Simple and Rapid Determination of Linear Alkylbenzene Sulfonates by In-Tube Solid-Phase Microextraction Coupled with Liquid Chromatography

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A simple and rapid method for the determination of linear alkylbenzene sulfonates (LAS) in aqueous samples was developed by using in-tube solid-phase microextraction (SPME) coupled with liquid chromatography (LC). Introducing a short section of open-tubular gas chromatography column as the extraction device, in-tube SPME method has been developed by Pawliszyn *et al*. for an effective on-line coupling of sample preparation and LC separation. As an open tubular capillary column for the in-tube SPME, we used a porous-layer open-tabular (PLOT) column with a porous layer on the inner wall and a larger surface area. The separation of LAS was carried out by using a mobile phase containing 65% acetonitrile and 35% sodium perchlorate aqueous solution (287 mM) with a Wakopak® WS AS-Aqua column. LAS in the sample solution was enriched through the capillary sequentially by using a microsyringe pump equipped with a gastight syringe and analyzed by using high performance liquid chromatography with fluorescence detection. The fluorescence excitation and emission were fixed at λ_{ex} = 221 nm and λ_{em} =284 nm. With the optimum conditions, linear calibration curves were obtained from 0.2 to 25 μg/l. The detection limits based on 3σ were between 0.02 and 0.10 μg/l and the relative standard deviations of the method were between 1.6 and 12% (n = 5). The method was successfully applied to the determination of LAS in natural water samples. This method was applied to the determination of LAS in some urban river waters where domestic wastewater was discharged.

Key words —— linear alkylbenzene sulfonates, in-tube solid-phase micro extraction, water, liquid chromatography, fluorescence detection

INTRODUCTION

The number of industrially produced chemical substances is estimated to be in the tens of thousands. Chemical substances have become indispensable in our daily lives, but on the other hand, they may affect human health and the ecosystem. Surfactants are the most versatile products among the chemical industry and widely used both industrially and domestically. There are anionic, cationic, nonionic and bothionic types in surfactants. Linear alkylbenzene sulfonates (LAS) are one of the major anionic surfactants (AS) and used as active ingredients in household and personal care products. The Japanese production of LAS was about 72078 t in the 2001 fiscal year.[1)]

The Pollutant Release and Transfer Register (PRTR) Law was enacted in Japan in 1999 and LAS is listed as Class I Designated Chemical Substances. According to the PRTR data in the 2001 fiscal year, the amount of LAS discharged to the environment was estimated to be about 33000 t.[1)] LAS degrades rapidly aerobically, whereas it does not degrade under anaerobic conditions. Their accumulation, or that of their biodegradable products, comprises an important fraction of dissolved organic carbon (DOC) in natural water. The aquatic prediction non-influenced concentration (PNEC) value of LAS is considered 250 μg/l by the Ministry of Environment in the Netherlands[2)] and the permissible limit of AS in drinking water prescribed by Ministry of Health,

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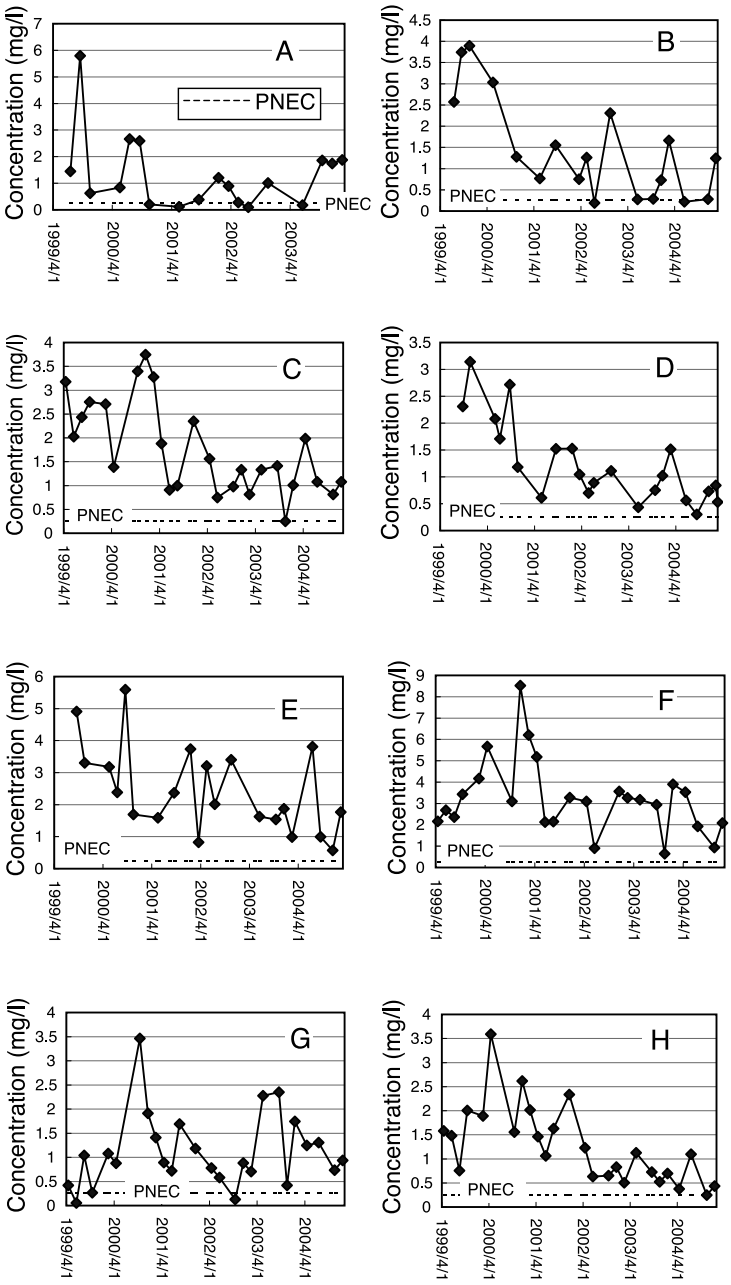

Fig. 1. Variation of MBAS Concentrations in the Shallow Streams of Chiba City
MBAS: methylene blue active substance. PNEC: PNEC of LAS (250 μg/l).

Labour and Welfare in Japan is 200 μg/l.[3)]

The methylene blue active substance (MBAS) method is one of the most popular techniques for the determination of AS in water.[4,5)] According to our investigation in the shallow streams of Chiba city, the concentrations of MBAS were in the range of 0.05–8.5 mg/l. Since the ratios of the concentrations of LAS to those of MBAS in river are estimated to 0.4–0.85,[6)] not a few surface waters of rural, urban, semi-urban and industrial areas are estimated to be contaminated with LAS beyond the PNEC value (see Fig. 1). Although LAS are not classified as dangerous compounds, their ecotoxicological properties may lead to the destruction of the natural flora and fauna.[7–11)] We must not neglect them and the monitoring of LAS in environment is important.

A large variety of analytical techniques have been proposed for determining LAS, such as molecular absorption spectrophotometry, liquid chro-

matography (HPLC) with ultraviolet (UV)[12,13] or fluorescence detection,[14,15] liquid and gas chromatography (GC)[16,17] with mass spectrometry (MS)[18–20] and biosensors.[21] However, they are tedious, complex, time consuming and require large amounts of organic reagents. Therefore, a new method is needed. Utilizing simple and inexpensive instrumentation with minimal maintenance is an important requirement.

Extraction and concentration process would be required for the samples containing low concentration of LAS. There are many enrichment techniques for trace analysis. Now, solid-phase extraction (SPE) is widely used, even more than liquid-liquid extraction. SPE have a remarkable advantage that one can avoid or minimize the use of harmful solvents. Styrenedivinylbenzene (SDB)[19] or octadecyl bonded silica (ODS)[22] have been the SPE material widely employed for extraction of AS. Recently, the HPLC method using SPE for enrichment was adopted as AS analysis method, with amendment of the water quality standard of tap water.[3] On the other hand, one of the demerits of SPE is the requirement of a large sample volume to attain a high enrichment. Applying a large volume sample solution through a solid-phase column wastes time and a large volume waste must be treated. We proposed high enrichment attainable separation techniques. In our previous study, golf course pesticides in water samples were efficiently enriched by using in-tube solid-phase microextraction (SPME).[23] The main advantage of in-tube SPME is good analytical performance combined with simplicity, low cost and does not need a special interface.[24–28]

In the present study, to develop a simple, rapid and eco-friendly in-tube SPME HPLC procedure that allows the detection of LAS in aqueous samples without tedious pretreatment steps, both chromatographic and instrumental parameters were optimized.

MATERIALS AND METHODS

Reagents and Chemicals —— The five Linear alkylbenzene sulfonates under the study, sodium decylbenzenesulfonate (C10), sodium undecylbenzenesulfonate (C11), sodium dodecylbenzenesulfonate (C12), sodium tridecylbenzenesulfonate (C13), and sodium tetradecylbenzenesulfonate (C14) were obtained as mixture standards from Wako Pure Chemicals (Osaka, Japan). The concentrations of these compounds were 1 mg/ml, respectively. Those of stock standard mixture were 50 μg/ml, respectively, by diluting with acetonitrile. Sodium perchlorate and HPLC grade acetonitrile were obtained from Wako Pure Chemicals. Pure water was purified with a Milli-Q Gradient system (Japan Millipore, Tokyo, Japan). The aqueous samples for limit of detection and linearity tests were prepared by spiking river water with the stock standard mixture or diluted standard mixtures reducing the concentration to suitable levels. Fresh aqueous samples were prepared before each experiment.

HPLC Conditions —— An LC10 series liquid chromatograph (Shimadzu, Kyoto, Japan) consisted of a pump, a fluorescence detector, a column oven, a Model 7125 valve (Rheodyne, Cotati, CA, U.S.A.) and a degassor. The separation was carried out by using Wakopak® WS AS-Aqua column (4.6 i.d. × 250 mm, 5 μm particle size; Wako Pure Chemicals). The mobile phase was prepared by mixing acetonitrile and sodium perchlorate aqueous solution at the ratio of 65/35 (v/v). This solution was containing 12.3 g/l sodium perchlorate. The flow-rate was set at 0.7 ml/min. The fluorescence excitation and emission wavelength were fixed at λ_{ex} = 221 nm and λ_{em} = 284 nm, respectively, and column temperature was controlled at 40°C. Data acquisition and processing were performed by using LC solution system (Shimadzu).

Sample Preparation —— Water samples were collected in 25 ml glass bottles (Shibata Scientific, Tokyo, Japan) and filtered through 47 mm diameter, 1 μm glass fiber filters (Toyo Roshi, Tokyo, Japan) to remove a particulate matter and excess oil before HPLC analysis. When a dilution was required, the sample was diluted with pure water.

In-Tube SPME Technique —— A GC capillary, Supel-Q PLOT (porous divinylbenzene polymer; 0.32 mm i.d. × 30 m) (Q-PLOT) was obtained from Spelco (Bellefonte, PA, U.S.A.). It was cut to 120 cm length and used as the in-tube SPME devices. The capillary was replaced with a sample loop of the valve and connected with a polyether ether ketone (PEEK) tubing (0.33 i.d. × 20 mm; GL Science, Tokyo, Japan),[27] a stainless nut and a stainless ferrule. The sample volume was 2.5 ml; it was preconcentrated by in-tube SPME. The first step in this method consists of rinsing the capillary with acetonitrile and pure water. At load position of the valve, the capillary was washed with 1 ml acetonitrile and 1 ml pure water by microsyringes. In the extraction step, a microsyringe pump KDS100 (KD Scientific, New Hope, PA, U.S.A.) equipped with 1005LTN gastight

syringe (HAMILTON, Nevada, U.S.A.) was employed to sequentially pump the sample solution through the capillary of the valve. And the capillary was washed with 1 ml pure water of a microsyringe and filled with 10 μl acetonitrile of another microsyringe. After the extraction step, the valve was switched to the inject position and the extracted analytes were desorbed from the capillary with acetonitrile. The sample was sent from the capillary loop to the analytical column by the mobile phase.

RESULTS AND DISCUSSION

Selection of Separation Columns

The separation was carried out by using Wakopak WS AS-Aqua column (4.6 i.d. × 250 mm, 5 μm particle size; Wako Pure Chemicals) which was a special column for the anionic surfactant analysis. LAS are a mixture of homologues and phenyl positional isomers, each containing an aromatic ring sulfonated at the para-position and attached to a linear alkyl chain of C10–C14 at any position except the terminal one. When LAS are separated by using ODS column, the isomers have been separated into many peaks in a chromatogram. As shown in Fig. 2, by using AS-Aqua column, the isomers were collected into one peak for each number of carbon atoms in a chromatogram. Since identification of peaks became easy and detection limits were improved, we used AS-Aqua column.

Selection of GC Capillaries for In-Tube SPME

Extraction and desorption were studied separately during the optimization of the in-tube SPME procedure. The selection of a suitable coating, based on the chemical nature of analytes, is the key step in the development of the extraction methods including the in-tube SPME. Many coatings are available for GC capillaries, and so the use of GC capillaries in the in-tube SPME method affords the higher levels of selectivity and sensitivity required for LAS. SDB[19] or ODS[22] have been the SPE material widely employed for extraction of anionic surfactants. The inner wall of Q-PLOT is coated with porous styrenedivinylbenzene which is the same material as the SDB-SPE used for SPE of anionic surfactants. Therefore, we selected this column by reason of its porous styrenedivinylbenzene structure and larger surface area. And we investigated the extraction efficiency of this column.

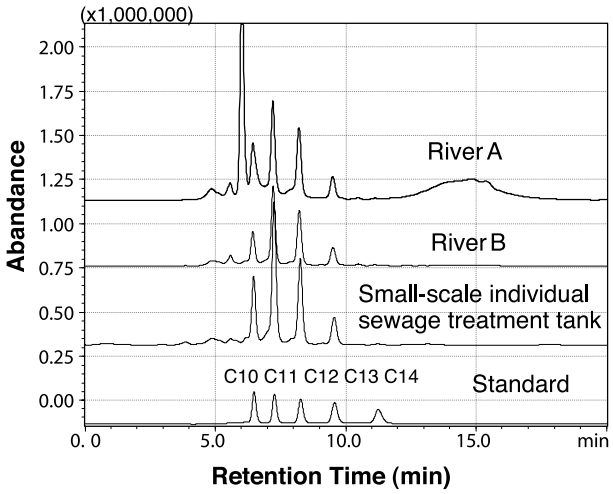

Fig. 2. Chromatograms of LAS by In-Tube SPME

Chromatograms were obtained by using in-tube SPME-HPLC-FL with AS-Aqua separation column. Peak identification: C10, Sodium decylbenzene sulfonate; C11, Sodium undecylbenzene sulfonate; C12, Sodium dodecylbenzene sulfonate; C13, Sodium tridecylbenzene sulfonate; C14, Sodium tetradecylbenzene sulfonate.

Desorption Solvent and its Volume

The desorption power of acetonitrile is very high. Considering the toxicity and cost of the solvents, acetonitrile is considered the better choice as desorption solvent. The solvent volume used in the desorption step can be manually controlled. The extraction efficiency in SPME can be evaluated by determining the amount of extracted analytes. The desorption efficiency varies with the different desorption volumes. The optimal desorption volume can be determined based on desorption curves. Figure 3 shows the desorption curves of the LAS studied. The total recovery can be calculated based on the peak area counts. Nearly full recovery was achieved when a desorption volume of 10 μl was used. When the acetonitrile volume was over 10 μl, the desorption efficiency was dramatically decreased. When the mobile phase was used as desorption solvent, it was very low. Figure 4 shows the chromatograms of the LAS with the different desorption volumes. When the acetonitrile volume was over 10 μl, the isomers have been separated into many peaks. This indicated that identification of peaks became difficult. Therefore we selected a desorption volume of 10 μl. The capillary was normally washed with 1 ml acetonitrile and 1 ml pure water after each injection. Using this procedure, no carry-over was detected.

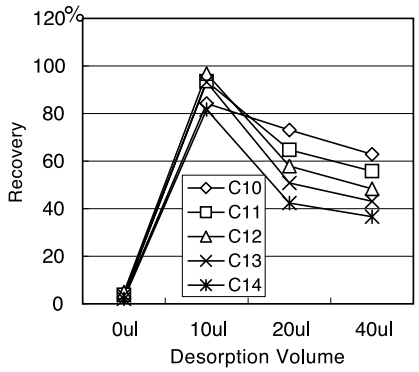

Fig. 3. Relationship between Recovery and Desorption Volume

Recoveries (%) are the percentage extracted amount of LAS per initial amounts (62.5 ng) in the 2.5-ml pure water using in-tube SPME. Desorption solvent were acetonitrile or mobile phase, and the desorption volume of acetonitrile was changed to 10, 20 and 40 μl. 0 μl denote only mobile phase.

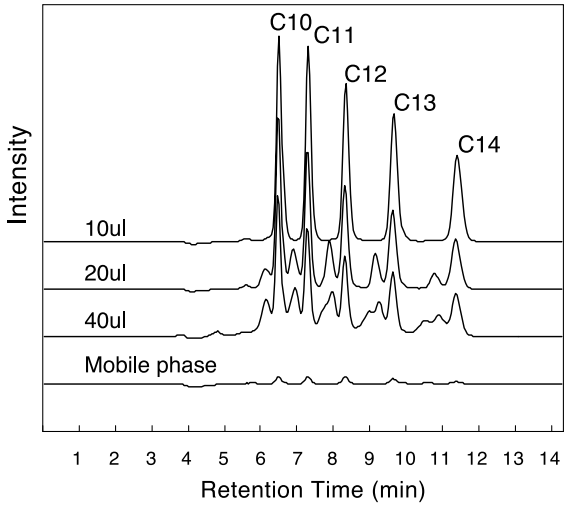

Fig. 4. Chromatograms of LAS by Different Desorption Volume

Chromatograms was obtained using in-tube SPME-HPLC-FL with AS-Aqua separation column. 62.5 ng each of LAS were spiked in each of the 2.5-ml purewater. Desorption solvent was acetonitrile or mobile phase, and the desorption volume of acetonitrile was changed to 10, 20 and 40 μl.

Relationship between the pH and the Extraction Efficiency

The relationship between the pH of the sample solution and the extraction efficiency of LAS was examined using pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 solutions. As shown in Fig. 5, excellent recoveries for the LAS studied were achieved at the conditions of pH 6.0 or higher. The pH of the sample solution was chosen to be between pH 6.0 and 9.0 in the work for safety purposes. A sample solution was adjustment between pH 6.0 and 9.0 with 0.1 M phosphoric acid or sodium hydroxide for further study.

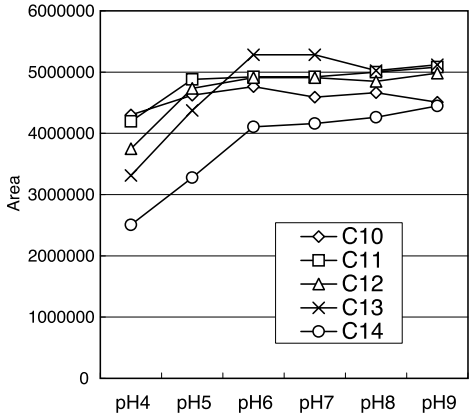

Fig. 5. Relationship between Recovery and pH of Sample Water

Recoveries (%) are the percentage extracted amount of LAS per initial amounts (62.5 ng) in the 2.5-ml purewater using in-tube SPME. The pH of sample water was changed between 4 and 9.

Table 1. Correlation between the Injection Speed and the Recovery

LAS	Injection Speed (μl/min)				
	100	150	200	250	500
					(%)
C10	86.4	88.5	79.4	79.3	70.3
C11	92.4	95.7	89.0	89.9	81.2
C12	97.4	98.3	94.3	94.3	86.1
C13	94.1	93.9	92.3	92.0	84.9
C14	81.4	82.1	83.1	81.6	76.5

Recoveries (%) are the percentage extracted amount of LAS per initial amounts (62.5 ng) in the 2.5-ml purewater using in-tube SPME. The injection speed of sample water was changed between 100 and 500 μl/min.

Relationship between the Injection Speed and the Recovery

The relationship between the injection speed and the recovery was established. A 2.5 ml aliquot of standard solution at 25 μg/l was extracted at the injection speed of 100, 150, 200, 250 and 500 μl/min. As shown in Table 1, the best recoveries were at the 150 μl/min injection speed. The recoveries of LAS slightly decreased when injection speed became faster. So we selected an injection speed of 150 μl/min.

Calibration Curve

The linearity at a large volume injection was in-

vestigated by using a 2.5 ml aliquot of sample solution. The amounts of each LAS in a sample solution were varied from 0.5 to 62.5 ng. As shown in Fig. 6, the method was linear for all the LAS studied with correlation coefficients (r^2) between 0.998 and 0.999. The reproducibility, using the optimal experimental conditions, was determined by analyzing seven replicate samples at the concentrations of the lower limit and the upper limit. The method was reproducible with precision between 0.8 and 3.1% relative standard deviation (RSD) at the upper limit and between 2.5 and 9.4% RSD at the lower limit, respectively.

Recoveries and Reproducibility by Using In-Tube SPME

The sensitivity of this analytical procedure was evaluated in terms of the limit of detection (LOD) and the limit of quantification (LOQ). For the calculation of them, a water sample from the Kashima River, in which traces of LAS were found, was spiked with 0.25 μg/l of the five LAS (C10–C14). The LOD was calculated as 3 times the standard deviation from analyses of 5 replicate samples and the LOQ was calculated as tenfold. As shown in Table 2, the LOD and LOQ of LAS by this method were in the range 0.02–0.10 and 0.06–0.35 μg/l, respectively.

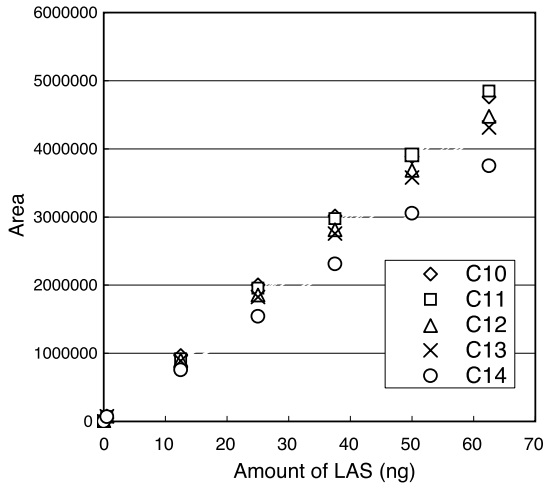

Fig. 6. The Linearity of Large Volume Injection by In-Tube SPME

They were investigated by using a 2.5 ml aliquot of sample solution. The amounts of each LAS were changed to 0.5, 12.5, 25, 37.5, 50 and 62.5 ng.

We conducted the recovery tests of river waters under the optimum conditions. The spiked-level of the five LAS (C10–C14) was 0.25 μg/l. As shown in Table 3, the recoveries of the spiked analytes ranged from 62.0 to 123%, and reproducibilities were found to be RSD 1.6–12% for five replicates. At the recovery tests of waste water, the spiked-level was 2.5 μg/l. The recoveries of the spiked analytes ranged from 65.0 to 104%, and reproducibilities were found to be RSD 2.8–5.9% for five replicates. Compared with the result depended on the SPE method, the almost same value was shown about C10 to C13. About C14, it was about 70% of value of the SPE method. It seemed that the influence which it had on the value of LAS was small since the abundance ratio of C14 was 5% or less of all the whole LAS.[1)]

Influence of Temperature on Degradation

The influence of temperature was studied with pure water including 0.025 mg/l LAS. 200 ml of LAS solution was put into a 300-ml flask for each of the working temperatures (5 and 20°C). During the experiments, the flasks were stopped with glass plugs. The aliquots for determining residual LAS concentration were collected with a pipette inserted to half the depth of the solution. Figure 7 shows per-

Table 2. Recoveries, LOD, and LOQ

LAS	River		Waste Water		LOD	LOQ
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	(μg/l)	(μg/l)
C10	85.1	5.5	89.6	4.3	0.04	0.11
C11	114	12	104	5.9	0.10	0.35
C12	123	1.6	101	2.8	0.02	0.06
C13	85.2	6.6	82.0	2.8	0.05	0.14
C14	62.0	3.9	65.0	3.6	0.02	0.06

Recoveries (%) are the percentage extracted amount of LAS per initial amounts in the 2.5-ml sample water using in-tube SPME. The spiked amounts were 0.625 or 6.25 ng. RSD (%) were measured at 0.625 or 6.25 ng ($n = 5$). The LOD was calculated as 3 times the standard deviation from analyses of 5 replicate samples and the LOQ was calculated as tenfold.

Table 3. LAS Concentrations in the Water Environments of Chiba City

	Main rivers	Shallow streams	Small-scale Individual Sewage Treatment Tank
			(mg/l)
Rural Areas	0.023	0.43	0.096
	0.024	0.48	0.20
	0.014	0.91	0.066
	0.024	1.9	0.36
	0.037		0.42
	0.012		0.058
Semi-urban Areas	0.088		
	0.033		
	0.016		
Urban Areas	0.020		
	0.080		

Investigation was conducted in spring of 2005.

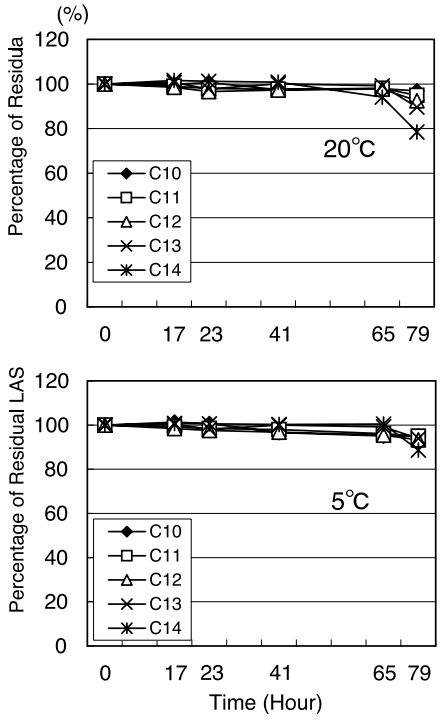

Fig. 7. Change with Time of Percentage of Residual LAS by Temperature

Recoveries (%) are the percentage extracted amount of LAS per initial extracted amounts in the 2.5-ml purewater using in-tube SPME.

centages of degradation reached on different hours of testing, for the two temperatures studied. It can be noticed that there was hardly any degradation for 3 days. However, fresh standard solutions should be prepared before each experiment.

Durability of the Extraction Column

We checked the decline of the extraction efficiency of the column. The extraction performance was calculated by comparison of the amount of compound extracted onto the capillary column with the average amount of them. As shown in Fig. 8, the extraction performance was only slightly decreased after the sequential sample extraction of more than 100 times. Since the column for in-tube SPME can be used about 100 times, the running cost of our method is very low. Furthermore, since this method uses a little solvent, it is environment-friendly.

Application to Practical Samples

In spring of 2005, LAS of several real water samples in Chiba city were investigated by using the developed method. The chromatograms which measured river water and drainage by in-tube SPME were shown in Fig. 2. Although the unknown peak was seen in river water A, it was dissociated with other peaks. About other river waters and drainages, there was hardly serious interfering. As shown in Table 3, LAS were found between 0.012 and 0.088 mg/l in main rivers. In a shallow urban stream receiving untreated household wastewater, they were found between 0.43 and 1.9 mg/l. And the survey of small-scale individual sewage treatment tank, LAS was found between 0.058 and 0.42 mg/l. Although part of them was detected only by low concentration, we need to continue this investigation further.

In conclusion, this system was successfully used to analyze the LAS. All the main parameters relating to both the extraction and desorption processes

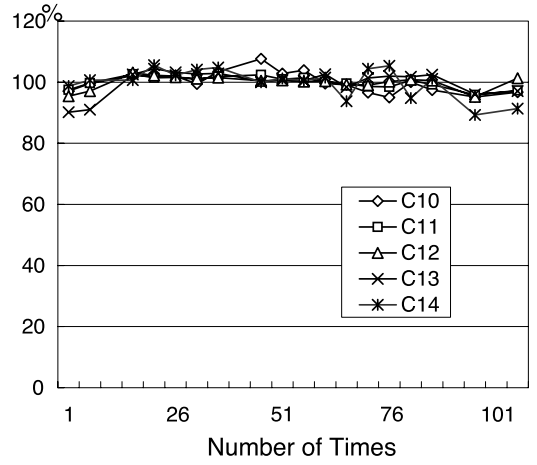

Fig. 8. Durability of the Extraction Column

Recoveries (%) are the percentage extracted amount of LAS per initial amounts (62.5 ng) in the 2.5-ml sample water using in-tube SPME.

of the in-tube SPME were investigated. The new in-tube SPME method presented the following advantages over the conventional in-tube SPME HPLC. A Q-PLOT column showed the better extraction efficiency for LAS and our method has realized the same recovery as a solid phase extraction method. It is a semi-automated method, requiring no sample manipulation between the extraction and the HPLC analysis, therefore offering a high efficiency and precision. This method has a reasonably low LOD for fluorescence analysis of the LAS studied. Since the column for in-tube SPME can be used about 100 times, the running cost is very low. This method requires no extracting solvent, and is eco-friendly.

Almost all the surface waters of rural, urban, semi-urban and industrial areas and shallow stream waters were found to be contaminated with LAS.

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