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Determination of nonylphenol polyethoxylates in household detergents by high-performance liquid chromatography

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Abstract

Preliminary survey results of the content of nonylphenol polyethoxylates (NPEOs) in various household detergents sold in Taiwan are presented. This survey was conducted to elucidate the concentration of NPEOs in household detergents and support pollution prevention and control programs. The concentrations of NPEOs in detergents and cleaners were determined by HPLC with a C_8 reversed-phase column and equipped with fluorescence detection. The accuracy and precision of the method was validated and was successfully applied to determine concentrations of NPEOs in household detergents. The results show that NPEOs were detected in 41% of 90 household detergents at concentrations from 0.2 to 21%. The highest concentration of NPEOs (21%) was detected in a laundry liquid especially designed for washing socks. Reversed-phase liquid chromatography connected with electrospray mass spectrometry confirmed the results.

Keywords: Surfactants; Nonylphenol ethoxylates; Ethoxylates

1. Introduction

Nonylphenol polyethoxylates (NPEOs) are a major class of non-ionic surfactants used and produced in Taiwan [1]. They are the most commonly used non-ionic surfactants in a range of industrial, household and commercial applications. The use of NPEOs has been banned or restricted in many European countries because one group of their degradation products, nonylphenol (NP) isomers, has been shown to persist and be estrogenic in a variety of both in vitro and in vivo bioassays [2–5]. In Taiwanese rivers and sediments concentrations of NPE-type residues higher than in other countries have been detected owing to Taiwan's deficient municipal wastewater treatment [6–9]. However, information on the amounts of NPEOs in most household detergents and cleaners in Taiwan is unavailable. Most non-ionic surfactant-containing detergents are used as laundry detergents for special purposes. The percentage of non-ionic surfactant in the detergents is unclear. Moreover, very few manufacturers declare that NPEOs are present in their household detergent products. Therefore, the concentration of NPEOs in household detergents and the associated environmental risk are not assessable, and concentrations of NPEOs in municipal effluents and sewage can not be evaluated. Although the possible estrogen-mimicking characteristics of some degradation products of NPEOs are well known and various household detergents are

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very likely to contain NPEOs as a major ingredient, no legal action has been taken nor limits yet established concerning their use in Taiwan. The widespread use of NPEOs, and the increasing public concern over human health and environmental issues have stimulated our interest to investigate the content and distribution of NPEOs in household detergents. This is an important area for study because NPEOs in wastewater directly discharged into the aquatic environment are a significant source of surface water contamination throughout developing countries due to deficient wastewater treatment.

Determinations of the concentrations of NPEOs for product and environmental samples have been reviewed elsewhere (see Refs. [10–12] and the references therein). Many methods of separation and identification have been applied to obtain information concerning the structures and quantities of nonionic surfactants in detergent formulations. High-performance liquid chromatography (HPLC) has been recognized as the preferred technique for evaluating a product's NPEOs content because many of these chemicals are not directly amenable to gas chromatography. In normal-phase HPLC, oligomers are separated according to their ethoxy chain length; the alkyl chain has virtually no effect on the chromatographic process. However, reversed-phase chromatography on C_8 or C_{18} packing allows the resolution of most alkylphenol polyethoxylates (APEOs) according to alkyl chain length. Therefore, each peak in reversed-phase HPLC represents a single alkyl chain length and contains the whole distribution of ethoxy chain length. This technique has been used for simply and rapidly determining the total concentration of APEOs in a complex matrix, since the smaller number of individual peak makes quantitation easier than normal-phase HPLC [13–17]. The detection of fluorescence is more selective and sensitive than UV–Vis detection, in that both excitation and emission wavelengths can be tuned for the particular molecule [13,15,18,19]. Therefore, we used a method introduced by Ahel and Giger [17], and modified by Marcomini and Giger [13] for the separation of NPEOs from linear alkylbenzenesulfonates (LASs) with fluorescence detection.

As part of a larger effort to characterize the impact of NPEOs on the environment, a simple and rapid method for routinely determining the total concentration of NPEOs in various household detergents and cleaners was developed. The method involves sample dilution and quantitation by isocratic reversed-phase HPLC with fluorescence detection. Reversed-phase liquid chromatography connected with electrospray mass spectrometry (LC–ESI-MS) confirmed the results. This work undertakes a preliminary study of the NPEO content in various household detergents and cleaners sold in Taiwan, to support surface water pollution prevention and control programs.

2. Materials and methods

2.1. Chemicals and reagents

Unless stated otherwise, all high purity chemicals and solvents were purchased from Aldrich (Milwaukee, WI, USA), Tedia (Fairfield, OH, USA) and Merck (Darmstadt, Germany), and were used without further purification. A pure commercial 4-nonylphenol polyethoxylate mixture (NPEO-9) with an average of nine and a range of 1–16 ethoxy units, and LAS mixture were provided by Taiwan Surfactant (Taiwan) and used as a standard mixture. 4-*tert*.-Octylphenol polyethoxylates (OPEOs, trade name Igepal CA-720) with an average of 12 ethoxy units was purchased from Aldrich. Stock standard solutions (1000 mg/l) of NPEOs, OPEOs and LAS mixture were prepared in methanol. Working standard solutions were obtained by diluting the stock standard solution with methanol to appropriate concentrations. Deionized water was further purified with a Millipore water purification device (Millipore, Bedford, MA, USA). The household laundry detergents, cleaners and dishwashing detergents in liquid or powder forms were purchased from local supermarkets or nationwide wholesale markets, and some dishwashing detergents were provided directly from restaurants. The liquid detergents were directly diluted with methanol. The appropriate amounts of powder detergents were dissolved in deionized water and then diluted with methanol. To prevent HPLC column blockage, all samples were filtered through a 0.45-μm membrane filter (Gelman Scientific, Ann Arbor, MI, USA) prior to injection.

2.2. HPLC analysis

The procedure used for HPLC analysis has been reported previously [17], and was used with minor modifications. Analyses were performed on an HP-1100 high-performance liquid chromatograph system coupled with a fluorescence detector (Agilent, Palo Alto, CA, USA). The fluorescence detection was achieved at an excitation wavelength of 228 nm and an emission wavelength of 305 nm. An XDB-C_8 column (15 cm×0.46 cm I.D., 0.5 μm packing, Agilent, Palo Alto, CA, USA) was used at a flow-rate of 0.5 ml/min at ambient temperature, and the injection volume was 20 μl. Isocratic elution was performed with a mixture of 80% methanol, 4% acetonitrile and 16% deionized water. As suggested previously no ion-pair reagents or sodium salts were added [20].

2.3. LC–electrospray mass spectrometry analysis

Electrospray mass spectra were obtained using an HP LC–MSD 1100 single quadrupole mass spectrometer equipped with an orthogonal-spray ion source with positive ionization detection mode (Agilent). Isocratic elution was performed with a mixture of 80% methanol, 4% acetonitrile and 16% deionized water, and no sodium salts were added to enhance the adduct ions. An XDB-C_8 column (15 cm×0.46 cm I.D., 0.5 μm packing) was used at a flow-rate of 0.5 ml/min, and the injection volume was 20 μl by autosampler system into HPLC. The MS system was operated in the unit resolution mode scanning the m/z 200–1000 range with a scan time of 5 s. Nitrogen was used as a drying gas (flow-rate as much as 10 l/min) and heated to 350 °C. The nebulizer pressure was maintained at 50 p.s.i., with capillary voltage of 4500 V (1 p.s.i. = 6.894.76 Pa).

3. Results and discussion

3.1. HPLC and LC–MS analysis

Fig. 1 displays C_8 reversed-phase LC chromatograms obtained by analyzing commercial LASs, NPEOs and OPEOs. Commercial LAS and NPEO mixtures were eluted as two major peaks, and OPEOs and NPEOs were baseline separated. The results indicated that the 5-μm octylsilica column was satisfactory for simultaneously and rapidly separating NPEOs from OPEOs and LASs, which chemicals may interfere with the determination of the concentration of NPEOs in household detergents. The column simplified the interpretation and quantitation of the chromatograms and enhanced detection limits by increasing the peak area (or height) corresponding to the NPEOs. Quick elution and efficient separation of NPEOs from LASs was achieved by adding acetonitrile (ACN) in the initial methanol:water (8:2) mobile phase reported previously [17], followed by increasing the ACN percentage to 4%. However, the separation of homologues or isomers of LASs was beyond the scope of this study.

The results were confirmed by determining the NPEO content by the LC–ESI-MS technique. Fig. 2 presents the total ion LC–ESI-MS chromatogram of NPEOs in commercial NPEO-9 standard mixture, and its corresponding ESI mass spectrum. The homologous series show approximately a statistically normal distribution. The ethoxy chain length for NPE varies from $n=5$ (m/z 463) to $n=13$ (m/z 810), and the most abundant homologue of NPE is 9 (m/z 639) representing $[M+Na]^+$ where M is $C_9H_{19}C_6H_4O(C_2H_4O)_9H$. Each major peak is separated from its neighbor by 44 u corresponding to a difference of one ethoxy unit. The manufacturer claims the EO mole number (number of moles of EO per mole of NPEO) to be between 8 and 9, which value is consistent with the results presented here. The ESI mass spectrum shows clearly the major and evenly spaced sodium adduct ions $[M+Na]^+$ for each homologue (i.e. m/z 507, 551, 595, 639, etc.), due to the ubiquity of sodium in the solvents, chemicals and metal surfaces. NPEO has a great affinity for alkali metal ions when using an aprotic solvent (i.e. acetonitrile) with the ESI interface [21]. The relatively intense $[M+NH_4]^+$ ions (i.e. m/z 502, 546, 590, 634, 678, etc.) may be also due to the impurity of NH_4^+ in the solvents and chemicals [21,22]. The protonated molecular signals $[M+H]^+$ (i.e. m/z 485, 529, 573, 617, etc.) were observed with a relatively smaller intensity.

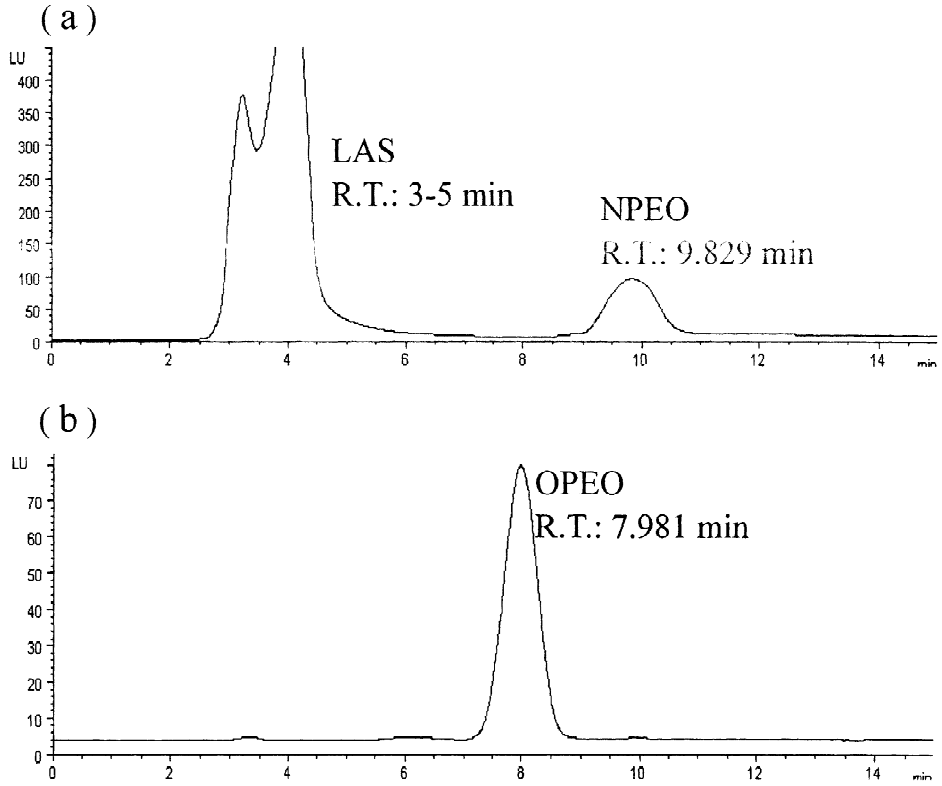

Fig. 1. C_8 RP-HPLC chromatograms of (a) LAS and NPEO-9 commercial mixtures, and (b) Igepal CA-720 (OPEOs). Injected samples contained 20 μl of methanol containing 10 ng/μl of each of the standard mixture. R.T., retention time.

3.2. Method validation

The analytical characteristics of the method, such as linear response range, reproducibility and quantitation limit, were investigated to evaluate the efficiency of the method and the possibility of application of the method to real samples. The reproducibility of the method was tested using three replicate injections of NPEO standards (0.1, 0.5, 1.0, 10 and 100 μg/ml) at each concentration. The RSD of the peak areas for each concentration was ~0.1–3.3%. The average retention time was 9.74 min, and the RSD of the retention times for each concentration ranged from 0.1 to 1.1%, as shown in Table 1. The linear response range for NPEOs was performed in the concentration covering the range from 0.1 to 100 μg/ml (five levels). The calibration curve was linear with coefficients of determination $r^2 \geq 0.999$. The precision of the curve as indicated by the RSD of calibration factors (CF, peak area/amount) was 1.6%. The detection limit ($S/N = 5$) of total NPEOs with fluorescence detection was 1.0 ng with respect to injected amount. The quantitation of NPEO was carried out using the external standard method. Validation of the method was also based on the separation in the fortified detergent samples (dishwashing detergent and laundry). Table 1 shows the good reproducibility of peak areas and retention times in fortified samples. These results demonstrate that the HPLC with fluorescence detection for NPEO analysis provides high reproducibility and excellent linearity.

3.3. Application to real samples

The described analytical method was used to determine the concentrations of NPEOs in laundry detergents, household cleaners and dishwashing detergents. Fig. 3 shows the typical C_8 RP-HPLC chromatograms obtained from (a) a dishwashing

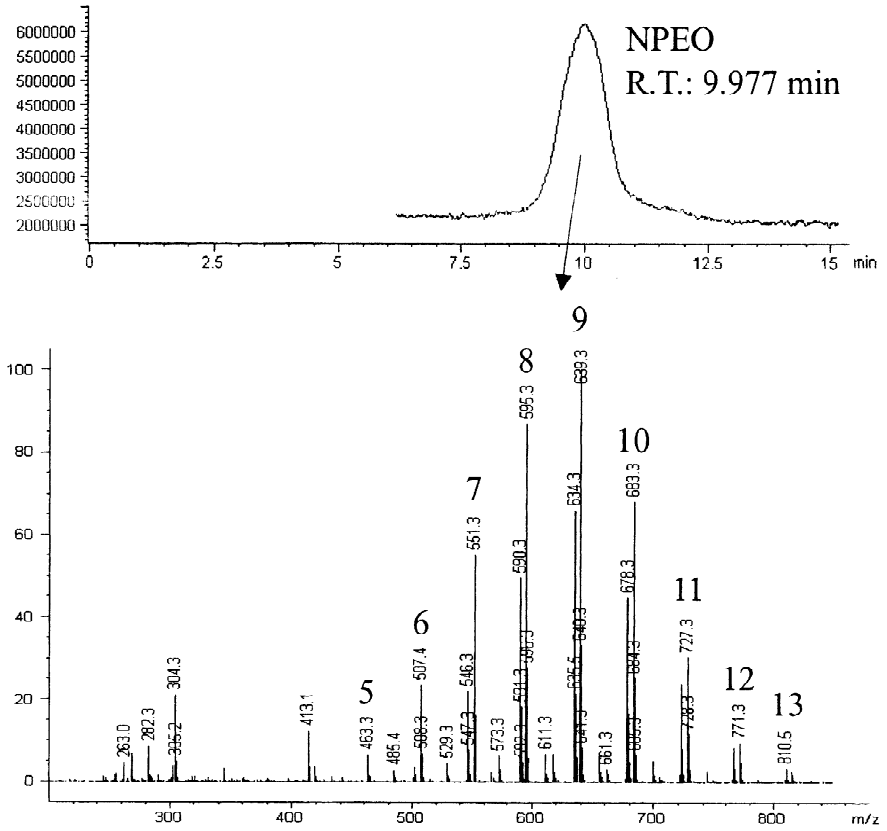

Fig. 2. Total ion LC–ESI-MS chromatogram of NPEOs from commercial NPEO-9 mixture, and its corresponding ESI mass spectrum. The numbers above the peaks indicate the number of the ethoxy units of NPEO.

detergent provide from a restaurant and (b) a heavy-duty kitchen cleaner. The peak was identified and quantitated using retention times and response factors, respectively. A total of 90 various household detergents were analyzed in this study. NPEOs were detected in 37 samples with concentrations from 0.2 to 21%, as shown in Table 2. The highest concentration of NPEOs (21%) was found in one of the

Table 1
Analytical reproducibility

NPEOs	Conc. (μg/ml)	Peak area, RSD (%)	Retention time, RSD (%)
Standard mixture	0.1	3.3	0.5
($n=3$ for each level)	0.5	2.0	0.1
	1.0	1.5	1.1
	10	0.9	0.1
	100	0.1	0.6
Fortified in dishwashing detergent ($n=3$)	10	1.2	0.2
Fortified in laundry ($n=3$)	10	2.6	0.5

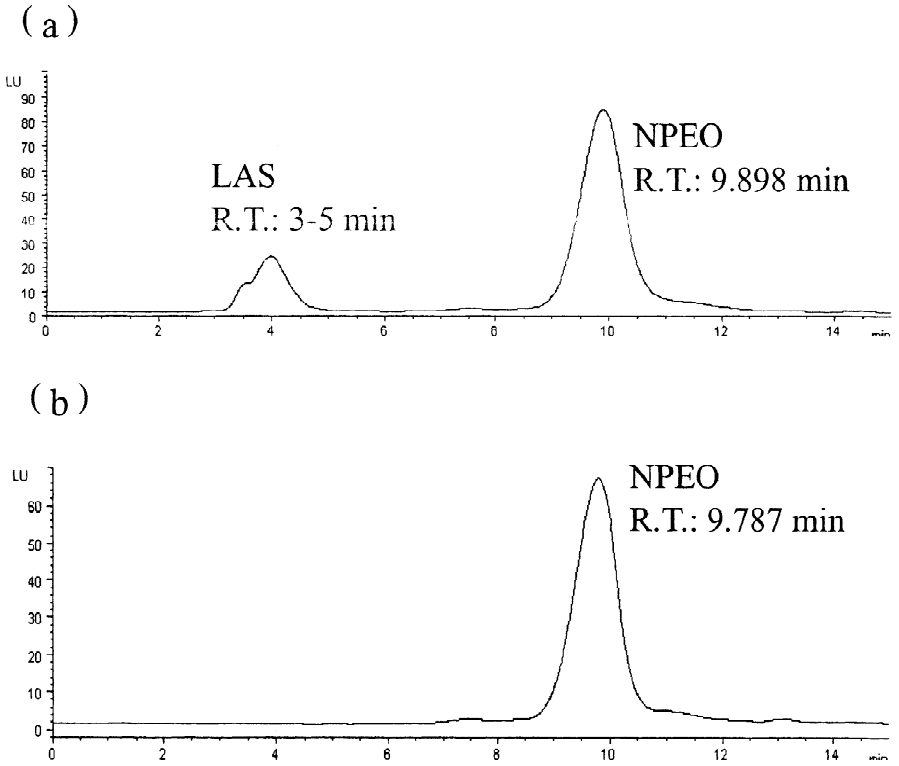

Fig. 3. Typical C_8 RP-HPLC chromatograms obtained from (a) a dishwashing detergent provide from a restaurant, and (b) a heavy-duty kitchen cleaner.

laundry liquids especially designed for washing socks. The highest detectable rate (57%) was found in dishwashing detergents. More than 12 of them included various concentrations of NPEOs, and some of them were used in local and famous fast-food restaurants. However, none of them was labeled NPEOs in the products by the manufacturers, and only a few products were labeled as containing ''non-ionic surfactants''.

The particular samples containing NPEOs were confirmed by LC–ESI-MS technique under positive ionization condition. Fig. 4 presents the ESI mass spectrum of NPEOs found in laundry liquid that was specially designed for washing socks. The spectrum shows characteristic patterns similar to those in Fig. 2, with major $[M+Na]^+$ ions for each homologue. Most examined household detergents were found to contain NPEOs with up to 14 ethoxy units similar to standard commercial NPEO mixture, according to their ESI mass spectra.

Table 2
Concentrations found in household detergents

	Laundry detergents	Dishwashing detergents	Bathroom and kitchen heavy-duty cleaners	Others (car wash, window and floor cleaners, etc.)
Sample number (*n*)	26	21	21	22
Number of NPEOs detected	11	12	10	4
Conc. range (%)	0.6–21	0.3–5.8	0.2–7.3	2.9–5.5

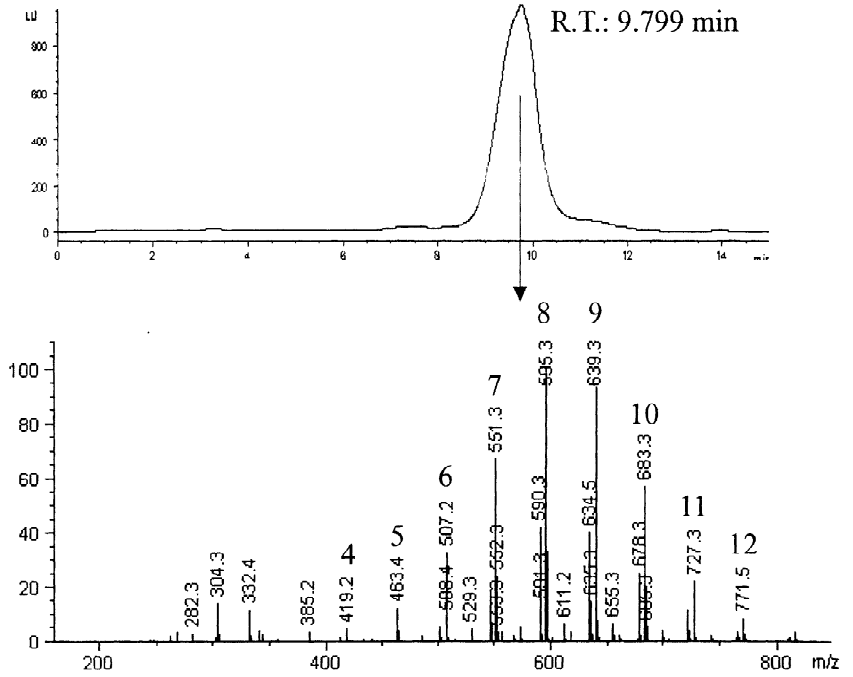

Fig. 4. The ESI mass spectrum of NPEOs found in a laundry liquid specially designed for washing socks.

4. Conclusions

The presented analytical procedure employing C_8 RP-HPLC is particularly suited for routine determination of total NPEO content in household detergents by taking many samples, because of its detection selectivity, simplicity, and short analysis time. Preliminary results in this study demonstrated that NPEOs are widely used in various household detergents in Taiwan, and the highest concentration of NPEOs was in laundry liquids for special purposes, such as for washing socks or collars. The increasing threat to the ecosystem, environment and health, imposed by the degradation products of NPEOs demands urgent preventive actions. The most appropriate action is to ban the use of NPEOs in detergent formulations, and household detergents in particular, by either a voluntarily phasing-out by the local detergent industry, or imposed by state regulation. This study provides further insights into environmental protection trends, promotes conservation, pollution control policies, and sustainable development in Taiwan.

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