Chapter 3

Fabrication of SERS substrate from naturally available diatom for detection and quantification of chemicals

This chapter discusses a low-cost method for development of SERS substrate from naturally available diatom frustules. The characterization of the substrate for Raman signal detection and analysis of common Raman active dye has been discussed. Using the designed SERS substrate for reliable detection of fluoride level concentration in drinking water has been demonstrated.

3.1 Introduction

Since its discovery, SERS technique has evolved to the point that using this technique it is now possible to detect single molecule [1, 2]. Due to its unique advantages over other detection methods, the technique became popular across the globe. The well established approaches to develop SERS substrates such as electrochemically roughened electrodes, colloidal metal nanoparticle solutions, lithographically generated substrates have their own merits and demerits [3]. For example, electrochemically roughened electrode SERS substrate has larger active sensing area whereas this kind of substrates yields low EF. Again, metallic colloidal solution yields a higher order of EF and it is relatively easy to

develop but the reproducibility of such substrate is poor. The lithographically obtained SERS substrates produce good degree of reproducibility, however, developing cost and requirement of sophisticated instruments and other laboratory facilities limit its popularity. In recent years, numerous works have been demonstrated related to development of SERS substrate that yields high enhancement factor, a good degree of reproducibility, longer durability which can be obtained at an affordable cost. Use of diatom frustule to obtain SERS substrate usher a way to develop SERS substrate from natural resources. The diatoms are single cell algae and frustule of diatom is of amorphous hydrated silica. The frustules are ornamented with some periodic pores. Diatoms are used in different field of applications such as solar cell, photoluminescence based bio-sensing, drug delivery system, electroluminescence/ photoluminescent devices, SERS based sensing etc. [4-10]. The use of diatom as a template for SERS based investigations has been observed to be cost-effective and time efficient [11-15]. However, the life span of such substrate with good degree of reproducibility has not been demonstrated so far. The usability of diatom platform SERS substrate for reliable SERS based sensing studies along with good reproducibility could give rise to a promising way towards fabrication of low-cost SERS substrate for detection of Raman active samples. In the present work, by utilizing the self-assembling property of AuNPs for attachment in the pores and on the surface of cleaned diatom frustules, SERS based studies have been carried out. Subsequently the proposed SERS substrate has been used to measure fluoride level in water. In the present study, diatom species namely cosmioneis hawaiiensis has been used as a template for development of the SERS substrate.

3.2 Experimental

3.2.1 Material

Sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7.2H_2O$) is procured from SRL, India, gold chloride trihydrate ($HAuCl_4.3H_2O$) is purchased from Himedia, India, Malachite green is purchased from sigma aldrich India, and H_2O_2, HCl, ethanol and sodium fluoride have been acquired from Marck, India. The procured chemicals are used as received without further processing. All the sample solutions are prepared in distilled water.

3.2.2 Diatom frustule processing

At first, living diatoms are collected from the Tezpur University lake. The collected diatoms are cultured in biochemical oxygen demand (BOD) incubator under controlled conditions. The day and night temperature cycle in the incubator is maintained at 25 ± 0.50°C and 20 ± 0.50°C respectively along with a photoperiod of 16 hours light (fluorescent lamps) and 8 hours of darkness. By slightly modifying the process suggested by Guillard and Lorenzen [16] diatoms are cultured in WC media. The pH level of the media is maintained between 7 to 6.23 and doubled the sodium metasilicate composition in the media. The diatoms are then cleaned by slightly modifying the procedure described by Mazumder et.al [17]. Initially, centrifuging the cultured media at 3000 rpm for 10 minutes the diatoms are separated from the media. The separated diatoms are then cleaned with distilled water for three times. To dissolve other impurities that may present in the frustule, the sample is treated with H_2O_2 and keep it in room temperature environment for 1 hour followed by washing with distilled water. To remove organic materials from the frustule, diatoms are treated with 35% HCl and then water bath treatment at 60°C for 30 minutes. To avoid any possible bacterial growth and other contamination, the cleaned diatom frustules are stored in ethanol medium.

3.2.3 SERS substrate preparation from diatom frustule

Synthesis of gold nanoparticle

Following Turkevich method 20 nm, 40 nm and 60 nm AuNPs are synthesized in the laboratory, where sodium citrate is used as a reducing agent. To synthesis 20 nm AuNPs, 100 mL of 0.01 wt% $HAuCl_4.3H_2O$ is heated to boil under stirring and 2.4 mL of 1 wt% $C_6H_5Na_3O_7.2H_2O$ is quickly added to the boiling solution under vigorous stirring condition. The color of the mixture is turned into wine red after 10-15 minutes, this indicates the formation of AuNPs. 40 nm AuNPs are synthesized by adding 360 μL of 1 wt% $C_6H_5Na_3O_7.2H_2O$ solution to 50 mL 0.01 wt% boiling $HAuCl_4.3H_2O$ under vigorous stirring condition. The mixture solution is stirred for 10-15 minutes and the solution slowly turns into light pink indicating the formation of AuNPs. For 60 nm AuNPs synthesis, 300 μL of 1 wt% $C_6H_5Na_3O_7.2H_2O$ solution is quickly added to 50 mL of boiling 0.01 wt% $HAuCl_4.3H_2O$ under vigorous stirring condition. After 10-15 minutes the color of the mixture solution become dark red which indicates the synthesis of AuNPs colloidal solution. All the synthesized AuNP solutions are allowed to cool down

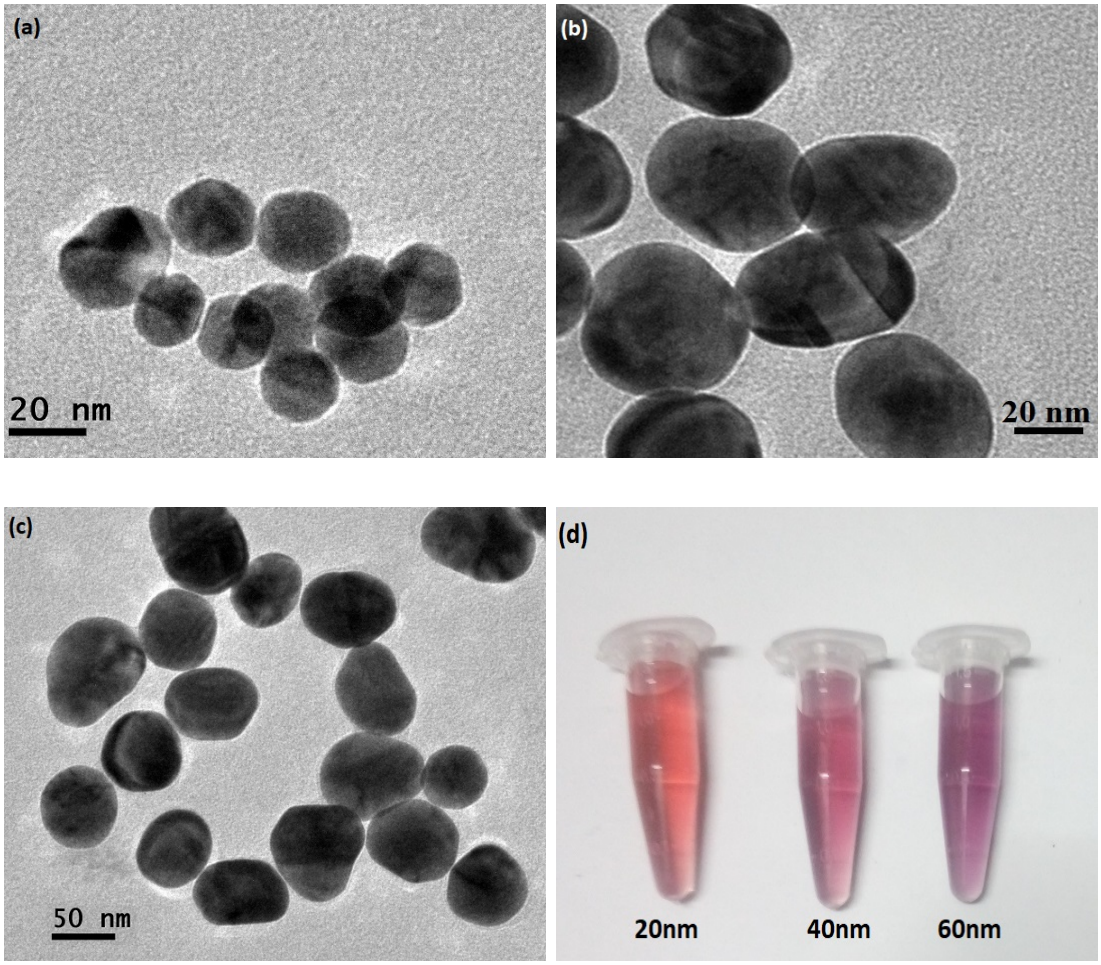

Figure 3.1: TEM images of chemically synthesized AuNPs of sizes (a) 20nm, (b) 40nm, (c) 60 nm and (d) photograph of synthesized AuNP colloidal solution.

at room temperature. Figure 3.1 (a), (b) and (c) shows the TEM images of 20 nm, 40 nm and 60 nm AuNP obtained from the above synthesized steps. Figure 3.1 (d) shows the photograph of synthesized AuNP colloidal solution.

Substrate preparation

20 μL of diatom frustules suspension is pipetted on a clean coverslip and allowed to dry at room temperature. Upon drying, the diatom attached coverslip is annealed at 400°C for 1 hour. To attach AuNPs with the pores of the diatom frustules, the substrate is dipped vertically into the AuNP colloidal solution for 12 hours in room temperature environment. The self-assembled AuNPs substrate is then removed from the solution and allowed to dry in room temperature environment. The FESEM image shown in figure

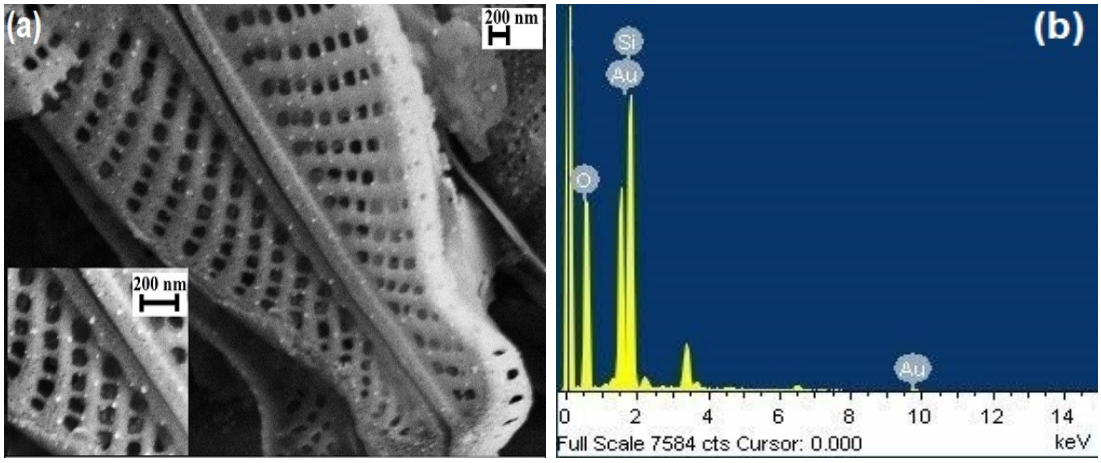

Figure 3.2: FESEM images showing distribution of AuNPs on (a) diatom frustule and (b) EDX data showing the elemental composition of the self-assembled AuNPs on the diatom frustule

3.2(a) confirms the AuNPs attachment and EDX data in figure 3.2(b) shows the elemental composition of the substrate. The average dimension of the pores are observed to be 100 nm while maintaining a periodicity of 300 nm in the lateral direction and 150 nm along the transverse direction.

3.2.4 Analyte sample preparation

10 μM stock solution of MG sample is prepared by dissolving proportionate amount of MG in distilled water. By diluting the stock solution, required lower concentration samples can be prepared. Following the standard procedure reported elsewhere [18, 19] different fluoride level concentration in water has been obtained. For detection of Raman signal of the considered chemicals, 10 μL of each sample is pipetted on the sensing region of the designed SERS substrates and allowed to dry in room temperature environment.

3.2.5 Simulation study of LSPR field magnitude of the proposed substrate

Prior to start of the experimental investigation, the magnitude of LSPR field generation for different dimensional self-assembled AuNPs in the pores and on the surface of diatom frustule has been studied using the simulation tool. For this study, diatom frustule is considered as a SiO_2 slab of thickness 250 nm and pores of diameter 120 nm with a

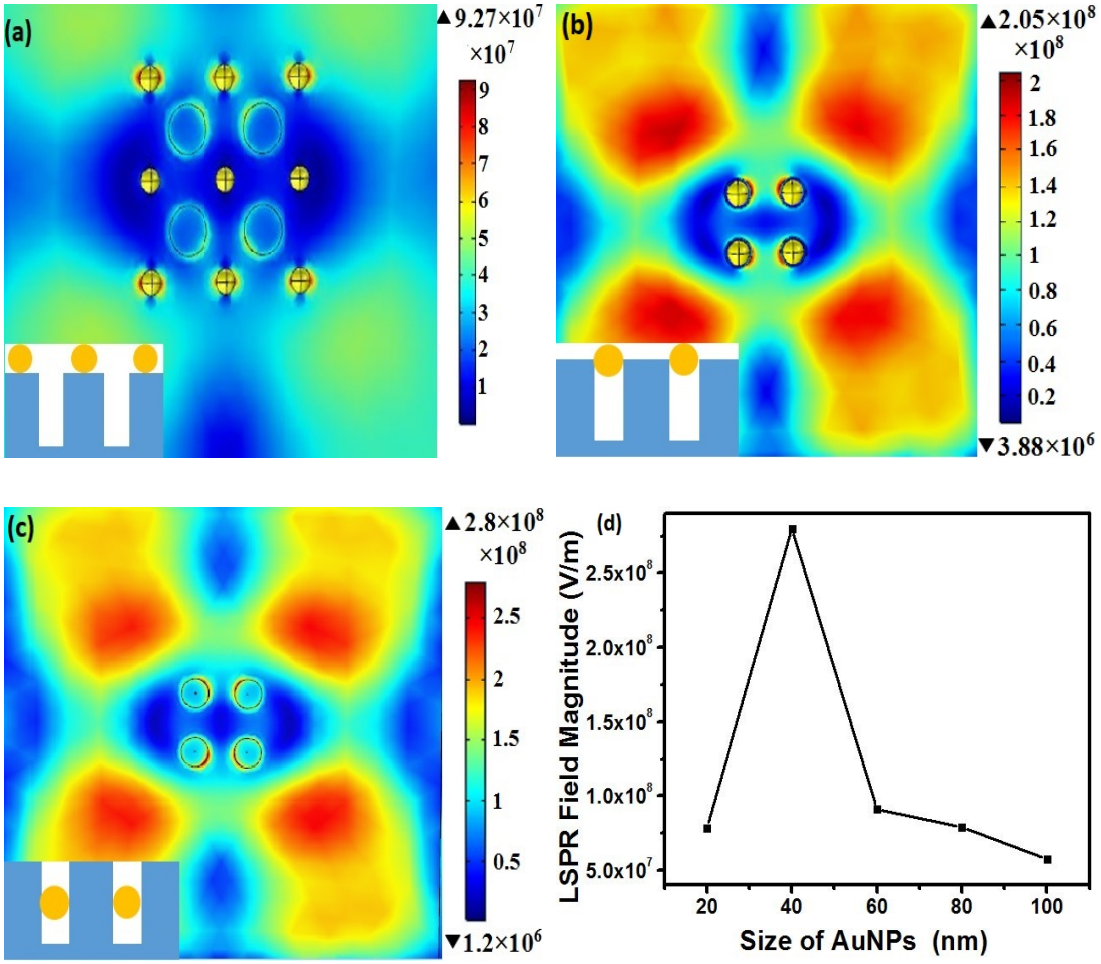

Figure 3.3: Simulation results showing the LSPR field enhancement in the proposed SERS substrate upon coupling of 785 nm plane polarized light. three different situations are considered: AuNPs assembled (a) on the surface of the diatom frustule, (b) just above the pores of the diatom and (c) inside the pores. (d) Variation in LSPR field magnitude with respect to size of the assembled AuNPs.

periodicity of 240 nm. A plane polarized light of 785 nm is allowed to incident normally on the designed substrate. Figure 3.3(a) shows the situation where AuNPs are assembled on the surface of the diatom and in the vicinity of the pores. For this case, an average field enhancement of the order of 10^7 has been observed. Figure 3.3(b) and 3.3(c) narrate the situations where AuNPs are assembled just above and inside the pores of the frustule. For these two situations, an average LSPR field magnitude of the order of 10^8 has been observed. Due to guided mode resonance (GMR) phenomenon [20] the field enhancement for assembled AuNPs on top and inside the pores of the diatom is found to be greater than that of the surface assembled AuNPs. The dependence of LSPR field magnitudes on the dimension of the assembled AuNPs has also been studied. Figure 3.3(d) shows the characteristics curve of maximum LSPR field magnitude within the pores of the frustule

for 20 nm, 40 nm, 60 nm, 80 nm and 100 nm AuNPs. In the present study, among the different dimension AuNPs, the maximum field coupling condition is obtained for 40 nm AuNPs assembled in the pores of diatom.

3.3 Results and discussions

3.3.1 Characterization of the substrate

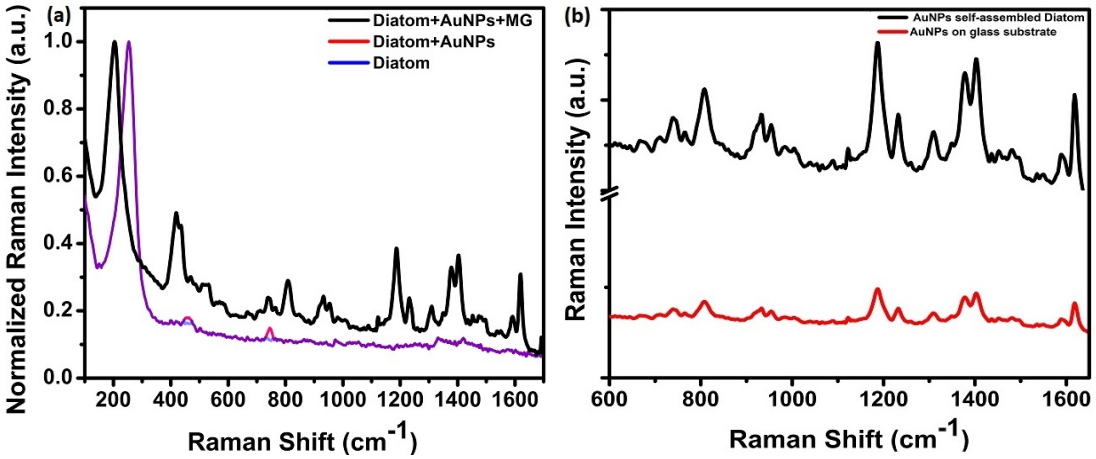

Figure 3.4: (a) Scattered Raman spectra for clean diatom frustule, AuNPs assembled diatom and MG treated SERS substrate, (b) comparison of Raman signal intensities of scattered from MG treated plane glass substrate and from the proposed SERS substrate.

The SERS investigation of the proposed substrate has been carried out using MG as test sample. 10 μL of 1 μM MG solution is treated with the proposed substrate. The scattered Raman signal from the substrate is recorded by the Raman spectrometer. The characteristics Raman signal of MG scattered from the SERS substrate is shown in figure 3.4(a). The Raman signals of bare diatom and AuNPs assembled diatom substrate are also included in this figure. With the designed SERS substrate, strong Raman peaks at 420 cm^{-1}, 1188 cm^{-1}, 1378 cm^{-1} and 1632 cm^{-1} are observed, which are attributed to out of plane vibration of phenyl-C-phenyl, in plane vibration of C-H ring, N-phenyl stretching and stretching of C-C ring respectively. Medium bands at 808 cm^{-1}, 1232 cm^{-1} and 1592 cm^{-1} correspond to out of plane vibration of C-H ring, C-H rocking and C-C stretching respectively [21-23]. In order to investigate the role of diatom frustule in the Raman signal enhancement, 10 μL of 1 μM MG treated separately with the proposed substrate and one with AuNPs coated plane glass substrate. The recorded Raman signals

scattered from these two substrates are shown in figure 3.4(b). As compared to the AuNPs coated on plane glass substrate, the Raman signal intensity scattered from the designed SERS substrate is estimated to be enhanced by ≈111 fold. This enhancement is mainly attributed to the periodic arrangement of the pores in the diatom frustule which contributes to the GMR of the incident laser beam and thus, generate strong LSPR field in the structure.

Effect of AuNP size on Raman signal enhancement

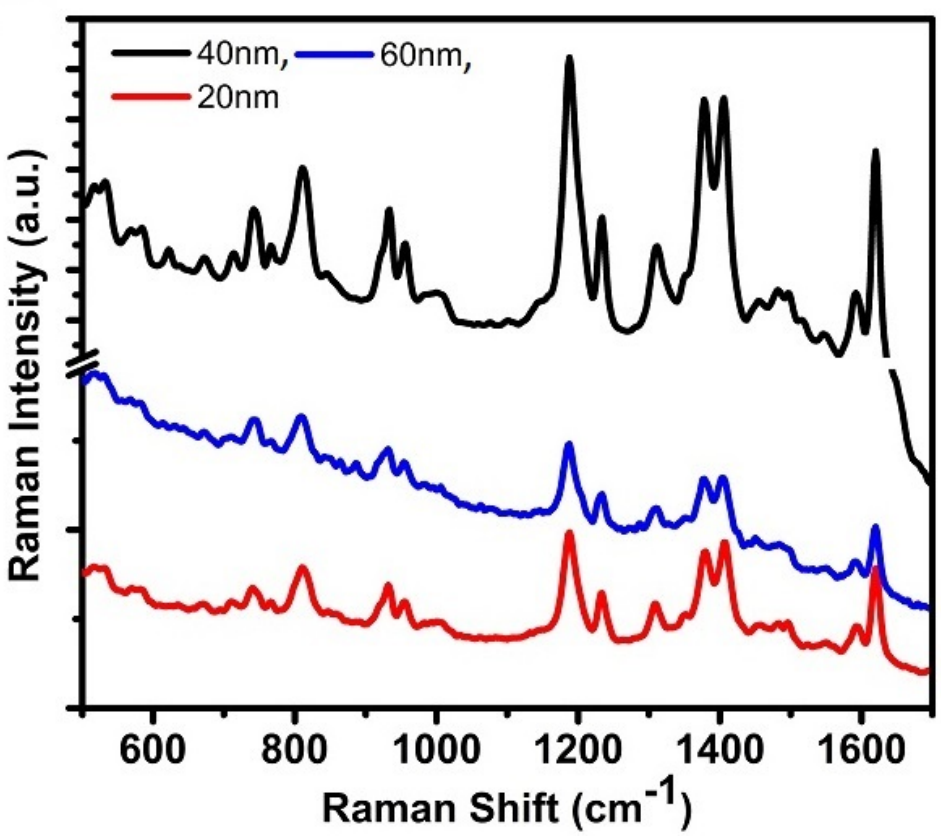

Figure 3.5: Comparison of Raman signal intensities of 2 μM MG scattered from three different substrates treated with 20 nm, 40 nm and 60 nm AuNPs.

The size of AuNP plays a crucial role in determining the magnitude of LSPR field which eventually affects the SERS signal intensity of the analyte molecule. To evaluate the effect of AuNP sizes on scattered Raman signal enhancement, synthesized AuNPs of dimensions 20 nm, 40 nm and 60 nm are allowed to assemble on three diatom decorated substrates separately. The substrates are then treated with 10 μL of 2 μM MG solution and back scattered Rama signals of MG from the substrates are recorded by the spectrometer. The obtained results are shown in figure 3.5. As shown in the

figure, the highest Raman signal intensity is observed for 40 nm AuNPs assembled SERS substrate, which is attributed to maximum coupling of the incident electromagnetic field with the LSP of 40 nm AuNPs. This has also been observed during simulation study for the proposed SERS substrate.

Minimum measurable sample concentration and EF estimation

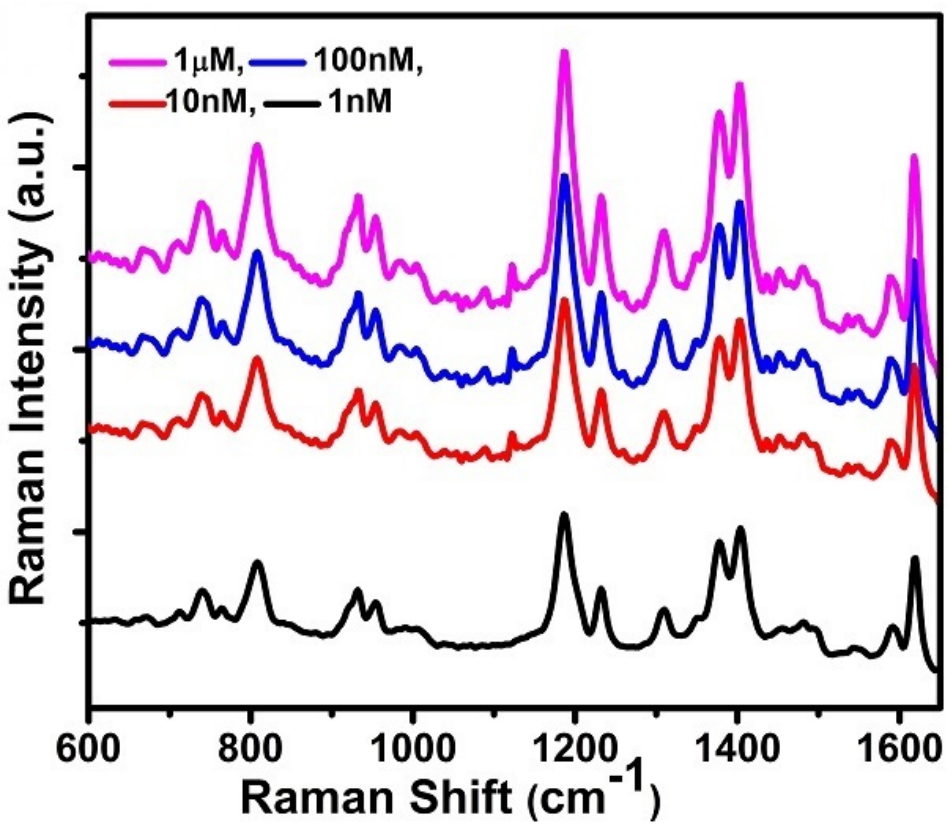

Figure 3.6: Raman signal intensities measured by the spectrometer for different concentrations of MG from the designed SERS substrate.

Four different concentrations of MG samples namely 1 μM, 100 nM, 10 nM and 1 nM, have been prepared and then treated with the proposed SERS substrates. Upon drying of the samples, the scattered Raman signals are recorded. The variation of peak signal intensities for different concentration MG samples are illustrated in figure 3.6. It is observed that with the decrements in MG concentration the intensities of signature Raman peaks of the sample also decreases proportionately. With the proposed SERS substrate, MG concentration as low as 1 nM can be detected reliably with the Raman spectrometer. In order to calculate the EF of the developed SERS substrate, a cleaned cover slip is considered as reference substrate where analyte molecule has been treated. The EF of the proposed SERS substrate is calculated using equation 1.22. I_{SERS} and

I_{REF} are obtained from the recorded data corresponding to Raman peak at 1186 cm^{-1}. In the present study, for MG, EF of 1.66×10^7 has been observed experimentally. While performing the simulation study for the designed structure the intensity of the incident electromagnetic field is considered as 1.94×10^4 V/m. The average local field enhancement due to LSPR field coupling is estimated to be of the order of 10^3. Considering $|E|^4$ approximation the average SERS EF of the order 10^{12} has been obtained. However, during experimental observations the practical EF of the order of 10^7 for MG and 10^6 for fluoride have been observed which is few order lesser than the simulation results. The size variation of AuNPs plays a critical role in determining the overall enhancement of Raman signal intensity. Furthermore, owing to the consideration of average Raman signal scattered from the sensing region of the substrate which covers both hotspot regions and non hotspot regions, the overall enhancement in the scattered signal intensity would be much more lower than the simulation results.

Reproducibility of the substrate

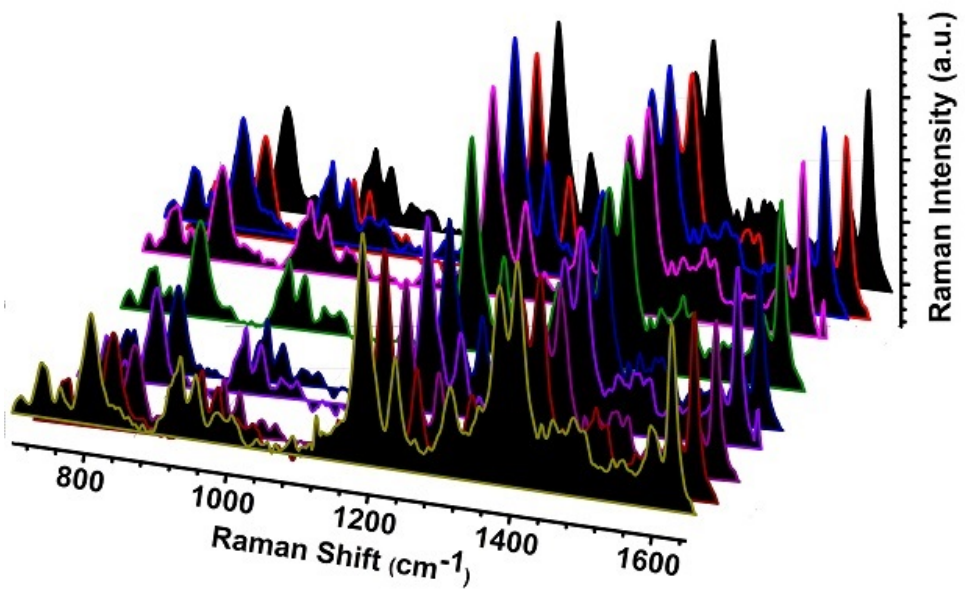

Figure 3.7: Back scattered Raman signal intensities of 1 M MG recorded from 10 different locations of the proposed substrate.

The reproducibility characteristics of the developed SERS substrate has been evaluated in the following manner. With 0.22 NA objective lens, the diameter of laser spot size is measured to be 4.353 μm. This ensures that the size of the beam is smaller than the average size of the diatom frustule. This implies that Raman signal of analyte can be obtained from different locations of the same diatom frustule by scanning the laser

beam over the substrate. The SERS substrate is mounted on an x-y-z translation stage of translational resolution of 5 μm. Scattered Raman signal of MG has been recorded from 10 randomly selected locations from the substrate. The corresponding characteristic signals are illustrated in figure 3.7. The average Raman signal intensities corresponding to the signature peaks at 808 cm^{-}1, 1186 cm^{-}1 and 1618 cm^{-}1 are observed to be fluctuated with RSD values of 6.81%, 7.57% and 4.74% respectively.

Life span evaluation of the SERS substrate

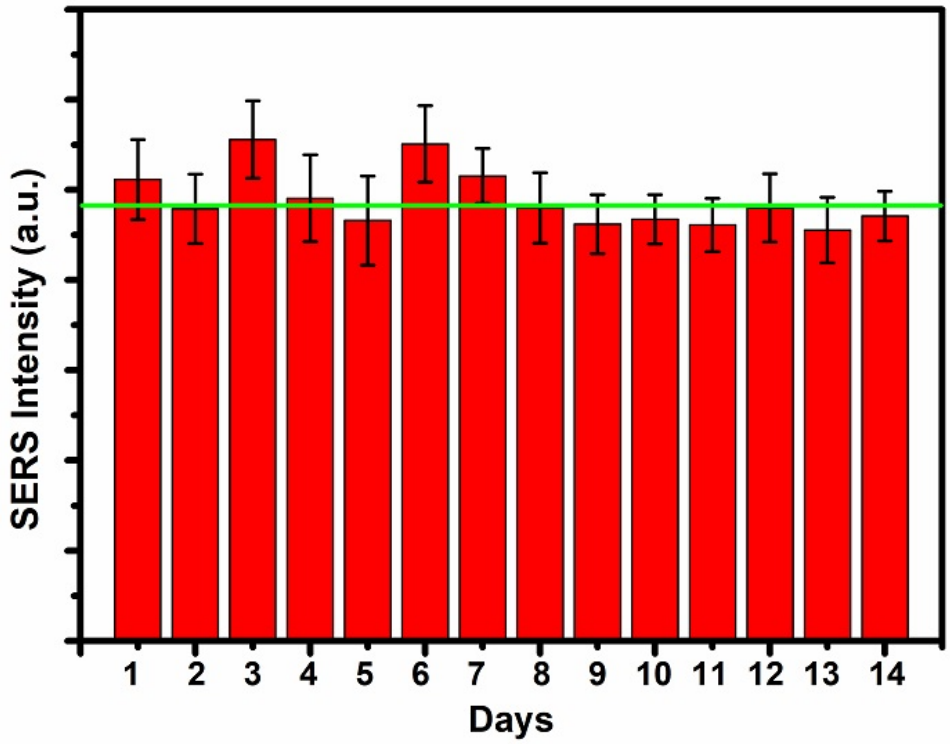

Figure 3.8: Stability study of the proposed SERS substrate through investigating the intensity fluctuations of Raman peaks (808 cm^{-1}, 1186 cm^{-1} and 1618 cm^{-1}) for 1 μM MG treated on it for two weeks.

To evaluate the durability of the substrate, the scattered Raman signals from MG treated substrates have been recorded for two weeks. The maximum intraday peak intensity fluctuation is observed to be 10.54% corresponding to the signature Raman peak at 1186 cm^{-}1. The maximum intensity fluctuation from the average value over the investigation period is 12% corresponding to the same peak position. This suggests a good life span and stability of the proposed substrate. The stability performance of the designed substrate is shown in figure 3.8.

3.3.2 Fluoride level in water

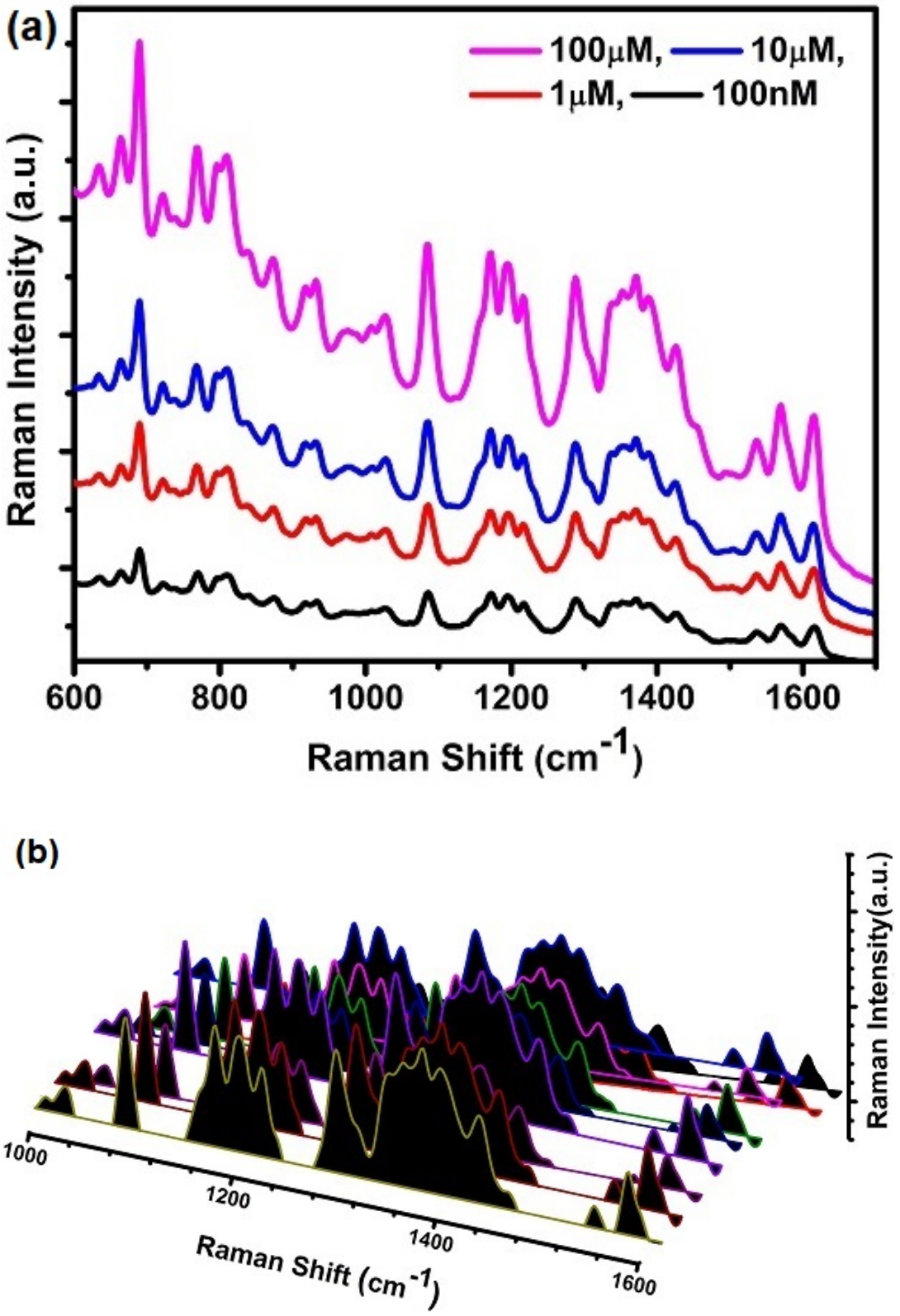

Figure 3.9: (a) Raman signal intensities scattered from the SERS substrates when treated with different fluoride level concentrations in water. (b) Back scattered Raman signal intensities of 10 μM fluoride recorded from 10 different locations of the proposed substrate.

In the final step of the present work, estimation of fluoride level concentration in water using the designed substrate is demonstrated. Four different concentration fluoride contained water samples have been prepared and treated with the developed substrates. The scattered Raman signal of fluoride samples have been recorded by the spectrometer. The characteristics Raman signals for the considered sample is shown in figure 3.9 (a). The prominent peaks at 1084 cm^{-1} and 1288 cm^{-1} which correspond to C-F stretching at different mode of vibrations indicate the presence of fluoride in water sample. Other peaks at 770 cm^{-1}, 1172 cm^{-1} and 1570 cm^{-1} correspond to C-Cl stretching, C-O stretching and due to bending N-H bond respectively. With the designed substrate fluoride level concentration as low as 100 nM can be measured reliably. Below 100 nM, the measured Raman signal yields poor signal to noise ratio. The reproducibility of the designed SERS substrate has also been evaluated for fluoride sample. 10 μM fluoride solution is treated with the designed substrate and back scattered Raman signals from the sensing region of the substrate are recorded from 10 randomly selected locations. Figure 3.9 (b) illustrates variation of the Raman signal intensity recorded from the considered locations of the substrate. Corresponding to the signature Raman peak of fluoride at 1084 cm^{-1} and 1288 cm^{-1} the RSD values are calculated to be 17.26% and 18.49% respectively. An EF of 1.05×10^6 corresponding to Raman shift 1084 cm^{-1} is observed for fluoride sample.

3.4 Summary

In summary, a simple and relatively low cost approach to obtain SERS substrate from naturally available diatom frustule has been demonstrated. With the designed SERS substrate, MG and fluoride level concentration as low as 1 nM and 100 nM have been detected reliably. The detectable limit of fluoride concentration is far below the danger limit (0.5 mg/L to 1.0 mg/L, as recommends by World Health Organization). The design SERS substrate yields EF of 1.66×10^7 for MG and 1.05×10^6 for fluoride. The developed substrate yields a good degree of reproducibility with maximum RSD of 7.57% for MG and 18.49% for fluoride. Furthermore, the designed SERS substrate has a relatively long life span of 14 days.

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