DEVELOPMENT OF 2D MATERIALS AND METAL NANOPARTICLES NANOCOMPOSITES FOR SERS BASED SENSING OF HAZARDOUS BIOMOLECULES AND DISEASES DETECTION VIA GLAD BASED Ag NANORODS

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by

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Submitted

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to the

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Dedication

This dissertation is dedicated to my parents **Mr. Chander Dutt Sharma** and **Mrs. Chander Prabha**. Their support, blessings, encouragement, and constant love have sustained me throughout my life.

CERTIFICATE

I am satisfied that the thesis entitled **"Development of 2D Materials and Metal Nanoparticles Nanocomposites for SERS based Sensing of Hazardous Biomolecules and Diseases Detection via GLAD based Ag Nanorods"** submitted by **Mr. Arvind** is worthy of consideration for the award of the degree of **Doctor of Philosophy** and is a record of original and bonafide research work carried out by him under our supervision. The results contained in it have not been submitted in part or full to any other university or institute for the award of any degree/diploma.

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ABSTRACT

The term sensing invokes curiosity in the entire scientific community as it plays a crucial role in every field. It involves sensing the presence of different biomolecules, infectious bacteria, explosives, toxic pollutants, poisonous gases and many other hazardous or non-hazardous components through various techniques. For example, electrochemical sensing, fluorescence microscopy, chemiluminescence, colorimetric sensing etc. are employed for detection of the above-mentioned molecules of interest. In recent years, all these methods have been proved highly effective in dedicated sensing of various probe molecules. However, most of these methods involve difficult sample preparations, poor sensitivity at ultralow concentrations, etc. Here, Raman spectroscopy becomes extremely efficacious as it produces the highly specific, ultrasensitive signals which are molecular fingerprints of the detecting species. To enhance the small intensity signals of Raman spectra, surface enhanced Raman spectroscopy (SERS) is employed preserving all the critical features of Raman spectroscopy.

This study aims to develop and explore the 2D material and their nanocomposites with metal nanostructures for SERS applications along with real world application of SERS by employing silver (Ag) nanorods for distinction between different stages of oral cancer.

In the first work, different morphologies of MoS_2 were synthesized for potential SERS applications. The morphology tunability was achieved by varying the growth parameters during the facile solvothermal method. The SERS

substrate prepared with the nanoplate morphology MoS_2 structures shows excellent detection up to 10^{-9} M concentration of Rhodamine B (RhB) dye. The MoS_2-Ag nanocomposite SERS substrates successfully detect an ultralow concentration of 10^{-15} M of RhB dye with an enhancement factor of 9.2×10^7. The contribution of Ag NPs to ultralow SERS detection is suggested in addition to a charge transfer mechanism between RhB molecules and MoS_2.

In next work, vertically oriented tungsten disulfide (WS_2) nanoflakes were synthesized on a silicon (Si) substrate using the pulsed laser deposition (PLD) technique. The fabricated nanoflakes of WS_2 were decorated with Ag NPs by employing thermal evaporation to enhance their SERS activity. The SERS performance of the WS_2-Ag nanocomposite was explored by using excitation lasers of 532 nm and 633 nm. Various environmental pollutant dyes such as Rh B (RhB), Methyl orange (MO), Methylene blue (MB) are detected at ultralow concentrations by using WS_2-Ag nanocomposite as SERS substrate. The detection limits achieved for RhB and MO are 10^{-16} and 10^{-17} M, respectively with 532 nm excitation laser, and 10^{-13} M is the detection limit for MB dye with 633 nm laser. The promising enhancement factors of the order of 10^8 were achieved for WS_2-Ag nanocomposite substrate.

In the following work, MoS_2-Au nanocomposite is fabricated for SERS application with optimized concentration of Au nanoparticles. The nanocomposites of MoS_2 and Au NPs are successfully used as highly sensitive SERS substrates for the detection of hazardous dyes RhB and N719, and infectious bacteria of E. coli and S. aureus. The MoS_2-Au nanocomposite SERS substrate successfully detects the RhB and N719 dye up to 10^{-15} and

10^{-9} M. The highest enhancement factor obtained for RhB and N719 dye is 5.2×10^7 and 2.1×10^7, respectively. Further, the nanocomposite substrate was employed for the sensing of infectious S. aureus and E. coli bacteria down to 10^2 cfu/ml.

In the final work of this thesis, glancing angle deposition-based label free Ag nanorods were employed for diagnosis of oral cancer in its pre-malignant phases, prior to its development into malignant tumors. All the SERS measurements were carried out on site in the field. The SERS spectra of pre-malignant and malignant samples exhibited distinct peaks corresponding to varying concentrations of inorganic metabolites and proteins. The multivariate analysis successfully separates the oral cancer positive and negative samples, as well as pre-malignant and malignant groups.

सार

संवेदन शब्द सम्पूर्ण वैज्ञानिक समुदाय में जिज्ञासा उत्पन्न करता है, क्योंकि यह प्रत्येक क्षेत्र में अत्यंत महत्वपूर्ण भूमिका निभाता है। यह विभिन्न जैव-अणुओं, संक्रामक बैक्टीरिया, विस्फोटक पदार्थों, विषैले प्रदूषकों, जहरीली गैसों और अन्य खतरनाक या गैर-खतरनाक घटकों की उपस्थिति का विभिन्न तकनीकों द्वारा पता लगाने की प्रक्रिया है। उदाहरणस्वरूप, इलेक्ट्रोकेमिकल संवेदन, फ्लोरेसेंस माइक्रोस्कोपी, केमिल्यूमिनेसेंस, और कलोरीमेट्रिक संवेदन जैसी विधियाँ उपरोक्त लक्षित अणुओं के विश्लेषण में प्रयुक्त होती हैं। हाल के वर्षों में, ये सभी विधियाँ विभिन्न खोजी अणुओं की समर्पित पहचान में अत्यंत प्रभावी सिद्ध हुई हैं। तथापि, इन तकनीकों में कठिन नमूना तैयारी, अत्यल्प सांद्रता पर कम संवेदनशीलता जैसी सीमाएँ होती हैं।

ऐसे में रमन स्पेक्ट्रोस्कोपी (Raman Spectroscopy) एक अत्यंत प्रभावी तकनीक बनकर उभरती है, जो विशिष्ट, अति-संवेदनशील संकेत उत्पन्न करती है जो विश्लेषण किए जा रहे अणु की आणविक पहचान होते हैं। रमन संकेतों की मंद तीव्रता को बढ़ाने के लिए सरफेस-एनहांस्ड रमन स्पेक्ट्रोस्कोपी (SERS) का प्रयोग किया जाता है, जो रमन स्पेक्ट्रोस्कोपी की सभी महत्वपूर्ण विशेषताओं को बनाए रखती है।

यह शोध, SERS अनुप्रयोगों के लिए द्वि-आयामी (2D) पदार्थों तथा उनके धातु नैनोसंरचनाओं के साथ बने नैनोसंयोजनों के विकास और अध्ययन पर केंद्रित है, तथा वास्तविक जीवन में SERS की उपयोगिता को भी प्रदर्शित करता है, विशेष रूप से चांदी (Ag) नैनोरॉड्स की मदद से मौखिक कैंसर के विभिन्न चरणों के बीच अंतर करने के संदर्भ में।

शोध के पहले भाग में, MoS_2 की विभिन्न रूपाकार संरचनाओं को SERS अनुप्रयोगों के लिए संश्लेषित किया गया। यह रूपाकार परिवर्तन, सरल सॉल्वोथर्मल विधि के दौरान वृद्धि मापदंडों को बदलकर

प्राप्त किया गया। नैनोप्लेट रूपाकार वाली MoS_2 संरचना से तैयार SERS सब्सट्रेट ने Rhodamine B (RhB) डाई की 10^{-9} M सांद्रता तक उत्कृष्ट पहचान प्रदर्शित की। MoS_2-Ag नैनोसंयोजन SERS सब्सट्रेट्स ने 10^{-15} M की अत्यल्प सांद्रता तक RhB डाई का सफलतापूर्वक पता लगाया, जहाँ संवर्द्धन गुणांक (enhancement factor) 9.2×10^7 रहा। इसमें Ag नैनोकणों का योगदान और RhB तथा MoS_2 के बीच चार्ज ट्रांसफर तंत्र की भूमिका प्रमुख रही।

अगले कार्य में, WS_2 नैनोफ्लेक्स को सिलिकॉन (Si) सब्सट्रेट पर पल्स्ड लेजर डिपोज़िशन (PLD) तकनीक द्वारा लंबवत रूप से उगाया गया। इन WS_2 नैनोफ्लेक्स को थर्मल एवपोरेशन द्वारा Ag नैनोकणों से सजाया गया जिससे SERS क्रिया को बढ़ाया जा सके। WS_2-Ag नैनोसंयोजन की SERS क्षमता का 532 nm और 633 nm उत्तेजना लेज़रों के साथ अध्ययन किया गया। विभिन्न पर्यावरणीय प्रदूषक डाई जैसे RhB, मेथिल ऑरेंज (MO), और मेथिलीन ब्लू (MB) की अत्यल्प सांद्रता पर पहचान संभव हुई। RhB और MO के लिए प्राप्त पहचान सीमा क्रमशः 10^{-16} M और 10^{-17} M रही (532 nm लेज़र के साथ), और MB के लिए 10^{-13} M (633 nm लेज़र के साथ)। इस सब्सट्रेट के लिए 10^8 के स्तर के प्रभावशाली संवर्द्धन गुणांक प्राप्त हुए।

इसके पश्चात, MoS_2-Au नैनोसंयोजन को उपयुक्त Au नैनोकण सांद्रता के साथ SERS अनुप्रयोग हेतु विकसित किया गया। यह संयोजन RhB और N719 जैसे विषैले डाई, तथा E. coli और S. aureus जैसे संक्रामक बैक्टीरिया की पहचान के लिए अत्यंत संवेदनशील SERS सब्सट्रेट के रूप में प्रयुक्त हुआ। RhB और N719 की 10^{-15} और 10^{-9} M तक की सांद्रता की सफल पहचान हुई, जहाँ अधिकतम संवर्द्धन गुणांक क्रमशः 5.2×10^7 और 2.1×10^7 रहा। इसके अतिरिक्त, इस सब्सट्रेट द्वारा E. coli और S. aureus बैक्टीरिया की 10^2 cfu/ml स्तर तक पहचान भी संभव हुई।

शोध के अंतिम भाग में, glancing angle deposition तकनीक से निर्मित लेबल-फ्री Ag नैनोरॉड्स का उपयोग मौखिक कैंसर के पूर्व-घातक चरणों का निदान करने के लिए किया गया, जो इसे पूर्ण रूप से घातक बनने से पहले पहचानने में सहायक है। सभी SERS मापन स्थल पर, क्षेत्र में ही किए गए। पूर्व-घातक एवं घातक नमूनों के SERS स्पेक्ट्रा में अकार्बनिक चयापचयों (metabolites) एवं प्रोटीन की सांद्रता में अंतर को दर्शाने वाले विशिष्ट शिखर (peaks) प्राप्त हुए। मल्टीवेरिएट विश्लेषण विधि ने कैंसर-सकारात्मक और नकारात्मक नमूनों के साथ-साथ पूर्व-घातक और घातक समूहों के बीच सफल पृथक्करण प्रदर्शित किया।

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ABBREVIATIONS

DOS	Density of states
EM	Electromagnetic enhancement
CE	Chemical enhancement
vdW	van der Waals
XRD	X-ray diffraction
FESEM	Field electron scanning electron microscopy
GLAD	Glancing angle deposition
OAD	Oblique angle deposition
XPS	X-ray photoelectron spectroscopy
SPR	Surface plasmon resonance
B.E.	Binding energy
MFC	Mass flow controller
PVD	Physical vapor deposition
2D	Two dimensional
3D	Three dimensional
EDX	Electron diffraction analysis
EPMA	Electron Probe Microanalyzer
TEM	Transmission electron microscopy
HRTEM	High-resolution transmission electron microscopy
AFM	Atomic force microscopy
TMDC	Transition metal dichalcogenides
PECVD	plasma-enhanced chemical vapor deposition
SERS	Surface enhanced Raman spectroscopy
OC	Oral cancer
JCPDS	Joint Committee on Powder Diffraction Standards
MO	Methyl orange
MB	Methylene blue
BPE	trans-1,2-bis(4-pyridyl)ethylene
EC	E. Coli
SA	S. Aureus
RhB	Rhodamine B

BSE	Backscattered electron
PLD	Pulsed laser deposition
PVD	Physical vapor deposition
PVA	Polyvinyl alcohol
EDS	Energy dispersive spectrometer
CB	Conduction band
VB	Valance band
SP	Surface potential
KPFM	Kelvin probe force microscopy
CCD	Charge coupled device
LOD	Limit of detection