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Thousand fold concentration of an alkaloid in capillary zone electrophoresis by micelle to solvent stacking

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ABSTRACT

In this paper, the co-solvent of methanol–water was used to facilitate the sodium dodecyl sulfate (SDS) micelles collapse, thereby inducing the on-line sample focusing technique of micelle to solvent stacking (MSS). To demonstrate this stacking method, the mechanism of micelles collapse in co-solvent was discussed. The details of the required conditions were investigated and the optimized conditions were: running buffer, 20 mM H_3BO_3 and 20 mM NaH_2PO_4 solution (pH 4.0); micellar sample matrix, 20 mM SDS, 20 mM H_3BO_3 and 20 mM NaH_2PO_4 solution (pH 4.0); co-solvent buffer, 20 mM H_3BO_3 and 20 mM NaH_2PO_4 in methanol/water (90:10, v/v). The validity of the developed method was tested using cationic alkaloid compounds (ephedrine and berberine) as model analytes. Under the optimized conditions, this proposed method afforded limits of detection (LODs) of 0.5 and 1.1 ng/mL with 300 and 1036-fold improvements in sensitivity for ephedrine and berberine, respectively, within 15 min.

1. Introduction

Capillary electrophoresis (CE) has been developed as a powerful analytical technique. Its advantages include high efficiency, short analysis time, and small sample requirements. It has been widely used in different areas of chemistry and biochemistry. However, because of the short optical path length across the capillary, one of the major limitations of CE is the low detection sensitivity with absorbance detection for trace analytes. Over past decades, a number of approaches have been developed to improve the detection sensitivity of CE, these investigations include extending the detection path length with a bubble cell [1] or z-shape capillary [2], using powerful detectors like laser-induced fluorescence, off-line and on-line sample preconcentration. The on-line sample preconcentration is based on focusing a large volume of injected sample to a minimum volume inside the capillary, requiring no modification of current commercial instrument. Therefore, on-line sample preconcentration is a useful technique to improve the concentration sensitivity.

Over the last decades, hundreds of articles were published on on-line sample stacking in CE [3,4], including field amplified sample stacking [5–10], transient isotachophoresis (tr-ITP) [11–13], dynamic pH junction [14,15] and transient moving reaction boundary (tMCRBM) [16] as well as sample sweeping [17–29], etc. Each method relies on creating difference between the background electrolyte (BGE) or buffer solution zone and the sample zone or a special inserted zone for enrichment. For example, the field amplified sample stacking relies on the changes in analytes' electrophoretic velocities caused by the mismatch in concentrations or electrical conductivities between the sample solution and the separation solution. tr-ITP uses an imposed electrophoretic mobility gradient to create concentrated analyte zones with nondispersing interfaces. The stacking effect of dynamic pH junction is based on pH discontinuity between the sample and the electrolyte, which causes significant changes in ionization states or electrophoretic velocities of the analytes. tMCRBM relies on non-steady-state isoelectric focusing. Sweeping is based on chromatographic partition, complexation or any other interaction between the analytes and additives, so the additives 'sweep' the long sample band into a narrow zone.

Recently, a new on-line focusing method termed as analyte focusing by micelle collapse (AFMC), has been developed by Quirino and Haddad [30]. Neutral analytes are associated to the sodium dodecyl sulfate (SDS) micelles in the sample matrix which contains

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anions of high electrophoretic mobility, and the running buffer contains high concentration of micelles but low concentration of the same anions in the sample matrix. When voltage is applied, the anionic micelles transport the associated sample molecules from the high conductivity zone to the low conductivity zone. Micelle dilution occurs when the micelles and electrolyte anions move into a dilution zone. Under the suitable conditions, the concentration of SDS falls below its critical micelle concentration (CMC), and the micelles collapse at the boundary of the two zones, thereby releasing and focusing the loaded molecules. This technique has been successfully applied to on-line concentration of some neutral analytes and the detection sensitivity was increased by one to two orders of magnitude [31–33]. Thereafter, micelle to solvent stacking (MSS) [34,35] has been introduced by the same group. In MSS, the sample was prepared in a micellar solution without organic solvent, the separation solution was modified by an organic solvent. The focusing was based on change in the effective electrophoretic mobilities of the analytes at the boundary between the micellar sample solution and the separation solution.

The change in electrophoretic mobility due to the presence of organic solvent in MSS [35] can also be caused by micelle collapse. This was demonstrated by Liu et al. [36], in their report, the sample was prepared in a 8.0 mM SDS micellar matrix, the running buffer was 75 mM H_3PO_4, 2% (v/v) Tween 20, 5% (v/v) methanol buffer, and a section of trapping solution composed of 50 mM H_3PO_4, 55% ethanol was inserted between the sample solution and the running buffer. The analytes change their electrophoretic mobilities in the trapping solution when released by SDS micelles collapse. After focused by MSS, the analytes were separated via micellar electrokinetic chromatography (MEKC). This technique afforded 113 and 123-fold improvements in the detection sensitivity for tetrandrine and fangchinoline, respectively. In this paper, methanol–water co-solvent was applied to induce micelles collapse, thereby leading to MSS. The micelles collapse in co-solvent and the required conditions were discussed in detail. Using the proposed MSS-CZE, good concentration sensitivity enhancements were obtained for the two test alkaloids (berberine and ephedrine).

2. Experimental

2.1. Instrumentation

All capillary electropherograms were recorded on a Beckman P/ACE MDQ system (Fullerton, CA), equipped with a diode array UV detector (190–600 nm). Data acquisition and instrument control were carried out using 32 Karat software (version 7.0). Electrophoresis was performed in fused silica capillaries of 50 μm i.d. and 375 μm o.d. obtained from Handan Xinnuo Fiber Chromatogram Co., Ltd. (Handan, China). All capillaries were 60.2 cm long with an effective length of 50.0 cm, and were thermostated at 18 °C.

2.2. Chemicals and reagents

All solvents and reagents were of analytical grade and were used without further purification. Berberine and ephedrine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Sodium dihydrogen phosphate, boric acid, methanol, ethyl acetate and sodium dodecyl sulfate (SDS) were products of Tianjin Chemical Reagent Factory (Tianjin, China). Redistilled water was used throughout.

2.3. Preparation of solutions and samples

Stock solutions of 0.4 M SDS, 0.4 M NaH_2PO_4, 0.4 M H_3BO_3 and 0.1 M H_3PO_4 were prepared in redistilled water. Running buffer was the mixture solution of 20 mM H_3BO_3 and 20 mM NaH_2PO_4 (pH 4.0), prepared by diluting the stock solutions of NaH_2PO_4 and H_3BO_3 with redistilled water. The micellar sample matrix comprised of 20 mM SDS, 20 mM H_3BO_3 and 20 mM NaH_2PO_4 solution (pH 4.0), prepared by diluting the corresponding stock solutions with redistilled water. pH was adjusted with 0.1 M H_3PO_4 using a PHS-3B pH meter (Shanghai Precision & Scientific Instrument Co., Ltd.). Co-solvent buffer (20 mM H_3BO_3 and 20 mM NaH_2PO_4 solution in methanol/water (90:10, v/v) was prepared by diluting 0.5 mL 0.4 M NaH_2PO_4 and 0.5 mL 0.4 M H_3BO_3 to 10 mL with pure methanol. The conductivities of the three solutions are determined by measuring the CZE electric current values using a CE instrument. The stock solutions of 0.50 mg/mL ephedrine and 0.50 mg/mL berberine were prepared in methanol/water (10:90, v/v) and stored in refrigerator at 4 °C. The standard test sample solutions at various concentrations were prepared by appropriate dilution of the stock solution with the micellar sample matrix.

Fresh urine was collected from a healthy volunteer, after frozen in a refrigerator overnight, the urine was unfrozen at room temperature and centrifuged at 1200 rpm for 3 min the supernatant was collected. Spiked urine samples at various concentrations were prepared by appropriate addition of stock solutions of 0.50 mg/mL ephedrine and 0.50 mg/mL berberine to 1.0 mL of the supernatant, which was extracted with 2.0 mL ethyl acetate three times, the ethyl acetate layer was collected and evaporated at 60 °C to dryness under nitrogen protection. Then the sample solution was obtained by dissolving the residues with 1.0 mL micellar sample matrix. All the sample solutions were filtered through a 0.45 μm syringe filter prior to CE experiments.

2.4. Procedures

Prior to use, new capillary was conditioned by flushing at 20.0 psi (1.0 psi = 56894.76 Pa) sequentially with methanol for 10 min, redistilled water for 3 min, 1.0 M NaOH solution for 20 min, redistilled water for 3 min, and running buffer for 20 min, and finally, equilibrated at 25 kV with running buffer for 60 min. At the beginning of each run, the capillary was rinsed at 20 psi sequentially with redistilled water (2 min), 0.5 M HCl (2 min), 0.1 M NaOH solution (2 min), redistilled water (2 min), and running buffer (3 min). Sample and co-solvent buffer introduction were facilitated by applying a certain pressure for a period of time. The approximate injecting length of sample plug and that of co-solvent buffer plug were calculated using BACKMAN EXPERT software.

3. Results and discussion

3.1. Design of SDS micelle collapse

As reported that the CMC is known to change in organic solvent [37], and as presented by Palepu et al. [38], the log(CMC) of surfactants in the solvent mixtures follows Eq. (1):

$$\log(\mathrm{CMC}) = \log(\mathrm{CMC})_{\mathrm{water}} + KC \quad (1)$$

where C is the solvent/water ratio in wt%, K is a constant and CMC is quoted in mol/L.

CMC at different solvent/water ratio was determined by CE method [39], the results were listed in Table 1. When log(CMC) was plotted against C, a straight line was obtained. The slope of the line was the K, which was equal to 0.02. From Table 1, it is obvious that the CMC increases with the increase in organic solvent content. The conclusion is in good agreement with previous reports that at higher organic solvent contents, the CMC is markedly increased, and the addition of significant amounts of organic solvent will likely cause the micelles to disintegrate [40]. Therefore,