

Surface Enhanced Raman Spectroscopic Studies of Analytes Using Various Approaches

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Abstract

Surface enhanced Raman spectroscopic studies have been carried out to overcome the weak Raman signals. Three different approaches have been applied to understand the mechanism and sensitivity of the technique. Chemically synthesized Silver nanoparticles using green precursor molecules provide good enhancement of the Raman signal from 4-nitrophenol. However, the static SERS method is limited by its reproducibility. This has been overcome by introducing automated microfluidic systems such as lab-on-a-chip (LOC). Preliminary SERS experiments using LOC on pyridine has been demonstrated in this study. Nano-fabrication method has also been explored to obtain background free, reproducible silver nanostructures and SERS of pyridine using structures shows promising results. Further, these can be incorporated into the microfluidic channels for LOC applications.

Introduction

Scientists are constantly trying to develop techniques which could enhance the weak Raman signals. In recent past, the techniques developed are Resonance Raman (RR), Surface-Enhanced Raman Spectroscopy (SERS), Surface-Enhanced Resonance Raman Spectroscopy (SERRS), stimulated Raman, Coherent Anti-Stokes Raman (CARS), Ultrafast Raman Loss spectroscopy (URLS) *etc.* SERS enhances the signal of the probed species placed on or nearby the surface of the metal (e.g. silver, gold) nano-particles due to surface plasmons¹. Since the discovery of the Surface Enhanced Raman scattering effect around four decades ago²⁻⁵, little had the discoverers fathomed the dormant potential this analytical tool carried underneath, and the vistas it would open in the field of research in Raman spectroscopy. The confluence of Raman Spectroscopy and nanotechnology overcame the inherent drawback of the weak signals produced in conventional Raman spectroscopy. Revival in the field was brought about in the past decade

by the enormous development of spectroscopic instrumentation and nanotechnology. From the first published report on the studies of pyridine on roughened silver electrode, this technique has matured and emerged as a state-of-the-art analytical tool for real world sensor applications. SERS spectroscopy has been employed for sensitive and selective molecular identification in biological and chemical sensing. This technique has been applied for the trace detection of pesticides, bio-hazards, bacteria, molecules of biological interest, and genetic diagnostics to name a few⁶⁻⁸. A portable and cost-effective SERS instrument holds the promise for trace analysis in field as well as in clinics.

Our laboratory is involved in SERS studies using different approaches. On one hand we synthesize silver (Ag) and gold (Au) nanoparticles using conventional chemical reduction method while on the other we are using Lab-on-a-chip (LOC) for SERS along with fabrication of Ag and Au films. In the following sections we shall be discussing each method briefly. In the first

part of the study, silver nanoparticles have been synthesized using various biosynthetic methods. Recent publications have revealed that biosynthetically prepared silver nanoparticles (AgNPs) are relatively easy to produce, time efficient, cost effective, green and stable for long time. SERS studies using biosynthetically prepared AgNPs have been demonstrated in static phase. The key concern with SERS experiments is the reproducibility; the nanoparticles tend to aggregate with time, and the aggregation of SERS substrates (NPs) cause variations in the enhancement behaviour. This variation in enhancement could also be due to non uniform mixing and human error. This limitation can be overcome by performing SERS spectroscopic measurements in flow cells like LOC, which does not allow aggregation of nanoparticles and provide automated mixing leading to improved reproducibility. LOC devices were introduced in chemistry and molecular biology to improve the output information from molecular systems by efficient analytical procedures. The miniaturization allows the reduction of the quantity of samples to gain similar information⁹⁻¹¹. The next section of the study presents the LOC set up with its utilization to SERS using Lee-Meisel silver colloids. The colloids can be synthesized by various methods and have inherent surface changes according to the chemicals used. Thus, it becomes difficult for the analyte of the similar charge to come in the vicinity of metal particles. This issue is overcome by using activating agents such as KCl, HCl, NaOH *etc.* However, addition of activating agents may enhance the background signals. On the other hand, roughened metal films provide the efficacy of background free detection. Metallic nanostructures provide electric field enhancement due to confinement effect and hence SERS substrates are prepared by fabricating metal nanostructures. The theory evolves from the early stages of SERS substrate fabrication by electrochemically roughened Ag electrodes². The fabrication methods since then have advanced with the technological innovation in micro and nano fabrication processes. Top-down approach

such as direct writing of nanostructures with electron beam or focused ion beam, has played a key role in fabricating controlled structures¹². Bottom-up architectures such as self-assembled structures enable large area substrate fabrication^{13, 14}. We have incorporated fabrication of Ag nanoparticles on Si<100> substrates by deposition of ultra thin film of the metal.

Experimental

Silver nitrate (AgNO_3), pyridine and *p*-Nitrophenol (PNP) were procured from SD Fine Chemicals. Clove and pepper were purchased from local market. The solutions were prepared using milliQ water (Bio cell Laboratories, Inc, Quantum® EX).

Raman spectra were recorded using Renishaw InVia Raman Microscope with a 50 mW green Ar ion laser with the excitation wavelength of 514.5 nm. The laser beam was set in position with a 50x long working distance (LWD) objective lens with a numerical aperture of 0.5 at a lateral spatial resolution of approximately $\sim 2 \mu\text{m}$ mounted on a Leica upright microscope (Leica Microsystems CMS GbmH Wetzlar, Germany) was employed for the experiments. Peltier cooled charge coupled device (CCD) array detector (1024 X 256 pixels) was used for detecting the signals from a 2400 grooves/mm grating light path controlled via Renishaw software (Wire 3.2), and were processed using Origin 8.5 (Origin Lab Corporation, MA, USA) software. The spectra were collected at a spectral resolution of 1 cm^{-1} . The acquisition time was 25 sec and number of accumulations was 1 for PNP. In case of static SERS and LOC experiments, $\sim 5 \text{ mW}$ incident power was used. The spectra for LOC experiments were acquired for 1s and accumulated only once.

SERS experiments on sputtered Ag films on Si were recorded on the substrate using LabRAM HR 800 equipped with 532 nm laser. Spectrum acquisition was performed by using 50 x LWD objectives to focus the laser beam ($\sim 20 \text{ mW}$) on the sample. The conventional Raman spectrum

of 0.1 M pyridine was acquired for 10s and the SERS spectrum was acquired for 1s.

SERS using biosynthesized Ag nanoparticles:

Silver nanoparticles (Ag NPs) were biosynthesized using previously reported methods using cloves and pepper as natural precursors^{15, 16}. Both cloves and pepper have eugenol as a component, in addition, pepper also contains piperine. Eugenol can release two electrons at a time which helps in the reduction of AgNO_3 . Briefly, 1.25g of cloves was soaked in 25ml of MilliQ water for 24h. The extract was collected after filtration and was used for further experiment. Synthesis of AgNPs was carried out by taking AgNO_3 as starting material. 3.33 mM AgNO_3 solution was stirred at 300 rpm and the temperature was maintained at 75° C, to which measured amount of clove extract was added. AgNO_3 to clove extract ratio was varied from 1:1 to 1:0.01 and the best yield was obtained for 1:0.25 ratio. The reduction was complete in about 2h which was indicated by the formation of greenish yellow colour of AgNPs. The same protocol was carried out for the biosynthesis of pepper reduced Ag colloids except that 2.5g of pepper was used to prepare the extract. Hereafter we refer silver nanoparticles obtained by the reduction of clove extract and pepper extract as C AgNPs and P AgNPs respectively. SERS samples were prepared by mixing equal amounts of biosynthesized Ag colloids described above. 50 mM HCl was added for activation of colloids. Aluminium substrate with cup shaped cavity was used for recording the SERS spectra.

Results and Discussion

Detailed literature survey reveals that biosynthesized silver nanoparticles have not been widely used for obtaining SERS of molecules. The simplicity of the synthesis procedure as well as the advantage over other reported methods was the motivation for using the biosynthesized route for SERS studies. *p*-Nitrophenol (PNP) was chosen as an analyte to demonstrate SERS. SERS studies were conducted on μM solution

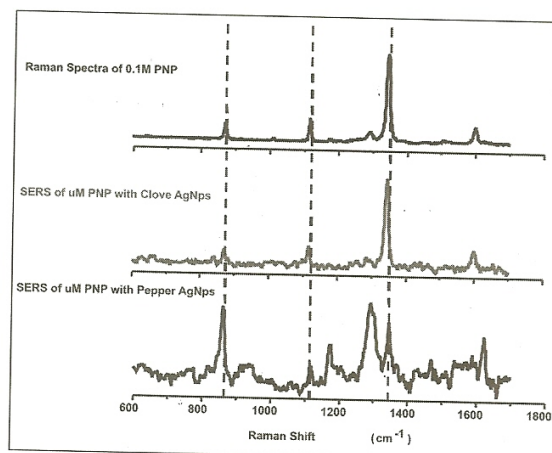


Figure 1: Raman and SERS Spectra of A) 0.1 M PNP, B) μM *p*-Nitrophenol obtained using Clove extract reduced AgNPs and, C) Pepper extract reduced AgNPs respectively.

of PNP with both C and P AgNPs respectively. Figure 1 shows the Raman spectra of 0.1 M PNP solution in milliQ water and SERS spectra of micro molar concentration of PNP using C and P AgNPs respectively. The conventional Raman spectra for 0.1 M PNP is observed at 867, 1116, 1289, 1342 and 1597 cm^{-1} respectively (Figure 1A). The observed bands correlate well with the literature values^{17, 18}.

The peak at 867 cm^{-1} has been attributed to CH out-of-plane bending motion. The peak at 1116 cm^{-1} corresponds to CH in-plane bending motion and NO_2 symmetric stretching mode. The 1289 cm^{-1} band can be attributed to CN stretching mode and NO_2 symmetric stretching mode. The 1342 cm^{-1} peak has been assigned for CH bending motion of the phenyl ring and CN stretching mode. The band at 1597 cm^{-1} has been attributed to ring deformation.

The SERS spectra obtained using C AgNPs matched well with the conventional Raman spectra of 0.1 M PNP while some variation in peak positions were observed in case of SERS of P AgNPs (Figure 1 B and 1 C). The peaks showing maximum variation for SERS of P AgNPs were observed at 867, 1116 and 1342 cm^{-1} . Notable SERS effect was observed for peaks centred at 1289 cm^{-1}

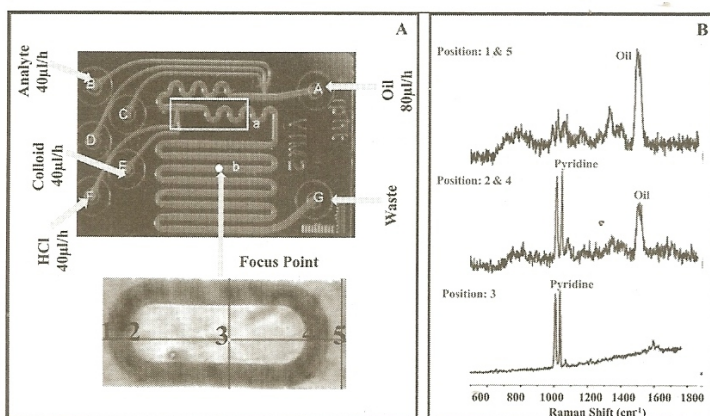


Figure 2. (A) Schematic of the lab-on-a-chip and the drop (enlarged) under investigation at point "b". (B) SERS spectra recorded at different position of the drop.

in case of P AgNPs. It is reported in the literature that the intensity changes observed in the SERS band can be different due to different adsorption site¹⁸. From these observations, it can be inferred that PNP interacts with the Ag NPs through the NO₂ group. However, the strong enhancement of the 1289 cm⁻¹ peak in case of P AgNPs can also be attributed to the background from the Ag colloids.

SERS study using Lab-on-a-chip:

To demonstrate the applications of LOC in SERS, preliminary experiment has been done using pyridine (0.1 M) as analyte. Silver colloids were synthesized by the reduction of silver nitrate solution using Lee-Meisel method¹². Briefly, 45 mg of AgNO₃ was dissolved in 250 ml of water and heated at 150 °C till boiling. 5ml of 1% trisodium citrate solution (aqueous) was added to boiling silver nitrate solution and left for boiling for 1 hour. The resulting colloids were filtered and stored in cool and dark place for SERS. The HCl (0.1M) solution was used for activation of silver colloids. Pyridine, HCl and colloid solutions were introduced into the chip at the same flow rate (40µl/hr). However, mineral oil was introduced at the rate of 80µl/hr. Mineral oil was used to create segmented flow for better mixing and also to prevent direct contact between the aqueous phase and the micro channel walls. The SERS

spectra were recorded at indicated focus point on the LOC (Figure 2A).

The spectra from positions 1 and 5 indicate oil which helps in the formation of drops. Positions 2 and 4 correspond to the interface between the oil and the mixture of colloids, pyridine and HCl. The mixture was maintained at 1:1:1 ratio by volume as the flow rates were kept same for all the three solutions. The position 3 represents the spectrum of pyridine recorded from the centre of the drop. As the flow was continuous, reproducible drops of identical size

could be obtained. SERS spectra from 50 different drops were recorded and all the spectra appeared similar to their corresponding positions on the respective drop. This demonstrates the high reproducibility of SERS using an automated flow system (LOC).

SERS on Ag Films:

Fabrication

As discussed earlier, SERS substrates are prepared by fabricating array of nanostructures which can be realized by lithographic techniques. In conventional photolithography technique, diffraction limit hinders the fabrication of structures in nanoscale range²⁰. In order to overcome the limitations, Electron beam lithography (EBL) is preferred over photolithography. EBL uses a narrow beam of electrons to directly write the desired patterns on a resist (PMMA). The beam diameter can be up to tens of nanometers depending on the accelerating energy. Though the writing is not diffraction limited, scattering determines the size. EBL is not only able to write nanostructures but also precisely control the shape and dimensions.

EBL is the most efficient technique to fabricate such substrates with high precision but large area fabrication remains a challenge. The cost and time involved in fabrication of such substrates are considerable. Self assembled process defines

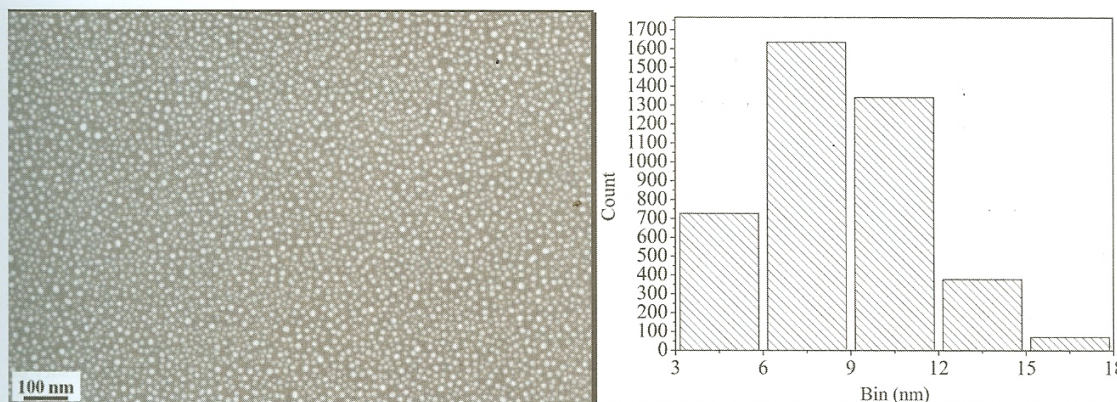


Fig.3. (A) SEM image of Ag nanoparticles formed on Si <100> substrate and (B) size distribution.

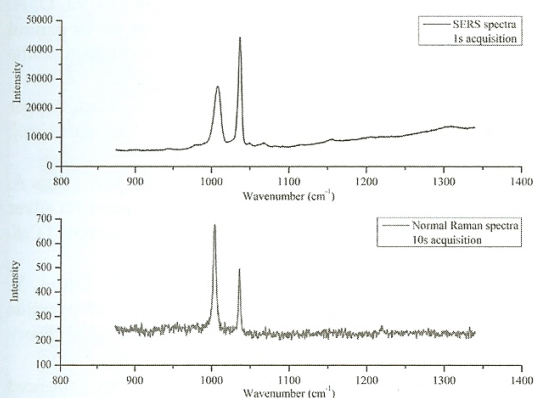


Figure 4. Comparison of conventional Raman and SERS spectra of 0.1 M Pyridine solution.

a new opportunity for fabrication of large area SERS substrates with significant optimization in cost and time. We develop SERS substrates by depositing metal to form nanostructures. A thin layer of metal, preferably less than 10 nm, forms nanoparticles on Si and many insulator surfaces. This is due to Volmer-Weber mode island growth mechanism involving high surface energy of the metal than the underlying substrates.

The nanoparticles are fabricated on Si <100> substrate by DC magnetron sputtering of silver (Figure 3). The sputtering time is controlled in order to get a thickness less than 5 nm. Substrate properties have effects on the plasmon response of the nanoparticles. Substrates can either

enhance or diminish surface plasmon resonance (SPR). Therefore, we fabricate substrates with dielectric layers and analyze the performance.

Methodology

In a typical SERS measurement, the analyte solution of a particular concentration is dispensed on the SERS substrate by using a micro-pipette. Laser is focused on to the sample and the z-axis of the microscope is adjusted so that the focal plane lies at the surface where the SERS spectrum can be observed. Figure 4 shows a SERS spectrum comparison with conventional Raman spectrum of 0.1 M pyridine solution, which also shows an average enhancement factor of $\sim 2 \times 10^3$.

Summary:

In this study we have shown SERS of various analytes using three different approaches. Static SERS is useful for optimizing right colloids and activating agents for particular analytes. LOC experiments can provide reproducible enhancements for the analytes. It also has the potential in point-of-care diagnostics, environmental monitoring *etc.* Further, SERS substrate fabricated using sputtering requires no sample preparation. These substrates have the capability of vapour phase detection. Due to the fabrication process involving Si wafers, microfluidic channels can be integrated for LOC applications.

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