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An integrated light emitting diode-induced fluorescence detector for capillary electrophoresis

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Abstract

An integrated light emitting diode (LED)-induced fluorescence detector was described and evaluated. The LED and its related components including lens and interference filter, the optical fiber used to collect fluorescence, and the capillary column are integrated into a substrate block, which eliminates the need of align procedure of the fiber and the capillary. Forty-fold enhancement of sensitivity was obtained compared with our previous work and the detection limit for fluorescein was 5 nM. Application of the detector for the analysis of FITC-labeled *Ephedrine* extract was demonstrated.

Keywords: Light emitting diode; Fluorescence detection; Capillary electrophoresis

1. Introduction

Laser-induced fluorescence (LIF) detection is frequently used in the ultra trace sample analysis in capillary electrophoresis (CE). Lasers have extremely high brightness and good spatial property, which is beneficial to fluorescence detection. In spite of these advantages, lasers, such as the Ar^+ and He–Cd are generally expensive, relatively bulky, high power consumption and have limited lifetime (∼3000 h), which hinder its use in miniaturized or portable instrumentation to a great extent. The limited choice of excitation wavelengths is another drawback of laser sources. Recently, LED-induced fluorescence detection (LED-IF) has aroused much interest [1–5]. The combination of high stability, small size, low cost, and very long lifetime makes the LEDs an attractive excitation source for fluorescence detection, especially to design miniaturized or portable setup. In addition, the LEDs emitting at different wavelength ranging from near-UV to near-IR are commercial and one can easily chose suitable excitation wavelength according to the properties of samples. Bruno et al. presented a LED-IF detector in CE using named pigtailing approach, to minimize losses and generation of stray light at the various optical interfaces [2]. Dasgupta and coworkers described the use of LED-FD combined with liquid core waveguide (LCW) technique in CE [3–5]. Su et al. determined riboflavin in beer using a blue LED-IF detector in CE combined with dynamic pH junction technique [6]. Recently, Yang et al. described a compact LED-IF detector based on collinear optical configuration in CE [7]. Zhang et al. presented a LED-IF detector in a LCW microfluidic CE system by synchronized dual wavelength modulation to improve the signal-to-noise level [8].

In our previous work, we described a simple LED-IF detector for CE [9]. In this setup, optical fiber with spherical end was used to collect the fluorescence. Low sensitivity (0.2 μM LOD for fluorescein) was obtained mainly arising from low excitation intensity, high background level and low collection efficiency for fluorescence. The present work describes an integrated LED-IF detector consisting of a brighter LED, and an optical fiber with bigger core diameter. In addition, to reduce the background level, an interference filter was used at the excitation axis. The use of a metal package miniaturized photomultiplier tube (PMT) and short optical fiber drastically reduces the size of the whole system. Compared to our previous work, ∼40-fold enhancement of sensitivity was obtained using fluorescein as model sample. The utility of this detector to analyze the fluorescein isothiocyanate (FITC)-labeled *Ephedrine* extract was demonstrated.

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2. Experimental

2.1. Reagents and apparatus

A homemade CE system, consisting of a fused silica capillary (50 cm × 100 μm i.d., 40 cm to the detector) and a high-voltage power supply (0–30 kV, Dongwen, China), was used to evaluate the detector. The working electrolyte for CE separation was $Na_2B_4O_7$ buffer (10 mM, pH 9.0). Sample injection was carried out by hydrodynamic technique. All reagents used were of reagent grade, and deionized (DI) water was used throughout.

Preparation of FITC-labeled *Ephedrine* extract Dried *Ephedrine* drug was crushed by disintegrator and then filtered. One gram powder was dissolved in 10 mL methanol. The solution was sonicated for 30 min, and left to cool down at room temperature for 10 min, and then centrifuged at 3000 rpm for 15 min. Take the supernatant and pass through 0.45 μm filters and add fresh methanol until its volume reaches 10 mL. The stock solution was stored at 4 °C prior to use. Stock FITC-labeled *Ephedrine* extract solution was prepared by mixing 50 μL stock solutions of *Ephedrine* extract solution with 750 μL of borate buffer and 200 μL of 1 mM FITC stock solution, and the resultant mixture was left in the dark overnight at room temperature. The labeled products were diluted with borate buffer prior to use.

2.2. Optical system

The optical arrangement of LED-FD was shown in Fig. 1. A blue LED (λ_{max}, 470 nm, 5 mW, Shifeng Corp., China) driven by a 5 V constant voltage source through a 100 Ω current-limiting resistor was used as the excitation source. LED light was collimated and focused with two quartz achromatic lenses (focal length is 15 and 10 mm, respectively) into the capillary. To reduce the scattering light from the capillary wall, an aperture of 0.2 mm was used to restrict the beam size. An interference filter (BP 470 nm, FWHM 20 nm, Huibo Optical Corp. Ltd., China) was inserted between two lenses to eliminate the interference of long wavelength from LED. A detection window on the capillary was formed by burning off the polyimide coating (5 mm in length) with an electrical coiled resistance. Fluorescence was collected with a right-angle geometry by an optical fiber (core 400 μm, cladding 480 μm, Chunhui, China) and passed through two blocks of interference filters (BP 530 nm; FWHM 30 nm; Huibo Optical Corp. Ltd., China). The fluorescence signal was then detected by a metal package miniaturized PMT (H5784, Hamamatsu, Japan). The signal from the PMT was acquired by chromatographic workstation (Dalian Sci-Tech Inc., China).

2.3. Construction of integrated block of optical system and the capillary column

The integrated block was similar to that of previous report except some modification [9]. Fig. 2 gives the schematic diagram of the experimental setup. The optical fiber and the capillary were integrated into the substrate block by using T-style rectangle grooves (1.6 mm × 1.5 mm, width × depth) machined onto the substrate. This design eliminates the need of alignment between the capillary and the fiber. The use of rectangle groove instead of V-groove can reduce the distance between the capillary column and LED, resulting in smaller beam size illuminated on the capillary. To restrict the size of the light beam, an aperture is drilled exactly at the center of the cross point of T-style grooves. LED and its related optical components including two achromatic lenses and a interference filter are placed inside an aluminum tube, which is mounted on the backside of the substrate block by fine screw. The distance between them can be optimized by rotating the aluminum tube until the highest fluorescence signal from the PMT is found, when 1 μM fluorescein continuously flowing through the capillary driven by gravity. All the alignment process is easy to perform even for an

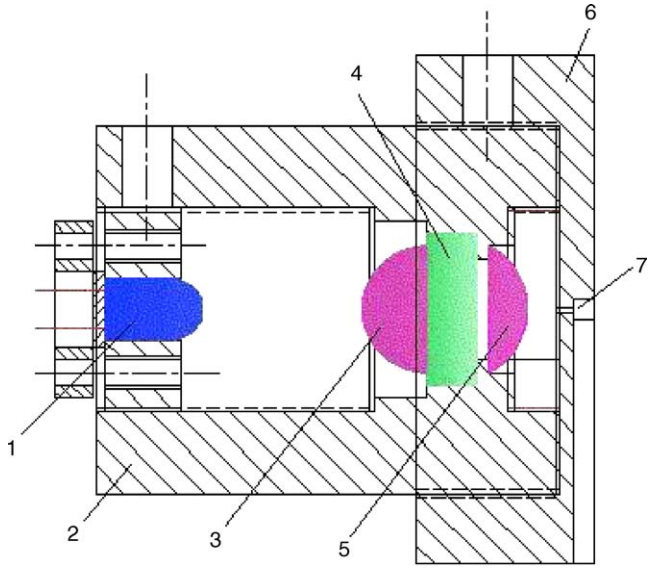

Fig. 1. Cross sectional view of the optical system: 1, LED; 2, aluminum tube; 3, 5, achromatic lens; 4, interference filter; 6, substrate block; 7, aperture.

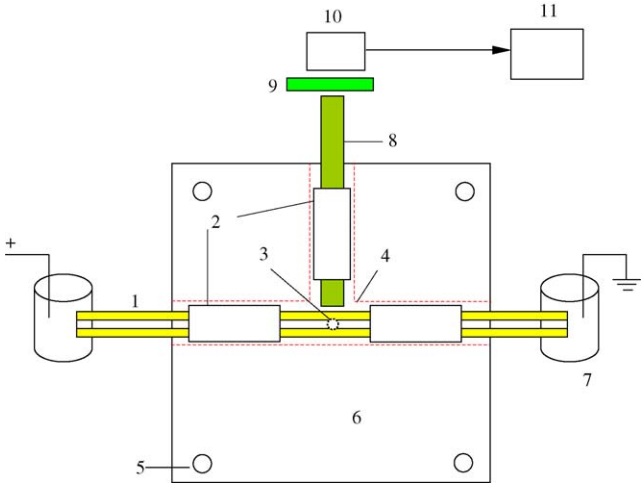

Fig. 2. Schematic of experimental setup: 1, fused-silica capillary; 2, Teflon tubing; 3, aperture; 4, rectangle groove; 5, fixed hole; 6, substrate block; 7, buffer vial; 8, optical fiber; 9, interference filter; 10, PMT; 11, PC.

inexperienced personal. The cover block is held to the substrate block by screws.

2.4. Experiments with flowing fluorescein

One end of the inlet capillary (0.32 mm i.d.) is immersed into a bottle filled with water and the other end is connected with the separation capillary column (0.1 mm i.d.). The water flows through the capillary for a few minutes by means of gravity in order to measure the baseline noise. The water is then replaced by a 1 μM fluorescein solution and flow through the capillary in the same way in order to measure the change of signal. Finally, water flows again through the capillary to check the consistency of the baseline.

3. Results and discussion

3.1. Effect of different optical setup

In on-column detection mode, it is essential to focus the excitation light into the center of the capillary in order to get higher sensitivity and lower background level. It is easy to focus the laser beam into a small spot (~20 μm), but is very difficult for LED source because of its incoherent characteristic. We examined three focusing manners, including (A) direct LED; (B) LED + lens, and (C) LED + Objective (20×), and compared the background level, noise, signal and signal to noise ratio (SNR) in each manner. The measurements were carried out according to the description in Section 2.4. Background level was determined by monitoring the PMT output difference with the LED on and off. The experimental results are listed in Table 1. The combination of LED and lens gives the best SNR, and at the same time yields the highest background signal and noise level, resulting from the highest excitation intensity on capillary column. We performed all the following experiments by using this manner. In addition, though the use of higher magnification of Objective (e.g. 40×) may give better focus effect, it was difficult to operate due to shorter working distance.

3.2. Effect of excitation filter

Compared to lasers, LED is a polychromatic source. Previous reports have indicated that the sensitivity can be severely degraded if the long wavelength LED light reaches the PMT [10,11]. In fact, relative broad emission spectrum of LED (30–100 nm) always overlaps with fluorescence spectrum and results in high background level and noise, as proven in our previous work [7]. Fig. 3 gives the emission spectrum of LED and the transmission curve of the interference filter used in this work. The effect of filter on background level, noise and SNR was shown in Table 2. It can be seen that the background level and noise can be reduced about 144 and 14.5-fold, respectively, when an interference filter is used at the excitation axis. Also, the SNR can be improved by four-fold.

Table 1
Comparison of different focusing manner

Types	Background level (mV)	Noise (mV)	Signal (mV)	SNR
Direct LED	226.1	0.23	64.2	279.1
LED + lens	328.6	0.32	102.6	320.6
LED + Objective	278.8	0.26	74.4	286.2

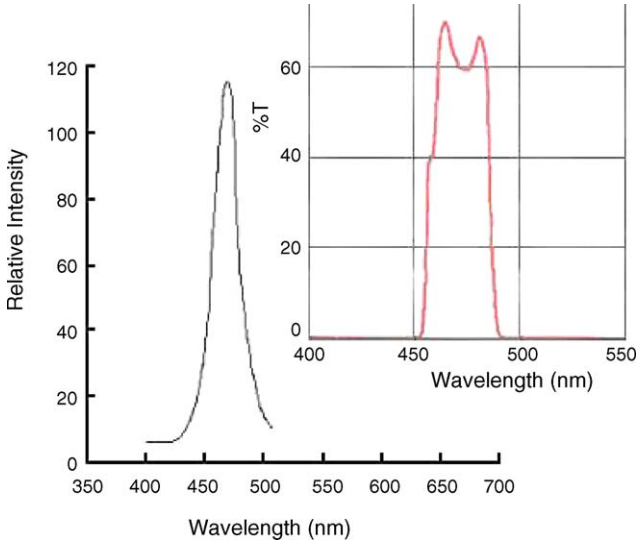

Fig. 3. Emission spectrum of LED and the transmission curve of the filter used in this work.

Table 2
Effect of interference filter placed at the excitation axis on the detection

Types	Background level (mV)	Noise (mV)	Signal (mV)	SNR
With no filter	328.64	0.32	102.6	320.6
With filter	2.28	0.022	24.83	1241.5

3.3. Effect of distance between the optical fiber and the capillary

In this setup, an optical fiber is perpendicularly placed to the capillary and used to collect fluorescence. Thus the distance *d* between the fiber and the capillary determines the sensitivity of the detector to a great extent. Fig. 4 shows this effect at *d*

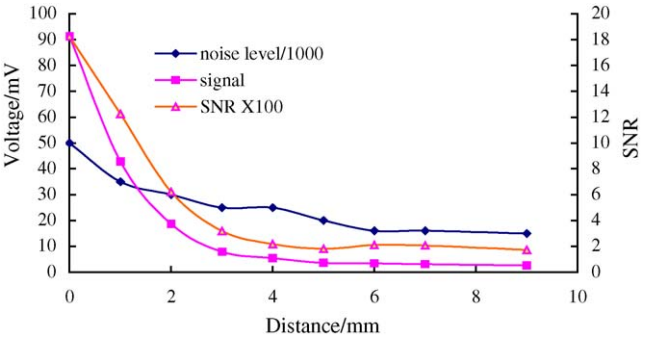

Fig. 4. Effect of distance between the optical fiber and the capillary.

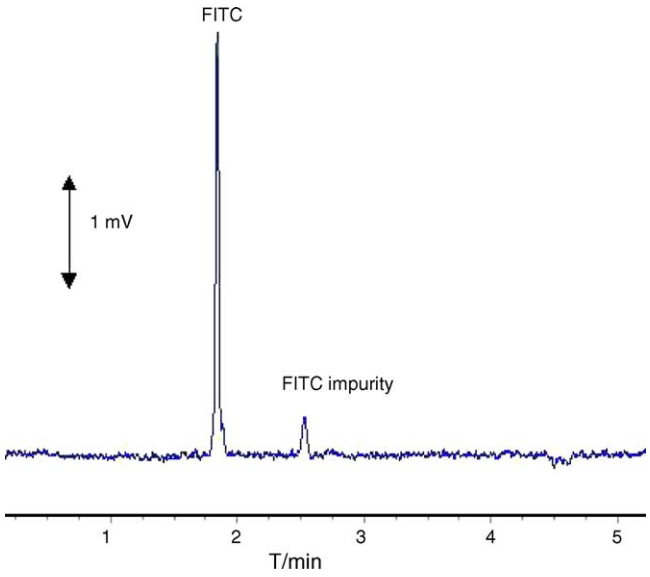

Fig. 5. Electropherogram of 0.1 μM fluorescein solution. Siphon injection, 10 cm × 10 s; separation voltage, 16 kV.

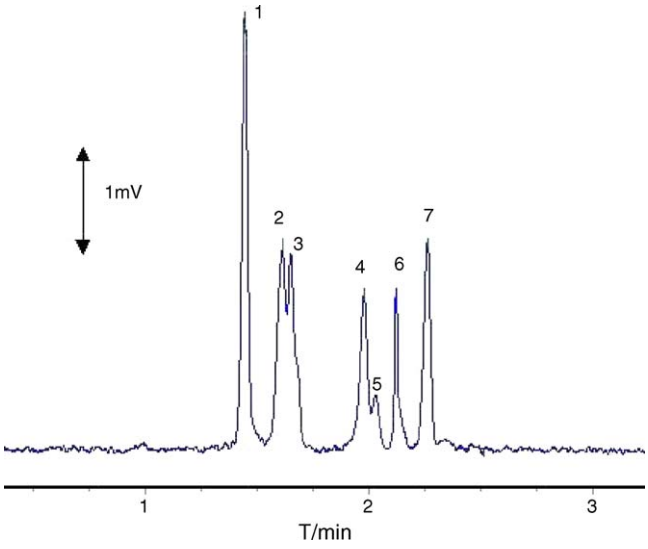

Fig. 6. Electropherogram of FITC-labeled *Ephedrine* extract. Siphon injection, 10 cm × 10 s; separation voltage, 18 kV; buffer solution, $Na_2B_4O_7$ buffer/acetonitrile (15/2); sample concentration, 0.1 μM; peak identification, 1–3, FITC-labeled *Ephedrine* extract; 4–7, excess FITC and its hydrolysis products.

between 0 and 9 mm. As expected, the shorter the distance, the higher the collection efficiency of fluorescence, and the higher the sensitivity. Considering the fragile optical window of the capillary, $d = 1$ mm distance is adopted in this setup.

3.4. System characteristics

The performance of the detector was evaluated by using fluorescein as the standard fluorophores. A typical electropherogram obtained for 0.1 μM fluorescein solution is illustrated in Fig. 5. At a signal-to-noise ratio (S/N) of 3, 5 nM of limit of detection (LOD) was achieved, which is 40 times lower than that of our previous report [9]. In addition, the dynamic range the detector is from 10 nM to 100 μM and exhibit a good linear response in the range of 0.01–5 μM ($R^2 = 0.998$). The reproducibility of the detector was tested by detecting 0.1 μM fluorescein and the RSD of peak height was 3% ($n = 10$), showing good stability of this detector. Although the detection limit obtained are much higher than those usually achieved by LIF detection or even conventional fluorometer, this detector exhibits advantages such as simple in structure, low cost and robust, light weighted, and very small in size, that make it contribute to the reduction of the cost of CE equipment. The size of the whole detector is only 5 cm × 12 cm × 6 cm ($W \times L \times H$), which is about 1% of the volume of conventional fluorometers or LIF system. Direct comparisons with commercial fluorometer or LIF detection were not made, but it can be made with that of LED-based fluorescence detectors. Dasgupta and co-workers [3,4] described a LED-FD combined with liquid-core waveguide technique in CE, achieving in 200-amole fluorescein of mass detection limit (injection volume 25 nL). The LOD value of this detector was comparable to that quoted by de Jong et al. (3 nM for fluorescein) [10].

To show the utility of this detector in CE, a representative application was implemented. Fig. 6 shows the analysis of FITC-labeled *Ephedrine* extract. To improve the separation effect, a little acetonitrile was added into the buffer solution (acetonitrile/buffer = 2/15). We did not make identification of the peaks in the electropherogram due to lack of standard *Ephedrine* sample. Other applications such as peptides and amino acids analysis were demonstrated in our previous work. These results showed that this LED-IF detector is suitable for routine analysis of some drugs and biomolecules.

4. Conclusion

An integrated LED-induced fluorescence detector for CE has been described. This integrated detector is very compact and the size is only 5 cm × 12 cm × 6 cm ($W \times L \times H$). Compared to our previous work, 40-fold enhancement of sensitivity can be obtained due to three improvements. They were: (A) use of a brighter LED and modification of integrated device of the capillary and optical system to increase excitation efficiency; (B) use of interference filter at the excitation axis to reduce the background level and noise; (C) use of optical fiber with bigger core diameter to increase the collection efficiency of fluorescence. This design eliminates the alignment procedure of the capillary and the fiber, which is always a tedious procedure in on-column detection. The detector shows reasonable high sensitivity and can be expected to routine analysis of protein, peptide, drugs and others compounds combined with CE. In addition, this detector can be applied to micro-LC and flow injection system.

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