Rapid detection of melamine in whole milk mediated by unmodified gold nanoparticles

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(Received 3 February 2010; accepted 3 March 2010; published online 30 March 2010)

A strategy for rapid and facile detection of melamine in food and other substances is described. Unmodified gold nanoparticles enable a colorimetric signal output that is amplified through integration by precipitation formation via a chemical component specific to melamine. The sensitivity was determined to be ~ 40 ppb with a two standard deviation cutoff. © 2010 American Institute of Physics. [doi:10.1063/1.3373325]

Melamine is an important industrial material which experienced wide application in plastic engineering and agriculture as fertilizer in the early 50s. Because of its high nitrogen content (66%) and low cost, it has been illegally added to food products in order to increase apparent protein content. Standard tests such as the Kjeldahl and Dumas tests¹ estimate food product protein levels by measuring the nitrogen content, which can be easily misled by adding nitrogen-rich compounds such as melamine.

Based upon this clear need for melamine detection, a technology that provides accurate, simple, and rapid readouts is required for areas with difficult testing conditions such as fields or rural areas. As food and complex fluids such as milk are the samples most commonly in need of melamine detection, there exists a significant demand for sample processing (e.g., protein separations) prior to the detection step. Therefore the measurement of melamine requires a combination of separation and detection processes. Currently, chromatography followed by mass spectroscopy,² enzyme-linked immunosorbent assay (ELISA),³ and spectroscopy⁴ are the major categories for melamine detection. Hydrogen-bonding based color change in cyanuric acid derivative-grafted gold nanoparticles (AuNPs) for visual detection of melamine has also been reported recently.⁵ Detection times have ranged from several seconds to several hours, while the sensitivity cutoff has been shown to vary from 6 parts per billion (ppb) to several hundreds of parts per million (ppm).⁶ However, these strategies are either time-consuming due to exhaustive separation procedures, and/or rely heavily on expensive instrumentation.

Here we introduce a simple, rapid and specific strategy for the detection of melamine which could overcome the major hurdles mentioned previously. In this work, colorimetric signal readouts and specific precipitation with AuNPs and cyanuric acid, respectively, are combined, resulting in accurate melamine detection. More specifically, quantitative colorimetric changes due to aggregation are among the many interesting properties associated with NPs. As illustrated in

the inset of Fig. 1(a), melamine has a strong interaction with AuNPs, which decreases the stability of citrate-stabilized AuNPs against the aggregation, thus causing dramatic and visible color changes and indicating the existence of melamine. This color change occurs within seconds and could be measured both by visual inspection and spectrophotometry. In addition, cyanuric acid has a specific reaction with melamine via strong hydrogen-bonding recognition, resulting in cocrystallization of white precipitants. Analogues of melamine were shown to not form this unique crystal. In order to increase specificity, cyanuric acid is introduced into the detection system to interact with melamine. With dual

FIG. 1. (Color online) (a) DLS demonstration of melamine concentration-dependent increases in particle size. Inset: Schematic of melamine-mediated AuNP aggregation (1: citrate ion; 2: melamine). (b) Spectrophotometric demonstration of color shift.

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FIG. 2. (Color online) Ultraviolet-visible (UV-vis) spectra of AuNP and the mixtures containing melamine, ATP, and Adenine in the absence (a) and presence (b) of cyanuric acid. [melamine]=0.5 mM (~ 62.5 ppm), [ATP]=0.5 mM, and [adenine]=0.5 mM.

signal readouts, melamine can be specifically detected in a rapid and straightforward process which is conducive toward translation.

Dynamic light scattering (DLS) analysis was performed to assess the interaction between the AuNPs and melamine. Findings revealed a melamine concentration dependent size increase in gold-melamine particle aggregation. Visually apparent color shifts from red to blue occurred in melamine concentrations between 0.12 and 0.49 ppm. As the average hydrodynamic particle diameter of bare AuNPs was ~ 35 nm, color changes occur at hydrodynamic diameters of approximately 50–100 nm. Previously reported results have indicated that this surface plasmon resonance based colorimetric change is dependent on aggregate size and the interparticle distances within.^{7–9} As the interparticle distance becomes greater or less than average particle diameters, the color will convert to red or blue, respectively [Fig. 1(b)].^{7,10} Figure 1(a) reveals the increase in particle sizes which is mediated by melamine-AuNP interactions.

The aggregation of AuNPs in the presence of melamine suggests a strong interaction between AuNPs and melamine. Previous studies have shown that amine binds strongly to AuNPs,^{11,12} and therefore the exocyclic amines in melamine could play an important role in the melamine-AuNP interaction. To examine the specificity of colorimetric changes of the AuNPs, additional control molecules containing exocyclic amines were also mixed with AuNPs. As seen from absorbance spectra in Fig. 2(a), a dramatic redshift was observed for melamine while no shift was observed for adenosine-5'-triphosphate (ATP) of the same concentration. This indicates that the colorimetric signal shows great selectivity for melamine over some amine-bearing molecules such as ATP which are shown to be insufficient to induce significant color changes to the same degree as that observed with melamine. However, some structurally similar molecules, such as adenine, were shown to be able to induce a similar redshift as melamine. Therefore, cyanuric acid, which is known to form precipitates with melamine via highly specific hydrogen bonds,¹³ was then introduced in the detection procedure. A comparison of Figs. 2(a) and 2(b) shows that the addition of cyanuric acid leads to a significant change in the absorbance spectra only for melamine. With colorimetric output from AuNPs and precipitation from cyanuric acid, a dual selectivity is achieved. Detection of melamine using cyanuric acid derivative grafted AuNP, which was reported recently,⁵ has the advantages of low cost and simplicity over other existing methods. Without involving any modifications on either the AuNP or cyanuric acid, the presently described

FIG. 3. (Color online) The ratio of absorbance readings at 660 nm to that at 518 nm vs the melamine concentrations in the presence of NaCl.

method of melamine detection offers an even more facile alternative.

As pointed out earlier, the colorimetric signal associated with the interaction between melamine and the AuNPs is due to the formation of AuNP aggregates. Therefore, we speculate that the sensitivity and dynamic range of the colorimetric signal is dependent on the resistance of AuNP to aggregation and could be adjusted by changing the buffer composition of the AuNPs. To demonstrate this, the effect of sodium chloride (NaCl), which is well-known to destabilize the AuNPs, was investigated. AuNPs were premixed with NaCl of different concentrations and the resulting mixtures were used to detect melamine (Fig. 3 and Fig. S1 of Ref. 15). As expected, a different sensitivity and dynamic range were observed for AuNPs containing different concentrations of NaCl. For example, a significant color change occurred between 78 and 39 ppb for AuNPs with 35 mM NaCl whereas a color change occurred between 313 and 156 ppb for AuNPs without NaCl. Here, with NaCl as an example, we demonstrate that it is possible to adjust the detection sensitivity and dynamic range to a desired concentration range by the addition of AuNP destabilizing/stabilizing reagents. The adjustability in sensitivity and dynamic range is very important toward practical applications. For example, to determine whether a sample contains melamine above a certain safety level, AuNPs can be preconditioned so that a significant color change occurs around the safety level and is added to the sample as a pre-screen. If no significant color change is observed, the sample is negative; if the significant color change is observed, cyanuric acid can then be added to eliminate the possibility of a false positive.

Milk is a complex system and its various components serve as potentially interactive species with melamine regardless of detection methodology (e.g., liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, ELISA, or colorimetric detection). The conventional ways for melamine detection from milk usually require complicated and time-consuming separation steps, including acetonitrile extraction and/or ion pair chromatography which can severely limit the application of on-site detection of melamine from milk. Here we provide a rapid strategy to extract melamine from milk. For the casein-based milk, the major interferent for colorimetric detection is casein. Since the casein exists in milk with the form of the water-soluble calcium salt of a phosphoprotein, acid treatment removes the calcium cation, leaving a water-insoluble phosphoprotein. By adjusting the pH, the casein forms dif-

FIG. 4. (Color online) Detection of melamine from milk after separation. (a) Visual color and turbidity changes of serials concentration of melamine under separation condition with different pH. (b) The colorimetric concentration profile (ratio of absorbance readings at 660 nm to that at 518 nm) with pH 3–4. The dotted line indicates the negative control. The concentrations of melamine spiked into milk are (from left to right) 1600, 400, 100, 25, 6.25, 1.56, 0.39, and 0 ppm.

ferent size of aggregates or gelatinous precipitate, therefore can be extracted from the milk, while leaving the melamine free from them.¹⁴ Hydrochloric acid (HCl) was added to the milk, generating precipitation in the form of a white, opaque solid. After centrifugation at 10000 rpm for 10 min with a Millipore filtration tube, the transparent liquid component was transferred for subsequent AuNP colorimetric detection (Fig. S2 of Ref. 15). In order to avoid filtration tube blockage from the precipitants, a pore size $\geq 5 \mu\text{m}$ was required. Similar to the results in de-ionized water, a high concentration of melamine resulted in both blue color and precipitation. Conversely, a low concentration of melamine resulted in a transparent and red-colored sample. pH dependence was also observed for the detection process. From the results shown in Fig. 4(a), low pH (<6) resulted in effective separation of milk and resultant sensitivity. With the optimized pH level of around 3–4, both color and turbidity changes could be observed above the 0.4 ppm melamine concentration [Fig. 4(b)]. When the pH levels were greater than 6, the separation efficiency was low and the interference from significant remaining milk components could not be removed, which resulted in poor sensitivity. When the pH levels were

lower than 0, the purple color observed in the absence of melamine indicated a significant decrease in the stability of AuNPs against aggregation, which also led to poor sensitivity. The complete detection methodology which included the acid-based milk component separation step as well as the observation of detection-based visual color and turbidity changes were completed in less than 15 min. This work presented a facile and accurate approach toward AuNP-mediated detection of melamine based upon a dual color and precipitation-based readout. By eliminating the need for costly/timely equipment and sample and reagent preparation steps toward sensitive and specific readouts, this study has realized a promising approach toward using nanomaterials for scalable chemical analysis.

F.W. and R.L. contributed equally to this work. F.W. gratefully acknowledges support from the School of Engineering and Applied Science (SEAS) Fund, University of California, Los Angeles. N.L. gratefully acknowledges start-up funds provided by the College of Engineering, University of Miami. D.H. gratefully acknowledges support from a NSF CAREER Award (Grant No. CMMI-0846323), NSF Mechanics of Materials program grant (Grant No. CMMI-0856492), V Foundation for Cancer Research V Scholars Award, NSF Center for Scalable and Integrated NanoManufacturing (SINAM) Grant No. DMI-0327077, Wallace H. Coulter Foundation Early Career Award in Translational Research, NSF National Center for Learning & Teaching in Nanoscale Science and Engineering (NCLT), and NIH Grant No. U54 A1065359.

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¹⁵See supplementary material at <http://dx.doi.org/10.1063/1.3373325> for materials and methods and data pertaining to pH-dependent milk processing.