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Article

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Low-Cost Gold Nanoparticle-Coated Aluminium Foil for SERS Detection of Chlorpyrifos in Honey

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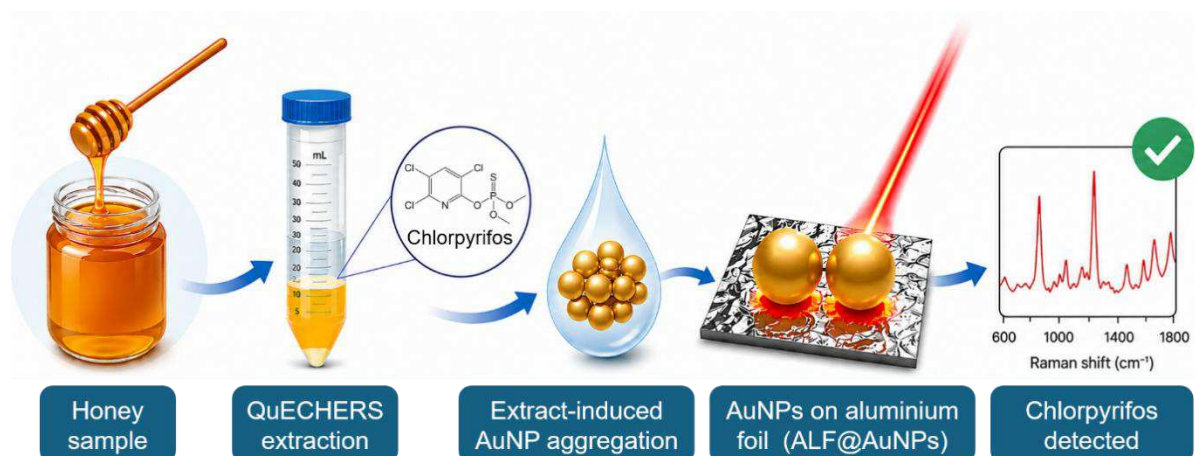
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Abstract

Rapid, low-cost detection of pesticide residues in honey remains challenging because its complex, sugar-rich matrix limits the performance of portable analytical techniques. Here, we integrate QuEChERS extraction with a surface-enhanced Raman spectroscopy (SERS) platform based on gold nanoparticles (AuNPs) deposited on commercial aluminium foil (ALF) to enable sensitive detection of chlorpyrifos (CPY) in honey. Aluminium foil coated with unmodified gold nanoparticles (ALF@AuNP) demonstrated superior Raman enhancement owing to plasmonic coupling, as confirmed by both simulations and experimental data. Coupling QuEChERS with the ALF@AuNP substrate achieved a detection limit of 4.8×10^{-7} M for chlorpyrifos for CPY while simultaneously reducing matrix interference. Residual extraction salts also promoted AuNP aggregation, increasing SERS hotspot density and improving analytical sensitivity. Principal component analysis further discriminated CPY from structurally related organophosphate pesticides in honey extracts. This simple, inexpensive and portable SERS–QuEChERS platform provides a practical approach for rapid pesticide screening in complex food matrices.

Keywords: SERS, Chlorpyrifos, QuEChERS, Aluminium foil substrate, pesticide detection

Graphical abstract



Schematic illustration of the QuEChERS-based extraction of chlorpyrifos (CPY) from honey and subsequent gold nanoparticle (AuNP) aggregation for trace detection on commercial aluminium foil.

Introduction

Blossom honey is a high-value natural food produced by honeybees from floral nectar, with a complex and highly variable composition influenced by botanical origin and environmental conditions. However, honeybees are frequently exposed to pesticides due to the proximity of their hives to treated agricultural fields, direct contact during foraging, or in-hive applications by beekeepers for mite control. These exposures can lead to the accumulation of pesticide residues in honeycombs, which may pose chronic health risks to bees and humans, even at low concentrations¹⁻⁴. Among these contaminants, chlorpyrifos (CPY), a widely used organophosphate insecticide, is of particular concern because of its neurotoxicity and adverse effects on both humans and honeybees^{5,6}. Although its agricultural use has been banned within the European Union since 2020, CPY continues to be applied extensively in many non-EU countries⁷. Given the EU's reliance on imported honey—approximately 208,000 tonnes annually, valued at €452 million, monitoring CPY residues in honey is essential for consumer safety⁸.

Reliable determination of pesticide residues in honey remains analytically challenging because of its highly viscous, sugar-rich matrix. Current analytical methods typically combine sample extraction with gas or liquid chromatography coupled to mass spectrometry^{1,9,10}. While providing excellent sensitivity and accuracy, these techniques require expensive instrumentation, skilled operators and time-consuming sample preparation, meaning they are heavily reliant on laboratory infrastructure and unsuitable for rapid or on-site screening. To mitigate the limitations of sample preparation, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) has emerged as one of the most widely adopted extraction methods, offering high efficiency while consuming considerably less solvent than conventional liquid-liquid extraction¹¹⁻¹³. Although QuEChERS is routinely coupled with GC-MS or LC-MS/MS for residue determination, these combinations still depend on centralised laboratory infrastructure¹³⁻¹⁵. Consequently, there remains a need to integrate QuEChERS with portable detection technologies capable of providing rapid screening without sacrificing analytical performance.

Surface-enhanced Raman spectroscopy (SERS) is a powerful, non-destructive technique that addresses this need by providing rapid molecular fingerprinting with ultrahigh sensitivity, typically enhancing Raman signals by factors of 10^6 to 10^{15} through the interaction of target molecules with plasmonic nanostructures^{16,17}. Its specificity, low operating cost, and minimal sample preparation requirements make it highly attractive for pesticide screening. For example, gold nanoparticle (AuNP)-coated paper and adhesive tape substrates have enabled CPY detection on fruit surfaces at sub-nanomolar levels^{18,19}.

Recent studies have demonstrated synergistic benefits of coupling QuEChERS extraction with SERS²⁰. In one case, QuEChERS extraction of spiked basmati rice led to recoveries between 83.4% and 115%, with SERS achieving lower limits of detection than corresponding solvent standards. This enhancement was attributed to

salt-induced aggregation of AuNPs, which promotes the formation of SERS "hot spots" by reducing interparticle spacing²⁰. However, previous applications have largely focused on matrices such as rice and fruit surfaces, whereas pesticide detection in complex, sugar-rich matrices such as honey remains comparatively underexplored.

A critical factor in optimising SERS performance is the choice of substrate. Deposition of citrate-stabilised AuNPs onto solid supports is commonly used, and recent work has shown that coupling between the surface plasmon resonance (SPR) of AuNPs and the substrate can significantly enhance Raman signals^{21,22}. While gold and silver substrates offer strong plasmonic activity, their high-cost limits widespread use. Aluminium foil (ALF), by contrast, is an inexpensive, readily available alternative that has demonstrated SERS activity when paired with AuNPs, achieving detection limits as low as 27 ppb for melamine²³.

To address these gaps, we hypothesised that integrating QuEChERS extraction with AuNP-coated commercial aluminium foil could simultaneously minimise honey matrix interference and enhance SERS performance through salt-induced nanoparticle aggregation and plasmonic coupling with the aluminium substrate. To test this hypothesis, we compared the performance of aluminium foil-supported AuNPs with conventional silicon-supported AuNPs using both experimental measurements and electromagnetic simulations. We further evaluated the analytical performance of the platform for chlorpyrifos detection in honey and investigated its selectivity against structurally related organophosphate pesticides using principal component analysis (PCA). This work demonstrates a simple, low-cost and portable SERS platform for pesticide screening in complex food matrices.

Results

Characterisation of synthesised AuNPs on ALF substrate

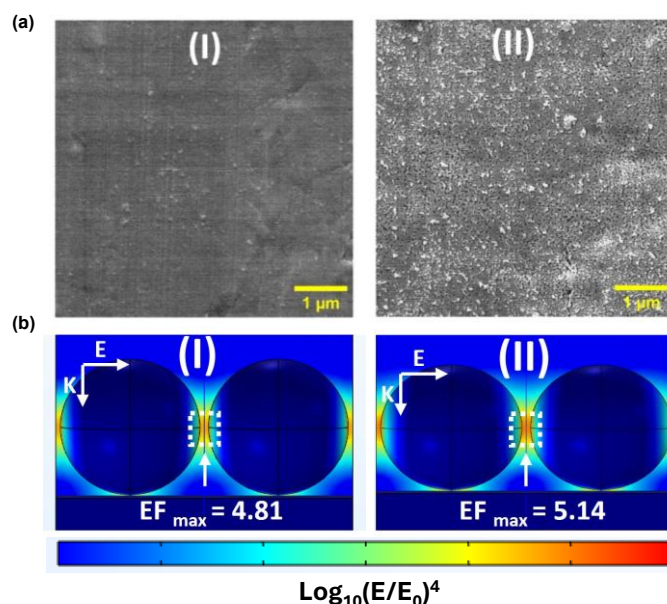


Fig. 1 (a) SEM images of (I) bare aluminium foil (ALF) and (II) ALF coated with gold nanoparticles (ALF@AuNPs), both at 1,000× magnification. (b) Computational simulations of the SERS substrates showing electromagnetic field confinement for (I) silicon (Si) and (II) aluminium foil (ALF).

To validate the effectiveness of a simple drop-casting method for depositing AuNPs onto ALF to fabricate a SERS-active substrate, SEM was performed on both uncoated ALF and ALF@AuNPs (Fig. 1a). The SEM images revealed dense and well-distributed clusters of AuNPs on the ALF@AuNPs surface (Fig. 1a.II), confirming that the straightforward deposition method yields a reproducible and cost-effective SERS substrate.

Given the inherent plasmonic activity of aluminium, third-generation SERS enhancement effects can be expected. Previous studies have demonstrated that such third-generation platforms exhibit strong electromagnetic enhancement due to plasmon coupling between metal nanoparticles and underlying plasmonic surfaces²³. However, SERS "hot spots" are highly localised, i.e. up to 80% of the signal can originate from as little as 0.64% of the analyte molecules²⁴. To better understand the influence of these localised enhancements, electromagnetic field simulations were carried out using the finite element method (FEM) in COMSOL Multiphysics for an interparticle hotspot formed between two adjacent AuNPs supported on both Si and ALF.

As shown in Fig. 1b, under a 785 nm incident laser, the maximum near-field enhancement (mNE) for the Si@AuNPs system was calculated to be 4.81 Log₁₀(E/E₀)⁴, while the ALF@AuNPs system exhibited a higher mNE of 5.14 Log₁₀(E/E₀)⁴. This increased enhancement on ALF is attributed to stronger plasmon

coupling between the AuNPs and the underlying metallic surface. These computational results are expected to correlate with the experimentally determined Raman enhancement factors presented in the following section.

To experimentally confirm this effect, the Raman and SERS performance of ALF and ALF@AuNPs were compared to that of Si and Si@AuNPs, respectively, as described in the subsequent section.

Comparison of the Raman and SERS performance between ALF@AuNPs and Si substrates

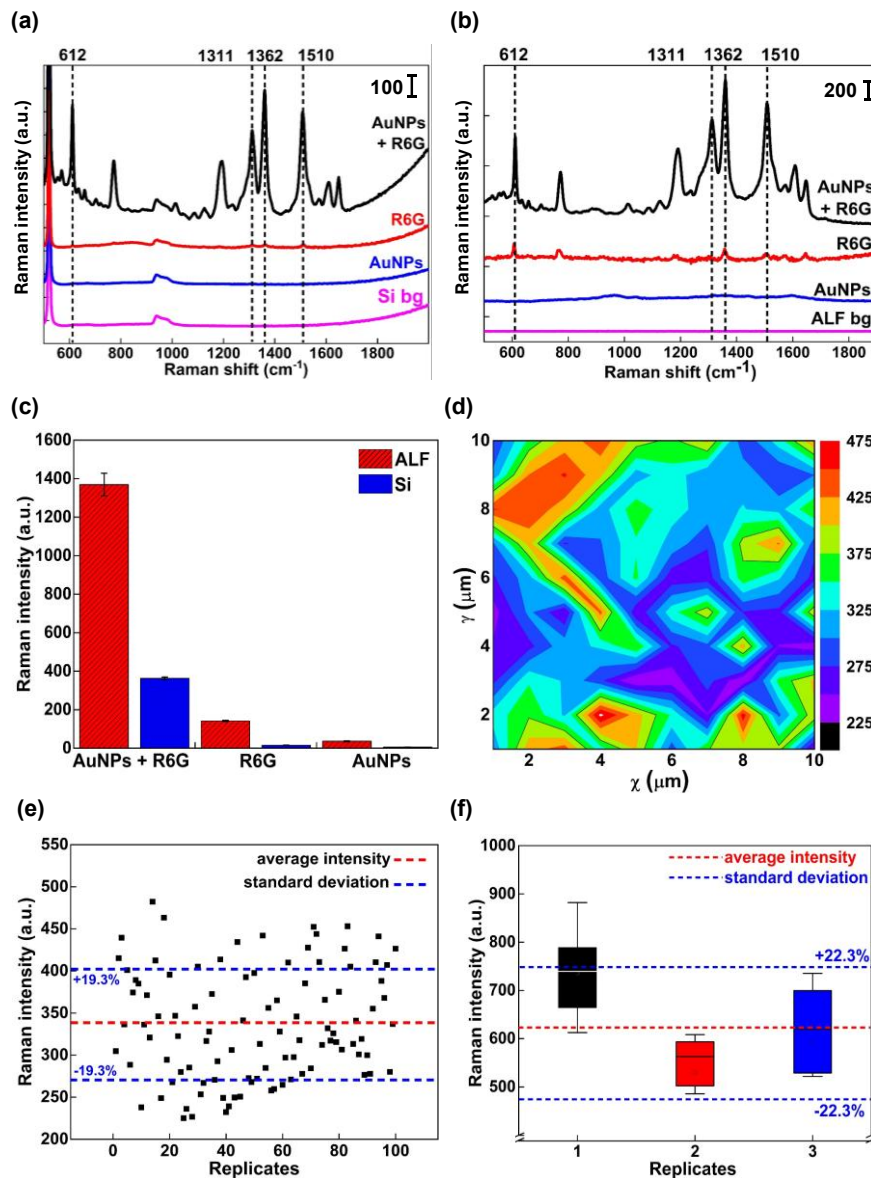


Fig. 2 Raman spectral analysis and SERS performance of Si@AuNPs and ALF@AuNPs substrates. (a): Raman spectra of Si (pink), AuNPs (blue), R6G (red), and R6G on Si@AuNPs (black). (b): Raman spectra of ALF (pink), AuNPs (blue), R6G (red), and R6G on ALF@AuNPs (black). (c): Comparison of average Raman intensity at 1362 cm⁻¹ ($n = 3$) for AuNPs, R6G, and AuNPs + R6G on Si and ALF substrates. (d): Raman mapping (10 × 10 μm², 1 μm step size) showing spatial distribution of

Raman intensity (a.u.) at 1362 cm⁻¹ for 10⁻⁵ M R6G on ALF@AuNPs. (e): Scatter plot of Raman intensities from mapping in (d), with mean intensity (red line) and relative standard deviation (blue line). (f): Box plot of Raman intensities at 1362 cm⁻¹ for 10⁻⁵ M R6G measured at 10 random points across three ALF@AuNPs substrates (η =30). Red line indicates mean; blue line shows relative standard deviation.

To compare the SERS performance of Si@AuNPs and ALF@AuNPs, rhodamine 6G (R6G), a well-characterised Raman dye, was used as a probe. As expected, R6G on a bare Si substrate, generally regarded as having negligible SERS activity, showed only weak Raman bands (Fig. 2a). In contrast, when R6G was deposited on Si@AuNPs, pronounced vibrational bands appeared at ~1362 cm⁻¹ and ~1510 cm⁻¹, corresponding to C–C and C–N stretching, respectively (full band assignments in Fig. S2 and Table S1). The peak at 1362 cm⁻¹, attributed to C–C stretching, was selected as the diagnostic peak for subsequent analyses.

Interestingly, Fig. 2b shows that the same R6G concentration (10⁻³ M) produced approximately twice the intensity on ALF@AuNPs compared with Si@AuNPs, suggesting a synergistic enhancement effect from the AuNPs and ALF. A comparative plot of average intensities at 1362 cm⁻¹ (Fig. 2c) confirms that R6G on ALF@AuNPs generated the strongest signal among tested groups (AuNPs, R6G, and AuNPs+R6G on Si and ALF). Linear dose response analysis of Raman intensity at 1362 cm⁻¹ versus R6G concentration for Si@AuNPs and ALF@AuNPs are shown in Fig. S3 and S4 respectively. Enhancement factors (EFs) were then calculated. On Si@AuNPs, the maximum EF was 5.97×10^2 , while ALF@AuNPs reached 1.94×10^4 relative to bare Si. This higher EF translated into a superior limit of detection (LOD) for R6G: 9.9×10^{-6} M on ALF@AuNPs, compared with 8.9×10^{-4} M on Si@AuNPs (Fig.S3 and Fig.S4). Thus, ALF@AuNPs improved sensitivity by nearly two orders of magnitude.

Reproducibility was also evaluated. Raman mapping of 10⁻⁵ M R6G across an ALF@AuNP surface (n=100 points) yielded a relative standard deviation (RSD) of 19.3% at 1362 cm⁻¹ (Fig. 2d–e), demonstrating acceptable intra-substrate uniformity. Measurements at 10 random locations on three separate ALF@AuNP substrates (η =30) gave an inter-substrate RSD of 22.3% (Fig. 2f), consistent with previous reports of ~20% for nanomaterials deposited on ALF²⁵.

These results reveal ALF provides an inherent enhancing effect compared with non-plasmonic Si. Coating ALF with AuNPs yields ALF@AuNPs, a simple and effective platform for producing sensitive SERS substrates with reproducibility within acceptable limits. To assess whether this enhancement translates into practical sensing, the LOD of ALF@AuNPs for chlorpyrifos (CPY) residues in solvent will be determined in the following section.

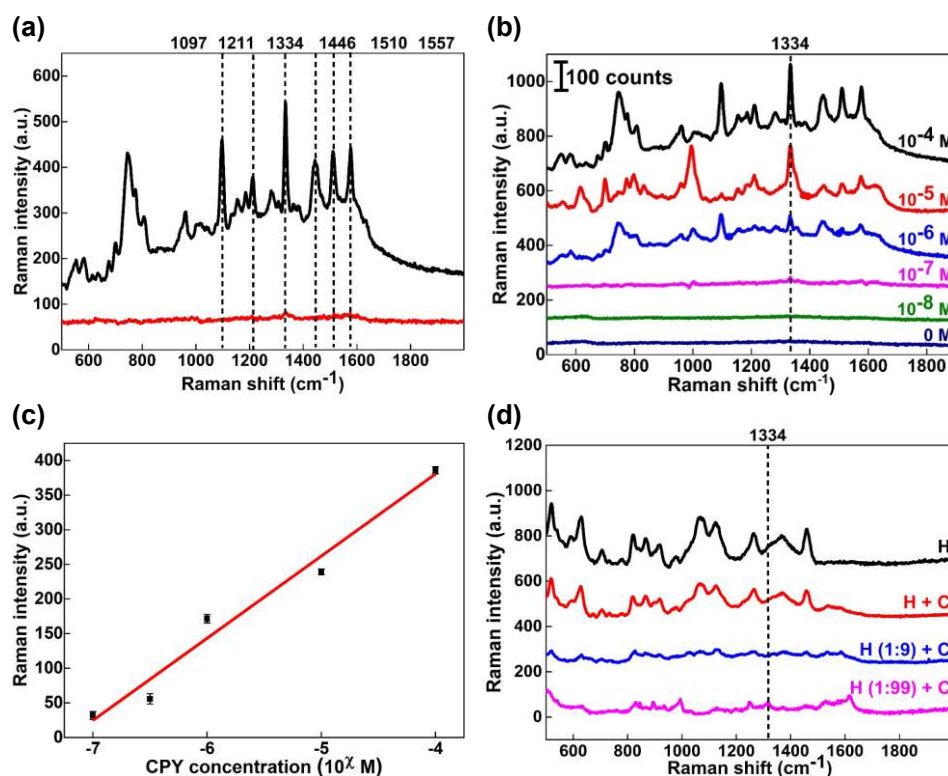


Fig. 3 (a) SERS spectrum of 10^{-4} M CPY in a 1:1 mixture of water (dH_2O) and ethanol (EtOH) on ALF@AuNPs (black line). The characteristic band assignments that do not overlap with the ALF@AuNPs background spectrum (red line), based on the CPY spectrum shown in Fig. S5 are provided in table S2. (b) SERS spectra of ALF@AuNPs with decreasing CPY concentrations (10^{-4}M to 10^{-8}M , black line to green line, respectively) in a 1:1 dH_2O :EtOH mixture. (c) Linear relationship between the SERS intensity at the 1334 cm^{-1} CPY diagnostic peak and CPY concentration on ALF@AuNPs. The standard deviation (σ) was calculated from triplicate samples. (d) SERS spectra on ALF@AuNPs for plain honey (H) (black line), honey spiked with 10^{-5} M CPY (H + C) (red line), honey spiked with 10^{-4} M CPY diluted in water in a 1:9 ratio (H (1:9) + C) (blue line) and honey spiked with 10^{-4} M CPY diluted in water in a 1:99 ratio (H (1:99) + C) (purple line) on ALF@AuNPs.

Initially, CPY was dissolved in a 1:1 mixture of water (dH_2O) and ethanol (EtOH), prior to characterisation on the ALF@AuNPs substrate. The resulting spectrum (Fig. 3a) revealed characteristic peaks, which are fully characterised in table S.2. The peak at the Raman shift of approximately 1334 cm^{-1} , corresponding to Cl-ring, σ (C-H), Vas (C=C), was selected as the diagnostic peak for CPY due to its consistently high Raman intensity.

Evaluation of varying CPY concentrations on ALF@AuNPs yielded a LOD of 5.2×10^{-6} M in a simple solvent. The SERS spectra for this concentration range are displayed in Fig. 3b, while the corresponding calibration curve at 1334 cm^{-1} is

presented in Fig. 3c. While these results were promising, the LOD exceeds the EU MRL for CPY in food (2.85×10^{-8} M) by over 2 orders of magnitude²⁶. The limited sensitivity is most likely explained by concentration dependant aggregation. While higher concentrations of CPY induce AuNP aggregation, thereby increasing SERS hot spot density and amplifying the SERS signal, this aggregation does not occur at lower concentrations. The limited aggregation reduces the density of hot spots formed and limits the LOD, a phenomenon supported by the UV-vis spectra in Fig. S6.

The challenge became more pronounced in a complex sample matrix. Direct spiking of 10^{-5} M CPY into honey required two successive serial dilutions to reduce matrix masking of the CPY signal (Fig. 3d), but these dilutions further decreased the analyte concentration available for SERS detection. To overcome this matrix interference and improve the practical utility of the ALF@AuNP platform, the sensor was subsequently coupled with QuEChERS extraction. It was hypothesised that QuEChERS would both reduce matrix suppression and introduce extraction salts capable of promoting AuNP aggregation even at low CPY concentrations, thereby improving the overall detection sensitivity.

Assessing the effectiveness of ALF@AuNPs coupled with QuEChERS extraction for the detection of CPY residues in honey

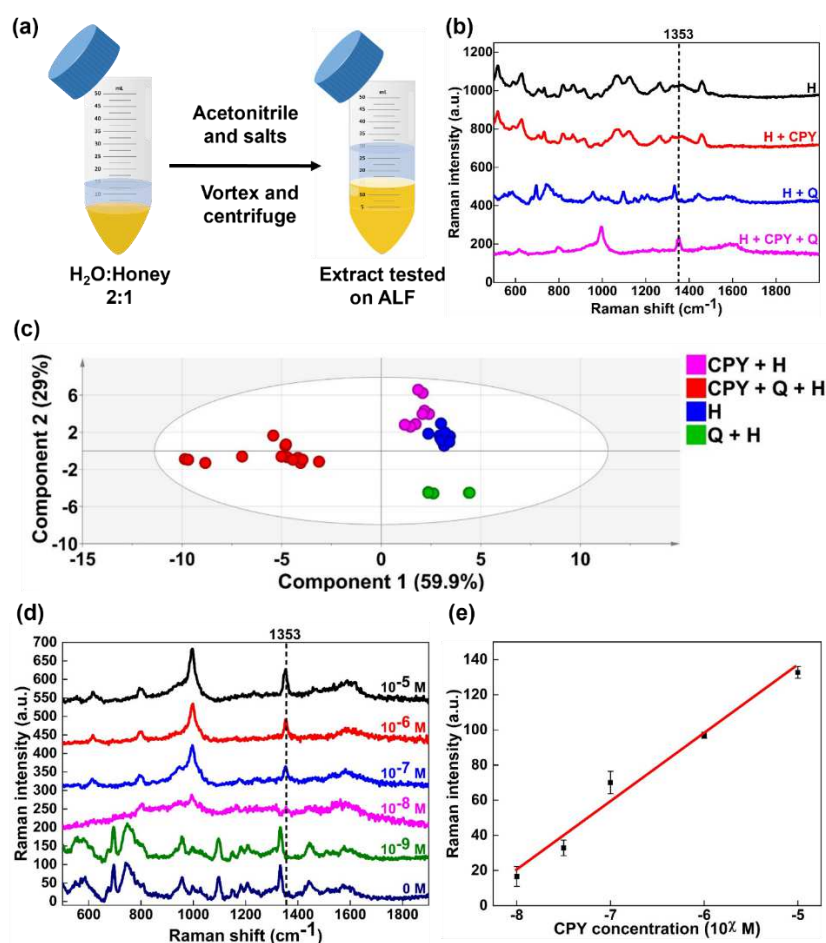


Fig. 4 (a) a schematic illustrating the QuEChERS extraction of CPY from honey, (b) SERS spectra on ALF@AuNPs for plain honey (H, black line), honey spiked with 10^{-5}

M CPY (H + C, red line), plain honey following QuEChERS extraction (H + Q, blue line), and honey spiked with 10^{-5} M CPY following QuEChERS extraction (H + CPY + Q, purple line) (c) PC1 vs PC2 scores plot summarising the principal components of spectra collected on ALF@AuNPs for plain honey (H), honey spiked with 10^{-5} M CPY (CPY + H), plain honey post QuEChERS extraction (Q + H), and honey spiked with 10^{-5} M post QuEChERS extraction (CPY + Q + H). (d) SERS spectra on ALF@AuNPs showing decreasing concentrations of CPY (10^{-5} M to 10^{-9} M, black line to navy line, respectively extracted from honey using QuEChERS (e) Linear relationship between SERS intensity at the 1353 cm^{-1} diagnostic peak and the concentration of extracted CPY on ALF@AuNPs. The standard deviation (σ) was calculated from triplicate samples.

Fig. 4a illustrates the QuEChERS–SERS workflow. To evaluate the QuEChERS efficacy, honey spiked with 10^{-5} M CPY was analysed pre- and post-extraction using the ALF@AuNP platform (Fig. 4b). The concentration-dependent spectra and calibration relationship are shown in Fig. 4d. and Fig. 4e., respectively. Compared with un-spiked honey, the diagnostic peak for CPY was clearly discernible, shifting slightly shift from 1333 cm^{-1} to 1353 cm^{-1} likely due to extraction conditions. Subsequently, a PCA was performed ($\eta > 30$) demonstrated clear clustering and separation between CPY-contaminated and non-contaminated honey, both pre- and post- extraction (Fig. 4c). The primary principal components accounted for a cumulative variance of 88.9% (PC1 = 59.9%, PC2 = 29.0%), underscoring the effectiveness of QuEChERS in mitigating the matrix effects of honey, justifying its use in subsequent experiments.

Honey samples spiked with varying CPY concentrations were extracted using QuEChERS and deposited onto ALF substrates. Following the addition of AuNPs, the characteristic CPY SERS peak at 1353 cm^{-1} remained visible down to 10^{-8} M and exhibited a strong linear correlation ($R^2 = 0.98$). While this experimentally observable concentration falls below the EU MRL for CPY in food (2.85×10^{-8} M), the formal statistical limit of detection (LOD) for the assay was calculated to be 4.8×10^{-7} M. Although this calculated LOD is currently higher than the stringent EU regulatory limit, it still represents a full order-of-magnitude improvement in sensitivity compared to CPY detection in a simple solvent matrix. This enhancement is likely attributable to the residual extraction salts that induce AuNP aggregation, as confirmed by UV-vis analysis (Fig. S7), which would increase hot spot density across the ALF@AuNPs surface and amplify the SERS signal.

Evaluating the selectivity of AuNP enhanced kitchen foil coupled with QuEChERS

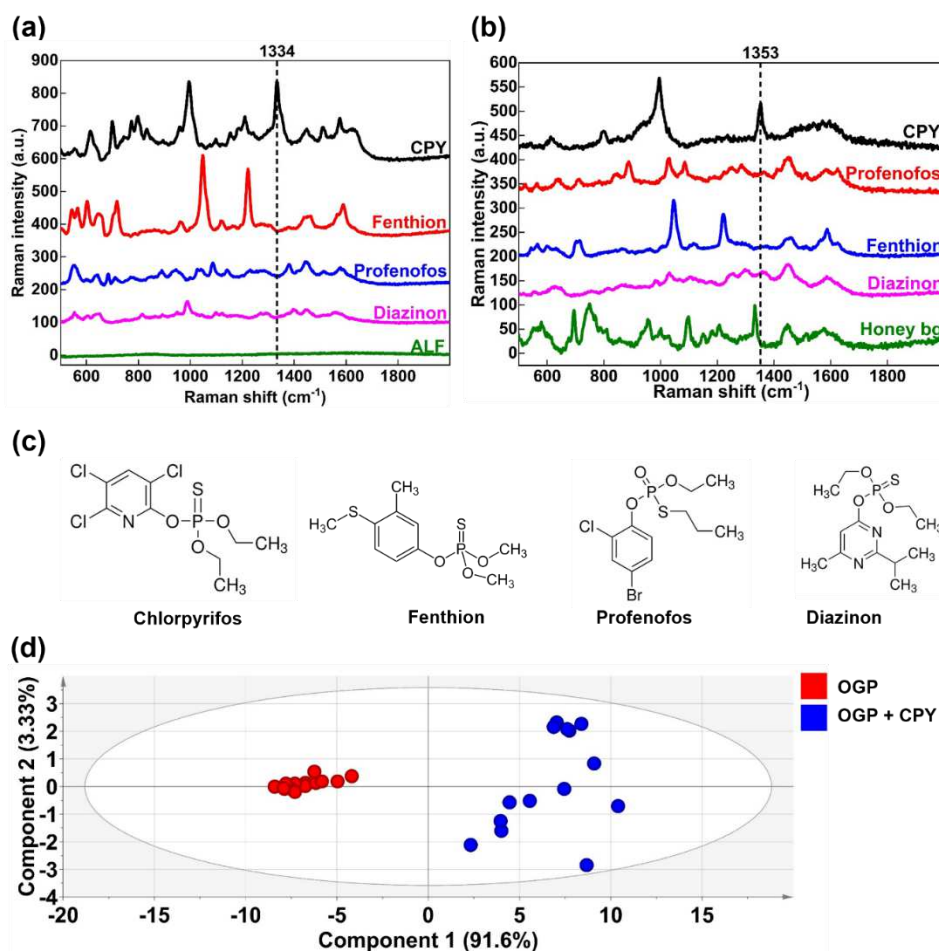


Fig. 5 (a) SERS spectra of 10⁻⁴ M CPY (black line), 10⁻⁴ M fenthion (red line), 10⁻⁴ M profenofos (blue line) and 10⁻⁴ M diazinon (purple line), in a 1:1 mixture of dH₂O and EtOH on ALF@AuNPs. (b) SERS spectra from honey spiked with 10⁻⁵ M CPY (black line), 10⁻⁵ M profenofos (red line), 10⁻⁵ M fenthion (blue line) and 10⁻⁵ M diazinon (purple line), alongside a blank sample (green line), following QuEChERS extraction on ALF@AuNPs. (c) Chemical structures of the selected pesticides. (d) PC1 vs PC2 scores plot comparing SERS spectra collected from a mixture of CPY and the other three organophosphates (OGP + CPY, blue) with spectra collected from a mixture of the organophosphates excluding CPY (OGP, red) on ALF@AuNPs. ($\eta=30$)

In agriculture it is unlikely that pesticides will be used in isolation. This is because farmers use a variety of pesticides to protect their crops from various pests and diseases²⁷. Therefore, the selectivity of the ALF@AuNP sensor coupled with QuEChERS was analysed. Three structurally similar organophosphate pesticides, namely fenthion, profenofos, and diazinon (chemical structures depicted in Fig. 5c) were selected for comparison. Individually, all four pesticides retained distinct, characteristic SERS peaks following extraction from honey (Fig. 5 a-b). However, when CPY was co-extracted alongside the other three pesticides, the resulting SERS spectra on ALF@AuNP were considerably more difficult to visually differentiate.

Although QuEChERS extraction successfully minimised honey matrix interference, residual co-extracted molecules occasionally masked trace CPY peaks. Unpredictable Raman shifts and hot spot variability inherent to SERS further complicated the direct classification of these structurally similar analytes²⁸. To resolve these spectral overlaps, chemometrics, which mathematically extract analytical information from a complex mixture of signals, was integrated with SERS^{29,30}. A PCA was performed on SERS spectra acquired from honey extract samples ($\eta=30$, Fig. S8).

The resulting scores plot (Fig. 5d) demonstrated clear discrimination between extracts containing only the fenthion, profenofos, and diazinon (OGP) and those containing the full mixture including CPY (OGP + CPY). The model proved to be highly robust, with the first two principal components capturing 94.93% of the cumulative variance (PC1 = 91.6% and PC2 = 3.33%). Although predictive multivariate models such as partial least squares discriminant analysis (PLS-DA) or regression (PLS-R) could be developed with a broader dataset, this initial PCA confirms that combining QuEChERS extraction with SERS and chemometrics effectively differentiates structurally similar compounds within a complex food matrix.

Discussion

This study demonstrates that the plasmonic activity of ALF significantly amplifies Raman and SERS signals when coupled with deposited AuNPs compared to non-plasmonic substrates such as Si. Simple drop-casting of AuNPs onto ALF provides an effective platform capable of detecting CPY in a solvent matrix with a LOD of 5.2×10^{-6} M. Substrate fabrication demonstrated acceptable reproducibility, exhibiting an intra-substrate RSD of 19.3% and an inter-batch RSD of 22.3%.

When coupled with QuEChERS extraction, the ALF@AuNP platform enabled quantitative and selective detection of CPY in complex honey matrices down to 4.8×10^{-7} M. QuEChERS extraction proved to be synergistic with SERS analysis; it not only mitigated matrix interference from honey but also utilises extraction salts to induce AuNP aggregation. This aggregation promoted the formation of densely packed SERS hot spots, significantly increasing the SERS response.

The use of aluminium kitchen foil coupled with QuEChERS extraction adds to an exciting field of utilising commonly available plasmonic materials in our households as potential optical sensors. With future work it has been envisioned that by changing the plasmonic material or by changing the surface of the kitchen foil to further concentrate the AuNPs and target analytes the detection limits of the approach can be further improved.

Methods

Materials and reagents

(3-Aminopropyl) triethoxysilane ($\text{C}_9\text{H}_{23}\text{NO}_3\text{Si}$, 99%), chlorpyrifos ($\text{C}_9\text{H}_{11}\text{Cl}_3\text{NO}_3\text{PS}$), magnesium sulphate (MgSO_4), ethanol ($\text{C}_2\text{H}_5\text{OH}$), acetonitrile ($\text{C}_2\text{H}_3\text{N}$), sodium citrate tribasic dehydrate ($\text{HOC}(\text{COONa})(\text{CH}_2\text{-COONa})_2\cdot\text{aq}$), sodium chloride (NaCl), gold(III) chloride trihydrate ($\text{HAuCl}_4\cdot 3\text{H}_2\text{O}$, 99.9%), N-propylethylenediamine, potassium hydroxide (KOH), lauric acid ($\text{C}_{12}\text{H}_{24}\text{O}_2$), rhodamine 6G, sodium acetate (CH_3COONa), acetic acid (CH_3COOH), cucurbit[5]uril hydrate ($\text{C}_{30}\text{H}_{30}\text{N}_{20}\text{O}_{10}\cdot x\text{H}_2\text{O}\cdot x\text{KCl}\cdot x\text{HCl}$), diazinon ($\text{C}_4\text{H}_7\text{Cl}_2\text{O}_4\text{P}$), profenofos ($\text{C}_{11}\text{H}_{15}\text{BrClO}_3\text{PS}$), fenthion ($\text{C}_{10}\text{H}_{15}\text{O}_5\text{PS}_2$) were all purchased from Sigma Aldrich (UK). 3,3',5,5'-Tetramethylbenzidine (TMB) was obtained from Thermo Fisher Scientific (UK). Rowse runny honey and aluminium foil (ALF) were purchased from Tesco (UK).

Analysis instrumentation

All SERS measurements were performed using a DXR2 Raman microscope (Thermo Fisher Scientific, UK) equipped with a 785 nm laser, operated at a power of 20 mW, with 10 $\times$ magnification and an acquisition time of 5 seconds. Optical absorbance spectra were recorded using a Cary 60 spectrophotometer (Agilent Technologies, USA). Scanning electron microscopy (SEM) images were obtained using a JSM-6500F microscope (JEOL, Japan) operated at 15 kV.

Electromagnetic field simulations were conducted using COMSOL Multiphysics. Raman peak assignment and mapping were performed using OMNICTM Series software, which includes the integrated AtIus function for Raman imaging and for measuring the diameter of deposited AuNPs and analytes used in SERS enhancement factor calculations (Thermo Fisher Scientific, UK). Multivariate analysis, including principal component analysis (PCA), was carried out using SIMCA[®] software (Sartorius, Germany).

Gold nanoparticle synthesis

AuNPs were synthesised using the Turkevich method with minor modifications³¹. Briefly, 500 μL of 100 mM $\text{HAuCl}_4\cdot 3\text{H}_2\text{O}$ was added to 49.5 mL of deionised water and heated under constant stirring until boiling. Once boiling, 5 mL of 1% sodium citrate solution was rapidly injected into the solution. The mixture was then allowed to cool gradually while stirring for an additional 30 min. A colour change from translucent yellow to wine red indicated successful AuNP formation.

The synthesised AuNPs were characterised by UV–Vis absorption spectroscopy (Fig. S1), and their concentration was calculated from the absorbance spectrum to be 8.19 nM using the method described by Haiss et al.³².

Comparison of Raman and SERS performance of AuNP-enhanced aluminium foil and silicon substrates: calculation of enhancement factor and limit of detection (LOD)

To evaluate the Raman performance of the two substrates, 1 µL of varying concentrations of Rhodamine 6G (R6G) ranging from 10^{-6} – 10^{-3} M were deposited onto ALF and Si surfaces and allowed to fully dry before Raman measurements were taken. For SERS performance assessment, 1 µL of R6G solutions ranging from 10^{-8} – 10^{-3} M were similarly deposited onto ALF and Si substrates and allowed to partially dry for 30 s. Subsequently, 1 µL of AuNPs (8.19 nM) was added on top of the R6G droplet and allowed to dry before spectral acquisition.

To calculate the Raman enhancement factor (REF) and the limit of detection (LOD), characteristic peaks in the collected spectra were identified and compared with literature values to select a suitable diagnostic peak. This peak characterisation is provided in the Supplementary Information (Fig. S2 and Table S1).

The Raman enhancement factor (REF) was calculated using the following equation:

$$REF = \frac{(I_{SERS})}{(I_{Raman})} \times \frac{(N_{Raman})}{(N_{SERS})}$$

LOD was calculated using the following formula³³:

$$LOD = LOB + 1.645 (SD_{low\ concentration\ sample})$$

$$LOB = mean_{blank} + 1.645 (SD_{blank})$$

Where:

LOD = Limit of Detection

LOB = Limit of the Blank

SD = Standard Deviation

To determine the LOB, matrix blank measurements were conducted by depositing 1 µL of deionised water (negative control) onto ALF, allowing it to partially evaporate for 30 s, followed by the addition of 1 µL of AuNPs. This procedure was repeated 10 times to obtain a robust estimate of signal variability.

Reproducibility analysis of SERS signal across ALF@AuNPs substrates and batches

To assess the reproducibility of Raman signals across the surface of the ALF@AuNPs substrate, 1 μL of 10^{-5} M R6G was deposited onto the ALF and allowed to partially dry for 30 s. Subsequently, 1 μL of AuNPs (8.19 nM) was added on top and allowed to dry before Raman measurements were taken. A $10 \times 10 \mu\text{m}^2$ Raman map was then collected using a 1 μm step size with the built-in Atlus function in the OMNICTM Series software. The Raman intensity at the diagnostic peak of R6G was used to calculate the relative standard deviation (RSD) across the mapped area, using the equation:

$$RSD = \frac{\text{standard deviation}}{\text{average}} \times 100$$

To evaluate batch-to-batch reproducibility, the same procedure was repeated on three independently prepared ALF@AuNPs substrates. For each substrate, Raman measurements were taken at 10 randomly selected locations. The resulting RSD values were calculated using the same formula to assess variation between substrate batches.

Determination of the LOD for chlorpyrifos in a solvent matrix using ALF@AuNPs

One μL of different concentrations of CPY, ranging from 10^{-8} M – 10^{-4} M, was deposited onto the ALF substrate and allowed to partially evaporate for 30 s. Subsequently, 1 μL of AuNPs (8.19 nM) was added to each spot and allowed to dry before Raman spectra were collected. To determine the LOD, the characteristic peaks in the CPY spectra were identified and compared with literature values to select an appropriate diagnostic peak. The LOD was then calculated using the same method described in a previous section.

Recovery of spiked CPY from honey using the QuEChERS extraction method

A modified QuEChERS extraction method was employed, based on the procedure described by Orso et al.¹¹. Briefly, 15 mL of sample (honey diluted with deionised water in a 1:2 ratio) was mixed with 15 mL of 1% acetic acid in acetonitrile. To this mixture, 6 g of MgSO_4 and 1.5 g of sodium acetate were added, followed by vigorous mixing for 30 s. The sample was then centrifuged at 1500 rcf for 1 min, and the upper acetonitrile layer was collected and dispensed into 1 mL aliquots.

For SERS analysis, 1 μL of the acetonitrile extract was deposited onto the ALF substrate and allowed to partially evaporate for 30 s. Then, 1 μL of AuNPs (8.19 nM) was added and allowed to dry before Raman measurements were performed.

Determination of the limit of detection of Chlorpyrifos from QuEChERS extracts using ALF@AuNPs

To ensure that the Raman spectra collected from the QuEChERS extract originated from CPY and not from residual matrix components, a comparative analysis was first performed. Honey samples spiked with 10^{-5} M CPY and non-spiked controls were both subjected to the same QuEChERS extraction method that has been previously described. From each extract, 1 μ L was deposited onto the ALF substrate and allowed to partially evaporate for 30 s. Subsequently, 1 μ L of AuNPs (8.19 nM) was added and allowed to dry before Raman measurements were taken.

Principal component analysis (PCA) was then performed (parameters detailed in a following section) to visually differentiate the spectral profiles of the spiked and non-spiked extracts. The PCA also included spectra from honey diluted with deionised water at 1:2 and 2:1 ratios, both spiked with 10^{-5} M CPY prior to extraction. Each of these diluted samples (1 μ L) was deposited onto ALF, allowed to settle for 30 s, followed by the addition of 1 μ L of AuNPs (8.19 nM), and dried before Raman spectra were acquired.

Subsequently, honey samples spiked with a range of CPY concentrations (10^{-9} M to 10^{-5} M) were processed using the same QuEChERS protocol detailed in a previous section. For each concentration, 1 μ L of the extract was deposited onto ALF, allowed to evaporate for 30 s, and overlaid with 1 μ L of AuNPs (8.19 nM). After drying, Raman measurements were collected. The LOD was then calculated using an equation which has been described in a previous section.

Determination of the selectivity of QuEChERS extraction for organophosphate pesticides in honey

To evaluate the selectivity of QuEChERS extraction for organophosphate (OGP) pesticides in honey, samples were spiked with a mixture of three structurally similar compounds: fenthion, diazinon, and profenofos, each at a concentration of 10^{-5} M. This mixture was labelled as "OGP". In parallel, another sample was spiked with the same OGP mixture along with 10^{-5} M of chlorpyrifos (CPY), referred to as "OGP + CPY". Both spiked honey samples were subjected to the same previously described QuEChERS extraction procedure. From each extract, 1 μ L was deposited onto the ALF substrate and allowed to partially evaporate for 30 s. Then, 1 μ L of AuNPs (8.19 nM) was added and allowed to dry before Raman spectra were collected. To assess selectivity, principal component analysis (PCA), as outlined in the following section, was conducted to determine whether CPY could be reliably differentiated from the other structurally similar organophosphates.

Principal Component Analysis (PCA)

Principal component analysis (PCA) was used to visualise and evaluate spectral differences between samples and to assess the selectivity of CPY detection in complex matrices. Two separate data matrices were constructed based on the experimental designs described in the previous sections.

The first matrix (corresponding to Fig. 4c, from the section entitled “Recovery of spiked CPY from honey using the QuEChERS extraction method”) consisted of 41 samples (rows) and 1,350 spectral variables (columns), while the second matrix (corresponding to Fig. 5d, used to differentiate between OGP and OGP + CPY mixtures in the section entitled “Determination of the selectivity of QuEChERS extraction for organophosphate pesticides in honey”) included 30 samples and 1,500 spectral variables. All spectral data were processed using Pareto variance scaling (modifier: 1), and linear transformation ($C1 \times 1 + C2$) was applied. Seven cross-validation groups were used to ensure model robustness.

PCA was performed using SIMCA® v14 software (Sartorius, Germany). All available wavelengths across the selected spectral region were included in the model construction. The resulting scores plots were used to assess sample clustering and distinguish CPY-specific spectral signatures from those of structurally similar organophosphates and background matrix components.

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Data availability

The datasets generated during the study are available from the corresponding author upon reasonable request.

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Ethics Declaration

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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