Surface-enhanced Raman scattering (SERS) spectroscopy: a versatile spectroscopic and analytical technique used in nanoscience and nanotechnology

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Abstract. Surface-enhanced Raman scattering (SERS) effect deals with the enhancement of the Raman scattering intensity by molecules in the presence of a nanostructured metallic surface. The first observation of surface-enhanced Raman spectra was in 1974, when Fleischmann and his group at the University of Southampton, reported the first high-quality Raman spectra of monolayer-adsorbed pyridine on an electrochemically roughened Ag electrode surface. Over the last thirty years, it has developed into a versatile spectroscopic and analytical technique due to the rapid and explosive progress of nanoscience and nanotechnology. This review article describes the recent development in field of surface-enhanced Raman scattering research, especially fabrication of various SERS active substrates, mechanism of SERS effect and its various applications in both surface sciences and analytical sciences.

Keywords: Surface-enhanced Raman scattering; Raman scattering cross section; nanoscience; nanostructured metal; nanoparticle; anisotropic; tip-enhanced Raman scattering

1. Introduction

The accidental and unexpected discovery of surface-enhanced Raman scattering spectroscopy (SERS) by Fleischmann and his group (Fleischmann *et al.* 1974) in 1974 at the University of Southampton has drawn a lot of attention to researchers due to the large enhancement of weak Raman signal and thereby facilitate convenient detection in chemical and biological systems. Later, with the advent of scanning probe microscopy technique (SPM) in the early 1980s, it was revealed that the order of surface roughness is in nanometer dimension. Therefore, SERS and nanoscience/nanotechnology are now closely related. SERS has emerged as a powerful technique not only for studying the molecules or ions at trace concentrations down to single molecule detection level but also for helping to understand the surface chemistry especially electrochemistry at the nanometer scale. The rapid development of nanoscience and nanotechnology in recent years boosted the scientific community and attracted a wide interest of SERS. 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 in electrochemistry. SERS is recognized as one of the most sensitive spectroscopic tools for highly sensitive chemical and biological detection. The advent of

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SERS has opened up a promising way to overcome the low sensitivity of traditional Raman spectroscopy. The versatility of SERS technique can be exploited from diverse applications in fields ranging from plasmonics to diagnostics. The rapid interest among researchers all over the world has elevated to a new height, which has been demonstrated from the recent publication of two high quality monographs by Aroca (2006) and Le Ru and Etchegoin (2009) that nicely cover the principles of SERS and its applications. In this review article, the recent advancement on SERS research has been discussed which covers the various SERS substrates, the various theories to explain the mechanism of SERS and its various applications. The discussion will start with a brief historical survey of SERS, followed by an overview on the different types of substrates developed for SERS. It will end by citing some recent applications of SERS. Due to space limitations, a complete review of all recent work on this important area of research is not possible. However, a few representative examples had been summarized to illustrate the recent development in SERS research.

2. Historical background and development of SERS

Raman spectroscopy is a spectroscopic technique 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 electromagnetic spectra. This effect was discovered by famous Indian physicist Professor C.V. Raman in the year 1928 (Raman and Krishnan 1928). The weak intensity of Raman signal observed in traditional Raman spectroscopy 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. With such a low scattering cross-section, from about 10^{12} to 10^{14} molecules cm^{-2}, usually present in a monolayer, is hardly going to be enough to be detected even by the most efficient modern and sophisticated Raman spectrometer. Raman spectroscopy is capable of obtaining fingerprint information of species by detecting the vibrational bands. However, the intensity of Raman signal of most systems is very weak and is only about 10^{-10} times the intensity of the incident laser. The signal becomes even weaker when the adsorbed species are concerned, because there are only monolayer or submonolayer species on the surface. The monolayers adsorbed on metallic surfaces can be detected by Raman scattering, which is particularly important in the context of spectroelectrochemistry, a special branch of electrochemistry. Spectroelectrochemistry deals with the electrochemical systems involving metallic electrodes in aqueous solutions to be probed by spectroscopic methods. Water gives a lower background in Raman, relative to IR absorption, and the use of visible radiation for excitation and detection are also important advantages for the application of Raman to study various electrochemical systems. Based on those advantages, a few spectroelectrochemists were trying very hard during the 1970s to obtain the Raman signal from adsorbed monolayers from metal surfaces under electrochemical control, but they were unsuccessful to obtain highly reproducible data. However, Fleischmann *et al.* were able to detect Hg_2Br_2, Hg_2Cl_2 and HgO in Hg electrode (Fleischmann *et al.* 1973) by utilizing the unusually high cross-section of mercury ion species. Later, Fleischmann and his group decided to try to increase the number of adsorbed molecules by using a roughened metal electrode surface. In 1974, they reported very high signal-to-noise ratio Raman spectra of pyridine adsorbed on electrochemically roughened Ag electrodes. The authors attributed the enhancement in the Raman intensity to an increase in the surface area of

the Ag electrode by the electrochemical roughening method.

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 surface-enhanced Raman scattering (SERS) spectroscopy in 1974 by Fleischmann, Hendra and Mcquillan of 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 a Ag electrode by using an electrochemical roughening method, they applied about 450 potential oxidation and reduction cycles (ORC) to a 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 the intense surface Raman signal of Py due to increased surface area. This was, in fact, the first SERS measurement and that roughened electrode was the first nanostructure exhibiting the SERS activity, although it was not recognized as such in 1974. Later, Jeanmaire and Van Duyne (Jeanmaire and Van Duyne 1977) from Northwestern University, USA, first realized that surface area is not the main point in the above phenomenon in 1977. Albrecht and Creighton (Albrecht and Creighton 1977) of University of Kent, UK, reported a similar result in the same year. 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 to 10^6 enhancement). 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 single molecule can be detected. The concept of enhanced optical effects in metallic surfaces was subsequently established by Moskovits in the year 1978 (Moskovits 1978). As a consequence, a significant amount of efforts were made by a large number of research groups, leading to a fast increase in the understanding of this SERS effect.

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 an electromagnetic effect on the enhancement. 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. This chemical enhancement theory relies on the charge transfer complex formation of the adsorbed molecule. However, it is very difficult to separate these two effects experimentally.

In the mid-1980s, the focus on SERS research began to move from the fundamental understanding of the SERS phenomenon to the exploitation of new and novel analytical applications of SERS. However, it was very difficult to obtain high-quality, highly reproducible, stable SERS spectra as shown by some of the studies in the mid-1980s as well as early 1990s. This kind of irreproducibility in SERS spectra can be attributed to the small variations in the fabrication of SERS substrates in a well controllable fashion. This drawback prevented for several years the expansion of SERS as a quantitative tool. Therefore, SERS active substrate fabrication is a very important field in SERS research to obtain highly uniform, stable and highly reproducible SERS signals.

In the mid-1990s, an important development in electrochemical surface-enhanced Raman spectroscopy (EC-SERS) was carried out by which significant surface Raman enhancements could be imparted to the VIIIB transition metals of importance for electrochemistry and catalysis. Professor Tian and his group at the Xiamen University, China developed several surface roughening procedures and demonstrated that SERS effect can be directly generated on transition metals such as pure Pt, Ru, Rh, Pd, Fe, Co and Ni electrodes, and their surface enhancements range in general from one to three orders of magnitude (Tian *et al.* 2002). 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 very promising class of highly SERS-active substrate. Up to now, molecular-level investigations by Raman spectroscopy on diverse adsorbates at various material electrodes had been realized. These advances have made Raman spectroscopy widely used in electrochemistry. Moreover, a systematic study on EC-SERS processes could be helpful for elucidating comprehensively SERS mechanisms.

Advances in Raman instrumentation and introduction of the confocal microscope and the holographic notch filter in the late 1990s caused drastic changes in the sensitivity of Raman spectrometer without any interference from the signal due to Rayleigh scattering and strong signal from the bulk phase.

The next major landmark in the development of the SERS research was the observation of SERS spectra from single molecules (SM-SERS) by two groups independently in the year 1997 (Nie and Emory 1997, Kneipp *et al.* 1997). The detection of single molecules elevated SERS to attain this ultimate limit of detection, together with, for instance, fluorescence spectroscopy and scanning tunneling methods. Single molecule detection gave a new dimension to the SERS research.

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.

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.

3. Enhancement mechanism observed in SERS effect

Despite numerous theoretical and experimental works on SERS and large number of research publications based on these works, the exact nature of the large enhancement in Raman intensity found in SERS is still a matter of controversy. However, it is generally accepted that two

enhancement mechanisms, one a long-range electromagnetic (EM) effect and the other a short-range chemical (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 consequence 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.

Localized surface plasmon resonance (LSPR), the lightning rod effect, and the image field effect have all been considered to contribute to SERS. Among them, LSPR makes the major contribution to the electromagnetic field enhancement and SERS. Anisotropic metallic nanostructures have all of the characteristics which make them excellent candidates as SERS substrates with high stability and reproducibility. Symmetry breaking allows for more complex plasmon propagation, potentially leading to more intense electromagnetic field generation from the structure and in gaps formed between these materials. Anisotropic nanostructures such as nanorods and nanoprisms have been shown to possess interesting size and shape-dependent properties, thus motivating interest in the controlled assembly of them into functional architectures for SERS. Anisotropic metal nanoparticles exhibit 'lightning-rod effect'(Liao and Wokaun 1982) another type of field enhancement refers to an enhanced charge density localization at a 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 these sharp tip or vertex with large curvatures, leading to large field enhancement in those regions. 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 (Orendorff *et al.* 2005, Hu *et al.* 2004). 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 play an important role on the overall SERS activity. Fan *et al.* synthesized nearly monodisperse single crystalline palladium (Pd) nanocubes and nanodendrites in aqueous solution at room temperature and used them as SERS active substrates (Fan *et al.* 2009). They prepared the SERS substrate by using a simple dropcoat method. A droplet of the Pd nanoparticles sol was dropped on a smooth Pd electrode surface and completely dried in air and recorded the SERS spectra of pyridine molecules adsorbed on these Pd nanocubes and nanodendrites. These Pd nanocubes exhibit better SERS activity in comparison to nanodendrites. The electromagnetic field coupling between nanocubes plays an important role causing an increase in electromagnetic enhancement. The effective area in the nanoparticle junction 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 nanocubes than that from 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, involves the photoinduced 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 $10\text{-}10^2$. EM is a long-range effect, which requires rough surface as SERS active substrate, while CM is a short-range effect occurring on the molecular scale. The two mechanisms ca EM and CM are not mutually exclusive, but work together in concert to produce the overall SERS effect. It is very 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.* had 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 (Pande *et al.* 2011).They also estimated the relative contributions of the electromagnetic (EM) and charge-transfer (CT) mechanism to the overall enhancement of the SERS bands of the PHEN molecule.

4. SERS substrates : an overview

SERS active substrate fabrication is a very important field in SERS research. The two most common SERS active substrates are metal colloids of coinage metals of 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 in three categories: (1) metal nanoparticles in suspension; (2) metal nanoparticles immobilized on solid substrates; (3) Nanostructures fabricated directly on solid substrates, which includes nanolithography and template based synthesis of nanostructures. Although, nanoparticles and nanoparticle film electrodes show very 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 inter-particle 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 to these, Langmuir-Blodgett technique can be used to fabricate highly-ordered SERS active substrates. Due to the rapid advances in nanotechnology, various methods are available now for fabrication of various metallic nanostructures of different size, shape as SERS active substrates. This has facilitated in a significant rise in the reports of new SERS active substrates in the last 10 years and further improvement are expected in the coming years.

Metal nanostructures of various shapes and sizes can be synthesized using a wide range of protocols as seen from the recent literature reviews. Spherical metal nanoparticles can be synthesized by various well established methods. Various methods, including both top-down and bottom-up approaches, have been applied for the synthesis of anisotropic nanoparticles. Bottom-up approaches are more popular in the synthesis of metal nanoparticles in comparison to top-down approach, as nanoparticles produced by top-down approaches have a relatively broad size distribution, varied particle shape or geometry, and use expensive synthetic pathways that are industrially non-scalable. Wet chemical synthesis is a potential bottom-up method to produce anisotropic nanoparticles in high yield and structural purity with variable size, shape, structure,

composition, and surface chemistry. The wet chemical synthesis (seed-mediated and polyol synthesis) of anisotropic metal nanoparticles can be carried out by selective adsorption of capping agents/stabilizing agents such as cetyltrimethylammonium bromide (CTAB), polyvinyl pyrrolidone (PVP) and several other selective adsorbates, which controls the growth process in a preferential direction. Preferential adsorption of capping agents to specific crystal facets can hinder or enhance the crystal growth in some directions. In certain cases, formation of surfactant micelles or structural defects would physically direct the anisotropic growth. In addition to these methods, biological (Shankar *et al.* 2004), photochemical (Kim *et al.* 2002, Esumi *et al.* 1995), electrochemical (Huang *et al.* 2006) and template synthesis (Hulteen and Martin 1997) have also been reported in the literature.

Metal nanoparticles in suspension is the simplest of all SERS substrates used so far, where SERS experiments are carried out in the presence of certain concentration of analyte (Raman probe molecule). However, aggregation of metal nanoparticles can hinder the reproducibility of SERS spectra. On the other hand, aggregation is sometimes required for the observation of high quality SERS signal (Nie and Emory 1997). In addition to this, MNPs suspension must be mixed with the analyte solution for SERS, a sampling requirement that might be limiting for some applications, such as in examining adsorbates on non-SERS active surfaces such as semiconductors and fruits. In spite of the reproducibility and potential sampling challenges, MNPs suspensions are used widely as SERS active substrate due to their high SERS-performance, good stability and ease of fabrication. In fact, this type of the substrate was used in the initial single molecule SERS experiments. The sampling drawback had been recently addressed by Professor Tian and his research group at the Xiamen University, China (Li *et al.* 2010). They developed a new generation of shell isolated MNPs as SERS enhancing smart dust, which was successfully applied to probe hydrogen adsorbed on single crystal Pt surface, and even directly detected pesticides residues that contaminated in citrus fruit like orange. This new borrowing SERS technique is known as shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS).

The variety of SERS substrates reported in recent years is enormous and it is not possible to review the whole field in a limited number of pages. To allow a critical assessment, the substrates covered in this review article will be limited to only three general types of substrates, classified according to their fabrication method: (1) MNPs immobilized in planar solid supports; (2) metallic nanostructures fabricated using nanolithographic methods; and (3) metallic nanostructures fabricated using template techniques.

4.1 MNPs immobilized in planar solid supports

The application of dispersed and aggregated MNPs as SERS substrate in real analytical problems is limited due to the poor reproducibility of enhancement factor. The reproducibility problem can be solved by immobilizing the MNPs on some kind of solid support. Since the first report of a SERS substrate consisting of MNPs synthesized by wet chemistry and subsequently immobilized onto a solid support (Freeman *et al.* 1995), the procedure gained popularity and several works have been published based on this approach and its variations.

Molecular self-assembly has been explored to chemically attach MNPs to solid substrates such as Si, glass, indium tin oxide (ITO), Au film using bifunctional molecules such as 4-aminothiophenol.The basic idea is to anchor the molecule to the surface by one of its functional groups, leaving the other functional group to bind the MNPs. The molecule forms a compact layer with the solid substrate with one moiety and another moiety interacts with the nanoparticles

through electrostatic or chemical interaction to form an ordered layer of nanoparticles. Various bifunctional molecules must be selected for different substrates. For example, a bifunctional molecule with thiol (SH) moiety and a N lone pair group can be selected for Au, Ag, Pt, and Pd substrates (Aroca *et al.* 2005). Special care should be taken for thiol, because thiol can be easily dimerized to form disulfur compounds and this leads to the annihilation of the functional group and a bad assembly effect. A Si or glass substrate should usually be completely degreased and activated to form a surface hydroxyl group, which can then react with the siloxane group of the bifunctional molecules. The MNPs are immobilized on solid substrates by a simple method. The surface of glass slides were first functionalized with either amino or thiol groups using a surface polymerization procedure, followed by immersing of the functionalized glass into MNPs suspension for a certain amount of time. Both electrostatic interaction and capillary force behave as driving mechanism for immobilization of MNPs on solid substrates.

4.2 Metallic nanostructures fabricated using nanolithographic methods

A highly ordered metallic nanostructures array can be fabricated using the top-down nanolithography and related nanoimprint lithographic techniques. Typically, a layer of polymeric photoresist (positive or negative) is cast on the solid substrates (such as Si, glass, or Au film), followed by patterning with ultraviolet (UV) light, an electron beam, or a focused ion beam directly on the photoresist surface or indirectly with the assistance of a mold. After exposure and development, the remaining photoresist can be used as a mold, on which SERS-active metals are deposited by physical vapor deposition at vacuum. A highly ordered nanostructured SERS-active substrate with a structure identical or complementary to that of the mold is formed after lifting off the mold. The nanolithography method allows the preparation of highly ordered and uniform SERS substrates with interparticle spacings below 10 nm with nanostructures having a wide diversity of shapes and geometries compared with nanosphere lithography. The use of focused ion beam (FIB) or electron beam (EB) lithography to make a SERS substrate of large area is still time-consuming and very expensive. Both, FIB and EB lithographic techniques can be employed to make molds for nanoimprint. Nanoimprint normally directly writes on the Si or quartz slide with an electron beam to produce the desired nanopattern as the mold. The mold is then aligned and pressed into the photoresist covering on the substrate. After curing, the mold is lifted off and the substrate is deposited with the desired metal. After removal of the photoresist, a highly ordered nanostructure with SERS activity can be obtained. Compared with nanolithography, nanoimprint has a much higher efficiency and lower cost.

4.3 Nanosphere lithography

NSL technique has been well developed by Van Duyne and his group (Willets and Van Duyne 2007, Haynes and Van Duyne 2001). In this technique, monodispersed polystyrene or SiO_2 nanospheres of the desired diameter are self-assembled on clean conductive substrate such as indium tin oxide (ITO) or evaporated metal substrate over glass to form a very 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 a controlled thickness. It will then result in three types of structured SERS substrate: 1) physical vapour deposition on the nanosphere template leads to the formation of Ag metal "film over nanosphere" (FON) surface; 2) the removal of nanospheres of the FON surface by sonicating in a

solvent results in surface confined nanoparticles with a triangular footprint; 3) electrochemical deposition followed by removal of spheres leaves a thin nanostructured films containing a regular hexagonal array of uniform segment sphere voids. The advantage of the nanosphere lithography method for fabricating the SERS active substrate is the control of the shape, size, and spacing of the nanostructures by tuning the size of the nanospheres and the thickness of the deposited metal so that the localized surface plasmon resonance (LSPR) position can be adjusted to match the excitation wavelength and to obtain an optimized SERS enhancement. Methods 1) and 3) will produce substrates with high SERS enhancement. Although, an ordered SERS substrate with an area of 10–100 μm^2 had been fabricated routinely by some experienced groups, the success rate of this method depends totally on the experience of the experimentalist and stringent maintenance of the experimental conditions of the fabrication method.

4.4 Ag/AAO systems for chemical and bio-sensing

Wang *et al.* had utilized porous AAO film as template to electrochemically deposit arrays of silver (Ag) nanoparticles with a precisely controlled gap upto 5 nm (Wang *et al.* 2006). 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). Various theoretical and experimental studies based on dependence of the enhancement factor on the gap between adjacent nanoparticles on a SERS active substrate indicate that the precise control of gaps between nanostructures on a SERS-active substrate in the sub-10 nm regime, which is extremely difficult to obtain by existing nanofabrication methods, is likely to be critical for the fabrication of substrates with uniformly high enhancement factors, and for understanding collective surface plasmons existing inside the gaps. 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). The experiment carried out by Wang *et al.* represents the first quantitative observation of the collective SERS effect on a substrate with precisely controlled 'hot junctions' in the sub-10 nm regime, and confirms the theoretical prediction of interparticle-coupling-induced Raman enhancement. This Ag/AAO based SERS substrate with highly uniform and reproducible SERS signals can be used as bio/chemical sensor with extremely high sensitivity and concentration upto picomolar level had been detected. This excellent SERS substrate can be further extended into single molecular regime increasing the detection limit and SERS sensitivity.

4.5 Langmuir-Blodgett technique

Chemical assembly of metal nanoparticles on solid substrates like glass, silicon, ITO can be used as ordered SERS substrates through electrostatic or chemical interaction to form an ordered layer of nanoparticles. However, this method still can not provide totally defect free SERS substrate over a large area of few hundred of cm^2.

The Langmuir-Blodgett (LB) technique can potentially solve this problem. The LB method was originally used to prepare a large-area surface film of amphiphilic molecules on solid substrates. In this method, the amphiphilic molecule dissolved in a volatile solvent immiscible with water, and dispersing the solution on the surface of the water phase, a monolayer of the amphiphilic molecule will form at the air/water interface after the 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 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 the evaporation of the solvent. As a result of compression of the layer through 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 LB technique (Tao *et al.* 2003). 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. Among them, the Ag nanocube exhibits the lowest enhancement, and the octahedral nanoparticle shows the highest enhancement, up to 10^7–10^8.

5. Applications of SERS

SERS is among the most sensitive techniques available both to surface science and nanoscience. This technique can be combined with a wide variety of other surface sensitive techniques to study various fundamental and applied research areas such as, corrosion, catalysis, advanced materials, biology and sensors.

Recently, Liu *et al.* used these SERS-active Ag/AAO nanostructured system to monitor antibiotic induced chemical changes in bacterial cell wall (Liu *et al.* 2009). The SERS profiles recorded by them are sensitive and stable and the 'chemical features' obtained from SERS spectrum of bacterial cell wall enables rapid identification of drug resistant bacteria within an hour. Moreover, characteristic changes in the SERS profile were noticed in the drug-sensitive bacteria at the early period (i.e., 1 hr) of antibiotic exposure, which could be used to differentiate them from the drug-resistant ones. The high-speed SERS detection represents a novel approach for microbial diagnostics. The SERS based novel technique was applied to a single bacterium. This high speed SERS detection makes possible 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 the determination of the identity of the isolate and an examination of the isolates responses 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 of the PCR or mass spectrometry based approaches can be applied to live bacteria to monitor their responses to antibiotics or to conduct functional tests. However, SERS based spectroscopic method solve 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

bacterium's different growth stages and of the bacterium's response to antibiotic treatment during early period of antibiotic exposure.

Nie and Emory (1997) carried out one of the earliest experiment on single-molecule SERS by combining SERS with the transmission electron microscopy (TEM) and scanning tunneling microscopy (STM) techniques. 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 as a versatile probing tool to study various biological molecules like virus, bacteria, protein, DNA and RNA.

The problem of surface generality of SERS had been overcome by the introduction of tip-enhanced Raman spectroscopy (TERS) in 2004 (Pettinger *et al.* 2004). This new technique is based on the enhancement of the Raman scattering intensity (Raman signal) by combining Raman spectroscopy with scanning probe microscopy such as atomic force microscopy (AFM) or STM. Its central element consists of an illuminated AFM or STM tip placed in a close vicinity of a substrate. The tip is operated in contact or tunneling mode. Upon illumination with a laser beam, a localized surface plasmons are excited in the tip-substrate gap, producing a large, local enhancement of electromagnetic field compared to the incident radiation. This new technique was invented by Pettinger *et al.* in 2004 to probe a dye molecule malachite green isothiocyanate adsorbed on Au(111) surface.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.

The non-destructive, highly surface-sensitive and *in-situ* nature of surface-enhanced Raman spectroscopy (SERS) can be utilized to detect reaction intermediates such as radical and radical ions on the electrode surface and elucidate the overall reaction mechanism. Recently, Tian and his group at Xiamen University, China reported the first *in-situ* electrochemical SERS (EC-SERS) study on the electrochemical reduction of $PhCH_2Cl$ in acetonitrile (CH_3CN) on Ag electrode (Wang *et al.* 2010). They detected benzyl radical anion as an intermediate and 3-phenylpropanenitrile as the major reaction product 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 Density Functional Theory (DFT) calculations, which can provide concrete evidence to identify the reaction intermediate and products.

Mulvihill *et al.* demonstrated that LB assemblies consist of different polyhedral Ag nanocrystals can be used as high quality SERS active substrates and can detect arsenate and arsenite in aqueous solutions with a detection limit of 1 ppb (Mulvihill *et al.* 2008), which is an order of magnitude below the current standard set by the World Health Organization (WHO). The SERS substrate can be used as chemical sensor, which is robust, reproducible, highly portable, and could be easily implemented in field detection. SERS technique can be used in environmental analysis. The target molecules analyzed by SERS included 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 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 (Shafer-Peltier *et al.* 2003). 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 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 the surface chemistry. The SERS substrate was modified with an alkanethiol partition layer to facilitate the glucose adsorption to the metal surface.

6. Conclusions

This review article illustrates a broad overview of some of the recent advances on the development of SERS research, especially on different SERS substrates and their applications. SERS has transformed into a very active area for fundamental analytical research and applications, with thousands of papers published in this field in the last 10 years. This review article just covered a fraction of those publications. Although, the theory to quantify the exact mechanism of SERS effect is yet to be developed, the 30 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. 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. We are optimistic that SERS will become increasingly general and indispensable versatile analytical, spectroscopic as well as surface-sensitive tool in fundamental studies and widespread applications ranging from sensors to diagnostics.

References

Albrecht, M.G. and Creighton, J.A. (1977), "Anomalously intense Raman spectra of pyridine at a silver electrode", *J. Am. Chem. Soc*., **99,** 5215-5217.

Aroca, R.F. (2006), *Surface enhanced vibrational spectroscopy*, Wiley, Hoboken, NJ.

Aroca, R.F., Goulet, P.J.G., Santos, D.S. dos, Alvarez-Puebla, R.A. and Oliveira, O.N. (2005), "Silver nanowire layer-by-layer films as substrates for surface enhanced raman scattering", *Anal. Chem.*, **77**, 378-382.

Esumi, K., Matsuhisa, K. and Torigoe, K. (1995), "Preparation of rodlike gold particles by UV irradiation using cationic micelles as a template", *Langmuir*, **11**, 3285-3287.

Fan, F.R., Attia, A., Sur, U.K., Chen, J.B. Xie, Z.X., Li, J.F., Ren, B. and Tian, Z.Q. (2009), "An effective strategy for room-temperature synthesis of single-crystalline palladium nanocubes and nanodendrites in aqueous solution", *Cryst. Growth Des.*, **9**, 2335-2340.

Freeman, R.G., Grabar, K.C., Allison, K.J., Bright, R.M., Davis, J.A., Guthrie, A.P., Hommer, M.B., Jackson, M.A., Smith, P.C., Walter, D.G. and Natan, M.J. (1995), "Self-assembled metal colloid monolayers: an approach to SERS substrates", *Science,* **267**,1629-1632.

Fleischmann, M., Hendra, P.J. and McQuillan, A.J. (1974), "Raman-spectra of pyridine adsorbed at a silver electrode", *Chem. Phys. Lett.,* **26**, 163-166.

Fleischmann, M., Hendra, P.J. and McQuillan, A.J. (1973), "Raman spectra from electrode surface", *J. Chem. Soc. Chem. Commn.,* 80-81.

Haynes, C.L. and Van Duyne, R.P. (2001), "Nanosphere lithography: a versatile nanofabrication tool for studies of size-dependent nanoparticle optics", *J. Phys.Chem. B.*, **105**, 5599-5611.

Hu, J.Q., Chen, Q., Xie, Z.X., Han, G.B., Wang, R.H. and Ren, B. (2004), "A simple and effective route for the synthesis of crystalline silver nanorods and nanowires", *Adv. Funct. Mater.*, **14**, 183-189.

Huang, C.J., Chiu, P.H., Wang, Y.H., Chen, W.R. and Mee, T.H. (2006), "Synthesis of the gold nanocubes by electrochemical technique", *J. Electrochem. Soc.,* **153**, D129-D133.

Hulteen, J.C. and Martin, C.R. (1997), "A general template-based method for the preparation of nanomaterials", *J. Mater. Chem.,* **7**, 1075-1087.

Jeanmaire, D.L. and Van Duyne, R.P. (1977), "Surface Raman electrochemistry part 1. Heterocyclic, aromatic and aliphatic amines adsorbed on the anodized silver electrode", *J. Electroanal. Chem.,* **84**, 1-20.

Kim, D., Park, J., An, K., Yang, N.K., Park, J.G. and Hyeon, T. (2007), "Synthesis of hollow iron nanoframes", *J. Am. Chem. Soc.*, **129**, 5812-5813.

Kneipp, K., Wang, Y., Kneipp, H., Perelman, L.T., Itzkan, I., Dasari, R.R. and Field, M.S. (1997), "Single molecule detection using surface-enhanced Raman scattering (SERS)", *Phys. Rev. Lett.*, **78**, 1667-1670.

Le Ru, E.C. and Etchegoin, P.G. (2009), *Principles of Surface-Enhanced Raman Spectroscopy and Related Plasmonic Effects*, Elsevier, Amsterdam, Boston.

Li, J.F. *et al.* (2010), "Shell-isolated nanoparticle-enhanced Raman spectroscopy", *Nature,* **464**, 392-395.

Lin, T.T., Lin, Y.H., Hung, C.S. Liu, T.J., Chen, Y., Huang, Y.C., Tsai, T.H., Wang, H.H., Wang, D.W., Wang, J.K., Wang, Y.L. and Lin, C.H. (2009), "A high speed detection platform based on surface-enhanced Raman scattering for monitoring antibiotic-induced chemical changes in bacteria cell wall", *PLoS ONE,* **4**, 1-9.

Liao, P.F. and Wokaun, A. (1982), "Lightning rod effect in surface enhanced Raman scattering", *J. Chem. Phys.,* **76**, 751-752.

Moskovits, M. (1978), "Surface roughness and the enhanced intensity of Raman scattering by molecules adsorbed on metals", *J. Chem. Phys.*, **69**, 4159-4161.

Mulvihill, M., Tao, A., Benjauthrit, K., Arnold, J. and Yang, P. (2008), "Surface-enhanced Raman spectroscopy for trace arsenic eetection in contaminated water", *Angew. Chem. Int. Ed.*, **47**, 6456-6460.

Nie, S. and Emory, S. R. (1997), "Probing single molecules and single nanoparticles by surface enhanced Raman scattering", *Science,* **275**, 1102-1106.

Orendorff, C.J., Gole, A., Sau, T.K. and Murphy, C.J. (2005), "Surface-enhanced Raman spectroscopy of self-assembled monolayers: sandwich architecture and nanoparticle shape dependence", *Anal. Chem.*, **77**, 3261-3266.

Pande, S., Chowdhury, J. and Pal, T. (2011), "Understanding the enhancement mechanisms in the surface-enhanced Raman spectra of the 1, 10-phenanthroline molecule adsorbed on a Au@Ag bimetallic nanocolloid", *J. Phys. Chem. C.*, **115**, 10497-10509.

Pettinger, B., Ren, B., Picardi, G., Schuster, R. and Ertl, G. (2004), "Nanoscale probing of adsorbed species by tip-enhanced Raman spectroscopy", *Phys. Rev. Lett.,* **92**, 096101-096104.

Raman, C.V. and Krishnan, K.S. (1928), "A new type of secondary radiation", *Nature,* **121**, 501-501.

Shafer-Peltier, K.E., Haynes, C.L., Glucksberg, M.R. and Van Duyne, R.P. (2003), "Toward a glucose biosensor based on surface-enhanced Raman scattering", *J. Am. Chem. Soc.*, **125**, 588-593.

Shankar, S.S., Rai, A. Ankamwar, B., Singh, A., Ahmad, A. and Sastry, M. (2004), "Biological synthesis of

triangular gold nanoprisms", *Nat. Mater.*, **3**, 482-488.

Tao, A., Kim, F., Hess, C., Goldberger, J., He, R.R., Sun, Y.G., Xia, Y.N. and Yang, P.D. (2003), "Langmuir-Blodgett silver nanowire monolayers for molecular sensing using surface-enhanced Raman spectroscopya", *Nano Lett.*, **3**, 1229-1233.

Tian, Z.Q., Ren, B. and Wu, D.Y. (2002), "Surface-Enhanced Raman scattering: from noble to transition metals and from rough surfaces to ordered nanostructures", *J. Phys. Chem. B*, **106**, 9463-9483.

Wang, H.H., Liu, C.Y., Wu, S.B., Liu, N.W., Peng, C.Y., Chan, T.H., Hsu, C.F., Wang, J.K. and Wang, Y.L. (2006), "Highly Raman-enhancing substrates based on silver nanoparticle arrays with tunable sub-10 nm gaps", *Adv. Mater.*, **18**, 491- 495.

Wang, A., Huang, Y.F., Sur, U.K., Wu, D.Y., Ren, B., Rondinini, S., Amatore, C. and Tian, Z.Q. (2010), "*In Situ* identification of intermediates of Benzyl chloride reduction at a silver electrode by SERS coupled with DFT calculations", *J. Am. Chem. Soc.,* **13,** 9534-9536.

Willets, K.A. and Van Duyne, R.P. (2007), "Localized surface plasmon resonance spectroscopy and sensing", *Annu. Rev. Phys. Chem*., **58**, 267-297.

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