NON-INVASIVE BIOSENSOR FOR NON-COMMUNICABLE DISEASES

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by

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in fulfillment of the requirements of the degree of Doctor of Philosophy

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Dedicated to

My Parents, Brother and my loving Husband

“Dream is not that which you see while sleeping, it is something that does not let you sleep”

- Dr. A.P.J. Abdul Kalam

CERTIFICATE

This is to certify that the thesis entitled **"Non-Invasive Biosensor for Non-Communicable Diseases"** being submitted by **Ms. Shweta Panwar** to the Indian Institute of Technology Delhi for the award of **Doctor of Philosophy** is a record of bonafide research work carried out by her. **Ms. Shweta Panwar** has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which, to my knowledge, has reached the requisite standard.

The results obtained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

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Shweta Panwar

ABSTRACT

Recent advancements in point-of-care technology have led to the development of handheld, cost-effective, and reusable biosensors designed for convenient, rapid, and daily monitoring of glucose level. These reusable biosensors also contribute to lowering the per-test cost price of glucose detection compared to conventional lab-based instruments. However, many existing biosensors rely on blood samples, causing discomfort and pain for patients. In recent years, non-invasive biosensors utilizing alternative body fluids like saliva, tears, sweat, and urine have emerged as superior alternatives. Despite these advancements, non-invasive biosensors face challenges such as poor correlation among body fluids, interference from surrounding molecules, high costs, and accuracy issues, limiting their impact in the biosensor market. Additionally, glucose devices and their strip cost for measurement of multiple times in a day are often expensive and, making them impractical for point-of-care settings. Therefore, there is considerable potential for developing a pain-free, affordable, easy-to-use, and reliable non-invasive glucose biosensor, particularly appealing in developing countries.

This doctoral research comprises six chapters aimed at developing a handheld, non-invasive glucose meter using a straightforward approach involving immobilization and miniaturization techniques. The thesis primarily focuses on the development of glucose biosensor with multiple features like non-invasive, cost-effective and pain free glucose biosensor using saliva samples, in a handheld portable form. Furthermore, the system has been clinically validated in AIIMS hospital, New Delhi.

The first chapter includes the introduction of diabetes and various diseases associated with it along with various methods of the measurement of glucose levels. While the chapter two extensively reviews literature on glucose biosensor technologies for both invasive and non-

invasive samples. It discusses the various generations of glucose biosensors and development in them and also outlines the future research directions.

Chapter three presents the design and development of a handheld optical reflectance-based instrument and optimization and fabrication of glucose strips, demonstrating its application in estimating glucose levels in PB buffer for blank line.

Chapter four presents the optical studies of glucose meter with three different chromogenic dyes i.e., O-Dianisidine, 4-AAP + Phenol and ABTS. The chapter includes simplified operating procedures suitable for point-of-care settings, interference studies, and shelf-life and reproducibility of the three developed instruments for three different chromogenic dyes.

Chapter five presents the clinical validation of the developed instruments. Clinical studies validate the system's performance for diabetic patients under fasting and non-fasting conditions, showing robust correlations. We did the comparison in Clarkes' Error Grid Model with the existing glucometer i.e., Accu Chek active which shows good correlation between saliva glucose levels and blood glucose levels in diabetic and non-diabetic patients. It also includes the comparison of the three developed instruments for three different chromogenic dyes. The aim was to develop a glucose meter which can potentially replace the traditional lab-based methods.

Chapter six focuses on the conclusion and future prospectives of the developed glucose biosensor in enhancing its design to diagnose other analytes such as HbA1c, cancer biomarkers etc. Improvement in strip design and manufacturing technology aim to reduce production time and enhance biosensor strip shelf life.

सार

पॉइंट-ऑफ-केयर तकनीक में हाल ही में हुई प्रगति ने ग्लूकोज के स्तर की सुविधाजनक, तेज़ और दैनिक निगरानी के लिए डिज़ाइन किए गए हैंडहेल्ड, किफ़ायती और पुन: प्रयोज्य बायोसेंसर के विकास को बढ़ावा दिया है। ये पुन: प्रयोज्य बायोसेंसर पारंपरिक प्रयोगशाला-आधारित उपकरणों की तुलना में ग्लूकोज का पता लगाने की प्रति-परीक्षण लागत को कम करने में भी योगदान देते हैं। हालाँकि, कई मौजूदा बायोसेंसर रक्त के नमूनों पर निर्भर करते हैं, जिससे रोगियों को असुविधा और दर्द होता है। हाल के वर्षों में, लार, आँसू, पसीना और मूत्र जैसे वैकल्पिक शारीरिक तरल पदार्थों का उपयोग करने वाले गैर-इनवेसिव बायोसेंसर बेहतर विकल्प के रूप में उभरे हैं। इन प्रगति के बावजूद, गैर-इनवेसिव बायोसेंसर शारीरिक तरल पदार्थों के बीच खराब सहसंबंध, आसपास के अणुओं से हस्तक्षेप, उच्च लागत और सटीकता के मुद्दों जैसी चुनौतियों का सामना करते हैं, जो बायोसेंसर बाजार में उनके प्रभाव को सीमित करते हैं। इसके अतिरिक्त, ग्लूकोज डिवाइस और एक दिन में कई बार माप के लिए उनकी स्ट्रिप की लागत अक्सर महंगी होती है और उन्हें पॉइंट-ऑफ-केयर सेटिंग्स के लिए अव्यावहारिक बनाती है। इसलिए, दर्द रहित, किफ़ायती, उपयोग में आसान और विश्वसनीय गैर-इनवेसिव ग्लूकोज बायोसेंसर विकसित करने की काफी संभावना है, जो विशेष रूप से विकासशील देशों में आकर्षक है। इस डॉक्टरेट शोध में छह अध्याय शामिल हैं, जिनका उद्देश्य स्थिरीकरण और लघुकरण तकनीकों को शामिल करते हुए एक सरल दृष्टिकोण का उपयोग करके एक हाथ में पकड़े जाने वाले, गैर-आक्रामक ग्लूकोज मीटर को विकसित करना है। थीसिस मुख्य रूप से लार के नमूनों का उपयोग करके एक हाथ में पकड़े जाने वाले पोर्टेबल रूप में गैर-आक्रामक, लागत प्रभावी और दर्द रहित ग्लूकोज बायोसेंसर जैसी कई विशेषताओं के साथ ग्लूकोज बायोसेंसर के विकास पर केंद्रित है। इसके अलावा, इस प्रणाली को नई दिल्ली के एम्स अस्पताल में चिकित्सकीय रूप से मान्य किया गया है। पहले अध्याय में मधुमेह और इससे जुड़ी विभिन्न बीमारियों के साथ-साथ ग्लूकोज के स्तर को मापने के विभिन्न तरीकों का परिचय शामिल है। जबकि अध्याय दो में आक्रामक और गैर-आक्रामक दोनों नमूनों के लिए ग्लूकोज बायोसेंसर प्रौद्योगिकियों

पर साहित्य की व्यापक समीक्षा की गई है। यह ग्लूकोज बायोसेंसर की विभिन्न पीढ़ियों और उनमें विकास पर चर्चा करता है और भविष्य के अनुसंधान दिशाओं की रूपरेखा भी बताता है। अध्याय तीन में एक हाथ में पकड़े जाने वाले ऑप्टिकल परावर्तन-आधारित उपकरण के डिजाइन और विकास और ग्लूकोज स्ट्रिप्स के अनुकूलन और निर्माण को प्रस्तुत किया गया है, जो ब्लैंक लाइन के लिए पीबी बफर में ग्लूकोज के स्तर का अनुमान लगाने में इसके अनुप्रयोग को प्रदर्शित करता है। अध्याय चार में तीन अलग-अलग क्रोमोजेनिक रंगों यानी ओ-डायनिसिडाइन, 4-एएपी + फिनोल और एबीटीएस के साथ ग्लूकोज मीटर के ऑप्टिकल अध्ययन प्रस्तुत किए गए हैं। अध्याय में तीन अलग-अलग क्रोमोजेनिक रंगों के लिए पॉइंट-ऑफ-केयर सेटिंग्स, हस्तक्षेप अध्ययन और तीन विकसित उपकरणों की शेल्फ-लाइफ और पुनरुत्पादकता के लिए उपयुक्त सरलीकृत संचालन प्रक्रियाएं शामिल हैं। अध्याय पांच विकसित उपकरणों की नैदानिक सत्यापन प्रस्तुत करता है। नैदानिक अध्ययन उपवास और गैर-उपवास की स्थिति में मधुमेह रोगियों के लिए सिस्टम के प्रदर्शन को मान्य करते हैं, जो मजबूत सहसंबंध दिखाते हैं। हमने क्लार्क के एरर ग्रिड मॉडल में मौजूदा ग्लूकोमीटर यानी एक्यू चेक एक्टिव के साथ तुलना की, जो मधुमेह और गैर-मधुमेह रोगियों में लार के ग्लूकोज के स्तर और रक्त ग्लूकोज के स्तर के बीच अच्छा सहसंबंध दिखाता है। इसमें तीन अलग-अलग क्रोमोजेनिक रंगों के लिए तीन विकसित उपकरणों की तुलना भी शामिल है। इसका उद्देश्य एक ग्लूकोज मीटर विकसित करना था जो संभावित रूप से पारंपरिक प्रयोगशाला-आधारित तरीकों की जगह ले सके। अध्याय छह में विकसित ग्लूकोज बायोसेंसर के निष्कर्ष और भविष्य की संभावनाओं पर ध्यान केंद्रित किया गया है, ताकि अन्य विश्लेषकों जैसे एचबीए1सी, कैंसर बायोमार्कर आदि का निदान करने के लिए इसके डिजाइन को बढ़ाया जा सके। स्ट्रिप डिजाइन और विनिर्माण प्रौद्योगिकी में सुधार का उद्देश्य उत्पादन समय को कम करना और बायोसेंसर स्ट्रिप की शेल्फ लाइफ को बढ़ाना है।

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Abbreviations

β-ME	β-Mercaptoethanol
δM	Delta M
µm	Microwave
µPADs	Microfluidic Paper Analytical Devices
1,5-DPC	1,5-Diphenylcarbazide
4-AAP	4-Amino Antipyrine
8-OHdG	8-Hydroxy-2’-Deoxyguanosine
ABTS	2,2'-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid)
ADA	American Diabetes Association
AKD	Alkyl Ketene Dimer
ANN	Artificial Neural Networks
APTES	3-Aminopropyltriethoxysilane
BGL	Blood Glucose Level
BIA	Batch Injection Analysis
BS	Bioimpedance Spectroscopy
BSA	Bovine Serum Albumin
CDs	Carbon Dots
CMC	Carboxymethyl Cellulose

CNTs	Carbon Nanotubes
CGM	Continuous Glucose Monitoring
CQDs	Carbon Quantum Dots
DC	Direct Current
DESI	Desorption Electrospray Ionization
DM	Diabetes Mellitus
DMSO	Dimethyl Sulfoxide
DNS	Dinitro salicylic Acid
DRA	Dielectric Resonator Antennas
DKA	Diabetic Ketoacidosis
EMS	Electromagnetic Sensing
ePAs	Electrochemical Paper-Based Analytical Devices
FAD	Flavin Adenine Dinucleotide
FBG	Fasting Blood Glucose
FBPDs	Ferrocene-Based Polymers and Derivatives
FDA	Food and Drug Administration
FDM	Fused Deposition Modeling
FIC	Ferricyanide
FRET	Fluorescence Resonant Energy Transfer
GBs	Glucose Biosensors

GBPs	Glucose Binding Proteins
GDH	Glucose Dehydrogenase
GelMA	Gelatin Methacryloyl
Gox/GOD	Glucose Oxidase
GO	Graphene Oxide
HbA1c	Glycated Hemoglobin
HBD	β-Hydroxybutyrate Dehydrogenase
HQ	Hydroquinone
HSV	Hue, Saturation, Value
I2C	Inter-Integrated Circuit
IoT	Internet of Things
ISF	Interstitial Fluids
LCD	Liquid Crystal Display
LC-MS	Liquid Chromatography-Mass Spectrometry
LDH	Lactate Dehydrogenase
LDR	Light-Dependent Resistor
LED	Light Emitting Diode
LOD	Limit of Detection
mDHB	m-Dihydroxybenzene
MALDI	Matrix-Assisted Laser Desorption/Ionization

M(ox)	Mediator Oxidized
M(red)	Mediator Reduced
MMC	Multimedia Memory Card
mmW	millimeter Wave
mPADs	Microfluidic Paper-Based Analytical Devices
MHC	Metabolic Heat Conformation
MI	Minimally Invasive
MIR	Mid-Infrared Spectroscopy
MNPs	Magnetic Nanoparticles
MNs	Microneedles
MPBA	Mercaptophenylboronic Acid
MPC-CHT	Chitosan-Supported Mesoporous Carbon
MTK	Michler’s Thioketone
MVC	Multivariate Calibration
MWCNT	Multiwalled Carbon Nanotubes
NIR	Near-Infrared
NIRS	Near-Infrared Reflectance Spectroscopy
NI	Non-Invasive
OCT	Optical Coherence Tomography
OCTS	OCT Signal Slope

ODB	O-Diaminobenzene
OP	Optical Polarimetry
oxODB	ODB oxides
PAA	Polyacrylic Acid
PADs	Paper-Based Analytical Devices
PAS	Photo-Acoustic Spectroscopy
PB	Phosphate Buffer
PCB	Printed Circuit Board
PCC	Precipitated Calcium Carbonate
PCR	Principal Component Regression
PDDA	Polydiallyldimethylammonium Chloride
POCDs	Point-Of-Care Diagnostic Devices
POCT	Point-Of-Care Testing Device
Pox/HRP	Horse Radish Peroxidase
PEG	Polyethylene Glycol
PLA	Polylactic Acid
PLL	Poly-L-Lysine
PLS	Partial Least Squares
PLSR	Partial Least Squares Regression
PVA	Polyvinyl Alcohol

QC	Quality Control
QCL	Quantum Cascade Lasers
QDs	Quantum Dots
RGB	Red-Green-Blue
Rh6G	Rhodamine 6G
RI	Reverse Iontophroresis
RMSE	Root Mean Square Error
RMSEP	Root Mean Square Error Of Validation
SEIRA	Surface-Enhanced Infrared Absorption
SERS	Surface-Enhanced Raman-Scattering
SGL	Salivary Glucose Level
SMBG	Self-Monitoring of Blood Glucose
SOP	Standard Operating Procedure
SPI	Serial Peripheral Interface
SPR	Surface Plasmon Resonance
SVR	Support Vector Regression
TCNQ	Tetracyanoquinodimethane
TES	Thermal Spectroscopy
THz-TDS	Terahertz Time Domain Spectroscopy
TMB	Tetramethylbenzidine

TOF	Time Of Flight
TTF	Tetrathiafulvalene
UV	Ultraviolet
WHO	World Health Organization
YSI	Yellow Spring Instrument Company
ZnO	Zinc Oxide