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Determination of nonylphenol polyethoxylates and their carboxylic acid metabolites in sewage treatment plant sludge by supercritical carbon dioxide extraction

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Abstract

A supercritical fluid extraction (SFE) method was developed for the extraction of nonylphenol polyethoxylate (NP*n*EO) non-ionic surfactants from dried sewage treatment plant sludge. Extraction was carried out at 80°C and 5100 p.s.i. with carbon dioxide using water as a modifier. The ethoxylates were analyzed by gradient high-performance liquid chromatography (HPLC) with an APS Hypersil column and a fluorescence detector (230 nm excitation and 300 nm emission). This SFE method was more time-efficient and it produced higher recovery than the traditional Soxhlet extraction and steam distillation techniques used for NP*n*EO in sewage sludge. The same procedure was also applicable to the coextraction of nonylphenoxyacetic (NP1EC) and nonylphenoxyethoxyacetic (NP2EC) acids, which were metabolites of the ethoxylates under aerobic conditions. Following an off-line methylation, analysis of the acids was achieved by GC–MS in selected ion monitoring mode. In a brief survey of sludge samples collected from nine sewage treatment plants across Canada, very high levels of nonylphenol mono- (NP1EO, 28–304 μg/g) and di-ethoxylates (NP2EO, 4–118 μg/g) were found. In contrast, the total polyethoxylate concentration (from 3 to 17 ethoxy units) was generally less than 50% of the sum of NP1EO and NP2EO in the same sample. NP1EC and NP2EC were found in only three of the seven samples tested, with concentrations ranging from 4 to 38 μg/g. © 1997 Elsevier Science B.V.

Keywords: Nonylphenol polyethoxylates

1. Introduction

Non-ionic surfactants based on nonylphenol polyethoxylates (NP*n*EO, *n* is the number of ethoxy units) are widely used in Canada by the textile and pulp and paper industries. The demand for NP*n*EO in Canada in 1990 was estimated to be 4.1 kton. Under aerobic conditions, the ethoxylates degrade in sewage treatment plants into nonylphenol and nonylphenol mono- (NP1EO) and di-ethoxylates (NP2EO), as well as nonylphenoxyacetic acid (NP1EC) and nonylphenoxyethoxyacetic acid (NP2EC) [1–4]. According to a study by Ahel et al. on eleven sewage treatment plants in the Glatt Valley, Switzerland, 46% of the nonylphenolic compounds were NP1EC and NP2EC while 22% were NP1EO and NP2EO, in secondary effluents [3].

A linear relationship between solubility and the number of ethoxy groups was established for nonylphenol ethoxylates, i.e., NP1EO and NP2EO were the least soluble oligomers [5]. The logarithmic

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octanol–water partition coefficients (log K_{ow}) for these two compounds were ca. 4.2 [6], so they are lipophilic and have a tendency to accumulate in sediment and aquatic organisms. In a study on the 48 h LC_{50} for Japanese killifish (*Oryzias latipes*), the toxicity of NP*n*EO was found to increase with decreasing length of the polyethoxy chain [7]. Data using responses to the yeast screen [8] and rainbow trout in vitro hepatocyte bioassay [9] indicated that NP2EO, NP1EC and NP2EC were weakly estrogenic. In fact, NP2EO and NP1EC were within a factor of 2 in relative estrogenic potency in comparison to nonylphenol. In contrast, NP9EO was ca. 30 times lower in estrogenic potency estimated by the bioassay [9].

The occurrence of NPnEO, NP1EC and NP2EC in treated and untreated sewage effluents at μg/l levels has been reported by Ahel et al. [1] and by Di Corcia et al. [10]. While there were more data for these contaminants in effluents, only limited results have been published so far for the ethoxylates in sludge and sediment [2,4,11]. Particularly, sludge and sediment data for NP*n*EO (for $n>2$) as well as NP1EC and NP2EC are not available.

The lack of sediment and sludge data for these contaminants is an indication of a shortage in analytical methods. To date, no analytical procedure has been published for the extraction of NP1EC and NP2EC in sludge. For the determination of NP1EO and NP2EO in sediment, a combined steam distillation–solvent extraction procedure has been used [2]. It was not known whether or not this method could also be applied to the determination of polyethoxylates with three or more ethoxy units. Previously, a supercritical fluid extraction (SFE) method for other non-ionic surfactants such as linear alkylbenzenesulfonates and secondary alkanesulfonates in sewage sludge was published by Field et al. [12]. While the analysis of polyethoxylated octylphenol and nonylphenol by supercritical fluid chromatography (SFC) has been reported [13,14], the application of SFE to the determination of NP*n*EO in sludge has never been exploited. Our present work describes an efficient technique which applies to the extraction of all NP*n*EOs (from $n=1$ to 17) as well as NP1EC and NP2EC from dried sewage sludge using supercritical carbon dioxide.

2. Experimental

2.1. Chemicals and reagents

NP1EO and NP2EO were purchased as a mixture called POE (1 to 2) nonylphenol from ChemService (West Chester, PA, USA). A calibrated mixture of NP*n*EO (No. 7427-20), with 1 to 17 ethoxy units, was provided by Dr. C. Naylor (Huntsman, Austin, TX, USA). This blend has the following composition, in weight%: nonylphenol (3%), distilled NP1EO (2.5%), surfonic N-40 (24%) and surfonic N-95 (70.5%). A 12 μg/ml working standard of this calibrated mixture was prepared by dilution of a stock solution with *n*-hexane–2-propanol (98:2, v/v). Nonylphenol ethoxylate preparations such as Imbentin-N/020, -N/7A, -N/11A and -N/20A, with ca. 1.5, 2.0, 3.0 and 5.0 ethoxy units, respectively, were also received from Dr. W. Kolb AG, Hedingen, Switzerland. NP1EC was synthesized by the oxidation of Imbentin-N/020 with Jones reagent [15]. A stock solution of NP1EC, at 1000 μg/ml, was prepared in methanol.

All organic solvents used were either distilled-in-glass grade (for extraction) or HPLC grade (for high-performance liquid chromatographic work). Supercritical carbon dioxide (SFE grade) was obtained from Air Products (Nepean, ON, Canada). 14% BF_3 in methanol was a reagent available from Supelco (Oakville, ON, Canada). Celite 545, non-acid washed, was a product of Fisher Scientific (Toronto, ON, Canada).

2.2. Collection and preparation of sludge samples

Digested sludge samples were collected from sewage treatment plants located in various municipalities across Canada. They were all air-dried, ground, homogenized and sieved through a 30-mesh screen prior to extraction.

2.3. SFE of NPnEO from dried sewage sludge

An extraction thimble was prepared by placing, at its bottom end, a piece of filter paper with the same diameter of the thimble and then 200 mg of Celite. Typically, a subsample from 100 mg to 1 g of an

air-dried sludge was extracted. Prior to the extraction, water was spiked to the sample as a static modifier at a rate of 2 ml per g sample. Another piece of filter paper was put on top of the sample. The void volume of the extraction thimble was filled with a glass rod of a suitable length and diameter. The sludge was extracted with supercritical carbon dioxide at 80°C and ca. 5100 p.s.i. (1 p.s.i.=6894.76 Pa) (0.79 g/ml) and at a flow-rate of 2 ml/min using a Hewlett-Packard 7680T supercritical fluid extractor. The extraction times were 10 min static and 15 min dynamic. The variable restrictor nozzle was kept at 60°C to prevent freezing. Sample extract was adsorbed onto an octadecylsilane functionalized silica gel (ODS) trap which was maintained at 10°C during extraction. When the extraction was done, the trap was heated to 60°C and rinsed with three 1.5 ml aliquots of methanol into 3 vials. The extracts were combined with acetone rinses and the solvent was evaporated down to 0.2 ml. The solvent was then exchanged into hexane and finally adjusted to 1.0 ml of the mobile phase (solvent A, see Section 2.6) for HPLC analysis. Blanks for this SFE procedure were obtained by the same extraction conditions except for the absence of a sludge sample.

2.4. SFE of NP1EC and NP2EC from dried sludge and off-line methylation of the acids

Carboxylic acid metabolites of nonylphenol ethoxylates in sewage sludge were extracted by the same method as for the ethoxylates with one difference: the static time was increased from 10 to 15 min. The methanol extract was combined and transferred to a screw cap tube with a PTFE liner. After the solvent was evaporated to <250 μl, 2 ml of a 14% BF_3 in methanol solution were added. The mixture was then heated at 85°C in a tube heater for 30 min. At the end of the reaction, the mixture was evaporated at 45°C to <500 μl before the addition of 3 ml of water. The methylated products were extracted by three 2-ml aliquots of petroleum ether (b.p. 30–60°C). After passing through an anhydrous sodium sulfate column prepared in a Pasteur pipet, the extract was evaporated and reconstituted into 1 ml of isooctane for GC–MS analysis.

2.5. SFE and in situ methylation of NP1EC and NP2EC from dried sludge

On-line SFE and methylation of NP1EC and NP2EC of the free acids from sludge was also attempted. In such cases, 200 μl of 14% BF_3 in methanol was used in place of water as a modifier as well as a methylating agent, and the other extraction conditions remained the same. At the end of the extraction, the derivatives were eluted from the trap by acetone. After the solvent was exchanged into isooctane, the extract was ready for GC–MS analysis.

2.6. HPLC analysis of NPnEO

All HPLC analyses were carried out with a system consisting of a Hewlett-Packard (HP) 1050 quaternary pump, a HP 1050 autosampler, a HP 1046A programmable fluorescence detector, a HP 35900 multichannel interface and data system. A HP APS Hypersil (5 μm, NH_2) 100×2.1 mm I.D. analytical column equipped with a 20×2.1 mm I.D. guard column packed with 5 μm ODS were employed. For the separation of a NP*n*EO mixture (from 1 to 17 ethoxy units), the following solvent mixtures were used: solvent A, *n*-hexane–2-propanol (98:2, v/v) and solvent B, 2-propanol–water (9:1, v/v). The initial mobile phase was 97% A and 3% B and was kept constant for the first 3 min. It was then linearly programmed from this composition to 43% A and 57% B in the next 22 min. A post-run equilibration time of 15 min was used between sample injections. The HPLC column was maintained at 40°C with a constant flow-rate of 0.3 ml/min. For the analysis of NP1EO and NP2EO only, the HPLC was operated under isocratic conditions using a mixture of *n*-hexane–2-propanol (98:2, v/v) as mobile phase. 10 μl of a sample was injected and the detector was set at wavelengths of 230 nm (excitation) and 300 nm (emission) with a PMT gain of 10.

2.7. GC–MS analysis of the methyl esters of NP1EC and NP2EC

Since a commercial standard for NP2EC was not available, a 25 μg/ml solution of Imbentin-N/7 A, a

non-ionic surfactant containing mainly NP1EO and NP2EO, was subject to microbial degradation under aerobic conditions to generate their carboxylic acid metabolites so that a qualitative standard for NP2EC could be obtained (Niimi and Lee, unpublished work). After an incubation period of 10 days at room temperature, an aliquot of the solution was acidified, extracted and methylated according to Ahel et al. [1]. The products were examined by full scan GC–MS (m/z 40 to 400) using a HP 5890 Series II gas chromatograph and a HP 5972 mass selective detector to establish the mass spectral characteristics and their retention times. A 30 m×0.25 mm I.D., 0.25 μm HP-5-MS column was used and 1 μl splitless injection was made by a HP 7673 autosampler. The GC oven program was 70°C initial (held for 1 min), increased to 160°C at 30°C/min and then to 290°C at 5°C/min. Injection port and detector interface temperatures were 250 and 280°C, respectively. Carrier gas (helium) linear velocity was held constant at 39.8 cm/s by means of an electronic pressure controller. The electron energy and electron multiplier voltage were 70 eV and 2200 V, respectively.

For the identification and quantitation of NP1EC and NP2EC in sludge extracts, GC–MS with selected ion monitoring was employed. The following characteristic ions, m/z 207, 221 and 292 (for NP1EC-Me isomers, from 13.2 to 14.2 min) and m/z 117, 265, 307 and 336 (for NP2EC-Me isomers, from 18.0 to 19.3 min), were monitored. Since an authentic standard for NP2EC was not available, its concentration in various samples was estimated by using the response factor generated for the synthesized NP1EC.

3. Results and discussion

3.1. Chromatographic determination of NPnEO and the methyl esters of NP1EC and NP2EC

In some earlier studies, capillary column GC procedures have been used for the analysis of NP*n*EO either directly [16] or after conversion into more volatile derivatives [17]. This approach was limited to ethoxylates with 1 to 5 ethoxy units because of the lack of volatility of the higher oligomers. In contrast, the combination of a normal-phase HPLC column and gradient elution provides an efficient separation of nonylphenol ethoxylate oligomers with 1 to ca. 17 ethoxy units [18–20]. Particularly, columns packed with modified silica such as the amino or cyano bonded phases have been demonstrated to be highly efficient for the separation of alkylphenol ethoxylates. Although the UV absorbance detector was more widely used for the detection of NP*n*EO, much higher sensitivity and better selectivity could be realized with a fluorescence detector operated at optimal wavelengths [21,22]. In this work, a narrow-bore, APS Hypersil column packed with 5 μm aminosilica and a fluorescence detector were chosen for the separation and detection of the ethoxylates. Using the solvent system and gradient described in Section 2.6, nonylphenol and all 17 NP*n*EOs in the calibrated standard were resolved in 25 min (Fig. 1). The advantages of using a smaller diameter (2.1 mm I.D.) analytical column were reduced solvent consumption (at a lower flow-rate of 0.3 ml/min) and a higher column efficiency. For the elution of NP1EO and NP2EO, however, isocratic runs with a mobile phase consists of 2% 2-propanol in hexane (v/v) provided more rapid analyses.

While LC and LC–MS methods have been used for the analysis of NP1EC and NP2EC [1,10,15,23], GC–MS analysis of their methyl esters was chosen in our work because of lower operating cost and higher selectivity of the detector. Instead of a single eluted peak observed for each carboxylic acid in the case of HPLC, the higher resolution of the capillary GC column produced two groups of peaks for the methyl esters of NP1EC and NP2EC due to the different isomers of the nonyl group [1]. This additional fingerprint information is extremely useful for the identification of the acids in a complex matrix such as sewage sludge. Full scan GC–MS analysis of the carboxylic acid methyl esters derived from the microbial degradation products of Imbentin-N/7A showed two major groups of peaks with retention times from 13.2 to 14.2 min (for NP1EC-Me) and from 18.0 to 19.3 min (for NP2EC-Me) (see Fig. 2). Also observed were two minor groups of peaks of retention times from 12.0 to 13.0 min as well as from 16.2 to 17.8 min. Presumably, the major and minor peaks were due to the 4- and 2-substituted isomers, respectively, of the alkyl side chain. The characteris-

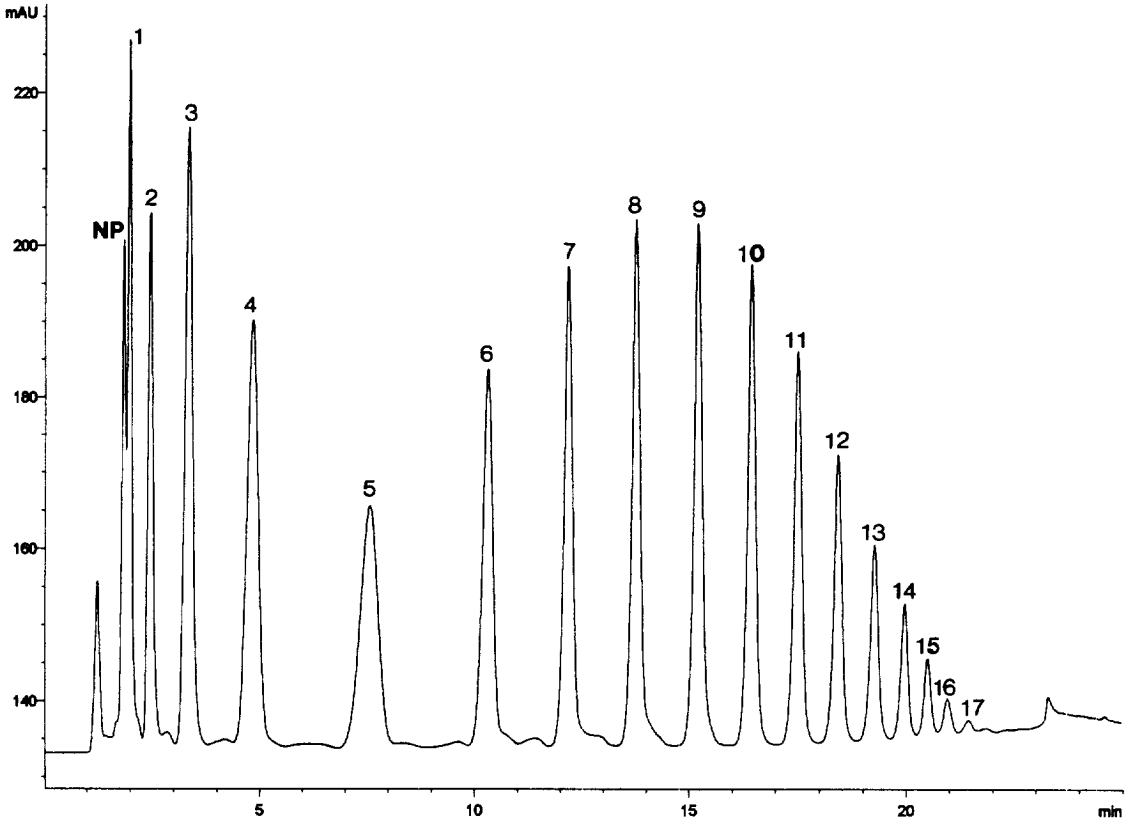

Fig. 1. HPLC separation and fluorescence detection of NP*n*EO (for $n=1$ to 17). Key: NP=nonylphenol, 1=NP1EO, etc.

tic ions of m/z 179, 193, 207, 221 and 292 (M^+), for NP1EC-Me, and ions of m/z 117, 265, 279, 307 and 336 (M^+), for NP2EC-Me, were consistent with those reported earlier by Ahel et al. for the same compounds [1]. In both cases, the molecular ions were weak (<5% of the base peak). Since ions of

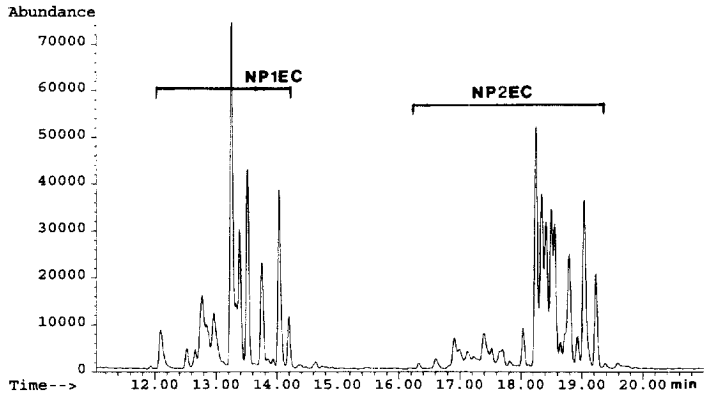

Fig. 2. GC–MS chromatogram of the methyl esters for a mixture of NP1EC and NP2EC derived from the carboxylic acid metabolites of Imbentin-N/7 A.

m/z 179, 193 were also noted for NP1EO and the ion of m/z 279 was observed for NP2EO, they were not used in the selected ion monitoring of the respective carboxylic acid esters.

3.2. Development of a SFE method for NPnEO from dried sewage sludge

A digested sludge collected from a municipal sewage treatment plant was dried, homogenized and used in the development of the SFE method. This sample had previously been extracted by the Soxhlet method using dichloromethane (DCM) and was shown to contain high levels of the ethoxylates. Since NP1EO and NP2EO are the major ethoxylates found in sewage sludge, they were used to monitor the extraction efficiency during the initial stage of the method development.

SFE of this sample was first attempted at 60°C by using pure CO_2 with a density of 0.86 g/ml (4997 p.s.i.). A dynamic extraction time of 15 min with a CO_2 flow-rate of 2 ml/min was also used. At the end of the extraction, acetone was used to elute the ethoxylates adsorbed on the ODS trap. For comparison, extraction was also carried out with the addition of DCM, water, and DCM–water (1:1) directly onto the sludge prior to the extraction, at a rate of 500 μl per g of sample. In those cases where a modifier was used, a static extraction time of 10 min was also included. As shown in Table 1, NP1EO and NP2EO in this naturally contaminated sample were readily extracted by pure CO_2 in 15 min. Addition of either DCM, water, or DCM–water (1:1, v/v) as static modifiers improved the recovery slightly, although the effect was more pronounced with water. In the case of water (at 50 μl/100 mg sample), 8 and 22% increases in recovery were observed for NP1EO and NP2EO, respectively. No further improvement in the recovery for these two compounds was observed when a larger amount of modifier, i.e., double or quadruple the amount of water, was introduced to the sample. For the remainder of this work, water was added to the sludge as a static modifier prior to the extraction of the ethoxylates.

Table 1
Effect of modifier on the SFE results of NP1EO and NP2EO from a sewage sludge with naturally contaminated NP*n*EO

Modifier	NP1EO (μg/g)	NP2EO (μg/g)
None	247	97
50 μl DCM	254	108
50 μl water	265	118
50 μl DCM–water (1:1)	259	105
100 μl water	263	117
200 μl water	253	109

Extraction conditions: 100 mg of sample was extracted, in each case, at 60°C with CO_2 at a density of 0.86 g/ml, in the presence or absence of a modifier. Extraction times: 10 min static, 15 min dynamic. Flow-rate of CO_2: 2.0 ml/min. Results were averages of two extractions.

The sludge sample was then extracted by supercritical CO_2 at 40, 60, 80, 100 and 120°C, in duplicate, to determine an optimal extraction temperature for the ethoxylates. For these extractions, the fluid pressure was kept at a relatively constant 5000±100 p.s.i.. While the recoveries were slightly (<15%) lower for the 40°C extractions, the results for NP1EO (from 265 to 285 μg/g) and NP2EO (from 113 to 119 μg/g) were essentially the same for the extractions done at temperatures from 60 to 120°C. A temperature of 80°C was then chosen for the extraction of the ethoxylates in sludge. It was also observed that a second extraction of the same sludge, at 80°C, did not improve the yield of NP1EO and NP2EO by any significant amount.

When this SFE procedure was applied to the extraction of the higher ethoxylates (n=3 to 17) on spiked Celite and sediment samples, less than quantitative recoveries were observed for these ethoxylates. Lower recoveries were caused by incomplete elution of the extracts from the ODS trap as well as incomplete extraction from the sediment. To correct these problems, the ODS trap temperature was increased from 45°C to 60°C during desorption and the rinse solvent was switched from acetone to methanol. Also, the amount of modifier (water) per g sample was increased from 0.5 to 2 ml. After these modifications, the recoveries of all NP*n*EO at fortification levels of 100 and 10 μg/g were close to quantitative (Table 2). This revised procedure was then applied to the extraction of the polyethoxylates in the sludge and the results are also listed in Table 2. Attempts such as higher extraction temperature, longer extraction time and larger amount of modifier

Table 2
Recoveries of NP*n*EO from spiked sediment and the comparative yields of NP*n*EO using SFE, Soxhlet extraction and steam distillation

Matrix	Spiked sediment	Spiked sediment	Sludge	Sludge	Sludge	Sludge
Spiking level (μg/g)	100[a]	10[a]				
Extraction method	SFE	SFE	SFE	Soxhlet A/H[d,e]	Soxhlet DCM[e]	Steam distillation[e]
No. of replicates	4	4	2	2	2	2
Recovery	(%)	(%)	(μg/g)	(μg/g)	(μg/g)	(μg/g)
NP1EO	93±4[b]	91±6	277	261	264	279
NP2EO	98±2	95±4	118	110	105	35
NP3EO	91±4	89±4	40	36	24	13
NP4EO	93±4	88±4	19	16	3	1
NP5EO	91±3	89±3	13	11	1	1
NP6EO	91±2	97±2	15	12	1	N.D.
NP7EO	96±6	96±5	13	10	N.D.	N.D.
NP8EO	93±4	92±4	11	8	N.D.	N.D.
NP9EO	95±4	93±4	11	10	N.D.	N.D.
NP10EO	99±2	93±7	10	9	N.D.	N.D.
NP11EO	102±3	99±6	9	7	N.D.	N.D.
NP12EO	105±7	103±8	9	6	N.D.	N.D.
NP13EO	101±6	100±5	10	8	N.D.	N.D.
NP14EO	98±5	94±5	11	10	N.D.	N.D.
NP15EO	93±4	89±7	N.D.[c]	N.D.	N.D.	N.D.
NP16EO	91±5	85±6	N.D.	N.D.	N.D.	N.D.
NP17EO	86±10	–	N.D.	N.D.	N.D.	N.D.

[a] Total concentration for all NP*n*EO (n=1 to 17).
[b] Mean±standard deviation.
[c] None detected (from <0.2 μg/g for NP1EO to <2 μg/g for NP17EO).
[d] 59:41 (v/v) mixture of acetone and hexane.
[e] All Soxhlet extractions were done with 1 g of sludge and 300 ml of the solvent for 8 h at 6–8 cycles/h. Steam distillations were carried out wih 1 g of sludge and 300 ml of water for 3 h according to Ahel and Giger [2].

all failed to further improve the yield for NP*n*EO in this sample.

Using the same sludge sample, this SFE method was also evaluated against traditional procedures such as Soxhlet extraction and steam distillation which have been used to extract NP1EO and NP2EO in the past [2,11]. While SFE and Soxhlet extraction with acetone–hexane (59:41, v/v) produced comparable results for all ethoxylates found in the sludge sample, the SFE results were 10 to 25% higher than the Soxhlet results for the ethoxylates with n=3 and higher. In contrast, the recovery of the ethoxylates by Soxhlet extraction using DCM dropped off rapidly from NP3EO and on, and did not recover any ethoxylates beyond NP6EO. Thus, the application of this procedure is limited to NP1EO and NP2EO. Similarly, poor recovery of all polyethoxylates by the steam distillation technique limited its application to the extraction of NP1EO only.

Based on the above results, it was concluded that the more efficient SFE procedure is a suitable substitute for the classical Soxhlet technique for the extraction of NPnEO from dried sewage sludge.

3.3. SFE of NP1EC and NP2EC from dried sludge

Supercritical CO_2 modified with a polar solvent has been successfully used to extract acidic compounds such as some phenoxy herbicides [24], underivatized phenols [25] and resin and fatty acids [26] from soils, sediments and other solid matrices. Since water was shown to be an effective modifier for the ethoxylates, the applicability of the same SFE procedure to the extraction of NP1EC and NP2EC from sludge was also evaluated. The SFE extracts of the sludge were then methylated and the resulting products were shown, by GC–MS, to contain the methyl esters of the nonylphenoxy carboxylic acids.

A second extraction of the same sludge with additional modifier yielded an additional 16% of NP1EC and less than 5% of NP2EC. In sediment samples spiked with NP1EC at 10 and 1 μg/g levels, the recoveries of this acid by the present SFE procedure were better than 90%.

Further attempts to improve the yield of NP1EC from sludge in the first extraction were made by the use of formic acid and hydrochloric acid as modifiers. However, no significant improvement in the recovery was observed when they were used in place of water. Since acids tend to cause corrosion problems for the SFE hardware, particularly the pin tubes, their usage has since been terminated.

3.4. SFE and in situ methylation of NP1EC and NP2EC from sludge

In situ methylation of fatty acids and a few phenoxy acid herbicides under supercritical carbon dioxide extraction conditions has been demonstrated [27]. Earlier, we developed an in situ acetylation–SFE method for the determination of nonylphenol in sewage sludge [28]. In this work, combination modifier–methylating agents such as BF_3 in methanol and HCl in methanol were used for the single step extraction and methylation of NP1EC and NP2EC. The presence of methanol in these reagents eliminated the use of water since the former has commonly been used for the extraction of polar organic compounds. While the in situ methylation procedure was feasible and faster, the extraction recovery was less than 40% for the NP1EC and NP2EC in comparison to the off-line methylation procedure. For this reason, the in situ methylation method was not used.

3.5. Application of the SFE procedure to Canadian sewage sludge samples

Sewage treatment plant sludge samples collected from nine cities across Canada were extracted by this new SFE technique and analyzed for NP*n*EO, NP1EC, and NP2EC. In all of these samples, NP1EO and NP2EO were invariably the most abundant ethoxylates, with concentrations (on dry weight basis) varied from 28 to 304 μg/g, and from 4 to 118 μg/g, for the nonylphenol mono- and di-ethoxylates, respectively (Table 3). These numbers were similar to those reported by Ahel and Giger [2] for activated and anaerobically digested sludge. The individual concentrations of the higher ethoxylates in these samples were much lower, with the total concentrations of NP*n*EO (from $n=3$ to 17) ranging from 9 to 169 μg/g. With only one exception, the level of NPnEO was less than 50% of the sum of NP1EO and NP2EO in the same sample. In contrast, NP1EC and NP2EC were only detected in three of the seven sludge samples tested. Their concentrations varied from 4 to 38 μg/g. A HPLC chromatogram of

Table 3
Levels, in μg/g, of NP*n*EO and NP1EC and NP2EC in sludge collected from some Canadian sewage treatment plants

City	NP1EO	NP2EO	NP*n*EO[a]	NP1EC	NP2EC
Burnaby	70	18	60	–[b]	–
Edmonton	277	118	169	25	38
Regina	304	106	58	N.D.[c]	N.D.
Winnipeg	102	22	30	N.D.	N.D.
Toronto	51	5	9	20	35
Hamilton	54	5	28	–	–
Burlington	28	4	9	4	21
Montreal	165	25	16	N.D.	N.D.
Charlottetown	90	11	17	N.D.	N.D.

Results were averages of two determinations.
[a] Total concentration of NP*n*EO for $n=3$ to 17.
[b] Analysis not performed.
[c] None detected (<0.5 μg/g).

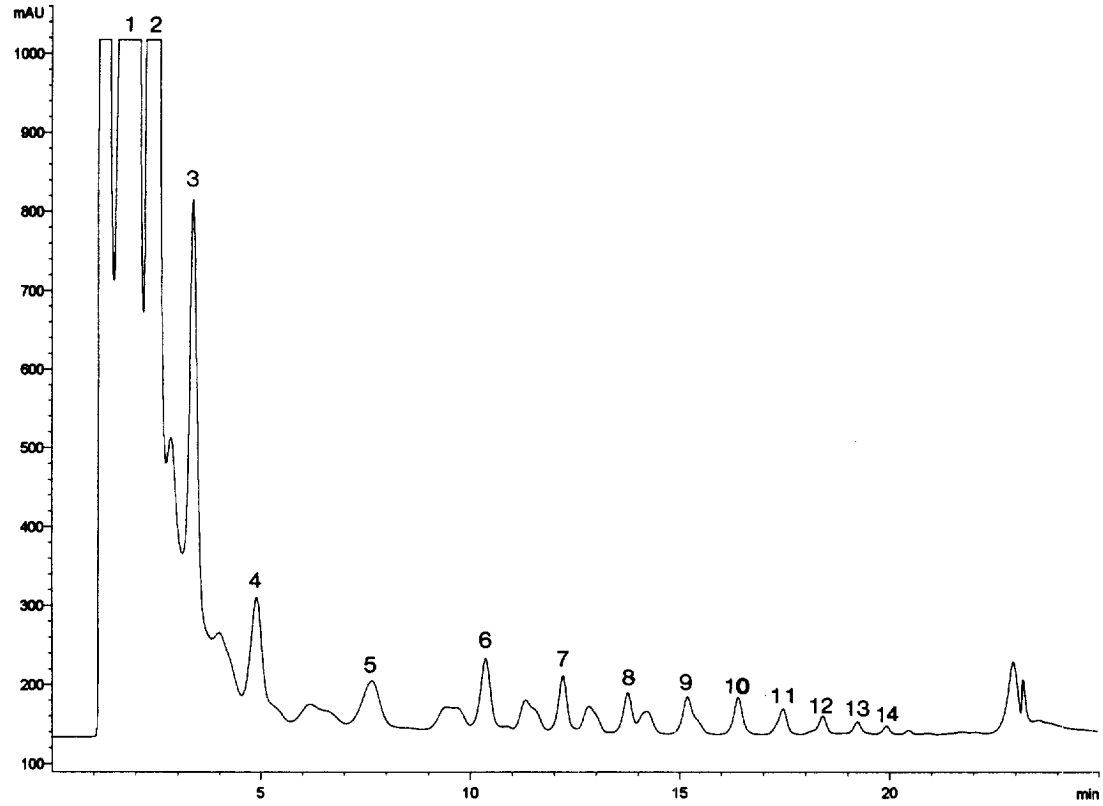

Fig. 3. HPLC chromatogram of a SFE extract derived from a sewage sludge with NP*n*EO. Key: 1=NP1EO, etc.

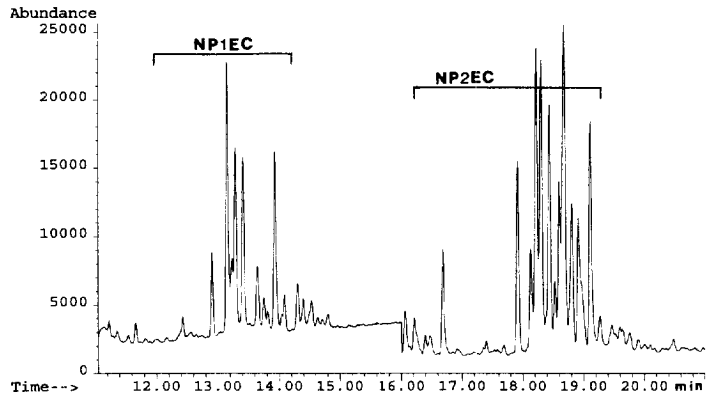

Fig. 4. GC–MS chromatogram of a methylated SFE extract derived from a sewage sludge with NP1EC and NP2EC.

a SFE extract depicting the presence of NPnEO in the sludge is shown in Fig. 3. A GC–MS chromatogram of another sludge extract containing the methyl esters of NP1EC and NP2EC is shown in Fig. 4. A comprehensive survey of the occurrence for these nonylphenolic compounds in Canadian sewage sludge and environmental samples is underway.

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