

DEVELOPMENT OF SCALABLE AND COST-EFFECTIVE SERS SUBSTRATES FOR BIO-PHOTONICS APPLICATION

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Development of Scalable and Cost-effective SERS Substrates for Bio-photonics Application

by

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Dedicated to my parents

Certificate

This is to certify that the thesis entitled ‘**Development of Scalable and Cost-effective SERS Substrates for Bio-photonics Application**’, submitted by **Sathi Das** to the Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy** in PHYSICS, is a record of the original, bona fide research work carried out by her under my supervision and guidance. The thesis has reached the standards fulfilling the requirements of the regulations related to the award of the degree.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma to the best of my knowledge.

Professor Dalip Singh Mehta
Department of Physics,
Indian Institute of Technology Delhi.

Date:

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In this proud moment of composing the acknowledgements for my thesis, let me share a story.

A person who always stood first in school became irresponsible and casual in learning, resulting in a disastrous career setback after high school. Consequently, the so-called meritorious student failed to secure a seat in reputable colleges for undergraduate studies. However, she gleaned valuable lessons from these failures, worked diligently, and secured a postgraduate seat in a reputable university, graduating with commendable grades. Upon attaining her postgraduate degree, she eagerly sought opportunities to embark on a flourishing career with profound enthusiasm and research aspirations. Eventually, through unwavering hard work and passion, she fulfilled her dream of pursuing research, remaining consistent with her goals. What is the underlying moral lesson conveyed by this narrative? To succeed, consistency is more crucial than intelligence, and intelligence thrives when supported by knowledge. Failure is okay, but being stuck in the concrete of failures is not okay! And indeed, that person is me. Despite feeling hopeless and shedding tears, I restarted everything from scratch to create meaningful work for myself and society. Hence, I first give credit to my honesty and determination to take action.

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Abstract

In recent years, the world has faced various public health and environmental sustainability challenges. Globally, there is a surge of infectious diseases due to increased international travel, urbanization, and the ability of pathogens to evolve and adapt antibacterial resistance genes. According to WHO, more than 1.2 million people died from severe bacterial infections in the respiratory, gastrointestinal, and central nervous systems in 2019. A healthy individual can acquire pathogens from other humans, the environment (contaminated food, water, and air), pets, and farm animals (zoonotic transmission). The pernicious spread of bacteria with antibiotic resistance poses a serious risk to the public's health. Traditional detection techniques, including polymerase chain reaction (PCR), flow cytometry, and enzyme-linked immunosorbent assay (ELISA), are time-consuming and laborious and require specific laboratory infrastructure and trained personnel. Thus, the culture-based detection technique precludes its use for rapid, ultralow detection of pathogens. Furthermore, regular exposure to antibiotics often drives the evolution of resistance via horizontal or vertical transfer of resistant genes, thereby enabling bacteria to escape antibiotic assaults. Consequently, the treatment of multidrug-resistant pathogens is becoming a challenge for clinicians. Thus, it is of utmost importance to determine the phenotypic characteristics of bacterial strains for rapid clinical diagnosis and initiation of a proper treatment regime in time. Thus, there is an ardent need to develop a fast, accurate detection method to diagnose bacterial infections. Apart from the diseases, air and water pollution have reached alarming heights due to industrialization and the increased pace of urbanization. These pollutants harm ecosystems, the climate, and human health. Chemicals are produced by various sources, including industrial emissions, agricultural, and urban areas. For a sustainable future, environmental contamination must be closely monitored and reduced.

This thesis has been inspired by an intense dedication to addressing the aforementioned issues and utilizing innovative technologies to offer practical solutions. The main goal of this study is to develop the methodology of molecular detection and evaluation, with the overriding objectives of enhancing public health and preserving the environment as its foundational values. The specific goals and justification for this undertaking are clarified by the purposes listed below: One of the primary goals of this research is to realize the enormous potential of Raman spectroscopy as a versatile analytical method. Raman spectroscopy has several advantages, including its non-destructive nature and real-time study of molecular vibrational

energy levels. Unlike many traditional procedures, it eliminates the need for time-consuming and laborious sample preparation, making it an essential tool for fast and accurate analysis. However, the inherently low Raman signal intensity limits its utilisation to detect analytes at the trace level. Thus, the technique requires an enhancement mechanism to identify molecular signatures for ultra-low detection.

Surface-enhanced Raman Spectroscopy (SERS) is an emerging detection method that addresses the inherent difficulty of low Raman scattering signal strength. This research intends to develop a robust platform for identifying compounds at ultra-low concentrations by relying on the unique features of SERS. The requirement for quick detection in the contexts of infectious pathogens, antibiotic resistance, and environmental monitoring has prompted the development of SERS. The SERS effect is based on the interaction of light with nanostructures. Hence, the investigation of enhancement activity and plasmonic tunability is explored. This study comprehensively investigates various SERS fabrication techniques, including top-down and bottom-up methods, using physical and chemical synthesis approaches. The objective is to determine the advantages and disadvantages of each technique and its applicability to diverse applications.

The evaluation of SERS performance in detecting complex biomolecules in suspension is critical to this research. The research includes various analytes, from biomarkers and infections to environmental pollutants. The versatility and applicability of SERS in detecting biological and chemical compounds are revealed through systematic examination, providing insights into its potential as a revolutionary tool.

सार

पिछले कुछ वर्षों में, दुनिया ने विभिन्न सार्वजनिक स्वास्थ्य और पर्यावरणीय स्थायित्व संबंधी चुनौतियों का सामना किया है। वैश्विक स्तर पर अंतर्राष्ट्रीय यात्रा एवं शहरीकरण में वृद्धि के कारण संक्रामक रोग और रोगजनकों की जीवाणुरोधी प्रतिरोधक जीन में भी वृद्धि हुई है। WHO के अनुसार 2019 में, श्वसन, जठरांत्र और केंद्रीय तंत्रिका तंत्र में गंभीर जीवाणु संक्रमण के प्रभाव से 1.2 मिलियन से अधिक लोगों की मृत्यु हुई है। एक स्वस्थ व्यक्ति अन्य मनुष्यों, पर्यावरण (दूषित भोजन, पानी, वायु आदि), घरेलू और कृषि जानवरों (जूनोटिक प्रसारण) द्वारा संक्रमित हो सकता है। हानिकारक एंटीबायोटिक प्रतिरोधक बैक्टीरिया का फैलाव सामान्य जनता के स्वास्थ्य के लिए एक गंभीर खतरा प्रस्तुत करता है। पारंपरिक तकनीकी पहचान, जैसे कि पॉलिमरेज श्रृंखला प्रतिक्रिया (PCR), फ्लो साइटोमेट्री, और एंजाइम-संबंधित इम्यूनोसोर्बेंट विश्लेषण (ELISA), अधिक समय लेती हैं और इनके लिए विशेष लैबोरेटरी संरचना एवं प्रशिक्षित कर्मचारियों की आवश्यकता होती है। इस प्रकार बैक्टीरिया कल्चर -आधारित पहचान तकनीक का उपयोग तेजी से, अल्प सांद्रता स्तर पर पैथोजनों की पहचान के लिए नहीं किया जा सकता है। एंटीबायोटिक संवाहक के साथ-साथ बैक्टीरिया के होरिजॉन्टल या लंबवत संक्रमण के माध्यम से प्रतिरोधी जीनों का विकास दिन-प्रतिदिन बढ़ता है, जिससे बैक्टीरिया एंटीबायोटिक हमलों से बच जाते हैं। इस परिणामस्वरूप, क्लिनिशियन्स के लिए समय-में सही चिकित्सा निर्देशों और शानदार चिकित्सा व्यवस्था की शुरुआत के लिए बैक्टीरियल स्ट्रेन की फेनोटाइपिक विशेषताओं को निर्धारित करना बहुत आवश्यक है। इसलिए, बैक्टीरियल संक्रमणों का त्वरित और सटीक पहचान करने के लिए एक तेज, सही पहचान पद्धति विकसित करने की एक तीव्र आवश्यकता

है। इस परिणामस्वरूप, बहुआयामी संक्रमणों का उपचार क्लिनिशियन्स के लिए एक चुनौती बन रहा है। बैक्टीरियल स्ट्रेन की फेनोटाइपिक विशेषताओं को त्वरित क्लिनिकल निदान और सही उपचार पद्धति की शुरुआत के लिए समय पर निर्धारित करना अत्यंत महत्वपूर्ण है। इसलिए, बैक्टीरियल संक्रमणों का निदान करने के लिए एक तेज और सटीक पहचान पद्धति विकसित करने की एक ज़रूरत है।

यह थीसिस पूर्व में उल्लिखित मुद्दों को संबोधित करने के लिए एक गहरे समर्पण से प्रेरित है और नवाचारी तकनीकों का उपयोग करके व्यावहारिक समाधान प्रस्तुत करने में। इस अध्ययन का मुख्य उद्देश्य मोलेक्युलर पहचान और मूल्यांकन की मेथडोलॉजी विकसित करना है, जिसका मुख्य उद्देश्य सार्वजनिक स्वास्थ्य को सुधारना और इसके मूलभूत मूल्यों के रूप में पर्यावरण को संरक्षित करना है। इस प्रक्रिया के उद्देश्यों और इस परियोजना के लिए न्याय देने के लिए नीचे सूचित उद्देश्यों द्वारा इस कार्य की विशेष उद्देश्य और व्यावस्थापन को स्पष्ट किया गया है: इस अनुसंधान का एक प्रमुख उद्देश्य रामन स्पेक्ट्रोस्कोपी की असीमित विशेषज्ञ विश्लेषण विधि की विशाल संभावना को साकार करना है। रमन स्पेक्ट्रोस्कोपी के कई फायदे हैं, जैसे कि इसकी अन्योदर्शी नैसर्गिकता और मोलेक्युलर वाइब्रेशनल ऊर्जा स्तरों का वास्तविक समय में अध्ययन। बहुत से पारंपरिक प्रक्रियाओं की तरह, यह समय लेने और मजबूती करने वाली नमूना तैयारी की ज़रूरत को हटाती है, जिससे यह तेजी से और सटीक विश्लेषण के लिए एक महत्वपूर्ण उपकरण बन जाता है। हालांकि, रमन सिग्नल की प्राकृतिक तरीके से कम तेजी इसे व्यक्तियों को निर्धारित करती है ताकि नमूने के नाकवांछक स्तर पर विश्लेषण किया जा सके। इस प्रक्रिया को अल्ट्रा-लो डिटेक्शन के लिए मोलेक्युलर संकेतों की पहचान के लिए एक सुधारणा तंत्र की आवश्यकता होती है।

सरफेस-एनहैंस्ड रमन स्पेक्ट्रोस्कोपी (SERS) एक उभरती हुई पहचान पद्धति है जो कम रमन बिखराव

सिग्नल शक्ति की मौजूदा मुश्किल का सामना करती है। यह अनुसंधान उल्लेखनीय दर्जे पर यूल्ट्रा-लो संघटनों की पहचान के लिए एक मजबूत प्लेटफ़ॉर्म विकसित करने का उद्देश्य रखता है, SERS की विशेषताओं पर भरोसा करके। संक्रमणकारी पैथोजनों, एंटीबायोटिक प्रतिरोध, और पर्यावरण मॉनिटरिंग के संदर्भ में त्वरित पहचान की आवश्यकता ने SERS के विकास को प्रेरित किया है। SERS प्रभाव पर प्रकाश के नैनो-संरचनाओं के साथ व्यापार पर आधारित है। इसलिए, एन्हांसमेंट गतिविधि और प्लास्मोनिक समायोजन का जांच किया जाता है। यह अध्ययन भौतिक और रासायनिक संश्लेषण दृष्टिकोण से टॉप-डाउन और बॉटम-अप विधियों सहित विभिन्न SERS निर्माण तकनीकों की व्यापक जांच करता है। इसका उद्देश्य हर तकनीक के फायदे और नुकसान तथा विभिन्न अनुप्रयोगों में इसकी अनुपातिता का निर्धारण करना है। इस अनुसंधान में सस्पेंशन में जटिल जीवाणुओं की पहचान में एसईआरएस के प्रदर्शन का मूल्यांकन महत्वपूर्ण है। इस अनुसंधान में विभिन्न विश्लेषक, जैसे कि जैविक संकेतक और संक्रमण से लेकर पर्यावरणीय प्रदूषकों तक, के विभिन्न अध्ययन शामिल हैं। एसईआरएस की व्यापकता और उपयोगिता को जीवन्त और रासायनिक यौगिकों की पहचान में दर्शाया गया है, सिस्टमात्मक परीक्षण के माध्यम से, जो इसे एक क्रांतिकारी उपकरण के रूप में उसकी संभावनाओं में अन्वेषण प्रदान करता है।

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Abbreviations

SERS	S urface E nhanced R aman S cattering
LSPR	L ocalised S urface P lasmonic R esonance
R6G	R hodamine 6g
EF	E nhancement F actor
CT	C harge T ransfer
HOMO	H ighest O ccupied M olecular O rbital
LUMO	L owest U noccupied M olecular O rbital
Ag	S ilver
Au	G old
Cu	C opper
Al	A luminum
Pt	P latinum
Si	S ilicon
NPs	N ano P articles
SM	S ingle M olecule
nm	N anometer
TSC	T risodium citrate
PEG	P olyethylene glycol
°C	D egree C elsius
EBL	E lectron B eam L ithography
PMMA	P olymethyl M ethacrylate
LIL	L aser I nterference L ithography
UV	U ltra V iolet
NIR	N ear I nfrared
UTI	U rinary T ract I nfection
TB	T uberculosis
WHO	W orld H ealth O rganisation

NCBI	N ational C enter for B iotechnology I nformation
CFU	C olony F orming U nit
R6G	R hodamine 6g
cm^{-1}	C entimeter ⁻¹
NC	N anocage
TiO_2	T itanium d ioxide
NRs	N anorods
GLAD	G lancing A ngle D eposition T echnique
MACE	M etal A ssisted C hemical E tching
Si NW	S ilicon N anowire
Si NP	S ilicon N anopillar
PS	P olystyrene
O_2	O xygen
DNA	D eoxyribonucleic acid
XRD	X ray diffraction
FESEM	F ield E mission S canning E lectron M icroscopy
TEM	T ransmission E lectron M icroscopy
FDTD	F inite D ifference T ime D omain
PDMS	P olydimethylsiloxane
microliter	μ L
M	M olar
E. coli	E scherichia coli
P. aeruginosa	P seudomonas aeruginosa
M. tuberculosis	M ycobacterium tuberculosis
K. pneumoniae	K lebsiella pneumoniae
E. cloacae	E nterobacter cloacae
S. aureus	S taphylococcus aureus
P. borealis	P aenibacillus borealis
B. clausii	B acillus clausii
B. subtilis	B acillus subtilis
X. autotrophicus	X anthobacter autotrophicus
P. citronellolis	P seudomonas citronellolis
WGS	W hole G enome S equencing
ng	N anogram
PML	P erfectly M atched L ayer
E	E lectric
LOD	L imit O f D etection
a.u.	A rbitrary U nit
s	S econd
μm	m icron
t-SNE	t -distributed S tochastic N eighbour E mbedding
NSL	N ano S phere L ithography
MIM	M etal I nsulator M etal
SAM	S elf A ssembled M onolayer

Abbreviations

SDS	Sodium d odecyl sulphate
sccm	Standard C ubic c entimetres
RIE	R eactive I on E tching