



DEVELOPMENT OF SURFACE ENHANCED RAMAN SPECTROSCOPY SUBSTRATES FOR PESTICIDE DETECTION.

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Abstract- Nanotechnology and the synthesis of nanoparticles are attractive in part because of interesting, tunable optical and electrical properties that are not observed with bulk material. Surface Enhanced Raman Spectroscopy (SERS) is one of the powerful analytical tool for chemical, biological and pesticide trace detection due to its high sensitivity and specificity. A new fabrication approach, to obtain SERS substrates for pesticide detection was carried out by depositing silver nanoparticles using modified mirror reaction and physical deposition method. The prepared substrates were examined using Field emission scanning electron microscopy (FESEM). The prepared slides were then used to detect an organophosphate pesticide, Malathion. Due to its excellent Raman enhancement, reproducibility of the average spectrum and sensitivity, modified mirror reaction process is suitable to form silver nanoparticles for SERS substrates and for various applications.

Keywords – Surface Enhanced Raman Spectroscopy (SERS), Modified mirror reaction, Field emission scanning electron microscopy (FESEM), Organophosphate pesticide.

I. INTRODUCTION

Pesticides are largely used in agriculture throughout the world and pesticides play a critical role in protecting fruit crops. Organophosphorus pesticides are a group of relatively small molecules that kills insects and mites by attacking the central nervous system [1]. They are neurotoxic due to their acetyl cholinesterase inhibitory property and are harmful to human and animal lives. Malathion, an organophosphorus compound is suspected to cause child leukemia, anemia, kidney failure, and human birth defects. Due to its low toxicity in humans and harmful effects to the environment, it is necessary to develop a simple, rapid and low cost method for the determination of organophosphates and detect trace amounts in samples. Surface enhanced Raman spectroscopy (SERS) is a well known analytical method for probing the chemical and structural properties of a compound on the microscopic scale. A great deal of SERS active substrates have been obtained, and are made from pure metallic nanostructures, in particular Ag and Au with different shapes [2-7].

Several methods for producing SERS substrates for the detection of insecticides and pesticides are available in literature. Silver cube-like nano structures of uniform size have been synthesized using rapid sulphide mediated polyol method [8]. In this method, SERS substrates were prepared by adding silver nanocubes collosol suspensions to different concentration of analytes, by dropping the mixture on to glasses and naturally drying at room temperature. Silver (Ag) hydrosols and gold (Au) nanoparticles can be obtained by citrate or hydroxylamine reduction method [9].

A compact Raman spectroscopy system, using silver nanorod film fabricated by magnetron sputtering is already in use for field portable pesticide detection [10]. It has been reported that nanoparticles of gold and silver are effective systems for the removal of Chlorpyrifos and Malathion from water [11]. The time dependent removal of these pesticides from water has been monitored by UV-Visible spectroscopy and the adsorption of pesticides on nanoparticles has been confirmed by infrared spectroscopy.

A new format of apta sensing (a particular class of biosensing where the biological recognition is a DNA or RNA aptamer) based on polymer gold nanoparticle composite microspheres for SERS detection of Malathion has already been performed [12]. A simple and rapid method using micro Raman spectroscopy for the determination of organophosphates pesticide residues have been reported [13]. Based on the results, it has been concluded that the method of using micro Raman spectroscopy can be utilized for the determination of pesticide residues on the surface

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of fruits. An analytical methodology using colloidal gold nanoparticles using Surface Enhanced Raman Imaging Spectroscopy and multivariate curve resolution have been reported [14].

In the present study, the substrates were prepared by modified mirror reaction (MMR) and by physical deposition using nanocluster deposition system (AgNC). The prepared substrates were then used to detect Malathion. The SERS spectra was recorded and compared with the already reported experimental Raman spectra.

II. EXPERIMENTAL DETAILS

A. Experimental Methods

The substrates for SERS detection were prepared using the modified mirror reaction. In this method, 20 μL of 3.2 wt.% KOH solution was added to 18 mL of a 2 wt.% AgNO_3 solution and this solution was mixed with a magnetic mixer to form a fine brown precipitate of Ag_2O . Ammonium hydroxide (29.5%) was added to the mixture drop by drop until all the precipitate dissolves. Then 6 wt.% AgNO_3 solution was added to turn the solution yellow. On adding a drop of 6 wt.% ammonium hydroxide solution, the solution turned colourless. The solution was then mixed with 3 mL methanol, 10 μL 10wt.% $\text{Na}_3\text{P}_5\text{O}_{10}$, and 40 μL 5wt.% Na_2HPO_4 consecutively to form a yellow colloid. Finally, 6 mL 35 wt.% glucose was added and mixed, which yielded a colloidal solution. Glucose acts as a fixing agent. Substrates were also prepared with another fixing agent, vanillin. Nanoparticles form even in the absence of fixing agents. Smooth glass surfaces usually need fixing agents otherwise the formed nano particles will be rubbed off from the slides surface. One disadvantage in using fixing agents is that analysis and detection of glucose or vanillin cannot be done with this method and these fixing agents become contaminants.

Modified mirror reaction method was changed to another form by adding 2-thienyl boronic acid to precipitate the nano particles. An advantage with this method is that there are no fixing agents. For this 20 μL of 3.2 wt.% KOH solution and 18 mL of a 2 wt.% AgNO_3 solution was mixed to get a brown precipitate of Ag_2O . Ammonium hydroxide (29.5%) was added drop by drop to dissolve the brown precipitate. Then 6 wt.% AgNO_3 solution was added to get a yellow colloid. To this a few drops of 2-thienyl boronic acid was added to get a colloidal solution and no fixing agent was added. The use of fixing agents was avoided by roughing glass slides by physically rubbing the slides surface with sand paper (zero number) and cleaning it thoroughly with Piranha solution (a mixture of three parts of concentrated H_2SO_4 and one part of H_2O_2 (29.5%)) and washing it over and over with Milli Q water (resistivity 18.2 M Ωcm).

Glass slides were cut in to the dimension of 7.5 cm x 2.5 cm and cleaned with Piranha solution for few minutes. To clean the glass slides further, it was sonicated in propanol, acetone and Milli Q water. The cleaned glass slides were immersed in the colloidal suspension for 24 hours at room temperature to form Ag nano film on them.

Substrates of silver nano particles were also made by physical deposition on glass slides using nanocluster deposition system. The Nanodep60 nanocluster deposition system from Oxford Applied Research UK produces nano particles with high purity. Nanoclusters are obtained in plasma medium and the deposition is based on the principle of inert gas phase condensation. Metal atoms are sputtered by a DC magnetron and the silver particles are moved in an inert gas atmosphere directly on to glass slide which acts as the substrate. The super saturated metal atoms while flowing through the inert carrier gas, nucleates and forms clusters of nanometer scale.

The nanoclusters are aggregated in the aggregation chamber and are made to flow through an aperture into the deposition chamber. The deposition chamber is maintained at a slightly low pressure than the aggregation chamber. The distance between the substrate and apertures was approximately 55 cm. A working pressure of 5×10^{-5} milli bar was maintained during the aggregation gas (Ultra high pure Argon of 99.999% purity) flow and the flow rate was 100 sccm (standard cubic centimeter per minute).

B. Instrumentation

The Raman measurements were performed using LABRAM HORIBA micro Raman Spectrometer equipped with TE cooled CCD detector and an Argon Ion Laser of 9 mW power with excitation wave length of 514.4 nm.

Micro structure of the deposited films of silver by modified mirror reaction and as deposited silver nanoclusters were characterized using the Carl Zeiss Ultra 55 FESEM. An excitation voltage of 5 kV was applied and a working distance around 5 mm was maintained for obtaining the SEM image.

III. RESULTS

After preparing the colloidal suspension and depositing on the slides, the prepared substrates were examined using FESEM. The examined glass slides were used to detect Malathion using SERS.

A.

FESEM CHARACTERIZATION

The morphology of Ag nanoparticles prepared by modified mirror method was examined using FESEM and is shown in Fig. 1. It was seen that the prepared slides which showed nano roughness had a particle size of the order of 20 nm.

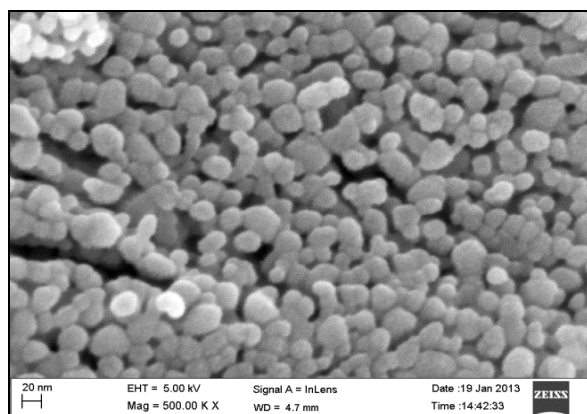


Fig. 1 FESEM image of the substrate prepared by Modified mirror reaction method.

To evaluate the SERS performance of the Ag nano film of the substrate, Crystal Violet was used as the probe molecule. For this Crystal Violet was diluted in milli-Q water by sequential dilution method and few drops were put over the prepared substrate using a micro pipette. Then the substrate was kept for drying inside the glove box and taken out only at the time of recording the SERS spectra. It was found that the observed peaks were more or less similar to that reported in literature [15]. This shows that the prepared substrate acts as a good SERS probe.

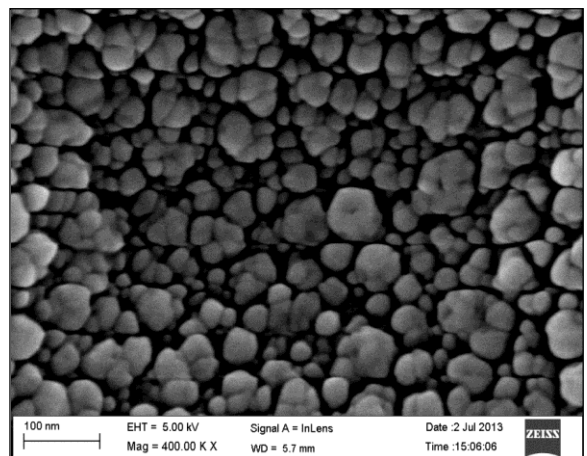


Fig. 2 FESEM picture of the substrate prepared by silver nano cluster deposition.

Silver nanoclusters were also obtained by physical deposition using Nanodep60 nanocluster deposition system. The FESEM of this substrate is shown in Fig. 3. It was seen from the FESEM image that the silver nano clusters were of the order of 40 nm.

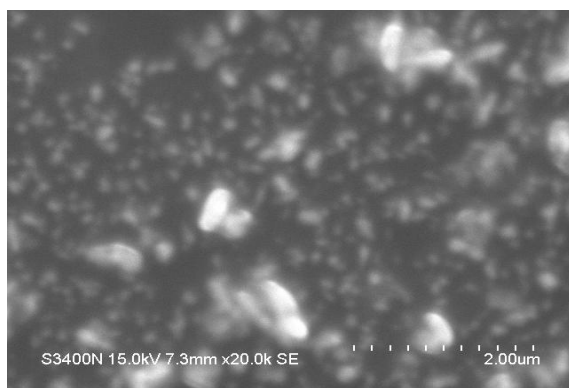


Fig. 3 FESEM image of the substrate prepared by Modified mirror reaction method with 2-thienyl boronic acid and vanillin.

Substrates prepared with 2-thienyl boronic acid and vanillin gave good results. The FESEM of the substrate using 2-thienyl boronic acid and vanillin is shown in Fig. 3. Uniformly distributed FESEM images were also obtained by using 2-thienyl boronic acid and is shown in Fig. 4

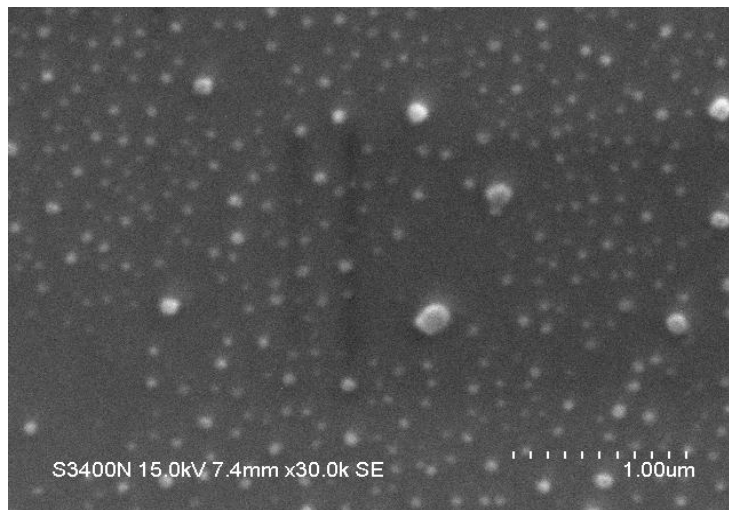


Fig. 4 FESEM image of the substrate prepared by Modified mirror reaction method with 2-thienyl boronic acid.

B. SERS DETECTION

SERS samples were made for millimolar (mM), micromolar (μM) and nanomolar (nM) concentrations of Malathion by taking an average of ten randomly selected locations. All SERS spectra were base line corrected. Base lines from data can be removed by subtracting a base line estimate from the data. The estimate is constructed by fitting a function such as a low order polynomial, to the data where it appears to contain no peaks. The resulting curve fit results are subtracted from the data. The difference then contains data peaks. In this work the base line points were selected manually and the points were used to fit a parabola

It was noted that Malathion gave good SERS spectra at different concentrations. The pesticide molecules may be adsorbed at different orientation of the silver substrate and the type of adsorption sites varies which contributes to significant broadening of the SERS signal. The molecular orientations and the types of adsorption sites when attached to the substrate affects the wavenumbers of Raman signals. This is because as the atoms in the molecule sticks to the silver substrate the cluster size increases so that the reduced mass increases which in turn decreases the frequency. A comparative graph of the SERS spectra of Malathion at different concentrations is shown in Fig. 5.

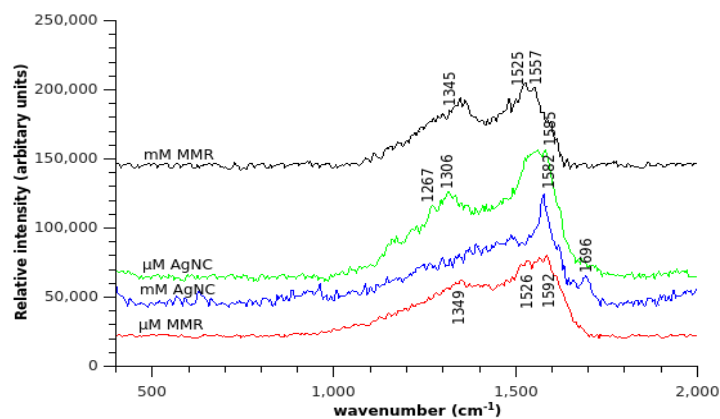


Fig. 5 Comparative figures of SERS spectra at different concentrations of Malathion.

From the reported Raman spectrum of Malathion P=S bond displays a strong band at 652 cm^{-1} [13]. The bands located at 496, 859, 1022, and 1733 cm^{-1} are due to P-S vibration, C-O-C stretching, C-C stretching and C=O stretching respectively [16]. Some of these bands were obtained as broad peaks in SERS with a shift in frequency due to the atoms of the pesticide molecule sticking to the substrate.

The first key element to a new SERS active substrate is sensitivity. From the SERS spectra it was found that the substrate exhibits good enhancement ability, highly sensitive and a detection limit of 0.002 ppm was obtained. The reproducibility of the substrate is another factor for SERS detection. The strong SERS signals suggest the presence of high density hot spots over the substrate, resulting in an average spectrum that shows reproducibility.

It is pertinent to mention here that the sensitivity obtained earlier from colloidal gold nanoparticles based on Lee-Meisel method for the detection of Malathion was 0.123 ppm [14]. In this respect modified mirror reaction method has a detection limit of 0.002 ppm and provides a better substrate to detect Malathion at very low concentration.

IV. CONCLUSION

Malathion was detected and analyzed using SERS substrates prepared using modified mirror reaction and physical deposition method. From the SERS spectrum obtained for this pesticide, the substrate was suitable as a highly sensitive SERS substrate with a detection limit of 0.002 ppm. Substrates prepared by modified mirror reaction and physical deposition are highly sensitive, reproducible and are cost effective. Thus SERS is a potential tool to determine the pesticide residues on food crops with rapid, non-destructive, low cost and eco-friendly advantages.

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