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An upconversion fluorescence resonance energy transfer nanosensor for one step detection of melamine in raw milk

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ABSTRACT

Here we report a nanosensor based on fluorescence resonance energy transfer (FRET) between upconversion nanoparticles (UCNPs) and gold nanoparticles (AuNPs) for melamine detection. The positively charged UCNPs as donor and the negatively charged AuNPs as acceptor bound together through electrostatic interaction, which caused the fluorescence quenching of UCNPs. Upon addition of melamine, AuNPs were released from the surface of UCNPs and aggregation due to the N-Au interaction between melamine and AuNPs, which results in the fluorescence of UCNPs gradually recovered. Under the optimal conditions including media pH (7.0), the concentration of AuNPs (1.23 nM) and incubation time (12 min), the fluorescence enhanced efficiency shows a linear response to the melamine concentration ranging from 32.0 to 500 nM with a detection limit of 18.0 nM. Compared with other fluorescence methods, the fluorimetric nanosensor shows high sensitivity of 0.968, ease of operation and can be used for the determination of melamine in raw milk samples.

1. Introduction

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton, which is mainly used in the production of plastic engineering, paint, adhesive and food packing materials [1,2]. However, melamine has been illegally added to milk powder, pet foods, and animal feeds to artificially increase the apparent protein content because of its nitrogen content as high as 66% by mass [3]. Since excessive ingestion of melamine beyond the safety limit (2.5 ppm in the United States and European Union, 1 ppm for infant formula in China) could lead to urinary system damage, kidney stone and ultimately death [4,5]. Thus, it is quite necessary to develop a simple and sensitive method for melamine detection.

Currently, various analytical methods have been reported for melamine assay, such as near infrared spectroscopy [6,7], gas chromatography (GC) [8], mass spectroscopy (MS) [9], gas chromatography-mass spectrometry (GC–MS) [10,11], high-performance liquid chromatography (HPLC) [12,13], enzyme-linked immunosorbent assay (ELISA) [14,15], surface-enhanced Raman scattering spectroscopy (SERS) [16,17]. Though some of the above methods have high sensitivity and accuracy, most of these are time-consuming and depend on professional technology, expensive instruments, and tedious sample pretreatment. Fluorescent methods have attracted great interests due to simple instruments and easy operations. Most of fluorescent methods for melamine detection mainly employ organic dyes compounds [18,19]. However, these currently used organic fluorophores and dyes are vulnerable to chemical and metabolic degradation and easily photobleached [20]. In addition, organic dyes often have narrow absorption and broad emission spectra with long tailing, and the excitation and emission wavelengths of them are not sufficiently stable and could easily change with the ambient environment (pH, temperature) [21]. All of these characteristics limit their application. To circumvent these restriction, many quantum dots (QDs) [3,22], have been used as fluorescent probe to detect melamine because of their bright photoluminescence, large Stokes shift, narrow emission and broad excitation [23,24]. Nonetheless, their inherent toxicity and chemical instability limit the application in the assay of biomolecules [25]. Moreover, both organic dyes and semiconductor quantum dots are down-conversion fluorescent materials which require excitation by short-wavelength ultraviolet (UV) light. The irradiation of hort-wavelength light may result in possible damage and the interference of protein fluorescence from biologic samples [26]. Thus, a simple, sensitive and suitable for complex samples approach is highly conceivable and needs further explorations.

Since the eighties of the 20th century, rare-earth (RE) doped upconversion nanoparticles (UCNPs) emitting higher-energy visible

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light when excited by low-energy NIR light have recently aroused considerable attention [27]. Compared with conventional fluorescent labels, the unique luminescence mechanism of UCNPs possess several advantages such as (1) improved detection sensitivity owing to no autofluorescence, (2) the minimum photodamage to living organisms due to deeper NIR light penetration. (3) good chemical and physical stability, and low toxicity [28,29].

On the basis of the advantages of UCNPs, a simple efficient fluorescence resonance energy transfer (FRET) system between positively charged UCNPs and negatively charged gold nanoparticles (AuNPs) was constructed to develop a nanosensor for melamine detection. As shown in Scheme 1, melamine could cause the aggregation of AuNPs by N-Au interaction, which influence the FRET system, and the fluorescence of UCNPs recovered. This method has been successfully applied to the determination of melamine in milk samples with satisfactory recovery from 98.8 to 102%.

2. Experimental

2.1. Apparatus

The size and morphology of UCNPs and AuNPs were characterized by transmission electron microscopy (TEM) images using a JEOL-1230 TEM (JEOL, Japan). The fluorescence spectra were measured using an F-4500 fluorescence spectrophotometer (Hitachi Ltd, Japan) with an external 980 nm laser diode (Hi-Tech Optoelectronic Co., Ltd. China) as the excitation source. Fourier transform infrared spectra (FT-IR) in the wavenumber range of 4000 to 400 cm^{-1} were recorded on a Nicolet Nexus 670 FT-IR spectroscope (Nicolet Instrument Co., USA). The crystalline phases of UCNPs were characterized using a Rigaku 2500 (Japan) X-ray diffractometer (XRD). The absorption spectra were collected on an UV-245 spectrophotometer (Shimadzu Co., Japan). A Nano-ZS Zetzsozer ZEN3600 (Malvern Instruments Ltd., U.K.) was used to measure the Zeta potential of UCNPs and AuNPs.

2.2. Materials

Rare-earth oxides used in this work, including yttrium oxide (Y_2O_3), ytterbium oxide (Yb_2O_3) and erbium oxide (Er_2O_3), were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and dissolved in hot nitric acid and then dissolved in deionized water to achieve final concentrations of 0.4 M, 0.2 M, 0.05 M, respectively. Chloroauric acid ($HAuCl_4$), hexadecyl trimethyl ammonium bromide (CTAB), melamine were obtained from Sigma (Shanghai, China). The buffer solutions with different pH were prepared by 0.01 M KH_2PO_4-Na_2HPO_4. All other chemicals (99%, Merck) used in this work were of analytical grade and without further purification, and Millipore Milli-Q ultrapure water (Millipore, ≥ 18 MΩ cm) was used throughout the experiments.

2.3. Preparation of UCNPs

The UCNPs of $NaYF_4$:Yb^{3+}, Er^{3+} were synthesized according to the previously reported method [30,31]. Briefly, 0.2925 g ethylenediaminetetraacetic acid disodium salt (EDTA) was added to the solution containing 1.315 mL of 0.4 M $Y(NO_3)_3$, 0.105 mL of 0.2 M $Yb(NO_3)_3$ and 0.105 mL of 0.05 M $Er(NO_3)_3$ under stirring and the pH was adjusted to 8.0, then 10 mL glycol and 0.0675 g CTAB were added to the solution, after the solution became clear under ultrasonic stirring, another aqueous solution containing 0.5 mL hydrofluoric acid (HF) was added dropwise to the above solution with vigorous stirring for 0.5 h. Finally, the mixture was transferred into a teflon-lined autoclave and heated to 180 °C for 18 h. Then, the solution was cooled to room temperature. The nanocrystals were precipitated from the solution by centrifugation. The precipitates were washed with deionized water at first and than washed with ethanol. This washing procedure was repeated for three times. The product was dried under vacuum before it was to be used.

2.4. Preparation of AuNPs

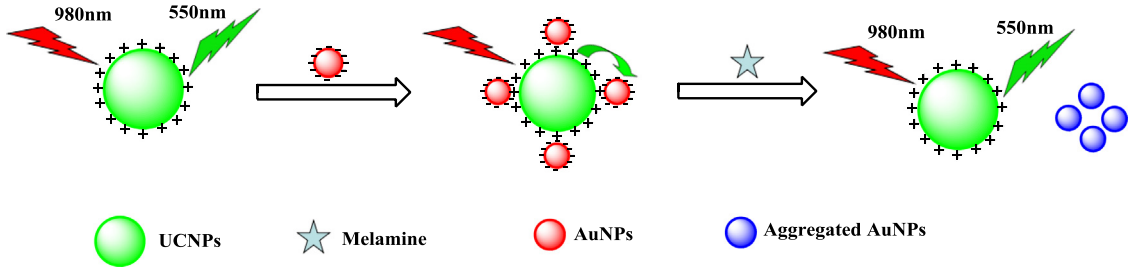
AuNPs were prepared by the citrate reduction of $HAuCl_4$ using the method described in the previous literature [32]. Typically, 100 mL chloroauric acid ($HAuCl_4$) solution (containing 0.5 mL 2% $HAuCl_4$) was firstly heated to boiling, and then 1.8 mL 1% sodium citrate solution was rapidly added to the boiled $HAuCl_4$ solution under vigorous stirring. The mixed solution was boiled for 10 min and further stirred without heating for another 15 min. The color of the solution changed from pale yellow to wine-red and the solution was cooled to room temperature and then stored in the refrigerator (4 °C) for further use.

2.5. Detection of melamine

60 μL of UCNPs and 200 μL of AuNPs were incubated in the KH_2PO_4-Na_2HPO_4 buffer solution (pH 7) for 10 min to form UCNPs-AuNPs complex in 2 mL tube. Then the melamine solutions (100 μL) with different concentrations were added to every tube. The mixture solution was diluted using KH_2PO_4-Na_2HPO_4 buffer to make the total volume of the solution 1 mL. The final concentration of UCNPs and AuNPs were 0.06 mg/mL and 1.23 nM, respectively. After incubation for 12 min at room temperature, fluorescence emission spectra were recorded with excitation at 980 nm.

2.6. Real sample pretreatment

Pretreatment of raw milk according to a previous report. Typically, 5 mL of raw milk was placed into 10 mL tube for centrifugation, and 1.5 mL of 2 M trichloroacetic acid was introduced. After 10 min' standing, the solution was centrifuged at 10,000 rpm for 10 min. The obtained supernatants were transferred into another tube and adjusted to pH 7.0 with NaOH and then filtered with a 0.22 mm filter.



Scheme 1. Schematic illustration of the "turn-on" fluorescence assay for melamine.