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Journal of Chromatography A, 1067 (2005) 161–170
JOURNAL OF CHROMATOGRAPHY A
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Determination of non-ionic polyethoxylated surfactants in wastewater and river water by mixed hemimicelle extraction and liquid chromatography–ion trap mass spectrometry

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Available online 28 November 2004

Abstract

The capability of hemimicelles-based solid phase extraction (SPE)/liquid chromatography/atmospheric pressure chemical ionisation in positive mode, ion trap mass spectrometry (LC/(APCI$^+$-IT)–MS) for the concentration, separation and quantitation of non-ionic surfactants has been investigated. Concentration was based on the formation of mixed aggregates of analytes [alkylphenol ethoxylates (APE, octyl and nonyl) and alkyl ethoxylates (AE, C_{12}–C_{16})] with the anionic surfactant sodium dodecyl sulphate (SDS) that is adsorbed on alumina. Parameters affecting SPE were investigated on the basis that hemimicelles are dynamic entities in equilibrium with the aqueous phase. The performance of ion trap mass spectrometry for MS and MS/MS quantitation of non-ionic homologues was assessed. Recoveries of analytes from wastewater influent and effluent and river water samples ranged between 91 and 98% and were found independent on the length of the alkyl chain under the optimised conditions. Anionic surfactants did not interfere to the levels found in environmental samples. The detection limits ranged between 14 and 111 ng/l for wastewater influent, 10 and 40 for wastewater effluent and 4 and 35 for river water, after concentration of 250, 500 and 750 ml of sample, respectively. The approach was applied to the determination of AE and APE in influent and effluent samples from four wastewater treatment plants and four river samples. The concentrations of individual non-ionic surfactants found ranged between 0.3 and 373 μg/l.

Keywords: Alkylphenol ethoxylates; Water analysis

1. Introduction

The non-ionic surfactants alkylphenol ethoxylates (APE, C_nH_{2n+1}-C_6H_4-$(OCH_2CH_2)_xOH$; $n = 8$, 9; $x = 1$–23) and alkyl ethoxylates (AE, C_nH_{2n+1}-$(OCH_2CH_2)_xOH$; $n = 12$–16; $x = 1$–23) are widely used in detergents, resins, plastics, stabilisers in the polymer industry and paints [1]. They are not completely removed in wastewater treatment plants (WWTPs) so, their monitoring is necessary in influents and effluents of WWTPs as well as in the surrounding natural environment in order to assess both the removal efficiency of non-ionics and their impact on aquatic life.

Several papers have discussed the different methods of sample preparation, chromatographic separation and detection systems used in the analysis of non-ionic surfactants in environmental samples [2–8]. Although a wide variety of extraction methods have been used [9–12], SPE with different sorbents such as alkyl-bonded silica [12], graphitised carbon black [5] and polymeric resins [6,13], has become the most popular sample preparation method. A common characteristic of the different SPE packing materials is that extraction efficiency for non-ionic surfactants decreases when the length of the hydrocarbon chain increases. Thus, it ranges from 97 to 85% and from 93 to 35% when using graphitised carbon black [5] and polymeric resins [8], respectively, for concentrating AE with carbon atom numbers varying between 10 and 18. The recoveries obtained using alkyl-bonded silica are very poor and therefore this pack-

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doi:10.1016/j.chroma.2004.11.017

ing material is not recommended for extraction of non-ionics [5].

Mass spectrometry detection is increasingly being used for the quantitation of non-ionics [14] because of the complexity of the technical AE/APE products. The limited volatility of the highly ethoxylated AE/APE restricts the use of GC/MS to the analysis of the short ethoxyl chain non-ionics unless derivatization is incorporated into the protocol. LC/MS is currently the most suited technique; LC separates AE and APE according to their alkyl chain length and MS allows the identification of oligomers that differ in the number of ethoxy units. Both, APCI [6] and ESI [5] have been used for ionisation of analytes, and quadrupole [2–8] and triple-quadrupole [15–19] mass spectrometers have been utilised for quantitative analysis and identification. Mass spectra of non-ionics are very characteristic with $M \pm 44$ ions corresponding to the entire ethoxylate series. MS/MS spectra contain characteristic fragments that constitute additional confirmation for non-ionics and usually provide higher selectivity in quantitative determinations.

One of the main problems at which mass quantification of non-ionics is confronted derives from the different distribution of oligomers in samples and standards. This fact forces to use the masses corresponding to all ethoxamers (usually $x = 1$–23) for quantitation and therefore full scan MS should be selected for working. We have previously proved [20] the influence of the number of masses considered for quantitation on the concentration of octylphenol ethoxylate found in a sewage sludge sample. Under- or overestimation of non-ionics will probably occur if all the ethoxymer masses are not used for calibration. For this purpose, ion trap mass spectrometry surpass to quadrupole mass filter instruments since similar sensitivity is obtained using SIM and Full scan [21]. On the other hand, ion trap mass spectrometers, with their ability to perform MS^n experiments, are well suited for MS/MS quantitative analysis and for many identification purposes.

This paper deals with the assessment of the performance of the ion trap mass analyser for the quantitative and qualitative analysis of AE and APE after separation of their homologues by LC. The capability of hemimicelles/admicelles to concentrate non-ionic surfactants from sewage and river water is also examined.

Hemimicelles and admicelles have been recently reported as excellent sorbents for the solid-phase extraction (SPE) of organic compounds of different nature such as hydrophobics [22], ionics [23] and amphiphilics [24]. These surfactant aggregates are produced by the adsorption of ionic surfactants on the surface of mineral oxides [25–32]. Hemimicelles consist of monolayers of surfactants adsorbing head down on the oppositely charged surface of the oxide, whereas admicelles have the structure of bilayers with the outer surface composed of the ionic head group of the surfactant. Some of the benefits obtained with the use of these sorbents have been high extraction yields, easy elution of analytes, high breakthrough volumes and high flow rate for sample loading [24]. Their capability to be used in on-line applications, with sorbent reusability, and the general considerations that should be taken into account for method development have been discussed [23].

Sodium dodecyl sulphate (SDS)-alumina hemimicelles and admicelles were examined for the concentration of APE and AE in environmental water samples, on the basis of the formation of non-ionic-anionic mixed adsorbed aggregates [33]. Reversed-phase liquid chromatography/atmospheric pressure chemical ionisation/ion trap mass spectrometry (LC/(APCI-IT)–MS) was used for the separation and quantitation/identification of AE and APE. Predominant factors influencing the formation of mixed analyte/sorbent aggregates, oligomers mass detection and product ions formation/detection were investigated. The feasibility of the method was proven by analysis of non-ionics in river water samples and sewage samples from various watewater treatment plants.

2. Experimental

2.1. Chemicals and materials

All reagents were of analytical reagent-grade and were used as supplied. Sodium dodecyl sulphate (SDS) was obtained from Aldrich (Milwaukee, WI). Hydrochloric, nitric and acetic acids, ammonia, and HPLC-grade acetonitrile and methanol were obtained from Panreac (Sevilla, Spain). All the individual polyethoxylated surfactants used as standards corresponded to pure homologues containing a mixture of oligomers with a determined average (x) of ethoxy units. Octylphenol ethoxylate (OPE_x, $x = 9$–10 Triton X-100) was obtained from Serva (Barcelona, Spain), nonylphenol ethoxylate (NPE_x, $x = 6$) from Masso y Carol (Barcelona, Spain), the alcohol polyethoxylates C_{12} ($C_{12}E_x$, $x = 4$) and C_{16} ($C_{16}E_x$, $x = 2$) were purchased from Sigma (Madrid, Spain), and C_{10} ($C_{10}E_x$, $x = 3$) and C_{14} ($C_{x4}E_x$, $x = 4$) from Fluka (Madrid, Spain). Stock solutions of the non-ionic surfactants were prepared in methanol. Alumina (γ-form, for column chromatography) was supplied by Sigma (St. Louis, MO). The physical properties of this mineral oxide were as follows: surface area (155 m^2/g), point of zero charge (pcz, 8.5), particle diameter range (50–200 μm, mean value 100 μm), mean pore size (58 Å), density (3.97 g/cm^3). Bond Elut Jr. cartridge columns filled with 500 mg alumina were obtained from Varian (Victoria, Australia).

2.2. Sample collection

Influent and effluent water samples were collected from different municipal WWTPs (Linares, Puentegenil and Morón de la Frontera) and a private WWTP (Progalectric factory) in the south of Spain in December 2003. Linares WWTP receives about the same levels of domestic and industrial wastewater, Puentegenil WWTP receives about 80% of industrial effluents (mainly from food industries) mixed

with about 20% domestic wastewaters, Morón de la Frontera receives mainly domestic water and Progalectric receives raw water from a factory of metals treatment. River samples were taken from various rivers flowing by Córdoba city (Pedroche and Rabanales), Córdoba province (Guadarramilla) and Seville province (Guadiana) in January 2004. Samples were collected in dark glass containers. They were immediately filtered through 0.45 μm filters (Whatman GF/F, Osmonics, France) in order to remove suspended solids and then they were adjusted to pH 2 by the addition of concentrated nitric acid. Finally, they were stored at 4 °C.

2.3. Admicelles extraction

The Bond Elut Jr. cartridge columns were conditioned with 10 ml of Milli-Q water. Then, hemimicelles were formed on the alumina by passing a 50 ml 0.01 M nitric acid solution containing 12.5 mg of SDS. Samples (250 ml of influent, 500 ml of effluent and 750 ml of river water) were preconcentrated on the hemimicelles and non-ionic surfactants were eluted with 1 ml of methanol. Solution and sample loading was performed by using a vacuum pump (Eyela A35, Rikakikai Co., Tokyo) at a flow rate of 20 ml/min. Aliquots of the eluate were injected into the LC/(APCI-IT)–MS system.

2.4. Liquid chromatography/mass spectrometry

A liquid chromatography–ion trap mass spectrometry system (1100 Series LC/MSD, Agilent Technologies, Waldbronn, Germany), equipped with an automatic injector, was used for separation and quantification/identification of analytes. The injection volume was set at 20 μl. The stationary phase column was a 15 cm Zorbax Eclipse XDB-C_8 column (5 μm particle diameter and 4.6 mm i.d.) supplied by Agilent. Chromatography was adjusted for eluting all the oligomers of AE and APE as single peaks. The mobile phase consisted of acetonitrile-methanol (50:50, solvent A) and water (solvent B), both containing 1.5% ammonium acetate. The gradient elution program was: isocratic conditions with 70% A:30% B for 5 min and then linear gradient from 30 to 5% B in 20 min. The flow-rate was set at 1 ml/min. The diver valve was programmed to send the mobile phase containing SDS and the most polar matrix compounds to waste. So, only 7 min after the beginning of the elution gradient program, the eluted components were sent to the ionisation source.

Surfactant analysis was carried out in the "APCI (+)" mode. Parameters affecting AE/APE ionisation and transport of ions were optimised by directly analysing the homologues (10 mg/l) in methanol using a KD Scientific, model 100, syringe pump (New Hope, MN, USA) at 400 μl/h. The set of parameters selected was as follows: capillary voltage 3.5 kV; corona discharge current 4000 nA; source and vaporizer temperature 300 and 350 °C, respectively; drying gas flow 1 l/min; nebulizer gas 50 psi; capillary exit voltage 180 V and skimmer voltage 25 V. Parameters influencing the performance of the ion trap were optimised by LC separation of AE/APE homologues. The selected values for MS or MS/MS measurements were: trap drive 50; ion charge control 20,000, resonance excitation 1 V for APE and 1.2 V for AE, fragmentation time 100 ms. MS quantification was carried out under full-scan conditions (*m/z* range 200–1200) by measuring the peak areas of the extracted molecular ion chromatogram for each homologue, at the *m/z* values corresponding to the $[M + NH_4]^+$ ions obtained for the 20 oligomers ($x = 1$–20) that typically can make up AE and APE homologues. So, the mass spectra for homologues showed equidistant signals with mass differences of 44 corresponding to the different oligomers present. Smooth chromatograms were obtained by using the Gauss function (width = 3 points, cycles = 1). The target mass for trapping ions was fixed to the *m/z* value corresponding to a central oligomer (e.g. $x = 10$). MS/MS quantification involved the isolation of 10 parent ions (the maximum permitted by the instrument, $x = 1$–10) from each homologue. The isolation width was set to 12 *m/z* units. Excitation of the ions was accomplished through collision with helium. The following characteristic product ion masses were used: $C_{12}E_x$ 257, $C_{14}E_x$ 285, $C_{16}E_x$ 313, NPE_x 291 and OPE_x 277. Correlation between peak areas and homologue concentrations (2–2000 ng absolute amounts) were determined by linear regression and were in the range 0.998–0.9992. The non-ionic surfactant $C_{10}E_x$ (200 g absolute amount injected), that is found in negligible amounts in commercial AE mixtures, was used as internal standard for quantification [5]. For this purpose, the peak area of the extracted ion chromatogram at the *m/z* corresponding to the $[M + NH_4]^+$ ions obtained for the 20 possible $C_{10}E_x$ oligomers was measured for MS quantitation. The characteristic product ion 229 was used for MS/MS measurements.

2.5. Adsorption studies

The adsorption isotherms of SDS on alumina in the presence of different amounts of non-ionic surfactants were obtained by adding variable amounts of SDS (0–300 mg) and a constant amount of non-ionics (total amount 0.5 and 15 mg; 0.1 and 3 mg of each non-ionic investigated) to an aqueous suspension of 1 g of alumina. The pH of the solution was adjusted to 2.0 with nitric acid and the solution made up to 25 ml with distilled water. After vigorous stirring of the solution for 5 min, the mixture was centrifuged at 5000 rpm for 5 min. The concentrations of SDS in the supernatants were determined by LC/(ESI-IT)–MS in the ESI negative mode. The mobile phase was methanol: water 80:20. Under isocratic conditions SDS eluted at 3.2 min. The operational conditions of the ESI interface were: capillary voltage 5.0 kV; source temperature 350 °C; drying gas flow 10 l/min; nebulizer gas 80 psi; capillary exit and skimmer voltage −100 and −40 V, respectively; trap drive 38; maximal accumulation time, 150 ms, and mass scan range 200–300 *m/z*. Quantification was carried out using the extracted molecular ion chromatogram, and the corresponding peak area was measured. Correlation between peak areas and SDS concentration was obtained in the

range 25–500 ng (absolute amount injected) with a correlation coefficient of about 0.998.

Adsorption of non-ionic surfactants on SDS admicelles as a function of SDS concentration was carried out at two non-ionic concentrations (total non-ionics 0.5 and 15 mg; 0.1 and 3 mg of each non-ionic, respectively) Bond Elut Jr. cartridge columns (500 mg alumina) were conditioned with 10 ml of Milli-Q water and loaded with varying amounts of SDS (0–150 mg) dissolved in 10 ml of 0.01 M nitric acid. Then, 25 ml of aqueous solutions containing 0.01 M nitric acid, non-ionics and an amount of SDS equal to the surfactant monomers in the aqueous phase for each adsorbed amount of SDS on alumina (which can be inferred from SDS adsorption isotherms) were prepared, passed through the cartridges and eluted with 1 ml methanol. Non-ionics were determined in the eluted solutions by LC/(APCI-IT)–MS, as specified above.

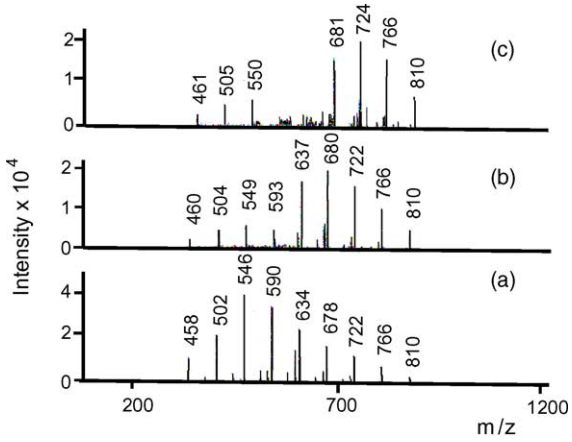

Fig. 1. Mass spectra obtained for NPE_x at different ion charge control (ICC) values fixed in the ion trap. (a) 50.000, (b) 70.000 and (c) 100.000; $[NPE_x] = 10$ mg/l.

3. Results and discussion

3.1. MS and MS/MS spectrometry studies

Ion trap mass spectrometry analysis of AE/APE homologues was based on the detection of $[M + NH_4]^+ \pm 44$ ions. The observed behaviour from the study of variables affecting APCI ionisation and transport of ions was similar to that found with quadrupolar mass filter instruments [2–8]. Below, the main parameters influencing the storage of parent ions into the trap are commented.

The ion charge control (ICC), parameter that fix the total ion current permitted in the trap during ion storage, increased the peak area measured for each homologue when its value raised from 5000 to 20,000, due to the higher number of ions stored in the trap, and then the peak area progressively decreased at higher ICC values. Space charge effects, which occur when too many ions are stored in the trap, were evident for ICC values from 70,000 [compare mass spectra for NPE in Fig. 1a (ICC 50.000) and b, c (ICC 70.000 and 100.000, respectively)]. Mass shifts of even three/four units were produced for the lower oligomers and although the intensity decreased for all of them, this decreasing was comparatively greater for the lower *m/z* oligomers so, the oligomeric distribution drastically changed. Similar behaviour was observed for all target compounds, so an ICC value of 20.000 is recommended for working.

The effect of reducing the scan range used for analysis was assessed for the standard $C_{14}E_x$ since its mass spectrum shows a single peak at *m/z* 408. The area of the chromatographic peak hardly decreased (about 5%) when the scan range varied from 200–1200 to 408 ± 5 *m/z* since reducing the acquisition range in an ion trap only shortens one part of the scan program (e.g. the time spent to production of the spectrum). The inherent high sensitivity of the ion trap working under full-scan conditions is one of the main advantages when compared with quadrupole mass filter instruments. The target mass, parameter that control the transport efficiency of oligomers to the ion trap, was fixed to a mass central value of the scan range (e.g. the mass of the oligomer with 10 ethoxy units) in order to have maximum sensitivity. The transport efficiency is maximal for the selected mass value $\pm$ 100 mass units; therefore the oligomer distribution obtained in the mass spectrum changes if the mass of a very short o high ethoxylated oligomer is selected as target.

Ion trap MS/MS spectrometry studies were conducted to determine the fragmentation pattern of AE/APE obtained by this technique. Due to instrumental restrictions, only molecular ions corresponding to 10 oligomers ($x = 1$–10) were isolated and fragmented from each homologue. These ions corresponded to the most abundant oligomers expected in environmental samples due to degradation. Fig. 2 depicts the full scan MS (a) and MS/MS (b) spectra obtained from NPE_x and a scheme showing the fragmentation behaviour. This pattern was similar for all AE/APE homologues and the fragment ions obtained were similar to those found by other mass techniques [15,18]. Parent ions corresponding to oligomers disappeared. The most abundant fragment ions (A- and B-type) resulted from charge site-initiated decomposition and subsequent loss of ethoxy units. A-type ion series were characteristic for the homologues. The base peak for all oligormers was observed for A-type ions at $x = 2$ and the masses of these peaks were selected for measurement of homologues (e.g. $C_{12}E_x$ 257, $C_{14}E_x$ 285, $C_{16}E_x$ 313, NPE_x 291 and OPE_x 277). On the other hand, formation of $[C_nH_{2n+1}]^+$ ions from chain alky breakdown (e.g. in Fig. 2b ions at *m/z* 85 and 99 corresponding to C_6, C_7 alkyl chains, respectively) was also observed, but their relative abundance was very low.

The abundance of fragment ions was dependent on the resonance excitation applied. The highest ion intensity signal occurred using resonance excitation between about 0.9 and 1.1 V for APE and 1.0–1.3 for AE. Maximal signal intensity was obtained for a fragmentation time around 100 ms.

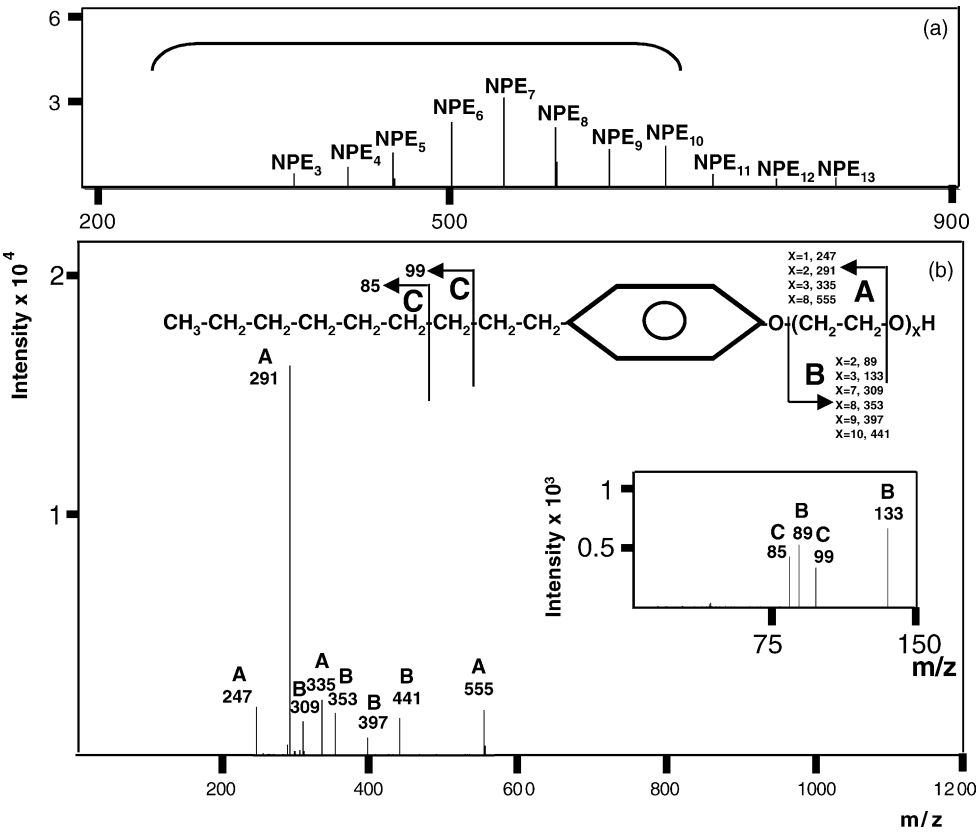

Fig. 2. (a) Mass spectrum from a NPE_x standard solution (10 mg/l) and (b) the corresponding product ion spectrum.

3.2. SDS hemimicelle-based SPE

3.2.1. Coadsorption of SDS and non-ionic surfactants on alumina

Method development involving hemimicelles/admicelles is only possible after knowledge of the adsorption isotherms of the surfactant used as sorbent (i.e. SDS) under the experimental conditions investigated [23]. Adsorption isotherms of anionic surfactants have been known to be modified in the presence of non-ionic surfactants for constant anionic/non-ionic molar ratios between 1 and 4 [34]. So, we investigated if the adsorption isotherm of SDS was modified in the presence of amounts of AE and APE in the range of analytical interest (e.g. at the maximal amount of non-ionics usually concentrated in SPE sorbents from environmental water samples, around 0.5–1 mg, and those of interest for fundamental studies using batch experiments, around 15 mg). The amount of SDS ranged between 0 and 300 mg, so the anionic/non-ionics molar ratio varied through the isotherm.

Fig. 3 shows the SDS experimental isotherms obtained in the absence (curve a, data from [24]) and the presence of 0.5 and 15 mg (curves b and c, respectively, data from the procedure specified in Section 2) of non-ionics. In the three regions suitable for SPE methods (hemimicelles, mixed hemimicelles/admicelles and admicelles), the SDS surfactant aggregates are in equilibrium with aqueous SDS monomers. Above the critical micellar concentration (cmc, 1417 mg/l at pH 2 and 25 °C), aqueous micelles are in equilibrium with admicelles which causes partition of analytes between both types of surfactant aggregates.

Coadsorption of non-ionics on the alumina produced different effects on the SDS adsorption isotherm (compare curves a, b and c in Fig. 3). Thus, the maximal amount of SDS adsorbed in the hemimicellar and admicellar region progressively decreased with increasing amounts of non-ionics. This effect was scarcely observable for 0.5 mg of non-ionics but clearly distinguishable for 15 mg of AE and APE. In the region where both hemimicelles and admicelles coexisted, the incorporation of SDS on the alumina occurred at two different rates, as it can be inferred from the different slopes found for the corresponding adsorption line (subregions I and II). The difference in values for these slopes was greater as the amount of non-ionics increased. The use of the admicellar region for SPE was dramatically restricted in the presence of non-ionics since two opposite effects occurred, namely, the total amount of SDS necessary to get constant SDS adsorption increased, which raised the amount of aqueous SDS monomers, and the cmc decreased (the cmc of SDS, at pH 2, 25 °C, and the presence of 0.5 and 15 mg/l of non-ionics, calculated by surface tension measurements, was 1385 and 1250 mg/l, respectively).

The adsolubilization of non-ionics (0.5 and 15 mg) on SDS-coated alumina as a function of SDS concentration was investigated. Fig. 4 shows the results obtained for 15 mg of

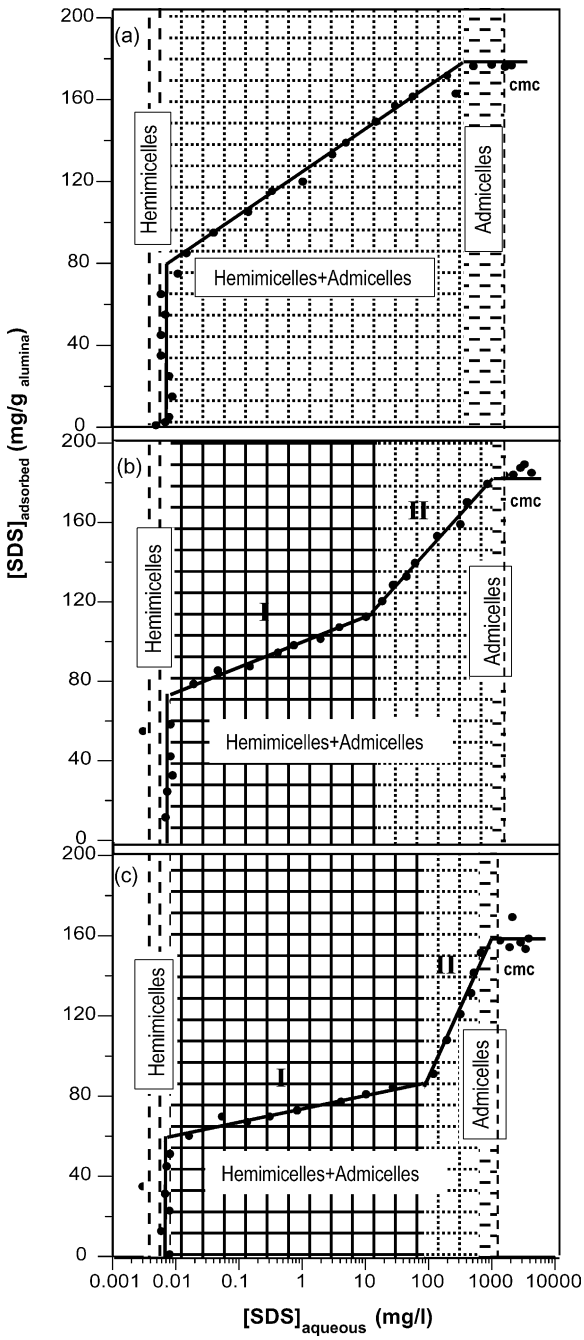

Fig. 3. Experimental adsorption isotherms for SDS on alumina at pH 2 in the absence (a) and the presence of 0.5 mg (b) and 15 mg (c) of AE and APE surfactants.

non-ionics. Similar results were obtained for 5 mg of AE and APE (data not shown). Non-ionics did not adsorb at all in the absence of SDS. Maximal adsolubilization occurred in the hemimicellar region; the maximum percentage of adsolubilization ranged between 94.1 for OPE_x and 98.3 for $C_{16}E_x$. The rate of adsolubilization was higher for the more hydrophobic surfactants (Fig. 4 inset). The amount of non-ionics retained on the alumina kept practically constant in the subregion I of the mixed hemimicelles/admicelles region, and then it progressively decreased in the subregion II (see

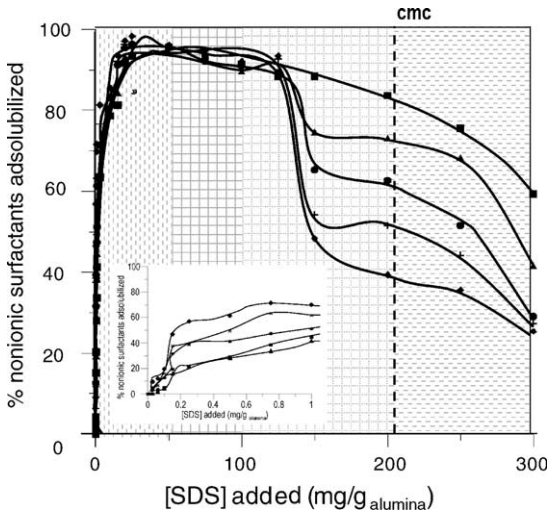

Fig. 4. Effect of the amount of SDS on the sorption of 15 mg of non-ionic surfactants on 1 g of alumina at pH 2: (♦) $C_{16}E_x$, (+) $C_{14}E_x$, (●) $C_{12}E_x$, (▲) NPE_x and (■) OPE_x. Amount of each non-ionic surfactant: 3 mg.

Fig. 3). This decrease was greater for the more hydrophobic non-ionics.

According to these results, the formation of AE, APE/SDS mixed aggregates was highly favoured in the hemimicellar region compared with the formation of pure SDS ones. This behaviour agrees with the stronger interaction reported for ethoxylated alcohols-anionic surfactants in aqueous solutions compared with those for pure surfactants [35]. In fact, when the non-ionic concentration increased (Fig. 3c), the maximal amount of SDS adsorbed in the hemimicellar region decreased, indicating that lower net alumina surface was available for the anionic surfactant. Once the alumina surface was saturated, the formation of admicelles occurred. In the subregion I, the presence of non-ionics on the alumina had a negative synergistic effect on the incorporation of SDS, and the slope of the isotherm decreased as the non-ionic concentration increased (Fig. 3 b and c). However, adsorption competition between non-ionics and SDS was established for amounts of SDS high enough, and as a result the amount of adsolubilized AE and APE decreased (Fig. 3, subregion II).

Both hemimicelles and the subregion I, where some admicelles have been produced, could be used as sorbents for concentrating these amphiphilic substances (Fig. 4). Hemimicelles were selected on the basis that the amount of SDS aqueous monomer in equilibrium with the adsorbed surfactant was high enough in the hemimicelles/admicelles region (Fig. 3b and c) to need a continuous supply of SDS during percolation of the sample in order to ensure that the amount of SDS on the alumina remained constant.

3.2.2. Optimisation

A minimal amount of SDS (about 20–25 mg SDS/g alumina) was necessary to get maximal adsolubilization independently on the amount of non-ionics tested in the range

0.5–15 mg. We selected 25 mg of SDS/g alumina for further studies.

The pH dramatically affects the charge density on the alumina surface (point charge zero: 8.5) and therefore, the amount of surfactant adsorbed on the oxide in both the hemimicellar and admicellar region. This amount decreases with increasing pH values, so it is essential to know the SDS adsorption isotherms as a function of the pH for the optimisation of this variable. We checked that in the range of pH comprised between 2 and 6 only hemimicelles were formed using 25 mg of SDS/g alumina. In this range, the percentage of adsolubilization of non-ionics kept constant. The positive charge of the oxide surface dramatically decreased around the pcz and as a result the amount of hemimicelles produced and the percentage of adsolubilization of non-ionics decreased, specially for the more hydrophobic compounds (e.g. at pH 8.5 the percentage of adsolubilization ranged between 35.2% for OPE_x and 9.1% for $C_{16}E_x$). Acid pH values (e.g. 2–3) are recommended for experimental work since breakthrough of the analytes should be delayed with decreasing pH because of the higher amount of hemimicelles formed on the alumina.

Desorption of non-ionic surfactants from the SDS-coated alumina column was studied using different organic solvents (acetonitrile, methanol), which are known to disrupt the hemimicelles. Quantitative recovery of non-ionics was observed from 1 ml of methanol, which was the organic solvent recommended for their elution.

The sample loading volume was determining by passing increasing volumes (0.025–1 l) of an aqueous solution at pH 2, containing 100 μg/l of non-ionics, through a 500 mg alumina cartridge loaded with 12.5 mg of SDS. The loading of the target analytes ranged between 2.5 and 100 μg. Quantitative recoveries were obtained up to 750 ml of solution containing 75 μg of non-ionics. The recoveries decreased for higher volumes (e.g. the recoveries for 100 μg of non-ionics contained in 1 l of solution were 75.3, 78.3, 81.9, 81.2 and 87.4% for OPE_x, NPE_x, $C_{10}E_x$, $C_{12}E_x$ and $C_{16}E_x$, respectively). The sample flow rate did not affect recoveries of non-ionics in the range comprised between 3 and 20 ml/min, which permitted a rapid treatment of samples.

3.3. Analytical performance

Calibration curves were run for each homologue in the range 2–2000 ng by using both MS and MS/MS responses. The surfactant $C_{10}E_x$ was used as internal standard in order to remove the between-day fluctuations in the sensitivity of the detector. The slope of the calibration curves was 0.0230 ± 0.0003, 0.0063 ± 0.0001, 0.0057 ± 0.0002, 0.0194 ± 0.0001 and 0.00295 ± 0.00006 for OPE_x, NPE_x, $C_{10}E_x$, $C_{12}E_x$ and $C_{16}E_x$, respectively, using MS detection, and 0.0214 ± 0.0002, 0.0058 ± 0.0001, 0.0042 ± 0.0001, 0.0121 ± 0.0001 and 0.00173 ± 0.00002 for OPE_x, NPE_x, $C_{10}E_x$, $C_{12}E_x$ and $C_{16}E_x$, respectively, using the MS/MS response from the characteristic fragment ion (see Section 2). The calibration slopes for homologues were lower using MS/MS spectrometry, mainly due to the instrumental restrictions in the number of masses that can be measured (MS $x = 1$–20; MS/MS $x = 1$–10).

The practical detection limits [36] were estimated from six independent complete determinations of analyte concentrations in typical wastewater influent and effluent and river water low-level materials. In cases where these materials could not be obtained, an estimate of the background signal was made at a representative part of the readout, adjacent to the analyte signal in the analyte-containing sample. Detection limits were calculated by using a signal-to-noise ratio of 3 (the ratio between the peak areas for each non-ionic and internal standard, and peak area of noise). The values found ranged between 14 and 111 ng/l for wastewater influent, 10 and 40 for wastewater effluent and 4 and 35 for river water.

Since environmental samples contain alkylbenzene sulphonates (LAS) at concentrations in the range 3–21 mg/l in wastewater influents and 0.09–0.9 mg/l in wastewater effluents [37], we examined their effect on both the adsorption isotherm of SDS and the determination of non-ionics. We checked that LAS adsorbed on alumina similarly to SDS and mixed anionic aggregates were produced on the oxide surface. However, the amount of LAS in the samples was low enough to no exceed the hemimicellar region and no changes on the percentage of adsolubilization of non-ionics were observed in the presence of LAS.

The possible interference of matrix components that could elute with non-ionics causing ion suppression or space-charge effects on the ion trap was assessed by comparison of the calibration curves obtained from standards and those obtained from wastewater influent and effluent and river water samples fortified with known amounts of non-ionics. Since the analytical characteristics of both type of calibrations were similar, we recommended using external calibration for determination of non-ionics in wastewater and river samples. However, when the matrix sample is expected to be very different to those investigated here, it is recommended to investigate its influence on the determination of non-ionics.

3.4. Analysis of environmental water samples

In order to check the suitability of the developed method, it was applied to the analysis of no-nionics in wastewater sam-

Table 1
Mean concentrations (μg/l) ± standard deviation (based on three replicates) of non ionic polyethoxylated surfactants in wastewater influent, analyzed by adsolubilization SPE/LC(APCI)–MS and SPE/LC(APCI)–MS/MS

Detection mode	OPE_x	NPE_x	$C_{12}E_x$	$C_{14}E_x$	$C_{16}E_x$
Lucena WWTP					
MS	17 ± 2	214 ± 12	5.2 ± 0.4	11 ± 1	2.6 ± 0.3
MS/MS	12 ± 1	185 ± 8	4 ± 1	6.9 ± 0.4	n.d.
Arahal WWTP					
MS	6.3 ± 0.4	161 ± 11	15 ± 1	2.1 ± 0.3	n.d.
MS/MS	4.1 ± 0.2	122 ± 7	8.1 ± 0.6	n.d.	n.d.

n.d.: not detected.

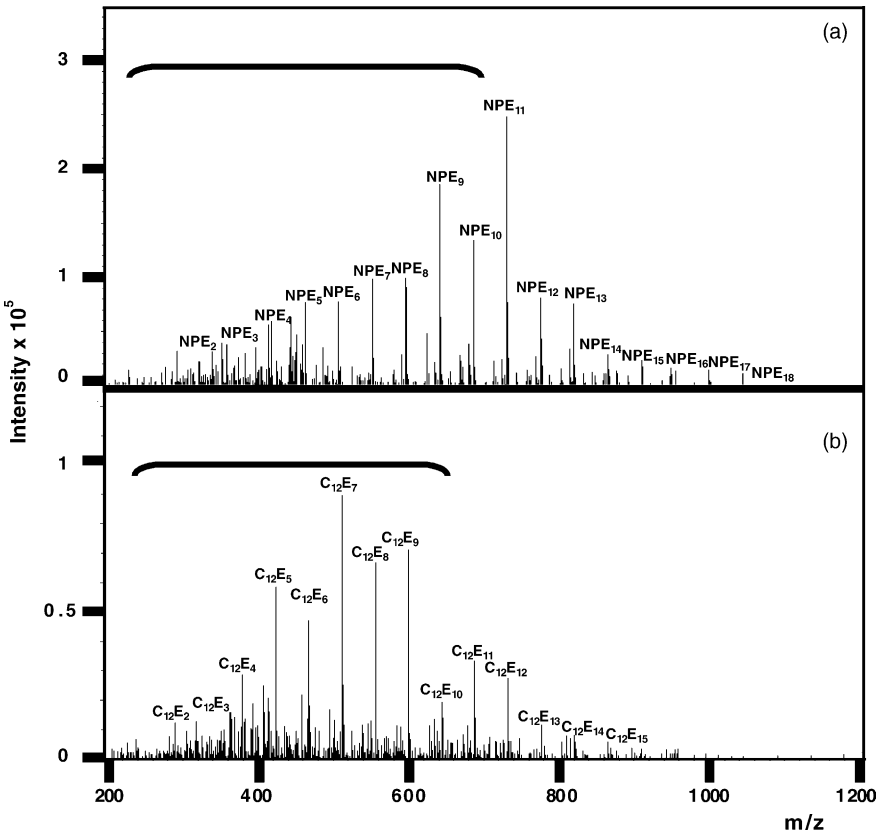

Fig. 5. Mass spectrum obtained for (a) NPE_x and (b) $C_{16}E_x$ from wastewater influent samples collected in (a) Lucena and (b) Arahal WWTPs.

Table 2
Concentrations (μg/l) ± standard deviation (based on three replicates) and recoveries (%) of target analytes found in wastewater influent and effluent samples and river water samples

Sample location	OPE_x	NPE_x	$C_{12}E_x$	$C_{14}E_x$	$C_{16}E_x$
WWTP Influent[a]					
Linares	30 ± 2	198 ± 2	188 ± 7	30 ± 2	15 ± 2
Puente Genil	17 ± 4	222 ± 17	373 ± 11	86.7 ± 0.4	44 ± 4
Morón de la Frontera	11 ± 2	167 ± 3	222 ± 8	73 ± 3	24 ± 1
Progalectric	1.4 ± 0.2	41 ± 4	157 ± 10	21 ± 2	< LOD
Spiked Linares sample[b] (200 μg/L)	96	94	92	95	97
WWTP effluent[c]					
Linares	0.6 ± 0.2	37 ± 5	54 ± 6	6 ± 1	< LOD
Puente Genil	3.9 ± 0.3	54 ± 5	117 ± 8	17 ± 1	9 ± 1
Morón de la Frontera	3.1 ± 0.4	34.6 ± 4	28 ± 4	4 ± 1	< LOD
Progalectric	0.6 ± 0.1	25 ± 4	16 ± 1	6.2 ± 0.4	< LOD
Spiked Puentegenil sample[b] (100 μg/L)	97	99	96	99	95
River water[d]					
Rabanales	7 ± 1	4 ± 1	9 ± 1	1.1 ± 0.2	< LOD
Guadarramilla	8 ± 1	12 ± 2	14 ± 3	2.9 ± 0.3	11 ± 1
Guadiana	0.3 ± 0.1	4 ± 1	6 ± 1	1.8 ± 0.3	< LOD
Pedroche	< LOD	5 ± 1	8 ± 2	0.8 ± 0.1	3 ± 1
Spiked Pedroche sample[b] (50 μg/L)	93	96	92	91	93

[a] Sample volume 250 ml.
[b] $n = 4$, range of R.S.D. values 2–8%; < LOD: lower than the detection limit.
[c] Sample volume 500 ml.
[d] Sample volume 750 ml.

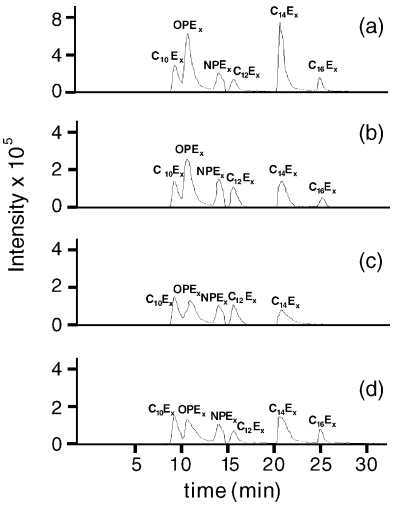

Fig. 6. LC/MS extracted ion chromatograms obtained from (a) a standard solution, (b) a wastewater influent sample (Linares WWTP in Jaén, Spain), (c) a wastewater effluent sample (Linares WWTP in Jaén. Spain) and (d) a river water sample (Guadarramilla, flowing by Córdoba province, Spain).

ples from different WWTPs and various river water samples. Table 1 shows, as an example, the concentrations found for the target compounds in two WWTP influents using MS and MS/MS detection. Lower concentrations were always found for all the homologues from MS/MS responses. These results were probably caused by the no quantitation of the higher oligomers ($x = 111–20$) which were also present in the samples analysed (Fig. 5). Although it is expected that similar results are obtained by MS and MS/MS detection when the amount of the higher oligomers is negligible, MS spectrometry will give more accurate results for most of the samples, and therefore this is the detection mode recommended for AE and APE in environment. Table 2 shows the concentration of homologues found in different samples using MS detection. The sample volume used for analysis of non-ionics in WWTP influent and effluent and river was according to the homologue concentrations expected. Lower concentrations were found for OPE_x, and $C_{16}E_x$ in all samples analysed, probably due to the lower use of OPE_x in commercial formulations and the strong adsorption of $C_{16}E_x$ in sewage sludge. Mostly, quantitation of $C_{16}E_x$ will require the use of higher sample volumes. Total method recoveries were assessed by analysing the same samples spiked with a standard mixture of analytes to give a final concentration of 200, 100 and 50 μg/l in influent, effluent and river water, respectively. As an example, the recoveries obtained for three samples are shown in Table 2. Recoveries above to 91% were obtained for all samples analysed. Fig. 6 shows the MS extracted ion chromatograms from a standard solution and different environmental samples.

4. Conclusions

The results obtained in this research establish the parameters affecting the determination of AE/APE using ion trap mass spectrometry and prove the ability of this mass filter analyzer for determining them in environmental samples. The different oligomer distribution in samples and standards forces to measure the masses of all oligomers present in the samples; otherwise, AE/APE concentrations will be under- or overestimated. Ion trap mass spectrometry, which features similar sensitivity in the full and SIM scan modes, should be the suited mass technique. Because only 10 masses are permitted for isolation and fragmentation in the MS/MS detection mode, this is not recommended for quatitation of AE/APE. However, the instrument can switch between full-scan MS and an ion product scan with no loss in signal, therefore, from the same chromatographic peak, you can quantify AE/APE with the maximum accuracy from single-stage mass spectrometry and corroborates their identity from MS/MS analysis.

The method developed surpasses the previous described ones [2–8] in terms of extraction efficiency (recoveries are higher than 91%). This high efficiency does not depend on the length of the alkyl chain under the optimised SPE conditions and it is a result of the strong interactions between anionic and non-ionic surfactants to form mixed hemimicelles. As consequence, lower limits of detection than in previous methods are achieved considering the same extraction sample volumes.

Acknowledgment

The authors gratefully acknowledge financial support from Spanish MCyT (Project BQU 2002-01017). They also thank to the personnel from the following municipal WWTPs for kindly collecting the sewage water samples: Linares (Jaén), Lucena and Montilla (Córdoba), and Morón de la Frontera and Arahal (Sevilla). We specially thank to the Environmental Management and Services Company (EGEMASA) from Puentegenil (Córdoba) and Progalectric factory from Montilla (Córdoba) their collaboration in providing us different type of wastewater samples.

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