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# SEPARATION AND QUANTITATIVE DETERMINATION OF LONG-CHAIN ALKYLTRIMETHYLAMMONIUM IONS BY REVERSED-PHASE ION-PAIR LIQUID CHROMATOGRAPHY USING ULTRAVIOLET-ABSORBING COUNTER IONS

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## SUMMARY

A fast and reliable high-performance liquid chromatographic method has been elaborated for the separation and quantitative determination of long-chain alkyltrimethylammonium ions. The separation was achieved on a Nucleosil CN column with methanol–water (55:45) containing 5 m*M* *p*-toluenesulphonic acid as the eluent. UV detection at 254 nm was possible owing to the added *p*-toluenesulphonic acid. The influence of various types of chemically bonded phases on the efficiency of the separation has been investigated and the more polar types such as bonded phenyl and bonded cyanopropyl were found to cause a better peak shape than bonded $C_8$ or $C_{18}$ alkyl chains. Furthermore, it was found that *p*-toluenesulphonate as the counter ion relative to 2-naphthalenesulphonate resulted in a better peak shape, albeit with a lower response. Mixtures of homologous alkyltrimethylammonium bromides, including the widely used disinfectant cetrimide, have been analysed.

## INTRODUCTION

During recent work on high-performance liquid chromatography (HPLC) on dynamically modified silica (*e.g.*, refs. 1–4) quantitation of long-chain alkyltrimethylammonium ions in solution was required. At an early stage ion-pair extraction followed by UV measurements was used[2]. According to this method, however, it was not possible to analyse the individual components in mixtures of homologues, nor was it possible to achieve reproducible results for the analysis of the compounds of greatest chain length at high concentrations. An HPLC method based on refractive index detection was subsequently used[3,4], but was not very sensitive.

Liquid chromatographic methods for the separation of alkyltrimethylammonium surfactants have been published previously, *e.g.* by Fudano and Konishi[5] who used salting-out chromatography and off-line detection by titrimetry. Even HPLC methods based on reversed-phase ion-pair partition have been published, *e.g.* by Nakamura and Morikawa[6]. None of these systems was considered to be well suited

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for the present purpose, however, owing to low speed of analysis[5] or to the use of the rather insensitive detection method of refractometry[6].

Recently, several papers have appeared dealing with detection in HPLC of non-absorbing, ionic compounds by means of reversed phase ion-pair chromatography using UV-absorbing counter ions (*e.g.* refs. 7–11). None of the published chromatographic systems were found to be well suited for the surfactant-type alkyltrimethylammonium ions, however, as these exhibited considerable peak tailing.

In the present paper the elaboration of a reversed-phase ion-pair liquid chromatographic method using a UV-absorbing counter ion and specially designed for the separation and determination of alkyltrimethylammonium surfactants is described.

## EXPERIMENTAL

### *Chemicals*

Dodecyltrimethylammonium (DTMA) bromide and tetradecyltrimethylammonium (TTMA) bromide were obtained from Sigma (St. Louis, MO, U.S.A.). Stearyltrimethylammonium (STMA) bromide was obtained from Fluka (Buchs, Switzerland). Cetrimide was of pharmacopoeial quality. Sodium naphthalene-2-sulphonate of technical grade (E. Merck, Darmstadt, G.F.R.) was purified with activated charcoal and recrystallized from water. All other reagents including cetyltrimethylammonium (CTMA) bromide were of analytical-reagent grade (E. Merck).

### *Chromatography*

A liquid chromatograph consisting of a Kontron Model 410 LC pump, a Pye Unicam LC UV spectrophotometer detector operated at 254 nm and a Rheodyne Model 7125 injection valve equipped with a 20-$\mu$l loop was used. Chromatograms were recorded on a Kipp & Zonen Model BD-8 recorder and retention data were measured and processed by means of a Hewlett-Packard Model 3353 A laboratory data system.

All experiments were performed on 120 or 250 × 4.6 mm columns (Knauer, Oberursel, G.F.R.) packed as earlier described[12] with Nucleosil silicas of 5- or 7-$\mu$m particle size bearing various chemically bonded phases (Macherey-Nagel, Düren, G.F.R.). The eluent consisted of methanol–water mixtures containing 0.4 m*M* sodium naphthalene-2-sulphonate and 10 m*M* phosphoric acid, or containing 5 m*M* *p*-toluenesulphonic acid.

### *Test and standard solutions*

Solutions for constructing the calibration curve were 0.016–16 m*M* solutions of each of DTMA, TTMA, CTMA and STMA bromides in the eluent.

The standard solution for quantitative determinations was a 0.8 m*M* solution of each of DTMA, TTMA, CTMA and STMA bromides in the eluent.

For the determination of the adsorbed amount of surfactant on the dynamically coated silica columns, the column was eluted with 50.0 ml of methanol–0.05 *M* phosphoric acid (1:1). The solution was diluted 1:10 with eluent.

The cetrimide soluting was a 0.125% solution in the eluent.

Samples of the solutions (20 $\mu$l each) were injected by means of the loop.

## RESULTS AND DISCUSSION

For the elaboration of the separation method the test solute was a mixture of four surfactant-type quaternary ammonium ions, DTMA, TTMA, CTMA and STMA, previously used as additives to the eluent in HPLC on dynamically coated silica[2]. The starting point for the investigations was the methods published by Schill *et al.*[9,11] which made it possible to separate several symmetrical tetraalkylammonium ions. These separations were performed on chemically bonded phenyl silica using aqueous phosphate buffers with the addition of 0.4 m*M* naphthalene-2-sulphonate as eluents. The surfactant-type quaternary ammonium ions of the present investigation, however, exhibit a far more pronounced affinity to the stationary phase than do the symmetrical tetraalkylammonium ions. Hence it proved necessary to add to the eluent a considerable amount of an organic modifier (methanol) to achieve a sufficiently fast elution. Even then an adequate separation was not achieved, as considerable peak tailing occurred. It is well known that amines and ammonium compounds may exhibit peak tailing in reversed-phase chromatography, possibly due to their affinity for residual silanol groups, and that this problem can often be overcome by the addition of unsymmetrical tertiary amines as so-called tailing reducers[13], *i.e.* cationic compounds of even higher affinity for the silanol groups than the solute. In this case the addition of tailing reducers is not a suitable solution for two reasons. First, it would be difficult to find compounds of higher affinity to silanol groups than the compounds here in question. Furthermore, such compounds would also exhibit a high affinity for the UV-absorbing counter ion and in this way compete with the solute ions resulting in a considerable decrease in the response factor.

It was investigated whether it was possible to improve the peak shape by changing the bonded-phase column material or the UV-absorbing counter ion. Table I shows the variations in peak shape and response for one of the four surfactant ions, STMA, using four different column materials and naphthalene-2-sulphonate or *p*-

TABLE I

INFLUENCE OF VARIATIONS IN BONDED PHASE MATERIAL AND COUNTER ION ON RESPONSE AND EFFICIENCY OF SEPARATION AS EXPRESSED BY NUMBER OF THEORETICAL PLATES ($N$) AND ASYMMETRY FACTOR ($A_s$) FOR THE PEAK CORRESPONDING TO STMA

| *Column* | *Dimension (mm)* | *N in test** | *Counter ion*** | *N (STMA)* | $A_s$ *(STMA)* | *Response**** |
|---|---|---|---|---|---|---|
| Nucleosil 5 $C_{18}$ | 4.6 × 120 | 3400 | NS | 72 | 2.7 | 33 |
| | | | TS | 300 | 3.2 | 21 |
| Nucleosil 5 $C_8$ | 4.6 × 120 | 4080 | NS | 60 | 3.1 | 38 |
| | | | TS | 410 | 2.2 | 14 |
| Nucleosil 7 $C_6H_5$ | 4.6 × 250 | 12,080 | NS | 245 | 2.8 | 138 |
| | | | TS | 765 | 2.5 | 25 |
| Nucleosil 5 CN | 4.6 × 120 | 5730 | NS | 410 | 4.6 | 90 |
| | | | TS | 770 | 1.9 | 38 |

* Measured on naphthalene peak when chromatographed with an appropriate methanol–water mixture as the eluent.

** NS = Naphthalene-2-sulphonate; TS = *p*-toluenesulphonate.

*** Area of STMA peak in mm$^2$.

toluenesulphonate as the counter ion. It appears from the table that the use of *p*-toluenesulphonate results for most columns in a lower response relative to that achieved with naphthalene-2-sulphonate, but on the other hand leads to a more efficient separation, *i.e.* an increase in the number of theoretical plates and a decrease in the asymmetry factor. Furthermore, it appears from Table I that the use of the more polar bonded phases (particularly the cyanopropyl) gives rise to more symmetrical peaks than do the apolar bonded alkyl materials.

The counter-ion-induced variation in response is partly due to the difference in concentration (5 m*M* and 0.4 m*M*, respectively, for *p*-toluenesulphonate and naphthalene-2-sulphonate) needed to reach the same absorbance level for the eluent. Thus the relative change in concentration caused by the solute ions is less for the counter ion of higher concentration (*p*-toluenesulphonate). It has been shown previously by Schill *et al.*[9,11] that the response may also depend on the relative polarities of solute ion and counter ion as well as that of the bonded-phase material. The above-mentioned alterations in peak shape might be a result of the changes in both polarity and in molecular size. However, further elucidation of the mechanism behind the separations has not yet been possible.

It was considered that the drawback due to the reduced response when using *p*-toluenesulphonate was sufficiently compensated for by the improved peak shape, and hence *p*-toluenesulphonate in connection with the cyanopropyl column was preferred for the separations. Fig. 1 shows chromatograms obtained using phenyl and

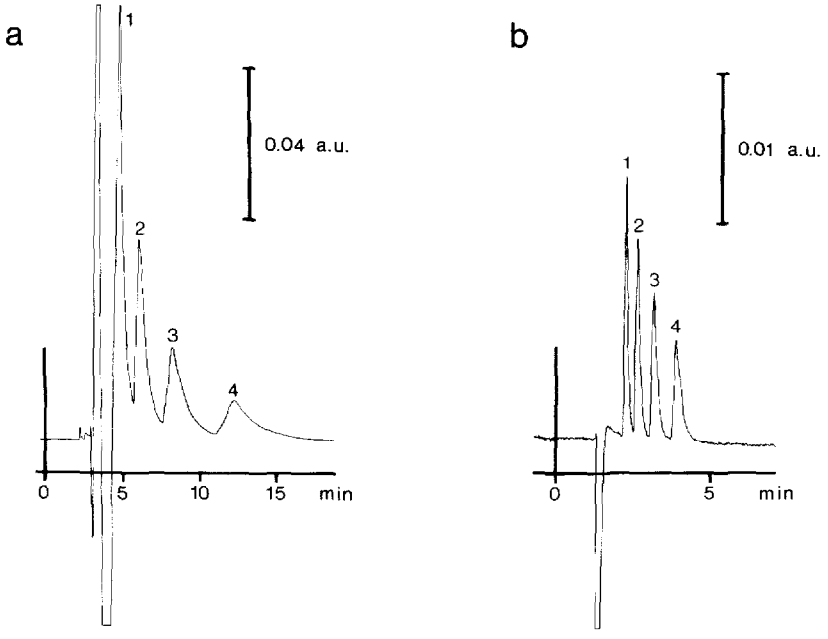


Fig. 1. Chromatograms of *ca.* 16 nmol of each of four alkyltrimethylammonium bromides. Columns: a, Nucleosil 7 $C_6H_5$ (250 × 4.6 mm); b, Nucleosil 5 CN (120 × 4.6 mm). Eluents: a, methanol–water (75:25) containing 0.4 m*M* sodium naphthalene-2-sulphonate and 10 m*M* phosphoric acid; b, methanol–water (55:45) containing 5 m*M* *p*-toluenesulphonic acid. Detection wavelength: 254 nm. Flow-rate: 1 ml/min. Peaks: 1 = DTMA; 2 = TTMA; 3 = CTMA; 4 = STMA.

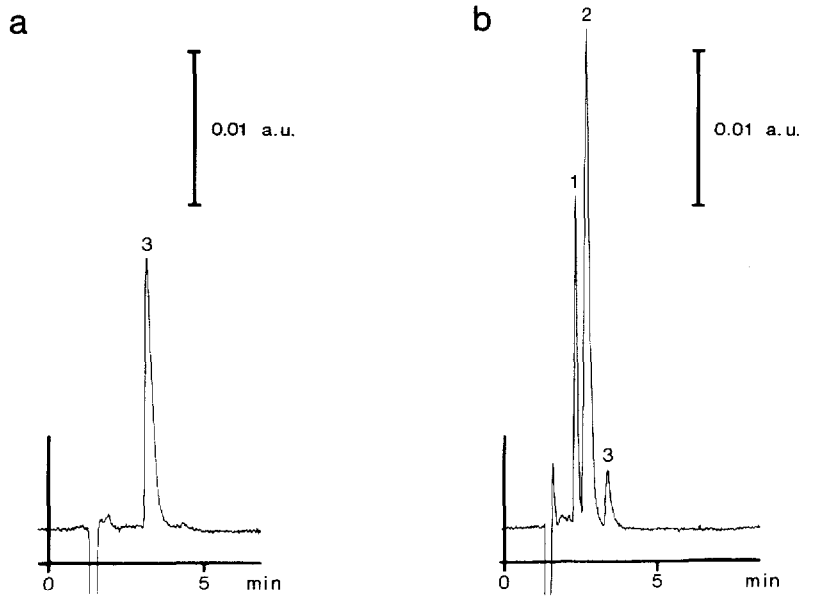


Fig. 2. Chromatograms of CTMA eluted from a dynamically modified silica column (a) and a sample of cetrimide (*ca.* 25 μg) (b). Chromatographic conditions and symbol key as in Fig. 1b.

cyanopropyl columns and the two different counter ions. It appears from the chromatograms that the negative system peak (*cf.* ref. 9) is eluted at the dead time.

Utilizing the chromatographic method described above the concentrations of various surfactants eluted from dynamically modified silica columns have been determined. Samples of cetrimide of pharmacopoeial quality have been analysed as well. For quantitation external standardization was used. Detector-response linearities for each of the four quaternary ammonium compounds, DTMA, TTMA, CTMA and STMA, were established for injected amounts ranging between 0.4 nmol (the detection limit) and 64 mmol. During the linearity experiments it was found, as previously reported by several authors (*e.g.* refs. 8 and 10), that the capacity factor, $k'$, decreases for increasing amounts of solute and hence that peak areas, and not peak heights, must be used for the quantitations. It was also confirmed, as reported by Parris[8], that the change in $k'$ with sample amount is much more pronounced when naphthalene-2-sulphonate is the counter ion rather than *p*-toluenesulphonate, which is yet an advantage to the latter. The precision of the method was investigated by analysing ten individually prepared solutions containing *ca.* 0.8 m*M* solutions of each of the four surfactants; the relative standard deviations ranged between 1.1 and 1.7%. Fig. 2 shows chromatograms of CTMA eluted from a dynamically coated silica column and of a sample of cetrimide.

## CONCLUSION

An HPLC method has been elaborated for the separation and determination of long-chain alkyltrimethylammonium compounds. The method is based on revers-

ed-phase ion-pair chromatography utilizing a UV-absorbing counter ion. By proper choice of the counter ion and of the bonded-phase column material it has proved possible to counteract the problem of peak tailing which is particularly pronounced for the surfactant-type solutes in question.

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