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Green synthesis of biologically benign SERS active gold nanoparticles

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ABSTRACT:

Recently, the focus of research has shifted to the creation of environmentally acceptable processes for the synthesis of metallic nanostructures. A surface-sensitive approach called surface-enhanced Raman scattering (SERS) increases Raman scattering by molecules adsorbed on rough metal surfaces. The development of biosensors and biocatalysts is known to be greatly influenced by the SERS properties of metal nanoparticles, particularly gold and silver nanoparticles. Here, we report the biosynthesis of gold nanoparticles with good SERS capabilities utilising the sweet orange (Citrus Sinensis) extract as a target molecule, enhancing Raman signals up to several times. By simply heating the mixture of tetrachloroauric acid solution ($HAuCL_4$) and orange extract at 80 ^{0}C for one hour, spherical mono-dispersed AuNPs with a very wide size range (5 - 50 nm) were produced. The characteristic plasmon resonance peak, morphological and aggregate characteristics, elementary composition, and other parameters were detected using the ultraviolet-visible (UV-vis) spectrophotometer, transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDS), x-ray diffraction analysis (XRD), and x-ray photoelectron spectroscopy (XPS). To identify the elements that contribute to the reduction and stability of AuNPs, the fourier transform infrared (FT-IR) spectrophotometer was used to assess the main functional groups in the extract. In order to determine the toxicity of the produced AuNPs, a hemolysis test for their aqueous solution was carried out. The results showed a low hemolysis percentage (less than 1 percent), which denotes their non-toxic nature. These "green" produced gold nanoparticles may find use in biological sensing and other biological applications, according to the data given here, because of their great biocompatibility.

KEYWORDS: Gold nanoparticles, green synthesis, SERS, hemolysis

INTRODUCTION:

Due to their uses in chemistry, electronics, medicine, and the pharmaceutical industry, metal nanoparticles can be regarded as one of the most adaptable forms of nanoparticles (1,2). Due to their biocompatibility, adjustable optical characteristics, and easily modifying surface chemistry, gold nanoparticles (AuNPs) stand out among the others (3,4). AuNPs are frequently used as medication and molecular carriers to enhance illness diagnostics and treatment because of their distinct physical-chemical features (5,6). However, the well-established synthetic methods typically employ poisonous materials and non-polar solvents, which have a negative impact on the environment and necessitate multiple stages of product purification, making the procedure expensive (7). Several biosynthetic routes have been suggested (8–10) for circumventing the difficulties associated with conventional techniques. Given that it uses non-toxic solvents like water, biosynthesis is thought of as a straightforward, inexpensive, and environmentally beneficial method (11). Furthermore, because the phytochemical substances used in the reaction also serve as stabilisers, biogenic synthesis yields greater quantities of very stable nanoparticles with better-defined sizes than some conventional approaches (12,13). Natural sources have already been used to produce metallic nanoparticles, indicating a potential synthetic pathway that needs to be investigated (14–17).

A factor of 10^6 or more can be added by surface-enhanced Raman spectroscopy (SERS) to the inelastic light scattering events (18). This improvement enables the development of exceedingly sensitive technologies for the quantification and identification of molecular biomarkers and tiny compounds. Through the use of signal augmentation techniques, SERS

kept attracting attention. Electromagnetic and chemical enhancement are the two mechanisms underlying the increased inelastic light scattering observed in SERS. The primary effect is electromagnetic amplification, which is caused by localised surface plasmon resonance (LSPR). LSPR is an optically excited electron wave resonance state on the surface of a nanostructure, which can increase both elastic (Rayleigh) and inelastic (Raman) light scattering from the sample (19). The molecular charge-transfer interactions between the molecule and the metallic surface are what lead to the chemical enhancement process (20, 21). When both techniques of improvement are combined, the Raman intensity is raised to the point where SERS can be employed for tasks requiring higher molecular sensitivity. Because of its ability to recognise fingerprints, high sensitivity, multiplex detection, and biocompatibility, SERS has emerged as a potent tool for biosensing applications. Surface-enhanced Raman scattering (SERS) is a useful method for examining surface/interfacial properties, and recent years have seen a significant increase in research into the interactions of biomolecules with metal surfaces (22). For instance, it has been demonstrated that the SERS properties of gold nanoparticles (NPs) in the detection of oral cancer cells were excellent (23).

The usage of sweet orange (Citrus Sinensis) extract in the biosynthesis of gold nanoparticles (AuNPs) using an inexpensive and environmentally acceptable approach has been investigated in this work. In order to investigate AuNPs' potential application in surface-enhanced Raman scattering (SERS). The Rhodamine 6G (R6G) was used as a target molecule, and the results clearly demonstrated that they had good SERS capabilities, enhancing Raman signals by up to several times. This study also assessed the toxicity of these nanomaterials to healthy blood cells and their structural characteristics. Hemolysis studies were performed to examine the toxicity of AuNPs. The sample had a modest

EXPERIMENTAL:

producing fruit extract

A medium sized orange was squeezed in a 250 ml beaker to obtain 61.5 g sweet orange juice (Citrus Sinensis), which was finally extracted with 20 ml deionised water and filtered through a sintered funnel. Within ten days, the juice was consumed after being kept at 4 °C.

Gold nanoparticle synthesis (AuNPs)

Chloroauric acid ($HAuCl_4$) (Sigma-Aldrich) was utilised to make gold nanoparticles. To 2 ml of orange extract, a 1 ml aqueous solution of $HAuCl_4$ 0.001 M was added. For one hour, the mixture was heated at 80 °C. 5 ml of deionized water was added once the mixture had been brought to room temperature, and it was then aged for almost 24 hours. The created gold nanoparticles were then disseminated in 10 ml of deionized water using an ultrasonic bath after being separated by centrifugation at a speed of 15000 rpm for 30 minutes. The particles were then evaluated using a variety of analysis techniques, such as UV-Vis, TEM, FT-IR, SERS, XRD, and XPS.

UV-Visible Analysis

A Perkin Elmer model UV-vis double beam spectrophotometer was used to analyse the qualitative measurement of produced nanoparticles from 300 to 800 nm with a 1 nm resolution. The light absorption band of a solution in the visible and nearby ranges was found using a UV-Visible spectrophotometer. It was specifically utilised to assess the gold nanoparticles' surface plasmon resonance (SPR) band, which aids in characterising the particles' absorption band. Before scanning the samples, it was necessary to run the corresponding baseline.

Transmission electron emission spectroscopy

A drop of the nanoparticle solution was placed on copper grids covered with carbon, and a sample for transmission electron microscopy (TEM) investigation was created by letting the solvent evaporate. A Tecnai F20 (Philips Electron Optics) device running at 100 kV was used to qualitatively characterise the nanoparticles. Selected area electron diffraction (SAED) patterns were also studied using TEM.

Analysis of Fourier Transform Infrared Spectroscopy

Investigating the vibration of bonding in ligand molecules conjugated to the surface of the created gold nanoparticles was done using Fourier Transform Infrared Spectroscopy (FT-IR). Nicolet iS10 FT-IR machine was used. In FT-IR analysis KBr plate was used as back ground. The sample was mixed with ground spectrometry grade grade KBr with the ratio of sample to KBr between 1:100 and 1:50.

Analysis of X-ray diffraction

Utilizing X-ray diffraction, the crystallinity of artificially created gold nanoparticles was assessed (XRD). The range of the X-ray diffractogram was 2θ from 30 to 75^0. XRD samples were prepared by dropping gold nanoparticles aqueous

solution on the 1 cm X 1 cm Si wafer. X'Pert PRO, PAaNalytical XRD machine was used with X-rays tension 45 kV, X-rays current 40 mA Ω 0.5^0.

Photoelectron spectroscopy with X-rays

In order to investigate the chemical and electronic states of the elements that are present in the material, X-ray photoelectron spectroscopy analysis (XPS) was performed. The aqueous solution containing gold nanoparticles was applied to a silicon wafer to create the XPS samples.Sigma Theta probe, VG ESCA Scientific XPS machine was used with X-ray source monochromatic A1Kα (1486.7 eV).

Surface Enhanced Raman scattering analysis

The Raman boosting capability of gold nanoparticles was assessed using surface enhanced Raman scattering analysis (SERS). The silicon wafer was cleaned, a drop of aqueous gold nanoparticle solution was put on it, and it was then dried under vacuum to create the SERS samples. A drop of a 0.1 mM solution in ethanol was employed as the probe molecule, which was Rhodamine 6G (R6G). In Raman scattering (SERS), a Kaiser machine laser with a wavelength of 785 nm, 131 mW of power, and a 3-second exposure duration was employed.

Hemolysis Test

To investigate the toxicity of AuNPs obtained by using orange fruit extract, hemolysis experiments were carried out. Firstly, the erythrocytes were collected from blood by centrifugation at 2000 rcf (relative centrifugal force) for 5 minutes followed by three times wash with PBS (phosphate buffer saline). Experimental matrix was then prepared as indicated in the table 1. The sample was heated to 37 ^{0}C and gently stirred at 40 rpm for 1 hour. They were then centrifuged at 2000 rcf for 5 minutes to obtain the supernatants, and the absorbance at 415 nm was used to measure them.

RESULT AND DISCUSSION:

The morphological and aggregate characteristics, elementary composition, and other parameters of the gold nanoparticles produced by sweet orange (Citrus Sinensis) extract were detected using the ultraviolet-visible (UV-vis) spectrophotometer, transmission electron microscopy (TEM), and energy-dispersive spectroscopy (EDS).

The inset of Figure 1 (b), which is Figure 1, contains images of a gold nanoparticle sample vial that corresponded to the peak created by gold nanoparticle solutions (b). The creation of gold nanoparticles was indicated by the purple colour. This colour developed as a result of the metal nanoparticles' surface plasmon oscillations being excited. In UV spectroscopy the surface Plasmon peak (λmax value) appeared at 565 nm for the Au nanoparticles. It is presumable that the orange-rich citrate components were the main reducing agents in the conversion of Au^{3+} ions to Au^0.

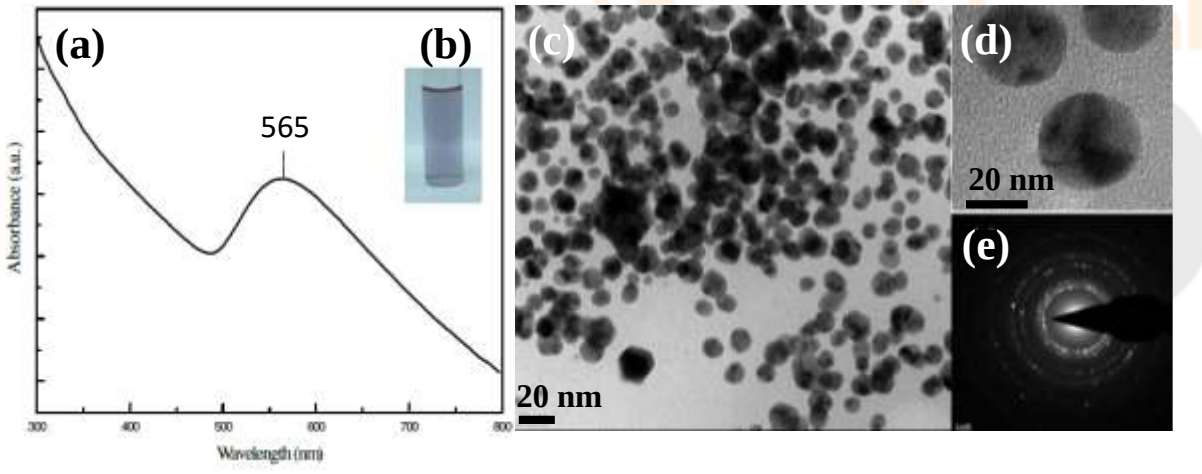

FIGURE 1. (a) The UV-visible spectrum of AuNPs produced in water with orange extract is obtained. (b) Picture of sample bottle containing aqueous dispersion of AuNPs has been shown in the in-set (c) TEM image of AuNPs synthesized using orange extract (d) HRTEM image and (e) selected area diffraction (SAED) pattern

It is obvious from the TEM image (Figure 1c) that the sweet orange was used to generate the monodispersed gold nanoparticles. The spherical nature of the AuNPs generated was clearly seen in the HR-TEM image (Figure 1d). The AuNPs' polycrystalline nature was revealed by the SAED pattern. X-ray diffraction (XRD) research was used to confirm that AuNPs are crystals. Figure 2 depicts the XRD pattern of AuNPs produced using orange extract. The facets with the numbers (111), (200), and (220) are represented by the diffraction peaks at 38.2^0, 44.4^0, and 64.6^0. Compared to the other peaks, the peak for the (111) plane was more powerful.

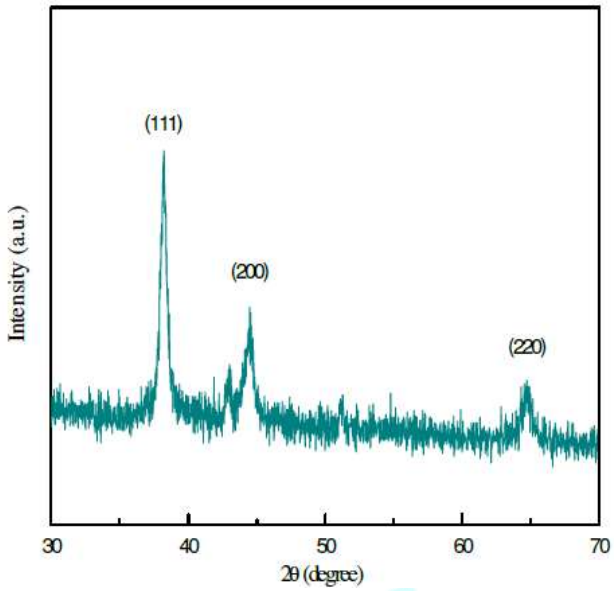

FIGURE 2. XRD pattern of AuNPs synthesized by orange extract

The fairly monodispersity of AuNPs obtained using orange extract indicated that capping molecules from the extract were bound on the nanoparticle surface. This is exhibited in Fourier transform infra-red (FT-IR) spectrum (Figure 3) in the regions of wave number of 1000-2000 cm^{-1}. The broad peak at 3409 cm-1, in particular, corresponded to the stretching frequency of the -O-H (found in -COOH), and the peak around 1731 cm^{-1}, to the C=O vibration for the carbonyl group in the ester of carboxylic acid. C=C stretching vibration had a peak at roughly 1653 cm^{-1}. The presence of the C-O band at 1062 cm^{-1}, which results from C-O-C symmetric stretching and C-O-H bending vibrations, was also demonstrated by the FTIR spectra.

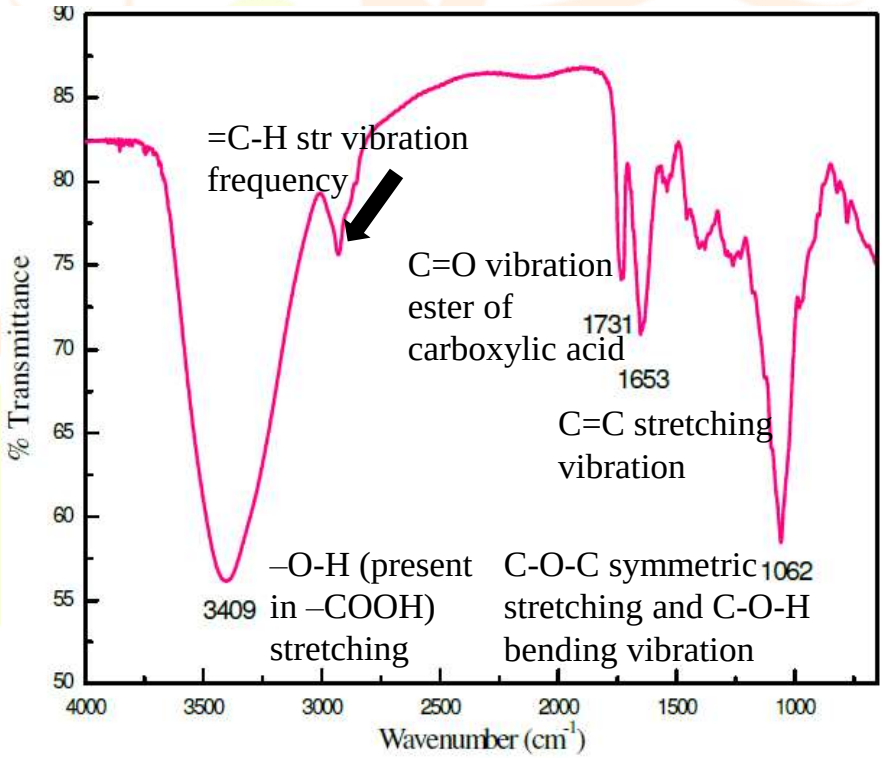

FIGURE 3. Fourier transform infra-red spectrum of AuNPs produced by orange extract

The existence of the capping molecules is further supported by X-ray photoelectron spectroscopic (XPS) analysis. Three main peaks of C1s were observed at 284.64 eV, 286.17 eV and 288.07 eV the binding energy of aliphatic or aromatic carbon, C-OH and COOH/O=C-N, respectively (Figure 4). The presence of AuNPs can also be confirmed through the Au4f peaks at the binding energy of 84.51 eV (4f7/2) and 88.18 eV (4f5/2) (Figure 4).

Raman scattering is able to identify many compounds because of their distinctive spectra. In Raman scattering, the incident light is dispersed on the molecules' rotational and vibrational states. Because of the inelastic nature of the scattering process, dispersed light might have a different energy from incident light. Complex molecules can be recognised because the energy shift is distinctive for the chemical structure where the scattering took place. If the molecules are adjacent to a gold surface with a high degree of curvature, as in small AuNPs, the amount of Raman

scattering is significantly increased. Surface-enhanced Raman scattering is the name given to this phenomenon (SERS). The concentration, shape, and aggregation state of the AuNPs as well as other variables affect how much the Raman signal is enhanced. The peaks corresponding to the R6G at 613, 770, 1311, 1362, 1507, and 1649 cm^{-1} were found in the SERS of AuNPs made from orange extract (Figure 5). The improvement may be related to the aggregation of nanoparticles across a vast region through Plasmon coupling. This is accurate in the case of AuNPs made from orange extract, which have the aggregated or collective particles shape seen in the preceding TEM image (Figure 1). The green source for the synthesis of tiny AuNPs was strengthened as a consequence of Surface Enhanced Raman Scattering (SERS) research. The initial studies of Raman signal amplification demonstrate their potential for use as a biosensor in biological applications (24–26).

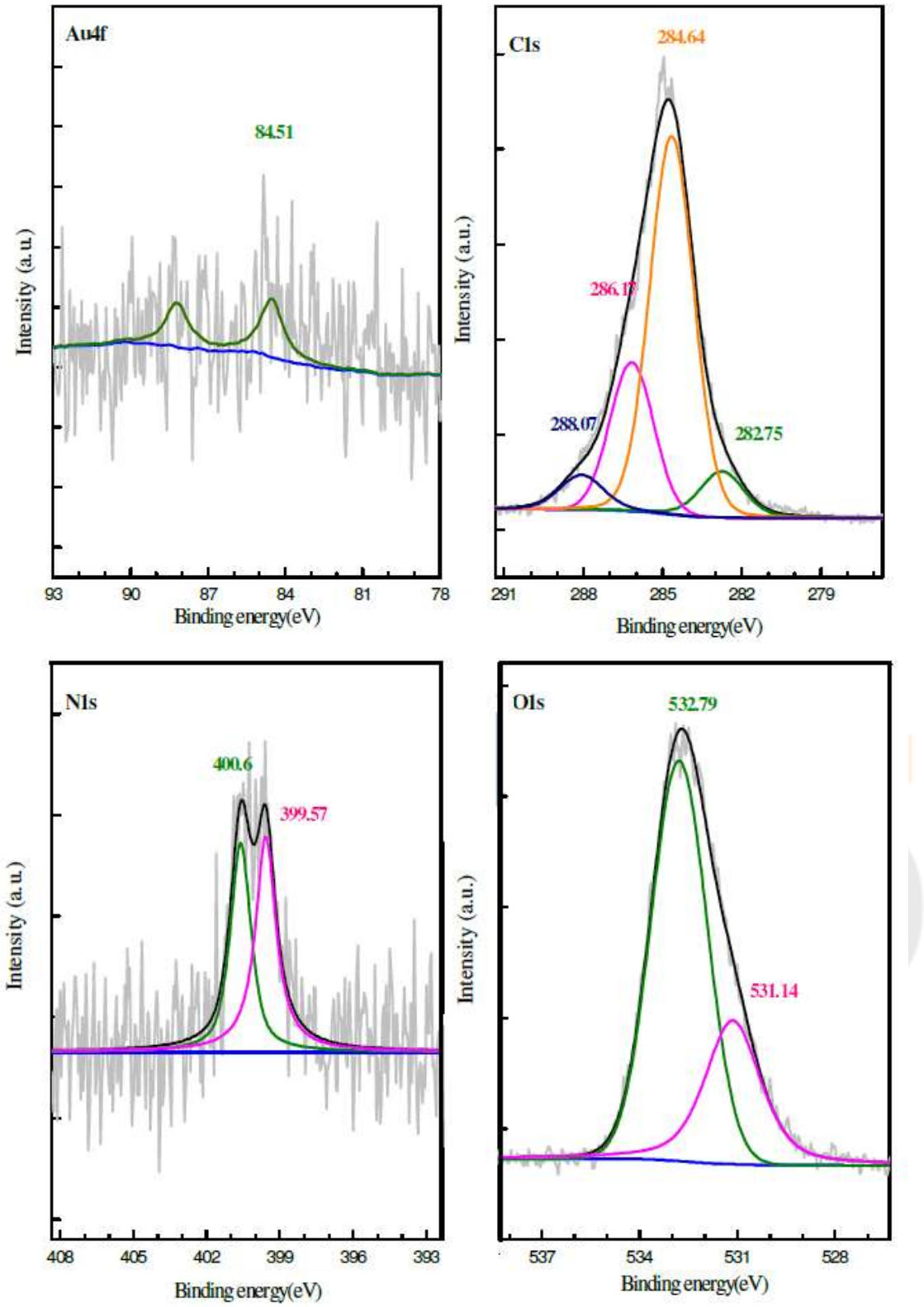

FIGURE 4. Au4f, C1s N1s and O1s XPS spectra for AuNPs synthesized by orange extract

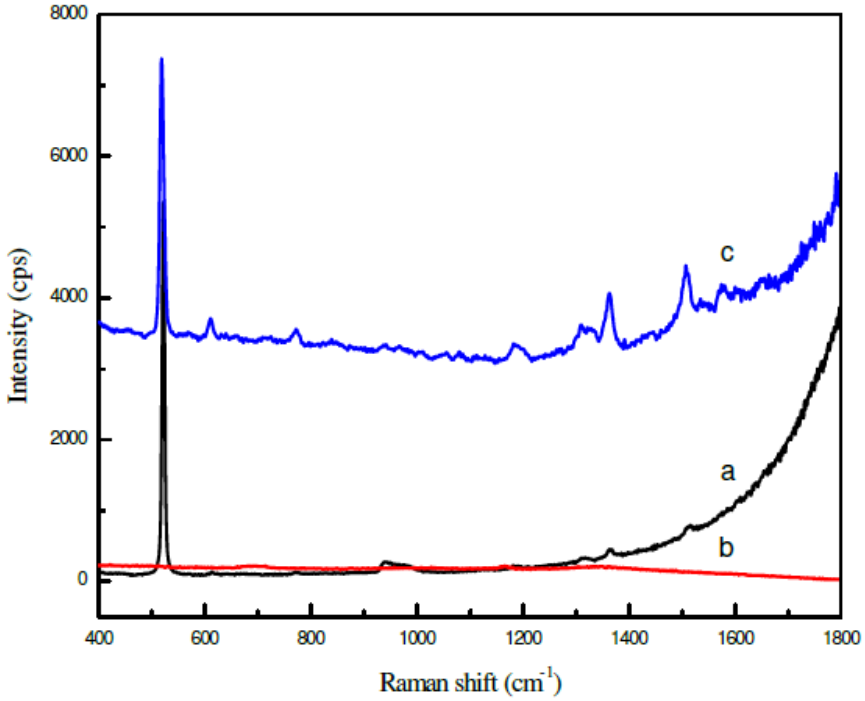

FIGURE 5. (a) Au thin film, (b) bare silicon, and (c) Au film of AuNPs manufactured using orange extract on silicon: SERS spectra of R6G obtained from substrates prepared by dropping 0.1 mM solution in ethanol.

Hemolysis is the breakdown of red blood cells, or erythrocytes, which causes the release of haemoglobin into the blood plasma. Anaemia is brought on by hemolysis and can endanger human life. That means, the higher percentage of hemolysis a matter gives, the more toxic it is considered to be. It is stipulated that if the hemolysis percentage is higher than 15%, the matter is toxic. Experimental procedure for hemolysis test has been described pictorially in Figure 6. The results of the hemolysis test with the titer 1:100 for the aqueous solution of AuNPs synhesized using orange extracts were executed. The result was shown in the following Figure 7 and in Table 1. It was clear that the sample had a low hemolysis percentage (less than 1%), which indicates their non-toxic property [27, 28]. With these results, the possibility for AuNPs obtained from orange extracts to be used in biological applications due to their high biocompatibility became enhanced. These results also consolidated our idea and objectives in using biological approach to synthesize AuNPs.

TABLE 1. Experimental matrix for hemolysis test

	PBS (ml)	Sample (µl)	Erythrocytes (µl)
Sample	2.45	50	100
Blank	2.55	50	X
100% Erythrocytes hemolysis	H_2O	x	100

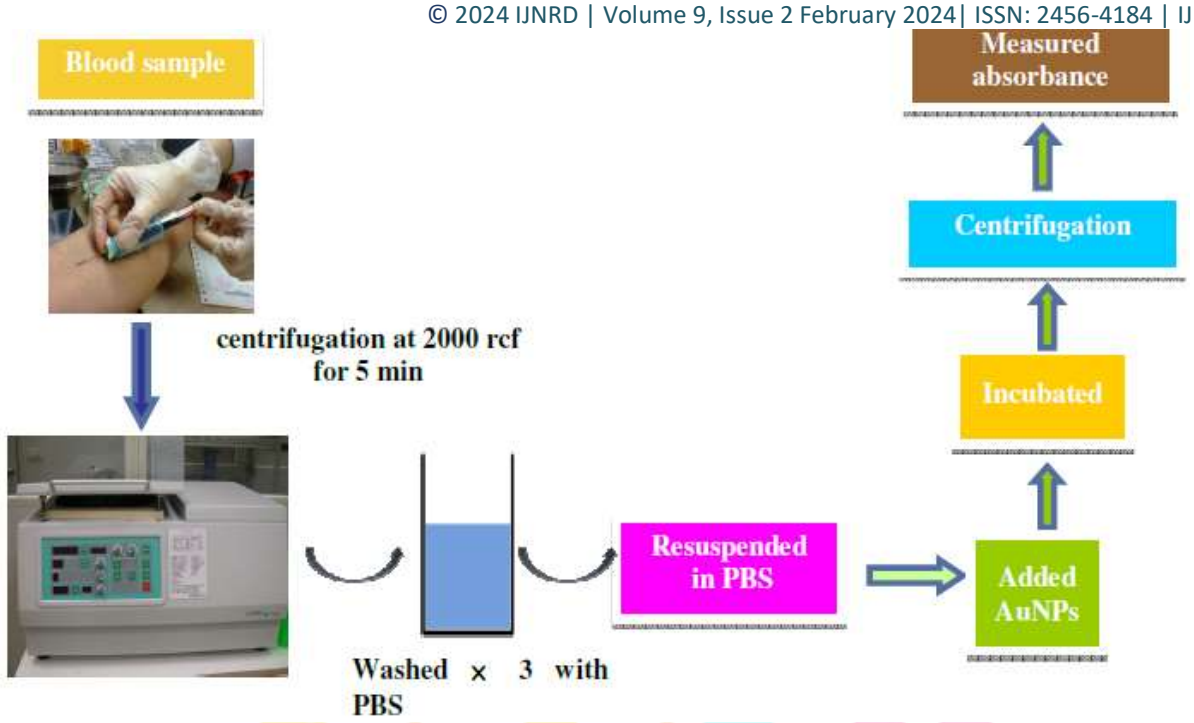

FIGURE 6. Experimental procedure for hemolysis test

AuNPs synthesized from orange extract

FIGURE 7. Hemolysis test for aqueous solution of AuNPs synthesized using orange fruit extract

CONCLUSION:

Using Rhodamine 6G (R6G) as a target molecule, it has been demonstrated in this study that biosynthesized gold nanoparticles exhibit good SERS properties, offering encouraging results and indicating that these greenly synthesised gold nanoparticles may have potential applications in sensor technology. Additionally, the sample's low hemolysis percentage (less than 1%), which supported their non-toxic nature, was clearly shown by the data. These "green" produced gold nanoparticles may be useful for additional biological applications and biological sensing, according to the results of this study.

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