A collinear light-emitting diode-induced fluorescence detector for capillary electrophoresis

Bingcheng Yang[1], Feng Tan, Yafeng Guan*

Department of Analytical Chemistry and Micro-Instrumentation, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116012, China

Abstract

A novel fluorescence detector based on collinear scheme using a brightness light-emitting diode emitting at 470 nm as excitation source is described. The detector is assembled by all-solid-state optical-electronic components and coupled with capillary electrophoresis using on-column detection mode. Fluorescein isothiocyanate (FITC) and FITC-labeled amino acids and small molecule peptide as test analyte were used to evaluate the detector. The concentration limit of detection for FITC-labeled phenylalanine was 10 nM at a signal-to-noise ratio (S/N) of 3. The system exhibited good linear responses in the range of 1×10^{-7} to 2×10^{-5} M ($R^2 = 0.999$).
© 2004 Elsevier B.V. All rights reserved.

Keywords: Light-emitting diode; Fluorescence detection; Capillary electrophoresis

1. Introduction

Capillary electrophoresis (CE) technique has aroused much interest in recent years. Among the detection modes commonly used in CE, the fluorescence detection as a highly sensitive and selective technology, are frequently used in routine biotechnological analysis. Lasers and discharge light sources are widely used as excitation sources in fluorescence detection [1,2]. The common limitations of these light sources are the bulky size, high power consumption and limited lifetime, which is one of the most expensive parts in CE equipment. In addition, the limited choice of excitation wavelengths may be the most important limitation of laser-induced fluorescence (LIF) detection.

The use of light emitting diodes (LED) as excitation source may provide a simple alternative [3–6]. The combination of extremely high stability, reasonably high intensity, small size,

low cost, and very long lifetime makes LED an attractive excitation source for spectroscopic detectors [7,8]. Several groups have made significant contributions to LED-based fluorescence detection used in CE. Bruno et al. [3] presented LED-induced fluorescence detector (LED-FD) in CE using named pigtailing approach, in which the optical components used for in this detector were assembled into a compact unit using refractive-index matching materials to minimize losses and generation of stray light at the various optical interfaces. Dasgupta and coworkers [4,8] described the use of LED-FD combined with liquid-core waveguide technique in CE, achieving in 200 amole fluorescein of mass detection limit. Recently, Hillebrand et al. [9] reported a LED-FD in CE using a pulsed mode-operating ultraviolet LED. Su and Lin [10] determined riboflavin in urine using a blue LED by CE combined with sample stacking. In addition, Wang and Morris [11] have demonstrated a continuous mode-operating UV LED in an analyte velocity modulation device applied for microfluidic devices. In previous reports, the optical scheme of LED-FD is usually based on orthogonal configuration for eliminating the interference of excitation beam [3,4]. Some reports described the use of confocal configuration of

* Corresponding author. Tel.: +86 411 83693515; fax: +86 411 83693510.
E-mail addresses: abing@dicp.ac.cn (B. Yang), kfguan@mail.dlptt.ln.cn (Y. Guan).
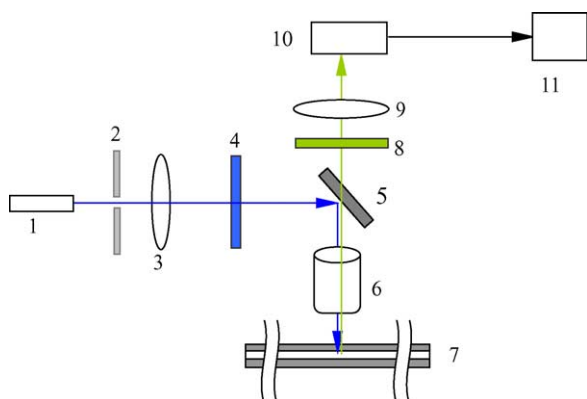[1] Tel.: +86 411 83693515; fax: +86 411 83693515.

Fig. 1. Schematic diagram of LED-induced fluorescence detection system: (1) LED; (2) iris; (3) lens (focal length 6 cm); (4), interference filter (BP 470 nm); (5) dichroic mirror; (6) objective; (7) capillary flow cell; (8) interfere filter (BP 530 nm); (9) lens (focal length 6 cm); (10) photomultiplier tube (PMT); (11) personal computer.

LED-FD [11,12], but details were not given. Compared with above two schemes, collinear configuration has the advantages such as simple, compact and effective. In this configuration, the choice of combination filters is vitally important to effectively reduce the interference of excitation light and background level. It is true especially to incoherent source such as lamp and LED.

Here we reported a novel LED-FD for CE based on collinear configuration. In this setup, a 470 nm high-brightness LED driven by direct current was used as the excitation source. FITC and FITC-labeled amino acids and peptides as test analyst were used to evaluate the performance of the detector.

2. Experimental

2.1. Reagents and apparatus

A homemade CE system, which constitutes a fused silica capillary (46 cm × 50 µm i.d., 34 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 (20 mM, pH 9.2). Sample injection was carried out by hydrodynamic technique. All reagents used were of reagent grade, and deionized (DI) water was used throughout.

2.2. Optical system

The optical arrangement of LED-FD is shown in Fig. 1. A blue LED ($\lambda_{max} \approx 470$ nm, optical power 2 mW, Shifeng Optic and Electronics Ltd., China) driven by a 5 V constant-voltage source through a 100 Ω current-limiting resistor was used as the excitation source. The LED light was shaped with a 2 mm iris and collimated with a 6 cm focal length achromatic lens. The collimated light passed through an in-terference filter (BP 470 nm, FWHM 20 nm, Huibo, China) and was reflected by a 45° 490 nm dichroic mirror (Huibo, China) and focused by a microscope objective (10 × 0.25, Jiapin, China) into the center of capillary flow cell. A detection window on the capillary column was formed by burning off the polyimide coating (5 mm in length) with an electrical coiled resistance. Fluorescence was collected through the objective, and passed through the dichroic mirror, a 530 nm interference filter (Huibo, China) and a lens (focal length 6 cm, Daheng New Epoch Tech. Inc., China), and then detected by a side-on photomultiplier tube (PMT) (R928, Hamamatsu, Japan) equipped with its own high-voltage power supply. The signal from the PMT was acquired by a chromatographic workstation (Dalian Sci-Tech Inc., China). To diminish light scattering and reduce background noise a hole was drilled on the capillary flow cell carrier opposite the detection area. The LED light went through the hole and was absorbed by a black cylinder underneath the hole.

3. Results and discussion

3.1. Emission spectrum of blue LED

A high brightness LED was used in this work and its FWHM is about 30 nm, which demonstrates acceptable monochromaticity. But extremely high background level was observed in our early experiment owing to the LED light interference, indicating that narrower spectrum was necessary to reduce the background in this setup. FITC was used to evaluate the detector performance and its absorption spectrum matches the LED emission spectrum.

3.2. Selection of filters

In collinear scheme, the interference of excitation light on the fluorescence detection is much stronger compared with the two optical schemes mentioned above. The situation is even worse for the incoherent light source such as lamp and LED. Significant background level was observed in our previous experiment owing to the long tail of LED emission spectrum. In this setup, an interference filter used to cutoff the long tail of LED spectrum was attempted to eliminate the excitation interference. In addition, dichroic mirror with 490 nm of cutoff wavelength was used to further reduce the excitation light level. An emission filter (central wavelength 530 nm, FWHM 40 nm) well matched the maximum of the emission wavelength of FITC and also contributed to the increase of sensitivity and the decrease of background noise. The results achieved proved that the combination of these filters effectively reduced the excitation light level.

3.3. System characteristics

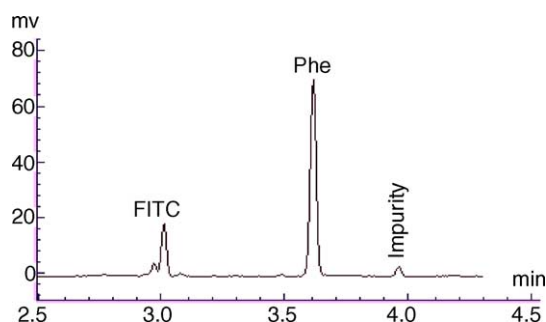A typical electropherogram obtained for 0.2 µM FITC-labeled phenylalanine solution is illustrated in Fig. 2. At a

Fig. 2. The electropherogram obtained for 0.2 μM FITC-labeled phenylala-nine. Conditions seen in the text.

Fig. 3. Electropherogram of FITC-labeled amino acid mixture. Amino acid concentration: 0.2 μM; separation voltage: 16 kV; hydrodynamic injection: $\Delta h = 10$ cm, $t = 15$ s; other conditions as in text.

Fig. 4. Electropherogram of FITC-labeled peptides mixture. Peptides con-centration: 0.1 μg/mL; separation voltage: 14 kV; hydrodynamic injection: $\Delta h = 10$ cm, $t = 10$ s; other conditions in text.

signal-to-noise ratio (S/N) of 3, 10 nM of limit of detection (LOD) was achieved for FITC-labeled phenylalanine solu-tion without any preconcentration procedure. In addition, the system exhibited good linear response in the concen-tration range of 1×10^{-7} to 2×10^{-5} M ($R^2 = 0.999$). This LOD is significantly better than the typical values encoun-tered in commercial equipment with absorbance detection, e.g. Li et al. achieved 195 μM LOD for phenylalanine at the absorbance wavelength of 200 nm [13] and Klampfl et al. provided 3 μM LOD for phenylalanine using 185 nm wave-length [14]. Although the detection limit obtained are much higher than those usually achieved by LIF detection and even conventional fluorometer, this detector exhibits many advan-tages over LIF and conventional fluorometer in that it is very simple, low cost and robust, and miniaturization, which make it contribute the reduction of the cost of CE equipment. Direct comparisons with commercial fluorometer or LIF detection were not made, but it can be made with that of LED-based fluorescence detectors. Obviously, the performance of this setup was greatly improved compared to our previous work in which optical fiber was used for the collection of fluores-cence [6]. The LOD value achieved of this detector is 100 times lower than that of the previous report in similar con-ditions [5]. Though the report demonstrated a miniaturized CE system, the separation capillary used was standard scale (48 μm i.d.). And the LOD was slightly better than that quoted by Dang et al. (19 nM for APTS-labeled glucose) using chip-based CE system [12].

3.4. Applications

To show the utility of this compact LED-FD in CE, a representative application is implemented. Fig. 3 shows a separation of three amino acids labeled by FITC. The 8, 10 and 15 nM of detection limits (S/N = 3) were achieved for ly-sine (Lys), phenylalanine (Phe) and tryptophan (Trp), respec-tively. In addition, the analysis of FITC-labeled dipeptides was also demonstrated and shown in Fig. 4. Three peptides including Pro–Met, Gly–Tyr and Gly–Thr can be separated baseline. These results showed that this LED-based fluores-cence detector is suitable for routine analysis of bio-technical samples. Considering the wide range of wavelength avail-
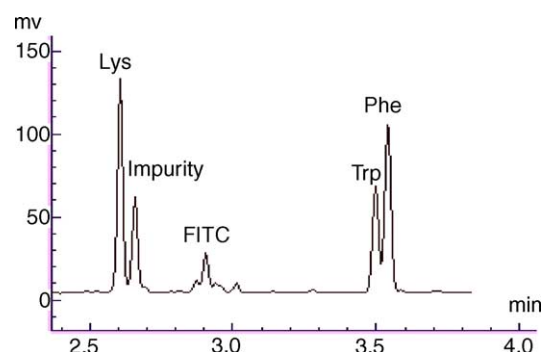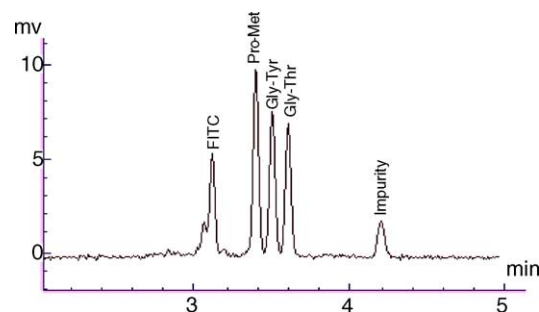
able of LED, it is convenient to select suitable LED and cor-responding filters to meet the detection needs for different fluorescent probe in visible spectrum range.

4. Conclusion

We have constructed a compact light-emitting diode-induced fluorescence detector for CE based on collinear scheme. The LOD for FITC-labeled phenylalanine was 10 nM without using any preconcentration procedure. Such LOD is quite satisfactory for many applications, consid-ering the relatively low optical power of LED. In addi-tion, the variety of LED emission wavelength can avoid the problem of limited choice of excitation wavelength of lasers in LIF detection. The miniaturized size and collinear optical scheme of this detector allows easy coupling with other microscale separation techniques such as microcol-umn liquid chromatography (μ-LC) and flow-injection anal-ysis (FIA) by on-column detection mode. Combined with sample preconcentration technique, the LOD can be fur-ther reduced to 1 nM or less [8]. Potential applications of the detector are routine analysis of protein, peptide, amino acids and others compounds combined with CE, HPLC or FIA.

Acknowledgement

References

[1] C. Gooijer, A.J.G. Mank, Anal. Chim. Acta 400 (1999) 281.
[2] E. Arriaga, D. Chen, X. Cheng, N.J. Dovichi, J. Chromatogr. A 652 (1993) 347.
[3] A.E. Bruno, F. Maystre, B. Krattiger, P. Nussbaum, E. Gassmann, Trends Anal. Chem. 13 (1994) 190.
[4] P.K. Dasgupta, G. Zhang, J. Li, C.B. Boring, S. Jambunathan, R. Al-Horr, Anal. Chem. 71 (1999) 1400.
[5] S. Wang, X. Huang, Z. Fang, Anal. Chem. 73 (2001) 4545.
[6] B. Yang, Y. Guan, Talanta 59 (2003) 509.
[7] P.A.G. Butler, B. Mills, P.C. Hauser, Analyst 122 (1997) 949.
[8] Q. Li, K.J. Morris, P.K. Dasgupta, I.M. Raimundo Jr., H. Temkin, Anal. Chim. Acta 479 (2003) 151.
[9] S. Hillebrand, J.R. Schoffen, M. Mandaji, C. Termignoni, H.P.H. Grieneisen, T.B.L. Kist, Electrophoresis 23 (2002) 2445.
[10] A. Su, C. Lin, J. Chromatogr. B 785 (2003) 39.
[11] S. Wang, M.D. Morris, Anal. Chem. 72 (2000) 1448.
[12] F. Dang, L. Zhang, H. Hagiwara, Y. Mishina, Y. Baba, Electrophoresis 24 (2003) 714.
[13] W. Li, D. Fries, A. Alli, A. Malik, Anal. Chem. 76 (2004) 218.
[14] C.W. Klampfl, W. Buchberger, M. Turner, J.S. Fritz, J. Chromatogr. A 804 (1998) 349.