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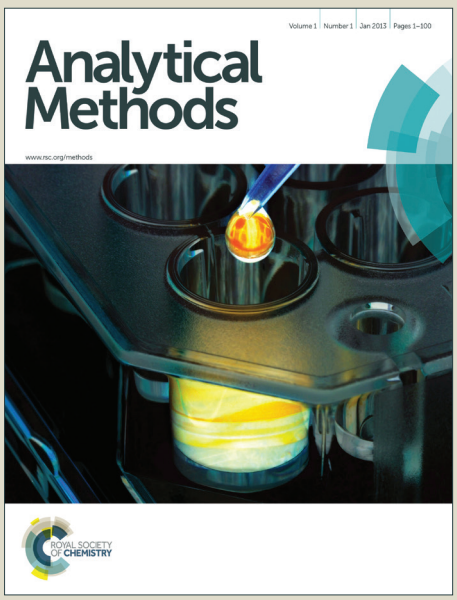

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Sensitive Colorimetric Detection of Melamine with 1, 4-Dithiothreitol Modified Gold Nanoparticles

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ABSTRACT

A colorimetric detection method of melamine based on 1, 4-dithiothreitol (DTT) functionalized gold nanoparticles (DTT-AuNPs) is reported. Hydrogen-bonding interaction between DTT and melamine resulted in the aggregation of AuNPs, consequent color changes of AuNPs from wine red to blue and red-shift of the surface plasmon resonance (SPR) peak of DTT-AuNPs. The concentrations of melamine could be determined with naked eye or a UV–vis spectrometer. FT-IR and TEM were used to investigate the modification of DTT-AuNPs. Results showed that the absorption ratio (A_{680}/A_{520}) was linear with the logarithm of melamine concentration in the range of 8.0×10^{-8} M to 6.0×10^{-7} M and 6.0×10^{-7} M to 1.5×10^{-6} M with linear coefficients of 0.993 and 0.999, respectively. The detection limit (S/N = 3) of the proposed method was 2.4×10^{-8} M, which was much lower than the safety limit. The coexisting substances did not affect the determination of melamine. Furthermore, the proposed method was applied for detecting trace melamine in real milk samples, with recoveries of 96%–103%.

Keywords: Melamine; Gold Nanoparticles; 1, 4-Dithiothreitol; Colorimetric Detection

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Introduction

The detection of melamine in food products has been the subject of much recent research. Melamine, a nitrogen-containing heterocyclic triazine compound, is an important organic chemical raw material, used in polymer resins or as a flame retardant, fertilizer and other products. Because of its high nitrogen contents (66.6% by mass) and low cost, melamine has been adulterated into human and animal food to produce an incorrectly high reading in the measurement of protein content by conventional standard Kjeldahl or Dumas tests. Although melamine has low toxicity, ingestion of melamine at levels above the safety limit (2.5 ppm in the USA and EU, 1.0 ppm for infant formula milk in China) can cause renal failure and even death. As it has been reported in 2008, thousands of Chinese babies were hospitalized because of the infant formula containing melamine [1]. Considering its potential toxicity, significant efforts have been made on the fast and effective detection of melamine. Up to now, many analytical techniques such as mass spectrometry [2], gas chromatography/mass spectrometry [3], high-performance liquid chromatography [4], liquid chromatography/mass spectrometry [5], electrochemistry, chemiluminescence [6, 7], near infrared spectroscopy[8], Raman spectrometry [9], capillary electrophoresis [10], and enzyme-linked immunosorbent assay [11] have been developed for the detection of melamine in milk and milk-based productions. However, most of the above strategies are time-consuming because complex pretreatment of samples is needed. Besides, expensive and sophisticated instrumentations operated by trained personnel are required. All of the issues above make them difficult to be implemented widely. Recently, chitosan [12], 1-(2-mercaptoethyl)-1,3,5-triazinane-2,4,6-trione [13], 3-mercapto-1-propanesulfonate [14], cysteamine [15], and riboflavin [16] modified AuNPs have been fabricated as colorimetric probes for visual detection of melamine in raw milk and infant formula. Most of these methods exhibit quite high sensitivity for melamine, but the complex modification and the unstability of AuNPs limits their

application to some extent. More simple methods have been developed by using citrate-capped AuNPs [17] or even bare AuNPs [18] as colorimetric probes. Furthermore, Ma et al. used cysteamine-modified gold nanoparticles for mercury (II) and melamine detections based on electrostatic attraction for melamine and the N–Hg^{2+}–N structure for Hg^{2+} under distinct pH conditions[19]. However, it is urgent to develop highly selective and sensitive method for the detection of melamine.

Considering that DTT is small and compact, it could therefore more easily penetrate surface corona to reach the AuNP surface. With two thiol groups, DTT has a high affinity for the Au surface [20, 21], therefore, DTT is much more reactive toward gold compared with most ligands of interest [22]. Besides, DTT could form a hydrogen-bond adduct with melamine [23] and the two end thiols of DTT could attach to the surface of AuNPs by the strong covalent interaction between Au and –SH [24]. Thus, the DTT-capped AuNPs will aggregate when melamine exists. Moreover, in this study, for real sample detection, the liquid milk bought from a local supermarket was diluted by Milli-Q-purified distilled water without any separation or extraction step, which means less time taken and less organic solvent used. The proposed method offers a rapid, selective, sensitive and environment-friendly melamine sensor.

Experimental

2.1 Chemical reagents

All chemicals used were of analytical reagent grade without further purification and solutions were prepared with Milli-Q-purified distilled water. DL-Dithiothreitol and Dicyandiamide were obtained from J&K Scientific Ltd. Melamine was purchased from Sigma (USA). $HAuCl_4 \cdot 4H_2O$ and uracil were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Ascorbic Acid was from Xilong Chemical Factory of Guangdong (Guangdong, China). Arginine and Tyrosine were from Zhongbei Linge Biotechnology Ltd. (Beijing, China). Glycerol was from

Sangon Biotech Co. Ltd. (Shanghai, China). Cyanuric acid and Phenylalanine were from Alfa Aesar (USA). $CaCl_2$, $MgCl_2$, glucose, lactose, Na_2HPO_4 and NaH_2PO_4 were all from Beijing Chemical Reagent Company (Beijing, China). The 0.01 M phosphate buffer solution (PBS) of pH6 was prepared by mixing the stock solutions of 0.01 M NaH_2PO_4 and 0.01 M Na_2HPO_4.

2.2 Apparatus

The UV–vis absorbance spectra were recorded with a UV-2550 spectrophotometer (Shimadzu, Japan) at ambient temperature (20 ± 2 ℃) with Quartz cuvettes. The photographs were taken by a camera DSC-W150 from Sony Corporation, Ltd. (Beijing, China). The Fourier transform infrared spectroscopy (FT-IR) characterization was carried out on a BRUKE Vertex 70 FT-IR spectrometer, and the samples were prepared in the form of pellets together with KBr. All the pH measurements were performed with PB-10 pH meter (Sartorius, Germany). Transmission electron microscopy (TEM) images were obtained by using a Philips EM-400 at 80kV.

2.3 Synthesis of DTT-AuNPs

AuNPs were prepared according to sodium citrate-mediated reduction of $HAuCl_4$ method [25]. Typically, 15 mL of trisodium citrate (38.8 mM) was rapidly injected into a boiling solution of 150 mL of $HAuCl_4$ (1 mM) under vigorous stirring, and the mixed solution was continually boiled under stirring for another 30 minutes to give a wine-red solution. The resulting solution was cooled to room temperature and filtered through a 0.45 μm Millipore syringe filter to remove the precipitate. The filtrate was stored in a refrigerator at 4 ℃ for further use. The size of AuNPs was about 13 nm and the concentration was 10 nM [26].

500 μL DTT aqueous solution (10^{-5} M) was added into 3 mL citrate stabilized AuNPs solution, shaken for 2 hours with a constant speed at room temperature. And

then the DTT modified AuNPs were obtained. Also, the solution was stored in a refrigerator at 4 ℃ for further use.

2.4 Colorimetric assay

The stock solutions of different concentrations of DTT and melamine were prepared daily with Milli-Q-purified distilled water. As pH had an effect on the stability of DTT-AuNPs [27], pH 6.0 was chosen for the entire detection in this paper for ensuring the stability of DTT-AuNPs within the entire experiment period. We carried out the experiments as follows: in one 5 mL centrifuge tube, 700 μL of the obtained DTT-AuNPs solution was diluted by a certain volume of PBS, then, 50 μL of different concentrations of melamine was added into the mixed solution. After that, the final solution with the total volume of 2 ml was allowed to react for 5 minutes at room temperature. UV-Vis absorbance spectra were used to characterize the above system and the color of Au colloid solution was recorded by digital camera. The solution was transferred into a 1 cm spectrophotometric cell to record the absorbance. The absorption ratio (A_{680}/A_{520}) was used to quantify the concentration of melamine.

For real sample detection, the liquid milk bought from a local supermarket was diluted by Milli-Q-purified distilled water. Then different concentrations of melamine were directly added into the milk without any separation or extraction step, and the other steps were the same as those described above.

Results and discussion

3.1 Sensing Mechanism

As well documented by the literature, the thiol-Au surface chemistry provides an effective route for the design and attachment of functional molecular structure [28]. Moreover, citrate-stabilized AuNPs have frequently been utilized as a precursor for

ligation with functional thiolated molecular species, for weakly bound citrate is easily displaced by the covalently bound thiol [29]. As DTT contains two end thiols, it could attach to the surface of AuNPs by the formation of Au–S bond. Considering that melamine molecule contains three exocyclic amino groups and a three nitrogen hybrid ring, it is easy to form hydrogen-bond adducts with melamine and DTT which contains two hydroxyl groups [23]. Besides, DTT is small and compact, and should therefore more easily penetrate surface corona to reach the AuNP surface. What is more, DTT has a high affinity for the Au surface with its two thiol groups [20, 21]. Therefore, DTT is much more reactive toward gold compared with most ligands of interest [22], and DTT-AuNPs would be obtained much more easier and stable. Therefore, we chose DTT to prepare the colorimetric probe for the detection of melamine. As shown in Fig.1, two end thiols of DTT were attached to AuNPs by the strong covalent interaction between Au and –SH [24]. Also we believed that melamine would form hydrogen-bond adducts with hydroxyl groups exposed of DTT-AuNPs through nitrogens from the exocyclic amino groups and the hybrid ring of its molecule. As individual spherical AuNPs came into close proximity (the center-to-center distance is normally smaller than 2.5 times of the diameter of AuNPs), the surface plasmon of individual AuNPs combined (interparticle plasmon coupling) [30], and the color changed from red to blue.

[Fig.1]

According to the report, melamine with a very strong binding ability to the surface of AuNPs could cause the color change of AuNPs without any addition of other analyte, attaining the visual detection of melamine [17]. Herein, we performed parallel experiment and proved that DTT modified AuNPs could improve the sensitivity of the colorimetric detection of melamine because of the formation of supramolecular hydrogen-bonded structure between DTT and melamine. Fig.2 showed the direct observation of the color change (A) and absorbance spectra (B) of unmodified AuNPs and DTT modified AuNPs in the absence and presence of 3.0×10^{-7}M melamine. In the presence of melamine, the color of DTT modified AuNPs changed from red to purple (d of Fig.2 (A)), indicated the aggregation of the AuNPs. However, the presence of

3.0×10^{-7} M melamine did not induced obvious change of unmodified AuNPs (c of Fig.2 (A)), in accordance with the results of UV-Vis spectroscopy (Fig.2 (B)). Moreover, the absorption spectrum of DTT modified AuNPs exhibited a large change. Especially, the absorption intensity at 520 nm decreased, and a new absorbance peak appeared at about 680 nm, indicating that the state of AuNPs changed from dispersion to aggregation. Therefore, DTT modified AuNPs showed a higher sensitivity to melamine.

[Fig.2]

3.2 Characterization

To investigate the modification of DTT-AuNPs, FT-IR spectroscopy was performed. From Fig.3, the spectroscopy showed that the S–C stretching vibration at 1409 cm^{-1} appeared in DTT-AuNPs, and the characteristic absorption peak of –SH at 2565 cm^{-1} in pure DTT disappeared in the FT-IR spectra of DTT-AuNPs, indicating that DTT had been successfully modified onto the surface of gold nanoparticles via the –SH group of DTT. Meanwhile, the spectroscopy also showed that the C–O stretching vibration at 1054 cm^{-1} and –OH stretch vibration at 3385 cm^{-1} appeared in DTT-AuNPs. From all of the above results, it could be concluded that –SH group was responsible for the formation of the functionalized DTT-AuNPs. The FT-IR results further proved the fact that the function group of –OH had been successfully attached onto the surface of AuNPs.

A direct evidence for melamine-stimulated aggregation of the DTT modified AuNPs could be supported by transmission electron microscopy (TEM) measurements. Fig.4 showed the TEM images of DTT-AuNPs in the absence and presence of 1×10^{-6}M melamine. DTT-AuNPs were well dispersed in aqueous solution (Fig.4A) in the absence of melamine, however, aggregation occured when melamine were added into the solution (Fig.4B). The result indicated that the addition of melamine could easily lead to the aggregation of DTT-AuNPs.

[Fig.3]

[Fig.4]

3.3 Optimization of experimental conditions

Since DTT was attached to AuNPs by strong covalent interaction, which might decrease the stability of AuNPs and cause visible color changes of AuNPs, it was crucial to control the concentration of DTT used for the modification. Fig.5 showed the color changes (A) and UV–vis spectrum (B) of AuNPs solution modified by different concentrations of DTT. When the DTT concentration increased over 5.0×10^{-7}M, the UV–vis spectrum of AuNPs changed a lot and the color changed to purple or even blue. Based on these results, 5.0×10^{-7}M DTT was chosen throughout the following experiments.

[Fig.5]

We recorded the absorption ratio (A_{680}/A_{520}) at different time in order to study the stability of DTT modified AuNPs. Fig.6 showed that there was no significant change of the absorption ratio (A_{680}/A_{520}) within 120 minutes, indicating that after the two hours of reaction time, DTT modified AuNPs could be stable long enough for our detection.

[Fig.6]

3.4 Colorimetric detection of Melamine

To examine this simple assay for the colorimetric detection of melamine, 50 μl of different concentrations of melamine were added into 1950 μl mixture which contained 700 μl DTT modified AuNPs and the others pH6 PBS. Fig.7 showed that the color of the DTT modified AuNPs changed from red to blue. And the absorption of DTT modified AuNPs was evaluated by UV-Vis spectra. As depicted in Fig.7, the absorbance of DTT modified AuNPs got gradual decrease in the surface plasmon resonance at about 520 nm with the increasing concentration of melamine, appearance of a new band at about 680 nm and gradual increase in the surface plasmon resonance

at about 680 nm, which were ascribed to the aggregation of AuNPs. It was found that the extinction ratio increased slightly with the melamine concentration in the range of 8.0×10^{-8} M to 6.0×10^{-7} M, corresponding to the slight absorption spectra and visible color changes (from red to purple). Then the extinction ratio followed a rapid increase in the melamine concentration range of 6.0×10^{-7} M to 1.5×10^{-6} M, corresponding to the significant changes in the absorption spectra and color (from purple to blue). And then the extinction ratio changed little with the continuous increase of melamine concentration. Moreover, as depicted in Fig.8, two linear correlations existed between the ratio A_{680}/A_{520} and the logarithm of melamine concentration in the range of 8.0×10^{-8} M to 6.0×10^{-7} M and 6.0×10^{-7} M to 1.5×10^{-6} M, with correlation coefficients of 0.993 and 0.999, respectively. Theoretical detection limits (S/N = 3) of the proposed method was 2.4×10^{-8} M, which was far below the safety limit, indicating that the proposed method based on DTT modified AuNPs definitely met the requirements of routine detection.

[Fig.7]

[Fig.8]

This work has successfully developed a simpler and more sensitive method for the colorimetric determination of melamine. When compared with the methods developed before (Table.1), our paper performed much higher sensitivity and suitable linear range.

[Table.1]

3.5 Selectivity of melamine detection

The selectivity of the proposed method for melamine was examined by evaluating the absorbance ratio A_{680}/A_{520} of DTT modified AuNPs in the presence of other interferences coexisting in the real samples (e.g., lactose, $CaCl_2$, and tyrosine) or having the similar structure (e.g., dicyanodiamide, cyanuric acid and uracil). Compared with the blank test, all the interferences except uracil had no significant changes with the concentration of 1.0×10^{-4} M which was 100-fold higher than

melamine, while with lower concentration (such as 10-fold), uracil had no influence on the test. Fig.9 showed that only the presence of melamine (1.0×10^{-6} M) resulted in a remarkable higher absorption ratio of A_{680}/A_{520}, although the concentration of each interference detected was 100-fold (1.0×10^{-4}M), indicating that the proposed method showed high selectivity for melamine over other interferences.

[Fig.9]

3.6 Practical Application

To investigate the practical application of this colorimetric method, the detection of milk sample was carried out by standard addition method. The liquid milk was diluted 1000 times with distilled water to reduce the effect of matrix before the measurement. Then different amounts of known concentrations of melamine were added into the sample to obtain the demanded concentrations and then the other steps were the same as Section 2.4. The recoveries of 1.2×10^{-7} M, 2.0×10^{-7} M, 4.0×10^{-7} M, 8.0×10^{-7} M, 9.5×10^{-7} M and 1.2×10^{-6} M of melamine were 99%, 99%, 96%, 103%, 99% and 98%, respectively, certifying that the proposed method had good reliability for trace melamine detection in liquid milk products.

Conclusion

By combining the rationally designable surface chemistry of AuNPs with supramolecular hydrogen-bonded structure between DTT and melamine, we have developed a novel, simple effective colorimetric method for the determination of melamine. In conclusion, the melamine-induced aggregation of DTT modified AuNPs results in a visible color change from wine red to blue. The absorbance ratio A_{680}/A_{520} obtained by UV-Vis spectrum was linear with the logarithm of melamine concentration in the range of 8.0×10^{-8} M to 6.0×10^{-7} M and 6.0×10^{-7} M to 1.5×10^{-6} M, and theoretical detection limits (S/N = 3) of the proposed method was 2.4×10^{-8} M.

The method developed was applied for detecting trace melamine in real milk samples. Furthermore, as DTT is much more reactive toward gold and DTT-AuNPs would be much stable, the probe might have the potential for detection of other substances.

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Figure Captions

Fig.1 (A) Possible supramolecular hydrogen-bonded structure between melamine and DTT. (B) Schematic illustration of possible procedure for the colorimetric detection of melamine.

Fig.2 (A) Visual color changes and (B) absorbance spectra of (a) AuNPs, (b) DTT modified AuNPs, (c) AuNPs in the presence of 3.0×10^{-7} M melamine and (d) DTT modified AuNPs in the presence of 3.0×10^{-7} M melamine.

Fig.3 IR spectra of DTT-modified AuNPs (A) and DTT (B).

Fig.4 TEM images of the DTT-AuNPs formed in the absence (A) and presence of (B) 1×10^{-6} M melamine.

Fig.5 UV-Vis spectra and colorimetric visualization of AuNPs modified with different concentrations of DTT: (a) 0, (b) 4.0×10^{-7}, (c) 4.5×10^{-7}, (d) 5.0×10^{-7}, (e) 1.0×10^{-6}, (f) 1.5×10^{-6}, and (g) 2.0×10^{-6} M.

Fig.6 The UV-Vis absorbance ratio A_{680}/A_{520} of DTT modified AuNPs recorded in the interval between 0 to 120 minutes.

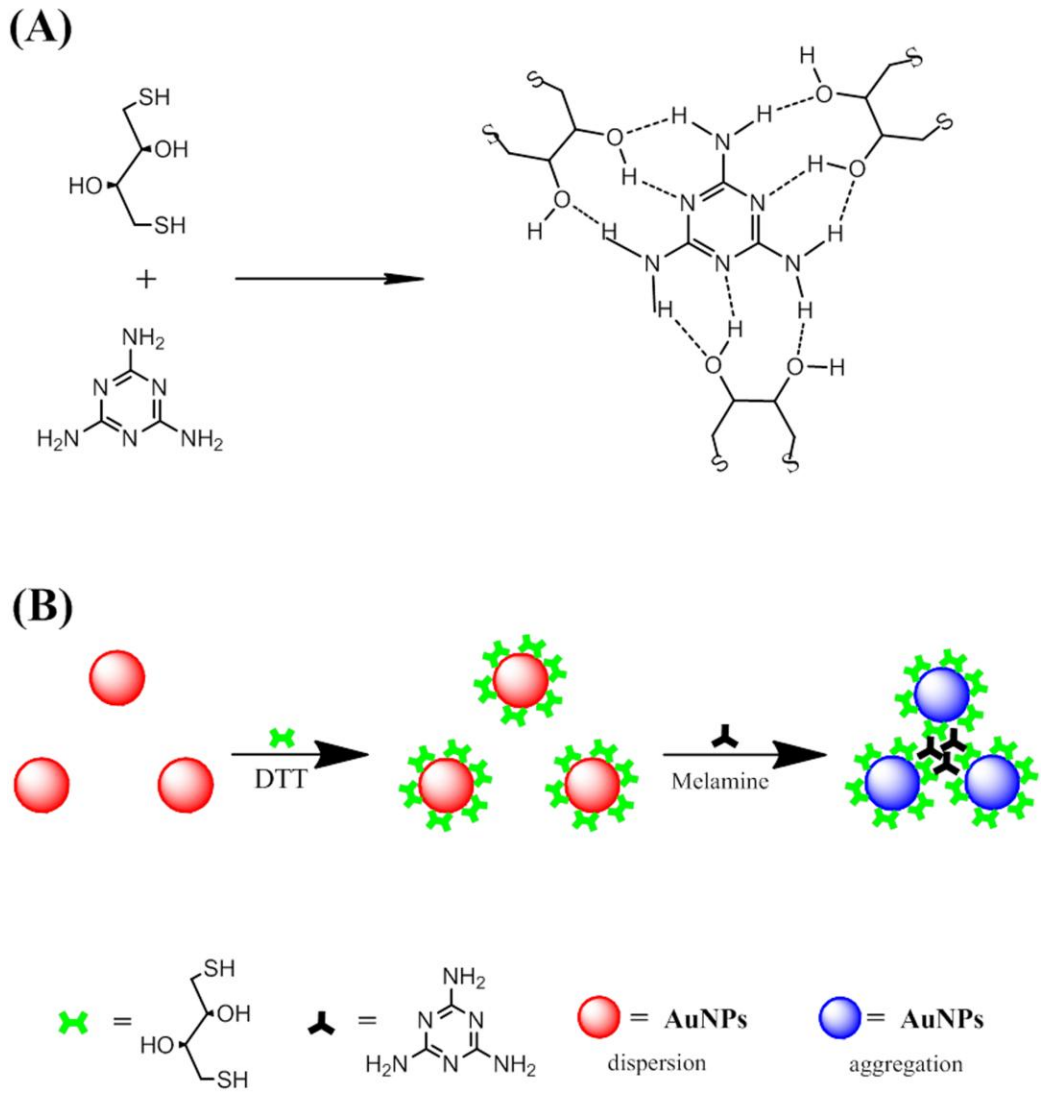
Fig.7 Visual color changes (A) and UV-Vis spectra (B) of the DTT modified AuNPs upon addition of melamine at different concentrations (from left to right: 0, 8.0×10^{-8}, 1.5×10^{-7}, 3.0×10^{-7}, 4.5×10^{-7}, 6.0×10^{-7}, 7.5×10^{-7}, 9.0×10^{-7}, 1.0×10^{-6}, 1.5×10^{-6} M).

Fig.8 Standard calibration curve of A_{680}/A_{520} against the logarithm of melamine concentrations from 8.0×10^{-8} M to 6.0×10^{-7} M and 6.0×10^{-7} M to 1.5×10^{-6} M. Error bars showed the standard deviations of measurements taken from three independent experiments.

Fig.9 Absorbance ratio A_{680}/A_{520} and photographs of DTT modified AuNPs in the presence of 1.0×10^{-6} M melamine or other interferences (1.0×10^{-4} M) (from a to m: blank, ascorbic acid, glucose, arginine, $CaCl_2$, $MgCl_2$, dicyandiamide, tyrosine, cyanuric acid, phenylalanine, lactose, glycerol and melamine). Error bars showed the standard deviations of measurements taken from three independent experiments.

Table Captions

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



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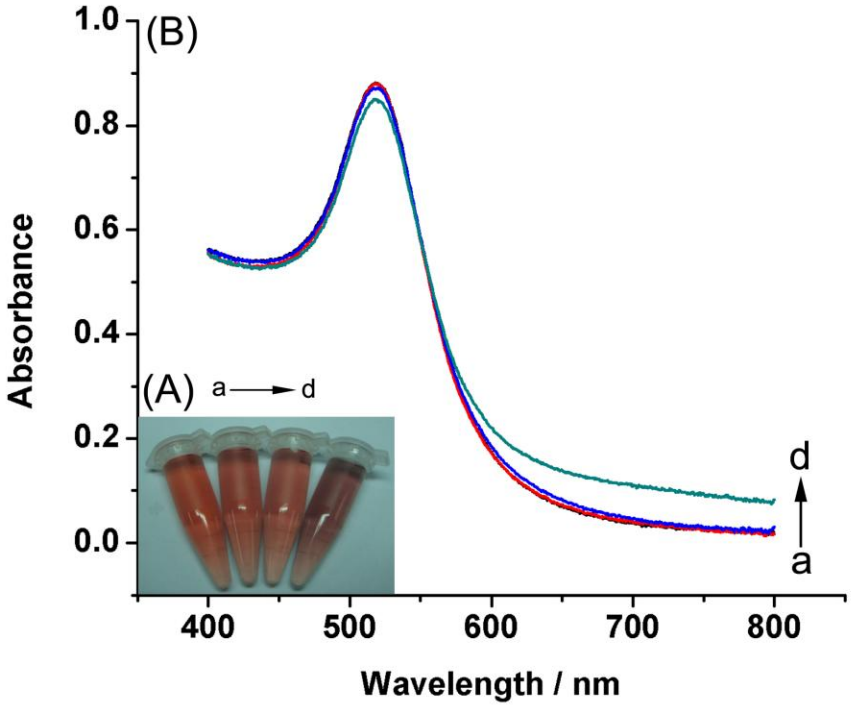

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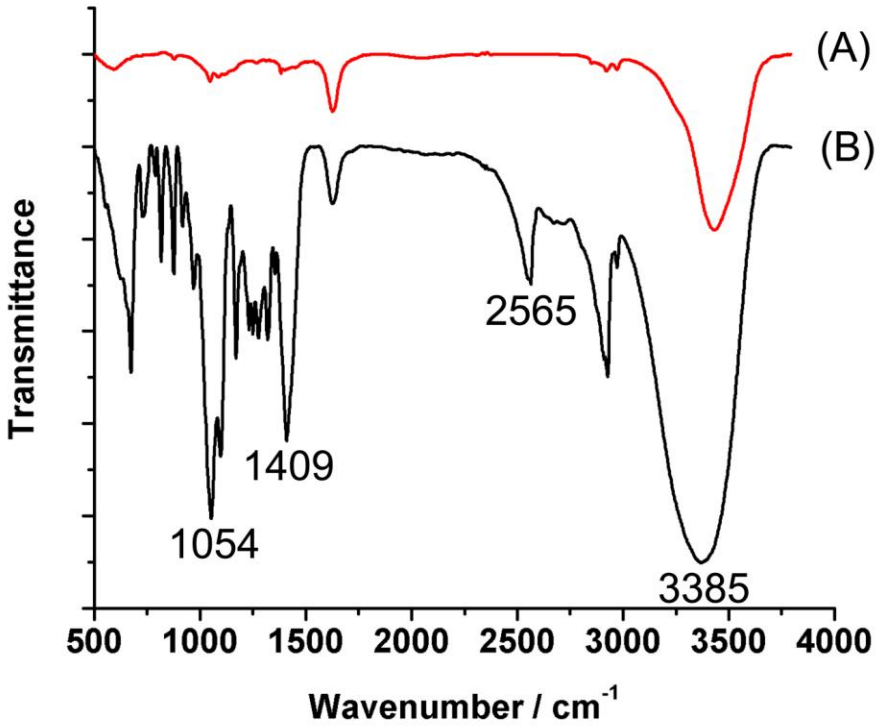

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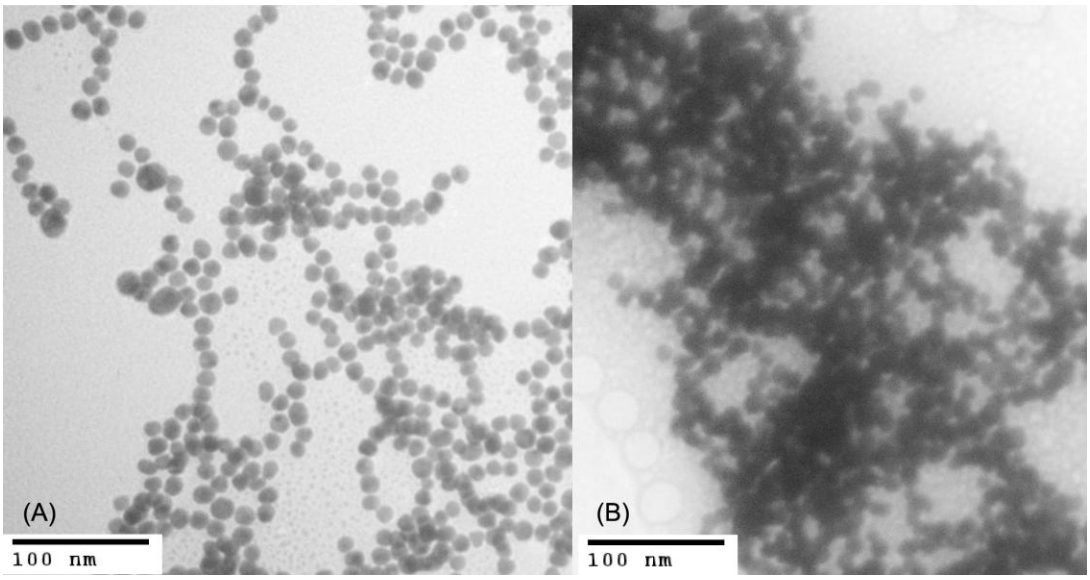

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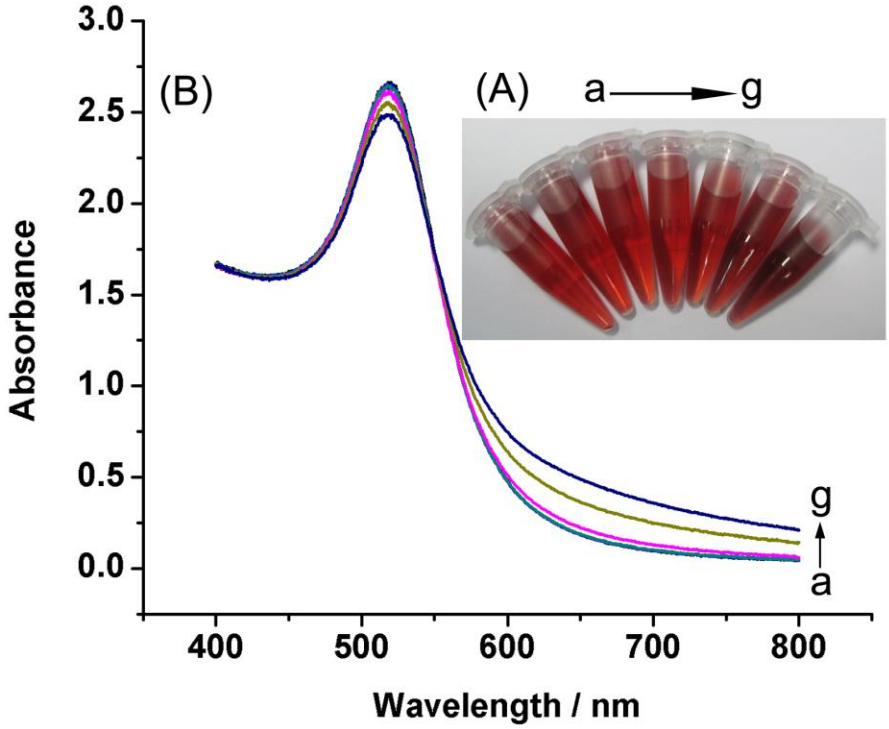

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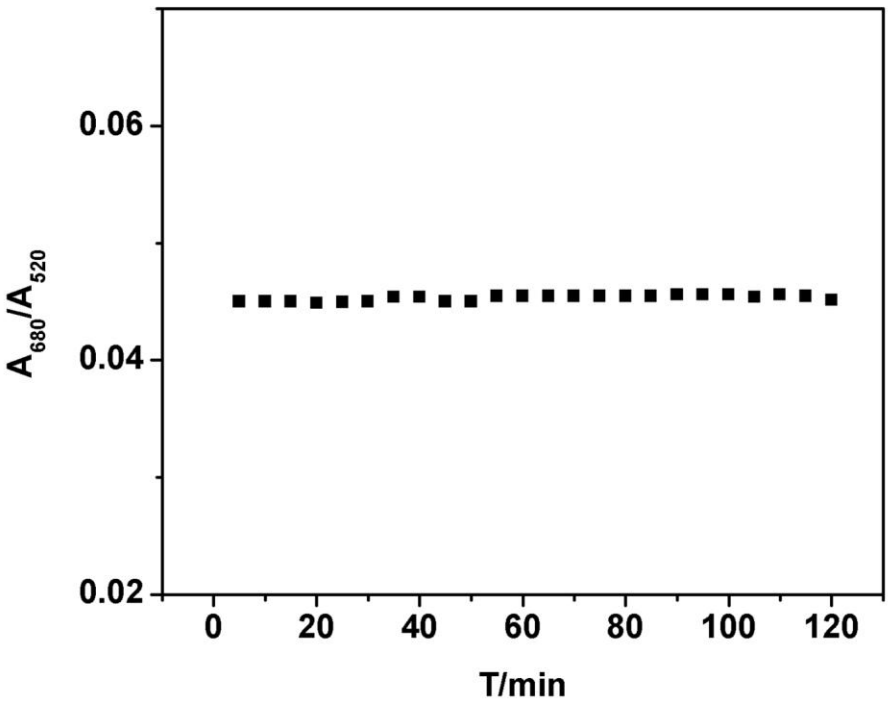

Fig.6

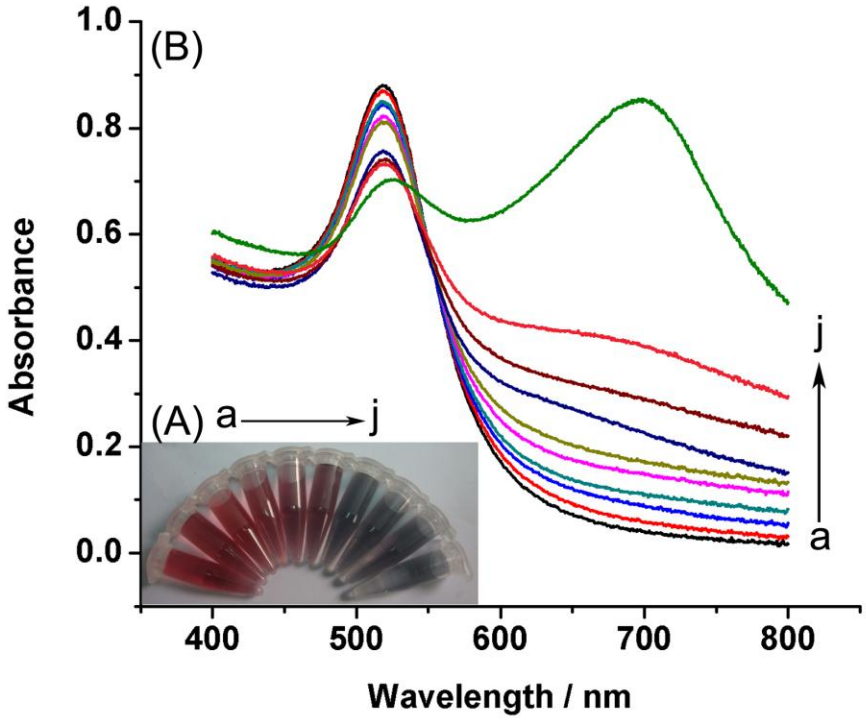

Fig.7

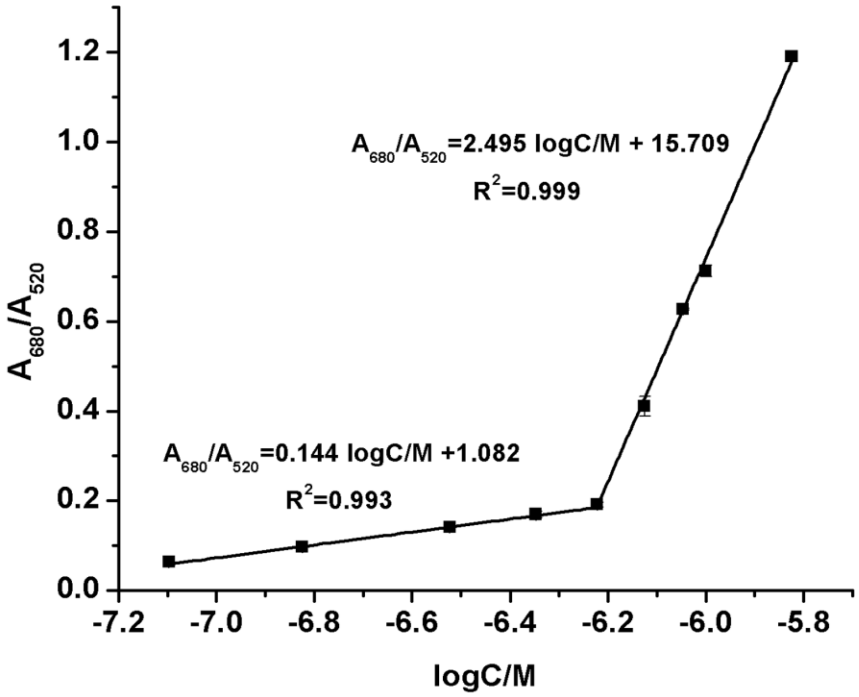

Fig.8

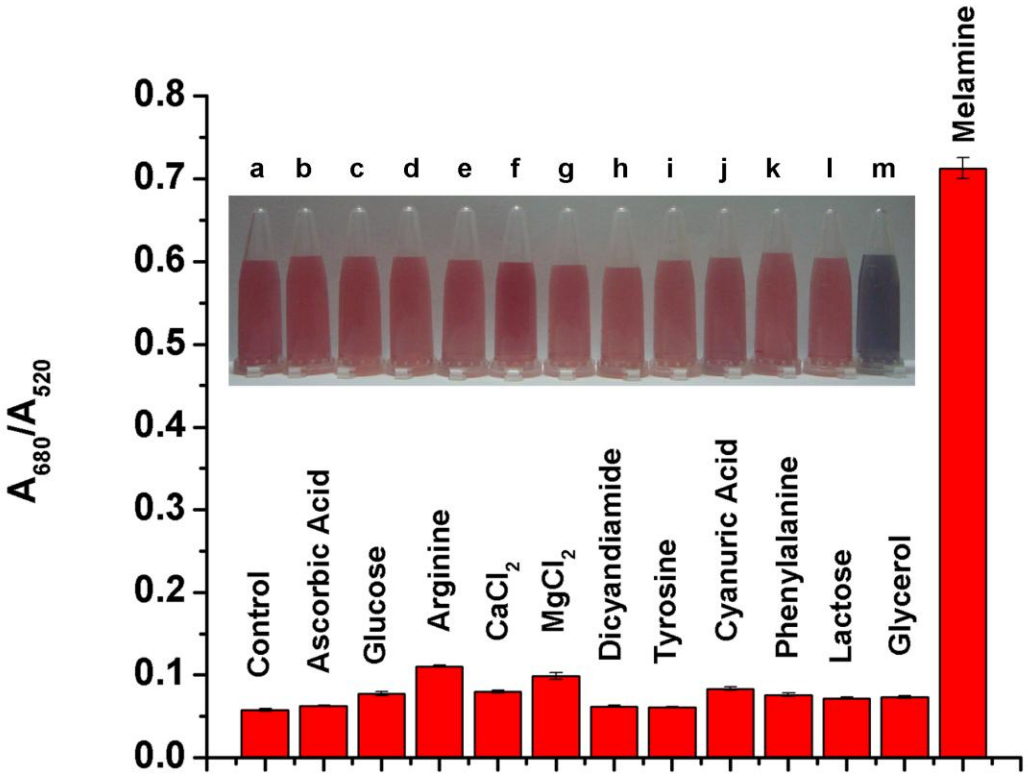

Fig.9

Table.1

Methods	Linear range (mol/L)	LOD (mol/L)	References
GC-MS	4.0×10^{-4} - 6.3×10^{-2}	2.1×10^{-7}	3
Fluoroimmunoassay	6.9×10^{-8}-9.0×10^{-7}	3.1×10^{-8}	6
Fluorescence	5.0×10^{-7}-1.0×10^{-5}	1.5×10^{-7}	7
Raman spectrometry	1.0×10^{-5}-1.0×10^{-4}	5.0×10^{-6}	9
Capillary electrophoresis	4.0×10^{-6}-1.6×10^{-5}	1.3×10^{-6}	10
Colorimetric sensing	8.0×10^{-8}-1.5×10^{-6}	2.4×10^{-8}	This work