

DESIGN AND DEVELOPMENT OF NON-INVASIVE MULTI-ANALYZER FOR CLINICAL APPLICATION

AMIT KUMAR SINGH



**CENTRE FOR BIOMEDICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY DELHI
MARCH 2020**

©Indian Institute of Technology Delhi (IITD), New Delhi, 2020

DESIGN AND DEVELOPMENT OF NON-INVASIVE MULTI-ANALYZER FOR CLINICAL APPLICATION

by

AMIT KUMAR SINGH

CENTRE FOR BIOMEDICAL ENGINEERING

Submitted

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



INDIAN INSTITUTE OF TECHNOLOGY DELHI

March 2020

Dedicated to
My Father (Late) Dr. S. K. Singh and family

*“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 '**Design and development of non-invasive multi-analyzer for clinical application**' being submitted by **Mr. Amit Kumar Singh** to the Indian Institute of Technology Delhi for the award of **Doctor of Philosophy** is a record of bonafide research work carried out by him. **Mr. Amit Kumar Singh** 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.

Dr. Sandeep Kumar Jha

Associate Professor

Centre for Biomedical Engineering

Indian Institute of Technology Delhi

Hauz Khas, New Delhi – 110016

India

ACKNOWLEDGMENTS

Successful completion of a well-cherished task in scientific research has its own reward. At the same time, remembering the kind guidance of caring teachers and everlasting encouragement from all the dear ones is an experience of its own kind. Without their continuous guidance and support, the study would not have seen the light of the day. I am incredibly grateful to all those persons and organizations that have kindly extended help in different ways during the course of my study. Acknowledging them for their noble deed is a matter of great pleasure for me.

*I want to express my sincere thanks to my doctoral supervisor **Dr. Sandeep Kumar Jha** for his valuable guidance, encouragement, and support at all stages of research as well as writing work. Working under his supervision has indeed been an enriching experience, and I had learned and mastered many techniques in his laboratory under guidance. It was my privilege and matter of pride to be an IIT Delhi student and having Dr. Sandeep Jha as my Ph.D. supervisor.*

*I would also like to thank my SRC members, **Prof. Veena Koul, Prof. Sneh Anand and Dr. Shalini Gupta** for their insightful suggestions and comments. I extend my sincere thanks to all faculty members and staff members of the Centre for Biomedical Engineering for their guidance and generous support.*

I am incredibly thankful to the Indian Institute of Technology Delhi for providing all the facilities and assistantship to carry out my research work. I am also thankful to the Department of Biotechnology (DBT) for providing me research assistantship to carry out my research work.

I am also thankful to the pathology lab staff of the Indian Institute of Technology Delhi Hospital, who helped me carry out clinical studies. I would also like to express my sincere gratitude to all the volunteers who participated in this study.

It is my immense pleasure to thank all my colleagues at CBME and all my lab members Appan Roychoudhury, Ms. Anuradha Soni, Tanu Bhardwaj, Rishi Raj, Marieshwaran, & Avinash Kaur for their help, moral support and friendly atmosphere throughout the research work.

I would also like to owe my deep sense of gratitude towards my late father and for my family for their tremendous moral support, constant encouragement, blessings, love and excellent guidance which always lighten up my task and my life.

Above all, I thank the master of ceremonies, the powerful Almighty God who gave me the confidence, power, blessings, skill, energy, and courage to accomplish this work.

Amit Kumar Singh

ABSTRACT

Advances in point of care technology have led to the development of handheld, low cost, and reusable biosensors for easy, fast, and daily monitoring of multiple analytes in different diseases like diabetes and chronic kidney disease. The reusable biosensor also helps in reducing per-unit cost of the analyte detection in the lab-based instruments. Most of them are blood-based, which cause pain and discomfort to subjects. In recent years, non-invasive biosensors using other body fluids such as saliva, tears, sweat, urine, etc. have emerged as better alternatives to invasive ones. Still, they suffer from various drawbacks such as weak correlation among body fluids, interferences from surrounding molecules, high cost, inaccuracy, etc. because of which they fail to create an impact in the biosensor market. Multi analyzer measurement of the multiple analytes are costlier and bulky therefore cannot be used in point of care technology. Hence, development of a pain-free, affordable, easy to operate, and reliable non-invasive multi-analyzer holds excellent commercial potential as well as can gain massive popularity among ordinary people, especially in developing countries.

In this regard, the present doctoral work consisting of six chapters aims to develop a handheld, non-invasive multi-analyzer using a simple strategy involving the critical techniques of immobilization and miniaturization. Most of the work presented in this thesis deals with reusable glucose biosensor, noninvasive glucose biosensor development using saliva sample and a handheld multi-analyzer instrument development on a strip based format and finally, the developed system has been tested for detection of other analytes such as urea and pH in saliva.

The first chapter of the thesis is devoted to the extensive literature survey technologies for the multi-analyzer instrument in existing, and upcoming technologies for invasive and non-invasive samples is also discussed. The reusability of the glucose biosensor is also discussed in this chapter, and the last chapter discusses the work summary along with the future outcomes of the research work.

The second chapter shows the development of reusable glucose biosensor using low-cost eggshell membrane in biological samples using the dissolved oxygen probe and LabVIEW. The proposed sensor was also demonstrated for the estimation of glucose in human blood samples.

The third chapter demonstrated the design and development of hand-held optical transmission based instrument and low-cost strip design and fabrication method for the development of the

glucose biosensor strips. The standard operating procedure for the measurement procedure for real samples was also simplified as per point of care technology. Interference study and clinical validation are also shown for this biosensor, and good correlation was found for diabetic and non-diabetic patients saliva glucose level to blood glucose level.

The fourth chapter deals with the improvement of instrument design to accommodate the multi-analyzer feature along with providing ambient temperature compensation for the enzymatic reactions. The strip design and manufacturing technology were also improved for decreasing the manufacturing time of the strips. Shelf life improvement in the biosensor strip and the ambient temperature compensation is also provided in the developed biosensor. The clinical results are also verified for diabetic and non-diabetic patients for fasting and non-fasting conditions and found good correlations for diabetic patients among the results.

The fifth chapter explores the feasibility of the developed system for testing other analytes such as urea and pH of saliva using the same strategy but with a low-cost strip having different enzyme (urease) dye combination and pH dye for pH detection. The successful implementation of the proposed system for urea and pH shows that our methodology for detecting multiple analytes via developed hand-held instrument is feasible and can be used as multi-analyzer detection in a non-invasive manner in future, thus replacing many lab-based traditional methods for multiple analyte detection.

सार

पिओसीटी में अग्रिमों ने मधुमेह और सीकेडी जैसे विभिन्न रोगों में कई विश्लेषणों के लिए आसान, तेज, और दैनिक निगरानी के लिए हाथ में, कम लागत और पुनः प्रयोज्य बायोसेंसर के विकास का नेतृत्व किया है। पुनः प्रयोज्य बायोसेंसर प्रयोगशाला आधारित उपकरणों में विश्लेषण का पता लगाने की प्रति इकाई लागत को कम करने में भी मदद करता है। उनमें से ज्यादातर रक्त आधारित हैं, जो विषयों के लिए दर्द और परेशानी का कारण बनते हैं। हाल के वर्षों में, शरीर के अन्य तरल पदार्थ जैसे लार, आँसू, पसीना, मूत्र, आदि का उपयोग करने वाले गैर-इनवेसिव बायोसेंसर लोगों के लिए बेहतर विकल्प बनकर उभरे हैं। फिर भी, वे विभिन्न कमियों से ग्रस्त हैं जैसे कि शरीर के तरल पदार्थ के बीच कमजोर संबंध, आसपास के अणुओं से हस्तक्षेप, उच्च लागत, अशुद्धि, आदि जिसके कारण वे बायोसेंसर बाजार में प्रभाव पैदा करने में विफल रहते हैं। मल्टीपल एनालाइजर का कई एनालिटिक्स का माप महंगा और भारी होता है, इसलिए पिओसीटी में इस्तेमाल नहीं किया जा सकता। इसलिए, एक दर्द मुक्त, सस्ती, संचालित करने में आसान और विश्वसनीय मल्टी-एनालाइजर का विकास उत्कृष्ट व्यावसायिक क्षमता के साथ-साथ आम लोगों के बीच, विशेष रूप से विकासशील देशों में व्यापक लोकप्रियता हासिल कर सकता है।

इस संबंध में, छह अध्यायों से युक्त वर्तमान डॉक्टरल कार्य का लक्ष्य है कि एक सरल रणनीति का उपयोग करते हुए स्थिरीकरण और लघुकरण की महत्वपूर्ण तकनीकों का उपयोग करके एक हैंडहेल्ड, गैर-इनवेसिव मल्टी-एनालाइजर विकसित करना। इस थीसिस में प्रस्तुत अधिकांश कार्य पुनः प्रयोज्य ग्लूकोज बायोसेंसर, लार के नमूने का उपयोग करते हुए बिनाइनवेसिव ग्लूकोज बायोसेंसर विकास और एक स्ट्रिप आधारित प्रारूप पर हैंडहेल्ड मल्टी-एनालाइजर इंस्ट्रूमेंट डेवलपमेंट से संबंधित है और अंत में, विकसित प्रणाली का परीक्षण अन्य विश्लेषणों जैसे कि लार में यूरिया और पीएच का पता लगाने के लिए किया गया है।

थीसिस का पहला अध्याय मौजूदा मल्टी-एनालाइजर इंस्ट्रूमेंट के लिए व्यापक साहित्य सर्वेक्षण प्रौद्योगिकियों के लिए समर्पित है, और इनवेसिव और गैर-इनवेसिव नमूनों के लिए आगामी तकनीकों पर भी चर्चा की गई है। इस अध्याय में ग्लूकोज बायोसेंसर की पुनः प्रयोज्यता पर भी चर्चा की गई है, और अंतिम अध्याय शोध कार्य के भविष्य के परिणामों के साथ-साथ कार्य सारांश पर भी चर्चा करता है।

दूसरा अध्याय डीओ प्रोब लैबव्यू का उपयोग करके जैविक नमूनों में कम लागत वाले अंडकोष झिल्ली का उपयोग करके पुनः प्रयोज्य ग्लूकोज बायोसेंसर के विकास को दर्शाता है। मानव रक्त नमूनों में ग्लूकोज के अनुमान के लिए प्रस्तावित सेंसर का भी प्रदर्शन किया गया था।

तीसरे अध्याय ने ग्लूकोज बायोसेंसर स्ट्रिप्स के विकास के लिए हाथ से संचालित ऑप्टिकल ट्रांसमिशन आधारित इंस्ट्रूमेंट और लो-कॉस्ट स्ट्रिप डिजाइन और फैब्रिकेशन विधि के विकास और डिजाइन का प्रदर्शन किया गया है। पिओसीटी के अनुसार वास्तविक नमूनों की माप प्रक्रिया के लिए एसओपी को भी सरल बनाया गया था। इस बायोसेंसर के लिए हस्तक्षेप अध्ययन और नैदानिक सत्यापन भी दिखाया गया है, और मधुमेह और गैर-मधुमेह के रोगियों एसजीएल से बीजीएल के लिए अच्छा सहसंबंध पाया गया।

चौथा अध्याय एंजाइमी प्रतिक्रियाओं के लिए परिवेश तापमान मुआवजा प्रदान करने के साथ-साथ मल्टी-एनालाइजर सुविधा को समायोजित करने के लिए डिजाइन के सुधार से संबंधित है। स्ट्रिप्स के विनिर्माण समय को कम करने के लिए स्ट्रिप डिजाइन और विनिर्माण प्रौद्योगिकी में भी सुधार किया गया है। बायोसेंसर स्ट्रिप में शेल्फ जीवन सुधार और विकसित बायोसेंसर में परिवेश तापमान कंपनसेशन भी प्रदान किया गया है। उपवास और

गैर-उपवास की स्थिति के लिए मधुमेह और गैर-मधुमेह के रोगियों के लिए नैदानिक परीणाम भी सत्यापित किए गए हैं और परीणामों के बीच मधुमेह के रोगियों के लिए अच्छे संबंध पाए गए हैं।

पांचवें अध्याय में समान रणनीति का उपयोग करते हुए यूरिया और लार के पीएच जैसे अन्य विश्लेषणों के परीक्षण के लिए कम लागत वाली पट्टी के साथ विकसित प्रणाली की व्यवहार्यता की खोज की गई है, लेकिन यूरिया का पता लगाने के लिए अलग एंजाइम (यूरेस) तथा डाई संयोजन और पीएच डाई प्रयोग की गई है। यूरिया और पीएच के लिए प्रस्तावित प्रणाली के सफल कार्यान्वयन से पता चलता है कि विकसित हाथ से आयोजित साधन के माध्यम से कई विश्लेषणों का पता लगाने के लिए हमारी कार्यप्रणाली संभव है और भविष्य में गैर- इनवेसिव तरीके से मल्टी-एनालाइजर डिटेक्शन के रूप में इसका उपयोग किया जा सकता है, इस प्रकार कई प्रयोगशालाओं में पारंपरिक तरीके से प्रयुक्त कई विश्लेषणों का पता लगाने के तरीके को बदला जा सकता है।

CONTENTS

Acknowledgments.....	i
Abstract.....	iii
List of Figures and Illustrations.....	xi
List of Tables.....	xvii
List of Abbreviations.....	xviii
Chapter 1: Introduction and literature review on noninvasive biosensors and multianalyzers	1
1.1. Introduction.....	1
1.2. Body fluids used in non-invasive biosensing.....	3
1.2.1. Blood.....	4
1.2.2. Urine	4
1.2.3. Sweat.....	5
1.2.4. Tears.....	6
1.2.5. Breath.....	7
1.2.6. Saliva.....	7
1.3. Historical perspective.....	8
1.4. Reusable biosensors	10
1.5. Transduction methods used in sensing	10
1.5.1. Noninvasive single analyte based sensing.....	11
1.5.1.1. Electrochemical techniques	11
1.5.1.2. Optical techniques	12
1.5.1.3. Other techniques.....	13
1.5.2. Noninvasive Multi-variate sensing.....	14
1.5.2.1. Electrochemical techniques	14
1.5.2.2. Optical techniques.....	16
1.5.2.3. Other techniques	17
1.6. State of commercialization of non-invasive multi-analyzer biosensors	19
1.7. Future of non-invasive multi-analyzer biosensors.....	22
1.8. Objectives of current research	24
1.9. References.....	26

Chapter 2: Reusable glucose sensor based on enzyme immobilized egg-shell membrane	36
2.1. Introduction.....	36
2.2. Experimental section.....	38
2.2.1. Materials	38
2.2.2. Immobilization of glucose oxidase on eggshell membrane.....	39
2.2.3. Preparation of flow-through the reaction vessel	39
2.2.4. Circuit design	40
2.2.5. Software design for the instrument and computer	42
2.2.6. Glucose sensing using fabricated DO probe.....	43
2.3. Results and discussion	45
2.3.1. Optimization of experimental conditions for membrane preparation.....	45
2.3.2. Reaction vessel, instrumentation, and software.....	46
2.3.3. Response Study.....	47
2.3.4. Sampling procedure	49
2.3.5. Membrane reusability and stability.....	49
2.4. Conclusion	51
2.5. References.....	52
Chapter 3: Fabrication and validation of a handheld non-invasive, optical biosensor for self-monitoring of glucose using saliva	55
3.1. Introduction.....	55
3.2. Experimental section.....	58
3.2.1. Materials	58
3.2.2. Biosensor strip design.....	58
3.2.3. Design of handheld instrument	59
3.2.4. Sample extraction protocol	62
3.2.5. Biosensor characterization studies and validation on clinical samples	63
3.3. Results and discussion	64
3.3.1. Biosensor strip development.....	64
3.3.2. Development of handheld Instrument.....	65
3.3.3. Biosensor characterization	66
3.3.4. Shelf-life studies, batch variation on strip and validation of biosensor under ambient interferences and on clinical samples.....	69
3.4. Conclusion	73
3.5. References.....	75

Chapter 4: Non-invasive temperature compensated biosensor for salivary glucose detection using multi-analyzer instrument	78
4.1. Introduction.....	78
4.2. Experimental section.....	81
4.2.1. Materials	81
4.2.2. Strip designs.....	82
4.2.3. Immobilization protocol of glucose biosensor.....	85
4.2.4. Development of handheld multi-analyzer instrument.....	86
4.3. Results and discussion	91
4.3.1. Development of handheld multi-analyzer Instrument	91
4.3.2. RGB profile of the glucose strips.....	93
4.3.3. Optimization of color gradient and shelf life of glucose biosensor strips	96
4.3.4. The temperature-compensated response of glucose biosensor	98
4.3.5. Interferents effects on glucose biosensor	101
4.3.6. Clinical validation.....	102
4.4. Conclusion	104
4.5. References.....	105
Chapter 5: Feasibility analysis for detecting urea and pH of saliva for multi-analyzer instrument	109
5.1. Introduction.....	109
5.2. Experimental section.....	114
5.2.1. Materials	114
5.2.2. Strip designs for urea and pH measurement	114
5.2.3. Immobilization protocol of the urea biosensor and the pH sensor	115
5.3. Results and discussion	117
5.3.1. RGB profile of the urea biosensor	117
5.3.2. Optimization of color gradient and shelf life of urea biosensor strips.....	120
5.3.3. Temperature-compensated biosensor response for saliva urea biosensor	122
5.3.4. Interferents effects on urea biosensor response	125
5.3.5. Clinical validation of urea biosensor	126
5.3.6. RGB profile of the pH strips	128
5.3.7. Optimization of the color gradient of pH strips	130
5.3.8. Interferents effects on pH sensor response and clinical results	132

5.4. Conclusion	135
5.5. References.....	136
Chapter 6: Conclusion and future perspectives	141
Publication made out of the thesis	147
Curriculum vitae	149

List of Figures and Illustrations

Figure number	Figure Title	Page number
Fig. 1.1	Ames reflectance meter: The first electronic meter for glucose determination.	9
Fig. 1.2	Cnoga's non-invasive MTX instrument.	19
Fig. 1.3	FISA for multiplexed perspiration analysis: wearable FISA on a subject's wrist.	20
Fig. 1.4	DRSA 2000 from ultimed for multiple drug screening in saliva and urine.	20
Fig. 1.5	Xtalline-CRP from OJ Bio Ltd. to detect multiple analytes in blood, urine and saliva.	21
Fig. 2.1	Dissolved Oxygen (DO) probe with BN connector and teflon membrane (A) flow through reaction vessel fitted with silicone tubes (B) buffer tank (C) magnetic stirrer (D) and syringe for sample injection (E).	40
Fig. 2.2	Schematic representation of the reaction vessel (A) and electronic circuit used for DO meter (B) with LabVIEW Interface (C).	41
Fig. 2.3	Block diagram of the designed LabVIEW program.	42
Fig. 2.4	Response studies of the fabricated sensor with respect to (A) low glucose concentration range (1 – 25 mM) and (B) entire glucose concentration range (1 – 1000 mM) in acetate buffer (50 mM, pH 5.1).	47
Fig. 2.5	Calibration curve as a function of the difference in initial and final DO level (Δ DO) for the entire glucose concentration range (1 – 1000 mM). Inset shows the calibration curve for clinically significant low glucose concentration range (1 – 25 mM).	48
Fig. 2.6	Block Diagram of the procedure.	49
Fig. 2.7	(A) Reusability curve of the membrane used as a function of percentage response with the number of uses in same experimental condition in acetate buffer (50 mM, pH 5.1) with 10 mM glucose sample and, (B) shelf-life curve of the membrane used as a function of percentage response with the number of days.	50

Fig. 2.8	Analysis of glucose concentration in real samples.	51
Fig. 3.1	Biosensor strip design: layers and mechanical design of strip.	59
Fig. 3.2	Circuit diagram of the instrument.	60
Fig. 3.3	Flow chart of the program for the microcontroller operation in the instrument.	61
Fig. 3.4	Block diagram of the proposed POCT device.	62
Fig. 3.5	Front view of the strip showing color change with respect to different spiked saliva samples containing glucose concentrations: (A: 10, B: 52, C: 137, D: 182, E: 272, F: 365 and G: 516 mg/dL).	63
Fig. 3.6	Internal View of the instrument.	65
Fig. 3.7	(A) The time response curve of the biosensor strip plotted for different glucose concentration vs. light intensity for 85 sec time interval (sample applied using bud). Where X corresponds to sample absorption point, Y is product formation period, A to G are the glucose concentrations (10, 52, 137, 182, 272, 365 and 516 mg/dL) respectively; (B) calibration curve: curve-A shows the calibration curve with reading taken for 15 sec after the absorption of the saliva onto the strip, while curve-B shows the calibration curve with readings taken for 60 sec after absorption of saliva on the strip, curve-C shows calibration curve with strips devoid of immobilized glucose oxidase (reference sample).	67
Fig. 3.8	(A) The shelf life of strip tested for 1 month using synthetic saliva sample (with 250 mg/dL glucose in the buffer). (B) Batch variation test of the biosensor strip tested for six batches. (with 250 mg/dL of glucose in the saliva sample).	69
Fig. 3.9	Interference curve of common acid variation found in saliva at 125mg/dL spiked concentration of glucose.	70
Fig. 3.10	(A) Ambient light variation response on the strip tested in different ambient light conditions such as darkroom, fluorescent light, sunlight and sunshade having light intensities of 0.07, 420, 5730 and 56500 lux. (B) Bar graph for wind speed variation using wall mounted fan (Havells) maintaining the distance of 48 Inches between instrument under test for two glucose concentration with 5% spiking of glucose concentration in saliva sample (35uL volume).	71
Fig. 3.11	Statistical correlation between SGL and BGL for pre-breakfast samples from (A) diabetic patients and (B) Non-diabetic	72

	subjects.	
Fig. 3.12	Clarke error grid showing a correlation between the BGL (glucose estimation using commercial Glucometer) and SGL (calculated using developed biosensor).	73
Fig. 4.1	Strip designs used for the biosensor (A) Represents the strip design (2) and (B) represents the design (3) of the glucose detection raw strip.	83
Fig. 4.2	Raw strips used for the biosensor (A) Front and back view of strip design (2) and (B) represents the front and back view of strip design (3) used for the glucose detection.	84
Fig. 4.3	The reaction for the formation of gluconic acid from glucose in the presence of GOx converts NY from dark blue to yellow.	86
Fig. 4.4	Circuit diagram of the multi-analyzer instrument.	87
Fig. 4.5	Flow chart of the program for the microcontroller operation, used in the instrument.	90
Fig. 4.6	Internal view of the developed instrument (A) Shows the bottom half of the instrument containing all the electronic part and (B) shows the top cover of the instrument.	92
Fig. 4.7	Glucose biosensor front view showing color change for different glucose concentration values from A-H (34, 44, 69, 84, 134, 184, 284, 384 mg/dL) respectively used for strip design (2).	93
Fig. 4.8	(A) Shows the response curve of biosensor strip design (2) at 620nm, (B) 530 nm and (C) 470 nm wavelengths respectively for different spiked glucose concentrations. (D) Shows calibration curve of red, green and blue color change for NY indicator for glucose biosensor for strip design (2) for time difference of 60 sec from the start of saliva absorbing time.	94
Fig. 4.9	(A) Calibration curve shows the red intensity change for different glucose concentrations with 7 sec integration time in different stabilizing agents: (X) shows assay without stabilizing agent (Y) with DTT and (Z) is with 2-ME, (B) Glucose biosensor strip front view color change for different glucose concentration values from D-K (0, 12, 37, 62, 112, 162, 262, 362 mg/dL) respectively tested at 25°C with 2-ME.	97
Fig. 4.10	The activity of GOx in different inhibitors and at different storing temperatures of the biosensor strips tested for 100mg/dL of synthetic glucose per week. The assay combination for curve A is GOx +2-ME, B is GOx +DTT, and	98

	C is GOx stored at 25°C respectively while curve D is GOx +2-ME, E is GOx +DTT, and F is GOx at 4°C respectively.	
Fig. 4.11	Response curve of glucose biosensor strips tested at different temperature in the spiked glucose concentration from 0-350 mg/dL up to 70 sec after sample detected: (A) GOx response at 14°C, (B) GOx response at 19°C, (C) GOx response at 25°C, (D) GOx response at 31°C, (E) GOx response at 37°C, (F) GOx response at 45°C (G) GOx response at 50°C, and (H) Response of the strip without GOx for LOD calculation respectively.	99
Fig. 4.12	(A) The 2D-Calibration curve at different temperature shows the red intensity change for different glucose concentrations for 7.35 sec (B) 3D calibration curve for the glucose biosensor strip at different ambient temperature, glucose concentration, and LII for 7.35 sec response time.	100
Fig. 4.13	(A) Interference tests for glucose strip in lactic acid, ascorbic acid and urea with 150mg/dL of spiked glucose (B) Biosensor response due to different sample volume tested at 250mg/dL spiked glucose level in saliva.	101
Fig. 4.14	Clinical results for glucose biosensor for (A) Diabetic pre-breakfast, (B) Diabetic-post breakfast (C) Non-diabetic subject pre-breakfast, and (D) Non-diabetic subject post breakfast.	103
Fig. 5.1	Basic strip design for the urea and pH biosensor strips.	115
Fig. 5.2	(A) Strips designed (4) shows the front and back view of raw urea strip and (B) Strip design (5) shows the front and back view of raw pH strip respectively.	115
Fig. 5.3	The reaction for the formation of ammonia from urea in the presence of urease converts PR from yellow to pink.	116
Fig. 5.4	Urea biosensor strip front view color change for different urea concentration from A-G (10, 35, 60, 110, 160, 260 and 360 mg/dL) respectively using PR indicator.	118
Fig. 5.5	(A) Shows the response curve of urea strip at 620nm, (B) 530 nm and (C) 470 nm wavelengths respectively for different spiked urea concentrations. (D) Shows calibration curve of red, green, and blue color change for PR indicator for urea biosensor for strip design (4) for time difference of 60 sec from start of saliva absorbing time.	119
Fig. 5.6	(A) Calibration curve shows the green intensity change for different urea concentrations with 3 sec integration time in different reducing agents: (X) shows enzyme with 2-ME (Y) with DTT and (Z) is without reducing agent (B) Urea biosensor	121

	strip front view color change for different urea concentration from D-H (8, 33, 53, 78 and 108 mg/dL) respectively tested at 25°C with 2-ME.	
Fig. 5.7	The activity of urease in different inhibitors and at different storing temperatures of the urea biosensor strips tested for 30 mg/dL of synthetic urea tested per week. The assay combination for curve A is urease + 2-ME, B is urease + DTT, and C is urease stored at 25°C respectively while curve D is urease + 2-ME, E is urease + DTT, and F is urease at 4°C respectively.	122
Fig. 5.8	Response curve of urea biosensor strips tested at different temperature in the spiked urea concentration from 0-100 mg/dL up to 50 sec after sample detected (A) urease response at 15°C, (B) urease response at 19°C, (C) urease response at 25°C, (D) urease response at 31°C, (E) urease response at 36°C, (F) urease response at 43°C, (G) urease response at 48°C, and (H) Response of the strip without urease for LOD calculation respectively.	123
Fig. 5.9	(A) The 2D-Calibration curve at different temperature shows the green intensity change for different urea concentrations for 3 sec (B) 3D calibration curve for the glucose biosensor strip at different ambient temperature, urea concentration, and LII for 3 sec response time.	124
Fig. 5.10	Urea biosensor strip front view color change for different urea concentration from A-H (0, 5, 20, 30, 45, 65, 90 and 120 mg/dL) respectively tested at 25°C with 2-ME.	125
Fig. 5.11	(A) Interference tests for urea strip in LA, KCl, NaCl, and NH ₄ Cl with 45 mg/dL of spiked urea (B) Biosensor response due to different sample volume tested at 45 mg/dL spiked glucose level in saliva.	126
Fig. 5.12	Clinical results for urea biosensor tested for (A) NCKD and (B) CKD subjects respectively.	127
Fig. 5.13	Front view of the pH strip color change for different pH values from A-I (3.79, 4.70, 5.61, 6.15, 6.68, 7.29, 7.81, 8.24 and 8.94) respectively tested on a spiked saliva sample.	128
Fig. 5.14	(A) Shows the response curve of pH strip at 620nm, (B) 530 nm and (C) 470 nm wavelengths respectively for different pH values. (D) Shows calibration curve of red, green and blue color change for the universal indicator for pH strip design (4) for time difference of 60 sec from the start of saliva absorbing time.	129
Fig. 5.15	Shows the response curve of pH strip (A) for X assay, (B) for	131

Y assay and (C) for Z assay at 470 nm wavelength for different pH values and (D) Shows the calibration curve of the pH strip at different pH for the three different indicator concentrations with universal indicator with PVA immobilized (X) one time, (Y) two time, (Z) three times.

Fig. 5.16 pH strip front view color change for different pH values from A-F (3.9, 5.0, 6.0, 7.0, 7.9 and 8.7) respectively for universal indicator with PVA immobilized three times. 132

Fig. 5.17 pH strip response due to different sample volume tested at 7pH of saliva, by maintaining the pH of saliva by spiking it with 1M NaOH and 1M LA stock solution 133

List of Tables

Table number	Title	Page number
Table 1.1	Different noninvasive multi-variate sensing techniques	23
Table 5.1	Average pH values of the healthy patients measured using standard electrode-based pH meter and the developed sensor	134
Table 5.2	Shows the four instrument average count, SD and percentage SD tested for 60 blank strip.	134
Table 6.1	Analytical performances of some non-invasive multi variate biosensors/ instruments including commercializable ones developed so far in comparison to our developed sensor.	144

List of Abbreviations

2-ME	2-Mercaptoethanol
μ-PAD	Paper-based analytical device
ADC	Analogue-to-digital converter
ASSURED	Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable
BGL	Blood glucose level
BiCMOS	Bipolar Complementary Metal-Oxide Semiconductor
BP	Bromocresol Purple
BUN	Blood Urea Nitrogen
BUL	Blood Urea Level
CKD	Chronic Kidney Disease
COMP	Cartilage Oligomeric Matrix Protein
CVD	Cardiovascular disease
DAQ	Data acquisition card
DO	Dissolved oxygen
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic acid
DTT	Dithiothreitol
DW	Distilled water
ELISA	Enzyme-linked immune sorbent assay
ESRD	End-Stage Renal Disease
FDA	Food and Drug Administration

FISA	Fully integrated sensor array
GDP	Gross Domestic Product
GFR	Glomerular filtration rate
GO _x	Glucose oxidase
GSM	Grams per Square Meter
H ₂ O ₂	Hydrogen peroxide
HRP	Horseradish peroxidase
HIV	Human immunodeficiency virus
I2C	Inter-integrated Circuit
IC	Integrated Chip
IDF	International Diabetes Federation
ISFET	Ion sensitive field-effect transistor
IR	Infrared
IL	Interleukin
KCl	Potassium Chloride
LA	Lactic Acid
LabVIEW	Laboratory virtual instrument engineering workbench
LCD	Liquid Crystal Display
LDR	Light Dependent Resistor
LED	Light Emitting Diode
LII	Light Intensity Integration
LOC	Lab on a chip
LOD	Limit of detection

MATLAB	Matrix Laboratory
MMC	Multi-media card
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NCD	Non-communicable diseases
NCKD	Non-Chronic Kidney Disease
NH ₄ Cl	Ammonium Chloride
NIR	Near-infrared spectroscopy
NY	Nitrazine Yellow
PB	Phosphate buffer
PCB	Printed Circuit Board
PHT	Phenol Hypochlorite Test
PIC	Programmable interrupt controller
POC	Point of Care
POCD	Point of Care Devices
POCT	Point of Care Technology
PR	Phenol red
PTH	Plated through-hole
PVA	Polyvinyl alcohol
QCM	Quartz crystal microbalance
RBC	Red blood cells
RGB	Red Green Blue
RNA	Ribonucleic acid

RSD	Standard deviation in sensor measurement
SAW	Surface acoustic wave
SD	Standard deviation
SD _x	Salivary diagnostics
SERS	Surface-enhanced Raman scattering
SGL	Salivary glucose level
SMT	Surface mount technology
S/N	Signal to noise ratio
SOP	Standard Operating Protocol
SPR	Surface Plasmon Resonance
SUL	Saliva urea level
UN	United Nations
USA	United States of America
US	United States
USB	Universal Synchronous Bus
VOCs	Volatile Organic Compounds
WBC	White blood cells
WHO	World Health Organization