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

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ABSTRACT

Two-step stacking of organic cations by sweeping and micelle to solvent stacking (MSS) in capillary zone electrophoresis (CZE) is presented. The simple procedure involves hydrodynamic injection of a micellar sodium dodecyl sulfate solution before the sample that is prepared without the micelles. The micelles sweep and transport the cations to the boundary zone between the sample and CZE buffer. The presence of organic solvent in the CZE buffer induces the second stacking step of MSS. The LODs obtained for the four beta blocker and two tricyclic antidepressant test drugs were 20–50 times better compared to typical injection.

1. Introduction

On-line sample concentration or stacking is an important research area to improve the detection sensitivity in capillary electrophoresis (CE) [1–3]. There are several well known stacking techniques, e.g., field amplified or enhanced sample stacking [4,5], transient isotachophoresis (t-ITP) [6–8], sweeping [9,10], and dynamic pH junction [11,12] and the few new ones transient trapping [13], analyte focusing by micelle collapse (AFMC) [14,15] and micelle to solvent stacking (MSS) [16–18].

The sequential use of two stacking techniques, referred to here as two-step stacking had also became popular for small molecules during the last decade [1–3]. For charged analytes, the first step is usually stacking by field amplification followed by a sweeping or t-ITP step. Cation [19] and anion [20,21] selective exhaustive injection–sweeping are two-step stacking techniques where the sample is electrokinetically injected under field amplified conditions and then the concentrated zone is focused further by sweeping prior to separation by micellar electrokinetic chromatography (MEKC) [22]. Other two-step techniques reported in MEKC were the combination of dynamic pH junction and sweeping [23] and of sweeping via borate complexation [24] and sweeping with nonionic micelles [25,26]. In capillary zone electrophoresis (CZE) [27], there is the two-step technique called electrokinetic supercharging [28], where the first step is electrokinetic injection under field amplified conditions and the second step involves the injection of a terminator to induce t-ITP.

Here, initial studies on the two-step stacking of organic cations in CZE by sweeping and MSS, as the first and second stacking steps, respectively are presented. Sodium dodecyl sulfate was used as the anionic micellar phase for sweeping and MSS while methanol was used as the organic solvent additive in MSS and CZE. Beta blocker and tricyclic antidepressant drugs that contain a basic nitrogen group were used as the cationic model test analytes.

2. Experimental

2.1. Equipment

Electrophoresis and stacking experiments were performed on fused silica capillaries of 50 μm i.d. and 375 μm o.d. obtained from Polymicro Technologies (Phoenix, AZ). The total length was 50 cm and the length from the inlet to the detector was 41.5 cm. All electropherograms were obtained with Agilent 3D capillary electrophoresis systems (Waldbronn, Germany) with detection set at 200 nm using the diode array detector. The temperature of the capillary was controlled at 20 °C. Water was purified with a Milli-Q system (Millipore, Bedford, MA). The pH was measured using an Activon Model 210 pH meter (Pennant Hills, NSW, Australia).

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2.2. Reagents and solutions

All reagents (sodium dodecyl sulfate (SDS) for electrophoresis, ammonium acetate (≥98%), USP grade acetic acid, sodium hydroxide (≥0.97%), and HPLC grade methanol) were purchased from Sigma Aldrich (St. Louise, MA). Stock solutions of 100 mM sodium dodecyl sulfate and 250 mM ammonium acetate (pH 6.2) were prepared every two weeks in purified water. Background solutions (BGS) were prepared each day by dilution of the ammonium acetate stock solution with appropriate amounts of purified water and methanol. The measured pHs of the BGSs with methanol were around 7. Micellar solutions (MS) and sample matrices were prepared each day by dilution of the SDS and/or ammonium acetate stock solutions with purified water. The pH of the MS or sample matrices was 6.3. All solutions were filtered through 0.45 μm filters from MicroScience (Dassel, Germany) prior to use. All the samples of the highest purity available were obtained from Sigma Aldrich (St. Louise, MA). Stock solutions of the beta blocker (alprenolol, propranolol, nadolol, and labetalol) and tricyclics antidrepressant (nortriptyline and clomipramine) drugs were prepared in methanol at a concentration of 10 mg/mL each. Care should be taken when handling these chemicals. Sample solutions (S) were prepared by appropriate dilution of the sample stock aliquots with the different matrices. The composition of each matrix is described in the text or figures.

2.3. General electrophoresis procedure

The capillary was conditioned (1 bar pressure) prior to use with 0.1 M NaOH (20 min), water (10 min), methanol (5 min), water (5 min), and finally BGS (10 min). The S and MS were injected into the capillary farthest from the detector end using pressure (50 mbar). Voltage (20 kV) was applied at positive polarity with the BGS at both sides of the capillary, until all peaks were detected. The capillary was conditioned, between consecutive analysis, with 0.1 M NaOH (1 min), purified water (1 min), and finally BGS (5 min). Other conditions are specified in the text or figures.

3. Results and discussion

3.1. Two-step stacking model

Fig. 1 shows the model for the two-step stacking of cationic analytes by sweeping followed by MSS using anionic SDS micelles in CZE. The movement of the cations (+), anionic SDS micelles (circles), and micelle to solvent stacking boundary (MSSB) in the presence of a homogenous electric field is illustrated. The electrokinetic velocities are positive and negative when movement is directed toward the cathode and anode, respectively. The velocity of the MSSB is the same as the electroosmotic flow (eof). The overall velocities of all zones are positive due to the strong eof. In the starting situation (Fig. 1A), the MS and the sample solution (S) both having similar conductivity as the background solution (BGS) are injected. Stacking and destacking effects due to differences in conductivity are absent. Upon application of voltage (Fig. 1B), the micelles from the anodic side of the MS zone sweep the cations [9]. This continues until all the cations are swept by the micelles. The direction of the electrophoretic mobility of the cations shift from positive to negative and the micelles transport the cations to the MSSB. The cations in the presence of SDS micelles depicted as + inside the circles in Fig. 1 have a negative effective electrophoretic mobility. When the micelles reach the MSSB (Fig. 1C), the second zone focusing due to MSS occurs. The effective electrophoretic mobility of the cations reverses from negative to positive due to the presence of organic solvent in the BGS. The analyte cations in the S bound to the micelles

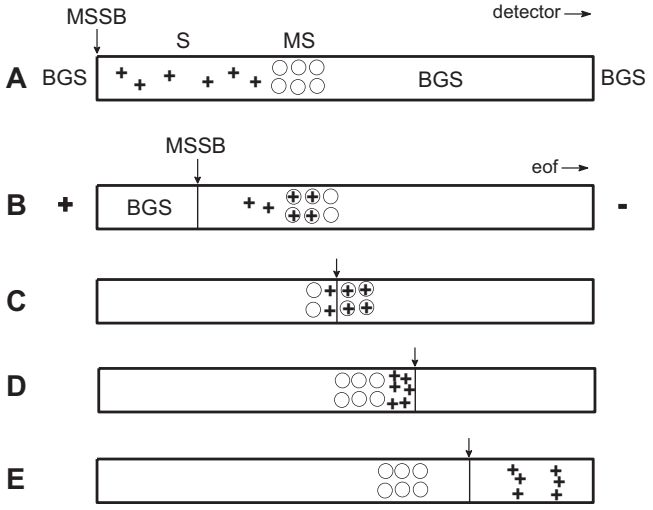

Fig. 1. Two-step stacking of cationic analytes by sweeping and MSS model. (A) The capillary is conditioned with the background solution (BGS) that contains an organic solvent. A MS is injected followed by the sample solution (S). The micelle to solvent stacking boundary (MSSB) is found at the inlet end of the S zone. (B) A voltage is applied with the anode and cathode at the inlet and outlet ends, respectively. A homogenous electric field across the capillary is assumed. The electroosmotic flow (eof) is directed toward the cathode. The MSSB moves with the velocity of the eof. The negatively charged SDS micelles (circles) sweep the cations (+). The effective electrophoretic velocity of the cations in the presence of SDS is directed to the anode. (C) The SDS micelles transport the cations to the MSSB. When the SDS micelles reach the MSSB, the second step of MSS occurs where the effective electrophoretic velocity of the cations reverses to the direction of the cathode. (D) The cations accumulate at the MSSB, and this stacking occurs until all the micelles from the injected MS zone traversed the boundary. (E) The two-stepped stacked cations separate by virtue of CZE and move toward the detector.

were electrophoretically attracted to the anode. Upon reaching the MSSB containing the organic solvent, the affinity of the analytes to the micelles were significantly lowered. This causes reduction of the retention factor k to the extent that the cations migrate toward the cathode and experienced an electrophoretic reversal resulting to analyte accumulation at the MSSB [16,18]. A theoretical consideration of MSS was given in ref. 18. The stacking process is complete when all the micelles traversed the MSSB (Fig. 1D). The two-step focused cations then separate by virtue of CZE and migrate to the detector (Fig. 1E).

3.2. Experimental verification of the two-step stacking

Fig. 2 shows electropherograms obtained from typical (A), non-stacking (B), one-step stacking (C), and two-step stacking (D) injections of the test beta blocker drugs in CZE. The beta blockers were cationic at the pH of the BGSs (pH ~ 7). The pKa of alprenolol, propranolol, nadolol, and labetalol is 9.34, 9.25, 9.00, and 8.8, respectively [29]. The effects of stacking and destacking due to conductivity differences were negligible since the measured CE currents obtained for the MS and/or S were within 60–100% of the current obtained for the pertinent BGS. A homogenous electric field was provided to remove stacking or destacking effects, and thus only demonstrated the effects of sweeping and MSS.

Fig. 2A is from a typical injection (3 s at 50 mbar) of the beta blockers prepared in the BGS. Fig. 2B is obtained from a long injection (60 s at 50 mbar) of the samples in Fig. 2A that was diluted 20 times with the BGS. Broad peaks are observed under these non-stacking conditions. Fig. 2C is from a long injection similar to Fig. 2B but the samples were diluted in 25 mM ammonium acetate and the BGS contains only 30% methanol. A short plug (15 s at 50 mbar) of