Chapter 6

SERS substrate realization using Blu-ray DVD and reliable detection of albumin, creatinine and urea in urine sample

This chapter discusses a simple, yet fairly reproducible SERS substrate by efficiently guiding localized surface plasmon resonance (LSPR) field of gold nanoparticle (AuNP) through trapping it in the nanochannels of a blu-ray digital versatile disc (BRDVD). The performance of the proposed substrate is initially realized by recording Raman signals of MG and BPE. Upon observing its performance for Raman active samples, detection of three clinically important chemicals have been demonstrated. The figure of merit of the BRDVD SERS substrate has been discussed at the end of this chapter.

6.1 Introduction

In the earlier works of the thesis work, SERS substrates fabrication from diatom, printing grade papers and Au coated PVA nanofibers using low-cost approaches have been demonstrated. All these substrates have specific merits from their application points of view. For example, GMR characteristics of diatom pores contribute to SERS signal enhancement and the periodic nature of pores helps in light confinement. Use of printing grade paper for development of SERS substrate, makes the technique extremely cost-effective.

Again, the use of PVA nanofiber to obtain SERS substrate enables biocompatibility of the substrate and hence without affecting the nature of analyte, sensing investigation can be performed. However, since the arrangement of these structures are random and depends on intrinsic factors such as size of the pores in diatom, paper quality and electrospinning parameters it is not always possible to develop a SERS substrate with same surface morphology using these approaches. Obtaining of SERS substrate from a fixed patterned structure at an affordable cost with high EF, durability and reproducibility could make SERS based study an interesting field of research. Keeping this issue in mind, the nanochannels of commercially available BRDVD has been exploited to obtain a SERS substrate. DVD and BRDVD based SERS substrates have been demonstrated by depositing Au film [1-5]. The Au films are coated by using sputtering and electrophoretic techniques. All commercially available BRDVDs comprises of periodic nanochannels of width 100 nm with a periodicity of 320 nm. Such structure is ideal for trapping of metal nanoparticles in the width of the channels. The sidewall of BRDVD nanochannels is effective for guiding the coupled em field with the trapped nanoparticles in the channel. The working of a BRDVD as a SERS substrate with reasonably good reproducible characteristic for detection and quantification of Raman active samples through correlating the Raman signal intensities from the substrate has been demonstrated. Initially, detection and estimation of two commonly used samples- MG and a non-fluorescent material BPE have been performed using the proposed SERS substrate. Upon noticing its reliable performance, real field application of the substrate has been realized through detection and analysis of different important chemicals namely albumin, creatinine and urea present in urine samples have been demonstrated.

6.2 Materials and Methods

6.2.1 Materials

BRDVD (25GB, 6X) manufactured from SONY Inc. is procured from a local stationery store. Albumin is purchased from Renkam, India. Creatinine and sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$) are procured from SRL, India. Sodium carbonate, sodium chloride, potassium chloride, calcium sulfate, sodium sulfate, magnesium sulfate, sodium dihydrogen phosphate and urea are procured from Merck, India. Gold chloride trihydrate ($HAuCl_4{\cdot}3H_2O$) is procured from Himedia, India. Procured chemicals are used without further processing. All the sample solutions are prepared in distilled water.

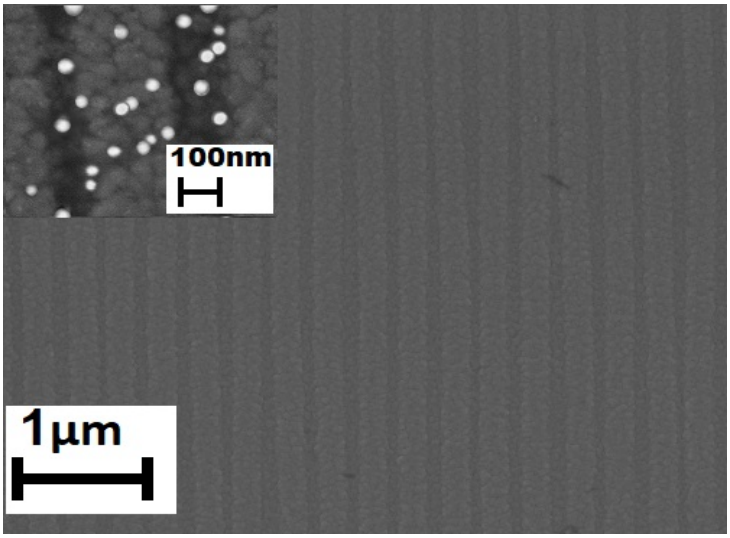

Figure 6.1: Field emission scanning electron microscope (FESEM) image of BRDVD substrate. The inset image shows the distribution of AuNPs on the substrate.

6.2.2 SERS substrate fabrication and Raman signal measurement system

Following the procedure described in the section 3.2.2 of chapter 3, AuNPs colloidal solution of particle dimension 20 nm, 40 nm and 60 nm are synthesized in the laboratory. Using a clean scissor, the BRDVD is initially cut into small pieces each of sizes 1 cm×1 cm and its protecting layer has been peeled-off using a tweezer. The BRDVD substrate pieces are then washed with ethanol in ultrasonic bath for 10 minutes followed by isopropyl alcohol bath for another 10 minutes. The substrate pieces are then allowed to dry at room temperature. Upon drying, 10 μL of the colloidal AuNP solution is pipetted on the substrate followed by drying at room temperature environment. Figure 6.1 shows the FESEM image of bare BRDVD substrate. The inset image in the same figure shows the FESEM image of AuNPs treated BRDVD substrate. The image depicts the distribution of AuNPs over the BRDVD sample. The trapped nanoparticles in the channel of BRDVD are found to be occupied either in single or few have positioned in close proximity. Upon irradiating of the SERS substrate with EM field, the generated LSP field in AuNPs within the channels of BRDVD will store for a longer time due to guided mode phenomenon of the channel and this would cause enhancement of the local field intensities. Subsequently, there would be an enhancement in scattered Raman signals from Raman active samples.

6.2.3 Electromagnetic (EM) simulation study

To study the LSPR field magnitude due to coupling of the incident EM field with the channel trapped AuNPs, a set of simulation study has been carried out using simulation tool. For this study a slab of 300 nm thick polycarbonate with channel width of 100 nm and spherical AuNPs of dimension 40 nm are placed on the structure. A plane polarized light with wavelength of 785 nm is defined as an optical source to study the magnitude of LSPR field in the present study. Single and two closely spaced AuNPs in the channel of BRDVD have been considered for the present study. The generated field magnitude has been compared between channel guided and unguided plane polycarbonate substrate. Figure 6.2 (a)-(d) illustrate the comparison of the field magnitude for these two situations. As compared to a plane substrate the field magnitude has been enhanced by ~ 2 fold for a single trapped AuNP in the channel, while for two closely spaced AuNPs this value is found to be increased by ~ 10 fold. The orientation of the coupled nanoparticles within the channel of the BRDVD plays a critical role on the magnitude of the local EM field. For AuNPs oriented along the length of the channel the maximum field intensity is observed to be 3.5×10^7 V/m while for laterally oriented AuNPs, the field magnitude is observed to be 1.6×10^8 V/m. Figure 6.2 (e) shows the magnitude of the generated local electric field for two closely spaced AuNPs oriented along the length of the channel. For any other orientations, the generated field magnitude will vary in between this range. Figure 6.2 (f) illustrates the maximum field coupling conditions for different sizes AuNP attached on the substrate. These simulation results reveal that with 40 nm size AuNPs, the proposed substrate yields the maximum field coupling condition when it is excited with 785 nm laser source. The spacing between AuNPs are assumed to be 10 nm while performing the simulation studies.

6.2.4 Analyte sample preparation

Stock sample solution of MG, albumin, creatinine and urea stock are prepared by dissolving proportionate amount of samples in distilled water. By diluting the stock solution required lower concentration samples have been obtained. The BPE stock solution is prepared in ethanol medium. Following standard procedure reported by Gu et.al [6], the artificial urine sample has been prepared. For detection of Raman signal of the considered chemicals, 10 μL of each sample is pipetted on the designed SERS substrates and allowed to dry in room temperature before analyzing the characteristic Raman signal from the substrate.

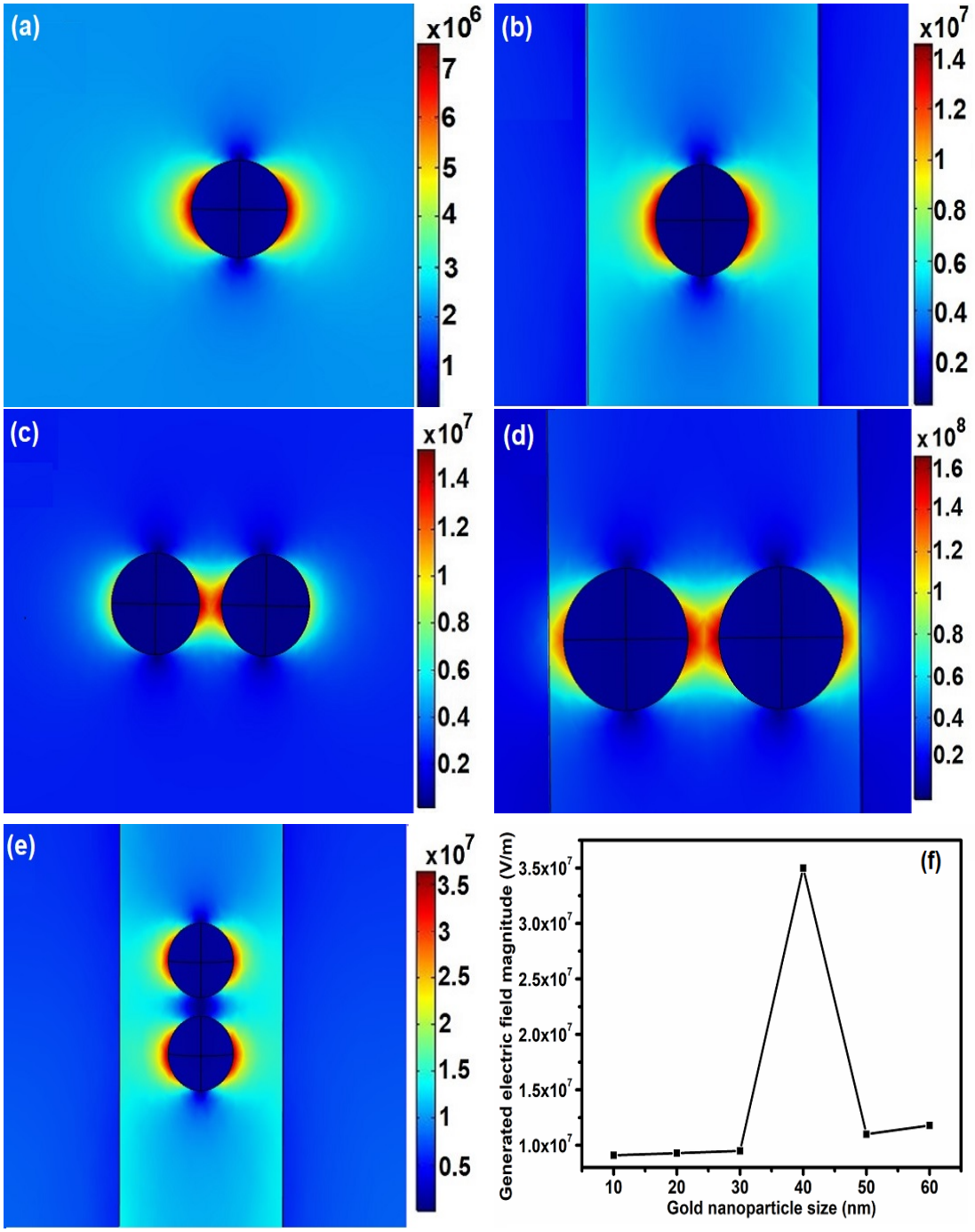

Figure 6.2: Comparison of LSPR field magnitude for a single nanoparticle when it is considered on (a) plane polycarbonate substrate and (b) within the channel of BRDVD substrate. The LSPR field magnitude has also been compared when two closely spaced AuNPs are considered on (c) a plane polycarbonate substrate and within the channels of BRDVD substrate oriented (d) laterally and (e) along the length of its channel. (f) Generated electric field magnitude variation for different AuNP size when oriented along the length of the channel. The particle spacing are maintained at 10 nm.

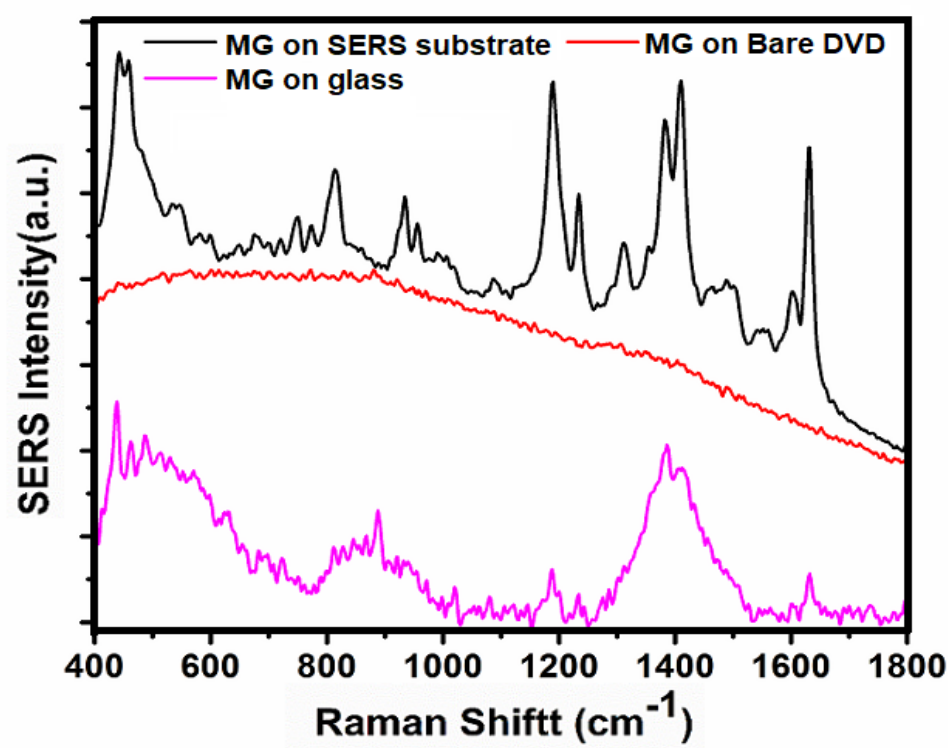

Figure 6.3: A comparison of Raman signal of MG scattered from developed SERS substrate, bare DVD and plane glass substrate.

6.3 Results and Discussions

6.3.1 Characterization of the substrate

To study the SERS investigation of the proposed substrate, 10 μL of 10 μM MG solution is treated with the substrate. Also the same amount of the sample has been treated with bare BRDVD and plane glass substrate. Upon drying of the sample, the back scattered Raman signal of MG from these substrates are recorded by the Raman spectrometer. Figure 6.3 shows the characteristic curves for the above considered substrates. The signature Raman peaks of MG at 440 cm^{-1}, 1188 cm^{-1}, 1408 cm^{-1} and 1632 cm^{-1} are attributed to the 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 [7-9]. It has been observed that as compared to the bare BRDVD and glass substrate, the proposed SERS substrate scatters intense Raman signal and strong prominent signature peaks of MG can be recorded easily.

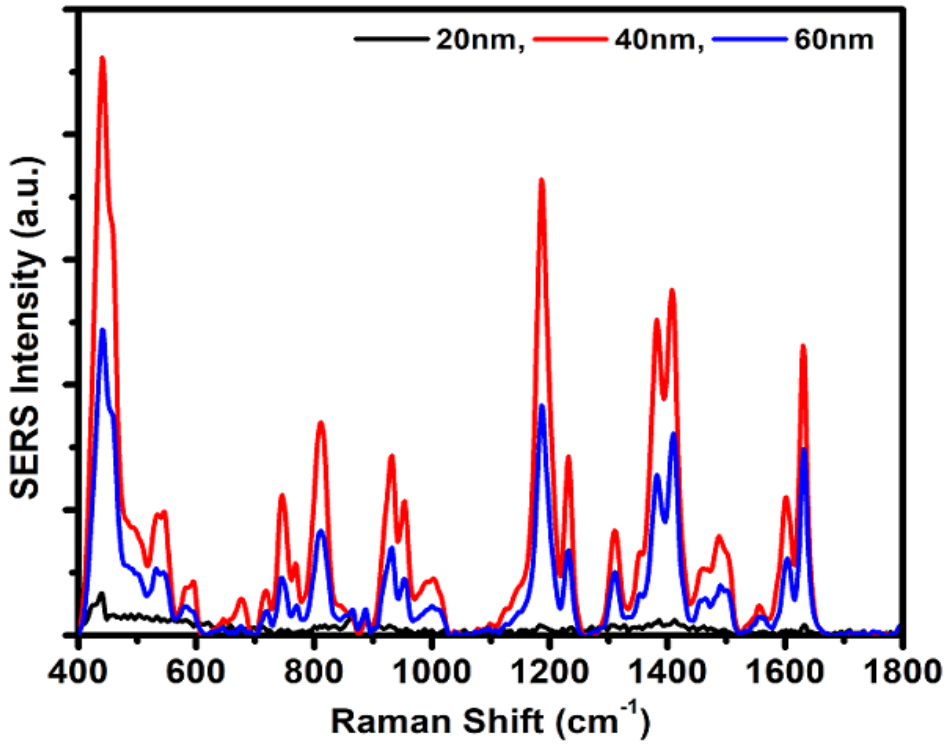

Figure 6.4: Characteristics curve of variation of scattered Raman signal intensity of MG, when substrate is developed by using three different size AuNPs.

Effect of AuNP size on Raman signal enhancement

The size of AuNPs greatly effects the LSPR field magnitude thereby effecting the scattered Raman signal intensity. Three different SERS substrates have been obtained by treating AuNPs of average sizes 20 nm, 40nm and 60 nm. 10 μL of 10 μM MG solutions are treated with each of the SERS substrate and back scattered Raman signals from the sample are recorded by the spectrometer. The obtained characteristic Raman signals are shown in figure 6.4. Clearly, as compared to 20 nm and 60 nm AuNPs treated substrate, the 40 nm AuNPs treated substrate scatters strong Raman signal. This is attributed to field coupling condition of incident EM field for 40 nm AuNPs treated BRDVD substrate would be the highest as compared to 20 and 60 nm AuNPs. These experimental data are well supported by the simulation results discussed in section 6.2.3.

Minimum measurable sample concentration and EF estimation

To study the minimum concentration of the Raman active samples which can be reliably measured by the proposed technique, different concentration of MG and BPE samples

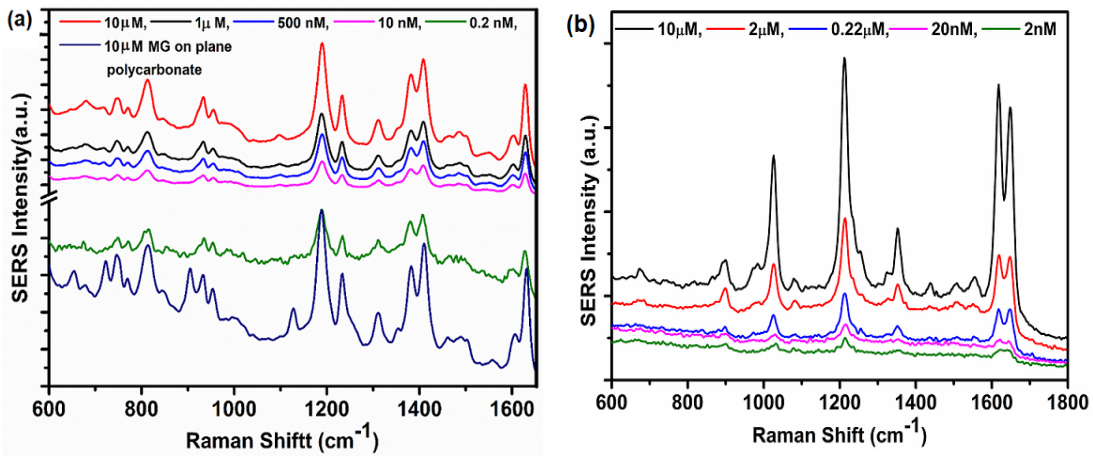

Figure 6.5: Characteristic Raman spectra of (a) MG and (b) BPE, scattered from the BRDVD SERS substrate at different concentrations.

have been prepared. MG samples with concentrations 10 μM, 1 μM, 500 nM, 10 nM and 0.2 nM have been treated with the proposed SERS substrate separately and the scattered Raman signal from the substrate are recorded by the Raman spectrometer. Figure 6.5 (a) shows the characteristic Raman signals of different concentrations of MG, recorded by the spectrometer. All the characteristics Raman peaks intensities are observed to be decreased with the gradual decrement in concentration of the samples. The effect of GMR on overall enhancement of the Raman signal intensity can be felt by comparing the results from AuNPs dispersed on plane polycarbonate substrate. Figure 6.5 (a) also includes the characteristic Raman signal of 10 μM MG scattered from a plane polycarbonate substrate with 10 μL AuNP colloidal suspension dispersed on it. Clearly, these characteristic Raman signals indicate that as compared to plane polycarbonate substrate the signal intensity at 1188 cm^{-1} is observed to be enhanced by a factor of 11.44 with the proposed SERS substrate. The minimum concentration of MG that can be measured reliably is 0.2 nM. Same investigation has been carried out for 10 μM, 2 μM, 0.22 μM, 20 nM and 2 nM BPE solutions. Figure 6.5 (b) depicts the Raman signal intensities measured by the spectrometer for different concentration of BPE samples. The signature Raman peaks of BPE at 1026 cm^{-1}, 1212 cm^{-1}, 1620 cm^{-1} and 1648 cm^{-1} are attributed to the carbon ring breathing mood, C-N stretching, C=C stretching and NH deformation respectively [10]. Here also gradual decrement in Raman signal intensities with decreasing concentrations of the samples has been observed. For BPE, the sample concentration as low as 2 nM can be reliably measured by the Raman spectrometer. To estimate the EF of the proposed substrate MG has been used as analyte sample. Using equation 1.22 the average EF for Raman peak at 1188 cm^{-1} is estimated to be 6.96×10^7.

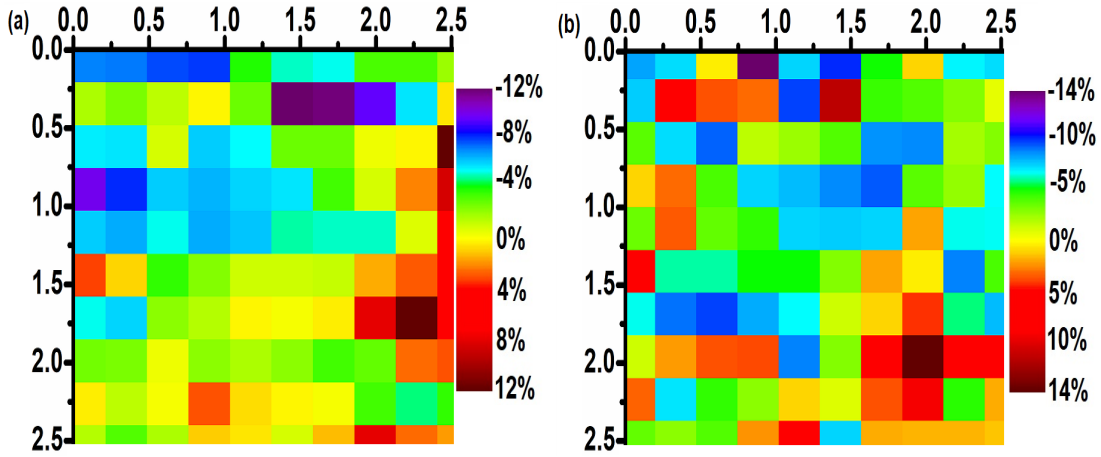

Figure 6.6: SERS signal intensity variation of (a) MG and (b) BPE, corresponding to the signature Raman peaks at 1188 cm^{-1} for MG and 1026 cm^{-1} for BPE over an area of 2.5 mm×2.5 mm on the sensing region of the substrate.

Reproducibility and spectral uniformity of the substrate

To evaluate the reproducibility characteristics of the BRDVD SERS substrate, Raman signal mapping over the sensing region of the substrate has been performed. 10 μL of 10 μM MG has been pipetted on the SERS substrate and upon drying of the sample, the back-scattered Raman signals from the region of area 2.5 mm×2.5 mm with an array of 11×11 have been recorded. Figure 6.6 (a) shows the characteristics mapping of the proposed SERS substrate, where, Raman peaks of MG at 1188 cm^{-1} has been considered. For the considered region the maximum fluctuation of SERS signal intensity is observed to be 12.05%. The same investigation has been carried out with BPE. Figure 6.6 (b) depicts the characteristics mapping of Raman signal intensity fluctuations for BPE at 1026 cm^{-1}. The figure implies that for the considered region of the SERS substrate the Raman signal intensity is fluctuated by a maximum value of 14.24%. These relatively low RSD values suggest that the designed SERS substrate is suitable for monitoring of both fluorescent and non-fluorescent Raman active samples with a good degree of reproducibility and signifies uniform distribution of AuNPs over the BRDVD substrate. During simulation study, the estimation of LSPR field magnitude is studied only for AuNPs trapped in a single channel. However in reality, with 0.22 NA objective lens, the diameter of the laser beam is measured to be 4.35 μm, which will cover almost 13 nearby channels of the substrate at a time. Thus, back-scattered Raman signal from the sample would then be an average signal intensity scattered from 13 channels and equal number of flat surfaces from the sensing region of the SERS substrate.

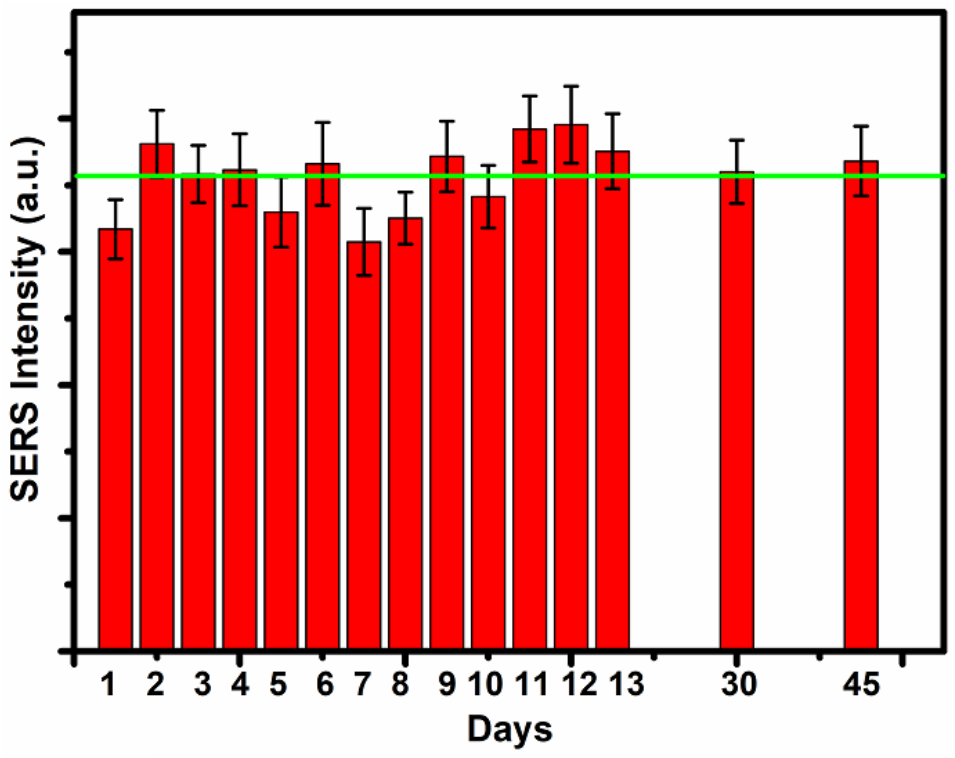

Figure 6.7: Variation SERS signal intensity of MG corresponding to its signature Raman peak 1188 cm^{-1} for the considered period of time.

Life span evaluation of the SERS substrate

In the next step of the present study, the life-span of the proposed SERS substrate has been evaluated. Again, 10 μL of 1 μM MG solution is treated with the substrate. Upon drying of the sample, characteristic Raman signal of MG has been recorded. Figure 6.7 shows the characteristic Raman signal intensity variation of MG at 1188 cm^{-1} where the signal intensities are observed for 45 days. It has been found that the designed SERS substrate yields reasonably stable signals over this period of time. The relative fluctuations of the signal intensity from its average value (indicated in green line in the figure) for this period is observed to be 13.85% while for intraday observations the maximum intensity variation is found to be 8.5% corresponds to 1188 cm^{-1} Raman peak.

6.3.2 Detection of albumin, creatinine and urea

Upon observing its reliable performance both for fluorescent and non-fluorescent Raman active samples, the field applicability of the proposed SERS substrate has been real-

ized through detection, quantification and analysis of urine sample. Three important constituents of urine namely- albumin, creatinine and urea have been considered in the present study. From clinical point of view, these constituents are important for proper diagnosis of kidney function of a patient [11, 12]. Albumin is a type of protein that normally presents in blood. Albumin helps in building body muscle, fight against infections and repairs tissue. A healthy kidney does not allow or allow very little amount of albumin (typically less than 30 mg/day i.e 0.15 μg/mL) to pass into the urine. Presence of albumin beyond the normal range in urine may be an indication of kidney disorder. Creatinine is another important constituent of urine which is a byproduct of muscle metabolism. This parameter is monitored to know the condition of renal health of human kidney. In normal condition, 0.955 g to 2.936 g (or 0.478 mg/mL to 1.468 mg/mL) of creatinine is released by male and 0.601 g to 1.689 g (or 0.3 mg/mL to 0.844 mg/mL) by female per day. Measuring creatinine concentration in urine sample provides the information about renal filtration function and health of the kidney. Urea is a nitrogenated metabolite which is produced due to protein degradation in the body. Normal range of urea concentration in human urine should be in the range from 6 mg/mL to 10 mg/mL. Generally, urea is not specified to diagnose renal disorder but it is an associated part to detect initial stage of renal disorder of a patient.

Prior to study the characteristics of the above chemicals in urine sample, Raman signal intensities of these chemicals have been studied individually in DI water. Albumin, creatinine and urea are dissolved separately in DI water at different concentration level and then treated with the substrate. The recorded Raman signals of the considered samples are shown in figure 6.8 (a)-(c). Raman peaks at 1208 cm^{-1}, 1370 cm^{-1} and 1646 cm^{-1} which corresponds to SO_2 symmetric stretch, CH bond deformation and amide-I respectively are the signature peaks of albumin. The signature Raman peaks intensities are also observed to be decreased with the decrement in concentration of albumin (6.8 (a)). Raman peaks of creatinine at 700 cm^{-1}, 864 cm^{-1}, 888 cm^{-1} and 1444 cm^{-1} are attributed to aromatic ring vibration, C-NH_2 stretching, C=O stretching and CH_3 anisometric deformation respectively which are again observed to be decreased with the decrease of concentration in the sample. A very strong peak at 1018 cm^{-1} corresponds to C-N stretching mode has been observed for urea. The intensity of this specific peak also decreases with the decrement of urea concentration in the sample. With the designed SERS substrate the minimum concentration of albumin, creatinine and urea that can be measured by the Raman spectrometer are found to be 0.1 μg/mL, 0.2 μg/mL and 0.6 μg/mL respectively.

To study the Raman signal characteristics of the above chemicals, different concentra-

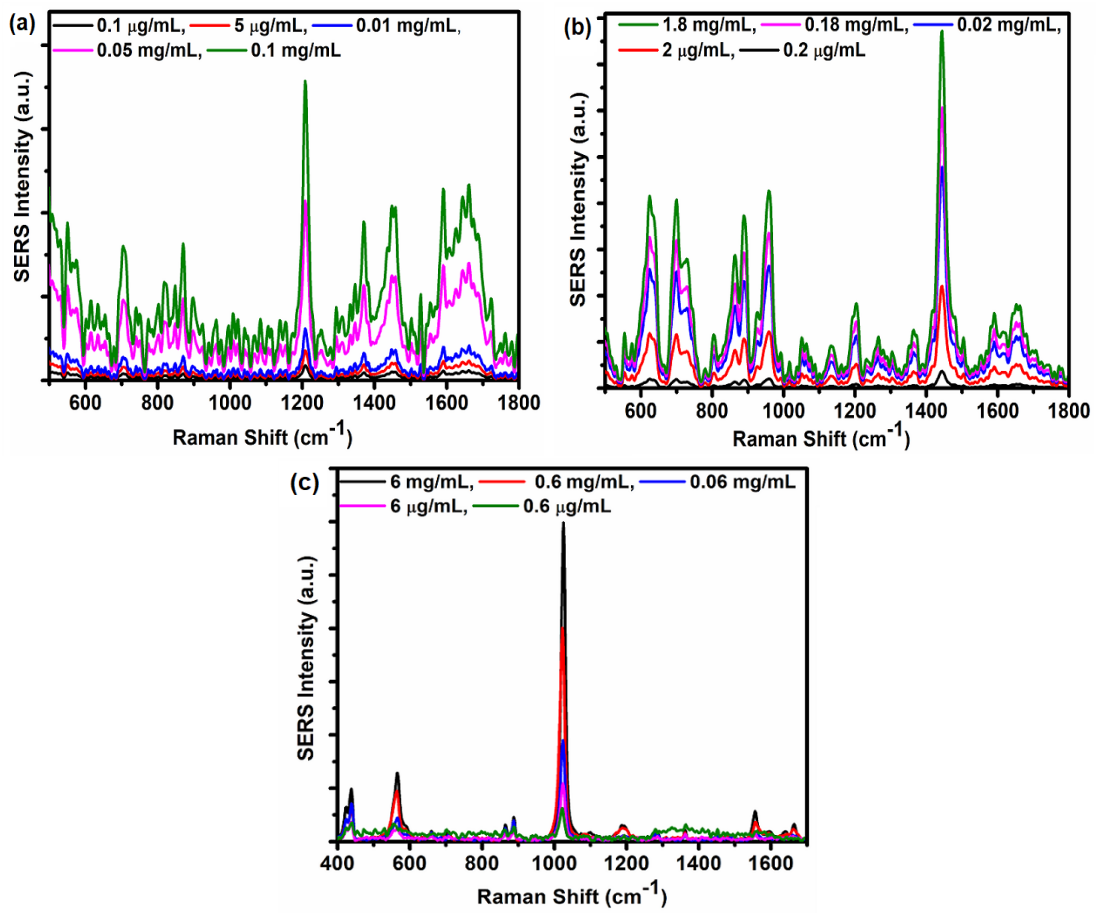

Figure 6.8: Raman signal characteristics of (a) albumin, (b) creatinine and (c) urea at different concentrations in DI water.

tions of albumin, creatinine and urea have been added to artificial urine sample separately. Figure 6.9 (a)-(c) show the characteristic Raman signals of albumin, creatinine and urea present in the urine samples. The strong Raman peak of albumin at 1208 cm^{-1} has been considered to correlate its concentration variation with peak intensity variation of the Raman signal from the substrate. Similarly for creatinine, the strong Raman peak at 1444 cm^{-1} and for urea the signature Raman peak at 1018 cm^{-1} have been considered. The zoomed-in figures adjacent to figure 6.9 (a), (b) and (c) clearly depict the correlation of Raman signal intensity variation with the decrement of concentration of these chemicals in the sample. For reference, all in figures of figure 6.9 include the characteristic Raman curve for bare urine sample.

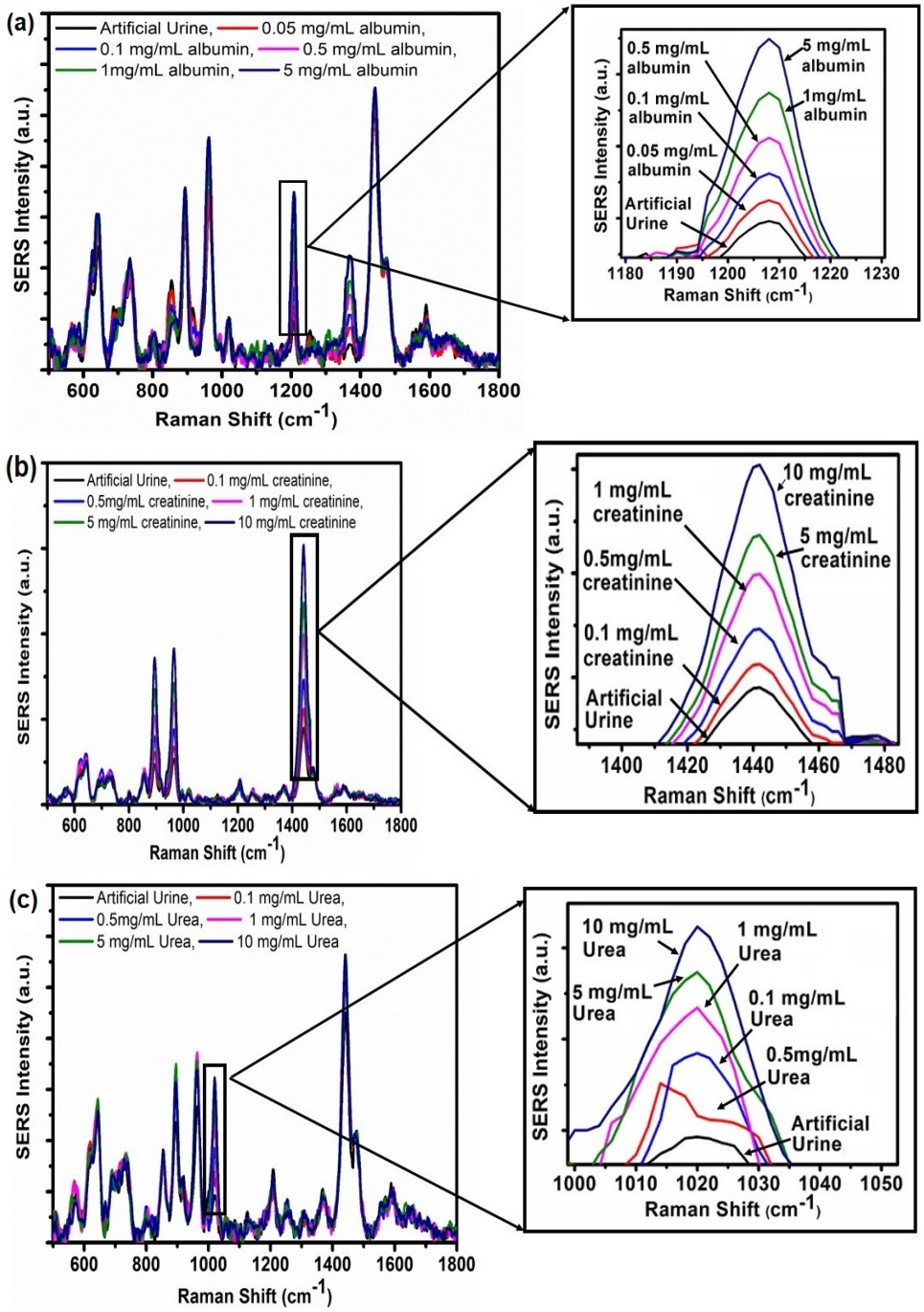

Figure 6.9: Characteristics Raman peaks of artificial urine sample measured by the Raman spectrometer for different concentrations of (a) albumin (b) creatinine and (c) urea present in the sample.

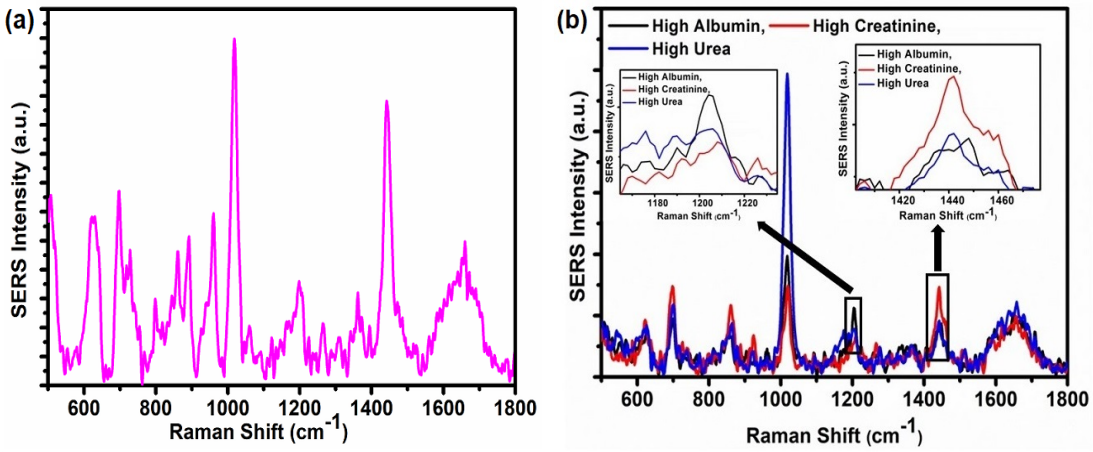

Figure 6.10: Characteristic SERS signal intensities scattered from the mixture of albumin, creatinine and urea when mixed at (a) same ratio and (b) in different ratio.

Multimodal sensing

In the next step of the present study, the multimodal sensing characteristics of the proposed technique has been evaluated. A mixture of albumin, creatinine and urea are prepared in DI water. Two situations have been considered for this study. In the first situation, all the chemicals are mixed in the same ratio i.e. 1:1:1 in DI water and record the scattered Raman signal upon treatment with the BRDVD SERS substrate. The scattered Raman signal of this compound is shown in figure 6.10 (a). It has been observed that all the strong Raman peaks of albumin, creatinine and urea are present in the scattered Raman spectra. In other situation, different ratios of the albumin, creatinine and urea in the mixture are considered. Three different samples have been prepared where albumin, creatinine and urea are added in the ratio, 3:1:1, 1:3:1 and 1:1:3 respectively. Figure 6.10 (b) illustrates the characteristic Raman spectra of these mixture samples scattered from the SERS substrate. As evident from the figure, few weak Raman peaks of a specific chemical are perturbed due to presence of other chemicals in the mixture. But, the strong Raman peak intensity of each of the individual component chemical in the mixture is observed to be prominent. For instance, for albumin the signature Raman peak at 1208 cm^{-1} is found to be varying linearly with its composition in the mixture. Similarly for creatinine, linear variation of Raman peak intensity at 1444 cm^{-1} with its composition in the mixture has been noticed and so is the case for urea. These studies suggest that with the proposed BRDVD SERS substrate, the considered important chemicals in the mixture can be detected reliably and specifically.

Estimation of LoD

The limit of detection (LoD) of the considered samples has been estimated for the proposed SERS substrate. To evaluate this parameter 6 different samples of each of the constituent chemicals have been prepared in DI water. The samples are then treated with the SERS substrates and the scattered Raman signal are recorded from 5 randomly selected locations of the substrate. The normalized SERS signal intensity variations at 1208 cm^{-1}, 1444 cm^{-1} and 1018 cm^{-1} of albumin, creatinine and urea respectively are shown in figure 6.11 (a)-(c). From these figures, the LoDs of the chemicals are calculated using standard equation.

$$LoD = \frac{3.3\sigma}{S} \tag{6.1}$$

where σ is the standard deviation of y-intercepts and S is the slope of the linear fitted line. The LoD of albumin, creatinine and urea are estimated to be 0.055 μg/mL, 0.032 μg/mL and 0.084 μg/mL respectively.

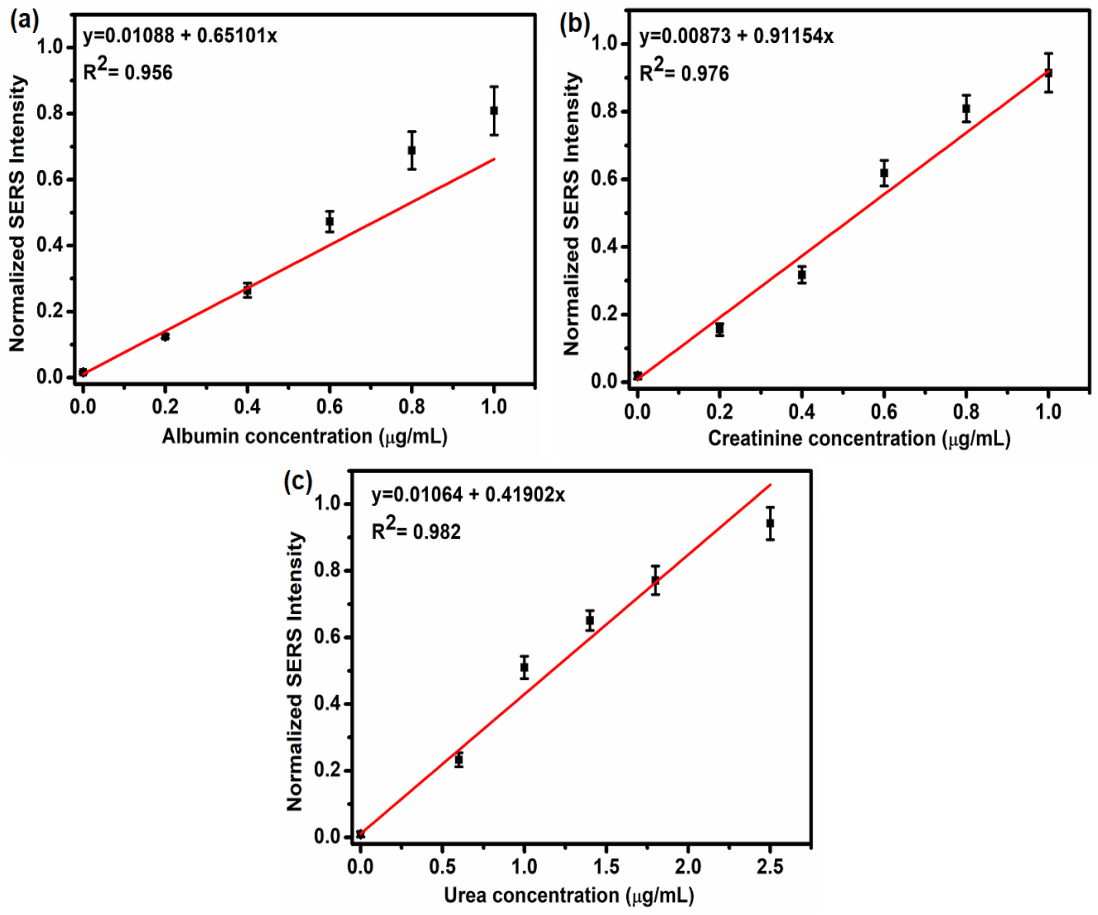

Figure 6.11: Normalized SERS signal intensity variation of (a) albumin, (b) creatinine and (c) urea solution corresponding to their signature Raman peaks at cm^{-1}, 1444 cm^{-1} and 1018 cm^{-1} respectively.

6.4 Summary

A SERS substrate has been realized by trapping AuNPs in the nanochannels of BRDVD substrate. MG and BPE Concentration as low as 0.2 nM and 2 nm can be detected reliably with the proposed BRDVD SERS substrate. An average field enhancement factor of 6.96×10^7 has been observed corresponding to the signature peak of MG at 1188 cm^{-1}. Considering the GMR effect of the designed SERS substrate the simulation result shows that for channel trapped AuNPs, the local field enhancement is found to be 5.26×10^2 which has caused an average EF of the order of 10^{10} for an ideal situation. The practical EF for the same substrate is however measured to be of the order of 10^7. The low EF for the experimental results could be due to consideration of average signal intensity scattered from a relatively large area (14.88 μm^2) which covers both guided and unguided resonant modes from the sensing region of the SERS substrate. The arrangement of AuNPs over the substrate also affects the overall EF while estimating this parameter experimentally. The substrate yields a good degree of reproducibility characteristics with RSD value of 12.05% for MG and 14.24% for BPE samples. Albumin, creatinine and urea in urine sample as low as 0.05 mg/mL, 0.1 mg/mL and 0.1mg/mL respectively can be detected reliably. The developed substrate shows a consistent result over a period of 45 days and a maximum intensity fluctuation of 13.85% has been observed for MG at 1188 cm^{-1}. LoDs for albumin, creatinine and urea that have been measured with the proposed sensing technique are estimated to be 0.055 g/mL, 0.032 g/mL and 0.084 g/mL respectively.

References

[1] Dou, X., Chung, P.-Y., Jiang, P., and Dai, J. Surface plasmon resonance and surface enhanced raman scattering sensing enabled by digital versatile discs. *Applied Physics Letters,* 100(4):041116, 2012.

[2] Giallongo, G., Pilot, R., Durante, C., Rizzi, G. A., Signorini, R., Bozio, R., Gennaro, A., and Granozzi, G. Silver nanoparticle arrays on a DVD-derived template: an easy & cheap SERS substrate. *Plasmonics,* 6(4):725, 2011.

[3] Leordean, C., Marta, B., Gabudean, A.-M., Focsan, M., Botiz, I., and Astilean, S. Fabrication of highly active and cost effective SERS plasmonic substrates by electrophoretic deposition of gold nanoparticles on a DVD template. *Applied Surface Science,* 349:190-195, 2015.

[4] Nieuwoudt, M. K., Martin, J. W., Oosterbeek, R. N., Novikova, N. I., Wang, X., Malmström, J., Williams, D. E., and Simpson, M. C. Gold-sputtered blu-ray discs: simple and inexpensive SERS substrates for sensitive detection of melamine. *Analytical and bioanalytical chemistry,* 408(16):4403-4411, 2016.

[5] Nieuwoudt, M. K., Martin, J. W., Oosterbeek, R. N., Novikova, N. I., Wang, X., Malmström, J., Williams, D. E., and Simpson, M. C. Gold sputtered blu-ray disks as novel and cost effective sensors for surface enhanced raman spectroscopy. In *Optical Diagnostics and Sensing XV: Toward Point-of-Care Diagnostics,* volume 9332, 933207. International Society for Optics and Photonics, 2015.

[6] 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.

[7] 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.

[8] 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.

[9] 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.

[10] Lambert, J. B., Shurvell, H. F., Lightner, D. A., and Cooks, R. G. *Introduction to organic spectroscopy.* Macmillan Publishing Company, 1987.

[11] Premasiri, W. R., Clarke, R. H., and Womble, M. E. Urine analysis by laser raman spectroscopy. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery,* 28(4):330-334, 2001.

[12] Saatkamp, C. J., de Almeida, M. L., Bispo, J. A. M., Pinheiro, A. L. B., Fernandes, A. B., and Silveira, L. Quantifying creatinine and urea in human urine through raman spectroscopy aiming at diagnosis of kidney disease. *Journal of biomedical optics,* 21(3):037001, 2016.