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Stationary, Continuous, and Sequential Surface-Enhanced Raman Scattering Sensing Based on the Nanoscale and Microscale Polymer-Metal Composite Sensor Particles through Microfluidics: A Review

Nikunjkumar R. Visaveliya,* Raminta Mazetyte-Stasinskiene, and Johann Michael Köhler

Surface-enhanced Raman scattering (SERS) is a label-free and accurate analytical technique for the detection of a broad range of various analytes such as, biomolecules, pesticides, petrochemicals, as well as, cellular and other biological systems. A key component for the SERS analysis is the substrate which is required to be equipped with plasmonic features of metal nanostructures that directly interact with light and targeted analytes. Either metal nanoparticles can be deposited on the solid support (glass or silicon) which is suitable for stationary SERS analysis or dispersed in the solution (freely moving nanoparticles). Besides these routinely utilizing SERS substrates, polymer–metal composite particles are promising for sustained SERS analysis where metal nanoparticles act as plasmon-active (hence SERS-active) components and polymer particles act as support to the metal nanoparticles. Composite sensor particles provide 3D interaction possibilities for analytes, suitable for stationary, continuous, and sequential analysis, and they are reusable/regenerated. Therefore, this review is focused on the experimental procedures for the development of multiscale, uniform, and reproducible composite sensor particles together with their application for SERS analysis. The microfluidic reaction technique is highly versatile in the production of uniform and size-tunable composite particles, as well as, for conducting SERS analysis.

1. Introduction

Green pigment chlorophyll of the plants interacts with sunlight to begin the natural photosynthesis process which ultimately converts light energy into chemical energy.[1,2] Inspired by these types of light-dependent natural processes, a wide range of technological processes based on photonics and optoelectronics phenomena were developed over the last century for efficient applications in the fields of healthcare, theranostics, sensing, and imaging, among others.[3–8] To deliver and implement the therapeutic procedure for curing diseases, for instance, an accurate diagnosis is a prerequisite.[6,7] In addition, minimally invasive or non-invasive techniques are desired to minimize the side effects during the theranostics applications.[3] In this way, not only for biological systems but to detect the trace amounts of various analytes on the food, in the air or water, etc., label-free and nondestructive analytical techniques are demanded that can identify the molecular structures of the toxins or other analytes at their lowest concentration level.[9,10] Considering these aspects of non-damaging light-mediated and label-free techniques, surface-enhanced Raman scattering (SERS) sensing is a very promising platform to meet the requirement with enhanced and accurate outcomes in various environments.[11–20] SERS is an inelastic scattering-based sensitive analytical technique that provides fingerprint information about molecular structures based on the vibration of molecular chemical bonds.[13]

In the SERS, plasmon-active substrates play the utmost role in interaction with light and targeted analytes. Since the discovery of SERS in the 1970s,[21–25] a broad range of plasmon-active substrates has been successfully fabricated and implemented to achieve the high enhancement factor for an ultimate limit of detection, that is, single-molecule.[19,26] As SERS is highly suitable for the aqueous phase too, the particle-based plasmon-active SERS substrates are very promising for the detection of analytes in solution.[27] In this regard, a broad library of various nanoscale plasmonic particles of various structures, shapes, and morphologies are available and have been synthesized via

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wet chemical solution-phase syntheses during the last two decades.[28] Nanoscale plasmonic particles possess a high surface area and allow the interaction of analytes, as well as, light at their surface three-dimensionally. In particular, sharp tips, corners, and edges of the structured metal nanoparticles, as well as, junctions and nanoscale gaps available in the controllably assembled nanoparticles, present the sites where concentrated plasmon resonance can be realized upon interaction with light.[29] These areas where light concentrate significantly called "hotspots" are the focus for significant enhancement of scattering signals.[30] Smaller-sized metal nanoparticles are freely dispersed in the solution. Because of the high surface area, nanoscale metal particles can potentially interact with analytes even at their lower concentrations and hence can be detected through SERS. Once the analytes are adsorbed on the nanoparticles during the SERS measurement, it is hard to remove them from the surface of the nanoparticles after measurement due to small size, aggregation possibility during centrifugation, and the possibility of oxidation of nanoparticles. Therefore, smaller-sized metal nanoparticles are very efficient for one-time use but difficult to regenerate for further use. In address, to protect the nanoparticles against aggregation and to regenerate them (removal of analyte during the first measurement) for further use, a very promising approach is to connect the metal nanoparticles with a cross-linked polymer network. Various materials such as, silica can be used to stabilize the metal nanostructures for potential use but cross-linked hydrogel polymers are promising because of their characteristics. Polymer particles are soft and swellable—particularly hydrophilic or hydrogel particles.[31] They are also relatively transparent due to their large water content and cross-linked swellable polymeric network.[20,32] The cross-linking network of the hydrogel polymers can accommodate smaller-sized metal nanoparticles and protect against aggregation. Suitable synthesis platforms need to be utilized that can allow the generation of polymer particles of suitable surface and interior characteristics for the accommodation of metal nanostructures.[31,33–35] On one hand, polymer particles are required to be relatively uniform in size, and on the other hand, they should be capable of forming/accommodating in situ metal nanostructures in their interior during the one-step process. In this regard, microfluidics is a very promising platform that can be able to produce metal-polymer composite particles of uniform size and composition.[36–39]

Microfluidics or lab-on-a-chip technique is a versatile reaction technique that deals with a small volume (droplets of picoliter or nanoliter) of liquid in the tiny microchannel.[40–42] Inside microfluidics, efficient reactant mixing and tunable composition can be realized that finally allow generation of uniform products with lower polydispersity.[43,44] Here, experimental development of the nanoscale and microscale polymer-metal composite particles via microfluidics supported processes for the SERS applications has been reviewed. In the polymer-metal composite particles, metals act as plasmon-active components, and polymers act as support to the metal nanoparticles. In general, plasmon resonance is a minimum requirement to experience the SERS effect that arises from the metal nanoparticles.[45] In this regard, to enhance the metallic content at the surface, further metal enrichment at the surface of polymer-metal composite particles can be achieved by a metal-catalyzed metal deposition process.[20] The enriched layer of the metal

at the surface is ligand-free, and hence useful for direct interaction with analytes of interest. The density of the enriched metals can systematically be tuned by applying various reaction parameters that are explained in detail in this paper. On one hand, a microfluidic platform is versatile in the production of uniform polymer-metal composite particles, and on the other hand, it is a powerful medium to perform SERS analysis in continuous as well as sequential flow manner.

Overall, the scope of this review article is to summarize the experimental works of the last decade from the authors' laboratory regarding the microfluidic-assisted synthesis of nanoscale and microscale polymer-metal composite sensor particles for SERS sensing in a stationary, continuous, and sequential manner. A brief schematic overview of the content of this review paper is provided in **Figure 1**. Initially, a basic concept of the SERS is provided. Afterward, the general idea of using polymer-supported sensor particles is described. Subsequently, the general rationale behind the synergy of microfluidics and SERS is provided. In the following, the microfluidic reaction strategy and their setups for the generation of nanoscale and microscale sensor particles are given. Afterward, a detailed synthesis process for the generation of nanoscale polymer particles via microfluidics, polymer-metal nanocomposite particles via electrostatic interaction, and metal enrichment process on the composite nanoparticles via metal-catalyzed metal deposition approach has been reviewed together with their application for SERS analysis. Here, the size range of the nanoscale particles is between 200 and 800 nm which are ideally defined as a sub-micron size range. But, distinguishing the sensor particles of size range 200–800 nm from microscale particles (size range: 30–800 μm), we have thoroughly considered the term "nanoscale sensor particles" in this review paper. In the following section, a detailed review of the microfluidic process for the generation of microscale sensor particles and their applications for SERS analysis is given. In the following, a setup and procedure for the sequential SERS analysis of multiple analytes (multiplexing) have been reviewed. In the last, a summary and outlook section of this review article is provided based on polymer-metal composite sensor particles for SERS.

2. General Features of Surface-Enhanced Raman Scattering

SERS is an advanced analytical technique that works on the principle of enhancement of the Raman scattering signals when molecules are located on the roughened metallic surface.[11,13,46–48] Various analytes such as, soil contaminants,[49] fluid contaminants,[50] trace amounts of pesticides on foods,[51] trace concentrations of air contaminants,[52–55] explosives,[56] etc., are dispersed in the diverse environment of liquid, solid, or gaseous phases. It is an important objective to detect such types of targeting analytes with high specificity and sensitivity for healthcare and other purposes. For this objective, SERS is a very promising and versatile tool that provides the possibility to detect the analytes at their ultimate detection limit of the single-molecule level.[19,57] Due to their working ability in a diverse environment, SERS can be broadly applicable for characterizations in the field of chemistry, materials, and life science.[11,58–60] The excitation source in the SERS experiments is intense,
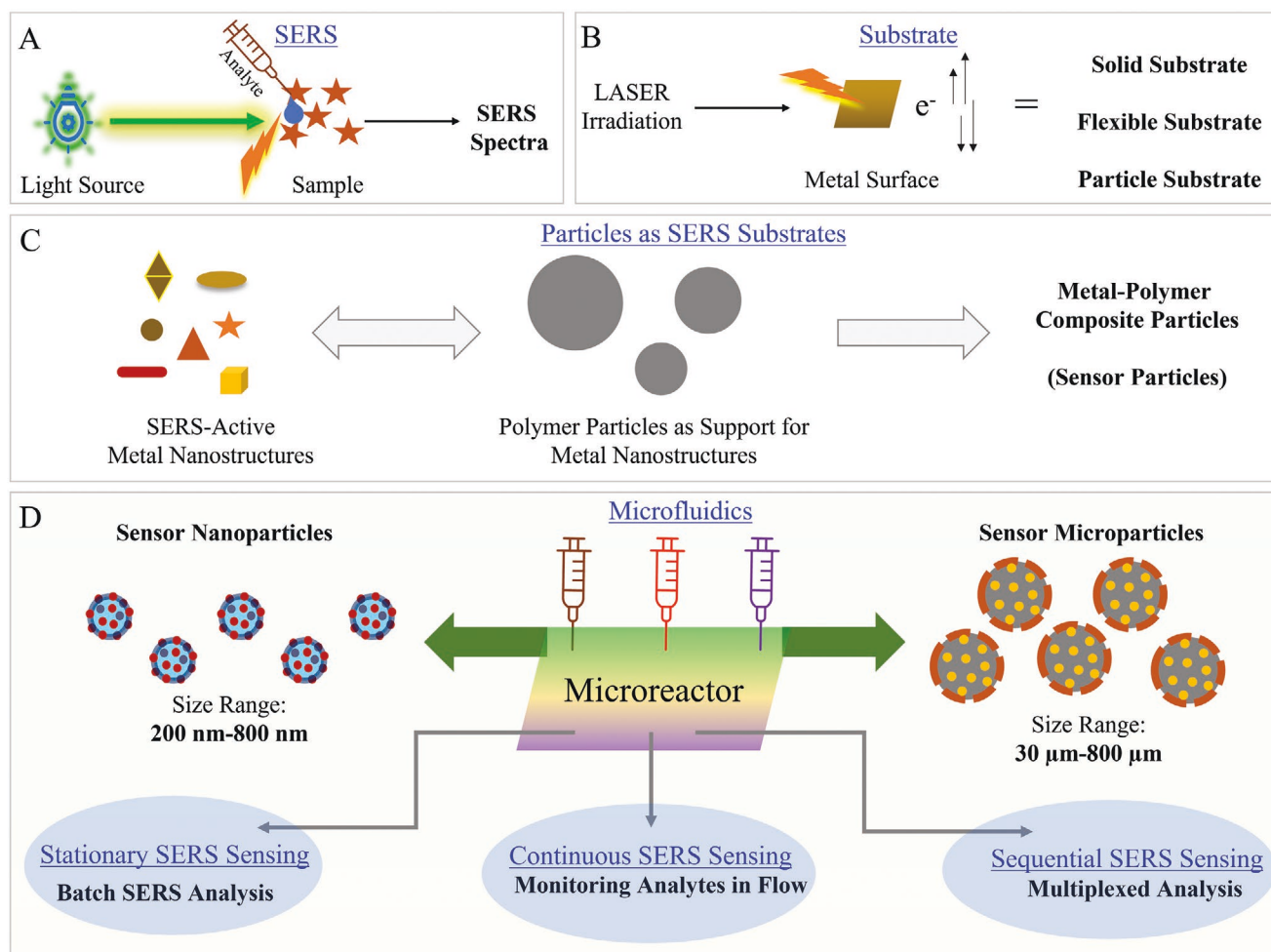
Figure 1. A brief schematic overview of the content described in this review article. A) A very basic concept of SERS analysis where analyte interacts with SERS substrate and hence SERS spectra observed. B) Substrate—an important component of the SERS measurement. C) Particles can be used as potential 3D SERS substrates where metal nanostructures are SERS-active components and polymers particles act as support to the metal nanostructures in the formation of metal-polymer composite sensor particles. D) Application of microfluidics for the synthesis of sensor nanoparticles and microparticles, as well as, SERS analysis of various analytes by sensor particles in the stationary mode (batch), continuous flow mode, and sequential analysis for multiplexed SERS sensing.

usually a laser of a defined wavelength such as 532 nm.[61] Excitation sources of various wavelength regions from the UV to IR range can be used depending on the types of substrate and SERS instruments.[11,46,61,62] SERS is an inelastic scattering method where scattered photons either lose or gain energy in order to excite the molecules of interest to their first excited vibrational state.[12,45,63] Moreover, because SERS is a label-free[64,65] and non-destructive[66,67] analytical technique, a wide variety of analyses for food safety and healthcare diagnosis can be performed in a sensitive, specific, and speedy way.[64–69] Compared to the simple Raman scattering which is an intrinsically very weak phenomenon,[70] the signals are strongly amplified in the SERS largely due to the electromagnetic and chemical effect.[71] A general arrangement for the detection of various analytes through SERS is schematically shown in **Figure 2**A. Advancements in nanofabrication techniques, as well as, the development of portable SERS instruments, have allowed the detection of diverse types of analytes from medical diagnosis to environmental protection. The development of portable

SERS instruments has also allowed sensitive detections in remote areas.[72,73] Usually, the targeted analyte molecules are interacting with SERS-active substrate for SERS enhancement. The plasmon-active metal substrates are the most prominent candidates where extremely high enhancement of the scattering signals of adsorbed analytes can be realized.[45] The field concentration or localization at the structured metal surface is most prominently realized due to the excitation of surface plasmon resonance which can be defined as a collective oscillation of surface electrons of the metals upon interaction with light (Figure 2B).[14,45,74,75] The concentration of electromagnetic field upon locating analyte molecules near metallic nanostructures that are equipped with nanoscale surface features (hotspots) is one of the most crucial aspects for observing the SERS signal enhancement.[76] As metallic nanostructures and hence surface plasmon resonance is the main focus in SERS, their fabrication with defined characteristics is a key interest in the progressive demands for using SERS in a wide range of analytical platforms. Either metal nanostructures are deposited on
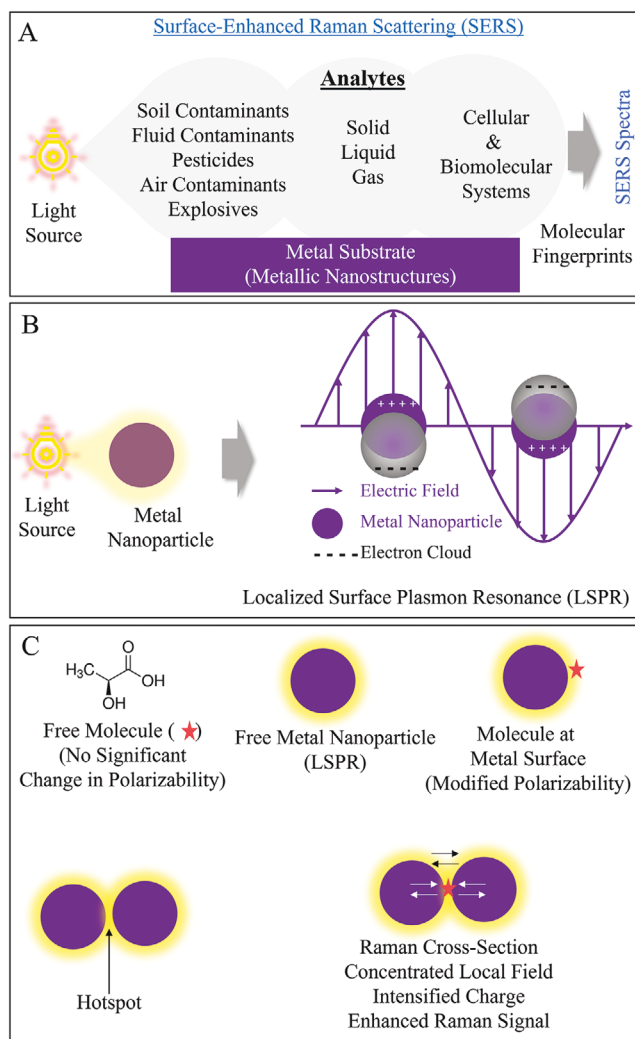
Figure 2. General features of SERS. A) SERS is an efficient analytical technique for the detection of various analytes upon irradiation by electromagnetic waves during their adsorption to the metallic substrate. B) Surface plasmon resonance is a key feature in the significant enhancement of signal. Reproduced with permission.[75] Copyright 2003, American Chemical Society. C) A schematic overview of the importance of hotspots in the SERS effect.

the solid support of silicon or glass, or freely-dispersed metal nanoparticles in water, the availability of the hotspots is a key requirement in observing highly enhanced signals.[77]

In the simple Raman analysis, molecules are considered as free and so-called in their original state. Once the molecules adsorb to the metal substrate either through physisorption (e.g., van der Waals interaction) or chemisorption (e.g., formation of chemical bond), their polarizability is believed to be modified concerning the electronic structure and molecular conformation. Consequently, the Raman cross-sections of the vibrational modes of the molecules are different than molecules that are not adsorbed on the surface. As laser light irradiates Raman cross-section of the molecules attached to the hotspots, it releases enhanced scattering signals full of information of the molecular vibrational bonds to the detector.[58] Based on the excited surface plasmons and Raman cross-section of molecules

at the hotspots, the enhancement factor is extremely high that can able to detect the limit of the single-molecule level.[19] Ideally, hotspots are generated between two nanoscale particles or between the nanoparticle and surface; in both cases, a nanoparticle is either very close to the other nanoparticle/surface or adsorbed/aggregated to it completely. When light irradiates nanoparticles, the electric field of light polarizes nanoparticles and generates excess charges (positive and negative) at opposite ends of the nanoparticles. The displacement of the charges is intensified when two nanoparticles are very close because the second nanoparticle is experiencing the electric field from light as well as from the induced dipole of the first nanoparticle.[46] Therefore, a molecule located at the hotspot region can experience a highly concentrated electric field and show enhancement in the SERS signal (Figure 2C).

3. Key Characteristics of Polymer-Metal Composite as Sensor Particles for Surface-Enhanced Raman Scattering Sensing

A key focus for the SERS effect in the identification of various analytes is a substrate.[26,78] Ever since the discovery of SERS,[21–25] a broad range of various SERS substrates have been fabricated by top-down approaches. Besides, the particle-based SERS substrates are very promising because of their dynamic movement in the solution (for instance, metal nanoparticles dispersion in water), small size, intense plasmonic features, and ability to bind analytes three-dimensionally.[79,80] During the last few years, three remarkable directions of progress in particle-based SERS sensing have been pursued. First, particle-based SERS sensing technique became more and more in the focus of interest for the detection of biomolecules or bioactive substances, drugs, and so on at low concentrations and measurements in microfluidic systems.[81–85] Second, metal/polymer composite microparticles were developed which are promising for the improvement of the sensitivity, re-use capability, and the handling of SERS-active particles for single-bead based sensor strategies.[31,86,87] Third, nanoparticles and special nanoparticle assemblies with special geometries were synthesized to achieve local strength of the electromagnetic field resulting in enhancements of the SERS effect.[88] Therefore, non-spherical metal nanoparticles, nanocubes, flat nanoprisms, particles with sharp edges and corners, and arrangements with nanometer-sized gaps have been developed for inducing an increased coupling of the electronic systems of adsorbed molecules and the metallic phase. The interaction of the electromagnetic field with the analyte molecules can be further improved by resonant Raman interaction. Therefore, the wavelength of the light source is adapted to the electronic resonance with high oscillation strength of the molecule, the metallic phase, or the complexes of both, respectively. The electronic resonance causes the intermediate formation of an electronically excited state. The Raman scattering is enhanced, typically, by two to three orders of magnitude in comparison with non-resonant Raman spectroscopy. The resonant Raman effect can also be applied in the case of shape-controlled plasmonic metal nanoparticles which are characterized by a specific optical resonance. Usually,

different localized resonance plasmonic modes are available in the anisotropic metal nanostructures. For instance, high oscillator strengths appear in the case of stretched nanorods and flat nanodiscs (geometries with high aspect ratios). Particularly in nanorods, two oscillation modes can be appeared, that is, longitudinal and transverse modes. Thus, such types of various nonspherical particles are in the focus of recent investigations on particle-based Raman spectroscopy.

Ideally, sensor particles are required to be equipped with a strong sensing function. The SERS sensor particles, therefore, should reveal a plasmonic feature, a key phenomenon for concentrating electromagnetic waves that generates the intense local field and assisting significantly enhanced scattering signal. Moreover, sensor particles should be tailored with a surface function that is capable of interacting with analyte molecules through various interfacial and physicochemical interactions.[60] As far as metal nanoparticles are SERS-active components,[14] their stability against uncontrolled aggregation is also a very important aspect because larger aggregates weaken the intense plasmonic characteristics that directly impact the SERS outcome. Freely moving metal nanoparticles in the solution phase (water) are small and challenging to chemically rinse to regenerate (removal of adsorbed analytes) after the first interaction with analyte type. It is a promising strategy where small nanoparticles regenerate and provide the platform for multiple analyte detection. Considering all of these requirements and promises such as, plasmonic function, surface function, stability, and regeneration, a versatile approach is to combine metal nanoparticles with polymer support.[20] Basic polymers such as polyacrylates and polyenes are not directly interacting with light, hence not contributing to the SERS effect.[20,89,90] However, polymer particles are efficiently useful as a powerful support to the SERS-active metal nanoparticles because polymer networks accommodate metal nanoparticles in their interior with the ability of binding and stabilizing the metal nanoparticles. The hydrogel-type polymeric network can be generated where a water-filled cross-linked network can particularly accommodate metal nanoparticles with thorough distribution without uncontrolled aggregation.[31] The optical transparency of the hydrogel polymer particles is particularly useful for penetrating the light to interact with metal nanoparticles. Moreover, hydrogel particles are filled with water and hence the network becomes more swellable when they are in water solution which allows transportation of molecular analytes through pores to interact with metal nanoparticles. Further characteristics of the polymers such as softness, flexibility, responsiveness, and mechanical tunability are very useful in dealing with metal nanoparticles toward the formation of polymer-metal composite particles. The combined properties of the polymer-metal composite particles reveal the extraordinary tailored sensing function for SERS sensing applications. Microfluidics is a powerful platform for the formation of tailored sensor particles in a uniform and reproducible manner. More details about the microfluidic application for sensor particles are provided in Sections 4 and 5, and microfluidic syntheses aspects with experimental conditions for the generation of nanoscale and microscale sensor particles are provided in Sections 6 and 7 below. Alternative to polymers, silica nanoparticles can also be used as the support to the metal nanoparticles. But, the utilization of hydrogel polymers is promising because of their cross-linking, optical transparency, swellability, and flexibility. Therefore, in this focused review, only a polymers as support to the SERS-active metal nanoparticles are considered. The details about the key features of polymer-metal composites particles as SERS sensor particles are provided in **Figure 3**.

As metal nanoparticles are a key interest in SERS, their enrichment at the surface is essential in a controlled manner. Therefore, it is an important strategy to apply additional metal with precise distribution at the surface of polymer-metal composite particles. In this regard, the embedded metal nanoparticles in the polymer matrix can act as catalysts for the reduction of the metal precursor by a reducing agent and simultaneously form a metal layer via a process called metal-catalyzed metal enrichment. With this process, a ligand-free metal surface is generated which is particularly important for the direct interaction of analytes that give rise to enhanced SERS outcomes with high sensitivity and specificity. The enforced metal nanoparticles are very well distributed at the surface because their formation is dependent on the previously embedded metal nanoparticles inside the polymer matrix which are well-controlled without aggregation. The density of the enforced metal nanoparticles can systematically be controlled depending on the concentration of metal precursor and reducing agent (more details in Sections 6 and 7). On one hand, a microfluidic platform is versatile for the development of sensor particles, and on the other hand, performing the sensor particle-based SERS sensing experiments in microfluidics is extraordinarily efficient which can sequentially allow detection of multiple analytes in flow arrangement as described in detail in Section 8 below.

4. The Combination of Surface-Enhanced Raman Scattering and Microfluidics

As explained above, SERS is proven to be a highly versatile analytical technique for sensitive detection of a broad spectrum of various analytes.[9,16,18,91] In further advances, the application of microfluidics during the SERS analysis is promising for in situ and real-time detection at lower ranges of analyte concentrations which, in principle, can enable detection of the ultimate limit down to the single-molecule level.[92–96] For various SERS substrates whether they are adsorbed on the solid support, freely movable nanoparticles in solution (water), or flexible substrates based on soft materials, their repeated use with sustained sensitivity is key for efficient sensing application of multiple analytes. In this regard, the microfluidic-mediated arrangement is very promising to meet the requirements because of its advantages not only for performing the precise experiment under a highly uniform reaction environment but also simultaneously integrating accurate characterization/detection.[97–102] In general, microfluidics is an advanced reaction strategy that deals with a very low amount of liquid in a tiny microchannel of micrometer length scales of various microreactor devices.[103] With regards to integrating SERS with microfluidics, the SERS-active substrates can be fixed to the microfluidic chip or channel during their fabrication for continuous and repeated detection of analytes.[104] On one hand, microfluidics is very suitable for the production of
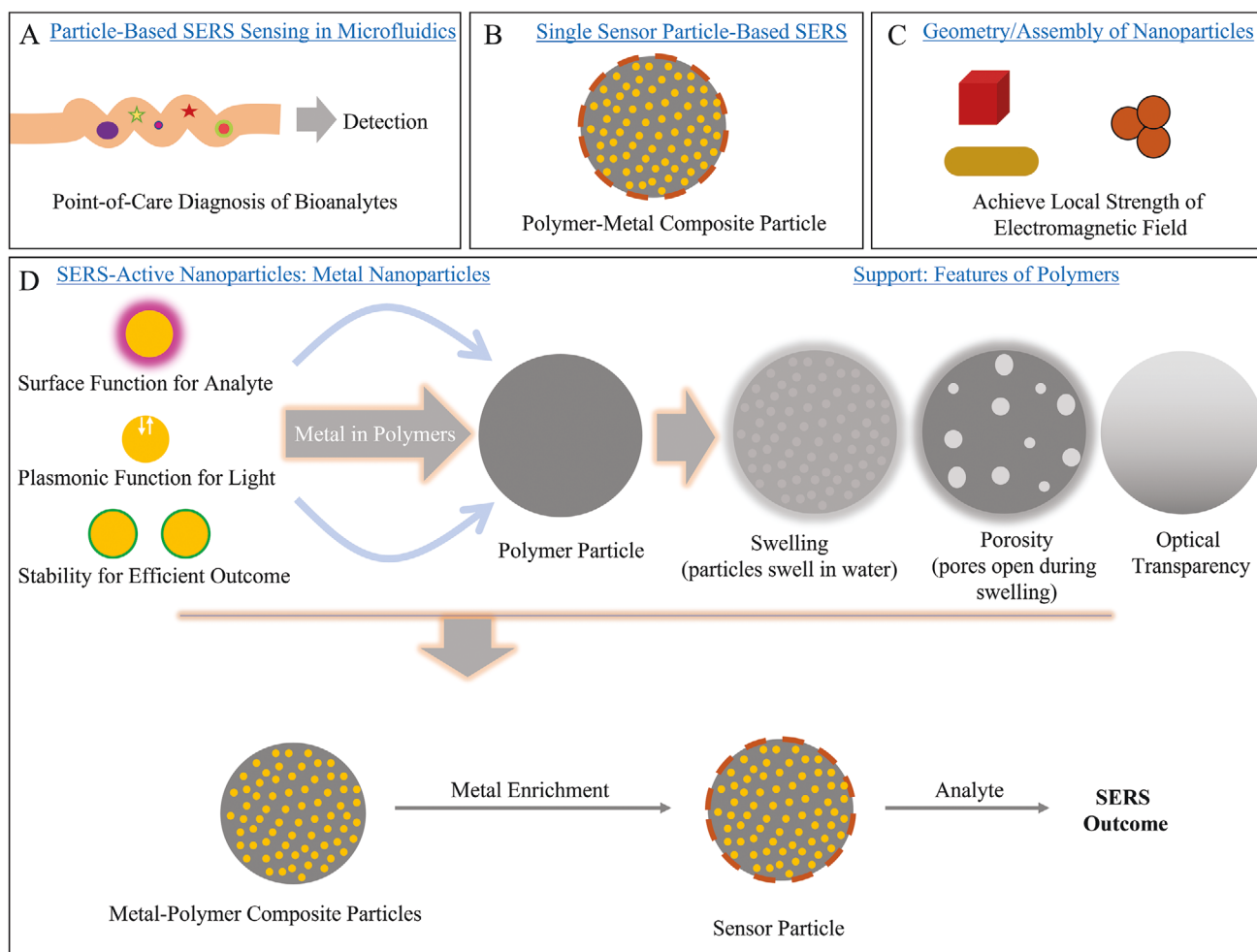
Figure 3. A general overview for the polymer-metal composite sensor particles and particle-based SERS sensing. A) A schematic of the microfluidic concept for detection of various analytes. B) A model cartoon for the microscale polymer-metal composite sensor particle for SERS sensing of analytes. C) A concept of the geometry-controlled and assembly of the nanoparticles for achieving local strength of the electromagnetic field. D) The key characteristics of the components of the sensor particles in which metal nanoparticles are SERS-active components and polymer particles act as support to the metal nanoparticles. The key features of the hydrogel polymer particles such as swelling (where particles swell in presence of water medium and allow transporting the analyte solution to interact with embedded metal nanostructures), porosity (hydrogel particles open the pores during swelling phase), optical transparency (suitable for penetrating the light below the surface of particles), etc., are promising for accommodating metal nanoparticles and SERS analysis.

sensor particles. On the other hand, the microfluidic platform is also very integrated for accommodating sensor particles for multiple sensing of analytes. The detection of low concentration of analytes, low liquid volume, rapid and multiplexed analysis, accurate detection, and cost-effectiveness are key features of modern bioanalysis and diagnostics applications.[37,105] Both fields of SERS and microfluidics are very promising to address all these requirements at a single and integrated platform. A most versatile aspect of microfluidics is its ability to handle fluid extraordinarily. On the other hand, it is possible to analyze samples in an aqueous solution by using SERS-based sensing, therefore physiological fluid analytes could be directly detected without excessive sample preparation.[106] Hence, integration of microfluidics with SERS techniques could be potentially applied for multiplex detection for analytical chemistry and biological applications, environmental sciences, or medicine.[107]

Lab on a chip—the miniaturized device—allow processing and analyzing low sample volumes (liquid solution) in microchannel typically in nanoliter or picoliter range in the continuous or segmented flow manner.[108] Alongside various components of the device such as valves, pumps, tubes, channels, etc., the heart of the microfluidics is a microreactor of different types. Microreactors can be made of various materials depending on the types of chemical and materials reaction or based on the requirement of specific applications in synthesis/detection integrated systems.[109] Usually, microreactors/microchips can be fabricated by using glass, silicon, or polydimethylsiloxane via various nanofabrication techniques such as optical or soft lithography, direct laser writing, 3D printing, etc.[37] Due to a tiny channel for fluid manipulation, microfluidics offers advantages of fast phase transfer, rapid mass transfer, and speedy heat transfer for time-sensitive reactions under high surface-to-volume ratios.[44] Moreover, pressure-controlled and

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light-mediated photochemical reactions can also be performed efficiently inside the microfluidic channels.[31,110] The high shear force and low Reynolds number for laminar flow provide precise and efficient reactant mixing conditions uniformly in a continuous flow manner.[44] Likewise, high reproducibility of the product and detection outcomes, as well as, high encapsulation efficiency are possible in microfluidic techniques.[34] More advantageously, digital microfluidics and droplet microfluidics provide a versatile platform in which the entire reaction can be performed in the droplets, and hence thousands of discrete reaction compartments are generated.[97,111] Such methods are highly useful for the high-throughput screening for drug discovery and single-cell analysis.[112] Microfluidic methods are operated through computer-controlled programs and hence automated processes allow high accuracy in chemical and materials composition that directly impact high uniformity and consistency in outcomes.[113] On one hand, there is always a challenge using microchannels because they can easily clog, and they are expensive. But it is possible to clean most of the microchannels just by applying a liquid flow of suitable solvents depending on the type of materials used. Overall, microfluidics is a versatile reaction strategy to perform accurate, precise, and multiple reactions within a short period, and can integrate multiplexed analysis functions.

As SERS is non-destructive, label-free, and of high spatial resolution technique, various SERS-active substrates can be tailored/fabricated in the microfluidic channel.[64,104] To obtain a SERS-integrated microfluidic platform,[104] SERS-active materials can be added during the fabrication of a microchip or microreactor. Plasmon-active materials are required to realize the SERS effect in the microchannel that can be deposited inside the microchannel through various methods such as Langmuir–Blodgett techniques, E-beam, or thermal evaporator, and laser deposition.[114] Though the fabrication procedure of the microreactor or microchip is expensive, the cost per analysis in the integrated microfluidic system is low and rapid. Multiple types of metallic nanostructures of various characteristics can be incorporated into the microreactor. In this way, many types of analytes can be detected by using a single platform equipped with a diverse range of metallic nanostructures (sensor particles) such as, those with sharp tips, edges, corners, joints, etc., as well as of various materials such as, silver, gold, or silver-gold composites. While integrating multiple types of metallic nanostructures in a single chip is advantageous for multiple detections, the regenerative method in which seerately prepared sensor particles can be fixed in the flow channel is very promising for sequential sensing of a wide range of various analytes in a continuous flow manner.[115] Such arrangement is very cost-reducing, easy handling, and without requiring fabrication in a clean room with sophisticated instrumentations. Considering the advantages of integrated SERS-microfluidics and sensor particle-based sensing, the strategy of sequential/alternative detection becomes more reliable. In general, one type of analyte strongly adsorb on the metal surface of sensor particles and their molecular fingerprint can be recorded. Subsequently, the adsorbed analyte molecules can be released by applying desorbing solutions such as diluted acid, and hence the sensor particles become renewed again and ready for detecting other analytes.[115] A scheme for the synergistic effect of the SERS

and microfluidics is shown in **Figure 4**. Here, it was shown that microfluidics is an essential reaction platform for the generation of polymer-metal composite sensor particles, as well as, for performing SERS analysis in the flow condition.

5. General Microfluidic Synthesis Strategies for the Generation of Sensor Particles

Polymer-supported nanoscale (sub-micron length scale) and microscale sensor particles for SERS sensing were produced by microfluidic-assisted syntheses.[20,89] Basic reaction arrangements for both nanoscale sensor particles and microscale sensor particles are shown in **Figure 5**.

In the nanoscale (ideally sub-micron) regime, fully usable sensor nanoparticles were prepared by the combination of two processes. In the first process, surface swellable core–shell-type polymeric nanoparticles were synthesized through a semi-microfluidic process[89] as shown in Figure 5A. The detailed synthesis procedure for the formation of core–shell polymer nanoparticles is provided in previous reports.[89,90] Polymeric nanoparticles do not interact with light and hence do not show the SERS fingerprint signature of analytes. Plasmon-active nanoparticles should be combined with polymer nanoparticles to realize the SERS effect. In this regard, a second sub-process has been applied to combining metal nanoparticles electrostatically to the surface of polymer nanoparticles to form the SERS-active sensor nanoparticles. A detail of the nanoscale sensor particles and the SERS activity is described in Section 6 below. Here, a microfluidic arrangement for the synthesis of polymer nanoparticles is shown (Figure 5A). A key component of the arrangement is the silicon holeplate incorporated microreactor chamber which has been fabricated through the nanolithography process and its details are provided in our previous report.[116] The chamber possesses three liquid transporting fixable nozzles connected through syringes. Two nozzles are used as inlets to carry continuous aqueous phase and droplet generating dispersed monomer phase inside the chamber where their precise mixing can be realized under the high shear force of continuous aqueous phase.[116] The third nozzle is used as an outlet for the emulsion solution to be collected in the vial which is arranged externally. One syringe contains the solution of an aqueous monomer (dissolved in water) and is fixed to the one syringe pump (Figure 5A) The second syringe contains the organic monomer (mixture of monomer, cross-linker, and initiator) and is fixed to another syringe pump for actuation. Both syringe pumps can be operated through the computer-controlled program for actuating the liquids at defined flow rates. The various flow rate ratio of both immiscible liquid phases was applied depending on the reaction condition for the formation of polymer nanoparticles (Section 6). 2.5 mL glass syringe for organic monomer phase and 5 mL glass syringe for aqueous monomer phase were used. The generated emulsion solution has been collected in a vial which is arranged at a heating block. The initiation and completion of the polymerization take place in a vial at 97 °C. Once the core–shell type polymer nanoparticles are formed, they can be used for accommodating the smaller-sized oppositely charged
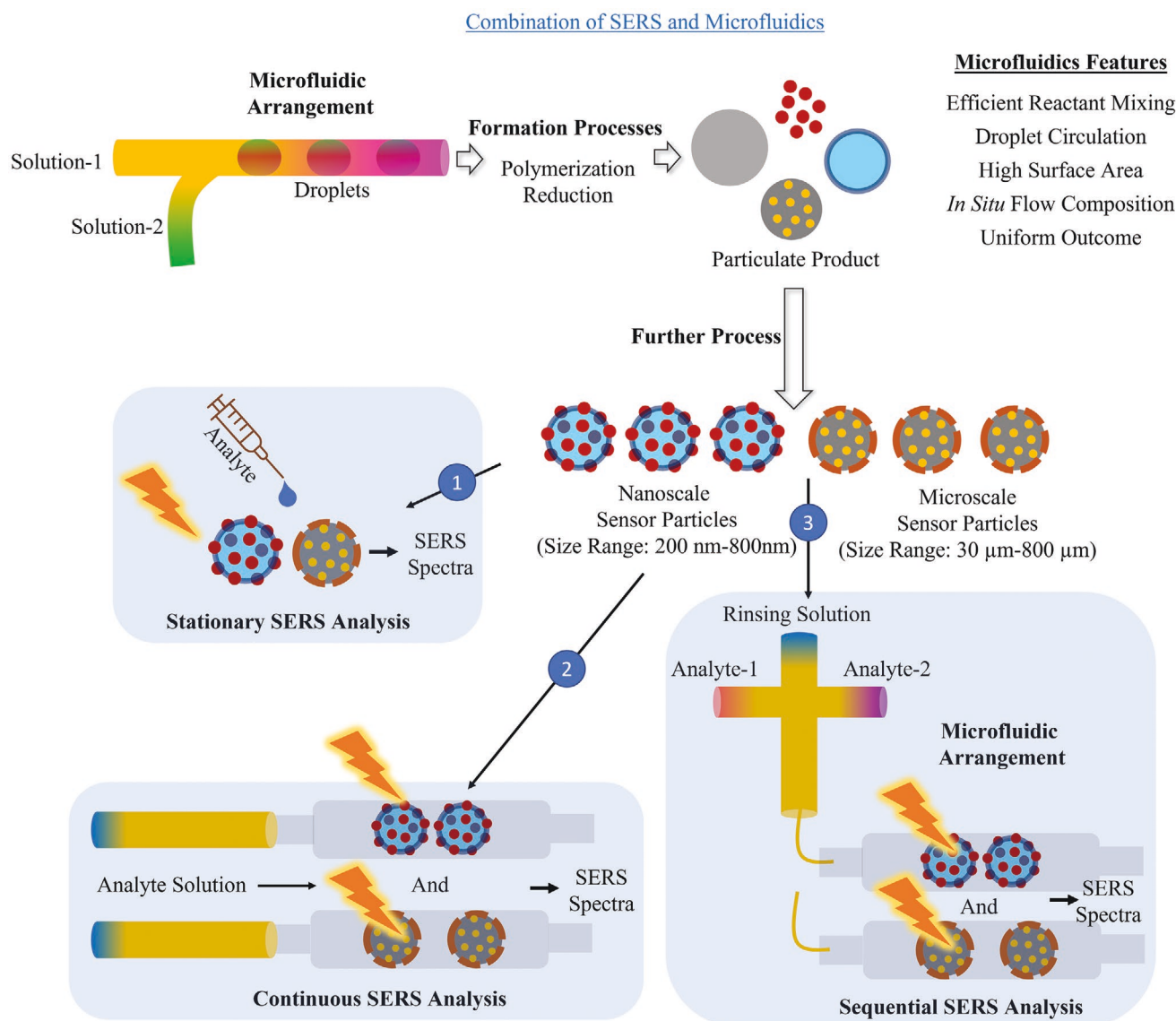
Figure 4. Synergistic advantages of the SERS and microfluidics. A microfluidic-supported reaction platform is highly essential in the generation of uniform, reproducible, and multifunctional particles. Further processing on the microfluidically prepared particles such as metal enhancement can be applied and versatile sensor particles (polymer–metal composite particles) can be generated which are useful for SERS sensing. The generated sensor particles via microfluidic-supported synthesis can be used for the stationary SERS analysis in batch conditions, continuous flow SERS analysis in the microchannel, and sequential SERS analysis of multiple analytes at a single platform in the flow capillary.

metal nanoparticles and further metallic enforcement process for utilizing as nanoscale sensor particles.

Besides the nanoscale sensor particles, microscale sensor particles were synthesized through droplet microfluidics in a single-step process. An arrangement for the synthesis of microscale sensor particles as shown in Figure 5B is very similar to nanoscale sensor particles. But a photopolymerization approach has been applied to solidify the generated droplets to form the sensor microparticles.[20] A detailed description of the sensor microparticles and their SERS activity is given in Section 7 below. Here, a basic microfluidic arrangement is shown in which a carrier phase contains the surfactant. The droplet generating phase contains the aqueous monomer, photoinitiator, and precursor for the in situ formation of metal nanoparticles.

Both syringes can be fixed to the syringe pumps which are operated through a computer-controlled flow rate program. The generated droplets of the aqueous phase can be solidified to form microgel particles through photopolymerization inside the microchannel under UV irradiation (Figure 5B). The fluorinated ethylene propylene (FEP) microtube (transparent) has been used which allows easy penetration of the UV light to the droplets inside microtubes.[20] The FEP microtubes of different internal diameters were used for the generation of different sized microparticles (same size as tubing internal diameter). Here, photopolymerization of the polymer matrix and photoreduction for the metal precursor takes place at the same time in the continuously flowing droplets to form the metal nanoparticles embedded polymer microgel particles in a single step.[20,31]
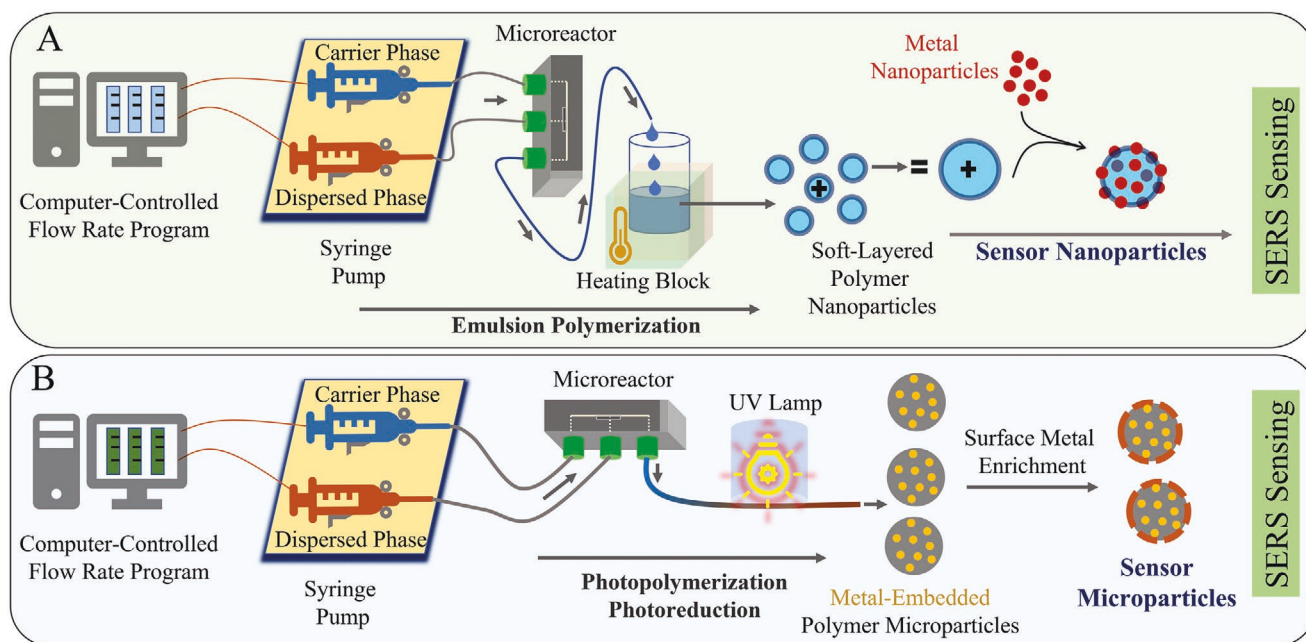
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Figure 5. Microfluidic reaction setups for the synthesis of A) nanoscale and B) microscale polymer particles toward the formation of sensor particles for SERS sensing applications. A) Reproduced with permission.[89] Copyright 2015, Wiley-VCH. B) Reproduced with permission.[20] Copyright 2015, American Chemical Society.

For utilizing the generated microparticles for SERS sensing, further metallic enforcement has been applied on the surface and its details are provided in Section 7 below.

Overall, a wide range of various nanoscale (the sub-micron length scale: 200–800 nm) and microscale sensor particles (30–800 µm) can be developed by using microfluidics as a synthesis platform. Regarding polymeric materials, various properties of polymers such as, hydrophilic polymer, hydrophobic polymer, block copolymer, etc., can be used depending on the requirement for support to the metal nanostructures. Besides, various metallic nanostructures in terms of shapes and compositions as SERS-active components can be used to bind with polymer particles. In the microfluidic setup, different types of microreactors/microchips can be used which can be fabricated from various materials such as, glass, silicon, polydimethylsiloxane, etc., by using various microlithographic techniques.

6. Surface-Enhanced Raman Scattering Sensing with Nanoscale Sensor Particles (Sub-Micron Length Scale)

In general, nanoscale particles possess a high surface-to-volume ratio and reveal a larger surface area to interact with analytes of interest for various interfacial interaction-based applications. As far as nanoscale sensor particle-based SERS sensing is concerned, the formation of relatively uniform nanoparticles (polymer-metal composite nanoparticles) with a systematic distribution of smaller-sized metal nanoparticles on polymer nanoparticles is desired for the uniform and sustained outcome. In this section, we reviewed the formation of the model nanoscale sensor particles in detail. Moreover, in the second part, a proof

of concept of the SERS sensing of various analytes by using the synthesized sensor nanoparticles is shown.

6.1. Formation of Polymer Supported Nanoscale Sensor Particles

Nanoscale sensor particles are made of two subsystems: i) Polymer nanoparticles and ii) metal nanoparticles. In addition, further enhancement of the metallic surface has been achieved through an additional metal deposition via metal-catalyzed metal enforcement. Finally, these sensor nanoparticles were used to detect analytes in SERS applications.

6.1.1. Synthesis of Core–Shell Polymer Nanoparticles: The Support

As a model subsystem, surface-active polymer nanoparticles were prepared via semi-microfluidic synthesis whose setup is shown in **Figure 6**A. To generate the swellable, porous, and soft surface layer (shell), a hydrophilic monomer diallyldimethylammonium chloride (DADMAC) was selected, on the one hand. On the other hand, the hydrophobic monomer methyl methacrylate (MMA) was chosen (Figure 6A) for creating relatively hard core support compared to the swellable shell layer. In the preparation of core–shell type polymer nanoparticles, a simultaneous copolymerization (hydrophilic–hydrophobic) process has been applied.[89] The primary monomer, cross-linker, initiator, and surfactant are required for polymer nanoparticle synthesis, in general. Initially, droplets of the primary dispersed monomer phase (hydrophobic) can be generated by shear forces of the flowing continuous aqueous phase (Figure 6A). A semi-microfluidic polymerization approach can be conducted in which
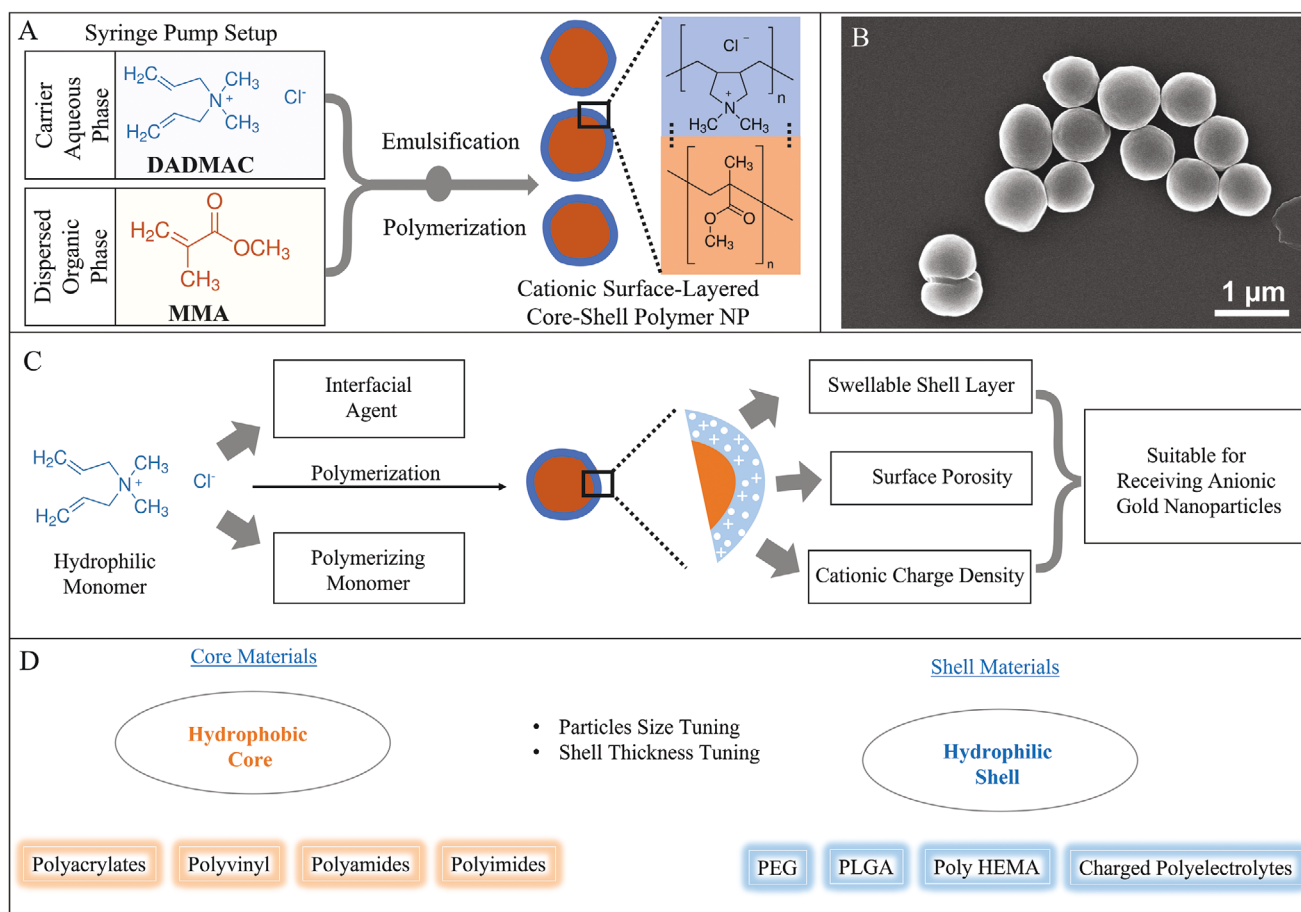
Figure 6. Synthesis of polymer nanoparticles (ideally sub-micron size range) for nanoscale sensor particles which are made of two sub-systems: Core–shell polymer nanoparticles and metal nanoparticles. A) A general scheme for the emulsification and polymerization process for the generation of soft and swellable layered core–shell type polymer nanoparticles. B) SEM image of the polymer nanoparticles obtained by using 5 mM diallyldimethylammonium chloride (DADMAC) in aqueous phase with aqueous to organic flow rate ratio 1200/70 µL min^{-1} in which core material is polymethyl methacrylate (PMMA) following the procedure provided in ref. [89]. C) Characteristics of the shell polymer layer that shows swellability, porosity, and high cationic charge density suitable for accommodating oppositely charged metal nanoparticles toward the formation of polymer-metal composite sensor nanoparticles. D) Tunable materials parameters for core and shell of the core–shell polymer nanoparticles.

emulsification of both immiscible liquid phases takes place in the microreactor and completion of polymerization occurred outside the microreactor.[117,118] As described in our previous report,[89] 1 mL of hydrophobic monomer phase is made up of 3.5 µg thermal initiator azobisisobutyronitrile, 10 µL cross-linker ethylene glycol dimethacrylate, and 990 µL MMA. Micrometer-sized droplets of the hydrophobic monomer phase can be generated in the continuously flowing aqueous phase which contains the positively charged hydrophilic monomer DADMAC. Usually, the role of an interfacial agent (surfactant) is to emulsify two immiscible liquids by forming shelter-like micellar formation at the surface of droplets of the opposite phase liquid (oil) in the larger liquid phase (water).[119–122] Here, a charged hydrophilic monomer plays a dual role at the same time: i) As a surface-active agent to stabilize the oil phase droplet and ii) as a hydrophilic polymerizing monomer to create a hydrophilic polymeric layer on the surface of hydrophobic core polymer nanoparticles. Once the emulsion solution entered a heating block of polymerization temperature, the thermal initiation process starts. Linking of MMA monomers through cross-linking can create a 3D hydrophobic polymethyl

methacrylate (PMMA) network. The polymerization takes place in the water phase, therefore, relatively harder PMMA core nanoparticles can be formed compared to the softer surface polymeric layer of poly(diallyldimethylammonium chloride) (polyDADMAC). The formation mechanism of the core–shell polymer nanoparticles is provided in our previous report.[89,90] Various concentrations of DADMAC between 1 and 20 mM have been applied in the aqueous phase to tune the surface thickness of the core–shell polymer nanoparticles.[89] In addition, the flow rate ratio of both liquid phases plays a key role in tuning the overall size and shell thickness of the core–shell polymer nanoparticles. The size of the core–shell polymer nanoparticles can be tuned between 200 nm and 1 µm depending on the concentration of DADMAC, flow rates, and flow rate ratios.[89] The hydrophobic core is compact in the aqueous solution but the surface layer of polyDADMAC is swelled when in water. PolyDADMAC surface layer also possesses a high positive surface charge density which is capable to bind negatively charged smaller nanoparticles in high amounts via electrostatic interaction. Moreover, the polyDADMAC surface polymeric layer creates many pores which

are particularly useful for entrapping smaller-sized metal nanoparticles not only at the outer surface but also at the deep level below the surface. Figure 6B shows a representative SEM image of the core–shell polymer nanoparticles, and Figure 6C reveals a scheme where surface shell layer is swellable, porous, and densely charged that is capable to bind the oppositely charged metal nanoparticles. The polymer does not directly contribute to the SERS effect because they are not potentially responding to light interaction. Besides, metal nanoparticles are key components for SERS-active material.[74] The higher density of metal nanoparticles without larger aggregation is crucial in enhanced SERS outcome upon analyte interaction.[14,18,123] In this regard, the porous, swellable, and highly charged polyDADMAC polymeric surface layer is very suitable to be used as potential support in the in situ formation or immobilization of the small metal sensor particles.

An important advantage of the hydrophobic-hydrophilic copolymerization approach is the formation of a swellable hydrophilic network.[124] The cross-linked matrix forms a stable core and hence provides efficient support to the incoming hard metal nanoparticles through the soft surface polymeric shell layer. Overall, various types of hydrophobic core polymeric networks can be formed/generated depending on the requirements.[125] PMMA and polystyrene (PS) are the most common polymers due to their easy formation process and ability to form robust polymeric covalent cross-linked networks.[126] Besides PMMA and PS hydrophobic cores, other polyacrylates, amides, imides, etc., can also be used by choosing a suitable combination with hydrophilic counterparts.[35,127] Like tuning hydrophobic core, hydrophilic surface polymers of different characteristics can also be formed. Hydrophilic monomers of different types can be selected based on their functions such as charge, solubility, molecular weight, and polymerization ability. Individual monomers can retain their properties in their polymeric network, hence useful in various interfacial interactions. As PolyDADMAC surface polymeric layer reveals high cationic charge density, other types of cationic polymers can also be selected such as those with quaternary ammonium functional groups. Likewise, an anionic charged hydrophilic surface polymeric layer on the core nanoparticles can also be generated. For instance, the 3-sulfopropyl methacrylate potassium salt is a potential hydrophilic anionic monomer for generating a swellable cross-linked polymer surface layer.[90] In the case of anionic core–shell polymer nanoparticles, overall nanoparticle size and shell thickness can systematically be tuned between 200 and 800 nm as reported in our previous article.[90] The size of nanoparticles can be controlled by flow rates and flow rate ratio of both immiscible liquid phases together with variable concentrations of charged monomers in the aqueous phase. Similarly, shell thickness is primarily tuned by a concentration of hydrophilic monomer. A general parameter of the core–shell polymer nanoparticles is shown in Figure 6D.

6.1.2. Metal Nanoparticles Assembling to Polymer Support

Once the size-tunable cationic core–shell polymer nanoparticles are formed, they can potentially be used for the formation of polymer-metal composite via electrostatic interaction with anionic metal nanoparticles. Negatively charged size-controlled gold or silver nanoparticles can be synthesized separately in either batch procedure or via microfluidics.[128,129] For instance, citrate-stabilized smaller nanoparticles of about 25 nm diameter were prepared by the Turkevich method.[130,131] Noble metal nanoparticles are plasmon-active and potentially interact with light.[74] Mostly due to their excellent crystalline characteristics, a wide variety of structural tunability of metal nanoparticles can be achieved in terms of shapes and morphologies.[132,133] Each distinct shapes and structures of metal nanoparticles present a unique characteristic of concentrating the electromagnetic waves for SERS enhancement upon analyte interactions.[14,45]

Here, we focused in detail on one type of simple gold nanoparticles in the development of model polymer-metal nanoassembly particles. Water dispersion of the citrate-capped negatively charged gold nanoparticles (zeta potential about −25 mV) was synthesized following the method[130,131] and can be filled in a 3 mL syringe. In another syringe, synthesized positively charged core–shell polymer nanoparticles were filled after washing through centrifugation to remove free monomers, unreacted reagents, and impurities (**Figure 7**A). When two oppositely charged nanoparticles meet together, an electrostatic interaction is being realized and polymer-metal nanoparticles were obtained. The purpose of conducting electrostatic interaction in the microfluidic channel (knot mixture) under continuous flow conditions is to provide a uniform environment for reactants interacting at a high surface area. Through microfluidics, the flow rate of one type of nanoparticle dispersion (for instance, anionic gold nanoparticles) can be increased and/or decrease while maintaining another constant. In this way, the density of surface adsorbing gold nanoparticles can be tuned. Second, the flow rate ratio of both nanoparticles dispersions (gold nanoparticles and core–shell polymer nanoparticles solution) can systematically be controlled to obtain the desired distribution of gold nanoparticles on the core–shell polymer nanoparticles precisely. Furthermore, the nanoparticle concentration (for instance, more diluted dispersion) can also be altered in both types of nanoparticles solution to realize even better distribution with desired density at the surface. Since most types of bio-analytes are water-soluble at some level, it is important to have a surface layer of polymer nanoparticles (support to metal nanoparticles) that is water compatible. Here, a polyDADMAC layer of core–shell polymer nanoparticles is water-compatible and swellable, as well as, fully capable to deal with an aqueous solution of various analytes and an aqueous dispersion of gold nanoparticles. The microchannel is tiny with an internal diameter of either 0.25 or 0.5 mm or 0.8 or 1 mm depending on flow rates, flow rate ratios, and nanoparticle concentrations. Also, a microchannel (tube) of tunable length can be used depending on how strong the zeta potential of nanoparticles is and the time required for the completion of electrostatic interactions of oppositely charged nanoparticles. Knot mixture or knitting/zigzagging of microtube can allow better circulation of the flowing liquid solution of nanoparticles, hence uniform assembling can be realized at all sides of nanoparticles. It is also noteworthy that the swellable layer of core–shell polymer nanoparticles can hold a large number of smaller-sized gold nanoparticles not only at the surface but also underneath the surface down to the hydrophobic PMMA core surface. In this
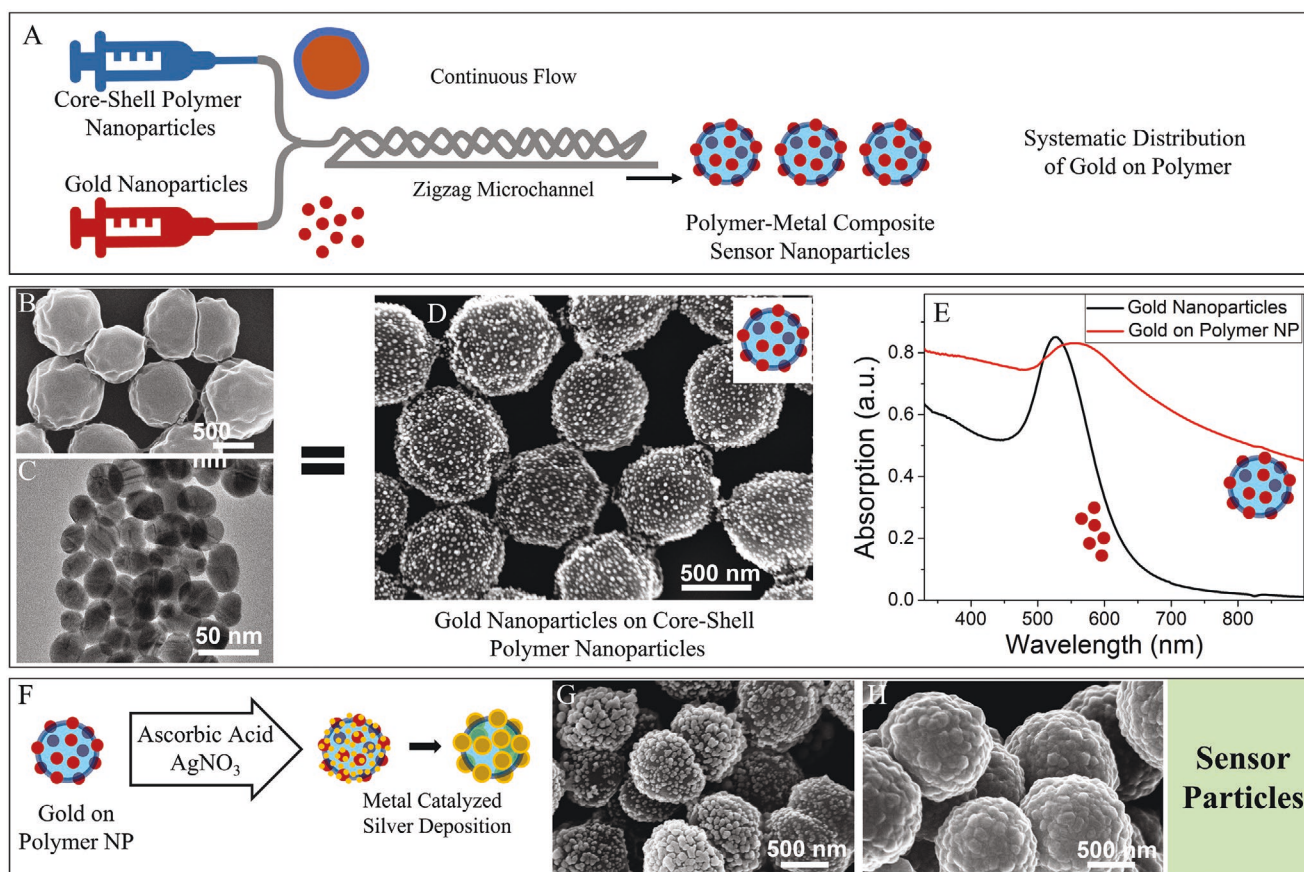
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Figure 7. Synthesis of the nanoscale sensor particles (sub-micron size range). A) A general scheme for the generation of polymer-metal composite sensor nanoparticles. B) SEM image of the polymer nanoparticles (cationic) obtained by using 6 mM DADMAC at flow rate ratio 1000/100 μL min⁻¹.[89] C) Citrate-capped gold nanoparticles (anionic) were obtained by using a slightly modified synthesis procedure.[131] D) SEM image of the polymer-gold composite nanoparticles obtained by electrostatic interactions.[89] E) UV–vis absorption spectra of the pure gold nanoparticles and gold nanoparticles attached to the surface of polymer nanoparticles.[89,90] F) A basic scheme for metal-catalyzed metal deposition at the surface of particles. G,H) SEM images of the sensor particles in which ligand-free silver deposited on the surface of polymer-gold composite nanoparticles obtained by using G) 3 mM and H)10 mM AgNO₃, respectively. B,D,E,G,H) Adapted with permission.[89] Copyright 2015, Wiley-VCH.

manner, a large density of gold nanoparticles is being accommodated without the uncontrolled aggregation of gold nanoparticles. If uncontrolled aggregation of plasmon-active gold nanoparticles into a larger cluster occurred, it made an adverse impact on signal enhancement during SERS measurement. To avoid such concern, a combined approach of microflow reaction, appropriate individual flow rates, suitable flow rate ratio, defined concentration of nanoparticles (dispersion), and using knot mixture type microchannel (microtube) can be emerged comprehensive. Consequently, systematically distributed gold nanoparticles on the surface of core–shell polymer nanoparticles (polymer-metal nanoassembly particles) were obtained as shown in Figure 7B–D. A characteristic UV–vis spectral peak of the smaller-sized pure gold nanoparticles is obtained at about 520 nm. Upon assembling with polymer nanoparticles, the significant redshifting, as well as, broadness of the plasmon peak of gold nanoparticles, can be realized largely due to the adsorption of gold nanoparticles on the polymer surface and a change in refractive index due to polymers (Figure 7E). In a very similar manner to gold nanoparticles, other types of noble metal nanoparticles such as silver nanoparticles and platinum

nanoparticles can also be applied electrostatically on the surface of core–shell polymer nanoparticles to form polymer-metal nanoassembly particles. Moreover, noble metal nanoparticles of other shapes and morphology such as nanocubes, nanorods, nanoprisms, etc., can be applied on the surface of polymer nanoparticles. Overall, uniformly distributed polymer-metal nanoassembly particles were obtained by using an integrated approach of surface swellable core–shell polymer nanoparticles, smaller-sized oppositely charged gold nanoparticles, and microflow arrangement for uniform interactions.

6.1.3. Metal Enforcement to Form Complete Nanoscale Sensor Particles

Two basic requirements for polymer-supported sensor nanoparticles are i) suitable surface layered polymer nanoparticles as support and ii) surface adsorbing metal nanoparticles as a SERS-active component. Despite both conditions has been fulfilled by applying colloidal metal nanoparticles on the core–shell polymer nanoparticles as shown in Figure 7D, the SERS

activity with these metal-polymer nanoassembly particles to detect various analytes was very weak even at higher analyte concentration (detailed results we have shown in our previous articles).[89,90] There could be three reasons for low SERS signals with a simple combination of core–shell polymer nanoparticles and metal (gold) nanoparticles: a) Availability of larger area of polymer which is not interacting with light, b) surface of gold nanoparticles is covered with ligand molecules, and also gold nanoparticles are to appear with low density during the swelling phase in aqueous solution, and c) no availability of sufficient hotspots (a key requirement in SERS enhancement) because spherical metal nanoparticles are systematically distributed in a hydrophilic gel-like polymer network. It is clear from the current development of SERS substrates that free metal nanoparticles (with sharp-edged and hotspots) dispersion are sufficient to reveal extremely enhanced SERS signals and able to detect the ultimate limit of an analyte such as single-molecule.[12,19,57] However, the recyclability and reusability of the colloidal nanoparticles are very challenging for the purpose of using sensor particles for the next analyte detection. The promising approach is to use polymer nanoparticles as support to strongly fix the metal nanoparticles at the surface as well as underneath the surface. Such particles are suitable for chemically cleaning and reuse. A detailed approach to the recycled and repeated use of the sensor nanoparticles has been explained in Section 8 below. While only electrostatically assembled polymer-metal nanoparticles are not sufficient to show enhanced SERS signatures, additional metal enforcement on polymer-metal nanoassembly particles in a controlled manner is a very promising approach. Additional metal can be deposited in a way that can provide larger metal surface exposure, reveal ligand-free surface, and show enormous hotspots. To fulfill all three conditions in a single process, metal-catalyzed metal enforcement has been implemented. Usually, in nanoparticles (particularly shape-controlled) synthesis, seeds (pre-formed nanoclusters) are used on which further growth of metal nanostructures occurred. In another form, smaller-sized pre-formed metal nanoparticles can be used as a catalyst to allow further formation and deposition of nanoscale metal on that.[89]

To apply the metal-catalyzed metal enforcement process, prepared polymer-metal nanoassembly particles (Subsection 6.1.2) can be filled in a small glass vial. Polymer-metal nanoparticles are heavy and hence settled down to the bottom of the vial within a minute. The water supernatant from the vial can be removed by a syringe needle of tiny diameter. For forming the metal nanoparticles from metal precursors, a reducing agent is required. In this regard, a solution of a weak reducing agent ascorbic acid has been poured onto the polymer-metal nanoassembly particles in a vial. Polymer-metal nanoparticles redispersed in the aqueous solution of ascorbic acid in the same way they dispersed in pure water without any color change. Afterward, the color of polymer-metal nanoparticles was immediately changed upon the addition of a diluted solution of silver nitrate indicating the deposition of a silver layer on the gold surface of polymer-metal nanoassembly particles.[89,90] Ascorbic acid reduces silver ions immediately within a second or few seconds and silver clusters are immediately formed on the surface of gold nanoparticles which are entrapped to the polymeric shell layer (Figure 7F). Though ascorbic acid reduces

metal precursor, the formation of a silver layer of nanoscale and simultaneous quick deposition can be directed by gold nanoparticles which play a catalytic role. Therefore, an electrochemical mechanism is assumed, in which ascorbic acid supplies in its oxidation process electrons to the gold nanoparticles, whereas adsorbing silver ions are reduced at the metal surface by electrons coming from the particle.[128] Thus, the growing particle act as an open-circuit mixed-electrode system with free-floating electrochemical potential. The density and size (thickness) of the enforced silver layer can be tuned by varying concentrations of silver nitrate (Figure 7G,H). The number of enforced silver nanoparticles (in other words density) on the gold nanoparticles of polymer-metal composite particles is dependent on the number of gold nanoparticles. The number of gold nanoparticles can be controlled during the electrostatic assembling process by applying a higher or lower amount of gold nanoparticles on core–shell polymer nanoparticles. Moreover, the thickness of the enforced silver nanoparticles on the gold nanoparticles can be controlled by the concentration of silver nitrate and ascorbic acid during the metal-catalyzed metal enforcement process. The UV–vis spectra can also be obtained as shown in ref. [90]. Here, an enforced silver layer creates a roughened metallic surface, providing a large number of hotspots, and revealing a ligand-free outer surface.[89] Consequently, these silver-enforced polymer-metal nanoassembly particles can act as complete sensor nanoparticles and are useful in SERS sensing of analytes. When sensor particles are in solution, they achieve the swelling phase which is very suitable for the transportation of analyte solution in and out during the SERS measurement. After depositing a ligand-free and roughened silver layer, the SERS signals of analytes were significantly enhanced which is shown below.

6.2. Representative Surface-Enhanced Raman Scattering Sensing of Analytes by Nanoscale Sensor Particles

Surface plasmon resonance is a minimum requirement in observing SERS enhancement.[11] Fabricated sensor nanoparticles are composed of three layers: a) Polymer support, b) electrostatically entrapped gold nanoparticles to polymer network, and c) roughened and ligand-free silver nanolayer on gold surface.[90] Plasmonic signature is being realized from the metallic layer at the top surface of sensor nanoparticles. Once fully functional sensor nanoparticles are ready, the supernatant can be removed, and sensor nanoparticles can be washed with pure water twice to remove all the unreacted reactants and impurities. A ligand-free metal surface is directly interacting with various analytes of amine or hydroxyl functional group through electron affinity. Various concentrations can be used depending on the types of analytes and their SERS spectra are obtained.

Figure 8A shows a basic schematic for SERS outcome upon interaction with laser light after sensor nanoparticles meet with analytes. Here, SERS analysis of a model analyte adenine is performed on sensor nanoparticles (Figure 8B). A swellable shell layer of polymer of sensor nanoparticles can be swelled with the addition of a diluted aqueous analyte solution. All processes can be conducted in an aqueous medium, hence the surface polymeric layer stayed swelled in all those conditions.
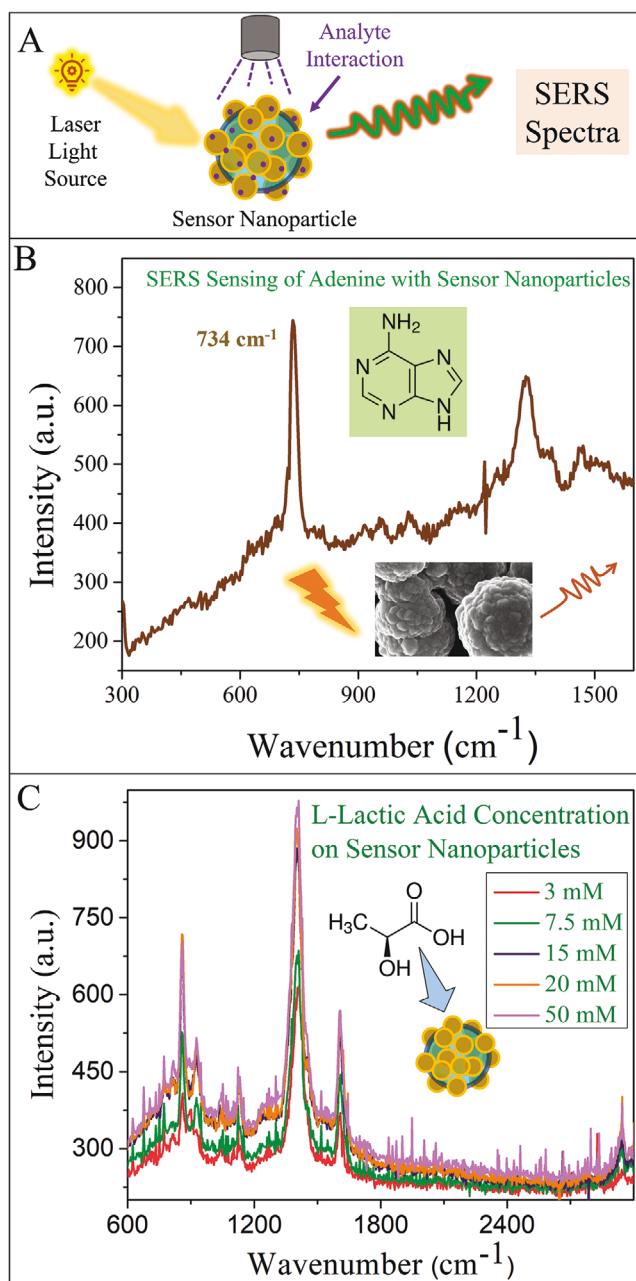
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Figure 8. SERS sensing of analytes with sensor nanoparticles. A) A general scheme for the analyte sensing with sensor nanoparticles. B) Adenine (0.1 mM) SERS spectrum after interaction with sensor nanoparticles. Reproduced with permission.[89] Copyright 2015, Wiley-VCH. C) SERS spectra of L-lactic acid of different concentrations with sensor nanoparticles. Reproduced with permission.[90] Copyright 2017, Wiley-VCH.

A large number of pores can be observed during the swelling of the hydrophilic shell polymer layer. Thus, gold nanoparticles can be entrapped at a deep level of the swelled polymeric layer and hence enforced silver layer too. For the batch SERS experiment, 300 µL sensor nanoparticles dispersion can be filled in the vial. Once the sensor nanoparticles settled down at the bottom of the vial, the supernatant can be removed by a syringe needle. Afterward, an analyte solution (500 µL) of interest can

be inserted in a vial. When a diluted solution of adenine was inserted in a vial where sensor nanoparticles are present (stationary SERS sensing), the adenine molecules also diffuse to swelled network and hence able to interact with a large number of metal hotspots. Consequently, it can reveal enhanced SERS signals and gives the possibility to detect the analyte at comparatively low concentrations. Figure 8B reveals the characteristic intense SERS peak at 734 cm⁻¹ which can appear due to the breathing of adenine under influence of light waves.[17] Peak intensity can be tuned by applying various concentrations of adenine analyte during SERS sensing.[89,90] Similarly, other analytes can be easily detected by using sensor nanoparticles through SERS sensing analysis. For instance, L-lactic acid of different concentrations was used, and their SERS spectra were recorded as shown in Figure 8C. The peak intensity can be tuned systematically with varying concentrations of L-lactic acid during the analysis.

Overall, polymer-supported sensor nanoparticles (submicron size range) can potentially be used to detect various analytes of low concentration. The versatile and sustained SERS outcome is the direct result of robust sensor nanoparticles which are integrated with a highly charged and swellable polymeric surface layer on the relatively hard hydrophobic core polymer nanoparticles, strongly bound metal nanoparticles in the swellable network of the hydrophilic polymer layer, and roughened and ligand-free surface silver layer at the outer surface. Though polymer-metal assembly-based sensor nanoparticles are not able to detect very low concentrations of analytes at the ultimate detection limit, their impact is important in sustained and regenerative use which is explained in detail in Section 8. In this way, SERS sensing of various biochemicals and other types of analytes, as well as, biological and biomolecular systems can be performed by using polymer-supported nanoscale sensor particles of sub-micron length scale as SERS substrates.

7. Surface-Enhanced Raman Scattering Sensing with Microscale Sensor Particles

On one hand, materials at the nanometer length scale are unique and highly advantageous due to their high surface-to-volume ratio, and high surface energy.[134] Also, they reveal unique properties upon interaction with light since their size is less than the light wavelength.[29] On the other hand, microscale materials are also versatile and promising in a wide range of various applications. For instance, the length scale of the biological cells falls into micrometers and hence the importance of microbiology is extraordinary.[135] Microscale scaffolds with porosity are useful in tissue engineering and regenerative medicine.[136] Moreover, the fabrication of microelectromechanical systems is very useful in microelectronics and energy applications. Likewise, fluid manipulation at micrometer lengthscale through microfluidics can generate microscale droplets and materials useful in biomedicine, drug discovery, sensing, labeling, and catalysis among the other applications.[113] In general, nanomaterials and micromaterials form the bridge between the molecular world and the macro world. As molecular and nanoscale colloidal systems experience high Brownian motion

in the solution phase, the micrometer-sized particles show certain properties of nanoscale functions and macroscale function. In this section, the synthesis procedure of a model system of polymer-supported microscale sensor particles is described in detail through microfluidics and their SERS analysis has been performed.

7.1. One-Step Synthesis of Metal Embedded Polymer Microparticles

Microfluidics manipulates a small volume of flowing liquids inside the microscale channel and creates microdroplets. Liquid manipulation can take place in terms of uniform-sized droplet formation, droplet circulation, better mixing inside droplets via circulation, and transportation of droplets inside microchannel or microtube.[111] Aqueous droplets and hydrogel microparticles can be generated in the continuous flow of immiscible liquid to it such as mineral oil, silicone oil (polydimethylsiloxane—PDMS), or fluorocarbon-based liquids such as perfluoromethyl-decalin (PP9) or Novec 7500.[20,31,34,36,118] Microscale droplets can be generated via various flow arrangements in the microreactor device such as cross-flow, co-flow, or flow-focusing with either jetting or dripping modes.[137] Here, a model cross-flow arrangement has been used as shown in Figure 5B for the generation of aqueous microdroplets and hence microparticles. In general, the hydrogel network of the microparticles is soft and cross-linked. The network is swellable in water and shrunk upon removal of water. The larger portion of hydrogel particles is water which, therefore, can serve as a platform to accommodate a wide range of water-compatible species such as active cargos, drug molecules, functional fluorescent agents, reporters, biomolecules, and functional nanoparticles.[32,34,138] Depending on the monomer to water ratio, the swelling profile of the hydrogel particles can easily be changed. Similar to nanoscale polymer particles (sub-micro size range) as shown in Subsection 6.1.1, microgel particles are not directly responding to light, that is, only hydrogel particles cannot be used as a SERS-active component. Plasmon-active SERS component is a minimum requirement in observing the SERS effect.[18] In this regard, in a first strategy, metal nanoparticles can bind to the surface of hydrogel microparticles post-synthesis. This strategy is interesting since a higher amount of metal is available at the surface which can efficiently respond to the light. However, in this case, the larger volume/area of the hydrogel core remains unused and does not contribute anything to the SERS effect. The purpose of using swellable and porous hydrogel particles is due to their ability to allow diffusion of analyte molecules at the deeper level in the core below the surface through pores during the swelled phase. But if metal nanostructures are not present inside the hydrogel core network, then the diffused molecules cannot give enhancement in the SERS signals. Therefore, a promising strategy is to distribute smaller-sized metal nanoparticles throughout the hydrogel core. Smaller-sized metal nanoparticles of typically about 5–10 nm size show intense plasmonic peaks.[74] But smaller sized metal nanoparticles can easily be aggregated within a short period to minimize the surface energy. The nanoparticles can be stabilized by applying a higher density and strongly charged surface-active agent at the surface through which interparticle repulsion can prevent uncontrolled aggregation.[139] Afterward, stabilized metal nanoparticles can be incorporated inside the hydrogel microparticles during their swelling phase. Here, smaller metal nanoparticles can diffuse to the hydrogel core, but evenly and systematic distribution can be a challenge. Moreover, post-synthetically incorporated metal nanoparticles are not permanently fixed to the hydrogel network. In this regard, to avoid the concern of uncontrolled aggregations, as well as, with the advantages of uniform distribution and efficient fixation, simultaneous in situ formation of metal nanoclusters inside hydrogel microparticles in one step is a very efficient strategy. Through this strategy, metal nanostructures smaller than 5 nm can be obtained inside the microgel particles. Various strategies to combining metal nanoparticles with polymer network is shown in **Figure 9**A. Thus, a droplet microfluidic approach (Figure 5B) via photopolymerization/photoreduction has been applied to obtain the uniformly metal-distributed homogeneous hydrogel microparticles where formation of metal nanoparticles takes place inside the hydrogel core thoroughly during the one-step microfluidic reaction and generate in situ composite particles in a single-step (Figure 9B).

Droplets of the monomer phase contain all the ingredients available in the generating metal-embedded hydrogel microparticles. Monomer, cross-linker, and photoinitiator are available for the photopolymerization process, as well as, metal salt for the formation of metal nanoparticles through photoreduction by the photoinitiator. In a model hydrogel system, around 67% (weight %) of water content is present in the hydrogel particles.[20] For this reaction, 0.6 gm monomer:crosslinker mixture of acrylamide:bis-acrylamide (19:1) has been dissolved in 2 mL of double-distilled water. In addition, a 3.8 mg silver nitrate ($AgNO_3$) solution has been added. Afterward, a little amount (40 µL) of photoinitiator 2-hydroxy-2-methylpropiophenone first dissolved in ethylene glycol was added to the monomer phase right before starting the microfluidic flow reaction. Droplets of the monomer phase were generated at the T-junction of the microreactor in which the embedded chip has been functionalized to make it compatible with the continuous phase as described in our previous report.[20] The continuous phase in this reaction was made up of Novec 7500 liquid in which picosurf surfactant has been added. The size of the microparticles is the same as the size of the droplets generates in the microtubes. Therefore, it is essential to control the size of the droplets of the monomer phase. The size of the microdroplets can be controlled/tuned by various routes such as surfactant concentrations, flow rates, flow rate ratios, and channel dimension.[20] When two immiscible liquid phases meet, high surface tension can be realized at the surface of generating droplets.[119] Surfactants can play a crucial role in reducing surface tension. Higher the concentration of surfactant, lower the surface tension. Hence, the generated microdroplets are of smaller size when the concentration of surfactant is relatively high.[140] The size of microdroplets was systematically controlled by applying various concentrations of picosurf in the carrier phase of Novec 7500.[20] Also, droplet size can be tuned by changing the flow rate of the carrier phase at constant surfactant concentration. The high flow rate of the carrier phase can create a high shear force and is capable to cut off the thread of the monomer phase early to form a smaller-sized droplet.[141] In this way, systematic
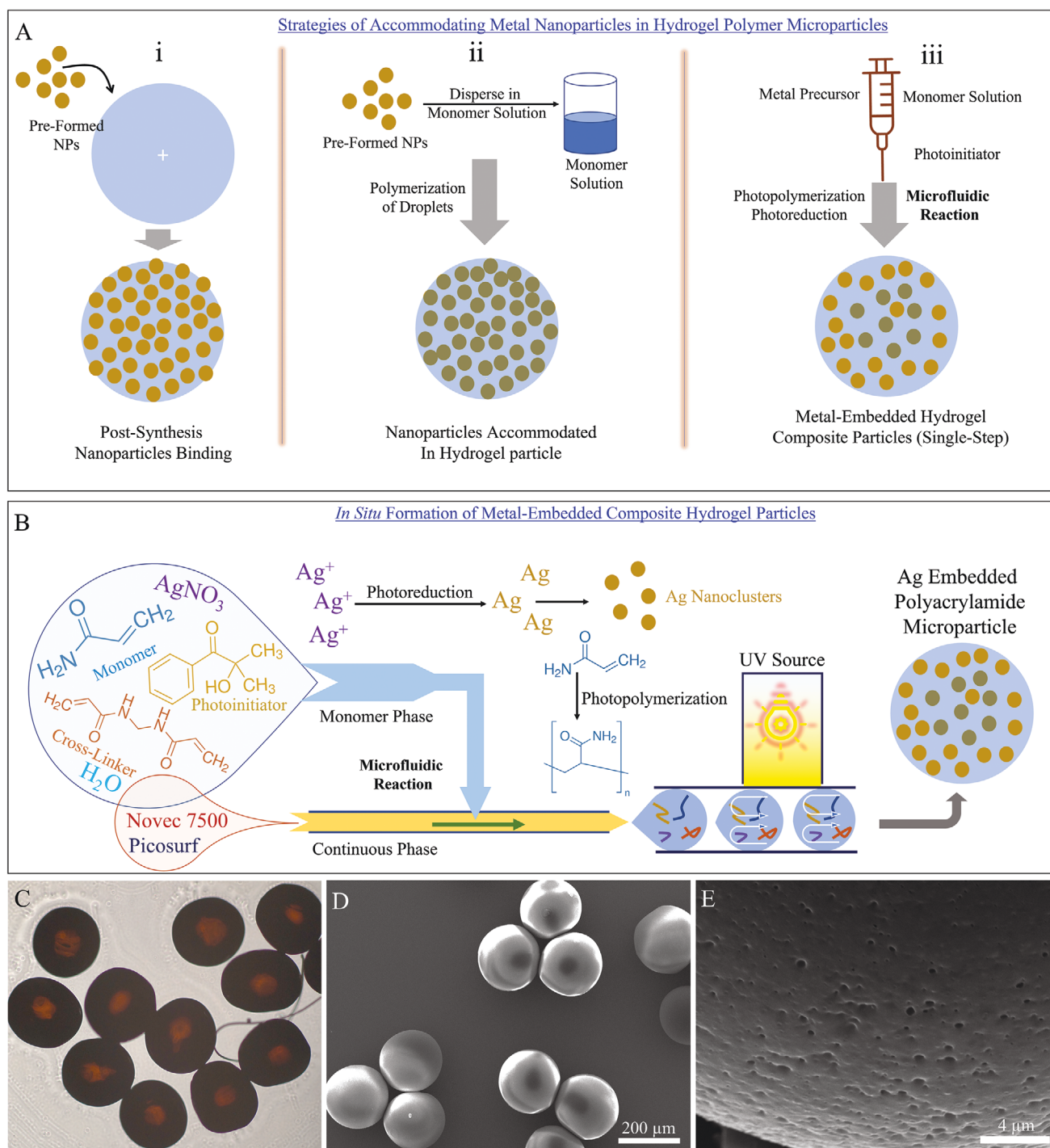
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Figure 9. Microfluidic synthesis of microscale sensor particles. A) Three basic strategies for linking metal nanoparticles to the polymer network: i) Post-synthesis electrostatic assembling, ii) pre-synthesized metal nanoparticles dispersed in the polymerizing solution, and iii) formation of metal nanoparticles and polymer particles takes place in a single step for the formation of polymer-metal composite microparticles. B) Reactants for the process of simultaneous photopolymerization and photoreduction for the production of silver nanoparticle-embedded polyacrylamide microparticles (single-step process). C,D) The light microscopy image and SEM image of the silver nanoparticles-embedded polyacrylamide microparticles synthesized by using 200/50 µL min⁻¹ carrier to monomer phase flow rate ratio and 0.125% picosurf in carrier phase (Novec 7500). E) SEM image of silver nanoparticles-embedded (80 mM) polyacrylamide particle possess porosity on the surface. C–E) Reproduced with permission.[20] Copyright 2015, American Chemical Society.

size control of the generating droplets can be achieved. Not only the flow rate of the carrier phase, but variation in the flow rate of the dispersed phase is also contributing the effect significantly to control the size of droplets. Overall, the size of

droplets with regards to the flow rate of individual flow rate or flow rate ratios can tune the size of droplets in the almost full spectrum of microscale. The flow rate of the carrier phase can be applied, for instance, 350 μL min⁻¹ and of dispersed phase 10 μL min⁻¹ to produce tunable sized microparticles between 40 and 300 μm at various concentrations of picosurf in the carrier liquid Novec 7500.[20] Likewise, one of the most important conditions to tune the size of microdroplets is channel diameter. In general, the diameter of the microdroplets is easily controlled by using a microtube (fluid carrier) of desired internal diameter at appropriate surfactant concentration and flow rate ratio. Most commonly, 250 μm, 500 μm, 800 μm, and 1 mm sized microdroplets can be produced by using microtubes of 250 μm, 500 μm, 800 μm, and 1 mm internal diameters, respectively (i.e., particles size same as internal diameter of microchannel).

Generated droplets in the microfluidic channel need to be solidified/polymerized to obtain polymer microparticles. Irradiation of UV light allows a quick photochemical reaction where monomer units form a polymeric network via photopolymerization (Figure 5B). For passing the UV light without hindrance, a transparent FEP microtube can be used. Residence time for the polymerization is less than 1 s which is sufficient to convert monomer droplets into solidified hydrogel network. Droplets of the monomer phase liquid also contain silver nitrate solution. Therefore, together with photopolymerization of monomers, photoreduction of silver nitrate form the smaller-sized metal nanoclusters throughout the droplet network which is initiated by the photoinitiator upon irradiation of UV light. Water content inside hydrogel microparticles can be tuned between as low as 50% and as high as up to 90% during photopolymerization reaction. Moreover, the droplet transportation inside the transparent tube is under high circulation in the flow of immiscible carrier liquid. Thus, the distribution of in situ forming metal nanoparticles inside the droplet is highly uniform. It can be observed during the experiment that photoreduction can start the metal formation immediately upon UV irradiation, but the ripening (or growth) process is continued until several hours post-reaction after hydrogel particles were formed. Such a process of continuous growth can be observed based on the color change. Initially, a color becomes very light yellow which is an indication of the formation of ultrasmall silver nanoparticles.[20] After 1 h, it is becoming a little more intense yellow, and intense yellow color has been observed after 6 h. Similar to the tunable content of water, the amount of silver content can also be tuned by using different concentrations of silver precursor during the photopolymerization/photoreduction one-step synthesis. Metal embedded hydrogel microparticles were obtained brown colored (Figure 9C) at higher concentrations of silver precursors, and microparticles were almost transparent with a slight yellow color at lower silver concentrations. As the formation of metal nanoparticles is taking place during polymerization of the liquid droplet, the metal nanoparticles are uniformly distributed without uncontrolled aggregation and fixed (stable) to the hydrogel network hence not leaching during swelling of hydrogel. Figure 9D,E presents the SEM images of the composite hydrogel particles at the lower and higher magnification, respectively. At higher magnification, the surface porosity can easily be visible. Similar to the model hydrogel system (polyacrylamide) shown here, other types of biocompatible hydrogel

materials can be used in the formation of metal-embedded hydrogel microparticles for the application in biological systems. For SERS sensing application, a stable hydrogel network is required which can permit for longer time use with repeatable analysis. However, it is a very promising strategy where a hydrogel network can develop with purpose adapted biodegradable properties, that is, the hydrogel can be degraded after their use.

7.2. Metal Enforcement on Hydrogel Microparticles

For utilizing metal embedded hydrogel polymer microparticles (composite particles) for SERS sensing of analytes, an additional metal deposition can be enforced in a similar procedure as explained in Subsection 6.1.3 above. Here too, embedded metal nanoclusters in the hydrogel can play the role of catalyst to form additional metal nanoparticles at the surface from the metal precursor. First of all, hydrogel composite particles need to be transferred into the aqueous phase from Novec 7500 by washing steps after the microfluidic photochemical reaction. The hydrogel network can swell in the aqueous phase which increases matrix permeability. Pure water is being replaced with a diluted solution of ascorbic acid, a mild reducing agent. Additional (enforced) silver nanoparticles were formed upon the addition of the metal precursor which is reduced by ascorbic acid and their formation process is catalyzed by embedded metal nanoclusters of the composite hydrogel particles (metal-catalyzed metal enforcement) (**Figure 10**). The density of the enforced metal nanoparticles can systematically be tuned by using various concentrations of ascorbic acid and ascorbic acid to the composite hydrogel particles.[20] As the hydrogel network in the aqueous phase is swelled, diffusion of ascorbic acid molecules and silver precursors takes place far below the surface. Resultantly, the deposition of the newly forming metal nanoparticles is not only at the outer surface of hydrogel microparticles but also inside the core network. Moreover, the enforced metal layer is applied without any ligand protection hence revealing a ligand-free metal surface which is particularly important for the interaction with the analyte of interest at their lower concentration. The UV–vis spectra of the composite microparticles before and after additional enforced silver layer can be measured as shown in our previous report.[20]

7.3. Surface-Enhanced Raman Scattering Sensing by Microscale Sensor Particles

Composite hydrogel microparticles can contain millions of small-sized metal nanoparticles in their cores which were formed during photochemical reactions (Subsection 7.1). In addition, the tunable enforced metal particles are not only available at the outer surface but also expected to be available below the surface through pores during swelling conditions in an aqueous solution that provides a ligand-free and metal-rich platform for direct interaction of analytes.[31] Moreover, a large content of the composite hydrogel particles is water and hence the hydrogel network is relatively transparent which can allow
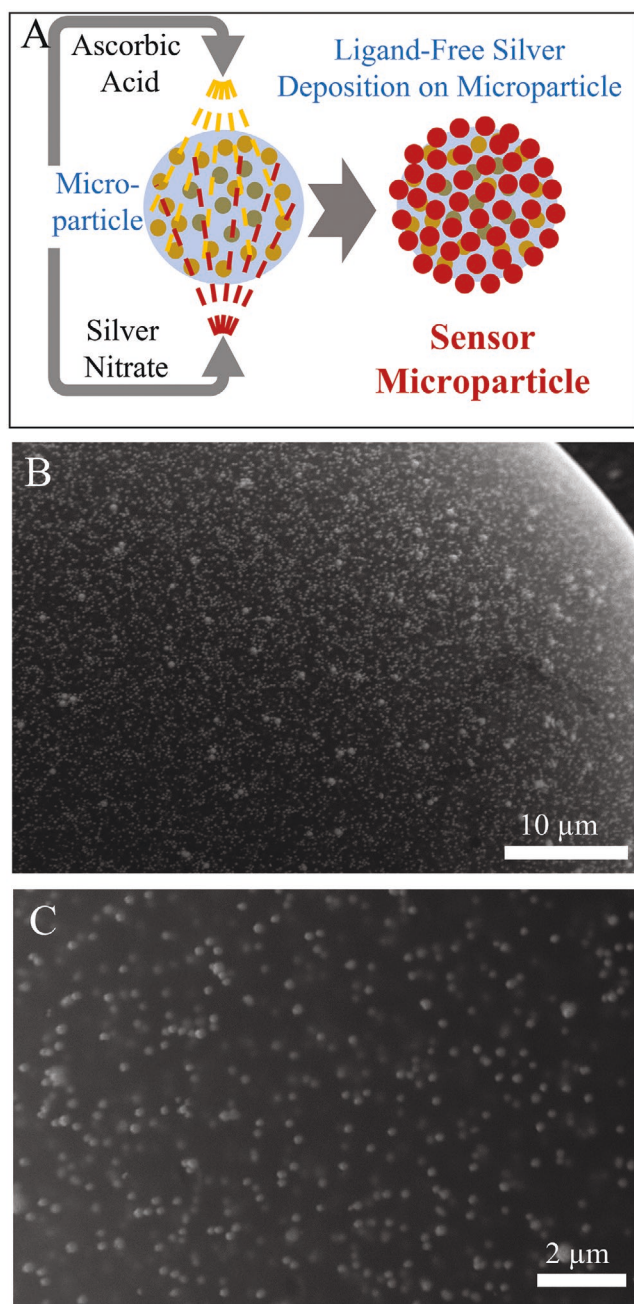
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Figure 10. Metal enforcement reaction on the polymer-metal composite particles. A) A general scheme for deposition of additional silver nanoparticles on the surface of silver nanoparticles-embedded polyacrylamide microparticles. B,C) The lower and higher magnified images of the silver enforced (10 mм) silver nanoparticles (60 mм)-embedded polyacrylamide microparticles, respectively. Reproduced with permission.[20] Copyright 2015, American Chemical Society.

penetrating light considerably below the surface (**Figure 11**A,B). Due to such characteristics of the microscale sensor particles, a relatively high outcome of the analyte interaction can be realized even at a low concentration which can be observed in SERS signals. After metal enforcement, the surface of the sensor particles is not smooth but obtained as roughened with a large number of gaps. The gaps and junction or controllably

aggregated metal nanoparticles create hotspots throughout the surface and below the surface. Such hotspots are crucial in concentrating electromagnetic light and give the SERS effect of analyte upon interaction with light. The SERS analysis of analytes through sensor microparticles can be performed by a portable compact SERS instrument. Sensor microparticles can be filled in the glass tube on which diluted solution of the analyte of interest is applied. During the experiment, 300 µL of the sensor microparticles dispersion was taken into the vial that typically contains about 10–12 sensor microparticles. Once the sensor particles settled down at the bottom of the vial, the water supernatant can be removed with a syringe needle. Afterward, 300 µL of the diluted analytes solution of interest can be added to the sensor microparticle-filled vial for SERS analysis. Laser light of defined wavelength (e.g., 532 nm) can be irradiated on the sample for variable integration time depending on the requirements such as 1 s, 3 s, etc., under a dark environment. The silver surface is reactive to a wide range of biochemicals and other analytes where analyte molecules interact efficiently based on affinity. Here, a model analyte, adenine, has been detected by using sensor microparticles (Figure 11C). The characteristic SERS peak of adenine has been observed, and its intensity was increased with increasing adenine concentration. Similarly, another analyte (L-lactic acid) has been identified by using sensor microparticles with SERS analysis as shown in Figure 11D. Recent development in the SERS substrates has allowed identifying the ultimate limit of the detection, that is, single-molecule with extraordinary high SERS enhancement factor. Polymer-supported microscale sensor particles are not able to detect such extremely low analyte concentration, but their robustness and regenerative ability are important for repeated use of sensor particles for detecting multiple analytes at one platform that is described with more details in below Section 8.

Overall, the microfluidic photochemical process is versatile to produce size and composition tunable hydrogel composite particles at one step. It is due to the advantages of microfluidics to provide an environment of uniform mixing of reactants, uniform distribution of embedding components, flow condition, and in situ droplet solidification that allows the formation of microscale hydrogel composite particles with a size range between 30 and 800 µm at various reaction conditions. Additional metal enforcement on hydrogel composite microparticles via metal-catalyzed metal deposition creates complete sensor microparticles with sensing functions in the form of silver nanoparticles with a ligand-free and roughened surface with a high number of hotspots. Due to the characteristics of swellability, porosity, metal-rich, and roughened surface, microscale sensor particles allow diffusion of analytes thoroughly and penetrate light efficiently to observe the SERS molecular fingerprints.

8. Continuous and Sequential Surface-Enhanced Raman Scattering Sensing in Flow Condition

Materials with sensing functions are valuable in identifying target molecules.[142,143] Therefore, the sensor should work with efficient function and for long-term use. In SERS sensing, metal nanoparticles play a central role in concentrating laser
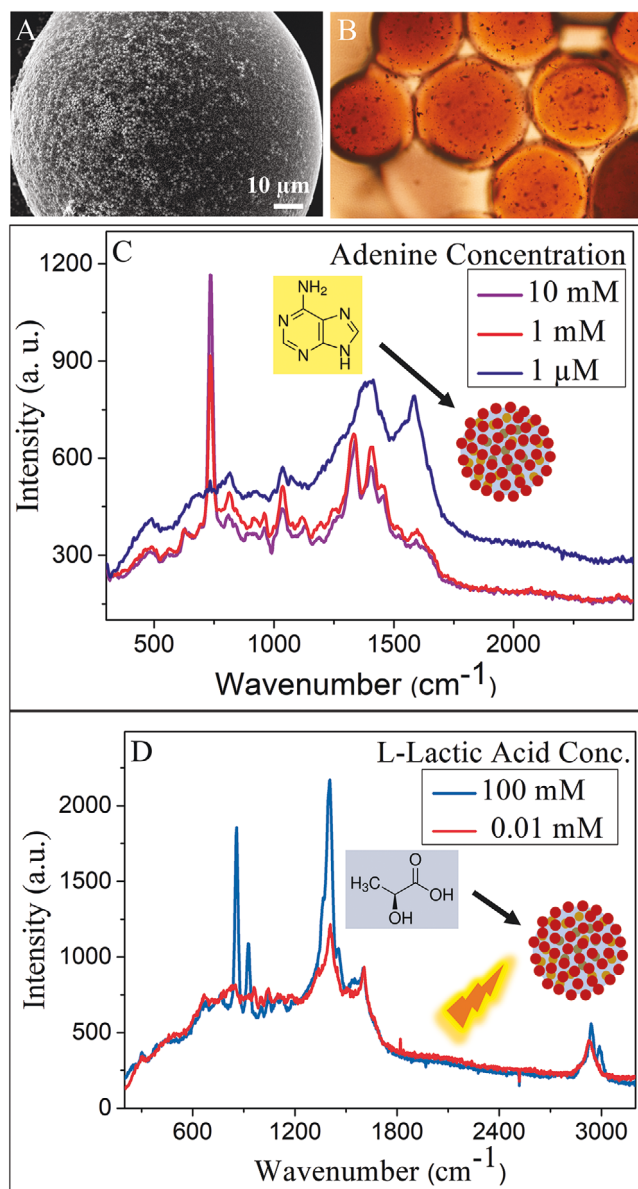
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Figure 11. SERS sensing of various analytes with sensor microparticles. A) SEM image of the silver enforced (50 mM) silver nanoparticles (60 mM)-embedded polyacrylamide microparticles. B) A light microscopy image of the silver enforced (25 mM) silver nanoparticles (40 mM)-embedded polyacrylamide microparticles. C) SERS sensing spectra of analyte adenine of different concentrations after interaction with sensor particles. D) SERS sensing spectra of analyte L-lactic acid of different concentrations after interaction with sensor particles. Reproduced with permission.[20] Copyright 2015, American Chemical Society.

light and significantly enhance the SERS signal during analyte interactions.[14] As explained above, nanoparticles possess high surface energy[134] and tend to aggregate in the solution phase to minimize surface energy. Hence, a surface protecting layer of suitable surfactant or ligand is required to stabilize nanoparticles against uncontrolled/undesired aggregation.[139] Metal nanoparticles strongly interact with suitable analytes of certain functional groups of high affinity to the metal surface.[144,145]

On one hand, the sensor particles can be used for stationary SERS sensing means the sensor particles can be filled in the vial and the analyte solution can be inserted while SERS measurement (Sections 6 and 7). The stationary SERS sensing method is efficient for one type of analytes detection quickly. On the other hand, sensor particles can be fixed to the capillary, and continuous flow of the diluted solution of analytes of interest can be applied. During the continuous SERS sensing, continuous monitoring of the analytes can be observed. The SERS spectra of the analytes can be obtained in the flow condition. A large volume of diluted analyte solution can be filled in the syringe which can be applied to the capillary where sensor particles are fixed. The continuous flow can be allowed to run in a simple flow arrangement with one syringe pump. The continuous flow concept is shown in Figure 4. Besides the stationary and continuous SERS sensing, an important strategy is sequential SERS sensing which can be performed also in the simple flow arrangement.

Once the analyte adsorbs on the metal surface, it is hard to remove by washing because of the very small size of nanoparticles (colloidal system). Also, even if desorption of analytes occurs through the chemical procedure, there is a possibility of aggregation of nanoparticles. In this regard, nanoparticle dispersion is good for single-time use for the detection of analytes which can give accurate results. A technique for re-using sensor particles is required that can enable detection of other types of analytes by using the same sensor after regeneration. Such possibility can be applied practically if metal nanoparticles are fixed to the polymer network which allows washing of the particles in a non-destructive manner. The development of the sensor particles described in Sections 6 and 7 are very suitable to use for continuous SERS sensing as well as sequential sensing of multiple analytes by a single platform. Polymer accommodates the metal nanoparticles and overall, the polymer-metal composite particles are mechanically strong. Water and molecular exchange can take place during the swelling phase. Moreover, metal nanoparticles can be strongly fixed to the polymer network, and hence not released/detached even during the swelling phase in an aqueous solution. This characteristic supports the washing of the sensor particles to release the adsorbed analyte molecule on a metal surface by the chemical process after the first analysis of the specific analyte. Subsequently, the fresh metal surface can detect another analyte and give fingerprint information in form of enhanced SERS signals. A general concept for sequential SERS sensing by polymer-metal composite sensor particles is shown in **Figure 12**A.

A basic schematic for sequential analysis by using sensor particles in the flow conditions is shown in Figure 12B. In general, a multifold connector with multiple inlets can allow connecting multiple syringes filled with different analyte solutions of low concentrations. One syringe can be filled with the ringing solution, that is, dilute sulfuric acid. The flow rate program can be set up in the software for each syringe that tends to deliver the liquid to the channel toward the detection zone of the location of sensor particles where laser light irradiates. Here, a set of sensor particles (typically about 10 microscale sensor particles) can be fixed into a small glass capillary of the approximate length of about 2.5 cm.[115] Once the sensor particles accommodate inside the capillary, soft cotton has been applied from both
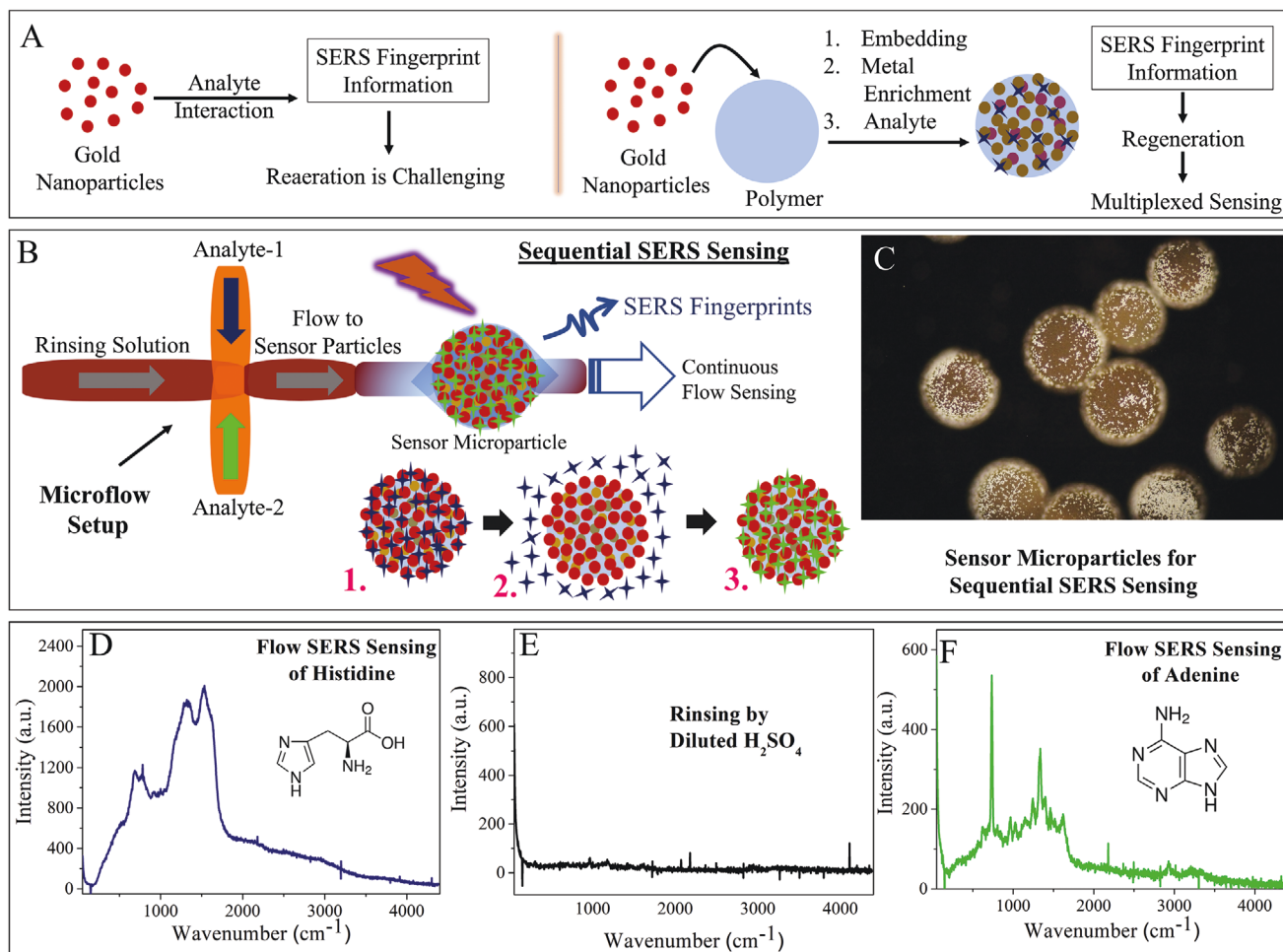
Figure 12. Sequential flow SERS sensing of different analytes by using a single set of sensor microparticles. A) General depiction of the importance of polymer-metal composite sensor particles for sequential SERS sensing. B) An overview drawing for the alternative flow of analyte-1 and analyte-2 followed by a rinsing solution that releases the attached analytes from the surface of sensor microparticles. C) Light microscopy image (darkfield) of the sensor microparticles used for analyte SERS sensing. D) SERS sensing spectrum of L-histidine (10 mM) by sensor microparticle. E) Rinsing of sensor microparticles by diluted sulfuric acid solution. F) SERS sensing spectrum of adenine (2 mM) by sensor microparticle. D–F) Adapted with permission.[15] Copyright 2015, Wiley-VCH.

sides and both ends of the capillary can be stretched to make thinner with glass blowing so that sensor particles do not transport (flow away) with the flow of the analyte solution. Thinner ends connect with microtubes from multifold through which analyte solution can be delivered to interact with sensor particles. Both types of sensor particles (nanoscale and microscale) can be used for SERS analysis of multiple analytes in different experiments. In the case of nanoscale sensor particles,[90] cotton protection in the capillary was very much required to protect the particles from flowing with a solution at a high flow rate. To fix the particles in the capillary, 0.5 mL aqueous dispersion of nanoscale sensor particles were inserted from one end of capillary whose second end is packed with cotton that only allows passing water and collects the sensor particles. Afterward, the second end of the capillary was also packed with little soft cotton. The focus of the laser is applied on the capillary at the zone where sensor nanoparticles are located.[115] For continuous analysis during the experiment, histidine solution has been applied for 2 min and integrated SERS spectra were obtained at

a time interval of every 3 s. It was realized that the intensity of the SERS peak was constant throughout 2 min in all individual spectra of every 3 s. After SERS sensing of histidine, the flow of diluted sulfuric acid was applied. No analyte-related SERS signal was obtained after the sulfuric acid rinsing process.[115] Subsequently, the flow of the second analyte adenine has been applied to the rinsed sensor nanoparticles. The characteristics peaks of the adenine were observed throughout the flow for 2 min with constant intensity. In this way, multiple cycles of histidine and adenine were measured sequentially.

Besides nanoscale sensor particles, microscale particles are large and can be manually moved with a soft tweezer. Only a set of 10 sensor microparticles of size about 800 µm can be fixed in the glass capillary of 1 mm internal diameter.[115] Microparticle size was large and hence there was no need to apply for cotton protection as stretched capillary end made thinned to about 500 µm inner diameter. A dark field image of the sensor microparticle is shown in Figure 12C. In a similar setup, analyte-1 solution (histidine) has been applied at the flow rate of 40 µL min⁻¹

for about 1 min. A representative SERS spectrum of the histidine is shown in Figure 12D. After histidine solution has been applied, a flow of diluted sulfuric acid is applied continuously for rinsing the particles. The rinsing process by diluted sulfuric acid can release the histidine molecules completely as no histidine-related SERS peak was observed after rinsing as shown in Figure 12E. Subsequently, the flow of analyte-2 (adenine) was applied, and their SERS spectra were obtained as shown in Figure 12F. In this manner, at least 6 cycles of alternative analyte sensing of histidine and adenine were applied by regenerative process of sensor microparticles in continuous flow conditions.[115] The intensity of the SERS signal was slightly decreased after every measurement cycle, but the clear characteristic peaks were obtained in both cases of histidine and adenine, and not even any small peaks were observed after the rinsing process with diluted sulfuric acid during every measurement cycle. This proof-of-concept proves the efficiency of polymer-supported sensor particles for sequential sensing of multiple analytes. Here, a proof-of-concept is shown with only two types of analytes alternatively during six cycles. Similarly, twelve analytes can be detected by using the same sensor particles with the regenerative process of sensor particles by diluted sulfuric acid in six pair of alternative cycles. Not only limited to six cycles and limited types of analytes, but further development of the multi-functional sensor particles can be able to detect hundreds of analytes through regenerative sequential analysis in the continuous flow arrangement at a single platform. Overall, microfluidic supported processes have the potential to produce uniform sensor particles and continuous microflow arrangement is very useful in performing sequential SERS analysis experiments of multiple analytes at a single platform by using the same set of sensor particles.

9. Conclusion and Outlook

The review of the polymer-metal composite particles for stationary, continuous, and sequential SERS sensing is presented here. The toxins, as well as targeted analytes, might be available in very dilute concentrations. It is required that the accurate identification of analytes be realized for the healthcare purpose of living organisms, as well as, the environment with food, water, and air safety. Concerning biological perspectives, the analytical techniques that promise the non-invasive ability are a top requirement for diagnostics and theranostics. In this regard, SERS—which is a non-destructive and label-free inelastic scattering-based powerful analytical technique—is becoming a highly essential tool for applications in the broad areas for sensitive and specific analysis. When an intense light source irradiates the roughened, corrugated, or structured metal surfaces featured with nanoscale gaps (hotspots), the enhanced scattering signals of the molecules which are closely located or adsorbed to the metal surface are being realized with significantly high enhancement factors and give accurate molecular fingerprint information. Plasmon-active metallic substrates concentrate the incident light and hence information regarding molecular vibration can emerge with enhanced scattering signal. Whereas bulk metallic substrates reveal surface plasmon resonance, the nanoscale metallic particles can

reveal concentrated localized surface plasmon resonance. The nanoscale features in the particles such as boundaries, tips, sharp corners, edges, and junctions are particularly essential in the generation of the intense local field. Therefore, these types of structured metal nanoparticles can potentially be used as SERS-active components in the solution phase. Whereas freely moving structured metal nanoparticles (nanoparticles dispersion in the solution) are excellent for SERS sensing, it is challenging to regenerate them for reuse after the first interaction with analytes because of their small size, strong binding ability, as well as the possibility of aggregation and oxidation during the chemical washing process. In the best scenario, it is possible to chemically rinse them, but it is a very time-consuming and laborious process. Advantageously, stabilizing metal nanoparticles, preventing uncontrolled aggregation, and the possibility of efficient chemical rinsing for regeneration, the strategy to combine metal nanoparticles in the cross-linked polymer network is very promising. Therefore, this review has focused on various syntheses strategies for the generation of polymer-metal composite sensor particles via microfluidic-supported methods.

Polymers, particularly hydrogels, are optically transparent and allow penetrating light. Moreover, their cross-linked network is soft, flexible, and responsive that allowing precise accommodation for plasmon-active and SERS-active metal nanoparticles. For a generation of the nanoscale polymer-metal composite sensor particles, a two-step process comprised of microfluidic-supported polymerization and electrostatic interaction between oppositely charged nanoparticles can be applied. First, to obtain the soft and swellable layer at the surface, co-polymerization of the hydrophobic core and hydrophilic shell has been applied. Here, the core polymer network is relatively hard (PMMA) and the swellable soft layer is made of polyDADMAC. Second, when polymer particles are dispersed in the water, the surface shell layer swells and creates lots of pores where anionic gold nanoparticles are entrapped with high density to the intense cationic shell layer to form the polymer-metal composite nanoparticles. The microfluidic platform allows systematically alters the parameters in a continuous flow manner, and hence the size of the core–shell particles and shell thickness can easily be tuned precisely. Moreover, the density of the gold nanoparticles can also be tuned depending on the requirement. While gold nanoparticles are covered with ligand (citrate), the selectivity for analyte molecular interactions can become limited. In this regard, achieving the ligand-free metallic surface layer with enrichment is a promising strategy to open the possibility of a broad interaction spectrum, intense plasmon resonance, and hence enhanced SERS outcomes. For this purpose, the metal-catalyzed metal enrichment approach has been applied as explained in Section 6 for the formation of efficient sensor nanoparticles for SERS sensing of analytes.

Beyond the nanoscale sensor particles, sensor microparticles are promising that provide the opportunity for single particle-based SERS sensing. Microparticles are large and allow accommodation of multi-millions of SERS-active metal nanostructures in their core cross-linked network which reveals high SERS sensing outcome. Droplet microfluidics is an excellent platform for the in situ formation of nano metal-embedded hydrogel microparticles in one step. The embedded metal

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nanoparticles in the hydrogel core are very small in size and distributed thoroughly without aggregation. To enhance the sensing outcome, metal enrichment with a ligand-free surface can be applied similarly to the process applied in the case of nanoscale sensor particles. Due to the large water content, pores during the swelling phase, high loading of metal nanoparticles, and ligand-free metal surface, polymer-metal composite microparticles emerged as potential sensors for SERS sensing of analytes (Section 7). The polymer can act as a potential support to accommodate the plasmon-active and hence SERS-active metal nanoparticles but the functional group of the polymers can also interact with metal nanoparticles that affect the SERS signature of the targeted analyte. To avoid that concern potentially, the additional metallic layer (ligand-free) can be applied to the polymer-metal composite particles. The additional enforced metal layer is capable to interact with the targeted analyte potentially.

On one hand, stationary SERS analysis is pivotal for detecting specific types of analytes, but the SERS analysis in a continuous flow manner provides the dynamic possibility to monitor analytes in the flow condition and able to measure multiple analytes in a sustained and sequential manner with high specificity, sensitivity, and speed. Because of the polymer support, polymer-metal composite sensor particles possess the ability to regenerate after chemical rinsing. Moreover, continuous flow arrangement provides a platform to deliver various analyte solutions after alternative rinsing processes in the flow. In this way, the detection of multiple analytes can be performed by using a single sensor microparticle or a single set of sensor particles fixed to the flow capillary (Section 8).

Overall, polymer-metal composite sensor particles are potential components for the SERS sensing of a diverse range of analytes in stationary, continuous, and sequential flow conditions. The support of the polymer is essential to control the location and stability of the SERS-active metal nanoparticles and hence combined characteristics can reveal efficient sensing functions to detect the analytes in diverse conditions/environments. Microfluidic supported arrangement is essential in both for the formation of sensor particles and performing the SERS experiments dynamically. SERS is ideally one of the highly efficient and accurate analytical techniques that can achieve the detection of the ultimate limit through specific types of substrates, particularly freely moving structured metal nanoparticles. Though polymer-metal composite sensor particles have not achieved the ultimate detection limit of single-molecule yet, further research efforts are required to implement functions to enhance the detection limit. On one hand, polymeric support is essential with huge advantages because ultimately it stabilizes the SERS-active nanoparticles and provides the regeneration possibility. On the other, the polymer-metal composite particles can become widely popular substrates with efficient sensing functions if the detection limit will be enhanced. In the presented content, the main focus for polymer-supported sensor particles is regarding their ability of the regeneration for detection of multiple analytes rather than the detection limit (i.e., extremely low concentration). It can be envisioned that future efforts will allow controlling the characteristics of the polymers that will be able to achieve a high detection limit. In this way, the SERS sensing through sensor particles can be applied to a broad range of targets including the resource-limited remote area with portable instruments alongside laboratory research, industrial monitoring, defense, environment, and monitoring of food, water, and air quality, etc.

Conflict of Interest

The authors declare no conflict of interest.

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