Chapter 4

Development of SERS substrate from printing grade paper and its application for monitoring of glucose and urine

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This chapter discusses an extremely low-cost technique to obtain SERS substrate from printing grade paper. By diffusing silver nanoparticles (AgNPs) in the pores of the paper, the SERS substrate can be obtained. The performance of the paper SERS substrate has been studied for different grade papers thoroughly. The applicability of the proposed SERS substrate for detection of Raman signals from two important samples namely glucose and urine have been realized.

4.1 Introduction

Designing of flexible SERS substrate by adopting low-cost approach has been a long standing goal for researchers. NIL is one of the promising technique to develop flexible SERS substrate on plastic sheet [1-3]. Other techniques such as screen and inject printing have also been used to develop low-cost flexible SERS substrates [4-10]. Use of paper to develop SERS substrate could reduce the fabrication cost to the lowest possible. There are several grades of paper commercially available that are used for different purposes such as for writing, packaging, painting, printing, sanitary use, etc. The grade of a pa-

per depends on the ratio of raw materials used during the manufacturing process. To quantify the amount of raw materials used during fabrication of a specific paper, manufacturer usually designate it with the term gram per square meter (GSM). Depending on its grade, thickness, porosity and roughness of a paper varies. Obtaining uniform surface morphology in microscale level on paper is not possible. The diffusion of metal nanoparticle in colloidal form would then be different for different GSM grade paper substrate. In the recent past, researchers have demonstrated the use of paper substrates as flexible electronic device and energy storage media such as supercapacitors [11-14]. Although metal nanoparticle printed paper has been used as SERS substrate, systematic study on the performance of SERS for different GSM paper substrate has not been reported so far. Herein, the working of a paper based SERS substrate obtained through the diffusion of PVP capped AgNPs on printing grade paper has been studied. The performance of the designed SERS substrate has been evaluated for different GSM grade papers. The developed SERS substrate yields a good degree of reproducibility, high order of EF and longer durability. Schematic representation of fabrication of proposed paper based SERS substrate and its application for detection of Raman signal from Raman active sample is shown in the figure 4.1.

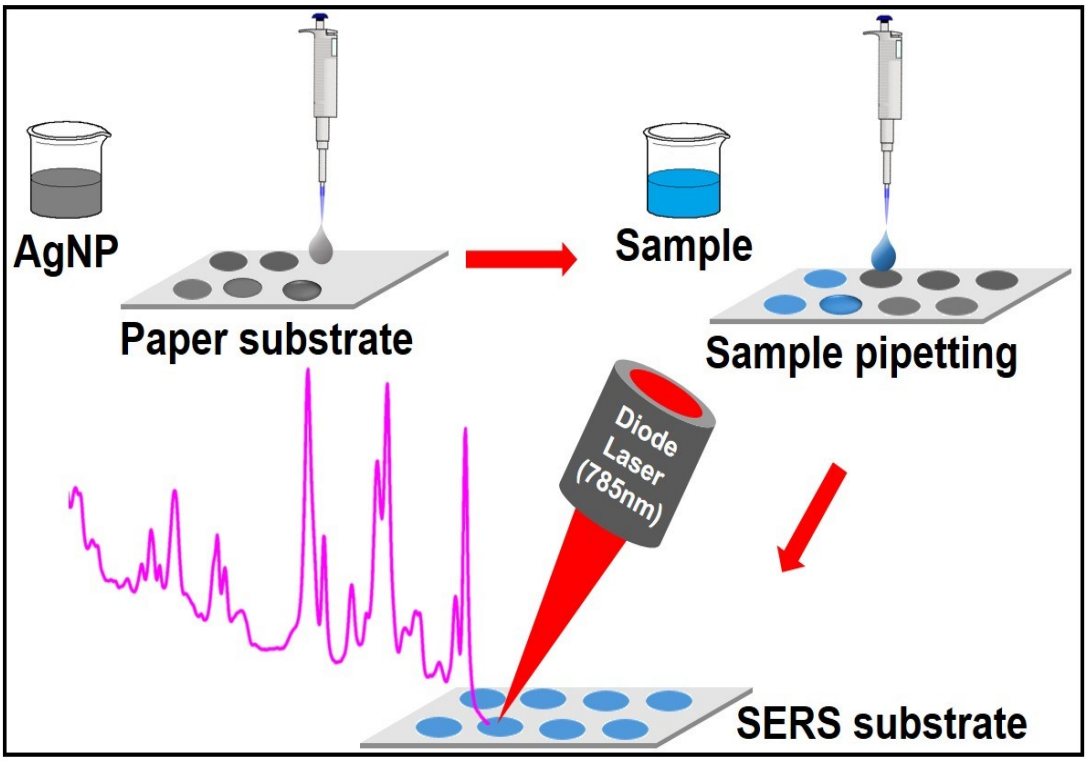

Figure 4.1: Schematic representation of the proposed paper based SERS substrate and its application for measuring of Raman signal from Raman active sample.

4.2 Experimental

4.2.1 Materials

Silver nitrate ($AgNO_3$) is procured from Rankem, India, MG and Polyvinylpyrrolidone (PVP, $(C_6H_9NO)_n$) of average molecular weight of 40,000 are procured from Sigma-aldrich. Creatinine, sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$) and rhodamine6G (R6G) are procured from SRL, India, while glucose, sodium carbonate, sodium chloride, potassium chloride, calcium sulfate, sodium sulfate, magnesium sulfate, sodium dihydrogen phosphate, urea and triethylamine are procured from Merck, India. Gold chloride trihydrate ($HAuCl_4{\cdot}3H_2O$) is procured from Himedia, India. All chemicals are used as received without further processing.

4.2.2 Synthesis of AgNPs colloidal solution

The synthesis procedure reported by Wu et.al [36] has been followed to obtain AgNPs in the present work. 0.0274 g of $AgNO_3$, 0.0135 g of glucose, 0.05 g of PVP and 0.00011 g of sodium carbonate are added to 50 ml of DI water and stirred it for 10 minutes. The mixture is then heated to boil under vigorous stirring condition. The color of the solution gradually turned into light blackish. 15 μL of triethylamine is added to the mixture which would change the color of the solution into brown. Finally, the solution is centrifuged at 2500 rpm for 20 min for four times by adding ethanol into it. The formation of AgNPs in the solution is confirmed from TEM image. Figure 4.2 (a) shows the TEM image of the synthesized AgNPs. The average size of the AgNPs is measured to be 60 nm.

4.2.3 SERS substrate preparation

In the present study, 5 different grade papers- 75 GSM, 85 GSM, 100 GSM, 140 GSM and 200 GSM have been considered. On each of the considered paper substrates, 10 μL of the synthesized AgNPs solution has been dispensed using micropipette and allowed it to dry. The photograph of the developed SERS substrates as obtained for different GSM papers is shown in figure 4.2(b). The effective sensing area on the different grade paper substrate is observed to be varied between 50.26 mm^2 to 73.62 mm^2. The FESEM images in figure 4.2 (c), (d), (e), (f) and (g) depict the distribution of AgNPs on 75 GSM, 85 GSM, 100 GSM, 140 GSM and 200 GSM paper substrates respectively. The grade of paper plays an

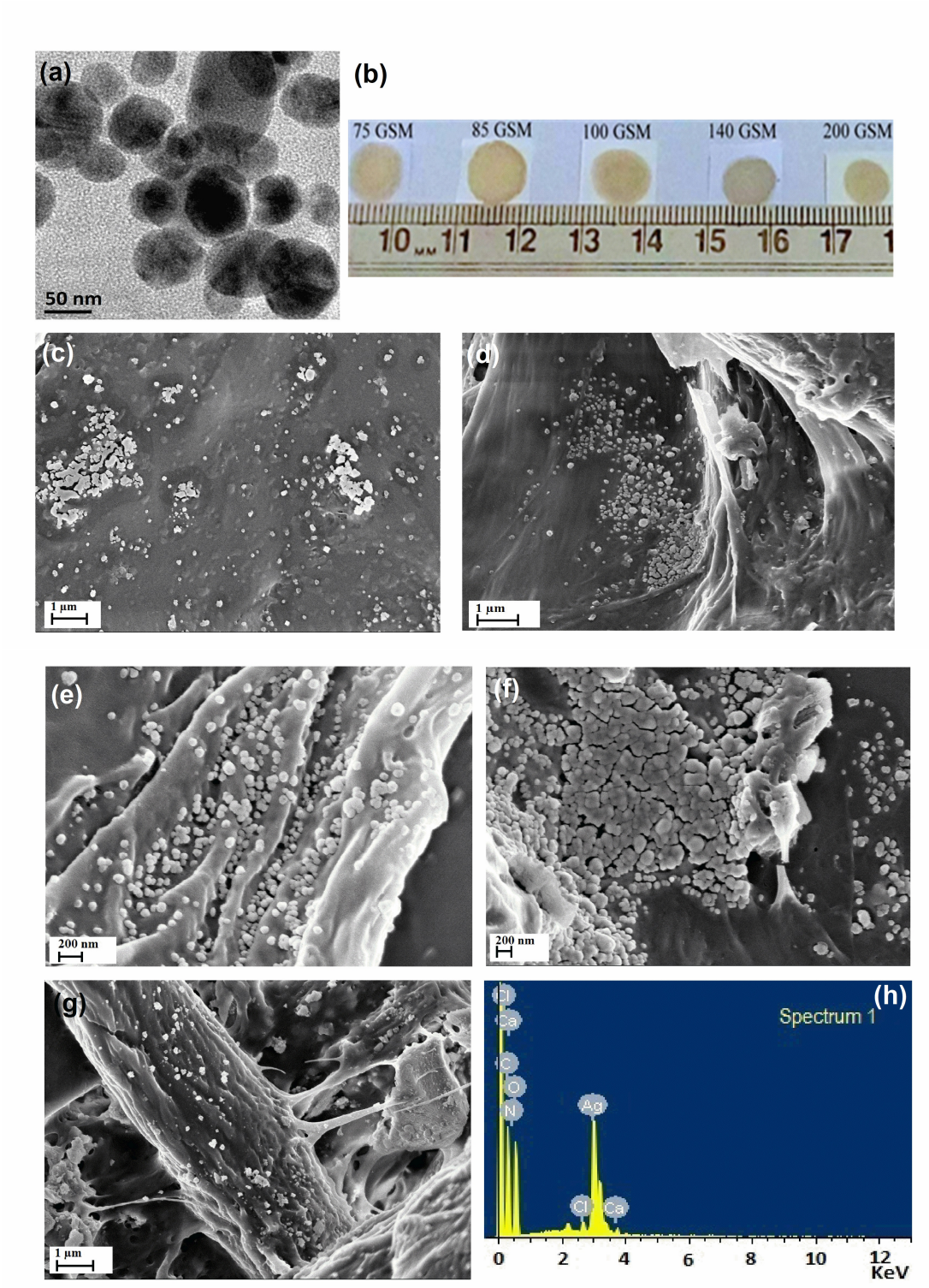

Figure 4.2: (a) TEM image of synthesized AgNPs, (b) Photo images of SERS substrates fabricated on different GSM papers. The dispersed area of AgNPs varies from 50.26 mm^2 to 73.62 mm^2. FESEM images of AgNPs distribution on (c) 75 GSM, (d) 85 GSM, (e) 100 GSM, (f) 140 GSM and (g) 200 GSM substrates. 10 μL of the synthesized AgNPs colloidal solution was pipetted on each paper substrate. (h) EDX data represent the elements composition on the sensing region of 100 GSM SERS substrate.

important role in controlling the diffusion of AgNPs on the paper substrates. This can be understood from the relative size of the diffused areas on the considered paper substrates shown figure 4.2 (b). Distribution of AgNPs on the paper substrate is primarily driven by pore size and surface morphology of the paper. Upon pipetting of AgNPs colloidal solution on the paper substrate, two possible modes of diffusion namely in-plane and lateral diffusion would occur. For lower grade GSM paper, the lateral diffusion is found to be dominant over in-plane diffusion, while for higher grade GSM paper substrates the in-plane diffusion dominants over the later. Among all the considered paper substrates, a relatively uniform distribution of AgNPs has been observed for 100 GSM paper. This is attributed to the optimized condition for lateral diffusion of AgNPs on the paper substrate due to surface morphology and porosity for this specific paper. The EDX data describing elemental compositions of the developed SERS substrate is shown in figure 4.2 (h).

4.2.4 Analyte sample preparation

10 mM stock solution of MG, R6G and glucose samples are prepared separately in DI water. By diluting the stock samples, required lower concentration samples can be obtained. Following standard procedure reported by Gu et.al [15] the artificial urine sample has been prepared in the laboratory. For detection and analysis of Raman signal for the considered chemicals, 10 μL of each the sample solution is pipetted on the designed SERS substrates and allowed to dry in room temperature environment for 2 hours.

4.2.5 Simulation study of LSPR field magnitude of the proposed substrate

Considering random arrangement of the AgNPs on paper substrate the average LSPR field magnitude of the designed SERS substrate has been studied using the simulation tool. For this study the paper substrate is considered as a plane cellulose slab of thickness 250 nm and AgNPs of dimension varying between 20 nm to 80 nm are considered to be distributed randomly with inter particle spacing between 30 to 80 nm. A plane polarized light of wavelength 785 nm defined as an exciting source is incident normally on the substrate. Under this consideration, the maximum LSPR field magnitude is observed to be 1.38×10^7 V/m. This would cause an EF of the order of 10^9 in the designed substrate. This suggests that the proposed paper based substrate can be used to record enhanced Raman signal from analyte samples through diffusing AgNPs on paper substrate. The smaller than the studied system.

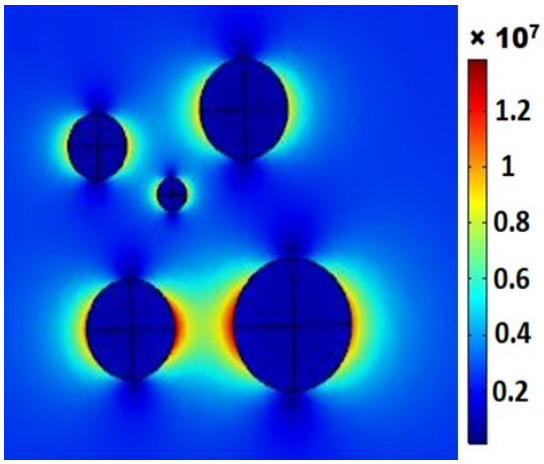

Figure 4.3: Simulation result of LSPR field magnitude for different dimensions of surface deposited AgNPs on 100 GSM paper substrate. The spacing between the particle are considered to be varying between 30-80nm.

4.3 Results and discussion

4.3.1 Characterization of the substrate

In order to evaluate the performance of the designed substrates on different grade paper, 10 μL of each of MG and R6G samples with 1 μM concentration are pipetted on the sensing region of each SERS substrate. Upon drying, the Raman signals of these samples are recorded by the Raman spectrometer. Figure 4.4 (a) and (b) show the relative Raman signal intensity of MG and R6G respectively. Among the considered different grade GSM paper substrates, the average Raman signal enhancement is observed to be the highest for 100 GSM paper substrate. As already shown in figure 4.2(e) a relatively uniform distribution of AgNPs on 100 GSM SERS substrate is observed and this is primarily responsible for producing the highest average LSPR field magnitude among all considered substrates. The scattered Raman signal intensity of Raman active samples would therefore be the highest for this specific substrate. In the present study, all investigations have been carried out under the same ambient conditions and investigation parameters. Owing to the fact that 100 GSM paper substrate yields the highest Raman signal intensity for the same analyte sample, in the subsequent step of the present study, the SERS based investigations have been performed with 100 GSM paper only.

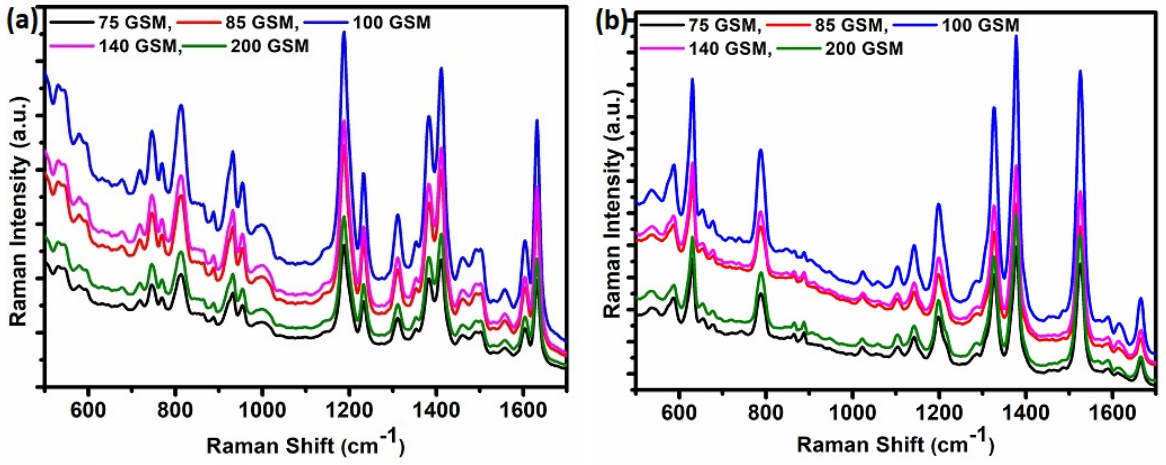

Figure 4.4: Relative Raman signal intensities of (a) MG and (b) R6G scattered from different GSM papers namely 75 GSM, 85 GSM, 100 GSM, 140 GSM and 200 GSM SERS substrate. 10 μL of both the samples each of concentration 1 μM has been dispensed on different GSM SERS substrate.

Minimum measurable sample concentration and EF estimation

To check the minimum sample concentration that can be detected with the developed SERS substrate, different concentrations of the considered samples have been treated with the substrates. Upon drying of the samples, the scattered Raman signals are recorded. Figure 4.5 (a) and (b) depict the relative Raman signal intensities for different concentration of MG and R6G respectively. For MG the minimum concentration of 0.1 nM and while for R6G 1 nM can be detected reliably from the designed SERS substrate. The figures also include the Raman signals of both the samples of concentration 1 mM, recorded from the bare paper substrate. With the proposed SERS substrate strong Raman peaks at 420 cm^{-1}, 1188 cm^{-1}, 1378 cm^{-1} and 1632 cm^{-1} have been observed for MG which is 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 [16-18]. Medium bands at 808 cm^{-1}, 1232 cm^{-1} and 1592 cm^{-1} corresponds to out of plane vibration of C-H ring, C-H rocking and C-C stretching respectively [16-18]. Also the strong peaks of R6G observed at 612 cm^{-1} correspond to C-C-C in-plane bending, 774 cm^{-1} correspond to C-H out of plane bending, 1127 cm^{-1} for C-H in-plane bending, 1310 cm^{-1} indicates C=C stretching, 1360 cm^{-1}, 1509 cm^{-1} and 1573 cm^{-1} for C-C stretching, and 1650 cm^{-1} for C=O stretching are in good agreement with earlier reported works [9, 19]. To estimate the EF of the proposed SERS substrate, Raman signal intensity of 10 mM MG scattered from bare 100 GSM paper substrate has been recorded for reference. Using equation 1.22 an average EF of the order of 10^7 has been observed experimentally.

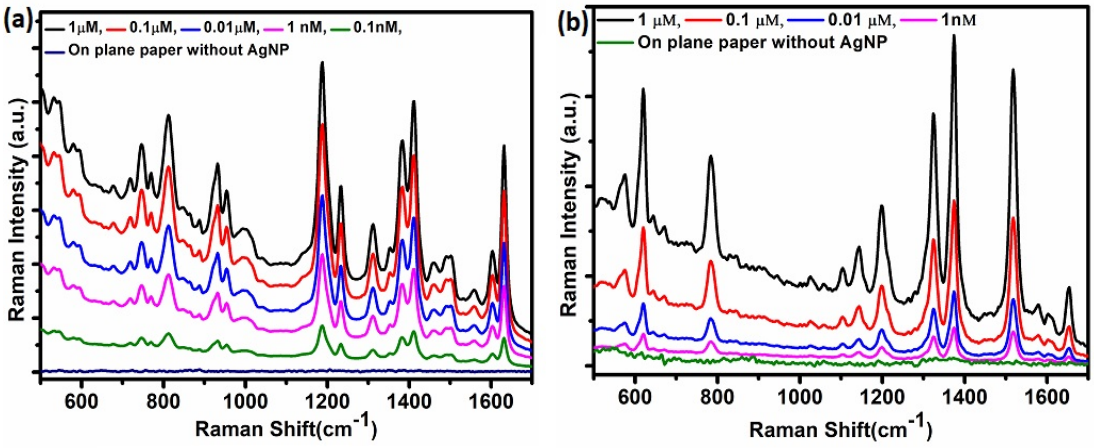

Figure 4.5: Recorded Raman spectra scattered from the substrate for different concentrations of (a) MG and (b) R6G. These figures also include the Raman signal intensities of the samples scattered from bare paper substrates.

Reproducibility and spectral uniformity of the substrate

A non-fluorescence chemical- 1,2-bis(4-pyridyl)ethylene (BPE) has also been considered in the present study to evaluate the reproducibility characteristics of the designed substrate. 1 μM BPE solution has been prepared in ethanol medium [20] and treated with the substrate. The back scattered Raman signals are recorded from 20 different randomly selected locations of the SERS substrate. Figure 4.6 (a) represents the reproducibility characteristic of the designed substrate. The signal intensity variation corresponding to Raman peak of BPE at 1214 cm^{-1} and 1620 cm^{-1} are observed to be varying with maximum relative standard deviation (RSD) of 8.76% and 8.14% respectively. Further, to study the spectral uniformity characteristics for the SERS substrate, the variation of Raman signal intensity corresponding to Raman peak at 1620 cm^{-1} is mapped over an area of 5 mm×5 mm with an array of 15×15 over the sensing region of the substrate. Figure 4.6 (b) shows the Raman signal intensity fluctuations for this specific peak over the mapping area. The maximum SERS intensity variation is observed to be 25%, however over 85% of the mapped area, the SERS signal intensity variation is found to be less than 15% . The low RSD values of Raman shift and lower fluctuations of signal intensity over a relatively large area signifies that the proposed substrate is highly reproducible and yields a good degree of spectral uniformity.

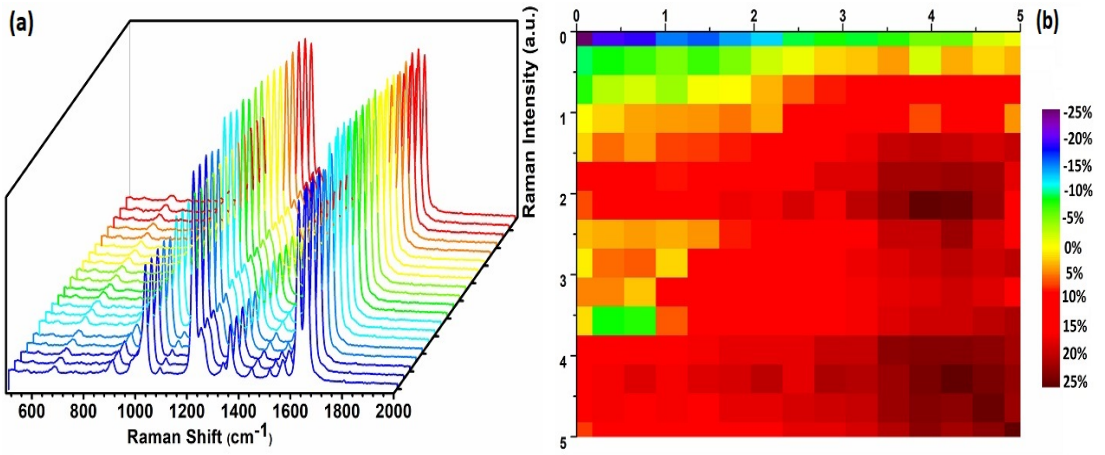

Figure 4.6: (a) Reproducibility characteristics of the developed substrate while BPE has been used as test sample and (b) Signal intensity variation in different locations of the substrate over an area of 5 mm×5 mm corresponding to the Raman peak at 1620 cm^{-1}.

Life span evaluation of the SERS substrate

The capping agent used in the present work for fabrication of SERS substrate inhibits the agglomeration of AgNPs and due to presence of such capping agent, the SERS substrate is relatively stable. The performance of the designed SERS substrate and bare AgNPs treated paper substrate has been evaluated and compared for a period of 7 days. Figure 4.7 (a) and (b) illustrate the time evaluation performance of this substrate for MG. The maximum intraday peak intensity fluctuations are observed to be 9.65% and 10.21% for PVP capped and bare AgNP diffused SERS substrate respectively and maximum intensity fluctuations from the average value (indicated in green line) during the investigation period for these two substrates are found to be 16.69% and 46.94% corresponding to Raman peak at 1188 cm^{-1}. Clearly, as compared to bare AgNPs diffused SERS substrates, the PVA capped AgNPs diffused paper substrate yields stable Raman signal over a considerable period of time. The gradual degradation of Raman peaks signal in bare AgNPs treated paper substrate is attributed to oxidation of metal nanoparticles, which eventually affects the LSPR field strength and thus the intensity of the scattered Raman signal from this substrate degrades gradually with time. Stability performance of proposed SERS substrate has also been compared with another substrate developed by diffusing 40 nm gold nanoparticles (AuNPs) on 100 GSM paper. The procedure of synthesizing 40 nm AuNP colloidal solution has been discussed in section 3.2.3. 1 μM MG is treated with the substrate and the intensity fluctuations of the scattered Raman signal from the substrate has been observed for two weeks. The maximum intraday peak intensity fluctuation is observed to be 9.28% and while maximum intensity fluctuations

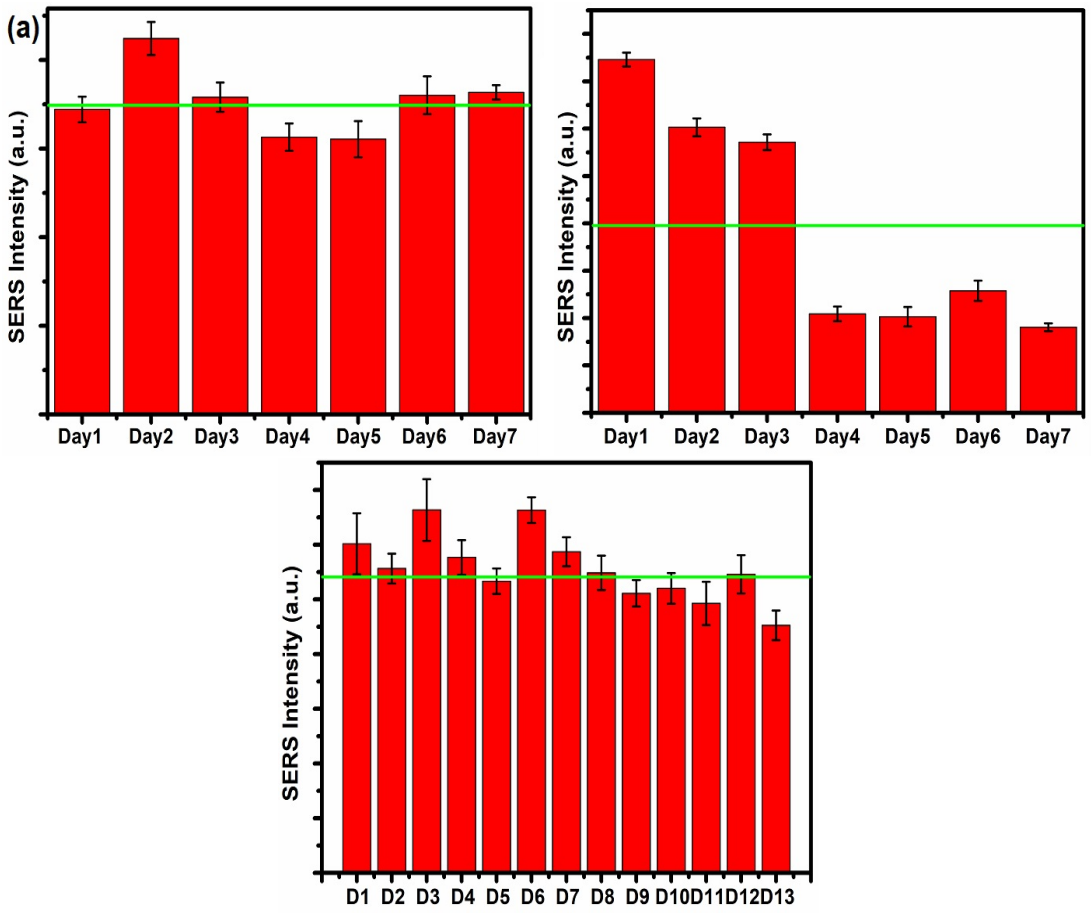

Figure 4.7: Time evaluation performance of PVP capped AgNPs (a) and bare AgNPs (b) diffused SERS substrate. MG has been taken as a test sample. Raman peak of MG 1188 cm^{-1} is considered for the present study (c) Raman signal intensities variation corresponding to the peaks same peaks of MG while AuNPs are diffused into the paper substrate.

from the average value (indicated in green line) over the investigation period is observed to be 15.87% corresponding to the signature Raman peak at 1188 cm^{-1}. The corresponding result is shown in figure 4.7 (c). The fairly stable reading in Raman shift value for AuNPs treated SERS substrate suggests that as compared to AgNPs based SERS substrate AuNPs diffused SERS substrate yields better stability. Though PVP capped AgNPs yields stable reading for seven days, it has been observed that after eight days the performance of the substrate degrades substantially and the Raman signal intensity from the substrate reduces significantly.

4.3.2 Glucose and artificial urine detection

The real field application of the proposed substrate has been realized through the detection of two clinically important samples- glucose and urine. In the present study, four different concentrations of glucose namely- 1 μM, 0.1 μM, 0.01 μM and 1nM have been prepared by dilution the stock sample. The SERS substrates are then dipped vertically into these samples for 12 hours to functionalize the glucose molecule with the substrates. The glucose functionalized substrates are then rinsed with distilled water and allowed to dry in room temperature. The Raman signals intensities of different glucose samples are recorded by the Raman spectrometer. Figure 4.8 (a) illustrates the relative Raman signal intensities measured for four different samples. The figure shows that with the decrement of sample concentration the relative intensities of the signature Raman peaks of glucose decreases gradually. For glucose, signature Raman peaks at 1123 and 1658 cm^{-1} are attributed to C-C stretching vibration and C=O stretching vibration mode respectively and the intensities of these peaks are varying gradually with concentration. Again, urine sample of different concentration has been prepared in the laboratory through dilution of the stock urine sample. Following the same procedure, different concentrations of urine sample are functionalized with the proposed SERS substrates. Figure 4.8 (b) illustrates the characteristics relative Raman signal intensities recorded by the spectrometer for different urine samples. Here also a gradual decrease in signal intensities has been observed with the decrement of concentration of the sample solutions. For urine, strong Raman peaks at 866, 950 and 1018 cm^{-1} are attributed to C-NH_2 stretching, CH_3 deformation and C-N stretching respectively and the intensities of these peaks are also observed to be varying with the concentration of the sample. These results indicate that the proposed SERS substrate can be reliably used for detection and quantification of any Raman active samples.

The proposed technique is extremely cost-effective, simple and the SERS substrate can be easily obtained within 30 minutes upon treatment of the paper with AgNP colloidal solution. To develop approximately 1 cm^2 SERS active area on paper substrate the net cost involved is less than INR 5.00 ($< \$0.07$) which is significantly lesser than the price of a commercially available SERS substrate. For large scale production, the cost would be reduced further. The size variation of metal nanoparticles deposited over of the paper substrate could lead to fluctuations of Raman signal intensities when measured from different locations on the substrate. With the commercially available AgNPs colloidal solution where the sizes of the particles vary within a small range, it is envisioned that the performance of the proposed SERS substrate would be enhanced further.

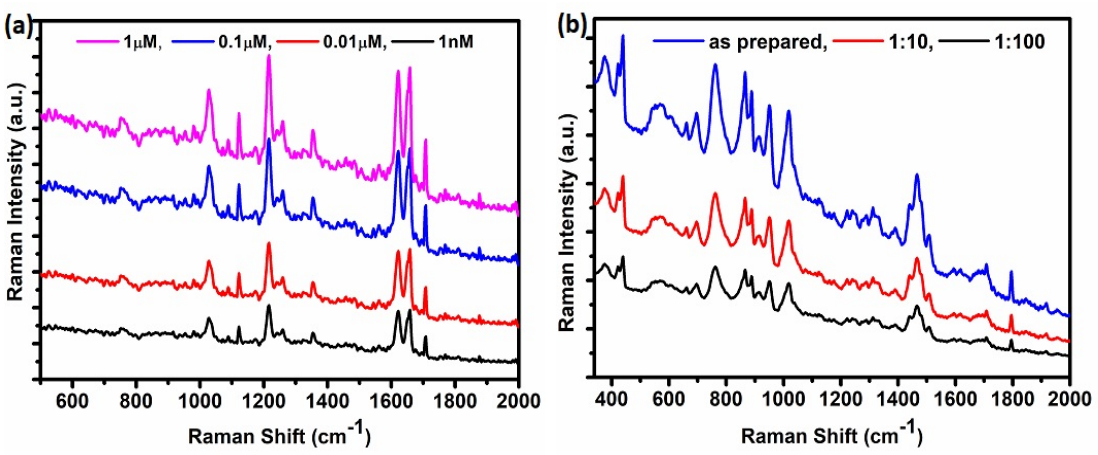

Figure 4.8: Relative Raman signal intiensities scattered from different concentrations of (a) glucose and (b) artificial urine sample solutions.

4.4 Summary

Realization of paper based SERS substrate by diffusing PVP capped AgNPs has been presented in the present chapter. Out of different grade GSM papers, 100 GSM papers yields the maximum enhancement in the scattered Raman signal. With the designed SERS substrate MG and R6G concentration as low as 0.1 nM and 1 nM can be detected reliably. The EF of the order of 10^7 has been observed for MG. The simulation results show an average local field enhancement of 2.06×10^2. Considering $|E|^4$ approximation the average SERS EF is estimated to be of the order 10^9. However, the experimentally obtained EF for the designed substrate is found to be of the order of 10^7. The simulation result is based on the generic pattern of the proposed SERS substrate where a part of the imaged pattern of the designed SERS substrate has been considered for simulation study. However, the actual distribution of AgNPs over the substrate is quite different from the simulated pattern which has caused different magnitudes of the coupled LSPR field over the sensing region of the SERS substrate. Usually, the value of the experimental EF is lesser than the simulated result, since EF for a specific SERS substrate is an analyte dependent phenomenon. The substrate yields a good degree of reproducibility with RSD value of 8.76 and 8.14% corresponding to BPE signature Raman peaks at 1214 cm^{-1} and 1620 cm^{-1} respectively. Also, a good spectral uniformity with an average intensity variation below 15% over 85% of the mapped area (5 mm×5 mm) of the substrate has been observed. Due to the presence of capping agent, the designed SERS substrate has a good life span (7 days). Field applicability of the proposed SERS substrate has been realized through detection and analyzing of Raman signal from two clinically important chemicals-glucose and urine. Owing to low-cost fabrication process involved, it is envisioned that

the proposed SERS substrate can be used for disposable purpose.

References

[1] Choi, C. J., Xu, Z., Wu, H.-Y., Liu, G. L., and Cunningham, B. T. Surface-enhanced raman nanodomes. *Nanotechnology,* 21(41):415301, 2010.

[2] Oo, S., Chen, R., Siitonen, S., Kontturi, V., Eustace, D., Tuominen, J., Aikio, S., and Charlton, M. Disposable plasmonic plastic SERS sensor. *Optics express,* 21(15):18484-18491, 2013.

[3] Liu, L., Zhang, Q., Lu, Y., Du, W., Li, B., Cui, Y., Yuan, C., Zhan, P., Ge, H., Wang, Z., et al. A high-performance and low cost SERS substrate of plasmonic nanopillars on plastic film fabricated by nanoimprint lithography with AAO template. *AIP Advances,* 7(6):065205, 2017.

[4] Wei, W. Y. and White, I. M. Chromatographic separation and detection of target analytes from complex samples using inkjet printed SERS substrates. *Analyst,* 138(13):3679-3686, 2013.

[5] Wei, W. Y. and White, I. M. Inkjet-printed paper-based SERS dipsticks and swabs for trace chemical detection. *Analyst,* 138(4):1020-1025, 2013.

[6] Hoppmann, E. P., Wei, W. Y., and White, I. M. Highly sensitive and flexible inkjet printed SERS sensors on paper. *Methods,* 63(3):219-224, 2013.

[7] Eshkeiti, A., Narakathu, B., Reddy, A., Moorthi, A., Atashbar, M., Rebrosova, E., Rebros, M., and Joyce, M. Detection of heavy metal compounds using a novel inkjet printed surface enhanced raman spectroscopy (SERS) substrate. *Sensors and Actuators B: Chemical,* 171:705-711, 2012.

[8] Miccichè, C., Arrabito, G., Amato, F., Buscarino, G., Agnello, S., and Pignataro, B. Inkjet printing Ag nanoparticles for SERS hot spots. *Analytical Methods,* 2018.

[9] Wu, W., Liu, L., Dai, Z., Liu, J., Yang, S., Zhou, L., Xiao, X., Jiang, C., and Roy, V. A. Low-cost, disposable, flexible and highly reproducible screen printed SERS substrates for the detection of various chemicals. *Scientific reports,* 5:10208, 2015.

[10] Ma, Y., Wang, Y., Duan, H., Li, D., Xu, H., Fodjo, E. K., et al. Rapid and sensitive on-site detection of pesticide residues in fruits and vegetables using screen-printed paper-based SERS swabs. *Analytical Methods,* 2018.

[11] Chinga-Carrasco, G. Exploring the multi-scale structure of printing paper-a review of modern technology. *Journal of microscopy,* 234(3):211-242, 2009.

[12] Zheng, G., Hu, L., Wu, H., Xie, X., and Cui, Y. Paper supercapacitors by a solvent-free drawing method. *Energy & Environmental Science,* 4(9):3368-3373, 2011.

[13] Nath, P., Hussain, I., Dutta, S., and Choudhury, A. Solvent treated paper resistor for filter circuit operation and relative humidity sensing. *Indian Journal of Physics,* 88(10):1093-1097, 2014.

[14] Tobjörk, D. and Österbacka, R. Paper electronics. *Advanced Materials,* 23(17):1935-1961, 2011.

[15] Gu, X., Wang, H., Schultz, Z. D., and Camden, J. P. Sensing glucose in urine and serum and hydrogen peroxide in living cells by use of a novel boronate nanoprobe based on surface enhanced raman spectroscopy. *Analytical chemistry,* 88(14):7191-7197, 2016.

[16] Kumar, P., Khosla, R., Soni, M., Deva, D., and Sharma, S. K. A highly sensitive, flexible SERS sensor for malachite green detection based on Ag decorated microstructured PDMS substrate fabricated from taro leaf as template. *Sensors and Actuators B: Chemical,* 246:477-486, 2017.

[17] Lee, S., Choi, J., Chen, L., Park, B., Kyong, J. B., Seong, G. H., Choo, J., Lee, Y., Shin, K.-H., Lee, E. K., et al. Fast and sensitive trace analysis of malachite green using a surface-enhanced raman microfluidic sensor. *Analytica chimica acta,* 590(2):139-144, 2007.

[18] Zhang, Y., Huang, Y., Zhai, F., Du, R., Liu, Y., and Lai, K. Analyses of enrofloxacin, furazolidone and malachite green in fish products with surface-enhanced raman spectroscopy. *Food chemistry,* 135(2):845-850, 2012.

[19] Wang, Y. Q., Ma, S., Yang, Q. Q., and Li, X. J. Size-dependent SERS detection of R6G by silver nanoparticles immersion-plated on silicon nanoporous pillar array. *Applied Surface Science,* 258(15):5881-5885, 2012.

[20] Xu, Z., Jiang, J., Wang, X., Han, K., Ameen, A., Khan, I., Chang, T.-W., and Liu, G. L. Large-area, uniform and low-cost dual-mode plasmonic naked-eye colorimetry and SERS sensor with handheld raman spectrometer. *Nanoscale,* 8(11):6162-6172, 2016.