

## 1.1 The need for molecular sensing

Over the past few decades, molecular sensing has emerged as one of the important area of research and has gained a wide attention across various disciplines that include physical sciences, chemical sciences, biological sciences, clinical diagnostics, environmental protection, defence and security, and other related fields. For human body, there is a need for the detection and analysis of disease biomarkers, proteins, antibody and genetic materials. Around us, there are large number of toxic and non-toxic compounds which are required to be monitored regularly. The industrial and agricultural wastes, micro-nanoplastics are among the few that have caused a huge risk to the aquatic lives [1-3]. Highly toxic pesticides, food preservatives, artificial food colours are causing serious health issues [4]. Again, in this globally interconnected world, there is a serious threat of spreading infectious diseases and pathogens, like the recent coronavirus disease (COVID-19) caused by the SARS-CoV-2 virus. In the field of medicine, there is a need for analysis of developed drugs, and their actions and reactions. The analytes can be a small molecules (like gases), ions, upto larger particles (like proteins) and living cells. Several techniques that are available to detect and identify the interaction between molecules, particles and interfaces are utilised in the development of sensors and in sensing applications. In the modern day, the analytical techniques like microscopy, spectroscopy, electrochemical methods, mass-spectrometry, microfluidics, and other tools are also evolved such that highly sensitive, rapid and reliable molecule sensing are possible. Among these techniques, Raman scattering has emerged as one of the important and widely used sensing tool mainly as it provide the unique fingerprint of a molecule.

## 1.2 Raman scattering

The phenomena of inelastic light scattering by molecules was first discovered by Sir C. V. Raman and K. S. Krishnan in the year 1928 [5]. When light interacts with a

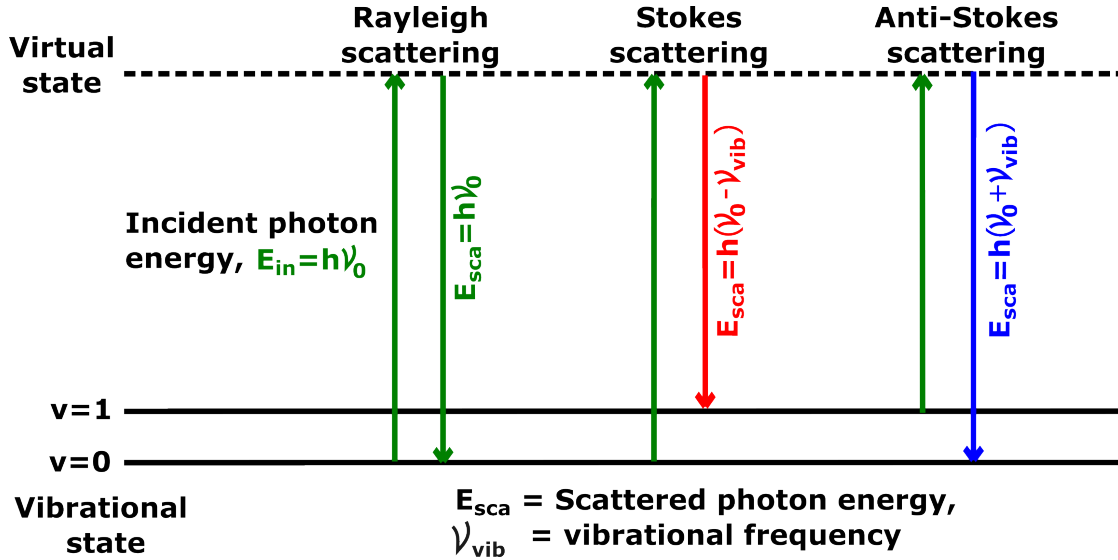


Figure 1.1: Energy level diagram for Rayleigh scattering, Stokes scattering and anti-Stokes scattering.

molecular sample, most of the light photons are scattered elastically with a frequency same as the incident light ( $\nu_0$ ), and it is called as Rayleigh light. In addition to this, few of the incident light photons undergo inelastic scattering with a shift in frequency ( $\nu_0 \pm \nu_{vib}$ ) from the incident light, and this phenomenon is called Raman scattering. Here,  $\nu_{vib}$  is the vibrational frequency of the analyte molecule. The frequency shift of the scattered photon from the incident light to lower frequency ( $\nu_0 - \nu_{vib}$ ) and higher frequency ( $\nu_0 + \nu_{vib}$ ) are called Stokes shift and anti-Stokes shift, respectively. For a particular molecule, these frequency shifts in the Raman spectrum represents the vibrational modes which is the unique characteristic of the molecule, and therefore, record the fingerprint of a specific molecule present in the sample [6].

### 1.2.1 Origin of the Raman spectra

According to classical theory, the origin of the Raman spectra can be explained from the change of polarizability of the molecule. Polarizability represents the molecules' ability to interact with the incident electric field, and is determined by the electron cloud. Electric field of an incident electromagnetic wave is represented as:

$$E = E_0 \cos(2\pi\nu_0 t). \quad (1.1)$$

Here,  $E_0$  represents the vibrational amplitude and  $\nu_0$  is the incident laser frequency. The induced electric dipole moment ( $P$ ) of a diatomic molecule can be expressed by the following equation when irradiated with this electromagnetic wave as:

$$P = \alpha E = \alpha E_0 \cos(2\pi\nu_0 t), \quad (1.2)$$

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where,  $\alpha$  represents the polarizability of the molecule. The molecular displacement ( $q$ ) of the molecule vibrating with a frequency  $\nu_{vib}$  can be written as [6]:

$$q = q_0 \cos(2\pi\nu_{vib}t). \quad (1.3)$$

Here,  $q_0$  represents the vibrational amplitude. For a small amplitude of vibration, polarizability ( $\alpha$ ) is a linear function of displacement ( $q$ ). It can be represented as:

$$\alpha = \alpha_0 + \left(\frac{\partial\alpha}{\partial q}\right)_0 q + \dots \quad (1.4)$$

Here,  $\alpha_0$  is the polarizability and  $\left(\frac{\partial\alpha}{\partial q}\right)_0$  is the rate of change of  $\alpha$  with respect to  $q$ , at the equilibrium position. From the equaltions 1.2, 1.3 and 1.4, P can be expressed as:

$$\begin{aligned} P &= \alpha_0 E_0 \cos(2\pi\nu_0 t) + \left(\frac{\partial\alpha}{\partial q}\right)_0 q_0 E_0 \cos(2\pi\nu_0 t) \cos(2\pi\nu_{vib}t) \\ &= \alpha_0 E_0 \cos(2\pi\nu_0 t) + \frac{1}{2} \left(\frac{\partial\alpha}{\partial q}\right)_0 q_0 E_0 [\cos\{2\pi(\nu_0 + \nu_{vib})t\} + \cos\{2\pi(\nu_0 - \nu_{vib})t\}]. \end{aligned} \quad (1.5)$$

From the classical electrodynamics, the first term in the equation 1.5 represents an oscillating dipole radiating a electromagnetic wave of frequency  $\nu_0$  (Rayleigh scattering). The second term represents two oscillating electric fields of frequencies  $\nu_0 + \nu_{vib}$  (anti-Stokes) and  $\nu_0 - \nu_{vib}$  (Stokes). For a molecule to be Raman active, rate of change of polarizability ( $\alpha$ ) with the molecular vibration or  $\left(\frac{\partial\alpha}{\partial q}\right)_0$  must be non-zero.

According to the equation 1.5, the intensity of Stokes and anti-Stokes Raman lines should be same. However, in the experimental Raman spectra the intensity of Stokes lines are found to be more compared to the anti-Stokes signal. This discrepancy can be addressed with the quantum mechanical approach of the origin of Raman signals. Here, the incident laser photon of frequency  $\nu_0$  and energy  $E_{in} = h\nu_0$ , excites the molecule to a virtual energy state. Three outcome from this situation are possible when the molecule relaxes to a lower energy state as describe in the figure 1.1. In the first case, if the molecule relaxes to the same ground state, it emits a scattered photon of energy  $E_{sca} = h\nu_0$ , same as the incident photon (Rayleigh scattering). In the second case, the excited molecule relaxes to a higher vibrational state emitting a photon of energy  $E = h\nu_0 - h\nu_{vib}$ , which is less than the incident photon (Stokes scattering). In the third scenario, the molecule is initially in the higher vibrational energy state and excites to the virtual state. If the molecule relaxes to the vibrational ground state, the emitted photon has the energy  $E_{sca} = h(\nu_0 + \nu_{vib})$ , which is higher than the incident photon (anti-Stokes scattering). At room temperature, as there are generally more molecules in

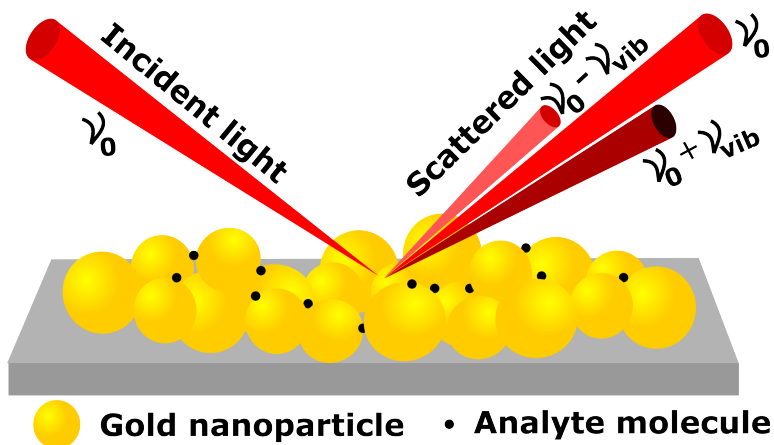


Figure 1.2: Schematic representation of SERS.

the ground state than in higher vibrational states, probability that a scattered photon will be anti-Stokes is significantly lower than the Stokes line. As a result, the anti-Stokes Raman signal has a lower signal intensity than the Stokes Raman scattered signal. For this reason, the majority of Raman instruments are made to record the Stokes Raman scattered signal.

Among the various analytical techniques, Raman spectroscopy is one of the important techniques for analysis of molecular structures. The main advantage of the Raman spectroscopy in molecular sensing is the observation of unique fingerprint as Raman spectral bands. In addition, Raman is rapid technique (only few seconds are needed to collect one spectrum), and very small sample volume is required as the diameter of the laser beam is very small (normally less than 1-2 mm). Water being a very weak Raman scatterer, it interferes very little in the Raman spectra collected from chemicals and biomolecules in aqueous solutions. But, due to the low scattering cross section  $\sim 10^{-30} \text{ cm}^{-2}$ , only few in a million of incident laser photons undergo inelastic scattering. This leads to an extremely low intensity Raman signal which hampers the direct application of Raman spectroscopy for the detection of low concentrations of analyte molecules. This specific limitation of Raman spectroscopy can be addressed with the development of surface-enhanced Raman spectroscopy where the low intense Raman signals are enhanced in the presence of noble metal nanoparticles. Figure 1.2 depicts the underlying phenomenon that is responsible for generating enhanced Raman signal from the analyte.

### 1.3 Surface-enhanced Raman scattering (SERS)

Surface-enhanced Raman scattering (SERS) is a commonly used sensing technique in which inelastic light scattering by molecules is greatly enhanced when the molecules are adsorbed onto roughened metal surfaces such as silver or gold nanoparticles [7–17].

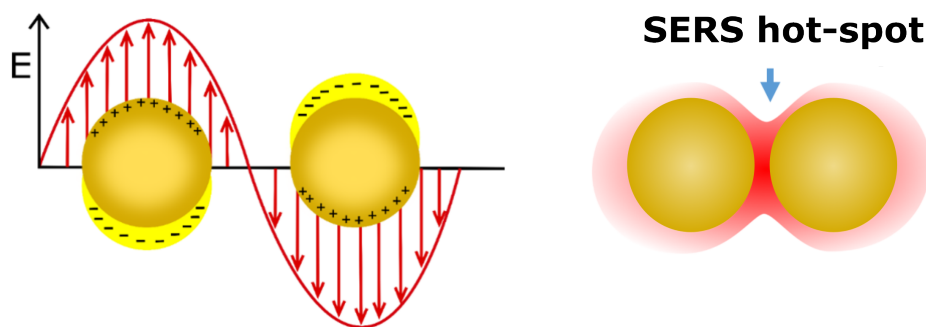


Figure 1.3: Illustration of the localized surface plasmon resonance effect and hot-spot.

The high enhancement of the Raman signal enables the detection and analysis of low concentrations of various chemicals and biomolecules which was difficult earlier with normal Raman spectroscopic technique.

After several decades of the discovery of Raman scattering, in 1974, Fleischmann and co-workers had discovered this interesting phenomenon related to Raman scattering process. Raman signal intensity of pyridine was found very high when adsorbed on electrochemically roughened silver electrode [18]. Initial assessment was that this high enhancement was due to increase in surface area of the roughened electrode. In the year 1977, this enhancement phenomenon was identified as occurring only in the presence of noble metal electrodes by two independent groups of Jeanmaire and Van Duyne [19], and Albrecht and Creighton [20]. Moskovits had connected the SERS intensities to enhanced electric fields arising due to the localized surface plasmons in nanostructured metals [21].

### 1.3.1 SERS enhancement mechanisms

The high enhancement of the Raman signals of a molecule adsorbed on a metal surface can be explained by two mechanisms: chemical enhancement and electromagnetic enhancement mechanisms. Chemical enhancement in SERS occurs due to the charge transfer from the adsorbed analyte molecule from the nanoparticle substrate, and is responsible for the SERS enhancement of the order of  $\sim 10 - 10^3$  [22–24]. Most of the SERS signal enhancement is due to the contribution of electromagnetic enhancement which is of the order of as high as  $\sim 10^{10} - 10^{11}$  [25]. The electromagnetic enhancement which is highly dependent on the plasmonic material, size and shape, orientation and distribution of the nanostructures, etc., is crucial for development of sensitive SERS substrates.

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## Localized surface plasmon resonance (LSPR)

When an electromagnetic wave interacts with the noble metals (gold and silver), the surface free electron on the metal starts oscillating due to incident electric field. According to the classical electromagnetic theory, this electron cloud oscillating with the positive core acts as an oscillating electric dipole and radiates electromagnetic wave. For a larger metal structures like metal nanofilm, this electric field propagates on the metal surface and is called surface plasmons. For metal nanostructures like nanoparticles as described in the figure 1.3, the generated electric field is localized near the nanoparticles and is termed as localized surface plasmon. In localized surface plasmon resonance (LSPR), the incident electric field frequency and the characteristic vibrational frequency of electrons are same, and a maximum electric field intensity is observed [26, 27].

## Electromagnetic enhancement mechanism

During the interaction of an electromagnetic wave with the metal surface, the electric field at the surface and near the metal is different from that found in the far field region. For a rough metal surface, the electromagnetic wave generates LSPR which results in an amplification of the electromagnetic field near the metal surface. This strong electric field enhances the intensity of the scattered Raman signal. This SERS enhancement mechanism is commonly known as electromagnetic enhancement mechanism, and is important for the understanding of SERS enhancement [23].

## $E^4$ Enhancement

The overall SERS intensity ( $I_{SERS}$ ) depends on both incident ( $\nu_0$ ) and scattered ( $\nu_0 - \nu_{vib}$ ) electric field as [13]:

$$I_{SERS} = I_{\nu_0} I_{\nu_0 - \nu_{vib}} = |E(\nu_0)|^2 |E(\nu_0 - \nu_{vib})|^2. \quad (1.6)$$

Therefore, for optimal SERS enhancement to occur, the metal nanostructure's plasmon peak must be in resonance with both the incident ( $\nu_0$ ) and Stokes Raman shifted ( $\nu_0 - \nu_{vib}$ ) radiation. For a particular Raman band, if incident and Stokes Raman frequency are close enough, the SERS intensity can be approximated as:

$$I_{SERS} \approx |E(\nu_0)|^4. \quad (1.7)$$

Thus, SERS signal intensity can be regarded as approximately the fourth power of the incident electric field [28].

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## Distance dependance

The electromagnetic theory of SERS indicates that the analyte molecule need not to be direct contact with the metal nanoparticle. If it is within a certain sensing volume, the SERS enhancement can be observed. Since the electric field around a nanosphere decays with distance ( $r$ ) according to  $E(r) = r^{-3}$ , the distance dependance of SERS intensity can be found using equation 1.7 as:  $I_{SERS} = r^{-12}$ . If two nanoparticles or nanostructures are close enough, the indivisual LSPR fields are coupled, and a very high electric field intensity can be observed in a particular position (hot-spot) as illustrated in the figure 1.3 [29].

### 1.3.2 SERS substrate

SERS substrate is a plasmon-resonance supporting structure that produces suitable Raman amplifications. After illumination with laser light, strong localized electric field are generated near the nanostructures. The analyte molecules adsorbed on this metallic structure provide enhanced Raman signal intensity as described in the section 1.3.1. Detail description of different SERS substrategies have been discussed in the Chapter 2.

### 1.3.3 Enhancement factor (EF)

One very important parameter in SERS experiment is the enhancement factor (EF) [30]. It represents how much a Raman signal is enhanced compared to normal condition (non-SERS). Measurement of EF of a SERS substrate is required for understanding the origin of SERS and physical mechanism behind the SERS enhancement [29].

#### Maximum enhancement factor ( $EF_{max}$ )

Maximum enhancement factor ( $EF_{max}$ ) is observed from hot-spot regions in a SERS substrate where the high electric fields are concentrated and the analyte molecules are adsorbed.  $EF_{max}$  for spherical nanoparticles can be of the order of  $\sim 10^6$ . Depending on the shape, size and material of the nanostructure, This value can be as high as  $\sim 10^{10} - 10^{11}$ . These substrates are usually employed in the single molecule sensing or other analytical techniques where a very high sensitivity is required [30].

#### Average enhancement factor ( $EF_{avg}$ )

While measuring average enhancement factor ( $EF_{avg}$ ), the enhancement is considered from all possible positions on the nanostructured metallic surface. Analyte molecules adsorbed randomly on the SERS substrate contributes to the signal enhancement. Typical values for  $EF_{avg}$  lies in the range of  $\sim 10^5 - 10^6$  for standard SERS substrates.

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It is possible to achieve the  $EF_{avg}$  as high as  $\sim 10^7 - 10^8$ , which are considered to be good SERS substrates [30].

$EF_{avg}$  of a SERS substrate can be estimated using the following equation:

$$EF_{avg} = \frac{\frac{I_{SERS}}{N_{SERS}}}{\frac{I_{Raman}}{N_{Raman}}}, \quad (1.8)$$

where,  $I_{Raman}$  and  $I_{SERS}$  represent normal Raman scattering and SERS intensities, respectively.  $N_{Raman}$  and  $N_{SERS}$  represents the number of analyte molecules probed for normal Raman scattering and SERS measurements, respectively.

### 1.3.4 Reproducibility

Another very important characteristic to be considered for a SERS substrate is reproducibility. It is the measure of signal intensity variation in the SERS substrates. After treating with a analyte molecule, the Raman spectrum is collected from several points on the SERS substrate. For a particular characteristic peak of the analyte, relative standard deviation (RSD) of the intensity values is collected. SERS substrate with RSD value less than 20% or reproducibility greater than 80% can be asumed to be a good SERS substrate. To check batch-to-batch reproducibility, the SERS substrates of the same batch when manufactured are treated with the analyte samples of same concentration. Then Raman signals from these samples are collected and RSD values of spectral intensity from the substrates are calculated [31, 32].

### 1.3.5 SERS probes

SERS probes are the molecules to be detected that are adsorbed on the SERS substrates. Raman signal intensities varies significantly for different probe molecules and incident laser wavelengths. For example, dye molecules with electronic energies close to the exciting laser energy provides high Raman enhancement. The probe molecule has to be adsorbed efficiently on the metal surface, such as molecule with thiol group has strong affinity with silver and gold. The probes can be attached through electrostatic interaction and surface functionalization of the SERS substrate. Raman active molecules can be attached with the SERS substrate (by chemical modification if required) and analysed using SERS techniques. But, maximum enhancement can not be achieved for different analytes with same SERS substrate. For a weak Raman active molecule, different SERS experimental parameters (material, nano structures, molecular adsorption, laser power and wavelength, etc.) can be adjusted and optimized for maximizing the signal enhancement and reliable detection [29].



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## 1.4 Statement of the research problem and research motivation

Design and development of SERS-based sensing systems, and their subsequent applications in the field of molecular sensing have emerged as a popular area of research in recent time [56, 57]. For a reliable SERS detection, the SERS substrate has to provide higher order signal enhancement, larger surface area for adsorption of analyte, and reproducible Raman signal. There are a large number of literature available that demonstrate different types of SERS substrates and fabrication strategies as discussed in Chapter 2. However, for the electrochemically roughened metal electrode SERS substrates, the fabrication procedure is very rapid and simple. But, these SERS substrates suffers from the low Raman intensity and poor signal reproducibility. Single molecule detection is possible with the colloidal nanoparticles that generate maximum SERS enhancement after appropriate substrate modification. The sensing system too suffers from low signal reproducibility. Although lithographic techniques can be used to create highly reproducible SERS substrates, these methods require specialized training for sample preparation and equipment operation, as well as expensive, sophisticated instruments and laboratory space. The complicated substrate fabrication procedure, the high cost of these substrates with the expensive Raman spectrometer setup limits the use of SERS as a sensing method to some extent compared to the colorimetric, fluorometric, and electrochemical sensing methods. However, keeping in mind all the benefits of SERS as a alternative sensing platform as discussed in the previous sections, research groups across the world are working extensively on the development of the SERS substrates utilizing the naturally available nanostructured materials. Such development will be extremely beneficial for countries with limited resources like India to solve various diagnostic, environmental and agricultural-related problems. Given the current state of the problem, present thesis work aims towards development of sensitive and reproducible SERS substrates on low-cost platforms that can be utilized for sensing chemicals and biomolecules.

## 1.5 Scope and contribution of the thesis

In this thesis work, SERS substrate fabrication strategies using inexpensive methods have been explored. The primary goal is to develop SERS substrates with high EF, enhanced sensitivity and good degree of reproducibility while utilizing the simple tools and resources possible. These substrates are envisioned to be emerged as a potential alternative to other sophisticated techniques.

In the first phase of this thesis work, simulation studies have been performed to get the information about the localized electric field generated due the interaction of

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metal nanoparticles and the incident laser. The intensity and distribution of this electric field depends on several parameters, like- size, shape, material, environment, separation between the particles, polarization of the incident light. All the simulation works have been performed using the finite element method (FEM)-based COMSOL Multiphysics software (Wave Optics Module).

In the next phase, transparent and flexible SERS substrates have been fabricated by drop-casting AgNPs on polyethylene terephthalate (PET) sheet. Malachite green (MG) has been used as a probe molecule to study the characteristics of the fabricated SERS substrates. SERS signals have been collected from the front surface and the rear surface of the transparent substrate. The intensity of the scattered light collected using the Raman spectrometer is found to be more when collected from the rear side of the substrate compared to the other case. This observation has been verified by studying the localized electric field distribution using COMSOL Multiphysics simulation software. These plastic-based SERS substrates have been utilized in sensitive detection of profenofos and cypermethrin pesticides in water samples.

After this, another SERS substrate has been proposed with better EF and reproducibility. The commercially available BR-DVD has well defined nanochannels where plasmonic nanoparticles could be trapped. In the substrate fabrication process, AuNPs have been drop-casted on the BR-DVD surface containing the periodic nanochannels. The developed substrates were then treated with MG and 1,2-bis(4-pyridyl)ethylene (BPE) to study the performance in terms of sensitivity and reproducibility. The substrates provide an  $EF_{avg}$  of  $3.2 \times 10^6$  and signal reproducibility of 94% with a relatively low fabrication cost INR 16 ( $\sim$  \$0.2) per substrate. The applicability of the substrates in biomolecule sensing have been explored by detecting rotavirus RNA samples in laboratory condition. The experimental results have been compared with a commercial-grade SERS substrate.

In the next work, printing-grade papers are explored to develop a sensitive, low-cost, flexible, and disposable SERS substrate for the regular monitoring of viral antigens. AgNPs are drop-casted over the commercially available 85 and 100 grams per square meter (GSM) papers to fabricate the SERS substrates. The performance of the substrates are initially realized by detecting and analyzing MG and rhodamine 6G (R6G). The 85 GSM paper SERS substrates have  $EF_{avg}$  of  $5 \times 10^6$  and signal reproducibility is 90% with a relatively low fabrication cost INR 5 ( $\sim$  \$0.06) per substrate. The substrates were utilized to detect rotavirus particles in clinical stool samples. The experimental results have been compared with a commercial-grade SERS substrate.

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## Bibliography

- [1] Morin-Crini, N., Lichtfouse, E., Liu, G., Balaram, V., Ribeiro, A. R. L., Lu, Z., Stock, F., Carmona, E., Teixeira, M. R., Picos-Corrales, L. A., et al. Worldwide cases of water pollution by emerging contaminants: a review. *Environmental Chemistry Letters*, 20(4):2311–2338, 2022.
- [2] Jadeja, N. B., Banerji, T., Kapley, A., and Kumar, R. Water pollution in india—current scenario. *Water Security*, 16:100119, 2022.
- [3] Sun, A. and Wang, W.-X. Human exposure to microplastics and its associated health risks. *Environment & Health*, 1(3):139–149, 2023.
- [4] Dey, S. and Nagababu, B. H. Applications of food color and bio-preservatives in the food and its effect on the human health. *Food Chemistry Advances*, 1:100019, 2022.
- [5] Raman, C. V. and Krishnan, K. S. A new type of secondary radiation. *Nature*, 121(3048):501–502, 1928.
- [6] Ferraro, J. R. *Introductory raman spectroscopy*. Elsevier, 2003.
- [7] Haynes, C. L., McFarland, A. D., and Van Duyne, R. P. Surface-enhanced raman spectroscopy, 2005.
- [8] Moskovits, M. Surface-enhanced raman spectroscopy: a brief retrospective. *Journal of Raman Spectroscopy: An International Journal for Original Work in all Aspects of Raman Spectroscopy, Including Higher Order Processes, and also Brillouin and Rayleigh Scattering*, 36(6-7):485–496, 2005.
- [9] Schatz, G. C., Young, M. A., and Van Duyne, R. P. Electromagnetic mechanism of sers. In *Surface-enhanced Raman scattering: physics and applications*, pages 19–45. Springer, 2006.
- [10] Stiles, P. L., Dieringer, J. A., Shah, N. C., and Van Duyne, R. P. Surface-enhanced raman spectroscopy. *Annu. Rev. Anal. Chem.*, 1:601–626, 2008.
- [11] Bell, S. E. and Sirimuthu, N. M. Quantitative surface-enhanced raman spectroscopy. *Chemical Society Reviews*, 37(5):1012–1024, 2008.
- [12] Lombardi, J. R. and Birke, R. L. A unified approach to surface-enhanced raman spectroscopy. *The Journal of Physical Chemistry C*, 112(14):5605–5617, 2008.
- [13] Schlücker, S. Surface-enhanced raman spectroscopy: Concepts and chemical applications. *Angewandte Chemie International Edition*, 53(19):4756–4795, 2014.

- 
- [14] Pérez-Jiménez, A. I., Lyu, D., Lu, Z., Liu, G., and Ren, B. Surface-enhanced raman spectroscopy: benefits, trade-offs and future developments. *Chemical science*, 11(18):4563–4577, 2020.
- [15] Ong, T. T., Blanch, E. W., and Jones, O. A. Surface enhanced raman spectroscopy in environmental analysis, monitoring and assessment. *Science of the total environment*, 720:137601, 2020.
- [16] Han, X. X., Rodriguez, R. S., Haynes, C. L., Ozaki, Y., and Zhao, B. Surface-enhanced raman spectroscopy. *Nature Reviews Methods Primers*, 1(1):87, 2021.
- [17] Tahir, M. A., Dina, N. E., Cheng, H., Valev, V. K., and Zhang, L. Surface-enhanced raman spectroscopy for bioanalysis and diagnosis. *Nanoscale*, 13(27):11593–11634, 2021.
- [18] Fleischmann, M., Hendra, P. J., and McQuillan, A. J. Raman spectra of pyridine adsorbed at a silver electrode. *Chemical physics letters*, 26(2):163–166, 1974.
- [19] Jeanmaire, D. L. and Van Duyne, R. P. Surface raman spectroelectrochemistry: Part i. heterocyclic, aromatic, and aliphatic amines adsorbed on the anodized silver electrode. *Journal of electroanalytical chemistry and interfacial electrochemistry*, 84(1):1–20, 1977.
- [20] Albrecht, M. G. and Creighton, J. A. Anomalously intense raman spectra of pyridine at a silver electrode. *Journal of the american chemical society*, 99(15):5215–5217, 1977.
- [21] Moskovits, M. Surface roughness and the enhanced intensity of raman scattering by molecules adsorbed on metals. *The Journal of Chemical Physics*, 69(9):4159–4161, 1978.
- [22] Lombardi, J. R., Birke, R. L., Lu, T., and Xu, J. Charge-transfer theory of surface enhanced raman spectroscopy: Herzberg–teller contributions. *The Journal of chemical physics*, 84(8):4174–4180, 1986.
- [23] Jensen, L., Aikens, C. M., and Schatz, G. C. Electronic structure methods for studying surface-enhanced raman scattering. *Chemical Society Reviews*, 37(5):1061–1073, 2008.
- [24] Valley, N., Greeneltch, N., Van Duyne, R. P., and Schatz, G. C. A look at the origin and magnitude of the chemical contribution to the enhancement mechanism of surface-enhanced raman spectroscopy (sers): Theory and experiment. *The Journal of Physical Chemistry Letters*, 4(16):2599–2604, 2013.

- 
- [25] Camden, J. P., Dieringer, J. A., Wang, Y., Masiello, D. J., Marks, L. D., Schatz, G. C., and Van Duyne, R. P. Probing the structure of single-molecule surface-enhanced raman scattering hot spots. *Journal of the American Chemical Society*, 130(38):12616–12617, 2008.
- [26] Hutter, E. and Fendler, J. H. Exploitation of localized surface plasmon resonance. *Advanced materials*, 16(19):1685–1706, 2004.
- [27] Willets, K. A. and Van Duyne, R. P. Localized surface plasmon resonance spectroscopy and sensing. *Annu. Rev. Phys. Chem.*, 58(1):267–297, 2007.
- [28] Le Ru, E. C. and Etchegoin, P. G. Rigorous justification of the  $e^{-4}$  enhancement factor in surface enhanced raman spectroscopy. *chemical Physics letters*, 423(1-3):63–66, 2006.
- [29] Le Ru, E. and Etchegoin, P. *Principles of Surface-Enhanced Raman Spectroscopy: and related plasmonic effects*. Elsevier, 2008.
- [30] Le Ru, E. C. and Auguie, B. Enhancement factors: A central concept during 50 years of surface-enhanced raman spectroscopy. *ACS nano*, 18(14):9773–9783, 2024.
- [31] Tantra, R., Brown, R. J., and Milton, M. J. Strategy to improve the reproducibility of colloidal sers. *Journal of Raman Spectroscopy: An International Journal for Original Work in all Aspects of Raman Spectroscopy, Including Higher Order Processes, and also Brillouin and Rayleigh Scattering*, 38(11):1469–1479, 2007.
- [32] Santos, J. J., Toma, S. H., Corio, P., and Araki, K. Key role of surface concentration on reproducibility and optimization of sers sensitivity. *Journal of Raman Spectroscopy*, 48(9):1190–1195, 2017.
- [33] Mosier-Boss, P. A. Review of sers substrates for chemical sensing. *Nanomaterials*, 7(6):142, 2017.
- [34] Asapu, R., Ciocarlan, R.-G., Claes, N., Blommaerts, N., Minjauw, M., Ahmad, T., Dendooven, J., Cool, P., Bals, S., Denys, S., et al. Plasmonic near-field localization of silver core-shell nanoparticle assemblies via wet chemistry nanogap engineering. *ACS applied materials & interfaces*, 9(47):41577–41585, 2017.
- [35] Lee, C.-W., Chia, Z. C., Hsieh, Y.-T., Tsai, H.-C., Tai, Y., Yu, T.-T., and Huang, C.-C. A facile wet-chemistry approach to engineer an au-based sers substrate and enhance sensitivity down to ppb-level detection. *Nanoscale*, 13(7):3991–3999, 2021.

- 
- [36] Shrestha, S., Wang, B., and Dutta, P. Nanoparticle processing: Understanding and controlling aggregation. *Advances in colloid and interface science*, 279:102162, 2020.
- [37] Israelsen, N. D., Hanson, C., and Vargis, E. Nanoparticle properties and synthesis effects on surface-enhanced raman scattering enhancement factor: an introduction. *The Scientific World Journal*, 2015(1):124582, 2015.
- [38] Burrows, N. D., Vartanian, A. M., Abadeer, N. S., Grzincic, E. M., Jacob, L. M., Lin, W., Li, J., Dennison, J. M., Hinman, J. G., and Murphy, C. J. Anisotropic nanoparticles and anisotropic surface chemistry. *The journal of physical chemistry letters*, 7(4):632–641, 2016.
- [39] Ortiz-Castillo, J. E., Gallo-Villanueva, R. C., Madou, M. J., and Perez-Gonzalez, V. H. Anisotropic gold nanoparticles: A survey of recent synthetic methodologies. *Coordination Chemistry Reviews*, 425:213489, 2020.
- [40] Jing, Y., Wang, R., Wang, Q., Xiang, Z., Li, Z., Gu, H., and Wang, X. An overview of surface-enhanced raman scattering substrates by pulsed laser deposition technique: fundamentals and applications. *Advanced Composites and Hybrid Materials*, 4(4):885–905, 2021.
- [41] Petti, L., Capasso, R., Ripa, M., Pannico, M., La Manna, P., Peluso, G., Calarco, A., Bobeico, E., and Musto, P. A plasmonic nanostructure fabricated by electron beam lithography as a sensitive and highly homogeneous sers substrate for bio-sensing applications. *Vibrational Spectroscopy*, 82:22–30, 2016.
- [42] Yang, J.-Y., Park, S.-G., Jung, S., Byeon, E.-Y., Kim, D.-g., Jung, H. S., Kim, H. J., and Lee, S. Sers substrates based on self-organized dimple nanostructures on polyethylene naphthalate films produced via oxygen ion beam sputtering. *Applied Surface Science*, 572:151452, 2022.
- [43] Fang, X., Zheng, C., Yin, Z., Wang, Z., Wang, J., Liu, J., Luo, D., and Liu, Y. J. Hierarchically ordered silicon metastructures from improved self-assembly-based nanosphere lithography. *ACS applied materials & interfaces*, 12(10):12345–12352, 2020.
- [44] Cai, J., Liu, R., Jia, S., Feng, Z., Lin, L., Zheng, Z., Wu, S., and Wang, Z. Sers hotspots distribution of the highly ordered noble metal arrays on flexible substrates. *Optical Materials*, 122:111779, 2021.
- [45] Yao, L., Dai, P., Ouyang, L., and Zhu, L. A sensitive and reproducible sers sensor based on natural lotus leaf for paraquat detection. *Microchemical Journal*, 160:105728, 2021.

- 
- [46] Chamuah, N., Hazarika, A., Hatiboruah, D., and Nath, P. Sers on paper: an extremely low cost technique to measure raman signal. *Journal of Physics D: Applied Physics*, 50(48):485601, 2017.
- [47] Martins, N. C., Fateixa, S., Fernandes, T., Nogueira, H. I., and Trindade, T. Inkjet printing of ag and polystyrene nanoparticle emulsions for the one-step fabrication of hydrophobic paper-based surface-enhanced raman scattering substrates. *ACS Applied Nano Materials*, 4(5):4484–4495, 2021.
- [48] Siebe, H. S., Chen, Q., Li, X., Xu, Y., Browne, W. R., and Bell, S. E. Filter paper based sers substrate for the direct detection of analytes in complex matrices. *Analyst*, 146(4):1281–1288, 2021.
- [49] Chamuah, N., Bhuyan, N., Das, P. P., Ojah, N., Choudhary, A. J., Medhi, T., and Nath, P. Gold-coated electrospun pva nanofibers as sers substrate for detection of pesticides. *Sensors and Actuators B: Chemical*, 273:710–717, 2018.
- [50] Wang, C., Wong, K. W., Wang, Q., Zhou, Y., Tang, C., Fan, M., Mei, J., and Lau, W.-M. Silver-nanoparticles-loaded chitosan foam as a flexible sers substrate for active collecting analytes from both solid surface and solution. *Talanta*, 191: 241–247, 2019.
- [51] Li, L., Yang, S., Duan, J., Huang, L., and Xiao, G. Fabrication and sers performance of silver nanoarrays by inkjet printing silver nanoparticles ink on the gratings of compact disc recordable. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 225:117598, 2020.
- [52] Chamuah, N., Saikia, A., Joseph, A. M., and Nath, P. Blu-ray dvd as sers substrate for reliable detection of albumin, creatinine and urea in urine. *Sensors and Actuators B: Chemical*, 285:108–115, 2019.
- [53] Li, Z., Huang, X., and Lu, G. Recent developments of flexible and transparent sers substrates. *Journal of Materials Chemistry C*, 8(12):3956–3969, 2020.
- [54] Bharati, M. S. S. and Soma, V. R. Flexible sers substrates for hazardous materials detection: recent advances. *Opto-Electronic Advances*, 4(11):210048, 2021.
- [55] Rebollar, E., Pérez, S., Hernández, M., Domingo, C., Martín, M., Ezquerro, T. A., García-Ruiz, J. P., and Castillejo, M. Physicochemical modifications accompanying uv laser induced surface structures on poly (ethylene terephthalate) and their effect on adhesion of mesenchymal cells. *Physical Chemistry Chemical Physics*, 16 (33):17551–17559, 2014.

- 
- [56] Cialla-May, D., Zheng, X.-S., Weber, K., and Popp, J. Recent progress in surface-enhanced raman spectroscopy for biological and biomedical applications: from cells to clinics. *Chemical Society Reviews*, 46(13):3945–3961, 2017.
- [57] Bell, S. E., Charron, G., Cortés, E., Kneipp, J., de la Chapelle, M. L., Langer, J., Procházka, M., Tran, V., and Schlücker, S. Towards reliable and quantitative surface-enhanced raman scattering (sers): from key parameters to good analytical practice. *Angewandte Chemie International Edition*, 59(14):5454–5462, 2020.