Fabrication process and characterization of AgNPs/PVA/cellulose as a SERS platform for in-situ detection of residual pesticides in fruit

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Keywords: AgNPs/PVA/ Cellulose, Raman spectrum, SERS spectrum, platform, detection of pesticides

Supplementary material for this article is available online

Abstract

In this work, AgNPs/PVA/Cellulose was used as a substrate material for surface Raman scattering enhancement. Silver nanoparticles (AgNPs) was synthesized by Lee and Meisel’s method with the average particles size of 15.4 nm. Then, this silver colloid was made a homogenous coating on polyvinyl alcohol and cellulose film and structural characteristics of this material were determined using Scanning Electron Microscopy (SEM). The findings demonstrated that the Raman shifts of the pesticide will be identified by the SERS method at 1660 cm⁻¹, 2234 cm⁻¹ (strong intensity), and at 3077 cm⁻¹, 1033 cm⁻¹, 1457 cm⁻¹ (medium intensity) when using the excited laser with wavelength of 532 nm. Under excited laser, the limit of chlorfenapyr detection is 1 ppm (mg l⁻¹), allowing determination of chlorfenapyr residue in food. Potential applications identified food samples containing chlorfenapyr residue for rapid detection, low cost, non-destructive nature and minimal sample preparation.

1. Introduction

Surface enhanced Raman scattering (SERS) is a highly-sensitive vibrational spectroscopy technique used to detect and identify the structure of trace analytes in samples. It works through the excitation of localized plasmons and thus causing the amplification of the local electromagnetic fields generated from such excitation [1]. The properties of these localized surface plasmons resonance have been linked to other physical properties of the noble metal nanoparticles used as plasmonic materials, including size, shape and surrounding media [2]. Plasmonic materials are chosen to preferably possess high surface area and special optical property in order to offer an amplified response. Gold and silver nanostructures have long been utilized, together with other alloys such as Au–Cu, Ag–Au, Ag–Zn, etc as substrates for SERS [3, 4]. In particular, silver nanostructures have received extensive attention in their applications for biological, optical and electrical sensing. However, the instability of the existing silver based substrates, leading to the requirement of strict conditions for preservation, has been the main issue limiting the utility of SERS in practice. Several stabilizers have been investigated, such as poly(N-vinylpyrrolidone) (PVP), carboxylic acid, sodium citrate, nano cellulose, starch, and protein [5–9]. The use of stabilizers, while improving the stability, also interfere with the scattering signals, which is extremely undesirable especially in quantitative analysis. Bearing this in mind, our study has proposed the synthesis of a silver nanoparticles based substrate on a cellulose membrane, covered with polyvinyl alcohol (PVA) (AgNPs/
PVA/cellulose) to enhance the physical stability, while still maintain a non-contaminated SERS spectrum. The synthesize substrate was aimed to be used as a SERS platform for in situ detection of Chlorfenapyr, a pesticide residue found in fruit and causes serious problems to humans and other organisms. To the best of our knowledge, till now, there is lack of reports on the synthesis of AgNPs/PVA/cellulose substrate for in situ detection of Chlorfenapyr.

Therefore, in our study, AgNPs/PVA/cellulose substrate was fabricated by coating a layer of PVA of various concentrations in deionized water on cellulose membrane. The synthesized PVA/cellulose base was further covered with solutions of silver nanoparticles of different ratios. The finished product possesses beneficial features, including environmental friendliness, cost effectiveness, and high enhancement of Raman scattering signals. It was tested for SERS performance on XploRA ONE™ at 532 nm, on produce samples with pesticides residue. Comparing with other methods such as HPLC/MS or GC/MS, this technique offers great benefits regarding sensitivity and expenditure [9].

2. Materials and methods

2.1. Materials
Silver nitrate AgNO₃ (>99%), trisodium citrate (TSC) (>99%), polyvinyl alcohol (>95%, Mw = 89 000–98 000) were obtained from Sigma Aldrich (USA). Chlorfenapyr 240SC was purchased from Ngoc Yen Ltd (Vietnam). Deionized water with resistance of 18 MΩ cm was used in all the experiments.

2.2. Synthesis of silver nanoparticles (AgNPs)
AgNPs were synthesized according to the procedure proposed by Lee and Meisel’s [10] (figure 1). Briefly, 0.5 ml of 0.1 M AgNO₃ (~8.5 mg) was added into 75 ml deionized water under constant stirring (1100 rpm) at 125 °C. As the solution started to boil, 0.1 ml TSC 0.05 M was added while maintaining the boiling for 90 min AgNPs were successfully formed when the solution turned from clear to yellow. The synthesized AgNPs were kept in the dark at room temperature for further use.

2.3. Stability study of AgNPs
The stability of AgNPs was evaluated by their size and UV–vis absorption after 1, 5, 10, 15, 20 and 30 days at room temperature. The sample was stored in a screw-capped, amber colored glass bottle for prevention of photooxidation.

2.4. Synthesis of AgNPs/PVA/cellulose substrate
Various concentrations of PVA solution were prepared in deionized water by dissolving different amounts (0.5, 1.0, 1.5 g) of PVA in 10 ml deionized water under constant stirring (800 rpm) at 80 °C. A thin layer of different PVA solutions was covered on strips of cellulose filter membrane with an active area of 2 cm × 6 cm by a dip coating, followed by drying at 50 °C.
An appropriate amount of the prepared AgNPs at different concentrations (2 ppm, 10 ppm and 50 ppm) was dipped on the top of the PVA/cellulose substrates. The experiments were carried out under inert atmosphere. The covered strips were kept at room temperature in dark and inert environment until being fully dried to obtain AgNPs/PVA/cellulose substrates for SERS measurement.

2.5. Conventional Raman and SERS measurements
20 μl of Chlorfenapyr solution at a certain concentration was placed on the active area of AgNPs/PVA/cellulose substrates. The dry sample was used for SERS assay measurements [11]. For comparison, conventional Raman spectra of the same Chlorfenapyr solution was also obtained without using the substrate. All Raman spectra were collected within the range of 500–3500 cm⁻¹ using 10× objective, 210 s exposure time, and 3 scans average per spectrum.

2.6. SERS detection limit determination
The detection limit of SERS assay using the synthesized substrate was determined by analyzing a series of Chlorfenapyr samples at different concentrations: 100 ppm, 10 ppm, 1 ppm, 100 ppb, and 10 ppb [12].

2.7. Characterization
The AgNP solutions were characterized with UV–vis absorbance method using a V-750 UV/Vis spectrophotometer (Jasco Co., Tokyo, Japan), dynamic light scattering (DLS) using a Zetasizer Nano ZS (ZEN 36000, Malvern Instruments, UK), transmission electron microscope (TEM) using a JEOL JEM-1400 (120 kV) and scanning electron microscopy (SEM) imaging using JEM-1400 (JEOL, Tokyo, Japan). Conventional Raman scattering and SERS measurements were performed on Raman microspectroscopy XploRA ONE™. Raman spectrometer was equipped with a diode laser emitting at 532 nm, a 600 grooves/mm holographic grating, and a Peltier–cooled CCD detector (1024 × 256 pixel).

3. Result and discussion

3.1. Synthesis of AgNPs
TEM was employed to study the morphology of AgNPs. The TEM image of the synthesized AgNPs shown in figure 2(a) revealed polydispersion of spherical AgNPs with the average size of 15 nm (figure 2(b)), which is consistent with the average size of (16.6 ± 0.8) nm determined by DLS (figure 2(c)). The polydispersity index of the AgNPs was determined as 0.528 indicating a polydisperse behaviour of AgNPs. The size distribution of AgNPs is in range of 10–100 nm, which is good for SERS signals [13]. Optical properties of spherical AgNPs were studied by UV–vis absorption spectroscopy. Obviously, the plasmon peak was observed at 404 nm (figure 2(d)) indicating the presence of nanosized Ag particles, which is in accordance with the aforementioned TEM and DLS results. The zeta potential of the AgNPs was calculated as −27.0 mV, which is closed to previous studies [14, 15]. The negatively charged surface of AgNPs could prevent their agglomeration due to generation of repulsion force between AgNPs, and thus, stabilizing the polydispersion of AgNPs in their colloidal solution. The zeta potential value of AgNPs in our study was found in range of −16 mV and −30 mV, which can be considered to be a state of dispersion [16].

3.2. Stability of AgNPs
Table 1 showed an increase in particle size with red shifts of λmax and a decrease in the absorbance, which is in accordance with the data reported by Agnihotri et al [17]. The stability of AgNPs was evaluated after 1, 5, 10, 15, 20 and 30 days at room temperature. The results revealed that AgNPs suspension can be stored stably within 10 days. After that period, extreme aggregation of AgNPs was found due to high surface energies of their suspension. The uncontrollable aggregation would lead to increased SERS intensity, which may cause significant spatial, time and sample-to-sample errors of SERS measurements [18]. Therefore, in our study, the deposition of AgNPs onto PVA/cellulose substrate was carried out right after the synthesis of AgNPs. When AgNPs was deposited on PVA/cellulose, without exposure to light, AgNPs can stay stably on PVA/cellulose.

3.3. Synthesis of AgNPs/PVA/cellulose
In order to determine the optimal PVA concentration to be used as the coverage on the cellulose membrane during the synthesis of AgNPs/PVA/cellulose, enhancement of SERS spectra of 10 ppm Chlorfenapyr samples deposited on AgNPs/PVA/cellulose substrates with different PVA concentrations was investigated. Figure 3 showed the SERS spectra from the assessed samples. In the case of the control sample where no PVA was covered on the surface of the cellulose membrane, no typical peaks for Chlorfenapyr were observed. However, for the other cases, the presence of most of the prominent Raman peaks was observed, including the C≡C and C–N
stretching in the pyrrole ring of Chlorfenapyr at 1660 cm$^{-1}$, the aromatic nitrile C≡N stretching at 3077 cm$^{-1}$, together with a C–O–C stretching at 1033 cm$^{-1}$ and a C–F stretching at 1457 cm$^{-1}$ [18]. The scattering intensity of these observed peaks varied according to the concentration of PVA. For the sample with no PVA coverage, the coating of AgNPs onto the cellulose filter membrane was not successful due to porous structure of the cellulose membrane, which did not facilitate any surface coating of the nanoscale AgNPs. The substrate with 0.5 g/10 ml of PVA started to give distinguishable peaks at typical positions, for the fact that PVA has worked as a surface filler and base for the attachment of AgNPs (as discussed later on figure 5). However, the intensity of the peaks was not optimal since lower concentration of PVA cannot thoroughly cover the surface of the cellulose membrane. In contrast, the Raman signal intensity of the samples with 1.0 g and 1.5 g/10 ml of PVA was

Figure 2. TEM image (a), particle size distribution determined by TEM (b) and DLS (c), UV–vis absorption spectra (d) and zeta potential measurement (e) of 50 ppm AgNP solution.
enhanced significantly but similarly. It can be ascribed to the fact that 1.0 g/10 ml of PVA concentration was enough to completely cover the membrane surface, leading to a little difference in the SERS enhancement effects of those two highest obtained PVA concentrations. Therefore, 1.0 g/10 ml of PVA concentration was chosen for further studies.

SERS performance at different AgNPs dilution ratios (2 ppm, 10 ppm and 50 ppm) was recorded in figure 4. In general, signal intensity was significantly enhanced in accordance to AgNPs concentration reached 50 ppm. In particular, without the coating of AgNPs, PVA/cellulose strips showed no enhancement of Raman scattering. At low concentration (2 ppm) of deposited AgNPs, the enhancement effect was extremely limited since the analytes shielded AgNPs on the substrate surface, leading to a decrease in the excitation of the surface plasmons. Typical signals for Chlorfenapyr at 1660 cm⁻¹ and 2234 cm⁻¹ were distinctively identified at the 10 ppm of AgNPs. The optimal amplification was observed at the 50 ppm of AgNPs, where signals indicating the stretching of C–O–C, C–F and aromatic nitrile were also observed [19–21]. The intensity of these enhanced signals was remarkable strengthened compared to the lower concentration of AgNPs, owing to the availability of substrate surface plasmons excitation. The results showed a downward trend of amplification ability as AgNPs concentration decreased from 50 ppm to 2 ppm. The 50 ppm concentration of AgNPs demonstrated the highest scattering intensity, and thus it was chosen as the optimal concentration for the substrate synthesis.

Top-view SEM images of the surface of cellulose filter membrane and AgNPs/PVA/cellulose substrate are shown in figure 5. A relatively uniform distribution of AgNPs on the surface of PVA/cellulose was observed after the deposition of AgNPs onto PVA/cellulose. The EDX results revealed that Ag, C, O are dominant elements of AgNPs/PVA/cellulose (figure S1 is available online at stacks.iop.org/RR/7/035019/mmedia). Once the cellulose filter was dip coated with PVA, cellulose fibres become thicker and the fibres in the inner layers become less visible. Therefore, the filter lost its porosity due to the completely pore-blocking behavior of the filter by a thick layer of polymer (figure S2).

Table 1. The stability data of AgNPs synthesized at room temperature.

| Day | Size (nm) | λ_max (nm) | Absorbance (a.u.) |
|-----|-----------|------------|-------------------|
| 1   | 15.5      | 404        | 1.18              |
| 5   | 16.1      | 405        | 1.07              |
| 10  | 21.8      | 410        | 0.74              |
| 15  | 29.5      | 411        | 0.68              |
| 20  | 30.7      | 411        | 0.68              |
| 30  | 31.3      | 418        | 0.65              |

Figure 3. SERS spectra of 10 ppm Chlorfenapyr using AgNPs/PVA/cellulose with various deposited amounts of PVA as substrates: (a) 1.5 g, (b) 1.0 g, (c) 0.5 g and (d) no PVA. Chlorfenapyr @ AgNPs/cellulose (no PVA) as a control sample. The concentration of AgNPs was controlled at 50 ppm for all samples.
3.4. Conventional Raman and SERS measurements
Chlorfenapyr as an analyte was prepared at 240,000 ppm and 1 ppm for conventional Raman microspectroscopy, while the same 1 ppm sample was analyzed using the synthesized AgNPs/PVA/cellulose substrate. Figure 6 reports the spectra from conventional Raman microspectroscopy and SERS assay with Chlorfenapyr samples at 1 ppm and 240,000 ppm concentration. The analyte was undetectable at 1 ppm using conventional Raman (without substrate). However, the same concentration showed remarkable detection using SERS with AgNPs/PVA/cellulose substrate (figure 7(c)), providing distinctive observation of various typical peaks. Chlorfenapyr sample at 240,000 ppm was readily detected with conventional Raman, yet the maximum allowance for Chlorfenapyr residue in food is <50 ppm. The results suggested high potential of SERS using AgNPs/PVA/cellulose substrate in detecting pesticide residues with exceptionally high sensitivity that conventional Raman is unable to offer.

3.5. SERS detection limit determination
SERS assays on an array of Chlorfenapyr from 10 ppb to 100 ppm were carried out to determine the limit of detection of AgNPs/PVA/cellulose substrate (figure 7). As shown in figure 7, typical peaks of Chlorfenapyr could be readily observed down to the concentration of 1 ppm, though signals for the C–O–C and C–F stretching [19] were lower. The two lowest concentrations, 100 ppb and 10 ppb, were undetectable as their spectra were identical to the negative control. The results further show the desirable scattering enhancement effect of AgNPs/PVA/cellulose substrate, as well as its high potential in food and pesticide regulating owing to high sensitivity with detection limit as low as 1 ppm (S/N = 3).
4. Conclusion

The successful synthesis of AgNPs/PVA/cellulose was demonstrated after optimizing different factors affecting the process. The prepared AgNPs were of the average size of 15 nm and coated on PVA/cellulose base at a 1:1 ratio, which gave the strongest scattering signals. The optimal concentration of PVA was found to be 1.0 g/10 ml of deH2O, resulting in a relatively high uniformity when coated on cellulose membrane. The synthesized AgNPs/PVA/cellulose showed localization of the AgNPs and the samples, preventing any leaking through the cellulose membrane (2 cm × 6 cm). With a detection sensitivity for Chlorfenapyr as low as 1 ppm, the synthesized SERS substrates allowed the detection and semi-quantification of pesticide residues in produce with lower cost, faster processing and analyzing time.

Acknowledgments

This research was supported by the Institute of Applied Materials Science, Vietnam Academy of Science and Technology.
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References

[1] Bhavya S, Renee R F, Anne-Isabelle H, Emilie R, Richard P and Van D 2012 SERS: materials, applications, and the future Mater. Today 15 16–25
[2] Bin T, Tian Z, Jun L, Ji Z, Young Y and Xunqai W 2017 Waste fiber powder functionalized with silver nanoprisms for enhanced Raman scattering analysis Nanoscale Res. Lett. 12 541
[3] Cecilia N 2007 Surface plasmons on metal nanoparticles: the influence of shape and physical environment J. Phys. Chem. 111 3806–19
[4] Huajun Q, Zhonghua Z, Xiron Y and Yinbo Q 2011 Dealloying Ag−Al alloy to prepare nanoporous silver as a substrate for surface-enhanced raman scattering: effects of structural evolution and surface modification Chem. Phys. Chem. 12 2118–23
[5] Huirong T, Dongmei F, Qiongqing L, Peng C, Jinpei G, Tao S, Xuan W, Jibran I and Yiping D 2012 Determination of tricyclazole content in paddy rice by surface enhanced Raman spectroscopy J. Food Sci. 77 105–9
[6] Hongjin J, Lingbo Z, Kyoung-sik M and Wong C P 2007 The preparation of stable metal nanoparticles on carbon nanotubes whose surfaces were modified during production Carbon 45 655–61
[7] Yudong L, Changji W, Ruiyun Y, Gang L, Youqiang C and Shangyuan F 2018 Ag-coated cellulose fibers as surface-enhanced Raman scattering substrates for adsorptive detection of malachite green Materials 11 1197
[8] Ying Z, Yuan T, Pinyi M, Aimin Y, Hanqi Z and Yanhua C 2015 Determination of melamine and malachite green by surface-enhanced raman scattering spectroscopy using starch-coated silver nanoparticles as substrate J. Analytical Methods 7 8116–1822
[9] Mehmet K, Ben N B and Sebastian W-H 2013 Hydrophobicity-driven self assembly of protein and silver nanoparticles for protein detection using surface-enhanced raman scattering Analyst 138 2906–13
[10] Lee P C and Meisel D 1982 Adsorption and surface-enhanced Raman of dyes on silver and gold sols J. Phys. Chem. 86 3391–5
[11] Liu Y, Ye B, Wan, Hao Y, Lan Y and Ouyang A 2013 Rapid quantitative analysis of dimethoate pesticide using surface-enhanced Raman spectroscopy Trans. ASABE 56 1043–9
[12] Han Z, Yan K, Ping L, Xin T, Jianwei P, Hui L and Yiping D 2016 Determination of pesticides by surface-enhanced raman spectroscopy on gold nanoparticle modified polydimethylacrylate Anal. Lett. 49 2268–78
[13] Ying Z, Yuan T, Pinyi M, Aimin Y, Hanqi Z and Yanhua C 2015 Determination of melanin and malachite by surface-enhanced Raman spectroscopy using starch-coated silver nanoparticles as substrate Anal. Methods 7 8116–22
[14] Vanitha G, Rajavel K, Veeravazhuthi V and Neelamegam P 2017 Physiochemical charge stabilization of silver nanoparticles and its antibacterial applications Chem. Phys. Lett. 669 71–9
[15] Salvioni L, Gahlbiati E, Colligo V, Alessio G, Avvakumova S, Corsi F, Tortora P, Prosperi D and Colombo M 2017 Negatively charged silver nanoparticles with potent antibacterial activity and reduced toxicity for pharmaceutical preparations Int. J. Nanomed. 12 2537–30
[16] Thomas M R 1968 Control of colloid stability through zeta potential zeta-meter Incorporated 1 1–372
[17] Agnihotri S, Mukherji S and Mukherji S 2014 Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy RSC Adv. 4 3974–83
[18] Fan M, Andrade G F S and Brolo A G 2019 A review on recent advances in the applications of surface-enhanced Raman scattering in analytical chemistry Anal. Chem. Acta 1097 1–29
[19] George Socrates 2004 Infrared and Raman Characteristic Group Frequencies: Tables and Charts (Middlesex, UK: The University of West London) (https://doi.org/10.1002/jps.1238)
[20] Tran D M, Lee B K and Nguyen L M T 2018 Methanol-dispersed of ternary Fe3O4@γ-APS/graphene oxide-based nanohybrid for novel removal of benzoic acid from aqueous solution J. Environ. Manage. 209 452–461
[21] Zhiyun Z, Huiyuan G, Thomas C, Arnab M, Amanda K, Jason C, White B X and Lili H 2016 Evaluation of postharvest washing on removal of silver nanoparticles (AgNPs) from spinach leaves J. Agric. Food Chem. 64 6916–22