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TECHNICAL PAPER

Dual confocal laser-induced fluorescence/moveable contactless conductivity detector for capillary electrophoresis microchip

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Abstract A new dual simultaneous detector was developed for capillary electrophoresis microchip. Confocal laser-induced fluorescence (LIF) and moveable contactless conductivity detection (MCCD) were combined together for the first time. The two detection systems shared a common detection cell and could respond simultaneously. They were mutually independent and advantageous in analyses of mixtures containing organic and inorganic ions. The confocal LIF had high sensitivity and the MCCD could move along the separation channel and detect in different positions of the channel. The detection conditions of the dual detector were optimized. Rhodamine B was used to evaluate the performance of the dual detector. The limit of detection of the confocal LIF was <5 nM, and that of the MCCD was 0.1 μM. The dual detector had highly sensitivity and could offer response easily, rapidly and simultaneously.

1 Introduction

In an effort to generate the lab-on-a-chip or Micro Total Analysis System (μ-TAS) (Manz et al. 1990), great developments have been made in microfluidic system in recent years due to its major benefits of speed, integration, portability, high performance, and reagent economy (Srinivasan et al. 2004). As one of the most important parts in microchip, many detection methods have been investigated, such as fluorescence detection, electrochemistry and mass spectrometry. Up to now, laser-induced fluorescence (LIF) detection has been widely used in microchip because of its high sensitivity, especially the confocal LIF mode (Jung et al. 2007). In the confocal LIF, there is a spatial pinhole filter (usually 100–500 μm) to eliminate out-of-focus light or flare scattering from the channel, so it has low background noise and high sensitivity. The total internal reflection fluorescence microscopy (TIRFM) using evanescent illumination is especially well suited for quality fluorescent imaging at the liquid/solid interface (Pennathur and Fygenson 2008) and can do real-time detection in small area (200–300 nm region) (Kang et al. 2007). But it is limited to observation at the inner microchannel/buffer interface. So the confocal LIF should be better for the microchip capillary electrophoresis (CE). In addition, conductivity detection is a sensitive and universal detection method and can be miniaturized on microchip. The contactless conductivity detection (CCD) has attracted considerable research interests, because it can separate electrodes from electrolyte to avoid the pollution of electrodes (Pumera et al. 2002). The moveable contactless conductivity detection (MCCD) performed using a moveable electrode plate can offer distinct improvements compared to common fixed-location CCD (Wang et al. 2003).

As the continuous developments, there are urgent needs for developing combined detection systems (Wang and Pumera 2002). Due to the diversity of components in real samples, it is usually difficult to determinate all components by using a single detection method, thus various dual detector schemes have been proposed for CE (Novotný et al. 2004; Tsukagoshi et al. 2007), while only a few coupled with CE microchips (Wang and Pumera 2002; Lapos et al. 2002; Qiu et al. 2005; Grabowska et al. 2007; Liu et al. 2008). Wang and Pumera (2002) described dual conductivity/amperometric microchip detection systems

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and employed them to detect electroactive and ionic species. Lapos et al. (2002) demonstrated simultaneous LIF and amperometric detection for CE microchip. Qiu et al. (2005) demonstrated an electrochemical and electrochemiluminescence detection scheme for both microchip and conventional CE systems. Grabowska et al. (2007) developed a dual optical and electrochemical detection system for making obtained results more reliable. Using a blue light-emitting diode (LED) as excitation source, Liu et al. (2008) recently reported a dual compact fluorescence and contactless conductivity detector. However, the confocal LIF method and MCCD method had not been combined in previous reports. The previous reports also either had two detection cells at different positions and for this reason their dual detection systems could not respond at the same time (Wang and Pumera 2002; Lapos et al. 2002; Grabowska et al. 2007; Liu et al. 2008) or had low limits of detection (Qiu et al. 2005; Liu et al. 2008).

In this paper, a novel dual detector with simultaneous confocal LIF and MCCD at the same place of the CE microchip was developed. A novel simple detection cell was designed through which the two detection systems could response simultaneously. To achieve high sensitivity, confocal LIF and MCCD with improved electronic circuit of were employed and the detection conditions were optimized. The coupling of the two detection systems enhanced the determining ability, which could offer simultaneous detection information of both ionic and fluorescent compounds.

2 Experimental

2.1 Reagents

The Borax run buffer solution (10 mM, pH 9.0) was prepared. High-purity deionized water (18.2 MΩ) was purified by a Millipore Simplicity for preparation of all aqueous solutions. Rhodamine B was obtained from Sigma-Aldrich Co. (St. Louis, MO). Borax was purchased from Beijing Reagents Co. (Beijing, China). Stock solution of rhodamine B was prepared by dissolving it in the run buffer. All solutions were filtered using 0.2 μm syringe filter before use. All reagents used were analytical grade.

2.2 Detection cell construction

The dual detector shared a common detection cell, shown in details in Fig. 1a. On the top of the detection cell, it was a simple cross-pattern PMMA chip with 50 mm of separation channel and 8 mm of sample channel. The shape of channel was trapezium whose widths were 125 and 75 μm, respectively, the depth is 25 μm, and one side of the channel was covered by a 50 μm-thin PMMA membrane.

On the underside of the cell, it was a moveable electrode plate made from PMMA. The electrodes fabricated on the plate by vacuum vapor deposition method were two rectangular-shaped, 10 μm-thick copper films (0.8 mm × 28.2 mm), with a distance of 800 μm between them. The laser focus was in the middle of the two electrodes. The electrodes were placed in an antiparallel orientation and the end side of the electrodes was widened to 4 mm to facilitate the electrical connection. Two copper wires were fixed at the end side of the electrodes using silver paint and then a quick-setting epoxy. The length of the wires was minimized to prevent induction of electric noise. The electrode plate was equipped with two clip holders at the ends to press mechanically the electrodes toward the bottom thin PMMA membrane of the microchip ensuring the separation channel would pass over the laser focus. The electrode plate could move along the separation channel, and detection in different positions of the channel would also be available.

2.3 Development of the dual confocal LIF/MCCD detector

The dual confocal LIF/MCCD detector was built in our laboratory and displayed in Fig. 1b. For the confocal LIF

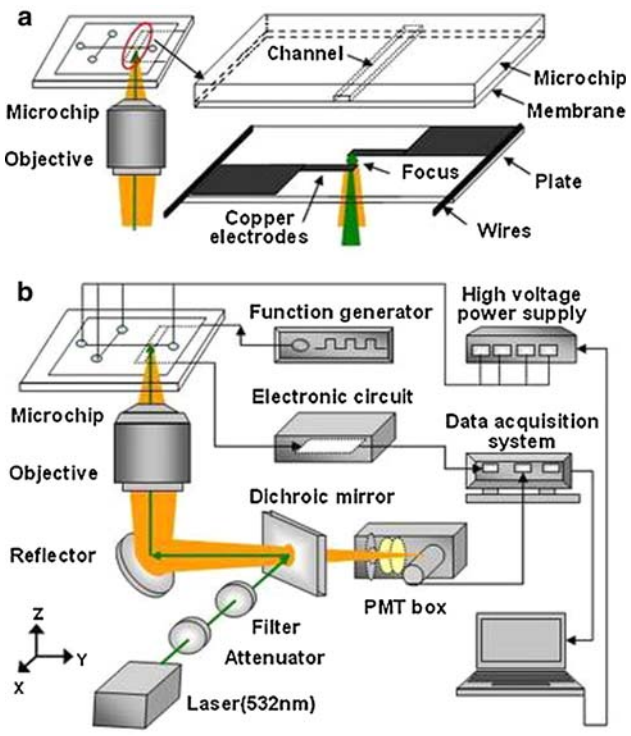

Fig. 1 Schemes of (**a**) the detection cell and (**b**) the dual LIF/MCCD detector

system, a 532-nm semiconductor laser (output power 50 mW) was used as the light source. The excitation laser power was adjusted by a circular variable attenuator. Then the laser beam was passed through a 532-nm band-pass filter (Shenyang HB Optical Technology Co., Shenyang, China), reflected by two mirrors (one was dichroic mirror) and focused by a relatively high NA objective (0.65 NA, 40×, 2.95-mm-long working distance, Chongqing MIC Optical & Electrical Instrument Co., Chongqing, China) to form a small spot (about 0.1 mm) in the separation channel. Alignment of the microchip position was adjusted by an X–Y–Z translation stage (Beijing Optical Instrument Factory, Beijing, China). The excited fluorescence was collected by the same objective, then passed though the same dichroic mirror (DM575, Shenyang HB Optical Technology Co.), a spatial filter (diameter 500 μm) and two pieces of 580 nm long-pass filters (Shenyang HB Optical Technology Co.) before being detected by a photomultiplier tube (PMT CR114, Hamamatsu, Japan). A high-voltage power supply with an adjustable voltage range between 0 and +5,000 V (College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan, China) was used for sample injection and CE separation.

For the MCCD system, the moveable electrode plate was positioned below the microchip tightly. An Agilent 33120A function generator (Agilent, Palo Alto, CA) was used for generating the sinusoidal AC waveform as the excited signal (usually with a frequency of 289 kHz and with peak-to-peak amplitude of 5 V). The excited signal was applied to one of the electrodes. The other one was connected to the input of the electronic circuit. The resulting current that passed through the detection cell was amplified and rectified by the electronic circuit, then measured by a data acquisition system. This current was a function of the conductance of aqueous solutions in the region between the electrodes (Fracassi da Silva and Do Lago 1998). The best frequency and the amplitude of the excited signal should be optimized, depending on the actual dimensions of the microchip and electrodes, as well as the quality of the electronic parts used. The electronic circuit was designed according to the reported scheme (Fracassi da Silva and Do Lago 1998) and completed by adding a low-pass filter followed by a changeable gain amplifier. There were three options for amplificatory multiple of inductive current: 300×, 600× and 1,500×. The electronic circuit was placed in a shielding box to protect the electronics from external electric fields. The signals from both systems were collected by a HP 34970A data acquisition system (Agilent, Palo Alto, CA) simultaneously and displayed using HP BenchLink DataLogger program.

2.4 Procedure

The channels of the PMMA chip were sequentially flushed with 0.1 M NaOH, deionized water, and 10 mM borate buffer before use. Sample reservoir was filled with the sample solution and others with run buffer solution. The chip was placed on a homemade Polyvinyl Chloride (PVC) platform fixed on the X–Y–Z stage. The chip position was adjusted to make the focus point of the laser in middle of the two electrodes. Pinched injection was carried out, the voltages applied to the sample, buffer, sample waste, and buffer waste reservoirs were 600, 300, 0, and 400 V in sample injection for 30 s, and then switched to 1,400, 1,600, 1,400, and 0 V in sample separation for 170 s.

3 Results and discussion

3.1 Confocal LIF detection

To develop our high-sensitivity confocal LIF system, various parameters were optimized. First, a relatively high NA objective (0.65 NA, 40×, WD 2.95 mm) to the long working distance was used for high collection efficiency of emitted photons. Second, a PMT was used, which efficiency is higher than charge coupled device. Third, the excitation laser power was also optimized so as not to overly saturate photon emission from fluorophores. A linear range of two orders of magnitude (from 8 nM to 0.8 μM; correlation coefficients, >0.998) was obtained for Rhodamine B, and the limit of detection (LOD) was <5 nM (run buffer, borax, 10 mM, pH 9.0; injection voltage, +600 V, for 30 s; separation voltage +1,600 V, for 170 s; signal-to-noise rate (SNR), 8; time RSD, 2.3%; peak value RSD, 7.4%; $n = 10$) (as shown in Fig. 2), which sensitivity was lower than that of photo counting model detection (8.5 pM) (Fister et al. 1998) and much higher than absorbance detection (0.95 μM) (Collins et al. 2007), LIF optical fiber detection (0.1 μM) (Lin et al. 2003) and photothermal technique detection (2 μM) (Katayama et al. 2007) for Rhodamine B. Because of the high background signals (Xu et al. 2007), the LOD of LED ($\lambda = 470$ nm) induced fluorescence detection (0.02 μM) (Liu et al. 2008) is still lower than that of LIF (1.1 pM) (Fu et al. 2006; Götz and Karst 2007) for fluorescein. The results demonstrated high reproducibility and sensitivity.

3.2 MCCD detection

Response of the MCCD was strongly dependent upon the frequency and amplitude of the excited signal. The optimum working frequency must be found experimentally

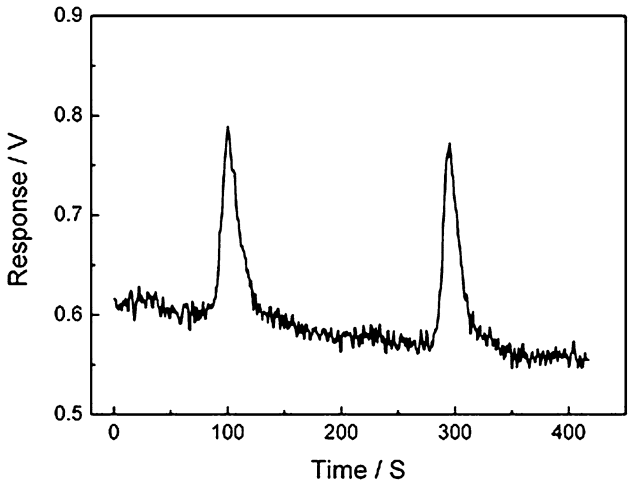

Fig. 2 Electropherogram of 5 nM Rhodamine B for two times with confocal LIF. Conditions were described in the text

for the given detector design and set of conditions. The influences of the applied ac voltage frequency on peak area and SNR at voltage amplitude of 5 Vp-p were shown in Fig. 3a. The curves were plotted over the 0–500 kHz range for Rhodamine B. The peak area increased slowly between 60 and 190 kHz, then more rapidly (reaching the maximum at 289 kHz), and decreased sharply thereafter. When the frequency was lower than 60 kHz, there was no response for any running buffer or sample. The SNR had the same tendency as the peak area (except at 240 kHz). High frequency or high voltage would induce unstable response and baseline drift, which could lead to the decrease of SNR. The effects of the applied ac voltage amplitude on the peak area and SNR at frequency of 289 kHz were illustrated in Fig. 3b. The peak area increased with the peak-to-peak amplitude of the applied ac voltage (Vp-p) from 2 to 8 Vp-p. When the voltage was lower than 1 Vp-p, there was no response. The SNR steadily increased between 1 and 5 Vp-p, and decreased after 5 Vp-p. The most favorable SNR characteristics were obtained at the frequency of 289 kHz and the voltage of 5 Vp-p.

The intensity of the response and the relative peak value would be remarkably increased with increasing the amplificatory multiple. The amplificatory multiple of 600× was chosen because the most favorable response characteristics could be achieved at this amplificatory multiple. A linear range of nearly two orders of magnitude (from 0.25 μM to 20 μM; correlation coefficients, >0.997) was obtained for Rhodamine B, and the LOD was 0.1 μM (SNR = 6), which sensitivity was higher than that of inorganic ions detection (about 1 μM) reported using CCD (Wang et al. 2003).

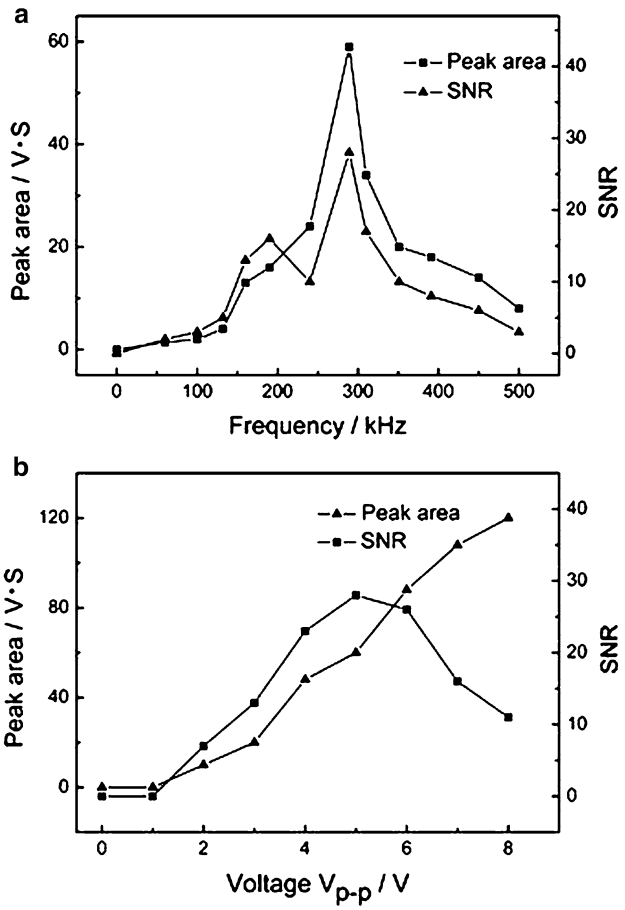

Fig. 3 Influence of the (**a**) frequency and (**b**) voltage amplitude upon the peak area and SNR of 80 μM Rhodamine B with MCCD

3.3 Simultaneous detection

In addition to the highly sensitivity of the two detection systems, the dual detector allows the attainment of two simultaneous signals for the same component. The electropherogram for the simultaneous LIF and MCCD detection of Rhodamine B was shown in Fig. 4. The simultaneous responses were obtained at the same time and the time RSD was 5.3% ($n = 9$). The sensitivity of LIF was higher than that of the MCCD which was more sensitive to ions detection. The generation of dual simultaneous responses offers several analytical advantages, for example, the simultaneous responses can be used for confirming the peaking identity, estimating the peak purity, or improving the reproducibility (Wang and Pumera 2002; Lapos et al. 2002).

4 Conclusions

The novel dual confocal LIF/MCCD detector for the CE microchip permits sensitive simultaneous detection of

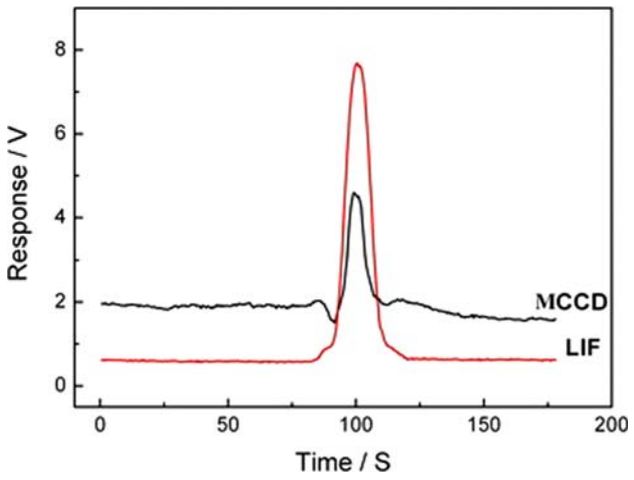

Fig. 4 Simultaneous detection of 2 μM Rhodamine B with dual confocal LIF/MCCD detector

mixtures containing both ionic and fluorescent compounds. This dual detector combines the respective response advantages of confocal LIF and MCCD methods to different analytes, thus it could simultaneously respond to mixtures and could offer more information of analytes. The dual detector offers the possibility of simultaneous determination of one analyte in two different ways and would be very useful in daily clinical analysis for making obtained results more reliable. Moreover, the two detection methods were mutually independent and provided opportunities to optimize performance for both detection and separation.

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