Detection of chlorpyrifos in apples using gold nanoparticles based on surface enhanced Raman spectroscopy

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Abstract: In this study, gold nanoparticles (AuNPs) were synthesized for rapid and sensitive characterization and quantification of chlorpyrifos in apples. Min-max signal adaptive zooming and second derivative transformation method were adopted to pre-process Raman spectral signal. The min-max signal adaptive zooming method showed a higher correlation coefficient than derivative transformation when developing linear calibration curve between chlorpyrifos pesticide and Raman spectral peak intensity. The present method had a high reproducibility with the relative standard deviation less than 15%. Regression models showed a good linear relationship (R=0.962) between intensity of characteristic spectral peaks (at 677 cm^{-1}) and chlorpyrifos concentration on whole apples ranging from 0.13 mg/kg to 7.59 mg/kg. The application of surface enhancement Raman spectroscopy (SERS) detected chlorpyrifos pesticide to the detection limit of 0.13 mg/kg, which can be applied further for lower concentration in the future. The method presented in this study can provide a way-out for detection of pesticide residue in whole apple to trace amount.

Keywords: surface enhancement Raman spectroscopy, apple, chlorpyrifos, gold nanoparticles, pesticide detection

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1 Introduction

Pesticides are defined by the UN Food and Agricultural Organization (FAO) as substances or mixtures intended to prevent, destroy, repel or mitigate any pest, including insects, rodents and weeds[1]. Chlorpyrifos, O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate, is a highly efficient and broad spectrum organophosphorus insecticide that has been widely applied around the world to control a number of insects on horticultural crops and ornamental plants. However, the presence of pesticides in vegetables and fruits has been reported, and thus, there has been an increasing concern about dietary intake of these pesticides residues in foods and their potential risks to human health. Currently, high performance liquid chromatography (HPLC)[2], liquid chromatography–mass spectrometry (LC/MS)[3], and gas chromatography-mass spectrometry (GC/MS)[4] are the main analytical methods used for detection of pesticides. Jiang et al.[5] developed electrochemical immunosensor method to reduce chlorpyrifos detection limit in agricultural products to 0.01 g/mL. Kim et al.[6] applied immunochromatographic assay to detection chlorpyrifos in agricultural samples, the detection limit was 50 ng/mL. Although these techniques can be used to detect trace amounts of chlorpyrifos residues, they are time consuming, sample destructive, and require sample pretreatment[7].

In recent years, there has been increasing interests in the use of vibrational spectroscopic methods such as Raman spectroscopy to evaluate food safety and quality[8].

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The advantage that Raman spectroscopy offers lies in the rich structural information and the saving of time and labor because of little or no sample preparation. Surface enhanced Raman spectroscopy (SERS) represents a great advance in the field of Raman spectroscopy during the 1970s. An incident laser light could excite the localized surface plasmons on the metal when properly matched to the nano-particles and substance under detection. This leads to redistribution of the local field and a great enhancement of the electromagnetic field at a specific position around the nanoparticles (called ''hot spots''). There are also reports of chemical enhancement, charge transfer between chemisorbed molecules and a metal surface, which provides Raman enhancement by one or two orders of magnitude. Recently, the enhancement factor of single molecule Raman signal at the nano structural junction was up to 10^{13}-10^{14} orders[9-11]. However, only free-electron-like metals such as Au, Ag and Cu with nanostructured surfaces can exhibit huge SERS activity[12]. To date, gold and silver are the two most frequently used metals for fabrication of SERS-active nano substrates[13]. Gold nanoparticles (AuNPs) are used in the research, due to the advantages of the oxidation resistance, stability and good biocompatibility.

This study aims to use SERS to detect chlorpyrifos in apples. AuNPs were used in the SERS measurements to acquire enhanced Raman signals of chlorpyrifos. Large AuNPs were prepared due to the fact that AuNPs diameter larger than 50 nm gives high enhancement[12]. The fluorescence background of SERS signals were removed by min-max signal adaptive zooming method[14] and second derivative transformation method. Linear relationships between the chlorpyrifos concentrations and the intensities of characteristic peaks of SERS spectra were developed to see the possibility of using SERS for quantitative analysis of chlorpyrifos.

2 Materials and methods

2.1 Apparatus

Raman instrument used in this study was designed as explained in our previous literature[15]. Raman spectrometer (Raman Explorer 785, Headwall Photonics, Fitchburg, MA, USA) equipped with a 785 nm diode laser source was utilized to collect Raman spectra. Raman scattering signal from the sample was detected via a 16 bit high performance spectroscopic CCD camera (Andor Newton DU920PBR-DD, Andor Technology, Inc., South Windsor, Conn.). All spectra were recorded with 450 mW laser power from 176 cm^{-1} to 2 400 cm^{-1} with a spectral resolution of about 2 cm^{-1}. Computer and Raman system was connected by a USB cable for data transfer and hardware control for collection of Raman spectral data from equatorial region of sample surface.

2.2 Materials

Solid chlorpyrifos (98.5%) was used to confirm Raman fingerprint of chlorpyrifos from Dr. Ehrensorfer GmbH (Augsburg, Germany). Commercially available chlorpyrifos solution (48% chlorpyrifos and remaining 52% other chemical compounds) was used to prepare pesticide solution. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from National Chemical Pharmaceutical Co., China. Sodium citrate ($C_6H_5Na_3O_7$), Nitric acid (HNO_3) and other reagents were of analytical grade. All solutions were prepared using double distilled water.

2.3 Gold nanoparticles preparation

$HAuCl_4$ (0.1 M, 0.25 mL) was added into 100 mL of ultrapure water. The mixture was heated to boiling with continuous stirring. After quickly injecting 1.5 mL of 1% trisodium citrate, the mixed solution was refluxed for about 30 min until it turned to wine red. After cooling to room temperature under stirring, the resulting solution was filtered through 0.22 μm Millipore membrane to obtain Au colloid which was then stored in a refrigerator at 4ºC for further use. The prepared Au colloid (4.0 mL) was put into a round-bottom flask as Au seeds. Ultrapure water (83 mL) and sodium citrate (1 wt%, 1 mL) were then added. After being stirred for 3 min, chloroauric acid (1 wt%, 0.9 mL) was added. And after 8 min stirring, hydroxylamine hydrochloride (10 mM, 1.4 mL) was added dropwise under continuous stirring. The gold nanoparticles were obtained after being stirred for another 1 h at room temperature. It was stored in an amber bottle at 4°C. The AuNPs concentration was about 0.02 nM.

2.4 Raman measurements of pesticide residues on apple peels

Briefly, 100 mL of AuNPs solution was centrifuged at 2000 r/min for 20 min to separate AuNPs. The obtained

solid was redispersed in 2 mL of ultrapure water by ultrasonication to obtain working solution. Apple samples were washed with ultrapure water. Apples were then cut into peels (1 cm × 1 cm × 0.6 cm) using a fruit knife. Then the spiked peels were prepared by the addition of pesticide solutions with eight different concentrations to cover the overall surface of peels and were completely dried at room temperature. Prior to the determination of pesticides, acetone (10 μL) was firstly dropped onto the surface of spiked peels to disassociate the pesticide molecules from peel matrix and then the working solution of AuNPs (10 μL) was added. After air dried for 10 seconds, Raman spectra were collected three times from one piece of fruit peels using 785 nm laser, 450 mW laser intensity and 3 s exposure time. Time interval between the addition of surface enhancement material and the collection of spectra is not good for surface enhancement effect. The 10 seconds was chosen for relatively satisfied enhancement effect based on experimental experience, and to save time and meet experimental operation time.

2.5 Raman measurements of chlorpyrifos on whole apples

Red Fuji apples with a slightly varying shape and size were used for experiment. The apples were cleaned with soap water and rinsed with ultrapure water to ensure that the sample apple surface is free from pesticide. The apples were then left to dry in laboratory at ambient temperature for 8 hours.

Commercially available chlorpyrifos pesticide (48%) was used as pesticide sample. It was mixed with acetone to prepare different concentrations of solution ranging from 3 600 mg/kg to 600 mg/kg. The apples, one at a time, were then completely dipped inside the pesticide solutions gently for a few seconds. The apple samples were then left to dry for 8 hours before collecting Raman spectral signal.

Prior to the detection of pesticides, acetone and AuNPs working solution were dropped onto apple surfaces successively. Spectral signal was collected from 20 different points on each sample surface using the developed system. After collection of spectral data, the samples were tested in Gas Chromatography (GC) to observe the pesticide residue in each sample.

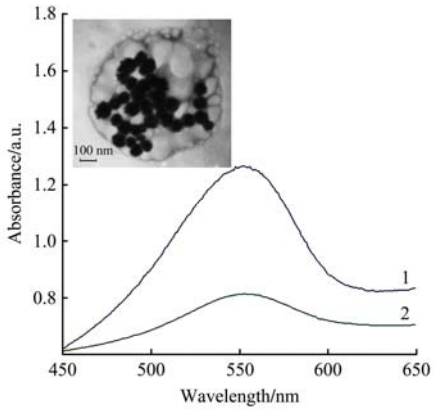
3 Results and discussion

3.1 UV-Visible spectroscopy of gold nanoparticles

To confirm the formation of AuNPs, UV-Visible spectroscopy analysis of the AuNPs was performed. In Figure 1a, the UV-Visible Spectroscopy showed a sharp plasmonic peak centered at 548 nm (curve 1), confirming the presence of plasmonic AuNPs[16]. As indicated by curve 2, the 200 μg/kg chlorpyrifos made AuNPs become aggregating which is evident by the decrease in the signal intensity at 548 nm. A series of concentrations of chlorpyrifos have been tested to figure out the aggregation point. When the chlorpyrifos concentration is lower than 75 μg/kg, the UV−visible absorbance of AuNPs remains unchanged, suggesting that no aggregation of particles occurs. However, when the chlorpyrifos concentration is larger than 75 μg/kg, the absorbance of AuNPs sharply decreases, suggesting the occurrence of AuNPs aggregation. Therefore, the aggregation point was about 75 μg/kg. Once aggregated, strong SERS signal can be observed immediately, so the prepared AuNPs showed good enhancement ability. The transmission electron microscope (TEM) image of the AuNPs shown in the inset indicated that the particles were homogeneous with an average diameter of about 75 nm.

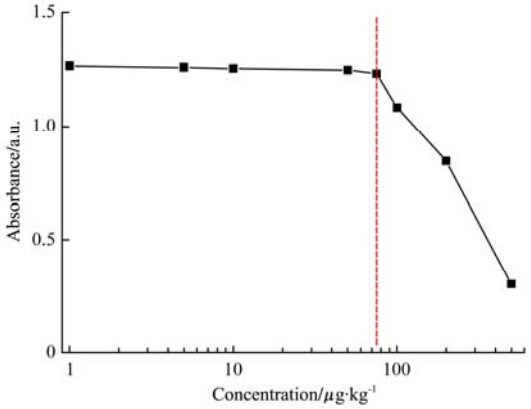
3.2 Spectral features of chlorpyrifos

Raman spectrum and SERS spectrum of chlorpyrifos are shown in Figure 2. Chlorpyrifos powder had very weak Raman response (curve a). Raman signal of chlorpyrifos with the concentration of 0.5 mg/L was enhanced dramatically by using the AuNPs, which demonstrated its excellent SERS effect. Relative intensity among characteristic peaks was different, though major vibrational transitions could be observed on both SERS and Raman spectra of an organic chemical. The most important bands present in the SERS spectrum of chlorpyrifos were those related to C-C stretching, P-O stretching, P=S stretching, C-Cl stretching and P-O-R stretching functional groups at 345 cm^{-1}, 536 cm^{-1}, 615 cm^{-1}, 677 cm^{-1} and 1099 cm^{-1} respectively[17]. In Raman spectrum, thus these five peaks can be taken as the Raman fingerprint of chlorpyrifos pesticide.



Note: The inset is the transmission electron microscope (TEM) image of the Au nanoparticles.

a. UV-Visible absorption spectra of AuNPs (1) and after reaction with chlorpyrifos at the concentration of 200 μg/kg (2)



b. Evolution of AuNPs absorbance at 548 cm^{-1} with the increase of chlorpyrifos concentration

Figure 1 UV-Visible spectroscopy analysis of the AuNPs

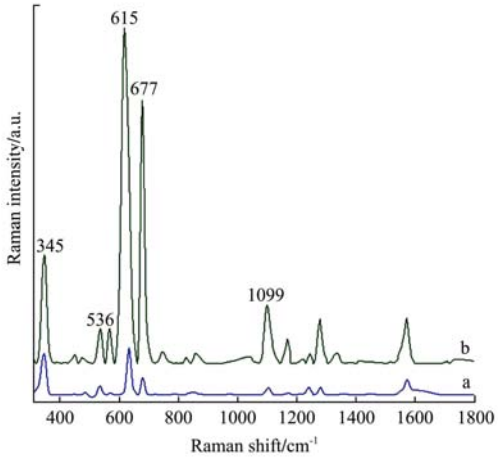
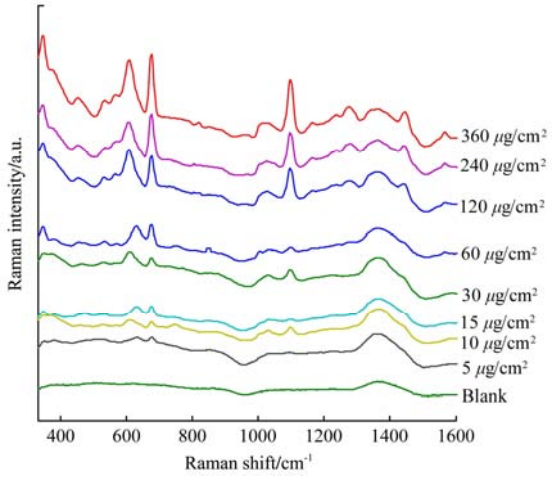


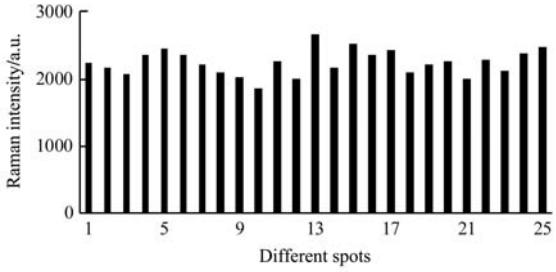
Figure 2 Raman spectrum (a) and SERS spectrum (b) of chlorpyrifos

3.3 Detection of chlorpyrifos on apples peels

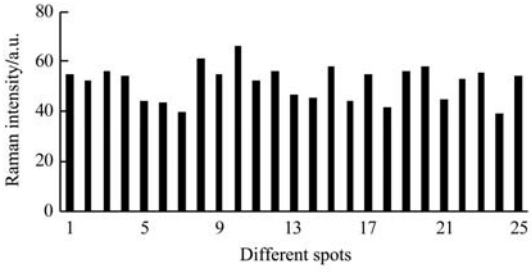
High Raman activity of AuNPs is particularly suitable for particle sensors to probe the residual molecules at various surfaces. Figure 3a shows typical Raman spectra collected from the peels of apples. The characteristic vibrating bands of chlorpyrifos molecules at the spiked peels appeared and became stronger with the increasing amount of chlorpyrifos residue on the peels. Even the chlorpyrifos residues at apples peels were 5 $\mu g/cm^2$, characteristic peaks at 615 cm^{-1} and 677 cm^{-1} were discernible. To investigate detection repeatability, SERS spectra of 25 apples peels with chlorpyrifos residues at 120 and 10 $\mu g/cm^2$ were collected. The 25 peels were obtained from 10 different apples randomly. For statistical analysis, the relative standard deviation (RSD) of the Raman intensity at 677 cm^{-1} was calculated. As shown in Figure 3b and Figure 3c, the RSD of spot to spot were 8.5% at 120 $\mu g/cm^2$ and 13.8% at 10 $\mu g/cm^2$. The results showed that the present method had a high reproducibility for the detection of chlorpyrifos pesticide and can be used for the further quantitative analysis.



a. Average SERS spectra signal of apples peels with different concentrations of chlorpyrifos



b. RSD of Raman intensity at 677 cm^{-1} for chlorpyrifos concentrations at 120 $\mu g/cm^2$



c. RSD of Raman intensity at 677 cm^{-1} for chlorpyrifos concentrations at 10 $\mu g/cm^2$

Figure 3 SERS spectra of apples peels with chlorpyrifos and the analysis of detection repeatability

3.3.1 Spectra pretreatment

Average SERS spectra of chlorpyrifos are shown in Figure 3, the SERS spectra of chlorpyrifos substances were highly influenced by fluorescence background. It is essential to remove background fluorescence from Raman signal for proper signal analysis. The min-max signal adaptive zooming and second derivative transformation method were used to remove fluorescence background.

The average SERS spectra signals were smoothed using Savitzky-Golay (SG) 5 points filter first, followed by background fluorescence removal using min-max signal adaptive zooming and second derivative transformation method, respectively. Min-max signal adaptive zooming method can determine the peak range by identifying the position of minimum and maximum signal, then reduce the inter-influence between adjacent peaks through the signal adaptive zooming calculation after zeroizing the minimum signals[14]. It can extract Raman spectra from high fluorescence, especially for low-density Raman characteristic peaks. Second derivative transformation is a powerful tool in analysis of spectral data, it can separate overlapping peaks, eliminate baseline effects, and enhance spectral resolution[6,18]. Figure 4a and Figure 4b illustrate the baseline-corrected SERS spectrum by min-max signal adaptive zooming and second derivative transformation methods for different concentrations of chlorpyrifos solutions on apple surfaces in the range of 310 cm^{-1} to 1 200 cm^{-1}. The characteristic Raman peak of chlorpyrifos at 677 cm^{-1} was monitored at different concentrations in Figure 4c and Figure 4d. The height of the Raman spectral peaks increased concomitantly with the increase of chlorpyrifos concentration. This peak was used for the quantitative determination of chlorpyrifos.

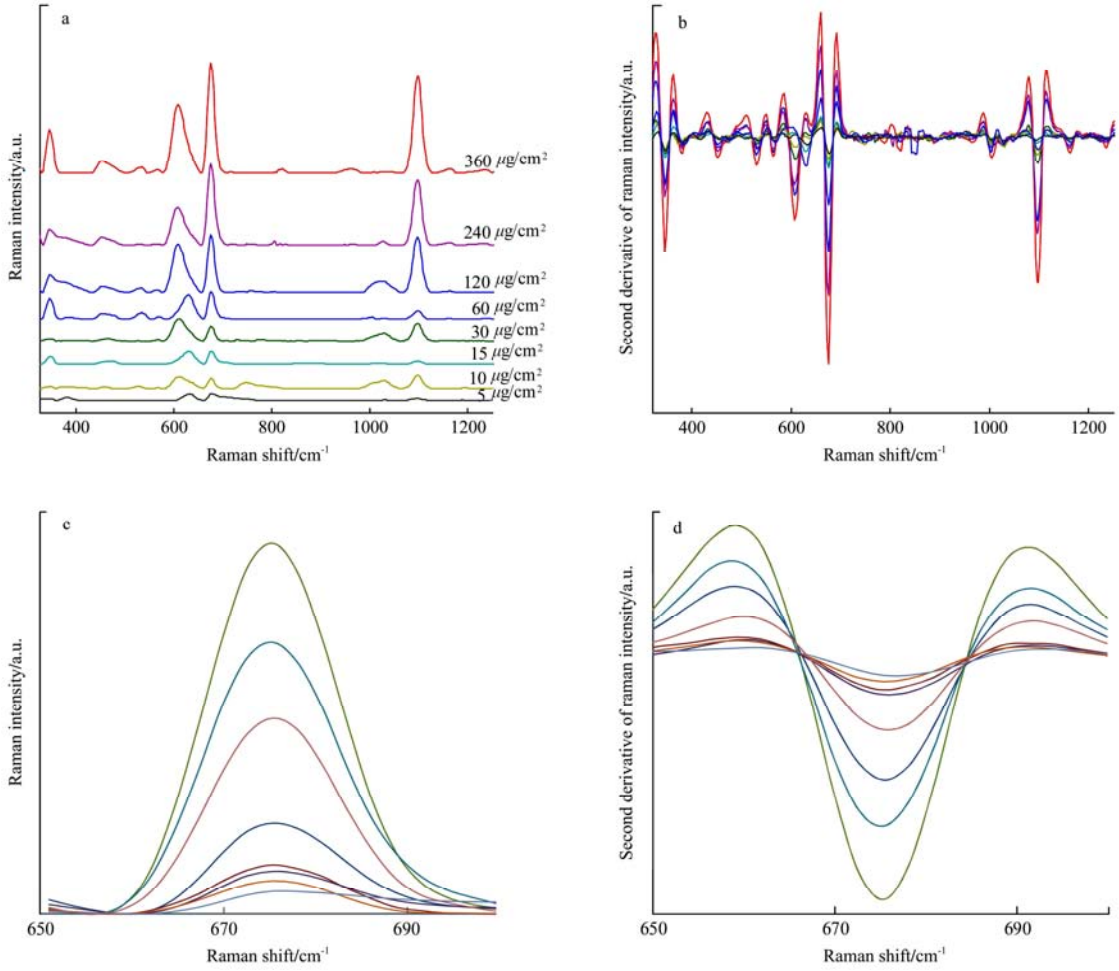


Figure 4 Average SERS spectra signal after fluorescence background removal by min-max signal adaptive zooming (a and c) and second derivative transformation method (b and d) at the range of 660-710 cm^{-1} and around the characteristic peaks of 677 cm^{-1}

3.3.2 Data analysis

SERS spectra pre-processing methods including min-max signal adaptive zooming and second derivative transformation method were applied to subtract baseline

shift and remove the high frequency noises. After the pretreatment of spectra, linear relationships were developed to predict chlorpyrifos residues respectively. As shown in Table 1, linear relationship between the chlorpyrifos residue amount on the peels and the Raman intensities of characteristic peaks at 677 cm^{-1} is clear, with R and standard error values of 0.988 $\mu g/cm^2$, 0.975 $\mu g/cm^2$ and 15.100 $\mu g/cm^2$ and 16.981 $\mu g/cm^2$ for min-max signal adaptive zooming and second derivative transformation methods, respectively. The cause of standard error might be the uneven distribution of pesticides on apple peels and the relatively few sampling points due to the complexity of acquiring signal from all spots where the pesticide gets scattered after applying. The linear relationships between chlorpyrifos residue amounts and the Raman intensities of 615 cm^{-1} were relatively poor. For min-max signal adaptive zooming and second derivative transformation methods, respectively, the R were 0.931 and 0.899, the standard error values were 19.201 $\mu g/cm^2$ and 20.812 $\mu g/cm^2$. The min-max signal adaptive zooming method showed a better linear calibration curve, so further studies will focus on this pretreatment method using the whole apple chlorpyrifos detection.

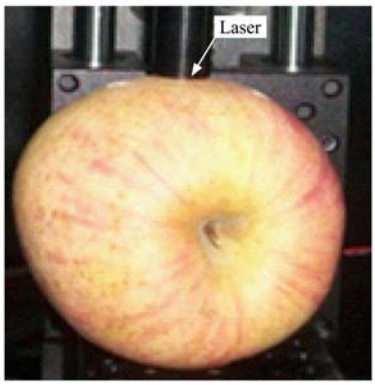
Table 1 Linear relationships between the chlorpyrifos residue amount on the peels and intensities at 677 cm^{-1} in the SERS spectra of apples

ERS spectra pre-processing method	Linear equation	R	SE/$\mu g \cdot cm^{-2}$
min-max signal adaptive zooming	y=0.08x-29.74	0.988	15.100
Second derivative transformation	y=0.7x-30.63	0.975	16.981

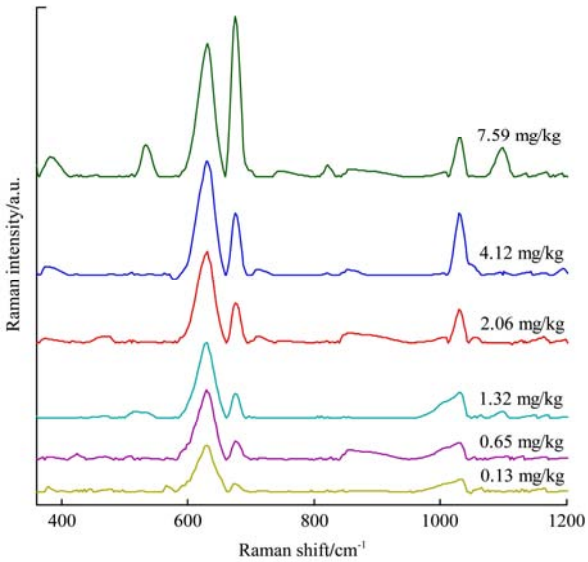
Note: x *is* the Raman intensity of Chlorpyrifos at 677 cm^{-1}; y is concentration of Chlorpyrifos solution.

3.4 SERS analysis of chlorpyrifos on whole apples

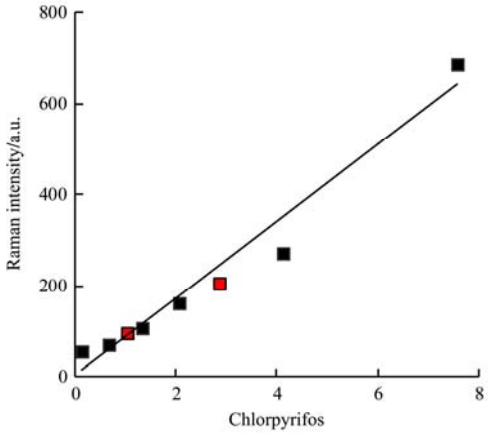
Six different chlorpyrifos concentrations were prepared and put into beakers (1 L). Six apple samples were submerged in chlorpyrifos solution for 3-4 seconds then left to dry in laboratory temperature. Raman spectral data at 20 different points were collected from the whole apple as Figure 5a. Figure 5b showed the average SERS spectra signals of six apples with different concentrations of chlorpyrifos after fluorescence background removal by min-max signal adaptive zooming.



a. Photograph of detection of chlorpyrifos on whole apples



b. SERS spectra on whole apples with different concentrations of chlorpyrifos



Note: the red points represent the positions of SERS intensities and corresponding pesticide residual amounts obtained by the GC test.

c. The linear relationship of peak intensity of SERS spectra at 677 cm^{-1} and the concentration of chlorpyrifos on whole apples

Figure 5 SERS analysis of chlorpyrifos on whole apples

Similar to the detection of chlorpyrifos on apples peels, the Raman intensities of peak at 677 cm^{-1} were linearly related to the concentrations of chlorpyrifos on the whole apple. The standard deviations of the 20 measured points for each apple were 15.7% to 19.8% due to uneven distribution of pesticide. Figure 5c indicated a linear

correlation between the intensity of the chlorpyrifos SERS spectral peak intensity and concentration of chlorpyrifos in the range of 7.59 mg/kg to 0.13 mg/kg (R=0.962). To further test the accuracy of the method, chlorpyrifos solution with the concentration of 0.48 g/kg (orchard actual amount) was prepared and sprayed onto cleaned apples, after a week under natural condition, SERS detection was conducted. The red spots in Figure 5c represent the detected SERS intensities and corresponding pesticide residual amounts obtained by the GC, which are close to the predicted values. Therefore, since the operation of the proposed method was simple and fast with a good sensitivity, this research showed a good prospect for practical application.

4 Conclusions

This study indicated the possibility of applying SERS technology for quantitative assessment of chlorpyrifos on apples. Present work proposed a facile SERS method coupled with AuNPs without sample pretreatment, rapid and accurate detection of chlorpyrifos at low concentrations on apples. The min-max signal adaptive zooming and second derivative transformation methods can be used efficiently for pre-processing of SERS spectra to eliminate baseline effects. After pretreatment of spectra, a linear relationship between the concentrations of chlorpyrifos solutions and the Raman intensities of characteristic peaks at 677 cm^{-1} was obtained. Moreover, the detectable concentration of chlorpyrifos pesticide on a whole apple by SERS was lower than 1 mg/kg, which met Chinese Standard. These results demonstrated that SERS coupled with AuNPs showed great potential for accurate and rapid detection of pesticide residues on fruits. With the use of SERS, it could significantly simplify sample preparation and make full use of rapid and sensitive features of SERS method for the detection of trace amounts of hazardous chemicals in various agricultural and food products.

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