Highly selective and ultrasensitive detection of nitrite based on fluorescent gold nanoclusters

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ABSTRACT

Near-infrared fluorescent gold nanoclusters (AuNCs) were prepared by sonochemical method and applied for the selective and sensitive detection of nitrite. The fluorescence of AuNCs could be selectively quenched by nitrite, and the mechanism was discussed in detail. The fluorescence intensity decreased linearly upon the increasing concentration of nitrite in a wide range of 2.0×10^{-8} M to 5.0×10^{-5} M. The detection limit was only 1.0 nM ($S/N = 3$), which was much lower than the maximum admissible concentration of 2.2 μM required by the European Community. The proposed method was also applied for the determination of nitrite in real water samples. The present method exhibited excellent analytical performances, such as wide detection range, good selectivity, high sensitivity, and the applicability in neutral medium.

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1. Introduction

Nitrite, as preservatives and fertilizing agents for food, widely exist in the environments. The increasing concentration of nitrite in real water has caused serious hazards to public health and environments [1–3]. It is reported that nitrite can damage the nervous system, spleen and kidneys, and have a strong correlation with high cancer levels when its concentration is higher than 4.5 mg mL^{-1} [4]. Therefore, it is very important to quantitatively detect nitrite in drinking water sources, waste water treatment, and food industry. Some methods such as chemiluminescence (CL) [5–7], titrimetric [8], chromatographic [9,10], capillary electrophoresis [11,12], and electrochemical methods [13–15] have been developed for the analysis of nitrite. However, some methods are unsuitable for the routine ultra-trace determination. For instance, spectrophotometric methods suffer from poor sensitivity and interference from other anions. Although chromatographic methods possess high sensitivity and selectivity, they are often time-consuming. Capillary electrophoresis has a complicated process and requires relatively expensive instruments. Compared with these methods, fluorescence method has attracted much attention with the advantage of low cost, simplicity, speed, high sensitivity, and low limit of detection [16,17].

Thus, it is highly desirable to develop sensitive and selective fluorescent sensors for the detection of nitrite.

Highly efficient and stable fluorescent probes are of great importance to fluorescence-based nitrite sensors. Intensive efforts have been focused on the exploration of new efficient fluorescent materials [18,19]. A variety of organic fluorophores have been successfully applied to detect nitrite [20,21]. Unfortunately, the performances of these sensors are limited by their poor sensitivity and stability [22,23]. Quantum dots with multifarious advantages over organic dyes such as excellent fluorescence properties, higher photochemical stability and excellent resistant to chemical degradation have attracted great attention and achieved encouraging developments in the analytical application [24,25]. Thus, QDs-based fluorescent sensors have great potential to overcome the problems encountered by organic fluorophore-based sensors. However, the inherent compositional toxicity limits their applications [26,27]. In addition, fluorescent probes emitting in the near-infrared window between 650 nm and 900 nm, have been extensively studied in some active field, benefiting from their attractive advantages such as improved tissue penetration, lower background interference, and reduced photochemical damage [28,29]. Thus, further explorations on novel fluorescent nitrite sensors in the near-infrared region are of great importance. Recently, gold nanoclusters (AuNCs), an attractive fluorescent probe [30–33], are of particular interest because of its low toxicity, excellent biocompatibility and stability, good solubility, and excellent luminescence properties [34]. AuNCs has been employed in some research fields, such as biological imaging [35,36], ions sensing [37–39], and

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biosensors [40,41]. To the best of our knowledge, the optical sensor for the detection of nitrite using AuNCs as the fluorescent probe has not been reported.

In our previous report, highly fluorescent and water-soluble AuNCs with near-infrared-emission were prepared via a facile and rapid sonochemical method under mild conditions [42]. The obtained bovine serum albumin-stabilized AuNCs (BSA–AuNCs) possess strong fluorescence signal, large stokes shifts, good photostability, and stimuli-responsive properties. All of these properties showed that AuNCs could be used as promising fluorescent probe candidates for optical sensor. In this work, BSA–AuNCs are used to construct nitrite sensor for the first time. The fluorescence of BSA–AuNCs was gradually decreased with the increase of nitrite concentrations, and the corresponding limit of detection was 1 nM. The fluorescence quenching mechanism of BSA–AuNCs was also discussed. Moreover, the sensor was successfully applied to natural water samples with precise and accurate results. The proposed sensor exhibited advantages of high selectivity, wide detection range, and low limit of detection, revealing the potential application of BSA–AuNCs in sensors.

2. Experimental

2.1. Reagents

BSA was purchased from Sigma-Aldrich. Chloroauric acid ($HAuCl_4$) was obtained from Shanghai Reagent Co. (Shanghai, China). All other reagents were of analytical reagent grade and used without further purification. Aqueous solutions were prepared with doubly distilled water at room temperature.

2.2. Apparatus

The UV–Visible absorption spectra were carried out on a Shimadzu 3600 UV–Visible spectrometer (Shimadzu, Japan). The fluorescence measurements were carried out on Edinburgh FLS920P fluorescence spectrometer (Edinburgh Instruments Ltd., UK). The high resolution transmission electron microscopy (HRTEM) images were observed by JEOL JEM-2100. X-ray photoelectron spectroscopy (XPS) was carried out on an ESCALAB MK II X-ray photoelectron spectrometer. Fourier transform infrared (FTIR) spectroscopic measurements were performed on a Bruker model VECTOR22 fourier transform spectrometer using KBr pressed disks.

2.3. Synthesis of BSA–AuNCs

The synthesis of BSA–AuNCs was performed according to the literature [42]. Briefly, $HAuCl_4$ solution (20 mL, 10 mM) was added into the BSA solution (20 mL, 50 mg mL^{-1}) under vigorous stirring. After reaction for 2 min, pH value was adjusted to 12 by the addition of NaOH solution (2 mL, 1 M). Then the mixture was exposed to high intensity ultrasonic irradiation (Xinzhi Co., China, JY92-2D, 10 mm diameter; Ti-horn, 20 kHz, 60 W cm^{-2}) under ambient air for a certain time. After the reaction, light brown solutions were obtained. The procedure for the synthesis of BSA–AuNCs and the fabrication of nitrite sensor were shown in Scheme 1. First, fluorescent BSA–AuNCs were obtained by one-step sonochemical method. Then the fluorescence of BSA–AuNCs was quenched dramatically in the presence of nitrite.

2.4. Fluorescence detection

Nitrite aqueous solutions with different concentrations were freshly prepared. For quenching studies, AuNCs and $NaNO_2$ with
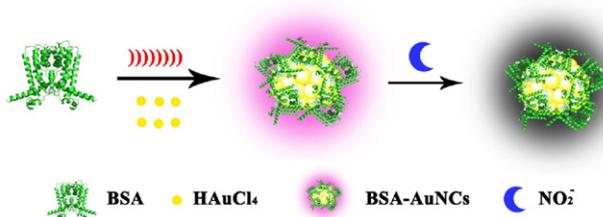
Scheme 1. Scheme of the synthetic strategy for BSA–AuNCs and the principle of nitrite sensing.

different concentrations were mixed in 50 mM phosphate buffer solutions (PBS, pH=7.4), and then equilibrated for 20 min. The fluorescence spectra were measured at 670 nm with the excitation wavelength at 350 nm.

2.5. Selectivity measurements

The following inorganic salts were used for selectivity experiments: sodium acetate, sodium chloride, sodium nitrite, sodium nitrate, sodium carbonate, and so on. 5 mM salt stock solution was prepared by ultrapure water. Subsequently, the salt solution was prepared by serial dilution with PBS (50 mM, pH=7.4), and then mixed with BSA–AuNCs solution in the absence and presence of $NaNO_2$.

2.6. Physical characterization of the fluorescence quenching

To get the HRTEM images of NO_2^--treated BSA–AuNCs, 5 μL of 1 mM $NaNO_2$ was added to 1 mL of 40 nM BSA–AuNCs solution. After incubation for 20 min, the suspensions were deposited onto copper grids with carbon support by slowly evaporating the solvent in air at room temperature.

3. Results and discussion

3.1. Characterization of the as-prepared BSA–AuNCs

The BSA–AuNCs were synthesized via sonochemical method and characterized by fluorescence spectra, photograph, HRTEM, FTIR and XPS, as shown in Fig. 1. The obtained BSA–AuNCs showed a deep brown color under visible light and emitted bright-red fluorescence under UV light. They exhibited a broad absorption at 500 nm and a strong emission peak at 670 nm (Fig. 1A). Compared with the phase solution method, a red shift of the emission wavelength from 620 nm to 670 nm for BSA–AuNCs was observed by sonochemical method. It suggested that the obtained BSA–AuNCs have higher potential in biosensor due to the low tissue absorption and scattering effects in the near-infrared region. Moreover, the as-prepared BSA–AuNCs showed stronger fluorescence than that by the phase solution method, which was consistent with our previous report [42]. The reasons may be as follows: when liquids are irradiated with ultrasonic irradiation, acoustic cavitation produces high temperature (5000 K), high pressures (20 MPa) and cooling rates (107 K/s), which can provide a platform for the growth of materials with an abundance of energy and extremely fast kinetics, thus promoting chemical reactions [43]. These results suggested the as-prepared BSA–AuNCs herein were favorable for bioapplications. Furthermore, the morphology of the BSA–AuNCs was characterized by HRTEM. As demonstrated in Fig. 1B, the obtained BSA–AuNCs were 1.8 nm in diameter with narrow size distribution. In addition, XPS was employed to investigate the oxidation states of the BSA–AuNCs. The Au $4f_{7/2}$ spectrum could be deconvoluted into