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Multi-residue method for the analysis of synthetic surfactants and their degradation metabolites in aquatic systems by liquid chromatography–time-of-flight-mass spectrometry

Pablo A. Lara-Martín[a,b,*], Eduardo González-Mazo[b], Bruce J. Brownawell[a]

[a] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794-5000, USA
[b] Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Campus Río San Pedro s/n, Puerto Real (Cádiz) 11510, Spain

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ABSTRACT

Synthetic surfactants are economically important chemicals, as they are widely used in household cleaning detergents, textiles, paints, polymers and personal care products. In this work we have developed a method capable of the isolation and analysis of the most widely used surfactants (linear alkylbenzene sulfonates, LAS, nonylphenol ethoxylates, NPEO, and alcohol ethoxylates, AEO) and their main degradation products (sulfophenyl carboxylic acids, SPC, nonylphenol ethoxycarboxylates, NPEC, and polyethylene glycols, PEG) in aqueous and solid environmental matrices. First, analytes were extracted by ultrasonic extraction from sediments and suspended solids using methanol at 50 °C as solvent and 3 cycles (30 min per cycle). Clean-up and pre-concentration of the extracts and water samples were carried out by solid-phase extraction (SPE), using Oasis HLB cartridges. Recoveries were generally about 80% for most compounds. Identification and quantification of target compounds were performed by liquid chromatography–time-of-flight-mass spectrometry (LC–ToF-MS), which has been much less used in the field of environmental analysis than other MS techniques. Examples which illustrate the possible advantages of this technique for multi-analyte analysis of target and non-target contaminants in environmental samples are provided. Finally, the methodology developed here was validated by measuring the concentration of surfactants and their metabolites in selected marine sediment and seawater samples collected in Long Island Sound (NY), and in influent and effluent wastewater from Stony Brook treatment plant (NY). This paper presents some of the first data relative to the occurrence of PEG in the environment, especially in sediments where concentrations were generally higher (up to 1490 μg/kg) than those for other classes of targeted surfactants and their metabolites.

1. Introduction

Synthetic surfactants and their metabolites are often found in wastewater effluents, receiving waters, and sediments, at very high concentrations compared to other targeted analytes [1], in spite of removal efficiencies in wastewater treatment plants (WWTPs) that are typically measured between 95 and 99% [2]. Used in a myriad of products such as household and industrial detergents, laundry products, and cleaners [3], the relatively high levels reported are due in part to the exceptionally huge volumes of surfactants produced every year, with worldwide surfactant production of above 10 million tonnes. This article is concerned with comprehensive analytical approaches as applied to the isolation, identification and trace level analysis of surfactants and their degradation intermediates in samples from aquatic environments. Identification and quantification of these compounds have relied upon a number of detection methods. Many of the first compound specific approaches employed gas chromatography–mass spectrometry (GC–MS) [4] or high performance liquid chromatography coupled to UV and/or fluorescence detectors (HPLC–UV–FL) [5]. LC-based approaches are often preferable for surfactant residues; e.g., many targeted analytes are not amenable to GC, at least without derivatization. Some surfactant classes (e.g., linear alkylbenzene sulfonates, LAS, and nonylphenol ethoxylates, NPEO) possess chromophores and can be analyzed spectrometrically directly or by using ion-pair or post-column derivatization methods. However, over the past decade, high performance liquid chromatography–mass spectrometry (HPLC–MS) has become the most important tool for analysis of surfactants and their metabolites, mainly due to the development of atmospheric pressure ionization, and particularly electrospray ionization (ESI). This is particularly true for polar organic compounds such as surfactants owing to the ease of ionization (ionic,

* Corresponding author at: Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Campus Río San Pedro s/n, Puerto Real (Cádiz) 11510, Spain. Tel.: +34 956 016159; fax: +34 956 016040.
E-mail address: pablo.lara@uca.es (P.A. Lara-Martín).

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acid or base functional groups, or easily formed ammonium or alkali metal adducts) that provides not only important parent ion information but often other characteristic ions for improved confirmation. As a consequence, LC–MS offers many advantages such as greater sensitivity; selectivity; and analyte confirmation, identification of unknown surfactants and their metabolites; and the ability to simultaneously measure multiple classes of compounds together. It may also be possible to employ simpler pre-concentration and purification protocols, given the selectivity of certain mass spectrometric methods. There are too many applications of LC–MS for analysis of small polar molecules and even surfactant residues to review in this introduction. Relevant to this work, González et al. [6] recently reviewed advanced LC–MS methods that have been applied to the analyses of surfactant fate in wastewater.

There are sensitive and selective methods for surfactant residue determination that employ single quadrupole LC–MS systems operating in selected ion monitoring (SIM) mode [7,8]. However, isobaric interferences lead to lower sensitivity and less resolution from major known or unknown interferences. These issues have been commonly solved by means of triple quadrupole [9] or ion trap MS detectors [10], where their respective MS–MS and MS^n capabilities allow scanning for daughter ions, increasing sensitivity and selectivity. One drawback of these types of MS detectors is related to the limited number of predetermined ions that can be monitored in SRM mode during a single experiment, as well as to the inherent difficulty of identifying non-target compounds from only their mass spectra, especially when such compounds exist at low concentrations compared to other co-occurring compounds. Less commonly used than other HPLC–MS instrumental approaches in the analysis of surfactant metabolites, liquid chromatography coupled to time-of-flight mass spectrometry (LC–ToF-MS) constitutes an alternative to address this issue. The full spectral sensitivity and improved mass resolution provide an extremely useful tool for the identification of polar organic compounds in all kinds of matrices, and the capability for estimating their elemental composition by accurate mass measuring of ions is useful for confirmation purposes. Most advances have been achieved in the medical field, as they are related to the identification of excipients, pharmaceutical products and their metabolites in medicaments [11] and human fluids [12]. More recently, some protocols, reviewed by Ferrer and Thurman [13], have also been developed to investigate the occurrence of polar organic contaminants in aquatic ecosystems, which are susceptible of contamination by a wide range of xenobiotic organic compounds due to discharges of treated and/or untreated wastewaters coming from adjacent populations. As example, pharmaceuticals and personal care products in wastewater and seawater [14] have been identified by HPLC–ToF-MS.

With respect to the identification of surfactants and their metabolites in aquatic environments by LC–ToF-MS, available literature is even scarcer. Willetts et al. [15] proposed to use matrix-assisted (MALDI) ToF-MS for the analysis of NPEO in seawater, after C_{18} solid phase extraction. However, limits of detection were too high (between 40 and 80 μg/L) compared to previous HPLC methods, which severely limited the applicability of this technique to environmental samples. More recently, quaternary ammonium compounds (a class of cationic surfactants) have been determined in marine sediments by our group using LC–ToF-MS [16]. Recognized in that study are the exceptionally high positive mass defects characteristic of these chemicals, effectively allowing facile discrimination of this compound class in complex mixtures. Finally, a wider range of surfactants and some of their degradation products (alkylphenols and their carboxylates) were identified in textile wastewater [17] using a tandem quadrupole-ToF (Q-ToF) detector. The present paper presents and validates a comprehensive methodology aimed to determine the most commonly used anionic (LAS) and non-ionic (NPEO and alcohol ethoxylates, AEO) surfactants and their main degradation intermediates in environmental matrices using LC–ToF-MS, and also discusses some of the potential advantages of this technique in the studies of the detection, identification, and environmental behaviour of these compounds in aquatic environments.

2. Experimental

2.1. Chemicals and standards

All solvents were of chromatography quality, purchased from Burdick and Jackson (Muskegon, MI). The 99% pure 2ΦC16 LAS internal standard used in negative ionization mode and the commercial LAS mixture were supplied by Petroquimica Española (PETRESA). A standard blend of commercial NPEO with a known ethoxymer distribution was provided by Huntsman Corporation. The internal standard in positive ionization mode (n-$NPEO_3$) was synthesized as described elsewhere [7]. The individual >98% pure polyethylene glycols (PEG) having 1, 2, 3, 4, 6 and 8 ethoxylated (EO) units, AEO ethoxymers (C_{12}, C_{14}, C_{16} and C_{18} homologues having 1, 2, 3, 6 and 8EO units), poly-L-phenylalanine, leucine enkephalin (lock mass), and a PEG 300 mixture were purchased from Sigma–Aldrich (Milwaukee, WI). The >98% pure C_5 to C_{13} sulfophenyl carboxylic acid (SPC) homologues, nonylphenol mono- and diethoxycarboxylates ($NPEC_1$ and $NPEC_2$) standards were kindly supplied by F. Ventura (AGBAR, Spain) and J.A. Field (Oregon State University, USA).

2.2. Sampling and sample preparation

Seawater and surface sediments were collected from Long Island Sound (NY) using pre-cleaned amber glass bottles and Van Veen grabs, respectively. Pore water was extracted from selected fresh sediment samples by centrifugation and later filtration using glass fiber filters. Wastewater was collected from the influent and effluent of Stony Brook WWTP (NY), a small facility equipped with secondary (oxidation ditch) treatment that treats approximately 8×10^6 L/day before releasing the final effluent into Long Island Sound via Port Jefferson WWTP (NY). Formaldehyde (4%) was added to aqueous samples; these, together with the sediment grabs, were kept at 4 °C during their transport to the laboratory, and later frozen until their analysis. Prior to analysis, sediment samples were freeze dried, then milled and sieved. Water samples were filtered through GF/F (0.7 μm nominal) glass fiber filters (Whatman, Maidstone, England) to separate particulate matter.

The extraction of dried sediments (0.5 g) and suspended solids was performed using ultrasonic irradiation at 50 °C during 3 cycles (30 min each). Methanol was used as solvent (30 mL). Blank sediment extractions, consisting of non-polluted muddy sediments were performed alongside actual samples. After extraction, solvent was separated from the samples by centrifugation and taken to 2 mL under a nitrogen steam. During the following stage, target compounds were isolated from these extracts and water samples using solid phase extraction (SPE) Oasis HLB 6 mL 500 mg mini-columns, supplied by Waters (Milford, MA). These cartridges were prerinsed with 8 mL of methanol and 5 mL of acidified Milli-Q water, prior to addition of sample. Water samples and sediment extracts reconstituted in 100 mL of Milli-Q water were acidified (pH 3) before being passed through the SPE cartridges. Sample vessels were rinsed two times with acidified Milli-Q water and one time with methanol (5% of total volume), and the rinses were also passed through the SPE cartridges. Field blanks, consisting of Milli-Q water were also extracted along with the rest of the samples. Cartridges

were rinsed with 5 mL of acidified Milli-Q water before being air-dried under vacuum. Finally, elution was performed with 8 mL of methanol and 4 mL of dichloromethane. Extracts were then taken gently to dryness under a nitrogen stream and reconstituted in 1 mL of methanol. Further dilutions were made in methanol/water 50:50 and spiked with internal standards (C_{16}LAS and n-$NPEO_3$) prior to LC–ToF-MS analysis.

2.3. Separation and determination of target and non-target compounds

Analysis of surfactants and their degradation metabolites was carried out by HPLC–ToF-MS. 10 μL of sample was injected in a Waters 2695LC HPLC system and separated by a Luna C_{18} (Phenomenex) 150 mm × 2 mm, 5 μm of particle size analytical column. LC conditions for anionic compounds were as follows: mobile phase A was acetonitrile and mobile phase B was 10 mM formic acid/10 mM ammonium formate buffer in Milli-Q water. Flow rate was constant (0.15 mL/min) and initial solvent composition was 5% A. A linear gradient was employed with a final solvent composition of 100% A in 20 min, which was held for another 20 min. The initial solvent conditions were then restored over a 3 min ramp and the column was allowed to re-equilibrate for an additional 7 min (total run time = 50 min). LC conditions for nonionic compounds were similar, but solvent composition of 100% A was held for 30 min instead of 20 min (total run time = 60 min).

A ToF detector (LCT from Micromass) was used under negative electrospray ionization mode (ESI−) for the determination of anionic surfactants (LAS) and their metabolites (SPC and NPEC), whereas ESI+ was used for AEO, NPEO and PEG. Cone voltage was optimized for every target compound to get the maximum signal and/or specific CID fragments. Typical values were −45 V and 20 V in negative and positive modes, respectively. Other MS parameters were: capillary voltage set to 2500 V (ESI−) or 2800 V (ESI+), desolvation and source temperature set to 250 and 150 °C, respectively, and 500 L/h as desolvation gas flow rate. External mass calibration was carried out everyday using poly-L-phenylalanine, and a 5 ng/mL leucine enkephalin solution was added post-column as a lock mass (to compensate for drift of the external calibration) at a rate of 1 μL/min. Mass resolution was always higher than 6000. Data files were internally mass calibrated using the lock mass and the all file accurate mass measure (AFAMM) software process. Identification of target compounds was based on accurate mass measurement of the quasimolecular ion $[M-H]^-$/$[M+H]^+$ or of different adducts (such as $[M+NH_4]^+$ and $[M+Na]^+$) with an error below 2 mDa, and confirmed by specific fragment ions and/or retention time of the compounds compared with that for a standard when available. Quantification was carried out by extracting the same ions that were using for identification of each compound using a 50 mDa window. A six-point calibration curve (from 1 to 500 μg/L) was constructed for each analyte and the analyte response was normalized to that for the internal standard.

2.4. Validation of the analytical procedure

Extraction recoveries of target compounds were determined for water and sediment samples spiked at two concentration levels (10 μg/L and 1 μg/L for water, 5000 and 500 μg/kg for sediments). For each matrix, recoveries were determined comparing the concentrations obtained with the initial spiking levels. In each case, samples were spiked and analyzed in triplicate. The precision of the method was expressed as the relative standard deviation (RSD) of replicate measurement.

Sediment, surface water and wastewater samples were analyzed in duplicate. The reproducibility and repeatability of the method were also evaluated carrying out three successive injections of the same sample and re-analyzing the same batch of samples one month after their first analysis, respectively. Limits of detection (LODs) were determined from spiked water and sediment samples, as the minimum detectable amount of analyte with a signal-to-noise ratio of 3. Ionization efficiency was evaluated for each compound by comparing the signal intensity of spiked analytes (100 μg/L) in both pure methanol and a methanolic extract from a non-polluted sediment sample.

3. Results and discussion

3.1. Extraction efficiency

Table 1 shows the recoveries obtained after the extraction of water spiked with target compounds using HLB cartridges, which were finally selected as they showed appreciably better recoveries for surfactant metabolites than C_{18}, used in previous works [10,18]. This was especially significant for short-chain SPC (C_4 to C_5) and PEG (EO_4 to EO_6), more hydrophilic metabolites for which recoveries were lower (≤30%) for C_{18} than for HLB sorbents. The recovery percentages for parent surfactants, however, were more comparable between the two types of sorbents (e.g. 104 ± 4, 94 ± 3, 83 ± 2, 78 ± 6 for C_{10}, C_{11}, C_{12} and C_{13}LAS, respectively, for C_{18}; see Table 1 for HLB). No significant differences were found between the two tested concentrations. Developing SPE methodologies for the simultaneous extraction and recovery of multiple compounds with widely variable properties typically results in incomplete recoveries for some analytes [18], so a compromise is usually made. In this case, those target compounds having the shorter alkyl/ethoxy chains (e.g., C_4SPC, PEG-EO_4) may interact poorly with the SPE sorbent, giving low recoveries, while those being most lipophilic (most AEO ethoxymers) become tightly bound to the packing material and are therefore hard to elute. With the method reported here, the recovery percentages obtained were satisfactory (above 70–80%) for most of the analytes, and excellent (nearly 100%) for LAS, NPEO and larger PEG ethoxymers.

Extraction of target compounds from solid matrices such as marine sediments was accomplished using an ultrasonic bath. Table 1 shows the recovery percentages obtained from the extraction of spiked sediments after they have been purified by SPE. Recoveries were similar to or slightly lower than to those obtained for spiked water samples, indicating that the extraction of analytes from sediments was almost complete after 3 extraction cycles (30 min per cycle) using methanol at 50 °C. This was confirmed by performing a fourth extraction with methanol and a fifth extraction with dichloromethane with field contaminated sediments samples. The additional amounts of analytes recovered from the combined extracts did not account for more than 8% of total LAS, 4% of total NPEC and 6% of total NPEO. No traces of most polar SPC and PEG were found in the fourth and/or fifth extractions but, as could be expected due to their higher hydrophobicity, up to 20% of total AEO were not extracted in some cases during the first 3 cycles. This issue could be partly overcome by including a forth extraction using dichloromethane in the method, which would increase the recoveries of nonionic surfactants. Drawbacks of this extra step include the use of a less environmentally friendly chlorinated solvent that also results in a greater amount of co-extracted organic matter that may increase isobaric interferences and/or affect ionization. A mixture of methanol and dichloromethane has been used in a previous work [18], but results from PLE have shown that extraction efficiency for AEO increased at the expense of lower recoveries of SPC. On the other hand, recoveries higher than 100% were observed for some analytes for both SPE and ultrasonic extraction, which was attributed to matrix-induced ionization enhancement of tar-

Table 1
Accurate masses, recoveries (%) and relative standard deviation (RSD) in spiked water and sediment, regression curves, coefficient of determination and limits of detection in water and sediment for target compounds.

Compound (homologue/ethoxymer)	Theoretical mass (m/z)	Recovery in water (%) (RSD)	Recovery in sediment (%) (RSD)	Calibration curve	R^2	LODs in water (ng/L)	LODs in sediment (μg/kg)
LAS	183.0116						
C_{10}	297.1524	100 (11)	101 (9)	$0.2222x + 1.0598$	0.9988	1.6	3.1
C_{11}	311.1681	100 (11)	93 (10)	$0.1508x - 0.3033$	0.9973	4.5	8.9
C_{12}	325.1837	95 (15)	81 (9)	$0.1126x - 1.0110$	0.9983	2.4	4.9
C_{13}	339.1994	83 (12)	68 (6)	$0.0163x + 1.3119$	0.9963	0.4	0.9
SPC	183.0116						
C_4	243.0327	43 (8)	37 (12)	$4.8495x + 8.3371$	0.9971	7.6	15.2
C_5	257.0484	73 (6)	74 (8)	$2.1725x - 0.3217$	0.9961	3.0	6.1
C_6	271.064	84 (9)	83 (8)	$1.2779x + 0.7517$	0.9949	1.1	2.2
C_7	285.0797	89 (11)	73 (14)	$0.8244x + 1.1173$	0.9983	1.3	2.6
C_8	299.0953	90 (9)	74 (14)	$0.7072x + 0.9520$	0.9984	0.3	0.7
C_9	313.1110	86 (10)	53 (13)	$0.7656x + 1.1491$	0.9982	2.3	4.6
C_{10}	327.1266	89 (11)	72 (16)	$0.7880x + 0.7728$	0.9986	0.8	1.6
C_{11}	341.1423	91 (7)	70 (10)	$0.7523x + 1.0598$	0.9982	1.4	2.8
C_{12}	355.1579	88 (11)	77 (15)	$0.4486x + 0.5358$	0.9986	2.5	5.0
C_{13}	369.1736	89 (15)	69 (13)	$0.4788x + 0.5022$	0.9980	0.4	0.8
NPEO							
EO_2	331.2249	65 (14)	60 (11)	$0.9497x - 05892$	0.9892	0.8	1.5
EO_3	375.2511	107 (4)	95 (13)	$0.0248x - 0.0362$	0.9987	0.2	0.4
EO_4	419.2773	98 (16)	95 (5)	$0.0218x - 0.2794$	0.9966	0.1	0.1
EO_5	463.3035	106 (12)	92 (11)	$0.0110x - 0.1053$	0.9980	0.1	0.1
EO_6	507.3297	95 (16)	96 (7)	$0.0156x - 0.0375$	0.9993	0.1	0.1
EO_7	551.3559	112 (11)	87 (14)	$0.0083x + 0.1858$	0.9992	0.1	0.1
EO_8	595.3821	104 (16)	92 (7)	$0.0075x - 0.0588$	0.9995	0.0	0.1
EO_9	639.4083	100 (15)	92 (14)	$0.0071x + 0.0869$	0.9995	0.1	0.1
EO_{10}	683.4345	108 (6)	95 (10)	$0.0072x + 0.1660$	0.9994	0.1	0.2
EO_{11}	727.4607	100 (13)	97 (11)	$0.0076x + 0.2246$	0.9987	0.1	0.1
EO_{12}	771.4869	99 (5)	102 (12)	$0.0086x + 0.0127$	0.9995	0.3	0.6
EO_{13}	815.5131	98 (13)	91 (6)	$0.0094x - 0.0088$	0.9977	0.3	0.7
EO_{14}	859.5393	106 (19)	93 (14)	$0.0089x + 0.1002$	0.9999	0.1	0.1
EO_{15}	903.5655	101 (14)	78 (6)	$0.0124x + 0.1447$	0.9977	0.1	0.1
NPEC	219.1749						
EO_1	277.1804	87 (6)	70 (5)	$0.3450x + 0.8802$	0.9995	1.3	2.6
EO_2	321.2066	91 (5)	84 (7)	$0.4593x + 0.5485$	0.9987	1.3	2.6
AEO							
C_{12}							
EO_2	275.2586	83 (10)	72 (8)	$1.0905x - 1.2861$	0.9898	0.8	1.6
EO_3	319.2848	65 (4)	72 (3)	$0.0163x + 0.0147$	0.9977	0.1	0.2
EO_6	451.3635	63 (5)	62 (1)	$0.0075x + 0.1192$	0.9973	0.1	0.1
EO_8	556.4425	67 (3)	47 (3)	$0.0121x + 0.0732$	0.9848	0.2	0.5
C_{14}							
EO_2	303.2899	80 (8)	69 (4)	$1.3127x - 1.1109$	0.9930	2.4	4.9
EO_3	347.3161	59 (3)	61 (3)	$0.0114x + 0.0013$	0.9976	0.1	0.2
EO_6	479.3948	62 (7)	61 (3)	$0.0069x + 0.0125$	0.9964	0.1	0.1
EO_8	584.4738	61 (8)	50 (4)	$0.0055x + 0.1539$	0.9968	0.1	0.2
C_{16}							
EO_2	331.3212	78 (10)	86 (5)	$1.5003x + 0.6463$	0.9955	2.8	5.6
EO_3	375.3474	62 (7)	75 (6)	$0.0121x - 0.2469$	0.9975	0.0	0.1
EO_6	524.4526	63 (8)	73 (2)	$0.0096x + 0.0721$	0.9975	0.1	0.3
EO_8	612.5051	61 (8)	52 (3)	$0.0057x + 0.1895$	0.9963	0.1	0.1
C_{18}							
EO_2	359.3525	58 (9)	96 (5)	$4.6894x + 2.7026$	0.9943	11.8	23.7
EO_3	403.3787	51 (6)	90 (6)	$0.0310x + 0.0517$	0.9960	0.1	0.2
EO_6	552.4839	51 (11)	74 (11)	$0.0212x + 0.1114$	0.9966	0.2	0.4
EO_8	640.5364	49 (5)	61 (9)	$0.0175x + 0.2833$	0.9925	0.1	0.2
PEG							
EO_4	195.1232	51 (10)	26 (6)	$0.0911x - 0.4743$	0.9904	0.1	0.3
EO_5	239.1495	73 (13)	49 (17)	$0.0462x + 0.2117$	0.9953	0.2	0.5
EO_6	283.1757	117 (9)	79 (11)	$0.0816x + 0.3769$	0.9996	1.4	2.7
EO_7	327.2019	115 (13)	99 (7)	$0.0953x + 0.7435$	0.9992	0.5	0.9
EO_8	371.2281	108 (13)	97 (3)	$0.0970x + 0.3020$	0.9993	0.1	0.1
EO_9	415.2543	112 (14)	99 (4)	$0.0907x - 0.0217$	0.9990	0.1	0.2
EO_{10}	459.2805	111 (22)	97 (6)	$0.0913x + 0.1539$	0.9993	0.1	0.2
EO_{11}	503.3076	106 (!3)	93 (16)	$0.1042x + 0.1064$	0.9994	0.1	0.2
EO_{12}	564.3595	113 (25)	93 (13)	$0.1186x + 0.0746$	0.9991	0.1	0.2
EO_{13}	608.3857	108 (17)	101 (3)	$0.1221x + 0.1285$	0.9973	0.1	0.2

get compounds during analysis. This enhancement was previously observed by Lee Ferguson et al. [19] for NPEO with ethoxy chain lengths greater than 3. Overall, advantages of the method proposed here for extraction of solid matrices for a very wide range of analytes include acceptable recoveries, low solvent consumption (<30 mL per sample), simplicity and low-cost. It also allows for processing a large number of samples within 3 h (typically, but not limited to 24 samples/batch). Other techniques such as PLE

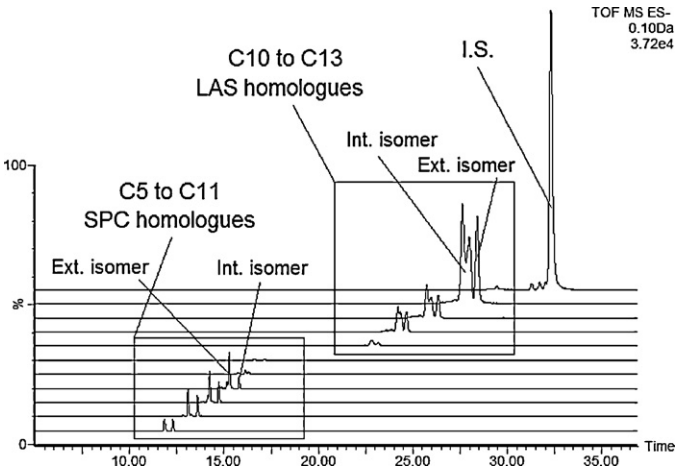

Fig. 1. LC–ToF-MS negative ion mode chromatograms showing the occurrence of different LAS and SPC homologues and isomers in a seawater sample. Internal standard C_{16}LAS is also displayed.

may be faster when fewer sample extractions are required (typical extraction time is 15–20 min per sample).

3.2. Chromatographic separation and detection by mass spectrometry

Fig. 1 shows an illustration of reconstructed ESI− chromatograms corresponding to different LAS and SPC homologues in a seawater sample. Linear alkylbenzene sulfonates are often sold as a complex mixture of homologues (the length of the alkyl chain ranges from C_{10} to C_{13}) and isomers (denominated as external or internal depending on the relative position of the benzosulfonic group with respect to the alkyl chain). Their degradation in the environment generates complex patterns of sulfophenyl carboxylic acids [10]. LC–ToF-MS can be very useful for analysis and confirmation of these homologue/isomer series. Retention times of both parent compound and metabolites increase as a function of the length of the alkyl chain. Due to the rapid degradation of LAS and long-chain SPC (C_{10} to C_{13}) occurring in seawater [20], C_6 to C_9SPC homologues were the metabolites found in greatest abundance (Fig. 1), which is in agreement with previous data on their environmental distribution [21]. SPC with up to 11 carbon atoms in their alkyl chains could also be identified by means of the accurate mass measurement of their molecular ions $[M-H]^-$ and the use of standards. Additional confirmation was provided by the specific CID fragment m/z 183.0116, diagnostic of both LAS and SPC (Fig. 2a and b) [10,21]. With respect to the retention time and order of elution of the different isomers, different behaviour is observed between LAS and SPC. Briefly, external LAS isomers elute later because the interaction of the molecule with the HPLC column phase takes place preferentially by the longer side of the alkyl chain with respect to the sulfophenyl group. Elution of SPC isomers is reversed because the presence of a carboxylic group in the alkyl chain enhances the interaction with the opposite side, which contains a higher number of carbon units in the case of internal isomers.

Fig. 3 shows ESI+ selected ion chromatograms obtained from the analysis of a wastewater sample. Individual AEO components are separated by alkyl chain and, partly, by ethoxylate chain length. This is highly desirable for assessing more accurately the environmental impact of this type of surfactant and metabolite mixtures, especially since aquatic toxicity varies with the alkyl and ethoxy chain lengths. A complete separation of all components, however, is virtually impossible with conventional reverse phase LC columns. By using several combinations of water with methanol or acetonitrile as mobile phase and octadecyl chemically bonded silica as a stationary phase, only partial separations of the various NPEO and AEO ethoxymers can be achieved [22]. Significantly better results in terms of complete separation of ethoxymers have been observed by using a mixed-mode (reverse phase and size exclusion) column [19], although the use of this type of packing materials may be not well-suited for a multi-residue screening approach such as the one presented here. Linear (n-alkyl) and monobranched AEO isomers can be separated by the current method, which can be also useful to distinguish from those surfactants coming from oleochemical and petrochemical sources [21]. This can be observed in Fig. 3, where smaller peaks corresponding to monobranched ethoxymers appear before the top of each C_{13}AEO linear ethoxymer. C_{16}AEO, however, is synthesized purely from oleochemical sources, so the presence of the branched isomers is not detected.

Alcohol and nonylphenol polyethoxylates, as well as polyethylene glycols lack charge or acid/base functional groups. The most widely used option for ionization is to create adducts as the oxygen atoms in the polyethoxylate chain can donate their free electrons to a selected cation agent and the flexible structure of the chain allows the molecule to "wrap" itself around that cation [23]. Thus, LC–MS detection of the ethoxylated compounds results in primarily molecular ions of the type $[M+NH_4]^+$ or $[M+Na]^+$, with some of the lower molecular weight compounds having a significant $[M+H]^+$ ion in our method where ammonium buffer is used. This can be observed in Fig. 2c–e, where typical mass spectra for NPEO, AEO and PEG are shown. The ability of a compound to form adducts depends on the nature and positions of oxygen functional groups present. In our case, the stability of NPEO, AEO and PEG complexes with cations is enhanced as the number of EO units increases [22], becoming unstable for compounds with less than four ethoxylate units [23]. Additionally, the ionization of less ethoxylated compounds may be suppressed by competition with co-eluting sample matrix or higher ethoxymers [19]. As a consequence, response of nonionic surfactants in positive ion mode greatly depends on both the length of the ethoxy chain and the applied cone voltage in the ESI source. At relatively low source cone voltage settings (10–20 V), maximum response was obtained for NPEO and AEO shorter ethoxymers (1–3EO), whereas those having greater ethoxylated chain length required higher cone voltages for an optimum signal (40–50 V for 5EO, 60–70 V for 8EO, and up to 90 V for >10EO). This trend was also observed for PEG, although the effect was less dramatic (optimal voltages were 10–20 V for 3–4EO, 25–35 for 5–8EO and 40–50 for >8EO). The detector response increased exponentially as the number of EO units increased from 2 to 6, while only a slight increase of the signal was observed after this (see how calibration curves in Table 1 are very similar for NPEO, AEO and PEG having 6 or more EO units). Ionization of $NPEO_1$ and AEO_1 was so poor that they were practically undetectable within the concentration range used in this study (from 1 to 500 μg/L). This issue can be solved by derivatization [24] if the objective of the study is aimed to the characterization of a specific ethoxylated species. Derivatization by-products, however, can interfere with the identification of non-target compounds, so it was ruled out when developing the multi-residue screening method proposed here.

3.3. Limits of detection and matrix effects

Limits of detection (LODs) were calculated for sediment (0.5 g) and water (1 L) samples (Table 1). Average LODs for LAS and SPC homologues were between 0.7 and 15.2 ppb in sediments, whereas they were usually below 0.5 ppb for most PEG, NPEO and AEO ethoxymers. In general terms, it was observed that ionization efficiency improved with decreased water content of the mobile phase. Therefore lower LODs were generally observed for those homologues eluting later in the chromatogram (e.g., C_{13}LAS or C_{10}SPC).

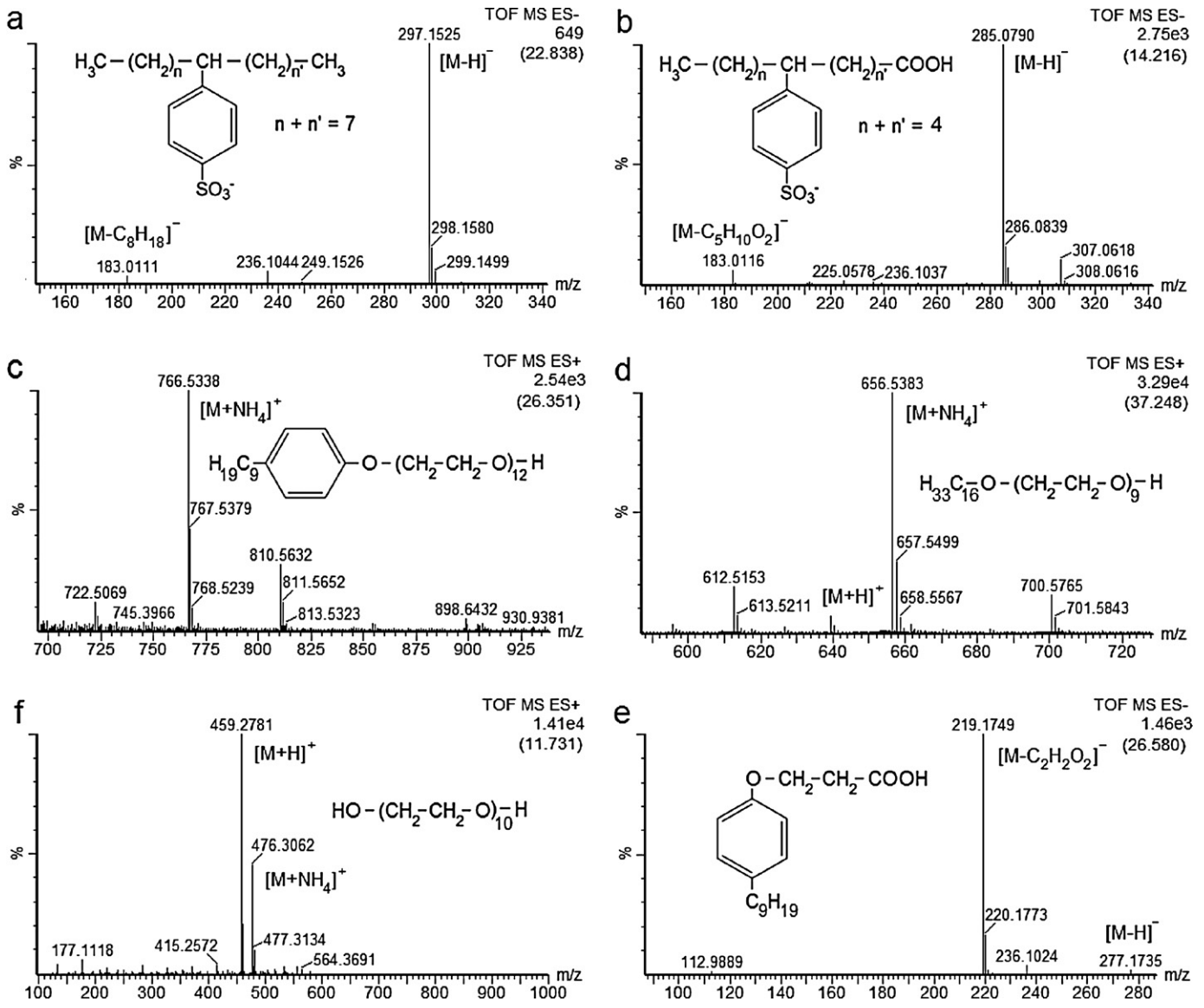

Fig. 2. LC–ToF-MS mass spectra from environmental samples corresponding to: (a) C_{10}LAS, (b) C_7SPC, (c) $NPEO_{12}$, (d) $C_{16}EO_9$, (e) PEG EO_{10}, and (f) $NPEC_1$. Signal intensity, retention time, molecular structures, molecular ions, adducts and/or their specific CID fragments are also indicated.

Greatest sensitivity was obtained for those ethoxymers having higher number of EO units in ESI+, with detection limits lower than 1 ppt in water and between 0.1 and 0.4 ppb in sediments. Shorter ethoxymers (<3EO) were detected with reduced sensitivity in all cases (LODs > 1.5 ppb in sediments and higher than 0.8 ppt in water). In any case, this was enough for the determination of these compounds in sewage impacted water and sediments. Additionally, when using time-of-flight detectors, it has to be considered that S/N is also affected by the mass window size selected. As the mass window is widened, isobaric interferences may appear, resulting in a noisier baseline and decreasing S/N values. The smallest window size achievable is limited by the mass resolution of the instrument. In our case, it was found that a 50 mDa window was optimal for most compounds.

LODs can also be affected by suppression or enhancement of the signal in the presence of co-eluting sample matrix. Target compounds were spiked in pure methanol and in a methanolic extract from a non-polluted sediment sample (not purified by SPE) to evaluate matrix effects by comparing their signal intensity. Signal suppression was up to 20% for LAS, 9% for SPC and 3% for NPEC, depending on the homologue considered; these compounds being analyzed in ESI−. On the other hand, we found that there was an average signal enhancement of 3% for PEG, 2% for NPEO and from 2 to 25% for AEO homologues in positive mode ionization, although the signal decreased for shorter ethoxymers (<4EO) between 5 and 17%. Two internal standards, C_{16}LAS (Fig. 1) and n-$NPEO_3$ (Fig. 3), were used in negative and positive ion modes, respectively, to account for matrix effects in real samples. Signal suppression or enhancement was almost negligible in water samples (<5–10%), but significant variations were detected among sediment extracts depending on their clay and organic carbon contents. Thus, ionization efficiency was reduced from 6 to 17% in ESI− and from 20 to 44% in ESI+. Most severe signal suppression cases, which generally belonged to samples from the most contaminated sampling stations, could be avoided by diluting the sample by a factor of 10.

Finally, overall precision of the analytical technique was evaluated by extraction and analysis of duplicate samples of seawater and marine sediment. In general, the resulting relative standard deviations (SDs) were excellent, being below 10% for all analytes. The reproducibility and repeatability of the method were also evaluated by realizing three successive injections of the same sample and re-analyzing a same batch of samples one month after

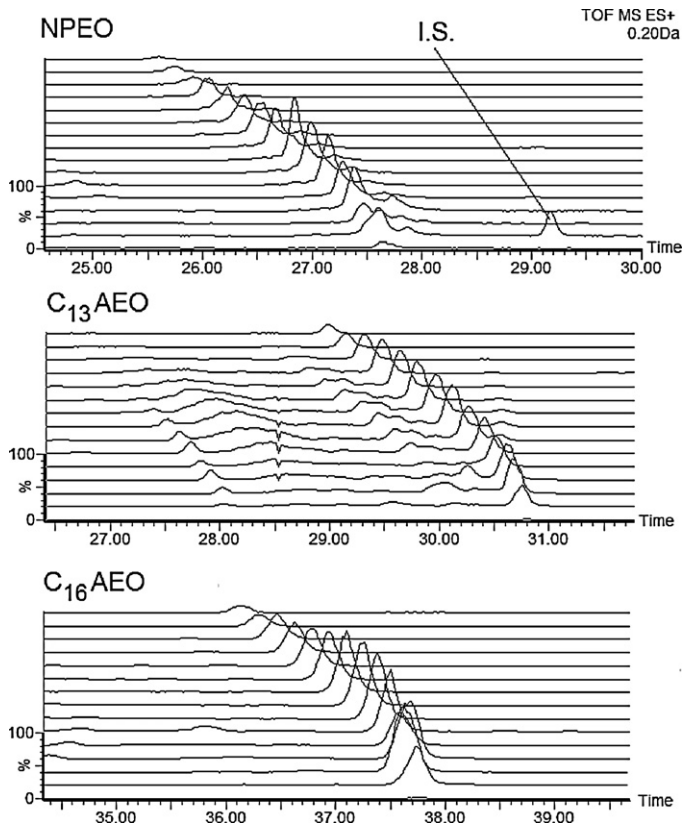

Fig. 3. LC–ToF-MS positive ion mode chromatograms showing the occurrence of NPEO, C_{13}AEO and C_{16}AEO ethoxymers in a wastewater sample. Internal standard n-$NPEO_3$ is also displayed.

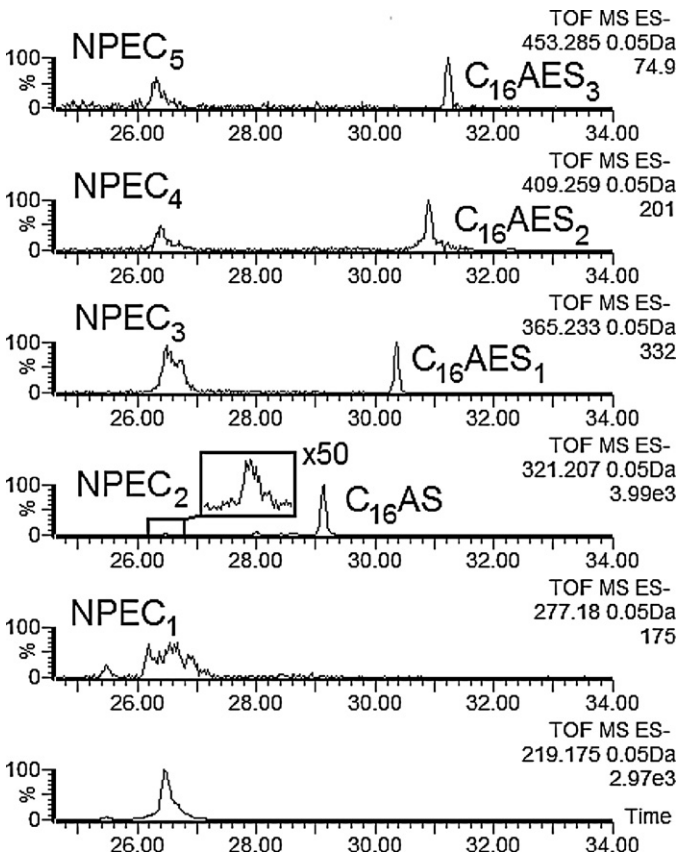

Fig. 4. LC–ToF-MS negative ion mode chromatograms showing the occurrence of different NPEC ethoxymers (m/z resolution = 0.05 Da) and their specific ESI-CID-MS fragment (m/z = 219.1749) in a pore water sample. A homologous series having the same nominal mass was also identified as alkyl sulfate (AS) and alkyl ether sulfates (AES) having a 16 carbon atom alkyl chain.

their first analysis. Resulting SDs were also below 10% for all analytes.

3.4. Advantages of using LC–ToF-MS for the analysis of environmental samples

Compared to most widely used instruments operating in MRM, ToF-MS has the ability to collect data over a wide range of m/z ratios without sacrificing sensitivity, and mass measurement accuracy, which is adequate for conformational purposes or determination of elemental composition, and to resolve interferences away from signals of interest. These capabilities also make LC–ToF-MS an attractive tool for identifying non-target compounds in environmental samples. In this study, detection was carried out in negative ionization mode for anionic compounds (LAS, SPC and NPEC) whereas NPEO, AEO and PEG were determined by separate HPLC–MS analysis conducted in positive mode. Therefore, full information for a wide range of accurate masses (up to m/z = 1000) in both ionization modes is available, which is extremely useful when screening for target and non-target compounds. An example of this is illustrated in Fig. 4, which shows the detection of NPEC in pore water from marine sediments. These metabolites have been described to form during the aerobic degradation of NPEO in surface water [9]. Due to their greater environmental persistence, only $NPEC_{1-2}$, identified as weak endocrine disrupting compounds (EDCs), were targeted compounds in this study. Full-scan analysis by ToF-MS of this sample (Fig. 4), however, not only revealed the presence of NPEC having more than 2EO units, but also a corresponding series of unknown ions with the same nominal masses as the NPEC series that were also separated by 44 mass units ($-CH_2CH_2O-$) when using a mass window of 0.05 Da around the target ions. Narrowing the mass window to 0.01 Da improved the separation of these series, and the presence of a specific CID fragment m/z 97 allowed us to identify them as alkyl sulfates (AS) and alkyl ether sulfate (AES) ethoxymers having 16 carbon units in their alkyl chain. In this case, the chromatographic separation of the different components helped to prevent mass overlaps that could be difficult to solve by only accurate mass measurement due to the proximity in mass of both NPEC and C_{16}AES (e.g., m/z 365.2328 and 365.2362 for $NPEC_3$ and $C_{16}AES_1$, respectively).

The full spectral sensitivity of ToF-MS provides other options for identification of important non-targeted environmental contaminants. One example is illustrated in Fig. 5, which shows a map representing peak intensity as a function of m/z and retention time in a filtered wastewater influent sample. Patterns of sequentially eluting homologous series separated by 44 or 58 Da can be observed. Some of the series are already target compounds, such as PEG, NPEO and C-even numbered AEO (C_{12} to C_{18}), that can be identified and quantified using commercially available standards. Other homologous series of compounds can also be observed using this feature. In the case of these non-target analytes, they can be easily identified as compounds previously reported to occur in surface waters [21,25]: octylphenol ethoxylates (OPEO), odd carbon numbered AEO and polypropyleneglycols (PPG).

Another advantage of full spectral sensitivity is that multiple confirmation measures can be obtained and, additionally, be used for quantification of target compounds. This approach consists in using several ions showing different signal intensities for the same compound. An example is illustrated in Fig. 6, displaying several adducts ($[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$) for PEG ethoxymers. The affinity of these compounds for the ammonium dissolved in the

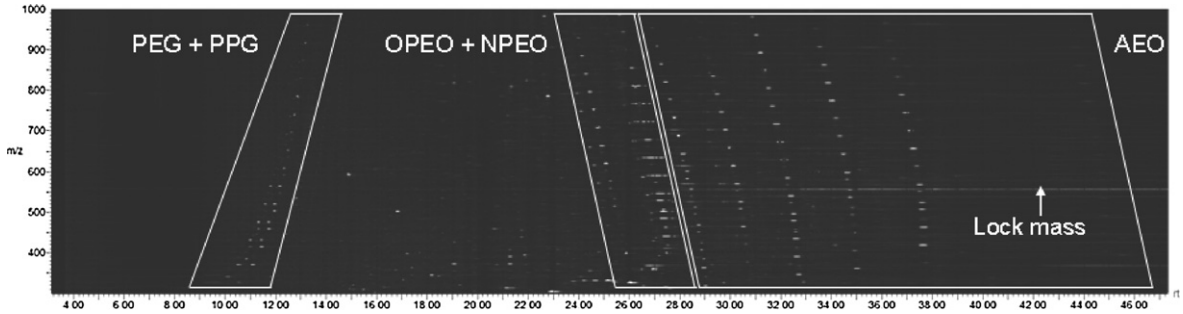

Fig. 5. LC–ToF-MS positive ion mode map showing the occurrence of different homologous series in a wastewater sample. Target compounds (PEG, AEO and NPEO) are indicated, but also other contaminants such as octylphenol polyethoxylates (OPEO) and polypropyleneglycols (PPG) were identified. Lock mass corresponds to $m/z = 556.2771$ (leucine enkephalin).

mobile phase changes depending on their number of EO units: as it was discussed above, it is higher for PEG having longer ethoxylated chains. The use of an ammonium buffer also minimizes the formation of sodium adducts and improves the signal of $[M+H]^+$ for shorter ethoxymers. As a consequence, $[M+NH_4]^+$ ions show the highest intensity for those PEG having more than 11EO units, whereas $[M+H]^+$ ions are more abundant when EO < 11. Identification and quantification are often carried out using these two adducts, but, when concentrations are high enough to exceed the linear region of the calibration curves, using third and fourth ions such as $[M+Na]^+$ or the ^{13}C isotope of the main ion $[M+NH_4]^+$ or $[M+H]^+$ becomes useful for obtaining a more reliable quantification (see Fig. 6).

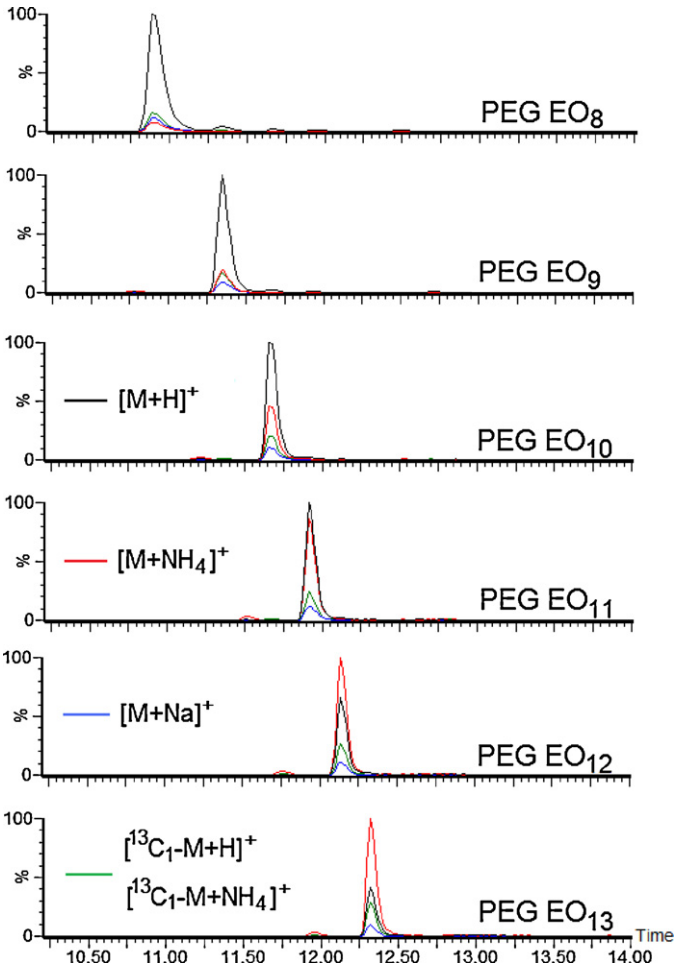

Fig. 6. LC–ToF-MS positive ion mode chromatograms showing the occurrence of PEG ethoxymers in a sediment sample. Relative signals for the molecular ions $[M+H]^+$, different adducts (NH_4^+ and Na^+) and ^{13}C isotopes are displayed.

3.5. Application of the method for the analysis of real samples

Presentation and discussion of the complete data set are beyond the scope of this work, therefore, we will focus on a comparison of the concentration of analytes in selected wastewater (influent and effluent), seawater, suspended solids and surface sediment samples (Table 2). First, we carried out a brief study on the removal efficiency of the sewage plant that serves our university (Stony Brook WWTP). Concentrations in the influent are approximately 1–2 orders of magnitude higher than those measured in the effluent (Table 2). Removal is very efficient for all target compounds, especially for surfactants, which agrees with more detailed works on this topic [2,3], showing removal percentages higher than 99% for all of them excepting the most hydrophobic C_{18}AEO, which is likely more resistant to biodegradation due to its limited bioavailability. Net removal of surfactant metabolites is also good for SPC (91.5%) and PEG (96.6%) in spite of these compounds also being produced during sewage treatment. In the case of NPEC, there is a net production of this contaminant (360%) as a consequence of the degradation of NPEO. In prior work [26] it has been estimated that carboxylated metabolites can account for more than 66% of all surfactant metabolites leaving the plants, whereas parent compounds are often found at lower concentrations, usually below 10 ppb for AEO and NPEO [22,24].

Samples were also taken in Long Island Sound, where Stony Brook WWTP discharges. Table 2 shows concentrations for target compounds in filtered seawater, suspended solids and surface sediments. In agreement to what we have observed in wastewater effluent samples, concentrations of degradation products such as SPC, NPEC and PEG are significantly higher than those for LAS, NPEO and AEO (<1 ppb). Their presence in natural waters is due to a combination of discharges from nearby WWTPs and in situ degradation of surfactants. Additionally, surfactant metabolites can sometimes degrade at slower rates than their parent compounds and persist at relatively high levels in aquatic environments. For example, concentrations up to 8.1 μg/L of NPECs have been measured in Dutch estuaries [27], and contamination of seawater by PEG was reported by Crescenzi et al. [28] in coastal seawater at distances up to 16 nautical miles from a sewage impacted estuary. In contrast, analysis of suspended solids and sediments shows that concentrations of surfactants in these matrices are higher than those for their degradation products. Thus, LAS, the most world-widely used anionic surfactant, presents values of 23,200 and 356 μg/kg in suspended solids and sediments, respectively, whereas their much more polar metabolites (SPC) were barely detected. This corresponds to the increased sorption of the relatively hydrophobic surfactants onto particulate materials. The most dramatic example is represented

Table 2
Concentration of target compounds (ppb = μg/L or μg/kg) in wastewater (influent and effluent from Stony Brook WWTP, NY), surface sediments, suspended solids and filtered seawater from Long Island Sound (NY). Average removal percentages after wastewater treatment and particulate-dissolved distribution coefficients (*K*) are also shown.

Compound	Influent	Effluent	Removal (%)	Sediment	S. solids	Seawater	log *K*
LAS	203.0	0.21	99.9	356	23237	0.82	4.5
SPC	41.1	3.50	91.5	<1	<1	11.01	–
NPEO	24.6	0.29	98.8	527	5696	0.74	3.9
NPEC	4.9	22.69	−360.2	204	1440	1.22	3.1
C_{12}AEO	4.8	0.05	99.0	41	1556	0.08	4.3
C_{14}AEO	8.8	0.03	99.7	49	500	0.01	4.8
C_{16}AEO	4.6	0.01	99.8	9	301	<0.01	5.0
C_{18}AEO	0.8	0.02	98.0	12	727	<0.01	5.4
PEG	35.8	1.23	96.6	1491	3439	0.63	3.7

by the different AEO homologues (Table 2), which are often not detected in waters (only C_{12}AEO, the most soluble homologue, was found by Krogh et al. [29] in a previous study). The abundance of AEO in suspended solids increases with the length of the alkyl chain, showing the highest distribution coefficient values between particulate and dissolved phases (log *K* ranges from 4.3 to 5.4 from C_{12}AEO to C_{18}AEO) among all target compounds. Finally, it is remarkable that NPEC and, especially, PEG, which are relatively polar metabolites, could be detected in suspended solids and sediments at concentrations that were comparable to those for NPEO and AEO. The presence of NPEC has been previously described in estuarine sediments by Jonkers et al. [27] in the Rhine and Scheldt estuaries, where values up to 185 and 239 μg/kg were found. In the case of PEG, however, these are the first data on their occurrence in sediments under our knowledge, and they are particularly significant taking into account that their concentration exceeds that for the rest of target compounds. Elevated levels of these weakly particle reactive compounds in sorbed phases can be explained by a combination of high production volume (PEG are not only AEO degradation products, but also are produced and used by themselves in a myriad of pharmaceuticals and personal care products), relatively slow degradation in seawater [28] and sorption enhanced by hydrophilic interactions of the ethoxylated chain with clays [30], although more research is currently under way.

4. Conclusions

This paper describes the development of a methodology aimed at comprehensive analysis of the most world-widely used surfactants and many of their main degradation metabolites in aqueous and solid environmental samples. It is especially focused on the use of ToF-MS, much less employed that other MS detectors for the determination of organic contaminants in aquatic systems. Several examples have been discussed here showing how to use the advantages of time-of-flight analyzers (high resolving power, accurate mass measurement, and full spectral sensitivity in a wide mass range) not only for identification and quantification of a very large number of target analytes, but also, for conducting survey of non-target compounds. LC–ToF-MS can thus constitute a reliable alternative or complementary approach for the environmental monitoring of target and non-target surfactants and their metabolites on which, in some cases like AEO and PEG, available information is scarce. With the method described, data on the occurrence of PEG in matrices such as sediment and suspended solids are provided for the first time.

Acknowledgments

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