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ABSTRACT

Although the concentration of plant hormone abscisic acid (ABA) in crops is very low, it can effectively regulate the growth of crops. Traditional ABA detection methods are limited by expensive instruments and cumbersome sample processing. Therefore, a new method for detecting ABA in ultra-low concentrations is urgently needed. In this paper, a new method for fast and accurate determination of ABA content based on surface enhanced raman spectroscopy (SERS) was proposed. In this method, a solution of silver-coated gold nanoparticles (Au@Ag) was self-assembled into dense monolayer under the action of capillary gradient force at the air/water interface, which could be transferred to PDMS wafers and filter paper as SERS active substrates. R6G was used as a probe molecule to characterize the Sensitivity and accuracy of PDMS SERS active substrates. When the PDMS active substrate was selected to detect ABA, the detection limit 1×10^{-11} M was obtained, indicating that PDMS supported core-shell precious metal monolayer substrate can be used as a effective active substrate for detecting ABA. This method is also expected to be applied to the detection of other plant hormones such as corn and soybean.

Keywords: Core-shell nanoparticles, SERS, Raman label, abscisic acid, PDMS, filter paper

1. INTRODUCTION

The plant hormone abscisic acid (ABA), as a regulator of plant responses to drought and cold, can effectively regulate root/crown growth, nutrition and reproduction, and play an important role in improving crop quality and yield [1]. Many methods have been used to detect ABA, among which the most widely used methods include gas chromatography/mass spectrometry (GC/MS) [2], high performance liquid chromatography/mass spectrometry (HPLC/MS) [3], and enzyme-linked immunosorbent assay (ELISA) [4]. However, these methods are generally limited to requiring cumbersome sample preprocessing and enrichment, as well as expensive laboratory equipment [5]. Therefore, the development of new methods for the detection of ABA at ultra-low concentrations is the basis for in vivo and on-line detection of plant hormones.

Surface-enhanced Raman scattering (SERS) has become a mature and powerful analytical technique, because it can effectively perform ultra-sensitive detection, which is derived from the fingerprint recognition ability of Raman spectrum and the enhanced sensitivity of plasma [6]. One of the keys to the application of SERS technology is the preparation of SERS substrates [7]. In the past two decades, researchers have devoted to various SERS enhanced substrates, among which flexible substrates have been especially frequently used in biological detection in recent years [8]. Both paper and PDMS substrates are common flexible substrates. The stickiness of PDMS and the flexibility of paper are very convenient in analyzing irregular surfaces. In this study, using the capillary gradient between air and water, the core-shell Au@Ag nanoparticles were induced self-assembly to a dense membrane of about 1 cm^2 in size distributed in a single layer. The dense membrane orderliness, closely arranged between particles, can be easily transferred to used the SERS substrate support. Raman enhancement of Au@Ag dense film was compared when common supporting matrix PDMS wafer and filter paper were used as supporting matrix in SERS experiment. R6G molecules were used as Raman probes to characterize the sensitivity, detection limit, repeatability and enhancement factors of two SERS substrates. Finally, the PDMS and paper supported Au@Ag core-shell dense membrane substrate were selected as the sensitive substrate for

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quantitative ABA detection, and the detection limit of ABA was determined as 1×10^{-11}M and 1×10^{-8}M respectively. The preparation of Au@Ag core-shell compact film supported by flexible material and its application in ABA detection provide a promising platform for the development and practical application of plant hormone biosensors.

2. THE EXPERIMENT PART

2.1 The preparation of silver coating gold nanoparticles

The preparation of silver coating gold nanoparticles can be divided into three steps. (1) Preparation of gold nanoparticles. Place 100 mL 0.01% chlorauric acid solution in the conical beaker on the magnetic heating stirrer, stir gently, set the heating temperature to 120°C, adjust the stirrer to intense stirring mode after the chlorauric acid solution boils, and quickly added 1 mL 1% trisodium citrate solution, after 10s, the solution turns gray, black and blue. Finally, it gradually turns purple red. After 10 minutes, turn off the heat source and continue to stir to room temperature. Refrigerated until further use. (2) signal molecules were modified on gold cores. 10 mL of the gold nanoparticles prepared above was taken into 20 mL glass bottle, then 5μL 1mM tetramercaptobenzoic acid (4-MBA) solution was dropped, stirring for 30 min, centrifuged to remove the supernatant, and disperse the precipitate in 10 mL deionized water again. (3) Coating silver shell. 10 mM ascorbic acid was added to Au-4MBA solution and stirred for 1 minute. After stirring, the stirring mode was adjusted to vigorous stirring mode. 150μL 20mM silver nitrate solution were added at the rate of 1 drop /3s, and the color of the solution gradually changed from purple-red to orange-red. The prepared Au@Ag NPs was stored in the refrigerator for later use.

2.2 Preparation of Au-4MBA@Ag nanomonolayer

12 mL Au@Ag core-shell nanoparticles was centrifuged, the supernatant was removed, and the sediment was added to a mixture of 950 μL n-butanol and 50 μL ethanol for 5 minutes by shaking. Fill a 50 mL beaker with deionized water and drop the mixture into the beaker with a pipette gun. N-butanol coated nanoparticles float on the surface of the water. After 3 hours, the water surface was completely still and a monolayer of Au @Ag core-shell nanoparticles formed on the water surface.

2.3 Sensitive detection of ABA

The nanomonolayer were transferred to support substrate filter paper and PDMS thin sheets, and then used as Raman substrates to test their enhancement. Nanomonolayer membrane was used as SERS substrate for the detection of plant hormone ABA. The linear relationship between the concentration and intensity of ABA was established by internal standard method, and the detection limit of ABA was calculated**.**

3. RESULTS AND DISCUSSION

3.1 Morphology characterization of Au@Ag nanoparticles

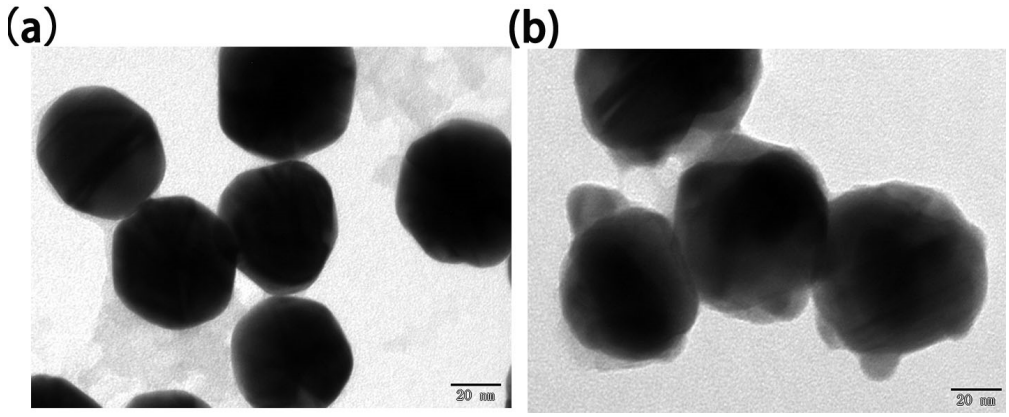

Figure 1. TEM photos of 49 nm AuNPs (a), the silver coated gold nanocomposites with 49 nm gold core and 9 nm Ag shell (b).

The transmission electron microscopy (TEM) of synthesized gold nanoparticles and silver coated gold core-shell nanoparticles are shown in Figure 1 (a) and (b). TEM images show that the size of gold nanoparticles was about 49 nm,

and the average thickness of silver shell was 9nm. Figure 2 (a) shows the Zeta potential changes of the synthesized Au nanoparticles and Au@Ag nanoparticles. Zeta potential characterization shows that the synthesized gold nanoparticles were negatively charged with a potential of -26MW, and the synthesized Au@Ag nanoparticles were negatively charged with a potential of -40.7 mW. Figure 2 (b) was Variation of the surface plasmon resonance (SPR) peak from Au nanoparticles to core-shell Au@Ag nanoparticles characterized by UV-Vis spectrophotometer. Uv-visible characterization indicated that the SPR peak of gold nanoparticles gradually disappeared with the silver shell gradually wrapping gold nanoparticles, while the SPR peak of silver gradually highlights, indicating that the silver shell successfully coated gold nanoparticles. The color of core-shell nanoparticles prepared is clear and bright, and the integrity of nanoparticles was good.

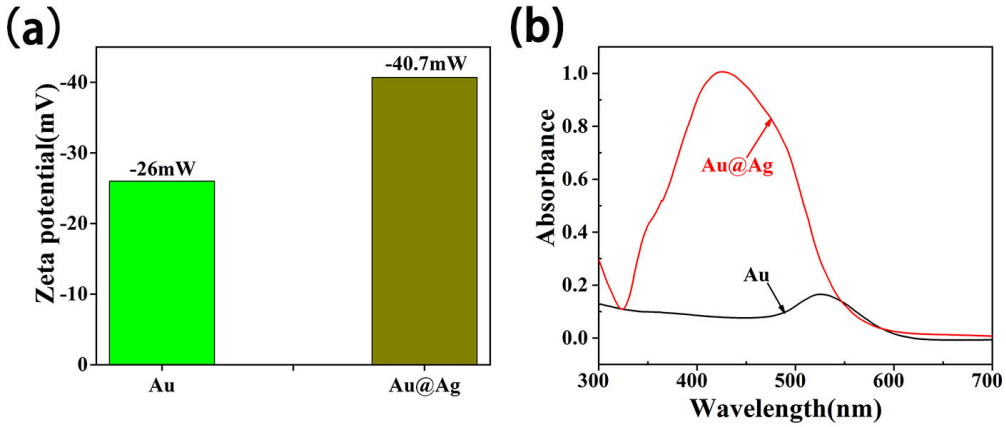

Figure 2. The ZETA potential (a) and The Uv-visible Spectrum and color (inset) (b) of AuNPs and Au@AgNPs.

3.2 Preparation of monolayer Au-4MBA@Ag nanoparticles

Figure 4 shows the monolayer film of Au-4MBA@Ag nanoparticles and the process of transferring nano particle membrane to PDMS. As can be seen from the figure, the appearance of the monolayer film formed with nanoparticles was golden yellow, with an area of about 1cm^2. It was very stable floating on the water surface, and was easily transferred to flexible substrates such as paper and PDMS flakes as the enhanced substrate for SERS. In addition, Only a tweezers was used to clamp the PDMS thin slice, gently go into the beaker, and lift the PDMS thin slice from bottom to top, the nano monolayer membrane could be easily transferred to the PDMS thin slice, forming a large area of SERS enhanced substrate.

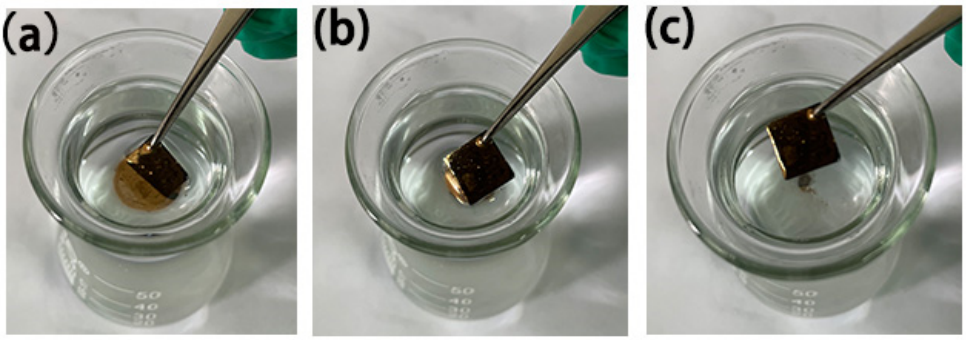

Figure 3. Au@Ag Transfer process of monolayer nanoparticles to PDMS film.

3.3 Sensitivity detection of flexible substrate

The sensitivity of SERS substrate was investigated using R6G as the target molecule. As shown in Figure 4, the Raman characteristic peaks of R6G were located at 1651 m^{-1}, 1512 cm^{-1}, 1361 cm^{-1}, 1312 cm^{-1}, and 1183 cm^{-1}, while the 4MBA's characteristic peak was 1589cm^{-1}. The SERS signal of R6G can still be distinctly clearly observed even if the concentration was reduced to 10^{-12} M, indicating the Au@Ag was an excellent SERS sensitivity substrate. The

relationship between the SERS intensity of the Raman peak centered at 1512 cm^{-1} and the logarithmic concentration of R6G revealed that SERS intensity of R6G had a high linear correlation (R2=0.9864) with its logarithmic concentration.

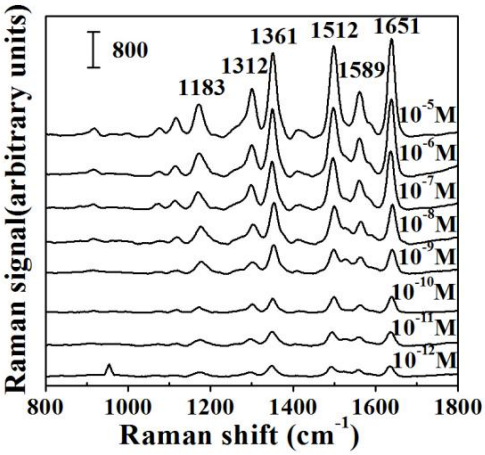

Figure 4. SERS intensities of R6G with different concentrations absorbed on Au@Ag nanocomposites.

3.4 Plant hormone ABA was detected by SERS of single membrane filter paper and PDMS substrate

10 μL ABA solution with different concentrations was dropped on PDMS and filter paper substrate, and SERS detection was conducted after drying. The SERS spectrum detected was shown in Figure 5 (a) and 6 (a). As can be seen from the figure, with the decrease of ABA concentration, the Raman characteristic peak of 4MBA at 1589 cm^{-1} does not change,while the characteristic peak of ABA at 1637 cm^{-1} decreases continuously. For filter paper substrate, The concentration of ABA could be detected at 1×10^{-8} M, and for.

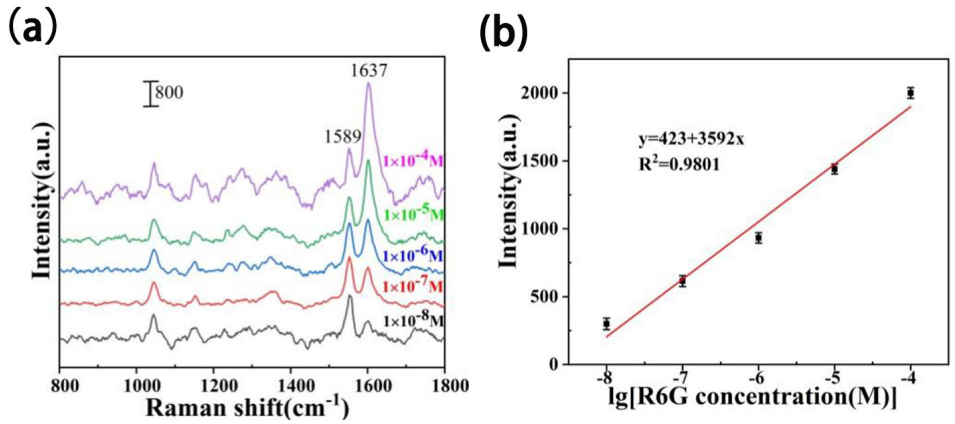

Figure 5. ABA was detected by filter paper substrate. The SERS spectral (a) and The linear relationship (b).

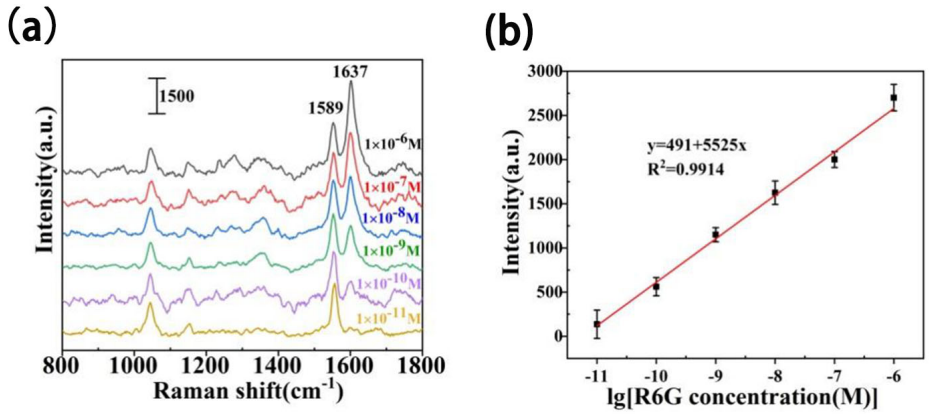

Figure 6. ABA was detected by PDMS substrate. The SERS spectral (a) and The linear relationship (b).

4. CONCLUSION

Driven by gradient capillary force on the liquid surface, the synthesized Au@Ag nanoparticle solution formed a monolayer at the water/liquid interface. The monolayer film was simple to prepare, the distribution of nanoparticles was uniform, and had a large area and good tension, which could be easily transferred to the flexible substrate as SERS active substrate. The experiment proved that the flexible substrate had good SERS enhancement and sensitivity. R6G molecule was used as probe molecule, and its detection limit was 1×10^{-12} M. The core-shell monolayer was used to detect plant hormone ABA for the first time, and very good detection limit and linear relation were obtained. The application of the nanoparticle membrane in ABA detection laid a foundation for SERS detection of other plant hormones. The transfer of the nanoparticle membrane to the flexible substrate PDMS and filter paper can provide technical support for the later online detection of plant hormones. In future studies, the recycling possibility of flexible SERS substrates will be further studied, and a more economical, fast and suitable for various contact surfaces will be developed.

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