

Pesticide residues detection by Characteristics

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The excessive use or abuse of pesticides leads to residues in food, which can threaten human health. Therefore, there is an extremely urgent need for multi-analyte analysis techniques for the detection of pesticide residues, which can be applied as screening techniques for food safety monitoring and detection. Recent developments related to rapid multi-residue detection methods for pesticide residues are reviewed herein. Methods based on the inherent characteristics of pesticides are described in detail. Enzymatic inhibition-based sensors, near-infrared spectroscopy, and SERS spectroscopy based on the inherent characteristics are discussed.

Pesticides residues

Inherent characteristics

Screening techniques

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1. Introduction

Pesticides and veterinary drugs are indispensable for increasing food production, as well as improving animal breeding and aquaculture ^[1]. Pesticides are widely applied in modern agriculture to control weeds and pests and regulate the growth of plants ^[2]. Veterinary drugs are commonly administered in farming practices for the prevention and treatment of diseases and promoting growth ^[3]. The application of pesticides and veterinary drugs can prevent devastating losses in agriculture and animal husbandry industries, enabling them to meet the demands of a rising global population ^{[4][5]}. However, the excessive use or abuse of pesticides and veterinary drugs can lead to drug residues in food and the environment, which can threaten human health through food chains ^{[6][7]}. Therefore, effective detection methods for residues have been developed for monitoring food safety and ensuring public health. The traditional methods for pesticide and veterinary drug residues determination are usually based on instrumental techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC), or chromatographic methods coupled with mass spectrometry (MS) detectors^[8]. These methods provide abundant qualitative and quantitative information of the residues with high accuracy. However, these systems are limited by complicated sample pre-treatments and require highly trained technicians and expensive equipment ^[8]. On the other hand, rapid methods such as immunoassays ^[9], spectroscopic analyses ^[10], and electrochemical techniques

[11] provide relatively convenient and highly sensitive strategies for the determination of pesticides and veterinary drugs. Although the accuracy and precision of rapid methods are not as good as those of instrumental techniques, these methods can be used complementarily to instrumental methods, particularly as pre-screening methods for detection in large-scale samples. Therefore, novel analysis methods for the rapid and sensitive detection of pesticides and veterinary drugs are highly desired. Common rapid methods are capable of detecting a single target with high specificity [12], whereas real food samples generally always contain more than one pesticide or veterinary drug. Therefore, multi-residue detection methods are more favorable for the actual analytical needs of end-users. Meanwhile, these simultaneous detection strategies are well-suited for screening analyses with the characteristics of ease of use, high-throughput, and low cost per sample [13].

2. Rapid Multi-Residue Detection Methods Based on the Inherent Characteristics of Pesticides and Veterinary Drugs

Aside from the above mentioned multi-residue detection methods with different recognition elements, enzymatic inhibition-based sensors, NIR spectroscopy, and SERS spectroscopy have also been used for the multi-analyte analysis of pesticide and veterinary drug residues, with the development of bio-neuroinformatics technology, spectroscopy technology, and nanotechnology. These detection methods were mainly developed based on the inherent characteristics of pesticides and veterinary drugs. Therefore, these methods can realize non-destructive, rapid, low cost, and environmentally friendly detection without the use of recognition elements. Enzymatic inhibition-based biosensors have been successfully applied in multi-residue screening and detection, based on the enzyme inhibition characteristics of pesticide residues. Due to the specific functional groups and chemical structures of pesticides and veterinary drugs, different absorption bands and spectral intensities are shown in the NIR or SERS spectrum. Thus, enzyme-based sensors, NIR spectroscopy, and SERS spectroscopy have become promising detection techniques for the multi-analyte analysis of residues.

2.1. Enzymatic Inhibition-Based Multi-Residue Detection

The activities of cholinesterases, acetylcholinesterase (AChE), and butyrylcholinesterase can be inhibited by OP and carbamate (CM) pesticides [14]. Based on this principle, enzymatic inhibition-based multi-residue detection can be applied in multi-analyte monitoring [15]. Quantitative detection can also be achieved, as the degree of inhibition is related to the concentration of pesticide residues [14]. The phosphorus atoms of the OPs can covalently bind to the hydroxyl group of the nucleophilic serine located at the active site of AChE [14]. Therefore, AChE is the most commonly used enzyme in the development of enzymatic inhibition-based multi-residue detection methods. Various detection methods based on enzymatic inhibition have been published, including colorimetric assays and electrochemical assays.

AChE can hydrolyze certain substrates to colored products, while the color development can be decreased in the presence of OP or CM pesticides [16]. Therefore, various colorimetric screening methods have been developed based on this principle. An AChE assay has been optimized and validated for carbofuran, carbofuran-3-hydroxy, and dichlorvos in lettuce and strawberry extracts [17]. Indoxyl acetate was used as a substrate which can be rapidly

hydrolyzed by AChE to produce blue hydrazine. The LODs of three kinds of OP and CM pesticides were found to be at the part per billion (ppb) level. Furthermore, this reaction was integrated into a double-film visual screening card for the rapid screening of OP or CM pesticides [18]. The LODs of six OP and CM pesticides were in the range of 0.04–0.5 mg/mL. With the development of nanotechnology, diverse nanomaterials with excellent enzyme immobilization properties have been used to develop electrochemical assays. An AChE electrochemical biosensor based on gold nanorods (AuNRs) was developed for the detection of OP pesticides. The LODs of paraoxon and dimethoate were 0.7 and 3.9 nM, respectively [19]. Graphene and transition metal carbides have also been used to modify an AChE biosensor for the detection of OP pesticides. The biosensor showed better catalytic performance compared to the biosensors without modification [17]. Furthermore, a robust and novel conjugated polymer and core-shell magnetic nanoparticle containing biosensor was applied to detect pesticides [20]. The biosensor revealed a rapid response of 5 s and an LOD of 6.66×10^{-3} mM.

The above detection methods can be used for the rapid pre-screening of OP or CM pesticides, in order to discriminate whether the sample contains pesticides or not. No qualitative or quantitative information about individual pesticides in the complex matrix can be obtained. Therefore, multi-sensor arrays combined with artificial neural networks (ANNs) have been developed for multi-residues quantitative analysis. Different types of native or genetically engineered AChEs portrayed different sensitivities to OP or CM pesticides, which were used to modify the multi-biosensors. Combined with the data processing of ANNs, the pesticide mixtures could be discriminated [15]. An AChE triple-biosensor array combined with an ANN was developed for the selective detection of chlorpyrifos and chlorfenvinfos [21]. Three different types of AChE, the wild-type from an electric eel, the genetically modified *Drosophila melanogaster* AChE B394 and B394, were used to modify the biosensors. The combined responses of the two pesticides were modeled by two different ANNs. Both pesticides could be quantified with low errors from a direct measurement step. Moreover, one-step AChE disposable biosensors based on genetically modified enzymes and ANNs has been developed to determine mixtures of three OP residues [22]. Two AChEs from *Drosophila melanogaster* (wild-type and genetically modified) were used to modify the biosensor array. The biosensor system successfully identified and quantified mixtures of chlorpyrifos oxon, chlorfenvinphos, and azinphosmethyl oxon.

Enzymatic inhibition based multi-residue detection methods have been applied in the rapid pre-screening of OP and CM pesticides. However, there are still some problems which need to be solved. One is how to retain the original catalytic activity of AChE after immobilization. Therefore, the immobilization surface should be well-designed. The second is the issue of the re-activation of an enzymatic layer complicating the operation of a biosensor. Therefore, a disposable biosensor with cheap production cost is desirable for field conditions. The third is a selectivity problem. It is difficult to determine which kinds of OPs inhibit the AChE activity in a particular sample. Although multi-sensor arrays combined with ANNs can provide a partial solution this problem. Due to the needs of different AChEs and the complex processing of ANNs, the practicality and operability need to be further improved.

2.2. Near-Infrared Spectroscopy based Multi-Residue Detections

NIR spectroscopy methods have been recognized as a non-destructive evaluation approach for the quality control of intact food [23]. Aside from quality assessment and food compounds measurement, NIR spectroscopy has been shown to have feasibility for the detection of pesticide and veterinary drug residues, in order to ensure food safety [24].

Typically, NIR absorption spectra are collected and the spectral information is analyzed for the assessment of residues. Jamshidi et al. [25] applied visible/near-infrared (Vis/NIR) spectroscopy in the range of 450–1000 nm to detect pesticide residues in intact cucumbers. Partial least squares-discriminant analysis (PLS-DA) models were developed to classify cucumbers with contents of diazinon below and above the maximum residue limits as safe and unsafe samples, respectively. Furthermore, Vis/NIR spectroscopy combined with a chemometric method has been developed for the detection of pesticide residues in cucumber samples [26]. Partial least squares (PLS) regression models and PLS-DA models were developed for the prediction of diazinon contents in the samples. For the convenience of users, a graphical user interface was created, based on the PLS and PLS-DA models, for the classification of cucumbers by the absence/presence of diazinon residues, respectively.

However, the spectra used in the above methods were collected from a small portion of the tested samples, which cannot guarantee the data accuracy and representativeness. Therefore, hyperspectral imaging technology has been used, in combination with NIR spectroscopy methods. Hyperspectral imaging integrated spectroscopy and imaging can be used to obtain both spectral and spatial information from samples [27]. An NIR-hyperspectral imaging technique and GC-MS were used to detect two pesticides (chlorpyrifos and imidacloprid) in jujube fruits in the spectral 900–1700 nm. Based on the extracted spectral data, a simplified E_S -AWLSGSD-RC-LWPLSR model was established with eight characteristic wavelengths, having correlation coefficients of cross-validation of 0.757 and 0.898 for chlorpyrifos and imidacloprid, respectively [28]. Moreover, an NIR-hyperspectral imaging system has also been used to map the distribution of pesticide residues in mulberry leaves [27]. The best successive projections algorithm multiple linear regression model was used to transfer the distribution of the pesticide residues to a visualization map. Furthermore, an NIR-hyperspectral imaging system (870–1780 nm) combined with a chemical molecular structure coupled with wavelet transform was proposed to detect five kinds of pesticide residues (dimethoate, acephate, phoxim, dichlorvos, and avermectin) on the surface of lettuce [29]. The calibration and prediction accuracy of the results were achieved at 100%.

NIR spectroscopy methods are capable of realizing multi-residue detection in intact food. In addition to the characteristic of non-destructive, NIR spectroscopy has the advantages of no sample preparation needed, fast response time, environmental friendliness, and low operating cost. Therefore, NIR spectroscopy methods have a great application potential in online monitoring and field detection.

2.3. Surface-Enhanced Raman Scattering based Multi-Residue Detections

SERS is a promising method, combining Raman spectroscopy and nanotechnology for the rapid and non-invasive detection of samples. The chemical bonds and vibrational properties of functional groups are represented by Raman spectral bands, providing a fingerprint for target analytes. With the enhancement effect of SERS

substrates, SERS can identify and quantify analytes with high sensitivity down to the single-molecule level. Various SERS substrates have been designed to functionalize the surface characteristics, in order to enhance the sensitivity of SERS [30]. Improved SERS substrates provided the possibility of multi-residue detection within a complicated matrix [31].

The sensitivity of SERS is influenced by the molecular intrinsic vibration of the targets and the interaction between targets and the SERS substrate. Pesticides and veterinary drugs with certain thiol or amine functional groups can bind strongly to Au and Ag substrates [32]. Therefore, various Au and Ag SERS substrates have been applied for the simultaneous detection of pesticide and veterinary drug residues. An in situ SERS method with 50 nm AuNPs as substrates was developed to discriminate three classes of pesticides on fresh tea leaves and apple peels [33]. In addition to single materials, silver-coated gold nanoparticles (Au@Ag NPs) have also been used as a substrate for the rapid detection of multiple OPs [34]. The Raman enhancement of composited Au@Ag NPs for OPs detection was stronger than that of single Ag and Au NPs. The aggregation of NPs caused by the connection between Au@Ag NPs and analytes could create hot spots, which showed significant Raman enhancement. The LODs of triazophos and methyl-parathion in peach were 0.001 mg/kg. For practical applications, flexible SERS substrates consisting of SERS active nanomaterials and flexible solid substrates are useful for the detection of surface residues. Wang et al. [35] constructed an Au-decorated dragonfly wing bioscaffold array as a flexible SERS substrate for the detection of three kinds of pesticides. The SERS substrate, fabricated by a 3D biomimetic array with high-density hot spots, showed greatly enhanced Raman activity (Figure 1A). The SERS platform was used for the detection of acephate, cypermethrin, and tsumacide, with respective LODs of 10^{-3} , 10^{-3} , and 10^{-4} ng/cm². A nanoporous cellulose paper-based AuNRs SERS substrate was constructed for the detection of three pesticides [36]. The LODs of thiram, tricyclazole, and carbaryl were 6, 60, and 600 ng/cm², respectively. Chen et al. [37] developed a jelly-like flexible SERS substrate based on nanocellulose decorated with Ag nanoparticles (Ag/NC substrate) for the detection of two types of pesticides on apple peels and cabbages (Figure 1B). The flexible SERS substrate facilitated good contact with the samples. The LODs for thiram and thiabendazole were 0.5 and 5 ng/cm², respectively.

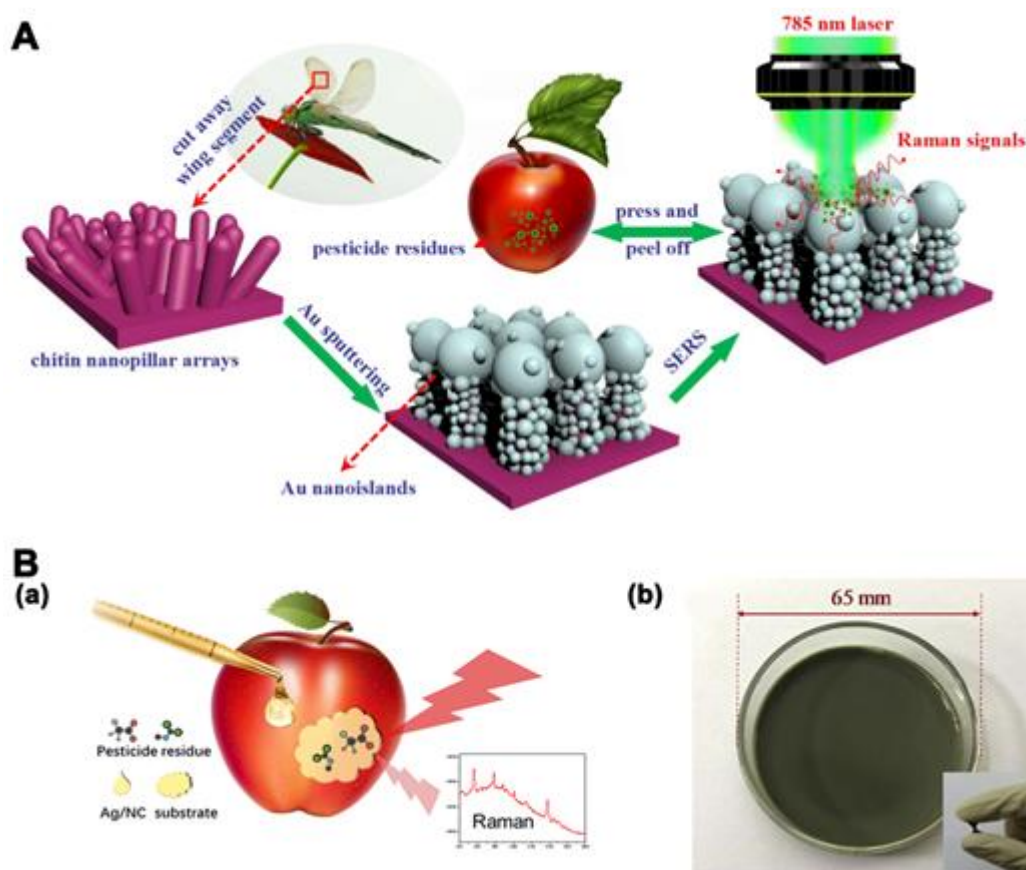


Figure 1. (A) Illustration of an au-decorated dragonfly wing bioscaffold array as a surface-enhanced Raman scattering (SERS) substrate by the Raman system . (B) a. Illustration of the SERS measurement of apples. b. Photograph of the Ag/NC substrate. Reproduced with permission from . Copyright MDPI, 2018. Reproduced with permission from . Copyright Elsevier, 2019.

SERS has demonstrated excellent sensitivity, simple sampling, and rapid test speed for the multi-residues detection of pesticide and veterinary drug residues. With facile design, SERS substrates with the advantages of flexibility, excellent adaptability, cost-efficiency, and suitability for large-scale production can be constructed for non-destructive practical applications. With the development of nanotechnology and the improvement of instruments, SERS can provide a routine and cost-effective detection method for the multi-analyte analysis of pesticide and veterinary drug residues.

3. Conclusions

The residues of pesticides threaten human health. Therefore, several screening assays have been developed for the rapid multi-residue detections of pesticides. Different broadly specific recognition elements have been prepared and applied for simultaneous analysis. In addition, enzymatic inhibition-based sensors, NIR spectroscopy, and SERS spectroscopy, based on the inherent characteristics of pesticides and veterinary drugs, can also realize multi-analyte analysis. With the development of genetic engineering and computer modeling technology, recognition elements with the desired broad specificity and high affinity to multiplex targets can be designed and

obtained. Meanwhile, the design of novel portable instruments and the synthesis of novel functional materials serve to promote the development of new detection methods with more target analytes and higher sensitivities. With the combination of rapid multi-analyte analysis methods and traditional instruments, efficient and accurate detection systems which feature simplified test processes and reduced determination time can be built.

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