*Analusis*, **1998**, *26*, 64-69


# Determination of nonionic aliphatic and aromatic polyethoxylated surfactants in environmental aqueous samples

A. Marcomini[1,*], G. Pojana[1], L. Patrolecco[2] and S. Capri[2]

[1] *Department of Environmental Sciences, University of Venice, Calle Larga S. Marta 2137, 30123 Venice, Italy*
[2] *Water Research Institute (IRSA-CNR), Via Reno 1, 00198 Rome, Italy*

**Abstract.** Nonionic surfactants of the polyethoxylate type are environmentally relevant because of their ubiquitous presence in raw and treated, municipal and industrial, wastewaters. Until today the standard methods currently used for the routine determination of nonionic surfactants in environmental matrices are cumulative methods (BIAS, CTAS, PPAS, TAS), which are poorly reliable, particularly in terms of accuracy, and do not supply any structural information on the ethoxymeric and homolog composition, as well as on the chain length of the hydrophobic moiety. This paper describes a specific analytical procedure for the simultaneous specific determination of polyethoxylate aliphatic (AE) and aromatic (APE) nonionics in aqueous matrices. The analytes were isolated from water samples by solid phase extraction with graphitized carbon black and then derivatized with 1-naphthylisocyanate (NIC). AE and APE were separated by reversed-phase HPLC combined with fluorescence detection (RP-HLPC/FL). The analytical procedure was applied to the monitoring of these two classes of surfactants in a municipal sewage treatment plant. The method allows rapid, precise and reliable determination of AE and APE in environmental samples at concentrations as low as 0.1 µg/L.

**Key words.** Aliphatic alcohol polyethoxylates – nonylphenol polyethoxylates – nonionic surfactants – reversed phase HPLC – environmental aqueous samples.

* Correspondence and reprints.
Received November 03, 1997; revised January 13, 1998; accepted January 14, 1998.

## Introduction

Aliphatic alcohol polyethoxylates (AE) and alkylphenol polyethoxylates (APE) are the two largest classes of nonionic surfactants currently used, accounting together for more than 80% of the overall marketed nonionic surfactants [1]. They represent the second and the third synthetic surfactants manufactured on a world basis [2], respectively.

Commercially available AE (Fig. 1) consist of homologs with an even number of carbon atoms ranging typically from 12 to 18 (so called oleochemical AE) or mixtures of even-odd linear and α-substituted alkyl chains (2-alkyl substituted or *oxo*-AE) with 11 to 15 carbons, respectively. Highly branched AE, synthesized via ethoxylation of "iso-tridecanol" (a mixture of $C_{10}$–$C_{13}$ homologs) obtained from oligomerization of butene and propene, are also marketed.

Each homolog shows an ethoxymeric distribution accounting typically for 1 to 30 ethoxy units with an average ethoxylation number in the range of 5 – 15; a significant amount, i.e. 2 – 10% on a molar basis, of unethoxylated alcohols is systematically present in the commercial blends.

Commercially available APE are mixtures of ethoxymers with 1 to 20 ethoxy units and with a branched alkyl chain of eight (OPE) or nine (NPE) carbons bonded in *para* position to the phenyl group (Fig. 1). NPE are by far the most utilized APE [3], particularly in the European market.

AE and APE are environmentally important because of their extensive use, leading to their ubiquitous presence in municipal and industrial wastewaters and to the potential to enter the aquatic environment [4,5].

Although the environmental acceptability of NPE has been recently strongly questioned [6-8], their excellent performances in terms of detergency, emulsification and wetting, warrant a worldwide continued usage in industrial cleaners.

Over the last decade, NPE were replaced by AE in household laundry products and, nowadays, NPE are used in Italy only by industry. Compared to NPE, AE show higher biodegradation rates because of the their completely aliphatic alkyl chains.

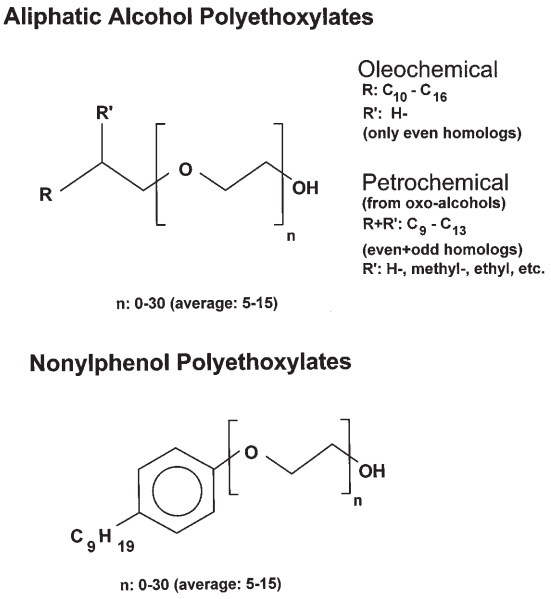


**Fig. 1.** Structures and acronyms of nonionic polyethoxylated surfactants.

The procedures proposed for the qualitative and quantitative determination of nonionic surfactants must cope with the structural complexity of these chemicals.

Until today, the regulatory methods currently applied to environmental matrices are the so-called collective or semispecific methods (BIAS, CTAS, PPAS, TAS) which provide only the total content of nonionic surfactants containing polyethoxy moieties [9-12]. These methods undergo however several limitations:

1) the low concentration levels of the compounds to be determined and the presence of interfering substances, at concentrations significantly higher than those of analytes, can often influence both accuracy and precision of the results;
2) the lack of sensitivity toward surfactant molecules with ethoxy units up to five;
3) structural information on the length of hydrophobic and hydrophilic chains of detectable compounds cannot be provided.

Environmental investigations carried out over the last decade prompted the development of several specific analytical procedures for the simultaneous determination of the major individual surfactants and their metabolites in environmental samples [13]. The most promising analytical procedures to determine selectively AE and NPE are based on chromatographic techniques. The gas chromatography is limited mainly by the scarce volatility of the higher ethoxylated derivatives and by the remarkable overlapping of the peaks corresponding to different homologs and oligomers [14-16]. Reversed-phase high performance liquid chromatography (RP-HPLC) combined with UV-absorption or fluorescence detection was instead recognized as a particularly valuable technique for analyzing NPE and AE compounds. Fluorescence detection, particularly, demonstrated to have the necessary sensitivity and specificity to perform adequate trace level determinations of these contaminants in environmental samples. While AE require a preliminary derivatization to be detected, NPE may be analyzed directly. Although the combination of HPLC with mass-spectrometer detection (HPLC-MS), at present the most adequate technique for the determination of AE in environmental samples, does not require preliminary derivatization [17], the high cost of the instrumentation and the scarce sensitivity toward the lower ethoxymers as well as the unethoxylated alcohols still limit its widespread diffusion.

This paper describes a specific, simple and reliable procedure, based on liquid chromatography, for the simultaneous extraction, enrichment, separation and determination of NPE and AE in liquid environmental samples such as STP influents and final effluents as well as natural waters.

## Materials and methods

### *Reagents*

Mixtures of AE with different high purity (98 to >99% active material) were used as reference materials. The reported compositions of the tested mixtures were those declared by the manufacturers. IMBENTIN 120/90 (Chemische

Fabrik W. Kolb AG, Hedingen, Germany) is a linear primary $C_{12}$AE with an average number of 9 ethoxy units. Marlipal 28/100 (Hüls, Marl, Germany) is a mixture of even linear primary $C_{12}$ (48 – 58% wt.), $C_{14}$ (19 – 24% wt.), $C_{16}$ (9 – 12% wt.) and $C_{18}$ (11 – 20% wt.) alcohol polyethoxylates characterized by an average number of 10.2 ethoxy units. LIALET 125/7 (Enichem Augusta, Milan, Italy) is a mixture of even and odd, linear and 2-alkyl substituted (i.e. mono-branched) $C_{12}$ – $C_{15}$ oxo-alcohol polyethoxylates with an average polyethoxy chain of 7 units. The content of mono-branched compounds in this blend was ca. 59% (w/w). LIALET 111/6 (Enichem Augusta) is a ca. 1:1 (w:w) mixture of $C_{11}$ linear and 2-alkyl substituted oxo-alcohol polyethoxylates, with an average of 6 ethoxy units in the polyethoxy chain.

Marlophen (99% purity, Chemische Fabrik W. Kolb AG) and NPE10 (> 99% purity, Carlo Erba, Milan, Italy) are mixtures of nonylphenol isomers polyethoxylates characterized by an average number of 9 and 10 ethoxy units, respectively. The derivatizing agent 1-naphthyl isocyanate (NIC), pure over 98%, was supplied by Aldrich (Milwaukee, USA).

All organic solvents employed for the preparation of standard solutions (acetone, methanol), the solid-phase extraction (methylene chloride, methanol), and the chromatographic separations (acetonitrile, methanol) were supplied by Baker (Deventer, The Netherlands) and were of HPLC grade.

Any water for chromatographic purposes was purified by a MilliQ system (Millipore, Bedford, USA).

Aqueous 30% (w/w) HCl employed for conditioning the solid-phase extraction was ultrapure grade from Merck (Darmstadt, Germany), while the dimethylformamide (DMF) (Aldrich), utilized as derivatization solvent, was of ACS-spectrophotometric grade.

The sorbing material, graphitized carbon black (GCB; grain size of 120 – 400 mesh) used for the solid-phase extraction (SPE) was purchased from Carbochimica (Rome, Italy). The SPE polypropylene tubes (6 mL volume), polyethylene frits, reservoirs (20 – 60 mL) and fittings, as well as the Visiprep-SPE Manifold extraction apparatus, were from Supelco (Bellefonte, USA).

### Derivatization reactions

All the derivatizations were performed in 1.8 mL teflon capped glass vials (Supelco). Reaction mixtures were heated in an oven with fine regulation of the temperature.

Triplicate series of aliquots of AE and NPE standard solution (100 – 600 μg/mL) were placed into screw teflon capped glass vial. The derivatization with NIC, after removal of the solvent by gentle heating under a mild nitrogen stream, was carried out redissolving the residue with 100 μL of DMF, adding 10 μL of pure derivatizing agent and heating the capped vial at 40 °C for 30 min. After reaction, DMF was removed by evaporation, and the residues redissolved in 1 mL of the mobile phase. The resulting mixture was then sonicated for 10 min and centrifugated at 2500 rpm for 5 min in order to separate the white precipitate resulting from the reaction of water with the excess of NIC.

### HPLC separations

The chromatographic apparatus (Hewlett Packard) consisted of a quaternary pump liquid chromatograph series HP 1050 equipped with a variable wavelength fluorescence detector mod. HP 1046A. A Chemstation HP 3365 Series II data system was used for the acquisition and handling of chromatograms. The samples were injected by a 100 μL syringe (Hamilton) in a manual 7125 injector (Rheodyne) equipped with a 20 μL loop.

The chromatographic separations were carried out, at room temperature, on a Lichrocart column (Merck) 125 × 4 mm i.d. containing Lichrospher 100 RP 18, 5 μm endcapped as stationary phases and on a Supelco LC-8 250 × 4.6 mm i.d. containing Supelcosil LC-8, 5 μm as stationary phase, both equipped with pre-columns of the same sorbing material. The homolog-by-homolog elution of the NIC derivatives of AE and NPE in standard solution and environmental extracts was attained by isocratic conditions on $C_{18}$-column by using premixed methanol-acetonitrile 90:10 (v:v) at a flow rate of 2 mL/min, or on $C_8$-column by using methanol-water with $NaClO_4$ 10 mM, 1 mL/min according to the following conditions of linear gradient elution:

| *t (min)* | *Methanol (%, v/v)* | *$NaClO_4$ 10 mM in water (%, v/v)* |
|---|---|---|
| 0 | 65 | 35 |
| 30 | 95 | 5 |

The detection conditions were 225 – 295 nm (excitation-emission wavelengths) for underivatized NPE and 228 – 358 nm for the AE and NPE derivatives. Both sets of wavelengths were employed in the simultaneous separation of unaltered and derivatized NPE by the variable wavelength detector.

Quantification of AE and NPE in environmental samples extracts was obtained from external calibration curves of AE and NPE standard solutions.

### Sampling and extraction of AE and NPE from environmental samples

Wastewater samples were collected from influents and final effluents of the Campalto sewage treatment plant, near Venice (Italy). Samples were preserved in 1% (v/v) formalin, stored at 4 °C and processed within 24 hours. Triplicate aliquots of ca. 20 mL of STP influent and 200 mL of final effluent, respectively, were processed under vigorous shaking to ensure adequate mixing and suspension of particulated material. After sample consumption, sample bottles (50 and 500 mL) were rinsed with 2 and 20 mL methanol-water 1:1 (v:v), respectively, and the washing solvent was passed through the cartridge. Recovery of analytes was evaluated by spiking the sample solutions with AE and NPE standards.

The analytes were extracted from the aqueous samples by solid phase extraction (SPE) on graphitized carbon black (GCB) at flow rates of about 3 mL/min under gentle vacuum.

The extraction cartridges were prepared by packing 1 g of GCB in 6 mL polypropylene tubes and placing polyethylene frits above and below the sorbing bed. The car-

tridges, before extraction, were conditioned with 7 mL of 10 mM tetramethylammonium hydroxide (TMAOH) in methylene chloride/methanol (90:10, v/v), 3 mL of methanol and 30 mL of MilliQ water acidified with HCl at pH 2, sequentially.

AE and NPE were desorbed from the SPE cartridge with 7 mL of methylene chloride/methanol 70:30 (v/v) and the extract was then concentrated to a volume of 1000 µL. A 100 µL aliquot of the resulting extract was completely blow-dried with nitrogen, redissolved in 100 µL of DMF and derivatized with 1-NIC. Both fractions, after evaporation of the solvents, were finally taken up with 100 µL of MeOH : $H_20$ 60:40 and re-unified for the simultaneous separation of the analytes.

The recovery of the commercial oleochemical $C_{12}$–$C_{18}$ AE mixture as well as of individual ethoxymers from STP influents and effluents and river waters resulted systematically higher than 96% with relative standard deviations of 2 – 7%, while the recovery of NPE were higher than 90% with relative standard deviations of 5 – 7% [18].

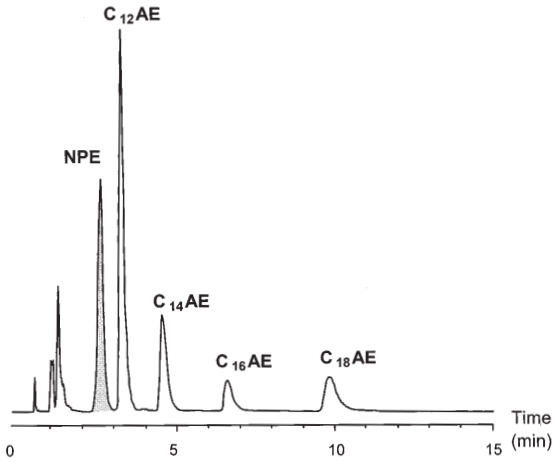


**Fig. 2.** Reversed-phase HPLC chromatogram of the NIC derivatives of standard mixtures of NPE and oleochemical $C_{12}$–$C_{18}$AE under isocratic conditions. Stationary phase: $C_{18}$-endcapped column (125 × 4 mm Lichrospher 100 RP–18, 5 µm). Mobile phase: Methanol-acetonitrile 90:10 (v:v). Detection: fluorescence at 228–358 nm.

## Results and discussion

The simultaneous presence in environmental aqueous samples of NPE and AE prompted the search of conditions for their simultaneous specific determination, with the aim to set a comprehensive analytical routine method to be used for the specific determination of nonionic surfactants.

Because of the lack of UV-absorbing or fluorescent groups, AE need a prior derivatization while NPE can be detected without further modifications. Owing to the structural similarity of NPE with AE, consisting of the terminal –OH groups in the polyethoxylic chain, all derivatizing agents suited for AE are expected to react with NPE too.

Fluorescent derivatizing agents are more suitable than UV-absorbing reagents for the environmental determination of trace level organic contaminants mainly because of their higher selectivity. In this work 1–naphthyl isocyanate (NIC) was applied as derivatizing agent for AE and NPE. This fluorescent reagent permits to attain quantitatively, with high sensitivity and reproducibility, AE, PEG and NPE derivatives suitable for RP-HPLC separation under fluorescence detection [19]. The derivatization by NIC, which permits to perform a homolog-by-homolog separation, results to be highly preferable to other fluorescent agents, since peaks identification and quantitation are easier and more accurate, allowing moreover to improve the detection limit.

A typical chromatogram of the NIC derivatives of fully linear, oleochemical, AE and NPE standard mixtures is shown in figure 2. After derivatization by NIC and under the chromatographic conditions previously found suitable for the separation of AE derivatives, the NPE adducts coeluted before the $C_{12}$AE homolog, so becoming a systematic potential interference for the determination of $C_{11}$AE homolog and possibly of $C_{12}$AE too, if the concentration of NPE in the sample were much higher than that of AE.

In order to overcome the coelution of $C_{11}$AE with NPE, the approach presented in figure 3 was adopted. It is based on the simultaneous separation of underivatized NPE and derivatized NPE and AE, after extraction and subsequent

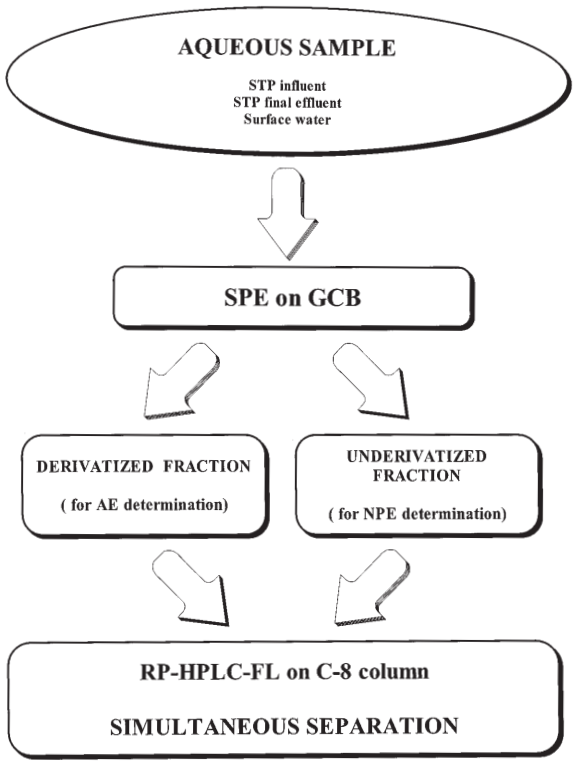


**Fig. 3.** Schematic diagram of the analytical procedure proposed for the routine determination of AE and NPE in environmental aqueous samples.

reaction of an aliquot of the final extract with NIC, which could be possible by splitting the extract in two fractions. A typical chromatogram is presented in figure 4.

The $C_8$-column (Fig. 4) was preferred to the $C_{18}$ one (Fig. 2) to maximize the coelution of the ethoxymers of each homolog without loosing a satisfactory inter-homolog resolution. The earlier elution of the unaltered NPE allows them to be accurately quantified. The resulting concentration of NPE is thereby subtracted from that obtained after

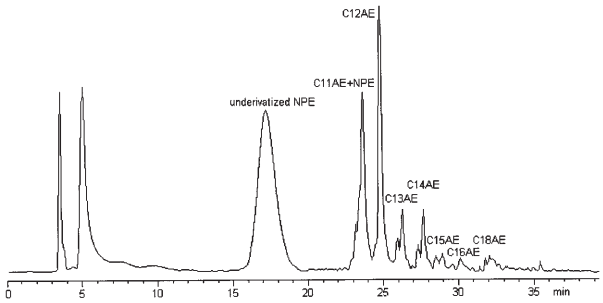


**Fig. 4.** Simultaneous reversed-phase HPLC chromatogram of a underivatized NPE standard mixture and NIC derivatives of standard mixtures of NPE and oleochemical and petrochemical $C_{11}$ – $C_{16}$ and $C_{18}$–AE. Stationary phase: $C_8$ column (250 × 4.6 mm Supelcosil LC–8, 5 μm). Gradient elution by Methanol-Water + $NaClO_4$ 10 mM. Detection: fluorescence at 228 – 295 nm (0 – 20 min) and 228 – 358 (20– min).

derivatization by NIC, the difference providing the concentration of the $C_{11}$AE homolog.

Since NPE and the NIC adducts of AE and NPE exhibit the maximum quantum yield at the same excitation wavelenght (228 nm), but at different emission wavelengths (295 and 358 nm, respectively), the proposed separation requires a variable wavelength fluorescence detector.

Despite of the longer time required by this elution of AE compared with the isocratic one (Fig. 2), the separation shown in figure 4 offers the great advantage of a sufficiently precise and accurate quantification of NPE and shorter AE homologs, a better baseline and a decreased influence of interfering substances.

Based on a signal-to-noise ratio of 3, the limit of detection for AE and NPE in the aqueous environmental samples was 0.1 μg/L.

### *Applications*

The proposed analytical procedure was applied to the monitoring of the Campalto municipal sewage treatment plant, collecting the sewage of Mestre (Venice). The determination of AE and NPE in influents and final effluents was carried out over a 5-months period.

A typical chromatogram of sewage extracts is shown in figure 5.

The influent and effluent concentrations of NPE and AE, found in 24h composite samples of influents and final effluents are presented in table I and visualized in figures 6(A,B).

Based on the intensity ratio of peaks corresponding to even and odd homologs, the values found in the influent reflect dominantly the domestic usage pattern of the oleochemical and petrochemical AE commercial mixtures. NPE is present to an extent of about 20% of the overall AE, on a molar basis, while the amount of $C_{11}$AE did not exceed 5% of the total AE, thus confirming the low percentage of this homolog in commercial detergents according to the declarations of industrial manufacturers. The $C_{11}$AE homolog contributed for no more than 15% to the cumulative peak of derivatized NPE and $C_{11}$AE.

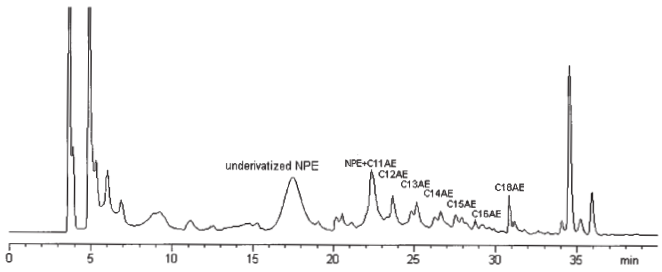


**Fig. 5.** Simultaneous reversed-phase HPLC chromatogram of a sewage extract. Stationary phase: $C_8$ column (250 × 4.6 mm Supelcosil LC–8, 5 μm). Gradient elution by Methanol-Water + $NaClO_4$ 10 mM. Detection: fluorescence at 228 – 295 nm (0 – 20 min) and 228 – 358 (20– min).

**Table I.** Concentrations of NPE and $C_{11}$–$C_{18}$AE in the influent and final effluent of a municipal sewage treatment plant.

| *Date sampling* | *February 1996* | | *April 1996* | | *June 1996* | |
|---|---|---|---|---|---|---|
| | *Influent* | *Effluent* | *Influent* | *Effluent* | *Influent* | *Effluent* |
| | *Concentration (μg/L)* | | | | | |
| **NPE** | 284 | 5.9 | 262 | 3.6 | 437 | 6.7 |
| **$C_{11}$–AE** | 37 | 0.9 | 38 | 0.2 | 47 | 0.1 |
| **$C_{12}$–AE** | 161 | 10.1 | 185 | 0.6 | 344 | 0.4 |
| **$C_{13}$–AE** | 153 | 2.5 | 148 | 0.7 | 302 | 0.6 |
| **$C_{14}$–AE** | 162 | 4.1 | 171 | 1.0 | 405 | 0.6 |
| **$C_{15}$–AE** | 178 | 0.9 | 137 | 0.4 | 344 | 0.3 |
| **$C_{16}$–AE** | 45 | 0.6 | 38 | 0.4 | 56 | < 0.1 |
| **$C_{18}$–AE** | 49 | 0.1 | 77 | 0.1 | 120 | < 0.1 |
| **$AE_{tot}$** | 785 | 19.2 | 794 | 3.4 | 1618 | 2.0 |

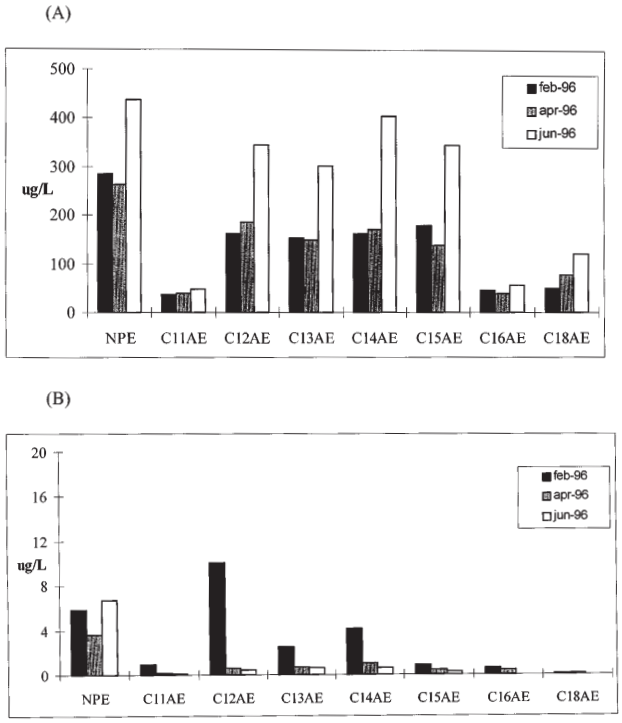


**Fig. 6.** Concentrations of NPE and $C_{11}$ – $C_{18}$ AE in the influent (A) and final effluent (B) of a municipal sewage treatment plant.

By comparing influent and effluent concentrations, NPE were removed systematically to an extent > 98%, while the removal of AE ranged from 98% for the $C_{12}$-homolog to 100% for the $C_{18}$AE homolog in all the analyzed samples. A closer examination of the values reveals an anomalous enrichment of NPE and AE in the influent during the summer, compared with the winter values, and an unexpected high concentration of the $C_{18}$ homolog in the raw sewage.

Moreover, by comparing the concentration of individual AE homologs in the influent and in the final effluent, it appears that, despite the higher concentrations of the compounds in the June influent, the highest values in the final effluent were found in the winter campaign, which points out the marked influence of the temperature on the primary biodegradation of AE.

As far NPE, on the other hand, the different ratio between the values found in February and June in the influent and final effluent, respectively, indicates that both the concentration levels in raw sewage and the temperature affect the primary biodegradation of NPE.

## Conclusions

RP-HPLC coupled with variable wavelength fluorescence detection allows the simultaneous separation of the nonionic surfactants AE and NPE, provided that a preliminary derivatization of the sample is performed in order to transform AE in fluorescent derivatives and unaltered and derivatized NPE are separated during the same chromatographic run. The proposed derivatizing procedure was successfully applied to both laboratory biodegradation studies [20] and trace level environmental monitoring [21].

### References


1. Tell, B. *Tenside Deterg*. **1991**, *28*, 385.
2. Greek, B. F.; Layman, P. L. *Chem. Engin. News* **1989**, *29*.
3. Marcomini, A.; Filippuzzi, F.; Giger, W. *Chemosphere* **1988**, *17*, 853.
4. Giger, W.; Ahel, M.; Koch, M.; Laubscher, H. U.; Schaffner, C.; Schneider, J. *Water Sci. Technol*. **1987**, *19*, 449.
5. Mc Evoy, J.; Giger, W. *Environ. Sci. Technol.* **1986**, *20*, 376.
6. Holt, M. S.; Mitchell, G. C.; Watkinson, R. J. *N. T.*, in: *The Handbook of Environmental Chemistry*, de Oude, Ed., **1992**, *89*, 3 Part F.
7. Ahel, M.; Giger, W.; Koch, M. *Wat. Res.* **1994**, *28*, 1131.
8. Ahel, M.; Giger, W.; Schaffner, C. *Wat. Res.* **1994**, *28*, 1143.
9. Wickbold, R. *Tenside Deterg*. **1972**, *9*, 173.
10. Greff, R. A.; Setzkorn, E. A.; Leslie, W. D. *J. Am. Oil. Chem. Soc*. **1985**, *42*, 180.
11. Favretto, L.; Stancher, B.; Tunis, F. *Int. J. Environ. Anal. Chem.* **1981**, *10*, 23.
12. Tsubouchi, M.; Yamasaki, N.; Yanagisawa, K. *Anal. Chem.* **1985**, *57*, 783.
13. Marcomini, A.; Zanette, M. *J. Chromatogr. A* **1996**, *733*, 193.
14. Sandra, O.; David, F. *J. High Resol. Chrom*. **1990**, *13*, 414.
15. Silver, A. H.; Kalivoski, H. T. *J. Am. Oil. Chem. Soc*. **1992**, *69*, 599.
16. Bruschweiler, H.; Felber, H.; Schwager, F. *Tenside Deterg*. **1991**, *28*, 348.
17. Crescenzi, C.; Di Corcia, A.; Samperi, R.; Marcomini, A. *Anal. Chem*. **1995**, *67*, 1797.
18. Di Corcia, A.; Samperi, R.; Marcomini, A. *Environ. Sci. Technol.* **1994**, *28*, 850.
19. Zanette, M. R.; Marcomini, A.; Marchiori, E.; Samperi, R. *J.Chromatogr. A* **1996**, *756*, 159.
20. Marcomini, A.; Pojana, G. *Analusis* **1997,** *7*, M35.
21. Marcomini, A.; Pojana, G. manuscript in preparation.