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Surface-Enhanced Raman Scattering

Ujjal Kumar Sur

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Abstract

The steady and fast development of surface and interfacial science have set up innovative openings for new diagnostic probes for analytical characterization of the adsorbates and determination of the microscopic structure of surfaces and interfaces. Regrettably Raman spectroscopy, being a weak scattering surface phenomenon, had seized no part in it, until the discovery and development of Surface Enhanced Raman Scattering (SERS) in the early 1970's that has opened up broad research fields both in the physics and chemistry of interfaces. The discovery of SERS by Fleischmann and coworkers in 1974 at the University of Southampton, United Kingdom is closely connected with the electrochemical systems. They reported an extraordinary million-fold enhancement of weak Raman signal from pyridine molecules adsorbed onto electrochemically roughened silver electrode compared to that from free molecules in liquid environment. In early 1976, Richard P. Van Duyne and David Jeanmaire at Northwestern University observed the effect and in early 1977, M. G. Albrecht and J. A. Creighton reported similar observation. This review article deals with the development of SERS research with special importance is given to the fabrication of various SERS-active substrates, mechanism of SERS effect and its various potential applications ranging from sensors to biomedical applications.

Keywords: Raman scattering, surface-enhanced Raman scattering, electromagnetic (EM) enhancement effect, hot spots, sensors, SERS active substrates

1. Introduction

Raman scattering arises as a result of interaction of electromagnetic radiation with matter resulting in the alteration of frequency or wavelength of the incident radiation. With the invention of strong, monochromatic, polarized and tunable lasers, the Raman spectroscopy has grown as a highly sensitive technique to probe structural details of a complex molecular structure. However, the applications of traditional or conventional Raman spectroscopy are restricted by the low scattering cross section involved with the Raman scattering process,

which are ~ 12–14 orders of magnitude lower than the fluorescence cross section for various biological and organic molecules, which are highly fluorescent in nature [1–7]. Therefore, the discovery of Fleischmann and coworkers from the University of Southampton, UK in 1974 [8], which demonstrated the unexpected high Raman signals obtained from pyridine molecules adsorbed on a rough silver electrode, has attracted considerable attention of researchers from various fields such as physics, chemistry, biology, mathematics, and engineering. In a published paper, Fleischmann et al. reported an extraordinary million-fold enhancement of Raman signal from pyridine molecules adsorbed onto electrochemically roughened silver electrode compared to that from free molecules in a liquid environment [8]. Surface-enhanced Raman scattering (SERS) effect deals with the gigantic amplification of the weak Raman scattering intensity by molecules in the presence of a nanostructured metallic surface [5–8]. The SERS enhancement factor can be defined as the ratio between the Raman signals obtained from a given number of molecules in the presence and in the absence of the metal nanostructure and this factor is dependent largely on the size and morphology of the nanostructures. In general, SERS enhancement value is around 10^6, but it may reach value as high as 10^{10} at definite highly effective subwavelength regions of the surface [5–8].

Since its discovery in the year 1974, surface-enhanced Raman scattering (SERS) has attracted significant interest of researchers [1–7]. The discovery of SERS has opened up a promising way to overcome the low-sensitivity problem associated with conventional Raman spectroscopy. Introduction of the SERS technique not only improves the overall surface sensitivity making Raman spectroscopy more applicable but also stimulates the study of the interfacial processes involving enhanced optical scattering from adsorbates on metal surfaces [9].

This review article covers the current development in SERS research along with brief discussion on the fabrication of various SERS active substrates, the various theoretical explanations of the mechanism of SERS effect and its various diverse applications in sensing, diagnostics, and catalysis. The article first deals with a short historical assessment of the SERS effect, followed by an overview on the preparation of various SERS active substrates. The article concludes with the citations of some recent applications of SERS from the literature. Due to insufficiency in space, a comprehensive review of all current work based on SERS is impossible. However, we have summarized a few representative examples including our own results to demonstrate the recent advancement in the SERS research.

2. Historical background and gradual development of SERS

Raman spectroscopy is a spectroscopic technique based on molecular vibration and is dependent on the inelastic scattering of monochromatic light, usually from a laser in the visible, near-infrared, or near-ultraviolet range of electromagnetic spectra. This effect was discovered by famous Indian physicist Professor C.V. Raman in 1928 [10].

Figure 1 shows the schematic diagram to explain the principle of Raman scattering. The weak Raman signal observed in conventional Raman spectroscopy can be explained by the low scat-

tering cross section (~10^{-30} molecule cm^{-2}). Therefore, Raman spectroscopy will provide low sensitivity in terms of signal and is the main reason for its inapplicability in practical fields for a long period [5–7]. For an extremely low scattering cross section in the range 10^{12}–10^{14} molecule cm^{-2}, usually present in a monolayer, it is very difficult to detect signal obtained from a Raman probe molecule even by the most effective modern and sophisticated Raman spectrometer. Raman spectroscopy is competent enough to obtain fingerprint information of species by detecting the vibrational bands. In Raman spectroscopy, the sample containing the Raman probe molecule is illuminated with a laser beam of appropriate wavelength. Wavelengths close to the laser line arises as a result the Rayleigh scattering is filtered out, while the rest of the unfiltered light is dispersed onto a detector. Spontaneous Raman scattering is very weak as compare to the Rayleigh scattering and identification and partition of these two signals are important to obtain a high quality Raman spectrum. Traditionally, holographic diffraction gratings are employed in Raman spectrometers to yield a high degree of laser rejection. On the other hand, modern instrumentation unanimously employs notch filters for laser rejection. Development of modern instrumentation along with the introduction of fast-Fourier transform (FFT)-based spectrometers, confocal microscopes, and charge coupled device (CCD) detectors has brought a new dimension in Raman instruments, providing very high sensitivity. **Figure 2** demonstrates the development of Raman instrumentation from the Raman spectrometer of C. V. Raman to the latest sophisticated Horiba-Jobin model of micro-Raman system.

However, the intensity of Raman signal obtained from most of the systems is very weak and is only about 10^{-10} times the intensity of the incident laser. Fleischmann and his group from the University of Southampton, UK, carried out Raman spectroscopic study with expected high intensity of signal by increasing the number of adsorbed molecules on a roughened metal electrode surface. In 1974, they reported very high quality Raman spectra of pyridine molecule (Raman probe molecule with high scattering cross section) adsorbed on electrochemically roughened Ag electrodes [8]. The authors attributed the enhancement in the Raman intensity to an increase in the surface area of the Ag electrode by the electrochemical
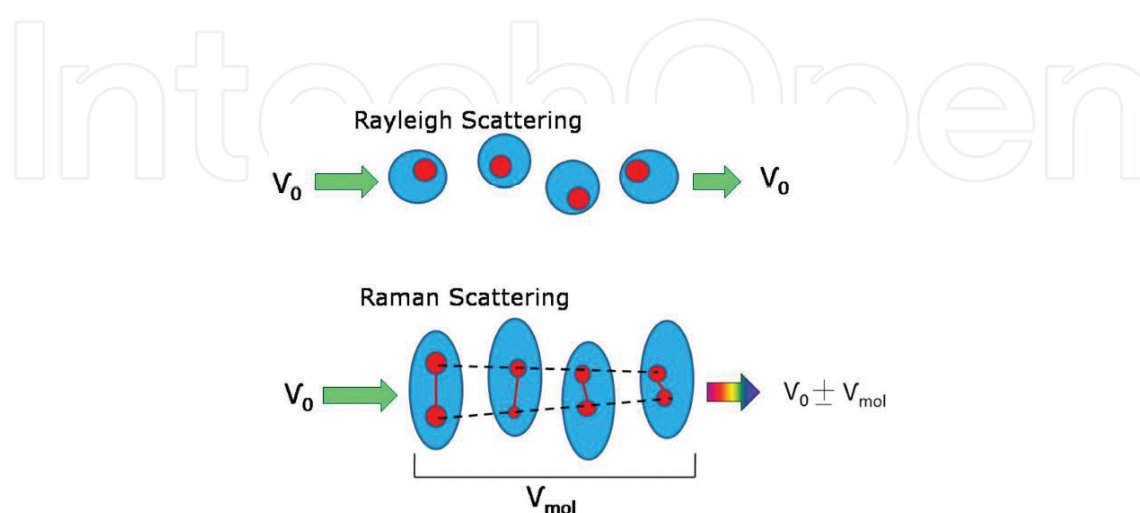
Figure 1. Schematic diagram to explain the principle of Raman scattering and Rayleigh scattering.

Figure 2. The development of Raman instrumentation from the Raman spectrometer of C. V. Raman to the latest sophisticated Horiba-Jobin model of the micro-Raman system.

roughening method. **Figure 3** illustrates the schematic diagram to explain the principle of SERS. The technique is so sensitive that even single molecule can be detected.

Figure 4 shows the photograph of Fleischmann, who invented SERS. Fleischmann, Hendra, and Mcquillan of University of Southampton, UK, discovered surface-enhanced Raman scattering (SERS) spectroscopy by chance when they tried to carry out Raman study with pyridine (Py) molecule having very high Raman cross section on the roughened silver (Ag) electrode [8]. The spectra were found to be dependent on the applied electrode potential.
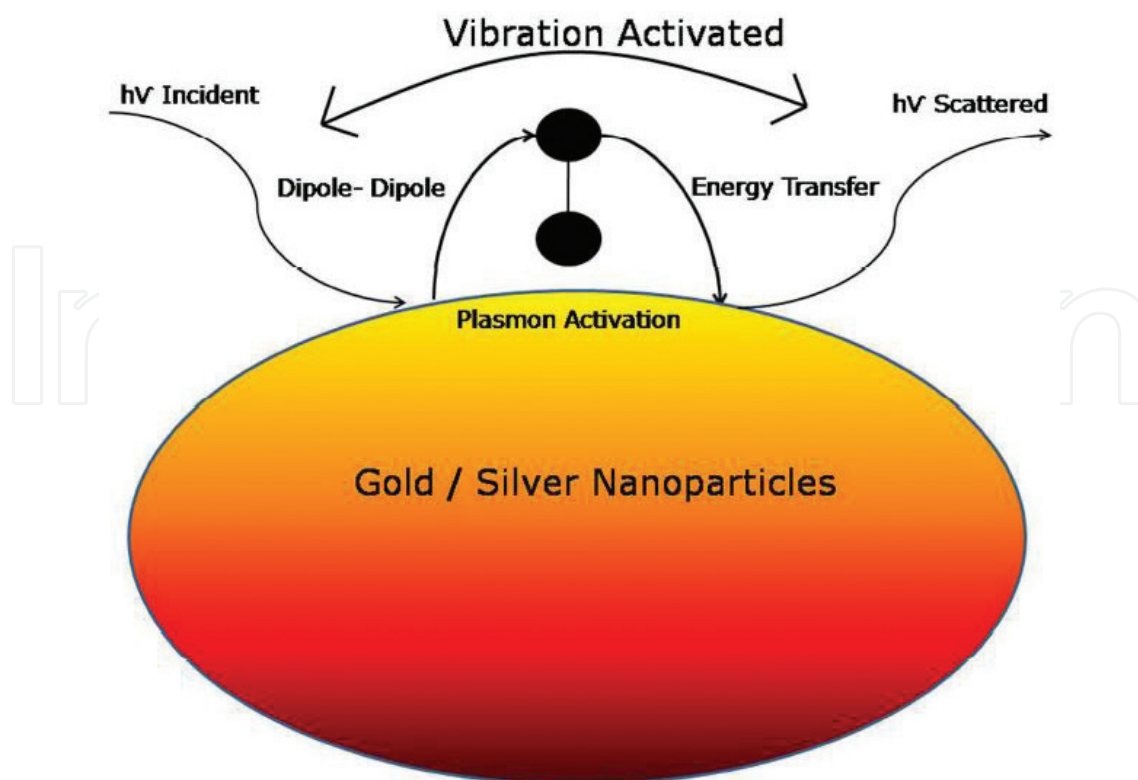
Figure 3. Schematic diagram to explain the principle of SERS.

Initially, it was thought that an increase of surface area to be responsible for the enhancement of Raman signal. Afterward, in 1977 Jeanmaire and Van Duyne [11], from Northwestern University, USA, first realized that the surface area is not the key point in the above phenomenon. Albrecht and Creighton [12] of University of Kent, UK, reported a similar result in the same year. Both the groups independently supported enough proofs to exhibit that the strong surface Raman signal must be created by an authentic augmentation of the Raman scattering efficiency (10^5 to 10^6 enhancement). Later, this effect was referred as surface-enhanced Raman scattering and now, it is a unanimously acknowledged surface analytical technique. In spite of the fact, the first SERS spectra were obtained employing an electrochemical system (Py + roughened Ag electrode), all significant reactions occurring on various surfaces like metal and semiconductors can be investigated by the SERS technique. The technique is so sensitive that even single molecule can be detected in addition to various electrochemical processes.

The precise mechanism responsible for the enhancement effect observed in SERS is still highly controversial as found from the literature. There are two major mechanisms that are responsible for the large enhancement of weak Raman signal obtained from pyridine molecules adsorbed on electrochemically roughened Ag surface. Jeanmaire and Van Duyne first proposed a theory based on the electromagnetic effect responsible for the enhancement of Raman signal [11]. This is known as the electromagnetic theory of SERS effect and is based on the excitation of localized surface plasmons (LSP). Albrecht and Creighton first proposed a theory

based on the charge transfer effect of the adsorbed molecule on the enhancement of Raman signal [12]. This is known as the chemical enhancement. This chemical enhancement theory depends on the charge transfer complex formation of the adsorbed molecule by absorption of photon of the suitable wavelength. Nevertheless, it is extremely complicated to separate these two effects experimentally and understand the overall mechanism of SERS.
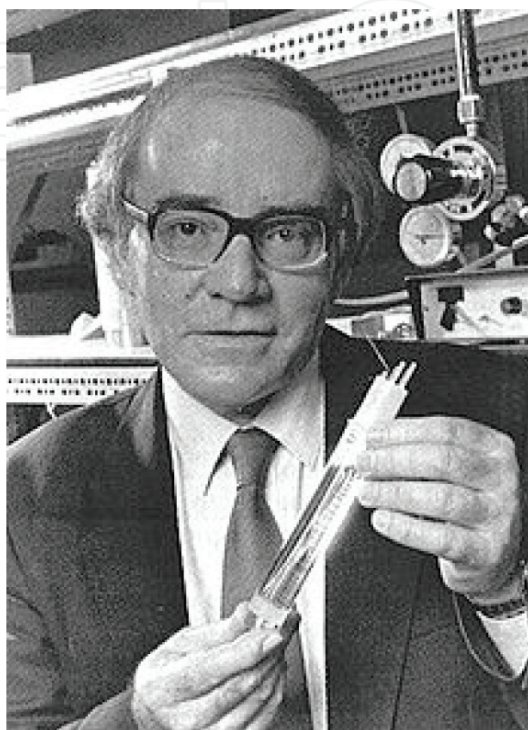
Figure 4. The photograph of Fleischmann, the inventor of SERS.

In the mid-1980s, the spotlight on SERS research diverted to the exploitation of SERS effect for new and novel analytical and biological applications from the basic understanding of the mechanism responsible for the SERS phenomenon. However, it was extremely difficult to record high-quality, highly reproducible and stable SERS spectra as demonstrated by some of the investigations carried out in the mid-1980s as well as the early 1990s. The SERS spectra obtained were highly irreproducible, which can be explained by the small variations in the fabrication of SERS substrates. This shortcoming has prevented the development of SERS as a quantitative tool for long period. For that reason, fabrication of an SERS active substrate is very important in SERS research so that highly uniform, stable, and highly reproducible SERS signals can be obtained.

In the mid-1990s, VIIIB transition metals were employed as SERS active substrates to carry out electrochemical surface-enhanced Raman spectroscopy (EC-SERS) and these SERS substrates were further utilized for electrochemistry and catalysis [13]. Professor Tian and his coworkers at the Xiamen University, China, first introduced quite a few surface roughening procedures and demonstrated that the SERS effect can be directly obtained from transition metals such

as pure Pt, Ru, Rh, Pd, Fe, Co, and Ni electrodes with a surface enhancement factor in the range between 1–3 orders of magnitude [14]. Since the early-2000s, the randomly roughened surfaces were replaced by the well-controlled nanostructures of both coinage (e.g., Au, Ag, and Cu) and transition metals due to the gradual and rapid development of nanoscience and nanotechnology. These nanostructures were considered as a very promising class of excellent SERS-active substrate. Up to now, molecular-level investigations by Raman spectroscopy on diverse adsorbates at various electrodes had been carried out.

The next major landmark in the field of SERS research was the observation of SERS spectra from single molecules (SM-SERS) by two independent research groups in the year 1997 [15, 16]. The detection of single molecules using the SERS technique and attainment of ultimate limit of detection in any analytical detection was possible by combining other techniques, for instance, fluorescence spectroscopy and scanning tunneling methods along with SERS technique.

Under suitable conditions, SERS enhancements in the order of 10^{14} can be obtained. It is important to mention here that special sites, sometimes referred to as "hot spots," are responsible for the observed enhancement in the SERS effect to a large extent. On the basis of these considerations, a great deal of the current research work in SERS is focused on the controlled and reproducible fabrication of metallic nanostructures which can create hot geometries like "hot spots" where the Raman probe molecules are correctly and inevitably located for gigantic Raman enhancement. This will provide new information in novel research areas like plasmonics.

3. SERS active substrates

The metals that are selected to be used as an SERS active substrate can be determined by the plasmon resonance frequency. Both, visible and near-infrared radiation (NIR) can generally be used for excitation of the Raman modes. Silver (Ag), gold (Au), and copper (Cu) are typically utilized for carrying out SERS experiments because their plasmon resonance frequencies are in the region of above-mentioned wavelength of electromagnetic spectrum, providing maximal enhancement for visible and NIR light [5–7]. Recently, transition metals such as platinum (Pt), ruthenium (Ru), palladium (Pd), iron (Fe), cobalt (Co), and nickel (Ni) have been utilized as SERS active substrates. These transition metals can display enhancements between 1–3 orders of magnitude and these enhancement factor values are very low compared to the enhancement factors obtained for metals such as Au and Ag. This can be explained by the fact that excitation of the surface plasmon resonances in the visible light region is extremely difficult. Nevertheless, large enhancement (~ 10^4) values can be obtained from the transition metals using excitation wavelength in the near ultra-violet (UV) region. The major features of the SERS technique are abridged briefly as follows:

- SERS is highly surface sensitive, nondestructive and *in-situ* vibrational spectroscopic analytical technique.

- SERS occurs as a result of bringing the Raman probe molecules closer within the few nanometers of the surface of SERS substrates of different morphologies.

- The SERS technique will exhibit exceptionally high spatial resolution, in which the enhancement range is several nanometers, effective for one or several molecular layers close to the SERS active substrate.

- SERS activity is strongly dependent on the type of metal and surface roughness of the SERS active substrate employed for the study.

Therefore, the fabrication of an SERS active substrate is a very important field from the point of view of SERS research. The two most commonly used SERS substrates are metallic colloids of Au, Ag, and Cu obtained from chemical reduction and the metal electrode surfaces roughened by one or more electrochemical oxidation-reduction cycles. Surface and substrate generality are the major limitations associated with the SERS effect and many research groups all over the world have tried to surmount these two major problems by obtaining SERS activity from other metallic surfaces other than Ag, Au, and Cu and from atomically flat (single crystal) surfaces rather than roughened surfaces. However, most of the metals used as SERS substrates will exhibit poor biological compatibility. For that reason, it is essential to provide new novel substrates for SERS study. For an ideal SERS substrate, the material should be economical, easily accessible, chemically inert, as well as biocompatible.

The SERS substrates can be approximately divided into three categories: (1) metal nanoparticles (MNPs) in suspension, (2) metal nanoparticles immobilized on solid substrates, and (3) metal nanostructures fabricated directly on solid substrates by nanolithography and template based synthesis [7, 17]. Although both nanoparticles and nanoparticle film electrodes can exhibit good surface uniformity, as a result difficulty in controlling the spacing of the nanoparticles will not be able to optimize the SERS activity. Only template-based fabrication methods can be employed to obtain highly ordered SERS substrates with controlled interparticle spacing. Amid different template-based methods, nanosphere lithography (NSL), and anodic aluminum oxide (AAO) films are most commonly employed to fabricate highly ordered SERS active substrates. The Langmuir-Blodgett technique can also be utilized to fabricate highly ordered SERS active substrates. **Figure 5** illustrates the different SERS active substrates used in recent years. As a result of fast advancement in nanoscience and nanotechnology, several methods are accessible now for the fabrication of various metallic nanostructures with different size, shape, which can be further utilized as SERS active substrates. This has facilitated in a significant enhancement in the citations of new SERS active substrates available in the literature in the last 5 years and we are expecting a further improvement in the upcoming years.

Metal nanoparticles in suspension are the simplest of all SERS active substrates used so far, where the SERS effect can be studied in the presence of definite concentration of analytes (Raman probe molecules). However, aggregation of metallic nanoparticles can prevent to obtain highly reproducible SERS spectra. Alternatively, aggregation is sometimes essential for obtaining high quality, highly reproducible SERS signal [15]. MNPs suspension must be mixed with the analyte solution for carrying out the SERS experiment, a sampling require-

ment that might be limiting for a few real-life applications, such as quantitative analysis of adsorbates on nonSERS active surfaces like semiconductors and fruits. In spite of the problems such as reproducibility in experimental results and potential sampling, MNPs suspensions are extensively employed as an SERS active substrate due to their high SERS-performance, good stability, and simplicity in production. Actually, this kind of substrate was employed in the early years for carrying out single molecule SERS experiments. The drawback of sampling has been recently overcome by Professor Tian and his research group at the Xiamen University, China [18]. They introduced a completely original shell-isolated MNPs as an SERS enhancing smart dust, which was successfully employed for probing hydrogen adsorbed on the single crystal Pt surface, and direct detection of pesticides residues in the form of contamination in citrus fruits such as oranges. This new borrowing SERS technique is referred by them as shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) [18].
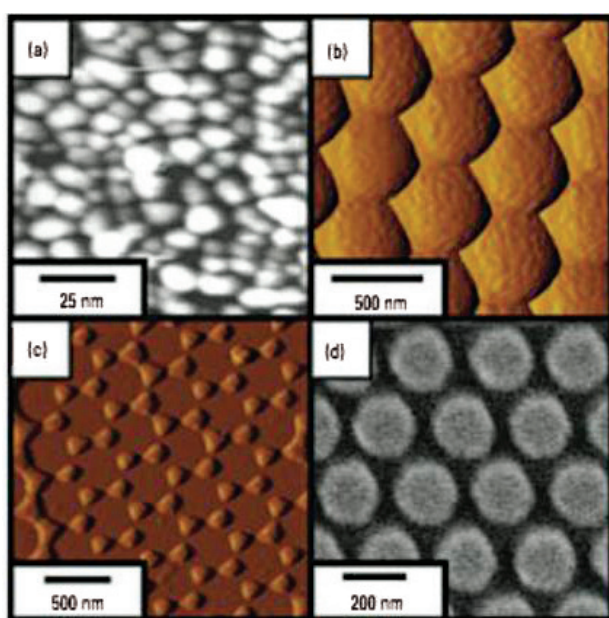
Figure 5. The different SERS active substrates fabricated by nanotechnology. Reproduced with permission from Haynes et al. [36]. Copyright @ American Chemical Society. (a) Rough silver film on glass; (b) Silver coating on top of PS beads; (c) Nanosphere lithography; (d) Silver column produced by e-beam lithography. after the word by nanotechnology

For crucial evaluation, the SERS substrates covered in this review article can be limited to only three general types of substrates, classified according to their fabrication technique: (1) MNPs immobilized in planar solid supports, (2) metallic nanostructures fabricated using nanolithographic methods, and (3) metallic nanostructures fabricated using template-based techniques.

The dispersed and aggregated MNPs cannot be used as a SERS active substrate in real analytical problems as a result of the poor reproducibility of SERS enhancement factor, which can be solved by immobilizing the MNPs on some kind of solid support. Since the first report of an SERS substrate consisting of MNPs synthesized by a wet chemical method and afterward immobilizing them onto a solid support [19], the process has become extremely popular and several papers have been published based on this process as found from the literature survey.

The top-down nanolithography and associated nanoimprint lithographic-based fabrication techniques were employed to fabricate highly ordered metallic nanostructures array. In this technique, a layer of polymeric photoresist (positive or negative) is cast on the solid substrates (such as Si, glass, or Au film). It was followed by direct patterning on the photoresist surface or indirect patterning with the assistance of a mold using ultraviolet (UV) light, an electron beam, or a focused ion beam. Afterward, the residual photoresist can be utilized as a mold, on which SERS-active metals are deposited by a physical vapor deposition technique under vacuum conditions. The mold was lifted off and a highly ordered nanostructured SERS-active substrate with a structure identical or complementary to that of the mold is formed. Highly ordered and uniform SERS active substrates with interparticle spacings below 10 nm can be produced by employing the nanolithographic method with a broad variety of shapes and geometries. However, nanolithographic techniques are still time-consuming and very expensive due to the use of high energy focused ion beam (FIB) or electron beam (EB) for the fabrication of the SERS substrate with a large area. Both, FIB and EB lithographic techniques can be employed to make molds for the nanoimprint lithography technique. In the nanoimprint lithographic technique, the desired nanopatterns are produced by direct writing on the Si or quartz slide by using an electron beam, which can be subsequently used as the mold. Next, the mold is aligned and pressed into the photoresist covering on the substrate and finally, the mold is lifted off after curing. Subsequently, the substrate is deposited with the desired metal to be used as an SERS active substrate. A highly ordered nanostructure with good SERS activity can be fabricated by completely removing the photoresist. Nanoimprint lithographic techniques are more efficient and inexpensive as compared to nanolithographic techniques.

Van Duyne and his group developed the nanosphere lithographic technique in 2001 [20, 21]. In this technique, highly ordered single or multi-layer colloidal crystal templates are produced from the self-assembly of monodispersed polystyrene or SiO_2 nanospheres of the desired diameter on clean conducting substrates such as indium tin oxide (ITO) or evaporated metal substrate over glass. Afterward, a metal layer is deposited by the physical vapor deposition or electrochemical deposition method on the substrate with a controlled thickness. Three types of structured SERS substrate can be produced by the nanosphere lithographic technique: (1) Ag metal "film over nanosphere" (FON) surfaces are formed due to physical vapor deposition on the nanosphere template; (2) surface confined nanoparticles with a triangular footprint are produced by the removal of nanospheres of the FON surfaces by sonication in a solvent; (3) thin nanostructured films containing regular hexagonal array of uniform segment sphere voids are formed by electrochemical deposition followed by removal of spheres. One can control the shape, size, and interparticle spacing of the fabricated nanostructures by tuning the size of the nanospheres and the thickness of the deposited metal with the ultimate objective that the localized surface plasmon resonance (LSPR) position can be adjusted to match the excitation wavelength with an optimized SERS enhancement.

Arrays of silver (Ag) nanoparticles with a precisely controlled gap up to 5 nm are electrochemically grown by Wang et al. [22] by utilizing the porous AAO film as a template material. This Ag/AAO system with tunable sub-10 nm interparticle gap can be further utilized as highly ordered, uniform SERS active substrate with high value of SERS enhancement factor (~10^8). It is extremely difficult to precisely control of interparticle gaps between nanostructures on an SERS-active substrate in the sub-10 nm regime as known from the

existing literature. Such studies are crucial for the fabrication of SERS active substrates with uniformly high enhancement factors, and for overall understanding of collective surface plasmons existing inside the gaps. The "hot junction" or "hot spot" located at the inter-particle gap of these nanostructure-based SERS substrates can enhance the SERS activity, which is an important aspect for large electromagnetic field enhancement and excellent SERS sensitivity. Wang et al. experimentally demonstrated the first quantitative study of the collective SERS effect on a substrate with precisely controlled "hot junctions" in the sub-10 nm regime, and confirmed the theoretical prediction of interparticle-coupling-induced Raman enhancement. This Ag/AAO-based SERS substrates fabricated by Wang et al. [22] with highly uniform and reproducible SERS signals can be utilized as both a biosensor and a chemical sensor with extremely high sensitivity. Using these SERS substrates, concentration up to picomolar level has been detected. This excellent SERS substrate can be further exploited for single molecule SERS study increasing the overall detection limit and SERS sensitivity.

The Langmuir-Blodgett (LB) technique can be employed for the large-scale production of a fully defect-free SERS substrate over a large area of few hundred of cm^2. The LB method was initially employed to form a large-area surface film of amphiphilic molecules on solid substrates. In this procedure, the amphiphilic molecules such as stearic acid is dissolved in a volatile solvent like benzene or chloroform, which is completely immiscible with water, and soon after, the solution is dispersed on the surface of the water phase. As a result, a mono-molecular film of the amphiphilic molecule will form at the air/water interface with complete evaporation of the volatile solvent. The film can be deposited on the substrate by the dipping and pulling method. Similarly, a nanoparticle film can be fabricated by the LB method. In this procedure, the surfaces of nanoparticles are modified with hydrophobic molecules and dispersed directly into a 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 complete evaporation of the volatile solvent. By compressing the layer through moving the barrier, one can control the density of the monolayer film. Subsequently, an ordered layer of nanoparticles will be formed on the surface. A systematic SERS study was carried out by Yang and his group using an SERS active substrate fabricated by the LB technique [23]. The LB technique was employed by them to successfully fabricate most uniform SERS substrates of films of nanorods, nanowires, and spherical, cubic, cuboctahedral and octahedral Ag nanoparticles [23].

4. Enhancement mechanism observed in the SERS effect

Even though, several theoretical and experimental research studies based on the SERS phenomena have been carried out and a great deal of research publications based on these works are well cited in the literature, the correct nature of the gigantic enhancement in Raman intensity found in the SERS effect is still controversial. However, it is generally accepted that two enhancement mechanisms, a long-range electromagnetic (EM) effect and a short-range chemical (CM) effect, are functioning simultaneously. The EM mechanism is based on the amplification of the electromagnetic field generated by coupling of the radiation field with the localized

surface plasmons (LSP) of the metal nanoparticles. The localized surface plasmon resonanace (LSPR) arises as a result of resonance condition between the incident wavelength of light and the electrons in the nanoparticles. This facilitates a combined oscillation of the conduction electrons and it will give rise to two main consequences. The first consequence is the absorption of the wavelengths of light selectively by nanoparticles, which is responsible for this collective oscillation. The second consequence is the enhancement of electromagnetic fields that extend from the nanoparticle surfaces. These fields are mainly responsible for the large enhancement observed in SERS. The enhancement is approximately proportional to $|E^4|$ and generally in the order of 10^8 or more, where E is the intensity of the electromagnetic field.

Localized surface plasmon resonance (LSPR), the lightning rod effect, and the image field effect, all these effects are responsible for the enhancement in SERS. Among them, LSPR contributes mainly to the electromagnetic field enhancement and SERS effect. Anisotropic metallic nanostructures have all of the characteristics of excellent SERS active substrates with good stability and high reproducibility. It has been demonstrated in the literature that anisotropic nanostructures such as nanorods, nanodisks, and nanoprisms will exhibit interesting size and shape-dependent properties. Anisotropic metal nanoparticles will exhibit "lightning-rod effect" [24], which is a new kind of field enhancement refers to enhanced charge density localization at a tip or vertex of a nanoparticle. The theory based on "lightning-rod effect" was developed by Liao and Wokaun in the year 1982. The excitation of the free electrons of a metallic tip by an electromagnetic field (laser light) will generate an extremely localized, sturdy electric field at these sharp tips or vertex with large curvatures leading to large field enhancement in those regions. This effect gives rise to high SERS activity of the anisotropic nanostructures. Anisotropic metallic nanostructures have been extensively utilized as an effective SERS active substrate with high SERS activity [25, 26] as found in the literature.

The chemical enhancement (CM) mechanism corresponds to the enhancement effect arises as a result of the chemical interaction between the adsorbates and the metal surface. The CM mechanism can also be referred 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 (LUMO) or vice versa. The enhancement factor of CM is generally in the order of $10–10^2$. EM has a long-range effect, for which rough metallic surfaces can be used as an SERS active substrate, while CM has a short-range effect taking place on the molecular scale. The two mechanisms of EM and CM are not reciprocally restricted, but these two effects work simultaneously to generate the overall SERS effect. However, it is very hard to differentiate CM from the EM effect. Several research groups all over the world have tried to solve this problem, but the problem is unsolved so far.

5. Applications of SERS

SERS is highly sensitive analytical spectroscopic technique available among the modern scientific communities and can contribute both to surface science and nanoscience. It is also associated with a broad range of other surface sensitive techniques to study various fundamental

and applied research topics such as, corrosion, catalysis, advanced materials, diagnostics, bio-medical applications, biological process, and sensing.

The SERS-active Ag/AAO nanostructured system as mentioned earlier in this chapter has been used by Liu et al. to investigate antibiotic-induced chemical changes in bacterial cell wall [27]. They recorded high quality, highly reproducible SERS spectra, which are also sensitive and stable. The "chemical features" observed from the SERS spectra of bacterial cell wall facilitates fast identification of drug-resistant bacteria within an hour both qualitatively and quantitatively. Furthermore, the characteristic changes in the SERS spectra were clearly observed in the drug-sensitive bacteria at the near the beginning (i.e., 1 hour) of antibiotic exposure, which could be utilized to differentiate them from the drug-resistant ones. The rapid detection of pathogens such as bacteria and viruses using the SERS technique provides a novel approach for microbial diagnostics. The SERS-based novel technique was applied to a single bacterium. This rapid SERS detection of pathogens makes possible direct analysis of clinical specimen as an alternative to a pure culture specimen. Traditional diagnostic protocols for diagnosing bacterial infections are based on the isolation of pure culture of the bacterium, which is fol-lowed by the resolving of the nature of the isolate and an extensive assessment of the isolates responses to several antibiotics proliferation or viability. For such biological assays, an incuba-tion period varying from days to weeks or even months is essential for the growth of bacteria with such a density that can be handled by the available diagnostic tools. Different PCR-based protocols have been inducted for the quantitative identification of bacteria. Mass spectrometry can also be alternatively utilized for culture-free bacterial diagnostics. Nevertheless, just like the PCR-based method, mass spectrometry-based protocol is dependent on the existing previ-ous information in the literature about the pathogens. Finally, neither of the PCR or mass spec-trometry-based protocols can be applied to a live bacterial sample to observe their responses to antibiotics or to carry out different functional tests. On the other hand, SERS-based spectro-scopic technique can resolve the limitation of PCR-based identification methods for pathogens. The SERS active substrates based on the Ag/AAO system can be utilized for the fine changes observed in the bacterial cell wall during different stages of bacterium's growth and also for the bacterium's response to antibiotic treatment during the early period of antibiotic exposure.

In a recent development, Ankamwar et al. [28] fabricated a highly stable and almost homogeneous SERS active substrate from silver nanoparticles synthesized from the leaf extract of *Neolamarckia cadamba* for the rapid detection of two strains of bacteria, Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. **Figure 6** demonstrates the TEM image of the as-synthesized silver nanoparticles along with their UV-visible spectrum, SAED pattern, and the resultant SERS spectra generated upon interaction with *S. aureus* and *E. coli*.

These silver nanoparticles upon interaction with bacteria can exhibit a large Raman enhance-ment factor ((3 ± 0.20) $\times 10^7$ and (5 ± 0.40) $\times 10^7$ for *S. aureus* and *E. coli* bacteria, respectively) with almost zero fluctuations. The SERS substrate developed by them is almost homogeneous with a relative standard deviation value of 6.32 calculated from 50 repeated measurements from various locations on the SERS substrate. In addition to this, the fabricated SERS substrates are extremely stable even after 3 months. Using this almost homogeneous, a stable SERS active substrate, Gram-positive bacteria can be differentiated from Gram-negative bacteria. The SERS

data presented in the above study is highly stable, uniform, and reproducible, which shows the versatility of the biosynthesized SERS active substrate. This SERS active substrate is capable of detecting extremely low concentrations (10^3 CFU ml^{-1}) of *E. coli* within a very short time of 1–5 s and also exhibits high sensitivity (see **Figure 7**). **Figure 7** demonstrates the SERS calibration curve obtained with SERS intensity of the peak at 1330 cm^{-1} (C–N stretching mode) as a function of concentration of bacteria *E. coli*. The 1330 cm^{-1} peak became detectable at 10^3 CFU/ml of *E. coli* concentration. The SERS intensity increases with the concentration of the bacterial solution, as it is exponentially correlated to the concentration of *E. coli* bacterial cells in the sample between 10^3 and 10^8 CFU/ml. Experiments were repeated five times with each bacterial concentration, and the standard errors of the mean for each concentration are also shown in **Figure 7**. The major intention of this SERS study using biosynthesized Ag nanoparticles was to develop a rapid fingerprinting method for the characterization of bacteria particularly *E. coli*, which is associated with urinary tract infection (UTI), a common disease among most people of all age groups in developed countries such as India and China.
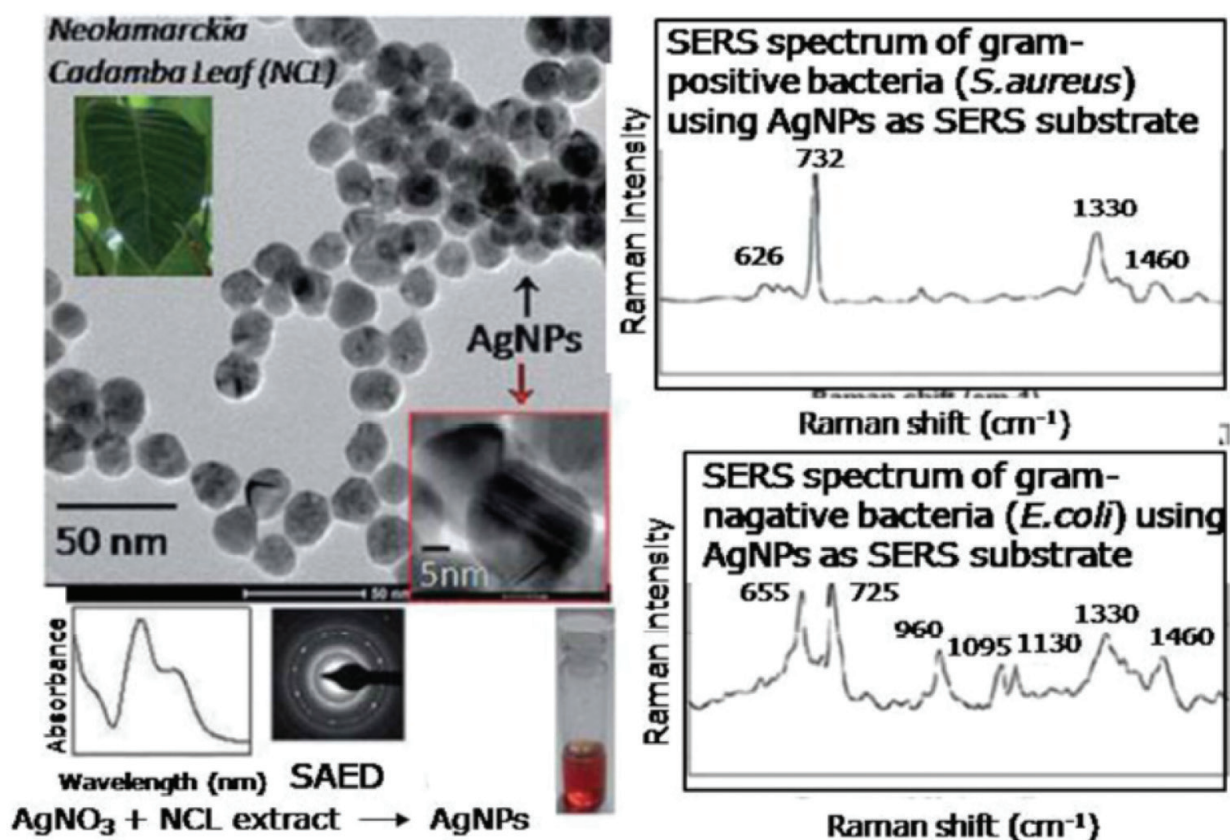
Figure 6. The TEM image of the as-synthesized silver nanoparticles along with their UV-visible spectrum, SAED pattern, and the resultant SERS spectra generated upon interaction with bacteria *S. aureus* and *E. coli*. Reproduced with permission from Ankamwar et al. [28]. Copyright @ Royal Society of Chemistry, Inc.

The SERS-based pathogen detection method is especially useful for the analysis of slow-growing bacteria, which typically may take few weeks during laboratory tests. The SERS spectra described above to detect and quantify bacteria lacks the molecular level specificity

compared to other commonly used techniques. The SERS-based technique demonstrates a novel approach for rapid microbial diagnostics, where SERS can be directly applied on the clinical sample rather than pure cultured bacteria.
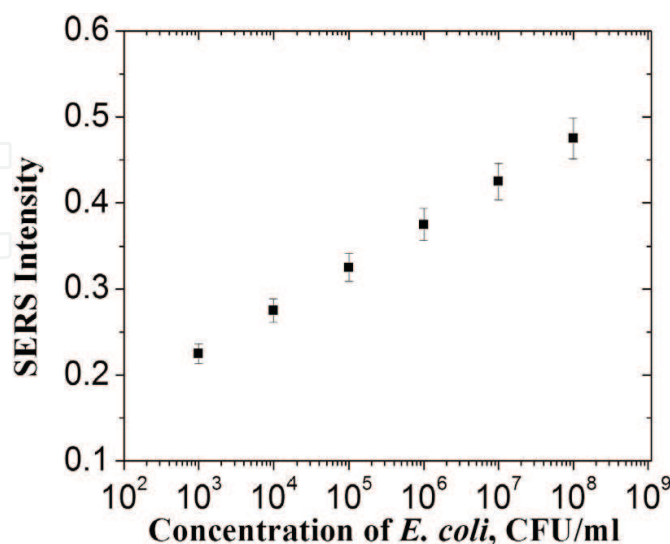
Figure 7. The SERS calibration curve obtained with SERS peak area or SERS intensity of the peak at 1330 cm⁻¹ (C–N stretching mode) as a function of concentration of bacteria *E. coli*. Reproduced with permission from Ankamwar et al. [28]. Copyright @ Royal Society of Chemistry, Inc.

Nie and Emory [15] carried out a single-molecule SERS experiment by employing the SERS technique along with the transmission electron microscopy (TEM) and scanning tunneling microscopy (STM) techniques. They observed surface Raman enhancement in the order of 10^{14}–10^{15} for single rhodamine 6G (R6G) molecule adsorbed on selected Ag nanoparticles. For the single-molecule SERS study, a single event was observed rather than an ensemble aver-aged value usually attained for traditional SERS measurements. Advancement of the single molecule SERS technique has brought a new aspect in biomedical research, as it can act as a versatile probing tool to investigate various biological molecules such as virus, bacteria, and protein.

The limitation arises out of surface generality of the SERS effect has been resolved by the invention of tip-enhanced Raman spectroscopy (TERS) technique in 2004 [29], which is a mod-ification of the conventional SERS technique. This new and novel technique is derived from the enhancement of the surface Raman scattering intensity (SERS signal) by merging Raman spectroscopy with a scanning probe microscopy technique such as atomic force microscopy (AFM) or scanning tunneling microscopy (STM). The experimental setup in the TERS tech-nique consists of AFM or STM tip placed in a nearby surrounding area of an ultrasmooth substrate, generally single crystal metal surfaces and illuminated by an electromagnetic radia-tion of suitable wavelength. The contact or tunneling mode of AFM/STM can be used in the experimental setup of TERS. Irradiating with a laser beam of suitable wavelength, a localized surface plasmons are excited in the tip-substrate gap, generating a huge, local enhancement of electromagnetic field in comparison to the incident radiation. TERS was used to probe malachite green isothiocyanate, a dye molecule adsorbed on the Au(111) surface [29]. TERS

has been used to study surface reactions on single crystal and smooth surfaces, as surface roughness of the substrate does not play any role in this enhancement.

Surface-enhanced Raman scattering spectroscopy (SERS) can be used for the identification short-live reaction intermediates such as radical and radical ions on the electrode surface and elucidation of the reaction mechanism in general. Tian and his research group at Xiamen University, China, carried out the first *in situ* electrochemical SERS (EC-SERS) investigation on the electrochemical reduction of $PhCH_2Cl$ in acetonitrile (CH_3CN) on the Ag electrode [30]. The benzyl radical anion as a reaction intermediate and 3-phenylpropanenitrile as the major reaction product were detected from the SERS study for the above surface reaction. The complete reaction mechanism enlightening the adsorption process of $PhCH_2Cl$ on the Ag surface and all other possible interactions including the solvent molecule have been determined from the systematic SERS study. The SERS results were further validated by quantum mechanical density functional theory (DFT) calculations, which confirm the detection of the reaction intermediate and products.

It was established by Mulvihill et al. that LB assemblies made of various polyhedral Ag nanocrystals can be utilized as high quality SERS active substrates for the high-sensitivity detection of arsenate and arsenite ions in aqueous solutions with a detection limit of 1 ppb [31]. The detection limit resulted from the analysis carried out by the SERS-based technique is an order of magnitude lower than the existing yardstick set by the World Health Organization (WHO). The SERS substrate can be used as a chemical sensor, which is simultaneously highly reproducible and portable, and therefore, this could be easily executed in field detection. The SERS technique can be further employed in environmental analysis. 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 [32, 33] can qualitatively and quantitatively analyzed by the SERS-based detection technique. The partition property of SERS substrates as well as surface chemistry facilitates the complete separation of pollutants and analysis of complex environmental samples in real environmental analysis and monitoring.

Immobilized metal nanoparticles in the form of SERS substrates can be used for biomedical diagnostics. For instance, the SERS substrate can be used as a glucose sensor to detect glucose in human blood. Although glucose is most commonly monitored by electrochemical-based sensors, a substitute protocol using SERS substrates fabricated by the NSL technique has been employed to detect glucose in blood [34]. In this new protocol, the SERS-based glucose sensor was developed by growing silver film over nanospheres (AgFON) surfaces prepared by the NSL technique. Nevertheless, glucose sensing on a bare AgFON surface was not successful and glucose was brought within the range of electromagnetic enhancement of the AgFON surface by formation of a self-assembled monolayer (SAM) on its surface to partition the analyte of interest, in a manner similar to the technique used to generate the stationary phase in high-performance liquid chromatography. Numerous SAMs were studied to partition glucose effectively to the AgFON surface and it was observed that both

straight-chain alkanethiols and ethyleneglycol-terminated alkanethiols partitioned glucose most effectively. The key for the detection of glucose by the SERS-based technique was the surface chemistry of alkanethiol molecule on the AgFON surface. The SERS substrate was modified with an alkanethiol partition layer to facilitate the glucose adsorption to the metal surface. Real-time sensing and quantitative detection of glucose in bovine plasma by SERS has been reported in the literature [35].

6. Summary

Even though, the appropriate theory and principles to elucidate the correct mechanism of the SERS phenomenon is yet to be developed, the 40 years of this versatile technique has reached a new height due to the increased efficiency of the modern Raman instrumentation and recent advancement in nanoscience and nanotechnology. Controlled and reproducible fabrication of SERS-active substrates [36] and understanding the in depth connection between the nanoparticle structure and SERS activity remains noteworthy challenges in this field of research. We believe that the fast and gradual development of nanoscience and nanotechnology will eventually allow an absolute understanding of the SERS effect and a broad range application of SERS in both analytical sciences and biomedical sciences.

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