



Original Article

Fabrication of SERS Substrates Via Green Synthesis of ZnO/Ag Hybrid Nanomaterial for Herbicide Detection

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Abstract: ZnO nanoparticles and ZnO/Ag hybrid nanomaterial were successfully synthesized via a green route using *Brassica juncea* leaf extract as a natural reducing and stabilizing agent. The effects of amount of 5, 10, and 15g dried leaves and Ag concentration of 1, 5, and 10 wt% on the structural and optical properties of the materials were investigated by UV-Vis spectroscopy, X-ray diffraction (XRD), and FE-SEM analysis. The results indicated that ZnO synthesized with extract from 5g dried leaves exhibited more improved crystallinity, lower impurity content, and more uniform morphology. Ag nanoparticles (AgNPs) were subsequently deposited onto the surface of ZnO nanoparticles using mustard leaf extract prepared from 5g of dried leaves. The ZnO/Ag 5 wt% hybrid nanomaterial showed enhanced optical absorption and a homogeneous distribution of Ag nanoparticles. The synthesized ZnO/Ag 5 wt% hybrid nanomaterials were applied as SERS-active coatings on paper substrates for the detection of 2,4-dichlorophenoxyacetic acid (2,4-D). Characteristic Raman signals of 2,4-D were clearly detected down to concentration of 10^{-10} M, demonstrating the high sensitivity of the SERS substrate. These findings suggest that green-synthesized ZnO/Ag hybrid nanomaterial paper-based SERS substrates are promising for low-cost and environmentally friendly pesticide sensing applications.

Keywords: Green synthesis, SERS, ZnO/Ag hybrid nanomaterial.

1. Introduction

Surface-enhanced Raman scattering (SERS) is an optical phenomenon in which Raman signals are significantly amplified when molecules are adsorbed onto plasmonic metal surfaces, owing to the

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localized surface plasmon resonance (LSPR) effect [1]. This phenomenon was first reported by Martin Fleischmann et al., in 1974 [2]. Since then, SERS has been widely applied in various fields, including optics, biomedicine, food safety, and environmental analysis [3-5].

Compared with conventional analytical techniques such as high-performance liquid chromatography (HPLC), mass spectrometry, UV-vis spectroscopy, and electrochemical methods, SERS exhibits superior advantages in terms of rapid detection, high sensitivity, and minimal sample preparation [6-9]. Moreover, this technique enables non-destructive analysis and shows great potential for detecting analytes at trace levels. In recent years, significant research efforts have been devoted to the development of ultrasensitive sensors based on SERS, aiming to achieve high sensitivity and accuracy, low cost, and reusability [10-12].

The SERS effect is generally explained by two main enhancement mechanisms. Those are the electromagnetic (EM) mechanism and the chemical (CM) mechanism. The EM mechanism plays a dominant role and originates from the LSPR effect of noble metal nanoparticles such as Ag, Au, and Pt [13-16], while the CM mechanism involves charge transfer between the metal surface and the analyte molecules [17, 18]. The combination of these mechanisms enables highly sensitive detection at trace concentrations. However, challenges such as high cost, poor stability, limited uniformity, and low reusability still hinder the practical application of SERS substrates [19,20]. In particular, noble metal nanoparticles like Ag are prone to oxidation and aggregation, leading to a decrease in enhancement performance.

To overcome these limitations, hybrid structures combining semiconductors and metals, such as ZnO, CuO, TiO₂, MnO₂, and Fe₂O₃, have been developed to improve charge transfer efficiency, uniformity, and sensitivity of SERS substrates [21-24]. Among these materials, ZnO has been widely used due to its low cost, high optical stability, good photosensitivity, biocompatibility, and reusability [25-28]. Conventional synthesis methods for ZnO/Ag material, including chemical reduction [29], hydrothermal [30], and evaporation techniques [31], can produce highly crystalline and uniform materials. However, these methods often require sophisticated equipment, toxic chemicals, and long processing times, making them less environmentally friendly and economically viable.

In this context, green synthesis using plant extracts as reducing and stabilizing agents has emerged as a sustainable and efficient approach. Several studies have reported the successful synthesis of ZnO/Ag hybrid material using biological sources such as *Carthamus tinctorius* L. [32], *Moringa oleifera*, mint leaves, citrus peel [33], and *Spilanthes acmella* [34].

However, studies utilizing Green mustard (*Brassica juncea*) for the synthesis of ZnO/Ag nanomaterials remain limited, particularly in the fabrication of SERS substrates. In this work, Green mustard leaf extract that rich in vitamin C, flavonoids, and polyphenols [35] was employed as a bioreducing agent to synthesize ZnO/Ag hybrid nanomaterial via an environmentally friendly approach. The obtained materials are expected to exhibit high stability, low cost, and excellent performance for SERS-based detection of trace-level analytes.

Table 1. Comparison of representative SERS substrates reported in the literatures and the present work.

S.no.	Structure	Fabrication	Analyte	LOD	Ref.
1	AuNPs	Citrate reduction	MG	$2.9 \times 10^{-6} \text{M}$	[16]
2	ZnO/Ag	Hydrothermal	Organic pollutants	10^{-7}M	[24]
3	Paper/Ag	Dip coating	Pesticides	10^{-8}M	[30]
4	AgNPs	Citrate reduction	2,4-D	10^{-7}M	[39]
5	Paper/ZnO/Ag	Green synthesis	2,4-D	10^{-10}M	Current work

2. Materials and methods

2.1. Materials

Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$), silver nitrate (AgNO_3), and sodium hydroxide (NaOH) were purchased from SBC Scientific and used without further purification. Filter paper (Newstar) was used for filtration. Fresh leaves of Green mustard was obtained from a local market. Deionized (DI) water was produced in the laboratory. A herbicide solution containing 2,4-D (2,4-dichlorophenoxyacetic acid) was used as the target analyte.

2.2. Preparation of Green Mustard

Fresh Green mustard leaves were thoroughly washed several times with tap water to remove dust and impurities. The cleaned leaves were air-dried for approximately 60 minutes and then oven-dried at 60°C for 20 hours to remove moisture while preserving bioactive compounds.

The extract process was prepared by grinding 5 g, 10 g, and 15 g of dried leaves into fine powder and dispersing them in 200 mL of DI water. The mixture was further homogenized and then subjected to ultrasonic treatment for 15 minutes to enhance extraction efficiency. The resulting solution was filtered to remove solid residues, and the filtrate was stored at 4°C for subsequent use.

2.3. Synthesis of ZnO Nanoparticles

In a typical synthesis, 2.19 g of $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ was dispersed in 50 mL of DI water and stirred at 80°C for 30 minutes using a magnetic stirrer. Subsequently, 100 mL of Green mustard leaf extract (with various amounts of 5, 10, 15 g of dried leaves) was added, and the mixture was continuously stirred for 2 hours. The pH of the solution was maintained at 7 by the gradual addition of 0.1M NaOH .

After completion of the reaction, the solution was allowed to cool naturally, followed by vacuum filtration and washing with DI water. The obtained product was dried at 80°C for 1 hour and then calcined at 500°C for 2 hours to obtain crystalline ZnO nanoparticles.

2.4. Synthesis of Ag Nanoparticles

Ag nanoparticles were synthesized via a green reduction method using mustard leaf extract. Briefly, 50 mL of 0.01M AgNO_3 solution was heated to 80°C under continuous magnetic stirring. Subsequently, 50 mL of mustard leaf extract was added dropwise into the AgNO_3 solution. The reaction mixture was maintained at 80°C for 1 hour until the solution color changed from colorless to dark brown, indicating the formation of AgNPs. The obtained suspension was centrifuged at 5000 rpm for 5 min, washed several times with deionized water and ethanol, and dried at 100°C for further characterization.

2.5. Deposition of Ag Nanoparticles on ZnO Nanoparticles Surface

ZnO nanoparticles (0.3 g) were dispersed in 50 mL of Green mustard leaf extract from 5g dried leaves and ultrasonicated for 10 minutes. Then, 2.8, 14.0, 28.0 mL of 0.01M AgNO_3 solution (corresponding to 1, 5, 10 wt% Ag) was added, and the mixture was heated at 80°C for 1 hour under continuous stirring.

After the reaction, the mixture was cooled to room temperature and centrifuged at 5000 rpm for 5 minutes. The collected solid was washed several times with DI water and dried at 100°C for 1 hour to obtain the final ZnO/Ag hybrid nanomaterial, denoted as ZnO/Ag 1%, ZnO/Ag 5%, and ZnO/Ag 10%.

3. Results and Discussion

3.1. UV-Vis Spectroscopy Analysis

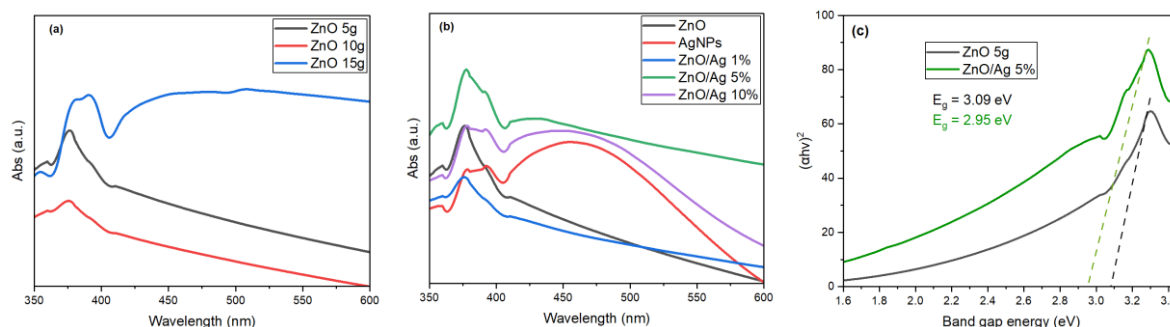


Figure 1. (a) UV-Vis spectra of ZnO nanoparticles with green mustard extract of 5 g, 10 g, and 15 g dried leaves. (b) UV-Vis spectra of ZnO nanoparticles, Ag nanoparticles, and ZnO/Ag synthesized using extract of 5g dried leaves. (c) Tauc plots for determining the optical band gap (E_g) of ZnO 5g and ZnO/Ag 5%.

The UV-Vis absorption spectra of ZnO nanoparticles synthesized using different mustard leaf extract of 5, 10, 15 g dried leaves are shown in Fig. 1(a). All samples exhibit an absorption edge at approximately 370-390 nm, characteristic of ZnO nanoparticles [36, 37]. Among the samples, ZnO synthesized with extract of 5 g dried leaves exhibits a sharper absorption edge and lower background absorption, indicating fewer residual organic species and a cleaner surface. In contrast, the 10g and 15 g samples show broader absorption features and higher baseline intensity, suggesting increased phytochemical residues. Therefore, the 5g sample was selected as the optimal ZnO nanomaterial for further Ag nanoparticles deposition on ZnO surface. The UV-Vis spectra of AgNPs and ZnO/Ag hybrid nanomaterials are presented in Fig. 1(b). AgNPs exhibit a surface plasmon resonance (SPR) band in the range of 410-460 nm, confirming the formation of silver nanoparticles [25, 32]. All ZnO/Ag nanomaterials display enhanced visible-light absorption due to the combined contribution of ZnO band-gap absorption and Ag plasmon resonance [25, 27]. Among them, the ZnO/Ag 5% sample shows the strongest absorption intensity, indicating a more effective distribution of Ag nanoparticles on the ZnO surface. The Tauc plots in Fig. 1(c) reveal optical band-gap energies of 3.09 eV for ZnO and 2.95 eV for ZnO/Ag 5%. The slight change in the band gap confirmed the presence of an interfacial charge transfer effect between ZnO and Ag, which contributed to the enhanced SERS performance through chemical mechanism. Therefore, ZnO/Ag 5% was identified as the optimal composition for subsequent SERS investigations.

3.2. X-ray Diffraction (XRD) Analysis

The XRD patterns of ZnO nanoparticles synthesized using different extract of 5g, 10g, and 15g dried leaves are shown in Fig. 2(a). All samples exhibit characteristic diffraction peaks at 31.7° , 34.4° , 36.2° , 47.5° , 56.6° , 62.8° , and 67.9° , corresponding to the (100), (002), (101), (102), (110), (103), and (112) planes of hexagonal wurtzite ZnO, respectively, in agreement with previous reports [36,37]. No impurity phases were detected, confirming the successful formation of crystalline ZnO. To further evaluate the crystallinity of the synthesized ZnO nanoparticles, the average crystallite size was estimated using the Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos \theta}$$

where D is the crystallite size, K is the Scherrer constant (0.9), λ is the X-ray wavelength (0.15406 nm for Cu K_{α} radiation), β is the full width at half maximum (FWHM) of the diffraction peak in radians, and θ is the Bragg angle. The crystallite size was estimated from the ZnO (101) diffraction peak because it is the most intense and well-resolved reflection. The FWHM values of the ZnO (101) peak decreased from 0.5326° for ZnO 15g to 0.4354° for ZnO 10g and 0.3354° for ZnO 5g, corresponding to crystallite sizes of 15.70, 19.21, and 24.94 nm, respectively. The progressive reduction in the FWHM of the ZnO (101) peak corresponds to an increase in crystallite size from 15.70 to 24.94 nm. As all samples were calcined under identical conditions, the difference is attributed mainly to variations in the nucleation and growth behavior during the green synthesis at various amounts of leaf extract, as the result, a slight difference in ZnO nanostructures can be seen in FE-SEM images (Fig. 3). Therefore, the ZnO synthesized using 5 g of dried mustard leaves, which exhibited the largest crystallite size, was selected for subsequent Ag deposition.

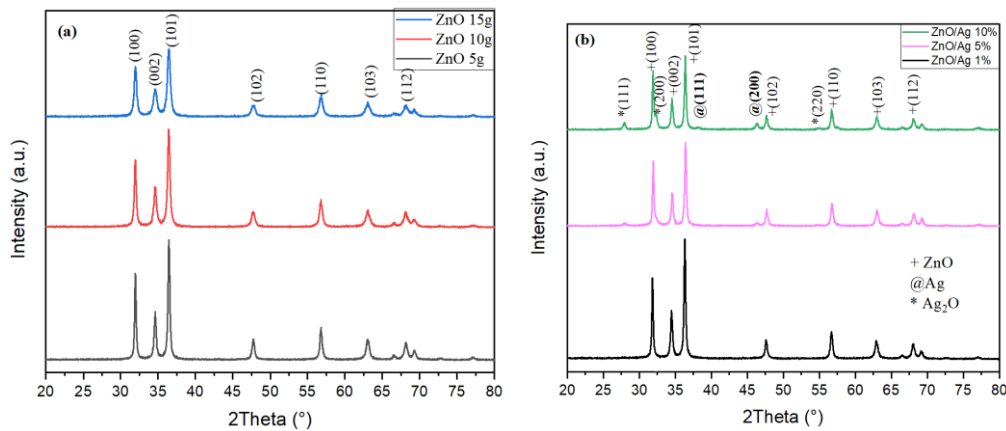


Figure 2. (a) XRD patterns of ZnO nanoparticles synthesized using different extract (5g, 10g, and 15g dried leaves), (b) XRD patterns of AgNPs and ZnO/Ag hybrid nanomaterials with 1wt%, 5wt%, 10 wt% of Ag.

The XRD patterns of AgNPs and ZnO/Ag hybrid nanomaterials with 1%, 5%, 10% of Ag are presented in Fig. 2(b). The characteristic diffraction peaks of wurtzite ZnO remain unchanged after Ag deposition, indicating that the crystal structure of ZnO is preserved. Additional diffraction peaks at approximately 38.1° and 44.3° are assigned to the (111) and (200) planes of metallic Ag, confirming the successful incorporation of Ag nanoparticles onto the ZnO surface [21, 23]. The crystallite size of the ZnO/Ag hybrid nanomaterials was also estimated using the Scherrer equation. The ZnO (101) diffraction peak exhibited FWHM values of 0.2624° , 0.2753° , and 0.2660° for the ZnO/Ag 1%, 5%, and 10% samples, corresponding to average crystallite sizes of 31.87, 30.33, and 31.42 nm, respectively. These similar crystallite sizes suggest that Ag loading has little effect on the crystal growth of ZnO. Furthermore, the characteristic diffraction peaks of hexagonal wurtzite ZnO remain unchanged after Ag deposition, confirming that the crystal structure of ZnO is preserved. For the Ag phase, the crystallite size was estimated from the Ag (200) diffraction peak. The ZnO/Ag 5% and ZnO/Ag 10% samples exhibited FWHM values of 0.4077° and 0.4871° , corresponding to Ag crystallite sizes of 20.73 and 17.34 nm, respectively. For the ZnO/Ag 1% sample, the Ag diffraction peak was too weak to allow reliable crystallite-size estimation because of the low Ag content. Overall, the ZnO/Ag 5% sample exhibited well-defined diffraction peaks without detectable impurity phases and possessed highly crystalline ZnO together with crystalline Ag nanoparticles, providing a suitable structural basis for efficient charge transfer and plasmonic enhancement in subsequent SERS applications.

3.3. Field Emission Scanning Electron Microscopy (FE-SEM) Analysis



Figure 3. The FE-SEM images of synthesized ZnO nanoparticles with different extract of 5 g, 10 g, and 15 g dried leaves (a-c).

The surface morphology of ZnO nanoparticles synthesized using different amounts of mustard leaf extract is presented in Fig. 3(a-c). It can be observed that all samples exhibit aggregated nanostructures, which is a common feature of ZnO nanoparticles synthesized via green methods due to the presence of phytochemical capping agents [29,36]. For the ZnO sample prepared with extract of 5g dried leaves (Fig. 3a), the particles show relatively uniform morphology with less severe aggregation and a more open structure. This indicates that a moderate amount of phytochemicals is sufficient to control nucleation and growth without excessively covering the particle surface. In contrast, the ZnO synthesized with extract of 10g dried leaves (Fig. 3b) exhibits increased aggregation, with particles forming larger clusters. This suggests that a higher concentration of organic compounds enhances particle-particle interactions, leading to partial agglomeration. The ZnO sample synthesized using 15 g of dried leaves (Fig. 3c) exhibits a more irregular and densely aggregated morphology, forming a compact structure. This behavior can be attributed to the excessive presence of phytochemicals, which may act as both reducing and binding agents, resulting in particle clustering and reduced surface accessibility.

Combining the SEM observations with UV-Vis and XRD results, the ZnO sample synthesized with extract of 5g dried leaves demonstrates a more favorable morphology with better dispersion, higher crystallinity, and fewer surface impurities. These characteristics are crucial for subsequent Ag nanoparticles deposition, as they provide more accessible active sites and facilitate stronger ZnO-Ag interaction, which is beneficial for enhancing SERS performance.

The SEM images of AgNPs and ZnO/Ag hybrid nanomaterials synthesized using 5g of mustard leaf extract are shown in Fig. 4(a-d). An aggregation of AgNPs, forming compact clusters with rough surfaces, is observed clearly in Fig. 4(a). After Ag deposition process onto ZnO surface, the morphology of ZnO/Ag 1% nanomaterial shows limited AgNPs coverage on the ZnO surface as shown in Fig. 4(b), which may result in a low density of plasmonic active sites. In contrast, the morphology of ZnO/Ag 5% nanomaterial in Fig. 4(c) exhibits a more uniform distribution of Ag nanoparticles while preserving the hierarchical ZnO structure. This morphology may contribute to enhanced SERS performance by promoting the formation of electromagnetic hot spots and increasing the available surface area for analyte adsorption. An increasing of the Ag concentration to 10% leads to particle agglomeration and partial coverage of the ZnO surface, which may reduce the accessibility of active sites as shown in Fig. 4(d). Therefore, the ZnO/Ag 5% sample is considered as optimal hybrid nanomaterial due to its homogeneous morphology and balanced Ag distribution, which are beneficial for enhancing SERS performance.

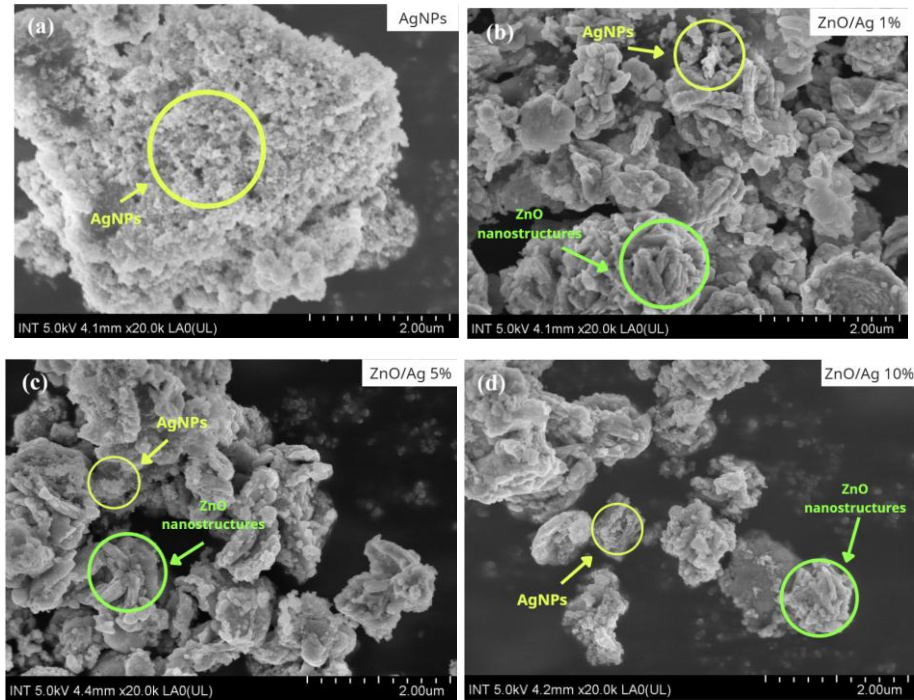


Figure 4. The SEM images of AgNPs (a), ZnO/Ag 1% (b), ZnO/Ag 5% (c), and ZnO/Ag 10% (d) synthesized using extract of 5 g dried leaves.

3.4. Strategy of SERS Detection

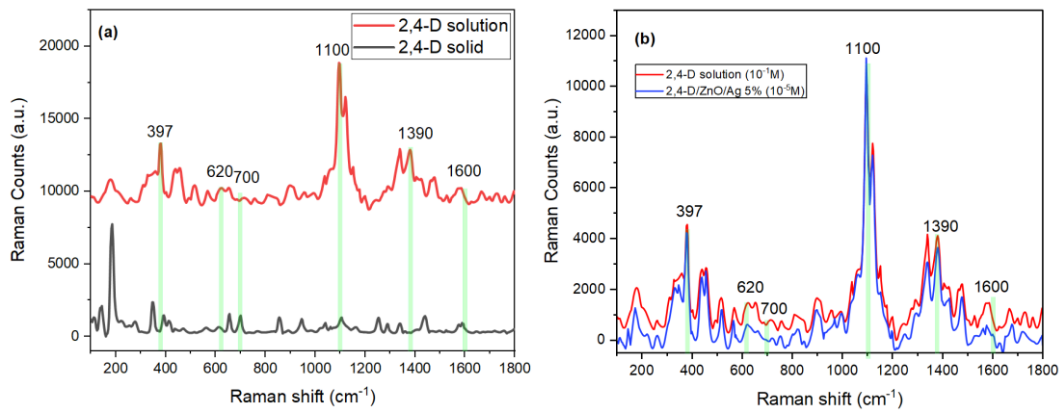


Figure 5. (a) Raman spectra of solid 2,4-D on a glass substrate and 2,4-D solution deposited on filter paper. (b) Raman spectra of 2,4-D solution deposited on filter paper and ZnO/Ag 5% SERS substrate.

Fig. 5(a) presents the conventional Raman spectra of solid 2,4-D and 2,4-D solution deposited on filter paper. The solid sample exhibits several characteristic vibrational bands of 2,4-dichlorophenoxyacetic acid, whereas the Raman signal of the solution is significantly weaker due to the low scattering cross-section and limited interaction with the excitation laser. This result highlights the difficulty of detecting trace levels of 2,4-D using conventional Raman spectroscopy. Figure 5(b) compares the Raman spectrum of 2,4-D solution (10^{-1} M) deposited on a bare paper substrate with that

obtained on the ZnO/Ag 5% (10^{-5} M) paper substrate. On the bare paper, only weak Raman bands can be observed because of the inherently low Raman scattering cross-section of 2,4-D and the absence of signal enhancement. In contrast, the ZnO/Ag 5% substrate produces a significant increase in Raman intensity while preserving the characteristic vibrational bands of 2,4-D at approximately 397, 620, 1100, 1390, and 1600 cm^{-1} . Among these bands, the peak at 1100 cm^{-1} exhibits the most pronounced enhancement, indicating the high SERS activity of the ZnO/Ag substrate. The substantial increase in signal intensity confirms that the incorporation of Ag nanoparticles effectively generates plasmonic hotspots and facilitates interfacial charge transfer with ZnO, leading to enhanced electromagnetic and chemical contributions to the SERS effect. These results demonstrate that the ZnO/Ag 5% substrate is highly effective for the sensitive detection of trace amounts of 2,4-D.

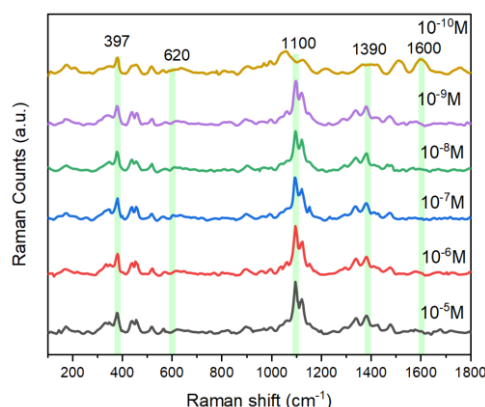


Figure 6. SERS spectra of 2,4-D at different concentrations (10^{-5} - 10^{-10} M) detected using the ZnO/Ag 5% coated paper substrate.

Fig. 6 shows the SERS spectra of 2,4-D recorded on the ZnO/Ag 5% paper substrate at concentrations ranging from 10^{-5} to 10^{-10} M. Characteristic Raman bands of 2,4-D remain observable even at low concentrations, demonstrating the excellent enhancement capability of SERS substrate based on the ZnO/Ag 5% hybrid nanomaterial. The major bands are located at approximately 397, 620, 1100, 1390, and 1600 cm^{-1} . The band near 397 cm^{-1} is assigned to the C-Cl deformation vibration, while the bands around 620 cm^{-1} originate from vibrations of the chlorinated aromatic ring. The prominent peak around 1100 cm^{-1} corresponds to C-O stretching and C-COO $^{-}$ vibrations, whereas the band at ~ 1390 cm^{-1} is attributed to the symmetric stretching vibration of the carboxylate group (COO $^{-}$). The peak near 1600 cm^{-1} is associated with the C=C stretching vibration of the aromatic ring. These assignments are consistent with previously reported Raman and SERS studies of 2,4-D herbicides on silver-based substrates [39].

The SERS intensity gradually decreases with decreasing analyte concentration. However, the characteristic bands remain distinguishable down to 10^{-10} M, indicating high detection sensitivity. At 10^{-10} M, the spectrum becomes noisier and several characteristic peaks exhibit reduced intensity, suggesting that the concentration approaches the practical detection limit of the substrate. The strong enhancement can be attributed to the localized surface plasmon resonance (LSPR) of Ag nanoparticles together with the charge-transfer interaction between ZnO and Ag, which promotes electromagnetic and chemical enhancement mechanisms. Similar enhancement behavior has been reported for Ag-based SERS substrates used in pesticide detection [19, 20, 38].

Overall, the ZnO/Ag 5% paper substrate demonstrates excellent SERS activity for 2,4-D detection, with a practical detection limit reaching approximately 10^{-10} M, indicating its potential application for rapid monitoring of herbicide residues in environmental and agricultural samples.

4. Conclusion

ZnO nanoparticles and ZnO/Ag hybrid nanomaterials were successfully synthesized via a green synthesis route using *Brassica juncea* leaf extract. Among the investigated conditions, the ZnO nanoparticles prepared with extract of 5g dried leaves exhibited superior optical properties, a higher crystallinity, and a more uniform morphology, making it suitable for subsequent Ag nanoparticles deposition. The incorporation of Ag nanoparticles significantly improved the optical response of ZnO, and the ZnO/Ag 5% hybrid nanomaterial demonstrated the most favorable structural and morphological characteristics.

The ZnO/Ag 5% hybrid nanomaterial was successfully applied as a paper-based SERS substrate for the detection of 2,4-D herbicide. Characteristic Raman signals of 2,4-D remained detectable down to approximately 10^{-10} M, indicating high sensitivity toward trace-level analytes. The enhanced SERS performance is attributed to the synergistic contribution of Ag-induced plasmonic enhancement and ZnO-assisted charge-transfer effects. The developed ZnO/Ag paper substrate offers a simple, low-cost, and environmentally friendly platform with potential applications in agricultural and environmental monitoring.

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