Surface-enhanced Raman scattering: overview of a versatile technique used in electrochemistry and nanoscience

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This article describes recent developments in the field of surface-enhanced Raman scattering (SERS) research. It begins with the early history of SERS as the first SERS spectrum was obtained from an electrochemical system of pyridine molecule adsorbed on roughened silver electrode, which led to the discovery of the SERS effect in the mid-1970s. The article also illustrates the various aspects of SERS research which includes SERS-active substrates, mechanism of SERS and its various applications. Special emphasis is given to the important features of electrochemical SERS, a special branch of SERS.

Keywords: Electrochemistry, nanoscience, pyridine molecule, surface-enhanced Raman scattering.

THE discovery of surface-enhanced Raman scattering (SERS) spectroscopy by Fleischmann and co-workers in 1974 at the University of Southampton, which is intimately connected with the electrochemical systems, has drawn the attention of researchers due to the large enhancement of the weak Raman signal thereby facilitating convenient identification in chemical and biological systems. Later, with the advent of scanning probe microscopy (SPM) technique in the early 1980s, it was revealed that the order of surface roughness is in nanometre dimension. Therefore, SERS and nanoscience/nanotechnology are now intimately related. SERS has now emerged as a powerful technique not only to study the molecules or ions at trace concentrations down to single-molecule detection level, but also help understand the surface chemistry, especially electrochemistry at the nanometre scale. The rapid development of nanoscience and nanotechnology in recent years has attracted wide interest in SERS among the scientific community. Thus, SERS has become an important branch of nanoscience and nanotechnology and also a strong analytical tool available both in surface chemistry as well as electrochemistry. Electrochemical SERS (EC-SERS), an special branch of SERS, is an important yet complex tool in electrochemistry as evident from the moderate number of publications on various aspects of SERS from electrochemical systems. As the SERS spectra can be recorded by the application of electrode potential, this makes EC-SERS one of the most complicated systems in SERS.

Although several review articles (both tutorial and critical) have been written on various aspects of SERS, none of them covers the prospective development of SERS in terms of substrates, methods, theory based on the mechanisms and applications, and its correlation with electrochemistry, nanoscience/nanotechnology and surface chemistry. There are few review articles on the development of EC-SERS. The aim of this article is to provide an updated and comprehensive review on various aspects of the SERS effect which include historical background and development, correlation with both electrochemistry and nanoscience, various SERS active substrates, various theories to explain the mechanism of SERS and its various applications. Due to space limitation, a complete review of all recent work on this important area of research in both electrochemistry/nanoscience is not possible. However, a few representative examples have been summarized to illustrate the recent developments in SERS research.

Historical background

Raman spectroscopy is a technique used in condensed matter physics and chemistry to study vibrational, rotational and other low-frequency modes in a system. It depends on the inelastic scattering, or Raman scattering of monochromatic light, usually from a laser in the visible, near-infrared or near-ultraviolet range of the electromagnetic spectrum. This effect was discovered by C. V. Raman in 1928 (ref. 1). The weak intensity of the Raman signal due to the low scattering cross-section ($\sim 10^{-30}$ cm^2 molecule^{-1}) is the biggest disadvantage of Raman spectroscopy with low sensitivity, which is the reason why it was not widely used for a long time.

The name 'surface-enhanced Raman spectroscopy' implies that it provides the same information that normal Raman spectroscopy does, simply with a greatly enhanced signal. The weak Raman signal can be greatly enhanced by the introduction of SERS spectroscopy in 1974 by

Fleischmann *et al.*[2] from the University of Southampton. It was accidentally discovered by them when they tried to do Raman with an adsorbate of very high Raman cross-section, such as pyridine (Py) on the roughened silver (Ag) electrode. The initial idea was to generate high surface area on the roughened metal surface. On the basis of their extensive experience in increasing the surface area of an Ag electrode using an electrochemical roughening method, they applied about 450 potential oxidation and reduction cycles (ORC) to an Ag electrode in an aqueous electrolyte comprised of 0.1 mol l^{-1} KCl + 0.05 mol l^{-1} Py. The Raman spectrum obtained was of unexpectedly high quality and evidently due to the adsorbed Py, and the spectra was found to be dependent on the electrode potential. They initially explained that the intense surface Raman signal of Py was due to increased surface area. This was, in fact, the first SERS measurement and the roughened electrode was the first nanostructure exhibiting the SERS activity, although it was not recognized as such in 1974. Later, in 1977, Jeanmaire and Van Duyne from Northwestern University, USA, first realized that surface area is not the main factor in the above phenomenon[3]. Albrecht and Creighton from the University of Kent, UK, reported a similar result in the same year[4]. These two groups provided strong evidences to demonstrate that the strong surface Raman signal must be generated by a real enhancement of the Raman scattering efficiency (10^5–10^6). The effect was later named as surface-enhanced Raman scattering and now, it is an universally accepted surface sensitive technique. Although the first SERS spectra were obtained from an electrochemical system (Py + roughened Ag electrode), all important reactions on surfaces including electrochemical processes can be studied by SERS. The technique is so sensitive that even a single molecule can be detected.

The exact mechanism of the enhancement effect of SERS is still a matter of controversy in the literature. There are two main mechanisms on the large enhancement effect of weak Raman signal from Py adsorbed on electrochemically roughened Ag. Jeanmaire and Van Duyne proposed the theory of electromagnetic effect on the enhancement[3]. The electromagnetic theory is based on the excitation of localized surface plasmons. Albrecht and Creighton proposed a theory based on the charge transfer effect of the adsorbed molecule on the enhancement efficiency[4]. This chemical enhancement theory relies on the charge transfer complex formation of the adsorbed molecule. However, it is difficult to separate these two effects experimentally.

In the mid-1990s, an important development in EC-SERS was carried out by which substantial surface Raman enhancement could be imparted to the VIIIB transition metals of importance for electrochemistry and catalysis. Tian and his group at Xiamen University, China, developed several surface roughening procedures and demonstrated that the SERS effect can be directly generated on transition metals such as pure Pt, Ru, Rh, Pd, Fe, Co and Ni electrodes, and their surface enhancement ranges in general from one to three orders of magnitude[5]. Since the early 2000s, the approach of replacing randomly roughened surfaces with well-controlled nanostructures of both coinage (e.g. Au, Ag and Cu) and transition metals has been introduced as a promising class of highly SERS-active substrate[6]. Up to now, molecular-level studies using Raman spectroscopy on diverse adsorbates at various material electrodes have been realized. These advances have made Raman spectroscopy a popular tool in electrochemistry. Moreover, a systematic study on EC-SERS processes could help elucidate comprehensively the SERS mechanisms.

The main features of the SERS technique are summarized briefly as follows.

(1) It is a highly surface-sensitive, non-destructive and *in situ* vibrational spectroscopic technique.
(2) SERS occurs when target molecules are brought closer within a few nanometres of the surface of SERS-active substrates of different morphologies.
(3) The excitation profile (scattering intensity versus excitation frequency) deviates from the fourth power dependence of normal Raman scattering.
(4) It has extremely high spatial resolution. The enhancement range is several nanometres, effective for one or several molecular layers close to the SERS-active substrate.
(5) SERS activity strongly depends on the nature of the metal and surface roughness.

The recent advancement in the field of SERS research is mainly focused in the following directions:

- Fabrication of new, novel and efficient SERS-active substrates to overcome the substrate generality of SERS.
- Single-molecule SERS to improve the molecule generality and enhance the detection limit of various adsorbates/species.
- Understanding the mechanisms involved in the SERS phenomenon.
- Use of SERS for imaging, sensing, diagnostics and further search for new applications.

General features of EC-SERS

EC-SERS is a special branch of the SERS technique, which generally consists of nanostructured electrodes and electrolyte. An electrochemical double layer is thereby formed in between, and is considered the most important and complicated interfacial region. Beyond the analytical aspect, EC-SERS has paid more attention to provide meaningful information for revealing the adsorption

configuration or the reaction mechanism for electro-chemistry[7].

Generally, it has been widely accepted that the SERS enhancement effect is contributed by the long-range electromagnetic field enhancement (EM) and short-range chemical enhancement (CM). The former is the main contributor to SERS signals in most SERS systems. However, in case of electrochemical systems, CM also plays an important role, especially in characterization of chemical species as it is closely associated with the chemical property of surface species and substrates. Both chemical and physical or electromagnetic field enhancements can be influenced to some extent by changing the applied electrode potential, i.e. the Fermi level of the metal and dielectric constant of the interfacial electrolyte in an EC-SERS system. The chemical enhancement can be strongly tuned by the potential, leading to extreme changes in interfacial structure and property. This makes EC-SERS one of the most complicated systems in SERS. Another important and common feature of EC-SERS is that the SERS intensity strongly depends on the electrode potential. The change in the electrode potential may result in a change in the coverage and/or adsorption orientation of the molecule, both of which will lead to a change in the SERS intensity.

In a typical EC-SERS set-up, the electrochemical system is studied while the electrode potential is tuned and the spectral response (including the intensity and frequency change or even the appearance of new bands) is recorded. In most cases, the experimental data will be interpreted by analysing the intensity or frequency change of some characteristic bands (vibrational modes), which may directly reflect a change in the surface coverage, orientation, structure, composition and morphology, and sometimes may indicate the involvement of a certain kind of surface enhancement. Figure 1 illustrates the schematic of a typical experimental set-up used in *in situ*
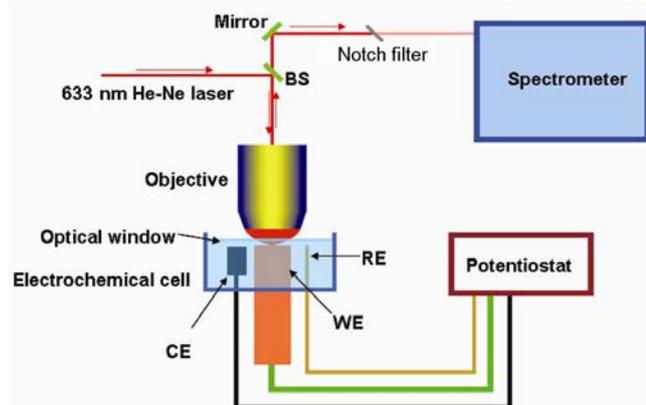

Figure 1. Schematic of a typical experimental set-up used in *in situ* EC-SERS, which includes a Raman spectrometer, a potentiostat or galvanostat, a computer and an EC-SERS cell. (Photo courtesy: Z. Q. Tian.)

EC-SERS, which includes a Raman spectrometer, a potentiostat or galvanostat, a computer and an ECSERS cell. The EC-SERS cell consists of three electrode configurations – a conductive SERS-active working electrode, an inert counter electrode and a reference electrode (usually a saturated calomel electrode (SCE) or Ag/AgCl electrode). The three electrodes should be assembled in a relative geometric position to allow both efficient Raman and accurate electrochemical measurements. Most of the systems which have been investigated by EC-SERS deal with the adsorption and reaction of inorganic species, organic molecules and biomolecules in aqueous solutions. In the case of EC-SERS, a collective analysis of the spectral features from the experimental and theoretical aspects is essential to obtain important information on chemical bonding, orientation and even electrochemical reaction of the molecules at the electrode surfaces, and thereafter to understand the overall interfacial structure at the electrode–electrolyte interface. Py was the first molecule which was used to demonstrate the SERS effect in the electrochemical system and since then, it has become an important probe molecule to verify the SERS activity and surface properties of a new SERS-active substrate. The relative intensities and frequency shifts of the major bands of Py are sensitive to the electrode potential and the surface properties of the metal surfaces used as SERS substrates. These phenomena cannot be solely interpreted with the EM effect; contribution of the CM effect should also be taken into account.

Tian and his group carried out systematic SERS studies of Py on different metal surfaces (both coinage and transition metals), illustrating how EC-SERS can be used to characterize the surface adsorption and molecule–metal interaction[8]. Their study also revealed that the frequencies and intensities of SERS of Py were correlated to the chemical interaction between Py and the metal surface and the adsorption orientation.

It is highly desirable to apply *in situ* SERS to obtain molecular-level information about the breaking and formation of bonds in the reaction, observe the reaction intermediate on the surface and finally, to distinguish the reaction products. The microscopic structure and dynamics of interfacial water molecules are important in understanding the electrode–electrolyte interface and is still an ongoing issue in electrochemistry as well as surface science. However, the interactions between water molecules and electrolyte ions as well as between water and electrode surface are complex. Recently, Tian *et al.* have characterized the surface water on Au core Pt group metal shell nanoparticles-coated electrodes[9]. They obtained the potential-dependent SERS spectra of water molecules adsorbed on Pt, Pd and Au electrode surfaces in the potential region of the hydrogen evolution reaction, wherein the band at around $1615 \, cm^{-1}$ is the bending vibration of water[9] (Figure 2). It is important to note that the intensity of bending vibration is almost the same as
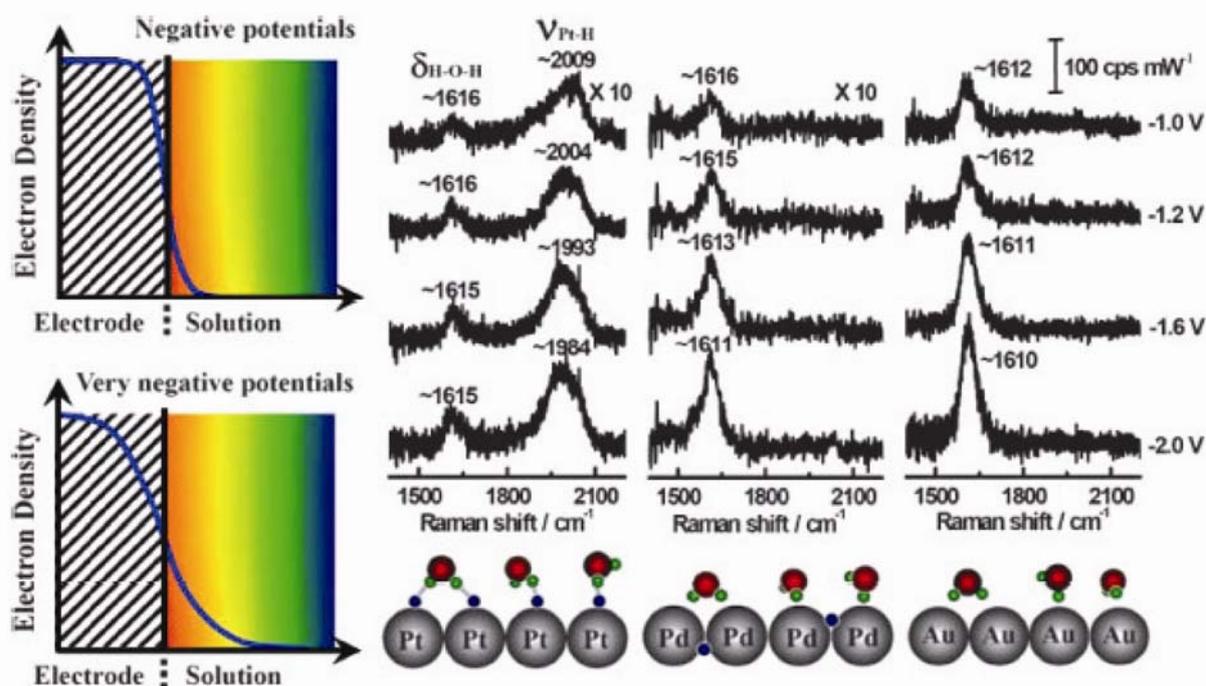
Figure 2. Potential-dependent SERS spectra of interfacial water molecules adsorbed on Au, Pd and Pt surfaces at negative potentials with the excitation wavelength of 632.8 nm. The suggested models (right bottom) for the adsorbed water on different electrode surfaces and the influence of potential on metal conduction electron are shown. (Reprinted with permission from Jiang et al.[9]. Copyright (2007) by Royal Chemical Society.)

the stretching one. The broad band at around 2000 cm^{-1} observed only on the Pt surface can be attributed to the Pt–H stretching vibration. The extent of frequency shift with applied potentials for the O–H stretching vibration is relatively low on Pt compared to both Au and Pd electrodes. They also proposed three adsorption models for the three metal electrodes (Pt, Au, Pd) to explain the different spectral features of water in the negative potentials. The schematic diagram representing the proposed model of adsorbed water on different electrode surfaces and the influence of applied potential on the metal conduction electrons has also been illustrated in Figure 2. It can be observed from the figure that the intensity of SERS signal of water is much stronger at the negative potentials than the potential of zero charge (PZC) and increases with the gradual negative shift of potentials. This anomalous phenomenon can be explained by the proposed model based on the influence of the electronic enhancement. The metal conduction electron has a high polarizability and the surface electronic tail penetrates into the solution to a distance of several angstroms at more negative potentials. In this condition, the surface water molecules are immersed under the influence of the 'electronic cloud' in addition to the local optical electric field, which could further enhance the SERS signal of the interfacial water.

Scientists are optimistic that SERS will become an indispensable tool in the study of interfacial structures and various surface processes involving interfacial water molecules without any interference from bulk water.

Weaver and his group carried out a combined study of EC-SERS and cyclic voltammetry (CV) to study the nitrobenzene surface reaction on the SERS-active Au surface[10]. It is well known that nitrobenzene can be reduced by three two-electron steps to form nitrosobenzene, phenylhydroxyamine and aniline:

$$C_6H_5NO_2 + 2e^- \rightarrow [C_6H_5NO] + 2e^-$$
$$\rightarrow [C_6H_5NHOH] + 2e^- \rightarrow C_6H_5NH_2$$
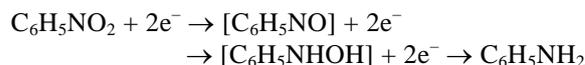
They acquired the SERS spectra during the potential sweep for the reduction of nitrobenzene at the SERS-active gold electrode and correlated the voltammetric and SERS results, and proved that the peak at –0.11 V can be assigned to the reduction of nitrobenzene to nitrosobenzene. Their study based on EC-SERS in combination with CV provides a new dimension in the investigation of electrochemical reaction on the electrode surfaces and identification of the intermediates and products of the reaction.

The non-destructive, highly surface-sensitive and *in situ* nature of surface SERS can be utilized to detect reaction intermediates such as radicals and radical ions on the electrode surface and elucidate the overall reaction mechanism. Recently, Tian and his group at Xiamen

University, China have reported the first *in situ* EC-SERS study on the electrochemical reduction of benzyl chloride ($PhCH_2Cl$) in acetonitrile (CH_3CN) on Ag electrode[11]. Figure 3 illustrates the potential-dependent SERS spectra of $PhCH_2Cl$ on an Ag electrode at different potentials ranging from –0.6 to –2.2 V versus SCE. When the cathode was held at –1.2 V, the SERS spectrum changed dramatically as shown in Figure 3. A strong and broad peak appeared at 800 cm^{-1} and reached its maximum intensity at –1.4 V. This indicates that a new species was formed at the electrode surface as soon as the electrochemical reduction occurred. A second drastic spectral change occurred when the potential was set beyond –1.6 V. A peak grew gradually at 1000 cm^{-1} and reached its maximum at –1.8 V. This new peak then is most likely due to the final reaction product(s) adsorbed at the electrode surface, its decay indicating a progressive desorption at more negative potentials. Benzyl radical anion was detected as an intermediate of the above surface reaction. The overall reaction mechanism revealing the adsorption process of $PhCH_2Cl$ on the Ag surface and all other possible interactions, including the solvent has been elucidated from the SERS study. The SERS results were confirmed by quantum mechanical density functional theory (DFT) calculations, which can provide concrete evidence to identify the reaction intermediate and products.

Enhancement mechanism in SERS

Despite numerous theoretical and experimental works on SERS and a large number of research publications based on these works, the exact nature of the gigantic enhancement in Raman intensity found in SERS is still a matter
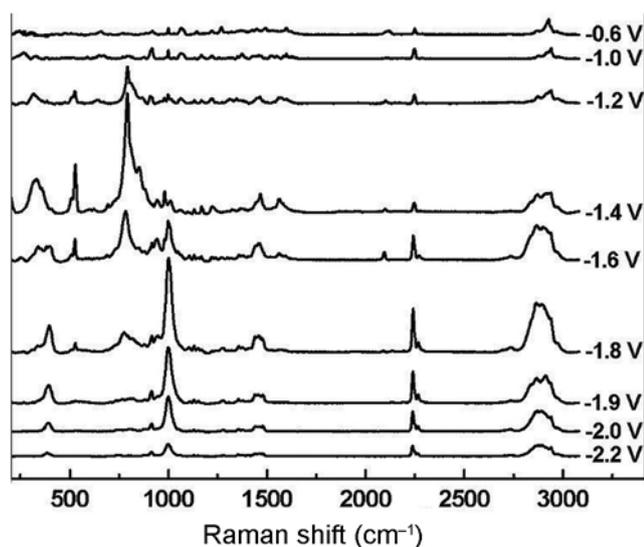
Figure 3. Potential-dependent SERS spectra of $PhCH_2Cl$ on Ag electrode at different potentials ranging from –0.6 to –2.2 V versus SCE (Reprinted with permission from Wang *et al.*[11]. Copyright (2010) by American Chemical Society.)

of controversy. However, it is generally accepted that two enhancement mechanisms, one a long-range EM effect and the other a short-range CM effect, are operative simultaneously. The EM mechanism is based on the amplification of the electromagnetic field generated due to coupling of the radiation field with the localized surface plasmons (LSP) of the metal nanoparticles. The localized surface plasmon resonanace (LSPR) occurs when a resonance condition between the incident wavelength of light and the electrons in the nanoparticles is achieved. This causes a collective oscillation of the conduction electrons and has two primary consequences. First, the wavelengths of light that cause this collective oscillation are selectively absorbed by the nanoparticles and can be monitored using UV–Vis spectroscopy. The second is the formation of enhanced electromagnetic fields that extend from the nanoparticle surfaces. These fields are responsible for a large portion of the enhancement observed in SERS. The enhancement is roughly proportional to $|E^4|$ and generally in the order of 10^8 or more, where E is the intensity of the electromagnetic field.

LSPR, the lightning rod effect, and the image field effect have all been considered to contribute to SERS. Among these, LSPR makes the major contribution to electromagnetic field enhancement and SERS. Anisotropic metal nanoparticles exhibit 'lightning-rod effect'[12], another type of field enhancement which refers to an enhanced charge density localization at the tip or vertex of a nanoparticle. When an electromagnetic field (laser light) excites the free electrons of a metallic tip, a highly localized, strong electric field develops at the sharp tip or vertex with large curvature, leading to large field enhancement in the regions[12]. This is the reason for the high SERS-activity of an anisotropic nanoparticle. Anisotropic metal nanoparticles had been used as an efficient SERS-active substrate with high SERS activity[13,14]. The high SERS activity of the anisotropic metal nanoparticles can be explained in terms of their unusual LSPR properties and the sharp edges on the surface of a nanoparticle being able to generate a greater localized electric field in comparison to isotropic (spherical) nanoparticles. Therefore, the shape of the nanoparticles plays an important role in the overall SERS activity. Fan *et al.*[15] synthesized nearly monodisperse single-crystalline palladium (Pd) nanocubes and nanodendrites in aqueous solution at room temperature and used them as SERS-active substrates. They prepared the SERS substrate using a simple drop-coat method. A drop of the Pd nanoparticles sol was coated on a smooth Pd electrode surface and completely dried in air, and the SERS spectra of pyridine molecules adsorbed on these Pd nanocubes and nanodendrites were recorded. These Pd nanocubes exhibit better SERS activity in comparison to the nanodendrites. The electromagnetic field coupling between nanocubes plays an important role causing an increase in electromagnetic enhancement. The effective area in the nanoparticle junc-

tion that can offer the largest SERS enhancement is much more limited in the case of dendrites compared to the cubes. This results in a stronger SERS signal from the nanocubes than that from the nanodendrites.

The chemical enhancement mechanism reflects the enhancement as a result of the chemical interaction between the adsorbates and the metal surface. Among the various types of chemical enhancements such as chemical bonding enhancement, resonance enhancement of a surface complex, and photon-induced charge-transfer enhancement (PICT), PICT is the most important one. Therefore, the CM mechanism is also known as the charge transfer (CT) mechanism, involving the photo-induced transfer of an electron from the Fermi level of the metal to an unoccupied molecular orbital of the adsorbate or vice versa depending on the energy of the photon and the electric potential of the interface. The enhancement of CM is usually in the order of $10–10^2$. EM is a long-range effect, which requires a rough surface as the SERS-active substrate, while CM is a short-range effect occurring on the molecular scale. The two mechanisms are not mutually exclusive, but work together to produce the overall SERS effect. It is difficult to separate CM from EM. Several research groups have tried to overcome this problem. Therefore, it is desirable to evaluate the relative contribution of these two mechanisms to the overall understanding of the SERS phenomenon. Recently, Pande et al.[16] have carried out SERS studies of 1,10-phenanthroline (PHEN) as a molecular probe adsorbed on a bimetallic nanocolloids as SERS-active substrate to elucidate the mechanism of SERS. They also estimated the relative contributions of the EM and CT mechanisms to the overall enhancement of the SERS bands of the PHEN molecule. Pradhan et al.[17] have synthesized highly branched Au flowers with sharp tips with high yield via galvanic replacement reaction and used them as an excellent and stable SERS substrate. The petal-shaped tips of the gold flowers provide an enhanced electric field, as visualized through three-dimensional finite difference time domain (FDTD) simulation studies. This new kind of SERS substrate contains a large number of embedded 'hot spots' hidden within the oriented petals and absence of surfactant or capping agent invites a probe molecule to show enhancement even for a concentration of 4-mercaptopyridine down to picomolar (10^{-12} M) level. The electric field distribution around the hot spot has been estimated from three-dimensional (FDTD) simulation studies.

Theoretical studies on optical excitations in metal nanostructures

The interaction of electromagnetic radiation of suitable wavelength (light) with the free conduction electrons in metal nanostructures causes coherent oscillation of electrons at the metal surface and generates surface plasmon resonance (SPR). Enhancement of optical signals can be achieved from various molecules (both organic and biological) in the vicinity of these metal nanostructures, caused by the excitation of localized surface plasmons in metal particles in the presence of optical fields. Under illumination by electromagnetic radiation, the metal nanostructures serve as optical antennas and generate enhanced electromagnetic fields in the vicinity. These regions of enhanced electromagnetic fields are known as 'hot spots'[18]. Therefore, molecules near the metal nanostructures can generate enhanced electromagnetic fields which affects several spectroscopic processes such as absorption[19], emission[20,21], Raman scattering[2-4], etc. This has led to the development of several surface-enhanced spectroscopic techniques such as metal-enhanced fluorescence, surface-enhanced infrared absorption and surface-enhanced Raman scattering spectroscopy. Theoretical studies on the optical responses of metal nanostructures can be used to understand the genesis of enhancements in all these enhanced spectroscopic techniques. Maxwell's equations are to be solved to understand the optical responses of nanomaterials theoretically. Several numerical techniques such as the FDTD method[22,23], finite element method (FEM)[24] and discrete dipole approximation (DDA)[25] are being used extensively in the last few years to calculate the optical responses of nanostructures to the incident electromagnetic radiation. Quantum-mechanical methods like time-dependent density functional theory (TDDFT)[26] are currently being utilized for simulations, though for small systems with few electrons, to get information about absorption, scattering, extinction cross-section and electric field distribution patterns in the near-field of the nanostructures. The electric field distribution patterns obtained from these simulation studies can be utilized to calculate the enhancement factors in various surface-enhanced spectroscopic techniques.

The 3D FDTD is a powerful grid-based, time-domain numerical technique, which can solve the Maxwell's equations numerically and simulates the distribution of the electromagnetic field around the illuminated nanoparticles or substrates with an arbitrary geometry[27]. The time-dependent Maxwell's equations (in partial differential form) are discretized using central-difference approximations to the space and time partial derivatives. The resulting finite-difference equations are then solved in either software or hardware in a leapfrog manner. The electric field vector components in a volume of space are solved at a given instant in time; then the magnetic field vector components in the same spatial volume are solved at the next instant in time; and the process is repeated over and over again until the desired transient or steady-state electromagnetic field behaviour is fully evolved. Since it is a time-domain method, the numerical solutions can cover a wide frequency range with a single simulation run[27].
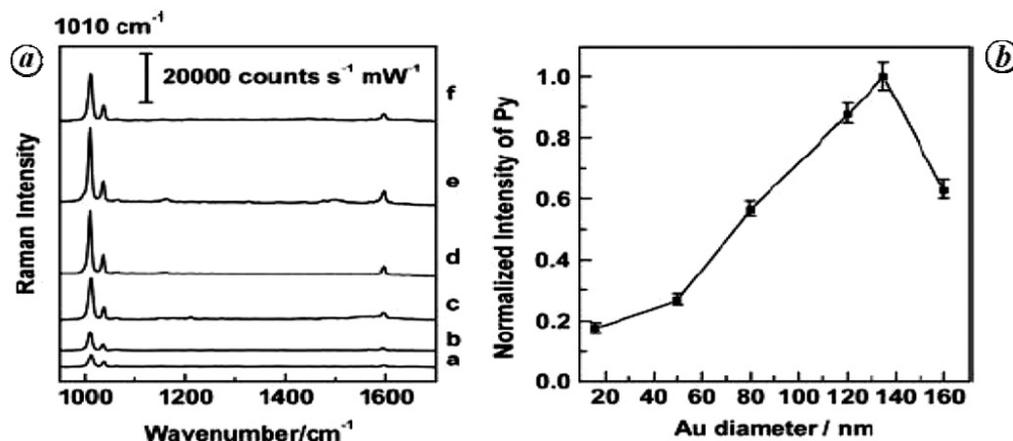
Figure 4. *a*, SERS spectra of pyridine molecule adsorbed on the glassy carbon electrode coated with Au nanoparticles of different sizes. *b*, Normalized SERS intensity of pyridine as a function of the size of Au nanoparticle. (Reprinted with permission from Fang *et al.*[30]. Copyright (2008) by Wiley Interscience.)

For the study of dispersive materials such as silver or gold, frequency-dependent complex permitivities of these metals have to be considered. Generalized Drude model may be explored to simulate the complex permittivities of silver and gold. The complex permittivity $\varepsilon(\omega)$ according to the generalized Drude model is represented as

$$\varepsilon(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + i\omega\tau} + \frac{\sigma}{i\omega\varepsilon_0}, \quad (1)$$

where ε_s, ε_∞, σ and τ represent static permittivity, infinite frequency permittivity, conductivity and relaxation time respectively, ω is the angular frequency and ε_0 is the permittivity of free space. The four parameters ε_s, ε_∞, σ and τ can be adjusted through curve-fitting technique to correctly match the complex permittivity. The generalized Drude model for the local dielectric function of metallic materials represents faithfully the optical response in a wide frequency range, ensuring the accuracy of the FDTD simulation[28].

Several groups are using the FDTD method to calculate the near-field and far-field optical properties of metallic nanostructures and comparing their experimental results to get an insight about the genesis of enhancement mechanism of various surface-enhanced spectroscopic techniques. The variation of electric field around the fabricated nanostructures used as SERS-active substrates of various sizes, shapes and different interparticle spacing can be analysed using 3D FDTD simulation study to elucidate the electromagnetic contribution of the overall enhancement by considering the spatial distribution of electric field around the fabricated SERS-active nanostructures. The size-dependent SERS activity of gold nanoparticles has been studied using 3D FDTD simulation[29,30]. Fang *et al.*[30] studied the dependence of SERS intensity on the size of Au nanoparticles using pyridine as the probe molecule. Figure 4 *a* shows a set of SERS spectra of Py molecule adsorbed on the glassy carbon electrode coated with Au nanoparticles of different sizes. Figure 4 *b* illustrates the normalized SERS intensity of pyridine as a function of the size of Au nanoparticles. It can be clearly observed that the particle size of about 135 nm exhibits the highest SERS activity when the excitation wavelength is 632.8 nm. Au nanoparticles with a diameter of about 120–135 nm could be considered as the optimal SERS substrate. They also carried out FDTD calculation to simulate the EM field enhancement around the illuminated nanoparticles with different sizes to quantitatively account for the size-dependent SERS activity of the above system. In general, the optimal size of metallic nanoparticles for SERS activity depends on the competing effects of radiation damping and surface scattering. It is well known that the dielectric constant of a metal particle becomes size-dependent when the particle size is smaller than the conduction electron mean free path. The SERS enhancement is strongly limited by surface plasmon damping, radiation damping and dynamic depolarization, which become more and more severe with increasing particle size. Figure 5 represents the calculation model and the 3D FDTD simulated electric field distribution for Au nanoparticles of different sizes. As a simple model, the SERS-active substrate was simulated as a gold nanoparticle dimer with a fixed interparticle distance of 2 nm. The 632.8 nm excitation laser is polarized along the axis connecting the two particles. The calculated data show that the maximum field enhancement (hot site) is at the junction of the two nanoparticles in all cases, and the enhancement value keeps on increasing with increase in the particle size up to 110 nm. After that, the radiation damping effect gets stronger, thereby decreasing the field enhancement and accordingly resulting in a weaker SERS activity. The calculation results show good agreement with the experimental data.
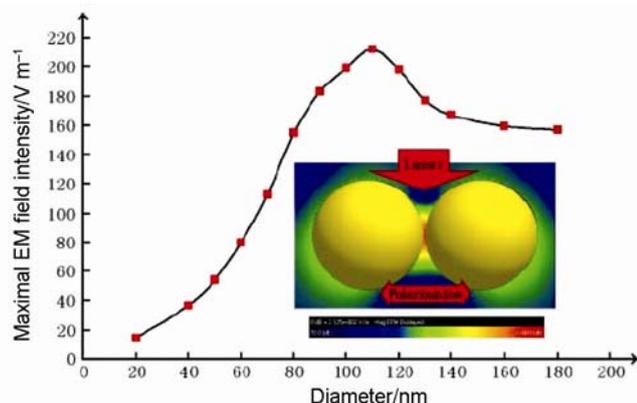
Figure 5. The calculation model and the 3D FDTD simulated electric field distribution for Au nanoparticles of different sizes. (Reprinted with permission from Fang *et al.*[30]. Copyright (2008) by Wiley Inter-science.)

In India too researchers are extensively using 3D FDTD simulation method to understand the near-field and far-field optical effects near metal nanostructures and the enhancement mechanism in various surface-enhanced spectroscopic techniques[31–33]. Recently, the 3D FDTD method has been successfully utilized by our research group for the simulation of the local electric field around Au@Ag nanocolloid of various core/shell ratios[16]. Figure 6 shows the SERS spectra of PHEN molecule adsorbed on Au@Ag nanocolloid of various core/shell ratios (for a fixed Au core and variable Ag shell ratios). Figure 7 illustrates the spatial distribution of the electric field around the illuminated Au@Ag nanocolloid, as obtained from the 3D FDTD simulation study. The results of the 3D FDTD calculations predict the maximum electric field enhancement around the Au@Ag nanocolloid of 29 nm size when core/shell ratio is 1 : 4. The electric field decreases with further increase in shell thickness of the colloid, which may be due to the dominance of the radiation damping effect resulting in a decrease in electric field enhancement. The simulated results discussed above allow us to correlate with the experimentally observed SER bands of the PHEN molecule adsorbed on the Au@Ag nanocolloid having fixed core and variable shell ratios. We also estimated the relative contributions of the EM and CT mechanisms to the overall enhancement of the SERS bands of the PHEN molecule. We obtained a moderate 5–7 orders of magnitude enhancement of almost all the Raman bands primarily representing the in-plane vibrations at ~408, 711, 1033 cm^{-1} and 1408, 1601 cm^{-1} belonging to A_1 and B_2 irreducible representations of the PHEN molecule. No significant enhancements from the out-of-plane vibrations belonging to A_2 and B_1 irreducible representations of the molecule were recorded. The apparent enhancement factor has been calculated for some selected bands, which helps us to evaluate the adsorption of PHEN on the Au@Ag surface and the orientation of the molecule with respect to the silver

shell. We find that PHEN molecules are adsorbed on the Ag shell surface through both the nitrogen atoms with the molecular plane almost perpendicular to the surface, which has been confirmed from Ag–N stretching vibration. The degrees of charge transfer in the SERS spectra of the PHEN molecule using variable core/shell ratios of the Au@Ag bimetallic nanocolloid have been reported by us. Finally, SERS spectra of the PHEN molecule have also been recorded at various adsorbate concentrations, and the high SERS sensitivity at lower concentration originating from a single or few PHEN molecules had been reported.

Substrate, surface and molecule generalities of SERS

When SERS was invented, it turned out that only a few 'free-electron-like' metals, mainly Ag, Au and Cu could provide a large SERS effect for roughened metal surfaces and colloidal metal particles with dimensions in the order of nanometres. As the SERS activity strongly depends on the nature of the substrates to be used for SERS effect, lack of suitable SERS substrates other than those mentioned above gave rise to lack of 'substrate generality' of SERS in the early 1990s. This means transition metals such as Ni, Pd, Pt, Ti, Co cannot be used as SERS substrates. Therefore, it is important to microscopically understand the origin of SERS from metal, especially metal nanoparticles. In general, electromagnetic field enhancement contributes to a large extent to the overall enhancement observed in SERS effect, which can be determined by the interaction of (incident and scattered) light and metal. It generates SPR at metal nanoparticles or surface nanostructures used as substrates. The electromagnetic field of the light at the surface can be greatly enhanced under conditions of SPR for 'free-electron' metals such as Au, Ag and Cu. The conduction electrons in these metal nanoparticles can be driven effectively by visible light to cause collective oscillation of these electrons, generating a strong electromagnetic field near the surface of metal nanoparticles. To meet the conditions of good SPR, the metal usually should have a low value of the imaginary component of the dielectric constant. This is impossible for a transition metal because in the visible light region the value of the imaginary part of the dielectric constant is large and interband excitation occurs as the Fermi level is located at the d-band. In addition, there is coupling between conduction electrons and interband electronic transitions. This can depress considerably the quality of the SPR of transition metals. Accordingly, the SERS activity of electrochemically roughened transition metal surface is in general quite low, with typical surface enhancement factor ranging from 10 to 10^3.

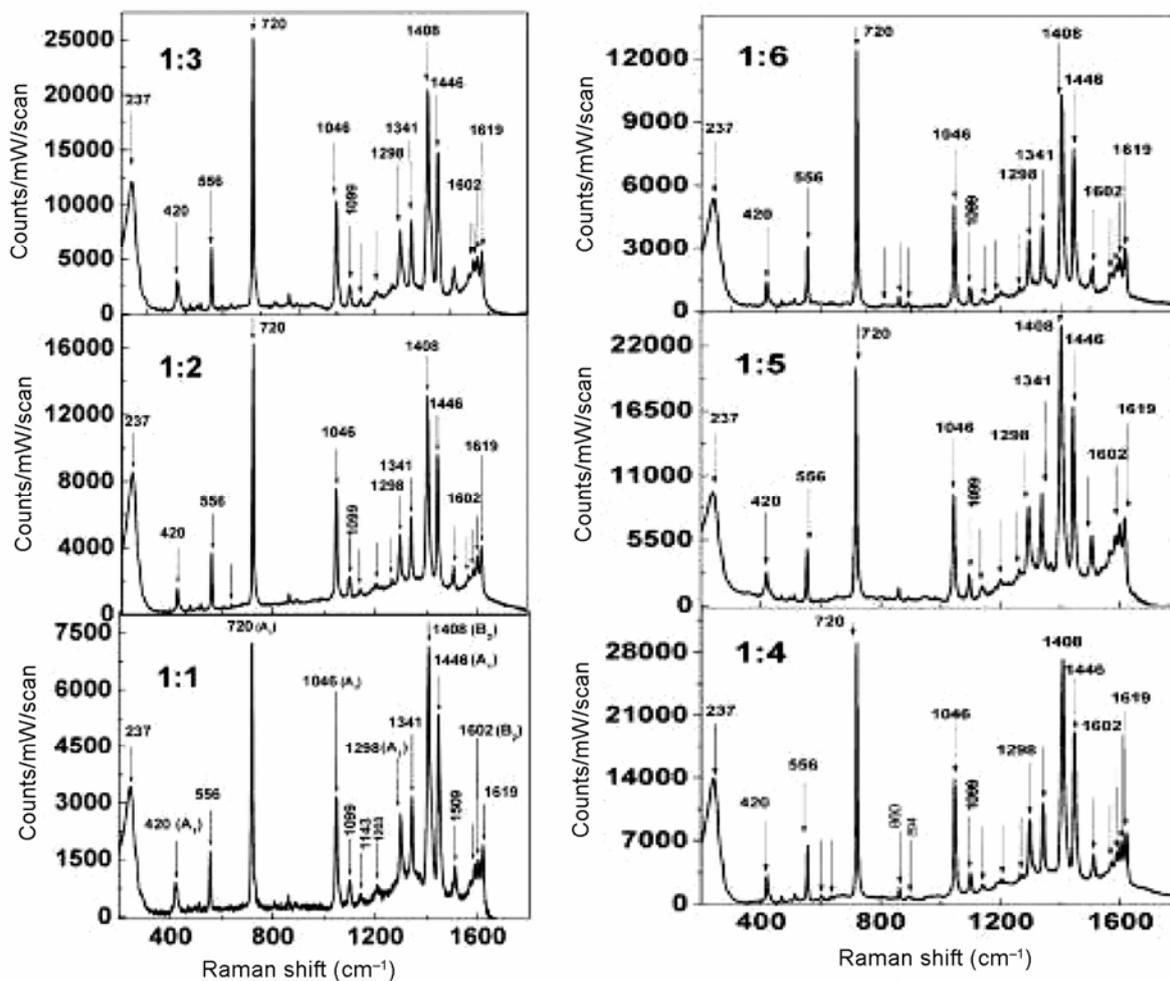When SERS was invented, it was thought that good SERS signals with large enhancement can be obtained

Figure 6. SERS spectra of PHEN molecule adsorbed on Au@Ag nanocolloid of various core/shell ratios (for a fixed Au core and variable Ag shell ratios). (Reprinted with permission from Pande et al.[16]. Copyright (2011) by American Chemical Society.)

only with electrochemically roughened metal electrodes with ill-defined surface morphology. Such surfaces are unacceptable in surface science. Therefore, surface scientists initially rejected SERS due to lack of atomically smooth single-crystal surfaces, which cannot be used as SERS substrates. However, these smooth surfaces are acceptable to them for various other surface-sensitive techniques such as STM and AFM. They termed it as the lack of 'surface generaility' of SERS. However, the extension of SERS substrates from the typical rough and ill-defined surface to the atomically flat and well-defined surface is important for SERS to develop into a powerful tool in surface science. Moreover, the well-defined single-crystal surface is completely different from the typical SERS substrates in which surfaces are ill-defined. Detailed comparison with the SERS signal from well-defined single crystal and typical rough surfaces can lead to better overall understanding of the SERS effect.

The word 'molecule generality' means getting good SERS signal and to improve the detection sensitivity of

SERS technique with a large variety of molecules. Therefore, it is challenging to obtain high-quality SERS spectra even from a single molecule adsorbed on SERS substrates. The overall improvement of SERS signal with a enhancement factor up to 10^{14} can give rise to high-quality single-molecule SERS spectra and can improve the detection sensitivity up to single-molecule level. The achievement of single molecular sensitivity of the SERS technique can improve the 'molecular generality'.

We can see that lack of substrate generality limited the practical applications of SERS in various materials, while lack of surface generality restricted SERS studies to surfaces with ill-define morphology that is unacceptable in surface science. Many groups had tried to overcome these two major limitations of SERS by obtaining SERS activity from metallic surfaces other than Ag, Au and Cu and from atomically flat (single-crystal) surfaces. However, most of those reported SERS spectra were found to be highly irreproducible. As a consequence of the lack of substrate and surface generalities and the difficulties to
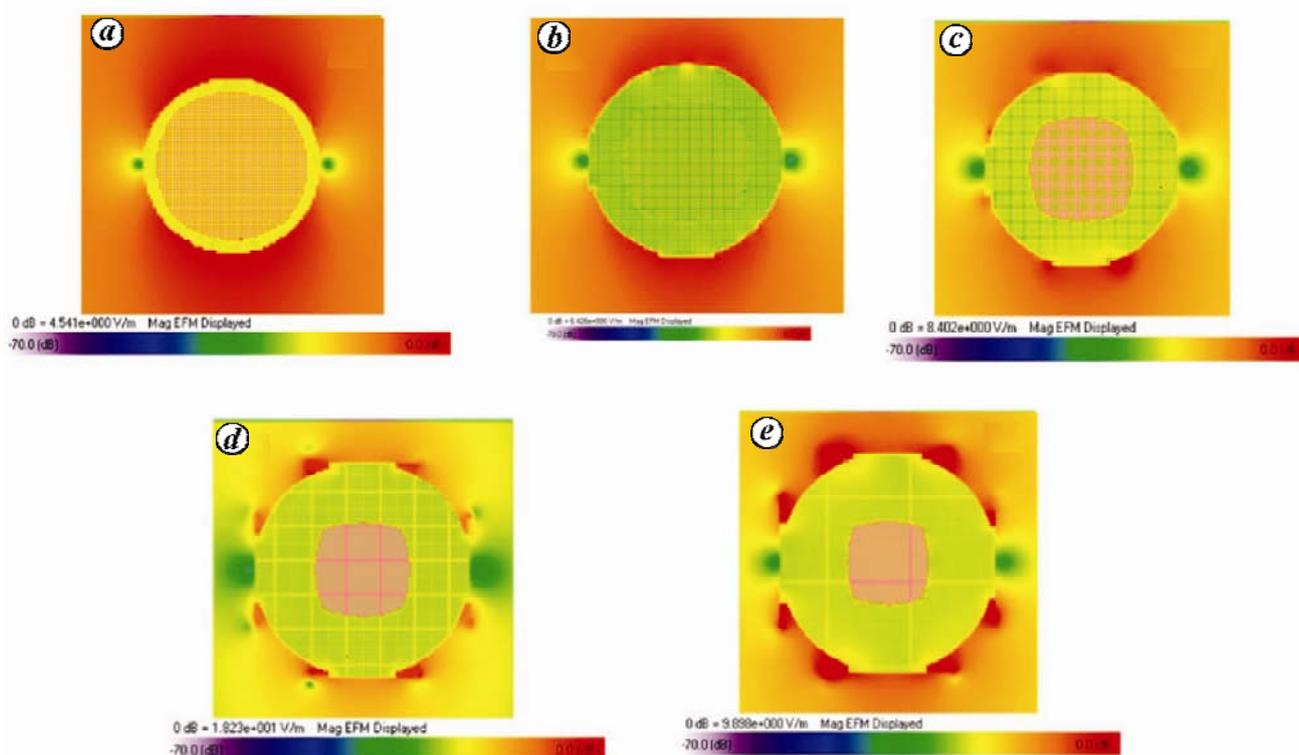
Figure 7. Spatial distribution of the electric field around the illuminated Au@Ag nanocolloid, as obtained from the 3D FDTD simulation study. (Reprinted with permission from Pande *et al.*[16]. Copyright (2011) by American Chemical Society.)

elucidate the SERS mechanism, many research groups gave up this field in the mid-1990s.

However, the rapid development of nanoscience and nanotechnology boosted the scientific community and attracted a wide interest in SERS again in the 2000s. Thus, SERS has become an important branch of nanoscience and nanotechnology. It has been found that SERS activity depends on the size, shape and aggregation of nanoparticles. Several techniques of nanoscience have been employed to fabricate and characterize SERS-related nanoparticles or nanostructures in a well-controlled fashion. Such efforts have led to the significant progress in this versatile field. High-quality SERS spectra from a single molecule adsorbed on well-characterized Au and Ag nanoparticles have been obtained with a high signal enhancement up to 10^{14}. The high-quality single molecular SERS spectra have improved the molecule generality and made SERS a promising tools for trace analysis in life and medical sciences as well as security and environment protection. In addition to this kind of sensitivity, the substrate generality of SERS has also been improved by demonstrating SERS effect directly on electrode surfaces made from transition metals such as Ni, Pd, Pt, Ti, Co and their alloys. The problem of surface generality of SERS has been solved by employing tip-enhanced Raman spectroscopy (TERS), which is described later in the article.

SERS substrates: an overview

SERS-active substrate fabrication is an important field in SERS research. The two most common SERS-active substrates are metal colloids of coinage metals like Au, Ag and Cu obtained from chemical reduction and the metal electrode surfaces roughened by one or more electrochemical oxidation–reduction cycles. The recent advancement of nanotechnology has been utilized to fabricate various nanostructures from nanoparticles to nanowires, which can be used as SERS-active substrates. The SERS substrates can be roughly classified into three categories: (1) metal nanoparticles in suspension, (2) metal nanoparticles immobilized on solid substrates and (3) nanostructures fabricated directly on solid substrates, which includes nanolithography and template-based synthesis of nanostructures. Although nanoparticles and nanoparticle film electrodes show good surface uniformity, it is difficult to control the spacing of the nanoparticles to optimize the SERS activity. Template methods can provide highly ordered SERS substrates with controlled interparticle spacing. Among various templates, nanosphere lithography (NSL) and anodic aluminum oxide (AAO) films have been most widely used for the fabrication of SERS-active substrates. In addition, Langmuir–Blodgett (LB) technique can be used to fabricate highly-ordered SERS active substrates.

Nanosphere lithography

NSL technique has been well developed by various groups[34,35]. In this technique, monodispersed polystyrene or SiO_2 nanospheres of the desired diameter are self-assembled on a clean conductive substrate such as indium tin oxide (ITO) or evaporated metal substrate over glass to form an ordered single or multi-layer colloidal crystal template or mask for metal deposition. Afterwards, a metal layer is deposited by physical vapour deposition or electrochemical deposition on the substrate with controlled thickness. It will then result in three types of structured SERS substrate: (a) physical vapour deposition on the nanosphere template leads to the formation of Ag metal 'film over nanosphere' (FON) surface, (b) the removal of nanospheres of the FON surface by sonicating in a solvent results in surface-confined nanoparticles with a triangular footprint and (c) electrochemical deposition followed by removal of spheres leaves a thin nanostructured film containing a regular hexagonal array of uniform segment sphere voids.

Ag/AAO systems for chemical and bio-sensing

Wang et al.[36] utilized porous AAO film as template to electrochemically deposit arrays of silver (Ag) nanoparticles with a precisely controlled gap up to 5 nm. This Ag/AAO system with tunable sub-10 nm interparticle gap can be used as a uniform SERS-active substrate with large enhancement factor (~10^8). The 'hot junction' present at the interparticle gap of this nanostructure-based SERS substrates can increase the SERS sensitivity (a key factor for large electromagnetic field enhancement and increased SERS sensitivity). This Ag/AAO-based SERS substrate with highly uniform and reproducible SERS signals can be used as bio/chemical sensor and concentrations up to picomolar level have been detected. This excellent SERS substrate can be further extended into single molecular regime increasing the detection limit and SERS sensitivity.

Langmuir–Blodgett technique

Chemical assembly of metal nanoparticles on solid substrates like glass, silicon and ITO can be used as ordered SERS substrates through electrostatic or chemical interaction to form an ordered layer of nanoparticles. However, this method still cannot provide totally defect-free SERS substrate over a large area of a few hundred square centimetres.

The LB technique can potentially solve this problem. The method was originally used to prepare a large-area surface film of amphiphilic molecules on solid substrates. In this method, the amphiphilic molecules are dissolved in a volatile solvent immiscible with water, and dispers-ing the solution on the surface of the water phase, a monolayer of the amphiphilic molecules will form at the air/water interface after evaporation of the volatile solvent. By changing the position of the movable barrier in the LB trough, one can change the density of the monolayer film. The film can then be deposited on the substrate by the dipping and pulling method. Similarly, a nanoparticle film can be fabricated by the LB method. At first, the nanoparticles should be modified with hydrophobic molecules and dispersed into highly volatile solvent, which is immiscible with water. By dispersion of the solution into the water phase, a layer of randomly distributed nanoparticles will be left at the interface after evaporation of the solvent. As a result of compression of the layer on moving the barrier, an ordered layer of nanoparticles will be formed on the surface. The LB technique has been shown to be a high-throughput, low-cost, rapid and easily integrated method for the controlled assembly and patterning of nanoparticles and nanowires.

Yang and his group had carried out systematic SERS studies using SERS substrate fabricated by the LB technique[37]. At present, the LB technique has been successfully utilized to fabricate most uniform SERS substrates of films of nanorods, nanowires, and spherical, cubic, cuboctahedral and octahedral Ag nanoparticles[37]. Among these, the Ag nanocube exhibits the lowest enhancement, and the octahedral nanoparticle shows the highest enhancement, up to 10^7–10^8.

Development of strategy of borrowing SERS activity

Lack of surface generality restricted SERS studies to surfaces with ill-defined morphology that is unacceptable in surface science. This can be overcome by developing a new strategy of borrowing SERS activity from atomically flat single-crystal surfaces.

Chen and Otto carried out SERS study on ultra-smooth single-crystal surfaces under electrochemical conditions using an attenuated-total-reflection (ATR) Raman cell with Otto configuration[38]. Another type of ATR Raman cell with Kretchmann configuration has also been used to obtain SERS spectra from an atomically smooth Ag(111) surface, as well as from Pt and Ni surfaces. However, there are only limited number of molecules with very weak SERS signals that have been detected by these two delicate ATR set-ups. The problem of surface generality of SERS was overcome in 2004 by the introduction of tip-enhanced Raman spectroscopy (TERS)[39]. This technique is based on the enhancement of the Raman scattering intensity (Raman signal) by combining Raman spectroscopy with SPM such as atomic force microscopy (AFM) or STM. Its central element consists of an illuminated AFM or STM tip placed in close vicinity of a substrate. The tip is operated in contact or tunnelling mode.

Upon illumination with a laser beam, the localized surface plasmons are excited in the tip–substrate gap, producing a large, local enhancement of electromagnetic field compared to the incident radiation. The technique was invented by Pettinger et al. in 2004 to probe malachite green isothiocyanate adsorbed on Au(111) surface[39]. Figure 8 illustrates the schematic diagram to explain the principle of TERS. The major drawback of TERS is that the total Raman scattering signal from the tip area is weak, thereby limiting TERS studies to molecules with large Raman scattering cross-sections. Moreover, the instrumentation of TERS technique is sophisticated and expensive for many practical applications. Recently, a new borrowing SERS approach, known as shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) has been reported[40]. In this technique, the Raman signal enhancement is provided by the gold nanoparticles with an ultra-thin silica or alumina shell. A monolayer of such nanoparticles is spread as 'smart dust' over the atomically smooth surface that is to be probed. The ultra-thin coating keeps the nanoparticles from agglomerating, separates them from direct contact with the probed material and allows the nanoparticles to conform to different contours of substrates. High-quality Raman spectra were obtained on various molecules adsorbed at Pt and Au single-crystal surfaces and from Si surfaces with hydrogen monolayers. This new technique was used in in situ probe of biological structures such as yeast cells and citrus fruits with pesticides residues. Yeast cells were selected because they are one of the most extensively studied model
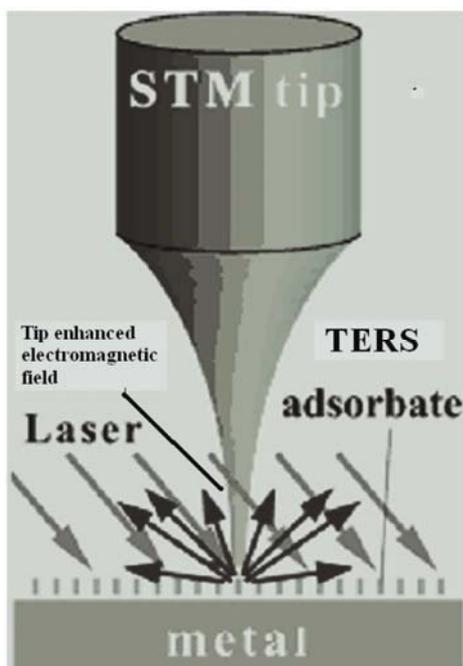

Figure 8. Schematic diagram to explain the principle of TERS. (Reprinted with permission from Sur et al.[55]. Copyright (2010) Indian Academy of Sciences).

eukaryotic organisms from genetics to biochemistry. Figure 9 illustrates the SHINERS spectra (curves I–III) from different locations of the cell wall of an yeast cell incubated with Au/SiO_2 nanoparticles. They are different from the normal Raman spectra (curve V) of yeast cells, but comparable to the SERS spectra of mannoproteins, which are considered to be the main components of the yeast cell wall. There are some other peaks attributed to amide, protein backbone and amino acid vibrations, related to the various activities of living cells such as protein secretion, movement, etc. These results demonstrate the potential of SHINERS as a safe and convenient technique for detection of cell-wall proteins and as a possible probe of the dynamics in situ and mechanisms of living systems.

Applications of SERS

SERS is among the most sensitive techniques available to surface science. It can be coupled with a wide range of other surface techniques to study various fundamental and applied research areas such as corrosion, catalysis, advanced materials, biology and sensors.

Recently, Liu et al.[41] used the SERS-active Ag/AAO system to monitor antibiotic-induced chemical changes in bacteria cell wall. The SERS profiles recorded by them are sensitive and stable and the 'chemical features' obtained from SERS spectrum of the bacterial cell wall enable rapid identification of drug-resistant bacteria within an hour. Moreover, characteristic changes in the SERS profile were noticed in the drug-sensitive bacteria during the early period (i.e. 1 h) of antibiotic exposure, which could be used to differentiate them from drug-resistant bacteria. The high-speed SERS detection represents a novel approach for microbial diagnostics. This technique was applied to a single bacterium. The high-speed SERS detection makes it possible for direct analysis of clinical specimen instead of pure cultured bacteria. It is important to mention here that conventional protocols for diagnosing bacterial infections based on the isolation of pure culture of the bacterium, followed by determination of the identity of the isolate and an examination of the responses of the isolate to various antibiotics in terms of proliferation and/or viability. For such biological assays, an incubation period ranging from days to weeks or even months is required in order for the bacteria to grow to a density that can be handled by the available diagnostic tools. Over the past decade, several PCR-based methods have been developed for the identification of bacteria. Mass spectrometry is another alternative technique which has the potential as a culture-free approach for bacterial diagnostics. However, like the PCR approach, mass spectrometry depends on the available prior knowledge on the pathogens, which may or may not exist. Lastly, yet importantly, neither the PCR or mass spectrometry based
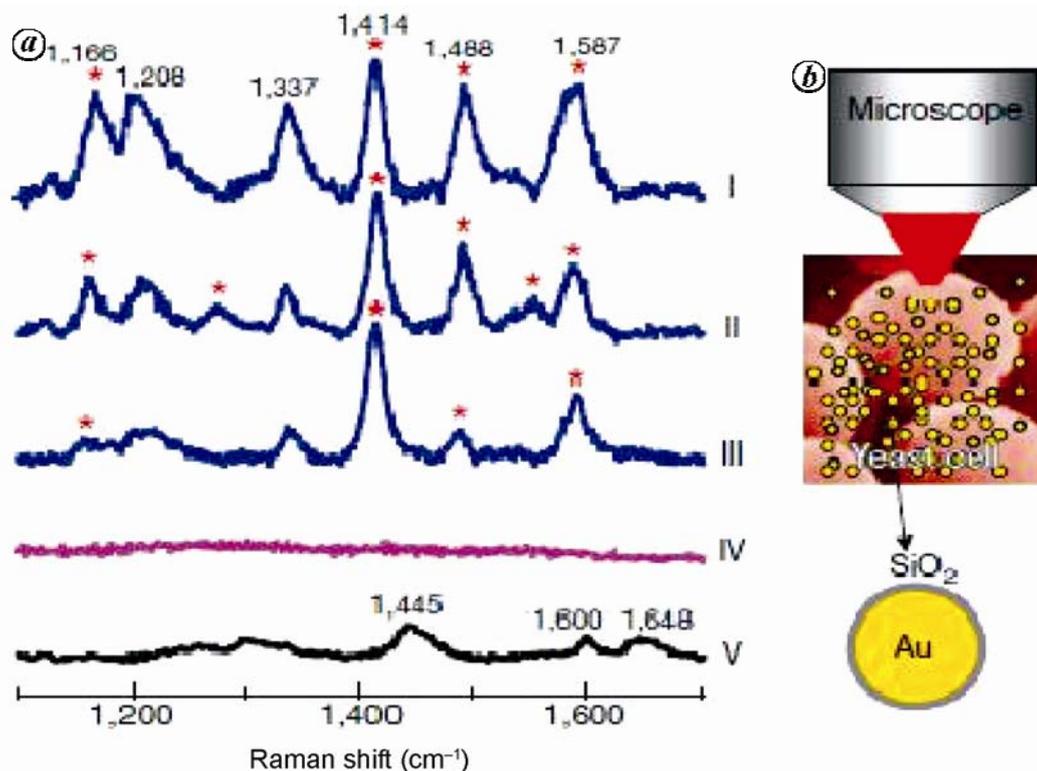
Figure 9. *a*, Curves I–III – SHINERS spectra obtained from different spots at the cell wall of an yeast cell incubated with Au/SiO$_2$ nanoparticles. Curve IV – the spectrum recorded from a substrate coated with Au/SiO$_2$, but without a yeast cell. Curve V – a normal Raman spectrum for yeast cells. The peaks marked with red asterisks are closely related to mannoprotein. *b*, Schematic of a SHINERS experiment on living yeast cells. Laser power on the sample was 4 mW. The data acquisition times were 60 s for curves I–IV and 600 s for curve V. (Reprinted with permission from Li *et al.*[40]. Copyright (2010) Nature Publishing Group.)

approach can be applied to live bacteria to monitor their responses to antibiotics or to conduct functional tests. However, SERS-based spectroscopic method solves the limitation of PCR-based methods. The newly developed SERS-active substrates based on Ag/AAO system can be used for the of fine changes in the bacterial cell wall during the different growth stages of the bacterium and its response to antibiotic treatment during early period of antibiotic exposure. Figure 10 demonstrates the SERS-based microbial diagnostics of a single Gram-positive bacterium, *Staphylococcus aureus*. Figure 10 *a* shows the individual bacteria under light microscopy as the SERS signal was recorded every 10 min for 90 min duration. It has been known that the SERS profile reflects the molecular compositions that are in close proximity with the SERS substrate, indicating that the spectra of bacteria should merely reflect the composition of the cell wall. The aromatic groups in the cell wall composition usually have relatively strong affinities towards silver nanoparticles in the SERS substrates, and are expected to be more probable candidates for SERS activity. The spectral repeatability shown here further demonstrates that these substances may not play any significant role in the observed SERS similar molecular composition, it is very

likely that these components all share similar Raman signatures. The most prominent features in the SERS spectra of Gram-positive bacteria are located at about 730 and 1330 cm^{-1}, which were assigned to the purine ring breathing mode and the C–N stretching mode of the adenine part of the lipid layer components of the cell wall. As the cell wall is made up of many components with similar molecular composition, it is very likely that these components all share similar Raman signatures. Figure 10 *b* shows the sequential SERS spectra of a single live bacterium of *S. aureus* on exposure to an antibiotic vancomycin, which is known to actively disrupt bacterial cell wall. The SERS profile indicates the susceptibility of the bacterium to the antibiotic.

Nie and Emory carried out one of the earliest experiments on single-molecule SERS by combining it with the transmission electron microscopy (TEM) and scanning tunnelling microscopy (STM) techniques[42]. This enabled them to study the relationship between the morphology of the SERS-active nanoparticles and SERS effect. They observed Raman enhancement as high as 10^{14} to 10^{15} for single rhodamine 6G (R6G) molecule adsorbed on selected Ag nanoparticles. In case of single-molecule SERS, they observed a single event rather than an ensemble-

averaged value generally obtained for conventional SERS measurements. Introduction of single-molecule SERS technique has brought a new dimension in biomedical research, with a versatile probing tool to study various biological molecules like virus, bacteria, protein, DNA and RNA.

TERS has been used to study surface reactions on single crystals and smooth surfaces, as surface roughness of the substrate does not play any role in this enhancement. The *in situ* and non-destructive nature of SERS has been utilized to study various physiological and biochemical processes *in vivo* in a living cell.

Mulvihill *et al.* demonstrated that LB assemblies of various polyhedral Ag nanocrystals can be used as highly active SERS substrates and can detect arsenate and arsenite in aqueous solutions with a detection limit of 1 ppb, which is an order of magnitude below the current standards set by the World Health Organization[43]. It is important to mention here that although the current technologies for laboratory analysis like inductively coupled plasma mass spectrometry, atomic fluorescence spectro-

scopy, and HPLC-mass spectrometry can be applied to detect arsenic below 10 ppb level, none of these techniques is readily available in the developing countries like India, Bangladesh, China and Thailand, where low-level arsenic contamination of drinking groundwater presents an public health crisis. In addition, on-site field detection is important for the reliable detection of arsenic in groundwater. There are no examples of chemical indicators available in the literature which can distinguish the two distinct oxidation states of the arsenic species. For exposure studies, this knowledge is necessary for toxicology, remediation and monitoring of the effects within the local populations. By developing a highly SERS-active substrate which can be used in combination with portable Raman technology, many of these challenges can be surmounted. Figure 11 demonstrates the SERS spectra indicating the chemical sensitivity and selectivity of PVP-coated silver nanocrystal arrays used as SERS-active substrate to distinguish between the two most common oxidation states of arsenic: arsenate (As^V) and arsenite (As^{III}). For an aqueous mixture of both arsenate and arsenite, both molecules can be detected simultaneously. A peak at 750 cm^{-1} corresponds to the (A_1) symmetric As–O stretching mode for arsenite, while the same stretching mode of arsenate appears at 800 cm^{-1}. The spectra were obtained for 18 ppb arsenate and arsenite solutions. Low arsenate concentrations (18 ppb) can be also detected even in the presence of high concentration of sulphates (10 mM) and phosphates (1 mM) using the SERS-based technique.

The SERS substrate can be used as a chemical sensor, which is robust, reproducible, highly portable and can be
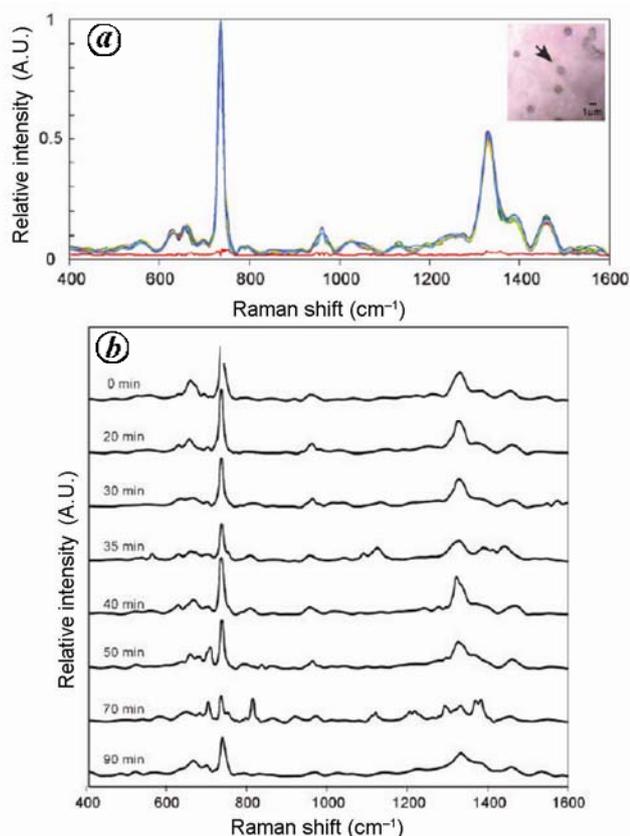

Figure 10. SERS-based microbial diagnostics of a single bacterium. *a*, Single *Staphylococcus aureus* under light microscopy (inset, arrow) was subjected to SERS detection every 10 min for 90 min (various coloured traces); the standard deviation among all recordings along the spectrum is shown in red. *b*, Sequential SERS spectral evolution of a single live bacterium of *S. aureus* on exposure to vancomycin, an antibiotic which is known to actively disrupt bacterial cell wall. (Reprinted with permission from Liu *et al.*[41]. Copyright (2009) *PloS One*.)
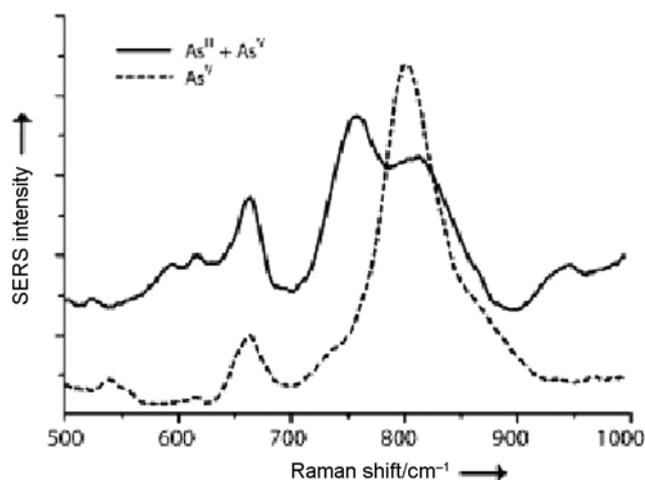

Figure 11. SERS spectra indicating the chemical sensitivity and selectivity of PVP-coated silver nanocrystal arrays used as SERS-active substrate to distinguish between the two most common oxidation states of arsenic: arsenate (As^V) and arsenite (As^{III}). The spectra were obtained for 18 ppb arsenate and arsenite solutions. (Reprinted with permission from Mulvihill *et al.*[43]. Copyright (2008) Wiley Interscience.)

easily implemented in field detection. SERS technique can be used in environmental analysis. The target molecules analysed by SERS include pesticides, herbicides, pharmaceutical chemicals in water, banned food dyes, aromatic chemicals in regular aqueous solutions and in sea water, chlorophenol derivatives and amino acids, chemical warfare species, explosives, and various organic pollutants.

Metal nanoparticles immobilized as SERS substrates can be used in biomedical diagnostics. For example, the SERS substrate can be used as a glucose sensor to detect glucose in blood. Although glucose is most commonly monitored by electrochemical-based sensors, an alternative protocol using SERS substrates prepared by NSL has been devised for the detection of glucose in blood[44]. In this protocol, the SERS-based glucose sensor was developed by growing a silver film over nanospheres (AgFON) surfaces prepared by NSL. However, glucose-sensing on a bare AgFON surface was unsuccessful. The inability to observe glucose signal on a bare AgFON surface can be attributed to the weak affinity of glucose molecule to the silver surface. To bring glucose within the range of electromagnetic enhancement of the AgFON surface, a self-assembled monolayer (SAM) can be formed on its surface to partition the analyte of interest, in a manner analogous to that used to create the stationary phase in high-performance liquid chromatography. Several SAMs were tested for their ability to partition glucose efficiently to the AgFON surface. Among these, straight-chain alkanethiols and ethyleneglycol-terminated alkanethiols partitioned glucose most effectively. However, decanethiol (DT) produced a hydrophobic surface, and partitioning glucose in an aqueous phase was not feasible. On the other hand, although tri(ethylene glycol)-terminated alkanethiol (EG3) partitioned glucose in a phosphate buffer environment, the complicated synthesis of this compound limited its availability. A new type of mixed SAM, based on two commercially available components, DT and mercaptohexanol (MH), produced an efficient partition layer for the SERS-based glucose-monitoring device. The key to detection of glucose by SERS was surface chemistry. The SERS substrate was modified with an alkanethiol partition layer to facilitate glucose adsorption to the metal surface.

SERS of anti-carcinogenic drug species and anthralin molecules at trace concentrations close to that encountered under physiological conditions in living systems has been reported with the aid of DFT and *ab initio* calculations[45,46]. The applications of *ab initio* and DFT calculations in general have enormously enriched the understanding of the molecule–metal interactions and CT contributions to SERS. Moreover, these calculations provide the structural details of free and adsorbed molecules, which allows one to extract maximum information from the SERS spectra.

Future prospects of SERS research

The remarkable works of Nie and Emory[42] and Kneipp *et al.*[47], later corroborated by the works of Xu *et al.*[48] and Michaeles *et al.*[49] have recently brought about a renaissance of interest in SERS research. They have reported that under appropriate circumstances, SERS enhancements as large as 10^{14} can be achieved. These studies indicate that special sites, sometimes referred to as 'hot spots', are considered to be responsible for a large portion of the observed enhancement.

On the basis of these considerations, a great deal of the current research effort in SERS is focused on the controlled and reproducible fabrication of metallic nanostructures that produce hot geometries where the molecules are appropriately and predictably located for giant Raman enhancement. With the above potential prospects of the proposed research field in mind and with the expertise we have achieved for more than a decade[11,15–17,50–55], our research group is geared up for further research in this exciting area of science. However, SERS is not a quantitative technique and limits its application in kinetic studies. Therefore, SERS cannot be used to obtain kinetic data of a chemical reaction, which can be further overcome by the use of time-resolved (TR)-SERS technique. Lombardi and his group are extensively using the TR-SERS technique[56] to study the CT reaction. However, the technique is not as popular as the ordinary steady-state SERS.

Conclusion

Although the theory to quantify the exact mechanism of SERS effect is yet to be developed, 30 years of using this versatile technique has reached a new height due to the increased efficiency of the modern Raman instrumentation and recent advancement in nanotechnology[55]. Thus, SERS offers a means to overcome the obstacles of sensitivity and surface/substrate selectivity, allowing one to obtain surface vibrational data *in situ* in diverse environments such as air, ultra-high vacuum and electrochemical environments. The SERS effect makes it possible as an *in situ* diagnostic probe for determining the detailed molecular structure and orientation of surface species applicable to electrochemical, biological and other ambient interfaces. Though considerable amount of work is being carried out throughout the world in this exciting area of research, controlled and reproducible fabrication of SERS-active substrates and understanding the detailed relationship between the nanoparticle structure and SERS activity remains significant challenges in this field of research.

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