Rapid Analysis of Ionic and Nonionic Surfactant Homologs by High Performance Liquid Chromatography

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ABSTRACT

Several ionic and nonionic surfactants were separated into their homologs and their homologous distributions were determined by high performance liquid chromatography on an octadecyl-silica (TSK Gel LS-410) column with a water-methanol-sodium chloride mixture as a mobile phase. The recommended condition for the separation was as follows: column size, 4 mm id x 250 mm; mobile phase, water/methanol (15/85, v/v) containing 0.4 M/L sodium chloride; column temp., 50 C. Under this condition, three nonionic, four anionic and one cationic surfactants were efficiently separated into their respective homologs. The addition of sodium chloride affected particularly the separation of strongly ionogenic surfactants such as sodium alkylsulfates and alkylbenzyldimethylammonium chlorides. Analytical results of homologous distributions of several commercial surfactants were in good accordance with those obtained by the conventional gas chromatography (GC) method.

INTRODUCTION

Surfactants are widely used in various industries as well as in household products and have important function in the cosmetic field as emulsifiers, solubilizers or dispersing agents according to their intended use. Commercial surfactants are usually mixtures of homologs, and their effectiveness and physical properties depend markedly on alkyl chain length in lipophilic groups. Their homologous distributions have been determined by gas chromatography (GC); however, this method requires their conversion into volatile derivative before analysis and hence is time consuming.

In recent years, several papers have been published on the analysis of homologous distributions by means of high performance liquid chromatography (HPLC) with porous microspherical poly (styrene-divinylbenzene) gels (1), and reverse phase packings containing octadecyl silane group chemically bonded to silica gel (ODS/Silica) (2,3). However, none have presented a convenient method applicable to nonionic, anionic and cationic surfactants with one mobile phase condition.

As reported in a previous paper (4), we separated the respective homologs of nonionic surfactants such as fatty acid alkylol amides and anionic surfactants such as sodium N-acyl-sarcosinates (SNAS), sodium N-acyl-N-methyl taurates (SMAT) and sodium N-acyl-L-glutamates (SNAG) by HPLC employing ODS/Silica as a column packing and a mixture of water-methanol containing phosphoric acid as a mobile phase (acid addition method). However, even though the mobile phase was adjusted to pH2.2, which was proximate to the acid resistance limit of ODS/Silica, strongly ionogenic sodium alkylsulfates (SAS) eluted in the dead volume of the column because of insufficient ion suppression and thus could not be separated into their homologs. Cationic surfactants such as alkylbenzyldimethylammonium chlorides (BzAC), on the other hand, did not elute from the column.

In this study, instead of phosphoric acid, sodium chloride was employed as an additive to develop a convenient analytical method applicable to surfactants in various ionogenic states (salt addition method). As a result, not only were various surfactants separated by the acid addition method, but SAS and BzAC also were separated into their homologs with one mobile phase condition. The homologous distributions obtained by the present method were in good accordance with those obtained by the conventional GC method.

EXPERIMENTAL PROCEDURES

Materials

Sodium salts of lauryl, myristyl, palmityl and stearyl sulfuric acids were all special grade reagents (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) and used without further purification. Lauryl, myristyl, palmityl and stearyl BzAC were obtained from Toho Chemical Ind. Co. (Tokyo, Japan). The purity of the alkyl chain length was determined to be >95% by GC. SNAS, SNAG, SMAT, fatty acid monoethanolamides (FME), fatty acid diethanolamides (FDE) and fatty acid monoisopropanolamides (FMP) with C_{12}, C_{14}, C_{16} and C_{18} alkyl chain length were

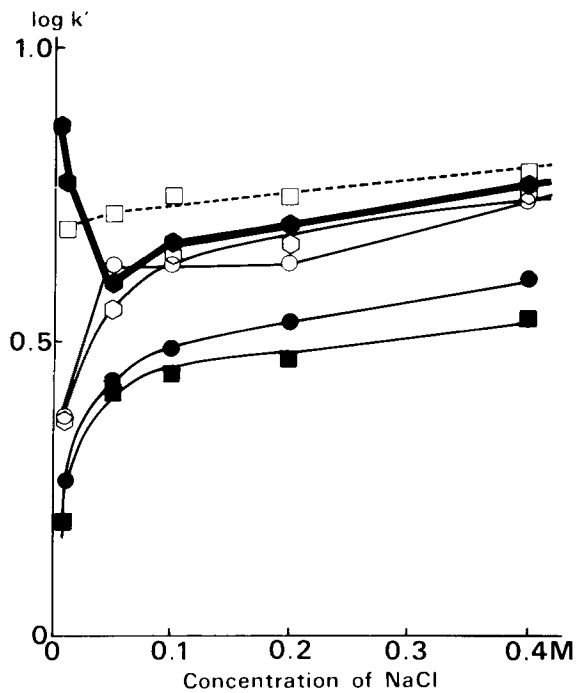

FIG. 1. Effect of concentration of NaCl on k' (□ Palmitoyl monoisopropanolamide, • Palmitylbenzyldimethylammonium chloride, ○ Sodium N-palmitoylsarcosinate, ○ Sodium palmitylsulfate, • Sodium N-palmitoyl-N-methyl taurate, and ■ Sodium N-palmitoyl-L-glutamate).

the same grade as used in the previous study. Other reagents used were all special grade.

HPLC Apparatus and Operating Condition

A Model ALC/GPC 201 liquid chromatograph (Waters Assoc., Milford, MA, USA) equipped with a Model SPD-1 variable-wavelength detector (Shimadzu Corp., Kyoto, Japan) and a Waters Model R401 differential refractometer were used in this study. The SPD-1 detector was operated at 210 nm. The R401 detector was used for the detection of SAS.

The chromatographic column (4 mm id x 250 mm) used was constructed of stainless steel, and warm water was run through the jacket to maintain the column temperature at 50 C. The column was packed with TSK-Gel LS-410 (5μ, spherically shaped ODS/Silica: Toyo Soda Manufacturing Co., Ltd. Tokyo, Japan) using tetrahydrofuran as a slurry-forming solvent and water/methanol (25/75, v/v) as a pressurizing solvent. (Packing pressure: 400-450 kg/cm^2)

All the samples were prepared as 0.1 to 1.0% methanol solutions, and 10-20 μl were injected into the HPLC column by means of a Waters Model U6K septumless loop injector. The mobile phase employed was a mixture of water-methanol containing sodium chloride (NaCl) and all the experiments were carried out under isocratic condition. Flow rate of the mobile phase was set at 1.5 ml/min. Capacity factor (k′) values were evaluated from the chromatograms by the usual method (5).

GC Apparatus and Operating Condition

A Shimadzu Model GC 6A-PTF gas chromatograph equipped with a flame ionization detector was also used to determine the homologous distributions of several commercial surfactants. The samples were converted into the corresponding volatile derivatives and analyzed as follows: SAS (ca. 1 g) was hydrolyzed with 6N hydrochloric acid aq. soln. (50 ml) for 6 hr at 100 C, and the extracted alcohols (as their trimethylsilyl ethers) were analyzed using a 3 mm id x 0.5 m glass column packed with Diasolid ZT (80-100 mesh). The column oven temperature was programmed from 100 to 300 C at a rate of 10 C/min. BzAC was analyzed by the pyrolysis GC method (6) using a 3 mm id x 2 m glass column packed with Shimalite W (AW, DMCS, 60-80 mesh) coated with 5% SE-30. BzAC was prepared as 10% ethanol solution, and 1 μl was injected into the gas chromatograph. The column oven temperature was programmed from 100 to 300 C at a rate of 10 C/min. Other surfactants were hydrolyzed with 6N hydrochloric acid aq. soln. for 6 hr at 140 C, and the extracted fatty acids (as

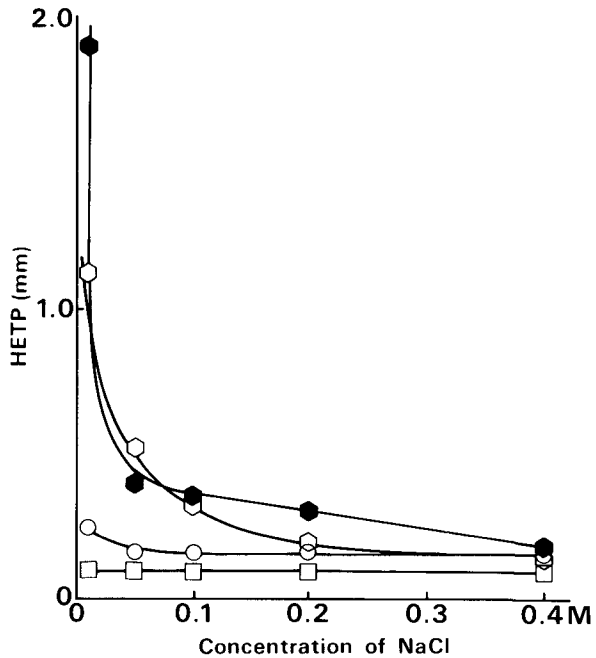

FIG. 3. Effect of concentration of NaCl on HETP. (Symbols are the same as in Fig. 1.)

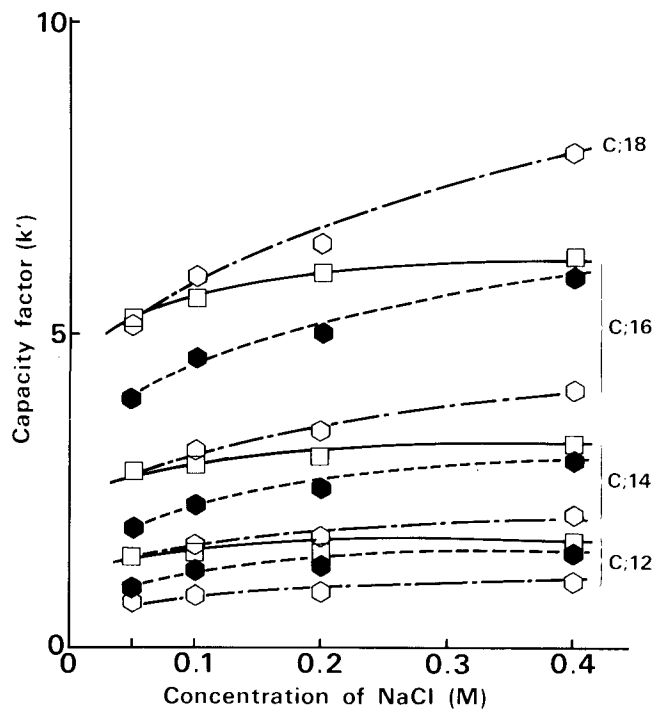

FIG. 2. Effect of concentration of NaCl on separation of homologs. (• BzAC, ○ SAS and □ FMP. C-12 means alkyl chain length in lipophilic group is 12.)

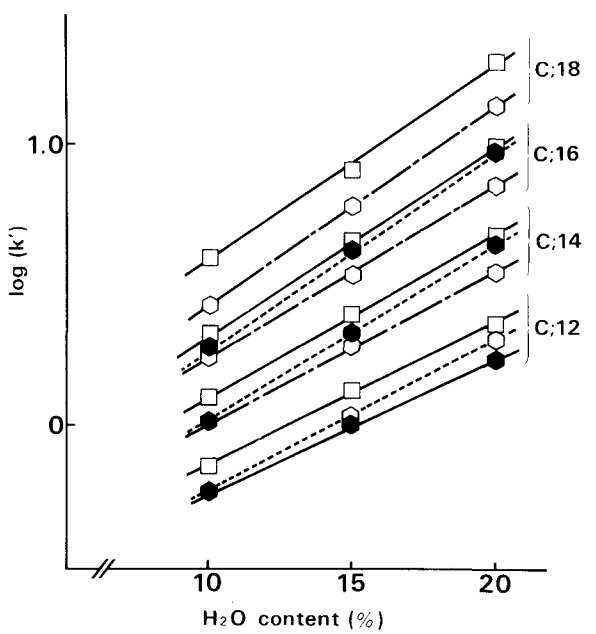

FIG. 4. Effect of water content in mobile phase on separation of homologs. (NaCl concentration 0.4 M/L, and symbols are the same as in Fig. 2.)

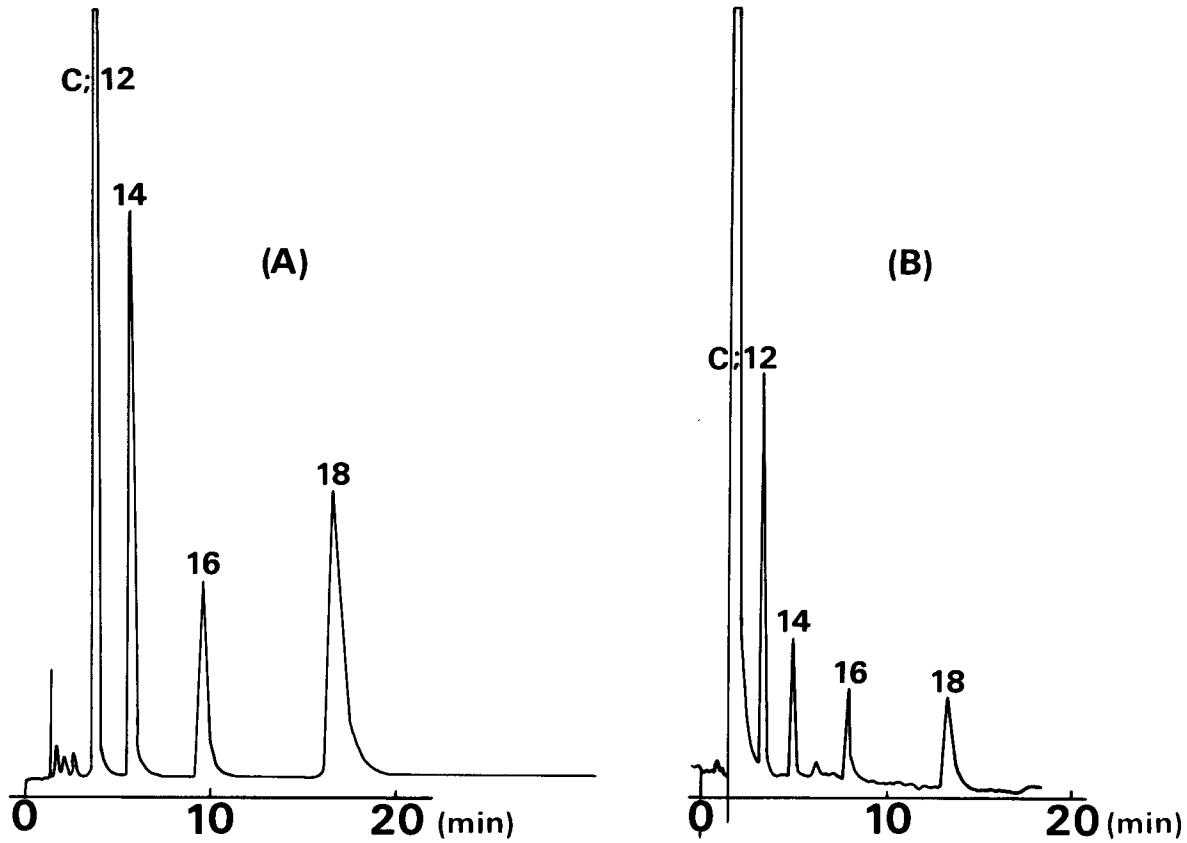

FIG. 5. Separation of BzAC (A) and SAS (B) homologs. (Mobile phase; water/methanol (15/85, v/v) containing 0.4 M/L NaCl; detector; (A): UV [210 nm], (B): RI.

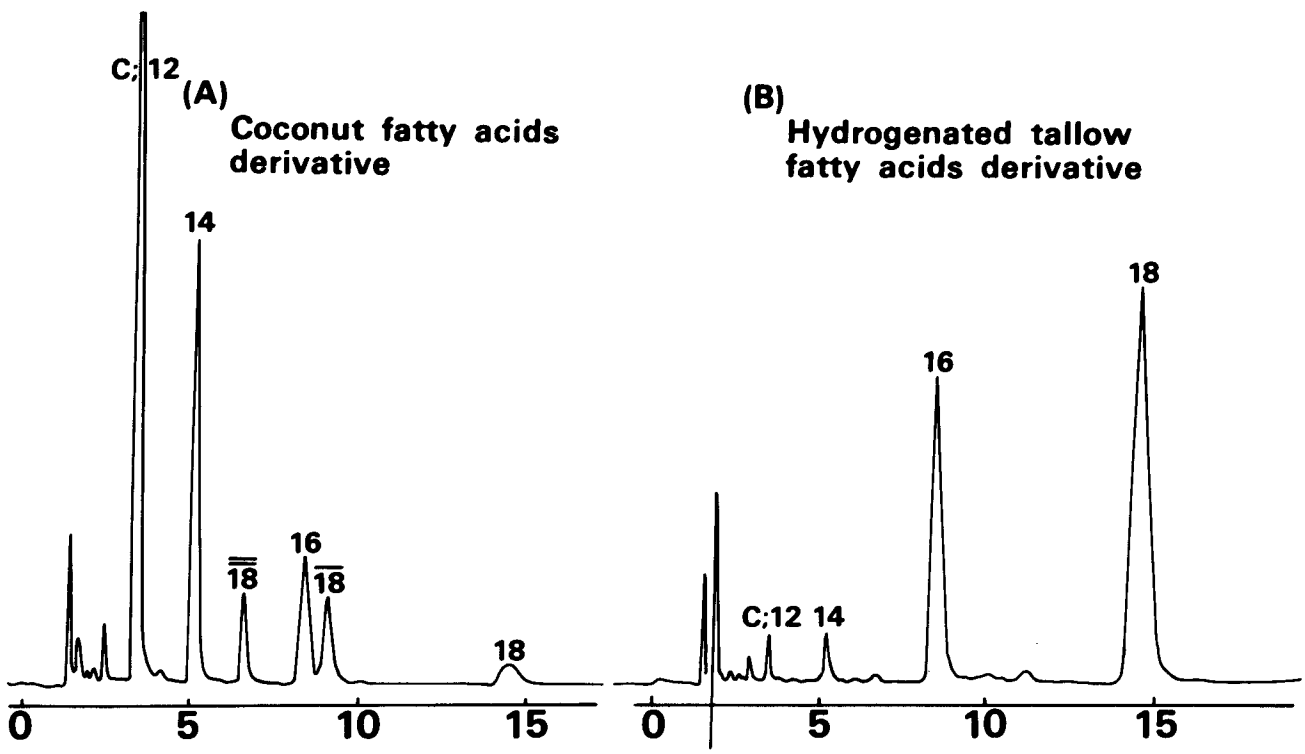

FIG. 6. Separation of commercial FME, from coconut fatty acids (A) and hydrogenated tallow fatty acids (B). (Mobile phase condition is the same as in Fig. 5, detector UV [210 nm].)

their methyl esters) were analyzed using a 3 mm id x 3 m glass column packed with Diasolid ZF (80-100 mesh). The column oven temperature was set at 190 C. All GC packings were obtained from Nihon Chromato. Co. (Tokyo, Japan). The homologous distributions were determined by calculating the peak area percentage.

RESULTS AND DISCUSSION

Optimization of HPLC Condition

Horvath et al. (7-9) reported that addition of inorganic salt to the mobile phase was effective in separating ionogenic substances by using ODS/Silica. Good separation was obtained for aliphatic organic acids (10) by adding inorganic salt. In this study, the results indicate that the different inorganic salts and their concentrations as well as the water contents in the mobile phase affect the separation of homologs. These factors necessitate that conditions be optimized.

First, NaCl, which is the most common inorganic salt, was examined. The effect of NaCl concentrations on the k′ values and the separation of homologs are shown in Figure 1 and Figure 2, respectively. The volume ratio of water to methanol in the mobile phase was 15/85. As in the case of FMP (Fig. 1), the k′ values of nonionic surfactants increased gradually by increasing the concentration of NaCl, and thus the separation of homologs was slightly improved as shown in Figure 2. The results indicate this improvement may be caused by a salting out effect from the addition of NaCl. The behaviors of anionic surfactants such as SNAS, SNAG, SMAT and SAS, on the other hand, were strongly affected by the addition of NaCl. Their k′ values increased by increasing the concentration of NaCl, and the separation of homologs was markedly improved. These phenomena are probably due to ion suppression effect by the addition of NaCl, which is strongly ionized in the mobile phase. BzAC behaved in an interesting manner on the addition of NaCl. When no NaCl was added, BzAC was strongly adsorbed by the column packings and did not elute from the column. With the addition of NaCl, BzAC began to elute and the k′ values decreased markedly by increasing the concentration of NaCl. When the concentration of NaCl exceeded 0.05 M/L, the k′ values began to increase by increasing the concentration of NaCl. As will be described later, this phenomenon may be attributed to the change in the charged condition of the packing surface and BzAC. The separation of homologs of BzAC was also improved by increasing the concentration of NaCl, as shown in Figure 2.

As in the case of homologous separation, HETP of each peak was greatly affected by the addition of NaCl. An example of the relationship between the concentration of NaCl and HETP values is shown in Figure 3. The HETP values decreased and thus the sharpness of each peak increased by increasing the concentration of NaCl. This effect was particularly marked with the strongly ionogenic SAS and BzAC, which were not separated by the acid addition method.

Other inorganic salts such as ammonium chloride and potassium chloride were also examined, but their effects were not as acceptable as that of NaCl. Thus, NaCl was chosen in this study. The concentration of NaCl was set at 0.4 M/L for the optimal separation of homologs and HETP values.

Since our previous study revealed that pH of the mobile phase strongly affected both the k′ values and the separation of homologs, the effect of the mobile phase pH on the k′ values was examined by adding phosphoric acid to the mobile phase (0.4 M/L NaCl in water/methanol [15/85]).

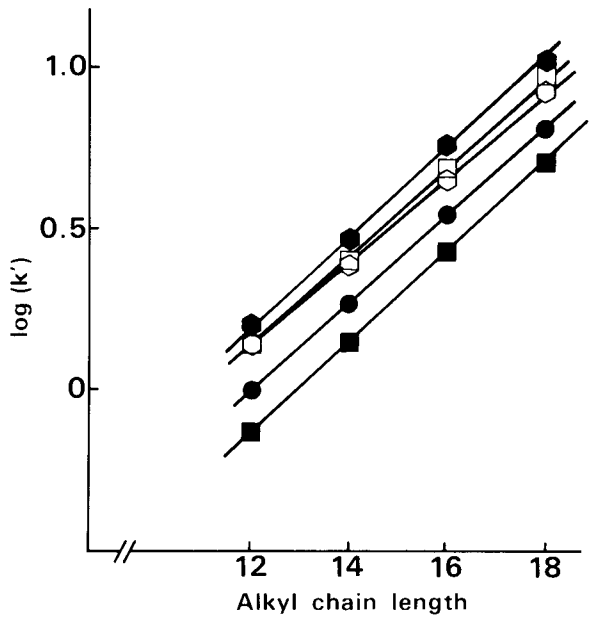

FIG. 7. Relationship between log (k′) and alkyl chain length in lipophilic group. (Symbols and mobile phase condition is the same as in Fig. 1 and Fig. 5.)

The k′ values of each sample, however, were fairly constant throughout the pH range 2.7-6.0. The separation of homologs was also found not to be affected by the mobile phase pH. These results may be attributed to the strong ion suppression effects upon the addition of NaCl.

Next, the relationship between the water content and the k′ values was examined. The results of FMP, SAS and BzAC are shown in Figure 4. As the water content in the mobile phase was increased, the k′ values increased, which resulted in improved separation of homologs. Similar results were obtained for other surfactants. A water/methanol mixture of 15/85 was chosen as the mobile phase in due consideration of the fact that it resulted in good separation of

TABLE I

Reproducibility of Homologous Distribution

Sample[a]	Alkyl chain length	Relative peak area[b]%	Cv[c]%
FME	12	35.0	0.79
	14	13.2	0.46
	16	19.1	1.12
	18	32.7	0.60
SNAG	12	38.1	3.10
	14	9.6	1.78
	16	17.6	2.16
	18	34.7	1.86
SAS	12	39.5	2.52
	14	15.2	0.60
	16	15.0	1.88
	18	30.1	2.42
BzAC	12	34.9	0.23
	14	19.3	0.23
	16	11.7	0.51
	18	34.1	0.29

[a]Samples are mixture of standard homologs.
[b]Taken as the average of 5 replicate analyses.
[c]Coefficient variation

TABLE II

Analytical Results of Commercial Surfactants by HPLC and GC Method

Sample	Method	Homologous distribution C_{12}[a]%	C_{14}%	C_{16}%	C_{18}%
Sodium laurylsulfate	HPLC[b]	62.1	26.6	11.3	
	GC[b]	60.7	27.7	11.8	
Stearoyl monoethanolamide	HPLC	1.2	2.6	27.9	68.3
	GC	0.7	2.2	27.4	69.1
Lauroyl monoethanolamide	HPLC	60.5	24.4	11.9	3.2
	GC	60.4	23.9	12.1	3.7
Sodium N-stearoyl-L-glutamate	HPLC	3.4	3.0	30.4	63.4
	GC	2.8	3.4	30.4	63.4
Laurylbenzyldimethylammonium chloride	HPLC	59.3	34.8	5.9	
	GC	60.1	33.1	6.9	

[a]Alkyl chain length in lipophilic group.
[b]Taken as the average of 2 replicate analyses.

homologs and shortened analysis time.

Figures 5 and 6 are typical chromatograms obtained under the final mobile phase condition. BzAC (Fig. 5A) and SAS (Fig. 5B) were readily separated into their homologs. As shown in Figure 6, the nonionic surfactants were also readily analyzed under the same mobile phase condition; commercial FME from coconut fatty acids and hydrogenated tallow fatty acids were successfully differentiated. Moreover, other surfactants used in this study were efficiently separated into their respective homologs.

Figure 7 shows the relationship between the alkyl chain length in the lipophilic groups and the logarithmic k′ values under the final mobile phase condition. A linear relationship was found as those generally obtained on reverse phase chromatography using ODS/Silica (11,12).

Determination of Homologous Distribution

The reproducibility and quantitative values obtained by the present method was examined. Standard mixtures of FME, BzAC, SANG and SAS, each having homologs of C_{12}, C_{14}, C_{16} and C_{18}, were prepared, and the relative peak area% for each homolog was measured. The results are summarized in Table I. A good reproducibility was obtained, and Cv(%) was equal to or lower than 3.1%.

The homologous distributions obtained by the present salt addition method were compared with those obtained by the conventional GC method. Several commercial surfactants were analyzed by GC as described in the experimental procedures. The same samples were prepared as 0.1 to 1.0% methanol solutions and analyzed by the present method: 10-20 μl of each solution was injected into the HPLC column, and the relative peak area% was calculated from the chromatograms obtained. Since each relative peak area% corresponds to the mole% of each component (1), the weight% was recalculated by using the corresponding molecular weights. As shown in Table II, the results obtained by both methods are in good agreement. Thus, the present method was proven to be a reliable and efficient method in determining the homologous distributions of surfactants in various ionogenic states with one mobile phase condition. Moreover, this method does not require any pretreatment such as conversion of the samples into their volatile derivatives, that is, the samples are directly dissolved in methanol and the resulting solution is simply injected into the HPLC column.

Effect of Unreacted Hydroxy Group on Packing Surface

As indicated in Figure 1, BzAC behaved in a distinctive manner upon the addition of NaCl. In the absence of added NaCl, they were strongly adsorbed on the column and not eluted. As this phenomena seemed to be caused by the effect of the unreacted hydroxy groups on the packing surface, "forward-phase chromatography" proposed by Scott et al. (13) was examined. The mobile phase was changed, in accordance with the solvent order reported by Scott et al., to nonpolar n-hexane, and then a mixture of benzene, benzylchloride and nitrobenzene was injected into the HPLC column. The chromatogram is shown in Figure 8. The nonpolar benzene eluted in the dead volume of the column, and polar benzylchloride and nitrobenzene were strongly retained on ODS/Silica. Since it has already been shown that the interaction between the stationary phase and the solutes under the nonpolar atmosphere is due to hydrogen bonding (14), it was concluded that the functional groups capable of forming hydrogen bonding, i.e., unreacted hydroxy groups, are present on the surface of the packings used. In polar atmosphere such as the mobile phase used in this study, these unreacted hydroxy

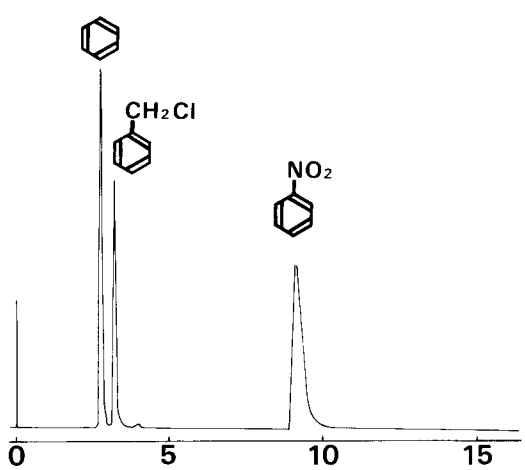

FIG. 8. Separation of benzene, benzylchloride and nitrobenzene by "forward-phase chromatography." (Column packing LS-410 [5μ], mobile phase n-hexane, flow rate 1.5 ml/min, Detector UV [254 nm].)

groups negatively charge the packing surface. Consequently, BzAC cations were electrostatically bonded on the packing surface and not eluted.

It was observed that the k' values of BzAC decreased greatly by increasing the concentration of NaCl, but increased when the concentration exceeded 0.05 M/L. This phenomenon may be caused by a combination of ion suppression and salting out effects on the addition of NaCl.

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Surface Active Properties of a Biosurfactant from *Corynebacterium lepus*

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ABSTRACT

A mixture of corynomycolic acids (R^1-CH(OH)-CH(R^2)-COOH) isolated from *Corynebacterium lepus* was shown to have excellent surfactant properties. It caused significant lowering of surface tension in aqueous solution and the interfacial tension between water and hexadecane at all values of pH between 2 and 10. A series of carboxylic acids and some hydroxy-carboxylic acids and alcohols were also studied as a comparison. None of these caused as large a lowering of the surface and interfacial tensions as the corynomycolic acids. The series of carboxylic acids studied showed that surfactant properties depend on the length of the alkyl chain and the pH of the solution in a manner consistent with the hydrophilic-lipophilic balance of these compounds. Hydroxyl substituents caused considerable enhancement of the surfactant properties of long chain carboxylic acids if they were located close to the carboxyl function.

INTRODUCTION

Corynebacterium lepus grown on kerosene produces a mixture of surface active agents (1). One of the major components in this mixture is a class of β-hydroxy-α-branched carboxylic acids (2) called corynomycolic acids (3,4). These acids, present in the *C. lepus* whole broth, have been shown to be effective agents to enhance bitumen recovery by a cold water extraction process (5,6). For this reason a study was undertaken of the surfactant properties of these acids as determined by surface and interfacial tension.

Spreading pressures of many carboxylic acids have been reported (7-13) as well as some determinations of the effect of pH changes (7,9,12,13). The spreading pressures of several hydroxy-carboxylic acids have also been measured (7,14-16).

This work considered the surfactant properties of the mixture of corynomycolic acids isolated from *C. lepus*. These were evaluated by measuring the surface tension and interfacial tension against hexadecane of saturated aqueous solutions over a wide range of pH. In an attempt to correlate the importance of structural features of the corynomycolic acids to their observed surface properties, similar studies were made of saturated carboxylic acids, hydroxy-carboxylic acids and alcohols.

MATERIALS AND METHODS

Materials

Most of the chemicals, including all of the saturated unsubstituted fatty acids were purchased from Sigma Chemical Co. (St. Louis, MO). The 16-hydroxy-hexadecanoic acid and hexadecanol were purchased from Aldrich Chemical Co. (Milwaukee, WI). Triacontanol was purchased from Polyscience Corp. (Evanston, IL). The 2-hydroxy-stearic acid was purchased from Pfaltz and Bauer Inc. (Flushing, NY).

The hexadecane was first cleaned by washing alternately with sodium hydroxide (4 N) and concentrated sulfuric acid and then distilled water until an interfacial tension with water of 45 dyne/cm was obtained.

Production, Isolation and Characterization of Corynomycolic Acids

The production, isolation and characterization of the corynomycolic acids (R^1-CH(OH)-CH(R^2)-COOH) from *C. lepus* are reported in full in other publications (1,2) and are only briefly summarized here. The organism was grown on

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