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DETERMINATION, AEROBIC BIODEGRADATION AND ENVIRONMENTAL OCCURRENCE OF ALIPHATIC ALCOHOL POLYETHOXYLATE SULFATES (AES)

GIULIO POJANA[a], GIORGIO CASSANI[b] and ANTONIO MARCOMINI[a,*]

[a]*Department of Environmental Sciences – University of Venice, Calle Larga S. Marta 2137, Venice, Italy;* [b]*Sasol Italy S.p.A. – Research & Technology Center, V. Reali, 4-20037 Paderno Dugnano (MI), Italy*

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A new analytical method was developed for the routine specific determination of the anionic surfactant Alcohol polyEthoxylate Sulfate (AES) in environmental aqueous samples. An enrichment/fractionation of the target analytes in water samples was performed by solid-phase extraction (SPE) on graphitized carbon black (GCB) (recoveries: 90–103%), followed by hydrolysis/derivatization with fluorescent reagents and separation/detection by reversed-phase high performance liquid chromatography coupled with fluorescence (HPLC-FLD). The developed procedure was applied to the study of the aerobic biodegradation of AES under laboratory conditions and to a ten-month monitoring of AES, as well as of linear alkylbenzene sulfonates (LAS), nonylphenol polyethoxylates (NPE) and alcohol polyethoxylates (AE) surfactants, in the Po river (Northern Italy). The residual concentrations found in the river waters were compared and used for a preliminary estimation of the annual average loads of monitored surfactants in the Adriatic Sea.

Keywords: Alcohol polyethoxylate sulfates; AES, Surfactants; Environmental behavior; HPLC-FLD

INTRODUCTION

The change of European consumers' habits during the 1990s led to a remarkable variation of anionic surfactant consumption. Linear alkylbenzene sulfonates (LAS), the most important surfactant in the 1970s and 80s, showed a significant decrease (−34% in the period 1991–98), while alcohol sulfates (AS) and alcohol polyethoxylate sulfates (AES, annual production: >360 000 tons in 1997), commonly used in many household and personal-care detergents such as shampoo and laundry detergents, exhibited a marked increase (+44% in the same period) [1]. The chemical structure of AES consists of an aliphatic hydrocarbon chain (the alkyl group) bonded to one or more ethoxylate groups and terminating with a sulfate group. The general formula for AES is $R–O–(CH_2–CH_2–O)_n–OSO_3^-\ M^+$, where R is an alkyl chain

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*Corresponding author. Fax: +39-041-234-8584. E-mail: marcom@unive.it

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(length range: 11 to 18 carbon atoms), n_{EO} is the number of ethoxylate groups, and M^+ is a cation, generally Na^+, Mg^+ or NH_4^+. Commercial AES consists of blends of individual AES isomers, with a short range of average n_{EO} value (1–3), compared to alcohol polyethoxylate (AE) blends, and typically contain also some level of AS ($n_{EO} = 0$).

These chemicals are generally discharged unaltered after use, and are extensively removed (83–99.9%) into municipal wastewater treatment plants (WWTPs) [2–4]. The biodegradation behavior of AES is well known and occurs in two stages, through a rapid degradation of the hydrophobic moiety followed by slower degradation of the residual hydrophilic group [5]. The toxicity of AES generally increases with increasing alkyl chain length and decreases with increasing ethoxy chain, as for AE [6]. In spite of the recent burst in consumption and incomplete removal during mechanical/biological sewage treatments, only a few data are available about the presence of residual AES in final effluents and surface waters [2,7]. Very sensitive and selective specific methods have to be applied to the determination of AES in environmental samples, because of the low concentration levels (a few micrograms per liter) and potential interference by other chemicals, such as LAS. Methods based on GC-FID [8], GC-MS [9], HPLC-UV [10] and HPLC-MS [11] have been proposed and applied to environmental investigations.

A method was developed in our laboratory for the routine specific determination of anionic (linear alkylbenzenesulfonate, LAS) and nonionic (alcohol polyethoxylate, AE, and nonylphenol polyethoxylate, NPE) surfactants in environmental aqueous samples [12]. In this work, we present modifications of this method in order to include AES for both laboratory and field investigation. The proposed method was applied to the determination of AES and potential biointermediates during aerobic biodegradation testing of AES under standardized conditions, as well as to a field monitoring for an evaluation of residual concentrations in surface waters.

EXPERIMENTAL

Chemicals

The tested AES mixtures (*COSMACOL 27-3-24 3EO*, a C_{12}–C_{14} AES blend with an average ethoxylation degree of 3, and *ISALCHEM 111/2EO*, a single homologue C_{11}-AES blend with an average ethoxylation degree of 2), both >98% purity, were kindly provided by SASOL (Milan, Italy). Standards of linear C_8–, C_{11}–, C_{12}–, C_{13}–, C_{14}– and C_{15}–OH alcohols, C_8–, C_{10}– and C_{12}– alcohol sulfates, individual alcohol polyethoxylate ethoxymer $C_{10}E_6$ (purity: >98%), as well as 1-naphthoyl chloride (NC), pyridine and dimethylformamide (DMF), were from Fluka (Buchs, Switzerland). Standard mixtures of nonylphenol polyethoxylates (average ethoxylation number: 10) and linear alkylbenzene sulfonates (LAS) were analytical grade reagents from Carlo Erba (Milan, Italy). 1-Naphthoyl isocyanate (NIC) was from Aldrich (Milwaukee, IL, USA).

All organic solvents employed were HPLC grade from Baker (Deventer, The Netherlands). Water was purified by a Millipore MilliQ system (Bedford, MA, USA). SPE sorbing material was graphitized carbon black (GCB, Carbograph-4, 120–400 mesh) from LARA (Rome, Italy). SPE polypropylene cartridges (6 mL), reservoirs (20–60 mL) and polyethylene frits were from Supelco (Bellefonte, USA).

TABLE I Recovery efficiencies for AES extracted by SPE from spiked water samples on GCB (triplicate determination)

Processed volume (mL)	*Tested compound*	*Aqueous matrix*	*Spiking conc. (μg/L)*	*Recovery (%)*	*RSD (%)*
100	C_{12-14}AES	Drinking water	100	86	6
500	C_{12-14}AES	Drinking water	100	78	9
500	C_{12-14}AES	Drinking water	10	65	13
100	C_{11}AES	Drinking water	100	103[a]	6
500	C_{11}AES	Drinking water	100	95[a]	8
500	C_{11}AES	Drinking water	10	91[a]	10
100	C_{11}AES	River water	100	90[a]	12
500	C_{11}AES	River water	100	92[a]	15
500	C_{11}AES	River water	10	90[a]	12

[a]Fraction B not eluted.

Biodegradation Tests

The biodegradation test of a C_{12}–C_{14} AES mixture was carried out in duplicate by applying the OECD 301E protocol [13]. A 1-mL aliquot of filtered final effluent from a municipal sewage treatment plant (STP) was added as inoculum to 1 L of test solution (initial concentration of substrate: 20 mg/L, 45 μmol/L). The screening tests were carried out in 3-L flasks at room temperature (22 ± 0.7°C) in half-light under continuous stirring for 28 days. Between 5 to 100 mL of test liquor were sampled 1–6 times per day, $HgCl_2$ added in the 100 ppm and stored at 4°C at dark before extraction. The latter was always performed within 24 h of the sampling.

Sample extraction, derivatization reactions, chromatographic separations and detection, and quantitation were performed after modifications of previously reported conditions [12,14]. A summary of results is reported in Table II.

Sampling of Environmental Samples

Grab samples (duplicate sampling) of river water samples were collected from the river Po (Northern Italy), near Pontelagoscuro (Rovigo) in May, June, July, October and December 2000 and in January 2001.

Sample Treatment

Analytes were extracted from water samples by means of a manual extraction apparatus (Visiprep-SPE-manifold) from Supelco. GCB cartridges (200 mg) were conditioned by sequential elution of 10 mL of a 5 mM NaOH in dichloromethane/methanol 8:2, 2 mL of methanol, 20 mL of water containing 10 mmol/L HCl and 20 mL of water. After addition of proper internal standards, aliquots from the biodegradation test (1–20 mL) and river samples (500 mL) were SPE-extracted on GCB at a flow of *ca.* 10 mL/min. Reservoirs were then washed with 1–10 mL of a methanol–water 50:50 mixture, and this solution was processed in GCB cartridges.

The first fraction (fraction A), containing neutral surfactants (AE, NPE) and their potential neutral metabolites (PEG, alcohols), was eluted with 10 mL of dichloromethane/methanol 80:20 (v/v). The second fraction (fraction B), containing potential carboxylated metabolites of AE, NPE and AES, was eluted, after inserting a Teflon

connector with a Vyton seal in the cartridge and reversing it, under *back-flushing* conditions, with 10 mL of dichloromethane/methanol 80:20 (v/v) acidified with 60 mmol/L formic acid. This elution step was not performed when river water samples were analyzed. The third fraction (fraction C), containing LAS and AES, was eluted under *back-flushing* conditions with 10 mL of dichloromethane/methanol 80:20 (v/v) basified with 5 mmol/L NaOH. After elution, this fraction was neutralized with 15 μL of a 10% methanolic solution of trifluoroacetic acid. All eluted fractions were concentrated by evaporation in test tubes by heating at 50°C in a sand-bath under a mild air stream, and the resulting concentrated solutions were then transferred into 2-mL screw-cap glass vials.

Derivatization Reactions

Prior to derivatization, extracts from fraction C were evaporated to dryness and treated with 500 μL of a 5% methanolic solution of trifluoroacetic acid for 30 min at 100°C to hydrolyse the AES terminal sulfate group to an alcohol group. The extracts of fraction A and C undergoing derivatization with NC were evaporated to dryness, then re-dissolved in 100 μL of acetonitrile, 25 μL of pyridine and 20 μL of derivatizing agent were added, and the vials were capped and heated at 80°C for 15 min [14]. The derivatization of hydrolysed extracts from fraction A and C with NIC was performed, after evaporation of the solvent, by adding 100 μL of dimethylformamide and 10 μL of derivatizing agent, then capping and heating the vials at 40°C for 30 min. After solvent removal, the residues were redissolved in 1 mL of acetonitrile–water 50:50 in the case of NC derivatization, and in 1 mL of methanol–water 60:40 in the case of derivatization by NIC. The white precipitates formed by hydrolysis of the excess of NIC and NC were separated by ultrasonication for 10 min followed by centrifugation for 5 min at 2500 rpm [14]. When information about LAS in the sample was needed, extracts from fraction C were reconstituted with 200 μL of methanol and divided into two sub-fractions, in order to ensure the HPLC/FL determination of LAS without derivatization.

Chromatographic Separation and Detection

The chromatographic apparatus consisted of a 1050 series liquid chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a model 1046A fluorescence detector (Hewlett Packard, flow cell volume: 8 μL). The samples were injected into a manual 7725 injector (Rheodyne, Rohnert Park, CA, USA) equipped with a 200-μL loop. NC derivatives of AE, AES and PEG were separated on a LUNA C18-2, 5 μm, 250 × 4 mm column (Phenomenex, Torrance, CA, USA) using linear gradient elution by acetonitrile–water at a flow rate of 1 mL/min. The initial mobile phase composition was 60% acetonitrile, which was increased to 95% in 50 min. NIC derivatives of AE, NPE, AES, as well as underivatized LAS, were separated on a Supelcosil C-8, 5 μm, 250 × 4.6 mm column (Supelco) using linear gradient elution by methanol–water containing 10 mmol/L $NaClO_4$ at a flow rate of 1 mL/min. The initial mobile phase composition was 60% methanol, which was increased to 95% in 30 min. The elution of NIC and NC adducts of analyzed compounds, as well as of LAS, was monitored by fluorescence detection under the following conditions of λ_{ex}–λ_{em} (nm), respectively: LAS, 228–295; NC, 228–365; NIC, 228–358.

Quantitation

Quantitation of AES and LAS was based on C_{10} alcohol sulfate (C_{10}-AS) and on the single homologue C_8-LAS, respectively, added to the sample as internal standards before SPE. Quantitation of AE and NPE were based on the individual linear AE ethoxymer $C_{10}E_6$ added to the sample as internal standard before SPE. The E_8 PEG oligomer was used as internal standard for the quantitation of released PEG from biodegradation tests. The NC derivatization procedure adopted is known to be insensitive to PEG oligomers with <3 ethoxy units [14]. The determined limits of detection (LODs, $s/n=3$) for individual AS homologues (C_8-AS, C_{10}-AS, C_{12}-AS) were found to be 20 ng (as injected amount). Determined limits of detection for AES, under oligomer-by-oligomer chromatographic conditions, were found to be 100 ng (as injected amount) while the LODs in river waters were 0.5 μg/L. The area linearity results were good ($R^2>0.99$) in the 50–2000 ng interval (as injected amount), while area reproducibility was 8.6 (RSD%, representing ten consecutive injections of 1000 ng of an AES standard mixture).

The biodegradation profiles obtained for the AES and their potential biointermediates are presented by plotting the residual molar concentration versus time.

RESULTS AND DISCUSSION

Analysis

Graphitized carbon black (GCB), commercially referred to as Carbopack or Carbograph, is a well-known sorbent material extensively used for the solid-phase extraction of a wide variety of analytes of environmental interest, acting as both a hydrophobic sorbent and a weak anionic exchanger, separating neutral from anionic compounds [15]. GCB, employed as the stationary phase in the enrichment/fractionation step, allowed efficient extraction of AES from aqueous environmental samples, such as spiked drinking waters, river waters and liquors from the biodegradation test. Moreover, AES were successfully isolated from nonionic surfactants and their carboxylated biointermediates. The fractionated elution of AES and AE is fundamental for their separation, since AE pose a potential interference after hydrolysis and derivatization steps when both surfactants are present in the sample. Moreover, the separation between B and C fractions is helpful in order to selectively divide carboxylated AES biointermediates from AES (in biodegradation pathway studies), as well as being a further clean-up step for the elimination, in real water samples, of carboxylated compounds that could interfere with derivatization reactions. Table II presents the recovery efficiencies obtained from triplicate spiking experiments of AES in drinking and river water samples. A significant improvement of recovery, from 65–86% to 91–103% in spiked drinking water samples, was obtained by avoiding the elution of fraction B. A partial hydrolysis of sorbed AES was thought to occur during the elution of the acidic fraction B, and it was confirmed by the presence of some (10–25% of spiked AES amount) AE in that fraction. Fraction B was therefore not eluted when spiked river water samples were examined. The potential interference from LAS, co-extracted by GCB with AES when present in the same sample, was overcome by the HPLC separation conditions. Under the chromatographic conditions adopted, a complete separation of LAS from derivatized AES was obtained. In principle,

TABLE II Analytical performances of the applied separation/detection procedures

Surfactant type	*Derivatizing agent*	*Chromatographic separation*[a]	*Retention time (min)*	*Fluorescence detection conditions (λ_{ex}–λ_{em}, nm)*	*Range of linearity (ng, as inj amount, with $r^2 > 0.99$)*	*Limit of detection ($\mu g/L$, $s/n = 3$)*	*Area reproducibility (RSD, %)*	*Ref.*
AES	NIC	hom.	28.7–34.2	228–358	20–2000	0.5	8.6	This work
AES	NC	eth.	41–82	228–365	100–2000	2.5	10.6	This work
LAS	–	hom.	13–19.5	228–295	100–2000	1.0	3.7	12
NPE	–	hom.	23–26	228–295	100–2000	0.6	3.6	12
AE	NIC	hom.	28.7–34.2	228–358	20–2000	0.5	3.0	14

[a]hom.: homologue-by homologue; eth.: ethoxymer-by ethoxymer.

LAS (λ_{ex} max: 228 nm; λ_{em} max: 295 nm) and derivatized AES (λ_{ex} max: 228 nm; λ_{em} max: 360 nm) can be detected simultaneously in the analyzed sample when a time-programmable fluorescence detector is employed, but a decrease of the signal-to-noise ratio was observed for LAS, owing to the presence of excess derivatizating agent partially coeluting with LAS.

The hydrolysis reaction step was optimized in order to avoid interferences with subsequent derivatization steps. A 5% solution of trifluoroacetic acid in methanol was found to be the best acidic medium for the transformation of AES to the corresponding AE. Figure 1 shows the HPLC separation of a commercial C_{12-14} AES mixture derivatized with NIC after hydrolysis, while in Fig. 2 the HPLC separation of the same

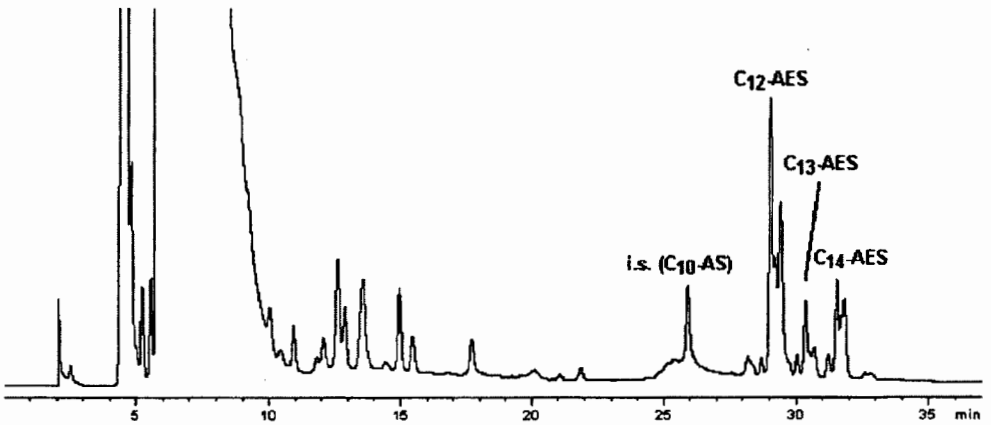

FIGURE 1 HPLC separation of a C_{12}–C_{14} AES commercial mixture after hydrolysis and derivatization with 1-naphthoyl isocyanate (NIC). Stationary phase: C-8 column. Mobile phase: linear gradient elution with methanol–water with 10 mM $NaClO_4$. Detection: fluorescence at $\lambda_{ex} = 228$, $\lambda_{em} = 360$ nm. i.s.: internal standard (sodium decylsulphate, C_{10}-AS).

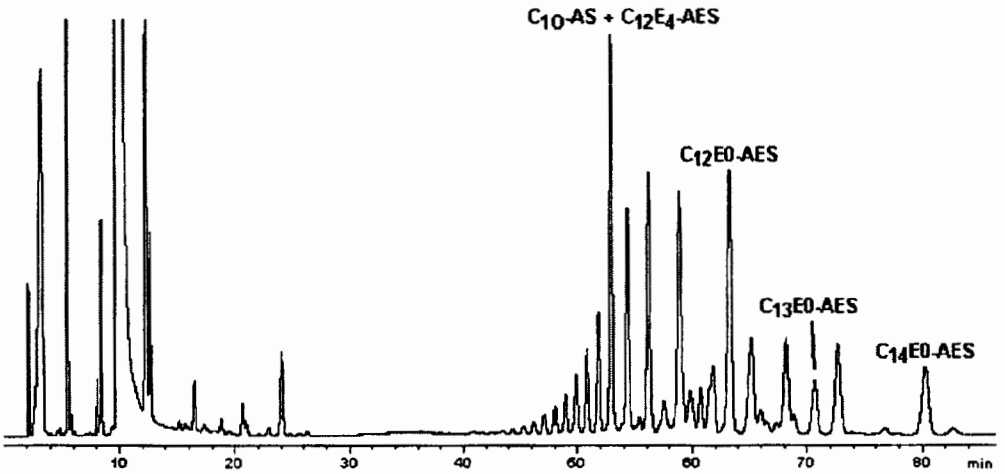

FIGURE 2 HPLC separation of a C_{12}–C_{14} AES mixture after hydrolysis and derivatization with naphthoyl chloride (NC). Stationary phase: C-8 column. Mobile phase: linear gradient elution with acetonitrile–water with 10 mM $NaClO_4$. Detection: fluorescence at $\lambda_{ex} = 228$, $\lambda_{em} = 360$ nm. i.s.: internal standard (sodium decylsulphate, C_{10}-AS).

mixture derivatized with NC is shown. As previously reported, derivatization with NIC allows a "homologue-by-homologue" separation, while an "ethoxymer-by-ethoxymer" separation can be attained after derivatization with NC [16]. The two chromatographic separations of the analyzed commercial mixture permitted the determination of its homolog and oligomeric composition. The resulting composition ($C_{12.8}E_{3.2}S$) was in good agreement with that provided by the supplier ($C_{13}E_3S$).

Biodegradation

The developed method was applied to the study of the aerobic biodegradation behavior of AES. A C_{12-14} AES commercial mixture was tested according to standardized conditions (OECD 301E biodegradation screening test). The disappearance of the parent compound and the formation of potential biointermediates was investigated and monitored for 28 days. The time profile of AES during the test is presented in Fig. 3. A fast removal of AES (complete disappearance after seven days), with no acclimation time, was observed with a resulting half-time ($t_{1/2}$) of 2.5 days. The observed $t_{1/2}$ value is much shorter than those observed for AE and LAS under the same experimental conditions [14,17], and is in good agreement with previous determinations [5]. No variation of the ethoxymeric distribution was observed during the performed test, which differed from what was observed for AE in a previous study [14]. In addition to the AES time-profile determination, potential biointermediates of AES were searched for in order to identify the main biodegradation pathway. No formation of compounds resulting from desolfatation (AE), central cleavage followed by desolfatation (PEG) or from desolfatation followed by oxidation (carboxylated AE) was observed during the performed test. This experimental finding excluded these mechanisms as main removal pathways accounting for the observed biodegradation of the tested AES mixture.

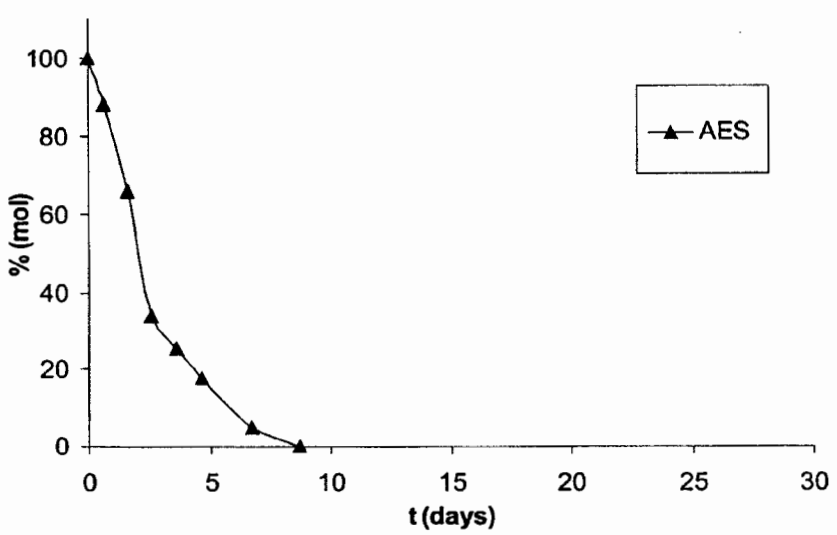

FIGURE 3 Time profile of AES during the biodegradation of a commercial C_{12-14} AES mixture under the OECD 301E test conditions; initial concentration: 20 mg/L.

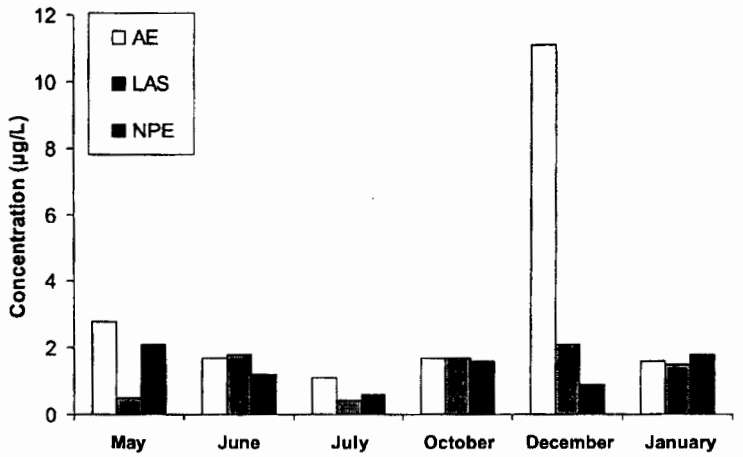

FIGURE 4 Concentrations (µg/L) of AE, NPE and LAS recorded in the Po river waters over the period May 2000–January, 2001. Note that AES were not detected in any analyzed samples.

Field Monitoring

The overall SPE-HPLC procedure was applied to the determination of anionic (AES, LAS) and nonionic (AE, NPE) surfactants in the river Po (Padania Valley, Northern Italy). Water samples were taken from a station (Pontelagoscuro, Rovigo) located approx. 80 km from the Adriatic Sea to avoid the influence of salt water intrusion. Figure 4 shows the concentrations determined in the samples analyzed (duplicate determination). Concentrations of AES were systematically < LOD (0.5 µg/L) in all analyzed samples.

Determined concentrations of other examined surfactants were very similar for LAS and NPE (0.5–1.7 and 0.6–1.8 µg/L, respectively), while AE concentrations were about double, in the 1.1–2.8 µg/L range, with no significant differences between summer and winter sampling sessions. The anomalous concentration (11 µg/L) recorded for AE in the December session could not be explained. Residual concentrations of LAS were approx. 8% of the mean concentration recorded in 1991 [18], indicating a quality improvement of Po river waters, due to both decreased consumption of LAS in household detergents and a more extensive treatment of wastewaters before release into the river Po. Given the average annual flow of the river Po over the sampling period, approx. $77 \times 10^9\ m^3$/year [19] and the mean concentrations of AE, NPE and LAS, that is 1.8 µg/L (anomalous concentration value not included), 1.4 µg/L and 1.3 µg/L respectively, the annual loads of monitored surfactants in the Adriatic Sea from this river were approximately estimated as 139 ton/year for AE, 108 ton/year for NPE and 101 ton/year for LAS. Assuming half of the determined LOD (0.25 µg/L) as mean concentration for AES, an average annual load of 19 ton/year could be roughly estimated.

CONCLUSIONS

The previously proposed method for the specific routine determination of nonionic and anionic surfactants in environmental water samples was successfully upgraded in order to include AES. The modified method permitted the attainment of a very sensitive,

as well as selective, determination of AES in environmental aqueous samples, with the advantage of distinguishing individual AES homologues and ethoxymers.

The method was successfully applied to infer the biodegradation behavior of AES in water, permitting the exclusion, under the applied experimental conditions, of some removal pathways proposed in the literature. Field monitoring confirmed the rapid degradation, in comparison with the other analyzed surfactants, of AES in natural waters, as previously reported.

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