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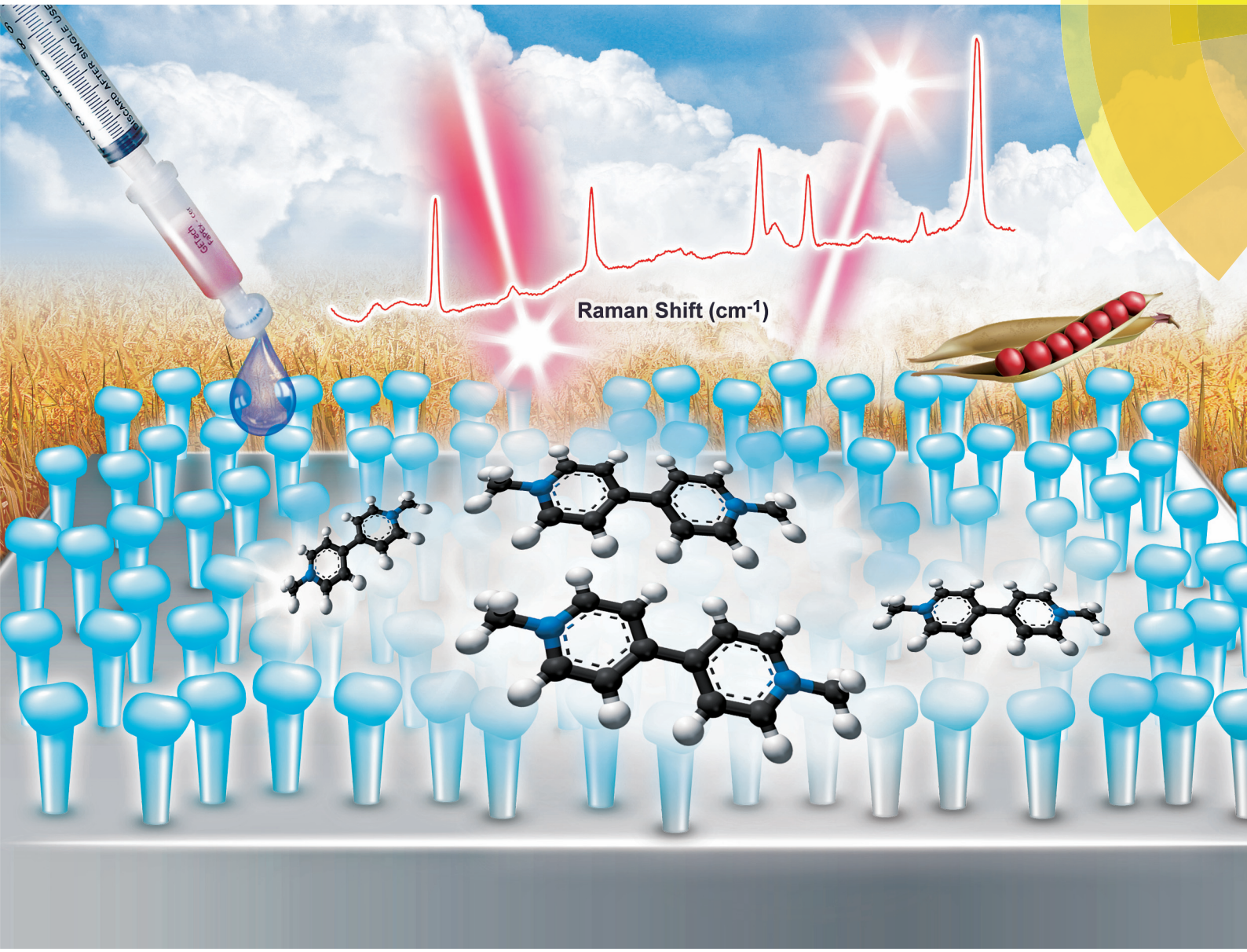

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PAPER
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A simple approach for the ultrasensitive detection of paraquat residue in adzuki beans by surface-enhanced Raman scattering

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Paraquat (PQ), a broad-spectrum contact herbicide, has been used in many countries for controlling weed growth in agriculture because of its quick-acting and nonselective contact with green plant tissue. PQ is also toxic to humans, and even contributes to the development of neurodegenerative diseases. However, PQ is generally excluded from pesticide residue monitoring programs due to the lack of suitable determination methods. Thus, this study developed a detection method combined with simple extraction and surface-enhanced Raman spectroscopy (SERS) to rapidly determine and quantify the PQ residue on legumes without destructive procedures and high-cost instruments. Following the extraction procedure of the QuPPe-method, however, we took whole adzuki beans (*Vigna angularis*) extracted *via* a mixture of methanol and 1% formic acid at room temperature and followed by a 1 min cleanup by SPE. The PQ values for adzuki beans determined by LC/MSMS showed that regardless of whether extraction was followed by the QuPPe-method or the method we proposed, a consistent and low relative standard deviation (RSD) below <22% was found. In this study, we proposed to extract PQ on the surface of the beans by shaking briefly with solvent, and then the PQ molecules were detected and quantified by depositing Ag nanoparticles (AgNPs) and performing SERS within 10 min. Using a coating of deposited Ag nanoparticles, SERS can achieve a limit of detection (LOD) for PQ on the order of 1 μg L^{-1} (~4 × 10^{-9} M) and a method detection limit (MDL) for adzuki beans of 0.8 μg kg^{-1} (~3.3 × 10^{-9} M). This sensitivity at the ppb level absolutely met the maximum residue limit (MRL) for PQ in dried beans as declared by most countries, including the US (0.3 mg kg^{-1}), Australia (1.0 mg kg^{-1}) and Taiwan (0.2 mg kg^{-1}). Taiwan will ban the use of PQ as a defoliating agent for harvest in adzuki bean fields in 2019; therefore, developing a method for detecting PQ residues in the field or in import markets is necessary for consumer health and for authorities. This study provided an opportunity to utilize SERS in the field of on-site pesticide residue screening.

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1. Introduction

Paraquat (PQ) is a broad-spectrum contact herbicide with fast-acting and nonselective activity as well as high soil binding potential. PQ has been used in many countries for controlling weeds in agriculture and aquatic weeds because it cannot leach into aquifers. This product is the second largest herbicide in the world (second only to glyphosate), and has been used on more than 100 crops in 100 different countries mainly for agricultural and horticultural weeding, on cotton, soybeans and so on. Since it is permitted in agriculture practice, PQ poisoning has been reported because of the unintended consumption of the herbicide. Exposure to PQ causes a high mortality rate because no specific drug is effective for treatment. Excessive intake of PQ can cause cell damage and necrosis in the brain, heart, lung, liver and kidney. Therefore, more than 20 countries have banned or strictly restricted its use. Taiwan will also prohibit the sale and use of PQ as a defoliating agent for harvest in 2019.

Because most pesticides are poisonous, food security departments worldwide strictly control the distribution and

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use of pesticides, and have established maximum residue limits (MRLs) for agriculture products according to the assessment of human health. Because of its modest toxicity, PQ is widely used in farmlands, and its permitted residue limit for legumes (*e.g.*, adzuki beans, black beans and soybeans) is set as low as 0.5 mg kg^{-1} ($\sim 2 \times 10^{-6}$ M) in many official regulations, including in Taiwan (0.2 mg kg^{-1}), the US (0.3 mg kg^{-1}), Australia (1.0 mg kg^{-1}) and Codex (0.5 mg kg^{-1}).

During the last two decades, a method called QuEChERS has been developed to detect multiple pesticides using various generic solvent extraction procedures in combination with liquid chromatography tandem mass spectrometry (LC/MS/MS).[1] However, some pesticides with distinctive physicochemical properties are not included in common multiple reaction monitoring (MRM). The challenge of PQ determination is that it is difficult to incorporate with other pesticides since a low pH is necessary for its molecular properties and due to its strong affinity to plant tissue. In fact, PQ is not able to be determined *via* MRM because of its permanent ionic character, high hydrophilicity ($\log K_{ow} = -4.5$) and tendency to strongly interact with various surfaces (*e.g.*, glass) *via* hydrogen, ionic and π–π bonds. With the lack of suitable detection methods, PQ is generally excluded from pesticide residue monitoring programs. Moreover, concerns about the adverse effect of PQ toxicity on farmers and the general population cannot be ignored.

An optimal detection method requires a high recovery yield, low matrix interference, and high sensitivity detection. Generally, extraction solvents employed for PQ detection in fruits, vegetables and grains include methanol (MeOH),[2,3] mixtures of MeOH with water (containing acetic acid or a buffer)[4–7,16] and aqueous hydrochloric acid (HCl).[8–13] To elevate the extraction yields of PQ, sulfuric acid was used in a lengthy reflux to obtain complete extraction of PQ.[14,15] In a few of these reports, clean-up was typically achieved *via* time-consuming solid-phase extraction methods.[7,8,12] In 2012, Kolberg *et al.*[17] developed a method with validation using acidified methanol to reduce the losses of PQ during the entire analytical procedure. Then, the QuPPe-method (quick polar pesticides method) was developed in 2014 for analyzing many highly polar pesticides, including PQ in foods of plant origin. Since the QuPPe-method was developed as QuEChERS, which makes the analytical process quicker, easier, cheaper, more effective, more rugged and more safe, we used the extraction procedures based on the QuPPe-method of the European Union Reference Laboratory for Pesticides Requiring Single Residue Methods (EURL-SRM)[18] to extract PQ from adzuki beans for residue screening. However, grinding and heating samples are necessary for extracting PQ in adzuki beans, which elongates the processing time, and is inconvenient to operate in farm fields.

Typically, detection methods for PQ analysis include ion pairing liquid chromatography (IP-LC)[19,20] and capillary electrophoresis (CE)[21] with various detectors such as ultraviolet (UV)[22] and mass spectrometry (MS).[23] Despite the fact that these analytical tools exhibit remarkable capacities for trace identification, the restrictions of the complex pretreatment procedure, the expense of the instruments, and the demand for an adept operator and adequate laboratory equipment cause difficulties for immediate application in field screening. In recent years, some new colorimetric assays were developed for PQ detection, which are easy to bring from the laboratory to the field, such as that by Prakit (2015),[24] who developed a simple light-emitting diode light-dependent resistor (LED-LDR) colorimeter for the determination of PQ in natural water. Weena *et al.* (2017)[25] also used negatively charged silver nanoparticles (AgNPs) to detect PQ residue in plants. However, the detection limits of the two above-mentioned methods are 0.15 and 0.1 mg L^{-1}, respectively. Taking the extract efficiency and the matrix interference in real plant samples, this sensitivity is not sufficient to meet the requirements of the official limits for field monitoring. Additionally, 0.01 mg L^{-1} is the minimum required detection limit for countries where PQ is banned. Consequently, it is worth developing a quick, inexpensive and sensitive method for PQ detection to cope with the irregular use in legume cultivations.

In the past decade, the surface-enhanced Raman scattering (SERS) technique has been considered a potential alternative for real-time detection with considerable sensitivity. Previous studies have shown that the detection of pesticides *via* SERS has produced peaks for general pesticide molecules. For instance, the sensitivity of fonofos is 10 ppm in dry-filmed AgNP SERS,[26] the sensitivity of omethoate is 2 ppm in aggregated AgNP SERS,[27] and the sensitivity of methamidophos in vegetables is 0.1 ppm in colloid AgNP SERS.[28]

Currently, we know that the detection limits of PQ in SERS varies with the matrix, such as 10^{-6} M in soil humic substances,[29] 2×10^{-9} M in water *via* a SERS-based microdroplet sensor,[30] 10^{-9} M on the peel of pears,[31] and 10^{-9} M on a monolithic column as a new type of substrate.[32] Nevertheless, these studies have shown the great sensitivity achieved in simpler matrixes compared with raw agricultural products. As an herbicide, the risk of PQ in beans is of concern. There have been few reports of a rugged method to screen the PQ residue in bean fields; thus, this work first reports a screening system based on Raman SERS coupled with simple filtration to reduce the bean matrix interference, and enhance the assigned peak signal of PQ *via* formic acid and methanol. This system can be applied to bean fields to screen PQ residues in harvested crops.

2. Materials and methods

2.1. Chemicals and suppliers

Acetonitrile (ACN; LC-MS grade) and formic acid (FA; p.a., 98%–100%) were obtained from Merck (Darmstadt, Germany), ammonium formate (p.a., 99%) was acquired from Fluka (Buchs, Switzerland), methanol (MeOH; LC-MS grade) was obtained from Roth (Karlsruhe, Germany), and water was deionized in the laboratory using a Millipore MilliQ water purification system (Billerica, MA). PQ dichloride (96.5%) was

obtained from Dr Ehrenstorfer (Augsburg, Germany). Sample grinding was carried out using a RT-02B pulverizing machine (Rong Tsong, Taiwan). Polypropylene centrifuge tubes (15 mL and 50 mL) with screw caps were from CORNING CentriStar™ (Corning, NY) and used in an Allegra® X-30R refrigerated centrifuge (Beckman Coulter, Brea, CA). A 10 mL solvent dispenser and micropipettes for handling volumes from 2 to 20 μL, 10 to 100 μL and 1 to 10 mL were from Brand® (Wertheim, Germany). Hydrophilic polypropylene syringe filters with a 0.45 μm pore size were GH Polypro (GHP) membrane disc filters (Pall Corp., NY). Then, 1 mL and 5 mL polypropylene (PP) syringes without needles were from Terumo (Tokyo, Japan). The 2 mL PP auto-sampler vials suitable for the LC auto-sampler were from MicroSolv Technology (Eatontown, NJ). Volumetric PP flasks with stoppers were from Vitlab (Großostheim, Germany).

2.2. Preparation of standard solutions

Since PQ tends to adsorb on glass, all stocks and working standard solutions of PQ were prepared and stored at 4 °C in PP bottles and in auto-sampler vials. The stock solution (1 mg mL^{-1} as a cation), taking the purity and the molecular formula of the salt form into account, was prepared by dissolving the standard reference materials in methanol. Working solutions were prepared from stock solutions diluted in methanol with 1% formic acid. The stability of PQ in the stock standard solutions could be preserved for over 24 months.

2.3. Sample preparation for PQ-immersed adzuki beans

Adzuki beans were harvested by hand *via* an organic, no-pesticide spray procedure and stored at ambient temperature. The sample of 2 kg of whole grain adzuki beans was immersed in a 2 ppm PQ solution at 4 °C for 1 hour, and the level of PQ in the adzuki bean was quantified by LC/MS/MS. The PQ-immersed adzuki bean sample was the adzuki bean sprayed with PQ on the farm.

2.4. The extraction procedure of PQ from adzuki beans

To evaluate the PQ absorbed in the PQ-immersed adzuki bean, a whole or ground sample was individually extracted according to the signature QuPPe-method. Two grams of the adzuki bean sample in a 50 mL centrifuge tube was followed by adding 2 mL of ddH_2O to thoroughly wet the beans. Then, the 10 mL extraction solution (MeOH (49%) + H_2O (50%) + FA (1%)) was added and thoroughly mixed by shaking for 1 min. The mixture was ultrasonicated for 30 minutes in an 80 °C water bath, shaken for 1 min, and cooled to room temperature. After centrifugation (4255*g*, 15 °C) for 3 min, a 0.9 mL aliquot of the supernatant was mixed with 0.1 mL of 1% FA/MeOH (v : v 1/1), then passed through a 0.45 μm syringe filter into a 2.0 mL plastic storage vial, and then determined directly using LC-MS/MS. The procedure for SERS detection is illustrated in Fig. 1, and the extraction procedure included adding 10 mL of the extraction solution (MeOH (49%) + H_2O (50%) + FA (1%)) to the 2 g of the adzuki bean sample. It was then mixed thoroughly by shaking for 1 min at room temperature. A

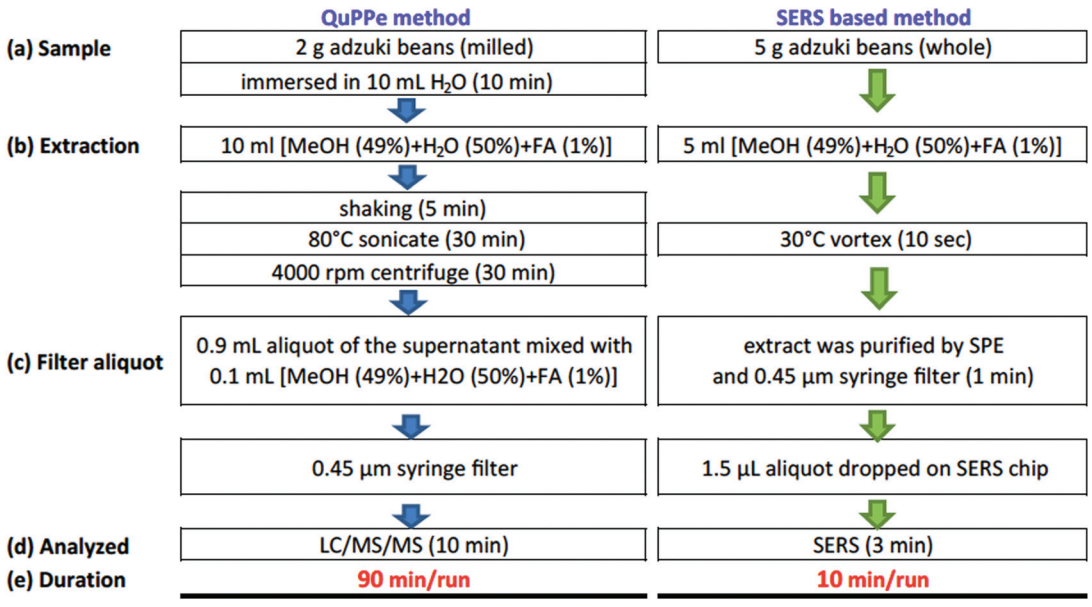


Fig. 1 Comparison of the pretreatment procedure of the QuPPe-method for LC/MSMS with the SERS-based detection method. (a) In the QuPPe-method, 2 g beans were ground to powder; in the SERS method, 5 g samples were kept as whole granules. (b) In the QuPPe-method, the mixture was ultrasonicated for 30 min in an 80 °C water bath and centrifuged; in the SERS method, the mixture required shaking 10 seconds at room temperature. (c) In the QuPPe method, the supernatant was mixed with the extractant for dilution suitable for LC/MS/MS; for surface extraction in the SERS method, the extract was purified *via* SPE. (d) One sample analysis required 10 minutes for LC/MS/MS, and SERS measured 1.5 μL of aliquot mixture cost only 3 minutes. (e) The total duration time for one sample is 90 min in the QuPPe-method and 10 min for the SERS-based detection method.

0.9 mL aliquot of the supernatant was passed through a cleanup SPE filled with 80 mg PSA and 5 mg magnesium sulfate ($MgSO_4$), and 1.5 μL of the purified solution was taken and mixed with 1.5 μL of deionized water, which was dropped onto the SERS chips. When the dropped solution was almost dry, the PQ molecules were theoretically evenly distributed at the surface and interacted with the AgNPs for the SERS.

2.5. Paraquat identification using SERS chips

The SERS chip used in this study is a AgNP-deposited silicon layer (US 8898811B2) fabricated by Phansco Scientific Ltd, (Hsinchu, Taiwan). In the manufacturing process, the substrate is rotated about the axis of substrate's normal at a constant speed in the electron-beam evaporation system. Fig. 2(a–c) show the top view diagram taken by SEM on the specimen of a single layer of silver nanopillars of three different thicknesses obtained by a process under a deposition speed of 1.2 nm s^{-1} and Θ_γ = 89 degrees collocating with a rotational substrate speed of 10 rpm. A surface enhanced Raman spectroscopy (SERS) sensing substrate comprised of a substrate of silver nanopillars that is capable of enhancing Raman signals. The metal nanopillars are randomly distributed on the silicon substrate and has a configuration in which the metal nanopillar structure is about parallel to a normal line with the following properties such as: (a) diameter of nanopillars: 80–380 nm, (b) distance of centers between two adjacent nanopillars, 10–250 nm, and (c) total height of nanopillars, 120 ± 10 nm 230 ± 10 nm and 450 ± 10 nm.

The single-mode laser diode was used to illuminate the Raman signal which was detected by using a spectrometer manufactured by Phansco Scientific Ltd (Hsinchu, Taiwan). The slit of the spectrometer is 50 μm and the resolution is 10 cm^{-1}. The laser wavelength was 785 nm with a laser power of 80 mW. The acquired time of the signal was 500 ms with 12 average times. The SERS signal of PQ was enhanced and detected when the laser excited the sample.

2.6. Determination of the PQ residue by LC-MS/MS

The residue level of PQ in adzuki beans was determined by LC/MS/MS. The chromatographic separation was performed at

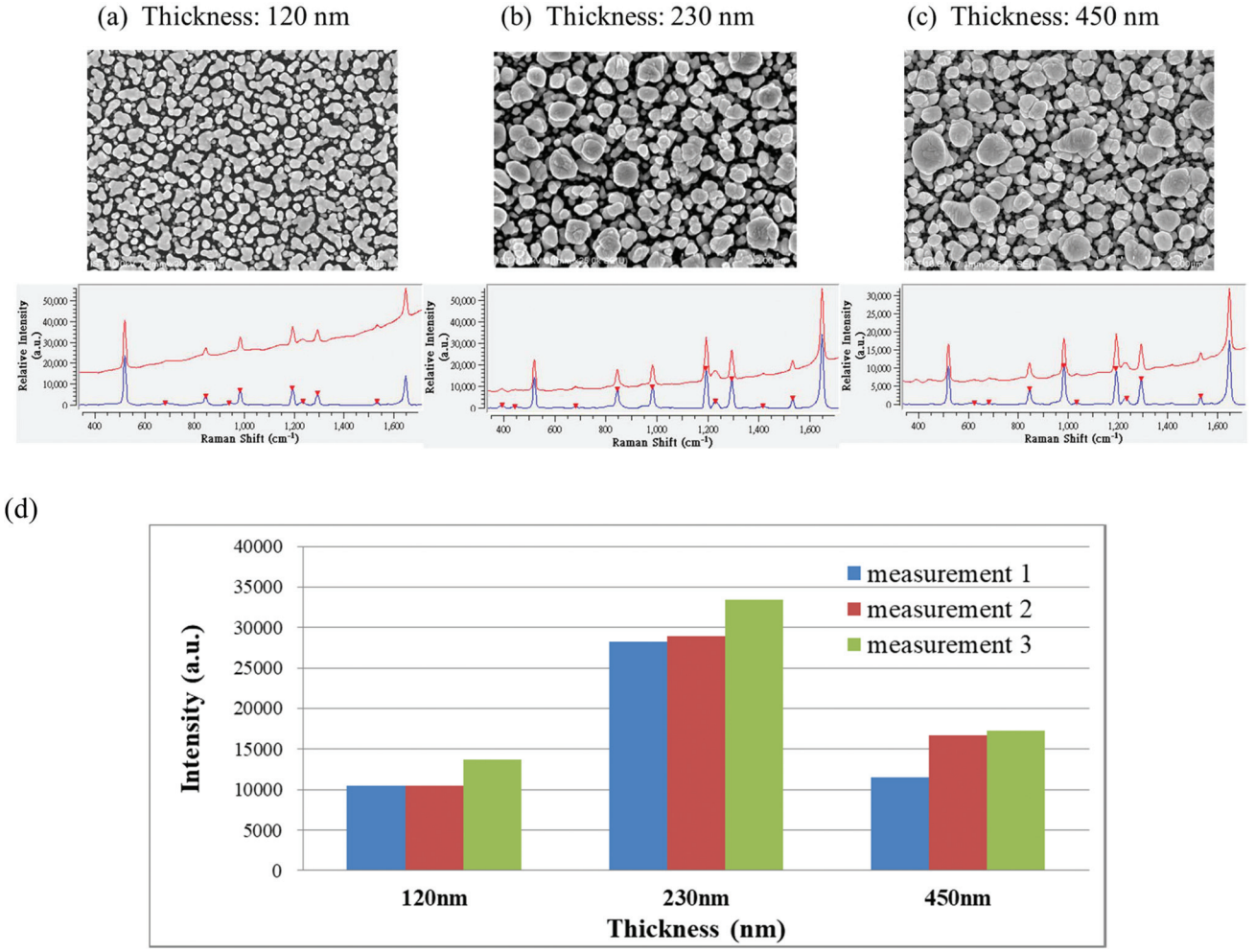


Fig. 2 Morphology and Raman signal intensity of silver nanopillars on SERS with different thicknesses. (a)–(c) SEM image at the top view of the SERS substrate used in this study. (d) The intensity of the peak at 1648 cm^{-1} measured three times for each thickness.

40 °C using an Agilent 1200 series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Palo Alto, CA) equipped with a Shiseido Capcell Pak ST column (150 × 2.0 mm ID, Shiseido, Tokyo, Japan). Solvents included 10 mM ammonium acetate in 0.1% (v/v), formic acid (eluent A), and acetonitrile (eluent B). All reagents used were HPLC grade. The gradient program was: 70% B at 400 μL min^{-1} (0 min), 10% B at 400 μL min^{-1} (1 min), 10% B at 500 μL min^{-1} (3 min), 10% B at 500 μL min^{-1} (6 min), 70% B at 400 μL min^{-1} (6.1 min), and 70% B at 400 μL min^{-1} (10 min). The volume injected was 10 μL. The molecular intensity was determined using a triple quadrupole mass spectrometer (Applied Biosystems 4000 QTRAP, Applied Biosystems, Warrington, UK) with the following features: ion-source: electrospray, positive mode; scan type: multiple reaction monitoring; ion-spray voltage: 5500; ion source temperature: 500 °C; curtain gas: 15; collision gas: high; nebulizer gas: 50; auxiliary gas: 60. Two transitions for PQ, 186/171 (declustering potential, DP: 40; entrance potential, EP: 10; collision energy, CE: 29; collision cell exit potential, CXP: 10) and 171/71 (DP: 86; EP: 10; CE: 55; CXP: 14) were selected and used as the quantitative transition and the qualitative transition, respectively.

3. Results and discussion

3.1. Characteristics of the Raman spectrum of PQ

We prepared wafers with three thicknesses (120, 230 and 450 mm) to measure 0.1 ppm PQ, and repeated the measurements three times. Fig. 2 shows that the wafer with 230 mm thickness presents the best signals, and the thinner (120 nm) and thicker (450 mm) wafers show decreased signal strength. The main mechanism of SERS enhancement upon interaction with paraquat (PQ) is the electromagnetic dipole accentuated by the shape of the silver nanopillars on SERS. The electromagnetic field localizes spatially at the edge of the silver nanostructures when the incident light becomes resonant with the plasmon.[33] Thus, the Raman scattered light from the adsorbed PQ then becomes resonant with the plasmon, and strong Raman scattered light is emitted.[34] According to the SEM images, the morphology of the wafers with various thicknesses presented the different diameters of the silver nanopillars layer-by-layer systematically, which formed fan-like porosity for the shadowing effect.[35] The larger diameter of the silver nanopillars would cause the lower porosity of the film to result in different detection performance.[36] The SERS spectrum of the 5 ng mL^{-1} PQ solution was used to determine whether any interfering signals from solvents or AgNPs were present in the SERS. As shown in Fig. 3(c), the SERS spectrum of the 5 ppb PQ solution dropped on the AgNP-deposited SERS substrate was acquired without any disturbance from the background and presented strong Raman peaks for the C–N stretching mode at 846 cm^{-1}, the C=C bending vibration mode at 1197 cm, the C–C structural distortion mode at 1293 cm^{-1} and the C=N stretching mode at 1648 cm^{-1}. The background spectra of the AgNP-deposited SERS substrate alone (shown in Fig. 3a) and MeOH coupled with the AgNP-deposited SERS substrate (shown in Fig. 3b) were quite weak and did not superpose the peaks from the PQ molecules. The SERS spectrum of PQ, coupled with the deposited AgNPs, was easily acquired, which indicated that a forceful cross interaction instantly occurred when PQ was absorbed onto the metal SERS surface. These characteristic peaks were consistent with previous studies,[30,31,37,38] which supported this proposed idea that SERS can be a qualitative and quantitative assay for PQ molecules.

3.2. Sensitivity of PQ determination in adzuki beans

This study applied SERS to detect PQ in real samples of adzuki beans. As shown in Fig. 3(d), even the wide baseline shift from 1000 to 1600 cm^{-1} was likely observed as a result of the luminescence from the quartz bottle and the complex matrix composition of the adzuki beans,[39,40] and we still obtained the reference peaks from 5 ng mL^{-1} PQ spiked in the extract of 2 g of adzuki beans, which indicated the high sensitivity of the SERS for the adzuki bean substrate. These results indicated that using SERS to determine the PQ level in adzuki beans is feasible even below 5 ng mL^{-1}. Although the detection limit of PQ detected by SERS in previous studies was as low as in this study, prior test matrixes were only limited to a simple matrix, such as a solvent, water or the peel of fruit, but they were not feasible for application to the whole sample. Gao *et al.* (2010)[30] used a SERS-based microdroplet sensor to detect PQ in water with an LOD estimated to be below 2×10^{-9} M (~0.5 ppb). Dao *et al.* (2016)[38] developed electrodeposited AgNP SERS to detect PQ in a solvent (20 mM $AgNO_3$ and 4.8 μM hydrofluoric acid) with a concentration as low as 0.01 ppm. Until 2015, Fang *et al.*[31] optimized an Ag nanoparticle colloid to reach an LOD of 10^{-9} M (~0.25 ppb) for the surface of fruits, such as pears or apples. The results from Fang *et al.* used the major peak at 1648 cm^{-1} alone to quantify, and the PQ level on the peels did not entirely represent the total PQ concentration in the fruit. In the supplement, they indicated that the LOD was calculated according to 20 measurements of blank samples and the approximate standard deviation of a blank (1×10^{-8} M PQ) solution, which provided the slope of the calibration curve. Thus, the LOD value was evaluated to be 3.4×10^{-9} M. Using this algorithm, in our study, 7 blank samples (1 ppb PQ solution) were measured, and the LOD of PQ detected *via* SERS was 3.3×10^{-9} M. This could be the lowest residue concentration of PQ that has been detected using SERS in a full sample rather than just on the surface of a fruit sample.

In this study, similar to the standard solutions of PQ, the Raman intensities of four main peaks at 846, 1197, 1293 and 1648 cm^{-1} were linearly related to the concentrations of PQ in adzuki bean extracts, although the regression results were not as good as those for the standard solutions (data not shown). These results showed the potential of using SERS for quantitative determination, but the presence of nontargeted compounds in adzuki bean extracts, which affected the sensitivity of qualitative identification and the accuracy of quantitative determination, may compete with PQ molecules on the

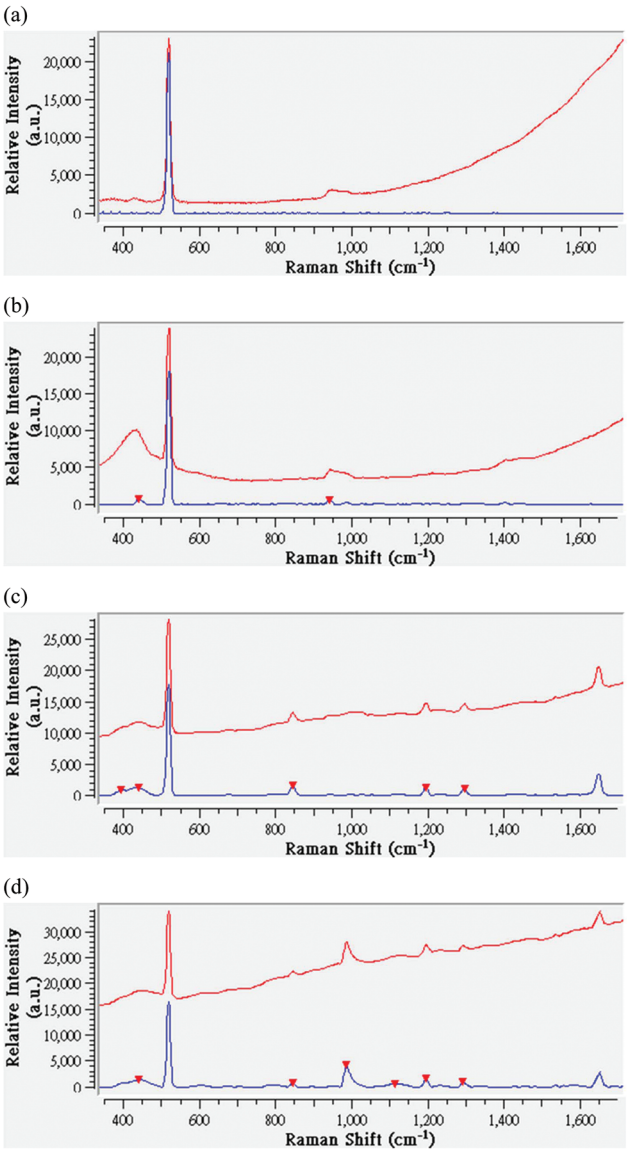


Fig. 3 The Raman spectra with normalization. The Raman spectra of (a) the AgNP-deposited SERS substrate, (b) the extractant: methanol/H_2O (v/v: 1/1) with 1% formic acid, (c) the PQ solution (5 ng mL^{-1}) in the extractant, and (d) the PQ solution (5 ng mL^{-1}) spiked in the adzuki bean extract. The scanning parameters for the Raman spectrometer were a wavelength of 785 nm, a power of 20% and a 4× lens. Red line: Raman spectra; blue line: normalization.

surface. These are the active spots of the AgNP-deposited SERS layer that generate the interference signal in the PQ spectrum and decrease the accuracy of quantitative SERS results. Due to the considerable sensitivity of SERS detection, the process of sample pretreatment required a reduction of the interference from nontargeted components (*e.g.*, organic acids, sugars, and pigments) before the residual PQ in adzuki bean tissues could be determined by SERS. Thus, SERS coupled with a sample pretreatment process could be a powerful approach for ultra-sensitive detection in trace amounts of organic chemicals. This study performed SPE filled with PSA and $MgSO_4$ for sample pretreatment to overcome matrix interference, which can enhance the sensitivity to determine the declared maximum residue level of PQ in adzuki beans.

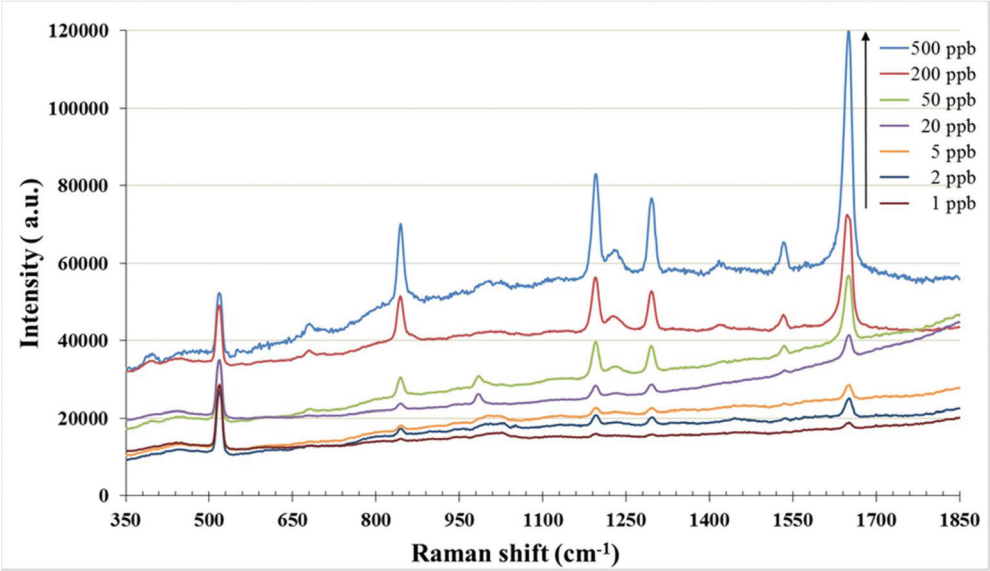
3.3. The matrix-matched calibration curve of the SERS intensity

The overlap plot of the PQ solution with different concentrations from 1 μg L^{-1} (ppb) to 1 mg L^{-1} (ppm) (Fig. 4a) showed that the peaks at 846 and 1648 cm^{-1} displayed a faster variation with concentration, but the relatively slower changes in the peaks were observed for 1197 and 1293 cm^{-1}. This may be due to the PQ molecule with various concentrations presenting different absorption abilities and orientations on the nanoparticle substrate surface.[41] We selected the peak with the highest intensity (1648 cm^{-1}) in the SERS spectra to plot as a function of the PQ concentrations to generate a standard curve for quantitative analysis of PQ. The linear working calibration was established by the matrix-matched calibration curve. The PQ standard solutions spiked with five concentrations (5, 20, 50, 200 and 500 ng mL^{-1}) of the adzuki bean extract were measured to establish the matrix-matched calibration curve. The calibration curve formed by the PQ concentration and the intensity of the Raman peak approached the natural logarithm; however, the explanatory power R^2 only reached 0.88 (Fig. 4b). If the calibration curve was established from both the logarithms of the PQ concentration ($\log C$) and the Raman spectrum ($\log I$), the explanatory power of the calibration curve could be R^2 = 0.98 (Fig. 4c). Therefore, we took the logarithm-adjusted matrix-matched calibration curve equation of ($\log I$) = 0.665 ($\log C$) + 2.9549 as the quantitative basis for detecting the test samples, which were immersed in the PQ solution with a known quantity. This quantification formulation is similar to that of Wang *et al.* (2016)[42] in which the calibration curves between Raman signal intensities and PQ concentrations were plotted by fitting Hill's equation. This is based on the assumption that the Raman signal intensity is proportionate to the molecule surface coverage that adsorbed on the SERS substrate. By fitting the data acquired from the SERS substrate, Wang *et al.* indicated that surface-bound PQ decreases the affinity of incoming molecules to the surface, meaning there is negative cooperative binding. Because the concentration for PQ sensing was far below the equilibrium constant, a log–log plot of the Raman signal intensity *vs.* the analyte concentration revealed a linear relationship.

3.4. Extraction efficiency of surface rapid extraction

To evaluate whether the sample types influence the extraction efficiency of the surface extraction and QuPPe method, we took 20 g of adzuki beans immersed in 20 mg L^{-1} of PQ solution and divided them into two groups depending on whether the beans were ground or kept whole. Both groups followed the extraction procedure of the QuPPe method: adding 10 mL of extractant (1% FA/MeOH (v : v 1/1)) to thoroughly mix the samples by shaking for 1 min, ultra-sonicating for 30 minutes in an 80 °C water bath, shaking for 1 min, and cooling down to room temperature. After centrifugation (4255*g*, 15 °C) for

(a)



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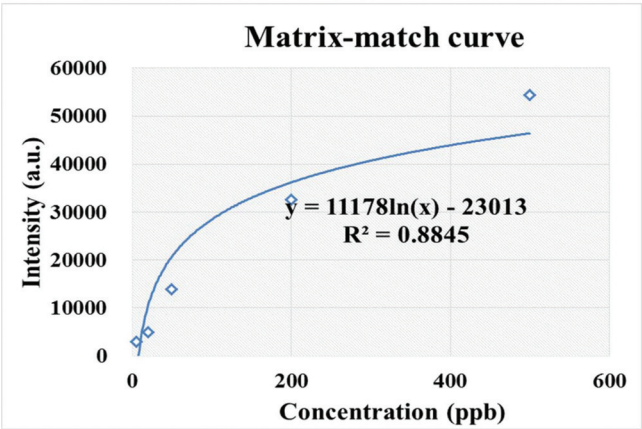

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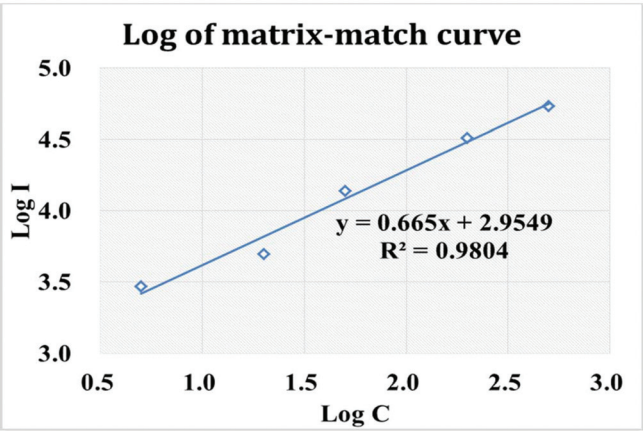

Conc. (ppb)	Intensity (a.u.)	log C	log I
5	2963	0.6990	3.4717
20	4976	1.3010	3.6969
50	13867	1.6990	4.1420
200	32614	2.3010	4.5134
500	54420	2.6990	4.7358
1000	113704	3.0000	5.0558

Fig. 4 Overlay spectrum and the standard curve of PQ for different concentrations. (a) SERS spectra of PQ dissolved in water from 1 ng mL^{-1} (ppb) to 1 mg L^{-1} (ppm). (b) The standard curve of the matrix matched SERS intensity as a function of the PQ concentration from 5 ng mL^{-1} (ppb) to 1 mg L^{-1} (ppm). (c) The standard curve of the log–log plot of the SERS signal intensity as a function of the PQ concentration from 5 ng mL^{-1} (ppb) to 1 mg L^{-1} (ppm), which revealed a linear relationship.

3 min, a 0.9 mL aliquot of the supernatant was mixed with 0.1 mL of extractant, then passed through a 0.45 μm syringe filter into a 2.0 mL plastic storage vial, and then directly determined *via* LC-MS/MS. The PQ values for the two samples, the milled bean powder and the whole adzuki bean, were 2.25 ng g^{-1} and 2.52 ng g^{-1} relative standard deviations (RSD) of

5% and 22.4%, respectively, for seven repetitions. This result indicated that regardless of whether the bean has been ground or intact, it did not affect the efficiency of extraction.

Moreover, the 20 g adzuki bean samples were immersed in the 20 mL PQ solution with three concentrations (1 ppm, 5 ppm and 20 ppm) for 1 hour, the sample was dried in the fume hood overnight, and finally we acquired samples with three residue levels. Then, we determined the PQ concentration of the extract following the QuPPe method: 2 g of dried adzuki bean samples immersed in 10 mL of water were extracted using methyl alcohol, and we acquired the average residue concentrations for the three samples (0.048 ppm, 0.27 ppm and 0.53 ppm, respectively). All had RSD values lower than 9.8%. To compare the efficiency of the surface extraction method, the 5 g sample prepared above was shaken in 5 mL of methyl alcohol, and the extract was eluted through SPE purification. The spectra of the extract from the three different immersed samples are shown in Fig. 5, and the PQ concentration of these three extracts was determined *via* LC/MSMS to be 0.04, 0.45 and 0.87 ppm, respectively, for the three different residue level samples with a RSD for two duplicate samples of below 7.2% (Table 1). The result showed that the simple surface extraction method was considerably stable. We assumed that the extracted samples were of the same weight (1 g) and the extractants were of the same amount (1 mL) to determine the equivalent value of the PQ extract. For instance, the equivalent concentration of the 1 g high-residue sample *via* the QuPPe method was 2.79 ppm, whereas the equivalent concentration *via* the surface extraction method was 0.87 ppm. Even though the extraction efficiency of the simple surface extraction method was one third of the efficiency of the QuPPe method, it was still sensitive enough to detect the trace PQ in the adzuki beans, which was because SERS has an excellent sensitivity with a LOD below 5 ppb for PQ.

Since systemic pesticides tend to become incorporated into starch, the temperature, extraction solvent, and small particle size of plant tissue used to extract starch granules are crucial for achieving good extraction yields. Additionally, PQ is commonly used to exfoliate bean pods during the harvest in adzuki bean fields; therefore, most of the PQ is removed when the bean pods fall off. To make the detection limit meet the tolerance requirement of the toxicological risk assessment, which is lower than sub ppm, it has been thought that it is necessary to perform several hours of extraction and only take sensitive LC/MSMS measurements with a regular analysis instrument. Kolberg *et al.*[17] indicated that heat should be used to speed up the extraction kinetics, which aids in the destruction of noncovalent interactions and the relaxation of the starch-rich matrix. Unlike other cereals with high-fiber contents, adzuki beans and various other legumes are abundant in starch. Starch molecules are arranged in granules, and have a distinctive range of granular starch sizes with each plant variety (*e.g.*, legumes are 16–21 μm). With the penetration of water into the starch grain, the breakdown of hydrogen bonds between the starch strains can cause the process termed gelatinization, accompanied by an increased temperature, which induces more water penetration into the starch granules, relaxes the semicrystalline structure, and makes the smaller amylose molecules leach out. Various types of starches have different gelatinization temperatures that are characteristic of the plant variety (*e.g.*, 60 °C to 90 °C for legumes, 62.5 °C for potato starch, and 67.5 °C for wheat starch).[43] Compared to the method described by Kolberg *et al.*,[17] the method used in this study did not require a heat-assisted step to elevate the extraction efficiency, and still met the sensitivity requirement for the limit. Thus, this method was more streamlined and applicable for the determination of PQ in adzuki beans.

In addition to heat aids for the extraction procedure, the clean-up assay is typically accomplished with the time-consuming procedure of solid-phase extraction. One method described by Anderson and Boseley[15] was the significant reference method, which involved refluxing over 5 hours with 0.6 M sulfuric acid and enrichment on a cation exchange resin. This time-consuming and laborious procedure is unsuitable for screening in the field. Zou *et al.* (2015)[44] developed a method for detecting PQ *via* LC/MSMS using an ultrasonic cleaner to promote sample extraction and weak cation exchange (WCX) cartridges for sample purification in which 2% TFA was selected as the final eluting solution due to its stronger acidity and significantly better eluting efficiency than formic acid. Grey *et al.* (2002) have illustrated the effect of pH on the extraction efficiency of PQ and diquat.[45] As the water/methanol (v/v: 1/1) solution became more alkaline (pH = 5, 7, 8.5, and 10), the recovery yield reduced from 80% to 40%. The adverse effect of alkaline pH can also be seen in our experiment results (Fig. 6a). When NaOH was added into the extractant, methanol/H_2O (v/v: 1/1) with 1% formic acid, to elevate the pH value, the intensity of the SERS signal of PQ solution purified by SPE was decreased. Furthermore, we found an interesting phenomenon that the SERS intensity of 0.1 ppm PQ solution at pH = 7 gradually decreases accompanied by the irradiation time in the measurement process (Fig. 6b). It is speculated that a sodium ion could compete with the adsorption of PQ for Ag particles on SERS. When the solution is just dropped on the wafer, the sodium ion is suspended, and the PQ becomes more capable of adsorbing Ag particles. As the solution gradually evaporates, the amount of sodium ion precipitated in the Ag particles also increases, which disturbs PQ from receiving incident light and scattering.

This result indicated that the higher pH solution was not suitable for extracting PQ. Therefore, Kolberg *et al.* (2012)[17] took aqueous methanol containing formic acid (actual pH = 2.52) to extract PQ in crops, the reason for which may be that the pH is quite acidic as 0.1 M of H_2SO_4 (theoretical pH = 0.7) and HCl (theoretical pH = 1) aqueous methanol solution were used, so the extraction efficiency of these three acid aqueous methanol solutions was quite good for PQ. Moreover, formic acid is safer than H_2SO_4 and HCl for operating experiments.

Replacing the usage of corrosive acids, such TFA, as the eluting buffer, we used 1% FA in MeOH/H_2O for eluting in the SPE column, which is safer and more suitable for field monitoring. In this study, SPE with PSA and $MgSO_4$ was utilized as

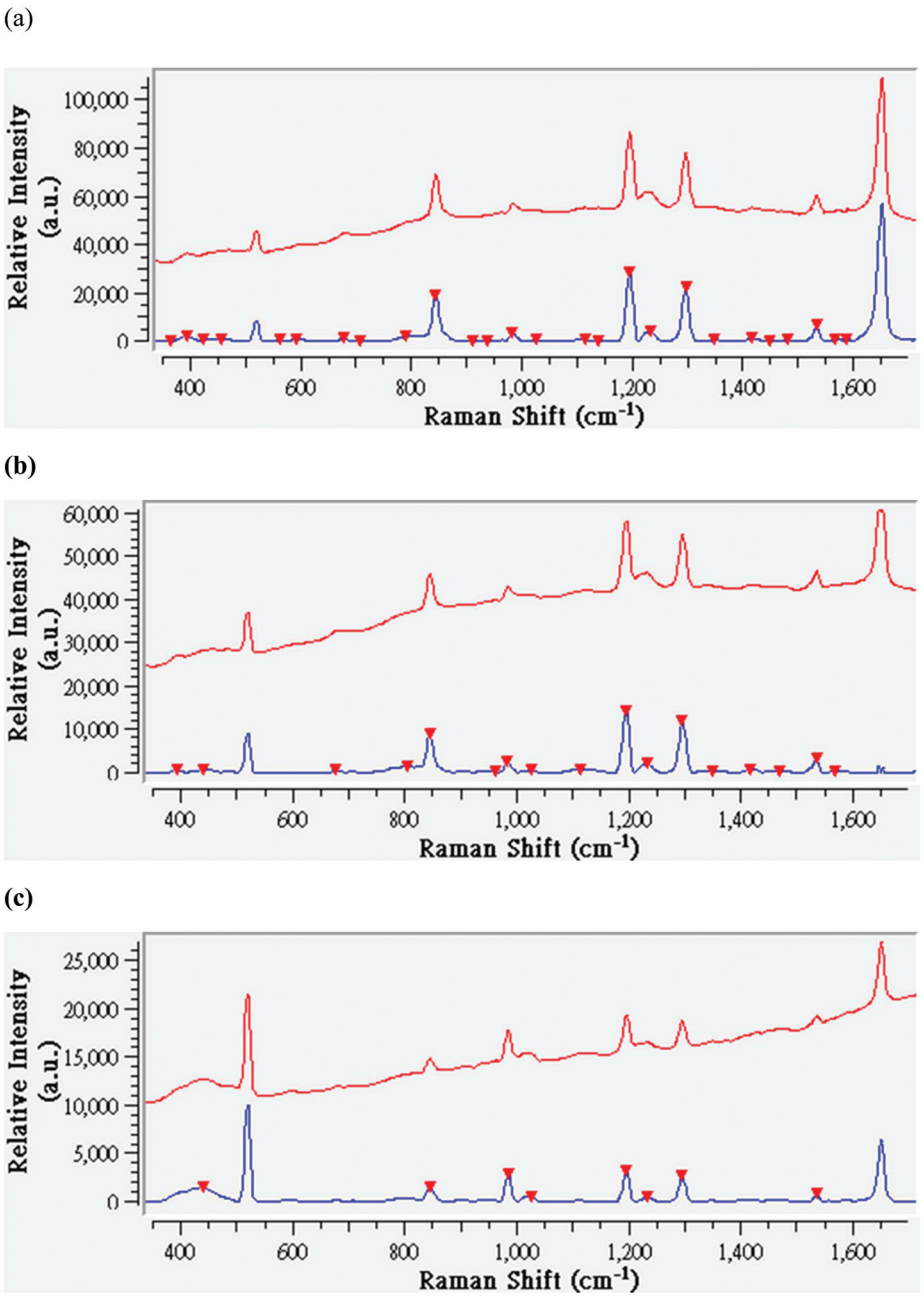

Fig. 5 The Raman spectra with normalization of the extract of whole beans immersed in the PQ solution. PQ solutions were at concentrations of (a) 10 mg L^{-1}, (b) 5 mg L^{-1}, and (c) 1 mg L^{-1}. Red line: Raman spectra; blue line: normalization.

the extraction and purification kit for chemical residue detection. In spite of the small amount of $MgSO_4$ mainly assisting in moisture absorption to avoid trace water from the plant extract that will affect the ionization efficiency of the gas chromatography system, the trace amount of $MgSO_4 \cdot 7H_2O$ will co-elute with the major extractant, aqueous methanol solution, and this heptahydrate sulfate mineral epsomite was the enhancer of SERS signals of PQ. In this study, PQ was extracted on the surface of the whole adzuki bean by shaking with mild acidic methanol solution without long-time heating and strong acid, which was sufficient to determine the PQ levels below the limit.

3.5. The incurred residue of the prepared sample

From the section above, the adzuki bean samples immersed in the PQ solution with three different concentrations (1, 5 and 10 ppm) were prepared as residue samples for validation. Then, the PQ residue concentrations in three level-immersed samples were 0.27, 1.50, and 2.79 ppm (Table 1), respectively, which shows that the adzuki beans immersed in equal weights

Table 1 Average values and RSDs for PQ determination in adzuki beans immersed in 1, 5, and 10 ppm PQ *via* the QuPPe or optimized extraction method

The PQ solution (20 mL) for the sample (20 g) immersed	PQ value *via* QuPPe	PQ value in the extract *via* simple extraction		
	Average value (mg kg^{-1}) determined by LC/MS/MS (RSD) (n = 3)	Determined by LC/MS/MS[a] (mg L^{-1})	Determined by SERS calibration (mg L^{-1})	SERS signal intensity (a.u.)
1 ppm	0.27 (9.2%)	0.04	0.037	5000
			0.027	8000
5 ppm	1.50 (7.2%)	0.45	0.304	40 000
			0.427	50 000
			0.328	30 000[b]
10 ppm	2.79 (6.6%)	0.87	0.864	80 000
			0.933	60 000[b]

[a] 5 g sample in 5 mL extractant, analyzed by LC/MS/MS. [b] 3 g sample in 5 mL extractant (1% FA in MeOH/water).

of PQ solution for 1 hour will absorb approximately 30% PQ from the solution on the bean surface. Thus, the PQ residue on dried adzuki beans immersed in 1 ppm of PQ solution for one hour is approximately 0.3 ppm. We compared this result with the immersion experiment of Zou *et al.* (2015),[44] which indicated that cabbage and lettuce immersed in a PQ solution for one hour will absorb PQ until the accumulated concentration is equal to that of the immersion solution *via* active transport and continues to absorb PQ and reaches a plateau after 3 hours. It is known that the seed coat structure of adzuki beans does not have a stomatal or active transport mechanism like vegetables to transport PQ into the inner structure, such as an endosperm or embryo. However, most PQ will tightly bind to the tissue immediately when it contacts the seed coat. Therefore, this could explain why the PQ residue of the adzuki beans immersed in the 20 mg L^{-1} PQ solution was the same as for the beans immersed in the 10 mg L^{-1} PQ solution. This was the result of PQ saturation for binding the seed coating of the beans. Except for the saturation, we can reasonably assume that most of the PQ residues are on the surface of the adzuki beans, and a real residue level acquired from the extract concentration is based on the relationship of extraction efficiency.

3.6. Validation of precision and accuracy in the optimized method

In order to verify the reliability of the SERS detection method, the prepared samples as section 3.5 with three incurred residues, over or below the limit, were taken for validation of the SERS assay. Adzuki bean samples immersed in the PQ solution of three different concentrations, 1, 5 and 10 ppm, incurred the residue of 0.27, 1.50, and 2.79 ppm (Table 1), respectively, determined by LC/MS/MS *via* the QuPPe method. Two samples that were immersed in a low concentration (in 1 ppm solution) were tested *via* simple surface extraction and SPE purification. The PQ value for the extract was calculated *via* the SERS matrix-matched calibration curve. The result showed that the Raman spectroscopy signal intensity of the samples immersed in a low concentration was lower than 10 000. According to the log–log calibration curve of the matrix-matched standard, the acquired concentrations of the two samples were 0.027 and 0.037 ppm, which were quite close to the average value of 0.040 ppm *via* LCMSMS analysis.

Additionally, two samples that were immersed in the 5 ppm solution with the residue of 1.5 ppm were selected to test whether SERS can identify the sample with a residue exceeding the maximum residue limit of 1.0 ppm for dried beans (as determined by the Australian government). After surface extraction and SPE purification, the PQ values of the extracts of these two samples calculated *via* the SERS calibration curve were 0.304 and 0.427 ppm. Considering that the signal intensity was close to the upper limit of the calibration curve, we downscaled the sample weight to 3 g as a dilution to check the value additionally and acquired the value of 0.328 ppm. All three values were not obviously different from the value of 0.45 ppm determined by LC/MSMS. The PQ value of the sample immersed in 10 ppm of solution determined *via* SERS and surface extraction was 0.864 ppm, which was over the Raman spectroscopy signal intensity upper limit (0.5 ppm) in the calibration curve. Thus, we downscaled the sample weight to 3 g as a dilution to fit the curve of the Raman signal inten-

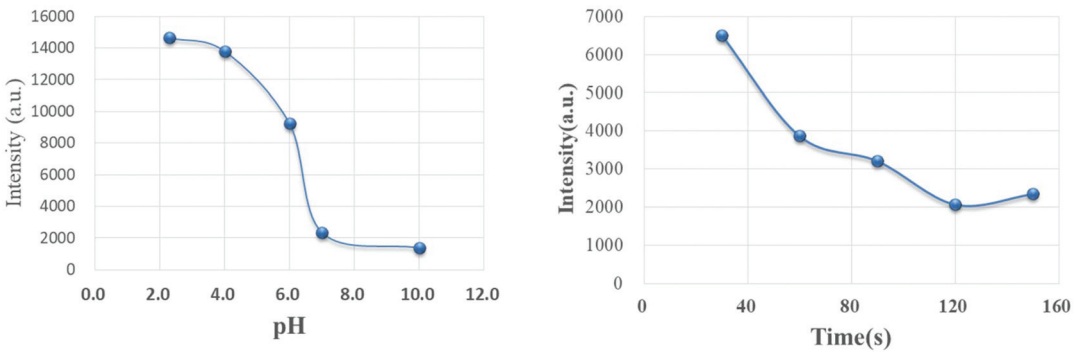

Fig. 6 (a) Raman signal intensity of SERS for 0.1 ppm PQ in various pH values. (b) Decay time of the signal intensity of SERS for 0.1 ppm PQ at pH 7.0.

sity and acquire the value of 0.933 ppm; both the SERS values, 0.864 and 0.933 ppm, were quite close to the analysis value of 0.865 ppm as determined *via* LC/MSMS.

According to the validation results, the SERS quantitative results of the adzuki bean extract presented a fairly reliable consistency with the quantitative results of LC/MSMS. We can further derive the residual PQ concentration in beans from the concentration of the surface extract. The PQ concentration of the extract, with the equal weight of the beans, is multiplied by ten times to be the PQ residue in the adzuki bean.

4. Conclusion

In this study, we developed an on-site detection strategy for PQ in adzuki beans using simple extraction coupled with SERS. The simple extraction includes shaking the whole beans without grinding and purifying the extract *via* SPE containing PSA and $MgSO_4$. The composition of the SPE effectively reduced the signal interference for high polarity chemicals because most nonpolar or low-polar pesticides do not dissolve well in polar extractants such as water or methanol. The concentration of the extract can be quantified by SERS, comprising one layer of Ag nanopillars with non-periodical structures and configuration parallel to a normal line, which is capable of enhancing Raman signals. The overall procedures only took approximately 10 minutes. We can characterize PQ molecules through the four characteristic peaks and quantify them by the characteristic peak at 1648 cm^{-1}. The sensitivity of AgNP SERS achieves a limit of detection (LOD) for PQ on the order of 1 μg L^{-1} ($\sim 4 \times 10^{-9}$ M), which was enhanced by the co-eluent of SPE and suitable for identifying agriculture products with PQ residues exceeding the allowed limit. Selecting the peak with the highest intensity (1648 cm^{-1}) in the SERS spectra to plot as a function of the logarithms of the PQ concentration ($\log C$) and the Raman spectrum ($\log I$), a log–log (Raman intensity *vs.* PQ concentration) calibration line was plotted for quantification between 5 and 500 ng mL^{-1} for the PQ quantitative assay. Regardless of whether the type of bean was ground or intact, it did not affect the extract efficiency. Hence, we took the whole adzuki beans for the extract by only simple surface shaking and SPE purification in this method, which showed reliable reproducibility. The PQ values of three residue level samples were consistent between our developed method and the QuPPe method. The strategy for PQ detection on-site achieved a good linear calibration range, sensitivity and reproducibility, which were significant factors for practical SERS application. This AgNP SERS-based PQ detection method was quick, inexpensive, eco-friendly and safe, so it provided an alternative approach for screening PQ residue in farm fields.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

Conflicts of interest

All authors declare no conflicts of interest.

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References

1 M. Anastassiades, S. J. Lehotay, D. Stajnbaher and F. J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, *J. AOAC Int.*, 2003, **86**(2), 412–431.
2 J. R. Startin, S. J. Hird, M. D. Sykes, J. C. Taylor and A. R. C. Hill, *Analyst*, 1999, **124**(7), 1011–1015.
3 M. Anastassiades, D. I. Kolberg, D. Mack, I. Sigalova, D. Roux and D. Fügel, EU Reference Laboratories for Residues of Pesticides, QuPPe-Method-Version 6, 2011, available at: http://www.eurl-pesticides.eu.
4 M. A. Aramendía, V. Borau, F. Lafont, A. Marinas, J. M. Marinas, J. M. Moreno, J. M. Porras and F. Urbano, *J. Food Chem.*, 2006, **97**(1), 181–188.
5 S. Riediker, H. Obrist, N. Varga and R. H. Stadler, *J. Chromatogr. A*, 2002, **966**(1–2), 15–23.
6 M. Vahl, A. Graven, R. K. Juhler and J. Fresenius, *Anal. Chem.*, 1998, **361**(8), 817–820.
7 X. Esparza, E. Moyano and M. T. Galceran, *J. Chromatogr. A*, 2009, **1216**(20), 4402–4406.
8 L. Grey, B. Nguyen and P. Yang, *J. Chromatogr. A*, 2002, **958**(1–2), 25–33.
9 M. Pateiro-Moure, E. Martínez-Carballo, M. Arias-Estévez and J. Simal-Gándara, *J. Chromatogr. A*, 2008, **1196–1197**, 110–116.
10 C. S. Evans, J. R. Startin, D. M. Goodall and B. J. Keely, *J. Chromatogr. A*, 2000, **897**(1–2), 399–404.
11 S. Riediker, H. Obrist, N. Varga and R. H. Stadler, *J. Chromatogr. A*, 2002, **966**(1–2), 15–23.
12 J. R. Startin, S. J. Hird, M. D. Sykes, J. C. Taylor and A. R. C. Hill, *Analyst*, 1999, **124**(7), 1011–1015.
13 M. H. Peeters, I. Defloor, J. Coosemans, J. A. Delcour, L. Ooms, R. Deliever and D. De Vos, *J. Chromatogr. A*, 2001, **920**(1–2), 255–259.
14 E. C. Guijarro, P. Yanez-Sedeno and L. M. Polo Diez, *Anal. Chim. Acta*, 1987, **199**, 203–208.
15 L. Anderson and A. D. Boseley, *ZENECA Agrochemicals SOP RAM 272/02*, 1997.

16 M. Vahl, A. Graven, R. K. Juhler and J. Fresenius, *Anal. Chem.*, 1998, **361**(8), 817–820.
17 D. I. S. Kolberg, D. Mack, M. Anastassiades, M. T. Hetmanski, R. J. Fussell, T. Meijer and H. G. J. Mol, *Anal. Bioanal. Chem.*, 2012, **404**(8), 2465–2474.
18 M. Anastassiades, D. I. Kolberg, E. Eichhorn, A. Benkenstein, S. Lukačević, D. Mack, *et al.*, *Quick method for the analysis of residues of numerous highly polar pesticides in foods of plant origin involving simultaneous extraction with methanol and LC-MS/MS determination(QuPPe-Method), Version 8.1*, EU Reference Laboratory for pesticides requiring single residue methods(EURL-SRM), Fellbach, Germany, 2015.
19 V. Y. Taguchi, S. W. D. Jenkins, P. W. Crozier and D. T. Wang, Determination of diquat and paraquat in water by liquid chromatography-(electrospray ionization) mass spectrometry, *J. Am. Soc. Mass Spectrom.*, 1998, **9**(8), 830–839.
20 K. C. Wang, S. M. Chen, J. F. Hsu, S. G. Cheng and C. K. Lee, Simultaneous detection and quantitation of highly water-soluble herbicides in serum using ion-pair liquid chromatography-tandem mass spectrometry, *J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci.*, 2008, **876**(2), 211–218.
21 M. C. Carneiro, L. Puignou and M. T. Galceran, Comparison of capillary electrophoresis and reversed-phase ion-pair high-performance liquid chromatography for the determination of paraquat, diquat and difenzoquat, *J. Chromatogr. A*, 1994, **669**(1–2), 217–224.
22 J. W. Munch and W. J. Bashe, *Method 549.2: Determination of diquat and paraquat in drinking water by Liquid-Solid Extraction and High-Performance Liquid Chromatography with ultraviolet detection; U.S. Environmental Protection Agency*, Environmental Monitoring and Support Laboratory, Cincinnati, OH, 1997.
23 C. Hao, X. Zhao, D. Morse, P. Yang, V. Taguchi and F. Morra, Optimized liquid chromatography tandem mass spectrometry approach for the determination of diquat and paraquat herbicides, *J. Chromatogr. A*, 2013, **1304**, 169–176.
24 P. Chuntib and J. Jakmunee, Simple flow injection colorimetric system for determination of paraquat in natural water, *Talanta*, 2015, **144**(29), 432–438.
25 S. Weena, S. Thachkor, C. Orawan and S. Kriangsak, *Talanta*, 2017, **174**, 448–453; W. Siangproh, T. Somboonsuk, O. Chailapakul and K. Songsrirote, Novel colorimetric assay for paraquat detection on-silica bead using negatively charged silver nanoparticles, *Talanta*, 2017, **174**(20), 448–453.
26 J. Vongsvivut, E. G. Robertson and D. McNaughton, Surface-enhanced Raman spectroscopic analysis of fonofos pesticide adsorbed on silver and gold nanoparticles, *J. Raman Spectrosc.*, 2010, **41**(10), 1137–1148.
27 L. Guerrini, S. Sanchez-Cortes, V. L. Cruz, S. Martinez, S. Ristorib and A. Feis, Surface-enhanced Raman spectra of dimethoate and omethoate, *J. Raman Spectrosc.*, 2011, **42**(5), 980–985.
28 Y. F. Xie, G. Mukamurezi, Y. Y. Sun, H. Y. Wang, H. Qian and W. R. Yao, Establishment of rapid detection method of methamidophos in vegetables by surface enhanced Raman spectroscopy, *Eur. Food Res. Technol.*, 2012, **234**(6), 1091–1098.
29 M. L. Roldán, G. Corrado, O. Francioso and S. Sanchez-Cortes, *Anal. Chim. Acta*, 2011, **699**(1), 87–95.
30 R. Gao, N. Choi, S. I. Chang, S. H. Kang, J. M. Song, S. I. Cho, D. W. Lim and J. Choo, Highly sensitive trace analysis of paraquat using a surface-enhanced Raman scattering microdroplet sensor, *Anal. Chim. Acta*, 2010, **681**(1–2), 87–91.
31 H. Fang, X. Zhang, S. Zhang, L. Liu, Y. M. Zhao and H. J. Xu, Ultrasensitive and quantitative detection of paraquat on fruits skins via surface-enhanced Raman spectroscopy, *Sens. Actuators, B*, 2015, **213**(5), 452–456.
32 Q. Q. Li, Y. P. Du, H. R. Tang, X. Wang, G. P. Chen, J. Iqbal, W. M. Wang and W. B. Zhang, Ultra sensitive surface-enhanced Raman scattering detection based on monolithic column as a new type substrate, *J. Raman Spectrosc.*, 2012, **43**, 1392–1396.
33 J. D. Driskell, S. Shanmukh, Y. Liu, S. B. Chaney, X.-J. Tang, Y.-P. Zhao and R. A. Dluhy, The use of aligned silver nanorod arrays prepared by oblique angle deposition as surface enhanced Raman scattering substrates, *J. Phys. Chem. C*, 2008, **112**, 895–901.
34 K.-i. Yoshida, T. Itoh, H. Tamaru, V. Biju, M. Ishikawa and Y. Ozaki, Quantitative evaluation of electromagnetic enhancement in surface-enhanced resonance Raman scattering from plasmonic properties and morphologies of individual Ag nanostructures, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 2010, **81**, 115406.
35 J. Yu and J. G. Amar, Dynamical scaling behavior in two-dimensional ballistic deposition with shadowing, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2002, **66**, 021603.
36 S. K. Srivastava, A. Shalabney, I. Khalaila, C. Grüner, B. Rauschenbach and I. Abdulhalim, SERS biosensor using metallic nano-sculptured thin films for the detection of endocrine disrupting compound biomarker vitellogenin, *Small*, 2014, **10**, 3579–3587.
37 H. R. Tang, Q. Q. Li, Y. L. Ren, J. P. Geng, P. Cao, T. Sui, X. Wang and Y. P. Du, Surfaceenhanced Raman spectroscopy signals of mixed pesticides and their identification, *Chin. Chem. Lett.*, 2011, **22**(12), 1477–1480.
38 T. C. Dao, T. Q. N. Luong, T. A. Cao, N. H. Kieu and V. V. Le, Application of silver nanodendrites deposited on silicon in SERS technique for the trace analysis of paraquat, *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, 2016, **7**(1), 015007.
39 A. D. Strickland and C. A. Batt, Detection of carbendazim by surface-enhanced Raman scattering using cyclodextrin inclusion complexes on gold nanorods, *Anal. Chem.*, 2009, **81**(8), 2895–2903.
40 F. A. Ibrahim and J. J. M. Nasr, Direct determination of ampicillin and amoxicillin residues in food samples after aqueous SDS extraction by micellar liquid chromatography with UV detection, *Anal. Methods*, 2014, **6**(5), 1523–1529.

41 T. H. D. Nguyen, Z. Zhang, A. Mustapha, H. Li and M. Lin, Use of graphene and gold nanorods as substrates for the detection of pesticides by surface enhanced Raman spectroscopy, *J. Agric. Food Chem.*, 2014, **62**(43), 10445–10451.

42 C. G. Wang, X. Z. Wu, P. T. Dong, J. Chen and R. Xiao, Hotspots engineering by grafting Au@Ag core-shell nanoparticles on the Au film over slightly etched nanoparticles substrate for on-site paraquat sensing, *Biosens. Bioelectron.*, 2016, **86**, 944–950.

43 J. A. Radley, In *Starch and its derivatives*, Chapman and Hall, London, 1968, vol. 2, pp. 311–348.

44 T. Zou, P. He, J. Cao and Z. Li, Determination of paraquat in vegetables using HPLC-MS-MS, *J. Chromatogr. Sci.*, 2015, **53**(2), 204–209.

45 L. Grey, B. Nguyen and P. Yang, liquid chromatography–electrospray ionization isotope dilution mass spectrometry analysis of paraquat and diquat using conventional and multilayer solid-phase extraction cartridges, *J. Chromatogr. A*, 2002, **958**, 25–33.

