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Short communication

Three-step stacking by field-enhanced sample injection, sweeping, and micelle to solvent stacking in capillary electrophoresis: Anionic analytes

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ABSTRACT

Three-step stacking by field-enhanced sample injection (FESI), sweeping, and micelle to solvent stacking (MSS) in co-EOF capillary zone electrophoresis (CZE) is presented for anionic analytes. Long FESI produced an overloaded stacked zone of analytes (four model penicillins). Sweeping of the FESI zone was by electrokinetic injection of cetyltrimethylammonium bromide (CTAB) micelles. MSS was by short injection of 60% methanol that released the swept analytes from CTAB micelles. The sensitivity enhancement factors were 146–279 and 519–954 for conductivity ratio of 10 and 100, respectively. The SEF enhancement factors (factor = SEF from three-step stacking/SEF from FESI) were 16–32 and 6–10, correspondingly. The LODs were between 6.6–13.2 ng/mL, repeatability (intraday and interday) was %RSD $\leq$ 5.4%, and linearity was $R^2 \geq 0.998$. Application to real sample was investigated using fortified plasma after liquid–liquid extraction.

1. Introduction

Sample concentration prior to capillary electrophoresis (CE) with UV detection is required to compensate for the poor detection sensitivity [1]. On-line sample concentration or stacking techniques have been developed and the details of these techniques can be found in numerous reviews [2–4]. A popular stacking technique is field-enhanced sample injection (FESI) where the sample in a lower conductivity solution compared to the background solution (BGS) is injected electrokinetically into the capillary. The maximum sensitivity enhancement factor (SEF) that can be reached with FESI is dictated by the conductivity ratio of BGS to sample solution (maximum SEF = conductivity ratio = conductivity of BGS/conductivity of sample) [5].

We recently reported a sequential three-step stacking strategy in order to increase the actual SEF of FESI [6]. Using model cationic analytes in capillary zone electrophoresis (CZE), we refocused the FESI produced overloaded sample zone using sweeping with sodium dodecyl sulfate (SDS) micelles [7] and micelle to solvent stacking (MSS) with SDS micelles and acetonitrile [8]. SEF values (calculated by dividing the peak height from stacking by the peak height from typical injection, and then multiplied by the dilution factor) from the three-step stacking were up to 891, 6463, and 6499 for conductivity ratio 10, 100, and 1000, respectively. To quantify the improvement produced by the sequential stacking strategy on FESI, we have defined the SEF enhancement factor which is equal to the SEF from three-step stacking divided by the SEF from FESI. The SEF enhancement factors of 11–161 were significant.

In this communication, we demonstrate this new three-step stacking approach to anionic analytes using penicillins as model analytes. The penicillins were chosen since they are commonly used antibiotics either in human and veterinary medicine [9,10]. Penicillin G, oxacillin and ampicillin are usually administrated parenterally (I.V. or I.M.). The concentration of these drugs in plasma a few minutes after bolus or infusion is in the range of 8–45 μg/mL. Amoxicillin is administrated orally and plasma concentration is about 8 μg/mL. The half-life of these penicillins is short (<1 h) and concentration in the plasma decreases dramatically (www.fda.gov). The FESI overloaded sample zone was refocused using cetyltrimethylammonium bromide (CTAB) for sweeping [11] and CTAB and methanol (MeOH) for MSS [12]. The mechanism of stacking was presented and confirmed. The SEFs and SEF enhancement

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factors were calculated. Analytical figures of merit such as linearity, LOD (S/N = 3), and repeatability (intraday and interday) were studied. In addition, spiked plasma samples were used to investigate sample matrix effect.

2. Experimental

The reagents, solutions, preparation of plasma samples, and coating and capillary conditioning procedures are described in Supp. info. The BGS was 150 mM ammonium acetate pH 9. For typical injection, the sample solution was injected at 50 mbar for 6 s. For three step-stacking, the sample was injected at −10 kV (anode at detector side), then micellar solution was injected at 10 kV (cathode at detector side), and finally, 60% MeOH was introduced at 50 mbar for 6 s. Separation was performed at −15 kV (anode at detector side). The identification of the analytes was by spiking the sample with penicillin standards. Other CZE conditions are specified in the text or figures.

3. Results and discussion

3.1. Three step stacking model

Fig. 1 presents the mechanism of three-step stacking (long FESI (A), sweeping (B), and MSS (C)) of anionic analytes in CZE. In Fig. 1A, the anionic compounds were injected electrokinetically at negative polarity. The analytes concentrated at the FESI boundary (FESIB) due to differences in analytes electrophoretic velocity at the BGS and sample zone. The long FESI created a long stacked zone of analytes (grey zone at right of the FESIB). The sample diluent was also introduced into the capillary by the EOF (light zone at left of the FESIB). In Fig. 1B, the positively charged micelles injected at positive polarity swept the FESI stacked anionic analytes at the sweeping boundary (SB). The sample diluent was pumped out of the capillary by EOF. In Fig. 1 C1, the MSS boundary (MSSB) was created between the MeOH (blue zone) and swept analyte zone with micelles (dark zone). In Fig. 1 C2, the separation voltage at negative polarity was applied that caused migration of the micelle bound analytes to the MSSB. When micelles reached the MSSB, they collapsed and released the transported anionic analytes. Finally, stacked analytes were separated by CZE when all the introduced CTAB micelles migrated through the MSSB.

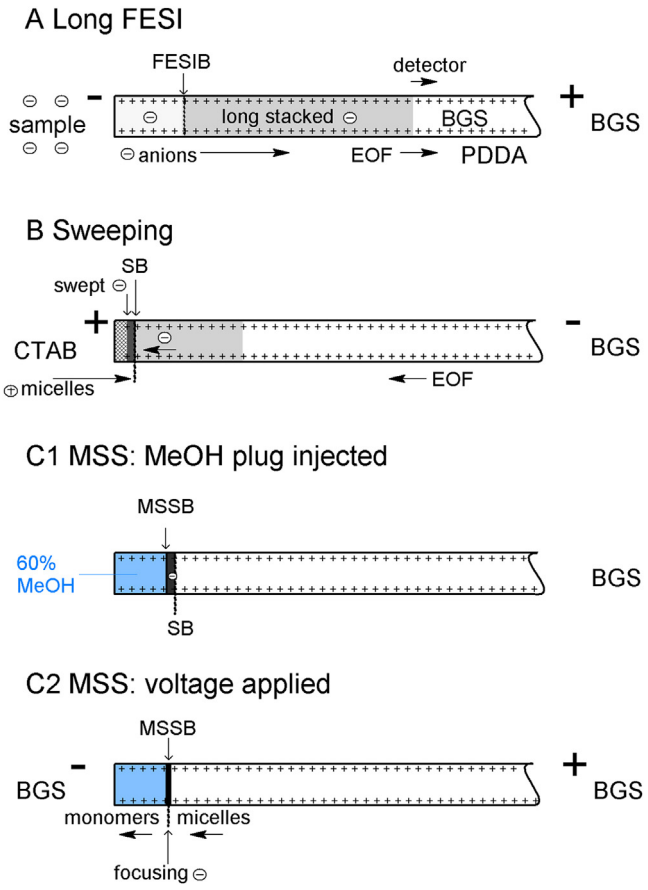

Fig. 1. Three-step stacking strategy by FESI, sweeping, and MSS of anionic analytes in CZE. Explanation in the text. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

3.2. Proof of concept

Fig. 2 shows the electropherograms achieved from typical injection (A), FESI (B), FESI and sweeping (C), and three-step stacking (D) of anionic penicillins in CZE. In Fig. 2B–D, the samples were prepared in 1/10 BGS to obtain field-enhanced conditions. The concentration of analytes in Fig. 2A was 10× higher than in Fig. 2B–D.

All analytes co-migrated and were detected as a broad peak in the FESI (Fig. 2B). FESI and sweeping also gave one broad peak but of a much shorter peak height (Fig. 2C). We did not expect this result since the swept analytes should have migrated out of the capillary during CZE. This broad peak was then attributed to the unbound analytes that migrated to the detector at negative polarity. We also observed the detection of bromide (injected by diffusion) when sweeping with CTAB was performed as in Fig. 2C. This was verified by CZE of the micellar solution where the inlet end of the capillary was dipped (no pressure or voltage) for 90 s. Fig. 2D demonstrated the concept of the three-step stacking of anionic analytes where sharp peaks were obtained for the tested penicillins.

The SEF was calculated by dividing peak height obtained from stacking by peak height from typical injection and then multiplied by the dilution factor. The SEFs for the conditions in Fig. 2D were between 86–139.

3.3. SEF determination for FESI

The maximum FESI time was studied for conductivity ratio 10, 100, and 1000 (see Supp. info. for more details). This was to obtain the SEF of FESI. The maximum FESI time that gave well separated peaks for all analytes using conductivity ratio 10 and 100 was 20 s. For conductivity ratio 10, the SEF for penicillin G, oxacillin, ampicillin, and amoxicillin was 9, 11, 9, and 9, respectively. For

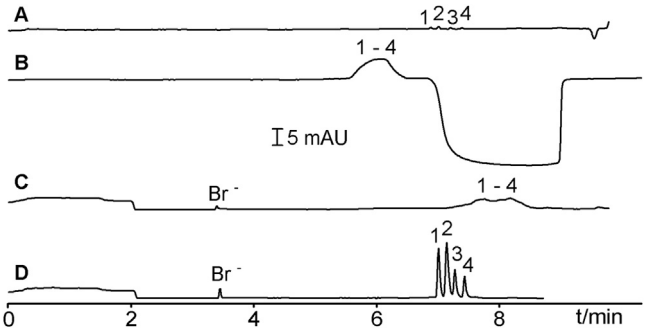

Fig. 2. Proof of concept—three-step stacking with 1/10 diluted BGS (conductivity ratio 10). Electropherograms obtained from typical injection (A), FESI (B), FESI and sweeping (C), and three-step stacking (D) of anionic analytes in CZE. The concentration of analytes in A was 10 μg/mL in BGS. The concentration of analytes in B-D was 1 μg/mL in 1/10 diluted BGS. FESI was 60 s. Sweeping was 90 s with 15 mM CTAB in the inlet. MSS was injection of 60% MeOH. Peak identification: penicillin G (1), oxacillin (2), ampicillin (3), and amoxicillin (4).