JCAMECH

Vol. 51, No. 2, December 2020, pp 501-509
DOI: 10.22059/jcamech.2020.307294.540

Review of the application and mechanism of surface enhanced raman spectroscopy (sers) as biosensor for the study of biological and chemical analyzes

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ARTICLE INFO

Article history:
Received: 31 July 2020
Accepted: 15 August 2020

Keywords:
Surface Enhanced Raman Spectroscopy (SERS)
Biological Nano-Sensor
Biological analytes
Chemicals, Metal Nanoparticles

ABSTRACT

Raman spectroscopy is an important method for the identification of molecules that is widely used to determine the chemical and structural properties of various materials. Many materials have special Raman spectra so that this phenomenon can it has become an effective tool for studying the structural and chemical properties of molecules. Since Raman spectroscopy can provide accurate information on the chemical and structural properties of biological compounds, this method is used in the field of science. Vital and especially in biological and medical studies is rapidly expanding. Raman is inherently weak and sometimes masked by noise and fluorescence. As a result, the study of low-concentration molecules is not feasible and the need to amplify the Raman scattering signal is clearly felt. . One of the efficient methods for studying low and even single molecular concentrations is the Surface Enhanced Raman Scattering (SERS) method. It uses gold, silver, copper and noble metal nanoparticles to enhance the Raman scattering signal. . SERS has been rapidly expanding over the past four decades, as applications for recognition in the fields of chemistry, materials sciences, biochemistry and biosciences are rapidly expanding. Advances in the manufacture of SERS-based biosensors are a major breakthrough in the detection of biological materials in which the electromagnetic field (effect) molecule is affected by the external field, this larger substitute field due to electromagnetic resonance near the metal surface is formed. Mechanisms of electromagnetic field (field effect) amplifiers mainly contribute to the development of SERS, which includes the study of detection performance, direct and indirect fabrication methods for the identification of biological and chemical analytes, Applications of biosensors, amplifiers, and SERS-based biosensor structures to detect biomolecules are briefly described.

1. Introduction

The application of Raman spectroscopy is primarily the identification of molecules. Nowadays, with the many advances in research equipment design, Raman spectroscopy has become more straightforward, more accessible, and cost-effective. Despite many advances in this field, interpreting Raman spectrums remains a significant challenge and requires special skills. Similar to all spectroscopy methods, the Raman spectrum contains information about the electromagnetic waves colliding the sample. After the electromagnetic beam collides the molecule, a part of it is scattered in all directions. Raman spectroscopy is employed to observe vibrational, rotational, and other low-frequency states in a system. This type of spectroscopy typically provides a special structural fingerprint that is used to identify different molecules. This kind of spectroscopy is based on the non-elastic scattering of monochromatic light (Raman scattering), and monochromatic beams of laser light in the visible region, near-infrared light, or Raman are usually near-ultraviolet light [1]. Raman scattering spectroscopy is one of the most important analytical methods for physical, chemical, and biological investigations. The interaction of electromagnetic waves or photons with an atomic and molecular device can be as follows: a) reflection of photons emitted from the surface of the material, b) passage of photons through the material, c) absorption of photons by material molecules and d) scattering of incident light from the surface of the material. The scattering of photons from material occurs due to elastic and non-elastic collisions, in which no energy is exchanged between photons and material molecules as a result of elastic collisions, and only the path of the photons is subjected to change. However, in non-elastic collisions, energy transfer between the incident photons and the material molecules is observed. Therefore, the Raman spectrum results from the non-elastic scattering of light from material molecules that can be used to obtain the vibrational spectrum of molecules and thus similar to fingerprints identify molecules and provide detailed information about the molecular structure of materials. Since the spectrum related to Raman scattering is inherently weak and sometimes invisible because of noise and the fluorescence effect, this method is not possible to detect molecules with low concentrations, and it is necessary to enhance the Raman scattering spectrum. One of the efficient methods for investigating low concentrations and detecting even single molecules is Surface-

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enhanced Raman Scattering (SERS). In this method, metal nanoparticles are used to enhance the Raman spectrum. SERS is a sensitive and selective method in which Raman scattering increases for molecules adsorbed on metal nanoparticles. By employing this method, information about molecule adsorption and the process of molecule interaction with the substrate surface can be obtained in addition to analyzing the molecular structures [2]. Since the Raman scattering intensity is proportional to the square of induced dipole moment, this process can be enhanced in two ways: enhancing the electric field and enhancing the polarization [3]. In the case that the molecule is located adjacent to the metal surface or physically adsorbed to metal nanoparticles, due to the interaction of molecules and surface plasmons (mass movement of conducting electrons under the oscillating field of the incident wave), an increase in intensity occurs in Raman signal, which is known as the electromagnetic effect. Alternatively, the molecule is chemically adsorbed in metal nanoparticles, and the intensity of the Raman signal is increased as electrons transfer from the metal to the molecule and return to the metal again. Metals such as silver, gold, copper, and platinum have been used to observe the surface-enhanced Raman scattering phenomenon in various experiments [4]. SERS has grown rapidly over the past four decades and is expanding as applications for detection in the fields of chemistry, materials science, biochemistry, and biological sciences. The advances in fabricating SERS-based biosensors is considered as a significant evolution in the detection of biological analytes and chemicals [5,98,99,104]. Detection performance, direct and indirect fabrication methods for detecting biological materials, biosensors applications, mechanisms of amplifiers, and SERS-based biosensor structures to detect biological molecules are discussed in the present study.

2. Basis of Raman Spectroscopy

Photons are often absorbed or scattered when they collide with a reflected molecule. In Raman spectroscopy, photons of monochromatic light (single-wavelength light) are scattered in different directions after colliding with a sample. In Raman spectroscopy, the scattered photons from the sample are important. Most of the photons that collide with the molecule are scattered elastically. This type of scattering is called Rayleigh Scattering, in which the photons scattered from the sample have the same energy or wavelength as the photons colliding with the sample. In the year 1928, an Indian physicist named Chandrasekhara Venkata Raman discovered a phenomenon called Raman. In this phenomenon, the energy or wavelength of a beam scattered by molecules differs from the initial beam wavelength that collides with the sample. This type of scattering of light beams is called non-elastic scattering. Approximately one in ten million photons are non-elastically scattered after colliding with the matter. Moreover, the rate of difference in energy or wavelength of non-elastic scattered light depends on the compound's molecular structure. Raman spectroscopy has been developed based on the analysis of these differences to determine the molecular structure of different compounds. The change in wavelength or initial radiation energy provides important information about the molecular movements within the system. In Raman scattering, the photon collides with the material, and its wavelength shifts to higher or lower wavelengths after scattering (Fig.1). In this type of beam scattering, transferring to higher wavelengths is dominant, called Raman Stokes Shift. Also, transferring to lower wavelengths is named Raman Anti-stokes Shift [6-7]. The ratio of the intensity of anti-stokes shift to stock shift has been reported to increase with increasing temperature. The input photon collides with the electron cloud of functional group bonds and excites the electrons to an implicit state. The electron then returns from the implicit state to an excited vibrational or rotational state (Fig.1). This phenomenon causes the photon to lose some of its energy and manifest as a Raman stokes shift. The lost energy is directly related to the functional group's chemical identity, the bonded molecular structure to the functional group, the type of atoms of molecules, and the environment. Therefore, the Raman spectrum is specific to each molecule and can be used like a "fingerprint" to detect chemical identity, molecular compounds inside a liquid, on a surface, or in the air [7-8].

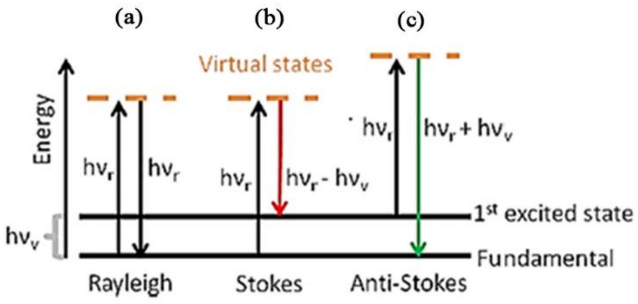


Figure 1. Diagram of energy levels in Raman spectrum scattering, (a) Rayleigh Scattering; (b) Raman stokes shift; and (c) Raman anti-stokes shifts; hvr is the energy of incident light, and hvv is the energy of vibrating light [8].

3. Main components of Raman spectroscopy

Raman device consists of four main parts: excitation source (laser), sample illumination system and light collection optics, wavelength selector (filter or spectrophotometer), and detector. After the laser light collides with the sample and scatters from its surface, the scattered light is collected by a lens and transmitted to the detector unit by a fiber. The wavelengths close to the laser wavelength (elastic or Rayleigh scattering) are absorbed by a special filter. Only Rayleigh beams are absorbed by a special filter. Only scattered beams that have changed in terms of energy or wavelength relative to the incident light can pass through and reach the detector (Fig.2).

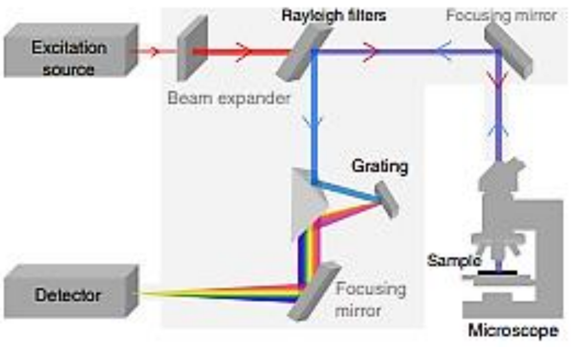


Figure 2. Internal components of the Raman device [1].

4. Applications of Raman Spectroscopy

Raman spectroscopy is used in many fields. This method can be applied for any project that requires non-destructive, microscopic, and chemical analysis as well as imaging. Whether the studied objective is quantitative or qualitative information, Raman analysis can easily and quickly provide key information. This technique can quickly characterize chemical compounds and samples with different phases, such as solid, liquid, gas, gel, and powder [9]. Another application of pharmaceutical criminology of Raman spectroscopy is the high power of drug characterization so that this technique can analyze and report cocaine, heroin, ecstasy, and other compounds of Phenethylamine ecstasy at a very high speed and within a few seconds [10]. In the field of antiquities research, each art object is unique, and sampling is normally prohibited, even if the sampling is in the size of 100 microns and takes minutes. Raman spectroscopy usually does not require sample preparation. For this reason, it is considered as a non-destructive technique. Therefore, Raman spectroscopy is a suitable tool for archaeologists, especially artwork researchers such as paintings, since this method is capable of investigating the pigments of the paintings with high accuracy. Thus, in recent years, many articles have been published on the use of a Raman microscope for pigment analysis of manuscripts and paintings. Besides, this method has been applied to analyze ancient inscriptions [11], potteries [12], and ancient glasses [13]. One of the most important non-destructive applications of Raman spectroscopy is the analysis of biological samples. This type of analysis can detect cancer from healthy tissue and identify cancer-prone cells. For instance, Kast et al. indicated that Raman spectroscopy is capable of detecting malignant tumors from a healthy breast sample and can detect early neoplastic changes in mice [14]. Since Raman signal measurements are often along with the characteristics of the analyzed compounds, Raman spectroscopy is capable of analyzing complex compounds, including in-vivo analysis.

Since the Raman signal can be obtained using a small probe, the optical fiber can be used as a probe. This feature makes it possible to easily shift and carry the spectrometer or test a Raman sensor inside the animal's body. One of the most important potential applications that can be mentioned for the Raman effect is the different techniques of this effect, each of which has many applications in different fields. Furthermore, since this type of spectrometer is cheap, small, and easy to use, it is very popular among scientists and researchers in various sciences [15]. Raman spectrum is the result of non-elastic scattering of light from the molecule of materials by which the vibrational spectrum of molecules can be obtained, and as a result, similar to fingerprints, molecules can be identified, and extremely detailed information about the molecular structure of materials can be obtained. Since the spectrum related to Raman scattering is inherently weak and sometimes invisible by noise and fluorescence effect, this method is not possible to detect molecules with low concentrations, and it is necessary to enhance the Raman scattering spectrum. SERS method is one of the efficient methods for studying low concentrations and detecting even single molecules [96].

5. Background of Surface-enhanced Raman Scattering (SERS)

Raman scattering spectroscopy is one of the most important analytical methods for physical and chemical studies and the detection of an extensive range of chemical and biological analytes. The inherent weakness of the Raman scattering cross-section prevents the achievement of a suitable detection for low dimensions, especially nanoscale single-molecule detection in different structures. SERS has been employed in several experiments to improve the sensitivity of the Raman method. Fleshmann et al. observed this phenomenon for the first time in 1974 for an electrochemically grown pyridine molecule adsorbed on the surface of silver electrodes. In 1974, for the first time, an unusually strong and increased enhancement of Raman scattering was reported for pyridine molecules. In this experiment, pyridine molecules had been adsorbed on the surfaces of silver electrodes that had been electrochemically roughened using oxidation-reduction cycles. In the early 1970s, primary research and extensive theoretical studies were conducted on the effect of SERS. From 1975 to 1980, the number of articles in this field was reduced, and no scientific application resulted because the effect of the increase of Raman was limited to a small number of molecules. Most basic studies had been limited to a small number of small polarizable molecules such as pyridine, benzoic acid, and its derivatives, or some ionic samples. In 1984, SERS' applicability was first reported as an analytical method for various types of chemicals and biological analytes [15-19]. This technology has shown many advantages in high-sensitivity spectroscopy and disposal of fluorescence radiation. Nowadays, the SERS method is applied to obtain information from molecules adsorbed on the surface of gold, silver, and noble metals. The discovery of SERS not only increases the sensitivity of Raman scattering detection but can also provide information on the absorption of the molecule and the process of molecule interaction with the substrate surface, which are considered in some studies [20-24].

6. Theories of Enhancement Mechanism for Surface-enhanced Raman Signal (SERS)

SERS is a sensitive and selective method in which Raman scattering is increased for molecules adsorbed on metal nanoparticles. By employing this approach, information can be obtained on the absorption of the molecule and the process of interaction of the molecule with the substrate surface in addition to analyzing molecular structures [25]. Since the intensity of Raman scattering is proportional to the square of induced dipole moment, it is possible to enhance this process in two ways [26]: 1) electric field enhancement, 2) polarity enhancement. Therefore, all theories related to SERS can be divided into two electromagnetic and chemical effects.

6.1. Electromagnetic Effect

In this case, the molecule is located near the surface or is physically adsorbed on metal nanoparticles, and the increase in the intensity of Raman scattering is due to the interaction of molecules and surface plasmons [26,97]. This effect is as a result of the development of enhanced electric fields on the uneven surface of metal due to plasmonic resonance of metal nanoparticles so that the replaced electromagnetic field resulted from the surface plasmon resonances is collected by the electromagnetic field of the excitation laser and causes the field to be amplified; therefore, the molecule becomes more polarized, and the Raman signal is amplified. The size, shape, and material of the metal nanoparticles' surface and surface roughness considerably influence the electromagnetic effect [27]. Consequently, it can be said that the electromagnetic mechanism is a guide for selecting the type of surface structure to achieve the appropriate enhancement for the Raman signal intensity at a specific excitation frequency. In other words, weak enhancers in the visible region may be excellent enhancers in the near-infrared region; in each case, optimization is required to increase the signal-to-noise ratio or the detector limit for a specific molecule, and of course, the SERS tests are different for each case [28]. As indicated in (Fig.3) and (Eq.1), in surface-enhanced Raman scattering, I_SERS (V_S) implies the intensity of the scattered signal, which is on the rate of the sample under N lighting, laser intensity of I(V_L), laser field enhancement factor of A(V_L), and the scattered Raman field enhancement factor of A(V_S) and the cross-section of Raman is dependent to the cross-section of the adsorbed molecules of Q_ads^R. An increase in the values of two factors of A(V_L) and A(V_S) is obtained by the surface plasmon resonances created on the surface of nanoparticles.

$$I_{SERS}(V_S) = N.I(V_L).(A(V_L))^2.(A(V_S))^2.Q^R_{ads} \qquad (1)$$

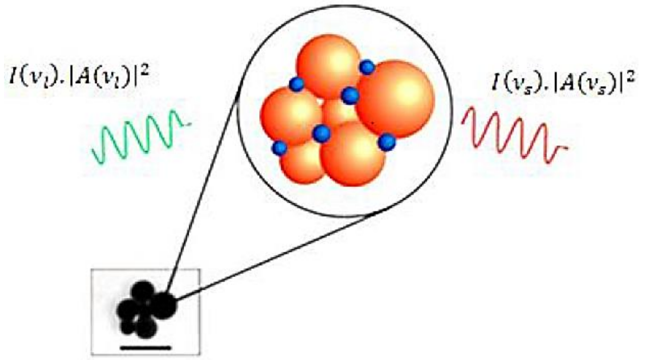


Figure 3. Demonstration of the interaction of electromagnetic light with a sample identified by small spheres and metal nanoparticles specified by large spheres [29].

In typical Raman scattering, the Raman signal's intensity is proportional to the Raman cross-section, the intensity of the laser, and the number of molecules in the probing volume [30].

$$I_{SERS}(V_S) = N.I(V_L).Q^R_{free} \qquad (2)$$

Although independent spherical nanoparticles provide an excellent pattern for accurately understanding the interaction between particles and electromagnetic radiation, many experiments show that these particles cannot be considered as best enhancers. According to many studies, non-spherical nanoparticles have a significant field enhancement compared to spherical nanoparticles, especially the enhancement is higher at sharp points. Moreover, the interaction of nanoparticles, which are close together, can cause more considerable enhancement in the field. The enhancement changes with shape and geometrical structure because of the aggregation of nanoparticles' physical and chemical properties, particularly the optical response and interaction with the target molecule [31-32]. The distance between two particles is a key factor in the rate of enhancement, and a high enhancement (approximately 10^{11}) can be achieved only at distances ranging from 1 to 2 nm [33]. High enhancements may also be made by combining the effect of a replaced field or near field and the effects of long-range coupling related to bipolar-bipolar coherence interactions between nanoparticles. Therefore, the arrangement of nanoparticles can be of great importance [33]. Electromagnetic enhancement is a function of the properties of the adsorbent surface, and its only relationship with the adsorbed molecule is the distance of the molecule from the surface. However, various experiments have confirmed the presence of another mechanism in the SERS process [34]. Hence, in order to interpret the SERS spectra, the interaction between the metal and the adsorbed molecule must also be considered. Indeed, there will be no signal without the electromagnetic mechanism, but the chemical mechanism interprets what was observed [28].

5.2. Chemical Effect or Charge Transfer

In this mechanism, it is assumed that the molecule forms a bond with the metal, and the transfer of electrons occurs between the metal and molecule. The charge transfer that takes place between the molecule and metal surface can be performed under three processes.

1. When a molecule interacts with a surface, the electron distribution and the polarization of the molecule will change. In the chemical effect, the enhancement resulted from a new electron state is due to bond formation.
2. The metal and molecule under investigation may form a surface complex that will change the molecule's polarization. Some of these surface complexes may even form new electron surfaces, and a phenomenon similar to intensified Raman occurs [27].
3. The third type, called "charge transfer process by electron excitation," is the most complicated type of charge transfer. This path occurs when the input laser energy is equal to the energy difference of "Highest occupied molecular orbital (HOMO)" or "lowest unoccupied molecular orbital (LUMO)" of surface molecules and "Fermi level" or surface alignment of the metal. This path can be associated with the excited state of the metal molecule system and the charge transfer between the molecule and the metal surface and cause a significant increase in the Raman intensity of the adsorbed molecule [35]. (Fig.4) demonstrates the load transfer processes [36].

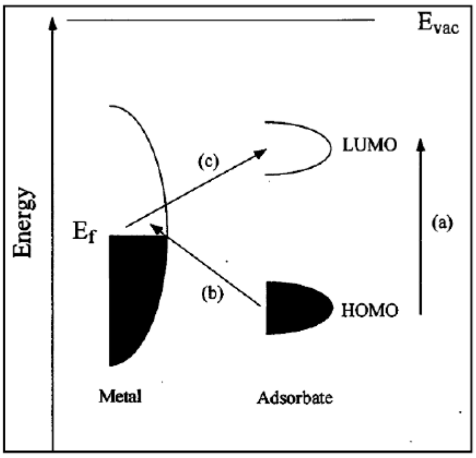


Figure 4. Schematic of the energy levels of the adsorbed molecule on the metal and possible charge transfers shown by states (a), (b), and (c) [36].

The chemical mechanism can be applied to explain Raman peaks' intensity changes for the change of wavelength of incident light [27]. In some cases, it has been reported that this effect usually causes the Raman intensity to increase 10 to 100 times; however, it should be noticed that the main factor in enhancing the Raman signal is the electromagnetic effect. The chemical effect is only possible for the first layer of the adsorbed molecule.

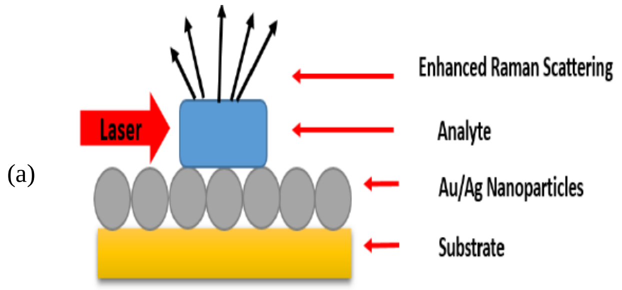
7. Mechanism of Metal Nanostructures for Sensitivity and Enhancement of SERS

Nowadays, the SERS method is applied to obtain information from molecules adsorbed on the surface of gold, silver, and noble metals. The discovery of SERS not only increases the sensitivity of Raman scattering detection but can also provide information on the absorption of the molecule and the process of molecule interaction with the substrate surface, which are considered in some studies [37-41]. At present, the SERS enhancing factor is known for two main phenomena: 1) The electromagnetic effect (field effect) in which the molecule is exposed to an external field. This larger replaced field is created by electromagnetic resonance near the metal surface, and 2) chemical effect (molecular effect) in which molecular polarization is affected by the interaction between the surface of the molecule and the surface of the metal [37]. By employing SERS, it is possible to study single molecules that enhance the Raman signal up to 10^{10} by binding nanoparticles to single molecules. [40-43]. Consequently, the detection of biological substances in small amounts and early recognition of them is of great importance. Nowadays, several methods such as electrochemistry [44], gas chromatography, and High-performance Liquid Chromatography (HPLC) [45], gravimetry [46-47] and optical spectroscopy [40-43] are used to determine the number of biological materials. These methods are destructive, difficult, polluting, and intra-laboratory, and require sample preparation, trained specialists, well-equipped laboratories, and high cost and time. Hence, it is essential to develop a non-destructive, simple to use, fast, low-cost, non-polluting, and portable method to be applicable outside the laboratory and to have less need for sample preparation. On the other hand, the identification and detection of minimal amounts of biological analytes and chemicals are also important. Biological materials and analytes can be detected using infrared spectroscopy and Raman spectroscopy, which are considered as fingerprint spectroscopy and evaluate the molecular vibrations of material [48-49]. In infrared spectroscopy, due to the active molecular vibrations of water, the identification of biological species is difficult, and the sensitivity of its detectors is low. Also, in Raman spectroscopy, the study of molecules with low concentrations is practically impossible because of the inherent weakness of the signal resulted from Raman scattering [50]. One of the methods to enhance the Raman signal is to use metal nanostructures, which can form a strong electric field near the nanostructures because of surface plasmon resonances or effectively enhance the scattering signal by increasing the rate of light scattering from these nanostructures, which is followed by the observation of molecular vibrations with better and greater signals. The SERS method, a sensitive and selective method, results in enhanced Raman scattering of molecules that are adsorbed on metal structures [51]. By irradiating light (laser) on the uneven metal surface, because of the surface plasmons resonance of metal nanostructures by the electromagnetic field of laser, enhanced electric fields are developed around the metal [52-53], as if the electric field resulted from the laser light is enhanced. Hence, the molecule that is placed in this enhanced electric field becomes more polarized, and thus its Raman signal is enhanced [54]. In this approach, when the studied biological analytes and chemicals are located adjacent to the metal surface or physically adsorbed to the metal nanoparticles, the intensity of the Raman signal increases as the result of the interaction of biological analytes, chemicals, and metal surface plasmons; hence, SERS can be applied to rapidly and accurately detect microbiological analytes. Increasing the electromagnetic field in the plasmonic resonance mode of the nanoparticles improves the excitation and emission of enhanced Raman in the SERS mode. In metal nanoparticles such as gold and silver nanoparticles and noble metals with proper shape and dimensions, the electromagnetic enhancement can be increased by a high factor called the hotspot and is directly related to the increase in sensitivity and enhancement of SERS. In order to improve the sensitivity of SERS-based nano-sensors and plasmonic resonance, nanostructured arrays can usually be adjusted so that many hotspots to be placed on them [54,55].

8. SERS as a Biosensor for Detecting Biological Analytes and Chemicals

In recent years, the surface plasmon of nanoparticles has attracted researchers' attention due to its rapid response and high resolution in sensors. Noble metal nanoparticles, particularly gold and silver, show an excellent optical feature in the calculation of surface plasmon resonance, which has received much attention in the electronics industry and biochemical and medical applications. In the case that the particle size reaches nanometers, a strong absorption is observed in the visible region, which is caused by the surface plasmon resonance occurring in the visible, ultraviolet spectrum and using to design biochips and biosensors. Surface plasmon resonance is a suitable tool for describing biomolecular interactions and is extensively applied in biosensors. Gold nanoparticles are used to distinguish cancer cells from healthy cells; placing these nanoparticles increases the absorption of unhealthy cells compared to intact cells. The width and position of the absorption peak of plasmon resonance depend on the size, shape, type of metal, the surrounding dielectric environment, and the distance between the nanoparticles, determined by the shift of the surface plasmon resonance peak over the visible spectrum. Surface plasmon resonance occurs when the frequency of incident light to metal nanoparticles is equal to the frequency of surface plasmon. In addition to the particle size, the adsorption peak of surface plasmon of nanoparticles also depends on the environment of the nanoparticles, and by changing the refractive coefficient of the environment, the peak shift of the surface plasmon resonance appears, which is applied to design chemical and biological sensors [56-61]. The films composed of noble metal nanoparticles (typically gold (Au) and silver (Ag)) are now popular and have caused a high interest in scientific research in the field of nanotechnology because of their remarkable optical features and surface plasmon resonance. When the electrons are shifted from their equilibrium position, the conductive electrons in noble metal nanoparticles lead to the light enhancement and have potential applications in many different fields, including biosensors [62-63], design of solar cells [64], and SERS [65]. Because of the extraordinary optical properties of noble metal nanoparticles, many existing methods for fabricating SERS biosensors have been proposed in the last few years [66,94]. Such methods can certainly be applied as a SERS biosensor. Raman scattering is the result of non-elastic scattering of light from the material, and considerably detailed information about the structure of a molecule can be obtained using this effect. Since Raman spectroscopy does not encounter the same problems as other spectroscopies, it is extensively used as a supplementary method. For instance, the identification of biological species is difficult, and the sensitivity of its detectors is low due to the active molecular vibrations of water in IR spectroscopy. Compared to electron- and ion-based spectrometers requiring a high vacuum, Raman spectroscopy not only provides molecular studies under normal conditions but can also be used to investigate catalytic processes and procedures in the metal-electrolyte interface [65]. However, the signal related to Raman scattering is inherently weak and makes its detection challenging [66]. One of the methods to enhance the Raman signal is to use metal nanostructures that can enhance the scattering signal because of surface plasmon resonance. This method is called SERS spectroscopy, which is a sensitive and selective method resulting in enhanced Raman scattering of molecules adsorbed on metal structures [67]. In 1974, this phenomenon was first observed in the case of the electrochemically grown pyridine molecule adsorbed on the surface of silver electrodes. Raman spectrum related to 6.25 mM solution of pyridine in silver colloidal solution compared to its 0.1 M solution is observed, and the enhancement of Raman lines [68] so that an enhancement in Raman signal resonance was observed in experiments in which the surface roughness of the electrode was as low as possible (approximately ten times surface increase) [69]. Over the last few decades, metals such as Au, Ag, Cu, and Pt have been applied to observe the SERS phenomenon in various experiments. Successful application of metal nanoparticles in SERS depends on the properties of the metal, such as morphology (shape, size, and aggregation mode) and the nature of the metal, which has been used as the substrate. Among the various metals, silver and gold have been mostly applied because of their wide plasmon resonance in the Vis-NIR region, high stability, and easy sample preparation [70]. (Fig.5-a) indicates the scattering pattern of SERS. Silver or gold nanoparticles placed on the substrate enhance the Raman spectrum. In this method, metal nanoparticles are used to enhance the Raman spectrum. SERS is a sensitive and selective method in which Raman scattering is enhanced for molecules adsorbed on metal nanoparticles. By applying this method, in addition to analyzing molecular structures, information about molecule absorption and the process of interaction of the molecule with the substrate surface can be obtained [71]. Then, gold and copper surfaces were also assessed; such metals are capable of enhancing the Raman spectrum; these metals are highly used because of the high stability of gold and silver [72]. Substrates and different methods were then applied to fabricate active SERS substrates to quickly and accurately investigate the biological and chemical samples. These substrates are made both physically and chemically [73-74].

Biological species and materials can be merely identified using SERS biosensors [75]. Various techniques such as HPLC [76], TMS (MS/MS)[1] [77], GC-MS[2] [78], amperometry [79], chromatography [80], magnetic resonance spectroscopy (MRS) [81], and SERS are applied to measure biological materials and molecules [82]. These techniques are less sensitive than the SERS method, and their use requires high costs. Raman spectroscopy is an excellent method to identify the composition of various materials, such as biological species; however, at low concentrations, the Raman signal of biological species is considerably low [83-84]. In this method, due to the interaction between metal plasmons of surface and species, the intensity of the Raman signal increases with placing the species adjacent to the surface or their physical adsorption on metal nanoparticles. Thus SERS can be applied to quickly and accurately identify biological species [85]. Another spectroscopy method is SERS, which is extensively applied in the investigation of low concentration and even single molecules due to its high enhancement coefficient in the scattering signal (10^3-10^6). This method is applied in studies of biomedicines, vitamins [86], biomolecules, viruses, and bacteria [87], analysis of explosives [88], drug-detecting [89], signaling environments [90], geology [91], etc. in addition to analytical sciences. (Fig.5-b) demonstrates a schematic of the applications of SERS active substrates for the detection of biological and chemical materials [92,93]. In order to present a comprehensive and brief definition of this effect, it can be said that SERS is a sensitive and selective method that results in the enhancement of Raman scattering for molecules adsorbed on metal nanoparticles. By applying this method, in addition to analyzing molecular structures, information about molecule absorption and the process of interaction of the molecule with the substrate surface can be obtained.



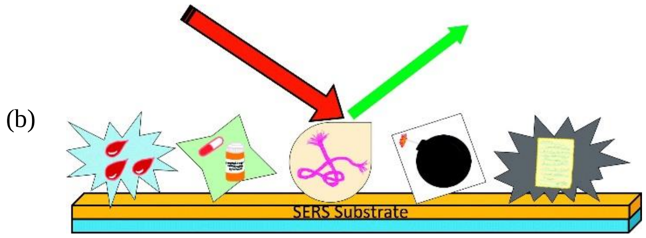


Figure 5. (a) surface-enhanced Raman scattering design. Silver or gold nanoparticles placed on the substrate enhance the Raman spectrum analyte [93], and (b) a schematic of the applications of SERS active substrates for the detection of biological and chemical materials [92].

9. Conclusion

The Raman spectroscopy method is based on receiving information from the light scattering phenomenon while colliding with the material. Nowadays, this approach has many applications in various research fields, which also provides important information about the structure of molecules so that Raman bands can be considered as a fingerprint of a composition. Since the Raman scattering spectrum is inherently weak and sometimes invisible to noise and the fluorescence effect, it is not possible to detect molecules with low concentrations by this method, and it is necessary to enhance the Raman scattering spectrum. Surface-enhanced Raman scattering (SERS) is one of the most efficient methods for studying low concentrations and detecting even single molecules. In the present study, first, the theories of enhancement mechanism for surface-enhanced Raman signal (SERS) were briefly discussed, and then the applications of SERS biosensor to detect biological analytes and chemicals were reviewed. This method is an effective way of investigating and diagnosing diseases in a non-destructive manner. Gold, silver, and copper nanoparticles were introduced as enhancement factors of Raman signal by the mentioned methods. SERS, after its discovery, was first used to study electrochemical reactions and the adsorption of molecular species on metal surfaces. SERS was an attractive tool for assessing molecules in small quantities in chemical decomposition because of the inherent molecular fingerprinting feature and the potential to detect single molecules. The fabrication of SERS active substrates is a modern method for detecting toxic substances, drugs, industrial toxic chemicals, biological analytics, and analysis of other biological and non-biological species with low concentrations due to the simplicity of the manufacturing process, low cost, and the capability of detecting materials with low concentrations. As a result, SERS can be highly applied in the field of internal security, medicine, and environmental monitoring.

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