Review of Surface Enhanced Raman Spectroscopic (SERS) Detection of Synthetic Chemical Pesticides

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Abstract

Pesticides are essential in modern agricultural practices. Detection of pesticides is an essential step in regulating and monitoring the levels of pesticides in the environment. Even though GC/LC-MS is often the gold standard method for pesticide detection, recent technological advancements has promoted the creation of alternative techniques, such as Surface Enhanced Raman Spectroscopy **(**SERS), that provide added advantages such as ultrasensitive detection, faster turnover, simpler protocols, *in situ* sampling, on-site capability and reduced cost. In this review, a comprehensive report of recent advances in SERS detection of synthetic chemical pesticides is given. The development and applications of the SERS technique for pesticide detection in both simple and complex matrices are discussed. The main advantages of using SERS for pesticide detection are highlighted, together with its limitations. Lastly, promising future trends and applications of SERS for pesticides detection are also discussed.

Keywords: SERS; pesticide; detection; substrate; method; matrix

Contents

1. Introduction

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. Because pests and diseases damage up to one-third of crops during growth, harvest or storage, pesticides are essential in modern agricultural practices. The use of pesticides in commercial agriculture has led to an increase in farm productivity[1]. According to the Environmental Protection Agency (EPA) report, the agricultural market shares of total pesticides resulted in $35.8 billion in 2006 and more than $39.4 billion in 2007 in spending expenditure respectively [2].Based on their chemical structures and functionality, synthetic pesticides are classified into five classes: organochlorine, organophosphate, carbamate, neonicotinoid and pyrethroid. Biopesticides, which is another category of pesticides, is derived from biological materials, and hence, their chemical compositions can be widely varied, such as a single protein to a microorganism that attacks pests.

Most chemical pesticides are designed to be toxic to pests, so by their very nature they pose risks to human beings, wildlife and the environment. Acute toxicity data of most pesticides are well documented. On the other hand,chronic toxicity data of pesticides are more difficult to obtain, and this has raised public health concerns nowadays. In order to monitor the amount of pesticides and metabolites accumulated in nature, a wide range of sample types, including forensic, crops, environmental (e.g. soil, water, air), food, beverage, biological (e.g. blood), plant and animal-derived products are needed to be tested routinely.

A critical requirement for pesticide monitoring is to have a well-established detection method. The detection technology of choice must possess several key characteristics, such as excellent

sensitivity and reproducibility. Since the 20th century, these important detection characteristic requirements have been fulfilled by various chromatographic based techniques, including LC/GC-MS, HPLCand TLC [3–6]. Even though these methods have been evolving gradually to improve on their detection capabilities, other detection technologies have also emerged that promise added benefits, such as faster detection times, simpler protocols, *in situ* sampling, portability and reduced cost. Surface enhanced Raman spectroscopy, or SERS, is one of these techniques, and will be the focus of this review for the detection of synthetic chemical pesticides.

SERS is essentially an agglomeration of two techniques, namely Raman spectroscopy and nanotechnology. Raman scattering was first discovered in 1928 by Raman and Krishnan, who observed the inelastic scattering of light which constitutes only about one in a million photons of incident light striking a surface. The rest reflect elastically, commonly known as Rayleigh scattering. It was further discovered that the frequency changes that occurred due to the inelastic scattering of light matched precisely with the differences in vibrational energy levels. This enables every type of molecule to yield distinct Raman spectral profiles since different functional groups possess different characteristic vibrational energies. Thus, the main advantage of Raman spectroscopy is the capability for molecular fingerprint specificity for every distinct molecule/analyte. However, this method was not applicable for sensitive detection due to the inherently weak Raman signals. In 1970, researchers discovered that Raman signals were enhanced by $10^4 – 10^5$ if the target analyte was placed in close proximity to a roughened noble metal substrate. Although the exact mechanism for this phenomenon is not clearly understood, two sets of theories have been well-received by the scientific community; namely the electromagnetic and chemical theory. In the electromagnetic theory, the enhancement of Raman

signals is thought to be due to the excitation of the localized surface plasmon resonance (LSPR) of nanoparticles when an incident light hits the surface of the target analyte close to the nanomaterial. In order to maximize the enhancement of Raman signals, the excitation frequency of the nanomaterials used has to resonate with that of the incident light (i.e. for noble metals, this is in the UV-vis range). This will then result in intensity peaks at particular Raman shifts with at least $10^4 – 10^6$ enhancements. In the chemical theory, the Raman signal enhancement occurs based on the assumption that the target analyte is adsorbed on the metal surface and that a charge transfer is in effect. Because of the chemisorption of target analyte on the substrate, the electronic state of the complex is shifted to a new absorption maximum, which allows it to resonate with the laser excitation frequency, and thus enhance the Raman signals. Several recent review articles are available to get deeper understanding of the mechanisms and theory of SERS[7–9].

Due to the potential of SERS as a detection technique, several scientific articles and review papers have also been published to highlight its possible applications. Examples include the detection of target compounds such as food chemicals [10], environmental pollutants [11], biomolecules[12] and cancer diagnostic agents [13], as well as to investigate broader topics such as forensic science [14],food safety [15] and integration with other technologies such as microfluidics [16]. General reviews of SERS applications are also available[17,18]. Although pesticides detection was covered briefly in some of these articles, a comprehensive report of recent developments in SERS technique specifically for pesticides detection has not yet been reported.

The earliest record of pesticide detection using SERS was performed in 1987 by Alak and Vo-Dinh [19]. In their article, eight organophosphorous pesticides were characterized using silver (Ag)-coated microspheres as substrates. Since then, a large number of scientific publications regarding the SERS detection of pesticides have been published (**Figure 1**). In this review paper, a comprehensive report of SERS for synthetic chemical pesticide detection is given. The initial development of SERS for pesticides detection mainly focused on the SERS substrate development, with demonstration of its detection capability using pesticides. In these studies, pesticides in simple matrices were tested. Hence, the first segment covers several aspects, including the sensitivity, reproducibility, selectivity, and portability of SERS that have been improved for pesticide detection mainly as a result of recent advances in substrate development. Another critical step for the advancement of SERS as a detection tool is the ability to selectively detect a target in any matrix. Therefore, the second segment focuses on applications of SERS for pesticide detection in complex matrices. Advantages and limitations of using SERS for pesticides detection are highlighted. Lastly, promising future trends and applications of SERS for pesticides detection are also discussed.

2. Development and applications of SERS for pesticides detection

2.1. Initial development of SERS for detection of pesticide in simple matrices

One of the main advantages of using SERS over traditional analytical techniques for the detection of pesticides in simple matrices (e.g. water or organic solvents) is the speed at which samples can be analyzed. The protocol can be as simple and quick as dropping a microliter sample onto a SERS substrate followed by a split-second laser integration time to obtain a SERS signal and comparing it against a reference spectrum. Other important considerations include sensitivity, reproducibility, selectivity, and portability. These factors have been improved over the years and are mainly driven by the development of SERS substrates.

2.1.1. Sensitivity

The limit of detection (LOD) is perhaps the most widely studied characteristic in a SERS study forthe sensitive detection of pesticides. The reported LODs of SERS for pesticides detection in simple matrices (e.g. water or organic solvents) mainly depends on the type of substrates and the pesticide molecular structure. Another important thing to remember is that the LODs for pesticides detection, which aregenerally reportedin the parts-per-million (ppm) or parts-per-billion (ppb) level, cannot be directly compared to the maximum residue levels (MRLs) allowed in agricultural produce and the environment, which are set by regulatory agencies such as EPA. The MRL for a specific pesticide is based on the sample matrix it is present in, while the LOD for the pesticide is usually based on the concentration of that pesticide in its solvent. Therefore, careful transition of the units and discussion of the relationship between LOD of the analytical method and the governmental requirement of the pesticide detection are critically needed.

From the substrate point of view, the variation in sensitivity of SERS detection can largely be attributed to the variations in “hot spots” on substrates [20]. These hot spots are found at the interstitial gaps between metallic nanoparticles,and are able to produce intense local field enhancements due to localized surface plasmon resonance (LSPR).The LOD of SERS methods can sometimes reach a single molecule detection level through a rational design of the SERS substrate and the experiment[21–24].

Thus, efforts have been made to increase the number, strength and location of hot spots to lower the LOD of pesticides. In the case of Ag or gold nanoparticles(Au NPs), i.e. most widely used SERS substrate, the addition of salt, such as NaCl, is used as an aggregating agent to increase the number of hot spots. In this way, the electric charges surrounding nanoparticles are neutralized by the salt and is able to pull both nanoparticles and the adsorbed target together, which will subsequently enhance the SERS peak intensities [25]. Pre-concentration of nanoparticles can also be done through physical means such as filtration. Yu and White showed the concept by trapping Ag NPs on filter membranes in order to form a SERS active substrate, after which a large volume (mL) sample containing low levels of malathionor melamine were concentrated onto the filter [26]. Another example is the coupling of electrokinetic pre-concentration with SERS, which was developed to detect small molecules such as antibiotics and phenols [27,28]. Besides increasing the number of hot spots, alternations of the shape and size of the nanoparticles can also increase the strength of localized electromagnetic field (hot spots) and/or increase the likelihood of the contact of target pesticides with the hot spots. Ag NPs that have aggregated forms of nanosize shapes such as flowers and leaves have been fabricated to take advantage of their SERS enhancement capabilities [29,30]. Unique nanoparticle shapes synthesized for SERS substrate applications include nanowires, nanocube and nanodishes [31–

33]. By increasing the probability of the target landing at the centroid position of a hot spot, ultrasensitive detection even at a single molecule has been achieved under optimal SERS conditions [21].

From the pesticide point of view, the sensitivity of SERS is different for each pesticidemainly because of the unique, intrinsic vibration of the molecules, the interaction between pesticide molecules and the substrate, as well as the compatibility of the pesticides when complexed with the substrate. For the intrinsic vibration, molecules with conjugated double bond system and symmetric vibrational modes are more active than the others. Therefore, certain pesticides such as crystal violet and malachite green are more popular as pesticide targets for the evaluation of a SERS substrate [34,35]. Pesticides with certain functional groups (e.g thiol, amine) that can bind Au and Ag substrates strongly are also good targets. For example, ferbam, thiram, thiabendazole and phosmet have been used for substrate evaluation partly for this reason [36–39]. Particularly for ferbam and thiram, since there is no GC/LC-MS method available to detect them directly, the capability of using SERS as the detection method show great promise [40,41]. Many pesticides intrinsically possess the two properties mentioned above, thus making them promising targets for sensitive SERS analysis. In the case of compatibility, if the affinity of the target to substrate is too weak for adequate adsorption, the sensitivity of SERS detection can also be dramatically reduced. For example, hydrophobic molecules such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) will not adsorb well on citrate stabilized Au or Ag colloids due to the incompatibility of the surface chemistry [42]. Hence, attempts have been made to overcome this limitation by modification of the substrate surface. In the case of hydrophobic molecule detection, colloidal hydrophobic films

have been placed onto Au NPs immobilized onto silanized quartz substrates to detect PAH [42]. Kubackova et al detected four organochlorine pesticides (i.e. Aldrin, dieldrin, lindane, and α-endosulfan) using a SERS substrate modified with several types of alkyl dithiols to increase the affinity of these liposoluble pesticides [43]. Due to the higher selectivity of these pesticides to the surface modified SERS substrate and the interparticle linkage induced by the thiol groups to produce more "hot spots", the LOD was able to reach 10^{-8} M. Zheng et al showed the advantage of using an oleate-modified Fe_3O_4 @Ag microspheres due to the selectivity of the oleate group to detect thiram through surface ligand exchange [44]. In their study, a gel reaction system containing oleate was used to avoid aggregation of Fe_3O_4 @Ag microspheres and to modify synthesized Ag NPs simultaneously during the generation process, thus increasing the sensitivity of the SERS substrates. Graphene, proteins and DNA fragments have also been conjugated onto SERS substrates to modify the surface chemistry to enhance the sensitivity of SERS [45–47]. Many of these studies show proof of concepts, but are not well studied in complex matrices such as food, hence, there is still a need for application based studies to evaluate SERS for practical applications of pesticide detection in real complex matrices

2.1.2. Reproducibility

Despite the huge advantage of SERS as an ultrasensitive detection tool, one of the main limitations that continue to be addressed in SERS studies is the reproducibility of results, particularly with regards to peak intensities. Thus, quantitative studies remain a challenge in SERS, not only in the pesticide detection area, but generally in all kinds of SERS applications. The origination of the variation mainly arises from the substrates and sample preparation that produce and control the hot spots. The hot spots of the traditional colloid SERS substrates

239 highly depend on their aggregation. Despite wide commercial availability and relative low cost,
240 they usually have difficulty in controlling aggregation, thus producing larger signal variation
241 compared to the well-ordered nanostructures. Therefore, the selection of detection spots cannot
242 be completely blind. With a Raman microscope, we are able to select certain areas on the
243 substrate for minimizing the variation. Core-shell based colloid nanoparticles have significantly
244 improved the signal variation of SERS, as their hot spots are not determined by aggregation [48].
245 Other studies that have shown to control the uniformity of nanoparticles into consistent patterns
246 include incorporation of colloids into a sol-gel [49,50] and integration with a microfluidic device
247 [34,51].
248 Information on the reproducibility of the well-ordered nanostructures made through top-
249 down and bottom-up techniques can be found in several review papers [52,53] and are not
250 discussed in this review. However, they are relatively expensive to fabricate and not as widely
251 available, which has limited their application for routine analysis in food and environmental
252 samples.
253 Another reason for the limitation on reproducibility is because each researcher often uses
254 a different Raman instrument system and employs a variety of possible configurations. For
255 example, four referenced publications using gold colloids as their SERS substrates[54–57]
256 usedRaman instruments that were completely set up differently. In addition to hardware
257 differences (i.e. LabRAM ARAMIS Raman, RamanStation 400F, Renishaw RM1000 Raman,
258 DXR Raman), their laser wavelength (i.e. 633, 780, 785 nm), laser power (i.e. 0.325, 5, 20, 250
259 mW), accumulation time (i.e. 2, 5, 10, 15 s) and spectral resolutions (1, 5 cm^{-1}) were varied. The
260 lack of standardization for SERS detection of pesticides makes it challenging to compare results
261 from one lab to the other.

2.1.3. Selectivity

When multiple pesticides are present in a sample, the selectivity of SERS to detect each of the specific pesticide in the mixture simultaneously is a big challenge, even if the matrix was just water or an organic solvent. In theory, one might think that SERS should be capable of detecting multiple pesticides from a sample mixture as long as each pesticide produces a distinct SERS peak. Especially with advanced chemomatrices, separation and detection of multiple pesticide peaks is possible. In reality, however, SERS studies have been limited to detecting less than five pesticides simultaneously each time [57,58]. This is probably because competitive adsorption to the SERS substrates occurs when multiple analytes are present. In other words, the target compounds with higher binding affinity to the substrate will have greater surface coverage adsorption on the substrates, thus the concentration ratios of target compounds present in the sample matrix would not be proportional to the SERS peak intensities generated. In some cases, the pesticides may not even produce a significant peak due to the presence of another compound with several orders of magnitude higher affinity to the substrate. Pre-separation of individual pesticides or pre-treatment to reduce the number of pesticides for a single SERS test can solve this problem, but this would inevitably increase the analytical time and complexity [59]. More discussion can be found in the next section when talking about the detection in real matrices.

2.1.4. Portability

One of the popular aspects of SERS development is the potential for it to become a field detection method, which is a great advantage when compared to the GC/LC-MS methods. In

pesticide applications, field detection is strongly needed for rapid screening and monitoring of pesticides in environment, agriculture production, and industrial processing. In order to have a portable detection system, it has to be rapid, light, easy-to-use and small.

The miniaturization and commercialization of hand-held Raman spectrometers has enabled portable SERS detection to become a reality. However, just as crucial as the instrument is the development of suitable substrates. For portable applications, traditional colloidal based substrates may not work well due to the additional steps needed to deposit the nanoparticles on a solid surface and to concentrate/aggregate the substrate. Hence, solid SERS substrates are more suitable for detection that requires portability. Another approach has been the development of paper-based [60–62] or fiber-based [63,64] SERS sensors. For example, one study screen printed SERS active nanoparticles onto a filter paper, which could then be used to screen many samples using high throughput analysis in a portable setting [62]. Not only were these substrates cost-effective, but they were able to exhibit reproducible results with less than 10% spot-to-spot variation. In another study, a template guided self-assembly of Au NPs into ordered arrays of uniform clusters was prepared on an optic fiber faucet [64]. These SERS enabled optic fiber showed high-performance of SERS as demonstrated by using crystal violet. This batch method approach may pave a way for low-cost, efficient SERS substrates. Continued research will be needed to examine the stability of the substrates and to apply it with wider varieties of target analyte.

2.2. Further applications of SERS for pesticide detection in real complex matrices

For any detection method development that will eventually be used in applications, the matrix in which the target is embedded in plays a crucial factor in influencing the sensitivity and reproducibility of the result. For example, for the detection of food contaminants, the matrix could be aqueous (e.g. milk, fruit juices, drinking water) or solids (e.g. meat, fruits, vegetable, cereal). Depending on the matrix, the interference of SERS signals can differ greatly. Since SERS can detect most compounds that are in close proximity to the substrate, non-targeted substances may produce substantial noise peaks to lower the sensitivity of the target analyte. Furthermore, several molecules may produce strong Raman peaks that are similar to the target, making it challenging for qualitative and quantitative detection. A summary of recent studies using SERS to detect pesticides in complex matrices are listed in **Table 1**. Here, we discuss several strategies that have been utilized in order to reduce the influence of complex matrices in the SERS method (**Figure 2**).

2.2.1. Extraction procedure

Depending on the nature of the target pesticides and the complexity of the sample matrix, in some studies, little or no extraction was applied for SERS detection of pesticides in complex matrices. For example, acetamiprid was detected in commercially bought apple juice spiked with acetamiprid with no prior sample treatment [65]. Even though there were significant peaks coming from apple juice, characteristic peaks attributed to acetamiprid were still observed. In this case, a sharp apple peak was observed in 610 cm^{-1} while a pure acetamiprid solution contained a sharp peak at 640 cm^{-1}. As the acetamiprid concentration in apple juice increased, the apple peak shifted towards 640 cm^{-1}, suggesting more displacement of the apple juice

component by acetamiprid on substrate surface. The LOD was 3 ppm. A similar study was also conducted to detect carbaryl using a different substrate [66]. The successful detection of pesticides without extraction really depends on the particular pesticides that can bind on the SERS substrate stronger than most of the matrix components, as well as a sharp and characteristic peak that can be easily distinguished from the matrix peaks.

For most applications in complex matrices, extraction or separation of target pesticides are necessary before applying SERS. As SERS is not a separation technique, but a detection technique, the integration of a separation technique like chromatography can greatly improve the selectivity of SERS. However, the integration of chromatography and SERS detection cannot compete with the current UV or mass spectroscopy detection systems in terms of instrumental automation and cost-effectiveness. Further development in instrumentation is needed for this type of method integration. On the other hand, a more practical strategy is to adopt and modify the sample clean-up procedures that are used for GC/LC-MS before applying SERS. Compared to chromatographic methods such as LC/GC-MS, a great advantage of SERS is that sample preparations do not require high purifications of samples, thus simpler extraction procedures can be applied to reduce time and resources. This is possible because there are no capillary tubes or filters to go through; hence clogging of the instrument is not an issue.

He et al demonstrated the simplicity of pesticide SERS detection in foods by using a simple swab method on apple peels [65]. A surgical swab pre-soaked in methanol solvent was used to swab a $1cm^2$ area on apple peels spiked with thiabendazole. They were able to successfully extract the pesticide and detect as low as 0.1 ppm. Kim et al also did a similar

extraction procedure for the detection of thiabendazole and chlorpyrifos by rinsing the spiked apple peels in water and dropping the rinse onto their substrate [38]. Similarly, Khlebstov et al reported the extraction of thiram for SERS analysis by immersing the spiked apple peels in methanol and dropping the extract directly on fabricated Au nanoisland films. Despite noise signals coming from other components on apple peels, the pesticide signals were distinguishable and sharp enough even at 30 ppb [67]. As we can see from all these examples, SERS is able to detect pesticides in lightly treated samples as long as the matrix components do not interfere with the pesticide signals. Other studies have also extracted pesticides in a similar manner [66,56].

2.2.2. Substrate functionalization

Another approach to detect specific pesticides in a complex matrix is through functionalization of the SERS substrates. The selectivity of SERS can be improved by using specific target capture agents such as aptamers (i.e. “nucleic acid antibodies”) and molecularly imprinted polymers (i.e. MIPs, “plastic antibodies”). Pang et al described an aptamer-based SERS method that was capable of detecting four organophosphate pesticides (phorate, profenofos, omethoate, isocarbophos) in apple juice. By using a Ag dendritic substrate conjugated with a thiol-modified ssDNA aptamer and a blocker molecule, the apple juice compounds were unable to adsorb onto the substrate surface, and thus did not contribute to the SERS signal [58]. In another study, an aptamer conjugated polymer-Au NP composite microsphere was used as a SERS substrate to selectively capture the pesticide malathion in tap water [68]. The controlled aggregation of Au NPs on the polymer microspheres also allowed for more reproducible SERS signal intensities.

Although functionalization of SERS substrates can improve the selectivity, additional signals can be produced from the capturers which may overlap with the target signals. Sometimes, due to the formation of a complex between the capturer and pesticide molecules, the SERS patterns may change significantly. Therefore, careful identification of the target pesticide signals is very important. In addition, due to the additional layer on the substrate, the target pesticide molecules are not directly on the surface of SERS substrate. This would inevitably decrease the sensitivity because the enhancement is strongly dependent on the distance between the target and substrate. The typical enhancement zone is within 10 nm [69]. Therefore, any capturer that is larger than that is not applicable to be used in the SERS substrate functionalization for pesticide detection.

Although functionalization of substrates for the detection of pesticides in several matrices have been explored and proven to be of potential, its detection capability is still in its infancy. More research would be needed to substantially claim the advantages of functionalization and to overcome possible limitations such as contribution of modifiers to spectral signatures and oversaturation of substrate surface coverage.

2.2.3. *In situ* sampling

Another popular trend in pesticide detection analysis is *in situ* sampling. This method requires little to no sample preparation since the pesticide is detected directly on the matrix itself. Coupled with a portable instrument, *insitu* sampling and detection find its market niche for rapid

screening of pesticides in agriculture applications. The various kinds of SERS substrates that have been used for *in situ* pesticides detection are presented in **Figure 3**.

One simple example of this application was illustrated in a detection assay that identified pesticides on fresh tea leaves and apple peels [57]. In this study, commercially available, citrate stabilized Au NPs were dropped onto the surface of the plant that was spiked with various amounts of organophosphates, pyrethroids, and neonicotinoids pesticides. After mixing the aqueous Au NPs solution and the spiked pesticide surface using gentle pipetting,the mixture was dried and a Raman spectro-microscope was used to focus directly on the surface of the leaves and peels to record their Raman signals. No pesticide Raman signals were observed when Au NPs were not dropped onto the sample surface whereas intense pesticide peaks were seen with the introduction of Au NPs, owing to the surface enhancement effect. Furthermore, no significant background interference was observed on both fresh tea leaves and apple peels, hence, low detection limits (ppb levels) for all the pesticides tested were achieved. In a similar study, an *in situ* method for detecting malathion pesticide was introduced using SERS imaging and multivariate curve resolution [55]. In this study, colloidal Au NPs solution was dropped onto spiked tomato peels and Damson plum surfaces, followed by addition of a NaCl solution. Then a mapping region was selected for SERS and data analysis. Liu et al proposed a different strategy for *in situ* detection, whereby Ag NP films were fabricated directly on contaminated sample surfaces using an *in situ* reduction method, followed by SERS analysis of that surface [70]. Based on this methodology, the pesticides paraquat and fenthion were detected *in situ* on the surfaces of capsicum, celery and cole, as well as malachite green on fish scales.

Another approach to the *in situ* sampling of pesticides on fruit surfaces was illustrated using a SERS derived concept termed as “SHINERS” (Shell isolated nanoparticle-enhanced Raman spectroscopy) [48]. In this detection assay, fabricated nanoparticles which consist of an ultrathin coating of silica or alumina (2 nm) on Au NPs were spread onto a sample surface as a “smart dust”. The coating prevented agglomeration of the nanoparticles and allowed monolayer distribution of the nanoparticles on the sample surface. To illustrate this concept, parathion was detected on an orange peel by simply spreading the “smart dust” on the surface. An advantage of this method is that it protects the SERS active metal nanostructure from touching directly onto the sample and allows it to conform to the structure of the sample surface. In a separate study, silver coated Au NPs (Au@Ag NPs) were characterized and shown to provide strong SERS signals when applied *in situ* onto pesticide spiked fruit peels [71]. An interesting observation was that the Raman intensity enhancements were dependent on the Ag shell thickness instead of the aggregation status of nanoparticles, suggesting that “hot spots” were not the main contributor in this particular SERS substrate. Rather, Au@Ag NPs acted as stand-alone-particle Raman amplifiers capable of detecting trace pesticide levels on complex matrix surfaces, similar to the mechanism of tip enhanced Raman spectroscopy (TERS) [72].

In a different attempt to perform *in situ* detection that was both sensitive and highly reproducible, Au NPs were grafted onto dendritic α-Fe_2O_3 to test thiram residues (5 μM) on tea leaves [73]. The dendritic nanostructures not only enabled the researcher to locate the test spot easily through an optical microscope, but provided a highly uniform substrate pattern that is a precursor to reproducible Raman enhancements.

Based on the studies exploring the application of SERS for pesticide detection *in situ*, it is evident that this method has potential for on-site screening capability and portability. However, more studies will have to be conducted such as testing it with a wide range of pesticides using commonly available or cost effective substrates in order for widespread applications to become a reality. Furthermore, the sample surfaces that were used were relatively clean and free of other interference compounds, hence it would be interesting to see the effect of this method in real matrices that may have more contamination or have been deliberately contaminated with other compounds.

3. Future trends and perspectives

3.1. Commercialization and method standardization

Just a few years ago, there were only two commercially available SERS substrates ready for use. Both of these, Klarite™ (Renishaw Diagnostics, Glasgow, UK) and Q-SERS (Nanova, Columbia, MO, USA) were manufactured based on electron beam lithography, a relatively expensive and time consuming method of production. As a result, the cost of each substrate was extremely high (<\$100/ in^2). Although the sensitivity and reproducibility of these substrates were satisfactorily good, the cost was the biggest hindrance to widespread usage such as for quality assurance and food industry applications. Studies have been conducted with these substrates to show the efficacy for the detection of pesticides, such as carbaryl, phosmet, and azinphos-methyl on apples and tomato surfaces using Q-SERS [56]. Klarite™ substrates have also been used to

detect phosmet on orange skin peels [74]. Other pesticide detection studies have also been conducted [75–77].

Due to the increasing popularity of SERS for trace detection, other commercial SERS substrates have also emerged. These include Ocean Optics SERS substrate (Ocean Optics, Dunedin, FL) which is basically Au NPs deposited onto a plain glass slide; SERStrate (Silmeco, Copenhagen, Denmark), a silica based substrate coated with Au or Ag with an overall pili nanostructure; Horiba Scientific SERS substrates (Horiba Scientific, Kyoto, Japan); P-SERS (Diagnostic anSERS, College Park, MD), an inkjet printed SERS substrate; EnSpectr SERS substrate (EnSpectr, Chernogolovka, Russia), a solid surface based substrate; and ATO ID^{TM} Ag and Au substrates (ATO ID, Vilnius, Lithuania), formed using ultrashort pulse laser on a soda-lime glass substrate. Aside from the business aspect of selling their own patented SERS substrate, these SERS substrates mostly utilize cheaper fabrication techniques to lower the cost of production, thus reducing the sales price tag. However, the SERS substrates are still relatively expensive, the cheapest running at around US$18/substrate with a surface dimension of 12.5x5mm. With such a small surface area per substrate, it might still not be feasible for high throughput analysis in industrial applications. Thus, it is certainly an area that is growing, seeing that the number of SERS publications focusing on substrate fabrication has continued to increase rapidly.

One possible area of growth in this field is the use of controlled wet chemistry to fabricate the SERS substrates, since these techniques do not often require expensive equipment such as laser cutting. By optimizing the parameters used to control nanoparticle growth, these

methods can potentially be used to create relatively cheap substrates. An example of this kind of SERS substrates are the dendritic nanostructures made from metal reduction methods such as from the reduction of ZnO or Al_2O_3 with $AgNO_3$. The resultant substrate is a leaf like dendritic nanostructure that are interlinked in even gaps with consistent spherical diameter (assuming optimized conditions are used) [78,79]. Another possible commercial use of SERS substrates for pesticides detection is filter-based substrates [80]. To fabricate this substrate, Ag NPs were embedded onto a filter paper using a silver mirror reaction method. Then, in order to test pesticide residues on fruits, the filter paper substrate was wiped onto the fruit surface. Limit of detection of thiram and paraxoan was between 10^{-7} to 10^{-9} M with less than 20% RSV, which is considerably reliable for SERS measurements. As more studies are being conducted to investigate the application of these cheaper alternative SERS substrates, the cost of commercializing SERS substrates will potentially decrease drastically to allow for widespread commercial applications.

In order to apply an analytical technique for practical and routine applications, it is important get accepted and approved by respected organizations such as AOAC International. Although there are thousands of different SERS substrates being fabricated and published, their performance are often tested on different targets. Hence, it is difficult to compare the performance between different substrates. Furthermore, the experimental methods and Raman instrument/specifications such as laser power, aperture size and integration times used are dependent on the researcher's choice since a standardized protocol is not available. Hence, there are very few instructions for a research lab to conduct standard pesticide detection analysis using SERS. This encourages them to look at more established methods such as GC/LC-MS. The

discovery phase for making SERS substrates is becoming more mature, and hence a possible future trend would be the implementation of good SERS substrates for standardized pesticide detection analysis.

3.2. Method integration and advance of instrumentation

SERS by itself holds many advantages for rapid, trace level detection of contaminants such as pesticides. However, like any analytical technique, there are limitations to the use of one technique to perform detection assays. To overcome such limitations, integration with other useful techniques can bring added advantages such as verification of results, better extraction procedures, and automation of detection.

Such integration studies have become increasingly popular in recent years. In one study, plasmonic nanoparticle modified capillary (NPMC) was fabricated and used for HPLC-SERS, which not only separated the target pesticide, thiram, from its matrix but also gave molecular specific fingerprints [81]. A similar separation procedure was integrated with SERS using thin layer chromatography to detect methadathion [59]. To validate the results of Au NP based colorimetric assays, SERS has been used since the nanoparticles when aggregated can form localized surface plasmon resonance effect to enhance the Raman signals [82]. The miniaturization of the SERS method in a confined space has also been investigated using the Ag NP based microfluidic SERS assay to detect the pesticides alachlor and carbofuran [83] and the pesticides methyl parathion and malachite green [84].

With more of these types of integration studies, the advantageous of SERS will be reflected in many more studies and potentially be expanded for wider applications. Significant advance in instrumentation is also critically important, in order to advance SERS as a routine and cost- effective analytical technique.

3.3. Internalized pesticides analysis

Another upcoming trend in the detection of pesticides using SERS is the ability to analyze pesticides that have been internalized or penetrated into cells of living tissues. Based on the physical behaviors of pesticides, they can be divided into two categories: systemic and non-systemic. Systemic pesticides are able to penetrate into the plant tissues and translocate from the site of applications to other parts of the plant. Non-systemic pesticides have little or no penetration ability. In all the *insitu* SERS studies and many SERS studies that applied a simple extraction procedure, their applications have been limited to pesticides on the surface. Therefore, more studies are needed for detection of internalized pesticides.

In order to observe the internalization of trace levels of pesticides over time, SERS can be conducted by adding penetrable nanoparticles that have strong adsorption affinity to the pesticides of interest. Coupled with a confocal Raman microscope which can scan layer by layer inside the plant tissue, it is able to study the penetration depth and location of the internalized pesticides[85]. Our preliminary data showed that with increase incubation times, thiabendazole, a systemic pesticide, can gradually penetrate further into a spinach leaf at 210 μm depth after 2 days of exposure (**Figure 4**).

By investigating the potential of using SERS for analyzing internalized pesticides, it opens the door for not only the *in situ* detection of internal pesticides, but also for the understanding of the mechanisms of pesticides behaviors and their fate. Understanding pesticide penetration behaviors and fate will help us to develop a better strategy to safely and effectively apply pesticides.

4. Conclusions

SERS is a multi-versatile detection tool that has evolved to become a highly potential rapid detection technique today. Its excellent sensitivity to the detection of a wide range of pesticides has promoted its use as an alternative detection technique for rapid pesticide analysis. The reproducibility, portability and detection in complex matrices have been improving as evidenced by the increase in scientific articles addressing these topics. The expansion of SERS as a tool for pesticides detection has also started to become a reality as the commercialization of more cost effective substrates has become more common. Furthermore, expanded usage of SERS through integration with other analytical techniques has broadened its usage scope. Lastly, new areas of research such as the analysis of internalized pesticides in plants *in situ* are poised to expand the popularity of SERS. Overall, the prospect of SERS as an alternative pesticide detection tool seems feasible and should continue to attract more studies in this area of research. Advancement in instrumentation and commercialization is expected to further apply SERS as a routine and cost-effective analytical method for pesticide detection.

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Figures/Tables

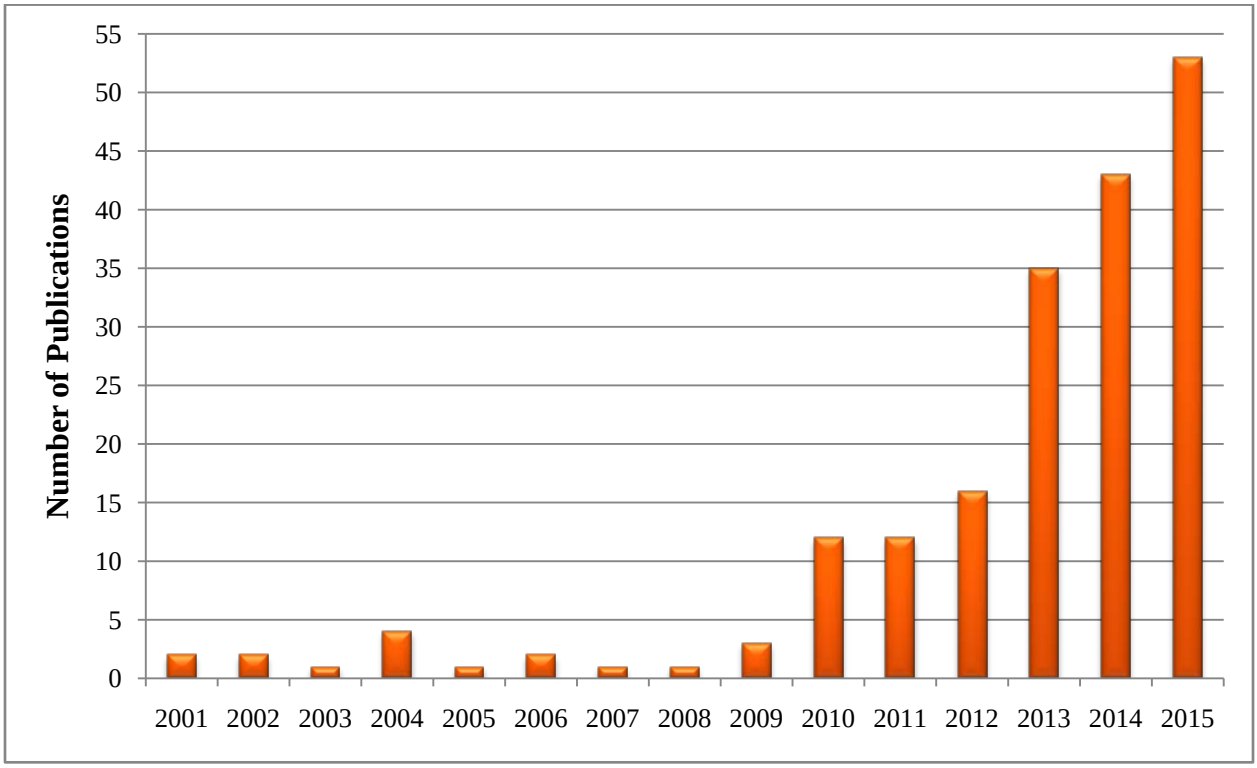

Fig. 1. Number of publications related to SERS detection of pesticides every year. Web of Science™ was used with the following search terms: "Pesticides" and "SERS". Date Accessed: 1/15/2015.

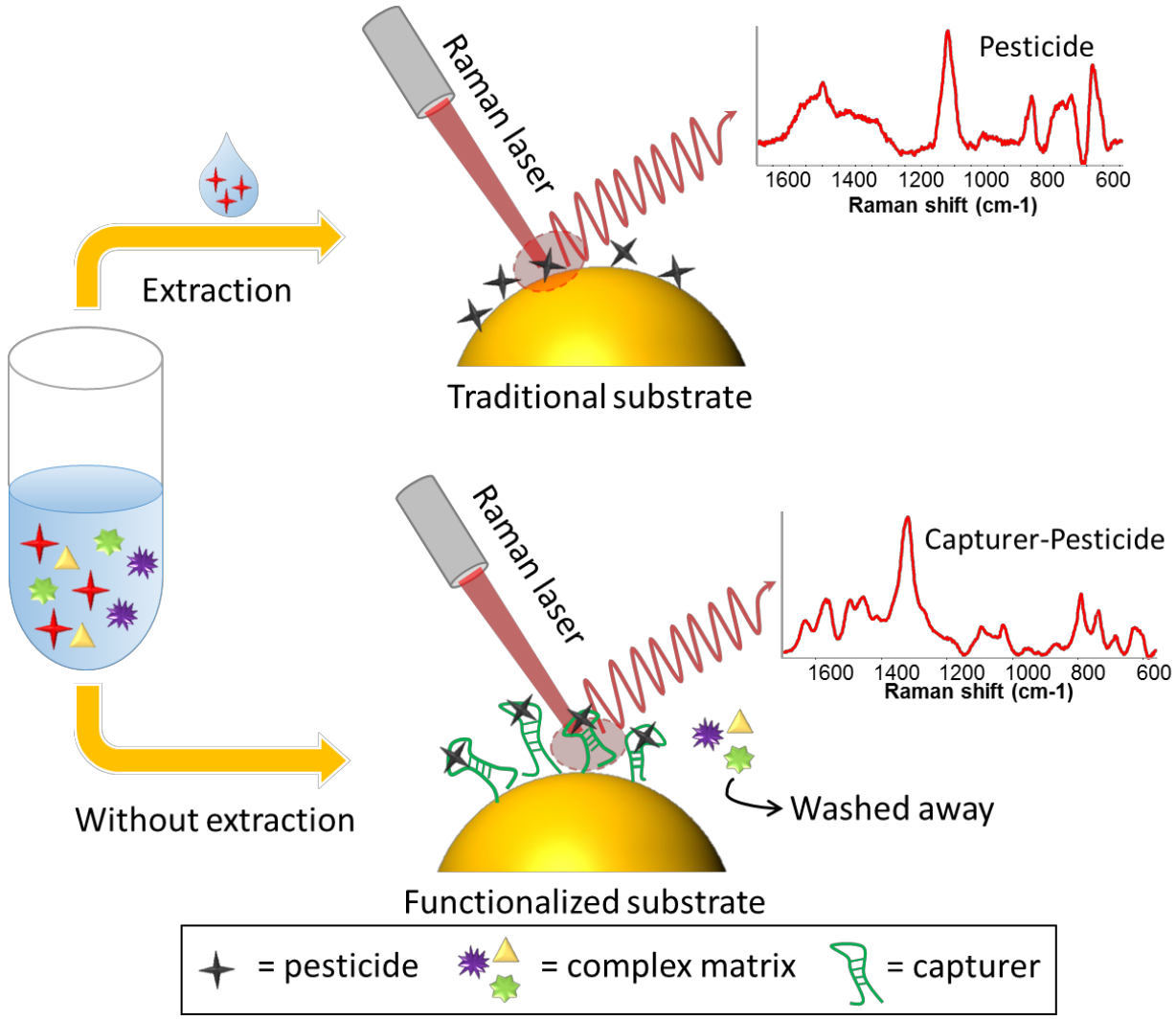

Fig. 2. Illustration of the detection of pesticides in real, complex matrix using extraction procedure and substrate functionalization respectively.

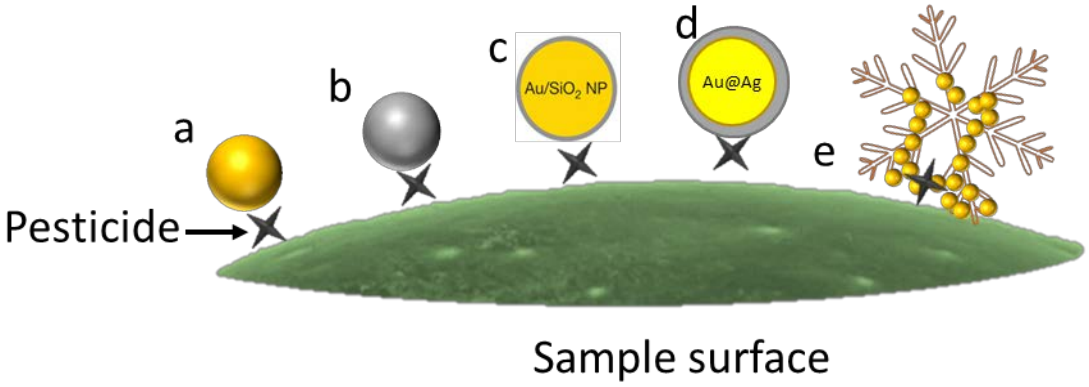

Fig. 3. SERS substrates applied for *in situ* pesticide detection. a. AuNPs [57] and [60], b. Ag NPs (fabricated *in situ*) [70], c. Au@SiO_2 NPs [48], d. Au@Ag NPs [71], e. Au NPs grafted on dendritic α-Fe2O3 [73].

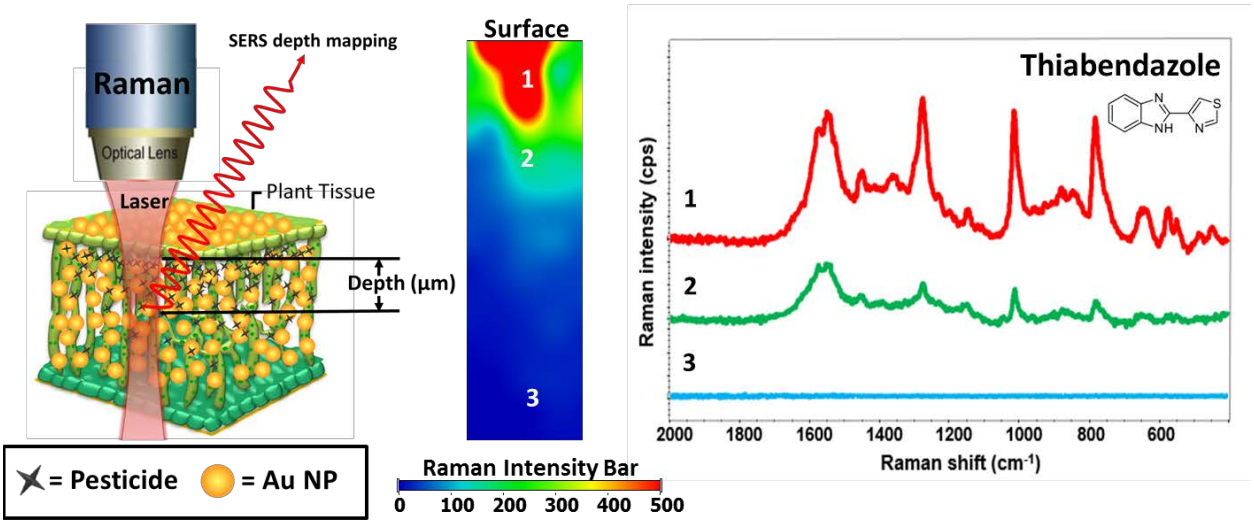

Fig. 4. Internalized pesticide analysis. Sample is immersed in Au NPs solution, and then SERS depth analysis is performed. Right below the surface is where the majority of pesticides were found (indicated in red). As the SERS spectra was taken deeper into the sample, the intensity of the peaks decreased.

Table 1

Summary of pesticides, detection matrices, nanosubstrates used, and the limit of detection (LOD).

Pesticide	Class	Matrix	Substrate	LOD	Ref
acephate	organophosphate	Urine	Au/Ag @Ti roughened	19 ppb	[86]
azinphos-methyl	organophosphate	Apples / Tomatoes	AuNPs	6.66 ppm / 2.94 ppm	[56]
carbaryl	carbamate	Apple juice / Cabbage	Au-nanorod on silicon slide	2.5 ppm / 2.5 ppm	[66]
carbaryl	carbamate	Apples / Tomatoes	AuNPs	4.51 ppm / 5.35 ppm	[56]
chlorpyrifos	organophosphate	Water / Apple skin	Au nanofinger chips	35 ppt	[38]
chlorpyriphos	organophosphate	Rice	OTR202 and 203 solution	0.5 ppm	[87]
deltamethrin	pyrethroid	Tea leaf / Apple peel	AuNPs	0.5 ppm / 0. 02 ppm	[57]
dimethoate	organophosphate	Honey	Klarite™	2 ppm	[88]
disulfoton	organophosphate	Orange	AuNPs decorated GMA–EDMA	1ppb, 39.7 mg/kg	[89]
imidacloprid	neonicotinoid	Tea leaf / Apple peel	AuNPs	0.5 ppm / 0. 02 ppm	[57]
isocarbophos	organophosphate	Tea leaf / Apple peel	AuNPs	0.25 ppm / 0. 01 ppm	[57]
malathion	organophosphate	Food peels	AuNPs	0.123 mg L^{-1}	[55]
methamidophos	organophosphate	Apple	Au@Ag NRs	440 μM	[90]
methyl parathion	organophosphate	apple	Filter paper withmultibranched Au nanoantennas (MGNs)	26.3 μg	[91]
paraquat	viologen	Fruit skins	AgNPs	1 nM	[54]
phorate	organophosphate	Tea leaf / Apple peel	AuNPs	0.25 ppm / 0. 01 ppm	[57]
phosmet	organophosphate	Apples / Tomatoes	AuNPs	6.51 ppm / 2.91 ppm	[56]
phosmet	organophosphate	Apple extract	Klarite™	1 ppm	[75]
phosmet	organophosphate	Orange	AuNPs decorated GMA–EDMA	5ppb, 8.25 mg/kg	[89]
thiabendazole	benzimidazole	Water / Apple skin	Au nanofinger chips	1 ppb / 7 ppb	[38]
thiabendazole	benzimidazole	Apple	Ag dendrites	0.1 ppm	[92]
thiram	dithiocarbamate	Apple skin	Au nanoisland film	5 ppb	[67]
thiram	dithiocarbamate	Orange	plasmonic nanoparticle-modified capillary (NPMC)	100 nM	[81]
thiram	dithiocarbamate	Tea leaf	optofluidic SERS	5 ppb	[84]
thiram	dithiocarbamate	Apple skin	Au nanorod-coated Fe3O4 microspheres	100 nM	[93]
thiram	dithiocarbamate	Apple	Au@Ag NRs	460 nM	[90]
thiram	dithiocarbamate	Apple skin	Ag nanoshells (Ag NSs) around silica NPs	38 ng cm^{-2}	[94]
thiram	dithiocarbamate	Grape	Au@Ag NPs/GO/Au@Ag NPs sandwich nanostructure	0.03 ppm	[95]
triazophos	organophosphate	Pear / Tree leaf / Plastic / Glass	Ag coated Sandpaper	~0.3 cm^2, 53.3 pM cm^{-2} / ~0.6 cm^2, 266 pM cm^{-2} / ~3 cm^2, 10.5 pM cm^{-2} / ~4 cm^2, 4.2 pM cm^{-2}	[96]