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One-step detection of melamine in milk by hollow gold chip based on surface-enhanced Raman scattering

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ABSTRACT

A hollow gold (HG) chip with high surface-enhanced Raman scattering (SERS) capability was fabricated and used to monitor the adulteration of milk with melamine. This chip was fabricated with self-assembled hollow gold nanospheres (HGNs) on glass wafers through electrostatic interaction. There are two important advantages for the use of this HG chip as a detection platform. First, HGNs show a strong SERS enhancement from individual particles due to their capability to localize the electromagnetic fields around the pinholes in hollow shells. Second, the HG chip improves the limit of detection through the enrichment effect. The characteristic SERS peak of melamine was used to distinguish it from other kinds of proteins or amino acids, and its intensity was used to monitor the percentage of melamine in milk. With its simple detection procedure (no pretreatment or separation steps), decreased processing time and low detection limit, this HG chip shows a strong potential for broad applications in melamine detection from real samples.

1. Introduction

Melamine (1, 3, 5-triazine-2, 4, 6-triamine) is an organic chemical used for the fabrication of melamine resins. In recent years, this molecule has been illegally added to milk products to produce high protein content readings. This is because the nitrogen content of melamine can reach 66% and the conventional Kjeldahl or Dumas test for total protein content in milk products cannot distinguish melamine from other proteins [1]. However, illegal adulteration of pet and dairy food products with melamine can induce the formation of kidney stones and renal failure in humans. It has even been closely related to deaths of pets in the United States [2,3]. Several analytical methods, including enzyme-linked immunosorbent assay (ELISA) [4], mass spectrometry [5,6], liquid chromatography [7,8], surface-enhanced Raman spectrometry [9–14] and colorimetric methods [15,16] have been employed for the accurate detection of melamine in food. However, some of the problems related to these analytical methods, such as a long sample pretreatment time, complicate separation process, poor limit of detection, length measurement time and indirect measurements, made these detection methods less attractive. In particular, only a few detection methods satisfy with the safety limits of 2.5 ppm in the United States and European Union and 1 ppm in China for infant formula [16]. Thus, a simple, fast and highly sensitive analytical method for on-site detection of melamine from real milk samples is still needed.

Recently, surface-enhanced Raman scattering (SERS)-based detection technique has been considered as a promising alternative for sensitive trace analysis of melamine in food. SERS is a process whereby the Raman signal is enhanced when molecules are confined within the range of electromagnetic fields generated upon excitation of the localized surface plasmon resonance (LSPR) of noble nanostructured metal surfaces [17,18]. The enhanced capability of SERS can reach 14 or 15 orders of magnitude in some special cases, which ensures that SERS can be applied for ultra-sensitive trace analysis down to the single-molecule level [19,20]. In addition, Raman bands are much narrower than fluorescence emission bands and each entity has its own characteristic Raman peaks [21,22]. Thus, the SERS technique can be applied for the detection of a specific target molecule in a mixed system (such as food or milk) if sample molecules are well adsorbed on SERS substrates.

In SERS applications for trace analysis, one important issue is the choice of a suitable SERS substrate. Up to date, plenty of SERS substrates, such as gold or silver colloids [23,24], core–shell nanoparticles [25,26], noble metal electrode [27] and patented arrays with nanostructures [28,29] have been developed. SERS

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substrates with strong enhancement capabilities should have the ability to create lots of "hotspots", which would be generated at the interstitial sites between two particles, or at locations outside sharp surface protrusions [24,30,31]. When molecules are located at these hotspot sites, their Raman signals would be greatly enhanced due to the strong local electromagnetic environment. However, it is difficult to precisely control the uniform distribution of hot spots in many cases. In quantitative analysis of target molecules using SERS, however, a linear relationship between the amount of target molecules and Raman signal intensity cannot be obtained without a precise control of these hotspots.

To resolve this problem, we fabricated a hollow gold (HG) chip and used it as a SERS substrate to monitor the adulteration of milk with melamine. This chip was fabricated using self-assembled hollow gold nanospheres (HGNs) on a glass wafer through electrostatic interaction. There are two important reasons for the use of this HG chip as a detection platform. One is that HGNs display a strong SERS enhancement from individual particles because of their capability to localize the electromagnetic fields through the pinholes in hollow shells. Accordingly, they can be used as reproducible sensing probes for the quantitative analysis of a target analyte. The other is that the HG chip substrate is effective for getting a low detection limit through the enrichment effect.

In the present work, the quantitative analysis of melamine adulteration in milk was performed using this HG chip. The characteristic SERS peaks of melamine were used to distinguish it from other kinds of proteins or amino acids in milk, and its intensity was used to monitor the percentage of melamine in milk. The detection limit of melamine using this chip could reach as low as 1 ppm, which meets the safety limit of infant formula in China. In addition, no separation or extraction step is needed. Our experimental results suggest that this HG chip is a promising on-site analytical tool for monitoring the adulteration of milk with melamine.

2. Experimental

2.1. Materials and reagents

Melamine (1, 3, 5-triazine-2, 4, 6-triamine) (99%), gold (III) chloride trihydrate (> 99.9%), sodium citrate dehydrate (99%), sodium borohydride, cobalt chloride hexahydrate, poly (diallyldimethylammonium chloride) (PDDA) (Mw = 200,000–350,000, 20 wt% aqueous solution) were purchased from Sigma (St. Louis. MO, USA), and used without further purification. Fresh milk was purchased from a local supermarket. All aqueous solutions were prepared using deionized water (DIW, 18 MΩ) obtained from a Milli-Q system (Millipore S.A., Bedford, USA).

2.2. Preparation of HGNs and gold nanoparticles (GNPs)

According to Schwartzberg's method [32], HGNs were prepared with minor modifications. A three-neck flask was filled with 50 mL of water, 500 μL of sodium citrate solution (0.1 M) and 100 μL of cobalt chloride solution (0.4 M). This solution was deoxygenated by ultrapure N_2 for 1 h. Then, 150 μL of freshly prepared sodium borohydride solution (1 M) was added during rapid magnetic stirring. This resulting solution was allowed to react for 45 min under constant N_2 flow. Then, the gold precursor solution (0.1 M) was added via 10 additions of 50 μL aliquots. Upon completion of the gold addition, N_2 flow was stopped and the solution was exposed to ambient conditions to oxidize any remaining cobalt metal. At the end of the reaction, the color of the solution changed to deep purple. GNPs were synthesized by the reduction of $HAuCl_4$ by sodium citrate [33]; 100 mL of 0.01% $HAuCl_4$ was heated to reflux with stirring, and then 2 mL of 1% sodium citrate was rapidly added. After that, the solution was kept boiling for another 30 min. Then, the mixed solution was cooled down to room temperature and a wine red solution of 30 nm GNPs was obtained.

2.3. Fabrication of self-assembled HGNs and GNPs on glass wafers

Glass wafers (1 cm in length and 1 cm in width) with a hydroxyl surface were immersed in 0.5% poly (diallyldimethylammonium chloride) (PDDA) solution. One hour later, these wafers were taken out and exhaustively rinsed with water and dried with nitrogen gas. The PDDA-coated glass wafers were soaked in a gold colloid (HGNs or GNPs) for 6 h to let the nanoparticles in the solution self-assemble on the glass. Then, these glass wafers with self-assembled gold nanoparticles were rinsed with water and dried with nitrogen gas. The SEM image and the digital picture of the HG chip were shown in Fig. S1.

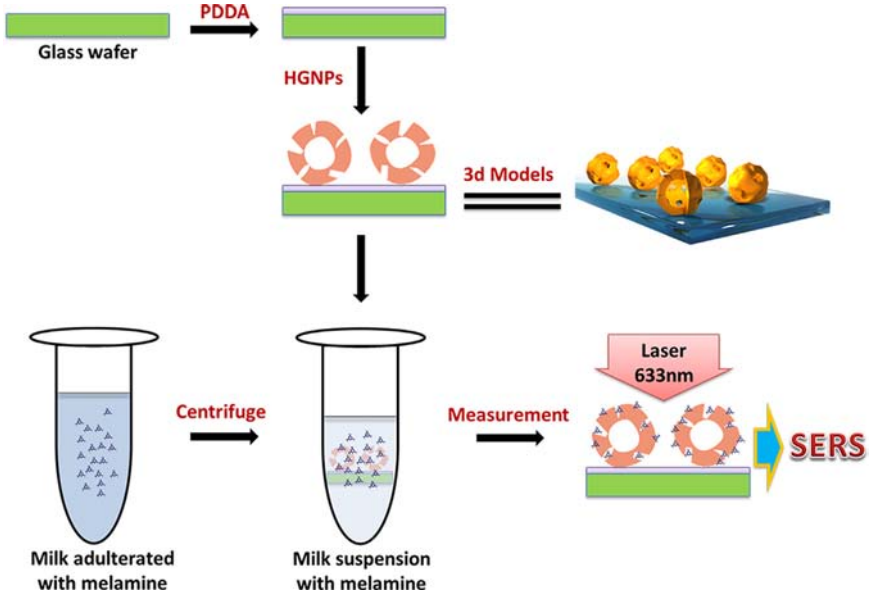

Fig. 1. Procedures of HG chip fabrication and detection procedures of melamine from real milk sample by HG chip.

2.4. Detection of melamine from aqueous solution and milk

Self-assembled HGNs and GNPs were immersed in an aqueous melamine solution (10^{-4}–10^{-9} M) or milk with different percentages (100–0 ppm) of melamine for 2 min. Then, these chips (as-treated self-assembled HGNs and GNPs) were rinsed with water and dried with nitrogen gas. Then SERS signals were measured. The HG chip fabrication procedures and melamine detection procedures from milk samples are depicted in Fig. 1.

2.5. SERS measurement

All normal Raman and SERS spectra were measured by a Jobin Yvon/HORIBA LabRam ARAMIS Raman spectrometer equipped with a HeNe laser (633 nm). The typical exposure time for each measurement in this study was 10 s with one time accumulation. Transmission electron microscopy (TEM) images of hollow gold nanoparticles and gold nanospheres were obtained by a JEM-2100 F microscope operated at 200 kV.

3. Results and discussion

In order to find the best analytical method for melamine, SERS spectra of melamine adsorbed on the traditional SERS substrates, gold colloids (Au NPs) and self-assembled gold nanoparticles on glass wafer (Au chip) were measured. In Fig. 2, two characteristic Raman peaks at 712 cm^{-1} and 985 cm^{-1} were assigned to the in-plane deformation and in-plane ring breathing modes of the triazine ring, respectively. These are characteristic Raman peaks of melamine, and they were not observed in other proteins or amino acids [14]. As shown in this figure, the SERS intensity of melamine for Au NPs (red) is much weaker than that measured for Au NPs on the chip (black). The enrichment effect in the Au chip contributes to these results. The total number of gold nanoparticles on a chip is considerably less than that in 1 ml of gold colloids. When we put the same amount of melamine on each substrate, the density distribution of melamine molecules for two substrates is different. Due to the high average amount of melamine on one gold nanoparticle on the Au chip, the SERS intensity on the Au chip was much stronger than that for Au NPs in solution. The SERS spectra of melamine were measured for both the Au NPs and the Au chip but stronger SERS peaks were observed for the Au chip.

When the SERS technique is applied for quantitative analysis, two factors should be carefully considered. One is how to create lots of so-called “hotspots” and the other is how to distribute them regularly throughout the whole substrate. In order to address these problems, the HG chip using the self-assembly of HGNs on a glass wafer was designed and fabricated. A transmission electron microscopy (TEM) image of HGNs is shown in Fig. S2B. According to our measurements, the diameter of the HGNs and the wall thickness were estimated to be 45 ± 5 nm and 15 ± 3 nm, respectively. It can be also seen from Fig. S2B, that there are some pinholes in the hollow shells of HGNs.

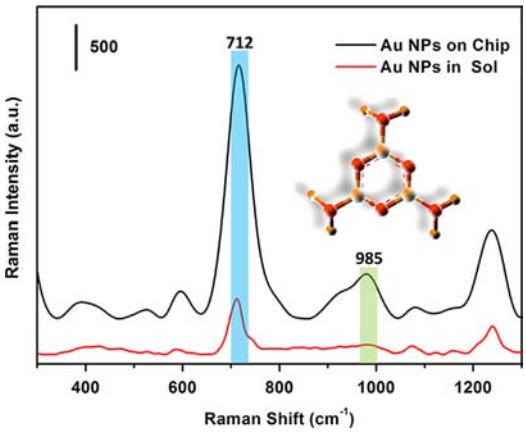

Fig. 2. SERS spectra of 10^{-5} mol/L melamine using Au NPs in sol (red line) and Au NPs on a chip (black line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Finite difference time domain (FDTD) calculations were performed to evaluate the local electric fields at the excitation wavelength of 633 nm for Au NPs and HGNs. A realistic dielectric function for Au was used in these calculations [34,35]. Electric field distributions for Au NPs and HGNs are shown in Fig. S3. The amplitudes of the electric fields were normalized relative to the amplitude of the input plane wave exciting the nanostructure. Electric field intensity around the pinholes on HGNs was very strong, and this fact suggests that HGNs displays a strong SERS enhancement from individual particles without any particle aggregation. HG chip was prepared using the self-assembled property of these HGNs on glass substrates. Due to the hotspots generated from individual HGNs and the enrichment effect of the chip substrate, the HG chip could be used as a highly sensitive and reproducible SERS substrate. Fig. 3 displays the SERS spectra of melamine for the substrates using Au NPs and HGNs. Here the HGNs show better detection sensitivity than Au NPs for melamine detection. In Fig. 4, the intensity of the characteristic peak of melamine at 712 cm^{-1} was plotted as a function of the melamine concentration for both the HG chip and Au chip. From the histogram, we can see that when using an HG chip as a SERS substrate, once the melamine concentration is higher than 10^{-6} mol/L, the intensity of the peak at 712 cm^{-1} is almost the same. When the concentration is below 10^{-6} mol/L, the intensity of the peak at 712 cm^{-1} weakens as the concentration decreases. Thus, the final detection limit for melamine with the HG chip is at least one order of magnitude lower than that of the Au chip.

After the limit of detection of melamine in water was investigated on HG chips, we analyzed melamine in real milk samples. The HG chip fabrication procedures and melamine detection procedures from milk samples are depicted in Fig. 1. First, certain amounts of melamine were added to fresh milk purchased from a local supermarket to create samples with different melamine percentages. Then, milk samples mixed with melamine were centrifuged at 5000 rpm for 20 min and the supernatant liquor gained after centrifugation was used in the following experiment. SERS signals for melamine could not be observed from real milk samples without the centrifugation step because many milk components, such as proteins and fats, prevent melamine from adsorbing onto the surface of HGNs. During this centrifugation step, all bio-macromolecules in the milk were separated out and the small molecules, such as amino acids, are left in the milk supernatant. Each melamine has six nitrogen atoms, each of which can interact with the gold surface through its lone pair electrons. Among all twenty amino acids, only arginine has four nitrogen atoms. Therefore, the binding affinity of melamine towards HGNs is much stronger than that of amino acids and other kinds of molecules in the milk supernatant. Finally, the HG chip was immersed in supernatant liquor for 10 min and then after it was washed with water and dried with N_2, the SERS spectrum was taken. Fig. 5A shows the percentage-dependent SERS spectra of melamine in fresh milk on the HG chip. The intensity of the characteristic melamine peak at 712 cm^{-1} gradually decreased as the percentage of melamine in milk decreased. The corresponding calibration curves obtained by SERS signal intensity and the percentage of melamine in milk based on HG chip are shown in Fig. 6, where each data point represents the average intensity at 712 cm^{-1} from five measurements. A good linear relationship was shown at the given percentage range, which indicates that a highly

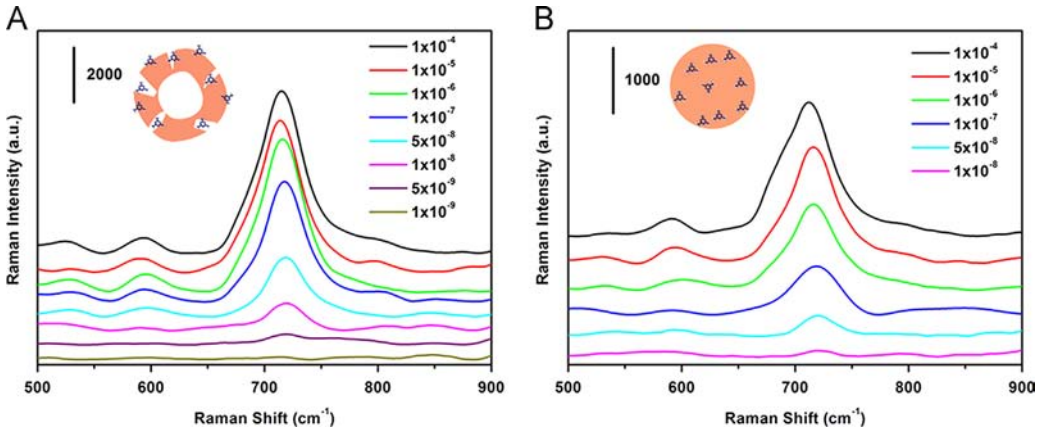

Fig. 3. (A) SERS spectra of melamine adsorbed on a HG chip. The concentrations of melamine solution were 1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7}, 5×10^{-8}, 1×10^{-8}, 5×10^{-9} and 1×10^{-9} mol/L from top to bottom. (B) SERS spectra of melamine adsorbed on an Au chip. The concentrations of melamine solution used were 1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7}, 5×10^{-8} and 1×10^{-8} mol/L from top to bottom.

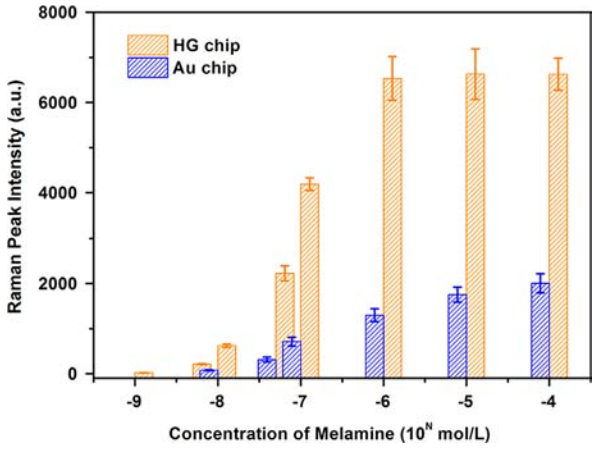

Fig. 4. SERS intensity of melamine at 712 cm^{-1} with concentrations determined using the HG chip (blue) and Au chip (orange). The error bars indicate standard deviations from five measurements for each sample. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

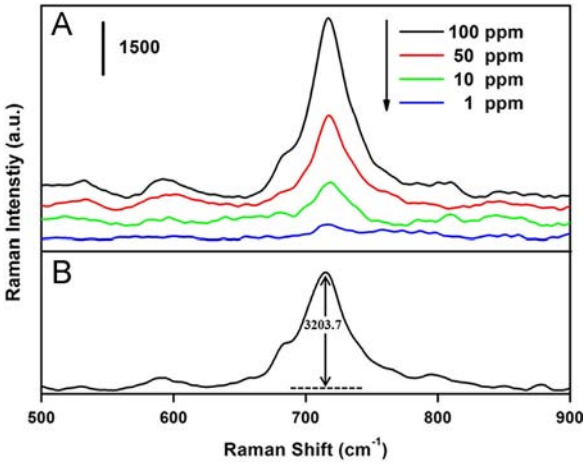

Fig. 5. (A) SERS spectra of melamine from real milk samples adsorbed on hollow gold chip. The percentages of melamine in milk were 100 ppm, 50 ppm, 10 ppm, 1 ppm and 0 ppm.

accurate quantitative evaluation of melamine can be achieved using this HG chip. The standard deviation of five measurements shown by the error bars is also depicted in Fig. 6. The detection

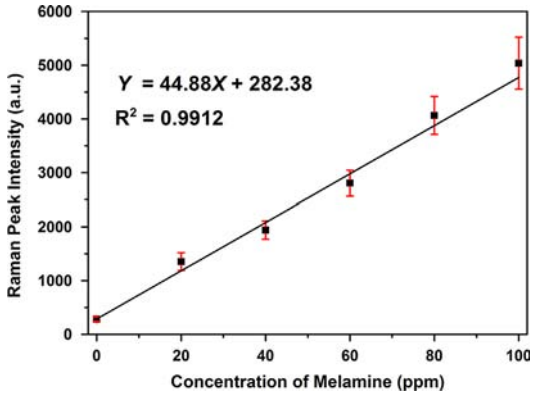

Fig. 6. The corresponding calibration curves obtained by SERS signal intensity and the percentage of melamine in a real milk sample based on hollow gold chip.

limit can reach as low as 1 ppm, which can meet the FDA's limits on melamine in infant formula.

In order to test the feasibility of this HG chip for melamine detection, we used one milk sample that was adulterated with 67 ppm melamine as the unknown sample. The HG chip was applied to detect the amount of melamine in milk. Based on the linear equation "$I_{SERS}=44.88C_{melamine}+282.38$" and intensity of peak 712 cm^{-1} from the unknown sample (Fig. 5B), we concluded that the deviation of melamine detection by this chip was 2.85%.

4. Conclusion

A one-step detection method of melamine in milk using SERS-based HG chip was developed. The HG chip was fabricated using the self-assembly properties of HGNs on glass substrates through electrostatic interactions. Based on the high SERS performance HG chip, a detection limit of 1 ppm for melamine in milk could be achieved without any sample pretreatment. The lack of pretreatment ensures that the whole detection procedure can be completed within 1 h. Here, the peak positions of the characteristic SERS peaks of melamine were used to distinguish it from other kinds of proteins or amino acids, and its intensity was used to monitor the percentage of melamine in milk. The fast detection procedures, low detection limit, no sample pretreatment make this SERS-based detection technique a promising method for monitoring the adulteration of melamine in milk under any circumstances. The HG chip is also expected to be used as a SERS substrate for the trace analysis of other toxic molecules.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.01.043.

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