Journal of Chromatography A, 1218 (2011) 5718–5724

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Potential of long chain ionic liquids for on-line sample concentration techniques: Application to micelle to solvent stacking

Joselito P. Quirino[a,b,*], Philippe Anres[b], Juliette Sirieix- Plénet[b], Nathalie Delaunay[b], Pierre Gareil[b,**]

[a] Australian Centre for Research on Separation Science, School of Chemistry, University of Tasmania, Hobart, Tasmania 7001, Australia
[b] Laboratory of Physicochemistry of Electrolytes, Colloids and Analytical Sciences, Chimie ParisTech, CNRS, UPMC Univ Paris 06, 11 rue Pierre et Marie Curie, 75231, Paris Cedex 05, France

ARTICLE INFO

Article history:
Received 9 April 2011
Received in revised form 16 June 2011
Accepted 18 June 2011
Available online 28 June 2011

Keywords:
Capillary zone electrophoresis
On-line sample concentration
Micelle to solvent stacking
Ionic liquids
Profens
Herbicides

ABSTRACT

The performance of micelle to solvent stacking (MSS) in capillary zone electrophoresis (CZE) was improved for anionic analytes using the long chain ionic liquid type cationic surfactant 1-dodecyl-3-methylimidazolium tetrafluoroborate (C_{12}-MIM-BF_4). The peak heights and corrected peak areas of the test profens and herbicides were enhanced up to 59 and 110-fold, respectively when compared to typical injection. These were up to 10 times better compared to the surfactant cetyltrimethyl ammonium bromide as MSS carrier. This performance was attributed to the properties of C_{12}-MIM-BF_4. MSS requires micelles in the sample for transport of bound analytes to a stacking boundary that contains an organic solvent for effective electrophoretic mobility reversal. The ionic liquid micelles provided better analyte transport properties that resulted from its hydrophobic and pi–pi interaction capabilities. The good solubility of the ionic liquid in high percentages of organic solvent also facilitated a more effective reversal of mobility. The LODs obtained for the test analytes were from 0.06 to 0.12 μg/mL. The linearity R^2 values in terms of peak height and corrected area were ≥0.99. The interday repeatabilities (%RSD, n = 10,) were 0.5–2.2% for retention time, 1.9–4.7% for corrected areas and 4.1–6.4% for peak heights.

1. Introduction

The unique properties of ionic liquids made them useful to separation science [1,2]. For example, the negligible vapor pressure, high density and viscosity made room temperature ionic liquids compatible stationary phases in gas chromatography [3,4]. Ionic liquids added into the mobile phase masked the residual silica silanols and reduced the peak tailing in liquid chromatography [5,6]. The cation of ionic liquids provided a positively charged capillary wall coating for the reproducible, pH independent and reversed electroosmotic flow (EOF) in capillary electrophoresis (CE) [7–9]. Ionic liquids were employed as chiral additives and pseudostationary phases in CE [10–12]. They were also used as sweeping carrier for on-line sample concentration or stacking in micellar electrokinetic chromatography (MEKC) [11].

Stacking techniques have enjoyed considerable success in the last decade and have expanded the use of CE techniques especially to the biological, environmental and forensic fields [13–20]. Long injections were allowed by simple manipulation of the sample solution (S) such that the injected analytes were compressed or focused into narrow zones prior to separation. Please refer to the review articles for discussions on the different stacking techniques [13–17]. One of the newer stacking technique, introduced in 2009 is micelle to solvent stacking (MSS) that works only for charged analytes (a') [21]. In the original configuration of MSS in capillary zone electrophoresis (CZE) [22], the S was prepared in a micellar matrix and the background electrolyte or solution (BGS) contained a sufficient percentage of organic solvent, so that analytes can be strongly desorbed from the micelles and their electrophoretic mobility change sign [21,23]. In addition, the analyte and additive have opposite charges.

The focusing in MSS relies on the a' transport to, and the reversal of the a's effective electrophoretic mobility ($\mu_{ep}(a')$) at the boundary (MSS boundary or MSSB) between the S and BGS. The effective electrophoretic mobility at the boundary ($\mu_{ep}(a')$ MSSB) was described in Ref. [23].

The $\mu_{ep}(a')$ MSSB is dependent on the effective electrophoretic mobility of the a' $\mu_{ep}(a')$, electrophoretic mobility of the micelle μ_{ep}(mc), and retention factor (k). For analyte transport to the MSSB, the k must be sufficiently high for analyte transport by the micelles. For the reversal in mobility, the amount of organic solvent in the BGS should be high enough to lower the k. This will lead to the

* Corresponding author at: Australian Centre for Research on Separation Science, School of Chemistry, University of Tasmania, Hobart, Tasmania 7001, Australia. Tel.: +33 614 57571970.
** Corresponding author.
E-mail addresses: jquirino@utas.edu.au (J.P. Quirino), pierre-gareil@chimie-paristech.fr (P. Gareil).

0021-9673/$ – see front matter
doi:10.1016/j.chroma.2011.06.071

change in the sign of the $\mu_{ep}(a')$ MSSB (i.e., from positive to negative or vice versa). The mobility reversal can also be facilitated by micelle collapse or $k = 0$ [23].

MSS has been applied to the analysis of cationic [21,24–26] and anionic [23,27] analytes in CZE and cationic [28] analytes in MEKC. It is noted that a plug containing the organic solvent was also shown to perform MSS in MEKC [28] and other variations of MSS might appear in the future. The additives studied so far were surfactant (i.e., sodium dodecyl sulfate (SDS) and cetyltrimethyl ammonium bromide (CTAB)) micelles typically used in MEKC [21,23–28].

Here, use of the long chain ionic liquid type cationic surfactant 1-dodecyl-3-methylimidazolium tetrafluoroborate (C_{12}-MIM-BF_4) was evaluated for the MSS of anionic compounds. The compounds chosen were biologically and environmentally important profens and herbicides. Method development parameters including the concentration of ionic liquid and electrolyte in the *S*, the type and amount of organic solvent in the BGS, as well as the *S* injection time were optimized. The analytical performance (repeatability, linearity) of the MSS CZE methods for the profens and herbicides were also determined. Moreover, the reversal of the $\mu_{ep}(a')$ was studied in MEKC using C_{12}-MIM-BF_4 and CTAB in the presence of methanol as organic modifier.

2. Experimental

2.1. Apparatus

Electrophoresis experiments were performed on Agilent 3D or 7100 capillary (when stated) electrophoresis systems (Agilent Technologies, Massy, France). Fused-silica capillary of 50 μm i.d. was obtained from Polymicro (Photonlines, Marly-Le-Roi, France). The effective and total length were described in the text or figures. Detection wavelength was set at 210, 214, and 240 nm. The capillary temperature was controlled at 20 °C. Ultra-pure water was delivered by a Direct-Q3 UV system (Millipore, Molsheim, France).

2.2. Reagents and solutions

C_{12}-MIM-BF_4 (≥99%) was synthesized according to procedures previously reported [29]. C_{12}-MIM-BF_4 (≥99%) was prepared by reaction of 1-methylimidazole and an excess of 1-bromododecane followed by metathesis with $NaBF_4$ using a procedure adapted from the method described [30]. To a solution of C_{12}-MIM-Br in acetone at room temperature was added an excess of $NaBF_4$. The mixture was stirred for four days, and then filtered through celite to remove the precipitated bromide salt and the organic phase reduced in vacuo. This was then re-dissolved in dichloromethane and washed several times with fresh water until the $AgNO_3$ test was negative. The organic layer was dried over $MgSO_4$, filtered and evaporated in vacuo. The residue was lyophilized and the C_{12}-MIM-BF_4 was obtained as white powder in 89% yield and characterized by ^{1}H NMR. Purity was at least 99%, as checked by NMR and MS. Stock solution of 25 mM C_{12}-MIM-BF_4 was prepared by dissolving with the aid of sonication an appropriate amount in ultra-pure water.

Ammonium acetate was purchased from VWR (Fontenay-sous-Bois, France). Hexadimethrine bromide used as EOF reversal agent [31] was purchased from Sigma–Aldrich (L'Isle d'Abeau, France). The test profens (naproxen and carprofen) and herbicides ((4-chloro-2-methylphenoxy)acetic acid and (*R*)-2-(2,4-dichlorophenoxy)propanoic acid (dichlorprop)), methanol (MeOH), acetonitrile (ACN), formamide and CTAB were also purchased from Sigma–Aldrich. 1 mg/mL individual profens and herbicides solutions were prepared by dissolution in 50:50 MeOH:ultra-pure water. 1% hexadimethrine bromide solution, and stock solutions of 300 mM ammonium acetate and 200 mM CTAB were prepared by dissolving an appropriate amount in ultra-pure water. The BGS and *S* matrix were prepared by mixing appropriate volumes of MeOH, ACN, ultra-pure water and stock solutions of ammonium acetate, CTAB, and C_{12}-MIM-BF_4. The pH of the BGSs (with and without organic solvent) and *S*s was 7. This was because ammonium acetate solutions produce solutions at neutral pH [32,33].

2.3. Electrophoretic procedures

Before first use, capillaries were conditioned by successive flushing with 1 M NaOH (20 min), ultra-pure water (10 min), 1% hexadimethrine bromide solution (60 min) and finally BGS (10 min), each under ~1 bar. Between each run, capillaries were rinsed with 1% hexadimethrine bromide solution (2 min), ultra-pure water (1 min) and finally BGS (5 min). This regimen ensured a constant EOF and repeatable analyses (more than 100 runs). Injections were performed at 50 mbar or using the flush mode (~940 mbar) of the Agilent systems. Voltages were applied at negative polarity for the stacking and separation. Fresh BGS was used to ensure a constant pH for each run. The running currents were below 20 μA. This low running current implied that electrolysis which could alter the pH of the BGS within the time frame of the experiments did not occur.

3. Results and discussion

3.1. Effect of C_{12}-MIM-BF_4 concentration on MSS CZE

For MSS to occur, the concentration of C_{12}-MIM-BF_4 in the *S* must be higher than the CMC in order to form micelles. This was assessed by using different concentrations (2.5 (B), 5 (C), 7.5 (D), 10 (E) and 15 (F) mM) of C_{12}-MIM-BF_4 in the *S* matrix using anionic profens (naproxen: pKa = 4.2 (20 °C), log *P* = 3.1, MW 230.3 g/mol; carprofen: pKa = 4.3 (20 °C), lop *P* = 4.1, MW 273.71 g/mol. Log *P* were calculated with Molinspiration software) as test analytes and the results are shown in Fig. 1. The BGS as matrix was also included for comparison in Fig. 1A. The BGS was 30 mM (final concentration in the water/methanol mixture) ammonium acetate with 50% MeOH, to induce reversal of $\mu_{ep}(a')$. The measured pH of the BGS with methanol was 7.0. Separation was in co-EOF mode where the velocities of analytes and EOF were directed to the anode by dynamic coating of the capillary with hexadimethrine bromide. The capillary length was 50 cm (41.5 cm effective), the on-line UV detection was at the anodic side of the capillary, the long injection of *S* was for 60 s at 50 mbar (approx. 10.5% of the capillary length) and the applied voltage was at −25 kV.

As shown in Fig. 1, broad peaks were produced with the BGS (see A), as well as with the 2.5 mM ionic liquid matrix (see B). MSS was effective using 5, 7.5 or 10 mM ionic liquid (see C–E). These results suggest that the CMC of C_{12}-MIM-BF_4 in 15 mM ammonium acetate was between 2.5 and 5 mM. For ionic liquid concentrations that were ≥5 mM, there were micelles in the *S* for analyte transport. There were no micelles in the 2.5 mM ionic liquid condition, and thus no MSS focusing was observed. However, the too high concentration of ionic liquid (i.e., 15 mM) did not properly induce MSS (see F), likely because there were no reversal of effective mobility in this latter case, due to too high *k*. The 7.5 mM condition was then chosen for further study (Fig. 1D). The sample matrix was detected after 7 min.

3.2. Elucidation of the $\mu_{ep}(a')$ reversal

In Fig. 1D, the mobility reversal may be caused by the collapse of the ionic liquid micelles [23]. The reversal was then investigated using hydrocortisone as neutral probe and the results are shown in Fig. 2. The conditions were as described in Section 3.1.

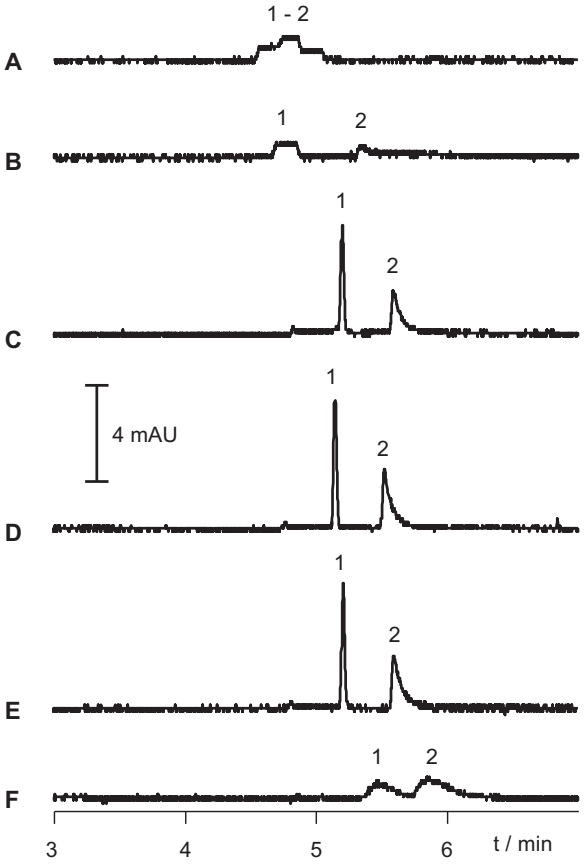

Fig. 1. Effect of ionic liquid concentration in the *S* on the MSS of profens. BGS: 30 mM ammonium acetate and 50% MeOH (pH 7). *S*: 0.5 μg/mL each of the profens in BGS (A), and in 2.5 (B), 5 (C), 7.5 (D), 10 (E) and 15 (F) mM ionic liquid and 15 mM ammonium acetate (pH 7). *S* injection: 60 s at 50 mbar. Applied voltage: −25 kV. Capillary: hexadimethrine bromide coated fused silica having 50 μm ID and 50 cm total (41.5 cm to the detector). Detection: 240 nm. Peak identity: naproxen (1) and carprofen (2).

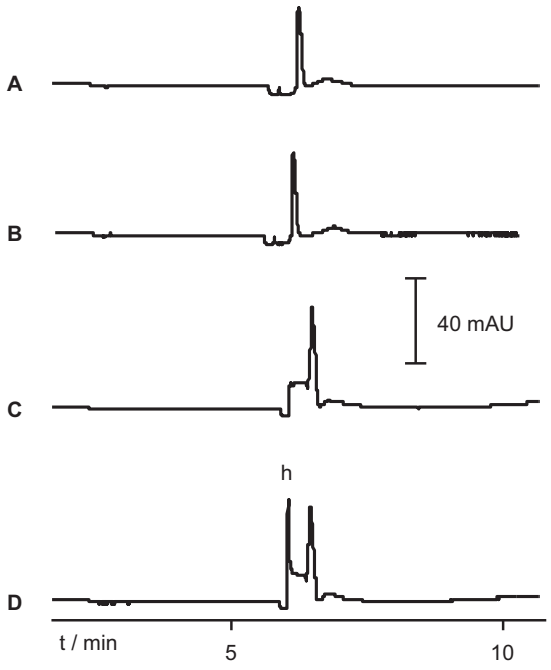

Fig. 2. MSS and AFMC using ionic liquid. BGS: 30 mM ammonium acetate and 50% MeOH (pH 7). Probe: hydrocortisone (h). MSS *S* matrix: 7.5 mM ionic liquid and 15 mM ammonium acetate. AFMC *S* matrix: 7.5 mM ionic liquid and 90 mM ammonium acetate. Injection: 60 s at 50 mbar of MSS *S* matrix (A), 10 μg/mL of probe in MSS *S* matrix (B), AFMC *S* matrix (C), and 10 μg/mL of probe in AFMC *S* matrix (D). Detection: 210 nm. Applied voltage: −25 kV. Capillary: hexadimethrine bromide coated fused silica having 50 μm ID and 50 cm total (41.5 cm to the detector).

The injection of *S* matrix (7.5 mM C_{12}-MIM-BF_4 in 15 mM ammonium acetate) with or without the probe (10 μg/mL) gave the same results (see Fig. 2A and B). This suggested that collapse did not occur in Fig. 1D and the mobility reversal was not due to $k = 0$. Neutral analyte focusing by micelle collapse (AFMC) [34,35] was however achieved by increasing the concentration of ammonium acetate to 90 mM as shown in Fig. 2D, where hydrocortisone (h) was detected as a sharp peak. In Fig. 2D, the concentration of ionic liquid was below the CMC at the dilution boundary between the BGS and *S*. The micelles collapsed and released the neutral probe at the boundary and continued electrophoresis caused the injected probe molecules to accumulate. The peak for the probe was confirmed by the injection of the matrix used in Fig. 2D (blank or without the probe) as shown in Fig. 2C. In addition, the UV spectrum of the sharp peak in Fig. 2D and of the peak from a typical injection of the probe (at a higher concentration prepared in the BGS) was similar.

3.3. Effect of type and concentration of organic solvent on MSS CZE

Mobility reversal in MSS using the ionic liquid depends on the amount, as well as the type of organic solvent in the BGS. The effect of different percentages (0, 10, 20, 30, 40 and 50%) of MeOH and ACN were then studied and the results are shown in Fig. 3. Other conditions were as described in Section 3.1. Broad peaks were observed when there was no organic solvent and MSS occurred when the % of organic solvent was increased. 10% ACN caused MSS of the

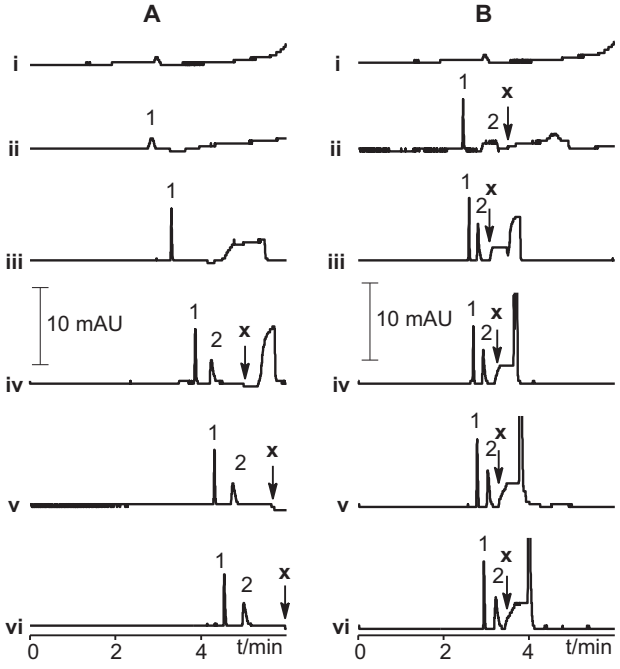

Fig. 3. Effect of organic solvent (MeOH (A) and ACN (B)) content on the MSS of profens. BGS: 30 mM ammonium acetate and different % (0 (i), 10 (ii), 20 (iii), 30 (iv), 40 (v) and 50 (vi)) of organic solvent. *S* matrix: 7.5 mM ionic liquid and 15 mM ammonium acetate. The *x* marks the start of detection of the *S* matrix. Other conditions and peak ID were the same as in Fig. 1.

least hydrophobic analyte naproxen (peak 1) while 20% MeOH was needed to achieve the same focusing. Note that ACN is a less polar solvent compared to MeOH. A higher % of organic solvent (i.e., 30% MeOH and 20% ACN) was needed to focus the more hydrophobic carprofen (peak 2). This was because a higher concentration of organic solvent was needed to effectively reduce the *k* of carprofen at the MSSB.

In addition, the use of MeOH conveniently slowed down the EOF. This caused a larger retention time difference between the focused analytes and the sample matrix (*x* in Fig. 3) (see Fig. 3A). The ionic liquid cation was detected after the sample matrix (not shown). On one hand, when ACN was used as solvent, the sample matrix was detected very close to the focused peaks and thus longer injections of *S* will not be tolerated (see Fig. 3B). Further optimization was done using 50% MeOH with the aim of injecting more *S*.

3.4. Effect of ammonium acetate concentration in the S

Before optimizing the injection time, the effect of ammonium acetate concentration in the *S* matrix was considered. In MSS, the conductivity of the *S* must be matched to a value lower than or equal to that of the BGS to avoid destacking effects [20,22]. Supplementary information Fig. 1 shows the effect of different concentrations (2.5 (A), 7.5 (B), 15.0 (C) and 30.0 (D) mM) of ammonium acetate in the *S* on the MSS of the profens. Other conditions were the same as described in Section 3.1 except the injection time was 90 s and the concentration of the profens were increased to 1 μg/mL for improved peak visualization. The sharp peaks obtained for all *S* matrices suggested that there were ionic liquid micelles for analyte transport. The CMC of C_{12}-MIM-BF_4 was below the concentration used (i.e, 7.5 mM). The best results were obtained using 7.5 mM and 10 mM ammonium acetate matrix because the conductivities of these matrices were close to the BGS (e.g., 72% of the BGS with the 7.5 mM matrix). The injection time or length was then optimized using the 7.5 mM matrix.

3.5. Effect of S injection time

Supplementary information Fig. 2 shows the effect of injection time (30 (A), 60 (B), 90 (C), 120 (D), and 180 (E) s) at 50 mbar on the MSS of profens (5.3%, 10.5%, 16%, 21.3%, 31.9% of the capillary length, respectively). Other conditions were as described in Section 3.1. The signals improved when the injection was increased from 30 to 90 s (see A–C). When the injection was increased beyond 90 s (see D–E), the peaks broadened with no further increase in peak height. The detection of the *S* matrix (*x* in Supplementary information Fig. 2) also became closer to the focused peaks. This means that the effective separation length after focusing decreased with the increase in injection time. It is noted that for quantitation, the focused analytes must be detected in the BGS zone and before this could happen the focused analytes must migrate through the *S* matrix zone. The optimum injection time for the test profens was chosen at 90 s.

3.6. MSS of herbicides

Another set of compounds (i.e., herbicides (4-chloro-2-methylphenoxy)acetic acid: pKa = 3.7 (25 °C), log *P* = 2.3, MW 200.6; dichlorprop: pKa = 3.0 (25 °C), log *P* = 2.9, MW 235) were tested using the conditions developed for the profens. The electrophoretic mobilities of the herbicides were higher than the profens and thus a longer 75 cm capillary was used in order to extend the capillary separation length. The applied voltage was also increased to −30 kV. Fig. 4 shows the effect of injection time (10 s (A), 20 (B), 30 (C), 35 (D), and 40 (E) s) at ~950 mbar, flush mode of the Agilent CE (23%, 45%, 67%, 78%, 89% of the capillary length, respectively). Flush mode

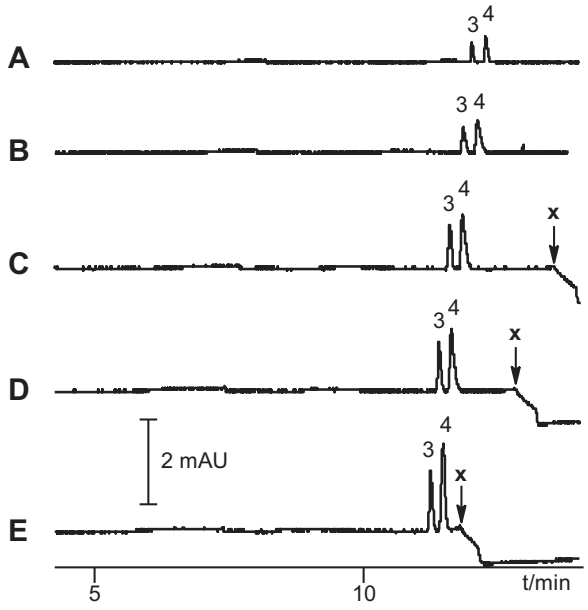

Fig. 4. Effect of *S* injection time on the MSS of herbicides. *S*: 0.5 μg/mL each of the herbicides in 7.5 mM ionic liquid and 7.5 mM ammonium acetate. Injection (flush at ~1 bar): 10 (A), 20 (B), 30 (C), 35 (D), and 40 (E) s. The *x* marks the start of detection of the *S* matrix. Peak ID: (4-chloro-2-methylphenoxy)acetic acid (3) and dichlorprop (4). Capillary length: 75 cm total (66.5 cm to the detector). Other conditions were the same as in Fig. 1.

was used to reduce the analysis time. Similar to the profen results, the effective separation length was reduced by the injection of *S*. This is shown in Fig. 4C–E, where the gap between the elution of the sample matrix (*x* in Fig. 4) and the focused analytes narrowed when the injection time was increased from 30 (C), 35 (D) to 40 (E) s. Injections longer than 40 s lead to the detection of analytes in the *S* matrix zone (data not shown). However, the peak heights and corrected peak areas (peak area/retention time) continued to increase up to an injection time of 40 s. This injection created a much longer plug of *S* and thus better signal improvements when compared to the profens. The reversal in mobility is easier for the less hydrophobic analytes (log *P* values) and this explained the better results obtained for the herbicides.

3.7. Analytical performance

The sensitivity enhancement factors (SEFs) were determined using Eq. (1).

$$\mathrm{SEF} = \frac{\text{peak response in MSS}}{\text{peak response in typical injection}} \times \frac{[\text{concentration typical injection}]}{[\text{concentration MSS injection}]} \quad (1)$$

where the peak response was peak height or corrected peak area. Fig. 5 shows representative typical (A and C) and optimized MSS (B and D) CZE electropherograms for the test profens (A and B) and herbicides (C and D). The profens and herbicides in typical injections were 25 and 50 times more concentrated, respectively compared to the MSS injections. The SEFs (height) for the profens naproxen (peak 1) and carprofen (peak 2) were 27 and 24, respectively. The SEFs (area) were higher at 40 and 29, correspondingly. For the herbicides (4-chloro-2-methylphenoxy)acetic acid (peak 3) and dichlorprop (peak 4), the SEFs (height) were 50 and 35, respectively. SEFs (area) were again higher at 92 and 79, correspondingly. For the herbicides, the analytical performance of the MSS injection shown in Fig. 4D (35 s at ~1 bar) was used. This was because in the

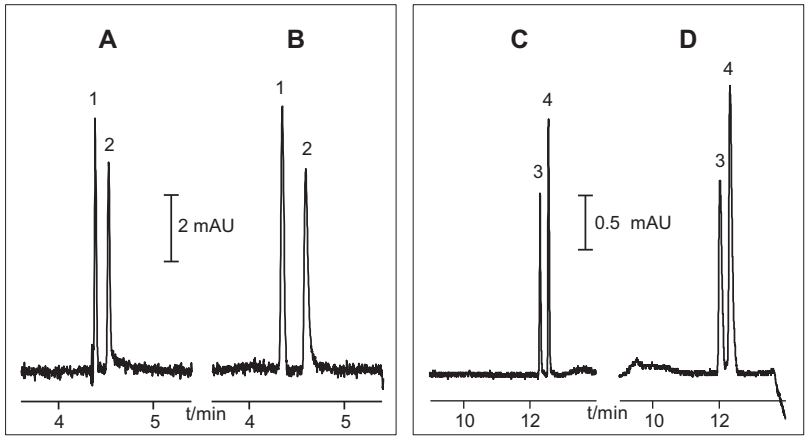

Fig. 5. Typical (A and C) versus optimum MSS (B and D) injection of profens (A and B) and herbicides (C and D). Profen concentration (each): 12.5 (A) and 0.5 (B) μg/mL. Herbicide concentration (each): 25 (C) and 0.5 (D). *S* matrix: 7.5 mM ionic-liquid and 7.5 mM ammonium acetate. Injection: 3 (A), 90 (B) and 5 (C) s at 50 mbar and 35 s at ~1 bar. Capillary length: 50 cm total and 41.5 cm effective (A and B) and 75 cm total and 66.5 cm effective (C and D). Instrument: Agilent 3D (A and B) and Agilent 7100 (C and D) Other conditions and peak ID were the same as in Figs. 1 and 4.

longer injection shown in Fig. 4E (40 s at ~1 bar), the *S* matrix zone was detected close to peak 4. If the 40 s MSS injection (Fig. 4E) was compared to the typical injection (Fig. 5C), the SEFs (height) were 59 and 49 for (4-chloro-2-methylphenoxy)acetic acid and dichlorprop, respectively. The SEFs (area) were 110 and 93, correspondingly.

Table 1 lists the linearity (concentration range, equation of the line for peak height and corrected peak area, R^2), LOD, and repeatability (intraday and interday for retention time, corrected peak area and peak height) results for typical and MSS injections in Fig. 5. The R^2 (area and height) of ≥0.99 for both typical and MSS injections were notable. The intraday and interday repeatabilities (%RSD $n = 10$, for retention time, area and height) obtained in typical injection were ≤6.9% and ≤6.5%, respectively. The values obtained in the MSS injections were comparable at ≤5.6% and ≤6.4%, correspondingly. The LODs obtained from the MSS injection compared to typical injection were 29, 28, 63, and 46 times better for naproxen, carprofen, (4-chloro-2-methylphenoxy)acetic acid and dichlorprop, respectively. The LODs obtained for the herbicides (i.e., 0.11–0.12 μg/mL) were higher than the profens (i.e., 0.06–0.07 μg/mL) because of the lower UV extinction coefficient of the herbicides.

3.8. C_{12}-MIM-BF_4 versus CTAB as micelle carriers for MSS

3.8.1. $\mu_{ep}(a')$ reversal in MEKC using C_{12}-MIM-BF_4 and CTAB

The use of C_{12}-MIM-BF_4 and CTAB as micelle carriers for MSS was compared. First, the reversal of $\mu_{ep}(a')$ was studied in MEKC mode using C_{12}-MIM-BF_4 and CTAB as pseudostationary phases and MeOH as organic modifier in the BGS. The four analytes were tested and the results are shown in Fig. 6A and B using CTAB and C_{12}-MIM-BF_4 in the BGS, respectively. The current optimum MSS conditions were obtained with 10 mM CTAB and 7.5 mM C_{12}-MIM-BF_4, and thus these concentrations were used for this study. It is emphasized that with these concentrations of CTAB or C_{12}-MIM-BF_4, there were micelles in the *Ss* for analyte transport. The ammonium acetate concentration in the BGS was kept at 7.5 mM that was the same as the concentration used in the optimized *S* matrix (see Fig. 5). The MeOH content in the BGS was varied at 10 (i), 20 (ii), 30 (iii), 40 (iv), 50 (v) and 60 (vi) %.

The analytes were detected after the EOF at low % MeOH and were detected before the EOF when the % MeOH was increased. The reversal in the $\mu_{ep}(a')$ was given by the detection of the charged analytes after the EOF with low % MeOH to the detection before the

Table 1
Analytical performance of typical and MSS injection CZE.

	Peak 1	Peak 2	Peak 3	Peak 4
Typical injection				
Linearity				
Range	2.5–50	2.5–50	10–100	10–100
Equation of line (height)	$y = 0.486x + 0.809$	$y = 0.440x + 0.620$	$y = 0.031x + 0.044$	$y = 0.042x + 0.105$
R^2	0.997	0.999	0.999	0.998
Equation of line (corr. area)	$y = 0.161x + 0.370$	$y = 0.169x + 0.385$	$y = 0.007x + 0.013$	$y = 0.010x + 0.013$
R^2	0.997	0.997	0.999	1.000
LOD (S/N = 3) μg/mL	1.75	1.94	7.37	5.07
Repeatability (%RSD, $n = 10$)				
Intraday (RT, corr. area, height)	0.2, 2.1, 3.1	0.3, 1.1, 3.9	0.2, 6.9, 6.3	0.2, 5.3, 4.6
Interday (RT, corr. area, height)	0.1, 4.4, 3.5	0.2, 2.9, 4.9	0.7, 4.9, 6.2	0.7, 6.5, 5.8
MSS				
Linearity				
Range	0.125–2	0.125–2	0.25–2	0.25–2
Equation of line (height)	$y = 12.938x + 1.533$	$y = 11.078x + 1.154$	$y = 1.378x + 0.204$	$y = 2.003x + 0.197$
R^2	0.997	0.996	0.995	0.985
Equation of line (corr. area)	$y = 7.500x + 0.592$	$y = 7.489x + 0.843$	$y = 0.872x + 0.061$	$y = 1.327x + 0.127$
R^2	0.992	0.994	0.998	0.999
LOD (S/N = 3) μg/mL	0.06	0.07	0.12	0.11
Repeatability (%RSD, $n = 10$)				
Intraday (RT, corr. area, height)	0.3, 3.5, 4.4	0.4, 3.9, 5.0	1.0, 2.5, 3.2	1.0, 2.9, 5.6
Interday (RT, corr. area, height)	0.5, 4.3, 5.1	0.6, 4.7, 6.4	2.1, 2.2, 4.1	2.2, 1.9, 4.2

Conditions are found in Fig. 5.

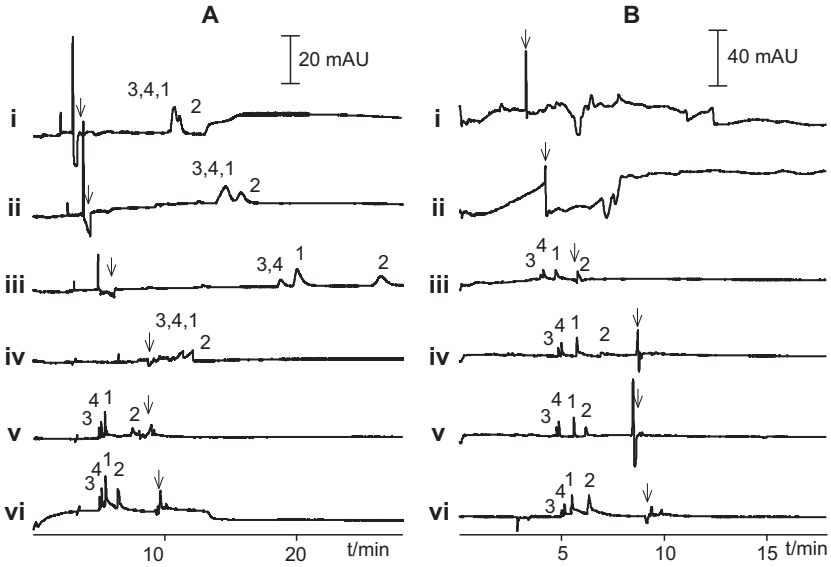

Fig. 6. MEKC profiles of the test analytes using a BGS that contained 10 mM CTAB (A) and 7.5 mM ionic liquid (B) and 7.5 mM ammonium acetate. The MeOH content was 10 (i), 20 (ii), 30 (iii), 40 (iv), 50 (v) and 60 (vi) %. *S*: 50 μg/mL of each test analyte in 10 mM CTAB, 7.5 mM ammonium acetate, 30% MeOH (A) or in 7.5 mM ionic liquid and 7.5 mM ammonium acetate and 30% MeOH (B). The pH of all solutions were neutral. Injection at 50 mbar: 3 s. Detection: 214 nm. Capillary length: 50 cm total and 41.5 cm effective. Applied voltage: −25 kV. Peak ID: see Fig. 5. Arrow denotes the EOF which was monitored by addition of formamide to the *S*.

EOF with higher % MeOH. The decrease in the k that reversed the $\mu_{ep}(a')$ could be due to the absence of the micelles in the separation solution.

With CTAB in the BGS, the test analytes were detected after the EOF (arrow) when MeOH was <50% (see Fig. 6A, i–iv). The direction of the effective mobility was to the cathode or driven by the cationic CTAB micelles. Note that UV detection was at the anodic side since the overall velocity was to the anode because of the strong anodic EOF produced by the cationic surfactant. The retention time of the analytes increased when the % MeOH was increased from 10 to 30 (see Fig. 6A, i–iii). This cannot be fully explained by the decrease in the EOF velocity (see arrows in Fig. 6A) and the cathodic velocity of the analytes seemed to increase with the increase in % MeOH. This may be due to the effect of organic solvent on the structure of the micelles [36].

The interaction between micelles and analytes weakened when the % MeOH was increased to 50 and caused the detection of analytes closer to the EOF (see Fig. 6A, iv). These interactions weakened further when MeOH was >50% (see Fig. 6A, v and vi). The analytes were detected before the EOF and this indicated the reversal of the $\mu_{app}(a')$s. The reversal of the $\mu_{app}(a')$s was most probably due to the absence of CTAB micelles at this high % MeOH. The migration order of the analytes obtained without CTAB was similar to that with CTAB containing >50% MeOH. The interaction between negatively charged analytes and CTAB monomers was not strong enough to afford detection of analytes after the EOF. Note that only positively charged molecules or those that significantly interact with the cationic CTAB will be detected after the EOF.

A different scenario was observed with the ionic liquid in the MEKC BGS. The analytes were not detected as peaks when the MeOH concentration was <30% (see Fig. 6B, i and ii). No peaks were found even after 30 min of electrophoresis. Stable baselines were produced and peaks were observed when MeOH was ≥30%. The stability was attributed to the improved solubility of the ionic liquid at higher amounts of MeOH [35]. With 30% MeOH, the effective mobilities of the faster analytes (peaks 3, 4 and 1) were reversed as evidenced by these peaks eluting before the EOF (arrow). The mobility of the most hydrophobic analyte (peak 2) did not reverse and thus this analyte eluted after the EOF. The mobilities of all the analytes reversed when MeOH was >30% (see Fig. 6B, iv–vi). The migration order of the analytes obtained without ionic liquid was similar to that with ionic liquid containing >30% MeOH.

3.8.2. MSS using CTAB

MSS of the four test analytes were studied using 10 mM CTAB and 7.5 mM ammonium acetate as *S* matrix and the results are shown in Supplementary Information Fig. 3. The CZE BGS contained 60% MeOH for efficient reversal of the mobility (see Fig. 6A, vi). There was no significant improvement in peak heights when the injection time was increased from 30 (A and C) to 60 (B and D) s (50 mbar) for both profens (A and B) and herbicides (C and D). The peaks broadened (B and D) and even overlapped (D) when the injection was increased. The SEFs obtained with the 30 s injection were <10.

3.8.3. C_{12}-MIM-BF_4 and CTAB comparisons

Longer injections were tolerated with C_{12}-MIM-BF_4 (i.e., 90 s at 50 mbar) as MSS carrier and thus the SEFs obtained were at least 6 times better compared to CTAB (i.e., 30 s at 50 mbar). In addition, the small peaks from the injected analyte molecules that were not transported to the MSSB for focusing (see Supplementary Information Fig. 3A and B) were not observed using the ionic liquid. The better performance was due to the properties of the ionic liquid. The imidazolium ring and the long chain of C_{12}-MIM-BF_4 served as targets for pi–pi and hydrophobic interaction, respectively. The former interaction was absent with CTAB and this interaction improved the transport of analytes to the MSSB in the presence of the ionic liquid. Second, the reversal in $\mu_{ep}(a')$ was easier with the ionic liquid since it occurred at lower % of MeOH (see Section 3.8.1). Finally, the reversal in $\mu_{ep}(a')$s was enhanced by the good solubility of the ionic liquid in organic solvents [37]. This solubility reduced the k or interaction between the analytes and ionic liquid micelles at the MSSB.

4. Conclusion

The long chain ionic liquid type cationic surfactant C_{12}-MIM-BF_4 was successfully employed as MSS carrier for the on-line sample concentration of test anionic small molecule profens and herbicides in CZE. The performance of C_{12}-MIM-BF_4 was found better

than CTAB due to the ionic liquid's unique properties. It will be interesting to test other ionic liquids and charged surfactants for the MSS of cationic and anionic solutes.

Acknowledgements

JPQ thanks Prof. Paul R. Haddad, ACROSS and the University of Tasmania for the support and the Australian Research Council for a Future Fellowship (FT100100213). JPQ is also grateful to Chimie ParisTech for providing a two-month invited professor position and supporting his research stay in Paris.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.06.071.

References

[1] S.A. Shamsi, N.D. Danielson, J. Sep. Sci. 30 (2007) 1729.
[2] P.P. Sun, D.W. Armstrong, Anal. Chim. Acta 661 (2010) 1.
[3] C.F. Poole, K.G. Furton, B.R. Kersten, J. Chromatogr. Sci. 24 (1986) 400.
[4] F. Pacholec, H.T. Butler, C.F. Poole, Anal. Chem. 54 (1982) 1938.
[5] W. Zhang, L.-J. He, Y. Gu, X. Liu, S. Jiang, Anal. Lett. 36 (2003) 827.
[6] W. Zhang, L.-J. He, X. Liu, S. Jiang, Chin. J. Chem. 22 (2004) 549.
[7] E.G. Yanes, S.R. Gratz, M.J. Baldwin, S.E. Robison, A.M. Stalcup, Anal. Chem. 73 (2001) 3838.
[8] T.-F. Jiang, Y.-L. Gu, B. Liang, J.-B. Li, Y.-P. Shi, Q.-Y. Ou, Anal. Chim. Acta 479 (2003) 249.
[9] T.-F. Jiang, Y.-H. Wang, Z.-H. Lv, J. Anal. Chem. 61 (2006) 1108.
[10] Y. François, A. Varenne, E. Juillerat, P. Gareil, J. Chromatogr. A 1155 (2007) 134.
[11] H. Su, M. Lan, Y. Hsieh, J. Chromatogr. A 1216 (2009) 5313.
[12] V.P. Schnee, G.A. Baker, E. Rauk, C.P. Palmer, Electrophoresis 27 (2006) 4141.
[13] S.L. Simpson Jr., J.P. Quirino, S. Terabe, J. Chromatogr. A 1184 (2008) 504.
[14] K. Sueyoshi, F. Kitagawa, K. Otsuka, J. Sep. Sci. 31 (2008) 2650.
[15] A.T. Aranas, A.M. Guidote Jr., J.P. Quirino, Anal. Bioanal. Chem. 394 (2009) 175.
[16] Z. Mala, A. Slampova, P. Gebauer, P. Bocek, Electrophoresis 30 (2009) 215.
[17] M.C. Breadmore, M. Dawod, J.P. Quirino, Electrophoresis 32 (2011) 127.
[18] M. Lagarrigue, A. Bossée, A. Bégos, N. Delaunay, A. Varenne, P. Gareil, B. Bellier, Electrophoresis 30 (2009) 1522.
[19] M. Lagarrigue, A. Bossée, A. Bégos, N. Delaunay, A. Varenne, P. Gareil, B. Bellier, J. Chromatogr. A 1178 (2008) 239.
[20] C. Sarazin, N. Delaunay, A. Varenne, J. Vial, C. Costanza, V. Eudes, J.-J. Minet, P. Gareil, J. Chromatogr. A 1217 (2010) 6971.
[21] J.P. Quirino, J. Chromatogr. A 1216 (2009) 294.
[22] J.W. Jorgenson, K.D. Lukacs, Science 222 (1983) 266.
[23] A.M. Guidote Jr., J.P. Quirino, J. Chromatogr. A 1217 (2010) 6290.
[24] J.P. Quirino, J. Chromatogr. A 1217 (2010) 7776.
[25] H.-D. Zhu, C.-L. Ren, S.-Q. Hu, X.-M. Zhou, H.-L. Chen, X.-G. Chen, J. Chromatogr. A 1218 (2011) 733.
[26] X.-F. Yin, Z. Li, S.-H. Zhang, C.-X. Wu, C. Wang, Z. Wang, Chin. Chem. Lett. 22 (2011) 330.
[27] J.P. Quirino, A.M. Guidote Jr., J. Chromatogr. A 1218 (2011) 1004.
[28] L. Liu, X. Deng, X. Chen, J. Chromatogr. A 1217 (2010) 175.
[29] L. Gaillon, J. Sirieix-Plénet, P. Letellier, J. Solution Chem. 33 (2004) 1333.
[30] P.A.Z. Suarez, J.E.L. Dullius, S. Einloft, R.F. De Souza, J. Dupont Polyhedron 15 (1996) 1217.
[31] M.T. Galceran, L. Puignou, M. Diez, J. Chromatogr. A 732 (1996) 167.
[32] R.J. Williams, C.M. Lyman, J. Am. Chem. Soc. 54 (1932) 1911.
[33] C.J. Schollenberger, J. J. Am. Chem. Soc. 54 (1932) 2568.
[34] J.P. Quirino, P.R. Haddad, Anal. Chem. 80 (2008) 6824.
[35] J.P. Quirino, Electrophoresis 30 (2009) 875.
[36] C.P. Palmer, S. Terabe, Anal. Chem. 69 (1997) 1852.
[37] M. Vaher, M. Koel, M. Kaljurand, J. Chromatogr. A 979 (2002) 27.