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Two-step stacking of cationic analytes by field enhanced sample injection and micelle to solvent stacking in capillary electrophoresis

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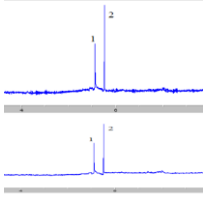
GRAPHICAL ABSTRACT



Electropherogram of blank river water samples. Peak (1) and (2) are unidentified peaks for both wavelengths (a) 195 nm and (b) 210 nm.

ABSTRACT

Capillary electrophoresis (CE) with ultraviolet (UV) detection suffers from a poor concentration sensitivity resulting in a significant obstacle for analysis of part per billion (ppb) levels. Therefore, in this study, a rapid and sensitive capillary zone electrophoresis (CZE) method with field enhanced sample injection and micelle to solvent stacking (FESI-MSS) was developed and validated for the determination of paraquat and bromhexine in river water without sample pre-treatment. The separation was carried out in an uncoated 40 cm × 50 µm fused-silica capillary with an applied voltage of 20 kV. The electrophoretic analysis was performed by 100 mM phosphate buffer with 20% acetonitrile at pH 2.5. Before sample injection, a micellar solution (10 mM sodium dodecyl sulphate; SDS in 80 mM phosphate buffer at pH 2.5) and an organic solvent rich solution (30% acetonitrile) was hydrodynamically introduced into the capillary. The detection wavelength was 195 nm for paraquat and 210 nm for bromhexine. From this study, the sensitivity of FESI-MSS technique compared with normal CZE was 1000-fold. Adaptability to real sample analysis was evaluated using spiked river water samples.

Keywords: *Field enhanced sample injection, micelle to solvent stacking, cationic analytes, capillary electrophoresis*

1. INTRODUCTION

Capillary electrophoresis (CE) have been used recently as a second separation technique after high performance liquid chromatography (HPLC) and gas chromatography (GC) in environmental analysis. This is because of the advantages of CE such as high efficiency, small sample requirement, and short time analysis [1]. Moreover, various separation modes can be used according to the analytes such as capillary zone electrophoresis (CZE) for charged analytes while micellar electrokinetic chromatography (MEKC) for neutral analytes. However, when ultraviolet-visible (UV-Vis) is being used as a detector, the sensitivity of CE is always limited [2-3] because of the small dimensions of CE capillaries, typically 25 – 150 µm i.d. and 40 – 80 cm in length, only very small sample volumes may be loaded onto the column and small detection window [4].

On-line sample pre-concentration or stacking techniques has become a popular approach to improve the poor detection sensitivity of CE and these techniques allowed for the expansion of CE to the real sample applications [4]. For example, field amplified/enhancement, the sample was prepared in a lower conductivity diluent (e.g. 10 × diluted than BGE) and caused the velocity in the sample to be faster than in background electrolyte (BGE). Then, the analytes slowed down at the boundary between the sample and BGE which help them to concentrate. Stacking by field enhancement with hydrodynamic and electrokinetic injection was called field amplified sample stacking (FASS) and field enhanced sample injection (FESI), respectively. While in transient isotachophoresis (t-ITP), the sample ions were squeezed between leading and terminating electrolyte. The samples were concentrated by the additive (e.g. micelle) that penetrated the sample zone during electrophoresis. The concentration effect relied on the analyte's affinity to the additive found in the BGE. In dynamic pH junction or large volume sample stacking (LVSS), a pH difference between the sample and BGS caused a change in the velocity of weakly acidic or basic analytes. Field amplified/enhancement are the simplest stacking techniques while comparing with t-ITP and LVSS. Nevertheless, recent studies have reported that a limitation of FESI is dependence of the sensitivity on the conductivity ratio of the BGE to the sample solution [3].

The combination of stacking mechanism has been developed in order to gain the sensitivity (e.g., up to million fold) and FESI is a common stacking technique that will be used to combine with other stacking due to its ability to introduce more sample ions but less sample diluent into capillary [5]. The combinations of FESI with sweeping or t-ITP have been proven to increase the sensitivity [6]. The FESI with t-ITP combination is known as electrokinetic supercharging [6] while FESI with sweeping combination is the cationic and anionic selective exhaustive injection sweeping. The most recent combination of stacking with FESI is micelle to solvent stacking (MSS) and it has been used for the analysis of various small anionic and cationic molecules. In MSS, the micelle was injected before the sample that dissolved in BGE or organic solvent and the boundary was formed between the sample solution and BGE (or solvent plug). The charge of the analyte should be opposite from the micelle. The direction of the effective electrophoretic mobility in the sample solution zone was dictated by the electrophoretic mobility of the micelle. The micelles transported the analytes to the boundary where the organic solvent reduced the interaction between the analytes and micelles. When a sufficient amount of solvent was present, the direction of the effective electrophoretic mobility was governed by the electrophoretic mobility between micelles and analytes due to their

charges. Therefore, this combination stacking techniques is unique from others because the difference in electric field did not participate in the analyte peak compression process. The amount of sample loaded was increased by FESI while analyte focusing was by MSS only. As a result, the peak height of the analytes have been improved up to thousands fold and low detection limits [5].

In this study, the FESI-MSS technique in CE was developed and validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, reproducibility, and recovery for the determination of paraquat and bromhexine. After validation, this new technique was applied for determination of paraquat and bromhexine in river water.

2. EXPERIMENTAL

Water was purified in a Milli-Q water system and used throughout the analysis. The pH of the BGE was measured using FiveEasy Plus pH Instrument (Mettler Toledo). The capillary zone electrophoresis experiments was carried out on a capillary electrophoresis system, model HP^{3D}CE (Agilent Technologies, Germany), equipped with source, destination and sample vials, power supply, electrode, and on-column diode-array detection (DAD). CE ChemStation Software was utilized for system control, data collection, and data analysis. A fused-silica capillary (Agilent Technologies, Germany) with inner diameter of 50 µm and a total length of 48.5 cm (40 cm effective length) was used. The capillary was thermostatted at 20˚C. New capillaries were conditioned with 1M NaOH for 15 min, followed by ultrapure water for 5 min and background electrolyte (BGE) for 15 min. At the beginning of the day, the capillary was conditioned with 0.1M NaOH for 15 min, followed by ultrapure water for 5 min, and then BGE for 10 min. In between each electrophoretic runs, the capillary was rinsed with 0.1M NaOH, ultrapure water, and BGE for 2, 2, and 5 min, respectively. In typical injection, the hydrodynamic injection (HDI) was used at 25 mbar/6 s. For FESI-MSS, the injection consists of three steps; (1) micellar solution (10 mM SDS in 80 mM phosphate buffer) at 50 mbar/100 s, (2) organic solvent (30% ACN) at 50 mbar/5 s, and (3) sample at 10 kV/150 s.

River water samples

Water samples were collected into 1.0 L amber glass bottles which were acid washed and rinsed in the laboratory with ultrapure water and later, on site, the bottles were rinsed with water sample to ensure no contamination to the water samples. The sampling point was at the raw water intake point of Syarikat Air Johor (SAJ) Water Treatment Plant at Sungai Skudai. The sample were brought back to UTM laboratory and filtered through 0.45 µm glass fibre filters to remove suspended particles. The sample was stored in the dark at 4˚C when not in used.

3. RESULTS AND DISCUSSION

The aim to enhance the sensitivity of CE with UV detector was accomplished when using FESI-MSS technique. This technique provided 215-fold for paraquat and 2534-fold for bromhexine improvements in peak height as shown in Figure 1 and 2.

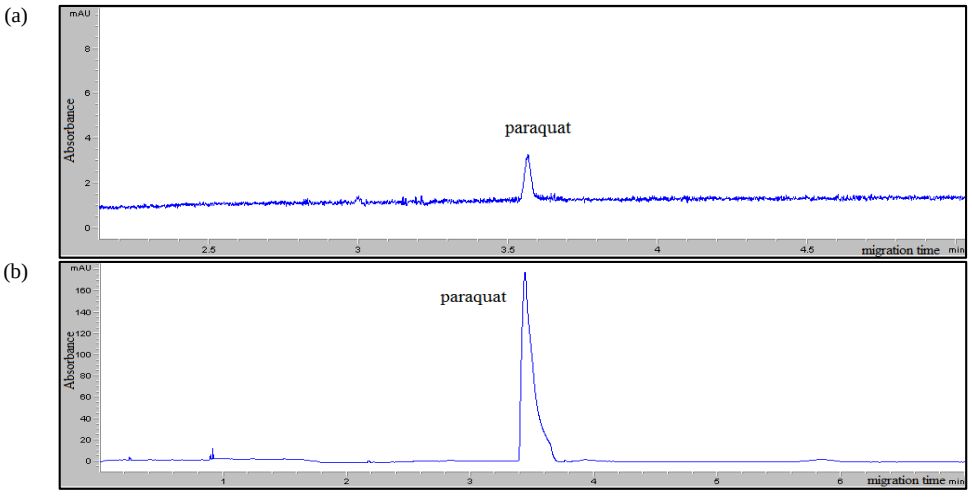


Figure 1 Electropherogram of typical injection in CE for (a) paraquat (5 mg/L) at 195 nm and (b) bromhexine (160 mg/L) at 210 nm. Conditions: fused silica capillary (50 µm i.d. × 40 cm); BGE: 100 mM phosphate buffer with 20% ACN (pH 2.5); separation voltage: +20 kV; capillary temperature: 20 ˚C; injection: 25 mbar for 6 s.

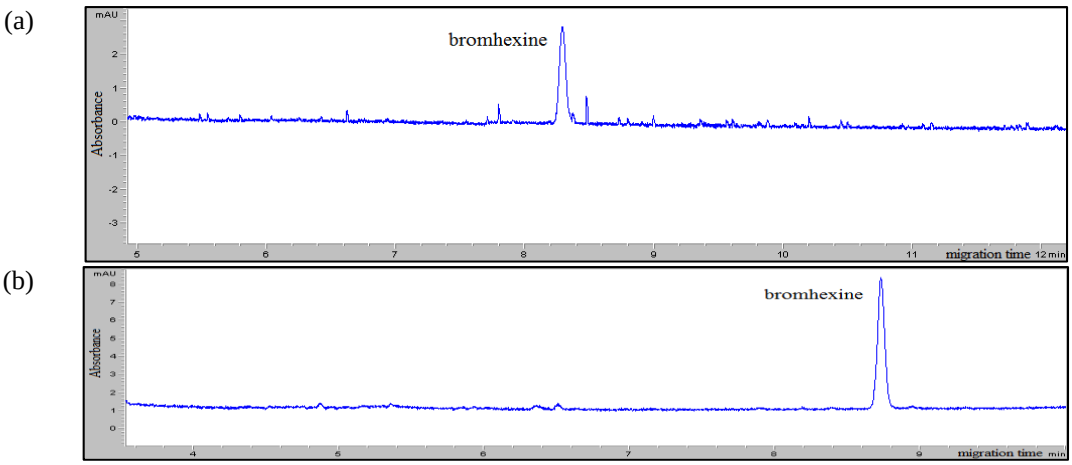


Figure 2 Electropherogram of FESI-MSS technique for determination of (a) paraquat (2 mg/L) and (b) bromhexine (60 µg/L). Injection: 50 mbar for 100 s (micellar solution), 50 mbar for 5 s (30% ACN), and followed by 10 kV for 150 s (sample). Other conditions are same as mentioned in Figure 1.

Figure 3 shows the linearity (r^2) and regression equation obtained using 30 µg/L of paraquat and 40 µg/L for bromhexine. For paraquat, the concentrations at higher than 50 µg/L starts to show big deviations from the linear curve. Therefore, it is better to conduct at below than 50 µg/L in order to obtain better correlation. The calibration curve for paraquat is below the expected response, not passing close to the (0, 0) marks. The same phenomenon is observed for bromhexine calibration curve but at higher expected response. The possible factor that affect these results is the low level of concentration used as linear range. However, from the regression equation, it showed a good correlation.

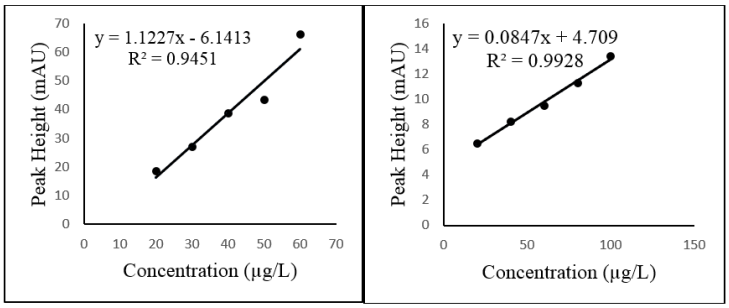


Figure 3: Calibration curves of (a) paraquat and (b) bromhexine

Paraquat and bromhexine were not detected when using this technique. It shows that the river water samples does not contaminate with these contaminate. However, from the blank river water (Figure 4), there are two unidentified peaks appeared at 5.6 and 5.7 mins. These two peaks are able to be identified by CE-MS because MS detection have better sensitivity compared to UV detection.

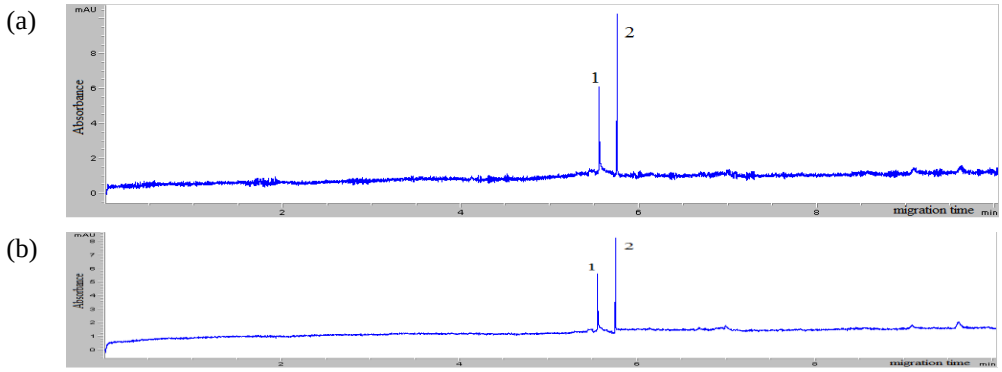


Figure 4: Electropherogram of blank river water samples. Peak (1) and (2) are unidentified peaks for both wavelengths (a) 195 nm and (b) 210 nm.

The blank water samples was spiked with 30 µg/L of paraquat and 40 µg/L of bromhexine. Both compounds gave a low recovery and it shows that there are some interaction between the matrices in water samples with paraquat and bromhexine.

4. CONCLUSION

In MSS, the injection of sodium dodecyl sulphate (SDS) micellar plug prior to the sample solution induced transient micellar phase extraction of cationic analytes in CE, whereas FESI allowed the larger sample loaded into the capillary. MSS was utilised by preparing the organic solvent while sample with low conductivity was prepared for FESI. As a result, the FESI-MSS provided until thousand-fold improvements in peak height when compared with typical injection of CZE in CE. Based on this study, FESI-MSS with CE-DAD shows better sensitivity to bromhexine analysis than paraquat. This technique has been applied for the determination of paraquat and bromhexine in river water samples. It shows that both compounds are absent in this water samples.

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