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On-line two-step stacking in capillary zone electrophoresis for the preconcentration of strychnine and brucine

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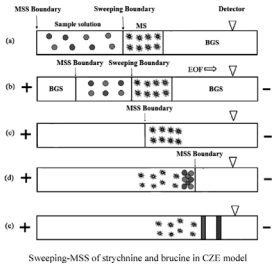
HIGHLIGHTS

- Two-step stacking in CZE was developed for the on-line preconcentration of alkaloids.
- The sensitivity of the method was much improved.
- The study expanded the application of the stacking technique to the analysis of more complex matrix samples.

GRAPHICAL ABSTRACT



Sweeping-MSS of strychnine and brucine in CZE model

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ABSTRACT

An on-line sample preconcentration method by two-step stacking i.e., sweeping and micelle to solvent stacking, in capillary zone electrophoresis (CZE) has been developed for the determination of strychnine and brucine in traditional Chinese herbal medicines. After experimental optimizations, the best separation was achieved by using 75 mM phosphate buffer (pH 2.5) with 30% methanol (v/v). Compared with normal CZE injection, 51- and 38-fold improvement in concentration sensitivity was achieved for strychnine and brucine, respectively. The calibration curve was linear in the range of 0.1–5.0 μg mL^{-1} for both strychnine and brucine, with the correlation coefficients of 0.9998 and 0.9997, respectively. The limits of detection ($S/N = 3$) for both alkaloids were 0.01 μg mL^{-1}. The inter-day ($n = 8$) and intra-day ($n = 5$) reproducibilities expressed as the relative standard deviations for corrected peak area were less than 9.5%. The method was applied to determine strychnine and brucine in two Chinese herbal medicines, with recoveries ranging from 94.2% to 105.4%. The results indicated that the method is simple, rapid, reliable, and can be applied to determine *strychnos* alkaloids in traditional Chinese herbal medicines.

1. Introduction

Capillary electrophoresis (CE) has developed into an attractive analytical technique due to its high separation efficiency, short analysis time, and small sample amount requirements and almost being organic solvent-free. However, because of the short optical path-length and small sample volume, the concentration sensitivity of CE is often insufficient for the determination of low concentrations of analytes in real samples. Currently, the most facile way to improve the concentration sensitivity in CE is on-line preconcentration technique since it is easy to execute and does not need to modify the commercial CE instrumentation.

There are several well-known on-line stacking techniques in CE, e.g., field amplification or enhanced sample stacking [1], dynamic pH junction [2], transient isotachophoresis (tITP) [3], sweeping [4], analyte focusing by micelle collapse (AFMC) [5] and micelle to solvent stacking (MSS) [6–15]. Sweeping is an effective and convenient on-line sample preconcentration technique in MEKC. It consists of the introduction of a large sample zone prepared in a matrix devoid of pseudo stationary phase (micelle), wherein the analytes are picked-up and accumulated by micelle that penetrates the

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sample. The usual procedure for sweeping includes a hydrodynamic injection step followed by the subsequent sweeping and MEKC separation processes.

During the last decade, the combination of other stacking techniques with sweeping or tITP, referred as two-step stacking, has been explored [16,17]. The first two-step technique reported in MEKC was the combination of sweeping with selective exhaustive injection [18]. The other reported two-step stacking techniques included dynamic pH junctiton-sweeping in MEKC [19,20], electrokinetic sample injection and tITP in CZE [21], and the combination of sweeping under electrokinetic injection with AFMC in two-dimensional CE [22].

MSS is a relatively new on-line focusing method which was developed by Quirino [6]. The crucial factor of the method was the reversal of the effective electrophoretic mobility at the MSS boundary between the sample solution zone and the background solution (BGS). In MSS, the sample was prepared in a micellar solution (MS) without organic solvent while the BGS was modified with an organic solvent. The analytes bound to the micelles were electrophoretically directed to the MSS boundary containing the organic solvent and then the affinity between the analytes and the micelles was significantly lowered. Therefore, the analytes then experienced an electrophoretic inversion and accumulated at the MSS boundary.

Recently, a novel two-step stacking strategy for organic cations or anions with sweeping and MSS as the first and second steps in CZE, respectively, was introduced by Quirino et al. [23,24]. In such strategy, the sample matrix was free of the micelles and a plug of MS was injected before sample solution in order to perform sweeping. After the first sweeping step, the swept analytes were bound to the micelles and the BGS which contained organic solvent induced the second MSS focusing step. This technique has been successfully applied to on-line preconcentration of some organic cations (β-blocker and tricyclic antidepressant drugs) [23] and anions (hypolipidaemic drugs, non-steroidal anti-inflammatory drugs, and herbicides) [24], and the detection sensitivities were increased by more than two orders of magnitude. Later, Wang et al. developed a long-chain ionic liquid-based sweeping and MSS in CZE for anionic compounds [25]. *N*-Cetyl-*N*-methylpyrrolidinium bromide (C_{16}MPYBr) was used as the cationic surfactant and 25–60 times sensitivity improvement in peak area or peak height for benzoic acid, 2-nitrophenol and 4-chlorophenol was obtained. However, the reported methods were only applied to the analysis of waste or environmental water samples. The two-step stacking technique has not been applied in the analysis of complex matrix samples until now.

The two *strychnos* alkaloids named strychnine and brucine are extremely toxic but they are frequently used as an important ingredient in traditional Chinese herbal medicines to treat nervous diseases, vomiting, arthritic and traumatic pains. When ingested, they could stimulate the central nervous system and make the sensory organs more sensitive. For this reason, strychnine was also included in the doping list of the prohibited substances by the Medical Commission of the International Olympic Committee [26]. At low doses, they exhibit high pharmacological activities. However, high doses of strychnine are known to be deadly poisonous, and sometimes can cause violent muscular convulsions [4]. Moreover, strychnine and brucine are highly toxic and the margin between therapeutic and toxic doses is very narrow; it was reported to be fatal to humans at the doses of 30–90 mg [27,28]. Therefore, to ensure their safe use, it is necessary to establish simple and efficient approaches for the determination of strychnine and brucine in Chinese herbal medicines.

In this work, a simple and convenient two-step stacking by the combination of sweeping with MSS was developed for the simultaneous on-line preconconcentration and determination of the *strychnos* alkaloids in CZE. To the best of our knowledge, this may be the first report of using sweeping-MSS in CZE for the determination of *strychnos* alkaloids in Chinese herbal medicines.

2. Experimental

2.1. Apparatus

All CE experiments were performed on a Beckman P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA, USA) equipped with an auto sampler and a DAD. An uncoated fused-silica capillary (Yongnian Ruifeng Optical Fiber Factory, Hebei, China) of 50 cm (effective length, 41.5 cm) × 75 μm id was used throughout the experiments. Data acquisition and instrument control were carried out using Beckman P/ACE MDQ 32 Karat software. A PHS-3C pH meter (Hangzhou Dongxing Insrument Factory, Hangzhou, China) was used for pH measurements.

2.2. Reagents, chemicals and materials

Strychnine and brucine (both >99%) were purchased from Chinese National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). *Fengshi maqian tablets* were purchased from Henan Blue Sky Pharmaceutical Co., Ltd. (Henan, China). *Yaotongning capsules* were bought from Chengde Jingfukang Pharmaceutical Group Co., Ltd. (Chengde, China). SDS was purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate, sodium hydroxide (NaOH), hydrochloric acid (HCl) and methanol (MeOH) were products of from Kaitong Chemcial Reagent Co., Ltd. (Tianjin, China). The MeOH was of HPLC grade and other reagents were of analytical grade. All reagents were used without further purifications. All the solvents were filtered through a 0.45 μm Micro Science membrane filter (Tianjin Automatic Science Instrument Co., Ltd. Tianjin, China). The water used throughout the work was double-distilled using SZ-93 automatic double-distiller (Shanghai Yarong Biochemistry Instrumental Factory Shanghai, China).

For sweeping-MSS, the sample matrix was 10 mM phosphate at pH 2.5. The MS was 10 mM SDS with 10 mM phosphate (pH 2.5). The BGS was 75 mM phosphate (pH 2.5) containing 30% MeOH (v/v).

A mixture stock solution containing 2.0 mg mL^{-1} each of strychnine and brucine was prepared in MeOH and stored in a refrigerator at 4 °C. A series of standard solutions were prepared by evaporating an appropriate amount of the stock solution under a gentle stream of air to dryness and then dissolving the residues with sample matrix. The BGS and MS were prepared fresh each day and sonicated for 10 min prior to use.

2.3. Sample preparation

Fengshi Maqian tablets and *Yaotongning capsules* were first ground, and then 400.0 mg of the resultant powder was weighed. The powder was wetted with 500 μL of 25% (w w^{-1}) ammonium hydroxide for 5 min. After being soaked in 10 mL chloroform for 30 min, the mixture was refluxed for 1 h, and then filtered. Chloroform was removed by reduced pressure distillation at 60 °C until dryness. The leftover was dissolved in 10.0 mL MeOH and then filtered through a 0.45 μm syringe filters. 250 μL of the resultant solution was evaporated under a gentle stream of air at 50 °C to dryness. Then the sample solutions were obtained by dissolving the residues with 10.0 mL sample matrix. All the sample solutions were filtered through a 0.45 μm syringe filter prior to the following CE procedures.