Synthesis of MBA-Encoded Silver/Silica Core-Shell Nanoparticles as Novel SERS Tags for Biosensing Gibberellin A₃ Based on Au@Fe₃O₄ as Substrate

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Abstract: A surface-enhanced Raman scattering (SERS) tag is proposed for high-sensitivity detection of gibberellin A₃ (GA₃). Silver nanoparticles (AgNPs) were synthesized using citrate reduction. 4-Mercaptobenzoic acid (MBA) was used for the Raman-labeled molecules, which were coupled to the surface of the AgNPs using sulfydryls. MBA was coated with silica using the Stöber method to prevent leakage. GA₃ antibodies were attached via the active functional groups N-Hydroxysuccinimide (NHS) and N-Ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) to construct a novel immuno-AgNPs@SiO₂ SERS tags. The captured SERS substrates were fabricated through Fe₃O₄ nanoparticles and gold nanoparticles (AuNPs) using chemical methods. These nanoparticles were characterized using ultraviolet-visible spectroscopy (UV–Vis), dynamic light scattering, Raman spectroscopy, transmission electron microscope (TEM), and X-ray diffraction (XRD). This immuno-AgNPs@SiO₂ SERS tags has a strong SERS signal based on characterizations via Raman spectroscopy. Based on antigen-antibody reaction, the immuno-Au@Fe₃O₄ nanoparticles can capture the GA₃ and AgNPs@SiO₂ SERS tags. Due to the increasing number of captured nanoprobes, the SERS signal from MBA was greatly enhanced, which favored the sensitive detection of GA₃. The linear equation for the SERS signal was y = −13635x + 202211 (R² = 0.9867), and the limit of detection (LOD) was 10⁻¹⁰ M. The proposed SERS tags are also applicable for the detection of other food risk factors.

Keywords: surface-enhanced Raman scattering; AgNPs@SiO₂ core-shell nanoparticles; Au@Fe₃O₄ nanoparticle; gibberellin

1. Introduction

Gibberellins (GAs) is a type of extensive plant growth regulator that promotes plant cell division, enhances plant growth and development, prolongs the preservation and freshness storage of fruit, and increases the output of seedless grapes [1,2]. Up to now, more than a hundred gibberellins have been isolated and determined in plants, fungi, and bacteria. Despite their structural similarity, only a small number of GAs display biological activity in plants, such as GA₃, GA₁, GA₇, and GA₄. Most of the GAs are considered to be precursors, intermediates and metabolites [3,4]. As is true for most pesticides, GAs may be harmful to humans who receive excessive exposure. Although such damage may not be developed over a short period, there is residual GAs in many fruit and vegetables.
Excessive ingestion of GAs may cause damage to the normal internal secretory system, chronic organ toxicity, and cancer [5–7]. There are several existing methods for GAs detection, including high-performance liquid chromatography (HPLC) [4], gas chromatography-mass spectrometry [8], liquid chromatography–electrospray tandem mass spectrometry (LC-MS) [9], and others [10]. However, liquid extraction and thin layer separation are needed for sample processing, which is complicated, time-consuming, and requires the abundant use of organic solvents.

SERS is a smart spectrum technology that has been developed over the past three decades. SERS can provide non-destructive and ultra-sensitive detection at the single molecule level, which is comparable with monomolecular fluorescence spectroscopy [11–13]. Due to the advantages of SERS—high sensitivity, high selectivity, and non-destructiveness—it has become a common tool in chemistry, biomedicine, and physics. It has been widely applied in many fields, including drug development, food safety, disease detection, explosives detection, imaging, minerals, and archaeology [14–17].

Immunoreactions provide a reliable, simple, and cheap method to recognize and perform quantitative analyses on specific antibodies or antigens. Therefore, several works have combined these two methods to construct a surface-enhanced Raman-immune detection system that has been extensively used to study small biological molecules, pathogenic bacteria, cells, and live imaging [18–20].

Herein, a quick detect, non-separated, high sensitivity, and cheap surface Raman-immuno detection system has been developed for the determination of GA3. Scheme 1 describes the fabrication process of the AgNPs@SiO2 SERS tags for GA3. The Raman reporter MBA were coupled on the surface of the AgNPs, and then were coated with SiO2 using the Stöber method to prevent leakage. GA3 antibodies were connected via active functional groups of NHS and EDC after amination of AgNPs@SiO2, and a novel immuno-AgNPs@SiO2 SERS tags was obtained. The captured SERS substrates (immuno-Au@Fe3O4 nanoparticles) were fabricated through Fe3O4 nanoparticles and AuNPs using chemical methods. Based on antigen-antibody reaction, immuno- Au@Fe3O4 nanoparticles can be captured the GA3 and AgNPs@SiO2 SERS tags. Due to the increasing number of captured nanoprobes, the SERS signal from MBA was greatly enhanced which favored the sensitive detection of GA3. The results suggested that the method possessed excellent potential for diagnostic immunoassay.

**Scheme 1.** Schematic representation of the synthesis procedure of the AgNPs@SiO2 SERS tags for the determination of GA3.
2. Materials and Methods

2.1. Reagents and Materials

Silver nitrate, trisodium citrate, chloroauric acid, ferric chloride, sodium acetate, polyethylene glycol, bovine serum albumin (BSA), isopropanol, and absolute ethylalcohol were obtained from Shanghai Sinopharm Chemical Regent Co. Ltd. Gibberellin A₃ (GA₃), forchlorfenuron, and the gibberellin antibody were purchased from Shanghai Yuanye biology Co. Ltd. 4-Mercaptobenzoic acid (MBA), Tetraethyl orthosilicate (TEOS), 3-aminopropyl-trimethylsilane (APTES), N-Hydroxysuccinimide (NHS), and N-Ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from Aladdin Chemicals Co. Ltd (Shanghai, China). For all aqueous solution, high-purity ultrapure water (18.18 MΩ cm) from a Millipore system was used for all experiments.

2.2. Measurements

All nanoparticles were characterized using transmission electron microscopy (TEM, Hitachi, Japan), scanning electron microscope (SEM, Hitachi, Japan), and X-ray diffraction (XRD, Rigaku, Japan). UV–Vis absorption spectra were acquired on a Cary 5000 spectrophotometer (Agilent, USA) with a 1.00 cm quartz cuvette sample holder. SERS were obtained using a Raman spectrometer 2000 (Renishaw, UK) equipped with a confocal microscope (Leica, Germany). A He-Ne laser (785 nm) excitation source was used in the experiment. A 20× objective lens was used to focus a laser spot on materials with a power of approximately 1 mW. The Raman spectra were collected with a 10 s exposure time and 2 accumulations from 400–2000 cm⁻¹.

2.3. Synthesis of Ag Nanoparticles

AgNPs were synthesized by dissolving 0.018 g of silver nitrate in 100 mL of ultrapure water. The mixture was slowly heated to 50 °C, after which 2 mL of 1% trisodium citrate solution was quickly added, and the solution was brought to a boil for 1 h. The solution was then cooled to room temperature. The obtained AgNPs were filtered with a 0.22 µm film and kept at 4 °C.

2.4. Synthesis of AgNPs@SiO₂ Core-Shell Nanoparticles

An 8 mL AgNPs was mixed with 40 µL of 10⁻³ M MBA and allowed to react for 30 min at room temperature. The mixture was centrifuged for 5 min at 9000 rpm and resuspended in 2 mL of ultrapure water. Then, 10 mL of isopropanol was added to achieve an alcohol-water ratio of 5:1. The pH was adjusted to ~ 9.5 using ammonium hydroxide. Then, 30 µL of TEOS was reacted with the nanoparticles for 30 min. The mixture was centrifuged for 5 min at 8000 rpm and rinsed twice using absolute ethyl alcohol. The prepared samples were then retained for later use.

2.5. Construction of Immuno-Ag@SiO₂ Core-Shell SERS Tags

A 4 mL Ag@SiO₂ core-shell nanoparticles and 40 µL APTES were mixed and reacted at room temperature for 30 min. The mixture was centrifuged for 5 min at 8000 rpm and resuspended in 2 mL of phosphate buffer saline (PBS). After, 100 µL of GA₃ antibody, conjugated to NHS and EDC, was added in core-shell nanoparticles and reacted for 60 min. Next, 200 µL of 1% BSA was added to block excess binding sites on the core-shell nanoparticles. The nanoparticles were centrifuged for 5 min at 8000 rpm and rinsed twice with PBS to obtain the core-shell SERS tags. The prepared core-shell SERS tags were retained for later use.

2.6. Characterized of the Obtained Nanoparticles by SEM and TEM

For SEM and TEM characterization, 2 µL of the obtained nanoparticles (Ag@SiO₂ and Au@Fe₃O₄) were put on a copper grid and dried at the room temperature. They were then transferred to detect on
the test platform and exhausted under vacuum. The morphology of nanoparticles was observed under 200 KV.

2.7. Synthesis of Fe$_3$O$_4$ Nanoparticles

Fe$_3$O$_4$ nanoparticles were synthesized according the group method [21]. 0.68 g of ferric chloride was dissolved in ethylene glycol (20 mL), followed by the addition of sodium acetate (1.8 g) and polyethylene glycol (0.5 g) under stirring vigorously, and transferred to a 25 mL Teflon lined stainless-steel autoclave. The autoclave was heated to and maintained at 200 °C for 7 h, and allowed to cool to room temperature. The black products were washed four times with ethanol and dried at 60 °C for 5 h.

A collection of 200 nm Fe$_3$O$_4$ nanoparticles (270 mg) were dispersed in 30 mL of methylbenzene and poured into a round-bottom flask. Then, 0.7 mL APTES was added and the mixture was stirred at 250 rpm at 80 °C for 8 h reflux. The reactants were rinsed with ultrapure water and ethyl alcohol alternatively until obtaining a neutral water solution. Impurities on the product surface were eliminated and the amino-Fe$_3$O$_4$ nanoparticles were stored in 4 °C ethyl alcohol for later use.

2.8. Synthesis of Au@Fe$_3$O$_4$ Nanoparticles

The dried, modified, amino-Fe$_3$O$_4$ powder (8 mg) and 110 mL of ultrapure water were mixed. After ultrasonic dispersion, 15 mL of 1.2 mmol chloroauric acid was added to the mixture and stirred evenly. Then, 570 µL of 0.2 M sodium citrate solution was added under ultrasonic conditions. Given a fixed frequency, the mixture was processed using ultrasonic processing at room temperature until the solution changed color from light yellow to black. At the end of the reaction, a permanent magnet was used for solution separation, which was cleaned with pure water three times until reaching a neutral pH.

2.9. Assembly of Immuno-Au@Fe$_3$O$_4$ Nanoparticles

A 10 mL sample of 0.5 mg/mL the Au@Fe$_3$O$_4$ nanoparticles was collected, and the pH was adjusted to 8.0. Then, 10 µL of 1 mg/mL GA$_3$ antibody was added and stirred via rotation for 0.5 h at room temperature. A 1.1 mL solution of 10% BSA was added to block the excess sites on the surface of Au@Fe$_3$O$_4$ nanoparticles. After centrifugation and dispersal, the immuno-Au@Fe$_3$O$_4$ nanoparticles were obtained and keep at 4 °C.

2.10. Immunoassay Detection of GA$_3$ Based on SERS Tags and Magnetic Nanoparticles

Ten pieces of eppendorf tubes were added to 50 µL of 0.1 mg/mL the immuno-Au@Fe$_3$O$_4$ nanoparticles. A series of different concentrations of GA$_3$, in 100 µL, was then added and reacted for 2 h at room temperature. After magnetic separation, 100 µL of PBS was added to disperse the sample. Next, 40 µL of the SERS tags and 100 µL of PBS were added and reacted for 2 h at room temperature. After performing magnetic separation twice, the sandwich complexes were formed and used for SERS detection.

3. Results and Discussion

3.1. UV–Vis Spectroscopy of AgNPs, MBA-AgNPs, Ag@SiO$_2$ Core-Shell Nanoparticles

To verify whether the Ag@SiO$_2$ core-shell nanoparticles were successfully synthesized, the obtained nanoparticles were characterized via UV–Vis spectroscopy, as illustrate in Figure 1. The maximum UV–Vis absorbance peak for the AgNPs was observed at 419 nm, which agrees with previous studies [22]. The peak width at half height is narrow and the peak shape is relatively symmetric, which indicates that the synthesized AgNPs have a relatively uniform shape and size. After an appropriate amount of MBA was added, the maximum absorbance peak of the solutions shifted to 420 nm, while the peak width at half height increased slightly. This indicates that the MBA was successfully coupled onto the AgNPs. The maximum absorbance peak of the obtained nanoparticles shifted from 419 to 460 nm when
silica was forming. It is very well known effect and from a simple model of the plasmon resonance in metal nanoparticles one can expect that coating with the material with the higher refractive induces the red-shift of the plasmon bands of metal nanoparticles [23,24].

3.2. TEM of AgNPs and Ag@SiO₂ Core-Shell Nanoparticles

To determine whether the synthesized nanoparticles have a core-shell structure and an appropriate size, their morphology was characterized using TEM. The TEM micrographs of the AgNPs and Ag@SiO₂ nanoparticles are shown in Figure 2. The synthesized AgNPs were well-dispersed and without aggregation from Figure 2A. The particle size was approximately 90 nm. After the silicon was coupled onto the AgNPs, the particle size increased. The particles have a clear core-shell structure, and the thickness of the surface silicon layer is around 20 nm from Figure 2B. Dynamic light scattering was used to confirm our result, as shown in Figure 3, zeta potential of AgNPs and Ag@SiO₂ core-shell nanoparticles is $-18.5 \text{ mV}$ and $-15.4 \text{ mV}$ because of reducing citrate on the surface of AgNPs, and the hydrodynamic diameter of Ag@SiO₂ was about 140 nm, while the hydrodynamic diameter of AgNPs is 95 nm.
3.3. UV–Vis Spectroscopy of Fe$_3$O$_4$ and Au@Fe$_3$O$_4$ Nanoparticles

Synthesis of the Au@Fe$_3$O$_4$ nanoparticles was preliminarily monitored using UV–Vis spectroscopy for Fe$_3$O$_4$ nanoparticles and Au@Fe$_3$O$_4$ nanoparticles, as shown in Figure 4. The Au@Fe$_3$O$_4$ nanoparticles have a maximum absorbance peak at 560 nm, while the Fe$_3$O$_4$ nanoparticles have no such peak. This suggests that gold enveloped the magnetic nanoparticles.

![UV–Vis Spectroscopy](image)

**Figure 4.** UV–Vis spectroscopy of Fe$_3$O$_4$ and Au@Fe$_3$O$_4$ nanoparticles.

3.4. Dynamic Light Scattering of Fe$_3$O$_4$ and Au@Fe$_3$O$_4$ Nanoparticles

During the synthesis of the Au@Fe$_3$O$_4$ nanoparticles, the intermediate products of each step were monitored using a dynamic light scattering instrument. The Fe atoms, which are exposed on the Fe$_3$O$_4$ surface, easily adsorb the OH$^-$ in water to form -OH functional groups. These functional groups have negative potentials as indicated by the increase in Zeta potential from $-$35.1 to $-$8.87 mV. The potential increased, indicating that the -NH$_2$ and -OH on the magnetic nanoparticles react to form amino functional groups. As shown in Figure 5, the hydrodynamic diameter of the amino- Fe$_3$O$_4$ was 237.4 nm in Figure 5A. When the Zeta potential increased to 30.6 mV, the magnetic nanoparticles were completely enveloped with gold to form Au@Fe$_3$O$_4$, the hydrodynamic diameter also increased to 275 nm in Figure 5B.

![Dynamic Light Scattering](image)

**Figure 3.** Dynamic light scattering of AgNPs(A) and Ag@SiO$_2$ core-shell nanoparticles (B).
3.5. X-Ray Diffraction Characterization of Au@Fe₃O₄ Nanoparticles

X-ray diffraction measurements were performed to determine the structure and morphology of the internal atoms and molecules of the materials. The characteristic peaks of gold were observed at 111, 200, 220, 311, and 222, indicating that the magnetic coating on the samples was made of elemental gold (Figure 6).

3.6. SEM Characterization of Fe₃O₄ and Au@Fe₃O₄ Nanoparticles

To determine the optimal synthesis conditions of the Au@Fe₃O₄ nanoparticles, the morphologies for the Fe₃O₄ and Au@Fe₃O₄ nanoparticles were compared under SEM. Figure 7 shows micrographs for (A) the 200 nm magnetic nanoparticles and (B) the Au@Fe₃O₄ nanoparticles. The magnetic nanoparticles have smooth surfaces while the Au@Fe₃O₄ surfaces are rough, which is caused by gold aggregation. These results are consistent with the zeta potential and XRD spectra. Therefore, gold is coated around the magnetic nanoparticle surface.
The analytical performance was assessed by measuring the SERS intensity at 1586 cm\(^{-1}\) separating. When GA was used as a blank, indicating that a small amount of SERS probe remained on the Fe\(_3\)O\(_4\) after magnetic separating. When GA\(_3\) was added, the SERS intensity at 1586 cm\(^{-1}\) obviously increased. From the Figure 10, a linear regression equation, y = 13,635x + 202,211 (R\(^2\) = 0.9867) was obtained between the SERS intensity of MBA and the concentration of GA\(_3\) using the 785 nm laser. There is a positive correlation between the concentration of GA\(_3\) and the Raman signal intensity of MBA. Figure 9 shows the SERS spectra for a concentration series of GA\(_3\). In the absence of GA\(_3\), a weak SERS signal was obtained (which was used as a blank), indicating that a small amount of SERS probe remained on the Fe\(_3\)O\(_4\) after magnetic separating. When GA\(_3\) was added, the SERS intensity at 1586 cm\(^{-1}\) obviously increased. From the

Figure 7. SEM characterization of Fe\(_3\)O\(_4\) (A) and Au@Fe\(_3\)O\(_4\) nanoparticles (B).

3.7. Raman Spectra of Solid MBA and AgNPs@SiO\(_2\) SERS Tags

To further determine whether the synthesized AgNPs@SiO\(_2\) tags show SERS activity, a Raman test on solid MBA and the probes was performed. The Raman spectra were obtained using the 785 nm laser, as shown in Figure 8A, the synthesized SERS probe shows a stronger Raman response compared to the Raman spectra of solid MBA. Peaks at 1076, 1173, 1394, and 1586 cm\(^{-1}\) correspond to the fingerprints of the MBA. The two Raman peaks at 1076 and 1586 cm\(^{-1}\) are attributed to the v\(_{12}\) and v\(_{8a}\) vibrations of the benzene ring on MBA [25,26]. The peak width at half height at 1586 cm\(^{-1}\) is relatively small and can be used to perform a quantitative analysis. For evaluating the SERS tags reproducibility, we measured SERS signals of MBA at 10 randomly chosen spots on the substrate, the relative standard deviation of the intensity at the 1586 cm\(^{-1}\) peak of MBA was calculated to be 15.55% (Figure 8B). The results indicated that the SERS tags had good reproducibility and stability during the preparation.

Figure 8. The Raman spectra of solid MBA and the AgNPs@SiO\(_2\) SERS probe (A). The SERS intensity of the AgNPs@SiO\(_2\) tags at the 1586 cm\(^{-1}\) peak of MBA (B).

3.8. SERS Detection of GA\(_3\)

The synthesized SERS tags was used to detect GA\(_3\). First, the immuno-Au@Fe\(_3\)O\(_4\) nanoparticles captured GA\(_3\) through antigen-antibody reactions. Second, the immuno-SERS probe was recognized automatically using the immunomagnetic nanoparticles to establish a sandwich detection model. The analytical performance was assessed by measuring the SERS intensity at 1586 cm\(^{-1}\) of MBA as a function of the GA\(_3\) concentration using the 785 nm laser. There is a positive correlation between the concentration of GA\(_3\) and the Raman signal intensity of MBA. Figure 9 shows the SERS spectra for a concentration series of GA\(_3\). In the absence of GA\(_3\), a weak SERS signal was obtained (which was used as a blank), indicating that a small amount of SERS probe remained on the Fe\(_3\)O\(_4\) after magnetic separating. When GA\(_3\) was added, the SERS intensity at 1586 cm\(^{-1}\) obviously increased. From the

\[
y = 13,635x + 202,211 \quad (R^2 = 0.9867)
\]
Figure 10, a linear regression equation, $y = -13,635x + 202,211$ ($R^2 = 0.9867$) was obtained between the negative logarithm of the concentration of GA$_3$, with a LOD of $10^{-10}$ M, where $y$ is the intensity of SERS peak at 1586 cm$^{-1}$ and $x$ is the negative logarithm of GA$_3$ concentration. This suggests that the proposed method is applicable for GA$_3$ detection. Compared to other methods, such as HPLC coupled with fluorescence detection [4], UPLC-MS [3,27], the proposed method had not only equivalent sensitivity, but also had advantage of fast detection, free-separated.

![SERS spectra for decreasing concentrations of GA$_3$.](image)

**Figure 9.** SERS spectra for decreasing concentrations of GA$_3$.

![Plot ratios of SERS intensities with the sample versus a series concentration of GA$_3$.](image)

**Figure 10.** Plot ratios of SERS intensities with the sample versus a series concentration of GA$_3$. Insert: the linear relationship of SERS intensity versus GA$_3$ concentration from $1 \times 10^{-6}$ to $1 \times 10^{-9}$ M, the error bars indicate standard deviations from three measurements.

4. Conclusions

Based on the facile separation of magnetic nanoparticles, a new method to detect GA$_3$ was developed based on SERS tags. SERS tags were constructed using AgNPs as a SERS substrate with embedded MBA by coating SiO$_2$. Au@Fe$_3$O$_4$ nanoparticles played a separation role, and SERS enhanced the functional role. Quantitative analysis of GA$_3$ was conducted using the SERS tags. The detection sensitivity was found to be $10^{-10}$ M. Compared with traditional detection methods, the developed method is fast, free-separated, and high sensitivity. It is a method that can be generalized and applied to other large and small single-biological molecules.
Author Contributions: Q.W. performed the sensor fabrication and Immunoassay; J.L., F.L. synthesized the core-shell SERS probe; C.W. detected SERS characterization; N.L. performed the material characterization; G.H., Z.L. supervised the work. All authors contributed to the writing and discussion of results.

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