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Capillary electrophoresis of cationic surfactants with tetrazolium violet and of anionic surfactants with adenosine monophosphate and indirect photometric detection

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Abstract

The compounds tetrazolium violet (TZV) and adenosine monophosphate (AMP) are characterized as electrolyte components for the respective capillary electrophoresis (CE) separations of quaternary ammonium compounds and sulfonated or carboxylated surfactants with indirect photometric detection (IPD). TZV is particularly effective for the visualization of C_{12}–C_{18} dialkyldimethyl quaternary ammonium compounds and *cis/trans* adamantane isomers. Detection limits are in the 0.25–1 mg/l range at 300 nm. Improved IPD of C_6–C_{18} sarcosine-type surfactants is found with AMP as compared to naphthalene monosulfonate. Detection limits for alkane sulfates and alkane sulfonates are in the 0.5–3 mg/l range. Both TZV and AMP are deemed particularly well suited for CE with IPD detection of long chain ($>C_{12}$) nonchromophoric surfactants.

Keywords: Indirect detection; Detection, electrophoresis; Surfactants; Tetrazolium violet; Adenosine monophosphate; Quaternary ammonium compounds; Adamantanes

1. Introduction

Surfactants are important additives having found widespread application as cleaning agents, emulsifiers, solubilizers and stabilizers. Anionic alkane sulfates and alkane sulfonates as well as cationic quaternary ammonium surfactants exist in many commercial formulations as homologues and isomers. Structurally, many of these compounds consist of an aliphatic hydrocarbon tail with a polar head group and nonchromophoric substituent. Mixed-mode reversed-phase ion chromatography (RPIC) with indirect photometric (IPD), fluorometric, non-suppressed or suppressed conductivity detection has been successfully used for the separation and detection of anionic surfactants [1–5]. Less work using RPIC with indirect detection has been directed toward cationic surfactants [6]. The reports on the development of capillary electrophoresis (CE) for the separate determination of nonchromophoric anionic surfactants [7–9] show that CE is a complementary technique to RPIC with respect to retention order. Minimal organic solvent consumption and inexpensive column replacement are practical benefits of CE besides its inherent good peak capacity. A literature survey revealed that the separation of non-UV-active cationic surfactants by CE is uncommon. There is one report of the separation of C_{12}–C_{16} trimethylammonium compounds by CE using benzyldimethyldodecylammonium as the IPD electrolyte in a phosphate running buffer with a tetrahydrofuran organic modifier [10]. However, this

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IPD reagent could be subject to interferences since it has strong absorbance bands only in the low-UV-wavelength region of 200–210 nm.

Previously, we have reported that naphthalene monosulfonate (NMS) is an effective IPD reagent for the CE separation of both alkane sulfates and alkane sulfonates from C_4–C_{18} in chain length [11,12]. In addition, ribonucleotide electrolytes such as adenosine monophosphate (AMP) are effective IPD reagents for the CE separation of inorganic polyphosphates and Dequest-type polyphosphonates [13]. Organic phosphates such as mono- and di-esters of C_1–C_6 phosphates can also be separated and detected by CE–IPD with AMP as the electrolyte [14]. Some work in the separation of cationic dialkyldimethylammonium compounds using benzylamine and ephedrine as IPD reagents has been recently completed [12]. In this article, we explore the utility of AMP for the CE separation and detection of alkane sulfates and alkane sulfonates. In addition, the IPD reagent tetrazolium violet (TZV) is introduced for the detection of cationic surfactants at moderate UV wavelengths (250–300 nm). Both AMP and TZV were found to be particularly suited for the CE separation and detection of longer chain surfactants.

2. Experimental

2.1. CE instrumentation and data collection

The CE instrument employed was an Applied Biosystems (Foster City, CA, USA) Model 270 A. Data acquisition was carried out with MacIntegrator I software with a Rainin Instruments (Woburn, MA, USA) interface connecting the CE system to the software. The leads of the signal cable connecting the interface and the CE instrument were reversed to obtain positive peaks for IPD. The CE detector time constant was set at 0.5 s and the data acquisition rate was 20 points/s. Full scale output was 100 mV; however, this could be attenuated to lower settings to improve detectability as noted on the *y*-axis of the figures. All data were collected and stored on a MacIntosh SE computer. The fused-silica capillary (Applied Biosystems) dimensions were 75 cm×50 μm I.D. with a 50 cm effective distance from the point of injection to the center of the detector cell. Because organic solvents were used in the run buffer, the acrylate cathode reservoir was replaced with a glass one and the acrylate manifold covered with PTFE tape. Both ends of the capillary were burned to remove the polyimide coating which can swell in the presence of organic solvents and affect injection reproducibility.

2.2. Reagents

TZV was obtained from Aldrich (Milwaukee, WI, USA) and AMP from Sigma (St. Louis, MO, USA). The sodium salts of alkane sulfates and alkane sulfonates of various carbon lengths were purchased from Lancaster Synthesis (Windham, NH, USA). The chloride salts having tetramethylammonium (TMA^+), chloroethyltrimethylammonium ($ClETMA^+$) and tetrabutylammonium (TBA^+) cations as well as the perchlorate salts having tetraethylammonium (TEA^+) or tetrahexylammonium (THA^+) cations were delivered from Aldrich. The didodecyldimethylammonium ($DDMA^+$) bromide salt was obtained from Eastman Kodak (Rochester, NY, USA). The Acrosoft (Adogen 442) sample was a gift from James Jasper of Witco Chemical (New York, NY, USA). The C_{12}–C_{18} sarcosine standards were sent from Alco Chemical (Chattanooga, TN, USA) and the Hamposyl samples were donated by Roland Kohl of W.R. Grace (Lexington, MA, USA). The azoniaadamantane sample was provided by Dow Chemical (Midland, MI, USA).

2.3. Preparation of electrolyte and analyte solutions

Using a 50 m*M* stock solution of either TZV or AMP, electrolyte solutions were prepared by appropriate dilution in 100 m*M* boric acid, adjusted to pH 6.0. Then the desired volume of methanol as the organic modifier was added. All the final operating electrolytes were filtered using 0.2 μm syringe filters from Gelman Science (Ann Arbor, MI, USA) by creating a vacuum inside the syringe. Stock solutions of anionic electrolytes up to C_{12} in chain length could be prepared in water but the longer chain compounds were dissolved in acetonitrile. The cationic surfactant stock solutions were prepared in

80% methanol–20% water. Aliquots of the 1000 mg/l stock solutions were diluted to the appropriate volume in water and these working solutions filtered before use.

2.4. CE procedures

Prior to first use, a new capillary was subjected to a standard wash cycle for 6 h using 1 *M* NaOH at 60°C. As a daily routine procedure, the capillary was flushed for 10 min with first 1 *M* H_3PO_4 and then NaOH with a triply deionized water wash (2 min) in between the acid–base treatment. Equilibration with the operating buffer for 10 min was done before any sample injections. The separation was initiated by applying a voltage (+30 kV) between the two capillary ends which were immersed in reservoirs containing the operating buffer. Replenishment of the inlet buffer solution was made after every run. When using TZV, the outlet electrode needed to be cleaned after every 5–10 runs. In between injections, the capillary was flushed for 2 min with each of the following solutions: 1 *M* H_3PO_4, water, 1 *M* NaOH, water again and then the operating buffer. This procedure resulted in improved peak shapes and the migration time reproducibility was ≤2.0% relative standard deviation (R.S.D.) for the cationic and anionic surfactants.

2.5. Electrophoretic mobility determination

Salicylanilide added to the standard mixture of cationic and anionic carrier electrolytes acted as the neutral marker for the electroosmotic flow (EOF) determination. The observed mobility (μ_{obs}) for each of the various cationic and anionic IPD reagents was determined in 100 m*M* H_3BO_3, pH 6.0, 50% methanol buffer. The parameters EOF or μ_{obs} were calculated by the following equation [15]: EOF or $\mu_{obs} = L_d L_t/(t_m V)$ [$cm^2/V/s$], where L_d and L_t are the length of the capillary to the detector and the total length of the capillary, respectively, V is the separation voltage and t_m is the migration time of the salicylanilide or IPD compound. The electrophoretic mobility (μ_{ep}) having the same units of μ_{obs} for each IPD compound was obtained by subtracting EOF from the μ_{obs}.

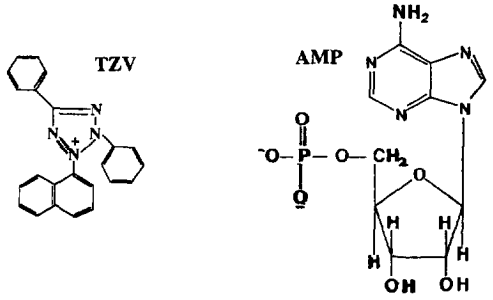

Fig. 1. Structures of tetrazolium violet (TZV) and adenosine monophosphate (AMP).

3. Results and discussion

The structures of TZV and AMP are shown in Fig. 1. TZV has an extended aromatic structure and a permanent +1 charge with a molar absorptivity (ε, l/mol cm) of 5800 at 300 nm. TZV has a broad absorption region from 200 to 300 nm with a λ_{max} at 254 nm (ε=18 000) and another absorbance band at 280 nm (ε=9700). The background absorbance of 10 m*M* TZV (the electrolyte concentration needed to prevent peak distortion of some samples) at these shorter wavelengths is quite high and not in the linear range of the detector. AMP also is a fairly bulky molecule with an effective −2 charge and a molar absorptivity of 9200 at 259 nm. The electrophoretic mobility values ($cm^2/V/s$) for TZV and AMP were found to be $1.63 \cdot 10^{-4}$ and $-1.25 \cdot 10^{-4}$, respectively. These values are significantly lower than $2.19 \cdot 10^{-4}$ for ephedrine (EP) and $-1.74 \cdot 10^{-4}$ for NMS, IPD reagents used previously for CE of surfactants. It is expected that long chain surfactants, because of closer mobility match [16] can be detected better using TZV and AMP. These reagents also have molar absorptivities in the 250–300 nm range comparable to those found for EP and NMS at a much lower wavelength of 205 nm. Therefore, TZV and AMP as IPD reagents should be less susceptible to absorbance effects caused by impurities in the running electrolyte or undesired sample components. The dynamic reserve value, calculated as the ratio of background absorbance to background noise, for TZV is 624 and for AMP is 612. These values are good, similar to those found for EP and NMS. In general, IPD reagents prepared

at a low concentration with a high dynamic reserve at a long wavelength are preferred [17].

The separation of both short and long chain quaternary ammonium compounds with TZV is shown in Fig. 2. Previously, we have found a 100 m*M* boric acid, pH 6.0 electrolyte is best for both reasonable migration time and good peak response [12]. As expected, cationic surfactants are detected in order of increasing number of carbon atoms and hydrophobicity, since positive polarity CE was used. This separation is similar to that found previously for EP using the wavelength of 204 nm [12] with the exception of the inversion of peak 1 (TEA^+) as a negative peak. Using pyridium-*p*-toluenesulfonate, negative peaks were also observed for TEA^+ and $ClTEA^+$ peaks. Since the polarity of the leads on the detector are reversed, this negative peak actually refers to an increased absorbance of the TEA^+ compound. Such unusual behaviour has been observed previously for the CE–IPD of short chain quaternary ammonium compounds [18]. One possibility is the mismatch of mobility between TEA^+ and TZV^+ and the lack of constancy of the Kohlrausch function with time [18]. The peak widths for the shorter chain surfactants are a little broader for peaks 2 and 3 using TZV as compared to EP. This is because the mobility match of EP with faster eluting surfactants is better. The TZV detection limits at 300 nm for these compounds ranged from 0.25–1 mg/l. These detection limits are slightly better than the approximately 1 ppm value reported previously at 210 nm using benzyldimethyldodecylammonium ion (C_{12}benzyl) as the IPD reagent [10]. This is reasonable since it is expected that the molar absorptivity of C_{12}benzyl at 210 nm (estimated to be 6500 from the measurement of a solution of benzyltributylammonium ion) is similar to that of TZV at 300 nm. Detection limits at the lower wavelengths of 280 or 254 nm are about two times and five times better, respectively (Table 1) but a TZV electrolyte concentration of no more than 5 m*M* is useable.

Table 1
Detection limit summary of cationic and anionic surfactants

Electrolyte[a]	Detection λ (nm)		Detection limits[b] (mg/l)		
			TBA^+	THA^+	$DDMA^+$
Cationic[c]					
TZV	254		0.20	0.05	0.10
	280		0.40	0.10	0.20
	300		1.0	0.25	0.50
Anionic[d]		C_6–C_8	C_{10}	C_{12}–C_{14}	C_{16}–C_{18}
AMP	259	1.0	2.0	3.0	0.50

[a]Using the optimized background electrolyte concentration of 5 m*M* with 100 m*M* H_3BO_3, 50% MeOH, pH 6.0.
[b]S/N>3 based on peak height.
[c]Vacuum injection 10 s.
[d]Vacuum injection 6 s; both sulfonate and sulfate type surfactants determined.

Fig. 3 shows that TZV is an effective IPD reagent

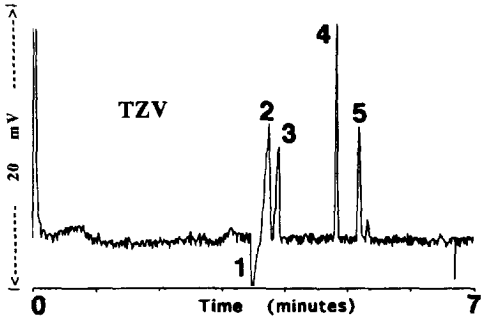

Fig. 2. The separation of a standard mixture of cationic surfactants. Electrolyte composed of 5 m*M* TZV with 100 m*M* H_3BO_3, pH 6.0, 50% methanol. Peak identification: 35 mg/l each of 1=TEA^+; 2=$ClETMA^+$; 3=TBA^+; 4=THA^+; 5=$DDMA^+$. Vacuum injection for 1 s, +30 kV, 2 μA. IPD at 300 nm with TZV.

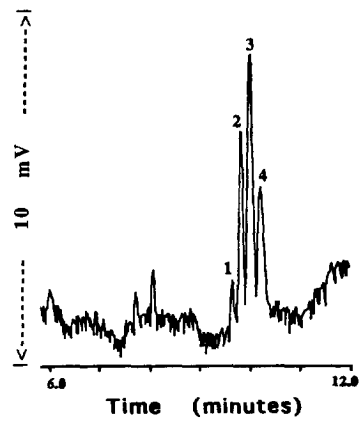

Fig. 3. Electropherogram of even numbered C_{12}–C_{18} dialkyldimethyl quaternary ammonium surfactants (peaks 1–4). Electrolyte composed of 10 m*M* TZV with 100 m*M* H_3BO_3, pH 6.0, 85% methanol. Sample is 0.1 g/100 ml of Adogen 442 dissolved in 85% methanol, filtered with IC syringe filter. Vacuum injection for 2 s. IPD at 300 nm.

for the detection of long chain quaternary ammonium type surfactants found in the commercial product Acrosoft (Adogen 442). To ensure sample solubility, the electrolyte methanol content was raised to 85% which slowed sample electrophoretic migration [13]; the $DDMA^+$ peak elutes at about 9 min compared to 5 min when 50% methanol was in the electrolyte. The mobility match of TZV with these long chain surfactants as shown by the peak shape is good. The alkyl chain distribution in this sample ranges from 12 to 18 and is consistent with the distribution of peaks in the electropherogram. The two small peaks around 7–8 min correspond to short chain cationic surfactants such as monoalkyltrimethyl or trialkylmethyl types [19]. This sample has not previously been analyzed by CE to the best of our knowledge.

The facile separation of *cis/trans* isomers is one of the key attributes of CE. Moore and Jorgenson [20] who succeeded in separating *cis/trans* isomers of some peptides by CE emphasized that, since a *cis–trans* equilibrium is a structural change, the two conformers would be expected to have slightly different electrophoretic mobilities. The *cis/trans* isomers of azoniaadamantane found in the commercial product Dowicil were effectively separated by CE and detected indirectly using TZV (Fig. 4). This adamantane compound is the active ingredient (69%) as an antimicrobial agent in paints, latex, metal working lubricants and other industrial formulations.

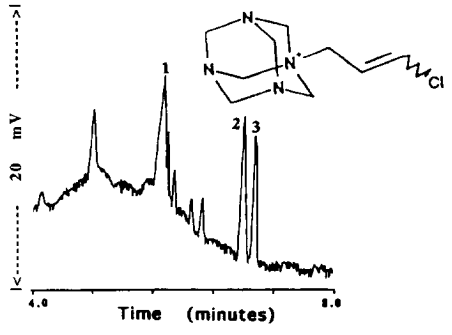

Fig. 4. Electropherogram of *cis/trans* isomers of azoniaadamantane-based preservatives. Electrolyte composed of 10 m*M* TZV with 100 m*M* H_3BO_3, pH 6.0, 85% methanol. Sample is 0.1 g/100 ml of Dowicil-75 dissolved in 50% methanol, filtered with IC syringe filter. Vacuum injection for 1.5 s. Peak identification: 1=hexamethylenetetraamine; 2=*cis*-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane; 3=*trans*-1-(3-chloro-allyl)-3,5,7-triaza-1-azoniaadamantane. IPD at 300 nm.

The identification of the *cis/trans* peak pair was established by spiking this sample with Dowicil 200 (about 95% *cis* isomer). The peak at around 6 min represents hexamethylenetetraamine, the starting material for the manufacture of this important antimicrobial product. Previously, Summers has reported a partial resolution of azoniaadamantane by reversed-phase cation-exchange chromatography with suppressed conductivity detection [21]. Our CE separation of the this *cis/trans* isomer pair is faster with better resolution.

The separation of C_4–C_{18} sulfonates using AMP as a visualization reagent is shown in Fig. 5a. Peaks

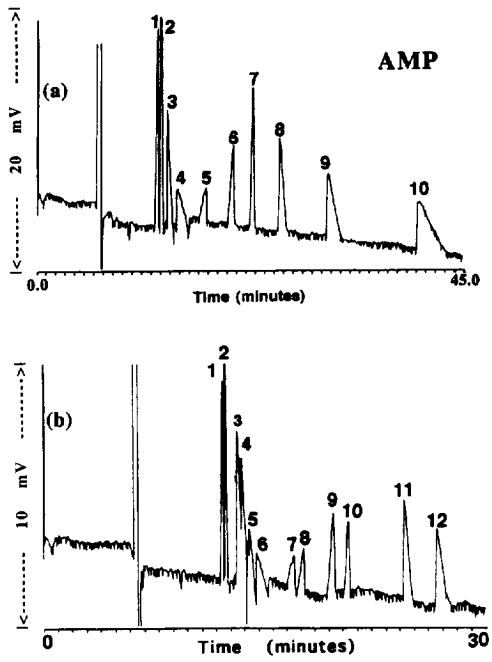

Fig. 5. (a) The separation of a standard mixture of anionic sulfonated surfactants. Electrolyte composed of 5 m*M* AMP with 100 m*M* H_3BO_3, pH 6.0, 50% methanol. Peak identification: 100 mg/l each of $1=C_{18}SO_3^-$; $2=C_{16}SO_3^-$; $3=C_{14}SO_3^-$; $4=C_{12}SO_3^-$; $5=C_{10}SO_3^-$; and 70 mg/l each of $6=C_8SO_3^-$; $7=C_7SO_3^-$; $8=C_6SO_3^-$; $9=C_5SO_3^-$; $10=C_4SO_3^-$. Vacuum injection for 3 s, +30 kV applied for separation, current 2–3 μA. IPD at 259 nm. (b) The separation of a standard mixture of anionic sulfonated and sulfated surfactants. Electrolyte composed of 5 m*M* AMP with 100 m*M* H_3BO_3, pH 6.0, 50% methanol. Peak identification: 100 mg/l each of $1=C_{18}SO_3^-$; $2=C_{18}SO_4^-$; $3=C_{14}SO_3^-$; $4=C_{14}SO_4^-$; $5=C_{12}SO_3^-$; $6=C_{12}SO_4^-$; $7=C_{10}SO_3^-$; $8=C_{10}SO_4^-$; and 70 mg/l each of $9=C_8SO_3^-$; $10=C_8SO_4^-$; $11=C_6SO_3^-$; $12=C_6SO_4^-$. Same conditions as in (a).

1, 2 and 3 representing the long chain compounds C_{18}-, C_{16}- and C_{14}-SO_3^- are well resolved and the peak shapes good. However, the remaining components could be better detected using NMS as the IPD reagent [12]. The separation of a mixture of anionic sulfonated and sulfated surfactants using AMP as the IPD reagent is shown in Fig. 5b. The heights for the first two peaks (C_{18}-SO_3^- and C_{18}-SO_4^-) are higher by about a factor of two than those seen previously for the same separation using NMS as the IPD reagent. Again, the slower electrophoretic mobility of AMP as compared to NMS provided a better mobility match for the longer chain surfactants and sharper peaks. However, the resolution of the first 5 peaks was better using NMS and the peak heights of peaks 5 to 12 were at least twice as high as those found for AMP. Detection limits for the C_6–C_{18} SO_3^-/SO_4^- pairs using AMP at 259 nm are in the 0.5–3 mg/l range. The best detection limits seen for C_{16}–C_{18} SO_3^-/SO_4^- peak pairs (Table 1) are 2 to 4 times better than those found using NMS. In general, all the detection limits reported in Table 1 are comparable to those found previously by ion chromatography [22,23].

Hamposyl sarcosinates, also known as N-acetylated amino acids are a class of surfactants used as speciality detergents for shampoos, cosmetics and the prevention of rust in various petroleum related products. Fig. 6 shows a comparison of electropherograms of Hamposyl C (50% active ingredient). Hamposyl C is a mixture of different fatty acids with a composition of 1% C_6-, 7% C_8-, 6% C_{10}-, 49% C_{12}-, 18% C_{14}-, 8% C_{16}-, 6% C_{18}- and 5% C_{20}-chain lengths. Peak 4 is believed to be the major component N-dodecyl sarcosine. This confirmation was made by spiking the mixture with N-lauryl sarcosine. The peak intensity and migration time is also consistent with the chemical composition of the surfactant blend. The electropherogram of sarcosine Hamposyl C by IPD using AMP at a moderate wavelength again showed substantially taller peaks for the long chained compounds (peaks 1–4) as compared to that using NMS even though the wavelength was now set in the low UV region. Although a system peak for AMP did obscure peak 7, this was not a problem in the NMS electropherogram. Slightly raising the percentage of methanol would likely alleviate this problem. Electropherograms of other commercial (Hamposyl) sarcosine samples using AMP are shown in Fig. 7. The one major peak in each electropherogram corresponds to either N-lauryl-, N-myristoyl-, or N-oleyl-sarcosine depending on the sample. Impurity peaks in the myristoyl and oleyl sarcosine electropherograms are evident. Oleic acid is listed on the material data sheet as a possible impurity in Hamposyl O. Although these Hamposyl surfactants could be separated by RPIC, only one broad peak was noted for each sample [24].

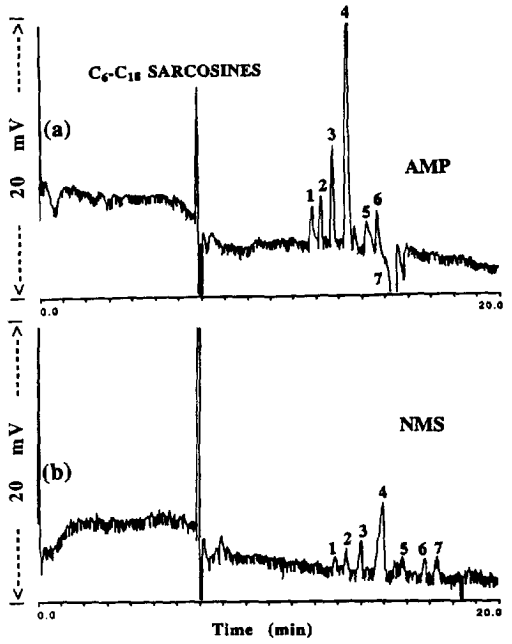

Fig. 6. Comparison of AMP (a) and NMS (b) for the separation of a commercial mixture of even numbered C_{18}–C_6 anionic sarcosinates (peaks 1–7). Electrolyte composed of 10 m*M* AMP or NMS with 100 m*M* H_3BO_3, pH 6.0, 50% methanol. Sample is 0.1 g/100 ml of 50% Hamposyl C solution dissolved in 70% methanol, filtered with IC syringe filter. Vacuum injection 0.5 s for (a) and (b). IPD at 259 nm with AMP and 206 nm with NMS at the same detector sensitivity.

In conclusion, it is recommended that indirect detection for CE of long-chain (greater than C_{12}) nonchromophoric surfactants be done using either TZV or AMP as compared to the higher mobility electrolytes EP or NMS. TZV has the advantage over

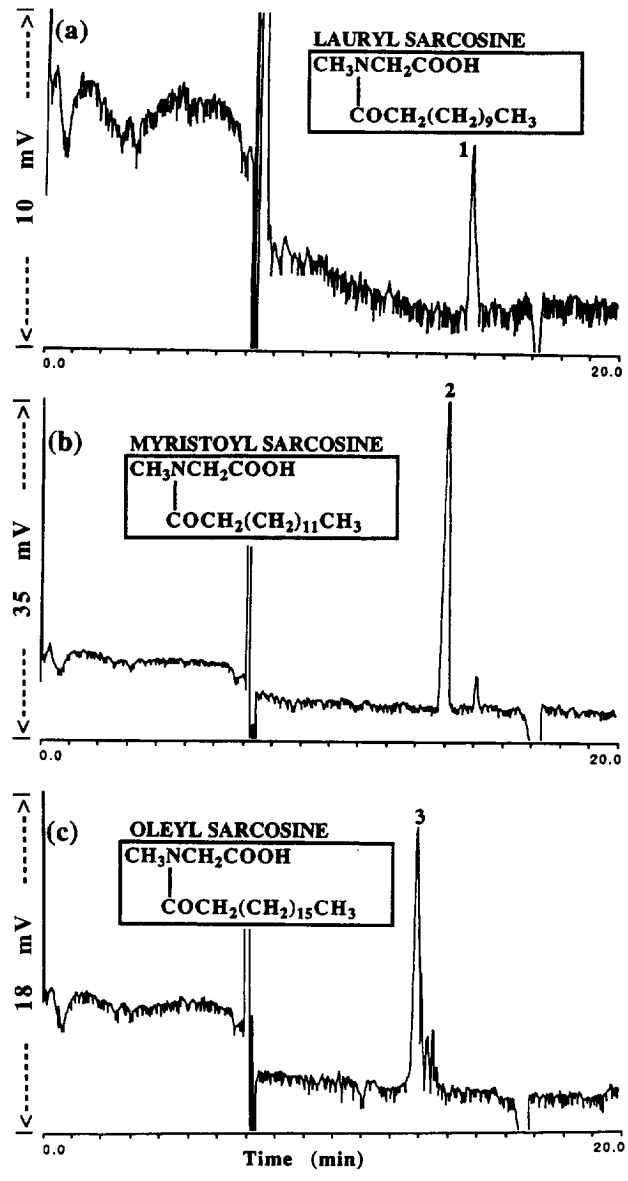

Fig. 7. Electropherograms of 0.1% Hamposyl L (a), Hamposyl M (b) and Hamposyl O (c) anionic sarcosinates. Electrolyte composed of 5 m*M* AMP with 100 m*M* H_3BO_3, pH 6.0, 50% methanol. Sample is 0.1 g/100 ml dissolved in 70% methanol, filtered with IC syringe filter. Vacuum injection for 1 s. IPD at 259 nm.

other cationic reagents of having good molar absorptivities at 3 wavelengths in the moderate UV region. This should prove useful in the analysis of real samples.

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