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Effect of drying on the degradation of cationic surfactants and separation performance in capillary zone electrophoresis of inorganic anions

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Abstract

Tetradecyltrimethylammonium bromide (TTAB) is used in capillary zone electrophoresis (CZE) to control the direction and magnitude of the electroosmotic flow and the migration time of analyte anions. Drying of the hygroscopic TTAB at 100°C overnight has been found to influence the final CZE separation by providing improved resolution, precision of migration times, and enhanced detection response for hydrogenphosphate. Chemical analysis of the dried TTAB using IR and GC–MS indicated the presence of small amounts of an unexpected tertiary alkylamine, tetradecyldimethylamine. This amine appears to contribute to the improved separation, probably by making more effective the masking of silanophilic activity at the capillary surface and/or generating a more stable double-layer.

Keywords: Inorganic anions; Surfactants; Tetradecyltrimethylammonium bromide; Tetradecyldimethylamine

1. Introduction

In capillary zone electrophoresis (CZE), the rate and direction of the electroosmotic flow (EOF) [1–4], the migration times of anions, and the separation selectivity [1–3,5–9] are influenced by the type and concentration of cationic surfactants (so-called ''EOF modifiers'') added to the electrolyte. Tetradecyltrimethylammonium bromide (TTAB) is the most widely used surfactant (e.g., [3,8–13]) in CZE. Work in our laboratory has shown that varying the concentration of TTAB by as little as 0.1 m*M* (2.5–2.6 m*M*) altered the separation selectivity of inorganic anions. It is therefore essential that an accurate amount of surfactant is used in order to generate reliable selectivity plots that are fundamental in optimising separations and calculating the mobilities [8] of anions. Moreover, it is critical that the absolute migration time (AMT) achieved is precise enough to permit reliable anion identification.

The separation of low-molecular-mass anions using electrolytes containing TTAB is common (e.g., [3,14,15]). When obtained as a solid, TTAB is normally dried before use and in this article we

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report some effects which can be produced by drying TTAB at 100°C; and on the separation of low-molecular-mass anions using a chromate-based electrolyte containing dried TTAB.

2. Experimental

2.1. Apparatus

A Waters Quanta 4000 capillary electrophoresis (CE) system fitted with an Accusep bare fused-silica capillary [60 cm (52 cm effective length)×75 μm I.D.×365 μm O.D.] was used. Sampling was performed hydrostatically (sample raised 10 cm for 30 s). The separation was performed using an applied voltage of −20 kV and a data acquisition rate of 20 Hz. Analytes were detected using indirect UV-absorbance at 254 nm. The capillary was conditioned by manually flushing with 0.3 ml 95% ethanol, 0.3 ml Milli-Q (Millipore, Bedford, MA, USA) water, 0.2 ml 0.5 *M* KOH, and 0.2 ml of the electrolyte.

Mass spectral analyses of dried and undried TTAB were undertaken using electron ionization (EI) and liquid secondary ion-mass spectrometry (LSI-MS) using a Kratos ISQ mass spectrometer. The conditions for EI-MS were 70 eV electron energy, 8 kV accelerating voltage, source temperature of 200°C and scanning at 2 s per decade between m/z 35–800. For the LSI-MS measurement, the sample was dissolved in *m*-nitrobenzylalcohol (*m*-NBA) as the liquid matrix on the probe tip and desorbed and ionized with a 10 kV Cs^+ ion primary beam, with an accelerating voltage of 5.3 kV. For LSI-MS, the dynamic resolution was 7000, the scan speed 5 s per decade, and the liquid matrix was used as an internal mass reference. Gas chromatography–mass spectrometry (GC–MS) analyses were conducted using a Hewlett-Packard 5890 GC system fitted with a HP5970 mass-selective detector. The carrier gas was helium (103 kPa), injections were made in the splitless mode with an injector temperature of 250°C, and the GC oven was programmed from 60°C to 290°C at 10°C min^{-1}. Cool on-column injections were also made to discriminate between products formed during flash injection and those formed during the experimental drying process. The separation was carried out on a HP1 column (25 m length×0.32 mm O.D., 0.52 μm film thickness). Mass spectra were recorded from m/z 35 to 550 at 1 scan s^{-1}. Infra-red scans of dried and undried TTAB were performed on a Bruker IFS 66 Fourier transform infrared (FT-IR) spectrophotometer.

2.2. Reagents

A test mixture containing the following anions was made from dried analytical-reagent grade salts (Ajax, Sydney, Australia) dissolved in Milli-Q water: chloride, nitrite, sulfate, nitrate, fluoride, bromate, hydrogenphosphate and carbonate. Cationic surfactants obtained as white powders from Aldrich (Milwaukee, WI, USA) were TTAB, cetyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB). TTAB, CTAB and DTAB were dried at 100°C for 0–8.5 days. Electrolytes were made freshly as required, filtered (0.45 μm) and degassed by ultrasound before use.

3. Results and discussion

3.1. Effects of drying the surfactant

The melting point range of TTAB is 245–250°C [16], so little physical or chemical change was expected to occur from drying this material at 100°C. However, drying overnight or longer caused a 0.26% loss in mass and the colour changed from white to light-brown, suggesting decomposition. Similar changes in colour were noted for CTAB and DTAB; both of which have melting points above 230°C [17]. The intensity of the colour change increased with the length of the drying period. Under either the typical conditions used in CZE for anion separation (e.g., [3,14,18–20]) or the conditions used here, the observed 0.26% mass loss for TTAB would not have any significant effect on the molar concentration (0.1 m*M*), so no significant change in EOF would be anticipated.

A chromate-based electrolyte was chosen for this study since this type of electrolyte is used commonly for the separation of anions by CZE (e.g., [3,8,9,21–24]). Some changes in behaviour were observed when the TTAB used to make up the electrolyte was dried. First, the separation selectivity for analyte

anions having similar mobilities was altered, especially for fluoride, bromate and hydrogenphosphate which were fully resolved with the dried TTAB but not with the undried material. In fact, the complete separation of these species with a TTAB–chromate electrolyte has not been reported previously. Second, the reproducibility of the detector response for hydrogenphosphate was highly variable [25], especially at low levels, with the undried TTAB but with dried TTAB a reproducible and enhanced detector response was observed, even from the first run (Fig. 1). Third, a study of the precision (RSD) of AMTs for analyte anions (monitored over two days) showed significant differences for the dried and undried TTAB. With the undried material migration times decreased with repeat injections (chloride: 1.4% RSD, $n=6$ on day 1) and on each day, the ''starting'' and ''stable'' migration times were different (Fig. 2). Typically, more than six repeat injections were needed to achieve precise migration times. These trends are in agreement with observations reported elsewhere [26] and result from a dynamic build-up of surfactant at the capillary–BGE interface, which in turn increases the double-layer thickness and zeta potential. Dried TTAB showed a trend similar to that of the undried TTAB but superior precision (chloride: 0.9% RSD, $n=6$ on day 1) was obtained immediately after equilibration of the capillary with the electrolyte.

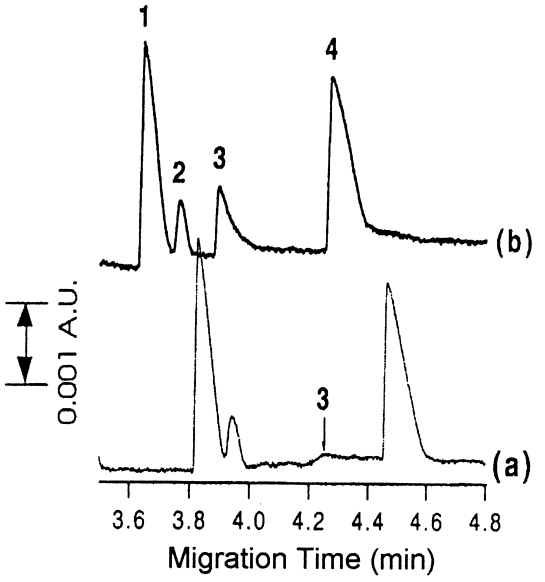

Fig. 1. Effect of drying of TTAB on migration time, resolution and detectability of selected anions. Other anions in the test mixture were fully resolved. Conditions: (a) run 1 using an electrolyte with 2.6 m*M* undried TTAB and 5 m*M* chromate at pH 8. Detection was in the indirect UV mode at 254 nm. Other conditions as in Experimental. The detector leads were reversed to make the peaks appear in the positive direction, and (b) run 1 using an electrolyte with dried (100°C overnight) TTAB. Other conditions as in (a). Electrolytes in (a) and (b) were prepared by diluting appropriate amounts of 100 m*M* chromate and 50 m*M* TTAB to 200.0 ml. The electrolyte pH was adjusted as appropriate with dilute mineral acid or KOH. Anions ($\mu g\ ml^{-1}$): 1=fluoride (5), 2=bromate (7), 3=hydrogenphosphate (12), and 4=carbonate (10).

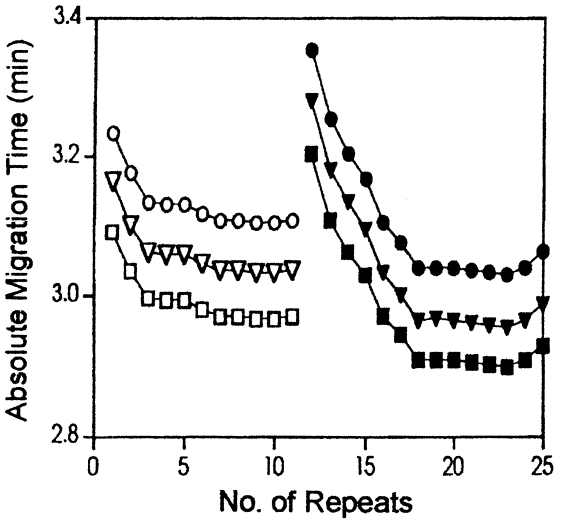

Fig. 2. Precision of absolute migration times over two days. Conditions as in Fig. 1a. Key: □=chloride on day 1, ○=bromide (system peak) on day 1, ▽=nitrite on day 1, ■=chloride on day 2, ●=bromide (system peak) on day 2, and ▼=nitrite on day 2.

3.2. Chemical analysis of the surfactants

The above results suggested that the chemical changes induced by drying exerted a beneficial effect on the separation. Attempts to identify the nature of these chemical changes were therefore undertaken. IR and MS determinations were performed on both dried and undried surfactants. Upon heating, quaternary ammonium surfactants (e.g., when present as the hydroxide or iodide salts) undergo Hofmann elimination at the β-carbon [27] to yield an alkene, amine and HX (where X=surfactant anion). For

TTAB, the expected products of this reaction are $CH_3(CH_2)_{11}CH{=}CH_2$, $N(CH_3)_3$ and HBr.

IR scans of TTAB, CTAB and DTAB dried at 100°C for varying lengths of time did not show detectable evidence of amines at 3100–3600 cm^{-1}, but a peak was noted at ~1725 cm^{-1} only for the dried surfactants. This peak increased in intensity with length of drying time and its likely identity is C=C or C=O [28]. Evidence of olefinic C–H at >3000 cm^{-1} was inconclusive. If an alkene was formed via Hofmann elimination, it may have been lost through volatilisation or by participation in a secondary reaction with the eliminated HBr. In summary, the IR spectra did not support the presence of products expected from a Hofmann-type elimination.

MS analysis of the dried and undried TTAB was then performed using EI-MS, LSI-MS and GC–MS to ascertain if any changes occurred as a result of drying. In order to enhance detectability, the TTAB was dried for an extended period (8.5 days). MS analysis of tetraalkylammonium salts using EI-flash desorption at >1000°C demonstrated that these salts can fragment to form, amongst others, R_4N^+ and R_4N groups [29]. As the surfactants used in this work were dried at 100°C, the investigation was geared towards determining if similar changes were occurring upon drying at the lower temperature. A further goal was to determine if there was a basis to link the drying process to the observed superior separation achieved using dried TTAB. EI-MS fragmentation patterns for dried and undried TTAB were similar but this did not necessarily mean that no changes had occurred upon drying because both the dried and undried TTAB were subjected to the same source temperature (200°C). However, there was evidence of a slow decomposition of the TTAB and diagnostic peaks were recorded for both dried and undried TTAB at: *m/z* 256, parent ion, $[CH_3(CH_2)_{13}N(CH_3)_3]^+$; *m/z* 241, tertiary amine, $[CH_3(CH_2)_{13}N(CH_3)_2]^{+\cdot}$ after CH_3Br abstraction; and *m/z* 94/96, $[CH_3Br]^{+\cdot}$. GC (splitless mode) for both dry and undried TTAB showed two well-resolved peaks at 14.028 and 14.610 min, respectively (Fig. 3). GC–MS spectra showed the first peak to have $M^{+\cdot}$ at *m/z* 241 and *m/z* 58, consistent with tetradecyldimethylamine; and the second peak to have typical alkyl fragments as well as a characteris-

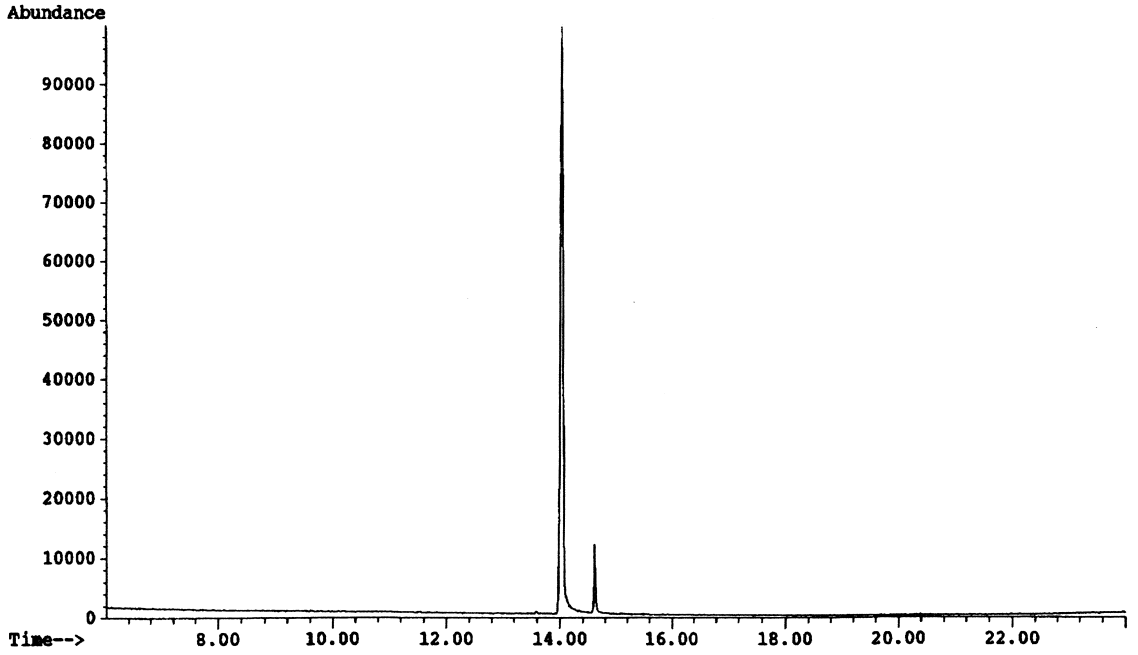

Fig. 3. GC analysis of TTAB using splitless injection. See Experimental for conditions. The chromatogram is for the undried TTAB. An identical response was obtained for the dried TTAB (not shown). Time scale in min.

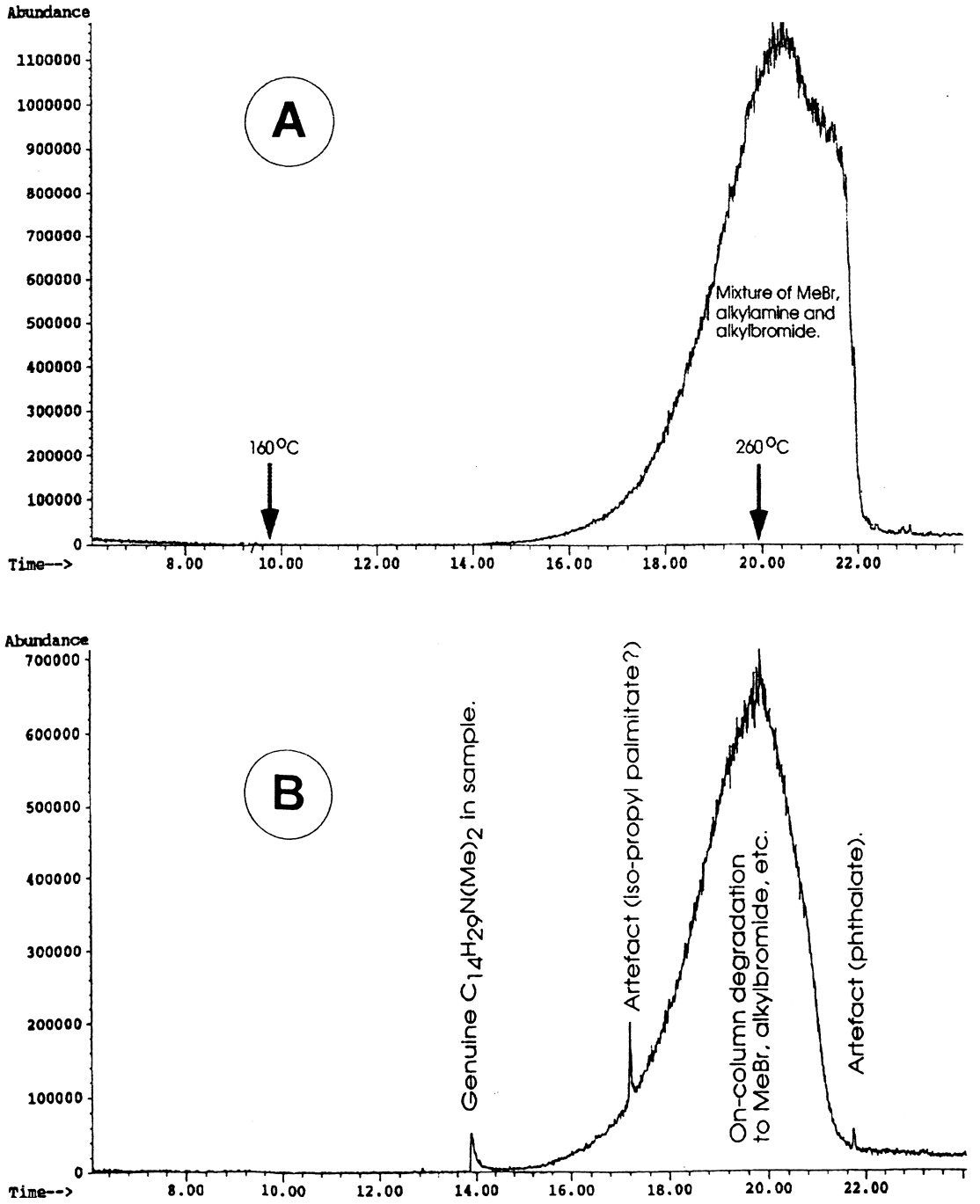

Fig. 4. GC–MS chromatogram with cool on-column injection. See Experimental for conditions. (A) Undried TTAB, (B) dried TTAB. Time scales in min.

tic bromine isotope pattern on other fragments. This peak was found to correlate with 1-bromotetradecane from the NIST library of mass spectra [30]. Considering the formula of TTAB and noting Markovnikov's rule, it is believed that the peak was due to the 2-bromotetradecane isomer. The GC–MS data also highlighted the absence of peaks for tetradecene, which supported the notion (from the IR discussion) that the alkene and HX generated via Hofmann elimination were reacting to give 2-bromotetradecane. This notion is likely because at 100°C, the alkene and HBr would readily undergo a saturation reaction to yield $CH_3(CH_2)_{11}CHBrCH_3$.

The above peaks (from the GC and GC–MS experiments) were presumed to be the result of the hot injection port temperatures causing rapid decomposition. This was confirmed with a series of cool on-column injections, which showed no tertiary amine or alkyl bromide peaks at the expected retention times in the undried sample (Fig. 4A) and a small but distinct peak for the tertiary amine at 14.028 min in the dried sample (Fig. 4B). The large broad peaks observed in the on-column injections were the result of the gradual decomposition of the quaternary amine to the tertiary amine, tetradecyl bromide and methyl bromide on the front of the column as the oven temperature was gradually increased. The LSI-MS spectra showed prominent ions at m/z 256 (parent ion) but almost no ions at m/z 241/242, i.e., $[M+H]^+$ for both undried and dried TTAB. The sensitivity in LSI-MS to the preionised quaternary species is much greater than for the neutral tertiary amine, so it was not unexpected that very low levels of genuine tertiary amine would be overshadowed in this analysis by the quaternary amine.

From these studies, it was concluded that alkylamines (and alkyl bromides) were formed in-situ in low yield when TTAB was dried. The alkylamine seems to be a product of C–N cleavage, a pattern noted for tetraalkylammonium salt fragmentation in mass spectrometry [31]. Moreover, the slow decomposition observed using EI-MS was also evident here. Similar results (i.e., in-situ formation of alkylamines) were observed for DTAB and CTAB. Alkylamines interact strongly with the silanol functionality [32] in reversed-phase ion-pair chromatography and their effectiveness in masking silanophilic activity (stabilising or reversing EOF) in CE has also been reported [29,33,34]. The present work has shown that drying generated an alkylamine (tertiary amine) in-situ, and it was this species which was contributing to the superior resolution, and AMT precision for separations using dried TTAB. Examination of this hypothesis using model alkylamines is being undertaken and will be reported later.

4. Conclusions

Shorter migration times, better AMT precision, improved detectability (particularly for hydrogenphosphate), and improved resolution for anions separated using CZE can result from the use of dried TTAB in electrolytes. Moreover, an unexpected tertiary alkylamine $[CH_3(CH_2)_{13}N(CH_3)_2]$ was found to be generated in-situ by drying at 100°C and appears to be contributing to the superior separation achieved with dried TTAB. It is likely that this effect was caused by effective and improved masking of silanophilic activity at the capillary surface.

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