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Separation of novel polyol surfactants on polystyrene and octadecylsilyl bonded silica columns

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ABSTRACT

The separation of a series of novel basic polyol surfactants, which exist as cations in aqueous solution, and their synthetic precursors have been compared using ion-suppression chromatography at pH 12 on a polystyrene–divinylbenzene column or ion-pair chromatography on an ODS column using hexanesulphonic acid as an ion-pair reagent. The latter method appeared to be the more versatile. Reversed-phase chromatography was also used for the separation of a related non-ionic diamide polyol surfactant.

INTRODUCTION

Surfactants play an important commercial role in many industries and their determination is important in quality control [1,2]. Many are ionised and contain either anionic sulphonic acid groups or cationic quaternary ammonium groups. The latter compounds are usually based on quaternary aliphatic amines, such as cetrimide (hexadecyltrimethyl ammonium bromide) or benzylalkyl ammonium compounds. Many of these products have caused concern as pollutants in water supplies and in recent years a number of biologically based surfactants [3] and carbohydrate- or polyol-based materials have been developed with greater biodegradability [4–6]. Many of these are industrial chemicals but polyol surfactants are now being developed for the biotechnology, biomedical and pharmaceutical fields. This has resulted in an increased interest in their purity and composition.

However, although a number of methods have been reported for anionic and non-ionic surfactants, so far there has been little work on the chromatographic analysis of cationic surfactants [1]. In most cases cation-exchange separation methods have been used but the absence of a chromophore in the quaternary alkyl ammonium compounds means that detection is a problem. Conductometric detectors [7,8], mass evaporative light-scattering detection [9] or post-column ion-pair extraction [10] have been used. In recent papers, capillary isotachophoresis [11] and capillary zone electrophoresis [12] of cationic surfactants have been described but detection was still a problem.

This paper compares ion-suppression and ion-pairing high-performance liquid chromatography (HPLC) for the determination of a series of recently developed basic polyol surfactants [4], (**I–V**), which are present as cations in aqueous solution, their synthetic precursor diepoxides (**VI–VII**) and the related non-ionic polyol amide surfactant [5] (**VIII**) (Fig. 1).

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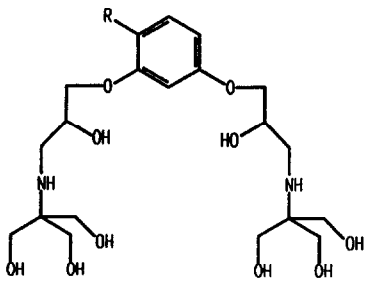
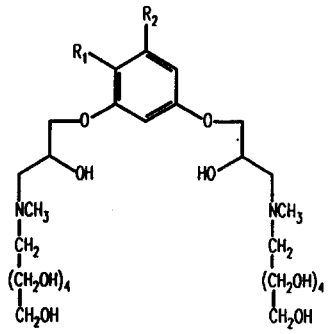


Hexohexool (I) $R = C_6H_{13}$

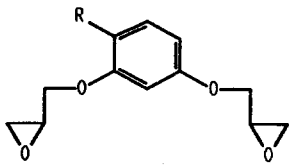
Dodecylhexool (II) $R = C_{12}H_{25}$



Hexyldecool (III) $R_1 = C_6H_{13}$ $R_2 = H$

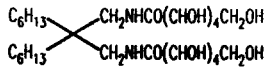
Dodecyldecool (IV) $R_1 = C_{12}H_{25}$ $R_2 = H$

Pentadecyldecool (V) $R_1 = H$ $R_2 = C_{15}H_{31}$



Hexyldiepoxide (VI) $R = C_6H_{13}$

Dodecyldiepoxide (VII) $R = C_{12}H_{25}$



Dihexyldecool amide (VIII)

Fig. 1. Structure of surfactants and related compounds.

EXPERIMENTAL

Chemicals and materials

The basic surfactants **I–V**, their precursors **VI** and **VII** and the non-ionic surfactant **VIII** were provided by Kodak Research Laboratory, Harrow, UK.

Methanol, acetonitrile and hexanesulphonic acids were of HPLC grade from Fisons Scientific, Loughborough, UK. Acetic acid, ammonium acetate and sodium hydroxide were of laboratory-reagent grade.

HPLC system

The liquid chromatograph consisted of a Waters M600A pump (Millipore, Milford, MA), a Shimadzu GTO-6A column oven (Kyoto, Japan) and a Philips PU4020 variable-wavelength detector (Cambridge, UK). The samples (10 μl) were injected using a Rheodyne 7125 valve (Cotati, CA, USA) fitted with a 20-μl loop onto either a PLRP-S polystyrene–divinylbenzene (PS–DVB) column (5 μm, 150 × 4.6 mm, Polymer Labs., Church Stretton, UK) or a 100 × 4.6 mm column packed with Hypersil 5 ODS (5 μm, Shandon

Scientific, Runcorn, UK). The eluent was degassed before use and was pumped at 1 ml min^{-1}. The peaks were recorded on a Hewlett-Packard 3390A integrator.

Separations using the PS–DVB column

The analytes were separated on the PS–DVB column at 45°C using acetonitrile–pH 12 aqueous sodium hydroxide solutions as the eluents and detection at 210 nm.

Separations using ion-pair reagents

The analytes were separated on the Hypersil ODS column at 35°C using acetonitrile–pH 4.5 buffer solutions containing 0.004 *M* 1-hexanesulphonic acid with detection at 210 nm. The pH 4.5 buffer solution contained ammonium acetate (3.13 g l^{-1}) and acetic acid (3 ml l^{-1}).

Separation of amide surfactants

The amide surfactant (**VIII**) was separated on the Hypersil ODS column at 35°C using acetonitrile–water (50:50) as the eluent and detection at 199 nm.

RESULTS AND DISCUSSION

The basic polyol surfactants (**I–V**, Fig. 1) studied in the present work have novel structures and contain both a prominent alkyl side chain and two secondary amino groups each substituted with a polyhydroxylated side chain. They had been synthesised from the corresponding alkyldiepoxides (*e.g.* **VI** and **VII**). The surfactants had a weak absorbance band at 280 nm but absorbed strongly at short wavelengths and 210 nm was selected as a suitable wavelength for detection.

The amino groups in these surfactants are strongly basic. In preliminary studies by HPLC on a reversed-phase column, it was found that they were protonated over the acceptable pH range (3–8) for ODS bonded silica-based column materials and were not retained even with a 100% aqueous eluent. Two approaches, ion-suppression chromatography at high pH on a polymeric column and ion-pair chromatography on an ODS-silica column, were therefore examined.

Separation using ion-suppression chromatography

When **IV** was examined on a PS–DVB column using acetonitrile–water (50:50) adjusted to pH 10.9 with ammonia as the mobile phase, it was found that the surfactant was retained but the peak shape was poor with considerable tailing. Increasing the basicity of the aqueous solution to pH 12 with 0.2 *M* sodium hydroxide gave a marked improvement in peak shape and reproducible retention times. Raising the column temperature to 45°C also improved the peak shape.

These conditions of acetonitrile–pH 12 sodium hydroxide solution (50:50) were then applied to the two dodecyl (**II** and **IV**) and the pentadecyl surfactants (**V**) and in each case the peak shapes were good. Surprisingly there was only a small difference between the retention times of **II** ($k' = 3.31$) and the more highly hydroxylated **IV** ($k' = 3.18$, Fig. 2a) and a mixture of the two compounds could not be resolved. Both were well separated from **V** ($k' = 9.71$). These results suggested that the retention was dominated by the interaction of the alkyl side chain with the PS–DVB column and that the hydroxyl groups played only a minor part. This would accord with previous studies, which have suggested that hydroxyl groups are strongly excluded from PS–DVB materials causing an enhancement of the

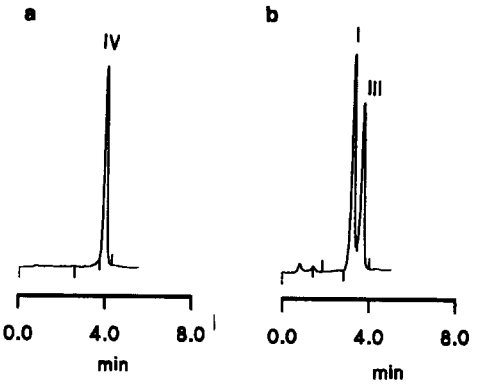


Fig. 2. Separation of the surfactants on a PS–DVB column using ion-suppression chromatography. Conditions: column, PLRP-S at 45°C; detection, 210 nm. (a) **IV**: eluent, acetonitrile–pH 12 sodium hydroxide solution (50:50). (b) **I** and **III**; eluent, acetonitrile–pH 12 sodium hydroxide solution (33:67).

differences between polar and non-polar analytes [13]. However, with these eluents the surfactants with a hexyl side chain (**I** and **III**) were eluted very close to the solvent front.

On increasing the proportion of the aqueous component of the eluent to give acetonitrile–pH 12 sodium hydroxide solution (33:76), these smaller compounds were retained but the order of elution of **I** ($k' = 2.53$) and **III** ($k' = 3.07$) surfactants (Fig. 2b) was unexpected as the latter with more hydroxyl groups was expected to be the more polar and thus to be eluted more rapidly.

A calibration curve was prepared for each of the analytes in the corresponding eluent for a reasonable retention. In each case a linear response was obtained over the concentration ranges 1 to 0.1 mg ml^{-1} with a correlation between 0.9993 and 0.9984 (Table I). However, the calibration curves did not go through the origin suggesting that a small but systematic loss was occurring, up to a maximum of 0.065 mg ml^{-1} for **IV**, although the reason for this could not be determined. The limits of detection corresponded to about 100–300 ng which is similar to the levels reported for other aromatic surfactants [14].

TABLE I

SEPARATION OF THE SURFACTANTS USING ION-SUPPRESSION AND ION-PAIR MODES

Ion-suppression mode conditions: column, PLRP-S; eluent, acetonitrile–pH 12 sodium hydroxide solution; detection, 210 nm. Ion-pair mode conditions: column, Hypersil 5 ODS; eluent, acetonitrile–pH 4.5 ammonium acetate buffer containing 0.004 *M* hexanesulphonic acid; detection, 210 nm. Correlations of calibration curve for solutions of surfactant over the range 0.1 to 1.0 mg ml^{-1}.

Compound	Modifier (%)	k'	Correlation
Ion-suppression mode			
I	33	2.53	0.9989
III	33	3.07	0.9984
II	50	3.31	0.9992
IV	50	3.18	0.9985
V	50	9.71	0.9993
Ion-pair mode			
I	30	4.43	0.9995
III	30	2.42	0.9996
II	50	8.11	0.9992
IV	50	3.62	0.9995
V	70	4.03	0.9945

In order to examine the purity of the surfactants it was also necessary to be able to determine their synthetic alkyldiepoxide precursors. These are relatively much less polar compounds and were highly retained under the conditions used for the corresponding surfactants. It was therefore necessary to employ much higher proportions of acetonitrile to obtain reasonable retentions. The hexyldiepoxide (**VI**) could be eluted with acetonitrile–pH 12 solution (75:25) ($k' = 7.65$) but the corresponding hexyl surfactants were very rapidly eluted (**I**, $k' = 0.60$; **III**, $k' = 0.75$) under these conditions. For **VII**, a stronger eluent acetonitrile–pH 12 solution (90:10) was required ($k' = 11.61$) and the dodecyl surfactants were rapidly eluted (**II**, k' 1.5 and **IV**, $k' = 1.1$). In both eluents it was not practical to quantify the starting materials and surfactants in a single isocratic run because the latter were eluted close to the major solvent disturbance caused by the aqueous solvent used to dissolve the sample.

Separation using ion-pair reagent

Although the PS–DVB column would give a good separation of the surfactants and their starting materials, the differences in their polarities meant that separate assay conditions would be needed. It was therefore decided to examine the potential of ion-pair separations, which might enable both products and starting materials to be assayed in a single determination.

Surfactants **II** and **IV** were chromatographed using an ODS-silica column with acetonitrile–pH 4.5 ammonium acetate buffer (50:50) containing 0.004 *M* hexanesulphonic acid. Unlike the ion-suppression separation **IV** ($k' = 3.62$) was eluted much more readily than **II** ($k' = 8.11$). The pentadecyldecaol (**V**) required a stronger eluent of acetonitrile–buffer (70:30) containing 0.004 *M* hexanesulphonic acid for elution ($k' = 4.03$).

By using a weaker eluent, acetonitrile–buffer (30:70) containing 0.004 *M* hexanesulphonic acid, the hexyl surfactants could be retained and again the decaol (**III**, $k' = 2.42$) was eluted more

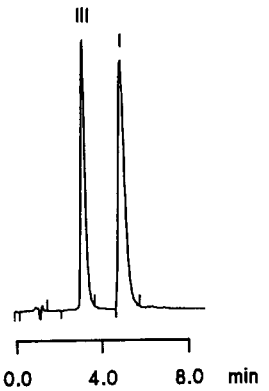


Fig. 3. Separation of hexyl surfactants **I** and **III** using an ion-pair reagent. Conditions: column, Hypersil 5 ODS; temperature, 35°C; eluent, acetonitrile–pH 4.5 ammonium acetate buffer (30:70) containing 0.004 *M* hexanesulphonic acid; detection, 210 nm.

rapidly than the hexaol (**I**, $k' = 4.43$) (Fig. 3). Thus under the ion-pairing conditions the analytes were behaving as expected with the more highly hydroxylated analytes being the more rapidly eluted in each case. For each surfactant a calibration curve showed a linear correlation (r = 0.9995–9945) over the concentration range 1 to 0.1 mg ml^{-1} and a limit of detection of 20–30 ng.

Acetonitrile–buffer (70:30) containing 0.004 *M* hexanesulphonic acid was needed to elute the hexyldiepoxide starting material (**VI**, $k' = 3.71$) but under these conditions the two hexyl surfactants were rapidly eluted with capacity factors of less then 0.5. For the dodecyldiepoxide a stronger eluent of acetonitrile–buffer (80:20) containing 0.004 *M* hexanesulphonic acid was required (**VII**, $k' = 12.12$). Examination of a deliberately impure sample of **IV** showed the product at $k' = 0.9$ and an impurity peak at $k' = 3.13$. When the purified surfactant product was examined this peak was absent but a trace of starting material was present. Thus it appears that ion-pairing separation conditions are better then the ion-suppression technique for the determination of impurities in these products. However, there were still significant differences between the retentions of the starting materials and the ion-paired products. The use of ion-pairing reagents with a longer alkyl side chain or in higher concentrations might give more comparable retentions if these were required.

Methanol–buffer eluents containing the ion-pair reagent were also examined for the chromatography of the surfactants. However, although by using methanol–buffer (65:35) containing 0.004 *M* hexanesulphonic acid the two hexyl polyols were retained, they were unresolved (**I**, $k' = 1.74$ and **III**, $k' = 1.95$) and the two dodecyl surfactants were unresolved in methanol–buffer (85:15) containing 0.004 *M* hexanesulphonic acid (**II**, $k' = 3.00$; **IV**, $k' = 2.94$). The peaks were also not as symmetrical as in the acetonitrile eluents.

Amide surfactants

The related aliphatic amide polyol surfactant (**VIII**) was also examined. Because of its limited chromophore, detection at 199 nm was employed. Using an eluent of acetonitrile–water (40:60) a good retention was obtained on the Hypersil 5 ODS column ($k' = 2.18$) with a symmetrical peak shape. A quantitative determination found a limit of detection of 260 ng. This is poorer than for the earlier surfactants because of the weaker chromophore.

CONCLUSIONS

Alternative methods could be employed for the chromatography of these surfactants. The choice will depend of the levels of detection required, the reason for the analysis and whether it is necessary to resolve the homologues.

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