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Note

Determination of alkylphenol ethoxylates in environmental samples by high-performance liquid chromatography coupled to fluorescence detection

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There is at present considerable interest in the determination and characterisation of non-ionic detergents in environmental samples. Much of this interest is centred on the increased market share of branched alkylphenol ethoxylates (APEOs) coupled to the uncertainty surrounding their ultimate biodegradability especially during the winter months[1]. The APEOs now comprise between 10 and 25% of the total non-ionic detergents present in domestic sewage influents across Europe and as a result there is a need for a rapid and sensitive method for the analysis of both parent compound and biodegradation products. The mechanism of APEO biodegradation involves shortening of the ethoxylate moiety by a stepwise cleavage of one ethylene oxide unit at a time[2], however, little is known regarding the biodegradation of the hydrophobic moiety. As a net result alkylphenoxyethoxyethanol has been identified by gas chromatography–mass spectrometry in the effluent from sewage treatment plants[3,4].

Current EEC legislation on the biodegradability of non-ionics (EEC directive 82/242) recommends the Wickbold method[5] (bismuth-active substances) for the determination of non-ionic surfactants but this method is limited by interferences from other organic compounds and by its decreased sensitivity to lower-chain-length ethoxymers. Other spectrophotometric methods based on complex formation reactions between the non-ionic surfactants and picric acid[6], potassium tetrathiocyanatozincate[7], malachite green[8] and Dragendorff reagent[9] are subject to the same limitations.

The simplest and most suitable analytical procedure for APEO analysis is high-performance liquid chromatography (HPLC), however the sensitivity of the procedure is dependent on the detection method. HPLC of APEOs has usually been coupled to UV detection[10–14], however only Ahel and Giger[13,14] have described its application to environmental samples. A review of HPLC of non-ionic surfactants has recently been published[15]. Only one report, to date, has been concerned with the use of HPLC coupled to fluorescence detection[16]. In this paper we describe a more sensitive and specific method for the analysis of parent APEOs based on normal-phase HPLC and fluorescence detection.

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EXPERIMENTAL

Analytical apparatus

The analytical HPLC equipment consisted of a Bruker LC/31 and LC/41 microprocessor-controlled solvent programmer linked to a Perkin-Elmer LS 5 fluorescence spectrometer with a 16-μl flow-through cell. The excitation wavelength was set at 230 nm and the emission wavelength at 302 nm with respective slit widths of 10 and 5 nm. The columns used were 250 mm × 4.6 mm I.D. packed with Zorbax NH_2 (DuPont) or Partisil 5 PAC (Whatman). Solutions of the surfactants were injected into the column using a Rheodyne 7125 injection valve. A linear gradient of 0.1% acetic acid in methyl *tert.*-butyl ether (MTBE) (solvent A) to 0.1% acetic acid in acetonitrile–methanol (95:5) (solvent B) over 30 min at 2 ml min^{-1} was used as eluent. A hold time of 15 min with 100% B was followed by a re-equilibration time of 10 min with 100% A before the next injection. The temperature was 20 ± 1°C.

Extraction of non-ionics

Extraction and purification of non-ionic surfactants was carried out on 250-ml sewage-influent samples and 1000-ml sewage-effluent samples by sublation, ion-exchange and alumina chromatography essentially as described by Waters *et al.*[17].

Separation of octylphenol ethoxymers (OPEOs)

Purification of individual OPEO homologues was achieved by subjecting aliquots of a standard mixture of 0.18 g Triton X-15, 0.36 g Triton X-35 and 1.96 g Nonidet P40 to preparative HPLC. The apparatus consisted of a Gilson Model 302 injector, two Model 303 pumps for the eluent, a Holochrome UV detector (set at 278 nm) and a 250 mm × 20 mm I.D. Partisil 10 PAC column (Whatman). The elution solvents were: MTBE (solvent A) and acetonitrile (solvent B). The gradient applied was 100% A for 3.5 min then linearly up to 50% solvent B at 0.5% min^{-1} at a flow-rate of 17 ml min^{-1}. The column was then flushed with acetonitrile–methanol (95:5) for 10 min followed by a re-equilibration period of 20 min with solvent A. Samples (5 ml) of the standard mixture (2.5 g in 50 ml MTBE) were injected. Fractions were collected from the centre of the peaks and evaporated to a low volume under a stream of nitrogen and transferred to pre-weighed 4-ml vials before being evaporated to dryness. Fluorescence (excitation wavelength 230 nm, emission wavelength 302 nm) weight response factors (WRFs) were calculated by dividing the amount (in ng) injected by the peak area. Relative weight response factors (RWRFs) were then derived for individual ethoxymers based on OP-12EO = 1.000. To quantify chromatograms the weights of individual ethoxymers are given by measured area × WRF. UV molar extinction coefficients were determined at 230 nm and 278 nm from the equation $E_m = A/[M \cdot p]$, where E_m is the molar extinction coefficient, M is the molar concentration, p is the cell path length in cm and A is the absorbance.

Chemicals

Nonidet P40 (octylphenol ethoxylate n_{av} = 8.5, where n_{av} refers to the average number of ethylene oxide units attached to the alkylphenol) was obtained from Shell Chemicals U.K., Carrington, U.K. Triton X-15 (n_{av} = 1.5) and Triton X-35 (n_{av} = 3.5) were obtained from Rohm and Haas, Philadelphia, PA, U.S.A. and HPLC-grade methanol, acetonitrile, MTBE and acetic acid from Rathburn, Walkerburn, U.K. All other chemicals were of analytical-reagent grade.

RESULTS AND DISCUSSION

Using the Perkin-Elmer LS 5 the excitation spectrum of Nonidet P40 with total emission > 290 nm shows a maximum at 225 nm. Absorption maxima are also found at 278 and 285 nm as shown in Fig. 1. Fig. 1 also illustrates the emission spectrum for Nonidet P40 at an excitation wavelength of 230 nm and shows a maximum emission at 302 nm. Similar spectra were obtained with octylphenol and octylphenol ethoxyethanol.

A typical chromatogram showing the separation of Nonidet P40 spiked with octylphenol and octylphenol 1–3EO using the Zorbax-NH_2 packing is shown in Fig. 2. The major components show baseline separation with the exception of octylphenol and octylphenol-1EO. For baseline separation of the shorter chain (EO 0–3) octylphenol ethoxylates we used Partisil 5 PAC which resulted in retention times of 2.2, 3.5, 5.5 and 6.9 min for octylphenol, OP-1EO, OP-2EO and OP-3EO, respectively (see Fig. 3).

The use of a Zorbax NH_2 column, coupled to a UV detector (276 nm), for the determination of ethoxymer distribution of alkylphenol polyoxyethylene surfactants has been reported by Rothman[18] and the use of fluorescence detection (excitation wavelength 280 nm, emission wavelength 310 nm) coupled to a 5-μm LiChrosorb Si 60 column has been reported by Kudoh *et al.*[16]. By gradient elution with MTBE, acetonitrile and methanol it is possible to use excitation wavelengths down to 230 nm and hence increase the degree of sensitivity. Furthermore, using MTBE as the start eluent separation of the shorter-chain-length ethoxymers (which have been identified as biodegradation products)[19] is enhanced such that this system can be used

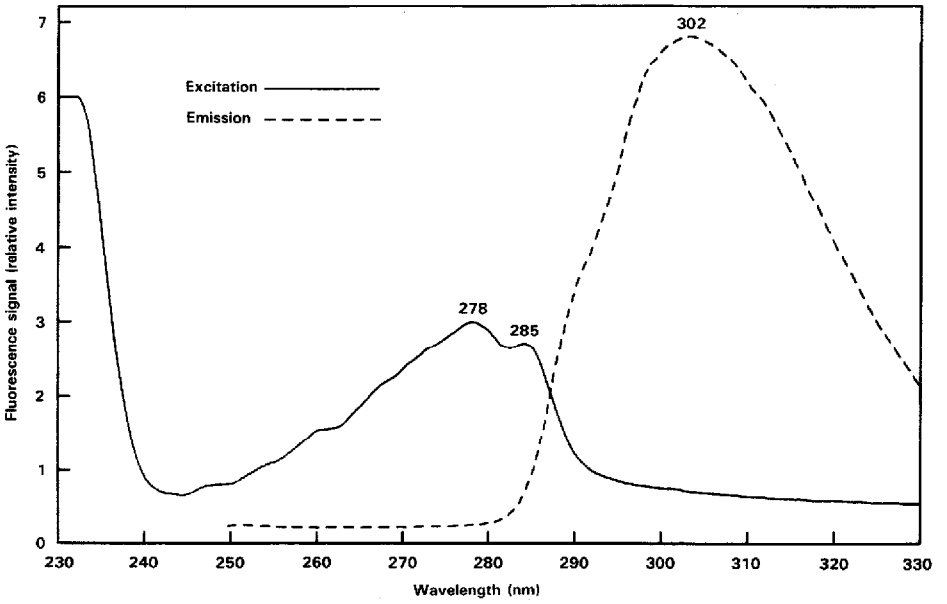

Fig. 1. Fluorescence spectra of Nonidet P40 in MTBE at 20°C; excitation scan monitoring total emission > 290 nm and emission scan using excitation wavelength of 230 nm.

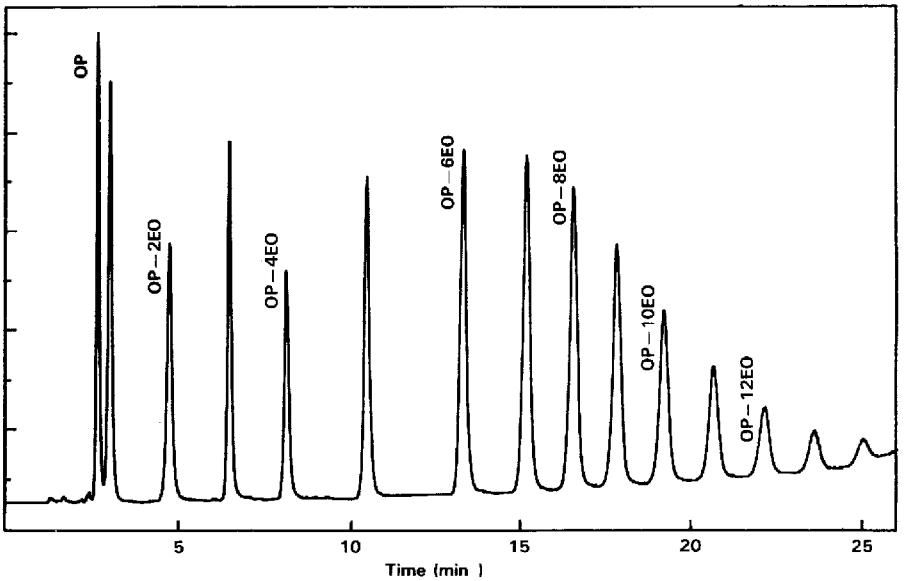

Fig. 2. HPLC trace of Nonidet P40 spiked with octylphenol, octylphenol-1EO, octylphenol-2EO and octylphenol-3EO. Linear gradient (30 min) of 100% A to 100% B at 2 ml min^{-1}, where A = methyl *tert.*-butyl ether + 0.1% acetic acid and B = acetonitrile–methanol (95:5) + 0.1% acetic acid; Zorbax NH_2 column.

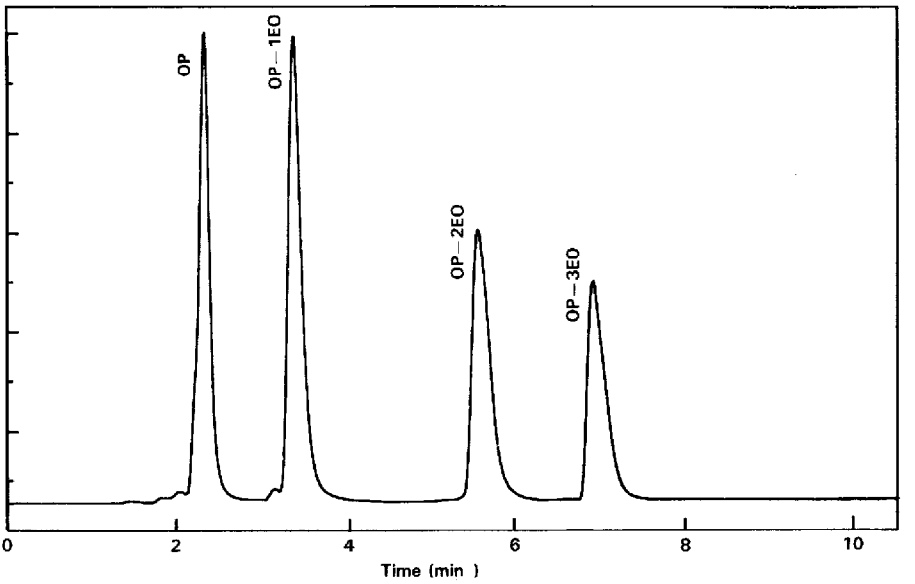

Fig. 3. HPLC trace of octylphenol, octylphenol-1EO, octylphenol-2EO and octylphenol-3EO. Linear gradient (9 min) of 100% A to 30% B at 2 ml min^{-1}, where A = methyl *tert.*-butyl ether + 0.1% acetic acid and B = acetonitrile–methanol (95:5) + 0.1% acetic acid; Partisil 5 PAC column.

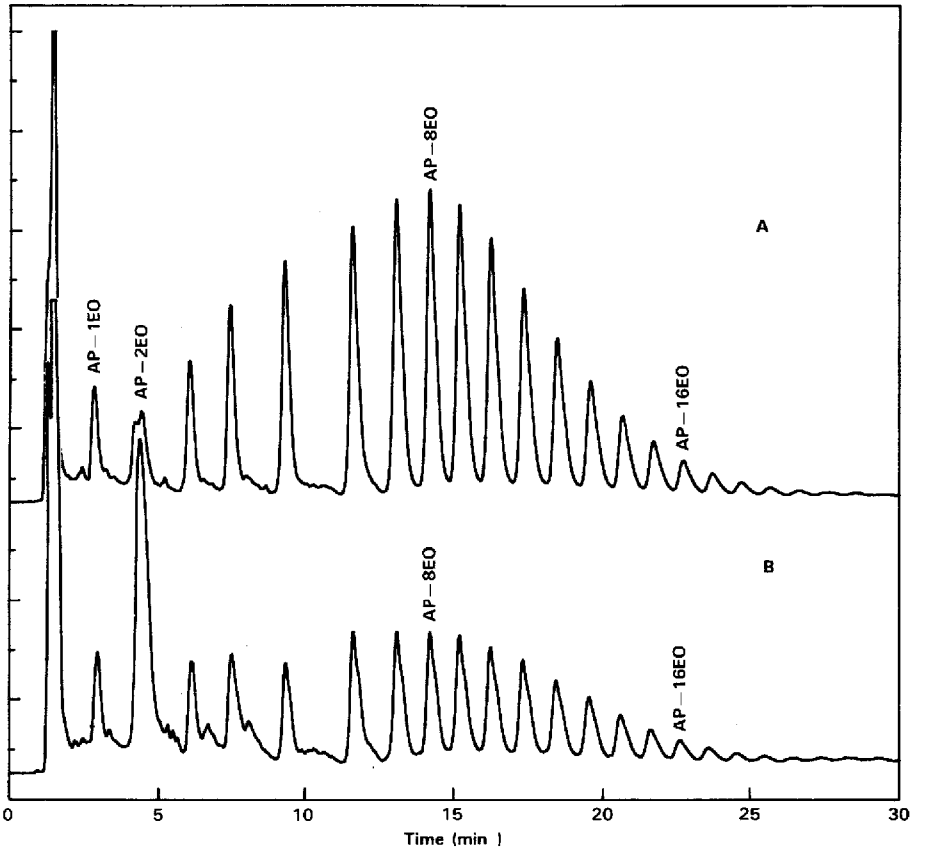

Fig. 4. Normal-phase high-performance liquid chromatogram (Zorbax NH_2 column) of solvent sublation extract. (A) raw municipal sewage; (B) effluent from an activated sludge sewage treatment plant.

for the analysis of biodegradation test liquors and environmental samples. Fig. 4 illustrates the HPLC of solvent sublation extracts of sewage influent and effluent.

Alkylphenol ethoxylates from AP-1EO up to AP-19EO were detected in both influent and effluent and confirmed by mass spectroscopy. AP-8EO was the major component of the influent and AP-2EO the major component of the effluent. Total APEO in influents from two sewage treatment plants in South East England varied from 126 to 410 $\mu g\ l^{-1}$ and effluent levels varied from 40 to 228 $\mu g\ l^{-1}$. The highest effluent levels being recorded during February (sewage temperature 9°C). The shoulders on each ethoxylate peak reflect the partial separation of the different alkyl chain lengths, branched isomers and substitution patterns on the phenol ring of these complex mixtures.

Using the settings and equipment described the WRFs were in the range 0.382 to 1.000 for OP-1EO and OP-12EO, respectively. An increase in ethoxylate chain length results in a lower specific absorbance and hence the RWRF is higher. These results are in good agreement with the results obtained by Ahel and Giger[13] for UV

response factors at 277 nm. The UV extinction coefficient at 230 nm was between $8.2 \cdot 10^3$ and $9.4 \cdot 10^3$ for OP-1EO to OP-12EO and between $1.5 \cdot 10^3$ and $1.8 \cdot 10^3$ at 278 nm and confirm that the molar extinction coefficient for ethoxylated octylphenols is independent of the ethoxylate chain length[18].

The minimum level of detection by this system is 0.2 ng of individual homologues of the APEOs. This method, using the extraction and clean up procedure of Waters *et al.*[17] together with chromatography on Zorbax NH_2, gives an improved analysis for parent alkylphenol ethoxylate surfactants in environmental samples. Due to the volatility of alkylphenols and their lower EO adducts care must be taken to avoid losses during the clean up of extracts. In this respect steam distillation[13] may be preferred for the extraction of the biodegradation products (AP, AP-1EO, AP-2EO) and improved separation is obtained using the Partisil PAC column.

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