7 Conclusions and future prospects

The concluding chapter encapsulates the significant findings of the present thesis work. The chapter also delves into the future directions of the research work and enlighten its scope to do further work in the future.

7.1 Summary of the present research work

The present thesis work primarily focuses on the development of various rapid and affordable routes to fabricate SERS substrates for sensing of various analytes. The applicabilities of the designed SERS platforms have been demonstrated through detection and analysis of drugs in water and various food matrices. With the incorporation of various chemometric and machine learning classification algorithms, rapid identification of the analyte in complex matrices have been possible through feature identification. The major findings of the thesis work are summarised below

1. A 100 GSM paper has been explored as a platform for fabrication of SERS substrates. The microporous structure of 100 GSM paper facilitates the diffusion of AuNPs thereby generating hotspot region of the coupled EM field. The optimized rate of in-plane diffusion over lateral diffusion supports the deposition of nanoparticles over the surface, thereby enhancing the scattered Raman signals. The fabrication process is rapid and simple, making the proposed substrate viable for infield sensing applications. As a swift alternative for analyte screening, 100 GSM paper proves advantageous over expensive commercial SERS substrates. In the first part of the thesis work, the application of this fabricated paper based SERS is showed. The applicability has been demonstrated through the trace detection of two pharmaceuticals, paracetamol and aspirin, in real water samples.

2. In chapter 3 of the thesis work, another affordable SERS platform that has been obtained from natural leaf surface of *AM* was illustrated. The naturally occurring micro/nanostructure in the leaf surface supports the formation of EM hotspots upon dispensing the metal nanoparticles over the leaf surface. The hydrophilic nature of the leaf facilitates uniform deposition of the nanoparticles over the substrate surface, making the Raman signature relatively uniform. The usability of the proposed SERS substrate is demonstrated through trace detection of antibiotics CEFTR and CEF-Na in cow milk. The incorporation of machine learning classification algorithm supports the quick identification of the chosen veterinary antibiotics in milk samples.

3. The traditional approach of SERS substrate fabrication by drop casting of nanoparticles over a plane surface suffers from issues like particle agglomeration and nonuniformity; making the sensing scheme ineffective for quantitative estimation of analyte samples. Therefore, a method that provides good control over the surface morphology is highly desired for deploying it for infield sensing applications. The electrochemical deposition method facilitates the deposition of metal nanostructures over a conducting surface. By properly tuning the applied voltage and current characteristics, a uniform deposition could be achieved. In the fourth chapter, a SERS substrate obtained through electrochemical deposition has been described. The technique involves chronoamperometry and cyclic voltametric process. The bimetallic Cu-Au nanostructures have been deposited over ITO substrate that facilitates a synergistic effect, thereby enhancing the plasmon characteristics of the fabricated SERS platform. The fabricated SERS substrate has been used for trace detection of two poultry antibiotics TCH and SFZ in egg samples. Furthermore, an optimized machine learning model has been implemented for identification of the analyte sample from a mixed medium.

4. For analysis of real samples and the fast screening of the analytes, a simple, low-cost yet biodegradable platform is required. PVA is a biodegradeable polymer that exhibits excellent physical and chemical properties. In chapter five of the thesis, the fabrication of an optimized PVA polymer-based SERS substrate using the ex-situ electrospinning technique was explored. By adjusting the voltage and rotor speed of the electrospinning setup, a sensitive SERS platform can be created. The fabricated SERS platform can then was used for trace detection of two widely used poultry antibiotics, DCH and ENX, in chicken meat samples. Additionally, a chemometric data analysis technique called PCA is used to segregate the analytes in real meat samples.

5. The ex-situ mode of nanofiber fabrication may generate residual harmful in-

termediates during the chemical synthesis of nanoparticles. The use of plasma for reducing the metal salts into the metal nanoparticles can be used to circumvent this issue. So, in the final part of thesis work O_2 plasma-treated bimetallic nanofibers have been used as a new platform of SERS based sensing studies. The presence of bimetallic Au-Ag nanoparticles further enhances the SERS characteristics due to the synergistic effect of nanoparticles. The reliability of the fabricated SERS platform has been demonstrated through trace detection of two antimicrobials FLU and LIN. Further, with the incorporation of the ANN deep learning model, the rapid identification of the said analytes has been carried out in mixed samples.

7.2 Limitations

In the present thesis work, various approaches for fabrication of SERS substrates have been explored. The usability of the developed SERS substrates have been demonstrated through trace detection and analysis of drugs in water and food matrices. Although the SERS substrates were fabricated with the aim of affordability, sensitivity, and reproducibility characteristics, they suffer from several limitations thereby leaving room for further improvement. The limitations of the present thesis work are summarised below-

1. During the fabrication process of 100 GSM paper SERS substrates, the chemical synthesis and dispersion of nanoparticles within the paper depends on various parameters that could effect the SERS performance. Variations in nanoparticle size alter hotspot formation, leading to signal variations. Additionally, the drop-casting method employed for nanoparticle dispersion over the micro-structured roughened surface may result in non-uniform deposition, further influencing scattered Raman signals. These fluctuations in the Raman bands present challenges for the quantitative detection of analytes.

2. In the case of AuNP treated *AM* leaf surface, there could be a significant fluctuations in the scattered Raman signals due to the non-uniformity of deposition of metal NPs, which further leads to inaccuracy in the quantitative estimation.

3. With the incorporation of an electrochemical deposition process, the issue of fluctuations could be resolved to a great extent. Non uniformity in deposition may still arise in the electrodeposition process; uneven deposition of nanoparticles may result in spatial variations in the scattered Raman signal intensity. With optimized deposition conditions, uniformity in substrate preparation can be assured. Additionally, during electrochemical deposition, residual ions from

the electrolyte solution can interfere with Raman signals, necessitating further chemical treatment to eliminate residual ions.

4. The issue of spatial variations in SERS signal intensity is also encountered in nanofiber-based SERS substrates due to the non-uniform distribution of analytes on the nanofiber surface. Here also, by carefully tuning the deposition parameters like rotor speed and applied voltage, such issues can be obviated. Furthermore, with the ex-situ fabrication of nanofibers, the presence of residual chemicals during the synthesis procedure may present and subsequently contribute to noise. This may perturb the SERS signal from the target analyte.

5. Plasma-based nanofiber approaches may be used to further enhance substrate properties. Being a green synthesis process, nanoparticles can be directly generated without producing residual compounds. However, with plasma treatment, the plasma exposure time is critical as prolonged exposure may damage the nanofibers, leading to degradation in SERS performance.

7.3 Future prospect

In the present thesis work, various approaches for SERS substrate fabrication have been explored, guided by criteria such as feasibility, affordability, field-portability, and reduced fabrication time. Additionally, sensitivity, uniformity, and reproducibility were considered as desired attributes during the fabrication of the SERS platforms. The various SERS substrates fabricated during the research work were found to be reliable and capable of producing a high EF. Micro-structured surfaces that are readily available such as 100 GSM paper and AM leaf were explored for SERS substrate fabrication. Furthermore, other naturally occurring nanostructured platforms such as flower petals, seashells, feathers, and butterfly wings can be explored in the future for fabricating SERS substrates. Upon observing consistent performance with standard samples, the proposed SERS substrates could be employed for sensing important chemicals and biological samples. While these substrates may exhibit a large signal fluctuation problem, they are well-suited for rapid screening of analytes in real field samples. For more detailed analysis of analyte samples, electrochemically deposited SERS substrates and nanofiber-based SERS substrates could be utilized. Due to their good sensitivity and reproducibility characteristics, the fabricated SERS platform could find applications in various emerging areas such as molecular detection, monitoring catalytic reactions, biomedicine, and in food safety.

In this thesis work, the applicability of the fabricated SERS substrates was realized through trace detection of drugs in water and various food matrices, indicating the field applicability of the designed SERS platforms. Addressing the crucial issue of

AR through trace determination of antimicrobial drugs in food matrices was also emphasized in present thesis work. The proposed SERS platform was demonstrated for detecting antimicrobials in various food matrices, aiding in monitoring AR. In the future, with proper modification of the substrate surface, resistant microbial strains can be trapped, which may facilitate trace detection of microbes, thus may address the issue of AR from a different perspective. The proposed sensing modalities could serve as viable alternatives to commercial counterparts in different sensing domains such as biomolecules, pesticides, and heavy metal detection. For quantitative estimation of analyte molecules, linear regression analysis was conducted for a known concentration range exhibiting nearly linear characteristics. In complex cases, other modalities such as Principal Component Regression (PCR) and Partial Least Squares Regression (PLS) can be applied for proper estimation of the analyte concentration. Additionally, machine learning-based regression models can be used to enhance the efficacy of the sensing scheme for estimating analyte concentrations in real complex matrices. Again, for preventing the overfitting of machine learning models, choice of the sample size is crucial. The minimum sample size for different ML algorithms can vary depending on several factors such as the complexity of the problem and the dimensionality of the data. This has been left as a future scope of work in the improvement of the ML models before deploying the sensing scheme in real sample analysis.