Rapid determination of melamine in milk using water-soluble CdTe quantum dots as fluorescence probes

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Rapid determination of melamine in milk using water-soluble CdTe quantum dots as fluorescence probes

Minwei Zhang¹, Hong Ping², Xianyi Cao², Hongkun Li³, Fengrui Guan³, Chunyan Sun⁴ and Jingbo Liu⁵

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Water-soluble CdTe quantum dots of different sizes capped with thioglycolic acid (TGA-CdTe QDs) were synthesised via a microwave-assisted method. It was found that melamine could quench the fluorescence emission of TGA-CdTe QDs in aqueous solution. Based on this, a novel method for the determination of melamine has been developed. Under optimum conditions, the fluorescence intensity of TGA-CdTe QDs versus melamine concentrations gave a linear response according to the Stern–Volmer equation. The proposed method has been successfully used to detect melamine in liquid milk with a detection limit of 0.04 mg L⁻¹, and the whole process including sample pre-treatment could be accomplished within 30 min. The obvious merits provided by this method, such as simplicity, rapidity, low cost and high sensitivity would make it promising for on-site screening of melamine adulterant in milk products. The possible mechanism involved in the interaction of melamine with TGA-CdTe QDs is discussed.

Keywords: CdTe quantum dots; melamine; fluorescence quenching; liquid milk

Introduction

Melamine, 1,3,5-triazine-2,4,6-triamine, is widely used as an industrial chemical in the production of polymer resins, which are used in laminates, glues, adhesives and plastics. It is not approved for use as a feed or food additive. However, because of its high nitrogen level (66% nitrogen by mass), melamine has been illegally added into feedstuffs or food to artificially distort their crude protein content (Sun et al. 2010). Without identifying nitrogen sources, the traditional Kjeldahl test measures total nitrogen content as an indication of protein levels and cannot differentiate melamine from protein molecules. Thus, melamine can be used to adulterate protein-rich diets (e.g. milk, infant formula) by unethical manufacturers to boost the nitrogen level and reduce costs (Chan et al. 2008; Ingelfinger 2008; Xin and Stone 2008). Melamine has low oral acute toxicity, but the combination of melamine with cyanuric acid leads to the formation of insoluble crystals in the kidneys. The chronic administration of high concentrations of melamine can induce renal failure and even death, especially in babies and children (Lam et al. 2009). A certain maximum level of melamine ingestion is officially fixed in foods, for example, 2.5 ppm in China, United States and European Union (EU) for adult foods, and 1.0 ppm for infant formula in United States and China (Ding et al. 2010).

The detection of melamine in food products has been the subject of much recent research (Sun et al. 2010; Tittlemier 2010). Common methodologies used for melamine detection are mainly based on high-performance liquid chromatography (HPLC) (Zhong et al. 2011), LC–mass spectrometry (MS) (Braekevelt et al. 2011; Gosciny et al. 2011), gas chromatography (GC)–MS (Miao et al. 2010), enzyme-linked immuno-sorbent assay (ELISA) (Liu et al. 2010) and capillary electrophoresis (Jin et al. 2010; Wen et al. 2010). Although the above methods are generally very sensitive and accurate, they are not suitable for routine analysis because of such drawbacks as expensive equipment, complicated and time-consuming procedures for sample pre-treatment, specific skills for operation. Recently, various methods including electrochemical (Zhu et al. 2010), surface-enhanced Raman spectroscopy (SERS) (Cheng and Dong 2011), infrared spectroscopy (Balabin and Smirnov 2011) and colorimetric sensor based on polydiacetylene (PDA) liposomes (Lee et al. 2011), have been investigated to detect melamine, some of which suffer from complicated chemical synthesis, high cost or...
poor sensitivity. Therefore, there is still an urgent need to develop a rapid, sensitive and facile method for the efficient detection of melamine in food. In recent years, significant efforts have been made on the development of nanoparticles (NPs)-based assay for the fast and effective detection of melamine. For example, Au and Ag NPs modified with different stabilisers have been used as colorimetric probes for melamine sensing in liquid milk and infant formula, through the hydrogen-bonding recognition (Ai et al. 2009; Qi et al. 2010), electrostatic interaction (Li et al. 2010; Ping et al. 2012), or electron donor–acceptor interaction (Han and Li 2010; Bera and Raj 2011) between melamine and the stabiliser at the nanoparticle interface. In addition, melamine in milk powder could be detected based on the fluorescence enhancement of Au NPs (Xiang et al. 2011). A sensitive “turn-on” fluorescent method for the detection of melamine has been developed by utilising Au NPs as a highly efficient quencher (Guo et al. 2011).

Luminescent semiconductor quantum dots (QDs), also called nanocrystals (NCs), have attracted considerable attention in the past decade because of their widespread applications ranging from electronics and photonics to chemical and biological assay. In comparison with organic dyes and fluorescent proteins, QDs exhibit unique optical properties, such as high quantum yield of fluorescence, broad excitation spectrum and narrow/symmetric emission spectrum, size- and composition-tunable emission wavelength, high photobleaching threshold and excellent photostability. Owing to these superior properties, QDs have attracted a significant and growing interest in the fields of biolabeling and bioimaging (Gill et al. 2008; Byers and Hitchman 2011). Meanwhile, these advantages of luminescence signal in its intrinsic sensitivity also have encouraged the application of QDs in the field of chemical sensing (Frasco and Chiantiotakis 2009). Fluorescence changes accompanying the addition of substrates to QDs have been utilised for the optical sensing of various biomolecules, for example, glutathione (Banerjee et al. 2009), cysteine (Negi and Chanu 2008), drugs (e.g. spironolactone; Liang et al. 2006), organic pollutants (e.g. paraoxon, Constantine et al. 2003; polycyclic aromatic hydrocarbons, Li and Qu 2007), metal ions (e.g. Cu²⁺, Ag⁺, Gattás-Asfura and Leblanc 2003; Liang et al. 2004; Cd²⁺, Banerjee et al. 2008; and cyanide ions, Jin et al. 2005).

To date, several fluorescent methods have been reported for the detection of melamine (Zhou et al. 2010; Attia et al. 2011; Guo et al. 2011; Xiang et al. 2011). To our knowledge, there has been no previous report on QDs-based luminescent probes for the detection of melamine in food. In the present work, water-soluble CdTe QDs of different sizes capped with thioglycolic acid (TGA) were synthesised via a microwave-assisted method. We investigated the interaction between TGA-CdTe QDs and melamine. It was found that melamine dramatically quenched the fluorescence of CdTe QDs. The quenched intensity of fluorescence was proportional to the concentration of melamine. Based on this phenomenon, a simple, convenient and sensitive fluorescence method has been established for the detection of melamine. Interference tests showed that some co-existing substances, such as carbohydrate, most ions and amino acids have little interference. The method with high sensitivity has been applied to the determination of melamine in real samples and satisfactory results were obtained. The possible mechanism of the proposed sensing method for melamine is also discussed.

Materials and methods

Apparatus

A WVFY-201 microwave reactor of 800 W power (Zhize Equipment Factory, Shanghai, China) was used in the experiments. All pH measurements were carried out with a Model pHS-3C (Chenghua Equipment Factory, Shanghai, China). The centrifugation was performed on a CR20B2 refrigerated centrifuge (Tokyo, Japan). The ultrasonic treatment was carried out on a 125 KQ-300DE ultrasonicator (Kunshan Ultrasonic Instrument Co., Shanghai, China). The absorption spectra were acquired on a 2500 UV–vis spectrophotometer (Shimadzu, Tokyo, Japan). The spectra and intensity of fluorescence were measured using a RF-5301 fluorescence spectrophotometer (Shimadzu) equipped with a 1-cm quartz cell. If not specifically stated, the fluorescence emission spectra of TGA-CdTe QDs were obtained with the excitation of 450 nm, and the exciting and emission slits were both set at 5 nm. All optical measurements were performed at room temperature (25°C) under ambient conditions.

Materials and reagents

Te powder, sodium borohydride (NaBH₄) and thioglycolic acid (TGA) were obtained from Sinopharm Chemical Reagent (Shanghai, China). Vitamin B₁, vitamin B₃ and vitamin C were purchased from Sigma. Melamine was purchased from Aladdin Reagent Company (Shanghai, China). Cadmium chloride (CdCl₂·2H₂O), lactose, glucose, chloroacetic acid and chloroform were purchased from Beijing Chemical Reagent Company (Beijing, China). Threonine, tryptophan, glycine, histidine and lysine were obtained by Huishi Biochemical Reagent Company (Shanghai, China). All chemical reagents are of analytical grade and were used as received without further purification. Doubly distilled water was used throughout the experiments. The liquid raw milk was purchased
from the local pasture, and different brands of milk were purchased from the local super market.

**Synthesis and purification of water-soluble TGA-CdTe QDs**

TGA-capped CdTe QDs were synthesised according to the procedure described previously with some slight modification (Wang et al. 2005; Li et al. 2006). Briefly, 0.0256 g Te powder and 0.0386 g NaBH₄ was first added to 1 mL water in a three-neck flask with a condenser attached, and reacted at 50°C for 45 min to get Te precursor (NaHTe). Cd precursor was prepared by mixing a solution of CdCl₂ (0.0913 g) with 66 μL TGA, and the solution was diluted to 100 mL, which was then adjusted to pH 11 by 1 M NaOH and deaerated with N₂ for 20 min. The Cd precursor was added into NaHTe solution while stirring vigorously at room temperature. The molar ratio of Cd²⁺:Te²⁻:TGA is 1:0.5:2.4. Under the protection of N₂ atmosphere, the mixed solution was stirred for 10 min and then heated with microwaves at 50% output power for different durations. The sizes of the TGA-CdTe QDs could be tuned by simply varying the heating time.

The crude CdTe QDs solution was washed with equal isopropanol and centrifuged to remove excess precursors. Then the QDs deposit was dried by vacuum desiccation. Finally, the prepared TGA-CdTe QDs were dispersed in water.

**Characterisation of TGA-CdTe QDs**

The particle size and concentration of TGA-CdTe QDs

According to the literature calculation method (Yu et al. 2003), the particle sizes of the as-prepared TGA-CdTe QDs were calculated in virtue of the following empirical polynomial:

\[
D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0064)\lambda - 194.84(\text{nm}).
\]

In the above equation, \(D\) (nm) is the particle size of a given QDs sample, and \(\lambda\) (nm) is the wavelength of the first excitonic absorption peak of the corresponding QDs.

The molar concentration of the obtained TGA-CdTe QDs was calculated using the Lambert Beer’s law:

\[
A = \varepsilon cl,
\]

where \(A\) is the absorbance of the first excitonic absorption peak of the corresponding QDs, \(c\) is the molar concentration (mol L⁻¹) of the QDs solution, \(l\) is the path length (cm) of the radiation beam used for recording the absorption spectrum and was set at 1.0 cm herein, and \(\varepsilon\) is the molar extinction coefficient of CdTe QDs at the first exitonic absorption peak. According to the literature (Yu et al. 2003), \(\varepsilon\) could be obtained by the following equation:

\[
\varepsilon = 10.043 (D)^{2.12}
\]

(3), in which \(D\) (nm) is the particle size of the QDs sample.

**Measurements of fluorescence quantum yield**

The gradient method (Deka et al. 2009) was adopted to estimate the fluorescence quantum yield (QY) of TGA-CdTe QDs, using Rhodamine 6 G (Rh 6 G, with QY of 95% in ethanol) as the reference fluorescent dye, and by exciting all the samples at 470 nm. Briefly, ethanol solutions at different concentrations of Rh 6 G were prepared, and their absorption and fluorescence spectra were recorded. The concentration range of these solutions was such that the absorbance at the excitation wavelength (470 nm) was between 0.01 and 0.1, to avoid self-absorption effects in the photoluminescence spectra. The integrated fluorescence intensity (in ordinates) and absorbance (in abscissas) were then plotted and fitted with a straight line of slope \(m_{\text{Dye}}\) and which in principle should have intercept equal to zero. The same approach was adopted for each TGA-CdTe QDs sample. The fluorescence QY from each QDs sample was then calculated using the following equation:

\[
\text{QY}_{\text{QDs}} = \frac{QY_{\text{Dye}} m_{\text{QDs}}}{m_{\text{Dye}}} \left( \frac{\eta_{\text{water}}}{\eta_{\text{ethanol}}} \right),
\]

where \(QY_{\text{Dye}}\) is the QY of Rh 6 G and \(\eta_{\text{ethanol}}\) and \(\eta_{\text{water}}\) are the refractive indexes of the solvents in which Rh 6 G and the QDs sample are dissolved, respectively.

**Analytical procedure**

Four millilitres of \(2.0 \times 10^{-7}\) mol L⁻¹ TGA-CdTe QDs solution (pH 8) was titrated by successive addition of 10 μL stock solutions of melamine (20 mg L⁻¹). Titrations were manually done by using micro pipette for the addition of melamine. After the sample was mixed thoroughly and allowed to equilibrate for 3 min at room temperature, the fluorescence emission spectra were recorded with the excitation of 450 nm. The calibration curve for melamine was established according to the ratio of fluorescence intensity, that is, \(F_0/F\), and \(F_0\) and \(F\) are the maximum emission intensities of the QDs in the absence and presence of certain concentrations of melamine, respectively.
Detection of melamine in liquid milk

In a 10 mL centrifuge tube, 2 mL of raw milk was respectively mixed with 2 mL of water or different concentrations of melamine, 1 mL of 10% chloroactic acid and 1 mL of chloroform under vortex for 1 min to deposit protein and dissolved organic substances in the matrix. The mixture was then ultrasonically treated for 15 min and centrifuged at 10,000 rpm for 10 min to separate the deposit. The supernatant was transferred to another centrifuge tube and adjusted to pH 8.0 by using 1 M Na₂CO₃. Then, the solution was centrifuged at 6000 rpm for 10 min to remove the deposit once again. The supernatant was filtered through a 0.45 µm GTTP (polycarbonate) filter membrane to obtain the final solution, which was used for detection according to the method described above. A certain amount of melamine was added into the raw milk, making the concentration of added melamine in the range 0.1–1.2 mg L⁻¹/C₀, and the spiked milk sample was pre-treated in accordance with the above procedure, then the extract was analysed.

Result and discussion

Absorption and fluorescence spectra of TGA-CdTe QDs

Using the microwave-assisted synthetic method, we prepared different-sized, water-soluble, TGA-stabilised CdTe QDs, and their absorption and fluorescence spectra are shown in Figure 1(A) and (B), respectively. These CdTe QDs possessed a relative well-resolved absorption maximum of the first electronic transition. According to the calculation method reported in literature (Yu et al. 2003), the particle diameters of the CdTe QDs prepared after microwave heating for 10, 20 and 45 min are respectively about 2.0, 2.5 and 2.8 nm, corresponding to the first excitonic absorption peaks of 495, 508 and 520 nm. The fluorescence spectra of these CdTe QDs exhibited the peaks at 532, 543 and 553 nm, respectively. As the consequence of quantum confinement effects, the absorption maximum and fluorescence peak shifted to longer wavelengths with increasing QD sizes. Besides, the fluorescence spectrum band is relatively narrow and symmetric (with full widths at half-maximum (FWHMs) about 50, 55 and 60 nm, respectively), which indicates that as-prepared CdTe QDs are nearly monodisperse and homogeneous. The fluorescence quantum yield of TGA-CdTe QDs was measured as discussed earlier, which is respectively 27%, 29% and 31% for the QDs with diameters of approximately 2.0, 2.5 and 2.8 nm.

The fluorescence quenching of TGA-CdTe QDs by melamine

The fluorescence emission spectra of 2.8 nm TGA-CdTe QDs in the absence (a) and presence (b) of melamine. The concentrations of TGA-CdTe QDs and melamine were respectively 2.0 × 10⁻⁷ mol L⁻¹ and 50 µg L⁻¹. λ_exc = 450 nm.

Figure 1. Absorption (A) and fluorescence emission spectra (B) of TGA-CdTe QDs with different sizes. λ_exc = 450 nm.

Figure 2. Fluorescence emission spectra of 2.8 nm TGA-CdTe QDs in the absence (a) and presence (b) of melamine. The concentrations of TGA-CdTe QDs and melamine were respectively 2.0 × 10⁻⁷ mol L⁻¹ and 50 µg L⁻¹. λ_exc = 450 nm.
centred at 553 nm (curve a). When melamine was added to the CdTe QDs, a significant decrease in QDs fluorescence emission was observed (curve b). Control experiments, including free CdCl₂, TGA and Cd(TGA) complex solutions with the same concentrations as those used in the synthesis of CdTe QDs, were also performed to explore the origins of the fluorescence quenching. The obtained results demonstrated that these substances had no contribution to the quenching effect of melamine on the luminescence of CdTe QDs. Therefore, the quenching effect was ascribed to the interaction of melamine with TGA-CdTe QDs.

Considering this significant quenching of fluorescence intensity, the possibility of developing sensitive methods for melamine sensing using TGA-CdTe QDs as probes has been evaluated.

**Optimisation of assay condition**

The performance of the developed melamine assay is strongly affected by the assay conditions such as media pH, reaction time and amount of QDs. Different assay conditions were optimised using 2.0 × 10⁻² mol L⁻¹ CdTe QDs with the diameter of about 2.8 nm.

**Effect of pH**

It is well known that the emission intensity of TGA-CdTe QDs was affected by the pH of the environment (Zhang et al. 2003). In addition, in the strong acidic or basic media (pH < 3.0 or pH > 12.0), melamine could be hydrolysed and gradually transformed into cyanuric acid (Bozzi et al. 2004). With 0.1 M NaOH or 0.1 M HCl for pH adjustment, the effects of the solution pH on the fluorescence intensity of TGA-CdTe QDs in the absence and presence of melamine were investigated, and the results are shown in Figure 3. From Figure 3(A), it can be seen that the maximum value of fluorescence intensity of TGA-CdTe QDs was obtained at the pH of 8. In the presence of melamine, as illustrated in Figure 3(B), ⁹₀/₀ is high and steady in the pH range of 7–8. Thus, the optimal pH was chosen to be 8 for further experiments.

**Effect of the reaction time**

At room temperature, the influence of reaction time on the fluorescence intensity of the system was examined. As shown in Figure 4, the fluorescence spectra of TGA-CdTe QDs with the presence of 80 µg L⁻¹
melamine were monitored every 30 seconds at real time. It can be seen that, after melamine was added to the QDs solution, the fluorescence intensity of the QDs gradually decreased by prolonging the reaction time, and the maximum fluorescence quenching was reached within 3 min. The plot of $F_0/F$ versus time kept constant after 3 min and could be stable for at least 3 h. Therefore, the reaction time was fixed at 3 min. This assay exhibits the characteristics of rapid reaction and good stability.

**Selection of the concentration of TGA-CdTe QDs**

The fluorescence of TGA-CdTe QDs can be quenched strongly by melamine. The amount of QDs in the solution will greatly influence the detection sensitivity and linear range for melamine assay. So different concentrations of QDs solutions were used to investigate the effect of QD amounts on the measurement. With the high concentration of QDs over $4.0 \times 10^{-7}$ mol L$^{-1}$, a wider linear range could be obtained at the expense of analytical sensitivity. Given the lower concentration of QDs than $2.0 \times 10^{-7}$ mol L$^{-1}$, the detection sensitivity could be improved with the relatively narrow linear range. Therefore, taking the high analytical sensitivity and wide linear range into account, the TGA-CdTe QDs solution in the concentration range of $2.0 \times 10^{-7}$ to $4.0 \times 10^{-7}$ mol L$^{-1}$ was chosen for the following experiments.

**Calibration and sensitivity**

Under the optimal conditions mentioned above, the emission spectra of TGA-CdTe QDs titrated by melamine were recorded, and the results are shown in Figure 5. It has been found that melamine quenched the fluorescence of QDs in a concentration dependence which is well described by a Stern–Volmer (SV) equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q],$$

where $F_0$ and $F$ are the maximum emission intensities of the QDs in the absence and presence of quencher (melamine), respectively. $K_{SV}$ is the Stern–Volmer quenching constant, and $[Q]$ represents the concentration of the quencher. For the prepared TGA-CdTe QDs with different sizes, the calibration plots of $F_0/F$ on concentrations of melamine are linear in the range of 10–100 μg L$^{-1}$, illustrated as the insets in Figure 5. All of the calibration plots display a good linearity. Using TGA-CdTe QDs with the diameters of about 2.0, 2.5 and 2.8 nm as fluorescence probes, the limit of detection (LOD) ($S/N = 3$) for melamine in water is respectively 1.55, 1.92 and 1.31 μg L$^{-1}$. The relative standard deviation (RSD) for six parallel determinations of 50 μg L$^{-1}$ melamine is 3.2%, 4.1% and 2.9%, respectively.

It is worth noting that even though the same experimental procedure is used for the QDs synthesis, many experimental variables would mean that each
Interference of other substances

To explore the specific detection of melamine in raw milk using TGA-CdTe QDs as fluorescence probes, the interferences of common ions and excipients in raw milk were investigated for determination of 50 \( \mu \text{g} \cdot \text{L}^{-1} \) melamine in water. The fluorescence spectra of 2.8 nm TGA-CdTe QDs with the presence of melamine pre-mixed with different substances are shown in Figure 6(A), and the corresponding ratios of \( F_0/F \) are shown in Figure 6(B), in which the \( F_0/F \) for melamine without addition of any other substance is defined as 1. It can be seen that the tolerable concentration ratios relative to concentration levels usually found in milk for interference at the 5% level were higher than 100 for vitamin B\(_1\) (0.44 g L\(^{-1}\)), glucose (30 g L\(^{-1}\)), threonine (143 g L\(^{-1}\)), \( K^+ \) (143 g L\(^{-1}\)), \( Na^+ \) (43 g L\(^{-1}\)), \( Cl^- \) (141 g L\(^{-1}\)); 10 for tryptophan (7.5 g L\(^{-1}\)), glycine (7.5 g L\(^{-1}\)), histidine (7.5 g L\(^{-1}\)), vitamin C (1 g L\(^{-1}\)); 12, Mg\(^{2+}\) (1 g L\(^{-1}\)); 13, Ca\(^{2+}\) (5.65 g L\(^{-1}\)); 14, PO\(_4^{3-}\) (10 g L\(^{-1}\)); 15, lactose (25 g L\(^{-1}\)); 16, NO\(_3^-\) (150 g L\(^{-1}\)); 17, vitamin B\(_1\) (0.44 g L\(^{-1}\)). \( F_0/F \) for melamine without addition of any other substance is defined as 1.

Detection of melamine in real samples

To validate the reliability of the proposed method for melamine detection in real samples, different amounts of melamine were added to the raw milk before sample
pre-treatment, and then the raw milk samples were processed and analysed according to the procedures described earlier with 2.8 nm TGA-CdTe QDs as fluorescence probes. The obtained results were shown in Figure 7. The observed quenching exhibited a good linear correlation (correlation coefficient $R^2 = 0.9908$) with melamine concentration in raw milk in the range 0.1–1.2 mg L$^{-1}$ (Figure 7(B)), and the detection limit ($S/N = 3$) was calculated to be 0.04 mg L$^{-1}$. The relative standard deviation for 11 parallel determinations of 0.5 mg L$^{-1}$ melamine was 2%. The proposed method was applied to analyse melamine in various milk samples spiked with melamine, including raw milk, breakfast milk, high calcium milk and reconstituted milk, and the recovery results are listed in Table 1. It can be seen that the recoveries vary from 102% to 109%. In addition, no melamine could be found in some kinds of brand milk available locally such as Mengniu, Yili, Guangming and Guangze, indicating that the proposed fluorescence sensing has promising feasibility for rapid detection of melamine in milk.

Compared with the existing methods, seen from Table 2, the detection limit of the proposed method was a little higher than that of conventional methods such as GC–MS/MS (Miao et al. 2010) or capillary electrophoresis (Jin et al. 2010; Wen et al. 2010) which could change the surface state of QDs. To explore the possible mechanism involved in the interaction of TGA-CdTe QDs with melamine, the UV–vis absorption spectra of melamine, TGA-CdTe QDs and TGA-CdTe QDs adding melamine were investigated (Figure 8). As shown in Figure 8, melamine had no absorption in the 300–650 nm wavelength range (curve a), so the quenching effect of melamine on the fluorescence of TGA-CdTe QDs is not due to an inner filter resulting from the absorption of the emission wavelength by melamine. No obvious change was observed for the TGA-CdTe QDs absorption spectra before and after adding melamine (curves b and c). In addition, only a slight red shift could be seen from Figure 5 with increasing concentration of melamine on the emission spectra maximum. This indicates that the presence of melamine mainly influences the surface and not the size of the TGA-CdTe QDs. For further investigation, cyanuric acid was used to carry out the control experiment as the substitute of melamine. In the molecular structure of cyanuric acid, three hydroxy groups (–OH) replace the three amine groups (–NH$_2$) of melamine. Both melamine and cyanuric acid could obviously quench the fluorescence emission of TGA-CdTe QDs, with no obvious shift of the maximum emission wavelength. Moreover, the presence of cyanuric acid could not induce any change of TGA-CdTe QDs absorption spectra. Based on these results, a possible mechanism was put forward to explain the interaction between TGA-CdTe QDs and melamine (Scheme 1). The CdTe QDs were capped with TGA and the $p$Ka of carboxyl group in thioglycolic acid is 3.53 (Zhang et al. 2003), so the surface of CdTe particles contains carboxyl groups that appear as negative charge at pH 8.

### Table 1. Application of the proposed method for analysis of various milk samples spiked with different amounts of melamine ($n = 3$).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of melamine (µg L$^{-1}$)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount added</td>
<td>Amount found</td>
<td></td>
</tr>
<tr>
<td>Raw milk 1</td>
<td>500</td>
<td>547 ± 11.2</td>
<td>109</td>
</tr>
<tr>
<td>Raw milk 2</td>
<td>700</td>
<td>733 ± 7.04</td>
<td>104</td>
</tr>
<tr>
<td>Raw milk 3</td>
<td>900</td>
<td>923 ± 11.4</td>
<td>102</td>
</tr>
<tr>
<td>Breakfast milk</td>
<td>500</td>
<td>540 ± 12.1</td>
<td>108</td>
</tr>
<tr>
<td>High-calcium milk</td>
<td>500</td>
<td>545 ± 11.5</td>
<td>109</td>
</tr>
<tr>
<td>Reconstituted milk</td>
<td>500</td>
<td>537 ± 10.9</td>
<td>107</td>
</tr>
</tbody>
</table>

Notes: RSD, relative standard deviation.

$^a$Average value of three determinations ± standard deviation.

### Mechanism of the interaction of TGA-CdTe QDs with melamine

Quenching the fluorescence emission of QDs may happen by energy transfer (Tang and Marcus 2006), charge diverting (Ji et al. 2005) and surface absorption (Dong et al. 2006) which could change the surface state of QDs. To explore the possible mechanism involved in the interaction of TGA-CdTe QDs with melamine, the UV–vis absorption spectra of melamine, TGA-CdTe QDs and TGA-CdTe QDs adding melamine were investigated (Figure 8). As shown in Figure 8, melamine had no absorption in the 300–650 nm wavelength range (curve a), so the quenching effect of melamine on the fluorescence of TGA-CdTe QDs is not due to an inner filter resulting from the absorption of the emission wavelength by melamine. No obvious change was observed for the TGA-CdTe QDs absorption spectra before and after adding melamine (curves b and c). In addition, only a slight red shift could be seen from Figure 5 with increasing concentration of melamine on the emission spectra maximum. This indicates that the presence of melamine mainly influences the surface and not the size of the TGA-CdTe QDs. For further investigation, cyanuric acid was used to carry out the control experiment as the substitute of melamine. In the molecular structure of cyanuric acid, three hydroxy groups (–OH) replace the three amine groups (–NH$_2$) of melamine. Both melamine and cyanuric acid could obviously quench the fluorescence emission of TGA-CdTe QDs, with no obvious shift of the maximum emission wavelength. Moreover, the presence of cyanuric acid could not induce any change of TGA-CdTe QDs absorption spectra. Based on these results, a possible mechanism was put forward to explain the interaction between TGA-CdTe QDs and melamine (Scheme 1). The CdTe QDs were capped with TGA and the $p$Ka of carboxyl group in thioglycolic acid is 3.53 (Zhang et al. 2003), so the surface of CdTe particles contains carboxyl groups that appear as negative charge at pH 8.
Table 2. Comparison between the proposed method and other reported methods for melamine analysis.

<table>
<thead>
<tr>
<th>Method</th>
<th>Pretreatment</th>
<th>Apparatus</th>
<th>Detection limit</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Immunoaffinity-based solid phase extraction</td>
<td>HPLC</td>
<td>15 ng g(^{-1})</td>
<td>Zhong et al. (2011)</td>
</tr>
<tr>
<td>GC-MS-MS</td>
<td>Derivation</td>
<td>GC-MS-MS</td>
<td>3.5 µg kg(^{-1})</td>
<td>Miao et al. (2010)</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Centrifugation</td>
<td>LC-MS</td>
<td>0.2 mg kg(^{-1})</td>
<td>Goscinny et al. (2011)</td>
</tr>
<tr>
<td>Ag NPs colorimetric analysis</td>
<td>Filtration</td>
<td>UV–vis</td>
<td>0.1 ppm (0.1 mg kg(^{-1}))</td>
<td>Han and Li (2010)</td>
</tr>
<tr>
<td>Au NPs colorimetric analysis</td>
<td>Centrifugation and filtration</td>
<td>UV–vis</td>
<td>2.5 ppb (2.5 µg kg(^{-1}))</td>
<td>Ai et al. (2010)</td>
</tr>
<tr>
<td>Au NPs colorimetric analysis</td>
<td>Centrifugation and filtration</td>
<td>UV–vis</td>
<td>0.4 mg L(^{-1}) (0.4 mg kg(^{-1}))</td>
<td>Li et al. (2010)</td>
</tr>
<tr>
<td>Fe(_3)O(_4) MNPs</td>
<td>Centrifugation</td>
<td>UV–vis</td>
<td>2.5 ppm (2.5 mg kg(^{-1}))</td>
<td>Ding et al. (2010)</td>
</tr>
<tr>
<td>ELISA</td>
<td>Solvent extraction</td>
<td>ELISA instrument</td>
<td>1.8 ng/g</td>
<td>Liu et al. (2010)</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>Centrifugation and filtration</td>
<td>CE instrument</td>
<td>0.05 ng mL(^{-1}) (50 ng kg(^{-1}))</td>
<td>Jin et al. (2010)</td>
</tr>
<tr>
<td>Electrochemical sensor</td>
<td>Centrifugation and filtration</td>
<td>Electrochemistry instrument</td>
<td>0.25 ppb (0.25 µg kg(^{-1}))</td>
<td>Zhu et al. (2010)</td>
</tr>
<tr>
<td>Raman spectroscopy based on gold nanosubstrate</td>
<td>Centrifugation and solid-phase extraction</td>
<td>Raman instrument</td>
<td>2.1 mg kg(^{-1})</td>
<td>Cheng and Dong (2011)</td>
</tr>
<tr>
<td>Infrared spectroscopy</td>
<td>–</td>
<td>FT-NIR</td>
<td>(0.76 ± 0.11 ppm) (0.76 ± 0.11 c)</td>
<td>Balabin and Smirnov (2011)</td>
</tr>
<tr>
<td>PDA liposomes colorimetric analysis</td>
<td>–</td>
<td>UV–vis</td>
<td>1 ppm (1 mg kg(^{-1}))</td>
<td>Lee et al. (2011)</td>
</tr>
<tr>
<td>Au NPs</td>
<td>–</td>
<td>Fluorescence spectrophotometer</td>
<td>6.1 × 10(^{-10}) M</td>
<td>Xiang et al. (2011)</td>
</tr>
<tr>
<td>CdTe QDs</td>
<td>Centrifugation and filtration</td>
<td>Fluorescence spectrophotometer</td>
<td>0.04 mg L(^{-1}) (0.04 mg kg(^{-1}))</td>
<td>This work</td>
</tr>
</tbody>
</table>

Notes: HPLC, high-performance liquid chromatography; GC, gas chromatography; MS, mass spectrometry; ELISA, enzyme-linked immunosorbent assay; NPs, nanoparticles; MNPs, magnetic nanoparticles; UV–vis, ultraviolet–visual; FT-NIR, Fourier transform–near infrared; PDA, polydiacetylene; CdTe QDs, cadmium telluride quantum dots.
Because hydroxy groups (–OH) and amine groups (–NH₂) can form hydrogen bonds with the carboxyl groups on the TGA-CdTe QDs surface (Chanu and Negi 2010; Zhang et al. 2011), melamine and cyanuric acid could bind to the surface of TGA-CdTe QDs like a cap through the hydrogen-bond interaction. Thus, we assume that melamine binding to the TGA-CdTe QDs made the surface of the particles change, which induced the quenching of QDs fluorescence (Liu et al. 2008; Negi et al. 2010).

It is worth noting that interference from cyanuric acid should be avoided when using TGA-CdTe QDs as fluorescence probes for melamine analysis. Melamine is a weak base with a pKa of 5.05. In strong acidic media and strong basic media, melamine can be hydrolysed and could gradually lose one, two and three amino groups, finally transformed into cyanuric acid (Bozzi et al. 2004; Li et al. 2010). Because both melamine and cyanuric acid could quench the fluorescence emission of TGA-CdTe QDs, the system was controlled at pH 8 to prevent melamine from transforming into cyanuric acid.

**Conclusion**

In summary, a novel and convenient technique for rapid melamine analysis has been developed based on melamine-induced fluorescence quenching of TGA-CdTe QDs. The possible quenching mechanism is due to the surface change of CdTe nanoparticles, induced by melamine binding through hydrogen bonding. This proposed method showed high precision and accuracy when applied to detect melamine in raw milk, with a detection limit as low as 0.04 mg L⁻¹. The whole procedure including sample pre-treatment takes about 30 min. The assay is characterised by simplicity, rapidity, low cost and high sensitivity. Furthermore, it appears to be a candidate for rapid screening of melamine contamination in other foods such as infant formula, eggs and animal feeds.

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**References**


