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## ANALYSIS OF NON-ULTRAVIOLET-ABSORBING DIALKYL-TYPE CATIONIC SURFACTANTS BY LIQUID CHROMATOGRAPHY WITH AN ON-LINE ION-PAIR EXTRACTION DETECTOR

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### SUMMARY

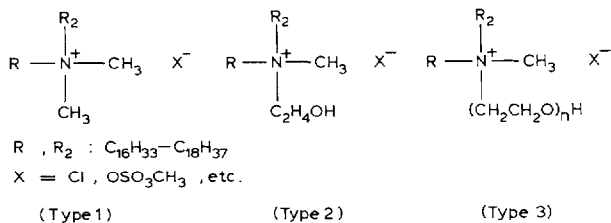
Dialkyl-type cationic surfactants, *i.e.*, dialkyldimethylammonium, dialkyl(2-hydroxyethyl)methylammonium and dialkylmethylpoly(oxyethylene)ammonium salts, were analysed by liquid chromatography with an on-line ion-pair extraction detector. The separation was achieved by reversed-phase chromatography with a eluent of 0.1 *M* ammonium acetate in methanol–acetonitrile–deionized water (60:60:5). The post-column detection system was based on the on-line ion-pair extraction of the cationic surfactants with a counter ion of Bromophenol blue into an immiscible organic phase of hexane which was monitored by a double-wavelength spectrophotometer (605 nm and 670 nm). By the proposed method, the type and alkyl chain distributions of the cationic surfactants in commercial products were distinguished within 15 min without any pre-treatment of samples and with a detection limit each of approximately 15 nmol.

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### INTRODUCTION

The ion-pair extraction technique has proved valuable for the determination of cationic surfactants by two-phase titrations<sup>1,2</sup> and/or by spectrophotometry<sup>3-5</sup>. Automated determination has been carried out by the AutoAnalyzer<sup>6</sup> and the flow injection analysis techniques<sup>7</sup>. The method is based on the ability of the surfactants to form ion-pairs with a counter ion.

Determination of the type and alkyl chain distribution of cationic surfactants have hitherto been carried out by thin-layer chromatography (TLC)<sup>8</sup>, gas chromatography (GC)<sup>9-14</sup> and liquid chromatography (LC)<sup>15-18</sup>. The GC techniques involved pre-treatment of samples to obtain pure cationic surfactants when the surfactants were formulated in commercial products. The liquid chromatographic techniques were convenient for the analysis of UV-absorbing cationic surfactants even in formulations, but the same pre-treatment of samples as in GC analysis is needed for the analysis of non-UV-absorbing cationic surfactants. The TLC techniques were advantageous for analysis of the types of cationic surfactants in formulations, but did not provide infor-



R, R<sub>2</sub> : C<sub>16</sub>H<sub>33</sub>–C<sub>18</sub>H<sub>37</sub>  
 X = Cl, OSO<sub>3</sub>CH<sub>3</sub>, etc.

Fig. 1. Structures of dialkyl-type cationic surfactants analysed.

mation on the alkyl chain distributions. Further, polyoxyethylated cationic surfactants could not be distinguished from other cationic surfactants.

Here we report an LC analysis of non-UV-absorbing cationic surfactants of the dialkyldimethylammonium and dialkylmethylpolyoxyethylated ammonium types listed in Fig. 1 using a post-column ion-pair extraction detector. The surfactants were separated by high-performance liquid chromatography (HPLC) and the resulting effluent was mixed with an aqueous solution of counter ion, Bromophenol blue (BPB), to form a coloured ion-pair. The solution was then mixed with an immiscible organic solvent and passed through a mixing coil, thus enabling the ion-pair to be extracted into the organic phase. The mixture was then directed to a membrane-type phase separator developed previously<sup>7</sup> where the aqueous solution and part of the organic phase were removed, while the remaining organic phase passed through the membrane and was air-segmented in the pathway of a flow cell of a double wavelength spectrophotometer where the coloured ion-pair was detected.

The proposed method gives information on both the type of surfactants and the alkyl chain length. The method has been applied successfully to the analysis of commercial products.

## EXPERIMENTAL

### Apparatus

A schematic diagram of the liquid chromatograph is shown in Fig. 2. Sample solutions were injected via a Rheodyne 7125 sample injector. A Hitachi 655 pump was used to force an eluent through the analytical column (controlled at 40°C) at a flow-rate of 1.0 ml min<sup>-1</sup>. Acid-resistant pumps (Nihon Seimitsu NSP-800, Tokyo, Japan) were used for the post-column reagents: a BPB aqueous solution at a flow-rate of 1.0 ml min<sup>-1</sup> and hexane at a flow-rate of 1.5 ml min<sup>-1</sup>. Hypersil 8C (3μm, Shandon, Great Britain) was slurry packed in 150 × 4.6 mm I.D. columns. The extraction coil was made of PTFE tubing (5 m × 0.5 mm I.D.) and the mixing tees and a membrane type phase-separator were as described previously<sup>7</sup>. The double-wavelength spectrophotometer (605 nm and 670 nm) was as described by Nakae and Tsuji<sup>19</sup>. A standard Technicon pump II was used both to withdraw the effluent from the flow cell and to generate air-segmentation. A Shimadzu Chromatopak CRIA was used both to print the chromatograms and to calculate the peak areas.

### Reagents

The eluent was 0.1 M ammonium acetate in methanol–acetonitrile–deionized water (60:60:5, v/v/v). The BPB aqueous solution comprised 0.01% (w/v) BPB in 0.05 M sodium carbonate–0.1 M sodium hydrogen carbonate aqueous solution.

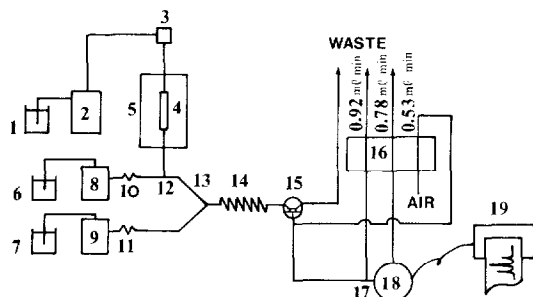


Fig. 2. Schematic diagram of the liquid chromatograph. 1 = Eluent ( $1 \text{ ml min}^{-1}$ ); 2 = pump; 3 = sample injector; 4 = analytical column; 5 = water-bath ( $40^\circ\text{C}$ ); 6 = BPB solution ( $1 \text{ ml min}^{-1}$ ); 7 = hexane ( $1.5 \text{ ml min}^{-1}$ ); 8/9 = acid-resistant pumps; 10/11 = pulse-suppressing coils; 12 = mixing tee (T-shape); 13 = mixing tee (W-shape); 14 = extraction coil; 15 = phase separator; 16 = peristaltic pump; 17 = debubbler; 18 = double-wavelength spectrophotometer; 19 = data processor.

The cationic surfactants listed in Fig. 1 were synthesized in our laboratories. Sample solutions for HPLC were prepared by dissolving the samples in 2-propanol and diluting appropriately in the solvent.

## RESULTS AND DISCUSSION

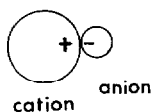
### *Ion-pair extraction detector*

The success of the proposed post-column detector depends on several factors including selectivity, sensitivity, rate of extraction, background absorbance and noise.

*Counter ions:* Before the post-column detection system was interfaced, counter ions commonly used in conventional spectrophotometric determination of cationic surfactants, *e.g.*, Orange II, BPB and dichlorofluorescein (DCF), were compared manually. The pH of the aqueous post-column reagent was chosen to be 9.3 buffered by 0.05 *M* sodium carbonate–0.1 *M* sodium hydrogen carbonate, to eliminate interferences from other amine compounds in formulations. The sample solution was prepared by dissolving the cationic surfactants in methanol–deionized water (50:50, v/v) containing 0.1 *M* ammonium acetate.

The analytical procedure was as follows: 3 ml of a 0.1 *mM* solution of cationic surfactant of type 1 in methanol–water (50:50, v/v) were mixed vigorously in a test-tube with 3 ml of the aqueous post-column reagent containing 0.01% counter ions (in excess over the cations) and with 6 ml of chloroform. After standing for about 10 min, the absorption spectrum of the chloroform layer was recorded. The results showed that the molar absorptivity of BPB at its maximum was twice that of Orange II and 1.5 times that of DCF. Further, the blank absorbance of BPB was the lowest of all the counter ions. Accordingly, BPB was preferred as the counter ion.

*Extraction solvent:* The solubility of the ion-pairs of BPB with the cationic surfactants listed in Fig. 1 was lower in chloroform than those of monoalkyl-type cationic surfactants. Insoluble ion-pairs were precipitated at the interface of the aqueous and organic layers, when the extraction was carried out with chloroform–water (50:50, v/v). The reason for this is as follows. The free charge density of the dialkyl type cationic molecule of the ion-pair is lower than that of the anionic counter ion, because the cation is large and lipophilic except for the positively charged centre and the exposed surface of the small anion would be expected to carry a relatively high negative charge per unit



Formed ion-pair

area. Therefore the anionic molecule of the ion-pair has high negative charged ionic character which results in the low solubility in the immiscible organic solvent. To enhance the extractability of ion-pairs into immiscible organic solvents, solvating agents such as alcohols have been added to the extraction system<sup>6,7,20</sup> to mask the ionic character of the bond between the two ions. In the proposed method, the eluent methanol-acetonitrile-deionized water (60:60:5) containing 0.1 M ammonium acetate was essential to distinguish surfactants of types 1 and 2. In this system, the extractability of the formed ion-pair was not very high, probably due to acetonitrile which almost transferred itself into the immiscible organic layer in the extraction. Some other immiscible solvents were tested in an attempt to enhance the extractability of the ion-pair, also some binary solvents, since hexane gave the best results it was employed as the extraction solvent in the post-column detection.

*Interface to the liquid chromatograph:* Improved sensitivity can be attained by modification of flow-rates of eluent, BPB and hexane, resulting in changes in both the time of extraction and the volume ratio of the aqueous and organic layers. A perfect phase separation can be attained when the immiscible solvent always overflowed the phase separator. The flow-rates of the post-column reagents given in Fig. 2 proved to be optimum. The debubbling unit in a flow cell assembly was found useful in avoiding influx of aqueous solution into the cell at the beginning of the operation.

#### Chromatographic separation of dialkyl-type cationic surfactants

The dialkyl-type cationic surfactants were separated by reversed-phase chro-

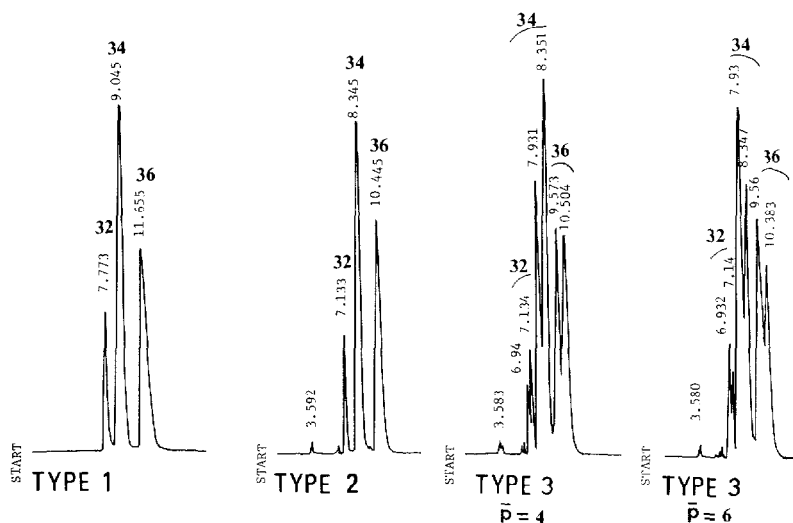


Fig. 3. Typical chromatograms of dialkyl type cationic surfactants obtained by the proposed method. The total alkyl chain lengths  $R_1 + R_2$ , and the retention times (min) are indicated on the peaks.  $\bar{p}$  represents an average number of  $n$  in Fig. 1.

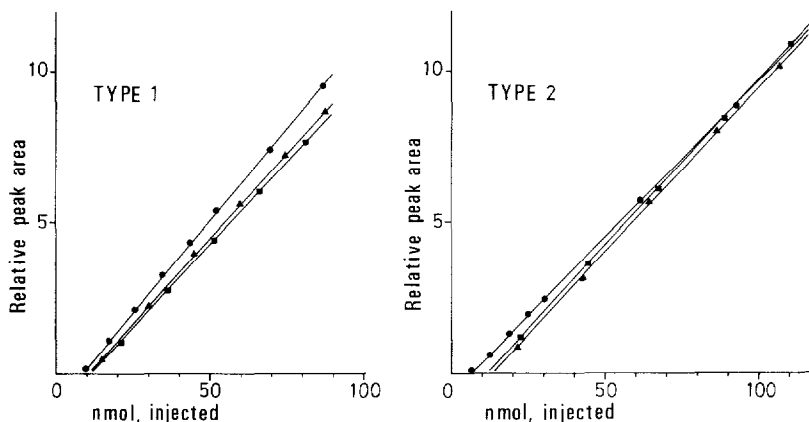


Fig. 4. Calibration curves of cationic surfactants of types 1 and 2. Total alkyl chain lengths,  $R_1 + R_2$ : (●) 32; (■) 34; (▲) 36.

matography in the presence of inorganic salts. Here, ammonium acetate was chosen as the salt in the eluent because it had less influence both on the blank absorbance of the post-column detection and on the extractabilities of the surfactants with BPB than did sodium perchlorate which is commonly used<sup>21-22</sup>.

Of the packing materials tested, only Hypersil C8(3  $\mu\text{m}$ ) separated the surfactant mixture of types 1 and 2 with the eluent methanol-acetonitrile-deionized water (60:60:5) containing 0.1 *M* ammonium acetate. Typical chromatograms are shown in Fig. 3. The results enabled analysis of the type of non-UV-absorbing dialkyl type cationic surfactants. The logarithms of the capacity factors of each homologous series of types 1 and 2 are directly proportional to the total alkyl chain length of the surfactants. Thus the eluted peaks of both homologous series can be identified by this relation. The dialkylmethylpolyoxyethylated ammonium salt (type 3) was eluted in the inverse order of its degree of ethoxylation but the separation was incomplete. However, a rough estimate of the degree of ethoxylation can be obtained from the chromatograms.

#### *Calibration curves and sensitivities*

Fig. 4 shows the calibration curves for surfactants of types 1 and 2. The calibration curve for each alkyl chain was linear but did not pass through the origin and the intercept was not negligible. Further, the response of each alkyl chain of the surfactants was not identical. Therefore calibration curves are needed in order to determine accurate alkyl chain distributions. The detection limit of each compound of types 1 and 2 was approximately 15 nmol.

#### *Application to commercial products analysis*

A commercial detergent powder containing about 3% (w/w) of cationic surfactants was analysed by the proposed method (Fig. 5). The detergent also contained linear alkylbenzenesulphonate (18.3%),  $\alpha$ -olefinsulphonate (18.3%), soap (0.4%) and poly (oxyethylene) nonyl phenyl ether + polyoxyethylene glycol (4.0%). The surfactants of type 1 are determined selectively without interferences from the other com-

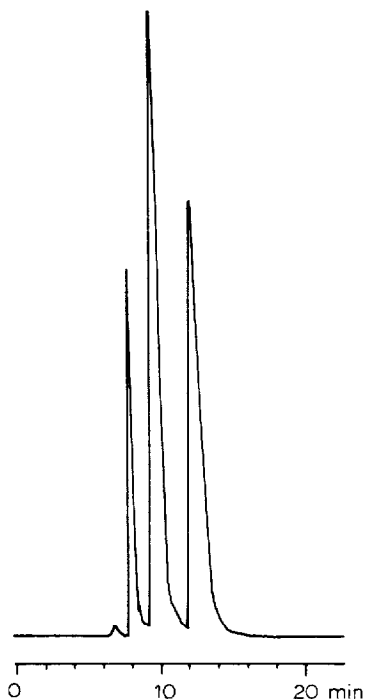


Fig. 5. Representative chromatogram of the commercial product analysis. The cationic surfactants found were of type I ( $R_1 + R_2 = 32, 34, 36$ ).

ponents and information on both the type and alkyl chain distribution is obtained without any pre-treatment of the sample.

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