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

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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

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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.

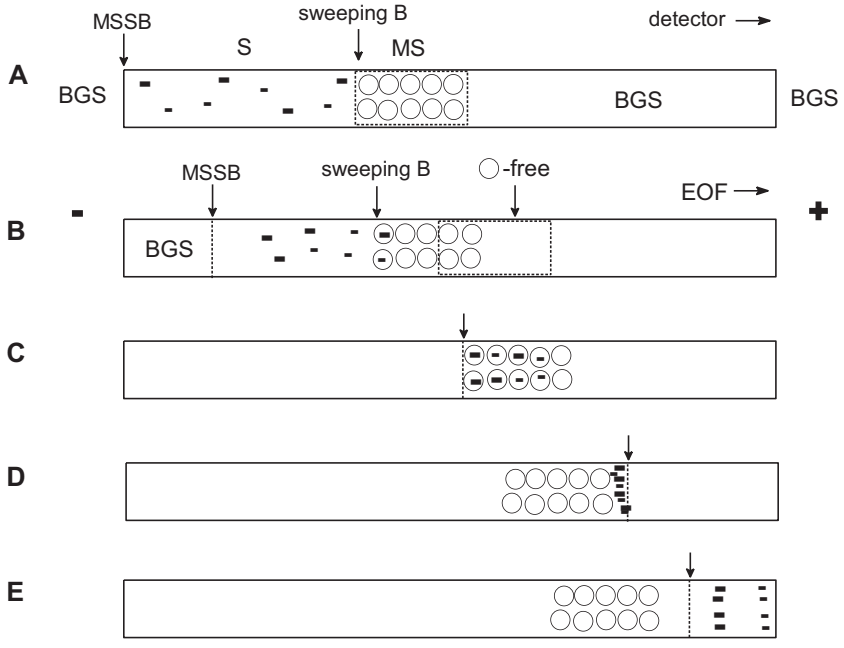

Fig. 1. Model for the two-step stacking by sweeping and MSS of organic anions in co-EOF CZE. (A) The positively charged coated capillary was first conditioned with a BGS that contained an organic solvent. This was followed by injection of the micellar solution (MS) of a cationic surfactant and then a 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.