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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.

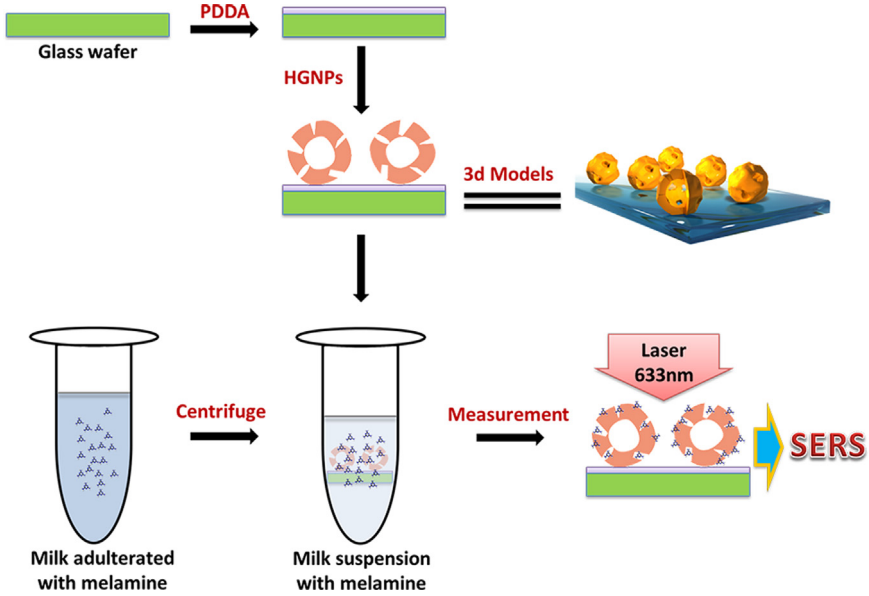

Fig. 1. Procedures of HG chip fabrication and detection procedures of melamine from real milk sample by HG chip.