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A carbon dot based biosensor for melamine detection by fluorescence resonance energy transfer

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ABSTRACT

In this paper, we constructed a fluorescence resonance energy transfer (FRET) system between amino-functionalized carbon dots (C-dots) and gold nanoparticles (AuNPs). In this system, C-dots were treated as energy donors, while AuNPs were treated as energy acceptors. We optimized some important factors including incubation time, AuNPs concentration and media pH, which would affect the efficiency of the FRET system. Under the optimized experimental conditions, melamine could be detected based on fluorescence intensity of C-dots. We could get a linear relationship between 50 nM and 500 nM and the detection limit was 36 nM. The proposed method was applied to the determination of melamine in milk samples with satisfactory results. Compared with previous reports, the proposed method manifested great advantages including high sensitivity, short analysis time, low cost and ease of operation.

1. Introduction

Melamine ($C_3H_6N_6$) is used primarily in the synthesis of melamine formaldehyde resins for manufacturing laminates, plastics, coatings, glues or adhesives, dishware, and kitchenware. However, because of its high nitrogen contents (66 mass%), it is known to be illegally added to dairy products and animal feed to artificially increase the measured protein content. Unfortunately, melamine will result in the formation of kidney insoluble crystals in kidney, ultimately causing the formation of kidney stones [1]. The ingestion of melamine at levels above the safety limit (20 μM in the USA and EU; 8 μM for infant formula in China) can induce renal failure and death in infants. Thus, development of a sensitive method for melamine detection and qualification is quite necessary.

Methods developed for melamine detection had been reported massively. Conventional methods for melamine analysis reported to date involved gas chromatography [2], liquid chromatography [3], liquid chromatography/mass spectrometry [4], capillary electrophoresis [5], NMR spectroscopy [6], enzyme-linked immunosorbent assay (ELISA) [7], surface enhanced Raman spectroscopy [8], colorimetry [9] and surface plasmon resonance [10]. However, because of their requirement of expensive, complicated instruments, tedious procedures for sample pretreatment or preconcentration, many of these assays are not adaptable to routine analysis.

Fluorescent method has been proved to be a powerful optical technique for the trace detection of analytes due to its high sensitivity, simple instruments, easy operation, and the ability to measure multiple fluorescence properties. Among them, a number of fluorescence-based assays rely on fluorescence resonance energy transfer (FRET) spectroscopic technique, which occurs when the emission spectrum of the donor and the absorption spectrum of the acceptor are overlapped to a certain extent. FRET has been widely used in various research areas, such as nucleic acid detection [11–13], immunoassay [14–16] and ion detection [17,18]. To date, there are only few reports regarding the FRET detection of melamine.

Most of these FRET-based melamine sensing systems are primarily based on the use of fluorescence from organic dyes [19,20]. However, most organic fluorophores suffer from photobleaching and poor stability in the ambient environment, which results in irreproducible fluorescence signals for analysis. To overcome these shortcomings, some types of nanomaterials, such as quantum dots (QDs) [21–23], have been used as signaling probe. Even though, this

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method still suffers from some drawbacks. For example, the preparation process is relatively complex and the application of quantum dots is still controversial due to the relatively high toxicity.

Carbon dots (C-dots), a newly emerging form of carbon nanomaterials have inspired intense research efforts in various fields which can be produced inexpensively by many approaches [24–27]. Compared to traditional QDs and organic dyes, photoluminescent C-dots are superior in terms of high aqueous solubility, robust chemical inertness, easy functionalization, high resistance to photobleaching, low toxicity and good biocompatibility [24,28]. The applications of C-dots in fluorescent sensing of cations and anions have also been widely reported [29,30]. To the best of our knowledge, FRET employing carbon dots as the fluorescence donor has rarely been reported.

In this work, a novel "turn-on" fluorescence sensor for melamine was developed based on melamine-induced decrease of the FRET efficiency between gold nanoparticles (AuNPs) and the C-dots. Upon addition of C-dots into AuNPs solution, the C-dots are prone to get close to the surface of AuNPs, resulting in the fluorescence quenching. Melamine contains amino groups, which can compete with the C-dots, leading the restoration of the fluorescence. This method shows apparent advantages in environmental protection, operability and sensitivity. What is more, the present method is also appropriate for the determination of melamine in real samples.

2. Methods and materials

2.1. Chemicals

Chloroauric acid tetrahydrate ($HAuCl_4{\cdot}4H_2O$) was acquired from Shanghai Chemical Reagent Co., Ltd (Shanghai, China). Melamine was purchased from Sinopharm Chemical Reagent Co., Ltd.). Vitamin C, Glycine, L-histidine and L-cysteine were purchased from Sigma–Aldrich. Trisodium citrate, Glucose, lactose, $Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, KNO_3, NaOH, $FeCl_2$, NaCl, $FeCl_3{\cdot}6H_2O$, Na_2SO_4 and $MgCl_2$ were obtained from Beijing Chemical Reagent Company. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs.

2.2. Apparatus

The fluorescence spectra were obtained using a PerkinElmer LS 55 luminescence spectrometer (PerkinElmer Co.). UV–vis absorption spectra were obtained with a Cary 500 UV–vis–NIR spectrophotometer (Varian, USA). X-ray photoelectron spectroscopy (XPS) measurements were performed on an ESCALAB MKII spectrometer (VG Co., UK) with Al Kα X-ray radiation as X-ray source for excitation. FT-IR spectra were measured on a VERTEX 70 Fourier transform infrared spectrometer (Bruker, Germany). Atomic Force Microscopy (AFM) image was acquired by Dimension Icon (Veeco Instruments, USA) in tapping mode.

2.3. Synthesis of AuNPs

AuNPs were prepared by the citrate reduction of $HAuCl_4$ using the method according to the literature [31]. All glassware used in the preparation of AuNPs was cleaned with freshly prepared 3:1 HCl/HNO_3. Typically, 100 mL aqueous solution containing 1 mM $HAuCl_4$ was firstly heated to boiling, and then 10 mL of 38.8 mM trisodium citrate was rapidly injected. The mixture was further refluxed for 30 min and cooled to room temperature under stirring. The obtained wine-red solution was stored at 4 °C in the refrigerator for further use.

2.4. Preparation of C-dots

Fluorescent C-dots were prepared by hydrothermal treatment of histidine. In a typical synthesis, 2.5 g histidine was dissolved in 25 mL NaOH (0.5 mol L^{-1}) solution and then transferred into a 50-mL Teflon-lined autoclave and heated at 180 °C for a period of 12 h. The C-dots were collected by removing the large dots through centrifugation and then dialyzed against ultra-pure water through a dialysis membrane (molecular weight cut-off = 1000) for 48 h to remove the excess precursors and resulting small molecules. The resultant C-dots was maintained at 4 °C for further characterization and use.

2.5. Quantum yield measurement

The quantum yield (QY) of the as-prepared C-dots was measured according to an established procedure [32]. Quinine sulfate in 0.1 M H_2SO_4 (literature quantum yield 0.54 at 360 nm) was chose as a standard. Absolute values are calculated using the standard reference sample that has a fixed and known fluorescence quantum yield value, according to the following equation:

$$\phi_x = \phi_{std}\frac{I_x}{A_x}\frac{A_{std}}{I_{std}}\frac{\eta_x^2}{\eta_{std}^2}$$

where φ is the quantum yield, I is the measured integrated emission intensity, η is the refractive index of the solvent, and A is the optical density. The subscript "std" refers to standard with known QY and "x" for the sample. In order to minimize re-absorption effects, absorbencies in the 10 mm fluorescence cuvette were kept under 0.1 at the excitation wavelength (360 nm).Procedures for the determination of melamine

A stock solution of melamine (10 mM) was prepared in water. Various concentrations of melamine were obtained by serial dilution of the stock solution. For the determination of melamine, 200 μL AuNPs (3.0 nM), 200 μL 10 mM (pH 8.0) HEPES buffer solution and 50 μL various amount of melamine solution were mixed in a series of 600 μL centrifuge tube. After stabilizing for 30 min, 50 μL 0.75 mg mL^{-1} fluorescent C-dots were introduced to the tube and further incubated for 5 min. The fluorescence intensity (at 438 nm) of the mixture solutions was recorded with an excitation at 350 nm. The procedures for all the optimization and control experiments are the same as described above.

Milk samples were prepared according to a previous report with a minor modification [22]. Typically, 5 mL of raw milk or 5.0 mg of milk powder was placed into a 10 mL centrifuge tube, and 1 mL of 2 M trichloroacetic acid was introduced. After precipitating the protein by sonication for 10 min, the solution was centrifuged at 10,000 rpm for 10 min. The supernatants were adjusted to pH 7.0 with NaOH, further filtered with 0.22 μm filter and diluted 25-fold before used for the detection.

3. Results and discussion

3.1. Morphological and spectral characterizations of AuNPs and C-dots

Fig. 1A displays the typical TEM images of the AuNPs in the absence of melamine. It can be seen that the as-prepared AuNPs are spherical in shape, monodisperse, and fairly uniform in size (about 13 nm). In the presence of a low concentration of melamine (500 nM), the AuNPs are still in a dispersed state (Fig. 1B). However, upon addition of a high concentration of melamine (2 μM), the AuNPs come into aggregation to some extent (Fig. 1C), which indicates the interaction between melamine and AuNPs.