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ANALYSIS OF AMPHOTERIC SURFACTANTS BY LIQUID CHROMATOGRAPHY WITH POST-COLUMN DETECTION

I. MONO- AND DIALANINE TYPE SURFACTANTS

JIRO KAWASE*, HIDEKO UENO and KAZURO TSUJI
Tokyo Research Laboratories, Kao Soap Co. Ltd., 2-1-3, Bunka, Sumida-ku, Tokyo (Japan)
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SUMMARY

β-Alanine type amphoteric surfactants were analysed by high-performance liquid chromatography with a novel post-column detection. The separation was performed on a reversed-phase column (Develosil ODS-3, 3 μm) with acetonitrile as an organic modifier in the eluent. The chromatographic behaviour of the surfactants (mono- and dialanines) was examined, and the specificity and sensitivity of post-column detection was demonstrated. The post-column detection system was based on a specific reaction with the amine structures of the surfactants. By this method, information on both the alkyl chain distribution and the degree of N-substitution could be obtained. The proposed method enables a direct and convenient determination of β-alanine type amphoteric surfactants in shampoos.

INTRODUCTION

The most important amphoteric surfactants have been generally classified according to types: alanines; glycines; imidazolines and betaine derivatives. These have been extensively applied to toiletry and household products such as shampoos, hair rinses and fabric softeners.

However little has been reported on their analysis. Takano *et al*[1] reported on the analysis of betaine type amphoteric surfactants by reaction gas chromatography. Dmitrieva *et al*[2] determined amphoteric surfactants by potentiometric titration. Sanders *et al*[3] and Keough *et al*[4] reported on the filed desorption mass spectrometry of carboxybetaine amphoteric surfactants. These analytical methods need pure mixtures solely of amphoteric surfactants and/or cumbersome sample preparation for the elimination of interferences. Therefore little information is available on the methods of analysis when the surfactants are present in a complicated matrix.

For reversed-phase high-performance liquid chromatographic (HPLC) analysis of amphoteric surfactants, Parris and co-workers[5,6] and Nakamura and Morikawa[7] used a differential refractive index detector and a UV absorption detector (at 210 nm) respectively. These detection systems were neither specific nor sensitive to the amphoteric surfactants.

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We report a new HPLC method for convenient and direct determination of amphoteric surfactants in commercial products by using novel post-column reaction systems. The post-column detection is based on the specific reaction with amine structures (primary, secondary and tertiary) of the surfactants. The amphoteric surfactants from an analytical column are first converted into the corresponding N-chloramines with hypochlorite ; the N-chloramines are then treated react with iodide to form triiodide which can be monitored at 355 nm.

This report is concerned with structurally confirmed mono- and dialanine type amphoteric surfactants. Their chromatographic behaviour and the specificity and sensitivity of the post-column reaction system were examined.

EXPERIMENTAL

Apparatus

A schematic diagram of the liquid chromatograph is shown in Fig. 1. Sample solutions were injected via a Hitachi sample injector. An Hitachi 635 pump was used to force eluent through the analytical column at a flow-rate of 1.0 ml min^{-1}. Acid-resistant pumps (Nihon Seimitsu NMP-2u) were used for post-column reagents each at a flow-rate of 0.4 ml min^{-1}. Develosil ODS-3 (3 μm ; Nomura Kagaku, Japan) was slurry packed in a stainless-steel column (150 × 4.6 mm I.D.). The post-column reaction coils were made of PTFE tubing (5 m × 0.5 mm I.D.). The effluent was monitored at 355 nm with a variable-wavelength detector (Hitachi 635-T, range 0.32) equipped with a flow cell (inner volume 8 μl, path length 10 mm) in combination with a multirange recorder (Hitachi Model 056) and a data processor (Shimadzu Chromatopac E1A).

Reagents

β-Alanine type amphoteric surfactants, sodium laurylaminoethylcarboxylate (R = $C_{12}H_{25}$), sodium alkylaminoethylcarboxylate (R = C_8H_{17}–$C_{18}H_{37}$), disodium laurylaminodi(ethylcarboxylate) (R = $C_{12}H_{25}$) and disodium alkylaminodi(ethylcarboxylate) (R = C_8H_{17}–$C_{18}H_{37}$), were made in our laboratories by a minor variant

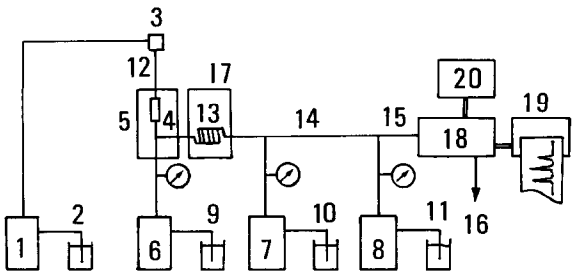

Fig. 1. Schematic diagram of the liquid chromatograph. 1 = pump ; 2 = eluent ; 3 = sample injector ; 4 = analytical column ; 5, 17 = water-bath ; 6–8 = acid-resistant pumps ; 9 = hypochlorite reagent ; 10 = nitrite reagent; 11 = iodide reagent ; 12 = 0.25 mm I.D. × 0.5 m stainless tubing ; 13 = 5 m × 0.5 mm I.D. PTFE reaction coil; (14/15) = 0.5 m × 0.5 mm I.D. PTFE tubing; 16 = 5 m × 0.5 mm I.D. PTFE suppressor tubing ; 18 = UV detector ; 19 = recorder ; 20 = data processor.

of the literature method[8]. In the reactions the molar ratio of ethyl acrylate to amine was 1.2:1 for monoalanines and 2.1:1 for dialanines:

$$\underset{\text{I}}{\text{R–NH}_2} + \text{CH}_2\text{CHCO}_2\text{C}_2\text{H}_5 \xrightarrow[\text{C}_2\text{H}_5\text{OH saponification}]{\text{Heat} \quad \text{NaOH/H}_2\text{O}} \underset{\text{II, monoalanine}}{\text{R–NHC}_2\text{H}_4\text{CO}_2\text{Na}} + \underset{\text{III, dialanine}}{\text{R–N(C}_2\text{H}_4\text{CO}_2\text{Na)}_2}$$

Sample solutions for HPLC were prepared by dissolving weighed samples in deionized water or in methanol. For HPLC analysis, the eluent was 0.2 *M* sodium perchlorate containing 60% acetonitrile (buffered at pH 2.5 by phosphoric acid). The post-column reagents were as follows : hypochlorite reagent, 0.25 *M* phosphate buffer (pH 8.0) containing 1% sodium hypochlorite; nitrite reagent, 0.5% sodium nitrite aqueous solution; iodide reagent, 0.5% potassium iodide aqueous solution.

RESULTS AND DISCUSSION

Post-column detection system

The mechanism of the proposed post-column reaction as follows :

Compounds N-chloramines

$$\underset{\text{I}}{\text{RNH}_2} \xrightarrow{\text{NaOCl}} \text{RNCl}_2 \xrightarrow{\text{KI}} 2\text{I}_3^-$$

$$\underset{\text{II}}{\text{RNHC}_2\text{H}_4\text{CO}_2\text{Na}} \rightarrow \text{RN(Cl)C}_2\text{H}_4\text{CO}_2\text{Na} \rightarrow \text{I}_3^-$$

$$\underset{\text{III}}{\text{RN(C}_2\text{H}_4\text{CO}_2\text{Na)}_2} \rightarrow \overset{+}{\text{RN(Cl)}}(\text{C}_2\text{H}_4\text{CO}_2\text{Na})_2 \rightarrow \text{I}_3^-$$

$$\underset{\text{(excess)}}{\text{NaOCl}} + \text{NaNO}_2 \rightarrow \text{NaCl} + \text{NaNO}_3.$$

The post-column detection system consisted of three reagents as described previously[9]. The active ingredients I–III of the mono- and dialanine amphoteric surfactants were first converted into the corresponding N-chloramines with hypochlorite. Then the excess of hypochlorite was selectively destroyed with nitrite, and the N-chloramines were treated with iodide to form triiodide which can be monitored at 355 nm.

In this system, 1 mole of compound I, II and III could be converted into 2 moles, 1 mole and 1 mole of triiodide, respectively provided that the overall reaction proceeds stoichiometrically. Therefore information on the degree of N-substitution is obtained by optimizing the conditions of post-column reaction.

Effects of organic modifier in the eluent

In this method, detection is influenced by both the type and the content of organic modifier in the eluent. Acetonitrile was more advantageous than methanol as a modifier for the post-column detection. In the case of methanol, detection could not

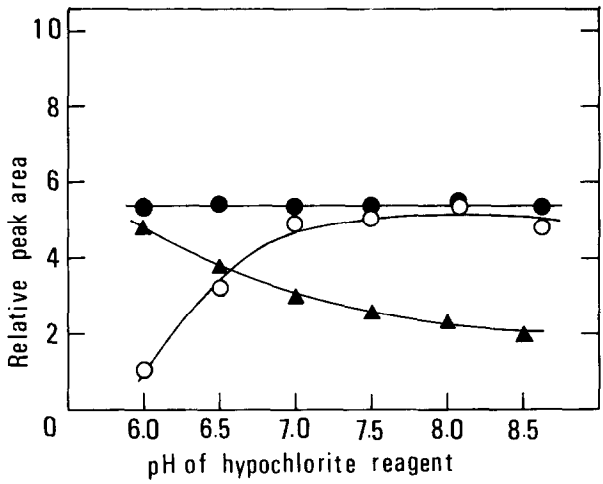

Fig. 2. Effects of pH of hypochlorite reagent on the peak areas: ▲, compound I (R = $C_{12}H_{25}$); ●, II (R = $C_{12}H_{25}$); ○, III (R = $C_{12}H_{25}$). The reaction temperature was fixed at 60°C.

be performed at all when its content was higher than 10% owing to the high blank absorbance caused by oxidation of methanol with hypochlorite. When acetonitrile was used the blank absorbance was low and the detection could be performed with up to 70% of acetonitrile in the eluent. Therefore acetonitrile was selected as the modifier.

Effects of the pH of the hypochlorite reagent

The effects of varying the pH of the hypochlorite reagent in the range pH 6.0–8.5, on the post-column reaction were tested. The eluent used was that given in *Reagents*.

Fig. 2 shows that the sensitivity varied with the pH of the hypochlorite reagent in different ways depending on the type of amine. For primary amines (I), the sensitivity decreased with increasing pH, for monoalanines (II), the sensitivities were fairly constant over the range examined, and the sensitivities of dialanines (III) increased with increasing pH. In practice, primary amines (I) were not found in the commercially available β-alanine amphoteric surfactants. Thus, the optimum pH of the hypochlorite reagent was found to be 8.0.

Effects of reaction temperature

Fig. 3 shows the effects of reaction temperature on sensitivity. With increase of reaction temperature, the sensitivity behaved in a similar manner to that in Fig. 2, except for primary amines(I). Above 70°C, a stable baseline could not be achieved due to the formation of air bubbles. Thus a temperature of 60°C is recommended.

On the basis of these results, it is assumed that the rate of reaction and the stabilities of the N-chloramines differ for each type of amine structure. Primary and secondary amines (I, II) would react with hypochlorite reagent faster than tertiary amines (III), and the N-chloramines derived from primary amines would be more unstable than those of the other amines at higher pH and at higher reaction temperature.

In our preliminary experiments, the post-column detection was applied to all sorts of amines, and it was possible to determine the structures of unknown amines

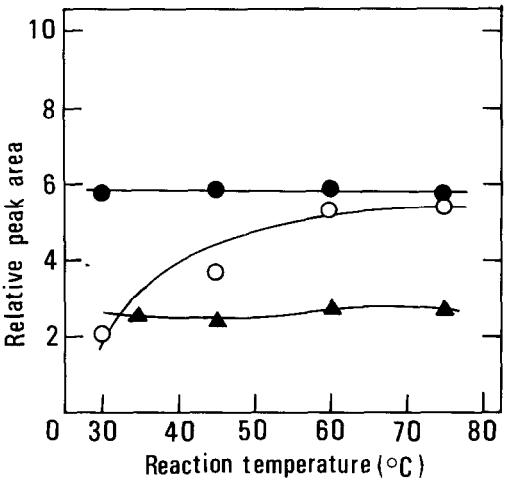

Fig. 3. Effects of reaction temperature on the peak areas. Compounds as in Fig. 2. The pH of the hypochlorite reagent was fixed at 8.0.

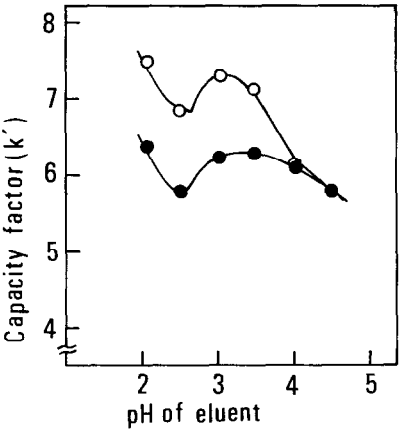

Fig. 4. Effects of eluent pH on k'. Compounds as in Fig. 2. Eluent : 0.2 *M* sodium perchlorate containing 60% acetonitrile (pH adjusted by phosphoric acid).

(such as primary, secondary and tertiary amines) by observing both the pH and temperature profiles of the compounds in the post-column detection.

Chromatographic separation of alanine type amphoteric surfactants

In the proposed method, β-alanine type amphoteric surfactants were separated by reversed-phase HPLC according to both the carbon number of the original fatty amines and the degree of N-substitution.

Nakamura and Morikawa reported the chromatographic behaviour of some amphoteric surfactants using methanol as an origanic modifier in the eluent. In the proposed post-column detection, methanol could not be used as the organic modifier, therefore we studied the chromatographic separation of β-alanine type amphoteric surfactants using acetonitrile as an organic modifer.

HPLC separation of ionic surfactants is usually achieved in the presence of inorganic salts[7,10]. Here, sodium perchlorate was chosen as the inorganic salt in the eluent. High concentrations of inorganic salts in the eluent lead to irregular baselines in the post-column detection. We found that the concentration of inorganic salt could be minimized while retaining the ability to separate ionic surfactants, 0.2 *M* sodium perchlorate was chosen.

Fig. 4 shows the effect of eluent pH on the capacity factor k'. Large changes in k' were not observed, but the peaks of alanines broadened and tailed with increasing pH. Thus, the eluent pH chosen was 2.5. The elution order was dialanine, monoalanine and fatty amine for the same alkyl chain.

The effect of column temperature on the separation was small. With increase of column temperature, the k' values decreased but the elution order of compounds I–III did not change. Therefore a column temperature of 40°C, was employed.

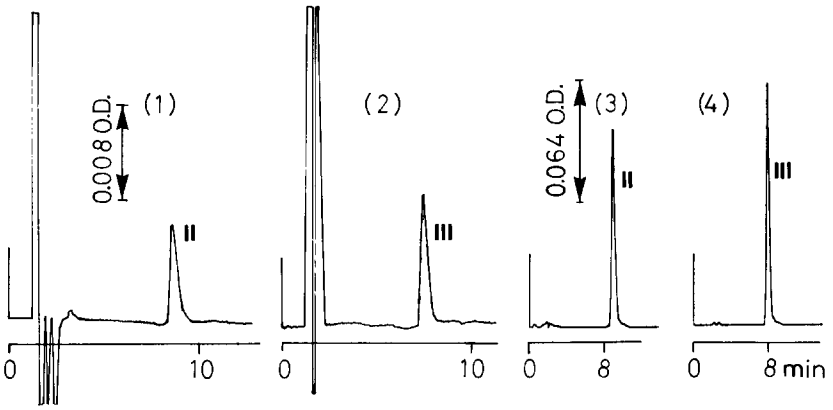

Fig. 5. Comparison of the relative responses to mono- and dialanines with UV and/or post-column detection. Compounds (R = $C_{12}H_{25}$) : 1, II, 40 μg (143.4 nmol), UV at 210 nm, 2, III, 34.92 μg (93.6 nmol), UV at 210 nm ; 3, II, 1 μg (3.58 nmol), post-column detection ; 4, III, 1.74 μg (4.66 nmol), post-column detection.

Sensitivity and selectivity

The sensitivity of the proposed method was examined by use of standard pure materials (mono- and dialanines : R = $C_{12}H_{25}$), see Fig. 5. Considering the stability of the baseline in the post-column detection, the practical range of the detector was fixed as 0.32 absorbance units full scale (a.u.f.s.), corresponding to about 3 μg of each alanine.

The relative molar response to mono-/dialanine (R = $C_{12}H_{25}$) was 105.1/100 in the post-column detection. Therefore the molar responses to mono- and dialanines were almost identical. This result shows that the degree of N-substitution can be calculated in the proposed method.

The detection limits of the method were about 80 times higher for a C_{12} monoalanine and about 30 times higher for a C_{12} dialanine compared with those achieved by UV detection (at 210 nm, 0.08 a.u.f.s.). Furthermore, the post-column detection has a higher selectivity compared to UV detection.

Determination of alkyl chain distribution and degree of N-substitution

Representative chromatograms obtained with the proposed method are shown in Figs. 6 and 7. The compounds were assigned by use of standard samples. From the results, both the alkyl chain distribution and the degree of N-substitution of the β-alanines could be obtained, provided that almost identical molar responses were yielded regardless of the length of the alkyl chain.

The results for the alkyl chain distribution obtained in the proposed method are compared with those from gas chromatographic analyses of the original fatty amines in Table I. The agreement between the two sets of results is good and the average molecular weight of the original fatty amines can be calculated conveniently in the proposed method.

In practice, the original fatty amines were not found in commercially available β-alanine amphoteric surfactants. Thus, the degree of N-substitution can be calculated from the peak area ratio of mono-/dialanine in the chromatogram, using the relative molar response(105.1/100) of the standard pure materials, as indicated previously.

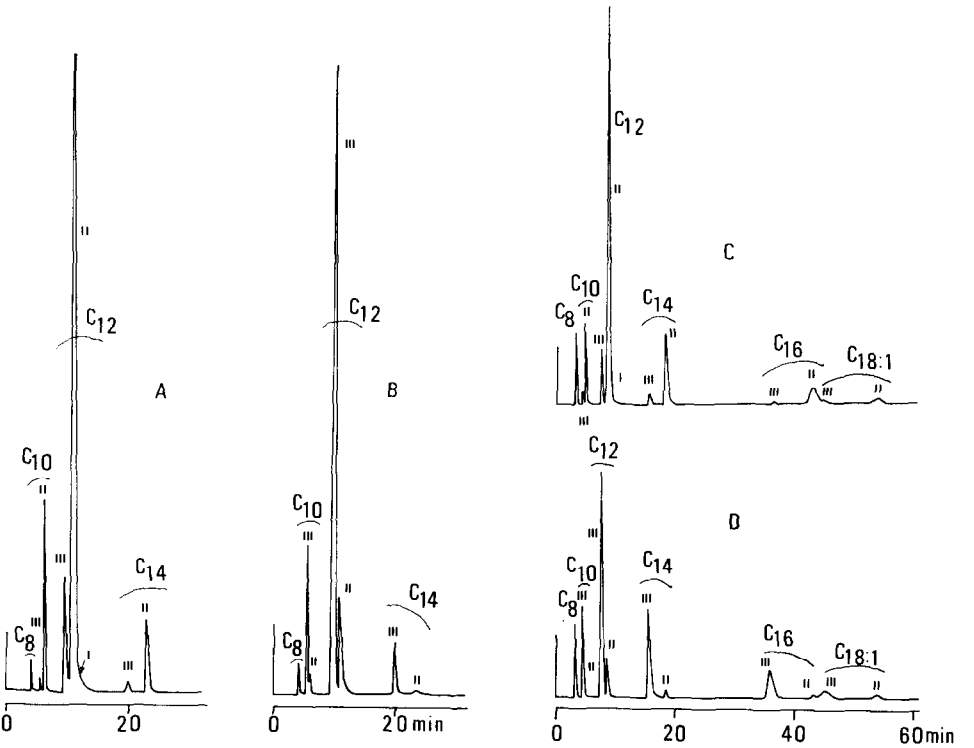

Fig. 6. HPLC of sodium laurylaminoethylcarboxylate (A) and disodium laurylaminodi(ethylcarobxylate) (B). The alkyl-chain distribution and the assignments of the compounds are indicated on the peaks. The surfactants were commercially available ones.

Fig. 7. HPLC of sodium alkylaminoethylcarboxylate(C) and disodium alkylaminodi(ethylcarboxylate)(D). Notation as in Fig. 6. The surfactants were commercially available ones.

TABLE I

COMPARISON OF THE PROPOSED HPLC METHOD WITH GAS CHROMATOGRAPHIC (GC) ANALYSES OF THE ALKYL CHAIN DISTRIBUTION

Sample : Surfactant C in Fig. 7.

Alkyl chain of original fatty amine	*HPLC method (mol %)**	*GC method*	
		*(wt. %)***	*(mol %)****
C_8	7.00	4.54	6.39
C_{10}	8.85	7.34	8.66
C_{12}	59.85	60.21	61.06
C_{14}	16.40	18.24	16.22
C_{16}	7.90	9.68	7.67

* Calculated by summing the peak areas (%) of compounds II and III, assuming that identical molar responses were yielded regardless of the length of the alkyl chain.

** GC–flame ionization detection response of acetylated original fatty amine.

*** Calculated from wt. %.

TABLE II

DETERMINATION OF MONO- AND DIALANINES IN A STANDARD SHAMPOO

1 = Sodium alkylaminoethylcarboxylate(R = C_8H_{17}–$C_{18}H_{37}$) ;
2 = disodium alkylaminodi(carboxylate) (R = C_8H_{17}–$C_{18}H_{37}$) ;
3 = sodium laurylaminoethylcarboxylate.

Compound	*Alanine type amphoterics*				
	Added(%)	*Found (%)*	*n*	*C.V. (%)*	*Recovery(%)*
1	5.00	4.84*	4	2.4	96.8
2	5.00	4.87*	4	2.1	97.4
3	2.50	2.49	4	1.3	99.6

* Calculated by summing the peak areas of C_8–C_{18} alkyl chains.

However, the separation between the mono- and dialanines of C_8 and C_{10} was not achieved. Therefore, the degree of N-substitution was calculated from the peak area ratio of C_{12} alanines, assuming that almost identical responses were yielded regardless of the alkyl chain length.

Thus the alkyl chain distribution and the reaction molar ratio of ethyl acrylate to fatty amines can be estimated from the chromatogram. The information obtained from the post-column detection were not affected by interfering substances even when the surfactants were formulated in shampoos.

Commercial products analysis

In the proposed method, the calibration curve of peak areas *vs.* concentrations was completely linear for each alkyl chain of the alanines (C_8–C_{18}) and passed through the origin. For example, the curves for both mono- and dialanines of C_{12} were linear in the range of 0.3–12 μg. Thus, the content of alanines in commercial products can be determined by summing the peak areas due to each alkyl chain. The precision and accuracy of the method were tested by adding known amounts of mono- and dialanines to a standard shampoo. Table II demonstrates that the proposed method can be applied to commercial products analysis.

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