# DEVELOPMENT OF CONDUCTIVE OXIDE BASED THIN FILM MODIFIED ELECTRODES AND BIOSENSORS APPLICATIONS

A Thesis Submitted to the Graduate School of İzmir Institute of Technology in Partial Fulfillment of the Requirements for the Degree of

### **MASTER OF SCIENCE**

in Biotechnology and Bioengineering

by Betül YURTTAŞ

> July 2021 İZMİR

### ACKNOWLEDGMENTS

First of all, I want to convey my thanks to my supervisor, Prof. Dr. Lütfi ÖZYÜZER, for accepting me into his laboratory and giving me the opportunity to explore the thin films field of physics. I would also like to thank him for his valuable time, experience, and support throughout my work in this field.

I would like to express my sincere thanks to my co-advisor Prof. Dr. Kadriye Arzum ERDEM GÜRSAN (nanoBioSens Lab, Ege University) while giving me the opportunity to develop the thin film electrodes and work on their applications in the field of biosensors. I want to thank for her valuable contributions, interest, and support throughout my thesis.

I would like to thank TEKNOMA Technological Materials Industrial and Trading Inc. for financial support during my master's thesis studies, and for enabling me to use of all the infrastructure for my experimental studies.

I would like to thank my thesis committee members Assoc. Prof. Dr. Engin ÖZÇİVİCİ, Prof. Dr. Çağlar KARAKAYA, and Asst. Prof. Günnur GÜLER for their participation and valuable comments.

I am also very thankful to José Enrique MARTINEZ MEDINA and Merve EKMEKÇİOĞLU for sharing their experiences with me, and all members of the OZYUZER research group who helped me when there is a need and created a friendly working environment.

I would also like to thank Meltem MARAL from the research group of my coadvisor Kadriye Arzum ERDEM GÜRSAN for her contributions to this thesis.

Finally, I would like to thank my family for always supporting, encouraging, and understanding me throughout my education life

## ABSTRACT

# DEVELOPMENT OF CONDUCTIVE OXIDE BASED THIN FILM MODIFIED ELECTRODES AND BIOSENSORS APPLICATIONS

From the first biosensor produced in 1956 to the present day, biosensors have been highly developed and diversified. In biosensor manufacturing, thin films have become a rapidly emerging field. Depending on the thin film material used, thin films have many advantageous properties for biosensors, such as high surface-to-volume ratio, conductivity, stability, specificity, biocompatibility, and good electrocatalytic activity. Dopamine is a neurotransmitter that has a significant impact on the emergence and treatment of certain diseases such as Alzheimer's and Parkinson's diseases. Dopamine monitoring is important for the prevention of these diseases, and it is a favorable option to use biosensors, which are useful and practical tools, instead of time-consuming and expensive conventional methods. For this purpose, in this thesis, a non-enzymatic electrochemical biosensor based on thin film electrodes was developed for monitoring dopamine levels. The electrodes were developed by deposition of Zn<sub>2</sub>SnO<sub>4</sub> (ZTO) thin film on ITO thin film substrate by DC magnetron sputtering technique. The properties of the electrodes were determined by thickness, optical transmittance, XRD and SEM analysis. Electrochemical analysis, namely CV, EIS and DPV measurements, were performed before and after the electrodes were sonicated and modified with APTES before their application to the voltammetric detection of dopamine. In addition, electrochemical measurements were performed before/after sonication, APTES modification. Dopamine was detected by a voltammetric method using DPV technique. Furthermore, experiments in the presence of interferents such as ascorbic acid (AA), uric acid (UA) etc. showed that the thin film electrodes can be successfully applied for voltammetric determination of dopamine. As a result, the biosensor technology developed in this study has the potential to be wearable in the future, enabling non-invasive monitoring of dopamine levels in body fluids such as saliva, tears and sweat.

# ÖZET

# İLETKEN OKSİT TABANLI İNCE FİLM MODİFİYE ELEKTRODLARIN GELİŞTİRİLMESİ VE BİYOSENSÖR UYGULAMALARI

1956 yılında üretilen ilk biyosensörden günümüze kadar geçen sürede, biyosensörler oldukça gelişmiş ve çeşitli alanlarda kullanılmaya başlamıştır. Biyosensör üretiminde ince filmlerin kullanılması ise hızla gelişen bir alan haline gelmiştir. İnce filmlerin sahip olduğu yüksek yüzey-hacim oranı, iletkenlik, kararlılık, özgüllük, biyouyumluluk ve iyi elektrokatalitik aktivite gibi özellikler, biyosensörler için oldukça avantajlıdır. Dopamin ise, Alzheimer ve Parkinson gibi bazı hastalıkların ortaya çıkması ve tedavisinde önemli bir etkisi olan nörotransmiterdir. Vücuttaki dopamin miktarının takibi bu hastalıkların önlenmesi için önemlidir. Dopamin miktarını belirlemek için kullanılan geleneksel yöntemler zaman alıcı ve pahalıdır. Bu yöntemlerin yerine kullanışlı ve pratik araçlar olan biyosensörlerin kullanılması daha uygun ve hayat kolaylaştırıcı bir seçenektir. Bu kapsamda tezde hazırlanan ince film elektrotlar kullanılarak enzime gerek duymaksızın dopamin tayinini mümkün kılan bir elektrokimyasal biyosensör geliştirilmiştir. Elektrotlar, DC mıknatıssal saçtırma tekniği ile ITO ince film substratı üzerine Zn2SnO4 (ZTO) ince filminin biriktirilmesiyle üretilmiştir. Üretilen elektrotların karakteristik özellikleri kalınlık, optik geçirgenlik, XRD ve SEM analizleri ile belirlenmiştir. Elektrot yüzeyleri sonikasyon ve APTES ile modifiye edilip dopamin ile muamele edildikten sonra ise elektrokimyasal analiz yöntemleri olan CV, EIS ve DPV ölçümleri yapılmıştır. Ayrıca elektrokimyasal ölçümler sonikasyondan önce, APTES modifikasyonundan önce ve sonra gerçekleştirilmiş olup ortamdaki dopamin miktar tayini DPV tekniğine dayalı voltametrik yöntemle gerçekleştirilebilmiştir. Ayrıca askorbik asit (AA), ürik asit (UA) vb. gibi girişim yapabilecek türlerin varlığında yapılan deneylerde, geliştirilen ince film elektrotların voltametrik dopamin tayini için başarılı bir şekilde uygulanabileceğini göstermiştir. Sonuç olarak, çalışmada geliştirilen biyosensör teknolojisi gelecekte giyilebilir hale getirilerek tükürük, gözyaşı ve ter gibi vücut sıvılarında dopamin seviyesinin invaziv olmayan bir yöntemle izlenmesini sağlama potansiyeline sahiptir.

# **TABLE OF CONTENTS**

LIST OF FIGURES	vii
LIST OF TABLES	X
CHAPTER 1. INTRODUCTION	1
1.1. Biosensors	1
1.2. Purpose of the Thesis	4
CHAPTER 2. BACKGROUND INFORMATION	5
2.1. Enzymatic and Non-Enzymatic Biosensors	5
2.2. Thin Films	6
2.3. Thin Film Biosensors	8
2.4. Magnetron Sputtering	9
2.5. Dopamine	11
2.6. X-Ray Diffraction (XRD)	12
2.7. Cyclic Voltammetry (CV)	14
2.8. Electrochemical Impedance Spectroscopy (EIS)	15
2.9. Differential Pulse Voltammetry (DPV)	17
CHAPTER 3. EXPERIMENTAL PROCEDURE	18
3.1. Deposition of ZTO Thin Films	18
3.2. Characterization of ZTO Thin Films	20
3.2.1. Thickness Measurement	21
3.2.2. Optical Transmission	22
3.2.3. X-Ray Diffraction (XRD)	22
3.2.4. Scanning Electron Microscopy (SEM)	22
3.3. Process of Sonication and APTES Modification of the Electrodes	22
3.4. Electrochemical Characterization of Electrodes	23
3.4.1. Cyclic Voltammetry (CV)	23
3.4.2. Electrochemical Impedance Spectroscopy (EIS)	23
3.5. Voltammetric Detection of Dopamine	23

CHAPTER 4. RESULTS AND DISCUSSION	25
4.1. Thickness Measurement	25
4.2. Optical Transmission	26
4.3. X-Ray Diffraction (XRD)	31
4.4. Scanning Electron Microscopy (SEM)	33
4.5. Cyclic Voltammetry (CV)	35
4.6. Electrochemical Impedance Spectroscopy (EIS)	42
4.7. Dopamine Detection with ZTO/ITO Electrodes	48
4.8. Selectivity of ZTO/ITO Electrodes	51
CHAPTER 5. CONCLUSION	55
REFERENCES	58

# **LIST OF FIGURES**

<u>Figure</u> <u>Pag</u>	<u>e</u>
Figure 1.1. Illustration of the biosensor components	1
Figure 1.2. Representation of the Clark electrode.	2
Figure 2.1. General comparison of thickness of thin films with some other materials	7
Figure 2.2. Images of the thin film biosensors. A) Thin film gold electrode. B) Thin	
film platinum electrode.	9
Figure 2.3. Illustration of the inside of the magnetron sputter coating chamber	0
Figure 2.4. Structure of the dopamine	1
Figure 2.5. Illustration of X-ray diffractometer instrumental setup.	3
Figure 2.6. Representation of a standard cyclic voltammogram	4
Figure 2.7. Illustration of a standard three-electrode electrochemical cell setup	5
Figure 2.8. Illustration of a Nyquist diagram	6
Figure 3.1. Image of the DC magnetron sputtering system.	8
Figure 3.2. Schematic representation of the DC magnetron sputtering system	9
Figure 3.3. Images of the samples after the cutting process. (A) ITO substrates. (B)	
ZTO/ITO electrodes	0
Figure 3.4. Image of the uncoated glass substrate with three drops of photoresist2	1
Figure 3.5. Image of the profilometer at Physics Department	1
Figure 4.1. Optical transmission graph of the ZTO samples deposited with different	
mass flow rates of O <sub>2</sub>	8
Figure 4.2. Optical transmission graph of the ZTO samples deposited at different	
times with 7.5 sccm O <sub>2</sub>	8
Figure 4.3. Optical transmission graph of the ZTO/ITO samples deposited with	
different mass flow rates of O2	9
Figure 4.4. Optical transmission graph of the ZTO/ITO samples deposited at different	
times with 7.5 sccm O <sub>2</sub>	9
Figure 4.5. Image of the ZTO/ITO electrodes. The oxygen concentrations of ZTO	
thin films during deposition are 7.5 sccm, 10 sccm, and 12.5 sccm (from	
left to right)	1

Page

Figure 4.6.	XRD graph of ZTO samples deposited with different mass flow rates of O <sub>2</sub> .	. 32
Figure 4.7.	XRD graph of ZTO samples deposited at different times with 7.5 sccm O <sub>2</sub> .	. 32
Figure 4.8.	XRD graph of the ZTO/ITO samples deposited at different times with 7.5	22
Eigura 4.0	SEM image of ITO	21
Figure 4.9.	SEM image of $TTO(7.5 \text{ source} O)$	. 54
Figure 4.10 $\Gamma^{*}$	SEM image of ZTO (7.5 secm $O_2$ )	. 34
Figure 4.11	1.5 EM image of 210/110 (10 sccm 0 <sub>2</sub> )	. 33
Figure 4.12	2. Cyclic voltammograms obtained by (a) 110, (b) $\geq$ 10/110 (7.5 seem	26
<b>D</b> : 4.12	$O_2$ ), (c) 210/110 (10.0 sccm $O_2$ ), (d) 210/110 (12.5 sccm $O_2$ ).	. 36
Figure 4.13	Cyclic voltammograms obtained by (a) 110, (b) 210/110, (c) sonicated	• •
	210/110.	. 36
Figure 4.14	A. (A) Cyclic voltammograms obtained by (a) sonicated ZTO/ITO, (b) 1%	
	APTES-modified ZTO/ITO, (c) 2% APTES-modified ZTO/ITO, (d)	
	5% APTES-modified ZTO/ITO. (B) Direct comparison of the CV	
	curves obtained by sonicated ZTO/ITO and 2% APTES and modified	
	ZTO/ITO.	. 38
Figure 4.15	5. Cyclic voltammograms obtained by (A) ITO electrodes: (a) ITO, (b)	
	sonicated ITO, (c) 2% APTES-modified ITO; (B) ZTO/ITO electrodes:	
	(a) ZTO/ITO, (b) sonicated ZTO/ITO, (c) 2% APTES-modified ZTO/ITO	40
Figure 1 16	E Nyquist diagrams of (a) ITO (b) Sonicated ZTO/ITO (c) ZTO/ITO	. 40
rigule 4.10	s. Nyquist diagrams of (a) 110, (b) Someated 210/110, (c) 210/110	
	electrodes. Inset Nyquist diagram snows the close-up view of a, b, and	40
D: 415	c at low impedance range.	.43
Figure 4.17	7. Nyquist diagrams of (a) sonicated $Z10/110$ , (b) 1% APTES-modified	
	ZTO/ITO, (c) 2% APTES-modified ZTO/ITO, (d) 5% APTES-	
	modified ZTO/ITO electrodes. Inset Nyquist diagram shows the close-	
	up view of a, b, c, and d at low impedance range	. 44
Figure 4.18	B. Nyquist diagrams of (A) ITO electrodes: (a) ITO, (b) sonicated ITO, (c)	
	20/ADTEC = 1.00 I ITO (D) 7TO (ITO 1 + 1 () 7TO (ITO (1)))	
	2% APTES-modified 110. (B) Z10/110 electrodes: (a) Z10/110, (b)	
	sonicated ZTO/ITO, (c) 2% APTES-modified ZTO/ITO. Inset Nyquist	
	2% APTES-modified ITO. (B) ZTO/ITO electrodes: (a) ZTO/ITO, (b) sonicated ZTO/ITO, (c) 2% APTES-modified ZTO/ITO. Inset Nyquist diagram shows the close-up view of a, b, and c at low impedance range.	

- Figure 4.19. (A) Differential pulse voltammograms obtained by using the 2% APTES-modified ZTO/ITO electrodes in the absence and presence of dopamine: (a) Control experiment in PBS (0.05 M, pH 7.0), (b) 0.1 μM DA, (c) 0.3 μM DA, (d) 0.5 μM DA, (e) 1 μM DA. (B) Calibration graph obtained by 2% APTES-modified ZTO/ITO electrodes in the presence of 0.1-1 μM DA. 49
- Figure 4.20. Differential pulse voltammograms presenting the oxidation signals measured by 2% APTES-modified ZTO/ITO. (A) (a) Control experiment in PBS (0.05 M, pH 7.0), (b) 0.1 μM DA, (c) 10 μM AA, (B) (a) 1 μM DA, (b) 10 μM AA, (C) (a) control experiment in PBS (0.05 M, pH 7.0), (b) 0.1 μM DA, (c) 10 μM UA, and (D) (a) 1 μM DA, (b) 10 μM UA.

# LIST OF TABLES

## Table

## Page

Table 3.1. Deposition parameters of ZTO thin films	. 20
Table 4.1. Data obtained from thickness measurement of ZTO thin films	. 25
Table 4.2. Data obtained from thickness measurement of ZTO thin films deposited	
in the presence of 7.5 sccm O <sub>2</sub>	. 26
Table 4.3. Optical transmission values of ZTO and ZTO/ITO samples at 550 nm	. 30
Table 4.4. The data obtained by using CV measurements of ITO, ZTO/ITO, and	
sonicated ZTO/ITO electrodes. The number of experiments (n) was 3	. 37
Table 4.5. The data obtained by using CV measurements of sonicated ZTO/ITO and	
APTES-modified ZTO/ITO electrodes. The number of experiments (n)	
was 3	. 39
Table 4.6. The data obtained by using CV measurements of ITO, sonicated ITO, and	
ITO electrodes modified with 2% APTES. The number of experiments	
(n) was 3	. 41
Table 4.7. The data obtained by using CV measurements of ZTO/ITO, sonicated	
ZTO/ITO, and ZTO/ITO electrodes modified with 2% APTES. The	
number of experiments (n) was 3.	. 41
Table 4.8. Average $R_{ct}$ and change % at $R_{ct}$ values measured by ITO, ZTO/ITO, and	
sonicated ZTO/ITO electrodes	. 43
Table 4.9. Average $R_{ct}$ and change % at $R_{ct}$ values measured by sonicated ZTO/ITO	
and APTES-modified ZTO/ITO electrodes.	. 45
Table 4.10. Average R <sub>ct</sub> and change % at R <sub>ct</sub> values measured by ITO, sonicated ITO,	
and 2% APTES-modified ITO electrodes	. 47
Table 4.11. Average $R_{ct}$ and change % at $R_{ct}$ values measured by ZTO/ITO, sonicated	
ZTO/ITO, and 2% APTES-modified ZTO/ITO electrodes.	. 48
Table 4.12. Comparison of the performances of APTES/ZTO/ITO electrodes over to	
the electrodes reported in earlier studies in terms of DA detection	. 50

# **CHAPTER 1**

# **INTRODUCTION**

#### 1.1. Biosensors

Interest in biosensors has been increasing over the years due to its advantageous usage properties. Production of a biosensor requires utilization of interdisciplinary fields such as biology, chemistry, physics, and engineering. Biosensor is a device that detects the concentration or presence of a biological material and generates electrical signals as a result of chemical and biological reactions occurred between the analyte and the bioreceptor (Bhalla et al., 2016; Mehrotra, 2016).



Figure 1.1. Illustration of the biosensor components. (Source: http://2018.igem.org/Team:Queens Canada/Background)

A biosensor consists of different components such as analyte, bioreceptor, transducer and detector (Figure 1.1). Analyte is the biomaterial that is wanted to detect. For instance, DNA, glucose, cholesterol, lactate, and dopamine are some of the analytes. The analyte is recognized by bioreceptor, and this process is called as biorecognition. The bioreceptor (such as antibodies, enzymes, immobilized cells, and nucleic acids) identifies the analyte, and binding between the bioreceptor and the analyte occurs. Signal is generated as a result of chemical and biological reactions, and the change of conformation. This signal become measurable with the help of transducer. The

unmeasurable signal obtained as an alteration of heat, pH, light, or charge is converted to a measurable signal by transducer. This conversion process is called as signalization. In the final step, the signals obtained proportional to the concentration of the analyte are processed and amplified by detector. In a system of electronic display, the signals are quantified, and the final data can be obtained as in several ways such as graphical, pictorial, and numerical (Parkhey & Mohan, 2018).



Figure 1.2. Representation of the Clark electrode. (Source: https://clinicalgate.com/additional-equipment-used-in-anaesthesia-andintensive-care/)

Although the basics of the biosensor date back to the early 1900s, the first biosensor was invented by Leland C. Clark in 1956. This amperometric biosensor, namely Clark electrode (it was named after Leland C. Clark), detects concentration of oxygen in a gas or a liquid such as blood (Heineman & Jensen, 2006). In an amperometric biosensor, current is measured which is obtained due to the application of voltage between the two electrodes (Mendelson, 2011). In a Clark-type electrode, there is an oxygen permeable membrane (made from teflon) between the part containing the electrodes and the sample (gas or liquid) containing oxygen (Figure 1.2). The electrodes which are platinum cathode and silver anode stay in an electrolyte such as sodium chloride (NaCl) and potassium chloride (KCl) (Chang et al., 1993). The oxygen passes through the membrane and

reaches to the platinum cathode. The oxygen molecules are reduced electrolytically at the cathode while oxidation of silver occurs at the anode. The catalysis is increased due to the application of voltage between the two electrodes. As a result of this voltage application, the obtained current is proportional to the oxygen concentration.

In 1962, Clark and Lyons also developed an amperometric electrode that is for the enzymatic detection of the glucose (Clark & Lyons, 1962). The basis of this electrode is the Clark oxygen electrode. They used a semi-permeable dialysis membrane to coat the oxygen electrode with the enzyme of glucose oxidase (GOx) (Davis & Higson, 2014). Thus, they did an enzyme immobilization. The concentration of glucose was detected with respect to the oxygen consumption from the sample containing glucose. The amount of oxygen consumption represented the concentration of glucose. This glucose electrode is further improved as a first commercial glucose biosensor by Yellow Spring Instruments, in 1975 (Yoo & Lee, 2010). They conducted the glucose detection with respect to amperometric measurement of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).

There are certain features that are expected to exist is biosensors. For instance, biosensors must have high selectivity, specificity, and sensitivity (Revathi & Rajendra Kumar, 2019). In other words, biosensors must have the ability to detect the desired biomaterial among the other unwanted substances successfully. Also, reproducibility and repeatability are the other important properties. Biosensors should be used multiple times in order to reduce the production cost and obtain the higher efficiency as much as possible from one biosensor device. The detection time, namely response time, of the biosensors should not be long. In general, the expected response time should not be more than 30 seconds. Moreover, the size of the biosensors should be small enough to make them easy to handle and also, make the fabrication process easier. Biosensors should have the ability to detect the desired analyte which has the volume as small as possible. In other words, detection limit of the biosensors should be low. In addition, stability is another significant feature when enzymes or proteins are used in biosensors (Gibson, 1999). The stability is related to activity retention of a protein, enzyme, or device. After the production of biosensors, either during the use or during the storage time, this activity retention must be increased.

Biosensors can be categorized in terms of analyte type, bioreceptor type, and mechanism of transduction (Ali et al., 2017). If the categorization is based on the analyte, there will be biosensors that detect dopamine, glucose, lactate, drugs, toxins, enzymes, and nucleic acids. According to the bioreceptor type, the biosensor types can be classified

as proteins, aptamers, DNA, enzymes, and oligonucleotides. The transduction mechanism used in the biosensors also determines the biosensor type such as mass dependent, sensitive to radiation, optical, thermal, piezoelectric, and electrochemical. These categorized biosensor types can be further increased. In this work, an electrochemical biosensor is developed for dopamine detection. An electrochemical biosensor converts the chemical signal coming from analyte-bioreceptor interaction to an electrical signal which are voltage or current (Jothi & Nageswaran, 2019). Conductometric, voltammetric, potentiometric, and amperometric biosensors are the sub-categories of the electrochemical biosensors. There are many studies on electrochemical biosensors in the literature, and various analytes can be determined by producing a wide variety of electrochemical biosensor electrodes (Erdem, 2007; Mathur et al., 2009; Alwarappan et al., 2009; Yilmaz et al., 2017; Erdem et al., 2015; Congur et al., 2019; Erdem & Eksin, 2019; Congur et al., 2021).

Biosensors have a wide range of usage area. They are used in environmental monitoring (e.g., pollutant detection), food and beverage control, agriculture, pharmaceutical industry (in terms of drug discovery or improvement), tissue engineering, plant biology, prosthetic devices, criminology disease monitoring (in terms of disease detection or treatment) such as detecting the markers and microorganisms that induce the diseases, etc. (Hasan et al., 2014). Body fluids such as saliva, sweat, urine, and blood are used for detection of the diseases. Foods, beverages, and agricultural products can be controlled by using biosensors if there exists any toxins or contaminants. Thus, the unwanted substances or microorganisms can be detected earlier, and the quality and safety of the products can be enhanced (Mehrotra, 2016).

#### **1.2.** Purpose of the Thesis

The purpose of this thesis is to develop the single-use electrodes based on thin films and apply them to determine dopamine voltammetrically. The electrodes were produced by coating Zn<sub>2</sub>SnO<sub>4</sub> (ZTO) on the In<sub>2</sub>O<sub>3</sub>:SnO<sub>2</sub> (ITO) substrate, by applying the DC magnetron sputtering method. Then, the surface of the electrodes was firstly sonicated and then modified by APTES. After all, these electrodes were applied for dopamine determination.

### **CHAPTER 2**

### **BACKGROUND INFORMATION**

#### **2.1. Enzymatic and Non-Enzymatic Biosensors**

Electrochemical biosensors can also be divided into another two groups which are enzymatic biosensors and non-enzymatic biosensors. In enzymatic biosensors, enzyme immobilization is done by applying different procedures such as entrapment, adsorption, cross linking, and covalent bonding (Nguyen et al., 2019; Revathi & Rajendra Kumar, 2019).

Entrapment is the method that the enzyme is immobilized in a polymer such as gel. This immobilization process is not done chemically and also, this method provides stability. However, the gel may have an unwanted effect on the active site of the enzymes which may affect the substrate binding. Adsorption is another immobilization method in which weak interactions such as van der Waals forces, hydrophobic interactions, and electrostatic interactions are used. Although adsorption is a cost effective and easy method, and not harmful for the enzymes, some disadvantages are present in which the differences occurred in the environmental conditions may negatively affect the enzymes due to the weak interactions. Furthermore, contamination may occur because adsorption of the unwanted substances may be occurred. It can be said that cross linking is also a chemical method since a supporting material is used, and the biomaterial is chemically connected to this supporting material. Although this process provides enhanced stability and efficiency due to the strong chemical bonding, the activity of the enzyme may be lost because of the application of some of the crosslinking reagents. Lastly, covalent bonding is a common method for enzyme immobilization because it provides stability due to its strong interactions. Low ionic strength, low temperature, and physiological pH conditions may be suitable for this method. However, this strong covalent bonding may affect the enzyme in a negative way.

To sum up, enzymatic biosensors offer high selectivity, high sensitivity, and high specificity due to unique enzyme-substrate interactions. On the other hand, because of the difficulties in the enzyme immobilization process, enzymatic biosensors may be unstable, have short shelf life, and high cost. For instance, the environment conditions of enzymes are important because enzymes can degrade under inappropriate conditions such as wrong pH and temperature values.

Biosensors have been developed to work without enzymes in order to overcome the challenges present in the enzymatic biosensors. Non-enzymatic biosensors are simple, reproducible, and stable because of the absence of the enzymes. Nanomaterials such as platinum (Pt), gold (Au), graphene, quantum dots, and carbon nanotubes (CNTs) are used in non-enzymatic biosensors (Revathi & Rajendra Kumar, 2019). These nanomaterials meet the requirements of suitable morphology, high surface area, and electron transport, and therefore, the non-enzymatic biosensors made of nanomaterials are selective, and provide to increase the electrocatalytic activity. Also, low cost metal oxide nanomaterials such as nickel oxide (NiO), zinc oxide (ZnO), tin dioxide (SnO<sub>2</sub>), and manganese dioxide (MnO<sub>2</sub>) are used in non-enzymatic biosensors. The catalytic activity of these metal oxide nanomaterials is high.

Besides the advantageous properties of the nanomaterials mentioned above, there may be still some issues when they used by themselves. Some of the characteristics of the biosensors are required to improve in terms of sensitivity, specificity, and selectivity. This issue has been managed by using chemically modified electrodes (CMEs). Chemical modifications such as covalent bonding and adsorption can be applied to the electrodes, such as gold and platinum. Furthermore, the interest has been increasing for using carbon materials, hybrid materials, polymers, enzymes, nanomaterials, etc. for biosensors in order to increase the response time, stability, sensitivity, specificity, and selectivity. However, especially for some non-enzymatic biosensors, there may be some issues such as working problems under physiological conditions, and the requirement for finding biocompatible materials for some types of biosensors such as wearable biosensors.

#### 2.2. Thin Films

Thin film is a material that can be deposited on either a surface or a substrate by a variety of deposition techniques. Tvarozek et al. suggested that thin films can be thought as two-dimensional (2D) materials since the thickness of the thin films are significantly low (Tvarozek et al., 1998). Thus, surface-to-volume ratio of thin films are high. Also, on this thin scale, they gain distinct properties when compared to thick bulk substances

having the same molecular formula (Mylvaganam et al., 2014). In general, thickness of the thin films is less than 1  $\mu$ m. According to the Figure 2.1, it can be said that foil is between the thin films and the thick films (having thickness of more than 1  $\mu$ m) in terms of thickness. Also, based on the Figure 2.1, the thinnest substances are atoms and molecules while the thickest substances are sheet and plate.



Figure 2.1. General comparison of thickness of thin films with some other materials. (Source: https://www.susumu.co.jp/germany/tech/know\_how\_02.php)

There are several thin film deposition techniques. Basically, there are three main categories which are physical deposition methods (PDM), chemical deposition methods (CDM), and solution based chemistry (Gould et al., 2017). PDM is generally called as physical vapor deposition techniques and requires vacuum conditions. First, the material used to generate the desired thin film becomes vapor, and then, it becomes solid due to condensation. Thus, the substrate is coated with the desired thin film. Thermal evaporation, pulsed laser deposition, molecular beam epitaxy, magnetron sputtering are the techniques that belong to the category of physical vapor deposition. CDM is also called as chemical vapor deposition process, the precursor is heated until it becomes gaseous. After that, the substrate surface and the hot gaseous precursor start to react. This reaction leads thin film to be produced. Chemical vapor deposition (CVD) techniques are divided into sub-categories which are metal organic CVD, low pressure CVD, atmospheric pressure CVD, and plasma enhanced CVD. In the category of solution based chemistry, chemical solutions are used to generate a variety of films such as thick films,

thin films, multilayered films, porous films, and dense films (Ukoba et al., 2018). Solution based chemistry includes the techniques of chemical bath deposition, sol-gel, spin coating, dip coating, screen printing, and spray pyrolysis.

#### 2.3. Thin Film Biosensors

There is a widespread usage of thin films in biosensors nowadays. The electrodes of the biosensors are modified with thin films. These thin film-modified electrodes become like a transducer having physicochemical properties in terms of electrical, magnetic, mechanical, and optical properties. Thus, the signal obtained as a result of the analyte-electrode interaction can be converted into a suitable signal that can be measured (Koydemir et al., 2013; Tvarozek et al., 1998). Gold (Au) is a common material used as an electrode or a transducer for sensors. Gold thin films provide high surface-to-volume ratio, good catalytic properties, amplification of electrochemical response, stability, and biocompatibility (Sharma et al., 2015). Gold thin films are favorable for enzymatic biosensors because they strongly bind to biomolecules and immobilize them on the surface covalently. Also, In<sub>2</sub>O<sub>3</sub>:SnO<sub>2</sub> (ITO) has a frequent usage in sensors since it has some preferred properties such as that it is electrically conductive, optically transparent, and colorless as a thin film (Pruna et al., 2017).

Other thin film materials for biosensor production includes metals such as platinum (Pt), silver (Ag), aluminum (Al), and titanium (Ti); allotropes of carbon such as diamond and graphene; polymers such as polyethylene (PE), Parylene C, polyimide (PI), PDMS, polypropylene (PP), and polymethylmethacrylate (PMMA); and metal oxides such as ZnO, NiO, titanium dioxide (TiO<sub>2</sub>); and transparent conductive oxides such as ITO (Koydemir et al., 2013). Images of the patterned thin film gold and platinum electrodes can be seen as an example, in Figure 2.2. If there is a requirement for a biocompatible material, some of the polymers such as Parylene C, some of the metal oxides such as TiO<sub>2</sub>, and ultra-nanocrystalline diamond will be suitable for this purpose. The biosensors made from biocompatible materials can be used for several purposes such as wearable devices and drug delivery applications. Also, some of the metals such as Au and Ti are the biocompatible materials. Diamond and graphene are the other materials used in biosensors because of their beneficial properties. Diamond has strength, good electrochemical activity, and thermal conductivity while graphene has high surface-to-

volume ratio, cost effectiveness, and simple processing. Chemical vapor deposition is an appropriate deposition technique to produce diamond thin films. If polymers are selected for biosensor fabrication, there will be a requirement of channeled/porous structure, and hydrophobicity or hydrophilicity. Also, polymers can increase specificity since they provide to eliminate the unwanted interactions between the analytes and surface of the material.



Figure 2.2. Images of the thin film biosensors. A) Thin film gold electrode. B) Thin film platinum electrode. (Source: https://www.micruxfluidic.com/en/electrochemical-solutions/thin-film-electrochemical-sensors/single-electrodes-se/)

In this work, Zn<sub>2</sub>SnO<sub>4</sub> (ZTO) thin film was deposited onto ITO thin film to prepare an electrode for biosensing applications. ZTO thin film was deposited with DC magnetron sputtering technique, by using a Zn<sub>2</sub>Sn (ZT) target and O<sub>2</sub> gas. ZTO thin film is an amorphous and dielectric material with a low conductivity and good transparency, while ITO thin film is electrically conductive, optically transparent, colorless, and has low sheet resistance (Ekmekcioglu et al., 2021; Demirhan et al., 2020). Recently, interest in semiconductor materials has been increasing. There are many studies in the literature about ZTO thin films, which is also the one of the semiconductor materials. ZTO has remarkable optical and electrical properties. Thanks to these features, ZTO is used in areas such as solar cells, lithium-ion batteries, photocatalyst materials, and gas sensors (Kim et al., 2011; Winkler et al., 2012; Kim et al., 2015; Jacob et al., 2020).

#### **2.4. Magnetron Sputtering**

Magnetron sputtering is one of the subcategory techniques of physical vapor deposition (PVD). It is an effective and economical technique. A substrate or a surface

such as glass, textile, polyester film, or solid polycarbonate sheet can be coated with metals, conductive oxides, insulating oxides, and alloys in the desired thickness. The coated surface can be either rigid or flexible. In general, the maximum thickness that magnetron sputter can deposit is 5  $\mu$ m (Hassan, 2017).



Figure 2.3. Illustration of the inside of the magnetron sputter coating chamber. (Source: Hassan, 2017)

Magnetron sputtering coatings are done under vacuum conditions in a chamber. Rough pump (RP) and turbo molecular pump (TMP) are used to provide high vacuum. In Figure 2.3, inside of the vacuum chamber can be seen. The desired type of target is placed in front of the magnets. The target which will be deposited on the surface of a substrate can be a metal or an alloy, in general. During the deposition process, a required amount of inert gas, which is usually argon or helium, is introduced into the magnetron sputtering system. Also, a required amount of power is supplied to the system via DC (Direct Current) or RF (Radio Frequency) power supply. The energetic ions of the gases collide to the target and detach the atoms from the surface of the target, and these atoms are deposited on the surface of the substrate (Hassan, 2017). Plasma is formed during this coating process. Moreover, cooling is applied with water because heat of the target increases while the deposition continues. As a result, a thin film is produced on the substrate surface. Magnetron sputtering is advantageous compared to conventional sputtering technique which can be called as "normal" sputtering. The working principle of normal sputtering is the same as magnetron sputtering, except magnetic field is not used during the coating process in normal sputtering. As a result of this, thin film deposition rates of normal sputtering are very low. For instance, when there is a requirement for 1  $\mu$ m or more than 1  $\mu$ m thin film thickness, the deposition process will continue for several days or weeks, which is a very long duration. Therefore, normal sputtering has been developed to overcome this issue, and its sophisticated version which is magnetron sputtering has been used for approximately more than 50 years (Braun, 2015).

#### 2.5. Dopamine

Dopamine is a significant neurotransmitter in the mammalian brain (Ashton, 2018). Molecular formula of the dopamine is C<sub>8</sub>H<sub>11</sub>NO<sub>2</sub>, and its structure can be seen in the Figure 2.4. Dopamine is an organic molecule consisting of a benzene ring with two hydroxyl groups and an amine group attached to an ethyl chain. Neurotransmitters are the chemical messengers in the brain that are synthesized from a neuron and attached to neurons or another cells, enabling the communication between the cells by carrying signals. After neurotransmitters are released from neurons, they travel between cells until they bind to receptor of another cell. Then, this message, carried by the neurotransmitter that binds to the receptor of the cell, is transmitted by this cell to neighboring cells (Breed & Moore, 2012).



Figure 2.4. Structure of the dopamine. (Source: https://chem.nlm.nih.gov/chemidplus/rn/51-61-6)

Different neurotransmitters are synthesized in each part of the brain. There are three parts in the brain where dopamine is synthesized mainly: the substantia nigra (SN), the ventral tegmental area (VTA), and the retrorubral field (RF). The SN, VTA, and RF are both located in the midbrain. The SN and VTA are also adjacent to each other. These three brain parts have a crucial role in movement, learning, and memory, which are related to cognitive and motor functions (Nair-Roberts et al., 2008).

Excessive or insufficient amounts of dopamine result in certain disorders such as Parkinson's disease, Alzheimer's disease, depression, and schizophrenia (Wolfe et al., 1990). Therefore, determination of abnormal levels of dopamine is significant for the diagnosis and treatment of these diseases. Dopamine levels in the body can be determined from the biological fluids such as urine, plasma, sweat, tear, and saliva.

There are several methods for detecting dopamine, including capillary electrophoresis, ELISA (enzyme-linked immunosorbent assay), HPLC (high performance liquid chromatography), mass spectroscopy, using fluorescent probes (such as quantum dots and organic dyes), and electrochemical analysis (Gong et al., 2020; Barth et al., 2006; De Benedetto et al., 2014; Carrera et al., 2007; Namkung et al., 2017; Nichkova et al., 2013; Wu et al., 2017; Wu et al., 2016).

Most of these detection techniques are time consuming, costly, and demanding. To overcome these problems, using electrochemical biosensors, which is a cost effective and faster method, is a favorable option. In this thesis, the single-use electrodes produced by deposition of ZTO thin film on ITO thin film were used as electrochemical biosensors to determine dopamine.

#### **2.6. X-Ray Diffraction (XRD)**

X-ray diffraction (XRD) is a non-destructive technique that is used to analyze the materials crystallographically. In other words, XRD measurement gives an information about whether an unknown material is crystalline or amorphous. In addition, the physical and chemical properties of the materials can be examined from XRD the measurement data (Sima et al., 2016). For instance, average grain size, strain, crystal defects, texture, and phases are some of the characteristics that can be understood from XRD measurement data (Bunaciu et al., 2015). XRD measurement can be done with a small amount or concentration of sample, and the sample is not damaged during the measurement. A wide variety of materials such as thin films, rocks, polymers, and crystallines can be examined by using XRD.

The working principle of XRD is based on refraction of X-rays in a specific order according to unique atomic arrangement of each crystalline phase. During the measurement, monochromatic X-ray beams scatter at particular angles from the sample that causes interferences. As a result of constructive and destructive interferences, XRD pattern is formed due to lower and higher intensities. If peaks are seen in a XRD graph, basically it can be said that this sample consists of crystalline structures. From the specific locations of peaks, the atomic arrangement of a sample can be identified, because XRD pattern is like a fingerprint for the substances (Kohli and Mittal, 2019).



Figure 2.5. Illustration of X-ray diffractometer instrumental setup. (Source: Hughes, 2008)

An X-ray diffractometer consists of three basic parts which are sample holder, Xray tube (X-ray source), and X-ray detector (Bunaciu et al., 2015). These parts can be seen in the Figure 2.5. As a result of heat application to a filament, X-rays are generated from the cathode tube, resulting in electrons being produced. After a voltage application, these electrons are directed to the target and bombard it. As a result of this bombardment, inner shell electrons are subtracted, and X-rays are generated and directed to the sample. Then, constructive interference happens. The X-ray detector converts the signal to a readable output. XRD graph is formed by using this readable data.

XRD is used in a wide variety of fields. Some of these fields are engineering, pharmaceuticals, physics, biology, electronics, and material science. In addition, the use of XRD in the fields such as glass industry, microelectronics, thin film coatings, forensic science, and pharmaceutical industry is significant when there is a requirement for identifying unknown materials.

#### 2.7. Cyclic Voltammetry (CV)

Cyclic voltammetry (CV) is a frequently used electrochemical measurement technique that measures current as a result of applied potential. The field of electrochemistry studies electron transfers in reactions. When chemical changes occur, electrons flow. In general, this chemical change occurs when a metal complex become oxidized or reduced. CV basically converts chemical information into electrical signal. One of the common uses of CV is to analyze the oxidation and reduction states of the materials (Wang et al., 2021). As a result of the data obtained from the CV measurement, a cyclic voltammogram is obtained (Figure 2.6). A cyclic voltammogram is plotted using the relationship between the applied potential and the resulting current flowing through the material being measured.



Figure 2.6. Representation of a standard cyclic voltammogram. (Source: https://www.comsol.com/blogs/analyzing-cyclic-voltammetry-at-a-microdisk-electrode-with-simulation/)

An electrochemical cell is often used in order to perform cyclic voltammetry (Figure 2.7). A standard three-electrode electrochemical cell setup consists of a working electrode, a counter electrode, and a reference electrode. During a CV measurement, the reference electrode is responsible for measuring the applied potential, and current flows through the working and counter electrodes.

On the surface of the working electrode, the electrochemical reactions to be measured occur. In this thesis, ITO, ZTO and ZTO/ITO electrodes were used as the working electrodes. During CV measurements, these electrodes were cycled until reaching a steady-state and stabilizing the cyclic voltammogram. Since the potential

applied to the electrochemical cell is measured by the reference electrode, it has a stable and good equilibrium potential. Counter electrode is like a complementary part of the CV system. In other words, counter electrode completes the electrical circuit. If oxidation occurs in working electrode, then reduction occurs in counter electrode and vice versa (Elgrishi et al., 2018).

It is significant to choose the appropriate electrolyte solution in CV measurement. Electrolyte solution consists of the solvent and supporting electrolyte which is a salt and dissolved in the solvent. Transfer of electrons occur as a result of redox reactions in CV measurement. The ion transfer in the solution provides the maintenance of electrical neutrality. The solvent must be stable during oxidation and reduction processes (Elgrishi et al., 2018). In this work, supporting electrolyte solution is  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  (potassium ferricyanide) containing 0.10 M KCl (potassium chloride).



Figure 2.7. Illustration of a standard three-electrode electrochemical cell setup. (Source: Elgrishi et al., 2018)

#### **2.8. Electrochemical Impedance Spectroscopy (EIS)**

Electrochemical Impedance Spectroscopy (EIS) is a sensitive and powerful electrochemical measurement technique. One of the usage areas of EIS is to analyze the electrodes electrochemically (Li et al., 2018). Electrochemical reactions occurred at the electrodes can be studied by applying the EIS method. In general, in order to measure the

electrochemical impedance, the current resulting from the application of AC (alternating current) potential is measured. EIS can be used in the fields such as electrode kinetics, photovoltaic systems, batteries, bioelectrochemistry, corrosion, and solid state electrochemistry.

Both CV and EIS use three-electrode electrochemical cell setup consisting of working electrode, reference electrode, and counter electrode. This electrochemical cell setup has been previously explained in the CV section, and Figure 2.7 shows the illustration of this setup.

EIS measurement data is commonly plotted in Nyquist plot (Figure 2.8). In a Nyquist plot, y-axis is negative. Also, low frequency values exist on the right side of the graph while high frequency values exist on the left side of the graph (Choi et al., 2020). Each point on the Nyquist diagram represents impedance at a frequency.

Although CV and EIS techniques look similar, they are different from each other. CV provides information about qualitative characteristics such as type of the reactions occurred, while EIS provides information about quantitative characteristics. For instance, EIS can be used for determining the exact rate of a reaction. Furthermore, in CV, the start and end potential values are determined and electrochemical reactions occur during the applied potential in this range. Then, a voltammogram is plotted using the data obtained by measuring the current produced in this process. On the other hand, different AC frequencies with a certain amplitude are applied during EIS measurement (Pajkossy, 2020).



Figure 2.8. Illustration of a Nyquist diagram. (Source: Choi et al., 2020)

#### 2.9. Differential Pulse Voltammetry (DPV)

Differential pulse voltammetry (DPV) is a sensitive voltammetric technique that is used for the electrochemical measurements. While performing DPV measurement, a base potential value is determined. DPV involves applying amplitude potential pulses on a linear ramp potential. A base potential value is selected at which is no faradaic reaction, and is also applied to the electrode (Simões & Xavier, 2017). This potential is increased equally between the pulses applied. The current is measured just before and immediately after the pulse is applied. The differential pulse voltammogram consists of these measured current differences and applied potentials. Peaks occur in the voltammogram due to the redox processes (Simões & Xavier, 2017).

Three-electrode electrochemical cell setup is also used for DPV measurements. As mentioned before, this electrochemical cell consists of working electrode, reference electrode, and counter electrode. The working principle of this three-electrode electrochemical cell has been previously explained in the CV section. Also, the illustration of this electrochemical cell can be seen in the Figure 2.7.

# **CHAPTER 3**

## **EXPERIMENTAL PROCEDURE**

#### 3.1. Deposition of ZTO Thin Films

In this study, ITO coated glass and uncoated glass were used as a substrate. Uncoated glass was basically used for two purposes. One of the purposes is to measure thickness of the thin films. The second purpose is to observe the differences between uncoated and ITO coated substrate use, and to compare them in terms of advantages or disadvantages. Same ZTO deposition procedure was applied with same parameters to both ITO coated and uncoated glasses. ITO coating procedure applied to glass was done with DC magnetron sputtering technique which was explained before (Chapter 2). The dimensions of the substrates were 75x25 mm. The schematic representation and real image of the DC magnetron sputtering system used for ZTO thin film deposition can be seen in the Figure 3.1 and Figure 3.2.



Figure 3.1. Image of the DC magnetron sputtering system.



Figure 3.2. Schematic representation of the DC magnetron sputtering system.

To prepare the substrates for the deposition process, they were cleaned with acetone in order to get rid of the unwanted materials such as dust. Unless uncoated substrates, a small area of the long edge of the ITO coated substrates were covered with aluminum foil. This process was done to obtain uncoated free ITO surface as an electrode for the electrochemical measurements. After that, the substrates with and without aluminum foil were placed in the center of the sample holder with the help of binder clips. The dimensions of the sample holder were 150x150 mm. The sample holder was placed into the chamber. The distance between the ITO coated substrates and the ZT target was adjusted to 7 cm. Rough pump and turbo molecular pump were used to obtain high vacuum conditions in the chamber. When  $8.0 \times 10^{-6}$  Torr was achieved as a base pressure, DC power supply was adjusted to 15 Watt and plasma was generated. The pre-sputtering process was done for 10 minutes in the presence of 30 sccm argon gas. The shutter staying above the target was kept closed during the pre-sputter process. Oxygen gas was released for 2-3 minutes after the pre-sputtering, in which plasma was still generated. The mass flow rate of the argon gas (30 sccm) was kept constant for all deposition processes while the mass flow rate of the oxygen gas was adjusted to different values for each deposition process (Table 3.1). Therefore, the working pressure values changed according to the mass flow rate of the oxygen. For the deposition process, the shutter was opened, and ZTO coating was applied for the required time. The durations of ZTO coatings were adjusted to the desired time for each deposition process.

Sample	Working Pressure (Torr)	Ar (sccm)	O <sub>2</sub> (sccm)	Power (W)	Deposition Time (min)
ZTO-0	1.5x10 <sup>-3</sup>	30	5.0	15	3
ZTO-1	1.6x10 <sup>-3</sup>	30	7.5	15	3
ZTO-2	1.6x10 <sup>-3</sup>	30	10.0	15	3
ZTO-3	1.7x10 <sup>-3</sup>	30	12.5	15	3

Table 3.1. Deposition parameters of ZTO thin films.

As a result of ZTO deposition on ITO coated and uncoated glasses, ZTO/ITO electrodes and ZTO samples were produced. Mass flow rate of the oxygen gas were adjusted to 7.5 sccm, 10 sccm, and 12.5 sccm for different thin film deposition processes. Therefore, according to oxygen concentration, three types of ZTO thin films were produced. ZTO/ITO electrodes were cut by a diamond cutter. The reduced size of each electrode was of 5x25 mm (Figure 3.3). Electrochemical measurements were done with these small samples.



Figure 3.3. Images of the samples after the cutting process. (A) ITO substrates. (B) ZTO/ITO electrodes.

### 3.2. Characterization of ZTO Thin Films

Physical, optical, and electrochemical characterizations of the bare ITO, ZTO, and ZTO/ITO electrodes were done after the ZTO thin film deposition by DC magnetron sputtering.

#### 3.2.1. Thickness Measurement

Thickness of the ZTO films depends on both thin film deposition time and concentration of the oxygen gas sent during the deposition. For thickness measurement, before the deposition process, a few drops of photoresist were dropped to different areas of the substrate surface (Figure 3.4). After the thin film deposition, the photoresist droplets were removed with acetone. As a result, small uncoated areas were obtained as well as large areas coated with ZTO.



Figure 3.4. Image of the uncoated glass substrate with three drops of photoresist.



Figure 3.5. Image of the profilometer at Physics Department.

Thickness measurements of ZTO thin films were determined by using a surface profilometer. The image of the profilometer can be seen in Figure 3.5. The instrument used for this purpose was Veeco DEKTAK 150 Profilometer. This surface profilometer has a diamond tipped stylus. This stylus moves in a straight line between coated and uncoated areas in order to determine the thickness of the films. The thickness values of ZTO films were obtained by applying this technique. The measurement results can be seen in Chapter 4.

#### **3.2.2.** Optical Transmission

Optical transmission measurements of ZTO and ZTO/ITO thin films were done by a double beam UV-VIS-NIR Perkin Elmer Lambda 950 spectrophotometer. During the measurements, the range of wavelength was adjusted to 2600 nm to 200 nm, data interval was 4 nm, and scanning speed was 993.61 nm/min. The permeability of ZTO and ITO/ZTO films was examined in Chapter 4 according to the results obtained.

#### **3.2.3. X-Ray Diffraction (XRD)**

Structure analysis of the ZTO and ZTO/ITO samples were performed by applying X-Ray Diffraction (XRD) with the device of Philips X'Pert. This X-Ray diffractometer has copper K- $\alpha$  radiation ( $\lambda$ =0.154 Å). The XRD data were recorded between 10 < 2 $\theta$  < 90. The working principle of XRD is explained in Chapter 2.

#### **3.2.4. Scanning Electron Microscopy (SEM)**

Surface morphology of the ITO, ZTO, and ZTO/ITO samples were determined by applying Scanning Electron Microscopy (SEM) with the device of FEI QUANTA 250 FEG at 15 kV. The SEM analysis was done before the surface modification of the samples.

#### **3.3. Process of Sonication and APTES Modification of the Electrodes**

ZTO/ITO and ITO electrodes which were cut to 5x20 mm size with a diamond cutter were sonicated in acetone for 10 minutes, in 0.1 M NaOH for 5 minutes, and in deionized water (DI water) for 15 minutes, respectively. Then, the electrodes were left to dry at 25°C during 15 minutes.

After then, the ITO and the ZTO/ITO electrodes were kept in APTES solution (1%, 2%, and 5%) prepared in acetone for 1 hour at 25°C. Then, the electrodes were washed with acetone and distilled water (dH<sub>2</sub>O), and they were left to dry at 25°C during

15 minutes. Thus, APTES-modified electrodes: APTES/ITO and APTES/ZTO/ITO were ready to use.

#### **3.4. Electrochemical Characterization of Electrodes**

After the sonication and APTES modification processes, electrochemical properties of the modified ZTO/ITO electrodes were analyzed by conducting electrochemical measurements with CV, EIS, and DPV methods.

#### **3.4.1.** Cyclic Voltammetry (CV)

After the sonication and modification step, cyclic voltammetry measurements were conducted. CV measurements were performed in a standard three-electrode electrochemical cell. The reference electrode was Ag/AgCl, and the counter electrode was platinum wire. The supporting electrolyte solution is 5 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  (1:1) containing 0.10 M KCl.

#### **3.4.2. Electrochemical Impedance Spectroscopy (EIS)**

EIS measurements were also performed in a standard three-electrode electrochemical cell. The reference electrode was Ag/AgCl, and the counter electrode was platinum wire. The supporting electrolyte solution is 5 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  (1:1) containing 0.10 M KCl.

#### **3.5.** Voltammetric Detection of Dopamine

After the surface of the ZTO/ITO electrodes were modified with APTES, voltammetric detection of dopamine (DA) was performed. Firstly, DA solutions in different concentrations were prepared by using phosphate-buffered saline solution (PBS; 0.05 M with pH 7.0). The solutions of DA were prepared varying from 0.1  $\mu$ M to 3  $\mu$ M. The electrodes modified with APTES were then immersed into the dopamine solution and kept for a while. Then, differential pulse voltammetry (DPV) technique was applied

in order to measure the dopamine oxidation signal. Voltammetric measurement was performed in the electrochemical cell containing a standard three-electrode system: APTES-modified ZTO/ITO electrode as working electrode, the reference electrode as Ag/AgCl and the counter electrode as platinum wire.

Selectivity of electrodes was tested in the presence of many substances such as, ascorbic acid (AA), uric acid (UA) as well as bovine serum albumin (BSA) and fish sperm DNA (fsDNA). The stock solution of AA and UA was prepared with 0.05 M PBS (pH 7.0). BSA solution was prepared with 0.05 M acetate buffer solution (ABS with pH 4.8), and it was diluted by using 0.05 M PBS (pH 7.0). fsDNA solution was prepared with ultra pure water and it was diluted with 0.05 M PBS (pH 7.0).

The dopamine solution was prepared fresh daily by using 0.05 M PBS (pH 7.0) and kept at +4°C protected from the light.

Other chemicals were in analytical reagent grade and were purchased from Sigma and Merck. Ultra pure and deionized water were used in all solutions. Milli-Q ultra-pure water was used to prepare all solutions freshly.

## **CHAPTER 4**

### **RESULTS AND DISCUSSION**

#### 4.1. Thickness Measurement

In this work, ZTO thin films with different rates of oxygen mass flow were deposited onto ITO substrates. As a physical property, thickness of these ZTO thin films were determined by using a surface profilometer. In Table 4.1 and Table 4.2, the thickness differences between ZTO thin film samples are shown. The thickness measurement values of all ZTO samples are under 100 nm. Also, the thickness of ITO thin film used as ITO substrate is 200 nm.

Sample	Deposition Time (min)	Ar (sccm)	O <sub>2</sub> (sccm)	Thickness (nm)
ZTO-0	3.0	30	5.0	76
ZTO-1	3.0	30	7.5	68
ZTO-2	3.0	30	10.0	63
ZTO-3	3.0	30	12.5	53

Table 4.1. Data obtained from thickness measurement of ZTO thin films.

There is not a significant difference in thickness between the samples, because oxygen content of the samples is very close to each other. In Table 4.1, ZTO samples containing 5 sccm, 7.5 sccm, 10 sccm, and 12.5 sccm O<sub>2</sub> are compared with each other. The thickness measurement of the ZTO-0 (the sample with 5 sccm O<sub>2</sub>) is included in the table for comparison purposes only. The deposition time of these samples are same, which is 3 minutes. It is observed from the table that thickness decreases as oxygen content increases. The thickest sample is ZTO-0 (containing 5 sccm O<sub>2</sub>) while the thinnest sample is ZTO-3 (containing 12.5 sccm O<sub>2</sub>). In Table 4.2, ZTO samples containing 7.5 sccm O<sub>2</sub>

are compared with each other. In this case, deposition times of the samples are different which are 3 minutes, 4 minutes, and 5 minutes. Parameters of ZTO-4 and ZTO-5 were not used for the production of electrodes, their measurements were made with the profilometer to see the thickness difference due to the change in the deposition time while the oxygen concentration was constant. Although their thickness is almost the same, the thickest sample is ZTO-5 (5 minutes) while the thinnest sample is ZTO-1 (3 minutes) as expected.

Sample	Deposition Time (min)	Ar (sccm)	O <sub>2</sub> (sccm)	Thickness (nm)
ZTO-1	3.0	30	7.5	68
ZTO-4	4.0	30	7.5	72
ZTO-5	5.0	30	7.5	78

Table 4.2. Data obtained from thickness measurement of ZTO thin films deposited in the presence of 7.5 sccm O<sub>2</sub>.

#### 4.2. Optical Transmission

ITO and ZTO used in this study are both transparent oxides. Optical properties of the samples were investigated by applying optical transmittance measurement. This measurement was made between 200-2600 nm wavelength. This range covers both the visible region (400 nm - 700 nm) and part of the infrared region (700 nm - 1 mm).

Figure 4.1 shows the changes in optical transmittance with the change of oxygen content in ZTO thin films. In these samples, deposition times are same while oxygen content varies from 7.5 sccm to 12.5 sccm. Also, the optical transmission data of the uncoated substrate is available in the graph. Table 4.3 indicates the optical transmission values at 550 nm, which is in the visible range. Although there is no significant difference between the ZTO samples, according to the table and the graph, the most transparent sample is ZTO-3 (with 12.5 sccm O<sub>2</sub>) while the least transparent sample is ZTO-1 (with 7.5 sccm O<sub>2</sub>) at 550 nm. It is understood that optical transmittance increases as oxygen concentration increases in ZTO samples. In addition, the uncoated substrate is 91.87%
optically transparent while the optical transmittance of ZTO samples is between 74-80%, in the visible range.

Figure 4.2 shows the changes in optical transmittance with the change of deposition time in ZTO thin films. The oxygen concentration of the samples in this graph are same however, deposition times are not same. At 550 nm (Table 4.3), optical transmittance values of the samples are very similar, which is an expected result because deposition time difference between each sample is only 1 minute. Additionally, the most transparent sample is ZTO-5 (5 minutes deposition). Furthermore, in terms of optical transmittance, the uncoated substrate is 91.87% transparent while the ZTO samples are 72-75% transparent. Due to the lower oxygen concentration applied during deposition, the optical transmittance values of ZTO-4 and ZTO-5 at 550 nm are lower than those of ZTO-2 and ZTO-3.

The last two optical transmission graphs were plotted with the measurement data obtained from ZTO/ITO samples. In Figure 4.3, the graph shows the comparison between the uncoated substrate, ITO coated substrate, and ZTO/ITO samples with different oxygen concentrations in ZTO. Optical transmission values at 550 nm are 77.69% for ITO substrate, 75.18% for ZTO/ITO-1, 84.10% for ZTO/ITO-2, and 85.28% for ZTO/ITO-3. The optical transmittance value of ZTO/ITO-1 is lower than that of ITO, because ZTO/ITO-1 has more metallic property due to the ZTO deposition process with lower oxygen concentration. The transmission values of the ZTO/ITO-2 and ZTO/ITO-3 samples are higher than the ITO sample because ZTO can absorb more light than ITO.

Lastly, the graph in the Figure 4.4 provides to observe the optical transmission differences of uncoated substrate, ITO substrate, and ZTO/ITO samples with different deposition times. Optical transmission values at 550 nm are 77.69% for ITO substrate, 75.18% for ZTO/ITO-1, 69.26% for ZTO/ITO-4, and 78.47% for ZTO/ITO-5. Compared to the ITO substrate, optical transmission of both ZTO/ITO-1 and ZTO/ITO-4 has lower values, because their metallic properties are more dominant due to low oxygen concentration of ZTO. Unlike ZTO/ITO-1 and ZTO/ITO-4 samples, the optical transmission value of ZTO/ITO-5 (5 min ZTO deposition) is slightly higher than that of the ITO substrate. The reason for this small difference is that the ZTO deposition time in ZTO/ITO-5 is longer than in other samples, despite the low oxygen concentration (7.5 sccm O<sub>2</sub>).



Figure 4.1. Optical transmission graph of the ZTO samples deposited with different mass flow rates of O<sub>2</sub>.



Figure 4.2. Optical transmission graph of the ZTO samples deposited at different times with 7.5 sccm O<sub>2</sub>.



Figure 4.3. Optical transmission graph of the ZTO/ITO samples deposited with different mass flow rates of O<sub>2</sub>.



Figure 4.4. Optical transmission graph of the ZTO/ITO samples deposited at different times with 7.5 sccm O<sub>2</sub>.

According to the test results obtained by using spectrophotometer, ZTO/ITO electrodes have good optical transmittance properties. This feature will be useful in the future when the electrodes are further developed for use in biosensor applications. Wearable and flexible biosensor production can be given as an example for the future development of the biosensor. Moreover, the good optical transmittance properties of ZTO/ITO electrodes can be useful for observing how the analyte moves through the biosensor with the human eye or microscope.

Sample	Deposition Time (min)	O <sub>2</sub> (sccm)	Optical Transmission at 550 nm (%)
Glass (Uncoated)			91.87
ITO			77.69
ZTO-1	3.0	7.5	74.13
ZTO-2	3.0	10.0	78.85
ZTO-3	3.0	12.5	79.43
ZTO-1	3.0	7.5	74.13
ZTO-4	4.0	7.5	72.66
ZTO-5	5.0	7.5	74.49
ZTO/ITO-1	3.0	7.5	75.18
ZTO/ITO-2	3.0	10.0	84.10
ZTO/ITO-3	3.0	12.5	85.28
ZTO/ITO-1	3.0	7.5	75.18
ZTO/ITO-4	4.0	7.5	69.26
ZTO/ITO-5	5.0	7.5	78.47

Table 4.3. Optical transmission values of ZTO and ZTO/ITO samples at 550 nm.

In addition, as it can be seen in Figure 4.5, ZTO/ITO electrodes with different tones of yellowish color were obtained as a result of different oxygen concentrations given during ZTO depositions. The darkest-colored ZTO/ITO electrode was obtained using 7.5 sccm of oxygen, while the lightest-colored electrode was obtained using 12.5 sccm of oxygen. Therefore, it can be said that the higher oxygen concentration given to the chamber, the more transparent resulting ZTO thin films, as long as the deposition times are the same.



Figure 4.5. Image of the ZTO/ITO electrodes. The oxygen concentrations of ZTO thin films during deposition are 7.5 sccm, 10 sccm, and 12.5 sccm (from left to right).

### **4.3. X-Ray Diffraction (XRD)**

XRD analysis of the ZTO and ZTO/ITO samples were done in order to determine whether the thin films in these samples were crystalline or amorphous. Figure 4.6 and Figure 4.7 show the data obtained as a result of XRD measurement of the ZTO samples while Figure 4.8 is related to the XRD results obtained from ZTO/ITO samples.

XRD patterns of bare ITO and ZTO/ITO samples can be seen in Figure 4.8. Since there are several peaks at specific positions, it is understood that ITO thin film has polycrystal structure. The peaks are marked on the XRD pattern. These peaks approximately at 21.6°, 30.6°, 35.5°, 37.8°, 50.6°, and 60.7° corresponds to planes of (211), (222), (400), (411), (440), and (622), respectively (Tuna et al., 2010; Erdogan et al., 2020; Ozbay et al., 2020; Koseoglu et al., 2015). These peaks indicates that ITO thin films are crystallize in a cubic structure (Prepelita et al., 2017). According to the XRD pattern, the preferential growth of the ITO thin film is the (400) orientation. This depends on the thin film deposition conditions (Ma et al., 1995).



Figure 4.6. XRD graph of ZTO samples deposited with different mass flow rates of O<sub>2</sub>.



Figure 4.7. XRD graph of ZTO samples deposited at different times with 7.5 sccm O<sub>2</sub>.



Figure 4.8. XRD graph of the ZTO/ITO samples deposited at different times with 7.5 sccm O<sub>2</sub>.

Since ZTO has amorphous structure, it is seen that there are only peaks belongs to ITO in the XRD patterns of ZTO/ITO samples. This confirms that there are ITO thin films underneath the ZTO thin films. In addition, it is observed that intensities of some peaks become smaller in the XRD pattern of the ZTO/ITO samples when compared to XRD pattern of ITO. This situation occurs when X-rays pass through the thin films and hit the substrate, if more than enough power is applied.

#### 4.4. Scanning Electron Microscopy (SEM)

Surface morphologies of the ZTO and ZTO/ITO samples were examined by performing SEM analysis. Since the SEM images of the samples are similar, only a few images were selected. The selected images belong to ITO, ZTO, and ZTO/ITO samples. When the SEM images below are examined, morphological differences between ITO and ZTO thin films can be seen.



Figure 4.9. SEM image of ITO.

Figure 4.9 shows SEM image of the ITO thin film surface. The grains forming the thin film are shown in the image. It is observed that size distribution of ITO thin film is uniform and there are no cracks throughout the film. This indicates that ITO thin film has good surface morphology. In addition, this SEM image is similar to the SEM images of ITO thin films in the literature (Heo et al., 2014; Jadhavar et al., 2015).



Figure 4.10. SEM image of ZTO (7.5 sccm O<sub>2</sub>).



Figure 4.11. SEM image of ZTO/ITO (10 sccm O<sub>2</sub>).

Surface morphology of ZTO thin film is shown in both Figure 4.10 and Figure 4.11. The SEM analysis showed that both ZTO and ZTO/ITO samples have almost identical morphology. According to the SEM images, it can be said that ZTO thin films have small grains. Also, ZTO thin films are uniform and lack of cracks. In other words, ZTO thin films have continuous surface morphology. This provides an uninterrupted flow of electric current. Moreover, this SEM images are similar to the SEM images of ZTO thin films in the literature (Han et al., 2015).

Consequently, XRD and SEM analysis results of ITO, ZTO, and ZTO/ITO thin films are consistent with each other. All of these measurement results confirm that the structure of the ZTO thin film is amorphous while the structure of the ITO thin film is crystalline.

### 4.5. Cyclic Voltammetry (CV)

CV experiments were performed for observing the electrochemical properties of the ZTO/ITO electrodes. Among the ZTO/ITO electrodes, ZTO thin film electrodes produced using 7.5 sccm O<sub>2</sub> were used in our study since the highest peak current was recorded by using these electrodes in combination with CV technique (Figure 4.12).



Figure 4.12. Cyclic voltammograms obtained by (a) ITO, (b) ZTO/ITO (7.5 sccm O<sub>2</sub>),(c) ZTO/ITO (10.0 sccm O<sub>2</sub>), (d) ZTO/ITO (12.5 sccm O<sub>2</sub>).

In order to apply these electrodes for voltammetric detection of dopamine, as we explained, first the electrodes were sonicated in acetone, NaOH, and DI water, respectively. After then, the electrodes were let to dried for a required time. APTES modification was then followed by using APTES in the concentrations of 1%, 2%, and 5% of APTES.



Figure 4.13. Cyclic voltammograms obtained by (a) ITO, (b) ZTO/ITO, (c) sonicated ZTO/ITO.

There are specific symbols shown in the Tables 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10,

- 4.11. The explanations of these specific symbols are given below:
- Ia: Anodic current
- Ic: Cathodic current
- Q<sub>a</sub>: Anodic charge
- Qc: Cathodic charge

Electrodes	I <sub>a</sub> (µA)	Q <sub>a</sub> (mC)	I <sub>c</sub> (μA)	Q <sub>c</sub> (mC)
ΙΤΟ	194.71 ± 19.70	$1.55 \pm 0.07$	$\begin{array}{r} 153.65 \pm \\ 35.89 \end{array}$	$1.45 \pm 0.21$
ZTO/ITO	$\begin{array}{c} 26.95 \pm \\ 31.20 \end{array}$	$0.39 \pm 0.30$	$\begin{array}{c} 16.95 \pm \\ 22.01 \end{array}$	$0.44 \pm 0.31$
Sonicated ZTO/ITO	71.47 ± 23.32	$0.60 \pm 0.08$	67.04 ± 22.25	$0.69 \pm 0.13$

Table 4.4. The data obtained by using CV measurements of ITO, ZTO/ITO, and sonicated ZTO/ITO electrodes. The number of experiments (n) was 3.

Figure 4.13 shows the CV curves obtained by ITO, ZTO/ITO, and sonicated ZTO/ITO. It was observed that there was a decrease at the peak current value from ITO to ZTO/ITO. The highest anodic peak current (I<sub>a</sub>) was measured as 194.71  $\pm$  19.70 µA (RSD%, 10.12%, n=3) using ITO electrode. On the other hand, the lowest I<sub>a</sub> was measured as 26.95  $\pm$  31.20 µA (RSD%, 115.79%, n=3) by ZTO/ITO electrodes (Table 4.4). The reason for this decrease can be explained by the fact that ITO is a conductor while ZTO is a dielectric material with a poor electrical conductivity. As ZTO is a dielectric, it has a high sheet resistance by resulting a decrease at current value. Then again, there is an increase at the peak current value from ZTO/ITO electrode to sonicated ZTO/ITO electrode. After the surface of ZTO/ITO electrodes was modified with sonication, the I<sub>a</sub> was measured as 71.47  $\pm$  23.32 µA (RSD%, 32.63%, n=3). Also, the relative standard deviation (RSD%) of ZTO/ITO electrodes were decrease after the sonication, from 115.79% to 32.63%, which was a good and expected decrease (Table 4.4). In a study in the literature, it was shown that ultrasonic irradiation causes an increase in the rate of charge transfer reactions in both anodic and cathodic reactions (Suliman et

al., 2006). Thus, the capacitance of the double layer at the metal-electrolyte interface increased due to sonication. It is known that if the capacitance increases, the current also increases, and vice versa (Campbell et al., 1998). In other words, current is proportional to capacitance. In conclusion, the increase in the peak current and decrease in the RSD% of the sonicated ZTO/ITO electrodes were due to the sonication process applied to the ZTO/ITO electrodes.



Figure 4.14. (A) Cyclic voltammograms obtained by (a) sonicated ZTO/ITO, (b) 1%
APTES-modified ZTO/ITO, (c) 2% APTES-modified ZTO/ITO, (d) 5%
APTES-modified ZTO/ITO. (B) Direct comparison of the CV curves obtained by sonicated ZTO/ITO and 2% APTES and modified ZTO/ITO.

Figure 4.14A shows CV curves obtained by sonicated ZTO/ITO electrodes, and APTES-modified ZTO/ITO electrodes in different ratios of APTES: 1%, 2% and 5%. According to the data (Figure 4.14A and Table 4.5) it was found that APTES modification

makes ZTO/ITO electrodes more conductive. The optimum APTES ratio was chosen as 2 % according to the CV data. It was observed that the peak current values of sonicated ZTO/ITO and 2% APTES-modified ZTO/ITO were higher than 1% APTES-modified ZTO/ITO. In Table 4.5, the average anodic peak current of sonicated ZTO/ITO was measured as 71477.00  $\pm$  23.32  $\mu$ A (RSD%, 32.63%, n=3), the one of 2% APTESmodified ZTO/ITO was measured as  $116892.83 \pm 31.01 \mu A$  (RSD%, 26.53%, n=3), and the one of 1% APTES-modified ZTO/ITO was measured as  $66065.75 \pm 36.13 \mu A$ (RSD%, 54.69%, n=3). Although there is an increase in the average peak current of 5% APTES-modified ZTO/ITO (197354.50  $\pm$  181.35  $\mu$ A with the RSD%, 91.89%, n=3), 2% APTES-modified ZTO/ITO electrode was selected for our further experiments due to the better reproducibility of 2% APTES-modified electrode. A direct comparison of the CV curves of sonicated ZTO/ITO and 2% APTES modified-ZTO/ITO can be seen in the Figure 4.14B. The reason at the increase in peak current could be explained that 2% APTES modification provides a higher surface area comparison to 1% APTES modification at electrode surface. Miecznikowski and Cox showed also that there was an increase at surface area as a result of APTES modification on the ITO surface by using SEM images. Also, adsorption of APTES allows electrons to bind to the electrode surface (Miecznikowski & Cox, 2020). There is an easy electron transfer between the positively charged amino group and the negatively charged [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> (Zhao et al., 2021). Therefore, it can be concluded that ZTO/ITO electrodes modified with 2% APTES are becoming more conductive and yield a high surface area that is suitable for dopamine binding.

Electrodes	I <sub>a</sub> (µA)	Q <sub>a</sub> (mC)	I <sub>c</sub> (μA)	Q <sub>c</sub> (mC)
Sonicated ZTO/ITO	$71477.00 \pm 23.32$	$0.60 \pm 0.08$	67.04 ± 22.25	$0.69 \pm 0.13$
1% APTES-modified ZTO/ITO	$66065.75 \pm 36.13$	$0.54\pm0.24$	62.75 ± 34.25	$0.70 \pm 0.34$
2% APTES-modified ZTO/ITO	$116892.83 \\ \pm 31.01$	$1.03 \pm 0.49$	$\begin{array}{r} 110.41 \pm \\ 32.33 \end{array}$	$1.15 \pm 0.41$
5% APTES-modified ZTO/ITO	$197354.50 \\ \pm 181.35$	$1.30 \pm 0.99$	164.89 ± 133.61	$1.50 \pm 0.42$

Table 4.5. The data obtained by using CV measurements of sonicated ZTO/ITO and APTES-modified ZTO/ITO electrodes. The number of experiments (n) was 3.

In solvent-based silanization, solution concentration affects the APTES growth on surface and morphology of APTES. Silanization is an effective method used for the surface modification of the materials (Boccafoschi et al., 2018). Surface of metal oxides can be silanized, and APTES is one of the silane-coupling agents. It is known that APTES can bind to oxidized substrates. Sonication with NaOH activates the hydroxyl groups on the electrode surface. This hydroxylated surface of the ZTO reacts with APTES and as a result, amine groups are introduced to the ZTO surface. However, the head and tail parts of the APTES molecule tend to interact with each other, causing APTES to form either monolayers or multiple layers on a surface. In other words, APTES has the ability of selfpolymerization (Arnfinnsdottir et al., 2020). Kim and Kwon showed that if the APTES concentration is more or less than sufficient, aggregation forms on the electrode surface due to the self-polymerization of APTES film can be obtained by using an appropriate APTES ratio. Thus, the optimum APTES% is chosen as 2% in our study.



Figure 4.15. Cyclic voltammograms obtained by (A) ITO electrodes: (a) ITO, (b) sonicated ITO, (c) 2% APTES-modified ITO; (B) ZTO/ITO electrodes: (a) ZTO/ITO, (b) sonicated ZTO/ITO, (c) 2% APTES-modified ZTO/ITO.

Table 4.6. The data obtained by using CV measurements of ITO, sonicated ITO, and ITO electrodes modified with 2% APTES. The number of experiments (n) was 3.

Electrodes	<b>Ι</b> <sub>a</sub> (μΑ)	Q <sub>a</sub> (mC)	Ι <sub>c</sub> (μΑ)	Qc (mC)
ІТО	194.71 ± 19.70	$1.55\pm0.07$	$\begin{array}{r} 153.65 \pm \\ 35.89 \end{array}$	$1.45\pm0.21$
Sonicated ITO	443.47 ± 12.15	$2.70\pm0.21$	427.95 ± 7.27	$2.70\pm0.21$
2% APTES-modified ITO	$\begin{array}{r} 462.02 \pm \\ 47.10 \end{array}$	$2.75\pm0.00$	$\begin{array}{r} 455.94 \pm \\ 43.69 \end{array}$	$2.85\pm0.21$

Table 4.7. The data obtained by using CV measurements of ZTO/ITO, sonicated ZTO/ITO, and ZTO/ITO electrodes modified with 2% APTES. The number of experiments (n) was 3.

Electrodes	I <sub>a</sub> (μA)	Q <sub>a</sub> (mC)	Ι <sub>c</sub> (μΑ)	Qc (mC)
ZTO/ITO	$\begin{array}{r} 26.95 \pm \\ 31.20 \end{array}$	$0.39\pm0.30$	$\begin{array}{c} 16.94 \pm \\ 22.01 \end{array}$	$0.44 \pm 0.31$
Sonicated ZTO/ITO	71.47 ± 23.32	$0.60\pm0.08$	67.04 ± 22.25	$0.69\pm0.13$
2% APTES-modified ZTO/ITO	$116.89 \pm 31.01$	$1.03 \pm 0.49$	$110.41 \pm 32.33$	$1.15 \pm 0.41$

The voltammograms obtained by using ITO, sonicated ITO, and 2% APTESmodified ITO can be seen in the Figure 4.15A, while the voltammograms obtained by using ZTO/ITO, sonicated ZTO/ITO, and 2% APTES-modified ZTO/ITO can be seen in the Figure 4.15B. The average values of current measured by each of ITO electrode in respect to from high to low, i.e: 2% APTES-modified ITO ( $462.02 \pm 47.10 \mu$ A with the RSD%, 10.20%, n=3), sonicated ITO ( $443.47 \pm 12.15 \mu$ A with the RSD%, 2.74%, n=3), and ITO ( $194.71 \pm 19.70 \mu$ A with the RSD%, 10.12%, n=3) (Table 4.6). Similar results were obtained using ZTO/ITO electrodes. 2% APTES-modified ZTO/ITO has presented the highest peak current ( $116.89 \pm 31.01 \mu$ A with the RSD%, 26.53%, n=3) while ZTO/ITO has given the lowest peak current ( $26.95 \pm 31.20 \mu$ A with the RSD%, 115.79%, n=3), and the CV curve of the sonicated ZTO/ITO is in the middle of these two samples ( $71.47 \pm 23.32 \mu$ A with the RSD%, 32.63%, n=3) (Table 4.7). The reason of the difference between sonicated and non-sonicated electrode samples is that ITO and ZTO/ITO become more conductive after the sonication step. The process of increasing conductivity after sonication was previously explained. Furthermore, the reason for the CV curve difference caused by the 2% APTES modification was also previously explained. Briefly, 2% APTES modification provides a higher surface area with conductivity. In addition, there was a dramatic decrease in the relative standard deviation of the 2% APTES-modified ZTO/ITO electrodes in comparison to the one of ZTO/ITO electrodes.

As a consequence, all of the cyclic voltammetry measurement results demonstrates that the processes of sonication and APTES modification were successfully applied to the electrodes. In other words, the changes at the peak currents refer also that the surface of the electrodes were successfully modified by using APTES.

When the CV results were compared, an increase about 63.54% was observed after APTES modification onto the surfaces of ZTO/ITO electrodes, while an increase of only 4% was recorded in the presence of APTES modification onto the surface of ITO electrodes. Accordingly, it was decided to test APTES modified ZTO/ITO electrodes for electrochemical monitoring of dopamine as a biosensor application.

#### 4.6. Electrochemical Impedance Spectroscopy (EIS)

EIS measurements were applied to determine other electrochemical properties of the ZTO/ITO electrodes (Randviir & Banks, 2013). EIS measurements were applied herein to determine electrochemical properties of the ZTO/ITO electrodes. EIS experiments were performed before/after sonication and APTES modification steps.

All data shown in each of Nyquist diagrams is the fitted versions of the related data obtained by EIS measurement.

 $R_s$ ,  $C_d$ ,  $R_{ct}$ , and W are the related parameters in the inset diagrams.  $R_s$  is the solution resistance.  $C_d$  is the double layer capacitance that depends on the void charge capacitance at the electrode/electrolyte interface.  $R_{ct}$  is the charge transfer resistance at the electrode/electrolyte interface. W is the Warburg impedance that results from mass transfer at the electrode surface.

The Nyquist plots are plotted by using the data obtained from EIS measurements. The portion represented by the electron transfer resistance in the higher frequency range is the semicircle. This portion gives information about the electron transfer kinetics of the redox probe which is at the electron surface. Also, the non-semicircular part of the Nyquist plots, that is the more linear part, corresponds to a lower frequency range, and corresponds the diffusion limited process (diffusion of ferricyanide  $[Fe(CN)_6]^{3-/4-}$ ). An increase in the diameter of the semicircle is a sign that the electron flow is blocked (Soylemez et al., 2016).



Figure 4.16. Nyquist diagrams of (a) ITO, (b) Sonicated ZTO/ITO, (c) ZTO/ITO electrodes. Inset Nyquist diagram shows the close-up view of a, b, and c at low impedance range.

Electrodes	Average R <sub>ct</sub> Value (Ohm)	Change % at R . Value (%)
ІТО	$2360.00 \pm 1994.04$	(According to
ZTO/ITO	264268.92 ± 849876.16	ZTO/ITO)
Sonicated ZTO/ITO	7702.82 ± 24131.42	97.00 decrease

Table 4.8. Average  $R_{ct}$  and change % at  $R_{ct}$  values measured by ITO, ZTO/ITO, and sonicated ZTO/ITO electrodes.

Figure 4.16 shows the Nyquist diagrams of bare ITO, ZTO/ITO, and sonicated ZTO/ITO. As mentioned before, ZTO thin film is a dielectric material with a poor conductivity. Since ZTO thin film deposited on ITO leads to decrease the conductivity, and it can act as a barrier for the electron transfer, the semicircle portion of the Nyquist plot is related to ZTO/ITO electrodes as expected. Accordingly, the highest average R<sub>ct</sub> value was measured as  $264268.92 \pm 849876.16 \Omega$  (RSD%, 321.59%, n=3) for ZTO/ITO electrodes (Table 4.8). The more linear part in the Nyquist plot is related to both ITO and sonicated ZTO/ITO. The lowest average R<sub>ct</sub> value was 2360.00  $\pm$  1994.04  $\Omega$  (RSD%, 84.49%, n=3) for ITO, and the average  $R_{ct}$  value of sonicated ZTO/ITO was 7702.82  $\pm$ 24131.42  $\Omega$ . Inset diagram shows more clearly the curves a and c in contrast to curve b. It can be seen that the curves are similar but not the same. Diameter of the ITO curve is smaller than the diameter of the sonicated ZTO/ITO curve as expected result due to the fact that ITO is more conductive than sonicated ZTO/ITO. On the other hand, this shows that sonication is effective step to increase the conductivity of ZTO/ITO electrodes so that the change at R<sub>ct</sub> value was resulted with a decrease as 97.00% (Table 4.8) after sonication step.



Figure 4.17. Nyquist diagrams of (a) sonicated ZTO/ITO, (b) 1% APTES-modified ZTO/ITO, (c) 2% APTES-modified ZTO/ITO, (d) 5% APTES-modified ZTO/ITO electrodes. Inset Nyquist diagram shows the close-up view of a, b, c, and d at low impedance range.

Electrodes	Average R <sub>ct</sub> Value (Ohm)	Change % at R <sub>ct</sub> Value (%)	
Sonicated ZTO/ITO	$7702.82 \pm 24131.42$		
1% APTES-modified ZTO/ITO	$5221.57 \pm 10513.80$	32.21 decrease	
2% APTES-modified ZTO/ITO	227.38 ± 167.79	97.04 decrease	
5% APTES-modified ZTO/ITO	$349.60 \pm 409.27$	95.46 decrease	

Table 4.9. Average R<sub>ct</sub> and change % at R<sub>ct</sub> values measured by sonicated ZTO/ITO and APTES-modified ZTO/ITO electrodes.

The EIS measurement data of sonicated ZTO/ITO and APTES-modified ZTO/ITO electrodes are shown in Figure 4.17. ZTO/ITO electrodes were prepared in different APTES concentrations: 1%, 2%, and 5%. The data obtained by Nyquist diagram is found as consistent with the data obtained by cyclic voltammogram (Figure 4.14A). It is also understood from this Nyquist plot that the 2% APTES concentration is appropriate for the ZTO/ITO electrodes, since ZTO/ITO electrodes modified with 2% APTES increase conductivity while also having the lowest relative standard deviation (227.38  $\pm$ 167.79  $\Omega$  with the RSD%, 73.80%, n=3) (Table 4.9) of all. Moreover, the change at Rct value after 2% APTES modification was measured as 97.04% decrease, which was the highest decrease of all. This can be better understood by looking at the inset diagram. Inset diagram shows also that 5% APTES-modified ZTO/ITO electrodes (349.60  $\pm$ 409.27  $\Omega$  with the RSD%, 117.07%, n=3) are more conductive than 1% APTES-modified ZTO/ITO electrodes (5221.57  $\pm$  10513.80  $\Omega$  with the RSD%, 201.35%, n=3) since 1% APTES-modified ZTO/ITO electrodes have the longest semicircular diameter of all. The reason for this difference may be that 2% APTES modification provides the higher electrode surface area that is also suitable for analyte binding, whereas 1% APTES concentration is less than sufficient, and 5% APTES concentration leads to selfpolymerization of APTES. Therefore, 1% APTES-modified ZTO/ITO electrodes exhibit more electron blocking behaviour than 5% APTES-modified ZTO/ITO electrodes. As a result, it has been confirmed once again that the APTES modification enables electrons

to bind to the electrode surface and makes the electrode surface more conductive. This confirmation is understood from the Nyquist diagram, because the semicircular radius of all APTES modified electrodes in the diagram appears to be smaller than the semicircular radius of the sonicated ZTO/ITO electrodes (7702.82  $\pm$  24131.42  $\Omega$  with the RSD%, 313.28%, n=3).



Figure 4.18. Nyquist diagrams of (A) ITO electrodes: (a) ITO, (b) sonicated ITO, (c) 2% APTES-modified ITO. (B) ZTO/ITO electrodes: (a) ZTO/ITO, (b) sonicated ZTO/ITO, (c) 2% APTES-modified ZTO/ITO. Inset Nyquist diagram shows the close-up view of a, b, and c at low impedance range.

Figure 4.18A shows the Nyquist diagram of the ITO, sonicated ITO, and 2% APTES-modified ITO electrodes, and Figure 4.18B shows the Nyquist diagrams of ZTO/ITO, sonicated ZTO/ITO, and 2% APTES-modified ZTO/ITO electrodes. Inset diagram in Figure 4.18B shows sonicated and APTES-modified electrodes in detail. As expected from the results, it was observed that the surface conductivity increased first

with sonication and then with APTES modification. Surface of the ZTO/ITO electrode has an insulating layer of ZTO which blocks the electron transfer to occur. In other words, ZTO thin film blocks the  $[Fe(CN)_6]^{3-/4-}$  (ferricyanide) diffusion towards to the electrode surface, and this causes an dramatical increase at the charge transfer resistance. Although the ITO thin film surface is conductive even in this state, its surface has become more conductive by applying the sonication/modification steps. Sonication causes an increase at the conductivity since ultrasonic irradiation provides to increase the rate of the charge transfer reactions. Therefore, the decrease at the semicircle diameter of the sonicated ZTO/ITO electrodes is the result of the ultrasonic irradiation application. Moreover, there is a further reduction in the semicircle diameter of the ZTO/ITO electrodes modified with 2% APTES according to the data shown in Nyquist diagram. The reason of this reduction is that modification of 2% APTES provides a higher surface area and thus, it allows more electrons to bind to the electrode surface. In Table 4.11, the average anodic peak current of ZTO/ITO was  $264268.92 \pm 849876.16 \ \mu A$  (RSD%, 321.59%, n=3), the one of sonicated ZTO/ITO was  $7702.82 \pm 24131.42 \mu A$  (RSD%, 313.28%, n=3), and the one of 2% APTES-modified ZTO/ITO was recorded as  $227.38 \pm 167.79 \mu A$  (RSD%, 73.79%, n=3). The Nyquist diagram of ITO electrodes shows that the sonicated and 2% APTESmodified electrode curves are almost identical since every ITO electrode already has conductivity. Also, the level of increase at the conductivity is almost same when surface modifications are applied. In Table 4.10, the average anodic peak current of ITO was  $2360.00 \pm 1994.04 \ \mu A \ (RSD\%, 84.40\%, n=3)$ , the one of sonicated ITO was  $49.20 \pm 3.81$ μA (RSD%, 7.76%, n=3), and the one of 2% APTES-modified ZTO/ITO was 0 μA (RSD%, 0%, n=3).

Electrodes	Average R <sub>ct</sub> Value (Ohm)	Change % at R <sub>ct</sub> Value (%)
ІТО	$2360.00 \pm 1994.04$	(According to ITO)
Sonicated ITO	$49.20 \pm 3.81$	97.91 decrease
2% APTES-modified ITO	0	100.00 decrease

Table 4.10. Average R<sub>ct</sub> and change % at R<sub>ct</sub> values measured by ITO, sonicated ITO, and 2% APTES-modified ITO electrodes.

Electrodes	Average R <sub>ct</sub> Value (Ohm)	Change % at R <sub>ct</sub> Value (%)
ZTO/ITO	$264268.92 \pm 849876.16$	(According to ZTO/ITO)
Sonicated ZTO/ITO	7702.82 ± 24131.42	97.08 decrease
2% APTES-modified ZTO/ITO	227.38 ± 167.79	97.04 decrease

Table 4.11. Average Rct and change % at Rct values measured by ZTO/ITO, sonicatedZTO/ITO, and 2% APTES-modified ZTO/ITO electrodes.

As a result, Nyquist diagrams obtained from EIS measurements are in a good agreement with voltammograms obtained from CV measurements.

Table 4.10 and Table 4.11 show the change at Rct and RSD % values that have changed after sonication and APTES modification (i.e, 100% decrease for ITO electrodes, and 97.04% decrease for ZTO/ITO electrodes). The reason for the significant decrease in the R<sub>ct</sub> value of the 2% APTES-modified electrodes is that the amino ends form a monolayer on the surface, allowing the diffusion of redox probe ( $[Fe(CN)_6]^{3-/4-}$ ) on the surface (Canbaz & Sezgintürk, 2014). In other words, electrodes modified with 2% APTES exhibit a lower resistance against the redox probe molecules to diffuse. As a result, the decrease at R<sub>ct</sub> value indicates that APTES modification onto the surface of ZTO/ITO electrodes was performed successfully.

### 4.7. Dopamine Detection with ZTO/ITO Electrodes

Differential pulse voltammetry (DPV) measurements were applied by using 2% APTES-modified electrodes (APTES/ZTO/ITO) in different concentrations of dopamine (DA). The electrodes were treated by using dopamine solutions in different concentrations: 0.1  $\mu$ M, 0.3  $\mu$ M, 0.5  $\mu$ M, 1  $\mu$ M, and 3  $\mu$ M. In addition, the control experiment was performed in the absence of dopamine in the solution. There has been an increase at the oxidation signal of dopamine when its concentration is increased from 0.1  $\mu$ M to 1  $\mu$ M (shown in Figure 4.19). However, a decrease at dopamine signal was recorded when its concentration increased from 1  $\mu$ M to 3  $\mu$ M (not shown).



Figure 4.19. (A) Differential pulse voltammograms obtained by using the 2% APTES-modified ZTO/ITO electrodes in the absence and presence of dopamine: (a) Control experiment in PBS (0.05 M, pH 7.0), (b) 0.1 μM DA, (c) 0.3 μM DA, (d) 0.5 μM DA, (e) 1 μM DA. (B) Calibration graph obtained by 2% APTES-modified ZTO/ITO electrodes in the presence of 0.1-1 μM DA.

Silanization, which is the introduction of amine groups at the electrode surface following by APTES modification, is a common method due to its low cost and robustness (Arnfinnsdottir et al., 2020). Silanization allows the preparation of active organic aminosilane monolayers onto the surface of metal oxide films. The monosilane monolayer allows the quick formation of covalent bonds between the metal oxide surface and the anchoring groups. In addition, the bulky alkoxy groups that APTES has provide the improvement of the stability (Hijazi et al., 2018). Thus, dopamine is absorbed by the surface of the ZTO/ITO electrodes as a result of silanization.

Figure 4.19A shows differential pulse voltammogram of the untreated and dopamine-treated APTES/ZTO/ITO electrodes. In this voltammogram, it can be seen that the oxidation peak current of DA observed at +0.5 V increases while increasing the concentration of DA. Also, Figure 4.19B shows the calibration curve obtained by the APTES/ZTO/ITO electrodes in different concentrations of DA. This calibration curve indicates that the oxidation peak current increases linearly when the DA concentration increases.

The LOD is related with the analytic sensitivity, and it is the smallest amount of the analyte that can be detected. The LOD (limit of detection) (Miller & Miller, 2018) of DA was calculated according to the equation obtained by using the calibration graph obtained between  $0.1 - 1 \mu M$  DA and found to be 0.013  $\mu M$ .

 Table 4.12. Comparison of the performances of APTES/ZTO/ITO electrodes over to the electrodes reported in earlier studies in terms of DA detection.

Electrode	Linear Range (µM)	LOD (µM)	Reference
ERGO/GCE	0.50 - 60	0.5000	(Yang et al., 2014)
18-BDD/Ta	0.40 - 600	0.1000	(Yang et al., 2020)
Hydrogel/GCE	0.08 - 250	0.0440	(Wang et al., 2019)
AuNPs/GCE	0.20 - 30	0.0026	(Zhu et al., 2017)
CNNS-GO/GCE	1.00 - 20	0.0960	(Zhang et al., 2014)
Pd/CNFs	0.50 - 160	0.2000	(Huang et al., 2008)
APTES/ZTO/ITO	0.10 - 1.0	0.0130	This work

In Table 4.12, there are abbreviations for the electrodes. The long names of these electrodes are written below.

- ERGO/GCE: Electrochemically reduced graphene oxide / Glassy carbon electrode
- 18-BDD/Ta: 18 sccm Boron-doped diamond / Tantalum
- Hydrogel/GCE: Hydrogel / Glassy carbon electrode
- AuNPs/GCE: Gold nanoparticles / Glassy carbon electrode

- CNNS-GO/GCE: Graphitic carbon nitride nanosheets Graphene oxide / Glassy carbon electrode
- Pd/CNFs: Palladium nanoparticle-loaded carbon nanofibers

The comparison of the APTES/ZTO/ITO electrodes over to other electrodes that are earlier reported in the literature in terms of DA detection, can be seen in the Table 4.12. Although the APTES/ZTO/ITO electrodes do not have a wide linear range, LOD of the APTES/ZTO/ITO electrodes appears to be lower in contrast to the ones of earlier studies shown in the table 4.12. Therefore, it can be said that APTES/ZTO/ITO electrodes have shown a good sensitivity for electrochemical detecting DA.

#### 4.8. Selectivity of ZTO/ITO Electrodes

DPV measurements were performed in which dopamine (DA), uric acid (UA), ascorbic acid (AA), bovine serum albumin (BSA), and fish sperm double-stranded DNA (fsDNA) were present even the concentration of interferents is higher (i.e 100 folds) than DA solution in order to indicate the selectivity of APTES-modified ZTO/ITO electrodes over to AA, UA, BSA and fsDNA.

Figure 4.20A and Figure 4.20B shows differential pulse voltammograms of 2% APTES-modified ZTO/ITO electrodes measured separately in 0.1  $\mu$ M DA, 1  $\mu$ M DA, and 10  $\mu$ M AA solutions. Similarly, Figure 4.20C and Figure 4.20D shows the voltammograms obtained by 2% APTES-modified ZTO/ITO electrodes individually in the presence of 0.1  $\mu$ M DA, 1  $\mu$ M DA, and 10  $\mu$ M UA. At both experiments, the control measurements were also performed after the electrodes were kept in PBS solution. According to the result of both experiments, it can be seen that DA is attached more onto the electrodes instead of AA and UA, even the concentration of DA is very low in comparison to the one of AA or UA. The peak current of DA is higher than the peak currents of AA and UA. Also, the oxidation signal of each compound is measured at different peak potential. It can be concluded that the 2% APTES-modified ZTO/ITO electrodes are selective in terms of DA detection since AA and UA do not have catechol and amine groups in their structure, unlike DA (Kim et al., 2013). Thus, the adhesion of AA and UA may not occur efficiently while using APTES-modified ZTO/ITO electrodes.



Figure 4.20. Differential pulse voltammograms presenting the oxidation signals measured by 2% APTES-modified ZTO/ITO. (A) (a) Control experiment in PBS (0.05 M, pH 7.0), (b) 0.1 μM DA, (c) 10 μM AA, (B) (a) 1 μM DA, (b) 10 μM AA, (C) (a) control experiment in PBS (0.05 M, pH 7.0), (b) 0.1 μM DA, (c) 10 μM UA, and (D) (a) 1 μM DA, (b) 10 μM UA.



Figure 4.21. Differential pulse voltammograms presenting the oxidation signals measured by 2% APTES-modified ZTO/ITO. (A) (a) Control experiment in PBS (0.05 M, pH 7.0), (b) 0.1 μM DA, (c) 10 μM BSA, (B) (a) 1 μM DA, (b) 10 μM BSA, (C) (a) control experiment in PBS (0.05 M, pH 7.0), (b) 0.1 μM DA, (c) 300 ppm fsDNA, and (D) (a) 1 μM DA, (b) 300 ppm fsDNA.

Figure 4.21A and Figure 4.21B shows differential pulse voltammogram of 2% APTES-modified ZTO/ITO electrodes measured separately in 0.1  $\mu$ M DA, 1  $\mu$ M DA, and 10  $\mu$ M BSA solutions. Figure 4.21C and Figure 4.21D shows the voltammograms measured by 2% APTES-modified ZTO/ITO electrodes individually in 0.1  $\mu$ M DA (equals to 0.0189 ppm), 1  $\mu$ M DA (equals to 0.189 ppm), and 300 ppm fsDNA solutions. At both experiments, the control measurements were done while the electrodes were in PBS solution. It can be seen in the Figure 4.21A that the oxidation signal of BSA measured at +0.25 V is slightly higher than the one of DA, and also in different peak position. This can be explained by that BSA and DA have different adsorption abilities onto the APTES-modified ZTO/ITO electrodes due to the large number of amino acid residues in BSA comparison to DA (Assadpour & Jafari, 2019; Chen & Huang, 2009). Their oxidation signals appeared at different peak positions. Therefore, it can be proposed that even if DA and BSA exist in the same medium, APTES modified ZTO/ITO electrodes presents much more selective behavior in terms of voltammetric DA detection.

In the presence of fsDNA, the possible binding of DNA was tested and compared to the signal of DA (Figure 4.21B). It is known that hybridization can occur between the DNA and a silanized electrode surface (Xu et al., 2001). The peak current of DA is measured higher than the one of fsDNA as well as at different peak potentials respectively at +0.5 V and +1.1 V. This also can be explained by the different adsorption abilities of DNA and DA onto the surface of APTES-modified ZTO/ITO electrodes. As a conclusion, APTES-modified ZTO/ITO electrodes can detect DA voltammetrically with a good selectively despite the presence of high concentrations of DNA molecules.



Figure 4.22. Differential pulse voltammograms presenting the oxidation signals measured by 2% APTES-modified ZTO/ITO. (A) (a) 1 μM DA, (b) Mixture of 1 μM DA and 1 μM AA, (B) (a) 1 μM DA, (b) Mixture of 1 μM DA and 1 μM UA.

Figure 4.22 shows differential pulse voltammograms of 2% APTES-modified ZTO/ITO electrodes measured in the mixture sample containing the solutions of 1  $\mu$ M DA and 1  $\mu$ M DA and 1  $\mu$ M UA. Even if there is substance in the same concentration level as much as DA, these electrodes recognize DA selectively.

# **CHAPTER 5**

# CONCLUSION

Studies conducted with biosensors have been increasing since the Clark electrode first appeared in 1956. Leland C. Clark, 'father of biosensors', took the lead for biosensor development by designing the Clark electrode that works for detecting oxygen. Since then, a great variety of studies have been done. A biosensor, that is for detecting a biological material (namely analyte), has four basic components: Analyte, bioreceptor, transducer, and detector. When the biomaterial is introduced to the biosensor, analytebioreceptor interactions occur. These interactions lead to signal formation. Then, the signals are transformed into a readable format with the help of transducer.

In this thesis, a non-enzymatic electrochemical biosensor with thin film electrodes was fabricated, characterized and then they were tested for monitoring of dopamine. It has a vital importance to monitor the concentration level of dopamine in the body since abnormal levels of dopamine can lead to serious diseases such as Alzheimer's disease, Parkinson's disease, and schizophrenia. As a result of studies conducted with people suffering from these common diseases, it has been seen that low dopamine levels in the brain can cause Alzheimer's disease and Parkinson's disease, however of this fact high levels of dopamine can cause schizophrenia (Lei et al., 2020). It is a favorable option to use dopamine biosensors, which is a useful and practical tool for early detection and treatment of these diseases. Dopamine levels in the body can be monitored by dopamine biosensors using the samples from urine, plasma, sweat, tears, and saliva.

Thin films have been become an emerging area, and they are generally used in the electrodes of the biosensors. Depending on the thin film material used, thin films can provide high surface-to-volume ratio, conductivity, stability, specificity, biocompatibility, and good electrocatalytic activity. These are the favorable features for biosensors. Also, thin film electrodes are like a transducer with physicochemical properties such as electrical, magnetic, mechanical, and optical properties. In this thesis, ZTO thin film was deposited on ITO thin film by DC magnetron sputtering technique and thus, thin film electrodes were obtained.

The idea of producing the ZTO/ITO electrodes used in this study was decided after researching the studies in the literature in which ITO was combined with ZTO. Optical transmittance and mechanical flexibility in these studies were found to be more improved than those in which ITO was used as a single layer (Kim et al., 2015). However, most of the studies using ZTO and ITO together are not related to biosensors. Therefore, it can be said that the study represented in this thesis is original according to the best of our knowledge, ZTO/ITO electrodes modified with APTES have been developed and applied for the first time on the electrochemical determination of dopamine.

The ZTO/ITO electrodes were fabricated by DC magnetron sputtering technique, which is a rapid, cost effective, and noncomplicated method for thin film deposition. Then, measurements of thickness, optical transmission, XRD, and SEM were made with the ITO, ZTO, and ZTO/ITO electrodes. The thickness values of different ZTO thin film samples were measured with a surface profilometer to see the difference in thickness depending on the oxygen concentration and the deposition time. As shown in Table 4.1 and Table 4.2, the thickness values of all samples were below 100 nm. Since the ITO substrate used has a thickness of 200 nm, the total thickness of the electrodes is close to 300 nm. In addition, optical transmission measurements have shown that when the appropriate oxygen concentration (above 7.5 sccm) is used, the optical transmittance of ITO (77.69%). This feature will be useful if it is desired to use light while developing the working principle of the electrodes. Moreover, XRD has proven that ZTO has an amorphous structure, while ITO has a crystalline structure in the produced ZTO/ITO electrodes. This morphological difference is clearly visible on the SEM images.

The surface of the electrodes was treated with sonication and APTES, respectively. Then, the electrochemical measurements were performed with the surface modified electrodes. As a result of the force produced by the sound waves sent during sonication, both the surface of the electrodes was cleaned, and the surface area of the electrodes increased by obtaining hydroxylated electrode surface. The APTES modification provides amine groups on the electrode surface thereby allowing diffusion for charge transfer reactions to occur. In addition, silanization with APTES allows organic molecules such as dopamine to bind to the surface by adsorption. When the CV and EIS results of electrodes with and without surface modification were compared, it was seen that the surfaces of ZTO/ITO electrodes were successfully modified by using APTES. It was determined that the appropriate APTES concentration was 2% in our study. Then,

the APTES/ZTO/ITO electrodes were applied for DA detection in its different concentrations. Moreover, voltammetric measurements in combination with APTES/ZTO/ITO electrodes were performed for DA as well as other substituents, such as: AA, UA, BSA and fsDNA. The results showed that dopamine binds to the surface of the APTES/ZTO/ITO electrodes and can be selectively detected by the electrochemical biosensor, however of the fact that other substituents (i.e possible interferents) have a higher concentration than DA. The selectivity of APTES/ZTO/ITO electrodes to DA was also tested in the mixture sample containing DA and AA or DA and UA in the same concentration level of these compounds. The results showed that dopamine can be sensitively and selectively detected by using APTES/ZTO/ITO electrodes, even if these interferents are in a higher concentration available in the medium in contrast to the one of DA. The LOD value of dopamine was calculated and found to be 0.013  $\mu$ M, that is comparable lower in contrast to the ones reported in earlier studies (Yang et al., 2014; Yang et al., 2020; Wang et al., 2019; Zhang et al., 2014; Huang et al., 2008).

To conclude, the surface-modified thin film electrodes was successfully designed, fabricated, characterized, and then implemented as a single-use electrochemical biosensor towards to voltammetric detection of dopamine levels. This biosensor has an easy fabrication procedure. Furtherly, it can be validated and easily manufactured after being claimed for its clinical use. Consequently, to detect the level of dopamine for health monitoring of patients with Alzheimer's or Parkinson's disease will be more cost effective and easier. In addition, this biosensor can be furtherly improved for non-invasive dopamine monitoring in body fluids (e.g., saliva, plasma, sweat, tear, and urine). Under this goal, the biosensor also be developed as wearable to allow patients to use it more easily. If flexible materials such as polyimide are used as substrate, because of the property of stretch, the biosensor can be mounted to or placed into the clothes and other accessories. Therefore, as people sweat, the dopamine detection process can occur automatically which will be a very advantageous feature for dopamine monitoring. As a subjective perspective, more attention should be paid to non-enzymatic dopamine biosensors based on thin films modified platforms. In that area, there is a noteworthy potential of electrochemical monitoring dopamine levels using such a kind of noninvasive methods which could easily be applied in the most appropriate potential body fluids; saliva, tear, and sweat.

# REFERENCES

Ali, Jazib, Jawayria Najeeb, Muhammad Asim Ali, Muhammad Farhan Aslam, and Ali Raza. 2017. "Biosensors: Their Fundamentals, Designs, Types and Most Recent Impactful Applications: A Review." Journal of Biosensors & Bioelectronics 08 (01): 1–9.

https://doi.org/10.4172/2155-6210.1000235.

- Alwarappan, Subbiah, Arzum Erdem, Chang Liu, and Chen Zhong Li. 2009. "Probing the Electrochemical Properties of Graphene Nanosheets for Biosensing Applications." Journal of Physical Chemistry C 113 (20): 8853–57. https://doi.org/10.1021/JP9010313.
- Arnfinnsdottir, Nina Bjørk, Cole A. Chapman, Ryan C. Bailey, Astrid Aksnes, and Bjørn Torger Stokke. 2020a. "Impact of Silanization Parameters and Antibody Immobilization Strategy on Binding Capacity of Photonic Ring Resonators." Sensors (Switzerland) 20 (11). https://doi.org/10.3390/s20113163.
- Ashton, Michael C. 2018. "Biological Bases of Personality." Individual Differences and Personality, 107–25. https://doi.org/10.1016/b978-0-12-809845-5.00005-6.
- Assadpour, Elham, and Seid Mahdi Jafari. 2019. "An Overview of Biopolymer Nanostructures for Encapsulation of Food Ingredients." Biopolymer Nanostructures for Food Encapsulation Purposes, January, 1–35. https://doi.org/10.1016/B978-0-12-815663-6.00001-X.
- Barth, Vanessa N., Eyassu Chernet, Laura J. Martin, Anne B. Need, Karen S. Rash, Michelle Morin, and Lee A. Phebus. 2006. "Comparison of Rat Dopamine D2 Receptor Occupancy for a Series of Antipsychotic Drugs Measured Using Radiolabeled or Nonlabeled Raclopride Tracer." Life Sciences 78 (26): 3007–12. https://doi.org/10.1016/J.LFS.2005.11.031.
- Benedetto, Giuseppe Egidio De, Daniela Fico, Antonio Pennetta, Cosimino Malitesta, Giuseppe Nicolardi, Dario Domenico Lofrumento, Francesco De Nuccio, and Velia La Pesa. 2014. "A Rapid and Simple Method for the Determination of 3,4-Dihydroxyphenylacetic Acid, Norepinephrine, Dopamine, and Serotonin in Mouse Brain Homogenate by HPLC with Fluorimetric Detection." Journal of Pharmaceutical and Biomedical Analysis 98 (September): 266–70. https://doi.org/10.1016/J.JPBA.2014.05.039.
- Bhalla, Nikhil, Pawan Jolly, Nello Formisano, and Pedro Estrela. 2016. "Introduction to Biosensors." Essays in Biochemistry 60 (1): 1–8. https://doi.org/10.1042/EBC20150001.
- Boccafoschi, F., L. Fusaro, and M. Cannas. 2018. Immobilization of Peptides on Cardiovascular Stent. Functionalised Cardiovascular Stents. Elsevier Ltd. https://doi.org/10.1016/b978-0-08-100496-8.00016-0.

- Breed, Michael D., and Janice Moore. 2012. "Neurobiology and Endocrinology for Animal Behaviorists." Animal Behavior, January, 25–65. https://doi.org/10.1016/B978-0-12-372581-3.00002-7.
- Bunaciu, Andrei A., Elena gabriela Udriștioiu, and Hassan Y. Aboul-Enein. 2015. "X-Ray Diffraction: Instrumentation and Applications." Critical Reviews in Analytical Chemistry 45 (4): 289–99. https://doi.org/10.1080/10408347.2014.949616.
- Canbaz, Mehmet Çetin, and Mustafa Kemal Sezgintürk. 2014. "Fabrication of a Highly Sensitive Disposable Immunosensor Based on Indium Tin Oxide Substrates for Cancer Biomarker Detection." Analytical Biochemistry 446 (1): 9–18. https://doi.org/10.1016/j.ab.2013.10.014.
- Carrera, Victoria, Esther Sabater, Eugenio Vilanova, and Miguel A. Sogorb. 2007. "A Simple and Rapid HPLC–MS Method for the Simultaneous Determination of Epinephrine, Norepinephrine, Dopamine and 5-Hydroxytryptamine: Application to the Secretion of Bovine Chromaffin Cell Cultures." Journal of Chromatography B 847 (2): 88–94. https://doi.org/10.1016/J.JCHROMB.2006.09.032.
- Ceylan Koydemir, Hatice, Haluk Külah, and Canan Özgen. 2013. Thin Film Biosensors. https://doi.org/10.1007/978-94-007-2592-8 8.
- Chang, S. C., J. R. Stetter, and C. S. Cha. 1993. "Amperometric Gas Sensors." Talanta 40 (4): 461–77. https://doi.org/10.1016/0039-9140(93)80002-9.
- Chen, Yan Shi, and Jin Hua Huang. 2009. "Electrochemical Sensing of Bovine Serum Albumin at Self-Assembled SWCNTs on Gold." Diamond and Related Materials 18 (2–3): 516–19. https://doi.org/10.1016/J.DIAMOND.2008.10.022.
- Choi, Woosung, Heon Cheol Shin, Ji Man Kim, Jae Young Choi, and Won Sub Yoon. 2020. "Modeling and Applications of Electrochemical Impedance Spectroscopy (Eis) for Lithium-Ion Batteries." Journal of Electrochemical Science and Technology 11 (1): 1–13. https://doi.org/10.33961/jecst.2019.00528.
- Clark, Leland C., and Champ Lyons. 1962. "Electrode Systems for Continuous Monitoring in Cardiovascular Surgery." Annals of the New York Academy of Sciences 102 (1): 29–45. https://doi.org/10.1111/j.1749-6632.1962.tb13623.x.
- Congur, Gulsah, Ece Eksin, and Arzum Erdem. 2019. "Chitosan Modified Graphite Electrodes Developed for Electrochemical Monitoring of Interaction between Daunorubicin and DNA." Sensing and Bio-Sensing Research 22 (February): 100255.

https://doi.org/10.1016/j.sbsr.2018.100255.

Congur, Gulsah, Ece Eksin, and Arzum Erdem. 2021. "Levan Modified DNA Biosensor for Voltammetric Detection of Daunorubicin-DNA Interaction." Sensors and Actuators, B: Chemical 326 (January): 128818. https://doi.org/10.1016/j.snb.2020.128818.

- Davis, F., and S. P.J. Higson. 2014. Carrier Systems and Biosensors for Biomedical Applications. Tissue Engineering Using Ceramics and Polymers: Second Edition. Woodhead Publishing Limited. https://doi.org/10.1533/9780857097163.2.270.
- Demirhan, Yasemin, Hasan Koseoglu, Fulya Turkoglu, Zemzem Uyanik, Mehtap Ozdemir, Gulnur Aygun, and Lutfi Ozyuzer. 2020. "The Controllable Deposition of Large Area Roll-to-Roll Sputtered Ito Thin Films for Photovoltaic Applications." Renewable Energy 146 (February): 1549–59. https://doi.org/10.1016/j.renene.2019.07.038.
- Ekmekcioglu, Merve, Nursev Erdogan, Aziz Taner Astarlioglu, Serap Yigen, Gulnur Aygun, Lutfi Ozyuzer, and Mehtap Ozdemir. 2021. "High Transparent, Low Surface Resistance ZTO/Ag/ZTO Multilayer Thin Film Electrodes on Glass and Polymer Substrates." Vacuum 187 (May): 110100. https://doi.org/10.1016/j.vacuum.2021.110100.
- Elgrishi, Noémie, Kelley J. Rountree, Brian D. McCarthy, Eric S. Rountree, Thomas T. Eisenhart, and Jillian L. Dempsey. 2018. "A Practical Beginner's Guide to Cyclic Voltammetry." Journal of Chemical Education 95 (2): 197–206. https://doi.org/10.1021/acs.jchemed.7b00361.
- Erdem, Arzum. 2007. "Nanomaterial-Based Electrochemical DNA Sensing Strategies." Talanta 74 (3): 318–25. https://doi.org/10.1016/J.TALANTA.2007.10.012.
- Erdem, Arzum, and Ece Eksin. 2019. "Zip Nucleic Acid Based Single-Use Biosensor for Electrochemical Detection of Factor V Leiden Mutation." Sensors and Actuators, B: Chemical 288 (June): 634–40. https://doi.org/10.1016/j.snb.2019.03.008.
- Erdem, Arzum, Ece Eksin, and Gulsah Congur. 2015. "Indicator-Free Electrochemical Biosensor for MicroRNA Detection Based on Carbon Nanofibers Modified Screen Printed Electrodes." Journal of Electroanalytical Chemistry 755 (August): 167–73. https://doi.org/10.1016/j.jelechem.2015.07.031.
- Erdogan, Nursev, Fuat Erden, A. Taner Astarlioglu, Mehtap Ozdemir, Salih Ozbay, Gulnur Aygun, and Lutfi Ozyuzer. 2020. "ITO/Au/ITO Multilayer Thin Films on Transparent Polycarbonate with Enhanced EMI Shielding Properties." Current Applied Physics 20 (4): 489–97. https://doi.org/10.1016/j.cap.2020.01.012.
- Gibson, T. D. 1999. "Biosensors: The Stability Problem." Analusis 27 (7): 630–38. https://doi.org/10.1051/analusis:1999270630.
- Gong, Xinghui, Tong Wang, Zhendong Zhang, and Xiao Wei. 2020. "A Novel and Non-Toxic Dopamine Detection Method Based on Self-Polymerisation of Dopamine." Journal of Experimental Nanoscience 15 (1): 233–41. https://doi.org/10.1080/17458080.2020.1757657.

- Gould, Robert D., Safa Kasap, and Asim K. Ray. 2017. Thin Films. Springer Handbooks. https://doi.org/10.1007/978-3-319-48933-9\_28.
- Han, Sangmin, Sang Yeol Lee, and Jun Young Choi. 2015. "Performance of Solution Processed Zn-Sn-O Thin-Film Transistors Depending on Annealing Conditions." Transactions on Electrical and Electronic Materials 16 (2): 62–64. https://doi.org/10.4313/TEEM.2015.16.2.62.
- Hasan, Anwarul, Md Nurunnabi, Mahboob Morshed, Arghya Paul, Alessandro Polini, Tapas Kuila, Moustafa Al Hariri, Yong Kyu Lee, and Ayad A. Jaffa. 2014. "Recent Advances in Application of Biosensors in Tissue Engineering." BioMed Research International 2014. https://doi.org/10.1155/2014/307519.
- Hassan, Mohammad M. 2017. "Antimicrobial Coatings for Textiles." In Handbook of Antimicrobial Coatings, 321–55. Elsevier. https://doi.org/10.1016/B978-0-12-811982-2.00016-0.
- Heineman, William R., and William B. Jensen. 2006. "Leland C. Clark Jr. (1918–2005)." Biosensors and Bioelectronics 21 (8): 1403–4. https://doi.org/10.1016/j.bios.2005.12.005.
- Heo, Kyong Chan, Phil Kook Son, Youngku Sohn, Jonghoon Yi, Jin Hyuk Kwon, and Jin Seog Gwag. 2014. "Characteristics of Ion Beam Assisted ITO Thin Films Deposited by RF Magnetron Sputtering." Molecular Crystals and Liquid Crystals 601 (1): 57–63. https://doi.org/10.1080/15421406.2014.940493.
- Hijazi, Mohamad, Valérie Stambouli, Mathilde Rieu, Vincent Barnier, Guy Tournier, Thomas Demes, Jean Paul Viricelle, and Christophe Pijolat. 2018. "Synthesis and Characterization of Tin Dioxide Thick Film Modified by APTES in Vapor and Liquid Phases." Journal of Materials Science 53 (1): 727–38. https://doi.org/10.1007/s10853-017-1541-4.
- Huang, Jianshe, Yang Liu, Haoqing Hou, and Tianyan You. 2008. "Simultaneous Electrochemical Determination of Dopamine, Uric Acid and Ascorbic Acid Using Palladium Nanoparticle-Loaded Carbon Nanofibers Modified Electrode." Biosensors and Bioelectronics 24 (4): 632–37. https://doi.org/10.1016/j.bios.2008.06.011.
- Hughes, TJ. 2008. "Plug Formation and Dissociation of Mixed Gas Hydrates and Methane Semi-Clathrate Hydrate ...." Ir.Canterbury.Ac.Nz, no. July. http://ir.canterbury.ac.nz/handle/10092/1579.
- Jacob, Jolly, U. Rehman, K. Mahmood, A. Ali, Khurram Mehboob, A. Ashfaq, S. Ikram, N. Amin, S. Hussain, and Fouzia Ashraf. 2020. "Improved Thermoelectric Performance of Al and Sn Doped ZnO Nano Particles by the Engineering of Secondary Phases." Ceramics International 46 (10): 15013–17. https://doi.org/10.1016/j.ceramint.2020.03.031.
- Jadhavar, Ashok Arjun, Ajinkya Bhorde, Adinath M Funde, and Amit Pawbake. 2015. "Synthesis of Indium Tin Oxide (Ito) As a Transparent Conducting," no. September.

- Jothi, Lavanya, and Gomathi Nageswaran. 2019. Plasma Modified Polymeric Materials for Biosensors/Biodevice Applications. Non-Thermal Plasma Technology for Polymeric Materials. Elsevier Inc. https://doi.org/10.1016/b978-0-12-813152-7.00015-9.
- Kim, Byung Kwon, Ji Young Lee, Jun Hui Park, and Juhyoun Kwak. 2013. "Electrochemical Detection of Dopamine Using a Bare Indium-Tin Oxide Electrode and Scan Rate Control." Journal of Electroanalytical Chemistry 708: 7–12. https://doi.org/10.1016/j.jelechem.2013.09.001.
- Kim, Dae Hyun, Han Kyeol Lee, Jin Young Na, Sun Kyung Kim, Young Zo Yoo, and Tae Yeon Seong. 2015. "ZnSnO/Ag/Indium Tin Oxide Multilayer Films as a Flexible and Transparent Electrode for Photonic Devices." Superlattices and Microstructures 83: 635–41. https://doi.org/10.1016/j.spmi.2015.04.002.
- Kim, Hojoong, and Jang Yeon Kwon. 2017. "Enzyme Immobilization on Metal Oxide Semiconductors Exploiting Amine Functionalized Layer." RSC Advances 7 (32): 19656–61. https://doi.org/10.1039/c7ra01615h.
- Kim, Ji In, Kwang Hwan Ji, Hong Yoon Jung, Se Yeob Park, Rino Choi, Mi Jang, Hoichang Yang, et al. 2011. "Improvement in Both Mobility and Bias Stability of ZnSnO Transistors by Inserting Ultra-Thin InSnO Layer at the Gate Insulator/Channel Interface." Applied Physics Letters 99 (12): 2009–12. https://doi.org/10.1063/1.3643054.
- Koseoglu, Hasan, Fulya Turkoglu, Metin Kurt, Mutlu D. Yaman, Fatime G. Akca, Gulnur Aygun, and Lutfi Ozyuzer. 2015. "Improvement of Optical and Electrical Properties of ITO Thin Films by Electro-Annealing." Vacuum 120 (October): 8–13. https://doi.org/10.1016/j.vacuum.2015.06.027.
- Lei, Yu, Derrick Butler, Michael C. Lucking, Fu Zhang, Tunan Xia, Kazunori Fujisawa, Tomotaroh Granzier-Nakajima, et al. 2020. "Single-Atom Doping of MoS2with Manganese Enables Ultrasensitive Detection of Dopamine: Experimental and Computational Approach." Science Advances 6 (32): 1–10. https://doi.org/10.1126/sciadv.abc4250.
- Li, Yuan, Pier Luc Tremblay, and Tian Zhang. 2018. Anode Catalysts and Biocatalysts for Microbial Fuel Cells. Progress and Recent Trends in Microbial Fuel Cells. Elsevier B.V. https://doi.org/10.1016/B978-0-444-64017-8.00009-9.
- Ma, H. L., D. H. Zhang, P. Ma, S. Z. Win, and S. Y. Li. 1995. "Preparation and Properties of Transparent Conducting Indium Tin Oxide Films Deposited by Reactive Evaporation." Thin Solid Films 263 (1): 105–10. https://doi.org/10.1016/0040-6090(95)06554-7.
- Mathur, Sanjay, Arzum Erdem, Christian Cavelius, Sven Barth, and Jessica Altmayer. 2009. "Amplified Electrochemical DNA-Sensing of Nanostructured Metal Oxide Films Deposited on Disposable Graphite Electrodes Functionalized by Chemical
Vapor Deposition." Sensors and Actuators, B: Chemical 136 (2): 432–37. https://doi.org/10.1016/J.SNB.2008.11.049.

- Mehrotra, Parikha. 2016. "Biosensors and Their Applications A Review." Journal of Oral Biology and Craniofacial Research 6 (2): 153–59. https://doi.org/10.1016/j.jobcr.2015.12.002.
- Mendelson, Yitzhak. 2011. "Biomedical Sensors." Introduction to Biomedical Engineering, 609–66. https://doi.org/10.1016/B978-0-12-374979-6.00010-1.
- Miecznikowski, Krzysztof, and James A. Cox. 2020. "Electroanalysis Based on Stand-Alone Matrices and Electrode-Modifying Films with Silica Sol-Gel Frameworks: A Review." Journal of Solid State Electrochemistry 24 (11–12): 2617–31. https://doi.org/10.1007/s10008-020-04697-w.
- Mylvaganam, Kausala, Yiqing Chen, Weidong Liu, Mei Liu, and Liangchi Zhang. 2014. "Hard Thin Films: Applications and Challenges." Anti-Abrasive Nanocoatings: Current and Future Applications, 544–67. https://doi.org/10.1016/B978-0-85709-211-3.00021-2.
- Nair-Roberts, R. G., S. D. Chatelain-Badie, E. Benson, H. White-Cooper, J. P. Bolam, and M. A. Ungless. 2008. "Stereological Estimates of Dopaminergic, GABAergic and Glutamatergic Neurons in the Ventral Tegmental Area, Substantia Nigra and Retrorubral Field in the Rat." Neuroscience 152 (4): 1024–31. https://doi.org/10.1016/j.neuroscience.2008.01.046.
- Namkung, Su Min;Choi, Jeong Su;Park, Ji Hyang;Yang, Man Gil;Lee, Min Woo;Kim, Suhng Wook; 2017. "Detection of Dopamine and Serotonin by Competitive Enzyme-Linked Immunosorbent Assay." Korean Journal of Clinical Laboratory Science 49 (3): 220–26. https://doi.org/10.15324/KJCLS.2017.49.3.220.
- Nee, Andrew Y.C. 2015. "Handbook of Manufacturing Engineering and Technology." HandBook of Manufacturing Engineering and Technology, 1–3487. https://doi.org/10.1007/978-1-4471-4670-4.
- Nguyen, Hoang Hiep, Sun Hyeok Lee, Ui Jin Lee, Cesar D. Fermin, and Moonil Kim. 2019. "Immobilized Enzymes in Biosensor Applications." Materials 12 (1): 1–34. https://doi.org/10.3390/ma12010121.
- Nichkova, Mikaela, Paul M. Wynveen, David T. Marc, Han Huisman, and Gottfried H. Kellermann. 2013. "Validation of an ELISA for Urinary Dopamine: Applications in Monitoring Treatment of Dopamine-Related Disorders." Journal of Neurochemistry 125 (5): 724–35. https://doi.org/10.1111/JNC.12248.
- Outline, Chapter. 2019. Methods for Assessing Surface Cleanliness. Developments in Surface Contamination and Cleaning, Volume 12. Vol. 12. https://doi.org/10.1016/b978-0-12-816081-7.00003-6.

Ozbay, Salih, Nursev Erdogan, Fuat Erden, Merve Ekmekcioglu, Mehtap Ozdemir,

Gulnur Aygun, and Lutfi Ozyuzer. 2020. "Surface Free Energy Analysis of ITO/Au/ITO Multilayer Thin Films on Polycarbonate Substrate by Apparent Contact Angle Measurements." Applied Surface Science 529 (November): 147111. https://doi.org/10.1016/j.apsusc.2020.147111.

- Pajkossy, Tamás. 2020. "Voltammetry Coupled with Impedance Spectroscopy." Journal of Solid State Electrochemistry 24 (9): 2157–59. https://doi.org/10.1007/s10008-020-04689-w.
- Parkhey, Piyush, and S. Venkata Mohan. 2018. Biosensing Applications of Microbial Fuel Cell: Approach toward Miniaturization. Biomass, Biofuels, Biochemicals: Microbial Electrochemical Technology: Sustainable Platform for Fuels, Chemicals and Remediation. Elsevier B.V. https://doi.org/10.1016/B978-0-444-64052-9.00040-6.
- Prepelita, P., M. Filipescu, I. Stavarache, F. Garoi, and D. Craciun. 2017. "Transparent Thin Films of Indium Tin Oxide: Morphology–Optical Investigations, Inter Dependence Analyzes." Applied Surface Science 424: 368–73. https://doi.org/10.1016/j.apsusc.2017.02.106.
- Pruna, Raquel, Francisco Palacio, and Manel López. 2017. "Towards Nanostructured ITO-Based Electrochemical Sensors: Fabrication, Characterization and Functionalization." Proceedings 1 (10): 288. https://doi.org/10.3390/proceedings1040288.
- Qian Wu, James L. Chu, Stanislav S. Rubakhin, Martha U. Gillette, and Jonathan V. Sweedler. 2017. "Dopamine-Modified TiO 2 Monolith-Assisted LDI MS Imaging for Simultaneous Localization of Small Metabolites and Lipids in Mouse Brain Tissue with Enhanced Detection Selectivity and Sensitivity." Chemical Science 8 (5): 3926–38. https://doi.org/10.1039/C7SC00937B.
- Randviir, Edward P., and Craig E. Banks. 2013. "Electrochemical Impedance Spectroscopy: An Overview of Bioanalytical Applications." Analytical Methods 5 (5): 1098–1115. https://doi.org/10.1039/C3AY26476A.
- Revathi, C., and R. T. Rajendra Kumar. 2019. Enzymatic and Nonenzymatic Electrochemical Biosensors. Fundamentals and Sensing Applications of 2D Materials. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-102577-2.00007-5.
- Sharma, Vimal Kumar, Frantisek Jelen, and Libuse Trnkova. 2015. "Functionalized Solid Electrodes for Electrochemical Biosensing of Purine Nucleobases and Their Analogues: A Review." Sensors (Switzerland) 15 (1): 1564–1600. https://doi.org/10.3390/s150101564.
- Sima, F., C. Ristoscu, L. Duta, O. Gallet, K. Anselme, and I. N. Mihailescu. 2016. Laser Thin Films Deposition and Characterization for Biomedical Applications. Laser Surface Modification of Biomaterials: Techniques and Applications. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-100883-6.00003-4.

- Simões, Fabio Ruiz, and Miguel Gustavo Xavier. 2017. Electrochemical Sensors. Nanoscience and Its Applications. Elsevier Inc. https://doi.org/10.1016/B978-0-323-49780-0.00006-5.
- Soylemez, Saniye, Bilal Demir, Gizem Oyman Eyrilmez, Seçkin Kesici, Aytül Saylam, Dilek Odaci Demirkol, Salih Özçubukçu, Suna Timur, and Levent Toppare. 2016. "Comparative Cell Adhesion Properties of Cysteine Extended Peptide Architectures." RSC Advances 6 (4): 2695–2702. https://doi.org/10.1039/c5ra23352f.
- Tuna, Ocal, Yusuf Selamet, Gulnur Aygun, and Lutfi Ozyuzer. 2010. "High Quality ITO Thin Films Grown by Dc and RF Sputtering without Oxygen." Journal of Physics D: Applied Physics 43 (5). https://doi.org/10.1088/0022-3727/43/5/055402.
- Tvarozek, Vladimir, Tibor Hianik, Ivan Novotny, Vlastimil Rehacek, Waldemar Ziegler, Rastislav Ivanic, and Marek Andel. 1998. "Thin Films in Biosensors." Vacuum 50 (3–4): 251–62. https://doi.org/10.1016/S0042-207X(98)00050-5.
- Ukoba, K. O., A. C. Eloka-Eboka, and F. L. Inambao. 2018. "Review of Nanostructured NiO Thin Film Deposition Using the Spray Pyrolysis Technique." Renewable and Sustainable Energy Reviews 82 (March): 2900–2915. https://doi.org/10.1016/j.rser.2017.10.041.
- Wang, Hsiang-Wei, Cameron Bringans, Anthony J. R. Hickey, John A. Windsor, Paul A. Kilmartin, and Anthony R. J. Phillips. 2021. "Cyclic Voltammetry in Biological Samples: A Systematic Review of Methods and Techniques Applicable to Clinical Settings." Signals 2 (1): 138–58. https://doi.org/10.3390/signals2010012.
- Wang, Meiling, Mingzhu Cui, Weifeng Liu, and Xuguang Liu. 2019. "Highly Dispersed Conductive Polypyrrole Hydrogels as Sensitive Sensor for Simultaneous Determination of Ascorbic Acid, Dopamine and Uric Acid." Journal of Electroanalytical Chemistry 832: 174–81. https://doi.org/10.1016/j.jelechem.2018.10.057.
- Winkler, Thomas, Hans Schmidt, Harald Flügge, Fabian Nikolayzik, Ihno Baumann, Stephan Schmale, Hans Hermann Johannes, et al. 2012. "Realization of Ultrathin Silver Layers in Highly Conductive and Transparent Zinc Tin Oxide/Silver/Zinc Tin Oxide Multilayer Electrodes Deposited at Room Temperature for Transparent Organic Devices." Thin Solid Films 520 (14): 4669–73. https://doi.org/10.1016/j.tsf.2011.10.122.
- Wolfe, N., M. L. Albert, A. Almozlino, R. Durso, L. Volicer, D. I. Katz, and M. C. Smith. 1990. "Neuropsychological Profile Linked to Low Dopamine: In Alzheimer's Disease, Major Depression, and Parkinson's Disease." Journal of Neurology, Neurosurgery and Psychiatry 53 (10): 915–17. https://doi.org/10.1136/jnnp.53.10.915.
- Wu, Dong, He Xie, Haifeng Lu, Wei Li, and Qunlin Zhang. 2016. "Sensitive

Determination of Norepinephrine, Epinephrine, Dopamine and 5-[Ag(HIO6)2]5--Luminol Hydroxytryptamine by Coupling HPLC with Chemiluminescence Detection." Biomedical Chromatography 30 (9): 1458-66. https://doi.org/10.1002/BMC.3704.

Xu, Jinzhong, Jun Jie Zhu, Qing Huang, and Hong Yuan Chen. 2001. "A Novel DNA-Modified Indium Tin Oxide Electrode." Electrochemistry Communications 3 (11): 665-69.

https://doi.org/10.1016/S1388-2481(01)00245-4.

- Yang, Lu, Dong Liu, Jianshe Huang, and Tianyan You. 2014. "Simultaneous Determination of Dopamine, Ascorbic Acid and Uric Acid at Electrochemically Reduced Graphene Oxide Modified Electrode." Sensors and Actuators, B: Chemical 193: 166-72. https://doi.org/10.1016/j.snb.2013.11.104.
- Yang, Zhen, Mingji Li, Hongji Li, Huavi Li, Cuiping Li, and Baohe Yang. 2020. "Polycrystalline Boron-Doped Diamond-Based Electrochemical Biosensor for Simultaneous Detection of Dopamine and Melatonin." Analytica Chimica Acta 1135: 73-82. https://doi.org/10.1016/j.aca.2020.08.042.
- Yilmaz, Nilay, Ece Eksin, Bilge Karacicek, Yasemin Eraç, and Arzum Erdem. 2017. "Electrochemical Detection of Interaction between Capsaicin and Nucleic Acids in Comparison to Agarose Gel Electrophoresis." Analytical Biochemistry 535 (October): 56-62. https://doi.org/10.1016/j.ab.2017.07.023.
- Yoo, Eun Hyung, and Soo Youn Lee. 2010. "Glucose Biosensors: An Overview of Use in Clinical Practice." Sensors 10 (5): 4558-76. https://doi.org/10.3390/s100504558.
- Zhang, Hangiang, Qitong Huang, Yihong Huang, Feiming Li, Wuxiang Zhang, Chan Wei, Jianhua Chen, et al. 2014. "Graphitic Carbon Nitride Nanosheets Doped Graphene Oxide for Electrochemical Simultaneous Determination of Ascorbic Acid, Dopamine and Uric Acid." Electrochimica Acta 142: 125-31. https://doi.org/10.1016/j.electacta.2014.07.094.
- Zhao, Peng, Sha Chen, Mei Yang, Yongzhong Wang, Huibo Luo, Danqun Huo, Zhong Ji, and Changjun Hou. 2021. "A Novel Multifunctional Platform Based on ITO/APTES/ErGO/AuNPs for Long-Term Cell Culture and Real-Time Biomolecule Monitoring." 122232. Talanta 228 (June): https://doi.org/10.1016/j.talanta.2021.122232.
- Zhu, Qing, Jing Bao, Danqun Huo, Mei Yang, Changjun Hou, Jianfeng Guo, Mei Chen, Huanbao Fa, Xiaogang Luo, and Yi Ma. 2017. "3D Graphene Hydrogel - Gold Nanoparticles Nanocomposite Modified Glassy Carbon Electrode for the Simultaneous Determination of Ascorbic Acid, Dopamine and Uric Acid." Sensors Chemical and Actuators, B: 238: 1316-23. https://doi.org/10.1016/j.snb.2016.09.116.