Journal of Chromatography A, 1218 (2011) 1004–1010

Contents lists available at ScienceDirect

Journal of Chromatography A

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

# Two-step stacking in capillary zone electrophoresis featuring sweeping and micelle to solvent stacking: II. Organic anions

Joselito P. Quirino [a,*], Armando M. Guidote Jr. [a,b]

[a] Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, University of Tasmania, Private Bag 75, Hobart, Tasmania 700, Australia
[b] Department of Chemistry, School of Science and Engineering, Loyola Schools, Ateneo de Manila University, Loyola Heights, Quezon City 1108, Metro Manila, Philippines

ARTICLE INFO

*Article history:*
Received 10 October 2010
Received in revised form
19 December 2010
Accepted 21 December 2010
Available online 30 December 2010

*Keywords:*
Capillary zone electrophoresis
On-line sample concentration
Sweeping
Micelle to solvent stacking
Herbicides
Hypolipidaemic drugs
Non-steroidal anti-inflammatory drugs

ABSTRACT

Two-step stacking of organic anions by sweeping and micelle to solvent stacking (MSS) using cationic cetyltrimethylammonium micelles in co-electroosmotic flow (co-EOF) capillary zone electrophoresis (CZE) is described. The co-EOF condition where the direction of the EOF is the same as the test anions was satisfied by positive dynamic coating of a fused silica capillary with hexadimethrine bromide. The strategy was as follows. After conditioning the capillary with the background solution (BGS), a micellar solution (MS) was injected before the sample solution (S). The BGS, MS and S have similar conductivities. Voltage was applied at negative polarity. The analytes in the micelle-free S zone were swept by micelles from the MS. The swept analytes were brought by the micelles to the MSS boundary where the second stacking step was induced by the presence of organic solvent in the BGS. Finally was the separation of concentrated analytes by CZE. The effect of electrolyte concentration in the S, injection time of the MS and the S and surfactant concentration in the MS were studied. A 20–29, 17–33 and 18–21 times increase in peak height sensitivity was obtained for the test hypolipidaemic drugs (gemfibrozil, fluvastatin and atorvastatin), non-steroidal anti-inflammatory drugs (diflunisal, naproxen, ketoprofen, indoprofen and indomethacin), and herbicides (mecoprop and fenoprop), respectively. The LODs (S/N = 3) were from 0.05 to 0.55 μg/mL. The intraday and interday repeatabilities (%RSD, $n = 12$) in terms of retention time, corrected peak area, and peak heights was less than 3.6, 8.9, and 10.8%, respectively. The application of sweeping and MSS in co-EOF CZE together with a simple extraction procedure to a waste water sample spiked with the test herbicides was also demonstrated.



## 1. Introduction

Stacking or on-line sample concentration techniques in capillary electrophoresis (CE) with ultraviolet (UV) detection are popularly employed due to poor UV detection sensitivity [1–8]. Tens to thousands-fold increases in sensitivity have been achieved and these techniques have been applied to a multitude of samples. The combination of stacking techniques had also received some attention [6–8]. The first reported combination was cation selective exhaustive injection – sweeping [9,10]. This two-step stacking approach is a combination of prolonged field amplified/enhanced sample injection [11,12] and sweeping [13,14]. This approach that yielded almost a million fold enrichment of cationic analytes was also developed for anionic analytes [15,16]. Other two-step stacking techniques featured dynamic pH junction [17,18] and sweeping [19] with separation using micellar electrokinetic chromatography (MEKC) [20,21] and field amplified sample injection and transient isotachophoresis (so-called electrokinetic supercharging) [22–25] with separation using capillary zone electrophoresis (CZE) [26,27].

A two-step stacking strategy for organic cations by sweeping and micelle to solvent stacking (MSS) [28–30] as the first and second steps, respectively using anionic SDS micelles was recently introduced in co-electroosmotic flow (co-EOF) mode CZE [31]. There are two basic conditions for this sweeping-MSS concentration strategy. First, the sample solution (S) must be free of the micelles in order to perform sweeping by injection of micellar solution (MS) before injection of S. Second, the micelle and analyte must have opposite charge and the CZE background solution (BGS) must contain a sufficient amount of organic solvent to induce MSS. In MSS, the analytes must be prepared in a micellar solution. This was satisfied by the first stacking step where the swept analytes were bound to the micelles. Here, we report this strategy for organic anions using cationic cetyltrimethylammonium bromide (CTAB) micelles for stacking and using hexadimethrine bromide (HDMB) for positive dynamic coating of a fused silica capillary for co-EOF CZE. The strategy was tested using three groups of organic anionic analytes, namely hypolipidaemic drugs, non-steroidal anti-inflammatory drugs (NSAIDs) and herbicides. These

* Corresponding author. Tel.: +61 3 6226 2529; fax: +61 3 6226 2858.
*E-mail address:* jquirino@utas.edu.au (J.P. Quirino).

0021-9673/$ – see front matter. 
doi:10.1016/j.chroma.2010.12.095

drugs and herbicides are widely used in the developed world. The analysis of these small molecules in the environment is an important topic in analytical chemistry and thus these compounds were chosen as test analytes. The mechanisms for stacking were proposed and verified, and the experimental variables that may affect the strategy were investigated. The technique was also tested to the analysis of the herbicides-spiked effluent water.

## 2. Experimental

### 2.1. Apparatus

An Agilent$^{3D}$ Capillary Electrophoresis System (Agilent Technologies, Germany) equipped with a fused-silica capillary (Polymicro Technologies, USA) of 50 μm i.d./50 cm (41.5 cm effective length) was used. Detection wavelength and capillary temperature were set at 214 nm and 20 °C, respectively. The pH meter used was an Activon Model 210 (Activon Scientific Products Co. Pty. Ltd., Australia).

### 2.2. Reagents and solutions

Water that was purified with a Milli-Q system (Millipore, USA) was used to prepare the BGS, S, and MS. The hypolipidaemic drugs (gemfibrozil, fluvastatin and atorvastatin) (all >98%) were purchased from Sequoia (Oxford, UK). 1 mg/mL stock solutions of the hypolipidaemic drugs were prepared with methanol. For the NSAIDs, naproxen was procured from Fluka (Buchs, Switzerland) while the rest: diflunisal, ketoprofen, indomethacin and indoprofen were from Sigma–Aldrich (St. Louis, MO, USA). The herbicides (mecoprop and fenofrop), alkylphenylketones (acetophenone, propiophenone, valerophenone, and hexanophenone) and thiourea were also from Sigma–Aldrich. 1 mg/mL stock solutions of the NSAIDs and herbicides were prepared in water/methanol solvents. All other reagents (HCl, $NH_4HCO_3$, CTAB, HDMB, methanol (MeOH), acetonitrile (ACN) and dichloromethane (DCM)) (analytical or USP grade) were purchased from Sigma–Aldrich. The BGS, MS, and S matrix were prepared by mixing appropriate volumes of MeOH, Purified water and stock solutions of 250 mM $NH_4HCO_3$ and 200 mM CTAB. The solutions were filtered with a 0.45 μm Micro-Science membrane filter (MicroAnalytix Pty. Ltd., Australia). The Ss were prepared by dilution of sample stock solution aliquots with the chosen S matrices described in the text. The BGS and MS were prepared each day and used after a 5-min sonication.

The effluent came from the sewage plant of Hobart, Tasmania, Australia. Duplicate samples (1.0 mL) were spiked to contain 0.9 and 1.8 μg/mL of fenoprop and mecoprop, respectively. Extraction was done by addition of 0.1 mL of concentrated HCl followed by 5 min sonication. Afterwards 2 mL of DCM was added to each replicate and blank and then sonicated for 10 min. The samples were afterwards centrifuged for 5 min at 3000 rpm. One mL of the DCM layer was isolated and then dried *in vacuo*. The samples were reconstituted with 20 mM $NH_4HCO_3$.

### 2.3. General electrophoresis procedure

HDMB was used for EOF reversal [25]. New capillaries were conditioned with 0.1 M NaOH (10 min), water (10 min), methanol (10 min), water (2 min) and then 1% HDMB (60 min). 1% of HDMB (10 min) was flushed through the column at the start of each day. After each run, the capillary was conditioned with 1% HDMB (2 min), water (1 min) and BGS (5 min). More than 100 reproducible injections can be performed as long as the conditioning regimen was followed. Applied voltage at negative polarity (anode at the detector end) was 18 kV in all experiments. The S and MS injections were done with 50 mbar pressure. Other experimental conditions are stated in the figures, tables or text.

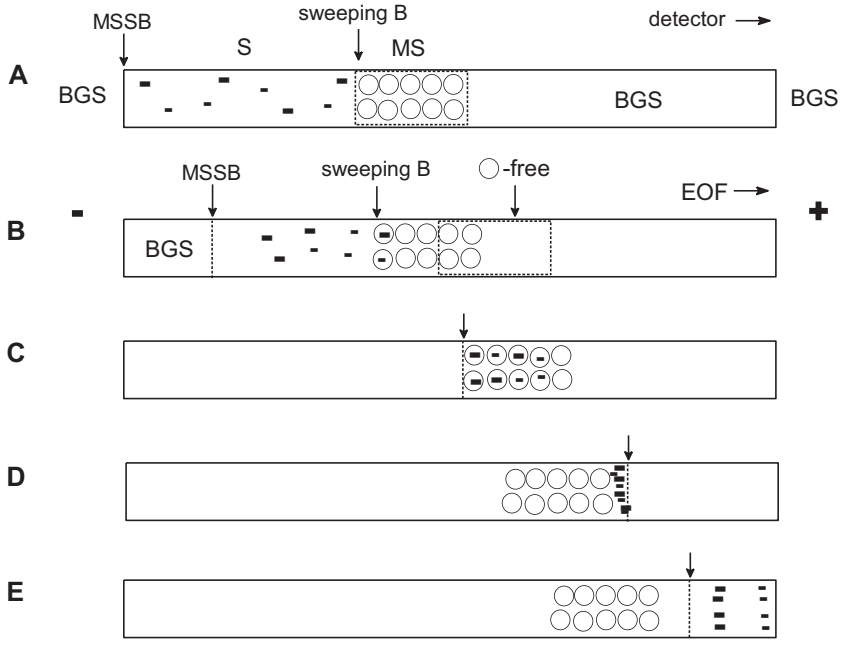


**Fig. 1.** Model for the two-step stacking by sweeping and MSS of organic anions in co-EOF CZE. (A) The positively charged coated capillary was first conditioned with a BGS that contained an organic solvent. This was followed by injection of the micellar solution (MS) of a cationic surfactant and then a long injection of the sample solution (S) which was devoid of micelles. The injected MS zone was also depicted with broken lines. The sweeping boundary (sweeping B) was found at the cathodic end of the MS close to the S zone. (B) When a negative voltage was applied, the cationic micelles swept and carried the analytes to the MSS boundary (MSSB). A micelle free section of the injected MS zone (anodic side of the MS zone) was also formed due to electrophoretic migration of the micelles to the cathode. (C) The analytes were completely swept into a concentrated zone and were about to cross the MSSB. (D) The swept analytes crossed the MSSB and formed a more concentrated zone at this boundary due to the second stacking step of MSS. (E) The two-step stacked analytes separated by CZE. Stacked analytes migrate through the injected S and MS zones and then the BGS where they were detected (not shown). More explanation in the text.

### 2.4. Measurement of retention factor (k)

The $k$ of the test analytes was determined using the method by Bushey and Jorgenson [32]. The alkylphenylketones and thiourea (EOF marker) were analysed by MEKC using 10 mM CTAB and 10 mM $NH_4HCO_3$ as BGS. The electrophoretic mobility of CTAB micelle was $2.73 \times 10^{-4}$ cm$^2$/V s.

## 3. Results and discussion

### 3.1. Sweeping-MSS of organic anions in co-EOF CZE model

The model for two-step stacking by sweeping and MSS of organic anions in co-EOF CZE is shown in Fig. 1. In Fig. 1A, the HDMB coated capillary was conditioned with the BGS that contained a buffer salt (i.e., $NH_4HCO_3$) and organic solvent (i.e., MeOH). The MS that contained CTAB micelles (circles) for sweeping and MSS was injected. Cationic micelles were used for the anionic analytes to satisfy a condition for MSS (see introduction) [28,30]. The MS injection was followed by long injection of the S that contained the anionic analytes (−). The S matrix was devoid of CTAB to satisfy the basic condition for sweeping [13,14]. The BGS filled vials were placed at both ends of the capillary. A similar conductivity of the BGS, MS, and S was assumed to provide a homogenous electric field across the capillary. In Fig. 1B, a negative voltage was applied and caused the cationic micelles and anionic analytes to electrophoretically migrate to the cathode and anode, respectively. The micelles then penetrated and swept the analyte zones. A micelle free section in the injected MS (dotted lines in Fig. 1A and B) was formed due to the electrophoretic migration of the micelles to the cathode. This first stacking step of sweeping created a concentrated sample zone with micelles (see Fig. 1C). The migration velocity of the micelles and analytes was toward the detector or anode due to the strong reversed EOF.

In Fig. 1D, the analytes were transported to the MSS boundary (MSSB) by the CTAB micelles and at the boundary experiences the second stacking step of MSS. The presence of micelles in the swept zone satisfied a condition for MSS [28,30]. Another condition for MSS was satisfied by the addition of organic solvent to the BGS [28,30], which reduced the interaction of the anionic analytes with the cationic micelles. This caused a reversal in the analytes' effective electrophoretic mobility. The effective electrophoretic mobility of the anion ($\mu^*_{ep}(a')$) is given by Eq. (1).

$$\mu^*_{ep}(a') = \frac{1}{1+k}\mu_{ep}(a') + \frac{k}{k+1}\mu_{ep}(mc) \quad (1)$$

where the electrophoretic mobility of the analyte ($\mu_{ep}(a')$) is negative and the electrophoretic mobility of the micelle ($\mu_{ep}(mc)$) is positive. In the presence of organic solvent, the $k$ decreases to a point where the direction of the $\mu^*_{ep}(a')$ in Eq. (1) reverses from positive to negative [30]. The collapse of micelles [33,34] leads to a $k = 0$ and this reverses the sign of $\mu^*_{ep}(a')$ as well. The reversal in direction caused the analytes to accumulate at the MSSB. The $\mu^*_{ep}(a')$ was directed to the cathode in the presence of CTAB micelles (after sweeping) and was to the anode in the presence of micelles and organic solvent at the MSSB. More information on the MSS of organic anions with CTAB micelles can be found in Ref. [30]. In Fig. 1E, the micelles from the MS were exhausted and the final focused band separate by virtue of CZE. In the CZE separation, the two-step focused analytes migrate through the injected S and MS zones before reaching the BGS zone where the analytes should be detected.

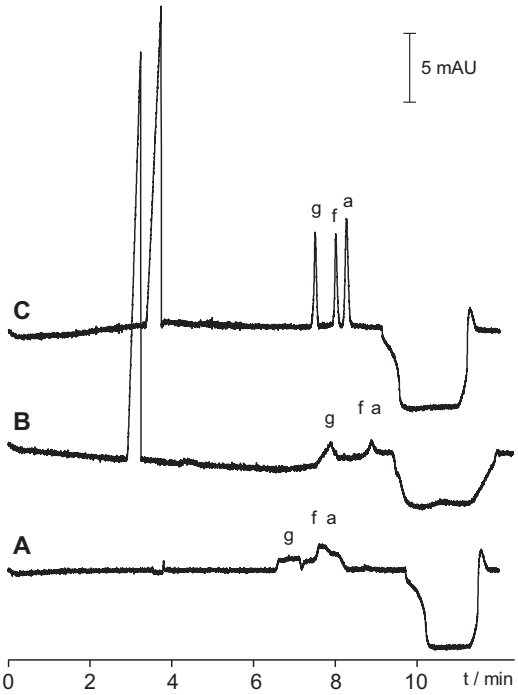


**Fig. 2.** Long injection of S under non-stacking (A), sweeping (B), and two-step stacking by sweeping and MSS (C) conditions in co-EOF CZE. BGS: 50 mM $NH_4HCO_3$ and 50% MeOH, pH 9.7 (A and C) and 50 mM $NH_4HCO_3$ and 25% MeOH, pH 9.5 (B). S: 1.9 μg/mL of each hypolipidaemic drug (g – gemfibrozil, f – fluvastatin, a – atorvastatin) in 20 mM $NH_4HCO_3$, pH 9.5. MS: 10 mM CTAB and 10 mM $NH_4HCO_3$. Injection scheme: 100 s of S (A) and 30 s of MS followed by 100 s of S (B and C). Other conditions in the experimental section.

### 3.2. Experimental verification of sweeping-MSS using test hypolipidaemic drugs

Fig. 2 shows the results obtained from the long injection of S (100 s) under non-stacking (A), sweeping (B), and two-step stacking by sweeping and MSS (C) conditions. The S was 1.9 μg/mL of each hypolipidaemic drug in 20 mM $NH_4HCO_3$. This is a basic condition for sweeping where the micelle forming agent was not added into the S. The BGS was 50 mM $NH_4HCO_3$ with 50% MeOH in Fig. 2A and C and with 25% MeOH in Fig. 2B. From preliminary experiments, MSS occurred when 50% MeOH was used in the BGS while MSS did not occur when 25% MeOH was used. Thus, the conditions using 50% and 25% MeOH in the BGS were excellent conditions to show the effect on the injected S of the two-step stacking procedure (sweeping and MSS) and sweeping alone, respectively. The drugs were negatively charged at the pH (i.e., 9.5 or 9.7) of the BGSs used. The MS (10 mM CTAB and 10 mM $NH_4HCO_3$) was injected for 30 s in Fig. 2B and C. The large peak detected before 4 min in Fig. 2B and C was the bromide from CTAB.

Under non-stacking and sweeping conditions (see Fig. 2A and B, respectively), the analytes came out as broad peaks. The last two peaks overlapped in the sweeping condition, but the peak for gemfibrozil (g) was obviously slightly narrow compared to the non-stacking condition. When the concentration of MeOH in the BGS was increased from 25% (see Fig. 2B) to 50% (see Fig. 2C), the MSS condition was satisfied and the swept zones in Fig. 2B were focused for the second time by MSS as shown in Fig. 2C. It was previously found that the BGS should contain at least 50% MeOH for the MSS of these hypolipidaemic drugs using a S matrix that contained 9 mM CTAB [30] which was similar to the concentration of CTAB (i.e., 10 mM) used in the MS here. Clearly, all the three analytes were successfully focused by the two-step stacking strategy (see Fig. 2C).

### 3.3. Optimization of the sweeping-MSS using test hypolipidaemic drugs

#### 3.3.1. Optimization of $NH_4HCO_3$ concentration in the MS

Supporting information Fig. 1 shows the effect of different concentrations of $NH_4HCO_3$ (i.e., 10 (A), 20 (B), 30 (C) and 50 (D) mM in the MS). The concentration of CTAB in the MS was fixed at 10 mM and the other conditions were the same as those in Fig. 2C. Supplementary information Table 1 provides the CE current obtained for the BGS, S, and different MSs. The conductivity ratio MS/BGS was approximated from the ratio of CE currents. Also, the CE current for the BGS = S, thus conductivity ratio MS/BGS = MS/S.

The peak shapes and heights improved when the concentration of $NH_4HCO_3$ was increased from 10 to 20 mM. The conductivity ratio was 0.8 and 1.3, respectively. The shorter peak height in the 10 mM condition may be caused by a stronger destacking at the MS and S boundary (cathodic side of the S zone) compared to stacking at the BGS and MS boundary (cathodic side of the MS zone). The reverse was in the 20 mM condition, a stronger stacking and weaker destacking correspondingly.

In 30 mM $NH_4HCO_3$ MS condition, the last two analytes (f and a) eluted as one sharp peak in the MS zone (see dip in the baseline from ~8.5 min), while in 50 mM condition, all the peaks eluted in the MS zone. The conductivity ratio was 1.8 and 2.8 in the 30 mM and 50 mM condition, respectively. Thus, the higher conductivity and lower electric field strength MS zone caused the slower electrophoretic migration and detection of these peaks in the MS zone. The field strength in the 50 mM $NH_4HCO_3$ MS was lowest and caused all the peaks to come out in the MS zone. This also increased the migration time for the analytes. The MS with 10 mM CTAB and 20 mM $NH_4HCO_3$ was then chosen for further optimization.

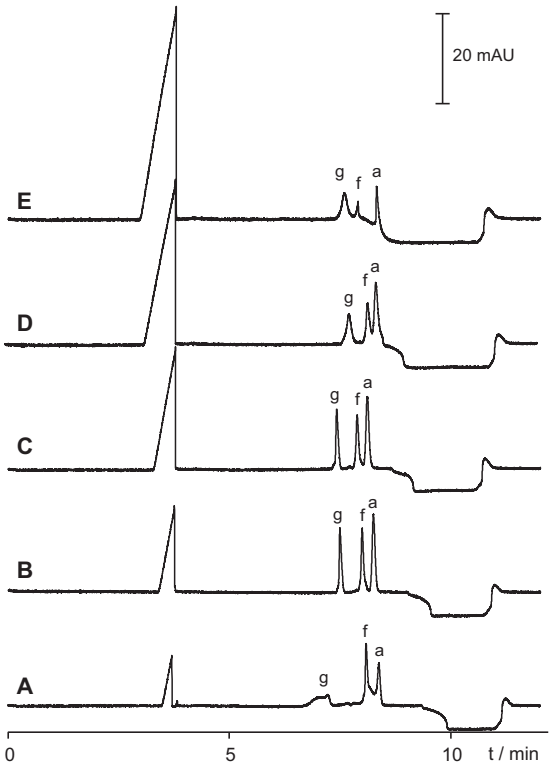


**Fig. 3.** Effect of MS injection time. MS: 10 mM CTAB and 20 mM $NH_4HCO_3$, pH 9.5 S: 3.7 μg/mL of each hypolipidaemic drug in 20 mM $NH_4HCO_3$. MS injection time: 5 (A), 15 (B), 30 (C), 60 (D), 90 (E) s. Other conditions and peak identity are the same as in Fig. 2C.

#### 3.3.2. Optimization of MS injection time

Fig. 3 shows the effect of MS injection time (5 (A), 15 (B), 30 (C), 60 (D), and 90 (E) s). The other conditions were the same as in Fig. 2C but the analyte concentrations were doubled for better visualization of the peaks. The effect of sweeping was less effective at the shortest injection time of 5 s and resulted to a broad peak for gemfibrozil (see Fig. 3A). A fronting peak for atorvastatin was also observed in Fig. 3A and the front overlapped with fluvastatin which gave the sharpest peak. This was quite surprising since the measured $k$ for gemfibrozil (10.5) and atorvastatin (3.7) were higher than fluvastatin (2.9). Note that sweeping works better for high $k$ analytes [13,14].

Narrower swept zones were formed for gemfibrozil and atorvastatin compared to fluvastatin, however the MS zone was too short and the swept molecules leaked and electrophoretically migrated away from the sweeping zone. These results can be explained by the effect of $\mu_{ep}(a')$ and $\mu^*_{ep}(a')$ on this first stacking step. The $\mu_{ep}(a')$ ($cm^2/V\,cm$) was fastest for gemfibrozil ($-9.9 \times 10^{-5}$) followed by fluvastatin ($-7.8 \times 10^{-5}$) and atorvastatin ($-6.6 \times 10^{-5}$). The $\mu^*_{ep}(a')$ ($cm^2/V\,cm$) calculated using Eq. (1) was also fastest for gemfibrozil ($2.4 \times 10^{-4}$) but followed by atorvastatin ($2.0 \times 10^{-5}$) and then fluvastatin ($1.8 \times 10^{-5}$). Mobility was negative and positive when directed toward the anode and cathode, respectively. The rate of broadening that can be related to the calculated $\left|\mu^*_{ep}(a')\right| + \left|\mu_{ep}(a')\right|$ values was indeed highest for gemfibrozil ($3.4 \times 10^{-4}\,cm^2/V\,cm$). In addition, all the injected gemfibrozil molecultes were also not brought to the MSSB as judged from the earlier migration time observed for this analyte (~7 min) compared to the longer injections of MS (>7.5 min) (see Fig. 3B–E). The calculated rate of broadening for atorvastatin however, did not account for the fronting of this peak. The rates were close at $9.6 \times 10^{-5}$ and $8.6 \times 10^{-5}\,cm^2/V\,cm$ for fluvastatin and atorvastatin, respectively. There could be poor accuracy in the measurements or another broadening mechanism (that may be investigated in the future) exists.

Leakage of the swept band was prevented by increasing the injection time to 15 or 30 s (see Fig. 3B and C, respectively) that resulted to sharp peaks for all the hypolipidaemic drugs. On one hand, increasing the time to 60 s (see Fig. 3D) produced broad peaks. At the time of 90 s (see Fig. 3E), there was a broad peak for gemfibrozil. Also at 90 s, the S and MS zones were brought very close to the detector and caused the detection of the last two peaks while still inside the MS zone. The broad peaks in Fig. 3D and the broad gemfibrozil peak in Fig. 4E were caused by the longer flux of micelles at the MSSB in the longer injections of MS. This longer flux of micelles also caused the longer migration time. For example, the migration time for gemfibrozil with a 15 or 30 s and 60 or 90 s injection of MS was 7.5 and 8.0 min, respectively. An MS injection time of 15 s (see Fig. 3B) was then chosen for further study.

#### 3.3.3. Optimization of S injection time

Fig. 4 shows the effect of S injection time (50 (A), 100 (B), 150 (C), 200 (D), and 250 (E) s). The other conditions were the same as in Fig. 3B. The peak heights increased when the injection was increased from 50 to 150 s. Detection of the peaks in the MS zone started when the injection time was increased to 200 s (see peak f and a in Fig. 4D). When the injection was increased to 250 s, all the peaks were detected in the S or MS zone (see Fig. 4E). At the 200 and 250 s injections, the focused peaks were already too close to the detector and were not allowed to migrate out of the S and MS zones before detection. When the 100 s was compared to a typical 3 s injection, there was a 16–22 times increase in peak height. The improvements of 20–29 times were better using the 150 s injection. The 100 s injection (see Fig. 4B) was chosen for the linearity and reproducibility studies since the resolution of the last two peaks was better compared to the 150 s injection (see Fig. 4C).

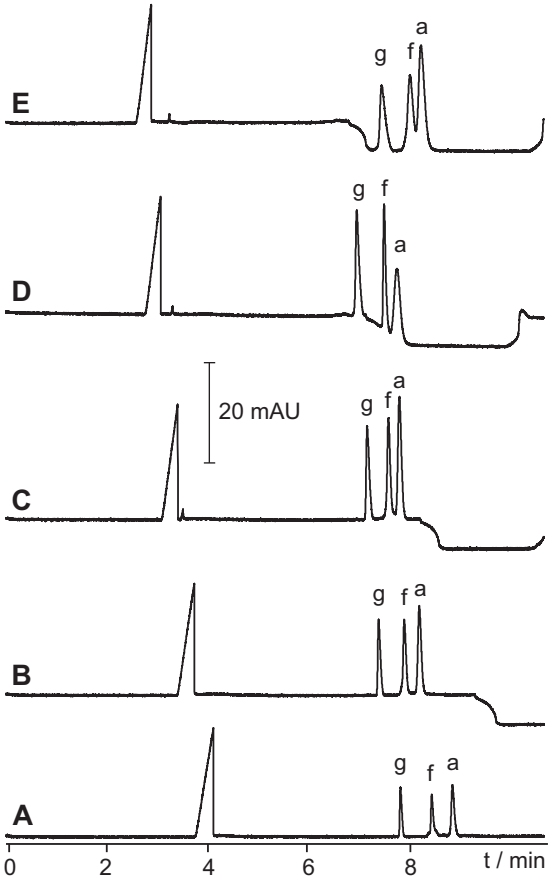


**Fig. 4.** Effect of S injection time at 50 (A), 100 (B), 150 (C), 200 (D), and 250 (E) s. Other conditions and peak identity are the same as in Fig. 3B.

### 3.3.4. Effect of the concentration of CTAB in the MS

The concentration of CTAB can affect the $k$ of the analytes in MEKC and thus an increase in the concentration CTAB in the MS may improve the first stacking step (i.e., sweeping). In the second stacking step (i.e., MSS), however, an increase in the concentration of CTAB may have a negative effect since a higher concentration of organic solvent is necessary to reverse the $\mu^*_{ep}(a')$ of a high $k$ analyte. The use of higher concentrations of surfactant in the MS (20 (A), 30 (B), 40 (C), 50 (D), and 60 (E) mM CTAB) was then investigated and the results are shown in Supplementary information Fig. 2. The MSs contained 5 mM of $NH_4HCO_3$ and have CE currents that were 60–100% of the currents obtained for the BGS or S. Each MS was injected for 5 s prior to the S. The S (3.7 μg/mL of each hypolipidaemic drug in 20 mM $NH_4HCO_3$) was injected for 100 s. The BGS was 50 mM $NH_4HCO_3$ and 50% MeOH. The results were slightly better using the lower concentrations (i.e., 20 (A) and 30 mM (B)). This could be explained by the opposite effects of CTAB concentration on sweeping and on MSS (see above).

A 20 and 30 mM CTAB may be used in the MS, but after optimization of parameters similar to that performed for the 10 mM CTAB (see Sections 3.3.1–3.3.3). For example, the effect of MS injection time (1–7 s) is shown in Supplementary information Fig. 3. It is noted that a 15 s injection of MS with 10 mM CTAB (see Fig. 3B) was needed to effectively stack a 100 s injection of S. On one hand, a shorter 4 or 5 s injection MS with 20 mM CTAB accomplished the same feat (see Supplementary information Fig. 3E and F).

### 3.3.5. Use of another organic solvent

A common organic solvent in CE ACN was also tested. MeOH was found better than ACN because MeOH also conveniently reduced the EOF. This was because in co-EOF CZE, the actual length for separation was reduced by the EOF since the velocity of the analytes and bulk flow was in the same direction. In effect, the reduction in the EOF by MeOH allowed longer injections of the S and MS in the two-step stacking procedure (Section 3.4). Application to two other groups of analytes (i.e., NSAIDs and herbicides).

Using the conditions used for the test hypolipidaemic drugs, the 100 s S injection time provided 26–33 times improvements in peak height for diflunisal, indoprofen, and indomethacin. However, there was a loss in resolution between the naproxen and ketoprofen peaks. The S injection time was then reduced to 75 s in order to resolve the five tested NSAIDs and this injection provided 17–25 times improvement in peak height when compared to a typical 3 s injection. Fig. 5A and B shows a typical injection and a sweeping-MSS injection, respectively of the NSAIDs. Note that the analytes in the typical injection were 5× more concentrated than in the two-step stacking injection.

The same approach was used for the test herbicides, and a 30 s injection of S was found optimum. This injection when compared to a typical 3 s injection yielded 21 and 18 times improvements in the peak height for fenoprop and mecoprop, respectively. Fig. 5C and D shows a typical injection and a two-step stacking injection, respectively of the herbicides. The analytes in the typical injection were 10× more concentrated than in the sweeping-MSS injection.

### 3.4. Repeatability, linearity, LODs

The current optimum sweeping-MSS conditions obtained for the different groups of analytes were presented in Figs. 4B and 5B, D. Some analytical figures of merit were summarized in Table 1 (hypolipidaemic drugs (A), NSAIDs (B), and herbicides (C)). Intraday repeatability that was performed by 12 successive injections of the standard samples (at least 1 μg/mL of each analyte) was acceptable. The %RSD ($n$ = 12) were 0.9–3.5%, 3.1–8.9%, and 1.7–10.8% for migration time, corrected peak area, and peak height, respectively. Good linearity values ($R^2$ > 0.99) for corrected peak area and peak height were also obtained for all the test analytes. The current linearity studies were designed only to cover at least one order of concentration magnitude. Interday repeatability (6 injections from day 1 to day 2) performed using the test herbicides only were also acceptable. The %RSD ($n$ = 12) were 1.6–3.6%, 4.4–4.8%, 4.9–9.8% for migration time, corrected peak area, and peak height, respectively.

The calculated LODs (S/N = 3) based on peak heights obtained from the typical 3 s injection of the hypolipidaemic drugs, NSAIDs, and herbicides were 1.75–2.16, 1.23–11.72, and 1.68–2.73 μg/mL, respectively. The analytical performance in terms of repeatability and $R^2$ of typical injections were comparable to the stacking methods. The slopes of the calibration lines in the two-step stacking methods were however higher due to improved sensitivity in the stacking methods. The LODs (S/N = 3) from the current optimized stacking injections were 0.09–0.10, 0.05–0.55, and 0.08–0.15 μg/mL, correspondingly. The improvements in concentration sensitivity were more than an order of magnitude for all the tested analytes.

### 3.5. Application to herbicides spiked waste water sample

The optimized condition (see Fig. 5D) for the herbicides was tested on a spiked waste water sample and the results are shown in Fig. 6. The blank injection (see Fig. 6A) shows that there were no interferences from the sample matrix. The spiked sample (see Fig. 6B) was successfully analysed using the method with a simple liquid–liquid extraction step. The recoveries were calculated by injection of one standard of the same expected concentration. The recoveries were acceptable at 94–100% and 98–107% for corrected peak area and peak height, respectively.

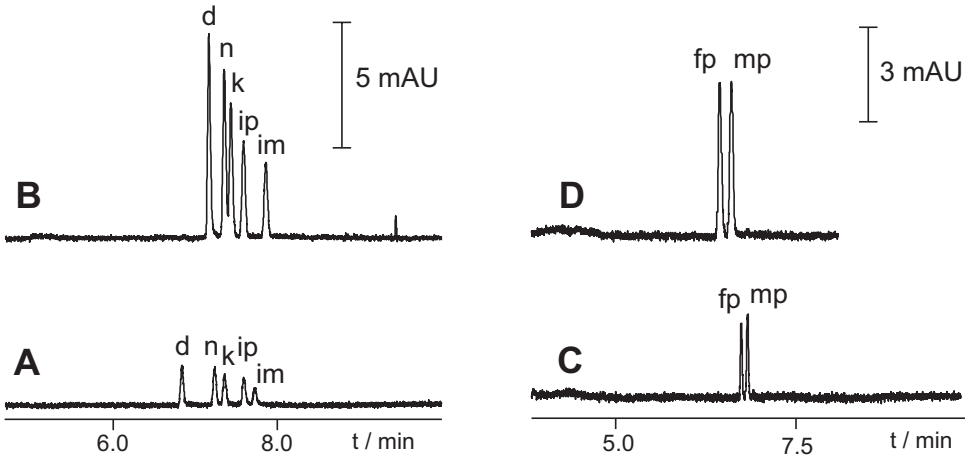


**Fig. 5.** Typical injection (A, C) versus sweeping-MSS injection (B, D) of the NSAIDs (A, B) and herbicides (C, D). S injection: 3 s (A, C), 75 s (B), and 30 s (D). MS injection: 0 s (A, C) and 15 s (B, D). Concentration of analytes: 5–20 μg/mL (A), 1/5 concentration dilution of A (B), 9.9–18.1 μg/mL (C), 1/10 concentration dilution of C (D). Other conditions are the same as in Fig. 3B. Peak identity: d = diflunisal, n = naproxen, k = ketoprofen, ip = indoprofen, im = indomethacin, fp = fenoprop, and mp = mecoprop.

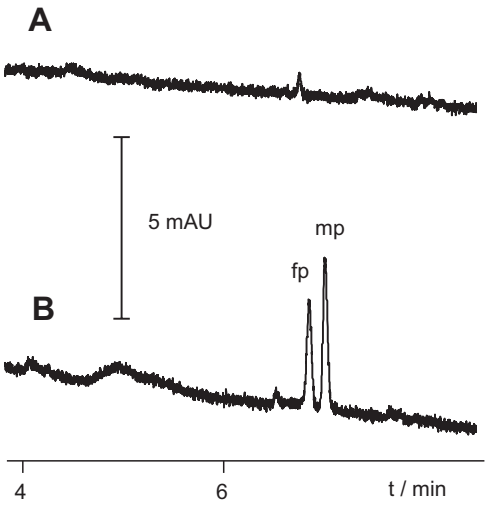


**Fig. 6.** Sweeping-MSS of blank (A) and herbicides spiked (B) waste water sample. Spiked sample before extraction contained 1 μg/mL of fenoprop (fp) and 2 μg/mL of mecoprop (mp). S injection = 30 s, MS injection = 15 s. Other conditions are similar to that in Fig. 3B. Explanation in the text.

## 4. Concluding remarks

The two-step stacking by sweeping and MSS in co-EOF CZE was successfully applied to a variety of organic anions (i.e., hypolipidaemic drugs, NSAIDs and herbicides) using cationic CTAB micelles and HDMB coated fused silica capillaries. The improvements in sensitivity for sweeping-MSS were two to three times better compared to MSS alone and the improvements in LOD were similar to that obtained for organic cations using anionic SDS micelles. The first two-step stacking method using SDS was applicable only to cationic analytes [31] while the current method using CTAB was only to anionic analytes. The applicability to only cationic or anionic analytes stems from the selective preconcentration of the second focusing step of MSS which requires that the analyte and micelle carrier should be of opposite charge. The methods should be useful for the selective analysis of anionic or cationic analytes in a sample mixture. Another advantage of the two step stacking methods includes compatibility to ESI-MS detection. This strategy can be combined with field enhanced sample injection (electrokinetic injection) for the three-step stacking of charged analytes. In addition, although the separation was not considered as partial-filling MEKC since the selectivity was not significantly affected by the micelles, the mechanisms of broadening and sharpening of sample zones shown here will find value in method development of partial-filling techniques using various pseudostationary phases in CE.

**Table 1**
Intraday reproducibility and linearity: hypolipidaemic drugs (A), NSAIDs (B), and herbicides (C).

| | Intraday RSD% ($n$ = 12) | | | Range (μg/mL) | Linearity ($R^2$) | |
|---|---|---|---|---|---|---|
| | Migration time | Corrected peak area | Peak height | | Corrected peak area | Peak height |
| A | | | | | | |
| Gemfibrozil | 2.3 | 5.2 | 10.8 | 0.1–1.1 | 0.998 | 0.998 |
| Fluvastatin | 2.7 | 3.8 | 3.8 | 0.1–1.1 | 0.996 | 0.995 |
| Atorvastatin | 2.8 | 4.2 | 6.5 | 0.1–1.1 | 0.999 | 0.997 |
| B | | | | | | |
| Diflunisal | 1.1 | 8.9 | 5.9 | 0.6–10.9 | 0.996 | 0.995 |
| Naproxen | 0.9 | 3.3 | 2.1 | 0.5–10.1 | 0.999 | 0.994 |
| Ketoprofen | 0.9 | 3.1 | 8.6 | 0.5–5.1 | 1.000 | 1.000 |
| Indoprofen | 1.0 | 3.8 | 7.3 | 0.3–5.2 | 0.997 | 0.999 |
| Indomethacin | 1.0 | 7.5 | 7.1 | 2.6–26.0 | 1.000 | 0.994 |
| C | | | | | | |
| Fenoprop | 3.5 | 4.1 | 2.9 | 0.3–10.0 | 0.998 | 0.994 |
| Mecoprop | 1.6 | 4.6 | 1.7 | 0.5–20.0 | 1.000 | 0.999 |

*Note*: corrected peak area = peak area/migration time. *Conditions*: see Figs. 5B and 6B, D for A, B, and C, respectively.

## Acknowledgements

AMG and JPQ are grateful for the support of Prof. Paul R. Haddad, ACROSS, and the University of Tasmania. JPQ also thanks the Australian Research Council for a Future Fellowship.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.12.095.

## References

[1] J.L. Beckers, P. Boček, Electrophoresis 21 (2000) 2747.
[2] G. Hempel, Electrophoresis 21 (2000) 691.
[3] J.P. Quirino, S. Terabe, J. Chromatogr. A 902 (2000) 119.
[4] Z.K. Shihabi, J. Chromatogr. A 902 (2000) 107.
[5] C.H. Lin, T. Kaneta, Electrophoresis 25 (2004) 4058.
[6] S.L. Simpson, J.P. Quirino, S. Terabe, J. Chromatogr. A 1184 (2008) 504.
[7] M.C. Breadmore, J.R.E. Thabano, M. Dawod, A.A. Kazarian, J.P. Quirino, R.M. Guijt, Electrophoresis 30 (2009) 230.
[8] A.T. Aranas, A.M. Guidote, J.P. Quirino, Anal. Bioanal. Chem. 394 (2009) 175.
[9] J.P. Quirino, S. Terabe, Anal. Chem. 72 (2000) 1023.
[10] J.P. Quirino, Y. Iwai, K. Otsuka, S. Terabe, Electrophoresis 21 (2000) 2899.
[11] R.L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 141.
[12] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) A489.
[13] J.P. Quirino, S. Terabe, Science 282 (1998) 465.
[14] J.P. Quirino, S. Terabe, Anal. Chem. 71 (1999) 1638.
[15] J.-B. Kim, K. Otsuka, S. Terabe, J. Chromatogr. A 932 (2001) 129–137.
[16] L. Zhu, C. Tu, H.K. Lee, Anal. Chem. 74 (2002) 5820–5825.
[17] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242.
[18] A.S. Ptolemy, P. Britz-McKibbin, Analyst 133 (2008) 1643.
[19] P. Britz-McKibbin, K. Otsuka, S. Terabe, Anal. Chem. 74 (2002) 3736.
[20] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[21] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[22] T. Hirokawa, H. Okamoto, B. Gas, Electrophoresis 24 (2003) 498.
[23] H. Okamoto, T. Hirokawa, J. Chromatogr. A 990 (2003) 335.
[24] Z.Q. Xu, E. Koshimidzu, T. Hirokawa, Electrophoresis 30 (2009) 3534.
[25] M. Dawod, M.C. Breadmore, R.M. Guijt, P.R. Haddad, Electrophoresis 31 (2010) 1184.
[26] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[27] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[28] J.P. Quirino, J. Chromatogr. A 1216 (2009) 294.
[29] L. Liu, X. Deng, X. Chen, J. Chromatogr. A 1217 (2010) 175.
[30] A.M. Guidote, J.P. Quirino, J. Chromatogr. A 1217 (2010) 6290.
[31] J.P. Quirino, J. Chromatogr. A 1217 (2010) 7776.
[32] M.M. Bushey, J.W. Jorgenson, Anal. Chem. 61 (1989) 491.
[33] J.P. Quirino, P.R. Haddad, Anal. Chem. 80 (2008) 6824.
[34] J.P. Quirino, Electrophoresis 30 (2009) 875.