

Form 1

2019 Report Form for Collaboration with Research Center for Biomedical Engineering

Year/month/date	
Number	

Date /Month/Year
date:

To Chairman, Board of Directors, Research Center for Biomedical Engineering

Applicant Affiliation: Discipline of Electrical Engineering, IIT Indore, India

Title: Associate Professor

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Report Form for Collaboration Research

Research Theme	Research and Development of SERS Based Sensor for Residual Fungicide Detection
Research Area	<ol style="list-style-type: none"> 1. Biomaterials 2. Bioengineering 3. Functional molecules 4. Chemistry/Electrical Engineering/Mechanical Engineering/Materials Science
Research Period	From: Date/month/Year To: Date/month/Year 0 1 / 0 6 / 2 0 1 9 ~ 3 1 / 0 3 / 2 0 2 0

Applicant Organization			
Name	Department	Title	Role
Dr. Vipul Singh	Discipline of Electrical Engineering, IIT Indore, Simrol, Khandwa Road, Indore, Madhya Pradesh, India.	Associate Professor	Overall management/execution of the research
Ms. Tejswini K Lahane	Discipline of Electrical Engineering, IIT Indore, Simrol, Khandwa Road, Indore, Madhya Pradesh, India.	Ph.D. student	Synthesis of Ag Nanoparticles and Ag Nanowire network
Mr. Anil Yadav	Discipline of Electrical Engineering, IIT Indore, Simrol, Khandwa Road, Indore, Madhya Pradesh, India.	M.S. (Research) student	Working on Research and development of SERS based sensor
Ms. Anitharaj Nagarajan	GSST, Shizuoka University, Japan	Ph.D. Student	FDTD Simulation, Raman Spectroscopy

Collaboration Partners in the Research Center	Prof. Hiroshi Inokawa, RIE, Shizuoka University Dr. Hiroaki Satoh, RIE, Shizuoka University
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Research Results (Including Purpose, Results, Figures, etc.)

Chlorpyrifos is one of the most widely-used organophosphate crystalline insecticide. It is used to protect more than 50 crops from insect, many of which are dietary staples. However, chlorpyrifos residues on fruits bring risks to human health e.g. affects nervous system, headache, diarrhoea, lung cancer etc. Therefore, it has become quite obligatory to detect and remove chlorpyrifos from the foodstuff before consuming. Numerous methodologies have been proposed to detect chlorpyrifos pesticide residues over the fruit surfaces, such as high-performance liquid chromatography (HPLC) [1], gas chromatography (GC), mass spectrometry coupled with gas chromatography (GC-MS) [2] or liquid chromatography, biosensor method [3] etc. All above mentioned techniques are costly and time consuming. Thus, it is required to develop simple and cost-effective techniques for efficient detection of chlorpyrifos. Surface Enhanced Raman Spectroscopy (SERS) has been emerged as a highly efficient and reliable technique, which is capable of detecting even a small trace amount of pesticide residues in food [4,5,6]. In this process, interaction between pesticide molecules and novel metal nanoparticles leads to significant enhancement in the Raman scattering. Raman scattering is the inelastic collision of photons with the molecules and the difference between the incident photon energy and emitted photon energy is equal to the difference between the vibrational energy levels. Raman spectroscopy gives the blueprint of a molecule with the shift in frequency of emitted photon known as Raman shift. But the intensity of Raman spectra is feeble as a very small number of photons i.e. one in million collides inelastically with the molecule. Metal nanostructures assisted Raman spectroscopy of molecules shows a significant enhancement of 10^6 in Raman intensity known as Surface Enhanced Raman Spectroscopy (SERS). The enhancement in Raman intensity can be product of two phenomenon occurring simultaneously, a) electromagnetic enhancement and, b) chemical enhancement. When incident photon interacts with the metal nanoparticle or rough metallic surface, it can enhance the local electric field through surface plasmon resonance phenomenon which leads to the enhancement in Raman intensity.

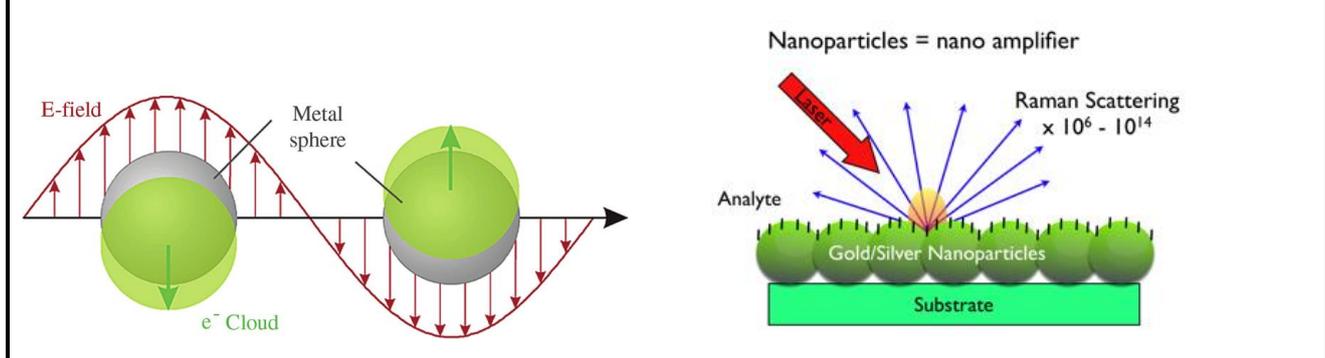


Fig. 1 (a) SPR Phenomenon and (b) The phenomena of Surface Enhanced Raman Scattering (SERS)

Localised Surface Plasmon Resonance (LSPR) enhances electromagnetic coupling at the interfaces of nanostructures within an extremely confined area. This phenomenon can provide many plasmonic “hot spots” in a unique nanostructure for surface-enhanced Raman scattering (SERS). Hotspots are locations in the vicinity of the plasmonic nanostructures where the local optical field is enhanced tremendously when compared to its surrounding [14]. Consequently, any molecule present in a SERS-active hot-spot will exhibit an immense enhancement in its Raman scattering signals. there by resulting in high sensitivity for the detection of target molecules with ultra-low concentration. Also, a significant time has been devoted by researchers in developing SERS substrates with high specific surface area and SERS hotspots.

To detect chemical analyte successfully, the following requirements must be met for SERS [15]:

- A good substrate is essential. It must have a roughened surface to give good enhancement as well as be reproducible and robust with a good lifetime.
- The analyte must absorb on the surface effectively. It should have a higher SERS cross-section than any likely interferents.

Various substrates are used for SERS application such as glass [16], silicon, porous silicon [17], PDMS [19]. SERS active substrates such as anodisc can also be used [18] add up in the enhancement of Raman intensity.

Here, we have used anodiscs with pore size of 100 nm, Fig 2 shows the FESEM (Field emission scanning electron microscope) image of anodisc which shows its porosity.

Metals such as Ag, Au, Cu, Pt etc. have negative real and small positive imaginary dielectric constants and can generate the LSPR phenomenon. Among them, Ag and Au nanoparticles have distinguishing features of LSPR, which can lead to their high sensitivity and excellent uniformity as SERS substrates. Ag shows stronger EM coupling than Au, therefore, Ag was the choice for fabricating metal based substrate for pesticide detection.

SERS coupled with silver colloidal nanoparticle was used to detect paraquat on fruit and vegetable skins [7]. It was used in the detection of phosmet residues in apple extracts with gold nanosubstrate [8]. Chlorpyrifos was detected in tea leaf apples, orange peels, rice, spinach, etc [12]. Rapid detection of omethoate residues in peach extract was performed with silver nanoparticle-based SERS [9]. Using SERS, the thiabendazole from the chemically treated citrus fruits skins were detected and monitored [10]. Carbaryl pesticide in apples extract was detected with combined SERS and chemometric method [11], whereas chlorpyrifos on tomato surfaces and in tomato extract was detected by surface-enhanced Raman spectroscopy (SERS) [13].

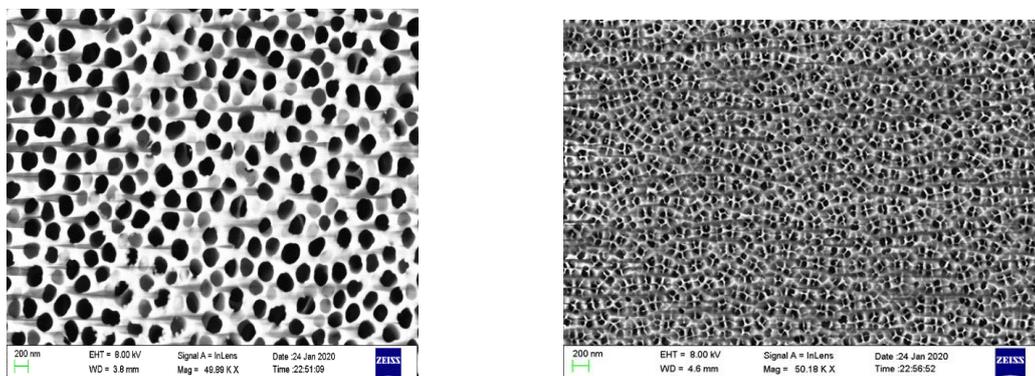


Fig.2 SEM images of Anodisc of front and back side.

Objective of our work is to optimize the synthesis of silver nanoparticles and silver nanowire and fabricate SERS sensor by using anodisc as substrate over which different silver nanostructure could be used to detect Pesticides.

Chemicals and Reagents Used:

Silver nitrate (AgNO_3 , 99.99%, Sigma Aldrich), Tri Sodium Citrate (Sigma Aldrich), DI Water, Ethylene Glycol (EG, 99.99%, Sigma Aldrich), Polyvinylpyrrolidone (PVP, MW 130000) NaCl, KBr (Sigma Aldrich), Reagent Ethanol and Acetone, Anodisc 100nm pore diameter.

Synthesis of Silver Nanoparticles:

There are many ways to synthesize silver nanoparticles (Ag NPs) such as physical, chemical and biological methods. Chemical reduction is most frequently applied for the preparation of stable, colloidal dispersions of Ag NPs in water or organic solvents.

Synthesis of AgNPs using sodium citrate:

AgNPs were synthesized by chemical reduction using Sodium citrate as reducing agent as well as stabilizer [5]. The average size, size distribution, morphology, composition and structure of particles were estimated by Field emission scanning electron microscopy (FESEM), EDX and UV/Visible absorption spectroscopy.

In this process 70mg of Silver nitrate were dissolved in 400ml DI water, after that it was continuously boiled and simultaneously stirred at 500 rpm. After boiling the silver nitrate solution, 1 wt. % aqueous sodium

citrate solution was rapidly injected into the flask. In fig. 4 the color changes after adding sodium citrate is shown which corresponds to different size of the Ag NPs during the growth process. After further boiling for 30 min, it was cooled down to room temperature. To remove excess citrate ions from AgNP solution it was centrifuged for 15 min with 3000 rpm. Overall growth mechanism of AgNPs is shown in Fig.5.

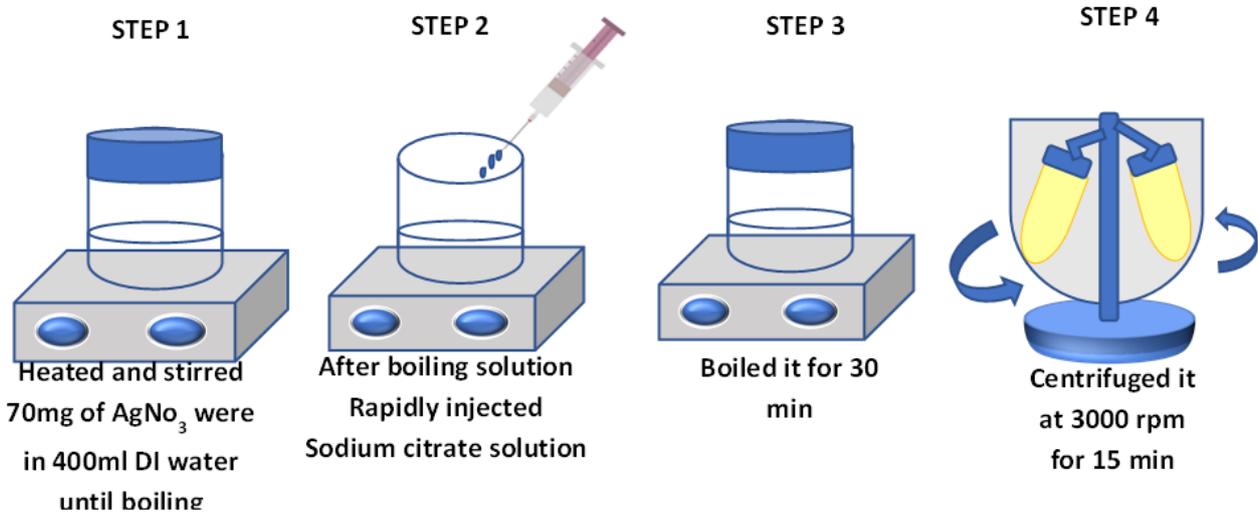


Fig. 3 Shows different steps involved in the synthesis of Silver Nanoparticles



Fig. 4 shows the changes in the color of the colloidal solution during synthesis

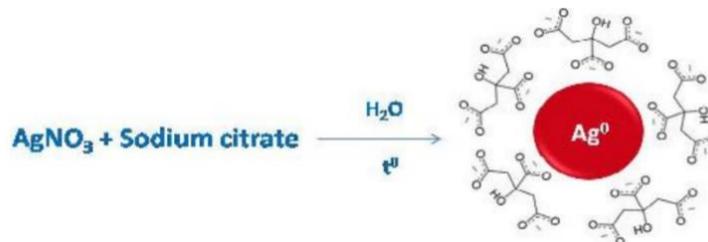


Fig.5 growth mechanism of Ag NPs capped by citrate ions [26].

FESEM-EDX analysis of silver NPs reduced by sodium citrate

FESEM was used to investigate the size and shape of silver nanoparticles. The SEM images of the AgNPs are shown in Fig.-6. Upon morphological investigations it was found that the spherical particles with average size of 30- 50 nm were formed. Here sodium citrate was used as reducing and capping agents. In growth solution. Moreover, from EDX spectra shown in Fig.-7 it is clear that silver nanoparticles were reduced by sodium citrate and has wt.% of 89.6%.

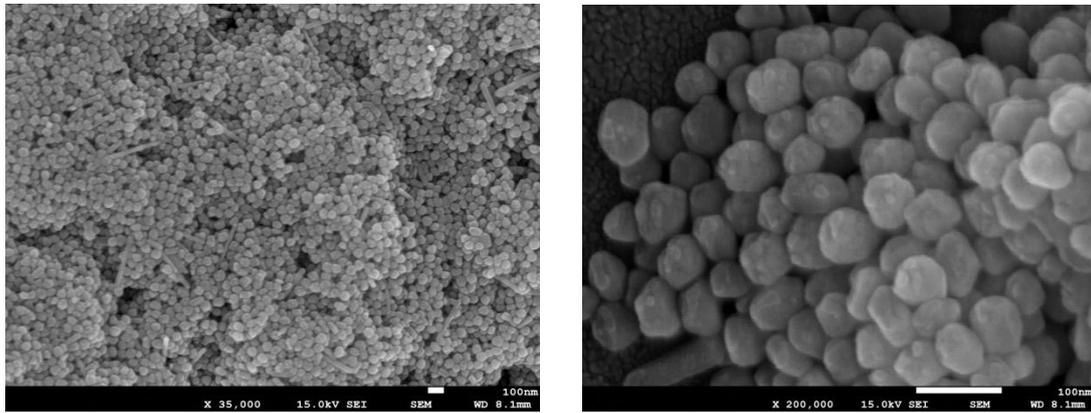


Fig. 6 Shows FESEM images of Ag nanoparticle synthesis using Sodium citrate

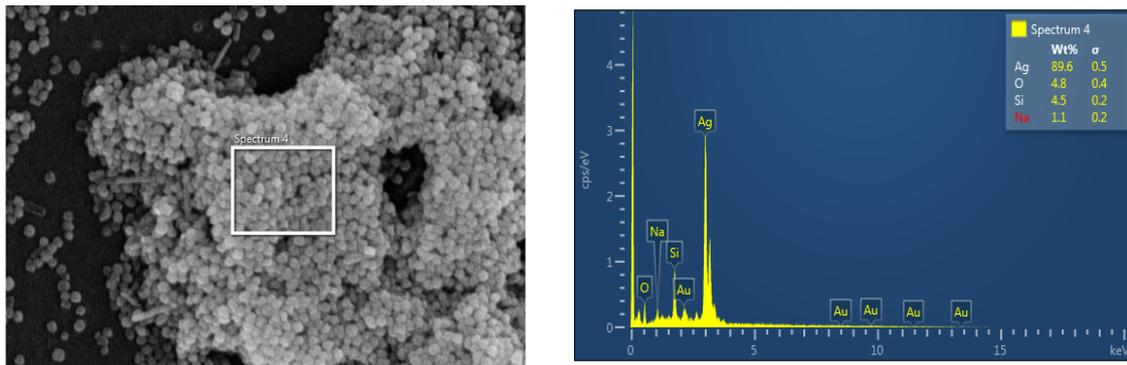


Fig. 7 EDAX data for Ag nanoparticle using Sodium citrate

UV-Vis of silver nanoparticles

The UV-Vis absorption spectra (Fig. 8.) shows a plasmonic peak at 414 nm which corresponds to the particle size of 30-50 nm. Hence, this investigation is in agreement with the previously estimated size by FESEM images. The full width half maximum (FWHM) of the peak is direct evidence of dispersity of the nanoparticles, where a large FWHM of can be attributed to polydispersity.

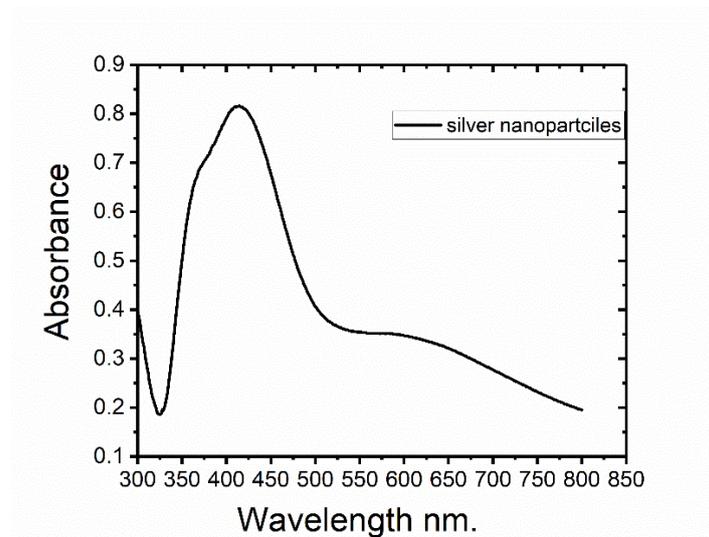


Fig. 8 UV-Vis Absorption spectra for silver nanoparticles dispersed on Anodisc

Synthesis of Silver Nanoparticles using polyol Method

Polyol method is used to synthesize monodispersed AgNPs. Size and shape control of silver nanoparticles can easily possible due to polyol method by varying molar ratio of PVP and silver nitrate. Size and shape of silver nanoparticles can be controlled effectively by the polyol method by varying molar ratio

of PVP and silver nitrate. The SERS phenomena is highly dependent on the size and shape of the nanoparticle. Therefore, polyol method was chosen to synthesize the Ag nanoparticles with control on size and shape.

In this method ethylene glycol is used as a solvent as well as reducing agent, whereas, Polyvinyl pyrrolidone is used as capping agent. To prepare the solution, 0.17g AgNO₃ and 1.2 g of PVP were dissolved in separate 10ml Ethylene Glycole (EG) solvent at room temperature. The PVP solution was continuously heating and stirring at 160 °C and 500 rpm for 1hr. Consequently, AgNO₃ solution was added drop by drop (1ml/5min) while continuous heating and stirring of the solution. Thereafter, the solution was kept for heating at 160 °C for 4hrs and allowed to cool at room temperature. The as-synthesized nanoparticles in the solution were ultrasonicated and centrifuged three times at 5000 rpm for a duration of 20 min each in acetone and ethanol. Finally, residue product was rinsed in ethanol solvent and kept in oven at 40°C for 5 hrs.

SEM-EDX analysis of silver NPs synthesized by Polyol method

As shown in the FESEM images (Fig. 9) the nanoparticles synthesized using Polyol method have monodispersed state with a particle size distribution in the range 90-100 nm. Whereas, the EDX spectra analysis shows of silver content to 97.1% by weight.

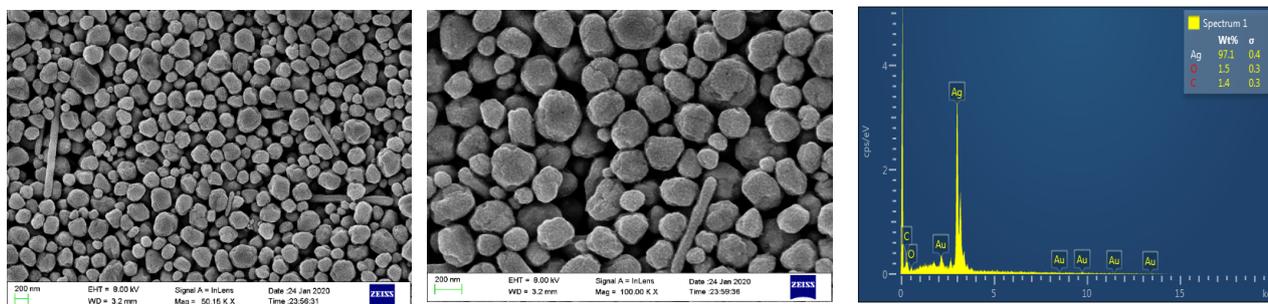


Fig. 9 FESEM Images and EDAX data of Ag nanoparticles synthesized using Polyol synthesis

Synthesis of Silver Nanowire (Polyol method)

AgNWs structure has high specific surface area with flexibility which can provide an effective adsorption surface for the target molecules. Therefore, AgNWs offer the possibility for use in SERS detection. However, the random spatial clusters of AgNWs cannot generate uniform electromagnetic (EM) coupling in the network structure. In contrast, AgNPs that are evenly distributed in the AgNW network can not only generate more hot spots but also improve the uniformity of EM coupling [21]. The perfect structure can generate plasmonic hot spots via both the strong EM coupling among AgNWs and the strong EM coupling among AgNPs and AgNWs [21].

In this work we have synthesized AgNWs using salt mediated polyol method for SERS based pesticide detection. Firstly, 75mg of PVP was dissolved in 10ml of EG with simultaneous heating and stirring the solution at 160 °C for 1hr. Thereafter, three solutions were prepared viz: NaCl with molarity of 3.5 mM, KBr with molarity of 1.8 mM and AgNO₃ of molarity 30.5 mM in 5 ml EG as solvent, respectively, for which were used as additives in the PVP solution. Subsequently, 40 µl of NaCl and 20 µl of KBr solutions were added in the PVP while continuous heating and stirring. After 20min-30min AgNO₃ solution was added drop by drop (1ml/5min) while the process of heating and stirring of the mixture at 160 °C was performed for 3hrs. Consequently, the solution was allowed to cool down at room temperature. To perform the purification, the as-synthesized NWs were ultrasonicated and centrifuged for 3 times at 5000 rpm for 20 min in acetone and ethanol, respectively. Finally, the purified colloidal solution of NWs was mixed in ethanol which was again dispersed on glass substrate for further characterization.

The Fig. 10 shows the FESEM images of the obtained NWs. From the analysis of the NWs images the aspect ratio of the synthesized NWs was estimated to be 500. This much high aspect ratio NWs has very diverse applications like in transparent electrodes.

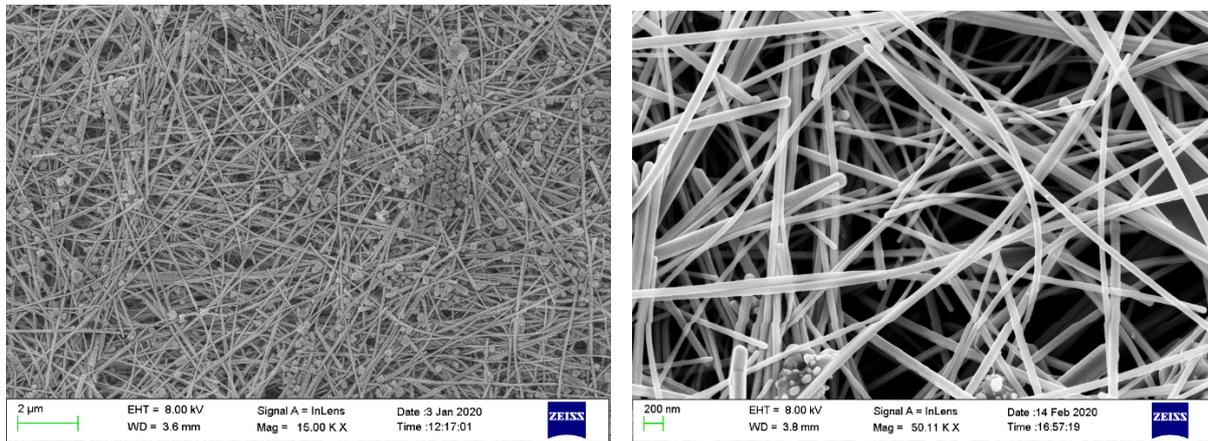


Fig. 10 FESEM Images is Silver Nanowire synthesized using Polyol

V- Vis of silver nanowire

The absorption spectra in Fig. 11 shows that the thin film has very feeble absorption corresponding to the visible light. Whereas, the absorption peak is observed to be at 370 nm. This peak may be assigned to the transverse plasmon mode of AgNWs. The exact position of this plasmon band is extremely sensitive to the nanoparticle size and shape.

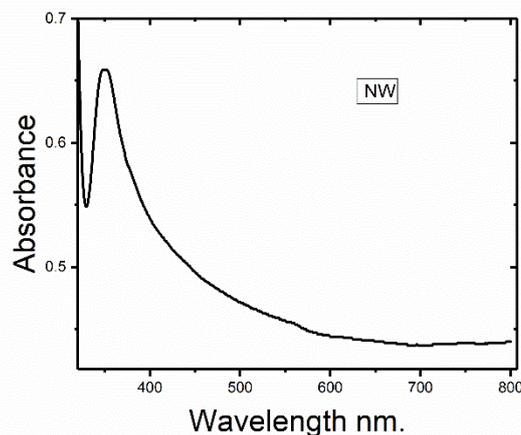


Fig.11 UV-Vis Absorbtion spectra for silver nanowire dispersed on glass

Fabrication of SERS sensor

The pesticide solution was prepared by adding 1 mM chlorpyrifos in 80 μ l of Methanol: DI (1:1) mixture. In order to fabricate pesticide sensor, colloidal solution of silver nanoparticles was dispersed over the anodisc and allowed to dried it at room temperature for 1hr. Afterwards, chlorpyrifos solution was dispersed on anodisc and dried it for 2 hr at room temperature.

Raman Spectra of silver nanostructures and pesticide adsorbed silver nanostructures

For the purpose of SERS based pesticide detection the NPs synthesized by sodium citrate assisted growth was preferred. The nanoparticles synthesised by this method has smaller feature size which is liable to provide more hot spots. Raman spectroscopy was performed for the pristine NPs dispersed over the anodisc and Fig. 12 shows the Raman spectra for the same. The Raman spectroscopy of chlorpyrifos, dispersed over AgNPs, results in the phenomena of SERS was performed. The Fig. 12 shows the spectra corresponding SERS phenomena.

In the Fig. 12 overall enhancement in Raman spectra of Ag nanoparticles with adsorption of chlorpyrifos pesticide can be attributed to SERS phenomena. Along with AgNPs peaks there are several other peaks in chlorpyrifos adsorbed AgNPs Raman spectra. These peaks might have emerged because of bond stretching

in pesticide assisted Ag nanoparticles. The peak at 340 cm^{-1} corresponds to N-cyclopropyl bending vibration and the peak at 411 cm^{-1} could be assigned to P–O–C stretch. Subsequently, a peak at 631 cm^{-1} with significant intensity is because of P=S stretching vibration [13]. Further, a feeble peak is also observed at around 1240 cm^{-1} could be assigned to Cl-ring vibrations in chlorpyrifos. Further as we can see all the peaks for Ag nanoparticles which are highly dependent on synthesis process and capping agents are also available in pesticide assisted Ag nanoparticles also.

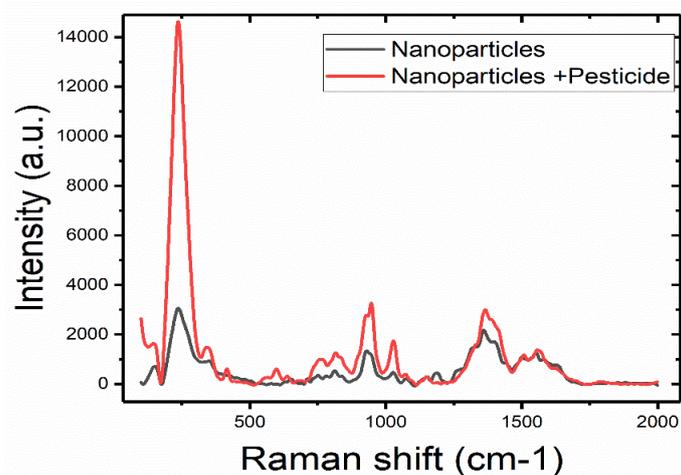


Fig. 12 Raman spectra of silver nanoparticles (synthesized by sodium citrate)and chlorpyrifos adsorbed on silver nanoparticles.

Surface Enhanced Fluorescence by Silver Nanoparticles

The PL from noble metal nanoparticles was reported for gold, copper and silver [22][23][24]. All these measurements revealed the maximum of the PL band close to the interband absorption edge and thus the PL was attributed to the interband radiative transitions [22-26]. The PL was observed only from nanoparticles smaller than 40 nm in diameter and from nanorods with a high aspect ratio, which suggests a strong size and morphology dependence of the PL spectra. When the size of the particles decreased, the intensity of the PL increased indicating a strong influence of the surface the nanoparticles on the PL spectra.

The strong local electric field close to the surface of the particles enhances the Raman scattering from molecules functionalized on its surface [25] and luminescence from molecules functionalized on the surface of the particles or close to a rough metal surface [26]. This suggests that the strong local electric field induced by the SPR excitation in the particle can also enhance the quantum yield of the photoluminescence from the particles.

In Fig. 13 the excitation spectra of AgNPs and chlorpyrifos adsorbed AgNPs are shown. The excitation wavelength was fixed at 325 nm and the emission spectra was recorded. Silver nanoparticles showed emissions peaks at 390 nm, 490 nm and 450 nm. Chlorpyrifos adsorbed on the surface of silver nanoparticles show enhancement and red shift of 490 nm emission peak by about 20 nm with respect to the AgNPs emission.

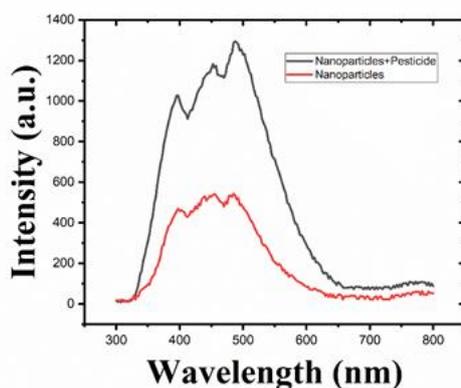


Fig.13. PL Spectra of silver nanoparticles synthesized by sodium citrate.

Conclusion(s):

- We have successfully synthesized silver nanostructures.
- Silver nanoparticles synthesized by sodium citrate reduction gives nanoparticles size in the range 30-50nm with agglomeration.
- Silver nanoparticles synthesized by polyol method gives monodispersed nanoparticles with size of nanoparticles 90-100 nm.
- Silver nanowire by salt mediated polyol method results in aspect ratios of 500.
- Anodisc as substrate gives porosity and roughness for silver nanoparticles which helps for Raman enhancement.
- The enhancement in Raman spectra was observed when chlorpyrifos adsorbed on silver nanoparticles.
- Hence, SERS mediated pesticide detection was achieved by AgNPs.
- Also, we have observed PL enhancement for silver nanoparticles.

Future Work:

- 1) Analysis of Raman spectra for AgNWs as well as combination of AgNPs and AgNWs.
- 2) Raman spectroscopic analysis of chlorpyrifos adsorbed over AgNPs synthesized by different methods.
- 3) Optimization of silver nanoparticle size and deposition on anodisc for improvement in enhancement of Raman scattering.
- 4) Detection of different pesticides and achieve repeatability.

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List of Publications Related to the Collaboration Research
The results are still in infancy we are working to improve further the performance of the SERS based sensor.
List of Presentations (Conference, Meeting, etc)
One Poster presented at the Annual meeting for the 2019 cooperative research at Research Center of Biomedical Engineering at Tokyo Institute of Technology. Abstract for the same was submitted.
List of Awards

Registration of research-theme continuation for next year Prior consent from the collaboration partner in the Research Center is necessary.	Yes	No
Research plan for the next year (from April 1, 2020 to March 31, 2021), if the collaboration research is continued.		
<p>Background: Pesticides and Fertilizers are widely used in modern day agriculture. In order to meet the demand for food for the booming world population, extensive usage of pesticides and fertilizers has become more common for getting high yield of crops by protecting them against attack of insects and diseases. An estimate suggests that nearly 3 billion kilograms of pesticides are applied every year around the world. Furthermore, due to lack of knowledge and proper enacting of laws in under developed countries farmers tend to apply pesticides in quantities which are more than the prescribed limits. These pesticide molecules can enter into the food chain through direct adsorption on the plant’s leaves, fruits or vegetables surfaces, or can also enter into the soil and thereby the underground water, leading to contamination of soil as well as water. Furthermore, the problem is more acute for countries like India, where the agriculture essentially relies on Monsoon or the underground water for irrigation purposes. The</p>		

long term effects of pesticides entering into the food chain can be highly detrimental [1-4]. In this work we propose to develop **SERS based sensor for detection of two pesticides molecules** namely **Diazinon** (an organophosphate insecticide) and **Thiram** (a Fungicide) which acts against fungal diseases in seeds and plants and also acts as an animal repellent. While Diazinon is used in agriculture to control insects on fruits, vegetables, nuts and field crops, however, it is also extensively used for household insects. Human exposure to Diazinon is known for its severe effects on the nervous system. On the other hand exposure to Thiram, especially upon inhalation can also lead to dizziness, nausea, headaches and other GI related complications. It is also reported to affect the thyroid, kidney and liver adversely.

Surface Enhanced Raman Scattering (SERS) as a phenomenon was first observed in 1973 [5], for Pyridine adsorbed on roughened Silver electrode. However, it was only in 1997 when single molecule SERS was observed and the technique gained attention of scientific community [6]. It is a unique technique that combines the modern laser spectroscopy with exciting optical properties of metallic nanostructures and thereby results in highly amplified Raman signals when molecules are attached to a nanometer sized Gold or Silver structures [7-10].

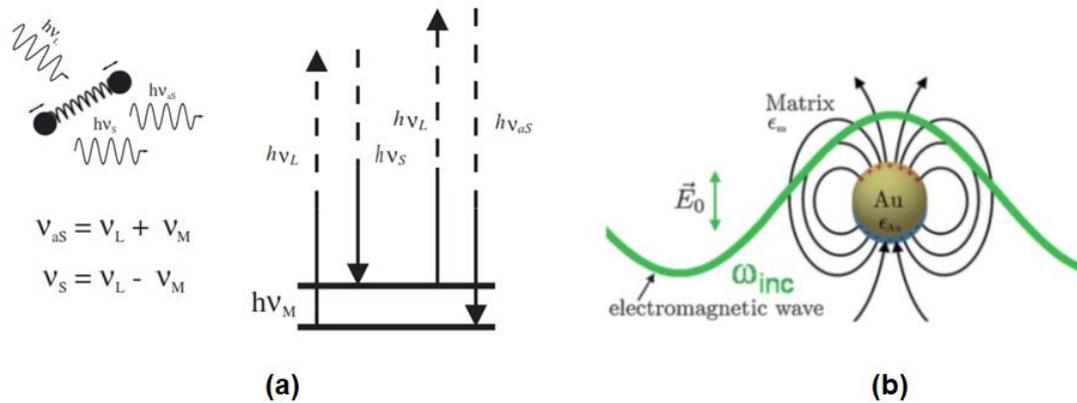


Fig.1 (a) Shows the Antistokes and Stokes shifted Raman spectroscopic signals (b) The effect of Localized Surface Plasmon arising due to coherent oscillations of electrons near a Gold nanoparticle.

The prime significance of the technique lies in the fact that it effectively combines the structural information content of Raman Spectroscopy (which typically consists of Stokes and Antistokes shifted signals, as shown in Fig. 1(a)), with localized surface plasmons giving rise to ultrasensitive detection limits. SERS is an analytical technique, that provides information on surface and interface processes, thus opens up exciting opportunities in the field of biomedical and biophysical spectroscopy. SERS as a technique also overcomes one of the major limitation of Raman spectroscopy emanating due to its extremely small cross-section of Raman process, which is nearly 12-14 orders below fluorescence cross sections. Surface Plasmon Polaritons (SPPs) are coherent oscillations of electrons that are excited by electromagnetic radiation at metal dielectric interfaces, which typically arises on the surface of coinage metals viz. Gold and Silver as shown in Fig. 1(b). The Localized Surface Plasmon Resonance (LSPR) effect involves the confinement of SPPs in nanostructures of dimension comparable to wavelength of light. LSPR exhibits significant enhancement of localized electromagnetic coupling at the interfaces of nanostructures within an extremely confined area. The overall intensity of a SERS signal depends on both the incoming field ω_{inc} and outgoing field ($\omega_{inc} - \omega_{vib}$). Thus the overall intensity of SERS signal is given by

$I_{SERS} = |E_{inc}(\omega_{inc})|^2 |E(\omega_{inc} - \omega_{vib})|^2$. Moreover since the incident radiation at ω_{inc} and the Stokes Raman shifted radiation ($\omega_{inc} - \omega_{vib}$) are in resonance with the plasmon peak of the metal nanostructure thus, $(\omega_{inc} - \omega_{vib}) \approx \omega_{inc}$. Hence the overall SERS scattering signal can be approximated as $I_{SERS} = |E(\omega_{inc})|^4$, and the above approximation has been found to hold good for blue and green region of electromagnetic spectrum. Further, it may be recalled that the electric field strength due to a dipolar radiation scales with $E(r) \propto \left(\frac{1}{r}\right)^3$, which directly

implies that the SERS signal intensity is given by $I_{\text{SERS}} \approx \left(\frac{1}{r}\right)^{12}$, indicating the fact that SERS is a highly surface selective effect. Infact the sensitivity of the technique is so high that even the orientation of molecules relative to the surface normal can be detected with ease. It also must be noted here that for a reasonable electromagnetic enhancement factor arising due to LSPR effect give by $\beta \sim 100$, the overall enhancement in the SERS signal is observed to be of the order of $\beta^4 \sim 10^8$, nearly a billion times enhancement of otherwise weak Raman signal.

Goal:

In this proposal we intend to develop a SERS based sensor. Some of the key requirements for the development of such a sensor are that it should be developed on an appropriate substrate viz. substrate that does not give Fluorescence, the enhancement of the signal as discussed above can be improved by efficient coupling of LSPR arising out of metal nanostructures and finally the chemical interaction of the molecule adsorbed on the metal nanoparticle substrate. **In this proposal we intend to utilize the Anodic Aluminum Oxide (AAO) as a template for SERS substrate.**

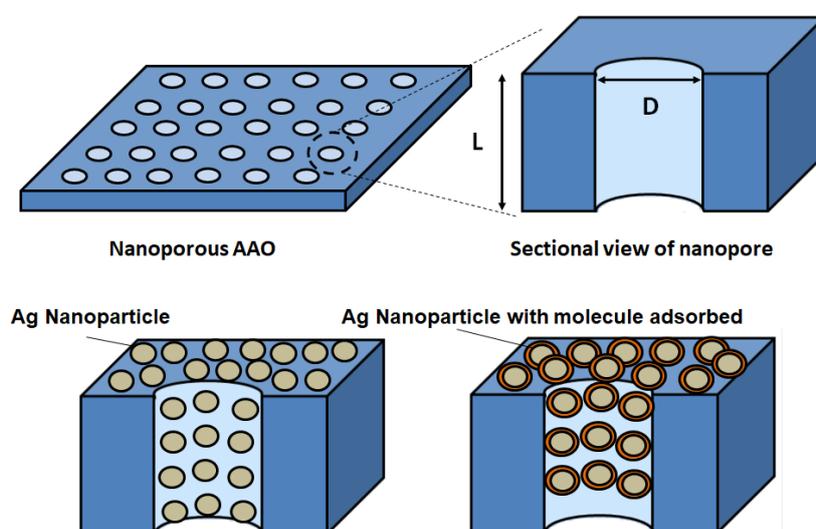


Fig. 2 Shows the nanoporous Anodized Aluminum Oxide (AAO) membrane, its sectional view and Silver nanoparticles dispersed over its surface and later adsorbed with molecules for development of proposed biosensor.

Objectives of the proposed work are the following:-

- 1) Electrochemical growth of porous AAO using Aluminum foil.
- 2) Optimization of growth parameters to obtain wide variety of aspect ratio of these nanoporous AAO membranes, to be used as SERS substrate.
- 3) Development of Ag nanoparticles of different shapes and sizes by chemical reduction method.
- 4) Adsorption of Thiram/Diazinon molecule on Ag nanoparticle.
- 5) Finally development of SERS based biosensor, and its optimization w.r.t. aspect ratio of the porous AAO substrates and size of metal nanoparticles.

Methodology:

The proposed methodology to achieve the above set objectives of the proposal includes electrochemical anodization of Aluminum foil, synthesis and dispersion of Ag/Au nanoparticles on the surface of AAO, adsorption of molecules on metal nanoparticles substrate and finally characterization of the sensor using SERS technique. It has been well reported in the literature that electrochemical anodization of Aluminum can be carried out to develop aluminum oxide giving rise to two different types of Al_2O_3 , namely the barrier type and the porous type. Well-ordered nanoporous AAO templates having honeycomb structure have been applied for the development of ordered Silver and Gold nanostructures as SERS-active substrate. These well-ordered 2D nanostructures of AAO have been utilized as a template for development of metal nanostructures, later used as an effective SERS

substrate. The honeycomb nanostructures of AAO can be easily grown by a single step electrochemical anodization of polished aluminum foil. The typical steps involved are electrochemical polishing of the Al substrate in a mixture of Perchloric acid (HClO_4) and ethanol, thereby resulting in smoothly finished Aluminum foil as shown in Fig. 3 (a). Later these electro polished Al substrates are taken in a two electrode electrochemical cell for the anodization of Al into Al_2O_3 [11-15]. Using sulphuric acid the electrochemical anodization of Aluminum proceeds to generate porous honeycomb network as shown in Fig. 3 (b). It has already been reported that due to the repulsive forces between the neighbouring pores resulting in mechanical stress drives the self-assembly process of formation of honeycomb nanostructures. By selectively controlling the electrochemical process parameters the aspect ratio and diameter of the pores can be selectively controlled to range a hexagonal close pack array of pores ranging in diameter from 20 nm – 500 nm. These would later be utilized for SERS substrate.

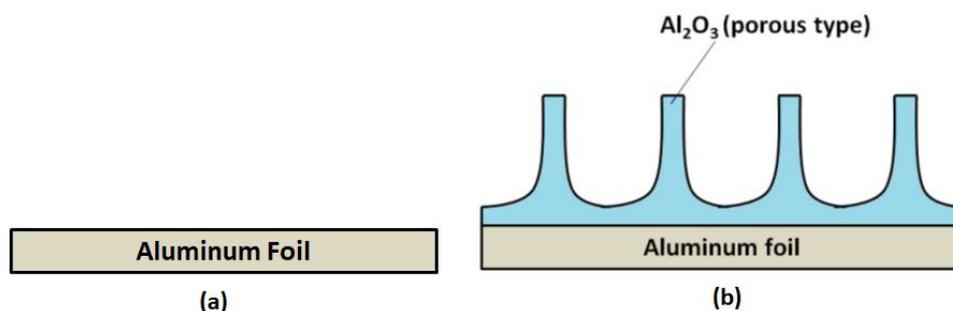


Fig. 3 shows schematic (a) electro-polished surface of Aluminum foil, (b) growth of hexagonal close pack honeycomb like structures of AAO.

Once a nanoporous AAO template is obtained, it would be dispersed with a colloidal solution of Ag nanoparticles. These nanoparticles in turn would be separately synthesized using chemical route. Although a wide variety of methods have been proposed for synthesis of Ag nanoparticles [16-19]. However, using the chemical reduction method called Turkevich method, in which AgNO_3 solution is reduced by using trisodium citrate. Using this type of bottom up approach Ag nanoparticle ranging in the size of 10 nm -100 nm can be readily synthesized. By Additionally using surfactants we can control the growth kinetics and stability of the Ag nanoparticles in the solution phase. Finally the device would be fabricated by dispersing the synthesized nanoparticles over the surface of the nanoporous AAO, over which the Thiram/ Diazinon molecules (in the form of their solutions in water/organic solvents) would be adsorbed. Upon excitation by CW laser the SERS signal can be obtained as is also shown in Fig. 4(a). The interface of Ag nanoparticle and the molecule and the underlying interfacial processes are shown in Fig. 4 (b & c) respectively. As mentioned earlier the SERS signal is strong enough owing to the LSPR coupling effects, it is this LSPR coupling that we sought to increase by using nanoporous structures offered by AAO template.

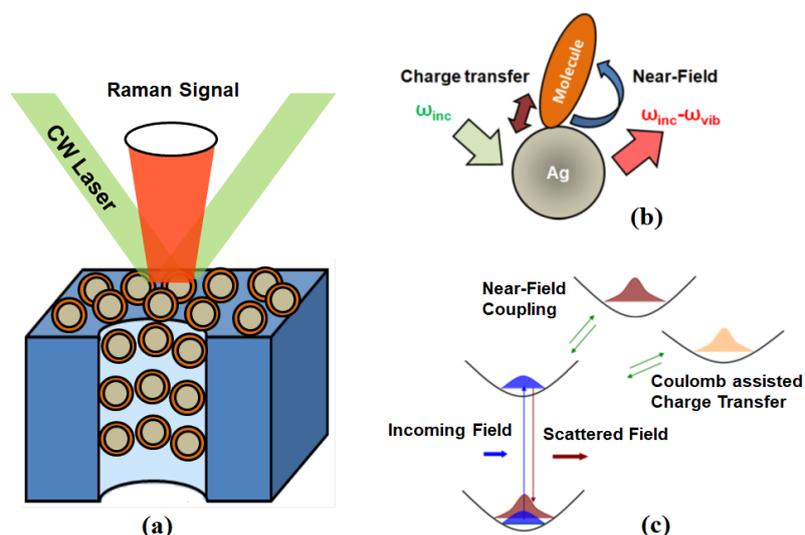


Fig. 4 (a) shows the Ag Nanoparticle coated with molecules dispersed on to the surface of AAO substrate utilized as a

SERS based sensing device, (b) the adsorbed molecule on the surface of Ag nanoparticle and (c) shows the Energy levels/transfer processes involved at the interface of Ag nanoparticle and the molecules adsorbed over it.

By controlled optimization of both the size of the nanopores of AAO and nanoparticles of Silver, we intend to obtain high sensitivity SERS substrate. Moreover the optimum aspect ratio of these nanoporous structures is expected to provide high enhancement factor, leading to high strength of the SERS signal. More recently researchers have shown the presence of hot spots to be critical for high enhancement of SERS signal. The hot spots are highly spatially localized regions exhibiting extreme field enhancements [1, 6]. Although the probability of finding hot spots is lesser. However, by utilizing nanoporous structured template the likelihood of hot spots consisting of Ag nanoparticles can be maximized. Hence high EM enhancement is expected. Furthermore dispersion of a mix of Ag nanoparticles and Ag nanowires could even lead to further enhancement of the SERS signal, thereby leading to improved limit of detection with enhanced sensitivity with improved uniformity.

Schedule/timeline of the project:

Activity	Time in quarters			
	June'20 – September'20	October'20 – December'20	January'21 – March'21	
Electrochemical polishing/ anodization of Al foil				
Characterization of nanoporous Al ₂ O ₃ substrates				
Synthesis & Optimization of Ag Nanoparticles				
Adsorption of Thiram/Diazinon molecule on Ag nanoparticle surface				
Fabrication & Characterization of SERS based Sensors				

The expected results/outcomes from the proposal are as follows:-

- Optimization of electrochemical growth conditions for Anodic Aluminum Oxide w.r.t. the aspect ratio of the nanoporous substrates thus obtained.
- Optimization of synthesis conditions for Ag nanoparticles to obtain nanoparticles with variety of diameters and shapes.
- Understanding the energy transfer mechanism i.e. Forster or Dexter between the metal nanoparticles and the adsorbed molecules on to its surface.
- Utilization of hot spots by using mix of Ag nanoparticles and Ag nanowires for large electromagnetic enhancement leading to strong SERS signal.

Demonstration of high sensitivity SERS based sensor with improved detection limit well below sub nM range is expected.

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List of Recent Publications of Dr. Vipul Singh (relevant to theme of LSPR and nanostructure synthesis that relates to the project proposal):-

1. J. Agrawal, T. Dixit, I. A. Palani and V. Singh, *Scripta Materialia*, Vol. 163 (2019) pp. 1.
2. P. Rajagopalan, M. J. Raj, N. Prasanth, V. Singh, I. A. Palani and S-J Kim, *ACS Appl. Mater. Interf.*, Vol. 11 (2019) pp. 6078.
3. T. Dixit, A. Tripathi, K. L. Ganapathi, I. A. Palani, M. S. R. Rao and V. Singh, *IEEE Electron Dev Lett.*, Vol. 40 (2019) pp. 255.
4. N. Patra, V. Singh, I. A. Palani, *Mater. Res. Exp.*, (2019, in press).
5. N. Patra, S. K. Karaturi, N. J. Vasa, D. Nakamura, M. Higashihata, V. Singh and I. A. Palani, *J. Alloy & Comp.*, Vol. 770 (2019) pp.1119.
6. J. Agrawal, T. Dixit, I. A. Palani, M. S. R. Rao and V. Singh, *Semicon. Sci. & Technol.*, Vol. 33 (2018) pp. 095021.
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9. T. Dixit, I. A. Palani and V. Singh, *RSC Adv.*, Vol. 8 (2018) pp. 6820.
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