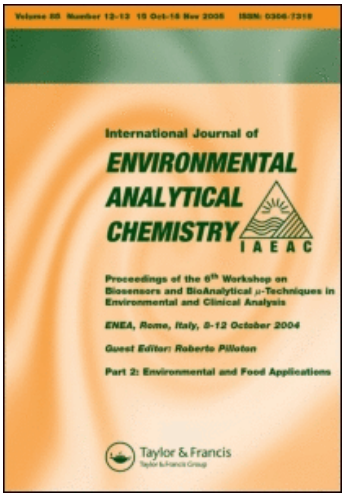

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DETERMINATION OF LINEAR ALKYLBENZENESULPHONATES AND ALKYLPHENOL POLYETHOXYLATES IN COMMERCIAL PRODUCTS AND MARINE WATERS BY REVERSED- AND NORMAL-PHASE HPLC

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Octadecylsilica was employed as reversed-phase HPLC packing material for the simultaneous separation and determination of linear alkylbenzenesulphonates (LAS) and nonylphenol polyethoxylates (NPEO). The role of $NaClO_4$ dissolved in the mobile phase on the separation of LAS and NPEO was investigated: it turned out to affect only the elution volumes of LAS. Alkylphenol polyethoxylates were exhaustively extracted from liquid commercial cleaning products with hexane-acetone mixtures. A procedure for the determination of all the NPEO oligomers enriched from marine waters is presented.

INTRODUCTION

The anionic and nonionic synthetic surfactants most largely employed worldwide are the linear alkylbenzenesulphonates (LAS) and the alkylphenol polyethoxylates (APEO).[1] The LAS in commercial use contain linear alkyl chains ranging from 10 to 14 carbons (C_{10}–C_{14}–LAS) in length, with phenyl groups placed at various internal positions in the alkyl chain. Commercially available APEO are mixtures of oligomers with 1 to 20 ethoxy units and with the branched alkyl chain having eight (OPEO) or nine (NPEO) carbons. As NPEO are by far the most utilized APEO,[2,3] we will neglect OPEO which are present in environmental samples at very low concentrations.[4] Both in LAS and NPEO the hydrophilic (the sulphonic and the ethoxylate groups, respectively) and hydrophobic (the linear and branched alkyl chains) moieties are bound to the benzene ring, in positions **1** and **4**.

These compounds are environmentally relevant because of their abundance in municipal wastewaters. Digested sewage sludges were found to contain unaltered LAS[5,6] and the NPEO biointermediates with one (NP1EO) and two (NP2EO) ethoxy units, as well as the completely deethoxylated product nonylphenol (NP),[4,7] at g/kg dry weight (d.w.) levels. Remarkably high concentrations of LAS were found in dated samples of sludge-only sanitary landfills.[8]

Selective procedures capable of distinguishing LAS and NPEO from other natural and synthetic surfactants on a routine basis have gained renewed interest

in the last years. The techniques best suited for this purpose are the chromatographic ones. Both high-resolution gas chromatography (HRGC),[9,10] often coupled with mass spectrometry (HRGC-MS),[5,11] and high-performance liquid chromatography (HPLC)[12-16] succeeded in selectively analyzing the LAS and NPEO components, as well as NP, in environmental samples. However, because LAS and the higher NPEO oligomers are not directly amenable to gas chromatography, HPLC has been recognized as a particular valuable method, especially when used in combination with fluorescence detection.[14,17-19] In addition, it has been recently shown that: (i) reversed-phase HPLC allows the simultaneous determination of LAS and NPEO by using octylsilica as stationary phase;[20] and (ii) simultaneous enrichment of LAS, NPEO, as well as NP, is feasible by employing octadecylsilica minicolumns.[21]

This paper reports on: the application of octadecylsilica for the simultaneous determination of LAS and NPEO; the role played by the salt present in the mobile phase on the separation of LAS and NPEO; procedures for the extraction of LAS and NPEO from liquid commercial products; the determination of all the NPEO oligomers, and NP, after their enrichment with LAS from surface marine waters.

EXPERIMENTAL

Reagents and Materials

Reagents were analytical grade; all solvents were HPLC grade. Reference LAS (Marlon A) and NPEO were supplied by Chemische Werke Huels (Marl, FRG) and Carlo Erba (Milan, Italy), respectively. Marlon A is a C_{10}–C_{13}–LAS mixture, whereas the NPEO standard contained an average number of 10 and a range of 1–16 ethoxy units. 4-Nonylphenol was obtained from Fluka (Buchs, Switzerland). All cleaned glassware was rinsed with methanol before use.

Samples

Liquid cleaning products marketed in Italy and in Switzerland were purchased from commercial sources in the late Spring 1987. Samples of surface marine water were taken from canals in the city of Venice and from the Venetian lagoon. The water samples were preserved by immediate addition of 1% of formaldehyde (37%, v/v) and stored at 4 °C.

Enrichment and Extraction

The enrichment of LAS, NPEO, and NP from surface marine waters of the lagoon of Venice (salinity: 2.5–3.5%) was accomplished by employing C18 minicolumn cartridges (400 mg, Sep-Pak, Waters Associates, Milford, CA, USA).

The cartridge was rinsed prior to use with 5 mL of acetonitrile followed by 3 mL of methanol and 3 mL of double-distilled water. Marine water samples (100–300 mL), previously centrifuged to remove particulate matter and fortified with 3% (w/w) NaCl, were passed through the cartridge at flow rates below 20 ml min^{-1}. One mL of double-distilled water was flushed through to remove salts and impurities retained in the column bed. The analytes were desorbed from the cartridge with 5 mL of acetone. The eluted acetone solution was split into two equal fractions, one of which was used for determining LAS, NPEO and NP by reversed-phase HPLC, after addition of 2.5 mL of water containing 1.4 g/L of sodium dodecylsulfate (SDS). The other fraction was completely blow-dried with nitrogen. Two mL of a hexane-acetone (75:25) mixture were added to the dry residue which was disaggregated and shaken. The resulting solution was centrifuged before injection into the normal-phase column.

The LAS and NPEO were batch-extracted from 100–600 mg of liquid cleaning products, under stirring for 30 min, with 20 to 50 mL of methanol-water (1:1) and determined by reversed-phase HPLC. The NPEO in the same products were analyzed by normal-phase HPLC either after batch-extracting 300–900 mg of liquid with hexane-acetone (75:25) under stirring for 30 min or after Soxhlet-extracting 30–100 mg of dry residue after the liquid was dried in an oven at 105 °C.

Instrumentation and Elution Conditions

The liquid chromatograph consisted of two single piston pumps with common drive (Hewlett Packard 1084 B) and a variable volume injector (5–200 μL) (Hewlett Packard 79841), connected in series with a Perkin–Elmer fluorescence spectrophotometer (MPF-44 B). Peak areas and retention times were recorded electronically. The fluorescence detector (flow cell volume: 8 μL; excitation and emission slit: 20 nm) operated at excitation-emission wavelengths of 225–295 nm for reversed-phase HPLC and at 225–304 nm for normal-phase HPLC.

Reversed-phase HPLC was carried out with spherical 3 μm octadecylsilica columns (Spherisorb S3 ODS II, 250 × 4 mm i.d.) and 10 μm irregularly shaped octylsilica columns (LiChrosorb RP8, 100 × 4 mm i.d.) connected to a 30 × 4 mm i.d. precolumn with the same bonded phase, which were purchased as prepacked columns from Knauer (Berlin, FRG). Eluents were acetonitrile (A) and an acetonitrile-water (25:75) mixture containing 10 g/L of $NaClO_4$ (B), at 40 °C. Gradient elution (flow rate: 1 mL min^{-1}) was carried out with both octadecyl- and octylsilica columns.

With the octadecylsilica column, the following elution program was applied:

0.0– 2.0 min		%A 15	%B 85
2.0–13.0 min	linear gradient to	%A 40	%B 60
13.0–14.0 min		%A 40	%B 60
14.0–17.0 min	linear gradient to	%A 70	%B 30
17.0–27.0 min		%A 70	%B 30
27.0–32.0 min	linear gradient to	%A 15	%B 85
32.0–35.0 min		%A 15	%B 85

With the octylsilica column the gradient elution program was:

0.0– 3.5 min		%A 15	%B 85
3.5–12.0 min	linear gradient to	%A 40	%B 60
12.0–12.5 min		%A 40	%B 60
12.5–15.5 min	linear gradient to	%A 55	%B 45
15.5–22.0 min	linear gradient to	%A 15	%B 85
22.0–25.0 min		%A 15	%B 85

Normal-phase HPLC was performed with columns packed with spherical 3 μm aminosilica (Hypersil APS 100 × 4 mm i.d., Knauer). The oligomeric distributions of NPEO were determined by applying, after minor modifications, the chromatographic conditions reported by Ahel and Giger.[15,16] The lower oligomers (NP1EO, NP2EO, NP3EO), and NP, were determined by using hexane (A) and hexane-isopropanol (98.8:1.2) (B) as mobile phase. After a 4-min elution of 50% A and 50% B, a linear gradient to 5% A and 95% B in 4 min was applied and followed by 6 min of 5% A and 95% B. A linear gradient to 50% A and 50% B re-established the initial conditions. The flow rate was 1.5 mL/min and the column was kept at room temperature.

Quantitation

The LAS and NPEO were quantified by external standard calibration curves in both normal-phase and reversed-phase HPLC. The standard solutions for the normal-phase determination were composed of nonylphenol (0.02–0.36 μg/μL) and the NPEO standard (0.09–1.14 μg/μL) dissolved in hexane-isopropanol (80:20). Standard solutions of the following substances were prepared in water–methanol (1:1) containing 1.4 g/L of SDS for the determination in the reversed-phase mode: Marlon A (0.03–1.27 μg/μL), nonylphenol (0.03–0.56 μg/μL) and the NPEO standard (0.04–0.93 μg/μL). All standard solutions and sample extracts were stored in the dark at 4 °C, to prevent photochemical degradation of the phenolic compounds. Calibration curves ($r = 0.9935$–0.9998) were plotted from measurements of peak areas versus known weights of each injected component.

RESULTS AND DISCUSSION

The simultaneous determination of LAS and NPEO by reversed-phase HPLC was previously obtained by employing octylsilica as stationary phase.[20] The LAS components with the same alkyl chain length and all of the NPEO oligomers were

eluted under the same peak. The mobile phase was fed by three solvent reservoirs consisting of water, acetonitrile and a water-acetonitrile mixture containing 2.5 g/L of $NaClO_4$. The present work was carried out by using two solvent reservoirs containing acetonitrile and water-acetonitrile (75:25) containing 10 g/L of $NaClO_4$, respectively. Fine (3 μm) octadecylsilica was employed to simultaneously determine LAS and NPEO and to investigate the interaction capabilities between the stationary phase and the NPEO components. The elution program reported in the experimental section allowed to obtain chromatograms like that shown in Figure 1A. The separation of LAS positional isomers belonging to the same homolog nicely matches the one obtained by HRGC.[5] The outer isomers were completely resolved and only the most inner isomers were poorly separated. Despite the higher hydrophobic properties and the smaller particle size of the C_{18} bonded-phase column, compared with the C_8 column previously used, the NPEO oligomers eluted again under the same peak. The completely deethoxylated biodegradation product NP also coeluted with the NPEO oligomers. This suggests that only the branched NPEO alkyl moiety is responsible for the interaction with reversed bonded-phases, and that the different branching of the NPEO components is not sufficient to discriminate any of these compounds by reversed-phase HPLC. Determination of LAS and NPEO with the C_{18} column is particularly convenient for the analysis of commercial products whose detailed LAS composition is required. Generally speaking, the performance and the applicability of a surfactant mixture depend on the content of differing homologs and isomers.

The oligomeric composition of NPEO can therefore be determined only by normal-phase HPLC (see Figure 3 below). If such a composition is known, however, and standards with similar composition are available, reversed-phase HPLC can be profitably used to quantify total NPEO since different oligomers show quite similar molar absorption coefficients.[14] Similar spectroscopic behaviour was observed for the LAS components.[12] This property can be used for simplifying the quantitation of LAS and NPEO. On the basis of the ratio amount/area obtained for the standard of one LAS homolog or NPEO oligomer (m_r/A_r), the other components can be quantitated by applying the following equation:

$$m_x = A_x/A_r \times m_r \times M_x/M_r \quad (1)$$

where m_x and m_r are the amount of the LAS, or NPEO, components corresponding to the unknown (x) and reference (r) peak, A_x and A_r are the respective peak areas and M_x and M_r the molecular weights. The availability of LAS reference homologs, for instance, with alkyl chain lengths lower than ten, or higher then thirteen, is not required for quantitation purposes.

The routine analysis of LAS and NPEO, as well as NP, in environmental samples such as surface water, sludge and sediment was attained more conveniently by using the 10 μm octylsilica column because of the shorter analysis time and its approximately two-fold lower price. A typical chromatogram resulting from the use of this column fed by two solvent reservoirs is shown in Figure 1B. Comparison of this chromatogram with the one of Figure 1A shows that the LAS

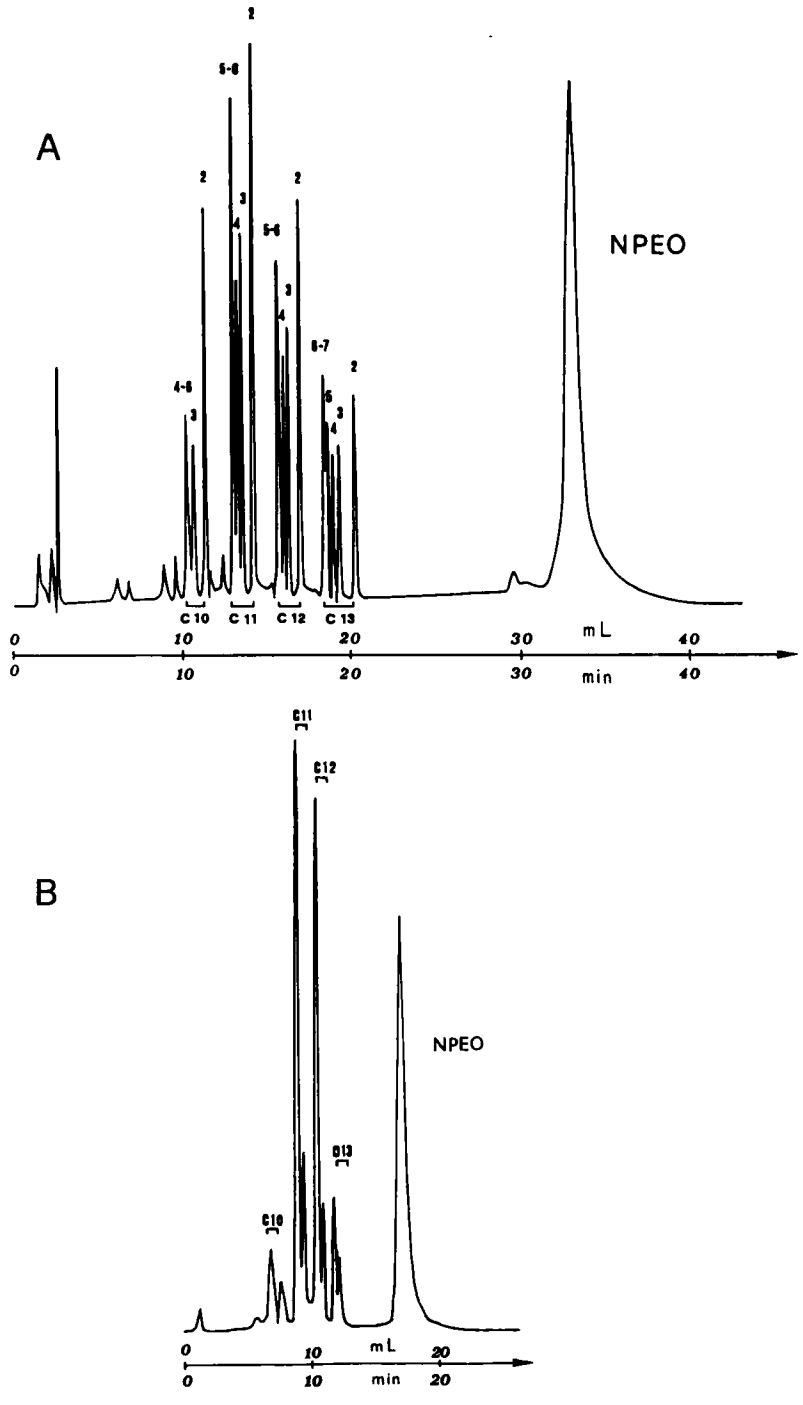

Figure 1 Reversed-phase HPLC chromatograms of LAS and NPEO extracted from a liquid detergent (A) and surface marine water (lagoon of Venice) (B). Columns: octadecylsilica Spherisorb S3 ODS II, 3 μm, 250 × 4 mm i.d., (A); octylsilica LiChrosorb RP8, 10 μm, 100 × 0.4 mm i.d., (B). C_{10}, C_{11}, C_{12}, C_{13}: LAS homologs. Numbers in chromatogram A refer to the LAS positional isomers, i.e. to the position of the LAS carbon atom bound to the benzene ring.

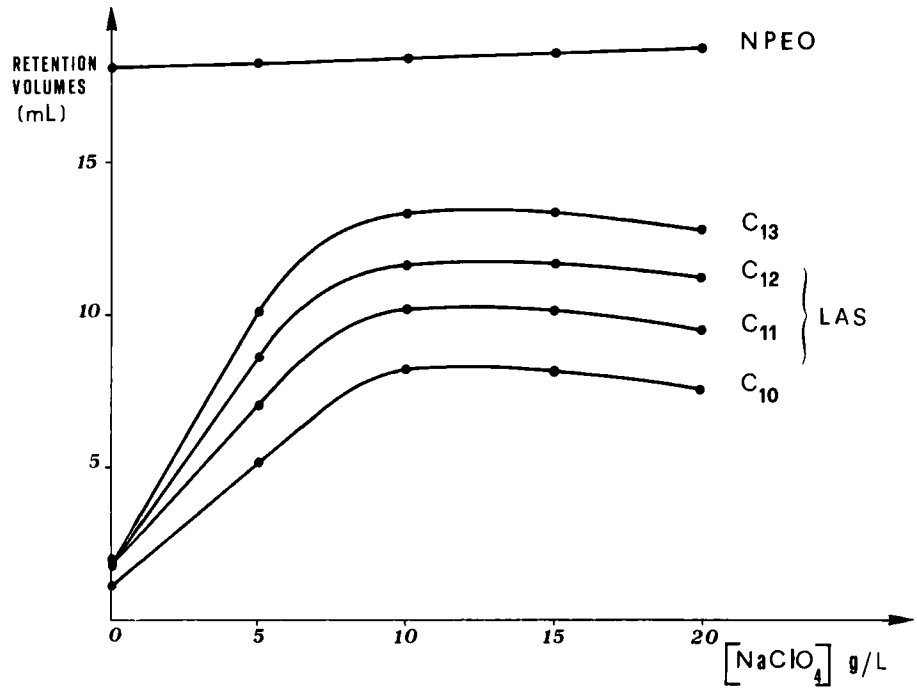

Figure 2 Effect of the $NaClO_4$ concentrations on the retention volumes of LAS and NPEO. C_{10}, C_{11}, C_{12}, C_{13}: LAS homologs.

small peak systematically following the bigger one corresponds solely to the outer positional isomer (2-C_n-LAS).

The effect of the $NaClO_4$ concentration on the LAS and NPEO separation, performed by the octylsilica column, is shown in Figure 2. As already pointed out,[13] any salt other than $NaClO_4$ can be used. This is particularly important when the liquid chromatograph is connected via the thermospray interface with the mass spectrometer and a volatile salt, e.g. ammonium acetate, must be used. From Figure 2 it appears that only LAS were affected by the salt. An increase of the salt concentration up to 10 g/L allowed separation of each LAS homolog via increasing retention. No further improvement was observed for concentrations of $NaClO_4$ up to 20 g/L. A simple salting out effect, i.e. a solubility decrease, cannot be solely claimed to explain the role played by $NaClO_4$. If so, it would influence both LAS and NPEO. A kind of ion-to-ion interaction should be considered that affects only charged molecules like LAS, and not NPEO. This would result in the orientation of the linear alkyl chains of LAS towards the hydrocarbon chains of the stationary phase. The observed stronger interaction of the longer LAS alkyl chains which leads to the separation of the LAS homologs further supports the hypothesis.

Table 1 Dry weight percentages of NPEO in commercial liquid cleaning products after extraction with hexane and hexane-acetone (75:25) of the liquid (Perlana) and of the dry residue (Tambour)[a]

	Perlana[b]		*Tambour*[c]	
	Hexane	*Hexane-acetone (75:25)*	*Liquid Hexane-acetone*	*Dried (75:25)*
NP1EO	0.02	0.01	0.20	0.06
NP2EO	0.04	0.02	0.36	0.18
NP3EO	0.08	0.05	0.66	0.57
NP4EO	0.16	0.12	0.83	0.81
NP5EO	0.15	0.18	1.24	1.41
NP6EO	0.10	0.26	1.76	2.34
NP7EO	0.05	0.36	1.83	2.84
NP8EO	0.02	0.44	1.87	3.77
NP9EO	–	0.48	1.47	3.46
NP10EO	–	0.45	0.63	2.88
NP11EO	–	0.36	0.21	2.11
NP12EO	–	0.26	0.08	1.23
NP13EO	–	0.12	0.03	0.76
NP14EO	–	0.05	–	0.28
NP15EO	–	0.02	–	0.11
Total	0.62	3.28	11.17	22.82

[a]Averages of three analyses: rel. S.D., 1–3%.
[b]Softner, commercialized in Italy.
[c]Hard-surface cleaner, commercialized in Switzerland.

Previous investigations on composition, and levels, of LAS and NPEO in commercial products were carried out by analyzing granular detergents.[2,3,20] The latter contain an average water percentage of 5–15%. LAS and NPEO were determined by reversed-phase HPLC after extraction with methanol, with the oligomeric distribution of NPEO obtained by normal-phase HPLC after extraction with hexane.[2,20] Quantitation data of NPEO by both methods were in good agreement. Table 1 reports the concentrations of individual NPEO oligomers extracted from liquid cleaning products by hexane and a mixture of hexane-acetone (75:25). The more polar NPEO oligomers were only poorly extracted in hexane. The increase of the extractant polarity obtained by adding 25% acetone was sufficient to exhaustively extract all of the NPEO oligomers from most of the examined products. Higher percentages of acetone provided extraction efficiency as good as those obtained in the presence of only 25%. The degree of extraction was checked by comparing the results obtained with the normal-phase and with the reversed-phase procedure. Such comparison disclosed poor extraction yields of NPEO from some hard-surface cleansers—one of which (Tambour) is reported in Table 1—irrespective of the composition of the hexane-acetone mixture. Full agreement between the reversed-phase and normal-phase determinations was achieved by analyzing the dry residue of these products. After the complete removal of water at 110 °C, NPEO were exhaustively extracted with the 75:25 hexane-acetone mixture.

In the previous investigation on LAS, NPEO and NP in wastewaters,[21]

Table 2 Determination precision and recovery of NPEO and NP in surface marine water[a]

Compounds	*Concentration* ($\mu g/L$)[b]	*Recovery* (%)
NP	0.20 ± 0.04	92 ± 4
NP1EO	0.73 ± 0.12	88 ± 5
NP2EO	1.10 ± 0.32	90 ± 3
NP3EO	3.04 ± 0.26	87 ± 6
NP4EO	1.68 ± 0.24	86 ± 4
NP5EO	2.02 ± 0.20	91 ± 5
NP6EO	2.14 ± 0.30	96 ± 4
NP7EO	1.88 ± 0.22	98 ± 3
NP8EO	1.94 ± 0.16	98 ± 4
NP9EO	2.10 ± 0.18	100 ± 3
NP10EO	1.36 ± 0.12	102 ± 2
NP11EO	0.68 ± 0.08	101 ± 3
NP12EO	0.44 ± 0.04	103 ± 4
NP13EO	0.24 ± 0.04	104 ± 6
NP14EO	-	101 ± 5
NP15EO	-	102 ± 7
NP16EO	-	102 ± 6
Total	19.55 ± 1.90	99 ± 4

[a]Averages and standard deviations are given.

[b]Triplicate analysis of a sample of surface marine water taken from the lagoon of Venice.

[c]Triplicate analysis of the previous surface marine water sample spiked with 100 μL of a standard solution containing 0.32 mg of NPEO in 1 mL of water-methanol (50:50).

prepacked octadecylsilica cartridges were successfully employed for the simultaneous enrichment of these compounds. Desorption from the cartridges was accomplished by acetone and the eluate was analyzed by reversed-phase HPLC. In order to determine the oligomeric distribution of NPEO, as well as NP, aliquots of the acetone eluate, diluted with methanol and acidified water containing NaCl, were extracted with 100–500 μL of hexane.[20] Only the more hydrophobic components, i.e., NP, NP1EO and NP2EO, however, were transferred to the hexane layer in good yield (80–92%). The NPEO oligomers with more than ten ethoxy units were not extracted at all. This problem was circumvented by splitting the acetone eluate into two equal fractions, or by extracting the water sample in duplicate, in order to use the acetone eluate separately for the reversed-phase and the normal-phase determination. In the latter case, the eluate was carefully and completely dried because the acetone eluate contained some water. The dry residue was then dissolved in hexane-acetone (75:25). The determination precision and the recovery of each NPEO oligomer, and NP, contained in marine water samples taken from the Venice lagoon are reported in Table 2. NP, NP1EO, and NP2EO were determined by applying the gradient program, reported in the experimental section, specially designed for the determination of NP and the lower NPEO oligomers.

The results in Table 2 show that all NPEO oligomers in the range 0.1–3.1 μg/L

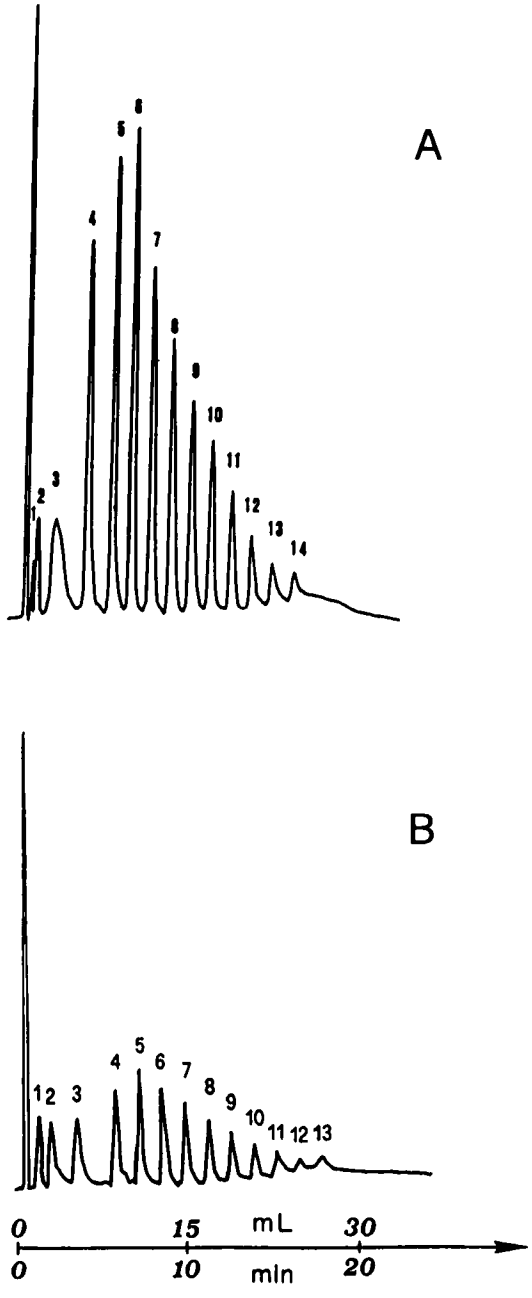

Figure 3 Normal-phase chromatograms of NPEO extracted from water collected in a secondary channel inside the city of Venice and in the surrounding lagoon. Volume of water analyzed (250 mL), final volumes of the extracts (1 mL) and injected volumes (200 μL) were the same for both marine water samples. Column: Hypersil APS, 3 μm, 100 × 4 mm i.d. Numbers refer to the number of ethoxy units.

were efficiently recovered. The relative standard deviation of the total NPEO oligomers was around 10%.

Normal-phase chromatograms of surface marine water extracts are reported in Figure 3A, B. Chromatogram A refers to water collected from a secondary channel inside Venice. Chromatogram B was obtained from an extract of water taken from the surrounding lagoon. Since the volumes of water, the final extract and the injected amounts were the same for both samples, the differences in signal intensity reflect the effect of dilution and degradation on the distribution and concentration of the NPEO components.

The environmental implications of the NPEO occurrence in the marine environment inferred by applying the proposed analytical procedures are discussed elsewhere.[23]

CONCLUSION

The extraction, enrichment and determination procedures for NPEO and LAS presented in this paper complement well the ones previously published[20,21] and further extend the potential of normal-phase and reversed-phase HPLC for the determination of these compounds in consumer products and aqueous environmental samples.

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