Research Paper

Composite MF@Ag-NPs microspheres for label-free quantitative detection of uric acid

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HIGHLIGHTS

- A convenient and easy-scalable synthetic strategy to achieve highly sensitive SERS substrates for single particle detection.
- Individual MF@Ag-NPs microspheres were screened under a microscope and were used to examine their single particle SERS properties by using four different probe molecules.
- The experimental results demonstrated the as-prepared SERS substrates possess a very high SERS activity and sensitivity for trace analysis of targeting molecules.
- The quantitative trace detection of uric acid (UA) as biomarker based on the label-free strategy has been successfully conducted.

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ABSTRACT

A convenient and easy-scalable synthetic strategy to achieve ultrasensitive surface enhanced Raman spectroscopy (SERS) substrates for single particle detection of target molecules is reported. Melamine-formaldehyde (MF) microspheres with variable sizes were synthesized and decorated with Ag nanoparticles (NPs) through an in-situ chemical deposition to obtain composite MF@Ag-NPs microspheres. Individual MF@Ag-NPs microspheres were screened under a microscope and used to examine their single particle SERS properties by using four different probe molecules. The experimental results demonstrated that as-prepared SERS substrates displayed a dramatically enhanced SERS activity and sensitivity as well as an extraordinary stability and reproducibility of the SERS signal. Moreover, we also showed a first example of single MF@Ag-NPs microsphere for quantitative trace detection of uric acid (UA) as biomarker based on the label-free strategy, making them a promising candidate as an ideal substrate for applications in fields of biosensor, trace analysis and biomedical diagnostics.

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1. Introduction

Surface enhanced Raman spectroscopy (SERS) is an ultrasensitive spectroscopic technique, which is currently the only way capable of simultaneously detecting a single molecule and provid-
ing its fingerprint chemical information [1]. SERS spectra combined with its label-free molecular specificity promise to be employed as high level quantitative analysis technique with the remarkable advantages of robust stability and ultrasensitivity towards analytes, which holds a great potential for next-generation convert security labels and biomarker trace detections [2–4].

Surface plasmons associated with noble metal nanoparticles can generate significant enhancement of local electromagnetic fields, which leads to intense SERS signals from Raman probe molecules in the vicinity of nanostructured substrates [5–9]. Notably, the enhancement factor and the stability of the SERS signal are strongly dependent on the respective SERS substrate. Such so-called SERS substrate with well-controlled “hot spot”, large area, and excellent spatial reproducibility would be desirable for SERS enhancement [10,11]. Moreover, the intensity of SERS signal could be highly affected by the surface physical state of the SERS substrates including the degree of aggregation, shape and size of gold or silver nanoparticles, as well as by the position and power of the focused laser beam, which may make it difficult to obtain quantitative SERS signals from the targeting molecules.

At present, there are many methods such as vacuum deposition [12,13], chemical reduction [14,15], chemical assembly [16], lithography [17] and nano-lithography [18] used for the preparation of high-performance SERS substrates. More recently, many new type of noble metal nanoparticles were developed for this purpose, such as core-shell structured nanoparticles [19–22], nanostructures [23] and bimetallic nanostructures [24,25], nano-arrays [26], nanochains [27,28], nanowires [29] and so on. Although the nano-structure design is very sophisticated, it is still difficult to position the laser beam precisely in order to distinguish the specific unit or group. Usually, Raman spatial resolution is about 1 μm, which means that the laser is usually focused on many particles and the Raman signal of different particles is simultaneously measured. However, the localized disordered stacking and alignment of the nanostructured SERS substrates will inevitably result in uneven distribution of the “hot spots”, and the reproducibility and stability of the Raman signal will be greatly reduced [30]. Thus, the use of nanoparticles as SERS substrate for the quantitative detection of probe molecules remain challenging and the practical application will be subject to many restrictions. Therefore, there is growing interest in the preparation of highly stable and uniform SERS substrate with simple, cost-effective and highly efficient methods with regard to practical applications of SERS quantitative detection.

To improve the accuracy of SERS quantitative detection, an internal standard method is often used [31–33]. In this case, a constant amount of a model substance is added to the sample and used for calibration of the analyte signal compared to the internal standard signal. According to the formula, the peak height or peak area, the relative correction factor, and the concentration of the sample can be obtained. To a certain extent, although the internal standard method eliminates the operating conditions caused by changes in the error, it is very difficult to find an appropriate internal standard substance in complex detection system, and moreover, the sample preparation is also very complicated. Therefore, the utilization of well-defined microspheres with metal nanoscale surface as SERS substrates would have clear advantages: (1) possess uniform structure and micron size to generate highly sensitive and reproducible SERS signal; (2) can be probed under an optical microscope to achieve single microsphere SERS detection.

Herein, we introduce a new class of single particle SERS detection of different probe molecules by using Ag nanoparticles decorated MF microspheres (MF@Ag-NPs microspheres) as highly efficient SERS substrates. MF microspheres with precisely adjustable sizes were synthesized through an easily controllable condensation polymerization technique (Fig. S1). The MF microspheres were subsequently decorated with Ag-NPs by using an in-situ chemical deposition method. The composite MF@Ag-NPs microspheres were thoroughly characterized by FESEM, XRD and TGA measurements. Moreover, due to the unique large diameters in the micron range as SERS substrates, herein prepared particles could be directly employed for SERS applications without transferring procedures and be easily distinguished and manipulated under a microscope, providing access to highly sensitive single particle SERS detection of different probe molecules, combined with a remarkable Raman signal enhancement effect, extremely high sensitivity as well as an extraordinary stability and reproducibility of the SERS signal. Furthermore, we demonstrated that this single MF@Ag-NPs microsphere has utility in label-free, quantitative trace detection of uric acid, illustrating their broad potential applications in areas of biological sensing and immunoassays (Fig. 1).

2. Experimental Sections

2.1. Materials

Melamine (C3H6N6, AR), silver nitrate (AgNO3, ≥99.8%), n-butylamine (CH3(CH2)3NH2, AR), 4-aminothiophenol (C6H5NS, ATP, AR), 4-methoxy-α-toluenethiol (CH3OC6H4CH2SH, MATT, AR), 4-chlorothiophenol (C6H5Cl5, CTP, AR) and 3,5-bis(trifluoromethyl)benzenethiol (C6H3F2S, FMBT, AR) were all purchased from Aladdin and used as received. Formaldehyde (HCHO, 37% in water), formic acid (HCOOH, AR), uric acid (UA, 99%) and ethanol (CH3CH2OH, 73.0–75.0 in water) were obtained from Sinopharm Chemical Reagent Co., Ltd. Deionized water was used in all experiments.

2.2. Synthesis of melamine resin (MF) microspheres

MF microspheres were synthesized using condensation polymerization. 0.32 g of C3H6N6 and 0.45 g of HCHO (molar ratio = 1:6 mol/mol) were added under stirring to 60 mL of deionized water at 70 °C. After 30 min, a certain amount of HCOOH was added. After polymerization at 70 °C for 1 h, the obtained dispersion was allowed to cool to room temperature. The dispersion was purified by centrifugation at 11000 rpm for 5 min, and the precipitated MF microspheres were re-dispersed in deionized water. A series of MF microspheres with different sizes were synthesized by changing the amount of HCOOH.

2.3. Preparation of composite MF@Ag-NPs microspheres

MF@Ag-NPs microspheres were synthesized using an in-situ deposition method. A typical procedure for the deposition of Ag
nanoparticles (i.e., Ag-NPs) onto MF microspheres was carried out as follows: 50 mg of MF microspheres were dispersed in 100 mL of an aqueous AgNO₃ solution (5 mg/mL) and then a certain amount of n-butylamine was added. The mixture was treated by ultrasonication for 4 min and vigorously stirred for 2 h at 70 °C. To ensure the complete reduction of AgNO₃, an excess amount of butylamine was used in all experiments, and the ratio between butylamine and AgNO₃ was kept constant (0.5 μL: 1 mg). After the synthesis, the composite MF@Ag-NPs microspheres were purified by adding ethanol under ultrasonication and then isolated by centrifugation.

2.4. Characterization and measurement

An OLYMPUS inverted fluorescence microscope (model BX51) was used to characterize the structure and particle size distribution of MF and MF@Ag-NPs microspheres. Field-emission scanning electron microscopy (FESEM) was performed on a Zeiss (model Ultra 55) at an operating voltage of 3 kV. Dispersions of the samples were drop-cast on mica plates at room temperature and sputter-coated with gold. Dynamic light scattering was conducted on a Nano ZS Zetasizer (model ZEN3600, Malvern Instruments) using a He-Ne laser at a wavelength of 632.8 nm. Fourier transform infrared (FT-IR) spectra of the samples were collected on a Magna-550 ( Nicolet, USA) spectrometer using KBr pellets. Powder X-ray diffraction (XRD) patterns were obtained using a X’Pert Pro (Panalytical, The Netherlands) diffraction meter with Cu Kα radiation at λ = 0.154 nm operating at 40 kV and 40 mA. Thermogravimetric analysis (TGA) measurements were performed on a Pyris 1 TGA instrument under air with a heating rate of 20 K/min. All TGA measurements were taken under a constant air flow of 40 mL/min. Ultraviolet-visible (UV-vis) absorption spectra were measured on a UV-3150 spectrometer (Shimadzu, Japan). Raman spectra were recorded on a HORIBA spectrometer (model XploRA) operating with 633 nm laser excitation and 1800 cm⁻¹ grating. The data acquisition time was usually 10 s, and peak intensities of the samples were normalized with respect to the standard of a silicon wafer at 520.7 cm⁻¹. For each measurement, the laser spot was randomly focused on a single composite microsphere to obtain the Raman spectra.

4-aminophenol (ATP) and 4-methoxy-o-toluenethiol (MAT) were utilized as model Raman probe molecules to evaluate the SERS signal reproducibility of single MF@Ag-NPs microsphere as substrate. Typically, 1 mg of composite MF@Ag-NPs microspheres was dispersed in 5 mL of aqueous ethanol solution containing 10⁻³ mg/mL ATP or MAT and incubated under gentle shaking at room temperature for 2 h. After that, the composite microspheres were separated by centrifugation, purified three-times with ethanol to remove excess ATP and MAT, and finally dispersed into 500 μL ethanol. By using the same procedure, solutions of 4-chloroanilinobenzothiazolines (CTP) and 3,5-bis(trifluoromethyl)benzenethiol (FMBT) in ethanol of different concentrations in the range from 10⁻³ mg/mL to 10⁻⁹ mg/mL were prepared. The sample of uric acid (UA) for SERS measurement was prepared accordingly by adding 0.1 mol/L of sodium hydroxide (NaOH) aqueous solution, and its final concentration was adjusted in the range from 10⁻⁵ mg/mL to 10⁻⁷ mg/mL. The spectra were obtained by averaging five readings at the point of each sample.

3. Results and Discussion

3.1. Preparation of melamine formaldehyde (MF) microspheres

Since the size and shape of the MF microspheres are critical for their practical applications, it is necessary to study the effects of the synthesis parameters onto the particle size and size distribution. Hence, the influence parameters of the reaction time, reaction temperature as well as the amount of formic acid as the reaction catalyst were characterized and evaluated in detail. In all syntheses, the molar ratio between melamine and formaldehyde was fixed to 1:6.

The changes of the particle sizes of uniform MF microspheres were measured with optimal microscopy. By altering reaction time from 0.25 to 3 h, the sizes of MF microspheres increased from 1.56 to 2.36 μm (Fig. 2a). After 3 h of reaction time, the diameter of the microspheres grew slightly, indicating that the monomers were almost completely reacted and the reaction apparently reached its limit after 3 h. When the reaction temperature varied, as shown in Fig. 2b, the sizes of MF microspheres first increased from 0.68 to 3.29 μm as the temperature raised from 40 °C to 85 °C, and then decreased when the temperature approaching to 100 °C, which can be attributed to an enhanced reaction rate, that is to say, the number of nucleation sites increased tremendously at higher temperatures (>85 °C) resulting in a larger number of particles and thus smaller particle sizes. We also found that the concentration of formic acid served as the catalyst had a significant effect on particle size and size distribution of MF microspheres. If the amount of formic acid was less than 0.5 μL, the uniform MF microspheres could not be obtained due to insufficient of catalytic sites and the resulting random nucleation. By applying sufficient amounts of catalyst, as demonstrated in Fig. 2c, the formation of secondary particles could be effectively prevented, leading to forming precisely controllable monodisperse MF microspheres from 4.28 to 0.69 μm with the increasing amount of catalyst from 0.5 to 50 μL. The corresponding MF microspheres morphology observed in FESEM images revealed homogeneous spherical nanoparticles (Fig. 3), providing further evidence for the remarkable uniformity of the MF microspheres preparation.

The chemical composition of MF microsphere was confirmed by FTIR spectroscopy (Fig. S2). The broad absorption peaks at 3415 cm⁻¹ and 3300 cm⁻¹ can be attributed to the −OH and −NH₂ stretching vibrations of hydroxymethyl (−CH₂OH) and secondary amine groups, respectively. A small absorption peak due to −CH stretching vibrations appeared at 2920 cm⁻¹. The characteristic bands at 1560 cm⁻¹, 1490 cm⁻¹ and 877 cm⁻¹ confirmed the presence of triazine rings associated with the stretching vibrations and bending vibrations of C=N groups, respectively. Furthermore, the sharp absorption peak at 1348 cm⁻¹ can be attributed to C=N stretching vibrations, while the absorption peak at 1160 cm⁻¹ can be clearly assigned to C=O symmetric and asymmetric stretching vibrations of ether groups.

3.2. Preparation of composite MF@Ag-NPs microspheres

The deposition of Ag nanoparticles (Ag-NPs) onto the surface of MF microspheres was carried out by chemical sediment method. In all experiments, the optimal mass ratio (w/w) of AgNO₃ to MF microspheres was set to 10:1. The optical microscope and FESEM images of the particles are shown in Fig. 4. Comparison of the optical microscopy images obtained before and after the Ag-NPs deposition (Fig. 4a and b) revealed the existence of individual non-aggregated MF@Ag-NPs microspheres with a mean diameter of ∼2 μm, while the initial spherical particle architecture of the MF microspheres could be obviously maintained. FESEM images of the resulting composite MF@Ag-NPs microspheres clearly displayed that the MF microspheres surface was evenly covered by Ag-NPs (Fig. 4c and d), and the sizes of Ag-NPs on the surface of MF microspheres are approximately 150 nm (Fig. S3). It is known that the Ag atoms can be easily deposited onto layers with the presence of abundant amino groups [31], the possible mechanism for the formation of Ag shell may be explained as follows: Ag⁺ ions were primarily adsorbed onto the surface of MF microspheres and form
a complex with the amino groups on the surface of MF microspheres; with the addition of reductant n-butylamine, Ag⁺ ions were reduced into nuclei in ethyl alcohol, thus the small Ag particles could be slowly grow up in the channels and finally deposited and immobilized onto the MF microspheres without further particle modification [14].

The deposition of Ag-NPs onto MF microspheres could be further confirmed by XRD as well as TGA measurements (Fig. 5). The XRD pattern of MF microspheres in Fig. 5a shows the characteristic amorphous halo (20 ~ 20–30°) generated from the amorphous phase of MF microspheres. XRD pattern of the composite MF@Ag-NPs microspheres, by contrast, additionally exhibited strong diffraction peaks at 20 values of 38.1°, 44.3°, 64.4° and 77.4° associated to the reflection of the (111), (200), (220) and (331) crystalline planes respectively, in good accordance with the standard spectrum (JCPDS 04-0783) [34], indicating the existence of Ag in the cubic phase. To gain further insights into the particle composition and accurately examine the final Ag content in the MF@Ag-NPs microspheres, the thermal decomposition was conducted and compared to the initial MF microspheres (Fig. 5b). The TGA measurements in the temperature range of 30–800°C revealed a three-step thermal decomposition behavior for both samples. The first step proceeded in the temperature range of 100–200°C resulted from the evaporation of low molecular weight products in the MF microspheres, while the second step proceeded in the temperature range of 350–450°C was caused by thermal decomp-

3.3 Evaluation of the SERS signal of individual composite microsphere

Four different probe molecules were used to study SERS characteristics of the MF@Ag-NPs microspheres and to evaluate the single particle SERS detection. The probe molecules 4-aminothiophenol (ATP), 4-methoxy-α-toluenethiol (MATT), 4-chlorothiophenol (CTP) and 3,5-bis(trifluoromethyl)benzenethiol (FMBT) were selected, considering similar structure and binding sites (thiols) accompanied with different chemical characteristics to confirm the reliability of the experiments.

In the first step, the UV–vis spectra of Ag-NPs and MF@Ag-NPs microspheres had been measured (Fig. 6a), which showed a redshift and broadened surface plasmon absorption (SPA) band due
Fig. 4. Optical microscope and FESEM images of (a, c) MF microspheres and (b, d) their corresponding composite MF@Ag-NPs microspheres with the mass ratio of 10:1 between AgNO$_3$ and MF microspheres, n-butylamine: AgNO$_3 = 5:1$ (µL: mg).

Fig. 5. (a) XRD patterns of MF and MF@Ag-NPs composite microspheres; (b) thermal gravity analysis of MF and MF@Ag-NPs composite microspheres.

to a Mie plasmon resonance excitation from the Ag-NPs, while the plasmon coupling between the deposited Ag nanoparticles and the interface structure between the Ag and MF microspheres may play a key role for the red shift and broadening of the SPA band. Furthermore, the intensity of SERS spectra of 10$^{-3}$ mol/L ATP based on MF@Ag-NPs composite microspheres as substrates under different excitation wavelength were operated with 532 nm, 633 nm and 785 nm laser excitation (Fig. 6b). Compared these three conditions, the SERS spectra of ATP showed strongest SERS activity under 633 nm laser excitation. Then, ATP and MATT were utilized as model Raman probe molecules to evaluate the SERS signal reproducibility by measuring and comparing the SERS signals of different individual MF@Ag-NPs microspheres under 633 nm laser excitation. For this purpose, fifteen different MF@Ag-NPs microspheres, which were incubated with aqueous analyte solutions of ATP and MATT at concentration of 10$^{-3}$ mg/mL, were identified through SERS measurement. The SERS spectra of resulting isolated composite microspheres in fifteen randomly chosen areas were detected, the individual composite microspheres possess superior SERS activity and extraordinary reproducibility of SERS signals of the particles among each other (Fig. S4), giving rise that herein prepared MF@Ag-NPs microspheres have great potential as an isolated SERS-active substrate for single particle detection.

Next, the sensitivity of composite MF@Ag-NPs microspheres for probe molecules was evaluated by measuring the SERS spectra of microspheres incubated with aqueous analyte solutions of CTP and FMFBT with different concentrations from 10$^{-9}$ mg/mL to 10$^{-3}$ mg/mL, respectively. The SERS spectra displayed in Fig. 7A and
Fig. 6. (a) UV–vis spectra of Ag-NPs and composite MF@Ag-NPs microspheres; (b) SERS spectra of composite MF@Ag-NPs microspheres under different excitation wavelength.

Fig. 7. SERS spectra of the composite MF@Ag-NPs microspheres treated with different concentrations of (A) CTP and (B) 3-FMBT. The blank spectrum was obtained from the single isolated MF@Ag-NPs microsphere without treating with any SERS probe. Fitting curve of dependence of the Raman intensities at (a) 1569 cm$^{-1}$ on the concentration of CTP; (b) 998 cm$^{-1}$ on the concentration of 3-FMBT.

B and the SERS intensity was progressively increased with concentration of CTP and FMBT. The plots of intensity at 1569 cm$^{-1}$ peak (Fig. 7A) and 998 cm$^{-1}$ peak (Fig. 7B) in the SERS spectra versus the logarithmic concentration (log (c)) of CTP and FMBT illustrated in Fig. 7a and b, can be fitted line with correlation coefficient of 0.976 (n = 5) and 0.951 (n = 5). The results show a good linear relation-
ship between SERS intensity and analyte concentration, revealing that the SERS detection based on single MF@Ag-NPs microsphere as substrate is extremely sensitive and highly reproducible, which is particularly advantageous for the quantitative detection of Raman molecular probes.

To evaluate the general applicability of composite MF@Ag-NPs microspheres for potential applications in bio-analytics, we continued to exploit the utility on single particle detection of uric acid as probe molecule. Uric acid is a powerful antioxidant and a scavenger of singlet oxygen and radicals. Under physiological condition, urate reduces the o xo-heme oxidant formed by peroxidase reaction with hemoglobin, protects erythrocytes ghosts against lipid peroxidation, and protects erythrocytes from peroxidative damage leading to lysis. In human blood plasma, the urate concentration is typically $3 \times 10^{-2}$ mg/mL$–7 \times 10^{-2}$ mg/mL for men and $2 \times 10^{-2}$ mg/mL$–6 \times 10^{-2}$ mg/mL for women [36]. Accordingly, the single MF@Ag-NPs microspheres were used as the substrate to measure the SERS spectra of concentration of uric acid from $10^{-4}$ mg/mL to $10^{-3}$ mg/mL. As shown in Fig. 8a, as expected, with the increase of uric acid concentration, the SERS intensity was clearly raised. The SERS spectra of uric acid can be characterized by the peaks at 1133 cm$^{-1}$, 1203 cm$^{-1}$, 1071 cm$^{-1}$, 1016 cm$^{-1}$, 641 cm$^{-1}$, 590 cm$^{-1}$ and 498 cm$^{-1}$, which can be assigned to $\nu$=O, C–H bending and C–C stretching vibrations, respectively, which is in good agreement with previous reports [37,38]. The plot of the intensity of 1133 cm$^{-1}$ peak in the SERS spectra versus uric acid concentrations illustrated in Fig. 8b also can be fitted a line with correlation coefficient of 0.950 (n = 5), indicating a good linear relationship between the SERS intensity and analyte concentration, making the single MF@Ag-NPs microsphere as an ideal SERS substrate candidate for quantitative and trace detection of uric acid.

4. Conclusions

In summary, monodisperse composite MF@Ag-NPs microspheres of controllable sizes have been successfully synthesized through a combination of precisely controllable condensation polymerization technique and subsequent in-situ chemical deposition of Ag NPs onto the surface of prepared MF microspheres. FESEM, XRD and TGA results confirmed that composite microspheres with remarkable uniformity were available by the presented method and that the surface of the MF microspheres was evenly covered with an adequate amount of crystalline Ag NPs. It could be demonstrated that herein prepared SERS substrates could be successfully used for the single particle SERS detection of different probe molecules. The MF@Ag-NPs microspheres possessed a very high SERS activity and sensitivity as well as an extraordinary stability and reproducibility of the SERS signal, which can be valuable in a wide range of applications in fields of sensor technology, trace analysis and biomedical diagnostics. Moreover, the quantitative trace detection of uric acid based on the herein presented single particle SERS detection could also be demonstrated.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfa.2017.03. 042.

References


