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Copper micro/nanostructures as effective SERS active substrates for pathogen detection

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Abstract. Surface-Enhanced Raman Scattering (SERS) spectroscopy is a multifaceted surface sensitive methodology which exploits spectroscopy-based analysis for various applications. This technique is based on the massive amplification of Raman signals which were feeble previously in order to use them for appropriate identification at qualitative and quantitative in chemical as well as biological systems. This novel powerful technique can be utilized to identify pathogens such as bacteria and viruses. As far as SERS is concerned, one of the most studied problems has been functionalization of SERS active substrate. Metal colloids and nanostructures or microstructures synthesized using noble metals such as Au, Ag and Cu are considered to be SERS active. Silver and gold are extensively used as SERS active substrates due to chemical inertness and stability in air compare to copper. However, use of Cu as a suitable alternative has been taken into account as it is cheap. Herein, we have synthesized air-stable copper microstructures/nanostructures by chemical, electrochemical and microwave-assisted methods. In this paper, we have also discussed the use of as synthesized copper micro/nanostructures as inexpensive yet effective SERS active substrates for the fast identification of micro-organisms like *Staphylococcus aureus* and *Escherichia coli*.

Keywords: bacteria; surface-enhanced Raman scattering; copper microstructures/nanostructures (micro/nanostructures); *Staphylococcus aureus*; *Escherichia coli*; SERS active substrates

1. Introduction

The Surface Enhanced Raman Scattering (SERS) spectroscopy makes use of Raman spectroscopic analysis with high enhancement in Raman signal to facilitate suitable as well as specific identification used in chemical as well as biological systems. It was invented by Fleischmann, Hendra along with McQuillan at the University of Southampton in 1974 (Fleischmann *et al.* 1974). There is moderate to large enhancement of Raman scattering intensity which were previously of weak intensity in case of Raman spectroscopy, with the help of SERS active metal micro/nanostructures like Au and Ag solving problems which used to arise due to less sensitive traditional Raman spectroscopy. Multifaceted nature of SERS has been exploited in various applications including plasmonics, sensing, diagnostics, etc., thus providing ultra-sensitive detection limits, including single molecule sensitivity (Tian and Ren 2003, 2004, Sur and Chowdhury 2013, Willets and Van Duyne 2007, Nie and Emory 1997). This powerful spectroscopic technique can be utilized to detect pathogens rapidly like bacteria qualitatively and quantitatively with high sensitivity up to single bacteria (Jarvis and Goodacre 2004, Sengupta *et al.* 2005, Efrima and Bronk 1998, Efrima and Zeiri 2009, Liu *et al.* 2009). The preference of metal used in the form of SERS substrate is dependent on the surface plasmon resonance frequency. Silver (Ag), gold (Au) as well as copper (Cu) have been reported to be noble metals used in SERS as the Surface Plasmon Resonance (SPR) frequencies of these metals are present in the range of visible and near-infrared radiation, providing maximal enhancement. Use of gold and silver has been extensively reported as SERS active substrates in the form of metal colloids and nanostructures as cited in the literature (Jeanmaire and Van Duyne 1977, Jiang *et al.* 2007, Pettinger *et al.* 2004, Li *et al.* 2010, Freeman *et al.* 1995, Wang *et al.* 2006, Moskovits 2005, Sharma *et al.* 2013, Betz *et al.* 2014, Gomez and Lazzari 2014), although there are a few reports on the use of copper colloids and nanostructures as SERS substrates (Dendisova-Vyškovska *et al.* 2012, Muniz-Miranda *et al.* 2011, Cejkova *et al.* 2009). Cu also possesses SPR and is a promising alternative of Ag and Au in SERS as it is cheap. However, copper colloids and nanostructures are not popular as SERS active substrates due to its poor chemical stability and spontaneous conversion into copper oxides. The high reactivity and poor stability in air has hindered its performance as a first-choice material for SERS active substrates. Efforts had been taken to overcome the limitation on the SERS activity of copper. Use of copper nanoparticles functionalized by ligands has been reported as effective SERS active substrates after eliminating the oxidative damage these nanoparticles cause by interfering with SERS analysis (Muniz-Miranda *et al.* 2011). Chemically synthesized copper nanoparticles with different morphologies have been used (Cejkova *et al.* 2009). Laser ablation technique has been applied to fabricate copper SERS active substrates (Muniz-Miranda *et*

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al. 2011, Liu *et al*. 2015). However, many such methods require a lot of time and money. In this paper, we have synthesized air stable Cu micro/nanostructures by employing chemical and electrochemical with, microwave-assisted methods without the use of any surfactant. The employed synthetic protocols are simple and cost effective. We have characterized micro/nanostructures by UV-visible absorption spectroscopy, FTIR spectroscopy, X-ray diffraction measurements, SEM, DLS along with zeta potential measurement. We have also used the synthesized micro/nanostructures as potent SERS substrates to detect bacteria such as *Staphylococcus aureus* and *Escherichia coli* rapidly.

2. Experimental

2.1 Materials

All these chemicals have been used as obtained without any further purification. MilliQ water has been used to prepare all aqueous solutions. $CuCl_2 \cdot 2H_2O$ (Emplura, 98.5%, Merck, India), starch (Merck, India), hydrazine hydrate (A.R. Grade, S.D. Fine Chem. Ltd., India) were purchased.

2.2 Fabrication of Cu micro/nanostructures

2.2.1 Chemical method

40 ml of 1 mM $CuCl_2 \cdot 2H_2O$ was mixed with 2 ml 1% starch in a 100 ml beaker followed by addition of 2 ml 100 times diluted 80% hydrazine hydrate solution. The resultant solution gradually becomes yellow colour indicating the formation of copper micro/nanostructures. The synthesized copper micro/nanostructures were investigated and analyzed for further characterization and applications.

2.2.2 Electrochemical method

Two silver electrodes have been immersed into a 100 ml beaker with 1 mM $CuCl_2 \cdot 2H_2O$ solution (40 ml) and connected to power supply. At the commencement of the electrolysis, current appeared 48 amp and voltage 75 voltage, while current increases and become steady and then decreases to 5.9 amp. The electrolysis was carried out for 15 min.

2.2.3 Microwave oven method

25 ml of 1 mM $CuCl_2 \cdot 2H_2O$ solution was mixed with 1.25 ml 1% starch solution followed by addition of 1.25 ml 100 times diluted 80% hydrazine hydrate solution. Controlled heating of the reaction mixture was carried out in a convective microwave oven at a temperature of 110°C for 120 sec. The resultant solution gradually becomes yellow colour indicating the formation of copper micro/nanostructures. The copper nanoparticles dispersion was subjected to centrifugation at the speed of 10,000 rpm for a time duration of 25 min thus facilitating separation of micro/nanostructures from reaction by-products, unreacted reagents and impurities present in the reaction mixture.

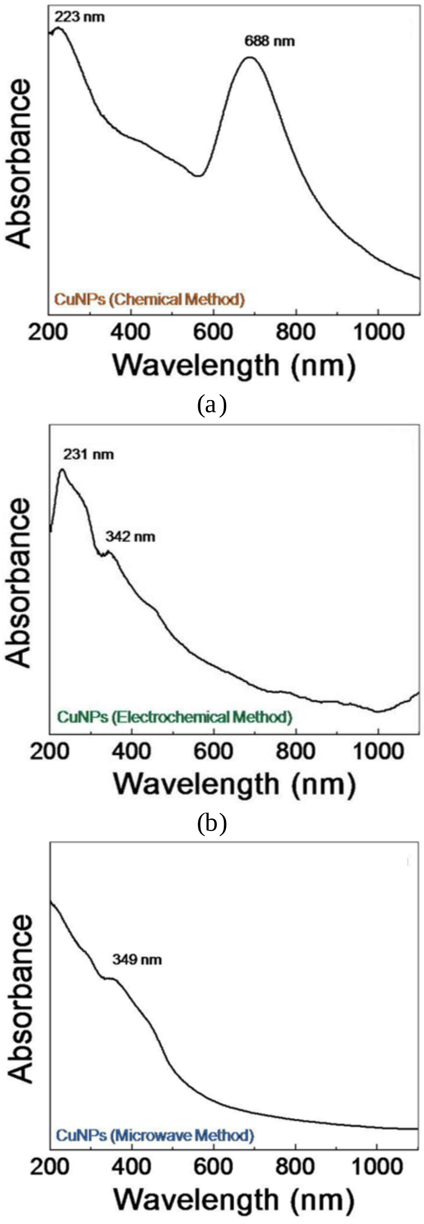
2.3 Characterization methods

The micro/nanostructures of copper were characterized using UV-vis spectrophotometer (Shimadzu UV-1800) from 200 nm to 1100 nm wavelength region. Field Emission Scanning Electron Microscopy (FESEM) (FESEM: Fei Quanta-450) of the copper nanoparticles was performed to test their morphology. Purified copper micro/nanostructures were loaded on Si (111) wafers in the form of thin films by air drying the sample drop which was casted upon the Si wafer. FTIR analysis of these samples was performed on a Platinum ATR Tensor 37 spectrophotometer. The X-ray diffraction analysis has been performed on Rigaku Ultima-IV X-ray Diffractometer operating at wavelength of 1.54 Å in the 2θ values within the range 40°-90° with a scanning speed of about 0.005°/sec by making use of CuK_α radiation. The particle size has been quantified by dynamic light scattering technique by calculating the effective diameter of the nanoparticles. The stability of the micro/nanostructures has been verified through measurement of zeta-potential by use of a dynamic light-scattering (DLS) (DLS; Model DLS-nano ZS, Zetasizer, Nanoseries, Malvern Instruments). Measurement of SERS signals have been performed using LabRam I confocal microprobe Raman system (Horiba JobinYvon, 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 as the source of excitation. Raman signals have been collected in between wave number 100 and 2000 cm^{-1} through integration time ranging from 1-5 sec.

2.4 Preparation of bacteria samples and Raman instrumentation/data processing

Microorganisms like *Staphylococcus aureus* as well as *Escherichia coli* have been taken as representative members of Gram positive and Gram-negative organisms respectively procured from Department of Microbiology, S.P. Pune University, Pune, India. The obtained micro-organisms have been preserved using nutrient agar slants and stored at a temperature of 4°C followed by intermittent sub culturing after 15-20 days for maintaining cell viability. The microorganisms have been inoculated into nutrient broth and kept for overnight incubation (12-18 hrs) at temperature 37°C under shaking conditions. These microorganisms which were grown in nutrient broth and further used as starter culture in subsequent inoculation procedures. After inoculating nutrient broth with starter cultures, they were grown for 4-6 hrs in shaker incubator at a temperature of 37°C under shaking conditions with speed 150 rpm. The number of cells was diluted to 10^3 CFU/ml for SERS analysis.

Bacterial samples whose density has been adjusted to 10^3 CFU/ml was allowed to interact with copper micro/nanostructures on a silicon wafer to carry out SERS experiments. Raman data were collected in the range of 100 and 2000 cm^{-1}. The raw SERS data sets were further processed using algorithms developed in our laboratory to remove excess noise.



(a)

(b)

(c)

Fig. 1 Representative UV-vis spectral analysis for (a) chemical, (b) electrochemical, (c) microwave-assisted synthesis of copper nanoparticles

3. Results and discussion

We have employed chemical, electrochemical and microwave-assisted methods to synthesize copper micro/nanostructures. All these methods are eco-friendly, cost effective and less time-consuming involving less reaction time and reaction steps. In the chemical, electrochemical and microwave-assisted methods, hydrazine hydrate has been used as reducing agent, while starch has been employed as stabilizing agents. We have not used any reducing or stabilizing agent in the electrochemical method. We have also not used any surfactant in our synthesis. The method is eco-friendly as it does not involve toxic chemicals or solvents as reagents or reaction media as reactions were carried out in aqueous media. Therefore, we can call our synthetic method as green synthesis of copper micro/nanostructures.

The formation of copper micro/nanostructures has been carried out with the help of UV-vis-NIR spectroscopy. Fig. 1(a) depicts UV-vis-NIR absorption spectrum obtained for the chemically synthesized copper micro/nanostructures while Figs. 1(b) and (c) show UV-vis-NIR spectra obtained from the electrochemically and microwave-assisted synthesized copper micro/nanostructures.

It is clearly observed in the case of chemically synthesized copper micro/nanostructures that the surface plasmon resonance band/peak appears at 688 nm, which is due to excitation of Localized Surface Plasmon Resonance (LSPR) present in the copper nanoparticles in the visible range present in the electromagnetic spectrum. The surface plasmon band of copper nanoparticles in aqueous conditions must exist approximately between 570-600 nm according to Mie theory (Kreibig and Vollmer 1995). Mie theory proposed that the LSPR bands exhibited by metallic nanomaterials in the visible range can be a result of transition of conducting electrons into excited state when it interacts with electromagnetic field and may deviate with respect to spectral position, intensity as well as width with the variation of the size, morphology, or dielectric environment of metal nanoparticles (Sur 2010). Other groups have also observed SPR band for copper nanoparticles in the same wavelength range (Kim *et al*. 2006, Salzemann *et al*. 2004). However, morphology, size range as well as the properties of capping and reducing agents might influence the exact position of the SPR band. The presence of red shift observed in Fig. 1 may be due to association which can be attributed to the interaction with the dissolved oxygen present in the colloidal solution when copper nanoparticles are synthesized followed by slow aggregation of synthesized nanoparticles. It implies that the chemically synthesized copper nanoparticles in our case undergo instantaneous oxidation, as an outcome of their high chemical reaction potential or due to increased proportions of copper nanoparticles because of aggregation. The presence of the surface oxide layer can transform the particle thus facilitating formation of core-shell structure, following by change in wavelength at which SPR band occurs, which is influences by dielectric constant as well as shell thickness. In case of copper micro/nanostructures obtained electrochemically, two peaks have been recorded at 231 nm as well as 342 nm. In case of copper micro/nanostructures obtained by microwave-assisted method one small peak was observed at 349 nm. However, no peak corresponds to the LSPR band of copper nanoparticles was monitored in the visible region for the synthesized copper micro/nanostructures by electrochemical or microwave-assisted methods. The presence of another peak at 223 nm can be explained by the copper Inter-Band Transition (IBT) in the short wavelength UV region. This may indicate the presence of Cu_2O along with copper micro/nanostructures (Yin *et al*. 2005, Borgohain *et al*. 2002). However, it is very difficult to come into final conclusion solely based from the results of UV-vis-NIR

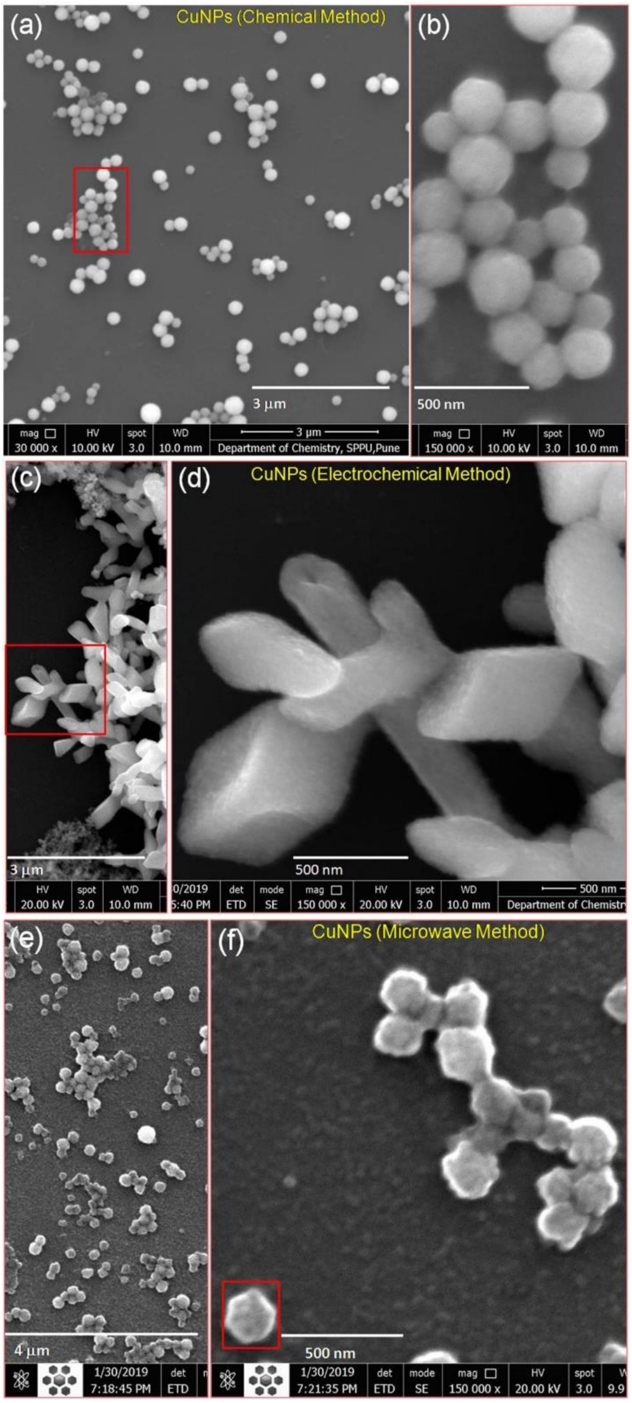
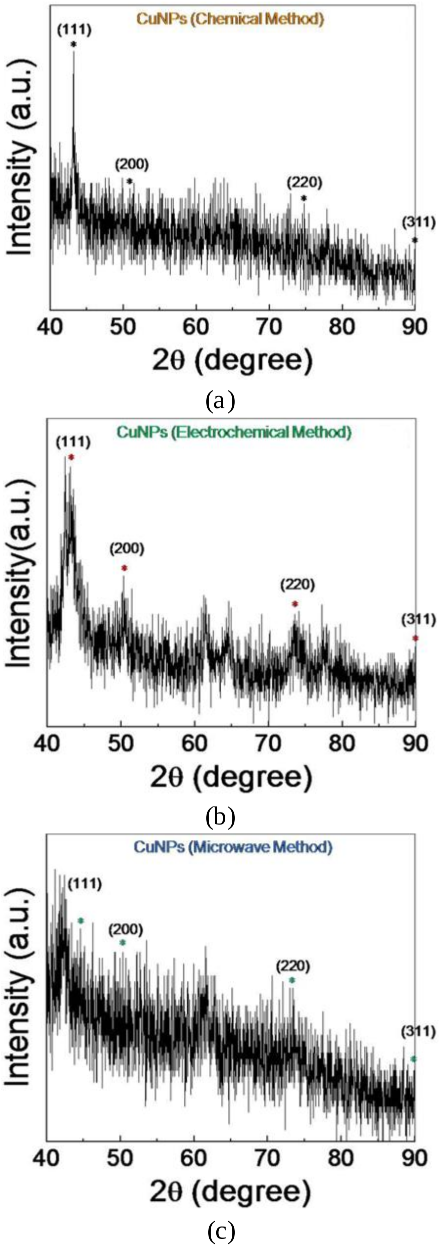


Fig. 2 Typical FESEM images of copper nanoparticles synthesized by chemical approach at (a) lower and (b) higher resolution, electrochemical approach at (c) lower and (d) higher resolution as well as using microwave-assisted methods at (e) lower and (f) higher resolution

absorption spectra.

The shapes and structures of the copper micro/ nanostructures synthesized using chemical, electrochemical or microwave-assisted methods were studied by FESEM technique. Fig. 2(a) illustrates the SEM image of the chemically synthesized copper micro/ nanostructures without using any surfactants, while Figs. 2(b) and (c) show the SEM image of the electro-chemical and microwave-assisted synthesized copper micro/nanostructures. The electrochemically synthesized copper micro/nanostructures were cubic in shape as observed from SEM image with dimension in the order of 500 nm, where copper micro/nanostructures obtained by microwave-assisted method was hexagonal in shape. Fig. 2(a) shows FESEM data from which it is clear that the diameter of the so-synthesized copper micro/nanostructures was approximately around 200 nm with nearly spherical morphology with monodisperse in nature.



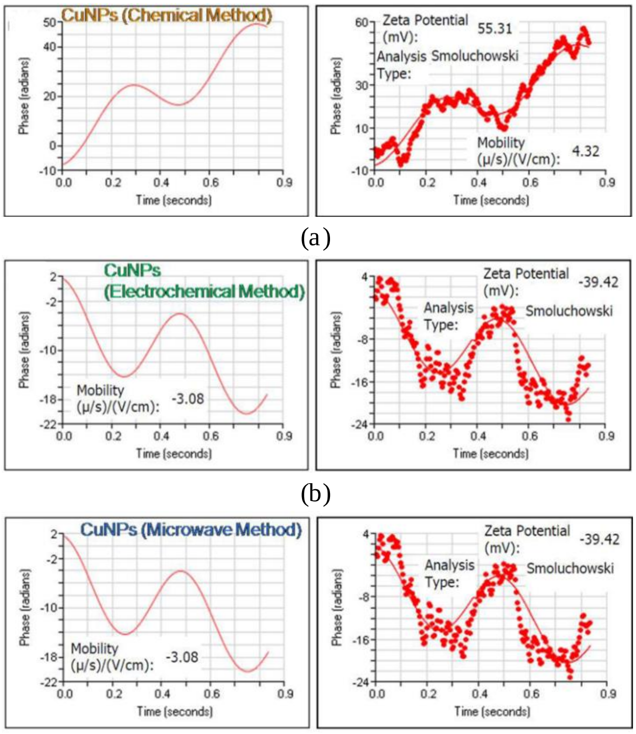
(a)

(b)

(c)

Fig. 3 X-ray diffraction patterns for copper nanoparticles synthesized by (a) chemical, (b) electrochemical, (c) microwave-assisted approaches

The XRD pattern of the chemically synthesized copper micro/nanostructures is illustrated in Fig. 4(a). Four diffraction peaks were observed with 2θ values 43.18°,



(a)

(b)

(c)

Fig. 4 Plots of zeta potential for copper nanoparticles synthesized by (a) chemical, (b) electrochemical, (c) microwave-assisted approaches

50.9°, 74.18° and 89.9° which correspond to (111), (200), (220) and (311) planes respectively of metallic Cu. This result signifies of face centered cubic structures of metallic Cu (JCPDS no. 03-1005). The XRD results confirm that the synthesized copper nanoparticles are highly pure and are present in the face centered cubic form (Khan and Rashid 2016, Mott *et al*. 2007, Klung and Alexander 1974). High degree of consistency has been observed in case of these three peaks in comparison with the peaks present in standard JCPDS File No. 03-1005 representing standard spectrum for the pure FCC (Face Centered Cubic) metallic Cu.

The broad peak corresponds to the nanocrystalline nature of the copper sample. We have also calculated the mean size (D) of the copper nanoparticles synthesized using chemical method by means of the typical Scherrer formula (Mahajan and Takwale 2014)

$$D = {}^{k\lambda}/_{\beta\ cos\theta} \tag{1}$$

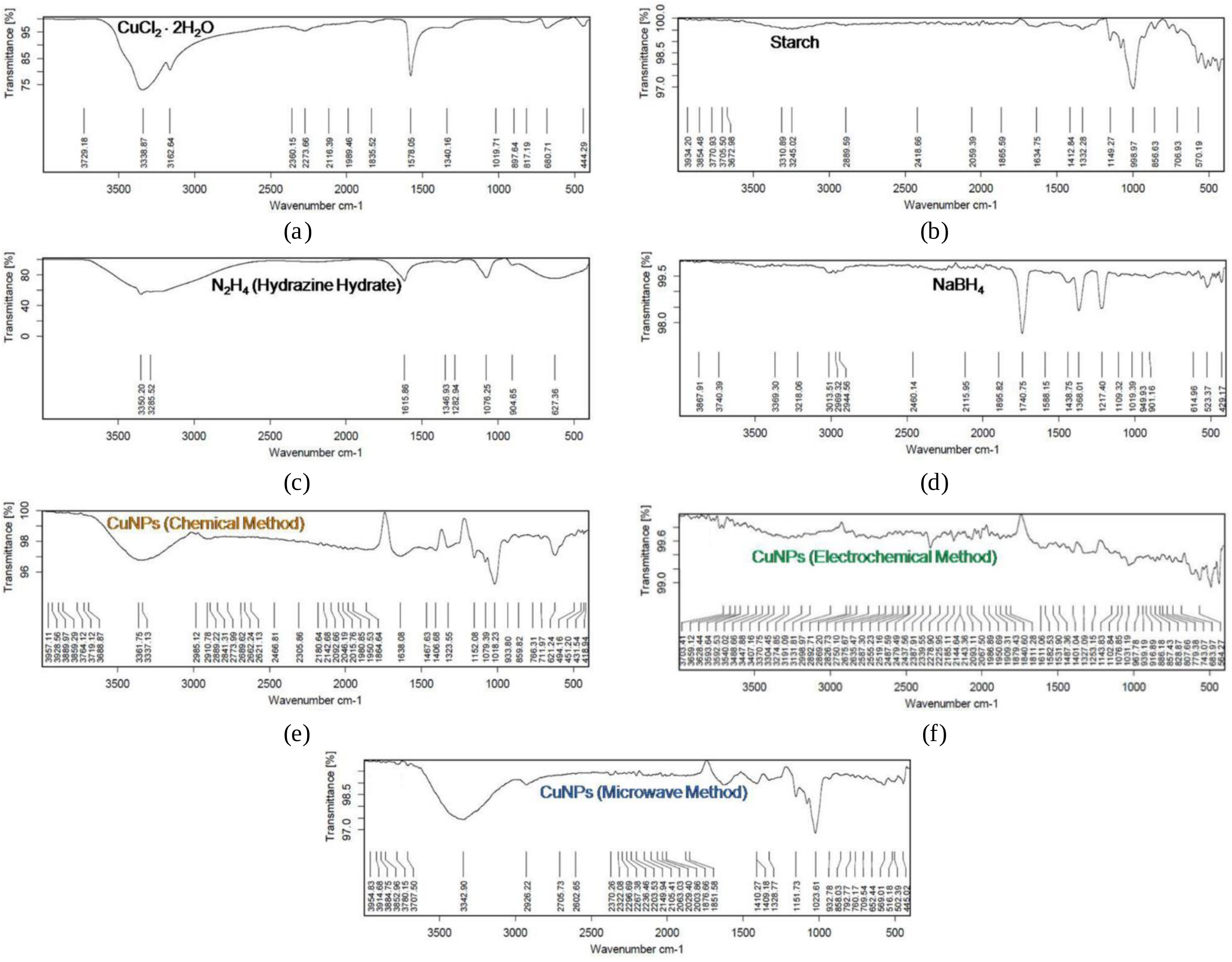
where, k is the constant which corresponds to the morphology factor usually equivalent to 1, λ corresponds to the wavelength of X-ray, θ corresponds to the Bragg's angle as well as β corresponds to the Full Width of the Half Maxima (FWHM). The mean size of the chemically synthesized copper micro/nanostructures crystal of sample was estimated to be around 2000 Å (200 nm).

The pattern was not completely clear, with the possibility of adulteration by copper oxides (CuO, Cu_2O). Similar results have been published by other groups for the synthesized copper nanoparticles using various synthesis methods in the literature (Panigrahi *et al*. 2006).

The XRD pattern of the electrochemically synthesized copper micro/nanostructures is depicted in Fig. 3(b), while Fig. 3(c) represents the XRD pattern of the copper micro/nanostructures synthesized by microwave method. Four diffraction peaks were observed with 2θ values of 43.12°, 50.12°, 73.58°, 89.66° and 43.38°, 50.38°, 73.44°, 89.82° in electrochemical and microwave assisted approach respectively which correspond to Cu (111), (200), (220) and (311) planes.

The stability of the chemically synthesized copper micro/nanostructures was determined by zeta-potential measurement. A positive zeta potential value of 55 mV for our copper micro/nanostructures was obtained from our measurement suggesting the formation of positively charged copper micro/nanostructures (See Fig. 4(a)). Aqueous solutions of metal nanoparticles such as Pt, Au and Ag usually exhibits negative zeta potential values, as a result of adsorption of hydroxide ions extracted from the aqueous environment or any negative ions from the stabilizing agent. In our case, the presence of starch molecules as stabilizing agent, results in interaction with the cationic Cu^{2+} ions thus forming complexes, which prevents the adsorption of the anionic hydroxide radicals. Zeta potential measurements show that the as synthesized Cu micro/nanostructures exhibit excellent stability. It has been observed that the copper micro/nanostructures do not aggregate even after three months. Our results obtained from the zeta potential measurements support the previous reports already present in the literature (Muniz-Miranda *et al*. 2011). However, we have obtained negative zeta potential values of -40 mV along with -30 mV for copper micro/nanostructures synthesized by electrochemical and microwave-assisted methods, respectively. The negative values of zeta potential for the synthesized copper micro/nanostructures suggest the formation of negatively charged copper micro/nanostructures due to adsorption of hydroxide ions during the electrochemical and microwave-assisted synthesis. The lower values of zeta potential for copper micro/nanostructures synthesized by electrochemical (Fig. 4(b)) and microwave-assisted methods (Fig. 4(c)) compare to chemical method (Fig. 4(a)) indicate that the copper micro/nanostructures are less stable. The observed low zeta potential values can be explained by the absence of stabilizing agent in case of electrochemical synthesis and decomposition and desorption of stabilizing agent in case of microwave-assisted synthesis at higher temperature.

FT-IR spectroscopic technique can be employed to detect the presence of any functional group of any organic molecule. Fig. 5 shows ATR-IR spectra of corresponding constituents such as copper chloride (a), starch (b), hydrazine hydrate (c), and sodium borohydride (d) which have been used for synthesis of nanoparticles and copper micro/nanostructures (e) synthesized using chemical method. Figs. 5(f) and (g) illustrate the corresponding ATR-IR spectra for the copper micro/nanostructures synthesized using electrochemical and microwave-based methods respectively. In this study, we want to confirm the surface



(a) (b)

(c) (d)

(e) (f)

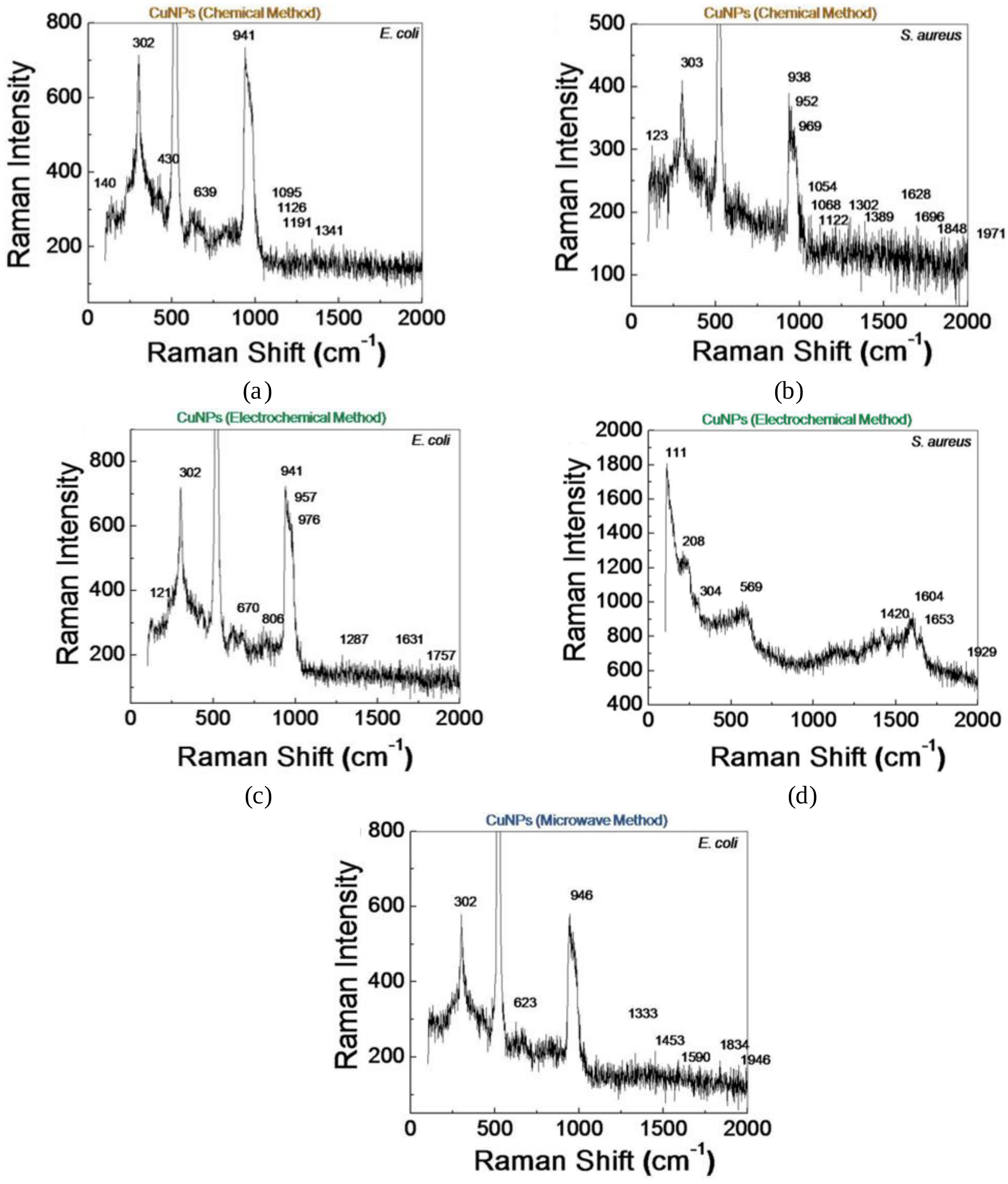
(g)

Fig. 5 ATR-IR spectra of corresponding constituents such as copper (a) chloride, (b) starch, (c) hydrazine hydrate, (d) sodium borohydride, (e) copper nanoparticles (CuNPs) synthesized by chemical, (f) electrochemical, (g) microwave-assisted approaches

functionalization of the copper nanoparticles by adsorption of ions from the stabilizing agent, aqueous media and also due to aerial oxidation. Presence of absorption peak in the FTIR spectra at low frequencies below 700 cm^{-1} signifies vibration of Cu–O bonds. The broad absorption band between 3300-3400 cm^{-1} represents the O-H stretching modes of –OH functional groups as well as water molecules. The bands at 1600-1650 cm^{-1} correspond to the O-H bending of water molecules.

We have also demonstrated the use of synthesized copper micro/nanostructures as SERS active substrate to detect bacteria rapidly. Earlier, we have carried out the fast identification as well as enumeration with the help of SERS using silver nanoparticles by bioreduction of silver nitrate solution with the help of *Neolamarckia cadamba* plant extract. SERS of Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) can be used for their differentiation (Ankamwar *et al*. 2016). SERS study has also been carried out for detection of *Mycobacterium tuberculosis*, which is the causative agent of tuberculosis with the help of silver nanoparticles, synthesized using Shikakai and Reetha (Sur *et al*. 2018).

Figs. 6(a) and (b) illustrate the SERS signals for detecting *E. coli* as well as *S. aureus* bacteria using chemically synthesized copper nanoparticles as SERS substrates. Good quality SERS spectra were recorded during the interaction of whole bacterium with the chemically synthesized copper micro/nanostructures and the obtained SERS data can be used to deduce details regarding membrane structures of the bacteria (Kahraman *et al*. 2007). We already know from our previous experience that there is difference of composition of bacterial cell wall in different bacterial species and these variations in bacterial cell wall can be clearly detected using SERS analysis. Raman photon of magnitude 10^4 counts on the order tens of thousands undoubtedly reveal the spectra as immediate result of the SERS phenomena relatively different from the conventional Raman scattering. Calculations have been carried out to determine Raman enhancement factor of the chemically synthesized copper micro/nanostructures used as the SERS substrate. The enhancement factor has been reported to be 1 ± 0.10 × 10^4 as well as 3 ± 0.20 × 10^4 for *S. aureus* and *E.*



(a) (b) (c) (d) (e)

Fig. 6 SERS spectrum was recorded using chemically synthesized copper nanoparticles as SERS substrates for detecting (a) *S. aureus* and (b) *E. coli*, electrochemically synthesized copper nanoparticles for detecting (c) *E. coli* and (d) *S. aureus* and by microwave-assisted method for detecting (e) *E. coli*

coli bacteria, respectively with more or less zero fluctuations. The SERS substrates fabricated by our employing chemically synthesized micro/nanostructures of copper are nearly homogeneous along with a relative standard deviation of 5 determined using 25 repetitive evaluation of a variety of positions present upon the SERS substrate. Additionally, the fabricated SERS substrates exhibit high levels to stability even after one month of storage. Using this almost homogeneous, stable SERS active substrate, we can use these SERS substrates to differentiate Gram-positive bacteria *S. aureus* from Gram-negative bacteria *E. coli*. The SERS data recorded has high stability, homogeneity as well as reproducibility is extremely stable, uniform and reproducible, which depicts the versatile nature of chemically synthesized copper micro/nanostructures as effective SERS active substrate. The sensitivity of this SERS substrate ranges up to 10^3 CFU ml^{-1} of *E. coli* as well as *S. aureus* indicating its highly sensitive nature. Normalization of the acquired SERS datasets has been performed using photon count of the highest peak obtained at 941 cm^{-1} for both Gram-positive bacteria (*S. aureus*) as well as Gram-negative bacteria (*E. coli*). Gram-negative bacteria (*Escherichia coli*) shows the SERS spectrum with peaks at 302, 430, 639, 941, 1126 and 1341 as well as 1460 cm^{-1} as observed in Fig. 6(a). For Gram-positive bacteria (*Staphylococcus aureus*), major peaks were observed at 303, 938, 952, 969 and 1302 cm^{-1} as shown in Fig. 6(b). There is a significant difference in observed SERS spectrum for Gram-negative *Escherichia coli* as compared to that of Gram-positive *Staphylococcus* *aureus* bacteria. However, typical strong band because of the presence of nucleic acid base adenine at 732 cm^{-1} was absent in our spectra, while peak at 1341 cm^{-1} was very weak. It is a well-known fact from the literature that some of the most remarkable trends in the SERS spectra of Gram-negative as well as Gram-positive bacteria (Figs. 6(a) and (b)) include peaks present at 730 cm^{-1} in addition to 1330 cm^{-1} which represent purine ring breathing mode of the purine ring as well as stretching mode of C–N bond present in the adenine region of the lipid bilayer components constituting the cell wall. The absence or presence of these peaks with low intensity observed in our case for the copper nanostructure may be due to the poor adhesion of bacterial cell wall component on the copper nanostructured surface. This suggests rather poor or moderate affinities of Cu nanoparticles compare to biosynthesized Ag nanoparticles as reported earlier with the aromatic rings which are part of bacterial cell walls (Ankamwar *et al*. 2016, Sur *et al*. 2018). The poor biocompatibility of our SERS active substrate can be further improved by suitable chemical functionalization. At present, we are involved in such research, which will be communicated in the near future.

Figs. 6(c) and (d) illustrate the SERS analysis for detecting *E. coli* as well as *S. aureus* bacteria using electrochemically and Fig. 6(e) microwave radiation assisted synthesized copper nanoparticles for detecting *E. coli* bacteria as SERS substrates. There was not much difference in the profiles of the SERS spectra with peak position at almost at same frequencies. These results suggest that SERS activity of electrochemically and microwave radiation assisted synthesized copper nanoparticles are almost same as the chemically synthesized copper micro/nanostructures.

It is important to mention here that this is the first report of the use of copper micro/nanostructures as an inexpensive and effective SERS active substrate for rapid identification of different bacteria as per our knowledge.

Consequently, we can discriminate between already reported novel micro-organisms rapidly in time duration of few seconds using the SERS data of bacterial cell wall as fingerprint using copper micro/nanostructures as an inexpensive yet effective SERS active substrates. Nevertheless, the use of SERS does not serve as a full proof method for detection of bacteria due to lack of differentiation within different strains of same bacterial species. This serves as the most important drawback of SERS based detection of micro-organisms employed by us.

4. Conclusions

- Green synthesis of copper micro/nanostructures has been reported by chemical, electrochemical and microwave-assisted methods without using any surfactant.
- The synthesized copper micro/nanostructures were subjected to characterization using different analytical techniques such as UV-vis absorption spectroscopy, FTIR spectroscopy, X-ray diffraction measurements, electron microscopy such as SEM, DLS and zeta potential measurements.
- Chemically synthesized copper micro/nanostructures do not aggregate even after storing them for three months.
- We have also utilized the synthesized micro/nanostructures as effective SERS substrates to detect bacteria such as *Staphylococcus aureus* and *Escherichia coli* rapidly. These nanoparticles can be used to differentiate Gram-positive bacteria *S. aureus* from Gram-negative bacteria *E. coli*.
- The SERS data recorded has high stability, homogeneity as well as extremely stable, uniform and reproducible, which depicts the versatile nature of chemically synthesized copper micro/nanostructures as effective SERS active substrate.
- The sensitivity of this SERS substrate ranges up to 10^{3} CFU ml^{-1} for both *E. coli* as well as *S. aureus* indicating its highly sensitive nature.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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