

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**PREPARATION OF MOLECULARLY IMPRINTED
NANOSENSOR FOR DOPAMINE DETERMINATION**

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March, 2022

İZMİR

PREPARATION OF MOLECULARLY IMPRINTED NANOSENSOR FOR DOPAMINE DETERMINATION

**A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylül
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by

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Ph.D. THESIS EXAMINATION RESULT FORM

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PREPARATION OF MOLECULARLY IMPRINTED NANOSENSOR FOR DOPAMINE DETERMINATION

ABSTRACT

Dopamine is a catecholamine class neurotransmitter found in the mammalian central nervous system and has a very important role in metabolic functions, cardiovascular, renal and hormonal systems. Rapid, easy, selective and low-cost analysis of dopamine can be achieved by the use of molecularly imprinted polymers, which are only dopamine-specific artificial receptors, in the preparation of nanobiosensors for dopamine determination for the early diagnosis of such diseases. Within the scope of this thesis, two separate sensitive, selective, inexpensive and miniaturizable sensor systems have been developed for dopamine determination by combining the advantages of nanotechnology with the selectivity of molecular imprinting technology and the sensitivity of biosensor systems. In the first system, an electrochemical impedance-based (EIS) sensor was designed by using molecularly imprinted fullerene modified pyrrole-pyrrole-3-carboxylic acid copolymers on a screen-printed carbon electrode. The performance parameters of the sensor system were determined by the calibration curve between 25- 250 ng/mL ($R^2= 0.9939$) and the LOD and LOQ were found to be 8.77 ng/mL and 26.6 ng/mL, respectively. The results obtained with the developed sensor were compared with the ELISA method and good correlation with $R^2= 0.979$ was observed between ELISA and developed sensor. In the second system, an EIS based sensor was prepared by using molecularly imprinted Cys-APBA-APBA modified Pyrrole-3-carboxylic acid copolymers on a screen-printed gold electrode. The performance parameters of the sensor system were determined by the calibration curve between 100- 600 pM ($R^2= 0.9517$) and the LOD and LOQ were found to be 30.34 pM and 95.82 pM, respectively. In the third system, an EIS based sensor was prepared by using molecularly imprinted Cys-PAMAM modified Pyrrole-3-carboxylic acid polymer on a screen-printed gold electrode for A β -42. The performance parameters of the sensor system were determined by the calibration curve between 0.5- 200 ng/ml ($R^2= 0.9837$) and the LOD and LOQ were found to be 0.14 ng/ml and 0.42 ng/ml, respectively.

Keywords: Dopamine, A β -42, EIS, MIP sensor

DOPAMİN TAYİNİ İÇİN MOLEKÜLER BASKILANMIŞ NANOSENSÖR HAZIRLANMASI

ÖZ

Dopamin, memeli merkezi sinir sisteminde bulunan ve metabolik fonksiyonlarda, kardiyovasküler, renal ve hormonal sistemlerde çok önemli bir role sahip olan katekolamin sınıfı bir nörotransmitterdir. Dopaminin hızlı, kolay, seçici ve düşük maliyetli analizi, nanobiyosensörlerin hazırlanmasında sadece dopamine özgü yapay reseptörler olan moleküler baskılanmış polimerlerin kullanılmasıyla sağlanabilir. Bu tez kapsamında, nanoteknolojinin avantajları ile moleküler baskılama teknolojisinin seçiciliği ve biyosensör sistemlerinin duyarlılığı bir araya getirilerek dopamin tayini için iki ayrı hassas, seçici, ucuz ve minyatürleştirilebilir sensör sistemi geliştirilmiştir. İlk sistemde, bir elek baskılı karbon elektrot üzerinde moleküler olarak damgalanmış fulleren modifiye pirol-pirol-3-karboksilik asit kopolimerleri kullanılarak elektrokimyasal impedans temelli bir sensör tasarlanmıştır. Geliştirilen sensör sisteminin performans parametreleri 25-250 ng/mL ($R^2= 0,9939$) arasında kalibrasyon eğrisi ile belirlenmiş ve LOD ve LOQ sırasıyla 8,77 ng/mL ve 26,6 ng/mL olarak bulunmuştur. Geliştirilen sensör ile elde edilen sonuçlar ELISA yöntemi ile karşılaştırılmış ve ELISA ile geliştirilen sensör arasında $R^2= 0.979$ ile iyi bir korelasyon gözlemlenmiştir. İkinci sistemde, moleküler baskılanmış Cys-APBA-APBA modifiye Pyrrole-3-karboksilik asit kopolimerleri altın ekran baskılı elektrot (SCE) üzerinde kullanılarak EIS tabanlı bir sensör hazırlanmıştır. Sensör sisteminin performans parametreleri 100-600 pM ($R^2= 0.9517$) arasındaki kalibrasyon eğrisi ile belirlenmiş, LOD ve LOQ sırasıyla 30.34 pM, 95.82 pM olarak bulunmuştur. A β 1-42 tayini için, Cys-PAMAM modifiye ekran baskılı altın elektrot üzerinde Pirol-3-karboksilik asit polimeri ile moleküler baskılanmış EIS temelli bir sensör geliştirilmiştir. Sensör sisteminin performans parametreleri, kalibrasyon eğrisi ile 0,5-200 ng/ml ($R^2= 0,9837$) arasında belirlenmiş ve LOD ve LOQ sırasıyla 0,14 ng/ml ve 0,42 ng/ml olarak bulunmuştur.

Anahtar kelimeler: Dopamin, A β -42, EIS, MIP sensör

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ABBREVIATIONS

AD	: Alzheimer's disease
AFM	: Atomic Force Microscope
AIBN	: Azobisisobutyronitrile
AM	: Acrylamide
AP	: Amyloid plaque
APBA	: Aminophenylboronic acid
APP	: Amyloid precursor protein
APS	: Ammonium persulfate
A β	: Amyloid beta peptide
CI	: Chronoimpedance
CSF	: Cerebrospinal fluid
CV	: Cyclic voltammetry
DA	: Dopamine
DPV	: Differential pulse voltammetry
EIA	: Enzyme immunoassays
EIS	: Electrochemical Impedance Spectroscopy
ELISA	: Enzyme-Linked Immunosorbent Assay
FRA	: Frequency response analyzer
GC/MS	: Gas chromatography-mass spectrometry
GCE	: Glassy carbon electrode
GNPs	: Graphene nanoparticles
HPLC	: High performance liquid chromatography
IS	: Impedance spectroscopy
LOD	: Limit of Detection
LOQ	: Limit of determination
LST	: Linear systems theory
MAA	: Methacrylic acid
MIP	: Molecularly imprinted polymers

MIT	: Molecular imprinted Technology
NFY	: Neurofibrillary tangle
OTA	: Ochratoxin A
PPy	: Polypyrrole
PPy-3-COOH	: Pyrrole 3 carboxylic acid
Py	: Pyrrole
QCM	: Quartz crystal microbalance
SEM	: Scanning Electron Microscopy
SPR	: Surface plasmon resonance
XPS	: X-ray Photoelectron Spectroscopy

CHAPTER I

INTRODUCTION

1.1 Neurodegenerative Diseases

Neurodegenerative diseases (Alzheimer and Parkinson), are defined as progressive diseases that do not go away and cause severe memory loss and physical dysfunction in the patient in advanced stages. Early diagnosis of both diseases is of vital importance, since no effective treatment has yet been found. Today, the diagnosis of these diseases is possible by measuring biomarkers such as dopamine, Amyloid β 42, Tau, Phosphorylated Tau and α -Synuclein, which increase or decrease in body fluids during the development of the disease.

The word dementia is derived from the Latin word 'mens' meaning mind, and 'demens' means loss of mind. Dementia is a brain disease that is characterized by progressive deterioration in many cognitive functions, especially memory, and leads to deterioration in occupational and social functionality. Alzheimer's disease (AD), which is one of the most common types of dementia, is an irreversible, progressive disease with an insidious onset, slow course, and other cognitive functions deteriorated with the inclusion of neocortical involvement (Galasko, D., Edland, S. D., Morris, J. C., Clark, C., Mohs, R., & Koss, 1995). Advances in medicine and technology have facilitated the diagnosis and treatment of many diseases, and as a natural result, the life expectancy of people has increased and the standard of living has increased. This has led to an increase in the elderly population. The increase in the elderly population has also brought along the problems that arise with aging (Selekler, K., B. Cangöz, 2010). This disease is the leading cause of death in individuals over 65 years of age in developed countries after cancer, stroke and heart diseases (Şenuzun Ergün, F., 2005). The clinical manifestation of the disease is variable. When Alzheimer's patients are clinically examined, deterioration in the ability to learn new information or remember previously learned information, speech disorder (for example, difficulty finding the right word), inability to perform motor activities even though motor functions are preserved, not recognizing objects although sensory functions are preserved, planning, organization It is observed that there is deterioration in functions such as focusing

attention, grasping what is under the appearance. These symptoms cause significant deterioration in the social and professional life of the person compared to the pre-illness. In the most advanced stage of the disease, the Alzheimer's patient may need 24-hour care. Patients may become unable to meet their nutritional, cleaning and toilet needs. AD is the most common form of age-related dementia, and its prevalence and incidence are highly correlated with age. In a study conducted in the United States, the incidence of AD progression was 4% under the age of 65, 13% between the ages of 65-74, 44% between the ages of 75-84, and 38% over the age of 85 years. The incidence of the disease (frequency of occurrence of new cases of a particular disease) is 1% in the 60-70 age group, while the incidence is 6-8% over the age of 85 years. It is known that there are more than 24.2 million Alzheimer's patients in the world and over 5 million new cases are reported every year. By 2050, it is predicted that there will be 106.23 million Alzheimer's cases worldwide (Gonçalves, Lima, Moraes, & Ribeiro, 2016). Although there is no comprehensive epidemiological study conducted in our country, it is estimated that there are approximately 500 thousand Alzheimer's patients.

Histologically, amyloid plaque (AP) and neurofibrillary tangle (NFY) formation, synapse and neuron loss, and marked atrophy in the brain are observed in Alzheimer's Disease. Both lesions can be seen both in normal aging and in some other neurodegenerative diseases; however, the lesions must show a certain neuroanatomical distribution and be in a certain amount for a definitive diagnosis of AD. The main component of NFYs is the 'hyperphosphorylated tau' protein. The tau protein is a protein from the microtubule-associated proteins (MAP) family located on chromosome 17. It plays an important role in stabilization of microtubules, cytoskeletal integrity and axonal transport. Pathophysiology of AD, hypoactive phosphatases or hyperactive kinases cause hyperphosphorylation of tau protein, impairing its ability to bind to microtubules. Unbound phosphorylated tau polymerizes into insoluble double-stranded filaments, and the newly formed modified form can be toxic. Affected neurons cause filament elongation and respond to toxic tau protein by synthesizing normal tau protein and wrapping and coiling abnormal hyperphosphorylated tau protein into neurofibrillary networks. These formations become intra-neuronal NFYs over time. NFY formation eventually causes cell death

by disrupting the integrity of the cytoskeleton and axonal transport. Amyloid plaques (AP), the second main neuropathological change in AD, may have different morphological structures; however, its main component is amyloid beta peptide ($A\beta$). $A\beta$ is a 40-42 amino acid peptide that formation of a proteolytic cleavage from amyloid precursor protein (APP), a transmembrane protein encoded on chromosome 21. In other words, $A\beta$ is one of the metabolism products of APP. Although no significant morbidity or mortality was observed in APP knock-out mice, there are studies indicating that APP may have neurotrophic and neuroprotective activity. As with all transmembrane proteins, APP has an intracellular carboxy terminus, a 28 amino acid segment traveling within the membrane, and an extracellular amino termini. $A\beta$ is a portion of APP that also contains the intramembrane 28 amino acid region.

APP is metabolized by cleavage a number of proteolytic enzymes. α -secretase cleaves APP about the middle of amyloid β , resulting in a new extracellular protein called sAPP instead of amyloid β . This molecule has been shown to have positive neurotrophic effects on neurons in cell cultures. Two other secretases (β -secretase and γ -secretase) cleave APP from its amino terminus (β -secretase) and carboxy terminus (γ -secretase) to form $A\beta$ as the product. The formed $A\beta$ s are 40-42 amino acids long, and the more amyloidogenic one is the 42 amino acid form, which is the first to precipitate. Following the precipitation process, $A\beta$ accumulates in the parenchyma and blood vessels as diffuse and neurotic plaques. This is known as the amyloid cascade hypothesis. Patients with early dementia have increased $A\beta$ -42 and amyloid oligomers in brain tissues and cerebrospinal fluid (CSF). Quantities of these structures are associated with cognitive decline. Abnormal phosphorylation of tau protein causes the formation of neurofibrillary tangles (NFT), which is a general characteristic of Alzheimer's and some other neurodegenerative diseases. In vitro studies have shown that the interactions of Apo-E isoforms with tau protein are different. Tau protein forms a stable complex with Apo-E3 instead of Apo-E4, abnormal phosphorylation of tau protein inhibits tau-Apo-E3 interaction. Apo-E3 can only bind to unphosphorylated tau protein. These data suggest that ApoE3 plays an important role in the tau-related process and may prevent the destabilization of the neuronal skeleton (Blennow et al., 2010). For the diagnosis of Alzheimer's disease, these biomarkers are measured by various methods.

For the determination of these biomarkers, enzyme-linked immunosorbent method (ELISA) (Hye et al., 2014), mass spectrometry (Doong, Lee, & Anitha, 2010) and surface plasmon resonance (Sparks et al., 2012) methods are used.

1.2 Dopamine

Dopamine is a catecholamine class neurotransmitter found in nervous system and crucial in metabolic functions, cardiovascular, renal and hormonal systems (S. Liu et al., 2012). Extreme abnormalities in dopamine levels can be a symptom of diseases as schizophrenia, Parkinson's and Alzheimer's. Monitoring the change in dopamine levels is a very effective way to control brain function (Yan, Feng, Chen, Li, & Zhu, 2008).

1.1.1 Biological significance of dopamine

Dopamine is a metabolite that has a very important role in monitoring the activity of the cardiovascular, hormonal, central nervous and renal systems. One of the causes of Parkinson's disease, which occurs when people lose the ability to perform controlled and smooth movements, is the loss of dopamine neurons in the nigrostriatal pathway (Sharma et al., 2013).

1.1.2 Dopamine determination methods

For dopamine determination, methods such as capillary electrophoresis (Bouri, Lerma-García, Salghi, Zougagh, & Ríos, 2012), chromatography (Britto-Júnior et al., 2021), high performance liquid chromatography (Sasa & Blank, 1977), flow injection (Nalewajko, Bort Ramírez, & Kojlo, 2004), spectrophotometry (Mamiński, Olejniczak, Chudy, Dybko, & Brzózka, 2005) and Enzyme-Linked Immunosorbent Assay (ELISA) (Kim, Jeon, Paeng, & Paeng, 2008) have been reported.

1.2.3.1 Capillary Electrophoresis

The migration of charged particles in a solution under the influence of an applied electric field is called as electrophoresis. These migration rates are different according to the charge and size of the particles, by making use of this feature, it is possible to separate the charged particles from each other by electrophoresis. In capillary electrophoresis, a device formed by immersing the two ends of a capillary column in which an electrolyte is placed into containers containing a Pt electrode and conductive solution is used. The charged particles in the sample given from one end of the capillary column are separated by the effect of the electrical field provided by the 2-30 kV direct current applied with the help of the electrodes, and they walk towards the other end of the capillary column and are also detected by a detector system there.

In their study, Zhang et al. determined dopamine by capillary electrophoresis method combined with amperometric determination. The linear detection range of the method was found to be 0.29 to 1.28 fmol (L. Zhang, Qv, Wang, & Cheng, 2003).

1.2.3.2 Chromatography

Chromatography is the process of moving and separating components in a sample over different time periods through mechanisms such as dispersion and adsorption by passing the mobile phase over a stationary phase. This method is technique that provides the separation and purification of the compounds in a mixture for quantitative and qualitative analysis.

Musshoff et al. used a gas chromatography-mass spectrometry (GC/MS) method after solid-phase extraction for the determination of dopamine. For this, N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) and chiral reagent (R)-(-)-2-phenylbutyryl chloride were used for the determination by making a two-step derivatization (Musshoff et al., 2000).

1.2.3.3 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is a technique based on the different interaction of molecules with adsorbent materials in a column, resulting in different elution times for certain compounds.

Zhao et al. developed a method for the determination of dopamine in which they combined reverse-phase HPLC with a fluorescent detector, called Fast, Easy, Inexpensive, Effective, Robust and Safe (QuEChERS). For this, a C18 column was used and the column derivatization was done with o-phthalaldehyde and 2-mercaptoethanol. The prepared system was used for the determination of dopamine in pig muscle. 60:40 methanol and deionized water (0.1% acetic acid, v/v) were used as mobile phase in my system, and the flow rate was determined as 0.8 mL/min. The detection limit was found to be 4 mg/kg (H. X. Zhao, Mu, Bai, Yu, & Hu, 2011).

1.2.3.4 Flow Injection Chemiluminescence

Chemiluminescence is a phenomenon observed as a result of the conversion of some of the energy released in an exothermic reaction between two molecules into radiation instead of heat. The main reason for this situation is that the energy released during the reaction makes the product electronically excited.

An electronically excited molecule can radiate as it recovers from its excess energy, or it can excite a different molecule to radiate.

Wabaidur et al. developed a sensitive flow injection-chemiluminescence method for dopamine determination. For this, it is based on examining the effect of dopamine on the chemiluminescence emission of potassium permanganate prepared in formaldehyde acid. With this method, the linear detection range and detection limit are found 3.1×10^{-8} – 1.7×10^{-5} mol/L and 1.0×10^{-8} mol/L (n=11, SD:2.1%), respectively (Wabaidur, Alothman, Alam, & Lee, 2012).

1.2.3.5 Spectrophotometry

Spectroscopy is defined as the measurement of the absorbed or emitted radiation during the transition of atoms, ions or molecules from the lower energy level to the higher energy level. This technique allows analysis based on measurements between light and matter.

Guo et al. performed the spectrophotometric determination of dopamine hydrochloride in the study they presented. They used potassium ferricyanide-Fe(III) for this and developed a sensitive and simple method. The principle of the method is based on the deoxidation of Fe(III) by dopamine hydrochloride to Fe(II) at pH 4.0 and then the reaction of Fe(II) with potassium ferricyanide to form a Prussian blue soluble compound. Dopamine determination was made by monitoring the absorbance of this compound at a wavelength of 735 nm with the help of a spectrophotometer. The apparent molar absorption coefficient for the indirect detection of dopamine hydrochloride was found to be $3.2 \times 10^{-4} \text{ L mol}^{-1} \text{ cm}^{-1}$. The application of the developed method has been made to a wide spectrum of samples, such as physiological, pharmaceutical and food (banana) (Guo, Zhang, & Li, 2009).

1.2.3.6 Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent (ELISA) is one of the enzyme immunoassays (EIA). EIA is a set of techniques for quantitatively determining antigen-antibody reactions, the presence and concentration of molecules in physiological fluids. The purpose of this test is to provide interaction with antigens on the plaque surface. Diluted serum samples are placed in areas containing antibodies. After waiting for the time required by the test, the serum or material in the environment is removed.

It is based on the principle of reducing the substrate as a result of an enzymatic reaction based on the antigen-antibody relationship in the medium and dependent on the secondary antibody and determining the color formed spectrophotometrically. The presence and density of the target molecule is directly proportional to its spectrophotometric absorbance (Aydin, 2015).

Kim et al. performed dopamine determination by ELISA method. The test was carried out using 5 $\mu\text{g mL}^{-1}$ BSA-DA and 1/7500 dilution of anti-DA antibody. The detection limit and linear detection range of the method were 1.0×10^{-9} M, 3.2×10^{-8} M to 3.2×10^{-7} , respectively. The applicability studies of the system were carried out using serum (Kim et al., 2008).

However, these methods for the determination and quantification of dopamine and metabolites often involve sample preparation, needs expensive equipment, and lower specificity (S. Liu et al., 2012). As an alternative to these methods, low cost, easy to prepare, high specificity electrochemical systems have taken their place in the literature. Dopamine is an electrochemically active molecule (Li, Zhao, & Wei, 2009). Despite the studies in the literature, studies are continuing to develop a method for the determination of dopamine in biological fluids, whose sensitive and selective measurement is very important for clinical diagnosis. Despite the selectivity of biosensor systems, the presence of dopamine in biological fluids together with ascorbic acid and uric acid and the large electrochemical oxidation potential of these molecules limit the electrochemical detection of dopamine. Molecularly imprinted affinity biosensor systems allow selective and sensitive analysis by creating structures specific to the target analyte.

1.3 Biosensors

Sensors are small sensing devices used to monitor target analytes in chemical or biological reactions (Turner, 1987). Biosensors are systems developed by combining highly selective biomolecules with analytical measurement methods and providing measurement in complex substances, and they have a wide area of use in the clinical field (Hilmiye Deniz Ertuğrul Uygün & Demir, 2020; Hasan et al., 2021; Mehrotra, 2016; Meza López, Khan, Picasso, & Sotomayor, 2021; H. Zhao, Wang, Quan, & Tan, 2013). The most important part of a sensor is the sensing element, where recognition takes place, as it affects the overall selectivity of the entire sensor.

Recognition occurs on the recognition element and transducer. The concentration changes its electrical signal with the appropriate transducer. The transducer is responsible for the sensitivity of the sensor and works with the sensing element to

create a dynamic concentration range. The detected signal by transducer is amplified by a device located near or integrated into the transducer (Malhotra & Chaubey, 2003). The elements that create a biosensor are shown in Figure 1.

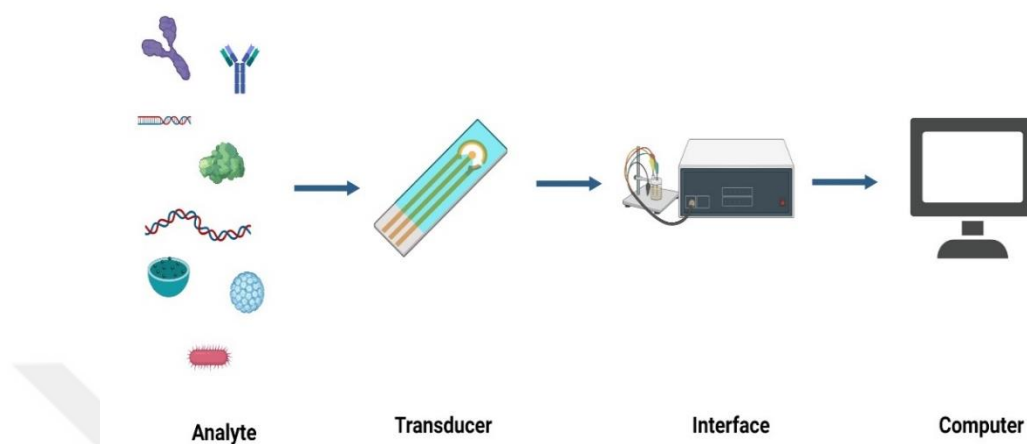


Figure 1.1 Schematic representation of biosensor elements

In these systems, enzymes, antibodies, receptors, DNA etc. molecules can be used as biorecognition agents (D'Souza, Kumar, Jha, & Kubal, 2013; Devasenathipathy et al., 2015; Ensafi, Rezaei, Amini, & Heydari-Bafrooei, 2012; Kaushik, Jayant, Tiwari, Vashist, & Nair, 2016; Zihni Onur Uygun & Dilgin, 2013). Biosensors are divided into two parts as bioaffinity and biocatalytic, according to the interaction feature of the biorecognition agent and the analyte molecule (Table 1.1). Bioaffinity-based biosensors are based on the affinity between the biorecognition molecule and the analyte molecule. With this affinity, the measurement system is performed by measuring the affinity of the analyte molecule to the biorecognition molecule.

Table 1.1 Classification of biosensors

BIOAFFINITY SENSORS		BIOCATALYTIC SENSORS	
RESEPTOR	ANALYTE	RESEPTOR	ANALYTE
Enzyme	Substrate,Inhibitor	Enzyme	Substrate
Apoenzyme	Prosthetic group	Microorganism	Cofactor
Antibody	Antigen	Organelle	Activator
Receptor	Hormone	Tissue section	Inhibitor
Lectin	Glycoproteins Saccharides Proteins		Enzyme

Sensor systems are also classified according to the characteristics of the signal converter (transducer) system used in the sensor system. Classification of chemical sensors by working principle of the signal converter is given in Table 1.2 (Hulanicki, Glab, & Ingman, 1991).

Table 1.2 Classification of sensors according to the signal converter used

OPTICAL	Absorbance Reflectance Luminescence Fluorescence Refractive Index Light Scattering Optothermal Effect
ELECTROCHEMICAL	Voltammetric sensors potentiometric sensors Chemically sensitive field-effect transistor (CHEMFET)
ELECTRIC	Metal oxide semiconductor sensors Organic semiconductor sensors Electrolytic conductive sensors Electrical Transmittance Sensors
MASS SENSITIVE	Piezoelectric Surface acoustic wave

Biosensor systems developed on the basis of bioaffinity are biosensor systems that based on the binding kinetics of biomolecules and analytes. In this type of biosensors, systems in which immune system molecules are used as biorecognition agents are known as immunosensors. It is possible to make highly specific diagnoses in the healthcare field with biosensors (Haji Mohammadi et al., 2021; Koo, Soda, & Shiddiky, 2021; Nguyen, Duong, Park, & Yeo, 2022; Q. Wang et al., 2022). Electrochemically developed affinity biosensors are generally used by receiving a signal from a secondary molecule or a marker molecule.

In these developed biosensor systems, measurement can be performed by a measurable change of the electrical current by a secondary molecule, in addition, the interaction of the bioreceptor-analyte molecule can also be measured by surface capacitance or impedance without using the secondary molecule (Z.O. Uygun & Ertuğrul Uygun, 2014).

1.3.1 Application areas of biosensors

Biosensors are capable of detect any molecules for field such as clinical, environmental and food analysis. Today, with the development of technology, factors that have the potential to adversely affect human health, from the food we buy to the electronic devices around us, are increasing rapidly. Although the developments in technology increase in the field of health, there is also an increase in threat parameters. Serious ailments occur with the impact of difficult life conditions, foods with a modified natural structure and environmental factors. For this reason, the need for accurate and rapid analyzes for clinical diagnosis and monitoring has increased. In recent years, studies have increased to respond to this need with biosensors. Looking at the historical background of biosensor technology, it is seen that the first studies in this field started with enzyme sensors. The "glucose oxidase enzyme electrodes" for glucose determination, reported by Clark and Lyons in 1962 and Updike and Hick in 1967, are the first examples in this regard. In the years since the first use of the Clark Oxygen Electrode sensor, there have been many improvements in the selectivity, sensitivity and reusability characteristics of biosensors (Monošik, Stred'ansky, & Šturdík, 2012). It is very important to monitor the concentration of glucose, which is an important marker in diabetes and endocrine disorders. The number of diabetes patients in the world (about 415 million) reveals the importance of monitoring this molecule (Onur Uygun & Ertuğrul Uygun, 2020). For this reason, biosensor development studies for glucose determination are frequently encountered in the literature. The first biosensor developed was L.C. It is an O₂ selective amperometric system used to monitor the oxygen level in the blood during surgery, prepared by Clark. Then, in 1962, it is an amperometric glucose biosensor prepared by immobilizing the glucose oxidase enzyme on the O₂ selective electrode surface (L C CLARK Jr, 1962).

Lactate is an important product of anaerobic metabolism. Clinical monitoring is very important. Because when the tissues are deprived of oxygen, lactate formed as a result of anaerobic respiration causes lactic acidosis. Lactic acidosis also causes shock, sepsis, and liver and kidney dysfunction. Animal products such as fermented food, milk, meat, fish, and pickled vegetables produce lactate. Therefore, lactate is an important marker in determining the degree of bacterial fermentation. Since this degree gives information about the freshness of the food, the determination of lactate is also very important in the food industry (Rathee, Dhull, Dhull, & Singh, 2016). The determination and monitoring of lactate, which is an important marker in the monitoring of heart failure, shock/trauma and metabolic disorders and food industry, is also possible with biosensors (Onur Uygun & Ertuğrul Uygun, 2020). There are many biosensor studies on this molecule in the literature (H. D. E. Uygun, Tinkilic, Attar, & Isildak, 2016). Hirst et al. designed a system for lactate determination using polyethyleneimine modified prussian blue printed electrodes. The measurement results obtained in the clinical peritoneal drainage fluid with the system using amperometry as the measurement method were compared with a commercial lactate assay kit (r_s 0.891, $P = 0.001$) (Hirst, Hazelwood, Jayne, & Millner, 2013). Zanini and her work group prepared a biosensor for the determination of lactate in food samples by immobilization of gold nanoparticles and lactate oxidase enzyme. Tracing of gold nanoparticle films was done with SPR and surface characterization was performed by CV and EIS measurements. The LOD of the system, in which the amperometry method was used as the measurement method, was found to be 0.47 μM (Paz Zanini et al., 2017).

In recent years, the number of high cholesterol patients is increasing with the increasing nutritional disorders and high stress factors. The risk of heart diseases, atherosclerosis and hypertension due to high cholesterol shows the importance of monitoring this molecule (Narwal et al., 2019). One of the commercialized biosensor systems is the cholesterol biosensor. Haritha et al. have designed an amperometric cholesterol biosensor that allows nanoscale measurement using multiple carbon nanotubes. The system was prepared by immobilization of cholesterol oxidase on glassy carbon electrode. Response time and detection limit of the biosensor were found to be 15 sec and 0.5 μM , respectively (Haritha, Kumar, & Rakhi, 2021).

Alagappan et al. determined cholesterol with the system they created using gold nanoparticles, ash, polypyrrole and multi-walled carbon nanotubes. Cholesterol oxidase was immobilized to the electrode surface and amperometrically measured cholesterol. The detection limit of the system applied in biological samples was found to be 1.0×10^{-3} M (Alagappan, Immanuel, Sivasubramanian, & Kandaswamy, 2020). Anirudhan and his working group prepared a cholesterol sensor based on molecular imprinting using silanated graphene oxide modified nanocellulose. Cyclic voltammetry and differential pulse voltammetry were used as electrochemical measurement methods. Response time and detection limit were found to be 10 minutes and $98.6 \mu\text{mol/L}$, respectively (Anirudhan, Deepa, & Binussreejayan, 2018).

The rapidly increasing number of cancer patients worldwide in recent years has increased the efforts to develop fast and reliable analysis methods for cancer detection. Biosensors are promising in this regard because they are fast, selective, low cost and practical enough to be used by the patient him/herself. Cancer types that occur in different tissues such as lung, prostate and colon have been reported as one of the most fatal conditions. Diagnosis is possible by monitoring biomarkers in blood, urine and different physiological fluids. All of the methods used are expensive methods that take time because they require expert personnel. Microscopic examinations of tissue taken with biopsy may miss cancerous cells at the beginning of the disease. Immunoassay tests such as ELISA are reliable methods, but they are time-consuming and expensive (Tothill, 2009). Biosensors can be used in the diagnosis of cancer with the advantage of point of care analysis (Rasooly & Jacobson, 2006). Besselink et al. developed a biosensor system for prostate-specific antigen detection in their study. In the study in which SPR was used as the measurement method, colloidal gold was used and the sensitivity of the system increased and the LOD was found to be 0.15 ng PSA/ml (Besselink, Kooyman, Van Os, Engbers, & Schasfoort, 2004). Sezginürk and Uygun performed the determination of vascular endothelial growth factor in their study. The linear determination range of the system prepared by covalent bonding of the PAMAM dendrimer to the cysteamine modified Au electrode was found to be $5 \text{ to } 125 \text{ pg/mL}$ (Sezginürk & Uygun, 2012). Zhao et al. developed an enzyme-based amperometric biosensor to detect hydrogen peroxide released from human breast cancer cells.

The LOD of the system prepared with Horse radish peroxidase (HRP)/peptide modification was found to be $3.0 \times 10^{-8} \text{M}$ and the linear determination range was $1.0 \times 10^{-7} \text{M}$ and $1.0 \times 10^{-4} \text{M}$ (J. Zhao, Yan, Zhu, Li, & Li, 2013).

Due to the increasing environmental pollution in recent years, monitoring of environmental elements such as soil, water and air gains more importance. Studies on the development of new alternative methods to the currently used chromatographic techniques have increased. Biosensors are useful devices that can be used in environmental analysis because they allow real-time analysis, are practical, portable and economical (Rodriguez-Mozaz, Alda, Marco, & Barceló, 2005). Microbial pollution can be detected in water with biosensor systems designed to measure biochemical oxygen demand (J. Liu & Mattiasson, 2002). Biosensor systems can be designed for the analysis of heavy metals in soil and water (Blake et al., 2001). Ayat et al. prepared an acetylcholine esterase-based electrochemical biosensor for the determination of pesticides, which are organophosphorus compounds. The linear detection range of the system was found to be 2-8 nM (Ayat, Ayouz, Yaddadene, Berouaken, & Gabouze, 2021). Radhakrishnan and Kumar developed a Single-stranded DNA (ssDNA) fluorescence biosensor for the determination of toxic Pb^{2+} ion and organophosphorus pesticide. The detection limit of the aptamer-based system was 0.6 nM for Pb^{2+} and 0.018 $\mu\text{g/L}$ for pesticide (Radhakrishnan & Kumar, 2022).

Biosensors are systems that can also be used for food safety and content monitoring. In the food industry, sensor systems can be designed for possible contaminants such as pesticides, additives, pathogen and drug residues (Yan Zeng, Zhu, Du, & Lin, 2016). Moghazy et al. developed an electrochemical aptasensor for OTA determination of toxic fungi in coffee and their secondary metabolite and ochratoxin A (OTA), one of the most harmful and poisonous mycotoxins. The detection limit of the sensor prepared using silanized nanofibers was found to be 0.81 pg/mL (El-Moghazy, Amaly, Istamboulie, Nitin, & Sun, 2020). Chen et al. developed an aptamer sensor based on electrochemical impedance spectroscopy for the determination of tetracycline. The response time of the system was 15 minutes and the detection limit was 1.0 ng/mL (D. Chen, Yao, Xie, & Liu, 2014).

Although biosensor systems seem to be highly advantageous and sensitive measurement systems due to the specificity of biological receptors for the analytes, the use of bioreceptors as sensing molecules limits the analysis in different environmental conditions. Optimum working conditions are required for efficient functioning of biological molecules. These are physical properties such as pH, temperature, pressure, light, ion strength, polarity of the measuring liquid. Even very small changes in these properties can affect a measurement. For example, acetylcholine esterase enzyme activity is extremely sensitive to pH changes. Another example is the regeneration of antigen-antibody based biosensors. In this process, disruption of antigen-antibody interactions is achieved by changing the ion strength, but in the meantime, molecules can be damaged. Another example is the effects of low ambient temperature. In this case, the activity of the enzyme used may not be observed/low observed. As in these examples, a number of interactions limit the use of biosensor systems and pose a major obstacle to practical applications. Artificial affinity-based sensor systems can be developed to overcome these disadvantages. With these systems, more stable sensing materials that are less affected by environmental conditions can be designed. The preparation of these artificial receptors is carried out by polymerizing monomers with special functional groups in such a way that they surround the analyte molecule according to their three-dimensional properties. Thus, it is possible to design specialized cavities on these polymers where certain molecules can enter. This artificial receptor creation technology is called molecular imprinting or molecular imprinting technology (MIT). Developed polymers of this type are also called molecularly imprinted polymers or molecularly imprinted polymers (MIP).

1.4 Molecular Imprinting Technology

Molecular imprinting technology (MIT) is the technology of producing cavities (structures) on a polymer to fit the three-dimensional structure of a specific molecule (target or template) in complex solutions. In other words, the “fingerprint” of the target molecule is created on a desired polymer molecule. Thus, polymers containing cavities that only this molecule can enter can also be used as artificial receptors. MIT has different functions such as molecular recognition, catalysis, chromatographic separation, chemical recognition (O. S. Ahmad, Bedwell, Esen, Garcia-Cruz, &

Piletsky, 2019; Beltran, Borrull, Marcé, & Cormack, 2010; Karsten Haupt and Klaus Mosbach, 2000; Martín-Esteban, 2013; Turiel & Martín-Esteban, 2010). There are two different approaches used when preparing molecularly imprinted polymers.

In the first method, a prepolymerization complex can be formed between the target molecule and functional monomers through noncovalent interactions (self-assembly).

The other method is the approach developed by Wulf et al. (Wulff, 1995) where the target molecule and monomers are covalently bonded. Because of the higher stability of covalent bonds, the covalent imprinting approach offers a more homogeneous binding site and binding efficiency than the non-covalent approach. However, the non-covalent approach developed by Mosbach et al. provides a more flexible working opportunity due to the wide range of functional monomers and target molecules used. In addition, the interactions in the processes in nature are also dominated by noncovalent interactions (Haupt & Mosbach, 2000). The methods used in the synthesis of molecularly imprinted polymers can be listed as bulk polymerization, surface grafting and electropolymerization (Sergey A. Piletsky* and Anthony P. F. Turner Institute, 2017). MIPs prepared by electropolymerization are frequently used in electrochemical sensors (Onur Uygun & Ertuğrul Uygun, 2020). The monomers of the artificial polymeric receptor to be formed are selected according to whether the monomer contains a functional group or groups that will interact with the target molecule to be attached. Polymers prepared by molecular imprinting have been successfully used for chiral separation of amino acid and sugar derivatives and drugs, for antibody-receptor-like proteins and protein analogs, as enzyme analogs in organic reactions and as ion-selective adsorbents. Molecular imprinted polymers are used as recognition elements in sensor technology, which is one of the application areas of molecular imprinting technology (Gao et al., 2011; Takeuchi, Murase, Maki, Mukawa, & Shinmori, 2006; Ünlüer, Ersöz, Denizli, Demirel, & Say, 2013).

1.4.1 Molecular Imprinted Polymer Synthesis Methods

Molecular imprinting is a method that a polymer network is formed by specific recognition for a template molecule (L. Chen, Xu, & Li, 2011). The monomers to be used to form this polymeric structure are selected according to the functional groups of the target molecule. Polymer structuring can occur by covalent or noncovalent bonds. After successful polymerization of the monomer and template molecule, the template molecule is removed from the polymeric structure with a suitable desorption agent. The structure formed after this process is a special and specific to the template molecule (Kryscio & Peppas, 2012). Molecularly imprinted polymers can be synthesized by mechanisms such as bulk polymerization, photopolymerization, sol-gel and surface etching polymerization (Vasapollo et al., 2011).

1.4.1.1 Bulk Polymerization

Most MIPs are prepared by dissolving the bulk polymerization monomer and crosslinker with a minimal amount of porogenic solvent. This type of polymerization is often used for the synthesis of step growth polymers that have a lower heat of polymerization and reactivity. The monomer and radical initiator used in this method should be in liquid form. The initiator molecule must be dispersed in the monomer (Vasapollo et al., 2011). It is also a very environmentally friendly polymerization method because purification is not necessary because the resulting polymers are of very high purity and the final product is a 100% solid resin. However, bulk polymerization has numerous limitations and disadvantages such as being not practical for very exothermic reactions, because of difficulty in the control of temperature and mixing problems created by growing viscosity. This method is the first strategy used to synthesize MIPs. With this procedure, grinding and sieving steps are inevitable, and the particles obtained after these steps have poor binding site accessibility for the target analyte and have heterogeneous particle size distribution (Martín-Esteban, 2013).

1.4.1.2 Photopolymerization

Photopolymerization is a frequently preferred method because it is an advantageous method. These advantages can be listed as not requiring solvent, efficient energy use, being economical and preferable for heat sensitive materials. The method is based on the use of UV light for the production of highly cross-linked polymers with multifunctional monomers in the presence of a suitable photoinitiator. Initiators used in this method can be grouped into three classes as free radical, cationic or anionic. Also, some initiators such as iodonium and sulfonium salts, arene complexes can use both free radical and cationic processes (Andrzejewska, 2001).

1.4.1.3 Free Radical Polymerization

Free radical (or chain growth) polymerization is a very useful synthetic method for built polymers from monomers and is widely used for the production of a number of commercially important plastics on a very large scale (quantities expressed in tons).

Many industrially important vinyl monomers, such as methyl methacrylate, styrene and ethylene can be polymerized very effectively with free radical polymerization methods in excellent yields. Free radical polymerizations can be performed in bulk or in solution under easy reaction conditions such as atmospheric pressures and room temperature. The free radical polymerization mechanism takes place in three stages: 1) initiation, (2) growth, and (3) termination. Many chemicals can be used as radical initiators such as azobisisobutyronitrile (AIBN) (Cormack & Elorza, 2004).

1.4.1.5 Suspension Polymerization

Suspension polymerization is a technique used in the production of very common thermoplastics such as polyvinylchloride and polystyrene. This method is a heterogeneous radical polymerization process that uses mechanical agitation in the aqueous phase to mix the monomer or monomer mixture, forming polymer spheres. The biggest advantage over emulsion methods is that it is easy to purify because surfactants that are difficult to remove from the final product are not used. This method, in which temperature and viscosity control is much easier, is also economical and environmentally friendly (Mayes & Mosbach, 1996).

1.4.1.6 Emulsion Polymerization

Emulsion is a radical polymerization that takes place in a system where the surfactant and monomer are dispersed in water. Spherical polymers can be obtained by emulsion polymerization. The most common type of emulsion polymerization, an oil-in-water emulsion is the most common type of polymerization and is the method in which oil droplets are emulsified in the water phase with surfactant. Emulsion polymerization consists of a monomer, initiator, a medium in which the emulsion takes place, and modifying agents. Its advantages listed as method is providing fast production and can be formed spherical particles Disadvantages can be listed as complex processes and use of stabilizers and surfactants. These structures are difficult to remove from polymer and can contaminate MIPs, for other polymerization adjuvants (additives) and dried polymers, removal of water is an energy intensive process (Cheong, Yang, & Ali, 2013).

1.4.1.7 Solution Polymerization

In solution polymerization, the monomer, initiator and the resulting polymer are all dissolved in the solvent or solvent mixture. The polymerization initiator, the monomer, is initiated by adding a small amount of catalyst to a suitable solvent medium. The reason for adding more solvent as the reaction progresses is to regulate the viscosity. The ratio of monomer used in solution polymerization to general concentration is higher than in emulsion polymerization. The end product as a result of this polymerization is generally lower molecular weight polymers. This situation can be considered as a disadvantage of the method. Another disadvantage of the method is the formation of a high viscosity medium. This method is generally preferred where polymer solutions are used directly as solvent-based coatings or (pressure sensitive) adhesives (Schmidt & Ray, 1981).

1.4.1.8 Precipitation Polymerization

Precipitation polymerization is a widely used method to obtain microsphere shaped polymers. The advantages of this method can be counted as not needing additives and being easy to prepare. This polymerization is a heterogeneous method that does not use surfactant, which contains monomer and crosslinker in a mixture of solvent and porogen. During polymerization, the growing polymer chains consume the monomers in the environment and micron-sized particles are formed. Monomers are selected from molecules with functional groups that will form non-covalent bonds with the template molecule. The most commonly used monomers are methacrylic acid (MAA) and acrylamide (AM) (Pardeshi & Kumar, 2016).

1.4.1.9 Electropolymerization

After the first study of the electrical conductivity of conjugated polymer (polyacetylene) was published by Shirakawa et al in 1977 (Shirakawa, Louis, MacDiarmid, Chiang, & Heeger, 1977), studies on conductive polymers increased rapidly. In electrochemically initiated polymerizations, conductive polymers generally begin to grow as a result of oxidation on the anode surface. A certain amount of polymer may also occur in the solution. Polymerizations occurring in the cathode region are less common. The oxidized monomer usually forms a radical cation, and after the addition of new monomer, as a result of electron transfer, positively charged polymer chains are formed on the surface. The structure, molecular weight, conductivity and physical durability of the obtained polymer vary according to the experimental conditions. Organic solvents with high dielectric constant but not nucleophilic character are generally preferred as solvents. Polymers produced by this method can be used in the preparation of sensors, light-emitting diodes, capacitors and various coatings.

Different techniques such as constant potential (potentiostatic) and constant current (galvanostatic) can be used to perform polymer synthesis (54). In 1979 (Kanazawa et al., 1979) electropolymerization of pyrrole became a widely used polymerization method with the production of polypyrrole by oxidizing pyrrole by electrochemical

method. Among the many conductive polymers prepared to date, polypyrrole has been studied a lot. The reasons for this intense interest in polypyrrole are certainly that the monomer (pyrrole) is easily oxidized, dissolves in water, and is commercially available. Therefore, polypyrrole is a widely used conductive polymer due to its advantages such as stability to environmental factors, good redox properties and ability to impart high electrical conductivity (Sadki, Schottland, Brodie, & Sabouraud, 2000).

Within the scope of this thesis, dopamine recognition imprinted surfaces on the electrode surface were prepared by electropolymerization.

1.4.2 Measurement Methods Used in Molecular Imprinted Sensors

1.4.2.1 Molecular Imprinted Electrochemical Methods and Application Examples

The basics of electrochemistry is to study the interaction between matter and electricity. This interaction provides information about the analyte and allows for quantitative measurement. Electrochemical techniques used in molecularly imprinted sensors are mostly used to measure surface properties, binding kinetics and polymer rearrangements of conductors (Zihni Onur Uygun, Ertugrul Uygun, Ermis, & Canbay, 2015). Various electrochemical methods can be developed according to the working method and the measurement principle of the analyte. Different systems have been developed with current, potential and resistance measurement under voltammetric techniques.

The cyclic voltammetry method is a three-electrode system. In this method, a forward voltage scan is performed that varies linearly with time. After the applied voltage reaches the desired value, the voltage is reversed and returned to the starting point.

Since the forward and reverse voltage scanning is performed, this method is also called a cyclic voltammogram for the response curve obtained from the cyclic voltammetry. One of the most used methods for the study of electroactive species is cyclic voltammetry (CV). The reason why it is widely used in the fields of electrochemistry, organic chemistry and biochemistry is the ease of the method. CV is the first experiment to recognize the compound or electrode being analyzed in

electrochemical studies. The advantages of the method can be counted as allowing fast measurement and working in a wide potential. The voltammogram obtained at the end of the measurement taken with the CV is just like a spectrum. This voltammogram provides information as a function of the energy scan. The CV measurement is taken by placing the electrode in a stationary solution and is based on measuring the alternating potential and the current generated. The 3-electrode system used in this method consists of a working electrode, reference electrode and counter electrode. Silver/silver chloride electrode (Ag/AgCl) and saturated calomel electrode are commonly used as reference electrodes. The task of the reference electrode is to control the potential of the working electrode. A cyclic voltammogram is obtained by measuring the resulting current generated by the application of potential to the working electrode (Kissinger & Heineman, 1983).

Da Silva et al designed a novel electrochemical sensor to detect trimethoprim by electropolymerization of pyrrole (Py) and molecular imprinted polymer (MIP) synthesized on a glassy carbon electrode (GCE) in aqueous solution using cyclic voltammetry. Graphene nanoparticles (GNPs) were used to increase the sensitivity of the sensor with an increase in electrochemical conductivity in their study. The linear operating range of the prepared sensor was found to be between 10^{-6} and 10^{-4} M and the detection limit was found to be 1.3×10^{-7} M (da Silva, Pacheco, MCS Magalhães, Viswanathan, & Delerue-Matos, 2014).

Another method used for electrochemical measurements is differential pulse voltammetry (DPV). The method is based on measuring the current before each potential change and plotting the current difference as a function of the potential. The effect of the charging current can be reduced by sampling the current before the potential change. The name of the method is differential pulse voltammetry due to current measurement after applying linear pulses to the working electrode.

Since these applied pulses are placed on top of each other like stair steps, it is a derivative of staircase voltammetry (Laborda, González, & Molina, 2014).

Aghoutane et al. developed a sensor system based on electrochemical impedance spectroscopy for the determination of malathion (MAL), an organophosphate pesticide in olive oil and olives. The imprinting was carried out via N group of the MAL and

the MIP formation was carried out by using acrylamide, TEMED and ammonium persulfate as initiator including MAL. MAL detection was carried out electrochemically by CV, EIS and DPV methods were also used for the electrochemical characterization of the surface. The detection limit was calculated as 0.06 pg/ml and calibration curve was between 0.1 to 1000 pg/mL (Aghoutane, Diouf, Österlund, Bouchikhi, & El Bari, 2020).

The method based on current measurement at a constant applied voltage is called the amperometric method. The precision of the method depends on the applied voltage value. In order to find the voltage value to be applied, it is necessary to take the polarogram or voltammogram of the substance, that is, its electrochemical behavior should be known. The voltage to be applied is selected from the plateau region of the reduction or oxidation peak of the substance. Since a constant voltage is applied, this method can also be thought of as electrolysis. As the electrolysis time progresses, the analyte concentration decreases and therefore the current decreases in direct proportion to the square root of the time. The amperometry method, in which the variation of the current with time at a constant applied voltage is measured, is called chronoamperometry. In the chronoamperometry method, the current-time relationship is observed in a stationary environment when the potential of the working electrode is suddenly changed. First, the E1 potential, where there is no reduction, is applied to the working electrode. Then the potential is abruptly changed to E2. The E2 potential is chosen so that the reduction is diffusion controlled (Skoog, Holler, & Crouch, 2017).

Xue et al. reported a gold nanoparticle (AuNP)-doped MIP-based electrochemical sensor for amperometric detection of dopamine. In this study, dopamine (DA) was used as template molecule, functionalized AuNPs as functional monomers and p-aminobenzethiol (p-ATP) as crosslinker.

The AuNPs modified electrode was sinked in the electrolyte solution and MIP modified-AuNPs sensor was prepared by electropolymerization at a constant potential of 1.0 V for 400 seconds. Next, the electrode sinked in 0.5 M H₂SO₄ was treated with a continuous potential of -0.5 V for 400 seconds to remove the template molecule, and finally the electrode was dried under nitrogen flow. It has been determined that the developed sensor detects dopamine with high sensitivity and specificity, and it has also

been reported to have a linear range of $0.02 \mu\text{mol L}^{-1}$ to $0.54 \mu\text{mol L}^{-1}$ and a detection limit of 7.8 nmol L^{-1} (Xue et al., 2013).

Conductivity measurement (conductometry) is a physicochemical measurement method used to determine the total ion content of aqueous solutions. With this method, electrolytic properties of ions such as transport, migration, diffusion and mobility are measured using conductive techniques. Measurement takes place as a result of the molecules leaving the electrode and interacting with the solution to change their dielectric constant (Su, Jia, Hou, & Lei, 2011).

In the study of Sergeyeva et al., molecularly imprinted polymer membranes that consists of artificial recognition residues for Atrazine were prepared by photopolymerization, methacrylic acid as functional monomer and tri (ethylene glycol) dimethacrylate as crosslinker. Oligourethane acrylate was added to the monomer mixture to obtain thin, flexible and mechanically stable membranes. Non-imprinted membranes were prepared in same mixture but. The printed membranes were tested using an atrazine-sensitive conductometric (conductivity) sensor as the recognition element. The developed sensor showed high selectivity and sensitivity with a detection limit of 5 nM for atrazine. The synthesized membranes showed the same recognition properties over a six-month period. It has been observed that the sensor response time is 6-15 minutes depending on the membrane thickness (Sergeyeva et al., 1999).

Warburg was the first to apply the concept of impedance to electrochemical measurements at the beginning of the 19th century (Warburg, 1899). The method was then ideally used to measure the capacitance of electrodes such as the mercury electrode and was a pioneer in the development of electrical interface models. With the invention of the potentiostat in the 1940s and then the production of frequency response analyzers in the 1970s, the use of EIS has become widespread in the investigation of electrochemical and corrosion mechanisms (MacDonald, 2006). The EIS method is a powerful tool for measuring the dielectric properties of materials, investigating the physical properties of electrodes, and studying electrochemical reaction mechanisms. The advantages of the method are that it is a linear technique, that the impedance contains all the information that can be obtained from the system

if it is used in the infinite frequency range, and that the experimental efficiency (experimental/theoretical ratio) is very high. The accuracy of the data obtained from the method can be easily determined using Kramers–Kronig transformations (integral transformation techniques) (MacDonald, 2006).

Impedance spectroscopy (IS) can be divided into two classes: dielectric IS and electrochemical IS (EIS). The major difference between them is the material to be studied. Dielectric IS is used for the analysis of dielectric materials. Otherwise, EIS is used in the study of electronic materials where ionic conduction is common. Examples of these materials are polymers and electrolytes (solid and liquid). In addition, EIS is also used in the study of electrochemical/photoelectrochemical cells such as corrosion and fuel cells or solar cells. The method is based on the application of an AC voltage (or current in galvanostatic measurement) to the system to be studied and the analysis of the AC current (voltage) as a function of frequency. It can be done using a frequency response analyzer (FRA) or potentiostat for measurement and with 3-electrode or 2-electrode systems in the presence of a reference electrode (Sacco, 2017).

The EIS method is a frequently used method for surface characterization. In addition, it is widely used in sensor systems with the advantage of providing label free analysis without the need for a secondary molecule for measurement. EIS is a very effective measurement method used in the examination of the electrolyte-electrode interface, measurement of mass transfer rates, and investigation of electrode reactions. It is a highly sensitive electrochemical technique based on electrical circuit components (Pajkossy & Jurczakowski, 2017).

All studied electrochemical cell-based studies form a circuit. This circuit contains resistance, capacitance and complex impedance elements. In the electrochemical systems formed by these circuit elements, the applied signal is in the ratio of the applied signal. During the application of these electrical circuit components to the system, the creation of the circuit is very important for the content of the electrical cell to be understood. Therefore, circuits should be constructed as simply and clearly as possible (Zihni Onur Uygun & Dilgin, 2013; Zihni Onur Uygun & Ertuğrul Uygun, 2014).

The measurement basis of impedance is based on Ohm's law. According to Ohm's law, resistance is defined as R (Z), potential as V (E), and current as I . This usage is suitable for systems showing DC current. The equation below shows the relationship between potential, current and resistance;

$$Z = \frac{E}{I} \quad (1.1)$$

But real electrochemical systems are not that simple, on the contrary, they contain more complex components. The electrochemical double layer adds a capacitive circuit element to this equation. The other electrode process is time dependent diffusion. For this reason, impedance is used instead of resistance. Since time-varying alternating current is used in the system, frequency measurement is considered as the main theme of the system. The resulting impedance is defined by the following equation:

$$Z = \frac{E_t}{I_t} = \frac{E_0 \sin(\omega t)}{I_0 \sin(\omega t + \Phi)} = Z_0 \frac{\sin(\omega t)}{\sin(\omega t + \Phi)} \quad (1.2)$$

Z impedance, E potential, I current; t is treated as a function of time. Since AC current has a sinusoidal character, the time-dependent difference between current and potential is explained by the sinus function. Accordingly, the system measures the AC impedance, thus it is called AC impedance spectroscopy. In measuring this system, impedance spectra are generally used in two different diagrams; Nyquist and Bode diagrams. Example Figures of these two diagrams are given in Figure 1.2 and Figure 2.5. Both of these diagrams present impedance as a function of frequency. Nyquist diagram creates a frequency curve against real and imaginary values at every point of the measurement at different frequencies (Yuan X-Z., Song C., Wang H., 2010).

Both diagrams perform a scan from high frequency to low frequency. Thus, the initial resistance can be found. The most widely used of these two representations are Nyquist diagrams, they contain more data than Bode diagrams, allowing for more accurate interpretations. Nyquist diagrams can be of different types, the schematic representation of these types is given in below.

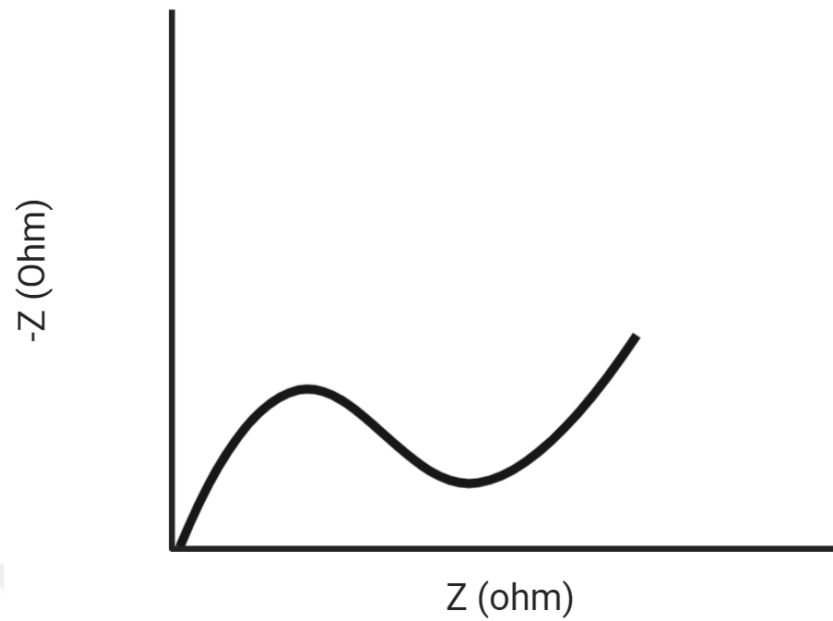


Figure 1.2 Schematic representation of Nyquist plot

In order to draw the circuit and fit the impedance curve on the circuit, the characterization of the interface should be done correctly and it should be well known which layer corresponds to which circuit element.

Impedance is defined as the effect that resists the movement of electrons. In the EIS system, resistance is defined as the electrode surface resistance that resists the transfer of electrons to the electrode surface. Depending on the potential applied to the electrode surface, an electrical double layer is formed on the electrode surface. This double layer consists of a charge balance on the electrode surface and ions collected on it. This double layer creates an electrical current. This is called capacitance. Along with these circuit elements, there are also frequency dependent elements. One of these elements is the Warburg impedance. This impedance is a resistance due to mass transfer. It is a circuit element that has resistance and capacitance on it. Another frequency dependent circuit element is the stationary phase element. Although it is not visible in these diagrams, it is a circuit element that is drawn constantly in the interval where the impedance curve first rises and is located on the X axis (Cai et al., 2010; Yuan X-Z., Song C., Wang H., 2010).

Bound Warburg and Bound Capacitance from other connected circuit elements are added to the system when a linear homogeneous diffusion layer of the double layer is formed. The impedance circuit elements are summarized in Table 1.3 (MacDonald, 2006).

Table 1.3 Representation of impedance circuit elements in the table

Circuit Elements	Definition
R	Resistance
C	Capacitance
W	Warburg
CPE	Constant Phase Element

Sundhoro et al., in their study, prepared a sensor for the detection of soy-based food allergens. MIP modified sensor has ability to detect food allergens in 100ppb concentration. The basis of the method is electrochemical surface investigation techniques such as CV and EIS. The method was also compared with food allergen lateral flow tests. In this study, genistein-specific cavities were formed by electropolymerization of o-phenylenediamine dihydrochloride at 1.2V in one minute. Imprinted occurred via N-H bonds. However, this study is an analyte centered redox method, therefore, quercetin and 7-hydroxyflavone redox potentials were used to detect food allergens on the screen-printed electrode in the presence of the genistein. The method was based on the non-covalent technique and performed successfully (Sundhoro et al., 2021).

In their study, Uygun et al. developed an EIS-based MIP sensor for cortisol detection. The molecularly imprinted sensor was prepared by polymerization of acrylamide with ammonium persulfate (APS) in the presence of cortisol. The linear detection range and detection limit of the sensor system were 0.5- 64nM ($R^2=0.9939$) 0.14nM, respectively (H.D. Ertuğrul Uygun, Uygun, Canbay, Girgin Sağın, & Sezer, 2020).

Tabrizi et al. developed an EIS-based sensor for the detection of SARS-CoV-2-RBD. For this, a macroporous imprinted gold electrode was used. The MIP surface on the gold electrode was prepared by electropolymerization of ortho-phenylenediamine in the presence of SARS-CoV-2-RBD. The linear range of the system was 2.0-40.0 pg mL⁻¹ and the detection limit was 0.7 pg mL⁻¹ (Amouzadeh Tabrizi, Fernández-Blázquez, Medina, & Acedo, 2022).

Moreira et al. prepared an EIS-based MIP sensor using carboxylated polyvinyl chloride (PVC-COOH) for myoglobin determination. The linear response range of the system was 0.852 -4.26 g mL⁻¹ and the limit of detection was 2.25 g mL⁻¹ (Moreira, Dutra, Noronha, & Sales, 2013).

1.4.2.2 Surface Plasmon Resonance (SPR)

The basis of the surface plasmon resonance method is to monitor the spectral shifts caused by the refractive index change that occurs as a result of the binding at the molecular level. This method is highly preferred for monitoring biomolecule interactions. The surface plasmons, which form the basis of the method, are generated by an electromagnetic wave propagating across the surface of a thin metal plate. Abbas et al. define the surface plasmon resonance as the collective oscillation of electrons located at the metal-dielectric interface (60). Three features that make the use of the SPR method interesting in the preparation of optical sensors can be listed as electric propagation length, field amplification and penetration depth. SPR-based sensors have a wide range of uses, such as antibody-antigen interactions, pharmacology, food and environmental analysis. Elements that make up an SPR sensor; light source, prism, transmission surface, detector, flow system, biomolecule. SPR sensor systems have two media, one of which is more optically dense, and an interface. When the light passing through the denser optical medium interacts with the less dense medium, a resonance amplitude change phenomenon occurs at the interface of the two mediums. A change in the refractive index causes a change in the SPR resonance angle. The change in refractive index is proportional to the change in mass as a result of antigen-antibody interaction. This indicates that the mass change of large biological molecules causes the refractive index change with the SPR resonance angle change (Xia, Zun-Zhong, Jian, & Yi-Bin, 2010).

Altıntaş et al., developed a nanoparticle-based optical sensor for pharmaceutical monitoring. The synthesized MIP nanoparticles, whose dimensions were determined as $\sim 132.3 \text{ nm} \pm 3.2$, were immobilized on the SPR gold sensor with EDC/NHS. The prepared sensor was used to monitor dichlorofenac in water and was verified by LC-MS method. Kinetic data analysis indicated a dissociation constant of $1.48 \times 10^{-9} \text{ M}$ was achieved (Altintas, Guerreiro, Piletsky, & Tothill, 2015).

1.4.2.3 Quartz Crystal Microbalance (QCM)

The working principle (QCM) of the Quartz Crystal Microbalance is based on the inclusion of a thin piezoelectric plate that exhibits acoustic resonance in the MHz range. When the crystal here interacts with the sample, its resonance properties change. QCM was first used by Sauerbrey in 1959. In this study, Sauerbrey showed that measuring the characteristic frequency of an oscillator circuit is an advantageous method. The changes in frequency were measured with a piezoelectric crystal. The very high sensitivity of the quartz crystal has shown that this method is very accurate and sensitive (63). The piezoelectric quartz crystal works in conjunction with a low frequency transistor oscillator. The frequency of the vibrating crystal is controlled by the counter. It is connected to the crystal oscillator (vibration) circuit. Frequency counter is also combined with this vibration device. The frequency counter records changes in frequency after each immobilization step and sample interaction. The advantages of the QCM method can be listed as monolayer sensitivity, surface specificity, usefulness for dilute concentrations, not requiring labeling, easy use and fastness. With this method, analysis at pictogram levels is possible. The fact that the detection limit is so low has increased the use of DNA-based biosensors and immunosensors of the QCM method (Uludağ, Piletsky, Turner, & Cooper, 2007).

In the study of Ermolaeva et al., molecularly imprinted polymers synthesized by precipitation method were used in the preparation of sensors for Ractopamine determination. Methacrylic acid was chosen as the monomer for MIP synthesis. The immobilization of the prepared nanoparticles on the electrode surface was done with cyanoacrylate. For this, MIP and cyanoacrylate solution was dropped on the piezoelectric crystal and spin coating method was used. The limit of detection in the

study was found to be 12 µg/mL (Ermolaeva, Farafonova, Chernyshova, Zyablov, & Tarasova, 2020).

1.4.3 Molecularly Imprinted Nanomaterials

Molecular imprinting is a very advantageous method for specific recognition reactions, and when combined with the advantages of nanotechnology, molecularly imprinted nanomaterials can be obtained that allow more sensitive and selective measurements. Molecularly imprinted nanomaterials are getting more and more interesting day by day with their advantages such as large surface area, physical and chemical stability, and low cost. Molecularly imprinted polymers have a wide range of uses and the advantages of nano-sized materials have improved the use of MIPs in related fields. Nanomaterials have been synthesized in many different ways, such as nanocapsules, nanorods, nanospheres, nanoshells, and nanofibers, depending on their use and purpose. The use of imprinted nanomaterials has also become widespread in sensor development studies. In particular, nanomaterials are used to increase the sensitivity, conductivity and selectivity of the transducer used. Different types of transducers have been used in sensor preparation by combining them with imprinted nanomaterials (Irshad et al., 2013). The new technology MIP's is not just a mixture of polymer and template. In recent years, the use of nanomaterials in sensor technology as polymer composites has increased as a result of increasing the surface area and physicochemical conductivity (Wackerlig & Schirhagl, 2016). With these advantages that nanomaterials add to sensor technology, sensor technology has advanced. They are very useful in reducing the potential required for electropolymerization, especially in studies where electrical conductivity is effective on polymerization. High surface area increases the quality of the polymer formed. Nanomaterial-modified polymers, which are generally very useful in terms of forming a thin layer to reduce limitations, also eliminate many disadvantages in sensor technology. MIPs have several disadvantages such as containing target molecule residues, limited selectivity and limited mass-carrying ability. Various methods have been used to overcome these disadvantages: thin film polymerization method has been used on the electrode surface to increase mass transport, nanomaterials has been used to increase surface stabilization and sensitivity of the method by increasing the surface area. In addition,

nanomaterials such as carbon nanotubes, graphene, fullerene help to increase reusability and surface conductivity (Caro et al., 2002).

Besides many nanomaterials such as gold nanoparticles, magnetic nanoparticles, quantum dots, carbon nanomaterials are very interesting materials due to their chemical and physical stability, mechanical and electrical properties. Due to their wide variety, they have applications in many different fields, from drug delivery to energy storage. Due to their efficiency in increasing the surface area and conductivity, the use of carbon nanomaterials in sensor technology has become quite common in recent years (Hilmiye Deniz Ertuğrul Uygün and Zihni Onur Uygün, 2019). Carbon nanoparticles are materials that can be found in a wide variety of conformations thanks to the sp^2 hybridization of carbon. It is possible to prepare a wide variety of carbon nanomaterials, from zero-dimensional to two-dimensional. Dimensional classification of the nanoparticle examples are carbon quantum dots and fullerene 0-D, single-walled and multi-walled carbon nanotubes 1-D, graphene 2-D (Mostofizadeh, Li, Song, & Huang, 2011).

Fullerenes are carbon-derived nanomaterials with a spherical and 0-D structure (Figure 1.3.). Unlike other carbon nanomaterials, it consists of pentagonal and hexagonal carbon structures. They have a large surface area because of their spherical structure. Since the large surface area increases the sensitivity of the system, the use of fullerenes in sensor systems has increased in recent years. Among all the fullerenes, C₆₀ is the most commonly used (Shetti, Mishra, Basu, & Aminabhavi, 2021). The 30 double bonds in Fullerene C-60 have the capacity to form 30 bonds. These bonds can be modified in many different ways to form fullerene derivatives (Hilmiye Deniz Ertuğrul Uygün and Zihni Onur Uygün, 2019).

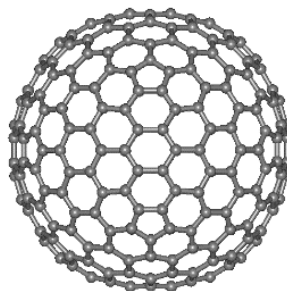


Figure 1.3 Fullerene structure

1.5 Literature Review

Liu et al. developed an electrochemical aptasensor based on graphene-polyaniline (GR-PANI) nanocomposite film using the square wave voltammetry (SWV) method. The aptasensor showed a good linearity for DA in the range of 0.007-90 nmol/L and a limit of detection was found 0.00198 nmol/L (S/N=3). The aptasensor has been tested using human serum (S. Liu et al., 2012).

Yan et al. developed a biosensor for dopamine determination using nafion film-coated glassy carbon electrode and silver chloride@polyaniline core-shell nanocomposite structures. Nanoparticles prepared for dopamine determination were characterized using SEM and TEM. The developed sensor is very sensitive. It can detect even a very low concentration of dopamine in the presence of 5000 times the concentration of ascorbic acid (Yan et al., 2008).

With the system they developed, Canbay and Akyılmaz prepared a multi-walled carbon nanotube (MWCNT)-Nafyon-Cysteamine modified tyrosinase biosensor and performed the determination of dopamine by differential pulse voltammetry method. In order to determine reproducibility for a 1- μ M dopamine concentration ($n=15$), an average value, standard deviation, and coefficient of variation were found as 1.026 μ M, ± 0.03975 , and 3.8%, respectively. The application of the developed sensor was made with drug samples (Canbay & Akyılmaz, 2014).

Mir et al. prepared a nanosensor modified with graphene oxide and gold nanoparticles containing poly (1,5-diaminonaphthalene) (poly-DAN) layer and performed amperometric monitoring of dopamine. The range of linear response of the sensor and the detection limit were found to be 10 nM- 1 μ M and 5.0 nM (± 0.01), respectively. The results showed that the developed biosensor can be used to monitor the effect of exogenous agents in living cells and drug efficacy tests (T. Ahmad et al., 2015).

Kan et al. prepared a sensor system for dopamine determination by combining molecularly imprinted polymers and multi-walled carbon nanotubes (MWNT). Methacrylic acid and trimethylolpropane trimethacrylate were used in addition to MWNT for polymerization and polymerization was carried out in the presence of

dopamine. The sensor prepared using a glassy carbon electrode is selective for dopamine in the presence of ascorbic acid. The linear operating range of the sensor is determined to be 5.0×10^{-7} to 2.0×10^{-4} mol/L (Kan, Zhao, Geng, Wang, & Zhu, 2008).

Maouche et al. prepared a sensor for dopamine detection on glassy carbon electrodes by electropolymerization of pyrrole. In this study, cyclic voltammetry and chronoamperometry methods were used comparatively for the polymerization of pyrrole. The non-imprinted NIP sensor was prepared without dopamine to show that molecular imprinting had taken place. The selectivity of the system was tested against 2-phenyl ethylamine (PEA), noradrenaline (NAD) and ascorbic acid (AA). The detection limit of the system using square wave voltammetry as the determination method was found to be 5.7 pmol/L (Maouche et al., 2012).

Qian et al. developed an electrochemical sensor for the in vivo determination of dopamine (DA) (MIP) with a molecularly imprinted composite prepared with carbon nanotubes and oxygen-containing polypyrrole (PPy). The linear response range of the prepared sensor was 5.0×10^{-11} – 5.0×10^{-6} M and the detection limit was 1.0×10^{-11} M (Qian et al., 2014).

Wang et al. prepared a molecularly imprinted polymer-based potentiometric sensor for the covalent determination of dopamine. In this study, boronate-based MIP spheres were used as recognition receptors. The prepared electrode was used for the in vivo determination of dopamine in the brain of living animals and the detection limit was found to be 2.1 mM (C. Wang, Qi, & Liang, 2021).

Li et al. prepared a sensor for dopamine detection by electropolymerization of o-aminophenol in the presence of dopamine. The sensor is selective for dopamine in the presence of high concentrations of ascorbic acid (AA). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used as measurement methods. After various optimization steps, the linear detection range and LOD of the prepared sensor were found to be 2×10^{-8} to 0.25×10^{-6} mol/L, 1.98×10^{-9} mol/L (S/N = 3), respectively (Li et al., 2009).

Song et al. prepared the MIP sensor for the determination of dopamine by electropolymerization method in their study.

For this, electropolymerization of o-phenylenediamine and resorcinol was carried out in the presence of the template molecule dopamine. When the MIP and NIP electrodes were compared, the dopamine response of the MIP electrode was observed to be higher. The linear detection range of the sensor was 5.0×10^{-7} – 4.0×10^{-5} M and the detection limit was 0.13 μ M (S/N=3). In addition, the selectivity of the sensor was studied against ascorbic acid and uric acid and it was found to be selective (Song, Chen, Xu, Yang, & Tian, 2010).

Ma et al. prepared a sensor for dopamine determination using polypyrrole (PPy), multi-walled carbon nanotubes modified graphene aerogels (MWCNT/GAs). When the prepared particles were characterized, it was determined that the MWCNT aerogels were well dispersed on the graphene walls. The use of MWCNTs has increased the conductivity and surface area of the system, and therefore its electrochemical performance. After the optimization of the sensor preparation steps, the detection range of the MIP/MWCNT/GAs electrode prepared under the best conditions was found to be between 5 nM and 20.0 mM, and the detection limit was found to be 1.67 nM (S/N $\geq$ 3). A serum sample was used for the application of the sensor (Ma et al., 2020).

Teng et al. prepared MIP Polypyrrole nanowires (PPyNWs) on a glassy carbon electrode (GCE) by electropolymerization for dopamine determination. MIP prepared by electropolymerization has a high surface area, which makes the electrode more selective. The linear operating range under optimum conditions is in the range of 50 nM to 100 μ M, and the detection limit is 33 nM (Teng, Liu, & Kan, 2017).

Zhang et al. prepared a selective and sensitive MIP sensor with polypyrrole (PPy)/ZIF-67/Nafion modified glassy carbon electrode (GCE) for the determination of dopamine (DA). The characterizations of the prepared materials were made using FT-IR, XRD, XPS, SEM, BET and AFM. EIS, CV and DPV methods were used for the electrochemical measurements of the system. The linear detection range of the sensor was found to be 0.08–100 μ M and 100–500 μ M (with DPV). The LOD of the PPy/ZIF-67-MIPs/Nafion/GCE sensor was found to be 0.0308 μ M (S/N=3). Sample application of the sensor system was made with human serum samples and recoveries were obtained (W. Zhang et al., 2018).

Vais et al. developed a MIP-based biosensor system for the determination of β -amyloid. The MIP surface was prepared by electropolymerization of polypyrrole in the presence of β -amyloid. The detection limit of the biosensor system was found to be 1.2 pg mL^{-1} (Vais, Yadegari, Heli, & Sattarahmady, 2021).

Özcan et al. developed a MIP-based system for the determination of Amyloid β . Before preparing the MIP surface, delaminated titanium carbide MXene ($\text{d-Ti}_3\text{C}_2\text{TX}$ MXene) and multi-walled carbon nanotubes (MWCNTs) composite structure were prepared. They imprinted Amyloid β on this composite by electropolymerization of pyrrole. The linear detection range of the system was $1.0 - 100.0 \text{ fg mL}^{-1}$ and the detection limit of 0.3 fg mL^{-1} was found (Özcan, Medetalibeyoglu, Akyıldırım, Atar, & Yola, 2020).

CHAPTER II

MATERIALS AND METHOD

2.1 Materials

2.1.1 Chemicals

(1,2-Methanofullerene C₆₀-61-carboxylic acid, Pyrrole, Pyrole-3-Carboxylic acid, Fe(CN)₆-3/-4, KCl, KH₂PO₄, Serotonin, Noradrenaline, H₂SO₄, HCl, NaOH Sigma Aldrich, Dopamine, Dopamine ELISA kit (colorimetric) was obtained from Novus Biologicals (KA1887).

2.1.2 Devices

Palme Sense 3 brand potentiostat was used for electrochemical tests. PSTrace 5.7 interface software was used to make the measurements and calculate the impedance curves. Screen Printed electrodes (DRP110 Carbon electrode) with a 3-electrode system (carbon working electrode, platinum counter electrode and Ag/AgCl reference electrode) were obtained from Drop sense company. EZODO brand PL-700PC pH meter was used in the preparation of buffer solutions. The pure water used in the studies was produced with Millipore ultrapure water device [Millipore Corporation (France) Elix 5 ® reverse osmosis unit, electrodeionization (E.D.I) module (Elix ® 5 Water System), organic/colloid removal ion exchange system]. COXEM EM30 model scanning electron microscope (SEM) and Thermo Scientific K-Alpha model X-ray Photoelectron Spectroscopy (XPS) was used for the characterization of the electrode surface. Atomic Force Microscope (AFM) data were obtained from BRUKER Dimension Edge with ScanAsyst.

2.2 Method

In this thesis, 3 different sensor systems based on molecularly imprinted impedance were developed for the determination of Dopamine (DA) and β -Amyloid molecules, which are clinically important to determine. The steps of the method to be followed separately for each sensor are listed below:

- Preparation of Dopamine/ β -Amyloid Specific Polymeric Surface on the Electrode Surface
- Determination of Sensor Performance Parameters
- Selectivity Studies
- Applicability Studies of the Sensor System

In this study, the Screen-Printed electrode system, which combines the working, reference and counter electrode triple system in a single electrode, was used (Figure 2.2). After each polymerization step, impedance data was obtained and it was examined whether the polymerization took place or not. After the polymerization was seen to be successful, a standard graph was drawn with the electrode.

2.2.1 Electrochemical Impedance Spectroscopy (EIS) Measurement of the Sensor Systems

Electrode surface characteristics, namely immobilization stages and dopamine-polymer binding and related concentration measurements were made by electrochemical impedance spectroscopy (EIS). The circuit in Figure 2.1 was used to calculate this coupling over impedance.

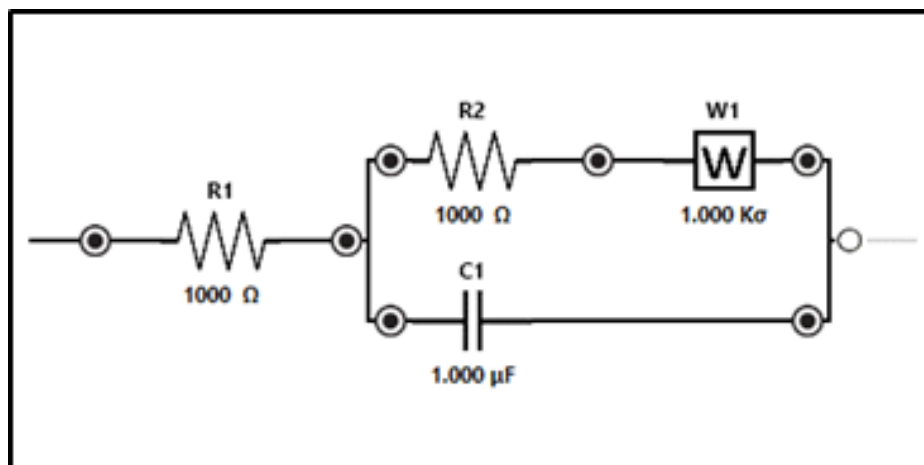


Figure 2.1 Electrochemical circuit diagram of the SCE surface

In this thesis, impedance parameters; pH=7 with 5mM $\text{Fe}(\text{CN})_6^{3-/4-}$ redox probes and 100mM KCl. The measurement will be performed by immersing the electrodes in 50mM PBS (Phosphate Buffer) buffer. It was determined as a potential application of 180 mV DC and 10 mV AC by frequency scanning between 10000 Hz and 0.05 Hz, and the $R_1(C(R_2W))$ model was used as the circuit diagram to determine the impedance change in ohms.

2.2.2 Cyclic Voltammetry Measurements of the Sensor Systems

Cyclic Voltammetry (CV) is a method that can be changed between two potentials in a certain time interval and is used to find the reduction oxidation types in the liquid in which the measurement is taken and to examine the kinetic parameters according to the electrode surface feature. In this thesis, CV was used as a characterization method in the control of immobilization steps and electropolymerization. It is determined as a scanning rate of 50 mV/s in the potential range of -0.1 to +0.5 V. CV is used for the surface characterization that is commonly used to detect redox probe on different surfaces. Therefore, our modifications were observed by redox probe electrochemical signals on different surfaces.

2.2.3 Electropolymerization CV Parameters

CV parameters for the PPy-PPy3COOH electropolymerization were determined as 0.1 and +0.5 V potential range with 50 mV/s scan rate for 5 cycles in 100mM H₂SO₄.

CV parameters for the APBA-PPy3COOH electropolymerization were carried out in the potential range between -0.1 and +1.0 V with 100 mV/s scan rate for 40 cycles in 200mM NaF including 100mM HCl.

2.2.4 Preparation of Carbon Electrode Based Dopamine Sensor

Carbon electrode (CE) was chosen as the working electrode (Figure 2.2). Before starting the study, the Carbon Screen Printed Electrode (SCE) was cleaned in ultrapure water for 2 minutes in an ultrasonic bath. After passing nitrogen gas over the cleaned electrode, CV and EIS measurements of the bare electrode were taken in Fe(CN)₆^{3-/4-} redox solution. In order to form –COOH or –OH groups on the electrode surface, the electrode was oxidized with H₂SO₄ and then these groups were converted to their active forms by using the EDC/NHS solution mixture (Figure 2.3). After this process, the electrode was incubated with fullerene for one hour and the Fullerene coated electrode was electropolymerized in a solution containing pyrrole, pyrrole-3-carboxylic acid and dopamine (target molecule). After this process, the electrode was gently washed in order to remove unpolymerized monomer molecules. In order to characterize the immobilization steps after each polymerization step, CV and EIS measurements were taken inside the Fe(CN)₆^{3-/4-} redox probe of the electrode.

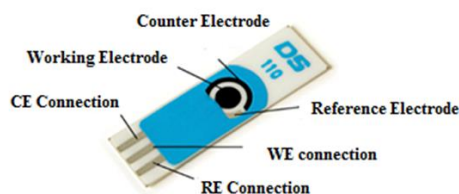


Figure 2.2 Screen printed electrode used in the sensor system

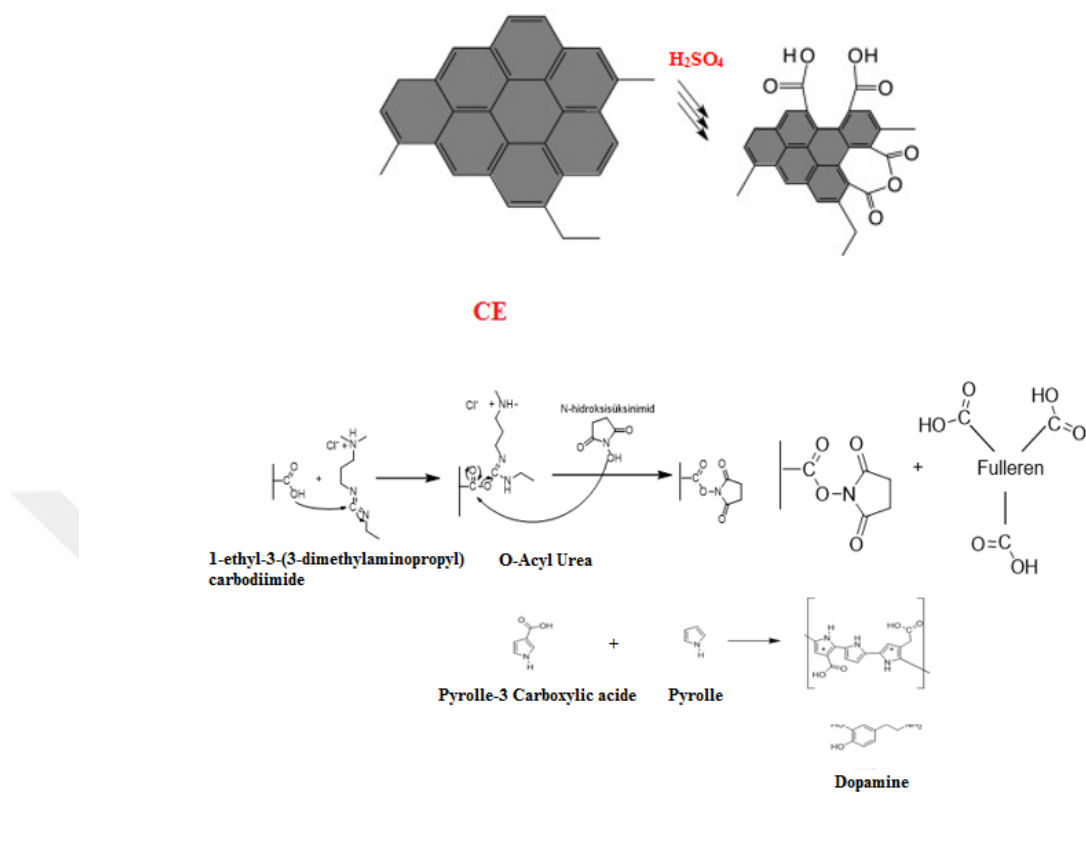


Figure 2.3 Activation chemical process of carbon electrode

Template removal was carried out after polymerization. For the desorption of the target molecule 1%, 2.5% and 5% acetic acid was studied as the desorption agent and 5% acetic acid solution was chosen as the desorption agent.

In order to see whether the template removal process was successful or not, CV and EIS measurements of the electrode were taken after this process. After template removal, standard graphics were drawn using dopamine standard solutions. For this, a series of standard solutions were prepared and 20 μl of the standard solution was dropped onto the electrode surface and CV and EIS measurements were taken after 30 minutes of incubation. In the performance steps, the lowest limit of determination (LOD) and limit of determination (LOQ), reproducibility, repeatability, selectivity, real sample testing were performed according to the linear measurement range of the calibration graphic.

2.2.5 Preparation of Gold Electrode Based Dopamine Sensor

A sensor system was prepared using screen-printed gold (Au) electrode for dopamine (DA) determination. Self-assembled monolayers (SAM) were formed by incubating the Au electrode in 100 mM cysteamine (Cys) solution for 2 hours. At the end of this period, the electrode was gently washed with distilled water and incubated in 5% glutaraldehyde solution for 45 minutes. Then, the electrode was kept in aminophenylboronic acid (APBA) solution at a concentration of 1 mg / ml for 1 hour. After this process, the electrode was electropolymerized in a solution containing APBA, pyrrole-3-carboxylic acid and dopamine (target molecule). CV and EIS measurements were taken in $\text{Fe}(\text{CN})_6^{3-/4-}$ after each polymerization step except after incubation with glutaraldehyde. This process was done for the characterization of immobilization steps. Template removal was carried out after the polymerization. Thus, 10mM and 50mM HCl solutions were used as desorption solution and 50mM HCl was chosen as the desorption solution for 30 minutes. After template removal, standard graphics were drawn using dopamine standard solutions. For this, a series of standard solutions were prepared and 20 μl of the standard solution was dropped onto the electrode surface and CV and EIS measurements were taken after 30 minutes of incubation. In the performance steps, the lowest limit of determination (LOD) and limit of determination (LOQ), reproducibility, repeatability, selectivity, real sample testing were performed according to the linear measurement range of the calibration graphic.

2.2.6 Preparation of Amyloid Beta Sensor

For the determination of Beta Amyloid ($\text{A}\beta$ 1-42), the recognition surface was prepared by modifying the gold electrode. For this, the gold electrode was kept in 100 mM cysteamine (Cys) for 2 hours. At the end of this period, the electrode was gently washed with distilled water and incubated in 5% glutaraldehyde solution for 45 minutes. Then, the electrode was kept in PAMAM G4 dendrimer solution for 1 hour. Then, electropolymerization was performed with $\text{A}\beta$ 1-42 and pyrrole 3 carboxylic acid (PPy-3-COOH) on the electrode incubated with a PAMAM G4 dendrimer. CV and EIS measurements were taken in $\text{Fe}(\text{CN})_6^{3-/4-}$ after each polymerization step except after incubation with glutaraldehyde. This process was done for the

characterization of immobilization steps. Template removal was carried out after the polymerization. Thus, 100mM and 500mM HCl solutions were used as desorption solution and 500mM HCl was chosen as the desorption solution for 30 minutes. After template removal, standard graphics were drawn using A β 1-42 standard solutions. For this, a series of standard solutions were prepared and 20 μ l of the standard solution was dropped onto the electrode surface and CV and EIS measurements were taken after 30 minutes of incubation. In the performance steps, the lowest limit of determination (LOD) and limit of determination (LOQ), reproducibility, repeatability, selectivity, real sample testing were performed according to the linear measurement range of the calibration graphic.

2.2.7 Non-Imprinted Sensor Preparation

The non-imprinted electrode was prepared without adding target molecule (dopamine and Beta amyloid) to the polymerization solution. Monomer amounts were used in the same way as the imprinted sensor. EIS measurements were taken with the electrode after polymerization. The desorption process was carried out in the same way with the MIP electrode. After template removal, chronoimpedance measurement was taken in phosphate buffer containing template molecule. The results were compared with the MIP sensor.

2.2.8 Chronoimpedance Measurements of The Sensor Systems

Chronoimpedance measurement was taken to determine the optimum binding time of targeted molecules on the molecularly imprinted electrode, which was prepared and desorbed as described before. Chronoimpedance measurement was performed under 200 mV and 100 Hz conditions for 1200 seconds. For this, the electrode was first tested to chronoimpedance measurement for 1200 seconds in pH 7.4 50 mM phosphate buffer, then 50 μ l 20 mM dopamine was added to the measuring cell for 1200 seconds.

2.2.9 Surface Characterization of The Sensor System

Surface characterization analyzes of the prepared sensor system were performed using SEM, AFM and XPS devices.

SEM analysis was performed to monitor the surface morphology of the electrodes. For SEM analysis, the electrodes were coated with a thin gold layer for better analysis. For this, an ion coater device was used. SEM analysis was performed under high vacuum using an application voltage of 20 kV. The appropriate spot size for the desired magnifications was determined as 12.

In the dopamine sensor study prepared with a gold electrode, XPS analysis was performed to determine the electrode element composition and surface chemistry. A Monochromatic Al-K α (1486.7 eV) X-ray source and a beam size of 400 nm in diameter were used for the XPS measurement. The survey scan of the XPS was carried out from -10 to 1350 eV with a scanning speed of 1 eV applying pass energy of 150 eV.

For AFM analysis, Tapping Mode was chosen as AFM mod, resolution was 256×256 pixels, ACLA was used as cantilever, and vibration was 1 V_{RMS}.

2.2.10 Determination of Sensor Performance Parameters

The lowest limit of determination (LOD) and limit of determination (LOQ) were calculated for the determination of performance of the sensor systems. LOD value is lowest after determination of calibration chart obtained as a result of repeated measurements of the concentration of the calibration chart. LOD and LOQ were calculated using these equations $LOD = 3.3 (S)/m$, $LOQ = 10 (S)/m$, respectively where S is the standard deviation of the response and m is the slope of the calibration curve.

2.2.11 Selectivity Studies of Sensor

Selectivity studies were carried out to show the specificity of the prepared sensors to the target molecule. In the sensor systems for dopamine, serotonin and neuradrenelin, which are dopamine-like molecules, were used for this. In addition, the selectivity of the system with uric acid and acetic acid found together with dopamine in physiological fluids was studied with the chronoimpedance (CI) method. With the prepared electrode, CI measurement was taken for 1200 seconds in an environment containing uric acid and acetic acid. Then, dopamine was added to the medium and the measurement was repeated. The selectivity of the sensor system prepared for amyloid beta was studied with Tau protein.

2.2.12 Applicability Studies of the Sensor System

For dopamine determination, blood samples collected in accordance with the ethics committee decision numbered 17-1/16 were collected in green capped heparin tubes as 2-3 ml. All samples were centrifuged at 2000 rpm and stored at -80°C until the plasma portion was used. Spot urine samples collected in sterile urine containers were used as urine samples. Urine samples were filtered with 0.45µm filter paper to remove particles and reduce interference before measurement, separated into 1 ml portions and stored at -20°C. Analysis was performed using the Dopamine ELISA Kit (KA1887) from Novus Biochemicals. Samples were added to the ELISA plate, first of all 25µL, separately for each well. Afterwards, 25µL of hydrochloric acid was added to the samples and incubated at 600rpm for 30 minutes at room temperature. After incubation, 50µL of dopamine antiserum was added to these mixtures and incubated for 2 hours at room temperature with shaking again at 600rpm. Afterwards, the wells were washed 3 times with 300µL of washing solution. Afterwards, it was conjugated with enzyme to each well, 100µL of antibody solution was added and incubated at 600rpm for 30 minutes at room temperature. The plate was washed 3 times with 300µL wash buffer again. 100µL of substrate solution was added to each well and incubated for 30 minutes at a shaking speed of 600rpm, 100µL of stop solution was added to these wells to stop the reaction, and reading was performed against the standards at 450 nm wavelength. The measurements performed with the ELISA and the sensor

system are shown in Table 2. Since the amount of dopamine in the blood samples did not fall within the linear range of our calibration chart, urine was preferred instead of blood as the real sample.

Real sample studies for the gold electrode-based dopamine sensor system and the amyloid beta sensor system were performed by adding standards to artificial serum (Sezgintürk & Uygun, 2012). The artificial serum was prepared using the ingredients and amounts in the reference article (Basiaga, Paszenda, Walke, Karasiński & Marciniak, 2014).

2.2.13 Statistical Comparison of Elisa and Sensor Results

Measurement results with Dopamine sensor and ELISA methods on real samples were compared by applying double-sided t-test. The t value calculated for the sensor system was compared with the critical t value found in the table (David Harvey, 2020) for the degrees of freedom (n-1).

CHAPTER III

ANALYSIS AND RESULTS

3.1 Preparation and Characterization of Carbon-Based Dopamine Sensor

The electrodes were prepared by the electropolymerization method as mentioned in the method section 2.2.4 (Figure 3.1). After each polymerization step, impedance data was obtained and it was examined whether the polymerization took place or not (Figure 3.2). After the polymerization, a standard graph was drawn with the electrode in the range of 25 -250 ng/ml (Figure 3.3-3.4). Successful polymerization can be seen from the decreasing CV peaks. Because decreasing peaks indicate that the amount of monomer molecule in the medium decreases and turns into polymer. SEM images were taken for the characterization of the prepared electrode (Figure 3.5). When these SEM datas are examined, it is seen that the surface area increases after the polymerization process.

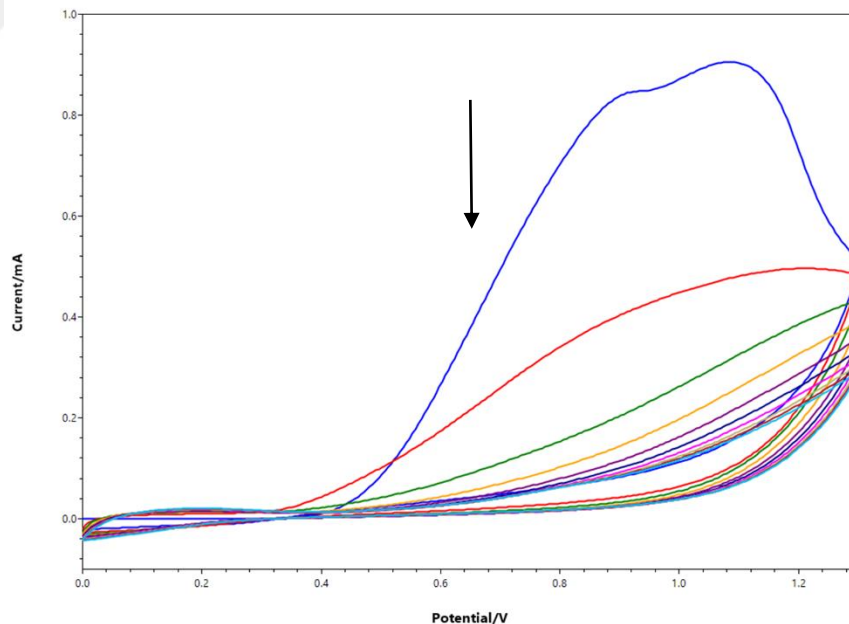


Figure 3.1 Electropolymerization of the electrode with pyrrole, pyrrole-3 carboxylic acid and dopamine. The direction of the arrow shows the decrease in the peaks with continued polymerization.

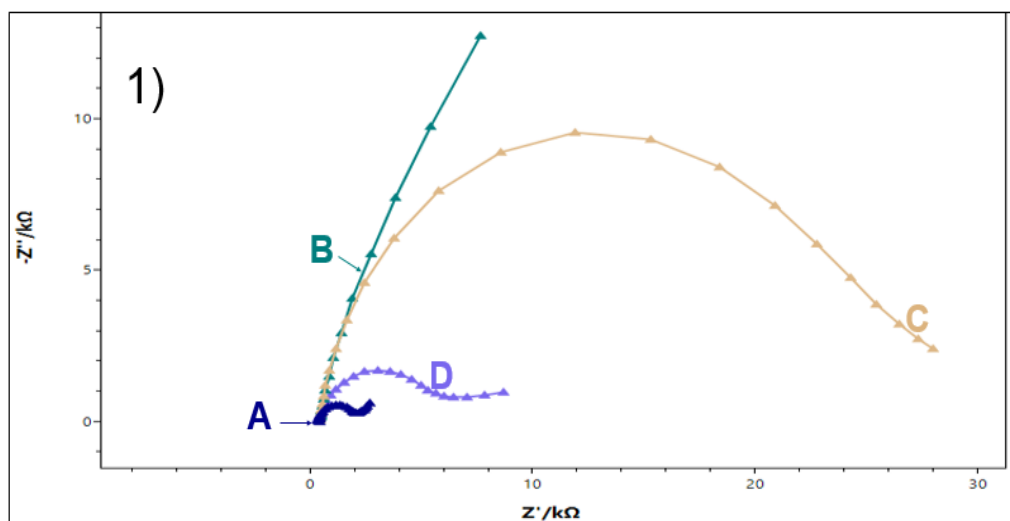


Figure 3.2 EIS data of electrode preparation steps. A) Bare CE B) CE/Fulleren C) CE-Fuleren-PPy-PPy3COOH-DA D) Template removed electrode CE-Fuleren-PPy-PPy3COOH

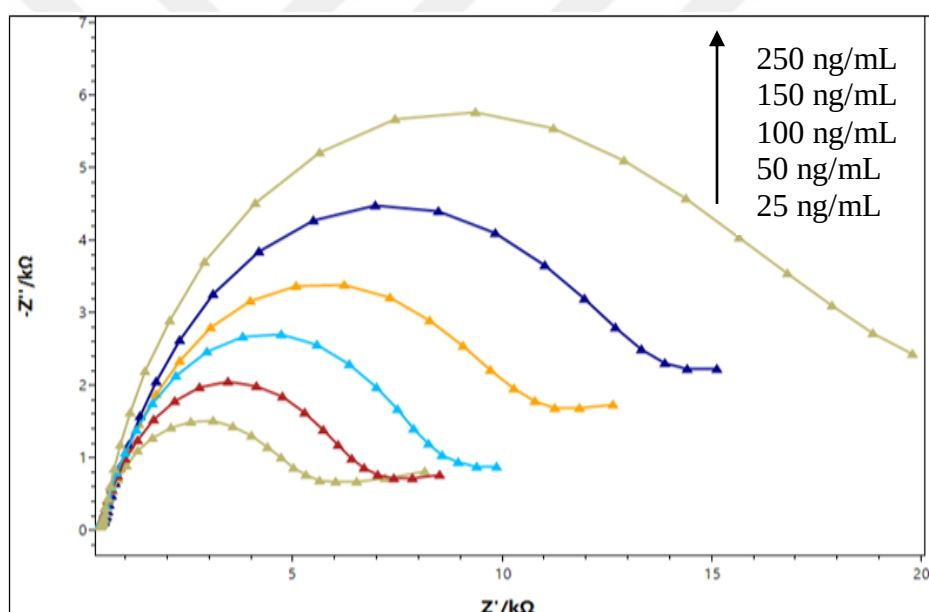


Figure 3.3 EIS data of the sensor system for dopamine determination

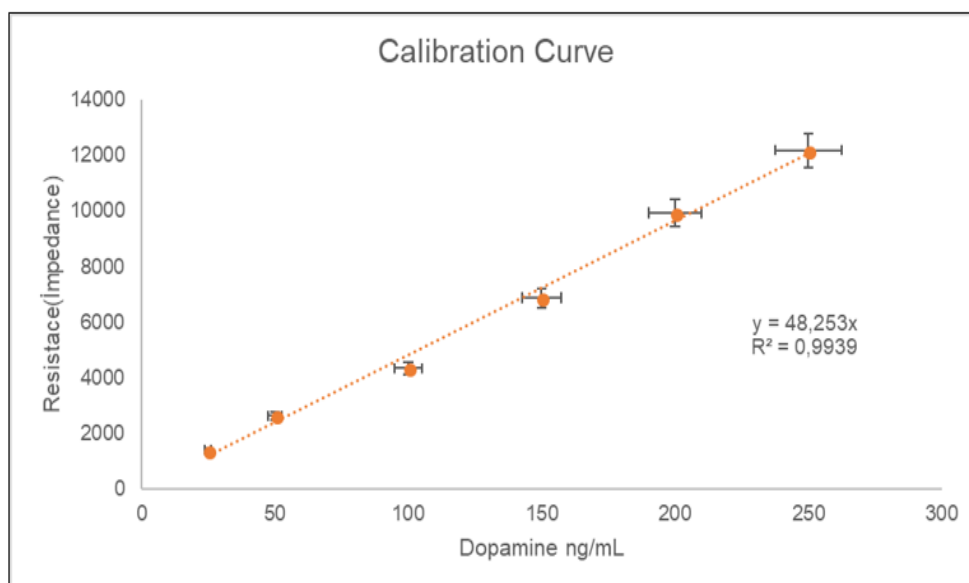


Figure 3.4 Calibration Curve

SEM analysis was performed for the surface characterization of the electrode. For this, bare CE and polymerized electrode images were taken. Comparison of images was made with images taken at the same magnification.

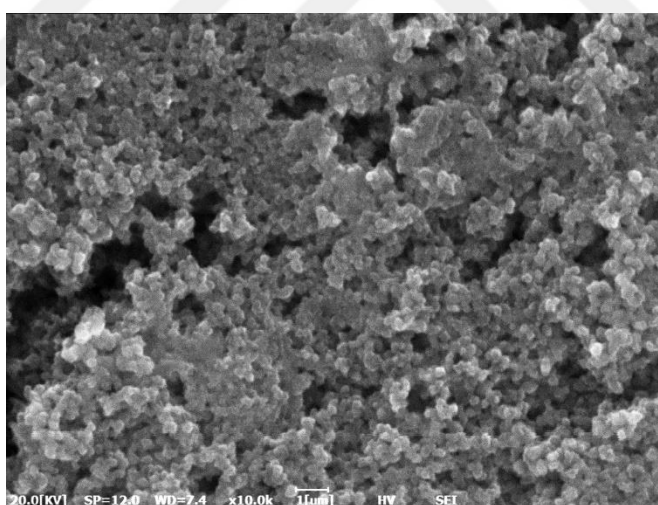


Figure 3.5 SEM Image of CE-Fuleren-PPy-PPyCOOH-DA electrode

When examined, it is clearly seen that the surface morphology has changed. The very rough nature of the surface indicates the width of the surface area, which supports the sensitive results of the method.

3.1.1 Optimization of the Monomers and Template Concentrations

Three sensors were prepared by using different concentrations (Table 3.11) of pyrrole and pyrrole-3-carboxylic acid which were the monomers used in the preparation of the sensor and target molecule dopamine (Figure 3.6).

Table 3.1 Monmer and template concentrations

Electrode Number	PPy (mM)	PPy3COOH (mM)	DA (mM)
1	20	20	20
2	10	10	20
3	30	20	20
4	30	10	20

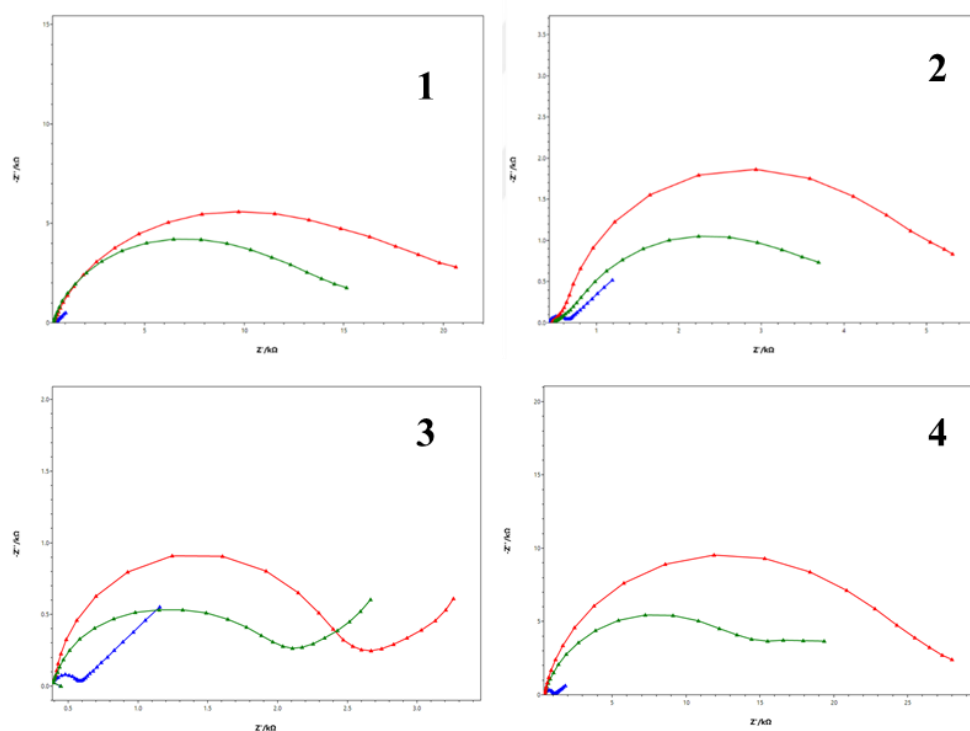


Figure 3.6 Prepared sensors with different concentration of monomers and template: Blue) Bare CE, Red) CE-PPy-PPy3COOH-DA, Green) After the desorption CE-PPy-PPy3COOH

The electrode with the best results was determined as electrode 3. The best monomer-template ratio was found by comparing the resistance errors of the EIS measurements taken after the CE-Fulerene-PPy-PPy3COOH-DA polymerization of

the electrodes. Since the electrode % error closest to the theoretical value is the lowest electrode, it was determined as the optimum electrode.

3.1.2 Performance of the sensor

In the performance steps, the lowest limit of determination (LOD) and limit of determination (LOQ), reproducibility, repeatability, selectivity, real sample testing were performed according to the linear measurement range of the calibration graphic. After the calibration curve, 13 calibration curves were prepared to evaluate the reproducibility of the sensor technology.

The characteristics of the sensors were evaluated using the means, the standard deviation of the R^2 values, and the slope of these calibration curves (Table 3.2). Calibration equation was found to be $y = 48,253x$. Here, y is the impedance and x is the concentration of dopamine in ng/mL. $R^2=0.9939$ indicates that linearity is quite good for this sensor.

Table 3.2 Performance characteristics of the sensor (SD: Standard Deviation, CV: Coefficient of variation, DA: Dopamine)

DA(ng/ml)	Ret(kOhm)	%CV	± S.D.	Δ Ret(kOhm)	± S.D. DA (ng/ml)	LOD (ng/ml)	LOQ (ng/ml)
25	5309	2,42	128,45	1397	0,60	8,77	26,8
50	6548	3,15	206,85	2636	1,58		
100	8254	2,74	226,48	4342	2,74		
150	10790	3,12	337,29	6878	4,69		
200	13850	3,97	549,84	9938	7,9		
250	16091	2,81	452,80	12179	7,0		

3.1.3 Selectivity of the Carbon-Based Dopamine Sensor

In this thesis study, the selectivity tests of the carbon based sensor system were performed by comparing the responses of the dopamine-like molecules serotonin and noradrenaline molecules (Fig. 3.6) with 100 ng/ml solutions of the surface prepared with the dopamine target molecule, relative to the same amount of standard solution of dopamine (Fig. 3.7-3.8).

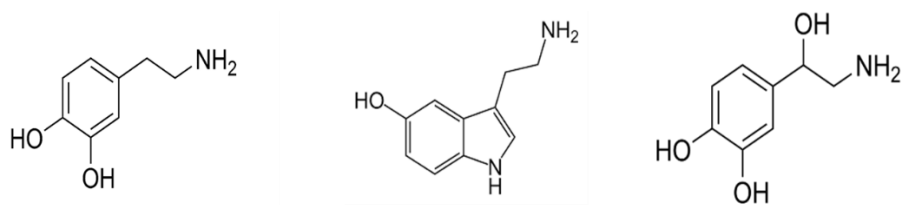


Figure 3.6 Molecular structures of Dopamine, Serotonin and Noradrenaline

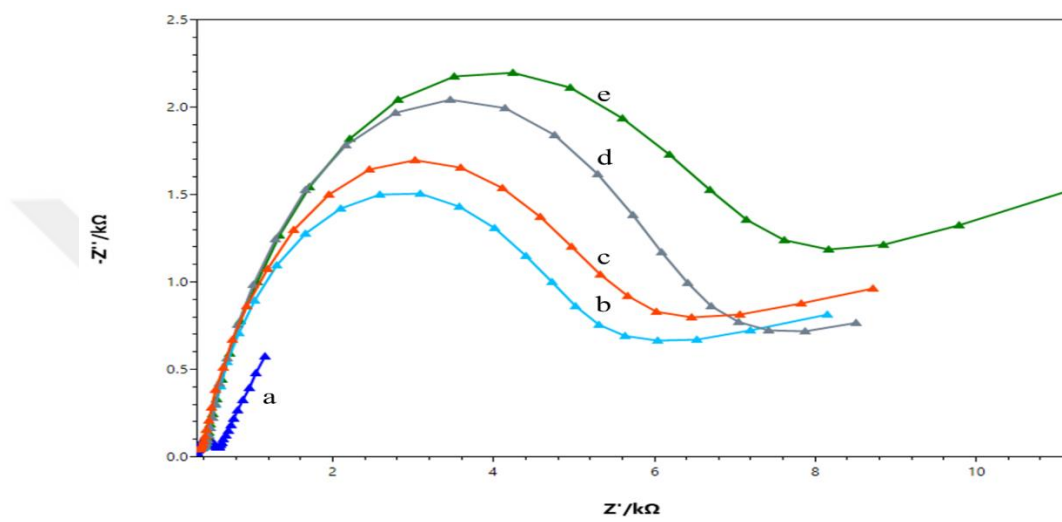


Figure 3.7 EIS curves. a) Carbon electrode b) DA removed electrode c) 100ng/mL serotonin d) 100ng/mL noradrenaline e) 100ng/mL dopamine

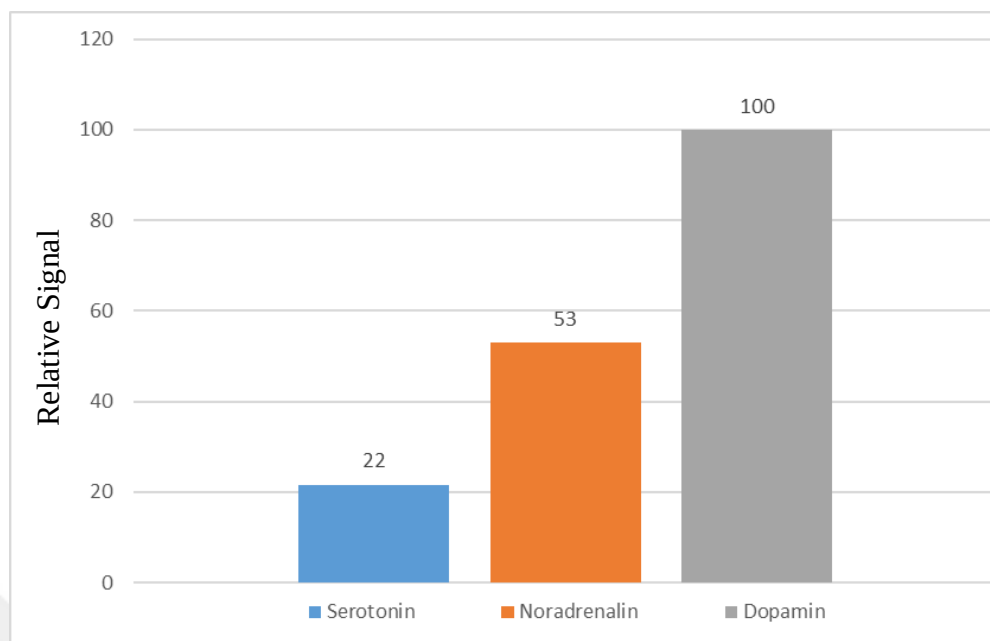


Figure 3.8 Relative comparison of sensor responses

Since the structure of noradrenaline and dopamine is very similar, interference effect was observed. Serotonin gave less signal. According to the results of the selectivity study, it was seen that the imprinted surface prepared for dopamine was specific for dopamine. The sensor's response to serotonin and neuradrenelin, whose molecular structures are quite similar to dopamine, was lower than dopamine.

3.1.4 Non-imprinted Carbon-Based Sensor for Dopamine

Non-imprinted surface was prepared by direct polymerization of PPy-Ppy3COOH to the carbon electrode without the target molecule dopamine. Then, after extraction, 500ng/mL dopamine was added (Fig. 3.9).

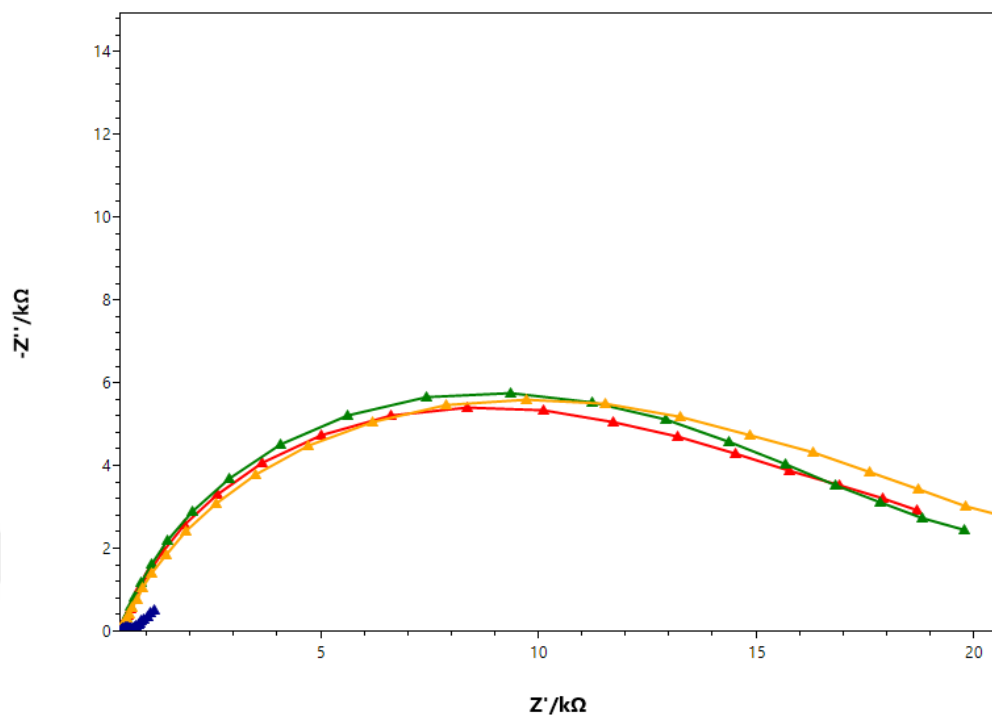


Figure 3.9 EIS curves of the non-imprinted surface. Blue) bare carbon electrode green) CE-PPyPPy-COOH, red) post-extraction, yellow) 500ng/mL dopamine administration

No significant increase was observed in dopamine application to the non-imprinted surface.

3.1.5 Carbon Based Dopamine Sensor Preparation without Fullerene

In the electrode preparation stage, the sensor system was prepared and dopamine was determined by applying the other polymerization steps one-to-one without using fullerene. It was observed that the use of fullerene increased the sensitivity of the sensor system (Fig. 3.10-3.11)

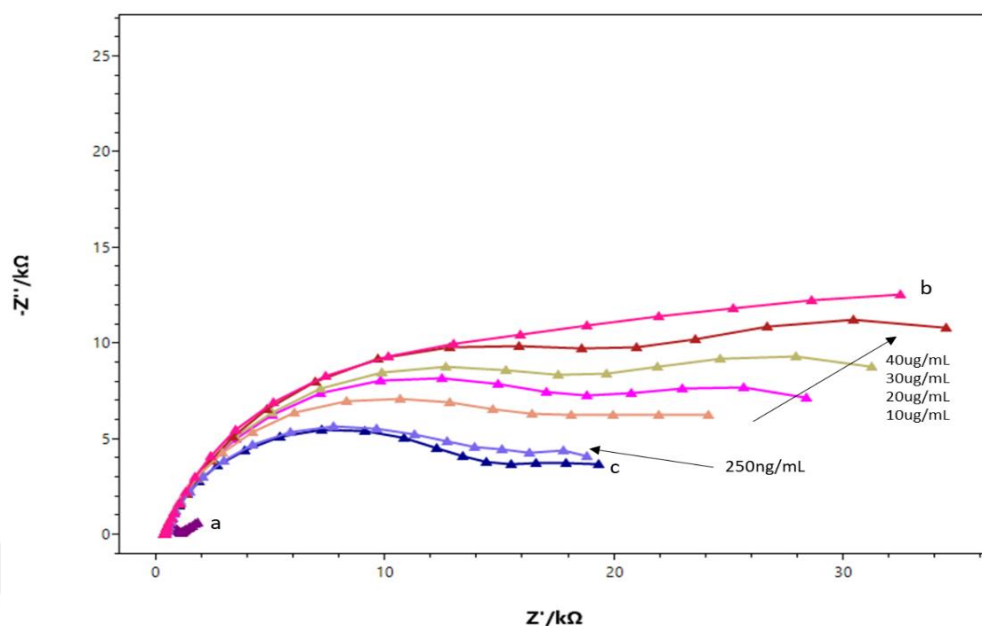


Figure 3.10 EIS curves of the fullerene-free system a) carbon electrode, b) polymer coated electrode, c) after extraction.

The Figure above shows the standard electrode graph produced without fullerene. Initially, a dopamine concentration of 250ng/mL was applied, since the increase in signal was low, 10 μ g/mL, 20 μ g/mL, 30 μ g/mL, and 40 μ g/mL were administered and the standard plot was drawn. However, measurements at these values are not possible and effective in a physiological environment, so Fullerene was added to this method and the sensitivity of the system increased and ng/ml concentrations were measured.

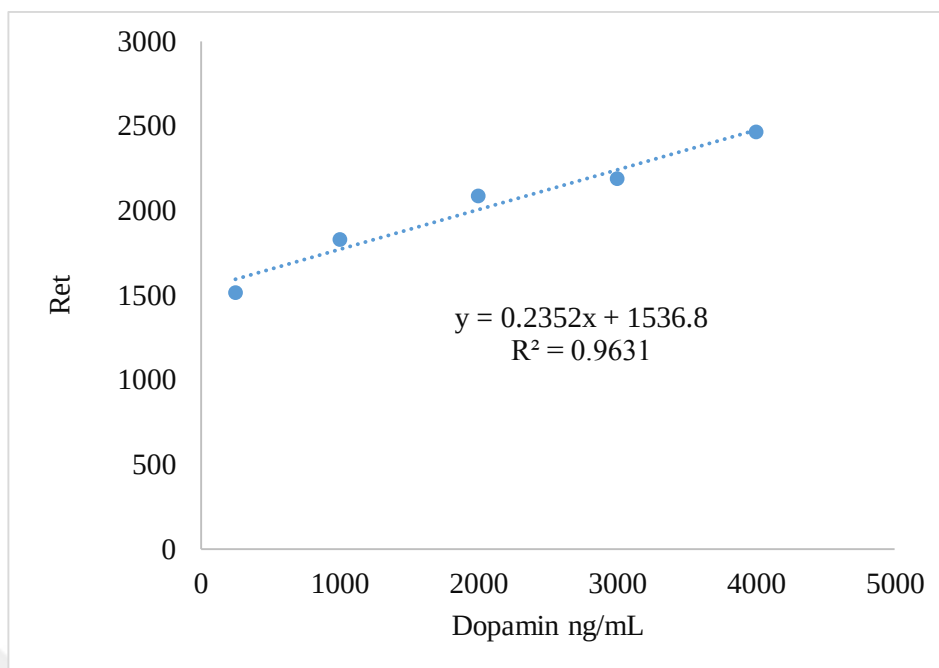


Figure 3.11 Calibration curve of the fullerene-free system

3.1.6 Applicability Studies of Carbon-Based Dopamine Sensor

After determining the appropriate parameters for the preparation of the sensor, dopamine determination was made using the real sample with the prepared sensor. For this, samples collected with the permission of the ethics committee numbered 17-1/16 from Ege University were used. ELISA method was used to compare the sensor results (Table 3.3).

Table 3.3 Comparison of ELISA and Sensor measurements

Sample	ELISA (ng/mL)	S	Sensor Response (n=3)	Relative Sensor Response (%)
1	125,6	1,102	139,07	10,72
2	189,8	1,803	198,20	4,43
3	144,6	0,462	156,07	7,93
4	150,0	1,905	155,00	3,33
5	156,0	3,418	162,80	4,36
6	162,8	3,855	168,43	3,46
7	170,0	0,902	178,17	4,80
8	178,8	0,520	185,80	3,91
9	208,9	1,305	199,97	4,28
10	110,8	2,879	115,10	3,88
11	124,1	0,529	138,60	11,68
12	139,0	1,650	147,73	6,28
13	155,8	7,414	163,07	4,66
14	174,5	2,000	197,20	13,01
15	176,4	2,407	186,67	5,82
16	185,6	3,327	196,44	5,84
17	195,9	6,865	188,18	3,94
18	151,2	2,881	167,30	10,66
19	166,9	4,610	181,01	8,49
20	198,9	3,581	210,27	5,71

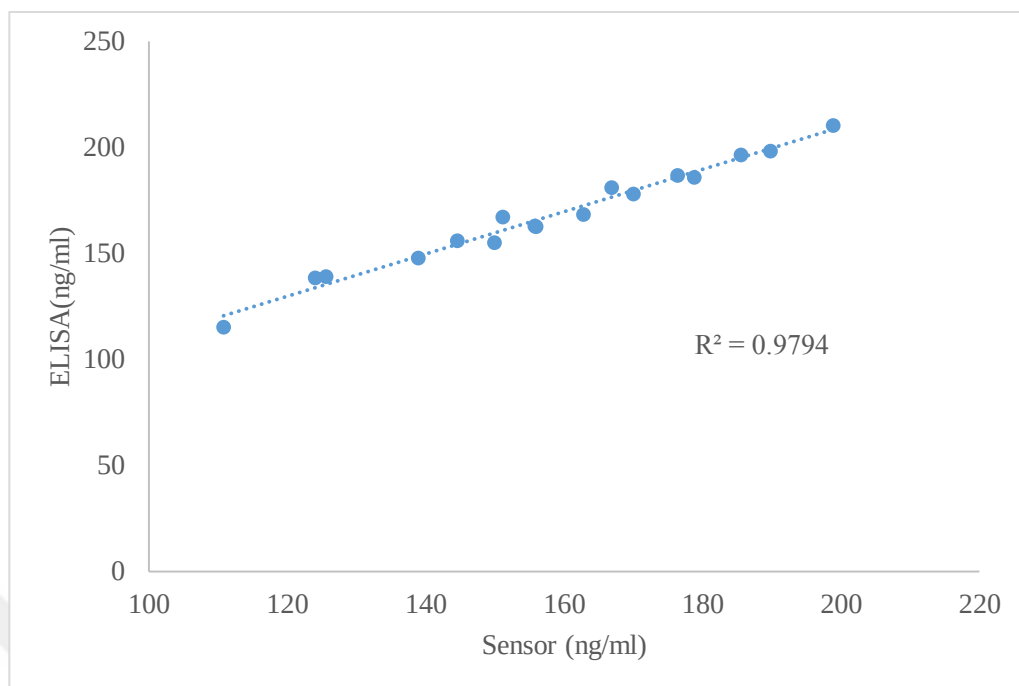


Figure 3.12 Regression analysis graph of ELISA and Sensor

The regression analysis of the two methods is shown in Figure 3.12. As can be seen, the sensor system for detecting dopamine levels in urine for real samples is usable compared to ELISA. Since the calculated R^2 value is very close to 1, we can say that there is a strong linear correlation ($R^2 = 0.9794$) between the sensor and ELISA results. The calculated t value (1.73) for the sensor system is smaller than the critical t value (2.093; $P=0.05$). This result shows that there is no significant difference between the results obtained by both methods. $T < t$, that is, the calculated value is less than the table value T, which shows that the methods can be used interchangeably.

In Table 3.4, the LOD value of this study and some studies in the literature are given.

Table 3.4 Comparison of the method with other methods

Detection Method	Detection (M)	Limit	References
GO/SiO ₂ -MIPs/GCE	3x 10 ⁻⁸		(Yanbo Zeng, Zhou, Kong, Zhou, & Shi, 2013)
Au@SiO ₂ -MIPs/GCE	2x 10 ⁻⁸		(Yu, Zeng, Qi, Zhou, & Shi, 2012)
AuNPs@MIES	8x 10 ⁻⁸		(Xue et al., 2013)
GSCR-MIPs hybrids	2x 10 ⁻⁷		(Mao, Bao, Gan, Li, & Niu, 2011)
<i>o</i> -aminophenol MIP	1.98 × 10 ⁻⁹		(Li et al., 2009)
CE-Fuleren-PPy-PPy3COOH	6x 10 ⁻⁸		This Work

3.2 Preparation and Characterization of AuE Based Dopamine Sensor

The electrodes were prepared by the electropolymerization method as mentioned in the method section 2.2.5. After each preparation step, impedance data was obtained and it was examined whether the polymerization took place or not (Figure 3.13). When these data are examined, it is seen that the surface area increases after the polymerization process. After the polymerization was seen to be successful, a standard graph was drawn with the electrode in the range of 100 -600 pM (Figure 3.14-3.15).

SEM images were observed as the surface structure changes and AFM results showed that topology of the surface was observed differently.

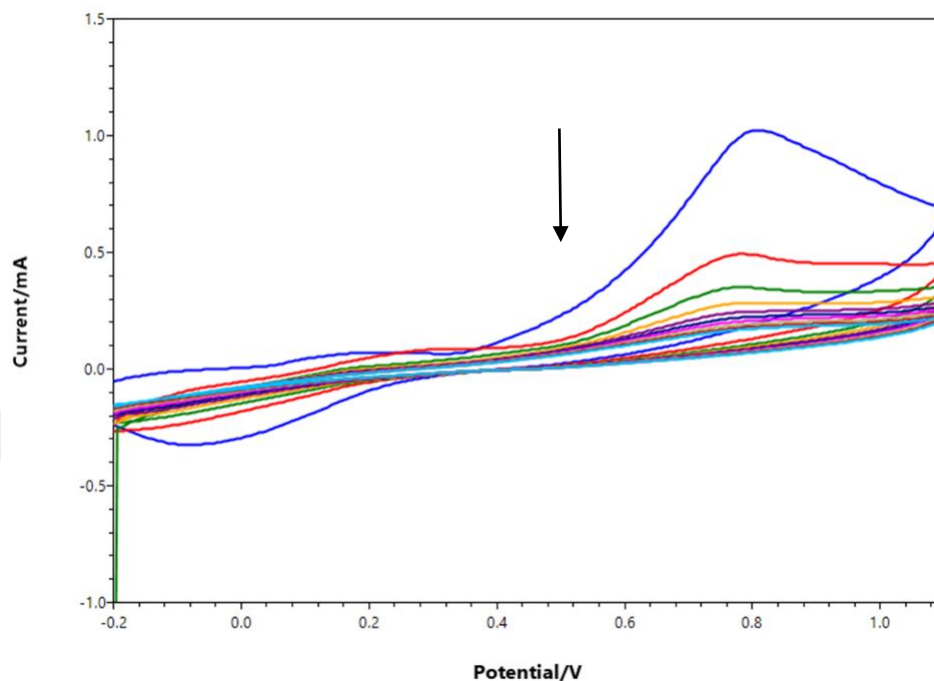


Figure 3.13 Electropolymerization of the electrode with APBA, pyrrole-3 carboxylic acid and dopamine. The direction of the arrow shows the decrease in the peaks with continued polymerization.

The decreasing peaks seen in Figure 3.13 show that the amount of monomer molecule in the medium decreases and turns into polymer.

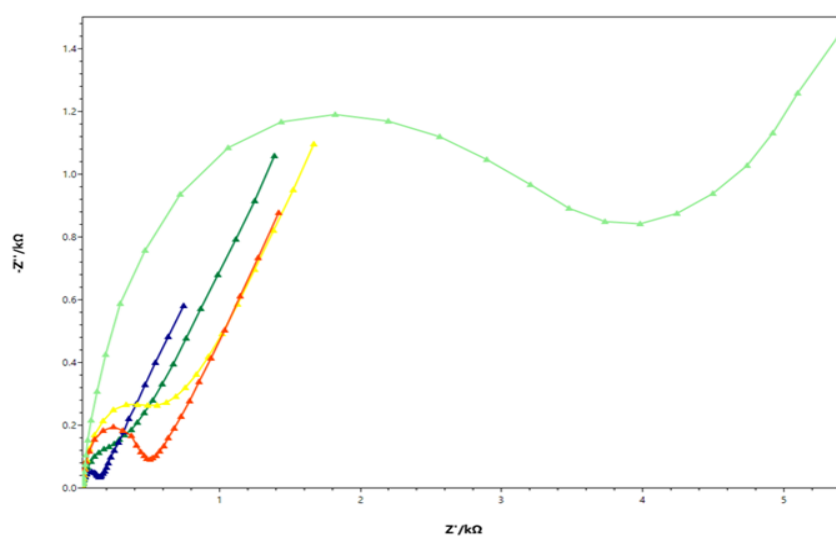


Figure 3.14 Preparation steps of Dopamine imprinted sensor. Blue) AuE Dark green) AuE-Cys Orange) AuE-Cys-APBA Light green) AuE-Cys-APBA-APBA-PPy3COOH-DA Yellow) AuE-Cys-APBA-APBA-PPy3COOH

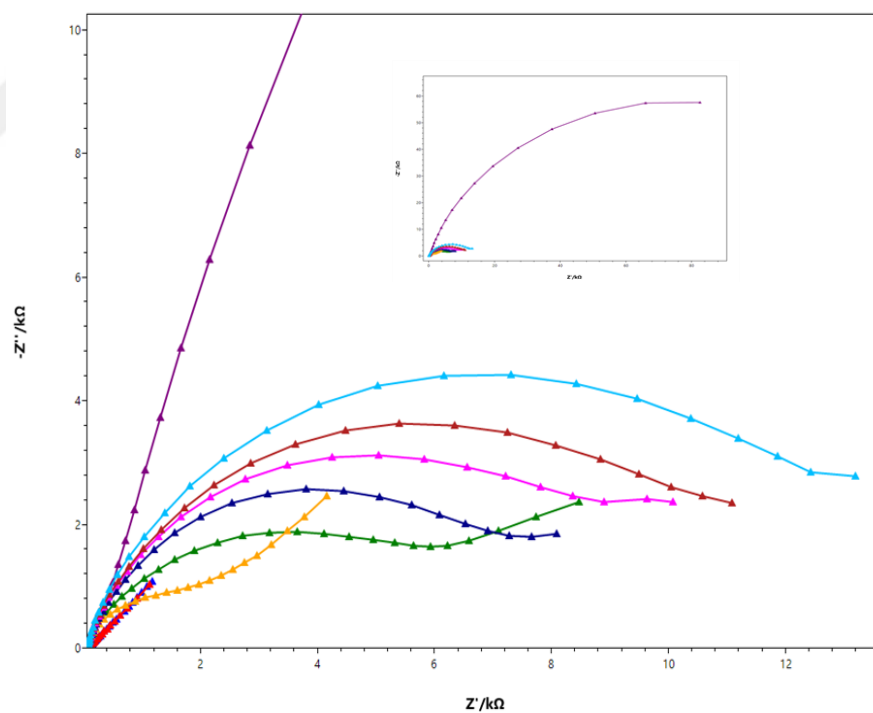


Figure 3.15 Calibration curve EIS values. Blue) AuE Red) AuE-Cys Purple) AuE/Cys/APBA/MIP (APBA-Carboxypyrrole-DA) Yellow) extracted, Others) Standart DA solutions, respectively increasing

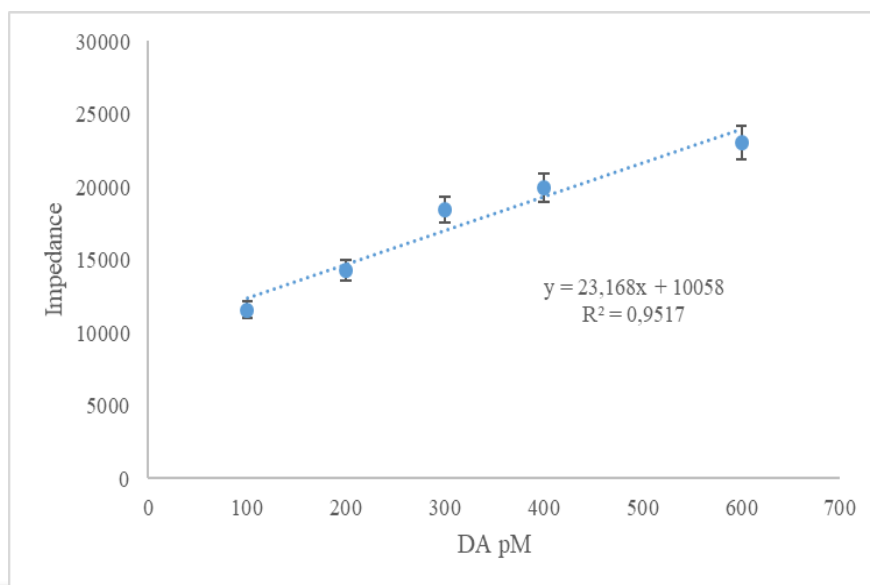


Figure 3.16 Calibration curve for DA sensor

Surface characterization analyzes of the prepared sensor system were performed using SEM (Figure 3.17), AFM (Figure 3.18) and XPS (Figure 20-24) devices.

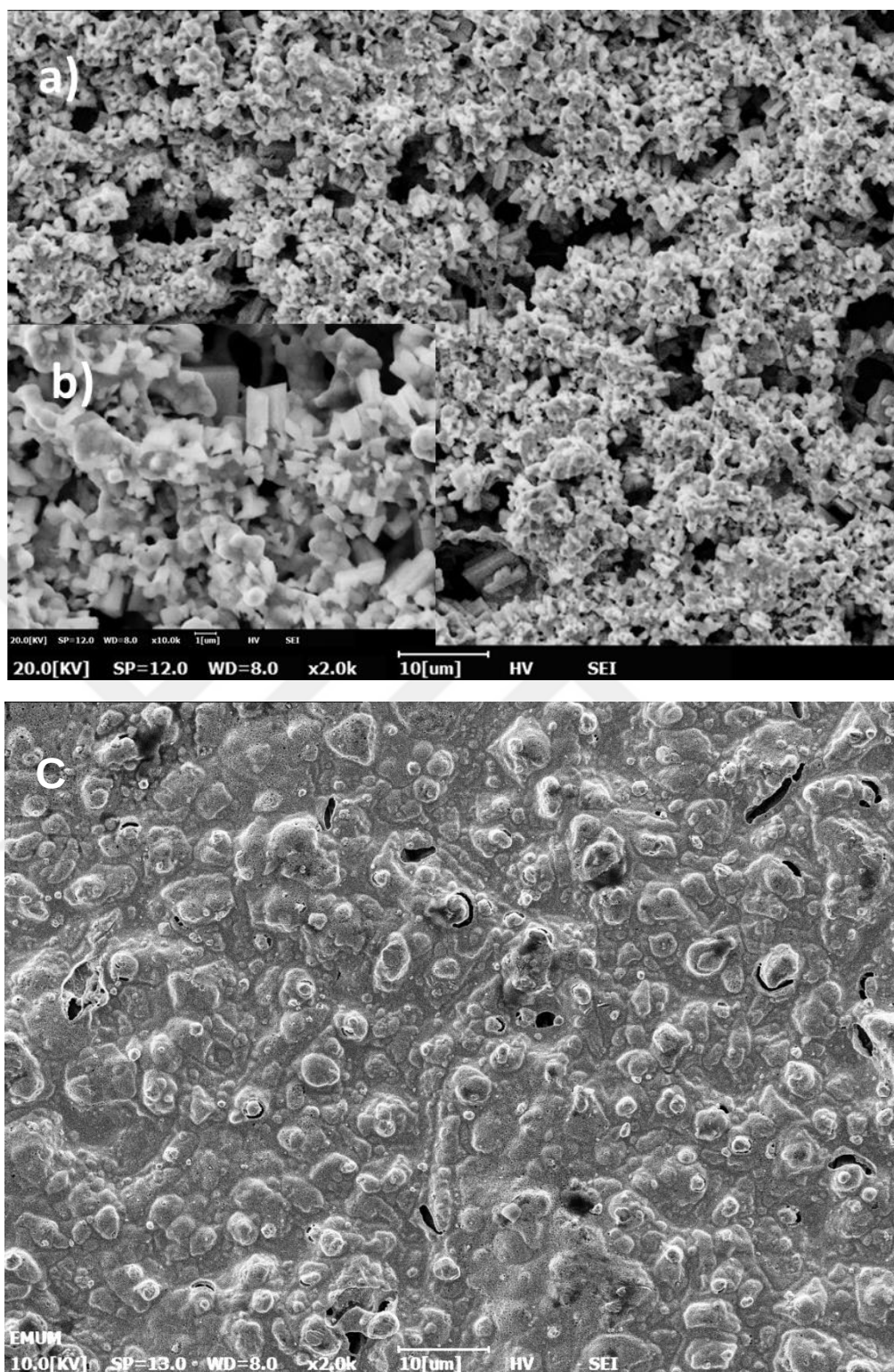


Figure 3.17 SEM images of the sensor surface: a) AuE/Cys/APBA/MIP(APBA-Carboxypyrrole-DA) 2k mag. b) AuE/Cys/APBA/MIP(APBA-Carboxypyrrole-DA) 10k mag. c) Bare AuE 2k mag.

SEM images of the bare electrode (Fig.3.17c) and the MIP electrode (Fig.3.17ab) are examined, it can be seen that the morphology of the electrode surface has changed and the surface area has increased. As a result of polymerization, the surface area of the electrode increased, which made the method more sensitive.

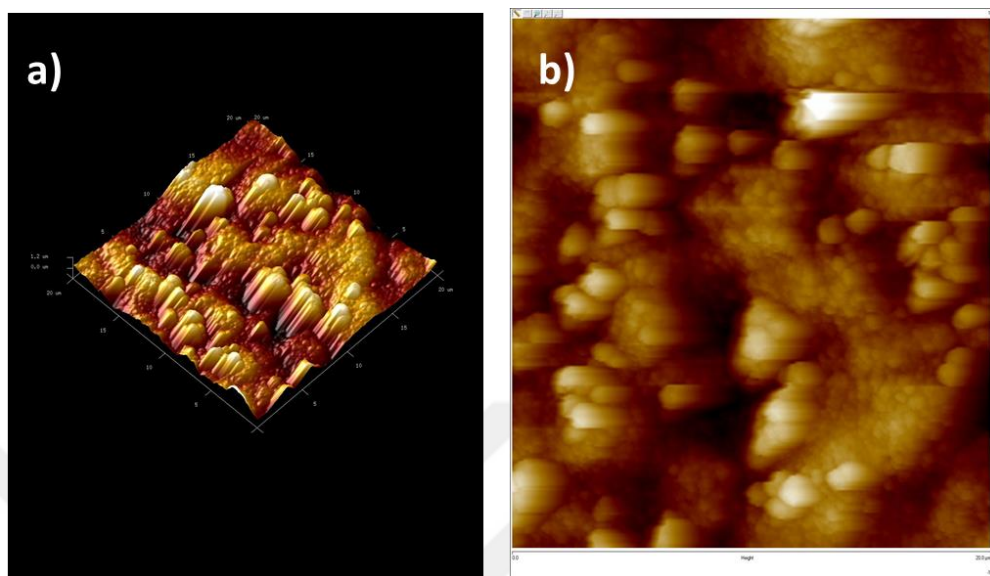


Figure 3.18 AFM images of the sensor surface: a)Height b)Topology

AFM images can be seen in Figure 3.18 and topology and the thickness of the surface changes by polymerization that approves the modification (Figure 3.18).

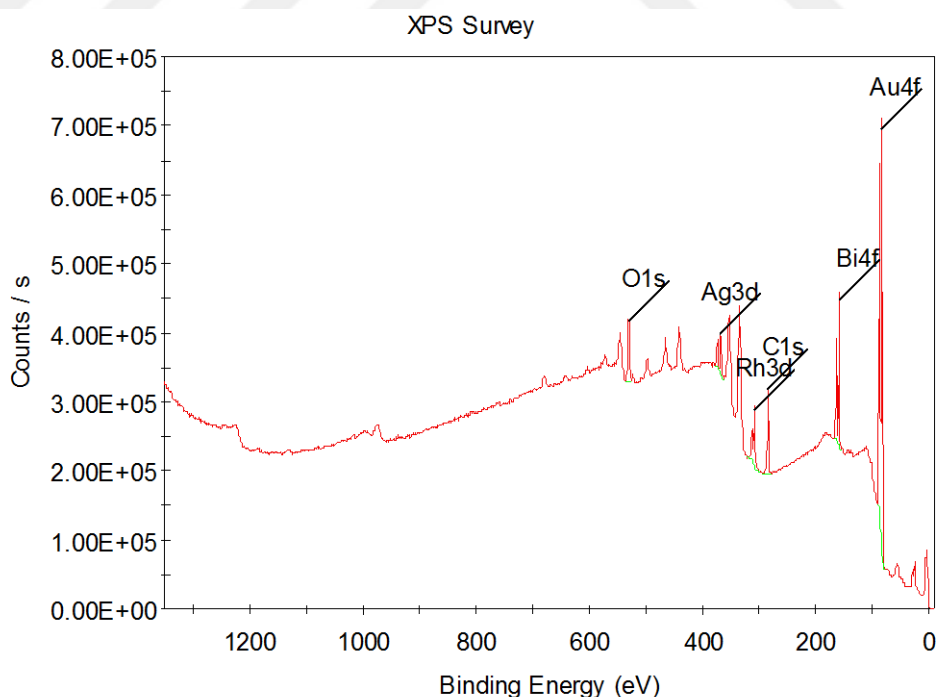


Figure 3.18 AuE XPS Survey

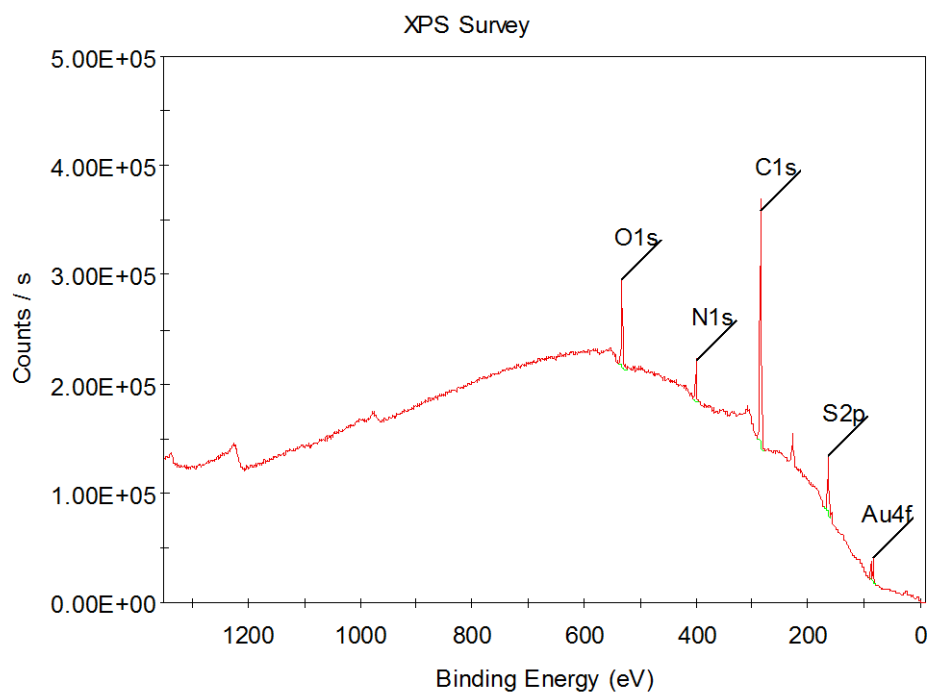


Figure 3.19 AuE-Cys electrode XPS Survey

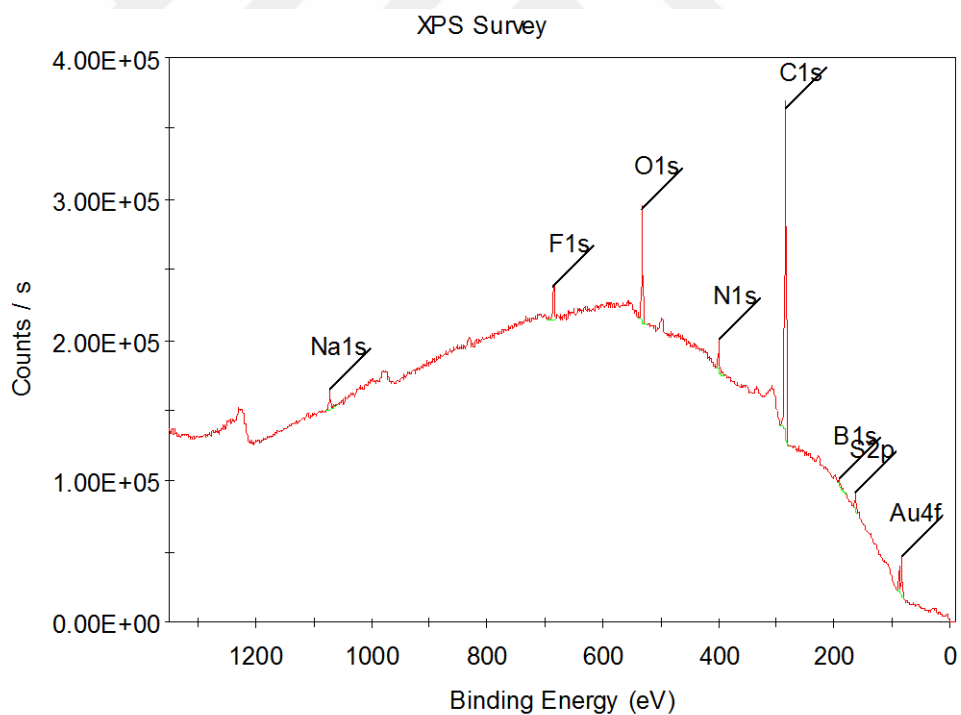


Figure 3.20 AuE-Cys-APBA electrode XPS survey

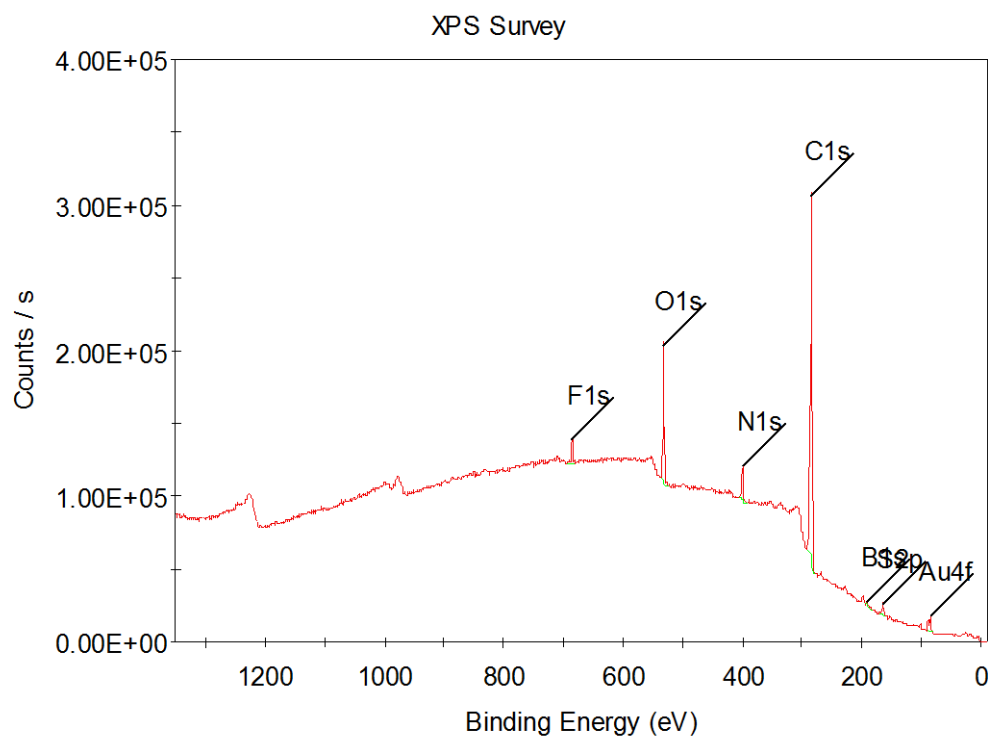


Figure 3.21 AuE-Cys-APBA-APBA-PPy3COOH-DA electrode XPS survey

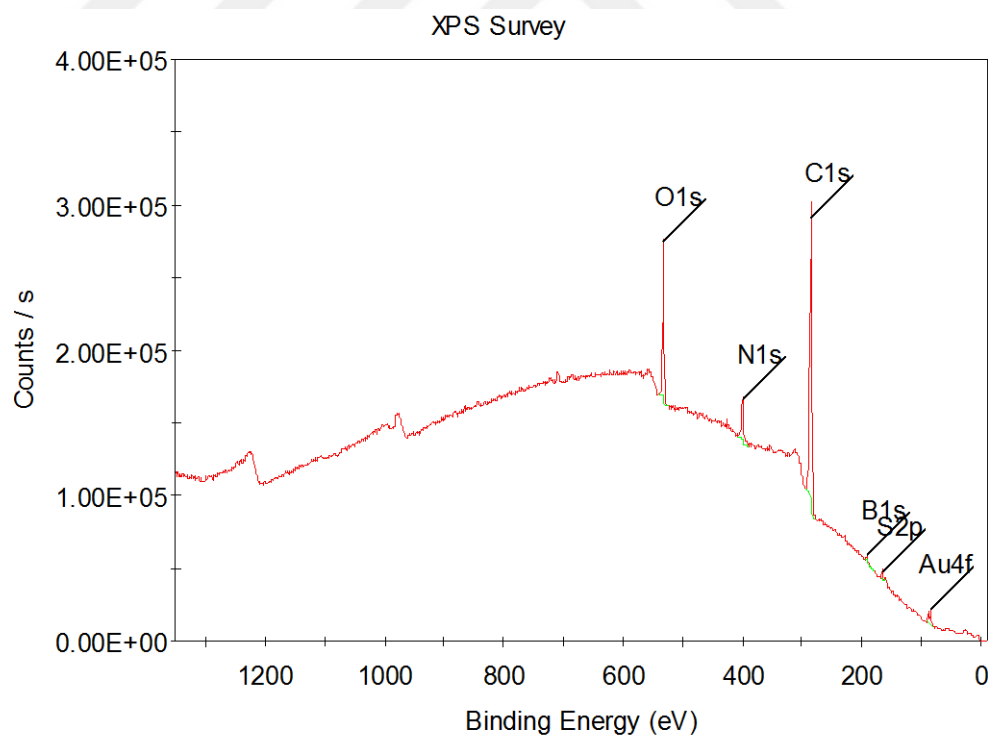


Figure 3.22 AuE-Cys-APBA-APBA-PPy3COOH electrode XPS survey

XPS analysis was performed after each electrode preparation step (Figure 3.18-3.22).

The survey spectras represented the peaks of C1s, N1s, O1s and Au4f at ~285 eV, 400 eV, 532 eV and 84 eV for AuE, AuE-Cys, AuE-Cys-APBA, AuE-Cys-APBA-APBA-PPy3COOH-DA, AuE-Cys-APBA-APBA-PPy3COOH electrodes. The % elemental distribution of each electrode is summarized in Table 3.5. With this analysis, it was seen that the electrode preparation and immobilization steps were successfully performed. It was observed that the percentage of gold in the bare gold electrode decreased as the electrode surface was coated. However, the amounts of C, N and O elements changed with each molecule added to the surface. The amount of C, which was 67.11% in XPS data obtained after dopamine imprinting, decreased to 61.58% after dopamine desorption. This indicates that desorption has taken place.

Table 3.5 % Weight of elements for each electrodes

Electrode	% Weight Au	% Weight C	% Weight O	% Weight N
AuE	57.70	12.16	7.64	
AuE-CYS	5.3	58.93	11.87	8.28
AuE-CYS-APBA	5.85	59.63	11.86	5.61
AuE-CYS-APBA-APBA-PPy3COOH-DA	3.42	67.11	14.36	5.52
AuE-CYS-APBA-APBA-PPy3COOH	3.09	61.58	17.37	8.27

Chronoimpedance measurement was taken to determine the optimum dopamine binding time of the molecularly imprinted electrode, which was prepared and desorbed as described before (Figure 3.23).

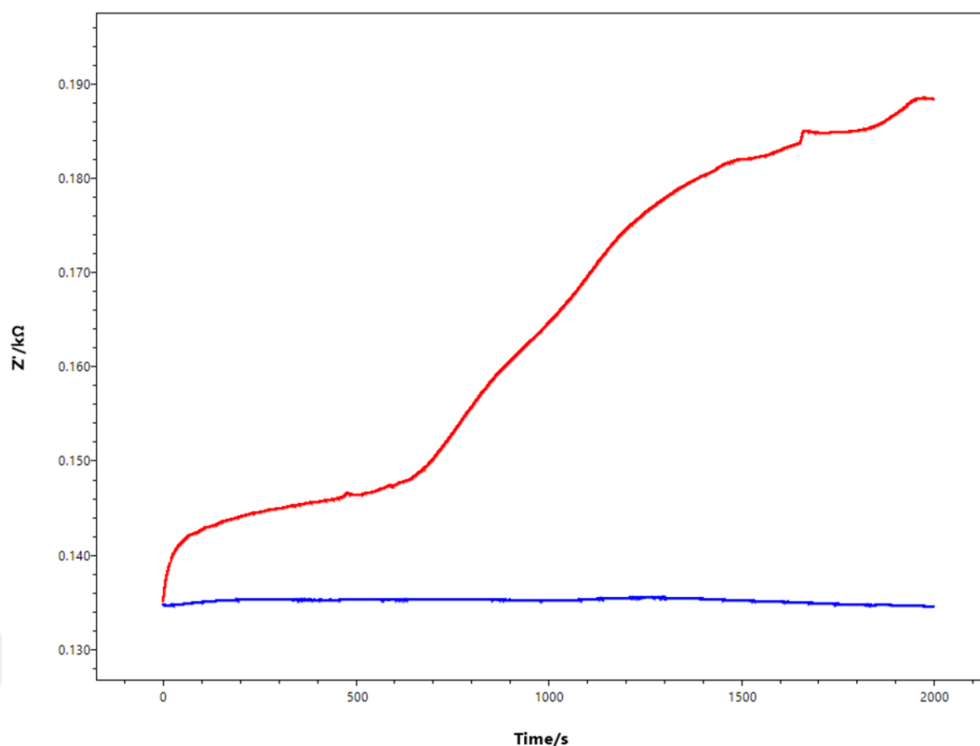


Figure 3.23 Chronoimpedance measurement of Dopamine imprinted sensor. Blue) in pH 7.4 50 mM phosphate buffer Red) 50 μ l 20 mM dopamine was added into pH 7.4 50 mM phosphate buffer

In the above Figure, first the measurement taken in phosphate buffer (blue), then the measurement taken after adding 50 μ l 20 mM dopamine into this buffer is given (red). Accordingly, the optimum binding time of the prepared electrode was determined as 200 seconds. Calibration curve was prepared after determining the bonding time by chronoimpedance measurement.

3.2.1 Optimization of the Monomers and template concentrations

For the optimization of the sensor surface, 4 different electrodes were prepared using different ratios of monomer (APBA and PPy3COOH). The ratios used were given in the table 3.5. The results of the optimization parameters were shown in Figure 3.24.

Table 3.6 Monomer and template concentrations

Electrode number	APBA (mM)	PPy3COOH (mM)	DA(mM)
1	30	30	20
2	40	20	20
3	20	40	20
4	20	20	40

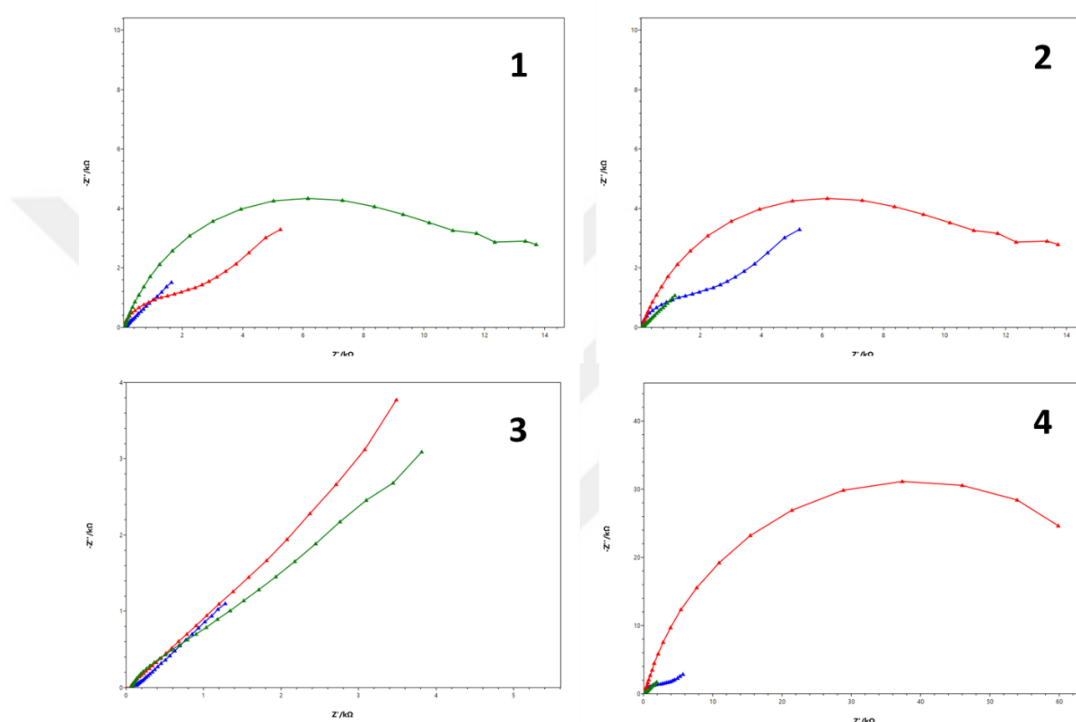


Figure 3.24 Prepared sensors with different concentration of monomers and template. Blue) Bare AuE, Red) AuE-Cys-APBA-APBA-PPy3COOH-DA Green) AuE-Cys-APBA-APBA-PPy3COOH

The electrode with the best results was determined as electrode 4. The best monomer-template ratio was found by comparing the resistance errors of the EIS measurements taken after the APBA-PPy3COOH-DA polymerization of the electrodes. Since the electrode % error closest to the theoretical value is the lowest electrode, it was determined as the optimum electrode.

3.2.2 Performance tests of the AuE Based Dopamine Sensor

In the performance steps, the lowest limit of determination (LOD) and limit of determination (LOQ), repeatability, reproducibility were performed according to the linear measurement range of the calibration graphic. After the calibration curve, 5 calibration curves were prepared to evaluate the reproducibility of the sensor technology.

The characteristics of the sensors were evaluated using the means, the standard deviation of the R^2 values, and the slope of these calibration curves (Table 3.7). From the calibration chart, $y = 23,168x + 10058$. Here, y is the impedance and x is the concentration of dopamine in pM. $R^2 = 0.9517$ indicates that linearity is quite good for this sensor.

Table 3.7 Performance parameters of the sensor

LOD pM	30.34
LOQ pM	95.82
Linearity	100-600 pM
R^2	0.9517
Repeatability (n=3)	93.70%
Reproducibility (n=5)	0.9517 ± 0.0288

3.2.3 Preperation of Non-Imprinted AuE Based Dopamine Sensor

The non-imprinted electrode was prepared without adding dopamine to the polymerization solution. Monomer amounts were used in the same way as the imprinted sensor (Figure 3.25). After the NIP sensor was prepared, the electrode was incubated with DA standard solution and EIS was measured (Figure 3.26). CI measurement was made on another NIP electrode prepared in the same way (Figure 3.27).

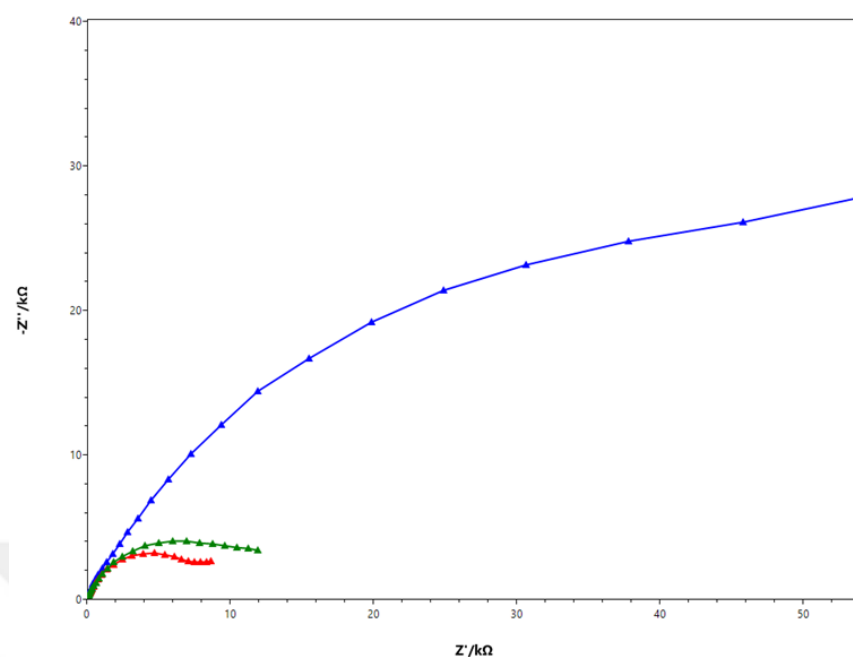


Figure 3.25 Immobilization steps of the NIP sensor: Blue: AuE-APBA-APBA-PPy3COOH Red) After Extraction Green) rebinding DA

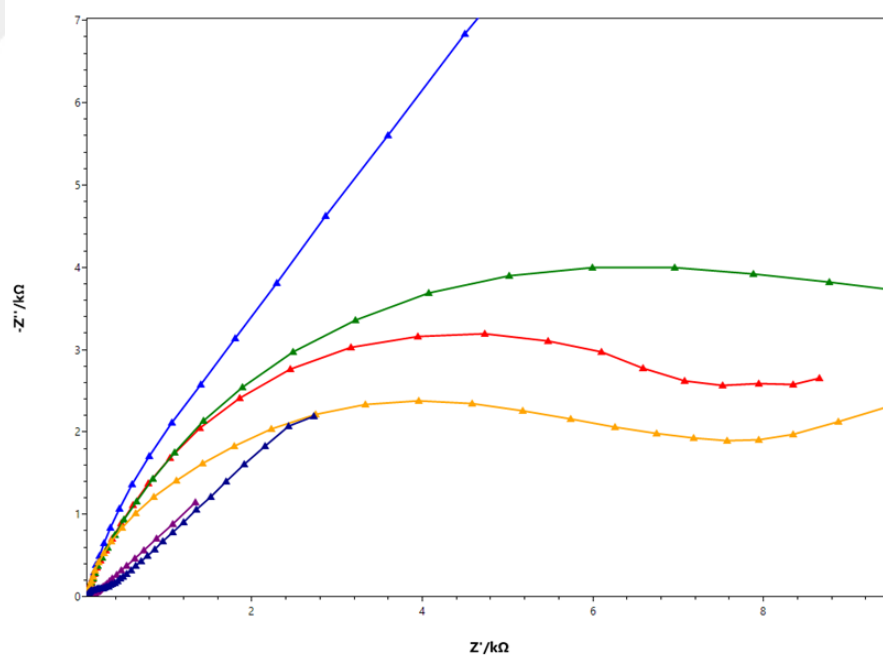


Figure 3.26 Dark blue) AuE Purple) AuE-Cys Yellow AuE-Cys-APBA Blue) AuE-Cys-APBA-APBA-PPy3COOH Red) After Extraction Green) Rebinding DA

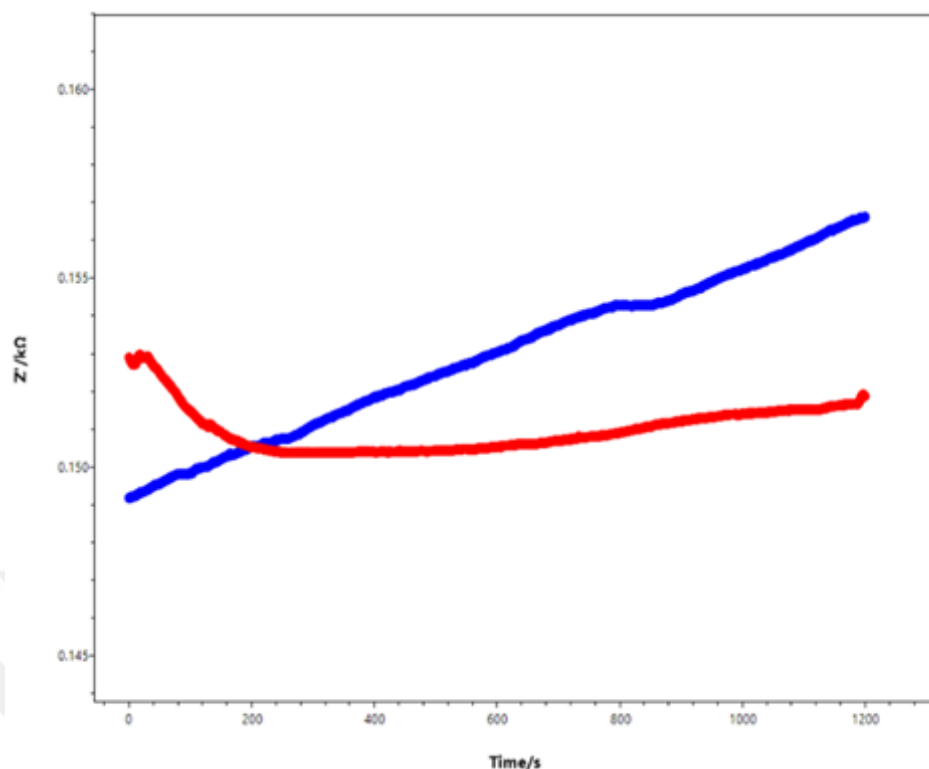


Figure 3.27 NIP sensor responses to dopamine at the same concentration

When the MIP and NIP sensor chronoimpedance results were compared, it was seen that the nonimprinted sensor did not respond to dopamine.

3.2.4 Selectivity of the AuE Based Dopamine Sensor

The selectivity of the sensor was studied with acetic acid and urea, which are known as interfering agents and frequently found together with dopamine in physiological fluids. The chronoimpedance method was used (Figure 3.28). It was observed that the sensor did not respond to urea and acetic acid and had a selectivity for dopamine. In addition, selectivity was also studied by using serotonin and noradrenaline, which are similar in structure to dopamine. Since the structure of noradrenaline and dopamine is very similar, interference effect was observed. Serotonin gave less signal. According to the results of the selectivity study, it was seen that the imprinted surface prepared for dopamine was specific for dopamine (Figure 3.29). The sensor's response to serotonin and neuradrenelin, whose molecular structures are quite similar to dopamine, was lower than dopamine.

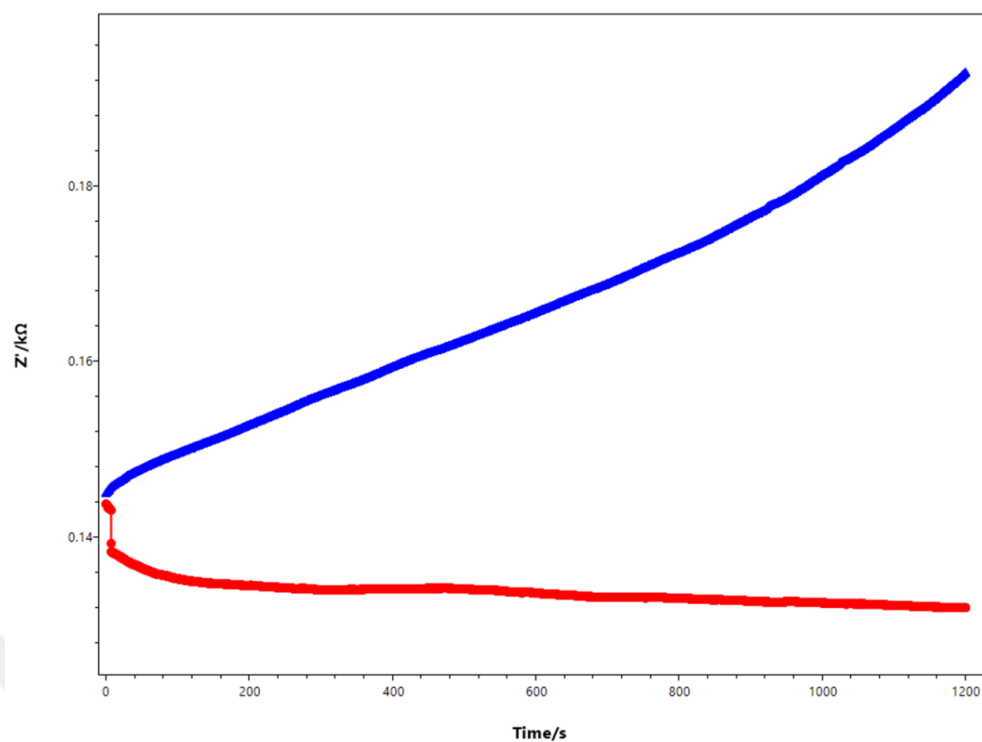


Figure 3.28 Selectivity of the sensor (Blue DA+Urea+A.A.) (Red Urea+A.A.)

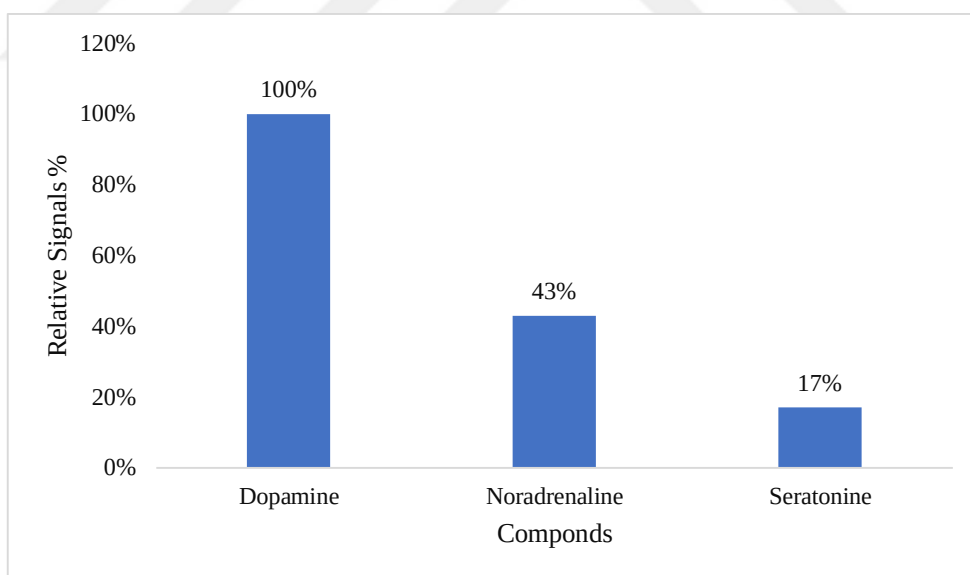


Figure 3.29 Relative signals of the DA sensor

3.2.5 Applicability Studies of AuE Based Dopamine Sensor

The applicability of the sensor to real samples was tested in serum and the results were compared with the ELISA method (Table 3.8).

Table 3.8 Real sample detection performance

Sample (1000x)	170 pM
Measurement 1	175,3 pM
Measurement 2	187 pM
Measurement 3	182,2 pM
Mean	181,5 pM
Standard Deviation	5,9 pM
% C.V.)	% 3,23

3.3 Preparation and Characterization of AuE Based A β 1-42 Sensor

For the determination of Beta Amyloid (A β 1-42), the recognition surface was prepared by modifying the gold electrode. For this, the gold electrode was kept in 100 mM cysteamine (Cys). Then, electropolymerization was performed with A β 1-42 and pyrrole 3 carboxylic acid (PPy-3-COOH) on the electrode incubated with a PAMAM G4 dendrimer. After each polymerization step, impedance data was obtained and it was examined whether the polymerization took place or not (Figure 3.20). After the polymerization was seen to be successful, a standard graph was drawn with the electrode in the range of 0.5-20 ng/ml (Figure 3.36). SEM and XPS analysis were carried out for the characterization of the prepared electrode.

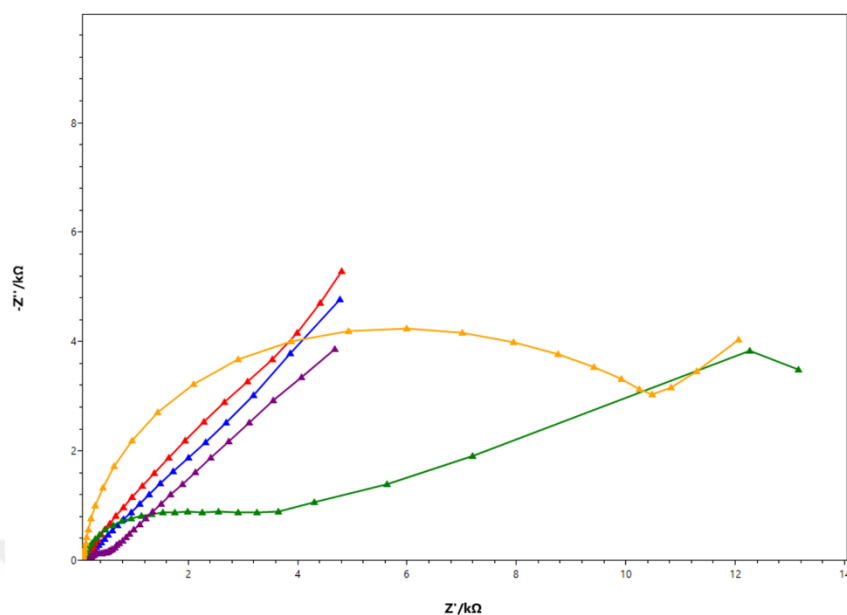


Figure 3.20 EIS data of A β 1-42 sensor electrode modification steps, Red) AuE Blue) Cys Green) AuE-Cys-PAMAM G4 Yellow) AuE-Cys-PAMAM- A β 1-42-PPy3COOH Green) After desorption

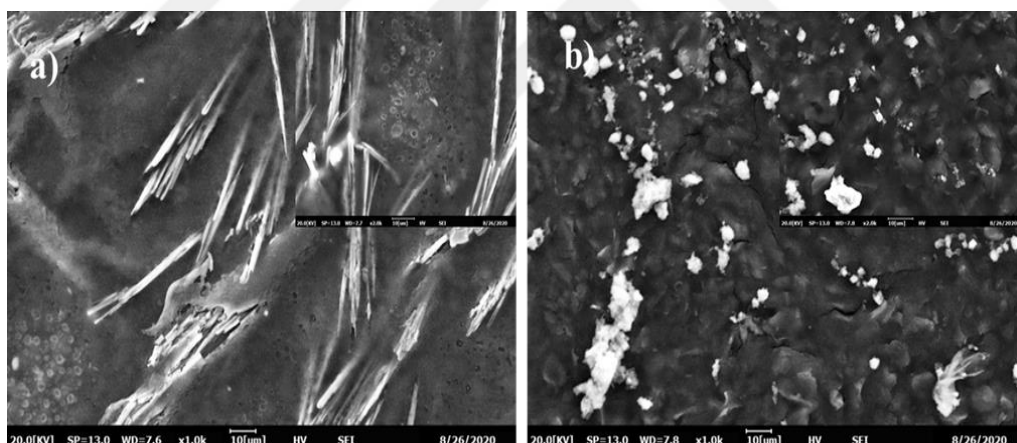


Figure 3.31 SEM analysis of Molecularly Imprinted Beta Amyloid Sensor. a) MIP sensor x1000 and x2000 magnification b) NIP sensor x1000 and x2000 magnification

SEM images of the imprinted and nonimprinted sensor surfaces are given in Figure 3.31. In MIP sensor, In MIP, it can be seen that the protein changes the polymer by entering the polymeric structure.

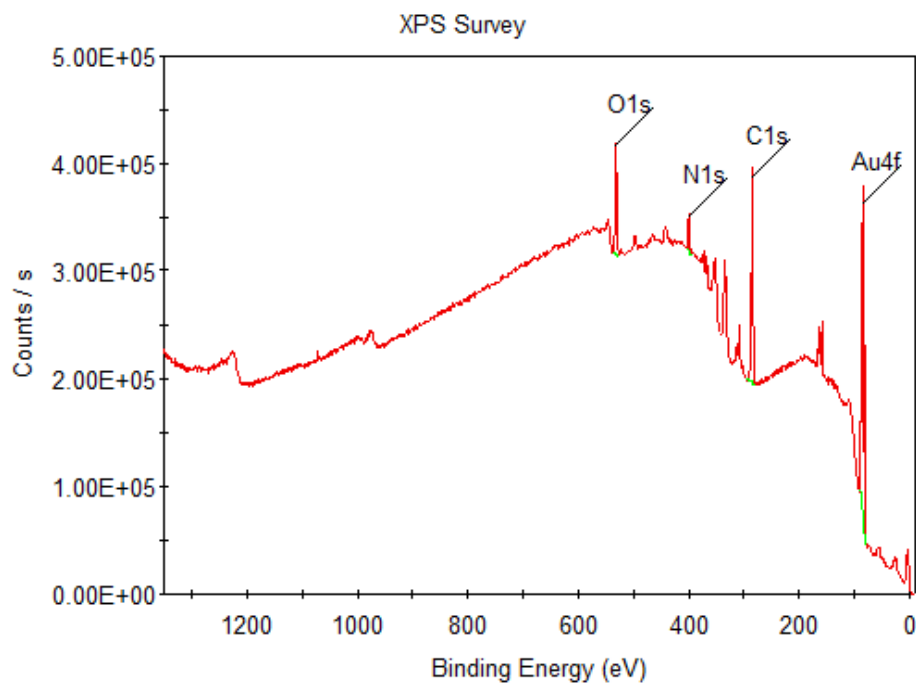


Figure 3.32 Au-CYS-PAMAM survey analysis

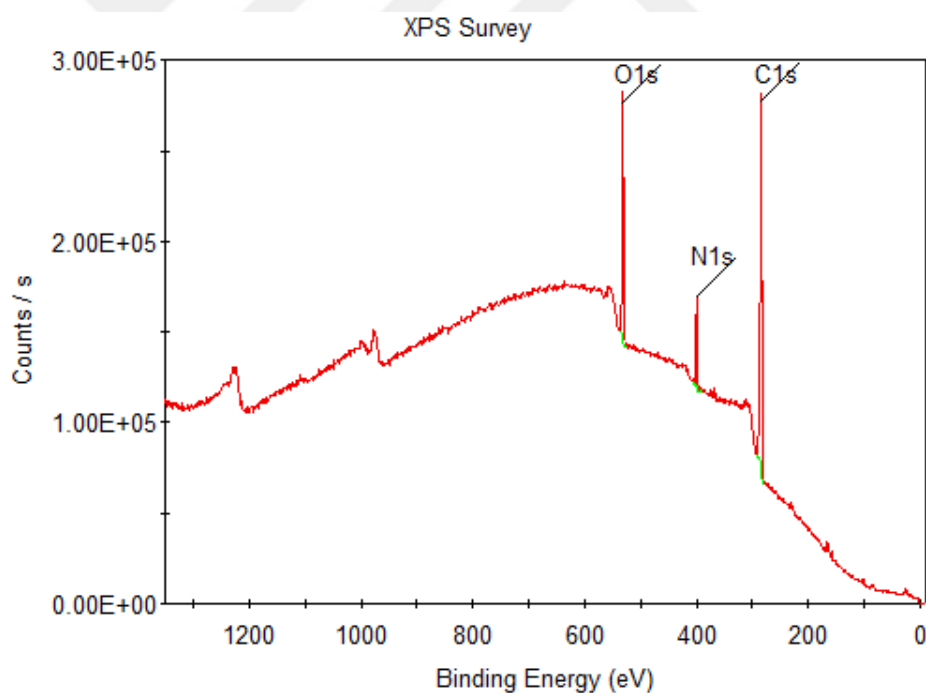


Figure 3.33 Au-CYS-PAMAM-A β 42 survey analysis

XPS analysis was performed after each electrode preparation step (Figure 3.31-3.32). The % elemental distribution of each electrode is summarized in Table 3.9.

With this analysis, it was seen that the electrode preparation and immobilization steps were successfully performed. It was observed that the percentage of gold in the bare gold electrode decreased as the electrode surface was coated. However, the amounts of C, N and O elements changed with each molecule added to the surface.

Table 3.9 % Weight of elements for each electrodes

Electrode	% Weight Au	% Weight C	% Weight O	% Weight N
AuE	57.70	12.16	7.64	-
AuE-CYS	5.3	58.93	11.87	8.28
AuE-CYS-PAMAM	48.64	33.61	12.32	5.43
AuE-CYS-PAMAM-Aβ42	-	66.15	22.64	11.22
AuE-CYS-PAMAM-PPy3COOH	2.26	53.69	19.43	11.85

3.3.1 Optimization of monomer and template molecule concentrations of the AuE Based A β 42 sensor

Four different electrodes were prepared using different ratios of monomer (PPy3COOH) for the optimization of the electrode preparation step of the sensor system prepared for the determination of A β 42 (Figure 3.33). The ratios used are given in Table 3.10.

Table 3.10 Monomer and template molecule concentrations

Electrode Number	PPy-3-COOH (mM)	A β 42 (ng/ml)
1	20	8
2	20	4
3	10	4
4	10	8

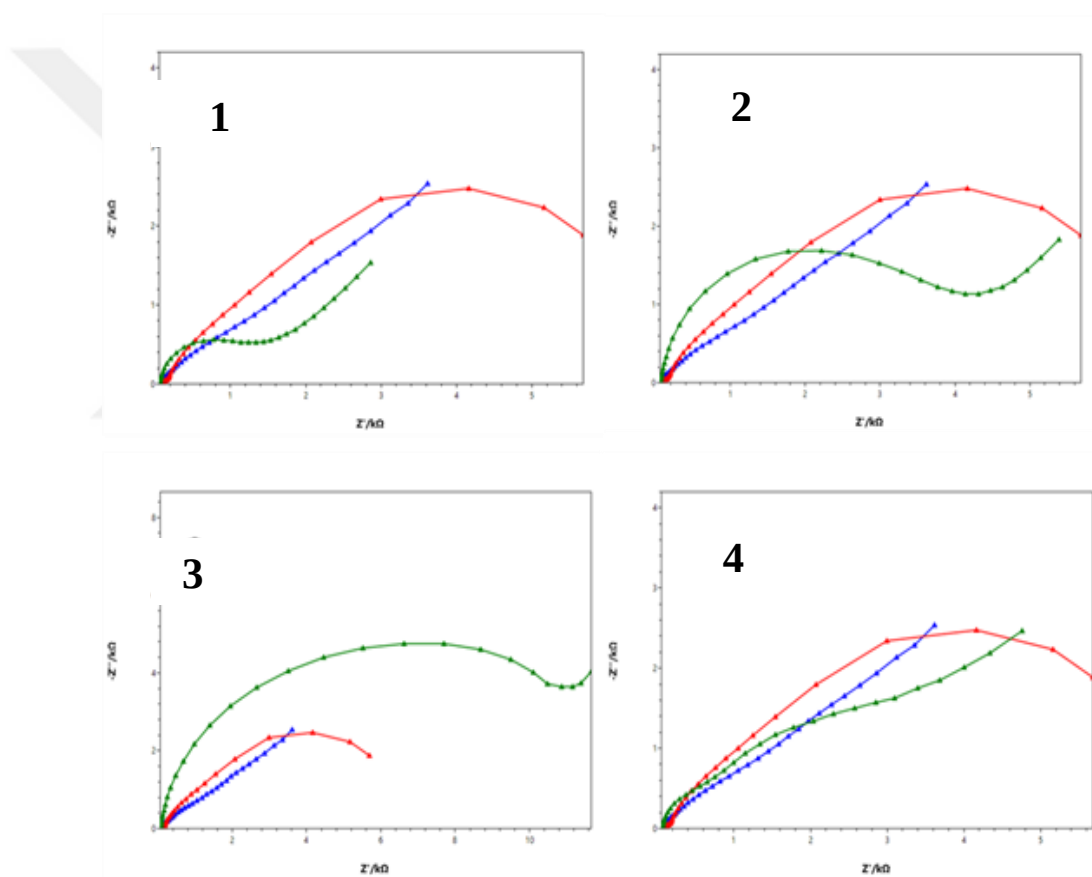


Figure 3.34 Prepared sensors with different monomer and template concentrations Red: Bare AuE Blue: AuE-PAMAM Green: Cys-PAMAM-PPy3COOH-A β 42

The electrode with the best result was determined as the number 2 electrode. The best monomer-template ratio was found by comparing the resistance errors of the EIS measurements taken after the Cys-PAMAM-PPy3COOH-A β 42 polymerization of the

electrodes. Since the electrode % error closest to the theoretical value is the lowest electrode, it was determined as the optimum electrode.

After the determination of the optimum electrode, chronoimpedance measurement was taken to determine the optimum A β 42 binding time of the molecularly imprinted electrode prepared and desorbed as previously described. Chronoimpedance measurement was made under 200 mV and 100 Hz conditions for 1200 seconds. For this, the electrode was first subjected to chronoimpedance measurement for 1200 seconds in pH 7.4 50 mM phosphate buffer, then 50 μ l of 66.7 ng/mL A β 42 was added to the measurement cell and chronoimpedance measurement was taken for 1200 seconds (Figure 3.35).

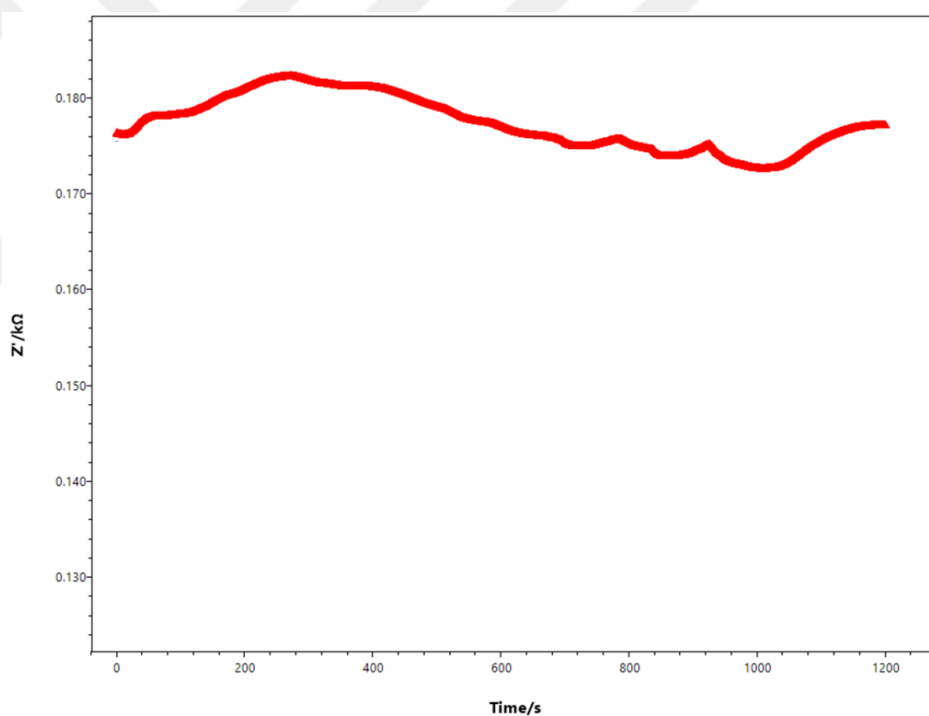


Figure 3.35 Chronoimpedance measurement of the A β 42 imprinted sensor

The optimum bonding time of the electrode prepared according to the chronoimpedance measurement was determined as 180 seconds.

3.3.2 Calibration Curve and Performance Characteristics of the A β 42 Sensor

Calibration curve with EIS performance parameters were given in Figure 3.36. LOD, LOQ, repeatability tests were also performed and showed in Table 3.11. Linear detection range was obtained between 0.5 to 20 ng/mL A β 42.

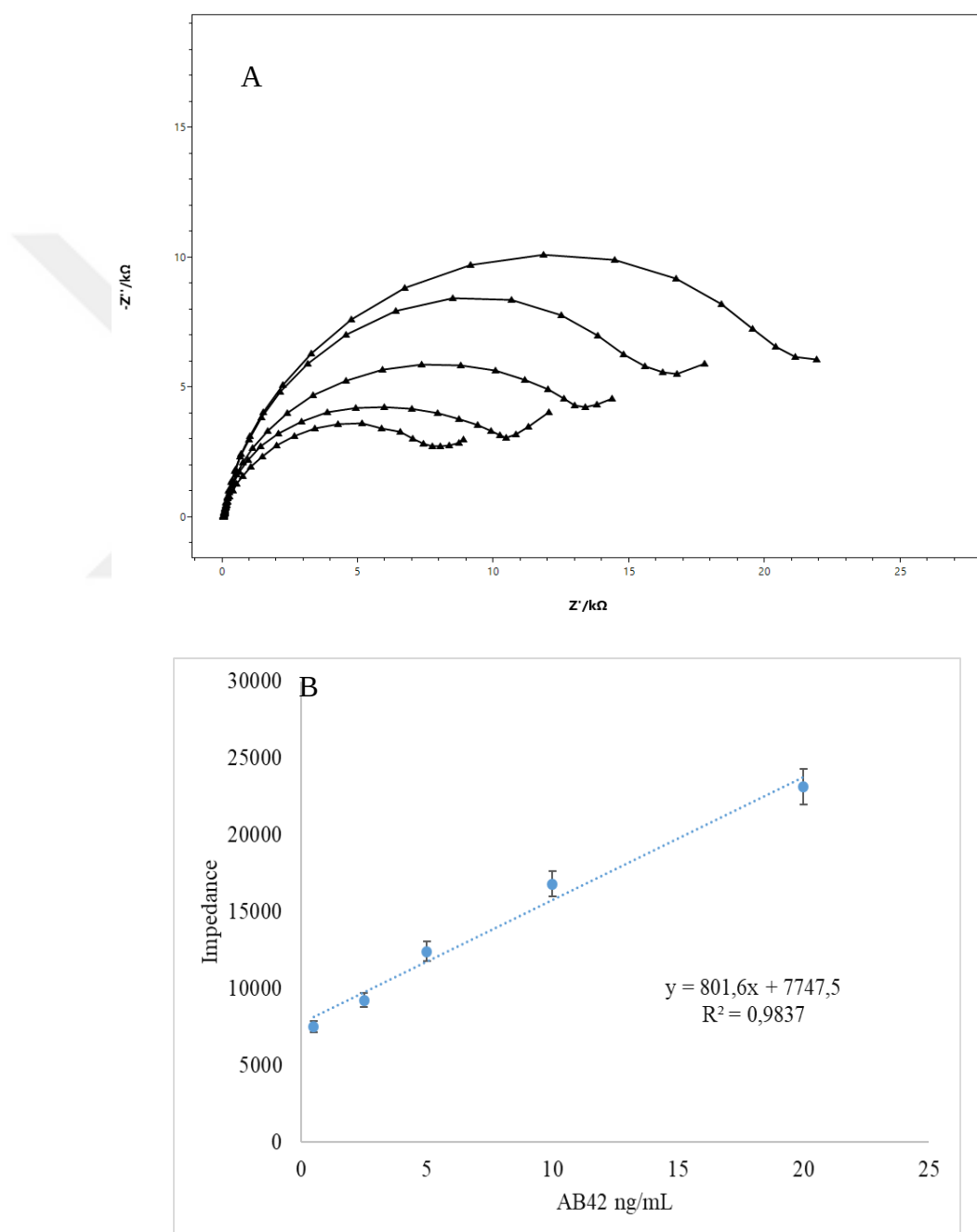


Figure 3.36 A: EIS Calibration curve value of the A β 42 sensor, B Calibration curve

Table 3.11 Performance characteristics of the A β 42 sensor

Linearity	0.5-20 ng/mL
R ²	0.9837
Reproducibility (n=4)	0.9837 $\pm$ 0.0089
Repeatability and Recovery (n=3)	
5 ng/mL	5.54 (111%)
20 ng/mL	18.4 (9%2)
LOD	0.14 ng/ mL
LOQ	0.42 ng/mL

3.3.3 Preparation of non-imprinted (NIP) AuE Based Sensor for A β 42

To verify the operability of the MIP electrode, the non-imprinted (NIP) electrode was prepared without adding the template molecule A β 42 to the polymerization solution (Figure 3.37). The monomer amounts were used in the same way as the imprinted prepared electrode. CI measurement was carried out also (Figure 3.38).

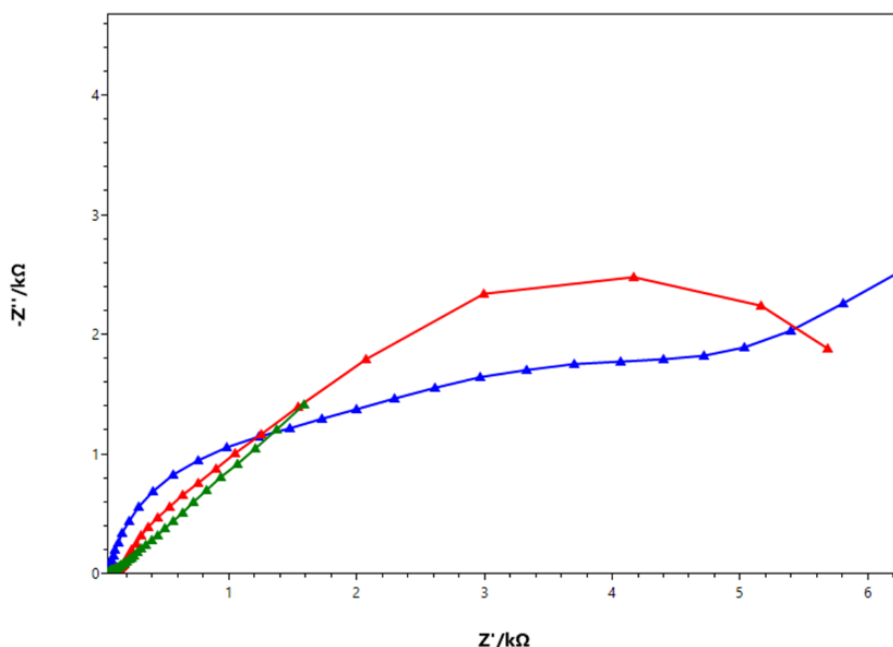


Figure 3.37 Immobilization steps of the NIP sensor: Green: AuE-Cys Red: AuE-Cys-PAMAM Blue: AuE-Cys-PAMAM-PPy3COOH

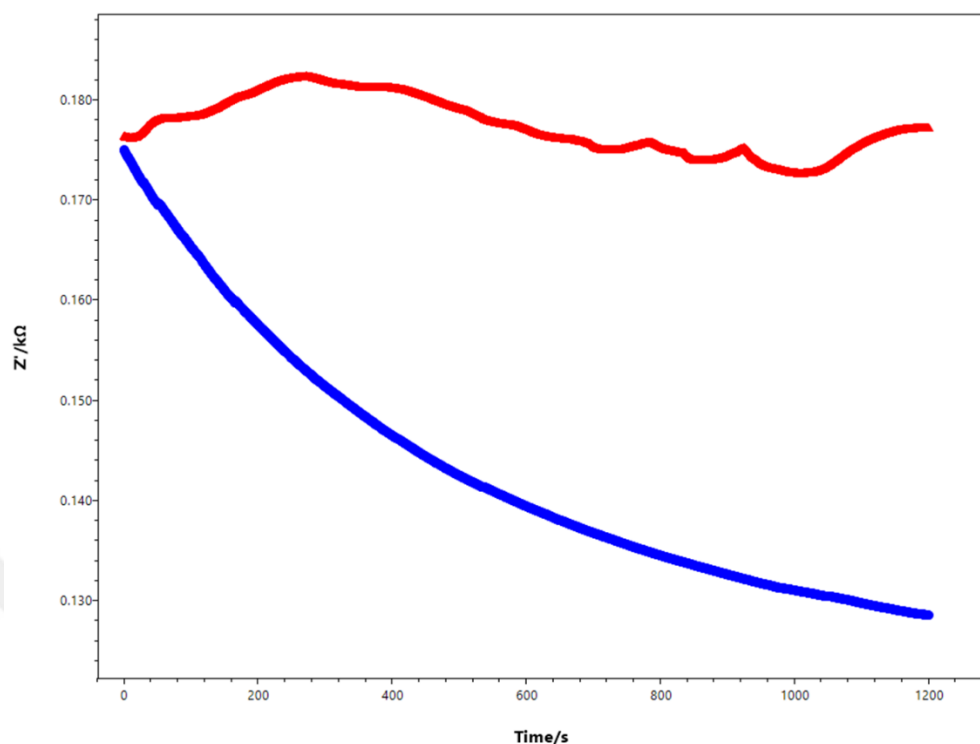


Figure 3.38 Comparison of A β 42 NIP (red) and MIP (blue) sensor responses at the same concentration

When the MIP and NIP sensor chronoimpedance results were compared, it was seen that the nonimprinted sensor did not respond to A β 42 in the response range of the imprinted sensor. The increase after 600 seconds is the increase due to the A β 42 molecule adsorbed on the surface.

3.3.4 Selectivity of the AuE Based Sensor for A β 42

The selectivity of the sensor was made with tau protein, another biomarker used in the diagnosis of neurodegenerative diseases. For this, chronoimpedance measurement was used. First, two electrodes were prepared as previously described. Measurements were taken with these electrodes separately in 3 ml pH 7.4 phosphate buffer for 1200 seconds (baseline). Then, by adding 10 μ l of stock protein solutions to the medium, measurement was taken again for 1200 seconds. The results were shown in Figure 3.39.

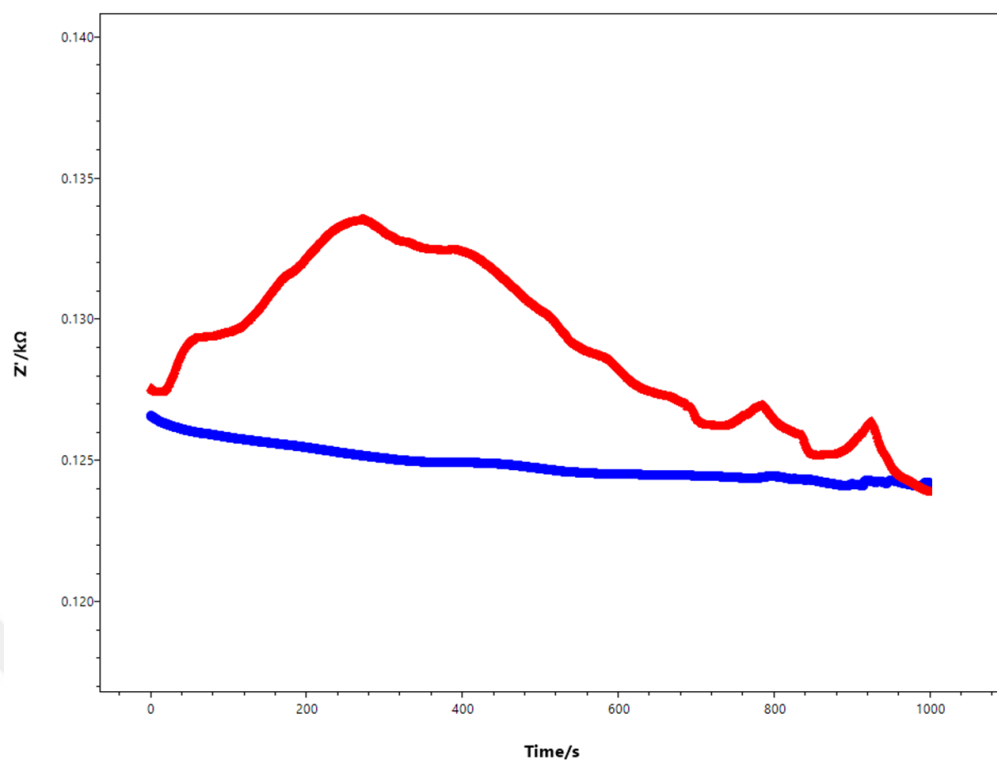


Figure 3.39 Selectivity of the sensor with Tau protein. Red) Response of the sensor to A β 42 Blue) Response of the sensor for Tau protein.

3.3.5. Applicability Studies of AuE Based Sensor for A β 42

The applicability of the sensor was tested in artificial serum. A β 42 was added to the serum with the standard addition method and the measurement was taken with the prepared sensor system.

Table 3.12 Real sample detection performance of the A β 42 sensor

Sample	110 ng/mL
Measurement 1	118.2 ng/mL
Measurement 2	120.0 ng/mL
Measurement 3	113.3 ng/mL
Mean	117.2 ng/mL
Standard Deviation	2.83 ng/mL

CHAPTER IV

CONCLUSION AND RECOMMENDATIONS

In this thesis, molecularly imprinted sensors were developed to measure different biomarkers used in the determination of neurodegenerative diseases such as dopamine and amyloid beta, those measurements were performed on these sensors. As it is known, dopamine is a neurotransmitter molecule, its measurement can be used to diagnose or monitor different diseases. Amyloid beta is known to be a biomarker of Alzheimer's disease. Therefore, the measurement of these two biomolecules is important. While measuring, or more precisely, when making a measurement in the biomedical field, the desired thing is to measure at low cost and without being affected by physical factors. Therefore, we conducted a study by taking these important points into account while developing a new generation measurement.

Although it is aimed to measure the same molecule by using different surfaces and transducers in the preparation of sensor systems, it is done in order to obtain more sensitive and advantageous sensors.

In this study, we prepared selective surfaces for dopamine using molecular imprinting technology. We increased the selectivity by imprinting the target molecules from different ends by producing a copolymer form as a monomer. By producing polymers with different structures by imprinting from different ends, they could be imprinted on the sensor surface with dopamine and amyloid beta, pyrrole, pyrrole-3-carboxylic acid and aminophenyl boronic acid monomers for different purposes. The N group on pyrrole, the carboxyl and boronic acid ends on the pyrrole-3-carboxylic acid were chosen for their ability to form hydrogen bonds. These electropolymerizable monomers were able to easily form polymers on the electrode surface. The potential to be reproduced in this way is also high, as production can be standardized. The proof of molecular imprinting is the verification of the sensor technology we produce with different methods. Here, electrochemical validations were performed on CV and EIS data. The redox probe, in which the electrode system is electrochemically characterized, is negatively charged. The movement of the redox probe to the surface changes with the change of charges on the electrode surface. Under normal conditions,

ignoring electrostatic interactions, the approach of the redox probe to the surface is by diffusion. However, if the surface is negatively charged, for example, if carboxyl groups or boronic acid groups are located on the surface, the redox probe is repelled by these negatively charged groups. On the other hand, if it is coated with pyrrole, it is attracted to the surface by N groups. As a result, more or less redox probes are found on the surface than diffusion and are electrochemically transformed, and in this way, the CV and EIS peaks change depending on the surface. At reverse loads, the CV peak increases, while the EIS decreases. Otherwise, the opposite situation occurs. With these interactions, electrode surface characterizations were carried out. Apart from this, it can be determined with which end of the target molecules interact. In the first sensor we developed, pyrrole and pyrrole-3-carboxylic acid monomers were coated on fullerene nanomaterial. EIS data decreased with the dominant and free pyrrole monomer seen here. In addition, the fact that the standard graph is between extraction and imprinting shows that the sensor is correctly and interested in the target molecule. This imprinting was also confirmed by scanning electron microscopy. The low adsorption characteristic of the sensor and its good linear range operation also have a high effect of the copolymer. Because it is important to cover the ends that will make adsorption with carboxylic acids. However, it shows that the surface area is increased with the addition of fullerene to the structure. In this way, the sensitivity is increased. Selectivity studies also show this situation and there is no signal on the non-imprinted sensor. Real sample trials with regression analysis also confirm this result. Considering similar studies, although the sensitivity is close to our system, better results were obtained in terms of selectivity and measurement time.

A gold electrode was used for another dopamine analysis. The important point here is the conductivity of the gold electrode. However, the gold electrode does not facilitate the adhesion of the polymer to the surface like the carbon electrode. Because the surface roughness is less and this makes it difficult for the polymer to stick. For this, the gold electrode surface is coated with self-assembled monolayers to form active tips and facilitate the interaction of the polymer with the surface. The fact that the gold electrode and the surface are loaded with positive charges is that the monomers used in dopamine imprinting can be stabilized by the positively charged SAM layer. Therefore, sensitivity at pM levels was achieved by creating a more

conductive area on the surface of the polymer. The regression value of 0.9517 also showed an acceptable level of linearity. This imprinting process, which was confirmed by AFM and XPS, was used to determine the measurement time of the target molecule chronoimpedimetrically. In the performance data obtained after the successful completion of the optimization stages, the LOQ value and the calibration graph being close to the starting point show that the imprinting process is successful. Chronoimpedimetric measurements also seem to confirm the selectivity. Repeatability and reproducibility data were also obtained in this way quite conveniently.

In the light of data obtained from gold electrode sensitivity, a new generation amyloid beta sensor has been developed. In this sensor, PAMAM and more monomer than normal were used to increase the SAM layer and surface area. Since the size of the amyloid beta molecule is high, the measurement was carried out at the nanogram level by using a high proportion of monomer.

As a result, in this doctoral thesis, new generation sensor systems with high selectivity were developed for use in the diagnosis of neurodegenerative diseases. In this way, the thesis study was completed in accordance with its aims.

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