

Detection and Analysis of Residual Pesticides Based On Surface Raman Spectra of Spinach

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Abstract

This paper with spinach as the research object, to chlorpyrifos pesticide as detection object, using Raman spectroscopy technique surface pesticides in spinach residual detection method and rapid detection research. Starting from the detection method, study of the classic method and method for rapid detection of current situation of the development, Raman spectroscopy detection method as a means of detection, the selected object of study; master the Raman spectroscopy for rapid detection of the basic principles and originpro 9.0 software acquisition function using, lay the foundation for the subsequent detection of. After the actual test to determine Raman spectroscopy instrument laser wavelength to 473nm, acquisition time 2s spinach sample testing effect Best result. After no medicine Raman spectra to obtain the surface of spinach were denoising analysis results show that wavelet de noise method compared to the adjacent averaging, savitzky Golay method smoothing, median method effect better. On Spinach with different concentrations of chlorpyrifos Raman spectroscopy analysis that accorded with the residual linear relationship. The peak intensity method were selected to establish models were detected in spinach surface of pesticide chlorpyrifos. Raman spectroscopy detection method can of spinach surface of pesticide residues detection analysis. It solves the traditional measurement method for sample pretreatment is complex and long detection time, low efficiency of the measured defect

Keywords: *Raman spectroscopy, pesticide residue analysis, quantitative detection*

1. Introduction

With the rapid development of science and technology, coupled with all the countries in the world began to concern for human health, food and health of people in daily life consumption requirements of increasingly stringent, especially in recent years for vegetable pesticide residue problems are particularly concerned, many countries began to on pesticide residue monitoring work should be paid more attention, and in detection of pesticide residue standards have been more and more stringent requirements. The world so much pesticide residue problem, it is the research and development of a more perfect instrument for detection of pesticide residue. The analysis has become an important branch of the pesticide residue detection of pesticide residues. Quick test technique is more suitable for the needs of the society. Analysis of residual pesticide in the future may be more complex, it is more difficult to analyze. So, must make the results in this study, the introduction of new knowledge as a support, such as: cell chemistry, fermentation chemistry, immunochemistry and polypeptide arrangement. The future of agricultural scientific development direction will be the innovation of chemical and biological technology and biological technology and the organic combination of modern chemical combination analysis method.

In the continuous development of agricultural science and technology today, the kinds

and amount of pesticide development grow with each passing day, the use of chemical pesticides has become a major means of prevention and control of pests and diseases. According to statistics, the year the world pesticide production reached more than 700, and sales to various countries. Everything has pros and cons, these pesticides are sprayed to crops, in addition to part is consumed outside, and a small part will remain to farming in the object. The residual pesticides in farming objects when the intake by the human body will cause adverse effects on human health, therefore, to farming in the object of the residue of pesticides into the agricultural experts have a common research objectives.

2. The Basic Principle of Raman Spectroscopy

Light scattering is a common phenomenon in nature. Scattering principle is: when a beam of light to medium, in addition to a portion of the light is scattered in all directions outside, in light of the other are medium to absorption, reflection or transmission of the absorbed. The scattering of light is divided into two types: elastic scattering and inelastic scattering. When the incident light and scattered light of the same frequency is called elastic scattering, for example, we usually see a clear blue sky. Otherwise known as non-elastic scattering. The main non elastic scattering is the microscopic structure of matter using the molecular rotation and vibration. The Raman reflection belongs to the range of non-elastic reflection.

For the scattering and elastic reflection occurs at the same time is also associated with the occurrence of inelastic scattering. In the process of elastic reflection is a most light to change the direction of but the frequency is not changed, which belongs to the elastic scattering. The remaining part of the light not only changes the direction and frequency also follows the change. This is what we mean by Raman scattering. It belongs to.1928 non elastic reflection Indian physicist Raman in the study to mercury lamp irradiation benzene liquid found diffuse reflection phenomenon.

Raman Effect exists in all substances. Raman spectroscopy as a structural material for a powerful analysis tool is not enough place is mercury lamp as Raman scattering of the excitation light source produces a spectral line is particularly weak. Raman spectroscopy is fast development is in the emergence of the laser in 1960 only after. At present, Raman spectroscopy again petroleum geology, criminal and many other areas until the application. The Raman shift is refers to the difference between the frequencies of incident and scattering. The structure information of the molecular scattering is the characteristic frequencies of functional groups or chemical bond in Raman spectra is reflected. The structure information is through the light scattering energy and Raman shift between to show the change of displacement, through the spectrum peaks, intensity and shape information is reflected. Raman scattering is instantaneous. The characteristics of Raman spectra is very narrow spectral lines, positive and negative frequency difference (same line pairs). As shown in figure 1.1. In the middle of the line called the excitation spectrum in the spectrum, the strongest of it. The frequency of ν_0 . In on both sides of the line excitation respectively for the Stokes and anti-Stokes lines, the Stokes line in the low-frequency side, the intensity should be lower than the excitation line, he and stimulate the line frequency difference namely Raman frequency shift for $\Delta \nu$. High frequency side of the anti-Stokes line, the Raman frequency shift and Stokes line the same is also $\Delta \nu$, anti-Stokes line in these three kinds of spectral line strength is the weakest, is not easy to be found. Stokes and anti-Stokes lines are often available in frequency $\nu_0 \pm \Delta \nu$ delta said.

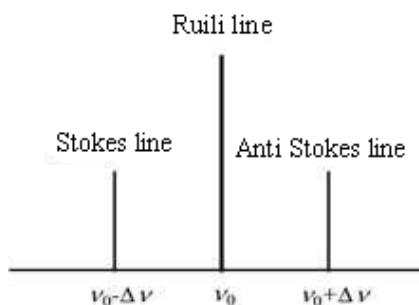


Figure 1. Diagram of Raman Spectrum

3. The Raman Spectrum Analysis Quantitative

3.1. The Theoretical Basis of Raman Spectroscopy Quantitative Analysis

The signal between the Raman spectra and the sample concentration ratio is:

$$I = k\phi^0 c \int e^{(-\ln 10)(k_0+k)z} h(z) dz \quad (3-1)$$

In (3-1), I , K , ϕ^0 , C , K_0 , $h(z)$, z , b are expressed as: For the sample surface is received by the optical system the Raman signal intensities, Raman scattering section area, the sample surface laser incident power, sample produce Raman scattering of the concentration of a substance, the width of the incident light and scattered light of the absorption coefficient and the angle of incident light and scattered light through the distance, the optical system transfer function and sample pool. From the formula (3-1) can be seen in certain conditions, the scattering intensity of Raman I with analyte concentration is proportional to C ,

$$\text{That is: } I \propto C \quad (3-2)$$

Due to the influence of the intensities of the Raman bands by factors such as temperature, sample in the environment of self absorption and the light source of the instrument power stability *etc.* Due to the influence of environment and instrument external factors, will produce certain error. Here we introduce two concepts, namely: the external standard method and internal standard method. Using these two methods can be established before the quantitative analysis model is modified. The following were introduce the concept of external standard method and internal standard method. The characteristic peaks of quantitative analysis of the actual reference external standard method for standard. When measuring the organic solution reference material usually use carbon tetrachloride (459cm⁻¹). This method is usually used for the determination of longitudinal coordinate error correction. The reason is due to the error between the material is very difficult to remove. Internal and external standard method used on the contrary, it is not in the characteristic peak of reference standard, but not to the Raman peaks with a stable peak measured Raman peaks overlap as standard. The internal standard method, the selection of chemical substances are relatively stable.

3.2. The General Process of Raman Spectroscopy Quantitative Analysis

As a kind of indirect measurement technology of Raman spectroscopy it is principle to stoichiometry. It gains to be measured parameters and properties of the samples is through the data processing, model building, correction equation obtained. Quantitative analysis of Raman spectrum is divided into two parts:., collecting data of the collected data to build the calibration model; second, by correcting the composition of the content and nature of

the composition of the model in the sample are measured. Specific processes as shown in Figure 2.

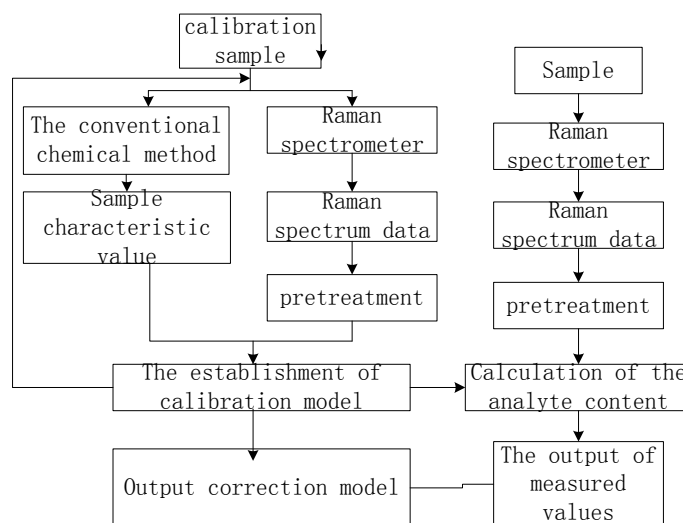


Figure 2. Schedule of Raman Spectroscopy Quantitative Analysis

4. The Data Acquisition System and Test Method

4.1. Test Materials

Test sample: buy directly from the market for fresh spinach.

Test of pesticides: chlorpyrifos buy Shandong Dacheng pesticide Limited by Share Ltd sold creamy white.

4.2. Test Methods

(1) when washing vegetables, wash, and then washed with pure and clean. Placed on glass slides.

(2) using distilled water as solvent, different concentrations of chlorpyrifos and water ratio, the ratio respectively in accordance with the 4800, 3200, 2400, 1200, 800, 400, 200 mg / kg configuration.

(3) will different concentrations of chlorpyrifos solution with medical disposable syringe sequentially drop coated on vegetable surface has been cleaned and indicate the label on the side of the paste, such as chlorpyrifos 200mg / kg, said vegetables are concentration of 200mg / kg chlorpyrifos pesticide.

(4) Suitable parameter settings of this experimental Raman spectrometer. This instrument spectral range $100 \sim 4200\text{cm}^{-1}$, resolving power 0.67cm^{-1} , The laser wavelength 473、633、785 cm^{-1} selection. According to the actual situation of the test in the spectrometer spectral and laser wavelength range to choose appropriate laser.

(5) the measured sample is placed in a loading platform. First, collect washed vegetables and chlorpyrifos pesticide sample spectra, and then dried samples (Note: the sample is before the test with different concentration ratio of pesticide droplets of solution in the measured sample) placed on a glass slide collection of Raman spectroscopy.

(6) according to the collected spectra of trichlorfon in spectral characteristics were analyzed, using origin 9.0 (Originlab 9.0 USA) and LabSpec 5 software of data were processed and analyzed.

4.3. The Hardware and Software System

The hardware device of the test using a Raman spectrometer made in the United States scientific research institute of information technology Heilongjiang Bayi Agricultural University. Using software is advantage of 532 software, the sample completed the collected field data acquisition, data (*.prn) format save, and then use the Origin9 software and LabSpec 5 software of data processing and data analyzing and operations to be performed on the collected data.

5. Analysis of Pesticide and Raman Spectra of Spinach

Chlorpyrifos usually dissolved in water, can also be made into powder and emulsion used is a low toxicity of organophosphorus pesticides, broad-spectrum, substitution is one of the main varieties of the high toxic organic phosphorus pesticide, with contact killing, stomach and fumigant poison, no ceiling effect. The drug in the soil to a long effective period, therefore, of the underground pests have very good control effect.

5.1. Raman Spectrum Analysis of Chlorpyrifos Pesticide

Chlorpyrifos characteristic Raman peaks obtained is of the pesticide (99%) were detected, such as Figure 3 shows the Raman spectra, from the figure can be seen, chlorpyrifos Raman activity is very strong, relatively abundant characteristic peak displacement as long as the concentration in the 150~400cm⁻¹ and 600~1100cm⁻¹ range. Therefore, can choose the two parts as the Raman spectrum information for identification of chlorpyrifos chlorpyrifos.

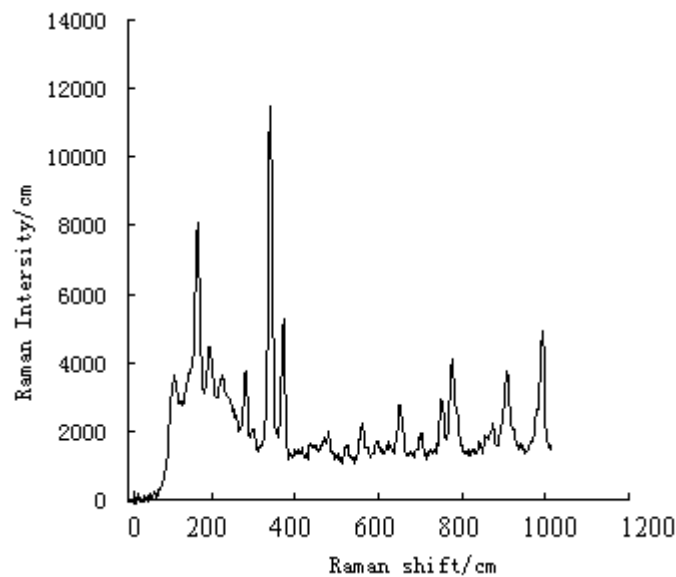


Figure 3. Raman Spectra of Chlorpyrifos

5.2. Spectral Analysis of Spinach

No original Raman spectroscopy such as spinach samples is shown in Figure 4 of pesticide residues collected.

The Raman spectra of spinach as long as the concentration in the range of 1100~1600cm⁻¹, in the range of 400~1100cm⁻¹ only detected Raman signal is weak, and the weak Raman signal is mainly reflected in the 744 cm⁻¹ position. Caused by spinach chlorophyll Lynn ring C-O-C and C=C double bond vibration peaks occurred mainly in

155cm-1 and 1157cm-1 two positions. Division for background fluorescence spectra curves of different spinach can be carried out in three areas: 400~900cm-1, 900~1500cm-1 and 1500~1900cm-1. The analysis found that different spectral lines with spinach fluorescence background of different downward trend difference. The major pesticide chlorpyrifos and Raman characteristic peaks in 632cm-1 and 637cm-1 two positions. Therefore, can choose 400~900cm-1 spectra as the analysis of the data area.

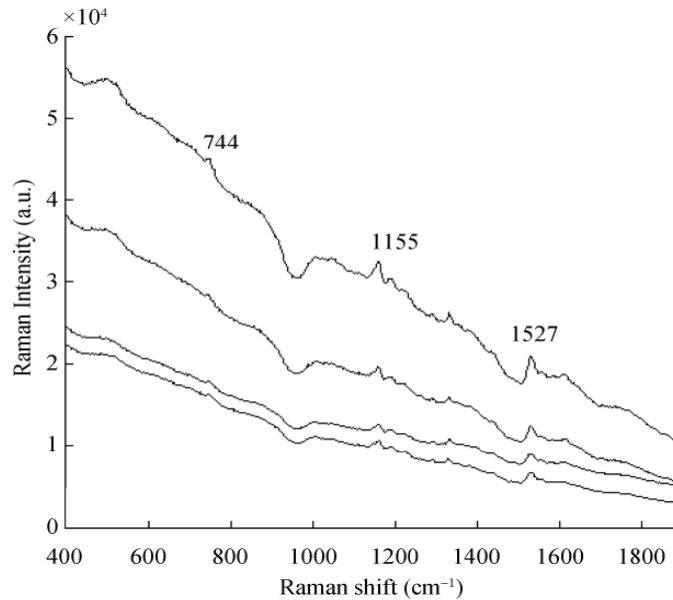


Figure 4. Raman Spectra of No Pesticide Apple

As shown in Figure 5, is the original spectra containing chlorpyrifos pesticide on spinach. As a result of the acquisition of system hardware and other reasons, the spectrum collected there may be some errors, there's some noise in the system itself. So, to deal with the noise of this experiment by using five point smoothing method for the collected spectra, to avoid interference to the data processing result is not true result. As shown in Figure 6, for spectrum after smoothing denoising, this method will not affect the original features of the peak size. Therefore, this method can improve the accuracy of data processing. Are usually processed by Raman spectrum data of the late Fu Liye transform method, wavelet transform method, polynomial fitting *etc.* As shown in Figure 7 is the result of fitting spectra obtained after polynomial fitting, and after peak after the baseline calibration curve is more obvious, as shown in Figure 8.

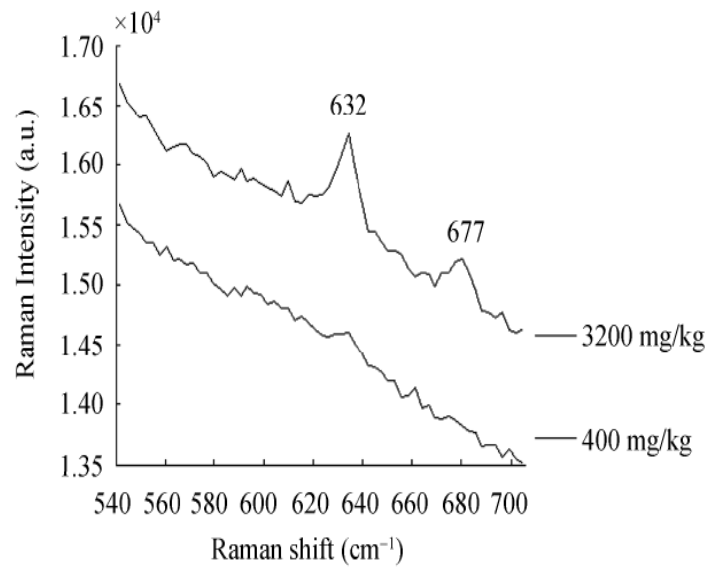


Figure 5. Contains the Original Spectra of Chlorpyrifos Spinach

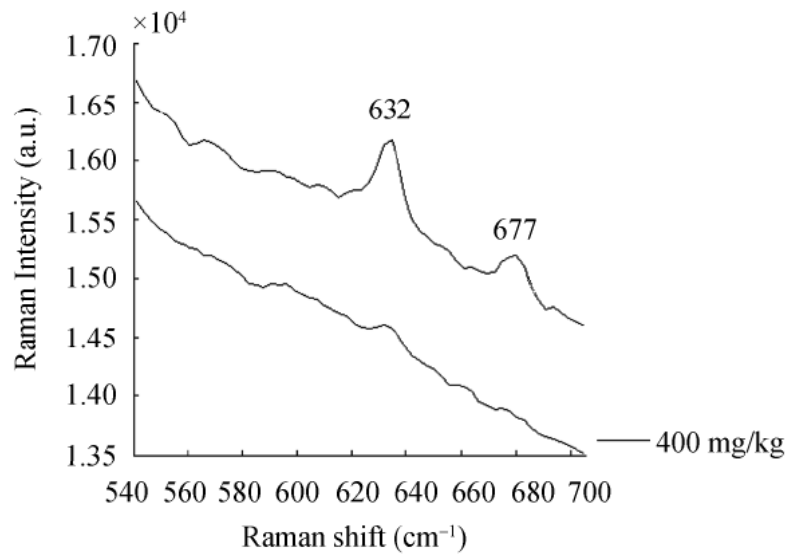


Figure 6. After Denoising Processing of the Raman Spectra

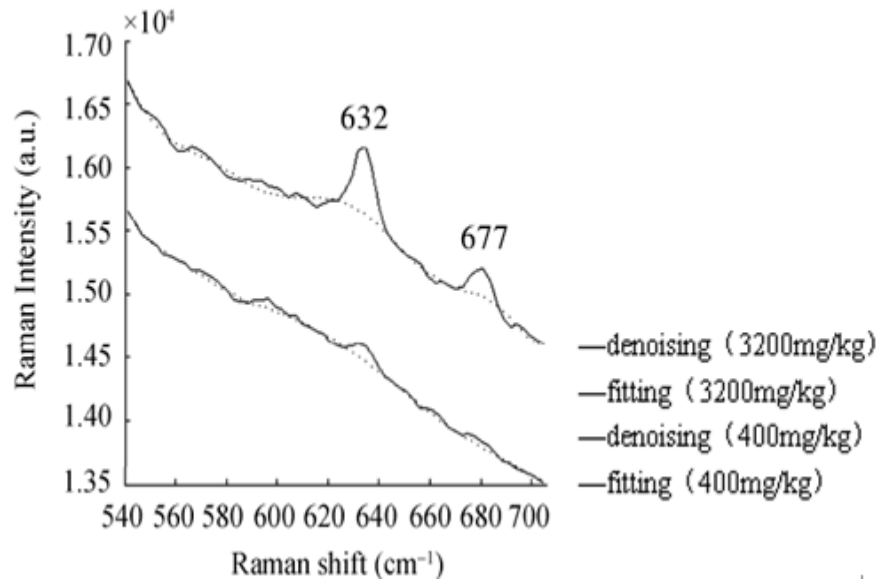


Figure 7. After A Polynomial Fitting of the Raman Spectra

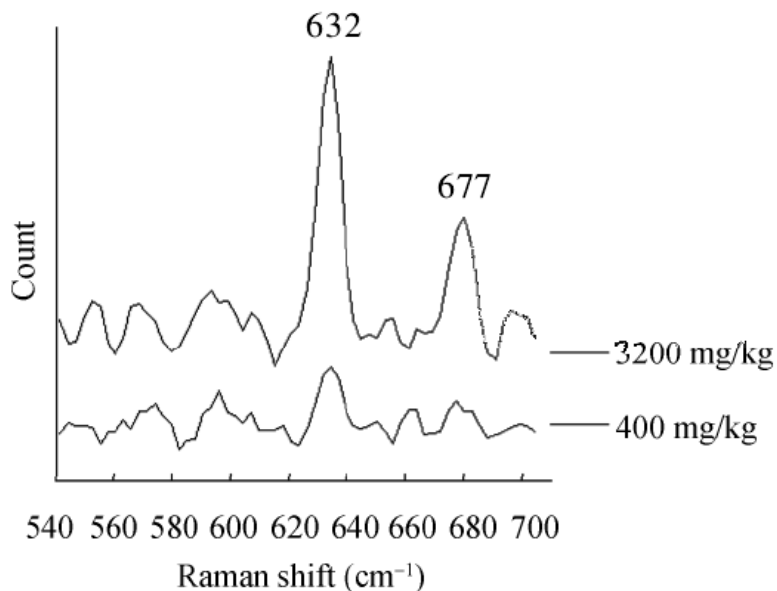


Figure 8. After Baseline Correction of Raman Spectra

5.3. The Surface of Different Concentrations of Pesticides in Spinach Raman Spectrum Analysis

The same samples of three different detecting points to the data were averaged, and then after baseline correction, calibration with the spinach spectral curve of different mass fractions of chlorpyrifos as shown in Figure 9.

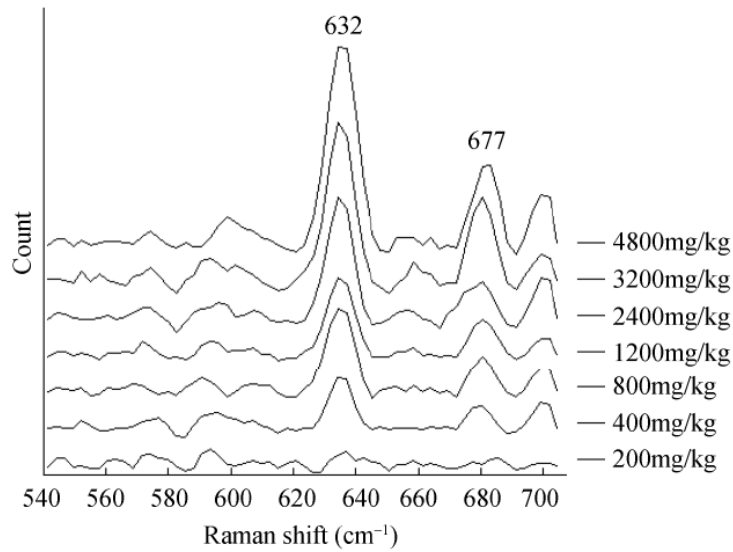


Figure 9. Apple Surface of Different Concentration of Trichlorfon Pesticides Trichlorfon

Chlorpyrifos pesticide in the 632cm⁻¹ 677cm⁻¹ appeared the main characteristic Raman peaks, but chlorpyrifos higher than 200 mg / kg, when the pesticide chlorpyrifos fell to 200mg / kg, the difference between 632cm⁻¹ 677cm⁻¹ two of the wave and noise almost is not obvious. So it is hard to judge the crest is spinach chlorpyrifos pesticide residues produced by Raman signal, so chosen in this experiment is 400mg / kg chlorpyrifos pesticide as object detection. At the same time, the all samples when the concentration decreased, the Raman signal intensity of 632cm⁻¹ and 677cm⁻¹ two has a decreasing trend. And, when the concentration of 4800mg/kg, 632cm⁻¹ and 677cm⁻¹ two of the Raman peaks are more stable than 677cm⁻¹ 632cm⁻¹, 4cm⁻¹ 677cm⁻¹ Raman shift phenomenon. Therefore, the selection of the Raman peak at 632cm⁻¹ is a linear relationship between the concentration of chlorpyrifos, the correlation coefficient is R²=0.92. This description can be relative intensity of the Raman signal according to the 632cm⁻¹ to establish the prediction model of surface of spinach chlorpyrifos pesticide. But in the future, still need to improve the system *etc.* method to improve the detection accuracy, as shown in Figure 10.

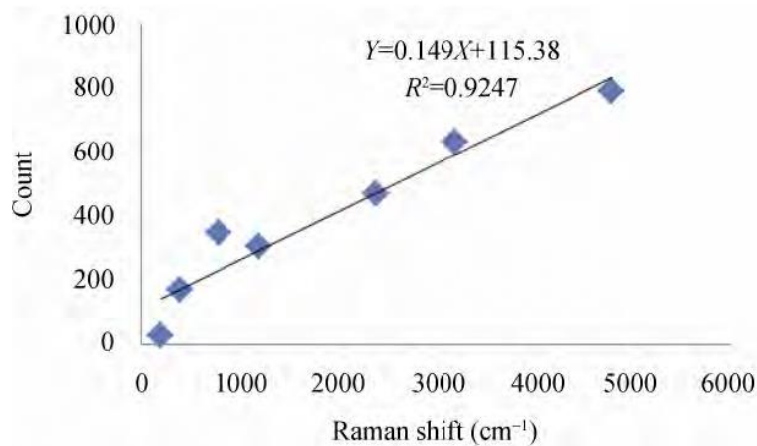


Figure 10. Relationship between Pesticide Concentration and Characteristic Peak Intensity

6. Conclusion

By reading a lot of literature, in summary of traditional pesticides determination method and rapid determination method in the research of agricultural products on the basis of the work, the theory of Raman spectroscopy detection technology and detection system composition, Raman spectroscopy detection principle, development process and characteristics of were learning, and master the use of Raman spectroscopy for rapid detection of basic principle, hr800 detection instrument flow and origin of 9.0 and LabSpec 5 acquisition software function, for the application of Raman spectroscopy detection technology for rapid detection of spinach surface residues chlorpyrifos pesticide to lay the theoretical foundation and experimental research based. The spinach as the carrier, chlorpyrifos as the measured object, the application of Raman spectroscopy detection technology, of Chlorpyrifos in vegetables surface residue was detected. The results show that:

1. After the actual test to determine the actual acquisition spinach Raman spectroscopy instrument testing conditions: laser wavelength 473nm, acquisition time 2s times, 3 times, through the adjacent averaging method, however, the conventional savitzky Golay method, median method, wavelet to denoising, five point smoothing method five de-noising methods after the selection of five point smoothing method on the acquisition of the Raman spectra denoising.

2. Through the experiment analysis of chlorpyrifos spectra, spinach spectra, that Raman spectroscopic characteristics of chlorpyrifos displacement mainly in the 150~400cm⁻¹ and 600~1100cm⁻¹ within the scope of the characteristic peaks of relatively rich; in 632 cm⁻¹, 637cm⁻¹ as chlorpyrifos detection feature displacement signal; the surface of the spinach no residual pesticide Raman spectroscopic characteristics of displacement is mainly manifested in: 744, 1155, 1527cm⁻¹.

3. When chlorpyrifos pesticide smell 400mg / kg, at this time of Chlorpyrifos in the most obvious Raman signal 632cm⁻¹, can be as spinach surface of chlorpyrifos pesticide residue detection. And this position, there is a good linear relation of residual Raman characteristic peaks and the relative intensity of spinach surface pesticide, the correlation coefficient is $R^2=0.92$. In the future, the Raman spectroscopy system further improved the detection accuracy, to establish a stable model for pesticide residue rapid implementation, lays the foundation for real-time detection.

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