

作为表面增强拉曼光谱基底的高度均匀 Au纳米棒的构筑及对福美双的检测

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摘要: 采用种子生长法制备 Au 纳米棒(GNRs), 随后进行组装和煅烧得到单层致密堆积的 GNRs 薄膜。在煅烧过程中, 组装所需有机物在煅烧过程中分解, 从而使得 GNRs 表面具有较高的清洁度。研究中发现, 煅烧前后金纳米棒表面的间隙进一步提高, 增强了其 SERS(表面增强拉曼光谱)活性。为了研究其 SERS 活性, 选择了 2 种探针分子以研究其灵敏度和均匀性, 发现其具有较高的灵敏度和高的信号稳定性。随后将所制备的 SERS 基底成功用于检测超低浓度的农药分子。

关键词: 表面增强拉曼光谱; 金纳米棒; 清洁表面; 快速检测; 农药残留

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Detection of Thirm Based on Highly Uniform Au Nanorods as Surface-Enhanced Raman Spectroscopy Substrate

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Abstract: A monolayer close-packed Au nanorods (GNRs) film has been prepared by an easy seed-growth approach, followed by self-assembling method with the surfactants and subsequent calcination treatment. During the calcination process, the surfactants were decomposed and eliminated completely, resulting in a clean surface of GNRs. Resultantly, these monolayer GNRs films explored as a SERS (surface-enhanced Raman spectroscopy) substrate have good uniformity and high sensibility for detecting probe molecules. Subsequently, the prepared SERS substrate was used to detect ultra-low concentration (10^{-9} mol·L⁻¹) of pesticide molecules.

Keywords: surface-enhanced Raman spectroscopy; Au nanorods; clean surface; rapid detection; pesticide residue

SERS (surface-enhanced Raman spectroscopy) detecting technique is an exciting non-destructive spectroscopic approach which could identify various

biochemical organic molecules adsorbed on the nanoscale noble metal surface, even up to single-molecule fingerprint-vibration signals. Because the

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surface plasmon resonance (SPR) surrounding the nanosized noble metal surface could generate enormous electromagnetic (EM) field and amplify Raman signals of probe molecules^[1-3], and the enhancement factor could reach up to $10^6 \sim 10^{15}$ ^[4]. So the SERS technique can be used as an effective analytical method in several fields, such as food safety, biochemical sensing, confirming of crime, and environmental engineering^[5-8]. In order to obtain the ideal performance of SERS techniques, it is essential to prepare uniform and sensitive SERS substrates.

So far, there are several routes for fabricating SERS substrates^[9-11]. Traditionally, Colloidal gold and silver nanoparticles (NPs) are available to detect some organic molecules arising from appropriate nanogaps between adjacent NPs, and sharp corners as well as edges of NPs. Furthermore, it still remains challenging to simply fabricate the SERS substrates with uniform enhancement response. Li and co-workers have prepared various periodic noble structural arrays based on colloidal templates, and their SERS reproducibility improved by physical deposition was further explored^[12]. Among these noble metal nanomaterials, Au possesses perfect oxidation resistance and stable chemical properties, which is necessary for use as SERS substrate to produce stable SERS signals. Many different shapes of Au NPs, including spherical, cubic-shaped, rhombic dodecahedral, and octahedral were served to fabricate periodic Au monolayer arrays by self-assembly method^[13-14]. However, during the assembly process, organic solvents were unavoidably used to stabilize the structural units, which also produce Raman signals simultaneously in detecting target molecules and bring interference to the desired SERS signals, and even lead to the failure in practical applications. Therefore, it is very meaningful to fabricate high-density and uniform hot-spot SERS substrates with clean surface. In the present study, highly monodispersed Au nanorods (GNRs) were fabricated by using typical seed growth consecutive two-step reactions. Then

high-quality methoxypolyethylene glycol thiol (PEG-SH) molecules were connected onto the GNRs to form close-packed GNRs film. Importantly, after assembly of GNRs, the heat treatment technology was used to remove the surfactant molecules, and thus naked clean GNRs were obtained. Then using the close-packed uniform naked GNRs as SERS substrate, high reproducible and sensitive SERS signals of some usual organic pollutant molecules can be rapidly detected. Here, thiram, a kind of frequently-used pesticides, was detected by the SERS as a quantitative analytical tool. In addition, the comprehension of enhancement theory from GNRs is helpful to further improve their Raman enhancement and important use for ecological environment and drinking water safety.

1 Experimental

1.1 Materials

Hydrogen tetrachloroaurate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), trisodium citrate and *L*-ascorbic acid (AA) were obtained from Alfa Aesar China (Tianjin) Co., Ltd. Cetyltrimethylammonium bromide (CTAB), sodium borohydride (NaBH_4), and silver nitrate (AgNO_3) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. PEG-SH with a molecular weight of 5 kDa was obtained from Shanghai Yare Biotech, Inc. In addition, rhodamine 6G (R6G), crystal violet (CV) and thiram were bought from Sigma Aldrich Ltd. All reagents were directly used without further purification. Deionized water with $18.2 \text{ M}\Omega \cdot \text{cm}$ was obtained by a Millipore Milli-Q gradient system.

1.2 Fabrication of GNRs

Typical seed growth route was employed to fabricate GNRs, as described briefly below, 0.5 mL of $20 \text{ mmol} \cdot \text{L}^{-1}$ HAuCl_4 was mixed with 10 mL of $0.2 \text{ mol} \cdot \text{L}^{-1}$ CTAB solution at 30°C . Subsequently, 0.6 mL newly fabricated NaBH_4 solution ($20 \text{ mmol} \cdot \text{L}^{-1}$) was dropped rapidly into the mixed solution described above to prepare seed solution. The growth solution was prepared as follow: 2 mL of $10 \text{ mmol} \cdot \text{L}^{-1}$ HAuCl_4 and 30 mL of $0.1 \text{ mol} \cdot \text{L}^{-1}$ CTAB

were added to $2 \text{ mmol} \cdot \text{L}^{-1} \text{ AgNO}_3$ solution at 30°C . Then 0.1 mL of $1.0 \text{ mol} \cdot \text{L}^{-1} \text{ HCl}$ and 0.08 mL of $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ AA}$ were successively added to the solution. Lastly, the prepared Au-seed solution with 20 mL was poured into the solution described above at 30°C to synthesize nanoscaled rod-shaped Au.

1.3 Assembly of GNRs film

The obtained Au NRs were conjugated with PEG-SH before assembly. Briefly, 2 mL of PEG-SH aqueous solution ($2 \text{ mmol} \cdot \text{L}^{-1}$) was injected to the GNRs colloidal aqueous solution, and kept for 8 h under stirring treatment at room temperature, followed by centrifugation. Then the GNRs were redispersed into deionized water. The GNRs aqueous solution was cast onto a Si wafer surface, and two-dimensional close-packed structures would be formed after self-assembly process. Then the heat treatment method with conditions of 400°C and 10 min was used to remove the surfactant from the surface of specimens.

1.4 Characterization

The surface morphology of the prepared samples was characterized by scanning electron microscopy (SEM, FEI Sirion FEG 206, 200 kV). The fine microstructure of individual GNRs was observed by transmission electron microscopy (TEM, JEOL, JEM 2100). Additionally, the optical absorbance properties were measured on a TU-1901 model UV-Vis spectrophotometer (Beijing 75 Purkinje, China) with resolution of 0.5 nm . The SERS signals were obtained from a confocal microscope Raman system (Horiba Jobin Yvon), and the

laser wavelength was 633 nm , and the laser power approximately was 1 mW .

2 Results and discussion

The colloidal GNRs were fabricated via the typical seed-growth route. Fig. 1a exhibits a typical SEM image of the highly monodispersed GNRs. It indicates that each structural unit had a rod-like shape, and its cross-section diameter was about 25 nm , and the longitudinal length was about 60 nm . According to the TEM image (Fig. 1b), it is obvious to see that there was dome-shaped surface at the both ends of the rod. The UV-Vis absorption spectrum of the GNRs displays two peaks, as shown in Fig. 1c. The peak located at 517 nm is originated from transverse SPR, and the peak around 675 nm is attributed to longitudinal SPR, respectively. These results indicate that the desired rod structure of GNRs has been successfully obtained. Moreover, the good optical absorption properties demonstrate that the samples have a regular structure and highly purified chemical components.

An ideal SERS substrate mainly embodied in below three aspects, high enhancement factor, stable and uniform signals, and facile preparing route. It is well known that the gap between two neighbouring GNRs at the desired SERS substrate must be less than 10 nm . Assembly of GNRs is an effective method to construct SERS substrate with good uniformity and small gaps. Hence, a very facile polymer self-assembly way may be introduced to combine multiple GNRs for construction of close arranged Au rods film. Moreover, to meet requirement of gigantic Raman signals enhancement effect, it is favourable to obtain sub- 5 nm

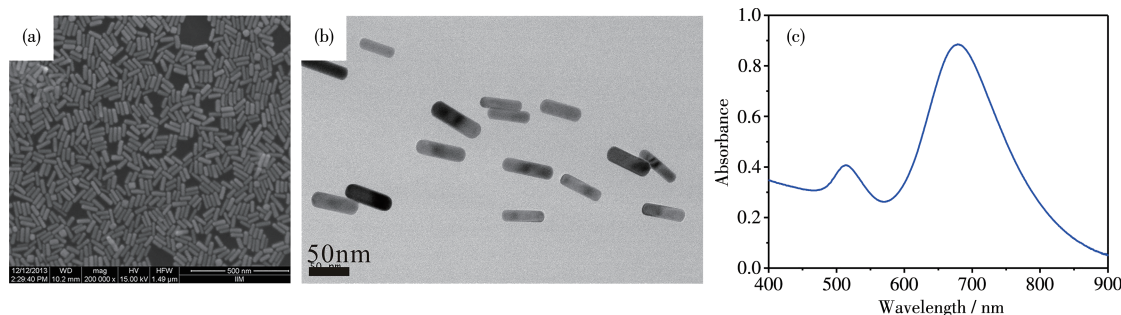


Fig.1 SEM image (a) and TEM image (b) of the uniform GNRs before calcination; (c) UV-Vis absorption spectrum of the fabricated GNRs

gaps between two neighbouring structural units^[15]. Here, PEG-SH molecules were used to combine with the GNRs in the polymer self-assembly approach. However, they would generate interference for the SERS signals of target molecules and greatly reduce the SERS activity, because they prevent the target molecules from accessing to hotspots. Therefore, it is necessary to clear the PEG-SH molecules away from the surface of GNRs by using heat treatment method. During the calcination process, it is very important to control the calcination temperature. If the temperature is lower than 350 °C, the surfactant will not be removed thoroughly, whereas high temperature, higher than 430 °C, will lead to formation of Si-rich Au metal complexes, because the surface tension of Si is lower than that of Au. The oscillation of surface electrons of such alloy and EM field coupling would be hampered due to covalent bonding of surface atoms^[16]. The SEM images of GNRs after calcination at 300, 400 and 450 °C are showed in Fig.S2A~S2C. And the SERS spectra of malachite green and thiram obtained from the corresponding GNRs as substrate in Fig.S2D and Fig.S3 demonstrated that the optimal temperature was 400 °C. Hence, the calcination temperature was determined to be 400 °C to thermal-decompose the surfactant, and close packed GNRs pattern with naked and clean surface was finally formed by comparing the SEM images before and after calcination (Fig.S1).

Fig.2a shows the SEM image of large scale of uniform GNRs film by self-assembling method after calcination process at 400 °C for 10 min. The close-packed GNRs film can be used as SERS active substrate due to strong SPR peaks of nanoscale gold and narrow gaps

with size of sub - 10 nm between two neighbouring GNRs. In order to estimate SERS sensitivity from the obtained GNRs, the R6G and CV molecules were chosen as the probe molecules. Fig.2b shows the SERS signals of R6G with concentrations from 10^{-7} to 10^{-10} mol·L⁻¹. The sharp and clear Raman signals of target molecules were obvious. More specifically, the peak located at 612 cm⁻¹ is attributed to the C—C—C ring in-plane vibration mode. And the peak located at 771 cm⁻¹ is attributed to the C—H out-of-plane bend vibration mode. In addition, the peaks located at 1 313 and 1 574 cm⁻¹ are assigned to the N—H in-plane bend vibration modes. The peaks located at 1 364, 1 511 and 1 650 cm⁻¹ are attributed to the C—C stretching vibration modes^[17-18]. Fig.2c shows the SERS signals of different concentrations of the CV from 10^{-8} to 10^{-11} mol·L⁻¹ adsorbed on the substrate. The peaks located at 1 619, 1 585, 1 535, and 1 444 cm⁻¹ are attributed to the aromatic C—C stretching vibration modes. Additionally, the peak located at 1 370 cm⁻¹ is attributed to *N*-phenyl stretching vibration mode and the peaks located at 1 171, 915, and 809 cm⁻¹ are attributed to aromatic C—H bending vibration modes^[19].

All SERS signals were obtained under the same measure conditions, including laser wavelength of 633 nm, laser power of 1 mW and acquisition time of 2 s. For further quantitative analysis, the Raman enhancement factor (EF) can be expressed by the following simple formula^[20]:

$$EF = (I_{\text{SERS}}/N_{\text{SERS}})/(I_0/N_0) \quad (1)$$

Where N_0 and I_0 represent the value obtained from the normal Raman measurement using Si wafers as substrate, and N_0 is the concentration of reference R6G

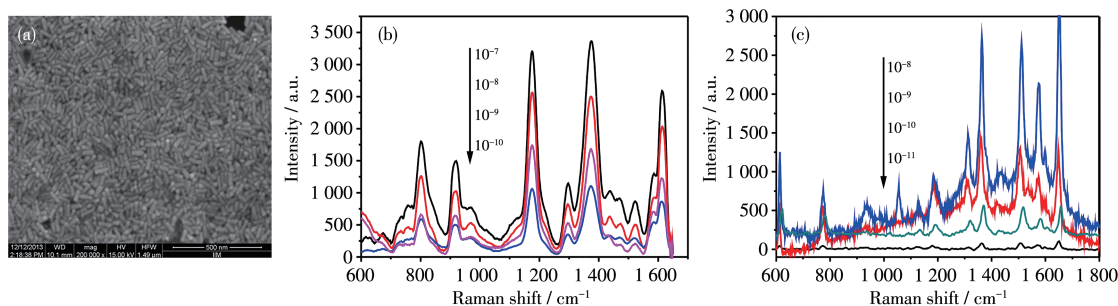


Fig.2 SEM image of the close-packed uniform GNRs after calcination (a) and SERS signals of R6G (b) and CV (c) with different concentrations adsorbed on the active substrate shown in (a)

solution ($0.1 \text{ mol} \cdot \text{L}^{-1}$), I_0 is the intensity of one typical peak (Fig.3a). N_{SERS} and I_{SERS} represent the value obtained from the SERS measurement using close-packed GNRs film as SERS active substrate (Fig.3b), and N_{SERS} is the concentration of diluted R6G solution ($10^{-9} \text{ mol} \cdot \text{L}^{-1}$), I_{SERS} is the according peak intensity. Based on the intensity of the carbon skeleton stretching vibration modes located at $1\,360 \text{ cm}^{-1}$, the calculation result of EF was 5.18×10^8 , demonstrating the excellent SERS activity of

the substrate^[21].

Besides sensitivity, uniformity is another important factor for SERS active substrate, and need to be validated. It is known that, based on good uniformity substrate, the SERS spectra obtained from different points on the substrate surface could exhibit high reproducibility, and the peak value fluctuated little, it is helpful to get intensity-related results quickly and accurately. In order to describe quantitatively the uniformity of the substrate, a parameter named relative standard deviation (RSD) was analyzed^[22]. Herein, CV molecules were used as probe molecule to test the uniformity among different points of the GNRs substrate. The SERS-RSD spectra were obtained from 50 points which randomly selected on the GNRs substrate film. Three major peaks ($1\,171$, $1\,370$, $1\,585 \text{ cm}^{-1}$) of CV Raman spectra were chosen to calculate the value of RSD. As shown in Fig.4, the RSD value from the three peaks was 12.9%, 13.8%, 11.4%, respectively. All of them were below 0.2, demonstrating that the GNRs film has good uniformity^[23]. This is because of close-arranged structural pattern and homogeneous chemical composition.

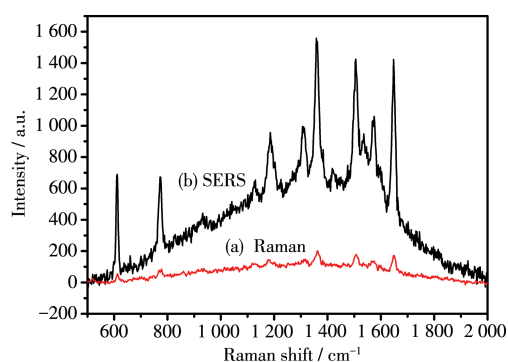


Fig.3 Raman signals of $0.1 \text{ mol} \cdot \text{L}^{-1}$ R6G reference solution (a) and SERS signals obtained from diluted R6G solution ($10^{-9} \text{ mol} \cdot \text{L}^{-1}$) using close-packed GNRs film as SERS active substrate (b)

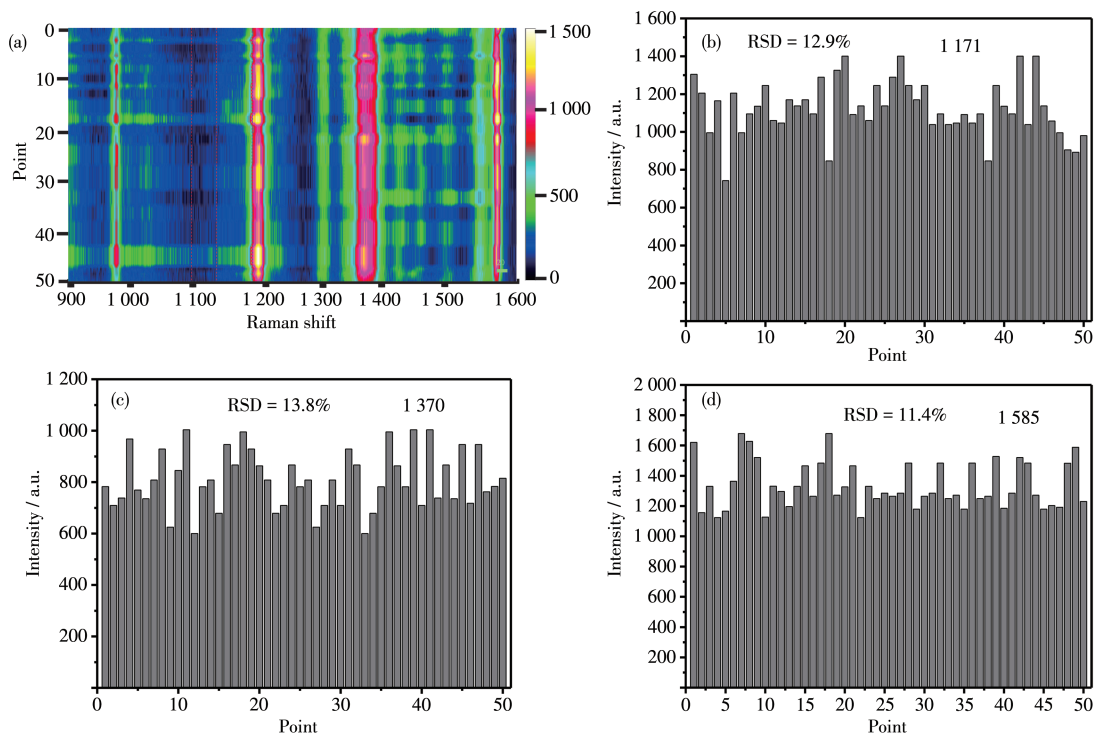


Fig.4 Two-dimensional color marked intensity of spatiotemporal SERS mapping (a) and intensities of the three main Raman vibration peaks ($1\,171$, $1\,370$, $1\,585 \text{ cm}^{-1}$) of CV molecules adsorbed on the surface of GNRs (b~d)

Furthermore, the practical application of the obtained close-arranged GNRs film has been further explored based on the SERS spectra. Thiram as a kind of pesticide is widely used in field crops so that its residues inevitably remain on the surface of crops. It is necessary to detect the amount of residues and to protect mankind from its toxicity. Fig.5 show typical SERS signals of different amount of thiram molecules using the naked GNRs as substrate. It indicated that the SERS spectra contained some obvious peaks. And the strongest peak was located at $1\,384\text{ cm}^{-1}$ which is attributed to the C—N stretching vibration mode and symmetric CH_3 deformation vibration mode, therefore, this peak intensity is regard as representative for identifying the amount of thiram. Furthermore, The C—N stretching mode also appeared at $1\,512\text{ cm}^{-1}$ as well as the rocking CH_3 mode, and they also occurred at $1\,149$

cm^{-1} . Additionally, the stretching CH_3N mode appeared at 930 cm^{-1} , and the band at 558 cm^{-1} is attributed to the S—S stretching vibration mode^[24-25]. The average SERS curves with thiram concentrations of $10^{-5}\sim 10^{-9}\text{ mol}\cdot\text{L}^{-1}$ were carried out to minimize photobleaching and also to minimize the variation of signal intensity, as shown in Fig.5a. Fig.5b illustrates the corresponding plot of I_{SERS} versus $-\lg c_{\text{thiram}}$, in which I_{SERS} means the SERS intensity of one thiram peak at $1\,384\text{ cm}^{-1}$. It is obviously demonstrated that there is a linear relationship about the intensity of SERS signals with concentrations of thiram aqueous solution. So it is easy to obtain the concentration of thiram solution by the intensity of SERS spectra.

For a practical application, the highly uniform GNRs was also used as SERS substrate for thiram analysis of real sample. First, $2\times 10^{-5}\text{ mol}\cdot\text{L}^{-1}$ thiram solution was sprayed on the surface of an apple. And then, the apple was washed with acetone after complete evaporation. The wash was collected and dropped on the GNRs SERS substrate. The primary vibrations of thiram were confirmed at $1\,149$, $1\,384$ and $1\,513\text{ cm}^{-1}$, which was shown in Fig.6. It is demonstrated the highly uniform GNRs can be applied to the determination of pesticide residue in real sample.

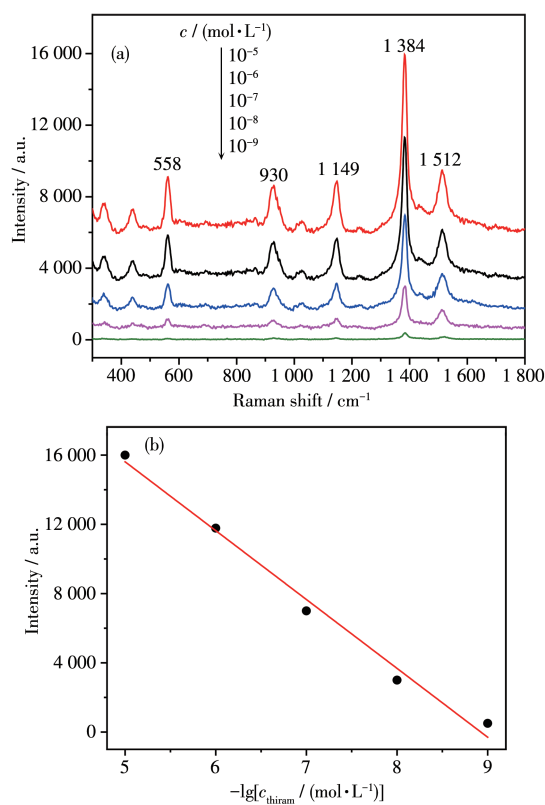


Fig.5 (a) SERS signals of thiram molecules with different concentrations of thiram from 10^{-5} to $10^{-9}\text{ mol}\cdot\text{L}^{-1}$ adsorbed on the active substrate of the GNRs; (b) Linear relationship of intensity of SERS peak at $1\,384\text{ cm}^{-1}$ versus logarithm of concentrations of thiram aqueous solution

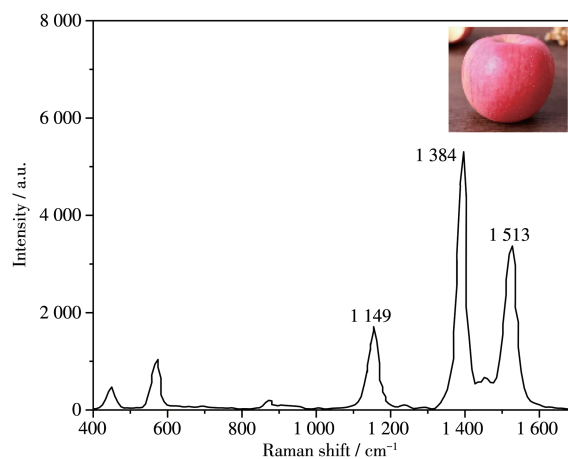


Fig.6 SERS signals of thiram which was washed from the surface of an apple

3 Conclusions

An effective and simple seed-growth method to fabricate GNRs with a cross-section diameter of *ca.* 25 nm and the longitudinal length of *ca.* 60 nm has been

presented. PEG-SH molecules were connected onto the GNRs to form close-packed GNRs film. After removing the surfactant by calcination process at 400 °C for 10 min, the close-packed uniform-size naked GNRs film can be used as SERS active substrate due to its strong SPR peaks of nanoscale gold and narrow gaps with size of sub-10 nm between two neighbouring GNRs. R6G and CV molecules were chosen to evaluate the SERS performance, and the results demonstrate that the GNRs film have high sensitivity and good uniformity. Moreover, it has been demonstrated that SERS spectra could offer an alternative method for identifying quantitatively highly diluted (10^{-9} mol·L⁻¹) thiram aqueous solution. This will open up a new opportunity for the actual testing ecological environment and drinking water safety.

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Supporting information is available at <http://www.wjhxxb.cn>

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