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Synthesis of Polyhedral Gold Nanostars as Surface-enhanced Raman Spectroscopy Substrates for Measurement of Thiram in Peach Juice

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Rapid detection of food contaminants using novel analytical methods in combination with nanomaterials has received much attention. This study aimed to synthesize polyhedral gold nanostars (AuNS) with multi-angled corners and develop surface-enhanced Raman spectroscopy (SERS) method coupled with AuNS to detect pesticide residues in juice products. AuNS are multi-branched three-dimensional metal nanostructures with rough surface features that can induce surface plasmon resonance. A facile synthesis of AuNS was achieved using a two-step method and as-prepared AuNS had much cleaner surfaces than gold nanoparticles. A Raman reporter molecule (4-aminothiophenol) was used to evaluate the performance of the SERS method, yielding fingerprint-like Raman spectra and the sensitivity of the SERS method could reach 10 ppb (μg/kg). SERS coupled with AuNS was used to detect thiram residues in peach juice and the detection limit was 50 ppb, which is 100 times more sensitive than using normal gold nanoparticles. These results demonstrate that AuNS are excellent substrates for SERS measurement, which has great potential for rapid detection of chemical contaminants in food products.

Introduction

Nano-scale gold structures have attracted much attention in recent years due to their unique optical properties in which free electrons on the surface of metal nanostructures interact with electromagnetic fields to generate surface plasmon resonance.[1] In particular, complex polyhedral gold nanostars (AuNS) exhibit unique optical properties owning to multi-angled corners and complex rough surfaces. AuNS have three-dimensional (3D) nanostructures with asymmetrical and multiple-edged shape. They mainly consist of a central core and a plurality of protruding branching angles. It is found that the presence of sharp edges and branching angles makes AuNS very sensitive to local dielectric environments, which can potentially be used in the Raman scattering.[2]

Current methods for the synthesis of AuNS are mainly based on the seed-mediated growth method in which the seed solution is synthesized first, then the seeds are used as the growth nuclei and a reducing agent is used to reduce chloroauric acid.[3] With the aid of the surfactant and using the gold seeds as a growth point, a plurality of branching angles are grown on the surface to form AuNS. For instance, AuNS could be prepared based on the synthesis of gold nanorods.[4] The seed is used as a nucleation growth point, and ascorbic acid is used to reduce chloroauric acid. Under the action of the surfactant cetyltrimethylammonium bromide (CTAB), the gold seed is oriented to grow into a gold nanorod with a certain aspect ratio. However, this approach has many drawbacks including the time-consuming synthesis cycles, complicated operations, and the difficulty in removing CTAB from the nanoparticle surface.[5]

Thiram is a type of sulphur fungicide that has been widely used to prevent fungal diseases in crops and fruits in the field and protect harvested crops from decaying during storage and transport.[6] However, thiram residues in foods can cause health problems in consumers such as nausea, vomiting, diarrhea, itchy skin, and rash.[7] Most current analytical methods for detection of thiram are chromatography-based methods.[8] However, these methods are time-consuming and labor-intensive. Thus, a new, rapid, and sensitive method is needed to detect and quantify thiram in complex food matrices.

Surface-enhanced Raman spectroscopy (SERS) is an emerging technology based on Raman spectroscopy in combination with nanotechnology.[9] There are two widely accepted enhancement mechanisms: electromagnetic enhancement and

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chemical enhancement. In the electromagnetic enhancement, the excitation of localized surface plasmon resonance (LSPR) on the nanostructure surface can enhance the Raman scattering signals for many orders of magnitude. While in the chemical enhancement, metal-to-molecule or molecule-to-metal charge transfer occurs, which dramatically changes the resonances of the system and contributes to around 10^2 of enhancement.[10, 11] The shape, size, and material type of substrate can influence the signal enhancement. SERS is a highly sensitive and reproducible technology that has been used in food safety[12] and environmental monitoring.[13] Compared to HPLC and other methods, SERS has many advantages such as high efficiency, high sensitivity, portability, and rapid measurement of samples.

This study aimed to develop a novel two-step seed-mediated growth method to synthesize polyhedral AuNS as a SERS substrate. The synthesis process was well controlled by changing the reaction conditions (concentration, temperature, time, pH of the reaction system, etc.). Thus, AuNS can be synthesized in different particle sizes and morphology[14]. Instead of CTAB, hydroquinone was used to prepare a cleaner AuNS solution. AuNS were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Peach juice spiked with thiram was detected by the SERS method coupled with AuNS substrate. Data analysis was conducted using multivariate statistical analysis (*i.e.* partial least squares (PLS) regression).

Materials and methods

Chemical and materials

Hydrogen tetrachloroaurate solution ($HAuCl_4$, 30 wt% in dilute HCl), thiram (analytical standard), 4-aminothiophenol (4-ATP, 97%), Hydroquinone and acetonitrile (HPLC Plus, ≥99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trisodium citrate dihydrate ($Na_3Cit{\cdot}2H_2O$), Acetone (HPLC grade) and Hydrochloric acid (Certified ACS Plus) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Organic peach juice was purchased from a local grocery store. All chemicals were used as received without further purification and Milli-Q water was used throughout all experiments.

Synthesis of seed solution

A volume of 300 μL of 1% chloroauric acid was added to 30 mL of deionized water, followed by stirring and heating to the boiling temperature. Then 100 mL of 1% sodium citrate solution was added in a flask and stirred continually until the color of the solution changed to light red wine colour.

Preparation of growth solution for synthesis of AuNS

A volume of 25 μL of 100 mM chloroauric acid solution was added to 10 mL of deionized water solution and stirred continuously. A volume of 50 μL of gold seed solution, 22 μL of 1% sodium citrate solution, and 1 mL of 30 mM hydroquinone solution were then added into the mixture and stirred at room temperature for 30 min until the colour of the solution changed to light blue.

Preparation of 4-ATP solutions

Different concentrations (10, 50, 100, and 500 ppb, 1 and 5 ppm) of 4-ATP were used as the Raman probe to measure the enhancement of AuNS.

Preparation of thiram in juice

Different concentrations (50 ppb, 100 ppb, and 1 ppm) of thiram in peach juice were prepared. Peach juice without thiram was used as the control group. The peach juice samples were filtered twice prior to the SERS measurement.

SERS measurement

A DXR2 Raman microscope with 785 nm laser source (Thermo Fisher Scientific Inc., USA) was used in this experiment. A volume of 2 μL of AuNS substrate solution and 2 μL of sample were mixed and dropped onto a gold plate at room temperature and then placed on a hot plate at 40°C for drying. During the =SERS measurement, a laser source (20 mW) was used to detect the samples on the substrate. Each sample was measured and its averaged spectra were acquired from 10 different spots on a substrate. Data were collected by the OMNIC software (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Data Analysis

The Delight Software Version 3.2.1 (D-Squared Development Inc., LaGrande, OR, USA) was used in the data analysis. Calibration curve was established by plotting the SERS intensity as a function of analyte concentration. Each point on the calibration curve represented an average value of ten replicate measurements. The following formula was used to calculate the limit of detection (LOD):

$$S_m = \overline{S_b} + k\sigma_{S_b}$$

where S_m is the minimum distinguishable signal, S_b is the Raman signal generated by a blank measurement of the SERS substrate in the absence of the analyte, σ_{S_b} is the standard deviation of blank measurements and k is the proportionality constant. The concentration value is determined by substituting the minimum distinguishable signals to the best-fit equation of the calibration curve to determine the LOD.

To determine the analytical enhancement factor (AEF) of AuNS as a SERS substrate, an aliquot of 3 μL of sample solution was deposited onto the spot of AuNS and a bare gold-coated glass slide, respectively. The AEF of SERS method was calculated according to the following equation:

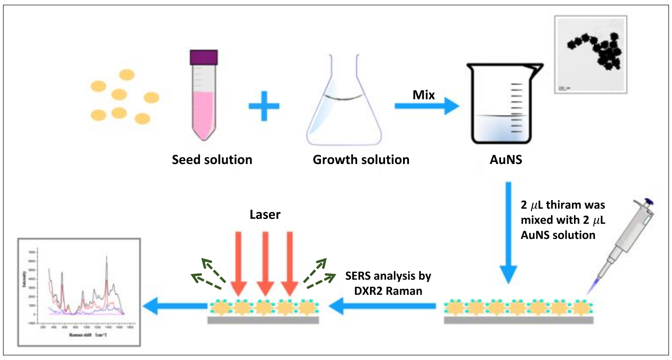
$$AEF = I_{SERS}/I_{RS} \times C_{RS}/C_{SERS}$$

where I_{SERS} and I_{RS} are the peak intensity in a SERS spectrum and a normal Raman spectrum, respectively. C_{SERS} and C_{RS} are the analyte concentration in the SERS measurement and the normal Raman measurement, respectively.

Results & Discussion

Synthesis and characterization of polyhedral AuNS

Scheme 1 shows the synthesis procedures of AuNS and the major steps of using AuNS in SERS measurement. Briefly, AuNS were prepared by a two-step seed-mediated growth method. Firstly, a certain concentration of sodium citrate was used to reduce chloroauric acid to form gold seeds in solution. Next, in the growth solution, sodium citrate acted as a reducing agent and hydroquinone was used as a morphological inducer. Although sodium citrate has a weak reducing ability, it can still reduce trivalent Au^{3+} in the reaction system to monovalent Au^{+}. Then hydroquinone, a strong reducing agent, continued to reduce the monovalent Au^{+} to Au, forming 3D irregular star-shaped nanostructures. Figure 1 shows the SEM images of AuNS at different magnifications, demonstrating that AuNS consist of a central core of ~80 nm and multiple protruding antennae with the size of 4-6 nm. The formation of AuNS resulted in a light blue transparent solution (Figure 1d).



Scheme 1. The mechanism and analysis process for AuNS.

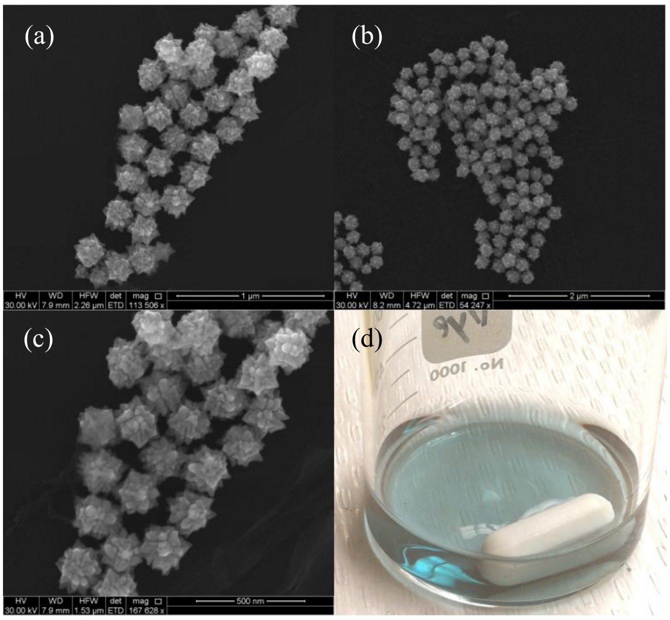


Figure 1. SEM images of AuNS substrate at different magnifications: 1 μm (a), 2 μm (b), 500 nm (c), AuNS solution (d).

TEM images (Figure 2) show that as-prepared AuNS are star-shaped nanostructures with multiple antennae. The synthesized AuNS is uniform in size with antenna size of around 4-6 nm. The surface of AuNS is very rough, which can adsorb more pesticide molecules and generate more hot spots than the smooth surface of gold nanoparticles. SEM and TEM images (Figure 1 & 2) indicate that, as compared with the AuNS solution synthesized by CTAB, the AuNS solution synthesized by hydroquinone was clearer and has fewer impurities, which eliminated the need to remove CTAB after synthesis.

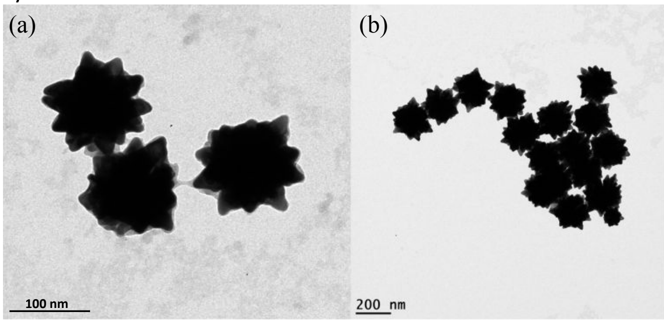


Figure 2. TEM images of AuNS substrates at different magnifications: 100 nm (a), 200 nm (b).

The performance of AuNS substrates in SERS measurement

To test the performance of synthesized AuNS, 4-ATP was used as a Raman reporter in this study. A previous study showed that 4-ATP is a good probe molecule that can be used in SERS coupled with gold nanoparticles.[15] In this study, a series of 4-ATP solutions from 10 ppb to 5 ppm were selected for the SERS measurement. Figure 3 clearly shows SERS spectra (n=6) in the wavelength range of 400-1900 cm^{-1}. The spectra of different concentrations of 4-ATP could be easily distinguished. Two characteristic peaks at 1076 and 1583 cm^{-1} can be clearly observed. In addition, there is a clear increasing trend of Raman intensity of both peaks at 1076 and 1583 cm^{-1}. The AEF of AuNS as a SERS substrate was calculated to be around 10^4. These results indicate that the AuNS is a suitable substrate that can be used in SERS measurement.

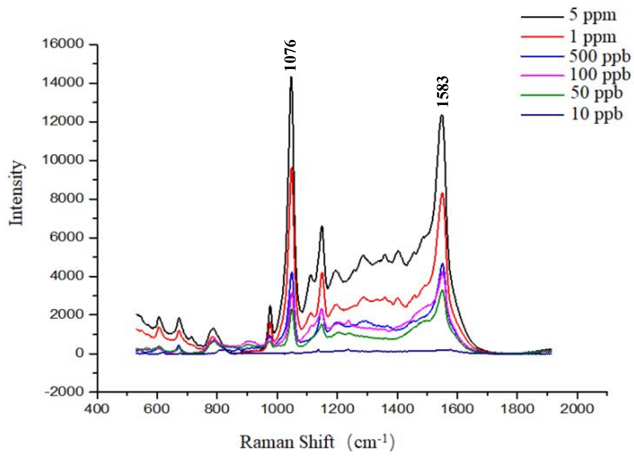


Figure 3. Raman spectra of 4-ATP in different concentrations acquired on AuNS substrate.

Detection of thiram in peach juice by SERS

To acquire characteristic Raman spectral features of thiram, thiram powders were deposited onto an AuNS substrate and tested by a Raman spectrometer. Figure 4 shows that there are more than 9 characteristic peaks of thiram in the range of 200 to 1700 cm^{-1}. It is noteworthy that a peak at 557 cm^{-1} is due to the S-S stretching mode, and a prominent peak at 1372 cm^{-1} arises from the C-N stretching mode.[16-18] LSPR is special phenomenon of noble nanoparticles. Strong electromagnetic fields are generated when the LSPR is excited by visible light. The Raman signals of the analytes being confined within these electromagnetic fields can be enhanced.[19 20] AuNS with unique structures can produce LSPR, resulting in greatly enhanced Raman signals of molecules on the AuNS surface.[21]

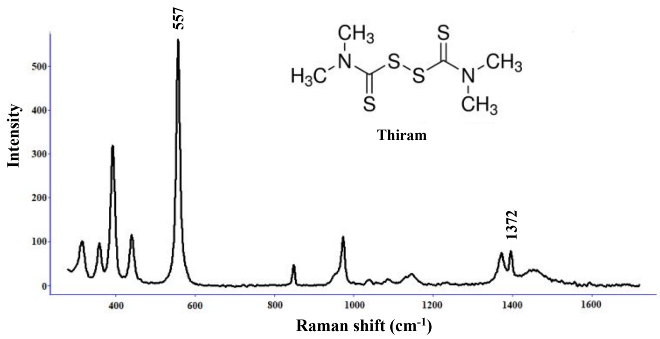


Figure 4. Raman spectra of pure thiram powders.

Figure 5a shows the average SERS spectra (n = 5) of difference concentration of thiram solution from 50 ppb to 1 ppm, demonstrating clear enhancement of Raman signals as compared to that of pure powders of thiram. The intensity of the peak at 1372 cm^{-1} of 1 ppm sample was 100 times higher than that of thiram powder. Figure 5b is the second derivative transformation of Raman spectra, which shows a clear increasing trend at 1372 cm^{-1}. Second derivative is commonly used to remove baseline offsets and enhance spectral differences. Figure 6 shows an optical image of AuNS and the corresponding SERS intensity map of 1 ppm of thiram. The black part of the optical image indicates AuNS structures with many hot spots (Figure 6a). The SERS intensity map shows that the darker the colour in Figure 6a, the high (redder) the SERS intensity in the map (Figure 6b).

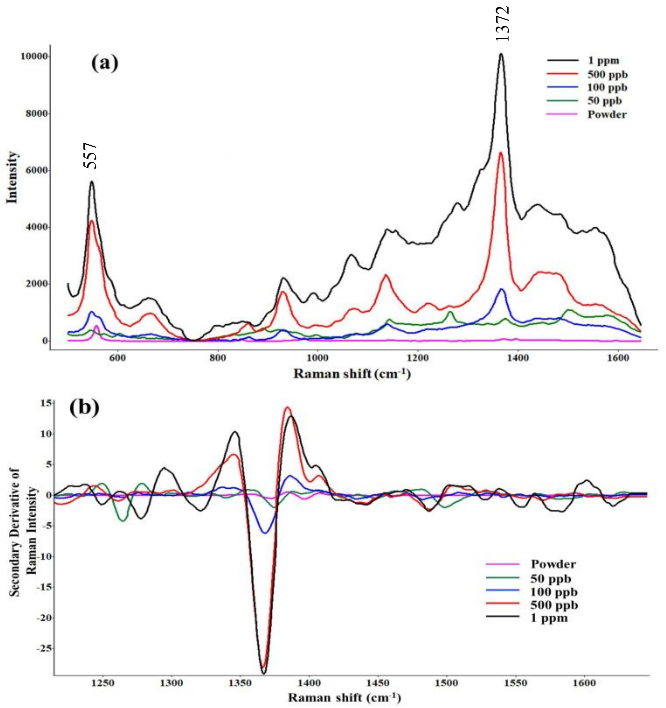


Figure 5. Average Raman spectra of thiram in different concentrations from 100 ppb to 1 ppm (a); second derivative transformation of Raman spectra (b).

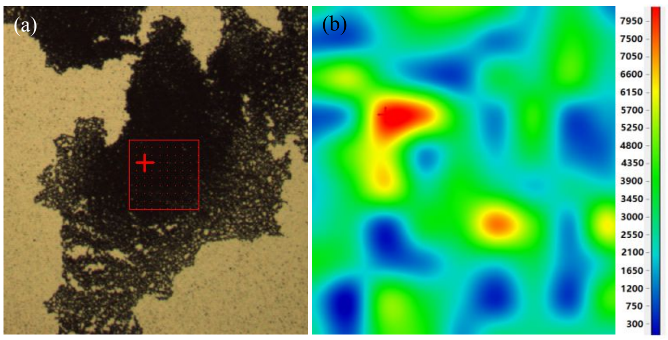


Figure 6. Optical images of AuNS and corresponding SERS intensity maps of 1 ppm of thiram.

Figure 7 shows the Raman spectra acquired from 10 randomly selected points in the area shown in the optical image (Figure 6a), indicating excellent consistency of Raman spectra including two peaks at 557 and 1372 cm^{-1}. These results reveal that AuNS can be used as a reliable substrate for the SERS measurement.

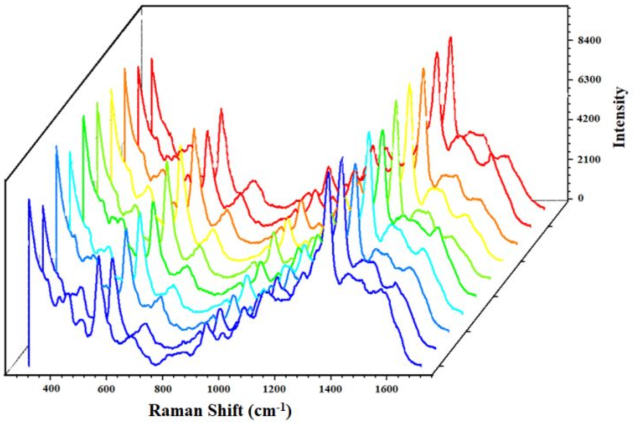


Figure 7. SERS spectra of 1 ppm of thiram collected from 10 randomly selected points from the area indicated by red lines in the optical images.

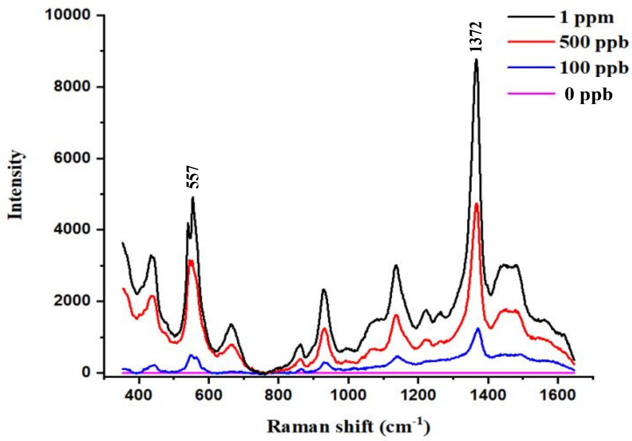


Figure 8. Average Raman spectra of thiram in peach juice in different concentrations (0, 100, 500 ppb and 1 ppm).

Peach juice contains more pectin and pulp components than other beverages. In this study, the juice samples were filtered to remove the interfering compounds prior to the SERS measurement. Figure 8 shows the Raman spectra of organic peach juice spiked with different concentrations of thiram from 100 ppb to 1 ppm. Characteristic peaks of thiram in peach juice are relatively consistent with that of the pure thiram solution. However, the intensity of characteristic peaks of the thiram pure solution is higher than that of peach juice samples, which is due to the fact that sugars and pectin in peach juice might interact and form complexes with pesticide molecules, thus reducing the number of pesticide molecules that interacted with AuNS. Nevertheless, a discernible peak can still be observed at a concentration of 100 ppb. The sensitivity of AuNS is 100 times higher than that of normal gold nanoparticles (Figure S1). Figure S2 shows the calibration plot of the Raman peak intensity at 1372 cm^{-1} and different concentrations of thiram solutions. SERS coupled with AuNS was used to detect thiram residues in peach juice and the LOD was 50 ppb (μg/kg), which is lower than the maximum residue limit (7 ppm) of thiram in fruit set by the U.S. Environmental Protection Agency.[22] These results indicate that AuNS can be used as a good substrate in the detection of pesticide residues in foods. In addition, previous studies reported using various types of nanomaterials with different shapes (normal round nanoparticle, dog bone, and triangle shape) for testing pesticides.[23-25] Compared with those nanomaterials, the AuNS surface is rougher, which can provide more “hot spots”. In this study, AuNS were synthesized in a high controlled manner with fewer impurities, which greatly improved the SERS measurement. AuNS have 3D asymmetric structures with multiple antennas on the surface, which provide more hot spots than gold nanoparticles.

Conclusions

In summary, this study established a facile method of synthesizing polyhedral AuNS using hydroquinone, which is more efficient than traditional method by eliminating the need for the elution of CTAB after synthesis. The results show that SERS coupled with AuNS substrate is a highly sensitive technique, which is 100 times more sensitive than using normal nanoparticles. These results demonstrate that AuNS has a great potential to be used as a SERS substrate for the detection of food contaminants.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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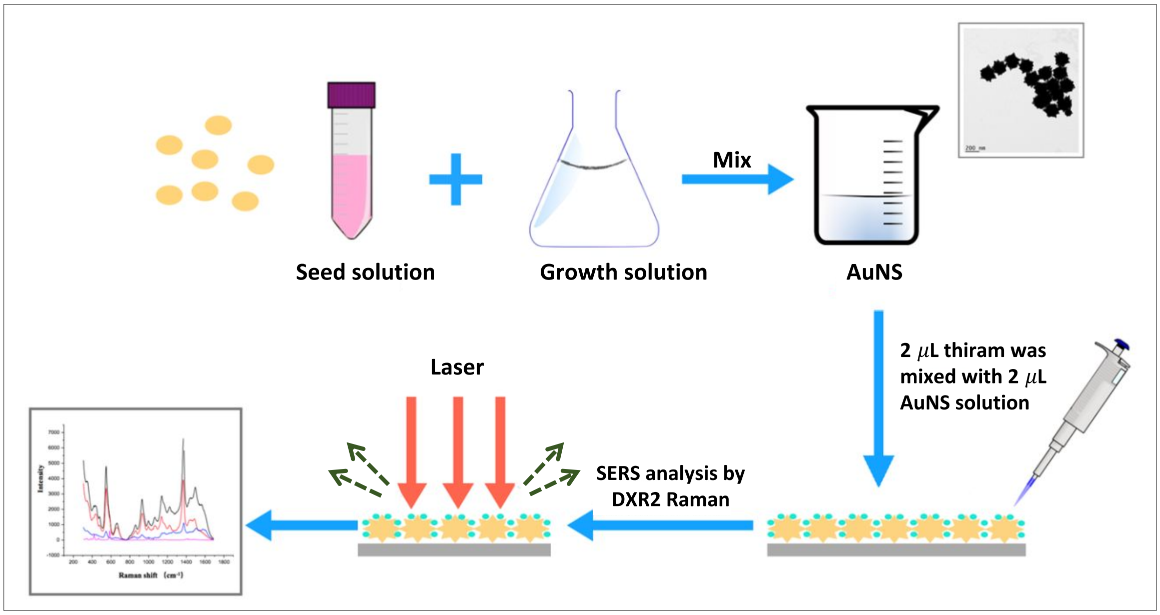

This study developed SERS method coupled with polyhedral gold nanostars to detect pesticide residues in juice products.