Determination of quaternary ammonium compounds by capillary electrophoresis using direct and indirect UV detection

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ABSTRACT

The determination of alkyl and alkylbenzyl quaternary ammonium compounds can be difficult owing to the polarity of the compounds, the formation of micelles by longer chain compounds and the lack of a chromophoric substituent for detection by standard separation techniques. The development of a free zone capillary electrophoresis method necessitated the use of organic modifiers in order to disrupt the formation of micelles by the longer chain ($> C_{12}$) surfactants. The selectivity obtained by using various levels of tetrahydrofuran as an organic modifier allowed the separation of a mixture of alkylbenzyl and alkylethylbenzyl quaternary compounds. The separation of alkyltrimethyl and dialkyldimethylammonium compounds was accomplished by the addition of a chromophoric cationic compound to the buffer system in order to allow the detection of the compounds with a standard UV absorbance detector. By varying the electrophoretic mobility of the indirect detection reagent, a variety of alkyl quaternary compounds, ranging from tetramethylammonium chloride to stearyltrimethylammonium chloride, can be detected with good peak shapes. The use of these methods for the determination of quaternary ammonium compounds will also be discussed. The use of internal standard methods and the linearity and linear range of the technique are considered.

INTRODUCTION

Capillary electrophoresis (CE) has been used to separate a variety of compounds, including proteins and peptides, DNA fragments, pharmaceuticals, inorganic ions and other organic compounds, and has been reviewed in several recent papers [1–3]. The technique provides high resolution and minimum solvent or buffer consumption and is appropriate to samples that are difficult to separate by gas or liquid chromatography. The successful application of CE requires that the compound of interest migrates freely through the column, with minimum adsorption or localized field effects, and that there be a suitable spectroscopic characteristic to detect the compound during the migration process.

The ability to find a suitable detection scheme for microcolumn separations, including CE, has been a major obstacle for many applications [4]. The UV absorbance detector is the most frequently used but limitations in terms of sensitivity and dependence on the absorptivity characteristic of the analyte can be limiting in the development of CE methods. Attempts to develop conductivity detectors have met with limited success [5,6] owing to the difficulty of measuring small changes in conductivity in the background electrolyte as a compound migrates through the column in the presence of the voltage which is driving the migration. The successful application of fluorescence and mass spectral detectors has been reported [7–11]. However, the universal application of these detectors is limited owing to their complexity and/or cost. Derivatization methods, both pre- and postcolumn, have been reported but require sample treatment and may have limitations based on the sample of interest [7,8,12,13]. Electrochemical detection methods have been re-

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ported, and essentially involve the very careful preparation of a micro electrochemical cell at the outlet of the CE column [14,15]. The construction of the end column cell, using a porous joint or a micromanipulator, makes the technique difficult to perform and still has limitations based on the electrochemical characteristics of the analyte of interest.

The application of indirect methods of detection for CE have been reviewed [16]. The technique was originally developed by Small and Miller [17] for ion chromatography using indirect UV detection. The measurement is based on having a concentration of UV-absorbing ion in the mobile phase, or in the buffer for CE, which provides a constant background. As the analyte moves through the column, the concentration of the background absorbing ion must be depleted in order to maintain electroneutrality. This depletion will cause a localized decrease in the concentration of the background absorbing ion and a subsequent decrease in the UV absorbance as the analyte band passes through the detector. The application of the method does not require any modifications to a basic CE instrument. The application of this technique of the CE analysis of inorganic ions has been reported by Jandik and Jones [18], of organic anions by Foret et al. [19] and of aliphatic alcohols by micellular electrokinetic chromatography by Szucs et al. [20]. Laser-induced fluorimetry offers a very favorable dynamic reserve for indirect detection and has been reported by Kuhr and Yeung [21]. The application of indirect amperometric detection has been demonstrated by Olefirowicz and Ewing [22]. The relationship between effective mobilities and peak areas, when using conductivity or indirect UV detection, has been defined by Ackermans et al. [23].

Alkyl and benzalkyl quaternary ammonium compounds are frequently used as surfactants and disinfectants in a variety of industrial and consumer applications. The analysis of these compounds is difficult owing to their polarity, ability to form micelles at low concentrations, thermal instability and the lack of a chromophoric substituent in the case of the non-benzyl types. The total concentrations are typically determined by the reaction with a compound which forms an ion pair with the quaternary compound [24]. Methods which use this process include two-phase titrations using chloroform, titra-

tions using surfactant electrodes for end-point detection and liquid–liquid extractions with UV detection. Gas chromatographic methods can be used to determine the alkyl chain distributions via an injection port pyrolysis reaction to the corresponding amine. These techniques provide useful information, but for the development of experimental compounds and the evaluation of the stability of these compounds in complex matrices, methods are required that can separate the mixtures of these compounds directly without modification. High-performance liquid chromatographic methods have been developed for benzalkyl quaternary compounds, but the analysis of the alkyl varieties is more problematic, requiring conductivity [25], refractive index [26] or indirect photometric detection [27]. The development of CE methods for the analysis of these compounds has provided a complementary technique which provides improved resolution, the ability to develop indirect UV detection for non-UV-absorbing analytes, minimum solvent consumption and inexpensive column replacement.

EXPERIMENTAL

The capillary electrophoresis system was constructed from various components. The power supply was a Model RHR30PN30/EI/OV/SS obtained from Spellman High Voltage Electronics (Plainview, NY, USA). The UV absorbance detector was a Model CV4 obtained from ISCO (Lincoln, NE, USA) and was operated at 210 nm. The electropherograms were acquired on either a Model 4270 integrator obtained from Spectra-Physics (San Jose, CA, USA) or a Model 941 interface using Access-*Chrom software from PE Nelson (Cupertino, CA, USA; on a VAX computer obtained from Digital Equipment (Maynard, MA, USA).

Phosphoric acid was of Baker Analyzed Reagent Grade. Sodium phosphate, monobasic, was obtained from Mallinckrodt. The following compounds were obtained from Aldrich (Milwaukee, WI, USA): benzyltrimethylammonium chloride (BTMACL), benzyldimethyldodecylammonium bromide (C_{12} Benzyl), benzyldimethytetradecylammonium chloride (C_{14} Benzyl), benzylcetyldimethylammonium chloride (C_{16} Benzyl), benzyldimethylstearylammonium chloride (C_{18} Benzyl), dodecyltrimethylammonium bromide (C_{12} Trimethyl).

myristyltrimethylammonium bromide (C_{14} Trimethyl), cetyltrimethylammonium bromide (C_{16} Trimethyl), octadecyltrimethylammonium bromide (C_{18} Trimethyl) and sodium dodecyl sulfate (SDS). Tetramethylammonium chloride (TMACL) was obtained from Eastman Kodak. Other quaternary compounds were obtained from various suppliers. Tetrahydrofuran (THF) was obtained from EM Science.

THF-containing buffers were prepared by first mixing appropriate volumes of THF and water. The mixture was heated on a steam-bath and and then vacuum filtered in order to degas. The appropriate volumes of solutions of sodium dihydrogenphosphate, phosphoric acid and, in the case of indirect detection, the alkylbenzyldimethylammonium bromide, were then added. The buffered THF–water solutions were also heated and vacuum filtered. The concentrations of THF–water reported reflect the volume percentages of the orginal amounts of THF and water that were actually mixed; loss of THF during filtration was noted.

The capillary tubing was uncoated fused silica of 50 μm I.D. and 360 μm O.D., obtained from Polymicro Technologies (Phoenix, AZ, USA). The capillary tubes were conditioned by treating them with a sequence of washes using air pressure applied to the ground-side reservoir. This procedure was performed on new columns and only when a problem in performance was suspected. A typical sequence would include 10 min water, 20 min 1.0 M NaOH, 10 min water, 20 min 1.0 M phosphoric acid, 10 min water and at least a 20-min rinse with the buffer to be used. The capillary columns were rinsed with buffer by applying pressure to the ground-side reservoir before each run.

Introduction of the sample on to the column was achieved by hydrostatic pressure, either by elevating the high-voltage end of the capillary tube, in contact with the sample solution, about 10 cm above the ground reservoir or by lowering the ground end of the capillary tubing about 16 cm, while the high-voltage end was immersed in the sample solution.

RESULTS AND DISCUSSION

The initial attempts to separate alkybenzyldimethyl ammonium bromides of chain lengths C_{12}–C_{18} were performed using a 0.05 M phosphate buffer. Separation was achieved in under 20 min, but the peaks tailed and the tailing worsened with increasing chain length. The signal obtained for the C_{18} quaternary compound was barely distinguishable from the baseline. We speculated that this may be due to the formation of micelles. The critical micelle concentrations (CMCs) for these compounds are given in Table I. It should be noted that the concentrations of the solutions introduced on to the CE column were of the order of 1 mM, which would indicate that the C_{12} quaternary compound was being introduced at around its CMC. The C_{18} quaternary compound was being introduced at a level several orders of magnitude above its CMC and would be present as a micelle. The behavior of a micelle as it passes through the column would continuously yield free quaternary molecules and could provide servere taling. A common way to disrupt micelle formation is to add organic solvents. The addition of methanol and acetonitrile did not improve the peak shape. The addition of tetrahydrofuran provided a sufficent alteration to the buffer to improve the peak shapes. A typical electropherogram obtained for a mixture of C_{12}, C_{14}, C_{16} and C_{18} alkylbenzyldimethyl ammonium bromides is presented in Fig. 1. The peak shapes are considerably improved, providing adequate separations of a number of quaternary compounds.

The theoretical plate number was calculated to be over 150 000 plates/m based on C_{12} Benzyl. The precision of the technique is summarized in Table II. The relative standard deviation of the measured area is *ca.* 5%, even with manual sample introduction. Calibration of the system showed a linear response from 10 to 80 ppm. The correlation coeffi-

TABLE I

CRITICAL MICELLE CONCENTRATIONS OF ALKYL-BENZYLDIMETHYLAMMONIUM CHLORIDE QUATERNARY COMPOUNDS [28]

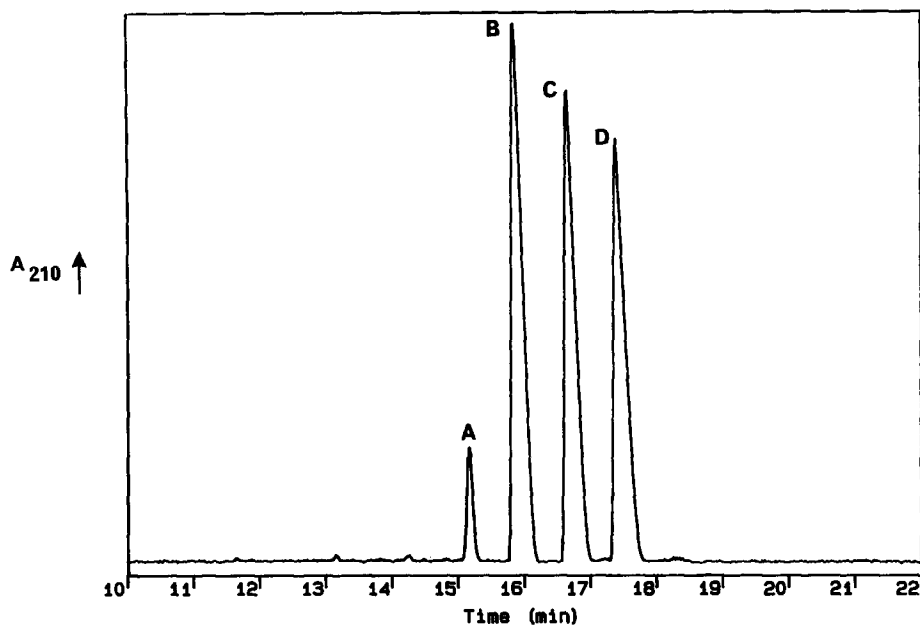| Alkyl chain length	Critical micelle concentration (M)
C_{12}	$3 \cdot 10^{-3}$
C_{14}	$3.7 \cdot 10^{-4}$
C_{16}	$2 \cdot 10^{-4}$, $4 \cdot 10^{-5}$
C_{18}	$8 \cdot 10^{-6}$

Fig. 1. Electropherogram obtained from the direct detection of (A) C_{12}, (B) C_{14}, (C) C_{16} and (D) C_{18} alkylbenzyldimethylammonium quaternary compounds using THF–Water (57.5:42.5) containing 44 mM phosphate at 25 kV/m on a 24 cm × 50 μm I.D. column.

cient was 0.999 for C_{14} Benzyl. The detection limit has been estimated to be less than 1 ppm. The application of this technique to the separation of a commercially available mixture of benzalkyl quaternary compounds revealed the ability not only to separate ethylbenzylalkyl from benzalkyl quaternary compounds but also to separate the *ortho* and *para* iso-

mers of the ethylbenzylalkyl quaternary compounds.

The initial attempt to develop an indirect detection mode involved the use of 0.01 M benzyltrimethylammonium chloride in THF–water (57.5:42.5). The separation of TMACL and C_{14} Trimethyl was attempted. The sensitivity of the sys-

TABLE II

PRECISION OF AREA AND RETENTION TIME FOR DIRECT DETECTION

	C_{14}		C_{16}		C_{18}	
	Area (counts)	Elution time (min)	Area (Counts)	Elution time (min)	Area (counts)	Elution time (min)
	79 053	15.828	68 552	16.624	67 999	17.365
	83 797	15.663	73 964	16.448	73 169	17.178
	81 390	15.565	71 065	16.352	69 553	17.082
	79 438	15.565	68 942	16.342	67 305	17.073
	91 405	15.497	78 226	16.273	75 452	17
	91 821	15.412	80 768	16.182	81 212	16.899
	82 826	15.446	72 607	16.208	72 686	16.928
	86 099	15.386	72 545	16.157	69 327	16.878
Mean	84 479	15.55	73 334	16.32	72 088	17.05
S.D.	4958	0.15	4277	0.16	4625	0.16
R.S.D. (%)	5.87	0.94	5.83	0.96	6.42	0.96

tem for TMACL was greater than that for C_{14} Trimethyl and the peak was more symmetric. The skewing of peaks in indirect CE has been attributed to the differences in electrophoretic mobility of the analyte relative to the electrophoretic mobility of the UV-absorbing background electrolyte [18]. Subsequent method modifications were made, and the final buffer consisted of THF–water (57.5:42.5), 3 mM C_{12} Benzyl, 3 mM SLS and 8 mM sodium phosphate, monobasic.

The electropherogram obtained for the mixture of alkyltrimethylammonium quaternary compounds is presented in Fig. 2. The resolution of the C_{12}, C_{14}, C_{16} and C_{18} compounds is easily obtained, although the asymmetry of the peaks increases with increase in chain length, as the difference in electrophoretic mobility of the analyte and C_{12} Benzyl increases. The calibration of the system is linear from 4 to 80 ppm and the correlation coefficients for each of C_{12}, C_{14}, C_{16} and C_{18} trimethylammonium quaternary compounds were greater than 0.99. The detection limit was estimated to be less than 1 ppm. The precision of the indirect detection mode is summarized in Table III. It is comparable to the precision obtained in the direct mode.

The ability to analyze real samples was the objective of this work. Fig. 3 displays the electropherogram of a quaternary in an experimental disinfectant product. The C_{14} Trimethyl has been added as an internal standard. Table IV summarizes the precision of the technique. The precision based on the area of the analyte of interest is 3.5% R.S.D., but when an internal standard ratio is used the precision is improved to about 2.5% (R.S.D.).

The ability to detect UV-absorbing components using the indirect detection mode had been observed earlier in the method development. Fig. 4 displays the signal obtained for a mixture of benzyldimethylalkyl quaternary compounds using the indirect mode. The sensitivity is reduced compared with the signal obtained in the direct mode for these components, and is lower than the sensitivity obtained for the alkyltrimethyl quaternary compounds in the indirect mode. These findings are reasonable considering the mechanism of the indirect mode.

CONCLUSIONS

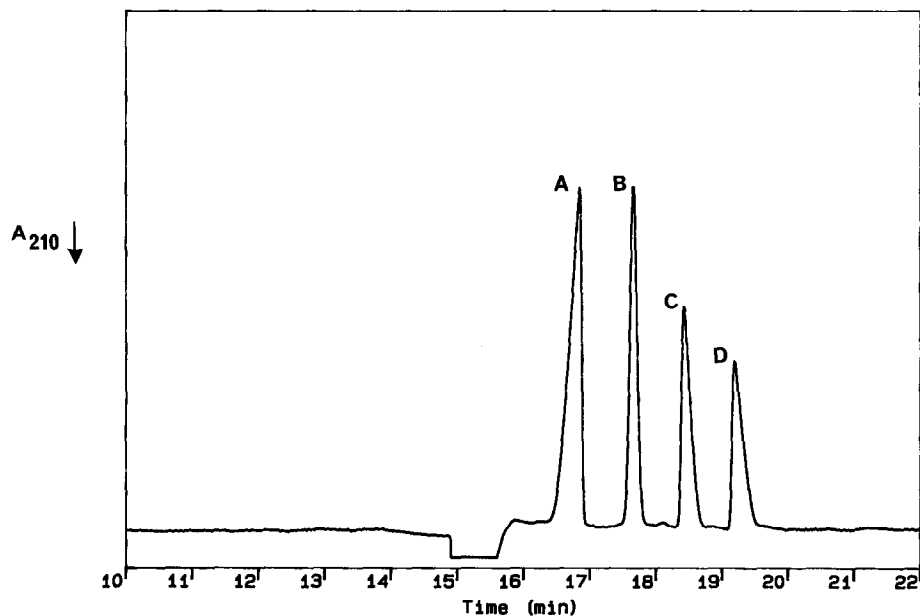The separation of long-chain cationic surfactants

Fig. 2. Electropherogram obtained from the indirect detection of (A) C_{12}, (B) C_{14}, (C) C_{16} and (D) C_{18} alkyltrimethylammonium quaternary compounds at 18 kV/m; buffer as described in text.

TABLE III

PRECISION OBTAINED DURING CALIBRATION FOR INDIRECT DETECTION

	Area (counts)				Elution time (min)
	4 ppm	20 ppm	40 ppm	80 ppm	
	9787	37 592	76 626	150 050	
	8189	38 647	76 830	155 305	
	8610	38 661	77 060	159 274	
	8826		77 371		
	8410		77 505		
			79 206		
Mean	8764	38 300	77 433	154 876	16.897
S.D.	618	613	928	4626	0.112
R.S.D. (%)	7.05	1.6	1.2	2.99	0.66

has been accomplished using capillary electrophoresis. The addition of THF to the buffer system has overcome the difficulty associated with micelle formation of these analytes during the the course of the analysis. The method is robust enough to develop indirect UV detection methods, which provide sufficient linear response and precision to determine these compounds in real samples. Capillary electrophoresis serves as a complimentary technique to other methods that have been reported and provides minimization of solvent consumption and an increased number of theoretical plates. The ability to separate these compounds in a bare silica capillary column provides a robust separation compart-
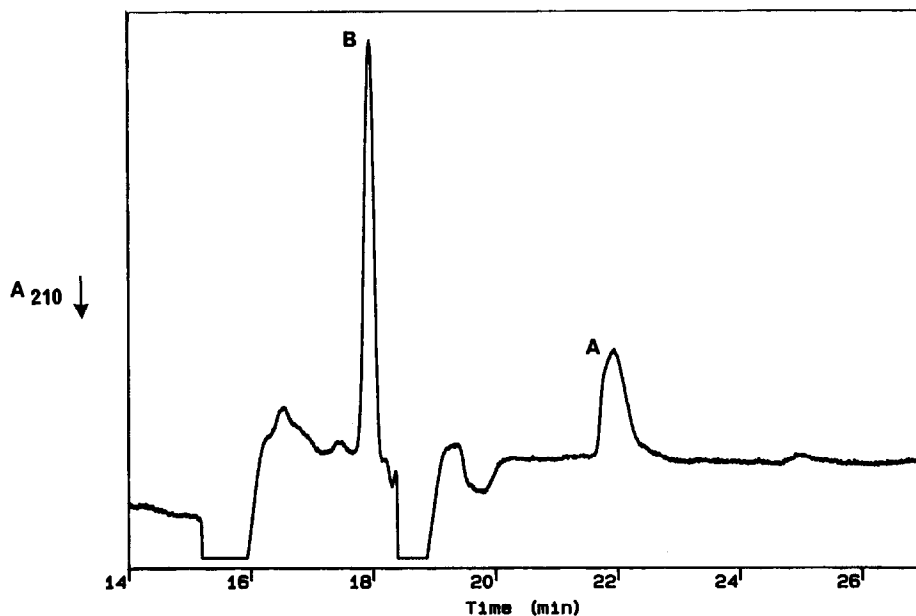

Fig. 3. Electropherogram obtained from the indirect detection of a quaternary compound (A) in an experimental disinfectant product with a C_{14} trimethylammonium quaternary (B) included as an internal standard.
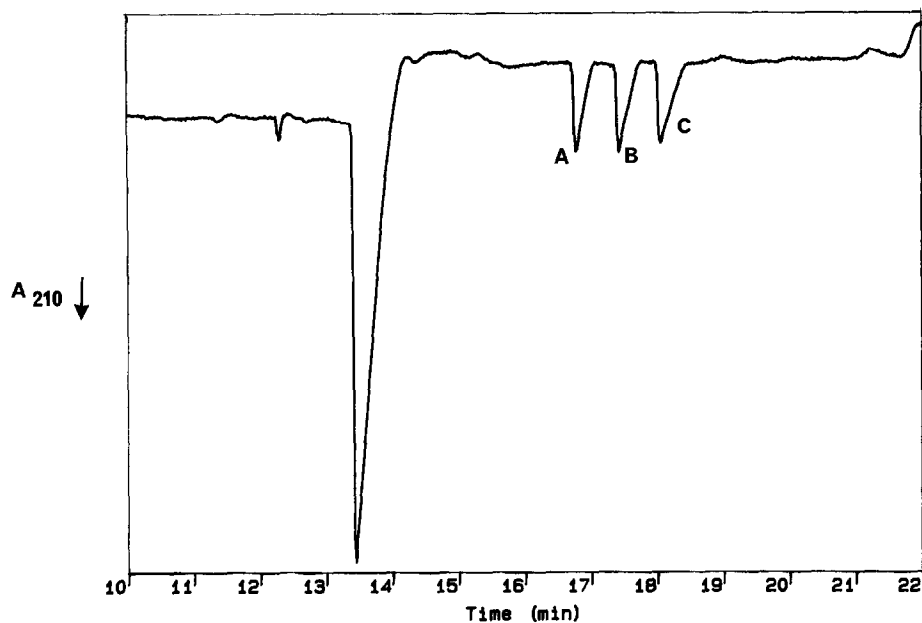
Fig. 4. Electropherogram obtained from the indirect detection of a mixture of (A) C_{14}, (B) C_{16} and (C) C_{18} alkylbenzyldimethylammonium quaternary compounds. Conditions as in Fig. 2.

TABLE IV

PRECISION OF DATA OBTAINED FROM APPLICATION OF INTERNAL STANDARD METHOD

	C_{14} Internal standard		Analyte		Area ratio
	Area (counts)	Elution time (min)	Area (counts)	Elution time (min)	
	52 951	17.948	35 312	21.3	0.67
	54 486	17.823	38 356	21.157	0.7
	55 362	17.834	39 464	21.214	0.71
	55 907	17.854	38 167	21.226	0.68
	56 052	17.773	38 123	21.116	0.68
	55 742	17.656	38 271	20.983	0.69
	56 621	17.658	38 350	20.982	0.68
	56 105	17.495	37 244	20.758	0.66
	56 911	17.318	39 269	20.558	0.69
	58 128	17.246	41 086	20.466	0.71
Mean	55 826.5	17.6605	38 364.2	20.976	0.69
S.D.	1395.53	0.24	1494.93	0.29	0.02
R.S.D. (%)	2.5	1.34	3.9	1.38	2.41

ment which can easily be cleaned and or inexpensively replaced, allowing for the development of methods for and analyses of difficult matrices with little concern for permanent damage to the column.

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