Gold and silver nanoparticles used for SERS detection of *S. aureus* and *E. coli*

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Keywords: silver and gold nanoparticles, chemical, electrochemical, microwave synthesis, SERS, *S. aureus*, *E. coli* detection

Abstract

Surface enhanced Raman scattering (SERS) is emerging as a robust analytical method used in sensing applications in chemical as well as biological systems. SERS has been reported to be used in fast detection of micro-organisms up to the specificity of strain identification. However, use of SERS is tricky because of difficulties involved in selection of SERS active substrate so as to give uniform, sensitive as well as reproducible results. We have synthesized silver and gold nanoparticles using chemical, electrochemical and microwave-assisted methods followed by their characterization. Uses of these nanoparticles in association with micro-organisms such as *S. aureus* and *E. coli* have been analyzed using SERS to generate signature spectra. This demonstrates use of so synthesized gold and silver nanoparticles as SERS active substrates for rapid detection of micro-organisms which pave way to find applications in disease diagnostics.

1. Introduction

In recent times, the field of nanotechnology has been exploited for synthesis of materials at nano scale showing unique properties in comparison with their bulk counterparts [1]. It is important to understand the dependence of shape, size, morphology, concentration and other aspects associated with the nanoparticles on their unique physical, optical, catalytic, magnetic as well as electronic properties in order to exploit them for applications in various fields [2, 3]. Extensive research is being carried out in understanding as well as tuning the properties of nanoparticles. Properties of nanoparticles can be modulated by controlling shape, size, morphology, etc of nanoparticles during the synthesis process [4]. Metal nanoparticles show optical properties which rely on surface plasmon resonance. It is because of the SPR absorption bands incident in visible spectra that gold as well as silver nanoparticles have been commonly used as sensitive detection as well as surface-enhanced Raman spectroscopy (SERS) [5, 6].

Like normal Raman spectroscopy, surface enhanced Raman spectroscopy (SERS) works on emission principle which consists of inelastic scattering of laser energy incident upon the sample. This results in formation of spectral peaks because of vibrational trends of the concerned molecule. However, efficiency as well as sensitivity of SERS is limited [7]. Raman effect can be remarkably enhanced if metal nanoparticles like silver, gold and copper have been adsorbed upon by the molecule or are in immediate vicinity [8, 9]. Localized surface plasmon resonance as well as charge transfer enhances SERS signal within the range of $10^3$ – $10^6$. Tremendous enhancement can be achieved up to $10^{14}$ – $10^{15}$ times under specific conditions. This level of enhancement is adequate for detecting single molecule [10, 11]. This approach is being actively applied for detection, characterization as well as analysis of biological moieties such as bacteria, fungi, viruses, different types of cells, biofilms, bio-macromolecules like proteins, carbohydrates, nucleic acids, etc [12–16].

For use of silver and gold nanoparticles as SERS active substrates, size and shapes of these nanoparticles have been reported to play crucial role in keeping them functional. Apart from these factors, other determinants affecting use of nanoparticles in SERS involve size distribution, surface charge as well as environment surrounding the nanoparticles [17]. Different synthesis approaches like solvothermal [18, 19], chemical [20],
spray pyrolysis [21, 22], electrochemical [23, 24], microwave-assisted [25] have been exploited for synthesizing silver as well as gold nanoparticles with different sizes as well as morphologies. Modulation of shape, size, surface area, electrical conductivity, etc of nanoparticles may be achieved via modification of the reaction conditions as well as chemicals used during chemical synthesis [20]. Nanoparticles can be synthesized on a large scale with long shelf life by electrochemical synthesis because of which it has been highly favored for industrial production [23, 24]. A great amount of attention is being given to microwave assisted synthesis as it ensures uniform heating, easy tuning of properties of nanoparticles as well as higher reproducibility in different batches of synthesis [26]. Here, synthesis of gold and silver nanoparticles by chemical, electrochemical as well as microwave-assisted methods along with its application as SERS active substrate has been reported.

2. Experimental

Silver nitrate (98%), ammonia (25%) and starch were procured from Merck. Chloroauric acid (49%) was procured from. Hydrazine hydrate (80%) was procured from SDFCL. All chemicals were used without any further purification. Milli Q water was used to prepare solutions using these chemicals which were further used during synthesizing nanoparticles. Glassware to be used for synthesis were first cleansed by aqua-regia which were further washed off multiple times using distilled water following which they were dried in hot air oven and were used further.

Silver nanoparticles (AgNPs) have been synthesized using three independent methods, viz., chemical, electrochemical as well as microwave-assisted which have been summarized as follows:

Chemical synthesis of silver nanoparticles: 40 ml silver nitrate solution (0.001M) which was prepared in Milli Q water was added to a beaker. 4 ml freshly prepared ammonia solution which has been diluted 1:40 times was then added to the beaker to maintain alkaline conditions. 2ml of 1% starch solution was added to the reaction mixture. Serial dilution of hydrazine hydrate solution was done 103 times following which 20 μl of the solution was added to the reaction mixture. Characterization of the so-obtained yellow colored solution has been performed.

Electrochemical synthesis of silver nanoparticles: 4 ml of freshly prepared ammonia solution which was diluted 1:40 times was added to the beaker to maintain alkaline pH of 40 ml silver nitrate solution (0.001M). Copper electrodes were dipped in the reaction mixture to be used as anode as well as cathode. Current of 23 A was applied initially. The voltage was adjusted to 82 V. The reaction mixture was stirred using magnetic stirrer. Deposition of black color was obtained on copper electrode acting as cathode. Black colored pellet deposited on the cathode was collected by multiple rounds of sonication. The pellet so obtained was used for further characterization.

Microwave-assisted synthesis of silver nanoparticles: 500 μl of 1% starch solution was added to the beaker containing 10 ml silver nitrate (0.001M). 1 ml of freshly prepared ammonia solution which has been diluted 1:40 times was then added to the beaker to maintain alkaline conditions. Hydrazine hydrate solution was diluted serially 103 times following which 500 μl of the solution was mixed into the beaker containing the reaction mixture. Controlled heating of the reaction mixture was done in microwave at temperature 110 °C for 120 s. The yellow colored solution so obtained was centrifuged at 10000 rpm for duration of 15 min. Pellet so obtained after discarding the supernatant was used for further characterization.

Synthesis of gold nanoparticles: Gold nanoparticles have been synthesized using three independent methods, viz., chemical, electrochemical as well as microwave-assisted which have been summarized as follows:

Chemical synthesis of gold nanoparticles: 1 ml of 1% starch solution was added to 20 ml chloroauric solution (0.001M) prepared in Milli Q water. Hydrazine hydrate solution was diluted serially 103 times following which 100 μl of the solution was added to the reaction mixture. Pale pink colored solution so obtained was used for further characterization.

Electrochemical synthesis of gold nanoparticles: 10 ml of 1mM HAuCl4 solution was taken in a beaker. Silver electrodes were dipped in the reaction mixture to be used as anode as well as cathode. Current of 37 A was applied initially. The voltage was adjusted to 42 V. The reaction mixture was stirred using magnetic stirrer. Deposition of black color was obtained on silver electrode acting as cathode. Black colored pellet deposited on the cathode was collected by multiple rounds of sonication. The pellet so obtained was used for further characterization.

Microwave-assisted synthesis of gold nanoparticles: 20 ml of 1 mM HApCl4 solution was taken in a beaker. 1 ml 1% starch solution was added to 20 ml HApCl4 solution (0.001M) prepared in Milli Q water. Hydrazine hydrate solution was diluted serially 104 times following which 1 ml of the solution was mixed into the beaker containing the reaction mixture. Controlled heating of the reaction mixture was done in microwave at temperature 110 °C for 120 s. Four rounds of intermittent addition of 1 ml hydrazine hydrate solution diluted 104 times were done to the reaction mixture. The reaction mixture was again kept in microwave at temperature
110 °C for 120 s. The pale pink colored solution so obtained was subjected to at the speed of 10000 rpm for a time period of 25 min. Pellet so obtained after discarding the supernatant was used for further characterization.

The so-synthesized silver and gold nanoparticles were characterized using UV–vis spectrophotometer (Shimadzu UV-1800) from 200nm to 1100 nm. Morphological analysis of both the nanoparticles was performed using Field Emission Scanning Electron Microscopy (FESEM) (FESEM: Fei Quanta–450), was performed. ATR-IR spectra of silver and gold nanoparticles as well as constituents used for synthesis process were obtained on instrument Platinum ATR Tensor 37. X-ray Diffraction analysis of silver and gold nanoparticles was done using Rigaku Ultima-IV x-ray Diffractometer by making use of CuKα, radiation at wavelength of 1.54 Å in the 2θ values ranging from 5° to 80° with a scanning speed of about 0.005° s⁻¹.

Micro-organisms like Staphylococcus aureus as well as Escherichia coli have been taken as representative members of Gram positive and Gram negative organisms respectively which have been obtained from the Department of Microbiology, Savitribai Phule Pune University, Pune, India. The so-obtained micro-organisms have been maintained on nutrient agar slants and stored at 4 °C followed by intermittent sub culturing after 15–20 days for maintaining cell viability.

The micro-organisms have been inoculated into nutrient broth and kept for overnight incubation (12–18 h) at temperature 37 °C under shaking conditions. These micro-organisms which were grown in nutrient broth were further used as starter culture in subsequent inoculation procedures. After inoculating nutrient broth with starter cultures, they were grown for 4–6 h in shaker incubator at a temperature of 37 °C under shaking conditions with speed 150 rpm. The number of cells was diluted to 10⁸ CFU/ml for SERS analysis. 5 μl of bacterial samples whose density has been adjusted to 10⁸ CFU/ml was allowed to interact with 5 μl of silver/gold nanoparticles on a silicon vapor. It was kept for air drying overnight and then subjected to SERS analysis. SERS experiments have been performed using Lab Ram I confocal microprobe Raman system (Horiba Jobin Yvon, France, Model no. HR 800) which consists of a He–Ne laser operating at wavelength of 632.8 nm along with 1.7 mW laser power to be used as the source of excitation. The low power density of the laser source helps in preventing adverse effects associated with illumination of laser source, such as localized heating, photo-oxidation as well as deformation of silver/gold nanoparticles. Raman signals have been collected in between 100 and 2000 cm⁻¹ through integration time ranging from 1–5 s.

3. Results and discussion

Silver and gold nanoparticles have been synthesized using three different methods, viz., chemical, electrochemical as well as microwave-assisted synthesis followed by characterization of the so-synthesized nanoparticles. Use of hydrazine hydrate as well as starch together for reduction of silver nitrate solution has been preferred over conventional reducing agents in sustainable synthesis of nanoparticles because they are cost effective as well as do not cause any environmental hazard [27–29]. Chemical, electrochemical as well as microwave-assisted synthesis routes trigger the process of nanoparticles’ synthesis.

Figure 1 depicts UV–vis spectral analysis indicating surface plasmon resonance (SPR) maxima for the so-synthesized silver nanoparticles using chemical (a), electrochemical (b) and microwave-assisted (c) methods. Similarly, UV–vis spectra of gold nanoparticles synthesized using chemical (d), electrochemical (e) and microwave-assisted (f) methods have also been demonstrated. Inset shows synthesized silver as well as gold nanoparticles using corresponding synthesis approaches. Chemically synthesized AgNPs show maximum SPR peak at 401 nm whereas AgNPs synthesis using microwave assisted approach shows SPR peak at 414 nm. AgNPs usually show maximum SPR peak within the wavelength range between 400–480 nm [30–32]. Figures 1(a) and (c) show shoulder at ~250 nm which signifies electronic transitions in metallic Ag⁰ form which is usually present in the range 250–330 nm. Presence of Ag⁺ ions results in incidence of absorption peak within the range 230–250 nm. Absence of that peak signifies complete conversion into silver nanoparticles [3]. Similarly, maximum SPR band for gold nanoparticles was observed at 547 nm for chemical synthesis as well as 578 nm for microwave-assisted synthesis. Gold nanoparticles have been reported to show maximum SPR band at 550 nm [33]. Broad SPR band along with absorption tails in longer wavelength was observed. This is characteristic for aggregation of spherical nanoparticles dependant on time, formation of anisotropic structures with increase in time or both [34, 35].

The morphologies of silver and gold nanoparticles synthesized using chemical, electrochemical and microwave-assisted methods were characterized by using FESEM as shown in figure 2. The lower as well as higher resolution FESEM images for silver and gold nanoparticles according to their corresponding synthesis approaches can be observed. Figures 2(a) and (b) show spherical silver nanoparticles at lower as well as higher resolution respectively which have been synthesized by using chemical method. Microwave-assisted synthesis of silver nanoparticles have also yielded nanospheres as depicted in figures 2(e) and (f) at lower and higher resolution respectively. Electrochemical synthesis of silver nanoparticles shows presence of anisotropic
structures. Along with spherical nanoparticles, it also shows presence of nanocubes evident from figures 2(c) and (d) depicting lower and higher resolution respectively. Similar trends have been recorded in case of gold nanoparticles. Figures 2(g) and (h) show spherical gold nanoparticles at lower as well as higher resolution respectively synthesized using chemical method. Gold nanospheres at higher and lower resolution were observed in microwave-assisted synthesis as depicted in figures 2(k) and (l) respectively. Electrochemical synthesis of gold nanoparticles has reported presence of anisotropic structures such as nanospheres, nanocubes, etc represented in figures 2(i) and (j) depicting lower and higher resolution respectively. Table 1 shows the size range of silver as well as gold nanoparticles synthesized using chemical (C), electrochemical (EC) as well as microwave-assisted (MA) methods.

Figure 3 demonstrates x-ray diffraction patterns for silver nanoparticles synthesized by chemical (a), electrochemical (b) and microwave-assisted (c) approaches. Similarly, x-ray diffraction patterns associated with gold nanoparticles synthesized using chemical (d), electrochemical (e) and microwave-assisted (f) approaches have also been represented. The XRD data of silver (AgNPs) as well as gold (AuNPs) nanoparticles synthesized using chemical (C), electrochemical (EC) as well as microwave-assisted (MA) methods has been well represented.
Figure 2. Representative FESEM images of chemically synthesized silver nanoparticles at lower (a) and higher resolution (b). Similarly, FESEM images of electrochemically synthesized silver nanoparticles at lower (c) and higher resolution (d) as well as silver nanoparticles synthesized using microwave-assisted methods show FESEM images at lower (e) and higher resolution (f). FESEM images of chemically synthesized gold nanoparticles at lower (g) and higher resolution (h). Similarly, FESEM images of electrochemically synthesized gold nanoparticles at lower (i) and higher resolution (j) as well as gold nanoparticles synthesized using microwave-assisted methods show FESEM images at lower (k) and higher resolution (l).
Figure 2. (Continued.)
in Table 2. Inset depicts solution cast film of the so synthesized silver as well as gold nanoparticles on glass substrate using corresponding synthesis approaches. Comparison of obtained 2θ values with standard values for silver (JCPDF File No.: 04-0783 for silver) as well as gold (JCPDF file no 04-0784 for gold) [36] confirms the

|                  | Chemical | Electrochemical | Microwave |
|------------------|----------|----------------|-----------|
| Silver nanoparticles | 50–75 nm | 20–80 nm        | 25–45 nm  |
| Gold nanoparticles  | 60–140 nm | 30–110 nm       | 90–170 nm |
presence of respective metals and thus successful synthesis of corresponding nanoparticles. These peaks could be assigned to the planes having the \((hkl)\) values of \((1\ 1\ 1)\), \((2\ 0\ 0)\), \((2\ 2\ 0)\) as well as \((3\ 1\ 1)\) respectively which have correctly matched with standard XRD peaks. The broader peaks are associated with different sizes as well as morphologies of nanoparticles.

ATR-IR analysis was done for silver nanoparticles synthesized using chemical (a), electrochemical (b) as well as microwave-assisted (c) methods represented in figure 4. Likewise ATR-IR analysis of gold nanoparticles synthesized by chemical (d), electrochemical (e) and microwave-assisted (f) approaches is also illustrated in figure 4. Figure 5 depicts ATR-IR spectra of corresponding constituents such as silver nitrate (a), chloroauric acid (b), ammonia (c), hydrazine hydrate (d) and starch (e) which have been used for synthesis of nanoparticles. This was done in order to decipher the functional groups from corresponding constituents responsible for synthesis as well as stabilization of nanoparticles.

Amongst these peaks, prominent peaks for chemical synthesis of silver nanoparticles are 2884.87 cm\(^{-1}\), 1623.89 cm\(^{-1}\), 1146.25 cm\(^{-1}\), 1077.06 cm\(^{-1}\) as well as 857.21 cm\(^{-1}\). The peak at 2884.87 corresponds to N–H stretching existing in amines, C–H stretching existing in alkanes as well as aldehydes. The peak present at 1623.89 cm\(^{-1}\) signifies C=O stretching of conjugated alkenes and N–H bending existing in amines. The peak present at 1146.25 cm\(^{-1}\) signifies C–N stretching in amines, C–O stretching in esters, tertiary alcohols, aliphatic ethers and S=O stretching existing in sulphones. The peak present at 1077.06 cm\(^{-1}\) signifies C–N stretching existing in amines, C–O stretching in primary alcohols, vinyl and aliphatic ethers. The peak present at 857.21 cm\(^{-1}\) signifies C–H bending out of plane in aromatic compounds and alkenes.

ATR-IR spectra for silver nanoparticles synthesized using electrochemical method show prominent peaks at 2170.29 cm\(^{-1}\), 1401.89 cm\(^{-1}\), 1075.34 cm\(^{-1}\) and 1028.76 cm\(^{-1}\). The peak present at 2170.29 cm\(^{-1}\) signifies S–C ≡ N stretching existing in thiocyanate. The peak present at 1401.89 cm\(^{-1}\) signifies O–H bending in
carboxylic acids and alcohols, S=O stretching existing in sulphates. The peak at 1075.34 cm\(^{-1}\) signifies C–N stretching existing in amines, C–O stretching in primary alcohols, vinyl and aliphatic ethers. The peak present at 1028.76 cm\(^{-1}\) signifies C–N stretching existing in amines as well as C–O stretching in vinyl ethers.

ATR-IR spectra for silver nanoparticles synthesized using microwave-assisted method show prominent peaks at 3248.04 cm\(^{-1}\), 1633.21 cm\(^{-1}\), 1332.84 cm\(^{-1}\), 1151.53 cm\(^{-1}\), 1079.02 cm\(^{-1}\) and 1024.93 cm\(^{-1}\). The peak present at 3248.04 cm\(^{-1}\) signifies O–H stretching existing in carboxylic acids and alcohols as well as N–H stretching in aliphatic primary amines. The peak present at 1633.21 cm\(^{-1}\) signifies C=C stretching existing in conjugated alkenes and N–H bending in amines. The peak present at 1332.84 cm\(^{-1}\) signifies N–O stretching existing in nitro compounds, O–H bending in phenols in addition to C–N bending in aromatic amines. The peak present at 1151.53 cm\(^{-1}\) signifies C–N stretching existing in amines, C–O stretching in esters, tertiary

### Table 2. The XRD data of silver (AgNPs) as well as gold (AuNPs) nanoparticles synthesized using chemical (C), electrochemical (EC) as well as microwave-assisted (MA) methods.

|   | a (C-AgNPs) | b (EC-AgNPs) | c (MA-AgNPs) | d (C-AuNPs) | e (EC-AuNPs) | f (MA-AuNPs) |
|---|-------------|--------------|-------------|-------------|--------------|--------------|
| 2\(\theta\) (hkl) | 2\(\theta\) (hkl) | 2\(\theta\) (hkl) | 2\(\theta\) (hkl) | 2\(\theta\) (hkl) | 2\(\theta\) (hkl) | 2\(\theta\) (hkl) |
| 37.98 (111) | 38.0 (111) | 37.98 (111) | 38.08 (111) | 38.18 (111) | 38.1 (111) |
| 44.16 (200) | 44.14 (200) | 44.16 (200) | 44.22 (200) | 44.38 (200) | 44.32 (200) |
| 64.36 (220) | 64.4 (220) | 64.48 (220) | 64.92 (220) | 65.68 (220) | 64.5 (220) |
| 77.38 (311) | 77.38 (311) | 77.22 (311) | 77.5 (311) | 77.72 (311) | 77.54 (311) |
alcohols, aliphatic ethers and S=O stretching existing in sulphones. The peak at 1079.02 cm⁻¹ signifies C–N stretching existing in amines, C–O stretching in primary alcohols, vinyl and aliphatic ethers. The peak present at 1024.93 cm⁻¹ signifies C–N stretching existing in amines as well as C–O stretching in vinyl ethers.

The aforementioned peaks of silver nanoparticles are compared with those of silver nitrate, ammonia, hydrazine hydrate and starch which together contribute to the synthesis of silver nanoparticles. The prominent peaks which silver nitrate shows are 2164.75 cm⁻¹, 1635.30 cm⁻¹, 1324.83 cm⁻¹, 1040.23 cm⁻¹ and 823.97 cm⁻¹. The prominent peaks which ammonia depicts are 3299.67 cm⁻¹ and 1636.56 cm⁻¹ whereas those observed in hydrazine hydrate are 1346.93 cm⁻¹ and 1076.25 cm⁻¹. The prominent peaks which starch depicts are 2889.59 cm⁻¹, 1332.28 cm⁻¹ and 1149.27 cm⁻¹. The lower shifts in these functional groups indicate their binding with silver nanoparticles.

ATR-IR spectra of chemically synthesized Au NPs show prominent peaks such as 3301.97 cm⁻¹, 1640.99 cm⁻¹, 1409.97 cm⁻¹, 1150.88 cm⁻¹ and 1078.71 cm⁻¹. The peak present at 3301.97 cm⁻¹ signifies O–H stretching existing in carboxylic acids and alcohols as well as N–H stretching in aliphatic primary amines. The peak present at 1640.99 cm⁻¹ signifies C≡C stretching existing in conjugated alkenes and N=C=S stretching in isothiocyanate compounds. The peak present at 1409.97 cm⁻¹ signifies O–H bending existing in carboxylic acids as well as alcohols, S=O stretching existing in sulphates. The peak present at 1150.88 cm⁻¹ signifies C–N stretching existing in amines, C–O stretching in esters, tertiary alcohols, aliphatic ethers and S=O stretching existing in sulphones. The peak present at 1078.71 cm⁻¹ signifies C–N stretching existing in amines, C–O stretching in primary alcohols, vinyl and aliphatic ethers.

ATR-IR spectra for gold nanoparticles synthesized using electrochemical method show prominent peaks at 3243.52 cm⁻¹, 2106.91 cm⁻¹, 1601.11 cm⁻¹, 1407.61 cm⁻¹, 1147.70 cm⁻¹ and 1020.66 cm⁻¹. The peak present at 3243.52 cm⁻¹ signifies O–H stretching existing in carboxylic acids and alcohols as well as N-H stretching in aliphatic primary amines. The peak present at 2106.91 cm⁻¹ signifies C≡C stretching existing in conjugated alkenes and N–H bending in amines. The peak present at 1409.97 cm⁻¹ signifies O–H bending existing in carboxylic acids as well as alcohols, S=O stretching existing in sulphates. The peak present at 1150.88 cm⁻¹ signifies C–N stretching existing in amines, C–O stretching in esters, tertiary alcohols, aliphatic ethers and S=O stretching existing in sulphones. The peak present at 1078.71 cm⁻¹ signifies C–N stretching existing in amines, C–O stretching in primary alcohols, vinyl and aliphatic ethers.

ATR-IR spectra for gold nanoparticles synthesized using microwave-assisted method show prominent peaks at 3289.10 cm⁻¹, 1621.11 cm⁻¹, 1407.83 cm⁻¹ and 1075.62 cm⁻¹. The peak present at 3289.10 cm⁻¹ signifies O–H stretching existing in carboxylic acids and alcohols as well as N–H stretching in aliphatic primary amines.
The peak present at 1621.11 cm⁻¹ signifies C=C stretching existing in conjugated alkenes and N-H bending existing in amines. The peak present at 1407.83 cm⁻¹ signifies O–H bending existing in carboxylic acids as well as alcohols, S=O stretching existing in sulphates. The peak present at 1075.62 cm⁻¹ signifies C–N stretching present in amines, C–O stretching in primary alcohols, vinyl and aliphatic ethers [37, 38]. The above mentioned peaks of gold nanoparticles are compared with those of chloroauric acid, hydrazine hydrate and starch which together contribute to the synthesis of gold nanoparticles. The prominent peaks which chloroauric acid shows are 3303.79 cm⁻¹, 2106.67 cm⁻¹, 1633.86 cm⁻¹, 1326.71 cm⁻¹ and 1038.74 cm⁻¹. The prominent peaks which hydrazine hydrate depicts are 3285.52, 1615.86, 1346.93 cm⁻¹ and 1076.25 cm⁻¹. The prominent peaks observed in starch are 3310.89 cm⁻¹, 1412.84 cm⁻¹ and 1149.27 cm⁻¹. The lower shifts in these functional groups indicate their binding with gold nanoparticles.

Rapid detection of micro-organisms responsible for diseases is extremely important for initiating treatment of the concerned disease. In addition to this, detailed analysis of metabolites involved in the biological process is necessary for successful treatment of the disease [39]. Because of this reason, a large amount of attention is being given to rapid, specific and sensitive detection methods. Use of SERS analysis in biological application has increased recently because of its high molecular specificity attributed by the optical properties of SERS active substrates [40, 41]. Gold and silver nanoparticles have been largely used as SERS active substrates for studying detection of micro-organisms as well as interaction of concerned biomolecules [42, 43].

A typical validation of use of silver and gold nanoparticles synthesized by chemical, electrochemical and microwave-assisted methods as SERS substrate for detection of micro-organisms such as S. aureus and E. coli has been represented in figure 6. SERS spectrum was recorded using chemically synthesized silver nanoparticles as SERS substrates for detecting S. aureus (a) and E. coli (b). SERS spectrum was recorded using electrochemically synthesized silver nanoparticles as SERS substrates for detecting E. coli (c). SERS spectrum was recorded using
silver nanoparticles synthesized by microwave-assisted method as SERS substrates for detecting *E. coli* (d). SERS spectrum was recorded using electrochemically synthesized gold nanoparticles as SERS substrates for detecting *S. aureus* (e) and *E. coli* (f). SERS spectrum was recorded using electrochemically synthesized gold nanoparticles as SERS substrates for detecting *E. coli* (g). SERS spectrum was recorded using gold nanoparticles synthesized by microwave-assisted method as SERS substrates for detecting *S. aureus* (h) and *E. coli* (i). It is clearly observed that the intensity SERS signals is stronger when electrochemically synthesized silver nanoparticles are used as SERS substrate than the rest of the nanoparticles. This is because electrochemical synthesis results in formation of strong electromagnetic coupling within the nanoparticles thus forming a large number of hotspots on the so synthesized AgNPs. These factors would ultimately favor their application as SERS-active substrates. Also, this enhancement is observed only for AgNPs as silver nanoparticles show higher enhancement factors as
compared to gold and copper nanoparticles. Due to this reason, electrochemically synthesized gold nanoparticles fail to show such enhancement [44, 45].

SERS spectra which were obtained after interaction of silver as well as gold nanoparticles synthesized using chemical, electrochemical and microwave-assisted methods as SERS substrate with micro-organisms such as *S. aureus* and *E. coli* have been recorded. As reported in the literature, significant SERS signal obtained after interaction of *S. aureus* with silver nanoparticles include 437, 545, 668, 727, 788, 915, 996, 1054, 1151, 1313, 1390, 1480, 1546, and 1621 cm$^{-1}$. Similarly, prominent SERS signal post interaction of silver nanoparticles and *Escherichia coli* O157:H7 are 437, 542, 689, 792, 918, 999, 1054, 1108, 1228, 1304, 1390, 1422, 1474, 1543, and 1621 cm$^{-1}$ [26]. It should be noted that SERS spectra generated post interaction of the bacteria and nanoparticles would reveal molecular composition up to 10 nm from the outermost envelope of the bacteria [31, 46]. Based upon the SERS analysis of biomolecules already available in the literature and the values obtained upon SERS analysis, we can say that vibrational peaks of proteins, polysaccharides as well as lipids from the bacteria are majorly responsible for the so obtained spectra [47]. The peaks observed at 1395 cm$^{-1}$, 1376 cm$^{-1}$ along with 1370 cm$^{-1}$ has been observed due to stretching interactions in carbohydrates present upon the bacterial surface. The intensity of these vibrations can be exploited for detailed analysis of detection of micro-organisms. In addition to this, carbohydrates from the bacteria are also responsible for peaks such as 1267 cm$^{-1}$, 1259 cm$^{-1}$ in addition to 1265 cm$^{-1}$ (COH, HCO, HCC bonding); 1108 cm$^{-1}$, 1109 cm$^{-1}$ as well as 1178 cm$^{-1}$ (C-C and C-O asymmetric ring breathing) and 520 cm$^{-1}$, 726 cm$^{-1}$ as well as 729 cm$^{-1}$ (glycosidic ring signature present in N-acetyl-D-glucosamine as well as N-acetylmuramic acid which from the framework of the bacterial cell wall) [48–51]. Bacterial proteins are responsible for peaks near 1270 cm$^{-1}$ (amide III), 1000 cm$^{-1}$ (phenyl alanine), 1577 cm$^{-1}$ (aspartic and glutamic acid) and 1178 cm$^{-1}$ (NH$_2$ twisting) [31, 46]. The peaks present near 730 as well as 1330 are attributed to breathing of purine ring as well as C-N stretching present in the adenine of peptidoglycan constituting the cell envelope of the bacteria [48–54]. Fine tuning of these SERS substrates can be further used for detection of various micro-organisms which can be extrapolated to disease diagnostics. However, fabrication of SERS active substrates along with its stabilization as well as optimization of the process has its use in various fields.
Obtaining SERS signals with appropriate substrates is always a tricky business considering reproducibility as well as signal enhancement. Chemically synthesized nanoparticles lack reproducibility but make up for the signal enhancement. Streamlining of synthesis techniques is necessary to have a control over shape and size which is essential for its use as SERS substrate. Moreover, detailed analysis is necessary for fine tuning reproducibility along with signal enhancement [55].

4. Conclusions

Silver as well as gold nanoparticles were synthesized by chemical, electrochemical as well as microwave-assisted methods and characterized by using UV–vis spectroscopy, FT-IR, FESEM and XRD. Interaction of so synthesized silver as well as gold nanoparticles by different methods with different micro-organisms such as S. aureus and E. coli has been checked using surface enhanced Raman scattering (SERS). Signals have been obtained signifying use of these nanoparticles as SERS active substrates in detection of micro-organisms. Herein, reported silver and gold nanoparticles can be further used for developing a fast and reliable method in disease diagnostics.

Acknowledgments

BA extends special gratitude to University Grants Commission-Department of Atomic Energy Consortium for Scientific Research (UGC-DAE CSR), R-5 Shed, Bhabha Atomic Research Centre, Trombay, Mumbai, India (Grant No. UDCSR/MUM/AO/CRS-M-248/2017/1169 dated 14.03.2017) for financial support to Major Research Project. Board of College and University Development (BCUD), for research grants (BCUD, Finance/2016-17/1596/ dated: 08/11/2016), SP Pune University, Pune, India is acknowledged by BA. BA acknowledges Mr Akshay Bhyor, Ms. Pooja Khapare and Mr Dhiraj Jagtap for their preliminary work. SG thanks Department of Chemistry, SP Pune University for the project under the supervision of BA.

Conflicts of interest

There are no conflicts to declare.

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