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Silver deposited polystyrene (PS) microspheres for surface-enhanced Raman spectroscopic-encoding and rapid label-free detection of melamine in milk powder

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ABSTRACT

Silver nanoparticles coated amino modified polystyrene microspheres (PS-NH_2/Ag NPs) with extremely high surface enhanced Raman scattering (SERS) activity and uniform surface morphology were created by precise controlling of deposition time. Nanojets that were formed underneath the individual microspheres could be used for nondestructive analysis of species adsorbed on the smooth gold or glass surface. A 10 fold enhancement of SERS was observed between PS-NH_2/Ag NPs and gold surface compared to glass surface due to more effective coupling of surface plasmonic resonance. These microsphere SERS substrates could detect 2-Mercaptopyridine down to 10^{-9}M. Four different thiol compounds have been successfully utilized as tags to prepare SERS encoded PS-NH_2/Ag NPs microspheres. Furthermore, the potential application of these SERS substrate for rapid detection of melamine in milk powder was explored. A linear relationship was observed between SERS intensity and logarithm of melamine concentrations with the limit of detection (LOD) of 1.9×10^{-8} mol/L. The promising advantages of easy sample pretreatment, low protein background interference, short detection time and low cost makes the PS-NH_2/Ag NPs substrate a potential detection tool in the field of food safety.

1. Introduction

Noble metal surface with ideal roughness can dramatically enhance the Raman scattering of analytes adsorbed on the surface [1–3]. Some novel substrate with enhancement factor of more than 10^{10} at so called "hot spot" allows the detection of analytes even at single-molecule level [4–7]. Due to its high sensitivity and finger-printing capability, Surface-enhanced Raman scattering (SERS) spectroscopy opens some new opportunities for biological sensing and trace analysis.

Over the past decade, many attempts have been made to produce favorable nanostructure substrates with high SERS activity, among which metal nanoparticle coated microspheres are popular [8–10]. These nanoparticle coated microspheres have uniform micrometer size while containing well defined nano structures on the surface. Polymeric microspheres provide excellent supports for producing the nanostructural SERS active surfaces. Nanosphere lithography is one of the most powerful techniques that use spin-coated microspheres layers as template for silver or gold deposition to produce substrate with high SERS activity, however the thermal evaporation needs special equipment [11,12]. Another widely used method to create microspheres are the in-situ (nanoparticles grown inside polymer microsphere) [13,14] or ex-situ (nanoparticles assembled or formed on the microsphere surface) [15,16] methods in order to prepare metal nanoparticles coated mono-dispersed microspheres. These metal coated microspheres are more favorable to homogenous SERS analysis in aqueous solution and the synthetic process does not require expensive instruments unlike the lithography. Thus, the synthesis and application of metal nanoparticles coated microsphere has drawn much attention in recent years.

Some researchers have reported the possibility of in-situ reducing gold or silver nanoparticles inside different types of microspheres, such as PEG grafted polystyrene(PS) [17], thermosensitive Poly(NIPAM-co-MAA) microsphere [18], poly(styrene-co-acrylic acid) microsphere [19] and silica nanosphere [20]. Nevertheless, the synthetic steps are usually time-consuming and low metal coverage on the microspheres frequently occurs. For ex-situ techniques, however, the distribution of size, and uniformity of the

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metal nanoparticle coated microspheres are easier to control than that of the in-situ techniques. Piao reported SERS-active PS microspheres with finely tuned Au network structure using stepwise electroless growth of Au NPs layers [21]. Silver coated microspheres also attracted much interest due to the stronger enhancement of Raman scattering comparing to gold. Tollen's test was the first attempt to create silver islands on dielectric beads [22]. Silver-coated silica microparticles with less than 25% metal coverage are transparent which can [23–26] be used as optically trappable Raman probes for SERS spectroscopy and bioimaging. Silver nanoparticle-embedded suffocated PS beads with a silica shell are more stable and biocompatible [27]. The Ag-Coated Fe_3O_4@-SiO_2 three-ply composite microspheres prepared through the well-known Ag-mirror reaction posses both ferromagnetic and SERS properties [28]. Yet the inhomogeneous enhancements and distributions observed on these Ag-coated microspheres also could not be ignored. Another study using self-assembly of dye-functionalized Ag nanoparticle tag onto SiO_2 microbeads via biotin-avidin binding shows highly uniformed SERS responses but the synthesis of the tags before assembly is a bit complicated [29]. Therefore, simple protocols that can easily control the morphology and density of silver nanoparticles on the microspheres with highly SERS activity in a reproducible manner needs to be explored.

Functionalized microspheres such as those containing amino groups allow favorable deposition of silver on the surface and can be easily obtained commercially. [21]. In this work, a PS-NH_2/Ag NPs SERS substrate is prepared using the sodium citrate reducing method for the deposition of silver nanostructure coating directly on the commercially bought amino modified PS microspheres. The self-assembled monolayers of thiol compounds on the PS-NH_2/Ag NPs could be easily detected. The relationship between deposition time, silver surface morphology, and SERS enhancement are studied in detail. The potential use of PS-NH_2/Ag NPs in preparing SERS encoded PS microspheres and ultrasensitive detection in real complex sample as melamine in a complex sample such as milk powder was demonstrated.

2. Experimental section

2.1. Materials and reagents

The major chemicals used in this work including silver nitrate and trisodium citrate dehydrate were obtained from Sinopharm Chemical Reagents (Shanghai, China) and have a purity of 99.5%. Amino polystyrene microsphere (PS-NH_2; 5% w/v; 5.0–5.9 μm) was purchased from Baseline ChromTech Research Centre (Tianjin, China); 2-mercaptopyridine (99%), 4-nitrophenylthiol (98%), 4-methyl-benzenethiol (98%) and 2-Naphthalenethiol (98%) were purchased from J&K Chemical Ltd. (Beijing, China). Melamine (99.8%) was obtained from Bodi Chemicals Inc., Ltd. (Tianjin, China) and milk powder was purchased from A. Best supermarket (Changsha, China). The other chemicals were all of analytical grade and used as received. The ultrapure water (18.32 MΩ) used was purified by Nanopure Infinity Ultrapure system (Barnstead/ thermolyne Corp, China).

2.2. Instrumentation

A Confocal Raman System Laboram 010 (Jobin Yvon Inc. USA) based on a 50 × long working-distance objective (8 mm) was used for the Raman spectra collection. A 632.8 nm He–Ne laser excitation (0.1 mW) was used as the laser source with the slit and pinhole setting at 100 and 1000 μm, respectively. The laser beam exposure time for the acquisition of SERS spectra was 15 s with 3 accumulations, which considered the balance between the signal-to-noise ratio, measurement time, and small sample volume on the SERS substrate. Usually 5–10 spots on the substrate were measured.

2.3. Preparation of silver-coated amino polystyrene microspheres

The preparation of silver coated amino polystyrene microspheres was according to the reference [30] with minor modifications: A 0.26 mL of 0.589 M silver nitrate solution was added to 47.5 mL of deionized water and stirred vigorously on a magnetic stirrer-hot plate and heated to 90–100 °C. A 5.0 ml of 5.0% (w/v) Polystyrene microspheres were added to the heated solution, and stirred for another 5 min. The initiation of the reduction reaction was initiated by the addition of 0.5 mL of 1.36 M sodium citrate solution to the mixture. The temperature was kept at 90–100 °C for different time before cooling down in ice water and the silver nanoparticles coated amino modified polystyrene microspheres (PS-NH_2/Ag NPs) were thus prepared. Different reduction time points as 1 min, 2 min, 4 min, 6 min, 8 min and 15 min were studied to optimize the silver coverage and SERS activity.

2.4. Nanojets formed between PS-NH_2/Ag NPs and smooth substrate for nondestructive detection

In order to study the influence of substrate material toward nanojets formed underneath individual PS-NH_2/Ag NPs, 2-mercaptopyridine was allowed to assembly on smooth gold or glass surface as a SERS probe layer, then PS-NH_2/Ag NPs microspheres were dispensed on top of the probe layer. For the preparation of smooth Au/ 2-mercaptopyridine/(PS-NH_2/Ag NPs), a polycrystalline gold (99.9%) electrode with a 2 mm diameter was first mechanically polished with 0.3 μm and 0.05 μm Al_2O_3 powder to a mirror finish, followed by ultrasonic cleaning with ethanol and ultrapure water for 2–3 times. For the preparation of glass/2-mercaptopyridine/ (PS-NH_2/Ag NPs), glass slide was washed carefully and then ultrasonic cleaning with ethanol and ultrapure water for 2–3 times. Then 10 μL 2-mercaptopyridine of 10^{-3}M was dropped onto the fresh gold or glass surface to form a probe layer. After the 2-mercaptopyridine layer was dried, 30 μL of PS-NH_2/Ag NPs (reduction time: 6min) was dropped on top of the probe layer. SERS spectra at different positions were collected to compare the enhancement effect of the substrate materials.

2.5. Preparation of surface-enhanced Raman spectroscopy encoded microspheres

A 1000 μl of silver nanoparticles coated amino modified polystyrene microspheres were mixed separately with 100 μL 10^{-9}M~10^{-3}M 2-mercaptopyridine while stirring. After self-assembly at room temperature for 12h, thin monolayer of thiol compounds would be formed on the silver surface. The thiol compounds labeled silver coated microspheres were collected by centrifuge at 4000 r/min for 3 min and the excess unassembled thiol compounds in the supernatant were discarded. The thiol compounds labeled microspheres were then washed three times with ultrapure water to remove the unabsorbed thiol compounds before resuspended into ultrapure water. For encoding, different thiol compounds as 2-mercaptopyridine (2-MP), 4-nitrobenzenethiol (4-NT), 4-methylbenzenethiol (4-MT) and 2-naphthalenethiol (2-NT) of 10^{-3}M were used to label the PS-NH_2/ Ag NPs using the same method. The SERS spectra obtained from each thiol compounds labeled microspheres were detected and used for the encoding of the silver coated microspheres.