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Research Paper

Fabrication of Surface-Enhanced Raman Scattering Sensors to Detect Antibiotic Residues in Muscle Foods Using Gold Nanoparticles

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Abstract: The surface-enhanced Raman scattering (SERS) is a precise technique for molecular structure analysis. This method relies on enhancing the Raman signal by using plasmonic nanostructures, such as gold and silver, which serve as substrate sensors. We used a fast and cost-effective approach to create a substrate for the SERS analysis. Gold colloidal nanoparticles in diameters of 50-80_{nm} were initially synthesized and deposited onto glass slides to create a uniform and rough substrate. To stabilize the gold nanoparticles, a sulfur compound called "1-dodecanethiol" was selected, increasing the contact angle of the sample to 45°. Florfenicol antibiotic residue in chicken meat was selected as an analyte. Spectrum acquisitions at various points on a single slide demonstrated acceptable substrate uniformity (RSD = 8.44%). Further experiments conducted on different slides confirmed the consistency of the results (RSD = 7.90%). Finally, the reliability of the results was confirmed through spectrum acquisitions over various time intervals (RSD = 1.26%).

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1. INTRODUCTION

Nowadays, food pollutants have become a serious concern for food specialists, consumers, and regulatory agencies. One example is the use of veterinary drugs, particularly antibiotics, in livestock and poultry farming. If these drugs are not completely eliminated within the appropriate timeframe, their residues can remain in the meat and pose a health risk to consumers. Surface-enhanced Raman spectroscopy (SERS) is a highly effective method for detecting trace amounts of chemical residues. In this method, a laser is directed onto the surface of a substrate containing metal nanoparticles such as gold, silver, or copper. This creates a strong electromagnetic field by enhancing surface plasmons, leading to an increase in the intensity of inelastic scattering (Figure 1).

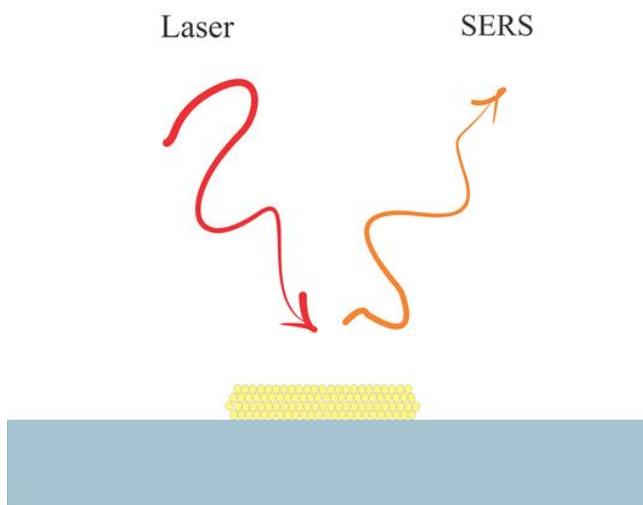


Fig. 1. Scheme of SERS using the gold nanoparticles substrate

The mass shape of gold is a natural element chemically, while its nanometer-scale particles are very active and able to perform a variety of complexes by forming bonds between the gold nanoparticles and other materials such as thiolate compounds (figure 2). In many of these compounds, the thiol group (-

SH) acts as an interface between metal molecules. Alkyl thiolates and aryl thiolates are among these compounds.

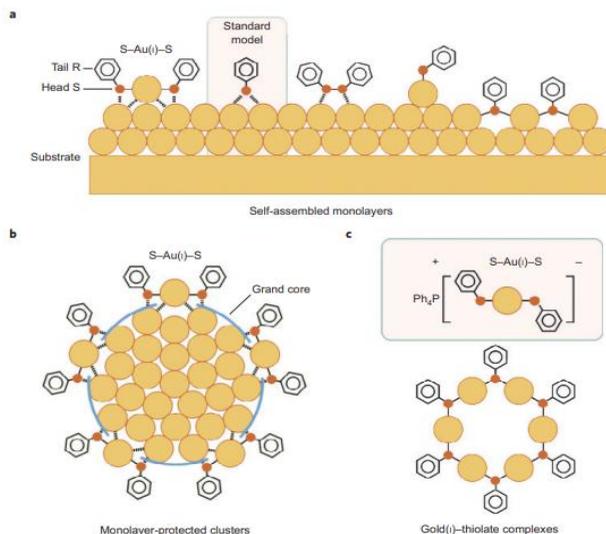


Fig. 2. Schematic shapes of the bonds between the thiolates and the gold nanoparticles;
 a) Self-arranged single layers b) Gold clusters protected with a single layer of gold c)
 Gold-Thiolate complex [1,2]

The SERS as a promising technology for the rapid and sensitive detection of biological samples [3], environmental pollutants [4], food contaminants [5,6], and even single molecule/particle [7], has attracted great interest (Figure 3). SERS can provide highly specific information about chemical and biochemical species with “fingerprint-like” spectrum patterns to detect, identify, and quantify chemicals within a complex biological matrix [8].

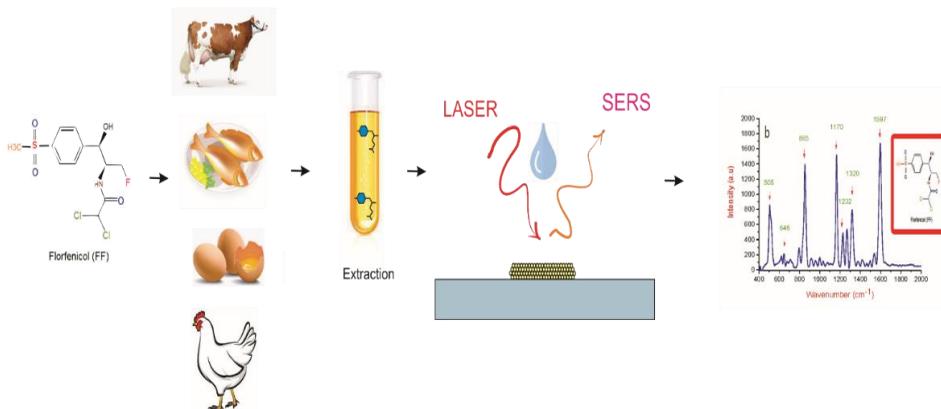


Fig. 1. The SERS is a rapid method to detect trace amounts of food contaminants such as the antibiotic florfenicol residues in animal source foods. The samples are extracted, and their SERS spectrums are investigated to find index peaks of antibiotic fingerprints.

In general, two mechanisms have been identified in Raman amplification: the electromagnetic (EM) effect and the chemical (CM) effect [9]. The dominant mechanism of SERS is characterized by locally enhanced electromagnetic (EM) fields occurring in the vicinity of the metal nanostructures owing to the localized surface Plasmon resonances (LSPRs) [10,11]. The spectrum positions of the LSPRs are functions of the dielectric constants of the surrounding media and structural information of the metal nanostructures, such as size, shape, and material [12]. According to theoretical calculations, the maximum enhancement factor (EF) using LSPR is around 10^{11} . Another mechanism for SERS is a chemical enhancement (EF of 10^1 – 10^3), which occurs via chemical interactions between the molecules and metal nanostructures [13]. Generally, the SERS enhancement is less than eight orders of magnitude [14].

Metals such as gold (Au) or silver (Ag) in colloidal forms and substrates are used to increase the Raman signal in the SERS. An active SERS substrate is a key component in SERS detection [15]. The performance of SERS noble metal nanomaterials depends largely on their size, morphology, composition, and structure. Noble metal nanomaterials with different morphologies, such as gold nanostars [16], silver nanodendrites [17], flower-like gold nanoparticles [18],

and bipyramidal gold nanoparticles [19] have been prepared as active SERS substrates. At present, much attention has been paid to metal nanoparticles due to surface energy, the surface-to-volume ratio, and many other properties [20] [21]. The widespread use of metal nanoparticles in biology, the pharmaceutical industry, and other research fields has led to an increase in demand for these nanoparticles and therefore there is a basic need for further production of such nanoparticles. Much research on gold nanoparticles has been used in the fabrication of SERS substrates [22-26]. Gold nanoparticles, like silver nanoparticles, have antimicrobial properties. The shape and size of gold nanoparticles play an important role in the antibacterial activity and its interaction with bacterial cells [27].

The synthesis of gold nanoparticles generally involves the reduction of ionic gold to atomic. One of the common methods involves dissolving gold tetrachloride in an aqueous solution and using reducing agents such as sodium citrate or borohydroxide, which is called the Turkevich method [28]. Among the factors affecting the particle size in the reduction system are the reduction rate and the amount of stabilizing agent. The higher reduction rate and the amount of stabilizing agent, the smaller the particle size. Biological, chemical, and physical approaches can be used to synthesize gold nanoparticles. [29-33].

Generally, a superior SERS- active substrate requires high- density hot spots over a large area with good uniformity, signal reproducibility, high enhancement factors, and low fabrication cost. [34]. Fabrication of pre-prepared substrates on smooth surfaces can be a way to reach this purpose. For the fabrication of cost-effective pre-made substrates, facile efficient approaches should be exploited to prepare a homogeneous plasmonic layer of nanoparticles with intensive SERS signals. Furthermore, reusable substrates can increasingly help to reduce costs. The development of recyclable SERS substrates is also an alternative strategy to realize cost- effective practical applications. Because of their self- cleaning characteristics, the SERS substrates are capable of being reused multiple times [34]. This study employed a simple and economical method to fabricate a SERS sensor by placing gold colloidal nanoparticles on smooth surfaces such as glass slides and fixing them with sulfur compounds.

2. MATERIAL AND METHOD

2.1. Chemicals and instruments

- Florfenicol with purity > 98% as the sample, made by Henan CoreyChem Co., Ltd. (CAS No.: 76639-94-6. Formula: $C_{12}H_{14}C_{12}FNO_4S$).
- Tetrachloroauric acid made by Alfa Aesar with purity 99.9% (CAS No.: 277988-77-8, formula: $HAuCl_4$ (III) [hydrogen tetrachloroaurate (III)], as the precursor of gold nanoparticles.
- Ultra-pure deionized distilled water for preparation of the solutions.
- Sulfur compounds to stabilize nanoparticles, all with analytical laboratory purities and without additional purification.
- Raman Confocal Microscope (model: X Plora Plus, made by Horiba Company, equipped with 532 and 785 nm laser sources, wavelength range: 0 – 4000 cm^{-1}) due to collect Raman spectrums.

2.2. Preparation of solutions

To prepare the test solutions, we followed a three-category approach.

I. Pure extract (EX): the breast meat was homogenized using a homogenizer. To extract the chicken meat, two solvents were used: ethyl acetate and deionized ultrapure water. The extraction process was carried out in three replications, and the resulting extracts were combined. In the ethyl acetate extraction, the following steps were followed: 20 g of sample was mixed with 10 ml of ethyl acetate and 40 ml of distilled water in a 100 ml test tube. The mixture was then mixed for 3 minutes using a mixer. After that, the test tube was placed in ice water and sonicated for 3 minutes using an ultrasonic homogenizer and catheter (with 10 seconds of work followed by 10 seconds of stopping). The next step involved centrifuging the mixture twice at high speed (16000 $gr = 12085$ rpm) for 10 minutes. The supernatants were separated and combined, and the volume of the extracted portion with distilled water was adjusted to 100 ml (20 g of meat per 100 ml of extract). For the extraction using ultrapure deionized distilled water, the same steps were repeated, except that only distilled water was used instead of ethyl acetate.

II. Aqueous solution of florfenicol (FF): we created a standard solution with a concentration of 1000 mg/L (2.8 mM) by dissolving pure FF powder in deionized distilled water at a temperature of approximately 40 °C. This solution was used to prepare other dilutions.

III. Spiked extract (FF + EX): we dissolved FF powder in the extract instead of water to obtain a standard spiked extract with a concentration of 1000 mg/L. This spiked extract was also used to prepare other dilutions.

2.3. Preparation of standard gold nanoparticles

The Turkevich - Frans method [35] was used to prepare a standard solution containing gold colloidal nanoparticles. A 10-fold coefficient was used to prepare the solutions To achieve the proper amount of nanoparticles. At first, HAuCl₄ solution (0.1% by weight = solution I) and Na₃-Citrate solution (1% by weight = solution II) were prepared using the ultrapure deionized distilled water. Then, the solution (I) was heated up to boiling point using a heater equipped with a mixer (500 rpm), and the solution (II) was added to it in desired values. The color of the solution changed to purple after about 25 seconds showing to form of the gold nanoparticles.

2.4. Preparation of SERS substrate

2.4.1. The gold layer

Two approaches were considered for this purpose:

i) Formation of a thin gold layer through the sputter coating method.

In this method, a very thin gold layer was coated on the surface of a glass slide using the “Desk Sputter Coater“. The gold particles are separated from the gold mass placed in the device reservoir, and If a barrier such as a glass slide is placed in the passage route, the particles will be placed on its surface. According to this method, a glass slide was placed in the designated place of the device, coating a layer of gold particles on the entire surface of the glass slide (Sputter Time = 200 s, Power = 200 Ma).

ii) Deposition of the colloidal gold nanoparticles

In the second method, the direct precipitation method of colloidal nanoparticles was employed. First, a droplet of the solution containing the colloidal gold nanoparticles was placed on the slide. After the evaporation of water, the solid content of the water droplet is deposited on the glass slide. Then the next droplet was gently dripped onto the previous deposit allowing the water to evaporate and dry again. This action was repeated 15-20 times. Spinning or vacuuming can speed up the drying process. By repeating the deposition and drying process, a thin, rough, and uniform layer of the gold nanoparticles formed on the slide surface provided the necessary platform for the SERS.

2.5. Method of stabilization of gold nanoparticles

Three groups of sulfur compounds were used to stabilize the formed layer. Among the aryl thiolates category, the compound of "2-Mercapto benzimidazole" was selected, and after dissolving it in distilled water was tested on the substrate. From the dithiols group, the "1, 3-Propanedithiol" was considered, and finally, from the alkyl thiolates group, the "1-Dodecanthiol" was investigated (Figure 4).

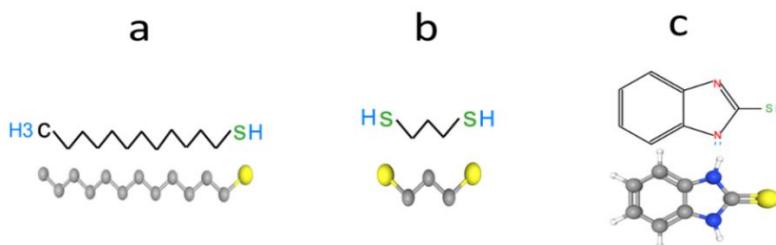


Fig. 4. The applied thiol Compounds; a) 2-Mercapto benzimidazole b) 1,3-Propanedithiol c) 1-Dodecanthiol

The high capability of sulfur compounds to form covalent bonds with the gold particles in nanometer dimensions causes the binding of the nanoparticles to each other and leads to stabilizing the formed gold layers on the smooth surfaces.

2.6. Raman spectrum acquisition

In general, two methods were used to collect Raman spectra. In the first method, without using the gold nanoparticles, normal Raman scattering spectrums were collected by direct laser irradiation to the sample's surface (laser wavelength 785 nm, collection radius 500 μm , acquisition time 2 s).

In the second method, the spectrums of the samples were collected through SERS, and three ways of using the gold nanoparticles, including; direct use of colloidal nanoparticles, gold sputter coating, and deposition and stabilization of nanoparticles on the glass slides, were compared.

3. RESULT AND DISCUSSION

3.1. Normal Raman spectroscopy

In this procedure, the spectrums of solid florfenicol, and 1000 mgr/L florfenicol solution, were obtained without using the gold nanoparticles by the normal Raman spectroscopy method. In the case of the solid florfenicol spectrum, the index peaks were clearly visible and had high intensities, while the florfenicol solution's index peaks were very weak and few (Figure 5). Therefore, the need to strengthen the peak intensities, using some methods like the SERS, was revealed.

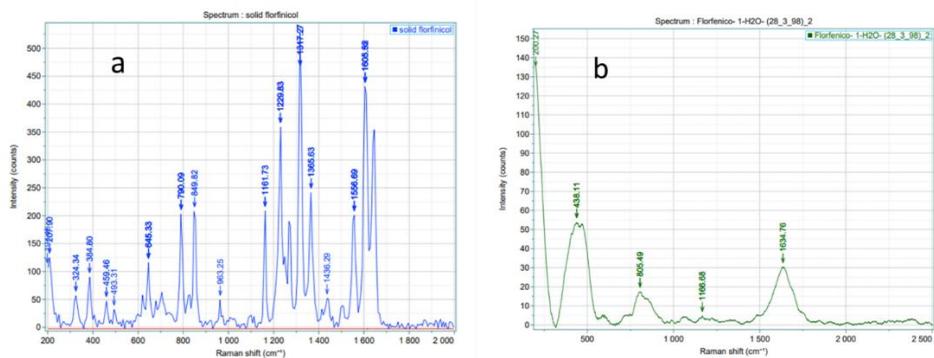


Fig. 5. The normal Raman spectrums of florfenicol; a) the solid powder b) the 1000 mgr/L solution

3.2. SERS

3.2.1. Comparison of the gold nanoparticles application methods

In order to appear the peaks and strengthen their intensities, the SERS technique was performed using the gold nanoparticles. In each stage of the experiments, the SERS spectrums of the sample were collected and compared with each other to determine their intensities and number of the peaks. The results of three different methods, applying the gold nanoparticles, to perform a suitable SERS substrate were as follows:

a) Direct use of the colloidal gold nanoparticles

The spectrums of three aqueous solutions of florfenicol with concentrations of 1, 10, and 100 mg/L, containing 0.05 ppm of the colloidal gold nanoparticles, were obtained; however, no significant peak was observed. Even when the concentrations of the gold nanoparticles were increased to 0.5, 5, and 25 ppm, no desirable peak was observed again. Therefore, probably the concentration of the gold nanoparticles had not been enough for the SERS, While increasing the concentration of nanoparticles to more than this amount did not seem economical.

b) Using gold sputtering

In order to spectroscopy, a standard florfenicol solution was placed on the substrate, but the substrate did not remain stable during the test, and the gold layer was washed from the slide's surface. Attempts to stabilize the gold layer with the sulfur compounds were unsuccessful. In addition, the gold particles sputtered over the entire surface of the slide without any control over the covered area, wasting a large amount of the gold. So this method did not seem cost-effective.

c) Using the colloidal gold nanoparticles in the substrate

This method effectively increased the number and intensity of peaks in the Raman spectrums. In the next step, this method was used simultaneously with the direct use of colloidal nanoparticles in the sample (method a), resulting in the maximum intensity and number of peaks, and used for the spectrum acquisition of other samples.

3.2.2 arrangement of the substrate layers

The main problem with this method was the instability of the deposited gold nanoparticles because the liquid sample resolved and washed up the substrate from the smooth glass slide's surface. In the case of drying the mixture of the gold nanoparticles and sample, quantitative measurements were no longer possible, so the performed layer had to be stabilized somehow.

Among the aryl thiolate categories, the compound "2- Mercapto benzimidazole" was selected, but it did not have desired stability. Among the dithiols group, the "1, 3-Propanedithiol" was tested on the glass slide, which due to its hydrophobic properties, after adding to the substrate, it could not dry to create the necessary adhesion and consistency, so it was not easy to place water-soluble samples on the substrat. In addition, a very unpleasant smell limited its use in laboratories. On the other hand, another sulfurous compound from the alkyl thiolates group, "1-Dodecanthiol ", with polar and non-polar properties, was a suitable choice. Generally, in alkyl thiols, by prolonging the alkyl chain and increasing the hydrophobic property, the material becomes oily and vice versa. This compound was used to fix nanoparticles, and after a few days of exposure to the air, it was turned into a semi-dried form and stabilized the layer completely.

Different arrangements were tested using four categories of materials including the gold nanoparticles, thiol compounds, silica (as a support material), and solvents (toluene and ethanol) to create the substrate on the surface of the glass slide (Figure 6), which they could be classified into two groups:

- i) In the first group of methods, firstly, the thiol compounds were fixed on the glass slide, and then the gold nanoparticles were placed on it. In some methods, silica is used as a support (methods 1 to 6 in Figure 6).
- ii) In the second group of methods, the gold nanoparticles were first deposited on the glass slide, and then thiol compounds were applied to cover them as a top layer. In some methods, silica is used as the support (methods 7 to 12 in Figure 6).

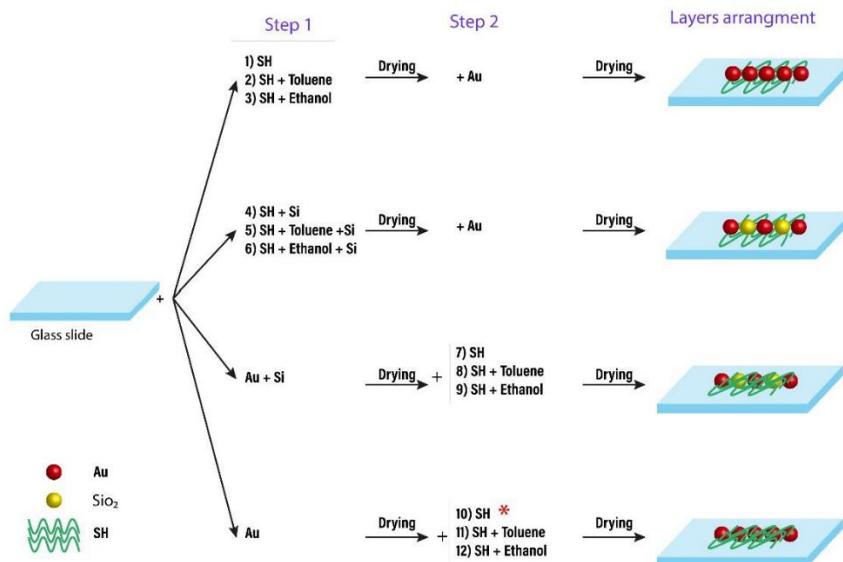


Fig. 6. Different methods were used to precipitate and stabilize the colloidal gold nanoparticles on the glass slides using silica and sulfur compounds. Method number 10 was selected as the optimal method.

Finally, method number 10 had the best performance and was selected as the best method to precipitate and stabilize the gold nanoparticles on the glass surface.

In the case of water-soluble samples, when the sample was placed on the substrate surface, the low hydrophobic property of the thiol compound made it behave like a liquid drop having a suitable surface tension with a wide contact angle (CA) that kept its spherical shape and didn't let the sample to flow. Thus it would be possible to adjust and focus the incident laser in the desired depth to get a good spectrum. Figure 7 shows the contact angle, under area, and the smooth edges of the sample droplet schematically.

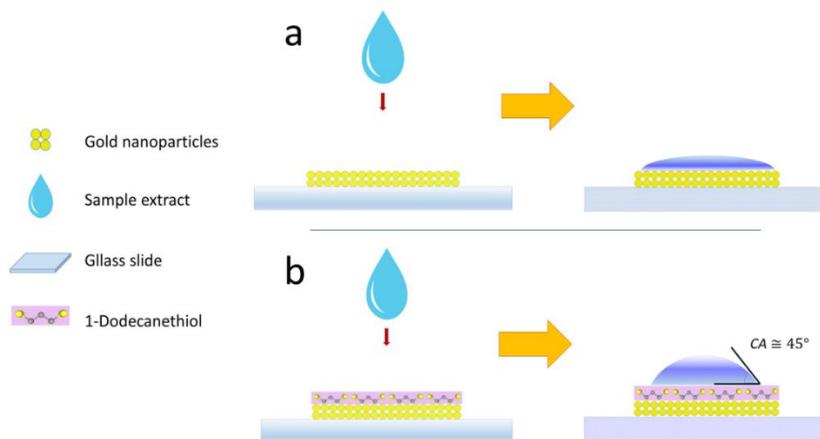


Fig. 7. The effects of using thiol stabilizer coating on the gold nanoparticles substrate include; a) the substrate containing a layer of deposited gold nanoparticles alone. After putting the drop on the substrate, the droplet spread on the surface without forming any significant contact angle. b) the substrate containing the gold nanoparticles stabilized by thiol compounds, the drop maintained its spherical shape leading to the formation of a detectable contact angle that facilitated spectrum acquisition.

The required numbers of pre-prepared glass slides were fabricated by employing the gold nanoparticle solution deposited and stabilized by the thiol compound on the glass surface (Figure 8), and the spectroscopy was performed by the SERS method.

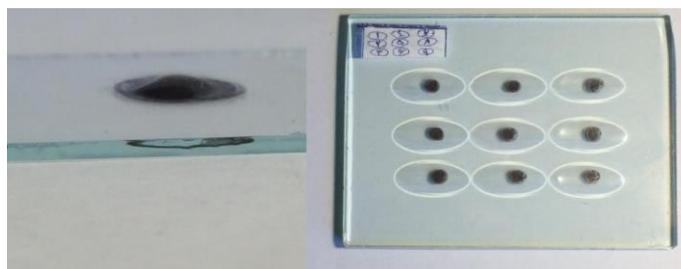


Fig. 8. A prefabricated SERS sensor

Table 1. Dimensions and colors of the colloidal gold nanoparticles in the different mixing ratios of solutions (I) and (II) by the Turkevich-Frans method

volume of solution I (ml)	Volume of solution II (ml)	Dimensions of nanoparticles (nm)	Color of Colloidal solution
50	1	16	Orange
50	0.75	24.5	Red
50	0.5	41	Red
50	0.4	50	Red to dark red
50	0.3**	71.5*	Red to dark red
50	0.21	97.5	Purple
50	0.16	147	Purple

* Approximately dimensions of the gold nanoparticles

** The required volume of solution II to prepare nanoparticles with a size of 70-80 nm

3.2.3. Optimization of the gold nanoparticles dimensions

In order to optimize the gold nanoparticles w in the dimensions of 50-80 nm in the Turkevich method, the proper mixing ratio of solutions (I) and (II) were determined (table 1), and the concentration of the colloidal gold nanoparticles solution was brought to 2.54 M (500 ppm for the gold nanoparticles). The DLS curve of the solution containing the colloidal gold nanoparticles was obtained and the average gold nanoparticles size was 78.5 nm (Figure 9).

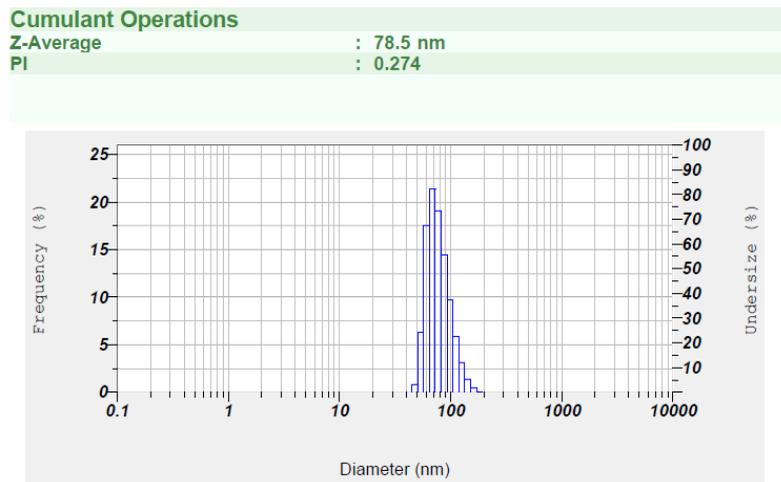


Fig. 9. The dynamic light scattering curve (DLS) of the monodisperse colloidal gold nanoparticles solution, the average size was 78.5 nm.

3.2.4. Calculation of the number of the gold nanoparticles

Based on the calculations, the weight of the gold nanoparticles in each circular spot on slides (with an average diameter of 8 mm) was equal to 1.10×10^{-2} mg /mm², which considering the approximate weight of each gold nanoparticle (with an average diameter of 80 nanometers) is 5.176×10^{-12} mg, the number of the gold nanoparticles in circular spots was equal to 2.125×10^9 Au NPs / mm².

3.2.5. SERS spectrum acquisition

For this purpose, a drop of florfenicol solution with a concentration of 1 gr/L was put on the substrate, and spectrum acquisition was done. As seen in Figure 10, the SERS spectrum quality is much better than the normal Raman spectrum (without using the gold nanoparticles), and the peaks had more numbers and greater intensity.

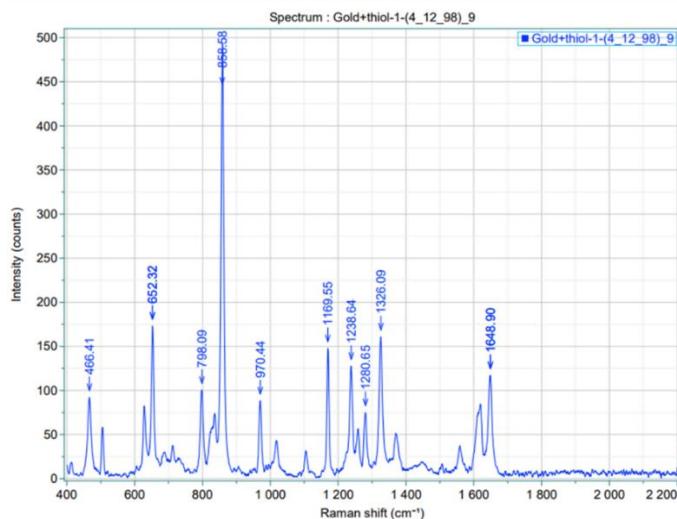


Fig. 10. The SERS spectrum of florfenicol solution with a concentration of 1000 mgr/L, using a substrate of the gold nanoparticles deposited on the glass slide and stabilized by thiol compound

3.2.6. Evaluation of the SERS substrate sensitivity.

In order to evaluate the fabricated substrates, the limit of detection (LOD) of FF was calculated and compared with its Maximum Residue Level (MRL) in chicken breast meat. The breast meats of organic chickens were extracted and spiked with different ratios of FF, and then their spectrums were collected by the SERS method. In spiked extract spectrums, FF index peaks (at 865, 1085, and $1175 \pm 3 \text{ cm}^{-1}$) were observed down to a concentration of 0.00025 mg/L (LOD = 0.25 $\mu\text{g/L}$) which was much lower than the permitted level of FF residue in chicken breast meat (MRL = 20 $\mu\text{g/L}$), showing an appropriate sensitivity of fabricated substrates to detect trace amounts of FF residues.

3.2.7. The SERS enhancement factor (EF) Calculation

The SERS Enhancement (EF) is determined as

$$EF = \frac{I_{SERS}}{I_{Normal}} * \frac{V_{Normal}}{V_{SERS}} * \frac{C_{Normal}}{C_{SERS}} \quad (1)$$

I_{SERS} and I_{Normal} are the peak intensities of FF-Au NPs and FF, respectively; V_{SERS} and V_{Normal} are probe volumes for the FF Au NPs and FF measurements, respectively; and C_{SERS} and C_{Normal} are FF concentrations contributing to SERS (I_{SERS}) and normal Raman intensity (I_{Normal}) respectively [36].

In normal Raman spectra of a 1% aqueous solution of FF, the intensity of the index peak at 1170 cm⁻¹ is about 2 a.u., which increases to 1560 a.u. in the SERS spectra. The increase in the intensity of the Au NPs can be considered as the reason.

$$(I_N)_{1170} = 2 \text{ (a. u.)}$$

$$(I_{SERS})_{1170} = 1560 \text{ (a. u.)}$$

The EF coefficient For the index peak at 1170 cm⁻¹ equals:

$$EF = \frac{I_{SERS}}{I_{Normal}} * \frac{V_{Normal}}{V_{SERS}} * \frac{C_{Normal}}{C_{SERS}} = \frac{1560}{2} * \frac{10}{10} * \frac{1}{1} = 780$$

The intensity of SERS signals increases up to 780 times.

3.2.8. Investigating the uniformity of substrates, similarity (or consistency) of the results, and reliability of experiments

The substrate used for SERS should be as uniform, rough, and fully coated on the glass as possible and free of empty spots. In this study, the residual amount of the florfenicol antibiotic in the chicken breast extract was investigated. Firstly, in order to investigate substrate uniformity in a single glass slide, the spectrums of different points of the glass slide (in the covered parts) were collected, and the intensity ratio of the index peaks (I_{1170} / I_{1365}) was calculated to perform spectroscopy (Figure 11), the (I_{1170}) is the intensity of index peak in the florfenicol solution (in the concentration of 1000 mg/L) as the

analyte, whereas the (I1365) is the intensity of index peak in the pure extract of chicken meat; and then the results were compared with each other (RSD = 8.44%).

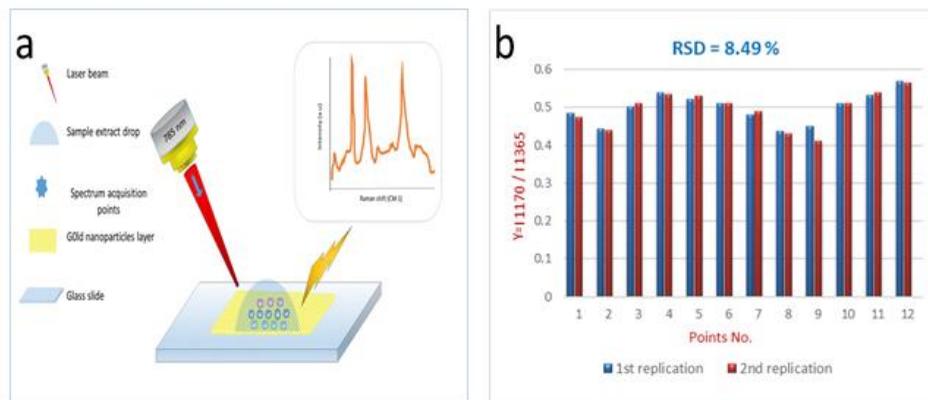


Fig. 11. a) Due to evaluate the uniformity of the substrate, the SERS spectrum acquisitions were performed at different points of a single slide. **b)** Comparison of the ratio of the florfenicol peak intensity to the extract peak intensity ($Y = I_{1170} / I_{1365}$) in the spectrums obtained from 12 points of the substrate of a single slide

Secondly, to check the similarity (or consistency) of the results on several different slides (Figure 12), the sample drops were placed on four slides separately, and their spectrums were obtained to compare the results (RSD = 7.90 %).

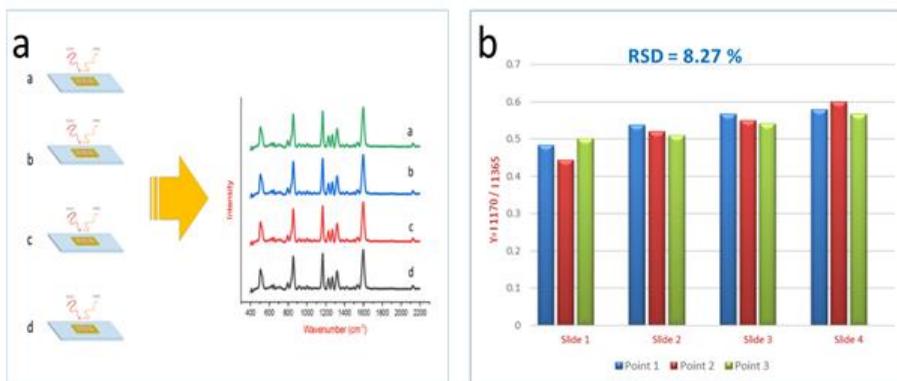


Fig. 12. a) Four pre-prepared SERS sensors were randomly selected, and their spectrums were plotted. **b)** statistical comparison showed acceptable similarity (or consistency) of the results in the slides.

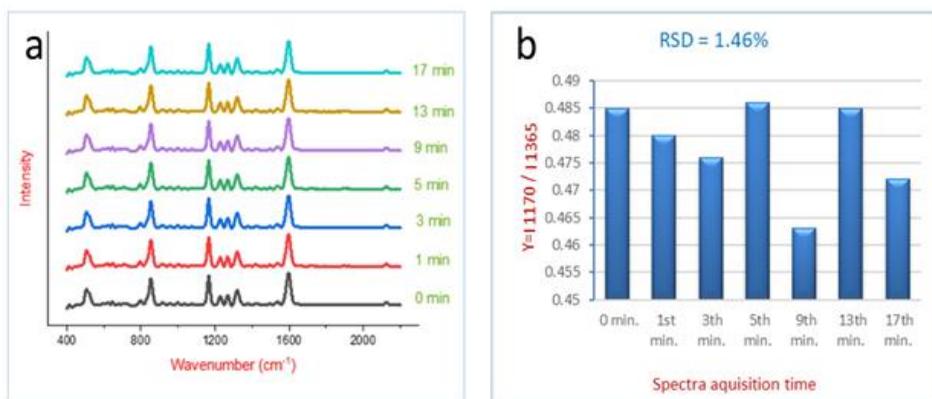


Fig. 13. a) The spectrums obtained at the time of sample placement, and also after 1, 3, 5, 9, 13, and 17 minutes on the slide. **b)** The statistical analysis showed acceptable reliability of the results.

Thirdly, to check the reliability of the results of the fabricated slide, the spectrum acquisitions were repeated at different times, and subsequently, the collected spectrums after 1, 3, 5, 9, 13, and 17 minutes were compared and analyzed statistically (Figure 13). The changes in the peak intensities ratio were calculated as a percentage showing an acceptable decrease/increase in the results (RSD = 1.26 %).

4. CONCLUSION

Today, nanotechnology has made significant advancements, leading to increased utilization of Raman spectroscopy and the SERS technique in various scientific fields. Raman spectroscopy involves the interaction of light with molecular vibrations, resulting in the scattering of photons. The physical principles of the SERS technique are based on enhancing the Raman signal by utilizing plasmonic nanostructures, such as gold or silver nanoparticles, which significantly amplify the Raman signal of molecules adsorbed on their surfaces. This enhancement is attributed to the excitation of localized surface plasmons, leading to an increased electric field at the molecule's vicinity. By exploiting this phenomenon, SERS enables highly sensitive and selective detection of analytes, making it a valuable tool for various applications, including chemical analysis and biomedical research.

These techniques are employed for qualitative and quantitative analyses of trace amounts of materials and for determining their molecular structures. The methods offer several advantages, including improved speed and accuracy of tests, the ability to analyze small sample sizes, and the potential use of portable devices. Prefabricated kits containing metal nanoparticles have a positive impact on experiment speed and ease, as well as reducing analysis time and cost. This study proposes using prepared kits that involve depositing gold nanoparticles on glass slides and stabilizing them with sulfur compounds. This method is advantageous due to the easy availability of necessary materials, cost-effectiveness, and simplicity in fabricating and using sensors as SERS substrates. Additionally, thiol compounds effectively stabilize the layers on slides, allowing for long-term storage of sensors and enabling multiple experiments to be conducted within a short period.

In this study, we investigated the use of depositing and stabilizing colloidal gold nanoparticles on the surface of a glass slide. The main objective was to create a cost-effective SERS sensor that is easily manufacturable and provides accurate and high-quality amplification of Raman signals using metal nanoparticles. Currently, the production of these sensors is monopolized by a few advanced countries, resulting in high prices that make the method uneconomical.

In our research, we first optimized the size of gold colloidal nanoparticles to be in the range of 50-80 nm and deposited them onto the glass slide surface. We tested various sulfur compounds to stabilize the gold nanoparticles on the glass surface, and found that the compound 1-dodecanethiol, which has both hydrophilic and hydrophobic properties, yielded the best results.

We used florfenicol as the analyte in our tests. This compound is a commonly used antibiotic in veterinary medicine, and its presence as residue in livestock and poultry meat, particularly chicken breast meat, poses health risks for consumers in Iran. By using pre-made sensors that amplify Raman signals in the SERS spectrum of florfenicol, we can quickly detect it at a low cost due to the affordability of the sensors. In contrast, conventional methods such as LC are expensive and time-consuming for antibiotic detection, while our method can be easily implemented in slaughterhouses and livestock and poultry supply centers due to its simplicity and speed.

Statistical tests conducted on the sensors, including uniformity of substrate surface, similarity of the results across different sensors, and similarity of the results over different time intervals, confirmed the optimal quality of the sensors. The deposition and stabilization method for gold nanoparticles on smooth surfaces can serve as a simple yet effective approach for fabricating pre-prepared sensors for SERS. This technique can be extended to other smooth surfaces as well. It is recommended to further enhance SERS quality through additional studies on different substrates, stabilizers, and their related parameters.

Conflict of interest

The authors declare no conflict of interest.

Ethics statement

This manuscript does not contain any studies with human participants, and all institutional and national guidelines for the care and use of laboratory animals were followed.

References

- [1] H. Häkkinen, *"The gold–sulfur interface at the nanoscale,"* Nature chemistry, 4 (2012) 443-455. Available: <https://doi.org/10.1038/nchem.1352>
- [2] R. L. Whetten and R. C. Price, *"Nano-golden order,"* Science, 318, (2007) 407-408. Available: <https://doi.org/10.1126/science.1150176>
- [3] Q. Wang, I. He, T. p. Labuza and B. Ismail, *"Structural characterisation of partially glycosylated whey protein as influenced by pH and heat using surface-enhanced Raman spectroscopy,"* Food Chem , 139 (2013) 313-319. Available: <https://doi.org/10.1016/j.foodchem.2012.12.050>
- [4] K. Kimm, J. W. Lee and K. S. Shin, *"Cyanide SERS as a platform for detection of volatile organic compounds and hazardous transition metal ions,"* Analyst, 138 (2013) 2988-2994. Available: <https://doi.org/10.1039/C3AN00105A>
- [5] L. He, N. J. Kimm, H. Li, Z. Hu and M. Lin, *"Use of a fractal-like gold nanostructure in surface-enhanced Raman spectroscopy for detection of selected food contaminants,"* Agic Foooc Chem , 56 (2008) 9843-9847. Available: <https://doi.org/10.1021/jf801969v>
- [6] H. Tang, D. Fang and Q. Li, *"Determination of tricyclazole content in paddy rice by surface enhanced Raman spectroscopy,"* Food Sci, 77 (2012) T105-T109. Available: <https://doi.org/10.1111/j.1750-3841.2012.02665.x>
- [7] J. Kneipp, H. Kneipp and K. kneipp, *"SERS—a single-molecule and nanoscale tool for bioanalytics,"* Chem Soc Rev, (2008) 649-662. Available: <https://doi.org/10.1039/B708459P>
- [8] M. Lin, *"The application of surface-enhanced Raman spectroscopy to identify and quantify chemical adulterants or contaminants in foods,"* Handbook of vibrational spectroscopy, New York, Jhon Wiely & Sons, 12

- (2020) 649-662. Available: <https://doi.org/10.1002/0470027320.s8965>
- [9] E. L. Ru, J. E. Blackie, M. Meyer and P. G. Etchegoin, "Surface enhanced Raman scattering enhancement factors. a comprehensive study," *Journal of Physical Chemistry C*, 111 (2007) 13794-13803. Available: <https://doi.org/10.1021/jp0687908>
- [10] W. Kim, S. Lee, J. Kim, Y. Ahn, Y. Kim, J. Yu and S. Choi, "Paper-Based Surface-Enhanced Raman Spectroscopy for Prenatal Diseases in Women," *ACS Nano*, 12 (2018) 7100-7108. Available: <https://doi.org/10.1021/acsnano.8b02917>
- [11] W. Wei, Y. Du, L. Zhang, Y. Yang and Y. Gao, "Improving SERS hot spots for on-site pesticide detection by combining silver nanoparticles with nanowires," *Mater. Chem.*, 6 (2018) 8793-8803. Available: <https://doi.org/10.1039/C8TC01741G>
- [12] K. Willets and R. Duyn, "Localized Surface Plasmon Resonance Spectroscopy and Sensing," *Annu. Rev. Phys.*, 58 (2007) 267-297. Available: <https://doi.org/10.1146/annurev.physchem.58.032806.104607>
- [13] D. Cialla-May, X. Zheng, K. Weber and J. Popp, "Recent progress in surface-enhanced Raman spectroscopy for biological and biomedical applications: From cells to clinics," *Chem. Soc. Rev.*, 46 (2017) 3945-3961. Available: <https://doi.org/10.1039/C7CS00172J>
- [14] H. Lai, F. Xu, Y. Zhang and L. Wang, "Recent progress on graphene-based substrates for surface-enhanced Raman scattering applications," *Mater. Chem. B*, 6 (2018) 4008-4028. Available: <https://doi.org/10.1039/C8TB00902C>
- [15] D. Zhang, H. Pu, L. Huang and D. W. Sun, "Advances in flexible surface-enhanced Raman scattering (SERS) substrates for nondestructive food detection: fundamentals and recent applications," *Trend in Food Science & Technology*, 109 (2021) 690-701. Available:

<https://doi.org/10.1016/j.tifs.2021.01.058>

- [16] Q. A. Jing, C. Xing, A. Yg, L. A. Rui and A. Al, "Gold nanostars-enhanced Raman fingerprint strip for rapid detection of trace tetracycline in water samples," *Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy*, 232 (2020) 118146. Available: <https://doi.org/10.1016/j.saa.2020.118146>
- [17] M. Tang, P. Zheng, Y. Wu and et al., "Silver dendrites based electrically conductive composites, towards the application of stretchable conductors," *Composites Communications*, 19 (2020) 121-126. Available: <https://doi.org/10.1016/j.coco.2020.03.010>
- [18] M. Ma, J. Sun and Y. Chen, "Highly sensitive SERS immunosensor for the detection of amantadine in chicken based on flower-like gold nanoparticles and magnetic bead separation," *Food and Chemical Toxicology*, 118 (2018) 589-594. Available: <https://doi.org/10.1016/j.fct.2018.06.013>
- [19] H. Wu, Y. Lou, C. Hou and et al., "Flexible bipyramid-AuNPs based SERS tape sensing strategy for detecting methylparathion on vegetable and fruit surface," *Sensors and Actuators B: Chemical*, (2019) 123-128. Available: <https://doi.org/10.1016/j.snb.2019.01.038>
- [20] K. B. Narayanan and N. Sakthivel, "Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents," *Adv. Colloid. Interface. Sci.*, 169 (2011) 59-79. Available: <https://doi.org/10.1016/j.cis.2011.08.004>
- [21] A. Sunday, C. Seyifunmi and S. Aderonke, "A review on synthesis, optimization, characterization and antibacterial application of gold nanoparticles synthesized from plants," *International Nano Letters*. 10 (2020) 237-248. Available: <https://doi.org/10.1007/s40089-020-00317-7>
- [22] S. Lin, X. Lin, X. Song, S. Han, L. Wang and L. Hasi, "Lab- on- paper surface- enhanced Raman spectroscopy platform based on self- assembled Au@Ag nanocube monolayer for on- site detection of thiram in soil,"

- Raman Spectrosc., 50 (7) (2019) 916-925. Available: <https://doi.org/10.1002/jrs.5595>
- [23] M. Park, H. Jung, Y. Jeong and K. H. Jeong, "Plasmonic Schirmer Strip for Human Tear-Based Gouty Arthritis Diagnosis Using Surface-Enhanced Raman Scattering," ACS Nano, 11 (1) (2017) 438-443. Available: <https://doi.org/10.1021/acsnano.6b06196>
- [24] C. Wang, B. Liu and X. Dou, "Silver nanotriangles-loaded filter paper for ultrasensitive SERS detection application benefited by interspacing of sharp edges," Sens. Actuators, B, 231 (2016) 357-364. Available: <https://doi.org/10.1016/j.snb.2016.03.030>
- [25] Y. Xu, P. Man, T. H. Ning, C. Li, B. Man and C. Yang, "Synthesis of the 3D AgNF/AgNP arrays for the paper-based surface enhancement Raman scattering application," Sens. Actuators, B., 265 (2018) 302-309. Available: <https://doi.org/10.1016/j.snb.2018.03.035>
- [26] C. M. Wang, P. K. Roy, B. K. Juluri and s. Chattopadhyay, "A SERS tattoo for in situ, ex situ, and multiplexed detection of toxic food additives," Sens. Actuators, B., 251 (2018) 218-225. Available: <https://doi.org/10.1016/j.snb.2018.01.146>
- [27] J. Bosse, Y. Li, T. Atherton, S. Walker, S. Williamson, J. Rojers and e. al., "Characterisation of a mobilisable plasmid conferring florfenicol and chloramphenicol resistance in Actinobacillus pleuropneumoniae," Vet Microbiol., 178 (3-4) (2015) 279-82. Available: <https://doi.org/10.1016/j.vetmic.2015.05.020>
- [28] F. Kashanian, M. S. Hoseinian, S. Khoshnevis and M. S. Hoseinian, "Gold Nanoparticles," 1st ed., Jahad daneshgahi Tehran, (2015) 132-135. ISBN: [978-600-133-201-2](https://doi.org/10.1016/j.snb.2018.01.146).
- [29] M. Koupaei, B. Shareghi, A. Saboury, F. Davar, A. Semnani and M. Evini, "Green synthesis of zinc oxide nanoparticles and their effect on the stability and activity of proteinase," K. RSC. Adv, 6 (48) (2016) 42313-42323.

Available: <https://doi.org/10.1039/C5RA24862K>

- [30] S. A. Akintelu and A. S. Florunso, "*Characterization and antimicrobial investigation of synthesized silver nanoparticles from Annona muricata leaf extracts*," *Nanotechnol. Nanomed. Nanobiotechnol.*, (2019). Available: <https://doi.org/10.24966/NTMB-2044/100022>
- [31] J. Paques, C. Vander, R. Van and L. Sagis, "*Preparation methods of alginate nanoparticles*," *Adv. Colloid. Interface Sci.*, 209 (2014) 163-171. Available: <https://doi.org/10.1016/j.cis.2014.03.009>
- [32] A. Umer, "*A green method for the synthesis of copper nanoparticles using L-ascorbic acid*," *Material. Rio Jan.*, 3 (2014) 197-203. Available: <https://doi.org/10.1590/S1517-70762014000300002>
- [33] A. Folorunso, S. Akintelu, A. Oyebamiji, S. Ajayi, A. Babawaie, H. Abdusalam and A. Morakinyo, "*Biosynthesis, characterization and antimicrobial activity of gold nanoparticles from leaf extracts of Annona muricata*," *Nanostruct. Chem.*, 9 (2019) 111-117. Available: <https://doi.org/10.1007/s40097-019-0301-1>
- [34] X. Kaichen, Z. Rui and T. Kuniharu, "*Toward Flexible Surface- Enhanced Raman Scattering (SERS) Sensors for Point-of-Care Diagnostics*," *Advanced Science*, 6 (16) 2019. Available: <https://doi.org/10.1002/advs.201900925>
- [35] M. C. Daniel and D. Astuc, "*Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology*," *Chemical reviews*, 104 (1) (2004) 293-346. Available: <https://doi.org/10.1021/cr030698+>
- [36] E. Leru, C. E. Blackie, M. Meyer and P. G. Etchegoin, "*Surface enhanced Raman scattering enhancement factors: a comprehensive study*," *physical chemistry*, 111 (37) (2007) 13794-13803. Available: <https://doi.org/10.1021/jp0687908>