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ARTICLE TYPE

Rapid Colorimetric Detection of Melamine by H_2O_2-Au nanoparticles

Miaolin Yin,[a] Lifang Zhao[a], Qin Wei[a] and He Li[*a,b]

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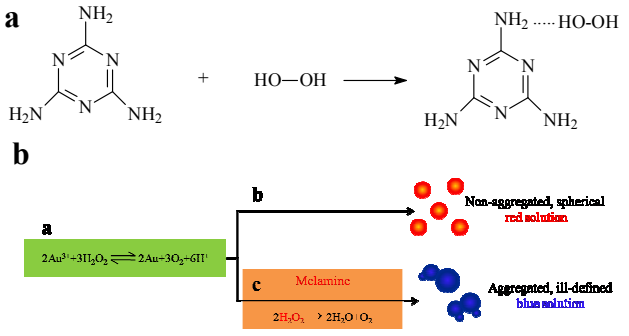
A simple, rapid and sensitive detection method for melamine in dairy products by naked eye is reported. Within 60 min, melamine can be detected as low as 0.4 μM by UV–vis spectrometer or naked eye observation, which is far below melamine safety limit in the USA and EU.

1. Introduction

Melamine (chemical formula: $C_3H_6N_6$) is a nitrogen-rich chemical which has been widely used in the manufacture of polymer resins, kitchenware and other products. Due to its high nitrogen content, melamine has been added illegally into infant formula, wheat gluten, pet foods to increase the measured protein content, which can easily mislead the value of the product.[1] However, the melamine could result in serious health issues related to kidneys and reproductive system, and even cause death, particularly for vulnerable individuals such as infants and young children.[2,3] Considering to these safety concerns, various methods for melamine analysis and related compounds in foods for human consumption and animal feed have been reported, such as gas chromatography/mass spectrometry (GC/MS), enzyme-linked immunosorbent assay (ELISA), terahertz time-domain spectroscopy,[4] surface-enhanced Raman scattering (SERS),[5] UV-SERS and electrochemical sensing methods.[6-13] However, all of these techniques are often required expensive and complicated instruments, well-trained operator to perform, time-costing, making on-site and real-time melamine testing difficult.[14] Accordingly, there is an urgent need to establish a simple, inexpensive, time-saving, reliable and highly sensitive method for the detection of melamine.

To address these challenges in melamine detection, we herein report a new strategy for rapid colorimetric sensitive detection of melamine in milk products based on H_2O_2-Au nanoparticles (Au NPs) system. Gold nanoparticles for signal generating have been widely employed for common colorimetric assay.[15] Usually, gold nanoparticles should be functionalized with sensing probes or chemical ligands firstly. Then the analytes will trigger the assemblies of gold nanoparticles by recognition process, resulting in an eye-catching color change, which can afford a simple colorimetric detection for various analytes. The functionalized step is often time consuming. Based on this strategy, some work have also been done for melamine detection.[16, 17] In Gao *et al* reporting,[16] melamine assay relied on the strong binding ability/affinity of melamine onto gold nanoparticles, enabling the assemblies of gold nanoparticles and producing visible color change. There is an issue that other analytes with similar chemical structure to melamine will bring an inevitable assay interference. In Zhao *et al* previous work,[17] the interruption of the synthesis of gold nanoparticles became an effective method for melamine assay. Ellagic acid (EA) can be taken as a reducer for the synthesis of gold nanoparticles. Due to the strong hydrogen-bonding interaction between melamine and EA, melamine will weaken the EA reducing function. As a result, the synthesis of gold nanoparticles will be interrupted and then produce color change. It should be noted that some metal ions may cause interferences for the assay because of the strong coordinate interaction with melamine. Herein, in the proposed method, hydrogen peroxide can reduce gold ions to generate quasi-sphere, non-aggregated Au NPs with a fast rate, yielding a red solution. In the presence of melamine, hydrogen peroxide will react with melamine to produce a stable addition compound.[18] With the consumption of hydrogen peroxide, the growth of Au NPs will be slow down, resulting Au NPs aggregations to give a blue solution, which is illustrated by Scheme 1. There is no need to prepare functionalized gold nanoparticles at all. Therefore, melamine can be fast, easily and affordable detected with naked eyes.



Scheme 1 The reaction of melamine and hydrogen peroxide (H_2O_2) (a), and the illustration of the detection mechanism employed (b), in the presence of H_2O_2, gold ions are reduced. High concentrations of H_2O_2 lead to the formation of non-aggregated, spherical Au NPs resulting a red solution. The consumption of melamine combining with H_2O_2 will decrease the concentration of H_2O_2, and then cause the formation of ill-defined Au NPs, yielding a blue solution.

2. Experimental section

2.1 Reagents and Materials.

MES (2-(N-morpholino) ethanesulphonic acid) was purchased from Shanghai Aladdin Reagent Co., LTD., (Shanghai, China). H_2O_2 (30wt %) was obtained from Tianjin Guang Cheng Chemicals Reagent Co. LTD (Tianjin, China), gold(III) chloride was purchased from Sinopham Chemical Reagent Limited Corporation (Shanghai, China). Other reagents were all analytical reagent grade and used as received without further purification. The milk powder and raw milk were purchased from the supermarkets (Jinan, China). Ultrapure water was used throughout the experiments.

2.2 Apparatus.

UV-vis measurements were performed using a UV–vis spectrometer (Tu-1901, Beijing Persee General Instrument Co. Ltd.). Transmission electron microscope (TEM) images were obtained from a JEM-2100 microscope (Japan). Pictures were taken from an Olympus E 620 camera (Japan).

2.3 Proposed procedure.

The raw milk and milk powder were purchased from the supermarkets and preferably detected in the same day. The milk samples were prepared as the following procedure. At First, for raw milk, 0.5 mL of trichloroacetic acid, 3.5 mL of water, and 0.5 mL of acetonitrile were put into 1.0 mL of raw milk; for milk powder, 0.5 mL of trichloroacetic acid, 4.5 mL of water and 0.5 mL of acetonitrile were put into 0.5 g of milk powder. Then the mixed milk sample was ultrasonically extracted for 15 min, following centrifuged at 7000 rpm for 15 min. The resulted supernatant was left for the following detected.

The analysis procedure for melamine was done as follows: hydrogen peroxide (100 µL, 120 µM) in MES buffer was added to the sample of the supernatant and incubated at 37°C for 20 min. Thereafter, freshly prepared gold (III) chloride (100 µL, 0.1 mM) in MES buffer was added to the incubated solution. The absorbance at 550 nm was recorded after 15 min with TU-1901 UV–vis spectrometer. Photographs were taken after 15 min after the addition of the gold precursor.

Calibration Curves of melamine was obtained as follows: Hydrogen peroxide (100 µL, 120 µM) in MES buffer was added to different concentrations of melamine (0.4 µM, 0.8 µM, 1.6 µM, 4 µM, 8 µM, 16 µM, 40 µM, 80 µM, 120µM , 160 µM, 100 µL). Then the same procedure as the detection of melamine was followed.

3. Results and discussion

As shown by Scheme 1, the color of testing solution relies on the amount of hydrogen peroxide. Therefore, the amount of hydrogen peroxide used should be optimized firstly.

In order to test the influence of the concentration of hydrogen peroxide on the morphology and optical properties of Au NPs, different concentrations of hydrogen peroxide were added into MES buffer solution (1 mM, pH 6.5), containing 0.1 mM gold ions. In Fig. 1a, the color of the solution changes from red to blue in a narrow concentration range between 120 to 100 µM, and the color intensity decreases with the concentration of hydrogen peroxide in a concentration range between 80 and 20 µM. It may be possible to control the color of the Au NPs solution to be blue when the melamine reduces the concentration of hydrogen peroxide below 120 µM.

A deeper insight into the process leading to the color change can be obtained from UV-vis spectra (Fig. 1b). Here, the spectra of the Au NPs dispersions getting broaden and red-shift when the concentration of hydrogen peroxide drops below 120 µM, this is attributed to the formation of clusters of Au NPs at low concentrations of hydrogen peroxide, which is confirmed by transmission electron microscopy (TEM) inspection (Fig. 1d, e) and it is also possible to monitor the growth of gold nanoparticles by measuring the absorbance at 550 nm with UV-vis spectra (Fig. 1c). However, this observation is attributed to the MES, which can be reduced the gold ions by hydrogen peroxide, MES is a mild reducing agent, which can generate blue-colored nanoparticle aggregated and red-colored nanoparticle dispersions. Therefore, we can tune the tonality of solution, which contains gold ions in MES buffer, by adding hydrogen peroxide.

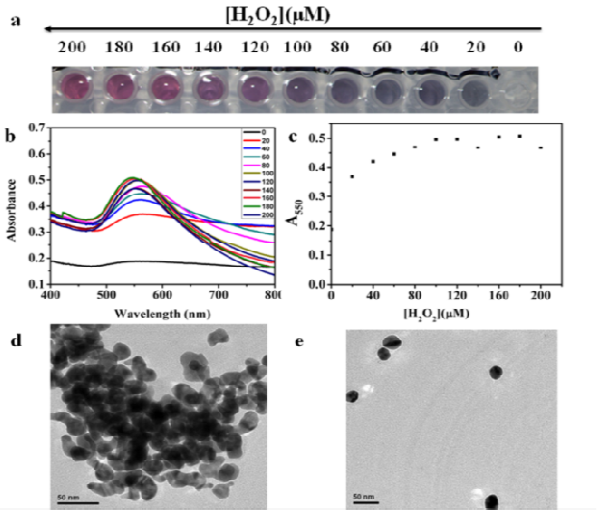


Figure 1. Generation of Au NPs solutions with different colors depends on the concentration of H_2O_2. Different concentrations of H_2O_2 were added to a solution containing gold ions (0.1 mM) in MES buffer (1 mM, pH 6.5). (a), Photograph showing the generation of nanoparticle solutions with different colors and intensities after 15 min. The tonality of the solution changes from red to blue between 120 and 100 µM. (b), UV-spectra for different H_2O_2 concentrations. The localized surface Plasmon resonance peak redshifts when the concentration of H_2O_2 is 100 µM or lower. (c), Graph showing that the absorbance of the solutions at 550 nm varies with concentration of H_2O_2. (d), TEM images of nanoparticles grown with H_2O_2 at concentration of 40 µM. (e), TEM images of nanoparticles grown with H_2O_2 at concentration of 200 µM.

During the detection process for melamine, the concentration of H_2O_2 of 120 µM was chose. In order to test the influence of melamine concentration on morphology and optical properties of Au NPs, different concentrations of melamine were added into MES buffer solution (1 mM, pH 6.5) containing gold ions (0.1 mM) and hydrogen peroxide (120 µM) (In Fig.2a). The color of the solution changed from red to blue in a narrow concentration

range between 8 and 16 μM for melamine. Hence, if we want to control the color of Au NPs solution to be blue, the concentration of melamine below 8 μM should be reduced. This is attributed to the formation of clusters of Au NPs at low concentrations of hydrogen peroxide, which is confirmed by TEM inspection (Fig. 2d and e). Furthermore, we monitored the growth of Au NPs by measuring the absorbance at 550 nm with a conventional plate reader (Fig. 2b). The growth of Au NPs is extremely sensitive to the concentration of melamine, which result in an abrupt change in the color of the solution from red to blue. These results indicate that H_2O_2-Au NPs system could be used for detection of melamine. In other words, the proposed H_2O_2-Au NPs system strategy for melamine detection is coupled with high selectivity.

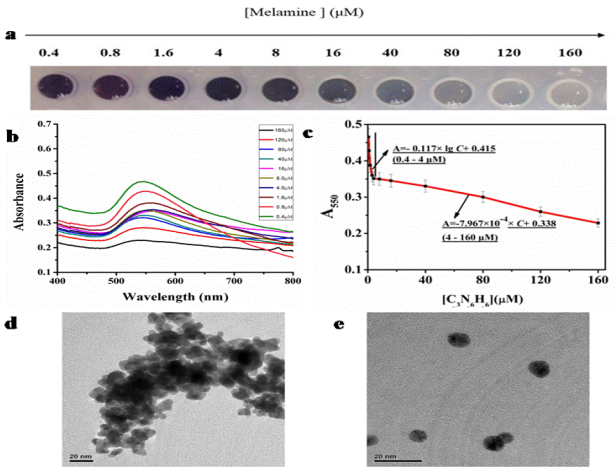


Figure 2. (a), Generation of nanoparticle-hydrogen peroxide solutions with different colors depends on the concentration of melamine. Photograph showing the generation of nanoparticle solution with different colors and intensities after 15 min. (b), UV- spectra for different melamine concentration. (c), Graph showing that the absorbance of the solution at 550 nm varies with concentration of melamine. (d), TEM images of nanoparticles grown with melamine at concentration of 80 μM. (e). TEM images of nanoparticles grown with melamine at concentration of 0.8 μM.

As shown in Table 1, different methods have been proposed to determine the concentration of melamine. Comparing with results by other various methods, this developed method presents the widest linear detection range and the lowest detection limit of melamine.

To verify the practicality of this analytical method, different concentrations of melamine were spiked into raw milk and milk powder, and then the samples were assayed according to the procedures described in Section 2.3. As shown in Table 2, it can be seen that good recoveries ranging from 90 % to 113.7 % were obtained for melamine concentrations of 0.8, 8.0, 40 μM. These same samples were also measured by HPLC/MS complying with the National Standard of China (GB/T22388-2008). Due to the sensitivity, melamine samples at 0.8 μM could not be detected by HPLC/MS. There was no obvious difference in results comparing with the two methods, which demonstrated that the accuracy and reliability of the colorimetric method for detecting melamine in practical applications. It is clear to see the obvious colorimetric differentiation from Figure 3 and 4.

Table 1 Comparison of different methods for the detection of melamine.

Method	Linear range (μmol/L)	Detection limit (μmol/L)	Reference
Raman spectroscopy	5-50	1.2	19
Fluorescence spectroscopy	7.9-47.6	6.35	20
Liquid Chromatography/ Tandem Mass Spectrometry	0.4-0.79	—	21
Electrochemical method	1.0-66.4	0.3	22
Colorimetric detection method	0.4-160	0.078	This work

Table 2 Results of the Determination of the Melamine by proposed colorimetric method and HPLC/MS in Milk Samples

Sample	Concentration of melamine(μM)		Recovery (%)	found by HPLC/MS
	Amount added	Amount found		
Raw milk				
1	8.0	8.96	112	8.25±0.23
2	40	41.11	100.17	39.84±1.31
3	0.8	0.91	113.7	—
Milk powder				
1				
2	8.0	8.76	109.5	8.10±0.19
3	40	40.34	100.9	39.08±1.17
	0.8	0.72	90	—

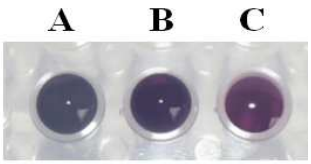


Figure 3. Typical photograph for melamine detection in the raw milk (from left to right: the concentration of melamine in the raw milk is A (40 μM), B (8 μM), C (0.8 μM)).

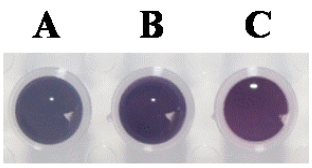


Figure 4. Typical photograph for melamine detection in the milk powder (from left to right : the concentration of melamine in the milk powder is A (40 μM), B (8 μM), C (0.8 μM)).

To evaluate the repeatability and reproducibility of the colorimetric method, a series of six melamine solutions at 3 μM were measured by the developed colorimetric method. The relative standard deviations (RSD) of the measurements for the six solutions were not more than 5.0 % (Figure 5), indicating that the repeatability and reproducibility of the proposed colorimetric method were acceptable.

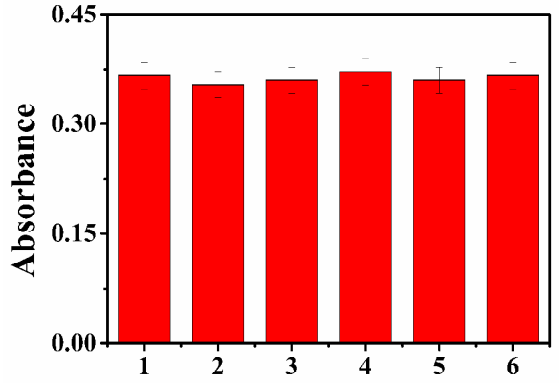


Figure 5. The repeatability and reproducibility of the proposed colorimetric method by recording the absorbance change of six melamine solutions (3 μM) at 550 nm.

Conclusions

In conclusion, we have demonstrated the detection of melamine with the naked eye by H_2O_2-Au NPs detection system was accurate, reliable, sensitive and convenient. By using this detection system, we can detect lower than 20 μM (the safety limit in the USA and EU) of melamine in real samples and be unambiguously detected in about an hour from the beginning of sampling to the final step of obtaining the results from the spectrophotometer. The reduced cost, ultrasensitive and rapid detection of melamine could be useful for the dairy products in resource-constrained countries. In the future, this convenient and reliable color analytical method could be applied in a real diary product.

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Notes and references

[a] *Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, China. Fax: +86 531 82765969; Tel: +86 13082738030; E-mail: lihecd@gmail.com*
[b] *School of Biological Science and Technology, University of Jinan, Jinan 250022, China*

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