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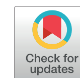
A novel label-free fluorescent sensor for highly sensitive detection of bleomycin based on nitrogen-doped graphene quantum dots



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HIGHLIGHTS

- A novel label-free fluorescent sensor for BLM has been established.
- The oxidative effect of BLM-Fe(II) on ssDNA was adopted in the detecting strategy.
- The proposed method exhibited a wide linear range, low detection limit, good selectivity, and anti-interference ability.
- The assay of BLM in human serum samples was realized with satisfactory results.

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ABSTRACT

In this work, we presented a novel label-free biosensor for rapid detection of bleomycinsulphate (BLM). The biosensor was based on the fluorescent "turn off-on" of nitrogen-doped graphene quantum dots (N-GQDs), which was prepared in a green way from citric acid and ammonia. The richness of carboxyl groups on the N-GQDs enabled strong adsorption of ssDNA to the surface of N-GQDs through π-π stacking interactions, resulting in the effective fluorescence quenching of N-GQDs system. The ssDNA underwent an irreversible cleavage event via the oxidative effect of BLM with Fe(II) as a cofactor, thus a turn-on fluorescence signal was observed. Thereby, the concentration of BLM can be quantitatively determined in a broad range from 0.34 nmol/L to 1300 nmol/L with a detection limit of 0.34 nmol/L. The presented method was applied to the determination of BLM in human serum samples with satisfactory results.

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1. Introduction

Cancer is a kind of disease characterized by the uncontrolled proliferation of cells that find their origin in genetic mutation [1]. The deaths from cancer worldwide are projected to continue rising, with an estimated 11 million deaths in 2030 [2]. Surgery is the main treatment, but is limited to accessible tumor. The use of antitumor drugs for cancer therapy has achieved considerable success in recent years [3]. The bleomycin sulphate (BLM) is a family of glycopeptide-derived antibiotics originally isolated from several streptomyces species. The antitumor activity of BLM is generally believed to relate with the ability of mediating the degradation of DNA, and possibly RNA, in the presence of oxygen and a redox-active metal ion in a low oxidation state [4]. BLM is currently used clinically in combination with a number of other agents for the treatment of several types of tumors, notably squamous cell carcinomas and malignant lymphomas [5]. In addition, BLM is less toxic to human body due to its specific advantages of low immunosuppression and low myelosuppression [6]. However, BLM also exhibits some serious dose-limiting side effects which are potential for pulmonary fibrosis and pneumonitis, as well as rigors and skin toxicity [7,8]. To the aim of the best treatment effect with the weakened toxicity of BLM, various reliable and sensitive methods for BLM detection have been developed, including liquid chromatography (HPLC) [9,10], enzyme immunoassay [11–13], radioimmunoassay (RIA) [14], microbiological assay [15] and fluorescent assay [2,16]. However, some of these methods suffer from complex instruments or complicated pretreatment, and many of them are also have the shortcomings of time-consuming, expensive, laborious or unsuitable for rapid assay. Therefore, further development of high sensitivity and selectivity method for BLM detection is necessary.

Nanotechnology has been playing a rapidly-growing role in biomedical technology in the last five years. Graphene and its

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derivatives are being investigated from biosensing to cancer therapy [17]. Graphene quantum dots (GQDs), as a kind of zero-dimensional nanomaterials, are widely applied in several fields including ion detection [18], photocatalysis [19], electrochemical biosensing [20], and bioimaging [21]. Compared with conventional semiconductor quantum dots, carbon-based GQDs present meritoriously optical and electrical characteristics due to quantum confinement and edge effects [22]. To increase optical and electrical properties of GQDs, researchers doped GQDs with heteroatoms like B, S and N atoms [23]. Nitrogen-doped graphene quantum dots (N-GQDs), as a new class of carbon nanomaterials, have potential application in sensor, fuel cells, optoelectronics field due to their stable photoluminescence (PL) and electrocatalytic activity [24].

In this work, we presented a novel label-free "turn-off-on" biosensor for rapid detection of BLM based on N-GQDs. As shown in Scheme 1, N-GQDs strongly absorbed the 18 mer ssDNA (5′-ATACCAGCTTATTCAATT-3′) to the surface of N-GQDs through π-π stacking interactions, forming N-GQDs-ssDNA complexes, resulting in the effective fluorescence quenching of N-GQDs [25]. The ssDNA underwent an irreversible cleavage by BLM-Fe(II), thus a recover of fluorescence signal of N-GQDs was observed. The change of the fluorescence intensity is proportional to the concentration of BLM. Thus a fluorescence turn-off-on method for the sensing of BLM was established. This system is simple, rapid, and avoids the complex process of the GQDs' modification or immobilization. To the best of our knowledge, this is the first time to apply the cleavage reaction of BLM to ssDNA for the label-free fluorescent BLM detection.

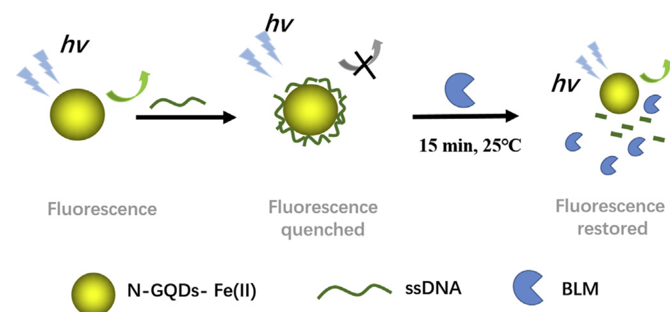
2. Experiment

2.1. Reagents and chemicals

All chemicals used were at least of analytical reagent grade and without further purification. Citric acid, $FeCl_2$, KCl, $CaCl_2$, alanine, urea, threonine, serine, glucose, cysteine were obtained from Beijing Dingguo Biotechnology Co. Ltd. Ammonia (30%wt), sodium dehydrogenized phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and sodium phosphate (Na_3PO_4) were purchased from Beijing Chemical Works. The ssDNA were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China), and their corresponding sequences are as follows: 5′-ATACCAGCTTATTCAATT-3′. The water used in all experiments had a resistivity higher than 18 MΩ/cm. The 10 mmol/L PBS buffered solution (pH = 7.4) was used as the medium for detection process.

2.2. Instruments

The fluorescence spectra were obtained by using a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a xenon lamp using right-angle geometry. UV–vis absorption spectra were obtained by a Varian GBC Cintra 10 e UV–vis spectrometer. In both experiments, a 1 cm path-length quartz cuvette was used. FT-IR spectra were recorded by a Bruker IFS66V FT-IR spectrometer equipped with a DGTS detector. Transmission electron microscopy (TEM) was conducted using a Hitachi H-800 electron microscope at an acceleration voltage of 200 kV. Powder X-ray Diffraction (XRD) was collected with a D8 ADVANCE (Germany) using Cu Ka radiation (λ = 1.5406 Å). Raman spectra were collected on an XploRA Raman spectrometer (Horiba Co., France). All pH measurements were made with a PHS-3C pH meter (Tuopu Co., Hangzhou, China).



Scheme 1. Schematic illustration of the N-GQDs-Fe(II) sensing system for the detection of BLM.

2.3. Synthesis of N-GQDs

N-GQDs were obtained from citric acid and, as carbon sources, and ammonia, as nitrogen sources [24]. In brief, 2 g citric acid and 0.3 mL ammonia were added into a Teflon-lined autoclave and heated at 210 °C for 6 h. 10 mL ultrapure water was added to the resultant dark brown mixture. Then, the pH of N-GQDs dispersion was adjusted to 7.0 by adding NaOH aqueous solution. The supernatant was centrifugated at 12,000 rpm for10 min in order to remove the large dots. Then the obtained liquid was diluted to 200 mL with ultrapure water. The concentration of acquired N-GQDs solution was 10 mg/mL. The as-prepared N-GQDs solution were stored at 4 °C for further use. The N-GQDs were precipitated by adding ethanol, and then centrifuged to collect solid sample, which was washed by ultrapure water for several times and dried under vacuum at 25 °C for 12 h. The acquired solid −state N-GQDs was used for FTIR tests.

2.4. BLM detection

For BLM detection, different amount of BLM were added into a series of 2 mL solution containing 2 mg/L N-GQDs, 33.33 μmol/L PBS buffer solution (pH = 7.4), 2 μmol/L Fe(II) and 50 μmol/L ssDNA. The solution was incubated at 25 °C for 15 min. The fluorescence spectra were recorded between 400 nm and 670 nm wavelength range at the excitation wavelength of 370 nm. The slit width of emission and excitation were set at 5 nm and 10 nm respectively.

2.5. Real sample detection

The blood samples of healthy persons were supplied by the Hospital of Changchun China, Japan Union Hospital. Some pretreatments to remove impurities are implied before experiment. First, we added acetonitrile to the blood samples (the volume of acetonitrile and blood was 1.5:1) in 5 mL centrifuge tube. After shaking for 2 min at room temperature, the product was centrifuged at 10,000 rpm for 10 min to remove protein. The supernatant was stored in −20 °C for future experiments. The obtained human serum samples were subjected to a 5-fold dilution, and 200 μL of them was added in the 2 mL testing system mixing with N-GQDs, Fe(II) and ssDNA. After that, different amounts of BLM were added into the mixture to prepare a variety of spiked samples. The fluorescence measurements were performed before and after the standard addition of BLM, respectively. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the writing of informed consent for all samples was obtained from human subjects.

3. Results and discussion

3.1. Characterization and feasibility

The N-GQDs was prepared according to the previous method

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