Sensors and Actuators B 212 (2015) 264–272

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

An ultrasensitive calcein sensor based on the implementation of a novel chemiluminescence system with modified kaolin

Zhihua Wang, Li Guo, Dongmei Han, Fubo Gu*

State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing 100029, China

ARTICLE INFO

Article history:
Received 12 December 2014
Received in revised form 9 February 2015
Accepted 10 February 2015
Available online 18 February 2015

Keywords:
Chemiluminescence
Calcein
Sensor
Modified kaolin
Peroxynitrous acid
CRET

ABSTRACT

In this work, a novel, rapid and ultrasensitive chemiluminescence (CL) method is established for sensing the fluorescent dye calcein in water, which is composed of peroxynitrous acid (ONOOH), calcein and modified kaolin. This is the first time that the modified kaolin is applied to CL analysis and used as catalyst and adsorbent in the ONOOH-calcein-modified kaolin CL system to enhance the signals. In this work, we have optimized the conditions and found that the enhanced CL intensity is proportional to the concentration of calcein in the range of 0.1–100 μM. The limit of detection is 0.01 μM. The proposed CL sensing method is successfully applied to determine calcein in simulative samples prepared from tap water and river water with recoveries of 96.9–105.2%. The primary CL mechanism is proposed based on CL spectra, powder X-ray diffraction (XRD), high-resolution transmission electron microscopes (HRTEM) and fluorescent spectra, etc. The modified kaolin has a porous structure along with abundant oxygen vacancies, which provides a desirable environment where CL can be catalyzed easily. The CL enhancement is also attributed to the increased efficiency of CL resonance energy transfer (CRET) and the greater ability of calcein to absorb energy and emit it through fluorescence. The newly established ONOOH-calcein-modified kaolin CL system provides not merely an ultrasensitive approach to sensing calcein but also the application potential in determinating the substances which could have coupling interaction with the reagents in the CL system.

1. Introduction

As a fluorescent dye, calcein has good water solubility and fluorescence property [1,2], and it is currently the subject of numerous studies to explore its efficacy for indicator, simulation of drugs, fluorescence labeling, etc. [3–5]. Many derivatives of calcein play an important role in the field of cellular biology, such as calcein-AM [6]. Furthermore, calcein is also used as a fluorescence probe to investigate the concentration of target objects, especially in the study of biology and medicine field [7,8]. Wider applications demand more sensitive and convenient methods to determinate the calcein. On the other hand, the frequent using and improper emissions of calcein would lead to water pollution, and the calcein can access to biological bodies and do harm to people's health through the enrichment from the organisms in water. Therefore, it is important to explore an accurate and rapid method for the on-line monitoring or determination of calcein. Currently, the ultraviolet analysis and fluorescence analysis are often employed to determine the fluorescent dyes [9]. However, the intensities of absorption and fluorescence spectra are easily affected by the environment and the condition of the samples, which is adverse to the accuracy of measurement. The proposed CL analysis in our work is an accurate and fast method, with good linear correlation and fast response time [10]. Compared to ultraviolet and fluorescence analysis, the proposed CL sensor exhibits lower requirement of the samples and higher sensitivity of the determination results. Besides, the CL instrument is more economical and low cost even with the flow equipment than the ultraviolet or fluorescence instrument. Moreover, of particular importance here is the application potential of the proposed CL system in the field of trace detection of other substances which have a coupling interaction with the reagents in the CL system.

There are papers concerning the spontaneous weak CL decomposition of ONOOH/ONOO$^-$, and the produced CL signals can be amplified by some catalysts or energy acceptors [11,12]. In this case, we choose the ONOOH CL as the model CL system, because the CL emission spectrum of the ONOOH at about 400–500 nm would vary along with the reaction environment, and the emission wavelength range in 400–500 nm can excite some fluorescent dyes (calcein, rhodamine, fluorescein, and etc.) [13,14]. It has been

---

* Corresponding author. Tel.: +86 010 64445927.
E-mail address: gufb@mail.buct.edu.cn (F. Gu).

http://dx.doi.org/10.1016/j.snb.2015.02.037
0925-4005/

observed that the interaction between the ONOOH and calcein can produce stronger CL than the ONOOH, in which CL resonance energy transfer (CRET) may occur. Nevertheless, the CL intensity cannot meet the detection requirement of calcein in practical application because of the poor sensitivity. Thus, suitable catalysts will be needed to enhance the CL intensity. Transition metal ions and their oxides are the common catalysts for the CL systems [15,16]. However, metal ions are not environmentally friendly. The linear range and sensitivity fail to satisfy the requirement to trace the calcein in our case. Therefore, it is an attractive research to develop a green and efficient catalyst for enhancing calcein CL reaction.

Kaolin is one of the well-known low-cost natural clays with the general formula $Al_2Si_2O_5(OH)_4$. It is a soft, earthy, usually white mineral (dioctahedral phyllosilicate clay), produced by the chemical weathering of aluminum silicate minerals [17]. Its crystalline structure consists of stacked layers of tetrahedral silica sheets and octahedral alumina sheets [18,19]. Kaolin has been widely studied thanks to its rich source, non-toxicity, thermo-stability, and environmentally friendly nature. A mass of derivatives from modified kaolin have also become essential industrial materials (e.g., mullite, metakaolin, zeolite molecular sieve). Scientists have explored many methods to improve its properties for better application, such as calcination, acidic and alkaline modification, stripping and intercalation, and surface modification [20–24]. Modified kaolin has been widely applied in ceramics, cosmetics, paints, fillers, and adsorbents in wastewater treatment, etc. [25–28]. Adsorption experiments using modified kaolin as the adsorbent have been reported on some metal ions and some basic dyes, such as methylene blue [29] and malachite green [30]. The researchers have observed the usability of modified kaolin as an adsorbent for the removal of dyes or metal ions, which reveals that kaolin has a huge potential in this field [31]. It has also been reported that modified kaolin was used as the catalyst to promote the reaction [32,33]. Thus, we choose modified kaolin as the catalyst and adsorbent in the CL system. Considering the wide material sources and simple modification method, in later work, the modified kaolin has huge potential to become a commercial material to serve as a universal catalyst or adsorbent.

In this work, we firstly established an ultrasensitive calcein sensor based on the implementation of a novel ONOOH-calcein-modified kaolin CL system by using flow injection analysis (FIA)-CL method. It is particularly worth mentioning that to the best of our knowledge, it is the first time the modified kaolin is applied to CL analysis and play the role of catalyst and adsorbent, which has economic value to become commercial product. The CL signals of the ONOOH-calcein system are amplified when the modified kaolin is added, and there is a good linear relation between the CL signal and the concentration of calcein in the range of 0.1–100 μM. Most common ions in common water have little effect on the ONOOH-calcein-modified kaolin CL. Under the optimum experimental conditions, the proposed method can be employed for the determination of calcein in natural aquatic environment. In order to control and employ the ONOOH-calcein-modified kaolin CL better and more widely, the primary mechanism has been discussed. In terms of the significance of the newly established ONOOH-calcein-modified kaolin CL system, it provides not merely an ultrasensitive approach to sensing calcein but also the application potential in determinating the substances which have a coupling interaction with the reagents in the CL system, such as some drug molecules and organic substances [34,35].

2. Experimental

2.1. Reagents

Kaolin was purchased from Tianjin Guangfu Chemical Reagent Company, chemical grade. NaOH was obtained from Beijing Chemical Reagent Company, analytical grade. A 0.02 M nitrite stock solution was prepared from 0.005 mol $NaNO_2$ (Tianjin Chemical Reagent Company, analytical grade) in 250 mL of deionized water. H_2O_2–HCl solutions were freshly prepared via the volumetric dilution of commercial H_2O_2 (30% (v/v), Beijing Chemical Reagent Company) and HCl (36% (v/v), Beijing Chemical Reagent Company) with ultrapure water, respectively. A 0.01 M stock solution of calcein was prepared by dissolving 0.1 mmol calcein (Sinopharm Chemical Reagent Limited Company, analytical grade) in 10 mL of deionized water. Other standard calcein solutions were obtained through diluting the 0.01 M stock solution of calcein to different concentrations.

2.2. Apparatus

The powder X-ray diffraction (XRD) measurements were performed on a Rigaku (Japan) D/max2500VB2+/PC X-ray diffractometer equipped with graphite monochromatized Cu/Kα radiation (λ = 1.54056 Å). The 2θ angle of the diffractometer was stepped from 5° to 70° at a scan rate of 0.2°/s. The surface morphology of the sample was obtained through high-resolution transmission electron microscopes (HRTEM, JEM-3010, JEOL). The CL detection was conducted on an Ultra-Weak Luminescence Analyzer, which was purchased from the Institute of Biophysics, Chinese Academy of Science, Beijing, China (Biophysics Chemiluminescence, BPCL). The CL spectra were obtained through the Ultra-Weak Luminescence Analyzer with a set of commercial optical filters. The fluorescence spectrum of calcein solution was obtained by using an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) at a scanning rate of 240 nm/min. The fluorescence lifetime was obtained by using an Edinburgh Instruments FLS980 spectrometer and measured by excitation wavelength at 473 nm with an EPL-470 picosecond pulsed diode laser. The percentage contribution of each lifetime component to the total decay was calculated with the F980 Edinburgh instrument software.

2.3. Modification of Kaolin

Commercial kaolin was modified by NaOH under heating. For example, 8.0 g kaolin was added to 60 mL NaOH solution (3.5 M) in a Teflon high pressure reactor. The reactor was heated in a vacuum drying oven at 110 °C for 8 h. The product was centrifuged and washed by using deionized water for 3–4 times, and then deionized water was added to a total volume of 20 mL. The concentration of the modified kaolin was 0.4 g/mL at this moment. The product was mixed with the sample and stock solution of calcein and injected into the flow injection system, and the concentration of the modified kaolin was 0.2 g/mL in the mixture.

2.4. Procedures for CL determination

The schematic diagram of the FIA-CL system is illustrated in the inset of Fig. 1. It is consisted of two peristaltic pumps (BT-100M, Baoding, China), a 110 mL loop injector, a six-way injection valve (Shimadzu, Tokyo, Japan), a flow cell, a photomultiplier (PMT) and a BPCL luminescence analyzer. When the catalytic activity of the modified kaolin on the ONOOH-calcein CL was investigated, the calcein solution was added to the modified kaolin colloidal solution to an appropriate concentration. The mixture was injected into the ONOOH solution which was generated from $NaNO_2$ solution (0.02 M) and acidulated H_2O_2 solution (0.05 M) on-line through a 110 mL loop-valve injector with the flow rate of 1.62 mL/min. The reaction liquid flowed via a spiral-shaped flow cell positioned on top of a PMT operating at 950 V, and the PMT signals were imported to the computer for data acquisition.