SEPARATION AND ANALYSIS OF MIXTURES OF CATIONIC SURFACE-ACTIVE AGENTS BY SALTING-OUT CHROMATOGRAPHY

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SUMMARY

The separation and analysis of mixtures of cationic surface-active agents by salting-out chromatography were studied. The conditions finally adopted were as follows: column, 25 mm I.D. and 410 mm long; resin, Amberlite CG-4B (200–400 mesh); eluent, 45% (or 55%) methanol–0.5 M sodium chloride solution; column temperature, 40.0°.

Not only a homologous series could be separated quantitatively, but the separation and determination of the constituents of mixtures of cationic surface-active agents also became feasible.

INTRODUCTION

Cationic surface-active agents have found increasing use as germicides, disinfectants, fabric softeners, emulsifying and dispersing agents, etc., and many methods have been reported for the analysis of mixtures of cationic surface-active agents. Although there are many references to the quantitative analysis of a single cationic surfactant or of the total amount of surface active constituents in a surfactant mixture, the investigation of the separation and determination of each component in a mixture of cationic surface-active agents has not been extended beyond methods involving paper chromatography[1,2], thin-layer chromatography[3,4] and gas chromatography[5-7]. In these methods, however, emphasis is laid on qualitative analysis, and many difficulties are involved if they are to be applied to the quantitative analysis of mixtures of cationic surface-active agents.

On the other hand, the technique of salting-out chromatography originally reported by Sargent and Rieman[8] has been applied to the analysis of ionic surface-active agents[9]. We have applied the technique of salting-out chromatography to the analysis of mixtures of anionic surface-active agents[9-13].

This paper describes the separation and analysis of mixtures of cationic surface-active agents by salting-out chromatography, and also discusses some aspects of the separation mechanism of salting-out chromatography.

EXPERIMENTAL

Apparatus

The column used was 25 mm I.D. and 450 mm long (Sephadex column, Model

K25/45 (jacketed), Pharmacia, Uppsala, Sweden). A constant-temperature water-circulating unit (Model NS/S15/22, Messgeräte, Lauda, G.F.R.) was used to control the column temperature. A Uvicord II detector (LKB, Stockholm, Sweden; wavelength 254 nm) was used for automatically recording the elution chromatograms of the compounds that showed a UV response. The effluent was collected with a fraction collector (Model SF-160K, Toyo Scientific Industry, Japan).

Distribution coefficients were measured by a batch method using a 50-ml conical flask with a stopper. The incubator (Model M-1N, Taiyo Scientific Industry, Japan) was used to obtain rapid adsorption equilibrium.

Reagents and samples

Amberlite CG-4B (200–400 mesh), a weakly basic anion-exchange resin, was used as a column support.

The following cationic surfactants were synthesized in our Company (except Hyamine 1622) and purified by recrystallization from acetone: alkyltrimethylammonium chloride (C_{12}, C_{14}, C_{16}, C_{18}); dialkyldimethylammonium chloride (C_{12}, C_{14}); alkylpyridinium chloride (C_{12}, C_{14}, C_{16}, C_{18}); alkylpyridinium iodide (C_{12}, C_{18}); alkyldimethylbenzylammonium chloride (benzalkonium chloride, C_{12}, C_{14}, C_{16}, C_{18}); and Hyamine 1622 (Rohm & Haas, Philadelphia, Pa., U.S.A.). All other reagents were of analytical-reagent grade.

Procedure

The ion-exchange resin was washed with methanol in order to remove impurities, then treated with aqueous 1 *N* sodium hydroxide solution and 1 *N* hydrochloric acid alternately. The resin was then finally converted into the hydroxide form followed by washing with 10 volumes of water per resin volume and air-drying under reduced pressure.

The procedure used to measure the distribution coefficients and the chromatographic procedure were similar to those described earlier[10], except for the composition of the eluent and the concentration of the sample solution (1 ml of solution contains 20 mg of surfactant).

The quantitative analysis of these surfactants was carried out by the two-phase titration method with bromophenol blue according to the following modification of the method of Hartley and Runnicles[14]. A 20-ml volume of chloroform, 20 ml of potassium hydroxide–sodium sulphate solution (25 g of anhydrous sodium sulphate dissolved in 0.05 *M* potassium hydroxide solution) and 1 ml of indicator solution (0.01% alcoholic bromophenol blue) were placed in a 100-ml conical flask fitted with a glass stopper. A sample solution (10 ml) was added to the flask and the mixture was titrated with 0.001 *N* sodium dodecylsulphate solution, shaking the stoppered flask each time and allowing the phases to separate. The end-point was reached when the indicator shifted from the lower to the upper phase.

An experiment to calculate the recovery of a sample was carried out as follows. The eluate of a section corresponding to an elution peak in the chromatogram was collected in a calibrated flask and diluted to volume with the eluent. A reference solution was prepared by diluting the same amount of sample solution to the same volume, and the volumes of titrant used with both solutions were measured. The recovery was calculated from the ratio of the titrant volume for the sample solution against that for the reference solution.

The chromatographic conditions finally adopted were as follows: resin, Amberlite CG-4B (200–400 mesh); column, Sephadex (25 mm I.D. and 410 mm long); eluent, aqueous 45% (or 55%) methanol–0.5 M sodium chloride solution; column temperature, 40.0°; flow-rate, 0.7 ml/min; determination, two-phase titration method with bromophenol blue as indicator.

RESULTS

Resin

Amberlite CG-400 (OH⁻ and Cl⁻), a strongly basic anion exchanger, and Amberlite CG-4B (OH⁻ and Cl⁻), a weakly basic anion exchanger, were investigated for their effects on the distribution coefficients of dodecyltrimethylammonium chloride, dodecylpyridinium chloride and C_{12}-benzalkonium chloride in aqueous 35% methanol–0.5 M sodium chloride solution in order to select the most suitable form of the resin. The results are presented in Table I, and show that the distribution coefficients of all the resins except Amberlite CG-4B (OH⁻) are small, that is, the surfactants are hardly adsorbed on the resin. Hence, Amberlite CG-4B (OH⁻) was adopted as being the most suitable resin in this study.

TABLE I

EFFECT OF RESIN FORM ON DISTRIBUTION COEFFICIENT IN 35% METHANOL–0.5 M SODIUM CHLORIDE SOLUTION AT 40°

Resin	Distribution coefficient		
	C_{12}-alkyltrimethyl-ammonium salt	C_{12}-pyridinium salt	C_{12}-benzalkonium salt
Amberlite CG-400 (Cl⁻)	0.8	1.0	1.3
Amberlite CG-400 (OH⁻)	0.9	1.3	1.5
Amberlite CG-4B (Cl⁻)	1.9	1.9	2.0
Amberlite CG-4B (OH⁻)	4.4	8.8	18.0

Eluent

After a preliminary examination, methanol was added in an aqueous salt solution in order to dissolve the surfactants completely.

The effects of various types of salts on the distribution coefficients of the C_{12}-pyridinium and C_{12}-benzalkonium salts were studied using aqueous 35% methanol–0.5 M salt solution, as shown in Table II. Table IIa shows the effects of cations on the distribution coefficients, with chloride as the anion in each instance. Little difference was found between the distribution coefficients, regardless of the charges on the ions. Table IIb shows the effects of anions on the distribution coefficients, with sodium as the cation. When using monovalent anions, little difference was found between the distribution coefficients. The effects of the bivalent anion, however, were greater than those of monovalent anions. Sodium chloride was selected as a suitable salting-out reagent as the counter ion of the surfactant was the chloride ion.

In order to examine the effects of the concentration of sodium chloride in the eluent on the elution volumes and peak resolutions of dodecyltrimethylammonium chloride and cetylpyridinium chloride, the elution behaviour of both samples was

investigated by using 55% methanol–sodium chloride as solvent, as shown in Fig. 1. It is apparent that the maximum elution volumes and the best peak resolutions were obtained when the concentration of sodium chloride was 1.0 M. The concentration of sodium chloride, however, was fixed at 0.5 M, as a higher concentration makes the elution of benzalkonium salts difficult.

TABLE II

EFFECT OF SALT ION ON DISTRIBUTION COEFFICIENTS IN 35% METHANOL–0.5 M SALT SOLUTION AT 40°

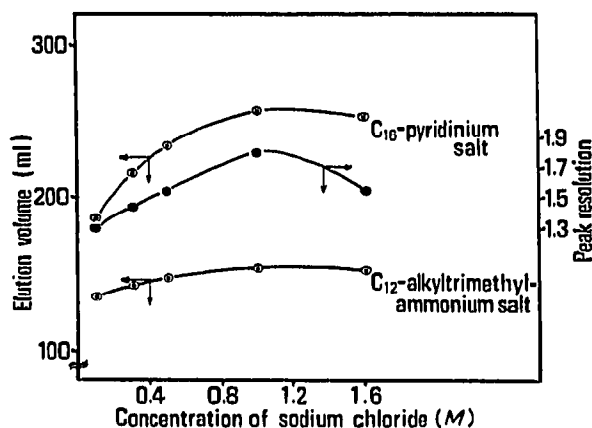| Salt	Distribution coefficient	
	C_{12}-pyridinium salt	C_{12}-benzalkonium salt
(a) Effect of cation		
KCl	10.8	16.7
NaCl	8.8	18.0
NH_4Cl	2.6	3.9
$MgCl_2$	5.8	10.9
$CaCl_2$	8.7	13.2
$SrCl_2$	11.3	15.4
(b) Effect of anion		
NaCl	8.8	18.0
$NaNO_3$	3.6	7.1
CH_3COONa	8.8	10.5
Na_2CO_3	32.7	60.0

Fig. 1. Effect of concentration of sodium chloride on elution volume and peak resolution. Column: 25 mm I.D. × 390 mm.

The effect of the concentration of methanol in the eluent on the elution volume of each surfactant was studied using the aqueous methanol–0.5 M sodium chloride solvent system. The results are presented in Fig. 2, which shows that the lower the concentration of methanol, the greater is the elution volume. Aqueous 45% methanol–

0.5 M sodium chloride solution was suitable as an eluent for a surfactant with 12 carbon atoms. However, either 45% methanol–0.5 M sodium chloride or 55% methanol–0.5 M sodium chloride solution was used as the eluent according to the combination of surfactants present, because the elution volume increases as the chain-length of the alkyl groups of the surfactant increases (see below).
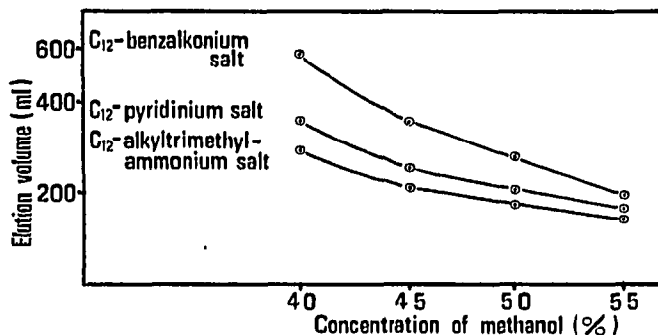

Fig. 2. Effect of concentration of methanol on elution volume in methanol–0.5 M sodium chloride.

Column temperature

It is presumed that an increase in the column temperature increases the diffusion rate of the sample molecules within the resins and accelerates adsorption equilibrium, thus improving the column efficiency. However, temperatures above 40° are not desirable owing to the thermolability of the resin, and the column temperature was consequently fixed at 40°.

Elution curves

Fig. 3 shows an elution curve obtained with a homologous series of alkyl-pyridinium salts (C_{12}–C_{18}) under the previously described conditions. Each homologous series of alkyltrimethylammonium and benzalkonium salts also showed a good resolution under the same conditions. The elution curve for a mixture of dodecyltrimethylammonium chloride and cetylpyridinium chloride is shown in Fig. 4, and it can be seen that the two peaks are completely separated. The peak resolution between dodecyltrimethylammonium chloride and C_{12}-benzalkonium chloride was also more than unity.
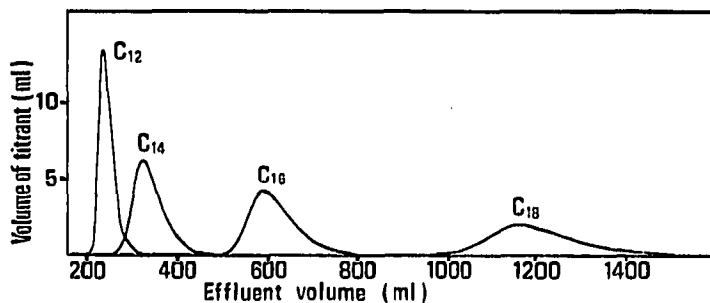

Fig. 3. Elution curves of a homologous series of alkylpyridinium salts. Column: 25 mm I.D. × 410 mm; resin: Amberlite CG-4B, 200–400 mesh; eluent: 45% methanol–0.5 M NaCl; sample size: 20 mg; column temperature: 40.0°; flow-rate: 0.7 ml/min.
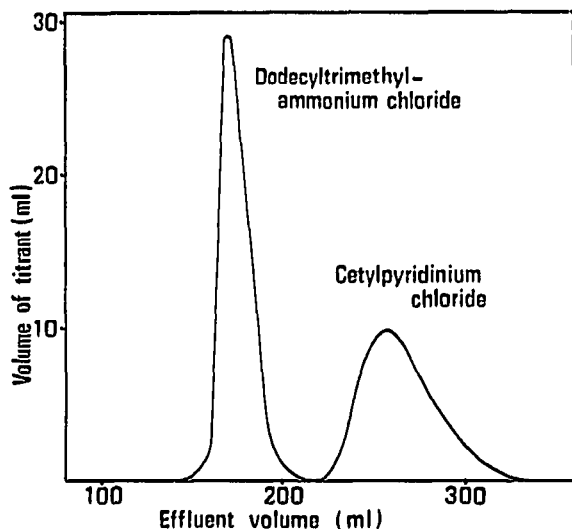
Fig. 4. Elution of C_{12}-alkyltrimethylammonium and C_{16}-pyridinium salts. Column: 25 mm I.D. × 410 mm; resin: Amberlite CG-4B, 200–400 mesh; eluent: 55% methanol–0.5 M NaCl; sample size: 20 mg; column temperature: 40.0°; flow-rate: 0.7 ml/min.
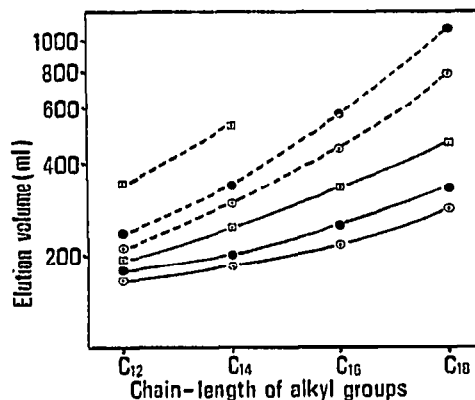
Fig. 5. Relationship between elution volume and chain-length of alkyl groups. ⊙, Alkyltrimethyl-ammonium salt; ●, alkylpyridinium salt; □, benzalkonium salt. Solid line, 55% methanol–0.5 M NaCl; dotted line, 45% methanol–0.5 M NaCl.

Recovery

The recoveries of known mixtures, prepared by mixing cationic surface-active agents in certain proportions, were investigated. Recoveries form a mixture of dodecyltrimethylammonium chloride and cetylpyridinium chloride were 99.1 and 101.4%, respectively, and from a mixture of dodecyltrimethylammonium choride and C_{12}-benzalkonium chloride, 99.4 and 98.5%, respectively. The recovery of Hyamine 1622 from the column was investigated independently, and was found to be 100.4%. The agreement between the calculated and determined values appears to be satisfactory.

Effect of length of alkyl groups

It is assumed that the elution behaviour in salting-out chromatography will change if the chain-length of the alkyl groups is changed. Therefore, the elution volumes of alkyltrimethylammonium, alkylpyridinium and benzalkonium salts with alkyl groups with different chain-lengths were studied, as shown in Fig. 5. As expected, the logarithm of the elution volume was proportional to the chain-length of the alkyl groups in a particular sample. Dialkyldimethylammonium salts with more than 12 carbon atoms were not eluted in 55% methanol–0.5 M sodium chloride solution.

State of sample molecules in column

In order to discuss the separation mechanism, it is important to decide whether the cationic surfactant molecules are present as dissociated ions or whether they are present as non-ionized molecules in the column. As there is a large amount of chloride ions from the salting-out reagent in the eluent, it is difficult to distinguish between the

chloride ions from the samples and those from the salting-out reagent by determining the chloride ions in the eluate. Therefore, in order to avoid this difficulty, use was made of alkylpyridinium iodides. The iodide ions in the eluate were detected by observing the colour developed by the addition of starch solution after oxidation of the iodide ions with 0.1 N potassium dichromate solution acidified with sulphuric acid. Fig. 6 shows the elution curves obtained with C_{12}- and C_{18}-alkylpyridinium iodides, C_{18}-alkylpyridinium chloride and sodium iodide. From Fig. 6, it appears that the elution volume of the alkylpyridinium cation is different from that of the iodide ion, and alkylpyridinium cations with alkyl groups with the same chain-length show the same elution volumes, regardless of the counter ion present. This fact shows that the added surfactant molecules are still present in an ionic state in the column.
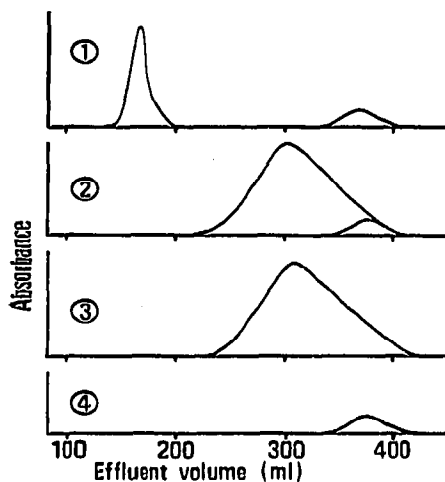

Fig. 6. Elution curves of alkylpyridinium iodide and chloride and sodium iodide. 1, Dodecyl-pyridinium iodide; 2, stearylpyridinium iodide; 3, stearylpyridinium chloride; 4, sodium iodide.

DISCUSSION

In the present study, not only a homologous series of cationic surface-active agents could be separated quantitatively, but the separation and determination of the constituents of their mixtures also became feasible.

It is presumed that cationic surfactant molecules are present in an ionic state and probably pass through the column accompanied by exchange of their counter ions for anions in the eluent.

The anion-exchange resins used in this experiment have a positive charge owing to the dissociation of ionogenic groups. This positive charge repels the cations formed by dissociation of cationic surfactants. However, it is assumed that the positive charges on both groups are neutralized electrically with the anions of inorganic salts in the eluent. Moreover, surfactant molecules are accumulated on the surface of the resin by the salting-out force of the ions of the inorganic salts, and thus each sample molecule is adsorbed on the resin[8]. The fact that larger distribution coefficients were obtained by using bivalent anions than by using monovalent anions, as shown in Table IIb, agrees with the statement by Jirgensons and Straumanis[15] that the higher the

surface charge density of hydrated anions the stronger is the electric neutralization power. In view of the fact that the effects of cations on distribution coefficients were not as large as those of anions, regardless of the charges on the ions, it seems reasonable to assume that the electric neutralization by anions in the eluent plays an important role in the adsorption of surfactants on the resin.

The pH of the eluent influences the dissociation of the ionogenic groups, and results in changes in the adsorptivity of surfactants on the resin. In this study, as the distribution coefficients were measured by a batch method, the pHs of the solutions differ after equilibrium, depending on the inorganic salts used as salting-out reagents. Consequently, it appears that the results shown in Table II do not agree strictly with the distribution coefficients in the column. In a batch method, the pH of the eluent corresponds to a weakly alkaline solution owing to the partial exchange of hydroxyl ions on the resin for chloride ions in the eluent. It can therefore be presumed that the electric neutralization of both metal cations and surfactant cations by hydroxyl ions occurs simultaneously, and apparent distribution coefficients become small (it is assumed that the electric neutralization power of chloride ions is stronger than that of hydroxyl ions).

The solubility of a surfactant in an eluent decreases as the chain-length of the alkyl groups in the surfactant increases. Surfactant molecules with long alkyl groups can be easily salted out and adsorbed on the resin, and they therefore have large elution volumes (Fig. 5). For this reason, dialkyldimethylammonium salts that have large hydrophobic groups were not eluted under the conditions mentioned above. The adoption of the gradient elution method, however, may not only enable these surfactants to be eluted successfully but also extend further the applicable range.

REFERENCES

1 H. M. Bregoff, E. Roberts and C. C. Delwiche, *J. Biol. Chem.*, 205 (1953) 565.
2 H. Holness and W. R. Stone, *Analyst (London)*, 83 (1958) 71.
3 H. K. Mangold and P. Kammerck, *J. Amer. Oil Chem. Soc.*, 39 (1962) 201.
4 K. Bey, *Fette, Seifen, Anstrichm.*, 67 (1965) 217.
5 L. D. Metcalfe, *J. Amer. Oil Chem. Soc.*, 40 (1963) 25.
6 H. P. Warrington, *Anal. Chem.*, 33 (1961) 1898.
7 T. Uno, K. Miyazima and T. Nakagawa, *Bunseki Kagaku (Jap. Anal.)*, 15 (1966) 584.
8 R. Sargent and W. Rieman, *J. Phys. Chem.*, 61 (1957) 354.
9 S. Fudano and K. Konishi, *J. Chromatogr.*, 77 (1973) 351.
10 S. Fudano and K. Konishi, *J. Chromatogr.*, 51 (1970) 211.
11 S. Fudano and K. Konishi, *J. Chromatogr.*, 62 (1971) 467.
12 S. Fudano and K. Konishi, *J. Chromatogr.*, 66 (1972) 153.
13 S. Fudano and K. Konishi, *J. Chromatogr.*, 71 (1972) 93.
14 G. S. Hartley and D. F. Runnicles, *Proc. Roy. Soc., Ser. A*, 168 (1938) 420.
15 B. Jirgensons and M. E. Straumanis, *A Short Textbook of Colloid Chemistry*, Pergamon Press, New York, 1962.