General Background of SERS Sensing and Perspectives on Polymer-Supported Plasmon-Active Multiscale and Hierarchical Sensor Particles

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Surface-enhanced Raman scattering (SERS) is one of the most powerful analytical techniques for the identification of molecules. The substrate, on which SERS is dependent, contains regions of nanoscale gaps (hotspots) that hold the ability to concentrate incident electromagnetic fields and effectively amplify vibrational scattering signals of adsorbed analytes. While surface plasmon resonance from metal nanostructures is a central focus for the SERS effect, the support of polymers can be significantly advantageous to provide larger exposure of structured metal surfaces for efficient interactions with analytes. Characteristics of the polymer particles such as softness, flexibility, swellability, porosity, optical transparency, metal-loading ability, and high surface area can allow diffusion of analytes and penetrating light deeply that can enormously amplify sensing outcomes. As polymer-supported plasmon-active sensor particles can emerge as versatile SERS substrates, the microfluidic platform is promising for the generation of sensor particles as well as for performing sequential SERS analysis of multiple analytes. Therefore, in this perspective article, the development of multifunctional polymer–metal composite particles, and their applications as potential sensors for SERS sensing through microfluidics are presented. A detailed background from the beginning of the SERS field and perspectives for the multifunctional sensor particles for efficient SERS sensing are provided.

1. Introduction

The technological advancement of the spectroscopic and microscopic techniques has allowed shifting the detection limit of various analytes with very high resolution down to the single-molecule level for chemical and biochemical analyses.[1–4] For instance, light microscopy (fluorescence microscopy) can detect and analyze single fluorescent molecules used as reporter tags in biological samples through imaging.[3] While the microscopic technique is efficient, a similarly efficient method is required that can detect trace amounts of toxic compounds, pesticides, biological analytes, etc., for applications in the field of healthcare, agriculture, the food industry, and the environment. In this regard, surface-enhanced Raman spectroscopy is a very promising and powerful technique for the identification of trace analytes and even at the level of single molecules through molecular spectral fingerprints.[5–7] Surface-enhanced Raman scattering (SERS) sensing is of particular interest for substances that show high enforcement factors. The preferential adsorption of molecules on the metal surface is an important focus for such strong enhancements. Amino groups, nitrogen heterocycles, thiols, thioethers, and disulfide compounds are known for their high affinity to gold and silver surfaces, which are well suited for SERS measurements.[8–9] These groups are found in such very important classes of biomolecules like proteins as well as nucleic acids, and in many other biomolecules, too.[10] Hence, the combination of molecular surface enrichment and the sensitive measurement of specific molecular structures and functions make SERS an invaluable tool in biomolecular diagnostics. Also, the Raman bands are much narrower than the fluorescence bands, therefore, the SERS can be applied for multiplexed analysis and fingerprint identifications in a wide range of various fields.[11]

The substrates for carrying the SERS-active material are playing a crucial role in the strength of the SERS effect.[7–12] It is required that substrates are made in a way that they present a relatively roughened (corrugated) metallic surface and contain nanometer-scale gaps (junctions) useful in concentrating an incident light and amplifying sensing signals of the proximate analytes.[10] Since the discovery of the SERS in the 1970s,[13–16] many substrates have been fabricated through nanofabrication techniques as well as via synthesis and self-assembling in the lab and are available commercially, too. Despite the accessibility of a diverse range of SERS substrates that are routinely

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utilized, the search is continued for dynamic substrates that are made of multifunctional characteristics and reveal extraordinary signal outcomes in addition to their suitability for using them in biological systems. Considering the requirement of dynamic nature, multifunctional characteristics, and parameter tunability, particulate polymers are very promising because of their softness, crosslinking, porosity, swellability, and enhanced surface area. Most polymers are not directly absorbing or scattering light and hence do not contribute to the SERS effect. However, polymer particles can serve as an excellent host (support) for the distribution and enrichment of metal nanostructures (plasmon-active SERS components) in their interior and at the surface. For instance, due to the crosslinking and large water content through hydrophilicity, hydrogel particles can systematically accommodate and distribute a large amount of smaller metal nanoparticles in their interior. In this perspective article, therefore, the synthesis strategies and applications of polymer-based sensor particles for SERS sensing of analytes are presented.

On one hand, sensor particles are essential to be relatively homogeneous in size and composition, as well as reproducible for uniform and sustainable SERS sensing outcomes. On the other hand, a suitable and robust platform is desired where SERS sensing experiments can be performed sequentially. For fulfilling both requirements, microfluidics is a versatile platform for the synthesis of uniform sensor particles and conducting sequential SERS analysis of multiple analytes. The semimicrofluidic emulsion polymerization approach is efficient that allows the formation of surface swellable nanoscale polymer particles of the charged surface. In addition, metal deposition at the surface can be initiated electrostatically and further application of metal enforcement (ligand-free) through metal-catalyzed metal deposition makes the polymer–metal composite nanoparticles ready as nanoscale sensor particles for SERS analysis. Besides, photopolymerization in droplet microfluidics can allow the in situ formation of metal nanoparticles embedded in hydrogel polymer particles of a micrometer length scale. Here too, ligand-free metal layer enforcement at the surface via metal-catalyzed metal deposition forms the microscale sensor particles.

Overall, the scope of this perspective article is to provide the background of the SERS field with progressive development since its discovery with particular focus on the polymer–metal composite sensor particles as SERS substrates and give the broad perspectives of using hierarchical architectures of multifunctional sensor particles for efficient SERS sensing through microfluidics. A schematic overview of the content of this article is highlighted in Figure 1. Initially, at the beginning of this perspective article, a brief introduction about SERS sensing is given followed by a brief description of the development of various SERS substrates with the advantages of flexible sensors (Section 2). Afterward, the importance and developments of the sensor particles together with their building block components are described for particle-based SERS sensing. In this regard, the characteristics of the metal nanoparticles, polymer particles, and composite sensor particles are provided (Section 3). More specifically in Section 3, a brief literature survey of various metal nanoparticle systems and how they are advantageous for significant enhancement of SERS signals are shown in Section 3.1. Section 3.2 describes key characteristics of the polymer particles useful for the development of versatile sensor particles. Section 3.3 gives the conceptual importance of the polymer–metal composite particles (sensor particles) for SERS sensing. In the following Section 4, a brief account of the importance of microfluidics for the generation of sensor particles and for performing multiplexed and sequential SERS sensing experiments in a sustained manner is described. In the following Section 5, broad perspectives on hierarchical development of the sensor particles for sustained, multiplied, and sensitive SERS analysis is given. A summary of the perspective articles is provided in Section 6. Overall, a key motivation of this perspective article is based on the research conducted on sensor particle-based SERS sensing mainly from the authors’ laboratory during the last decade.

2. SERS Sensing

Traditional Raman scattering is a very useful technique for molecular identification, but its detection limit and weak signal intensity are a concern (drawback) for sensitivity and low concentration of analytes. Advantageously, SERS is an efficient vibrational spectroscopy technique that offers an enhancement of orders of magnitude in Raman intensity. In special cases, the SERS intensity enhancement factor can be as high as $10^{14}$–$10^{15}$ that is sufficient to detect a single molecule with a concentration as low as $10^{-14}$–$10^{-15}$ M using Raman scattering. In the SERS, the amplification of electromagnetic fields (intensity enhancement) is not only generated by increasing the concentration of the sample (target analyte) but mainly due to the excitation of localized surface plasmons at a metal surface. Ideally, the surface of noble metals such as silver or gold can be fabricated with nanoscale roughness where adsorption of analyte molecules takes place, which allows concentrating incident light and enhances scattering outcome.
2.1. General Concept and Background of SERS Effect

Light has been an extremely essential source of inspiration as well as a tool in the development of various technical devices to understand the molecular and materials properties based on optical phenomena throughout the history of modern science and technology.[12–37] In particular, a large range of light-based discoveries has been observed and occurred in the last few decades of the 19th century and an initial couple of decades of the 20th century. The understanding and importance of the Rayleigh scattering,[18] i.e., elastic scattering of light by particles where scattered light has the same energy with regards to frequency, wavelength, and color as of incident light, has been widespread in optical instrumentations and applications. Progressively, through lab experiments on dust-free liquids by sunlight, C. V. Raman in 1928 has observed modified scattered radiation of lower frequency than incident beam (inelastic or Raman scattering) alongside the same wavelength scattered radiation (Rayleigh scattering).[33] It was difficult to observe this fascinating inelastic scattering effect largely because inelastic scattering (Raman effect) is a very weak effect where only one photon from one million can be observed with a different wavelength than the wavelength of the incident photon. To amplify the effect of scattered light, an intense light source was demanded.

In general, Raman spectroscopy is one of the most important methods for the investigation of the structure of molecules.[39] In analogy to infrared (IR) spectroscopy,[38] it supplies information about oscillatory resonances, which are strictly related to bond strengths, bond lengths, and masses of involved atoms. In contrast to optical spectroscopy (UV/vis),[40] the resonance bands are smaller and much more characteristic for specific molecular structures, functional groups, and molecular geometries in Raman spectroscopy. Patterns of Raman scattering can be used as fingerprints for the identification of single substances or special substance mixtures. The intensity of Raman signals is dependent on the concentration of substances and can, therefore, also be used in quantitative analyses. The narrow bands allow accurate characterization of small resonance shifts, which indicate changes in bond strength or molecular geometries due to chemical modification of molecules, dimer or aggregate formation, changes in intermolecular forces, or solvent effects. Water is not a suitable solvent for oscillation spectroscopy with IR radiation (middle IR range), because of the intensive and broad absorption band of the O–H oscillation, which is related to different association states of water. In contrast to IR spectroscopy, Raman spectroscopy can be applied in aqueous solutions, too. The Raman effect allows gaining oscillation information with visible, UV-, or near-IR radiation, which are not or much less affected by the water resonances. This difference between IR and Raman spectroscopy is particularly important for biomolecules and cellular systems that are existing and work in aqueous environments.[41–43] Thus, Raman spectroscopy became an important tool for biomedical, biochemical, and cell biological investigation.[44–45] The schematics of the basic principles of IR, UV–visible, and Raman spectroscopy are shown in Figure 2.

Unfortunately, the sensitivity of classical Raman spectroscopy is low. This low sensitivity is the reason for the main application of Raman spectroscopy for the characterization of pure substances or substance mixtures at high concentrations, for example, for investigations in organic, inorganic, and coordination chemistry, on the one side. On the other side, classical Raman spectroscopy is less suited for the investigation of biological systems due to the typically low concentrations of investigating biomolecules. With the fascinating introduction of the laser in the 1960s,[46–47] it was possible to supply the intense light source for the Raman experiments which was the expectation since the first experimental observation of the Raman effect in 1928. The laser delivers the intense monochromatic light to the sample, which can allow detection of enhanced Raman scattering outcomes at the detector. In further advance, Fleischmann et al. in 1974 observed the amplified Raman signal by orders of magnitude when pyridine molecules have been placed on the surface of roughened silver electrodes.[33] In the following, Jeanmaire and Van Duyne,[48] as well as Albrecht and Creighton,[49] have confirmed in 1977 the amplification of the Raman signal when the molecules are proximate to the roughened metal surface. With those experiments, it was strengthened that the signal amplification was not only due to the increase in the concentration or number of molecules excited by the laser. Instead, it was claimed that the amplification of the Raman signals was possibly due to the generation of intense electric fields at the surface of the metal.[14] Moreover, it is also a possibility that the amplification of the signals can probably be realized because of the formation of a Raman resonant molecule–metal complex.[50] This effect of the amplified Raman scattering signals upon placing molecules on the roughened metal surface has been termed as SERS. In general, the electronic systems of the molecules are in strong interaction with the electrons of the metal upon irradiation by the laser that enhanced the scattering signals.[51] It is hard to observe in the case of adsortion of molecules on oxide, salt or hydroxide films as they are formed spontaneously on the surfaces of less noble metals. But it is found typically on the surfaces of noble metals, whereby the adsorbed molecules are directly connected with the metal surface atoms.[52] Thus, particularly high SERS effects can be found on nonoxidized silver surfaces. Instead of molar concentrations in classical Raman spectroscopy, SERS is applicable for concentrations in the micromolar, nanomolar, or even in the picomolar and femtomolar range.[2] Very strong signals at low solvent concentrations can be achieved if molecules are locally enriched by high affinity to the metal surface. Biomolecules containing amino and thiol groups are preferably adsorbed on electron-rich metal surfaces. Enforcement factors for Raman scattering up to about $10^{11}$ to $10^{14–15}$ have been reported in the case of using this effect.[53] This enforcement of Raman signals makes Raman spectroscopy particularly promising for the application of biomolecular systems alongside a wide range of other analytes in a diverse environment. Meanwhile, SERS measurements are well introduced in biomolecular analytics, biomedical investigations, and biomolecular screenings.[54] The strong increase in sensitivity of the scattering signals with high enhancement factors can allow detection of the single-molecule,[55] hence SERS is an invaluable analytical tool finds applications in a wide spectrum. A brief overview with features regarding IR, UV–visible, Raman, and SERS is provided in Table 1. In addition, a brief timeline for the milestone developments with regards to Raman scattering is shown in Figure 3.
2.2. Overview of SERS Substrates

The successful and accurate sensing of the analytes by SERS is influenced by many factors, but possibly the most important factor is the SERS substrate.\(^7\) Substrate—a component with active surface characteristics—can be defined as solid support on which chemical reaction occurs. For instance, biomolecules with a thiol functional group interact with a gold surface (Figure 4A). In general, the SERS substrates are designed with a metal surface that has nanoscale features (Figure 4B). On one hand, it was possible to develop portable analytical instruments largely due to the advancement of the field of optics.\(^{50–52}\) On the other hand, the advancement of nanofabrication techniques has allowed fabricating various types of SERS substrates ranging from simple to complex properties for their applications to detect diverse analytes in various conditions.\(^7\) The detailed observations during analysis and proposed mechanisms for the signal enhancement during the last four decades have established understanding that
Table 1. A brief overview of the key features of the main spectroscopy techniques (IR, UV–visible, Raman, and SERS) for the identification of various molecules and compounds of interest.

| Spectroscopy | Light source | Detection | Type       | Features                                                                 |
|--------------|--------------|-----------|------------|---------------------------------------------------------------------------|
| IR           | IR radiation | Transmission | Vibrational | • The vibrational transition occurred upon absorption of the IR radiation, hence called vibrational spectroscopy |
|              |              |           |            | • IR Frequency matched with the frequency of molecular vibration that gives the peak |
|              |              |           |            | • Gives information of oscillatory resonances, which are strictly related to bond strengths, bond lengths, and masses of involved atoms |
|              |              |           |            | • IR bands correspond to the characteristic functional groups and bonds of the molecules or compounds |
|              |              |           |            | • Electronic transition does not occur due to less energy of IR radiation than UV light |
|              |              |           |            | • Absorption of IR radiation is restricted to substances that have small energy differences in their vibrational and rotational states |
|              |              |           |            | • Water is not a suitable solvent for IR spectroscopy due to the broad absorption band of O–H oscillation |
| UV–visible   | UV–visible radiation (tungsten lamp for visible region and deuterium lamp for UV region) | Absorption | Electronic | • Uses light in UV and visible range of the electromagnetic spectrum |
|              |              |           |            | • Excitation of electrons in the atoms and molecules from the ground state to excited states realized where absorption by molecules takes place at the wavelength specific to particular molecules |
|              |              |           |            | • The electronic transition takes place due to high energy of UV and visible radiation than IR radiation |
|              |              |           |            | • It works based on Beer–Lambert law[17] |
|              |              |           |            | • High resonance peak observed |
| Raman        | Laser (visible or IR radiation) | Scattering | Vibrational | • Gives information of oscillatory resonances, which are strictly related to bond strengths, bond lengths, and masses of involved atoms |
|              |              |           |            | • Resonance bands are small and more characteristic for specific molecular structures, functional groups, and molecular geometries |
|              |              |           |            | • Inelastic light scattering |
|              |              |           |            | • Suitable for aqueous solution |
|              |              |           |            | • Suitable for analysis of biomolecules and cellular systems, which deals with the aqueous phase |
|              |              |           |            | • The Raman effect allows gaining oscillation information with visible, UV-, or near-IR radiation |
|              |              |           |            | • Limitation: weak signal intensity and sensitivity is low |
| SERS         | Laser (visible or IR radiation) | Enhanced scattering | Vibrational | • A surface plasmon is a minimum requirement for enhanced scattering |
|              |              |           |            | • SERS solid substrate shows surface plasmon polariton |
|              |              |           |            | • Nanoparticles present localized surface plasmon resonance with significantly enhanced SERS signals |
|              |              |           |            | • Hotspots concentrate electromagnetic light efficiently and significantly enhance scattered signals of analytes |
|              |              |           |            | • Enhancement factor: as high as $10^{14}$–$10^{15}$ |
|              |              |           |            | • Detection limit: single-molecule with concentration as low as $10^{-13}$–$10^{-14}$ M |
|              |              |           |            | • Label-free and nondestructive analytical technique |
|              |              |           |            | • Sensing (detection) of amino groups, nitrogen heterocycles, thiols, thioethers, disulfide compounds, etc. |

the SERS enhancement is mainly originated from two effects: chemical effect and electromagnetic effect (Figure 4C,D).[9,53–54] The electromagnetic effect originates when laser light interacts with the roughened metal nanostructured surface, which excites the surface plasmons.[54] The surface plasmon resonance is in a more confined and concentrated space near the hotspot, which concentrates the electromagnetic field and releases significantly enhanced scattering signal of the analyte molecules revealing information of vibrational fingerprints of molecules.[53] This effect is also a chemical effect in which the Raman cross-section of a molecule located on a metal surface leads to the formation of a metal–molecule complex.[9]

Various methods are commonly utilized to fabricate substrates ranging from top-down fabrication to bottom-up synthesis and self-assemblies. Initially, the electrochemically roughened electrodes have shown great promise during the next two decades of the discovery of SERS due to its easy preparation, simplicity, and low cost.[13–14,53–56] Immediately after the SERS discovery, it was investigated that enhancement in the SERS intensities is due to the localized surface plasmons arising from the metal nanostructures with a roughened surface on which molecules of interest can be adsorbed.[15] Also, the advancement in high-resolution electron microscopy has enabled capturing nanoscale images of the substrate and suggested that the presence of junction-like nanoscale areas called hotspots are responsible to concentrate the light and plays a key role in the SERS enhancement factor.[37–38] Furthermore, the continued emergence of the SERS field with classiness in
Figure 3. A brief overview of the key milestones in the development of Raman scattering effects. In 1871, a concept of the elastic scattering of light by particles had been proposed by Rayleigh. Adolf Smekal in 1923 had theoretically predicted the scattered light of different energy than the energy of incident light. In 1928, a major milestone was achieved when C. V. Raman had experimentally observed a new type of secondary radiation, which later became famous as Raman scattering. Raman effect is a very weak effect where one in million photons reveals an inelastic scattering. Raman realized the need for an intense light source. In this regard, a technique for the generation of monochromatic IR radiation had been proposed by Schawlow and Townes in 1958. In a quick time in the following year 1959, two papers discussed the electron-excited gaseous systems by Javan and Sanders. Based on the proposals of these 1958 and 1959 papers, T. H. Maiman had proactively developed the first laser in 1960. Laser has provided the intense monochromatic light source, which accelerated the use of Raman scattering in a broad range of applications. In 1974, Fleischmann et al. observed the amplified scattered signals when they placed pyridine molecules on the roughened silver electrode. In 1977, two research groups had confirmed the amplification of the scattering signals of the molecules in close contact to the roughened metal surface and provided the explanation that the amplification is not only due to the increase in the number of molecules but mainly due to the generation of an intense electric field at the metal surface or the formation of a Raman resonant molecule–metal complex. Afterward, Moskovits in 1978 had proposed that the origination of the large signal is due to the optical excitation of collective oscillations of the electrons in nanoscale featured metal surface. This effect of the signal enhancement by the surface has been termed as surface-enhanced Raman scattering (SERS) and became an extremely important tool as a label-free and nondestructive analytical technique for applications in a wide range of different areas including biological samples. With further progress, a first report on the single-molecule detection (ultimate limit) by SERS had been reported in 1997 by Kneipp et al.
instrumentation (benchtop and portable) and advanced substrates has subsequently allowed detecting the trace amount of analytes at the ultimate limit of the single-molecule detection.\textsuperscript{[49,59–60]} Various nanofabrication methods can be employed where noble metal nanostructures can be bound, for instance, on the silicon wafer for the formation of SERS substrates.\textsuperscript{[7,57]} In addition to the silicon wafers, various glass-based solid supports can also be used for depositing metal nanostructures on the surface.\textsuperscript{[61]} To achieve a high binding reproducibility, the surface of base materials and metal colloids can be functionalized with various ligands which possess functional groups such as amine, thiol, and cyanide (Figure 5A).\textsuperscript{[62]} On the other hand, a wide range of lithographic techniques (top-down approaches) such as soft lithography, photolithography, electron beam lithography, etc., can be used to form ordered arrays of the nanoparticles on the solid base.\textsuperscript{[9,63]} As shown in Figure 5B, the nanopillars with deposited metal nanoparticles at the top and arrays with sharp tips can be useful to provide a SERS-active area for signal enhancement toward the detection of analytes.

Besides nanofabrication methods, bottom-up wet chemical syntheses of metallic nanoscale particles with structured characteristics can be developed for the generation of SERS-active nanoparticles. Here, nanoparticles themselves can act as substrates in the solution phase mainly. The structured nanoscale metal particles possess a light-concentrating area in the form of sharp tips, corners, or edges. In such regions of the nanoparticles, the surface plasmon resonance is intense. When analyte molecules are placed on these structured metal nanoparticles, enhanced SERS signals with molecular fingerprint information can be observed. More details about various types of structured metal nanoparticles for the SERS analysis are provided in Section 3.1 below. A representative scheme for the bottom-up approaches of the solution-based metal nanoparticles is shown in Figure 5C.

On one hand, structured nanoparticles such as nanostars or nanocubes possess a light-concentrating area for the SERS effect. On the other hand, potential hotspots can be generated by linking isolated nanoparticles in a controlled manner. As far as nanoscale gaps and junctions are important in SERS substrate for light concentration, the nanoparticle aggregation strategies are very promising approaches that can form aggregated nanoparticles equipped with junctions. Usually, the nanoscale particles possess a high surface-to-volume ratio and high surface energy. To achieve a thermodynamically stable state, nanoparticles can aggregate randomly if the surface is not properly protected with surface-active agents. The random, uncontrolled, and larger aggregate can exhibit weak plasmonic resonance, and hence directly impacting SERS intensity to lowered down. In address, various approaches for the controlled

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**Figure 4.** Concept of the origin of SERS effect when molecules are placed near the metal surface (substrate). A) A general chemical reaction on a basic substrate where chemical interaction between molecule and substrate takes place. B) SERS-active substrates are nanostructured metals equipped with nanoscale features such as roughness, nanoscale pattern, or nanogaps that can concentrate electromagnetic fields for enhancing the SERS signals of molecules. C) One of the two key effects for the origin of SERS signal enhancement: electromagnetic effect in which excitation of the surface plasmon is realized upon the interaction of the metal nanostructure with light. Reproduced with permission.\textsuperscript{[84]} Copyright 2003, American Chemical Society. D) Chemical effect in which molecule forms the complex with metal and is excited to the vibrational state upon interaction with the light source. The polarizability of the molecules becomes modified upon interaction with the metal substrate.
nanoparticles assembly (aggregation) can be applied such as based on the DNA-mediated assembly and controlled electrostatic assembly.\cite{64-65} The desired length of complementary strands of DNA can be bound on the surface of nanoparticles during the synthesis that brings two nanoparticles closure and forms nanoparticles assembly. Similarly, the assembly or controllably aggregated nanoparticles can be generated when two oppositely charged metal nanoparticles of controlled charge density are mixed in the solution. Such types of aggregated or assembled nanoparticles can act as potential SERS substrates to accommodate and identify analyte molecules. A basic scheme of the assembly nanoparticles is shown in Figure 5D.

Overall, the SERS substrates need to be equipped with the metallic surface with nanoscale gaps, junctions, and surface roughness for efficiently concentrating the incident electromagnetic field and allowing amplifying SERS signals of vibrational molecular fingerprints.

2.3. Flexible SERS Sensors Overview

In the current time, portable, wearable, and wireless devices are of particular interest specifically for healthcare purposes not only in remote and resource-limited areas but also for every household and individual uses.\cite{66-67} As the SERS technique is label-free and capable of molecular detection with high sensitivity, its key component—the substrate—can be developed with flexible characteristics.\cite{68-69} In Section 2.2, it was explained that the SERS substrates are usually rigid, either dispersed in solution (metal nanoparticles) or fixed to the solid support such as silicon or glass plate. Alternatively, polymers can be used as a support to the metal nanostructures that can provide both flexibility and optical transparency, which are capable of penetrating/passing the light.\cite{70-71} A flexible SERS sensor, for instance, is very useful in environmental toxin monitoring and trace pesticide detection on the surface of fruits and vegetables.

Figure 5. A brief overview of various representative fabrication techniques for the generation of SERS-active substrates. Four basic strategies are shown. A) General approaches where electrochemically roughened metal electrodes\cite{13-14} act as potential SERS substrates. B) Top-down approaches where various lithographic techniques can allow the formation of nanoparticle arrays in high order and of reproducible morphologies.\cite{9} C) Bottom-up approaches where solution-phase wet-chemical synthesis of the metal nanoparticles of tunable size and shape can be generated from metal precursors by using a suitable reducing agent and capping agent.\cite{96,98} Freely moving metal nanoparticles can act as SERS substrates. D) Controlled nanoparticles assemblies (SERS substrates) via DNA-mediated interaction and electrostatic interaction.\cite{64-65}
for practical applications.\[68\] Also, key advantages of the flexible SERS substrates include that they are not introducing any destruction or damage to the surface of interest, they can be detached after measurements, and they can be reused for the detection of other analytes. Various methods can be implemented to prepare flexible SERS substrates. For example, a flexible SERS substrate can be prepared by depositing tunable-sized metal nanoparticles onto the stretchable polydimethylsiloxane (PDMS) film.\[72–74\] Similarly, a bioinspired SERS substrate can be prepared by silver (Ag) coating on a structured surface of PDMS substrate using textured nano/microstructured Taro leaf as a template that can exhibit high adhesion properties and hydrophobic characteristics, and also provides plasmonic hotspots for detection of low concentration of analytes.\[75\] Also, PDMS film embedded with gold nanostar assemblies can be prepared as a deformable, transparent, and flexible SERS active substrate.\[76\] Such types of PDMS-supported flexible substrates can be prepared by combining soft lithography and nanosphere lithography processes.\[77\] Besides the lithography techniques, flexible, transparent, and active SERS substrate can be prepared by applying the self-assembly process of Au nanoparticles on the surface of a polymethylmethacrylate template.\[71\] While polymers provide flexible support, the SERS signal can be enhanced by developing high-density hotspots at their surface. In this regard, a 3D structured array such as a nanohump array on a polymer film can provide a larger surface area on which Ag nanoparticles can be grafted, for example, by sputtering.\[70\] Likewise, pyramidal plasmonic structures on polymethylmethacrylate (PMMA) templates can be fabricated for SERS active substrate.\[78\] A key purpose for using flexible SERS substrate is to have their biocompatible nature and comfortability during their use. For such purpose, bacterial nanocellulose-based flexible SERS substrates have been developed by adsorbing a dense layer of plasmonic nanostructures on the surface of bacterial nanocellulose representing the typical case of a biomaterial.\[79\] Similarly, paper-based cellulose templates can potentially be used for preparing an active SERS substrate.\[80\] Besides, a simple approach has been employed in which adhesive tape can be used together with biosilica plasmonic nanocomposites.\[81\] Overall, the characteristics of the flexible SERS substrates (mostly the flexible and transparent films) are highly promising for practical applications because they can be wrapped or swabbed on various nonplanar surfaces for the efficient collection and detection of analytes present in their low concentrations and they are reusable. An overview of the flexible SERS substrates is shown in Figure 6.

3. Particle-Based SERS Sensing

Section 2 above has presented a brief overview of the bulk substrates such as solid/hard supports (e.g., silicon and glass plates) and flexible/soft support (e.g., polymer films) for SERS sensing of analytes. This section presents a detailed description of the particle-based SERS substrates (sensor particles) and shows how they are useful in the detection of trace analytes.

![Figure 6. Flexible SERS substrates: their representative fabrication methods and flexible substrate types in which metallic nanostructures are attached to the flexible base materials.\[68–71,75–79,81\]](image-url)
of various types. In general, simple sensing by SERS in the original sense of sensing is possible if the surface binding of analyte molecules (the substance to be analyzed) is a spontaneous reversible process. Reversibility means adsorbed molecules are quickly released from the metal surface if the solution concentration is lowered. Such reversibility can be expected in the case of high rates of desorption which means binding constants cannot be very high. Thus, high concentrations of analyte molecules on the metal surface can only be expected at high analyte concentrations in solution. This fact contradicts the desire of proving and characterization of molecules at low concentrations. This problem can be solved by pursuing a sensor concept in an extended sense of sensing. This concept responds to the necessity of nonreversible molecular surface bonding on the one hand and the desire for repeated measurements on the other hand by introducing SERS-active sensor particles. These particles integrate the possibility of the feeding of the analytical system by small sensor units as miniaturized consumables with the presence of metal solids showing the SERS effect.

Various types of metallic particles and polymer particles can be developed with desired structures and for well-defined targeted functions, hence showing an excellent structure–function relationship. Also, particles can be considered as miniaturized individual systems. Nanoscale particles, for instance, can exhibit the properties of both domains, molecular and macroscale materials. With transducer characteristics, particles can perform the tasks of receiving signals, signal transduction, and emission. Overall, the particles can act as efficient objects to convert the chemical and biomolecular information into a physically readable signal for contactless transmission. Sensor particles are very promising for identifying molecular structures through SERS sensing. This section presents the characteristics of the sensor particles useful for the SERS analysis. So-called plasmonic nanoparticles are marked by an electronic excitation, which is usually interpreted by a localized surface plasmon resonance (LSPR). This interaction of incident light with particle electrons can strongly enforce the SERS effect. Therefore, the fundamental features of the plasmonic metal nanoparticles are briefly described and key plasmonic nanoparticle systems with the characteristic of efficient hotspots are highlighted in Section 3.1. Besides, polymers show extraordinary characteristics of softness, swellability, porosity, flexibility, and transparency. Hence, the features and advantages of polymer particles to entrap and stabilize metal nanoparticles are described in Section 3.2. Furthermore, the description of how the composition of polymer particles and metal nanoparticles in a systematic manner can form efficient sensor particles for SERS sensing applications is given in Section 3.3.

### 3.1. Noble Metal Nanoparticles: Component for LSPR, Analyte Binding, and SERS Enhancement

The minimum requirement for experiencing the SERS effect is the excitation of surface plasmon resonance (SPR) that can be originated from the metallic surface upon interaction with light. Surface plasmons are essentially light waves that can be concentrated on the metallic surface because of their interaction with free surface electrons of the metal. When light waves pass through the metallic surface, the free electrons of metals respond collectively by oscillating in resonance. Hence, the resonant interaction of the oscillation of surface electrons upon irradiation by the electromagnetic field of the light creates surface plasmons and therefore generates unique optoelectronic properties. In general, the frequency of the incident light wave is in resonance with the characteristic frequency of the delocalized surface electrons of the metals that motivates the collective/coherent electron oscillation. While relatively larger and flat surfaces of metals can generate so-called continuous surface plasmon resonance waves, the nanoscale metal particles are considered to form LSPR. Excitation of LSPR can generate a local electromagnetic field—called a near field—at the surface of nanoparticles, which is then scattered with the same wavelength. In particular, the near field can be increased in the case of nons isotropic, shape-controlled, and structured metal nanoparticles such as those with corners, boundaries, edges, and tips, as well as high aspect ratios, where the near field can be concentrated in a highly confined space. The LSPR wavelength is dependent on the dielectric function of the metal nanoparticles as well as their surroundings. Metal nanoparticles, particularly gold nanoparticles, are known to be utilized since ancient times. However, the first scientific report on the experimental relations of gold nanoparticles to light by Michael Faraday’s landmark paper has appeared in the middle of the 19th century. Later on, the fantastic theoretical rationalization of the plasmon has been reported by Gustav Mie in the early 20th century. Following the pioneering work of Ritchie in the 1950s, SPR was widely recognized in the field of surface science. In the last several decades, a broad spectrum of synthesis techniques for the formation of simple to complex metal nanoparticles of tunable size, shape, morphology, and surface properties have been developed. A brief timeline for metal nanoparticles is shown in Figure 7. The optical readout—characteristic absorption band—of the LSPR of metal nanoparticles can be observed through UV/visible spectroscopy and is strongly dependent on the size, shape, morphology, and composition of the metal nanoparticles. Tuning in the properties of metal nanoparticles including size, shape, and morphology, as well as local refractive index surrounding the particles directly impact the variability in absorption peak position and their broadness/sharpness, as well as spectral shifts.

Gold and silver nanoparticles are among the most popular types of noble metal nanoparticles that show extraordinary physicochemical and surface properties. Various wet chemical syntheses such as Turkevich synthesis, polyol synthesis, Brust–Schiffrin synthesis, and many other syntheses can be employed to synthesize the size and shape-controlled gold and silver nanoparticles. Metal nanoparticles are crystalline, and their growth is directed by energy-driven crystal planes and facets. The seed-mediated approach of the shape-controlled metal nanoparticles is most established not only because they allow the formation of uniform shapes but also in high yield. Different types of ligands and surface-active agents are required during the synthesis of metal nanoparticles to stabilize them in the solution phase against undesired aggregation because of the high surface energy of the nanoparticles. In particular, a surface directing agent...
since then, a wide range of synthetic methodologies has been developed for the synthesis, properties, and applications of metal nanoparticles.\textsuperscript{91–93} It is well known that a famous lecture “There is plenty of room at the bottom” by Richard Feynman in 1959 is a landmark for the nanotechnology field. Such polyvinylpyrrolidone (PVP) is useful to passivate the growth of the low-energy facet and allow the growth of nanoparticles toward high-energy facets that generate the shape-controlled metal nanoparticles.\textsuperscript{103} By applying various types of surface-controlled strategies during the growth, different types of shape-controlled metal nanoparticles can be generated. For example, 1D nanorods, 2D nanodiscs, 3D nanocubes, and 0D quasispherical nanoparticles can be generated by the applications of various surface-directing growth strategies. Not only limited to simple shapes but more complex shapes such as metal nanorice, nanostar, nanocage, octahedra, bipyramid, and core–shell nanoparticles can also be synthesized. In addition, a controlled self-assembly approach can be applied to generate the assembled nanoparticles such as silver nanoparticle dimers that can create a hotspot useful in enhancing the electromagnetic SERS signals.\textsuperscript{104} An overview of the representative synthesis strategies is given in Figure 8A. Usually, a low concentration of the analytes of interest can be mixed with metal nanoparticle solution in a spectroscopy cell and their SERS spectra can be analyzed thoroughly. Shape- and morphology-controlled metal nanoparticles play key roles in the significant optimization of the Raman scattering enhancement by the plasmonic effect. As far as LSPR is important for SERS enhancement, the modes of plasmon resonance can systemically be tuned based on the shapes of the metal nanoparticles. For instance, nanorods can show two absorption peaks in the UV–vis spectrum contributed to the longitudinal mode and transverse mode.\textsuperscript{103,105} The tuning in diameter of the spherical nanoparticles can only change a peak position slightly but a large variation in absorption peaks can be observed by slightly altering dimensions of the shape-tuned anisotropic metal nanoparticles such as diameter or length of the nanorods that are particularly advantageous for SERS.\textsuperscript{95} Once the molecules are very close to or attached to the surface of metal nanoparticles, the synergistic mutual excitation of LSPR and molecules can lead to enhanced Raman scattering with shifted frequency.\textsuperscript{85} Shape-anisotropic metal nanoparticles allow enhancement in the plasmon-supported electromagnetic field that directly contributes to increasing the SERS intensity significantly.\textsuperscript{99} In the case of 1D nanorods, for instance, the high electromagnetic field enhancement can be realized at the reactive ends that are equipped with high-energy facets during longitudinal excitation, which resultantly reveals high SERS activity.\textsuperscript{105–106} Besides 1D nanorods, 2D nanostructures such as triangular plates or hexagonal plates show high enhancement because the electromagnetic field can be confined at their sharp corners and edges.\textsuperscript{107} Metal nanocubes, the 3D nanostructures, have six flat faces (sides) together with 12 sharp edges and 8 sharp corners.\textsuperscript{108} The enhanced local electromagnetic field can be concentrated or localized at their edges and especially at their sharp corners due to strong and tunable LSPR, hence a significant SERS enhancement factor of the molecules of interest attached to nanocubes can be realized. A better understanding of the nucleation and growth processes of the nanoscale particles has allowed the designs and development of even more diverse types of nanostructures with controlled tips, corners, and edges. Sharper tips in some unusual shapes such as nanostars and nanoflowers can exhibit an excellent optical response that in turn show strong LSPR and SERS signals. As multitrip-based nanoparticles can show stronger LSPR, they are particularly useful in the detection of very low concentrations of various analytes, possibly up to a single-molecule level.\textsuperscript{108,85} An overview of the SERS-active plasmonic nanostructures and their use for SERS analysis of analytes is shown in Figure 8B.C.
In general, the key morphological parameters, i.e., shape-tuned and structured individual nanoparticles such as nanorods, nanocubes, nanoprisms, nanobipyramids, and nanostars possess areas called intrinsic hotspots, which are located at their edges, tips, corners, and boundaries showing enhanced and tunable LSPR effects and hence a significant enhancement in SERS signals. On the other hand, extrinsic hotspots can be generated by applying self-assembly or directed assembly processes during the synthesis or postsynthetically in the case of nanoparticles that are not sufficiently equipped with in-built hotspot regions. For instance, nanoparticle dimers and controllably aggregated nanoparticles are capable of strongly enhancing the SERS signals of the adsorbed analytes.
that can generate an enhanced local electromagnetic field. A strong SERS signal can be realized when molecules are attached to or placed near the junction. Besides the dimers and controllably assembled nanoparticles, a core–shell architecture is an important configuration that can be utilized as an efficient SERS substrate for high enhancement factors. In this regard, tunable-sized Au–Ag core–shell nanoparticles were synthesized by either varying the size of Au core or Ag shell, and their SERS activities were performed by different laser lines. Besides, Au–Ag nanoshuttles with sharp tips and tunable shell thickness were synthesized by using Au nanorods as core materials, and applying Ag shell on its surface in presence of glycine buffer shows improved SERS activity. In another study, Au–Ag nanorods of tunable lengths were prepared by applying Ag precursor on the nanobipyramidal Au core and their SERS activity was performed. Also, various types of bimetallic combinations in the case of core–shell nanorods can be developed such as Au–Ag, Au–Pd, and Au–Pt, and their structural and morphological control is particularly useful for the strong enhancement of Raman signals. A spray-like deposition/formation of shell layer on the core nanorod can form the corn-like bimetallic core–shell superstructure that can create a more roughened/structured metallic surface, which is particularly advantageous for achieving enhanced SERS signals. A similar type of structured core–shell architecture could be developed in the case of nanotriangles where smaller-sized Ag nanoparticles can be deposited/formed on the surface of micelle-like catanionic surfactant-covered flat Au triangles. Such Au–Ag structured triangles present tunable LSPR and hence SERS activity under the resonant condition can show significant SERS enhancement factors. Nanostars are particularly very interesting geometry for the enhanced SERS activity in the nonaggregated state because of the strong enhanced electromagnetic fields realized at their branches/tips. In addition, bimetallic nanostars with core–shell architectures are even more promising for tunable LSPR and SERS signals. In this regard, core–shell nanostars were synthesized in which coating of Ag has been applied on the Au nanostars that reveal resonant SERS properties. Likewise, in the alternative study, Au shell was applied on the Ag nanostar core and their SERS activity has been performed. Similar to these types of key nanostructures, a broad library of various nanoparticles equipped with nanoscale features can potentially be used for the SERS sensing experiments for detecting various analytes in a diverse environment. A SERS enhancing area (hotspots) in various nanostructures is highlighted in Figure 8D.

3.2. Polymer Particles: Support for Holding and Stabilizing Metal Nanoparticles

While polymers are available ubiquitously in nature in various forms ranging from cellulose and starch to proteins and nucleic acids, their bioanalogous, semisynthetic, and synthetic counterparts can be tailored with defined functions. Polymers can be made via linking of monomeric units shows extraordinary physicochemical, structural, and surface characteristics and can play an excellent supporting role in the formation of efficient sensor particles for SERS sensing of analytes. Whereas bulk polymers are useful in a wide range of various applications because of their mechanical properties and other advantages, their particulate counterparts are particularly useful in nanotechnological applications and for spectroscopic and microscopic techniques for a variety of functions. Ideally, polymer particles are 3D crosslinked networks and can be prepared via emulsion polymerization, suspension polymerization, miniemulsion polymerization, nanoprecipitation, and photopolymerization. Additional processes can be applied to tune the properties of generated polymer particles such as through solvent evaporation, surface functionalization, etc., depending upon their requirements for specific applications. Polymer particles are mostly amorphous or semicrystalline. Depending on the type of the monomers and polymerization process, the polymer particles can be hydrophilic, hydrophobic, or amphiphilic. Moreover, polymer particles can be consist of either homopolymers, copolymers, or block copolymers. Whether synthetic or natural polymer particles, mechanical properties are their most attractive aspects. For instance, polymer-supported hierarchically structured composites (biological materials made of inorganic minerals and organic polymers) found in nature’s materials such as wood, bone, and nacre are very rich in mechanical properties, i.e., toughness, stiffness, and strength. The size tunability of the polymer particles in the range from sub-100 nm up to higher microscale and millimeter length scale can be obtained by controlling reaction parameters. For instance, the size of spherical PMMA nanoparticles can be tuned between 70 and 600 nm by the application of various concentrations of anionic and cationic molecular surfactants. Because of the amorphousness, nonspherical shapes of polymer particles at the nanoscales are challenging to control via a single-step process. However, suitable interfacial interactions driven by polyelectrolytes support in situ assemblies during polymerization can able to form the nonspherical polymer nanoparticles such as with ellipsoidal, dumbbell, necklace-type, branched, and flower shapes.

Besides the size and shape, polymer particles are soft and flexible. Recently, research focus has attracted significant attention for the development and applications of flexible sensors. In this regard, the softness and flexibility of the polymer particles are very promising. Softness is an essential mechanical feature of the polymer particles that also reveal elasticity through which polymer particles can be deformed upon requirement. Furthermore, softness can be measured by the ratio between elastic and thermal energy through which the colloidal behavior of the particles can be regulated. Similarly, the elasticity of polymer particles can control their swelling and wetting behavior. Therefore, the elasticity of polymer particles is a crucial characteristic to distinguish hard particles such as PMMA colloids from soft and ultrasoft microgel particles. Here, surfactant-protected dense polymer nanoparticles are defined as hard colloids and water-filled microgel particles are soft colloids, but overall the class of polymer particles can be considered as soft nanoparticles compared to hard metal nanoparticles. Hence, the softness and flexibility of polymer particles are essential to forming so-called particle-based flexible sensors for SERS sensing of analytes. Alongside the softness, polymeric microgel particles are equipped with a crosslinked
network that can swell in the aqueous environment and alternatively deswell in response to environmental conditions, such as temperature and concentration. Here, the properties such as swelling ratio, osmotic pressure, thermodynamic phase behavior, viscosity, and pair structures can make hydrogel particles useful for regulating analytes. The responsiveness of polymer particles by various external stimuli reveals their dynamic functions. In general, the responsive polymer particles possess switchable properties, which are particularly useful in burst release as well as a sustained release at targeted sites during drug delivery applications, for instance. Polymer particles, therefore, can be developed that allow spatial, temporal, and dosage-controlled delivery of drugs in response to chemical, physical or biological stimuli. Various endogenous stimuli such as pH or enzyme, as well as exogenous stimuli such as light, temperature, ultrasound, or magnetic field, can be employed for stimuli-responsive activities. In addition, biocompatibility is a key property of polymers for using them in biological applications. Various polymeric materials can be tailored with biocompatibility and purpose-adapted biodegradability by carefully selecting the monomeric building blocks. In modern biomedical applications such as tissue engineering, targeted drug delivery, endocytosis, and biosensing, a primary requirement is to have the utilizing biomaterials be biocompatible. Though complete biocompatibilities of the polymeric biomaterials and healthcare devices is a challenging to achieve, a wide range of various biomaterials such as supramolecular biomaterials and resorbable polymers with remarkable biocompatibility have been produced and widely utilized. The selection of the biocompatible polymers in the development of metal–polymer composite sensor particles allow to use them in biological applications to detect various bioanalytes.

Besides, porosity and wrinkling are other important features of the polymer particles useful for surface adsorption, adhesion, and wetting functions during their interaction with targeted sites. Pores at the surface of particles can allow analyte molecules to diffuse below the surface and inside the core polymeric structure. Moreover, basic hydrogel particles are transparent that is useful in the penetration of incident light below the surface. Hence, the porosity and transparency of the polymer particles allow penetrating both the analyte and light by which enhanced sensing outcome can be realized. Key features of the polymer particles are shown in Figure 9. All of these features of polymer particles are highly useful in the formation of polymer–metal composite particles (sensor particles) for SERS sensing of various analytes in potential manners. As mentioned in Section 3.1 that metal nanoparticles are SERS-active components, and here polymer particles can act as a potential support to the metal nanoparticles and allow the formation of versatile polymer–metal composite sensor particles.

3.3. Composite Sensor Particles and SERS Sensing

Sensor particles can efficiently bind the analytes, receive incident laser light, and allow releasing vibrational scattering signals full of molecular information to the detector in the SERS process. In this regard, polymer–metal composite particles can emerge as efficient sensor particles. Metal nanoparticles (Section 3.1) are conductive and efficiently interact as well as respond to the incident light whereas polymer particles (Section 3.2) are nonconductive and do not directly respond to light. When polymer combines with metal, a synergetic effect is realized, and properties of the metal nanoparticles such as LSPR and polymers such as flexibility are aligned. Here, metal nanoparticles play a role in dealing with receiving and scattering the light as well as chemically linking analyte molecules on their surface whereas polymer particles act as efficient support to hold, distribute, and stabilize the metal nanoparticles. Metal–polymer composite sensor particles (Figure 10A) can be prepared in a large size spectrum ranging from mid-nanometer to higher micrometer and millimeter length scales. Also, the systematic development of the polymer–metal composite particles provides mechanical strength to their soft yet versatile particulate structure. The concept of sensor microparticles connects the local specific interaction of chemical sensing with the universal noncontact read-out of optical measurement. The sensor particle acts as a primary transducer, which translates the presence of molecules.
into the specific feature of surface-enforced Raman response by adsorption and chemo-electronic interaction between analyte molecule and metal nanoparticle. This Raman characteristic is read-out by application of a monochromatic laser beam and registration of the intensity distribution over a wavelength of the scattered light that represents the second transduction step by the universal noncontact optical process (Figure 10B).

The first essential requirement for efficient SERS sensing is the presence of metal-rich structured surfaces for electronic interaction between the electronic states of the metal and the electrons of molecules resulting in the intended electronic enforcement. The second essential requirement is the presence of a sufficiently high number of molecules on the metal surface. This can be achieved by preferential adsorption or specific interaction between the metal surface and the analytes (chemical enforcement).

The selective polymer matrix of the composite sensor particles is relatively transparent, porous, and swellable because of its gel-like nature. Therefore, they can receive the analytes comprehensively as well as allow penetrating light at the deep level below the surface of particles. The presence of polymer matrix as support in the sensor particles provides flexibility and softness, which is particularly useful in diverse analysis types in a nondestructive manner. The in situ formation of metal nanoparticles embedded in hydrogel microparticles is an excellent strategy for the systematic distribution of metal nanoparticles throughout the core of the polymer matrix without uncontrolled aggregation. Therefore, an in situ formation strategy can be implemented via a droplet-based microfluidic process in the synergetic photopolymerization-photoreduction reaction in a continuous manner. Through continuous progress on the polymeric materials with control over their mechanical strength and biocompatibility and purpose-adapted biodegradability at the same time, hydrogel particles with more than 95% water content can also become possible to prepare. In this case, the polymer matrix is very transparent and allows exchanging and transportation of the water-soluble molecules in an easy manner during the swelling phase in an aqueous solution through pores. Consequently, high molecular (analyte) interaction with dispersed and embedded metal nanostructures can be

Figure 10. Polymer–metal composite particles (sensor particles) for SERS sensing of analytes. A) A schematic depiction of the formation of polymer–metal composite particles for SERS sensing application. B) The general concept of using sensor particles for the identification of various analytes through various transducing steps.
realized inside the gel polymer matrix, and laser light can thoroughly penetrate efficiently, hence enhanced SERS signals can be detected. Depending on the requirement, types of polymers, the water content in the hydrogel, tunable amount of metal nanoparticles, size of composite particles, and their surface functionality can be controlled. All these aspects can be controlled at a single platform in a one-step microfluidic process.

Generally, colloidal metal nanoparticles with sharp tips, edges, and boundaries can cause an extraordinarily high electromagnetic SERS enhancement.\[58\] These smaller nanoparticles can act in a colloidal state in analogy to other dissolved aqueous molecules in the solution phase and hence analyte solution can easily be mixed with them. Subsequently, analyte molecules bind to the surface of nanoparticles irreversibly if the bindings are based on strong molecular interactions through high electron affinity. In other cases, analyte molecules can just proximate very close to the surface of nanoparticles but not strongly binds irreversibly yet in mutual interaction. Either analyte molecules bind to the nanoparticle surface or stay in very close contact without a strong bond, they can reveal strong SERS signals. However, whether analytes bind to the surface of metal nanoparticles weakly or strongly, it is hard to separate them from the nanoparticles after one use. It can be separated via dissociation processes, i.e., deactivated by weak acid or base, but the collection of nanoparticles by centrifugation and washing is a very time-consuming process with the possibility of oxidation of metal nanoparticles over time. In the case of the spontaneous reversible process of analyte binding, high concentrations of the analytes in the solution are required.

SERS is known for its high sensitivity and detectability of lower concentrations. Therefore, the case of reversibility may be a concern for the enhancement factor. Alternatively, for the non-reversible binding of the analyte to the metal surface and yet the advantage of repeated use for multitime detection in multiplexed analysis, polymer–metal composite particles are very promising. Such types of polymer–metal composite particles can be prepared via a single-step microfluidic process.\[20\] These composite sensor particles work as SERS transducers and concentrate analyte molecules for SERS analysis. While a huge amount of smaller metallic nanoparticles are embedded and available at the surface, a cumulative Raman signal can be read out by an outside optical system in a nondestructive manner (Figure 10B).

In particular, silver shows strong surface plasmon resonance and hence a strong SERS enhancement factor compared to gold. However, silver has lower chemical stability and can be oxidized in the air or aqueous solution over time. Besides, gold has better chemical stability and its LSPR region can be extended up to red-NIR wavelength.\[131\] Depending on the type of laser wavelength and measurement requirements, either silver or gold metal nanoparticles can be used as a primary component of the composite sensor particles. The roughened metallic surface, nanoscale gaps, junctions, and highly controlled nanoparticle assemblies can significantly increase the possibility of SERS signal enhancement of the adsorbed analytes at that region. In this regard, the metal amount can be increased controllably in the metal–polymer composite particles by metal-catalyzed metal deposition at the surface.\[20,27\] Here, initially, embedded metal nanoparticles (that can be developed during the one-step photopolymerization-photoreduction) in the core of polymer particles can act as a seed or catalyst for deposition of additional metal nanoparticles layer at the surface. The additional deposition can take place controllably depending on the utilized precursor metal amount, as well as the type and strength of the reducing agent. Also, metal deposition layers can be formed in a structured manner and hence efficient SERS-active areas can be generated.

Overall, the polymer–metal composite particles can act as highly versatile sensor particles for analyzing various analytes through SERS. By incorporating plasmon-active and hence SERS-active metal nanostructures in the core of the polymer matrix, a wide range of advantages can be optimized. First of all, the decaying rate/oxidation of the metal nanostructures can be reduced significantly after embedding them inside water-filled hydrogel polymer particles. Second, aggregation is a major concern for the smaller-sized metal nanoparticles due to high surface energy which directly impacts SERS outcome; the aggregation can potentially be prevented when metal nanoparticles are fixed into the crosslinking network of the polymer matrix. Moreover, due to the porosity and optical transparency of the polymer matrix in the solution phase, the analyte molecules can diffuse and interact with a large number of metal nanostructures and light can penetrate thoroughly. Consequently, high Raman signal enhancement even at low analyte concentration can be realized. In addition, when metal nanoparticles are dispersed in the solution, it is hard to regenerate them for reuse. But, it is more convenient to apply the regeneration process when smaller-sized metal nanoparticles are embedded in the larger polymer matrix. In this manner, polymer–metal composite particles can be used for the multiplexed analysis as well as sequential analysis of multiple analytes. For such types of sequential analysis of multiple analytes at a single platform, the microfluidic arrangement is very promising, which is described briefly in Section 4 below. In general, the combination strategy of microfluidics platform and polymer–metal composite particles as sensor particles is highly versatile for SERS sensing analysis.

4. Microfluidics: Generation of Sensor Particles and SERS Experiments

Sensor particles are made of polymer–metal composite\[20,27,119–121,156\] that allows efficient interaction with analytes and reveals the sensing function for the identification of molecules. To achieve a uniform outcome, sensor particles need to be prepared with a systematic distribution of the metal nanoparticles in the polymer matrix. Considering this requirement, microfluidics can play a significant role in the development of one-step polymer–metal composite particles in an in situ manner.\[20,26,126–127,157–160\] Microfluidics is an advanced reaction technique that can manipulate fluids in the microchannel in a highly precise manner.\[23\] Due to the high surface area in the microchannel, better mixing of reactants (dissolved or dispersed in the solution) can be realized, which directly contributes to the formation of uniform chemical or materials products.\[161\] Advantageously, the distribution of molecules or nanoparticles can take place precisely in the droplets which
experience high circulation during the flow.\cite{22,25,162–164} In general, the droplet is the key focus in microfluidics because the droplet acts as a microreactor.\cite{162} In the formation of polymer particles, the droplet can be polymerized (solidify) and form the polymer particles.\cite{123} Polymer particles obtained via microfluidics possess a larger amount of water and smaller-sized metal nanoparticles (preformed). If the metal nanoparticles can be prepared in situ during the process of polymerization of droplets, even better distribution, much smaller size, nonaggregation, and the ligand-less surface can be obtained.\cite{20} The in situ formed polymer–metal composite particles in the one-step microfluidic process is particularly advantageous for the detection of analytes at their lower concentration. Moreover, to achieve the enhanced sensing outcome, the additional metal layer can be deposited where already embedded metal nanoparticles (in situ formed) can support the formation of ligand-free metal layer at the surface of sensor particles.\cite{20} Overall, microfluidics is a very promising platform for the generation of polymer–metal composite particles (sensor particles) for SERS sensing. A general scheme for the microfluidic formation of sensor particles is shown in Figure 11A.

As a microfluidic platform is very useful in the formation of sensor particles, it is also very promising to conduct the sequential SERS analysis of multiple analytes in a continuous flow manner in a microfluidic setup by using microfluidically synthesized sensor particles. As described in Section 3.3 that metal nanoparticles are systematically caged and efficiently surface-bounded to the polymer matrix; therefore, they can be chemically rinsed after the first analysis of analyte and hence regenerate for reuse to detect another analyte. Chemical rising means the adsorbed analytes to the metal surface can be released by applying a deactivated chemical such as diluted acid. Once the ringing is completed, the particles are ready for interaction with another analyte at the surface. The systematic identification of various analytes can be observed in the SERS spectra during flow measurements. When sensor particles are fixed in the microfluidic channel, it is convenient to apply various analytes and rinsing solutions sequentially in a continuous flow manner.\cite{156} A basic scheme for sequential SERS analysis in the microfluidic channel is shown in Figure 11B. Overall, a microfluidic platform is very promising for both purposes: generation of sensor particles and performing the sequential SERS analysis of multiple analytes.

5. Multifunctional Hierarchical Sensor Particles: Perspectives

A wide range of materials is available in nature with a hierarchical organization such as those found in wood, nacre, and bone alongside the extracellular matrix of the biological tissues.\cite{134} Hierarchical architectures, in general, are made from small yet functionally energetic building blocks that reveal extraordinary functions based on their graded structures. In the case of molecular architectures, for instance, various atoms such as carbon, nitrogen, hydrogen, and oxygen attached with other atoms based on electron sharing ability through covalent bonds to form small/simple molecules such as water (H₂O), carbon dioxide (CO₂), and ammonia (NH₃) as well as large molecules like tannic acid (C₇₆H₅₂O₄₆) and more complex vitamin B₁₂ (C₆₃H₈₈CoN₁₄O₁₄P). Similarly, nanoscale building blocks such as gold and silver nanoparticles can be synthesized of well-defined structures and surface functionality, and their hierarchical architectures can be prepared where nanoscale building blocks retain their functions during the formation of larger hierarchical architectures.\cite{165–166} Beyond nanoparticles retaining individual functions, hierarchical architectures of nanoparticles show completely new and advanced functions in the form of integrated and/or collective properties which are not possible to realize/harvest by nanoscale building blocks alone. Moreover, the properties of nanoparticles are not only determined based on their chemical compositions, but primarily based on their size, shape, and morphological structures.\cite{84} For instance, silver nanoparticles of the same size but different shapes show completely different plasmonic characteristics.\cite{95} Spherical silver nanoparticles show a single plasmonic peak at...
about 420 nm whereas silver nanorods show two characteristic plasmonic peaks, one attributed to the longitudinal mode (red-shifted) and the other for transverse mode (blue-shifted).[^86] Not only plasmonic properties, but other material-based properties are largely dependent on the nanoscale structures. For example, the arrangement of the carbon atoms in different structures in carbon-based nanomaterials such as carbon nanotubes, graphene, diamond, and graphite determine their conductive, mechanical, optical, and thermal properties.[^167] In this way, it is understood that the same type of materials (or material compositions) can demonstrate completely different properties which are largely dependent on their size and most importantly shapes (structures) at the nanoscale. Therefore, hierarchical architectures based on size and shape-controlled nanoscale building blocks possess remarkably advanced functions useful for multidisciplinary aspects for performing/analyzing multipurpose tasks. Functionally graded hierarchical structures made up of the nanoscale building blocks can be prepared in the whole spectrum of size range between smaller individual nanoparticles of about 10 nm and larger composite particles of about 1 mm.[^168]

The main motivation for the formation of hierarchical architecture for SERS sensing is to tailor multifunctional characteristics in sensor particles that allow multiplexed analysis. As the size, shape, and surface chemistry of the nanoparticles are key during their functional applications, various syntheses procedures can be developed to control these aspects toward the formation of their hierarchically assembled network. Alongside the interparticle self-assembly, electrostatic assembly, and other interfacial assemblies, the promising strategy is to introduce swellable hydrogel polymer particles that bind, hold, and stabilize smaller SERS-active building blocks without their uncontrolled aggregation for the sustained outcome. Ideally, a specific type of nanoparticles or small set of nanoparticles with different properties can be embedded in polymer nanoparticles to make polymer–metal nanocomposites. Different types of polymer–metal nanocomposite particles concerning their size, shape, and mechanical properties can be enclosed in the larger polymer hydrogel. In this manner, the desired level of hierarchical order can be developed and multifunctional super particles can be produced. Such hierarchically assembled particles can reveal promising ability for multichannel sensing and SERS microimaging, in general. Further future developments can be expected from constructing complex composed sensor particles and sensor array particles. Such particles and arrays could be useful for simultaneous measurement of several analytes by differently surface-functionalized SERS nanoparticles. The functionalization should be related to the specific chemical affinity of analytes to get preferential adsorption or chemical binding on one or a few types of nanoparticles. Thus, different analytes can be imaged by different optical resonances of nanoparticles using resonance SERS. After incubation with the analyte mixture, the particles or arrays are imaged by excitation light of different wavelengths. Consequently, different Raman scattering light patterns are obtained concerning the presence and concentration of substances in the analyte mixture. For this purpose, particles with hierarchical spatial organization or arrays of sensor microparticles are needed. A general schematic formation architecture of the particles in hierarchical order is shown in Figure 12. Various parameters need to be considered for the formation of multifunctional hierarchical sensor particles, which are described in Sections 5.1 to 5.6 below.

### 5.1. Syntheses Strategies

For constructing hierarchical architectures, various synthesis procedures can be applied. Whereas top-down methods can allow fabricating structured materials with sharp edges and roughened morphologies that provide SERS hotspots, bottom-up approaches are advantageous where each level of hierarchical structures can be equipped with specific functions and their collective properties can precisely be tailored based on controlled interfacial interactions.[^7,169–171] Top-down fabrication methods can generate nanoscale features in the solid substrate and allow deposition of various materials layers in 2D sheet-like structures (roughened).[^7] In this way, multicomponent exposure to laser light at the same time can be challenging due to the nontransparency of the material layers even though they are thin. Solution-based bottom-up synthesis strategies, on the other hand, can generate freely moving nanoparticles of diameters much smaller than the light wavelength. Nanoscale particles have a high surface area and their size, shape, and morphology play key roles in the medium where they interact or involve.[^171] It is possible to control the individual type of nanoparticles with desired characteristics in the solution-based methods.[^86] As the formation of the nanoscale particles takes place by combining/diffusing atomic and molecular components during the growth, there is a chance to synthesize hybrid nanoparticles by the composition of various materials in a single step such as gold-silver bimetallic nanoparticles, and various dopants can systematically be incorporated in the nanoparticles. The most prominent synthesis approach for the generation of a wide variety of nanoparticles is via wet-chemical syntheses in batch. In the solution phase, each different type of nanoparticle has a well-defined structure, desired ligand molecules and surface functional groups, and suitable surroundings where nanoparticles are stable. After the synthesis of the individual type of different nanoparticles, their assembling structures can be prepared depending on the requirement. Through solution-based methods, various levels of hierarchy can be achieved ranging from two to ten levels or even more depending on the sensing systems and requirements in a diverse environment.

An application of the microfluidic platform for the syntheses of functional nanoparticles is highly advantageous in many ways. Microfluidics provides a very uniform reaction environment which results in homogeneous nanoparticles formation.[^161] Therefore, wet chemical synthesis of structured metal nanoparticles can be applied inside the microfluidic channel. Though there is a challenge to provide the temperature-sensitive reaction which requires a growth process of a longer time, it is possible to supply fast heat transfer, rapid mass transfer, and efficient phase transfer in many cases. On one hand, therefore, the microfluidics process is suitable for controlling morphological and surface parameters of the metal nanoparticles.[^172] It is also very promising, on the other hand, to produce the size and surface-controlled polymer particles.[^160]
droplet-solidification method via photopolymerization can produce in situ metal embedded polymer composite particles in a single step without uncontrolled aggregation.\textsuperscript{[20]} Furthermore, the multiplexed microfluidic platform forms desired architectures of the sensor particles with the hierarchical organization in a graded manner. In the hierarchically structured sensor

Figure 12. A schematic overview of the formation strategies for hierarchically structured sensor particles for multiplexed SERS sensing. A) Various shape-controlled metal nanoparticles such as gold nanotriangles and gold nanorods can be enclosed in the lower micrometer hydrogel polymer particles to form gel-like composite microparticles. Embedding of the composite microparticles in larger microparticles during droplet microfluidic photopolymerization can form multifunctional superparticles. B) Formation scheme for the multifunctional microarray where different size- and shape-controlled functional metal nanoparticles can incorporate in polymer arrays.
particles, each level of hierarchical units can be precisely functionalized and tuned with various aspects through continuous flow in microfluidics (Figure 13A). In the end, the hierarchical architecture of sensor particles (multifunctional superparticles) should reveal the multiplied sensing function in the form of surface interaction with diverse types of analytes, interact with light, and for sustained and repeated analysis through regeneration. The future efforts in the development of the multifunctional hierarchical architectures will be useful promisingly for multiplexed analysis.

5.2. Tailoring of Interface

The primary contact of nanoparticles with any active objects such as biological cells, functional molecules, light, other types of nanoparticles, solid surfaces, or any other objects occurs through their surface or interface.[173] The interface is defined here where nanoparticles interact with another object in the liquid phase. On one hand, it is required to apply a molecular layer of the ligand at the surface of nanoparticles for the protection of nanoparticles to prevent their undesired aggregation.

Figure 13. A general overview of various aspects for the formation of hierarchically structured sensor particles for multiplexed SERS sensing. A) Synthesis strategies for the development of sensor particles. B) Various schemes for tailoring SERS-active metal nanoparticles with functional surfaces. C) Tailoring plasmonic characteristics/functions by metal nanostructure shapes and assemblies that possess hotspots. D) Characteristics of polymers, i.e., softness, optical transparency, swellability, and porosity, which are very useful to stabilize SERS-active metal nanostructures. E) Regeneration process of the sensor particles where adsorbed analyte molecules can be released with rinsing solution (dilute acid) and can be reused for detection of other analytes. F) Continuous flow setup for multiplexed analysis with multiple types of analytes delivery at the single platform via flow measurement.
in the solution due to high surface energy. On the other hand, suitable surface-active agents with desired functional groups are essential in initiating controllable and systematic interaction with other types of nanoparticles to assemble toward the formation of hierarchical particulate architecture. Most analytes such as biomolecules or pesticides possess specific functional groups in their molecular structures. It is required that the surface of the SERS substrates (sensor particles) holds an affinity for analyte interaction efficiently. On one hand, the ligand-free metallic surface is special to interact with the molecules that possess thiol, hydroxyl, or amine functional groups in a direct interaction via affinity. On the other hand, the attachment of the charged molecules (ligands) at the surface of nanoparticles such as those that possess citrate, sulfate, or tertiary ammonium groups is useful for stabilizing the particles through interparticle repulsion and initiating electrostatic interaction with oppositely charged molecules (analytes). Besides, the functionalization of nanoparticles with biomolecules such as biotin, vitamins, or peptides is particularly interesting to detect target biomolecules via secondary interaction such as biotin–streptavidin and biotinylated antibody–antigen in a lock-and-key manner. Besides the ligand-free metallic surface, which is very powerful for selective types of analyte interactions, the surface of the nanoparticles with functional molecules (ligands) can be tailored mainly in three manners: a) Ligand-binding during the synthesis of nanoparticles, which is the most common and widely acceptable method. b) Postsynthesis ligand exchange in which desired types of functional molecules can be exchanged with the original ligand attached to the surface. c) Surface functionalization through layer-by-layer modification where charged molecular or polyelectrolyte layers can be bound to the outer surface via electrostatic interactions. The surfaces of various types of metal nanostructures (different shape-controlled particles) can be tailored with a diverse range of functional characteristics for the interaction of a wide range of various analytes (Figure 13B). When these types of functionally active metal nanoparticles are available in one hierarchical architecture, a multiplexed analysis of analytes through SERS can be allowed at a single platform.

### 5.3. Tailoring Plasmonic Characteristics/Functions

A surface plasmon is the most important feature in the SERS process. Surface plasmon resonance is realized by metal nanostructures. Though the surface plasmon resonance upon interaction with UV-visible light is common in the case of metallic materials, different shapes, morphologies, and structures of metals at the nanoscale reveal different plasmonic modes depending on their orientations. Also, the SERS intensity is dependent on how concentrated plasmons are excited. Therefore, it is important to tailor the structural characteristics of the plasmonic nanostructures in the formation of multidimensional hierarchical architecture. A huge Raman enhancement is realized from electromagnetic enhancement and chemical enhancement effects. For the resonant Raman effect, it is necessary to use a laser wavelength (e.g., 532 nm) that is compatible with the chosen SERS metal where Raman cross-section of molecules being excited and gives enhanced scattering effect. Tailoring plasmonic characteristics in metal nanostructures can be realized by tuning in size, shape, and composition. When nanoparticles are under light irradiation, surface electrons can collectively oscillate. The oscillation of electrons in spheres is uniform at all dimensions and hence single plasmonic peaks can be realized. The peak broadness and redshifting are largely dependent on the size of nanoparticles. Both broadness and redshifting are enhanced with enhancement in the size of spherical metal nanoparticles. On the other side, plasmonic resonance can significantly be tuned by tuning the shapes of metal nanoparticles in three dimensions, two dimensions, one dimension, and zero dimension ranging from nanocubes, nanobipyramids, nanotriangles, nanodisks to nanoshuttles, nanorods, and nanorice. The details about various shapes and their applications for plasmonic resonance and SERS is provided in Section 3.1 above. Various syntheses methods can be applied where shape control of nanoparticles can be achieved and hence the nanoscale features such as corners, edges, tips, boundaries, gaps, and junctions of anisotropic nanostructures allow concentrating light efficiently and shows enhanced plasmonic resonance. The nanoscale features of plasmonic nanostructures create hotspots, which are key in realizing the enhanced SERS effect when molecules of interest are proximate to the hotspot area. The formation process (nucleation and growth) of the nanostructures during their synthesis are driven by the energy facets due to their crystalline nature. The seeds play an important role in defining the shape, hence a wide range of seed-mediated methods for the precisely tunable shaped nanostructures were developed. Besides, seedless synthesis methods also produce anisotropic metal nanostructures. Specific types of plasmonic nanostructures give specific molecular signatures based on their LSPR (Figure 13C). When various types of functionalized shape-controlled metal nanoparticles are available in the hierarchical architecture, it is possible to achieve multiplexed analysis outcome.

### 5.4. Encapsulation and Protection by Polymers

Assembling the metal–metal nanostructures in an extended manner can create larger metallic structures. Characteristics of the plasmon resonance become weak with the increasing size of plasmonic aggregates beyond the limit. It is necessary that if smaller plasmonic nanoparticles are not structured then they need to assemble in a controlled manner that can create plasmonic hotspots for realizing efficient SERS enhancement. Not only for the concern of undesired larger plasmonic aggregates but an efficient strategy is also required that can prevent metallic nanostructures from chemical and photodegradation. Usually, nanoparticles are covered with molecular surface-active agents. However, adsorbed ligand layer is not considered as a robust protecting layer to stabilize nanoparticles against all conditions for a long time. The metal nanoparticles are SERS-active components, therefore, their stability and sustained utilization is a key focus. In this regard, polymers can act as a potential support to handling metal nanostructures with regards to binding and stabilizing in their network (Figure 13D). Three main characteristics of the polymers, i.e., swellability, porosity,
and optical transparency are very promising in the formation of polymer–metal composite particles for SERS sensing application. During the swelling phase, a large number of pores can be opened where transportation of the aqueous solution of analytes can take place, and hence interaction between analyte molecules and embedded metal nanostructures can be realized. Moreover, due to optical transparency, light can penetrate thoroughly in the hydrogel particles that can reveal enhanced scattering signals with molecular fingerprint information. For multiplexing SERS analysis, a wide range of various metal nanostructures together with their diverse functional surfaces are required. When they aggregate uncontrollably in a larger structure, their surface plasmon efficiency becomes weak. Hence, polymers can provide essential protection and stabilize them for longer use. Moreover, micrometer-sized polymer particles can accommodate millions of smaller-sized metal nanostructures. Droplet microfluidic is a promising platform for the formation of reproducible metal-loaded hydrogel polymer particles. In general, the polymer can act as support and carrier of the metal nanostructures. Furthermore, the key aspect of the polymer is its softness. Because of the softness, the polymer network is very flexible. Therefore, polymer-supported sensor particles can create a particle-based flexible sensor. While there is an increasing demand regarding the flexible sensor for accurate monitoring of various analytes in a diverse environment is growing, flexible sensor particles can be very promising because of their 3D interaction ability with high surface area compared to the bulk substrate. Various levels of size domains of metal-embedded polymer particles can construct the hierarchical network where each level of the graded structure is equipped with sensing functions. Overall, the microfluidic platform can allow the generation of a hierarchically organized metal–polymer sensing network equipped with a wide range of sensing functions. Therefore, polymer-supported hierarchical architectures are very promising for multiplexed SERS analysis of multiple analytes at a single platform.

5.5. Regenerative/Reusable Substrate for Multiple Analytes

Regarding a broad perspective, a versatile and sustained sensor is required to continuously monitor various analytes, for instance, in water flow and air in the environment. Air and water, in general, carry a large number of various analytes of different types. A key element of the SERS sensor surface plasmon resonance at which analyte of a high-affinity functional group interacts and enhanced SERS signal can be observed. Usually, the adsorption of the molecules on the metallic surface is strong based on affinity. Due to high affinity, it is hard to desorb the measured analyte so that the sensor can be able to detect another analyte of the same type or other types during continuous monitoring. Therefore, the feature of the regeneration or reuse of sensors needs to be developed in widespread uses of SERS-based sensors as the SERS sensing strategy is one of the most powerful analytical techniques useful in a diverse environment. A concern of the regeneration of freely moving metal nanoparticles can be realized due to their small size. Nanoparticles possess high surface energy. During the washing process for desorption of attached analytes, there is a change that nanoparticles are randomly aggregated. In addition, some metal nanoparticles are air-sensitive and tend to oxidize if not properly protected. Moreover, the process of the metal nanoparticles through centrifugation is time-consuming. All of these concerns can be addressed when smaller-sized metal nanoparticles are embedded properly inside the hydrogel polymeric network. As explained above, the hydrogel network is swellable and porous which allows the transportation of liquid. Because metal nanoparticles are fixed properly in the swellable hydrogel network, they can be chemically rinsed and still maintain stability. After rinsing, the metal surface inside the hydrogel network is free to interact with other types of analytes. In this way, a sequential analysis of various analytes can be possible (Figure 13E). Regeneration of sensor particles is not only useful for the detection of multiple analytes at one platform, but it is also a promising approach for time-sensitive speedy analysis. The hierarchical architectures of the sensor particles are essential where selective types of metal nanostructures are tailored in a way that binds specific types of analytes and can rinse them accordingly with suitable rinsing solutions such as weak acid or weak base. In general, the polymer–metal composite-based sensor particles are an excellent system that can be regenerated and hence allow the detection of multiple analytes sequentially.

5.6. Flow Entrapment and Continuous Measurement

Substrate maintenance and SERS arrangement are strategic for the analysis of various analytes at their lower concentrations. The bulk substrates are based on solid chips and freely moving small nanoparticles of about size range 50 to 200 nm are well dispersed in the solution. It is possible to anchor structured metal nanoparticles at the surface of solid substrate and fix them to a microfluidic chip for continuous analysis. In addition, metal nanostructures can be packed into soft support such as PDMS chips or paper-based support. Here, direct chemical interaction of analytes with SERS-active components can be challenging. Besides, freely moving small nanoparticles are difficult to entrap in the microchannel without any hard or soft support. Alternatively, metal embedded polymer composite particles are of microscale, capable to handle fluid dynamically due to softness, and reveal the efficient possibility of regeneration. Therefore, these particles can strongly be entrapped in the microfluidic channel for continuous flow measurements of different analytes (Figure 13F). Flow capillary can be selected depending on the size of composite particles, which allow flowing the analyte solution through a microfluidic flow setup. The hierarchical architectures of the sensor particles are large, which contains multimillions of small-sized metal nanostructured functionalized with different surface chemistries, shape tunability, and metal composition. Therefore, hierarchical particles can entrap in flow capillary and hence very promising for continuous flow SERS measurement of various analytes. Future development is needed to construct multifunctional hierarchical architectures of the sensor particles for flow analysis via a continuous microfluidic setup.

Overall, SERS is a powerful tool for sensitive and specific detection of analytes with high accuracy and enhancement factor. The general terminologies useful for the SERS are
highlighted in Figure 14, which are also thoroughly used in this perspective article. Advantageously, a hierarchical sensor architecture is highly versatile and promising for the analysis of a wide range of various analytes through flow measurements.

6. Summary

The fascinating early principles of Rayleigh scattering have motivated huge development in the field of optics and light-based technologies. However, the experimental observation in 1928 by C. V. Raman and K. S. Krishnan regarding the secondary radiation, which is based on inelastic scattering has inspired significant progress and advanced developments in photonics. This observation has been popularly recognized as the Raman scattering phenomenon. Yet, Raman scattering is a very weak effect where only one out of about one million photons reveals the inelastic effect (either gain or lose energy). To recognize the high population of the inelastically scattered photons, therefore, an intense light source was strongly required. The development of the laser in the 1960s has met this requirement and the Raman scattering effect has become widely popular in the detection of chemicals and materials in a broad range of various fields. Furthermore, the breakthrough effect has been observed in the 1970s where the scattering signal was significantly enhanced when molecules proximate to the roughened metallic surface. It was quickly identified that the surface plasmon resonance—collective oscillation of the surface electrons upon interaction with light—is realized from the metal surface, the understanding of the formation of nanoscale metal structures, their optical/photonic properties, and their advanced applications in modern science and technology has been exponentially progressive path so far. The fascinating color shining from the glasses dates back to ancient times and the middle age was generated from the metal nanoparticles, particularly gold nanoparticles. However, it was 1857 when Michael Faraday’s experimental synthesis of gold nanoparticles has appeared which can be considered as the start of the nanoscale materials era. Later, the theoretical rationalization of the optical properties of the metal nanoparticles through Mie theory is considered as a versatile solution in light–matter interaction. The pioneering work of Ritchie in the 1990s on the surface plasmon resonance has a great impact on the progressive development of the field. The advanced impact of the surface plasmon resonance had been identified in the 1970s when it had been found out that surface plasmon resonance from roughened metallic surfaces played a key role in the significant enhancement of the SERS signals. Since then, advanced synthesis strategies for the generation of simple to more complex metal nanostructures regarding their size, shape, and morphology were developed in the last three decades particularly. Not only metal nanoparticles are sufficient, but the requirement of the nanoscale features in the form of sharp edges, tips, corners, boundaries, junctions, and gaps is primary that concentrates electromagnetic waves significantly and allow enhanced scattering outcomes. As SERS is the perfect platform for the analysis of solution-based analytes alongside solid and gaseous analytes, the dispersion of nanoparticles (solution) is highly useful as an efficient substrate that reveals LSPR.

The nanoscale metal particles with available hotspots show enormous enhancement factors for the SERS sensing applications. However, it is difficult to regenerate/rinse the nanoparticles after adsorption of analytes to metal nanoparticles for reuse purposes because of the small size of nanoparticles, their risk of aggregation, and the possibility of oxidation. If nanoparticles can be regenerated after one use, they are essential for detecting multiple analytes at a single platform. In this regard, the combination of SERS-active metal nanoparticles with polymeric support is a promising strategy where particle-based SERS sensing and the possibility of particle regeneration after one use are satisfied. The characteristics of the polymer particles as described in this perspective article are useful for the fixing, stabilization, and regeneration of metal nanoparticles for reusing to detect multiple analytes in a sustained as well as sequential manner. Optical transparency of the polymers allows penetrating light thoroughly and porosity of the hydrogel.
particles during their swellable phase is essential for diffusion of analytes below the surface which results in enhanced SERS outcome. Moreover, the crosslinked hydrogel polymeric network can support the systematic binding (distribution) of the metal nanoparticles inside the core network thoroughly and stabilize the metal nanoparticles against interparticle aggregation. For distributing small nanoparticles with high density and without aggregation inside the polymer core, an ideal strategy is an in situ approach where large polymer particles and small metal nanoparticles form simultaneously in one step. For this purpose, droplet microfluidics is an excellent reaction platform. Liquid droplets that are solidified to form composite particles contain all the ingredients required for the polymerization to form a polymer network and reduction to form metal nanoparticles simultaneously. In this way, microfluidically formed polymer–metal composite sensor particles are very promising substrates for analyzing various analytes through SERS sensing applications.

The hierarchical particulate structure that can be formed by graded construction can provide a multifunctional platform for detecting multiple analytes of diverse functionality and properties at a single platform. Particles are, in general, the miniaturized systems that connect the bridge between the molecular and macro counterparts and show the properties of both. Particles can be designed with diverse surface functions due to their large surface-to-volume ratio and being movable in the solution phase that is useful for efficient molecular interaction. Each level of the nanoparticles toward the formation of hierarchical architectures can be functionalized with a specific type of surface molecular function that can be able to interact with various types of analytes through molecular interactions. Polymeric support can play a pivotal role in holding and stabilizing metal nanoparticles of different sizes, shapes, and surface functionalities. In this way, multifunctional superparticles can be formed which are equipped with diverse functions and able to detect multiple analytes at their lower concentrations. On one hand, a microfluidic platform is essential in the formation of uniform metal nanoparticles, polymer particles, polymer–metal composite particles, as well as different levels of hierarchical particles. Moreover, continuous flow arrangement through microfluidics is essential to perform particle-based SERS sensing experiments. Overall, polymer–metal composite particles combine the characteristics of the metal nanoparticles as SERS-active components and polymers as powerful support, which reveal the role of potential SERS substrates for the detection of diverse analytes dynamically through a microfluidic platform.

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Conflict of Interest

The authors declare no conflict of interest.

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