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Development of Gold Nanoparticle-Based Rapid Detection Kit for Melamine in Milk Products

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ABSTRACT: A reliable and sensitive kit for the rapid detection of melamine (Mel) was developed. The kit is based on gold nanoparticle (Au NP) probe and includes a standard colorimetric card. The Au NPs were prepared by sodium borohydride reduction and characterized by transmission electron microscopy, which revealed particle sizes of approximately 5 nm. The performance of the kit in terms of aggregation kinetics, cross-reactivity, anti-interference, and sample pretreatment was investigated. The standard colorimetric card was then fabricated by chromatic aberration of a series of standard Mel-spiked milk reacts with the 5 nm Au NPs. The working range of the kit is 1−120 mg/L, and its performance is visibly more rapid and reliable by comparison with the standard colorimetric card. As low as 1 mg/L of Mel levels in milk can be determined, with the assay taking only about 10 min, including sample pretreatment. The kit can be stored for a year at room temperature. Samples were also detected by the kit, yielding results close to those obtained by high-performance liquid chromatography/mass spectrometry. Thus, the kit is applicable to qualitative and semiquantitative field detection, as well as naked-eye screening without the aid of any instrumentation.

KEYWORDS: melamine (Mel), gold nanoparticles (Au NPs), rapid detection kit

INTRODUCTION

Melamine (Mel; 1,3,5-triazine-2,4,6-triamine), which contains 66% nitrogen by mass, is used in various industrial processes, as well as plastics for the production of tableware and kitchen utensils, flame retardants, can lining, etc. In 2007, Mel contamination of pet food was associated with many clinical cases of canine and feline nephrotoxicity and, in some cases, mortality. In 2008, high levels of Mel in milk and other dairy products were reported, and in July 2009, contamination related to this compound was still prevalent in many Chinese provinces such as Gansu, Qinghai, and Jilin. Guan found that children exposed to high-melamine formula were seven times more likely to suffer from kidney stones than unexposed children, while preterm infants are 4.5 times as likely to develop stones as full-term infants.[1] The ingestion of melamine at levels above the safety limit (2.5 ppm in the USA and EU; 1 ppm for infant formula in China) can induce renal failure and death in infants.[2] For the accurate detection of Mel in food and water, the methods currently used include high performance liquid chromatography (HPLC),[3,4] mass spectrometry (MS),[5−8] liquid chromatography/mass spectrometry (LC/MS),[9] gas chromatography/mass spectrometry (GC/MS),[10,11] capillary zone electrophoresis/mass spectrometry (CE/MS),[12] and enzyme-linked immunosorbent assay (ELISA).[13−16] Other technologies such as electrochemical sensor,[17] micellar electrokinetic chromatographic methods,[18−20] and molecularly imprinted silica sol−gel film[21] were also applied for the detection of Mel. However, these methods require complicated matrices and derivation of mass, entail lengthy sample pretreatment, cause antigen−antibody reaction, etc. Hence, a fast, low-cost, highly sensitive, and selective method for trace Mel detection in real samples is urgently needed.

Recently, colorimetric technologies based on gold nanoparticles (Au NPs) have attracted considerable attention because of their unique properties such as color rendering, biocompatibility, stability, and high extinction coefficients.[22] Given that the ring nitrogen of hybrid aromatics exhibits much stronger binding ability/affinity to Au NPs, the pyridine-like compounds are often used as phase transfer agents for Au NPs.[23] Mel has multiple binding sites, including three exocyclic amino groups and a three-nitrogen hybrid ring; it can strongly coordinate to Au NPs by ligand exchange with weakly surface-bound citrate ions, and cross-link Au NPs.[24] It enables aggregation and causes a significant shift in the extinction spectrum as the solution color changes from red to purple.[25−27] The clearly distinguishable color change facilitates simple sensor readout that can be performed by the naked eye.[28]

There are a few studies on Au NP-based colorimetric detection of Mel; in these studies, aggregation is induced by interparticle cross-linking.[24,29−35] For rapid Mel detection, label-free Au NPs should be used in on-site or field screening of raw milk and milk products. In the present study, we developed a novel 10 min Mel detection kit that employs 5 nm Au NPs and a colorimetric card.

MATERIALS AND METHODS

Synthesis of Au NPs. All the glassware used in the procedures was soaked and cleaned in a bath of freshly prepared 3:1 HNO_3−HCl for 24 h, rinsed thoroughly in pure water, and dried in air prior to use. Colloidal Au NPs with different diameters were synthesized by sodium borohydride and sodium citrate reductions. The procedure is described as follows: 100 mL of 0.01% (w/w) $HAuCl_4$ solutions was mixed with 1 mL of 1% trisodium citrate and 1 mL of 0.075% $NaBH_4$-1% trisodium citrate

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solution under vigorous stirring at room temperature (RT) for 30 min. Then, 1 mL of 0.02% NaN_3 was added into the Au NP solution for antisepsis and stabilization. The solution was filtrated using 0.22 μm filters and then stored at 4 °C. Au NPs of another size were prepared by citrate reduction, according to published protocols.[36] The sizes of the Au NPs were then examined by transmission electron microscopy (TEM).

Selection of Au NP Size. Aliquots (2 mL) of the prepared NPs were placed in microtubes (150 μL/tube). The prediction of aging performance using accelerated aging tests was based on Arrhenius theory; the aliquots were stored at 37 °C for 7, 14, 28, 56, 77, and 91 days. The Au NPs were used to detect the same concentration of Mel in the solutions stored at the aforementioned periods. Color change and absorbance were recorded using a UV−vis spectrometer. To select the appropriate nanoparticle size, reaction performance and aggregation kinetics were also investigated.

Pretreatment of Milk Samples. Milk products could be classified as liquid and solid states. Three drops (approximately 0.15 mL) of 300 g/L trichloroacetic acid were added into the microtube containing 2.0 mL of liquid milk such as raw milk, pure milk, pasteurized milk, yogurt, etc.; the tube was shaken several times by hand to thoroughly mix the solution. A white deposit was immediately observed. After being left to stand for 1−2 min, the mixture was filtrated using filter paper, and a drop of 3 M NaOH was added to adjust the pH to 7 (measured using a pH testing strip). Finally, the filtrate was refiltered using a syringe and 0.22 μm filters. The same procedure was followed for the pretreatment of solid milk products (including powdered infant formula, cheese, milk candy, etc.), except that a 0.4 g sample was dissolved in 2 mL of water to which 4 drops of trichloroacetic acid were added. The filtrate obtained was used for Mel detection.

Fabrication of the Mel Detection Kit. Pretreated milk filtrate without Mel (tested by HPLC/MS beforehand) served as the blank control. Standard solutions were prepared by adding a series of Mel standards to milk samples to come up with final concentrations of 120, 60, 30, 15, and 1 mg/L. Au NPs (300 μL) were added into the series of 150 μL solutions, including the blank control; the resultant mixture was left to stand for 2 min at RT. Color was recorded using a digital camera. The procedure was repeated three times, and the standard colors were determined to compare chrominance at the same concentration. The colors of the Mel standard solutions, which can be distinguished by the naked eye, were regarded as standard levels. These standard levels were printed in polyvinyl chloride boards and cut into strips to serve as the standard colorimetric cards. After the standard colorimetric cards were prepared, the Mel detection kit was assembled using a series of reagents (Au NP solution, NaOH, and trichloroacetic acid) and materials (pH testing strip, plastic straw, syringe, microtube, filter, etc.). The reagents and material that make up the kit are shown in Figure 1. It can approximately detect 25 samples.

Validation Experiments. Milk samples spiked with Mel at concentrations of 1, 12.5, 25, and 50 mg/L were prepared and pretreated using the procedures mentioned in Pretreatment of Milk Samples. The samples were added to 300 μL of Au NP solution, and each sample was evaluated against the standard colorimetric cards after a 3 min reaction at RT. Mel in the samples could be semiquantitatively determined by the nearest chrominance of the standard colorimetric card representing certain original standard concentrations. The same samples were extracted and detected by HPLC/MS in accordance with the National Standard of China (GB/T22388-2008). The blank control was also subjected to the two methods (the rapid kit and HPLC method) to validate reliability.

RESULTS AND DISCUSSION

Selection of the Particle Size of Au NPs. *Stability.* TEM image observation is the favored method in assessing the formation, shape, diameter, and uniformity of Au NPs. The ideal particle should be circular and equirotal, not oval or polygonal. The particle sizes of the Au NPs prepared by sodium borohydride and sodium citrate reductions were approximately 5 and 18 nm, respectively, as determined by TEM (Figure 2).

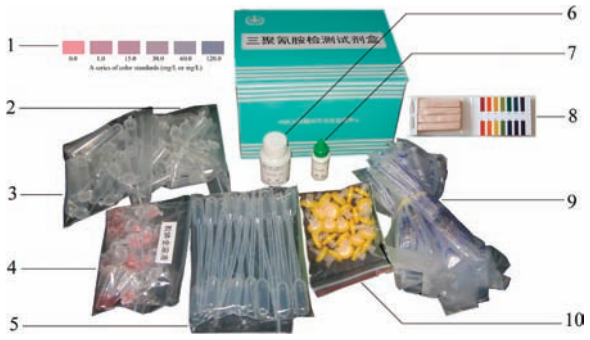

Figure 1. Composition of the rapid detection kit for Mel. The kit is composed of the following reagents and materials: (1) standard colorimetric card; (2) microtube (2 mL); (3) microtube (5 mL); (4) Au NP solution (300 μL/tube); (5) plastic straw (1 mL); (6) trichloroacetic acid (300 g/L); (7) NaOH (3 M); (8) pH testing strip; (9) syringe (1 mL); (10) filter.

The two Au NP aliquot solutions were stored at 37 °C for three months. At the end of this period, the 5 nm Au NPs demonstrated good dispersibility and stability, as observed by the naked eye or by TEM (Figure 2A). Using the Arrhenius equation, the calculated shelf life at RT is one year (storage at 37 °C after 91 days equals one year when stored at RT).[37] For the same concentration of Mel, the detection time and change in color were similar to those of the fresh solutions. Through naked-eye observation, sediments were found at the bottom of the microtube in the 18 nm Au NP solution; visible aggregation was observed by TEM (Figure 2B). Given the citrate electrostatic repulsion on the surface of Au NPs, the monodispersed Au NPs in aqueous solution are not likely to be aggregated. However, as diameter increases, the stability of electrostatic repulsion between Au NPs is difficult to maintain. In this study, a tendency toward aggregation, or even collision between particles, was observed. This formed overlapping aggregations that led to deposition.

Reaction Performance of the Prepared Au NPs. Different concentrations of Mel (0−10 mg/L) were simultaneously added into the two solutions of Au NPs with different particle sizes to compare their detection performance by the naked eye (Figure 3).

When the final concentration of Mel reached 0.06 mg/L, as in Figure 3B, the change in color was visibly recognized by the naked eye; however the change was not recognized until the final concentration reached 0.2 mg/L in Figure 3A. Moreover, the chromatic aberration in Figure 3B is more significant than that in Figure 3A. We repeated the experiment mentioned above and obtained the same result. The findings suggest that the 5 nm Au NPs are more sensitive and capable of detecting low concentrations of Mel than are the 18 nm sized Au NPs. This result may be attributed to the fact that the surface energy of nanoparticles increased with the decrease in diameter,[38] a result consistent with those of previous studies.[30] However, Au NPs with diameters less than 2.5 nm are light-colored and infeasible for visual study. Hence, the 5 nm Au NPs showed better performance; that is, the Au NPs showed improved sensitivity and long shelf life.

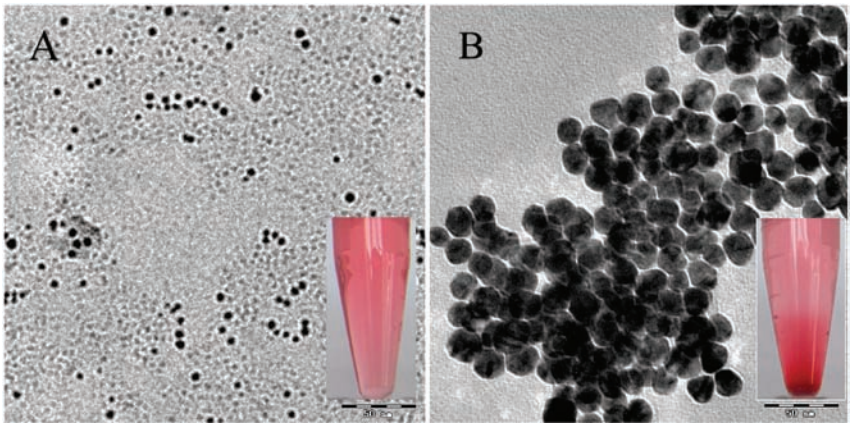

Figure 2. TEM images of the Au NPs: (A) prepared by sodium borohydride and (B) citric acid reductions. Insets are the corresponding photographs of the Au NP solutions after storage in 37 °C for 3 months.

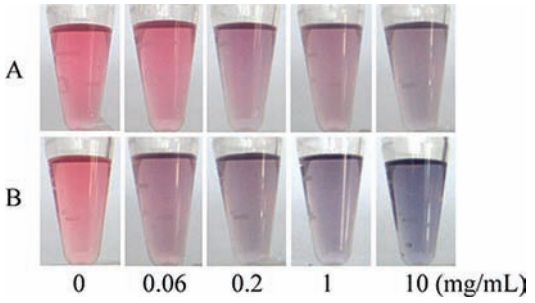

Figure 3. Chromatic illustrations of different concentrations of Mel (150 μL) dissolved in water added to 300 μL Au NP solutions: (A) 18 nm Au NPs and (B) 5 nm Au NPs.

Aggregation Kinetics of the Au NPs in the Presence of Mel. Given the rapid response of Au NPs and Mel at RT, the kit is applicable to on-site and real-time detection of Mel in milk products after pretreatment. We examined and evaluated the aggregation kinetics of the two kinds of Au NP probes in the presence of 0.2 mg/L Mel by measuring the absorbance ratios at RT.

Figure 4A shows that the absorbance ratio (A_{560}/A_{510}) curve of the 5 nm Au NPs tended to stabilize within 100 s, suggesting that the aggregations caused by hydrogen bonds can be completed within this duration. Nevertheless, after 0.2 mg/L Mel was added in the 18 nm Au NP solution, the absorbance ratio (A_{680}/A_{520}) curve exhibited a gradual increase during the first 500 s to reach equilibrium, and a slow increase during the succeeding 1300 s. Considering that free monodispersed Au NPs were immediately consumed at the initial stage, the 5 and 18 nm Au NPs took 100 and 500 s, respectively, to reach equilibrium. The result suggests that the smaller Au NPs are more sensitive to Mel, and that they can detect Mel concentrations more rapidly than larger sized Au NPs. This finding may be attributed to the increase in surface energy of the Au NPs as the diameter decreased, and the tendency of the smaller Au NPs to combine more strongly with Mel.

With the overall considerations on the stability, reaction performance, and aggregation kinetics of the two kinds of Au NPs, we chose the 5 nm Au NPs as the detection probe.

Evaluation of Cross-Reactivity. To validate the specificity of the kit for Mel detection, 30 mg/L Mel analogues such as cyanuric acid, Mel-Cl, and atrazine were investigated by UV−vis spectroscopy (Figure 5A).

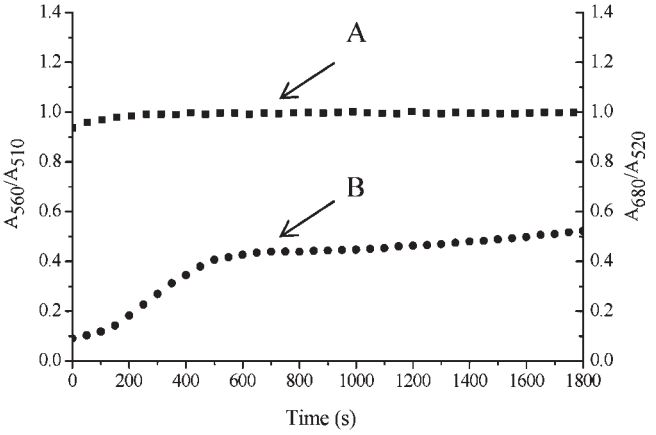

Figure 4. Time-dependent absorbance ratio changes upon the interaction of the (A) 5 nm (A_{560}/A_{510}) and (B) 18 nm (A_{680}/A_{520}) Au NPs with 1 mg/L Mel. The absorbance peaks of 5 and 18 nm Au NPs were at 510 and 520 nm, respectively; after adding Mel to the solution, the absorbance peaks shifted to 560 and 680 nm, respectively. The absorbance ratio between the two values exhibits the aggregation kinetics of Au NPs.

To compare the absorbance ratios of the analogues, we subtracted the absorbance ratio of the Au NPs, i.e., the background value (A_0). Their absorbance ratios ($A_{560}/A_{510} - A_0$) are shown in Figure 5B. Under the same concentration, Mel exhibited the highest absorbance ratio (approximately 0.6), whereas the analogues showed relatively lower values (<0.1). This result suggests that the kit enables highly specific Mel detection.

Anti-Interference Performance of the Kit. Nutritional ingredients, such as common ions, excipients, proteins, lipids, etc., in milk products are complex. Proteins and lipids can be removed by trichloroacetic acid, but lactose and ions cannot be eliminated. Figure 6 shows the absorbance ratios ($A_{560}/A_{510} - A_0$) of Mel and other interferences.

Among the chemicals, the 2.5 mg/L Mel exhibited the greatest absorbance ratio, indicating that the Au NPs are mostly influenced by Mel. In addition, the other interferences, including KCl, NaCl, CaCl, $CuSO_4$, $MgSO_4$, $ZnSO_4$, $Fe_2(SO_4)_3$, lactose, glucose, etc., all exhibited a concentration of 30 mg/L (10 times higher than that of Mel). This concentration, however, did not

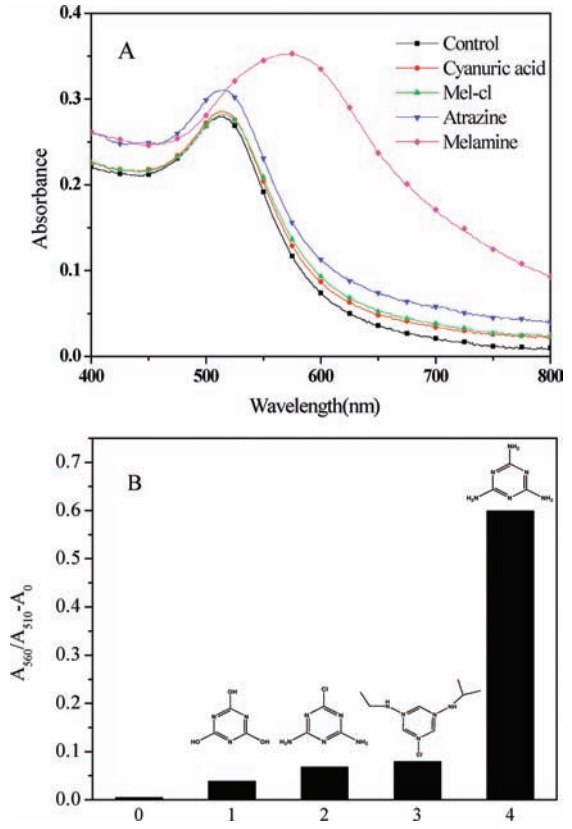

Figure 5. (A) UV−vis spectra of the Au NP solution in the presence of different analogues. (B) The absorbance ratio (A_{560}/A_{510}) of the Au NP solution in the presence of different analytes: 0, blank control; 1, cyanuric acid; 2, Mel-Cl; 3, atrazine; 4, Mel. The concentration of all the analogues was 30 mg/L.

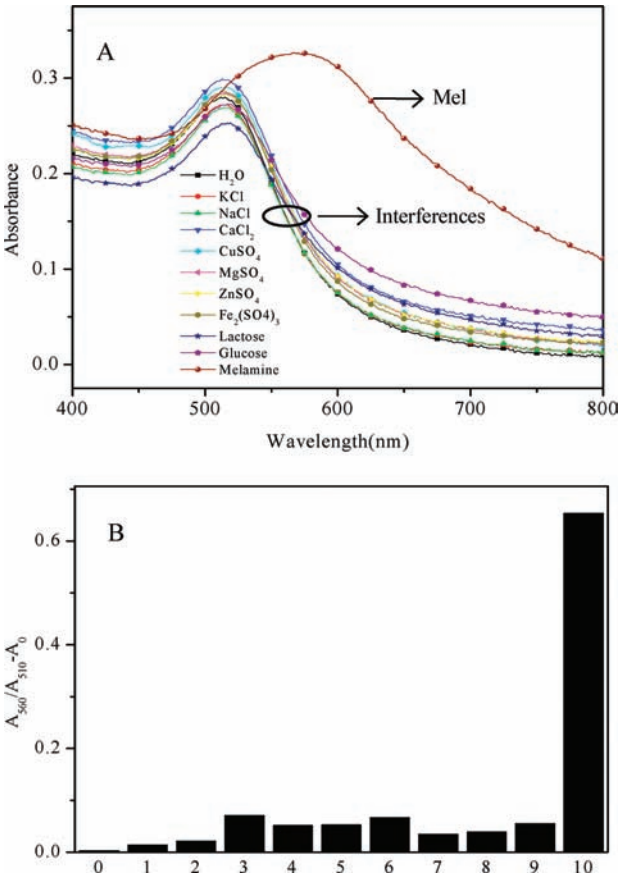

Figure 6. (A) UV−vis spectra of the Au NP solution in the absence and presence of different interferences. (B) The absorbance ratio ($A_{560}/A_{510} - A_0$) of the Au NP solution in the absence and presence of different analytes: 0, control; 1, KCl; 2, NaCl; 3, CaCl; 4, $CuSO_4$; 5, $MgSO_4$; 6, $ZnSO_4$; 7, $Fe_2(SO_4)_3$; 8, lactose; 9, glucose; 10, Mel. The concentration of Mel was 2.5 mg/L while that of the others was 30 mg/L.

affect Mel detection, indicating that Au NPs are highly selective to Mel and have no obvious interference with common ions and small organic molecules in milk.

Sample Pretreatment. Currently, Mel pretreatment is based primarily on the National Standard of China (GB/T22388-2008), which requires extraction, purification, condensation, etc.,[24,29] as well as the use of a centrifuge, ultrasonic apparatuses, etc.[30,31] Although the sensitivity improves, such procedures are complex, time-consuming, and cannot be operated without a laboratory platform. The on-site and rapid screening and detection of Mel is currently limited by the aforementioned disadvantages. In terms of rapidity, high efficiency, and sensitivity, the extraction reagent should satisfy two requirements. First, it should extract the target through the addition of a small volume of extraction reagent with little imports. Second, it should not induce aggregation and interfere with the detection of monodispersed Au NPs. When we used 0.15 mL 300 g/L trichloroacetic acid to treat 2.0 mL liquid milk, proteins and greases in milk products could be basically and quickly removed by simple process and required no aid of any instrumentation in field or in lab. The process, which benefits from the optimization of the reaction agent and operational procedures, took less than 5 min in obtaining the filtrate without inspissation. This process can be used to directly detect Mel by the rapid kit.

The sensitivity of the kit at the same concentration of Mel in milk was not as good as that in water. The LDL of Mel in water was 0.06 mg/L (Figure 3B); significant changes in color were not observed until the concentration reached 1 mg/L or 1 mg/kg in the milk samples. The performance of the kit may be attributed to the matrix effect, in which some of the proteins and greases in milk products do not precipitate completely by such a simple pretreatment. This phenomenon weakens Au NP aggregation and reduces sensitivity.

Lowest Detection Limit (LDL) and Detection Ranges of the Kit. Given the relationship between the degree of aggregation of the Au NPs and the changes in color with varying Mel concentrations, we fabricated a standard colorimetric card by collecting the chromatic diversification induced by the various concentrations of Mel that were added in the milk. When the original Mel concentration was as low as 1 mg/L, the chroma was close to that of the matrix and could still be differentiated. Similarly, at a concentration higher than 120 mg/L, no apparent change in color was observed because the reaction tended toward saturation. The chromatic gradients between 1 and 120 mg/L, whose changes in color were recognized by the naked eye, were used in preparing the settings of the Mel concentrations. Therefore, the working range of this kit is 1−120 mg/L: the LDL is 1 mg/mL while the highest detection limit is 120 mg/L. The additional volume of trichloroacetic acid (0.15 mL) was negligible compared with that of the sample (2 mL); nevertheless, when 150 μL of filtrate was added into 300 μL of Au NP solution, the

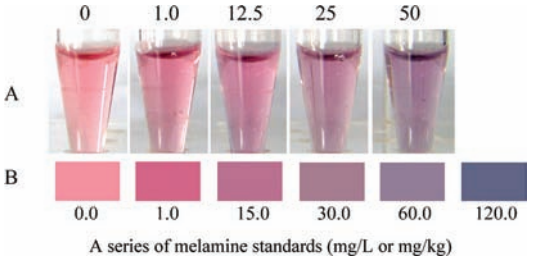

Figure 7. Results of the standard colorimetric card for Mel detection. (A) Mel concentrations in actual samples: 0, 1.0, 12.5, 25, and 50 mg/L. (B) Standard colorimetric card (0.0, blank control; others are the color levels of the original concentrations from 1 to 120.0 mg/L Mel in milk).

Table 1. Mel Detection (mg/L) in Milk Samples by Rapid Kit and HPLC/MS (n = 3, mg/L)

concn	found by rapid kit	found by HPLC/MS
0	0, 0, 0	0.01 ± 0.01
12.5	15, 15, 15	12.20 ± 0.38
25.0	30, 30, 30	23.57 ± 2.36
50.0	60, 60, 60	44.25 ± 2.09

concentration of Mel was diluted 3-fold. The dilution of Mel has not been discussed in previous studies;[25,29−31] the concentration detected was the diluted concentration of Mel and not the original concentration in the samples. In the present work, all the corresponding Mel concentrations marked in the card are the actual concentrations of Mel in the samples, without any mathematical conversion to the original concentrations. Thus, the card is convenient and easy to qualitatively and semiquantitatively apply in screening and field detection.

Validation of the Kit with HPLC/MS and Application in Real Milk Products. To validate whether the kit can be used to detect Mel in milk products, we tested unknown samples and conducted comparison experiments. As demonstrated in Figure 7, the color of the Au NPs was kept stable by the addition of the blank milk filtrate. With the increase in Mel concentration from 1 to 50 mg/L, the color visibly changed from orange to violet within 2 min. The same samples were also examined by HPLC/MS.

The results for the 1, 15, 30, and 60 mg/L samples, as evaluated against the standard colorimetric card, are displayed in Table 1. The results are close to those obtained by the HPLC/MS method recommended by the National Standard of China (GB/T22388-2008). The results indicate that the kit is reliable for the rapid detection of Mel. Although the rapid kit cannot qualitatively detect Mel and achieve the accuracy observed in HPLC/MS, it is appropriate for field detection and does not require the use of professional techniques. Nearly everyone can use it. Rapidity, convenience, and simplicity are the advantages of the kit over the HPLC/MS method.

Spiked Mel milk samples were used to validate the effectiveness of the kit. Nevertheless, the LDL of the kit satisfies the national standard requirement, i.e., 1 mg/kg in powdered infant formula and 2.5 mg/kg in milk products. Except for the Au NP solution, the reagents and materials in the kit, including the standard colorimetric card, are readily available and easy to prepare in general laboratories. Additionally, the cost of synthesizing Au NPs is considerably lower than that incurred in other kinds of ELISA-based kits. Moreover, the shelf life of our kit is much longer than that of the ELISA kits and immunochromatographic testing strip;[39] and stringent storage requirements are not necessary. It is also different from immunogold chromatographic strip test based on a monoclonal antibody as the capture probe.[40] First, monoclonal antibody for Mel is very costly and very difficult to prepare in the lab for a long time. The cost of synthesizing Au NPs as the probe is considerably lower than preparation or purchasing commercial monoclonal antibody. Second, the preparation method for the kit was much simpler than that of immunochromatographic strip. Third, the preparation methods of colloidal gold were different by different sizes and different capture means. Another great advantage is the one-year-long shelf life at RT. This kit is available for rapid screening of large batches of milk products; it is not time-consuming and is economical, making it a promising product.

In this study, we have developed a kit for rapid detection of Mel by naked eye in comparison using the standard colorimetric card without the aid of any instrument. It can be employed for most milk products, and can be used in the dairy industry and quality assurance departments, as well as by supermarket managers, customers, etc. The ease of use presented by the kit for qualitative and semiquantitative examinations in large-scale screening and field detection makes it a promising product amid the growing commercialization of Mel detection kits.

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Author Contributions

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ABBREVIATIONS USED

Au NPs, gold nanoparticles; CE/MS, capillary zone electrophoresis/mass spectrometry; ELISA, enzyme-linked immunosorbent assay; GC/MS, gas chromatography/mass spectrometry; HPLC, high performance liquid chromatography; HPLC/MS, high performance liquid chromatography/mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; LDL, lowest detection limit; Mel, melamine; MS, mass spectrometry; NaN_3, sodium azide; PVC, polyvinyl chloride; RT, room temperature; TEM, transmission electron microscopy

REFERENCES

(1) Guan, N.; Fan, Q.; Ding, J.; Zhao, Y.; Lu, J.; Ai, Y.; Xu, G.; Zhu, S.; Yao, C.; Jiang, L.; Miao, J.; Zhang, H.; Zhao, D.; Liu, X.; Yao, Y. Melamine-contaminated powdered formula and urolithiasis in young children. *N. Engl. J. Med.* **2009**, *360*, 1067–1074.

(2) Zhu, L.; Gamez, G.; Chen, H.; Chingin, K.; Zenobi, R. Rapid detection of melamine in untreated milk and wheat gluten by

ultrasound-assisted extractive electrospray ionization mass spectrometry (EESI-MS). *Chem. Commun.* **2009**, 559–561.

(3) Wei, R.; Wang, R.; Zeng, Q.; Chen, M.; Liu, T. High-performance liquid chromatographic method for the determination of cyromazine and melamine residues in milk and pork. *Chromatogr. Sci.* **2009**, *47*, 581–584.

(4) Sun, H.; Wang, L.; Ai, L.; Liang, S.; Wu, H. A sensitive and validated method for determination of melamine residue in liquid milk by reversed phase high performance liquid chromatography with solid phase extraction. *Food Control* **2010**, *21*, 686–691.

(5) Campbell, J. A.; Wunschel, D. S.; Petersen, C. E. Analysis of melamine, cyanuric acid, ammelide, and ammeline using matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOFMS). *Anal. Chem.* **2007**, *40*, 3107–3118.

(6) Tang, H. W.; Ng, K. M.; Chui, S. S. Y.; Che, C. M.; Lam, C. W.; Yuen, K. Y.; Siu, T. S.; Lan, L. C. L.; Che, X. Y. Analysis of melamine cyanurate in urine using matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* **2009**, *81*, 3676–3682.

(7) Yang, S. P.; Ding, J. H.; Zheng, J.; Hu, B.; Li, J. Q.; Chen, H. W.; Zhou, Z. Q.; Qiao, X. L. Detection of melamine in milk products by surface desorption atmospheric pressure chemical ionization mass spectrometry. *Anal. Chem.* **2009**, *81*, 2426–2436.

(8) Venkatasami, G.; Sowa, J. R., Jr. A rapid, acetonitrile-free, HPLC method for determination of melamine in infant formula. *Anal. Chim. Acta* **2010**, *665*, 227–230.

(9) Andersen, W. C; Turnipseed, S. B.; Karbiwnyk, C. M.; Clark, S. B.; Madson, M. R.; Gieseker, C. M. Determination and confirmation of melamine residues in catfish, trout, tilapia, salmon, and shrimp by liquid chromatography with tandem mass spectrometry. *J. Agric. Food Chem.* **2008**, *56*, 4340–4347.

(10) Zhu, X.; Wang, S.; Liu, Q.; Xu, Q.; Xu, S.; Chen, H. Determination of residues of cyromazine and its metabolite, melamine, in animal-derived food by gas chromatography-mass spectrometry with derivatization. *J. Agric. Food Chem.* **2009**, *57*, 11075–11080.

(11) Xu, X.-M.; Ren, Y.-P.; Zhu, Y.; Cai, Z.-X.; Han, J.-L.; Huang, B.-F.; Zhu, Y. Direct determination of melamine in dairy products by gas chromatography/mass spectrometry with coupled column separation. *Anal. Chim. Acta* **2009**, *650*, 39–43.

(12) Vo, T. D. T.; Himmelsbach, M.; Haunschmidt, M.; Buchberger, W.; Schwarzinger, C.; Klampfl, C. W. Improved analysis of melamine–formaldehyde resins by capillary zone electrophoresis-mass spectrometry using ion-trap and quadrupole-time-of-flight mass spectrometers. *J. Chromatogr., A* **2008**, *1213*, 83–87.

(13) Garber, E. A. E. Detection of melamine using commercial enzyme-linked immunosorbent assay technology. *J. Food Prot.* **2008**, *71*, 590–594.

(14) Liu, J.; Zhong, Y.; Liu, J.; Zhang, H.; Xi, J.; Wang, J. An enzyme linked immunosorbent assay for the determination of cyromazine and melamine residues in animal muscle tissues. *Food Control* **2010**, *21*, 1482–1487.

(15) Lei, H.; Shen, Y.; Song, L.; Yang, J.; Chevallier, O.; Haughey, S.; Wang, H.; Sun, Y.; Elliott, C. T. Hapten synthesis and antibody production for the development of a melamine immunoassay. *Anal. Chim. Acta* **2010**, *665*, 84–90.

(16) Wang, Z.; Ma, X.; Zhang, L.; Yang, W.; Gong, L.; He, P.; Li, Z. Screening and determination of melamine residues in tissue and body fluid samples. *Anal. Chim. Acta* **2010**, *662*, 69–75.

(17) Cao, Q.; Zhao, H.; He, Y. J.; Ding, N.; Wang, J. Electrochemical sensing of melamine with 3,4-dihydroxyphenylacetic acid as recognition element. *Anal. Chim. Acta* **2010**, *675*, 24–28.

(18) Hsu, Y.-F.; Chen, K.-T.; Liu, Y.-W.; Hsieh, S.-H.; Huang, H.-Y. Determination of melamine and related triazine by-products ammeline, ammelide, and cyanuric acid by micellar electrokinetic chromatography. *Anal. Chim. Acta* **2010**, *673*, 206–211.

(19) Wang, J.; Jiang, L.; Chu, Q.; Ye, J. Residue analysis of melamine in milk products by micellar electrokinetic capillary chromatography with amperometric detection. *Food Chem.* **2010**, *121*, 215–219.

(20) Wu, W.-C.; Tsai, I.-L.; Sun, S.-W.; Kuo, C.-H. Using sweeping-micellar electrokinetic chromatography to determine melamine in food. *Food Chem.* **2011**, *128*, 783–789.

(21) Yu, J.; Zhang, C.; Dai, P.; Ge, S. Highly selective molecular recognition and high throughput detection of melamine based on molecularly imprinted sol–gel film. *Anal. Chim. Acta* **2009**, *651*, 209–214.

(22) Wu, Z. J.; Zhao, H.; Xue, Y.; Cao, Q.; Yang, J.; He, Y. J.; Li, X. J.; Yuan, Z. B. Colorimetric detection of melamine during the formation of gold nanoparticles. *Biosens. Bioelectron.* **2010**, *20*, 2140–2144.

(23) Gittins, D. I.; Caruso, F. Spontaneous phase transfer of nanoparticulate metals from organic to aqueous media. *Angew. Chem., Int. Ed.* **2001**, *40*, 3001–3004.

(24) Chi, H.; Liu, B.; Guan, G.; Zhang, Z.; Han, M.-Y. A simple, reliable and sensitive colorimetric visualization of melamine in milk by unmodified gold nanoparticles. *Analyst* **2010**, *135*, 1070–1075.

(25) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. Selective colorimetric detection of poly-nucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* **1997**, *277*, 1078–1081.

(26) Park, S. J.; Taton, T. A.; Mirkin, C. A. Array-based electrical detection of DNA with nanoparticle probes. *Science* **2002**, *295*, 1503–1506.

(27) Lin, C. X.; Xie, M. Y.; Chen, J. J. L.; Liu, Y.; Yan, H. Rolling-circle amplification of a DNA nanojunction. *Angew. Chem., Int. Ed.* **2006**, *45*, 7537–7539.

(28) Cai, M.; Li, F.; Zhang, Y.; Wang, Q. One-pot polymerase chain reaction with gold nanoparticles for rapid and ultrasensitive DNA detection. *Nano Res.* **2010**, *3*, 557–563.

(29) Ai, K.; Liu, Y.; Lu, L. Hydrogen-bonding recognition-induced color change of gold nanoparticles for visual detection of melamine in raw milk and infant formula. *J. Am. Chem. Soc.* **2009**, *131*, 9496–9497.

(30) Li, L.; Li, B.; Cheng, D.; Mao, L. Visual detection of melamine in raw milk using gold nanoparticles as colorimetric probe. *Food Chem.* **2010**, *122*, 895–900.

(31) Guo, L.; Zhong, J.; Wu, J.; Fu, F.; Chen, G.; Zheng, X.; Lin, S. Visual detection of melamine in milk products by label-free gold nanoparticles. *Talanta* **2010**, *82*, 1654–1658.

(32) Kuang, H.; Chen, W.; Yan, W. J.; Xu, L. G.; Zhu, Y. Y.; Liu, L. Q.; Chu, H. Q.; Peng, C. F.; Wang, L. B.; Kotov, N.; Xu, C. L. Crown ether assembly of gold nanoparticles: Melamine sensor. *Biosens. Bioelectron.* **2010**, *26*, 2032–2037.

(33) Qi, W. J.; Wu, D.; Ling, J.; Huang, C. Z. Visual and light scattering spectrometric detections of melamine with polythymine-stabilized gold nanoparticles through specific triple hydrogen-bonding recognition. *Chem. Commun.* **2010**, *46*, 4893–4895.

(34) Qin, Z. H.; Zhao, H. W.; Huang, C. Z.; Wu, L. P. Simple determination of melamine based on self-assembly of citrate-capped gold nanoparticles. *Chem. Lett.* **2009**, *38*, 470–471.

(35) Wei, F.; Lam, R.; Cheng, S.; Lu, S.; Ho, D. Li, Na. Rapid detection of melamine in whole milk mediated by unmodified gold nanoparticles. *Appl. Phys. Lett.* **2010**, *96*, 133702-1–133702-3.

(36) Grabar, K. C.; Freeman, R. G.; Hommer, M. B.; Natan, M. J. Preparation and characterization of Au colloid monolayers. *Anal. Chem.* **1995**, *67*, 735–743.

(37) Gasa, J. V.; Liu, Z.; Shaw, M. T. Relationship between density and elongation-at-break of naturally and artificially aged cable materials used in nuclear power plants. *Polym. Degrad. Stab.* **2005**, *87*, 77–85.

(38) Jiang, Z. L.; Feng, Z. W.; Li, T. S.; Li, F.; Zhong, F. X.; Xie, J. Y. Resonance scattering spectroscopy of gold nanoparticle. *Sci. China, Ser. B: Chem.* **2001**, *44*, 175–181.

(39) Peng, F. H.; Wang, Z.; Zhang, S. H.; Wu, R. W.; Hu, S. S.; Li, Z. L.; Wang, X. L.; Bi, D. R. Development of an immunochromatographic strip for rapid detection of H9 subtype avian Influenza viruses. *Clin. Vaccine Immunol.* **2008**, *15*, 569–574.

(40) Li, X. M.; Luo, P.; Tang, S.; Beier, R. C.; Wu, X.; Yang, L.; Li, Y.; Xiao, X. Development of an immunochromatographic strip test for rapid detection of melamine in raw milk, milk products and animal feed. *J. Agric. Food Chem.* **2011**, *59*, 6064–6070.