

# RAPID QUANTITATIVE ANALYSIS OF DIMETHOATE PESTICIDE USING SURFACE-ENHANCED RAMAN SPECTROSCOPY

Y. Liu, B. Ye, C. Wan, Y. Hao, Y. Lan, A. Ouyang

**ABSTRACT.** Pesticides are widely used in agriculture, and pesticide residues have become a public concern. So far, no analytical method has been available for the rapid and quantitative analysis of most food pesticides. In this study, the application of micro-surface-enhanced Raman spectroscopy (SERS) for analysis of the typical organophosphorus pesticide dimethoate is demonstrated. Huge enhanced Raman signals of pesticides at low concentrations of 0.5 to 10  $\mu\text{g mL}^{-1}$  were acquired by confocal Raman micro-spectrometry with Klarite substrate. The observed spectra were analyzed by comparison with the normal Raman spectra of dimethoate. Partial least squares (PLS) regression combined with different data preprocessing methods and wavelength selection was applied to develop quantitative models for dimethoate solutions. The best model, with the highest correlation coefficient (0.969) and the lowest root mean square error of predictions (0.626), was achieved with the first derivative combined with standard normalized variate (SNV) spectra and the wavelength bands of  $1845.5\pm 1186.9\text{ cm}^{-1}$  and  $1023\pm 199.5\text{ cm}^{-1}$ . This study indicated that SERS coupled with a nanosubstrate is a potential tool for rapid quantification of pesticide residues at  $10^{-6}$  concentration levels.

**Keywords.** Dimethoate, Nanosubstrate, Partial least squares, Quantitative analysis, Surface-enhanced Raman spectroscopy.

Organophosphate pesticides (OPPs) play an important role in increasing agriculture productivity and have become one of the most commonly used means to prevent, destroy, repel or mitigate pests, including insects, rodents, and weeds, in agriculture throughout the world. OPPs are a group of relatively small molecules that act as acetylcholinesterase inhibitors. Their acute toxicity and widespread use have attracted intensive worldwide concern about trace amounts of residues on agricultural products that might cause long-term health effects (Vongsivut et al., 2010). OPPs are widely used in fruit and vegetable crops (Kegley and Wise, 1998). Dimethoate (O,O-dimethyl S-(N-methyl carbomoyl-methyl) phosphorodithioate) is highly effective and is among the most widely used OPPs. Dimethoate is both an insecticide and acaricide with both contact and stomach action. Dimethoate residues in food pose potential health risks, such as depression, anxiety, or irritability, and long-

term exposure to high levels can lead to aplastic anemia and cancer (Fleming et al., 2012). In recent years, there has been growing concern among consumers about pesticide contamination in fruits. Therefore, rapid, reliable, and consistent detection methods for OPPs, especially dimethoate, are crucially needed.

A variety of analytical methods suggested by the Collaborative International Pesticides Analytical Council (CIPAC) for quality control of pesticide formulations are based on chromatographic analysis, such as gas chromatography (GC) (Ortelli et al., 2005; Rawn et al., 2006; Yan et al., 2010), high-performance liquid chromatography (HPLC) (Banerjee et al., 2007; Cheng et al., 2009; Tsochatzis et al., 2010; Tuzimski, 2011), liquid chromatography with mass spectrometry (LC-MS) (Liu et al., 2005; Grimalt et al., 2007), and liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Frenich et al., 2004; Wong et al., 2004). In recent years, new analytical methods for detection of fruit pesticides have been reported, including fluorescence polarization immunoassay (Kolossova et al., 2003), multi-enzyme inhibition assay (Walz and Schwack, 2007), and biosensors (Valdés-Ramírez et al., 2008). Although, these methods can be used to detect trace amounts of pesticides, they are time-consuming, labor-intensive, and expensive, making these analytical approaches less attractive and limiting laboratory, real-time, and field detections to some extent (Tang et al., 2011). Therefore, reliable, accurate, and rapid detection of pesticides is important for protecting the environment and human health (Sato-Berrú et al., 2004).

Raman spectroscopy is a highly specific detection method that enables identification of molecules through their specific molecular fingerprints. Unfortunately, the

---

Submitted for review in November 2012 as manuscript number FPE 10009; approved for publication by the Food & Process Engineering Institute of ASABE in April 2013.

Mention of company or trade names is for description only and does not imply endorsement by the USDA. The USDA is an equal opportunity provider and employer.

The authors are **Yande Liu**, Professor, **Bin Ye**, Graduate Student, **Changlan Wan**, Graduate Student, and **Yong Hao**, Lecturer, School of Mechanical and Electronic Engineering, East China Jiaotong University, Nanchang, China; **Yubin Lan**, **ASABE Member**, Research Engineer, USDA-ARS Southern Plains Agricultural Research Center, College Station, Texas; **Aiguo Ouyang**, Professor, School of Mechanical and Electronic Engineering, East China Jiaotong University, Nanchang, China. **Corresponding author:** Yande Liu, School of Mechanical and Electronic Engineering, East China Jiaotong University, Nanchang 330013, China; phone: +86-791-87046173; e-mail: jxliuyd@163.com.

sensitivity of Raman spectroscopy is quite low, so it cannot be used for analysis of samples at low concentrations. However, this weakness can be overcome through the use of surface-enhanced Raman scattering, which can enhance the weak Raman signal up to  $10^6$  or even  $10^{14}$  times, dramatically extending the range of available applications (Zamuner et al., 2009). This technique does not require the pretreatment that is required by other analytical techniques. It allows obtaining high-quality vibrational spectra of samples adsorbed onto nanostructured metal surfaces, including colloids, electrodes, and thin films (Aroca et al., 2005). Surface-enhanced Raman spectroscopy (SERS) has already been applied for characterization of adsorbate-metal surface interfaces (Smitha et al., 2011), biological samples (Manno et al., 2010), single molecules or particles (Tan et al., 2011), and detection of environmental pollutants (Liu et al., 2011b).

Many studies have indicated the great potential of SERS as an analytical tool for food contaminants and some adulterants, including detection of organophosphate and carbamate pesticides in fruit using gold nanostructures (Liu et al., 2012), determination of chloramphenicol and crystal violet in seafood (Lai et al., 2011a; He et al., 2008), analysis of restricted sulfonamide residues and the banned food dye Sudan 1 (Lai et al., 2011b; Cheung et al., 2010), quantitative detection of carbaryl with silver nanoparticle-coated silicon nanowire arrays (Wang et al., 2012), analysis of fonofos pesticide on silver and gold nanoparticles (Vongsvivut et al., 2010), quantification of trace amounts of melamine and its analogs with gold nanosubstrates (He et al., 2008; Liu et al., 2010), and study of the adsorption of the insecticide cyromazine on silver colloid (Mukherjee et al., 2001).

The goal of this study was to investigate the feasibility of using SERS technology with a Klarite substrate for quantitative analysis of dimethoate. Standard solutions of the pesticide were used to gain a better understanding of the detection limit. Different preprocessing methods and wavelength bands were compared to the PLS calibration models. This study could be used as the basis for further studies on detecting pesticide residues in fruit.

## MATERIALS AND METHODS

### MATERIALS AND REAGENTS

Dimethoate (O,O-dimethyl S-[2-(methylamino)-2-oxoethyl]; 98.6% purity) was purchased from Accu-Standard, Inc. (New Haven, Conn.). Due to its hydrophobicity and the limited solubility of the analyte in water, a stock solution of  $100 \mu\text{g mL}^{-1}$  dimethoate was prepared by subsequent dilution in high-purity methanol (HPLC reagent, Sigma-Aldrich, St. Louis, Mo.). Methanol was recommended as a solvent for dimethoate to prepare a series of standard solutions. Mixtures of methanol and ultra-pure water at a ratio of 1:1 were used to prepare a range of standard solution concentrations from 0.5 to  $10 \mu\text{g mL}^{-1}$  at  $0.5 \mu\text{g mL}^{-1}$  intervals. The SERS process requires a  $0.2 \mu\text{L}$  dimethoate solution. To acquire the spontaneous Raman spectra, solid pesticide powder was deposited on a glass slide and then pressed to form a thin film.

### INSTRUMENT AND RAMAN SPECTRA ACQUISITION

A Nicolet DXR microscope Raman spectrometer (Thermo Fisher Scientific Corp., Madison, Wisc.) was used to collect the confocal Raman micro-spectra and SERS spectra with a CCD array detector. The excitation wavelength was 780 nm. This system has an Olympus optical microscope with 10 $\times$ , 20 $\times$ , 50 $\times$ , and 100 $\times$  power, a high spatial resolution ( $<1 \mu\text{m}$ ), and a 14 mW maximum power diode laser. The resolution of the confocal depth was  $2 \mu\text{m}$ .

The SERS spectra were collected using Klarite SERS-active substrates (Renishaw Diagnostics, Ltd., Glasgow, U.K.) with an active area of  $4 \text{ mm} \times 4 \text{ mm}$ . These substrates were fabricated on silicon wafers coated with gold and had regular arrays of inverted square pyramid subunits that were about  $1 \mu\text{m}$  deep and  $1.8 \mu\text{m}$  long, while the distance between subunits was about  $0.4 \mu\text{m}$  (Liu et al., 2012). Klarite has shown potential in detection of trace amounts of melamine, cyanuric acid, and melamine cyanurate, as well as *Bacillus* spores (Szeghalmi et al., 2007; He et al., 2008).

To acquire the surface-enhanced Raman spectra,  $0.2 \mu\text{L}$  of dimethoate solution was placed onto the active area of the Klarite substrate. The solvent was then evaporated at room temperature, leaving a thin film of analytes at the surface. Fifteen Raman spectra of dimethoate were collected at different locations on the substrate. Spectral data were collected using Omnic software of (Thermo Nicolet Corp., Madison, Wisc.). In this study, all Raman spectra collections were carried out from 200 to  $2000 \text{ cm}^{-1}$  with the same experimental conditions ( $5 \text{ cm}^{-1}$  resolution, 17 mW laser power on the sample, and 5 s exposure time), and spectra at 15 representative positions were recorded for all samples.

### DATA PROCESSING AND ANALYSIS

Raman and SERS spectral data sets were analyzed using Opus software (ver. 6.5, Bruker Optik GmbH, Ettlingen, Germany). Partial least squares (PLS) regression was used to develop the analysis models. To compare the effects of preprocessing on the prediction performance of the PLS calibration models, data preprocessing algorithms, including first and second derivative transformation, standard normal variate (SNV), and multiple scattering correction (MSC), were used to remove background interference and increase the signal-to-noise ratio (Liu and Ying, 2006).

The prediction accuracy of the models was evaluated using the correlation coefficient (R), root mean square error of cross-calibration (RMSECV) or prediction (RMSEP), bias, slope, and offset. The R and RMSEP values were the main parameters used to evaluate model performance in this study. The slope and bias should be taken into consideration for distinguishing systematic errors and measuring the average difference between reference and predicted values. Generally, a good model should have a high R value and low RMSECV, RMSEP, and bias values (Liu and Ying, 2006).

## VARIABLE SELECTION

Wavelength selection involves selecting a subset of spectral regions with which the established calibration model produces the minimum errors in prediction. Wavelength selection not only enhances the stability of the model, resulting from the collinearity of multivariate spectra, but also helps in interpreting the relationship between the calibration model and the sample compositions. The selection of appropriate portions of the spectra is also crucial to the performance of the calibration model.

In this study, according to the SERS spectrometer, the SERS spectra were divided into three regions, and calibrations were performed for each spectral region as well as for combinations of them: wavelength band  $1845.5 \pm 199.5 \text{ cm}^{-1}$  (according to the Raman and SERS spectra) and wavelength bands  $1845.5 \pm 1186.9 \text{ cm}^{-1}$ ,  $1023 \pm 199.5 \text{ cm}^{-1}$ ,  $1022.0 \pm 199.5 \text{ cm}^{-1}$ , and  $1681.5 \pm 199.5 \text{ cm}^{-1}$  (based on the Opus software). For each region, PLS models with 1 to 20 factors were investigated. Model performance was evaluated by comparing the standard error of calibration and the standard error of prediction.

## RESULTS AND DISCUSSION

### SPECTRAL FEATURES OF DIMETHOATE

Figure 1 shows the molecular structure and SERS spectrum of dimethoate. The pesticide molecules may be adsorbed onto the Klarite substrate at different orientations, and the types of adsorption sites on the substrate also varied, which contributed to significant broadening of the Raman signals. The molecular orientations when attached to the substrate and the types of adsorption sites also affect the wavenumbers of the Raman signals (Steven and Narayana, 2008). The same major peaks in the Raman spectrum of dimethoate, such as the peaks at 650 and  $766 \text{ cm}^{-1}$ , were prominent in the SERS spectrum. However, some prominent peaks (such as 257 and  $498 \text{ cm}^{-1}$ ) in the spontaneous Raman spectrum of dimethoate may not be prominent peaks in the SERS spectrum. It was reported that peak splitting observed at 407, 498, and  $1166 \text{ cm}^{-1}$  for dimethoate can be respectively assigned to P–O–C

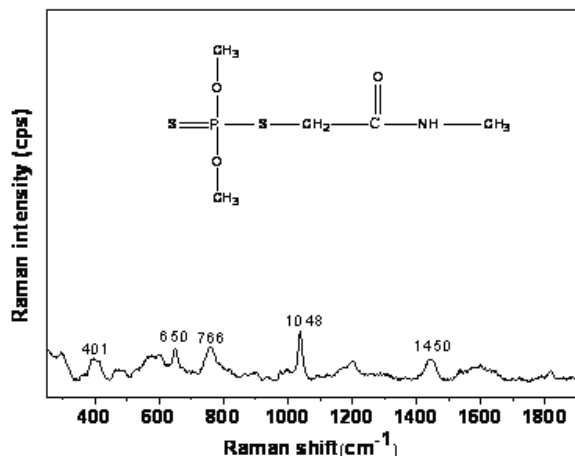


Figure 1. Molecular structure and SERS spectrum of dimethoate.

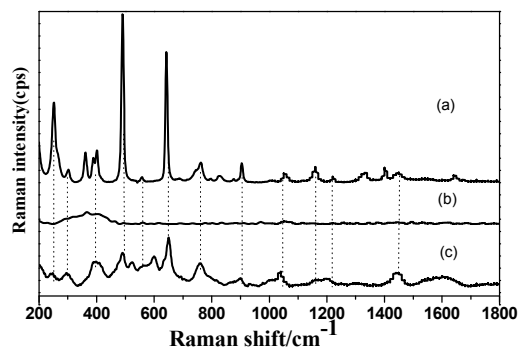


Figure 2. Raman spectra of (a) solid dimethoate, (b) dimethoate solution ( $10 \mu\text{g mL}^{-1}$ ), and (c) SERS spectrum of dimethoate ( $10 \mu\text{g mL}^{-1}$ ).

deformation,  $\text{CH}_3$  torsion vibration, and C–C stretching vibration (Thomas, 1974; Lin-Vien et al., 1991).

Figure 2 shows the Raman spectra of solid dimethoate, of dimethoate solution at  $10 \mu\text{g mL}^{-1}$ , and the SERS spectrum of dimethoate solution at  $10 \mu\text{g mL}^{-1}$ . As shown in figure 2, a strong Raman signal can be found in the spectrum of solid dimethoate, but no detectable signal was found at low concentrations ( $10 \mu\text{g mL}^{-1}$ ) of dimethoate solution, which was due to the low sensitivity of Raman spectroscopy. However, when this concentration was deposited on the Klarite substrate, the characteristic SERS spectrum is clearly observed (fig. 2c). Figure 2 clearly shows that the enhanced SERS signals on the active area were stronger, compared to no Raman signal on the inactive area.

The main wavenumbers of these spectra as well as the assignments derived from the calculation are listed in table 1. According to the Raman spectrum of solid dimethoate, major peaks were located at several positions, including  $1449 \text{ cm}^{-1}$  caused by O=C–N I or O=C stretching,  $1227 \text{ cm}^{-1}$  caused by  $\rho(\text{P–O–CH}_3)$ , and 913, 834, 766, 650, 498  $\text{cm}^{-1}$ , etc. Compared with the spectrum of solid dimethoate, the SERS spectrum of dimethoate solution included some closely similar major peaks at 258, 368, 401, 497, 650, 766, 913, and  $1450 \text{ cm}^{-1}$ . Most of the peaks were greatly enhanced by SERS.

Table 1. Vibrational peaks of dimethoate and assignments.<sup>[a]</sup>

Dimethoate Peaks ( $\text{cm}^{-1}$ )		Assignments	
Raman Shift	SERS Shift	Raman Shift	SERS Shift
257 (s)	258 (w)	Lattice	Lattice
368 (m)	368 (w)	-	-
407 (m)	401 (vw)	$\delta(\text{P–O–C})$	$\delta(\text{P–O–C})$
498 (vs)	497 (s)	$r(\text{CH}_3)$	$r(\text{CH}_3)v$
563 (vw)	550-565 (vw)	$\delta(\text{O=C–CH}_2)$	$\delta(\text{O=C–CH}_2)$
650 (vs)	650 (vs)	$v(\text{P=S})$	$v(\text{P=S})$
766 (m)	766 (m)	$\nu_s(\text{P–O–C})$	$\nu_s(\text{P–O–C})$
834 (w)	-	$\delta(\text{C–C=O})$	-
913 (m)	910-913 (vw)	$v(\text{P–O})$	$v(\text{P–O})$
1166 (w)	1160-1165	$v(\text{C–C})$	$v(\text{C–C})$
1227 (vw)	-	$\rho(\text{P–O–CH}_3)$	-
1338 (w)	-	$v(\text{S–CH}_2)$	-
1449 (w)	1450 (vw)	$v(\text{O=C–N})$ I	$v(\text{O=C–N})$ II

<sup>[a]</sup> vs = very strong, s = strong, m = medium, w = weak, vw = very weak, v = stretching,  $\delta$  = deformation, r = torsion, and s = symmetric.

Finally, according to the literature on specific vibrations found in dimethoate, specific vibrations were found for the basic P=S stretching vibration in the region 750 to 535  $\text{cm}^{-1}$ , for P-S stretching vibration in the region 613 to 440  $\text{cm}^{-1}$ , and for P-O-CH<sub>3</sub> in the region 1088 to 1015  $\text{cm}^{-1}$  (Thomas, 1974; Lin-Vien et al., 1991). Our measured dimethoate bands are similar to the data found in the literature.

## QUANTITATIVE ANALYSIS OF DIMETHOATE FORMULATIONS

### SERS Spectral Pretreatment

Generally, noise and systematic behavior are undesirable features in SERS spectra. To solve these problems, the original spectra were preprocessed with MSC, first and second derivatives, SNV, and first derivative combined with SNV. Figure 3 shows the first derivative and SNV preprocessed SERS spectrum of the dimethoate solution. Table 2 shows the results of the different pretreatment methods for the calibration models in the whole spectral region. The highest R value was achieved by using the first derivative combined with SNV. The highest correlation coefficient of calibration ( $R_C$ ) was 0.979, and the lowest RMSEC value was 0.821, while the correlation coefficient of prediction ( $R_P$ ) for the validation model was 0.968, and the lowest RMSEP value was 0.626. Figure 4 is a scatter plot of the best validation model built by PLS with the best preprocessing method, which shows the correlation between the reference and predicted values of dimethoate solution concentrations. The offset was 0.092.

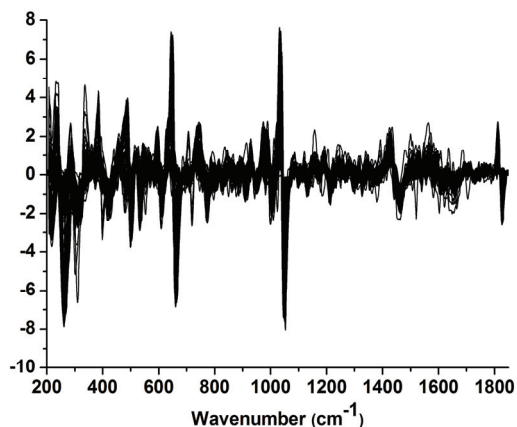


Figure 3. SERS spectra of dimethoate solution preprocessed with first derivative + SNV.

Table 2. Comparison results for dimethoate solution concentrations developed by PLS with different preprocessing methods.

Pretreatment	Calibration Set		Validation Set	
	$R_C$	RMSEC	$R_P$	RMSEP
None	0.970	1.08	0.933	0.959
First derivative	0.974	1.35	0.947	0.819
Second derivative	0.969	1.05	0.925	0.991
MSC	0.939	1.45	0.861	1.40
SNV	0.959	1.27	0.938	0.887
First derivative + SNV	0.979	0.821	0.968	0.626
First derivative + MSC	0.974	0.897	0.952	0.786

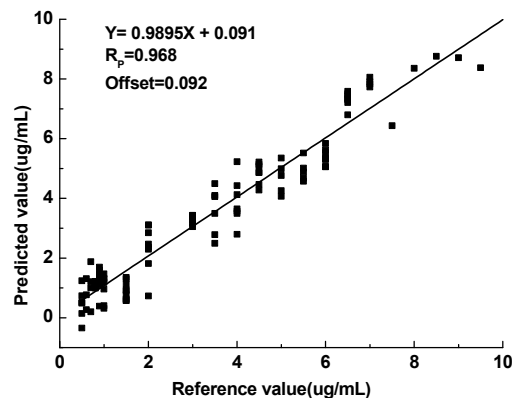


Figure 4. Comparison between reference values and predicted values of dimethoate solutions using the PLS models with first derivative and SNV in the whole spectral region based on SERS spectra.

Table 3. Comparison results for dimethoate solution concentrations developed by PLS with different spectral ranges.

Spectral Range ( $\text{cm}^{-1}$ )	Calibration Set		Validation Set	
	$R_C$	RMSEC	$R_P$	RMSEP
1845.5±199.5	0.979	0.821	0.968	0.626
1845.5±1515.7 and 1351.8±199.5	0.978	0.830	0.968	0.627
1681.5±199.5	0.978	0.840	0.964	0.669
1845.5±1186.9 and 1023±199.5	<b>0.979</b>	<b>0.816</b>	<b>0.969</b>	<b>0.626</b>
1845.5±1022 and 658.1±199.5	0.976	0.869	0.967	0.639

### Variable Selection and Analysis Model Construction

The SERS spectra were measured in the range of 2000±199.5  $\text{cm}^{-1}$  and the valid wavelength range is 1845.5±199.5  $\text{cm}^{-1}$ . In order to compare the results of different wavelength ranges, the ranges of 1845.5±199.5  $\text{cm}^{-1}$ , 1845.5±1022  $\text{cm}^{-1}$ , 1351.8±199.5  $\text{cm}^{-1}$ , 1023±199.5  $\text{cm}^{-1}$ , and 1681.5±199.5  $\text{cm}^{-1}$  are discussed in this study. The calibration set was selected with the aim of providing strong calibration for the dimethoate solution concentrations by maximizing the variability among sample compositions and obtaining a wide range of spectra. Selection of the optimum wavelength range for the best prediction model was done by PLS analysis with the preprocessing method of first derivative and SNV. The prediction results for the different wavelength ranges are present in table 3. The optimum wavelength ranges for the best performance of the predictive model were 1845.5±1186.9  $\text{cm}^{-1}$  and 1023±199.5  $\text{cm}^{-1}$ . These regions achieved the best calibration performance results.

### Determining the Best Analysis Model

Based on the above results, calibration analysis models were built in the wavelength ranges of 1845.5±1186.9  $\text{cm}^{-1}$  and 1023±199.5  $\text{cm}^{-1}$  for first derivative and SNV SERS spectra using the PLS technique. The best analysis model was obtained with high correlation coefficients ( $R_C = 0.979$  and  $R_P = 0.969$ ) and low RMSEC (0.816) and RMSEP (0.626) values. Figure 5 shows a scatter plot of the best model built by PLS with first derivative and SNV in the optimal spectral range. The offset is 0.036.

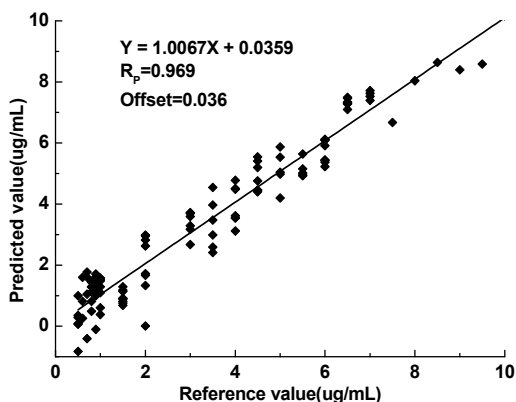


Figure 5 Comparison between reference values and predicted values of dimethoate solution using the PLS models with first derivative and SNV in the regions of  $1845.5 \pm 1186.9 \text{ cm}^{-1}$  and  $1023 \pm 199.5 \text{ cm}^{-1}$  based on SERS spectra.

### COMPARISON TO OTHER RESEARCH

Some studies have been reported about the detection of pesticides in fruit and vegetable and indicated the great potential of applying SERS as an effective analytical tool for fruit contaminants. Armenta et al. (2004) reported two vibrational spectrometry methods (FT-IR and FT-Raman) for cyromazine determination of solid pesticide formulations, and detection limits of 0.2% and 0.8% (w/w) were obtained for FT-IR and FT-Raman, respectively. Zhang et al. (2006) used Raman spectrometry at 514.5 nm and 1064 nm and FT-Raman to study several vegetable and fruit pesticides (abamectin petroleum oil, chongmanjue, cypermethrine, and zhibaoboshi). The Raman spectra of fruits and pesticides were successfully recorded, and pesticides on the surfaces of fruits could be identified conveniently using the FT-Raman spectra.

Valdés-Ramírez et al. (2008) presented the construction of an amperometric biosensor for highly sensitive detection of the organophosphorus insecticide dichlorvos. The pesticide was measured in the presence of 5% acetonitrile, and the biosensor was used to quantify dichlorvos on a sample of apple skin. Vongsvivut et al. (2010) studied the SERS spectra of fonofos and organophosphorus pesticide. The spectra were recorded using citrate-reduced silver (Ag) and gold (Au) colloidal nanoparticles in the form of dried films. In this study, significant enhancements were achieved with fonofos concentrations down to approximately  $10 \times 10^{-6}$  with the Ag colloids, demonstrating the potential of the technique in the analysis of fonofos residues.

Wang et al. (2012) reported that a surface-enhance Raman scattering sensor made of Ag nanoparticle-coated silicon nanowire (SiNW) arrays was fabricated for the quantitative detection of carbaryl (nitrogen pesticide). The wire arrays exhibited a superior detection sensitivity of  $10^{-17} \text{ M}$  for Rodamine 6G with high reproducibility. Liu et al. (2011a) studied the shell thickness-dependent Raman enhancement of silver-coated gold nanoparticles (Au@AgNPs) for identification and detection of pesticide residues on various fruit peels. Liu et al. (2012) used SERS with gold-coated SERS-active nanosubstrates to detect and characterize pesticides extracted from fruits surfaces. Three types of pesticides (carbaryl,

phosmet, and azinphos-methyl), widely used on apples and tomatoes, were selected. They concluded that SERS was able to detect all three types of pesticides extracted from fruit samples at the parts per million level, and the detection limit demonstrated that, at a 99.86% confidence interval, SERS could detect carbaryl at  $4.51 \times 10^{-6}$ , phosmet at  $6.51 \times 10^{-6}$ , and azinphos-methyl at  $6.66 \times 10^{-6}$  on apples, and carbaryl at  $5.35 \times 10^{-6}$ , phosmet at  $2.91 \times 10^{-6}$ , and azinphos-methyl at  $2.94 \times 10^{-6}$  on tomatoes.

The studies mentioned above indicate the potential applications of SERS with chemometric methods for determination of dimethoate. However, limitations of the above-mentioned studies in comparison to the present study include the following: different types of Raman spectrometer, different SERS-active nanosubstrates, different types of pesticide, different levels of pesticide concentration, and different sample extraction and chemometric methods. Therefore, the performance of the models in terms of quality or quantification lacks of robustness and stability.

This study is the first to report micro-surface-enhanced Raman spectroscopy (SERS) using analysis of the typical organophosphorus pesticide dimethoate. Huge enhanced Raman signals at low concentrations of  $0.5$  to  $10 \mu\text{g mL}^{-1}$  were acquired by confocal Raman micro-spectrometry with Klarite substrate. Combined with the PLS method, different data preprocessing methods and wavelength selection, quantitative models for dimethoate solutions were developed and validated. The analysis model had the highest correlation coefficient (0.969) and the lowest root mean square error of prediction (0.626) with the first derivative combined with standard normalized variate (SNV) SERS spectra and the wavelength bands of  $1845.5 \pm 1186.9$  and  $1023 \pm 199.5 \text{ cm}^{-1}$ . This study indicates that SERS coupled with a nanosubstrate is a potential tool for rapid quantification of pesticide residues at  $10^{-6}$  concentration levels.

However, the prediction by the analysis model in our study was affected by the following factors: first, the small sample size and the limited dimethoate concentration level, which ranged from  $0.5$  to  $10 \mu\text{g mL}^{-1}$ ; second, the cost of the substrate and the heterogeneous nature of Klarite, as well as the complicated mechanism of SERS enhancement (Lai et al., 2011a). Finally, the molecular orientations and binding sites of dimethoate absorbed onto the substrate influenced the SERS enhancement and resulted in shifts in band positions, which caused difficulty in applying SERS for quantitative analysis (Sajan et al., 2008; Smitha et al., 2011). In this study, a relatively simple system (dimethoate solution on a substrate) was used in the quantitative analysis. In future research, we should apply SERS methods to test for chemical samples on fruit and find extraction standards for the fruit samples. We also need to use different kinds of organophosphate pesticides and different concentration levels.

### CONCLUSIONS

This study demonstrated application of the SERS technique coupled with a Klarite substrate in trace analysis

of a hazardous pesticide (dimethoate) at  $10^{-6}$  concentration levels. Huge enhanced Raman signals of the pesticide at low concentrations of 0.5 to  $10 \mu\text{g mL}^{-1}$  were acquired by confocal Raman micro-spectrometry with the Klarite substrate. The observed spectra were analyzed by comparison with the normal Raman spectra of dimethoate. PLS regression combined with different data preprocessing methods and wavelength selection was applied to develop quantitative models for the dimethoate solutions. The results of the PLS models suggested that Klarite was a good SERS-active substrate for qualitative and quantitative analysis of dimethoate, as well as for practical applications in pesticide residue analysis of food. This study indicated that SERS coupled with a nanosubstrate is a potential tool for rapid quantification of pesticide residues at  $10^{-6}$  concentration levels.

#### ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by the National Science and Technology Support Program (31160250, 61178036), the Technological Expertise and Academic Leader Training Program of Jiangxi Province (2009DD00700), and the Leading Talent of GanBo “555” Engineering Program of Jiangxi Province.

#### REFERENCES

- Armenta, S., G. Quintas, S. Garrigues, and M. De la Guardia. 2004. Determination of cyromazine in pesticide commercial formulations by vibrational spectrometric procedures. *Anal. Chem. Acta* 524(1-2): 257-264.
- Aroca, R. F., R. A. Alvarez-Puebla, N. Pieczonka, S. Sanchez-Cortez, and J. V. Garcia-Ramos. 2005. Surface-enhanced Raman scattering on colloidal nanostructures. *Adv. Colloid Interface Sci.* 116(1-3): 45-61.
- Banerjee, K., D. P. Oulkar, S. Dasgupta, S. B. Patil, S. H. Patil, R. Savant, and P. G. Adsule. 2007. Validation and uncertainty analysis of a multi-residue method for pesticides in grapes using ethyl acetate extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* 1173(1-2): 98-109.
- Cheng, J., M. Liu, Y. Yu, X. Wang, H. Zhang, L. Ding, and H. Lin. 2009. Determination of pyrethroids in porcine tissues by matrix solid-phase dispersion extraction and high-performance liquid chromatography. *Meat Sci.* 82(4): 407-412.
- Cheung, W., I. T. Shadi, Y. Xu, and R. Goodacre. 2010. Quantitative analysis of the banned food dye Sudan-1 using surface-enhanced Raman scattering with multivariate chemometrics. *J. Phys. Chem. C* 114(16): 7285-7290.
- Fleming, G. D., F. Celis, A. Aracena, M. Campos-Vallete, A. E. Aliaga, and R. Koch. 2012. Vibrational and scaled quantum chemical study of O,O-dimethyl S-methyl carbamoylmethyl phosphorodithioate, dimethoate. *Spectrochim. Acta Part A* 89: 222-230.
- Frenich, A. G., J. L. Vidal, T. L. López, S. C. Aguado, and I. M. Salvador. 2004. Monitoring multi-class pesticide residues in fresh fruits and vegetables by liquid chromatography with tandem mass spectrometry. *J. Chromatogr. A* 1048(2): 199-206.
- Grimalt, S. O., J. Pozo, J. V. Sancho, and F. Hernández. 2007. Use of liquid chromatography coupled to quadrupole time-of-flight mass spectrometry to investigate pesticide residues in fruits. *Anal. Chem.* 79(7): 2833-2843.
- He, L. L., Y. Liu, M. S. Lin, J. Awika, D. R. Ledoux, H. Li, and A. Mustapha. 2008. A new approach to measure melamine, cyanuric acid, and melamine cyanurate using surface-enhanced Raman spectroscopy coupled with gold nanosubstrates. *Sensing and Instrum. for Food Qual. and Safety* 2(1): 66-71.
- Kegley, S. E., and L. J. Wise. 1998. *Pesticides in Fruits and Vegetables*. Sausalito, Cal.: University Science Books.
- Kolosova, A. Y., J. H. Park, S. A. Eremin, S. J. Kang, and D. H. Chung. 2003. Fluorescence polarization immunoassay based on monoclonal antibody for the detection of the organophosphorus pesticide parathion-methyl. *J. Agric. and Food Chem.* 51(5): 1107-1114.
- Lai, K. Q., Y. Y. Zhang, R. Du, B. A. Rasco, F. L. Zhai, and Y. Q. Huang. 2011a. Determination of chloramphenicol and crystal violet with surface-enhanced Raman spectroscopy. *Sensing and Instrum. for Food Qual. and Safety* 5(1): 19-24.
- Lai, K. Q., F. L. Zhai, Y. Y. Zhang, X. C. Wang, B. A. Rasco, and Y. Q. Huang. 2011b. Application of surface-enhanced Raman spectroscopy for analyses of restricted sulfa drugs. *Sensing and Instrum. for Food Qual. and Safety* 5(3-4): 91-96.
- Lin-Vien, D., N. B. Colthup, W. G. Fateley, and J. G. Grasselli. 1991. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*. San Diego, Cal.: Academic Press.
- Liu, B., M. Lin, and H. Li. 2010. Potential of SERS for rapid detection of melamine and cyanuric acid extracted from milk. *Sensing and Instrum. for Food Qual. and Safety* 4(1): 13-19.
- Liu, B. H., G. M. Han, Z. P. Zhang, R. Y. Liu, C. L. Jiang, S. H. Wang, and M. Y. Han. 2011a. Shell thickness-dependent Raman enhancement for rapid identification and detection of pesticide residues at fruit peels. *Anal. Chem.* 84(1): 255-261.
- Liu, B., G. Han, Z. Zhang, R. Liu, C. Jiang, and S. Wang. 2012. Shell thickness-dependent Raman enhancement for rapid identification and detection of pesticide residues at fruit peels. *Anal. Chem.* 84(1): 255-261.
- Liu, M., Y. Hashi, Y. Song, and J. M. Lin. 2005. Simultaneous determination of carbamate and organophosphorus pesticides in fruits and vegetables by liquid chromatography-mass spectrometry. *J. Chromatogr. A* 1097(1-2): 183-187.
- Liu, Y. D., and Y. B. Ying. 2006. Rapid non-destructive determination of maturity in Fuji apple using FT-NIR spectroscopy. *Trans. ASABE* 49(1): 91-95.
- Liu, Y., G. Su, B. Zhang, G. Jiang, and B. Yan. 2011b. Nanoparticle-based strategies for detection and remediation of environmental pollutants. *Analyst* 136(5): 872-877.
- Manno, D., E. Filippo, R. Fiore, A. Serra, E. Urso, A. Rizzello, and M. Maffia. 2010. Monitoring prion protein expression in complex biological samples by SERS for diagnostic applications. *Nanotech.* 21(16): 1-7.
- Mukherjee, K., S. Sanchez-Cortes, and J. V. Garcia-Ramos. 2001. Raman and surface-enhanced Raman study of insecticide cyromazine. *Vibr. Spectros.* 25(1): 91-99.
- Ortelli, D., P. Edder, and C. Corvi. 2005. Pesticide residues survey in citrus fruits. *Food Addit. Contam.* 22(5): 423-428.
- Rawn, D. F. K., S. C. Quade, J. B. Shields, G. Conca, W. F. Sun, G. M. A. Lacroix, M. Smith, A. Fouquet, and A. Bélanger. 2006. Organophosphate levels in apple composites and individual apples from a treated Canadian orchard. *J. Agric. Food Chem.* 54(5): 1943-1948.
- Sajan, D., G. D. Sockalingum, M. Manfait, I. Hubert Joe, and V. S. Jayakumar. 2008. NIR-FT Raman, FT-IR, and surface-enhanced Raman scattering spectra, with theoretical simulations on chloramphenicol. *J. Raman Spectrosc.* 39(12): 1772-1783.
- Sato-Berrú, R. Y., J. Medina-Valtierra, C. Medina-Gutiérrez, and C. Frausto-Reyes. 2004. Aplicación de la espectroscopia Raman para la caracterización de pesticidas organicos. *Rev. Intl. Contam. Ambient.* 20(1): 23-30.
- Smitha, S. L., K. G. Gopchandran, T. R. Ravindran, and V. S.

- Prasad. 2011. Gold nanorods with finely tunable longitudinal surface plasmon resonance as SERS substrates. *Nanotech.* 22(26): 265705.
- Steven, E. J. B., and M. S. S. Narayana. 2008. Quantitative surface-enhanced Raman spectroscopy. *Chem. Soc. Rev.* 37(5): 1012-1024.
- Szeghalmi, A., S. Kaminskyj, and K. M. Gough. 2007. A synchrotron FTIR microspectroscopy investigation of fungal hyphae grown under optimal and stressed conditions. *Anal. Bioanal. Chem.* 387(5): 1779-1789.
- Tan, E., P. Yin, and L. Li. 2011. Single-molecule surface-enhanced Raman scattering of R6G in aqueous environment under non-resonance conditions. *Chinese Optics Letters* 9(8): 082901.
- Tang, H., Q. Li, Y. Ren, J. Geng, P. Cao, T. Sui, X. Wang, and Y. P. Du. 2011. Surface-enhanced Raman spectroscopy signals of mixed pesticides and their identification. *Chinese Chem. Letters* 22(12): 1477-1480.
- Thomas, L. C. 1974. *Interpretation of the Infrared Spectra of Organophosphorus Compounds*. Mississauga, Ontario, Canada: John Wiley and Sons.
- Tsochatzis, E. D., U. Menkissoglu-Spiroudi, D. G. Karpouzas, and R. Tzimou-Tsitouridou. 2010. A multi-residue method for pesticide residue analysis in rice grains using matrix solid-phase dispersion extraction and high-performance liquid chromatography-diode array detection. *Anal. Bioanal. Chem.* 397(6): 2181-2190.
- Tuzimski, T. 2011. Determination of analysis in medical herbs extracts by SPE coupled with two-dimensional planar chromatography in combination with diode array scanning densitometry and HPLC-diode array detector. *J. Separation Sci.* 4(1): 27-36.
- Valdés-Ramírez, G., D. Fournier, M. T. Ramírez-Silva, and J. L. Marty. 2008. Sensitive amperometric biosensor for dichlorvos quantification: Application to detection of residues on apple skin. *Talanta* 74(4): 741-746.
- Vongsvivut, J. E., G. Robertson, and D. McNaughton. 2010. Surface-enhanced Raman spectroscopic analysis of fonofos pesticide adsorbed on silver and gold nanoparticles. *J. Raman Spectrosc.* 41(10): 1137-1148.
- Walz, I., and W. Schwack. 2007. Multienzyme inhibition assay for pesticide parathion-methyl. *J. Agric. Food Chem.* 55(26): 10563-10571.
- Wang, J., S. Zhu, J. He, Y. Cheng, C. Fan, G. Cai, and E. J. Liang. 2012. Surface-enhanced Raman scattering properties of Klarite chips. *Chinese J. Light Scat.* 24(1): 17-22.
- Wong, J. W., M. G. Webster, D. Z. Bezabeh, M. J. Hengel, K. K. Ngim, A. J. Krynitsky, and E. E. Susan. 2004. Multiresidue determination of pesticides in malt beverages by capillary gas chromatography with mass spectrometry and selected ion monitoring. *J. Agric. Food Chem.* 52(21): 6361-6372.
- Yan, H., F. Qiao, M. Tian, and K. H. Row. 2010. Simultaneous determination of nine pyrethroids in indoor insecticide products by capillary gas chromatography. *J. Pharm. Biomed. Anal.* 51(3): 774-777.
- Zamuner, M., D. Talaga, F. Deiss, V. Guieu, A. Kuhn, P. Ugo, and N. Saojic. 2009. Fabrication of a macroporous microwell array for surface-enhanced Raman scattering. *Adv. Function Mat.* 19(19): 3129-3135.
- Zhang, P. X., X. Zhou, A. Cheng, and Y. Fang. 2006. Raman spectra from pesticides on the surface of fruits. *J. Physics* 28(1): 7-11.