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Short communication

Rapid sensing of melamine in milk by interference green synthesis of silver nanoparticles

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ABSTRACT

A highly sensitive, selective, and rapid interference green synthesis based determination of potential milk adulterant melamine has been reported here. Melamine is a nitrogenous compound added to milk for mimicking proteins, consumption of which leads to kidney stones and renal failures. Melamine interacts with ascorbic acid (AA) through strong hydrogen-bonding interactions, thus resulting in an interference/interruption in the formation of silver (Ag) nanoparticles which was confirmed by UV–Vis spectroscopy and Transmission Electron Microscopy (TEM). The corresponding benchmark validations for melamine spiked milk samples were performed using High Performance Liquid Chromatography (HPLC). This interference in the formation of Ag nanoparticles resulted in color change that varies with concentration of melamine, thereby enabling in-situ rapid sensing of melamine from milk to a lower limit of 0.1 ppm with a linear correlation coefficient of 0.9908.

1. Introduction

Food adulteration has become a serious concern in recent years. Increased awareness of health benefits as well as the consequences of bad health within the global populous has lead researchers to direct their attention towards developing portable detection techniques [1–3], for monitoring food quality. Among the various concerns of present day society, milk adulteration is perhaps the most widespread. Different kinds of adulterants are being added to milk and dairy products on a daily basis worldwide. The adulteration of one of the most basic staples poses a significant and direct threat towards healthy sustainable living envisioned by the general population. Adulteration of milk affects the quality of milk and can even make it hazardous to consume.

Out of the identified milk adulterants, melamine (1,3,5-Triazine-2,4,6-triamine), has been known to be added to milk in order to give an incorrect high readout of apparent protein content levels due to its high nitrogen content [4] and is associated with kidney stones and renal failures in mammals [5–7]. In 2008, the Chinese milk scandal lead to the death of 6 infants and a total of 300,000 babies were reported to fall ill due to the consumption of milk products adulterated with melamine [8]. The current melamine detection methods include high-end sophisticated instruments such as Gas Chromatography (GC–MS) [9], Liquid Chromatography Mass Spectroscopy (LC-MS) [10] and High Performance Liquid Chromatography (HPLC) [11]. Due to their sheer size and complex handling, these instruments and methods are out of the reach of distributors and consumers. A point-of-care, in-situ sensing mechanism that can deliver rapid and timely analysis of milk purity, is the need of the hour.

Nanotechnology has long been touted as the next level of technological evolution and in the last few decades, glimpses of its vast potential have been witnessed in various fields such as catalysis [12,13], fuel cells [14], lithium ion batteries [15,16] etc. With nanotechnology taking the forefront [17,18], there has been gradual but significant development in the fabrication of miniaturized devices for sensing of melamine and other milk adulterants using various metal plasmonic nanoparticles such as silver (Ag) [19–22] and gold (Au) [23–26]. For instance, Kaleeswaran et al. [15] studied the aggregation induced recognition of Ag nanoparticles by sodium D-gluconate for sensing of melamine and the time required for synthesis/sensing was reported to be more than 2 h with an average particle size around 12–14 nm. Another study by Ni et al. [18] using gold nanoparticles reported an improved peroxidase-like activity of bare Au nanoparticles for visual detection of melamine with a detection limit of 0.5 μM in milk and the time required for synthesis/sensing was reported to be approximately 30 min with an average particle size of 10 nm. Recently, Atar et al. proposed a novel voltametric sensor based on carbon nitride nanotubes for estimation of melamine with a detection limit of 1.0×10^{-11} M [27].

In all the above cases, the principle of sensing primarily relies on the pre-synthesized nanoparticles followed by the modification/functionalization and aggregation of previously synthesized nanoparticles due to the presence of analyte (melamine), which is a time consuming process. The development of a simple, low-cost and rapid detection technique for melamine which can be used even by an inexperienced or unskilled personnel, by realization of a portable device is highly desirable. Through this paper, a rapid way of determining melamine by

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means of interference green synthesis of silver nanoparticles utilizing ascorbic acid as reducing agent is reported.

2. Experimental

2.1. Materials

Silver nitrate (Merck), Ascorbic acid (S D Fine-Chem Limited), Sodium hydroxide pellets (S D Fine-Chem Limited) and Melamine (Thomas Baker) were used as received without further purification. Stock solutions were made and kept in amber colored bottles to limit the amount and spectral range of light which enters the bottle thereby eliminating any chance of photochemical reactions. Ultrapure water obtained using an ultrafiltration system (Milli-Q, Millipore) with a measured resistivity above 18 MΩ cm at 25 °C was used throughout for all the experiments.

2.2. Preparation of melamine sensor and its characterization

1 mM silver nitrate was used as a precursor [28] and 10 mM ascorbic acid (AA) as reducing agent. The particular concentration of AA was chosen on the basis of optimization experiments performed using different concentrations of AA for the synthesis of Ag nanoparticles (Fig. S3). 200 μL of different analyte concentrations were added to 500 μL of the silver nitrate precursor solution. 10 μL of the AA was added to the above solution. 10 μL of 0.85 M NaOH was added to all the microtubes for maintaining the pH of solution. The nanoparticle solutions with different concentrations of melamine were analyzed using UV–Vis absorption spectroscopy scan from 275 nm to 800 nm using the DeNovix microvolume spectrophotometer (FX 100, USA). The melamine was spiked according to the standard addition procedure followed by the milk distributors [22,29]. The pre-processed milk was prepared using 30% tricholoroacetic acid and 3 M NaOH. The different concentrations of melamine spiked milk were validated using High Pressure Liquid Chromatography (Thermo Finnigan Surveyor HPLC, USA). Transmission Electron micrographs (TEM) were recorded on a Tecnai G2 F30 S-TWIN HR-TEM (FEI, Hillsboro, USA) operating at 200 kV. The samples were drop casted into 400 mesh carbon coated copper grid and it was allowed to dry before imaging. The detection limit or limit of detection mentioned in the manuscript, is the lowest quantity/amount of analyte that can be distinguished from the control (sample having no analyte).

3. Results and discussion

In the current work, a new sensing strategy is reported for the first time through a single-step interference synthesis process of using ascorbic acid (AA) as a reducing agent in the formation of Ag nanoparticles. AA is a green chemical, non-toxic and environment friendly. Hence, it provides a green sensing platform. Interference/interruption of the synthesis of nanoparticles for sensing analytes is one of the least explored fields [30,31]. Single-step sensing process is realized with the interference synthesis of Ag nanoparticles, using AA as the reducing agent, which has a specific selectivity towards its interaction with the adulterant melamine in raw milk. Although there are many proteins and other chemicals present, AA does not react with them if the milk pre-processing step is done. To eliminate any further need for bringing in selectivity, an inherent selectivity towards the analyte was introduced based on the choice of reducing agent in the interference synthesis of Ag nanoparticles. Hence, there is no additional requirement of chemical modification to incorporate selectivity in interference sensing when compared to conventional two-step sensing process, wherein functionalizing the already synthesized nanoparticles to the analyte for incorporating the selectivity is imperative. Here, a simple visual inspection enables detection of the presence of melamine by the color change induced.

This proposed sensing mechanism is instantaneous with a limit of detection up to 0.1 ppm. The allowed limit for melamine levels in milk varies country wise. The increase in concentration of melamine interferes with the synthesis and subsequent spectral change can be monitored by UV–Vis spectroscopy (Fig. 1). Also, this sensing strategy gives a colorimetric change which is visible to naked eye.

Previously, the sensing of melamine and other analytes were only reported for already prepared nanoparticles, followed by a chemical modification and aggregation leading to sensing [32–34], whereas, a new sensing scheme in which the synthesis itself is altered leading to the change in size and morphology of the formed nanoparticles is being reported here. In the conventional two-step sensing process, size and morphology of the nanoparticles are already stabilized but in the proposed single-step sensing, the size and shape gets altered/modified during the interference synthesis process itself. Fig. 1 inset shows the colorimetric change of different concentrations of melamine from 0.1 ppm to 1000 ppm. It depicts the color change during the interaction of analytes with the formation of Ag nanoparticles. The Ag nanoparticles showed a sharp surface plasmon resonance (SPR) peak at 398 nm. The change in the SPR can be determined spectrophotometrically. The decrease in absorbance is due to the formation of lower number of nanoparticles.

To confirm the interference of melamine towards Ag nanoparticles, a selectivity test was carried out by possible chemicals found in milk that may interfere with the synthesis. The selectivity was done against 100 ppm concentration of dextrose, glycine, leucine, citric acid, lactose, zinc, sodium, melamine and control (water). Fig. 2 shows the bar diagram depicting the selectivity test carried out using other analytes against absorbance at 398 nm. Melamine interfered green synthesis resulted in an observable color change while rest of the analytes did not show much difference from the control (Fig. S1). When melamine is added, the synthesis gets disrupted with an intensity variation in absorbance according to the concentration of melamine.

The interference in the synthesis of Ag nanoparticles may be attributed to the strong hydrogen-bonding interaction between the reducing agent and analyte. The schematic representation of the deduced mechanism of interference green synthesis based sensing is presented in Scheme 1. Melamine is a strong nucleophile which contains nine hydrogen-bonding sites. Thus, melamine would specifically interact with AA through a hydrogen-bonding interaction to interfere in the synthesis of Ag nanoparticles. The hydrogen-bonding interaction consumes a certain amount of AA thereby not having enough reducing agent to reduce Ag ions, resulting in the weakening of AA's reducing ability, thus

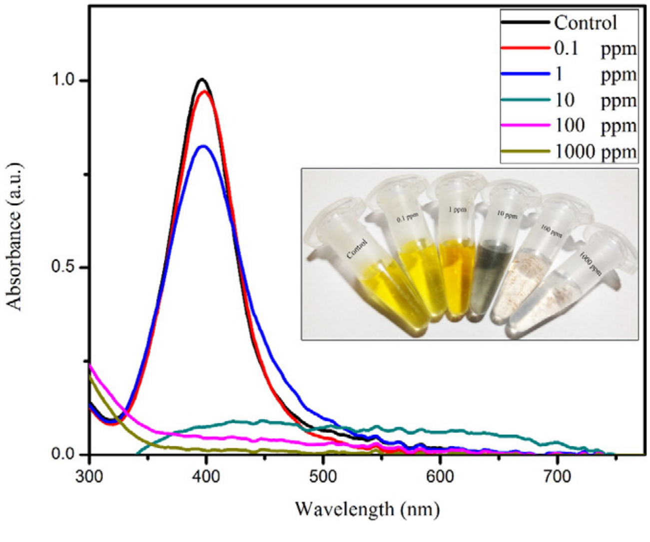

Fig. 1. UV–Vis absorption spectra of interference green synthesized Ag. The inset shows the photograph of interference green synthesized Ag nanoparticles with different concentration of melamine (0.1 to 1000 ppm, Control: 0 ppm).