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Characterization of surfactants and their biointermediates by liquid chromatography–mass spectrometry

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Abstract

To determine complex mixtures of surfactants in both commercial and environmental matrices, semi-specific and insensitive methods based on colorimetry and complexometry are no longer acceptable. Several liquid chromatographic (LC) methods were developed in the past for analyzing surfactants in a variety of matrices. The recent introduction of robust and sensitive interfaces to couple LC to mass spectrometry (MS) has given a new impulse to elaboration of LC–MS methodologies able to identify unequivocally complex mixtures of isomers, oligomers and homologues of surfactants as well as their biodegradation intermediates in environmental samples at trace levels. The actual state-of-the-art in the analysis of the above compounds is reviewed in this paper. Particular attention is paid to those methods involving the two currently most used interfaces, i.e. thermospray and electrospray. © 1998 Elsevier Science B.V.

Keywords: Reviews; Interfaces, LC–MS; Thermospray interface; Particle beam interface; Electrospray interface; Surfactants

Contents

1. Introduction

In 1993, the worldwide production of synthetic surfactants was estimated to be larger than 7.2 million tons. Of these, about 30% were non-ionic, 55% anionic and 15% cationic. General formulae and acronyms of the most widespread surfactants are shown in Table 1. After use, surfactants are usually disposed to sewage treatment plants (STPs). Here, (bio)degradation processes and/or adsorption on

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Table 1
General formulae and acronyms of the most widely used surfactants

Common name (acronym)	Hydrophobic group	Hydrophilic group
Linear alkylbenzenesulfonates (LAS)	$C_6H_4–C_nH_{2n+1}–$ n=10–14	SO_3^-
Alkylethoxysulfates (AES)	$C_nH_{2n+1}–$ n=12–15	$–(OCH_2CH_2)_n–OSO_3^-$ n=1–8
Alkylsulfates (AS)	$C_nH_{2n+1}–$ n=12–15	OSO_3^-
Nonylphenolethoxylates (NPEO)	$C_9H_{19}–C_6H_4–$	$–(OCH_2CH_2)_nOH$ n=1–23
Octylphenolethoxylates (OPEO)	$C_8H_{19}–C_6H_4–$	$–(OCH_2CH_2)_nOH$ n=1–23
Linear alcoholethoxylates (AEO)	$C_nH_{2n+1}–$ n=12–18	$–(OCH_2CH_2)_nOH$ n=1–23

sludge particles remove these chemicals from waste waters to a greater or lesser extent, depending on the particular nature of the surfactant molecule. After treatment, residual surfactants, related byproducts and (bio)degradation products (DPs) dissolved in STPs effluents or adsorbed on sludges are discharged into the environment. By several transport mechanisms, these chemicals can penetrate and contaminate any environmental compartment [1–10]. Analytical methodologies able to determine low ppb levels of surfactants and related compounds in environmental matrices are required to validate mathematical models designed to predict environmental concentrations of target materials as well as quantify target materials in their natural environment in support of a wide range of biodegradation and ecotoxicological studies. Moreover, surfactants and related products, which are probably the largest class of technical products of domestic use, are potential candidates as tracers of urban sewage pollution. Simple and effective analytical methods are also required by industrial surfactant manufacturers to characterize complex mixtures of surfactants and related byproducts and for supplying data from field and laboratory biodegradation studies in support of applications for the registration of surfactants.

The chemical complexity of surfactants, which are often mixtures of homologs, oligomers and isomers, has led in the past to development of simple and inexpensive substance-class-specific determination methods based on gravimetry, complexometric and acid–base titrations and colorimetry. These approaches are no longer acceptable, as they lack sensitivity and suffer from interferences by naturally occurring compounds. Moreover, they are unable to distinguish individual surfactant molecules and often, surfactants from related DPs. More sophisticated spectroscopic approaches, such as infrared and nuclear magnetic resonance, provide information on the identification of surfactants [11–15]. Even these techniques are, however, limited by the matrix and their inability to separate and quantify homolog distribution.

Surfactants are invariably low volatile species. Several of them are ionogenic in nature and some of them are thermolabile. In spite of these unfavourable characteristics, major developments in identification and quantitation of surfactants and related products have been achieved by high resolution gas chromatography–mass spectrometry (GC–MS), after formation of suitable derivatives [16–22]. These methods are able to determine individual surfactant homologs, oligomers and isomers with a high specificity and sensitivity. However, they require intensive sample pretreaments and conversion of surfactants into volatile derivatives, which makes this technique rather time consuming.

Chromatographic analysis of surfactants and their DPs is a problem which is typically solved by liquid chromatography (LC). LC with fluorimetric (FL) detection has been extensively used for direct analysis in a variety of environmental matrices of linear alkylbenzenesulphonates (LAS) [23–27]; LAS and related side products formed during their synthesis,

e.g. dialkyltetralinsulfonates (DATS) [7]; OPEO and NPEO [28,29]; the latter ones simultaneously with LAS [30]; LAS, NPEO and related DPs of these two surfactant classes [31]. Non-UV-absorbing AEO have been analyzed in STP influents and effluents by LC with UV detection after suitable derivative preparation [32].

However, a serious weakness of all these methods is that they lack sufficient specificity for testifying without doubt to the presence of traces of surfactants in complex matrices. Even when analyzing commercial mixtures of surfactants, MS data can be of support for assigning a definitive identity to a chromatographic peak. Thus, coupling LC to MS is the key for the future of many LC methods. Several government legislatures in both the US and Europe have planted the seeds to attempt to pursue and protect water and food qualities. Research in new methodologies in MS, notably LC–MS, has greatly benefited from such an international need and now can serve to fulfil the goals initially sought by such a technique, that is monitoring non-volatile and polar target compounds with the specificity and sensitivity similar to GC–MS. In the past 20 years, a large variety of interfaces have been developed to make the high vacuum of the mass analyzer compatible with the large amounts of liquids exiting from the LC column. LC–MS has been extensively reviewed in the past years. Several books [33–36] and review papers [37–39] devoted to illustrate principles, instrumentations and applications of LC–MS have been published.

It is general opinion that, among the various interfaces developed, thermospray, particle beam and mainly, electrospray are the most effective ones for LC–MS analysis. Here, we describe typical characteristics, qualities and faults of these three devices.

1.1. Thermospray (TS) interface

The TS interface can accommodate reversed-phase solvents and any volatile buffer. Among the three most commonly used interfaces for LC–MS, TS is the only one that exhibits the optimum efficiency at 100% water. TS sensitivity is analyte dependent and difficult to predict. Usually, detection limits lie in the 1–50 ng range, under full scan conditions.

Typically, ion evaporation shows $[M+H]^+$ ions for compounds having a high proton affinity, such as those basic in nature. Otherwise, $[M+NH_4]^+$ ions are formed. In negative detection, $[M-H]^-$ or [M− buffer or solvent ion] ions are observed. When using the electric discharge, gas-phase chemical ionization (CI) spectra are generated. Due to the soft ionization process, TS spectra often do not display fragment ions. In some cases, fragmentation can be induced by using the ‘discharge-assisted thermospray’ technique or using higher repeller voltages. By this expedient, the kinetic energy of the ions is increased and collision-induced decomposition (CID) processes generate fragment ions. However, high repeller voltages are not compatible with an optimal transmission efficiency, hence sensitivity is decreased. That TS spectra rarely show fragment ions is a well defined drawback of this interface and precludes its use for definitive confirmation of a target compound. The specificity of methods involving the TS interface can be greatly increased by making use of tandem MS. However, a sensitivity loss of a factor of 10 has to be expected due to ion scattering, and the cost of such instrumentation cannot be afforded by many environmental and research laboratories.

1.2. The particle beam (PB) interface

The lack of fragment ions for unequivocal determination of a certain analyte by the TS-MS arrangement has encouraged several researchers to consider the use of the PB interface for detection of several classes of analytes, as it is able to generate classical electron-impact (EI) spectra. The ion source is the same as that used by GC–MS and EI spectra are equal to those reported in spectra libraries. This is a well-defined advantage of the LC–PB-MS technique. Another positive feature of the PB interface is that it can handle common solvents for RP-LC, including volatile buffers, at flow-rates of up to 1 ml/min.

A serious drawback of the LC–PB-MS system is that it is unsuitable to analyses of very low volatile compounds, such as the majority of surfactants and their biointermediates, as the interface is incapable of transferring them from the liquid to the gas-phase.

Another well established drawback of the LC–PB-MS system is the relatively low sensitivity, because the transmission efficiency is not higher than 1%.

Even with selected ion monitoring (SIM) detection, typical quantification limits are as high as 100 ng.

1.3. The electrospray (ES) interface

The ES interface is the newest device introduced for LC–MS coupling. ES has opened new and exciting perspectives to the LC–MS technique. It is sufficient to say that the ES interface enables LC–MS analysis of compounds having molecular masses up to 400 000 u (unified atomic mass unit) [40], as the ES process is able to form multiple charged ions depending on the acid–base chemistry and hydration energy of the molecules. The ability to increase charge (z) permits the analysis of large molecular masses on a conventional quadrupole limited to m/z or 2000 u for singly charged ions. The versatility of this interface is making it extremely popular among both analytical chemists and biochemists.

The most serious drawback of the ES-MS system is that it cannot accommodate LC effluents containing relatively high concentrations of salts. With such liquids, signal instability, or even worse, electrical breakage is obtained. Another trouble deriving from the presence of high amounts of salts in the LC effluent is that the small orifice of the sample cone can be rapidly plugged. These problems have not yet been completely resolved, however, good performances of the LC column, in terms of selectivity and efficiency have been obtained in the author's laboratory with mobile phase containing the necessary buffers and other additives in concentrations up to one order of magnitude less than those proposed in earlier works [41].

In the ES process, gas-phase ions are softly generated leading to formation of $[M+H]^+$ (positive-ion mode) or $[M-H]^-$ (negative-ion mode) even for the most thermally labile and non-volatile compounds. Not rarely, spectra from non-basic analytes display intense signals for Na^+, K^+, NH_4^+ adduct ions. These cations are always present as impurities in organic solvents used as organic modifiers of the LC mobile-phase.

A very interesting option offered by the ES-MS system, which is not often practised, is that, by raising the electrical field in the desolvation chamber, the quasi-molecular ions can be accelerated to such a point that multiple collisions with residual molecules from the drying gas generate characteristic fragment ions. Provided the target compound is not coeluted with non-target compounds, the resulting collision-induced dissociation (CID) spectra closely resemble those obtained by the more costly tandem MS technique [42]. Unlike the MS–MS technique, structural information on the analyte can be achieved at little or no expense of sensitivity, as little collisional scattering occurs.

Similarly to the TS ion source, the instrumental sensitivity of the ES-MS arrangement is strictly dependent on the chemical characteristics of the analyte. Under full scan conditions, we recently showed that limits of detection of 45 pesticides ranged between 0.02 and 2 ng of analyte injected into the column, by extracting ion current profiles (ICPs) of related adduct or fragment ions from the total ion current (TIC) chromatogram [43].

2. Applications of LC–MS to surfactant analysis

2.1. Ionic surfactants

LAS are the most commonly used synthetic surfactants. Because of their widespread use, monitoring LAS in various environmental matrices receives particular attention. In spite of this continuous interest, very few analytical methods based on LC–MS have been proposed. This could be due to the fact that LAS have a native fluorescence and thus sufficiently selective analysis can be performed by well-established procedures based on LC with FL detection.

Escott and Chandler [44] proposed a LC–TS-MS procedure for analyzing simultaneously LAS and polyethoxylate-based non-ionic surfactants. They pointed out that the use of ammonium acetate had a dual role: as a chromatographic ion-pair forming agents for anionic species, such as LAS, and as a volatile electrolyte for TS-MS detection. No practical application was reported by the authors.

Similarly to Ref. [44], Scullion et al. [45] developed a LC methodology involving SPE with a C_{18} cartridge and based partly on FL detection and partly on APCI-MS detection for the simultaneous determination of LAS, OPEO and NPEO. Contrary to expectations, the authors found that LAS, which are

natural candidates to detection in the negative-ion mode, were five times more sensitively detected in the positive-ion mode as MNa^+ ions. Very surprisingly, authors assigned a peak at m/z 626 appearing in the spectrum from $OPEO_8$ with eight ethoxy units (n_{EO}=8, M_r=558 u) to $[M+3Na]^+$ adduct ion with only one positive charge. More realistically, the signal at m/z 626 appearing in the non-background-subtracted spectrum should be attributed to the sodium adduct ion of $OPEO_9$, which was eluted very close to $OPEO_8$. When the method was applied to analysis of the two surfactant classes in river water, the original object of the authors was not pursued. In fact, LAS were purposely removed from the sample extract together with unknown interfering acidic compounds by a strong anion exchanger material. The authors did not explain which kind of difficulties were encountered in detecting LAS by the APCI-MS detector in the presence of coeluted acidic species, presumably fulvic acids.

LC of ionic surfactants usually requires the addition of an ion-pair forming agent to the mobile phase. This chromatographic condition is incompatible with most forms of MS ionization. Conboy et al. [46] found an ingenious solution to this problem by interposing an ion suppression membrane between the LC column and the ES-MS system. For example, when anionic surfactants are separated by ion-pair reversed-phase (RP) LC with a cationic ion-pair agent, a suppressor with a cation exchanger membrane able to subtract 99.9% of the ion-pair agent from the LC effluent was used. By this system, both cationic and anionic surfactants could be structurally characterized by tandem mass spectrometry. No application to real samples was reported by the authors.

A method for monitoring four AS and thirty six AES in STP influents and effluents as well as receiving waters using LC–ES-MS has been developed by Popenoe et al. [47]. Detection was performed in the ion-negative mode. Chromatographic separation was attained by a RP C_8 column with gradient elution. Although the mobile phase contained only 0.3 m*M* ammonium acetate, sufficiently well shaped peaks for the negatively charged analytes were obtained. Surfactants of interest were isolated from the aqueous matrices by solid-phase extraction (SPE) with a C_2 chemically bonded silica cartridge. Analyte recoveries were equal or better than 90%, except for effluents at high spike level. The effectiveness of using the ES-MS arrangement as a LC detector was fully evidenced by the fact that, although coeluted with AS, quantitation of AES could be achieved by extracting ICPs at m/z relative to their $[M-H]^-$ ions from the TIC chromatogram (Fig. 1). In this work, author claimed that a mathematical model, which fits experimentally measured concentrations to an expected distribution of AES components, can enable prediction of total AES concentration from measurements of a few selected compounds in cases where expected distribution are known with sufficiently accuracy. Limits of detection for AES in river waters were reported to be about 500 and 10 ng/l in the full-scan and SIM modes, respectively.

Schröder [48] utilized both solvent extraction and SPE with C_{18} cartridges followed by flow-injection analysis (FIA) TS-MS–MS or, alternatively, LC–TS-MS–MS for monitoring surfactant residues leaving a STP. LAS were detected in the positive-ion mode as MNH_4^+ adduct ions. Final evidence was obtained by generating negative fragment ions by the CID process, resulting in only the structure-significant fragment at m/z 183. Later, the same analytical procedure was extended to qualitative estimation of LAS in the Elbe river [49].

A complex mixture of the anionic surfactants AES, non-ionic surfactants NPEO, as well as ethoxylated tallow amines having the general formula $[RHN(CH_2-CH_2OH)]^+X^-$ and polyethylene glycols (PEGs) were separated by normal-phase (NP) on an aminopropyl silica column. Detection was carried out using both UV and TS-MS in positive and negative-ion mode. The non-ionic surfactants and PEGs were identified as MNH_4^+ adduct ions. Detection conditions of the TS-MS system were optimized by varying the discharge voltage and the ion source temperature [50].

Ditallowdimethylammonium chloride (DTDMAC), a cationic surfactant commonly used as active ingredient in fabric softener products, was chosen by Lawrence [51] as model compound to demonstrate the effectiveness of coupling a normal-phase fused-silica capillary LC column to a magnetic sector MS by using continuous flow fast atom bombardment (FAB). By this instrumentation,

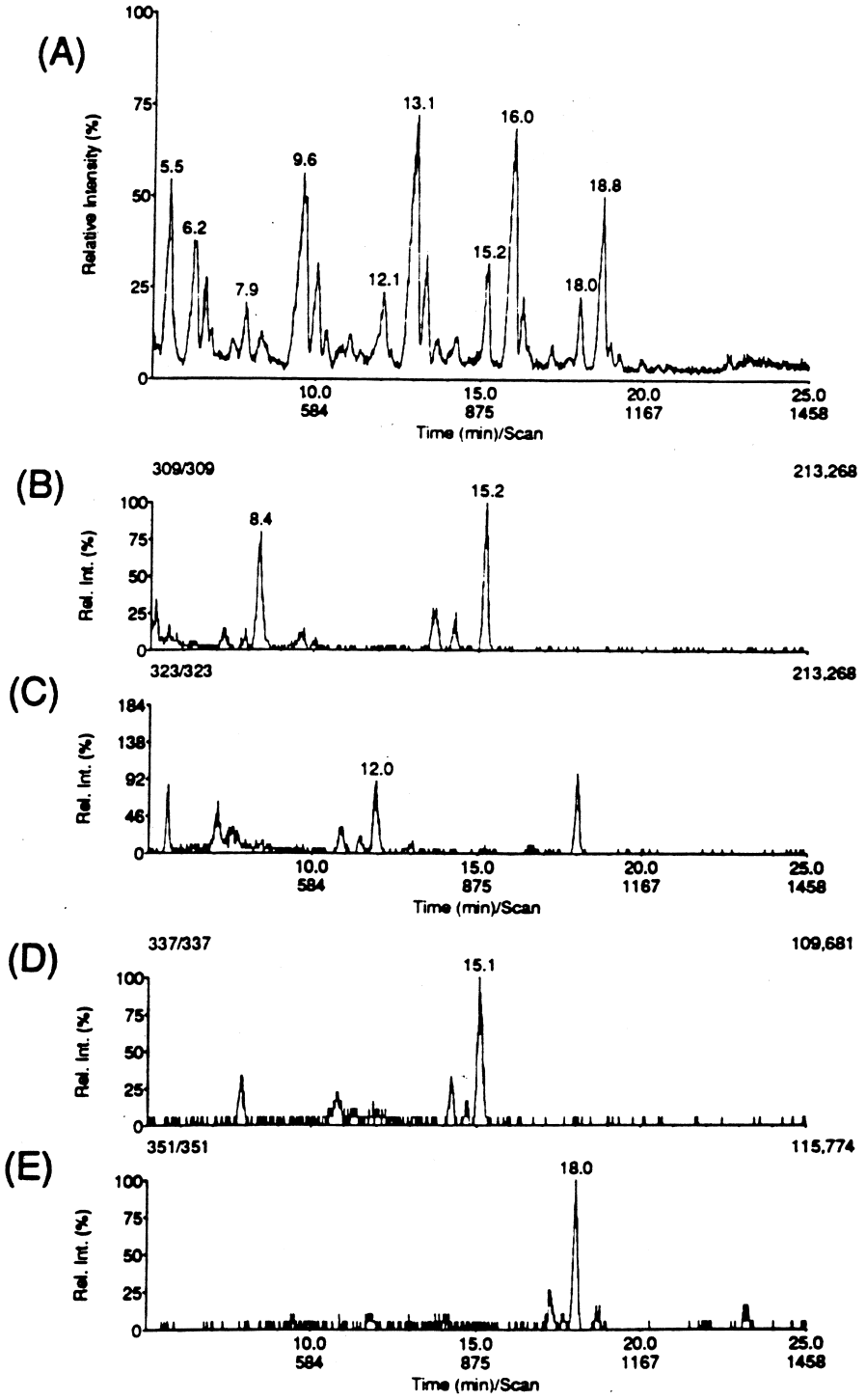

Fig. 1. Mass chromatograms of an unspiked sewage treatment plant sample. (A) TIC chromatogram, $200 \leq m/z \leq 800$ (B) m/z 309 extracted, corresponding to $A_{12}EO_1S$ (C) m/z 323 extracted, corresponding to $A_{13}EO_1S$ (D) m/z 337 extracted, corresponding to $A_{14}EO_1S$ (E) m/z 353 extracted, corresponding to $A_{15}EO_1S$. Peak labels represent scan time in minutes. Reproduced with permission from Ref. [47].

DTDMAC was identified in a commercially available fabric softener product with accurate mass and tandem MS.

LC–ES-MS has been used to demonstrate that, under certain chromatographic conditions, peaks for a typical byproduct of LAS, i.e. DATS, could be completely resolved from the much more abundant peaks for LAS (Fig. 2), thus showing that simultaneous monitoring of these two classes of compounds can be routinely performed by LC with FL detection [7].

2.2. Non-ionic surfactants

Among surfactants, analysis of non-ionic ones, mainly OPEO, NPEO and AEO, has received the most attention after introduction of LC–MS instrumentation. In particular, the lack of any chromophore group in the molecular structure of AEO makes mass detectors extremely useful for LC analysis of this surfactant class at trace levels.

In the mid-1980s, Levsen et al. [52] analyzed OPEO, NPEO and AEO by LC–MS with electron impact (EI) ionization. As interface, a moving-belt device was used. While EI spectra from aromatic surfactants showed well distinct peaks for molecular ions, these were absent in spectra from the non-aromatic AEO surfactants. For these species, the EI ionization process produced abundant amounts of fragment ions with the general formula $[(CH_2CH_2O)_nH]^+$. Branched NPEO could be distinguished from linear ones, as decomposition of the former compounds produced abundant amounts of a fragment at m/z 131 due to the loss of the hexyl radical. Abundant production of quasi-molecular ions from the surfactants considered was achieved by a much softer ionization technique, such as chemical ionization (CI) with isobutane as reagent gas. For

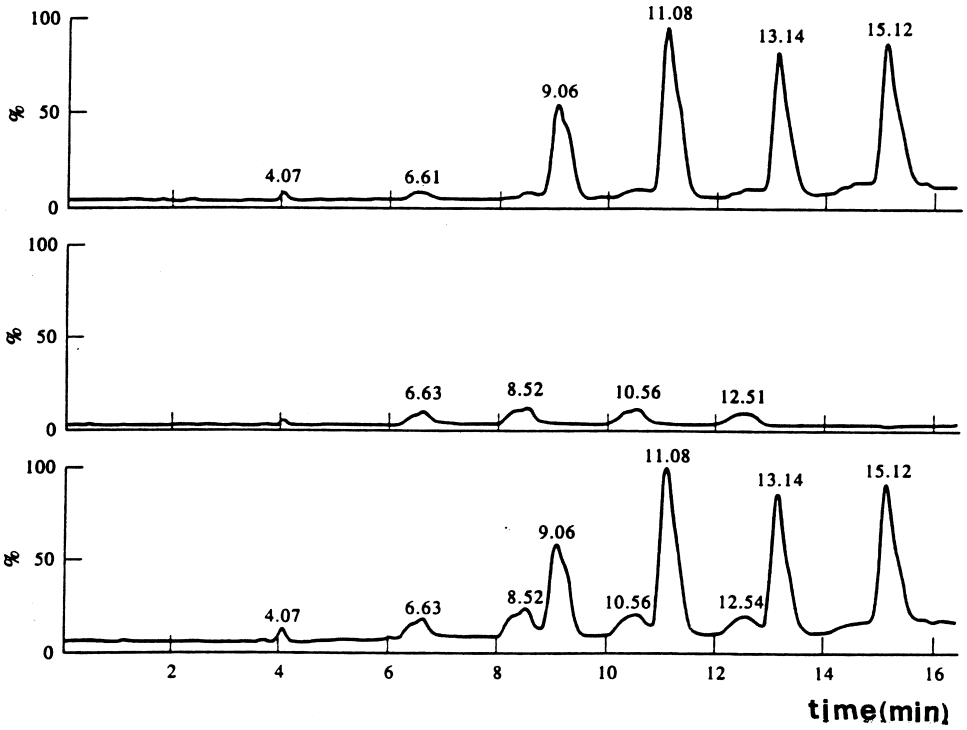

Fig. 2. LC–ES-MS chromatograms for a standard solution of C_{10}–C_{13} LAS containing related typical side products, e.g. dialkyltetralinsulfonates (DATS) as impurities. Peak labels represent scan time in minutes. TIC chromatogram (bottom); sum of extracted ion current profiles of MH^+ adducts relative to C_{10}–C_{13} DATS (middle); sum of extracted ion current profiles of MH^+ adducts relative to C_{10}–C_{13} LAS (top). Small and flat peaks appearing just before every peak for LAS homologs in the upper chromatogram are for branched LAS. Reproduced with permission from Ref. [7].

NPEO and OPEO homologues with up to six ethoxy units, CI spectra displayed intense signals relative to daughter ions formed as a result of the loss of nonene (NPEO) or octene (OPEO). The CI spectra of higher homologs showed fragment ions at m/z 291 (NPEO) and 277 (OPEO) formed by the cleavage of the C–O bond at the level of the second oxyethylene unit. However, by looking at the total ion current profile for OPEO, remarkably weaker signals than expected can be noted for high-mass oligomers. Conceivably, the moving-belt interface, which involves a thermal desorption step from the belt to the ion source, discriminated analytes on the basis of their volatilities, this resulting in distortion of the apparent relative abundances of the various oligomers. The moving-belt interface is no longer used today. In any case, the same MS data could be achieved by using a commercially available interface, e.g. PB, since even this interface serves to link LC with an EI ion source. Probably, however, the same negative effect cited above could again occur with the PB interface.

One of the first successful applications of LC–TS-MS was dealing with analysis of a non-ionic surfactant mixture in product testing [53]. The surfactant mixture consisting of diethanol amides of lauric, myristic, palmitic, oleic and stearic acids were separated on a Nucleosil C_{18} column. Detection was performed in parallel by refraction and TS-MS operated in the positive-ion mode. The diethanol amides were identified as $[M+H]^+$ and in dimer form $[2M+H]^+$. The different compounds were detected in commercial detergent materials by selected ICPs and TIC chromatograms.

Microcolumn LC coupled with FAB was applied to the analysis of OPEO and PEGs mixtures. A porous frit attached to the top of the capillary fused-silica tubing worked as LC–MS interface. OPEOs were separated according to the ethoxy chain length by using a reversed-phase C_{18} material as stationary phase and a water–acetonitrile mixture (containing 5% glycerol, which acts as a suitable FAB matrix solvent) as mobile phase. The elution order of the various oligomers was reversed with respect to their molecular masses, that is lighter oligomers were eluted later than heavier ones [54].

LC–MS with a direct liquid introduction (DLI) interface and a quadrupole mass analyzer (upper m/z 1000) was used by Liang et al. [55] to analyze OPEOs commercial mixtures with increasing average number of ethoxy units (n_{EO}). The DLI interface splits ~1–4% of the LC effluent into the mass spectrometer for analysis and the LC solvent which enters the ion source acts as a chemical ionization reagent gas for mass spectral analysis. The authors showed that, when analyzing OPEOs with a high condensation degree, LC–MS gave anomalously low ion signals for the high-mass oligomers. Fragmentation of molecular ions produced in the chemical ionization process and sensitivity decrease of the quadrupole mass filter towards the high end of the mass range were deemed responsible for this failure. Accurate estimation of the molecular mass distribution without the need for prior chromatographic separation was obtained by laser desorption Fourier transform ion cyclotron resonance mass spectrometry (Fig. 3). It has to be said, however, that negative results obtained by the authors with the LC–MS technique were due to limitations of the particular instrumentation used, rather than to an intrinsic weakness of the LC–MS technique. Moreover, the DLI interface is no longer used and is replaced by the TS one.

Effluents from three STPs in New Jersey were analyzed for non-ionic organic compounds by SPE with the XAD 2 resin followed by LC–PB-MS [56]. The advantage of using this instrumentation over GC–MS was demonstrated by the fact that 46 additional compounds could be detected by the former technique. Some of the new contaminants identified by interpretation of EI spectra were non-ionic surfactants of the NPEO and OPEO classes.

LC separation coupled to FAB-MS was used [57,58] for the analysis of commercial AEO blends and provided identification of individual components similar to that obtained by LC–TS-MS.

Environmental applications of TS-MS–MS were proposed by Schröder [4,59] for the analysis of various non-ionic surfactants containing oxoethylene or oxopropylene chains and their related metabolites, i.e. PEGs or polypropylene glycols, in various aqueous matrices. The comparison between the results obtained by a prior injection on the LC column and those from direct injection (FIA), led Schröder to conclude that the chromatographic separation does not contribute significantly to the information attain-

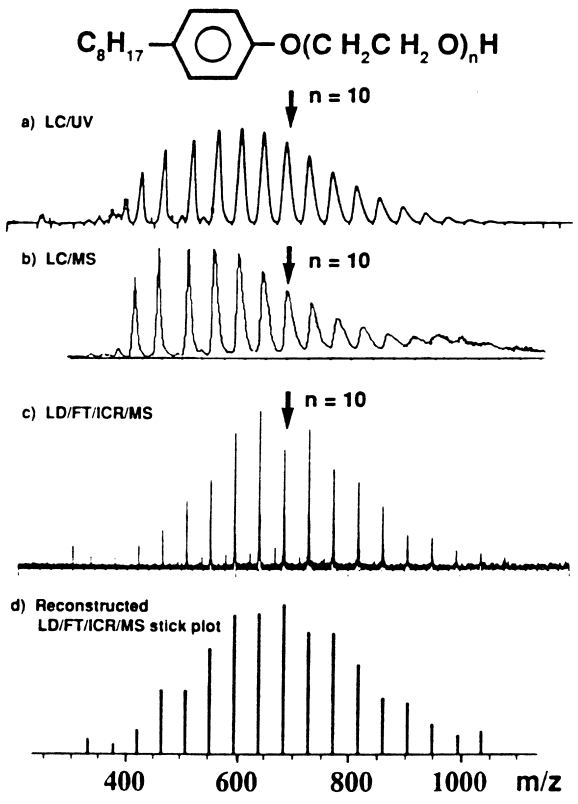

Fig. 3. (a) LC–UV chromatogram of a Triton sample (average of ten ethoxy units) (b) LC–MS (quadrupole; upper m/z 1000) chromatogram of the surfactant material (c) laser desorption Fourier transform ion cyclotron resonance of the surfactant material obtained from a single time-domain data set (d) data of Fig. 3c replotted as the product of peak height and width, to give approximate relative peak areas for a better representation of relative ion abundances. Reproduced with permission from Ref. [55].

able by FIA of the sample extract with TS-MS–MS and is time consuming. Later, the author contradicted himself [60] on affirming that coupling LC to MS–MS (or to a single MS) offers some distinct and important advantages over FIA, which are as follows:

1. Larger volumes can be injected into the LC column. This coupled with LC focusing results in larger analyte amounts per time unit introduced in the ion source and available for MS identification.
2. Provided the LC column is able to separate compounds having the same m/z ratio, misinterpretation due to development of mixed CID spectra by the MS–MS device is avoided.
3. Last but not least, simultaneous introduction into the TS (or ES) ion source of compounds having very different proton affinities can depress or even extinguish signals for these compounds having low proton affinities due to competition effects. This unwelcome effect can be avoided by separating compounds on a LC column.

According to the hydrophilic chain length, alcohol propoxylated surfactants were separated on C_{18} material [61]. In this way, one definite molecular mass could be assigned to each chromatographic peak. The MNH_4^+ ions appearing at m/z 250–714 ($\Delta m/z$ 58) have propoxy units ranging between two and ten.

To determine AEO in river waters and effluents from STPs, Evans et al. [62] developed a method based on LC–TS-MS. Samples were extracted by a C_8 silica cartridge and aliquots of the final extracts were then injected into a C_{18} LC column, which was operated isocratically with a mobile phase composed of a water–tetrahydrofuran (55:45, v/v) solution. By the use of this mobile phase, the column was able to separate AEOs according to the lengths of both the alkyl and ethoxy chains as well as the highly branched AEO compounds from essentially linear ones, as shown in Fig. 4. A continuous post-column addition of aqueous ammonium acetate was done before TS-MS detection. Individual AEOs were identified by both MH^+ and MNH_4^+ adduct ions. To compensate for variations in the instrumental response due to ionization efficiency, quantification was performed by an internal standard procedure. An $A_{11}EO$ mixture with an average of 9 ethoxy units, which is virtually absent in commercial detergent formulations, was used as internal standard. Although not applied to analysis of unspiked real water samples, this method has the potential to quantify concentrations of AEOs at levels of 25–100 ppb and less than 3 ppb for individual homologs.

A different strategy for analyzing surfactants containing ethoxylate chains by LC–MS is that of eluting each oligomer constituent of each homolog component into a single peak. This goal can be reached by RP-LC with methanol as organic modifier [6,59], as shown in Fig. 5A. Besides simplifying quantification, lumping all of the oligomers into a

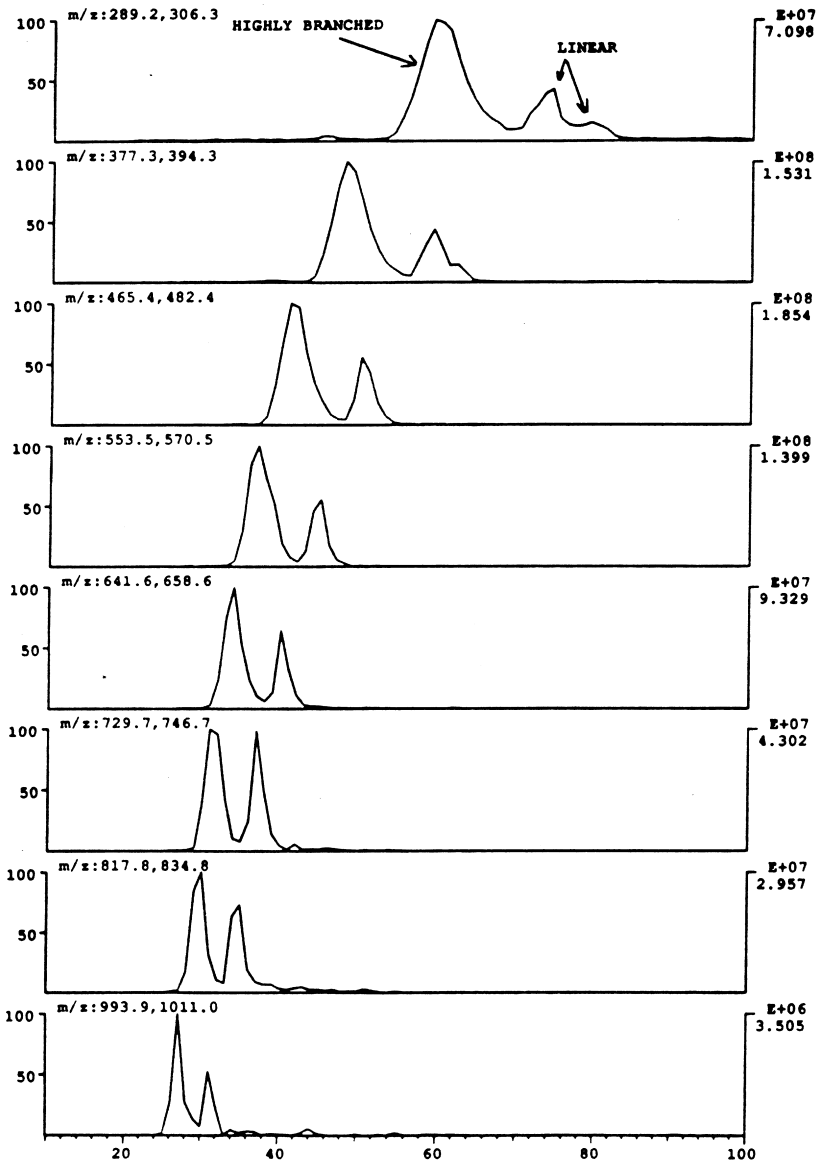

Fig. 4. Extracted ion current profiles of $MH^+ + MNH_4^+$ adduct ions relative to AEOs with a C_{13} alkyl chain and varying ethoxylate chain lengths of a mixture of linear and branched $A_{13}EO$ obtained from the LC–TS-MS analysis of a mixture (1:1) of propylene-based and linear AEOs. Reproduced with permission from Ref. [62].

single peak offers the advantage of increasing the peak intensity, thus providing a means of determining very low concentrations of NPEOs and AEOs, such as those encountered in some types of aqueous environmental samples. If desired, quantitative information on the oligomer distribution of NPEO and AEO homologs in actual water samples can be readily obtained by extracting ICPs for selected m/z from the TIC chromatogram, or by spectra taken from the average of chromatographic peaks for each alkyl homolog (Fig. 5B). However, a drawback of this approach is that, when using ES or TS as ion sources, long-chain oligomers can depress signals for coeluted short-chain oligomers. As a consequence,

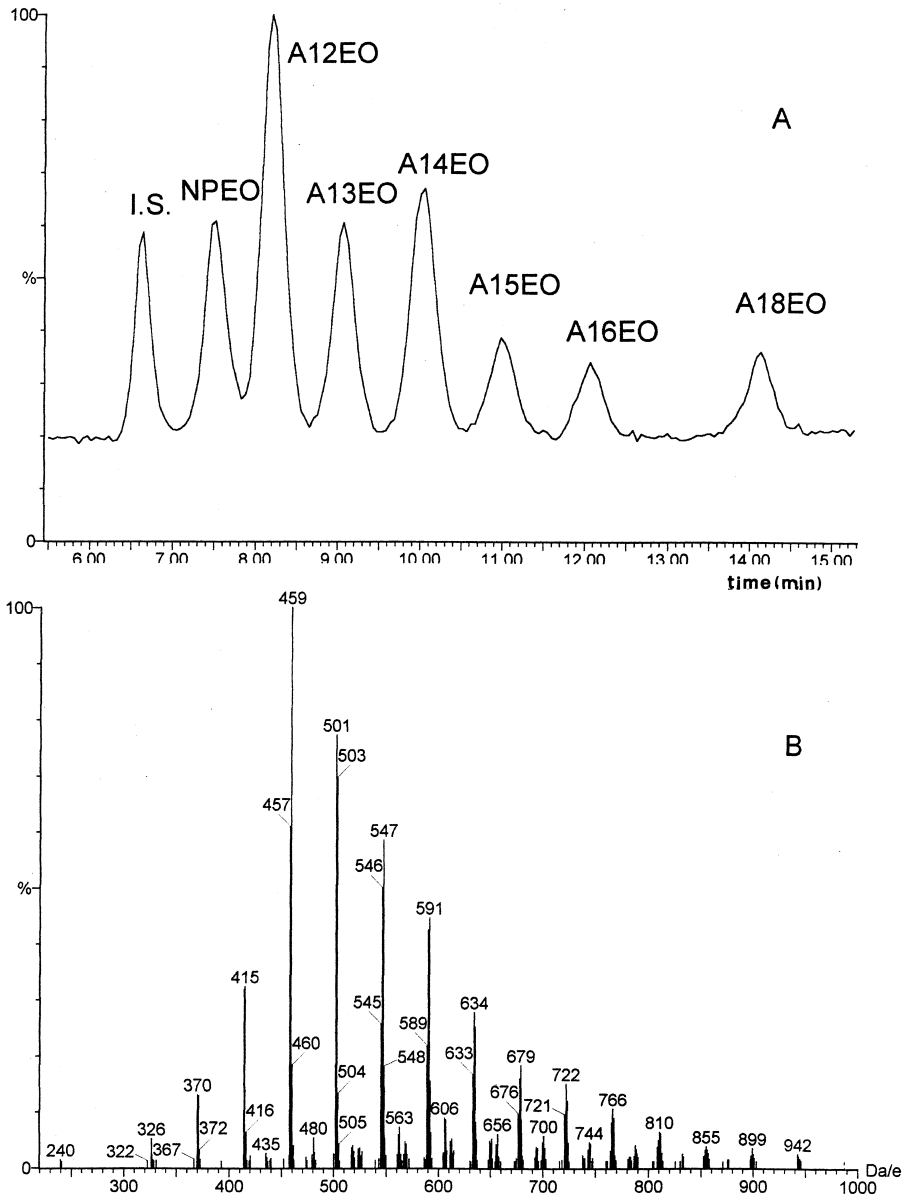

Fig. 5. (A) LC–ES-MS chromatogram obtained by injecting a composite standard solution containing NPEO and AEO. I.S. (internal standard), $A_{10}EO_6$ (B) mass spectra of MNa^+ adduct ions from the $A_{14}EO$ oligomers eluted as a single peak. Reproduced with permission from Ref. [6].

distortion of the oligomer distribution can be observed.

In the author's laboratory, a very sensitive analytical procedure for determining AEO and NPEO in aqueous environmental samples by SPE with a graphitized carbon black (GCB) cartridge followed by LC–ES-MS has been developed [6]. Although the mobile phase was acidified with 0.1 m*M* of a strong acid, analytes were detected as MNa^+ adduct ions. This behavior can be explained by considering that polyethoxylate-containing compounds have a great tendency to form stable complexes with inorganic

cations. Chromatography was adjusted for eluting all the oligomers of NPEO and AEO homologs as single peaks. Analyte quantification was performed by the internal standard method, using $A_{10}EO_6$ as internal standard. This compound is found in negligible amounts in commercial AEO mixtures. Under these conditions, limit of detection (S/N=3) was estimated to be 20 pg/component injected into the column or 0.6, 0.02, 0.02 and 0.0002 μg/l of each analyte in the influents and effluents of STPs, river water, and drinking water, respectively. Analysis of a municipal water sample revealed the presence of NPEO and AEO at ppt levels (Fig. 6). In the same paper, it was pointed out that a fairly good linear response could be obtained by injecting into the column analyte amounts not larger than 20 ng and that partial saturation of the ES-MS detector took place on injection of 400–500 ng of each individual analyte. The restricted linear dynamic range of the ES-MS detector is perhaps its major fault. Moreover, the strict dependence of the detector response on the number of ethoxy units in the surfactant molecules was outlined. While a weak signal was obtained for AEOs homologs having $n_{EO}=1$, the response of the ES-MS detector increased exponentially as the n_{EO} increased from 1 to 6. Only a slight increase was observed passing from $A_{12}EO_6$ to $A_{12}EO_8$. This result was explained by considering that (1) the ability of a molecular species to form adducts with cations in the electrosprayed solution is deeply influenced by both the nature and the number of polar sites eventually present in the molecule and (2) polyethoxylate species can form increasingly stable complexes with cations as the n_{EO} increases.

3. Application of LC–MS to analysis of surfactant biointermediates

Several laboratory biodegradation tests of OPEO and NPEO led to conclusion that these surfactants are mainly degraded via shortening of the ethoxy chain leading to formation of $NPEO_1$ and $NPEO_2$. Subsequently, the terminal alcoholic group of these products of the primary biodegradation can partially be oxidized giving NPE_1C and NPE_2C. Only traces of NPE_3C were found by Ahel et al. [20] on analyzing effluents from various treatment plants in the area of Zürich. Vice versa, LC–ES-MS analysis of a STP effluent extract [31] evidenced the presence of large amounts of NPEC with $n_{EO}>3$. (Fig. 7). This demonstrated that, under certain conditions, an alternative route for NPEO biodegradation is that of the initial oxidation of the terminal alcoholic group. Thereafter, the biodegradation process should proceed by depolymerization of the ethoxy chain with loss of C_2 units, leading ultimately to persistent short-chain NPEC.

Short-chain NPEO ($n_{EO}=2–5$) from non-oxidative depolymerization of the ethoxy chain of NPEO were monitored in extracts of effluents from three municipal STPs by LC–TS-MS with positive discharge chemical ionization [63]. Analyte separation for quantitation was accomplished by RP-LC with gradient elution. Semiquantitative estimation of these biointermediates was achieved by comparing the peak area for an internal standard (2-fluoro-9-fluorenone) with those for the analytes.

On developing a LC–TS-MS–MS method for analyzing pollutants in STP effluents, Schröder [48] identified AEO acidic metabolites, abbreviated as AEC, by their CID spectra (Fig. 8). These metabolites usually originate from the oxidation of the ethoxy chain of branched AEO. These surfactants can be present in commercial detergents for industrial use or as minor components in domestic detergents. Quantitative determination of AEC was made by the standard addition procedure. AEC standards were obtained by submitting an AEO mixture to biodegradation and isolating acidic metabolites from precursor compounds by differential elution with a C_{18} SPE cartridge.

The chemical nature of metabolites which can be formed by biodegradation of an alcohol propoxylate surfactant was investigated [59,61]. For this purpose, the surfactant mixture was introduced in a waste water treatment plant on a semi-technical scale. At intervals, effluent samples were taken and extracted by a C_{18} SPE cartridge. Final extracts were analyzed by LC–TS-MS. After a lag time of several days, effluent analyses showed that, in addition to intact surfactant compounds which were detected as MNH_4^+ adduct ions at m/z 250–772 ($\Delta m/z$ 58), new compounds left the small-scale treatment plant. These metabolites formed MNH_4^+ adduct ions at m/z 248–770, and differed by −2 u from ions of the

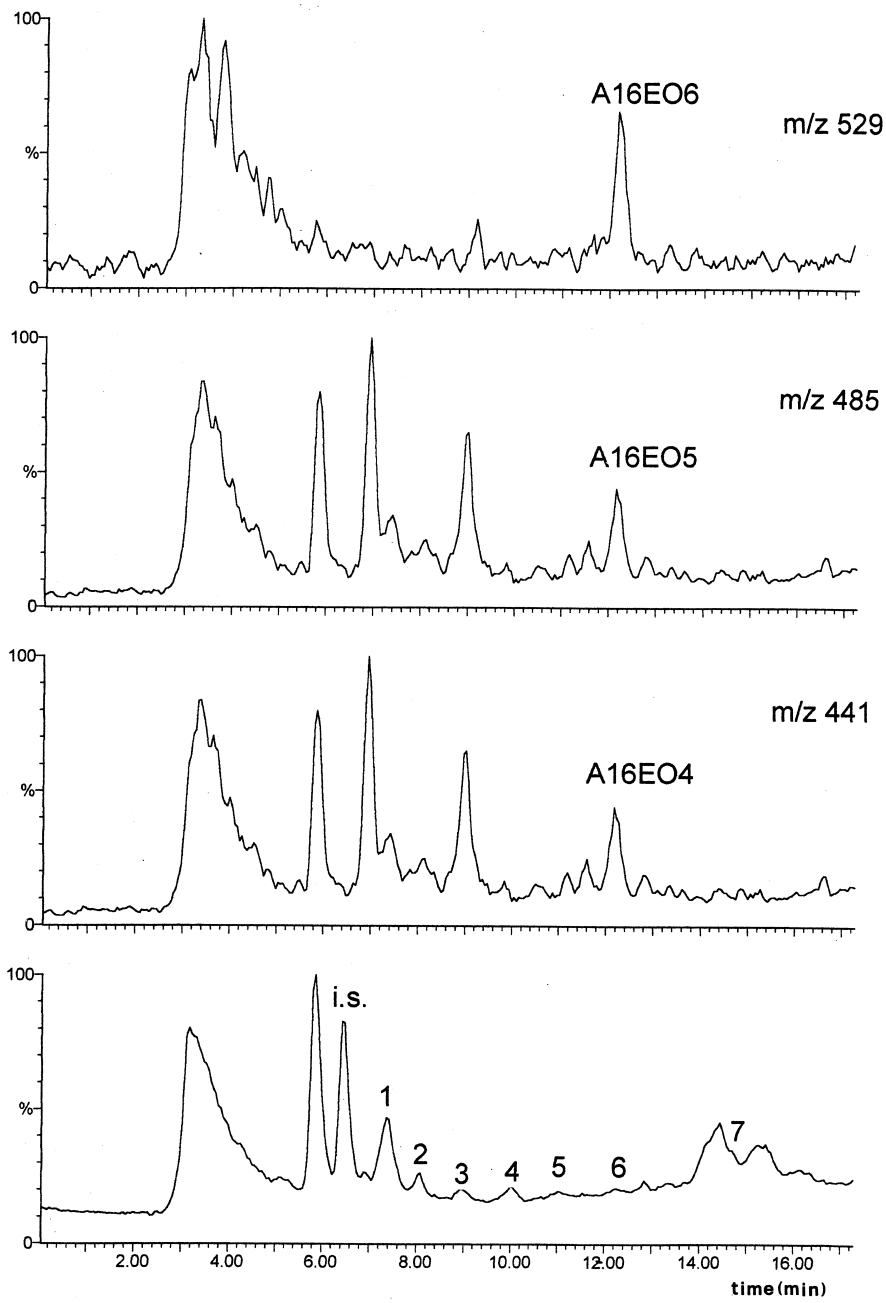

Fig. 6. TIC LC–ES-MS chromatogram (bottom) and extracted ion current profiles (top) relative to MNa^+ adducts of three $A_{16}EO$ oligomers. These chromatograms were obtained by analyzing 4 l of a municipal water. Peaks 1 to 6 (measured analyte concentrations in ng/l are reported in parentheses) are respectively for: (1) NPEOs (61) (2) $A_{12}EOs$ (10) (3) $A_{13}EOs$ (5.6) (4) $A_{14}EOs$ (5.1) (5) $A_{15}EOs$ (1.7) (6) $A_{16}EOs$ (1.2) (7) $A_{18}EOs$ (0.5). Reproduced with permission from Ref. [6].

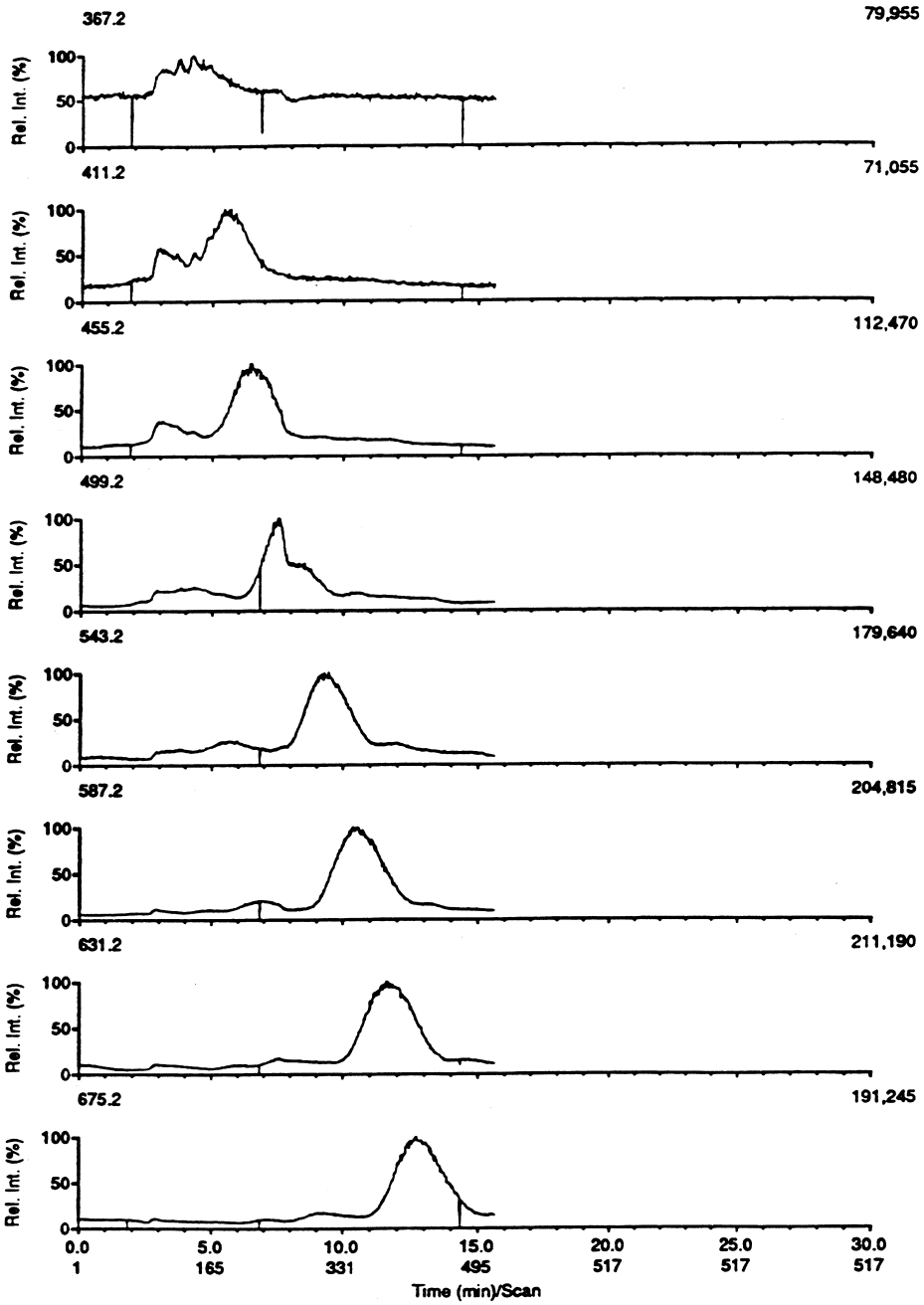

Fig. 7. Extracted ion current profiles relative to selected MNa^+ adduct ions obtained by analyzing the acidic extract of a sewage treatment plant effluent showing the presence in the sample of NPE_3C through $NPE_{10}C$. These acidic metabolites were formed by bacterial oxidation of the ethoxy chain of NPEOs. Reproduced with permission from Ref. [31].

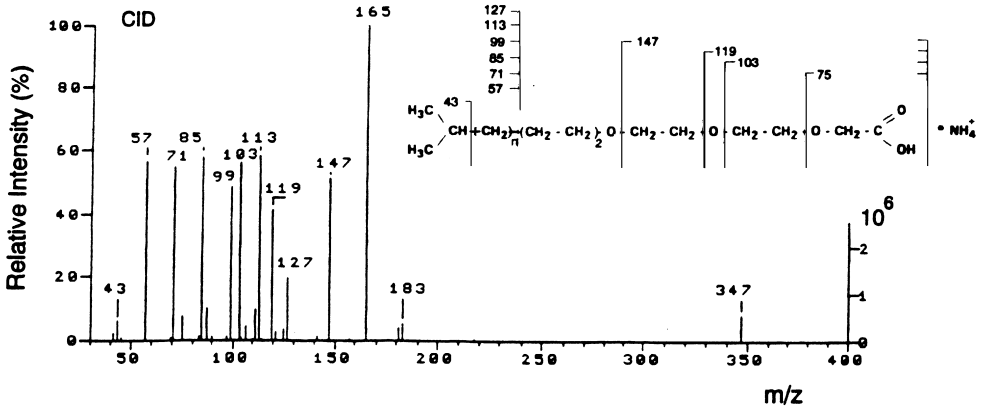

Fig. 8. TS-MS–MS (daughter) ion mass spectrum, structure and fragmentation scheme of a non-ionic surfactant metabolite (m/z 364) from waste water extract. Reproduced with permission from Ref. [48].

precursor compounds. From the interpretation of CID spectra obtained by MS–MS, it was postulated these metabolites originated from terminal alcohol oxidation to give aldehyde derivatives.

Linear AEOs are rapidly biodegraded in water. There is abundant evidence [64] that the initial biodegradation of linear alkyl chain AEO occurs by cleavage at the ether bridge between the alkyl group and the polyoxyethylene moiety, following which both PEGs and aliphatic alcohols are degraded independently of one another. PEG biodegradation proceeds by successive depolymerization of the ethoxy chain via non-oxidative and oxidative cleavage of C_2 units leading to formation of shorter chain neutral PEGs as well as mono-(MCPEGs) and dicarboxylated PEGs (DCPEGs). Depending on the particular aquatic system in which polyethoxylated species are dissolved, their half-lives can vary from weeks to months. For the reasons mentioned above, PEGs are considered one of the most abundant classes of contaminants in water. Monitoring PEGs and carboxylated PEGs in various aquatic systems is of interest for assessing their impact on aquatic life. Moreover, they could be effective markers of potable water contamination from anthropogenic sources.

Investigation on the levels of PEG-type compounds in the environment is made very difficult by the lack of simple and effective detection methods at trace levels. Less than 10% of six ethoxy unit-containing PEG (PEG 6) can be recovered from water by solvent extraction. The highly polar nature of neutral and acidic PEGs as well as the absence of any chromophore group in the structure of these analytes makes them not amenable to direct analysis by both GC and LC.

By using LC–TS-MS, Schröder [48] identified abundant amounts of PEGs in a STP effluent. PEGs were fractionated by RP-LC with a C_{18} column and gradient elution. Both methanol and acetonitrile were used as organic modifiers. A continuous post-column addition of aqueous ammonium acetate was done before TS-MS detection.

In the author's laboratory, a procedure for determining PEGs and their related acidic metabolites in a variety of aqueous matrices has been very recently elaborated [8]. This method involves analyte extraction from 50 ml raw sewage, 100 ml treated sewage, 500 ml riverwater and seawater, 2000 ml groundwater and municipal water by a 0.5-g GCB (Carbograph 4) extraction cartridge. Isolation of acidic analytes from coextracted neutral ones was accomplished by differential elution. During removal of the HCl-acidified CH_2Cl_2–CH_3OH eluent mixture, carboxylated PEGs were purposely allowed to convert into their methyl esters. This expedient offered the advantage of analyzing all the species considered by the same instrumental arrangement. Both extracts were analyzed by LC–ES-MS operated in the positive-ion mode. For fractionating both PEGs and methyl esters of acidic PEGs, the phase A

was in-glass distilled methanol and the phase B was water. Both solvents contained recrystallized 10 μ*M* NaCl.

Although the upper limit of the mass scan range was set at 900 *m*/*z*, neutral and carboxylated PEGs with molecular masses considerably larger than 900 u could be analyzed by the ES-MS system. This is due to the known ability of the ES process to generate multiply charged adduct ions from large molecules. In particular, we observed that PEG homologs were able to pick up an additional sodium ion for every increment of 12 ethoxy units, starting from PEG 12. As an example, spectra from some selected PEGs found in a STP influent are shown in Fig. 9.

Under full-scan conditions, analytes could be quantified in real water samples down to few dozens of ng/l, while data acquisition in the SIM mode afforded limits of quantification of 0.1–0.2 ng/l. Analyses of influents and effluents of STPs showed

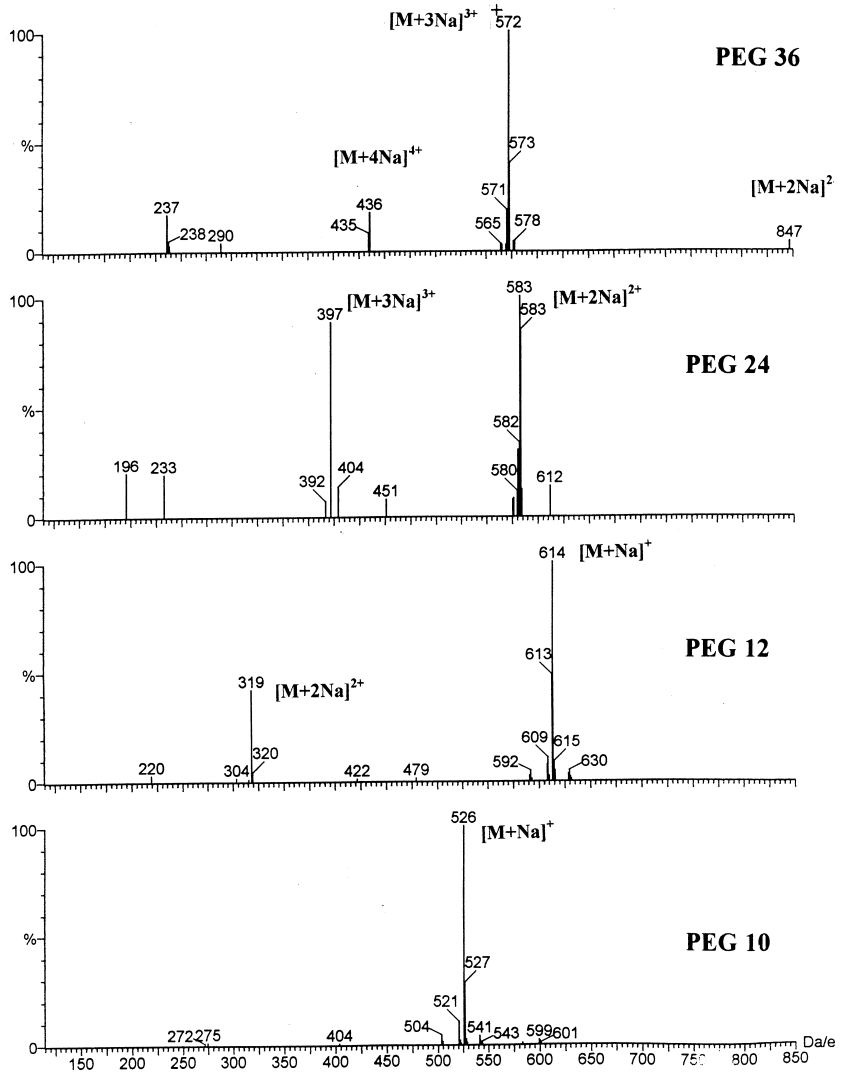

Fig. 9. ES-MS spectra from some PEGs with n_{EO}=10, 12, 24, 36 detected in raw sewage. Depending on the ethoxy chain length, spectra display signals for $[M+nNa]^{n+}$ adduct ions with n=1–4.

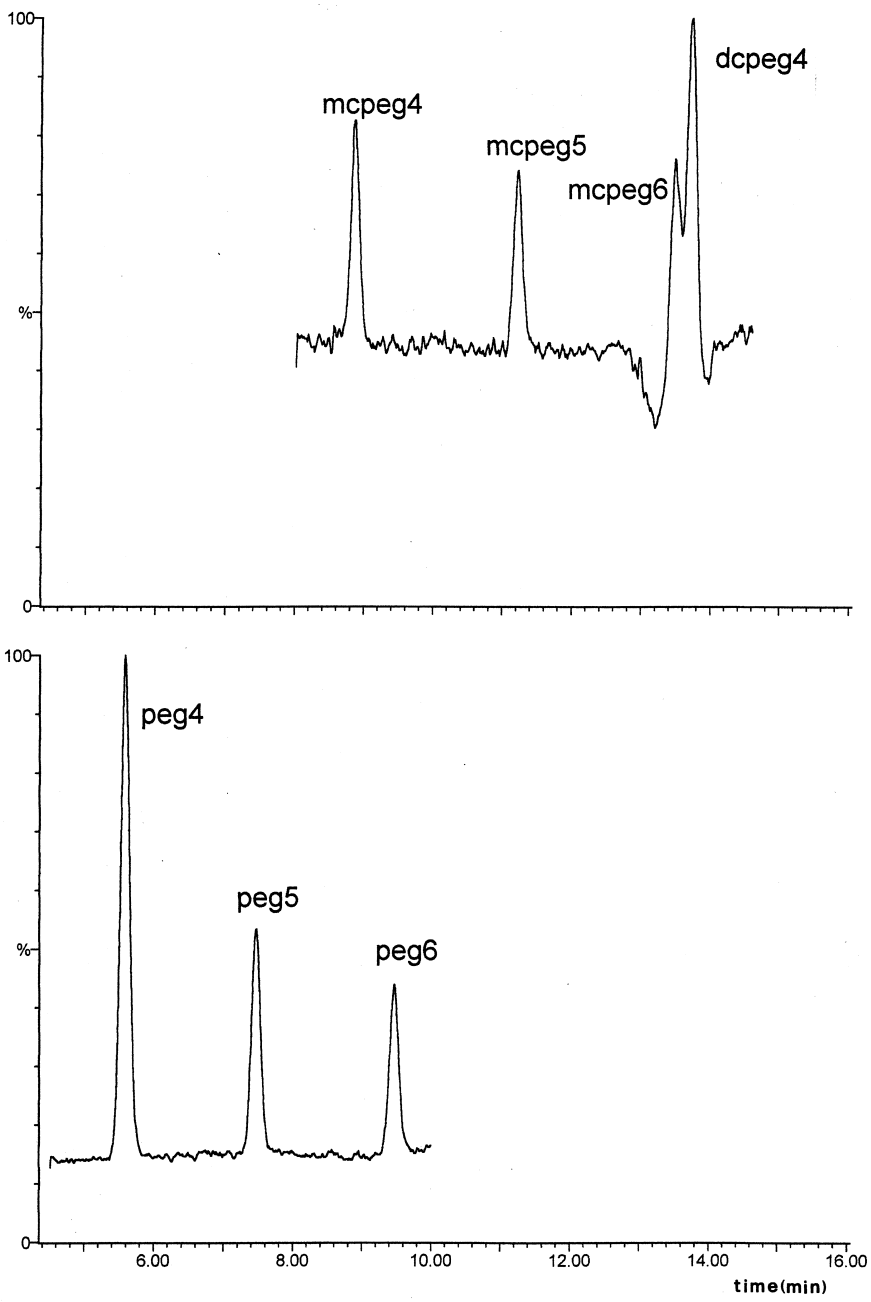

Fig. 10. Initial views of time-scheduled SIM LC–ES-MS chromatograms obtained by analyzing neutral (bottom) and acidic (top) extracts of a 2-l sample of a municipal (Rome) water. Peak labels are acronyms of the analytes. For example, mcpeg4 is for monocarboxylated PEG with 4 ethoxy units. Measured analyte concentrations (ng/l) were as follows: peg4, 4.3; peg5, 1.9; peg6, 1.8; mcpeg4, 1.6; mcpeg5, 1.7; mcpeg5, 1.3; mcpeg6, 1.6; dcpeg4, 2.8.

that even high-molecular mass PEGs, so far deemed rather resistant to biodegradation, were very efficiently removed from sewages. The ubiquitous nature of PEG-type compounds was well evidenced by the fact that they were detected at parts per trillion levels in municipal water (Fig. 10), in five groundwaters at depths ranging between 60 and 208 m and in marine water 16 sea miles from the coast.

In our laboratory, LC–ES-MS has demonstrated to be a powerful tool for identifying and quantifying metabolites formed as the result of biodegradation of NPEO and a branched AEO mixture synthesized from 2-butyldodecanol [65]. These two surfactant mixtures were separately submitted to biodegradation according to an OECD test. At intervals, the test solutions were analyzed by following an analytical scheme analogous to that of [8]. Briefly, we observed that the first degradation step of branched AEO was that of the oxidation of the terminal alcoholic group. Then, bacterial attack was addressed to both the main and side alkyl chains of both oxidized and residual neutral AEO with formation of complex mixtures of mono-, di- and tricarboxylated acids via ω-/β-oxidations. Thereafter, shortening of the alkyl chains was accompanied by slow depolymerization of the ethoxy chain. These latter metabolites appeared to be highly persistent, as they were found in the test solution after more than two months from the beginning of the biodegradation test. Fig. 11A, B and C show three mass chromatograms of the acidic fraction representative of three different moments of the biodegradation process.

Results from the biodegradation study of NPEO corroborated the hypothesis that an alternative

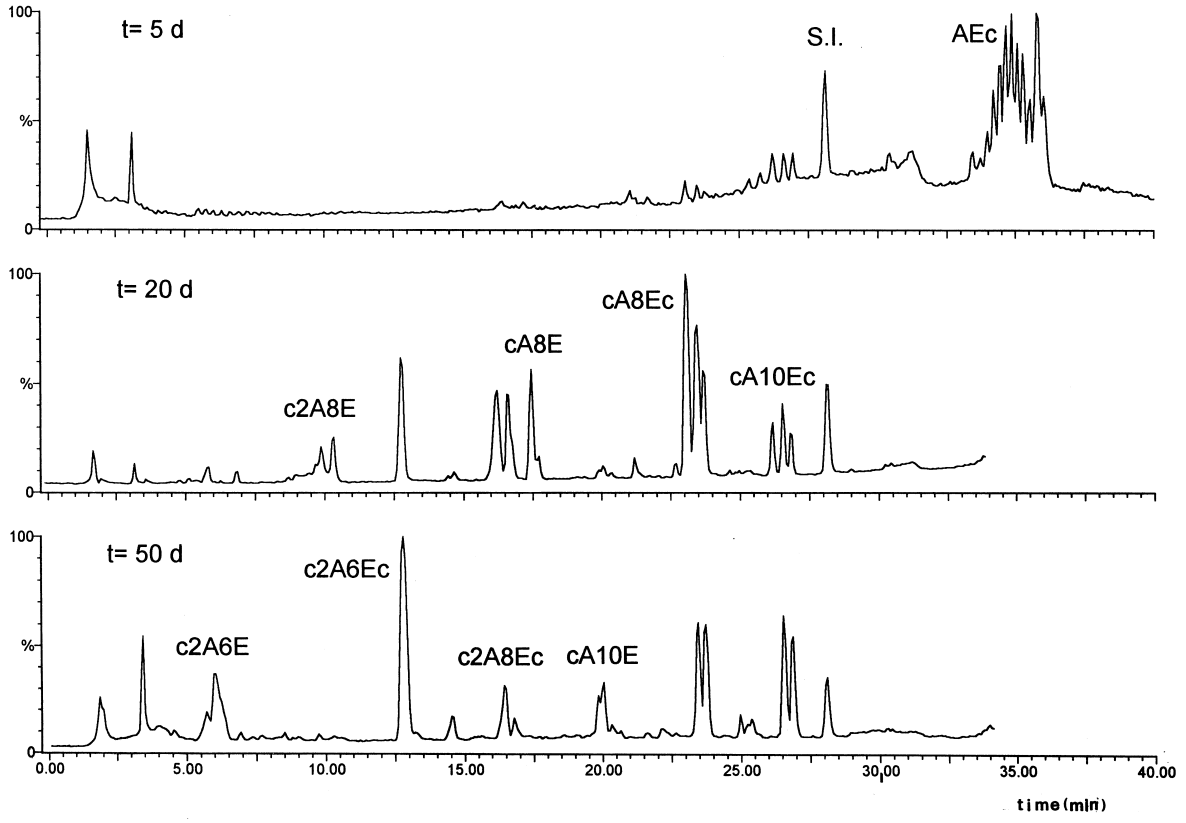

Fig. 11. TIC LC–ES-MS chromatograms obtained by analyzing the acidic extracts of aliquots of the biodegradation test solution of a 2-butyl branched $A_{12}EO$ mixture. Aliquots were taken after 5, 20 and 50 days after the beginning of the test. Labels over the peaks are acronyms of the carboxylated metabolites of the surfactant. The letter 'c' just preceding or following acronyms indicates carboxylation respectively of the alkyl and ethoxylate chains. The term 'c2' indicates metabolites formed by oxydation of both the main and side alkyl chains.

biodegradation route of this class of surfactants is that of the oxidation of the terminal alcohol group with formation of long-chain NPEC (Fig. 12). Both neutral and acidic NPEO were chromatographed on a C_{18} column with a gradient elution. Acetonitrile was chosen as organic modifier. Interestingly, under these

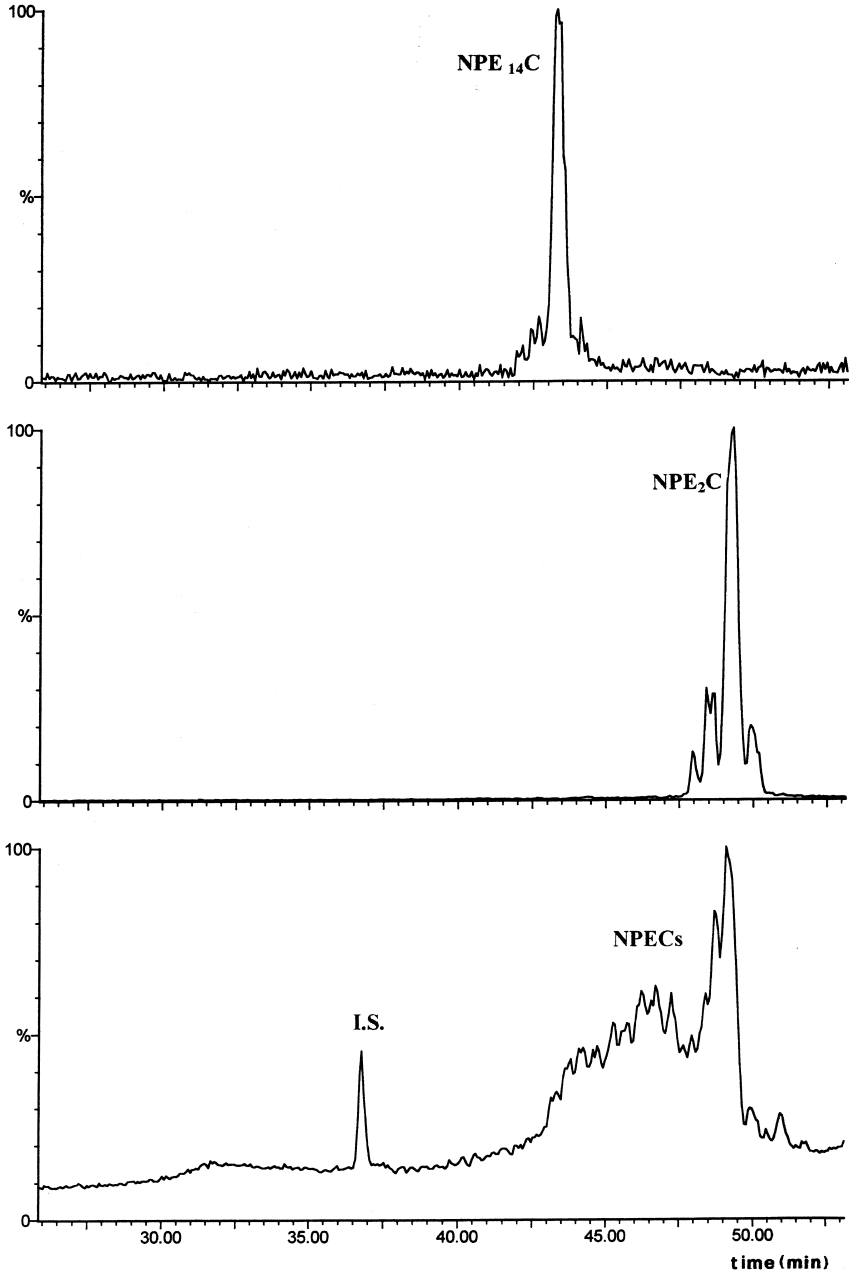

Fig. 12. TIC LC–ES-MS chromatogram (lower) obtained by analyzing the acidic extract of an aliquot (t=8 days from the beginning of the test) of the biodegradation test solution of NPEO after conversion of the acidic metabolites to methyl esters. Extracted ion current profiles (upper) of MNa^+ adduct ions relative to NPE_2C and $NPE_{14}C$ methyl esters.

conditions, the LC–ES-MS instrumentation was able to show isomers of both NPEO and NPEC differing from each other as to the extent of branching of the alkyl chains (see extracted ICPs in Fig. 12).

4. Conclusions

This discussion has shown that analysis of surfactants and their biodegradation intermediates can greatly benefit from the use of the LC–MS technique. The electrospray ion source is considered to have the most promising future. It is expected that the recent introduction of less expensive easy-to-use bench-top LC–ES-MS instrumentation will further stimulate elaboration of analytical methodologies enabling monitoring of the above mentioned compounds in a variety of environmental samples. These field data will serve to definitively understand the fate of surfactants and related products in the environment and their impact on the ecosystem. In the past, efforts to elucidate detailed mechanistic pathways for surfactant biodegradation have been limited by the complexity of the commercial products. It is likely that newer approaches based on LC–MS will find increasing use in identifying biodegradation intermediates of surfactants in both laboratory biodegradation tests and environmental samples.

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