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Separation and determination of quaternary ammonium compounds by high-performance liquid chromatography with a hydrophilic polymer column and conductometric detection

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Abstract

An HPLC method has been developed for separation and determination of long alkyl chain quaternary ammonium compounds. A column packed with a hydrophilic polymer packing, Shodex Asahipak GF-310 HQ, and a water–acetonitrile mixture containing 4,4'-bipyridyl and hydrochloric acid were used to depress hydrophobic adsorption of the quaternary ammonium compounds and increase the sensitivity of the conductometric detection with a micromembrane suppressor. Dodecyltrimethylammonium, cetyltrimethylammonium, tetradecyldimethylbenzylammonium and stearyltrimethylammonium ions can be completely separated from one another and quantified at 0.1 nmol level. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Quaternary ammonium compounds containing at least one long alkyl chain act as cationic surfactants and are widely used as fabric softeners, disinfectants and human hair cosmetics. After usage, the surfactants are generally discharged via waste water treatment facilities to surface waters. Hence they can disturb the ecological balance due to their toxicity to

aquatic organisms [1,2]. In this context reliable analytical methods for determination of quaternary alkylammonium compounds are required in the analysis of environmental samples as well as in the quality control of manufactured products.

The most widely used techniques are solvent extraction followed by spectrophotometry and two-phase titration, both of which are based on the formation of ion-pair complexes with anionic dyes [3–9]. However, these methods usually need the use of toxic chlorinated organic solvents such as chloro-

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form and they lack specificity so that they suffer from many interfering compounds and are not able to differentiate individual homologues. Furthermore the procedures are tedious and the sensitivity is not very high.

HPLC is currently the most promising method for the analysis of complex cationic surfactant mixtures. Although capillary electrophoresis (CE) can also be an efficient separation method of ionic surfactants [10–14], analysis of cationic surfactants by CE is somewhat problematical because of their adsorption onto the fused-silica capillary wall [11,12]. It has been reported that CE is inferior to HPLC with respect to detection limit expressed in concentration and reproducibility for the determination of cationic surfactants [10,12].

Many quaternary ammonium compounds lack UV absorbance, and hence a direct photometric detection cannot be used. This can be overcome by using indirect UV detection technique or conductometric detection. Larson and Pfeiffer [15] adopted indirect UV detection technique for determination of aliphatic quaternary ammonium compounds by ion-exchange chromatography, where an aromatic quaternary ammonium ion is employed as a chromophore. They reported that the detection limit calculated as 2.5 times short-term noise was 0.6–0.8 μg for various quaternary alkylammonium compounds.

Conductometric method appears to offer higher sensitivity for the detection of cationic surfactants. Wee and Kennedy [16] used a cyano–amino-bonded silica column and chloroform–methanol (92:8) as mobile phase and showed that the absolute detection limit based on 2 times the standard deviation of background noise for several mono- and dialkylammonium compounds was approximately 0.01 μg . Levsen et al. [17] obtained the detection limit of 0.03–0.04 μg by using a Lichrospher 100 Diol column and methanol–tetrahydrofuran–water (45:45:10) containing hydrochloric acid. They reported that a separation of the cationic surfactants without the addition of hydrochloric acid was not possible. Presumably the use of non-aqueous solvents and the addition of hydrochloric acid to the mobile phase are necessary to elute hydrophobic cationic surfactants and to eliminate the adsorption of the surfactants onto the residual silanol groups on the surface of the silica-based column packing,

respectively. Since Wee and Kennedy [16] first presented this technique, all the HPLC methods with conductometric detection which have so far been reported utilize non-aqueous media as mobile phase and detection system without suppressor [16–19]. If possible, however, the use of toxic organic solvents such as chloroform should be avoided.

Coupling of a liquid chromatograph and a mass spectrometer may represent a powerful method for determination of trace amounts of cationic surfactants. For example, Yakata et al. [20] reported that stearyltrimethylammonium ion could be determined at ppb level by using HPLC with fast-atom bombardment mass spectrometry. However, the equipment is expensive and not commonly available.

This paper presents an HPLC method for the separation and determination of quaternary alkylammonium ions using a hydrophilic organic polymer packing as a separation medium instead of silica-based materials. This polymer packing exhibits only moderate hydrophobic adsorption and allows us to use essentially aqueous media as the mobile phase. This also means that the enhancement of the detection sensitivity can be achieved by means of suppressor system, which converts the counter anions to hydroxide ion, the anion which has the highest ionic mobility in aqueous media [21,22]. We have found out that a mixture of 4,4'-bipyridyl and hydrochloric acid is effective for the elution of cationic surfactants. It will be shown that some commonly used quaternary alkylammonium ions can well be separated primarily according to the length of their alkyl chains and determined at 0.1 nmol level by using conductometric detection system accommodated with micromembrane suppressor.

2. Experimental

2.1. Chemicals

Dodecyltrimethylammonium chloride (DTMA) and tetradecyldimethylbenzylammonium chloride (TDDBA) were obtained from Kanto Chemicals (Tokyo, Japan). Cetyltrimethylammonium bromide (CTMA) and stearyltrimethylammonium chloride (STMA) were from Wako Chemicals (Osaka, Japan) and Tokyo Kasei (Tokyo, Japan), respectively. These

quaternary ammonium compounds were used without further purification. 4,4'-Bipyridyl was analytical reagent grade obtained from Tokyo Kasei. HPLC-grade acetonitrile and methanol were from Kanto Chemicals. Hydrochloric acid used was Suprapur-grade purchased from Merck (Darmstadt, Germany). Aqueous tetramethylammonium hydroxide solution (15%) used for the preparation of regenerating solution was received from Wako Chemicals. Mobile phase and sample solutions were prepared using water purified by a Milli-Q system (Nihon Millipore, Tokyo, Japan).

The column packings used in this study were MCI GEL CQP 06 (alkylene glycol methacrylate gel, particle size 10 μm , Mitsubishi Chemical, Tokyo, Japan), Bio-Gel P-2 (polyacrylamide gel, 200–400 mesh, Bio-Rad Laboratories, Richmond, CA, USA), TSKgel Toyopearl HW-40S (poly(vinyl alcohol) gel, 20–40 μm , Tosoh, Tokyo, Japan), Shodex Asahipak GS-520 7G, GS-320 HQ and GF-310 HQ (poly(vinyl alcohol) gel, 6 μm , Showa Denko, Tokyo, Japan). The former three packings were slurry packed into a 100 \times 4.6-mm polyetheretherketone (PEEK) column in the laboratory after they had been allowed to swell for 24 h in water, whereas the latter three were prepacked columns (100 \times 4.6-mm stainless steel column) manufactured by Showa Denko.

2.2. Apparatus

The HPLC instrumentation employed was a Dionex M-14 ion chromatograph (Nihon Dionex, Tokyo, Japan) equipped with an injection valve with a 50- μl loop, a CMMS-II micromembrane suppressor and a conductivity detector. Data analysis was carried out on a CDS86 data processing system (LA Soft, Tokyo, Japan).

2.3. Preparation of analyte solutions

The analyte solutions of quaternary ammonium compounds were prepared by taking appropriate portions of the 1.0 mM stock solutions and diluting them with the mobile phase solution to be used. All the solutions prepared were kept in glass bottles as some quaternary alkylammonium ions strongly adsorb onto the inner wall of plastic bottles.

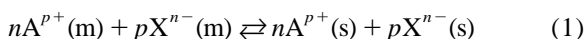
3. Results and discussion

3.1. Column packing

All the column packings investigated in this study are neutral hydrophilic polymer gels which do not possess any ion-exchange groups basically, although some of them may have a small amount of carboxyl groups. These packings are usually used for aqueous size-exclusion chromatography. It was thus expected that their ability to sorb quaternary alkylammonium ions was not so strong and accordingly the concentration of organic modifier in the mobile phase could be lowered. However, MCI GEL CQP 06 and Toyopearl HW-40S strongly adsorb the quaternary ammonium ions and even with the mobile phase described in the following section they could not be eluted from the columns. On the other hand, the adsorption of the surfactants on Bio-Gel P-2 was so weak that the individual quaternary compounds could not be separated. Shodex Asahipak GS-520 7G and GF-320 HQ exhibited skewed peaks with strong tailing. Therefore, complete separation of the four cationic surfactants could not be achieved and the detection sensitivity was not satisfactory. Only Shodex Asahipak GF-310 HQ showed moderate retention and high resolution for the analyte compounds.

3.2. Mobile phase electrolyte

The retention of the cationic surfactants on the neutral hydrophilic polymer columns may result from their partition into the packing gel matrix or adsorption onto the surface of the gel particles. It is very important to choose a suitable mobile phase electrolyte because the type of the background electrolytes strongly affects the retention of analyte ions in partition and adsorption chromatography. Shibukawa and Ohta [23] have clarified the effect of the electrolyte in the mobile phase on the retention of analyte ions in partition and adsorption chromatography by assuming that the partition or adsorption equilibria of ionic solutes are expressed as



where A^{p+} is analyte ion, Y^{m+} and X^{n-} are mobile phase cation and anion and (m) and (s) denote the mobile and the stationary phases, respectively. Based on this ion partition model, the retention factor of analyte ion A^{p+} , $k_A'^{YX}$, when eluted with the mobile phase containing Y^{m+} and X^{n-} is given by

$$\ln k_A'^{YX} = \ln k_A'^{WZ} + \frac{p}{m+n} (\ln k_X'^{WZ} - \ln k_Y'^{WZ}) \quad (3)$$

where WZ denotes an arbitrary mobile phase electrolyte. This equation indicates that the cationic analyte will be less retained when a mobile phase electrolyte consisting of strongly retained cation and weakly retained anion is used.

The mobile phase electrolyte must be also easily neutralized by passing through the membrane suppressor. Small et al. [24] used anilinium ion for the separation of some quaternary alkylammonium ions with relatively short alkyl chains such as tetraethyl- and tetrabutylammonium ions in their first work of suppressed ion chromatography. Therefore, we at first attempted to use a mixture of aniline or butylaniline and hydrochloric acid for the elution of the cationic surfactants. Butylaniline showed the elution ability greater than that of aniline, but its elution power was still insufficient to elute TDDBA and STMA. In addition butylaniline is very susceptible to oxidation by oxygen dissolved in solution. Then we tried to use 4,4'-bipyridyl instead of aniline and its alkyl derivatives. It was found that a mixture of 4,4'-bipyridyl and hydrochloric acid exhibits sufficient ability of eluting the analyte surfactants used in this study. Although 2,2'-bipyridyl has nearly the same elution power as that of 4,4'-bipyridyl, it may dissolve stainless steel by forming a complex with Fe^{2+} ion and cause deterioration of the column. Actually we have found that the column top was colored red after a mobile phase containing 2,2'-bipyridyl had been used for several weeks.

As described above, one should also employ the mobile phase anion of which the retention is as weak as possible in order to lower the retention of cationic analytes. We used hydrochloric acid to make 4,4'-bipyridyl divalent cation, which means that the anion is chloride ion. It has been reported that the retention of fluoride ion is less retained than chloride ion on some hydrophilic polymer columns [23,25]. In order to introduce fluoride ion into the mobile phase,

however, we have to use hydrofluoric acid instead of hydrochloric acid and this would make it difficult to use glasswares. Consequently, a mixture of 4,4'-bipyridyl and hydrochloric acid was adopted as the eluting agent in this study. Nitric acid or perchloric acid should not be used because the retention of nitrate and perchlorate ions is stronger than that of chloride ion.

3.3. Organic modifier

The basic material of Shodex Asahipak GF-310 HQ is poly(vinyl alcohol) and the column does not show very strong hydrophobic adsorption. However, it was necessary to add organic modifiers to the mobile phase in order to elute longer chain surfactants even when the mobile phase containing 4,4'-bipyridyl-hydrochloric acid was used. Fig. 1 shows the dependence of the retention times of the quaternary ammonium ions on the concentration of acetonitrile in the mobile phase. As can be seen from the figure, the retention time of the each analyte decreases with increase in the concentration of acetonitrile.

Methanol also exerts a similar effect to that of acetonitrile although higher concentration is needed to elute the quaternary ammonium ions at nearly the same times as those obtained with acetonitrile. However, the addition of methanol to the mobile phase greatly decreased the sensitivity of the conductometric detection. For example, 35% (m/v) methanol and 18% (m/v) acetonitrile gave nearly the same retention times for the analytes, while the peak areas obtained when the former mobile phase was used were about half those obtained with the latter. This may be attributed to the difference in viscosity between the two mobile phases. The equivalent conductivity of an ion i at infinite dilution, λ_i , is given by [26]

$$\lambda_i = \frac{F|z_i|e}{6\pi\eta r_i} \quad (4)$$

where F is Faraday constant, $|z_i|$ the magnitude of the charge on the ion, e electronic charge, η the viscosity of the medium and r_i the radius of the ion, respectively. The r_i values of the quaternary am-

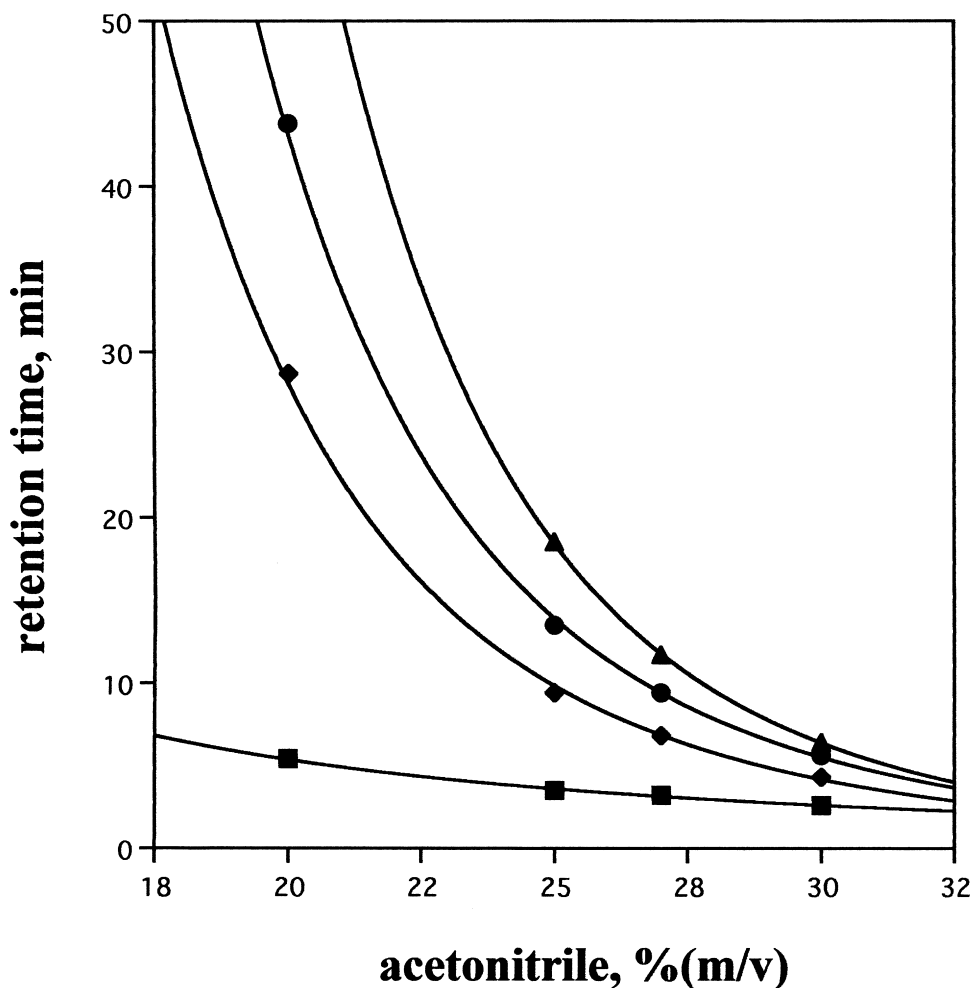


Fig. 1. Effect of acetonitrile concentration on retention time of quaternary ammonium ions. Column, Shodex Asahipak GF-310 HQ (100×4.6 mm); mobile phase, 0.4 mM 4,4'-bipyridyl, 0.8 mM HCl, acetonitrile concentration variable; flow-rate, 0.5 ml/min. Symbols: (■) DTMA; (♦) CTMA; (●) TDDBA; (▲) STMA.

monium ions can be assumed to be independent of the kind of the solvents because any molecules of water, methanol and acetonitrile do not seem to solvate the surfactant ions strongly. The ion-pair formation of the quaternary ammonium ions with counter anions may be negligible in acetonitrile–water or methanol–water medium so that the charge of them can also be regarded as being independent of the concentration of the organic modifiers. Therefore, the low detection sensitivity observed when methanol–water mixture was used as the mobile phase can

be ascribed to its high viscosity compared to that of mixture of water and acetonitrile.

3.4. Optimum composition of mobile phase

The retention of the quaternary ammonium ions can also be reduced by increasing the concentrations of 4,4'-bipyridyl and hydrochloric acid. However, the increase in concentration of the mobile phase electrolytes increases the background conductivity and then necessitates the increase in the concen-

tration of the regenerating agent, which would result in the baseline fluctuation. The optimum mobile phase was determined as 27% (m/v) acetonitrile–

water containing 0.4 mM 4,4'-bipyridyl and 0.8 mM hydrochloric acid. The concentration of tetramethylammonium hydroxide, the regenerating agent,

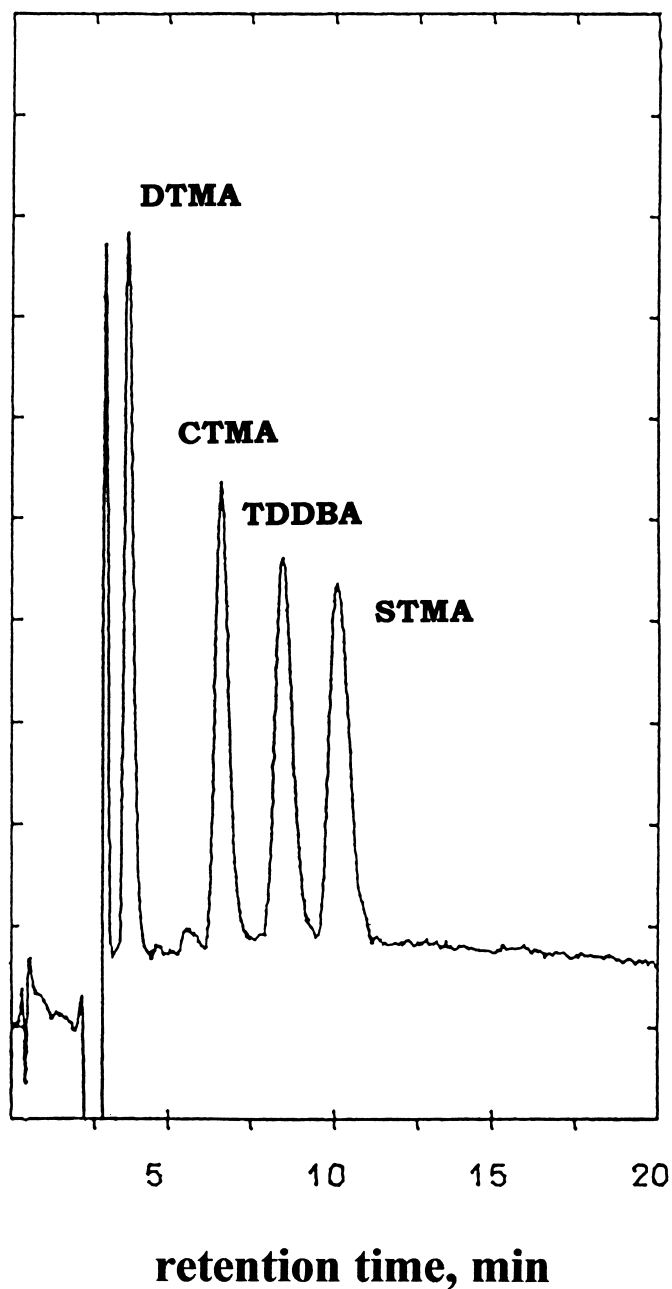


Fig. 2. Chromatogram of quaternary alkylammonium compounds. Column, Shodex Asahipak GF-310 HQ (100×4.6 mm); mobile phase, 0.4 mM 4,4'-bipyridyl, 0.8 mM HCl, 27% (m/v) acetonitrile; flow-rate, 0.6 ml/min; analyte concentration, 1.0 μ M; injection loop, 50 μ l.

was kept at 4.0 mM. Fig. 2 demonstrates an HPLC chromatogram of the quaternary ammonium ions used.

3.5. Linearity of calibration plots and detection limit

For CTMA, TDDBA and STMA it was found that adsorption occurred onto the inner wall of Teflon connecting tubes in the injection system if the

analyte solutions were prepared by dissolving with water. Therefore, all the analyte solutions were subsequently prepared in the chromatographic mobile phase. Calibration plots are given in Fig. 3 for small amounts of the analyte cationic surfactants. The calibration plots are linear up to at least 40 μM (the correlation coefficients obtained for the plots were 0.9987–0.9998). As can be seen from the plots, the peak area does not appreciably depend on the quaternary ammonium compounds, which is

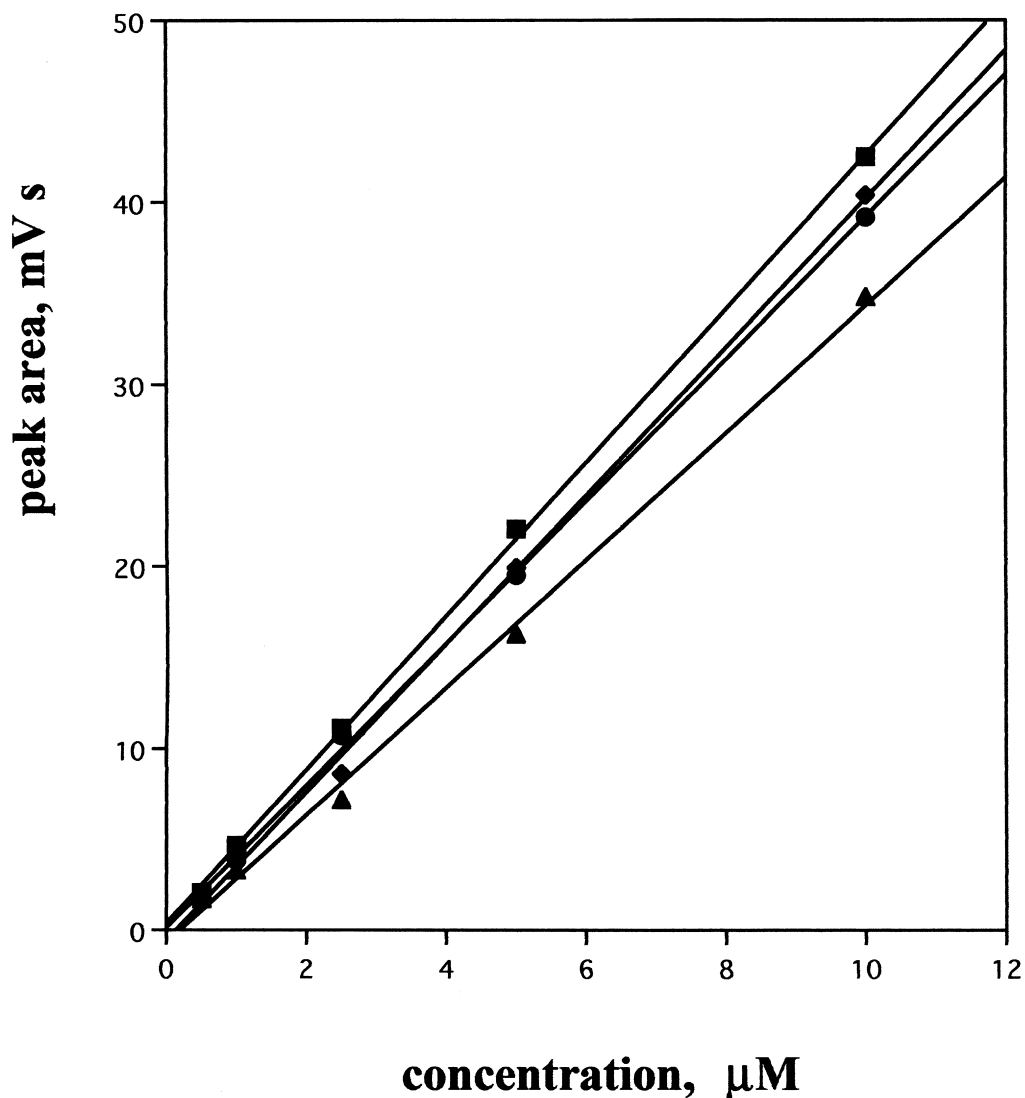


Fig. 3. Calibration plots for quaternary alkylammonium compounds. Symbols: (■) DTMA; (♦) CTMA; (●) TDDBA; (▲) STMA.

different from the results reported in literature [17,19]. The detection limit calculated as 3 times the standard deviation of the background signal is 0.03–0.05 μM for the four quaternary ammonium ions, which corresponds to 0.4–0.8 ng for 50 μl of injected solution. These results reveal that the sensitivity of the present method is much higher than those of the HPLC methods with conductometric detection which have so far been reported for determination of cationic surfactants.

4. Conclusion

The separation of quaternary alkylammonium compounds was accomplished by HPLC with a column packed with a hydrophilic polymer packing. Using a mixture of water and acetonitrile containing 4,4'-bipyridyl and hydrochloric acid as the mobile phase, the analysis time was minimized and the sensitivity of conductometric detection was maximized.

The present HPLC method could be applied to the determination of cationic surfactants in environmental samples. Work is currently in progress in this regard.

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