Concave gold nanoparticles on aluminum as surface enhanced Raman spectroscopy substrate for detection of thiram

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Abstract
We report a simple preparation of surface enhanced Raman spectroscopy (SERS) substrates with gold concave nanocubes of different sizes, deposited onto aluminum slides. The SERS substrates were characterized using 4-Aminothiophenol. Resulting in that the substrate prepared with 55 nm nanocubes exhibits the higher analytical enhancement factor (AEF), \(1.5 \times 10^6\). Moreover, the SERS substrates’ performance was also evaluated for thiram detection using a series of water and tomato juice samples spiked with the pesticide. Our results show that the excellent performance of the substrate allows the detection of thiram with high sensitivity. The substrates were able to detect thiram with a limit of detection of 7 ppm and 14 ppm, respectively, in water and tomato juice. Our preliminary results open the possibility of using our methodology to detect diverse pollutants of interest.

Keywords
Aluminum slide, food, gold concave nanocubes, pesticides, surface enhanced Raman spectroscopy, thiram

Date received: 6 September 2021; accepted: 30 January 2022

Introduction
In recent years, there has been extensive use of chemical pesticides in agriculture to avoid losses in production, and one of the most used compounds is dimethyl dithiocarbamate, commonly used in the production of pesticides like thiram (tetramethylthiuram disulfide),\(^1\)\(^2\) utilized as an animal repellent, protective agent for crops, bactericide, and sunscreen. Yet, the use of pesticides has also entailed extensive environmental problems\(^3\) and side effects that include health risk factors for producers and consumers of agricultural products.\(^4\)\(^5\) It is well-documented that some pesticides can remain in food and water for a very long time before they can be decomposed into less harmful compounds.\(^1\)\(^6\) Hence, the monitoring of hazardous molecules in water is critical to prevent health risks. Nowadays, analytical methods for pesticide detection include high-performance liquid chromatography, gas chromatography-mass spectroscopy, and liquid chromatography-tandem mass spectrometry.\(^7\)\(^9\) However, these methods require exhaustive and time-consuming sample preparation and the use of expensive equipment. One of the most promising alternatives is surface enhanced Raman spectroscopy (SERS).\(^4\)\(^10\) SERS has proven to be a powerful tool for the detection of a wide range of compounds, including pesticides, with high sensitivity and selectivity.

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Raman spectroscopy (SERS), because it exhibits rapid response and high sensitivity for the detection of diverse compounds, and is an up-and-coming technology that significantly impacts the science and technology of materials, biology, medicine, and environmental monitoring. One of the main challenges for SERS is the design and optimization of suitable substrates that have good features, such as excellent reproducibility, good uniformity, portability, and a high enhancement factor (EF). It has been shown that SERS substrates can be developed with a variety of metal NPs that display localized surface plasmon resonance, increasing the SERS spectroscopy sensitivity. For example, Fu G et al. carried out the in-situ detection of thiabendazole on the surface of contaminated apple peel by depositing gold silver core-shell nanorods (Au@Ag NRs). Khlebstov B. N. et al. used anisotropic nanoparticles, such as nanocubes, nanostars, and films with gold nanoislands, for thiram detection on apple peel; the authors reported an analytical enhancement factor (AEF) of 4.5 × 10^7 for the case of nanostars. Zhu Y et al. detect pesticide residues in tomatoes skin using filter paper decorated with silver nanoparticles.

Even though all the above results are relevant, SERS substrates suitable for analyzing or detecting a broad analyte under various conditions are not available yet. However, it is encouraging that novel SERS substrates for specific applications and good performance can be designed and fabricated, yielding significant advantages over traditional detection techniques. In this work, water and tomato juice contaminated with thiram at concentrations between 300 μM and 10 μM were prepared to evaluate our SERS substrate performance; they were fabricated by means of drop-casting concave gold nanocubes (CGNCs) onto the surface of functionalized aluminum slides.

Materials and methods

Chemical reagents

Hexadecyltrimethylammonium chloride (98%, CTAC), Tetrahydroauric (III) acid (99.9%, HAuCl₄), Sodium borohydride (99, 99%, NaBH₄), silver nitrate (99.0%, AgNO₃), hydrochloric acid (HCl), 4-Aminothiophenol (97%, 4-ATP), 3-Aminopropyl)triethoxysilane (99%, APTS) and Thiram were purchased from Sigma-Aldrich, while L-Ascorbic acid (99.9%) was acquired from Alfa Aesar. Ultra-pure water from a Milli-Q system (Milli pore America, resistivity <18MΩcm) was used throughout the experiments.

Concave gold nanocubes

CGNCs were synthesized through the seed-mediated method, which consists in preparing 5 mL of a 0.1 M CTAC solution in a glass beaker of 25 mL, then adding 130 μL of a 10 mM HAuCl₄ solution and 380 μL of a 10 mM NaBH₄ solution (ice-cold), all under magnetic agitation. The color of the solution changed from yellowish to brownish after the sodium borohydride was added, and then, the colloid was set to rest for 2 hours of aging; after this time, a UV-Vis absorbance spectrum was acquired, and the colloid’s absorbance was set at 0.4OD. The colloid of seeds was used to perform the synthesis of the CGNCs. The CGNC growth solution was prepared by sequentially adding 10 mL of CTAC (0.1 M), 0.5 mL of HAuCl₄ (10 mM), 48 μL of AgNO₃ (20 mM), 200 μL of HCl (1.0 M) and 120 μL of ascorbic acid (100 mM), all under magnetic stirring; in order to control the size of the CGNCs, different volumes of seeds were added to the growth solution (5, 10, 20, 40, 60, 90 μL). The color of the resulting colloids was navy-blue for the smaller CGNCs and pale green for the bigger ones. The colloids were characterized by means of UV-Vis spectroscopy and scanning electron microscopy (SEM, model JSM-7800FM JEOL), using 3.0 mm in working distance, 15 KeV, and 100,00X in amplification as parameters.

Aluminum slides and the surface enhanced Raman spectroscopy substrate

The aluminum slides were prepared following the method reported by Martinez-Garcia. First, aluminum 6063 alloy (Al) slides were prepared by mechanically polishing them to a mirror-like finish on their surface. The slides were then subject to electrochemical polish in a solution of perchloric acid and ethanol (1:4), under constant voltage (15 V) with 3 A of current. Once the slides were correctly neutralized and cleaned, we functionalized each one by soaking it in a 10% APTS-methanol solution. Finally, the slides were dried in an oven at 120 °C for 10 min. Clean CGNPs were deposited by drop-casting using 3 μL of the CGNC colloids on the surface of the treated Al slides.

Aminothiophenol Raman reporter

We prepared a stock solution of 4-ATP at 7.6 M in methanol, and then, two additional solutions, one at 0.1 M and the other at 1.0 × 10⁻⁶ M, were prepared in water. One 3 μL drop of the first solution was poured onto an aluminum slide for normal Raman analysis, and one 3 μL drop of the second solution was poured onto the SERS substrates, and we proceeded to acquire the SERS Raman spectra.

Water spiked with thiram

For pesticide detection, a stock solution of pristine thiram of 1.0 mM in acetone was prepared and sonicated until the reagent was dissolved. Then, solutions at concentrations of 300, 250, 200, 150, 100, 30, 20, 10 × 10⁻⁶ M were prepared in water.
Tomato juice spiked with thiram

A piece of tomato was meticulously washed to remove wax and preservative residues. This piece of tomato was blended with 100 mL of Milli-Q water, to later be passed through filter paper of 11 μm in pore size to eliminate all solid residues. Finally, we prepared the tomato juice with the pesticide solution under sonication, to obtain final concentrations of 250, 200, 150, 100, 50, 40, and 30 × 10⁻⁶ M.

Substrate Raman analysis

SERS substrates were prepared in triplicate under the conditions as mentioned above and characterized by using 3 μL of the corresponding probe solution (4-ATP, thiram-spiked water, and thiram-spiked tomato), which were deposited onto the substrates and analyzed with a micro-Raman spectroscopy system (inVia Renishaw) employing a diode laser at 785 nm as excitation source. The average laser power focused on the samples was 4 mW with a 20X objective (NA = 0.4), a time exposure of 2 s and 10 accumulations.

Results and discussion

The seed-mediated method followed in this work produced CGNCs with reasonable control in size and shape to prepare SERS substrates. Figure 1 shows the SEM image, the

![Figure 1](image)

**Figure 1.** Micrographs and sizes histograms of the concave gold nanocubes: (a) 36 ± 5 nm, (b) 42 ± 5 nm, (c) 46 ± 7 nm, (d) 55 ± 9 nm, (e) 63 ± 5 nm, and (f) 85 ± 8 nm.
micrographs were analyzed using ImageJ, we observed a high yield synthesis of monodisperse nanocubes. The estimated average sizes were (a) 36 ± 5 nm (90 μL), (b) 42 ± 5 nm (60 μL), (c) 46 ± 7 nm (40 μL), (d) 55 ± 9 nm (20 μL), (e) 63 ± 5 nm (10 μL), and (f) 85 ± 8 nm (5 μL), respectively. When a small volume of seeds is added to the growth solution, large-sized CGNCs grow, and conversely, smaller sized CGNCs were obtained using a large volume of seeds. The size of CGNCs as a function of seeds shows an exponential behavior (see Supplementary Figure S1 in the supplementary material).

Figure 2 shows the normalized optical extinction spectra of the six CGNC colloids. Maximum absorbance is located at 634.7, 640.6, 656.7, 764.2, and 774.2 nm, for cubes of 36, 42, 46, 55, 63, and 85 nm, respectively. The nanocubes of 63 nm and 85 nm in size exhibit a widened plasmonic band. The optical scattering phenomena play a predominant role in the extinction spectra of this kind of NPs. It has been reported that the electric field is significantly enhanced at the concave faces and at sharp corners of concave nanoparticles, making CGNC excellent candidates for SERS applications. The six different SERS substrates were tested using a 4-ATP as a Raman reporter to evaluate their performance. The typical Raman spectrum of 4-ATP at 1 × 10−6 M is shown in Figure 3(a), and the mean SERS spectra of 4-ATP at 1 × 10−6 M of the six substrates appear in Figure 3(b). The mean SERS spectra were calculated using 65 spectra acquired per substrate. Supplementary Figure S2 in the supplementary material shows the raw SERS spectra and a Raman intensity map at 1078 cm−1 with a relative standard deviation (RSD) of 19% estimated from 12 × 8 points equivalent of a scanned area of 258 μm × 50 μm, which shows the uniformity of the SERS substrate prepared with cubes of 55 nm. All samples were analyzed in the liquid phase to obtain the Raman spectra. The most intense peaks of the fingerprint for the average normal Raman spectra of the pristine 4-ATP are located at 1088 and 1593 cm−1, and they are assigned to ν(C-S) and ν(C=C) modes, respectively.

From Figure 3(b), the reader can see the mean spectrum of each substrate. Now, there are three strong-intense peaks located at 1078 cm−1 ν(C-S), 1181 cm−1 δ(C-H), and 1587 cm−1 ν(C-C), which are consistent with previous reports of SERS analysis using 4-ATP.

We selected the two most intense peaks, at 1078 and at 1587 cm−1, to analyze the SERS signal’s behavior. The scatter plot of Figure 4 clearly shows the relationship between the SERS signal’s maximum intensity and the size of the nanocube. After carefully analyzing our data, we have established that the SERS substrate prepared with 55 nm CGNCs (CGNCs-55) displays the most intense Raman signal, indicating that this substrate produces the best enhanced Raman signal. The mean AEF calculated for each substrate were 0.4, 0.3, 0.6, 1.7, 0.5, and 0.4 × 106, Supplementary Table A in the supplementary file shows the complete information of the calculated AEF for the six substrates. The higher enhancement factor for both selected peaks happened for the SERS substrate prepared with nanocubes of 55 nm, showing that the substrate prepared with CGNCs-55 (SERS-CGNCs-55) has the best performance. The detection of molecules with similar characteristics like 4-ATP or molecules that exhibit good affinity for CGNCs are good candidates to be monitored with the SERS substrate. Previous reports showed the excellent affinity of thiram for gold nanoparticles.

To test our SERS substrate’s performance in pesticide detection, first, a solution of pristine thiram in methanol at 1.0 × 10−2 M was prepared as a stock solution. Then, several dilutions of the pesticide were prepared in water to get final concentrations of 300, 200, 150, 100, 30, 20, and 10 μM. A series of normal Raman spectra of pristine thiram (powder) were acquired, and Figure 5(a) shows the average Raman spectra. The reader can notice that the most intense peak is located at 557 cm−1, assigned to ν(S-S) vibration, and followed in intensity by that at 441 cm−1, assigned to ν(CH3NC) and ν(C=S). For SERS analysis, 3 μL of each sample of thiram was dropped onto the SERS-CGNC-55 substrate, in which the SERS signal was acquired from the liquid samples; a total of 96 spectra were acquired by scanning an area of 12 × 8 points by substrate. For example, Supplementary Figure S3 in the supplementary material shows the SERS spectra (raw data) and the Raman intensity map for the thiram concentration of 30 × 10−6 M; the map shows the excellent uniformity of the substrate with an RSD = 16% estimated from an area of 166 μm × 10 μm.
The raw SERS spectra of thiram are shown in Figure 5(b); Raman peaks are located at 563 ν(S-S), 1145 ν(CH3), and ν(C−N)), and at 1380 cm⁻¹, assigned to δ(CH3) and ν(C=N). The calibration curve was obtained using the peak at 1380 cm⁻¹, which is shown in Figure 6. Calibration curves exhibit a linear behavior between Raman intensity and pesticide concentration. The best fit to the data is depicted by a gray line with a correlation coefficient of R² = 0.98. SERS analysis was carried out within a wide range of concentrations of the pesticide. This result shows the potential application of our SERS substrate to the detection of thiram in contaminated water samples. Samples do not require exhaustive preparation to be examined, such as reported in other works. Where the solvent is evaporated to increase the analyte’s concentration and improve the SERS sensitivity.

Our substrate can be used to detect thiram in water at concentrations in the range of tolerance concentration, and it is shown in Figure 6; the lowest concentration we detected was 10 μM (3 ppm), but, the estimated limit of detection (LOD) was calculated by using LOD = 3σ/m, where m is the slope of the calibration curve, and σ is the standard deviation of response. For this case, the estimated LOD was 7 ppm. Water contamination is becoming a problem in every country in which pesticides can be used in large quantities by the agricultural industry, producing contamination of ground, subsoil, and aquifers. Techniques such as SERS, which can quickly detect pesticide contamination, can help determine contamination and, therefore, maintain aquifers at safe levels. We also evaluated the performance of our substrates in another actual practical application. A series of samples of tomato juice spiked with thiram were prepared. First, tomatoes were selected from a local market and washed with soap and plenty of water. Then, one tomato was chosen to be blended with 100 mL of Milli-Q water. Finally, the juice was filtered using a Whatman filter paper of 11mµ particle retention. After that, we prepared, by triplicate, seven samples of tomato juice with thiram at final concentrations of 250, 200, 150, 100, 50, 40, and 30 μM. One drop of 3 μL of each sample was deposited onto the SERS substrate. Ninety-six Raman spectra were measured from the liquid sample (Supplementary Figure S4 shows the Raman intensity map at 1380 cm⁻¹ with an RSD = 19% and Raman raw spectra acquired tomato juice spiked with thiram at the concentration of 30 × 10⁻⁶ M). Figure 7(a) shows the average normal Raman spectra of tomato juice. The three most intense peaks of the tomato are located at 1008 δ(C−CH3) in-plane rocking, 1157 ν(C−C) stretching, and 1520 cm⁻¹ ν(C=C) in phase stretching.

On the other hand, Figure 7(b) shows the average SERS spectra of tomato juice spiked with thiram for each concentration, and the plot shows the three most intense peaks located at 560, 1143, and 1380 cm⁻¹. Comparing the position of the peaks of the Raman spectrum of pure thiram (Figure 5(b)) with the spectrum of thiram mixed with tomato juice (Figure 7(b)), a slight shift is observed.
Figure 5. (a) Raman spectra of pristine thiram powder. (b) surface enhanced Raman spectroscopy spectra of thiram prepared in water at concentrations from 300 to 10 μM.

Figure 6. Calibration curve of thiram for concentrations from 300 to 30 μM in water, for the Raman intensity peak at 1380 cm⁻¹.

Figure 7. (a) Raman spectra of pure tomato juice without any dilution. (b) surface enhanced Raman spectroscopy spectra of the mixture of thiram with tomato juice for concentrations ranging from 250 to 30 μM.

Figure 8 shows the calibration curve of the SERS intensity signals using, in this case, the peak located at 1381 cm⁻¹ as a function of thiram concentration. We observed a linear behavior, the gray line representing the best linear fit of the data, with a correlation parameter of R² = 0.94. For the case of a thiram spike in tomato juice, we measured
the Raman spectra at the lowest concentration of thiram at 30 μM (9 ppm), and the estimated LOD was 14 ppm.

**Conclusions**

Here, we propose the fabrication of SERS substrate using CGNCs deposited onto functionalized aluminum slides. Aluminum does not exhibit Raman signal that can interfere with the analyte signal. On the other hand, concave gold nanoparticles display strong surface plasmon resonance within a wide range of wavelengths, among the six sizes of synthesized nanocubes. The SERS substrate prepared with CGNCs of 55 nm shows the highest AEF (1.7 × 10^6), good reproducibility, and uniformity. For this reason, SERS-CGNC-55 was selected to carry out the analysis and detection of thiram in samples of water and tomato juice. SERS-CGNC-55 was able to detect the pesticide in a wide range of concentrations. The maximum residue level of pesticides is in the range of 3–8 ppm. Here, we detected the pesticide at 3 ppm and 9 ppm in water and tomato juice, respectively. These results illustrate the potential of SERS in applications for detecting contaminants in liquids by using substrates made with CGNCs.

**Acknowledgements**

Authors thanks for the facilities provided by the CIO on the use of the scanning electron microscope. Also, thanks to Ing. Ma Cristina Albor and Jose Bante Guerra for their technical help on SEM analysis and Mario A Ruiz Berganza for proofreading the manuscript.

**Author contributions**

JL Pichardo-Molina, and M M Martinez-Garcia contributed to conception and design, contributed to acquisition, analysis, and interpretation, drafted the manuscript, and critically revised the manuscript. JJ Alvaro Gil and N Arzate-Plata contributed to analysis and interpretation and critically revised the manuscript.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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**Supplemental Material**

Supplemental material for this article is available online.

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