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Short communication

Micelle to trapping solution stacking in micellar electrokinetic chromatography

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ABSTRACT

An analytical strategy micelle to trapping solution stacking (MSS) was developed in acidic buffer in micellar electrokinetic chromatography (MEKC). The stacking mechanism is based on the transport, release, capturing of molecules bound to micelle carriers that are made to collapse into trapping solution (TS) to serve as the medium to contain and stacking the analytes. Tetrandrine and fangchinoline were selected as model mixture using sodium dodecyl sulfate (SDS) micelles as carrier to demonstrate this stacking method. The experiments by MSS-MEKC were carried out and further compared with those by normal MEKC. The results reveal that 113–123-fold improvements in the detection sensitivity was obtained for the analytes, and separation and determination of tetrandrine and fangchinoline in *Stephaniae tetrandrae* S. Moore and Fengtongan capsules were finished under optimum conditions using the sample matrix containing 8.0 mM SDS and TS containing 50 mM H_3PO_4–55% (v/v) ethanol.

1. Introduction

In capillary electrophoresis (CE), a smaller inside diameter (ID) capillary is preferred for highly efficient separations in a shorter time by applying higher voltages [1]. However, the relatively poor concentration sensitivity associated with the limited optical path length for online ultra-violet (UV) detection and the small amount of sample loaded into the capillary are most often demand further efforts for improvement [2,3]. A number of strategies have been developed to improve the sensitivity of CE through the use of online concentration. These include field amplified sample stacking [4–7], large-volume sample stacking [8,9], isotachophoresis [10,11], sweeping [12,13], transient-trapping [14], pH-mediated stacking [15], dynamic pH junction [16], the transient moving chemical reaction boundary method [17] and so on. Each method relies on specific modification of the composition of electrolyte relative to the background electrolyte (BGE) used for separation. In general, sweeping is effective for both charged and neutral analytes while the other techniques work for charged analytes only. Analyte focusing by micelle collapse (AFMC) was first introduced by Quirino in 2008 [18]. And the operating parameters that affect the performance of AFMC to neutral analytes in normal migration micellar electrokinetic chromatography (MEKC) are examined [19]. Recently, micelle to solvent stacking (MSS) was introduced by Quirino, the focusing effect relies on the reversal in the effective electrophoretic mobility at the boundary zone between the micellar matrix and the BGE modified with organic solvent [20]. However, the AFMC and MSS have not been applied to the analysis of real world samples with UV detection. In this paper, trapping solution, serve as the medium, was added between sample solution and BGE and expanding applying scope of MSS. Our goal in this present study was the development of an analytical strategy based on MSS for the simultaneous separation and sensitive determination of analytes in real samples. Tetrandrine and fangchinoline were selected to provide a model mixture to demonstrate the feasibility of this stacking method.

2. Experimental

2.1. Apparatus

All CE experiments were performed on a CL1030 high-performance CE apparatus with a UV detector (Beijing Cailu Instrumental Co., Beijing, China). Uncoated fused-silica capillaries purchased from Yongnian Optical Fiber Factory (Hebei, China) were used. The dimensions of the capillary are 50 cm × 50 μm I.D. × 375 μm O.D. The effective length of the capillary is 41.5 cm from the injection end of the capillary. The data acquisition was carried out with an HW-2000 Chromatography Workstation (Shanghai Qianpu Software Company, Shanghai, China). UV detection was carried out at 214 nm. Samples were introduced into the capillary by hydrodynamic injection, where the sample vial was raised by 15.5 cm. A PH-3C acidity meter (Shanghai Hongyi Instrument Co., Ltd., Shanghai, China) was used for the pH measurement and the pH was apparent pH (pH*).

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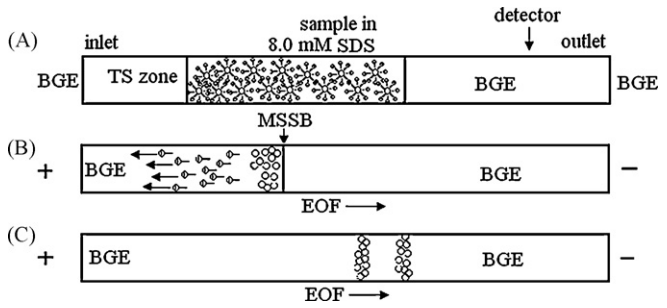

Fig. 1. Transport, release, accumulation and separation of analytes in MEKC by during and after MSS. The MSSB is the micelle to solvent stacking boundary. (A) hydrodynamic injection of analytes prepared in a 8.0 mM SDS micellar matrix and TS in turn after conditioning the capillary with BGE; (B) voltage is opened, the surfactant micelles migrate rapidly into TS zone and then collapse, thereby releasing and accumulating the transported molecules and (C) focused bands separate by virtue of MEKC.

Before use, the new capillary was flushed with 100 mM sodium hydroxide for 30 min and then with redistilled water for 10 min. At the beginning of each working day, the capillary was flushed sequentially with distilled water (5 min), 100 mM NaOH (5 min) and distilled water (5 min), followed by running buffer (5 min). Simultaneously the CE instrument was warmed up until a stable baseline was achieved. Between consecutive analysis, the capillary was rinsed for 1 min with fresh buffer. The column was left filled with distilled water overnight.

2.2. Chemicals and reagents

Tetrandrine and fangchinoline were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, China. The standards were used as received. The crude drug of *Stephaniae tetrandra* was purchased from local drug stores. Fengtongan capsule (brand name: Yinnuoke) was purchased from Changchun Yinnuoke Pharmaceuticals Co., Ltd (Changchun, China). Fengtongan capsule (brand name: Weifengtong) was purchased from Siping City Jite Pharmaceuticals Co., Ltd (Siping, China). Ethanol and phosphoric acid were purchased from Guangzhou Chemical Plant (Guangzhou, China). Methanol was purchased from Tianjin Fuyu Fine Chemical Co. Ltd (Tianjin, China). Sodium dodecyl sulfate (SDS) and polyoxyethylene sorbitan monolaurate (Tween 20) were purchased from Guangdong Guanghua Chemical Factory Co., Ltd (Guangzhou, China). All chemicals were of analytical reagent grade and were used as received. All solution and buffer were made in distilled water.

2.3. Solutions preparation

Standard stock solutions (500 μg/mL) of tetrandrine and fangchinoline were prepared in 50% (v/v) aqueous ethanol. The solutions at various concentrations were prepared by appropriate dilution of the stock solution with 50% (v/v) aqueous ethanol for 5 s hydrodynamic injection. The standard solutions at various concentrations were prepared by appropriate dilution of the stock solution with SDS (100 mM)—distilled water. The running buffer was 75 mM H_3PO_4–2% (v/v) Tween 20–5% (v/v) methanol buffer (pH* 3.0). The buffer was prepared daily from stock solution of 1 M H_3PO_4, methanol and Tween 20, and then adjusted to the desired pH using 2 M NaOH. All solutions were filtered through 0.45 μm syringe filters before use. *S. tetrandra* (1 g) and Fengtongan capsule (five capsules) were extracted by ultrasonic treatment with ethanol (each 10 mL) for 1 h, and filtered through 0.45 μm syringe filters, and then distilled water of equal volume was added to the filtrate. Sample solutions were prepared by appropriate dilution with the distilled water and 0.1 M SDS. The sample matrix tested contained a constant concentration of 8.0 mM SDS. All solutions were filtered through 0.45 μm syringe filters before use.

3. Results and discussion

3.1. Focusing theory

In the current work, TS is composed of 50 mM H_3PO_4–55% ethanol wherein the sample is captured with the aid of SDS micelles in sample solution. In this case, we introduce MSS online concentration technique performed in 75 mM H_3PO_4–2% Tween 20–5% methanol (pH* 3.0) buffer solution. Analytes were prepared in 8.0 mM SDS solution. A simple model to describe the stacking process is developed (see Fig. 1). Firstly, the capillary was filled sequentially with BGE (without SDS), sample matrix (8.0 mM SDS), TS (without SDS) followed by BGE from the capillary inlet using hydrodynamic injection (Fig. 1A). Secondly, when voltage is applied from the anodic inlet toward the cathodic outlet, the TS moves toward the cathode with same velocity of electroosmotic flow (EOF). The sample molecules inside micelles SDS move toward the anode depending on micelles' negative charge, and the micelle concentrations are slightly above the critical micelle concentration (CMC). The SDS micelles are continuously collapsed into TS zone where the concentration of the SDS micelle drops below its CMC, thereby releasing and accumulating the transported analytes (Fig. 1B). Lastly, the analytes are positively charged in acidic pH, and separated in the MEKC mode (Fig. 1C). At moderate and steady sample concentration, good peak shape and repeatability were obtained under acidic conditions. The CMC for Tween 20 in pure water is reported to be 0.06 mM [21], in this study, although the presence of methanol in BGE may change CMC significantly, it can be reasonably estimated that 2.0% (v/v) Tween 20 (almost 26 mM) is far exceeding its CMC, so Tween 20 micelles exist predominantly in the BGE solution, leading to the separation mode should be MEKC. The result of the experiment showed there is no sweeping as a second preconcentration, and tetrandrine and fangchinoline almost overlapped without Tween 20 in BGE. This is proved by comparing electrochromatograms using a separation solution with and without Tween 20.

Table 1
LOD, RSD of peak area regression equation, correlation coefficient, linear range for MEKC system with normal way and MSS method ($n = 7$).

	Tetrandrine		Fangchinoline	
	5 s hydrodynamic injection	MSS	5 s hydrodynamic injection	MSS
Regression equation[a]				
a	203.28	14831	197.3	357.16
b	386.01	14753	907.22	1010.7
Correlation coefficient	0.9990	0.9987	0.9983	0.9956
LOD (S/N = 3) (μg/mL)	1.8	0.016	3.2	0.026
Linear range (μg/mL)	5–300	0.05–2.5	5–300	0.05–2.5

[a] $y = ax + b$; y, peak area; x, standard concentration (μg/mL).

Table 2
Results for the determination of the two components in sample extracts ($n = 3$).

Sample	Ingredient	Content	Concentration spiked (μg/mL)	Concentration found (μg/mL)	Recovery (%)	Average (%)	CV[a]
Stephania tetrandra S. Moore	Tetrandrine	1.39 mg/g	0.15	0.14	93	102	7.9
			0.3	0.31	103		
			0.45	0.49	109		
	Fangchinoline	0.57 mg/g	0.15	0.16	107	98	8.3
			0.3	0.29	97		
			0.45	0.41	91		
Yinnuoke	Tetrandrine	0.079 mg/capsule	0.45	0.47	104	101	3.5
			1.0	1.0	100		
			0.3	0.32	107		
	Fangchinoline	0.025 mg/capsule	0.45	0.42	93	95	7.1
			1.0	0.9	90		
			0.3	0.31	103		
Weifengtong	Tetrandrine	0.011 mg/capsule	0.10	0.13	104	103	7.3
			0.125	0.11	110		
			0.075	0.071	95		
	Fangchinoline	0.009 mg/capsule	0.10	0.10	100	106	5.7
			0.125	0.14	112		
			0.075	0.080	107		

[a] CV, coefficient of variation.

3.2. Optimization of the stacking conditions

The effect of increasing the injection time was investigated in the range 60–420 s using 75 mM H_3PO_4–2% Tween 20–5% methanol (pH* 3.0) buffer solution, 8.5 mM SDS (sample matrix), 100 mM H_3PO_4–55% ethanol TS, 90 s introduction time of TS, 22 kV applied voltage. The result showed that the peak height and area of the analytes increased with the injection time increasing from 60 s to 240 s. With further increasing the injection time, the peak area increased all through, but the peak height of tetrandrine and fangchinoline slightly increased, and the peaks shape of tetrandrine and fangchinoline became gradually broad and tailed, which may be the maximum injection length that can be concentrated by the method. Additionally, good peak shape of the analytes was obtained at 240 s. Therefore, the injection time was chosen as 240 s to get compromise between the peak height and area.

To determine how the injection time of TS affected sensitivity, a more exhaustive study between 0 s and 180 s of hydrodynamic injection was performed. As expected, no peaks were observed if TS was not introduced into capillary. An increase in the response was observed with increasing the introduction time, the reason for this could be due to the fact that, when a large volume is injected, the TS would be able to associate with more analyte molecules. However, at introduction time higher than 90 s, the decrease in the magnitude of the peak height and area can be explained in part by an increase in the volume of the rich phase within the collection column as the percentage of the rich phase increases, leading to a dilution of the analytes. So, 90 s was selected as the optimum introduction time of TS.

As we know, the CMC of SDS in water is around 8.0 mM. If micelles can be formed in the nonaqueous ethanol solution, the CMC will be much higher than in aqueous media because ethanol is less polar than water [22]. According to the method proposed by Cifuentes et al. [23], by plotting the electric current values, as measured by a CE instrument, versus the SDS concentration with a range of 1–20 mM, a good linearity has been obtained with the relative coefficient of 0.9996, supporting that micelles are absent within the range of investigations in 50% ethanol solution. The effect of concentration of SDS in the sample matrix was investigated in the range 6.0–13.0 mM. The peak area and height of tetrandrine and fangchinoline increased with increasing SDS concentration until it reached a maximum value at 8.0 mM SDS. Further increasing SDS concentration, the peak area and height decreased. These results suggest that for more effective MSS, the sample must be prepared in a matrix that contains concentrations of surfactant closer to the CMC. Thus, we selected 8.0 mM SDS for the investigation of the limit of detection (LOD).

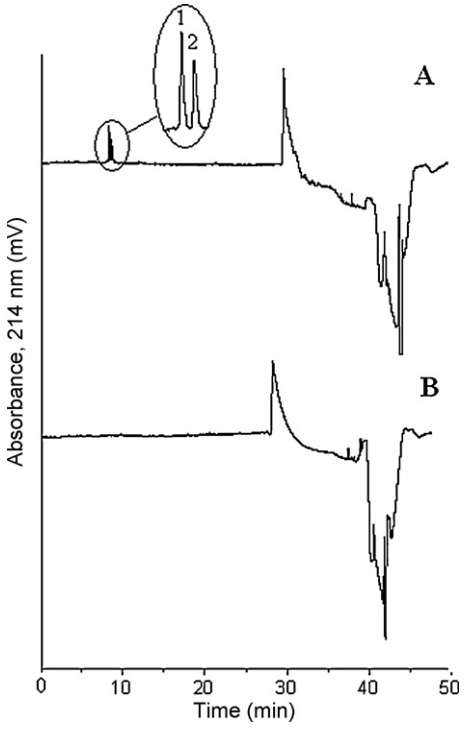

Fig. 2. Electrochromatograms of a standard mixture of tetrandrine and fangchinoline and blank injection. (A) standard mixture, concentration of tetrandrine and fangchinoline: 0.2 μg/mL and (B) blank injection. Conditions: 50 μm I.D. × 375 μm O.D. × 50 cm length (41.5 cm effective length), uncoated; sample matrix: 8.0 mM SDS solution, TS: 50 mM H_3PO_4–55% ethanol, BGE: 75 mM H_3PO_4–2% Tween 20–5% methanol buffer (pH* 3.0), introduction time of the TS: 90 s, injection time of the sample solution: 240 s, 22 kV voltage, 214 nm UV detection. Peaks: 1 = tetrandrine, 2 = fangchinoline.

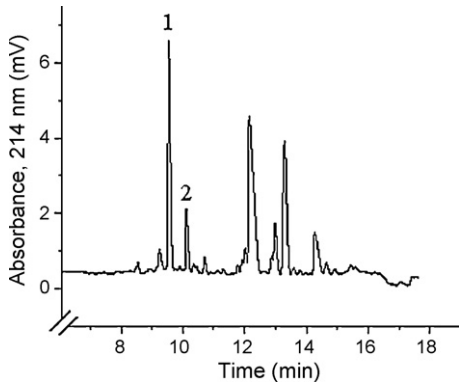

Fig. 3. Electrochromatogram of Fengtongan capsule (Yinnuoke). Other conditions as in Fig. 2.

3.3. Linearity, repeatability and LOD

Under the optimum conditions, the linear relationships between the peak area of the analytes and the corresponding concentrations are shown in Table 1. The repeatability of the method was determined by repeated ($n = 4$) injection of the standard mixture solutions at the concentration levels of 0.9, 1.2, 2.0 μg/L for all analytes. The RSDs of the migration times and peak areas were 0.23–0.79%, 0.78–3.3% (intra-day, $n = 4$), and 1.8–2.6%, 1.9–4.5% (inter-day, $n = 4$), respectively. The peak areas were employed for quantification. The standard and blank solution electropherograms are shown in Fig. 2. The performance of this method is summarized in Table 1. The LOD was calculated as the peak height at a signal-to-noise ratio of 3 (S/N = 3). As a comparison, the linearity and limits of detection using the 5 s hydrodynamic injection are also given in Table 1.

3.4. Application

To test the applicability of the developed method based on MSS, real samples were analyzed. Quantitative analysis was performed under the optimum conditions obtained from the experiments described above. This method was applied to the analysis of tetrandrine and fangchinoline in *S. tetrandra* and Fengtongan capsules. The peaks were identified by the standard addition methods. The contents of the analytes found in the different kinds of herbs are given in Table 2. The electropherogram of Fengtongan capsule (Yinnuoke) is shown in Fig. 3. The accuracy of the methods and the potential matrix effects were established by analyzing spiked samples. The results are presented in Table 2.

4. Conclusions

The online preconcentration approach based on MSS with SDS served as carrier of samples in acid buffer using MEKC has been proven a feasible and attractive way for improving the sensitivity of the detection in CE. This technique further exploited the potential of online focusing methods in CE. The method is sensitive with LODs of 16 ng/mL tetrandrine and 26 ng/mL fangchinoline. The LOD of the method was improved about 113–123-fold in comparison with conventional hydrodynamic injection.

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