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SEPARATION AND DETERMINATION OF MIXTURES OF ANIONIC SURFACE-ACTIVE AGENTS BY SALTING-OUT CHROMATOGRAPHY

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SUMMARY

The optimum chromatographic conditions for the separation and determination of mixtures of anionic surface-active agents by salting-out chromatography were determined. The conditions finally adopted were as follows: column, 25 mm I.D. and 410 mm long; resin, Amberlite CG-50 (200–400 mesh); eluent, aqueous 30% isopropanol–0.2 *M* sodium chloride solution; column temperature, 40.0°.

The elution behaviour of various types of surface-active agents was examined under these conditions and the surfactants were classified by their elution volumes in order to establish separable combinations.

INTRODUCTION

The technique of salting-out chromatography has been applied to the separation and determination of organic non-electrolytes[1,2] and surface-active agents[3–6] since it was proposed by SARGENT AND RIEMAN[7].

We have developed this technique in order to establish a new analytical method for the separation and determination of ionic surface-active agents. Previously, analytical investigations of the following combinations have been reported: linear alkylbenzene sulphonates (LAS) and branched alkylbenzene sulphonates (ABS)[3]; hydroxyalkane and alkene sulphonates in α-olefin sulphonates (AOS)[4]; alkylsulphates (AS) and LAS[5]; and AS and soap[6]. However, in the analyses of these two-component mixtures, the chromatographic conditions used for the different combinations were not identical. Accordingly, the relative elution volumes of these surfactants were obscure, so that it was difficult to apply the information obtained from previous work to the analysis of mixtures with more than three components or of mixtures in which the constituents were unknown.

Therefore, the optimum salting-out chromatographic conditions for the separation of two-component mixtures were investigated and the separation and determination of the constituents of mixtures with more than three components under these conditions were also studied.

The anionic surface-active agents used were *p*-toluenesulphonate (PTS), AS, LAS, ABS, alkanesulphonates (SAS), AOS, di-2-ethylhexylsulphosuccinate and soap.

The surface-active agents were classified according to the elution volume obtained under the optimum chromatographic conditions.

EXPERIMENTAL

Apparatus

The column used was of 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. A constant-flow pump (Model P_2, Japan Electron Optics Laboratories Co. Ltd., Japan) was used to regulate the flow-rate of the eluent. A Uvicord II detector (LKB-Produkter AB, Sweden) was used for recording the elution chromatogram of PTS.

Reagents and samples

Amberlite CG-50 (200–400 mesh), a weakly acidic cation-exchange resin, was used as a column substrate. C_{15}-AOS, AS and soap were synthesized in our laboratory and purified by recrystallization from an aqueous methanolic solution. LAS and ABS were manufactured by our company and purified by the usual method[3]. SAS, di-2-ethylhexylsulphosuccinate and AOS, except C_{15}-AOS, were manufactured by our company and were used without further purification. All other reagents were of analytical-reagent grade.

Procedure

The conditioning of the ion-exchange resin and the chromatographic procedure were similar to those described earlier[3,6]. Quantitative analyses of these surface-active agents were carried out by the two-phase titration method with bromocresol green according to MILWIDSKY AND HOLTZMAN's method[8]. In the analysis of PTS, the absorbance of each fraction was measured at 261 nm because of the difficulty in carrying out the quantitative analysis by the two-phase titration method. An experiment to calculate the recovery of a sample was carried out according to the following method. The eluate of a section corresponding to an elution peak in the chromatogram recorded 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. The volume of titrant or the absorbance at 261 nm of both solutions were measured, with the eluent being used as a blank solution. The recovery was calculated from the ratio of the results on the sample solution against the results on the reference solution.

The chromatographic conditions finally adopted were as follows: resin, Amberlite CG-50 (200–400 mesh); column, Sephadex column (25 mm I.D. and 410 mm long); eluent, aqueous 30% isopropanol–0.2 *M* sodium chloride solution; column temperature, 40.0°; flow-rate, 0.7 ml/min; determination, two-phase titration method with bromocresol green as indicator.

RESULTS AND DISCUSSION

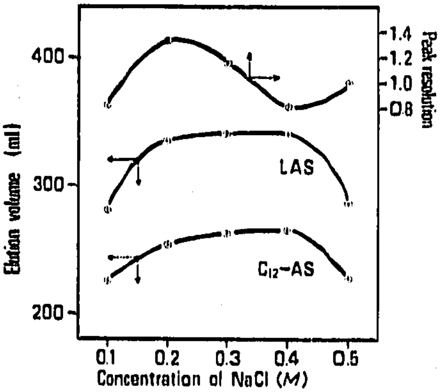
In order to determine the optimum chromatographic conditions, the elution behaviour of C_{12}-AS and LAS was first investigated because the elution volumes of these surfactants are similar.

Eluent

As soap is almost insoluble in a salt solution, isopropanol was added to the eluent in order to increase the solubility of soap in the eluent. From a preliminary examination, sodium chloride was selected as the salting-out reagent.

The effects of the concentrations of isopropanol on the elution curves of C_{12}-AS and LAS with isopropanol–0.5 *M* sodium chloride solution used as the solvent were examined. The concentrations of isopropanol tested were 20, 25, 28 and 30%. The lower the concentration of isopropanol, the greater were the elution volume and peak resolution. At the same time, the tailing of the peaks also increased and the width of the peaks broadened when the concentration of isopropanol decreased. Therefore, the concentration of isopropanol was fixed at 30% in consideration of the peak resolution and tailing.

In order to examine the effects of the concentration of sodium chloride in the eluent on the elution volumes and peak resolutions of C_{12}-AS and LAS, the elution behaviour of both samples was investigated by using 30% isopropanol–sodium chloride as solvent, as shown in Fig. 1. It is apparent that the best peak resolution was obtained when the concentration of sodium chloride was 0.2 *M* although the elution volumes of both samples were at a maximum when the concentration was 0.4 *M*. From these results, 30% isopropanol–0.2 *M* sodium chloride solution was adopted as being the most suitable eluent.



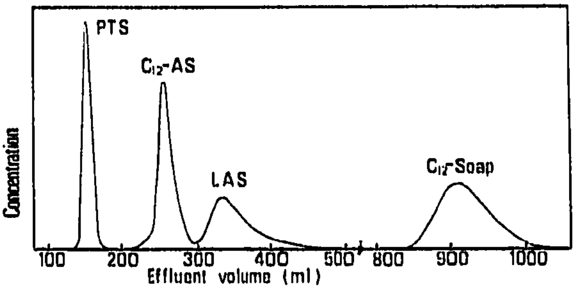


Fig. 1. Effects of concentration of sodium chloride on elution volume and peak resolution of C_{12}-AS and LAS. Concentration of isopropanol = 30%.

Fig. 2. Elution curve of PTS, C_{12}-AS, LAS and C_{12}-soap. Column, 25 mm I.D. × 410 mm; resin, Amberlite CG-50, 200–400 mesh; eluent, 30% isopropanol–0.2 *M* sodium chloride solution; Flow-rate, 0.7 ml/min; temperature, 40.0°; sample size, 25 mg.

TABLE I

RECOVERIES OF SAMPLES FROM COLUMN

Sample	*Recovery (%)*
PTS	98.6
C_{12}-AS	100.9
LAS	99.5
C_{15}-AOS (hydroxyalkane)	99.0
C_{15}-AOS (alkene)	98.2
C_{12}-Soap	98.7

Flow-rate

The effect of flow-rate on the number of theoretical plates was studied by changing the flow-rate from 1.9 to 0.35 ml/min. At flow-rates of 1.9 and 0.35 ml/min, the number of theoretical plates was 202 and 264, respectively, thus showing that the lower the flow-rate, the better is the separation. However, the use of a very low flow-rate is time consuming, and consequently the flow-rate of the eluent adopted was 0.7 ml/min.

Elution curves

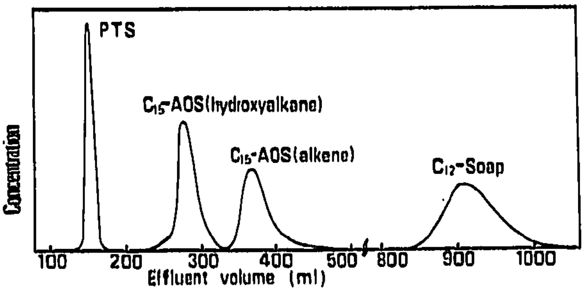
Fig. 2 shows an elution curve obtained with a four-component mixture prepared by mixing PTS, C_{12}-AS, LAS and C_{12}-soap under the above conditions. The elution curve of PTS was recorded by the absorbance at 261 nm. The elution curve for a mixture of PTS, C_{15}-AOS (hydroxyalkane and alkene sulphonates) and C_{12}-soap is shown in Fig. 3. The peak resolutions were satisfactory. The elution of soap can be speeded up by using the gradient elution method described earlier[6].

Recoveries

The recoveries of the surface-active agents from the column were investigated. The results in Table I show that the recoveries are satisfactory.

Effect of chain-length of alkyl groups

In salting-out chromatography, a sample of lower solubility is generally well adsorbed on the resin and tends to give a large elution volume. It is assumed that there



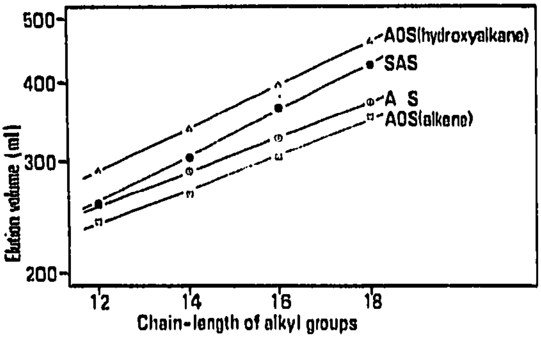


Fig. 3. Elution curve of PTS, C_{15}-AOS and C_{12}-soap. Conditions as in Fig. 2.

Fig. 4. Relationship between elution volume and chain-length of alkyl groups.

TABLE II

CLASSIFICATION OF SURFACE ACTIVE AGENTS BY ELUTION VOLUME

Group	*Surface-active agent*
1	PTS
2	C_{12}-AS C_{16}-AOS (hydroxyalkane) C_{12}-SAS Di-2-ethylhexylsulphosuccinate
3	C_{16}-AOS (alkene) LAS, ABS C_{16}-SAS, C_{18}-SAS
4	Soap

is a difference in solubility and elution volume if the chain-lengths of the alkyl groups of the surface-active agents are different. Therefore, the elution volumes of AS, AOS and SAS having different chain-lengths of alkyl groups were studied, as shown in Fig. 4. It appears that the logarithm of elution volume is proportional to the chain-length of the alkyl groups in the sample, which indicates that surfactant molecules that have long-chain alkyl groups can be easily salted out and adsorbed on the resin.

Classification of surfactants by elution volume

Table II shows the classification of the surface-active agents by their elution volumes. PTS in Group 1 is eluted first and soap in Group 4 is eluted last. A four-component sample mixture, prepared by mixing surfactants selected from each group, can be separated under the above conditions.

CONCLUSION

The optimum salting-out chromatographic conditions for the separation of mixtures of anionic surface-active agents were determined. Various types of surfactants were classified by their elution volumes in order to establish separable combinations of surface-active agents.

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