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Determination of Nonylphenol Polyethoxylates and Their Lipophilic Metabolites in Sewage Effluents by Normal-phase High-performance Liquid Chromatography and Fluorescence Detection

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Analytical procedures using normal-phase high-performance liquid chromatography (HPLC) with fluorescence detection, suitable for the routine determination of alkylphenol polyethoxylate (AP*n*EO) surfactants and their lipophilic metabolites in sewage effluents at sub-microgram per litre level, have been developed. The parent nonylphenol polyethoxylates (NP*n*EO), covering oligomer range of 1–18 ethoxy units per molecule, were extracted from water samples using solid-phase extraction on graphitised carbon black (GCB), while their lipophilic metabolites, including nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO) and nonylphenol (NP), were extracted by liquid-liquid extraction into n-hexane. The extracts were analysed by normal-phase HPLC using amino-silica columns and fluorescence detection at 228/305 nm. This allowed reliable quantitative determination of individual oligomers down to 0.1 μg/l. Analyses of sewage effluents collected in several sewage treatment plants of the Canton of Zürich, Switzerland, indicated that NP*n*EO occur at significant concentrations (96–430 μg/l) despite the partial ban introduced in 1986. Oligomer distributions found in analysed sewage samples revealed that the

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major part of toxic lipophilic metabolites, including NP, still originate from detergent-derived NP*n*EO.

Key words: nonylphenol polyethoxylates, nonionic surfactants, nonylphenol, normal-phase HPLC, sewage effluents.

INTRODUCTION

Alkylphenol polyethoxylates (AP*n*EO, Figure 1) with the world annual production rate of about 500 000 are among the most widely used surfactant classes,[1] while nonylphenol polyethoxylates (NP*n*EO) are the strongly prevalent sub-group of AP*n*EO. NP*n*EO find their application in households as ingredients of laundry detergents and cleaning agents but they are also very popular components of various formulations used in industrial processes such as emulsification, dispersion and flotation. Several studies conducted in the last 15 years (see Refs. 2 and 3 for review and references) showed that NP*n*EO are rather widespread in various types of wastewater and sewage effluents. Although the parent NP*n*EO themselves are not highly toxic and can efficiently be removed from wastewater by the common sewage treatment,[4] they represent a compound class of high environmental concern. The reason for this is a very complex biotransformation pattern of NP*n*EO in the aquatic environment characterised by the formation of numerous stable metabolites, including nonylphenol (NP), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO) and their carboxylated analogoues.[4–9] These metabolites were detected at considerable

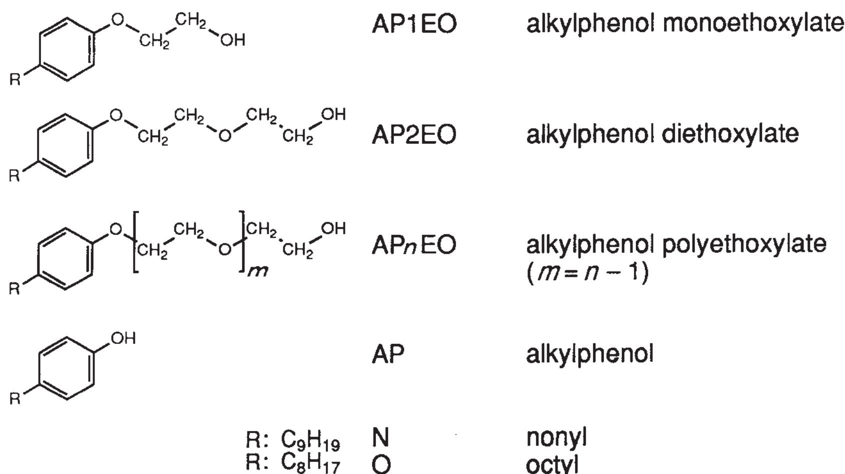

Figure 1. Structures and acronyms of alkylphenol polyethoxylates and their lipophilic metabolites; n = number of ethoxy units per molecule.

concentrations in various receiving waters[2,3] and represented the highly predominant percentage of the total nonylphenolic compounds in a polluted river.[10] NP2EO, NP1EO and, especially NP, were shown to exhibit a rather high lethal and sub-lethal toxicity to various aquatic organisms,[11,12] while some more recent reports have indicated that these compounds have a significant endocrine-disrupting potency.[13] Moreover, due to their pronounced lipophilic properties (log K_{ow} values above 4),[14] NP, NP1EO and NP2EO accumulate in aquatic organisms, including mussels, fish and algae.[15,16]

A number of different analytical methods were developed for the determination of NP*n*EO and their lipophilic metabolites in aqueous samples (see Ref. 3 for review). Gas-chromatographic methods are not suitable for the determination of higher NP*n*EO oligomers due to their low volatility and high polarity, but they were successfully applied for the determination of different metabolic products of NP*n*EO.[5,8,17–20] However, the most popular methods for the determination of both the parent NP*n*EO and their metabolites make use of reversed-phase or normal-phase HPLC techniques.[21–27] A very promising approach for the analysis of environmental samples is the application of electrospray liquid chromatography/mass spectrometry, which allows otherwise very difficult confirmation of NP*n*EO at low concentrations.[28]

Intensive investigations of NP*n*EO in sewage effluents, sludges and natural waters in early 80's revealed significant exposure levels of surfactant-derived nonylphenolic compounds in the aquatic environment. As a consequence, the use of NP*n*EO in laundry detergents was banned in Switzerland in 1986, while in several other countries the risk reduction was achieved by a voluntary agreement of detergent manufacturing companies. Further restrictions, including industrial cleaning applications, are foreseen to follow in Europe in 2000. This has been strongly disputed by some manufacturers, especially in the USA.[1] Additional data showing the results of the imposed risk reduction measures are therefore highly desirable to allow better assessment of the environmental impact of NP*n*EO.

The aim of this work was to develop analytical procedures that are suitable for the routine determination of NP*n*EO and their lipophilic metabolites at the levels of 1 μg/l and 0.1 μg/l, respectively, following basically the same approach that was applied in our earlier studies in Switzerland.[7]

EXPERIMENTAL

Materials

Commercial mixture of NP*n*EO Marlophen 810 (Chemische Werke, Hüls, Marl, Germany), having an average number of EO-units per molecule of 10, was used as a reference material for the HPLC-determination of individual oligomers in the range

from 1 to 18 EO units. Another two commercial mixtures of NP*n*EO, Marlophen 83 (Chemische Werke, Hüls, Marl, Germany) and Imbetin N/7A (Dr. Kolb, Hedingen, Switzerland), contain chiefly lower oligomers (NP1EO to NP3EO) and were applied as reference materials for the determination of lipophilic metabolites (NP1EO and NP2EO). Technical 4-nonylphenol (Fluka, Buchs, Switzerland) was applied as received from the supplier. The content of 2-nonylphenol and other typical impurities in the 4-nonylphenol[29] was found to be 15%. 2,4,6,-trimethylphenol (TMP) (EGA Chemie, Steinheim, Germany) and 4-*tert*-butylphenol (4*t*BP) (Fluka) were applied as internal standards.

Stock solutions (1–5 mg/ml) of the standard materials reported above were prepared in n-hexane, n-hexane/2-propanol (8/2) or methanol. Various working standard solutions were prepared by diluting and mixing stock solutions in appropriate solvents suitable for a given chromatographic procedure.

The solvents used for the extractions (dichloromethane, methanol, and hexane) were of *p.a.* quality (Fluka) and were used without further purification. HPLC-eluents (n-hexane, 2-propanol and water) were HPLC grade (Fluka).

GCB (120–400 mesh, surface area 100 m^2/g) and other consumables necessary to prepare solid-phase extraction (SPE) cartridges were supplied by Supelco, Belfonte, USA. The extraction cartridges were prepared by packing 500 ± 10 mg of GCB into a polypropylene tube (6.7 × 1.3 cm i.d.) supplied by polyethylene frits below and above the sorbent bed.

Samples

Samples of primary and secondary effluents from several sewage treatment plants located in the Canton of Zürich were obtained in October 1997. Aliquots of 24-hour composite samples collected under a flow proportional regime were taken into 1-liter glass bottles and immediately preserved with 10 ml of formaldehyde (36%) to prevent bacterial degradation and stored at 4 °C until analysis.

Extraction

NP*n*EO oligomers with 1 to 18 EO units per molecule were extracted from water samples using a modified procedure by Di Corcia *et al.*[26] Unfiltered effluent samples were percolated over SPE-cartridges containing 0.5 g of graphitised carbon black (GCB), while acidification to pH = 3 was omitted since we were not interested in analysing concurrently the carboxylated metabolites. Before percolation, bottles containing 1 l of effluent sample were vigorously shaken to ensure homogenous resuspension of particles and sample aliquots (10–100 ml) were quickly transferred to calibration flasks. Six GCB cartridges were fitted to a Supelco-box percolation unit. Before extracting the samples, the cartridges were cleaned up by washing them subsequently with 5 ml of dichloromethane/methanol (7/3), 3 ml of methanol and 10 ml of HPLC-grade water. Precautions were taken not to allow GCB adsorbent to get dry before and during the percolation of the water sample. The glass tubes used to collect solvent wastes from washing the cartridges were taken out from the box and discarded. The samples were then poured into the cartridges and percolated through them at a flow rate similar to 10–15 ml/min using a slight vacuum. The calibration flask that contained the sample was rinsed with 10 ml of pure water and this washing was also percolated through the cartridge. Finally, the cartridge reservoir itself

was rinsed with 5 ml of water. After percolation, the GCB cartridge was allowed to get dry under vacuum for 1 min. To remove the last water residues from the sorbent, exactly 1 ml of methanol was passed slowly through the cartridge. Subsequently, the GCB trap was air-dried under vacuum again. Clean glass tubes (12 ml) were put in the percolation box under the cartridges and the analytes were desorbed from the sorbent by 7 ml of dichloromethane/methanol (7/3), while the flow rate was adjusted to < 5 ml/min. Before elution, this portion of eluting solvent was used to rinse calibration flasks that contained the sample as well as walls of the cartridge to minimise adsorption losses.

The collected extracts were evaporated to dryness using rotary evaporation and transferred, using multiple washing with dichloromethane, into a 1.8-ml glass vial equipped with a Teflon-lined screw cap. The sample was brought to dryness under the stream of nitrogen and redissolved in an exact volume (typically 1000 μl for primary effluents and 300 μl for secondary effluents) of hexane/2-propanol (8/2). Such final extract was directly analysed by normal-phase HPLC as described below.

Lipophilic NP*n*EO (NP1EO and NP2EO) as well as fully de-ethoxylated metabolite NP were extracted by liquid-liquid extraction with hexane. An aliquot (100 ml) of the original (non-filtered) effluent sample was transferred from the storage bottle to a separatory funnel, followed by addition of 5 g of NaCl to increase the partitioning into the organic phase. Such sample was extracted 3 times with 2 ml of n-hexane. A known amount (typically 50 ng) of TMP was added to the sample during the first extraction from a TMP solution (1 ng/μl) prepared in hexane. During the later phase of the method development, a second internal standard, 4-*tert*-butylphenol, was also added to the sample. After each extraction, the hexane phase was collected in a 10 ml-flask. The combined extracts were dried over anhydrous sodium sulphate and collected into a clean flask. The extracts were evaporated gently down to 0.5 ml under a stream of nitrogen and transferred into a 1.8-ml glass vial equipped with a Teflon-lined screw cap. A blank sample, containing nanopure water instead of an effluent sample, was processed with each sample series following identical procedures as for the real samples.

High-performance Liquid Chromatography

The HPLC analyses were carried out using a Hewlett Packard Series II liquid chromatograph (Model 1090) consisting of an automatic injector, built-in diode array detector and serially coupled programmable fluorescence detector (HP 1046 A) equipped with a cut-off filter (295 nm). A Hewlett Packard HPLC Chemstation software supported the system. Columns containing amino-silica material were applied for all chromatographic separations.[21,22]

For the determination of NP*n*EO oligomers in the range from 3 to 18 EO units, a 125 × 3 mm i.d. column packed with 5-μm particles of Nucleosil 100-5 NH_2 (Macherey-Nagel, Germany) was used. A binary gradient was applied for the elution. The mobile-phases A and B were mixtures of n-hexane/2-propanol (98/2) and 2-propanol/water (98/2), respectively. A linear gradient program from 97% A and 3% B (with 1 min. isocratic hold) to 25% A and 75% B in 20 min at a flow of 0.6 ml/min was used. The fluorescence detector was set at excitation and emission wavelengths of 228 nm and 305 nm, respectively. Acquired chromatograms were analysed for individual oligomers by integrating the corresponding peak areas using the HPLC Chem-

station software. The reference solution for daily performance testing of the HPLC system was prepared in hexane/2-propanol (8/2) at a concentration of 5.4 ng/μl.

For the determination of lipophilic metabolites of NP*n*EO (NP, NP1EO and NP2EO), a simpler chromatographic procedure using an isocratic elution was applied. A 125 × 3 mm i.d. column packed with 5-μm particles of Hvoersil APS (Knauer, Germany) was used. The mobile-phase (hexane/2-propanol, 98.5/1.5) was pumped through the column at a flow rate of 0.6 ml/min, which allowed the separation of analytes within 10 min. The reference solutions for quantitative determination were prepared by adequately mixing pure solutions of Marlophen 83 or Imbetin N/7A with NP and the two internal standards, TMP and 4*t*BP.

Quantification

The quantification of higher oligomers was performed using the external calibration with standard solutions of Marlophen 810 covering the wide concentration range of 3–4 orders of magnitude. The exact mass fraction of individual oligomers in Marlophen 810 was determined using the same normal-phase HPLC system as described above but coupled with UV-detection at 277 nm.[21, 22] Consequently, this allowed accurate determination of response factors (RF) for each individual oligomer present in the mixture for the system using fluorescence detection as described below in *Results and Discussion*. RF was defined as peak-area counts per ng of injected compound.

The oligomer composition of Marlophen 83 and Imbetin N/7A were also previously determined using UV-detection. By analysing the prepared reference solutions that contained also TMP and 4*t*BP as internal standards it was possible to determine relative response factors (RRF) for each oligomer as well as for NP for the system using fluorescence detection. RRF of a given oligomer is defined as the ratio between the RF of internal standard and RF of that compound.

Consequently, concentration of each oligomer in a water sample was calculated applying the internal standard procedure from the following expression:

$$C_n = A_n / A_{\mathrm{is}} \times (\mathrm{RRF})_n \times C_{\mathrm{is}} \qquad (1)$$

where C_n is the concentration of oligomer n, A_n and A_{is} are peak areas for oligomer n and internal standard, $(\mathrm{RRF})_n$ is the relative response factor of oligomer n and C_{is} is the concentration of internal standard in the water sample.

Following, the previously developed procedure,[22] TMP was used first as the internal standard but towards the end of our study 4*t*BP was found more suitable.

RESULTS AND DISCUSSION

HPLC Separations

Figure 2 shows normal-phase HPLC chromatograms of commercial NP*n*EO surfactant Marlophen 810 (A) and a reference mixture containing lipophilic NP*n*EO oligomers (NP1EO and NP2EO), NP, and two internal

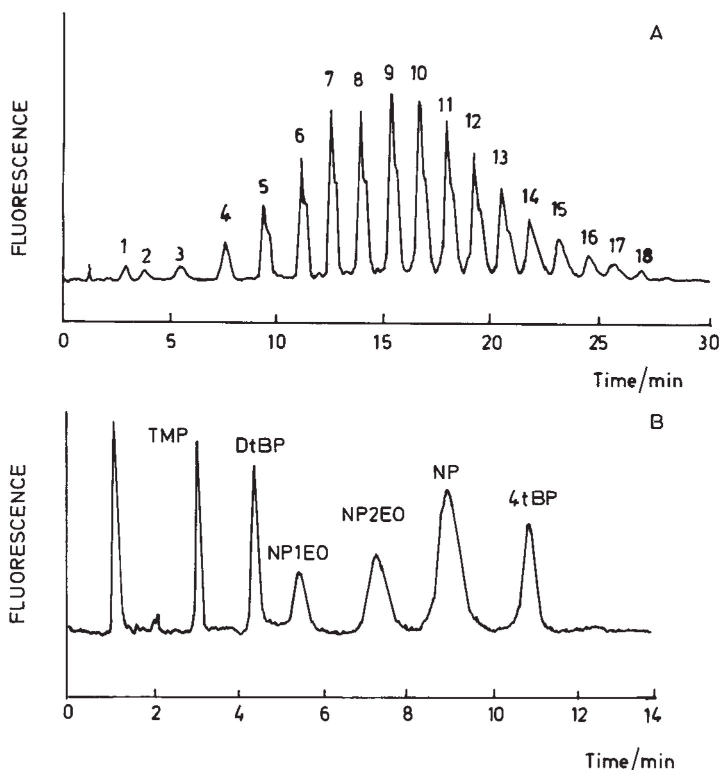

Figure 2. Normal phase high-performance liquid chromatograms of (A) commercial NP*n*EO mixture Marlophen 810 typically used in detergent formulations and (B) a mixture containing nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) from Marlophen 83, nonylphenol (NP), and 2,4,6,-trimethylphenol (TMP), 2,4-di-*tert*-butylphenol (D*t*BP) and 4-*tert*-butylphenol (4*t*BP). Exact chromatographic conditions are given in the experimental section. Numbers in Figure 2A refer to the corresponding NP*n*EO oligomers.

standards TMP and 4*t*BP (B). As can be seen, the first procedure enables separation of oligomers in a wide molecular weight range,[21] however, it does not allow separation of NP from NP1EO and NP2EO. To achieve such a separation with a normal-phase system, the elution should be optimised using less polar solvents. The application of columns packed with irregularly shaped 10-µm amino-silica required a very careful gradient elution,[22] while columns packed with finer 3 or 5-µm material allow satisfactory separation under isocratic conditions, as shown in Figure 2B. Di Corcia *et al.*[26] have developed a very elegant chromatographic technique able to separate NP from the NP*n*EOs applying a gradient reversed-phase system, but in that case distribution of oligomers remains unknown because all of them elute under

the same peak. The information about the exact distribution of individual oligomers in an environmental extract is rather important as a clue to identifying the major source of NP*n*EO as well as to assess the degree of degradation of the original surfactant after disposal into the aquatic environment.[4,10]

Response Factors

One of the key prerequisites for an accurate analysis is the availability of pure reference compounds. Unfortunately, such standards that would allow direct determination of specific response factors for each NP*n*EO oligomer are not commercially available and could be isolated only by time-consuming preparative isolations from the complex commercial mixtures.[21,22] To overcome this problem, an alternative procedure was applied in this work. The exact composition of each commercial mixture of NP*n*EO used as reference material was determined using UV-detection at 277 nm as described earlier.[21,22] It was, namely, shown that molar response factors of individual oligomers are rather constant. This allows determination of their mass fractions in various commercial mixtures from the normal-phase HPLC/UV chromatograms using the following equations:

$$M_t = M_1 + M_2 + \dots + M_{n-1} + M_n \quad (2)$$

$$M_t = a_1 \times M_{r,1} + a_2 \times M_{r,2} + \dots + a_{n-1} \times M_{r,n-1} + a_n \times M_{r,n} \quad (3)$$

$$a_n = A_n / A_1 \times a_1 \quad (4)$$

where M_t is the total mass of the material injected into HPLC, M_n is the mass of individual oligomer n, a_n is the amount (moles) of oligomer n, $M_{r,n}$ stands for the relative molecular mass (molecular weight) of the oligomer n, while A_n and A_1 represent peak areas of the corresponding oligomers. Substituting the expression (4) for a_n into the equation (3) one gets the solution for the amount of NP1EO in the injected sample:

$$a_1 = M_t / (M_{r,1} + A_2 / A_1 \times M_{r,2} + \dots + A_{n-1} / A_1 \times M_{r,n-1} + A_n / A_1 \times M_{r,n}) \quad (5)$$

Subsequently, this allows calculation of mole fractions for all other oligomers. Furthermore, conversion of mole fractions into mass fractions allowed determination of mass fractions for different oligomers. Based on that, response factors were determined for each oligomer using fluorescence detection. The RRFs of individual oligomers (relative to RF of NP1EO = 1) plotted *versus* the number of EO units per molecule (Figure 3) showed a

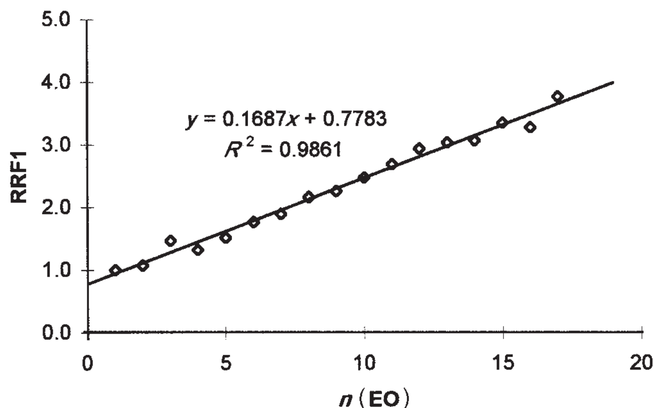

Figure 3. Dependence of relative response factors, RRF1, of nonylphenol polyethoxylates on the number of ethoxy units per molecule, *n*(EO), obtained using gradient HPLC with fluorescence detection. RF of NP1EO is arbitrarily assumed to be 1.

highly correlated linear relationship (R^2 = 0.9861). It should be mentioned that RRFs of individual oligomers increase considerably with increasing molecular weight (from 1.0 to 3.8), which indicates the importance of knowing the exact oligomer composition when determining the total NP*n*EO concentration by reversed-phase systems.[23,26]

Relative response factors for the analytes which were determined using an isocratic HPLC system were obtained using internal standards TMP and/or 4*t*BP. It was observed that TMP-based RRFs of NP and especially of NP1EO and NP2EO could vary significantly depending on the composition of eluent, *i.e.* percentage of 2-propanol in hexane. As can be seen in Figure 4, increase of the percentage of 2-propanol in the eluent causes an increase of RRFs of NP, NP1EO and NP2EO and the reason is an enhancement of RF for TMP at higher 2-propanol concentrations. It is therefore important to determine the exact RRFs of analytes daily with each series of samples and with each new HPLC eluent portion. The variations are much smaller when using 4*t*BP as an internal standard (< 5%) and therefore 4*t*BP was considered more suitable than TMP.

Interferences

The problem of interfering co-extracted compounds is one of the most critical issues in the determination of both parent NP*n*EO and their lipophilic metabolites, particularly at concentrations in the low μg/l range. The problem could be very site-specific and therefore it is important to assess each type of sample to be analysed routinely by HPLC-techniques for pos-

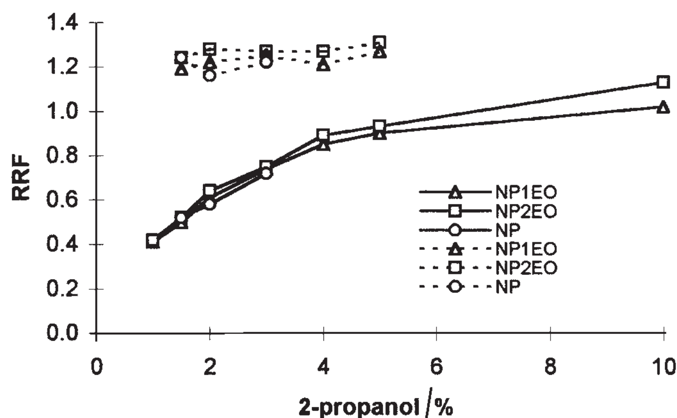

Figure 4. Dependence of the relative response factors (RRF) of nonylphenol (NP), nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) as related to 2,4,6-trimethylphenol (solid line) and 4-*tert*-butylphenol (dashed line) on the composition of mobile phase during isocratic HPLC with fluorescence detection.

sible interferences. The analyses of samples from numerous municipal sewage treatment plants in Switzerland showed that the same typical interfering peaks occur in most STPs with only few exceptions. The chromatograms presented in Figure 5 illustrate typical interferences found when using the methods applied in this work. Compared with the originally proposed procedure using UV-detection,[21] the application of fluorescence detection provides a significant improvement with respect to the detection of NP1EO and NP2EO in the presence of early eluting compounds of lower polarity, such as polycyclic aromatic hydrocarbons and phthalates.[7,23,24] However, in some samples, significant interfering shoulder peaks co-eluting with NP7EO, NP8EO and NP15EO were observed. The clue to detecting possible presence of interfering substances on the chromatogram is the distribution pattern of NP*n*EO oligomers which, as a rule, does not show large sudden changes in the concentrations of subsequent oligomers but rather shows a trend of gradually changing concentrations.[4,7] Therefore, it is very likely that the peak assigned 14 in Figure 5A contains a significant contribution of some unidentified interfering compound which prevented accurate determination of NP14EO. According to Di Corcia *et al.,*[26] it is unlikely that the extract obtained by eluting GBC with dichloromethane/methanol contained any carboxylic compounds, but it is very likely that this fraction contained a significant contribution of phenolic compounds, most probably biogenic polyphenols. Unfortunately, no positive identification of these interferences was possible, so far, by GC/MS techniques. A promising method could be possibly derivatisation with PFBT followed by negative ionisation MS[18, 20] or HPLC coupled

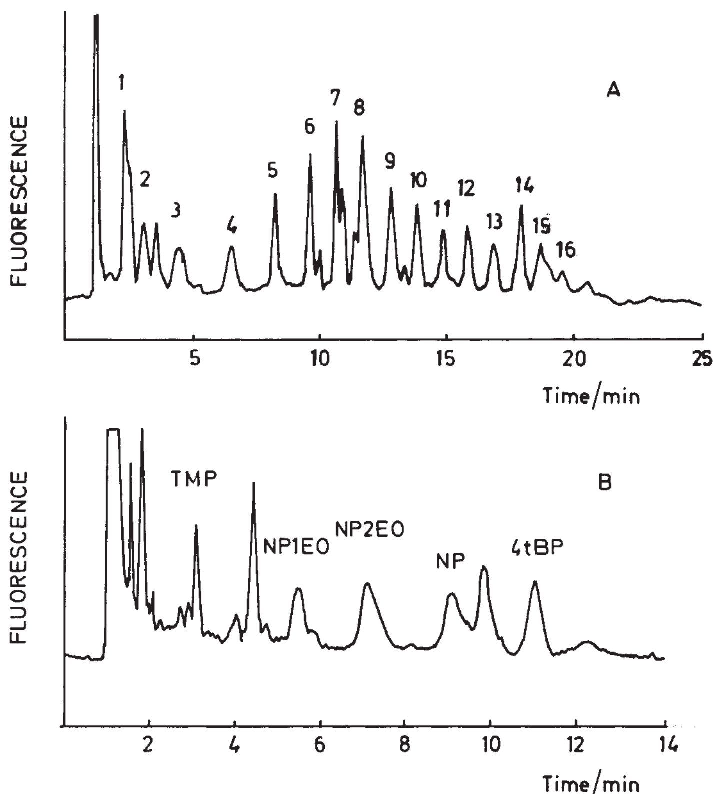

Figure 5. Normal phase high-performance liquid chromatograms of (A) GCB-extract of primary effluent sample obtained from the sewage treatment plant Zürich-Glatt and (B) a hexane-extract of the river water sample collected downstream from the sewage treatment plant Zürich-Glatt. Numbers in Figure 2A refer to the corresponding NP*n*EO oligomers. NP1EO = nonylphenol monoethoxylate, NP2EO = nonylphenol diethoxylate, NP = nonylphenol, TMP = 2,4,6-trimethylphenol, 4*t*BP = 4-*tert*-butylphenol. Exact chromatographic conditions are given in the experimental section.

with ES/MS.[28] An interesting approach to solving the problem of interfering compounds present in GCB-extracts of effluent samples by using acetylation prior to HPLC analysis has been reported.[27]

The most common compounds interfering with determination of NP, NP1EO and NP2EO are other alkylphenols, which are a numerous and widespread group of organic compounds, having both anthropogenic and biogenic origins. Most of them show a strong response when using the fluorescence detection at 228/305 nm and therefore sample treatment and chromatographic separation on the amino-silica column are decisive steps for a reliable, interference-free determination of individual nonylphenolic compounds. Ex-

amination of the chromatographic behaviour of a number of alkylphenolic compounds using the system applied in this study showed that potential interferences are *o*-cresol, which co-elutes with NP, 2-*tert*-butylphenol, which partially overlaps with NP1EO and 2,5-dimethylphenol, which overlaps with NP2EO. Due to the very low partition coefficients into hexane, *o*-cresol very probably does not represent a serious problem unless present at a very high concentration. When analysing highly anaerobic samples, a possible interference by skatol that partially co-elutes with NP1EO should be also considered.[29]

Finally, it must also be stressed that neither of the two applied normal-phase HPLC techniques is able to separate nonyl- from octyl-homologues of AP*n*EO and therefore no concurrent presence of OP*n*EO-surfactants in the extracts can be detected. In case of doubt, the reversed-phase HPLC system should be applied to check the nature of the alkyl substituent.[21,23] It was established earlier that OP*n*EO represent < 10% of the total AP*n*EO in Swiss sewages.[21]

Accuracy, Precision and Sensitivity

Recovery and reproducibility of the determination of parent NP*n*EO were assessed by analysing samples of both pure HPLC grade water and primary effluents spiked with a known amount Marlophen 810. The results presented in Table I show a total recovery of 79–92% with a relative standard deviation of 3–15%. The recovery of oligomers NP1EO to NP10EO was nearly quantitative, while somewhat lower recoveries were obtained for the highest oligomers (80%). The reproducibility of the determination of individual oligomers depended upon the concentration to be determined. The reproducibility for the most abundant oligomers at the concentration levels of 100 μg/l of the total NP*n*EO varied from 3 to 10%, while for some minor oligomers (NP2EO, NP14–18EO) it could reach 20%. At lower concentrations (10 μg/l of the total NP*n*EO), the reproducibility was significantly lower (10–20% for major oligomers and up to 42% for minor oligomers). It was established that the most critical part of the quantification was a proper integration of complex chromatograms, especially for primary effluent samples. A baseline subtraction feature was found necessary for an appropriate integration when reprocessing the acquired chromatograms.

Various sample volumes from 10 to 100 ml were processed to check the adsorption capacity of GCB-sorbent to retain NP*n*EO from the real effluent samples. It was shown that percolation of larger volumes (50–100 ml) might cause a partial breakthrough of about 10–15% when analysing primary effluent samples. This is in agreement with the observation by Di Corcia *et al.*[26] that humic material can hamper successful enrichment of NP*n*EO on

TABLE I

Reproducibilty and recovery of the determination of nonylphenol polyethoxylates (NP*n*EO) in fortified water samples

n(EO)[a]	Concentration / µg l^{-1}			
	Nanopure water[b] + 10 µg/l NP*n*EO	Nanopure water[b] + 100 µg/l NP*n*EO	Primary effluent[b]	Primary effluent[b] + 216 µg/l NP*n*EO
1	0.15 ± 0.02 (11)	0.9 ± 0.08 (9.4)	4.3 ± 0.2 (4.2)	4.8 ± 0.6 (11.7)
2	0.07 ± 0.03 (42)	0.6 ± 0.10 (18)	1.4 ± 0.29 (21)	2.2 ± 0.3 (11.5)
3	0.12 ± 0.03 (24)	1.0 ± 0.11 (12)	5.5 ± 0.09 (1.6)	6.8 ± 0.3 (4.9)
4	0.24 ± 0.04 (16)	1.9 ± 0.06 (3.4)	2.4 ± 0.18 (7.2)	5.6 ± 0.3 (5.5)
5	0.25 ± 0.04 (15)	2.9 ± 0.17 (6.0)	4.9 ± 0.36 (7.5)	10.6 ± 0.3 (3.3)
6	0.45 ± 0.05 (10)	4.6 ± 0.10 (2.2)	7.0 ± 0.32 (4.5)	15.3 ± 0.3 (1.7)
7	0.63 ± 0.10 (16)	6.7 ± 0.27 (4.1)	9.9 ± 0.77 (7.8)	29.6 ± 4.3 (14)
8	0.81 ± 0.11 (14)	8.4 ± 0.54 (6.4)	12.3 ± 1.21 (9.8)	31.1 ± 1.1 (3.4)
9	0.87 ± 0.14 (16)	10.2 ± 0.52 (5.1)	9.8 ± 0.43 (4.4)	33.1 ± 1.0 (3.0)
10	0.91 ± 0.16 (17)	11 ± 0.63 (5.7)	7.8 ± 0.77 (9.9)	31.7 ± 1.4 (4.3)
11	0.91 ± 0.12 (13)	10.6 ± 0.6 (5.6)	7.4 ± 0.61 (8.2)	32.6 ± 1.5 (4.6)
12	0.77 ± 0.12 (16)	9.4 ± 0.56 (6.0)	7.3 ± 0.50 (6.9)	28.8 ± 1.4 (4.9)
13	0.57 ± 0.07 (12)	7.7 ± 0.36 (4.6)	10.1 ± 0.75 (7.4)	27.0 ± 1.6 (5.9)
14	0.41 ± 0.05 (12)	5.7 ± 0.19 (3.4)	5.6 ± 0.23 (4.0)	18.0 ± 0.7 (4.0)
15	0.31 ± 0.04 (12)	3.7 ± 0.26 (6.9)	6.1 ± 0.63 (10.4)	16.3 ± 1.4 (8.9)
16	0.20 ± 0.02 (11)	2.2 ± 0.10 (4.4)	5.2 ± 0.59 (11.3)	9.0 ± 0.7 (7.3)
17	0.14 ± 0.01 (6)	1.2 ± 0.08 (7.1)	2.3 ± 0.05 (2.0)	4.0 ± 0.3 (8.1)
18	0.10 ± 0.03 (30)	0.6 ± 0.02 (3.3)	1.4 ± 0.14 (9.8)	2.6 ± 0.3 (10)
Total	7.91 ± 1.18 (15)	89.3 ± 3.9 (4.4)	111.9 ± 4.48 (4.0)	309.6 ± 9.6 (3.1)
Recovery / %	79.1	89.3		91.5

[a] n(EO) – number of ethoxy units per molecule; given values refer to the arithmetic average, standard deviation and relative standard deviation (in parentheses) of analyses performed in triplicate.
[b] Nanopure water as well as primary effluent from the sewage treatment plant Werdhölzli (6th October 1997) was spiked with Marlophen 810.

GCB-cartridges. Therefore, a sample volume of 25 ml was routinely processed to determine NP*n*EO in primary effluents.

The blank value of the analytical procedure was typically in the range of 0.1–0.5 µg/l of the individual oligomer with the most pronounced interferences coeluting with NP1EO, NP2EO. The determination limit (signal/noise, S/N ratio = 10) for an individual oligomer based on a 50-ml sample was 0.2–0.6 µg/l, depending on its RF.

Accuracy and precision of the determination of lipophilic metabolites was determined by spiking river water with Marlophen 83 and NP at concentrations of individual analyte in the range from 0.1–2.6 μg/l. The data presented in Table II show that for the Glatt River sample quantitative recoveries were obtained for all analysed compounds with the relative standard deviation in the range of 8–10%. This is in agreement with high partition coefficients for the n-hexane/water system exceeding 2000 for all analysed compounds.[16] Somewhat lower recoveries were obtained for the Rhine River sample (77–94%), probably due to the enhanced concentration of suspended solids (non-filtered samples were analysed), which caused emulsion formation during the extraction. The blank values determined in triplicate with each series of samples were in the range 0.01–0.1 μg/l for NP and NP1EO, while for NP2EO they were usually lower (< 0.05 μg/l). The detection limit from 100 ml-samples was estimated at 0.03 μg/l (S/N = 3), while the determination limit was 0.08 μg/l (S/N = 10). This limit of determination is comparable to the one obtained using UV-detection[22] but the analysed sample is 20 times smaller. Moreover, the daily sample-throughput was considerably improved by replacing lengthy extractions (3 hours per sample) in a steam distillation/extraction unit[22] by a simple liquid-liquid extraction in a conventional separatory funnel.

TABLE II

Recovery and reproducibility of the determination of nonylphenol (NP), nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) in fortified water samples

Sample	Recovery / %		
	NP	NP1EO	NP2EO
Glatt River[a]	105 ± 8 (7.6)	99 ± 8 (8.1)	107 ± 11 (10.3)
Rhine River[b]	94 ± 5 (5.3)	77 ± 5 (6.5)	86 ± 7 (8.1)

[a] Sample collected on 3rd March 1998 containing 0.12 μg/l NP, 0.11 μg/l NP1EO and 0.31 μg/l NP2EO;
[b] Sample collected on 26th May 1998 containing 0.07 μg/l NP, = 0.03 μg/l NP1EO and 0.08 μg/l NP2EO. Both samples were spiked with NP, NP1EO and NP2EO at 5 different concentrations in the range from 0.2 to 3 μg/l. Given numbers refer to the arithmetic average, standard deviation and relative standard deviation of the recoveries obtained from these experiments.

Applications

The summed concentrations of NP*n*EO, covering an oligomer range from NP1EO to NP18EO, in primary and secondary effluents of 8 major mechanical-biological STP located in Canton Zürich, Switzerland, are presented in

TABLE III

Nonylphenol polyethoxylates (NP*n*EO) in primary and secondary effluents from the major sewage treatment plants of the Canton of Zürich, Switzerland[a]

Sewage treatment plant	Concentration / $\mu g\ l^{-1}$	
	Primary effluent	Secondary effluent
Bassersdorf	96	5.2
Bülach	110	4.3
Dübendorf	192	2.0
Fällanden	214	4.8
Opfikon	430	5.3
Uster	111	6.6
Werdhölzli	363	8.0
Zürich-Glatt	157	2.5

[a] Sampled on 16th October 1997.

Table III. Primary effluents contained total concentrations of NP*n*EO in the range from 96 μg/l to 430 μg/l. These concentrations are about 5–10 times lower than the levels measured before the ban introduced in 1986 by the Swiss ordinance for environmental pollutants,[4] however, they indicate that the wastewater-relevant usage of these surfactants is still significant. The concentrations of the total NP*n*EO in secondary effluents are significantly lower (2.0–8.0 μg/l), suggesting an efficient transformation and/or elimination of these compounds during the biological stage of sewage treatment.[4] It is interesting to note that there was no correlation between the primary and secondary effluent concentrations. Very similar concentration ranges of the total NP*n*EO were reported for the raw and treated sewage from the area of Rome, Italy, using the same enrichment method but applying a reversed-phase HPLC approach.[26,28]

It should be stressed that the accuracy of the determination of NP*n*EO by the reversed-phase HPLC is highly dependent on the similarity of the oligomer composition in the reference material and in the analysed samples. The composition of oligomers in real samples can vary, due to different source materials and, even more importantly, due to the alteration of the original oligomer composition by biological and physico-chemical reactions in the sewage treatment.[4] This is clearly documented in Figure 6, which illustrates oligomer compositions in primary and secondary effluents from 4 STPs analysed in this study. As can be seen, the distribution in all primary effluents is characterised by an oligomer distribution peaking at NP7EO-NP8EO. This distribution is typical of the detergent-derived NP*n*EO-mixture

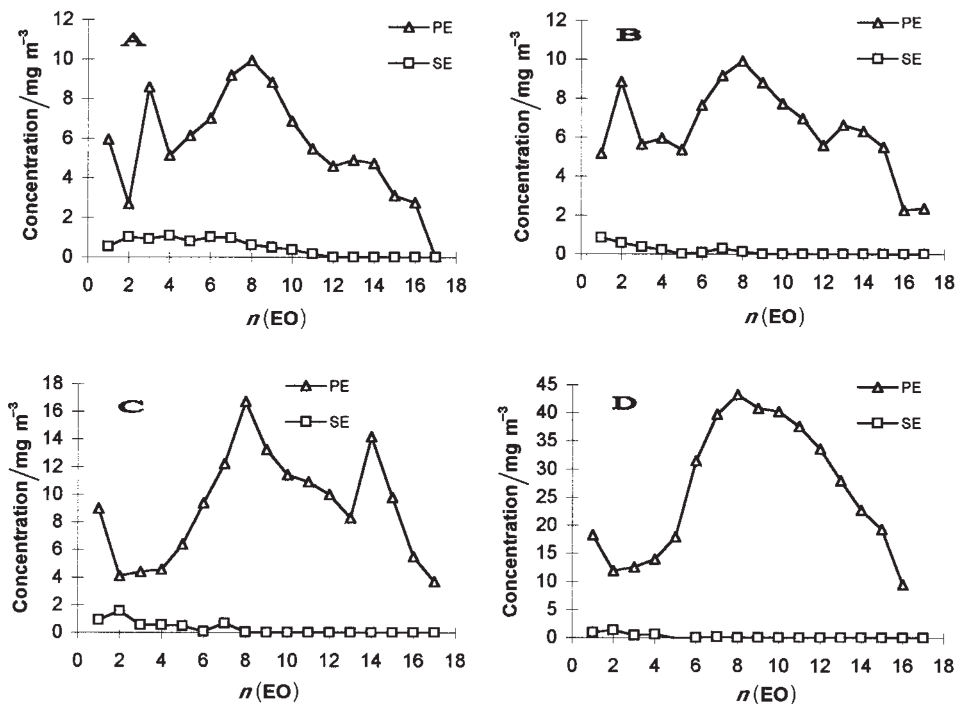

Figure 6. Distribution of nonylphenol polyethoxylate (NPnEO) oligomers in primary (PE) and secondary effluent (SE) samples collected in sewage treatment plants located in the area of Zürich, Switzerland: A) Bassersdorf, B) Bülach, C) Zürich-Glatt and D) Opfikon. Samples were collected in October 1997. n(EO) = number of ethoxy units per molecule.

that was slightly altered by biological degradation.[4,7] Another important feature, indicative of biological degradation, is the occurrence of a second maximum at lower oligomers (NP1EO to NP3EO) which are considered stable metabolic products of NPnEO. After biological treatment, most of the secondary effluents virtually contain only these lipophilic products. Since the RF values of NP2EO and NP8EO differ by a factor of 2, it obviously means that the total concentration of NPnEO by the reversed-phase HPLC cannot be accurately assessed unless the oligomer composition of the sample is known. On the other hand, it must be pointed out that the normal-phase approach to NPnEO analysis applied in this study does not allow selective determination of NP in the presence of NP1EO and NP2EO.

Since NP is the most toxic metabolite derived from NPnEO-surfactants, it is particularly important to determine its concentration in secondary effluents to control the emissions from STPs into receiving natural waters.

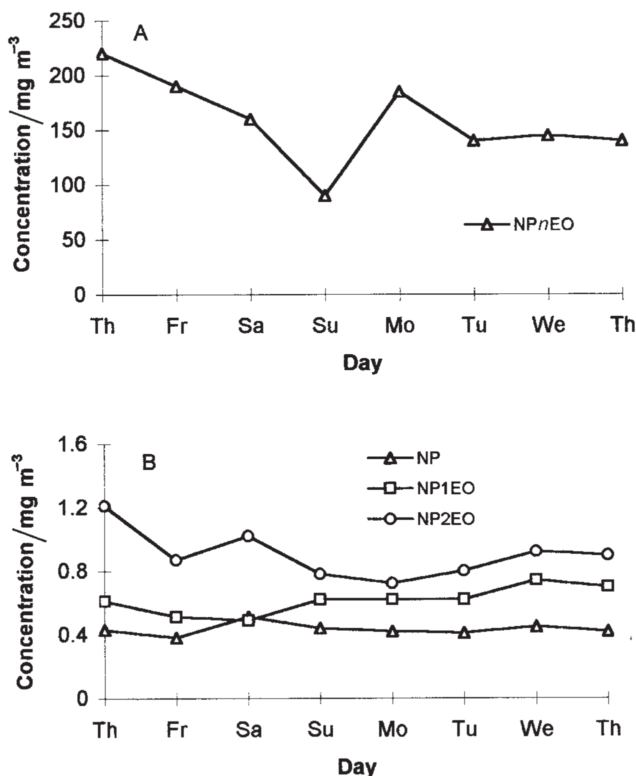

Figure 7. Variability of the concentrations of (A) nonylphenol polyethoxylates (NP*n*EO) in the primary effuent and (B) nonylphenol (NP), nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) in the secondary effluent of the sewage treatment plant Zürich-Glatt, Switzerland. Samples were collected from 16th to 23rd October 1997.

Furthermore, elimination of higher oligomers in a mechanical-biological STP is so efficient that there is little need for extensive surveys of natural waters for these compounds. Therefore, a second normal-phase HPLC procedure developed in this work was optimised for accurate routine determination of NP, NP1EO and NP2EO. This procedure allowed extensive monitoring of NP*n*EO-related emissions *via* secondary effluents in a study that is currently being carried out in Switzerland. The data presented in Figure 7 show weekly dynamics of NP*n*EO-related compounds in STP Zürich-Glatt. Variability of the NP*n*EO concentration in the primary effluent reflects the well-known feature of a decreased input to that STP during weekends (Figure 7A), while the concentrations of lipophilic metabolites in the secondary effluent remain relatively constant (Figure 7B).

CONCLUSIONS

Despite the recent development of elegant methods suitable for the concurrent routine determination of NP*n*EO and their lipophilic metabolites by reversed-phase techniques,[26] the application of normal-phase HPLC coupled with fluorescence detection still represents an attractive alternative with some clear advantages. The most important advantage is the information on oligomer distribution that is highly indicative of the type of NP*n*EO-emission into the wastewater (*e.g.* surfactant-derived *versus* emulsifier-derived NP*n*EO) as well as of biological alteration of the original material after release into the environment. Fluorescence detection offers a significant improvement of the originally developed normal-phase HPLC procedures using UV-detection[21,22] with respect to selectivity and sensitivity. This allows processing of much smaller samples (< 100 ml) to achieve the required sensitivities of 1 μg/l for the total NP*n*EO and 0.1 μg/l for the individual lipophilic metabolites. The methods developed in this work proved to be suitable for the routine application and are currently being applied in an extensive monitoring study carried out in Switzerland.

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SAŽETAK

Određivanje nonilfenol-polietoksilata i njihovih lipofilnih razgradnih produkata u otpadnim vodama primjenom tekućinske kromatografije visoke djelotvornosti i fluorometrijske detekcije

Marijan Ahel, Walter Giger, Eva Molnar i Slavica Ibrić

Razvijen je analitički postupak prikladan za rutinsko određivanje tenzida iz skupine alkilfenol-polietoksilata (AP*n*EO) i njihovih lipofilnih razgradnih produkata u otpadnim vodama pri koncentracijama ≤ 1 μg/l primjenom tekućinske kromatografije visoke djelotvornosti (HPLC). Osnovni sastojci tenzidne smjese, oligomeri nonilfenol-polietoksilata (NP*n*EO) koji sadrže 1–18 etoksi-jedinica, ekstrahirani su iz vodenih uzoraka propuštanjem kroz kolonicu ispunjenu grafitiziranim ugljikom, dok su njihovi lipofilni razgradni proizvodi, nonilfenol-monoetoksilat (NP1EO), nonilfenol-dietoksilat (NP2EO) i nonilfenol (NP), ekstrahirani izmućkivanjem uzoraka s n-heksanom. Ekstrakti su analizirani tekućinskom kromatografijom normalnih faza uz upotrebu kolona punjenih amino-silikagelom i fluorimetrijsku detekciju pri 228/305 nm. Ta je tehnika omogućila pouzdano kvantitativno određivanje pojedinih oligomera NP*n*EO niskih koncentracija (0,1 μg/l). Analize uzoraka sakupljenih u uređajima za pročišćavanje otpadnih voda kantona Zürich, Švicarska, uputile su na znatnu prisutnost NP*n*EO (96–430 μg/l) u otpadnim vodama koje su prošle samo mehanički stupanj obradbe iako je još od 1986. godine njihova primjena zakonom ograničena. Raspodjela oligomera NP*n*EO u analiziranim uzorcima potvrdila je da najveći dio toksičnih lipofilnih metabolita nonilfenolnog tipa potječe od upotrebe NP*n*EO kao tenzida.