ABSTRACT

Drugs play a central role in the modern healthcare system and are widely used for treating various ailments and infections. However, they can also cause significant harm to the environment by polluting water bodies. Residues from over-the-counter drugs are often found in natural water resources, originating from both household and industrial waste [1]. Moreover, the use of antimicrobial drugs in animal farming is rapidly increasing. Under-dosing of antibiotics is often used to boost muscle growth and increase farming yield [2]. However, this practice can lead to antibiotic resistance (AR), as trace amounts of antibiotics from various food sources such as milk, eggs, and meat can cause AR. Therefore, detecting trace amounts of antimicrobial drugs in food samples is crucial. Traditionally, different chromatographic techniques such as liquid chromatography and mass spectrometry (LC-MS) [3], gas chromatography and mass spectrometry (GC-MS) [4] and high-performance liquid chromatography (HPLC) [5] have been used to detect these drugs . However, these techniques have several drawbacks, such as long sample preparation times and being unsuitable for field use.

Raman spectroscopy is an analytical technique that is known for its high specificity and ability to fingerprint molecules. With the advent of new state-of-the-art laser systems, this technique is now being used in various sensing applications [6]. However, Raman spectroscopy has low scattering correction, making it ineffective for recording spectra at low concentrations. Surface-enhanced Raman Spectroscopy (SERS) offers the benefits of Raman spectroscopy with the added advantage of high sensitivity [7, 8]. SERS is observed when the Raman signals are recorded near the metal nanostructured surface. Owing to the phenomena of Localized Surface Plasmon Resonance (LSPR), the Raman bands are enhanced manifold, thus enabling the trace detection of the analyte of interest. In the current thesis work, different modalities of substrate fabrication have been explored and the application has been carried out in the trace detection of drugs in water and food matrices.

Different low-cost methods are already explored in available literatures for detection analysis of analyte samples. Paper substrates provide an excellent platform for detecting analytes in trace concentrations. The microporosity of the surface facilitates the generation of electromagnetic hotspots, which enables the trace probing of analytes of interest. Recently, different paper-based SERS substrates have been reported for probing molecular elements in trace concentrations. Chamuah et al. reported the sensitive detection of glucose and urine using nanoparticle-deposited printing paper as a SERS platform [9]. Sallum et al. reported using silver nanoparticle-coated filter paper to determine the amount of acetylsalicylic acid in commercial tablets [10]. In this study, the silver nanoparticles were prepared using Tollens reagent. This approach offers several advantages such as a low-cost synthesis, minimal residual materials, and the use of readily available reagents and support. Again, natural surfaces, such as leaves, are an excellent choice for SERS substrate fabrication because they have naturally imprinted nanostructures that support the formation of hotspots, which enhance the scattered Raman signals [11–13]. Several groups have reported the fabrication of hydrophobic as well as hydrophilic SERS substrates based on the leaf surface. Hydrophobic surfaces have a dense distribution of nanoparticles in a narrow area, which facilitates a high intensity of the Raman bands in that region. However, with hydrophilic SERS substrates a uniform distribution of nanoparticles could be achieved and the sensing can be performed in a comparatively larger surface area compared to the hydrophobic counterparts. Huang et al. have reported a superhydrophobic SERS chip based on an Ag-coated natural taro leaf [14]. Natural taro leaves exhibit ordered micro-papillae, which provide superhydrophobicity for analyte enrichment. Additionally, the natural taro leaf contains secondary crossed nanoplates that support the SERS hotspots. The designed SERS platform achieved a LoD of 10^{-8} M. Further, for sensitive and reliable detection of analytes a highly uniform and reproducible SERS substrate is desired. The electrochemically deposited SERS substrates emerge as a convenient option while considering the uniformity and reproducibility of the signals [15–18]. Wang et al. have reported the fabrication of ITO-rGO/Ag NPs nanocomposite by two-step chronoamperometry electrodeposition method. With optimized experimental settings, LoD as low as 10^{-11} M and enhancement factor (EF) $\sim 5.9 \times 10^8$ was demonstrated for rhodamine-6G (R6G); the results are significantly reliable compared to the ITO-Ag NPs substrate. With the ITO-rGO/AgNP platform, the obtained EF is 24 times higher than that for the ITO-Ag NPs platform. Again electrospun polymer-based SERS platforms are widely used for obtaining enhanced SERS characteristics [19]. Poly Vinyl Alcohol (PVA) based polymers are widely used because of their biocompatible nature, easy fabrication process, and cost-effectiveness. Chamuah et al. reported the use of gold-coated electrospun PVA nanofibers as SERS substrates for the trace detection of pesticides [20]. With the designed platform the detection of deltamethrin, quinalphos, and thiacloprid has been successfully demonstrated.

In the present thesis work, various low-cost SERS platforms like paper, natural leaf surface, electrochemically deposited substrates and nanofiber have been explored for fabricating reliable and sensitive SERS platforms. Performance of the fabricated substrates have been initially evaluated with standard Raman active samples MG, R6G and BPE. Upon optimizing the fabricated platform with standard samples, the designed platforms have been deployed for trace detection and quantification of drugs in real field samples like water and food matrices. Alongside the sensing scheme, multivariate methods and machine learning (ML) based classification algorithms are implemented for rapid detection of the analyte molecules in complex matrices. The chapter wise organization of the present thesis work is illustrated below-

Chapter 1

Chapter 1 provides an overview of the proposed sensing work, focusing on the need for trace detection of drugs and food adulterants. The available analytical methods, such as LC-MS and GC-MS, have been briefly discussed along with their limitations. Additionally, the mechanism of Raman spectroscopy and SERS would be explained in detail. The chapter will also include a discussion on various ML and deep learning algorithms used for analysis.

Chapter 2

In Chapter 2, it is planned to demonstrated the trace sensing of paracetamol and aspirin in real water samples using a paper substrate. Our proposed paper-based SERS substrate is a cost-effective alternative to commercially available SERS substrates. These substrates can be utilized as disposable platform, which is useful for rapid identification of trace concentrations of analytes. With the designed substrate it is possible to achieve good reproducibility and uniformity characteristics.

Chapter 3

In Chapter 3, the working of agles marmelos leaf as a SERS substrate has been demonstrated. The natural nanostructures in the leaf provide excellent SERS results due to the generation of EM hotspots. Trace determination of ceftiofur sodium and ceftriaxone has been investigated in real samples. Also, the ML-based classification was performed for real samples. Further, an optimized ML classification model was performed for accurate identification of the analytes in real milk samples

Chapter 4

In Chapter 4, the working of an electrochemically deposited SERS substrate has been investigated. Herein, bimetallic nanoparticles are deposited in the ITO-glass platform using cyclic voltammetry and chronoamperometry techniques. Further, trace determination of two antibiotics drugs tetracycline hydrochloride (TCH) and sulphamethoxazole (SFZ) has been performed. Upon noticing the reliable signals in lab samples the SERS determination of the antibiotics in the real farm egg samples has been demonstrated. The sensing scheme is again coupled with an optimized ML classification algorithm for accurate classification of the TCH and SFZ in real egg samples.

Chapter 5

In Chapter 5, the process of desigining a SERS substrate using electrospun PVA nanofibers has been described. The nanofibers were treated with AuNP using an ex-situ synthesis protocol and fabricated using the electrospinning technique. This sensing platform was then used to detect two commonly used antibiotics, Doxycycline hydrochloride (DCH) and Enrofloxacin (ENX), in chicken meat. Additionally, the presence of these antibiotics in real meat samples was identified using PCA.

Chapter 6

In this chapter, a different modality of the fabrication of electrospun PVA nanofiber SERS substrate has been explored. Plasma-based synthesis is a green synthesis and provides a good alternative to the chemical reduction process. Herein, the O_2 plasma treatment has been employed for enhanced generation of plasmonic metal nanoparticles. Further, the substrate has been optimized with plasma etching time and the applied voltage to achieve reliable SERS signals. The fabricated SERS platform has been used for detecting two antimicrobials fluconazole (FLU) and lincomycin (LIN). In the final step of the proposed sensing work, the ANN algorithm of the deep learning model has been used for the classification of the antimicrobials FLU and LIN.

Chapter 7

It covers the concluding remarks of all the chapters and future scope of the work.

Keywords:

Raman Spectroscopy, SERS, Antibiotic Resistance, LSPR, Machine Learning, Deep Learning

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