoxymer recoveries form Milli-Q water (based on four extractions).

5.8.11 Analysis of Seawater Samples by MALDI-TOF

The entire sample employed for the initial studies by MALDI had been consumed; therefore, new samples had to be obtained. Surface water samples were collected from Langstone Harbour in Portsmouth.

The extraction procedure used for these samples was based on the C_{18} SPE method developed by a previous research student [26]. This was chosen instead of the GCB SPE method used in chapter three of this thesis because it was much simpler and took less time to perform, and because anionic surfactants were not important in this extraction. Following extraction, the dried extract was reconstituted in 1 mL of methanol and 200 μL of this solution was mixed with 40 μL of DHB solution (100 mg/mL) and 10 μL of lithium chloride solution (10 mg/mL).

Analysis of the resulting extract by MALDI-TOF did not produce any recognisable signals for NPEO. Instead, the spectrum (Figure 5.21) exhibited an envelope of peaks representing $[M+Na]^+$ for Triton X-100 internal standard. This spectrum highlights two obvious problems; no observable signal for NPEOs and the formation of sodium rather than lithium adducts of the Triton X-100.

The formation of sodium adducts is not so surprising when considering the matrix from which the samples were collected. The high concentration of sodium chloride in seawater must be responsible for the formation of sodiated rather than lithiated species. The fact that the sodium adduct is formed rather than the lithium adduct is surprising considering the large concentration (10 mg/mL) of lithium chloride added to the solution before crystallisation. Perhaps this type of surfactant has a greater affinity for sodium than it does for lithium.

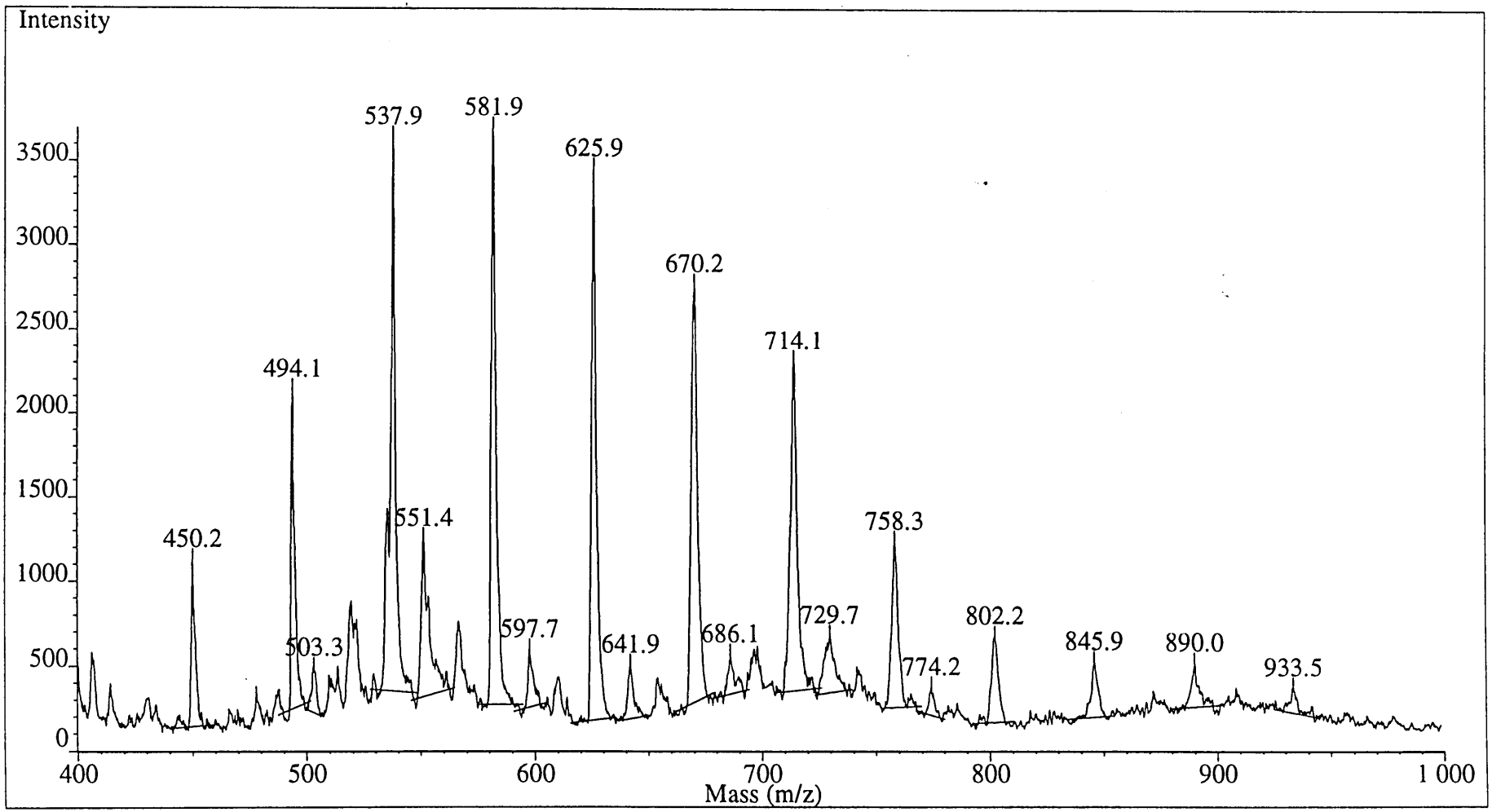


Figure 5.21 MALDI-TOF spectrum of an extracted seawater sample spiked with Triton X-100 as an internal standard

The absence of peaks for NPEO is probably due to their level in the sample being lower than the limit of detection of the method. It may also be a result of the high concentration of Triton X-100 added which has the affect of suppressing any signal due to NPEOs. This phenomenon of signal suppression has been observed in work by Gusev *et al.* [28] during an investigation into the quantitative analysis of peptides using MALDI-TOF mass spectrometry. In their paper, they reported a reduction in linearity at the higher end of the calibration curve with respect to analyte concentration. In other words, the signal due to the internal standard was suppressed at relatively higher concentrations (with respect to the concentration of internal standard) of analyte. This effect was attributed to two factors; when high analyte concentrations of analyte were used the (internal standard + analyte)/matrix molar ratio was relatively low, below the optimum level which could lead to degradation of signal. Another reason for the effect could be that although the internal standard and analyte were both chemically similar, the large differences in concentration may have caused differences in the crystallisation process and incorporation of analyte and internal standard into the matrix crystal structure. Either or both of these explanations may be the cause of the failure to observe NPEO in the spectrum.

However, repeating the experiment without the addition of Triton X-100 as internal standard, produced a spectrum without any peaks for NPEO. This result indicates that signal suppression by the internal standard was not responsible for the absence of analyte peaks.

It is possible that the solid phase extraction method was unable to extract NPEO from the matrix in the presence of OPEO. To investigate this possibility, a 250 mL sample of seawater was spiked with Synperonic NP9 (200 μg/L) and Triton X-100 (188

μg/L) which would give concentrations of 50 mg/L and 47.1 mg/L, respectively, after extraction / preconcentration.

The C_{18} functionalised silica used in the solid phase cartridges has the potential to extract many different organic species from surface water. Triton X-100 and various organic substances extracted from water with the surfactants may bind to all of the active sites on the cartridge, leaving no sites left to retain the NPEO in the sample. This would especially be true if the C_{18} phase has a greater affinity for OPEO than for NPEO. However, the resultant spectrum (Figure 5.22) of the sample spiked with both Triton X-100 and Synperonic NP9 proves that this is not the case. The spectrum shows typical envelopes of peaks for both nonylphenol and octylphenol ethoxylates; again each peak represents the $[M+Na]^+$ adduct of each ethoxymer.

The limit of detection for the method was found to be 100 μg/L for NPEO (Figure 5.23). Therefore the concentration of NPEO in the samples obtained from Langstone Harbour would appear to be <100 μg/L based on a 250 mL sample.

As noted previously, spectra of extracted seawater samples exhibited $[M+Na]^+$ adducts despite the addition of a large amount of lithium chloride to the sample before crystallisation on the target. The reason for the addition of an excess of lithium ions during sample preparation was to allow the resolution of octylphenol and nonylphenol ethoxylates without the complication of a mixture of sodium and potassium adducts. However, due to the large amount of sodium ions present in seawater, only sodium adducts were observed rather than lithium adducts. This is not a problem as quantification can take place using the sodium adducts.

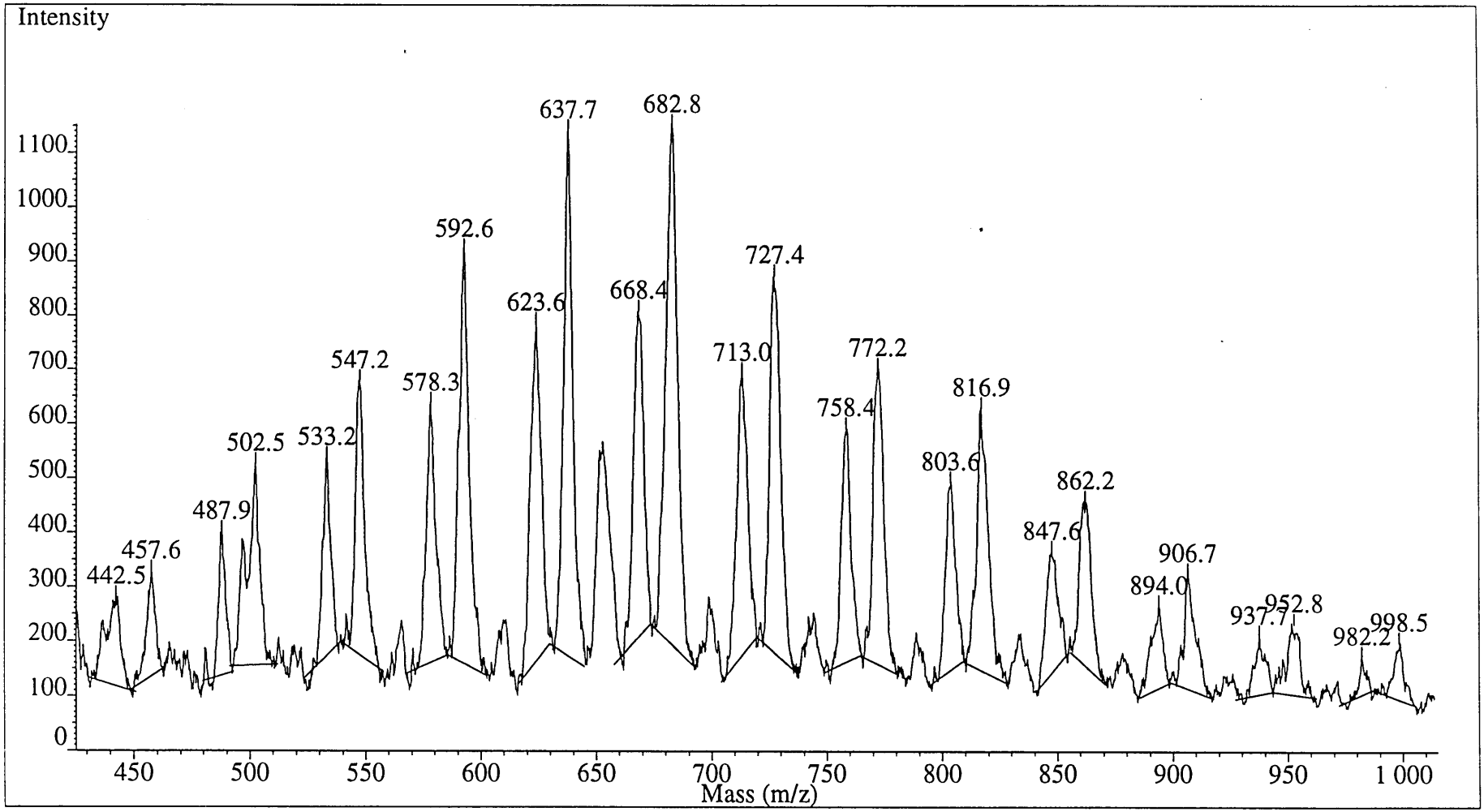


Figure 5.22 MALDI-TOF spectrum of an extracted seawater sample spiked with Triton X-100 (188 µg/L) and Synperonic NP9 (200 µg/L)

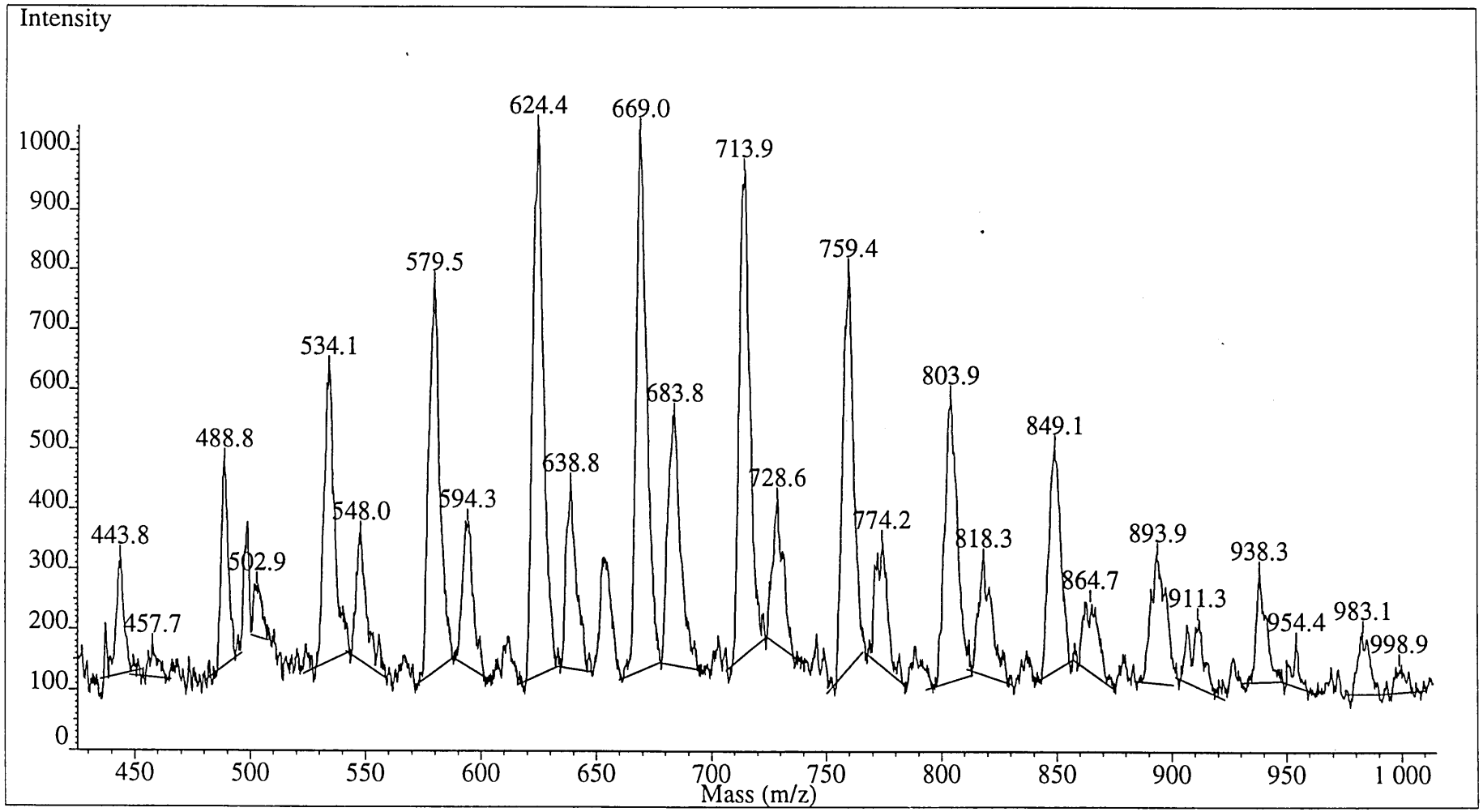


Figure 5.23 MALDI-TOF spectrum representing the limit of detection for the method of 100 µg/L NPEO

5.8.12 Addition of a Large Excess of Lithium to Seawater Extracts

Although the formation of sodium adducts would still enable quantification to be performed, there remains some uncertainty in differentiating the $[M+K]^+$ adduct for OPEO from the $[M+Na]^+$ adduct for the corresponding NPEO, as there is only a mass difference of two between the two species. The formation of solely lithium adducts would make identification more certain; therefore experiments were carried out using different concentrations of lithium chloride.

A 250 mL water sample from Langstone Harbour was spiked with Triton X-100 and extracted as described before. 10 μL of lithium chloride solution were added during sample preparation at concentrations of 100, 200 and 500 mg/mL.

Previous spectra obtained using lithium chloride at a concentration of 10 mg/mL show intense peaks for the sodium adducts of the internal standard and much smaller peaks representing the potassium adducts. The addition of 10 μL of a 100 mg/mL solution of lithium chloride (Figure 5.24) produced two peaks of roughly the same intensity for each ethoxymer. These correspond to the lithium and sodium adducts for OPEO. The addition of a 200 mg/mL solution of lithium chloride (Figure 5.25) produced more intense lithium adducts, but the sodiated species are still present. Figure 5.26 shows the results of the addition of a 500 mg/mL solution of lithium chloride. In this spectrum the only peaks present are the lithium adducts of Triton X-100. Although addition of lithium chloride at 500 mg/mL suppressed totally the formation of sodium and potassium species, this large excess of lithium has caused a corresponding decrease in spectral quality. Another interesting point is the fact that as lithium chloride concentration was increased, so did the intensity of the peak at m/z 487, the origin of which is unknown at the time of writing.

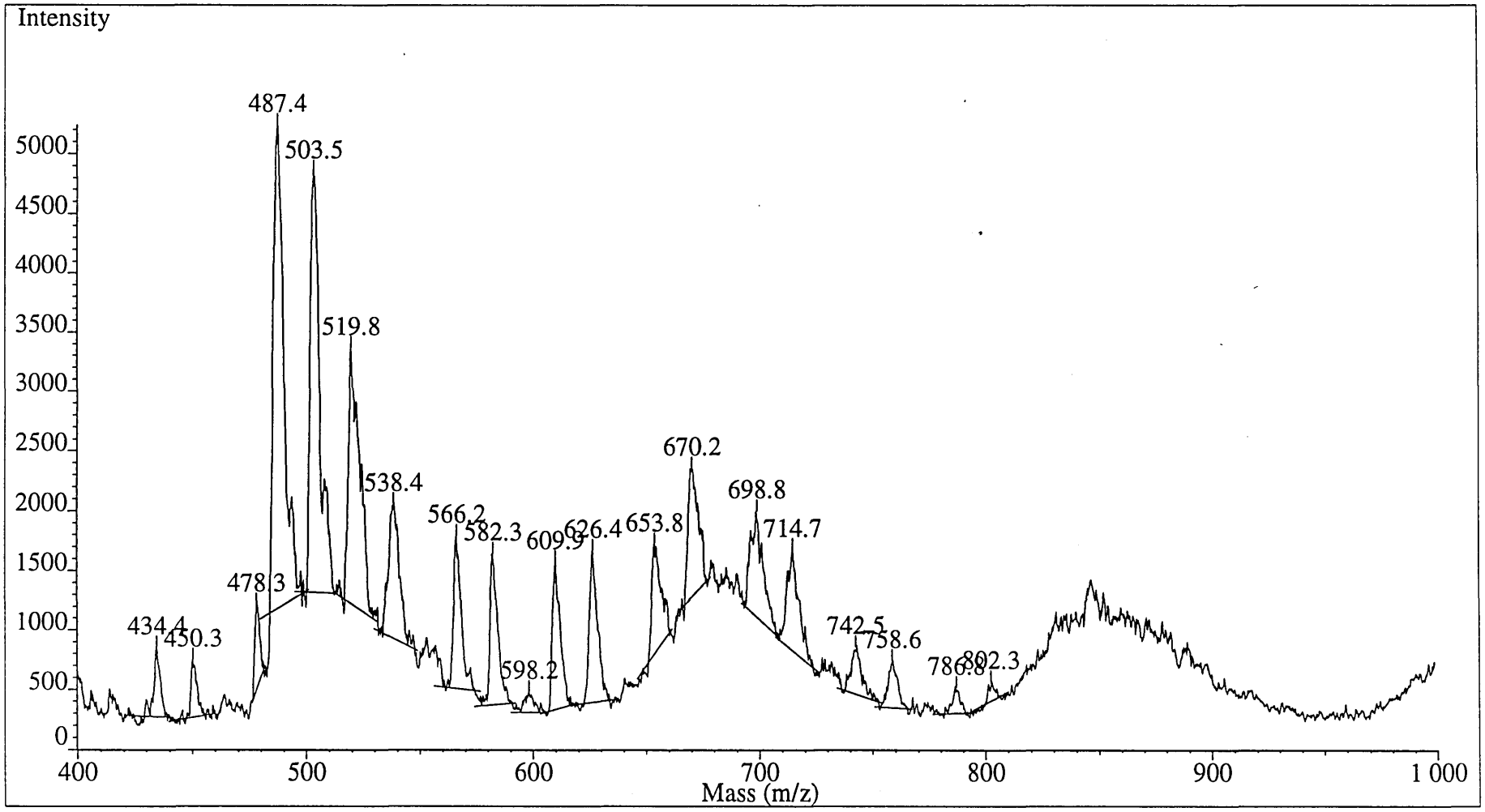


Figure 5.24 MALDI-TOF spectrum of a seawater sample spiked with Triton X-100 and 10 μL of a 100 mg/L LiCl solution

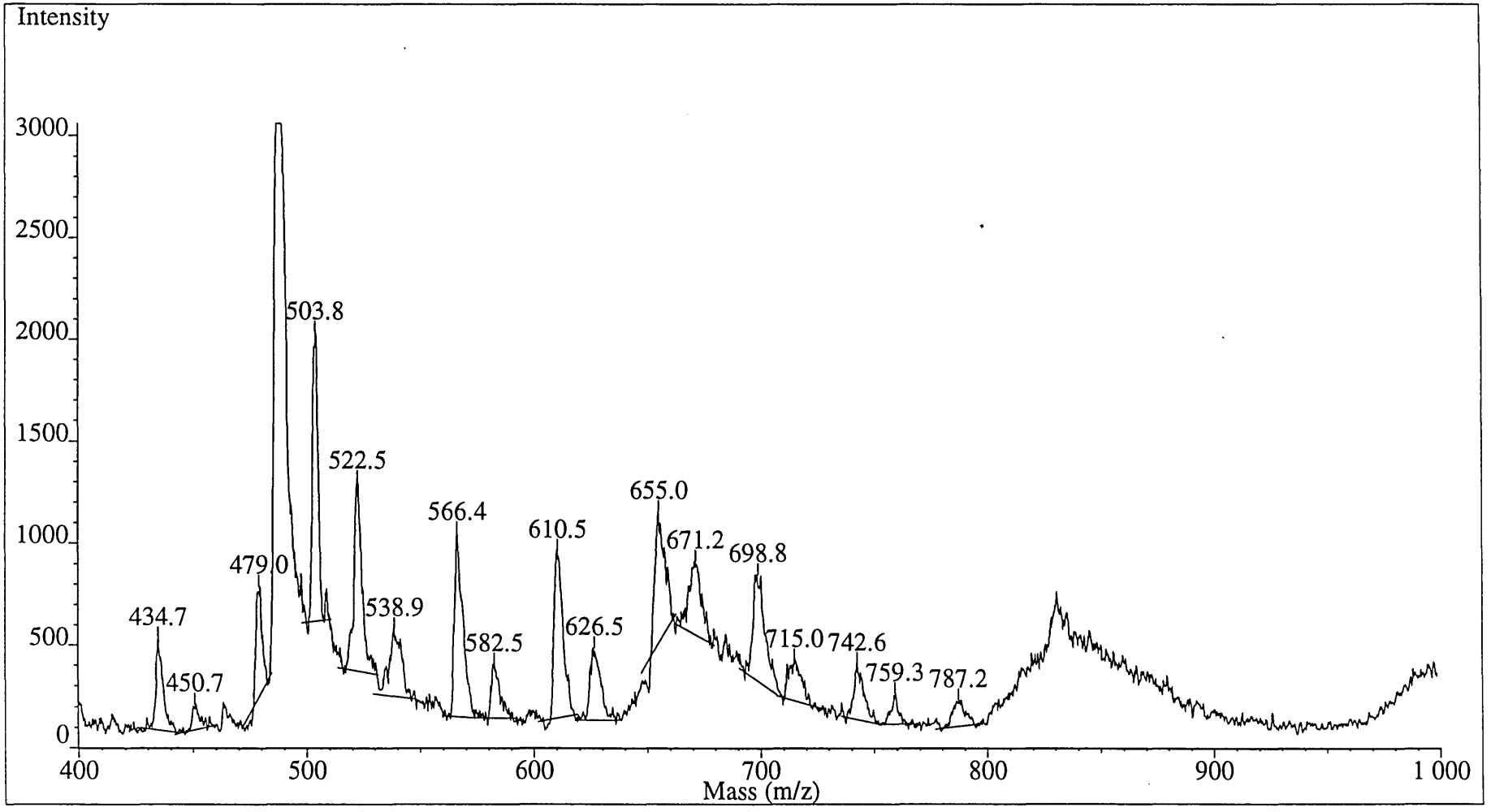


Figure 5.25 MALDI-TOF spectrum of a seawater sample spiked with Triton X-100 and 10 μL of a 200 mg/L LiCl solution

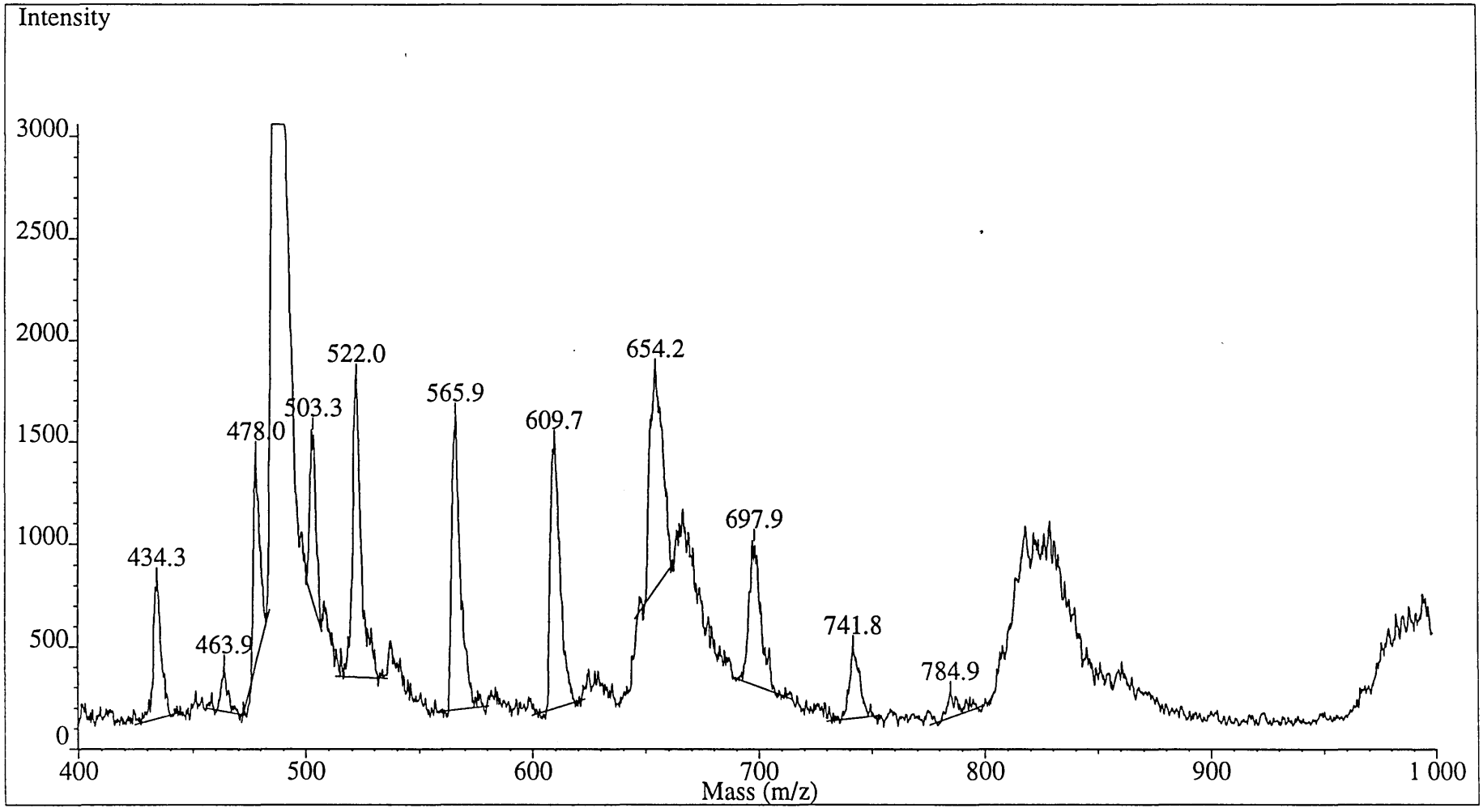


Figure 5.26 MALDI-TOF spectrum of a seawater sample spiked with Triton X-100 and 10 µL of a 500 mg/L LiCl solution

5.8.13 Analysis of River Water Samples by MALDI-TOF

The concentration of NPEO in the sea water samples was below the limit of detection for this method (100 μg/mL); however, initial work on extracts from the River Don showed levels of NPEOs that were detectable by MALDI-TOF. Therefore, more samples from the River Don were collected and extracted.

Unlike previous samples obtained from the River Don, analysis of these samples by MALDI-TOF did not seem to show any peaks for NPEO (Figure 5.27). The peaks in the spectrum would appear to correspond to the lithium, sodium, and in some cases, potassium adducts for the internal standard Triton X-100. As mentioned above, it is not always obvious whether a peak is due to an octyl or NPEO adduct. However, repeating the extraction without the addition of internal standard produced a spectrum with no peaks corresponding to NPEO. From these data, it would appear that the new samples from the River Don do not contain as large a concentration of NPEO as samples analysed previously.

800 mL of river water was extracted using the C_{18} SPE method described above; after removal of the solvent the final extract was reconstituted in 100 μL of methanol. This gives an overall concentration factor of 8000, which is thirty-two times greater than that of previous extractions.

Figure 5.28 shows the spectrum of the 800 ml sample from the River Don with the addition of 10 μL of lithium chloride (10 mg/mL). The spectrum contained a lot of noise, but it is possible to distinguish peaks for $[M+Li]^+$ adducts for NPEOs at m/z 580.7, 624.3, 668.6, 710.9, 756.1 and 843.6. However, some or all of these peaks could also be $[M+Na]^+$ adducts for the internal standard, especially as a closer look at the peaks

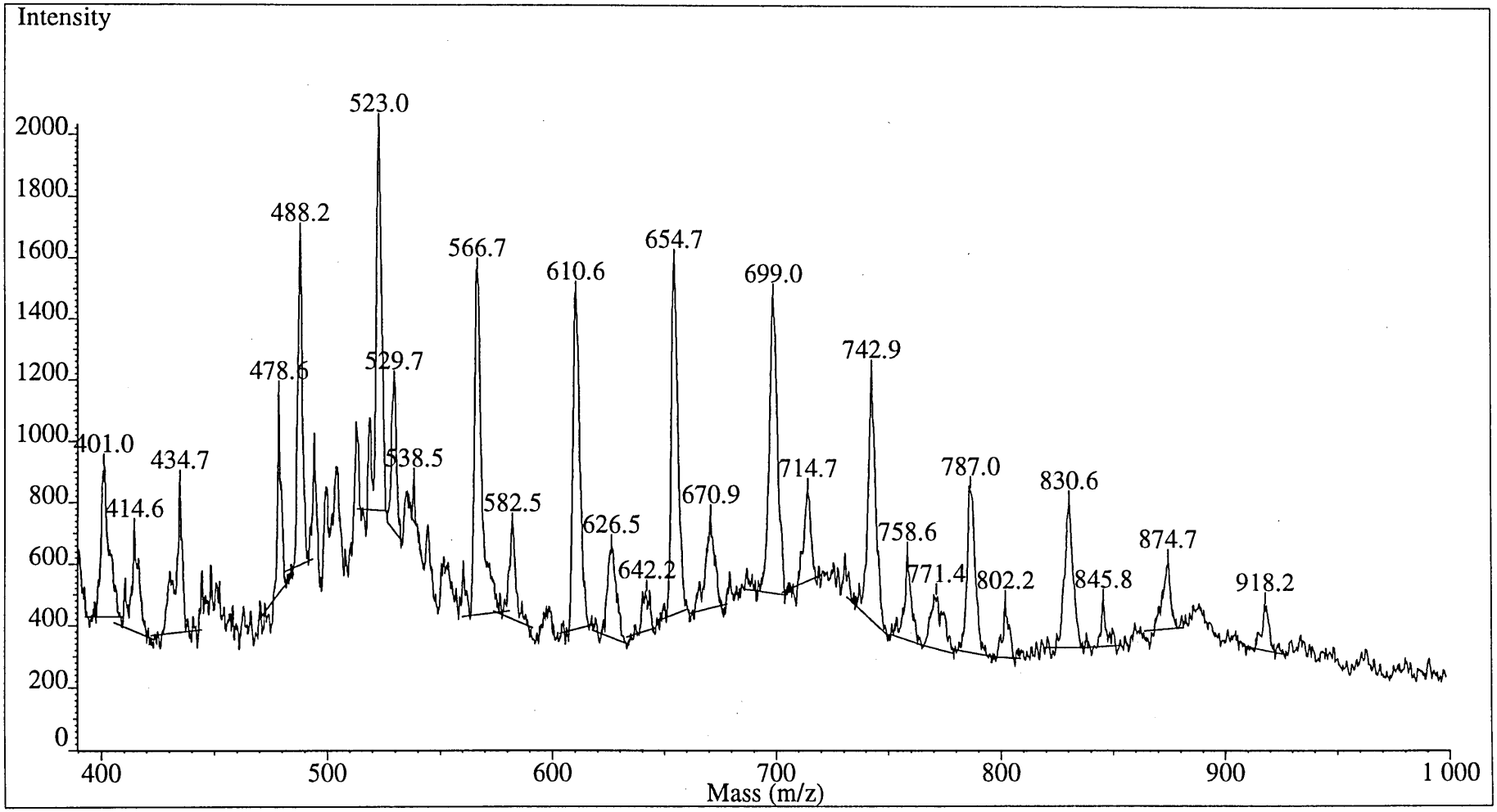


Figure 5.27 MALDI-TOF spectrum of a River Don extract spiked with Triton X-100

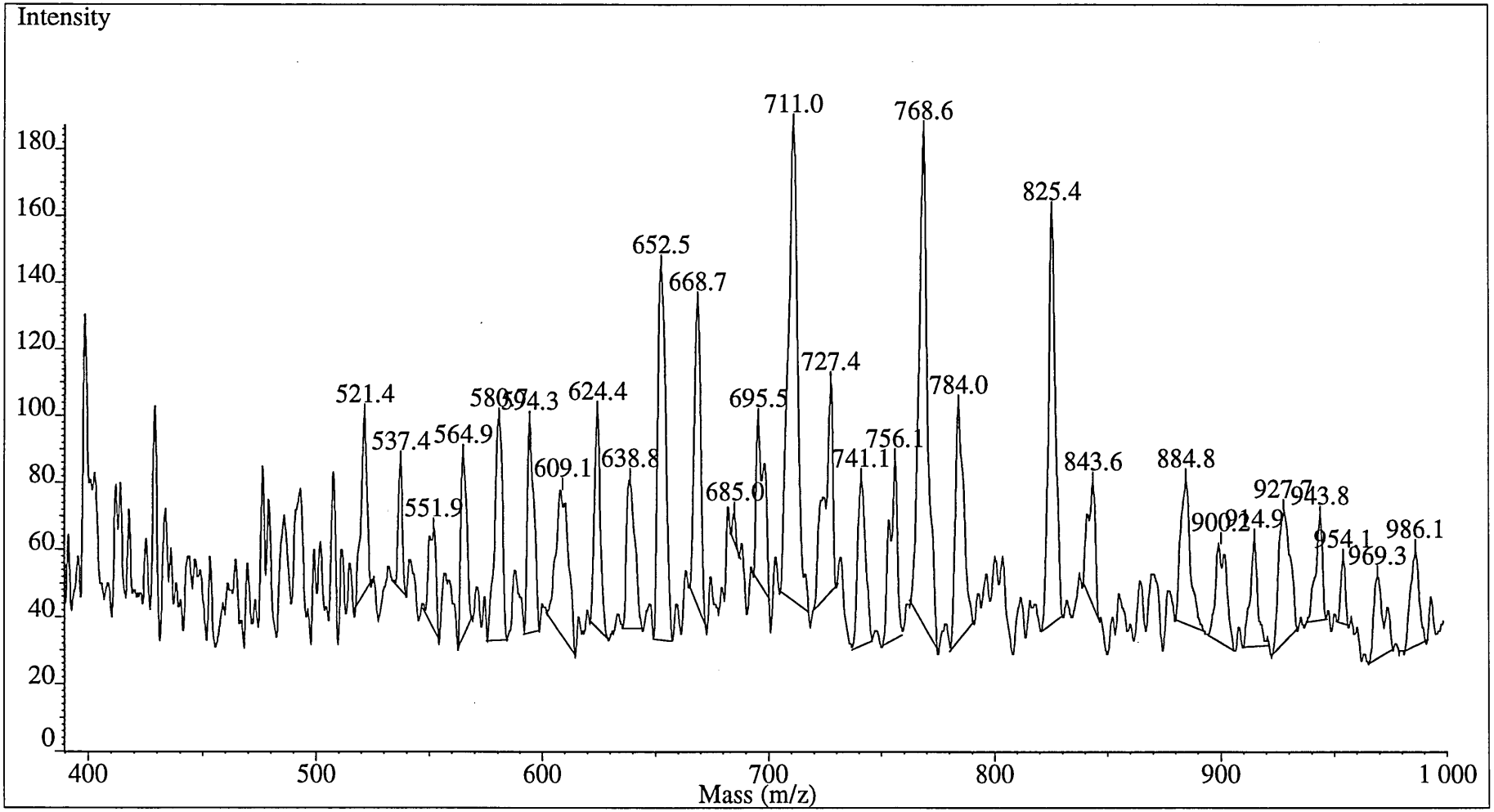


Figure 5.28 MALDI-TOF spectrum of a 800 mL extract of the River Don

suggests that some are composed of more than one peak. $[M+Na]^+$ adducts for NPEOs are present at m/z 594.3, 638.8, 683.0 and 727.5. Lithium adducts for Triton X-100 are present in the spectrum at m/z 521.4, 565.0, 608.8, 695.5, 741.0 and 784.0. Sodium adducts may also be present but, as mentioned above, it is difficult to distinguish these from lithium adducts of NPEO.

5.10 Conclusion

To the author's knowledge, this is the first time that MALDI-TOF mass spectrometry has been used for the qualitative and quantitative analysis of NPEO surfactants from environmental matrices.

Analysis of various formulations of alkylphenol ethoxylate surfactants using the matrix, 2,5-dihydroxybenzoic acid, showed intense sodium and potassium adducts. A similar situation was observed in extracted surface water samples from the River Don. The spectra exhibited an envelope of peaks whose masses corresponded well with those for sodium and, in some cases, potassium adducts of NPEO. However, at the time, no method was available to determine the concentration of these surfactants by MALDI-TOF MS.

Quantitative analysis by MALDI-TOF MS is complicated due to an inherent lack of shot-to-shot reproducibility. This lack of reproducibility is largely due to the non-uniform (and poorly understood) nature of the crystallisation of the matrix/analyte complex, and hence the extremely variable nature of the fine structure of the analyte on the target surface. Therefore, it is often the case that different intensities of analyte will be seen in different areas of an individual crystal. Thus, quantitative measurements by MALDI-TOF require

the use of an internal standard, which is as similar as is possible to the analyte, both chemically and physically. The ideal solution is to use an isotope-labelled version of the analyte itself, which would exhibit almost identical crystallisation properties to that of the analyte. Isotope labelling of NPEO was not considered feasible for this work due to the cost and complexity. The 'next best' solution is to use a structural analogue of the analyte. In this work, OPEO were chosen as an internal standard as they only differ from NPEOs by a methylene unit (14 Da) and therefore exhibit very similar crystallisation properties.

The addition of lithium chloride as a source of lithium ions during sample preparation was shown to produce solely $[M+Li]^+$ adducts, suppressing the formation of sodium and potassium adducts. This produced a much simpler spectrum and allowed the use of OPEO as an internal standard without interference from sodium and potassium adducts. Plots of nonylphenol / OPEO ratio against increasing concentration of NPEO gave good, linear relationships over the ranges 100-500 and 10-50 mg/L NPEO.

Seawater samples (250 mL) from Langstone Harbour in Portsmouth were extracted using a C_{18} solid phase extraction method developed in a previous project [26]. Analysis of the resulting spiked extract by MALDI-TOF MS using the method described above produced a spectrum consisting of solely peaks due to the internal standard; no recognisable signals were observed for NPEO. The peaks due to the internal standard were not the lithiated species expected from the addition of lithium chloride during sample preparation; instead sodium adducts were observed. The formation of sodium adducts was attributed to the high concentration of sodium chloride that is naturally present in seawater. The reason for the preferential formation of sodium adducts over lithium adducts, despite the addition of a large concentration of lithium ions, was not

clear. It is possible that these ethoxylated surfactants have a greater affinity for sodium than they do for lithium. Experiments carried out using an even larger excess of lithium chloride showed that solely lithiated species were observed at a lithium chloride concentration of 500 mg/mL. However, this was accompanied by a corresponding reduction in the quality of the spectra produced.

As surface water samples obtained from the River Don in previous work showed MALDI-TOF spectra containing characteristic envelopes of peaks for NPEO further samples from the river were collected. The spectra of the resulting extracts did not show peaks corresponding to NPEO; instead, as with the seawater samples, only signals due to the internal standard were present in the spectrum. Unlike the seawater samples, however, peaks due to the internal standard were present as their lithium, sodium and in some cases their potassium adducts.

Increasing the concentration factor from 250 to 8000 by using a larger sample volume and reconstituting in a smaller volume of methanol created a very complicated looking spectrum. The very noisy spectrum appeared to contain peaks representing lithium and sodium adducts of both nonylphenol and OPEO. The identity of some of these peaks was uncertain due to the small mass difference between the lithium adduct of a particular NPEO and the sodium adduct of the corresponding OPEO. The complexity seen in this spectrum and in some of those mentioned above stem from the inability of the LaserTof 1500 to resolve ions of only a few mass units difference. This is particularly the case when considering spectra of 'real' samples that suffer from a considerable amount of noise.

Despite the problems observed in low level concentrations of analyte such as those used

for the majority of this work, to date MALDI-TOF has been shown to be capable of producing spectra of NPEO in more polluted surface water samples such as those samples taken from the River Don in the initial stages of this work. Also, a quantitative method has been developed for the determination of NPEO surfactants in water samples, with good linearity seen over the entire envelope of ethoxymers. Although the MALDI-TOF appears to lack the sensitivity of HPLC; the current standard method for the determination of NPEO in surface water, it does have an important advantage of rapid analysis time. The equivalent HPLC method requires approximate analysis time of one hour per sample compared with five to ten minutes per sample for MALDI-TOF. This method could prove useful for the rapid screening of more polluted samples such as those from sewage treatment plants.

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Chapter 6

Conclusions and Future Work

6.0 Conclusions

The vast quantity of surfactants that are used today make them major environmental pollutants. Of the four classes of surfactant used, anionic and non-ionic are the most environmentally significant, as these are more widely used than the cationic and amphoteric type.

The non-ionic surfactant nonylphenol ethoxylate (NPEO) and its biodegradation products have been shown in the literature to be weakly oestrogenic in nature. NPEOs along with other pollutants such as DDT, bisphenol A and tributyltin chloride are thought to disrupt the endocrine systems of a wide variety of animals and fish, and may be related to recent problems in human reproductive health. While NPEOs have been largely replaced by alcohol ethoxylates, which are thought to be less damaging to the environment, NPEOs still find use in some industrial cleaning processes, such as in the woollen industry, and hence still find their way into the environment.

Work by a previous Ph.D. student involved the development of a HPLC method for the simultaneous determination of linear alkylbenzene sulphonates (LAS) and alkylphenol ethoxylate surfactants in surface water [1]. However, following clean up / preconcentration using C_{18} solid phase extraction cartridges, the resulting chromatograms showed a large anionic interference that co-eluted with, and masked any LAS that may have been present in the sample.

In the work described in this thesis, an attempt was made to remove this anionic species that is now thought to be humic acids. A different extraction procedure was investigated to determine whether the humic acids could be fractionated from the LAS. The method from the literature which was employed [2] used graphitised carbon black (GCB) SPE

cartridges. While recovery results showed the method to be slightly better than the C_{18} method [1], the resulting chromatograms still showed a large peak eluting at the same time as LAS.

Therefore, a phase-switching method was developed to try to separate the LAS and humic acids chromatographically. The phase-switching set up allowed the LAS / humic acid portion to be directed to a C_{18} column following elution from the C_1 column. The alkylphenol ethoxylates were allowed to separate as usual, and then the C_1 column was removed from the flow and the humic acids were separated from the LAS on the C_{18} column. While this method worked well with standards, the results from an extracted river water sample were very inconclusive. The identities of the peaks observed in this chromatogram were not obvious and it was decided that LC-MS would be needed to characterise the peaks properly; the instrumentation for this was not available at the time.

During this work, another problem occurred involving the ability of new C_1 columns to adequately resolve the NPEO ethoxymers. The original method was developed on a Spherisorb S5C1 column obtained from Hichrom Ltd. During the development of the method, and in subsequent work, it was shown that C_1 columns from Supelco and Hypersil were unable to attain the same resolution of ethoxymers as produced with the Spherisorb column. However, later batches of the Spherisorb column were also unable to produce the same resolution as that observed in earlier batches.

This problem was investigated using elemental analysis and x-ray photoelectron spectroscopy to determine the percentage bulk and surface carbon, respectively, of the column. Bulk and surface carbon results revealed that the column which provided the best resolution of NPEO ethoxymers had the lowest surface carbon coverage. This

suggested that the resolution of the ethoxymers was effected by the remaining surface hydroxyls on the surface of the silica, whereas the presence of the trimethylsilyl group actually hindered the separation. This conclusion was supported by the fact that a method has been published that described the resolution of NPEO ethoxymers on a Spherisorb silica column [3]. The authors of this work also found that only Spherisorb silica was able to achieve adequate resolution.

Separation is based on increasing ethylene oxide chain length; the species with the shortest ethoxy chain elutes first from the column. If increasing hydrophilicity is taken to be equivalent to increasing polarity, then the fact that the most hydrophilic species eluted last, along with the evidence that the separation worked best on the column with the least carbon, showed that the separation might follow an adsorption or normal phase mechanism. However, increasing the organic component of the mobile phase decreased the retention time, which is typical of a reverse phase or partition mechanism. Therefore, the resolution of NPEOs on a C_1 column showed evidence of both normal and reverse phase mechanisms. It is important to note that the trimethylsilyl moiety is essential for the separation of the LAS homologues and therefore essential to the simultaneous nature of the method.

A new method was developed for the qualitative and quantitative determination of NPEO surfactants in surface waters by matrix-assisted laser desorption / ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Samples were mixed with a concentrated solution of either 2,5-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxycinamic acid (HCCA) as a matrix. Approximately 1 μL of this solution was applied to a stainless steel target and the solvent was allowed to evaporate, leaving matrix / analyte crystals. Analysis of the resulting crystals showed spectra of intense

$[M+Na]^+$ and $[M+K]^+$ adducts for NPEOs. Analysis of surface water samples from the River Don in South Yorkshire also produced characteristic spectra of NPEOs, with peaks corresponding to sodium and potassium adducts. While these were excellent results, showing the method to be very capable of the qualitative determination of alkylphenol ethoxylate surfactants in surface water, there was no quantitative aspect to the results.

The lack of shot-to-shot reproducibility inherent to MALDI-TOF MS makes quantitative determination difficult. As the surfactant octylphenol ethoxylate differs from NPEO by a single methylene unit (14 Da) it was decided that this would be an ideal candidate for an internal standard, as it would provide a reference peak for each analyte peak. The internal standard was added either before extraction or with the matrix; and a concentrated solution of lithium chloride was also added before crystallisation as a source of lithium ions. The addition of lithium ions led solely to the formation of $[M+Li]^+$ adducts, and created much cleaner, less complicated spectra essential for inclusion of the internal standard. Excellent linear relationships were achieved down to 10 mg/L NPEO (equivalent 200 μg/L before extraction). However, spectra of extracted samples from Langstone Harbour in Portsmouth and the River Don did not show any signals corresponding to NPEOs. The levels of NPEOs in these new samples must have been below the limit of detection for this method (100 μg/L). The extraction of a large quantity of water from the River Don produced very noisy spectra that contained many peaks, some of which could possibly, but not definitely, be attributed to NPEOs. Peaks for NPEOs were observed in spiked surface water samples indicating that the method was capable of measuring these surfactants in surface water at higher concentrations. This method could prove useful for the rapid screening of more polluted environments

such as sewage treatment plants as its analysis time of approximately ten minutes per sample compares well with the established HPLC method which has analysis times of more than thirty minutes.

6.1 Future Work

It would be interesting to repeat the phase-switching method but instead coupling the system to a mass spectrometer via an electrospray or APCI interface. It might then be possible to determine whether or not the peaks in the latter half of the chromatogram are due to LAS in the sample. However, the use of a mass spectrometer for detection and identification of unknown components of the chromatogram would add a large amount of complexity and cost to the method.

In order to investigate the retention of NPEO on C_1 stationary phases further it would be interesting to pack some columns with Spherisorb silica containing different surface concentrations of the trimethylsilyl moiety. Using XPS it would then be possible to determine the optimum concentration needed for effective resolution of NPEO ethoxymers and LAS homologues.

The MALDI method could be extended by trying to lower the limit of detection, possibly by further investigation of the optimum matrix / analyte ratio. It would also be interesting to analyse some samples that are known to be more polluted with NPEOs such the influent and effluent from sewage treatment plants. The method could also be extended to the determination of linear alkylbenzene sulphonates and possibly even biodegradation intermediates of both non-ionic and anionic surfactants. However, the low molecular weight of these molecules may mean they are subject to interference from matrix ions.

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Appendix 1. Table of Masses Used in Chapter 5.

NPEO

EO	M	M+Li	M+Na	M+K
1	264	271	287	303
2	308	315	331	347
3	352	359	375	391
4	396	403	419	435
5	440	447	463	479
6	484	491	507	523
7	528	535	551	567
8	572	579	595	611
9	616	623	639	655
10	660	667	683	699
11	704	711	727	743
12	748	755	771	787
13	792	799	815	831
14	836	843	859	875
15	880	887	903	919
16	924	931	947	963
17	968	975	991	1007
18	1012	1019	1035	1051
19	1056	1063	1079	1095
20	1100	1107	1123	1139

OPEO

EO	M	M+Li	M+Na	M+K
1	250	257	273	289
2	294	301	317	333
3	338	345	361	377
4	382	389	405	421
5	426	433	449	465
6	470	477	493	509
7	514	521	537	553
8	558	565	581	597
9	602	609	625	641
10	646	653	669	685
11	690	697	713	729
12	734	741	757	773
13	778	785	801	817
14	822	829	845	861
15	866	873	889	905
16	910	917	933	949
17	954	961	977	993
18	998	1005	1021	1037
19	1042	1049	1065	1081
20	1086	1093	1109	1125

Appendix 2. Meetings and Symposia Attended.

April 1996	**Environmental Analytical Chemistry**, Vienna, Austria Poster Presentation
Sept 1996	**Royal Society of Chemistry R and D Topics**, Nottingham Trent University Poster Presentation
Mar 1997	**Oestrogenic Substances in the Environment**, Royal Society of Chemistry, London
Sept 1997	**BMSS - Environmental Mass Spectrometry Special Interest Group**, University of Salford
April 1998	**Three-day Laboratory Accreditation Course.**
Sept 1998	**BMSS – Annual Conference**, University of Warwick Poster Presentation

Appendix 3. Paper Published in Rapid Communications in Mass Spectrometry

Willetts M, Clench MR, Greenwood R, Mills G and Carolan V. *Rapid Commun. Mass Spectrom.* 13 (1999) 251.

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. **13**, 251–255 (1999)

The Determination of Non-ionic Surfactants in Surface Waters by Matrix-assisted Laser Desorption/Ionisation Time-of-flight Mass Spectrometry

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Matrix-assisted time-of-flight mass spectrometry (MALDI-TOFMS) has been shown to be a useful tool for the analysis of a wide range of biological and synthetic polymers. This has included the analysis of some commercial formulations of some non-ionic surfactants. But, as yet, no one has reported the use of MALDI-TOFMS for the analysis of surfactants in environmental matrices. Here we report the use of MALDI-TOFMS for the qualitative and quantitative determination of nonylphenol ethoxylate surfactants in environmental surface waters. Following extraction/preconcentration by C_{18} solid phase extraction, the sample is mixed with a standard MALDI matrix (2,5-dihydroxybenzoic acid). The mixture is then crystallised on a stainless steel target for introduction into the mass spectrometer. The limit of detection is 40 μg/L based on a 250 mL sample.

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Surfactants are a widely used group of chemicals, both industrially and domestically. Hence, they have become ubiquitous in the environment. Present worldwide surfactant usage is approximately 10 million tonnes per year.[1] There are three main classes of surfactant: anionic, such as linear alkylphenol sulphonates (LAS); non-ionic such as alkylphenol ethoxylates (APEO); and cationic surfactants, such as quaternary ammonium salts.

Recent work by Sumpter *et al.*[2] has shown that APEOs and their associated degradation products are weakly oestrogenic in nature. This work stemmed from results published by Soto[3] which showed that nonylphenol (a biodegradation product of nonylphenol ethoxylates) demonstrates an oestrogenic response with breast cancer cells. Furthermore, Sharpe *et al.*[4,5] have linked these compounds, along with other environmental pollutants, to the apparent decrease in sperm production and an increase in sexual reproductive problems observed throughout the Western Hemisphere.

Identification and quantification of surfactants is usually performed by gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC). The work by Stephanou[6] typifies the current GC/MS methodology. A variety of HPLC methods have been published.[7–9] Both normal and reversed-phase separations have been used in conjunction with ultraviolet (UV), fluorescence and mass spectrometry detection. The above methods have been used, with cleanup and preconcentration steps such as solid-phase (SPE) and Soxhlet extraction, for the determination of anionic and non-ionic surfactants in a variety of water and sewage systems.

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Only a few papers have been published on the MALDI-MS of surfactants. Just and co-workers[10] compared the use of MALDI with supercritical fluid chromatography (SFC) for molar mass determination of some APEO surfactants. They produced good MALDI spectra of these surfactants using 2,5-dihydroxybenzoic acid (DHB) as the matrix. Spectra of the APEOs showed a mixture of $[M + Na]^+$ and $[M + K]^+$ adducts. Direct comparison between SFC and MALDI-MS proved that the latter provided much better differentiation in the higher mass range but seemed to cause some discrimination in the lower mass region, with lower intensities than expected observed for lower molecular weight ethoxymers.

Thompson *et al.*[11] produced MALDI mass spectra for three classes of surfactant – non-ionic, anionic and cationic. The anionic surfactants sodium dodecylsulphate (SDS) and sodium dodecylbenzene sulphonate were successfully analysed to produce negative ion spectra, either simply as an aqueous solution allowed to dry on the metal probe, as in the case of SDS, or dispersed in ethylene bis[3-(2-naphthyl)acylate], as in the case of the latter anionic surfactant. Cationic surfactants, cetyldimethylethyl ammonium bromide, cetylpyridinium chloride and benzalkonium chloride produced good quality, intense spectra either neat or dispersed in DHB. Good spectra of the NPEO surfactants, IGEPAL CO-850, CO-880 and CO-890, were produced as their sodium adducts by dispersal in DHB with NaCl added as a source of cations.

Parees *et al.*[12] have briefly compared electrospray, fast atom bombardment (FAB) and MALDI ionisation techniques for the analysis of some commercial nonylphenol ethoxylate (NPEO) surfactants. Their findings seem to indicate that the data obtained from the two methods are comparable for lower average molecular weight surfactant mixtures. However, at higher average molecular weights,

CCC 0951–4198/99/040251–05 $17.50

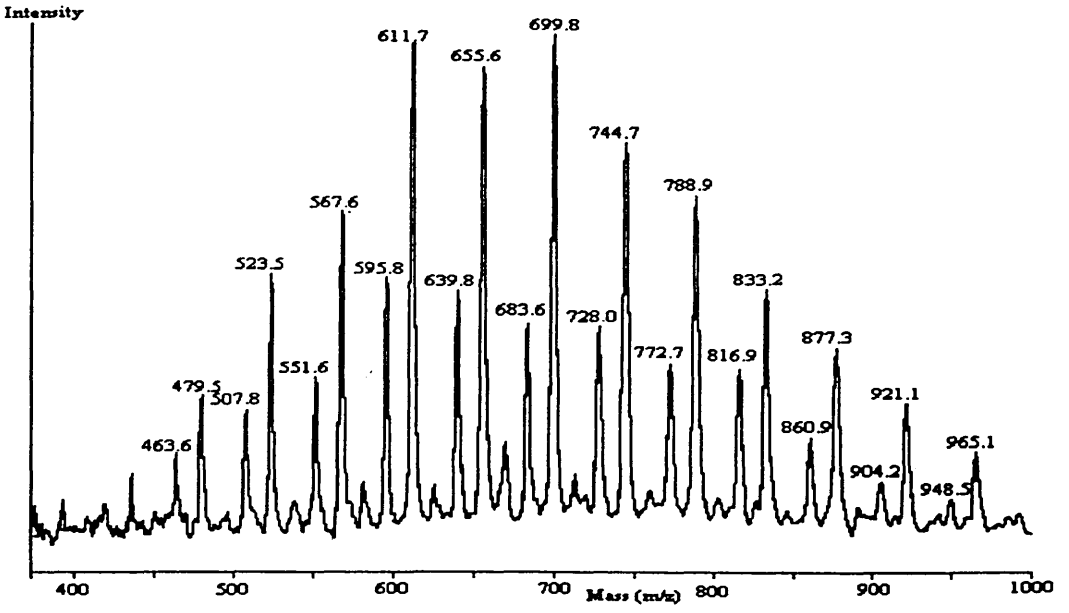

Figure 1. MALDI-TOF mass spectrum of a 100 mg/L Synperonic NP9 standard without the addition of LiCl.

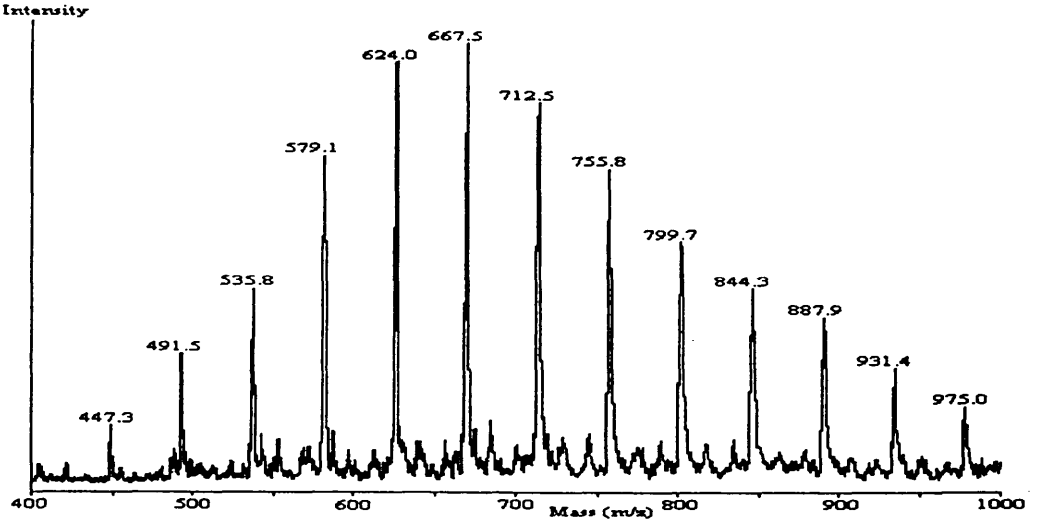

Figure 2. Spectrum of a 100 mg/L Synperonic NP9 standard with the addition of LiCl.

although the electrospray and MALDI results were similar, the FAB data exhibited distinctly lower molecular weight distributions than electrospray and MALDI, possibly due to fragmentation.

Another comparison has been made between MALDI, reversed-phase HPLC and thin-layer chromatography (TLC) for the analysis of non-ionic surfactants. In this work Cumme and co-workers[13] compared the molecular weight information given by MALDI, TLC (using mass spectrometry to analyse the fractions) and HPLC. The MALDI spectra were obtained using DHB as the matrix, and the HPLC analysis was performed using a C_{18} column with an isocratic iospropanol/water (45:55) mobile phase containing 0.1% trifluoroacetic acid (TFA). The results from these experiments did not show any major discrepancy between the average molecular weight found by HPLC and TLC with that found by MALDI. However, the authors[13] state that for MALDI, ethoxymers with masses below 405 Da were not included in the results because of interferences from matrix ions.

Here we present, for the first time to our knowledge, a quantitative method for the determination of non-ionic surfactants in surface waters by MALDI-TOFMS. Internal standard calibration is achieved by using octylphenol ethoxylate as the internal standard.

EXPERIMENTAL

Materials and Instrumentation

MALDI matrix 2,5-dihydroxybenzioc acid (Aldrich, Dorset, UK) was used as received. All solvents used were HPLC grade (Fischer, UK). Bakerbond light octadecyl SPE cartridges were obtained from J. T. Baker (Phillipsburg, NJ, USA).

All MALDI-TOFMS experiments were performed using

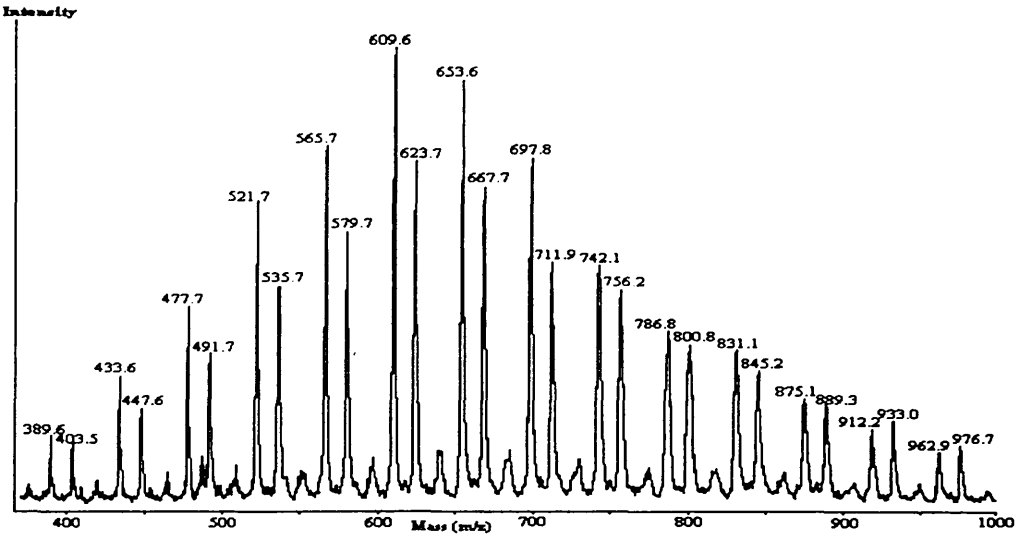

Figure 3. Spectrum of a mixture of Synperonic NP9 (100 mg/L) and Triton X-100 (100 mg/L) with the addition of LiCl.

a LaserTof 1500 instrument (SAI, Manchester, UK) which uses a N_2 laser at 337 nm. Experiments were carried out using a 20 kV extraction voltage. All spectra were the result of the cummulative acquisition of 32 shots.

Standards

Synperonic NP9, a NPEO surfactant with an average ethoxymer distribution of 9, was a gift from ICI Materials Research Centre (Wilton, UK). Triton X-100, an octylphenol ethoxylate surfactant with an average ethoxymer distribution of 9, was obtained from Aldrich (Dorset, UK).

Samples

Sea water grab samples were obtained from Langstone Harbour, Portsmouth, UK. Prior to extraction all samples were stored in polyethylene bottles at +4 °C using 1% formaldehyde as a preservative.

Extraction

Prior to extraction Triton X-100 (octylphenol ethoxylate) (114.4 μg/L) was added as internal standard. Extraction/preconcentration was then carried out by SPE using C_{18} cartridges based on the method described by Scullion *et al.*[14] The cartridges were first conditioned with methanol (7 mL) followed by water (7 mL). The sample was then passed slowly through the cartridge; after the sample stage the cartridge was washed with water/methanol (70:30) (12 mL). Elution was carried out with methanol (5 mL). Extracts were then evaporated to dryness under a steady stream of N_2 and redissolved in 1 mL of methanol.

MALDI Sample Preparation

Standards were dissolved in methanol. DHB (90 mg/mL) was dissolved in 0.1% TFA in methanol. LiCl (10 mg/mL) was dissolved in methanol. For analysis 200 μL of sample or standard, 40 μL of matrix and 10 μL of LiCl were mixed and ~1 μL of the resulting solution deposited on the stainless steel target.

RESULTS AND DISCUSSION

Figure 1 shows a spectrum of a 100 mg/L Synperonic NP9 standard without the addition of LiCl. The spectrum shows the characteristic envelope of peaks, typical for non-ionic ethoxylate surfactants, over the range *m/z* 400–1000. This includes the ethoxymers NP4EO to NP17EO. Ethoxymers below this range were masked by matrix interferences. The spectrum exhibits intense $[M+K]^+$ and $[M+Na]^+$ adducts. These adducts are probably formed from sodium and

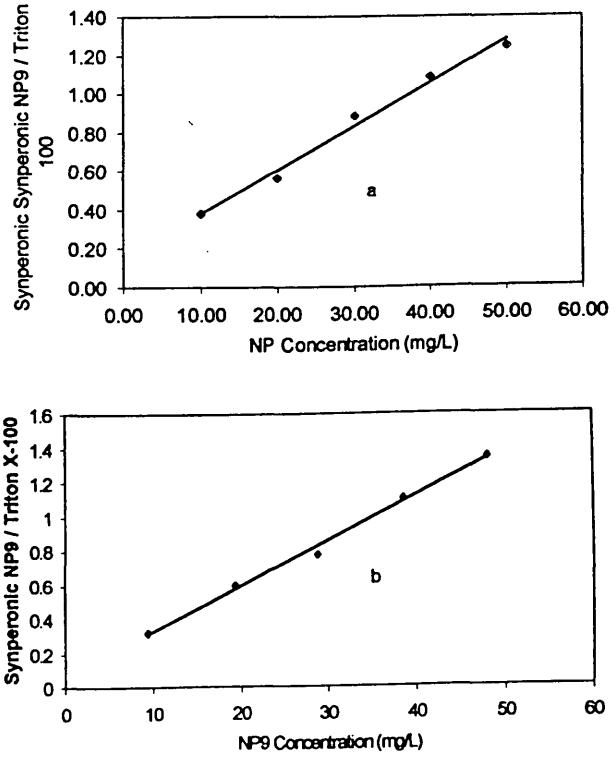

Figure 4. (a) Standard curve for Synperonic NP9 concentration against Synperonic NP9/Triton X-100 ratio ($R^2 = 0.99$); (b) Example of a calibration curve of Synperonic NP9 concentration against Synperonic NP9/Triton X-100 ratio for the NP5EO ethoxymer.

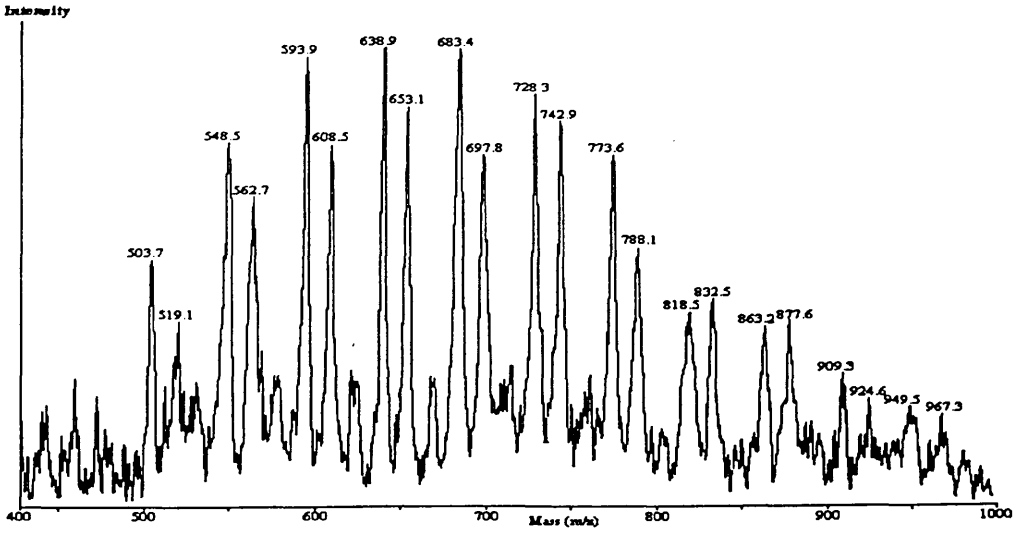

Figure 5. Spectrum showing spike recovery at 200 µg/L Synperonic NP9, in a sample from Langstone Harbour, with 28.6 mg/L Triton X-100 internal standard.

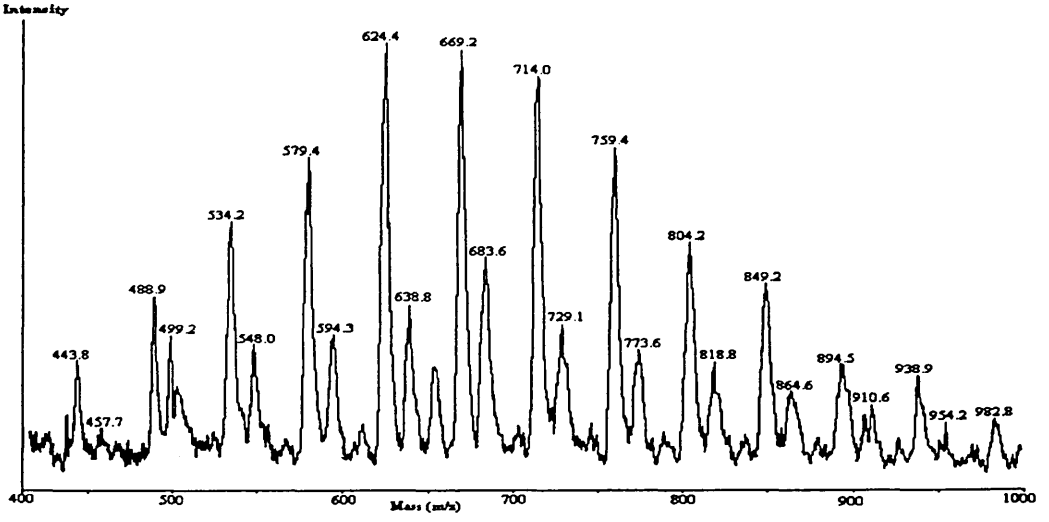

Figure 6. Spectrum of 80 µg/L Synperonic NP9 spike, the limit of detection.

potassium contamination of the matrix or standard. The appearance of both sodium and potassium adducts leads to an over-complicated spectrum, and makes the calculation of signal intensities due to each ethoxymer particularly difficult.

Figure 2 shows a spectrum of a 100 mg/L Synperonic NP9 standard with the addition of LiCl. The addition of an excess of Li^+ ions (in the form of LiCl) produces a spectrum containing almost solely $[M + Li]^+$ adducts with no obvious deterioration in peak shape or resolution. This effect of the suppression of sodium and potassium adducts was also observed by Just and co-workers.[10] The appearance of solely $[M + Li]^+$ adducts produces a much less complicated spectrum, and ensures that the area under each peak represents the entire signal due to that particular ethoxymer.

Quantification in MALDI has been addressed by relatively few workers.[15–18] The inherent lack of shot-to-shot reproducibility makes quantitative determinations particularly difficult. This difference is largely due to variation in local matrix/analyte concentration arising from the crystallisation process. Hence, upon irradiation, detector response does not always relate to analyte concentration. To overcome this problem, the use of an internal standard is proposed. A suitable internal standard should possess physical and chemical properties as similar as possible to those of the analyte. The most suitable candidates are stable-isotope-labelled versions of the analyte as described by Duncan *et al*,[17] which have almost identical chemical and physical properties to those of the analyte. Isotopically labelled versions were not available for this work and therefore a 'next-best' solution was chosen. Octylphenol ethoxylates exhibit chemical and physical properties very similar to those of NPEOs, since these structures only differ by one methylene unit. The use of structurally similar analogues for internal standard quantification in MALDI has been reported by several workers.[16–18] A spectrum of a mixture of Triton X-100 (100 mg/L) and Synperonic NP9 (100 mg/L) is shown in Fig. 3. It would seem from these data that the detector response for Triton X-100 is slightly higher than for Synperonic NP9. A plot of Synperonic NP9 concentration against the ratio of peak areas for Synperonic

NP9/Triton X-100 gave a linear curve ($R^2 = 0.99$) over the range 10–50 mg/L (Fig. 4a). Quantification of NPEO surfactants is further complicated by the uncertainty of the ethoxymer distribution in environmental samples. It cannot be assumed that environmental distributions will follow the same gaussian profile exhibited by Synperonic NP9 and Triton X-100 standards. Therefore, quantification must take place on an ethoxymer by ethoxymer basis and the results summed to obtain a value for the total concentration. To achieve this the percentage of each ethoxymer in the standard was calculated by dividing each individual area by the sum of all the areas. Then individual calibration curves were constructed for each ethoxymer in the standard over the range 10–50 mg/L Synperonic NP9. R^2 values ranging from 0.89 to 0.99 (see Fig. 4b) were obtained for these calibration curves.

The extracted sea water samples from Langstone Harbour used in this study did not give recognisable signals for APEOs when analysed by MALDI-TOFMS. This result is not unexpected, as studies[19] have shown levels of APEOs in sea water to be generally less than 40 μg/L. The lowest standard used for calibration (10 mg/L Synperonic NP9) is equivalent to 40μg/L before extraction. Figure 5 shows a spectrum of a sample from Langstone Harbour spiked with 200 μg/L Synperonic NP9 and 28.6 mg/L Triton X-100 internal standard. As can be seen in this data, for marine samples the addition of LiCl at 10 mg/L does not lead to the preferential formation of $[M + Li]^+$ adducts, this presumably being due to the very high concentrations of Na in the original sample. This is, however, not a problem as in this case the $[M + Na]^+$ adducts can be used for quantitation. A limit of detection study suggests that the lowest reliable extractable level from this matrix is 80 μg/L (Fig. 6). This compares unfavourably with current HPLC methods[7–9,14] where a typical limit of detection would be 10 μg/L. However, the present method is rapid and simple to perform.

CONCLUSIONS

MALDI-TOFMS has been shown to be a new and useful method for the analysis of APEO surfactants in surface waters. If required, spectra can be simplified by the addition of LiCl to produce solely $[M + Li]^+$ adducts. For quantification, the addition of octylphenol ethoxylate as an internal standard has overcome the problem of matrix discrimination, and ratios of the equivalent ethoxymers are constant across the entire mass spectrum. The limit of detection of 80 μg/L is higher than that of HPLC-fluorescence. However, the rapid analysis time of this technique would make it ideal for use in screening.

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