Analysis of Ionogenic Surfactants by HPLC with Ion-pair Extraction Detector

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A technique for developing a selective and highly sensitive detection method was studied, which responds to particular ionogenic surfactants using a system of connecting the post-column reaction detector with a high performance liquid chromatograph (HPLC).

HPLC conditions used for the separation were: column packing and size, TSK-LS410 (5μ) and 6 mm i.d. × 250 mm; mobile phase, a mixture of methanol, water, sodium perchlorate and phosphoric acid. A Technicon AutoAnalyzer II system was used as the post-column reaction detector. Orange II was used to form the hydrophobic ion-pair complexes for cationic and amphoteric surfactants, and Methylene blue for anionic surfactants.

By this method, cationic, amphoteric and anionic surfactants were easily identified in the chromatograms. The analytical results of their homolog distributions were in good agreement with those obtained by conventional gas chromatographic (GC) methods.

Surfactants are widely used as emulsifiers and solubilizers in cosmetics, foodstuffs and pharmaceutical products. Generally, they are classified into nonionic, anionic, cationic and amphoteric surfactants, according to their ionogenic properties.

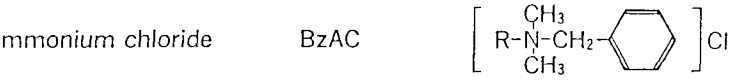
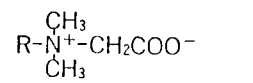
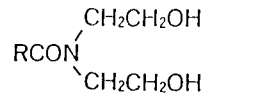
Recently, high performance liquid chromatography (HPLC) has been used successfully to separate these surfactants analytically into each ionogenic type and discriminate within a group (i.e. homologs and isomers).

In the analysis of surfactants by HPLC, spectrophotometry (UV-Vis), refractive index (RI), fluorophotometry (FL) and flame ionization detection have been used. In the case of the complicated mixtures of various kinds of surfactants these detectors have shortcomings in both selectivity and sensitivity.

In recent years, several papers have been published on the application of the post-column reaction detector based on the extraction of ion-pairs. Frei et al. (1,2) reported the application of this detector first to normal-phase HPLC and second to reversed-phase HPLC for the separation of anionic surfactants. Nakae and Tsuji (3) and Kawase et al. (4) reported the application of this detector to reversed-phase HPLC for the homolog separation of anionic and cationic surfactants. However, there are few reports on the

TABLE 1

Chemical Structures of Surfactants Studied

Surfactant name	Symbolic name	Chemical structure
Cationic surfactant (A) Alkylpyridinium chloride	APC	
(B) Alkyltrimethylammonium chloride	ATC	
(C) Alkylbenzyldimethylammonium chloride	BzAC	
Amphoteric surfactant (D) Alkyldimethylaminoacetic acid betaine	ADB	
(E) Sodium alkylaminopropionate	SAP	$RNHCH_2CH_2COONa$
Anionic surfactant (F) Sodium alkylsulfate	SAS	$ROSO_3Na$
Nonionic surfactant (G) Fatty acid diethanolamide	FDE	

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technique in which cationic, amphoteric and anionic surfactants are detected selectively with the same mobile phase condition.

We have been studying the separation of surfactant homologs by HPLC using a reversed-phase packing containing an octadecyl silane group chemically bonded to silica gel (ODS/Silica) (5-7). In our previous paper (6), nonionic, anionic, cationic and amphoteric surfactants were separated into their individual homologs and simultaneously distinguished from each other using a mixture of methanol, water, sodium perchlorate ($NaClO_4$) and phosphoric acid as the mobile phase. This method has the disadvantage that the chromatograms are generally complicated due to the charactor of the RI detector, which responds to a wide range of compounds. The RI detector is non-selective and of low sensitivity.

In order to develop a selective and highly sensitive detection method which responds to particular ionogenic surfactants, a combination of the mobile phase conditions described in our previous paper and the post-column detection technique based on the extraction of ion-pairs was studied in this paper. The Technicon AutoAnalyzer II system was used as the post-column reaction detector. Orange II was used to form the hydrophobic ion-pair complexes for cationic and amphoteric surfactants, and Methylene blue for anionic surfactants. By optimizing several factors of the AutoAnalyzer II system, cationic, amphoteric and anionic surfactants separated by reversed phase HPLC using one set of mobile phase conditions and detected with a post-column reaction detector were easily identified in the chromatograms; the analytical results of their homolog distribution were in good agreement with those obtained by conventional gas chromatographic (GC) methods.

EXPERIMENTAL PROCEDURES

Materials. Homologs of seven typical surfactants were used in this study. Their chemical structures and symbolic names are illustrated in Table 1. The homologs were n-dodecyl, n-tetradecyl, n-hexadecyl and n-octadecyl derivatives.

The purities of all the surfactant homologs were >95% by GC. APC, BzAC, ADB, SAP, SAS and FDE were the same grade as used in previous studies. The ATC was a special reagent grade (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) and was purified by recrystallization from ethanol. Other reagents and solvents used were all analytical grade, and the shampoos used were commercially available.

Apparatus. The liquid chromatograph consisted of a Model 6000A pump (Waters, Milford, Massachusetts), a Waters Model 730 Autoinjector, a Waters Model R401 differential refractometer and an AutoAnalyzer Model II ion-pair extraction detector (Technicon, Tarrytown, New York). The ion-pair extraction detector consisted of a proportioning pump, a manifold and an UV-Vis spectrophotometer. Chromatograms were recorded with a Model 056 recorder (Hitachi, Tokyo, Japan).

A chromatographic column (stainless steel, 6 mm i.d. × 250 mm) was packed with TSK Gel LS-410 (5 μ, spherically shaped ODS/Silica, Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan), by the method described previously (6). The mobile phase was a mixture of water and methanol containing $NaClO_4$, and its pH was adjusted with phosphoric acid. The flow rate of the mobile phase was set at 1.5 ml/min, and the column temperature was maintained at 50 C. All experiments were performed under isocratic conditions. All the samples were prepared as 0.01-0.1% methanol solu-

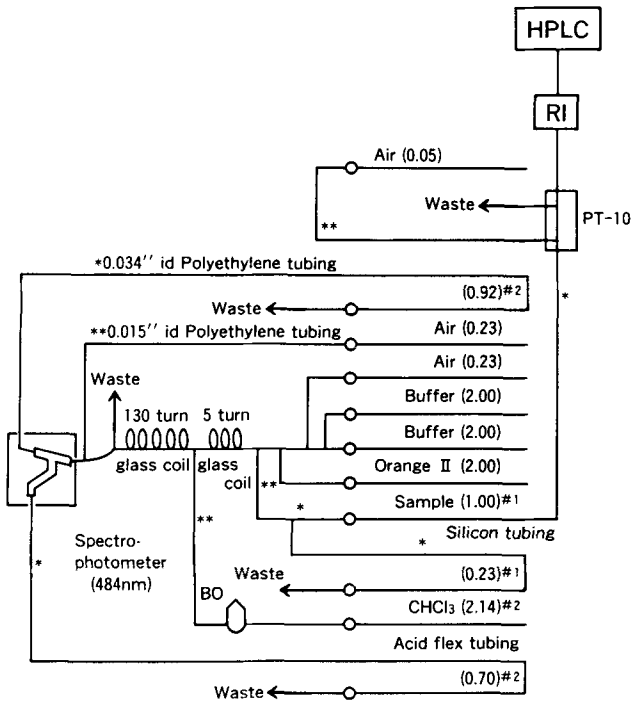

FIG. 1. Schematic representation of the ion-pair extraction detection system of the Orange II methd.

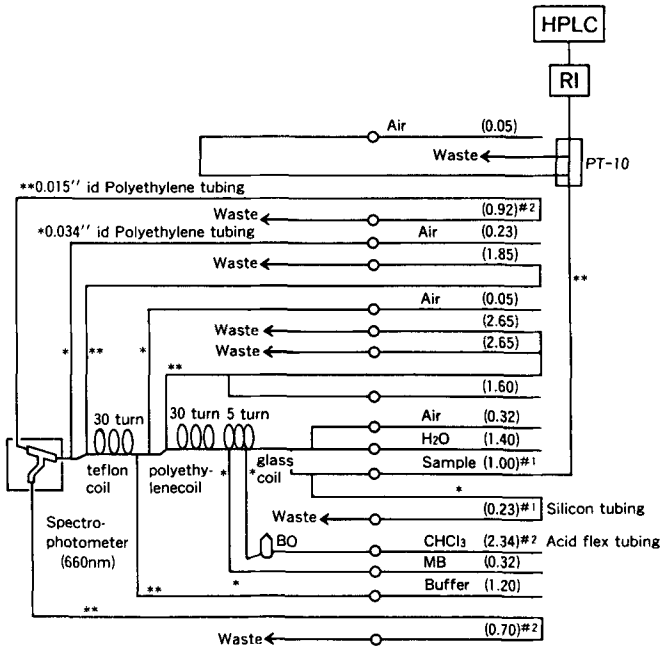

FIG. 2. Schematic representation of the ion-pair extraction detection system of the MB method.

tions, and 10-20μl were injected into the HPLC column.

Orange II was used in the detection of cationic and amphoteric surfactants (Orange II method). The ion-pair extraction system of the Orange II method is shown schematically in Figure 1. The mobile phase was fundamentally segmented with air as shown in Figure 1. The mobile phase through the HPLC column was first mixed with buffer aqueous solution and Orange II solution, and then mixed with chloroform. After the phase separation, the chloroform phase was monitored spectrophotometrically at 484 nm. Methylene blue was used in the detection of anionic surfactants (MB method). The ion-pair extraction system of the MB method is shown in Figure 2. The mobile phase is segmented with air as in the Orange II method. The mobile phase was mixed with water, chloroform and MB solution. In the MB method, two-step phase separations were necessary to separate chloroform from aqueous solutions due to a high $NaClO_4$ content. The chloroform phase was monitored spectrophotometrically at 630 nm.

RESULTS AND DISCUSSION

Orange II method. The Orange II method (8) has been used in conventional spectrophotometric determination of cationic and amphoteric surfactants. Kawase and Yamanaka (9) reported that the Orange II method could be successfully automated using the AutoAnalyzer. Kawase et al. (4) reported on the post-column detection system, which was based on the ion-pair extraction of the cationic surfactants with a counter-ion of Bromophenol blue into an immiscible organic phase of n-hexane.

In preliminary experiments, the following anionic dyes and extractants were investigated for the extraction of cationic and amphoteric surfactants in the form of ion-pairs into an organic layer: Orange II, Resorcine brown B, G and Lithol red R as anionic dyes, and chloroform, n-hexane and dichloromethane as extractants. Eventually it was determined that the combination of Orange II as anionic dye and chloroform as extractant gave the most stable baseline.

In the Orange II method, the results of detection and determination of surfactants were affected by the diameter of a tube from the HPLC to the ion-pair extraction system, the concentration of the Orange II solution and the buffer pH.

Figure 3 shows the effect of tube diameters from HPLC to the ion-pair extraction system on response intensities and coefficient variations (Cv%) of a typical cationic (ATC) and amphoteric surfactant (ADB). The results indicate that the response intensity increases with increasing tube diameter. Cv% had small values at 0.051 inch for both ATC and ADB. Therefore, a 0.051-inch tube was chosen from the standpoint of reproducibility.

The concentration of Orange II solution was investigated in the range between 25 and 100 mg/ml, and was set at 50 mg/ml for reproducibility and the stability of the baseline.

The buffer pH of the ion-pair extraction system drastically affected the detection of cationic and amphoteric surfactants. Figure 4 shows the effect of

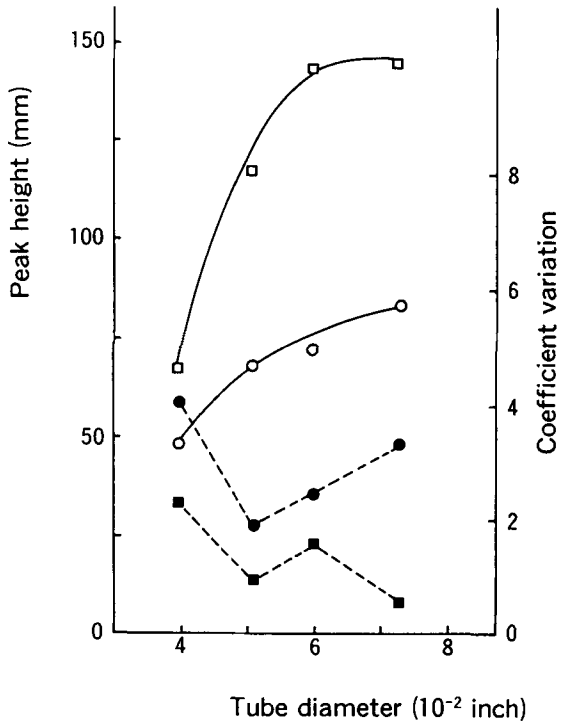

FIG. 3. Effect of tube diameters from HPLC to the ion-pair extraction system on response in the Orange II method. [6 mm i.d. × 250 mm, water/methanol (15:85) containing 1.0 M $NaClO_4$ and adjusted to pH 2.5 with phosphoric acid, 1.5 ml/min. All samples are n-dodecyl derivatives; ○, ATC; □, ADB for peak height and ●, ATC; ■, ADB for Cv%].

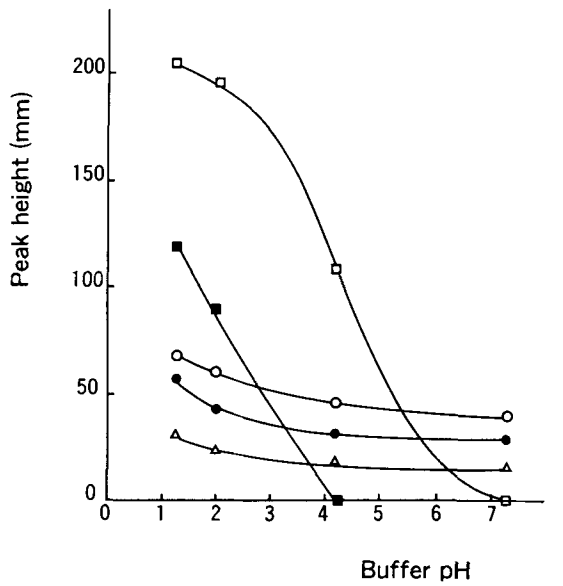

FIG. 4. Effect of the buffer pH on response in the Orange II method. (HPLC conditions are the same as in Fig. 3. All samples are n-dodecyl derivatives. ○, ATC; ●, APC; △, BzAC; □, SAP, and ■, ADB).

the buffer pH in the ion-pair extraction system on the response intensities. The buffer pH was adjusted with 0.1 N potassium chloride and 0.1 N hydrochloric acid in the pH range 1.0–3.0, 0.1 N acetic acid and 0.1 N sodium acetate from 3.0–5.0, 0.1 N potassium dihydro-

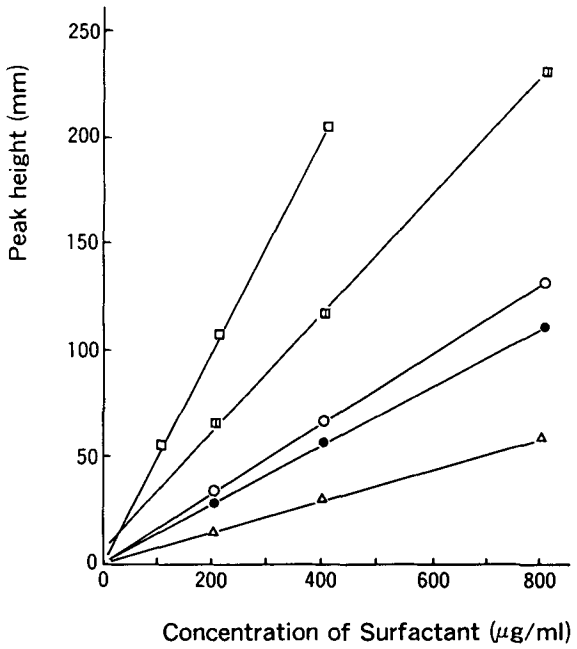

FIG. 5. Effect of surfactant concentration on response in the Orange II method. (HPLC conditions, samples and symbols are the same as in Fig. 3.)

gen phosphate and 0.1 N disodium hydrogen phosphate from 5.0–7.3. The response intensities of cationic surfactants examined were nearly constant in all pH ranges, but the intensities of amphoteric surfactants drastically decreased with increasing pH values in the ion-pair extraction system. This behavior of the amphoteric surfactants is attributed to their electrostatic properties, which act as cationic surfactants in an acidic environment. As shown in Figure 4, cationic and amphoteric surfactants studied were simultaneously detected at pH 1.0–4.0, and cationic surfactants detected selectively over pH 7.3.

Figure 5 shows the relationship between the concentration of n-dodecyl derivatives and the corresponding peak intensities at pH 1.3. The peak intensities were linearly proportional to the concentration of respective surfactants. For all other derivatives, the same linear relationships were obtained. Their peak intensities changed with the buffer pH, but these were directly proportional to the concentration at all other pHs. As shown in Figure 5, the concentrations of surfactants were from 100 μg/ml to 800 μg/ml. These concentrations were about 1/10 of that needed when using the RI detector.

Commercial surfactants usually are mixtures of homologs, and their effectiveness and physical properties depend largely on alkyl chain length as lipophilic groups. Their homolog distributions have been determined by the proposed method. Analytical results of homolog distributions of commercial ATC by the Orange II method are shown in Table 2 (a). The response intensity varied with the homologs of ATC. Therefore, for quantitative analysis, it was necessary to use the calibration curves of n-dodecyl, n-tetradecyl, n-hexadecyl and n-octadecyl derivatives. The results were in good agreement with those obtained by

TABLE 2

Analytical Results of Homolog Distributions of Commercial ATC and SAS

Alkyl chain	Proposed method (%)	GC
(a) ATC		
C_{14}	2.3	2.3
C_{16}	20.2	19.3
C_{18}	77.5	78.4
(b) SAS		
C_{12}	61.8	64.4
C_{14}	28.2	26.7
C_{16}	10.0	8.9

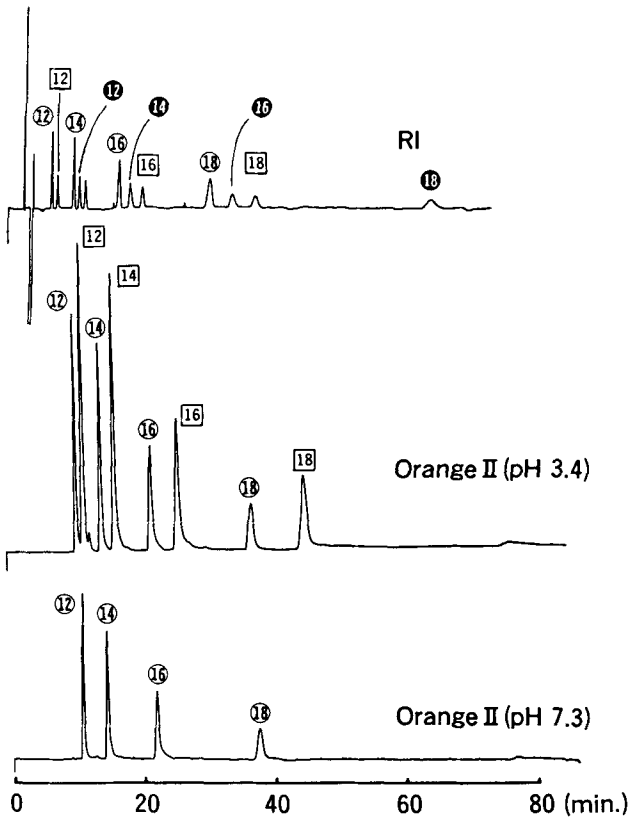

FIG. 6. Identification chromatograms of standard cationic and amphoteric surfactant by the Orange II method. (HPLC conditions are the same as in Fig. 3. Arabic numbers mean alkyl chain length. ○, ATC; □, SAP, and ●, SAS.)

conventional GC methods (5).

In the GC method, the samples were converted into the corresponding volatile derivatives and analyzed as follows: ATC was analyzed using a 3-mm i.d. × 1-m glass column packed with Shimalite W (AW, DMCS, 60-80 mesh) coated with 5% SE-30. ATC (ca. 1 g) was prepared as 2-propanol (20 ml) solution, and 1 μl was injected into the gas chromatograph. The column oven temperature was programmed from 100 to 300 C at 10 C/min.

MB method. Methylene blue was selected as a

cationic dye because it has been used in the conventional spectrophotometric determination of anionic surfactants containing sulfonic and sulfuric groups. As shown in Figure 2, two phase separation steps were necessary to remove the interference of the excess $NaClO_4$ which makes the baseline unstable. Other conditions were optimized in the same manner as in the Orange II method.

The peak heights were linearly proportional to the concentration, and the results were similar to those shown in Figure 5. The analytical results of homolog distributions determined by this method were also in good agreement with those obtained by conventional GC methods as shown in Table 2 (b).

In the GC method, SAS (ca. 1 g) was hydrolyzed with 6N hydrochloric acid aqueous soln (50 ml) for 6 hr at 100 C, and the extracted alcohols (as their trimethylsilyl ethers) were analyzed using a 3-mm i.d. × 0.5-m glass column packed with Diasolid ZT (80-100 mesh). The column oven temperature was programmed from 100 to 300 C at 10 C/min.

Application. As described above, anionic surfactants were selectively detected by the MB method, and cationic and amphoteric surfactants were selectively detected by the Orange II method, even if a sample solution contained all ionogenic surfactants. Furthermore, only cationic surfactants were detected at the buffer pH of 7.3 by the Orange II method. From these results it seems that the peaks in the complex chromatograms detected by the RI detector could be easily identified.

Figure 6 shows the analytical chromatograms of a standard surfactant mixture containing n-dodecyl, n-tetradecyl, n-hexadecyl and n-octadecyl derivatives of ATC, SAP and SAS detected by the RI detector and the Orange II method. All components were detected by means of the RI detector, and the chromatogram was complex. Using the Orange II method at a buffer pH of 3.4, the shadow peaks which were SAS homologs were eliminated, and ATC and SAP homologs were detected simultaneously. Furthermore, ATC homologs were detected selectively at a buffer pH of 7.3. Therefore, these techniques seem to be useful for identifying surfactants in commercial shampoos. Commercial shampoos are generally formulated with several surfactants which have different ionogenic properties and homolog distributions. Results of analyses of a commercial shampoo detected by the RI detector, the MB method and the Orange II method are shown in Figure 7. Surfactants were extracted according to the previous method (7). As shown in Figure 7, a chromatogram produced by the RI detector was also complex. Peaks No. 4, 5 and 6 were detected by the MB method. These results indicate that these components are anionic surfactants. In comparison of the retention times and the patterns with those of standard materials, it was determined that peaks No. 4, 5 and 6 were sodium n-dodecyl sulfate, polyoxyethylene (POE) sodium n-dodecyl sulfate and sodium n-tetradecyl sulfate, respectively. On the other hand, peak No. 1 was detected only by the Orange II method with a buffer pH of 3.4. As this peak was not detected at a buffer pH of 7.3, this peak component was identified as an amphoteric surfactant. The retention time of peak No. 1 coincided with that of the n-dodecyl derivative of ADB. Peaks No. 2 and 3 were detected by neither the MB method nor the Orange II method. This indicates that these components are nonionic surfactants. Peaks No. 2 and 3 were finally identified as dodecanoyldiethanolamide and dodecanoylmonoethanolamide, respectively.

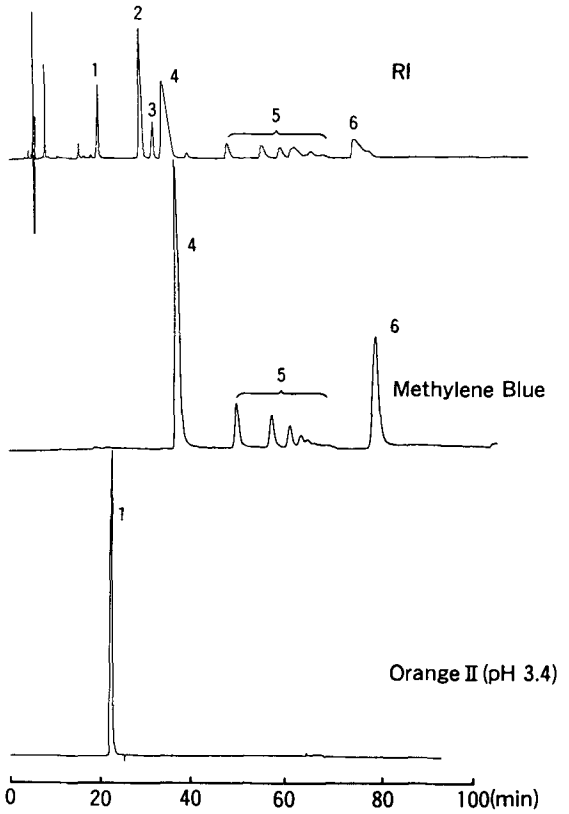

FIG. 7. Identification chromatograms of ionogenic surfactants in a commercial shampoo by both the Orange II method and the MB method. (HPLC conditions are the same as in Fig. 3. 1, n-Dodecyl dimethylaminoacetic betain; 2, dodecanoyldiethanolamide; 3, dodecanoylmonoethanolamide; 4, sodium n-dodecyl sulfate; 5, POE sodium n-dodecyl sulfate; 6, sodium n-tetradecyl sulfate.)

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