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DETERMINATION OF TRACE AMOUNTS OF ALCOHOL AND ALKYLPHENOL ETHOXYLATES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORIMETRIC DETECTION

MEGUMU KUDOH*, HITOMI OZAWA, SHINTARO FUDANO and KAZURO TSUJI
Wakayama Research Laboratories, Kao Corporation, 1334 Minato, Wakayama-shi, 640 (Japan)
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SUMMARY

Trace amounts of alcohol and alkylphenol ethoxylates were determined by high-performance liquid chromatography (HPLC). Alcohol ethoxylates were determined by reversed-phase HPLC after fluorescent derivatization using 1-anthroylnitrile and alkylphenol ethoxylates were determined by normal-phase HPLC without derivatization. The ethylene oxide distributions of the determined alcohol and alkylphenol ethoxylates were also obtained. The lower limit of determination was 0.05 ppm for alcohol ethoxylates and 0.2 ppm for alkylphenol ethoxylates.

INTRODUCTION

Trace amounts of alcohol and alkylphenol ethoxylates have been determined by spectrophotometry with cobalt thiocyanate[1,2], potentiometric titration[3–5] and atomic-absorption spectrometry[6]. Spectrophotometric methods based on extraction of the complexes between cobalt thiocyanate and alcohol and/or alkylphenol ethoxylates have long been used but often give erroneous results owing to interferences by many organic materials. Spectrophotometric results, therefore, have been represented not in terms of alcohol and alkylphenol ethoxylates but of cobalt thiocyanate-active substances. Potentiomatric titration (Wickbold's method) requires several tedious procedures and has a disadvantage that there is a decrease in sensitivity at lower degrees of ethoxylation. Atomic-absorption spectrometry based on the measurement of bismuth after precipitation of alcohol and alkylphenol ethoxylates with Dragendorff reagent shows good sensitivity. However, the results of these potentiometric and atomic-absorption methods are also often erroneous owing to interferences from bismuth-active substances.

High-performance liquid chromatography (HPLC) is the most suitable method for non-volatile alcohol and alkylphenol ethoxylates. The HPLC methods reported have mainly been applied to alkylphenol ethoxylates using ultraviolet detection[7,8] and there is no report of an HPLC method for the determination of trace amounts of alcohol ethoxylates, because alcohol ethoxylates have no specific ultraviolet-active or fluorescent functional groups in their molecules. Moreover, there are only a few

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good fluorescent derivatization reagents for hydroxy groups, such as 7-methoxycoumarin-3-carboxylic acid chloride[9], but they are not generally commercially available.

In this paper, we describe two methods for the determination of trace amounts of alcohol and alkylphenol ethoxylates. One is based on derivatization of the terminal hydroxy group in alcohol ethoxylates using a fluorescent derivatization reagent and separation by reversed-phase HPLC. The other method is suitable for the determination of alkylphenol ethoxylates, which are separated by normal-phase HPLC and are detected fluorimetrically without derivatization.

EXPERIMENTAL

HPLC apparatus

The liquid chromatograph consisted of a Shimadzu LC-4A high-pressure pump with a six-port injection valve, a Shimadzu RF-530 fluorescent spectrometric detector and a Shimadzu C-R1B data processor (Shimadzu, Kyoto, Japan).

Reagents

Deionized water was further purified through an octadecylsilane column. Nonylphenol ethoxylate [average degree of ethoxylation (EO) = 9], octylphenol ethoxylate (average EO = 10), lauryl alcohol ethoxylate (average EO = 8) and decyl alcohol ethoxylate (average EO = 8) were obtained from our company. Standard layryl alcohol ethoxylates with EO = 1, 3, 5 and nonylphenol ethoxylate with EO = 5 were used for peak assignments. All other reagents were of analytical-reagent grade.

Preparation of alcohol ethoxylate samples

The fluorescent derivatization reagent was synthesized in the Tochigi Laboratories of our company. A scheme of the synthesis is illustrated in Fig. 1. The reaction product, 1-anthroylnitrile, reacts with primary alcohols and produces fluorescent derivatives of alcohol ethoxylates. The derivatization procedure was as follows: 1–250 μg of alcohol ethoxylate, 5 ml of a 0.2% acetonitrile solution of triethylamine (base catalyst) and 5 mg of 1-antrhoylnitrile were heated at 45°C for 2 h, then the solution was cooled to room temperature and diluted to 0.1–2.5 μg/ml using the mobile phase.

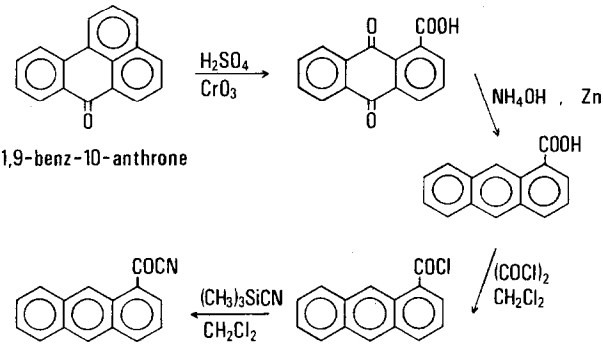

Fig. 1. Synthesis of 1-anthroylnitrile.

Alkylphenol ethoxylate samples

Solutions of alkylphenol ethoxylates in chloroform of concentration 0.5–50 μg/ml were prepared.

Chromatographic procedure for alcohol ethoxylate derivatives

Analyses of alcohol ethoxylate derivatives were carried out on a 150 × 6 mm I.D. column (ERMA Optical Works, Tokyo, Japan) packed with 3 μm Hypersil ODS (Shandon, Cheshire, U.K.) using acetonitrile–water (7:3) as the mobile phase. The column effluent was monitored at an excitation wavelength of 395 nm and an emission wavelength of 450 nm. The flow-rate was maintained at 2.0 ml/min and the injection volume was 250 μl.

Chromatographic procedure for alkylphenol ethoxylates

The column used for the separation of alkylphenol ethoxylates was 150 × 4 mm I.D. packed with 5 μm LiChrosorb Si 60 (Merck, Darmstadt, F.R.G.) by the balanced density method. The elution of alkylphenol ethoxylates was performed by gradient elution. *n*-Hexane was delivered first from the pump and after 60 min was replaced with ethanol–tetrahydrofuran–water (60:40:1). The gradient profile was linear. The column effluent was monitored at an excitation wavelength of 280 nm and an emission wavelength of 310 nm. The flow-rate was 2.0 ml/min and the injection volume was 250 μl.

RESULTS AND DISCUSSION

Derivatization of lauryl alcohol ethoxylate

The optimum conditions for the fluorescent derivatization of alcohol ethoxylates were determined. Fig. 2 shows the effects of reaction temperature and reaction time on the derivatization of lauryl alcohol ethoxylate. The fluorescent intensity of the lauryl alcohol ethoxylate derivative obtained decreased in proportion to the increase in reaction temperature. As shown in Fig. 2, the optimum reaction temperature was 45°C. The fluorescent intensity also increased in proportion to the increase in

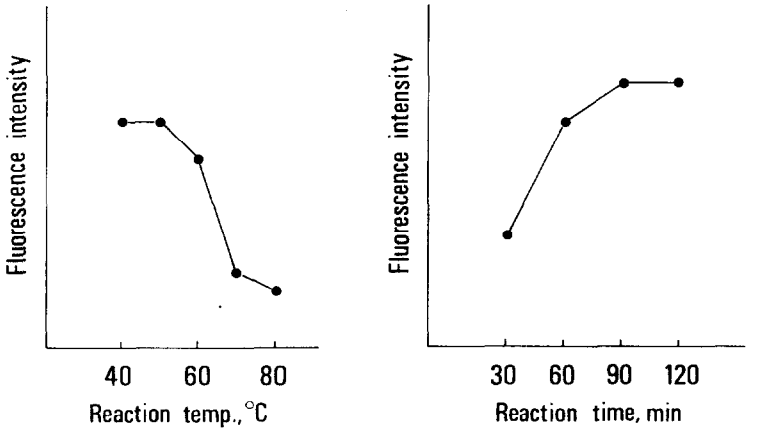

Fig. 2. Effects of reaction temperature and time on the derivatization of lauryl alcohol ethoxylate.

reaction time and the fluorescence intensity became a plateau after 90 min. This fluorescent derivatization reagent, 1-anthroylnitrile, tends to decompose at high temperatures, and reacts with impurities in the base catalyst (triethylamine), so it would be better to use a relatively low temperature and a long reaction time.

Fluorescent spectra of lauryl alcohol ethoxylate derivative and nonylphenol ethoxylate

When a component of lauryl alcohol ethoxylate derivative or nonylphenol ethoxylate was eluted in the flow-through cell of the detector, the flow-rate of the mobile phase was stopped and both excitation and emission wavelengths were measured. Fig. 3 shows the fluorecence spectra of lauryl alcohol ethoxylate derivative and nonylphenol ethoxylate, both with EO = 5. Under these conditions, lauryl alcohol ethoxylate derivative absorbed light of maximum wavelength 395 nm and fluoresced at a maximum wavelength of 450 nm, while the maximum excitation and emission wavelengths of nonylphenol ethoxylate were 280 nm and 310 nm, respectively. These excitation and emission wavelengths shifted slightly with other alcohol ethoxylate derivatives and alkylphenol ethoxylates, but the decrease in sensitivity was small.

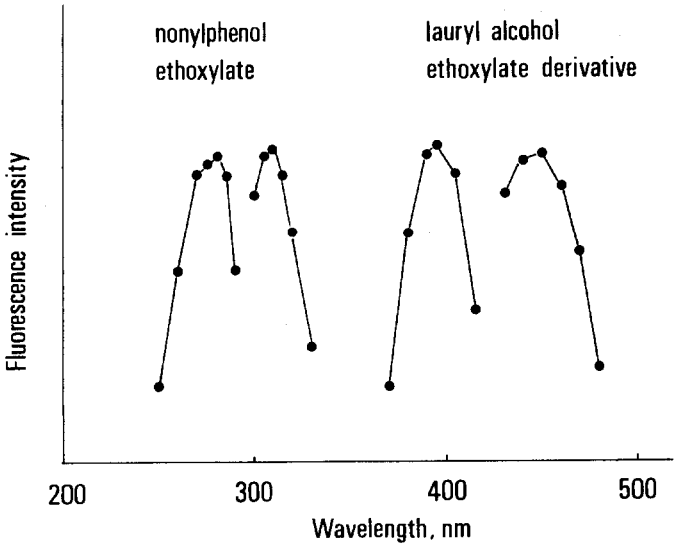

Fig. 3. Fluorescence spectra of lauryl alcohol ethoxylate derivative and nonylphenol ethoxylate.

Chromatography of alcohol ethoxylate derivatives

Although the separation of alcohol and alkylphenol ethoxylates in accordance with the EO distribution has usually been performed by normal-phase HPLC[10–12], the separation of the fluorescent derivative of lauryl alcohol ethoxylate under normal-phase conditions was not suitable, because excess of derivatization reagent eluted in region of the alcohol ethoxylate derivatives eluted and interfered. To eliminate the excess of derivatization reagent, other pre-treatments such as thin-layer chromatography and chromatography using a small disposable column have been tried. However, these procedures were tedious and it was difficult to eliminate completely the interference from the excess derivatization reagent, and moreover there was a pos-

sibility of losing the alcohol ethoxylate derivatives during these procedures. We therefore decided to use reversed-phase HPLC in order to separate alcohol ethoxylate derivatives in accordance with the EO distribution.

Figure 4a shows a typical chromatogram of a trace amount of lauryl alcohol ethoxylate derivative (0.1 ppm) and Fig. 4b is a chromatogram of decyl alcohol ethoxylate derivative. It can be seen that the alcohol ethoxylate derivatives were separated in accordance with the EO distribution and there was no interference from excess of derivatization reagent. The limit of determination of lauryl alcohol ethoxylate derivative is 0.05 ppm.

Chromatography of alkylphenol ethoxylates

Reversed-phase HPLC with fluorescent derivatization could not be applied to the separation of branched alkylphenol ethoxylates such as nonylphenol and dodecylphenol ethoxylate in accordance with their EO distribution. Fig. 5a and b show chromatograms of nonylphenol and octylphenol ethoxylate derivative, respectively. The branched-chain nonylphenol ethoxylate was not separated according to the EO groups, but the unbranched octylphenol ethoxylate showed a good separation in accordance with the EO distribution. To separate the branched-chain alkylphenol ethoxylates in accordance with the EO distribution, normal-phase HPLC was selected and the poor normal-phase HPLC for the laury alcohol ethoxylate derivative was overcome. Because the detection of alkylphenol ethoxylates was based on their own

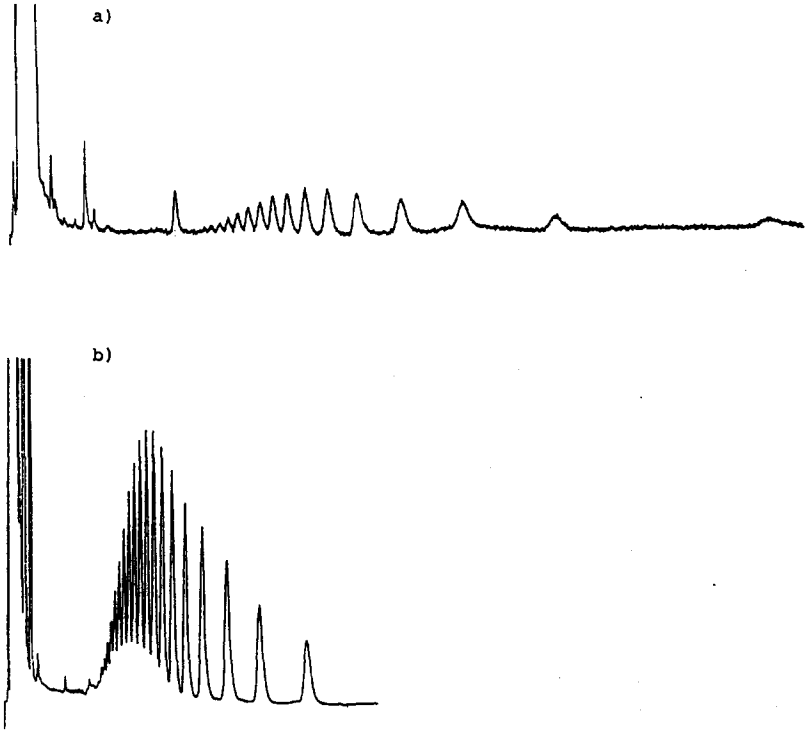

Fig. 4. (a) Typical chromatogram of a trace amount of lauryl alcohol ethoxylate derivative (0.1 ppm); (b) chromatogram of decyl alcohol ethoxylate derivative.

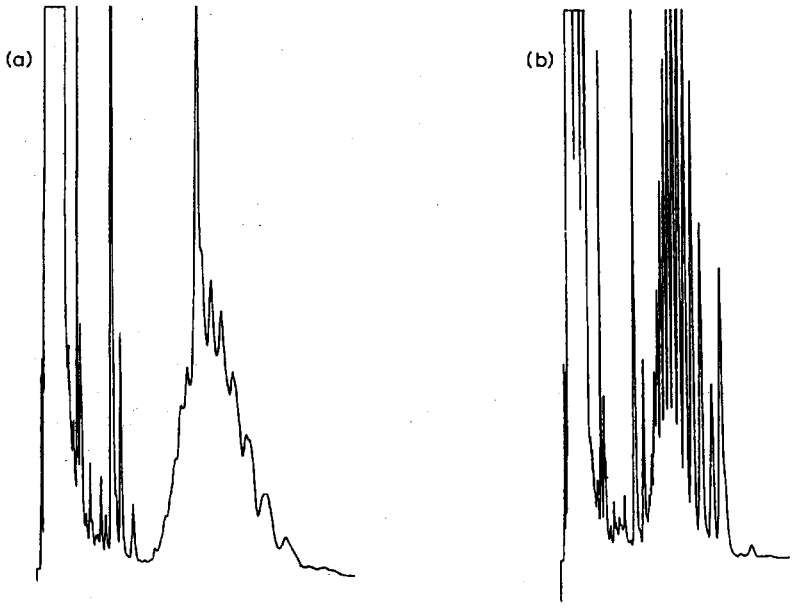

Fig. 5. Chromatograms of (a) nonylphenol ethoxylate derivative and (b) octylphenol ethoxylate derivative.

fluorescence, no derivatization was required. There was another problem when the separation of alkylphenol ethoxylates was carried out by normal-phase HPLC, namely that in order to elute alkylphenol ethoxylates gradient elution was required, and when the fluorescences from each mobile phase were very different, the baseline was drifted away during the gradient elution. Gradient elution using *n*-hexane and ethanol–tetrahydrofuran–water (60:40:1) was suitable for the separation of branched and unbranched alkylphenol ethoxylates.

Fig. 6a and b show chromatograms of nonylphenol ethoxylate and octylphenol ethoxylate, respectively, at concentrations of 25 ppm, in which each EO group was separated. These alkylphenol ethoxylates were separated satisfactorily in accordance with the EO distribution.

Fig. 7 shows a chromatogram of a trace amount of nonylphenol ethoxylate (0.5 ppm). The limit of determination was about 0.2 ppm.

Determination of an alcohol ethoxylate and nonylphenol ethoxylate in river soil

In the fluorescent derivatization using 1-anthroylnitrile the presence of water should be avoided, because the derivatization reagent reacts with water. Also, the normal-phase HPLC separation of alkylphenol ethoxylates using a silica gel column was affected by the presence of water, which caused a decrease in column efficiency. Therefore, when an aqueous sample was used, prior to measurement or derivatization water had to be eliminated under mild conditions such as by freeze-drying or vaporization of water under a stream of nitrogen at room temperature.

Table I shows the results of the determination of lauryl alcohol ethoxylate and nonylphenol ethoxylate in river-bed soil. One litre of an aqueous solution of lauryl alcohol ethoxylate or nonylphenol ethoxylate was added to about 3 g of soil and the

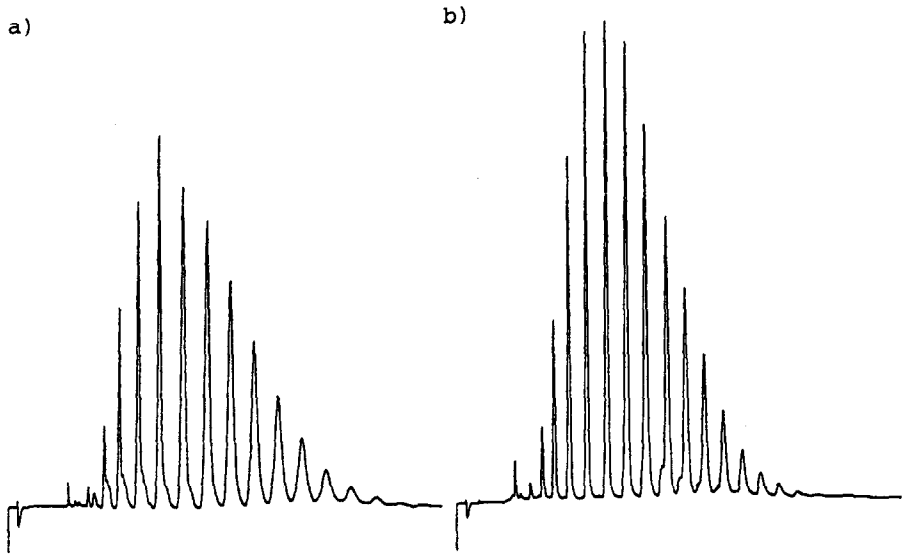

Fig. 6. Chromatograms of (a) nonylphenol ethoxylate and (b) octylphenol ethoxylate under normal-phase conditions.

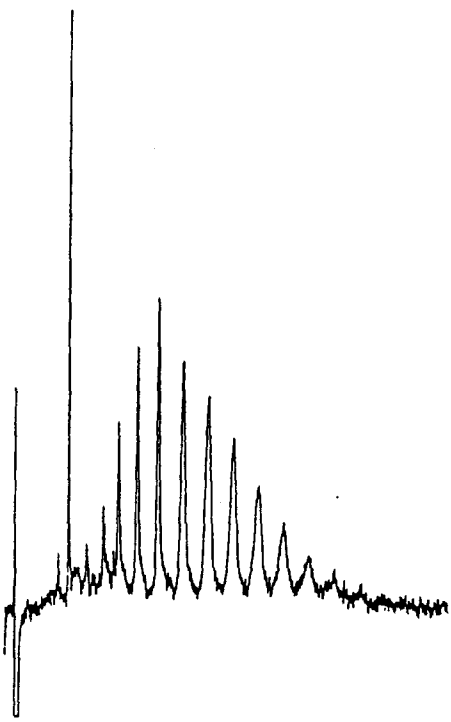

Fig. 7. Chromatogram of a trace amount of nonylphenol ethoxylate (0.5 ppm).

TABLE I

DETERMINATION OF LAURYL ALCOHOL ETHOXYLATE AND NONYLPHENOL ETHOXYLATE IN RIVER SOIL

Sample	*Time*	*Cobalt thiocyanate method (μg/g)*	*HPLC method (μg/g)*
Lauryl alcohol ethoxylate	Start	584 (406*)	31 (2*)
	30 days	754 (436*)	4 (2*)
Nonylphenol ethoxylate	Start	617 (469*)	27 (2*)
	30 days	418 (506*)	5 (2*)

* Blank value.

solution was stirred for 30 days. Methanol extracts of the soil were used as sample solutions. To confirm the chromatographic results, results obtained by cobalt thiocyanate method are also given. Table I shows that HPLC gives considerably lower values than those obtained by the cobalt thiocyanate method, as the former method is less subject to interferences from organic and inorganic materials in the methanol extracts.

In conclusion, the results in this paper indicate that using the proposed HPLC methods, trace amounts of alcohol and alkylphenol ethoxylates can be simply determined. These methods could be applicable to biodegradation tests of alcohol and alkylphenol ethoxylates and have advantages over the conventional cobalt thiocyanate method.

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REFERENCES

1 N. T. Crabb and H. E. Persinger, *J. Amer. Oil Chem. Soc.*, 41 (1964) 752.
2 R. A. Greff, E. A. Setzkorn and W. D. Leslie, *J. Amer. Oil Chem. Soc.*, 42 (1965) 180.
3 R. Wickbold, *Tenside Deterg.*, 9 (1972) 173.
4 R. Wickbold, *Tenside Deterg.*, 10 (1973) 179.
5 R. Wickbold, *Tenside Deterg.*, 11 (1974) 137.
6 S. Setsuda, S. Ito, A. Utsunomiya and S. Naito, *Eiseikagaku*, 25 (1979) 199.
7 K. Kobayashi and H. Numata, *Zenkoku Kogai Ken Kaishi*, 4 (1979) 58.
8 A. Otsuki and H. Shiraishi, *Anal. Chem.*, 51 (1979) 2329.
9 W. Baker and C. B. Collis, *J. Chem. Soc.*, (1949) 12.
10 M. C. Allen and D. E. Linder, *J. Amer. Oil Chem. Soc.*, 58 (1981) 950.
11 H. Bruschweiler, *Mitt. Geb. Lebensm. Hyg.*, 68 (1977) 46.
12 K. Nakamura and I. Matsumoto, *Nihon Kagaku Kaishi*, (1975) 1342.