



Original Article

# Fabrication of SERS Substrates Using Au Nanoparticles Prepared by Laser Ablation in Distilled Water and Detection of Tetracycline at Low Concentrations

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**Abstract:** We studied to produce SERS substrates using gold (Au) nanoparticles (AuNPs) prepared by pulse laser ablation (PLA) in water. The colloidal Au NPs with average size of 23nm were deposited on a silicon wafer to form AuNPs/Si SERS substrate. Malachite green was chosen as a test analyte to examine the sensitivity of the SERS substrates. The SERS enhancement factor of the AuNPs/Si was found to be about  $10^6$ . The high sensitivity of the AuNPs/Si substrates was confirmed by the SERS spectra of malachite green detected with high quality at concentrations of 0.1ppm. The SERS substrates can detect SERS spectra of tetracycline at low concentrations of around 1ppm.

**Keywords:** Surface enhanced Raman scattering (SERS), pulse laser ablation, enhancement factor (EF), SERS substrate.

## 1. Introduction

Surface enhanced Raman scattering (SERS) was first discovered in 1974. Because of its huge sensitivity in spectroscopy, the investigations of SERS and its applications have developed ceaselessly. The electromagnetic (EM) enhancement mechanism of SERS was based on the amplification of the incoming and scattered radiation the by the excitation of localized surface plasmon resonances (LSPRs) involving plasmonic nanostructures [1-5]. Gold (Au), silver (Ag) are often used as the plasmonic materials because they have LSPRs that cover most of the visible and near infrared wavelength range where most Raman measurements occur [6,7].

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Because the enhanced EM field decreases rapidly with distance from metallic surface, the close contact between analyte molecule and the metallic nanoparticles is required to get the large EF for SERS [1-3]. Furthermore, the field enhancement is increased in nanostructured gaps between two or more neighboring structures [2,8]. There were various ways to produce plasmonic nanostructures for SERS (SERS substrates) including assemblies of metallic nanoparticles of different shape, electrochemically roughened metal surfaces, optical fiber sensors etc. [8]. For reproducible and uniform SERS substrates, the ordered arrays of nanoparticles were proposed and developed such as electron beam lithography arrays metal island films, metallic films over nanospheres/nanostructures and nanosphere lithography arrays [1, 2, 8]. However, the last are both highly complicated and expensive. Thus, low cost SERS substrates based on periodic structured surface (grating pattern surface) of commercial recordable compact discs (CDs), digital video discs (DVDs) and Blu-ray discs (BDs) were proposed [9-11]. The AuNPs or Ag NPs were deposited by different methods on the grating pattern surfaces to form SERS substrates.

In this work, we prepared SERS substrates from AuNPs synthesized by pulse laser ablation of pure Au pieces in distilled water. The Au NPs synthesized without surfactant in high purity are expected to give larger enhancement factor for SERS, suitable for Raman spectroscopy analysis. To prepare AuNPs/Si SERS substrates, we deposited colloidal AuNPs on silicon wafers by “drop-cast” method. Because of the weak interaction of AuNPs and silicon with organic materials, the AuNPs/Si substrates were expected for detection of antibiotics such as tetracycline, amoxicillin at low concentrations. A simple method that was used to rapidly produce SERS substrates of a high sensitivity is presented.

## 2. Experimental

A pure (9,99%) Au piece was placed at the bottom of a beaker filled with 10ml of distilled water. A laser beam from a Q-switched Nd: YAG pulsed laser (Quanta Ray Pro 230 - USA) was focused on the Au piece by a lens (focal length  $f = 150\text{mm}$ ). The Nd: YAG pulsed laser gives laser pulses with 8 ns pulse width in 10Hz repetition rate at 1064 nm fundamental wavelength. The average laser power was set at 450 mW. During the laser ablation, the beaker support system was rotated (8 rpm) by a motor. After several tests, the laser irradiation time of 15 min was chosen. The UV-Vis absorption spectrum (UV-2450 Shimadzu) of the colloidal AuNPs was shown in Figure 1 where one can see the characteristic plasmon resonance peak of AuNPs at 526nm. The morphology of Au NPs was observed by transmission electron microscope (TEM, JEOL JEM-1010). The AuNPs were nearly spherical in shape (Figure 2a) and their sizes range from 5 to 45 nm with an average size of 17 nm (Figure 2b). The concentration of Au NPs in water determined by Atomic absorption spectroscopy (AAS) was about 96mg/L.

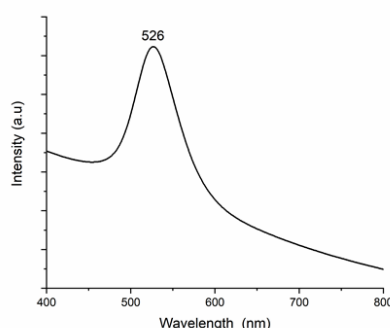


Figure 1. UV-Vis Absorption spectrum of the colloidal AuNPs in water.

A silicon (Si) wafer was cut in square-shaped plates (10x10mm) and then rinsed carefully with ethanol and distilled water. A pipet (10 -100  $\mu\text{l}$ ) was used to drip a drop of 15  $\mu\text{l}$  colloidal AuNPs on the Si plate and let dry in a heater at 70° C. The Si plates were placed on a horizontal plane which was adjusted and controlled by a bubble level. The distance between the tip of pipet and Si surface was fixed at 3mm. After dripping, the colloidal AuNP droplet forms a nearly circular spot of 6mm diameter on the Si surface. Because of surface tension and capillary flow effects, the density of AuNPs on Si surface increases from center to the rim of the circular AuNP spot (the “coffee ring” effect). After several experiments to determine suitable active area for SERS we chose the area of 700  $\mu\text{m}$  size near the rim of the circular AuNPs spot. The size of the chosen SERS active area was large enough when taking SERS spectra by Micro Raman spectrometer with laser spot of 2 $\mu\text{m}$ .

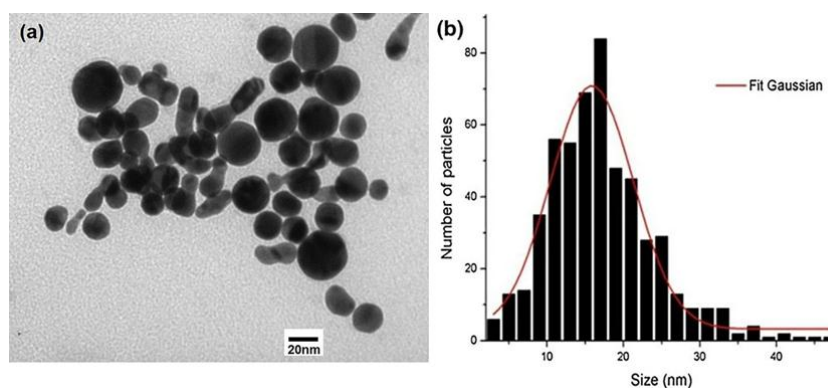


Figure 2. TEM image (a) and size distribution (b) of Au NPs prepared in distilled water.

This procedure can be repeated to get more layers of Au NPs on Si plates. The SERS substrates prepared by this procedure are denoted by AuNPs/Si. We prepared substrates with 2, 4 and 6 AuNPs layers on Si plates and denoted by 2AuNPs/Si, 4AuNPs/Si and 6AuNPs/Si substrates, respectively. The SEM image (Nova Nanosem 450-FEI) of 4AuNPs/Si substrates was shown in Figure 3. Malachite green ( $\text{C}_{23}\text{H}_{25}\text{ClN}_2$ ) solutions were prepared in distilled water to be used as analytic samples to examine SERS effect of the SERS substrates. Raman spectra of the samples were taken by a micro-Raman spectrophotometer (LabRAM HR 800 HORIBA JobinYvon) using the 632,8nm excited wavelength.

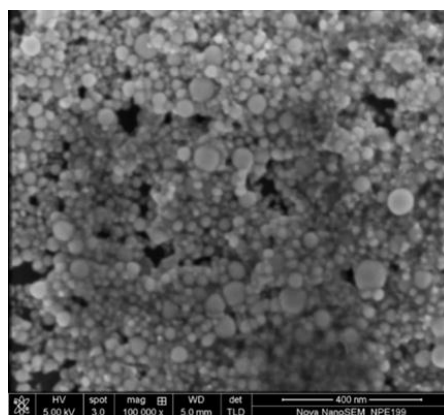


Figure 3. SEM image of the 4AuNPs/Si substrate water.

### 3. Results and Discussion

For comparison, in Fig. 4 the SERS spectrum of malachite green (MG) at concentration of 10ppm on the 4AuNPs/Si (Fig.4a) is attached to the SERS spectrum of MG at concentration of  $10^5$  ppm on the Si plate without AuNPs.

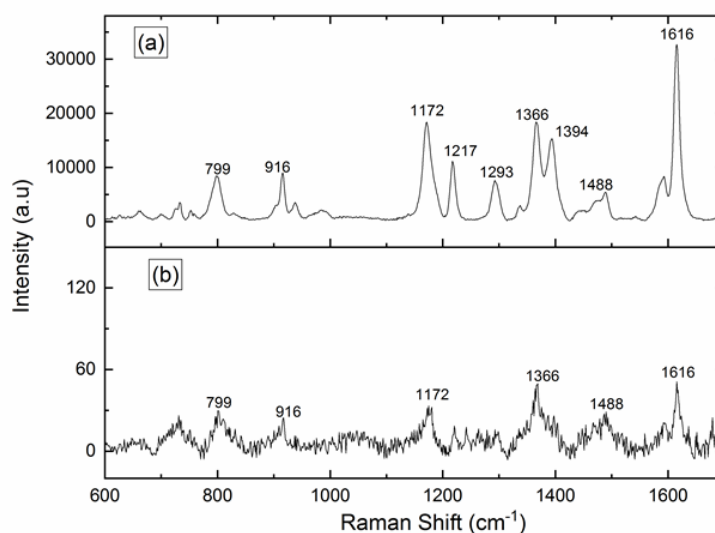


Figure 4. SEM image of the 4AuNPs/Si substrate water SERS spectrum of MG at 10ppm concentration from the 4AuNPs/Si substrate (a) and Raman spectrum of MG at  $10^5$  ppm concentration from Si plate without AuNPs (b)

The Raman spectrum of MG at the very high concentration on the Si plate is very weak with some MG characteristic peaks. Meanwhile, most characteristic SERS peaks of MG are clearly observed from the 4AuNPs/Si substrate with high intensity in agreement with Raman spectra of MG reported in the literature [12,13]. The strong peaks of MG at  $1172\text{ cm}^{-1}$  is caused by in-plane vibrations of ring C -H. The other strong peak at  $1616\text{ cm}^{-1}$  and the peaks at  $1594\text{ cm}^{-1}$ ,  $1488\text{ cm}^{-1}$  are assigned to the vibrations of ring C – C. The  $1394\text{ cm}^{-1}$  and  $1366\text{ cm}^{-1}$  peaks are assigned to N-phenyl stretching mode. The distinguished  $1293$ ,  $1217$ ,  $916$ , and  $799\text{ cm}^{-1}$  peaks are attributed to C-C ring, C-H rocking, C-H out-of-plane bending, ring C -H out-of-plane bending and in-plane stretching of Benzene rings respectively [12,13]. The result shows that the 4AuNPs/Si substrate gives high SERR enhancement.

We observed SERS spectra of MG at the same concentration on the SERS substrates with different numbers of AuNPs layers to determine the suitable AuNPs density for SERS. Figure 5 shows the SERS spectra of MG at the concentration of 10ppm from the 2AuNPs/Si, 4AuNPs/Si and 6AuNPs/Si substrates.

The SERS spectrum intensity increases clearly when the numbers of Au NPs layers increases from 2 to 4 layers. However, when the number of AuNPs layers increases from 4 to 6, the SERS spectrum intensity increases slowly. Thus, the 4AuNPs/Si substrate was chosen for the next experiments.

The average SERS enhancement factor (EF) of the 4AuNPs/Si substrate was estimated following a definition of EF given by Eric C. Le Ru et al. [22]:

$$EF = \frac{I_{SERS}/N_{Surf}}{I_{RS}/N_{Vol}} \quad (1)$$

where  $N_{Vol} = c_{RS} \cdot V$  is the average number of molecules in the scattering volume  $V$ ,  $c_{RS}$  is the concentration of analyte for the Raman (non-SERS) measurement, and  $N_{Surf}$  is the average number of adsorbed molecules in the same scattering volume for the SERS experiments.

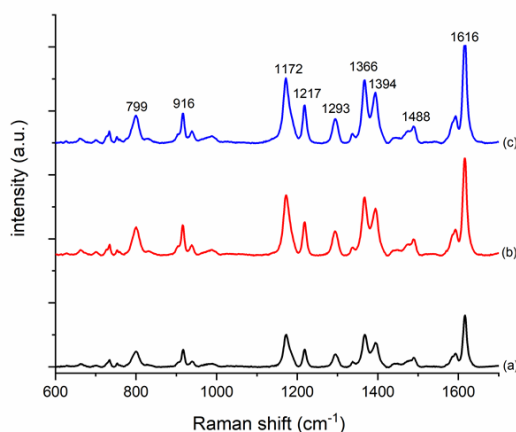


Figure 5. SERS spectra of MG at 10ppm concentration from 2AuNPs/Si (a), 4AuNPs/Si (b) and 6AuNPs/Si substrates. (c)

In approximation we take  $N_{Surf} = C_{SERS} \cdot V$  where  $C_{SERS} = 10\text{ppm}$  is the concentration of MG dropped on the SERS substrate with the same volume as non-SERS measurement. In fact, the average number of adsorbed molecules in SERS substrate  $N_{Surf} < C_{SERS} \cdot V$ . The concentration of MG dropped on the Si plate without Au NPs is  $C_{RS} = 10^{-5}\text{ppm}$ .

The EF was calculated for MG at the characteristic Raman peak of  $1616\text{cm}^{-1}$ . By the same condition for Micro Raman measurement we recorded Raman spectra and evaluate the intensity of SERS peak  $I_{SERS}$  and the intensity of Raman peak  $I_{RS}$  at the same peak  $1616\text{cm}^{-1}$ . The EF of the 4AuNPs/Si calculated by formula (1) is around  $10^6$ .

In order to examine the sensitivity of the AuNPs/Si substrates we analyzed SERS spectra taken when using the 4AuNPs/Si substrate of MG at different low concentration. The SERS spectra of MG at concentrations of 10ppm, 1ppm and 0.1 ppm from the 4AuNPs/Si substrate were given in Figure 6.

When the MG concentration decreased from 10ppm to 0.1ppm the peak intensities of MG spectra decreased quickly, however most characteristic peaks of MG remains in good resolution in the SERS spectrum of MG at concentration of 0.1ppm. This result showed a large EF of the 4AuNPs/Si substrate.

We used the 4AuNPs/Si substrate to detect tetracycline hydrochloride at different concentrations in water to investigate the potential applications of the SERS substrates for detection of antibiotic residues. Figure 7 shows the SERS spectra of tetracycline at concentrations of 100ppm, 10ppm and 1ppm.

The characteristic peaks of tetracycline hydrochloride ( $C_{22}H_{24}N_2O_8$ ) at low concentrations were detected in good agreements with SERS spectra of tetracycline reported in the literature [14-19]. Tetracycline is a derivative of naphthacene according to the complicated chemical structure of tetracycline (Figure7a). Raman spectrum of tetracycline depends strongly on several factors such as pH,

temperature, solvent, live time and the interaction with SERS substrates that results in a band shifts on the order of several  $\text{cm}^{-1}$  [14,15].

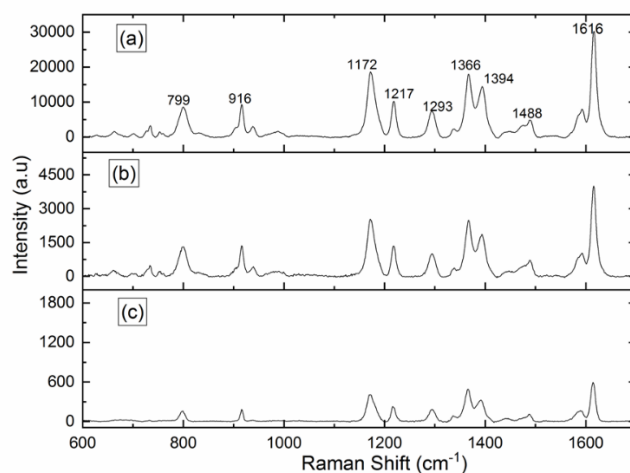


Figure 6. SERS spectra of MG at concentrations of 10ppm (a) 1ppm (b) and 0.1 ppm (c) from the 4AuNPs/Si substrate.

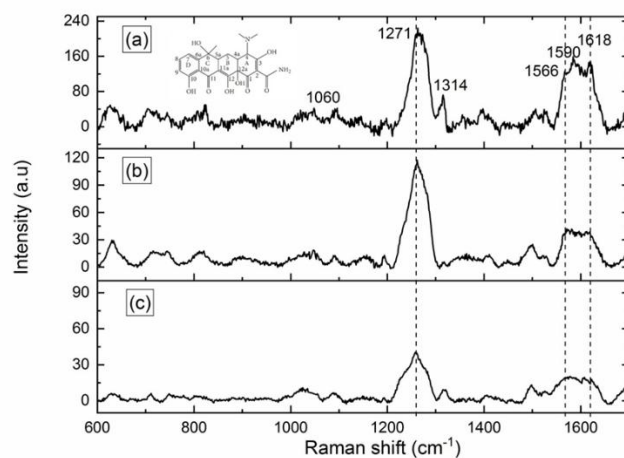


Figure 7. SERS spectra of tetracycline at concentrations of 100ppm (a) 10ppm (b) and 1ppm (c) from the 4AuNPs/Si substrates

The strong peak at  $1271\text{ cm}^{-1}$  was attributed to bending vibration of  $\text{CH}_{4,4a,5,5a}$ ,  $\text{OH}_{12}$ , and amid-NH and stretching vibration of  $\text{CO}_{10}$ ,  $\text{CO}_3$ ,  $\text{CH}_{7,8,9}$ , amid-NC,  $\text{C}_{4a}\text{C}_5$ , and benzene ring D [20,21]. The peak at  $1314\text{ cm}^{-1}$  was assignable to ring breathing  $\nu(\text{C}_{10}\text{-C}_{10a})$ ,  $\nu(\text{C}_{6a}\text{-C}_7)$ ,  $\nu(\text{C}_9\text{-C}_{10})$  relative to the planar  $\text{C}_9$  to  $\text{C}_{12}$  moiety. The peaks at  $1572\text{ cm}^{-1}$  and  $1590\text{ cm}^{-1}$  were assignable to bending and stretching mode  $\delta(\text{OH}_{10,12})$ ,  $\nu(\text{D})$ ,  $\nu(\text{C}_{11a}\text{C}_{12})$ . The characteristic peak at  $1618\text{ cm}^{-1}$  was ascribed to amide stretching and bending modes  $\nu(\text{O-C}_1)$ ,  $\nu(\text{C}_2\text{-C}_3)$ ,  $\delta(\text{amide-CO})$ ,  $\delta(\text{amide-NH})$ . The peak at  $1060\text{ cm}^{-1}$  was attributed to stretching vibration of  $\text{CO}_3$  [14-16].

The SERS peak intensity decreased with concentration of tetracycline. In the SERS spectrum of tetracycline at concentration of 1ppm, some tetracycline peaks disappeared, however the characteristic peaks at 1271 and 1618  $\text{cm}^{-1}$  are remaining and discrete for tetracycline identification.

#### 4. Conclusion

The SERS substrates using AuNPs synthesized by pulse laser ablation in distilled water were prepared. The advantages of laser ablation method help to produce rapidly AuNPs of high purity that is suitable for Raman analysis. The AuNPs synthesized without surfactant in high purity enable AuNPs/Si substrates to attain a large enhancement factor for SERS. The high sensitivity of the AuNPs/Si substrates was confirmed by SERS spectrum obtained from 0.1ppm-concentration MG sample. The detection of tetracycline at low concentrations by the AuNPs/Si substrates suggested a potential application of the SERS substrates for detection of antibiotic residues.

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