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Integration of colorimetric and SERS detection for rapid screening and validation of melamine in milk†

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Various analytical methods have been developed for detecting melamine in milk. Herein, we developed a novel method which integrated two gold nanoparticle (Au NP) based techniques, colorimetric and surface enhanced Raman spectroscopic (SERS) analyses, for rapid screening and validation of melamine in milk. The colorimetric method, which utilizes the color change of Au NPs from red to blue or purple upon interaction with melamine, was used for rapid screening. However, the colorimetric method presents false positive and inaccurate quantitative signals in the presence of interfering compounds. To overcome these limitations, the SERS method, which can directly utilize Au NPs from the colorimetric method, was employed as a rapid validation tool. In order to optimize the integration of these two methods, three sizes (15, 30, and 50 nm) of Au NPs were evaluated, and the 30 nm Au NPs were determined to be the best size for both colorimetric and SERS methods based on limits of detection (LODs) and quantification capability of melamine. By using the developed colorimetric–SERS method, we were able to rapidly screen and validate as low as 0.25 ppm melamine in milk within 20 min. Integrating colorimetric and SERS methods exploits the advantages of both methods, and provides a more rapid, accurate, and cost-effective way for monitoring melamine contamination in large amounts of food or feed products.

Introduction

Melamine (1,3,5-triazine-2,4,6-triamine) is an industrial compound with 66% nitrogen by mass. Since the scandal revealing its illegal adulteration in dairy products as a fake protein, various detection methods have been developed to meet the maximum residual level (MRL) of 2.5 ppm melamine in milk and 1 ppm in infant formula in the US.[1,2,3] Methods based on high performance liquid chromatography (HPLC),[4] gas chromatography (GC),[5] liquid chromatography-tandem mass spectrometry (LC-MS/MS),[6] and enzyme-linked immunosorbent assay (ELISA)[7] have been used as gold standards. However, these methods are limited to more sophisticated lab facilities as they require expensive equipment and extensive sample preparation.

A colorimetric method, which is based on Au NP aggregation upon the addition of melamine, has been developed to provide a much simpler and more straightforward way to detect melamine. Optical results can be realized without the use of a large instrument, which advances this technique for on-site application. The melamine molecule contains multiple amino ligands and can bind strongly to the surface of Au NPs. After interacting with trace amounts of melamine, Au NPs cross-link to these amino ligands, aggregate quickly, and show an obvious change in color from red to blue or purple due to the shift in the surface plasmon band to a longer wavelength. This phenomenon establishes the foundation for a simple, easy and rapid method for detecting melamine. Many different formats of this colorimetric method have been proposed, such as using 1-(2-mercaptoethyl)-1,3,5-triazinane-2,4,6-trione (MTT), thiol-functionalized cyanuric acid (CA) derivative stabilized Au NPs,[8] unmodified citrate-stabilized Au NPs,[9–11] aptamer-modified nanogold probes,[12] and bare gold nanoparticles prepared by the borohydride reduction method.[13]

Although colorimetric methods look promising for on-site measurements of melamine, they can be greatly influenced by the sample matrices, resulting in high rates of false positive and false negative data. Therefore, tedious sample preparation steps are usually needed to remove the matrix effect and achieve desirable sensitivity and accuracy. In real application, these methods are limited to screening and another method is required for validation and quantification of samples.

Surface enhanced Raman spectroscopy (SERS) has shown great feasibility in the identification and quantification of various analytes.[14–18] SERS utilizes noble metallic nanostructures, such as Au NPs, to enhance the Raman signals of analytes and to identify compounds based on their chemical signatures. SERS has been applied to detect and quantify

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melamine at ppb levels using a variety of substrates, such as SERS-active gold nanosubstrates,[19] Au NPs,[20] 4-mercaptopyridine-modified gold nanoparticles,[21] Ag-coated Fe_3O_4@SiO_2 microspheres,[22] Ag micro- and nanostructures created by the galvanic displacement of Cu,[23] silver nanorod arrays,[24] Ag nanoparticle coated poly(styrene-*co*-acrylic acid) nanospheres,[25] and so on. The main limitation of SERS when compared to the colorimetric method is that a Raman spectrometer is needed, which can be costly. In addition, the performance of SERS on melamine detection highly depends on the type of SERS substrate. The lack of a standardized SERS substrate greatly limits this method to be applied as a routine method for melamine detection. Therefore, SERS substrates that are commercially available and cost-effective, such as the citrate coated Au NPs, will be ideal for real application of SERS for melamine detection.

Herein, we aimed to integrate colorimetric and SERS methods based on the fact that both utilizes Au NPs. The colorimetric method will be applied first to screen for positive samples. Then, SERS will be applied to the same sample used in the colorimetric method for validation and quantification. A similar colorimetric and SERS dual-mode detection method was proposed for the telomerase activity.[26,27] For melamine detection, the integration of these two methods can serve as a practical approach to screen melamine in milk samples on-site, and to subsequently validate and quantify melamine in a lab with a bench-top Raman instrument, or more conveniently on-site with a portable Raman instrument.

To implement this integration, two key studies were performed. First, the size of Au NPs was optimized so that it would work for both colorimetric and SERS methods. The optimal size was determined based on the limit of detection (LOD) and the quantification capability of both the colorimetric and SERS methods. Second, a suitable sample pretreatment method was developed to cater to both methods. The sample pretreatment had to be simple and rapid, without the use of any bench-top equipment, in order to facilitate on-site measurements of milk products. To the best of our knowledge, this is the first study that attempts to integrate colorimetric and SERS methods for rapid screening and validation of melamine in milk. With this approach, melamine contamination in a large number of food and feed products can potentially be detected accurately, quickly, and efficiently.

Experimental

Materials and apparatus

Melamine (purity 99%, $C_3H_6N_6$, Acros Organics) and acetoguanamine (purity 98%, 6-methyl-1,3,5-triazine-2,4-diamine, Sigma-Aldrich) were used as received. Three kinds of Au NPs with diameters of 15 nm, 30 nm and 50 nm were purchased from Nanopartz™. The weight concentrations were all 50 ppm and were all citrate capped. 2% reduced fat raw milk (Big Y, MA, USA) was purchased from a local grocery store. Acetonitrile, sodium chloride, and syringe filters fitted with a Mixed Cellulose Ester (MCE) membrane (pore size 0.22 μm) were purchased from Fisher Scientific. All reagents were of analytical grade. Ultra-pure water (18.2 MΩ cm) was used in all experiments.

Absorption spectra were measured using a UV-Vis spectrophotometer (SpectraMax M2e from Molecular devices). All samples were added into 96 well plates, and the absorption ratio coefficient was measured using the wavelength range of 400–800 nm.

The Raman spectra were measured using a DXR Raman Spectro-microscope (Thermo Scientific) with the following parameters: a 50× confocal microscope objective (1 μm spot diameter, 4.7–8.7 cm^{-1} spectra resolution), 780 nm excitation wavelength, 5 mW laser power and 50 mm slit width for 2 s integration time. OMNIC™ software version 9.1 was used to control the Raman instrument. More than 10 spots were selected randomly for each sample within the spectral range of 400–3000 cm^{-1}.

A sonic dismembrator (Fisher scientific model 50), equipped with a tapered microtip was used to homogenize the samples. The maximum power was 50 watts. 20% amplitude was used.

Melamine detection assay

First, 2 mg of melamine was spiked into 4 ml of double distilled water to obtain a 500 ppm stock solution. The standard stock solution was sequentially diluted by water into a series of concentrations, typically 0.025 ppm, 0.05 ppm, 0.1 ppm, 0.25 ppm, 0.5 ppm, 1 ppm and 2.5 ppm. Double distilled water was used as the control (0 ppm).

Pure melamine, acetoguanamine and mixtures of melamine and acetoguanamine at 2 : 1, 1 : 1, and 1 : 2 concentration ratios at 0.25 ppm each were prepared using double distilled water.

Melamine was spiked into the 2% reduced fat raw milk, and then diluted to a series of different concentrations, typically 0.25 ppm, 2.5 ppm and 25 ppm. Milk with no melamine was used as the control.

170 μl of Au NP solution was premixed with varying concentrations of sodium chloride and incubated for 10 min to optimize its sensitivity. Then, 20 μl of target samples were added into Au NPs and incubated for an additional 5 min. 100 μl of the mixture was then transferred into a 96 well plate and the absorbance was measured at 520 nm and 640 nm. For Raman analysis, 5 μl of the mixture from the colorimetric method was deposited onto a gold slide to dry. The dried samples were then analyzed under the DXR Raman spectro-microscope. All colorimetric samples were tested in triplicates while SERS was tested with at least 5 spots per sample.

Melamine extraction from milk samples

Milk samples were deproteinized with acetonitrile at a ratio of 1 : 2 (milk–acetonitrile, v/v). After a quick vortex, the samples were sonicated for 3 min at 10 W. Then, the treated samples were spun down for 5 min with a portable mini centrifuge (Fisher Scientific). Finally, the supernatant was taken out and filtered with the MCE filter. The whole process took less than 10 min.

Statistical analysis

The collected SERS data were analyzed using TQ Analyst software (version 8.0) from Thermo Scientific. The SERS data gathered from different spots were averaged and then normalized using a Raman peak representing Au NPs located between 2000 and 2200 cm^{-1}. The Raman peaks representing melamine and acetoguanamine were both between 600 and 800 cm^{-1}, and therefore the statistical analysis was narrowed to that range. Second derivative Raman spectra were recorded and analyzed. The smoothing of spectra was achieved using a Norris derivative filter, with the segment length and gap between segments being 9 and 9, respectively.

Results and discussion

Size optimization of Au NPs for the integration of colorimetric and SERS methods

In order to integrate the colorimetric and SERS methods, the size of Au NPs was optimized. Three Au NP sizes were evaluated for this study; 15 nm, 30 nm, and 50 nm. These sizes were chosen because the highest SERS enhancement was previously reported to be between 50 and 80 nm (ref. 28) whereas typical colorimetric assays using Au NPs reported those to be less than 20 nm.[8–13] The limit of detection (LOD) and quantification capability of the colorimetric and SERS methods were compared using these three different Au NP sizes.

LOD and quantification capability of the colorimetric method using three different Au NP sizes

Several mechanistic variations of the colorimetric method have been reported previously.[8–13] In this study, a simple model was chosen whereby melamine interacted directly with Au NPs through its multiple amine groups, inducing a bridging effect/aggregation of Au NPs that changed its color from red to blue or purple. Citrate stabilized Au NPs were used in this study because they can be easily fabricated and are widely commercially available. In order to promote the ligand exchange between citrate and melamine at the surface of Au NPs, a predetermined amount of salt (sodium chloride) was mixed in before the addition of melamine. To determine the optimum NaCl concentration, a preliminary study was performed to find the highest concentration of NaCl at which no Au NP aggregation would occur without the addition of melamine. Different concentrations of NaCl were incubated with Au NPs for 10 min, and the absorbance at varying wavelengths was measured, as shown in Fig. S1.† The optimum NaCl concentration was 50 mM (final concentration), 30 mM and 30 mM for 15 nm, 30 nm and 50 nm Au NPs, respectively.

Next, different concentrations of melamine were incubated with the three Au NP sizes, and their visual images were captured (Fig. S2†). Visible color changes from red to purple were observed as the concentration of melamine increased; proving that melamine induced the aggregation of Au NPs. Sample measurements using a UV-vis spectrophotometer also showed a peak shift in the absorption spectrum to a longer wavelength. The evolution of UV-vis absorbance spectra of Au NP suspension with different concentrations of melamine is presented in Fig. S3–5.† The absorption ratio (A_{640}/A_{520}) was then calculated to quantify the change in color. As shown in Fig. 1, all three sizes of Au NPs showed an increase in the absorption ratio when melamine was added, signifying an increase in Au NP aggregation. This is interesting to note because the Au NP sizes tested were relatively larger than those typically used for colorimetric measurements in most studies, but nevertheless, yielded promising results. Our results showed that the limit of detection (LOD) for 15 nm, 30 nm and 50 nm Au NPs was 0.5 ppm, 0.25 ppm, and 0.25 ppm, respectively. Furthermore, a broader concentration dependent trend was observed with the 30 nm Au NPs (*i.e.* 0.1 to 1 ppm melamine) when compared to the 50 nm Au NPs (*i.e.* 0.1 to 0.5 ppm melamine). These results suggests that 30 nm Au NPs have the best characteristics for a colorimetric detection assay in terms of sensitivity (*i.e.* LOD) and quantification capability (*i.e.* concentration dependent range).

LOD and quantification capability of the SERS method using three different Au NP sizes

After performing the colorimetric assay, a small volume (5 μL) of the same sample was deposited onto a gold coated slide, quickly dried and analyzed under a Raman microscope. Raw SERS spectra are shown in Fig. S6.† The Raman peak intensity ratio of melamine (around 710 cm^{-1}) and Au NPs (around 2100 cm^{-1}) was calculated to determine the LOD and quantification capability of the SERS method. The peak intensity ratios of melamine and Au NPs were used instead of using the melamine peak alone in order to minimize the variations caused by different aggregation states of Au NPs.

As shown in Fig. 2, all three Au NPs sizes had an LOD of 0.025 ppm melamine and exhibited a linear trend over a wide concentration range (0–2.5 ppm), demonstrating the superior

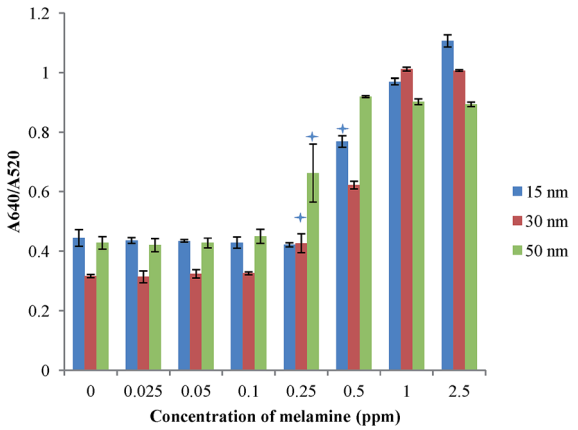

Fig. 1 The absorption ratio (A_{640}/A_{520}) *versus* different concentrations of melamine in water (0 ppm, 0.025 ppm, 0.05 ppm, 0.1 ppm, 0.25 ppm, 0.5 ppm, 1 ppm and 2.5 ppm) when the diameters of Au NPs were 15 nm, 30 nm and 50 nm, respectively. The limit of detection (LOD) of melamine for 15 nm, 30 nm and 50 nm Au NPs was 0.5 ppm, 0.25 ppm, and 0.25 ppm, respectively, as indicated by a blue star on the graph (based on the *T*-test).

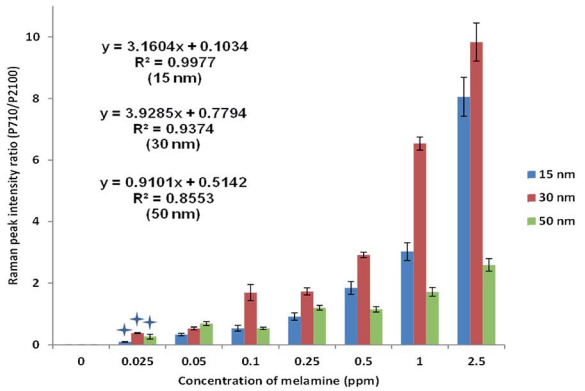

Fig. 2 The SERS peak intensity ratio of the melamine peak to the Au NP peak (P710/P2100) *versus* different concentrations of melamine in water (0 ppm, 0.025 ppm, 0.05 ppm, 0.1 ppm, 0.25 ppm, 0.5 ppm, 1 ppm and 2.5 ppm) when the diameters of Au NPs were 15 nm, 30 nm and 50 nm, respectively. The limits of detection (LODs) for 15 nm, 30 nm and 50 nm Au NPs were all 0.025 ppm, as indicated by a blue star (based on the *T*-test).

sensitivity and quantification capability of the SERS method over the colorimetric method. The 15 nm Au NPs provided the best linearity ($R^2 = 0.9977$), followed by the 30 nm Au NPs ($R^2 = 0.9375$) and the 50 nm Au NPs ($R^2 = 0.8553$). In terms of relative enhancement (*i.e.* Raman peak intensity ratio), the 30 nm Au NPs performed better than the 15 nm Au NPs. Based on the results obtained from the colorimetric and SERS methods, the 30 nm Au NPs were determined to be the best size for the integration of these two methods.

Detection of melamine in the presence of an interfering compound using the colorimetric–SERS method

Colorimetric methods employing the aggregation of Au NPs for color change has been shown to be simple, straightforward and quick. However, when interfering compounds (*i.e.* compounds that can interact with Au NPs) are present, false positive signals and inaccurate quantification results become inevitable. In order to demonstrate this concept, the absorption ratios (A_{640}/A_{520}) of melamine and acetoguanamine, two similar looking amino compounds, at different concentration ratios were tested. The rational to test acetoguanamine as the interfering compound is based on the study by Chi *et al.* (2010) that acetoguanamine was the most interfering compound for melamine in the colorimetric assay.[9] Fig. 3A shows the absorption ratios of three samples: 0.5 ppm melamine (M), 2.5 ppm mixture of melamine (M) and acetoguanamine (A) with a 1 : 4 concentration ratio, and 2.5 ppm acetoguanamine (A). All three samples exhibited varying degrees of Au NP aggregation as shown by their absorbance ratio (A_{640}/A_{520}), demonstrating that the colorimetric method alone could not provide accurate information about what was being added. These results suggest that the colorimetric method can merely be treated as a rapid screening method, and would require an additional validation method in order to accurately determine and quantify the presence of melamine. To do this, SERS measurements were

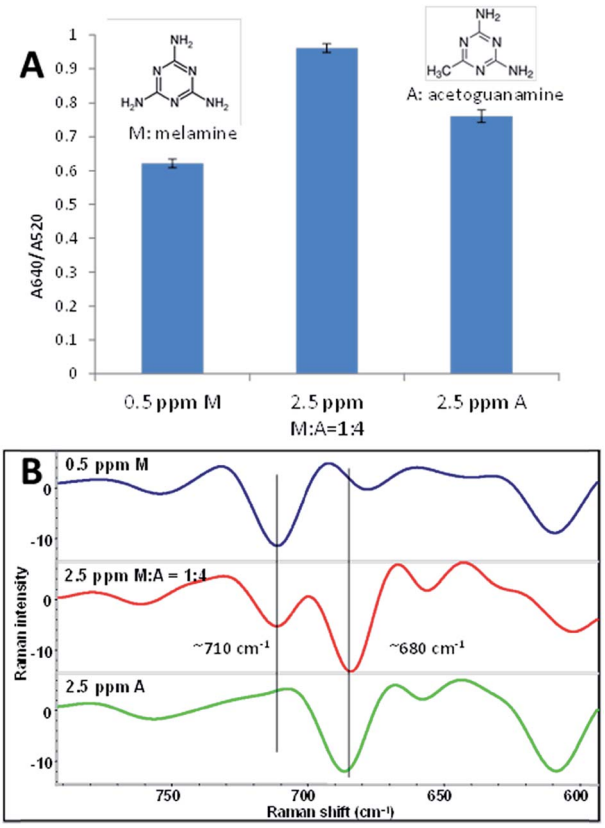

Fig. 3 (A) The absorption ratio (A_{640}/A_{520}) of three samples: 0.5 ppm melamine (M), 2.5 ppm mixture of melamine (M) and acetoguanamine (A) with a 1 : 4 concentration ratio, 2.5 ppm acetoguanamine (A); (B) The second derivative SERS spectra of three samples: 0.5 ppm melamine (M), 2.5 ppm mixture of melamine (M) and acetoguanamine (A) with a 1 : 4 concentration ratio, 2.5 ppm acetoguanamine (A). The SERS peak of melamine is around 710 cm^{-1}, whereas the SERS peak of acetoguanamine is around 680 cm^{-1}.

performed immediately on the same Au NP samples that were used in the colorimetric method.

Fig. 3B shows the SERS spectra of the three samples reported in Fig. 3A. A clear differentiation is visible between these three SERS spectra. In particular, the melamine peak at 710 cm^{-1} is clearly separated from the acetoguanamine peak, which is situated at 686 cm^{-1}. Therefore, unlike colorimetric methods, SERS can easily discriminate different amino compounds, and thus provide more accurate information. It is worthy to note, however, that a smaller melamine peak is present at 678 cm^{-1}, which may have caused the acetoguanamine peak to shift from 686 cm^{-1} to 684 cm^{-1}. Nevertheless, this slight overlap does not limit the capability of the SERS method to differentiate melamine from acetoguanamine.

Since SERS validates the existence of specific compounds based on its chemical signature, it has the potential to quantify melamine in the presence of interfering compounds. In order to demonstrate this, five samples containing 0.25 ppm of total compounds (*i.e.* pure melamine, pure acetoguanamine, and mixtures of melamine and acetoguanamine with 1 : 2, 1 : 1 and 2 : 1 concentration ratios) were tested. Their second derivative

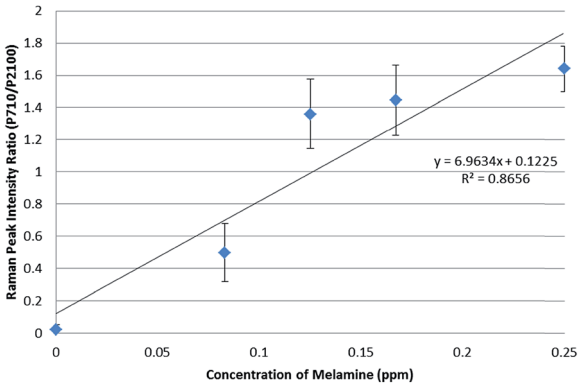

Fig. 4 A concentration standard curve showing the effect of melamine (M) concentration on the Raman peak intensity ratio (P710/P2100) in the presence of acetoguanamine (A) [M + A = 0.25 ppm for all samples].

SERS spectra are shown in Fig. S7.† Fig. 4 shows the effect of melamine concentration on Raman peak intensity ratios (*i.e.* P710/P2100 for melamine) in the presence of acetoguanamine. A fairly decent concentration dependent peak intensity trend was observed for melamine even at low concentrations (up to 0.25 ppm), which demonstrated the quantitative capability of SERS for melamine in the presence of an interfering compound ($R^2 = 0.8656$). The relatively large error bars may be due to the low concentrations and narrow concentration range tested.

Detection and validation of melamine in milk samples using the colorimetric–SERS method

Since many ingredients in milk, including proteins, lactose, lipids, vitamins and minerals, would interfere with the aggregation of Au NPs, a pretreatment of milk samples was necessary. The extraction of melamine from a complex food matrix is not easy. Some studies used complicated and time consuming solid phase extraction (SPE) purification.[8,9] Others mixed milk with trichloroacetic acid or acetic acid to remove proteins and lipids, and then adjusted the pH of the supernatant with NaOH.[11,12] Initially, the trichloroacetic acid method was attempted.

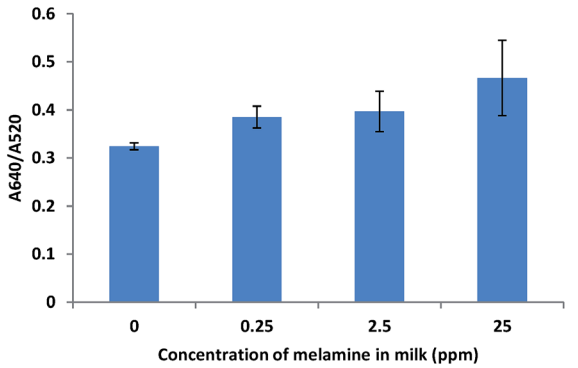

Fig. 5 The absorption ratio (A_{640}/A_{520}) of milk samples containing different concentrations of melamine (0 ppm, 0.25 ppm, 2.5 ppm and 25 ppm).

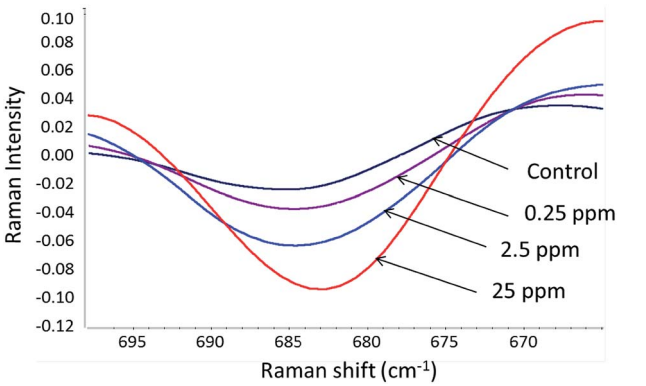

Fig. 6 The second derivative SERS spectra of milk samples containing different concentrations of melamine (0 ppm, 0.25 ppm, 2.5 ppm and 25 ppm).

Unfortunately, the colorimetric results were not very reliable as the pH greatly influenced Au NP aggregation, and subsequently, the amount of added NaOH varied considerably due to the heterogeneity of milk. Eventually, an extraction procedure was standardized, as provided under the Experimental section. This simple and rapid pretreatment procedure was developed to quickly screen samples by the colorimetric method. To achieve this, the type of filters used and the volume ratio of milk to acetonitrile were optimized. The whole process took less than 10 min.

Melamine was spiked in milk at four different levels, 0 ppm, 0.25 ppm, 2.5 ppm and 25 ppm. Then, the pretreatment step and colorimetric method were performed. As shown in Fig. 5, a statistically significant difference was observed between the control (0 ppm) and 0.25 ppm melamine in milk. This concentration is significantly lower than the MRL of 2.5 ppm established in the US. However, the sensitivity and quantitative capability of this method was not as good as other procedures that required tedious separation steps and/or large equipment. In spite of this limitation, the simplicity of our pretreatment and screening procedure makes it ideal for rapid on-site applications, especially when it is integrated with SERS.

Positive results, as determined by the colorimetric assay, can be validated directly by SERS with minimal preparation. Fig. 6 shows the Raman spectra of melamine spiked in milk at four different levels, 0 ppm, 0.25 ppm and 2.5 ppm and 25 ppm. The melamine peak was at 683 cm^{-1} in milk compared to 710 cm^{-1} in water. This may be due to the interaction between melamine and proteins, which shifted the melamine peak. This result, however, revealed the importance to determine the matrix effect when applying the SERS method. When different matrices are tested, the shift of target peaks need to be pre-determined using standards. By observing the melamine peak, we can validate the presence of melamine in milk.

Conclusion

In conclusion, this is the first report of a colorimetric–SERS method that is able to detect trace amounts of melamine in milk within a short time (*i.e.* 20 min). The colorimetric method,

by itself, can be used for rapid screening of large amounts of samples. SERS can be performed immediately to validate the results obtained from the colorimetric method. The integration of these two methods leverages the advantages of both techniques, providing a rapid and accurate on-site method for (1) quality control of incoming ingredients and (2) on-site safety evaluation and monitoring. More studies are needed to further improve the extraction procedure for milk.

Author contributions

The manuscript was written through contributions from all authors./All authors have given approval to the final version of the manuscript.

References

1 FAO – News Article: International experts limit Melamine levels in food http://www.fao.org/news/story/en/item/43719/icode/, accessed 2 Oct 2014.
2 Codex alimentarius commission, Geneva, Switzerland, http://www.codexalimentarius.org/input/download/report/744/al33REPe.pdf, accessed 14 Feb 2014.
3 Y. Liu, E. E. D. Todd, Q. Zhang, J. Shi and X. Liu, *J. Zhejiang Univ., Sci., B*, 2012, **13**, 525–532.
4 X.-T. Peng, Z.-G. Shi and Y.-Q. Feng, *Food Anal. Method.*, 2010, **4**, 381–388.
5 B. Jurado-Sanchez, E. Ballesteros and M. Gallego, *J. Agric. Food Chem.*, 2011, **59**, 7519–7526.
6 M. Ibáñez, J. V. Sancho and F. Hernández, *Anal. Chim. Acta*, 2009, **649**, 91–97.
7 E. A. E. Garber, *J. Food Prot.*, 2008, **71**, 590–594.
8 K. Ai, Y. Liu and L. Lu, *J. Am. Chem. Soc.*, 2009, **131**, 9496–9497.
9 H. Chi, B. Liu, G. Guan, Z. Zhang and M.-Y. Han, *Analyst*, 2010, **135**, 1070–1075.
10 H. Xing, Y. Wu, S. Zhan and P. Zhou, *Food Anal. Method.*, 2013, **6**, 1441–1447.
11 L. Li, B. Li, D. Cheng and L. Mao, *Food Chem.*, 2010, **122**, 895–900.
12 H. Xing, S. Zhan, Y. Wu, L. He and P. Zhou, *RSC Adv.*, 2013, **3**, 17424.
13 W. Chen, H.-H. Deng, L. Hong, Z.-Q. Wu, S. Wang, A.-L. Liu, X.-H. Lin and X.-H. Xia, *Analyst*, 2012, **137**, 5382–5386.
14 G. Han, R. Liu, M.-Y. Han, C. Jiang, J. Wang, S. Du, B. Liu and Z. Zhang, *Anal. Chem.*, 2014, **86**, 11503–11507.
15 W. Xie and S. Schlücker, *Phys. Chem. Chem. Phys.*, 2013, **15**, 5329–5344.
16 M. M. Harper, K. S. McKeating and K. Faulds, *Phys. Chem. Chem. Phys.*, 2013, **15**, 5312–5328.
17 Y. Chen, Z.-P. Chen, S.-Y. Long and R. Yu, *Anal. Chem.*, 2014, **86**, 12236–12242.
18 S. Pang, T. P. Labuza and L. He, *Analyst*, 2014, **139**, 1895–1901.
19 L. He, Y. Liu, M. Lin, J. Awika, D. R. Ledoux, H. Li and A. Mustapha, *Sens. Instrum. Food Qual. Saf.*, 2008, **2**, 66–71.
20 Z.-Q. Wen, G. Li and D. Ren, *Appl. Spectrosc.*, 2011, **65**, 514–521.
21 T. Lou, Y. Wang, J. Li, H. Peng, H. Xiong and L. Chen, *Anal. Bioanal. Chem.*, 2011, **401**, 333–338.
22 H. Hu, Z. Wang, L. Pan, S. Zhao and S. Zhu, *J. Phys. Chem. C*, 2010, **114**, 7738–7742.
23 J. F. Betz, Y. Cheng and G. W. Rubloff, *Analyst*, 2012, **137**, 826–828.
24 X. Du, H. Chu, Y. Huang and Y. Zhao, *Appl. Spectrosc.*, 2010, **64**, 781–785.
25 J.-M. Li, W.-F. Ma, C. Wei, L.-J. You, J. Guo, J. Hu and C.-C. Wang, *Langmuir*, 2011, **27**, 14539–14544.
26 S. Zong, Z. Wang, H. Chen and Y. Cui, *Small*, 2013, **9**, 4215–4220.
27 S. Zong, Z. Wang, H. Chen, G. Hu, M. Liu, P. Chen and Y. Cui, *Nanoscale*, 2014, **6**, 1808–1816.
28 S. E. J. Bell and M. R. McCourt, *Phys. Chem. Chem. Phys.*, 2009, **11**, 7455–7462.