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Colorimetric detection of melamine in pretreated milk using silver nanoparticles functionalized with sulfanilic acid

Juan Song, Fangying Wu∗, Yiqun Wan, Lihua Ma

Department of Chemistry, Nanchang University, Nanchang 330031, China

Abstract Multiple methods have been published to detect melamine, only a few offer sensitivities below 50 nM with complicated procedures and sophisticated equipments. We demonstrate here a simple, rapid and lower-cost assay with high sensitivity for the melamine detection in milk samples using sulfanilic acid-modified silver nanoparticles (SAA-AgNPs). Due to the special chemical structures, SAA shows similar response to its analogues, which reveals that the selectivity and sensitivity of SAA itself is poor. However, the formation of SAA-AgNPs dramatically improves the selectivity of SAA and only melamine reacts with SAA-AgNPs. The possible mechanism is discussed. The interaction between exocyclic amine of melamine and SAA induces rapid aggregation of SAA-AgNPs accompanied by a naked-eye visible color change, resulting in precise quantification of melamine that can be monitored by a simple UV-visible spectrometer. Metal ions, amino acids and sugars that are common in milk have negligible interference influences. The extinction ratio (A\text{620nm}/A\text{390nm}) is correlated with the melamine concentration over the range of 0.1-3.1 µM. The detection limit is 10.6 nM, which is much lower than the safety limits (8 µM for infant formula in China, 20 µM in both the USA and EU and 1.2 µM in the CAC review for melamine in liquid infant formula). The method is applied successfully to determine melamine in pretreated milk products, indicating the potential practical use for the products suspected of melamine exposure.

Key words: Colorimetric assay; Melamine; Silver nanoparticles; Sulfanilic acid; Milk samples.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triazine) is a trimer of cyanamide that is commonly found in

∗ Corresponding author: Fangying Wu, Tel: + 86 79183969882; Fax: + 86 79183969514; E-mail address: fywu@ncu.edu.cn
flame-resistant products, textile industries and the production of pesticides. In addition, although it is not registered as a fertilizer in the U.S., melamine has been used as a fertilizer in some parts of the world. In recent years, melamine has been intentionally and illegally adulterated in the milk, infant formula and pet foods due to its low cost and high nitrogen content (66%, by mass) that can contribute to the protein content measured by the conventional standard Kjeldahl and Dumas tests (Serdiuk, Skryshevsky, Phaner-Goutorbe, & Souteyrand, 2010). Addition of 1% melamine in food causes protein content to artificially boost more than 4%. The metabolism of melamine in vivo is inert and it has low oral acute toxicity. However, melamine can be hydrolyzed to cyanuric acid which in turn associates with melamine to form reticulations, resulting in the formation of kidney stones depending upon urine pH that might cause organs failure and even death in humans and animals (Manzoori, Amjadi, & Hassanzadeh, 2011). In 2007, US scientists confirmed that pet food adulteration with melamine was the cause of illness and death of many cats and dogs (Tyan, Yang, Jong, Wang, & Shiea, 2009). In 2008, consumption of melamine-contaminated infant formula and related dairy products in China likely caused more than 51,900 infants and young children to suffer from urinary problems, including six child deaths (Xu, et al., 2009). To assure food safety and protect public health, many countries have restricted maximum residue limits (MRL) for melamine in various products. The European Union (EU) set melamine MRL in dairy products and high-protein foods at 20 µM, whereas the US Food and Drug Administration (FDA) set it as 2.0µM in milk and dairy products but stressed that infant formula sold to US consumers must be completely free of melamine (Filazi, Sireli, Ekici, Can, & Karagoz, 2012). The Ministry of Health of China published new dairy safety standards and emphasized that food should not be contaminated with melamine (Guo, et al., 2011).

The most commonly used approaches that have been published are chromatography-based methods including high performance liquid chromatography (HPLC) (Zhang, et al., 2014), gas-chromatography mass spectrometry (GC–MS) (Li, Qi, & Shi, 2009), and LC–MS/MS (He, et al., 2014). Others also have tried immunoassay (Lei, et al., 2010), electrochemiluminescence (ECL) (Guo, et al., 2011), fourier transform infrared spectroscopy (FTIR) (Jawaid, Talpur, Sherazi, Nizamani, & Khaskheli, 2013), surface-enhanced Raman spectroscopic (SERS) (Zhao, et al., 2013) and molecular imprinting (MIP) (Jin, Yu, Yang, & Ma, 2011) to detect melamine in foods. Some of the methods possess high sensitivity and accuracy. Nevertheless, most of these technologies are time consuming, labor-intensive, instrumental expensive and well-trained scientists are needed to perform sample
pretreatment and to operate the instruments.

Owing to their unique chemical and physical properties, metal nanoparticles (NPs) have been favorably selected to design novel sensors for detecting biologically or environmentally important analytes by various optical methods such as absorption (Sun, Zhang, & Li, 2012), fluorescence (Su, Chen, Sun, & Ai, 2012), resonant light scattering (RLS) (Navarro & Werts, 2013), surface-enhanced raman scattering (SERS) (Zhou, et al., 2010) and so on. Due to the extremely high extinction coefficient and strongly distance-dependent optical property, silver nanoparticles (AgNPs) were utilized as an ideal color reporter for colorimetric sensors. Up to now, AgNPs-based colorimetric methods have been established to detect proteins, ions and other small molecules (Li, Qiang, Vuki, Xu, & Chen, 2011; Miao, et al., 2013; Yang, Gao, Zhang, Shen, & Wu, 2014). Melamine detection using AgNPs without label has been published before and achieved only 2.32 µM LOD (Ping, et al., 2012). In order to facilitate aggregation and increase the water solubility, the AgNPs are always covalently modified with functional molecules (Lee, Lytton-Jean, Hurst, & Mirkin, 2007). Our team also reported chromotropic acid-modified AgNPs for the visual detection of melamine with detection limit of 36 nM (Song, Wu, Wan, & Ma, 2014).

Sulfanilic acid (SAA) is applied in quantitative analysis of nitrate and nitrite ions (Narayana & Sunil, 2009). It is commercially available, inexpensive, environmentally benign, stable and incompatible with strong oxidizing agents. It shows similar UV-visible spectral responses to melamine and its structural analogues. However, we found out that after the AgNPs was modified with SAA, the SAA-AgNPs dramatically changed the selectivity and sensitivity of SAA and it only reacted with melamine among the analogues. The affinity of melamine towards the functional group of SAA leads to a notable aggregation of SAA-AgNPs and a clear and rapid color change from bright yellow to blue-green was observed by the naked eye. Based on the above discovery, we established a colorimetric assay for melamine (Scheme 1) with detection limit as low as 10.6 nM in aqueous solution and successfully applied it to the milk products with satisfactory recoveries.
Scheme 1 Schematic illustration of possible mechanism for the colorimetric response of SAA-AgNPs to melamine.

2. Experimental

2.1. Chemicals and Materials

Sulfanilic acid was purchased from the third Shanghai reagent factory. (Shanghai, China, http://6379.cn.Toocle.com). Silver nitrate (AgNO$_3$), sodium borohydride (NaBH$_4$), trisodium citrate, melamine and the other metallic ions (NaCl, MgCl$_2$, ZnCl$_2$, FeCl$_3$, CaCl$_2$, KNO$_3$, Na$_2$CO$_3$, Na$_4$P$_2$O$_7$) were purchased from Shanghai Qingxi Technology Co. Ltd. (Shanghai, China, www.ce-r.cn/sites/qingxi/). All the carbohydrate including D-fructose, D-glucose and sucrose were purchased from Shanghai Lanji Technology Co. Ltd. (Shanghai, China, http://lj80414.bioon.com.cn/). Aniline compounds and phenol compounds including o-phenylenediamine (o-PDA), m-phenylenediamine (m-PDA), p-phenylenediamine (p-PDA), phloroglucinol, cyanuric acid, trimethy-1,3,5-triazine, and amino acids including lysine (Lys), tryptophan (Try), methionine (Met), leucine (Leu), isoleucine (Ile), phenylalanine (Phe) and valine (Val) were purchased from Shanghai Jingchun Technology Co. Ltd. (Shanghai, China, http://www.aladdin-reagent.com/).

2.2 Instruments

UV-vis absorption spectra were recorded using UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan, http://www.shi-madzu.com) with a 1.0 cm quartz cell at room temperature. For the Transmission
electron microscopy (TEM) analysis, the solution of SAA-AgNPs was dropped onto the carbon-coated copper grid and the microscope used was a JEM-2010 TEM (JEOL Ltd. Japan, http://www.jeol.cn).

The FTIR spectra were collected in Nicolet 380 FTIR Spectrometer (Nicolet, USA, http://www.thermonicolet.com) at room temperature. For FTIR characterization, the solution of SAA-AgNPs was centrifuged. Upon removal of the supernatant the solid deposit was rinsed with DI water and then placed in a vacuum dryer (DZF-6030A) at 40°C and full pressure (133 pa) for 12 hours. The dried AgNPs was mixed with KBr, and pelleted by macro KBr die kit. Pellets were loaded into a Nicolet 380 FTIR spectrometer and the spectra were taken in the wavenumber range from 400 to 4000 cm$^{-1}$ with a resolution of 4cm$^{-1}$. For comparison, FTIR spectrum for pure SAA was also recorded.

Atomic emission spectra were recorded by ICP-AES OPTIMA 5300DV (PE, USA, http://www.perkinelmer.com)

2.3. Preparation of SAA-modified AgNPs

The bare AgNPs were prepared by reducing AgNO$_3$ according to the method reported previously (Li, et al., 2010). All pieces of the experimental glassware used were cleaned in a bath of freshly prepared aqua regia solution (HCl: HNO$_3$, 3:1) and then rinsed thoroughly with milli-Q-purified distilled water prior to use. Briefly, 2.0 mL of 0.01 M AgNO$_3$ and 4.0 mL of 0.01 M sulfanilic acid were added into 94 mL double distilled water under stirring. Then 8.8 mg of NaBH$_4$ was quickly added into the mixture solution under vigorous stirring. The resulting yellow silver colloidal solution was stirred for 2 h in the dark at room temperature. The SAA-AgNPs was stored at 4°C in dark undergoing no change within 7 days (shown in Fig. S1) and used directly in the following experiments. The concentration of testing AgNPs solution was estimated to be 14 nM according to extinction coefficient on particle diameter ($\varepsilon=Ad^2$, for AgNPs which diameter is less or equal to 38 nm, $A=2.3\times10^5$ M$^{-1}$cm$^{-1}$, $\gamma=3.48$) (Navarro, & Werts, 2013). In our experiment, the average of AgNPs size is 6.7 nm and the absorbance of testing solution is 2.4.

2.4. Milk Samples pretreatment

The milk samples were pretreated according to the general procedure (Ma, Niu, Zhang, & Cai,
2011). In short, proteins were removed by mixing with 1.5 mL 10% trichloroacetic acid, 5.0 mL acetonitrile and then 375 µL 1.0 M Na₂CO₃ for 2.0 g milk. The mixture solution was transferred to centrifugal tube to be sonicated for 10 min and then centrifuged at 12,000 rpm min⁻¹ for 15 min. The supernatant was filtered through a 0.22 µm membrane to remove lipids. The pH of filtrate was adjusted to 6.8, and the filtrate was filtered again after centrifugation. The filtered liquid was diluted with water to 10 mL for further analysis. The concentration of calcium ion before and after the treatment were measured by the ICP-AES to be 1500 mg/L and 29 mg/L, respectively, indicating that this procedure is suitable for the purpose of effective calcium ions removal.

2.5. General procedure of colorimetric detection of melamine

The colorimetric detection of melamine was performed at room temperature. A 1.0×10⁻⁴ M aqueous solution of melamine was prepared. In a typical experiment, 50 µL of melamine solution with various concentrations was added to the 3 mL SAA-AgNPs solution (14 nM), and the mixture was incubated at room temperature for 5 min. The photographs of resulting solutions were taken and the UV–visible absorption spectra of the mixtures were recorded immediately. The absorbance intensity ratio of A₆₂₀nm/A₃₉₀nm acted as the quantitative index for melamine. The spiked-recovery detection of melamine in pretreated samples was manipulated with the same procedure.

3. Results and discussion

3.1. Characterization of SAA-AgNPs

To elucidate the stabilization mechanisms and to gain further insight into the interactions between various functional groups of SAA and AgNPs, FTIR measurements were carried out. Fig.S2 compared the characteristic stretching frequencies for SAA (b) and SAA-AgNPs (a). It was noteworthy that the S=O stretching vibration band at 1235-1109 cm⁻¹ of sulfonic acid that is obvious for SAA disappears for SAA-AgNPs. Furthermore, the bands at 1640-1560 cm⁻¹ and 900–650 cm⁻¹ those are the characteristic peaks of primary aromatic amines N–H bending and wagging vibrations, respectively are obviously shown in the spectra of both SAA-AgNPs (Fig.S2a) and SAA (Fig.S2b). The above
observation based on S=O and NH groups implied that SAA was bound to the surface of AgNPs via the sulfonic acid and suggested that SAA-AgNPs were successfully synthesized. TEM was used to characterize the size and shape of AgNPs. The TEM image of SAA-AgNPs in the absence of melamine was shown in Fig.1. It was clear that the SAA-AgNPs were dispersed uniformly with spherical shape. The average size was 6.7 nm with relative standard deviation as 14% (shown in Fig.S3).

3.2 Recognition of melamine by AgNPs and the selectivity of the assay

In the aqueous media, the affinity of melamine towards the functional groups of SAA lead to a notable aggregation of SAA-AgNPs and a color change (presented in Fig.1). The free SAA-AgNPs with bright yellow color showed a typical peak around 390 nm (photographic image (a) and curve (a)) resulting from the surface plasmon resonance with extremely high extinction coefficient. In the presence of melamine, the SAA-AgNPs aggregated rapidly (Fig.1b), and the intensity of peak at 390 nm decreased and a broad peak at 620 nm appeared, accompanied by a visible color change from bright yellow to blue-green (as shown in photographic image (b) and curve (b) of Fig.1).

**Fig.1** UV-vis spectra (left) (insert image is colorimetric response) and TEM images (right) of SAA-AgNPs in the absence (a) and presence (b) of 0.9 µM melamine.

In order to examine the specificity and selectivity of the interaction between melamine and SAA-AgNPs, we performed two types of parallel experiments including the effect of the potentially
interfering cation, anion, some amino acids, sugars (shown in Fig. 2) and the responses of SAA-AgNPs
to other chemical analytes (arginine, histidine, guanidine hydrochloride, cyanuric acid,
trimethy-1,3,5-triazine, phloroglucinol, \( m \)-dihydroxybenzene, \( o \)-phenylenediamine(\( o \)-PDA),
\( m \)-phenylenediamine(\( m \)-PDA), \( p \)-phenylenediamine(\( p \)-PDA) shown in Fig.3A), the structures of which
were illustrated in Fig.3B. As shown in Fig.2, the presence of following amounts of ions and
compounds resulted in less than ±10% error: 50 times the concentration of Lys, Try, Met, Leu, Ile, Phe,
Val, glucose, fructose, Sucrose, 300 times the concentration of Ca\(^{2+}\), 500 times the concentration of
\( Mg^{2+}\), \( Zn^{2+}\), \( Fe^{3+}\) and 1000 times the concentration of \( Na^{+}\) and \( NO_{3}^{-}\), pyrophosphate, citrate, \( CO_{3}^{2-}\),
EDTA. And the SAA-AgNPs were closely non-responsive to melamine analogues as shown in Fig.3A.
But His and Arg interfered the assay to some extent. Because these two amino acids are not common in
liquid milk and formula, together with the different color change, the influence can be ignored in the
practical use.

![Fig.2](image)

**Fig.2** The intensity ratio (\( A_{620nm}/A_{390nm} \)) of SAA-AgNPs upon addition of 0.9µM melamine with
the coexistence of interference (1-21: control, 50 times Lys, Try, Met, Leu, Ile, Phe, Val, 1000 times
\( NO_{3}^{-}\), pyrophosphate, citrate, \( CO_{3}^{2-}\), EDTA, 300 times of \( Ca^{2+}\), 500 times of \( Mg^{2+}\), \( Zn^{2+}\), \( Fe^{3+}\), 1000
times of \( Na^{+}\), glucose, fructose, sucrose, respectively
Fig. 3 (A) The intensity ratio ($A_{520\text{nm}}/A_{390\text{nm}}$) and photographs of SAA-AgNPs in the presence of 0.9 µM melamine and other analytes. (B) Structures of melamine analogues and amino acids His and Arg.

3.3. Optimization of the assay for melamine

3.3.1. Effect of the SAA concentration

Since the concentration of SAA could have effect on SAA-AgNPs detection capability, the stoichiometric ratio between SAA and AgNO$_3$ were explored. In our work, SAA-AgNPs were synthesized based on four different molar ratio ($n_{\text{AgNO}_3} / n_{\text{SAA}}$), intensity ratios ($A_{520\text{nm}}/A_{390\text{nm}}$) of which were compared (insert in Fig.4). The result indicated that SAA-AgNPs prepared at lower molar ratio was more sensitive to melamine. However, the concentration of SAA can’t be too high because the excess of SAA may lead to SAA-AgNPs aggregation to some extent as shown in the absorption spectra of Fig.4. When the molar ratio was 1:4, the absorbance at 390 nm was weak and became insensitive to melamine. Therefore, molar ratio 1:2 that gave the best sensitivity to melamine was selected as the optimized condition.
Fig. 4 Absorption spectra of SAA-AgNPs prepared with various molar ratio ($n_{\text{AgNO}_3}: n_{\text{SAA}}$). Insert is the absorption intensity ratio ($A_{620\text{nm}}/A_{390\text{nm}}$) of these different types of SAA-AgNPs as a function of melamine concentrations.

3.3.2. Effect of pH and reaction time

Influence of the media pH (4.0-11.5) and reaction time on the assay were also investigated. pH 9.5 was found to be the optimal condition for the further experimental. It was noticed that the reaction took place in less than several seconds and underwent slight increase in 5 min. To obtain the maximum response, 5 min was selected as the incubation time for the following experiments.

3.4. Analytical applications

3.4.1. Colorimetric assay of melamine in water

Under the optimized detection conditions and at room temperature, the sensitivity of the sensor was investigated. Several calibration samples with various concentrations of melamine were analyzed. As shown in Fig. 5A, with increasing the melamine concentration, the absorbance of SAA-AgNPs at 390 nm decreased gradually and the absorbance at 620 nm increased. The variation could be visualized by the naked eyes as shown in photographs in Fig. 5A. Two linear ranges were observed, resulting in two calibration equations, one was $y = 0.00669 \times [C] + 0.00644$ when melamine concentration was in the range of 0 to 0.9 µM, the other was $y = 0.02697 \times [C] - 0.2112$ when melamine concentration was from 0.9 to 3.1 µM (Insets a and b of Fig. 5B). The limit of detection (LOD) is defined by the equation $\text{LOD} = 3S_0/K$, where $S_0$ is the standard deviation of blank measurements ($n=10$) and $K$ is the slope of calibration line. The LOD of the proposed method was 10.6 nM according to the lower concentration range.
Fig. 5 (A) Absorbance spectra of the SAA-AgNPs in the presence of melamine with various concentrations at 0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, 1.9, 2.1, 2.3, 2.5, 2.7, 2.9 and 3.1 µM, respectively. Inset is the selected photograph. (B) The plot of intensity ratio \( \frac{A_{620nm}}{A_{390nm}} \) of SAA-AgNPs versus the concentration of melamine. Insets are the two linear fitting curves.

### 3.4.3. Detection of melamine in milk samples

To validate the reliability of the proposed colorimetric method in practical applications, the detection of melamine in milk products was examined. A big challenge for detecting melamine in milk products is how to decrease the potential interference from macromolecular compounds (such as protein). In our study, milk products samples were pretreated by a sample extract procedure and these samples were spiked with different concentrations of melamine. In the pretreatment process, proteins were precipitated by trichloroacetic acid, and lipid micelles were removed by repeating filtrations. In order to increase the tolerance of Ca in the assays, we use Na\(_2\)CO\(_3\) to get rid of most calcium. The Ca concentration was measured by ICP-AES before and after treatment. The detailed procedures were shown in the milk samples pretreatment (seen part of 2.4). Even though there may be trace calcium exist, the lower amount of calcium would not interfere with the response of melamine to the SAA-AgNPs sensor. As shown in Fig. S4, the UV-vis spectra of SAA-AgNPs, the intensity ratio plot \( \frac{A_{620nm}}{A_{390nm}} \), the calibration equations in the presence of melamine were similar in different matrix such as liquid and solid milk, suggesting that this colorimetric assay can detect melamine without being affected by the complex environments. The recoveries for liquid and solid milk were from 96% to 109% and 97% to 108%, respectively (Table 1). Therefore, it turns out to be a promising method for rapid screening of melamine contamination in milk products with high.
reproducibility.

Table 1 Recovery test results of the assay in liquid and solid milk samples with spiked melamine

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (10^-7 M)</th>
<th>Average (10^-7 M)</th>
<th>RSD (% , n=3)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid milk</td>
<td>9.0</td>
<td>9.8</td>
<td>1.9</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>21.6</td>
<td>1.3</td>
<td>103</td>
</tr>
<tr>
<td>Solid milk</td>
<td>9.0</td>
<td>9.7</td>
<td>1.5</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>20.3</td>
<td>0.9</td>
<td>97</td>
</tr>
</tbody>
</table>

3.5. Possible Mechanism of the sensor

The UV-visible spectra, visible color changes, TEM images explained the aggregation of SAA-AgNPs in the presence of melamine. Based on the FTIR, we concluded that SAA was modified on the surface of AgNPs through the sulfonic acid and therefore it was the -NH_2 group on the outer surface of the SAA-AgNPs that was responsible for sensing melamine based on hydrogen bonding (Ma, Niu, Zhang, & Cai, 2011). The results implied that the selectivity and the sensitivity of the SAA-AgNPs were significantly enhanced as compared with the free SAA (Fig.3 and Fig.S5), which were possibly attributed to both hydrogen bonding and the shape complementarity between SAA-AgNPs and melamine (Liu et al., 2011). By anchoring on the surface of AgNPs, multiple SAA may assemble to provide more hydrogen binding sites and leave more spaces that are favored by melamine molecules. It has been reported that melamine has a specific structure which could form ordered structures, it might be reasoned that the neighbor melamine coated AgNPs could be cross-linked with melamine molecules (Guan, Yu, & Chi, 2013; Silly, et al., 2008) (as shown in Scheme1), which in turn significantly improve the responses of SAA-AgNPs to melamine compared with that of free SAA.

4. Conclusions

In summary, an ultrasensitive and selective detection of melamine using SAA capped AgNPs based on the interaction between melamine and –NH_2 group of SAA was proposed. The presence of
melamine induced the aggregation of SAA-AgNPs through cooperative hydrogen bondings, resulting
in a color change from yellow to blue-green that is easily observed with the naked eye or the UV-vis
spectrometer. At the optimized conditions, the SAA_AgNPs based detection system has an excellent
selectivity to melamine comparing with other analogues. This method is easy to be operated with lower
cost (0.5$ per sample). In particular, the proposed low-cost and rapid-reaction method is successfully
applied to determine melamine in milk products with LOD of 10.6nM, which is lower than most
existing methods without the aid of expensive instruments (as shown in Table S1), allowing the
potential use of this system for fast quality screening of milk products.

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nanoparticle–oligonucleotide conjugates based on DNA with triple cyclic disulfide moieties.
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Captions

Scheme 1 Schematic illustration of possible mechanism for the colorimetric response of SAA-AgNPs to melamine.

Fig.1 UV-vis spectra (left) (insert image is colorimetric response) and TEM images (right) of SAA-AgNPs in the absence (a) and presence (b) of 0.9 µM melamine.

Fig.2 The intensity ratio \( \frac{A_{620nm}}{A_{390nm}} \) of SAA-AgNPs upon addition of 0.9µM melamine with the coexistence of interference (1-21: control, 50 times Lys, Try, Met, Leu, Ile, Phe, Val, 1000 times NO\(_3^\), pyrophosphate, citrate, CO\(_3^{2-}\), EDTA, 300 times of Ca\(^{2+}\), 500 times of Mg\(^{2+}\), Zn\(^{2+}\), Fe\(^{3+}\), 1000 times of Na\(^{+}\), glucose, fructose, sucrose, respectively.

Fig.3 (A) The intensity ratio \( \frac{A_{620nm}}{A_{390nm}} \) and photographs of SAA-AgNPs in the presence of 0.9 µM melamine and other analytes. (B) Structures of melamine analogues and amino acids His and Arg.

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Table 1 Recovery test results of the assay in liquid and solid milk samples with spiked melamine.
Research Highlights

An easy-operate colorimetric assay for melamine (MA) based on AgNPs was presented.

The color change can be observed via naked-eyes when only 1.0µM MA exists.

The assay was applied to detect MA in milk sample.

The detection limit as 10.6 nM for MA is much lower than the safety limits.
Electronic Supporting Materials

Colorimetric detection of melamine in pretreated milk using silver nanoparticles functionalized with sulfanilic acid

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Fig.S1 The UV-vis spectra of SAA-AgNPs stored at 4°C in different time

Fig.S2 IR spectra of SAA-AgNPs (a) and pure SAA (b)

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**Fig.S4** Relationship between the absorbance ratio ($A_{620nm}/A_{390nm}$) of SAA-AgNPs and the concentration of melamine with water (black), liquid milk (red) and solid milk (blue) samples.

**Fig.S5** The UV-vis spectra of SAA (2.0×10^{-4} M) in the presence of melamine and its analogues (18μM).
Table S1 Comparison of linear range and detection of limit of melamine obtained by different methods.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Detection limit (mol L(^{-1}))</th>
<th>Linear range (mol L(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>3.2×10(^{-9})</td>
<td>4.0×10(^{-9})-1.6×10(^{-6})</td>
<td>(Zhang, et al., 2014)</td>
</tr>
<tr>
<td>LC-MS</td>
<td>2.1×10(^{-8})</td>
<td>7.9×10(^{-8})-8.0×10(^{-6})</td>
<td>(He, et al., 2014)</td>
</tr>
<tr>
<td>GC-MS</td>
<td>8.0×10(^{-9})</td>
<td>8.0×10(^{-9})-7.9×10(^{-3})</td>
<td>(Li, et al., 2009)</td>
</tr>
<tr>
<td>FTIR</td>
<td>2.0×10(^{-8})</td>
<td>4.0×10(^{-8}}-7.9×10(^{-8})</td>
<td>(Jawaid, et al., 2013)</td>
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<tr>
<td>ECL</td>
<td>2.4×10(^{-9})</td>
<td>7.9×10(^{-8}}-7.9×10(^{-6})</td>
<td>(Guo, et al., 2011)</td>
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<tr>
<td>SERS</td>
<td>1.9×10(^{-8})</td>
<td>1.0×10(^{-8}}-1.0×10(^{-3})</td>
<td>(Zhao, et al., 2013)</td>
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<tr>
<td>MIP-based sensors</td>
<td>1.3×10(^{-9})</td>
<td>1.0×10(^{-9}}-9.0×10(^{-7})</td>
<td>(Jin, et al., 2011)</td>
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<tr>
<td>Citrate capped AgNPs</td>
<td>2.3×10(^{-6})</td>
<td>4.0×10(^{-6}}-1.7×10(^{-4})</td>
<td>(Ping, et al., 2012)</td>
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<tr>
<td>p-nitroaniline modified AgNPs</td>
<td>7.9×10(^{-7})</td>
<td>5.0×10(^{-8}}-1.0×10(^{-4})</td>
<td>(Han &amp; Li, 2010)</td>
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<tr>
<td>Dopamine modified AgNPs</td>
<td>7.9×10(^{-8})</td>
<td>7.9×10(^{-8}}-1.0×10(^{-3})</td>
<td>(Ma, et al., 2011)</td>
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<tr>
<td>Crown ether modified AuNPs</td>
<td>4.7×10(^{-8})</td>
<td>7.9×10(^{-8}}-4.0×10(^{-6})</td>
<td>(Kuang, et al., 2011)</td>
</tr>
<tr>
<td>Cysteamine modified AuNPs</td>
<td>7.9×10(^{-6})</td>
<td>7.9×10(^{-8}}-1.6×10(^{-3})</td>
<td>(Liang, et al., 2011)</td>
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<tr>
<td>Chitosan-stabilized AuNPs</td>
<td>4.8×10(^{-8})</td>
<td>7.9×10(^{-8}}-7.9×10(^{-5})</td>
<td>(Guan, et al., 2013)</td>
</tr>
<tr>
<td>CTA capped AgNPs</td>
<td>3.6×10(^{-8})</td>
<td>1.0×10(^{-7}}-1.5×10(^{-6})</td>
<td>(Song, et al., 2014)</td>
</tr>
<tr>
<td>SAA capped AgNPs</td>
<td>1.1×10(^{-8})</td>
<td>1.0×10(^{-7}}-3.1×10(^{-6})</td>
<td>Our method</td>
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</table>

References


Chromatogr A, 1216(29), 5467-5471.


Table 1 Recovery test results of the assay in liquid and solid milk samples with spiked melamine

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added ($10^{-7}$M)</th>
<th>Average ($10^{-7}$M)</th>
<th>RSD (% , n=3)</th>
<th>Recovery (%)</th>
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<td>Liquid milk</td>
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<td>1.9</td>
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<td>21.0</td>
<td>21.6</td>
<td>1.3</td>
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<td>Solid milk</td>
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<td>1.5</td>
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<td>21.0</td>
<td>20.3</td>
<td>0.9</td>
<td>97</td>
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