Capillary electrophoresis of cationic and anionic surfactants with indirect conductivity detection

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Abstract

Anions such as F^-, Cl^-, Br^- and SCN^- and cations such as Li^+ and Na^+ have been investigated as electrolytes for the capillary electrophoresis (CE) separation of aliphatic surfactants with indirect conductivity detection (ICD). The ICD results have shown that the signal-to-noise order is $F^->SCN^-=Br^-=Cl^-$ for the anion electrolytes and $Na^+>Li^+$ for the cation electrolytes. A mixture of C_{12}-, C_{10}-, C_8- and C_6-SO_3^- can be separated in less than 5 min using 10 mM F^- and 1 mM triethanolamine (TEA) prepared in triply distilled water. A mixture of cationic quaternary ammonium surfactants can be separated in less than 3 min using 10 mM Na^+ and 1 mM TEA prepared in triply distilled water. Detection limits (about 6 mg/l) for the surfactants by ICD are presently not as favorable as those previously obtained by indirect photometric detection. Use of NaF as the electrolyte did permit the simultaneous CE separation of anionic and cationic surfactants with ICD in the presence of an aromatic non-ionic surfactant.   © 1997 Elsevier Science B.V.

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1. Introduction

Anionic surfactants such as alkane sulfonates and cationic quaternary ammonium surfactants exist in many commercial formulations including cleaning agents, emulsifiers and stabilizers. Capillary electrophoresis (CE) offers outstanding resolution of structurally related anionic compounds. Minimal organic solvent consumption and inexpensive column replacement are practical benefits of CE. Direct UV detection of aliphatic charged surfactants after separation by CE is not possible due to lack of a chromophore. However, CE with either indirect photometric detection (IPD) or indirect fluorometric detection has been shown to be an important approach for the separation and detection of these

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aliphatic surfactants. In particular, alkane sulfonates have been detected by IPD using naphthalene monosulfonate [1,2] or adenosine monophosphate [3] in the CE electrolyte solution. Adenosine monophosphate was recommended over naphthalene monosulfonate for long chain ($>C_{12}$) non-chromophoric surfactants because of a better mobility match [3]. Other reagents such as p-toluene sulfonate in high methanol content mobile phases have also been used for CE with IPD of aliphatic sulfonated surfactants [4]. Flavin mononucleotide, as a visualizing reagent for anions including surfactants, has a good wavelength match for a commercial CE instrument with laser induced fluorometric detection [5]. Less work has been done for non UV active cationic surfactants by CE. Using benzyldimethyldodecylammonium ion as the IPD electrolyte, separation of C_{12}–C_{16} trimethylammonium compounds by CE with UV de-

tection in the 200–210 nm region was shown [6]. Recently, we have shown tetrazolium violet is a useful IPD reagent in the moderate UV region (250–300 nm) for aliphatic quaternary ammonium compounds after CE separation [3].

Conductivity was first introduced as an on-column detector for CE by Huang et al. [7] and then as an end-column detector [8] which made its design simple and more accessible. Although CE with conductivity detection of inorganic cations and anions as well as organic acids is documented [9], little work has been done on surfactants. We have found that direct conductivity detection of the bulky surfactants after CE separation does not provide adequate detection limits or resolution. The anion electrolytes Cl^- and Br^- have been shown useful for CE of aliphatic anionic surfactants with ICD [10]. However, a complete study of both anion and cation electrolytes for CE of surfactants with ICD has not been done to the best of our knowledge. In this work, we have shown that F^- and Na^+ are respectively, simple but effective electrolytes for CE of aliphatic sulfonates and quaternary ammonium compounds with ICD. The separation of both classes of anionic and cationic surfactants in a single run using NaF is demonstrated.

2. Experimental

2.1. Chemicals

Reagent grade sodium fluoride (NaF); sodium chloride (NaCl); sodium thiocyanate (NaSCN); sodium benzoate ($NaC_6H_5CO_2$); potassium ferricyanide [$K_3Fe(CN)_6$] and sodium acetate ($CH_3COONa\cdot 3H_2O$) were from Fisher Scientific (Pittsburgh, PA, USA). The lithium chloride (LiCl); sodium bromide (NaBr); magnesium sulfate ($MgSO_4$); triethanolamine (TEA); 1-hexanesulfonic acid, sodium salt [$CH_3(CH_2)_5SO_3Na$]; 1-decanesulfonic acid, sodium salt [$CH_3(CH_2)_9SO_3Na$]; (2-chloroethyl)-trimethylammonium chloride ($ClETMA^+$); tetrahexylammonium bromide (THA^+) and tetraethylammonium bromide (TEA^+) were from Aldrich (Milwaukee, WI, USA). The potassium chloride (KCl) and ferric chloride ($FeCl_3$) were from MCB (Cincinnati, OH, USA). The sodium 1- octanesulphonate

monohydrate [$CH_3(CH_2)_7SO_3Na\cdot H_2O$] and sodium 1-dodecanesulphonate [$CH_3(CH_2)_{11}SO_3Na$] were from Lancaster Synthesis (Windham, NH, USA). The tetrabutylammonium iodide (TBA^+) was from Eastman (Rochester, NY, USA) and the Triton X-100 was from Sigma (St. Louis, MO, USA).

2.2. Preparation of electrolyte and analyte solutions

The electrolyte solution was prepared fresh each day and according to the specifications for each table or figure. The electrolyte solution was suction filtered before each use. The analyte solutions were prepared in triply distilled water from 1000 mg/l stock solutions. The anionic mixture was prepared by taking appropriate portions of the 1000 mg/l $C_6SO_3^-$, $C_8SO_3^-$, $C_{10}SO_3^-$ and $C_{12}SO_3^-$ stock solutions, diluting and mixing. The cationic mixture was prepared by taking appropriate portions of the 1000 mg/l $ClETMA^+$, TBA^+, TEA^+ and THA^+ stock solutions, diluting and mixing. The analyte solutions were filtered using an Acrodisc LC PVDF 0.2 μm syringe filter obtained from Gelman (Ann Arbor, MI, USA).

2.3. CE instrumentation

The CE instrumentation employed was a Crystal CE system made by Thermo CE (Franklin, MA, USA) with a Crystal 1000 CE conductivity detector. The conductivity cell of the detector connects the ConCap I fused-silica capillary to the ConTip I sensor. The ConTip I sensor unites both electrodes on the same surface as the sensor. The ConTip I sensor has a detection surface that consists of a center Pt electrode (150 μm O.D.) surrounded by a polyimide insulator, then an electrode spacer, and finally the stainless-steel second electrode (375 μm I.D.). The ConCap I connector houses the fused-silica capillary and the contact surface contains two grooves which allows rapid flushing with electrolyte. The distance between the ConCap I and ConTip I connectors has been optimized to be 24.1±1.2 μm by the manufacturer and determines the detector cell volume (less than 3 nl) [11]. The ConCap I fused-silica capillary dimensions were 60 cm×50 μm I.D. Due to the design of the ConCap I fused-silica

capillary, the effective capillary length is equal to the total capillary length (60 cm). A potential of 25 kV was used for all of the data collected. The sample injection was obtained by a pressure of 25 mBar for 12 s giving an injection volume of 7.7 nl. The detector output was set to invert the peaks. Data acquisition was obtained with a Rainin Instruments (Woburn, MA, USA) interface connecting the CE system to the MacIntegrator I software. At the beginning of the day, the conductivity detector cell was purged with the electrolyte and the capillary flushed with the electrolyte. The autocell voltage and the background electrolyte conductivity in microsiemens were recorded. At the end of the day, the capillary was flushed with water for 2 min, 1.0 M NaOH for 10 min and water for 10 min; the conductivity detector cell was also purged with water. One problem encountered was the required ConCap I fused-silica capillary had a limited lifetime of approximately 3 weeks. The depreciation of the capillary was observed by the increase in the electro-osmotic flow (EOF) marker peak mobility increasing and by the baseline deteriorating until a stable baseline could no longer be achieved. Many different cleaning methods such as NaOH, KOH and HNO_3 have been tried to rejuvenate the capillary but all were unsuccessful.

3. Results and discussion

3.1. Determination of the most effective electrolyte type

To determine the most effective electrolyte for CE of surfactants with ICD, the signal-to-noise ratio was determined for many different indirect conductivity

reagents using 10 mM electrolyte, 1 mM TEA, 100% water as previously recommended [10]. We found that the TEA was a necessary component and with the omission of TEA to the electrolyte solution an unstable baseline occurred. The following electrolytes NaF, NaBr, NaCl, NaSCN, $NaC_7H_5O_2$, $K_3Fe(CN)_6$ and $CH_3COONa \cdot 3H_2O$ were all investigated for the separation of anionic surfactants. For the latter three electrolytes, an adequate separation or a stable baseline could not be attained. For example, the neutral peak marking the EOF was about 3 times larger and the analyte peak was 3 times smaller using acetate rather than fluoride. However, NaF, NaBr, NaCl and NaSCN each gave an adequate separation and a stable baseline and the results of this study are given in Table 1. The S/N data for NaBr, NaCl and NaSCN were all comparable in magnitude. It was found that the NaF signal-to-noise ratio was approximately 5 times larger than those for the other three electrolytes with all four electrolytes indicating similar relative standard deviations (R.S.D.s) of about 10%.

For the separation of cationic surfactants, the electrolytes NaCl, LiCl, KCl, $MgSO_4$ and $FeCl_3$ were all investigated. For the latter three compounds, an adequate separation or a stable baseline could not be attained. Data for NaCl and LiCl are shown in Table 2. For LiCl only two of the analytes (TBA^+ and THA^+) in the cationic mixture were easily detected. For TEA^+, it was difficult to make reproducible measurements and the $ClETMA^+$ peak was lost in the baseline noise. For NaCl, an adequate separation and a stable baseline were obtained and the R.S.D. for the signal-to-noise ratio was lower at about 5%.

Overall it was determined that NaF was the most effective electrolyte for the separation of surfactants.

Table 1
Effect of the electrolyte on the signal-to-noise (S/N) ratio for the anionic surfactants

Analyte 50 mg/l (7.7 nl injected)	10 mM electrolyte, 1 mM TEA, 100% water ($n=3$)							
	NaF		NaBr		NaCl		NaSCN	
	S/N	R.S.D. (%)	S/N	R.S.D. (%)	S/N	R.S.D. (%)	S/N	R.S.D. (%)
C_6	56.5	10.8	10.7	9.2	9.6	2.9	10.8	8.2
C_8	64.2	10.2	11.3	13.8	11.1	4.1	11.8	11.4
C_{10}	67.8	9.1	11.8	8.2	11.1	2.7	12.2	10.2
C_{12}	63.6	8.4	10.9	9.8	11.0	1.7	11.3	9.4

Table 2
Effect of the electrolyte on the signal-to-noise (S/N) ratio for the cationic surfactants

Analyte 50 mg/l (7.7 nl injected)	10 mM electrolyte, 1 mM TEA, 100% water ($n=3$, $n=1$[a])			
	NaCl		LiCl	
	S/N	R.S.D. (%)	S/N	R.S.D. (%)
CIETMA$^+$	4.8	3.0	–	–
TEA$^+$	5.6	3.5	2.3[a]	–
TBA$^+$	10.1	5.2	6.3	8.1
THA$^+$	5.8	3.7	7.3	15.8

In general, this choice is consistent with the idea that a high conductivity eluent is desirable when detecting by ICD low mobility analytes. The rest of the research was aimed to optimize the conditions for the NaF electrolyte so that both anionic and cationic surfactants could be separated in the same run.

3.2. Effect of the concentration of the electrolyte on the effective mobility (μ_e) and peak height

The effective mobility (μ_e) of the anionic (Fig. 1A) and cationic (Fig. 1B) surfactants was investigated from 5–40 mM NaF. Low mM electrolyte solutions such as 1 mM gave an autocell detector excitation of greater than 10 V; this can cause damage to the ConTip sensor. A 50 mM NaF, 1 mM TEA, 100% water electrolyte solution was investigated; however, a reproducible electropherogram could not be obtained. The results for both plots were as expected; as the concentration increased, the effective mobility (absolute value) increased. The change in effective mobility for the anionic surfactants was not crucial to the optimum conditions because the anionic mixture was adequately separated even at 10 mM NaF. However, the change in effective mobility for the cationic surfactants was critical to determine the optimum conditions because the peaks for CIETMA$^+$ and TEA$^+$ are very close in retention as are those for TBA$^+$ and THA$^+$. Therefore, as the concentration increased the separation between these sets of peaks also increased slightly.

The peak heights of the anionic surfactants (Fig. 2A) increased proportionally with the concentration of electrolyte. The peak heights for the cationic mixture (Fig. 2B) increased between 10 and 20 mM
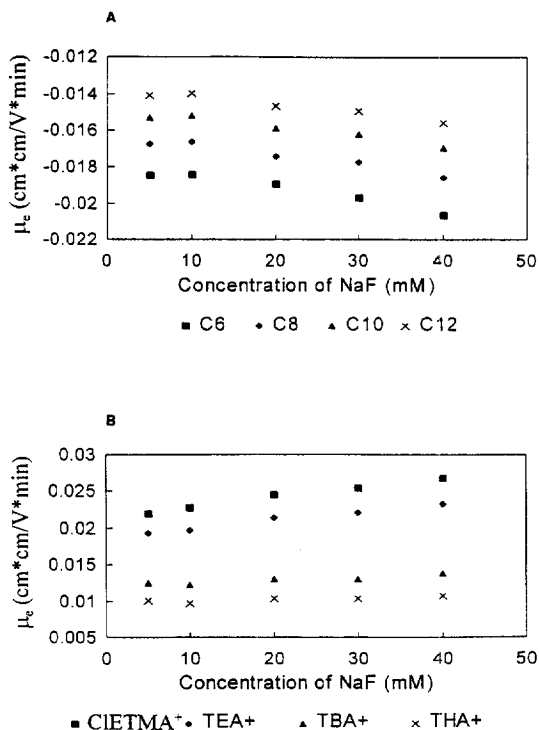

Fig. 1. (A) Optimization of the effective mobility (μ_e) of the anionic surfactants as a function of the concentration of NaF. Electrolyte: X mM NaF, 1 mM TEA, 100% water. (B) Optimization of the effective mobility (μ_e) of the cationic surfactants as a function of the concentration of NaF. Electrolyte: X mM NaF, 1 mM TEA, 100% water.

NaF and between 30 and 40 mM NaF but were approximately the same between 5 and 10 mM NaF and between 20 and 30 mM NaF. The standard deviation (S.D.) at 40 mM NaF was 0.125 for TBA$^+$ compared to 0.0818 for TBA$^+$ and 0.0125 for TBA$^+$ at 20 and 30 mM NaF, respectively.

Overall it was determined that the optimum concentration of NaF was 30 mM if the solvent was 100% water. The electrolyte at 30 mM NaF gave increased peak heights with good S.D.s and increased effective mobility. Representative electropherograms are shown in Fig. 3A and B. The electropherograms show that the anionic and cationic mixtures were reproducibly separated giving an average peak height R.S.D. of 2.1% for C_6, 1.1% for C_8, 2.2% for C_{10}, 2.9% for C_{12}, 0.07% for CIETMA$^+$, 1.8% for TEA$^+$, 0.6% for TBA$^+$, and 2.8% for THA$^+$ for three replicate injections. The
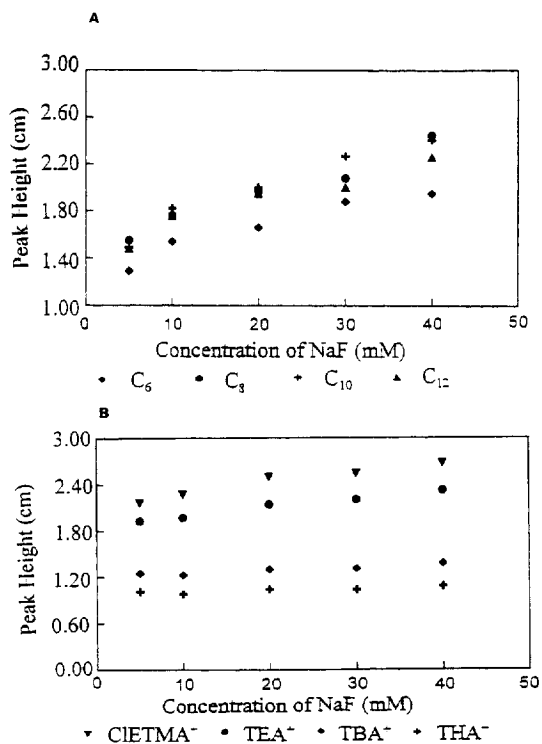
Fig. 2. (A) Optimization of the peak height of the anionic surfactants as a function of the concentration of NaF. Electrolyte: X mM NaF, 1 mM TEA, 100% water. The standard deviation for all of the data represented is very similiar and within the area of the symbol. (B) Optimization of the peak height of the cationic surfactants as a function of the concentration of NaF. Electrolyte: X mM NaF, 1 mM TEA, 100% water. The standard deviation for all of the data represented is very similiar and within the area of the symbol.
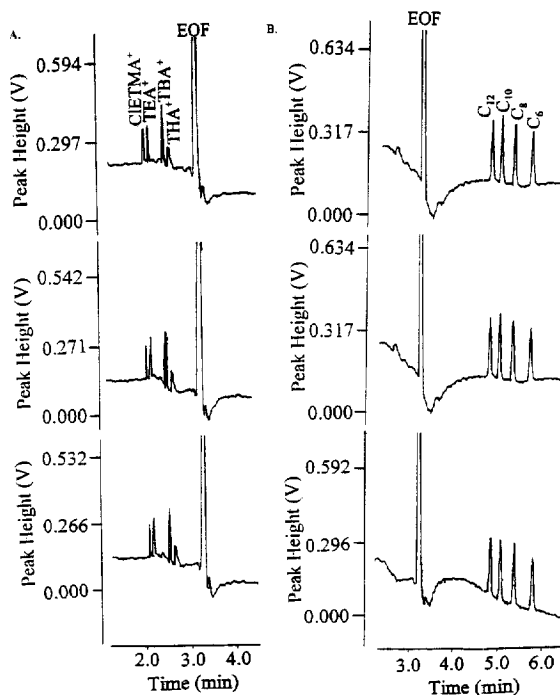

Fig. 3. (A) Replicate electropherograms of 50 mg/l anionic surfactant mixture. Electrolyte: 30 mM NaF, 1 mM TEA, 100% water. (B) Replicate electropherograms of 50 mg/l cationic surfactant mixture. Electrolyte: 30 mM NaF, 1 mM TEA, 100% water.

day-to-day reproducibility was only 7.9% R.S.D. for C_8; however, it is believed that if acetonitrile was added to the electrolyte solution that the day-to-day reproducibility would improve particularly for the longer chain surfactants.

3.3. Effect of solvent in the electrolyte on the effective mobility (μ_e) and peak height

The optimum solvent ratio was investigated by looking into water–methanol, water–acetonitrile and water–tetrahydrofuran solvent ratios. The development of an unstable baseline occurred at 30 mM NaF, 1 mM TEA with the addition of any organic solvent. The water–tetrahydrofuran solvent ratio

could not be investigated due to the inability to obtain a stable baseline. It was also determined that only 20% of methanol or acetonitrile could be added to 10 mM electrolyte before an unstable baseline developed. The effective mobility for the anionic surfactants (Fig. 4A) decreased as the amount of methanol increased. We have noted this same trend in our CE study of phospholipids [12]. The effective mobility for the acetonitrile (Fig. 4B) increased slightly with 10% acetonitrile and decreased with 20% acetonitrile. Substitution of 10% methanol with 10% acetonitrile has been shown to decrease the separation times of phospholipids by about 25% [12]. The peak height decreased with the addition of either methanol (Fig. 5A) or acetonitrile (Fig. 5B). However, the decrease from 100% water to 90% water–10% acetonitrile was minimal as compared to the decrease from 100% water to 90% water–10% methanol. Overall it was determined that the optimum conditions for this method are 20 mM NaF, 1
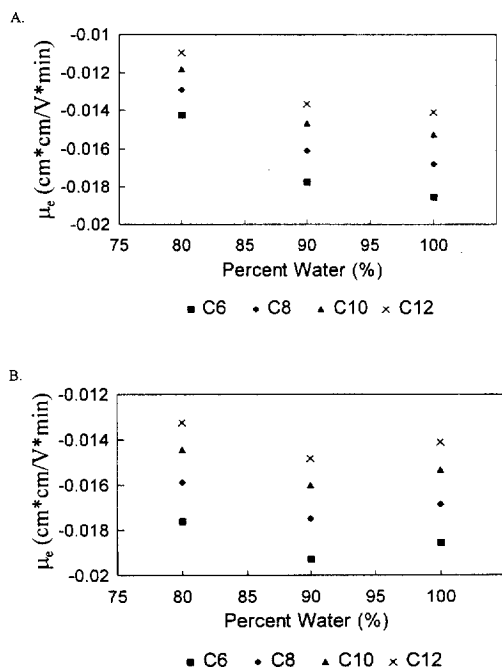
Fig. 4. (A) Optimization of the effective mobility (μ_e) of the anionic surfactants as a function of the percentage of methanol in the electrolyte solution. Electrolyte: 10 mM NaF, 1 mM TEA, X% water, 100−X% methanol. (B) Optimization of the effective mobility (μ_e) of the anionic surfactants as a function of the percentage of acetonitrile in the electrolyte solution. Electrolyte: 10 mM NaF, 1 mM TEA, X% water, 100−X% acetonitrile.

mM TEA, 90% water, 10% acetonitrile. These conditions allow increased peak height and effective mobility and the addition of acetonitrile to ensure sample solubility. The optimum conditions also improved the day-to-day reproducibility giving an average peak height R.S.D. of 1.48% for C$_8$.

3.4. Linearity and detection limit

A linearity study (Table 3) for the anionic mixture was completed using the optimum conditions. The upper limit of the linear range is at least 50 mg/l. This upper limit could most likely be extended to 100 mg/l; however, there is loss of linearity up to 200 mg/l. Similar linearity data was found for the cationic surfactants. For example, the TEA$^+$ had a linear range of 20–40 mg/l with a correlation coefficient of 0.999. The correlation coefficients
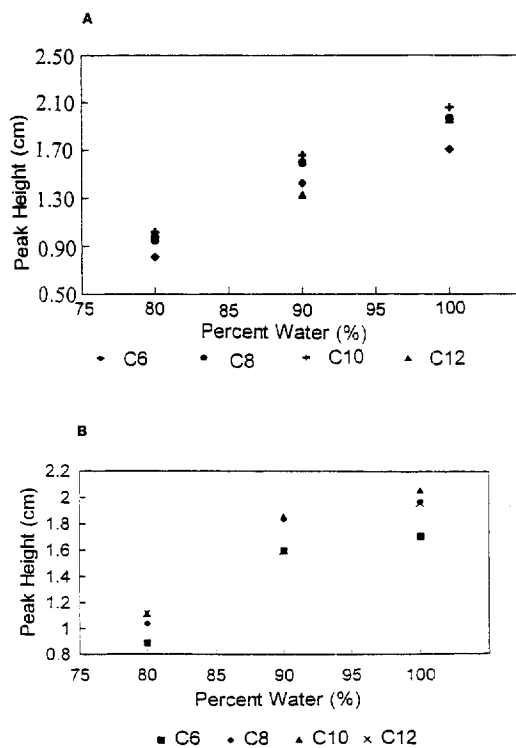

Fig. 5. (A) Optimization of the peak height of the anionic surfactants as a function of the percentage of methanol in the electrolyte solution. Electrolyte: 10 mM NaF, 1 mM TEA, X% water, 100−X% methanol. The standard deviation for all of the data represented is very similiar and within the area of the symbol. (B) Optimization of the peak height of the anionic surfactants as a function of the percentage of acetonitrile in the electrolyte solution. Electrolyte: 10 mM NaF, 1 mM TEA, X% water, 100−X% acetonitrile.

were not as good for TBA$^+$ and THA$^+$. It was observed that the peaks for these cationic surfactants could vary in peak width and at times there were no peaks at all for THA$^+$. It is believed that this was a solubility problem that could be alleviated by the addition of acetonitrile to the stock solutions. The detection limits for the indirect conductivity method are about 6 mg/l for the anionic compounds (Table 4). This detection limit is comparable although not as low to that (2 mg/l) found by CE of the same compounds using IPD [3]. Detection limits of cationic surfactants are similar to those found for the alkyl sulfonates (Table 4). These cation detection limits are about 1–2 orders of magnitude higher than

Table 3
Linearity of response for the anionic surfactants

Analyte 7.7 nl injected	20 mM NaF, 1 mM TEA, 90% water, 10% acetonitrile			
	Data points	Linear range (mg/l)	Least squares equation $y = mx + b$	Correlation coefficient
C_6	10	20–50	$m = 4.8 \cdot 10^{-2}$ $b = -2.3 \cdot 10^{-1}$	0.998
C_8	13	10–50	$m = 4.5 \cdot 10^{-2}$ $b = 8.4 \cdot 10^{-2}$	0.997
C_{10}	10	20–50	$m = 4.8 \cdot 10^{-2}$ $b = 1.1 \cdot 10^{-2}$	0.999
C_{12}	9	30–50	$m = 1.7 \cdot 10^{-3}$ $b = 1.0 \cdot 10^{-1}$	0.998

those found by CE with IPD using tetrazolium violet [3].

3.5. Advantages and future work

The simultaneous separation of anionic and cationic surfactants in a single run using NaF by CE with ICD was a straightforward extension (Fig. 6). Finally, the selectivity of CE with ICD as compared to IPD for the separation of surfactants in the presence of aromatic neutral matrix compounds such as ethoxylated alkylphenols is a potential advantage. No change in the peak width of the EOF marker peak in Fig. 6 was observed by the addition of 1% Triton X-100 to the analyte solvent. A concentration this high of an aromatic matrix sample component

Table 4
Detection limit summary

Analyte 7.7 nl injected	20 mM NaF, 1 mM TEA, 90% water, 10% acetonitrile		
	(mg/l)	μM	fmol
C_6	6.0	31.9	245
C_8	6.0	27.7	214
C_{10}	5.0	20.5	158
C_{12}	6.0	22.0	170
ClETMA$^+$	6.0	38.0	292
TEA$^+$	6.0	28.5	220
TBA$^+$	6.0	16.2	125
THA$^+$	10.0	23.0	177

would undoubtedly increase the EOF marker peak width using IPD obscuring either long chain cationic or anionic surfactant peaks. Present work is addressed at developing a replaceable capillary with the ConCap connector.
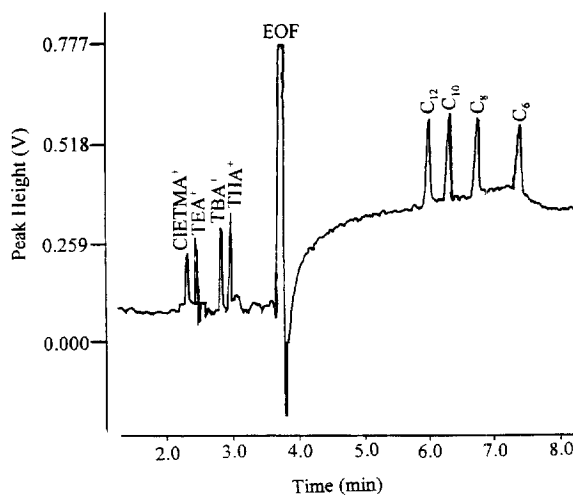
Acknowledgments

Fig. 6. Electropherogram of 37.5 ppm anionic and cationic surfactant mixture. Electrolyte: 20 mM NaF, 1 mM TEA, 90% water, 10% methanol.

References

[1] J. Romano, P. Jandik, W.R. Jones, P.E. Jackson, J. Chromatogr. 546 (1991) 411.

[2] S.A. Shamsi, N.D. Danielson, Anal. Chem. 66 (1994) 3757.

[3] S.A. Shamsi, N.D. Danielson, J. Chromatogr. 739 (1996) 405.

[4] H. Salimi-Moosari, R.M. Cassidy, Anal. Chem. 68 (1996) 293.

[5] S.A. Shamsi, N.D. Danielson and I.M. Warner, in preparation.

[6] C.A. Weiss, J.S. Hazlett, M.H. Dalton, J. Chromatogr. 608 (1992) 325.

[7] X. Huang, T.K. Pang, M.J. Gordon, R.N. Zare, Anal. Chem. 59 (1987) 2747.

[8] X. Huang, R.N. Zare, S. Sloss, A.G. Ewing, Anal. Chem. 63 (1991) 189.

[9] C. Haber, W.R. Jones, J. Cap. Electrophor. 3 (1996) 1.

[10] W.R. Jones and J. Soglia, presented at the 8th International Symposium on High-Performance Capillary Electrophoresis, Orlando, FL, 22–25 January 1996, Paper P508.

[11] Crystal 1000 CE Conductivity Detector Users Manual Version 1.1, ATI Unicam, 1994, 61.2.

[12] F. Haddadian, S.A. Shamsi, J.P. Schaeper and N.D. Danielson, J. Chromatogr., submitted for publication.