

# Low Cost Paper-based SERS Sensor using Gold Nanoparticles for Molecular Detection

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**Abstract.** SERS (Surface Enhanced Raman Spectroscopy or Surface Enhanced Raman Scattering) is a surface sensitive technique and enhanced form of Raman spectroscopy. In this technique, nanostructures provide hotspots for the analyte detection, which enhances the Raman signal  $10^{10}$ -  $10^{11}$  times higher signal compared to the normal Raman spectroscopy. SERS is a very powerful technique for quantitative and qualitative analysis of various molecules. The technique is highly sensitive and can detect even a single molecule of the sample. These sensors can be used to detect a variety of biological and chemical samples. This research work presents the development of the SERS substrate using gold nanoparticles. Rhodamine B, a marker molecule is used to determine the intensity enhancement capability of the substrates. Raman spectrometer and Uv-vis spectrophotometer is used for measurement. Gold nanoparticles, sized 40-45 nm, were deposited on the porous cellulose paper; developing the Nano-gaps between the particles. Electromagnetic coupling occurs in the Nano-gaps which is responsible for electromagnetic enhancement. Availability of large number of hot-spots on the substrate leads to better enhancement. We used  $10^{-6}$ M Rhodamine samples to characterize the SERS substrate.  $10^5$  fold enhancement in Raman signal with uniform intensity was achieved. These type of low cost paper sensors will find numerous biochemical and bioanalytical application.

**Keywords:** SERS, Gold Nanoparticle, Rhodamine B.

## 1. Introduction

Raman spectroscopy is an optical technique that used to provide information of the molecular Structure as well as the chemical composition of a sample. This technique is highly specific due to the fingerprint information that gives of a sample, and to determine quantitatively the amount of a substance in a sample. Its major disadvantage is weak signal due to low scattering, which limits the application in sample at low concentrations.

The optical signal from Raman scattering is usually very weak and hard to be detected. In order to enhance the weak signal of Raman scattering, scientists have contributed great efforts to develop different kinds of surface enhanced Raman Scattering (SERS) substrate based on various Nano-structures of noble metals, such as gold and silver. The development of SERS provides Raman technique with the potential for trace detection of chemical substances.

Surface-enhanced Raman spectroscopy (SERS) is a technique for molecular detection and characterization that relies on the enhanced Raman scattering of molecules that are adsorbed on, or near, SERS-active surfaces, such as nanostructured gold or silver. SERS was first discovered in the 1970s by Fleischmann and co-workers through the enhanced signal of pyridine on electrochemically roughened silver electrodes [1].

The enhancement of the Raman scattering is as of yet still not fully understood. However, two main mechanisms have been proposed to provide the enhanced characteristic. Electromagnetic enhancement is the dominant mechanism that causes enhanced signal by SERS, usually in the range of  $10^6$  -  $10^8$ . The enhancement comes due to the characteristics exhibited by metals, which possess free conduction electrons around ions within the structure. These generally remain in a neutral oscillating state, where the plasmon exist by themselves in a neutral zero charge [2, 3]. Metals also possess a negative real and small positive imaginary dielectric constant upon the surface, such as in thin films. Therefore, when an incident wavelength interacts with these free conduction electrons and there is coherence between the oscillation of the conduction electrons and the electromagnetic wavelength, surface plasmon polarizations of the electrons will occur, causing the electrons to have additional energy and oscillate between the metal and the dielectric interface, also known as surface plasmon resonance (SPR). With regards to localized surface plasmon resonance (LSPR) it typically occurs in metal nanostructures, whereby the incident electromagnetic wave interacts with a particle which is

much smaller than the wavelength and this causes plasmon to oscillate locally around the nanostructure at a frequency known as LSPR [4].

The wavelength-dependent effect of surface plasmon resonance causes an enhancement of the incident electric field within the range of  $10^2$  -  $10^4$  in the vicinity of the metal surface [5, 6]. The magnitude of the electromagnetic field outside of the particle depends quite significantly on the type of metal, the wavelength used and the dielectric material or environment the metal is in. Chemical enhancement is also noted as one of the two mechanisms that contribute to the SERS effect, providing an enhancement factor of  $10^2$  for the overall SERS intensity.

Recently, flexible SERS platforms such as glass slide, polymer material and paper material emerged as an alternative for flexible SERS substrate. Flexible SERS substrate offers non-invasive and nondestructive detection of analytes [7, 8]. Various types of flexible SERS substrates have been described in literatures such as adhesive tape, flexible polymer film, polydimethylsiloxane (PDMS), Cellulose paper and etc. J Chen et al. have demonstrated the flexible SERS based substrate of Au nanoparticles which is limited to 2D structures [9, 10]. Cellulose Paper is widely used in daily life as well as in research. Here, we have demonstrated the Gold nanoparticles (AuNPs) on flexible cellulose paper. Metal NPs on Paper surface provide high density of hot spots in compare to 2D structures. Further, Rhodamine B sensing at low concentration was demonstrated on the developed flexible SERS platform.

## 2. Experimental

### 2.1. Materials

Chloroauric acid ( $\text{HAuCl}_4$ ), Trisodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ ), Sodium borohydrate ( $\text{NaBH}_4$ ), Hydrochloric acid (HCl) and nitric acid ( $\text{HNO}_3$ ), Rhodamine B were purchased from *Sigma-Aldrich*. Deionized water was used for all solution preparation ( $18\text{M}\Omega\text{cm}$  resistivity).

### 2.2. Synthesis of Gold nanoparticles

Gold nanoparticles (AuNPs) were synthesized by using chemical approach. To synthesize the AuNPs 300ml of 0.05mM aq. gold chloride solution is heated at 100 degree centigrade and after that slowly mixed 30 ml of aq. 38.8 mM trisodium citrate solution. Reaction was carried out at 2000 RPM. Here trisodium citrate works as reducing agent as well as stabilizers. At 100 degree centigrade we got the colloidal solution of AuNPs. After 20 minutes we get the wine red color of the AuNPs that was uniform. Stored the AuNPs at 4 degree Celsius for further use.

### 2.3. SERS active substrate Preparation

SERS substrates were made, employing paper as substrate, to deposit the nanoparticles. In Raman spectra, silicon presents a strong peak at  $520\text{cm}^{-1}$  but does not present fluorescence signal; making it suitable for Raman analysis. But silicon substrates has the disadvantage of a high cost, while common glass is a cheap material, however glass presents high fluorescence signal, that can overlap the Raman signal of the sample. Paper is low cost material and doesn't show any strong peak of Raman signal and fluorescence signal is also not present. To fabricate the SERS active substrates, we used the colloidal solution of gold nanoparticles. Cellulose paper were used to make SERS active substrate. Cellulose papers were cut into  $2\times 2\text{cm}^2$  pieces and  $20\ \mu\text{l}$  of AuNPs solution was poured on the paper substrates and dried it. Diameter of SERS active area was around 0.75cm as shown in figure 1.

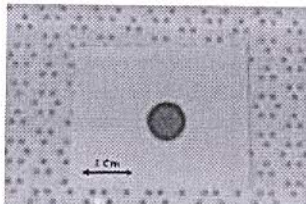


Fig 1. Synthesized AuNPs deposited on paper substrate.

#### 2.4. Sample Preparation

Rhodamine B ( $C_{28}H_{31}ClN_2O_3$ ) is a dye that is basically used in biological application such as ELISA, flow cytometry and fluorescence microscope. In SERS analysis, Rhodamine B is used as model dye to probe the nature of SERS substrate. It shows extreme fluorofore in visible light. To test the substrates different concentration of Rhodamine B is prepared i.e.  $10^{-6}$ ,  $10^{-9}$ ,  $10^{-12}$ ,  $10^{-15}$  and  $10^{-18}$  molar. All samples were prepared by serial dilution techniques.

For SERS substrate analysis, 5  $\mu$ l of each sample is transferred on the substrate and dried, it in open environment for 2 hours. Raman spectra is measured using Renishaw's Raman spectroscopy equipment at 785nm.

### 3. Results and discussion

#### 3.1. Characterization of Gold Nanoparticles

Reaction between Chloroauric acid and Trisodium citrate were carried out at 100°C and 2000 RPM that turns in red wine color after 20 minutes. No further color changes was noticed after Gold nanoparticles synthesis. Red wine color shows the colloidal solution of Gold nanoparticles. On passing the laser through this solution, laser was completely absorbed by the nanoparticles.

**Uv-Vis Spectroscopy:** Gold nanoparticles exhibit localized surface plasmon resonance (LSPR) that is the collective oscillation of electrons in the conduction band of gold nanoparticles, in resonance with a specific wavelength of incident light. Due to LSPR, Gold nanoparticles show strong absorbance band in the visible region (500 nm-600 nm), which can be measured by UV-Vis spectroscopy. The spectrum is dependent both on the size and shape of gold nanoparticles. The peak of absorbance of the sample, correlates linearly to the concentration of nanoparticles in solution. In our study, highest peak of absorbance was at 546nm. By using the standard data of Uv-Vis spectroscopy, for gold nanoparticles, AuNPs diameter was confirmed which was around 40-50nm. We can control the size of NPs using molar ratio of reactant.

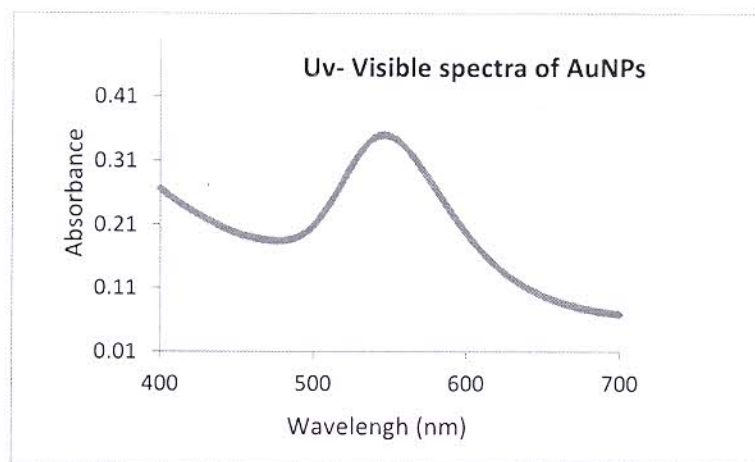


Fig 2. UV-Visible absorption spectrum of Gold nanoparticles samples

#### 3.2. Characterization of SERS substrate

Optical images were captured using Zeta optical microscope. That shows nanoparticles randomly dispersed on paper surface and some nanoparticles aggregated some places. Fig. 3. shows the roughness of surface. A roughened surface of substrate have more number of hot spot areas of different size that provide the enough space for binding of analytes. Different size of gaps between the nanoparticles helps to identify different size of molecules. Energy dispersive spectroscopic (EDS) analysis (Fig. 4.) of SERS substrate shows the presence of Au NPs.

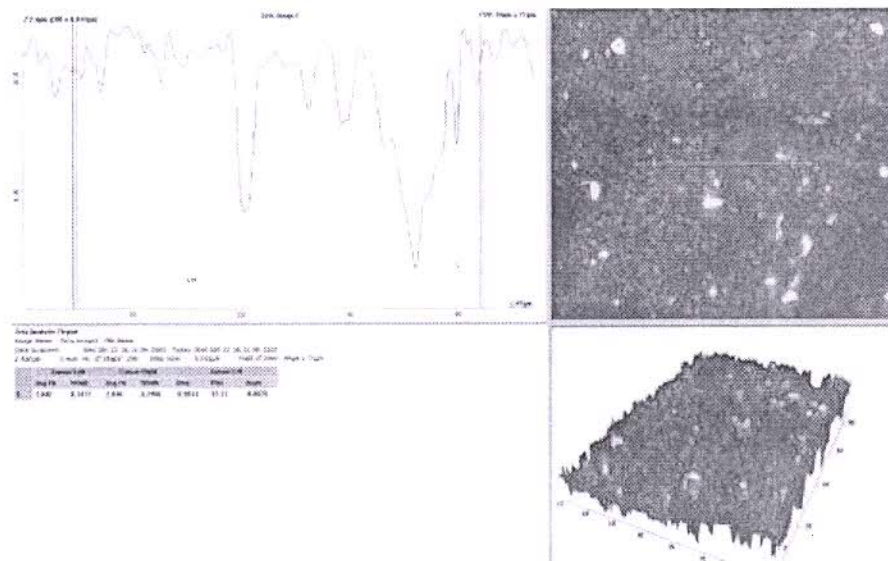


Fig. 3. Optical image of paper SERS substrates using Zeta optical microscope shows the roughness of substrates.

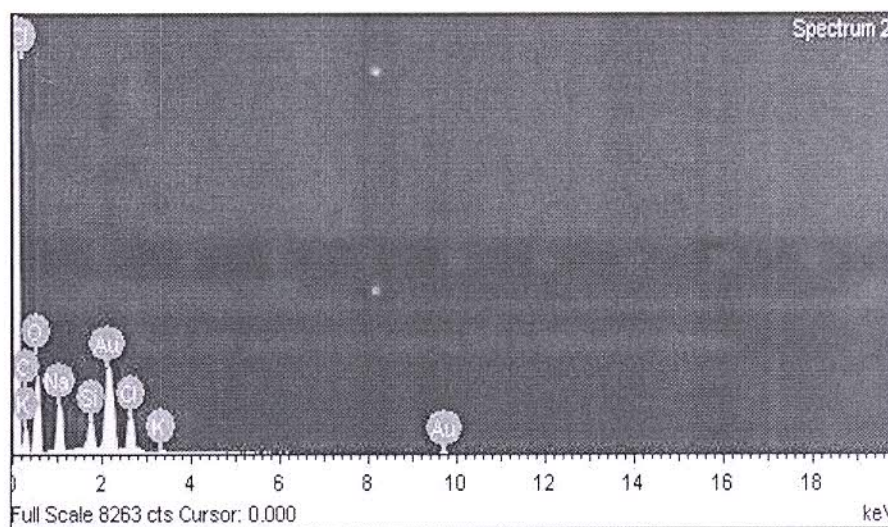


Fig. 4. EDS spectra of transferred Au NPs on SERS active substrate

### 3.3. SERS spectra analysis

Rhodamine B is used as a model molecules to study the SERS substrate. SERS spectra is measured using Renishaw Raman spectroscopy tool. 785nm wavelength of laser is used and 3mW power is used. All samples were scanned in the range of 400 - 2000 Raman shift/cm-1 and with 20x objective. Exposure time was 20 seconds.

Raman spectra is showing below for paper substrate. In the substrate  $10^{-6}$  M Rhodamine B is used. During the experiment all the parameters were same. In both, two different substrate is used and captured the spectra on same concentration. Both the sample shows the approximately same excitation peak. One reference sample is also used to compare the spectra.

SERS spectra of Rhodamine B on AuNPs on paper is shown in Fig. 5. Raman bands at around  $620\text{ cm}^{-1}$  shows the presence of C-C-C ring in a plane. At  $1190\text{ cm}^{-1}$  shows C-C stretching vibrations. The other Raman bands  $1360\text{ cm}^{-1}$ ,  $1510\text{ cm}^{-1}$ ,  $1599\text{ cm}^{-1}$ , and  $1651\text{ cm}^{-1}$  shows the aromatic C-C stretching vibrations of RhB. In

Figure 5 Raman spectra of RhB showing all the corresponding peaks. Peak at  $620\text{ cm}^{-1}$  is used for comparison of concentration and enhancement of sample. Further we can calculate the enhancement factor for comparison of different concentration and enhancement using this formula:

$$EF = (I_{\text{SERS}}/C_{\text{SERS}}) / (I_{\text{Raman}}/C_{\text{Raman}})$$

Where,  $C_{\text{SERS}}$  and  $I_{\text{SERS}}$  is concentration and peak intensity for SERS sample and  $C_{\text{Raman}}$  and  $I_{\text{Raman}}$  is concentration and peak intensity for normal Raman sample.

The SERS enhancement factor is calculated by comparing the SERS signal excited the RhB adsorbed on the SERS platform and the Raman signal of bulk RhB for control measurement. For paper substrate measurement, intensity of rhodamine RhB peak ( $\sim 620\text{ cm}^{-1}$ ) measured in SERS experiment from an aqueous solution ( $10^{-6}\text{M}$ ) of RhB was compared with control Raman measurement of bulk RhB without SERS substrate. The enhancement factor was calculated to be approximate  $10^5$  for the developed SERS platforms.

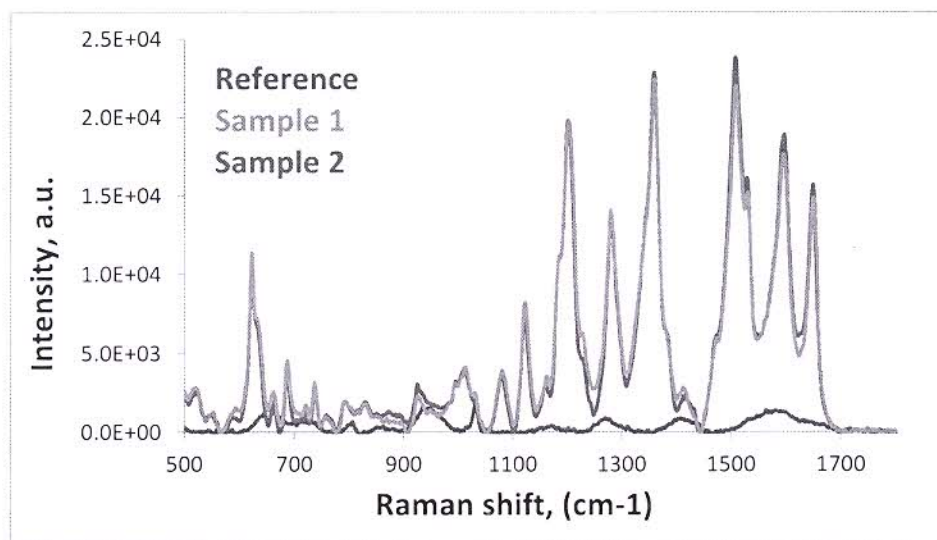


Fig 5. SERS spectra of  $10^{-6}\text{M}$  Rhodamine B solution on AuNPs on Paper substrate

#### 4. Conclusions

The work demonstrates a novel approach to realize SERS active substrates. Fabrication process is comparatively low cost and feasible for mass production. In SERS substrate, abundant hot spots are developed due to small variation in size of nanoparticles. Electromagnetic coupling occurred in these closely separated Nano-gaps that are responsible for electromagnetic enhancement. The substrate detected Rhodamine B molecules at low concentration i.e.  $10^{-6}\text{ M}$  indicating  $10^5$ -fold enhancement in Raman signal. The developed substrates showed the uniform intensity all over the area hence they can be utilized for numerous chemical and bioanalytical analysis.

#### References

1. C. Zhang, P. Yi, L. Peng, X. Lai, J. Chen, M. Huang M, Ni J. Continuous fabrication of nanostructure arrays for flexible surface enhanced Raman scattering substrate. *Scientific reports*, 7 (2017), pp. 39814.
2. A. Kumar, J. Agrawal, A. K. Sharma, V. Singh, and A. Agarwal, A cost-effective and facile approach for realization of black silicon nanostructures on flexible substrate. *Journal of Materials Science: Materials in Electronics* 30 (2019), 16554–16561.
3. Chang Feng, Ajay Agarwal, E. Widjaja, Marc Garland, S.M. Wong, L. Linn, N. M. Khalid, S. M. Salim & N. Balasubramanian, Metallization of Silicon Nanowires and SERS Response from a Single Metallized Nanowire, *Chem. Mater.* 21 (2009), pp 3542–3548.

4. Coluccio ML, Das G, Mecerini F, Gentile F, Pujia A, Bava L, Tallerico R, Candeloro P, Liberale C, De Angelis F, Di Fabrizio E, Silver-based surface enhanced Raman scattering (SERS) substrate fabrication using nanolithography and site selective electroless deposition. *Microelectron Eng* 86 (2009), 1085–1088.
5. Nguyen BH, Nguyen VH, Tran HN, Rich variety of substrates for surface enhanced Raman spectroscopy. *Adv Nat Sci Nanosci Nanotechnol* 7 (2016), 033001.
6. Hansang Cho, Brian Lee, Gang Liu, Ajay Agarwal and Luke P. Lee, Label-free and highly sensitive biomolecular detection using SERS and electrokinetic preconcentration, *Lab chip*, 9 (2009), pp. 3360.
7. E. Wiercigroch, E. Szafraniec, K. Czamara, M. Z. Pacia, K. Majzner, K. Kochan, A. Kaczor, M. Baranska, and K. Malek, "Raman and infrared spectroscopy of carbohydrates: A review". *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 185 (2017), pp. 317.
8. Fang, C., Agarwal A., Ji H., Karen, W. Y., Yobas, L., Preparation of a SERS substrate and its sample-loading method for point-of-use application, *Nanotechnology*, 20 (40) 2009, Article Number: 405604.
9. Jing Chen I, Gaowu Qin, Jiansheng Wang, Jiangyu Yu, Bo Shen, Song Li, Yuping Ren, Liang Zuo, Wen Shen, Biswajit Das, One-step fabrication of sub-10-nm plasmonic nanogaps for reliable SERS sensing of microorganisms. *Biosens Bioelectron* 44 (2013), 191-7.
10. Dinish U. S, Fu Chit Yaw, Ajay Agarwal and Malini Olivo, Development of Highly Reproducible Nanogap SERS Substrates: Comparative Performance Analysis and its Application for Glucose Sensing, *Biosensors and Bioelectronics*, 26 (2011), pp. 1987-1992.