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Optical Technologies for Determination of Pesticide Residue

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1. Introduction

Pesticides are essential for agricultural and horticultural crops production. Pesticides are commonly classified as insecticide, fungicide, herbicide, rodenticide, etc. These pesticides act against insects, rodents, weeds which are harmful in agricultural or horticultural planting. Normally, farmers use the pesticides following the instruction written in the package. In most cases, the pesticides are mixed with water and sprayed over the plants. Basically, after spraying fruits or vegetables with pesticide, a period of 10 to 14 days is required to allow the chemical to degrade. However, the full degradation of pesticide is not always achieved. In recent years, some farmers ignored to use the pesticide correctly and rationally. In order to chase a better insecticidal effect and the economic interests, the phenomenon of using pesticide excessively, or selling the fruits or vegetables just after spraying the pesticide in few days are not difficult to see. Moreover, the pesticides overdosing also have the potential to contaminate the soil, air, and river.

Currently, several different technologies such as gas chromatography (GC), high-performance liquid chromatography (HPLC), thin-layer chromatography, supercritical fluid chromatography, chromatography-mass spectrometry, capillary electrophoresis, enzyme inhibition method, immunoassay method, and bio-sensor method are used to determine the concentration of pesticide residue. The accuracy of these technologies such as GC and HPLC is best (Gambacorta et al., 2005). However, these analysis methods have limitations of time and labor for controlling individual products. Normally, at least hours are needed to measure the pesticide concentration in a single sample because of the complication in the testing process. These instrument analysis methods as such, can be used only in laboratory for accurate analysis and statutory inspection (Luypaert et al., 2003). Biological and chemical analysis methods were developed in recent years, but there are also some flaws, such as the pre-treatments are needed and the demanding of experimental conditions.

Compare with the growing public requirement of food security, the traditional pesticide detection technologies are not competent because of the shortcomings such as longer detection cycle, complex testing process, testing process, lagged nature of nature of detection results, etc. Therefore, development of fast, reliable detection method or equipment of pesticides residue is imperative. And it is vital to control the pesticide concentration on agricultural products for maintaining public health conditions and protecting the entire environment.

Development of safe, fast, reliable and low-cost analytical methods for the determination of pesticide residue that avoids the use of organic solvents, and reduces the contact of operator with the toxic substances is growing interest at present. In recent years, spectroscopy based procedures is regarded as a potential method which could solve the above problems. Spectroscopy analysis methods have been widely used in chemical industry, agriculture, medicine and other areas (Peng et al., 2008, 2009; ElMasry et al., 2007).

A NIR spectroscopic method and an optical imaging technology for prediction of organophosphorus pesticide are introduced as follows.

2. NIR spectroscopy for pesticide determination

Among the optical analysis methods, near-infrared (NIR) spectroscopy is the most popular method because of its non-destructive nature, the low operating cost and the fast response times (Armenta et al., 2007), and it also has been successfully applied to quality control in food (Pi et al., 2009; Leroy et al., 2003; Subbiah et al., 2008), petrochemical, pharmaceutical, clinical and biomedical and environmental sectors (Ripoll et al., 2008). Near-infrared (0.7-2.5μm; 12900-4000cm^{-1}) spectroscopy is further classified into NIR reflectance spectroscopy and NIR transmission spectroscopy. NIR can be non-dispersive (filter-based instrumentation), dispersive and use Fourier transform-based instrumentation. Table 1 lists some NIR spectroscopic applications suitable for pesticides determination. All these researches have shown the possibility and reasonability for determination of pesticide concentration using NIR spectroscopy.

Instrumental method	Determination attribute	Reference
Mid- and near-infrared	Metribuzin in agrochemicals	Khanmohammadi et al., 2008
NIR	Pesticide determination in commercially formulations	Armenta et al.,2007
Fourier transform infrared spectroscopy	Propamocarb in emulsifiable pesticide concentrate formulations	Quintås et al., 2008
NIR	Pesticide phoxim residues	Shen et al., 2009
IR spectroscopy	Classification of Pesticide Residues in the Agricultural Products	Makio et al., 2007
Mid- and near-infrared	Describing diuron sorption in soils	Forouzanhohar et al., 2009
NIR/ Dry extracts	Determination of acephate, dichlofluanid and tetrachloro-isophthalonitrile	Sarawong et al., 2007
NIR	Determination of soil content in chlordecone	Brunet et al., 2009
NIR–Raman	Quantitative analysis of methyl-parathion pesticide	Ysacc Sato-Berrú et al., 2004
NIR	Detecting the chlorpyrifos content	Liu et al., 2009
NIR	Determination of active ingredient of agrochemicals	Xiong et al., 2010

Table 1. Near-infrared spectroscopy to determinate pesticides concentration.

The following example presents the methodology for determination of chlorpyrifos based on NIR.

Samples

Pesticide solution: A commercial pesticide, containing 40% chlorpyrifos (Noposion, China) was used. Chlorpyrifos is an organophosphorus pesticide, normally used in the paddy, wheat, cotton, fruit trees, and vegetables. Distilled water was prepared in order to provide the solutions with different concentrations. A total of 24 concentration levels, from 1 mg/kg to 400 mg/kg of active ingredient were diluted based on the amount of chlorpyrifos. After preparation, the solutions were kept in conical flasks and preserved in a cool place in order to prevent chemical degradation and contamination.

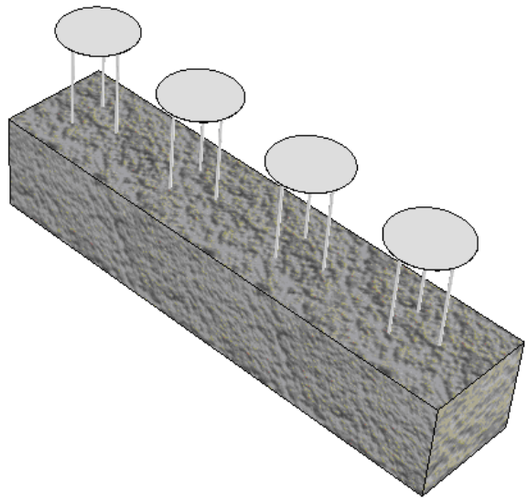


Fig. 1. Platform for filter paper.

Filter paper samples: It is well known that the control level of pesticide residue does not lie at the percent level but at the 10^{-6} level, even 10^{-9} level. It is hard to obtain a satisfactory result by the use of NIR spectroscopy to determine the concentration of pesticide solution. The reason being that water has several strong absorption peaks in near-infrared bands; as a result it is difficult to get the information of pesticide compared to water in the solution. In order to obtain the absorption of trace chemicals, a special method to concentrate the amount of chemicals on samples was developed. Filter paper was used as substrate, water was removed from wet substrate by drying, and then the NIR measurement was performed on the dried substrate.

Normal filter papers (Shuangquan, China), 9 cm diameter were selected. Initially, every piece of 9 cm filter-papers were sheared into four pieces each 30 mm diameter by using a special mold. Then the filter paper were kept into a special platform, which was made of polystyrene foam and pins (Figure 1). Each platform ware almost 20 cm long and 5 cm wide, and four pieces of filter paper could be placed on each platform. After putting the filter papers onto the platform, 200μL of pesticide solution was gently pipetted onto each filter paper (the amount of 200μL is the volume absorbable by filter paper without any overflow). Several pieces of filter paper samples were prepared for each concentration level. A total of 99 filter paper samples were prepared.

Drying filter paper samples: Platform with filter paper samples were carefully moved into the vacuum drying oven, at room temperature for 1 hour. After drying, samples were stored into vacuum packing bags immediately and marked with different concentrations.

Spectrum acquisition

An Antaris FT-NIR spectrometer (Thermo Nicolet, Waltham, Massachusetts, USA), equipped with an InGaAs detector was used. The filter paper sample was placed in a specially modified sample cell. The spectra were acquired in the range of 4000 cm^{-1} to 10000 cm^{-1} at 8 cm^{-1} interval. For each sample, three points were chosen randomly for the NIR measurement, and 32 scans were co-added for each point. The sample was then removed, and the spectra were collected again in the same manner. Three spectra were obtained for each sample at the same state, and averaged spectra were calculated for further evaluation. To prevent the interference of water vapor in the air, the spectra of samples were acquired immediately after taking out from the vacuum packing bags.

Pre-processing method and data analysis

The Matlab 7.0 software (MathWorks, USA) was used for all calculations. A total of 99 filter paper samples were divided into two groups, 75 samples were selected as calibration set; the left 24 samples in each concentration level were put into validation sample set. Partial least squares regression (PLSR) was used to develop a prediction model. Multiplicative scatter correction (MSC) and standard normal variate (SNV) were used in PLSR for pre-processing of spectral data. MSC efficiently eliminates the base line drift of the spectra which in turn reflects the more detailed characteristics of the spectra, and also removes additive and/or multiplicative signal effects (Brunet et al., 2009). The main advantage of SNV is to avoid attributes in greater numeric ranges dominate those in smaller numeric ranges. The PLSR model basing on all variables of the spectra is complex, thus a special algorithm uninformative variable elimination (UVE) was used as a method for variables selection of NIR spectral data in order to develop the effective PLSR prediction model for determination of pesticide the concentrations in each sample.

UVE is an algorithm based on the regression coefficient b of PLSR (Chen et al., 2005; Wu et al., 2009). In the PLSR-NIR prediction model, there is a relationship between X (spectral matrix) and Y (concentration matrix):

$$Y = Xb + e \tag{1}$$

where b is the regression coefficient vector, e is the error vector. The following five steps were taken to get a new spectral matrix with fewer wave bands:

1. PLSR was used to develop a prediction model in the entire wave range from 4000 cm^{-1} to 10000 cm^{-1}. Cross validation was applied to the calibration set. Each time, one sample was taken out from the calibration set. A calibration model was established for the remaining samples and the model was then used to predict the sample left out. Thereafter, the sample was placed back into the calibration set and a second sample was taken out. The procedure was repeated until all samples have been left out once. The root mean square error of cross validation (RMSEcv) was calculated for each of all wavelength combinations. The best principal component (PC) number with the highest Rcv (correlation coefficient of cross validation) and lowest RMSEcv value was selected.

2. A random matrix was developed which has the same dimension size as spectral matrix X. Then X and Ra were joined together to be a new matrix XRa.
3. Partial least squares regression (PLSR) was used again. Leave one out cross validation was carried between the new matrix XRa and concentration matrix Y. After each step of leave one out cross validation, a regression coefficient *b* was obtained.
4. Analyzing the stability of *C* value which is the ratio of the mean value of vector *b* and the standard deviation of vector *b*:

$$C_i = \frac{mean(b_i)}{std(b_i)} \tag{2}$$

5. Absolute value of C_i was used to discriminate if each spectra variable is effective or not. All effective variables were selected to create a new independent variable matrix, and then this new matrix and Y were used to establish a new PLSR prediction model.

NIR spectra

Original NIR spectra of total 99 filter samples are shown in figure 2, and the spectra of samples after pre-processing with MSC are shown in figure 3. It is obviously seen that the base line drift of the spectra is reduced in the figure 3 compared to figure 2 by the application of MSC.

Results of PLSR in full bands

For the total sets, two spectrum pre-processing methods MSC and SNV were used. Figure 4 illustrates the results of the cross validation when MSC and SNV were used as the spectrum pre-processing method.

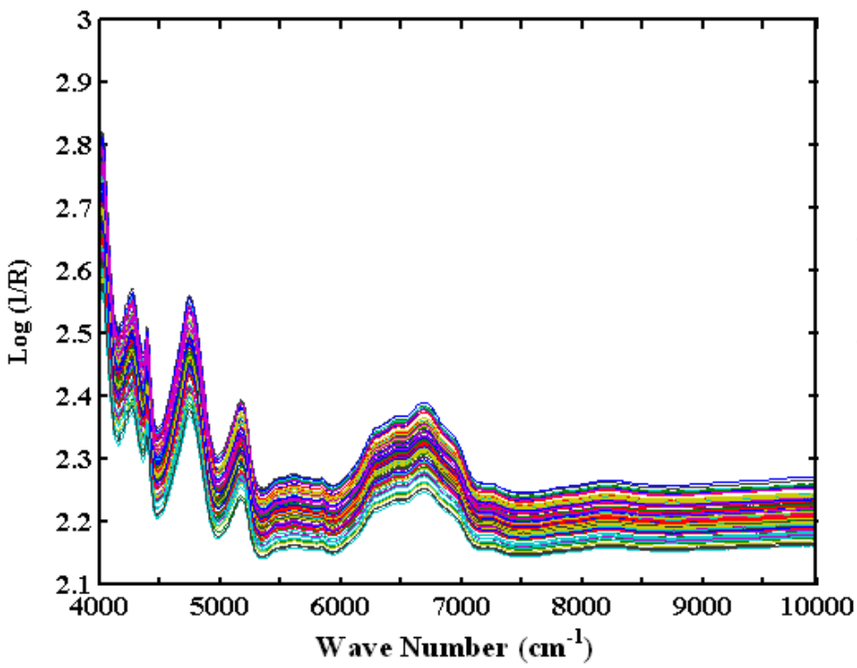


Fig. 2. NIR transmittance spectra of filter-paper samples with different chlorpyrifos content.

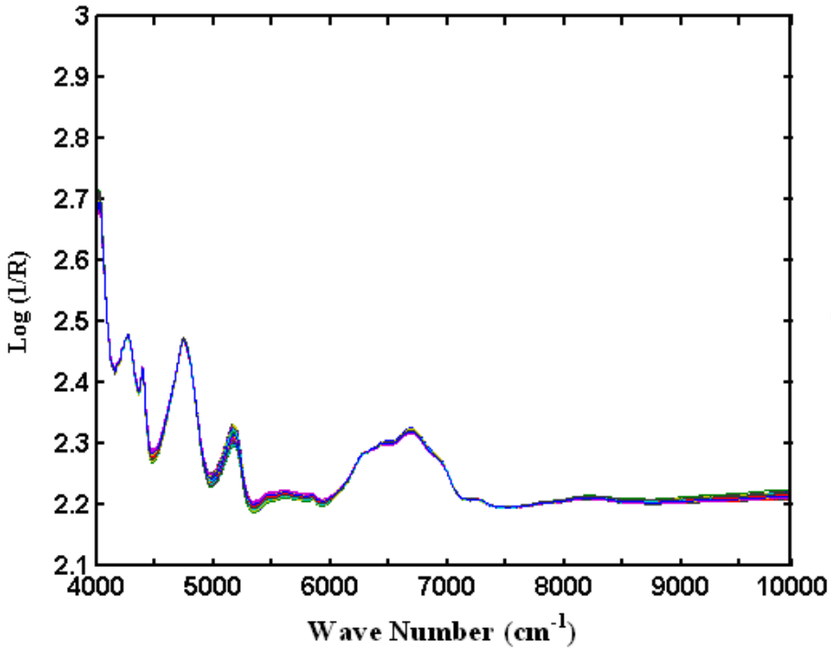


Fig. 3. NIR transmittance spectra of filter-paper samples after MSC.

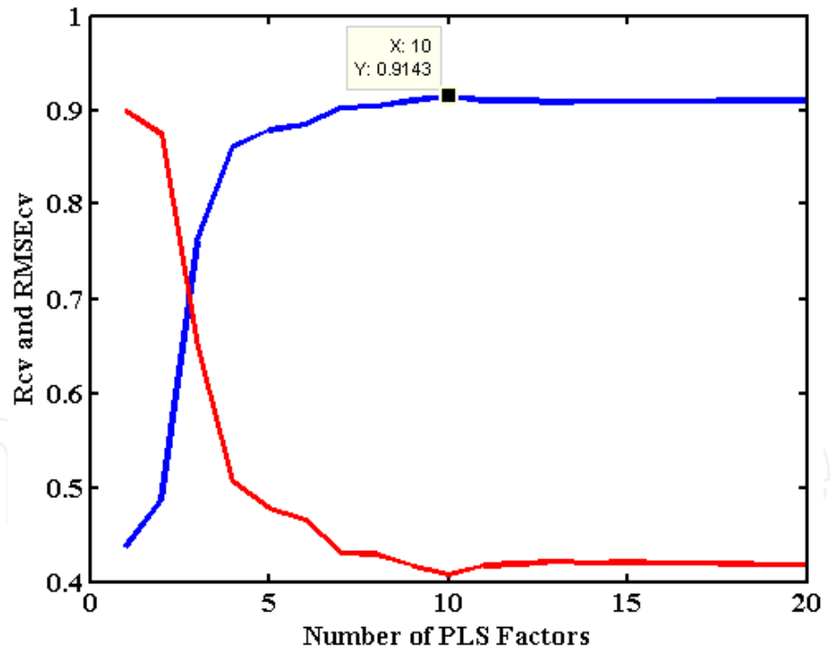


Fig. 4. Optimal PC number of prediction model for filter-paper samples.

The total sample sets were separated into calibration set and validation set. Cross validation was first used in calibration sample set to find the optimal principle component number. From figure 4 we can see the best principle component number to be 10 with corresponding highest Rcv of 0.91 and lowest RMSEcv of 0.41. Model accuracy was then evaluated on the validation set using the root mean square error of prediction (RMSEP), correlation

coefficient (R) between predicted and actual data. The results obtained are shown in table 2 corresponding to R = 0.95 and RMSEP= 0.32 mg/kg.

Results of UVE-PLSR

From table 2 we can see that PLSR method do get satisfied prediction results. However, PLSR method using full bands of the spectra for developing calibration model are time-consuming while running the computer program. Some variables in the full bands of samples' spectra are effective while some are not. As such determining effective spectra from the full band spectra is very essential. A special algorithm, namely uninformative variable elimination (UVE) was used in this research to find out the effective variables. The variables with useless information were eliminated.

Pre-processing method	LV	Rcv	RMSEcv (mg/kg)	R	RMSEP (mg/kg)
MSC + SNV	10	0.91	0.41	0.95	0.32

LV: the optimal principal component (PC) number used in cross-validation
Rcv: correlation coefficient of cross validation
RMSEcv: root mean square error of cross validation
R: correlation coefficients in validation set
RMSEP: root mean square error of prediction

Table 2. Calibration and validation results for chlorpyrifos concentration by using PLSR method.

MSC and SNV were used as the pre-processing method. According the result in table1, the optimal principal component number was chosen as 10. Then UVE algorithm was used to select the effective variables. The results are shown in figure 5.

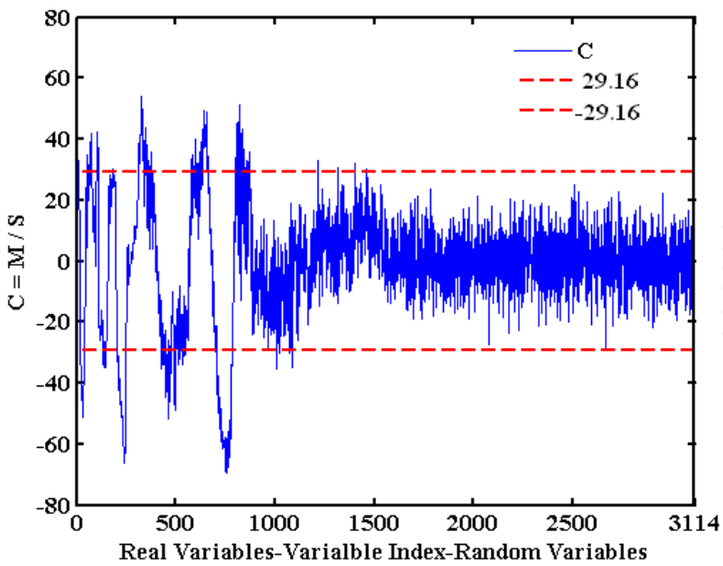


Fig. 5. Variables selected by UVE.

In figure 5, the dotted line indicates the threshold of variables selection. In the range of [1, 1557], the variables corresponding the *C* value within the threshold range are not effective,

and 368 variables left after ineffective variables were eliminated. A new PLSR prediction model was developed by using these 368 variables. The results showed that the correlation coefficient (Rcv) in cross validation is 0.91, the root mean square error of cross validation (RMSEcv) is 0.42 mg/kg, and the correlation coefficient (R) in validation set is 0.94, the root mean square error of prediction (RMSEP) is 0.36 mg/kg. Compared with the results of the PLSR used in full bands, the UVE-PLSR could get similar results but using fewer wave bands. In the UVE algorithm, the random matrix which was added into the original matrix was different each time, so the results would be different in every prediction model. In order to prove the stability of UVE algorithm, another 4 times of UVE-PLSR was used. The results of a total of five times UVE-PLSR are shown in table 3.

Model	Number of Variables	LV	Rcv	RMSEcv (mg/kg)	R	RMSEP (mg/kg)	Variables Thresholds
UVE-PLSR-1	368	7	0.91	0.42	0.94	0.36	±29.16
UVE-PLSR-2	281	7	0.90	0.47	0.94	0.37	±31.31
UVE-PLSR-3	395	7	0.90	0.43	0.94	0.36	±27.61
UVE-PLSR-4	379	7	0.90	0.43	0.94	0.37	±28.23
UVE-PLSR-5	330	7	0.90	0.43	0.94	0.36	±30.27

LV: the optimal principal component (PC) number used in cross-validation
Rcv: correlation coefficient of cross validation
RMSEcv: root mean square error of cross validation
R: correlation coefficients in validation set
RMSEP: root mean square error of prediction

Table 3. Prediction results of UVE-PLSR methods.

Figure 6 shows the variables selection results by the use of another 4 times UVE-PLSR based on different random matrix. As the results shown in table 3, the differences between each UVE-PLSR are small. The number of variables ranged from 281 to 395, and the prediction results were almost identical to each other. Considering the different random matrix, the Rcv (correlation coefficient of cross validation) range from 0.90 to 0.91, RMSEcv (cross validation) range from 0.42 to 0.47 mg/kg, R (validation set) is 0.94, RMSEP range from 0.36 to 0.37mg/kg which MSC and SNV were used as the pre-processing method. It could be concluded that the differences of random matrix have very weak affection in the process of developing a prediction model, and the numbers of variables used in UVE-PLSR could be declined by more than 70%. These results indicated that the prediction capability of UVE-PLSR is similar as the PLSR used in full bands. So, it can be concluded that NIR determination of pesticide is a low cost, an environment friendly and a potential method compared to the traditional methods, and the UVE-PLSR algorithm is an efficient method to select the effective variables of spectra and develop a prediction model of pesticide concentration with fewer wave bands.

3. Optical imaging technology for pesticide determination

In recent years, optical imaging technology has become popular. Hyperspectral imaging as one of optical imaging technology has been used in agriculture, biomedicine, food industry

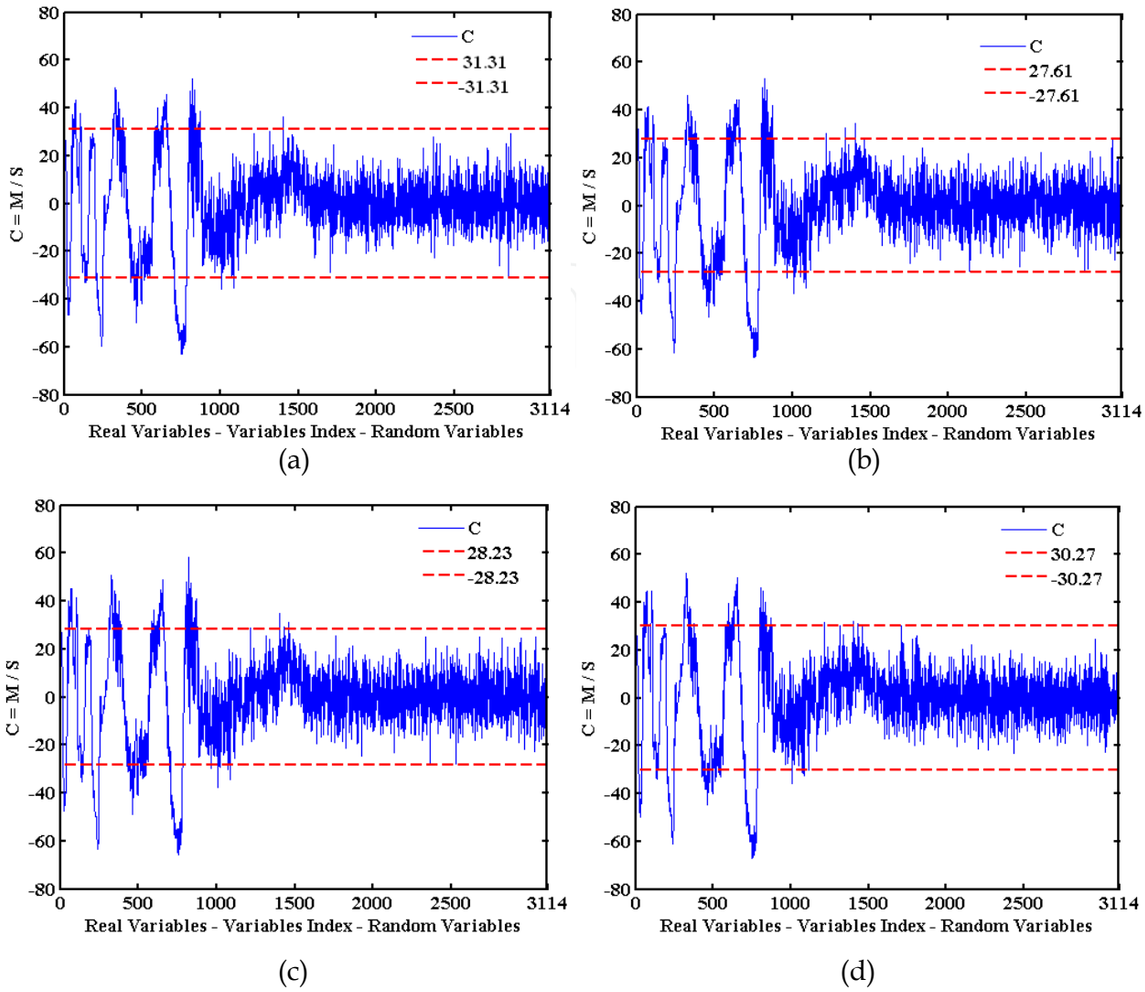


Fig. 6. Variables selected by UVE with different random matrix.

etc. Hyperspectral imaging is a powerful tool for acquiring both spectral and spatial information from an object at contiguous wavelengths over a wide spectral range. According to determination of pesticide, hyperspectral imaging combined with fluorescence stimulate technology could acquire a satisfactory result.

The following example presents the methodology to determinate chlorpyrifos based on hyperspectral fluorescence imaging technology.

Samples

Pesticide solution: A commercial pesticide, containing 40% chlorpyrifos (Noposion, China) was used. Methanol was prepared in order to provide the solutions with different concentrations. Five concentration levels, 0.5, 1, 2, 8 and 16 mg/kg of active ingredient were diluted based on the amount of chlorpyrifos.

Vegetable samples: Pollution-free rapes bought from local market were used. After washing up all the surface of rape samples by the use of distilled water, pesticide solutions were sprayed evenly on dry rape samples' surface.

Data acquisition

A hyperspectral fluorescence imaging system (Figure 7) was used which mainly consisted of a high-performance back-illuminated charge coupled device (CCD) camera (Sencicam QE Germany), an imaging spectrograph (ImSpector V10E, Spectral Imaging Ltd., Finland), and a light unit with fluorescence lamps as the light source (Foshan, China). The camera, with spatial resolution of 1376×1040, was fitted with a 25mm lens (Computar, Japanese), the spectrograph had an effective spectral region from 400 to 1100nm with a 2.8 nm spectral resolution. The light source used in this study was a pair of fluorescence lamps which had the spectral region from 340 to 600nm. The whole system was shielded with a close chamber avoiding the interferences of external lights from outside. Hyperspectral fluorescence images were acquired and ENVI 4.3 software was used for data analysis.

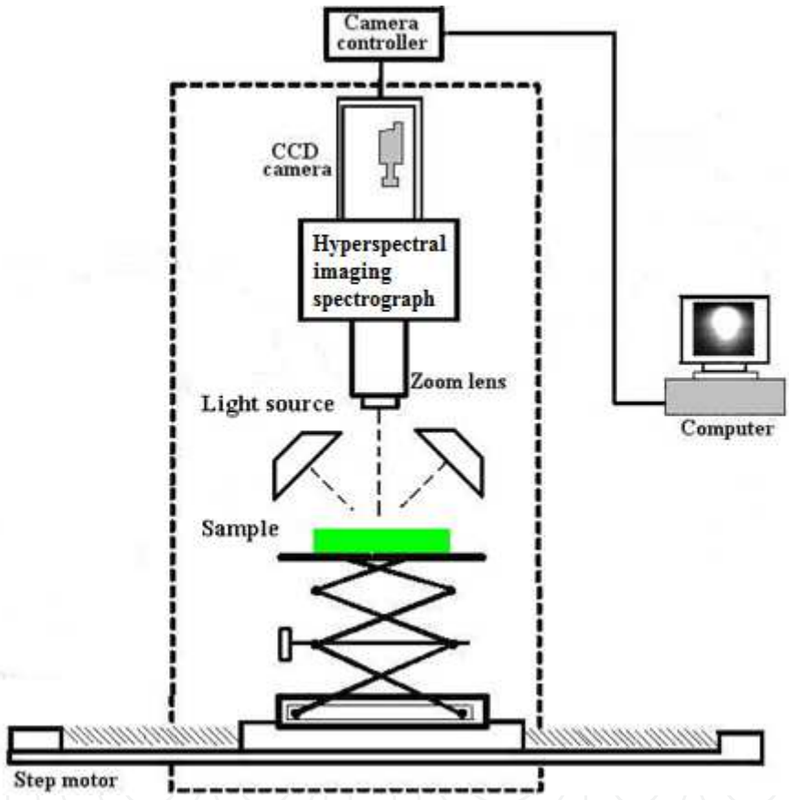


Fig. 7. Hyperspectral fluorescence imaging system.

Data analysis

Figure 8 shows the hyperspectral fluorescence image of 8mg/kg sample which was composed by the use of ENVI 4.3 software. In this picture, the white regions are the fluorescence of chlorpyrifos solutions. Threshold segmentation method was used to acquire the Region of Interest (Figure 9, red regions). Then average spectral curves of different samples in Region of Interest were calculated in whole wave bands (Figure 10). According to figure 10, chlorpyrifos has strong fluorescence characteristic when methanol is used as solvent. The emission spectrum of chlorpyrifos indicates that it has the peak emission at the wavelength of 437 nm, and chlorpyrifos samples with different concentration have different fluorescence

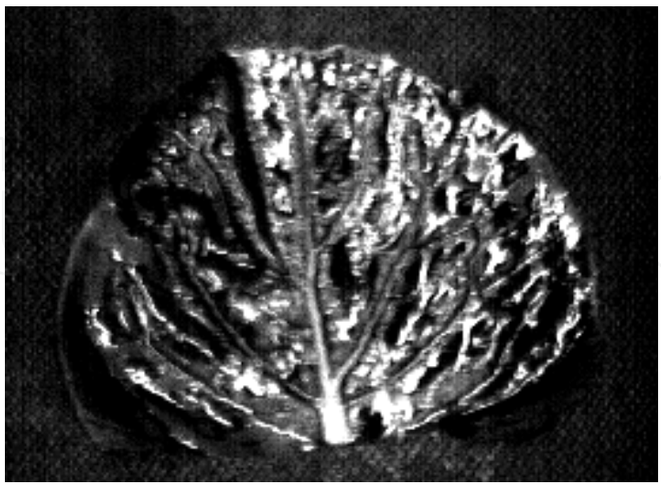


Fig. 8. Hyperspectral fluorescence image of 8mg/kg sample.



Fig. 9. ROI image of the sample with the chlorpyrifos concentration of 8mg/kg sample.

emission spectral intensity at the peak. It can also be seen in figure 10 that the fluorescence emission peak value reduces when the concentration of chlorpyrifos decreases. The peak emission at the wavelength 524nm might be the effect of other organic elements present in the commercial composite pesticide used in this research. The results can be used as theoretical basis for developing rapid detection instrument for vegetable pesticide residue.

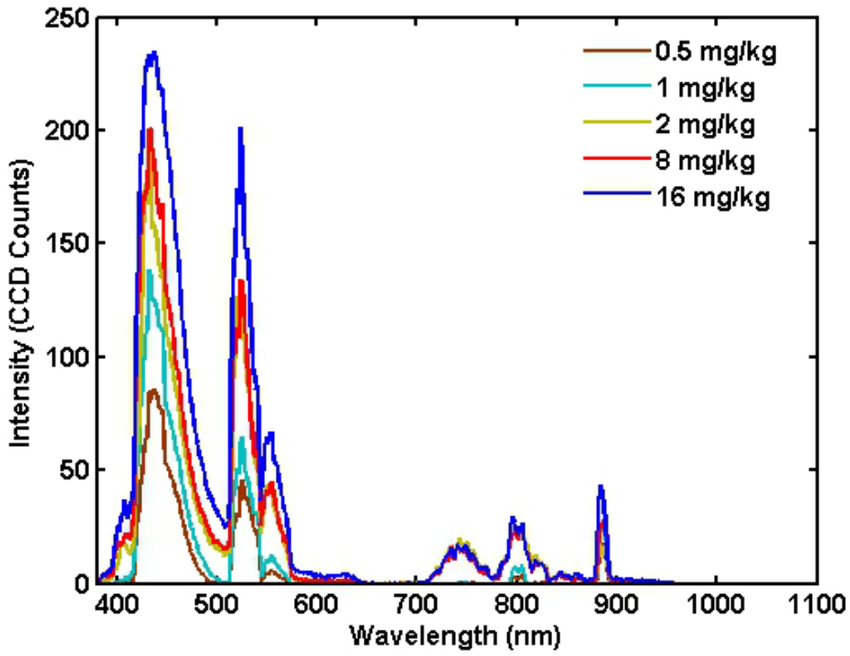


Fig. 10. Emission spectrum of samples with different chlorpyrifos concentration.

4. Conclusion

Pesticide concentration can be readily measured with NIR spectroscopy and optical imaging technology. However the accuracy and precision could be improved. There is a need to develop rapid optical techniques for pesticide determination which could be used in the future for agro-food safety assurance. The optical technique could be one of the most useful tools along with the advancement of spectral instrument for determination of pesticide residue.

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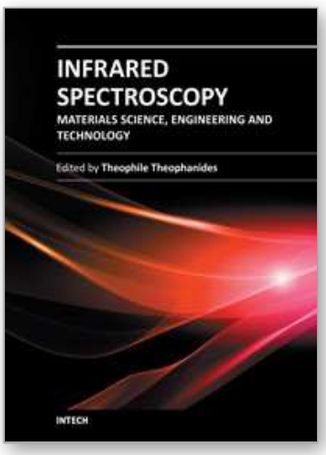
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The present book is a definitive review in the field of Infrared (IR) and Near Infrared (NIR) Spectroscopies, which are powerful, non invasive imaging techniques. This book brings together multidisciplinary chapters written by leading authorities in the area. The book provides a thorough overview of progress in the field of applications of IR and NIR spectroscopy in Materials Science, Engineering and Technology. Through a presentation of diverse applications, this book aims at bridging various disciplines and provides a platform for collaborations among scientists.

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