ABSTRACT

Due to its high nitrogen content and low cost, melamine, a widely used material in plastics industry, has been illegally added to food products to boost apparent protein content. We’ve previously developed a colorimetric method to detect melamine. This method utilizes the melamine-concentration dependent gradual color shift of gold nanoparticles (AuNPs) and an absorbance reader is usually required for reliable quantification of melamine. Herein, we present a novel strategy for reliable visual quantification of melamine without any equipment. First of all, we further explored the mechanism of the melamine-induced AuNP aggregation with various experimental techniques. Secondly, based on the findings from the mechanism studies, we’ve developed and demonstrated a critical coagulation concentration (CCC)-based salt titration as a reliable means to visually quantify the melamine. This has allowed us to accurately quantify melamine at a level as low as 30 ppb without any equipment.

Keywords: melamine, gold nanoparticles, colorimetric, critical coagulation concentration

1 INTRODUCTION

Melamine is a nitrogen-rich compound widely used in plastics industry. Due to its high nitrogen content and low cost, it has been illegally added to food products to boost apparent protein content, thus misleading conventional protein tests that are based on total nitrogen content[1]. Exposure to high levels of melamine poses adverse health concerns [2].

The necessity for melamine detection has generated various techniques, including ELISA (enzyme linked immunosorbent assay) [3] and chromatography/spectroscopy-based [4-7]. These methods are usually reliable and sensitive; however, they tend to require expensive instrumentation and/or labor-intensive procedures, thus limiting their use in resource-limited settings. We’ve previously developed a simple and rapid colorimetric method to detect melamine [8]. This method utilizes the finding that melamine causes the unmodified, citrate-capped gold nanoparticles (AuNPs) to aggregate, thus resulting in a red-to-blue color shift of AuNP. The melamine-concentration dependent gradual color shift could be observed with unassisted eyes; however, an absorbance reader would be still required for reliable quantification of melamine concentration. Herein, we present a novel strategy for reliable visual quantification of melamine without any equipment.

2 EXPERIMENTAL

2.1 Materials

All the chemicals were purchased from Sigma-Aldrich, unless otherwise specified. The gold nanoparticles (AuNPs) were synthesized with Turkevich method, where gold hydrochlorate is reduced by citrate [9]. The melamine stock solution was prepared by dissolving melamine powder in deionized water.

2.2 UV-Vis Absorption Spectroscopy

AuNPs were first mixed with serial dilutions of melamine stock solution in a volume ratio of 1:1. The absorption spectra (400nm-800nm) were then collected on a Synergy 2 microplate reader (Biotek). To quantify the extent of melamine-induced color change of AuNPs, the ratios of absorbance at two characteristic wavelengths (660nm and 520nm) were calculated.

2.3 Dynamic Light Scattering and Zeta Potential Analysis

Dynamic light scattering (DLS) and zeta potential analysis were performed with a Delsa Nano C particle analyzer (Beckman Coulter). The DLS analysis was performed in a disposable plastic cuvette with a 165° scattering angle. The resultant hydrodynamic size was the cumulant mean of 100 accumulation cycles. The zeta potential analysis was performed in a quartz flow cell under a fixed voltage (60 V). The resultant zeta potential was calculated from average of 10 measurement cycles.

2.4 Transmission Electron Microscopy

Transmission electron microscopy (TEM) was carried out on a SM-200 transmission electron microscope (FEI). The TEM samples were prepared by loading and air-drying the solution on a nickel TEM grid that is covered with silicon nitride.
3 RESULTS AND DISCUSSIONS

3.1 Mechanism of Melamine-Induced AuNP Aggregation

Previous studies have shown that there is a strong interaction between noble metal surfaces and molecules bearing exocyclic amino groups and/or ring nitrogen atoms [10, 11]. Melamine carries three exocyclic amino groups and ring nitrogen atoms. Therefore, it’s speculated that melamine could have a strong interaction with AuNPs and this strong interaction could cause the surface stabilizer (i.e. negatively charged citrate ions) to be displaced from AuNPs, resulting in the aggregation of AuNPs. In order to verify the speculation, the melamine-aggregated AuNPs were systematically characterized. The optical property was characterized by UV-Vis absorption spectroscopy. The hydrodynamic size and zeta potential were determined by dynamic light scattering and electrophoretic light scattering, respectively. As shown in Figure 1 (a) and (b), with increase of melamine concentration, the hydrodynamic size and the ratio of absorbance at 660 nm to 520 nm both increase. This reveals the melamine concentration dependent aggregation of AuNPs and consequential red-to-blue color shifts. As shown in Figure 1 (c), with increase of melamine concentration, the zeta potential decreases. This agrees with the above speculation that melamine displaces the negatively charged citrate ions from AuNPs, thus causing the decrease in the surface charge of AuNPs.

3.2 TEM of the Melamine-Induced AuNP Aggregates

TEM imaging was performed on three AuNP samples: in the absence of melamine, in the presence of melamine, and in the presence of salt. It can be seen in Figure 2 that AuNP is well-dispersed in the absence of melamine and aggregation occurs in the presence of melamine as well as in the presence of salt. However, the highly disordered structure of the melamine-induced AuNP aggregates is significantly different from the structure of salt-induced AuNP aggregates. This observation has eliminated the possibility that melamine could cause AuNPs to aggregate by decreasing the thickness of electrical double layer as salt.
Figure 2: TEM imaging: (a) Well-dispersed AuNPs in the absence of melamine; (b) AuNP aggregates formed in the presence of 0.7 ppm melamine; (c) AuNP aggregates formed in the presence of 0.1M NaCl.

3.3 Critical Coagulation Concentration (CCC)-Based Salt Titration

Based on the findings from the mechanism studies, we’ve developed and demonstrated a critical coagulation concentration (CCC)-based salt titration as a reliable means to visually quantify the melamine concentration. More specifically, the samples to be tested for melamine were premixed with AuNPs. The CCC-based titration was performed by adding sodium chloride (NaCl) to the premixtures until a rapid red-to-blue color shift was visually observed, and the NaCl volume added was then recorded. The NaCl volume needed for a rapid color shift and the derived CCC were found to quantitatively correlate with melamine concentrations (Figure 3a and 3b). This has enabled an equipment-free method to not only detect, but also accurately quantify melamine at a level at least one order of magnitude lower than that achieved previously with a spectrometer. Furthermore, a comparison between CCC and zeta potential (Figure 3c) has revealed that CCC is a more sensitive and quantifiable indicator of melamine-induced AuNP aggregation than zeta potential. This observation agrees with the prediction from DLVO theory of colloid stability[12].

Figure 3: CCC-based salt titration for visual quantification of melamine: (a) Titration volume needed for a rapid red-to-blue color shift; (b) CCC derived from titration volume; (c) Zeta potential.
4 CONCLUSIONS

In this paper, we further explored the mechanism of the melamine-induced AuNP aggregation. The melamine-aggregated AuNPs were systematically characterized with various experimental techniques, including UV-Vis absorption spectroscopy, dynamic light scattering (DLS), zeta potential analysis, and transmission electron microscopy (TEM). The results suggest that the strong interaction between melamine and AuNPs could cause the negatively charged surface stabilizer to be displaced from AuNPs, thus resulting in the aggregation of AuNPs. Furthermore, inspired by the findings from the mechanism studies, we’ve demonstrated a simple and reliable strategy to visually quantify the concentration of melamine. More specifically, the critical coagulation concentration (CCC)-based salt titration is performed on the pre-mixtures of AuNPs and samples containing melamine. The salt titration volume needed for a rapid color shift is recorded and used to quantify the melamine concentration. Without extensive optimization, this CCC-based quantification strategy has allowed us to accurately quantify melamine at a sensitivity of tens of ppb, which is at least one order of magnitude lower than that achieved previously with a spectrometer. With the simplicity and sensitivity, this new strategy is highly suitable for using as a screening tool in low-resource settings.

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