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Highly Sensitive Chemiluminescence Detection for PDMS/Glass Micro-chip Electrophoresis

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Abstract: This paper described a highly sensitive chemiluminescence detection system for micro-chip electrophoresis (MCE) based on luminol-hydrogen peroxide reaction catalyzed by the metal ions. The micro-chip was composed of poly(dimethylsiloxane) (PDMS) and glass, and was fabricated by micro-machining technology. The surface of channels was dynamically modified by polydimethylacrylamide (PDMA) in order to eliminate unhomogeneous electroosmotic flow (EOF) of the PDMS/glass chip, adsorption of molecules, and improve hydrophobicity on PDMS surface. The detection modes, reagent mix procedures and reaction conditions were optimized and the detection limit of 5 x 10^{-11} mol/L for cobalt (II) was achieved by MCE with chemiluminescence detection, which was about four orders of magnitude more sensitive than that reported in the reference.

Keywords: Micro-chip, electrophoresis, chemiluminenscence, poly(dimethylsiloxane).

Chemiluminescence (CL) is a highly sensitive detection method widely used in flow injection analysis, liquid chromatography and immunoassay. Due to its simple optical system and low background nature this method is uniquely suited to on-line detection for capillary electrophoresis (CE) and microchip electrophoresis (MCE). CE combined with CL (CE-CL) has been successfully used for analysis of amino acids, proteins, catecholamines, ATP, catalytic and non-catalytic metals ions, and polyamines [1-3]. The ultra-sensitive detection limit down to10^{-12} mol/ L was obtained in CE-CL system. CL is also an attractive promising detection in MCE, and has been used for detection of amino acids, DNA, metal ions. To our knowledge, so far the sensitivity of CL detection in MCE is only about 10^{-8} mol/L, which is about ten thousand times lower than that in CE [4]. According to our experiences in CE-CL, we think that the main reasons of CL detection in MCE may be attributed to unreasonable detection modes, reagent mix modes and reaction conditions.

In this letter, we reported a highly sensitive MCE-CL system on the PDMS/glass chip based on luminol-hydrogen peroxide reaction catalyzed by metal ions.

Experimental

Acrylamide and ammonium peroxydisulfate were obtained from Sigma Chemical Co. (St.

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Louis, MO). N, N′-Dimethylacrylamide was from Aldrich-Chemie (Germany). Luminol was purchased from Fluka (Switzerland). Poly(dimethylsiloxane) (PDMS) prepolymer and curing agents were generously gifted by Rhodia Silicon Co (Shanghai). Cobalt (II) nitrate, copper (II) nitrate were obtained from Shanghai Reagents Co. (Shanghai, China). Hydrogen peroxide was from Taopu Chemical Factory (Shanghai, China). Sodium acetate and acetic acid were products of Guangzhou Reagents Co. (Guangzhou, China). Lactic acid was obtained from Beicheng Chemical Factory (Zhengjiang, China). All chemicals were of analytical grade or better grade. The stock solutions of metal ions ($1x10^{-3}$ mol/L) were prepared by dissolving their salts in water. Ultra-pure water (18.2 MΩ), double-distilled and purified on Millipore Simplicity, was used for preparation of all aqueous solutions. Poly(dimethylacrylamide) (PDMA) was synthesized in water according to the reference [5].

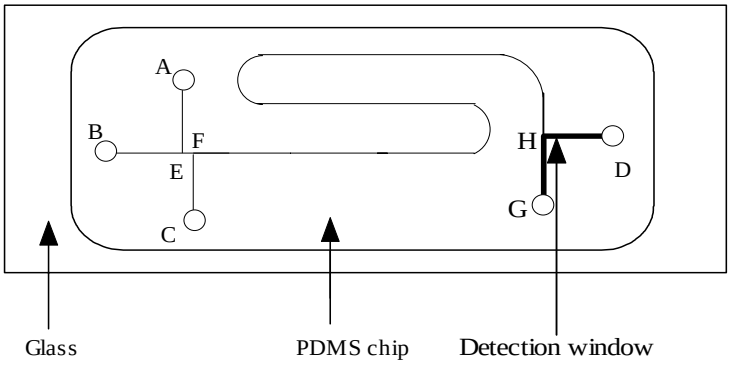
MCE-CL setup

The MCE-CL system was built in our laboratory and the microchip used was depicted in **Figure 1**. The microchip was composed of PDMS (upper) and glass substrates (bottom) and irreversible sealing was formed by UV irradiation. A high voltage supply (Institute of Nuclear Science, Shanghai, China) was employed to offer potentials for sample injection and electrophoresis separation. A home-built switch box similar to that reported in the reference [6] was connected to the high voltage supply, which was used to alternate between injection and separation modes. This unit provided counter voltage to the sample and sample waste reservoirs when applied in the separation mode, and to buffer and buffer waste reservoirs in the injection mode. A vacuum was created to pull the reaction reagents through the detection window by the syringe pump modified (MD-1001, BioAnalytical System Inc., IN, USA). The flow rate of the reaction solution was controlled by an in-house-built control device. A highly sensitive photomultiplier tube (PMT) (Model H5784-04, Hamamatsu, Japan) integrated with an amplifier closely situated in the detection window and transferred chemiluminesence signal into electric signal. The output from the amplifier was not further amplified, and directly fed to a computer. We used Caesar software (version 4.0) for data collection from Prince Technologies (Emmn, The Netherlands).

Electrophoresis Procedure

The channels for a new chip were rinsed successively with 0.1 mol/L hydrogen peroxide, water and electrophoresis buffer. Sodium acetate-lactic acid-luminol solution with PDMA was used as electrophoresis buffers. The samples were introduced by electrokinetic injection using double T model, and electrophoresis was performed at positive polarity mode under the conditions specified in the figure legends. A syringe pump was used to deliver the reaction solutions containing luminol and H_2O_2.

Figure 1 Schematic layouts of MCE system coupled with CL detection



A. Sample reservoir, B. Buffer reservoir, C. Sample waste reservoir, D. Buffer waste reservoir, G. Reaction reagents containing luminol and hydrogen peroxide. AE=BE=CF=GH= 5 mm, HD= 10 mm, EF = 2 mm, FD = 7cm, effective length, 6.2 cm; the width and depth of the injection and separation channel network were 70 and 40 μm, the width and depth of GH and HD were 200 μm and 40 μm. The buffer waste reservoir (D) was connected to a syringe pump.

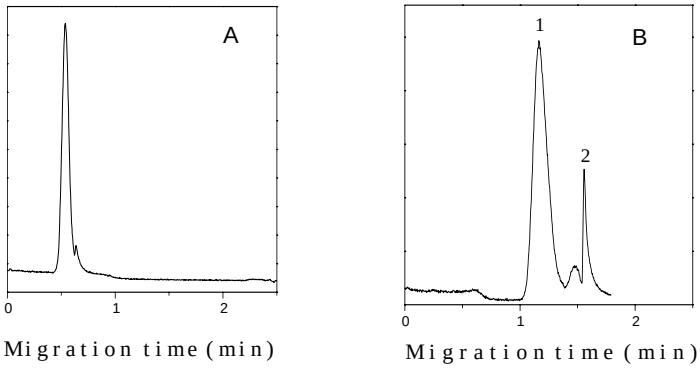
Results and Discussion

Some transition metal ions, such as cobalt (II), copper (II), have catalytic effects on the luminol-hydrogen peroxide reaction widely used in conventional CL analysis. **Figure 2A** shows the electropherogram of cobalt (II) using MCL-CL system. In sodium acetate-lactic acid buffer (pH 4.4), cobalt ion had a sharp peak and the detection limit was less than $5x\ 10^{-11}$ mol/L (S/N=10), which was ten thousand times more sensitive than that reported in the reference [4]. **Figure 2B** illustrated that rapid separation of cobalt (II) ion and copper (II) ion was successfully obtained by MCE-CL system.

We found that the sensitivity of chemiluminescence detection for MCE was dramatically dependent on the surface of microchannels, reaction conditions, reagent mix modes and electrophoresis conditions.

Microchips made of PDMS have low EOF and hydrophobic surface with respect to glass, when using unmodified PDMS/glass microchip, samples were very difficult to be introduced into microchannels. After adding a tiny amount of PDMA to running buffer, we found that successful sample injection and the good separation were achieved, and the sensitivity was remarkably increased, mainly due to the dynamic coating of PDMA to the microchannel surfaces. This coating improved hydrophobic PDMS surface, and eliminated unhomogeneous EOF and analyte adsorption on all sides of channels on PDMS/glass chip. Compared to the end detection mode commonly used, we found that on-line detection mode, the metal ion such as cobalt (II) were extremely sensitive and showed sharp symmetry peaks.

Figure 2 Electropherograms of metal ions by MCE-CL



Running buffer, 5 mmol/L sodium acetate-lactic acid containing 0.5 % PDMA (pH 4.4); reaction mix, 5 mmol/L sodium acetate containing 1.5×10^{-3} mol/L luminol and 5×10^{-3} mol/L hydrogen peroxide (pH 11.8). Applied voltages in **Figure 2A** and **2B** were 3000 V and 2000 V. 1. Co^{2+} (1×10^{-9} mol/L), 2. Cu^{2+} (5×10^{-5} mol/L).

Acknowledgments

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