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Visible and fluorescent detection of melamine in raw milk with one-step synthesized silver nanoparticles using carbon dots as the reductant and stabilizer

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ABSTRACT

Using carbon dots as the reductant and stabilizer, a visible and fluorescent method was developed for melamine detection in raw milk with one-step synthesized silver nanoparticles. In this work, carbon dots (C-dots) were applied to reduce silver ions and stabilize the nanoparticles, resulting in the formation of silver nanoparticles (AgNPs). As a result, the inherent fluorescence emission of C-dots was significantly reduced after the formation of AgNPs. However, in the presence of melamine, silver ions could interact with the nitrogen atoms in amine and triazine groups of melamine. With 0–2 μM melamine, aggregated AgNPs were found after the reduction by C-dots, resulting in color and absorbance changes. With further increase of melamine (2–20 μM), both formation and aggregation of AgNPs were inhibited, and the fluorescence was gradually increased. This optical platform was optimized for melamine detection and then was applied to detecting melamine in raw milk samples. The results for melamine assay based on visible and fluorescent method showed the requisite sensitivity with a low detection limit of 30 nM, as well as high selectivity.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine) is an organic compound that serves as raw material for the production of melamine resins. As melamine contains a high level of nitrogen (66% by mass), the illegal addition of melamine to dairy products can lead to significant enhancement of protein content that is measured by using the Kjeldahl method. The continuous ingestion of melamine over the safety limit (2.5 ppm in USA and EU; 1 ppm for infant formula in China) could result in the formation of kidney stones, kidney failure, and even death in infants [1–4]. As recorded in 2008 Chinese milk scandal [5], melamine-tainted infant formula caused an estimated 300,000 victims in total, which involved the death of several children. Melamine concentrations in the adulterated milk products were as high as ~3300 ppm, posing an extreme danger to consumers [6]. Thus, it is becoming increasingly important to obtain highly selective and sensitive determination of melamine in dairy products [7]. Recent works on visual detection of melamine have provided an attractive route to achieving simple methods that afford requisite sensitivity and high selectivity [8–11].

Silver nanoparticles (AgNPs) have been widely used as their optical properties can be tuned by controlling particle size and shape [12–14]. When AgNPs get together in close proximity, their surface plasmons interact to shift resonant excitation, resulting in color variation from yellow to brown (or pale red). AgNPs have been utilized to visually detect melamine and these strategies generally follow the steps: synthesis of AgNPs, further modification of AgNPs, and melamine determination [10,15,16]. Achieving modified AgNPs usually requires toxic reagents, complex procedures, and time-consuming process, leading to the limited applications of these sensors. Thus, establishing a more facile and rapid assay is still in need for one-step melamine sensing in raw milk samples.

Recently, tremendous attention has been paid to the water-soluble fluorescent carbon dots (C-dots) owing to their tunable photoluminescence, high photostability, and biocompatibility, which render C-dots great potential for sensing, bio-imaging, drug delivery, and photocatalysis [17–19]. The functional groups on their surface can be tailored based on the source and procedure during the synthesis process. C-dots can act as both the reducing and stabilizing reagent by virtue of the abundant hydroxyl moieties on their surface [20–23]. In 2009, Wang and co-workers reported that the

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silver ions could be reduced to elemental silver by irradiating an aqueous solution of C-dots and $AgNO_3$ [24]. Additionally, employing C-dots as the reducing and stabilizing reagent has been recently reported for the synthesis of AgNPs [20].

In this assay, one-step synthesized silver nanoparticles with C-dots as the reducing and stabilizing reagent were employed for visible and fluorescent detection of melamine in raw milk. C-dots played an important role in reducing silver ions and stabilizing the nanoparticles. When C-dots were added to the silver ions in alkaline solution, monodispersed AgNPs would be obtained, resulting in remarkably reduced fluorescence emission. Aggregated AgNPs were observed when melamine was present. Visual color changes corresponding to the UV–vis absorption spectra were observed. Moreover, the fluorescence signals would be increased with the further increase of melamine. As a result, a dual optical platform combined UV–vis absorption and fluorescence signals could be achieved through the addition of melamine into the system.

2. Materials and methods

2.1. Materials and reagents

Silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), phenylalanine (Phe), glutamine (Glu), leucine (Leu), lysine (Lys), cysteine (Cys), melamine, and ethylene glycol were purchased from Aladdin Chemistry Co., Ltd., Shanghai, China. Glucose and lactose were purchased from Sigma–Aldrich Co., Ltd., Buchs, German. The raw milk was obtained from the local supermarket. Trichloroacetic acid, acetonitrile, and other reagents were analytical grade and used without further purification. Ultrapure water (specific resistance of 18.2 MΩ cm) was used throughout the whole experiment.

2.2. Apparatus

UV–vis absorption spectra were measured using a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence measurements were carried out on a Hitachi F-4500 spectrofluorophotometer (Tokyo, Japan). The fluorescence emission spectra in the 380–600 nm range were determined by monitoring fluorescence emission under excitation at 360 nm. The morphologies of nanoparticles were collected on a JEOL-2100 system operated at 200 kV (Japan). The Fourier transform infrared (FT-IR) spectra were recorded with a Bruker IFS 113v spectrometer (Germany).

2.3. Preparation of C-dots

Carbon dots (C-dots) were hydrothermally prepared according to a previous report with some modifications [20]. Briefly, 10 mL of ethylene glycol and 600 μL of sodium hydroxide solution (1 M) were mixed together, and the mixture was thereafter heated at 180 °C for 2 h in a 25 mL Teflon equipped stainless steel autoclave. After cooling down to room temperature, the obtained pale yellow C-dots solution was collected and dialyzed against water for two days to remove excess reagents through a dialysis film (Mw 1000). The resulting nanoparticles were lyophilized and stored in a refrigerator at 4 °C before use.

2.4. UV–vis absorption and fluorescence detection of melamine

In the melamine sensing assay, 30 μL of $AgNO_3$ (1 mM), 50 μL of melamine with different concentrations, and 370 μL of water were added together. After that, 25 μL of C-dots (2.4 mg mL^{-1}) and 25 μL of NaOH (56 mM) were added and the mixture was incubated at 37 °C for 30 min. Finally, the UV–vis absorption and fluorescence spectra were recorded, respectively. The fluorescence intensity was measured at the maximum excitation and emission wavelengths (λ_{ex} = 360 nm, λ_{em} = 450 nm).

2.5. Preparation of raw milk samples

The raw milk samples were prepared according to a reported work with few modifications [25]. Briefly, 15 mL of 61 mM trichloroacetic acid and 5 mL of acetonitrile were added to 2 g of raw milk to remove the proteins. Then, these samples were sonicated for 10 min and stirred for 10 min. The obtained solution was centrifuged at 12,000 rpm for 10 min, and the supernatant was adjusted to pH 6.8 and filtered through a 0.22 mm nylon filter for three times to remove lipids. For recovery experiments, the raw milk samples were spiked with different concentrations of melamine before the extraction procedure, and these samples were treated with the same extraction experiment as the blank raw milk sample.

3. Results and discussion

3.1. Characterization of C-dots and AgNPs

Optical characterization of C-dots produced from ethylene glycol is shown in Fig. S1. The UV–vis absorption spectrum exhibits strong peak absorption at 261 nm and a shoulder at about 320 nm because of the π–π* transition of C=C bonds and n–π* transition of C=O bonds, respectively [26–28]. When the C-dots are excited at 360 nm, their fluorescence emission spectrum shows a peak centered at 450 nm (Fig. S1A). Additionally, a significant peak shift of the emission spectrum is discovered upon increasing excitation wavelength from 330 to 400 nm (Fig. S1B). The morphology of C-dots was characterized using TEM. These C-dots are well dispersed and have an average size around 3 nm as shown in the TEM image in Fig. 1A. The functional groups on C-dots were evaluated by FTIR spectrum (Fig. 1B). The characteristic peaks at 3414 and 1415 cm^{-1} could be assigned to O—H bonds [29,30]. The rich hydroxyl groups on C-dots are thought to be originated from hydroxyl groups of ethylene glycol. The peaks centered at 1595 and 1082 cm^{-1} signify the C=O and C—O bonds, respectively [31]. These functional groups provide the ability for the C-dots to act as both the reducing and stabilizing reagent.

By virtue of the aforementioned functions of C-dots, AgNPs were successfully synthesized in alkaline solution. The TEM image of AgNPs shows spherical nanoparticles with the diameter around 15 nm as shown in Fig. 1C. Furthermore, the HRTEM image of AgNPs (Fig. 1D) displays a 0.24 nm lattice spacing, which corresponds to the (111) metallic Ag lattice space [32]. Additionally, significant changes in both the UV–vis absorption and fluorescence emission spectra of C-dots were seen (Fig. S2). As shown in Fig. S2A, the strong peak absorption appears at around 415 nm, which agrees with the typical surface plasmon resonance absorption of dispersed AgNPs. The decreased fluorescence of C-dots also demonstrates the reduction of silver ions with C-dots (Fig. S2B). Ding et al. reported that C-dots could emit strong fluorescence mainly because of the radiative recombination of their surface-confined electrons and holes [33]. With C-dots participating in the reduction of silver ions, the nucleation began at the surface of C-dots that hold the electrons, and thus the radiative electron–hole recombination on their surface was inhibited, resulting in reduced fluorescence emission [21,34,35].

3.2. Design strategy

Recent reports have described that hydroxyl-rich C-dots can reduce silver ions to AgNPs without any other reducing reagents [20,36]. In this work, C-dots functioned as both the reductant and