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Research Article

Capillary electrochromatography-mass spectrometry of cationic surfactants

The CEC-MS of alkyltrimethylammonium ($ATMA^+$) ions with chain lengths ranging from C_1–C_{18} is optimized using an internally tapered column packed with mixed mode reversed phase/strong cation exchange stationary phase. A systematic study of the CEC separation parameters is conducted followed by evaluation of the ESI-MS sheath liquid and spray chamber settings. First, the optimization of CEC separation parameters are performed including the ACN concentration, triethylamine (TEA) content, buffer pH and ammonium acetate concentration. Using 90% v/v ACN with 0.04% v/v TEA as mobile phase, the separation of longer chain C_6–C_{18}-TMA^+ surfactants could be achieved in 15 min. Lowering the ACN concentration to 70% v/v provided resolution of shorter chain C_1, C_2-TMA^+ from C_6-TMA^+ although the total analysis time increased to 40 min. Furthermore, variation of both the ACN and TEA content as well as ionic strength has found to significantly influence the retention of longer chain surfactants as compared to shorter chains. The optimum CEC conditions are 70% v/v ACN, 0.04% v/v TEA, pH 3.0 and 15 mM ammonium acetate. Next, the optimization of the ESI-MS sheath liquid composition is conducted comparing methanol to isopropanol followed by the use of experimental design for analysis of spray chamber parameters. Overall, the developed CEC-ESI-MS method allows quantitative and sensitive monitoring of $ATMA^+$ from $\leq$10 μg/mL down to 10 ng/mL. Utilizing the optimized CEC-ESI-MS protocol, the challenging analysis of commercial sample Arquad S-50 $ATMA^+$ containing *cis-trans* unsaturated and saturated soyabean fatty acid derivatives is demonstrated.

Keywords: Alkyltrimethylammonium halides / Cationic surfactants / CEC-MS / ESI / Internal taper DOI 10.1002/elps.200600301

1 Introduction

Cationic surfactants are surface-active agents utilized as chemical intermediates and as functional ingredients in numerous formulations. Generally, the chemical structure contains both a hydrophobic alkyl chain and a hydrophilic group, which is often a quaternized nitrogen functionality that renders the cationic surfactant overall permanent positive charged in solution. Moreover, there are a diverse group of cationic surfactants that have different substitutions around the nitrogen atom including mono-, di-, and trimethyl, alkyl and benzylated configurations. Alkyltrimethylammonium ($ATMA^+$) compounds with chloride, bromide or iodide counterions are one of the major types of non-chromophoric quaternary ammonium surfactants, which are widely used in industrial applications as well as in cosmetics and pharmaceutical preparations. For example, longer chain $ATMA^+$ are utilized in the personal care industry due to a unique ability to impart detangling, conditioning, and static control properties in hair care formulas including crème rinse, mousses, sprays, shampoos and conditioners. In addition to being good cleansing agents, they also possess germicidal properties, which make them useful in hospitals as disinfectants.

Atypically, the manufacture of fatty amine nitrogen derived surfactants including $ATMA^+$ first utilize natural feedstock raw materials such as animal fats and vegetable-based oils (*e.g.*, palm, kernel, and coconut oil based). As a consequence, the products contain a homologous range of alkyl chain lengths typically C_9–C_{22}

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Abbreviations: ATMA$^+$, alkyltrimethylammonium; **EIC**, extract ion chromatogram; **IPA**, isopropanol; **SCX**, strong cation exchange, **TEA**, triethylamine

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whereupon C_{12}–C_{18} is predominant. Since these surfactants containing different alkyl chain lengths are added as ingredients to end products such as shampoo and conditioners, it is of importance to develop analytical separation/detection methods in order to monitor the composition that can ultimately affect the performance of these end products. To date, the analysis of $ATMA^+$ has been accomplished using different methods such as GC-MS requiring conversion to tertiary amine [1–3], HPLC utilizing various detection such as chemiluminescent nitrogen [4], ESI-MS [5–7], and conductometric detection [8]. In addition, CE methods using indirect photometric detection [9–13] or MS detection [14] have also been employed for the analysis of $ATMA^+$.

As a recent alternative to these traditional methods of analysis, CEC is gaining recognition as a powerful separation technique that combines both electrophoretic separation based on charge-to-mass ratio, along with HPLC retention mechanisms including ion-exchange and hydrophobic/hydrophilic interactions with a stationary phase. These attributes therefore make CEC well-suited for analysis of cationic surfactants due to differences in electrophoretic mobility and chain length inherent to these molecules. In particular, CEC coupled to MS offers highly efficient separation along with sensitive detection (*e.g.,* ng/mL). Recent reviews of the numerous CE-MS modes and applications demonstrate the growing acceptance of this hyphenation technique in the analytical community [15, 16]. Several very recent publications from our group have shown the utility of packed column CEC-ESI-MS with a specialized internally tapered column for sensitive analysis of achiral and chiral compounds [17, 18]. Overall, CEC-MS is eventually expected to surpass CZE-MS due to inherent advantages of the former that includes the ability to separate both charged and neutral compounds (with improved selectivity for homologues) as well as the higher sample capacity (resulting in improved limit of detection). On the other hand, lower solvent consumption and higher separation efficiency are the obvious advantages of CEC-MS as compared to HPLC-MS [16]. Therefore, the application of CEC-MS for separation of cationic surfactants is important not only for fundamental understanding of the operating variables, but also to provide a powerful and capable alternative technique to traditional CZE-MS and HPLC-MS.

This manuscript describes a novel CEC-ESI-MS optimized method utilizing an internally tapered tip column for analysis of C_1–C_{18} $ATMA^+$ compounds (Fig. 1). Typically, only the longer chain C_6–C_{18} are classified as surfactants which aggregate to form micelles at a concentration greater than the CMC, whereas the short chain cationic compounds of chain length C_1 and C_2 are included in this

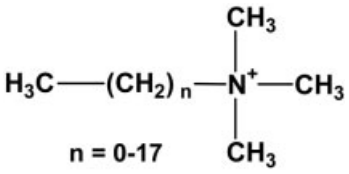

Figure 1. Generalized chemical structure of the $ATMA^+$ surfactant.

work in order to better characterize the developed CEC-MS method. A commercially available non-encapped mixed mode packing consisting of C_6 alkyl chain and sulfonate strong cation exchange (SCX) functionality (*i.e.*, CEC-C_6/SCX) was used for in-house packing of the CEC-MS column. The influence of several critical mobile phase parameters including ACN concentration, addition of triethylamine (TEA), ionic strength effect, and pH are studied. This is followed by a strategy for the ESI-MS optimization including sheath liquid and spray chamber optimization using experimental design for the latter. A study of the system durability and performance proved that excellent interday and intraday reproducibility of migration time and chromatographic resolution is possible for greater than 165 runs over 111 h of continued use. Finally, utilizing the optimized CEC-ESI-MS method protocol, the application to commercial Arquad S-50 $ATMA^+$ detergent sample containing soyabean fatty acid derivatives is next presented. The presence of *cis*/*trans* geometrical isomers for unsaturated $ATMA^+$ is found to occur in the S-50 sample. The developed CEC-MS method is not only suitable for the challenging separation of *cis*/*trans* isomers, but also provides high degree of selectivity for simultaneous separation of saturated and unsaturated $ATMA^+$ as compared to the manufacturer method based on acid/base titrations.

2 Materials and methods

2.1 Chemicals

Tetramethylammonium chloride (98%), ethyltrimethylammonium iodide (99%), oleyl chloride and trimethylamine hydrochloride (98%) were purchased from TCI America (Portland, OR, USA). The *n*-hexyltrimethyl ammonium bromide (98%), *n*-octyltrimethyl ammonium bromide (97%), *n*-decyltrimethylammonium bromide (98%), *n*-tetradecyltrimethyl ammonium bromide (98%), and *n*-hexadecyltrimethyl ammonium bromide (98%) were obtained from Lancaster Synthesis (Pelham, NH, USA). The *n*-octadecyltrimethyl ammonium bromide (99%), *n*-dodecyltrimethyl ammonium bromide (99%), and ammonium acetate (NH_4OAc) (molecular biology grade) were purchased from Sigma (St. Louis, MO,

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USA). TEA (99.5%) was supplied from Aldrich (Milwaukee, WI, USA). A commercial sample, Arquad S-50 (batch SR576363X) was kindly donated from Akzo Nobel (Illinois, USA). ACN, methanol (MeOH) and isopropanol (IPA) of HPLC grade were purchased from Burdick and Jackson (Muskegon, MI, USA). Ammonium hydroxide, acetic acid, and NaCl were supplied by Fisher Scientific (Springfield, NJ, USA). Acetone of HPLC grade was purchased from EM Science (Gibbstown, NJ, USA). Finally, water was purified by a Barnstead Nanopure II purification system (Barnstead International, Dubuque, IA, USA).

2.2 Buffer, sheath liquid and sample preparation

The CEC running buffer was prepared using 100 mM NH_4OAc adjusted to the appropriate pH using acetic acid. An appropriate aliquot of stock solution was then combined with ACN, triply deionized H_2O and TEA using volumetric pipettes, followed by 25 min sonication, filtering using 0.45 PTFE membrane syringe filters and finally degassing for 10 min. The MS sheath liquid was prepared by mixing appropriate volume fractions of MeOH and H_2O followed by sonication and degassing. Stock solutions of C_1–C_2-TMA^+ and C_6–C_{18}-TMA^+ were dissolved at a concentration of 20 mg/mL and 10 mg/mL, respectively, in 65/35 v/v ACN/H_2O. A test mixture containing equal aliquot of each TMA^+ was then combined to constitute the final injection solution.

2.3 CEC-MS column preparation

The mixed mode CEC-C_6/SCX stationary phase (ReliaSil 100Å, 3 μm) was purchased from Column Engineering Inc. (Ontario, CA, USA). In the literature, this type of mixed-mode packing has been utilized for both applied [19–22] and fundamental studies [23] in CEC. A fused-silica capillary (75 μm id, 362 μm od) was obtained from Polymicro Technologies Inc. (Phoenix, AZ, USA). The fabrication of the CEC-MS column including slurry pressure packing and internally tapered outlet tip is the same as documented in our previous work [17, 18] with several important technical differences which are summarized as follows: (i) overnight packing at 200 bar using 70% ACN/30% H_2O; (ii) manual syringe injection of 10 mM NaCl in 70% ACN/30% H_2O, followed by flushing at 200 bar for 3 h; (iii) frit construction using neochrome wire burner while the column was still under 200 bar pressure; (iv) removal of the NaCl solution using running buffer (containing 70% ACN/30% H_2O, pH 3.0, 15 mM NH_4OAc, 0.04% TEA). Thus, the aforementioned modified procedure showed no shrinkage in CEC-C_6/SCX packed bed length.

2.4 CEC-ESI-MS instrumentation

All CEC-ESI-MS experiments were performed on an Agilent $HP^{3D}CE$ system (Agilent Technologies, Waldbronn, Germany), which was interfaced to an Agilent 1100 series MSD quadrupole mass spectrometer, equipped with a CE-MS adaptor kit and a sprayer kit. Sheath liquid was delivered by an Agilent 1100 series HPLC pump equipped with a 1/100 split flow. The ChemStation software (v. 10.02) was used for data processing. The CEC-MS column was installed into the nebulizer according to manufacturer specification with one important difference. It was found experimentally that the optimum positioning of the column tip should be almost flush (protrude ~0.01 mm) with the nebulizer tip instead of protruding ~0.1 mm typically used in open-tubular CE-MS. For CEC-ESI-MS, this correlates to turning the nebulizer adjustment screw counter clockwise by only ¼ mark instead of conventional 2 marks as described in the manufacturer setup manual for the installation of Agilent CE ESI-MS Sprayer Kit (G1607A).

2.5 CEC-ESI-MS conditions and capillary conditioning

The new CEC column was first installed into the MS cartridge followed by preparation of the inlet tip, which involve diamond cutting, and removal of 2 mm polyamide coating using the homemade burner. A manual syringe pump was then attached allowing mobile phase to precondition and equilibrate the column for at least 2 h. For CEC-ESI-MS conditioning, 12 bar pressure was applied to the inlet end, and then the voltage was sequentially raised in increment as follows: 2 kV/20 min, 5 kV/20 min, 10 kV/20 min, 15 kV/20 min, and finally 18 kV/15 min. The maximum separation voltage was 18 kV as higher voltage was found in some cases to create unwanted arcing and shortened the column lifetime. Injection was performed electrokinetically at 6 kV for 6 s. The ESI measurements were conducted in positive ion mode. Nitrogen obtained from a nitrogen generator was used for both nebulizing and drying gas. The mass range in full scan mode was set between 50–1500 *m/z*. For SIM mode, the masses were monitored as group SIM with low resolution and a gain setting 4. The direct infusion of C_8-TMA^+ was conducted using the following settings: sheath liquid: 70/30 MeOH/H_2O, 10 mM NH_4OAc, pH 6.7, pump flow = 0.5 mL/min. Spray chamber: drying gas flow 6 L/min, nebulizer pressure 10 psi, drying gas temp. 300°C, V_{cap} 4000 V, fragmentor 125 V.

3 Results and discussion

Initially, the direct infusion of one representative cationic standard (C_8-TMA^+) was conducted in order to obtain SIM ions for on-line CEC-ESI-MS method development.

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The full-scan (50–1500 *m/z*) MS spectra generated from direct infusion of C_8-TMA^+ showed that only the protonated molecular ion $[M^+ H]^+$ (*m/z* 172) was formed in highest abundance (*e.g.*, Signal. Abundance = 965 000). Therefore, $[M^+ H]^+$ was used for sensitive SIM monitoring for all standard cationic surfactants.

3.1 Optimization of the CEC separation

3.1.1 Effect of ACN

As a starting condition, the separation of the nine standards (*e.g.,* C_1-,C_2-C_6-,C_8-, C_{10}-, C_{12}-,C_{14}-,C_{16}-, and C_{18}-TMA^+) was first investigated using a mobile phase of 60% v/v ACN, 30 mM KH_2PO_4, pH 3.0. The selection of this mobile phase conditions was based on previous studies by Ye *et al.* for the analysis of protein and peptides [24]. Slight modifications to these conditions were made considering the mixed mode C_6/SCX stationary phase and MS detector used in this work. Hence, a greater percentage of 90% ACN v/v was used as a starting condition in order to counteract increased hydrophobic interaction due to presence of alkyl chains on both analyte and stationary phase. Furthermore, for better MS compatibility, KH_2PO_4 was replaced with volatile NH_4OAc (10 mM).

The corresponding electrochromatograms in Fig. 2 show that 90% ACN v/v provides separation of all long chain C_6–C_{18}-TMA^+ cationic surfactants in less than 30 min. However, the inset extract ion chromatogram (EIC) at 90% v/v for shorter chains C_1-TMA^+ (*m/z* 74) and C_2-

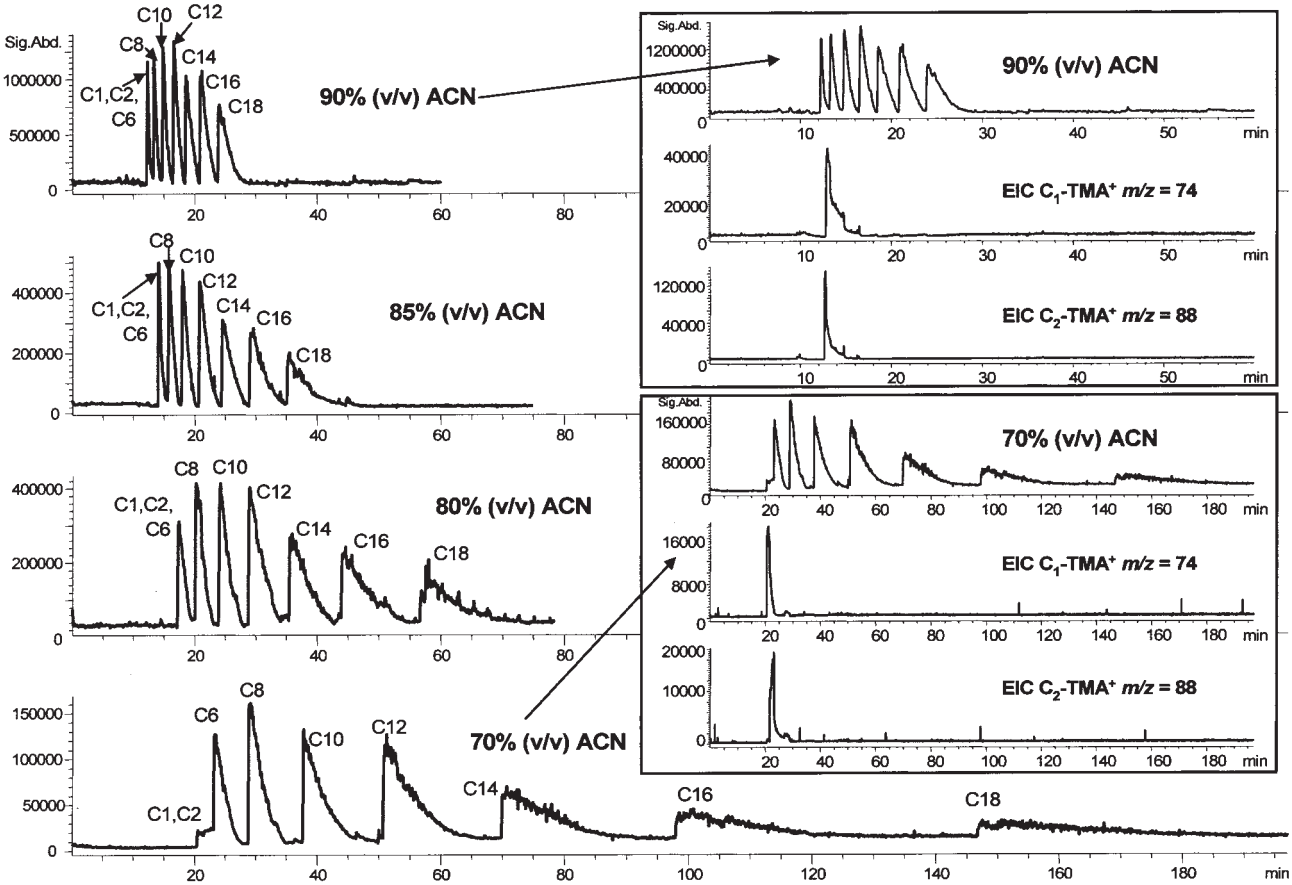

Figure 2. Electrochromatograms showing the effect of ACN volume fraction on the separation of nine alkyltrimethylammonium cationic compounds. Inset shows the EIC for short-chain C_1- and C_2-TMA^+ at (top) 90% ACN v/v and (bottom) 70% ACN v/v. Conditions: column 1: 64 cm long, 22 cm packed bed length, 75 μm id capillary tapered internally (*ca*. 8 μm) packed with 3 μm CEC C_6/SCX stationary phase; mobile phase: ACN% v/v varied, 10 mM NH_4OAc, pH 3.0. Sheath liquid; 70/30 MeOH/H_2O, 10 mM NH_4OAc, natural pH 6.7, pump flow = 5 μL/min. Spray chamber: drying gas flow 6 L/min, nebulizer pressure 10 psi, drying gas temp. 300°C, V_{cap} 4000 V, fragmentor 125 V. Electrokinetic injection: 10 kV, 10 s, 16 kV run voltage, ESI SIM positive ions (9 ions) monitored as group SIM at *m/z* 74, 88, 144, 172, 200, 228, 256, 284, 312. Analytes: C_1–C_2-TMA^+ 20 mg/mL, C_6–C_{18}-TMA^+ 10 mg/mL all in 65/35 v/v ACN/H_2O.

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TMA^+ (*m/z* 88) suggests that these shorter chain quats are co-eluting with C_6-TMA^+. Therefore, the % v/v ACN was lowered sequentially in order to better resolve the short chain C_1- and C_2-TMA^+. Decreasing the ACN fraction from 90–80% v/v ACN was observed to have the greatest influence on the longer chain surfactants, which were retained much longer due to hydrophobic interaction with the stationary phase. Interestingly, the shorter chain C_1- and C_2-$TMAs^+$ still co-eluted with C_6-TMA^+. Further decreasing the ACN from 80 to 70% v/v provided significantly longer retention for longer chains as well as peak tailing. However, the EIC showed no separation between C_1- and C_2-TMA^+ at 70% v/v ACN but improved resolution from neighboring C_6-TMA^+. Overall, 70% ACN v/v was chosen based upon resolution of short chain C_1- and C_2-TMA^+ from C_6-TMA^+, whereupon the problems of increased retention and peak tailing of longer chain cationic surfactants were next addressed.

3.1.2 Effect of TEA

It is well known that the protonated form of TEA competes with the cationic analytes for the sulfonic acid cation exchange sites on the stationary phase, ultimately reducing the retention of cationic analytes. Thus, it was decided to add small increments of TEA to the running buffer to decrease the retention of longer chain surfactants while still achieving resolution of C_1- and C_2-TMA^+ from C_6-TMA^+. First, as an experiment of interest, TEA was added in the range of 0.005–0.08% v/v to 90% v/v ACN to further enhance the separation of C_1- and C_2-TMA^+ . Over the entire range of TEA additive, no separation of C_1- and C_2-TMA^+ from each other or from C_6-TMA^+ was achieved (data not shown). However, baseline separation of C_6–C_{18} $ATMA^+$ in under 30 min was observed even with no added TEA. Addition of 0.04% v/v TEA was found to significantly reduce peak tailing especially for longer chain surfactants along with a decrease in total analysis time. At greater 0.08% v/v TEA, a higher CEC operating current (*e.g.*, 24 μA) was generated causing excess joule heating of the capillary and unstable baseline. Upon repeating the TEA experiment at 70% ACN v/v shown in Fig. 3, even a small volume increment of TEA (*i.e.*, 0 to 0.01% TEA) provided significant reduction in retention for all surfactants. In particular, the retention effects were more pronounced for longer chains. Overall, increasing the TEA content over the range of 0.01 to 0.04% at 70% ACN v/v still provided no resolution between C_1- and C_2-TMA^+, but their separation selectivity with C_6-TMA^+ was maintained. In addition, the total analysis time decreased nearly five-fold and peak tailing of longer chains (*e.g.,* C_{16}- and C_{18}-TMA^+.) were dramatically improved. Similar to the trend observed at 90% v/v ACN, a higher concentration of TEA resulted in higher current which makes the CEC column unstable. Therefore, a maximum of 0.04% v/v TEA at 70% ACN v/v was chosen for further study.

3.1.3 Effect of buffer pH

Next, the effect of varying the buffer pH over the range pH 2.5–5.0 was investigated maintaining previously optimized 70% v/v ACN and 0.04% v/v TEA. Adjustment of the pH using acetic acid was expected to influence mainly the EOF and ion exchange process rather than analyte or stationary phase ionization. This is because the alkyl-TMA^+ ions maintain a non-reversible constant positive charge due to quaternization of nitrogen by surrounding alkyl groups (Fig. 1), whereas the sulfonic acid group of the stationary phase is negatively charged at pH$>$1. The results showed that modification of the buffer pH over the range of 2.5–4.0 provides almost identical separation and detection. However, increasing the pH to 5.0 lengthens the analysis time from 35 min to 60 min along with loss in signal for short chain C_1- and C_2-TMA^+ and excessive peak tailing of longer C_{16}- and C_{18}-TMA^+ ions (data not shown). In order to better understand the elution trends, thiourea was used as a to marker to monitor the EOF. Triplicate runs were performed with %RSD less than 1.1%. As the p*K*a of thiourea is 1.44 ± 0.5 (Science Finder Scholar, v.2002), this was expected to be neutral over pH 2.5–5, and therefore deemed acceptable to be used as a t_0 marker. In general, an increase in retention of thiourea with decreasing pH was observed due to added ionic strength at lower pH resulting in lower EOF from decrease in double layer thickness (data not shown). Overall, a pH of 3.0 was selected due to fastest separation and enhanced S/N of cationic surfactants.

3.1.4 Effect of ammonium acetate concentration

Continuing the CEC optimization utilizing conditions of 70% v/v ACN, 0.04% v/v TEA and pH 3, the effects of varying the ammonium acetate (NH_4OAc) concentration over the range of 2.5–20 mM were next examined. The corresponding electrochromatograms are presented in Fig. 4a. Several trends are worth mentioning. First, at a lower NH_4OAc concentration of 2.5 mM there are not enough NH_4^+ ions present in the mobile phase for adequate ion exchange, hence a longer separation and loss of signal for several longer chain cationic surfactants is observed similar to the previous trend at pH 5. Second, upon increasing the NH_4OAc concentration to 5 mM, the ion exchange is improved and overall better separation and sensitivity is provided. Third, increase in NH_4OAc concentration to 10 mM was shown to enhance S/N as

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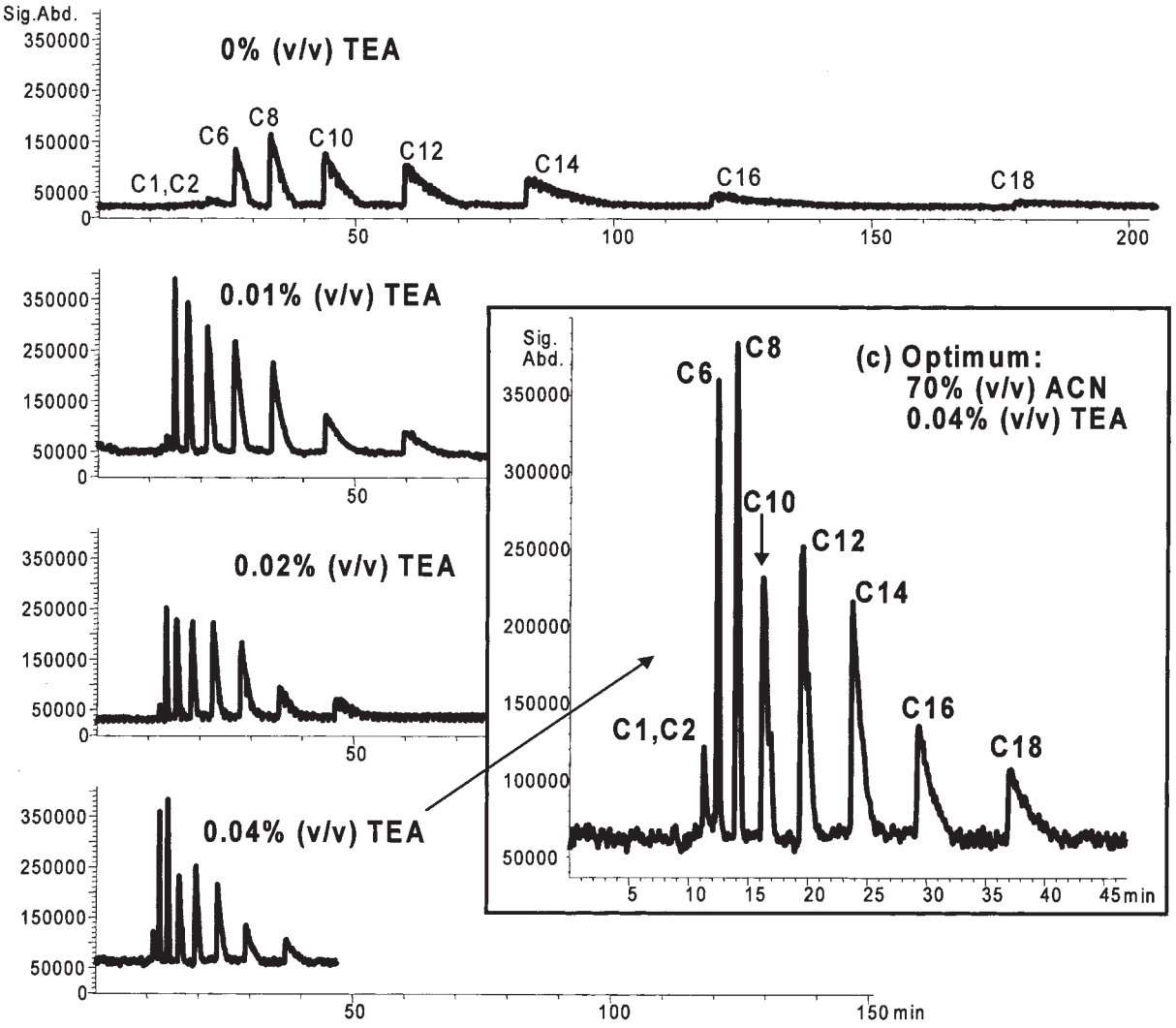

Figure 3. Electrochromatograms showing the effect of TEA volume fraction on the separation. Conditions are the same as in Fig. 2, except % TEA varied at 70% ACN v/v on Column 2 packed bed = 27 cm, total length 70 cm with 18 kV runs. Inset shows the optimum separation achieved using 0.04% TEA. Spray chamber and sheath liquid conditions are the same as in Fig. 2.

observed from a stable baseline with a significant decrease in the analysis time for longer chain (*e.g.*, C_{12}–C_{18}-TMA^+) surfactants. However, very slight change in migration times were observed for short chains (*e.g.*, C_1–C_{10}-TMA^+) as compared to longer chains aforementioned. The same trend was seen when increasing NH_4OAc ionic strength over the range 10–20 mM, whereupon shorter chains exhibited very minor decrease in retention times compared to longer chains, which continued to significantly reduce the retention times of the latter.

In order to better understand the elution trends, thiourea was again monitored as t_0 marker as shown in Fig. 4b. Only duplicate runs were performed because the %RSD was found to be less than 0.5%, hence error bars are too small to be visible on plot. The general trend clearly shows that t_0 is increased. Hence, EOF is slowing down with increasing ionic strength. This is in accordance with previous pH discussion that states for increased BGE ionic strength increases the double layer thickness as well as higher viscosity reduces the EOF. However, as described earlier when pH was decrease from 4.0–2.5, it caused an increase in ionic strength. Consequently, the ion exchange *vs*. EOF effects ultimately counteracts each other causing no change in net retention time for surfactants. In contrast, the effect of increasing NH_4OAc concentration shown in Fig. 4a clearly shows that ion exchange is predominating over EOF for longer chains

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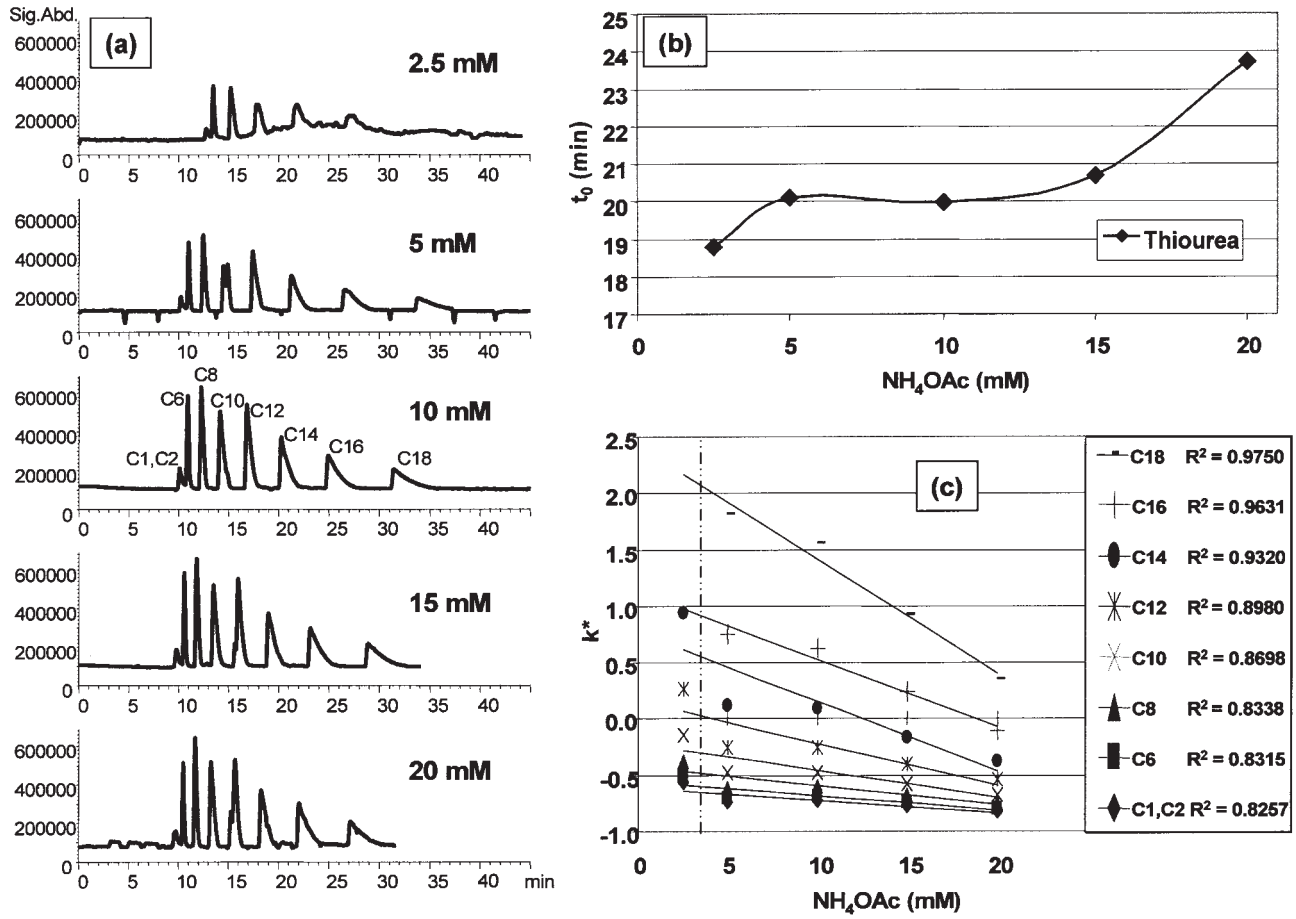

Figure 4. Electrochromatograms (a) and plots (b–c) showing the effect of buffer NH_4OAc concentration (mM) on the separation, (b) thiourea retention trend (t_0) and (c) CEC capacity factor (k^*) *vs.* NH_4OAc concentration (mM) with correlation value (dashed line indicates 2.5 mM is omitted from R^2). CEC conditions are the same as in Fig. 3, except the mobile phase pH = 3.0 and column 4 = 25 cm packed, 67 cm total length. Spray chamber and sheath liquid conditions are the same as in Fig. 2.

(*e.g.*, C_{12}–C_{18}-TMA^+) shown by decrease in retention times. On the other hand, short chains (*e.g.*, C_1–C_{10}-TMA^+) exhibit no change suggesting counteracting effects. Therefore, a plot of electrochromatographic capacity factor (k^*) was constructed in Fig. 4c to better understand these trends, considering both the contributions from electrophoresis *vs.* chromatographic retention using the Eq. (1) set forth by Ye *et al.* as follows [24]:

$$k^* = (k' - \mu_{ep}/\mu_{eo})/(1 + \mu_{ep}/\mu_{eo}) \quad (1)$$

where k' is the chromatographic capacity factor $(t_R\text{-}t_0)/t_0$, μ_{ep} is the electrophoretic mobility and μ_{eo} is the electroosmotic mobility. It was decided to omit 2.5 mM from the best-fit line and corresponding equation as outlying data, which considerably lowered the correlation factors (R^2) for short chains (data not shown). This is because this lower ionic strength does not provide adequate operating conditions, hence the dashed vertical line on the k^* plot is to omit 2.5 mM. Overall, for longer chain C_{18}- and C_{16}-TMA^+, the k^* generally decreased with increasing ionic strength over the entire range of 5–20 mM. In addition, reasonably high correlation factors are observed which suggests that retention due to ion-exchange is predominant. For shorter chain length, (*e.g.*, C_1–C_{10}-TMA^+), the k^* was essentially the same at lower ionic strength of 5–10 mM. However, over the range of 15–20 mM the k^* showed gradual decline which was more pronounced for mid-chain length (*e.g.,* C_{14}, C_{12}-TMA^+) surfactants. Note, that the correlation (R^2) factors were higher for longer chain (*e.g.,* C_{18}- and C_{16}-TMA^+) but then falls off gradually with decrease in chain length of cationic surfactants. In general, it can be concluded that for shorter chain cationic surfactants, the separation is controlled by electrophoresis rather than chromatographic retention. This is supported by negative k^* corresponding

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to elution before thiourea, as well as minor influence of ionic strength with respect to retention times. In contrast, for longer chain surfactants, the separation is controlled by chromatographic as well as ion-exchange retention as shown by a significant decrease in retention time with increasing ionic strength as well as positive k* values due to their elution following the EOF marker. Although 20 mM NH_4OAc offered the shortest analysis time, this was coupled with increased current (24 μA), which could in turn shorten the column lifetime and promote joule heating. Therefore, 15 mM was selected as final NH_4OAc concentration.

3.2 Optimization of the ESI-MS detection

3.2.1 ESI-MS sheath liquid tuning

Utilizing optimized CEC separation conditions of 70% v/v ACN, 0.04% v/v TEA, pH 3 and 15 mM NH_4OAc, the sheath liquid composition and flow rate were next investigated to study the effects on MS signal abundance and S/N utilizing two representative surfactants (*e.g.,* C_6- and C_8-TMA^+). A comparison of IPA volume fraction over the range of 20–70% *versus* using 30–90% MeOH all containing 10 mM NH_4OAc is presented in Figs. 5a–d. The

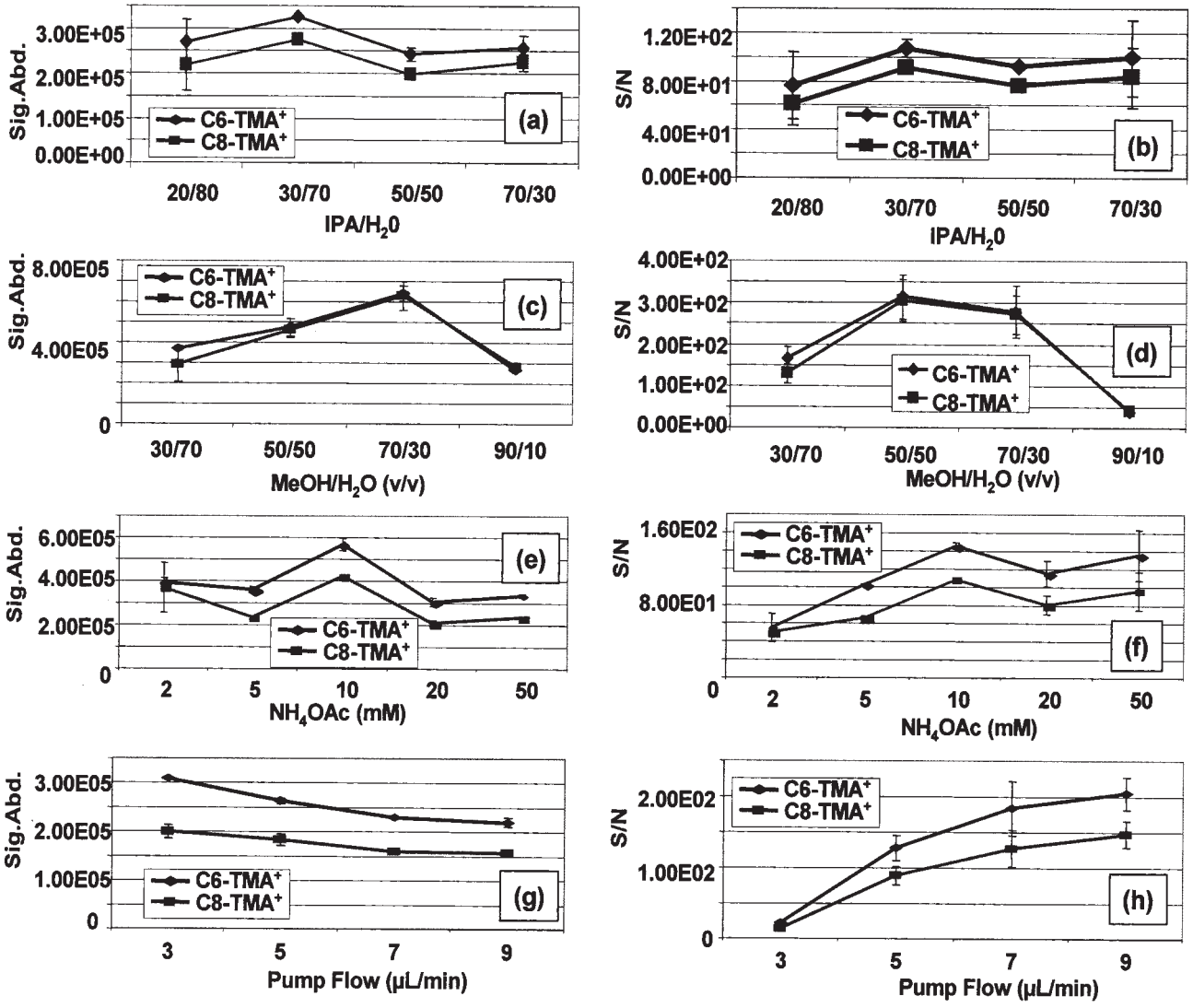

Figure 5. Sheath liquid tuning plots. Effects of IPA volume fraction on (a) MS signal, (b) S/N, (c) MeOH volume fraction on (c) MS signal, (d) S/N. NH_4OAc concentration effects on (e) MS signal (f) S/N using 50/50 MeOH/H_2O (g) pump flow effect on (g) MS signal (h) S/N using 10 mM NH_4OAc. Conditions: same as in Fig. 4, except CEC mobile phase contains 15 mM NH_4OAc and 15 kV runs using 3000 V_{cap}.

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results showed that for IPA, a smaller volume fraction of 30% provided highest abundance for both C_6- and C_8-TMA^+, although consideration of the error bars suggests only minor effects of IPA on signal abundance over the range studied. A similar effect of IPA on the S/N was observed that followed the same trend as the aforementioned signal abundance. The use of %MeOH in the sheath liquid showed that both signal and S/N are higher in the range of 50–70% v/v for both C_6- and C_8-TMA^+ions. Since S/N with %MeOH at its optimum composition (Fig. 5b) is more than twofold higher compared to the optimum for IPA (Fig. 5d), it was decided to select 50% v/v MeOH for increased sensitivity.

Next, the effect of addition of NH_4OAc to the sheath liquid over the range of 2–50 mM was examined for further improvement of MS signal and S/N. The corresponding plots shown in Figs. 5e–f demonstrate that similar response for both surfactants was achieved over entire concentration range, however, 10 mM NH_4OAc provided highest signal (Fig. 5e) abundances as well as improved S/N (Fig. 5f). Finally, the variation of sheath liquid pump flow on both signal intensity and S/N in the range of 3–9 μL/min is illustrated in Figs. 5g–h. Opposite trends for signal *vs*. S/N were observed. The signal continually decreased with increasing pump flow probably due to the dilution effect with the sheath liquid. On the other hand, the S/N continually increased over the same flow rate settings due to increased electrospray stability and reduction in background noise. Overall, 7 μL/min was selected as a compromise for stable and sensitive ESI-MS detection.

3.2.2 ESI-MS spray chamber tuning

First, the effects of fragmentation voltage (*V*) and MS capillary voltage (V_{cap}) on MS signal and S/N was evaluated utilizing previously optimized CEC conditions and sheath liquid containing 50/50 v/v MeOH/H_2O, 10 mM NH_4OAc delivered at 7 μL/min. Briefly, a fragmentor of 50 V was chosen as this provided the highest S/N and a V_{cap} equal to 3000 V was selected as a compromise between MS signal, S/N and stability of the CEC column.

For spray chamber parameters including nebulizer pressure, drying gas flow rate and drying gas temperature, our previous work has shown that the use of structured or tailored experimental design are an efficient and effective means of optimizing these settings [17]. The corresponding plots showing effects of aforementioned spray chamber parameters on the MS signal and S/N for representative C_8-TMA^+can be seen in Figs. 6a–b. First, the design calls for maintaining a drying gas temperature (*e.g*., 150, 200, and 250°C) while varying the drying gas flow and

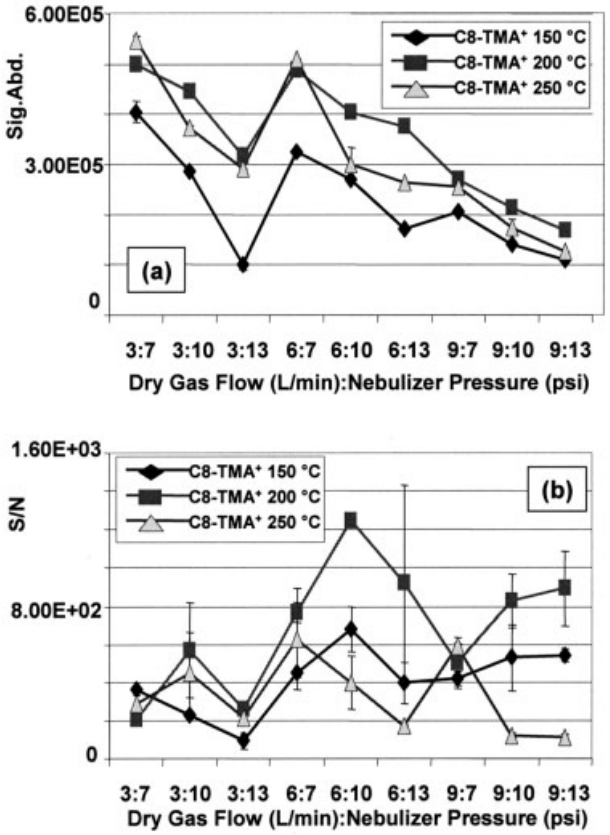

Figure 6. ESI spray chamber optimization. Structured experimental design calls for maintaining two of three spray chamber parameters constant for analysis of trends when varying the drying gas temperature, drying gas flow rate, and nebulizer pressure. For the representative surfactant C_8-TMA^+, plots show effects on (a) MS signal, (b) S/N. Conditions: same as Fig. 5 except sheath liquid 50/50 MeOH/H_2O, 10 mM NH_4OAc, 7 μL/min and fragment voltage = 50 V.

nebulizer pressure in a systematic fashion. For example, in Fig. 6 at each drying gas temperature the design calls for maintaining the drying gas flow (*e.g*., 3, 6 or 9 L/min) then varying the nebulizer pressure in the range of 7–13 psi. This format allows optimization while minimizing time between changing spray chamber settings.

Overall, the trends can be summarized as follows. Variation in the drying gas temperature showed that lower drying gas temperature of 150°C always provided lower MS signal (Fig. 6a) as compared to higher temperatures of 200 and 250°C, which were very similar in intensity. A moderate drying gas temperature of 200°C provided a slightly higher MS signal in some cases (*e.g*., Fig. 6a, 3:10, 6:10, 6:13). Comparison of the three drying gas temperature effect on S/N shown in Fig. 6b demonstrate that 200°C overall provided the highest S/N as compared to low 150°C and high 250°C temperature. Next, analysis

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of the nebulizer pressure maintaining constant drying gas temperature and drying gas flow revealed that in all cases the MS signal decreases with increase in nebulizer pressure. For example, in Fig. 6a increasing the nebulizer pressure from 7–13 psi at each drying gas flow rate (3, 6 or 9 L/min) and each drying gas temperature (150, 200 and 250°C) decreases the signal abundance. The effect of nebulizer pressure on S/N suggested that in several cases moderate setting at 10 psi was slightly higher than lower 7 psi or higher 13 psi (*e.g.,* Fig. 6b at 200°C drying gas temperature, increasing nebulizer pressure from 3:7 to 3:10 to 3:13, as well as 150°C increasing from 6:7 to 6:10 to 6:13). It was also observed that higher nebulizer pressure of 13 psi provides a large deviation in S/N (*e.g.*, Fig. 6b, 6:13) indicating that higher nebulizer pressure is not well suited for CEC-MS. Finally, for effect of varying the drying gas flow rate from 3–6–9 L/min showed general systematic decline in MS signal with increase in flow (*e.g.* compare 3:7, 6:7, 9:7, Fig. 6a) although at 10 psi the signal was similar in some cases (*e.g.,* 3:10, 6:10, Fig. 6a). The effect of drying gas flow rate on S/N suggested that moderate setting at 6 L/min provided the highest S/N (*e.g.,* 6:10). Thus, it can be concluded that the optimum spray chamber settings for ESI-MS detection of cationic surfactant are 6 L/min, 10 psi, and 200°C.

3.3 LOD and LOQ

The linearity, LOD and LOQ of the CEC-ESI-MS system were evaluated utilizing the optimized CEC separation and MS detection conditions. As the longer chain cationic surfactants including C_{12}–C_{18}-TMA^+ are most frequently used in commercial formulations, these analytes were quantitated while choosing C_8-TMA^+ as an internal standard. For $n = 5$ runs, the concentration of C_{12}–C_{18}-TMA^+ was varied over the range of 5 ng/mL –10 μg/mL at eight concentration levels while C_8-TMA^+ concentration was held constant at 300 ng/mL. Overall, 10 ng/mL–3 μg/mL was found to be the widest concentration range that provided acceptable R^2 (data not shown). For the LOD and LOQ, Table 1 provides a summary of the calibration parameters over the linear range of 10–3 μg/mL. The satisfactory linear regression coefficients indicate that the cationic surfactant responses were linear over the concentration range studied and that these lines pass through the origin. Using three times S/N value, the LOD was determined for longer chain C_{18}-TMA^+ to be 5 ng/mL, although higher S/N was observed for shorter chain C_{12}–C_{14}-TMA^+. For example, at 5 ng/mL the EIC for C_{12}-, C_{14}-, C_{16}- and C_{18}-TMA^+ provided S/N of 30, 20, 6 and 4, respectively. The LOD estimated for cationic surfactants based on the aforementioned S/N is tabulated in Table 1. This data suggests that the LOD for shorter chain TMA^+ should be much lower than the longer ones. For example, at 5 ng/mL C_{12}-TMA^+ and C_{18}-TMA^+ provided a S/N = 30 and 4, respectively. Thus, LODs for C_{12}-TMA^+ and C_{18}-TMA^+ was estimated to be 500 pg/mL and 5 ng/mL, respectively whereas the LOQ was determined experimentally at ten times S/N. A concentration of 10 ng/mL was established as the LOQ for all C_{12}–C_{18}-TMA^+.

3.4 Precision

The precision of the optimized CEC-MS method was evaluated regarding the repeatability. For this, the separation of a mixture of nine cationic standards was run continuously over a one week period in order to simulate running the system in a rigorous industrial laboratory setting. In all, the system was run for seven consecutive days approximately 16 h per day, 40 min each run. The result of

Table 1. Linearity, LOD and LOQ of C_{12}–C_{18}-TMA^+ surfactants[a)]

Analyte (TMA^+)	Linearity range	Calibration parameters			LOD[b)]	LOQ[b)] (ng/mL)
		Slope	Intercept	r^2		
C_{12}	10 ng/mL–3 μg/mL	0.0023	0.0236	0.9994	500 pg/mL	10
C_{14}	10 ng/mL–3 μg/mL	0.0013	0.0185	0.9966	500 pg/mL	10
C_{16}	10 ng/mL–3 μg/mL	0.0005	0.0038	0.9905	2.5 ng/mL	10
C_{18}	10 ng/mL–3 μg/mL	0.0002	0.0023	0.9872	5 ng/mL	10

a) Optimum CEC separation conditions: 70% ACN, 0.04% TEA, pH 3.0 and 15 mM NH_4OAc. Sheath liquid optimized to 70/30 MeOH, 10 mM NH_4OAc delivered at 7 μL/min, ESI–MS operating and spray chamber parameters: fragmentor voltage = 50 V, V_{cap} = 3000 V, drying gas flow rate 6 L/min, nebulizer pressure 10 psi, drying gas temperature 200°C.
b) Determined experimentally for $n = 5$ runs.
c) Estimated from S/N at 5 ng/mL.

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the experiment proved that the system is durable for greater than 167 runs, over 111 h which was deemed as an acceptable stopping point. System durability and repeatability for one representative cationic surfactant C_8-TMA^+ is demonstrated in Table 2 which shows the intraday and interday precision of the migration time, peak area, normalized peak area, efficiency and resolution.

First, for consideration of the migration time reproducibility shown in column 3, all individual day %RSD was acceptably less than 2%. Similarly, both the t_R intraday (n = 22) and interday (n = 167) %RSD was impressively less than 1.5 and 5%, respectively. Next, an evaluation of the peak area response shown in column 4 exhibited a slightly higher error with %RSD as high as 10% (Day 1). Moreover, intraday and interday %RSD was as large as 21%. This larger deviation is expected due to the absence of internal standard and variation in MS response. Therefore, the following column 5 shows the NPA using C_6-TMA^+ as an internal standard. The NPA provides significantly improved in-day %RSD lower than 4%, with much improved intraday %RSD of 2.9% and interday %RSD of 4.3%. The peak efficiency data provided in column 6 showed %RSD in the range of 4.6–9.5% with intraday and interday %RSD of 7.1 and 10.3, respectively. Lastly, the reproducibility for R_s shown in column 7 demonstrates very low deviation for all seven days, intraday %RSD of 2.9, and impressive 4.2% RSD for interday (n = 167 injections). Overall, the experimental data demonstrates a precise yet rugged and durable CEC-MS system suitable for applications in an industrial setting.

3.5 Application to commercial sample

Next, the developed CEC-ESI-MS method was applied for analysis of a commercial cationic detergent Arquad S-50. This soyabean fatty acid derivative or alkyl trimethylquaternary ammonium compound has the general formula $[R\text{-}N(CH_3)_3]^+Cl^-$ where R represents a straight alkyl chain mainly consisting of a mixture C_{16}–C_{18}. The uses of the product include microbiocides as well as oil field industrial applications. According to the manufacturer product guide (Product Care Guide, 2002 Akzo Nobel Surface Chemistry, Publication SC02–01), the alkyl chains present in S-50 include both saturated R = C_{12}, C_{14}, C_{16}, and C_{18} as well as unsaturated R = C_{16}, C_{18}' (one double bond), and C_{18}" (two double bonds). Similar

Table 2. Intraday and interday precision of the migration time, peak area, normalized peak area (NPA), efficiency (N) and resolution (R_s)[a)]

	t_R (min)	Peak area	NPA[b)]	N	R_s
Day 1 avg.	13.0	3 100 000	1.3	31 000	5.5
n = 22%RSD	1.9	10.0	2.9	5.9	2.8
Day 2 avg.	12.9	2 500 000	1.3	30 000	5.4
n = 23%RSD	1.5	4.3	2.6	4.6	1.9
Day 3 avg.	13.1	1 900 000	1.3	29 000	5.4
n = 30%RSD	0.7	8.9	2.2	6.7	2.9
Day 4 avg.	13.1	2 000 000	1.4	27,000	5.2
n = 25%RSD	0.8	7.9	3.9	9.5	3.4
Day 5 avg.	11.9	1 900 000	1.3	27 000	5.3
n = 22%RSD	0.9	7.1	3.7	7.7	3.0
Day 6 avg.	12.0	1 800 000	1.4	25 000	5.1
n = 30%RSD	1.4	7.5	2.5	8.0	3.4
Day 7 avg.	11.8	2 200 000	1.4	26 000	5.1
n = 15%RSD	0.5	8.5	2.6	7.5	3.0
Intraday[c)] avg.	12.5	2 200 000	1.4	28 000	5.3
$n_{avg.}$ = ~22%RSD	1.1	7.7	2.9	7.1	2.9
Interday[d)] avg.	12.6	2 200 000	1.4	28 000	5.3
$n_{avg.}$ = 167%RSD	4.6	20.6	4.3	10.3	4.2

a) Conditions: same as Table 1.
b) NPA = peak area C_8-TMA^+/peak area C_6-TMA^+.
c) Calculated using the average values of day 1–7.
d) Calculated using all 167 injections.

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to previous analysis of the standards, ESI direct infusion of the S-50 was first conducted in order to obtain the ion spectra. The results showed high abundance of *m/z* 312 (C_{18}), *m/z* 310 (C_{18}') and *m/z* 308 (C_{18}") followed by less intense *m/z* 284 (C_{16}). Therefore, it was decided to monitor the SIM molecular ions $[M^+H]^+$ corresponding to the respective chain lengths (*e.g.* C_{12}–C_{18}) surfactants reported in the manufacturer product guide.

Figure 7 presents the corresponding electrochromatogram of S-50 along with the EICs for all respective alkyl chains included in the SIM method. Most interestingly, the EIC shows that for unsaturated compounds more than one peak was observed. For example, the EIC of C_{16}'-TMA^+ and C_{18}'-TMA^+ showed two peaks as compared to only one for both C_{16}-TMA^+ and C_{18}-TMA, respectively. Similarly, C_{18}"-TMA^+ shows the presence of four peaks. Therefore, it was hypothesized that these multiple peaks observed for unsaturated cationic surfactants were representative of the *cis*/*trans* composition arising from the double bond configuration. This is illustrated in Fig. 7 which shows the separation of four C_{18}"-TMA^+ isomers and two C_{18}'-TMA^+ isomers in S-50. As expected C_{18}"-TMA^+ isomers with two double bonds are more hydrophilic, and they eluted faster than C_{18}'-TMA^+ isomers with one double bond. In addition, a comparison of the experimental% alkyl distribution for most of the chain length using ChemStation v.10.02 was found to correlate to the manufacture product guide (Table 3).

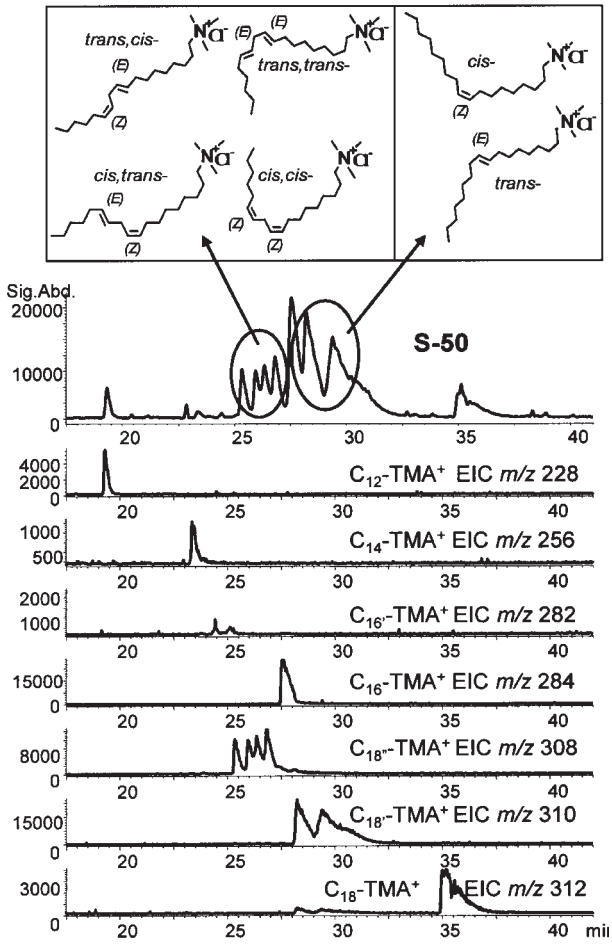

Figure 7. CEC-ESI-MS analysis of Arquad S-50 industrial sample. Conditions were the same as in Fig. 6 except sample concentration was 15 μg/mL and injection was 6 kV for 8 s. Top shows SIM group method monitoring *m/z* for C_{12}–C_{18}-TMA^+ , while the bottom shows EIC of each ion, four peaks in the region of 25–27 min (left circle) corresponding to separation of four *cis-trans* isomer for C_{18}"-TMA^+ with two degrees of unsaturation; two peaks in the region of 28–30 min (right circle) corresponding to separation of two *cis-trans* isomer for C_{18}'-TMA^+ with one degree of unsaturation.

3.5.1 Synthesis of *cis*-C_{18}'-TMA^+ and spiking into S-50 commercial sample

In order to validate the separation of *cis*/*trans* isomers shown in Fig. 7, it was decided to obtain a pure (1) *cis*- or (2) *trans- isomer of* C_{16}–C_{18}-TMA^+ surfactant then spike the S-50 sample followed by comparison of the peak area. However, a search for these compounds from all chemical manufacturers showed that only the carboxylic acid form was available in *cis* form (*e.g., cis*-oleic acid), not the trimethylated ammonium surfactant. Therefore, an alternate route was approached which involved in-house synthesis of representative *cis*-C_{18}'-TMA^+ with hopes of identifying the C_{18}' (*cis*) component presented in Fig. 7.

Following the synthesis of *cis*-C_{18}-TMA^+, a spiking study of S-50 was next undertaken with hope of identifying the *cis*- component C_{18}'. The corresponding electropherograms for the spiking study are presented in Figs. 8a–c. Duplicate runs are shown for qualitative reproducibility including the EIC for comparison of *cis*- *trans* peak ratios. In Fig. 8a, both EIC for C_{18}' component in S-50 shows that the *cis-trans* peak ratio is very similar in height. However, for the synthesized *cis*-C_{18}-TMA^+ shown in Fig. 8b, the *cis-trans* ratio is much higher as evident from the increase height of the left peak which indicates that *cis* C_{18}'-TMA^+ elutes before the *trans* C_{18}'-TMA^+. Similarly, when the *cis*-C_{18}-TMA^+ is spiked into the S-50 commercial sample shown in Fig. 8c, an increase in *cis* height was observed. Although the qualitative differences in peak height are clearly established in the Figs. 8a–c, for proper quantification and identification it was decided to repeat the experiment running each sample six times ($n = 6$) replicated on two more columns and monitor the differences in peak area rather than peak height.

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Table 3. Composition of S-50 sample, experimental *vs.* manufacturer value[a)]

TMA^+	C_{12}	C_{14}	C_{16}'	C_{16}	C_{18}	C_{18}'	C_{18}"
Soya alkyl percent (manufacturer)	0.5	1.0	1.0	16.0	15.0	49.5	13.0
CEC-ESI-MS (% peak area)	2.5±1.5	0.9±3.1	0.3±7.8	19.6±1.1	6.6±1.5	47.2±1.4	22.9±2.2

a) Manufacturer method uses back titration of base acids from which amines were derived.

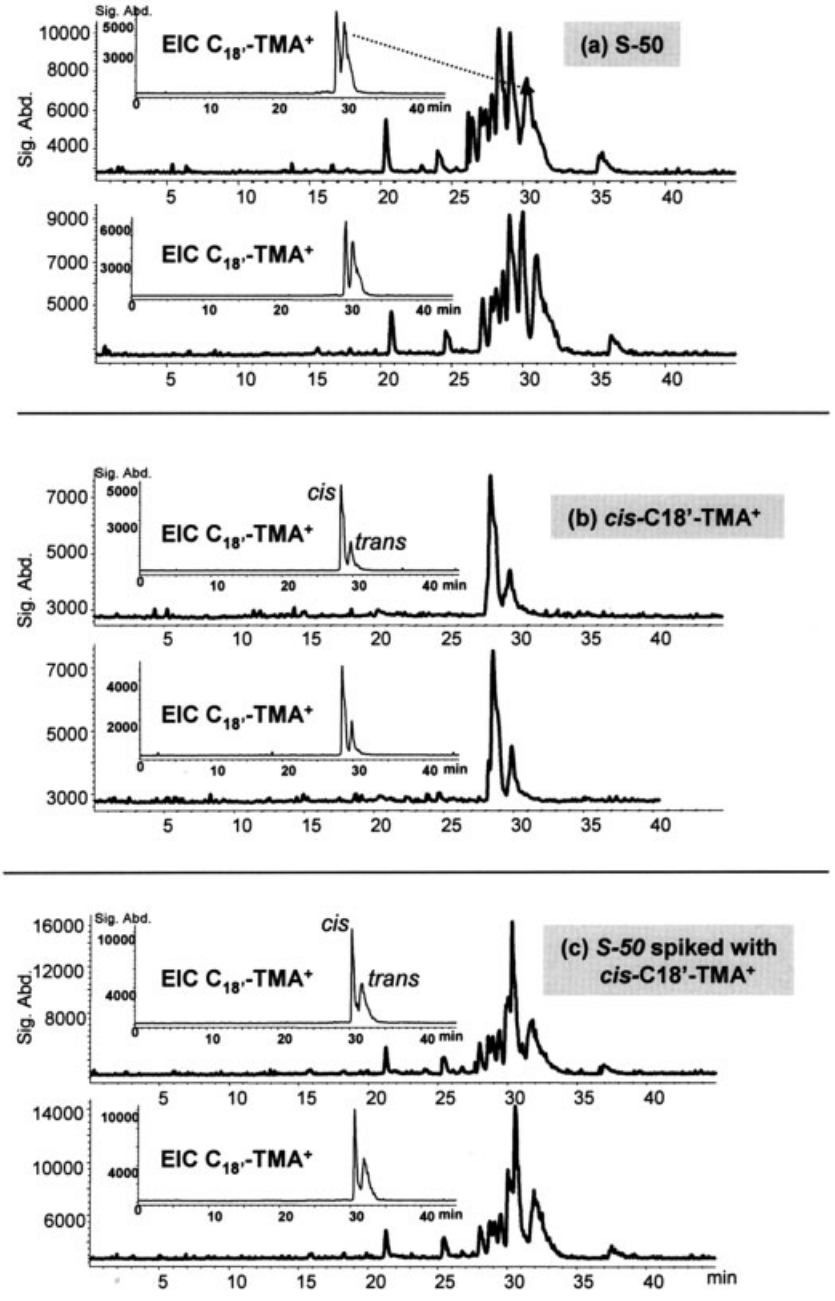

Figure 8. Spiking study of the *cis-trans* C_{18}' component present in Arquad S-50 sample. Duplicate injections for each study are shown for reproducibility. Conditions are the same as in Fig. 7. (a) SIM analysis of S-50 sample. The inset EIC shows extracted *m/z* corresponding to C_{18}'. (b) Analysis of synthetic *cis*-C_{18}'-TMA^+ injected at 10 μg/mL with corresponding EIC (shown as inset) for C_{18}'-TMA^+. (c) spiking of synthetic *cis*-C_{18}'-TMA^+ into Arquad S-50 using two vial injections both 6 kV 8 s. SIM ions and analyte concentrations are same as in (a) and (b).

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Table 4. Comparison of the peak area percent for C_{18}' *cis*/*trans* isomers in Arquad S-50 *versus* S-50 spiked with *cis*-C_{18}'-TMA^+

Trial 1	S-50 (15 μg/mL)		*cis*-C_{18}, -TMA^+ (10 μg/mL)		S-50 spiked +*cis*-C_{18},-TMA^+	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*
	26.6	73.4	60.5	39.5	33.7	66.3
	34.6	65.4	60.6	39.4	32.9	67.1
	29.9	70.1	61.5	38.5	35.9	64.1
	27.7	72.3	59.7	40.3	33.9	66.1
	31.0	69.0	63.3	36.7	33.8	66.2
	28.3	71.7	62.3	37.7	37.1	62.9
Avg.	29.7	70.3	61.3	38.7	34.6	65.5
SD	2.9	2.9	1.3	1.3	1.6	1.6
%RSD	9.7	4.1	2.2	3.4	4.6	2.4

Trial 2	S-50 (15 μg/mL)		*cis*-C_{18}, -TMA^+ (15 μg/mL)		S-50 spiked +*cis*-C_{18},-TMA^+	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*
	31.9	68.1	61.4	38.6	39.8	60.2
	27.3	72.7	61.9	35.2	35.0	65.0
	28.3	71.7	59.1	40.9	40.9	59.1
	30.6	69.4	60.6	39.4	40.1	59.9
	32.0	68.0	58.5	41.5	41.7	58.3
	29.8	70.2	59.0	41.0	43.6	56.4
Avg.	30.0	70.0	60.1	39.4	40.2	59.8
SD	1.9	1.9	1.4	2.3	2.9	2.9
%RSD	6.4	2.7	2.3	5.9	7.2	4.8

Conditions: same as in Fig. 8.

Table 4 presents the data when monitoring the *cis-trans* isomeric peak area for two trials, both $n = 6$. The data was obtained using EIC for C_{18}'-TMA^+ *m/z* 310 and % peak area in the ChemStation software. In Trial 1, the concentration of S-50 was maintained at 15 μg/mL while synthesized *cis*-C_{18}'-TMA^+ was injected at 10 μg/mL. Overall, the average of six runs on S-50 sample shows that the *cis-trans* % peak area ratio is ~30/70, for *cis*-C_{18}'-TMA^+ ~60/40, and for the spiked sample ~35/65. Therefore, the increase in *cis* ratio is ~5% in the spiked sample. A similar result was achieved for S-50 and the synthesized *cis*-C_{18}'-TMA^+ in Trial 2 where injection of each was performed at slightly higher concentration (15 μg/mL). However, the spiked sample shows increase in *cis-trans* % area. For example, the spiked sample (Trial 2, column 3) shows increase in *cis*-C_{18}'-TMA^+ to ~40 from 30% and decrease in *trans* from 70 to 60%. These results suggest that indeed the challenging separation of *cis-trans* geometrical isomers is occurring in the CEC-MS system. Furthermore, the *cis* isomer is shown to elute faster than the *trans* isomer most likely due to steric interactions. Overall, the elution order is consistent with previous work on CZE separation of *cis*/*trans* adamantane isomers where *cis*- eluted before *trans*-isomer [12].

4 Concluding remarks

We have developed a simple, robust and reproducible CEC-MS method for C_1–C_{18}-TMA^+ utilizing an internally tapered column packed with a mixed mode CEC-C_6/SCX stationary phase. The CEC separation was first optimized by varying the volume fraction of ACN and TEA, as well as pH and NH_4OAc concentration. The results of TEA study at both 90 and 70% v/v ACN showed that separation of longer chain cationic surfactants (C_6–C_{18}-TMA^+) can be performed with 90% ACN in 15 min, but simultaneous separation of short chain cationic compounds (C_1–C_2-TMA^+) with longer chain (C_6–C_{18}-TMA^+) requires a much weaker solvent (*e.g.,* 70% ACN) that in turn increases the total analysis time to 40 min. Despite a decrease in EOF when the pH of NH_4OAc was decreased from 5.0–2.5, the retention time first decrease up to pH 4.0 due to ion-

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exchange mechanisms. Further decrease in pH form 4.0–2.5 results in no significant drop in retention times of cationic surfactants, which suggest that ion-exchange and EOF effects compete with each other over this pH range. Optimization of the buffer ionic strength was found to mainly influence the longer chain C_{10}–C_{18}-TMA^+ surfactants. A comparison of the *k** values suggested that these C_{10}–C_{18}-TMA^+ were retained under chromatographic mechanism, while the shorter chain C_1–C_8-TMA^+ compound migrated mainly due to difference in electrophoretic mobility. The optimum CEC separation conditions were 70% ACN, 0.04% TEA, pH 3.0 and 15 mM NH_4OAc.

Optimization of the ESI-MS sheath liquid composition showed that similar ionization is achieved using higher volume fraction of MeOH (*e.g.*, 70%) as compared to lower volume of IPA (*e.g.*, 30%). The sheath liquid was optimized to 70/30 MeOH, 10 mM NH_4OAc delivered at 7 μL/min. The ESI-MS operating voltages and spray chamber parameters were next investigated utilizing experimental design for the latter. The settings that provided highest sensitivity were as follows: fragmentor voltage = 50 V, V_{cap} = 3000 V, drying gas flow rate 6 L/min, nebulizer pressure 10 psi, drying gas temperature 200°C. These conditions were then utilized to determine the linearity and LOQ. The LOD was estimated to be as low as 500 pg/mL for the shortest chain C_6-TMA^+. The durability and performance of the system proved reproducible for greater than 165 runs over 111 h. Finally, the developed CEC-ESI-MS method was applied for analysis of S-50 sample whereupon the simultaneous separation of saturated and unsaturated cationic surfactants (containing *cis*/*trans* geometrical isomers) was achieved. The identity and elution order of the *cis*/*trans* isomer of C_{18}'-TMA^+ in S-50 sample was confirmed *via* synthesis of *cis* isomer and spiking this isomer in S-50 sample.

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5 References

[1] Tsai, P. -C., Ding, W. -H., *J. Chromatogr. A* 2004, *1027*, 103–108.

[2] Ding, W. -H., Tsai, P.-C., *Anal. Chem.* 2003, *75*, 1792–1797.

[3] Hind, A. R., Bhargava, S. K, Grocott, S. C., *J. Chromatogr. A* 1997, *765*, 287–293.

[4] Harrison, C. R., Lucy, C. A., *J. Chromatogr. A* 2002, *956*, 237–244.

[5] Radke, M., Behrends, T., Förster, J., Herrmann, R., *Anal. Chem.* 1999, *71*, 5362–5366.

[6] Di Corcia, A., *J. Chromatogr. A* 1998, *794*, 165–185.

[7] Fernández, P., Alder, A. C., Suter, M. J.-F., Giger, W., *Anal. Chem.* 1996, *68*, 921–929.

[8] Shibukawa, M., Eto, R., Kira, A., Miura, F., *et al*., *J. Chromatogr. A* 1999, *830*, 321–328.

[9] Liu, H. -Y., Ding, W.-H., *J. Chromatogr. A* 2004, *1025*, 303–312.

[10] Wycherley, D., Rose, M. E., Giles, K., Hutton, T. M., Rimmer, D. A., *J. Chromatogr. A* 1996, *734*, 339–349.

[11] Heinig, K., Vogt, C., Werner, G., *J. Chromatogr. A* 1997, *781*, 17–22.

[12] Shamsi, S. A., Danielson, N. D., *J. Chromatogr. A* 1996, *739*, 405–412.

[13] Heinig, K., Vogt, C., *Electrophoresis* 1999, *20*, 3311–3328.

[14] Buchberger, W., Schöftner, R., *Electrophoresis* 2003, *24*, 2111–2118.

[15] Klampfl, C. W., *J. Chromatogr. A* 2004, *1044*, 131–144.

[16] Barceló-Barrachina, E., Moyano, E., Galceran, M. T., *Electrophoresis* 2004, *25*, 1927–1948.

[17] Norton, D., Zheng, J., Danielson, N., Shamsi, S. A., *Anal. Chem.* 2005, *77*, 6874–6886.

[18] Zheng, J., Norton, D., Shamsi, S. A., *Anal. Chem.* 2006, *78*, 1323–1330.

[19] Steiner, F., Lobert, T., *Chromatographia* 2003, *58*, 207–211.

[20] Spikmans, V., Lane, S. J., Tjaden, U. R., v. d. Greef, J., *Rapid Commun. Mass Spectrom.* 1999, *13*, 141–149.

[21] Jiskra, J., Claessens, H. A., Cramers, C. A., *J. Sep. Sci.* 2003, *26*, 1305–1330.

[22] Klampfl, C. W., Buchberger, W., Haddad, P. R., *J. Chromatogr. A* 2001, *911*, 277–283.

[23] Smith, N., Evans, M. B., *J. Chromatogr. A* 1999, *832*, 41–54.

[24] Ye, M., Zou, H., Liu, Z., Ni, J., *J. Chromatogr. A* 2000, *869*, 385–394.

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