

Determination of fenamidone residues by surface-enhanced Raman spectroscopy

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Abstract. The purpose of this study is to establish a novel, simple and rapid surface-enhanced Raman spectroscopy (SERS) detection technology for fenamidone. Fenamidone is a fungicide widely used with curative activity against oomycete diseases. Gold nanoparticles (AuNPs) were prepared by improved trisodium citrate reduction method of chloroauric acid. As a surface-enhanced Raman scattering (SERS) active colloid, the optimized product had the advantages of high sensitivity, reproducibility and chemical stability. The Results showed that the detection limit of fenamidone adsorbed on AuNPs was 0.01 mg/kg. The linear response between SERS intensity and logarithmic concentration was $R^2 = 0.9562$. The method was preliminarily applied in tobacco sample and showed its potential to serve as an alternative rapid quantification method of fenamidone residues with simplicity, efficiency, high accuracy and precision.

1. Introduction

Fenamidone is a new imidazolinone fungicide with high activity against downy mildew caused by oomycetes in grapes, vegetables, tobacco and other crops. It also has control effect on other pathogenic bacteria such as ascomycetes and *Alternaria alternata*. Fenamidone is widely used in the control of various crop diseases and insect pests such as downy mildew, blight and pear black rot [1-3]. Imidazolinone is less toxic, but all countries have strict limits on its residue, such as the EU's recommendation on the residue limit of fenamidone of 2 mg/kg [4]. In order to deal with the import and export of agricultural products trade and quality and safety supervision needs, it is urgent and of great practical significance to establish a simple, accurate and highly sensitive detection method for the analysis of fenamidone residues in natural products.

At present, the main methods for detecting fenamidone residues are gas chromatography [5], gas chromatography mass spectrometry [6-7] and liquid chromatography mass spectrometry [8-10]. Chromatographic methods have great limitations in real-time monitoring since they require high operating environment, complex and time-consuming analysis process, expensive equipment and the participation of professionals. Surface-enhanced Raman spectroscopy (SERS) is a powerful analytical tool, which plays an irreplaceable role in material composition identification and molecular structure analysis [11]. SERS technology uses gold, silver, copper and other metals with roughened surfaces as active substrates, which significantly enhances Raman scattering signals. SERS

technology has the advantages of less sample preparation, short measurement time, relatively high sensitivity and accuracy, which is suitable for rapid and onsite detection, and is expected to become a new method of pesticide analysis [12-14].

In this paper, colloidal gold nanoparticles (AuNPs) were used as SERS substrate to make fenamidone pesticide adsorbed on the surface of Au NPs, which amplified the Raman signal, and realized the rapid and on-site detection of fenamidone residues in tobacco.

2. Materials and Methods

2.1 Instruments and Reagents

EZ Raman-SSR 3000 Raman portable spectrometer (Nanjing, China) collects the spectral information of fenamidone, with the wavelength of 785 nm, the integration time of 2 s, the laser power of 350 mW, and the collection range from 500 to 2000 cm^{-1} . H2500R high-speed centrifuge (Hunan Xiangyi Experimental Instrument Development Co., Ltd.). XH-C vortex oscillator (Jiangsu Tianyi Instrument Co., Ltd.). MLtrapure water meter (Millipore Company, USA.). Fenamidone standard substance (99.9% purity from Aladdin Reagent Shanghai Co., Ltd.). Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), acetonitrile, sodium citrate, and L-ascorbic acid (AA) from Shanghai Yuanye Biotechnology Co., Ltd.

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2.2 Colloidal Gold Nanoparticles Synthesis Method

In this study, the seed growth method was used to synthesize the colloidal gold nanoparticles. Specifically, 200 μL of 1% chloroauric acid was added to 20 mL of water in a round-bottomed flask. After stirring in an oil bath at 130°C for 20 minutes, 100 μL of 0.1 mol/L sodium citrate was then slowly dropped in and stirred for 10 minutes. At this time, the solution color was burgundy. Continuously adding 50 μL sodium citrate (0.1 mol/L) and 100 μL 1% chloroauric acid into the solution while heating and stirring for 8 min. Repeated this operation for three times, and then took out the solution and stored in the refrigerator at 4°C for later use.

2.3 Raman Spectrum Acquisition of Fenamidone Standard

Prepared different concentrations of fenamidone standard solutions (0.01, 0.05, 0.10, 1.00 mg/L). Mixed 200 μL of each standard solution with 200 μL AuNPs surface enhancer. Under the conditions of 785 nm excitation light, 500 mW laser power and 10 s scanning time, the Raman spectrum was collected by portable Raman spectrometer.

2.4 Sample Processing and Determination

The tobacco samples were baked at 40°C for 15 h, crushed, sieved with 40 mesh sieve and sealed for storage. Accurately weighted 1 g of tobacco powder, put it into a 50 mL conical flask, added 20 mL of acetonitrile, ultrasonically extracted at room temperature for 30 min, filtered, concentrated the filtrate with rotary evaporator at 50°C to dryness, dissolved the residue with acetonitrile, and at last set the volume to 10 mL.

3. Results and discussion

3.1 Characterization of AuNPs

SERS enhancement ability is related to the size and distribution of AuNPs. The morphology and particle size of AuNP were identified by UV-vis spectrum and electron microscope. Figure 1 showed a high-resolution TEM image of AuNPs of a core-shell sphere structure, with a core size of 28.5 ± 0.5 nm and a shell thickness of 6.2 nm. In addition, the colloid exhibits an absorption spectrum in the region of 460 ~ 620 nm, with a characteristic band at 530 nm. These Results showed that the required AuNPs were successfully obtained, and the eminent light absorption performance indicates that AuNPs had regular structures and purified chemical components.

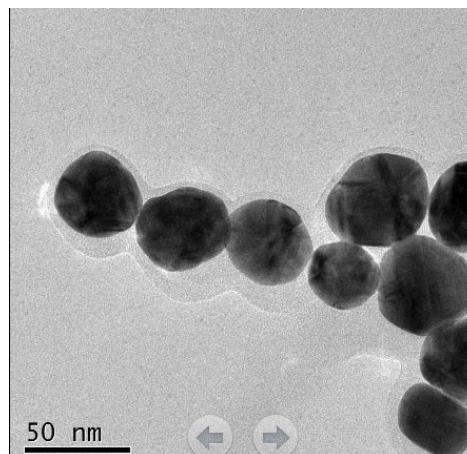


Figure 1. TEM images of AuNPs

3.2 Performance of AuNPs as SERS Substrate

R6G molecule was used as Raman probe to evaluate SERS enhancement performance of AuNPs substrate. As shown in the Figure 2, when the concentration of R6G was 0.05 mg/mL, mixed with the gold nanoparticles by 1:1 volumely, Raman characteristic peaks of R6G were observed at 611, 771, 1125, 1189, 1308, 1360, 1649 cm^{-1} [15,16]. Correspondingly, in the absence of AuNPs, the Raman characteristic peaks of R6G were almost not able to observed, proving that the AuNPs synthesized in this study had the effect of Raman signal enhancement.

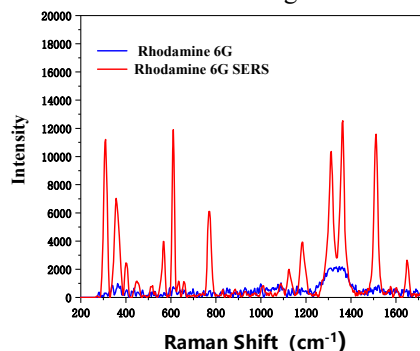


Figure 2. SERS spectra of R6G aqueous solutions

3.3 Fenamidone Raman Spectroscopy and the Quantitative Determination of Fenamidone

From the SERS spectra of fenamidone series standard solutions (Figure 3), we observed the main characteristic peaks of fenamidone in SERS are 671, 750, 1368, and 1557 cm^{-1} , and further, the intensity of characteristic peaks varies with the concentration of standard solutions (5, 4, 3, 5, 3, 2.5, 2, 1.5, 1, 0.1 and 0.01 $\mu\text{g/mL}$). The apparent characteristic peaks could still be observed at the concentration as low as 0.01 mg/L, which indicated that the detection limit of fenamidone by this method could be as low as 0.01 mg/L. With the concentration of fenamidone increased from 0.1 to 5 mg/L, the peak intensity at 1368 cm^{-1} changed more obviously than other characteristic peaks. Therefore 1368 cm^{-1} was more suitable for quantitative determination of fenamidone. Fig. 4 showed the curve of logarithmic peak intensities of fenamidone (1368 cm^{-1} , Figure 3) changing with the

logarithmic concentrations. The logarithmic SERS intensity at 1368 cm^{-1} had a linear relationship with the logarithmic concentration of fenamidone in the range of 0.1 to 5 mg/L. The linear equation was $y = 2723.91x + 4222.79$, and the correlation coefficient R^2 was 0.9562. In addition, the concentration of fenamidone was also detected in the range of 0.01 to 0.1 mg/L, but there was no linear relationship with the above curve. Further, using the same method, we measured fenamidone in tobacco samples and obtained the Raman spectrum of actual sample. As shown in Figure 4, the most significant peak changed with the concentration was also at 1368 cm^{-1} . A standard addition experiment was performed at the spiked concentration of 3 mg/mg with 10 times repetitive measurements. The relative standard deviation (RSD) values of the 10 measurements were 1.38% at 1368 cm^{-1} , revealed this method of fair repetitions.

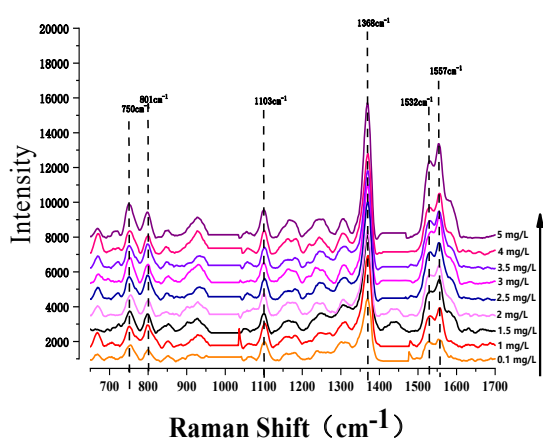


Figure 3. SERS spectra of fenamidone standard

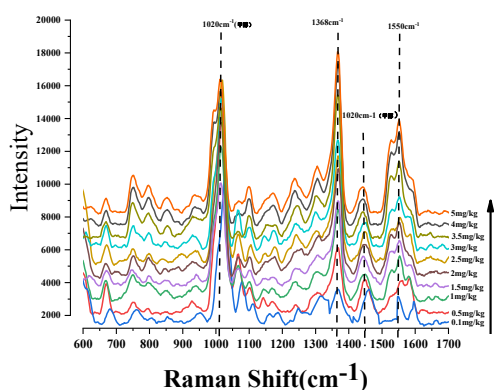


Figure 4. SERS spectra of fenamidone in tobacco extraction

4. Conclusions

In this study, we optimized the AuNPs colloid synthetic method, and used portable Raman spectrometer combined with SERS technology for the quantitative determination of fenamidone residues. The synthesized AuNPs colloid had obvious Raman signal amplification effect on fenamidone. The limit of detection of fenamidone standard was 0.01 mg/kg and the operation time consumption is within 10min. Considering that the MRLs of fenamidone for different plants in the food standards of

China were different, from the lowest 0.02 mg/kg in potato and the highest 40 mg/kg in celery [17], this method was potentially able to fulfill the test requirements and alternatively served as a simple, efficient, and accurate rapid quantification method of fenamidone residues.

Acknowledgments

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