SERS study of molecules on Ag nanocluster films deposited on glass and silicon substrates by cluster deposition method

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1. Introduction

Soon after the discovery of Surface Enhanced Raman Scattering (SERS) as a tool for the detection of organic/bio molecules at the ultra low level concentration, it has been growing as one of the promising areas of research in nanoscience and technology due to its power as an analytical tool for sensitive and selective detection of molecules adsorbed on noble metal (Au, Ag, Cu etc.) nanostructures [1–3]. The commonly used SERS-active substrates have been prepared by employing many chemical and physical routes. The chemical route is based on aggregated Ag and Au colloids and these metal colloid solutions of Ag or Au particles of nanometer size have shown high SERS enhancement factors [4–8]. The problem involved in this method is that the tendency for colloidal particles to aggregate spontaneously upon addition of analytes leads to poor stability of system and irreproducibility in the magnitude of SERS signals [9,10]. The substrates made of electrochemically roughened gold and silver surfaces, are relatively easy to produce and present good SERS enhancement, however, their reproducibility is poor [11]. But these problems were slightly overcome in the physical route. SERS effects of various nanostructures, for example, flowerlike Ag nanostructures [12], Ag nanowire bundles [13], Ag nanorod arrays [14], Ag nanoparticles [15], Ag nanowell and nanopore array [16,17] and bimetallic (Ag–Au) substrates [18] were reported in the literature. Among them, the lithographic technique provides highly organized nanostructures that lead to high sensitivity and reproducibility [19,20]. However, this technique is quite expensive and requires elaborate preparation methods. Both the chemical and physical routes have their advantages and drawbacks and hence both of them are currently used for the preparation of highly efficient SERS substrates. In this context, there is a need to develop cheaper and reliable substrates for enhancing the sensitivity and reproducibility of the SERS signals.

The present work is carried out to fabricate a suitable SERS substrate using a physical route namely cluster deposition method with Ag as target. This method offers ease of fabrication and also provides nanoparticles with high purity. It also allows fabricating variety of nanoparticles using various target metals like Au, Cu, and Pd etc. From the literature it is well established that the SERS intensity depends on the excitation of the localized surface plasmon resonance (LSPR), which in turn depends upon many factors such as shape, size and inter particle spacing of the material. Based on these points we optimized the Ag nanoclusters (from hereafter it is referred to as Ag NC’s) size and density through a cluster deposition method. The present work is carried out to fabricate a suitable SERS substrate using a physical route namely cluster deposition method with Ag as target. This method offers ease of fabrication and also provides nanoparticles with high purity. It also allows fabricating variety of nanoparticles using various target metals like Au, Cu, and Pd etc. From the literature it is well established that the SERS intensity depends on the excitation of the localized surface plasmon resonance (LSPR), which in turn depends upon many factors such as shape, size and inter particle spacing of the material. Based on these points we optimized the Ag nanoclusters (from hereafter it is referred to as Ag NC’s) size and density through a cluster deposition method. Our recent report [21] shows that this preparation method can generate SERS substrates suitable to detect ultralow concentrations of molecules. Following the research line of this work, we were motivated to take up the present work to study the suitability of an efficient substrate for SERS.

Very few studies have been published on the influence of the nature of the substrates of nanoparticles in SERS measurements [22,23]. Thus it seems dependency of the nature of the substrates has been ignored or given less priority in the field of SERS. There-
fore, we kept the aforementioned aspect in the mind and carried out the present work to study the nature of the substrates (p-type Si (100) and glass) on SERS signal enhancement and compare their signal intensities generated by both the substrates. A special focus is also been given on the reproducibility and repeatability of these substrates. The SERS activity of the Ag NCs/p-type Si (100) and Ag NCs/glass substrates was evaluated with CV as the probe due to its high Raman scattering cross-section.

2. Experimental

2.1. Preparation and characterization of Ag nanocluster films/substrates

The microscope glass slides and p-type Si (100) wafers (hereafter it is referred to as Si) were used to prepare substrates for Ag nanocluster deposition. These were cut into 1 × 2 cm² pieces and surface contamination was removed by sonication for ten minutes, first in methanol, then in ethanol and finally in Milli-Q water. The slides/wafers were subsequently dried with N₂ gas at room temperature before placement in the vacuum chamber. Silver nanoclusters were deposited on these substrates at a base pressure of 2 × 10⁻⁶ Torr using a cluster deposition system (NANODEP 60 from Oxford Applied Research, UK). The power of the magnetron was maintained at ~70 W (with a voltage of 350 V and a plasma current of 200 mA). The Ag atoms were grown in the form of clusters while they traversed towards the substrate placed at a distance of few centimeters from the exit of the magnetron chamber. Ag NCs were deposited on glass as well as Si substrates for different exposure times (6, 7 and 8 min) by maintaining all other deposition parameters such as Argon gas flow rate, power etc. at the same levels. This was done to achieve the variation in the cluster sizes. Further these substrates were annealed at 300 °C for 2 h under Ar environment to grow desired and homogeneous nano size clusters. Field Emission Scanning Electron Microscope (FESEM) images of as-deposited and annealed substrates were recorded using Carl-Zeiss Ultra 55 system. The UV–Vis spectra of the present substrates were measured at room temperature in the range of 200–800 nm using UV–Vis spectrophotometer (JASCO V-670).

2.2. Surface Enhanced Raman Scattering (SERS) studies

SERS spectra of CV adsorbed on these substrates were recorded using micro-Raman spectrometer (LABRAM-HR) using laser excitation lines of 514.5 nm (Ar⁺), and 632.8 nm (He–Ne) at room temperature. The reason for recording SERS spectra with these excitation wavelengths was to confirm the resonance and non-resonance SERS effect of adsorbate on Ag nanoclusters. All measurements were made in a backscattering geometry, using a 50× microscope objective lens with a numerical aperture of 0.75. Typical laser power at the sample surface was 2.4 mW with a spot size of 2 μm diameter. CV of 100 and 1 μM concentration was prepared by dissolving the required amount of CV in water. For each test, 10 μL of the CV solution was dropped onto each of the SERS substrates (1 × 2 cm²) and dried at room temperature.

3. Results and discussion

Fig. 1A shows the FESEM image of Ag NCs on glass substrate (8 min deposited) that was annealed at 300 °C for 2 h. It is observed from the image that this substrate has irregular nanoclusters or aggregates with large size distribution (poly dispersed). Similarly, Fig. 1B shows the FESEM image of Ag NCs on Si substrate (8 min deposited) that was annealed at 300 °C for 2 h. We can observe less poly dispersity of Ag NCs on Si substrate compared to glass. The insets of Fig. 1A and B show the surface morphology of pristine glass and silicon substrates. It is seen that the surface roughness is more for glass substrate compared to silicon. From the obtained FESEM images, the histograms were plotted to represent the size distributions of the nanoclusters. One such histogram is shown in Fig. 2 for 8 min deposited on glass and its inset shows the histogram obtained for Si substrate (annealed) for the same time of deposition. The average size of the Ag NC on the glass was estimated to be approximately 157 nm with a standard deviation of 119 nm. Similarly, the same method was adopted for all other substrates and the estimated nanocluster sizes were approximately 95 ± 78 nm, 118 ± 77 nm for 6 and 7 min deposition respectively. For Si substrate the estimated average nanoclusters sizes were approximately 73 ± 65 nm, 94 ± 71 nm, 110 ± 67 nm for 6, 7 and 8 min deposition respectively.

Fig. 3 show the UV–Vis spectra of annealed Ag NCs/glass substrates for 6 and 8 min deposition. We found that the surface plasmon resonance (SPR) wavelengths for all Ag NCs/glass substrates lie in the range of 360–480 nm. The spectra have peaks at ~360 nm and ~470 nm, corresponding to small (<100 nm) and large Ag NCs (>100 nm) respectively [21]. From the literature it is known that SPR frequency is sensitive to the particle size as well as the dielectric behavior of the substrate [24]. The inset of Fig. 3 shows the reflectance spectra of annealed Ag NCs/Si substrates for 6 and 8 min deposition. Under the assumption that the measured reflectance (R) is equal to the total reflectance and the transmission through the Si substrate is zero (opaque material), the absorbance (A) can be estimated as A = 1 − R. Thus the absorption spectrum has a peak at ~400 nm corresponding to the SPR of Ag NCs on Si substrate. Due to the smaller size of Ag NCs (~73–110 nm) on Si and dielectric nature of Si (the dielectric constant is ~11–12), the SPR on Ag NCs/Si substrates is found to be at shorter wavelength as compared to the glass substrate with an average size of Ag NCs (~95–157 nm) and having a dielectric constant in the range of 3.7–10. In addition to this, the SPR tunability is found to be broad on glass substrate as compared to silicon. The wide size distribution of Ag NCs on glass than that of silicon is thought to be responsible for SPR broadening.

The laser excitation (514.5 nm) was chosen in such a way that the excitation wavelength falls within the SPR absorption band of Ag NCs on the glass and Si substrate but is away from the CV absorption as its absorption band maximum is located at ~590 nm. Besides this, the excitation wavelength of 632.8 nm was also chosen for recording SERS spectra as it matches well with the electronic absorption band of CV (~590 nm) but it matches at the tail end of the SPR peak resulting in Resonant Raman Scattering (RRS). In accordance with the literature it is known that electromagnetic contribution to Raman signal is dominant (5 or 6 orders higher) always over the chemical contribution (due to the molecule electronic resonance and charge transfer state). Therefore the excitation wavelengths of 514.5 and 632.8 nm produces SERS enhancement, RRS enhancement respectively.

The SERS spectra were recorded with the excitation wavelengths of 514.5 nm and 632.8 nm for CV with concentration of 100 and 1 μM adsorbed on both Ag NCs/glass and Ag NCs/Si substrates. We observed from the results that the maximum SERS signal enhancement occurs for the average size of 157 ± 119 nm on glass substrate and 110 ± 67 nm on Si substrate. The literature also suggests that the Ag nanoclusters in the size range of 100–150 nm are expected to be most efficient for surface Raman enhancement when the excitation wavelength is around 500 nm [25–27]. The wavelength dependence on SERS enhancement effects for Ag nanoparticles is described elsewhere [10,28–30]. Therefore we have compared the SERS signal enhancements on the two substrates of glass and silicon that has Ag NCs of the size 157 and 110 nm respectively.
A series of experiments were performed with wide range of concentrations of CV (100 μM–10 nM) to identify the substrate effect on SERS enhancement. Fig. 4 and 5 and their insets shows the comparison of SERS spectra of CV of 100 and 1 μM adsorbed on Ag NC's/glass as well as Ag NC's/Si recorded with 514.5 nm and 632.8 nm, respectively. The peaks of CV appeared at \( \nu = 208, 338, 420, 807, 912, 1177, 1293, 1371, 1530, 1587 \) and \( 1620 \) cm\(^{-1}\) are in accordance with the reported values [31].

When we compared the sensitivity of Ag NC's/glass and Ag NC's/Si substrates, Ag NC's/Si substrate could not detect Raman peaks of CV 10 nM but Ag NC's/glass detected Raman peaks of CV 10 nM (given as supplementary data). This indicates that Ag NC's/glass substrate is more sensitive than that of Ag NC's/Si substrate at very low concentration. From Fig. 4 and 5 it can be seen that the intensities of the characteristic Raman peaks of CV at \( \nu = 420, 807, 912, 1177, 1587 \) and \( 1620 \) cm\(^{-1}\) are significantly enhanced.

The largest enhanced Raman peaks of 1 μM CV such as \( \nu = 418, 809, 912, 1177 \) and \( 1587 \) cm\(^{-1}\) on both Ag NC's/glass and Ag NC's/Si substrates are taken for their intensity comparison. This was done by fitting a Lorentzian shape to the spectral line and finding the area under the peak and the results are shown in Fig. 6 for the excitation wavelength of 514.5 nm. The inset of Fig. 6 shows

### Fig. 1.
FESEM images of 8 min deposited (A) Ag NC's/glass substrate and (B) Ag NC's/Si substrate annealed at 300°C for 2 h. The insets show the surface morphology of pristine glass substrate and silicon wafer.

### Fig. 2.
Histogram of Ag NC's/glass as a result of cluster deposition for 8 min (average size = 157 nm with standard deviation of 119 nm). The inset shows the histogram of 8 min deposited Ag NC's/Si, the average size = 110 nm with standard deviation of 67 nm.

### Fig. 3.
and the inset shows the UV–Vis absorption and reflectance spectra of the annealed Ag NC's on glass and Si substrates deposited for 6 and 8 min respectively.

### Fig. 4.
The SERS spectra of 100 μM concentration CV on (A) Ag NC's/glass of average size 157 ± 119 nm and (B) Ag NC's/Si of average size 110 ± 67 nm recorded with 514.5 nm. Inset shows the same spectra for a laser wave length 632.8 nm.
the comparison for the excitation wavelength of 632.8 nm. From the results it is observed that the SERS signal intensity of every peak is almost 8–11 times of magnitude higher for Ag NC's/glass substrate when compared to Ag NC's/Si substrate. We also tried to see whether the same behavior is observed with Ag NC's/glass and Ag NC's/Si substrates of almost comparable size of nanoclusters. For this purpose we compared the SERS signal intensities of drop coated 10 µl of CV on Ag NC's of 118 nm size on glass substrate and Ag NC's of 110 nm size on silicon substrate. Interestingly we observed that Ag NC's/glass substrate offer 5–6 times higher SERS signals than that of Ag NC's/Si substrate (given as Supplementary data). Therefore we strongly believe from the observed results that the Ag NC's on glass provides better SERS signal enhancement when compared to on Si substrate.

The significant enhancement in the SERS signal intensities for Ag NC's/glass substrates can be explained in this way. One of the important factors could be due to the difference between the electric field distribution around the nanoclusters on Si and glass substrates. And also it is seen from Fig. 1A and B that the nanoclusters formation is more spherical and of uniform size distribution due to the smoothness of the Si surface, while due to the roughness on the glass substrate the nanoclusters are of different shapes and sizes. This large variation leads to smaller inter-particle distance and higher density of the nanoclusters. The inter-particle separation for Ag NC's/glass substrates are in the range of 20–150 nm. The closeness of Ag NC's on glass surface suggests that it can be treated as a 3D structure because of the roughness of the surface. Therefore we expect the hot spots to have higher field enhancement on glass compared to the Si substrate. Whereas on Si surface one sees that the few number of smaller particles that are in between the uniformly sized nanoclusters are of small size might not be contributing the resonant surface plasmon coupling leading to weaker field enhancement. Fig. 2 confirms that the number density is also higher for the nanoclusters on glass substrate than on the Si surface. One can also realize higher field enhancements due to different shapes and closer inter particle distance leading to near bow–tie antenna situations [32,33]. Moreover light-trap or absorption is more in Si rather than in glass [24] and this might alter the coupling between the plasmons more on Si than on glass. Hence the field enhancement is reduced on Ag NC's/Si than that of glass substrate. Thus the present study confirms that the substrate efficiency is related to the Ag particle morphology, which can be tailored in order to match the excitation frequency with the surface plasmon resonance related to the nanoparticles.

Besides the particle distribution and the inter-particle distance, opaque nature and oxidation of Si may also have an effect on the surface plasmon formation. It is well known that Si may easily get oxidized. The oxygen atoms could form some Si–O–Si bonds by breaking existing Si–Si bonds. During annealing of as deposited Ag NC's/Si substrate the deposited Ag atoms arriving at Si–O–Si and Si–Si bonds forms Si–O–Ag and Si–Ag (covalent bond) respectively. The reason is the Pauling electronegativity of Si (1.9) is close to that of Ag (1.93). These bond formations occur at several places on Si substrate. This mechanism might inhibit the growth rate of nanoclusters. We observed the oxidization of Ag NC's/Si substrate to a small quantity through energy dispersive X-ray spectrometry (EDS) spectrum while performing FESEM study. This could be due to the high Ag coverage at low annealing temperature (300 °C), most of the oxygen X-rays are absorbed by the Ag clusters leading to a very low detection probability of oxygen.

The peak observed at around ~208 cm⁻¹, as shown in Fig. 4 and 5, suggests that the CV molecule is coordinated to the Ag surface via the nonbonding electrons of the nitrogen atom. Because of this interaction part of the fluorescence energy of CV molecule could be transferred to Ag metal surface. Thus the fluorescence intensity is reduced while the SERS signal is amplified. In accordance with literature, when the fluorophore is placed near the Ag nanocluster and the excitation light is resonant with surface plasmon, enhanced local electric fields are generated at the position of the fluorophore, resulting in an enhancement of the excitation efficiency [34,35]. In the emission process, both the radiative and nonradiative decay rates are modified in the presence of metallic nanostructures. However, if an exited fluorophore is placed very close to a metallic surface (within ~20 nm), the non radiative energy transfer to the metal takes place. This results in the quenching of fluorescence, which changes the polarizability of CV molecule and increases the Raman scattering cross-section. It is well known that the local field intensity around a metal nanoparticle decays rather rapidly away from the particle surface. The decay length of the local electric fields is of the order of the particle size [35]. This suggests that the decay length of the electric field is larger on glass substrate as compared to silicon because of the nanocluster size difference. Due to this, the larger size of Ag NC's on glass substrate

![Fig. 5. SERS spectra of 1 µM CV on (A) Ag NC's/glass of average size 157 ± 119 nm and (B) Ag NC's/Si of average size 110 ±2nm recorded with 514.5 nm and inset shows for the laser wave length 632.8 nm.](image)
may interact with relatively more CV molecules by forming complex Ag–N bonds than Ag NC’s on Si substrate, which leads to the relatively high SERS signal enhancement on Ag NC’s/glass substrate. On the other hand the formation of some Ag–O bonds could de-activate the Ag–N interaction and so they do not contribute to the SERS process. The oxidation of Ag would therefore reduce the SERS signals from the adsorbed CV molecule on Si substrate.

In the field of SERS one of the major problem is the reproducibility (it refers to the ability to produce enhanced signal at various parts of the substrate with as minimum as possible intensity variation) and repeatability (it refers to the minimum batch to batch variation of substrate) of the SERS spectra, as the spectra depends on the shape, size of the metal nanoparticles and the orientation of adsorbed molecule on the metal nanoparticle. These are the two major factors that determine the efficacy of SERS substrates. We have recorded the SERS spectra at different positions at the edges and in the center of Ag NC’s/glass as well as Ag NC’s/Si substrates with both excitation wavelengths 514.5 and 632.8 nm. In order to determine the exact variation in the intensity of Raman peaks, we recorded Raman spectra at 25 different locations for both the substrates and calculated the intensity variations for each individual peak. We find for the glass substrate, a variation of 34%, 22%, 23%, 28% for the peaks at 418, 809, 912, 1177 and 1587 cm$^{-1}$ respectively. Similarly for the Si substrate we find a variation of 25%, 27%, 22%, 23%, 28% for the peaks at 418, 809, 912, 1177 and 1587 cm$^{-1}$ respectively. The maximum intensity variations for the Si and glass substrates are 25% and 34% respectively from their averages. These results suggest that the substrates employed in the present are highly reproducible. The above procedure was adopted for quantifying the results which were obtained with 632.8 nm, respectively. Similarly for the Si substrate we find a variation of 25%, 27%, 22%, 23%, 28% for the peaks at 418, 809, 912, 1177 and 1587 cm$^{-1}$ respectively. The maximum intensity variations for the Si and glass substrates are 25% and 34% respectively from their averages. These results suggest that the substrates employed in the present are highly reproducible. The above procedure was adopted for quantifying the results which were obtained with 632.8 nm, respectively. Similarly for the Si substrate we find a variation of 25%, 27%, 22%, 23%, 28% for the peaks at 418, 809, 912, 1177 and 1587 cm$^{-1}$ respectively. The maximum intensity variations for the Si and glass substrates are 25% and 34% respectively from their averages. These results suggest that the substrates employed in the present are highly reproducible. The above procedure was adopted for quantifying the results which were obtained with 632.8 nm, respectively. Similarly for the Si substrate we find a variation of 25%, 27%, 22%, 23%, 28% for the peaks at 418, 809, 912, 1177 and 1587 cm$^{-1}$ respectively.

From Fig. 7, the intensities of Raman peaks at $\sim$418, $\sim$809, $\sim$912, $\sim$1177, and $\sim$1587 were quantified. In order to determine the exact variation in the intensity of Raman peaks, we recorded Raman spectra at 25 different locations for both the substrates and calculated the intensity variations for each individual peak. We find for the glass substrate, a variation of 34%, 22%, 23%, 28% for the peaks at 418, 809, 912, 1177 and 1587 cm$^{-1}$ respectively. Similarly for the Si substrate we find a variation of 25%, 27%, 22%, 23%, 28% for the peaks at 418, 809, 912, 1177 and 1587 cm$^{-1}$ respectively. The maximum intensity variations for the Si and glass substrates are 25% and 34% respectively from their averages. These results suggest that the substrates employed in the present are highly reproducible. The above procedure was adopted for quantifying the results which were obtained with 632.8 nm, respectively. Similarly for the Si substrate we find a variation of 25%, 27%, 22%, 23%, 28% for the peaks at 418, 809, 912, 1177 and 1587 cm$^{-1}$ respectively. The maximum intensity variations for the Si and glass substrates are 25% and 34% respectively from their averages. These results suggest that the substrates employed in the present are highly reproducible. The above procedure was adopted for quantifying the results which were obtained with 632.8 nm, respectively.

In summary, we have demonstrated that the enhancement in the SERS signals is higher for Ag NC’s/glass substrates as compared to Ag NC’s/Si substrates. This could be due to the variations in the surface density, roughness, the size distribution, inter particle distance and yield of hot spots of Ag NC’s on glass compared to Si. The SERS enhancement of optimized nanostructures on glass substrate was found to be one order of magnitude larger than that of Si substrate. We have demonstrated that the preparation of the SERS-active substrate by cluster deposition is reliable because it offers high purity of Ag nanoclusters as compared to chemical routes, better control on preparation and high stability. This method also offers a variety of nanoclusters using different targets instead of Ag.

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Appendix A. Supplementary material


References