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On-line sample concentration of organic anions in capillary zone electrophoresis by micelle to solvent stacking

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ABSTRACT

Micelle to solvent stacking (MSS) is a new on-line sample concentration technique for charged analytes in capillary zone electrophoresis (CZE). Sample concentration in MSS mainly relies on the reversal in the effective electrophoretic mobility of the analyte at the boundary zone between the sample solution (S) and CZE background solution (BGS) inside the capillary. The basic condition for MSS is that the S is prepared in a matrix that contains an additive (i.e., micelles) which interacts with and has an opposite charge compared to the analytes. In addition, the BGS must contain a sufficient percentage of organic solvent. MSS was first reported for organic cations using anionic dodecyl sulfate micelles as additive in the S and methanol or acetonitrile as organic solvent in the BGS. Here, theoretical and experimental studies on MSS are described for organic anions using cationic cetyltrimethyl ammonium micelles as additive in the S and methanol as organic solvent in the BGS. Up to an order of magnitude improvement in concentration sensitivity was obtained for the test hypolipidaemic drugs using MSS in CZE with UV detection. The optimized method was also evaluated to the analysis of a spiked wastewater sample that was subjected to a simple extraction step.

1. Introduction

Capillary electrophoresis (CE) using ultraviolet (UV) detection has been gaining wider use over the years despite its limitations in sensitivity. This is partly due to the development of on-line sample concentration or stacking techniques [1–4] that have increased the sensitivity despite the limited amount of sample introduced and the short optical path length due to the minute size of the capillary. Some of these stacking techniques are field amplification or enhancement [4,5], pH mediated stacking [6], dynamic pH junction [7,8], transient isotachophoresis (tITP) [9],, acetonitrile stacking [10], electrokinetic supercharging [11] and sweeping [12,13] or sweeping in combination with other techniques [13–17]. The more recent techniques include transient trapping (tr-trapping) [18], analyte focusing by micelle collapse (AFMC) [19–21] and micelle to solvent stacking (MSS) [22].

MSS was introduced in 2009 and was done by preparing the sample solution (S) with micelles and the background solution (BGS) with an organic solvent [22]. In the initial paper on MSS, separation was achieved through capillary zone electrophoresis (CZE). A similar concentration mechanism was used by Liu and co-workers in micellar electrokinetic chromatography where they injected a plug of trapping solution containing an organic solvent after the injection of S [23]. In both studies, the analytes were focused at the boundary between the S zone and the zone containing an organic modifier (BGS or trapping solution).

In MSS with CZE, the samples were cationic β-blocker drugs and the surfactant used was the anionic sodium dodecyl sulfate [22]. The analyte cations bound to the micelles were electrophoretically attracted to the anode but upon reaching the micelle to solvent boundary (MSSB) containing the organic solvent, the affinity of the analytes to the micelles were significantly lowered. The cations then moved towards the cathode and experienced an electrophoretic inversion or reversal resulting to analyte accumulation at the MSSB. The sample is thus concentrated as a result of the presence of the organic modifier. This inversion in eletrophoretic mobility was demonstrated by observing the elution of the elecroosmotic flow (EOF) marker and the test cationic analytes in MEKC mode using the S matrix as BGS. In MSS, the S matrix and BGS had similar conductivities, ruling out field enhancement as the mechanism behind the results. It was established that this is a new technique working for analytes that can be electrophoretically inverted. An order of magnitude increase in concentration sensitivity had been realized.

Here, the application of MSS to anionic analytes, using hypolipidaemic drugs in their anionic form as model analytes and using cationic cetyltrimethyl ammonium micelles is presented. The principle is discussed and a theoretical explanation is pro-

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posed. The application of MSS-CZE with a prerequisite anodic EOF is experimentally demonstrated. The inversion of the electrophoretic mobility of the organic anion is shown. The results in the optimization of the technique for the hypolipidaemic drugs: gemfibrozil, fluvastatin and atorvastatin as well as the results of the system reproducibility are presented. In addition, evaluation of the optimized MSS-CZE method to spiked wastewater sample is shown.

2. Experimental

2.1. Apparatus

An Agilent[3D] Capillary Electrophoresis System (Agilent Technologies, Germany) with fused-silica capillaries (Polymicro Technologies, USA) of 50 cm length (41.5 cm effective length) and 50 μm i.d. was always utilized for this study. Temperature was set at 20 °C and a voltage of -18 kV was applied all throughout this work. Unless otherwise stated, the detection wavelength was set at 214 nm. The pH meter used was an Activon Model 210 (Activon Scientific Products Co. Pty. Ltd., Australia).

2.2. Reagents and solutions

All reagents for the electrolytes: NH_4HCO_3, cetyltrimethylammonium bromide (CTAB), methanol, hexadimethrine bromide (HDMB) were of >99% purity and were all purchased commercially. The hypolipidaemic compounds (statins): gemfibrozil (g), fluvastatin (f) and atorvastatin (a) (all >98%) were procured from Sequoia (Oxford, UK). Milli-Q water (Millipore, USA) was used all throughout. The statins were prepared from 1 mg/mL stock solutions with methanol as solvent. All solutions used for the BGS were filtered with a 0.45 μm MicroScience membrane filter (MicroAnalytix Pty. Ltd., Australia). The BGS was prepared fresh everyday and sonicated for 5 min prior to use.

The effluent came from the sewage plant of Hobart, Tasmania, Australia. The sample (1.0 mL) was spiked to contain 1 μg/mL of the statins. The extraction was done by the addition of 0.1 mL of concentrated HCl and 2 mL of dichloromethane (DCM). The mixtures were shaken for 5 min and centrifuged for 3 min at 3000 rpm. The DCM layer was isolated and then dried *in vacuo*. The samples were reconstituted with9 mM CTAB and 10 mM NH_4HCO_3.

2.3. General electrophoresis procedure

HDMB (1%) was used for EOF reversal [24]. 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 the day. For each run, the order of preconditioning was 1% HDMB (2 min), water (1 min) and BGS (5 min). Specific experimental conditions are stated in the figures and in Section 3.

3. Results and discussion

3.1. MSS anion focusing model and theory

3.1.1. Model

Presented in Fig. 1 is the model for the on-line sample concentration of organic anions by MSS in CZE with anodic EOF. The starting situation is A where the capillary is first conditioned with the BGS containing the electrolyte and organic modifier. The sample solution (S) which is in a matrix that contains the electrolyte and cationic micelles but excludes the organic modifier is then injected. At B upon application of a negative voltage, the effective

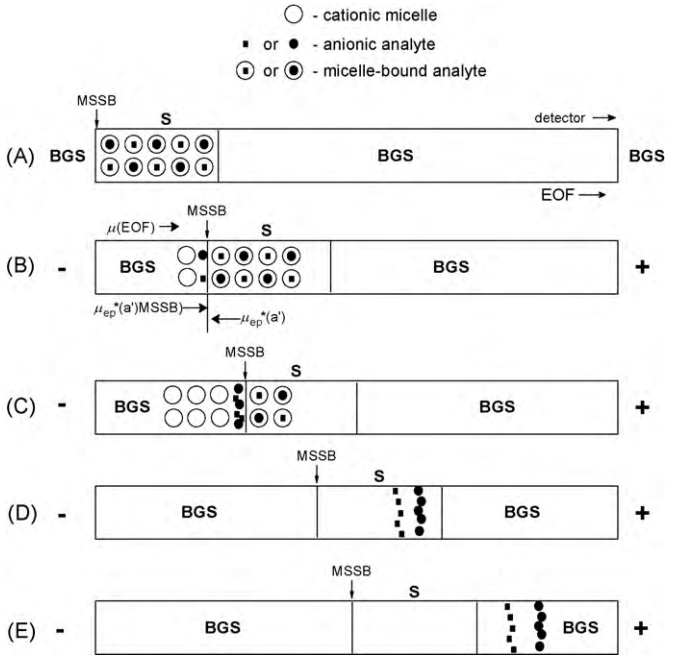

Fig. 1. Model for the on-line concentration of organic anions by MSS in CZE with anodic EOF. The background solution (BGS) was an electrolyte that contains an organic modifier and the sample (S) solution includes two anionic analytes prepared with an electrolyte that contains a cationic surfactant above the cmc. (A) Starting situation: The capillary was conditioned with BGS and then the S was injected. (B) Application of voltage: The micelles cross the MSSB boundary where their affinity to the analytes is lowered due to the organic modifier. (C) The analytes were focused as more and more micelles traverse the MSSB and the analytes are accumulated along the boundary. (D and E) The analytes were successfully separated by CZE. More explanation given in the text.

electrophoretic mobility of the analyte in the S zone (μ_{ep}*(a′)S) is directed towards the cathode due to the micelles. The cationic micelles transport the analytes to the MSSB, which is the boundary between the S zone and BGS at the cathodic end of the capillary. At the MSSB, the analytes have less affinity toward the micelles due to the organic solvent. This causes a decrease in the retention factor (k) to the extent that there is an inversion of the effective electrophoretic mobility with the effective electrophoretic mobility of the analyte in the MSSB (μ_{ep}*(a′)MSSB) directed to the anode. The anionic analytes are accumulated near the MSSB. At C, with continuous electrophoresis, more of the cationic micelle-bound analytes migrate to the cathode and cross the MSSB, and more analytes are accumulated resulting to the enrichment of the sample. The analytes that inversed mobility can penetrate back into the micellar zone. Afterwards at D when all the micelles have traversed the MSSB, CZE follows and the analytes move into the original S zone. This continues in E, as the analytes migrate out of the S zone to the BGS zone and then to the UV detector. It is important that the conductivities of the S and BGS zones be similar since a significant difference would result to destacking (i.e., if the conductivity of the S is higher compared to the BGS).

3.1.2. Theoretical consideration

The change in the effective electrophoretic mobility of a charged analyte at the boundary between the S and BGS is the main driving force in MSS sample concentration. The S must be in a matrix that contains an additive which interacts with and has an opposite charge compared to the analytes and the BGS must contain an organic solvent. Here, the additive (i.e., micelle) and analyte, is positively and negatively charged, respectively.

The effective electrophoretic velocity of an anionic analyte in the *S*, (ν_{ep}*(a')*S*), in the presence of the positively charged micelle

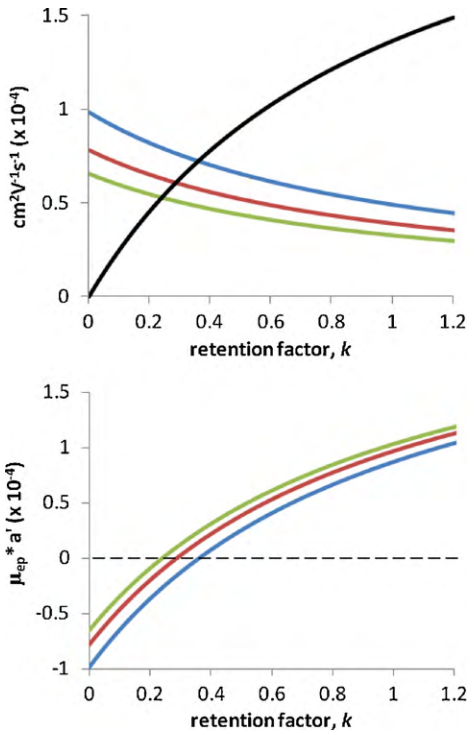

Fig. 2. Effect of the retention factor, k, to effective electrophoretic mobility (μ_{ep}*(a′)). The lines for gemfibrozil (blue), fluvastatin (red) and atorvastatin (green) are for the contribution of the analytes, $[1/(1+k)]\mu_{ep}$*(a′) and the line for the micelle (black) is for the contribution of the micelle, $[k/(k+1)]\,\mu_{ep}$*(mc) to the effective electrophoretic mobility, μ_{ep}*(a′). The lower graph is the μ_{ep}*(a′) for the three analytes after summation of $[1/(1+k)]\mu_{ep}$*(a′) and $[k/(k+1)]\mu_{ep}$*(mc). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

is given by Eq. (1) [25].

$$v_{ep}{}^{*}(a')S = \left[\frac{1}{1+k}\mu_{ep}(a') + \frac{k}{k+1}\mu_{ep}(mc)\right] E \quad (1)$$

where $\mu_{ep}(a')$ is the electrophoretic mobility of an analyte a′; $\mu_{ep}(mc)$ is the electrophoretic mobility of the micelle; k is the retention factor; and E is the electric field strength.

The sum of the expression within the bracket in Eq. (1) is equivalent to the effective electrophoretic mobility of the analyte, μ_{ep}*(a′).

The sample is injected as a long plug and a boundary (i.e., MSSB) between the S and BGS is created at the cathodic end of the S zone. At this boundary, the effective electrophoretic mobility of the analyte (μ_{ep}*(a′)MSSB) is given by Eq. (2).

$$\mu_{ep}{}^{*}(a')MSSB = \frac{1}{1+k}\mu_{ep}(a') + \frac{k}{k+1}\mu_{ep}(mc) \quad (2)$$

Once in the MSSB, because of the presence of the organic solvent, k can become a very small number or zero. There is a limit to k such that the term for $\mu_{ep}(a')$ is dominant and will be bigger than the term for $\mu_{ep}(mc)$ so that the analyte enrichment occurs.

Using calculated electrophoretic mobilities of the analytes from this experiment and that of the cetyltrimethyl ammonium micelle approximated from the chloride salt [26], a theoretical limit to k can be predicted that will result in the occurrence or non-occurrence of MSS. With $\mu_{ep}(\text{gemfibrozil}) = 9.85 \times 10^{-5}$ cm^2/V s, $\mu_{ep}(\text{fluvastatin}) = 7.82 \times 10^{-5}$ cm^2/V s, $\mu_{ep}(\text{atorvastatin}) = 6.57 \times 10^{-5}$ cm^2/V s, and $\mu_{ep}(mc) = 2.73 \times 10^{-4}$ cm^2/V s, a plot (Fig. 2) is shown involving:

$$\frac{1}{1+k}\mu_{ep}(a') \quad (3)$$

vs.

$$\frac{k}{k+1}\mu_{ep}(mc) \quad (4)$$

This plot predicts that the limit to k is the intersection of Eqs. (3) and (4) (see Fig. 2A) and the intersection with the value of zero for μ_{ep}*(a′) (see Fig. 2B) for each analyte. The limit to k for gemfibrozil, fluvastatin and atorvastatin are 0.35, 0.28 and 0.23. At k values below these, inversion of electrophoretic mobilities will occur.

At k equals zero where the micelles have collapsed, the effect of the micelle on the effective electrophoretic mobility disappears and μ_{ep}*(a′) relies solely on the electrophoretic mobility of the analyte. Thus, Eq. (2) is reduced to:

$$\mu_{ep}{}^{*}(a')MSSB = \mu_{ep}(a') \quad (5)$$

The necessary conditions for MSS to occur are: (1) In the S zone, the $\mu_{ep}(mc)$ is large compared to $\mu_{ep}(a')$ and proceeds at the opposite direction to that of the $\mu_{ep}(a')$. (2) In the MSSB, the k should be sufficiently small or zero so that the contribution of the micelle to μ_{ep}*(a′)MSSB is negligible. Condition (1) is necessary for the micelle-bound analyte to migrate to the cathode when in the S zone and condition (2) is necessary for the organic anion to have less affinity toward the micelle and migrate to the anode once it has crossed the MSSB.

3.2. Experimental model and theory of MSS verification for organic anions

3.2.1. MSS-CZE analysis of organic anions: effect of methanol addition on the CZE BGS

The presence of methanol in the BGS is necessary for MSS to occur and the amount of methanol is important for the peak profile to be optimal. The effect of the percentage of methanol in the BGS on the analysis of organic anions by CZE using a micellar S matrix is shown in Fig. 3. Different amounts of methanol (0, 30, 40, 50, 60%) were varied to form the BGS which also consisted of 50 mM NH_4HCO_3. All these solutions have a pH range from 9.3 to 9.4. Note that the injection (30 s at 50 mbar) was longer than typical injection. From Fig. 3, it can be seen that at 0% methanol, no peaks can be detected or organic anions were not focused. At 30% methanol, peaks were still indistinguishable from the baseline. This amount of methanol was not enough to decrease the affinity of the organic anions toward the micelles. With 40, 50 and 60% methanol, the peaks were seen but the peaks for the 40% MeOH were smaller compared to the 50 or 60% MeOH. The 40% methanol was not enough a concentration to lower the k at the MSSB. The 50 and 60% methanol BGSs resulted to a lower k for the analytes that was necessary for MSS.

3.2.2. Reversal of electrophoretic mobility in the presence of organic modifier

In order to verify the reversal of effective electrophoretic mobility of the organic anions in the system in Fig. 3 using 50% methanol in the BGS, thiourea as EOF marker and one of the statins (i.e., fluvastatin) were analysed using two BGSs that mimic the S and MSSB zones (see Supporting Information Fig. S1). The BGS1 to mimic the S zone was 9 mM CTAB with 10 mM NH_4HCO_3 (Fig. S1A and B). The BGS2 was 9 mM CTAB with 50 mM NH_4HCO_3 and 50% MeOH was for the MSSB zone (Fig. S1C and D). Thiourea was monitored at a wavelength of 254 nm (see Fig. S1A and C). Fluvastatin was eluted after and before the EOF marker using BGS1 and BGS2, respectively (see Fig. S1B and D). This provided clear evidence that the reversal of electrophoretic mobility at the MSSB had occurred.

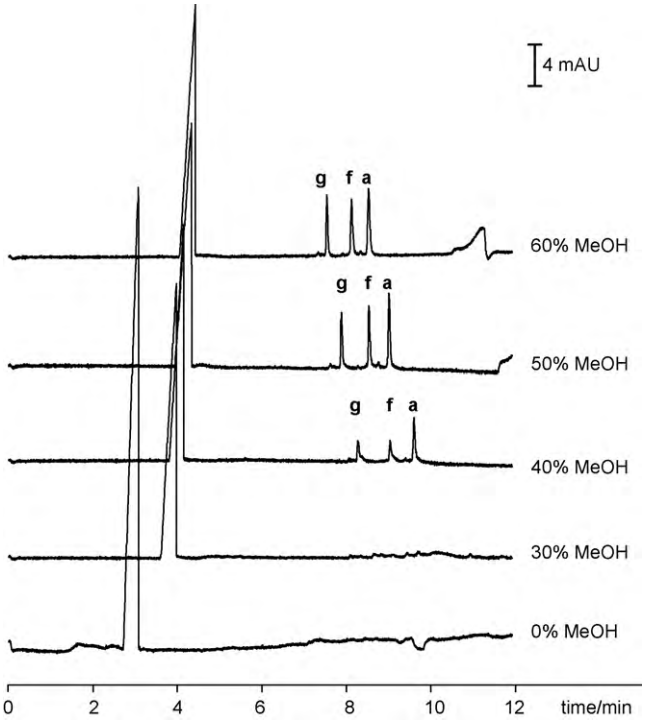

Fig. 3. Effect of the amount of methanol in the BGS to the on-line concentration by MSS and separation by CZE with anodic EOF of organic anions. The BGS consisted of 50 mM NH_4HCO_3 and methanol (0%, 30%, 40%, 50%, 60%). The S (3.7 μg/mL statins: g – gemfibrozil, f – fluvastatin, a – atorvastatin) was prepared in 9 mM CTAB and 10 mM NH_4HCO_3. The injection time was 30 s at 50 mbar pressure.

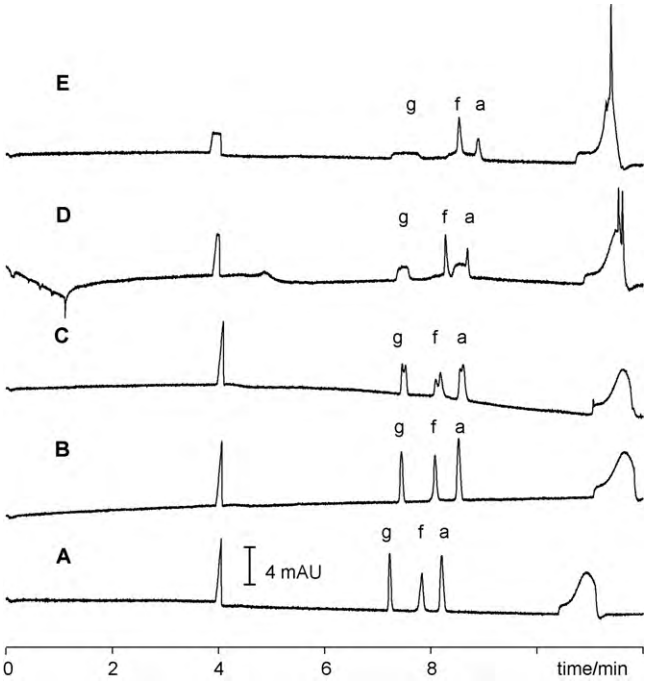

Fig. 4. Effect of the amount of NH_4HCO_3 in the sample matrix to the on-line concentration by MSS and separation by CZE with anodic EOF of organic anions. The BGS consisted of 50 mM NH_4HCO_3 and 50% methanol. The S (3.7 μg/mL statins: g – gemfibrozil, f – fluvastatin, a – atorvastatin) was prepared in 9 mM of CTAB and varying amounts of NH_4HCO_3 (A – 5, B – 10, C – 15, D – 30, E – 60 mM). The injection time was 30 s at 50 mbar pressure.

3.2.3. Verification of non-collapse of the cetyltrimethyl ammonium micelle

When *k* goes down to zero, this may be as a result of micelle collapse [11]. The possible collapse of the micelles in Fig. 3 using 50% methanol in the BGS was therefore studied. The BGS consisted of 50 mM NH_4HCO_3 and 50% MeOH. The S was hydrocortisone (50 ppm) prepared in two S matrices (9 mM CTAB with 10 mM NH_4HCO_3 and BGS). These samples and a blank (using S matrix of 9 mM CTAB with 10 mM NH_4HCO_3) were injected for 30 s (50 mbar) and the separation conditions were similar to that used in Fig. 3. The results (see Supporting Information Fig. S2) showed that the blank had no hydrocortisone peak. The BGS with steroid had a small broad peak while the steroid in the S matrix had a bigger peak that was not sharp. If the peak produced using the S matrix was sharp, micelle collapse could have happened [19–21]. The possibility that micelle collapse occurred was ruled out under these conditions. The collapse of the micelle was not necessary to induce the reversal in the charged analytes' effective electrophoretic mobility.

3.3. MSS condition optimization

3.3.1. Effect of the concentration of surfactant in the sample matrix

Varying concentrations of the surfactant, CTAB (1.1 (A), 2.2 (B), 4.5 (C), 9.0 (D), 18 (E), 36 (F) mM) were prepared, each with 10 mM of NH_4HCO_3 (pH range: 9.3–9.5) to observe the effect of the concentration of CTAB in the S matrix (see Supporting Information Fig S3). The BGS was composed of 50 mM NH_4HCO_3 and 50% methanol. The ratios between current of the BGS and the S matrix with varying CTAB concentration, 1.1, 2.2, 4.5, 9.0, 18 and 36 mM were 1.8, 1.7, 1.6, 1.4, 1.2 and 0.9, respectively. Therefore, there were negligible differences in conductivity between the S and BGS. The effect of field amplification was not relevant in this case.

With 1.1, 2.2 and 4.5 mM CTAB, the peaks for gemfibrozil (g) were broad. Gemfibrozil had less affinity (low *k* value) to the micelles and some molecules migrated out of the S zone heading towards the anode and thus the broadening. The best peak profiles were those with 9.0 and 18.0 mM CTAB. At 36.0 mM CTAB, the peak heights decreased, the peaks became broader and were eluted later. At this concentration, the reversal in the effective electrophoretic mobility at the MSSB did not occur for all the micelle-bound analytes.

3.3.2. Effect of the amount of NH_4HCO_3 in the sample matrix

The importance of the concentration of the NH_4HCO_3 in the S matrix is exhibited in Fig. 4. The BGS was 50 mM NH_4HCO_3 with 50% methanol. The S matrix had 9 mM CTAB and varying amounts of NH_4HCO_3 (5 (A), 10 (B), 15 (C), 30 (D), and 60 (E) mM). These had a pH range from 9.2 to 9.5. At 5 and 10 mM NH_4HCO_3, the peak qualities were good. With an increased amount at 15 mM NH_4HCO_3, the peak profiles deteriorated. The quality of the peaks at 30 and 60 mM NH_4HCO_3 were even worse with the middle peaks being a mixture of f and a.

The current ratio between the BGS and S matrix with 5, 10, 15, 30, 60 mM NH_4HCO_3, were 2.1, 1.4, 1.0, 0.6 and 0.3, respectively. At increased concentration of the NH_4HCO_3 in the S matrix, there was an increased difference (ratio going down) in conductivity between the S and BGS zone. This resulted to destacking at the anodic end of the S zone and the unacceptable separations. Destacking occurs after MSS and migration of the analytes through the S zone. Note that broadening or destacking also occurred for the bromide peak found at the migration time of 4 minutes. For the S matrix with 15 mM NH_4HCO_3, the current ratio is almost 1 and there is no broadening of the bromide peak (see Fig. 4C), however the ionic concentration in the S was still high causing the splitting of the analyte peaks. The current ratio should therefore not be the only factor considered when optimizing the MSS conditions. With the 5

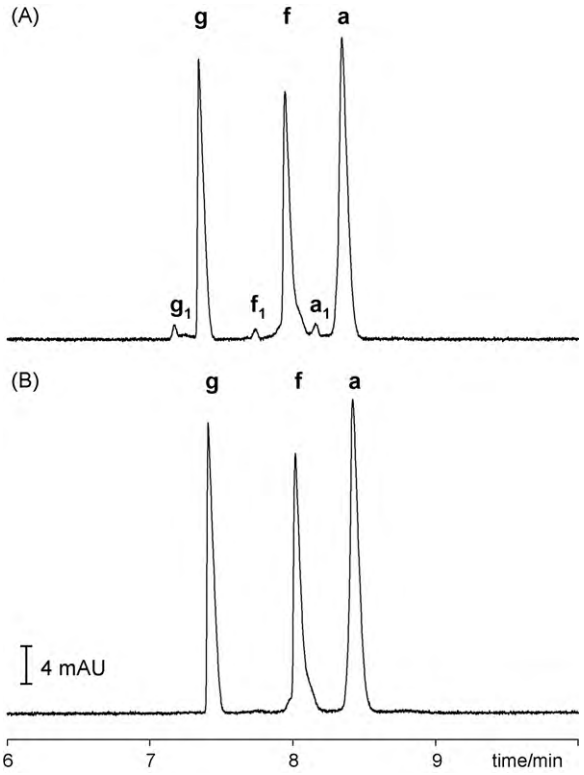

Fig. 5. Effect of the injection of sample matrix prior to sample injection. The S matrix was 9 mM CTAB and 10 mM NH_4HCO_3 while the BGS was composed of 50 mM NH_4HCO_3 and 50% methanol. For both A and B, the S (35 μg/mL statins: g and g_1 – gemfibrozil, f and f_1 – fluvastatin, a and a_1 – atorvastatin) was injected for 30 s at 50 mbar pressure. For B, a short S matrix plug was injected for 5 s at 50 mbar prior to the sample.

and 10 mM NH_4HCO_3 conditions, the peak responses were similar. Field amplification at the S zone (conductivity of the BGS or MSSB is greater than the S zone) using these conditions was not enough to cause a change in the concentration effect by MSS.

3.3.3. Effect of the concentration of electrolyte in the BGS

The electropherograms obtained by changing the concentration of the NH_4HCO_3 (20, 40, 50, 60, and 80 mM) in the BGS while keeping methanol at 50% generally looked the same (see Supporting Information Fig. S4). The pH range of these solutions was from 9.3 to 9.4. The S consisted of 3.1 mg/mL of the 3 statins in 9 mM CTAB and 10 mM NH_4HCO_3. The current ratio of the BGSs with the 20, 40, 50, 60, and 80 mM NH_4HCO_3 and S matrix were 0.5, 1.1, 1.4, 1.6, and 2.2, respectively. The BGS with 40, 50 and 60 mM NH_4HCO_3 had comparable current with that of the S matrix. The effect of field enhancement in the S or BGS zone with the 20 and 80 mM NH_4HCO_3 conditions, respectively did not affect MSS. Considering all the other conditions mentioned preceding this section, the BGS with 50 mM NH_4HCO_3 and 50% methanol was chosen as optimal.

3.3.4. Effect of the sample injection time

The effect of S injection time (3, 10, 20, 30, 40, 50 and 60 s) at 50 mbar pressure was studied. The BGS consisted of 50 mM NH_4HCO_3 and 50% methanol. The S (3.7 μg/mL statins: g, f, a) was prepared in 9 mM of CTAB and 10 mM NH_4HCO_3. There was no further increase in peak heights at 40, 50 and 60 s injection compared to 30 s. The 30 s injection was chosen as optimum injection time.

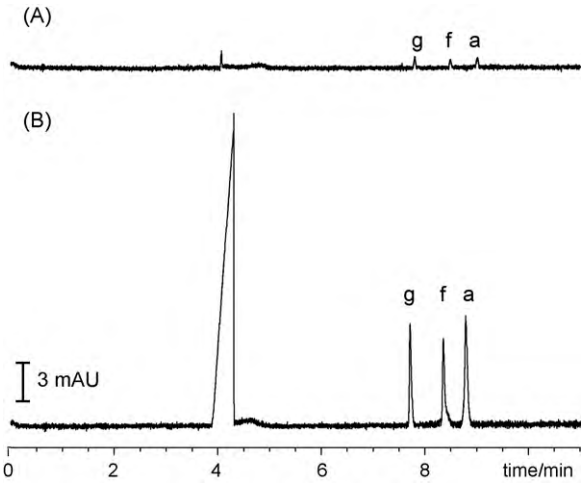

Fig. 6. Enhancement achieved in MSS compared to a regular CE injection. The S (1.9 μg/mL statins: g – gemfibrozil, f – fluvastatin, a – atorvastatin) was prepared using an S matrix that is the same as the BGS which consisted of 50 mM NH_4HCO_3 and 50% methanol (A – regular CE injection) and the S was also prepared in an S matrix that consisted of 9 mM of CTAB and 10 mM NH_4HCO_3 (B – MSS). The injection time was 3 and 30 s at 50 mbar pressure for A and B, respectively.

3.4. Effect of sample matrix injection prior to sample injection

In Fig. 5A, using the optimized S matrix and BGS, small peaks prior to the statin peaks can be seen. A high concentration of each analyte was used to easily visualize the small peaks. These small peaks disappeared in Fig. 5B with the injection of the S matrix prior to S injection. The small peaks, g_1, f_1 and a_1, in front of the tall peaks correspond to the same analytes g, f, and a, respectively. This "anomaly" can be explained by looking at the model in Fig. 1. Some anionic analyte molecules, especially those near the boundary between the S zone and BGS at the anodic side, migrated out of the S zone toward the anode or detector. This was remedied by the injection of a plug of S matrix prior to the S. The micelles in this plug picked-up the anodically moving analytes and thus the disappearance of the small peaks in Fig. 5B and an improved electropherogram profile. Different injection times (5, 10, and 15 s) of the S matrix plug were tried and though the small peaks disappeared, there was no change in the peak heights. The 5 s injection of S matrix was chosen as optimal.

3.5. Enhancement and limit of detection

Fig. 6 shows the enhancement achieved in MSS compared to a regular CE injection. The regular S injection was for 3 s and a concentration of 1.9 μg/mL for each analyte was used with the BGS (50 mM NH_4HCO_3 and 50% methanol) as S matrix. Injections longer than 3 s resulted into broad peaks with the same peak height. This was because there was no concentration effect when the analytes were prepared in the BGS. With the same concentration of each analyte using MSS at 30 s injection, an approximate 10-fold increase in peak height was observed for all three peaks.

3.6. Reproducibility and linearity

The reproducibility ($n = 10$) for intraday migration time and peak height were below 8.0%. Intraday migration time RSD% for g, f, and a, were 1.6, 1.8, and 1.9, respectively. The intraday peak height RSD% for g, f, and a, were 5.7, 7.4 and 4.4. Intraday corrected peak areas were also computed by dividing the peak areas with the corresponding migration time. The intraday corrected peak area RSD% for g, f, and a, were 5.6, 12.5, and 6.0, respectively.

Interday reproducibility for migration time, peak height, and corrected peak area were all acceptable. The reproducibility of interday migration time RSD% for g, f, and a, were 2.3, 2.5, and 2.7. The interday reproducibility of peak height RSD% for g, f, and a, were 4.7, 3.0, and 4.0, respectively. In the case of interday reproducibility of corrected peak area RSD%, for g, f, and a, the values obtained were 5.5, 7.6 and 5.6.

The linearity range studied for the 3 statins was 0.37–18.52 μg/mL. The linearity for the three statins was obtained by averaging the peak heights or corrected peak areas at each concentration. The r^2 using peak heights for g, f, and a, were 0.989, 0.995, and 0.996, respectively. The r^2 using corrected peak heights for g, f, and a, were 0.999 for all cases.

3.7. Evaluation to the analysis of a spiked wastewater sample extract

Water samples from the Hobart sewage plant were spiked with the statins (1 μg/mL), acidified and then extracted with DCM and then centrifuged. It was difficult to get all the DCM without any water being included. At best, only 75% of the DCM was obtained. When water was also in the recovered DCM, the ions left after drying *in vacuo* interfered with the analysis. The peaks for the three statins can be clearly distinguished but they were at the limit of detection although below the limit of quantitation (∼0.4 μg/mL, S/N = 10) (see Supporting Information Fig. S5).

4. Conclusion

A model and theoretical consideration of MSS as well as its application to organic anions using a cationic micelle has been demonstrated. MSS occurs because of the presence of the organic modifier in the BGS. This organic modifier is responsible for the reversal of the effective electrophoretic mobility of the analyte at the MSSB. The ease of reversal is dependent on the retention factor k which then affects the direction of the effective electrophoretic mobility of the analyte, $\mu_{ep}{}^*(a')$, which is composed of two terms: $[1/(1+k)]\mu_{ep}(a')$ and $[k/(k+1)]\mu_{ep}(mc)$. The first and second terms must have opposite signs and the second term must be dominant when in the S zone. When in the MSSB, the first term will predominate when k is of a sufficiently low value resulting to the inversion of the $\mu_{ep}{}^*(a')$ and the accumulation of the analyte at the MSSB. This theory also applies to the MSS of organic cations using anionic surfactants.

The concentrations of the reagents in the BGS and S matrix are important. If there is not enough organic modifier in the BGS, broad peaks result. The concentrations of the surfactant in the S matrix should be optimized. The S matrix and BGS should have relatively similar conductivities such that destacking will not occur. The optimum analysis requires the injection of a short plug of S matrix prior to the injection of the S to scavenge escapee analytes. A 10-fold peak height enhancement factor or a magnitude improvement in concentration detection sensitivity compared to typical injection was achieved with acceptable reproducibility and linearity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.07.077.

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