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SEPARATION AND DETERMINATION OF ALKYL SULFATE AND SOAP BY SALTING-OUT CHROMATOGRAPHY

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SUMMARY

The separation and determination of alkylsulfate and soap in their mixtures were investigated by salting-out chromatography. The analytical conditions were as follows: column, 25 mm I.D. and 325 mm long; resin, Amberlite CG-50 (200-400 mesh); eluent, aqueous 30% isopropanol-0.5 M sodium chloride solution; column temperature, 40.0°.

The separation and determination of C₁₂-alkylsulfate and C₁₂-soap were satisfactory. The gradient elution method was used to analyze commercially available soap and alkylsulfate in their mixtures.

INTRODUCTION

Since the technique of salting-out chromatography was proposed by SARGENT AND RIEMAN¹, it has been applied to the separation of organic non-electrolytes^{2,3} and surface-active agents⁴⁻⁷.

The present paper, one of a series⁴⁻⁷ of studies on the application of this technique to the analysis of anionic surface-active agent mixtures, describes the separation and determination of alkylsulfate (AS) and soap, and also discusses some aspects of the separation mechanism of salting-out chromatography.

EXPERIMENTAL

Apparatus

The distribution coefficient was measured by a batch method using a 50-ml conical flask with a stopper. The incubator (Model M-1N, Taiyo Scientific Industry Co. Ltd., Japan) was used to obtain the adsorption equilibrium rapidly. The column used was 25 mm I.D. and 450 mm long (Sephadex column, Model K25/45 jacketed, Pharmacia Fine Chemicals, Sweden). A constant temperature circulating unit (Model NS/S15/22, Messgeraete-Werk, Lauda, G.F.R.) was used to control the column temperature. The effluent was collected by a fraction collector (Model SF-160K, Toyo Scientific Industry Co. Ltd., Japan). Quantitative analysis was carried out using a

Reagents and samples

Eight kinds of standard AS and soaps, the carbon number distribution of which was 12, 14, 16 and 18, were synthesized in our laboratory and purified by recrystallization from methanol or an aqueous methanolic solution. Instead of commercially available soap, soap having a range of alkyl groups (C_{10} - C_{18} -soap) was synthesized from fatty acid prepared by mixing tallow acid and coconut acid.

Amberlite CG-50 (100-200 and 200-400 mesh), a weakly acidic cation-exchange resin, was used as a column support.

All other reagents were of analytical-reagent grade.

Procedure

The ion-exchange resin was washed with methanol using a Soxhlet extractor and treated with aqueous 3 *N* sodium hydroxide and 3 *N* hydrochloric acid solutions. The resin was then treated with 30 volumes of 3 *N* sodium chloride followed by washing with 5 volumes of water and drying at 105° for 5 h. The measurement of distribution coefficients and the chromatographic procedure were similar to those described earlier⁴ except for the composition of the eluent. Quantitative analyses of these surfactants were carried out by the two-phase titration method with bromocresol green according to MILWIDSKY AND HOLTZMAN's method⁸. The solubility of AS and soap was represented as the clear point, which is the temperature at which the clear sample solution becomes a white turbid solution. This temperature was measured by the following procedure. A solution of the surfactant was prepared in a 10-ml volumetric flask. The flask was immersed in water, the temperature of which was kept constant by the thermostat, and was shaken occasionally. The water temperature was decreased by 0.5° per hour.

The gradient elution of C_{10} - C_{18} -soap was carried out by the following procedure. A 40-ml volume of aqueous 30% isopropanol-0.5 *M* sodium chloride solution was placed in a 50-ml reservoir, which was connected to the column with polyethylene tubing. Simultaneously with the start of elution, 30% aqueous isopropanol solution was fed into this reservoir from another one. Consequently, the concentration of salt in the eluant decreased gradually as the elution progressed.

The chromatographic conditions determined finally were as follows: resin, Amberlite CG-50 (200-400 mesh), H-form (partially Na-form); column, Sephadex column (Model K25/45 jacketed); eluent, aqueous 30% isopropanol-0.5 *M* sodium chloride solution; column temperature, 40.0°; flow-rate 0.3 ml/min; determination, two-phase titration method with bromocresol green as indicator.

RESULTS

Organic solvent

A sample molecule must be dissolved completely in the eluent in order to carry out salting-out chromatography. As soap is almost insoluble in a salt solution, the solubility must be increased by adding an organic solvent. Therefore, the solubility of soap in various types of mixed organic solvent-water systems was investigated.

The solubility of C_{12} -soap in pure organic solvents, without water, was first studied. Methanol could dissolve more than 5% of C_{12} -soap at room temperature.

N,N'-dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and acetonitrile were all less than 0.05 %. However, in mixed organic solvent–water solvent systems, the solubility of soap increased greatly in comparison with the value in pure organic solvent.

Fig. 1 shows the solubility of C_{12} -soap, represented as the clear point, in isopropanol–water. The isopropanol–water system gave the largest dissolving power of all the organic solvent–water systems tested. In the case of homologous series of alcohols, the dissolving power decreases in the order isopropanol > ethanol > methanol.

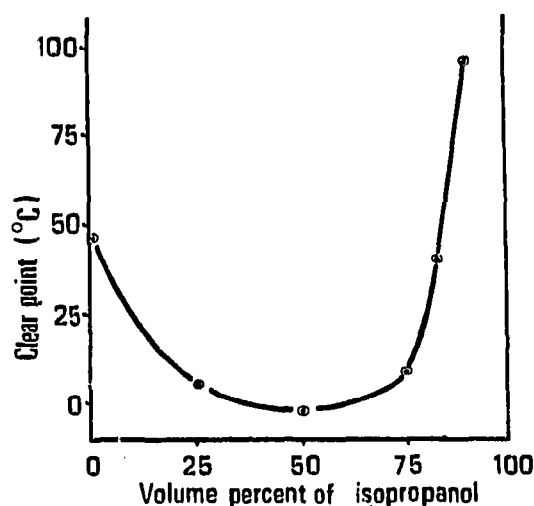


Fig. 1. Relationship between clear point of C_{12} -soap and solvent composition of isopropanol–water mixed solvent (C_{12} -soap concentration 800 mg/10 ml).

Table I shows the distribution coefficients of C_{12} -AS and C_{12} -soap on the resin in various aqueous 30 % organic solvent–0.5 *M* sodium chloride solutions. From Table I, it was found that the distribution coefficients of AS and soap in 30 % isopropanol–0.5 *M* sodium chloride were the smallest of all, and this system was most suitable for separating both surfactants by salting-out chromatography.

From these results, isopropanol was selected as the most suitable organic solvent.

TABLE I

EFFECT OF ORGANIC SOLVENT ON DISTRIBUTION COEFFICIENTS OF C_{12} -AS AND C_{12} -SOAP IN 30 % ORGANIC SOLVENT–0.5 *M* NaCl SOLVENT SYSTEM AT 40° ON AMBERLITE CG-50

Organic solvent	Distribution coefficient	
	C_{12} -AS	C_{12} -soap
Acetonitrile	23.6	713.0
DMSO	39.0	388.7
DMF	14.7	560.1
Acetone	11.7	522.1
THF	4.1	440.9
Methanol	45.5	553.6
Ethanol	19.7	109.6

TABLE II

EFFECT OF SALT ION ON DISTRIBUTION COEFFICIENTS OF C_{12} -AS AND C_{12} -SOAP IN 30% ISOPROPANOL-0.5 M SALT SOLVENT SYSTEM AT 40°

Salt	Distribution coefficient	
	C_{12} -AS	C_{12} -soap
<i>(a) Effect of cation</i>		
LiCl	3.4	19.5
NaCl	3.7	21.2
KCl	5.6	20.1
NH ₄ Cl	4.3	22.4
<i>(b) Effect of anion</i>		
NaNO ₃	3.5	10.9
NaCl	3.7	21.2
CH ₃ COONa	2.7	7.9
Sodium tartrate	3.0	8.5
(NH ₄) ₂ SO ₄	4.8	23.4

Salting-out reagent

It is thought that the type of salting-out reagent present in the eluent is one of the factors that influence the separation mechanism. Therefore, the effects of various types of salts on the distribution coefficients of C_{12} -AS and C_{12} -soap were investigated using aqueous 30% isopropanol-0.5 M salt solution as shown in Table II. Table IIa shows the effects of cations on the distribution coefficients of C_{12} -AS and C_{12} -soap. In this case, the chloride ion was used as the anion in each instance. When using univalent cations such as lithium, sodium, potassium and ammonium, little difference was found between the distribution coefficients of C_{12} -AS or C_{12} -soap. A salt having a bivalent cation cannot be used as a salting-out reagent because it may cause formation of an insoluble complex with a soap. Table IIb shows the effects of anions (nitrate, chloride, acetate, tartrate and sulfate) on the distribution coefficients, with sodium as the cation in each instance (except for ammonium sulfate). It was found that the effect of anions was not as large as that of cations, regardless of the charges on the ions.

From these results, sodium chloride was selected as the most suitable salting-out reagent.

Table III shows the effects of the concentrations of sodium chloride on the

TABLE III

EFFECT OF CONCENTRATION OF SODIUM CHLORIDE ON DISTRIBUTION COEFFICIENT OF C_{12} -AS AND C_{12} -SOAP IN 30% ISOPROPANOL-SODIUM CHLORIDE SOLVENT SYSTEM AT 40°

Concentration of NaCl (M)	Distribution coefficient	
	C_{12} -AS	C_{12} -soap
0.1	1.3	19.7
0.5	1.5	21.2
1.0	1.0	18.7

distribution coefficients of C_{12} -AS and C_{12} -soap using 30% isopropanol-sodium chloride as the solvent system. From Table III, aqueous 30% isopropanol-0.5 M sodium chloride solution was selected as the most suitable eluent.

Resin particle size

It is generally claimed that separation and the peak width of the elution peaks are greatly influenced by the particle size of the resin. Therefore, the effects of the particle sizes of the resin on the separation of C_{12} -AS and C_{12} -soap were investigated by using 100-200 mesh and 200-400 mesh resin under constant conditions. With 100-200 mesh resin, the theoretical plate number of C_{12} -soap was 131 and the peak resolution between C_{12} -AS and C_{12} -soap was 1.99, while in the case of 200-400 mesh resin these values were 592 and 5.29, respectively. From these results, 200-400 mesh resin was used in these experiments.

Elution curve

Fig. 2 shows an elution curve obtained with C_{12} -AS and C_{12} -soap under the conditions mentioned above. The two peaks are completely separated.

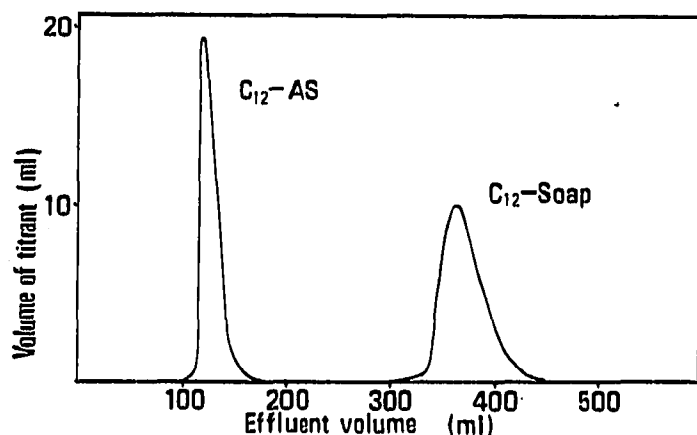


Fig. 2. Elution curve of C_{12} -alkyl sulfate and C_{12} -soap. Column 25 mm I.D. \times 235 mm; resin, Amberlite CG-50, 200-400 mesh; eluent, 30% *i*- C_3H_7OH -0.5 M NaCl; sample size, C_{12} -AS 20 mg, C_{12} -soap 20 mg; flow-rate, 0.3 ml/min; temperature, 40.0°.

TABLE IV

RECOVERIES OF KNOWN MIXTURES OF C_{12} -AS AND C_{12} -SOAP

Sample	Taken (mg)	Found (mg)	Recovery (%)
AS	40.28	40.44	100.4
Soap	40.41	40.77	100.9
AS	20.01	19.99	99.9
Soap	40.41	40.05	99.1
AS	40.28	41.17	102.2
Soap	20.03	19.85	99.1
AS	30.08	29.72	98.8
Soap	20.03	20.45	102.1
AS	20.01	20.71	103.5

Recovery

The recoveries of known mixtures, prepared by mixing C_{12} -AS and C_{12} -soap in certain proportions, were analyzed and the results are shown in Table IV. The agreement between calculated and determined values appears to be satisfactory.

Effect of length of alkyl groups

Single-carbon C_{12} -AS and C_{12} -soap were used as standard samples for the basic investigation. However, it is assumed that the solubility and elution volume will change if the chain-length of the alkyl groups is changed. Therefore, the relationship between the elution volume of AS and soap having alkyl groups of different chain-length and the solubility of these samples in the eluent was studied, as shown in Fig. 3. It appears that the elution volume is proportional to the solubility of a sample.

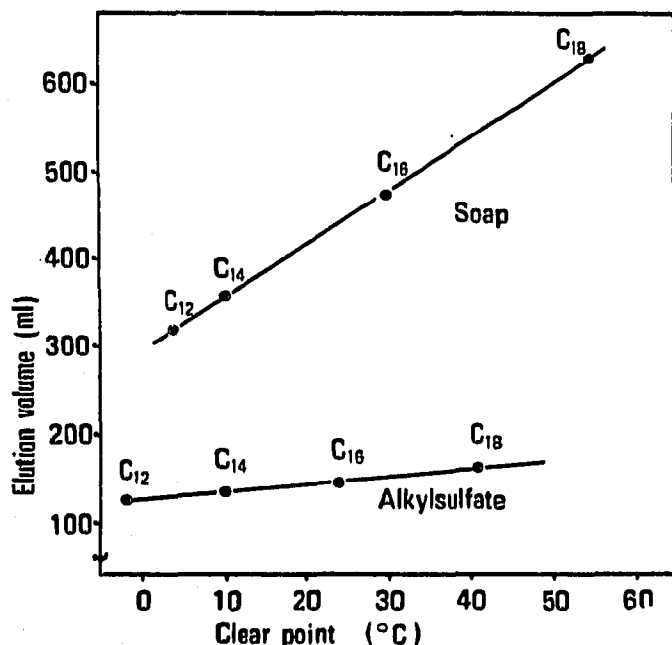


Fig. 3. Relationship between elution volume and clear point of AS and soap.

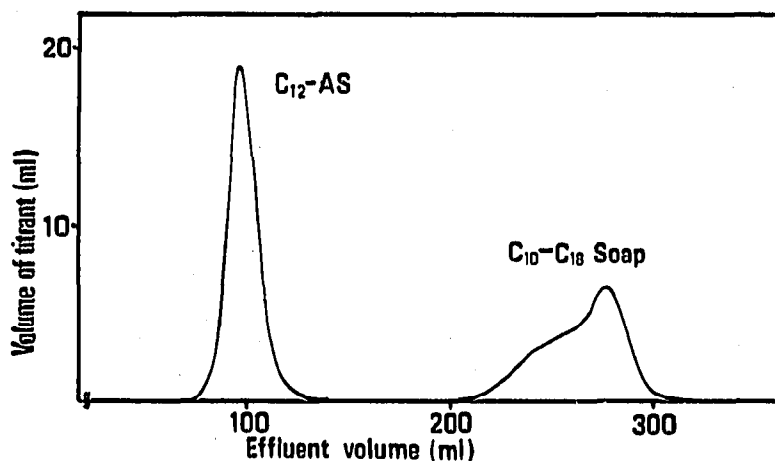


Fig. 4. Gradient elution chromatogram of C_{12} -AS and C_{10} - C_{18} -soap. Column, 25 mm I.D. \times 235

Gradient elution

Commercially available soaps usually have a carbon number distribution in the range 10-18. The elution of soap having a large carbon number is too time-consuming. As C_{18} -soap is less soluble in the eluent, the soap in the effluent may crystallize at the outlet of the column. Therefore, the gradient elution method was used to analyze commercially available soaps and AS in their mixtures.

Fig. 4 shows the gradient elution chromatogram of C_{12} -AS and C_{10} - C_{18} -soap. The separation between both samples is satisfactory and the rate of elution of soap is increased.

DISCUSSION

Soaps have low solubilities in comparison with other anionic surface-active agents, such as sulfates and sulfonates. The soap molecules must be dissolved completely in the eluent in order to carry out salting-out chromatography. In the present study, this problem was resolved by mixing isopropanol in an aqueous salt solution. Compared with the low solubility of C_{12} -soap in the pure isopropanol, soap was readily soluble in the mixed isopropanol-water solvent system. This fact can be explained according to the concept of "co-solvency" introduced by PALIT⁹. The water molecules in the solvent interact with the polar carboxyl groups of the soaps due to hydrogen-bonding. In contrast, isopropanol molecules in the solvent mainly interact with the hydrocarbon parts of soap molecules. Thus, when the soap molecules can interact with each component in the mixed solvent, they are very soluble in this mixed solvent. The fact that dodecane is more soluble in isopropanol than in DMF, DMSO or acetone shows that isopropanol mainly interacts with the hydrocarbon parts of the C_{12} -soap molecule. Thus, as the organic solvent added interacts with the hydrocarbon parts of soap molecules, it is desirable to use solvents having a lower degree of polarization than that of water. The dipole moment of isopropanol (1.66 Debye) is less than that of water (2.35 D). The dipole moments of DMF, DMSO and acetone are 3.96, 3.96 and 2.88 D, respectively, and these values are larger than that for water. In fact, C_{12} -soap was less soluble in these latter solvents. In the case of the mixed alcohol-water solvent system, the co-solvency power for C_{12} -soap decreased in the order isopropanol > ethanol > methanol. This fact suggests that the alcohols added interact with the hydrocarbon parts of soap molecules and the co-solvency power increases as the chain-length of the alkyl groups of alcohols increases. From these results, it is presumed that the organic solvents added in the eluent must not only have a lower dipole moment than that of water but must also be completely miscible with water.

Surface-active agents form micelles in aqueous solution. When two types of surface-active agent are present in an aqueous solution, mixed micelles are formed. A separation of both surface-active agents will not occur if the sample passes through the column in the form of mixed micelles. It is therefore necessary that the mixed micelles are dissociated to single molecules and pass through the column in that state. It is assumed that isopropanol added to the eluent serves this purpose.

The solubility of the surface-active agent in the eluent decreases as the chain-length of the alkyl groups of the surfactant increases. Surfactant molecules that have

ment, the gradient elution method was successfully adopted for these soaps. It is thought that the gradient elution method is useful for salting-out chromatography in which the number of theoretical plates is generally not so large.

As the separation is achieved as a result of Van der Waals' forces between the resin skeleton and the sample molecules in salting-out chromatography, the particle size of the resin influences the width of peaks to a great extent. When using a coarse resin as a column support, the resistance to mass transfer is increased by the diffusion of the sample molecule into the interior of the resin, and this may cause a broadening of the peak width. Accordingly, the smaller the particle size of resin, the larger is the number of theoretical plates of the column. In this experiment, the number of theoretical plates obtained with finer resin (200-400 mesh) was about three times that obtained with coarse resin (100-200 mesh).

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