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Hydrogen-Bonding Recognition-Induced Color Change of Gold Nanoparticles for Visual Detection of Melamine in Raw Milk and Infant Formula

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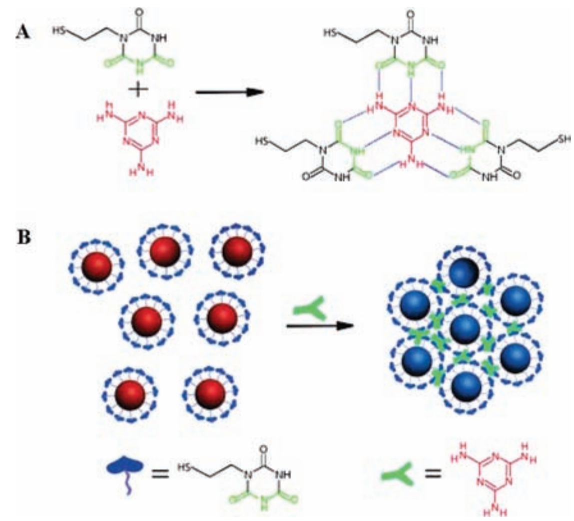
Melamine is a chemical compound used primarily for the production of melamine resins. Because of its high nitrogen level (66% by mass), melamine is illegally added to milk products to produce an incorrectly high reading in the measurement of protein content based on total nitrogen. Ingestion of melamine at levels above the safety limit (2.5 ppm in the USA and EU; 1 ppm for infant formula in China) can induce renal failure and even death in infants.[1] Therefore, it is important to develop a reliable and highly sensitive sensor that can provide on-site and real-time detection of melamine in milk products. However, current analytical techniques for melamine in milk products, such as a low-temperature plasma probe combined with tandem mass spectrometry (LTP/MS), all require expensive and complicated instruments, making on-site and real-time melamine sensing difficult.[1−3]

It is well-known that reaction of melamine (M) and cyanuric acid (CA) or its derivatives forms a stable complex CA·M through the interaction between diaminopyridine and diimide moieties, exhibiting three complementary NH···O and NH···N hydrogen bonds (Scheme 1A).[4−6] Such triple hydrogen bonding is considered to be particularly useful for controlling molecular self-assembly due to the reversibility, specificity, directionality, and cooperative strength of this class of interactions.[4−6] Therefore, we reason that when combined with CA derivatives, gold nanoparticles may find important application in detecting melamine because it is possible for melamine to control the assembly status of CA derivative-stabilized gold nanoparticles through hydrogen-bonding recognition, allowing the tuning in optical properties of gold nanoparticles.[7−11] The change in optical properties can be directly reflected by the color change,[8−10] and thus CA derivative-stabilized gold nanoparticles may be ideally suited as a colorimetric sensor for detection of melamine in milk products.

Based on the above consideration, we demonstrate herein for the first time that the color change induced by the triple hydrogen-bonding recognition between melamine and a CA derivative grafted on the surface of gold nanoparticles can be used for reliable detection of melamine. Since such a color change can be readily seen by the naked eye, the method demonstrated here enables on-site and real-time detection of melamine in raw milk and infant formula without the aid of any advanced instrument. With this colorimetric sensor, we could identify 2.5 ppb melamine within 1 min even with the naked eye.

We synthesized 1-(2-mercaptoethyl)-1,3,5-triazinane-2,4,6-trione (MTT), a kind of thiol-functionalized CA derivative,[12] and prepared the MTT-stabilized gold nanoparticles (12 nm) by ligand-exchange reaction using MTT and citrate-stabilized Au nanoparticles. Initially, the MTT-stabilized gold nanoparticles were well dispersed in distilled water, and the color of the uniform colloid was wine red due to its strong surface plasmon resonance (SPR) at 519 nm (see Supporting Information). Upon exposure of the MTT-stabilized gold nanoparticles to melamine, hydrogen-bonding recognition between melamine and MTT resulted in the aggregation of gold nanoparticles (Scheme 1B), and the wine red color of the gold colloid was accordingly changed to a blue color, as demonstrated in Figure 1A. The melamine-stimulated aggregation of gold nanoparticles was evidenced by TEM images that revealed monodisperse nanoparticles in the absence of melamine and significant aggregation of nanoparticles in the presence of 1.5 μM melamine (Figure 1B, C).

Scheme 1[a]



[a] (A) Hydrogen-bonding recognition between melamine and cyanuric acid derivative. (B) Colorimetric detection of melamine using the MTT-stabilized gold nanoparticles.

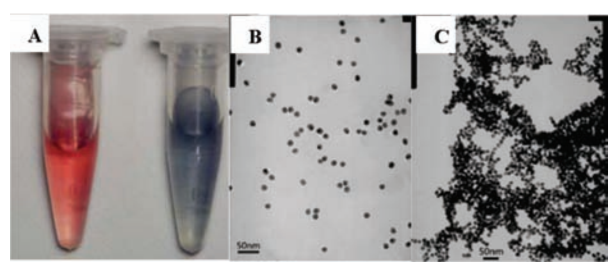


Figure 1. (A) Visual color change of the MTT-stabilized gold nanopaticles upon addition of melamine (from left to right: 0, 1.5 μM). (B and C) The corresponding TEM images (0 and 1.5 μM, respectively).

UV−vis spectroscopic measurements were performed to determine the optimal detection conditions (see Supporting Information). To demonstrate the performance of the optimized sensor for naked-eye detection of melamine by the mechanism mentioned above, different amounts of melamine were added to an aqueous solution of the MTT-stabilized gold nanoparticles, and the results were presented in Figure 2. Upon addition of increasing concentrations

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of melamine, the color of MTT-stabilized gold nanoparticles gradually changed initially from wine red, then to purple, and finally to violet blue within 1 min, which was consistent with the change in UV−vis spectra indicated in Figure S5. Even the addition of 20 nM of melamine (2.5 ppb) caused a color change that could be unambiguously distinguished from that of the initial suspension, which is ∼3 orders of magnitude lower than the safety limit of melamine permitted by the U.S. Food and Drug Administration (FDA). The limit of detection (LOD) achieved with the naked eye was about the same order of magnitude as that obtained by using the LTP/MS technique.[3] Furthermore, this method remained much simpler than the existing methods without the requirement of expensive and complicated instruments.

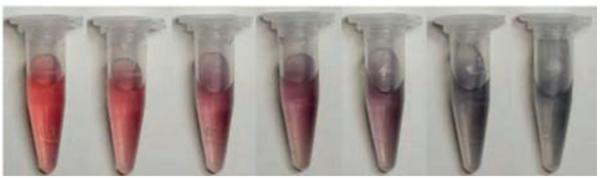

Figure 2. Visual color change of the MTT-stabilized gold nanoparticles upon addition of melamine with different concentrations under the optimized conditions (from left to right: 0, 20, 60, 100, 200, 280, 400 nM).

The selectivity of the optimized sensor for melamine was evaluated by monitoring the extinction ratio ($A_{700/519}$) response in the presence of other molecules with similar structures. Our results showed excellent selectivity for melamine over cytosine, uracil, and thymine (Figure S6). This excellent selectivity was mainly attributed to the high specificity of triple hydrogen-bonding recognition between MTT and melamine.[4−6] Moreover, the specific topology of melamine also could be an important factor which contributed to this excellent selectivity.[4]

To determine whether this colorimetric sensor could be applied to melamine detection in real milk products, we first prepared a raw milk sample tainted with melamine, and this sample was pretreated using an extract recipe (see Supporting Information). As shown in Figure 3A, the sole addition of extract from blank raw milk to the melamine sensor solution did not lead to a distinguishable color change. However, when 8 μL of the samples with 1, 2.5, and 5 ppm melamine were added to 1 mL of the sensor solution, respectively, a red-to-violet blue color change could be clearly observed, coinciding with the SPR shift in UV−vis spectra demonstrated in Figure 3B.

More importantly, such a colorimetric sensor also could find potential application in the detection of melamine in infant formula. A big challenge for detecting melamine in commercially available infant formula was how to decrease the potential interference from its ingredients including all 20 standard amino acids, lactose, soluble vitamins and minerals, etc. In this case, amino acids and most of the lactose could be removed through an extraction procedure. Although the extract from infant formula contained some residual ingredients (Figure S7), their presence did not lead to the distinct color change of the sensor solution (Figure S8), indicating that this melamine sensor had high selectivity against these residual ingredients.

In summary, we take advantage of the color change of gold nanoparticles induced by hydrogen-bonding recognition to develop a novel nanoparticle sensor for visual detection of melamine. This new method offers several advantages over current melamine

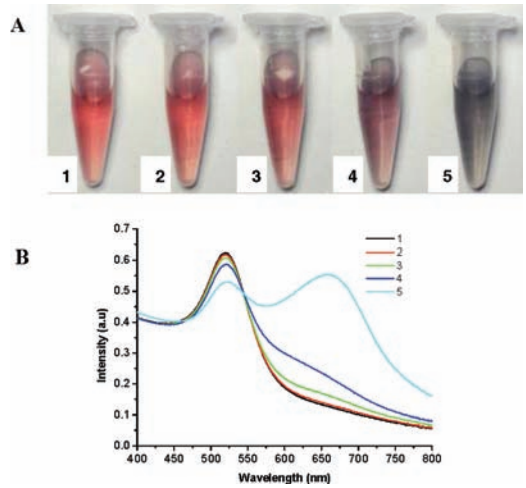

Figure 3. (A) Visual color change of the optimized sensor: (1) without any addition; (2) with the addition of the extract from blank raw milk; (3) with the addition of the extract containing 1 ppm (final concentration: 8 ppb); (4) with the addition of the extract containing 2.5 ppm (final concentration: 20 ppb); (5) with the addition of the extract containing 5 ppm (final concentration: 40 ppb). (B) The corresponding extinction spectra.

detection techniques. First, the method does not require expensive and complicated instruments, which simplifies operations and reduces the cost. Second, the method allows a detection concentration as low as 2.5 ppb to be achieved with the naked eye within 1 min, useful for rapid and ultrasensitive detection of melamine in milk products. Finally, this sensor exhibits excellent selectivity for melamine over other molecules with similar structures and the residual ingredient in the extract from raw milk and commercially available infant formula. These advantages substantially make this method quite promising for on-site and real-time detection of melamine in raw milk, infant formula, and other milk products.

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Supporting Information Available: Synthetic and experimental details. This materials is available free of charge via the Internet at http://pubs.acs.org.

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