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Effect of pH on the Stability of Gold Nanoparticles and Their Application for Melamine Detection in Infant Formula

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Abstract: *A simple and sensitive colorimetric gold nanoparticle probe for detection of melamine in infant formula was developed by the reduction of Au(III) salt with sodium citrate. This method is rather simple that does not involve any surface modification of the nanoparticles or multistep sample treatment. This technique is based on the fact that the optical properties of gold nanoparticles depend on distance between particles. Gold nanoparticles are aggregated in a neutral media in the presence of melamine; this causes an easily measurable change in the absorption spectrum of the particles, which can be monitored with the naked eye or UV-vis spectrophotometer. Here, the color of gold nanoparticles changed from wine red to blue in the presence of melamine and no color change was observed before melamine was introduced into the milk sample. The observed color change is the result of the coupling of the surface plasmon resonance (SPR) between particles in close proximity. This method is also sensitive and a limit of detection of 0.90 ppm of melamine was obtained with the UV-vis spectrophotometer in aqueous solution and 0.46 ppm of melamine was detected visually with the naked eye in a solution of infant formula.*

Keywords: *Gold nanoparticles (AuNPs), infant formula, melamine, pH stability, Surface plasmon resonance (SPR), TEM, UV-vis*

I. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine, $C_3H_6N_6$) is an organic base known for its fire retardant properties and other industrial applications. In recent years, two global incidents of kidney related diseases in infants and pets have been reported in China and USA. It has been found that the cause was a deliberate addition of melamine to milk, infant formula and pet foods to falsely increase the "apparent-protein" content in these products since melamine has a high nitrogen content (66.6% nitrogen by weight) [1,2]. High intake of melamine for prolonged time would result in the formation of melamine cyanurate crystalline complex that could damage the renal cells causing kidney failure and eventually death. Because of this, the European Union, Canada, FDA of USA and the World Health Organization (WHO) set a tolerable limit of melamine [3].

Since melamine is found in a number of industrial products and the illicit activities of some companies to add melamine in food products, there is a need to have reliable analytical techniques for its detection. The common methods currently used for melamine detection in milk and other food products are GC-MS [4,5], LC-MS [6,7], CE-MS [8,9] and HPLC [10,11]. These techniques are all expensive, time consuming that require complex sample treatment and well trained personnel. Therefore, there is a need for a fast, cheap, sensitive and potentially field portable analytical method for detection of melamine in food products at lower concentration limits set by different safety and health regulatory institutions or organizations.

Gold nanoparticles and other metal nanoparticles lend themselves as colorimetric sensors for detection of various chemical systems because of their unique surface plasmon resonance (SPR) [12-15]. Gold nanoparticles, in particular, have intrinsically strong surface plasmom resonance absorption band and high molar absorptivities (10^8-10^{10} $M^{-1}cm^{-1}$) in the visible portion of the electromagnetic spectrum [13]. This unique SPR depends on size, shape, the refractive indices of the surrounding media and the distance between particles [13]. The solutions of smaller gold nanoparticles appear wine red in their dispersed state and larger particles or in their aggregate forms appear purple or blue. The observed color changes are associated with the shift in the surface resonance band to longer wavelengths in the aggregated state and can easily be observed visually with the naked eye. We take advantage of these analyte-induced color changes of gold nanoparticles for visual detection of melamine in infant formula without the need for an expensive and complex instrumentation.

In this report, we studied the effect of pH on the stability of gold nanoparticles and their application for the detection of melamine in infant formula. As stated above, the method of detection depends on color change of gold nanoparticles from wine red to blue when mixed with melamine. This method involves simple treatment of the infant formula with trichloroacetic acid to remove proteins which otherwise may interfere with the detection of melamine. Furthermore, the method is fast and highly sensitive.

II. Experimental

2.1 Reagents and Materials

$HAuCl_4$ was purchased from Sigma-Aldrich. Sodium citrate tribasic dihydrate was also purchased from Sigma-Aldrich. The milk powder sample was made from Similac® Advanced® baby formula which was purchased at the local grocery store. Trichloroacedic acid was purchased from Fisher Scientific. Melamine was purchased from Acros Organics. All glassware was soaked in aqua regia and then rinsed in milliQ (18 MΩ cm) water and dried prior to use. pH was adjusted with either NaOH or HCl.

2.2 Apparatus

Characterization and stability of the gold nanoparticles were done using UV-vis spectroscopy and transmission electron microscopy (TEM). A Bodenseewerk Perkin-Elmer GmbH UV-vis spectrometer (made in Germany) was used for UV-vis spectroscopy. The size of nanoparticles were estimated using transmission electron microscopy (TEM). TEM was performed on a FEI Tecnai F20 XT transmission electron microscope at the University of Kansas. pH was measured with HI 2211 pH/ORP meter from HANNA Instruments.

2.3 Preparation of Gold Nanoparticles

Gold nanoparticles were synthesized using the Turkevich method [16]. In an Erlenmeyer flask, 250 mL of 0.01% gold chloride solution was heated to boiling on a heating/stirring plate. Aluminum foil was placed over the neck of the Erlenmeyer flask to prevent excess evaporation and boil over. 10 mL of 1% (w/v) sodium citrate was added to the boiling gold chloride and the solution was boiled for 10 minutes under rigorous stirring. After 10 minutes, the solution was transferred to a room temperature heating/stirring plate and allowed to continue stirring for an additional 15 minutes. The red wine colored gold nanoparticle solution was then allowed to cool to room temperature.

2.4 Preparation of Milk Sample

The milk samples were prepared from Similac® Advanced® baby formula purchased from local retail stores. A solution was made by dissolving 2.98 g of the powder in 20 mL of MilliQ water. The solution was mixed thoroughly using a stir bar on a heating/stirring plate. Samples of milk solution and trichloroacetic acid (30% w/v) were mixed in a 5:3 ratio by volume in test tubes to remove proteins. The test tubes were vortexed for 5 min and then centrifuged for about 2 minutes. The supernatant was taken using a syringe fitted with 25-gage needle and brought to a neutral pH with NaOH. The neutral solution was then filtered using a syringe fitted with a 0.22 μm filter. This filtrate, called the milk matrix, was then used to make spiked melamine solutions with different concentrations.

III. Results and Discussion

3.1 Effect of pH on the Stability of Gold Nanoparticles in Aqueous Solutions

Gold nanoparticles prepared using citrate as a reducing agent are relatively stable and uniformly dispersed in solution. The citrate functions as a capping agent to prevent aggregation of particles due to the negative surface charges. The electrostatic repulsions between neighboring nanoparticles because of the negative surface charge of the citrate layer keep them remain dispersed in solution. However, any factor that affects this negative surface charge will result in aggregation of the nanoparticles.

One of the factors that disrupts this negative charge on citrate is pH. Here we studied the effect of pH in the range from pH =3 to pH= 11 on the stability of gold nanoparticles. We used visual observations and TEM imaging for this purpose. At pH < 7, the color of the gold solution was changed from wine red to purple indicating aggregation of the particles. This is because at lower pH values, citrate is fully protonated and thus the number of surface negative charges is greatly reduced. The decrease in negative surface charges results in aggregation and hence decreased stability of the gold nanoparticles. At neutral pH, well above the pKa values of citric acid, the aqueous solutions of the nanoparticles maintained the red wine color indicating no aggregation took place. This is because, the citrated is fully deprotonated and there are more negative surface charges that result in repulsion between nearby gold nanoparticles and thus little or no aggregation was observed. Therefore, we chose neutral pH (pH = 7) for all experiments that involve detection of melamine. These observations are also supported by TEM images (Fig. 1).

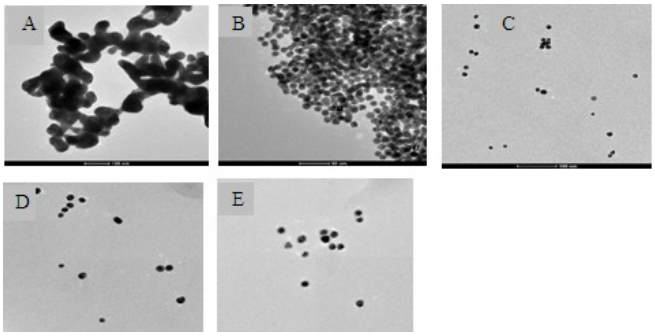

Figure 1. TEM images of gold nanoparticles at different pHs: A) pH = 3 B) pH = 5, C) pH = 7, D) pH = 9 and E) pH = 11

3.2 Characterization of AuNPs in Aqueous Solution in the Presence and Absence of Melamine

Since the method of detection in this report is based on the measurement of AuNP's aggregation in the presence of melamine, we first studied the behaviors of AuNPs in aqueous solution before evaluating their degree of aggregation in solution of infant formula. As shown in Fig. 2A, the color of the well dispersed AuNPs is wine red and λ_{max} for its surface plasmon resonance (SPR) is centered at about 521 nm (Fig. 2E). The TEM images in Fig. 2C show that the size of the dispersed gold nanoparticles is about 15-20 nm. As shown in Fig. 2D the AuNPs undergo aggregation in the presence of melamine and there is a clear shift of the absorption peak to longer wavelengths and broadening of the SPR band (Fig. 2E). Previous reports showed that the peak position of SPR is dependent on the distance between the nanoparticles [13]. Similarly, the color of AuNPs changed from wine-red (dispersed state) to blue (aggregated state) (Figs. 2A & B). Fig. 2C is the TEM images of AuNPs in their dispersed state and Fig. 2D represents the TEM images of AuNPs in the presence of melamine.

Figs. 3A & B show that the SPR absorption is red-shifted and the degree of aggregation, here defined as the ratio of absorbance at 721 and 521 ($A_{aggregation}/A_{disperrsion}$) increases with increasing concentration of melamine. Furthermore, there is a good correlation between the degree of aggregation of AuNPs and concentration of melamine (R^2 = 0.9973, Fig. 3B, inset) over the range of 0 to 0.23 ppm melamine concentration. However, after 0.23 ppm of melamine concentration, this ratio becomes constant indicating that the surface of the AuNPs is saturated and no more responds to increasing melamine concentration.

The melamine saturated AuNPs have a λ_{max} of 721 nm compared to the λ_{max} of 521 for dispersed gold nanoparticles. This red-shifted and relatively broadened peak compared to that of AuNP solutions confirms the aggregation of AuNPs caused by melamine. Addition of cynauric acid, an analog of melamine or glucose or other salts resulted in no change in λ_{max} of the SPR, indicating that these substances if present in milk will not able to induce aggregation of the AuNPs.

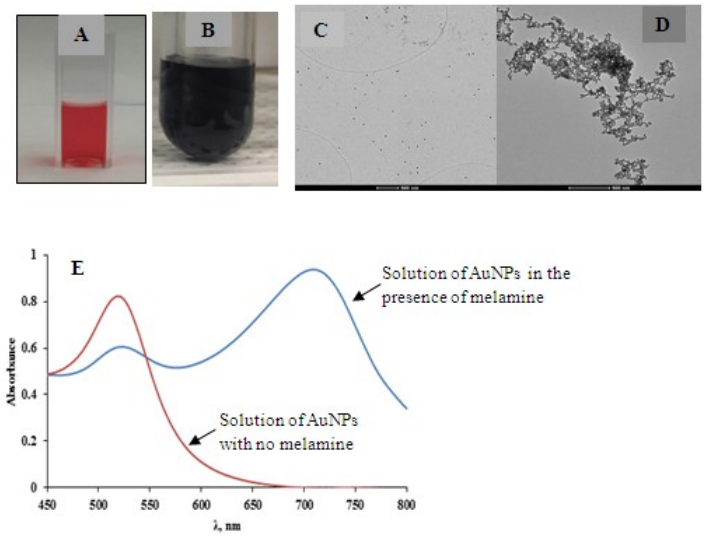

Figure 2. A) Photograph of a solution of gold nanoparticles without melamine B) photograph of a solution of gold nanoparticles in the presence of melamine C) TEM images of gold nanoparticles without melamine D) TEM images of gold nanoparticles in the presence of melamine and E) UV-vis spectra of gold nanoparticles with and without melamine.

3.3 Detection of Melamine in Infant Formula

Figs. 4A & B show the UV-vis spectra and photographs of AuNPs taken by regular smart phone in a solution of infant formula to which different concentrations of melamine were added. With the addition of 0 to 15 ppm of melamine, the absorbance of AuNPs at 521 nm decreases and the absorbance around 650 nm increases. The shift to a longer wavelength due to aggregation of the AuNPs is similar to what was observed in aqueous solution (section 3.2) with two clear differences. The first one is that, more melamine concentration was required to reach saturation compared to AuNPs in aqueous solutions. The degree of aggregation increases with increasing melamine concentration and remains constant after addition of 15 ppm of melamine. There is also a good correlation between the degree of aggregation and melamine concentration over the range of 0-15 ppm (R^2 = 0.9973, Fig. 4C, inset). The second difference is that he shift in the SPR band of AuNPs in a solution of infant formula upon addition of melamine occurred at a relatively shorter wavelength (650 nm) compared to that in aqueous solution (720 nm). Based on the method in this report, the limit of detection was found to be 0.90 ppm, which is lower than the limit set by FDA and WHO [3]. The limit of detection was calculated from the regression equation of the inset graph in Fig. 4B. It is determined using the equation, LOD = 3 times standard deviation of the intercept of the calibration curve divide by the slope of the calibration curve [17]. Though, we were not able to see distinction in the UV-vis spectra of AuNPs in infant formula solution with melamine concentration below 0.71 ppm and those of AuNPs with no melamine added, there is a clear blue color at the interface for visual detection as low as 0.46 ppm final concentration of melamine in the infant formula (Fig. 5). When this solution was mixed well for UV-vis measurement, its spectrum is similar to that of gold nanoparticles without melamine (Fig. 4A).

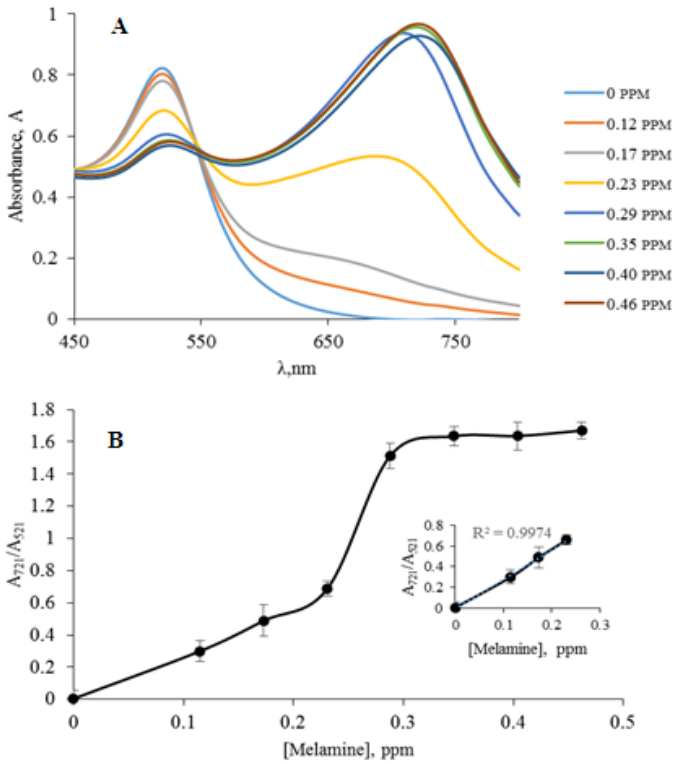

Figure 3. A) UV-vis spectra of gold nanoparticles in the presence of different concentrations of melamine and B) Plot of absorption ratio of A_{721}/A_{521} *vs.* melamine concentration. Inset in B shows the linearity of the curve of the A_{721}/A_{521} *vs.* melamine concentration. Error bars represent standard deviations from three experiments.

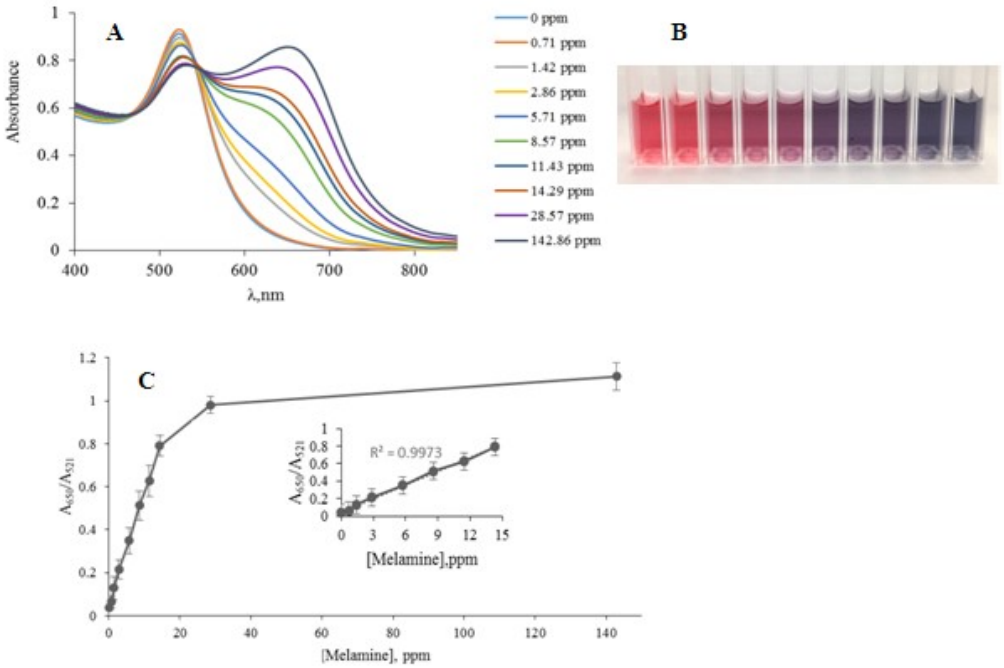

Figure 4. A) UV-vis spectra of gold nanoparticles in the presence of different concentrations of melamine that has been spiked in infant formula and B is the corresponding photographs C) Plot of absorption ratio of A_{650}/A_{521} *vs.* melamine concentration. Inset in C shows the linearity of the curve of the A_{650}/A_{521} *vs.* melamine concentration. Error bars represent standard deviations from three experiments.

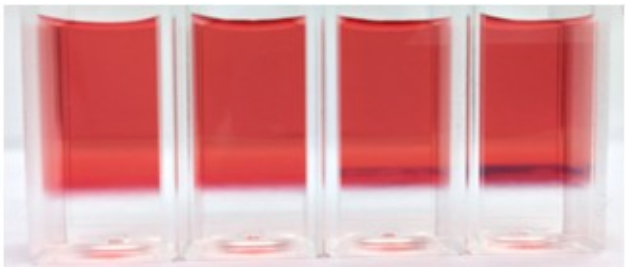

Figure 5. Visual color change of AuNPs with milk extract spiked with different concentrations of melamine (left to right: 0.00 ppm, 0.23 ppm, 0.46 ppm, 0.69 ppm). The limit of detection achieved visually was determined to be 0.46 ppm.

IV. Conclusion

A quick and easy method for the visual detection of melamine in milk and milk powder using gold nanoparticles was presented in this study. In this report, we showed that when using gold nanoparticles as analyte-induced colorimetric probe for detection of chemical systems, the effect of pH has to be taken into account. The optical properties of the gold nanoparticles and their affinity to aggregate in the presence of melamine have proven to be sensitive enough to show visual detection of melamine below the current limit set by different organizations. Because the process is so easy and relatively inexpensive compared to other methods, it could be developed into a product for on-sight detection of melamine for quality assurance and food safety.

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