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Microchip capillary electrophoresis coupled with an end-column electrochemiluminescence detection

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Abstract

An easy and universal wall-jet configuration for microchip CE-ECL detection system was constructed and investigated in this work. Two detection modes of pre-column and post-column were applied to the above system. TPA, tramadol and lidocaine were chosen as model analytes to estimate the system in both modes. The important operational parameters such as the concentration of luminescent reagent and the distance between the separation outlet and the working electrode were optimally obtained and compared for the first time.

Keywords: Microchip electrophoresis; Electrochemiluminescence; Tris(2,2′-bipyridyl)ruthenium(II)

1. Introduction

Many compounds such as oxalate, amino acids, amines, pharmaceuticals or protein can be sensitively detected via electrochemiluminescence (ECL) based on tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) [1–5], thus meaning that this detection strategy may have widespread application in a variety of important areas[6–13].

Recently, some efforts have been reported to integrate capillary electrophoresis (CE) with the ECL detection [14–24]. Aqueous ECL of $Ru(bpy)_3^{2+}$ was often used to combine with CE to detect analytes due to its convenience. The appropriate mixing of $Ru(bpy)_3^{2+}$ and the separation buffer is critical in order to achieve good separation efficiency at maintaining high sensitivity all the while. Forbes et al. reported on-line electrogenerated $Ru(bpy)_3^{3+}$ chemiluminescent detection of β-blockers separated with CE [14]. Bobbit et al. reported in situ cell electrogenerated $Ru(bpy)_3^{3+}$-based chemiluminescence detection in CE [15,18]. Wang and co-workers reported a new technique for CE directly coupled with end-column electrochemiluminescence detection [19,20]. For these studies, two detection modes, pre-column mode and post-column mode, were applied for CE-ECL. For the pre-column mode, $Ru(bpy)_3^{2+}$ is added to the running buffer. For the post-column mode, $Ru(bpy)_3^{2+}$ is directly added to the cathodic cell. For common CE, the major shortcoming of the above modes is the excessive consumption of expensive $Ru(bpy)_3^{2+}$ reagent. To develop a solid-state ECL sensor in which $Ru(bpy)_3^{2+}$ is immobilized may offer an effective approach to resolve the above problem [25,26], however, its stability is a severe challenge. Meanwhile, the process of fabricating ECL sensors is tedious.

Microchip CE may offer another possibility to resolve such a problem. For microchip CE-ECL detection system, the separation time is much shorter than that for conventional CE-ECL, based on this fact, a series of the influences resulted from the variation of the experimental conditions such as the change of EOF in pre-column mode, a dilution of $Ru(bpy)_3^{2+}$ in post-column mode as well as excessive consumption of $Ru(bpy)_3^{2+}$, etc. should be minimized.

Up to now, reports on the microchip CE with ECL detection are very limited [25–31]. Manz and his co-workers reported a microfluidic system with indirect $Ru(bpy)_3^{2+}$ ECL detection of amino acids, based on a "U"-shape floating platinum electrode placed across the separation channel [29]. Wang and co-workers reported an ECL detector with microchip CE, which consisted of a poly(dimethylsiloxane) (PDMS) layer containing separation and injection channels and an electrode plate with an ITO electrode fabricated by a photolithographic method

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[25,30,31]. However, these microchip CE-ECL detection systems were complicated and to construct these detection systems was not only time-consuming and tedious but also need expensive special instruments. It is surprising that there are few reports on a simple, low-cost, end-column, wall-jet detector, which is widely used in electrochemically amperometric detection, for microchip CE-ECL.

In this work, a simple and universal wall-jet configuration for the microchip CE combined with an end-column ECL detection system was constructed and both detection modes, i.e. pre-column mode and post-column mode were applied to the microchip CE-ECL system. A 400 μm diameter glassy carbon disc electrode used as a working electrode and aligned with the outlet of separation channel electrochemically oxidizes the $Ru(bpy)_3^{2+}$ to the active $Ru(bpy)_3^{3+}$ form, which then reacts with analytes at the cathodic cell and produces light.

Since tramadol and lidocaine are often used for analgetic and local anesthetic, respectively, and with tertiary amine structures for $Ru(bpy)_3^{2+}$ ECL reaction, fast analysis of tramadol and lidocaine is of clinic importance for understanding the patient's medical process. Meanwhile, the $Ru(bpy)_3^{2+}$–TPA system has been well studied and shown to give rise to high ECL intensity. Therefore, TPA, tramadol and lidocaine were chosen as model compounds to characterize this microchip CE-ECL detection system in both modes.

2. Materials and methods

2.1. Chemicals and apparatus

All reagents used were commercially available and of analytical grade. Tripropylamine (TPA) and tris(2,2′-bipyridyl)ruthenium(II) chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used as received. Tramadol and lidocaine were obtained from Nanjing Pharmaceutical Institute (Nanjing, China). All solutions were prepared with twice-distilled water. The stock solutions were stored in the refrigerator (at 4 °C). All standard solutions and phosphate buffers (PBS) were prepared and filtered through a 0.22 μm membrane (Jasco, Dailian, China) before use.

Electrochemical measurements were performed with a three-electrode system comprising a platinum wire as auxiliary electrode, an Ag/AgCl (sat. KCl) electrode as reference electrode, and the glassy carbon disk electrode with a diameter of 0.4 mm as working electrode. The electrodes were connected to a CHI630A electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China). All microchip CE-ECL emission detections were obtained by a Model MPI-A CE-ECL Analyzer Systems (Xi'an Remax Electronic High-Tech Ltd., Xi'an, China). The photomultiplier tube (PMT) was biased at 1000 V.

2.2. Applications of the microchip CE-ECL detection

Fig. 1 is the schematic diagram of the experimental setup and the detector was of the wall-jet configuration. The construction of the microchip CE-ECL platform is shown in Fig. 2. The platform body was made of polymethyl methacrylate material. The working electrode was aligned at the exit of the electrophoretic separation channel. No decoupler was employed for isolating the high separation electric field in this system. The glass chip shown in Fig. 2 consisted of a glass plate, with a 50 mm long separation channel and a 10 mm long injection channel (between the sample reservoir and the sample waste reservoir). The two channels crossed each other halfway between the sample and the sample waste reservoir and 45 mm from the outlet to yield a separation channel with an effective length of 45 mm. The channels were 60 μm width and 20 μm depth.

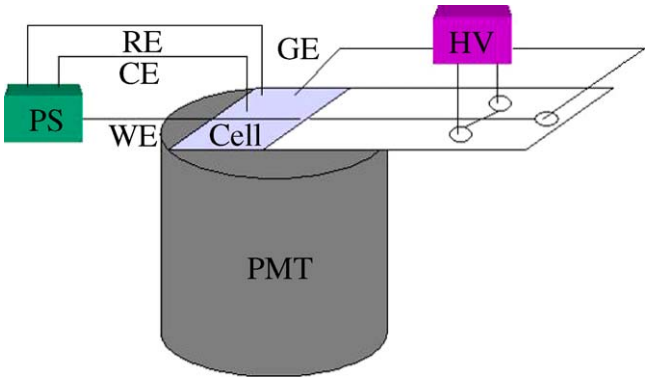

Fig. 1. Schematic diagram of the experimental setup: (WE) working electrode, (CE) counter electrode, (RE) reference electrode, (GE) ground electrode, (PS) potentiostat, (HV) high voltage, (PMT) photomultiplier tube. WE was a glassy carbon disk electrode with diameter of 0.4 mm; CE, GE were platinum wires; RE was Ag/AgCl (sat. KCl) electrode.

Alignment of the working electrode with the outlet of separation channel was quite important in the experiment. To accomplish alignment, the working electrode was aligned with the outlet of separation channel and fixed by a precisely three-dimensional adjustor (Shanghai Lian Yi Instrument Factory of Optical Fiber and Laser, Shanghai, China) with the precision of ±1 μm in each direction under the microscope with ruler. Meanwhile, the end of separation channel outlet of the glass microchip was rubbed away to a wedge shape with the thick-

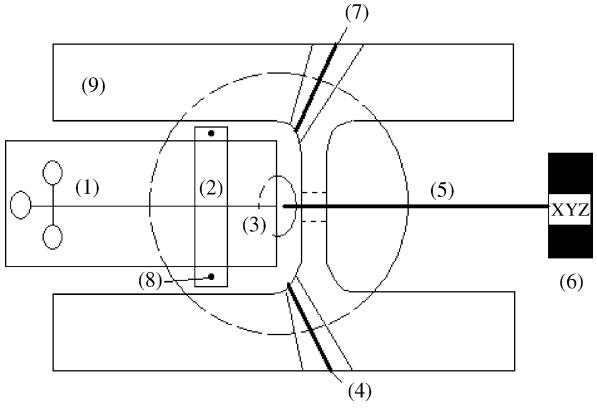

Fig. 2. The schematic diagram of the microchip CE-ECL platform by end-column mode. (1) glass microchip; (2) a piece of polymethyl methacrylate; (3) UV transparent optic glass; (4) counter electrode; (5) working electrode; (6) three-dimensional adjustor; (7) reference electrode; (8) screws; (9) polymethyl methacrylate body.