

Flexible label-free SERS substrate with alginate-chitosan@silver nanocube for in situ nondestructive detection of thiram on apples

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ABSTRACT

The rapid in situ detection of pesticide residues in real samples based on surface-enhanced Raman spectroscopy (SERS) remains a challenge, necessitating an urgent need for a feasible solution that addresses issues such as sample complexity, reproducibility, and SERS substrate stability. This paper proposes a flexible SERS substrate, which consists of a composite gel made of sodium alginate-chitosan loaded with silver nanocubes (SA-CTS@AgNCs). The flexible nature of the SERS substrate enables the analysis of irregular surfaces of apples, dispensing with laborious pretreatment and promoting an effective contact with target molecules. By utilizing the SA-CTS@AgNCs substrate in conjunction with a portable Raman instrument, an exceptional sensitivity was achieved with a detection limit of 0.055 mg/L for thiram in apples. In addition, the stability, homogeneity, and batch-to-batch reproducibility of the substrates were evaluated. The experimental results showed that after 45 days of storage, the substrate still maintained more than 84.40 % SERS activity, demonstrating long-term stability. Within a single substrate, the point-to-point relative standard deviation (RSD) was only 4.2 %, while among different batches of substrates, the RSD was as low as 6.8 %, displaying better homogeneity and reproducibility. Hence, this flexible SERS substrate provides a reliable and convenient platform for rapid detection and on-site monitoring of food safety.

1. Introduction

Apples, as one of the most commonly cultivated and consumed tree fruits globally, are prone to various pests and diseases throughout their growth cycle. These challenges result in substantial economic losses for growers. To ensure the yield and quality of apples, the use of pesticides is essential [1]. Thiram (THR), a widely employed pesticide in apple cultivation, is utilized to control downy mildew, blight, anthracnose, and seedling yellow blight. However, excessive reliance on pesticide application by fruit growers has led to the accumulation of THR residues in the environment. This accumulation poses significant risks to aquatic organisms and exhibits cytotoxic effects on humans, thereby having a significant negative impact on agricultural trade and consumer food safety [2].

Continuous research and development efforts have been dedicated to

exploring efficient and effective methods for detecting pesticide residues. Traditional detection methods, including high-performance liquid chromatography (HPLC), capillary electrophoresis, liquid chromatography-mass spectrometry (LC-MS/MS), and enzyme-linked immunosorbent assay (ELISA), are widely employed and have demonstrated high detection accuracy [3–5]. However, these methods are associated with certain drawbacks, such as being time-consuming, labor-intensive, expensive, requiring large quantities of reagents, and involving complex sample pretreatment. In recent years, emerging detection techniques such as electrochemistry [6], fluorescence [7], and surface-enhanced Raman spectroscopy (SERS) have been successfully applied to pesticide residue detection. In particular, SERS technology, due to its excellent molecular fingerprinting properties, can achieve rapid, stable, and nondestructive identification of various target molecules, making it widely used in monitoring food quality and safety [8].

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For example, for pesticide residue detection, Qu et al. developed a detection platform based on a floating SERS substrate to achieve self-assembly at the water-air and water-oil interfaces [9]. They synthesized stable gold nanoparticles using the seed growth method and prepared AuNPs-coated hollow silica microspheres (HSMs) through electrostatic adsorption. The obtained HSM@AuNPs were used for SERS detection of pesticide residues in beverages. Besides, Yang et al. achieved in situ real-time detection of organophosphorus and carbamate insecticides on fruits and vegetables with different roughness by preparing AgNWs@ZIF-8 core-shell nano-chains [10]. The SERS technique is commonly used for the detection of mycotoxins such as aflatoxin B1 (AFB1) and patulin (PAT) in agricultural products [11,12].

The SERS effect represents the advancement and extension of Raman scattering. The enhancement mechanism primarily arises from the electromagnetic enhancement (EM) of localized surface plasmon resonance (LSPR) in noble metals such as gold and silver, as well as the chemical enhancement (CM) originating from charge transfer [13]. Undoubtedly, the selection of a suitable SERS substrate is essential for achieving significant enhancement effects. Traditional precious metal colloidal substrates, prepared in various shapes such as nanoparticles [14], nanorods [15], nanoflowers [16], and nanowires [17], have been extensively employed. These substrates serve as a solid foundation for SERS enhancement, but they often necessitate additional sample pre-treatment steps to ensure adequate contact between the substrate and the analyte. Furthermore, colloidal SERS substrates, in particular, exhibit poor stability and aggregation tendency, resulting in lower signal repeatability [18]. Given the significant importance of the stability of the SERS signal, rigid SERS substrates have sparked considerable interest. Materials such as glass [19], quartz [20], silicon wafers [21], and other stiff materials have been used as platforms for immobilizing metallic nanoparticles. However, despite the stable advantages of rigid SERS substrates, their limited flexibility and adaptability to complex sample surfaces have prompted the exploration of flexible materials as alternatives.

With the advancement of nanotechnology, the combination of gels and nanoparticles has become a viable option in flexible SERS substrate construction. Hydrogel is a novel encapsulation material with a three-dimensional network structure and a high specific surface area, among which marine polysaccharides have attracted significant attention as an inexpensive and widely used natural biomass material in pharmaceuticals, water purification, and food science [22–24]. Natural polysaccharide materials such as sodium alginate (SA) and chitosan (CS) are appealing in terms of their versatility, biodegradability, biocompatibility, and nontoxicity. However, past studies have mainly used single-component gels combined with nanoparticles to construct SERS substrates [25,26], and this approach has shown limitations in terms of stability. In contrast, by exploiting the electrostatic interactions between SA and CTS, cationic chitosan and anionic sodium alginate were able to form polyelectrolyte complexes without the addition of toxic chemical cross-linkers [27]. This complex not only retained the biological properties of sodium alginate and chitosan but also overcame the limitation of the poor stability of individual components [28]. Inspired by this, we further explored the use of SA and CTS composites to prepare flexible SERS substrates.

Herein, the purpose of this study is to investigate a method for loading silver cubes (AgNCs) with uniform shape, high density, and uniform distribution into SA-CTS composites to create high-performance SA-CTS@AgNCs flexible SERS substrates. SA-CTS and AgNCs were mixed in a specific ratio to create a slurry-like gel, and after freeze-drying, the water was changed into ice crystals and evaporated at low temperatures, preserving the gel's shape and forming a porous network structure. At the same time, freeze-drying preserved the AgNCs in the gel, ensuring equal distribution without aggregation or precipitation. Furthermore, the reduced water content in gel enhances its stability, while lightweight and compact nature simplifies storage and transportation. We employed the SA-CTS@AgNCs flexible SERS substrate in

conjunction with a portable Raman spectrometer to detect trace THR in apples. Satisfactory results revealed that this substrate is convenient, reliable, and capable of quick detection. Thus, the SA-CTS@AgNCs substrate is expected to facilitate the commercialization of SERS technology for sensitive pesticide residue detection in food, as well as lay the groundwork for the future development of robust, non-destructive, field, and portable SERS tests.

2. Materials and methods

2.1. Materials and instruments

Cetyl trimethylammonium bromide (CTAB) and glucose ($C_6H_{12}O_6$) were supplied from Macklin Biochemical Co. Ltd. (Shanghai, China). Aqueous ammonia ($NH_3 \cdot H_2O$, $\geq 25\%$ – 28%), silver nitrate ($AgNO_3$, 99%), rhodamine 6G (R6G), and ethanol (99.5%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Thiram (THR) was received from Beijing InnoChem Science & Technology Co. Ltd. (Beijing, China). Sodium alginate (SA) and chitosan (CTS) were obtained from Shanghai Bide Pharmatech Co. Ltd. (Shanghai, China). Ultrapure water was used for the preparation of all solutions ($18.2\text{ M}\Omega\text{ cm}$).

A "Bruker XFlash 6/60" scanning electron microscopy (SEM) equipped with an energy dispersive spectroscopy (EDS) system was used for morphology and elemental composition analysis, operated at 15 kV . Transmission electron microscopy (TEM) images were captured using a Hitachi HT7800 transmission electron microscope. Particle size and zeta potential analysis of the samples were performed using a Malvern Zetasizer Lab instrument. A handheld Raman spectrometer equipped with a 785 nm laser from "Shenzhen Well Optics Co. Ltd. in China" was used to collect SERS spectra.

2.2. Synthesis of AgNCs

The synthesis of AgNCs was carried out using a typical hydrothermal method [29]. Initially, 42.5 mg of $AgNO_3$ was dissolved in 2 mL of ultrapure water. Subsequently, $NH_3 \cdot H_2O$ (25% – 28%) was slowly added until the brown solution became clear, and the volume was adjusted to 5 mL by adding water. Following that, 5 mL , 75 mM CTAB aqueous solution, and 10 mL , 1.5 mM glucose solution were added, and the mixture was stirred for 5 min . The resulting solution was then transferred into a 50 mL reactor lined with polytetrafluoroethylene and subjected to a reaction at 120°C for 8 h . The obtained product was washed twice by centrifugation (6000 rpm , 20 min) and stored in the refrigerator at 4°C .

2.3. Preparation of sodium alginate-chitosan composite gel

Building upon previous research, the production process of the sodium alginate-chitosan composite gel was enhanced [30]. In this process, prepare a solution containing 4% sodium alginate (SA) and another with 2% chitosan (CTS) first. Subsequently, the dissolved SA and the dispersed CTS were combined in a separate container and subjected to magnetic stirring to ensure the homogeneity of the mixture, resulting in the preparation of the sodium alginate-chitosan composite gel (SA-CTS).

2.4. Fabrication of flexible SERS substrate

In brief, the synthesized AgNCs were blended with the composite SA-CTS gel at various ratios and subjected to magnetic stirring for a duration of 2 h . Following this, the resulting mixture was transferred into a mold, which was subsequently covered with a film and subjected to freeze-drying for 24 h at a temperature of -40°C .

2.5. In situ THR detection platform based on a portable Raman spectrometer

The objective of this research was to provide a streamlined, user-friendly, eco-friendly, and cost-effective synthesis process for fabricating flexible SERS substrates, enabling in situ online detection of complex biological molecules while eliminating the need for intricate pre-treatment stages. This was achieved through the integration of a portable Raman spectrometer with a rationally designed sodium alginate-chitosan porous structure loaded with silver cubes. The integration of these components led to the efficient enhancement of Raman signals, facilitating the detection of target analytes in intricate biological samples. To begin, hydrothermal reduction was used to create silver cubes, which exhibited significant localized surface plasmon resonance (LSPR) properties. Furthermore, the right side of Fig. 1 demonstrates the binding process of sodium alginate and chitosan gels. This biopolymer gel has unique properties such as biocompatibility, biodegradability, and the ability to form porous network structures [31]. The porous framework, formed through electrostatic interactions between sodium alginate and chitosan molecules, not only provides favorable support for the efficient loading of silver cubes but also maximizes the interaction between the analytes and the active surface sites, thereby enhancing the detection sensitivity. Moreover, the portability of the Raman spectrometer allows for real-time monitoring in the field and fast and convenient THR detection on apple surfaces.

A commonly recognized spiking method was used to detect THR in real time on curved surfaces, as outlined below: Initially, for making spiked samples, 20 μL of an ethanol solution containing various concentrations of THR (0.5, 2, 5, 7.5, 10, 15, and 20 mg/L) were applied to

the surface of an apple and allowed to dry naturally at 25 $^{\circ}\text{C}$. An ethanol solution of 20 μL was then applied to the apple surface to extract the targeted THR. Subsequently, the SA-CTS@AgNCs substrate was covered on the peel and the Raman test was performed directly. The experimental parameters were configured as follows: The excitation wavelength was set to 785 nm, the scanning range spanned from 400 cm^{-1} to 1600 cm^{-1} , the laser power was maintained at 150 mW, the scanning time was set to 3 s, and a single cycle was performed. The average SERS spectra were promptly acquired from 10 randomly selected points to ensure representative data collection.

2.6. SERS spectral data processing

Principal Component Analysis (PCA) is a popular approach for multivariate data analysis. The goal is to minimize information loss by choosing the direction of the largest variance to create a new coordinate system that maps n-dimensional characteristics to k-dimensions [32]. By conducting PCA analysis, the raw SERS spectral data can be transformed into a new set of principal components. Examining the contributions and correlation coefficients of these principal components enables the identification of differences among various pesticide concentrations.

3. Results and discussion

3.1. Characterization of AgNCs

High-purity AgNCs were successfully synthesized using the conventional hydrothermal method and employing CTAB as a stabilizer. TEM analysis confirmed the characteristic truncated cubic shape of the

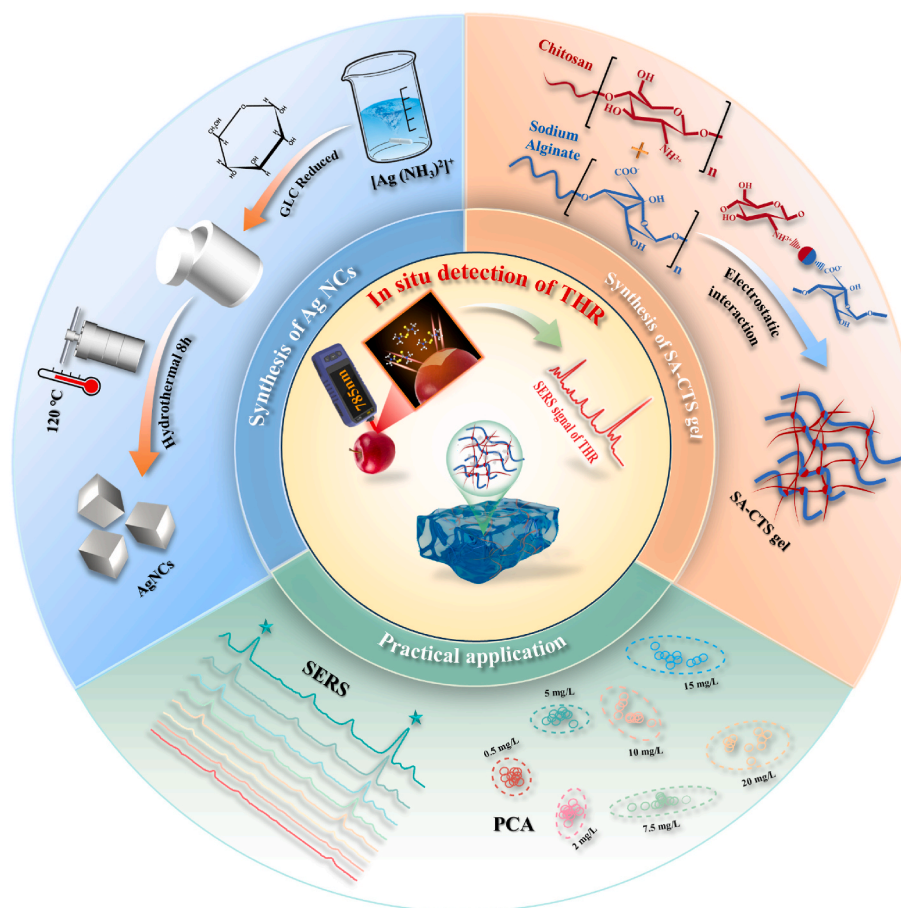


Fig. 1. Schematic illustration of SA-CTS@AgNCs-based flexible SERS substrate combined with portable Raman spectrometer for on-site thiram detection on apple surfaces.

AgNCs, displaying uniform sizes and regular morphology as shown in Fig. 2a. The particle sizes predominantly ranged between 50 and 90 nm, as depicted in Fig. 2b. SAED images further validated the well-crystallized state of the AgNCs, with diffraction spots corresponding to the {200} and {220} crystallographic planes, where the characteristic lattice spacing of the {200} crystal plane is 0.228 nm, indicating the ordered nature of its crystal structure (Fig. 2c) [33].

3.2. Characterization of SA-CTS@AgNCs flexible SERS substrate

The UV absorption spectra of AgNCs and SA-CTS@AgNCs flexible SERS substrates are shown in Fig. 2d. The absorption peaks measured for the nanocubes are similar to prior findings in the literature [34] showing absorption at 350, 386, and 464 nm, respectively. According to the literature, quadrupole local surface plasmons (LSP) create the greatest peak at 464 nm, whereas the combined action of dipole and quadrupole LSP results in lesser absorption at 350 and 386 nm, demonstrating the relationship between nanocube size and absorption spectrum [35]. Compared to AgNCs, the UV absorption peaks of flexible SERS substrates had wider profiles and exhibited a minor redshift.

SA and CTS are two forms of oppositely charged ionic polysaccharides that may easily form SA-CTS polyelectrolyte complexes via electrostatic interactions [36]. The SEM images in Fig. S1 show linked porous structures. This well-organized porous network structure provides the gel material with a large surface area and superior pore performance. To elucidate the functional groups and chemical bonding in the composite gels, the FTIR spectra of SA, CTS, and SA-CTS composite gels were analyzed (Fig. 2e). The FTIR spectra of SA revealed two unique absorption peaks at 1595 cm^{-1} and 1411 cm^{-1} , representing the asymmetric and symmetric stretching vibrations of carboxyl groups. The FTIR spectrum of CTS demonstrated characteristic peaks at 1644 cm^{-1} and 1587 cm^{-1} , which correspond to the stretching vibrations of C=O and N-H, respectively [37]. After combining SA and CTS to generate the SA-CTS composite, the FTIR analysis revealed two notable discoveries. On one hand, the peak at 1644 cm^{-1} , which corresponds to the N-H bending vibration, was almost completely gone. This is caused by the overlapping impact of the polyelectrolyte on the stretching vibration of NH_2 . On the other hand, the SA-CTS composite gel exhibited a distinct

absorption peak at 1591 cm^{-1} , demonstrating the conversion of the NH_2 group of CTS to NH_3^+ under acidic environments [38]. The stretching vibration of OH in the SA-CTS composite gel exhibited a broadening in the range of 3600–3000 cm^{-1} . This observation is likely attributed to the formation of intermolecular hydrogen bonds between SA and CTS [39]. As a result, it can be concluded that the NH_3^+ groups of CTS and the carboxyl groups of SA are connected through electrostatic interactions.

Moreover, zeta potential tests were performed to verify the successful construction of the substrates, and the zeta potential findings are presented in Fig. 2f. According to the test results, the zeta potentials of sodium alginate and chitosan were -46.31 mV and $+14.61$ mV, respectively, which was consistent with their properties as natural cationic and natural anionic polysaccharides [40]. When sodium alginate and chitosan were connected by electrostatic interactions, the SA-CTS composite gel exhibited a negative potential of -31.09 mV. The cationic surfactant cetyltrimethylammonium bromide (CTAB) was utilized in the production of silver cubes, and therefore the surface of AgNCs was positively charged. When AgNCs were loaded onto the composite gel, the entire SA-CTS@AgNCs composite had a negative potential, which might be linked to the presence of free carboxyl groups in the sodium alginate gel.

The morphology of the SA-CTS@AgNCs flexible SERS substrates was examined by SEM, as demonstrated in Fig. 3a. The prepared flexible substrates have a typical porous network structure as illustrated in the inset digital photograph of the flexible SERS substrate. Furthermore, high magnification SEM photos show the loading of silver cubes. The elemental mapping shown in Fig. 3b indicated that Ag atoms were uniformly distributed in the AgNCs region, and C, O, and N elements were from the SA-CTS composite gel. Furthermore, EDS image analysis of the flexible substrate of SA-CTS@AgNCs revealed the presence of C, O, N, and Ag atoms (as shown in the inset in Fig. 3b).

3.3. Optimization of the experimental conditions

Since the SA-CTS@AgNCs flexible SERS substrate was built from a combination of AgNCs solution and SA-CTS gel, the content of the composite SA-CTS gel and AgNCs has an impact on the performance of the substrate. The signal enhancement capability of the SERS substrate

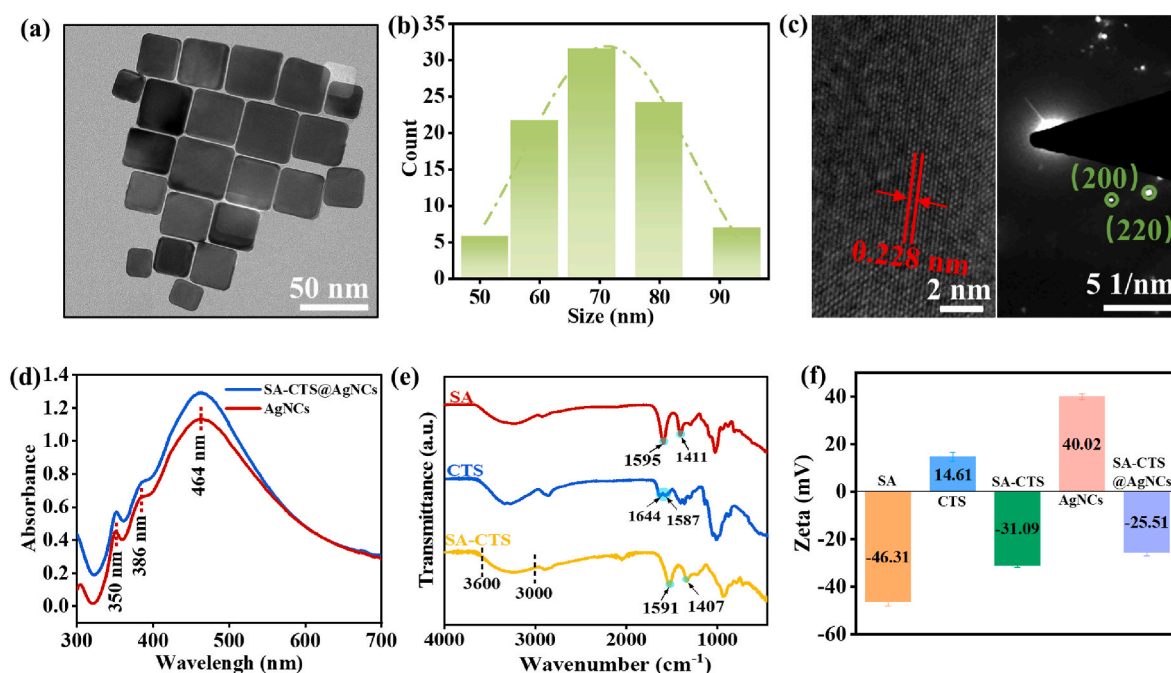


Fig. 2. (a) TEM, (b) Particle size distribution histogram, (c) HRTEM and SAED images of AgNCs; (d) UV-vis absorption spectra of AgNCs and SA-CTS@AgNCs substrate; (e) FTIR spectra of SA, CTS, and SA-CTS; (f) Zeta potential of SA, CTS, SA-CTS, AgNCs, SA-CTS@AgNCs.

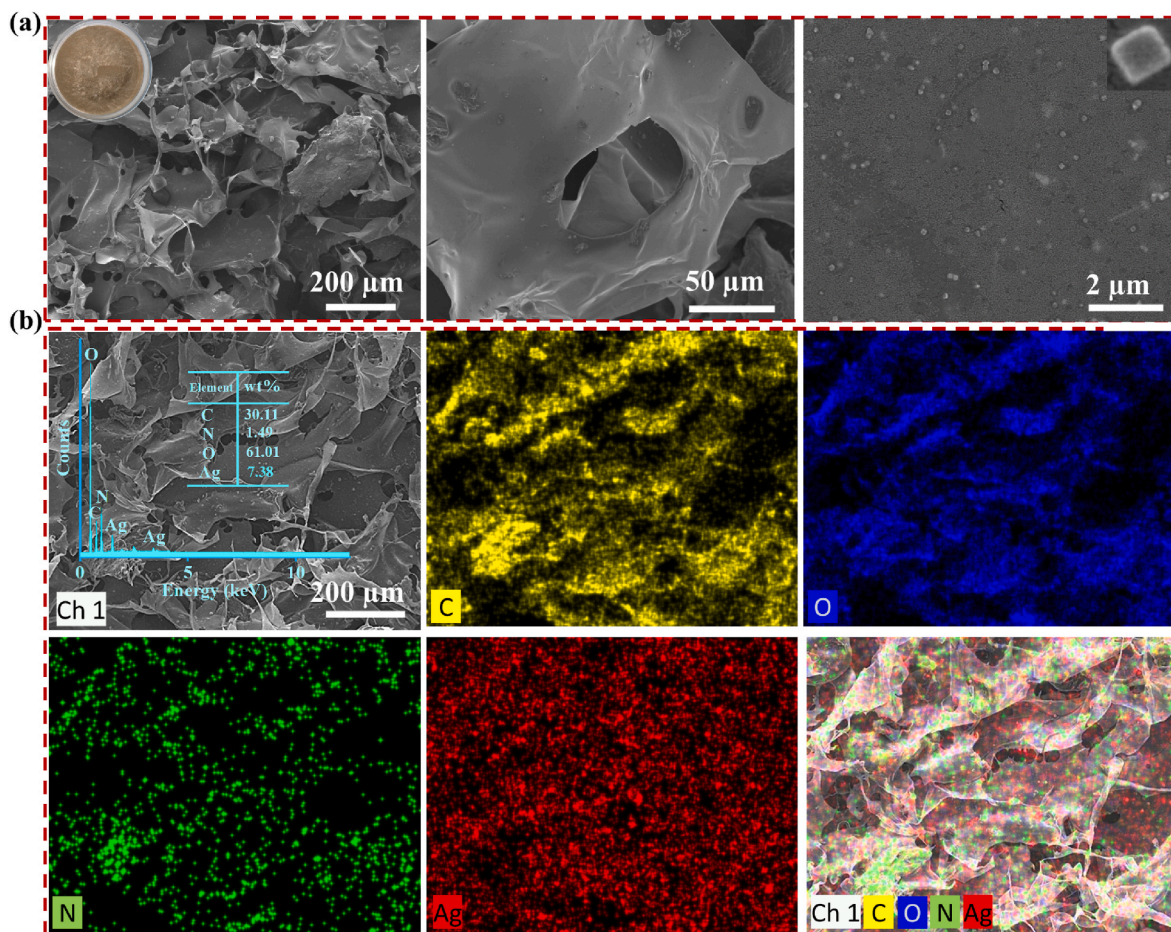


Fig. 3. (a) Photographic morphology and SEM images; (b) Elemental mapping of the SA-CTS@AgNCs flexible SERS substrate.

was investigated in relation to the relative volume ratio of gel and AgNCs, and the results are depicted in Fig. 4a. With THR as the detection target, it was observed that as the volume ratio of gel to AgNCs was reduced from 2:1 to 1:3, the enhancement performance of the flexible SERS substrate tended to increase before subsequently declining. In particular, the SERS signal of THR reaches its greatest value at a volume ratio of 1:2. Moreover, they were all generally better than the 2:1 vol ratio (Fig. 4b). This tendency could be attributed to the rise in SERS signals brought on by the increased content of AgNCs. While at a 1:3 vol ratio, the SERS signals abruptly dropped, which was probably the result of the decrease in gel content and made it difficult to efficiently prevent oxidation or erosion of the interior encapsulated AgNCs. As a result, the enhanced performance of the proposed SERS substrate gradually decreased due to the corrosion denaturation of AgNCs. It is worth mentioning that the improved performance of the SERS substrate mainly comes from the proportion of AgNCs in the gel, as shown in the inset of Fig. 4a, where the color of AgNCs dispersed in the gel solution gradually deepens with the increase of AgNCs content.

However, in addition to influencing SERS sensitivity, the gel content also had a significant effect on the stability of the proposed SERS substrates. According to the results in Fig. 4d, at a lower gel content (1:3 vol ratio of gel to AgNCs), the enhanced performance of the obtained SA-CTS@AgNCs flexible SERS substrate gradually decreased with the extension of the storage time, and after a period of time, the SERS activity was retained at only 60.61 % of the original level. In contrast, the obtained SERS substrate at a gel-to-AgNCs volume ratio of 1:2 showed excellent stability even after 45 days of storage, retaining 84.46 % of the original activity (Fig. 4c and e). These findings indicated that a higher gel concentration increased the stability of the SERS substrate, and a

larger AgNCs content increased the sensitivity of the substrate. Based on these findings, a 1:2 vol ratio of gel to AgNCs was chosen for the subsequent experimental investigation to produce a flexible SERS substrate with high sensitivity and test stability.

3.4. Performance assessment of flexible SERS substrate

To assess the SERS activity of the flexible SERS substrate, the enhancement factor (EF) of the substrate was calculated using the formula $EF = (I_{SERS}/N_{SERS}) \times (N_{Raman}/I_{Raman})$. Where, I_{SERS} is the SERS intensity with 50 μL of 10^{-5} M THR added to the SERS substrate, and I_{Raman} is the normal Raman intensity in the absence of the substrate. N_{SERS} and N_{Raman} are the corresponding numbers of molecules in the SERS measurements and in the normal Raman measurements without substrate, respectively. Thus, the EF of SA-CTS@AgNCs flexible SERS was calculated as 1.34×10^6 .

The homogeneity and reproducibility of the flexible SERS substrate of SA-CTS@AgNCs were comprehensively evaluated using rhodamine 6G (R6G) solution with a concentration of 10^{-4} M as a Raman probe molecule. After dropping 5 μL of R6G onto the flexible SERS substrate, SERS spectra were collected at 20 randomly selected points (Fig. 5a), revealing characteristic peaks of R6G at 1504 cm^{-1} . The homogeneity of the substrate was assessed by the relative standard deviation of the selected SERS intensity at 1504 cm^{-1} , as illustrated in Fig. 5b. The obtained RSD value of 4.2 % at 1504 cm^{-1} , which is significantly lower than that of commercial substrates and other flexible SERS substrates reported in literature [41,42], suggests that the substrate demonstrates exceptional uniformity.

In addition, the reproducibility of six batches of different substrates

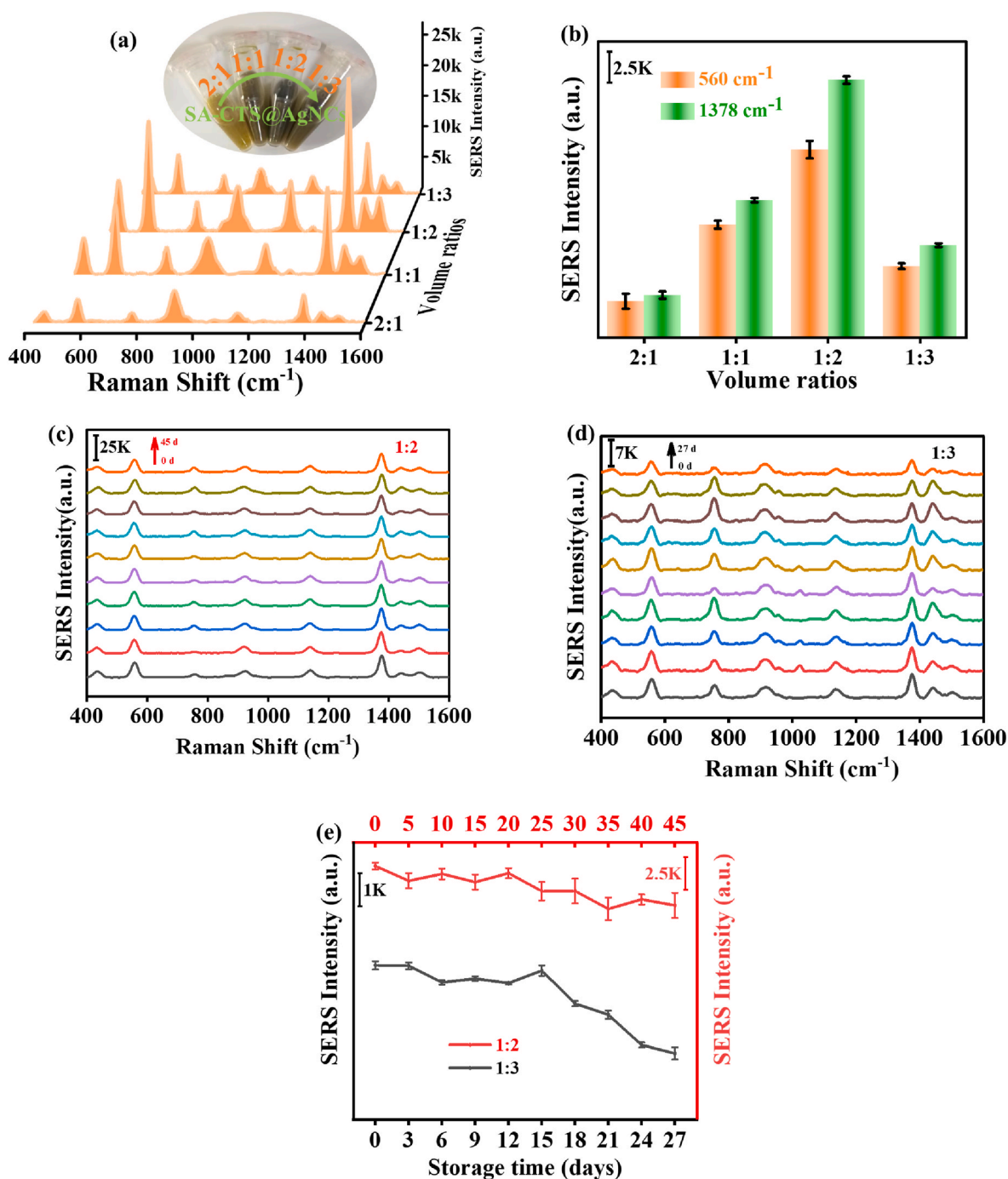


Fig. 4. (a) The SERS spectra of THR (5 mg/L) molecules collected on SERS substrates with varying levels of AgNCs, in the inset are the corresponding optical photographs; (b) The corresponding signal intensity at 560 and 1378 cm^{-1} ; SERS spectra of THR located on (c) SERS substrates (1:2) and (d) SERS substrates (1:3) kept in natural conditions for different days; (e) Corresponding SERS intensity variation at 1378 cm^{-1} with different time.

was tested, with five points being selected for each batch, resulting in a total of 30 Raman spectra, as seen in Fig. 5c. Encouragingly, all SERS spectra showed consistent peak patterns. To evaluate the reproducibility of the SERS substrate, we calculated the RSD values of the SERS intensities at 1357 cm^{-1} and 1504 cm^{-1} . The results revealed that these RSD values were as low as 6.8 % and 7.5 %, respectively (Fig. 5d). These results indicate the better reproducibility of the prepared SERS substrate. Based on these results, the remarkable stability and reproducibility presented by this flexible substrate provide substantial support for the reliability and credibility of the experimental results.

3.5. SERS spectral analysis of thiram pesticide

In the initial phase, the Raman spectrum of the powdered THR pesticide was acquired (Fig. 6a), and the inset depicts the chemical formula of THR. Notably, the Raman spectrum of THR revealed significant spikes at various wavenumbers. Specifically, peaks were observed at 560 cm^{-1} , indicating S-S symmetric stretching. Another peak appeared at 972 cm^{-1} , corresponding to C-S-S anti-symmetric stretching. Additionally, a peak at 1378 cm^{-1} was observed, which was associated with CH_3 rocking and C-N stretching [43]. Other peaks corresponding to THR were observed at 441 cm^{-1} , 848 cm^{-1} , 1036

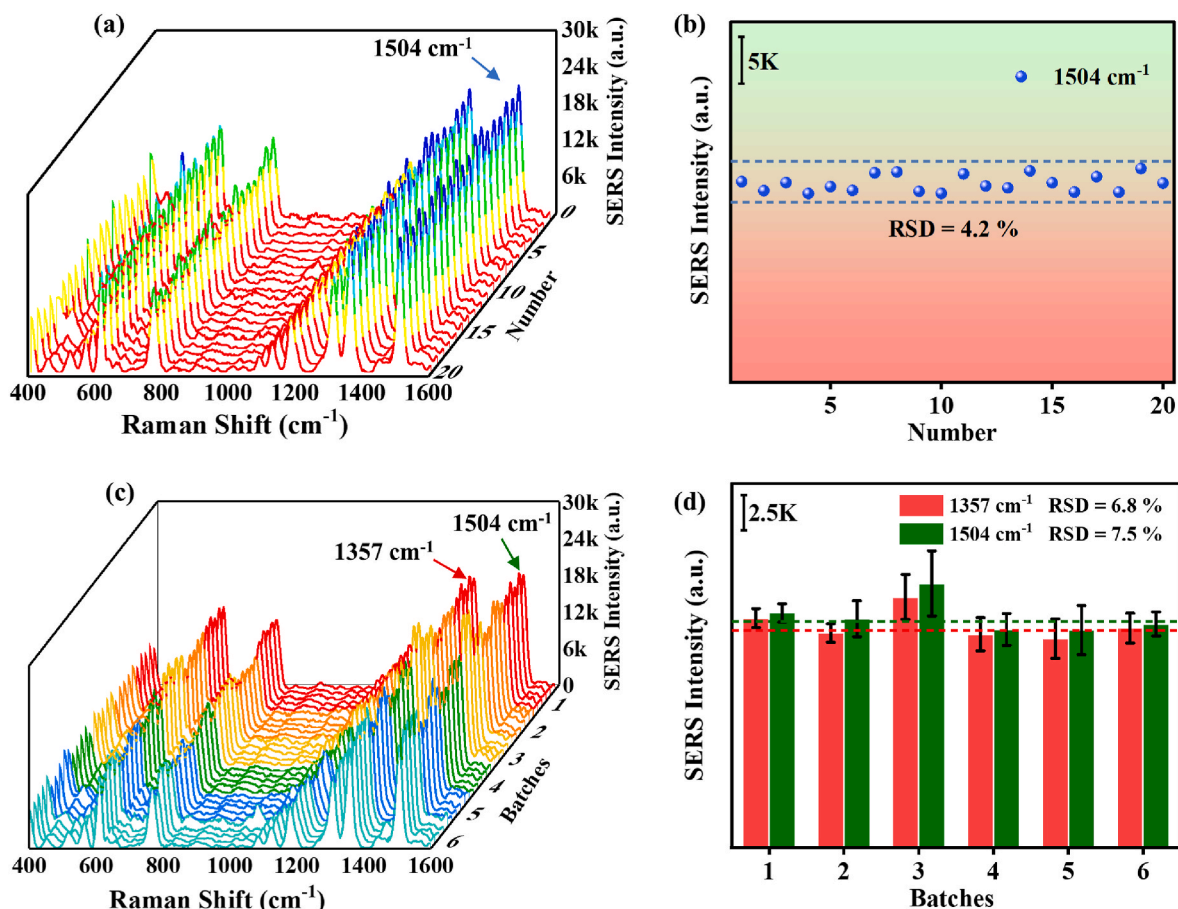


Fig. 5. (a) SERS spectra obtained from 20 arbitrarily selected positions of the SA-CTS@AgNCs substrate; (b) R6G peak intensity distribution at 1504 cm^{-1} ; (c) SERS spectra of R6G (10^{-4} M) obtained from 6 batches of SA-CTS@AgNCs substrates; and (d) R6G peak intensity distribution at 1357 and 1504 cm^{-1} .

cm^{-1} , 1084 cm^{-1} , 1145 cm^{-1} , 1395 cm^{-1} , and 1460 cm^{-1} , with their respective vibration modes presented in Table 1.

3.6. In situ quantitative detection of THR on apple surface

The Raman detection of 5 mg/L THR solution was performed on SA-CTS@AgNCs substrate, and the results confirmed that the substrate did not influence the THR characteristic peaks, as there was no significant overlap observed between the distinctive peaks of THR and the blank SA-CTS@AgNCs substrate. Meanwhile, for further quantitative analysis, a THR standard solution was used to estimate the sensitivity of SA-CTS@AgNCs and to compare the SERS enhancement performance of this flexible SERS substrate with that of silver cubes alone. The enrichment effect of the gel on the AgNCs resulted in superior SERS enhancement performance for the flexible SERS substrate. Notably, the peak intensity at 1378 cm^{-1} was observed to be the highest, thus guaranteeing optimum performance during further quantitative analysis.

The practical applicability of the proposed SERS substrate was validated through real-time in situ detection of pesticide residue THR on the surface of apples. Fig. 6c displays the handheld Raman spectrometer used for in situ detection in the field, which can rapidly and accurately detect pesticide residues on the surface of apples with the advantages of portability and real-time, as well as the convenience of exporting and analyzing spectral data. Based on the previous optimal experimental parameters, the SERS intensity at 1378 cm^{-1} was investigated for different concentrations of THR. As shown in Fig. 6d, the SERS signal intensity at the pesticide characteristic peak at 1378 cm^{-1} increased steadily with the increase of the THR concentration in the range of

$0.5\text{--}20\text{ mg/L}$. The linearly fitted calibration curve with error bars is shown in Fig. 6e, accompanied by the linear equation " $y = 2096.38x + 74.32$ " and a coefficient of determination R^2 of 0.992. The limit of detection (LOD) calculated at $3\sigma/k$ was 0.055 mg/L (σ represents the standard deviation of the SERS intensity from ten blank samples, and k denotes the slope of the calibration curve) [44]. In order to evaluate the SERS performance of SA-CTS@AgNCs for pesticides, spectral analysis was carried out using a chemometric method. Fig. 6f shows a two-dimensional principal component analysis (PCA) plot using the SERS spectra of THR within the Raman shifts range of $400\text{--}1600\text{ cm}^{-1}$. The clusters are clearly separated, even at low concentrations, which demonstrates the ability of the flexible substrate of the SA-CTS@AgNCs to quantitatively detect THR. Of note, the analytical performance of this SERS substrate was compared to other previously published methods, and this work demonstrated superior performance in terms of lower detection limits (Table S1). The accuracy of the substrate for detecting pesticide residues on apple surfaces was validated through recovery calculations of spiked samples. The results were presented in Table 2, with recoveries ranging from 83.13% to 105.17% and the highest RSD recorded at 7.34% ($<10\%$). Compared to the standard HPLC approach, the findings were within an acceptable range.

In addition, the prepared flexible substrate was also used for in-situ detection of THR on pear surfaces to verify the universality of the substrate. As shown in Fig. S2, the strength of SERS varied in response to THR concentration changes. The linear relationship between characteristic peak and concentration of pesticide at 1378 cm^{-1} is shown in Fig. S2b, where LOD of pesticide on pear surface reaches 0.079 mg/L and coefficient of determination $R^2 = 0.9872$. To further investigate the detection sensitivity of the proposed flexible substrate, THR and R6G

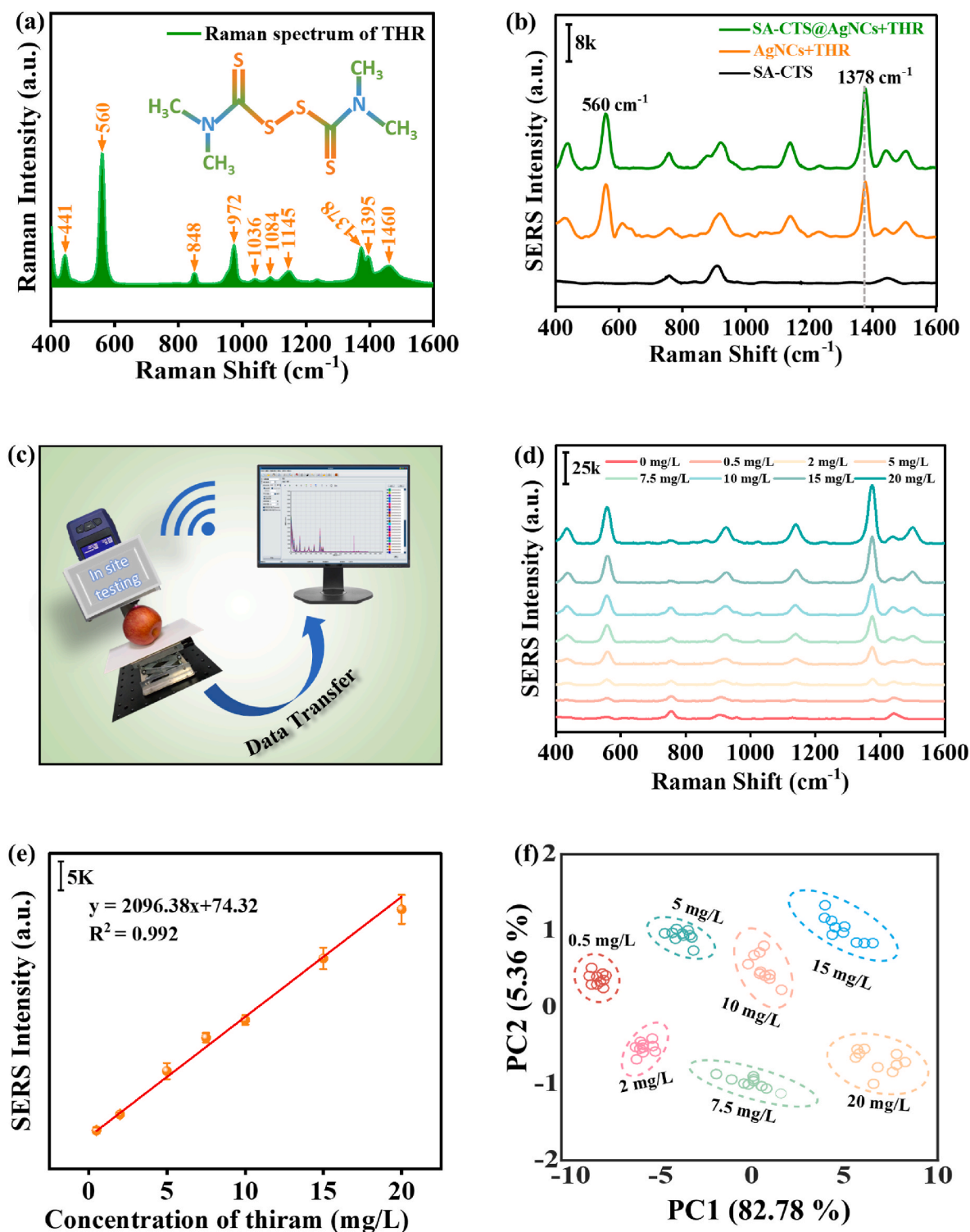


Fig. 6. (a) Raman spectrum of THR powder, and its molecular structure is inset; (b) SERS spectra of AgNCs and SA-CTS@AgNCs for THR detection; (c) Schematic representation of portable Raman-based in situ thiram detection on apple surfaces; (d) SERS spectrum of THR at different concentrations; (e) Plot of SERS intensities at 1378 cm^{-1} versus THR concentrations; (f) 2D PCA plot for the SERS spectrum of THR with different concentrations.

standard solutions were used as beacon molecules to evaluate the signal from the sensor reaction to various targets [45]. The concentrations of THR and R6G, along with the SERS intensity, correlated with the gradient change (Fig. S3 a, c). The linear relationship between the SERS intensity and the concentration of the characteristic peaks of THR and R6G at 1378 cm^{-1} and 1504 cm^{-1} respectively, was shown in Fig. S3 b, d. The limits of detection reached 0.048 mg/L and 1.43×10^{-8} M,

respectively. It is shown that the proposed flexible substrate is capable of in situ detection of THR on the surface of different samples while also exhibiting high sensitivity and wide applicability to different targets, providing a reliable platform for the detection of different samples and analytes.

Table 1
Distribution of the Thiram pesticide Raman peaks.

Raman band (cm ⁻¹)	Vibrational modes
441	C=S stretching mode and CH ₃ NC deformation
560	SS stretching mode
848	CH ₃ N stretching vibrations
972	CSS anti-symmetric stretching
1036	CH ₃ N and -CH ₃ stretching vibrations
1084	CH ₃ N stretching vibrations
1145	CH ₃ N and C=S stretching vibrations
1378	CH ₃ Rocking mode and C-N stretching vibrations
1395	C-N stretching and -CH ₃ bending vibrations
1460	C-N stretching vibrations and -CH ₃ Rocking mode

Table 2
Spiked recovery test of THR residue on apple surface.

Additive quantity mg/L	Flexible SERS substrate			HPLC		
	Recovery amount mg/L	Recovery %	RSD %	Recovery amount mg/L	Recovery %	RSD %
2	2.05	102.63	6.03	2.06	103.06	4.16
	1.83	91.41		2.16	107.86	
	2.01	100.28		2.24	112.02	
5	4.52	90.45	4.36	5.04	100.83	3.38
	4.16	83.13		4.72	94.41	
	4.43	88.64		4.82	96.38	
10	9.22	92.24	7.34	10.65	106.54	4.34
	10.52	105.17		11.32	113.19	
	9.35	93.54		11.60	116.00	

4. Conclusion

In this study, a label-free SA-CTS@AgNCs flexible SERS detection platform was effectively developed. To accomplish AgNCs adsorption and enrichment, the platform used the excellent pore structure of sodium alginate-chitosan composite gel as a carrier. Meanwhile, the surface-enhanced Raman scattering effect of AgNCs was utilized to achieve high sensitivity detection. The better homogeneity and batch-to-batch reproducibility of the substrate guaranteed the validity of the experimental results. Moreover, the time lag between sample collection and laboratory analysis in the conventional method was successfully addressed by integrating this flexible SERS platform with a portable Raman spectrometer, leading to a shorter detection cycle and improved convenience for food safety monitoring. In addition, this method was effectively utilized for the in situ detection of thiram on apples, with a detection limit as low as 0.055 mg/L. This is significantly lower than the maximum residue limits (MRLs) of pesticides established by pertinent government agencies in China (GB2763—2021) and the United States (40 CFR 180.132). The established SERS analytical platform has a wide range of potential applications and provides a novel idea for the in situ detection of other pesticide residues. Future studies can further optimize the performance of the method and explore its applicability to different food samples for practical applications.

CRediT authorship contribution statement

Zhiming Guo: Supervision, Project administration, Funding acquisition, Conceptualization. **Yuxia Zheng:** Writing – original draft, Software, Investigation. **Chen Wang:** Resources, Methodology. **Heera Jayan:** Writing – review & editing. **Limei Yin:** Methodology, Data curation. **Hesham R. El-Seedi:** Writing – review & editing. **Yunyun Gong:** Writing – review & editing. **Xiaobo Zou:** Resources, Formal analysis.

Declaration of competing interest

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2024.127168>.

Data availability

Data will be made available on request.

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