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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION AND SELECTIVE DETECTION OF ANIONIC SURFACTANTS

APPLICATION TO COMMERCIAL FORMULATIONS AND WATER SAMPLES

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SUMMARY

Reversed-phase liquid chromatography combined with post-column ion-pair extraction detection was investigated for the analysis of C_{10}–C_{18} homologues of sulphonate and sulphate type surfactants. The separations were carried out on Hypersil SAS and Hypersil ODS as the packing and water–acetone mixtures as the mobile phase. For the separation of more than five successive homoloques, an acetone gradient was applied.

Anionic surfactants were detected as ion pairs by mixing of the effluent with chloroform and a solution of acridinium chloride. The ion pairs formed between the anionic solutes and acridinium ion were extracted into the chloroform phase. After phase separation the chloroform phase was monitored fluorimetrically. The detection system behaved linearly for up to 4 μg of anionic surfactant injected. The detection limit ranged between 1 and 5 ng, independent of the alkyl chain length of solutes investigated.

The suitability of the method for the analysis of commercial surfactant formulations and the determination of anionic surfactants at parts per billion levels in water samples, after on line on column pre-concentration, is demonstrated.

INTRODUCTION

Surface-active substances have found widespread application in cleaning agents and as emulsifiers, solubilizers and stabilizers in foodstuffs, pharmaceutical products and in many manufacturing processes. By far the most frequently applied surfactants are the anionic surface-active substances of the sulphonate or sulphate type. Commercial surfactant formulations usually consist of mixtures of homologues and isomers.

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For the anlysis of anionic surfactants, various techniques have been applied such as extraction[1,2], paper[3,4] and thin-layer chromatography[5], electrophoresis[6] and ion exchange[7]. Most of these techniques classify the surfactants according to the type of anionic group but fail to discriminate the compounds within a class (*i.e.*, homologues and isomers). For the analysis of compounds within a class, gas chromatography[2,8] and high-performance liquid chromatography (HPLC)[9–11] have proved to be more successful. For the analysis of anionic surfactants that do not contain a chromophoric group we reported previously a selective and sensitive post-column ion-pair extraction detection system adapted for normal-phase liquid chromatography[12].

In this paper we report the results of an investigation to apply this ion-pair extraction detection system to reversed-phase chromatography for the analysis of homologues and isomers of sulphonate and sulphate type surfactants in commercial formulations. The applicability of the system for the analysis of anionic surfactants in water samples at the parts per billion* level by means of on-line pre-concentration on a small column is also demonstrated.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of an LC-3 gradient pump (Perkin-Elmer, Norwalk, CT, U.S.A.), a Rheodyne 7010 high-pressure sampling valve equipped with 100-μl loop, a Fluorichrom fluorimetric detector (Varian, Palo Alto, CA, U.S.A.) and a stainless-steel column (150 × 4.6 mm I.D.). For the pre-concentration of anionic surfactants from water samples, a small stainless-steel column (10 × 4.6 mm I.D.) was installed in the sample loop (see Fig. 1). To pump the water samples, use was made of a reciprocating pump (Orlita, Giessen, G.F.R.). All feed lines were constructed of stainless-steel 316 tubing.

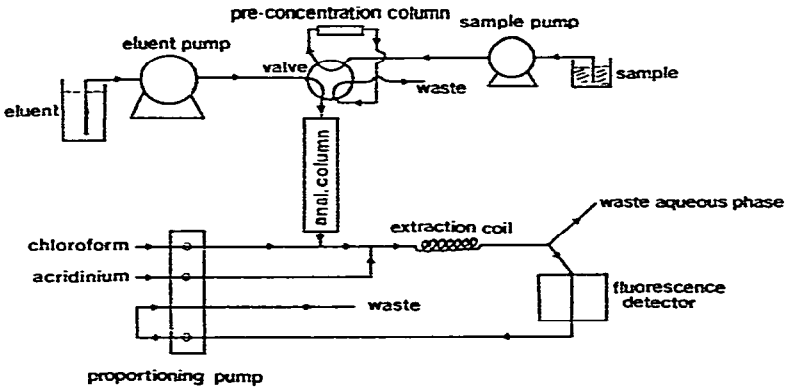

Fig. 1. Schematic representation of the HPLC–ion-pair extraction detection system. For the analysis of test mixtures and commercial surfactant formulations the pre-concentration column was replaced by a 100-μl sample loop. Extraction coil: 10 turns, 70 cm × 1.2 mm I.D., polyethylene. Phase separator: glass, Technicon, PTFE insert. Flow-rates: analytical column, 1.5 ml/min; chloroform, 1 ml/min; acridinium, 0.4 ml·min; detector, 0.6 ml/min; sample, 10 ml/min.

* Throughout this article the American billion (10^9) is meant.

The ion-pair extraction system is shown schematically in Fig. 1. The column effluent is first solvent segmented with chloroform and then mixed with an aqueous solution of acridinium chloride. After phase separation the chloroform phase is monitored fluorimetrically (excitation 400 nm; emission 470 nm cut-off).

The acridinium solution and chloroform were delivered by means of a peristaltic pump (Technicon Pump III proportioning pump), which was also used to control the chloroform flow through the fluorimetric detector.

Materials

The reversed-phase column packing materials used were Hypersil SAS and Hypersil ODS (Shandon, London, Great Britain). Sodium alkylsulphonates were obtained from Eastman (Rochester, NY, U.S.A.) and acridinium chloride from Merck-Schuchardt (Munich, G.F.R.). Serdet DCK (C_{12}–C_{14} alkyl ether sulphate (3Mol EO) was obtained from Servo (Delden, The Netherlands) and SAS 30 (C_{13}–C_{17} secondary alkylsulphonates from Hoechst (Amsterdam, The Netherlands).

Procedures

The analytical and pre-concentration columns were slurry packed using methanol–tetrachloromethane (1:20) as dispersing and pressurizing liquid. The columns were washed with methanol and conditioned with the mobile phase until constant retention was achieved (usually 100–150 ml). The acridinium solution was prepared daily by dissolving 96 mg/l of acridinium chloride in 1 *M* phosphoric acid. The capacity ratios (k_i') were calculated from the retention times of the solutes, corrected for the delay in the reactor, and the retention time of perchlorate, which was considered to be non-retained. The solutes and commercial sufactants were dissolved in water–acetone (7:3), containing 0.05 *M* sodium dihydrogen phosphate. The water samples used in the pre-concentration study were prepared by dissolving the solutes in distilled water containing 0.05 *M* sodium dihydrogen phosphate. The effect of the acetone content of the mobile phase on the extraction efficiency (*i.e.*, the height of the signal) was determined by plug injection of solutions of the alkylsulphonates directly into the extraction system fed with varying water–acetone mixtures and peak-height measurements.

RESULTS AND DISCUSSION

Choice of polarity modifier

In a previous paper[12] we reported the separation of anionic surfactants into different classes by means of normal-phase liquid chromatography. For the detection of anionic surfactants use was made of an on-line post-column ion-pair extraction system and fluorimetric detection[12,13]. For this purpose the organic effluent was segmented with an aqueous solution containing the fluorigenic cation acridinium. The anionic surfactants form ion pairs with the acridinium cation that preferentially dissolve in the organic phase. The extraction of the ion pairs depends on the hydrophobic moiety of the surfactant[12]. After phase separation the organic phase is monitored fluorimetrically.

Although the selectivity of normal-phase systems toward the different types of anionic surfactants was reasonably useful, the selectivity within a class (*i.e.*, compounds with the same acidic group but with different alkyl chains) was poor.

Reversed-phase liquid chromatography with alkyl-modified silicas has proved to be eminently suitable for the separation of compounds with different hydrophobic moieties. It seems therefore obvious to apply reversed-phase systems to the separation of compounds within a class of surfactants. However, when combining reversed-phase systems with the ion-pair extraction system, the set-up has to be changed because in reversed-phase system aqueous eluents are applied. To detect the anionic surfactants as acridinium ion pairs, the column effluent is first solvent segmented with chloroform and then mixed with an aqueous acridinium solution. After phase separation the chloroform phase is monitored fluorimetrically. In principle it should be possible to add the acridinium cation to the mobile phase[14], which avoids an extra mixing step, but this alternative has not yet been investigated.

From previous reports on the separation of homologues on reversed-phase systems[15] and on the performance of the ion-pair extraction system[12,13] it can be expected that sometimes one will have to find a compromise between separation and detection, because the anionic surfactant formulations usually contain C_{10}–C_{18} alkyl chains. These large hydrophobic moieties demand a large organic modifer content in order to elute these compounds in a reasonable time and with acceptable dilution. Moreover, the increase in k' with increasing length of the alkyl chain is so large that usually only a limited number of successive members (four or five) or a homologous series can be separated isocratically. To separate more members in one chromatographic run, gradient elution has to be applied. However, it was found previously[12,13] that large organic modifier concentrations affect the extraction efficiency unfavourably. This is particularly so with methanol as organic modifier and less so with acetonitrile. However, acetonitrile is toxic and was therefore rejected as an organic modifier.

As methanol is a relatively weak modifier on the reversed-phase elution strength scale, more favourable results can be expected with lower concentrations of more hydrophobic, water-miscible, organic solvents. In this respect acetone was found to be useful for two reasons: first, the acetone concentration in the mobile phase can be much lower than the methanol concentration for the same retention, and second, the partition coefficient of acetone between chloroform and water is about 5, which implies that a significant part of the acetone present in the effluent is extracted into the chloroform phase. Despite the dilution effect, this favours significantly the extraction efficiency compared with water–methanol as the mobile phase as the proportion of acetone in the aqueous phase is less. As the mobile phase composition also influences the background owing to coextraction of acridinium salts in the absence of anionic surfactants, one sometimes has to find a compromise between separation and detection.

In order to determine the optimal conditions, a number of experiments were carried out. For various alkylsulphonates the capacity ratio (k') was measured as a function of the acetone content and the salt concentration in the mobile phase using Hypersil SAS as the stationary phase. The results of these measurements are given in Figs. 2 and 3. In accordance with theory, k' of alkylsulphonates decreases with increasing acetone content of the mobile phase. Fig. 3 shows the relationship between log k' and the length of the alkyl chain of the sulphonates at different acetone contents of the mobile phase. In agreement with earlier reports on the retention of homologues on reversed-phase materials[15], a linear relationship is found. Also, it can

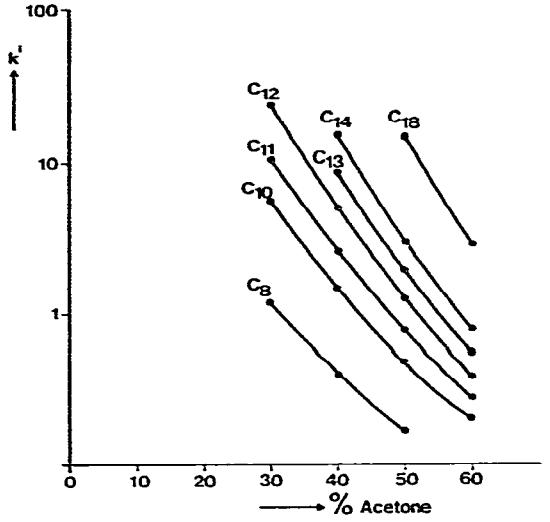

Fig. 2. Effect of acetone content of the mobile phase on the capacity ratio (k') of linear alkylsulphonates (LAS). Stationary phase: Hypersil SAS. Mobile phase: 0.05 M NaH_2PO_4 + 30–60% acetone. Solutes: C_8-C_{18} alkylsulphonates.

be seen that on adjusting k' between 2 and 10 (which is usually the practical range) only a limited number of homologues can be separated isocratically. As many surfactant formulations contain homologues in the range C_{10}–C_{18}, in most instances an acetone gradient has to be applied in order to separate the individual surfactants in mixtures in one run and in a reasonable time. An advantage of gradient elution is the peak contraction, which favours the detection limit.

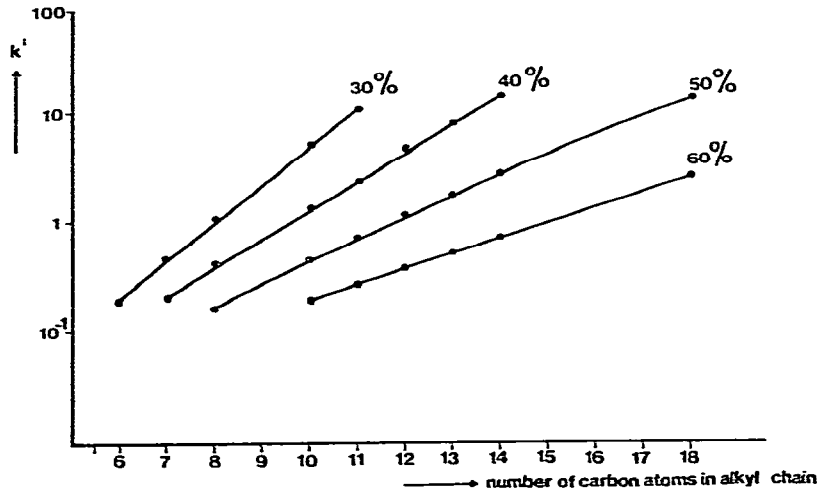

Fig. 3. Relationship between log k' and the number of carbon atoms in the alkyl chain of LAS. Conditions as in Fig. 2.

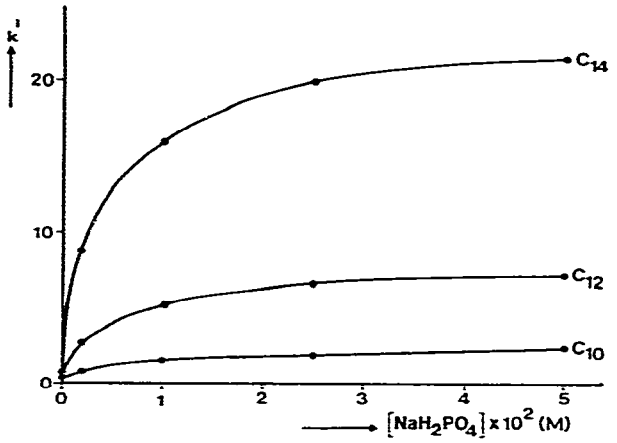

Fig. 4. Effect of salt concentration on k' of LAS. Stationary phase: Hypersil SAS. Mobile phase: ($2 \cdot 10^{-3}$–$5 \cdot 10^{-2}$) *M* NaH_2PO_4 + 40% acetone. Solutes: C_{10}, C_{12}, C_{14} alkylsulphonates.

Optimization of salt concentration and column packing

As can be seen from Fig. 4, the salt concentration significantly influences the retention; k' increases steeply on adding only 0.002 *M* sodium dihydrogen phosphate to the mobile phase and this increase continues with increasing salt concentration but levels off at very large concentrations. The same effect on k' was noticed with other electrolytes such as sodium sulphate and sulphuric acid. The effect of the salt concentration on the retention is still not well understood, but apart from ion-pair equilibrium effects electrostatic exclusion effects are also involved, as was noticed when using salts as unretained markers[16]. It was noticed that the retention volume of perchlorate, used in this study as an unretained solute, increases with increasing salt concentration in the mobile phase. As can be seen from Fig. 4, the effect of the salt concentration on k' can be used to produce gradients and can be exploited to improve the pre-concentration of surfactants in water samples on small reversed-phase columns.

The influence of the type of reversed-phase packing (alkyl chain) was investigated by comparing the retention on a short-chain (Hypersil SAS) and a long-chain modified silica (Hypersil ODS). As expected, the retention on the long-chain modified silica is larger than on the short alkyl chain support (by a factor of about 2.5). However, the dependence of k' on the mobile phase composition coincides on both reversed-phase packings. On adapting the acetone content both packings show about the same separation power.

Effect of mobile phase composition on detection

The effect of the acetone content of the column effluent on the extraction efficiency was determined for alkylsulphonates as described under *Procedures* and is shown in Fig. 5. The peak height of the test solutes decreases with increasing acetone content (the peak broadening of the extraction system was found to be independent of the acetone content). This decrease is caused by the fact that acetone is extracted into the chloroform phase and so increases the volume of the organic phase. When accounting for this dilution effect, the extraction efficiency is distinctly less influenced

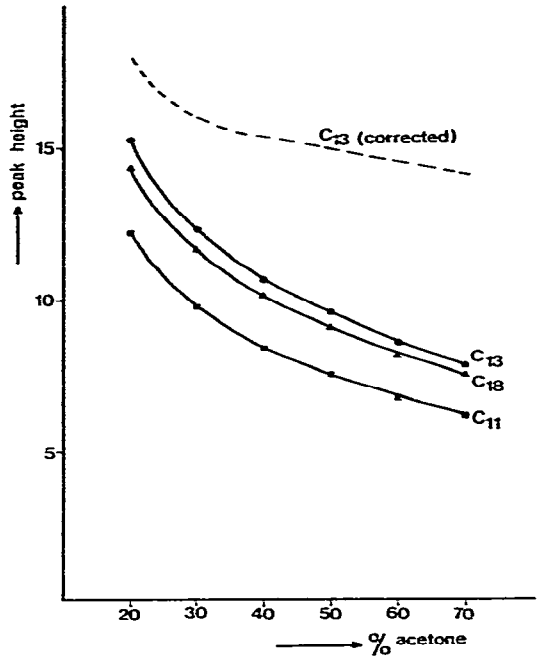

Fig. 5. Effect of acetone content of the mobile phase on the response of the ion-pair extraction detection system for a number of alkylsulphonates. Solutes: C_{11}, C_{13}, C_{18} alkylsulphonates.

by the acetone content of the mobile phase (see broken line in Fig. 5). The background caused by the extraction of acridinium salts in the absence of surfactants was found to be almost independent of the acetone content and even decreases at larger acetone contents because of the dilution effect. This behaviour of the ion-pair extraction system allows the application of an acetone gradient.

Analytical aspects

On the basis of the results in Figs. 2–5, one can determine the chromatographic conditions for the separation of alkylsulphonates. For a limited number of successive members of homologues an isocratic system can be used, as is shown in Fig. 6.

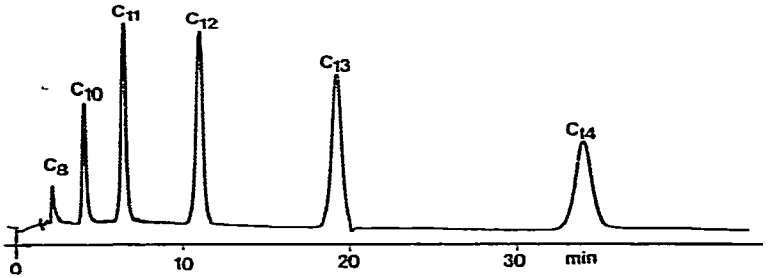

Fig. 6. Isocratic separation of a test mixture of linear alkylsulphonates. Stationary phase: Hypersil SAS. Mobile phase: water–acetone (6:4) + 0.05 *M* NaH_2PO_4. Solutes: C_8–C_{14} alkylsulphonates.

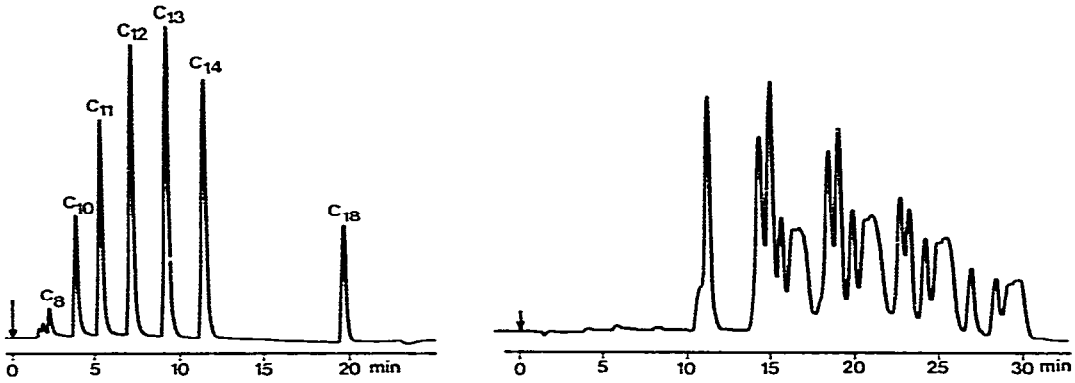

Fig. 7. Separation of a test mixture of linear alkylsulphonates by using gradient elution. Stationary phase: Hypersil SAS. Mobile phase: eluent A, water–acetone (6:4) + 0.05 *M* NaH_2PO_4; eluent B: water–acetone (4:6); gradient starting from eluent A, to eluent B in 20 min with a linear gradient. Solutes: C_8–C_{18} alkylsulphonates.

Fig. 8. Chromatogram of a commercial surfactant formulation of alkyl ether sulphates (3Mol EO). Stationary phase: Hypersil SAS. Mobile phase: gradient as in Fig. 7. Solute: Serdet DCK 30.

However, for the separation of mixtures containing C_{10}–C_{18} alkyl chains excellent results are obtained by using an acetone gradient, as is shown in Fig. 7. The analytical aspects of the method were investigated by injection of solutions containing known concentrations of alkylsulphonates and measuring peak heights. Under the selected conditions the calibration graph was found to be linear up to 4 μg alkylsulphonate injected. At higher concentrations the signal deviates from linearity. The calculated detection limit, defined as four times the standard deviation of the background noise, ranges between 1 and 5 ng (C_{18}–C_{10}), in agreement with earlier reports[12].

Applications

Commercial surfactant formulations. The applicability of reversed-phase chromatography combined with ion-pair extraction detection for the analysis of the constituents within a class of anionic surfactants is demonstrated in Figs. 8 and 9. The separation of a commercial mixture of C_{12}–C_{14} alkyl ether sulphates (3Mol EO) is shown in Fig. 8, from which it can be seen that a regular cluster of peaks appear, probably owing to isomers with similar carbon contents but varying numbers of ethylene oxide (EO) groups attached to the alkyl chains. Fig. 9 is a chromatogram of a commercial surfactant mixture of secondary alkylsulphonates with C_{13}–C_{17} alkyl chains. Also in this chromatogram a regular cluster of peaks can be seen, which probably are isomers with the same carbon content.

Figs. 8 and 9 demonstrate clearly the potential of the developed HPLC–ion pair detection system for the characterization of commercial surfactant formulations.

Water samples. The determination and characterization of the type of surfactants in waste and surface waters is important in tracing possible pollution sources and in particular for following the biodegradability of surfactants. As the concentration level of surfactants in surface water is usually at the ppb level, a pre-concentration step has to applied prior to the analysis by HPLC. The most effective means of preconcentrating solutes present in water samples is by adsorbing them on a small pre-

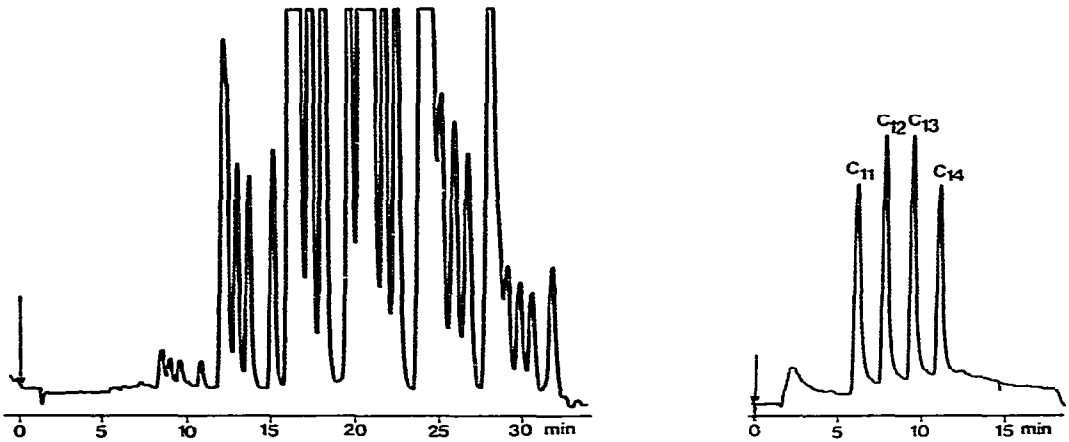

Fig. 9. Chromatogram of a commercial surfactant formulation of secondary alkylsulphonates. Stationary phase: Hypersil ODS. Mobile phase: eluents A and B as in Fig. 7; gradient starting with 99% A + 1% B in 35 min to 5% A + 95% B and then in 5 min to 1% A + 99% B. Solute: SAS 30.

Fig. 10. Chromatogram of a water sample spiked with C_{10}–C_{14} alkylsulphonates (20 ppb of each) after on-line pre-concentration of 50 ml on a small reversed-phase column. Stationary phase: analytical column, Hypersil ODS; pre-concentration column, Hypersil SAS. Mobile phase: eluent A, water–acetone (55:45) + 0.05 *M* NaH_2PO_4; eluent B, water–acetone (35:65); gradient. Starting with 75% A + 25% B, then in 10 min to 10% A + 90% B.

concentration column[17]. The volume of water sample that can be handled on such columns and thus the enrichment factor depend on the degree of retention of the solutes on the selected column packing when water is the mobile phase and on the adsorption capacity of the packing.

The pre-concentration of surfactants on small columns (10 × 4.6 mm I.D.) was investigated with water samples spiked with a number of alkylsulphonates. From Fig. 2 it can be seen that on extrapolating k' to zero acetone content, the retention of the alkylsulphonates on the reversed-phase support becomes very large. It is therefore logical to select a reversed-phase packing for the isolation of the surfactants. As k' was found to be 2.5 times larger on Hypersil ODS than on Hypersil SAS, the former packing is the obvious choice. However, from the chromatographic point of view it is advantageous, when applying on-line pre-concentration, to use a packing for the pre-concentration column that is less retentive than the packing used for the analytical column[18]. Therefore, in this study Hypersil SAS was selected as the packing for the pre-concentration column and Hypersil ODS for the analytical column. Fig. 10 shows a chromatogram obtained after on-line concentration of 50 ml of a water sample spiked with C_{11}–C_{14} alkylsulphonates (20 ppb of each solute). In order to determine the recovery of the isolation procedure, 50-ml water samples spiked with varying amounts of alkylsulphonates were pre-concentrated and analysed by HPLC. The recovery ranged from 70% for C_{10} to 90% for C_{14} in the range 4–80 ppb. It must be noted that serious losses occurred when the water samples came into contact with synthetic materials such as PTFE tubing, as the surfactants adsorb irreversibly on these materials. This effect is larger the lower is the surfactant concentration.

The reproducibility of the on-column concentration was also investigated by concentrating five times 50-ml volumes of a standard solution containing 20 ppb of

alkylsulphonates. The relative standard deviation of the recovery ranged between 0.5 and 1.6%, which indicates that despite the losses, the method is reproducible.

CONCLUSIONS

Reversed-phase liquid chromatography with water–acetone mixtures as mobile phase and post column ion-pair extraction detection is very useful for the analysis of homologues within a class of anionic surfactants. Acetone seems to be a favourable organic modifier in combination with ion-pair extraction systems because of its solubility in chloroform which is used as extraction solvent. This permits the application of an acetone gradient and thus the separation of a large number of homologues of surfactants in one chromatographic run.

The HPLC–detection system developed can be used for the characterization of commercial surfactant formulations and for the analysis of surfactants in water samples at ppb level after on line pre-concentration on a small reversed-phase column. This latter application looks promising to follow the biodegradability of anionic surfactants.

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REFERENCES

1 G. F. Longman, *The Analysis of Detergents and Detergent Products*, Wiley, Chichester, 1975, p. 157.
2 M. Uchiyama, *Water Res.*, 11 (1977) 205.
3 C. M. Coyne and G. A. Maw, *J. Chromatogr.*, 14 (1964) 552.
4 F. Püschel and D. Prescher, *J. Chromatogr.*, 32 (1968) 337.
5 R. Takeshita, N. Jinnai and H. Yoshida, *J. Chromatogr.*, 123 (1976) 301.
6 D. Frahne, S. Schmidt and H. G. Kuhn, *Fette, Seifen, Anstrichm.*, 79 (1977) 32 and 122.
7 H. König, *Z. Anal. Chem.*, 254 (1971) 337.
8 E. L. Sones, J. L. Hoyt and A. J. Sooter, *J. Amer. Oil Chem. Soc.*, 56 (1979) 689.
9 A. Nakae and K. Kunihiro, *J. Chromatogr.*, 152 (1978) 137.
10 P. W. Taylor and G. Nickless, *J. Chromatogr.*, 178 (1979) 259.
11 D. Thomas and J. L. Rocca, *Analusis*, 7 (1979) 386.
12 C. P. Terweij-Groen, J. C. Kraak, W. M. A. Niessen, J. F. Lawrence, C. E. Werkhoven-Goewie, U. A. Th. Brinkman and R. W. Frei, *Int. J. Environ. Anal. Chem.*, 9 (1981) 45.
13 J. F. Lawrence, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr.*, 185 (1979) 473.
14 C. van Buuren, J. F. Lawrence, U. A. Th. Brinkman, I. L. Honigberg and R. W. Frei, *Anal. Chem.*, 52 (1980) 700.
15 E. Tomlinson, H. Poppe and J. C. Kraak, in E. Reid (Editor), *Blood, Drugs and Other Analytical Challenges*, Ellis Horwood, Chichester, 1978, p. 207.
16 G. E. Berendsen, P. J. Schoenmaker, L. de Galan, G. Vigh, Z. Varga-Puchony and J. Inczedy, *J. Liquid Chromatogr.*, 3 (1980) 1669.
17 C. E. Werkhoven-Goewie, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 53 (1981) 2072.
18 J. Lankelma and H. Poppe, *J. Chromatogr.*, 149 (1978) 587.