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Sensitive determination of neurotransmitters in urine by microchip electrophoresis with multiple-concentration approaches combining field-amplified and reversed-field stacking

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ABSTRACT

Microchip electrophoresis (MCE) is particularly attractive as it provides high sensitivity and selectivity, short analysis time and low sample consumption. An on-line preconcentration strategy combining field-amplified stacking (FASS) and reversed-field stacking (RFS) was developed for efficient and sensitive analysis of neurotransmitters in real urine samples by MCE with laser induced fluorescence (LIF) detection. In this study, the multiple-preconcentration strategy greatly improves the sensitivity enhancement and surpass other conventional analytical methods for neurotransmitters detection. Under optimal conditions, the separation of three neurotransmitters (dopamine, norepinephrine and serotonin), was achieved within 3 min with limits of detection (S/N = 3) of 1.69, 2.35, and 2.73 nM, respectively. The detection sensitivities were improved by 201-, 182-, and 292-fold enhancement, for the three neurotransmitters respectively. Other evaluation parameters such as linear correlation coefficients were considered as satisfactory. A real urine sample was analyzed with recoveries of 101.8–106.4%. The proposed FASS-RFS-MCE method was characterized in terms of precision, linearity, accuracy and successfully applied for rapid and sensitive determination of three neurotransmitters in human urine.

1. Introduction

The content of monoamines such as catecholamines (like dopamine, norepinephrine) and serotonin (5-hydroxytryptamine) in biological fluids plays important physiologically roles both related to nervous function in the body and to various diseases [1,2]. Dopamine (DA), for example, is a key neurotransmitter affecting brain processes that control motion, emotional response, and ability to experience pleasure and pain [3,4]. Furthermore, a low concentration or abnormal metabolisms of DA may lead to various neurological diseases, such as Parkinsonism [5], schizophrenia [6] and Alzheimer's [7] disease. Norepinephrine (NE) is an important catecholamine neurotransmitter in the mammalian central nervous system, which is associated with many disorders including multiple sclerosis [8] and Parkinson's disease [9,10]. The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT), for example, is thought to play a crucial role in the emotion system, such as the regulation of the mood [11] and the sleep [12]. Based on these findings, it is necessary to accurately determine DA, NE and 5-HT contents in pharmaceutical and biological samples.

In view of these challenges, it is important to use reliable and sensitive analytical methods to identify and quantify neurotransmitters in complex matrices. Until now, HPLC [13] coupled with various detection methods, such as GC detection [14], electrochemical detection [15], and laser-induced fluorescence detection [16], have been generally used for the determination of monoamine neurotransmitters, particularly for DA, NE and 5-HT. Moreover, capillary electrophoresis (CE) has been developed as a powerful analytical technique in analyzing neurotransmitters, because of its lower consumption of sample and better separation efficiency [17,18]. Recently, microchip electrophoresis (MCE), conserving the advantages of low sample consumption and high analysis speed, is increasingly being viewed as a successful alternative to the CE technology for rapid analysis. When compared to CE, MCE proves to be more accommodating for the miniaturization of the analytical instrumentation [19,20].

In order to achieve higher detection sensitivity, online sample stacking is the most common strategy to improve the concentration sensitivity in MCE, including field-amplified sample stacking (FASS) [21], reversed-field stacking (RFS) [22], large-volume sam-

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ple stacking (LASS) [23], isotachophoresis (ITP) [24], sweeping [25], solid-phase extraction [26] among others. However, a single-step stacking method for MCE sometimes cannot meet the needs of the test because of the very low analyte concentration. Therefore, these multiple-concentration techniques with high concentration factors inspired us to investigate the possibility of applying a multiple-concentration method for achieving an efficient test at lower concentrations of sample with the MCE system. For example, Pan et al. reported a highly effective on-chip preconcentration method combining field-amplified sample injection and bovine serum albumin sweeping methods for ultrasensitive detection of green fluorescent proteins on a simple cross-channel microchip device [27]. Our group has reported a multiple stacking strategy combining chitosan sweeping, field-amplified sample stacking and reversed-field stacking for the ultrasensitive detection of bacteria using microchip electrophoresis with laser-induced fluorescence (MCE-LIF). This strategy afforded 6000-fold improvements in peak height [28].

The aim of this paper was to find a suitable combination of concentration approaches for the detection of low-abundant 5-HT, NE and DA by MCE in biological samples. After a series of experiments, satisfying efficiency values were obtained by combining field-amplified stacking (FASS) and reversed-field stacking (RFS) and were compared to the values obtained with classical CE. We employed an on-line multiple-preconcentration approach combining FASS and RFS for the simultaneous analysis of three neurotransmitters including 5-HT, NE and DA by MCE with laser-induced fluorescence (LIF) detection. To the best of our knowledge, this is the first report combining FASS and RFS techniques for the simultaneous determination of 5-HT, NE and DA with MCE. Under optimal conditions, 5-HT, NE and DA were successfully focused and well separated within 3 min. This FASS-RFS-MCE strategy allows for the determination of these compounds at very low concentrations and can yield 182- to 292-fold increase in detection sensitivity. Another major goal of this work was to develop and validate a simple and rapid method for the analysis of 5-HT, NE and DA in human urine by MCE with multiple-concentration. The experimental results indicated that this approach can be used for the simultaneous analysis of low-abundance neurotransmitters in urine sample.

2. Materials and methods

2.1. Instrumentation and reagents

All MCE experiments were conducted using a sp-800 model MCE system coupled to a laser-induced fluorescence (LIF) detection device (Shanghai Spectrum Ltd. Co., Shanghai). Briefly, a diode laser (5 mW) was used to generate an excitation beam at 635 nm. The fluorescence signal was spectrally isolated using an edge filter and was subsequently collimated with an achromatic lens before being focused onto the photomultiplier tube (PMT). The variable high voltage power unit (0 ± 6 kV) was used for the on-chip sample injection and for the zone electrophoretic separation. The amplified current was transferred through a 10 kΩ resistor to a 24 bit A/D interface at 10 Hz (Borwin, JMBS Developments, Le Fontanil, France) and stored in a personal computer.

5-Hydroxytryptamine (5-HT) was purchased from J&K Scientific Ltd. (Shanghai, China), and dopamine (DA) was obtained from Alfa Aesar Co., Ltd. (Johnson Matthey, Tiajin, China). Norepinephrine (NE) was purchased from Sigma (St. Louis, MO, USA). Sulfoindocyanine succinimidyl ester (Cy5) was obtained from GE Healthcare Company (Shanghai, China). Sodium hydroxide, sodium tetraborate, hydrochloric acid and acetonitrile were provided by Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All solutions were prepared by ultrapure water from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Preparation of solutions

A stock solution of Cy5 (100 μM) was prepared in anhydrous acetonitrile. Mother solutions of 5-HT, DA, and NE with a concentration of 100 mM in ultrapure water were prepared separately, diluted to 100 μM with the derivatization buffer and ascorbic acid was added to prevent oxidation. The running buffer solution was prepared by dissolving sodium tetraborate in ultrapure water to a concentration of 100 mM, whereas the sample buffer and the derivatization buffer solutions were made by dissolving sodium tetraborate in water to a concentration range of 10–40 mM and 10–50 mM, respectively. Above solutions were sealed and stored at +4 °C.

2.3. Procedure of derivatization

The standard solutions (10 mM) of 5-HT, NE, and DA were diluted to 100 μM with the derivatization buffer (20 mM borate solution, pH 9.24). Derivatization of each compound was performed by mixing the corresponding diluted solution with Cy5 (100 μM) in a 1:1 (v:v) ratio in a 1.5 mL centrifuge tube. The three solution mixtures were left to react in darkness for 6 h at room temperature, and then diluted to the required concentrations with the sample buffer (20 mM borate solution, pH 8.50) before use.

2.4. Preparation of the urine samples

Urine samples were obtained from healthy volunteers from the East China Normal University Infirmary. The pH value of the fresh urine samples were immediately adjusted to pH 3 with a hydrochloric acid solution (6 M) and then stored in a refrigerator until analysis. Ascorbic acid was added to prevent oxidation (0.015 g of ascorbic acid for 10 mL of urine sample). Before purification, the pH of the solution was adjusted to 8.5 with a solution of sodium hydroxide (1 M). The samples were centrifuged for 10 min (12,000 rpm) in order to precipitate the proteins. The supernatant was transferred into another 10 mL vial, and then immediately passed through a SPE alumina B column (Dikma, China), which had been priorly activated with methanol (3 mL) and then with water (3 mL). The cartridge was washed with water (5 mL), and the analytes were eluted with acetic acid (5 mL, 1 M). The processed urine samples were then filtered through a polypropylene acrodisc syringe filter (0.22 μm) and diluted two fold with the sample buffer solution (100 mM, pH 8.5). The diluted samples were then derivatized following the procedure in Section 2.3 [29].

2.5. MCE conditions

The design of the glass microchip used in the present work consisted of a simple cross channel. The separation channel was 60 mm in length and 45 mm from the injection intersection to the detection point. All other channels had a length of 10 mm measured from the channel intersection. Microchannels were etched to a depth of 25 μm and a width of 70 μm. Platinum electrodes were inserted into the reservoirs, providing electrical contact from the power supply to the electrolyte solutions. All experiments were running in full filling mode.

Before starting the experiments, the microchannels were rinsed respecting the following order hydrochloric acid (1.0 M) for 20 min, copious amounts of ultrapure water for 10 min, sodium hydroxide (1.0 M) for 10 min, ultrapure water for 10 min, and last with the running buffer during 5 min. Before each injection, the microchan-