REPUBLIC OF TURKEY
YILDIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

A NOVEL POTENTIOMETRIC BORATE SELECTIVE BIOSENSOR FOR REAL TIME DETECTION OF GLUCOSE, GLYCATED ALBUMIN AND GLYCATED HEMOGLOBIN LEVELS

YİĞİT TANYERİ

MSc. THESIS
DEPARTMENT OF BIOENGINEERING
PROGRAM OF BIOENGINEERING

ADVISER
PROF. DR. İBRAHİM IŞILDAK

İSTANBUL, 2019

REPUBLIC OF TURKEY

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

A NOVEL POTENTIOMETRIC BORATE SELECTIVE BIOSENSOR FOR REAL TIME DETECTION OF GLUCOSE, GLYCATED ALBUMIN AND GLYCATED HEMOGLOBIN LEVELS

A thesis submitted by Yiğit TANYERİ in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** is approved by the committee on 31.07.2019 in Department of Bioengineering, Bioengineering Program.

Thesis Adviser

Prof. Dr. İbrahim Işıldak

Yıldız Technical University

Approved By the Examining Committee

Prof. Dr. İbrahim IŞILDAK

Yıldız Technical University _____

Assoc. Prof. Didem ÖZÇİMEN

Yıldız Technical University _____

Asst. Prof. Burcu YÜCEL

Istanbul Medeniyet University _____

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) Grant No: 116S758.

ACKNOWLEDGEMENTS

I would like to put my gratitude forward to Prof. Dr. İbrahim IŞILDAK, my esteemed professor, who I always benefited from his valuable knowledge, ideas and experiences at all stages of my graduate studies and thesis studies.

I would also like to thank my Tanyeri family, especially to my mother Yelda Zerrin Tanyeri, my father Şah Tanyeri and my brother Baris Tanyeri for their continuous support during my studies.

I would like to thank all my colleagues who helped me in every stage of my graduate studies and thesis studies. I would especially like to thank Özlem Tavukçuoğlu, Selim Işıldak, Ayşenur Ertunç, Mustafa Niğde, Gökay Vardar, Rıdvan Yıldırım for helping me in this study whom I enjoyed working together very much in the laboratory and wish them all the success in the future.

July, 2019

Yiğit TANYERİ

TABLE OF CONTENTS

Page

LIST OF SYMBOLS viii

LIST OF ABBREVIATIONS ix

LIST OF FIGURES x

LIST OF TABLES xii

ABSTRACT xiii

ÖZET xv

CHAPTER 1

INTRODUCTION 1

1.1 Literature Review 1

1.2 Objective of the Thesis 2

1.3 Hypothesis 3

CHAPTER 2

GENERAL INFORMATION 4

2.1 General Information on Diabetes Mellitus 4

2.1.1 Symptoms 5

2.1.2 Diagnosis 5

2.1.3 Treatment 6

2.1.4 Prevention 6

2.2 Boron Sources of Turkey 6

2.3 Boron Measurement Methods 8

2.3.1 Electroanalytical Sensing Methods 8

2.3.1.1 Potentiometric Techniques 9

2.3.1.2 Amperometric Techniques 9
2.3.1.3 Conductometric Techniques 9
2.3.1.4 Voltammetric Techniques 10
2.3.1.5 Calorimetric Techniques 10
2.3.1.6 Fluorometric Techniques 11
2.4 Potentiometry and Flow Injection Analysis Technique 11
2.4.1 Potentiometry technique and measurement 11
2.4.2 Ion selective electrodes (ISE) 12
2.4.3 Reference electrodes 13
2.4.4 Flow injection analysis 14
2.4.5 Performance characteristics of an ion selective biosensor 15
2.4.5.1 Ion selectivity 15
2.4.5.2 Response time 16
2.4.5.3 Linear Working Range 17
2.4.5.4 pH Working Range 18
2.4.5.5 Lifetime 18
2.4.5.6 Repeatability 18
2.4.5.7 Advantages and disadvantages of ion selective electrodes 18
2.4.5.8 Applications of ion selective electrodes 19
CHAPTER 3
MATERIAL AND METHODS 20
3.1 Chemicals Used 20
3.2 Equipments Used 21
3.3 Methods 22
3.3.1 Preparation of solutions 22
3.3.2 Preparation of silver borate 22
3.3.3 Preparation of CNT based borate composite electrodes 23
3.3.4 Preparation of GO based borate composite electrodes 25
3.3.5 Preparation of CNT-Ag_3BO_3 and GO-Ag_3BO_3 26
3.4 Working Method 28
3.5 Purpose of the Study 28
CHAPTER 4

RESULTS AND DISCUSSION 29

4.1 Stagnant solution measurements 29

4.1.1 Potentiometric performance characteristics of the developed borate selective electrode 29

4.1.1.1 Ion selectivity 29

4.1.1.2 Response time 31

4.1.1.3 pH working range 33

4.1.1.4 Repeatability 34

4.1.2 GO-Borate Composition Results 36

4.1.3 CNT-Borate Composition Results 38

4.1.4 Detection of glucose, glycated albumin and glycated hemoglobin levels through flow injection analysis by the developed CNT based borate selective electrode 45

4.2 Discussion 48

REFERENCES 51

CURRICULUM VITAE 54

LIST OF SYMBOLS

E	Electrode potential
F	Faraday constant
R	Gas constant
Z	Ion charge
µl	Microliter
M	Molarity
mV	Millivolt
T	Temperature

LIST OF ABBREVIATIONS

CNT	Carbon nanotube
DM	Diabetes Mellitus
GO	Graphite oxide
ISE	Ion selective electrode
mV	Millivolt
SCE	Standard calomel electrode
SHE	Standard hydrogen electrode
THF	Tetrahydrofuran

LIST OF FIGURES

Page

Figure 1.1 A Figure representation of biosensors being divided into bio-element and sensor element components [2] 1

Figure 2.2 Visual representation of the World's largest boron reservoirs [10] 8

Figure 2.3 Vision from an ongoing experiment where a working electrode is being tested potentially in numerous different concentrated borate solutions 12

Figure 2.4 Close look of the multichannel potentiometer 12

Figure 2.5 Electrochemical cell for making a potentiometric measurement with an Ion Selective Electrode [24] 13

Figure 2.6 Visual of SCE and Ag/AgCl reference electrode [25] 14

Figure 2.7 Flow injection analysis setup 15

Figure 2.8 Calibration graphic of selectivity 16

Figure 2.9 A representation of an electrical output balancing itself after a certain time (x axis is the time axis and y axis is the potential axis 17

Figure 3.10 Preparation of CNT-Ag3BO3 and GO-Ag3BO3 27

Figure 4.11 Selectivity measurement of the electrode number 36 30

Figure 4.12 Electrode number 101 is being tested to see the response time of the electrode 31

Figure 4.13 Electrode number 111 is being tested to see the response time of the electrode 32

Figure 4.14 4 different electrodes being tested for their potential outputs under 3 different pH levels 33

Figure 4.15 pH calibration graph 33

Figure 4.16 Electrode number 101 is being tested for its repeatability on 10-2 and 10-3 M solutions 35

Figure 4.17 Electrode number 111 is being tested for repeatability 36

Figure 4.18 GO borate measurement with the 154 numbered electrode 37

Figure 4.19 GO borate measurement with the 154 numbered electrode 37

Figure 4.20 Borate measurement of electrode number 38 38

Figure 4.21 Borate measurement of electrode 35 and 36 39

Figure 4.22 Repeatability measurement of electrode number 101 40

Figure 4.23 Measurements of 22-24-25 numbered electrodes on borate ions 41

Figure 4.24 Borate measurement of the electrode numbered as 91 42

Figure 4.25 Ion selectivity measurement of #35 electrode 42

Figure 4.26 Ion selectivity measurement of #38 electrode 43
Figure 4.27 Ion selectivity measurement of electrode numbered as 81 44
Figure 4.28 Ion selectivity measurement of electrode numbered as 38 45
Figure 4.29 Electrode 102 responding to hba1c solution 46
Figure 4.30 Electrode number 102 responding to glycated human serum albumin solution 47
Figure 4.31 Electrode number 102 responding to glucose 47

LIST OF TABLES

Page

Table 2.1 The table of the total million tones of boron distribution for top countries [10] 7
Table 3.2 Chemicals and average amounts used for the electrode compositions 21
Table 3.3 Compositions of the electrodes built for the thesis 23
Table 3.4 Solid contact material composition 24
Table 3.5 Electrode composition when GO was used as a replacement for CNT 26
Table 4.6 Potentiometric response of some pH values. 34
Table 4.7 Composition of the best built electrode that showed necessary characteristics for this project 48
Table 4.8 Electrode's best parameter distributions 49

ABSTRACT

A NOVEL POTENTIOMETRIC BORATE SELECTIVE BIOSENSOR FOR REAL TIME DETECTION OF GLUCOSE, GLYCATED ALBUMIN AND GLYCATED HEMOGLOBIN LEVELS

Yiğit Tanyeri

Department of Bioengineering

MSc. Thesis

Adviser: Prof. Dr. İbrahim Işıldak

Diabetes Mellitus (DM) is a metabolic disease and affects about 8.3 percent of the world's adult population. Regular control of blood sugar levels of the patients is of great importance in order to prevent complications that may occur in patients. Glycemic control can be achieved either by measurement of glycosylated proteins or by measurement of sugar on an empty stomach. Blood glucose measurement on an empty stomach has to be taken when the patient hasn't eaten anything in the morning, but glycosylated hemoglobin, albumin and fructosamine gives evidence without anyone fasting or depends on other parameters. Hba1c is the most common type of hemoglobin and is only one of the non-enzymatic non-glycosylated proteins. Hemoglobin is a source that provides an average of 3 months before the day it was measured, and so has been studied frequently in recent years and many methods have been developed. Particularly in chromatographic and immunochemical methods, a method of measurement has been developed using differences in structure differences or load differences but these are expensive techniques that also requires lots of preliminary procedures.

The last protein designed for use in this study is serum albumin. In cases where Hba1c measurement cannot provide a definite result, serum albumin which gives an average value until the last 2-3 weeks is being used. The half-life of serum albumin (HSA) in the blood ranges from 12 to 21 days. The methods developed for this protein are boronate affinity chromatography, enzymatic methods and immunoassay techniques.

The aim of this thesis is to develop a borate selective biosensor that will be used in the simultaneous measurement of glucose, glycated albumin and glycated hemoglobin. This measurement will be designed collectively in a flow injection system. In this method of analysis, there will be a mini-colon unit to provide separation and a detection unit to include a borate selective sensor and reference electrode to detect the borate. Simultaneous measurement of glucose, glycolized albumin and glycolized hemoglobin will be provided in the flow system to be passed within the whole system.

The part that differentiates this project from other studies is that a system with which these three parameters can be measured together is not created and it is foreseen that it will include economic, simple and few preliminary procedures.

Keywords: Diabetes mellitus, HbA1c, borate-selective electrode, flow-injection analysis, glycosylated hemoglobin, glycated albumin, glucose

ÖZET

GLİKOZ, GLİKOLİZE HEMOGLOBİN VE GLİKOLİZE ALBÜMİNİN GERÇEK ZAMANLI ÖLÇÜMLERİNİN SAĞLANMASI AMACIYLA POTANSİYOMETRİK YENİ BİR BORAT SEÇİCİ BİYOSENSÖRÜN GELİŞTİRİLMESİ

Yiğit Tanyeri

Biyomühendislik Anabilim Dalı

Yüksek Lisans Tezi

Tez Danışmanı: Prof. Dr. İbrahim Işıldak

Diabetes Mellitus metabolik bir hastalıktır ve dünya yetişkin nüfusunun yaklaşık yüzde 8,3'ünü etkilemektedir. Hastaların kan şekerlerinin düzenli kontrolü hastalarda oluşabilecek komplikasyonların önüne geçmek için büyük önem arz etmektedir. Glisemik kontrol ise ya glikozlanmış proteinlerin ölçümü sonucu veya aç karnına şeker ölçümü ile sağlanabilmektedir. Aç karnına şeker ölçümünün hastadan sabah hiçbir şey yemeden kan alınması zorunluluğu bulunur fakat glikolize olmuş hemoglobin, albumin ve fruktozamin değerleri açlık tokluk veya başka parametrelerden etkilenmeyen bir bulgu sağlamaktadır. Hba1c hemoglobinin en sık bulunan çeşididir ve enzimatik olmayan glikozlanmaya uğramayan pek çok proteinden sadece bir tanesidir. Hemoglobin ölçüldüğü günden 3 ay öncesine dair bir ortalama sunan bir kaynaktır ve bu yüzden de son yıllarda sıkça çalışılmıştır ve birçok yöntem geliştirilmiştir. Özellikle kromatografik ve immünokimyasal yöntemler yapı farklarındaki farklılıklardan veya yük farklılıklarından ölçüm yöntemi geliştirilmiştir fakat bu yöntemler oldukça pahalı ve zor işlemler olduğundan dolayı bir alternatif yönteme ihtiyaç duyulmuştur.

Projede kullanılması tasarlanan son protein ise serum albüminidir. Hba1c ölçümünün sağlıklı sonuç veremeyeceği durumlarda son 2-3 hafta öncesinde kadar ortalama değer veren serum albümin kullanılmaktadır. Serum albümininin (HSA) kanda yarılanma süresi 12 ila 21 gün arasında değişmektedir. Bu protein için geliştirilen yöntemler ise boronat afinite kromatografisi, enzimatik yöntemler ve immunoassay teknikleridir.

Bu tezin amacı, glikoz, glikolize albümin ve glikolize hemoglobinin eşzamanlı ölçümü için kullanılabilen bir borat seçici elektrotu geliştirmektir. Bu ölçüm bir akış enjeksiyon sisteminde toplu bir şekilde tasarlanacaktır. Bu analiz yönteminde ayırımı sağlamak üzere bir mini kolon ünitesi ve borat algılamak üzere bir borat seçici sensör ve referans elektrot içerecek algılama ünitesi olacaktır. Bütün sistem dahilinde geçirilecek akış sisteminde glikoz, glikolize albümin ve glikolize hemoglobinin eş zamanlı ölçümü sağlanacaktır.

Bu çalışmayı diğer çalışmalardan ayıran kısım ise daha önce bu üç parametrenin beraber ölçülebileceği bir sistemin oluşturulmamış olmasıdır ve yapılması dahilinde ekonomik, basit ve az sayıda ön prosedür içereceği öngörülmüştür.

Anahtar Kelimeler: diabetes mellitus, HbA1c, borat-seçici elektrot, akış-enjeksiyon analiz, glikolize hemoglobin, glikolize albümin, glikoz.

CHAPTER 1

INTRODUCTION

1.1 Literature Review

People have been sensing it's environment via their enzymes, tissues, cells and antibodies etc. since they existed. This receptive mechanism along human body inspired bio-sensing technologies. Biosensors are devices that can convert a biological analyte's specific feature measurement to an electrical output. Biosensor field does include lots of various engineering studies such as chemistry, biology, materials science. Biosensor field depending on various engineering studies makes this subject easier to be developed as breakthrough improvements are observed in these subjects [1].

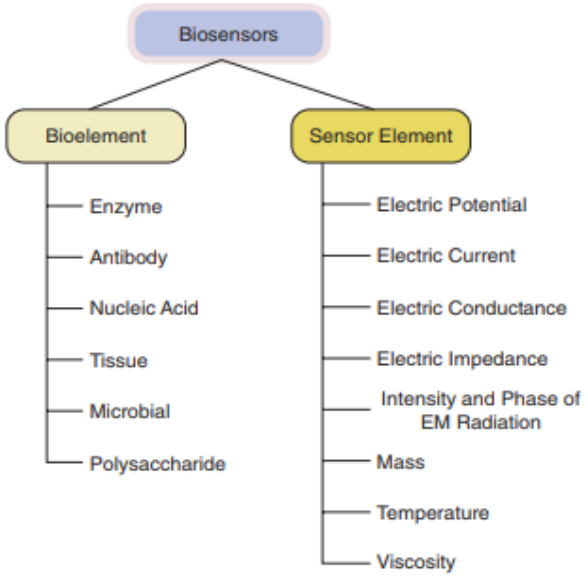


Figure 1.1 A Figure representation of biosensors being divided into bio-element and sensor element components [2]

Biosensors are devices that are the integration of two components which are bioreceptors and transducers. Bioreceptors are parts that are responsible for the recognition of the bioelements therefore can be considered as the living part of these devices. Bioreceptors can be anything from an enzyme, antibody, nucleic acid, microorganism, cell, tissue to immunoagents. Transducers are the part that is responsible from converting the recognition event into an electrical signal. These devices can be electrochemical biosensors (potentiometric, amperometric, voltammetric and conductometric), optical biosensors, calorimetric (thermal) biosensors, piezoelectric biosensors or acoustic (sound or location) biosensors. Transducers are often named as physicochemical converters as it converts the analyte's output to an electronic signal. At the end of the transducer part of the biosensors, there is usually a screen that displays the converted electronic signal into a numerical data that can be read by the user.

For any biomedical diagnosis, biosensors play a huge role. Not only for biomedical diagnosis but biosensors are also being widely used in environmental monitoring, disease progression, drug discovery, food control and forensics [2].

Biosensors especially in the 21st century are very much desired to be as small as possible especially when they are designed to be used inside the body. Biosensors being developed in nano-scaled structures allows it to be implemented easily on computer chips. Therefore advances on nanotechnology and implementing those new features allows the biosensors to be more accurate, durable and cheap in terms of production costs. Biosensors will continue to develop as more advances on nanotechnology is observed [3].

1.2 Objective of the Thesis

Thesis's goal is to produce a borate selective electrode that will be used to measure glucose level, mid-term glucose level and long term glucose levels in blood simultaneously within the flow injection analysis. Glycated proteins will be forming a complex structure with the combination of these proteins and borate structures, therefore a borate selective electrode will be able to help detecting these molecules.

Within the projected measurement of glycated proteins, there is a separation column cell that will allow the injected solution to reach detection column where the reference and borate selective electrode is present. Separation column will include the filling material that will do the separation. This miniature design will be able to enable the detection of

the bioanalytes simultaneously in a short period of time. Projected design only needs the blood sample to be centrifuged prior using. Acquired plasma through centrifuge will be injected to the column. Red blood cells will be lysed and the acquired lysate will also be injected into the flow column. Therefore the design of this column is projected to measure the analyte in a simple and fast way.

Within the projected measurement of the blood sample, hospitals that doesn't automatic analyze systems will be able to detect and measure glucose, glycated hemoglobin and glycated albumin simultaneously and therefore they will be able to provide these data sets for their patients in a simple, economical way with a faster approach.

1.3 Hypothesis

Studying on this thesis will consist of developing a borate selective electrode. A composite electrode that can select borate ions will be used as a detection tool for glucaose and glycated proteins.

Electrode should possess performance characteristics in order to be used as a detection tool for glucose, glycated hemoglobin and glycated albumin. If the borate selective electrode manages to detect these substances, it will be used inside the flow injection system where one of the stream becomes a borate solution where the other stream becomes the solution of these 3 substances.

Due to the affinity they have on borate ions, they will form a complex which will lead to the borate concentration decreasing which will be picked up by the electrode and this data will be interpreted and correlated to the concentration of these 3 substances inside the streams.

CHAPTER 2

GENERAL INFORMATION

2.1 General Information on Diabetes Mellitus

Diabetes Mellitus is a chronic disease that can be distinguished into 2 types. It can be either acquired or inherited. The disease is defined as either the ineffectiveness of the insulin hormone produced or the deficiency of the insulin hormone production. The deficiency of the hormone production inside the body leads to the glucose level staying high on blood vessels therefore high glucose level caused harm becomes greater in organs, nerves and blood vessels of people with the disease.

Type 1 was formerly known as insulin dependent. This is the type of the disease where people generally acquire when they are young and it starts to increase over age. This is the type that pancreas fails to produce insulin hormone. This situation was formerly known as low insulin resistance.

Type 2 is formerly known as non-insulin dependent. This is the more common type of Diabetes Mellitus among human which is 90% of the patient cases worldwide [4][5].

Due to the global diabetes community database based in the United Kingdom [6], there are 3.5 million people that are diagnosed with diabetes disease and 549 thousand people are estimated to have the disease but yet to be diagnosed.

When global numbers are examined, 415 million people (roughly 9% of the total population) are living with the disease around the World and global diabetes community believes that this number will reach to 642 million people by the year 2040 by examining the numbers throughout the years [6]. This data set and the estimation also shows how the diabetes prevelance increases over the years.

Symptoms, diagnosis, treatment and prevention methods from diabetes mellitus are explained below.

2.1.1 Symptoms

Symptoms of this condition has a disadvantage of not always being too obvious. Therefore getting a regular glisemic control even becomes more important. Symptoms also do change depending on the type of Diabetes Mellitus as well.

In type 1 diabetes, symptoms are excessive and frequent secretion of urine, thirst, weight loss and tiredness.

In type 2 diabetes, symptoms are almost the same with lesser effects. Generally when not being regularly checked, diagnosis can take several years.

With addition to symptoms in general, when a person with high glucose level on blood or low insulin resistance, intakes glucose, tiredness or sleepiness occurs in the body momentarily which can be used as an indicator if a person is experiencing these issues [4][5].

2.1.2 Diagnosis

World Health Organization provides diagnostic values for blood glucose concentration [5]. Either fasting blood glucose concentration of glycated protein measurement helps diagnosing this disease.

As fasting blood glucose level measurement has its disadvantages, studies continue on the measurement of glycated protein measurements as this study is solely focused on using a borate selective electrode.

American Diabetes Association recommends people with certain conditions to be tested for diabetes mellitus more frequently than others: anyone with a mass index higher than 25 (regardless of age), anyone who is older than 45 years old, women with gestational diabetes in their past and anyone who has been diagnosed with pre-diabetes. Unlike people without these conditions, people who are associated with conditions explained above should have more frequent tests [7].

2.1.3 Treatment

To be able to prevent glucose level from rising in the body, dietary and physical activity is suggested to anyone or patients that are already diagnosed with diabetes mellitus.

About 40% of the patients does require insulin injections recommended by their doctors. Only type 1 patients with prolonged diabetes mellitus are recommended with this treatment method as the goal of the method is to increase insulin resistance as their body becomes incapable of handling glucose. Insulin injections on a daily basis does help patients with long time survival.

Type 2 form patients which are the majority of the diabetes patients does not need insulin injections as well as type 1 patients but injections help them for reducing their glucose levels in blood [4][5].

2.1.4 Prevention

As explained above in the treatment section, dietary and exercising 30 minutes a day helps people reducing glucose levels in their blood. As the treatment methods are expensive and only for providing long time survival, preventing the disease gains importance. Monitoring glucose levels as frequently as possible and compare them with the minimum and maximum interval of blood glucose level provided by World Health Organization is very important.

Insulin is an expensive item and can be a hard to reach substance in many poor countries, therefore in these examples, prevention is very important as well before diagnosis.

As the cases of diabetes mellitus patients increasing worldwide, public and professional awareness is crucial. Explaining risk factors, symptoms of diabetes can help increase awareness on public regarding this issue that allows either preventing or at least keeping it under control [4][5].

2.2 Boron Sources of Turkey

When compared to the other countries, Turkey holds the largest boron reserve which is approximately %60 of the Worlds boron reservoir [8-10]. Visible, possible and total million tonnes distribution is represented below in Table 2.1.

Table 2.1 The table of the total million tones of boron distribution for top countries [10]

Country	Visible million tones	%	Possible million tones	Total million tones	%
Turkey	375	66.3	483	858	58.2
USA	60	10.6	149	209	14.2
Kazakhstan	54	9.5	82	136	9.2
Russia	28	5	112	140	9.5
China	27	4.8	9	36	2.4
Iran	1	0.13	1	2	0.14
Serbia	3	0.53	0	3	0.20
Chile	8	1.41	33	41	2.8
Bolivia	4	0.71	15	19	1.3
Peru	4	0.71	18	22	1.5
Argentina	2	0.35	7	9	0.61
Total	566	100	909	1475	100

Even though Turkey holds the largest amount of boron reservoir, United States of America converts more of its reservoir into boron products. This outcome results United States to convert this material into high value added products which significantly increases the profit when used commercially.



Figure 2.2 Visual representation of the World's largest boron reservoirs [10]

Within the figure provided, Worlds largest boron reservoirs can be observed. California state of the USA leads its country also there are an important part of boron reservoir in South American region. From the figure, no visible boron reservoirs are present in the Europe and Africa regions.

2.3 Boron Measurement Methods

2.3.1 Electroanalytical Sensing Methods

Cremer developed a glass electrode in the year 1906 which is defined as the first electrochemical sensor that was built. Haber and his student later Klemensiewicz maintained the subject and now holds the majority of the literature on the field [11][12]. As of today, electrochemical sensors are being widely used as an analytical role in environmental field, disease control and prevention and industrial analysis. There are lots of parameters listed below that defines an electrochemical sensor's ability to convert a chemical information into an analytic signal. According to Thomson Reuters Web of Knowledge, there are more than 2000 peer reviewed academic papers that was published in the field of electrochemical sensors. These papers are published in order to obtain a optimization on these electrochemical sensors in terms of cost, rapid response, sensitivity, selectivity, detection limits and miniaturizing possibilities [13].

There are very distinguished parameters a biosensor should hold in order to provide meaningful data to the researcher. These parameters are all analyzed under the performance parameters of an ion selective biosensor section. These parameters are

response time, ion selectivity, linear working range, pH working range, lifetime of a biosensor, repeatability [13].

Besides of these parameters, when biosensors are designed to be used in vivo or generally in direct contact with biological fluids or tissues such as cell cultures, the structure should also hold the compatibility characteristic. If they don't hold this characteristic, direct contact of these materials to human body can cause biological response [14].

2.3.1.1 Potentiometric Techniques

Potentiometric biosensing occurs through the potential difference between the reference electrode and the working electrode. Driving force of a potential difference when an analyte is being measured is the concentration of the analyte. There is a correlation between the concentration and the potential output. The sensor part of the potentiometric biosensors are generally an ion selective electrode or a gas-sensing electrode (Bobacka et al., 2008; Lei et al., 2006a). When the working electrode holds a selective attitude and the reference electrode is an accurate and a stable reference electrode which holds a fixed potential, results of a potentiometric biosensing can be implemented into studies [15].

2.3.1.2 Amperometric Techniques

Amperometric biosensors gets operated at a potential that lies between the potential of the reference electrode and the working electrode [16]. Concentration of the analyte gets associated within the given output signal. By the time a new analyte is going to be measured, the correlation is being used to correlate the result. The way that the amperometric detection works is that, an electroactive metabolic product either gets reduced or oxidized on the surface of a working electrode (Ding et al., 2008). Amperometry measurement can be enhanced by the use of microbial biosensors in terms of sensitivity of the current measurement.

2.3.1.3 Conductometric Techniques

Conductometry is a technique where the results is gathered through conductivity. Conductometry can be used to measure whenever there is a production or consumption of an ion inside a solution therefore by the metabolic activity (Lei et al., 2006a; Shulga

et al., 1994). Conductivity measurement has 2 big advantages over other biosensing techniques. Conductometric sensing being a faster method with modern analytical techniques when compared to the others makes conductometry very desirable. The other very big advantage is the unnecessity of a reference electrode (Shulga et al., 1994). Unnecessity of a reference electrode is a big feature for a system that is desired to be miniaturized.

Specific selectivity is very important for all biosensing techniques as the result should only be related to the analyte that is being tested. Unfortunately for conductometric biosensors, there are lots of charge carriers that can interfere with the measurement. Selectivity of the conductometric biosensors are a field that needs some improvement (Mikkelsen and Rechnitz, 1989).

2.3.1.4 Voltammetric Techniques

Voltammetry being able to measure potential and current makes it function as a potentiometry and amperometry. Specific chemicals in voltammetric measuring provides specific peaks that can be implemented on the measurement of required analyte's voltammetry. As well as some of the other biosensing techniques, voltammetry can also provide simultaneous measuring of many analyte at once. Being able to measure numerous analyte at once eases the measurement process as it lowers down the process time. Moreover, the electrochemical stripping analysis step which is a pre concentration step makes voltammetric biosensing technique much more desirable as it's sensitivity increases [17].

2.3.1.5 Calorimetric Techniques

Driving force of the calorimetric measuring method is the observed enthalpy change on an enzymatic reaction. Nearly all of enzymatic reactions are exothermic therefore an increase on heat is observed during these reactions. There is a linear correlation between the enthalpy change and the concentration of the substrate.

There are many studies that researchers tried to integrate calorimetric biosensors to microfluidic devices to monitor concentrations on a real time basis [18].

Therefore all forms of measurement techniques are desired to have the ability to be integrated with microfluidic devices and to make real time detection as this study was projected to ensure.

2.3.1.6 Fluorometric Techniques

Fluorescent biosensors are being used for specific ions that allows the sensor to fluoresce when the targeted ion is present.

It is generally believed that the fluorometric techniques hold the highest sensitivity rates on ions when compared to the other techniques [19]. (Scott, et al.) Researcher claims that when the background is stable enough, sensitivity of a fluorometric read increases as the distance between the grounds are kept longer and therefore superimposed detection is assured [19].

There are other applications of fluorescent techniques are used where sensors are based on fluorescent proteins to measure pH in cells which detection plays a vital role in intracellular biology [20][21].

2.4 Potentiometry and Flow Injection Analysis Technique

2.4.1 Potentiometry technique and measurement

Potentiometry techniques driving force is the ion concentration difference. This technique involves a working electrode and a reference electrode. Potentiometry gives information about the electrochemical change in solution, depending on the potential value measured in the electrochemical cell composed of the comparative electrode and a suitable second electrode. Analytical methods based on potential measurement are called potentiometric methods [22][23].

Potentiometry technique involves the equipment of a computer with the suitable software that can detect and graph output values, a multichannel potentiometer, a reference electrode, one or more working electrodes and analyte solutions as shown in Figure 3.3.

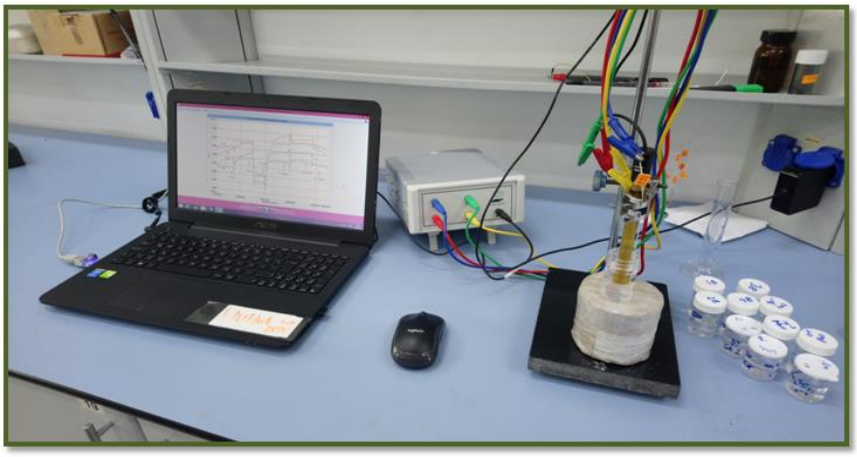


Figure 2.3 Vision from an ongoing experiment where a working electrode is being tested potentially in numerous different concentrated borate solutions

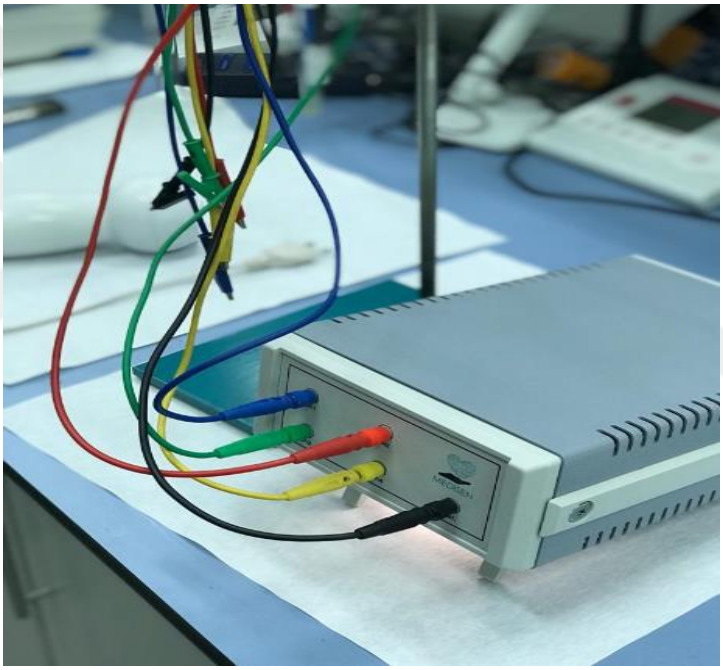


Figure 2.4 Close look of the multichannel potentiometer

This device shown in Figure 2.4 was built by Medisen Research and Development company located inside Yıldız Technical University. 4 different cables with 4 different colors represents the working electrodes as this device ensures 4 different electrodes to be tested at the same time. Black cable on the right end of the device gets connected to the reference electrode.

2.4.2 Ion selective electrodes (ISE)

Ion selective electrodes are electrodes that possess high selectivities due to their membrane structure. They are designed within their composition inside the membrane

so that they only allow the uptake of a specific ion, either an anion or a cation. Therefore the selectivity characteristic of an electrode is directly related within the composition of the membrane on the ion selective electrode. Ion exchange processes occur in potentiometry readings that involve ISE's rather than redox processes [24].

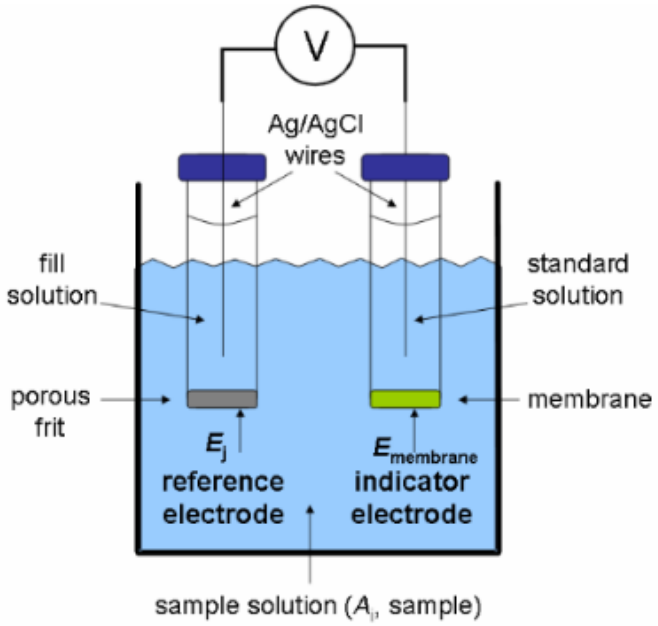


Figure 2.5 Electrochemical cell for making a potentiometric measurement with an Ion Selective Electrode [24]

2.4.3 Reference electrodes

There should be at least 2 electrodes for a potential measurement to occur and one of them is the reference electrode. The other electrodes is either called working electrode or the indicator electrode. Indicator electrode is built in a certain way that it reflects when a change occurs in analyte's activity while reference electrode does not. The standard reference electrode was the standard hydrogen electrode (SHE) but due to the hydrogen gas being very hard to maintain there are 2 common types of reference electrodes. These are standard calomel electrodes (SCE) and Ag/AgCl reference electrode [25].

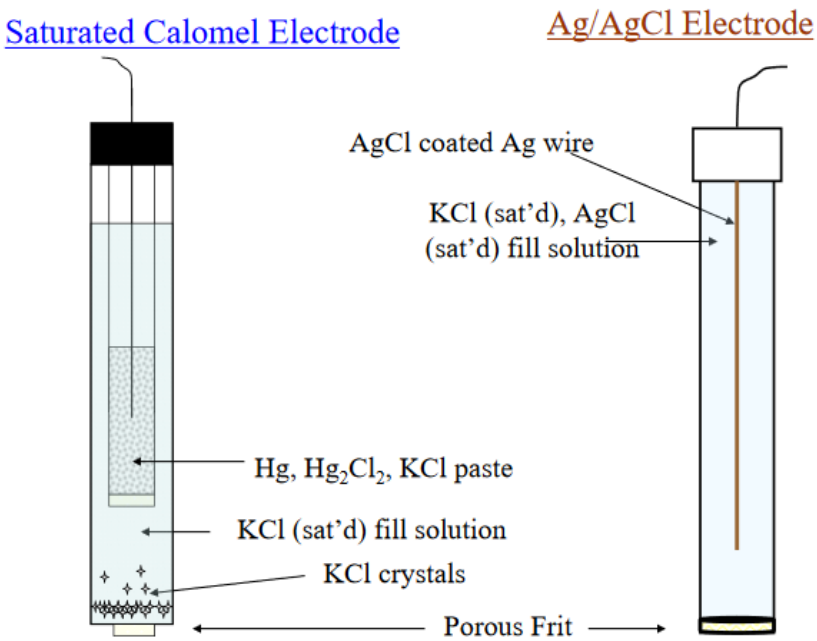


Figure 2.6 Visual of SCE and Ag/AgCl reference electrode [25]

Both of the reference electrodes hold a porous frit at the bottom and they are filled with saturated KCl solution however SCE also contains KCl crystals unlike Ag/AgCl reference electrode.

The half reaction of SCE is,

$$Hg_2Cl_2 + 2e^- <=> 2\ Hg(l) + 2\ Cl^-\ (sat'd) \quad (2.1)$$

with a E_0 value of +0.244 V.

The half reaction of Ag/AgCl is,

$$AgCl(s) + e^- <=> Ag\ (s) + Cl^-\ (sat'd) \quad (2.2)$$

with a E_0 value of +0.222 V.

2.4.4 Flow injection analysis

Flow injection analysis is an analysis type where a continuous stream meets a second batch stream. When a continuous stream is started, the electrode starts to show the

potential of the stream that the electrode is sensing. The potential output is desired to be as steady as possible. When other streams are also provided with desired volume and flow rate ranges, concentration of ions change and the ion selective electrode detects the difference by the potential difference.

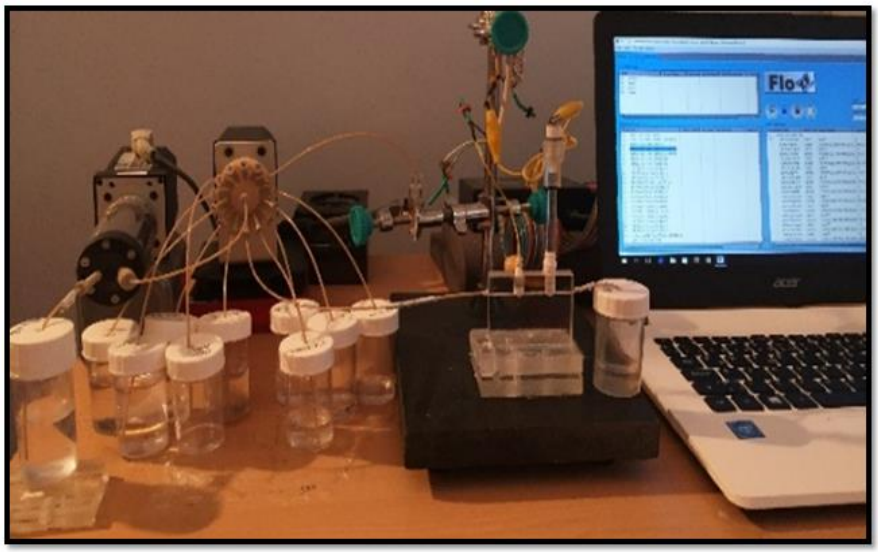


Figure 2.7 Flow injection analysis setup

Figure 2.7 shows the software inside the computer, cell where the electrodes are present (in the middle), solutions (different concentrations) and flow injection analysis pump. Electrodes inside the cell are connected to the potentiometer when measurement starts. Pumps on the left back ensures syringing solutions through the cell in the middle where the liquid is sensed by both of the working and the reference electrode. Within the software of the flow injection analysis system, total volume and the flow rate of the syringe solution can be manually implemented. Therefore results on the potentiometer software can be interpreted accordingly.

2.4.5 Performance characteristics of an ion selective biosensor

2.4.5.1 Ion selectivity

It is desired for a biosensor to measure the respective solution of an ion as accurate as possible. When the solutions differ, response of the biosensor is expected to change as well according to the Nerst equation[26]. In terms of selectivity, it is desired for an electrode to measure a specific ion and that ion only. It is never desired for a borate biosensor to provide the same electrical output when applied against a borate solution

and a chloride solution. If a biosensors response to 2 different solution appears to be the same, it means that the biosensors selectivity appears to be failed. Sodium ion selectivity leads to the biosensor to have very low accuracy.

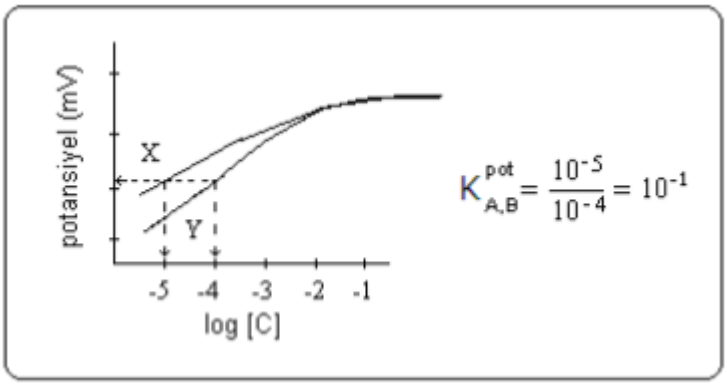


Figure 2.8 Calibration graphic of selectivity

In order to measure selectivity, a calibration graphic (Figure 2.8) can be drawn for calculating selectivity coefficient.

2.4.5.2 Response time

It is desired for an electrode to have a quick response time as much as possible. A biosensor with a quicker response time will be able to display the measurement in a shorter amount of time and will need smaller amounts of solutions that needs to be run on the electrode. As if an electrode with a 2 second response time will not need the entire 100 mL stock solution with a flow rate of 2 mL/s.

Smaller amount usage will significantly lowers down the time required to measure during experiments and will ease the use by patients or employees in the health industry. Using 2 mL solution instead of 20 mL will significantly lower down the costs for the solution preparation as well.

For the definition of IUPAC, response time can be measured when the potential change becomes smaller than 1 mV or 95% interval of the mV value of the equilibrium stand. Graphical representation of this definition is shown below in Figure 2.9.

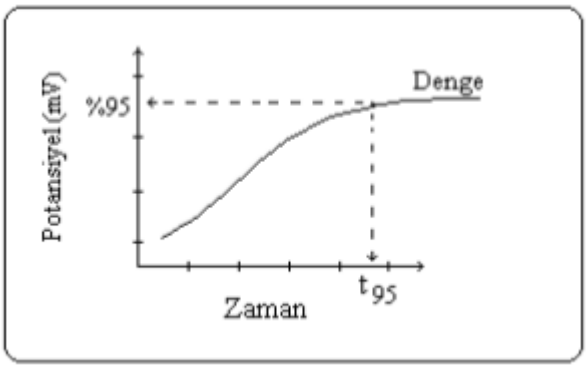


Figure 2.9 A representation of an electrical output balancing itself after a certain time (x axis is the time axis and y axis is the potential axis

As can be observed from the Figure 2.9, the electrode requires a certain amount of time before it settles in the x axial. Higher conductivity achieved in the electrode, faster response time is observed.

Response time being low is one of the most desired features of an electrode therefore shortening the response time of an electrode is very essential when finishing the production of an ion selective electrode.

In order to shorten the response time of an electrode, composite solution should be mixed very well and the area of the electrode that is inside the solution should be as small as possible.

In order to shorten the response time in a flow injection reading, flow rate might be increased to ensure shortening response time.

When an electrode is being tested, first used solution to be measured should be the most diluted solution. If an electrode is being measured for a solution through 10^{-1} M to 10^{-5} M, first solution selected should be 10^{-5} M and. With every 10 fold solution measurement, the ion selective and the reference electrode should be washed thoroughly with ultra pure water in order to prevent any solution that is left over in the area.

2.4.5.3 Linear Working Range

The linear operating ranges of the ion-selective electrodes can be calculated from the plotted calibration graphs. The linear operating range of the electrode is defined as the range in which the data points on the calibration curve do not deviate more than 2 mV from linearity either from the top or from the bottom. Linearity of concentration-

potential change in the studied range value can be calculated using the calibration graph's equation and R squared value [27][28].

2.4.5.4 pH Working Range

As well as all the other parameters a biosensor have, they also have a specific desired range of pH that they work. Especially when a biosensor is designed is developed in order to use it to measure a substance in a blood sample, the biosensor is desired to work under the pH of the blood. Therefore it is desired for an electrode to show similar mV value outputs when tested against same concentrated solutions with different Ph values.

2.4.5.5 Lifetime

Lifetime is also a very crucial characteristic for an ion selective electrode. It defines the electrode to provide the same potential outputs with a high accuracy when used on a long period of time. It is always desired for an electrode to provide very similar almost equal measurements when used on a daily, weekly or sometimes even on a monthly basis. An electrode to work for a very long time as long as possible is desired which is considered as the lifetime of an electrode and the electrode providing very similar almost equal results defines repeatability.

2.4.5.6 Repeatability

It is desired for a biosensor to provide the same results over time as well. Even when an electrode is being tested for several hours, the peaks that the electrode provides within the highest and lowest concentration levels should be the same or similar levels with small margin of errors.

2.4.5.7 Advantages and disadvantages of ion selective electrodes

There are many advantages of using ion selective electrodes when compared to other types of electrodes.

They can be miniaturized and mobilized thus they are eligible to be used in almost any location and under lots of circumstances.

They do provide a linear response to the targeting ions.

They generally do have a low response time.

They are easy to operate and generally have low production costs.

No contamination occurs with using ion selective electrodes.

However, they do have some disadvantages as well.

An ion selective electrode can sometimes measure a non-targeted ion. This event can cause a misleading situation when an electrode is being tested, therefore an electrode must be tested many times or even on a daily or weekly basis and should be compared to each other.

Their lifetimes are generally not as long as it is desired for them to have. Within their fragile structural developments, they are not very suitable to be used inside flow injection cells but using hardeners on the outer surface of an electrode can neglect this disadvantage.

Precision of ion selective electrodes are the most crucial disadvantage. Within the same composition being used for an electrode, they do not always give the same results. For this thesis study there are more than 900 electrodes are produced for 40-60 different compositions. Homogeneity of an electrode, a contamination in the composition or saturation levels and many other parameters can all change the output of an ion selective electrode provides [29].

2.4.5.8 Applications of ion selective electrodes

There are many fields that ion selective electrodes can be implemented into. These electrodes are being implemented into microfluidic devices and being used in health, agriculture, environmental engineering, bioengineering, biomedical engineering industries and many others.

To get into more detail, they can be used for pH measurement, water hardness or mineral measurement, hydrogen ion measurement (higher range of usage area), determination of biomarkers (health industry) [30].

CHAPTER 3

MATERIAL AND METHODS

3.1 Chemicals Used

There are some chemicals used for the production of the borate selective electrode. These are CNT, GO, CNT-silver borate, Cu_2S, Ag_2S, epoxy resin, THF and acrylic. During the process of creating the best composition all the ratios of these chemicals were changed from time to time which sometimes led to better results and sometimes led to negative results. Increase in CNT and GO ratios for example lowered the response time which was also observed when the epoxy resin ratio was decreased. On the other hand, epoxy resin ratio was seen to be very important for the composition to hold its solidity.

Apart from the chemicals used in the composition of the electrode, few solutions were prepared to test the electrode's selectivity. Some of these solutions are sodium bicarbonate, sodium chloride, disodium tetraborate, sodium sulfate, potassium chloride.

For the preparation of borate stock solution, disodium tetraborate solution was used.

The project was designed to use carbon nanotube within the composite electrode composition as the CNT enhances the electrochemical reactivity. Their remarkable electrical, chemical, mechanical and structural properties makes it favorable for any electrochemical sensing. Therefore they are highly being used in most forms of electrochemical biosensors [30][31].

Table 3.2 Chemicals and average amounts used for the electrode compositions

Chemicals Used	Amount used
CNT	100 mg
CNT-Ag_3BO_3	10 mg
Cu_2S	30 mg
Ag_2S	80 mg
Epoxy resin	200 mg
THF (purified)*	10-20 drops
Acrylic liquid**	10-20 drops

***THF Solution was purified prior usage. Argon gas is also passed through the solution prior usage in order to prevent any oxygen gas addition from the solution to the borate composite electrode.**

****Some of the compositions included acrylic liquid instead of THF. Table 4 identifies the electrodes**.

3.2 Equipments Used

- Centrifuge
- Flow injection pump
- Manual and automatic industrial and lab size press machines
- Electric drills
- 3d printer (flow injection analysis cells)
- Potentiometry and flow injection analysis softwares
- Hanna instruments reference electrode
- Microcomputer brand pH meter

3.3 Methods

3.3.1 Preparation of solutions

For selectivity measurement of the electrode, NaCl, $NaNO_3$, Na_2So4, Ag_3BO_3, $NaHCO_3$ solutions were prepared. All of these chemicals were prepared by diluting their respective 10^{-1} M stock solutions. To be able to dilute these solutions from 10^{-1} M to 10^{-2} M, 10^{-3} M, 10^{-4} M and 10^{-5} M only a graduated cylinder and ultra pure water was used. To be able to dilute a solution 10 times, 1 mL of the stock solution gets mixed with 9 mL of ultra pure water. The stationary measurement solutions for this experiment was chosen to be 20 mL solution containers.

For the pH measurement of the electrode, 6-7 different pH solutions were prepared by adding a few drops of NaCl or NaOH to be able to create a variance in the measurement. Since the blood of the pH is between 7 and 7.4, the gaps of the sample dots gets smaller when approaching that range.

3.3.2 Preparation of silver borate

0.255 grams of silver nitrate is blended in 50 mL of ultra pure water in order to get 0.03 M of silver nitrate solution. In another beaker, 0.57 grams of disodium tetraborate is blended in 50 mL of ultra pure water in order to get 0.03 M of disodium tetraborate solution. Once both solutions are prepared Argon gas is passed through both of these solutions in order to get rid of oxygen gas inside the beakers in order to prevent silver from being oxidized. Once both solutions are ready, silver nitrate solution gets distilled over disodium tetraborate solution over a 2-3 minute time. The 100 mL solution is getting constantly stirred in order to prevent the ions from getting precipitated. It also increases the chances of the ions to encounter and form silver borate. Once the solution is all mixed up, it starts to get centrifuged. After a 8-10 minute period of centrifuging, liquid inside gets emptied and the remaining solid gets left do dry. Approximately 1 gram of silver borate is acquired for this process conditions. Using Argon gas and preventing direct light reflection into the beakers are very essential for this procedure. Aluminum foil wrapping outside the beakers and centrifuge tubes helps solving the light issue.

3.3.3 Preparation of CNT based borate composite electrodes

CNT, CNT-silver borate, silver sulfide and copper sulfide solution gets mixed with the epoxy resin and either with THF liquid at the end or the acrylic liquid. The compositions of these materials are changed throughout the study but there was a final composition found which appeared to present the best results for this electrode. Different set of compositions were built for the project due to the fact that every different composition led to different results in terms different characteristic results.

Table 3.3 Compositions of the electrodes built for the thesis

Electrode number	CNT (mg)	CNT-Ag_3BO_3 (mg)	Ag_2S (mg)	CU_2S (mg)	Epoxy (mg)
22	100	-	85	10	800
25	100	-	85	10	800
35	100	10	80	10	800
36	100	10	80	10	800
38	100	10	80	10	800
81	10	10	80	10	600
82	10	10	80	10	600
83	100	10	80	30	300
91	100	10	80	30	200
101*	100	10	80	30	600
102**	100	10	80	30	200
111*	100	10	80	30	160
154	1 GO	1 Silver borate	90	10	200

***Compositions that contained acrylic liquid instead of THF as a solvent for epoxy resin.**

****102 was used for the measurement of glucose, glycated albumin and glycated hemoglobin.**

Table 3.3 presents the compositions that was used since the beginning of the electrode development for this project. It does not involve all the numbers as some of the

electrode numbers were executed with changing a variable that did not provide a good result. Some of the compositions did provide good results for a number of characteristics but at the end, a electrode composition was found that led to the measurement of glucose, glycated albumin and glycated hemoglobin.

It is very crucial to mix all the solid chemicals in this process and break down big pieces and mix it well with the rest of the chemicals. After mixing the entire preparate, epoxy resin is added to this solution with few drops of THF. The solution should resemblance the fluidity of dough. While epoxy losing its viscosity and thf evaporating at the same time the process of putting the solution inside a tube should be handled as fast as possible. For the next 2 weeks, the solution inside the pipe should be constantly pressed and released as frequently as possible as it is desired for the solution to be pressed but to be able to get the THF evaporate a little faster than usual rate, the rods on both ends of the plastic tube can be released every once in a while. When the sharp smell of THF evaporates, next step of the process can be initiated. After solidifying the composite solution, it can be converted into an electrode by combining the composite with a copper wire with the help of solid contact material. Solid contact materials composition is 50% (w/w) graphite, 35% (w/w) epoxy resin and 15% liquid hardener as shown in Table 3.4.

Table 3.4 Solid contact material composition

Solid contact material	Composition (w/w)
Graphite (<100 nm granule size)	50
Epoxy resin	35
Liquid hardener	15
THF*	10-20 drops (depends on liquidity and sample size)

***As an alternative to THF solution which is being used as a solvent to epoxy resin, acrylic liquid was also used in this study.**

Copper wire on one end is dipped into the solid contact material and adhered to the composite material. By the help of the liquid hardener, solid contact material that wraps around the copper wire concretes. The time for the solid contact material to turn into a solid material can take seconds to minutes depending on the composition. After waiting a few hours or 12-24 hours, process can continue with the next step.

After solidifying the solid contact material wrapped around the copper wire, pattex is being applied. Pattex is being used in order to strengthen the connection between the copper wire and the composite borate sensing electrode. When pattex is applied to strengthen the connection it also gives water impermeability to this part. Therefore when the electrode is being measured in a solution the copper wire gets the electrical current through the composite borate electrode only.

After the electrode development is complete, the electrode gets saturated with borate ions. In order to saturate the borate electrode, the electrode can either be dumped in the 10^{-1} M borate solution for a couple of hours or in 10^{-2} M borate solution overnight. For longer periods of saturation 10^{-3} M solution can be used. Therefore the electrode gets prepared for the measurement. Borate composite electrode gets measured in borate solution first and if it responds well against borate ions, it is being tested in other solutions to check ion selectivity.

3.3.4 Preparation of GO based borate composite electrodes

Just as CNT based composite electrode is built, an alternative borate composite electrode was built using GO instead of CNT. GO, GO-silver borate, silver sulfide and copper sulfide solution. This solution was mixed with THF liquid and the epoxy resin just as it was used for the compositions that included CNT rather than GO. The compositions of these materials are changed throughout the study but there was a final composition found which appeared to present the best results for this electrode.

Preparation of GO-AG_3BO_3 is explained under the section 3.3.5. Preparation of CNT-AG_3BO_3 and preparation of GO- AG_3BO_3 appeared to be the same yet with different materials.

Just as GO- AG_3BO_3, GO was also manufactured rather than using it commercially.

Table 3.5 Electrode composition when GO was used as a replacement for CNT

Chemical	Amount (mg)
GO	100
GO-Ag_3BO_3	10
Ag_2S	80
Cu_2S	30
Epoxy resin	200-250
THF	10-20 drops depending on fluidity

3.3.5 Preparation of CNT-Ag_3BO_3 and GO-Ag_3BO_3

Within this study, CNT-Ag_3BO_3 and GO-Ag_3BO_3 was also been added to the composition of the borate composite electrode respectively when producing CNT based composite electrode and GO based composite electrode.

To be able to produce CNT-Ag_3BO_3 and GO-Ag_3BO_3, a beaker was fully covered with epoxy-CNT and epoxy-GO respectively on the inside.



Figure 3.10 Preparation of CNT- Ag_3BO_3 and GO- Ag_3BO_3

Within the same procedure of gathering Ag_3BO_3, silver borate is produced inside this beaker with the addition of 1 gram of CNT or GO respectively for the desired composite production but using an electric current on the beaker this time. 1.5 V of electric is being applied to the beaker on the plus end and the negative end is being applied to the solution through a platinum rod. While only $AgNO_3$ solution is present inside the beaker with the CNT or GO, the positive and the negative ends gets changed every 10 minutes for 30 minutes. After the addition of borate solution to the beaker, positive and negative ends gets changed in every 5 minutes for another 30 minutes. After 1 hour of constant stirring this GO or CNT based silver borate solution, the solution is centrifuged and let dried for a couple of days. CNT or GO based silver borate is gathered after drying.

3.4 Working Method

Thesis study starts with the development of a borate electrode. The electrode is desired to have a fast response, be selective for borate ions only, with a long lifetime, and provides decent potentiometric performance that copes with Nerst equation standards. The developed borate electrode will be tested in flow injection analysis after stationary solution reads are as expected. The flow injection system will contain a continuous and an intermittent flow of a solution with a flow injection cell that consists Ag/AgCl reference electrode and a borate selective electrode. Continuous flow will be selected as 5.10^{-4} M borate solution and the intermittent solution will vary from 10^{-1} M to 10^{-5} M borate solution changing 10 fold every time. Every time the intermittent solution is injected through the system, associated electrical peaks started to exist.

3.5 Purpose of the Study

Purpose of this study is making use of one of the most abundant raw materials of Turkey and successfully develop a borate selective electrode that will be used to measure glucose, glycated hemoglobin and glycated albumin levels in blood samples. Thesis study involved developing a borate sensitive electrode that will show the best characteristics of an ion selective electrode and use the electrode to help detecting glucose, glycated hemoglobin and glycated albumin levels in blood samples.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Stagnant solution measurements

Before the electrode to be tested in flow injection analysis to measure glucose, glycated albumin and glycated hemoglobin, electrodes are being tested in stagnant solutions to check for their characteristics.

When electrodes show good results on stagnant solutions in terms of repeatability, response time, lifetime etc, they are being used in flow injection analysis where there are 2 streams and the response of the electrode is observed.

4.1.1 Potentiometric performance characteristics of the developed borate selective electrode

4.1.1.1 Ion selectivity

Developed borate selective sensor was tested for selectivity. Figure 4.11 shows the characteristic of ion selectivity of the borate selective sensor number 36 against sodiumsulfate and sodiumchloride solutions.

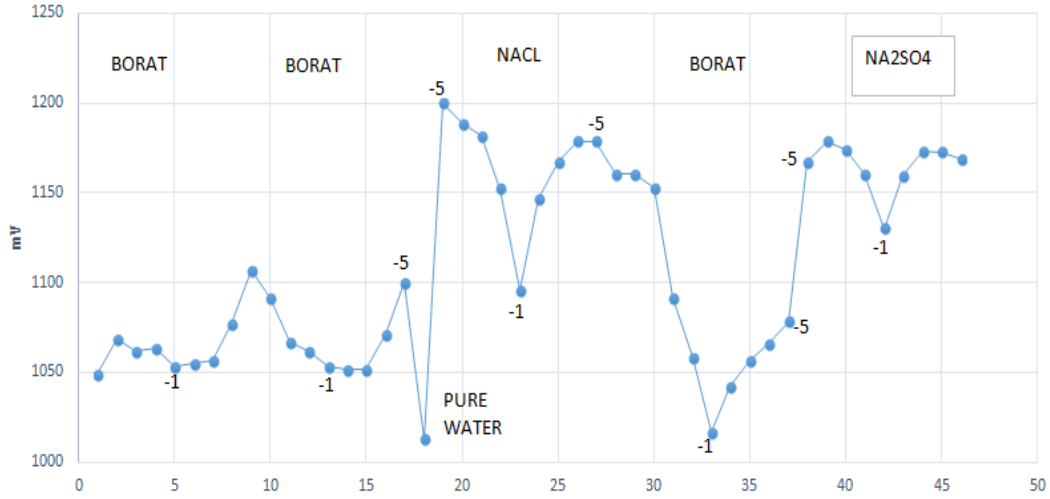


Figure 4.11 Selectivity measurement of the electrode number 36

As the number 36 electrode was identified with its compositions in Table 3.3, the composition of this electrode was ideal and widely used in this Project as it provided good results for selectivity measurements, due to its high content of epoxy response time of this electrode was not ideal therefore following number of electrodes involved lesser epoxy ratios in their compositions.

This data set was gathered from the software directly and graphed in Microsoft Excel. First solution always gets selected as 10^{-5} M and gets increased to 10^{-1} M with every step changing the concentration 10 fold. At first a borate solution was tested and seen that 10^{-1} M solution hits a mV low value around 1050. Other borate solution measurements confirms that the electrode shows a value around 1030-1050 mV when a 10^{-1} M borate solution is tested. To be able to interpret the reading better, ultra pure water is also tested. Testing other solutions that can be predicted beforehand gives an advantage to understand if the potentiometer is working as accurate as possible. Apart from borate solutions Figure 11 also includes the readings under sodium chloride and sodium sulfate measurements. For these borate sensitive electrodes, sodium bicarbonate, sodium chloride, potassium chloride, sodium sulfate etc. was tested during these experiments to see if the borate sensitive electrode is also sensitive to other ions. As seen from Figure 11, when sodium chloride and borate ion is present (10^{-1} M) in a solution, the borate sensitive electrode will not pick up the sodium chloride ions and lead to misinterpretation. Therefore this electrode was considered as an electrode that is

sensitive to borate ions and safe and accurate to use in applications where sodium chloride may be present.

4.1.1.2 Response time

Developed borate selective sensor is also tested for response time.

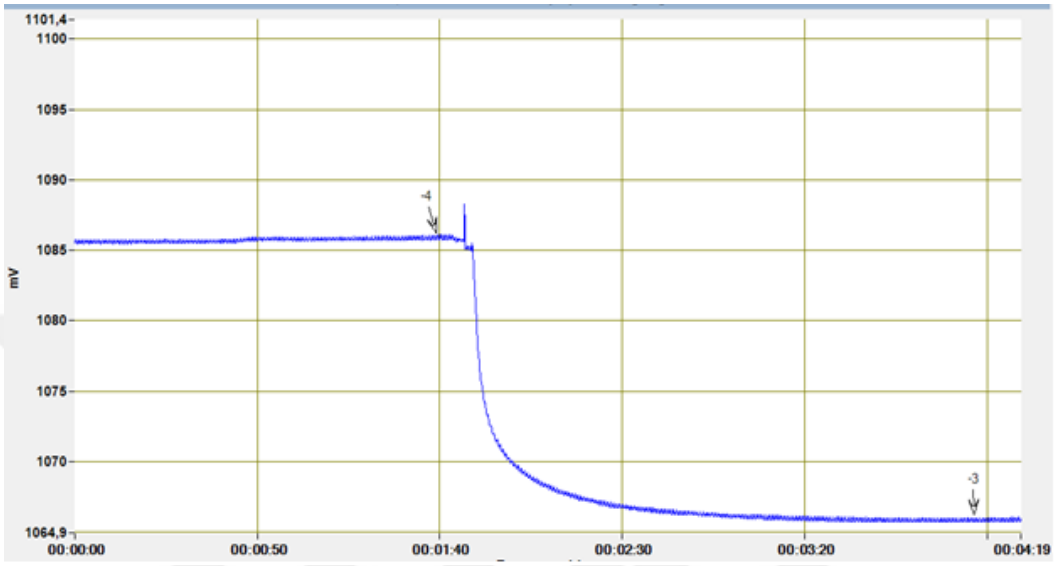


Figure 4.12 Electrode number 101 is being tested to see the response time of the electrode

Figure 4.12 belongs to the response time measurement of the electrode 101. Its composition is also provided in Table 3.3. Due to the fact of 101 having lesser epoxy content when compared to the other compositions, this electrode provides better results for response time measurements. It was clearly observed in this project is that when epoxy content is low, the electrode tends to have a faster response time as the CNT ratio stays higher in these compositions that leads to better conductivity.

Response time is measured by looking at the two interval of times where the signal is settled to parallel lines. -1 and -3 represents two solution with 2 different concentrations. As seen above, the stability on the line started to fade and the mV value starts dropping around 01:50 and it can be interpreted that the line gets stable again around 03:10. This shows that this electrode has a response time of 80 seconds. As explained earlier, electrodes are desired to have as low as possible response times and 80 seconds for 1 concentration change (10 M fold) can be considered as a bad feature for this electrode.

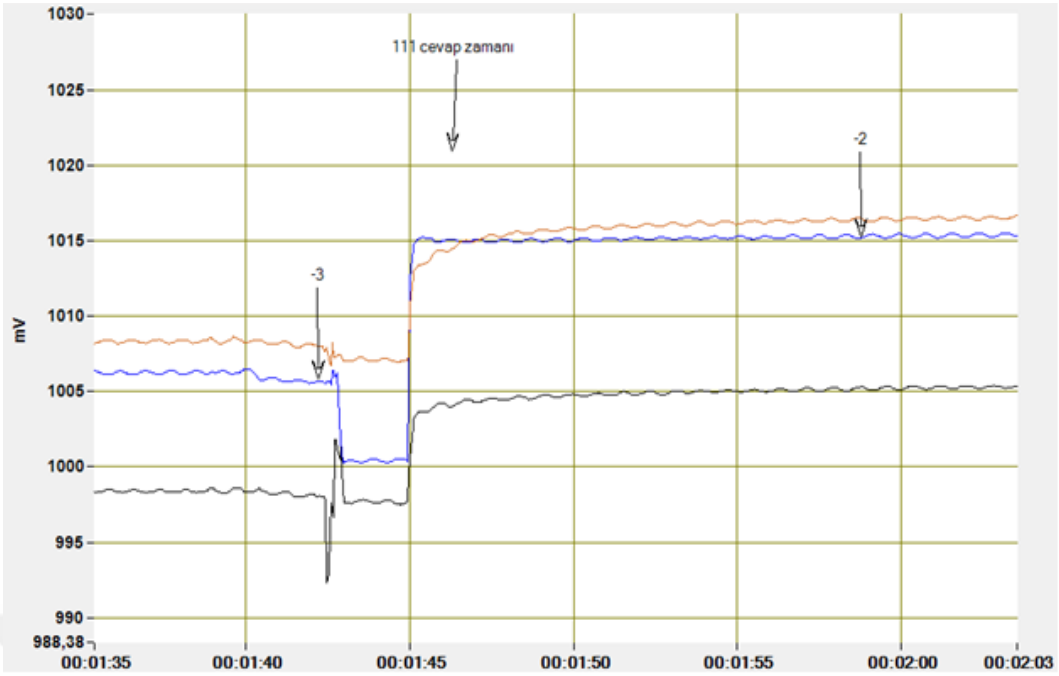


Figure 4.13 Electrode number 111 is being tested to see the response time of the electrode

As seen above from the Table 3.4, electrode number 111 also has a low epoxy content that leads to the electrode having a higher ratio of CNT which leads to better conductivity resulting the electrode to have a faster response time.

In the figure 4.13, 3 different electrodes (#111) with same compositions are being tested for the response time. As seen above in the figure, electrodes leave the 10^{-3} M solution around 01:42 and starts to settle inside the 10^{-2} M solution around 01:48. This response time of 6 seconds between 1 concentration change can be considered as very good. When an electrode provides such a good response time it can quickly be tested with 5-6 or even more different ion solutions and can be implemented into the flow injection analysis system if the electrode achieves selectivity.

4.1.1.3 pH working range

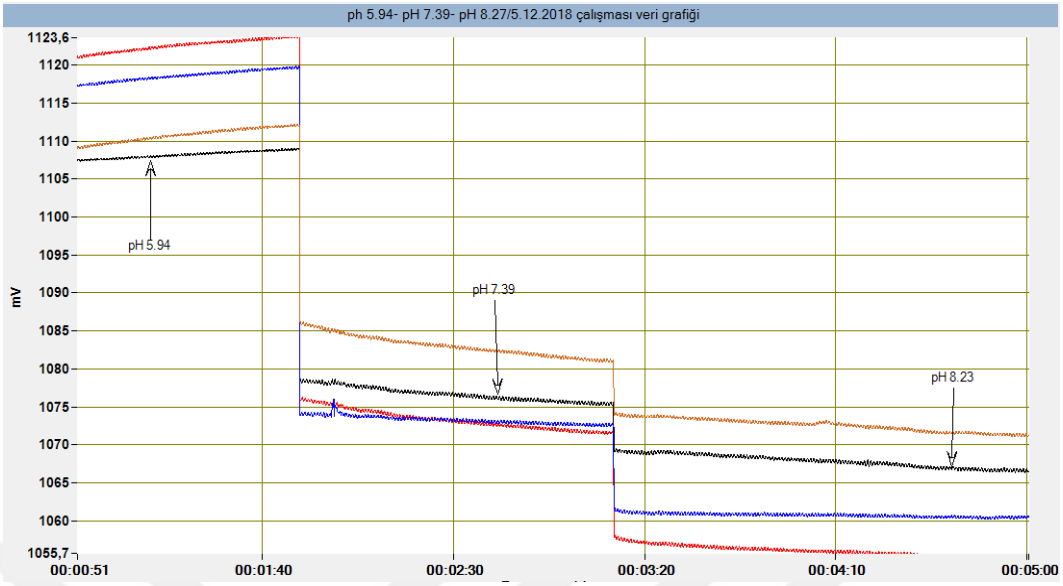


Figure 4.14 4 different electrodes being tested for their potential outputs under 3 different pH levels

Figure 4.14 represents 4 different electrodes with the same composition and how their outcomes change when the pH levels change under same concentrated solutions. The electrodes does not provide close results when compared to each other as the electrode marked with red color changes the most through pH change.

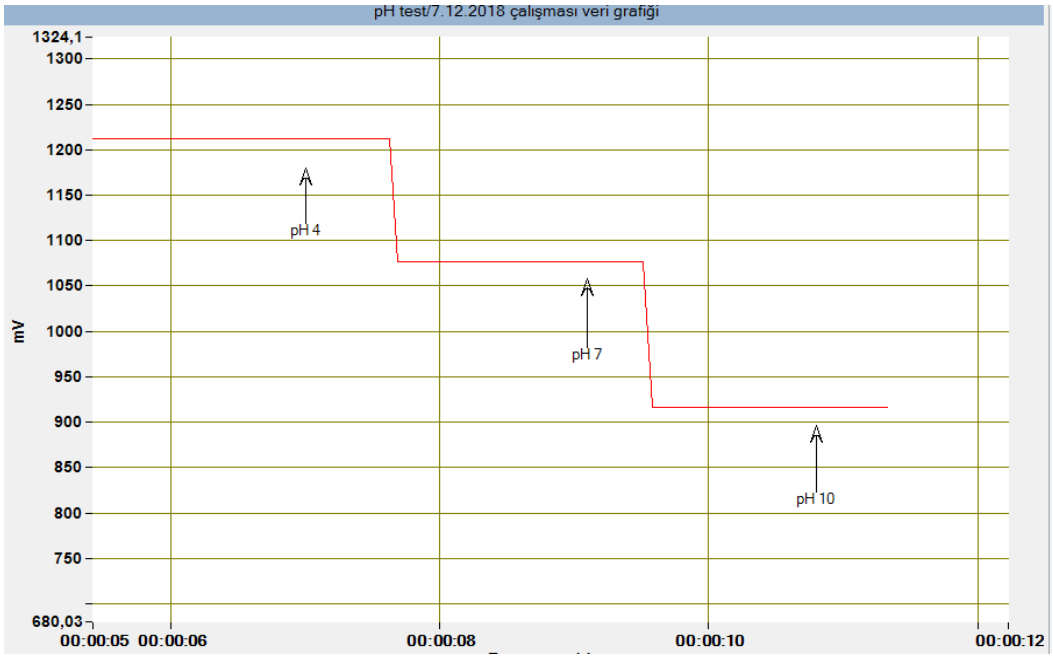


Figure 4.15 pH calibration graph

pH test of a non-commercial pH electrode that was built inside the lab to be used within the flow injection analysis testing. This electrode was squeezed into the same yellow tube. Using a commercial pH electrode would not be able to be squeezed to fit the flow injection cell.

Table 4.6 Potentiometric response of some pH values.

Number	pH	Potentiometric result (mV)
1	4	1211.5
2	4.7	1172
3	6.3	1106
4	7	1076.5
5	7.6	1010
6	8.7	960
7	10	917

Table 4.6 belongs to a measurement of potentiometry when a borate solution under different pH values were tested. As explained above in the pH section, an electrode should show significantly close mV values for the desired working pH range. This electrode provides close values for the pH value of the blood therefore it is projected to work under the pH level of blood samples.

4.1.1.4 Repeatability

The developed electrode also tested multiple times to check if the potentiometric outcome stays constant or does not change significantly when tested at the same concentration levels. For this repeatability test, 10^{-2} M and 10^{-3} M borate solutions were

used. The lower mV value belongs to the 10^{-2} M solution measurements and higher mV value belongs to the 10^{-3} M solution measurements.

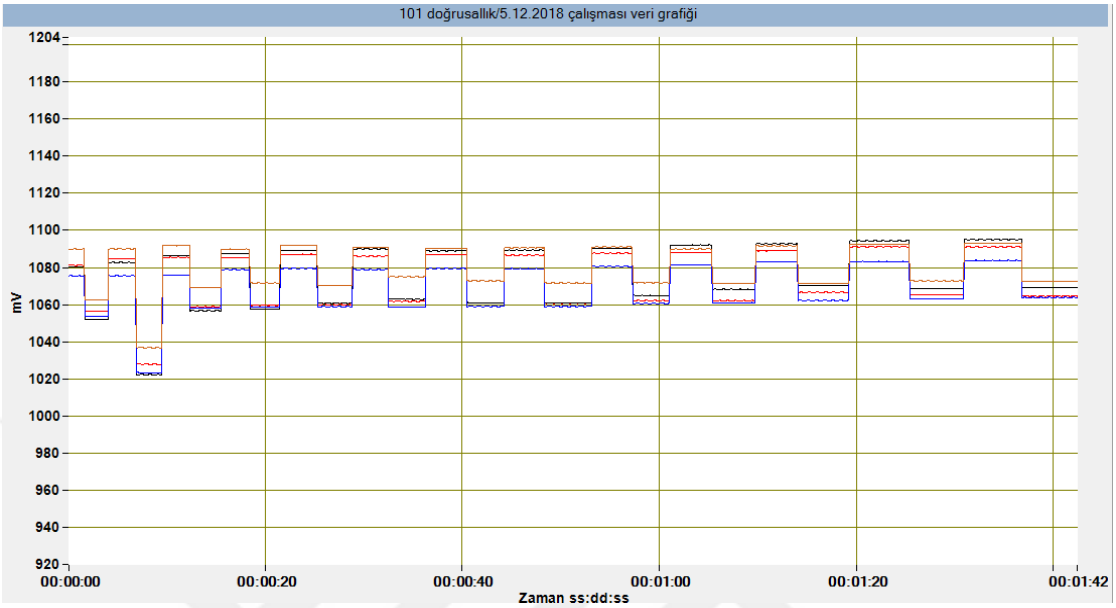


Figure 4.16 Electrode number 101 is being tested for its repeatability on 10-2 and 10-3 M solutions

As can be seen from the Figure 4.16, the electrode is being tested for its repeatability. The electrodes are being inserted into 2 different solutions (10^{-2} and 10^{-3} M solutions) for many times in a row to see if the electrode will provide the same or almost equal values every time. Except for 1 outliar, the electrode in Figure 16 managed to provide almost equal results every time when tested. All the lines with distinguished colors matched the same mV values from earlier measurements.

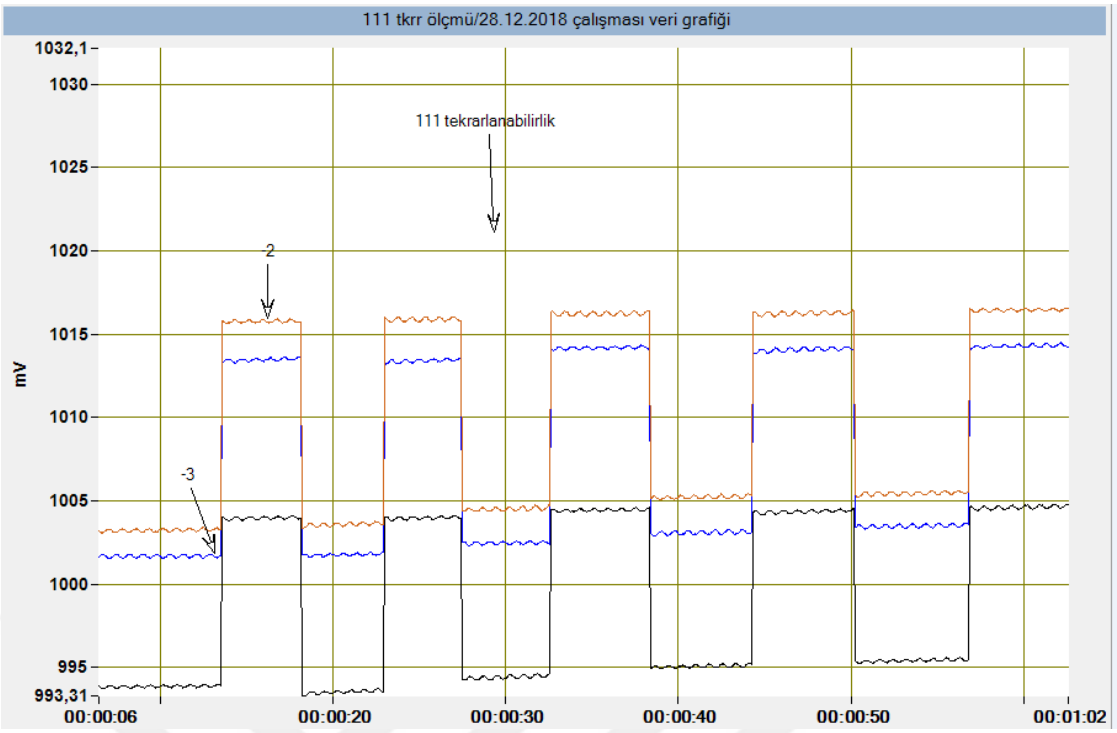


Figure 4.17 Electrode number 111 is being tested for repeatability

The number of trials are 5 in this figure however the output is zoomed in can be observed that the mV values are almost identical with each other therefore one can interpret that this electrode has the repeatability feature. This measurement is one of the best result that is observed for this project.

Electrode numbers 101 and 111 which were identified in Table 4 provided good results for repeatability which was expected.

4.1.2 GO-Borate Composition Results

GO was tested in some of the composite borate compositions. GO was used as a substitute for CNT. They are both conductive materials which was the reason GO was intended to be used as an alternative. GO containing high thermal and chemical stability, electrochemical properties of graphene made GO a perfect candidate to be used as a material inside electrochemical biosensors[32][33]. It is becoming widely popular in the electrochemical sensor fabrication field as the chemical and structural features of graphene is expected to perform very well in terms of stability, precision and sensitivity [13].

However electrodes that were prepared with GO didn't provide good examples for a borate selective biosensor for this project.

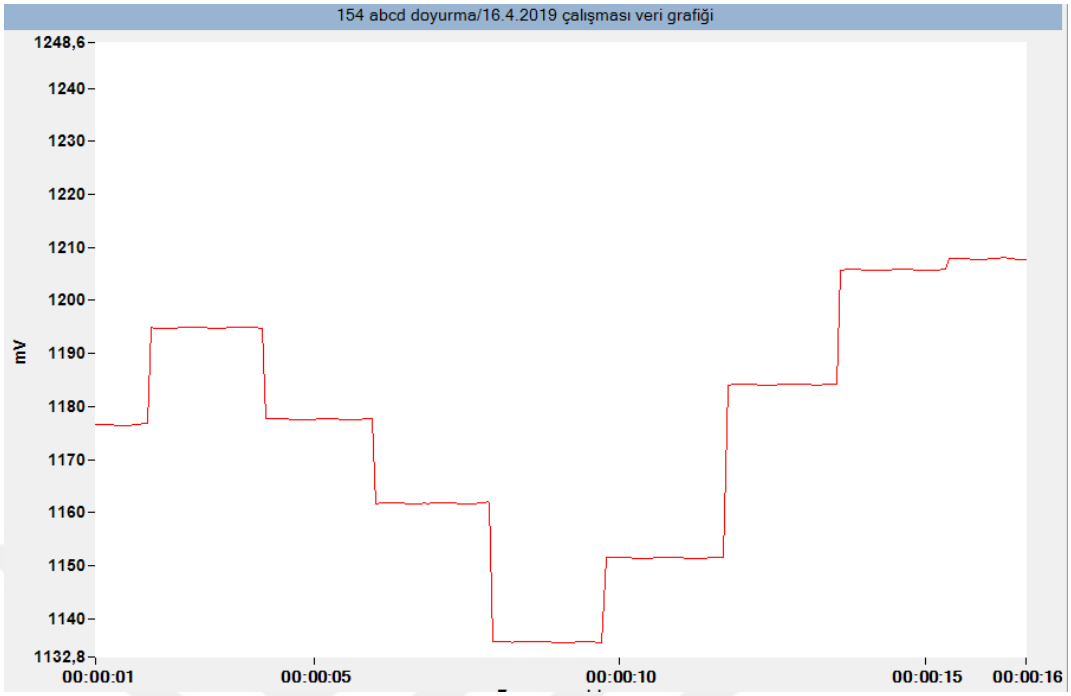


Figure 4.18 GO borate measurement with the 154 numbered electrode

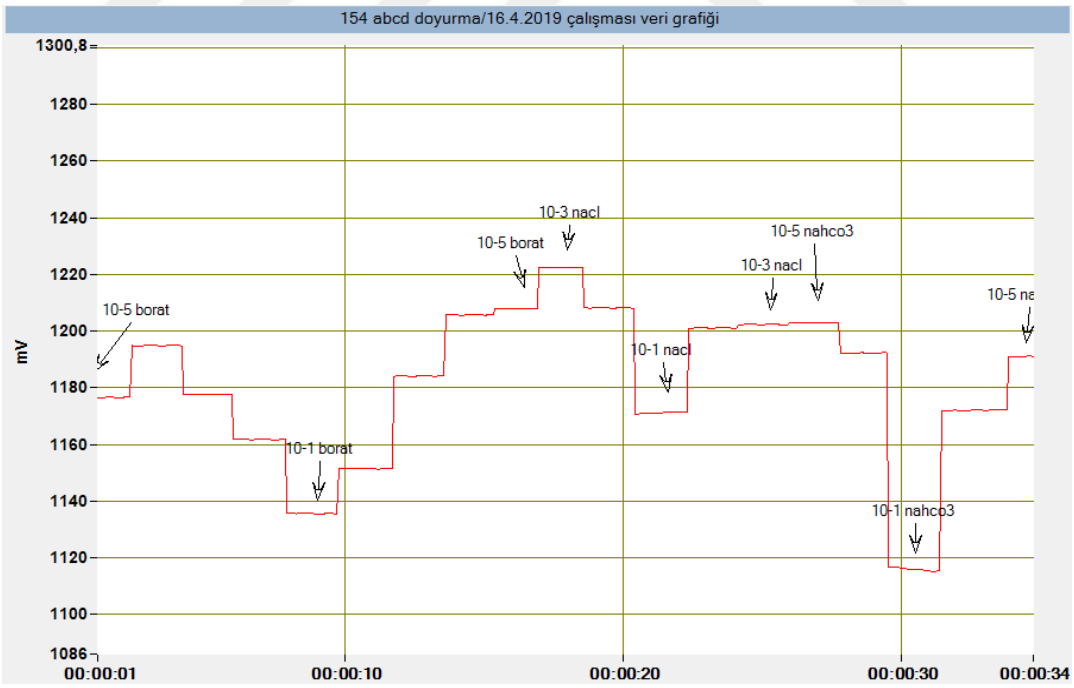


Figure 4.19 GO borate measurement with the 154 numbered electrode

As can be seen from the figures 4.18 and 4.19, the electrode tested has not provided the ladder formation therefore not consistent within the same concentration levels. This

substitution between CNT and GO failed as the samples from GO compositions didn't provided any characteristic feature of an ion selective electrode.

4.1.3 CNT-Borate Composition Results

Most of the electrodes built for this project included CNT-borate compositions. Some of the composite electrode compositions managed to provide an ideal potential output for borate solutions as well as when being tested for selectivity.

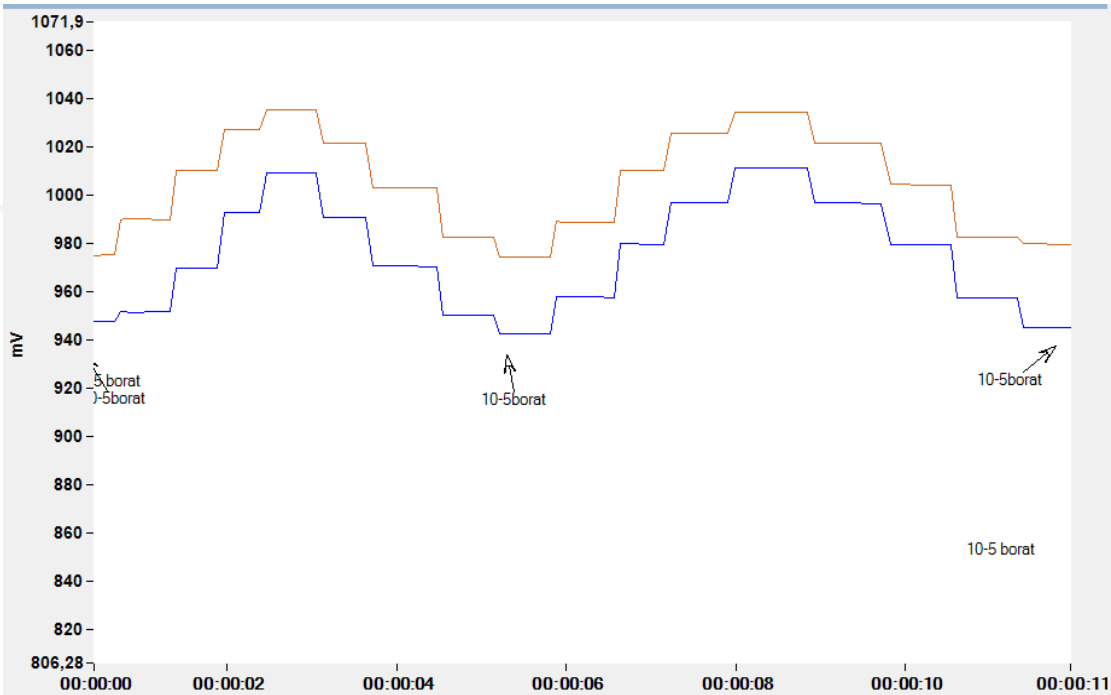


Figure 4.20 Borate measurement of electrode number 38

This figure belongs to electrode number 38 that responds well against borate ions. 10^{-1} M measurements are the top of the ladders and while the 10^{-5} M measurements are on the bottom of the ladders. The ladder formation is very visible in the photo where 10^{-5} M borate solutions provide mV values around 940's and 10^{-1} M borate solutıons provıde mV values around 1040's. Can be seen that same levels of concentrations provide similar results and accuracy is very good when mV jumps between all levels are observed. Depending on the Nerst equation, ions with positive or negative 3 charge should show approximately 20 mV difference between concentration levels.

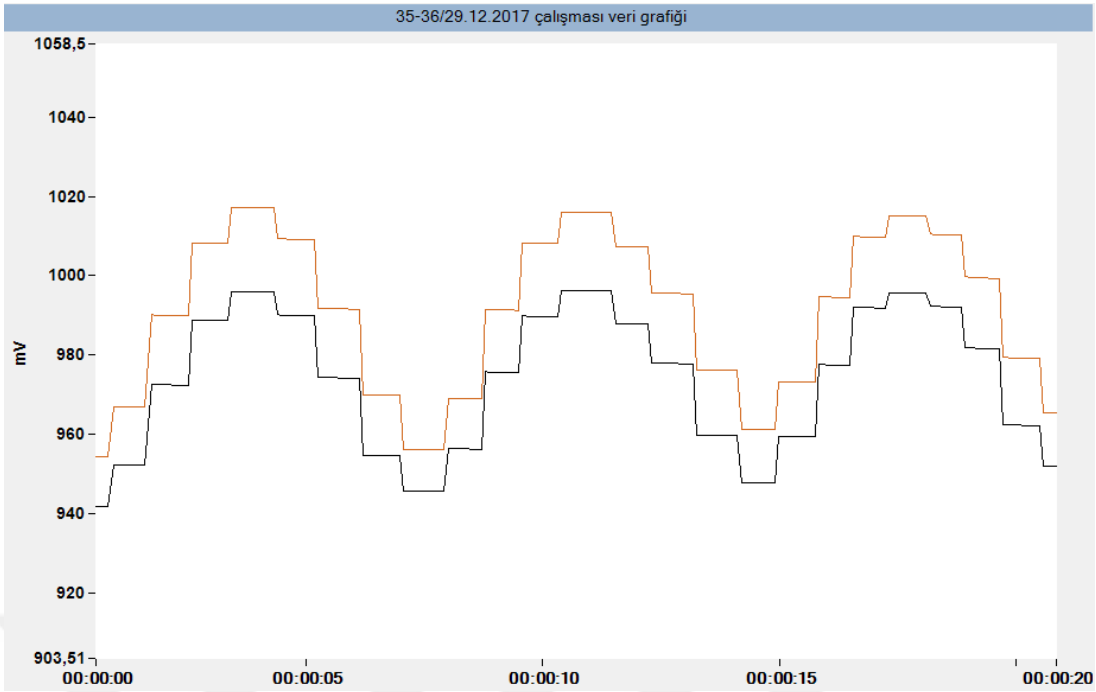


Figure 4.21 Borate measurement of electrode 35 and 36

35 and 36 involves the same composition as they showed very similar results when tested against borate ions. The ladder formation is very visible as it is in the previous figure.

This electrode in Figure 4.21 was one of the first electrodes that was built for the project that provided accurate and desired results when tested in stagnant measurements. Ladder is visible. The only parameters missing for this electrode at this time were repeatability and selectivity on different ions.

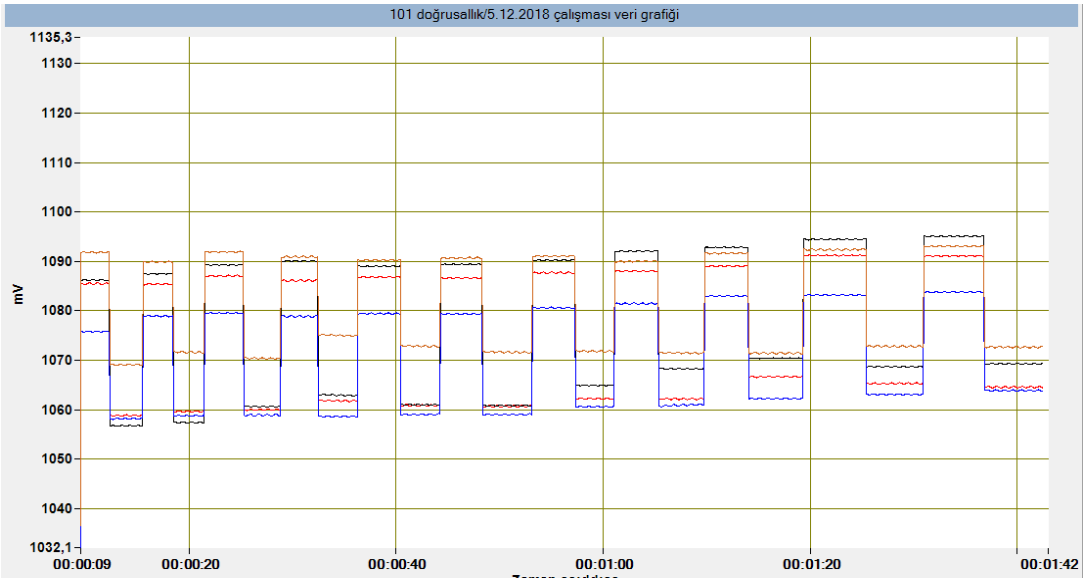


Figure 4.22 Repeatability measurement of electrode number 101

As the composition of the 101 electrode presented in the Table 3.3, this electrode provided very good results for every characteristic an ion selective electrode should have. A repeatability test here is applied on this electrode. 2 different concentrations (10^{-2} and 10^{-3} M) of borate solutions were used

The same 4 electrodes are being tested through same concentrations to check if they provide the same results for same concentrations over and over again. Electrode presented in Figure 4.22 contained sustainable results over time therefore can be considered as an electrode with repeatability.

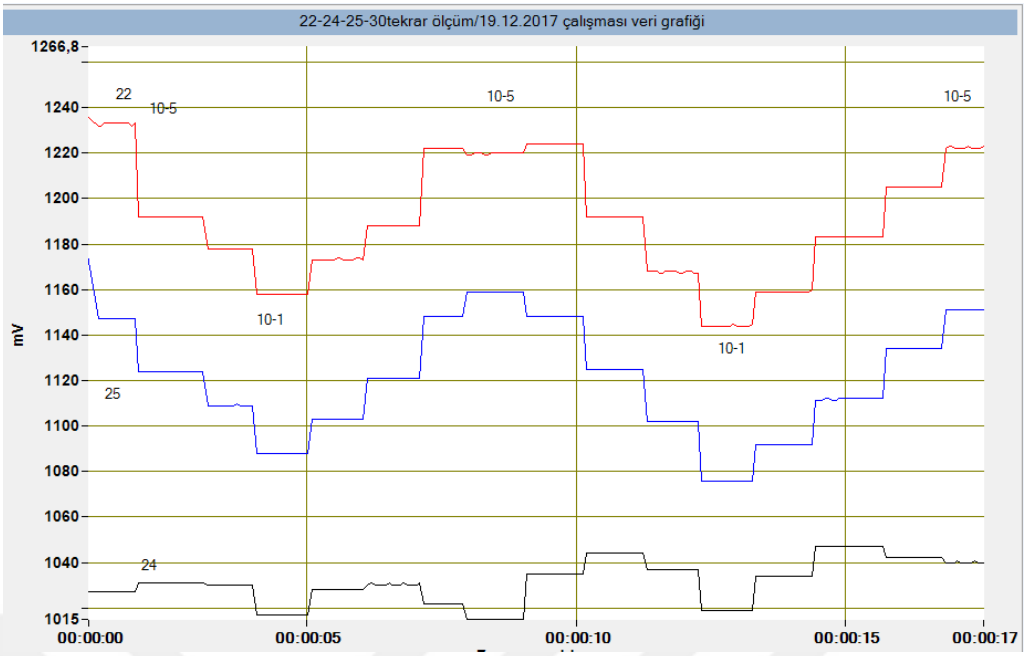


Figure 4.23 Measurements of 22-24-25 numbered electrodes on borate ions

Electrode 22 and 25 includes the same compositions as listed in Table 3.3 led to very similar results. Red line in figure 23 represents the 22 numbered electrode while blue belongs to the 25 and black belongs to the 24. Even though all of these electrodes have the same composition, black markered electrode showed different results than the others which leads to the conclusion of a possible physical mistake that was made when 24 was being built.

The electrode in figure 4.23 showed different results when compared to figure 20 and figure 4.21. While figure 4.20 and 4.21 showed that the mV value was higher when 10^{-1} M solution is being tested however the ladder in figure 4.20 is upside down as it shows higher mV values for diluted concentrations. Within more than 150 different kinds of electrodes built and tested for this project, electrodes that provided higher mV values for more diluted concentrations didn't show any selectivity when tested for other ions.

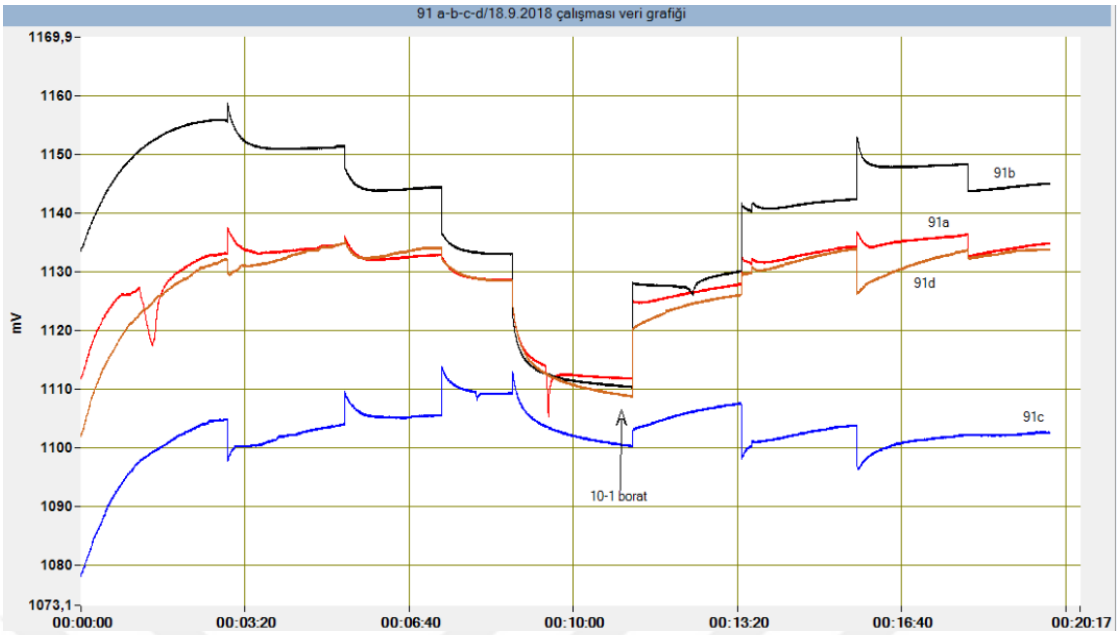


Figure 4.24 Borate measurement of the electrode numbered as 91

This measurement belongs to the electrode numbered as 91. Measurement starts with 10^{-5} M borate solution which is the most diluted and ends with the same concentration while reaching to 10^{-1} M concentration in the middle of the measurement. However in this measurement, unlike other figures, potentiometer does not being stopped while the solutions are being changed therefore response time of the electrodes also becomes visible as well. As far as it can be seen from the figure, response time is not very quick and also not very ideal mV jumps but 3 out of 4 electrodes (marked as brown, red and black) does respond to different borate solution concentrations.

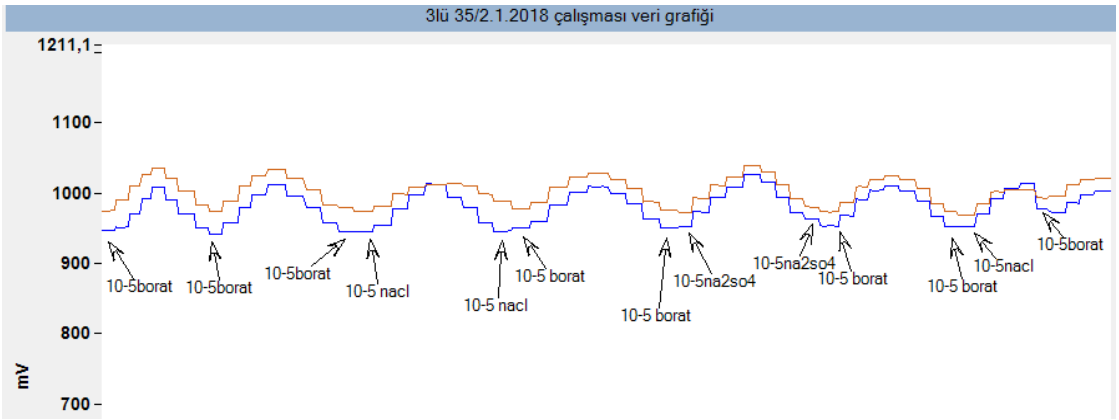


Figure 4.25 Ion selectivity measurement of electrode numbered as 35

While Figure 4.25 shows good characteristics against borate ions as the ladder occurs, this electrode does not provide ion selective results. As the peaks of the sodiumsulfate and sodiumchloride are higher than it provides for borate ions, one can interpret as this

electrode would not be able to detect borate ions when these ions are all present in the same solution. This is not an acceptable behavior a user would expect from a borate ion selective electrode.

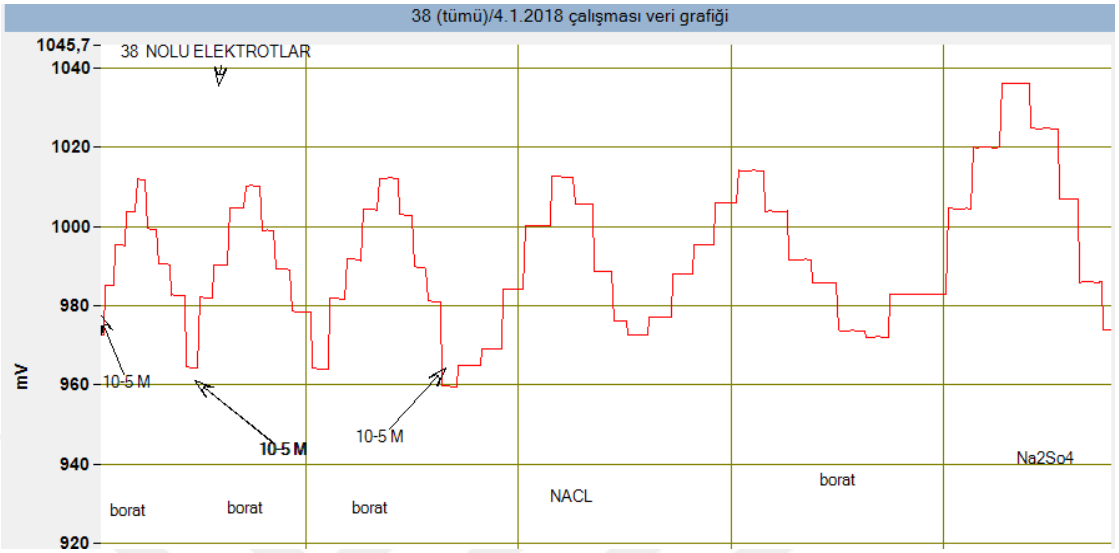


Figure 4.26 Ion selectivity measurement of electrode numbered as 38

Figure 4.25 and 4.26 includes ion selectivity measurements of #35 and #38 electrode respectively. When electrodes respond well against borate solutions with good response times, they are being checked against other solutions to check selectivity. Even though both of these compositions responded well against borate solutions, they didn't provide good results against other solutions as the peaks of the outputs exceeds the peaks that belongs to the borate solutions. This statement can be interpreted as when other ions and borate ions are both present in an anonymous solution, it will be not certain that the electrode will pick up borate ions, therefore the electrodes can't be trusted to detect borate ions.

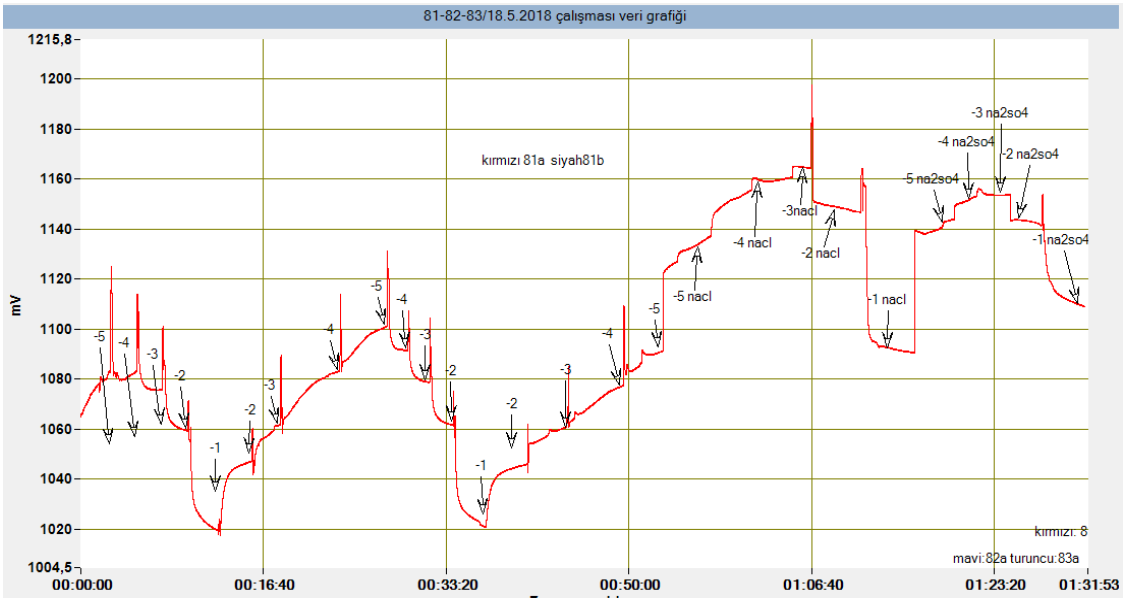


Figure 4.27 Ion selectivity measurement of electrode numbered as 81

Figure 4.27 represents an electrode that responds well to brate ions and also successful when tested against other ions and therefore can be confirmed as an ion selective electrode. Even though the ladder formation is not well consistent for borate ions, 10^{-1} M sodiumchloride and sodiumsulfate peaks does not not exceed to the peak that belongs to the borate ions. This statement can be interpreted as when borate ions and other anonymous ions are present in a solution, this electrode will be able to pick up borate ions.

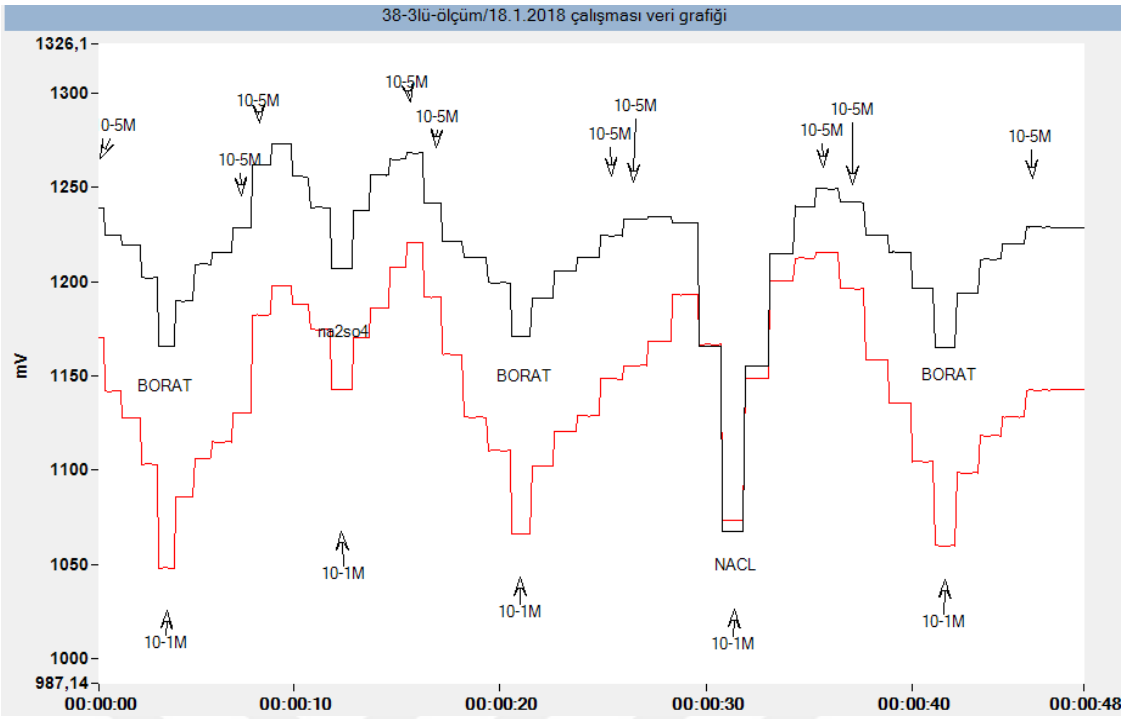


Figure 4.28 Ion selectivity measurement of electrode numbered as 38

Figure 4.28 also displays an electrode that can be interpreted as an ion selective electrode. Even though the compositions are the same for the red and black electrode, black electrode is not ion selective as the peak for the sodiumchloride exceeds the peak for borate ion measurement. Electrode that is indicated as red peaks the most for borate ions even when compared with sodiumchloride and sodiumsulfate ions.

Further in taking a look over the borate selective electrode, it is also capable of measuring glucose glycated albumin and glycated hemoglobin levels as this was the project's goal.

4.1.4 Detection of glucose, glycated albumin and glycated hemoglobin levels through flow injection analysis by the developed CNT based borate selective electrode

Developed CNT based borate selective electrode used for the detection of glucose, glycated albumin and glycated hemoglobin.

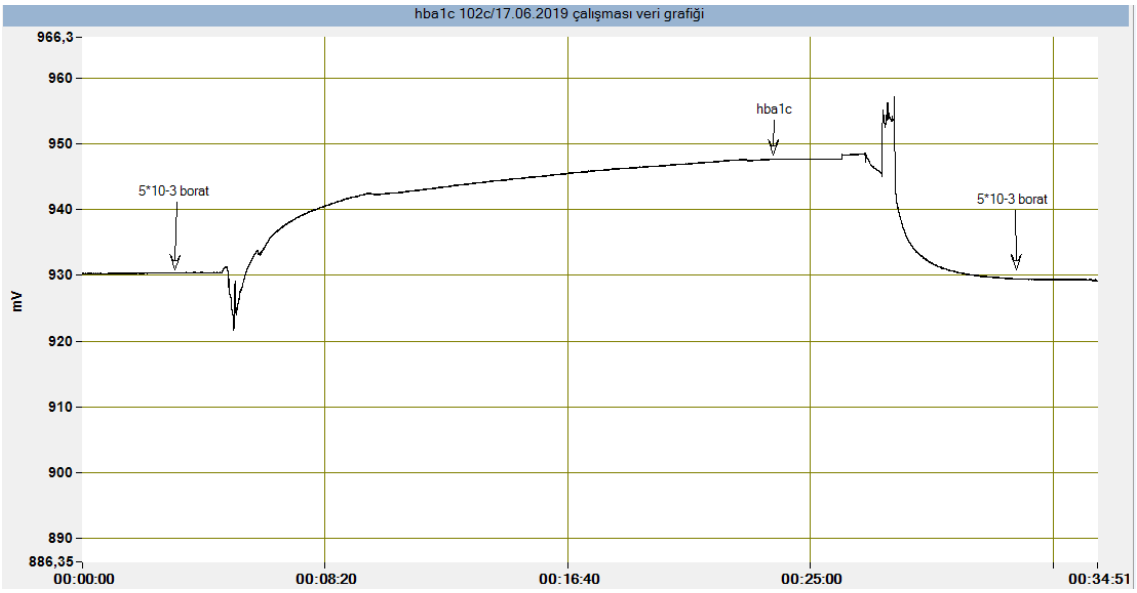


Figure 4.29 Electrode 102 responding to hba1c solution

Figure 29 through 31 involves the detection of glucose, glycated albumin and glycated hemoglobin using the electrode 102. 102's composition is presented in Table 3.3.

Figure 29 represents the change that occurs on output when glycated hemoglobin is involved. When only borate solution is being tested, 5.10^{-5} M borate solution has a mV value of 930. When glycated hemoglobin is involved, concentration of borate ions decrease therefore mV value increases. This confirms hemoglobin gets involved with borate ions to form a complex molecule as predicted. When mathematical correlation is calculated for these mV values, this electrode will be able to determine the glycated hemoglobin concentration by looking at the change in borate concentration that is picked up by the borate sensing electrode.

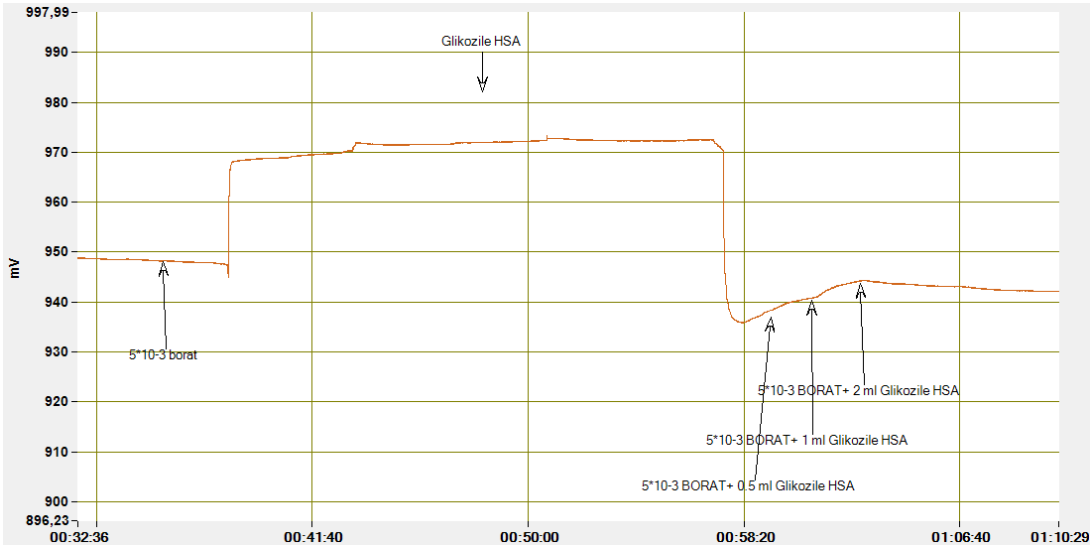


Figure 4.30 Electrode number 102 responding to glycated human serum albumin solution

Figure 4.30 shows the representation of mV change when glycated human serum albumin is involved in the solution. As observed in figure 4.30, when glycated HSA is added with different amounts (0.5 ml, 1 ml, 2 ml glycated HSA respectively added in Figure 4.30), mV value changes. This figure also confirms that the borate ions interact with glycated albumin therefore via an equation, this electrode will also be to detect glycated albumin via the mV change in the output which will be interpreted as a concentration change in borate ion concentration in the solution. Second part of the figure also confirms that the borate ion concentration changes when more glycated albumin is added into the solution.

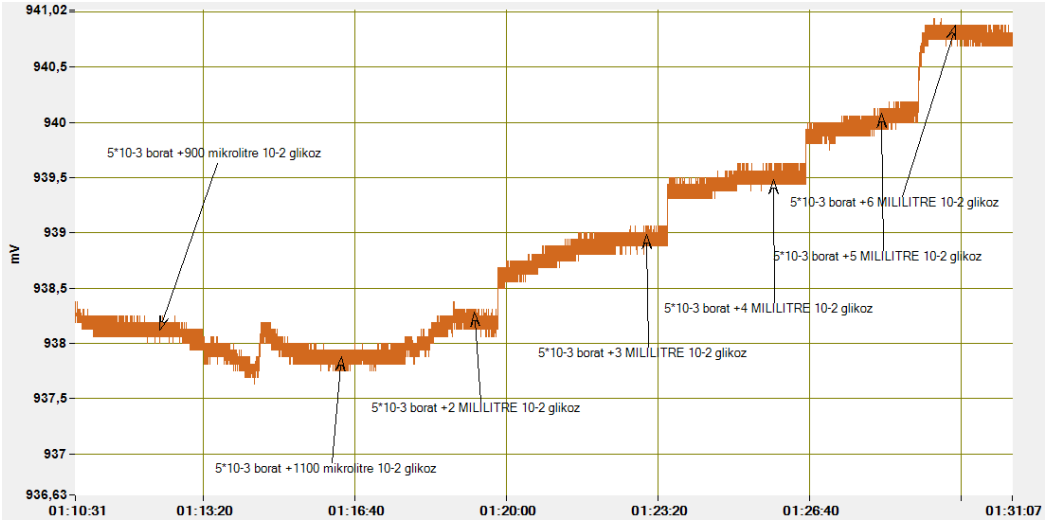


Figure 4.31 Electrode number 102 responding to glucose

Figure 4.31 also confirms that this electrode can also be used to measure glucose just as it can be used to measure glycated albumin and glycated hemoglobin. As the borate ions interact with glucose and gets glycated, borate ion concentration starts to drop. When the amount of glucose is increased in the solution, mV value goes up which confirms that the borate concentration drops. This electrode can also be used to measure glucose level in a solution as the mV change can be correlated with borate ion concentration change.

4.2 Discussion

A novel potentiometric borate sensing electrode was built for this project. Electrodes were tested for repeatability, ion selectivity, response time and carefully reviewed within the important parameters of an electrode that must have. Different set of compositions for the electrodes provided different results. Table 4.7 shows the composition of one of the best built CNT based borate selective electrode that was also used for measuring glucose, glycated hemoglobin and glycated albumin.

Table 4.7 Composition of the best built electrode that showed necessary characteristics for this project.

Chemical	Amount (mg)
CNT	100
CNT-Ag_3BO_3	10
Ag_2S	80
Cu_2S	30
Epoxy resin	160-250
THF	10-20 drops depending on fluidity

Table 4.8 Electrode's best parameter distributions

Parameters	Results
Response time	5-6 seconds
pH range	5.5-8
Lifetime	1 month
Repeatability	More than 20 trials
Selectivity on NaCl	10^4 fold
Selectivity on Na_2SO_4	At least 100 fold
Selectivity on HNO_3	At least 100 fold

Figure 4.29-4.31 are the figures that gathered by using this best built electrode through the flow injection analysis. These are results from glucose, glycated albumin and gylcated hemoglobin readings. The electrode was used to detect glucose, glycated hemoglobin and glycated albumin. The continuous stream is borate solution and the feed stream became glycated hemoglobin, glycated albumin and glucose solutions respectively.

Developed borate selective electrode for this project is able to detect glucose, glycated albumin and glycated hemoglobin. These measurements can be made when they are mixed with borate solutions as borate ions interact with them to build complex structures. This structure build up will change the borate concentration in the solution. An electrode that is selective for borate ions will be able to detect concentration changes on borate ions. This concentration change does reflect on mV output in the potentiometer which can be correlated to measure glucose, glycated hemoglobin and glycated albumin levels. This potential change is used as an indication to detect if the second stream consists any of these substances in a sample.

First findings of this project (figure 4.29-4.31) is very successful. To further study on this subject, real time simultaneous detection of these molecules will be studied.

REFERENCES

[1] Mehrotra, Parikha. “Biosensors and Their Applications – A Review.” Journal of Oral Biology and Craniofacial Research, 6, no. 2, 2016, pp. 153–159.

[2] Mohanty, Saraju P., and Elias Kougianos. "Biosensors: a tutorial review." Ieee Potentials 25.2 (2006): 35-40.

[3] Lee, Hakho, et al. "Chip–NMR biosensor for detection and molecular analysis of cells." Nature medicine 14.8 (2008): 869.

[4] Centers for Disease Control and Prevention. "National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011." Atlanta, GA: US department of health and human services, centers for disease control and prevention 201.1 (2011): 2568-2569.

[5] “Diabetes.” World Health Organization, World Health Organization, www.who.int/news-room/fact-sheets/detail/diabetes, 4 May 2019.

[6] “Diabetes Prevalence.” How Many People Have Diabetes - Diabetes Prevalence Numbers, www.diabetes.co.uk/diabetes-prevalence.html.

[7] “Diabetes.” Mayo Clinic, Mayo Foundation for Medical Education and Research,www.mayoclinic.org/diseases-conditions/diabetes/diagnosis-treatment/drc-20371451, 8 Aug. 2018.

[8] Roskill, 1999; Garrett, 1998; Kalafatoğlu and Örs, 2001.

[9] Demir, Caner. Bor minerallerinin enerji kaynağı olarak değerlendirilmesi. Diss. Selçuk Üniversitesi Fen Bilimleri Enstitüsü, 2006.

[10] Tübitak Bor Raporu, 2003.

[11] Cremer M, (1906), Über die Ursache der elektromotorischen Eigenschaften der Gewebe, zugleich ein Beitrag zur Lehre von den polyphasischen Elektrolytketten, Zeitschrift fur Biologie, 47: 562 - 608.

[12] Haber F and Klemensiewicz Z, (1909), Über elektrische Phasengrenzkräfte Zeitschrift für Physikalische Chemie, 67: 385 - 431.

[13] C., Aoife, and Aoife Morri. “Electroanalytical Sensor Technology.” Electrochemistry, 2013.

[14] Thévenot, Daniel R., et al. "Electrochemical biosensors: recommended definitions and classification." Analytical Letters 34.5 (2001): 635-659.

[15] Libretexts. “11.2: Potentiometric Methods.” Chemistry LibreTexts, Libretexts, chem.libretexts.org/Bookshelves/Analytical_Chemistry/Book:_Analytical_Chemistry_2.0_(Harvey)/11_Electrochemical_Methods/11.2:_Potentiometric_Methods, 5 June 2019

[16] “Enzyme Technology.” Edited by Martin Chaplin, Amperometric Biosensors, 2014, www1.lsbu.ac.uk/water/enztech/amperometric.html.

[17] Gupta, Vinod K., et al. "Voltammetric techniques for the assay of pharmaceuticals—a review." Analytical biochemistry 408.2 (2011): 179-196.

[18] Zhang, Yuyan, and Srinivas Tadigadapa. "Calorimetric biosensors with integrated microfluidic channels." Biosensors and Bioelectronics 19.12 (2004): 1733-1743.

[19] Scott, R.P. W. “Chapter 3 The Fluorometric Detector.” Elsevier, 11, 1977, pp. 121–130.

[20] R Bizzarri, et al. “Green Fluorescent Protein Based PH Indicators for in Vivo Use: a Review.” Anal Bioanal Chem, 393, no. 4, ser. 1107-2, Feb. 2009. 1107-2.

[21] Tantama, Mathew, Yin Pun Hung, and Gary Yellen. "Imaging intracellular pH in live cells with a genetically encoded red fluorescent protein sensor." Journal of the American Chemical Society 133.26 (2011): 10034-10037.

[22] Covington A. K., 1974. Ion-Selective Electrodes, Anal. Chem., CRC Critical Reviews in Analytical Chemistry, 355-406.

[23] Skoog D. A., West D. M. and Holler F. J., 1996. Fundamentals of Analytical Chemistry, 7th Edition, Saunders College Publishing, Rinehart and Winston Inc. USA.

[24] Libretexts. “Ion-Selective Electrodes.” Chemistry LibreTexts, Libretexts, 5 June 2019,chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Analytical_Sciences_Digital_Library/JASDL/Courseware/Analytical_Electrochemistry:_Potentiometry/03_Potentiometric_Theory/03_Ion-Selective_Electrodes, May 4 2019.

[25] Libretexts. “Reference Electrodes.” Chemistry LibreTexts, Libretexts, 5 June 2019,chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Analytical_Sciences_Digital_Library/JASDL/Courseware/Analytical_Electrochemistry:_Potentiometry/03_Potentiometric_Theory/04_Reference_Electrodes, May 4 2019.

[26] Libretexts. “The Nernst Equation.” Chemistry LibreTexts, Libretexts, 3 June 2019,chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Analytical_Sciences_Digital_Library/JASDL/Courseware/Analytical_Electrochemistry:_Potentiometry/03_Potentiometric_Theory/05_The_Nernst_Equation, May 4 2019.

[27] TOPCU, Cihan. “Bor Seçici Elektrot Geliştirilmesi, Toprak Ve Su Numunelerinde Bor Ölçümü İçin Uygulanması.” Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, 2013.

[28] Singh, Shweta. “Ion Selective Electrodes.” LinkedIn SlideShare, www.slideshare.net/ShwetaSingh44/ion-selective-electrodes-18411285, 8 Apr. 2019.

[29] “Application of ISE.” Monokrystaly, www.monokrystaly.cz/en/fields-of-application-ise.html, May 4 2019.

[30] Wang, Joseph. "Carbon- nanotube based electrochemical biosensors: A review." Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis 17.1 (2005): 7-14.

[31] Fogg A. G., Pathan A. S., Thorburn Burns E. D., 1974. A Liquid-State Tetrafluoroborate Ion –Selective Electrode Based on Brilliant Green Tetrafluoroborate, Analytical Letters, 7, 545-551.

[32] Zhu, Chengzhou et al. “Electrochemical sensors and biosensors based on nanomaterials and nanostructures.” Analytical chemistry, 87,1 (2015): 230-49.

[33] Shetti, Nagaraj P., et al. “Graphene–Clay-Based Hybrid Nanostructures for Electrochemical Sensors and Biosensors.” Graphene-Based Electrochemical Sensors for Biomolecules, 2019, pp. 235–274.

CURRICULUM VITAE

PERSONAL INFORMATION

Name Surname : Yiğit Tanyeri

Date of birth and place : 08/11/1991 Istanbul, Turkey

Foreign Languages : English, German

E-mail : tanyeriyigit@gmail.com

EDUCATION

Degree	**Department**	**Institution**	**Date of Graduation**
Bachelor's	Bioengineering	Istanbul Technical University- Montana State University	2016
High School	-	Sisli Terakki High school	2009

WORK EXPERIENCE

Year	**Corporation/Institute**	**Enrollment**
2019	Golden Horn	Customer representative
2016	Careem	Customer representative

2013	Shell UK	Customer representative

PUBLISHMENTS

Conference Papers

1. Akış Enjeksiyon Sistemde Glikolize Protein Tayini İçin Borat Seçici Elektrot Geliştirilmesi, Yiğit Tanyeri, Duygu Üner-Bahar, İbrahim Işıldak, 9. Ulusal Analitik Kimya Kongresi, 19-23 Eylül 2018, NOVOTEL Convention Center, Konya.