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Characterization and Determination of Linear Alkylbenzenesulfonates in Environmental Water Samples by High-Performance Liquid Chromatography with a Hydrophilic Polymer Column and Electrospray Ionization Mass Spectrometric Detection

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A liquid chromatography–mass spectrometry (LC/MS) method was developed for the separation and determination of linear alkylbenzenesulfonates (C_{10} – C_{14} LAS) in environmental water samples using a hydrophilic polymer column (Shodex Mspak GF-310 4D). This method involves a solid-phase extraction of the LAS samples with a Sep-Pak PS-2 cartridge. The LAS components were separated on the column with a mobile phase of 29% (w/v) acetonitrile–water containing 0.8 mM di-*n*-butylammonium acetate and 0.2 M acetic acid, and were detected by mass spectrometry with electrospray ionization. Detection limits of the developed method based on selected ion monitoring (SIM) technique for the C_{10} – C_{14} LAS standards were 13 – 47 ng L^{-1}. The concentrations of the C_{10} – C_{14} LAS in the environmental water samples ranged between 5 – 317 μg L^{-1} for a river water sample and 0.4 – 6.4 μg L^{-1} for a seawater sample. Linear relationships between the logarithms of retention factors and the alkyl chain lengths for each phenyl positional isomer of LAS could successfully be used for the identification of the isomer peaks.

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Introduction

Linear alkylbenzenesulfonates (LASs) are the anionic surfactants most widely used in detergent formulations. Commercial LASs are usually the mixtures of homologues of C_{10} – C_{14} alkyl compounds, and each homologue contains phenyl positional isomers. Their toxicity towards aquatic organisms has been proven to be rather serious,[1,2] so that a sensitive and precise analytical method for the determination of their residual levels in environmental waters is greatly required in ecotoxicological studies.

Identification and determination of LAS in environmental samples are often difficult because LAS are present at trace levels in complex sample matrices. Colorimetric methods[3] based on ion-pair extraction with Methylene Blue have been routinely used to measure the amount of total anionic surfactants in aqueous samples. However, these methods are not specific for LAS and are not able to identify individual homologues.

Recently, for the specific characterization and quantitation of individual LASs, analytical methods based on gas chromatography (GC) and liquid chromatography (LC) have been reported. GC has very high efficiencies for the analysis of individual components of LAS homologues and isomers. Particularly, GC combined with mass spectrometric (MS) detection provides low detection limits at the ppt level and allows us to identify homologues and isomers of LAS in real samples such as sediments, river water, and seawater.[4-6] However, some derivatization steps to convert LAS into a volatile compound[7,8] are unavoidable for GC procedures, because LASs are not volatile enough to permit direct measurement by GC. LC has widely been applied to the determination of LAS in various environmental samples without any derivatization using ultraviolet (UV),[9-11] fluorescence (FL),[12-16] and MS[17-19] detection. Nakae *et al.*[12,13] obtained an excellent separation of many LAS components (homologues and isomers) by reversed-phase chromatography with FL detection. They determined LASs at the sub-ppm level in river water samples without any pretreatment. However, in the determination of trace amounts of LASs in environmental samples, some pretreatments of the samples for concentration of LASs and elimination of co-existing interfering components are necessary. Generally, a solid-phase extraction technique is employed for the preconcentration and separation of LASs from various environmental matrices.[20,21]

The development of LC/MS techniques has enabled more sensitive and selective determination of LASs. Moreover, LC/MS makes it possible to identify biodegradation intermediates of LASs, which escaped from the analysis based on GC/MS after derivatization.[19] Several studies have been published on the determination of LASs in sewage and river water by LC/MS.[17-19] However, the separation is usually carried out only on the homologue level, so that discussions about their isomer concentrations are not yet available.

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The aim of this work is to propose a sensitive and selective method for identification and determination of homologues and isomers in trace amounts of LAS in environmental water samples. This method is divided into two steps: (a) solid-phase extraction of LASs from the sample with a polystyrene cartridge and (b) separation and determination of individual LAS by LC/MS using a hydrophilic polymer gel column with a gradient elution of acetonitrile–water.

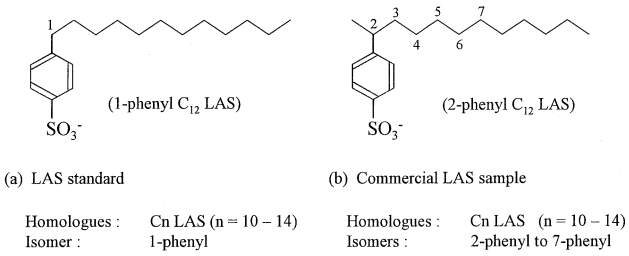

Fig. 1 Structures of linear alkylbenzenesulfonates (LASs) used in this study.

Experimental

Reagents

LAS standard solution and sodium *n*-dodecylbenzenesulfonate (commercially-available LAS sample) were obtained from Wako Pure Chemicals (Osaka, Japan). The LAS standard solution consisted of a mixture of homologues of C_{10} – C_{14} alkyl chain length having only a 1-phenyl isomer (Fig. 1a). This solution was used as a calibration standard. On the other hand, the commercially-available LAS sample contained C_{10} – C_{14} homologues with many phenyl positional isomers (Fig. 1b). These reagents were used without further purification. Di-*n*-butylammonium acetate was obtained from Tokyo Kasei (Tokyo, Japan). HPLC-grade acetonitrile was from Kanto Chemicals (Tokyo, Japan). Acetic acid and hydrochloric acid were purchased from Wako Pure Chemicals. Water purified by an AUTOPURE WD 500 (Yamato Science, Tokyo, Japan) was used throughout the present experiments.

Sample preparation

Environmental samples were collected from the Ebi river (Funabashi, Chiba, Japan) and from Tokyo Bay into 500-mL glass bottles. In order to prevent further microbial degradation of LASs, 12 M hydrochloric acid was immediately added to the samples collected, and then the resultant acid concentration was 0.1 M. After sampling, we performed all the experiments in a class-100 clean room to avoid contamination of LASs from the laboratory environment. The water samples were homogenized by sonication at 25°C for 10 min and filtered through a Kiriyama filter paper (Tokyo, Japan). LASs in the filtrate were extracted using a Sep-Pak Plus PS-2 cartridge (Waters, MA, USA). The cartridge was conditioned prior to use with 5 mL of acetonitrile followed by 10 mL of water. Through the cartridge, 20 mL (river water sample) or 100 mL (seawater sample) of the samples were passed at a flow rate of 10 mL min^{-1}. After the cartridge was rinsed with 10 mL of water and dried using a stream of air, the extracted LASs were eluted with 10 mL of acetonitrile. The eluted solution was evaporated up to about 1 mL on a hot plate. The solution was transferred into a 10-mL (river water sample) or 5-mL (seawater sample) volumetric flask, diluted to the mark with the mobile phase of acetonitrile–water (29% (w/v) containing 0.8 mM di-*n*-butylammonium acetate and 0.2 M acetic acid), and then subjected to LC/MS.

LC/MS analysis

A Waters Alliance 2690 HPLC instrument was used for the separation of LASs. The column used was a Shodex MSpak GF-310 4D (4.6 mm i.d. × 150 mm, 6 μm; Showa Denko, Tokyo, Japan). The chromatograpy was performed at 40°C with a flow-rate of 0.25 mL min^{-1}. A gradient program was used with acetonitrile–water 29% (w/v) containing 0.8 mM di-*n*-butylammonium acetate and 0.2 M acetic acid (pH 2.7) (A) and acetonitrile (B). After injection of 20 μL of the sample, the isocratic elution with 100% (A) was used for 15 min, followed by gradient elution to 30% (v/v) (B) over 10 min, then the isocratic mode for 15 min. Finally, the solvent system was brought back to the initial composition and the column was equilibrated for 10 min.

The MS detection was carried out using a Waters ZMD 4000 mass spectrometer equipped with an electrospray ionization (ESI) interface. The mass spectrometer was operated in the negative ion mode for the detection of LASs. The operating conditions of mass spectrometry were optimized by direct injection of the LAS standard sample. The operating working conditions for ESI were as follows: capillary voltage at –3 kV; cone voltage at –39 V; source block temperature at 90°C; desolvation temperature at 390°C; cone gas flow at 55 L h^{-1}; desolvation gas flow at 350 L h^{-1}. Full-scan data acquisition was performed from a range of 250 – 400 *m/z* in centroid mode and with a cycle time of 0.5 s and an interscan time of 0.1 s. In selected-ion monitoring (SIM) mode, the molecular anions, $[M]^-$, were used for the detection of C_{10} – C_{14} LAS homologues (*m/z* 297, 311, 325, 339, and 353), with a dwell time of 0.3 s and an interchannel delay of 0.02 s. Data analysis was carried out with a Waters Mass Lynx software Ver. 3.4.

Quantitation

A calibration curve was obtained by using samples prepared by dilution of the standard LAS solution with the mobile phase in the concentration range of 0.025 to 1 mg L^{-1}. The SIM peak areas were used for the quantification of LAS components. The recovery experiments were carried out by spiking the LAS standard samples into the water samples.

Results and Discussion

Optimization of mobile phase composition

The basic material of Shodex MSpak GF-310 4D is a poly(vinyl alcohol) gel possessing a small amount of carboxyl groups.[22] Therefore, the retention of LAS is strongly influenced not only by the concentration of organic modifiers in the mobile phase but also by the acidity of the mobile phase. We first tried to use an isocratic elution with acetonitrile–water. However, no evidence of the separation of any positional isomers was obtained. Then, we tried to add a volatile cationic ion-pairing reagent, di-*n*-butylammonium acetate (DBAA), to the mobile phase in order to provide sufficient retention for the individual isomers. The addition of DBAA to the mobile phase caused the change of peak profiles in the SIM chromatograms due to some positional isomers for C_{12} and C_{13} LAS homologues. However, the positional isomers of the C_{10} and C_{11} homologues were still poorly resolved due to their relatively weak retention. The separation of these isomers was improved by adding acetic acid to the mobile phase. Acidification of the mobile phase

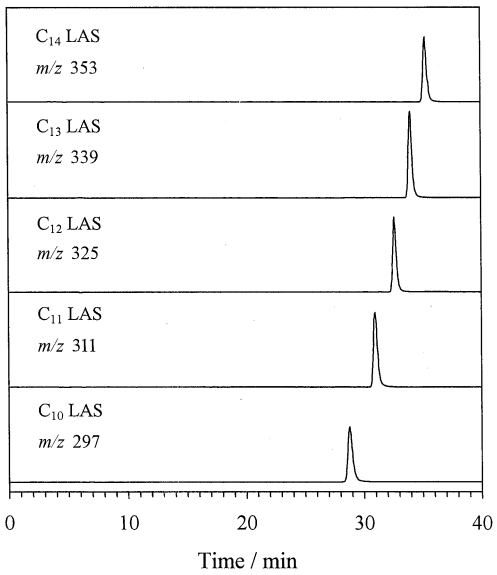

Fig. 2 SIM chromatograms of a LAS standard mixture. Conditions: column, Shodex MSpak GF-310 4D (150 × 4.6 mm); mobile phase, a gradient elution with 29% (w/v) acetonitrile–water containing 0.8 mM di-*n*-butylammonium acetate and 0.2 M acetic acid (pH 2.7) (A) and acetonitrile (B); gradient program, at first 100% (A) was used for 15 min, followed by gradient elution to 30% (v/v) (B) over 10 min, then the isocratic mode for 15 min; flow rate, 0.25 mL min^{-1}; column temperature, 40°C; injection volume, 20 μL; ion mode, electrospray negative; cone voltage, –39 V; sample concentration, 0.5 mg L^{-1}. $[M]^-$ were used for the detection of C_{10} – C_{14} LAS homologues (*m/z* 297, 311, 325, 339, and 353).

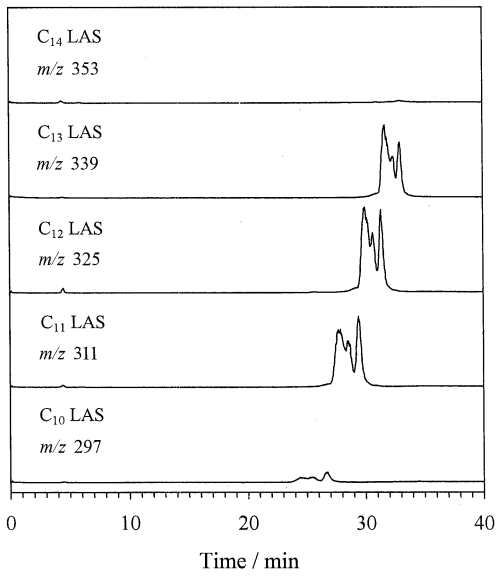

Fig. 3 SIM chromatograms of a commercial LAS sample. Sample concentration: 3 mg L^{-1}. Experimental conditions were the same as in Fig. 2.

suppressed the dissociation of the carboxyl groups on the surface of gel particles. The ion exclusion effect exerted on LAS molecules was thus depressed, so that the retention and the resolution of LAS species were enhanced. Finally, we obtained a good resolution of LAS isomers by a gradient elution with 29% (w/v) acetonitrile–water containing 0.8 mM DBAA and 0.2

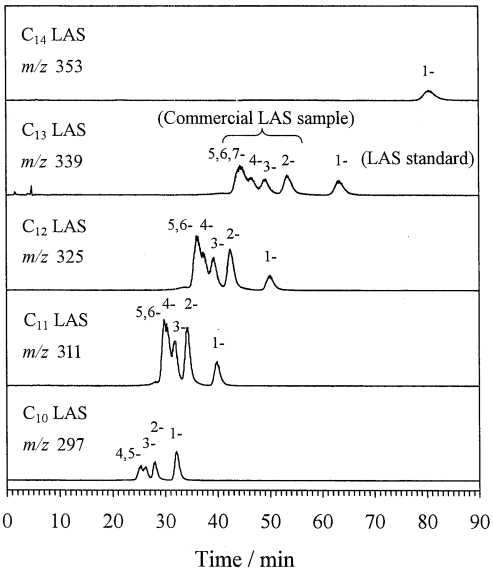

Fig. 4 SIM chromatograms of a mixture of the LAS standard and the commercial LAS in an isocratic elution. Mobile phase: 29% (w/v) acetonitrile–water containing 0.8 mmol L^{-1} DBAA and 0.2 mol L^{-1} acetic acid. Sample: a mixture of 0.5 mg L^{-1} of LAS standard and 3 mg L^{-1} of commercial LAS. Other experimental conditions were the same as in Fig. 2. The numbers indicate the positions of the phenyl group on the alkyl chain of LAS isomers.

M acetic acid (A), and acetonitrile (B), as described in the Experimental section.

Figures 2 and 3 show the SIM chromatograms obtained for the LAS standard and the commercial LAS sample solutions, respectively. As is clearly seen from the figures, the retention times of LAS homologues increased with increasing the number of carbon atoms in the alkyl side-chain. It was also found that each homologue of the LAS standard provided a single peak (Fig. 2) in its SIM chromatogram, while the homologue of the commercial LAS sample gave several peaks (Fig. 3). These peaks were assumed to correspond to the phenyl positional isomers.

Identification of LAS isomers

Nakae and Kunihiro[23] demonstrated that the logarithms of the retention factors (*k*) of the individual LAS isomers were directly proportional to their alkyl chain lengths in the isocratic elution system. To identify the isomer peaks obtained from the SIM chromatograms, we also performed the analysis of LASs with an isocratic elution mode. Figure 4 shows the SIM chromatograms of a mixture of the LAS standard and the commercial LAS sample obtained with 29% (w/v) acetonitrile–water containing 0.8 mM DBAA and 0.2 M acetic acid. The chromatograms show essentially the same isomer peak patterns as those obtained with the gradient elution. Each LAS homologue exhibited four to five isomer peaks. Assuming that the each homologue of the commercial LAS sample contained all possible phenyl positional isomers except 1-phenyl isomer, the plots of log *k* against the carbon number of alkyl group of LAS were constructed for each isomer (Fig. 5). As is shown in Fig. 5, each plot was quite linear. These results indicate that the commercial LAS sample is a mixture of C_{10} – C_{13} homologues and that each homologue contains all possible phenyl positional isomers except 1-phenyl isomer as expected. Although a small peak for C_{14} homologue was also detected, its

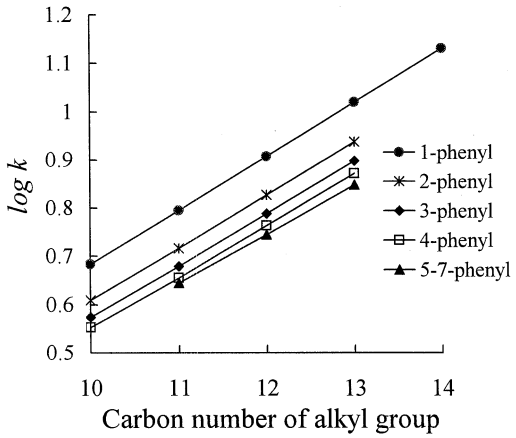

Fig. 5 Relationships between log *k* and carbon number of alkyl group of LAS for each isomer. See text for discussion.

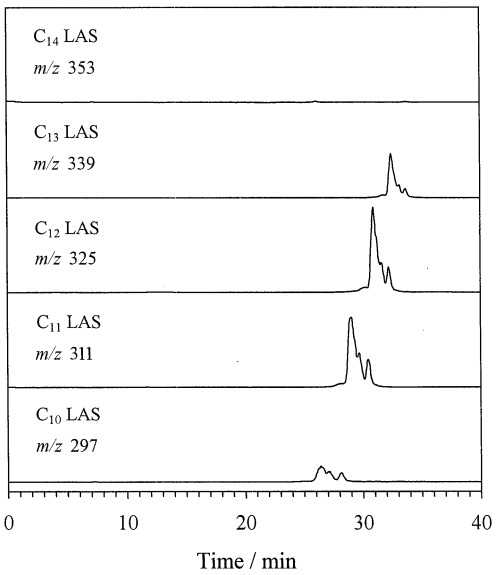

Fig. 6 SIM chromatograms of LAS obtained from a river water sample. Sample: Ebi river water. Other experimental conditions were the same as in Fig. 2.

positional isomers could not be identified. These results again indicate that the LC/MS technique developed in the present study enables us to identify and determine the individual LAS isomers.

Calibration plots and detection limits of LASs

Calibration plots were constructed from the SIM peak areas obtained by injecting the LAS standard solutions in the concentration range of 0.025 to 1 mg L^{-1}. Calibration data are summarized in Table 1. A linear relationship passing through the origin was obtained for the each LAS homologue. The detection limits calculated as 3 times the standard deviation of the background signal were 13 to 47 ng L^{-1}.

Determination of LAS in river and seawater samples

Figure 6 shows the SIM chromatograms obtained for the river water sample. Almost the same chromatographic profiles were obtained for the seawater sample (chromatograms not shown). These environmental water samples provided chromatograms

Table 1 Calibration data for LAS determination

Alkyl chain length	Equation	Regression coefficient	Detection limit/ ng L^{-1}
C10	$y = 1.20 \times 10^6 x + 1.31 \times 10^4$	0.9990	13
C11	$y = 1.32 \times 10^6 x + 1.39 \times 10^4$	0.9995	19
C12	$y = 1.13 \times 10^6 x + 0.80 \times 10^4$	0.9997	47
C13	$y = 1.35 \times 10^6 x + 1.78 \times 10^4$	0.9990	40
C14	$y = 1.30 \times 10^6 x + 1.43 \times 10^4$	0.9993	25

Table 2 Determination of individual LAS homologues and isomers in river and seawater samples

Sample	Homologue	Isomer	Concentration/ μg L^{-1}		Recovery[a], %
Ebi river	C10	3- to 5-phenyl	83	104	95
		2-phenyl	21		
	C11	3- to 6-phenyl	258	305	96
		2-phenyl	47		
	C12	3- to 6-phenyl	284	317	95
		2-phenyl	33		
	C13	3- to 7-phenyl	111	122	95
		2-phenyl	11		
	C14	2- to 7-phenyl		5.0	90
	Total			853	
Tokyo bay	C10	3- to 5-phenyl	0.3	0.4	88
		2-phenyl	0.1		
	C11	3- to 6-phenyl	2.9	3.6	87
		2-phenyl	0.7		
	C12	3- to 6-phenyl	5.7	6.4	81
		2-phenyl	0.7		
	C13	3- to 7-phenyl	2.0	2.2	73
		2-phenyl	0.2		
	C14	2- to 7-phenyl		n.d.[b]	70
	Total			12.6	

a. Average of the recovery obtained by spiking LAS standard samples to the water samples. The spike levels are 100 to 400 μg L^{-1} and 1.25 to 12.5 μg L^{-1} for Ebi river and Tokyo bay water samples, respectively.
b. n.d. = not determinable.

similar to those for the commercial LAS sample. However, the 2-phenyl isomer contents found for both environmental samples were significantly lower than those for the commercial LAS sample. It has been reported that the biodegradation rate of LASs depends on the carbon number of the alkyl group and on the position of the phenyl group on the alkyl chain. The longer alkyl chain length and the position of the phenyl group closer to the end of the alkyl chain provided the larger degradation rates of LASs.[24] Therefore, the present results may be ascribed to the biodegradation of LASs in environmental waters. The concentrations of LASs in the river and seawater samples obtained are shown in Table 2. The total concentrations of LASs were 853 and 12.6 μg L^{-1} for the Ebi river and the Tokyo bay samples, respectively. Replicate analyses were performed on the same sample to determine the precision of the method. The relative standard deviations (n = 3) for the Ebi river and the Tokyo bay samples were found to be less than 7% and 11%, respectively. Extremely low LAS concentrations were found in the seawater sample (about 1/70 of the river water sample). This result can be explained by the effect of dilution and biodegradation of LASs in seawater.

Conclusions

The analytical procedure developed in the present study demonstrated that the LC/MS method using a hydrophilic polymer column was an effective approach for the determination of LAS homologues and isomers in environmental water samples. The isomeric separation of LAS by GC/MS has been reported in recent years. However, it has not yet been accomplished by the LC/MS methods. In the proposed method, the isomeric separation was achieved by the addition of a cationic ion-pairing reagent to the mobile phase. As a result, the 2-phenyl isomers of LAS were determined in the environmental water samples at low concentration levels (0.1 to 0.7 μg L^{-1} for seawater). The LC/MS method developed needs only a simple pretreatment of environmental water samples without derivatization steps and gives the low detection limits (13 to 47 ng L^{-1}), as compared to UV or FL detections.

Further works on the distribution patterns of the homologues and isomers of LASs may provide further information on their behaviors in the environmental water samples.

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