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Chemiluminescence flow biosensor for glucose using Mg-Al carbonate layered double hydroxides as catalysts and buffer solutions

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ABSTRACT

In this work, serving as supports in immobilizing luminol reagent, catalysts of luminol chemiluminescence (CL), and buffer solutions for the CL reaction, Mg-Al-CO_3 layered double hydroxides (LDHs) were found to trigger luminol CL in weak acid solutions (pH 5.8). The silica sol–gel with glucose oxidase and horseradish peroxidase was immobilized in the first half of the inside surface of a clear quartz tube, and luminol-hybrid Mg-Al-CO_3 LDHs were packed in the second half. Therefore, a novel CL flow-through biosensor for glucose was constructed in weak acid solutions. The CL intensity was linear with glucose concentration in the range of 0.005–1.0 mM, and the detection limit for glucose (S/N=3) was 0.1 μM. The proposed biosensor exhibited excellent stability, high reproducibility and high selectivity for the determination of glucose and has been successfully applied to determine glucose in human plasma samples with satisfactory results. The success of this work has broken the bottleneck of the pH incompatibility between luminol CL and enzyme activity.

1. Introduction

Diabetes mellitus is a chronic disease characterized by high blood sugar (glucose) either because the body does not produce sufficient insulin, or cells do not respond to the produced insulin. It is a major and growing public health problem across the world. If diabetes is not adequately controlled, the patient has a significantly higher risk of developing complications, such as hypoglycemia, ketoacidosis, and metabolic disruption (Skyler, 2004). The International Diabetes Federation estimates that about 360 million people worldwide are living with diabetes, which is expected to rise to 552 million by 2030 if no urgent action is taken (Veerapur et al., 2012). Therefore, the determination of glucose is of importance in the monitoring of diabetic diets. The majority of conventional glucose sensors rely on an enzymatically based sensing scheme (Amerov et al., 2005; Hua et al., 2012; Li et al., 2010; Tierney et al., 2009).

In the past few years, chemiluminescence (CL) flow-through sensors have received increasing attention because of their operational convenience, instrumental simplification, environmental friendliness, low cost, resource savings and increased accuracy (Li et al., 2001; Yu et al., 2010; Zhou et al., 2005). The luminol CL reaction usually occurs in a strongly basic medium (pH 10–11); while glucose oxidase (GOD) retains its maximum activity at pH 5.5. Therefore, the known and well assessed luminol CL flow-through glucose sensors are generally fabricated by immobilizing GOD for the formation of H_2O_2 accompanying mobile luminol (Lan et al., 2008; Li et al., 2008; Lin et al., 2001). Due to the pH incompatibility issues, it is still a great challenge to achieve a micro-fabricated device immobilizing luminol and enzyme in one column.

Layered double hydroxides (LDHs) are an important class of host–guest materials consisting of positively charged metal hydroxide sheets with charge-balancing intercalated anions and water molecules (Bastianini et al., 2012; Evans and Duan, 2006). Mg-Al-CO_3 LDHs are extensively studied as actual catalysts, catalyst precursors, catalyst supports and optical materials due to their high stability and convenient synthesis (Chitrakar et al., 2011; Shi and He, 2011; Xu et al., 2010). Recently, we observed that Mg-Al-CO_3 LDHs can greatly catalyze the luminol–H_2O_2 CL reaction, and the CL intensity was in proportion to the concentration of Mg-Al-CO_3 LDHs; however, higher concentrations of Mg-Al-CO_3 LDHs were able to clog the tubing, which is becoming a significant limiting factor in their further applications (Wang et al., 2011a, 2011b).

In this study, the silica sol–gel with GOD and horseradish peroxidase (HRP) was immobilized in the first half of the inside surface of a clear quartz tube, and the luminol-hybrid Mg-Al-CO_3 LDHs were packed in the second half, which was constructed a CL flow cell, adjacent to a photomutiplier tube (PMT). In this case, Mg-Al-CO_3 LDHs served as supports in immobilizing luminol

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reagent, catalysts of luminol chemiluminescence (CL), and buffer solutions for the CL reaction (Cheng et al., 2009; Mandal et al., 2009; You et al., 2001). Therefore, using this simple micro-fabricated device, a stronger light can also emit in weak acid or neutral medium. This novel CL flow-through biosensor has been successfully applied to determine glucose in human plasma samples with simple procedures, shorter response time, high selectivity and wide pH compatibility.

2. Experimental

2.1. Chemicals and solutions

Analytical grade chemicals including $Mg(NO_3)_2 \cdot 6H_2O$, $Al(NO_3)_3 \cdot 9H_2O$, Na_2CO_3, glucose and NaOH were purchased from Beijing Chemical Reagent Company, and were used without further purification. A 0.01 M stock solution of luminol (3-aminophthalhydrazide) was prepared by dissolving luminol (Acros, USA) in 0.1 M NaOH solution without purification, and was used after about two weeks in order to make the luminol solution stable. GOD (from *Aspergillus niger*, $\geq$264 U/mg) and HRP (1000 U/mg) were obtained from SERVA Electrophoresis GmbH (Germany). The GOD solution was prepared by dissolving 5.4 mg GOD in 1.0 mL phosphate buffer (pH 7.4) and the HRP solution was prepared by dissolving 10.8 mg HRP in 1.0 mL phosphate buffer (pH 7.4). Glucose stock solution was prepared with deionized water and stored for at least 48 h before use to allow mutarotation to take place. Working solutions of H_2O_2 were prepared daily from 30% (v/v) H_2O_2 (Beijing Chemical Reagent Company, China). All solutions were prepared with deionized water (Milli Q, Millipore, Barnstead, CA, USA).

2.2. Apparatus

The powder X-ray diffraction (XRD) measurements were performed on a Bruck (Germany) D8 Advance X-ray diffractometer equipped with graphite-monochromatized Cu/Kα radiation (λ=1.54178 Å). The 2θ angle of the diffractometer was collected in the range from 5° to 75°. The Raman spectra were collected by a Renishaw Micro-Raman Spectroscopy InVia Raman system (England). A Renishaw red laser excitation at 785 nm was employed as the excitation source. Hitachi (Japan) S-4700 field-emission scanning electron microscope (SEM) was used for obtaining the scanning electron microscopy images. The CL spectra were obtained using a F-7000 fluorescence spectrophotometer (Hitachi, Japan) at a slit of 10.0 nm with a scanning rate of 1200 nm/min. UV–vis spectra were measured on a USB 4000 miniature fiber optic spectrometer in absorbance mode with a DH-2000 deuterium and tungsten halogen light source (Ocean Optics, Dunedin, FL). The CL detection was conducted on a BPCL luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China).

2.3. Procedures

2.3.1. Synthesis of Mg-Al-CO_3 LDHs

Mg-Al-CO_3 LDHs were prepared by the coprecipitation method at constant pH under low supersaturation conditions. $Mg(NO_3)_2 \cdot 6H_2O$ (0.045 mol) and $Al(NO_3)_3 \cdot 9H_2O$ (0.015 mol) were dissolved in 60 mL deionized water. The pH of the solution remained 10 by drop-wise addition of 60 mL alkali liquor (0.12 mol NaOH and 0.0075 mol Na_2CO_3). The resulting slurry was aged for 24 h at 60 °C. The product was centrifuged and washed with deionized water 3–4 times and dried at 60 °C for 24 h. Then the slurry was dried for 24 h at 60 °C. The as-prepared Mg-Al-CO_3 LDHs were grinded and passed through a standard sieve to get the size from 0.45 to 0.80 mm.

2.3.2. Preparation of luminol-hybrid LDHs

To prepare luminol-immobilized sensor, 0.20 g Mg-Al-CO_3 LDHs were packed into a quartz tube (5 cm in length and 0.6 cm i.d.) and furnished with glass wool at both ends to prevent the loss of Mg-Al-CO_3 LDHs. 0.01 M luminol solution was pumped into the column at 1.0 mL/min for 15 min. The as-prepared column was washed with deionized water until the baseline kept steady. Finally, the light yellow luminol-immobilized column was obtained (Fig. 1).

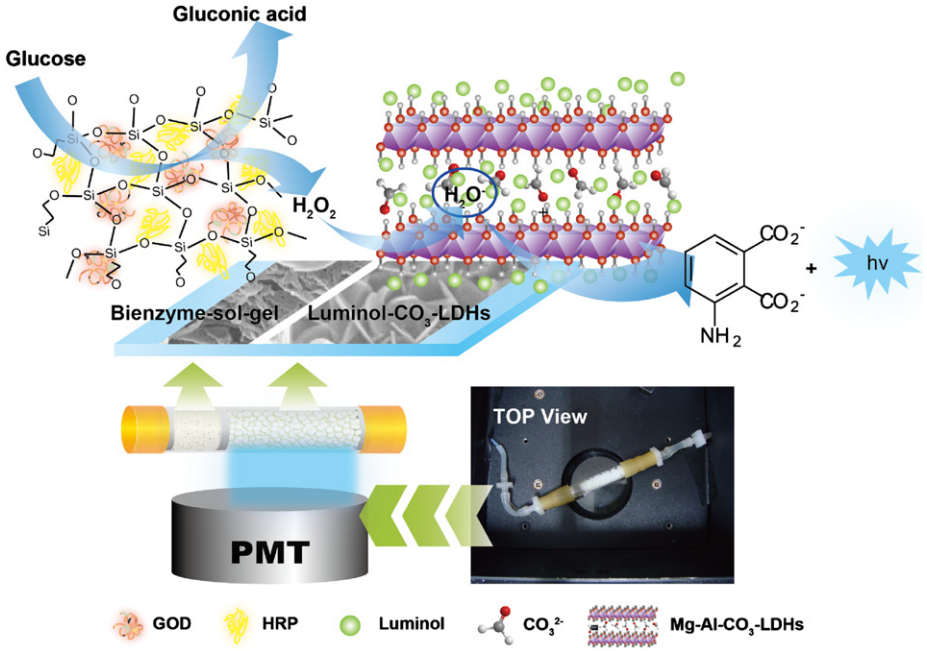

Fig. 1. Schematic illustration of platform fabricated by bienzyme and luminol-hybrid Mg-Al-CO_3 LDHs. Top: illustration of the experimental procedure of the biosensor for glucose. Left down: scheme of the CL flow-through biosensing device. Right down: photo of the CL flow-through biosensing device.