

Evaluation of Gold and Silver Nano Spherical Efficiency for Enhancing Raman Scattering of Explosives

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Abstract

Surface-enhanced Raman spectroscopy (SERS) is sensitive analytical technique for detection of organic molecules. A wide range of nanostructure substrates were used as SERS substrate for explosive detection that gave various efficiencies. In this work, gold and silver nanoparticles (spheres) were used to evaluate the enhancement of Raman signals of the most widely used explosives 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitroperhydro-1,3,5-triazine (RDX) and Pentaerythritol tetranitrate (PETN). SERS studies were carried out using three concentrations of explosives 10-5, 10-7 and 10-9 M to determine the limit of detection. The enhancement factor was estimated as ≈ 104 for 10-5 M samples and ≈ 106 for 10-7 M samples.

Keywords: Surface-enhanced Raman spectroscopy; Gold and Silver nanoparticles; Explosives, Enhancement factor; TNT; RDX; PETN

Introduction

Nanotechnology has become a very important segment of science and research. Today, it is applied in medicine, industrial productions and in catalytic processes. Different properties of nanomaterials including their shapes, sizes and chemical properties are very crucial for future applications [1]. Gold and silver nanoparticles are metallic and there are different ways through which they can be synthesized. The methods of synthesis are either based on physical or chemical formulations. The most commonly used chemical methods include the use of metallic salts, micro emulsion process, thermal decompositions, and alcohol reduction procedures among others [2]. On the other hand, physical methods of synthesis include plasma, microwave irradiation, and laser ablation among others.

Surface Enhanced Raman Spectroscopy (SERS) is a method used to enhance the magnitude of Raman intensity to detect explosives and other targeted molecules. SERS of gold and silver nanoparticles investigates different properties of the

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explosives including their shapes, sizes and chemical properties [3]. Previous studies have proven that both Au and Ag nanoparticles have great SERS properties which make them appropriate for use as substrates for explosives [4-5]. A wide range of SERS methodology were reported for characterization and identification of explosives with various detection qualities and detection sensitivity [4, 6-8]. Overall, Au and Ag nano-scale structures provide a strong signal for SERS enhancement among other substrates, and they are stable and less overlapped signals with any analyte signals [9].

In this work, SERS measurements of explosives were conducted using traditional Au and Ag nanoparticles (spheres) as substrates. These experiments were carried out to examine the efficiency of Au and Ag nanoparticles for routine SERS measurements and to evaluate their limit of detection. The Ag and Au nanoparticles were prepared from a mixture of analyte and colloids solutions. The colloidal suspensions of the nanoparticles were air dried and placed on the slides to test them for explosives. Air drying contributes to the aggregation and creation of SERS "hotspots" in the samples. It is important to note that the use of spectroscopic techniques in the augmentation of nanostructured metals has become very popular in the last couple of years [10]. The new development can be attributed to the ability of the method to enable Raman scattering of light, which is generally inelastic for certain molecules in nanostructures that are either roughened or discontinuous. The increase of the Raman signal can be explained as having been triggered by electromagnetic interaction model (EM) or resulting from charge transfer which is a chemical process [11].

Experimental section

Chemicals and Materials

Silver nitrate (AgNO_3 , 99%), sodium hydroxide (NaOH , 98%), Gold(III) chloride trihydrate (HAuCl_4 , reagent, $\geq 49.0\%$), nitric acid (HNO_3 , 99%) and ethanol (reagent grade) were all obtained from Sigma-Aldrich and used as received. All aqueous solutions were prepared using deionized water (18.3 M Ω). TNT, RDX and PETN samples in methanol (10^{-3} M) were provided by Cranfield University. Samples were dried under a stream of nitrogen to recrystallize the solid explosive for original Raman measurements as a reference. The samples were dispersed in ethanol to different dilution concentrations for the SERS experiments.

Synthesis of Gold Nano spheres

Gold nanoparticles were synthesized using the Turkevich-Frens procedure to give an average size of 26 ± 5 nm [12-13]. In a typical procedure, 10 mg of HAuCl_4 (chloroauric acid) was dissolved in 20 mL of deionized water and boiled. A solution of 1% sodium citrate was prepared by dissolving 20 mg in 20 mL of deionised water, and then added to the HAuCl_4 solution. Boiling was continued for 1 hour, while the mixture was stirred, resulting in a deep red colour solution. The maximum absorption peak was found at 520 nm, corresponding to that of AuNPs.

Synthesis of Silver nanospheres

AgNPs were also produced using the standard Turkevich method. Initially, (0.1 M) of sodium hydroxide NaOH was used to adjust the pH to 7.7 of an aqueous solution of tri-sodium citrate solution (7 mM). The solution was then heated until boiling, then 1 mL of 0.1 M aqueous silver nitrate was added. The reaction was stirred for 5 minutes, then HNO_3 was added to adjust the pH to 6.1 in order to slow down the reaction and obtain better particles shape and size distribution. After 30 minutes, the reaction was completed, and particles of 58 nm with standard deviation (s.d.) of 14 nm were obtained, these showed an absorption peak at 460 nm, corresponding to that of AgNPs.

SERS substrate Preparation

In the preparation of the SERS substrate, both silver and gold nanoparticles were used. First, about 100 μL of the analyte were mixed with 50 μL of the colloid. The mixture was then cast drop by drop on the microscope slides. In preparation for the Raman measurement, the mixture was exposed to air and left to dry. Thereafter, the dried mixture was aggregated into different clusters and aggregates. The process helped in the creation of spots where the molecules of the analyte could be adsorbed into the nanoparticles. This resulted in high scattering of the incident radiation.

Characterisation techniques

Raman spectroscopy studies were carried out using a Renishaw 1000 spectrometer coupled to a microscope with 50 \times objective lens and equipped with a 633-nm laser. The laser spot size was ca. 4.4 μm^2 . The Raman system was calibrated using a silicon reference. The acquisition time was 10 s with one accumulation for all measurements. UV/vis spectroscopy was carried out using a Perkin Elmer Lambda 125 UV/Vis/NIR instrument. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) were carried out using a Jeol JSM-6700F. Transmission electron microscopy (TEM) images and selected area electron diffractograms (SAEDs) were obtained using a high resolution TEM Jeol 2100 with a LaB-6 source operating at an acceleration voltage of 200 kV. Micrographs were recorded on a Gatan Orius Charge-coupled device (CCD).

Result and Discussion

Various measurements were conducted for SERS with silver and gold nanoparticles at different concentrations of the analytes. During the synthesis, the optical properties and surface plasmon resonance (SPR) of both silver and gold were determined. It was observed that different concentrations of the colloidal particles had different sizes and shapes which ultimately affected the sensitivity of the SERS process. R6G was used as a probe in the evaluation of the activities of Ag and Au substrates. The explosives tested included Trinitrotoluene (TNT), Cyclotrimethylenetrinitramine (RDX) and Pentaerythritoltetranitrate (PETN).

Characterisation of the nanoparticles

UV-Vis spectra for Au and Ag nanoparticles colloidal were recorded for the 300 -800 nm region for 50 μL of the colloidal solution in 4 mL of DI water, as shown in **Figure 1**. Also recorded was the maximum absorption of silver and gold colloids which were about the average size of the particles. It was also determined that the Au colloids had a maximum absorption band of about 520 nm. This corresponded to the SPR of the gold nanoparticles which had a full width at half maximum (FWHM) of 50 nm. Such recordings were indicators of a low dispersion of the particle sizes in the solution. Additionally, the absorption of gold nanoparticles at longer wavelengths was non-existent.

For the silver colloids, the same procedure was followed and a maximum absorption band at a λ_{max} of 460 nm was measured. The colloid had a FWHM of 124 nm. This was an indication that there were large particles in the solution. This can possibly be attributed to the aggregation that occurred in the silver nanoparticles.

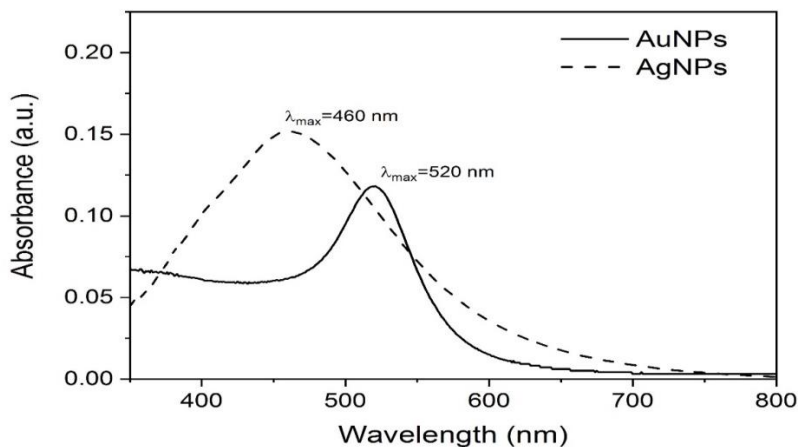


FIG. 1. UV-Vis absorption spectra| UV-vis spectra of gold and silver nanoparticles. The surface plasmon resonances (SPR) absorption peaks are 460nm and 520 nm for AgNPs and AuNPs respectively. All spectra were obtained at room temperature using

Transmission Electron Microscopy (TEM) images were obtained for AuNPs and AgNPs. These images showed that the gold nanoparticles were uniform in size and shape. AuNPs were found to have an average size of $26\text{nm} \pm 5\text{nm}$, as shown in Figure 2 (a & b), while silver nanoparticles were, on average larger, at $58\text{nm} \pm 14\text{nm}$ **Figure 2 (c & d)**.

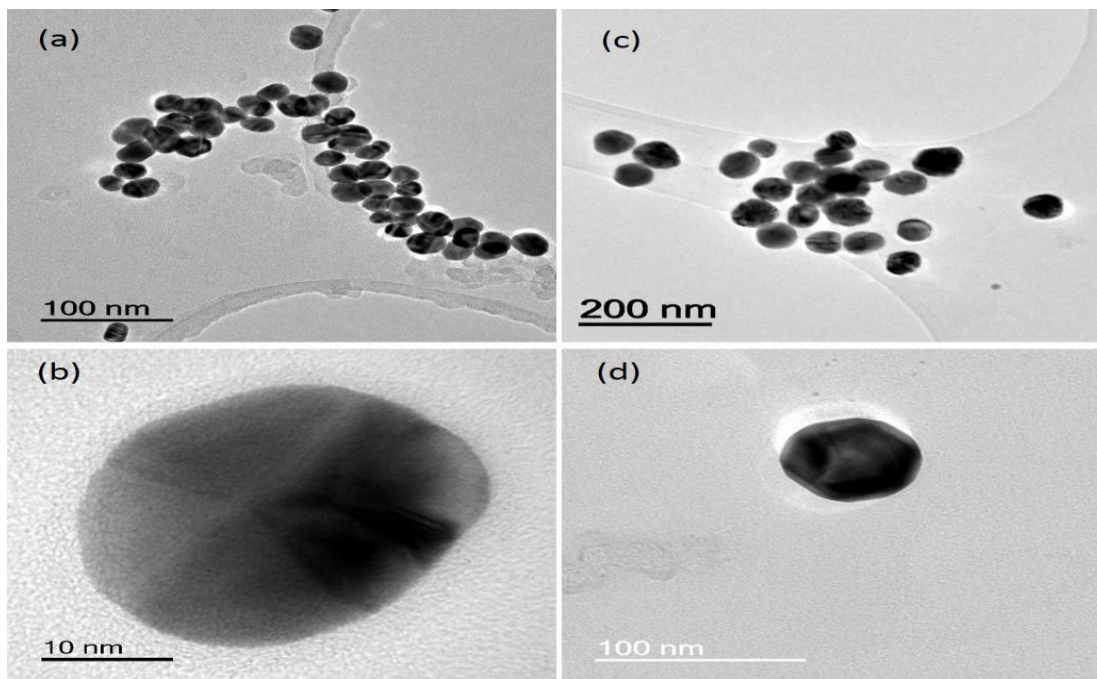


FIG. 2. Transmission Electron Microscope images| TEM images (a & b) for gold nanoparticles AuNPs, and (c & d) for silver nanoparticles AgNPs.

SEM images also were collected for AuNPs deposited on glass substrate, to show the distribution and the aggregation of the nanoparticles, (**Figure 1**), as the nanoparticles distributed randomly with formation of some clusters of nanoparticles, which are potential hotspots. Statistical analysis of the gold and silver nanoparticles was carried out using TEM images to show the average particle size distribution, (**Figure 2**)

SERS measurements

The SERS spectra were recorded for the explosives using both the Au and Ag colloidal solutions as the substrate materials. An excitation wavelength of 633nm (He-Ne) from the source of the laser was applied with an exposure time of 10 seconds. Prior to recording of the Raman and SERS spectra of R6G and explosive samples, recordings were done for the Raman spectra of the 50 μ L of the gold and silver colloidal solution on the microscope slide to determine the effect of impurities and aggregation on the Raman signals, which showed no clear signals in the vibrational fingerprint region of the analytes, makes the chosen substrate suitable for enhancing Raman signals of explosives.

Characterisation of SERS activity of Ag and Au nanoparticles

The SERS activities of each of the substrates were characterised using unique Raman active modes. Rhodamine-6G has unique Raman active modes, therefore it used for the characterization of the SERS performance here. Solutions of R6G were prepared at different concentrations they were then mixed with colloidal solutions of Au and Ag mixed with ethanol. The resulting mixture was in the ratio of 2:1 for the R6G: colloidal solution. This was used to develop final concentrations of the analyte of 10⁻⁵, 10⁻⁷ and 10⁻⁹M. The resulting mixture was drop cast on glass slides and then air dried for SERS measurements. The intensity of the signal decreases in a linear manner as the levels of concentration decreases. The **Figure 3** shows the Raman spectrum for solid R6G as a reference and SERS at concentrations of 10⁻⁵ M, 10⁻⁷ M and 10⁻⁹ M.

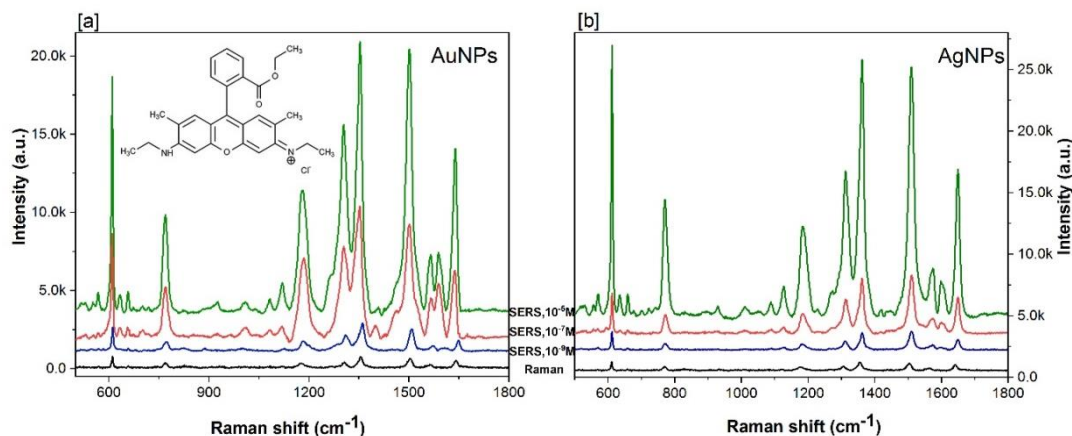


FIG. 3. Raman and SERS of R6G| Raman spectrum of solid R6G and SERS spectra of 10⁻⁵, 10⁻⁷ and 10⁻⁹M of R6G solutions on [a] AuNPs [b] AgNPs substrates.

The SERS measurements for R6G indicated a very strong level of enhancement where the peaks had similar signatures in all the samples in both cases Au and Ag NPs substrates. This can be attributed to the fact that R6G tends to have a large Raman cross-section which resulted in massive scattering of the noble-metal nanoparticles. A reduction in the concentration levels of the Au and Ag nanoparticles results in the reduction of the number of molecules being adsorbed into the substrate. This eventually affects the levels of enhancement of the Ag nanoparticles. The observed SERS enhancement indicates that this

method of detecting trace samples is sufficient at some point and that dependent on several parameters such as vapour pressure of the analyte as well as the molecules adsorption in nanoparticles sites.

Measurement of the SERS of Trinitrotoluene (TNT)

SERS measurements were conducted for Trinitrotoluene (TNT) solutions at molar concentrations of 10^{-5} , 10^{-7} and 10^{-9} M. **Figure 4** shows the Raman spectrum of neat solid TNT and SERS spectra of TNT samples with AuNPs and AgNPs substrates.

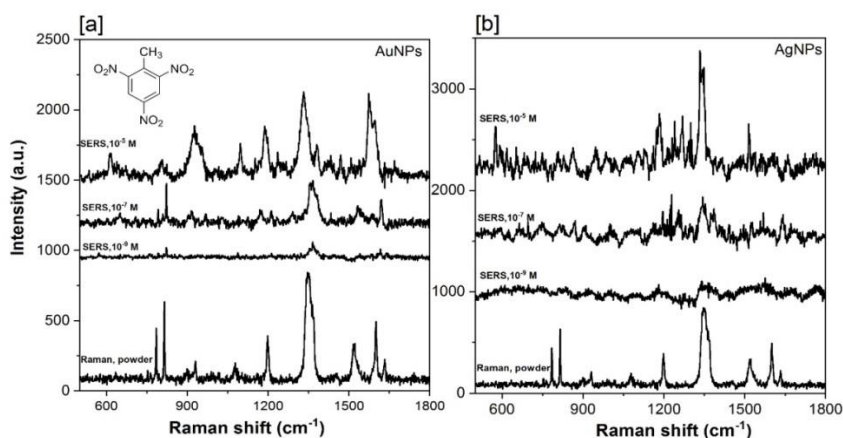


FIG. 4. Raman and SERS spectra of TNT | [a] on AuNPs and [b] on AgNPs for analyte concentration of 10^{-5} M, 10^{-7} M and 10^{-9} M of ethanolic solutions of TNT, and Raman spectrum of solid sample of TNT as a reference.

From **Figure 4**, it is indeed clear that 10^{-5} M of TNT had the highest enhancement at both substrates Au and AgNPs. Strong enhancements were also evident on the weak modes, a phenomenon which was as a result of the out of plane bending of the C-H. Peaks with lower intensities were also observed at 791 and 822 cm^{-1} in the SERS spectrum at 10^{-5} M on AuNPs. The lower intensity can be attributed to their orientation towards the adsorbed molecules on the Au nanoparticles. This was the point at which the spectrum was recorded. At a concentration of 10^{-7} M of TNT on AuNPs, the SERS spectrum showed a number of enhanced signals with strong Raman active modes in the spectrum. For the 10^{-9} M on AuNPs, the SERS measurements showed small enhancements on the peaks which included 823 cm^{-1} , 1360 cm^{-1} and 1615 cm^{-1} . This occurrence was mainly as a result of symmetric and asymmetric stretching of nitrogen dioxide. 10^{-9} M solution showed the lowest concentration and showed enhanced Raman signals. TNT SERS measurements were mainly dependent on factors such as the orientation of the analyte and nanoparticles, as if the molecule located in the nanoparticle site where the modified electric field maximised, high enhancement of the vibrational signals will be observed.

On the other hand, no clear enhancement was observed for the sample at 10^{-9} M on Ag nanoparticles, **Figure-4b**. Several other peaks were observed whereby some matched the active Raman modes while others corresponded to the inactive modes. At 10^{-7} M, the signal intensity was much lower than that of the 10^{-5} M sample which was mainly as a result of the adsorbed molecules on the Ag nanoparticles. The higher enhancement observed in the Au substrates compared to the Ag nanoparticles was mainly as a result of the red-shift of the SPR of the nanoparticles after they had been mixed with the analyte.

Measurement of the SERS Cyclotrimethylenetrinitramine (RDX)

Cyclotrimethylenetrinitramine (RDX) is a well-known explosive used in military operations and in terror attacks. In this measurement, SERS spectra were collected from different concentrations of RDX solutions on Au and Ag nanoparticles

substrates. The results showed that at a 10⁻⁷M, the sensitivity levels were reasonable but below this level, the substrates struggled. The same procedure followed in the previous measurements was applied. The concentrations of the RDX samples were at 10⁻⁵M, 10⁻⁷ M and 10⁻⁹ M. **Figure 5** shows the recorded spectra of the RDX samples.

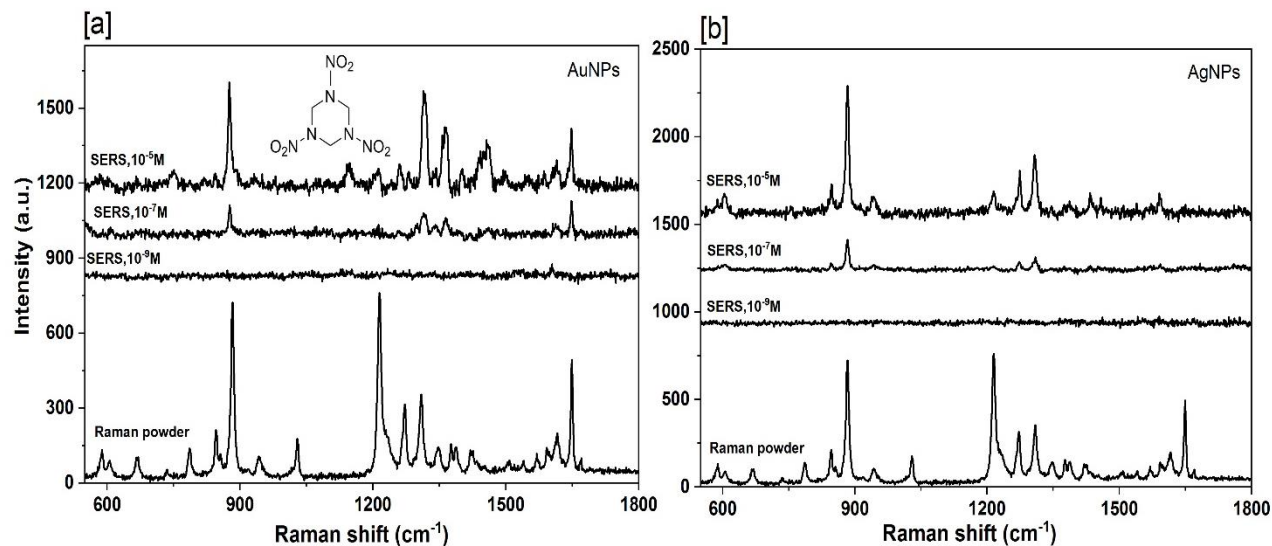


FIG. 5. Raman of solid sample and SERS spectra of RDX | [a] AuNPs and [b] AgNPs substrates from different concentrations in ethanol down to nanomolar range.

From SERS spectra on the AuNPs substrate, the 10⁻⁵M sample showed a good enhancement of the signature peaks in its SERS spectra of the RDX. The most intense peak was observed at approximately 882 cm⁻¹ that attributed to the asymmetric stretch breathing mode of the RDX ring which was strongly enhanced. There was a small shift at 877 cm⁻¹ with another strong peak being recorded at 1215 cm⁻¹. However, it was not as enhanced as the previous one. Other enhanced peaks were recorded at 1316 cm⁻¹ (CH₂ wagging) and 1364 cm⁻¹ v (NO₂). Low intensity peaks for the SERS spectrum of the 10⁻⁷ M of RDX showed strong enhancements for peaks at 880, 1315, 1364 and 1648 cm⁻¹. There were no peaks observed for 10⁻⁹ M except a very weak one at 1610 cm⁻¹. On the other hand, AgNPs showed good enhancements for the samples with concentrations of 10⁻⁵ M and 10⁻⁷ M but no enhancement was observed at 10⁻⁹ M, as shown in **Figure 5-b**.

Reproducibility of SERS is a crucial parameter, especially for trace-level detection. Therefore, the SERS reproducibility for a RDX sample on AgNPs was evaluated by collecting SERS spectra at different spots. AgNPs substrate showed sufficient reproducibility for the 10⁻⁵ M sample of RDX, (Supplementary **Figure 3**)

Measurement of the SERS of Pentaerythritoltetranitrate (PETN)

Pentaerythritoltetranitrate (PETN) is also a well-known explosive that is widely used for terrorism attacks. With AuNPs substrate, a reasonable amount of enhancement was observed for the 10⁻⁵ M PETN sample. At 10⁻⁷M, the sample showed limited sensitivity and had three different characteristic peaks with low intensity. The 10⁻⁹ M concentrated sample had a poor sensitivity for the SERS spectrum, as only a small peak was recorded at 871 cm⁻¹ as shown in **Figure 6-a**.

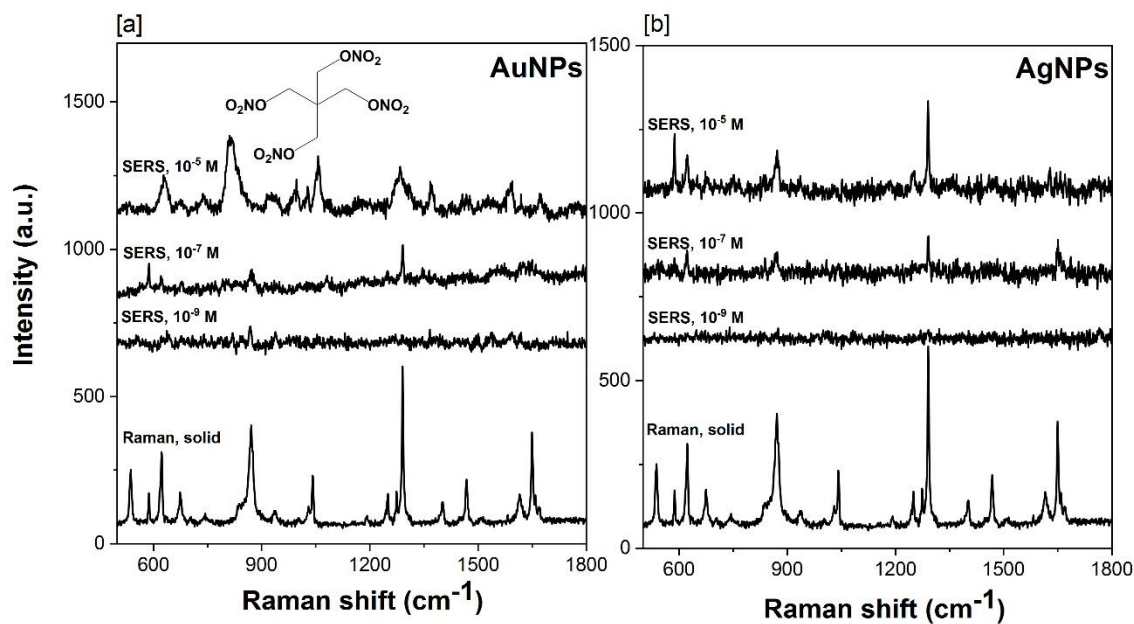


FIG. 6. Raman of solid sample and SERS spectra of PETN | [a] on AuNPs and [b] on AgNPs substrates from different concentrations in ethanol, 10-5, 10-7M and 10-9M range, and Raman spectrum of solid RDX crystals as a reference.

From **Figure 6a**, for the sample 10-5 M of PETN, the SERS spectrum showed specificity and reasonableness. It enhanced most of the characteristics of the Raman active modes of PETN. Small shifts were observed at 871 cm^{-1} and 1290 cm^{-1} , a phenomenon that results from C-C-C deformation and CH₂ Wagging vibrational modes 14. Also observed was enhancement of four signals at 588, 622, 870 and 1290 cm^{-1} for the SERS spectrum of 10^{-7} M of PETN on AuNPs. They all had strong active Raman modes, except for the peak observed at 588 cm^{-1} . There were only two peaks with high frequencies and their Raman active modes matched at 1650 cm^{-1} , a value which corresponded to the asymmetric stretching of NO₂. Finally, only a small intensity peak at 871 cm^{-1} was observed for the nanopolar concentrated sample of PETN on AuNPs.

On the other hand, SERS enhancement was seen for PETN samples with concentrations of 10-5 M and 10-7 M, while no signals were seen for the sample with a concentration of 10-9 M, as shown in **Figure 6b**.

Overall, there are a number of factors that affect the sensitivity and reproducibility of SERS. The most important one discussed in this work are the properties of the nanoparticles. The shape and size of the nanoparticles as distributed in the substrate affect the sensitivity of the SERS 15-17. In the tests conducted, the nanoparticles of Ag and Au were randomly distributed and later deposited on the glass slides for air drying and SERS measurements. As a result, the detection sensitivity of the samples was reduced with the limit being 10-9M for the R6G and 10-7M for explosives. Since R6G molecules are large, they resulted in a high scattering with a lower cross section area of the molecules 18.

The sensitivity of the SERS could be improved through close packing of the nanoparticles. An example of this can be seen on the SERS spectra for 10-5 M of RDX on the Ag nanoparticles which showed this characteristic. The enhancement of the nanoparticles is mainly affected by electromagnetic enhancement mechanism (EM) and chemical enhancement (CT). The two processes involve the generation and transfer of energy of the nanoparticles across the analyte. For lower concentrations, the enhancement factors were recorded to be very high. It is a common phenomenon to have EFs that are higher with low

concentrated solutions 19. This can be attributed to the nature of the EF formula 20. The recorded values for the concentrations show varying values of the EF across different concentrations, as shown in Supplementary Table 1.

Conclusions

The objectives of this work were attained. Measurements for various variables were taken and analysed accordingly. It is important to point out that among the colloids measured; only the 10⁻⁷M solutions were found to be good candidates for the explosives. Reasonable enhancements were observed high-to-medium concentration. For solutions less than 10⁻⁷ M, it was difficult to achieve both the specificity and the sensitivity. In RDX, it was show that the reproducibility is highly dependent on the molecules and the concentrations. The sensitivity of the SERS efficiency can be improved by considering parameters such as the shape, size and distribution of the particles.

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REFERENCES

1. Kneipp K, Kneipp H, Itzkan I, et al. Surface-enhanced raman scattering and biophysics. *Journal of Physics: Condensed Matter*. 2002;14
2. Orendorff CJ, Gearheart L, Jana NR, et al. Aspect ratio dependence on surface enhanced Raman scattering using silver and gold nanorod substrates. *Physical Chemistry Chemical Physics*. 2006;8:165-170.
3. Kneipp K, Haka AS, Kneipp H, et al. Surface-enhanced raman spectroscopy in single living cells using gold nanoparticles. *Applied Spectroscopy*. 2002;56:150-154.
4. Hakonen A, Andersson PO, Schmidt MS, Rindzevicius, T.; Käll, M., Explosive and chemical threat detection by surface-enhanced Raman scattering: A review. *Analytica chimica acta* 2015.
5. Wackerbarth H, Salb C; Gundrum L, et al. Detection of explosives based on surface-enhanced Raman spectroscopy. *Applied Optics*. 2010;49:4362-4366.
6. Finot E, Brulé T, Rai P, et al. In Raman and photothermal spectroscopies for explosive detection, Micro-and Nanotechnology Sensors, Systems, and Applications V. International Society for Optics and Photonics. 2013.
7. Ben-Jaber S, Peveler WJ, Quesada-Cabrera R, et al. Photo-induced enhanced Raman spectroscopy for universal ultra-trace detection of explosives, pollutants and biomolecules. *Nature Communications* 2016;7.
8. Aber SSB, Peveler WJ, Cabrera RQ, et al. Sensitive and specific detection of explosives in solution and vapour by Surface-Enhanced Raman Spectroscopy on silver nanocubes. *Nanoscale* 2017.
9. Sharma HS, Carmichael E, McCall D. Fabrication of SERS substrate for the detection of rhodamine 6G, glyphosate, melamine and salicylic acid. *Vibrational Spectroscopy*. 2016;83:159-169.

10. Leopold N, Lendl B. A new method for fast preparation of highly surface-enhanced Raman scattering (SERS) active silver colloids at room temperature by reduction of silver nitrate with hydroxylamine hydrochloride. *The Journal of Physical Chemistry B*. 2003;107:5723-5727.
11. Le Ru EC. Principles of surface-enhanced raman spectroscopy and related plasmonic effects. Elsevier Science: Amsterdam, London. 2009.
12. Meisel PCLD. Adsorption and surface-enhanced raman of dyes on silver and gold sols'. *The Journal of Physical Chemistry*. 1982;86:3391-3395.
13. Kimling J, Maier M, Okenve B, et al. Turkevich method for gold nanoparticle synthesis revisited. *The Journal of Physical Chemistry B*. 2006;110:15700-15707.
14. Hatab NA, Eres G, Hatzinger PB, et al. Detection and analysis of cyclotrimethylenetrinitramine (RDX) in environmental samples by surface-enhanced Raman spectroscopy. *Journal of Raman Spectroscopy*. 2010;41:1131-1136.
15. Zhang CL, Lv KP, Cong HP, et al. Controlled assemblies of gold nanorods in pva nanofiber matrix as flexible free-standing sers substrates by electrospinning. *Small*. 2012;8:648-653.
16. Wustholz KL, Brosseau CL, Casadio F, et al Surface-enhanced Raman spectroscopy of dyes: from single molecules to the artists' canvas. *Physical Chemistry Chemical Physics*. 2009;11:7350-7359.
17. Lee S, Chon H, Lee M, et al. Surface-enhanced Raman scattering imaging of HER2 cancer markers overexpressed in single MCF7 cells using antibody conjugated hollow gold nanospheres. *Biosensors and Bioelectronics*. 2009;24:2260-2263.
18. Le Ru, EC, Blackie E, Meyer M, et al. Surface enhanced raman scattering enhancement factors: A comprehensive study. *The Journal of Physical Chemistry C*. 2007;111:13794-13803.
19. Li W, Camargo PH, Lu X, et al. Dimers of silver nanospheres: facile synthesis and their use as hot spots for surface-enhanced Raman scattering. *Nano letters*. 2008;9:485-490.
20. Mahmoud M, El-Sayed M. Comparative study of the assemblies and the resulting plasmon fields of Langmuir-Blodgett assembled monolayers of silver nanocubes and gold nanocages. *The Journal of Physical Chemistry C*. 2008;112:14618-14625.