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Light emitting diode induced chemiluminescence and its application as a detector for high performance liquid chromatography

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ABSTRACT

Some categories of compounds, including quinones, coumarins, flavins, and xanthene dyes, were found to produce strong chemiluminescence (CL) signals with luminol in sample solution under the irradiation of light emitting diodes (LED) with proper wavelengths. Based on this phenomenon, a compact photochemical reactor was constructed to develop a novel LED induced CL detector for high performance liquid chromatography (HPLC). The effects of related parameters including LED wavelength, luminol concentration, flow rate, pH, and eluents of HPLC were investigated in detail. Under the optimized conditions, the limits of detections (LODs) were in the range of 0.2–80 ng mL^{-1}. The applications and accuracy of the proposed method were validated by analyzing food samples such as milk powder, beer, candy and beverage with satisfactory results.

1. Introduction

Chemiluminescence (CL) has been recognized as one of the most useful analytical techniques owing to its low background, high sensitivity, simplicity, and low cost of instrumentation and maintenance. Thus, it has enjoyed widespread applications for environmental, pharmaceutical, biological, clinical and food analysis [1,2]. However, a major drawback of conventional CL is that only a limited number of target compounds can be detected [3]. Several approaches have been suggested to expand the range of detectable analytes including photo-induced chemiluminescence (PI-CL) [4–16], cataluminescence (CTL) [17–21] and dielectric barrier discharge-induced chemiluminescence (DBD-CL) [22,23]. The demand of gas phase condition in the CTL reactions and the DBD-CL makes them incompatible with liquid chromatography (LC), and subsequently, a nebulizer is needed to couple them with LC [22]. PI-CL, on the contrary, is suitable for liquid phase reaction and ease of coupling to HPLC, and this makes it a potential HPLC detector.

PI-CL detection is mainly based on the following modes: (i) photo-decomposing an analyte into a reductive with small molecules to be oxidized by an oxidative CL regent [4–10]; (ii) generating an oxidative species to react with reductive CL regents [11–15] or (iii) indirectly detecting analytes by the varied background CL values due to their inhibition or enhancement effect on photochemical reaction [16]. Therefore, it can effectively extend the detectable range of CL without tedious derivation steps, and several successful attempts have already been reported for the analysis of pharmaceuticals [4], pesticides [5–9], herbicide [10], quinones [11], aromatic compounds [12], organic peroxides [13], chemical oxygen demand (COD) [14], and saccharides [15].

In the reported luminol-based PI-CL systems, mercury lamp was the most frequently utilized light source, but it often leads to self-oxidation of luminol and accordingly, high background. In order to avoid the self-oxidation, luminol is often added after the light irradiation [13,14,24], but this demands a process of carrying the active species generated in photoreaction to the reaction cell to react with luminol. The loss of active species during the transport process leads to the decrease in sensitivity. Also, the application of a luminol-based PI-CL system is rare in HPLC for the reason that luminol can react with the radical generated from methanol contained in HPLC eluent under mercury lamp irradiation, resulting in a high background [24]. In some cases, a cooling unit is needed to eliminate the heat from the lamp, complicating the instrumentation [11,14,15]. Therefore, luminol-based PI-CL systems gain few applications in HPLC.

Light emitting diodes (LED) provide several advantages of low cost, long life-time, high intensity, wide available wavelengths ranging from violet to infrared, small sizes, low energy consumption, and ease of operation. In this work, utilizing LEDs with proper wavelengths as light sources, it was found that some categories of compounds can produce strong CL signals in the presence of lumi-

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nol with low background, and the two frequently used eluents in HPLC, methanol and acetonitrile (ACN), led to no increase of background. Therefore, LED was used to induce CL to establish a novel LED induced CL (LED-CL) detector for HPLC. It has several unique advantages over conventional luminol-based PI-CL systems such as simplicity, small size, low background, and low energy consumption.

2. Experimental

2.1. Reagents

Riboflavin, erythrosine, phloxine B, rose bengal, eosin, fluorescein, rhodamine B, hymecromone, vitamin K_3, and anthraquinone sulfonic acid sodium (ASAS) were used as analytes to evaluate the performance of the proposed method. Erythrosine and phloxine B are products of Sigma (St. Louis, MO, USA) and Alfa (Lancaster, USA), respectively. Riboflavin, fluorescein sodium, rhodamine B and vitamin K_3 were purchased from Kelong Reagent Co. (Chengdu, China), and ASAS and rose bengal from Guoyao Reagent Co. (Shanghai, China). The stock solutions of analytes were made in methanol and kept in a refrigerator at 4 °C. The working solutions were prepared by diluting the stock solution to desired concentration level with mobile phase.

Luminol (Sigma) was used as the CL reagent, and the stock solution of 0.01 mol L^{-1} was prepared by dissolving 0.4454 g powder in 250 mL doubly distilled water (DDW). HPLC grade methanol and ACN used as mobile phases were obtained from Dima Technology Inc. (Richmond Hill, Canada). The phosphate reagent used to prepare phosphate buffer solution in HPLC was provided by Kelong Reagent Co. (Chengdu, China).

2.2. HPLC system

The HPLC system consisted of a quaternary pump (L-2130, Hitachi, Japan), a Luna reverse phase C-18 column (150 × 4.6 mm, Phenomenex, USA), a post-column reactor and a computerized ultraweak CL analyzer (Xi'an Remax Co., Xi'an, China). The injections were carried out with an injector (7725i, Rheodyne, USA) equipped with a 100-μL loop. The flow rate through the column was maintained at 1.0 mL min^{-1}. The mixture of ACN (or methanol) and 10 mol L^{-1} phosphate buffer solution was used as mobile phase and 1 mol L^{-1} HCl and NaOH was utilized to adjust the phosphate buffer to the desired pH.

2.3. Photochemical reactor

The photochemical reactor consists of four LEDs (5 mW) and a quartz irradiation/reaction tube (2 cm in length × 2 mm i.d). The four LEDs were placed on both sides of the irradiation/reaction tube with their focus tips lying on the tube surface, as shown in Fig. 1. The size of the entire photochemical reactor is about 3 cm in length × 2 cm in width × 1 cm in height. The CL regent, luminol was pumped by a peristaltic pump (Qingpu Huxi Instrument Factory, Shanghai, China) and mixed with the eluent from the column through a three way connector. The mixed solution was irradiated in the irradiation/reaction tube by LEDs.

2.4. Sample preparation

For milk powder, 1.0000 g sample was accurately weighed into polypropylene tubes and 5 mL ACN was added. The sample was vibrated continuously to extract for 10 min. Then, 5 mL phosphate buffer of 0.01 mol L^{-1} at pH 5.0 was added. After homogenization and centrifugation at 4500 rpm for 10 min, the supernatant was filtered through 0.45 μm nylon filter and diluted appropriately before injection. For candy sample, 10 tablets were weighed and dissolved in 25 mL mobile phase (30% ACN + 70% 10 mol L^{-1} pH 7.0 phosphate buffer). After filtered through 0.45 μm nylon filter, the solution was diluted appropriately for analysis. The liquid samples such as beer and beverage were directly filtered through 0.45 μm nylon filter and diluted when necessary before injection.

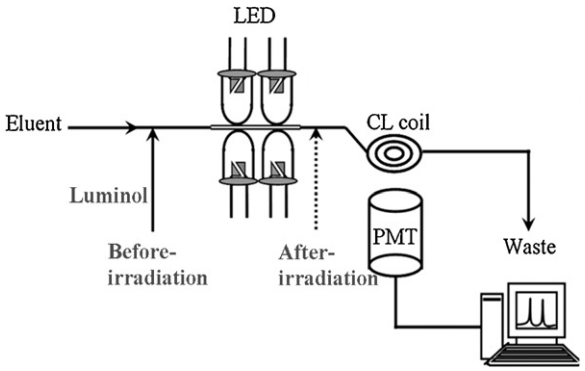

Fig. 1. Schematic diagram of the HPLC–LED-CL system.

3. Results and discussion

3.1. Design of LED-CL system

In the preliminary experiments, luminol was added before-irradiation or after-irradiation (solid line and dash line in Fig. 1, respectively) by a flow-injection CL system. It was found that the CL intensity in before-irradiation mode was much stronger than that obtained in after-irradiation mode. This may arise from different photo-oxidation mechanisms. Two photochemical reaction pathways, Type I and Type II, for luminol have been reported [25–27]. Type I pathway is initiated by the electron transfer from luminol to the analyte in triple state with the generation of luminol radical which subsequently reacts with oxygen in the solution to produce CL; Type II reaction is induced by energy transfer from excited triple state of the analyte to molecular oxygen to form singlet oxygen, which reacts with luminol. When luminol was added after-irradiation, only Type II reaction could happen since luminol was absent during irradiation; and when luminol was mixed with analyte prior to irradiation, both pathways could happen. The much

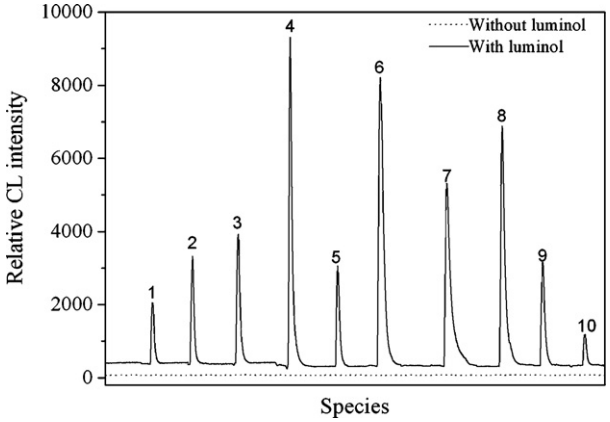

Fig. 2. CL signals with flow-injection CL system of (1) hymecromone; (2) vitamin K_3; (3) ASAS; (4) riboflavin; (5) erythrosine; (6) phloxine B; (7) rose bengal; (8) eosin Y; (9) fluorescein; and (10) rhodamine B. Conditions: luminol pH 11.0; luminol flow rate, 2.9 mL min^{-1}; luminol concentration, 1×10^{-5} mol L^{-1}; LED color, violet for (1), (2) and (3), white for the others; number of LEDs, 1; and concentrations of analytes, 5 μg mL^{-1} for (1), (2) and (3), 0.5 μg mL^{-1} for others.