DEVELOPMENT OF ELECTROCHEMICAL SENSOR BASED ON MODIFIED ELECTRODE FOR THE DETERMINATION OF CARBENDAZIM

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This thesis titled DEVELOPMENT OF ELECTROCHEMICAL SENSOR BASED ON MODIFIED ELECTRODE FOR THE DETERMINATION OF CARBENDAZIM has been prepared and submitted by Fayha MOHAMED ADAM HAMID in partial fulfillment of the requirements in "Anadolu University Directive on Graduate Education and Examination" for the Degree of Master's in Chemistry Department has been examined and approved on 16/01/2020.

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ABSTRACT

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In this study, the development of an electrochemical sensor was examined based on the preparation of a modified electrode in order to produce a method highly sensitive, selective, simple and rapid determination of (CBZ) fungicide. Firstly, nanocomposite consisting of fumed silica decorated with silver nanoparticles (FS@Ag) was synthesized. Secondly, the carbon paste electrode was modified with the prepared modified agent and a voltammetric method was proposed for carbendazim quantification. Modification agent and modified electrode surface were characterized by X-ray diffraction, EDX mapping and scanning electron microscopy analysis. Modified electrodes were also electrochemically characterized using cyclic voltammetry and electrochemical impedance spectroscopy analysis. The modified electrode showed an electrocatalytic effect in the oxidation of carbendazim. Development of the method for the determination of carbendazim was involved optimizing experimental parameters including the percentage of modification agent, pH and accumulation time. The performance of the prepared electrochemical sensor has been investigated in real samples such as river water, orange and apple juice, and tomatoes samples. With this proposed method, a low limit of detection (0.000940 μM) and a low limit of quantification (0.003134 μM) and a wide linear range (0.05 – 10.0 μM) were obtained for CBZ analysis. The results showed that the electrochemical sensor prepared here can be used as an alternative to current analytical methods used for the measurement of carbendazim.

Keywords: Carbendazim, Nanocomposite, Modified electrode, Silver   nanoparticles, Sensor.

ÖZET

KARBENDAZİM TAYİNİNE YÖNELİK MODİFİYE ELEKTROT TEMELLİ ELEKTROKİMYASAL SENSÖR GELİŞTİRİLMESİ

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Kimya Anabilim Dalı

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Bu çalışmada, fungisit karbendazimin (CBZ) oldukça hassas, seçici, basit ve hızlı bir şekilde belirlenmesi için modifiye edilmiş bir elektrodun hazırlanmasına dayanan bir elektrokimyasal sensör geliştirilmiştir. İlk olarak, gümüş nanoparçacıkları ile modifiye edilmiş tütsülenmiş silikadan oluşan nanokompozit sentezlenmiştir. İkinci olarak, karbon pasta elektrot hazırlanan nanokompozit ile modifiye edilmiş ve karbendazimin belirlenmesi için voltametrik bir yöntem önerilmiştir. Nanokompozit, taramalı elektron mikroskopisi (SEM) ile karakterize edilmiştir, ayrıca hazırlanan modifiye elektrot yüzeyi, X-ışını kırınımı, EDX haritalaması ve taramalı elektron mikroskopisi ile karakterize edilmiştir. Modifiye edilmiş elektrotlar, elektrokimyasal olarak dönüşümlü voltametri ve elektrokimyasal empedans spektroskopisi kullanılarak karakterize edilmiştir. Modifiye edilmiş elektrot karbendazimin yükseltgenmesinde bir elektrokatalitik etki göstermiştir. Karbendazimin belirlenmesi için geliştirilen yöntem, yüzde modifikasyon ajanı, pH, tarama hızı ve bekleme süresini içeren deneysel parametrelerin optimizasyonunu içermektedir. Bu çalışmada hazırlanan elektrokimyasal sensörün performansı nehir suyu, portakal suyu, elma suyu ve domates numunelerinde test edilmiştir. Önerilen bu yöntemle CBZ analizi için düşük bir gözlenebilme sınırı (0,000940 μM), düşük bir tayin sınırı (0,003134 μM) ve doğrusal ölçüm aralığı (0,05 − 10,0 μM) elde edilmiştir. Elde edilen sonuçlar hazırlanan elektrokimyasal sensörün karbendazimin ölçümü için kullanılan mevcut analitik metotlara alternatif olarak kullanılabileceğini göstermiştir.

Anahtar Sözcükler: Karbendazim, nanokompozit, gümüş nanoparçacıklar, sensör.

ACKNOWLEDGEMENTS

STATEMENT OF COMPLIANCE WITH ETHICAL PRINCIPLES AND RULES

I hereby truthfully declare that this thesis is an original work prepared by me; that I have behaved in accordance with the scientific ethical principles and rules throughout the stages of preparation, data collection, analysis and presentation of my work; that I have cited the sources of all the data and information that could be obtained within the scope of this study, and included these sources in the references section; and that this study has been scanned for plagiarism with "scientific plagiarism detection program" used by Anadolu University, and that "it does not have any plagiarism" whatsoever. I also declare that, if a case contrary to my declaration is detected in my work at any time, I hereby express my consent to all the ethical and legal consequences that are involved.

Fayha MOHAMED ADAM HAMID

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GLOSSARY OF SYMBOLS AND ABBREVIATIONS

CBZ	:	Carbendazim
FS	:	Fumed silica
CPE	:	Carbon paste electrode
FS@Ag	:	Fumed silica decorated silver nanoparticles
CV	:	Cyclic voltammetry
DPV	:	Differential pulse voltammetry
EIS	:	Electrochemical impedance spectroscopy
ELISA	:	Enzyme-linked immunosorbent assay
SEM	:	Scanning electron microscope
XRD	:	X-ray diffraction
EDX	:	Energy dispersive X-ray
HPLC	:	High performance liquid chromatography
GC	:	Gas chromatography
M	:	Molar
mM	:	Millimolar
μM	:	Micro molar
V	:	Volt
mV	:	millivolt
A	:	Ampere
μA	:	Microampere

1. INTRODUCTION

One of the current environmental problems which the world is facing is the contamination of natural resources by toxic chemical compounds, such as pesticide residues. Pesticides are a group of chemical compounds that are specifically produced and developed for agricultural productivity and enhancing food quality. The extensive and uncontrolled using of pesticides can lead to an accumulation of pesticide residues in the environment. Pesticides residues can make serious damage to ecological safety, the health of human beings, the quality of foods and the water ecosystem [1]. Monitoring the trace levels of pesticides in the environment and food samples is a very important task to reduce the risks of pesticides.

Carbendazim is used in the prevention and control of different plant diseases such as citrus black spots of crops and vegetables. Plants absorb carbendazim through roots, leaves, and seeds; also, carbendazim persists for a long time periods in soil without degradation and contaminates water resources. Recently several toxicology studies have been reported that carbendazim may have a harmful effect on human health and animals [2].

Determination of low levels of carbendazim is a very important subject due to its high toxicity to humans and animals; therefore, it is essential to develop an accurate and simple determination method. Nowadays, different analytical techniques are used in carbendazim detection. Most of these studies are based on chromatography and spectroscopy, but these methods have some disadvantages such as expensive instrumentation, complicated sample preparation procedures, and long analysis periods. Electroanalytical methods may be an alternative to common methods used for the determination of carbendazim. Electroanalytical methods have advantages such as simple instrumentation, high sensitivity, low cost, short analysis time and easy operation.

Modification of bare electrode surfaces with different substances brings different additional advantages such as increased selectivity, high sensitivity, and a decrease in the potential of the electrochemical reaction [3]. Recently silver nanoparticles (Ag NPs) have been used to prepare modified electrodes due to its excellent conductivity and convenience to functionalization. These studies showed that silver nanoparticles have significant electrochemical reactivity [4].

Fumed silica (FS) is a form of silicon oxide, consist of agglomerates of chain-like three dimensional particles. Properties of fumed silica are the non- porous structure, low density

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and high surface area. Due to these criteria, fumed silica is considering a good candidate as a modification agent used in the preparation of nanocomposite for different modified electrodes [5].

In this study, we have prepared a nanocomposite based on the formation of silver nanoparticles on fumed silica (FS@Ag) and it was used as a modifier for the preparation of a chemically modified carbon paste electrode (CPE/FS@Ag). The modified electrode was used for the determination of CBZ by differential pulse voltammetry. The modification of CPE with the nanocomposite has improved the performance of the CPE by increasing the electron transfer rate, effective surface area and functionality of the electrode. Optimization of parameters such as modifier amount, accumulation time and pH of supporting electrolyte were performed. The characterization of the modified electrode (CPE/FS@Ag) was performed by scanning electron microscopy, X-ray diffraction and electrochemical impedance spectroscopy. The detection limit and linear range of CBZ were determined. Analytical application of CPE/FS@Ag was tested by performing the determination of CBZ in real samples including river water, orange and apple juice, and tomatoes samples. The results revealed that the prepared modified electrode can be safely used in the determination of CBZ at different samples.

2. BASIC CONCEPTS

2.1. Pesticides

Pesticides are chemical compounds that are used to destroy pests, including rodents, insects, weeds or diseases caused by fungi and bacteria [6]. The remaining pesticides can enter the food chains through the soil, water, and air and lead to different potential health problems to the ecosystem, animals, birds, and human beings. The health problems that arise from pesticides can be cytogenic or carcinogenic. They can lead to hormone disorder, nerve disorder, bone marrow diseases, immunological and respiratory problems and infertility. To avoid these risks, the European governments and the USA have imposed new laws. Enforcement of this requires reliable monitoring of the environment for the presence of harmful compounds, which can affect human health and the local ecosystem. The highest allowable levels for different pesticides in water for human use are varied from 0.3 to 400µg/L [7].

2.1.1. Classification of pesticides

The classification of pesticides can be performed taken into account many different properties. According to their high toxicity, pesticides are classified as highly dangerous, moderately dangerous, and slightly dangerous. Classification based on their target organisms such as bacteria, fungi, and viruses...etc to fungicides, herbicides, insecticides, miticides, nematicides, and rodents. Pesticides are classified as chemical pesticides and biopesticides according to their sources. Biopesticides are generally based on the natural substances derived from living organisms such as fungi, viruses, bacteria, and plants. Biopesticides are divided into biochemical pesticides, microbial pesticides, and plant-incorporated protectants. According to chemical structures, pesticides are classified into several groups, including organophosphates, organochlorines, and carbamate and pyrethroid pesticides [8]. A general classification of pesticides is shown in Figure 2.1. Fungicides are a specific group of pesticides, which are defined as chemical substances that control the fungal disease by inhibiting or killing the fungi causes diseases [9]. Carbendazim is one of the widely used fungicides to control fungal diseases. Therefore, it was selected as a target molecule in this study.
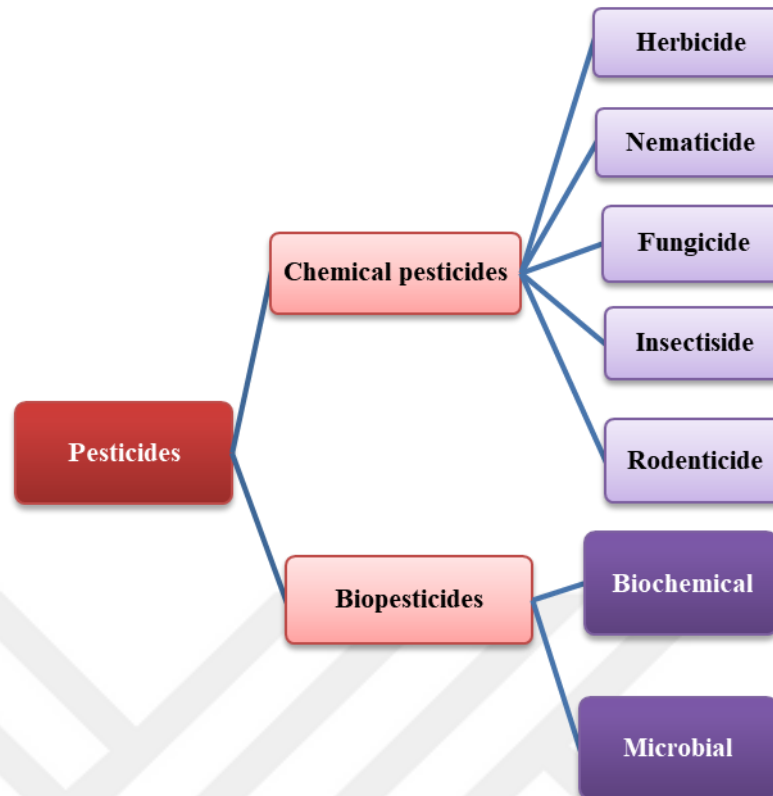
Figure 2.1. *Classification of pesticides*

2.1.2. Characteristics of pesticides

Characteristics of pesticides can help in determining a pesticide's interaction and understanding the behavior of pesticides and their movement in the environment. The first property is solubility which is a measure of the ability of a pesticide to dissolve in water. The second one is the adsorption process in which pesticides bind to the soil surface, including organic and microscopic inorganic materials. The third property is persistence defined as the ability of pesticides to remain in the soil, in the active and original form without degrading. The persistence of pesticides is measured by half-life. Volatility is the tendency of a pesticide to form a gas vapor into the atmosphere. Volatility depends on temperature, speed of the wind and humidity [10].

2.1.3. Determination methods of pesticides
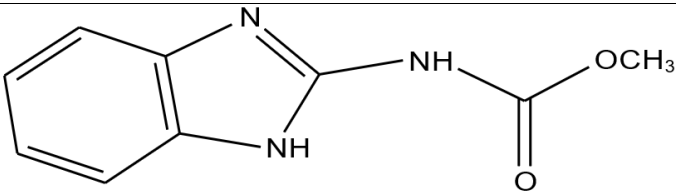
The common analytical methods used for the analysis of pesticides contain ELISA, gas chromatography, high-performance liquid chromatography (HPLC), fluorescence spectrophotometry, mass spectroscopy or coupled techniques such as GC-MS and LC-MS [11, 12]. The classical methods are reliable, sensitive and precise, but the disadvantages of these methods are high-cost instrumentation, long analysis time, multiple phases of sample

preparation and requirement to a highly skilled technician [13]. The simple and helpful alternative methods are electroanalytical methods. Electroanalytical methods have been preferred in pesticide detection because of their high sensitivity, short analysis time, simplicity, easy operation and low cost [14, 15].

2.2. Carbendazim

In this study, an electrochemical sensor based on a modified electrode has been developed for the determination of carbendazim, which is a widely used pesticide in agricultural activity. Carbendazim (CBZ) is a systematic broad-spectrum fungicide belonging to the family of benzimidazole methyl N-(1H-benzimidazole-2-yl) carbamate (MBC). It has a solid white crystalline form, insoluble in water and melts at approximately 250°C. It is stable under normal storage conditions [16]. The properties and structure of carbendazim are shown in Table 2.1. Normally carbendazim found in combination with other fungicides like fenamiphos and has been widely used in agriculture for pre- and post-harvest, mainly in crops of fruitsand cereals to control several fungal plant diseases such as spot, mildew, mold, rot and scorch [17]. Carbendazim suppresses fungal growth by disrupting tubulin polymerization [18]. This fungicide has been also used for the preservation of different materials like films, fibers, leather, rubber and polymers [19].

Table 2.1. *Properties and structure of carbendazim*

Structure	
Common name (ISO)	Carbendazim.
Chemical name (IUPAC)	Methylbenzimidazol -2-yl carbamate.
Structure molecular formula	$C_9H_9N_3O_2$
Molecular weight	191.19 g/mol

2.2.1. Toxicity of carbendazim

Inhalation of CBZ through the respiratory system and contact to the derm can cause skin inflammation and eye irritation, being related to dermatitis and responsible for provoking burns in the skin [20]. In addition, a small amount of carbendazim can lead to critical damage in the endocrine system and lead to hormonal disorder. It also affects the function of some organs and exhibit teratogenicity and mutagenicity on animals [21]. Carbendazim also shows

a serious effect on the male mammalian reproductive system result in infertility [22]. The toxicity produces rapid effects on meiotic spermatocytes and latent effects on spermatids, leading to morphological abnormalities and failure of spermatogenesis [23]. Because of the high toxicity of carbendazim and harmful effects on the health of human beings and animals, many countries prevent the use of carbendazim.

2.2.2. Methods used for determination of carbendazim

Rapid determination of trace amount of carbendazim is very important because of its high toxicity, and to prevent and reduce the effect of exposure to human health. New analytical techniques must be needed to provide simple, cheap, easy, rapid, sensitive and low-cost analysis to enhance the detection of pesticides. The common classical methods are used for detection of CBZ include high-performance liquid chromatography [24], UV spectroscopy [25], fluorescence spectroscopy [26], mass spectroscopy [27] and chemiluminescence [28]. Electroanalytical methods have been considered alternative techniques for the determination of carbendazim because of the high sensitivity, selectivity, low cost, simplicity, short-time analysis and easy portability [29, 30].

2.3. Electrochemical Sensor

The sensor term was defined as a transducer according to the American national standard institute (ANSD). The sensor is a device that provides usable output in response to specific measured and output was defined as electrical quantity (ISA, 1975) (Instrument Society of America, 1975). Chemical sensors can be divided into many categories. Electrochemical sensors are one of the subgroups of chemical sensors. The aim of the electrochemical sensor is to form an electrical signal that is directly related to the concentration of a specific analyte. Electrochemical sensors contain a transducer that transforms the response into a measurable signal (Fig 2.2). Electrochemical sensors are considered attractive due to low cost, simplicity, and stability. They are used in many different fields in industry and in monitoring environmental pollution and pharmaceuticals. The electrochemical sensor represents an essential subclass of a chemical sensor, uses an electrode as a transduction element. Electrochemical sensors are devices that convert the information associated with electrochemical cell reaction (the reaction between an electrode and analyte) into a measurable signal. The produced signal is proportional to the analyte concentration. Electrochemical sensors are commonly divided into three types voltammetric/amperometric, conductometric and potentiometric [31]. The application of the electrochemical sensor

includes many important fields of health care, food industry and monitoring environmental, industrial, and agricultural substances. The ideal sensor should be highly sensitive, highly selective, easy to use, simple, repeatable, reproducible, and free from interference and has a short analysis time. Potentiometric sensors are devices which measure the potential difference between two electrodes under the condition of zero current flow. The measured potential is used to determine the analytical quantity of analyte interest. Potentiometric sensors generally based on the Nernst equation [32]. Conductometric sensor measures the changes that occur in the electric conductivity of bulk material. Voltammetric sensors are characterized by the application of potentials (E) to an electrode and monitoring of the resulting current flowing through the electrochemical cell. The applied potential is varied, and the current is monitored over a period of time. Voltammetric techniques are usually used for the quantitative determination of a variety of dissolved organic and inorganic substances [33].
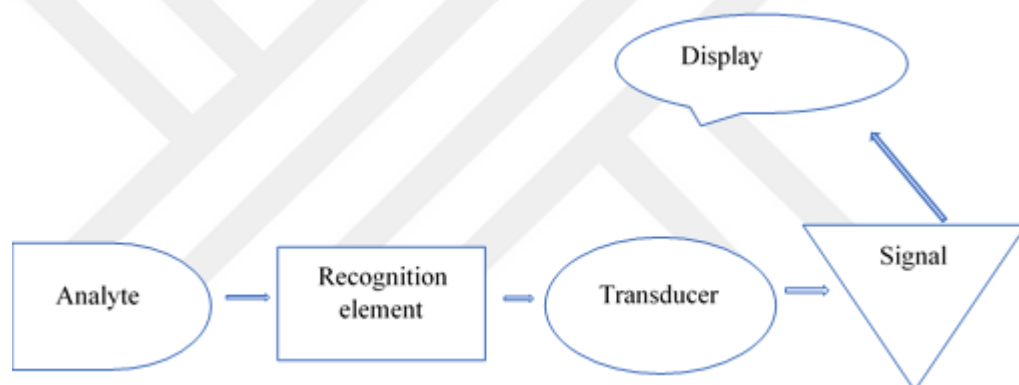
Figure 2.2. *Principle of sensor*

2.4. Design of The Electrochemical Cell

Electrochemical tests are generally performed in an electrochemical cell shown in Figure 2.3. The three-electrode cell is commonly used in electrochemical experiments; cells are selected according to the nature of the sample and the type of the experiments. The cells commonly used are produced from glass due to transparency, low cost, impermeability, and chemical inertness. Other materials used are Teflon and quartz. Cells may take volume 5-50 mL and also consist of three electrodes including a working electrode, a counter electrode (an auxiliary electrode) and a reference electrode that is immersed in a solution containing supporting electrolyte to provide conductivity [34].
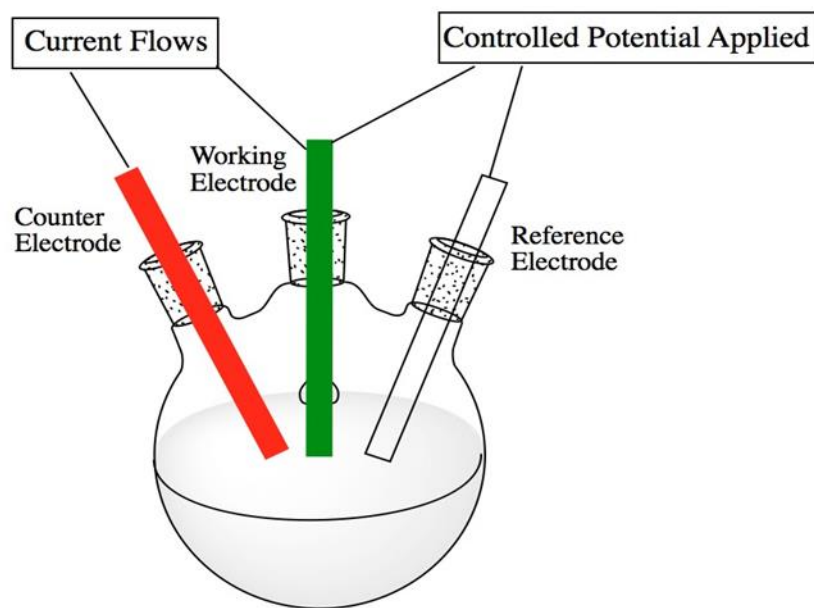
Figure 2.3. *An electrochemical cell assembly (three-electrode system)*

2.4.1. Working electrode

A working electrode is an electrode at which the analyte is oxidized or reduced. The potential between the working electrode and the reference electrode is controlled, but electrolysis current passes between the working electrode and the counter electrode. The dimensions of the working electrode are kept small to improve its tendency to become polarized. Commonly working electrodes which are used in voltammetric techniques are metallic conductors like inert metal, such as gold, platinum and silver; and semiconductors such as indium or tin oxides, or a metal coated with a film of mercury, and also carbon-based materials such as carbon paste, pencil graphite, and glassy carbon. Some of the commonly used working electrodes are shown in Figure 2.4.
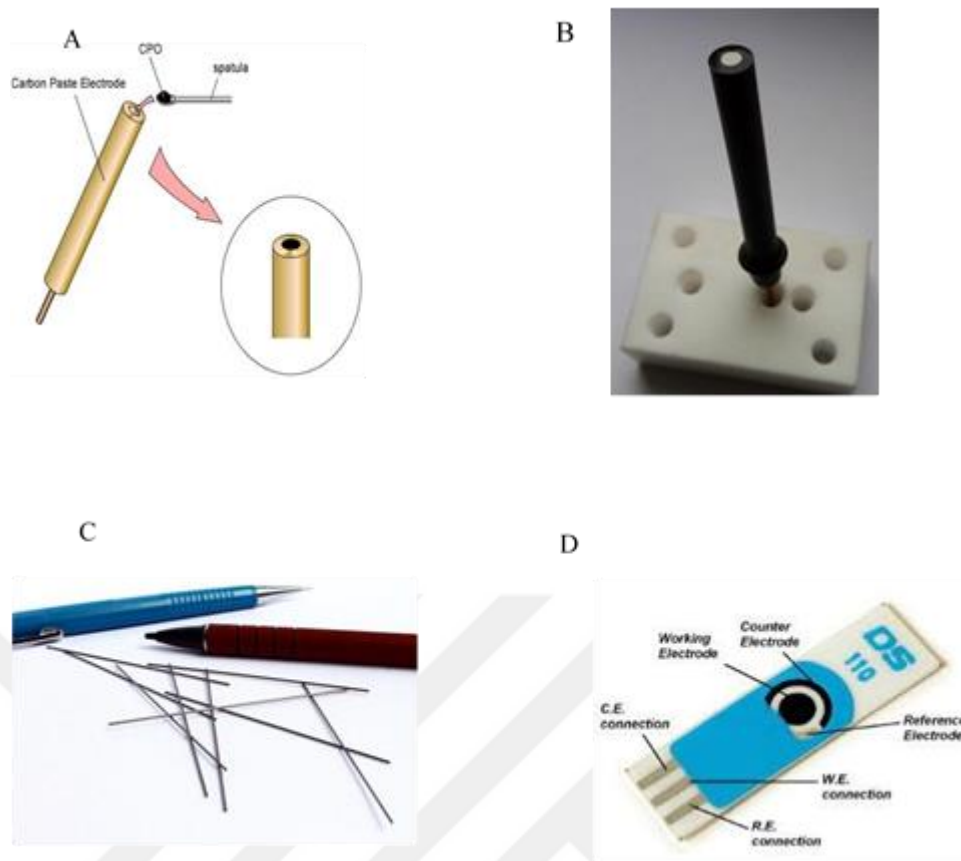
Figure 2.4. *A) Carbon paste electrode (CPE) B) glassy carbon electrode (GCE) C) pencil graphite electrode (PGE) D. screen printed electrode*

2.4.2. Reference electrode

A reference electrode is desirable in most electroanalytical applications. An ideal reference electrode must be reversible, and it supplies a potential that is constant with time. It has to obey the Nernst equation, exhibits little hysteresis with temperature cycling and retains to its original potential after being subjected to small current. One of the commonly used reference electrodes is the calomel reference electrode consists of mercury in contact with a solution that is saturated with mercury (I) chloride (calomel) and that also contains a known concentration of potassium chloride (Fig. 2.5.a). Another commonly used reference electrode is silver/ silver chloride electrode containing a solution of potassium chloride that has been saturated with silver chloride (Fig. 2.5.b). Silver/silver chloride electrodes can be used at temperatures greater than 60 ºC, while calomel electrodes cannot. To avoid contamination of electrode solution and reaction of the analyte solution with silver or mercury (I) ion from the inner solution, the level of the internal liquid must be kept above that of the sample solution.

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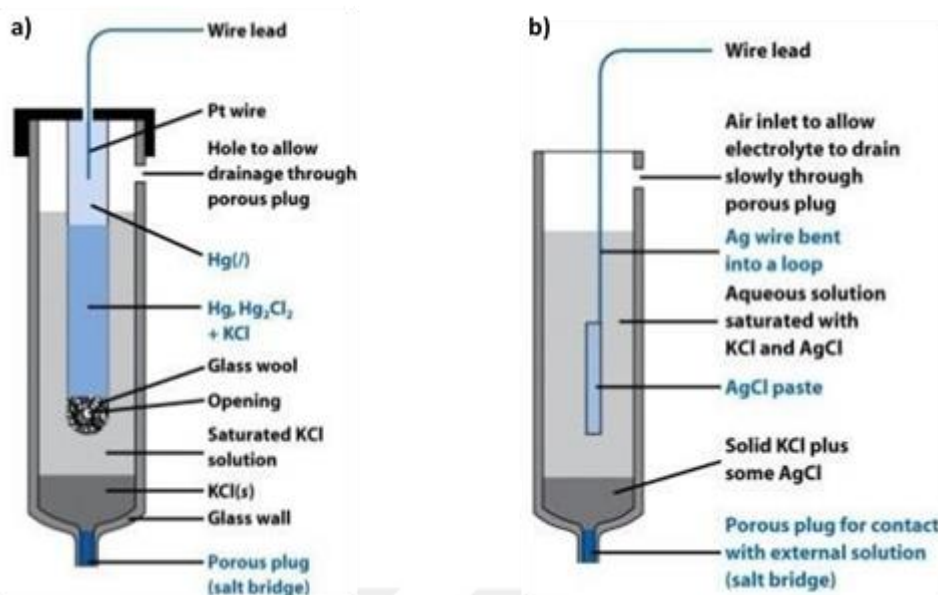
Figure 2.5. *Commonly used reference electrodes. a) Calomel reference electrode. b) Silver/silver chloride reference electrode*

2.4.3. Counter electrode

The counter electrode also called auxiliary electrode is used in the electrochemical cell for the voltammetric reaction, the electrode is coupled to the working electrode but plays no part in determining the magnitude of the potential being measured. The current in the cell passed between the working electrode and the counter electrode. Counter electrodes are made of inert conducting materials such as gold, a coil of platinum wire, a pool of mercury, or graphite rods. They are commonly used as the current-carrying electrode, conduct electricity from the source through the solution to the working electrode. Some of the commonly used counter electrodes are shown in Figure 2.6.
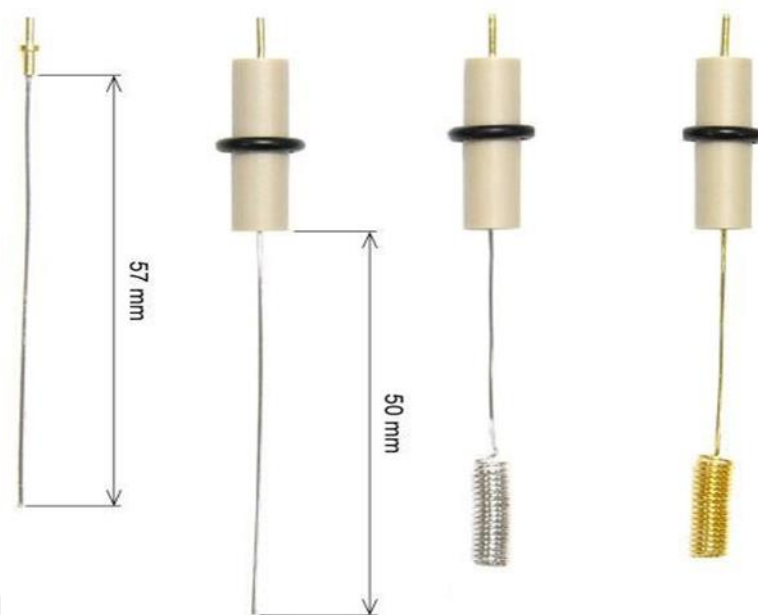
Figure 2.6. *Gold and a coil of platinum wire counters electrodes*

2.4.4. Supporting electrolytes

Electrochemical experiments are performed in a medium containing supporting electrolytes. Supporting electrolytes are required for controlled potential measurement to decrease the resistance of the solution, maintain a constant ionic strength and eliminate electromigration effects [35]. Supporting electrolytes in voltammetry could be simple salt (an alkali metal salt), base or acid [36]. Supporting electrolyte is selected according to the solubility of the analyte, redox activity and the properties of solvent such as electrochemical activity, chemical activity, and electrical conductivity. The supporting electrolyte solution does not react at the working electrode at potential being used to determine the analyte and should not react with the analyte or product of the reaction and also should have high ionic conductivity and low electrical resistance. The most common solvent used in the electrochemical experiments is water compared to other protic solvents like propylene carbonate, acetonitrile, and methanol. The inert supporting electrolytes consist of mineral acid, inorganic salt, and buffer solutions such as citrate, acetate, and phosphate when pH control is necessary [37].

2.5. Electroanalytical Techniques

Electroanalytical techniques detect analytes by the measurement of electrical quantities such as potential, charge or current in an electrochemical cell containing the analyte. The electroanalytical methods are used for many applications including environmental monitoring,

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industrial quality control and biomedical purposes [38]. The electroanalytical methods include voltammetry, potentiometry, coulometry, oxidation/reduction titrimetry, and electrogravimetry.

2.5.1. Voltammetry

Voltammetry consists of a group of electroanalytical methods in which information about analyte is obtained from the measurement of current as a function of applied potential under conditions that promote polarization of a working electrode. Voltammetry is also widely used for nonanalytical purposes including basic studies of redox processes (oxidation, reduction) in different media, electron transfer processes in chemically modified electrode surfaces (CME) and adsorption studies on the electrode surfaces [39]. Various types of experiments could be performed to obtain information in voltammetry including cyclic voltammetry (CV), linear sweep voltammetry, stripping voltammetry and square wave voltammetry (SWV) [40].

2.5.1.1. Excitation signals in voltammetry

In voltammetry, the voltage of the working electrode is varied systematically while the current response is measured. In the voltammetry the most common excitation signals are four waveforms including linear scan voltammetry in which the applied potential to the cell increased linearly as a function of time, differential pulse voltammetry, square wave voltammetry, and cyclic voltammetry. The commonly used excitation signals are shown in Figure 2.7.
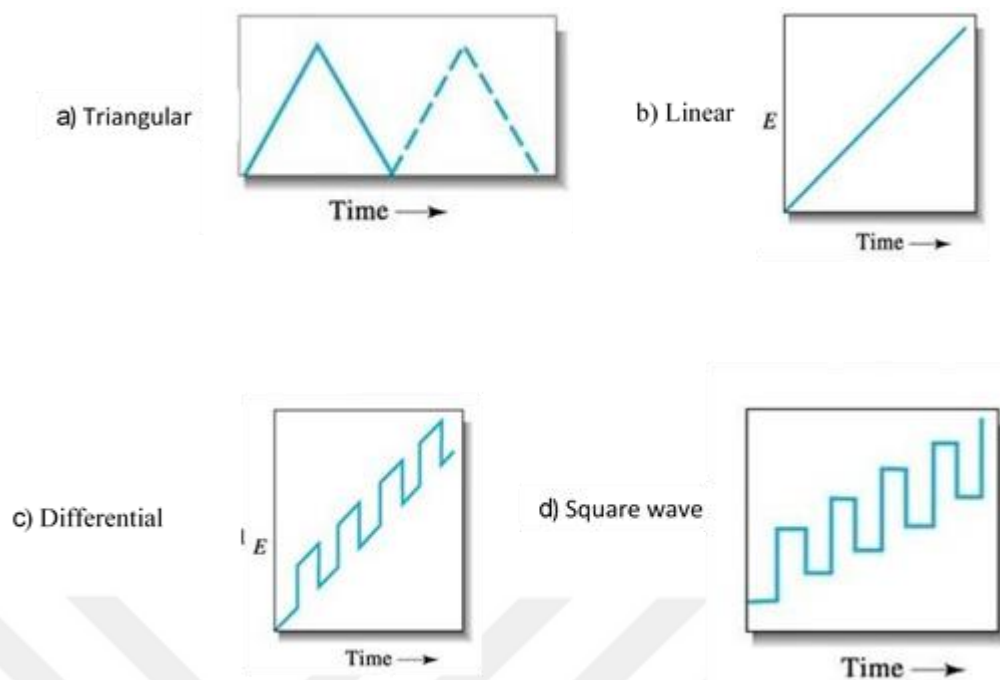
Figure 2.7. *Excitation signals that are used in voltammetry*

2.5.1.2. Cyclic voltammetry

Cyclic voltammetry (CV) is the most common voltammetric technique used for studying the electrode reactions. In cyclic voltammetry, the current response of a small stationary electrode in an unstirred solution is recorded by applying a triangular potential waveform shown in Figure 2.8. The triangular potential produces the forward and then the reverse scan. Cyclic voltammetry results provide information about the thermodynamics of redox reactions, adsorption process or coupled chemical reactions and also provide considerable information in the kinetics of the electron transfer process and the detection of reaction intermediates. This technique commonly used for the qualitative measurements rather than the quantitative measurements in the electrochemical processes, particularly in the organic and metalorganic systems. The characteristic peaks in cyclic voltammograms are generated by forming a diffusion layer nearby the electrode, immobilization of the electroactive layers on the surface of the electrodes. Cyclic voltammetry is commonly used for investigating the performance and behavior of chemically modified electrodes. Cyclic voltammetry is a perfect tool for investigating the mechanism of the electrode reactions and is commonly not used for chemical sensing, but rather for characterization purposes. One of the important cyclic voltammetry applications is used for the qualitative examination of the chemical reaction of the redox process. Reaction mechanisms are classified by letters E and C (redox and chemical steps, respectively). The occurrence of chemical reactions directly affects the available surface

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concentration of electroactive species. Changes in shapes of cyclic voltammograms are due to the change takes place in the chemical compositions of the electrochemical reactant or product, and this useful for reaction pathways and supplying reliable chemical information for the reactive intermediates [41].
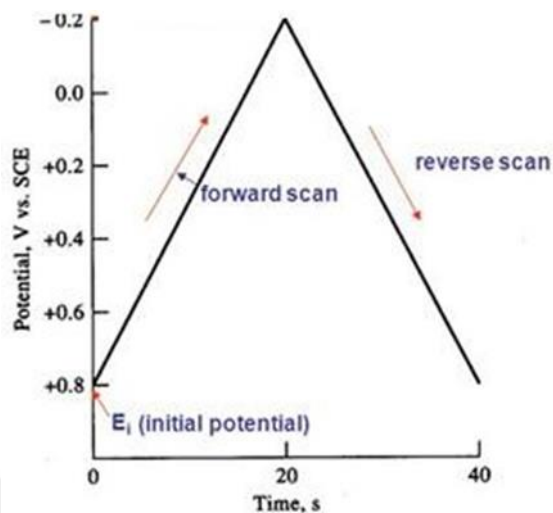
Figure 2.8. *Chemical formula of paracetamol*

2.5.1.3. Pulse voltammetry

The aim of pulse voltammetry is to decrease the detection limit of voltammetric measurements, by increasing the ratio between the faradaic and nonfaradaic currents. Pulse voltammetry allows decreasing quantitative detection limit until the 10-8 M concentration level. The difference between several pulse voltammetric methods is the current sampling regime and the excitation waveform.

Normal pulse voltammetry contains a series of square wave voltage pulses of increasing magnitude is superimposed upon a constant dc voltage signal. In this technique, the cell current was measured as a function of potential and function of time between the working electrode and the counter electrode

Differential pulse voltammetry (DPV) is a very helpful electrochemical technique for the detection of trace levels of organic and nonorganic materials, the ability to achieve detection limit is 10-7 M [42]. In the differential pulse voltammetry technique, fixed magnitude pulses superimposed on linear potential ramps applied to the working electrode at a certain time period (Fig. 2.9). Measurement of current was sampled in two- point, first point before the pulse application and the second point is at the ending of the pulse. The resulting differential pulse voltammograms contain peaks, the height of peaks is directly proportional

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to the concentration of interested analyte. Peak shaped response of the DPV measurements results in improved resolution between the redox potential of two kinds. In different states, peaks separated by 50 mV could be measured, in which quantitation not only depends on the similar peak potential but also depends on the widths of the peaks. The widths of the peaks (in half-height) were related to the electron stoichiometry. In addition to improvements in the resolution and the selectivity, the technique could be supplied information about chemical form in which the analyte show (Oxidation state, complexation...etc) [43].
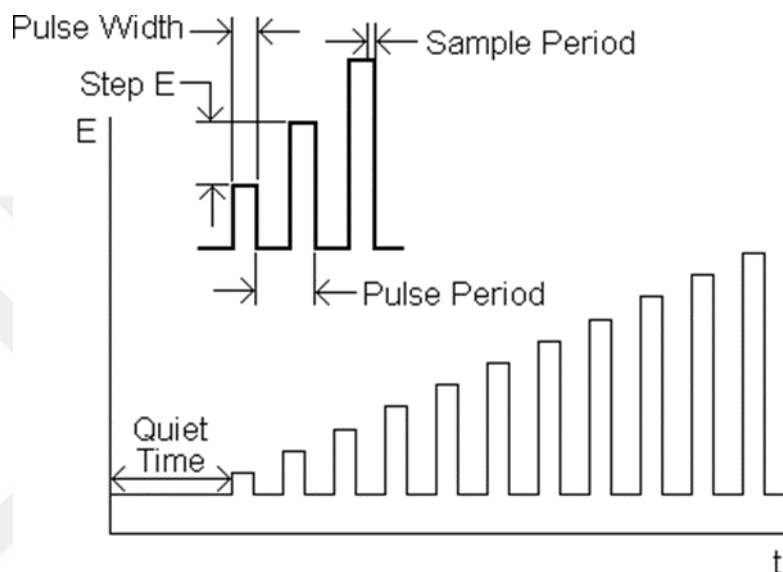
Figure 2.9. *Excitation signal that is used in DPV measurement*

2.5.1.4. Square wave voltammetry

Square wave voltammetry is which a waveform composed of a symmetrical square wave, superimposed on a base staircase potential, is applied to the working electrode [44]. Square wave voltammetry is performed on a single mercury drop; a new drop is dispensed for each analysis cycle. Plotting the difference between the current observed at forward current and that at reverse current produce current voltage peaks with height proportional to the concentration of the species in solution. Detection limits for square wave voltammetry are in the range of 10-7 to 10-8 M. Square wave voltammetry generally used in detectors for liquid chromatography.

2.6. Modified Electrode

A modified electrode is defined as an electrical conductor or semiconductor that has its surfaces modified with an ionic, monomolecular, multi-molecular, or polymeric film for various electrochemical functions. The modified electrode represents a modern approach to

the electroanalytical system. The main purpose of the electrode modification is to obtain new surfaces of completely different properties on the surface of the working electrode, this enables the determination of electroactive species or non- electroactive species. Modifying electrode surfaces can lead to an improvement in the performance of electrode, these include prevention of electrode fouling, acceleration of electron- transfer reactions, selective binding, and can impart higher sensitivity, selectivity and stability to the electrode. Modified electrodes have been used for the analysis of organic and inorganic substances..

2.6.1. Modification methods

2.6.1.1. Adsorption

The principle of this method that uses the same type of valence forces involved in formation of chemical compounds, which the film strongly adsorbed or chemisorbed, spontaneously to the surface of the electrode. A good example of this approach is the interaction of organic species such as those containing double bonds, which are usually hydrophobic and strongly adsorb from aqueous solutions on carbon or platinum surface [45].

2.6.1.2. Covalent bonding

Chemical agents are used to create a covalent bond between the electrode surface and one or more monomolecular layers of the modifying agent. A carbon surface is considered efficient for covalent modification because of their alterable functionalities [46]. Carbon can be oxidized so the surface can be considered as consisting of hydroxyl group to form covalent bonds.

2.6.1.3. Preparation of Composites

Composite substances are formed by mixing two or more materials that have different properties. In the electroanalytical method, the modifier agent (electron–transfer mediator) are combined with an electrode component material such as carbon particles in CPE (the electrode matrix) [45].

2.6.1.4. Polymers film coating

In this method, polymeric modifying layers on the surfaces of the electrode are formed from immersing the electrode into a solution containing a dissolved polymer. Spin coating is considered better methods than dip- coating because of producing more uniform film and also widely used in the production of semiconductor chips. Polymer layers can be also coated by electrodeposition or by inducing the polymerization of monomers at the electrode surface.

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Some polymer itself is electroactive and can undergo redox reactions. İn some cases, the polymer is a polyelectrolyte, that is a material contain ionic groups can extract charged ions from the solution and hold them by electrostatic binding (Nafion) [46].

2.7. Modification Agents Used In This Study

2.7.1. Fumed silica

Fumed silica is a form of silicon oxide, synthesized by high- temperature flame hydrolysis of silicon tetrachloride (SiCl4) in oxygen- hydrogen flame. Fumed silica is characterized by a high specific surface area in the form of an amorphous structure. It contains many silanol groups that have the potential to interact with many different materials. Due to its wide surface area and nonporous structure, fumed silica has been used in many fields in cosmetic, paint, coating industries and in the clinical field. Fumed silica can be considered as a great modifying agent in the preparation of modified electrodes and promising material for electrochemical sensor studies. Fumed silica may affect the rate of electrochemical reaction, may increase the surface area and may provide surface hydroxyl groups for molecular interactions. In a previous study, fumed silica was used in the preparation of a nanocomposite to prepare a modified GCE which was tested in the determination of fenitrothion [47]. In another study, it was used in the preparation of a modified electrode for the estimation of the 17 –β estradiol [48]. Fumed silica was also used as a modifier in the preparation of modified electrodes for the determination of propham [49], and clopyralid [50]. These studies showed that fumed silica has a great potential in the preparation of electrochemical sensors.

2.7.2. Silver nanoparticles

Electroactive nanoparticles such as metal, semiconductor nanocrystal, etc are widely used in the preparation of electrochemical sensors for detecting biological substances and heavy metals [51]. These electroactive nanoparticles could be quantitatively modified onto electrode surfaces and also can demonstrate strong and stable electrochemical signals. The metal nanoparticles have significant advantages such as easy to surface functionalization, facile synthesis, effective catalytic role in electrochemical reaction and improvement of electron transfer processes [52, 53, and 54]. Silver nanoparticles (AgNPs) have high quantum properties, large specific surface area, and small granule diameter. Silver nanoparticles also have effective catalytic behavior, high conductivity, stability, excellent biocompatibility, and lead to amplified electrochemical signals [55, 56, and 57]. Based on these advantages, silver nanoparticles were selected as a modifier agent in this thesis. As for as we know, there is no

previous study dealing with the preparation of fumed silica decorated silver nanoparticles (FS@Ag). Therefore, we have synthesized this nanocomposite for the first time. This nanocomposite combines the advantages of fumed silica and silver nanoparticles in a single substance and it provides new insights in the preparation of electrochemical sensors.

2.8. Literature Review for the Determination of Carbendazim

YuXie and co-workers (2019) have synthesized neodymium molybdate wrapped with multi-walled carbon nanotubes ($Nd_2Mo_3O_9$/MWCNTs) composite to prepare modified glassy carbon electrode [58]. The modified electrode was used for the quantification of CBZ. The linear range was obtained between 0.05 to 9, 0 μM with a detection limit of 0.016 nM.

Shuxiao and co-workers (2019) developed a novel sensor based on nitrogen and sulfur doped hollow Mo_2C/C spheres and molecularly imprinted polymer (MIP). The prepared electrochemical sensor was used for the determination of carbendazim [59]. The developed novel sensor was characterized by using a transmission electron microscope, scanning electron microscopy, X-ray diffraction, Fourier transforms infrared spectroscopy, cyclic voltammetry and electrochemical impedance spectroscopy. A linear range was obtained between the concentrations of 1×10^{-12} to 8×10^{-9} M with detection limit of 6.7×10^{-13}.

Adriene and co-workers (2019) developed an enzymatic biosensor by using microbial lipase. The enzyme was immobilized on lamellar zinc hydroxynitrate decorated gold nanoparticles and then incorporated into carbon paste with multi-walled carbon nanotube [60]. Square wave voltammetry was used for the determination of pesticide carbendazim. The prepared biosensor response for the carbendazim was linearly increased in the range from 10 to 100 μgL-1 with a limit of the detection value of 3.13 μgL-1 and limit of quantification vale of 9.50 μgL-1.

Elizabeth and co-workers (2018) developed a modified carbon paste electrode with recrystallized zeolite. It was used for the simultaneous determination of carbendazim and thiram fungicides in the sample of fresh grape juice, agricultural formulation, and honey [61]. The modified electrodes were characterized by XRD, FT-IR, SEM-EDS, and TG-DSC. It was reported that oxidation peak current of carbendazim linearly varied in the concentration range from 0.10 to 2.35×10^{-6} mol L-1, with a limit of the detection value of 3.51×10^{-9} mol L-1 and limit of quantification value of 11.71×10^{-9} mol L-1.

Wei and co-worker (2017) have synthesized gold nanoparticles using lactase produced by Pleurotus ostreatus as reducing agent [62]. Synthesized gold nanoparticles used for the modification of screen-printed electrodes (SPE). The modified electrode (SPE) was used for the analysis of carbendazim in vegetable samples by linear sweep voltammetry. Gold nanoparticles were characterized by X- ray photoelectron spectroscopy, X- ray diffraction spectroscopy, and scanning electron microscopy. The linear detection range and detection limit were obtained between 0.05-50 µM and 10 nM, respectively.

Yu Ya and co-workers (2015) have synthesized nitrogen-doped graphene oxide by pyrolysis of graphene oxide and urea to prepare a modified glassy carbon electrode [63]. The modified glassy carbon electrode was characterized by X-ray photoelectron spectroscopy and transmission electron microscopy. Fast electron transfer and large surface area of the modified electrode were determined by cyclic voltammetry and electrochemical impedance spectroscopy. Differential pulse voltammetry was used for the measurement of CBZ in PBS (pH: 4.0) containing a different concentration of CBZ at the accumulation time of 240s. Oxidation peak current was obtained at +0.90 V. The oxidation peak current for carbendazim linearly varied in the concentration range of 5.0-850 µg/L with a limit of detection value of 1.0 µg/L.

Claudia. A and co-workers (2015) developed an electrochemical sensor based on the modification of glassy carbon electrode with a thin film of mesoporous silica/multiwalled carbon nanotubes to determine carbendazim [64]. The phosphate buffer solution was used as a supporting electrolyte solution (pH= 8.0). The linear range was obtained between 0.2 to 4.0µM and the detection limit was calculated as 0.056 µM.

Peeyanun and co-workers (2014) proposed a single drop analysis method using a graphene-based electrochemical sensor for detection of carbendazim and isoproturon in water, soil and vegetable samples [65]. The graphene-based electrochemical sensor was fabricated by three printing step. Square wave voltammetry was used for the analysis of the sample. The linear range was determined between 0.50 to 10.0mg/L and the detection limit value was calculated as 0.11mg/L.

As can be seen from the presented literatures, there was no study related to the preparation of fumed silica decorated silver nanoparticles and its use in the determination of CBZ. This reveals the originality of the presented study.

3. MATERIAL AND METHODS

3.1. Chemical Substances And Reagent

Table 3.1. *Chemical substances used in study.*

Chemical	Molecular formula	Company
Carbendazim (99%)	$C_9H_9N_3O_2$	Aldrich
Fumed Silica	SiO_2	Sigma –Aldrich
Acetonitrile	C_2H_3N	Riedel de Hâen
Potassium dihydrogen phosphate (99.5%)	KH_2PO_4	Fluka
Silver nitrate	$AgNO_3$	Sigma –Aldrich
Nitric acid	HNO_3	Sigma –Aldrich
Sodium borohydride	$NaBH_4$	Sigma –Aldrich
Ascorbic acid (99.5%)	$C_6H_8O_6$	Sigma –Aldrich
Citric acid	C_6H_8O	Sigma –Aldrich
Zinc nitrate (98%)	$Zn (NO_3)_2$	Fluka
Methanol	CH_3OH	Fluka
Nujol mineral oil	-	Riedel de Hâen
Graphite powder	-	Riedel de Hâen
Sodium nitrate	$NaNO_3$	Sigma –Aldrich
Copper (II) chloride	$CuCl_2$	Sigma –Aldrich
Potassium chloride	KCl	Sigma –Aldrich
Bisphenol A	$C1_5H_{16}O_2$	Sigma –Aldrich
Para-aminophenol	C_6H_7NO	Sigma –Aldrich
Ammonium sulfate	$(NH_4)_2SO_4$	Sigma –Aldrich
Phosphoric acid	H_3PO_4	Sigma –Aldrich
Potassium hydroxide	KOH	Sigma –Aldrich
Potassium ferrocyanide	$K_4Fe(CN)_6$	Sigma –Aldrich
Potassium ferricyanide	$K_3Fe(CN)_6$	Sigma –Aldrich
Sodium hydroxide	$NaOH$	Sigma –Aldrich
4-Aminophenol	$H_2NC_6H_4OH$	Sigma –Aldrich
Fenitrothion	$C_9H_{12}NO_5PS$	Riedel de Hâen

3.2. Apparatus

Electrochemical measurements were performed by Autolab PGSTAT240 model potentiostat equipped with NOVA 2.0.2 version software (EcoChemie, the Netherland). The experiments were carried out by an electrochemical cell containing a conventional three-electrode system. CPE and CPE/FS@Ag were used as working electrodes. A home-made Pt

electrode was used as an auxiliary electrode and a saturated calomel electrode (SCE, Gammry Instruments, and the USA) was employed as a reference electrode. Measurement of CBZ was carried out scanning the potential from 0.55V to 1.25V with pulse amplitude of 0.05V. The supporting electrolyte was phosphate buffer solution (pH = 2.0 to 10.0). The ultrasonic bath (ISOLAB model) was used for mixing and solubilization processes. Deionized water (SartoriusArium ComfortI-1-UV-T model) was used for the preparation of the solutions. Magnetic Stirrer with Heater (Velp Scientifica model) was used for mixing solutions. An analytical electronic sensitive balance (OHAUS model) used for the weighing process. Characterization of electrodes was performed with an ultra-high-resolution field emission scanning electron microscope (ULTRAFE-SEM) (Zeiss Ultra plus). Energy dispersive X-ray (EDX) analyses were performed with a BRUKERAXS energy-dispersive X-ray detector. X-ray diffraction (XRD) analyses were performed with the BRUKER D8 advance X-ray diffractometer using Cu Kα radiation (λ= 0.154056nm) (Panalytical Empyrean, Netherlands). The scan was recorded with a scan rate of 0.02º s-1. In addition, electrochemical impedance spectroscopy (EIS) used for the characterization of modified electrodes; measurements were performed using the CHI 660D Electrochemical Workstation.

3.3. Synthesis of Fumed Silica

1g of the fumed silica was weighted and then added into 4M HNO_3 (100 mL) aqueous solution, then the mixture was stirred for a night to remove any remaining impurities. After that, the mixture was replaced in a membrane filtration system (0.45μm) and washed with deionized water until the pH of filtrate reached to 7. Then the precipitate was dried in an oven at 70ºC under vacuum (600mbar).

3.4. Synthesis of Fumed Silica Decorated Silver Nanoparticle

The preparation of fumed silica decorated silver nanoparticles (FS@Ag) was performed by three steps. In the first step, 0.00857 g of $AgNO_3$ was weighted and dissolved in 50 ml deionized water and mixed well, the concentration was 1.0 mM. In the second step, 0.1 g of acid-treated FS was added to the solution and ultra-sonicated for 1 hr, then the mixture was placed in a water bath. The temperature of the solution was kept constant at 60º C and stirred for one hour. In the third step, 10 ml (0.01M) an aqueous solution of sodium borohydride was added into the solution drop by drop. After that, it was magnetically stirred for 1hr. The solid phase was obtained by washing several times and filtered with a membrane filter. The precipitate (FS@Ag) was dried in an autoclave oven at 60ºC under vacuum (600mbar).

3.5. Preparation of Carbon Paste Electrodes

The carbon paste electrode is a mixture made of blending conducting graphite powder and a hydrophobic binder (nujol oil). Carbon paste electrodes are suitable for composite electrode preparation and chemical modification. Carbon paste electrodes are soft and non-compact, and surface could be renewed manually, also surfaces can be restored reproducibility after spoiling or loss of catalytic activity.

The unmodified carbon paste electrode (CPE) was prepared by hand mixing; graphite powder and nujol oil and then mixed well in a petri dish until to obtain a homogeneous mixture (Fig. 2.1). The modified electrodes (CPE/FS@Ag) were prepared by hand mixing of 0.3 g of graphite powder, 0.076 g of modifier material (FS@Ag) and 150 μL nujol oil. The mixture was mixed carefully until becoming a homogeneous paste. A glass tube which has an internal diameter of 3.0 mm was used to convert the prepared paste into a working electrode. The paste was firmly packed into the glass tube and electrode contact was achieved by dipping a copper wire opposite side of the tube. The surface of the electrode was smoothed and polished with a weighing paper, and then used to measure the CBZ in the solution.

Figure 3.1. *Homogenous carbon paste mixture containing 0.3 g of graphite powder, 0.076 g of modifier material (FS@Ag) and 150 μL nujol oil*

3.6. Investigation of Electrochemical Behavior of Modified Electrode (CPE/FS@Ag) in The Presence of Carbendazim

Standard solution of carbendazim was prepared by the following procedure; 0.0019 g of carbendazim powder was weighted, then 20 mL acetonitrile (ACN) was added, then ultra-sonicated for 1 hour and then 5 mL of deionized water was added to the solution and mixed for one hour. Voltammetric performances of the modified electrodes (CPE/FS@Ag and CPE@FS) and of the unmodified carbon paste electrode (CPE) were examined by the differential pulse voltammetry measurements in a 0.1 M phosphate buffer solution (PBS: pH 2) containing 10.0 μM CBZ in the potential range of +0.55 V to +1.25 V.

3.7. Characterization of Modified Electrode

Methods used for characterization of modified electrode are cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), energy dispersive X-ray (EDX), X-ray diffraction (XRD) and scanning electron microscopy (SEM).

3.7.1. Cyclic voltammetry (CV)

Cyclic voltammetry measurements were performed with CPE, CPE@FS, CPE/FS@Ag electrodes to examine the electrochemical behavior of 2.5mM $Fe(CN)_6^{3-/4-}$ in 0.1M KCl solution in the potential range of -0.3V and +1.25V. The same measurements were repeated with 1.0 mM $Ru(NH)^{3+}$ in the potential range of +0.4 and-0.6 V. CV measurements were also performed in 0.1M KCl solution.

3.7.2. Electrochemical impedance spectroscopy (EIS)

The CPE and CPE/FS@Ag electrodes were characterized by electrochemical impedance spectroscopy. Measurements were obtained in 0.1M KCl solution containing 2.5mM $Fe(CN)_6^{3-/4-}$, at set potential of +0.21V voltage and frequency range of 0.01-10^5 Hz. The data were analyzed by fitting using an electrical equivalent model circuit including solution resistance (R_s), charge transfer resistance (R_{ct}), Warburg element (W) and double layer capacitance (C_{d1}). The electron transfer resistance (R_{ct}) was calculated from the diameter of the semicircle in the Nyquist diagram and provides information about the electron transfer rate at the electrode and electrolyte interface.

3.8. Optimization of the Parameters

Parameters affecting the voltammetric performance of the CPE, CPE@FS, and CPE/FS@Ag were evaluated. The first parameter is the effect of the various percentages of

modifiers (FS-Ag) on the oxidation peak currents of carbendazim. The second parameter is pH of measurement solution. The effect of pH in oxidation performance of carbendazim was examined by adding the same amount of carbendazim to 0.1M of phosphate buffer solution having different pH values (2, 3, 4, 5, 6, 7.4, 8.5, and 10). The third parameter is the accumulation time (15, 30, 45, 60, 75, 90, 120, 150 sec).

3.9. Investigation of Interference Effects

The effects of possible interference substances were examined by performing DPV measurements of CBZ in the presence of suspected substances. For this purpose, the effects of ascorbic acid, acetic acid, Salicyic acid, tebuthiuron, ammonium sulfate, azinphos-methyl and bisphenol-A were tested. In addition, the effects of inorganic molecules such as $CuCl_2$, $Na(NO_3)_2$, KCl, $Zn(NO_3)_2$, and $NaNO_3$ were investigated. For this purpose, DPV measurements were performed with the modified electrode in a solution containing a certain amount of CBZ and increasing concentration of these species.

3.10. Preparation of Real samples

In the investigation of the validity of the proposed method, the CPE/FS@Ag electrode was tested for the measurement of CBZ in real samples. Different real samples were used including orange and apple juice, tomatoes, and water. Two commercial apple and orange juices were taken from the local market and then samples were passed through a membrane filter (0.45μm). The river water sample was collected from the Porsuk River in Turkey and was filtered through a membrane filter which has a porosity of 0.45μm. Tomato sample was taken from the local market and prepared according to the following procedure; tomatoes were grinded in a blender and then the sample was passed through a membrane filter (0.45μm). The obtained filtrate was used in the experiment by diluting with a required amount of PBS.

3.11. High Performance Liquid Chromatography (HPLC) Analysis

CBZ spiked samples were also analyzed by using high-performance liquid chromatography (HPLC) to test the validity of the proposed method. The analysis was performed with an Agilent 1100 liquid chromatography system which was equipped with a reversed phase analytical column (Inertsil ODS- 3, 5-μm, 4.6mm X 250mm) and a diode array detector. A mixture of 0.1 M of phosphate buffer solution (pH=2) and methanol were used as the mobile phase. The flow rate was selected as 0.8 ml/min and an injection volume of 15μL was used in the analysis. Detection wavelengths were set to 240 nm and 280 nm.

4. RESULTS AND DISCUSSION

In this thesis, a nanocomposite was synthesized as part of an electrochemical sensor prepared for the determination of CBZ a widely used pesticide. Silver nanoparticles were chemically formed on the surfaces of fumed silica (FS@Ag) to prepare the nanocomposite. Electrochemical sensor was designed by the modification of the carbon paste electrode with FS@Ag nanocomposite. A voltammetric method was developed by using the electrochemical sensor.

4.1. Selection of Modifier Agent for The Determination of CBZ

Voltammetric performances of the bare CPE and the modified CPE with different modifier agents such as FS, FS@Pt, FS@Au, and FS@Ag were investigated by performing differential pulse measurements in a 0.1 M phosphate buffer solution (PBS: pH 7.4). The results are presented in Figure 4.1, the oxidation of 10μM CBZ took place at the potential value of +0.68 V on the surfaces of bare CPE, CPE/FS, CPE/FS@Pt, and CPE/FS@Au. Also, the oxidation of CBZ took place at the potential value of +0.73 V when CPE/ FS@Ag was used as a working electrode. The oxidation potential of CBZ shifted slightly to a more positive value. The highest oxidation peak current was observed when the CPE/FS@Ag was used in the measurements. The FS@Ag showed a great electrochemical response toward CBZ compared to the other modifier agents. Therefore, fumed silica decorated silver nanoparticles (FS@Ag) was selected as a modifier and used in the subsequent studies.
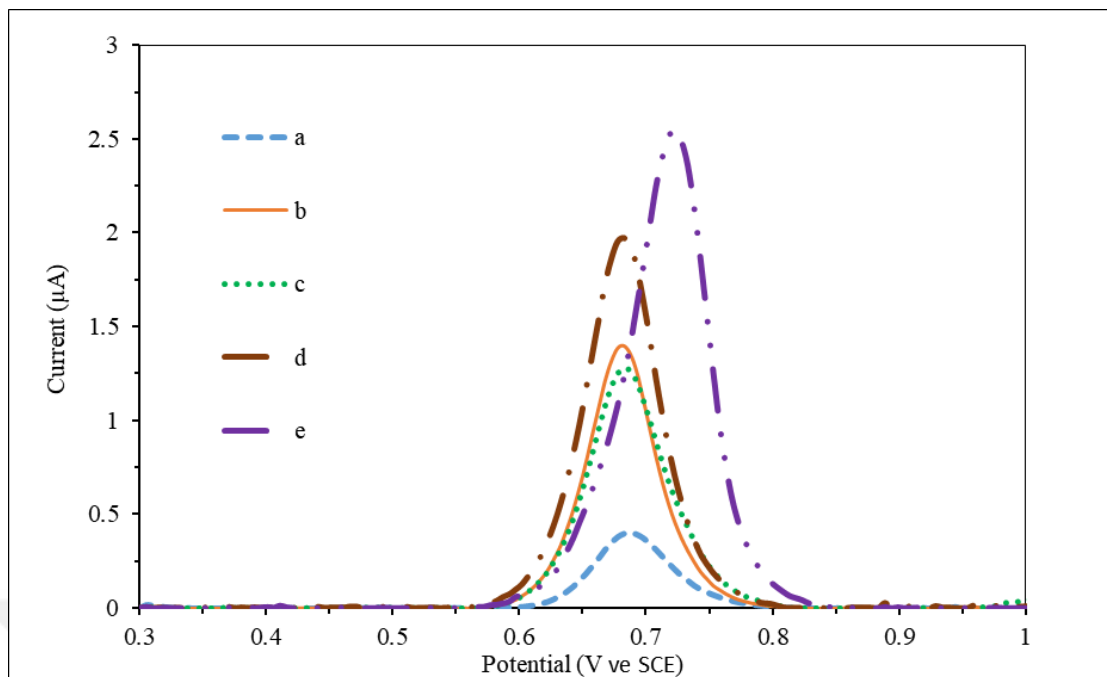
Figure 4.1. *Baseline-corrected differential pulse voltammograms of 10μM CBZ in 0.1 M PBS (pH 7.4) obtained with (a) bare CPE (6%), (b) CPE/FS (6%), (c) CPE/FS@Au (6%), (d) CPE/FS@Pt (6%), (e) CPE/FS@Ag (6%). Accumulation time 60 sec. Stirring rate 370rpm*

4.2. Characterization of Modified Electrodes

Characterization of the surface-modified electrode was performed by different methods including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersive X-ray (EDX) analysis.

4.2.1. Cyclic Voltammetry Measurements

Cyclic voltammetry study was performed at CPE, CPE/FS, and CPE/FS@Ag in 0.1 M KCl solution (Fig.4.2). A clear anodic peak was observed at the potential value of +0.102V on the CPE/FS@Ag and no oxidation peaks were observed in bare CPE and CPE/FS. Therefore, the obtained oxidation peak may arise from the oxidation of silver nanoparticles found in the structure of the modified electrode. The reduction peak was observed at the

potential value of -0.3V on the CPE/FS@Ag may also verify the presence of silver nanoparticles. Similar results were also reported in the literature [66].
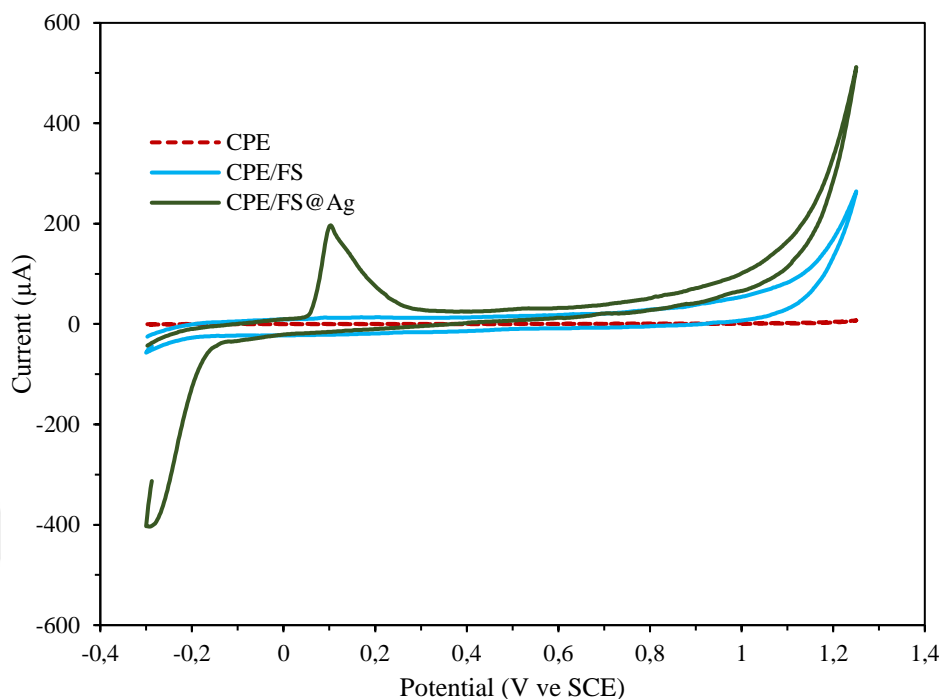
Figure 4.2. *Cyclic Voltammograms of CPE, CPE/FS, and CPE/FS@Ag obtained in 0.1M   KCl. Scan rate: 50 mVs $^{-1}$*

Electrochemical behaviors of CPE, CPE/FS and CPE/FS@Ag electrodes were investigated via cyclic voltammetry measurements in 0.1M KCl solution containing 2.5 mM $Fe(CN)_6^{4-/3-}$ or 1.0 mM $Ru(NH_3)_6^{3+}$ solution. Cyclic voltammograms of 2.5 mM $Fe(CN)_6^{4-/3-}$ are shown in Figure 4.3. The oxidation of $Fe(CN)_6^{4-}$ on the unmodified CPE took place at the potential value of +0.307 V and reduction at the potential value of +0.116 V. On the CPE/FS, peaks were observed at the potential values of +0.222 V and +0.129 V. On the CPE/FS@Ag two oxidation peaks were observed at the potential values of +0.210 V and +0.591 V, and reduction at the potential value of +0.139 V. The oxidation peak observed at +0.210 V may belong to the oxidation of both Ag nanoparticles found in the structure of the electrode and redox specie of $Fe(CN)_6^{4-}$. The second oxidation peak observed at +0.51 V may arise from the oxidation of an intermediate specie which is possibly formed during the simultaneous oxidation of Ag nanoparticles and $Fe(CN)_6^{4-}$. The difference between the oxidation and reduction potentials were calculated as +0.191 V, +0.093 V, +0.071 V for CPE, CPE/FS, CPE/FS@Ag, respectively. The results indicate that the modifier in the electrodes increases the electron transfer rate of the oxidation-reduction behaviors of $Fe(CN)_6^{4-/3-}$ redox couple.

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The peak currents increased in presence of modifier on CPE. The highest peak current was obtained with CPE/FS demonstrating the efficiency of the modification procedure.

Figure 4.3. *Cyclic Voltammograms of (a) CPE, (b) CPE/FS, and (c) CPE/FS@Ag obtained in 0.1M KCl solution containing 5mM Fe(CN)$_6$ $^{3-/-4}$. Scan rate: 50 mVs $^{-1}$*

The electrochemical characterization of CPE, CPE/FS, and CPE/FS@Ag was continued using a positively charged redox species 1mM Ru(NH$_3$)$_6$$^{3+}$ (Fig.4.4). As can be seen, the bare CPE showed redox couple which have oxidation and reduction peaks at the potential values of -0.154 V and -0.215 V, respectively. CPE/FS electrode showed an oxidation peak at the potential value of +0.045 V and reduction peak at the potential value of -0.164 V. On the other hand, CPE/FS@Ag showed two well- defined peaks at the potential values of +0.104 V and -0.098 V in addition to the redox behavior of Ru(NH$_3$)$_6$$^{3+}$. These peaks belong to the oxidation and reduction of Ag nanoparticles. The results obtained on the CPE/FS@Ag showed a great increase in peak currents. This improvement may be explained by the presence of silver nanoparticles and fumed silica on electrode surfaces. Fumed silica contains surface

silanol group (Si-OH) which can be ionized to form negatively-charged surface, which accelerates the adsorption of positively-charged $Ru(NH_3)_6^{3+}$ molecule.
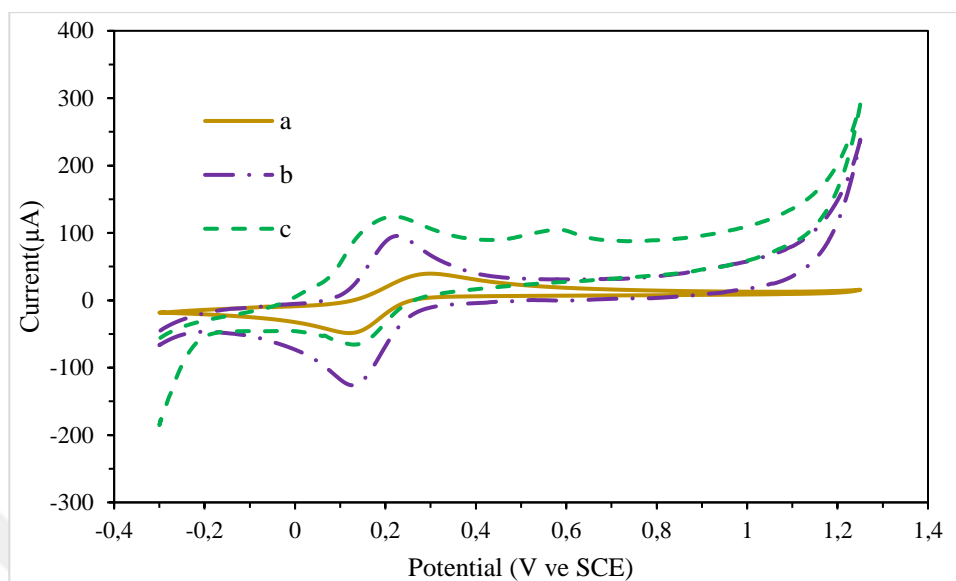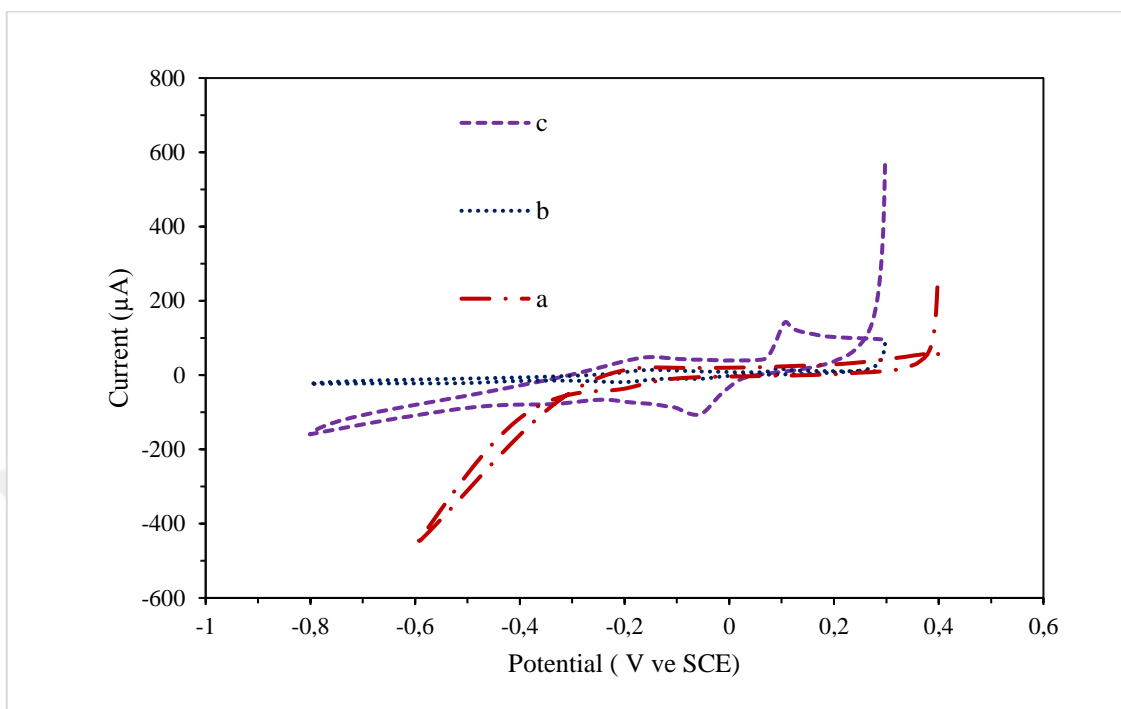

Figure 4.4. *Cyclic Voltammograms of (b) CPE, (a) CPE/FS, and (c) CPE/FS@Ag obtained in 0.1M KCl solution containing $Ru(NH_3)_6^{3+}$ .Scan rate: 50 mVs $^{-1}$*

4.2.2. Electrochemical impedance spectroscopy (EIS) measurements

The effect of fumed silica decorated silver nanoparticles on the interfacial characteristics of the carbon paste electrode was investigated via electrochemical impedance spectroscopy (EIS). Nyquist plots of the CPE and CPE/FS@Ag were obtained in 0.1M KCl solution containing 2.5 mM $Fe(CN)_6^{3-/4-}$. Solution resistance (R_s), double-layer capacitance (C_{dl}), and electron transfer resistance (R_{ct}) parameters were determined to monitor the electron transfer performance of the electrodes. These three parameters were calculated by modeling the experimental results with a Randles electrical circuit given in Figure 4.5. The charge transfer resistance (R_{ct}) values of the electrode were calculated by taking the diameter of the semi-circle found in Nyquist plots. Rct values measure the electron transfer rate at the electrode and electrolyte interface. Rct value for the unmodified carbon paste electrode was 938.3 Ω and the Rct values of the modified electrode (CPE/FS@Ag) was 153.6 Ω. The obtained values

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indicated that FS@Ag reduces Rct values, which leads to an increase in the electron transfer rate of the electrode.
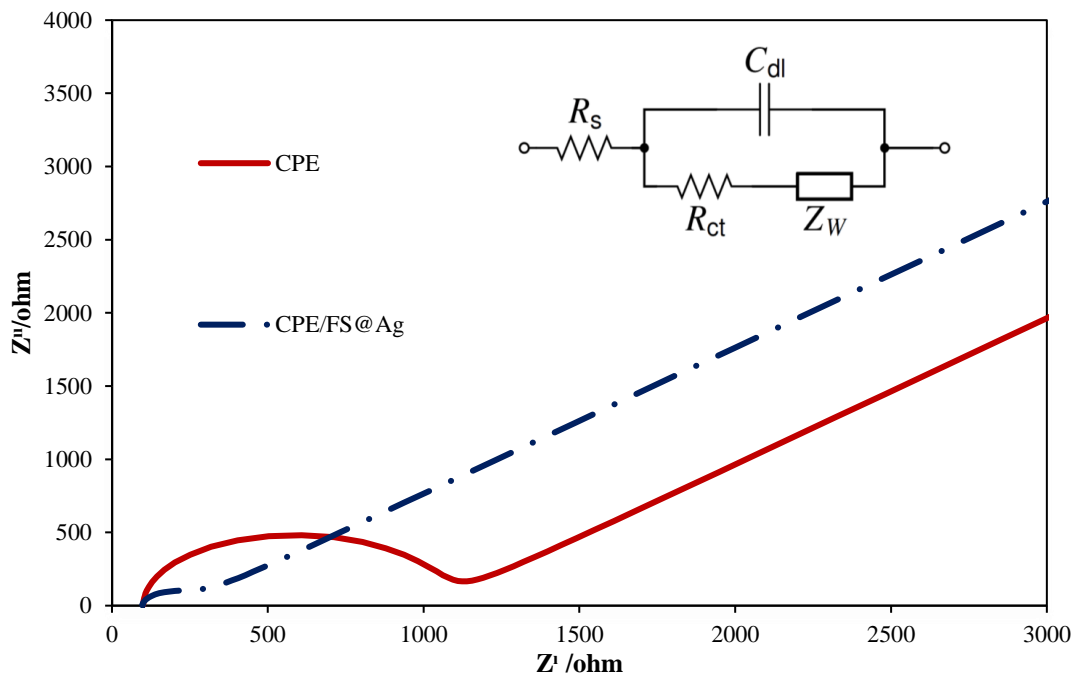
Figure 4.5. *Nyquist plots of bare CPE, and CPE/FS@Ag obtained in 0.1M KCl solution containing 2.5mM Fe(CN)$_6$ $^{4-/3-}$. Frequency range: 0.01-100 Hz. Set potential: +0.21V. Raw data were fitted according to Randles circuit given as inset.*

Table 4.1. *Values of impedance circuit parameters for electrodes obtained by equivalent circuit*

Electrode	R_{ct} (Ω)	R_s (Ω)	C_{dl}(F)	W
CPE	938.3	100.4	4.801×10^{-7}	0.0006182
CPE/FS@Ag	153.6	97.95	2.409×10^{-6}	0.000452

4.2.3. Scanning electron microscopy (SEM) images

Scanning electron microscopy (SEM) was employed to analyze the surface morphology and microstructure of the synthesized modification agent, and morphological characterization of CPE/FS and CPE/FS@Ag. SEM images of the modification agent of FS and FS@Ag are given in Figure 4.6. The hyperbranched nanofiber structure of FS are obviously seen in the SEM image shown in Figure 4.6. (A). FS@Ag also showed similar structure with that of FS but the main difference between them is the presence of small aggregates of silver nanoparticles on FS. A very homogenous distribution of silver nanoparticles on FS was

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observed (Fig.4.6. B). these pictures indicate that FS was successfully modified with Ag nanoparticles.
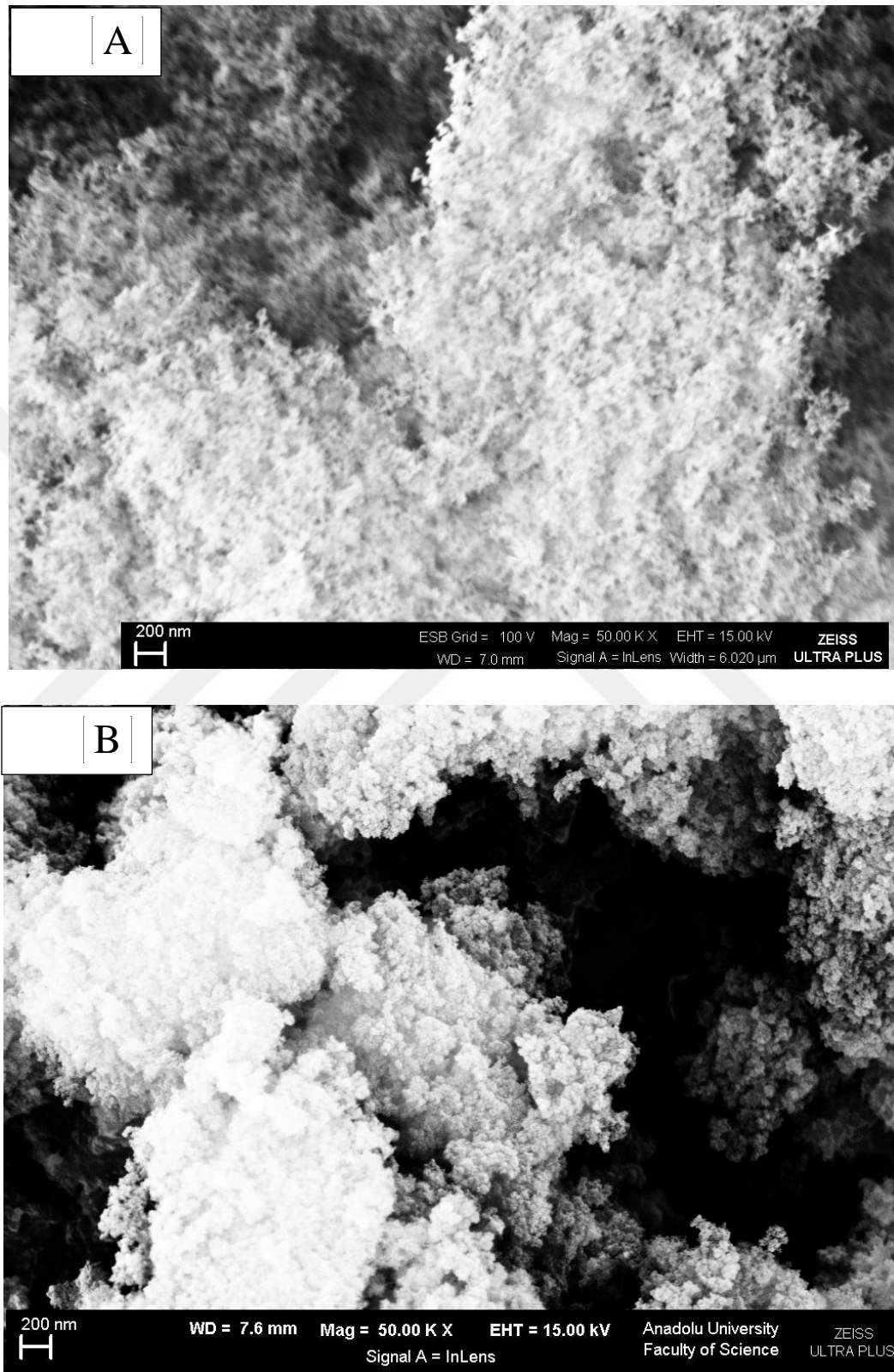
Figure 4.6. *SEM images of synthesized modification agent FS (A), FS@Ag (B)*

The SEM images of the CPE/FS and CPE/FS@Ag electrodes were also obtained to examine the morphologies of the electrode surface (Fig.4.7.). SEM images of the CPE/FS and CPE/FS@Ag showed a compact surface and some white aggregates belongs to the modification agents (FS and FS@Ag) (Fig.4.7.A). In higher magnified images of CPE/FS and CPE/FS@Ag, there are small gray dots homogeneously distributed in the electrode matrix on the surface of CPE/FS@Ag (Fig.4.7.C, D and E). These dots belong to small granules FS@Ag

nanoparticles (Fig.4.7.D and E). Therefore, it can be said that FS and FS@Ag homogeneously distributed on the surface of electrode.
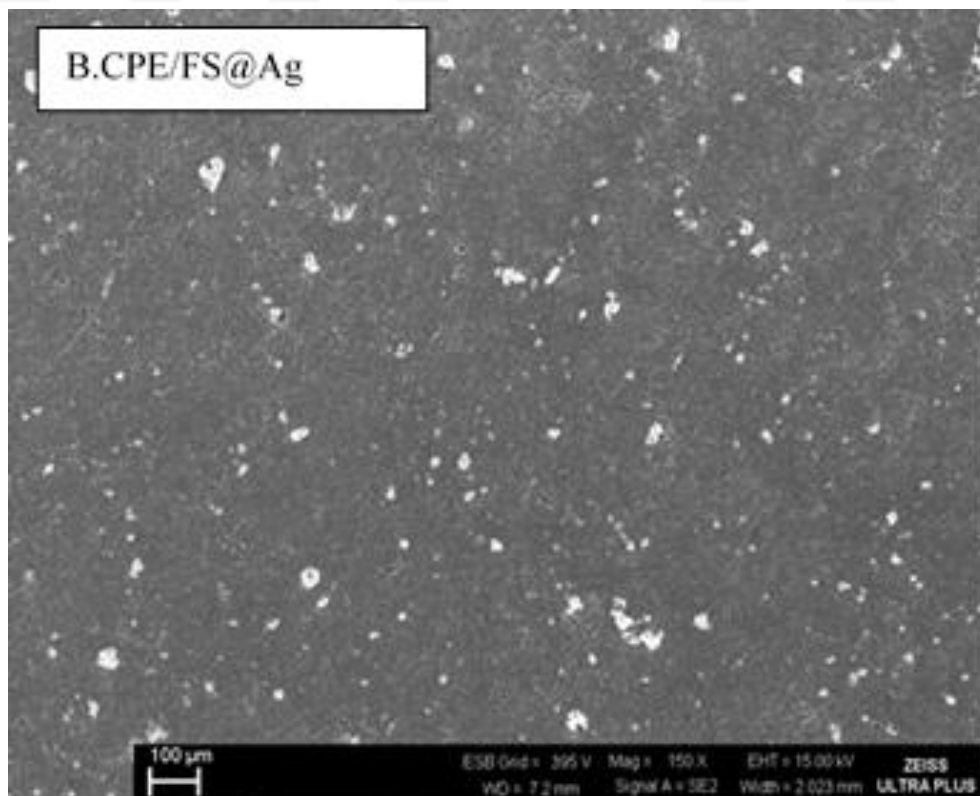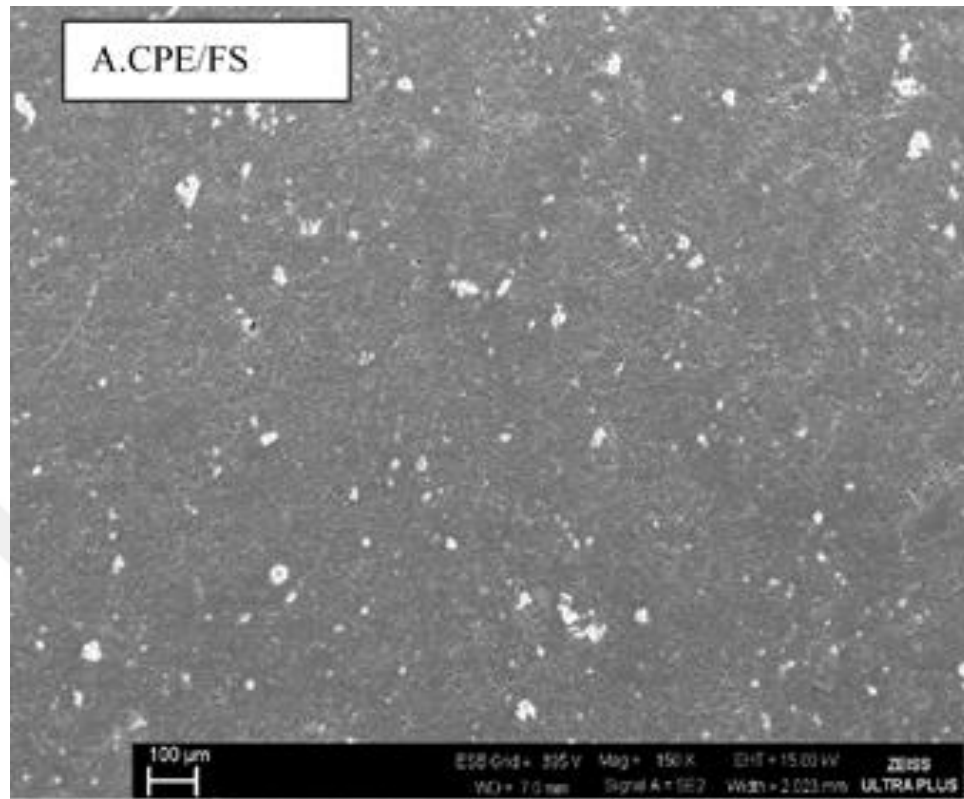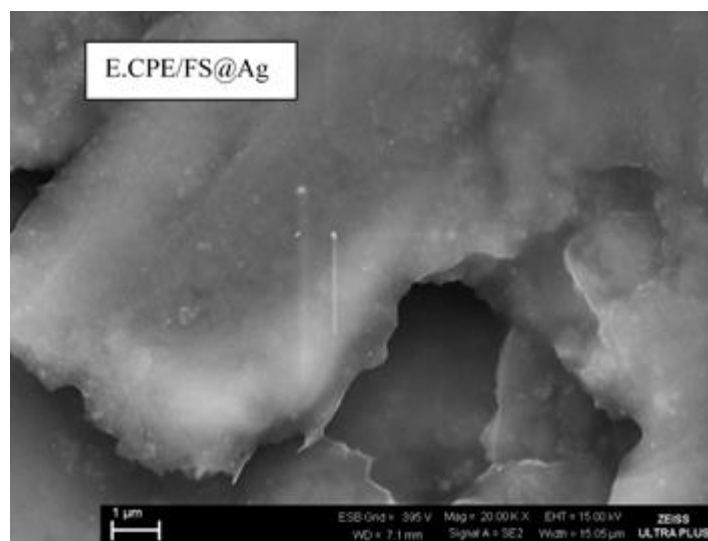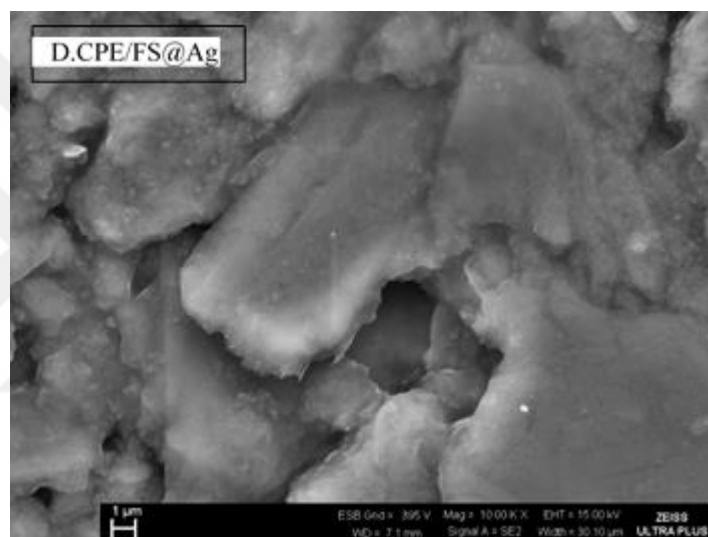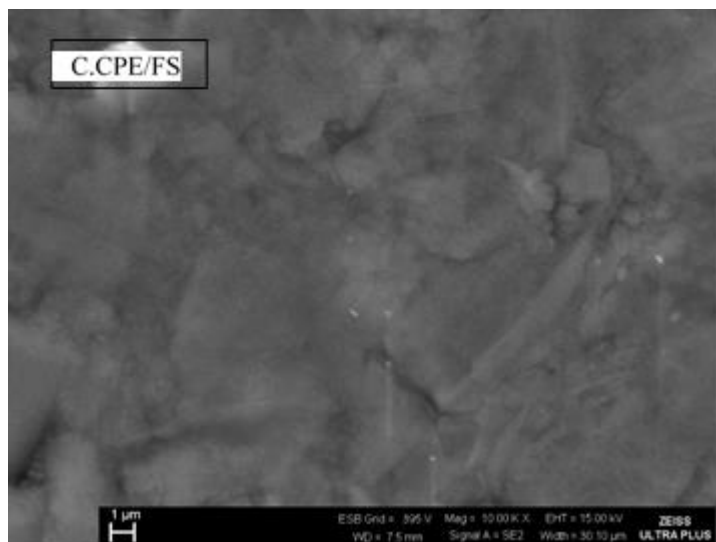
Figure 4.7. *SEM images of modified electrodes CPE/FS(A and C), and CPE/FS@Ag (B, D and E)*

4.2.4. Energy dispersive X-ray (EDX) analysis

The elemental composition of FS@Ag was characterized by using energy dispersive X-ray (EDX) detector coupled to the SEM. Weak signals were observed at 1.8 KeV and 3.00 KeV, which correspond to the presence of elemental silicon and silver nanoparticles, respectively (Fig.4.8). A similar finding was observed in the previous study [67]. A strong signal was also observed at 0.3 KeV due to the presence of carbon. Oxygen produced a weak signal at 0.5 KeV. EDX mapping elements was also performed to examine the distribution of the modifier in the CPE surface (Fig. 4.9). As seen, there is a homogenous distribution of Si, Ag, and O and C atoms on the surface of CPE / FS@Ag. This result verified the homogenous distribution of FS@Ag in the CPE matrix. Mapping image also shows that silver nanoparticles were spread homogenously through the surface matrix. Quantitative elemental composition (atom and weight %) was obtained from the EDX spectrum (Fig. 4.8).
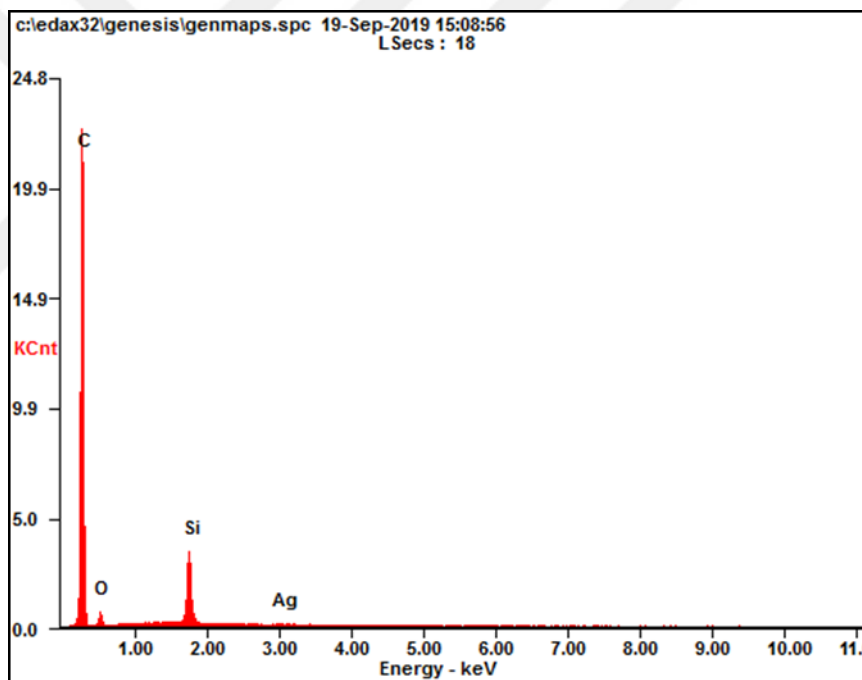
Figure 4.8. *EDX for purity analysis of silica and silver nanoparticles. AgNPs, Si, C, O was observed in EDX spectrum*

a.Si

b.Ag
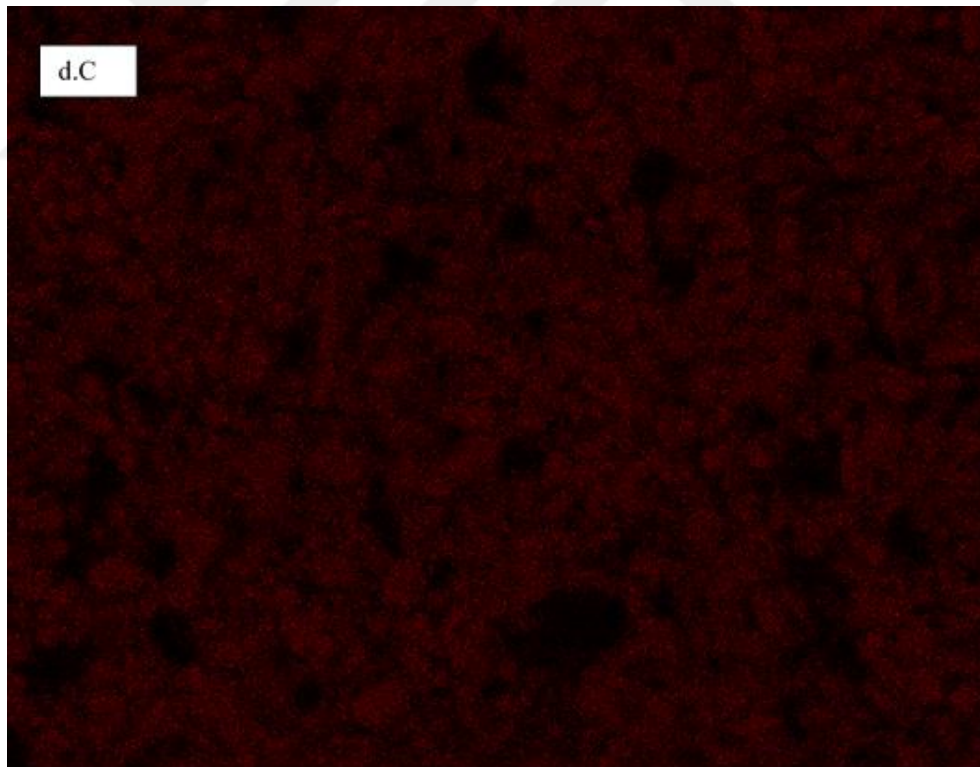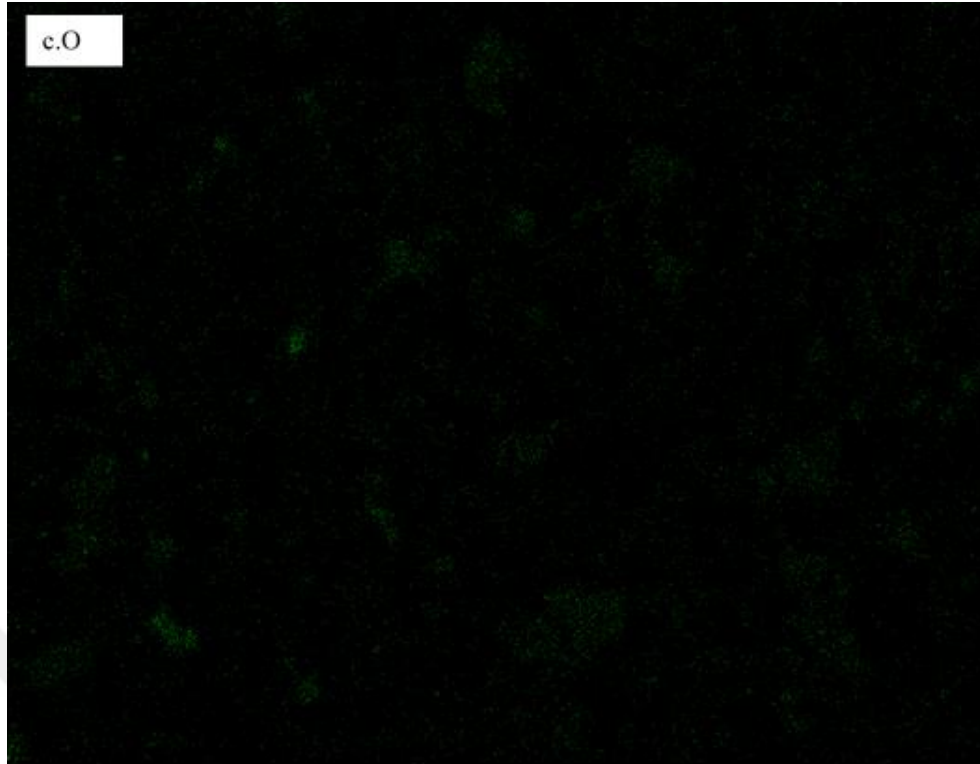
Figure 4.9. *EDX of the distributions of defined elements, silicon (a), silver (b), oxygen*

(c) and carbon (d)

4.2.5. X-ray diffraction (XRD) analysis

Characterization of FS and FS@Ag were also performed by X-ray diffraction (XRD) analysis to determine the crystal structure of FS and FS@Ag nanoparticles (Fig.4.10). FS showed a clear XRD pattern including only one broad peak at $2\theta = 21.1°$. There were five Bragg's well-defined characteristic diffraction peaks at 2θ values of 38.2° (111), 44.23° (200), 64.59° (220), 77.53(311), 81.3 (222) after modification of FS with silver nanoparticles. At 2θ value of 38.2° (111), there is a strong peak indicating the formation of pure crystalline silver nanoparticles.
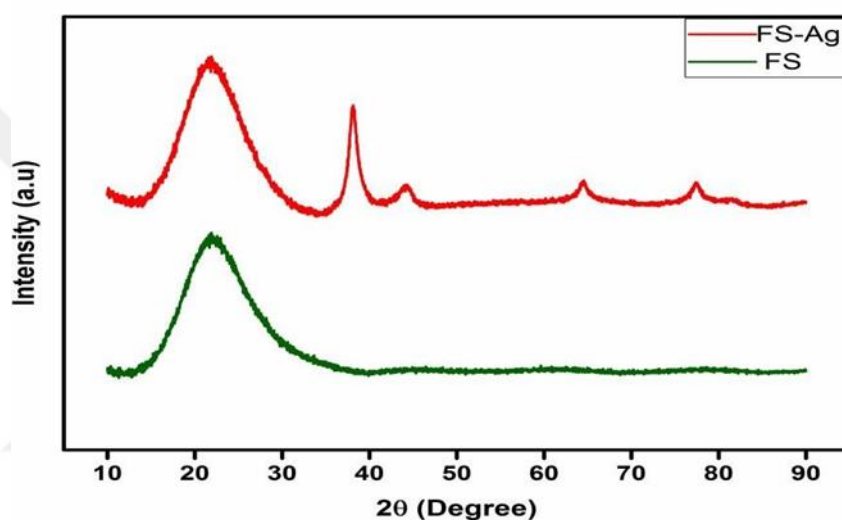

Figure 4.10. *XRD patterns of synthesized FS and FS@Ag*

4.3. Evaluation of the Voltammetric Responses of CBZ in The Modified Electrodes

Voltammetric performances of carbon paste electrode (CPE) and CPE/FS@Ag were tested in the differential pulse voltammetry measurement of 10 µM CBZ in 0.1 M phosphate buffer solution (PBS: pH 2.0). An oxidation peak of CBZ was observed at the potential value of +1.0 V at the bare CPE and CPE/FS@Ag (Fig .4.11). The high oxidation peak current was obtained when the CPE/FS@Ag was used. The oxidation peak current of CBZ increased tremendously with CPE/FS@Ag compared to bare CPE.

This indicates that the FS@Ag nanocomposite shows a synergistic effect and increases clearly the voltammetric behavior of CBZ. The improvement in the voltammetric responses of CBZ on CPE/FS@Ag may arise from the following reasons; the modification of CPE with FS may increase the surface area of the electrode due to nanostructure of fumed silica. This may increase the oxidation peak currents of CBZ. The interaction between CBZ and surface

hydroxyl groups of FS may lead to an increase in the adsorption of CBZ which also increases the oxidation peak currents of CBZ. Another reason may be the high electron transfer rate of the electrode in the presence of FS because the presence of fumed silica increases the electron transfer rate of redox species, which was reported many times in the literature [48]. The modified electrode also contains Ag nanoparticles in addition to FS. The presence of Ag nanoparticles may enhance the conductivity due to the properties of silver; it's an excellent conductor of electricity. Interact with CBZ, and this may increase the adsorption behavior of CBZ. Finally, it can be said that the Ag nanoparticles may show electrocatalytic activity toward the oxidation of CBZ because Ag nanoparticles show electroactivity at a potential value lower than that of CBZ.
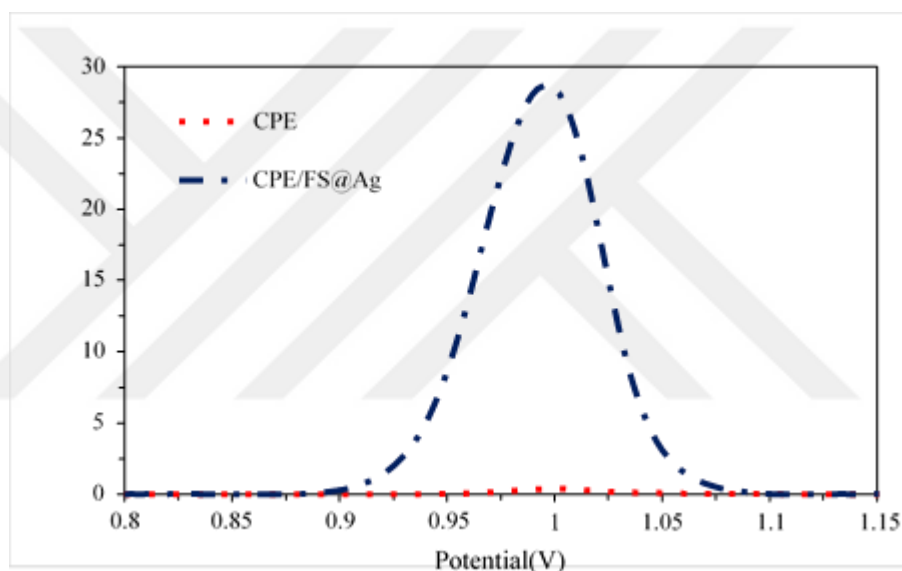
Figure 4.11. *Baseline-corrected differential pulse voltammograms of 10.0μM CBZ with unmodified carbon paste electrode (CPE) and modified carbon paste electrode (CPE/FS@Ag) in 0.1M PBS (pH :2). Accumulation time 120 s. Stirring rate 370rpm.*

4.4. Electrochemical Redox Behavior of CBZ

Cyclic voltammetry measurements was performed to investigate the electrochemical behavior of CBZ on the CPE and CPE/FS@Ag electrode. CV measurements were performed in PBS (pH = 2) in the absence and presence of 15 μM of CBZ (Fig.4.12). In the absence of CBZ, CPE/FS@Ag electrode showed a broad oxidation peak at +0.468 V and a broad reduction peak at +0.25 V. This redox couple may arise from the oxidation of Ag nanoparticles and subsequent reduction of oxidized Ag+ ion to Ag nanoparticles on the electrode surface. In the presence of CBZ, CPE/FS@Ag electrode showed a well-defined oxidation peak at +1.044 V in addition to discussed peaks. This peak belongs to the oxidation of CBZ. In the

reverse scan, there was no reduction peak related to the redox behavior of CBZ. This indicates that the oxidation of CBZ on the CPE/FS@Ag surface is an irreversible process. The oxidation of CBZ at bare CPE took place at +1.052 V. As can be seen, there was a large difference between the peaks currents of CBZ obtained at both electrodes. This reveals that the presenc of FS/Ag nanocomposite in the CPE structure gradually increased the electrochemical behavior of CBZ.
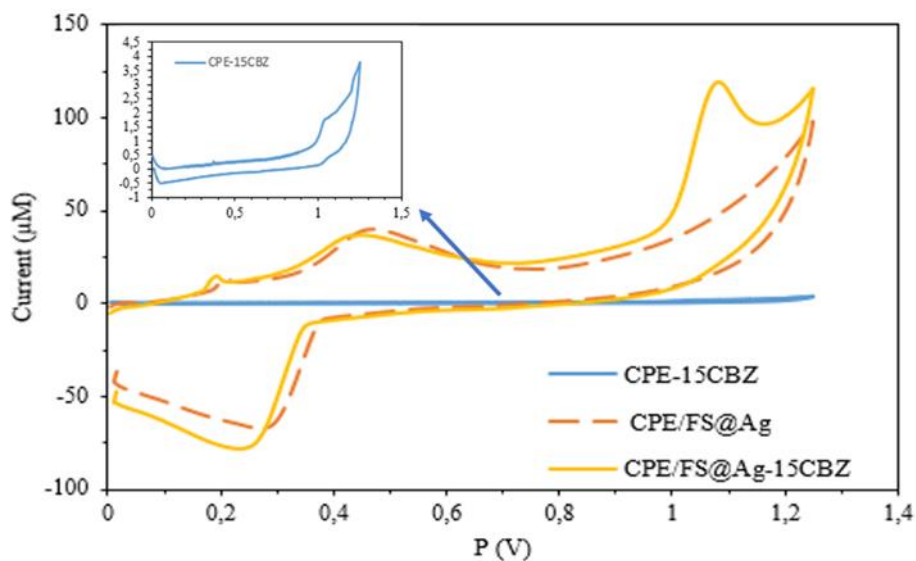
Figure 4.12. *Cyclic voltammograms of CPE and CPE/FS@Ag in PBS (pH = 2) in the presence and absence of 15 μM of CBZ. Scan rate 50 mV s-1*

The effect of scan rate on the electrochemical oxidation behavior of CBZ was investigated to obtain information about the electrochemical oxidation mechanism of CBZ and the kinetic process on the modified electrode surface. Measurement was performed in a 0.1 M phosphate buffer solution (pH = 2) at different scan rates ranging from 15 to 180 mV/s (Fig.4.13 .a). Modified electrode showed an obvious oxidation peak, and no reduction peaks were observed in the reverse scan. This result shows that the electrochemical oxidation reaction is an irreversible process. The peak currents of CBZ were linearly proportional to the scan rate from 10 to 250mV/s (Fig.4.13.b), this reveals that the oxidation of CBZ on CPE/FS@Ag is mainly an adsorption-controlled process ($R^2 = 0.9926$). The determination coefficient (R^2) was calculated as 0.9821 when the oxidation peak current was plotted against the square root of the scan rate (Fig.4.14). This confirms that the oxidation of CBZ is an adsorption-controlled process on the CPE/FS@Ag surface. In addition, peak potential shifted

to more positive value with an increase in scan rate, which supported the irreversibility of the electrode reaction.
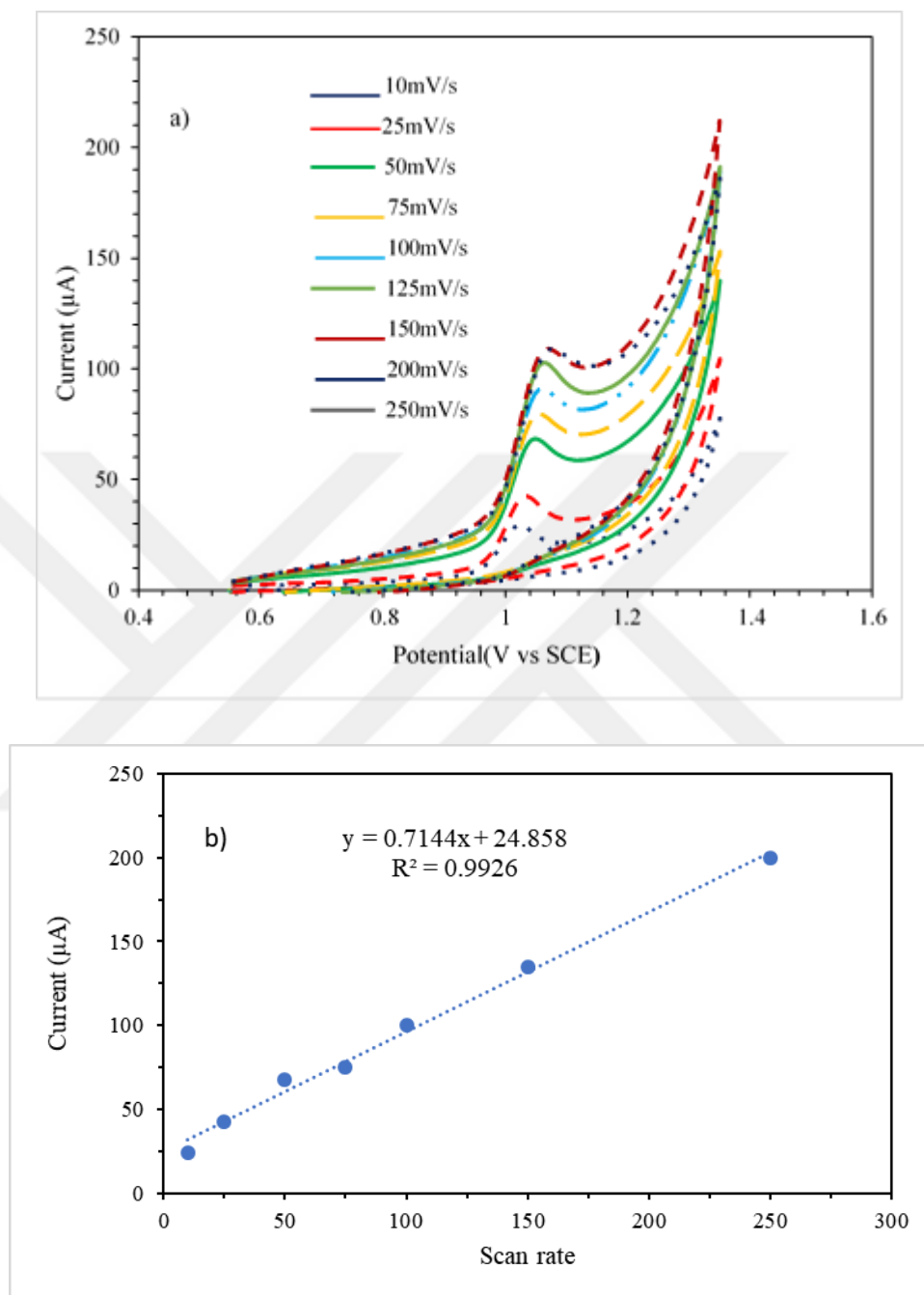
Figure 4.13. *a) CVs of 10.0 μM CBZ in 0.1 M PBS (pH:2) obtained by CPE/FS@Ag at different scan rates values. b) plot of the oxidation peak current of CBZ versus scan rate*

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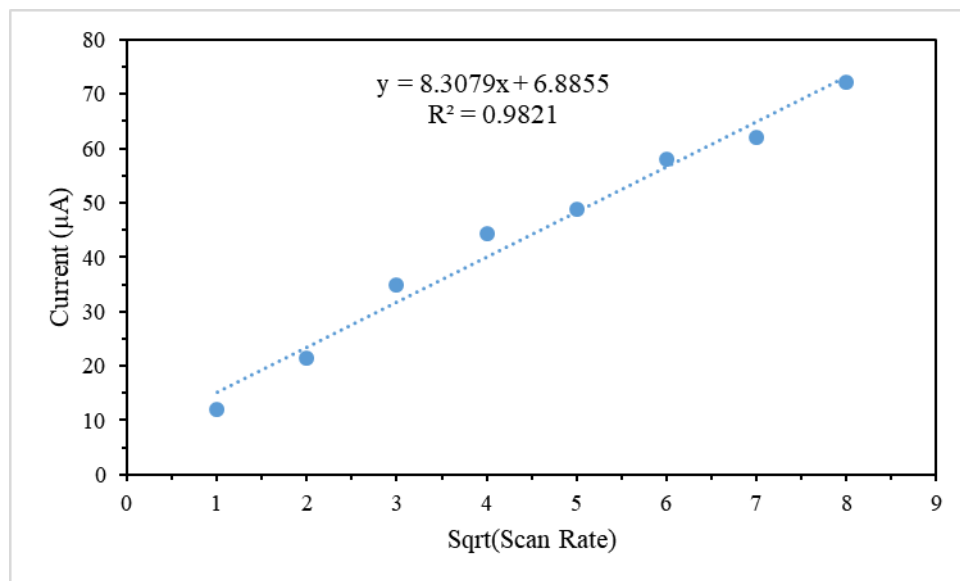
Figure 4.14. *Plot of the oxidation peak current of CBZ versus square root scan rate*

4.5. Optimization of Parameters

4.5.1. Optimization of the amount of FS@Ag

Parameters affecting the voltammetric performance of the modified electrode (CPE/FS@Ag) were examined to find the optimal values. The oxidation studies were completed in three steps. In the first step, the effect of the amount of modifier (FS@Ag) on the oxidation peak current of CBZ was determined with the electrodes containing different percentages of FS@Ag (Fig4.15). The oxidation peak currents increased with the increase of modifier in the order of 3%, 6%, 9%, 12%, and 15%. The highest oxidation peak current of CBZ were observed with the electrode containing 15% of FS@Ag compared to other

percentages. Therefore, 15% was selected as the optimum modifier amount and used in the rest of the study.
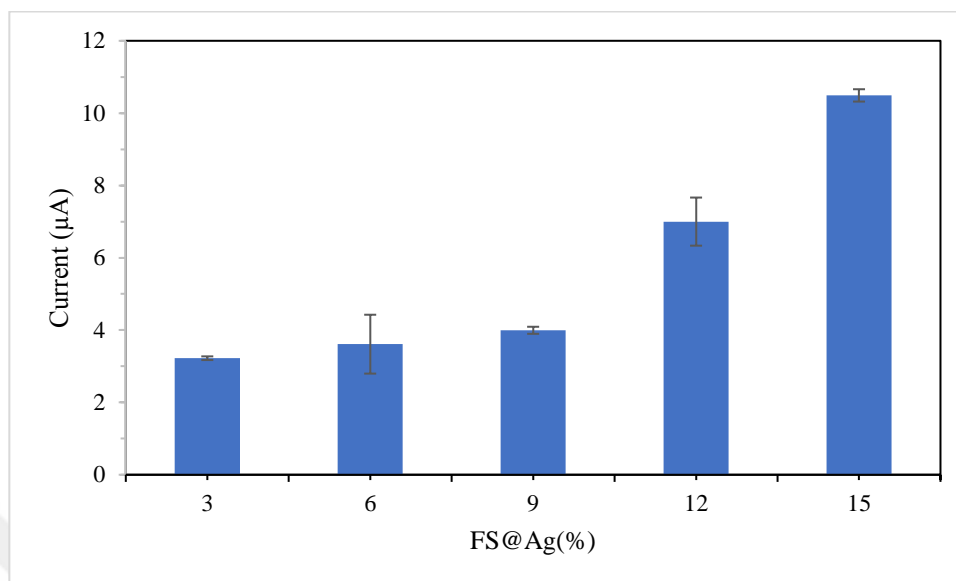
Figure 4.15. *The effects of the amount of FS@Ag percentage on the voltammetric response of 10.0 μM CBZ in PBS (pH 7.4). Accumulation time: 60 s, stirring rate: 370 rpm*

4.5.2. Optimization of the pH of measurement medium

In the second step, the optimization of the pH value of measurement medium. The electrochemical oxidation behavior of CBZ on the surface of CP/FS@Ag electrode was examined by performing differential pulse voltammetry measurement in the presence of 10.0 μM of CBZ in 0.1 M PBS having different pH values (2, 3,4,5, 6, 7.4, 8.5, 10). A clear oxidation peak was observed in all pH values indicating oxidation of CBZ in a large pH range (Fig.4.16 .A). The highest oxidation peak current was noted at a pH value of 2.0. Therefore, this value was chosen as optimal pH to detect CBZ to achieve high sensitivity. In different study, researcher reported a pH value of 5 as optimal pH to detect CBZ concentration [68].

As shown in the graph (Fig.4.16.B), there were two linear regions. First linear region was observed between oxidation peak potentials of CBZ and pH values between 2 and 5 having an equation of (E_{CBZ}= -0.0636 pH + 1.1261; R^2 =0.9934). Second linear region was observed between oxidation peak potentials of CBZ and pH values between 6 and 10 having

an equation of (E_{CBZ}= -0.0252 pH + 0.928; R^2 =0.997)The relationship between peak potential and pH relative to the Nernst equation can be explained by the following formula [48].

$$\frac{dEpa}{dpH} = -\frac{0.059m}{n}$$

(4.1)

Here, m represents the number of protons involved in the electrochemical reaction, and n represents the number of electrons. The obtained value (-0.0636) in acidic region is very close to the theoretical value (-0,059) found in the Nernst equation. This result shows that the equal numbers of protons and electrons took place during the electrochemical oxidation of

CBZ. According to these results and studies in the literature, a mechanism for the electrochemical oxidation of CBZ can be proposed [69, 70 and 71] (Fig.4.17).
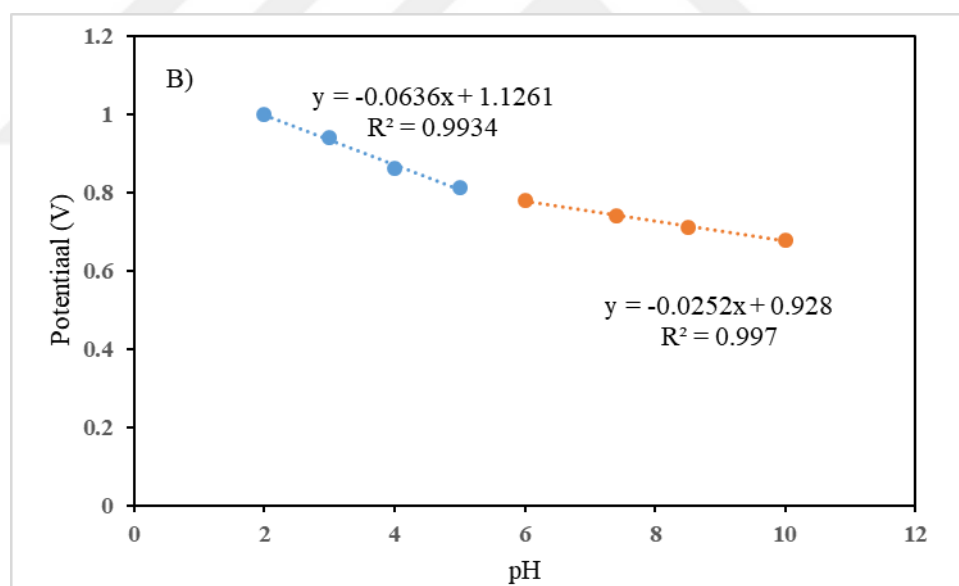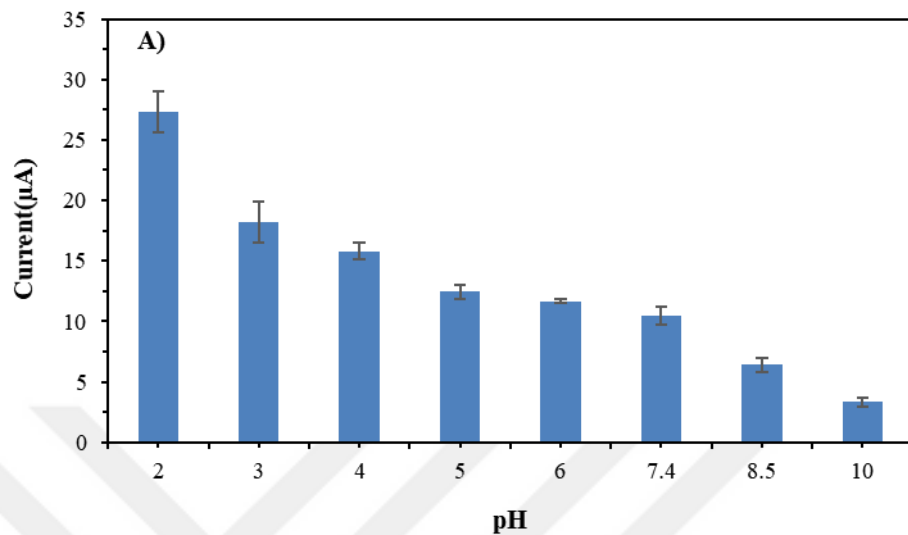

Figure 4.16. *(A)DPV of oxidation peak currents of 10.0 μM CBZ obtained by CPE/FS@Ag at different pH values. B) The relationship between oxidation peak potentials and pH of thesolutions*
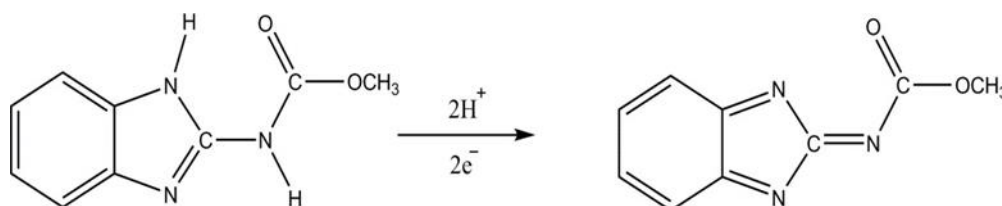
Figure 4.17. *Electrochemical oxidation mechanism of CBZ*

4.5.3. Optimization of the accumulation time

The oxidation of CBZ on CPE/FS@Ag is an adsorption-controlled process. Therefore, the experiments were designed to optimize accumulation time value to determine the optimal contact time of electrode with CBZ. The modified electrode was immersed in a solution containing 2.5 µM CBZ for different time periods (15 s, 30 s, 45 s, the 60 s, 90 s, 120 s, 150 s), and then differential pulse voltammetry measurements were performed (Fig.4.18). The oxidation peak currents of CBZ gradually increased with the increase of accumulation time up to 120 s and remained almost constant after this time value. This indicates that surface saturation takes place when the accumulation time reaches to 120 s. Therefore, this value was selected as the optimal value.
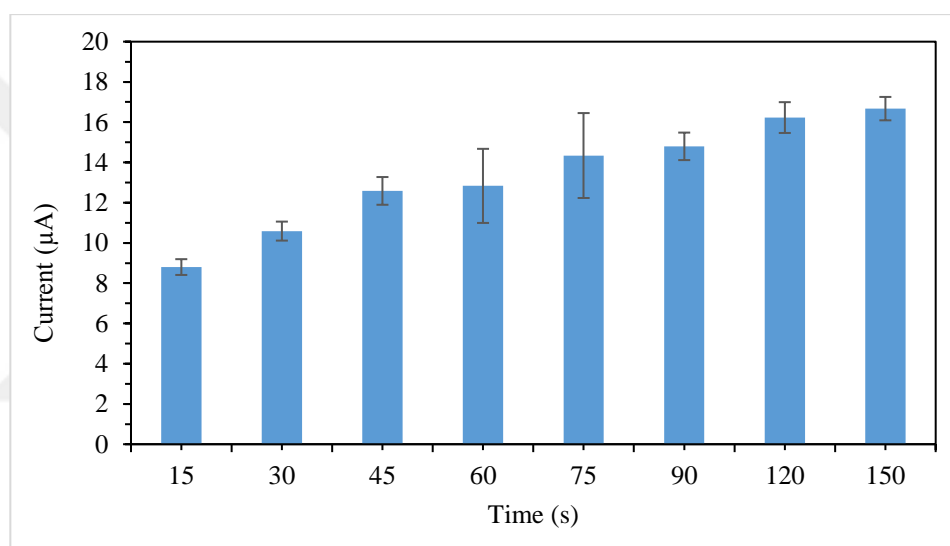
Figure 4.18. *The oxidation peak current values of 2.5 µM CBZ in PBS (pH 2.0) obtained by CPE/FS@Ag at different accumulation time periods. Stirring rate: 370 rpm*

4.6. The Effect of Potential Interfering Substances on Electrochemical Behavior

The selectivity of the fabricated modified carbon paste electrode (CPE/FS@Ag) was tested by the measurement of 2.5µM CBZ in the presence and absence of specific various interfering substances. The measurement was conducted by differential pulse voltammetry in a 0.1 M phosphate buffer solution (pH =2). The interference substances were added to the solution containing CBZ for the measurement. Firstly, the effect of inorganic ions was investigated including Zn^{2+}, K^+, Cl^-, NO^{2-}, CO_3^{2-} and Na^+. There was no significant effect in the voltammetric response of the 2.5µM CBZ. The other potential interfering substances such as ascorbic acid, citric acid, Salicyic acid, and pesticides such as tebuthiuron fenitrothion, azinphos-methyl and ammonium sulfate, bisphenol A and 4- aminophenol (Fig.4.19) were

also investigated. The results demonstrate that there was no potential effect on the differential pulse voltammetry signal of CBZ. The maximum allowed concentration of interfering substances was determined and given in Table 4.2. Some interference substance such as 4 aminophenol and copper (II) chloride leads to a decrease or an increase in the oxidation peak currents of CBZ, this change may arise from the competitive adsorption behavior of the interfering substances and CBZ. The results reveal that the CPE/FS@Ag has enough selectivity for the estimation of CBZ without interfering from the signal of other coexisting substances.

Table 4.2. *Maximum allowed concentration of interfering substances and peak current variation percentages in the presence of interference species*

Interfering substances	MA/μM	RCP $= \frac{Ip2-Ip1}{Ip1} x100\%$
Ascorbic acid	2000	-3.5
Citric acid	2000	-1.4
Tebuthiuron	25	5.8
Ammonium sulfate	1000	1.8
Bisphenol A,	10	-27.9
4-aminophenol,	10	-27.5
Fenitrothion	10	0.7
Azinphos-methyl	50	-6.0
Salicyic acid	10	-0.8
KCl	500	-1.06
NaNO$_3$	1000	1.1

RCP : Relative change percent in oxidation peak current of CBZ

MA : Maximum allowed concentrations of interfering substance

Ip1 : Oxidation peak current of 2.5 μM CBZ in the absence of interfering substance Ip2 : Oxidation peak current of 2.5 μM CBZ in the presence of interfering substance
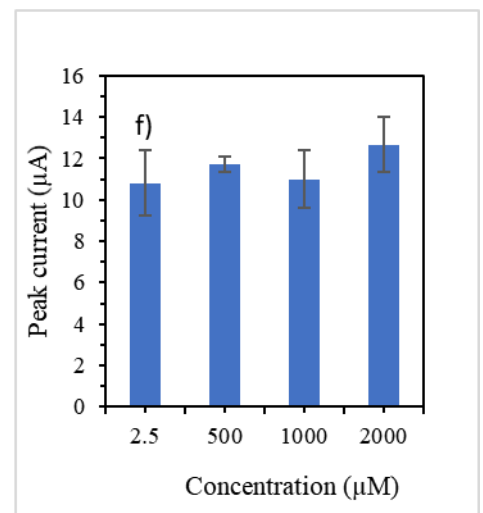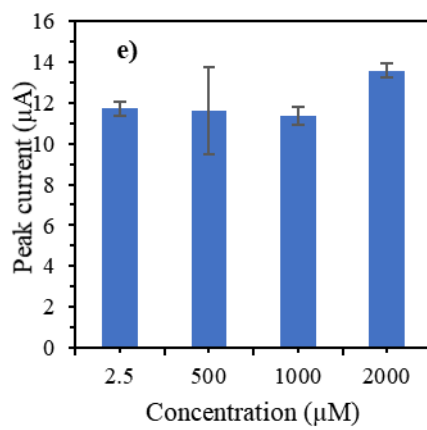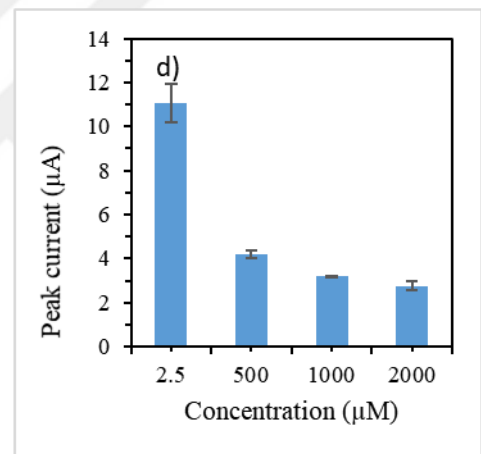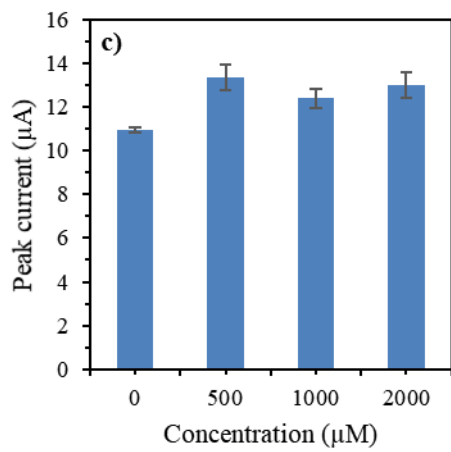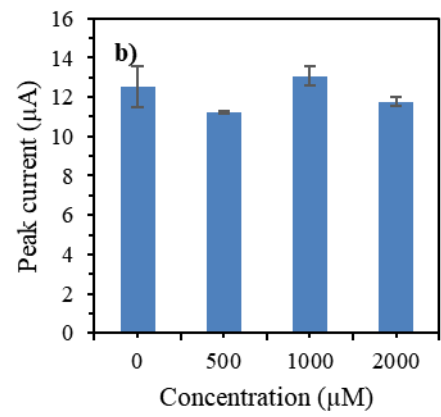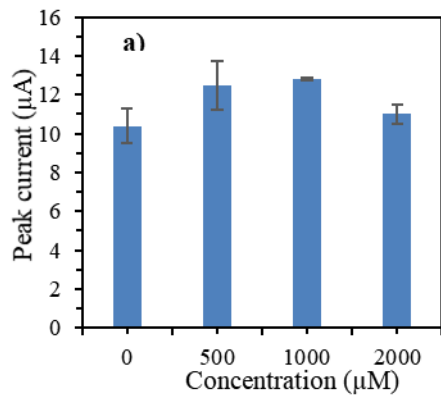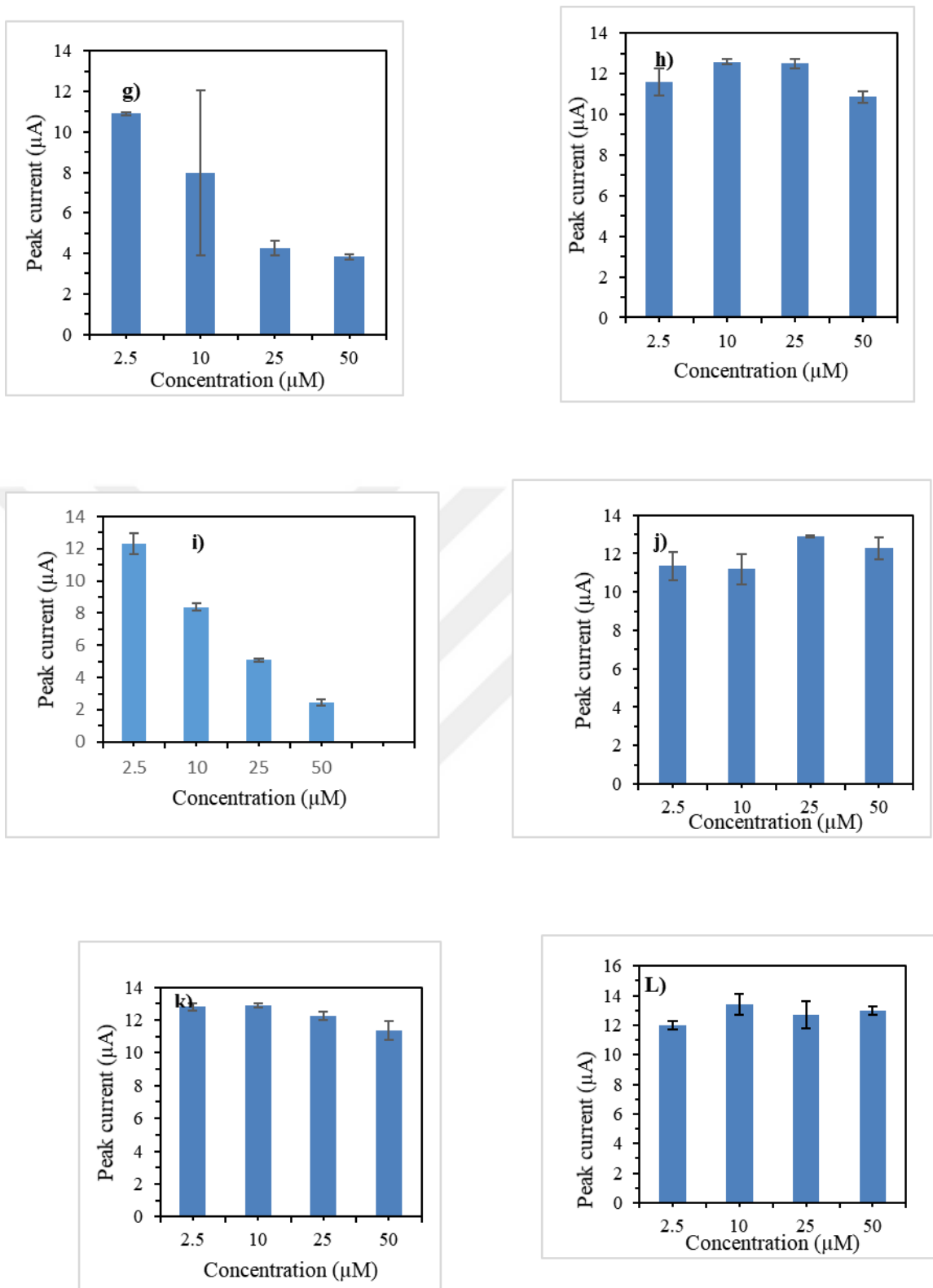
Figure 4.19. *The oxidation peak current values 2.5 µM CBZ in PBS (pH 2.0) obtained by CPE-FS-Ag in the absence and presence of different interference substances (a) ascorbic acid, (b) citric acid, (c) Na⁺, (d)CuCl₄, (e)KCl, (f) (NH₄)₂SO₄, (g) 4-aminophenol, (h) azinphos, (i) bisphenol A, (j) Salicyic acid (k) fenitrothion, (l) tebuthiuron*

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4.7. Determination of analytical parameters of CBZ detection

The analytical parameters such as linear range, limit of detection, limit of quantification, and repeatability were determined for the determination of CBZ with the prepared modified electrode. Measurements were conducted with differential pulse voltammetry in 0.1 M phosphate buffer solution (pH=2.0). The oxidation peak current of CBZ showed two linear regions with the increasing concentration of CBZ (Fig.4.20.A). First linear range, oxidation current of CBZ increases linearly in a concentration range of 0.05 μM to 3.0 μM. The calibration line has an equation of Ipa (μA) = 7.0013 C_{CBZ} + 0.1338 with a linear regression constant (R^2) value of 0.9993 (Fig.4.20.B). The detection limit (LOD) of CBZ was determined by measuring 10 blank measurements with the CPE/FS@Ag electrode, and its value was calculated as 0.000940 μM and the limit of quantification (LOQ) was 0.003134 μM taking the signal to noise ratio as 3.0. Second, linear range lies between the concentration value of 3.0 μM to 10.0 μM with linear equation of Ipa (μA) = 1.6758 CCBZ + 17.941 and a linear regression constant (R^2) value of 0.9923 (Fig.4.20.B.).The measurements were also repeated with bare CPE. The calibration line of bare CPE has an equation of Ipa (μA) = 0.036 CCBZ +0.0047 with a linear regression constant (R^2) value of 0.9948 (Fig.4.21.a and b). On the other hand, obtained result by CPE/FS@Ag showed a much lower detection limit and quantification limits compared to the results of LOD value of 0.18285 μM and LOQ value of 0.60953 μM obtained by bare CPE. The oxidation peak current of CBZ in bare CPE is irregular and there is a small shift in the oxidation potential value of CBZ. The repeatability of the electrode was estimated by measuring 6 freshly prepared CPE/FS@Ag electrodes; the measurement was performed with differential pulse voltammetry in 0.1 M phosphate buffer solution containing 10.0 μM CBZ. The relative standard deviation (RSD) value was detected as 5.3%, the high repeatability of FS@Ag. The analytical parameters of this study were compared with the

previous studies (Table 4.3). The CPE/FS@Ag showed lower detection limit value and a wide linear range compared to other literature studies.
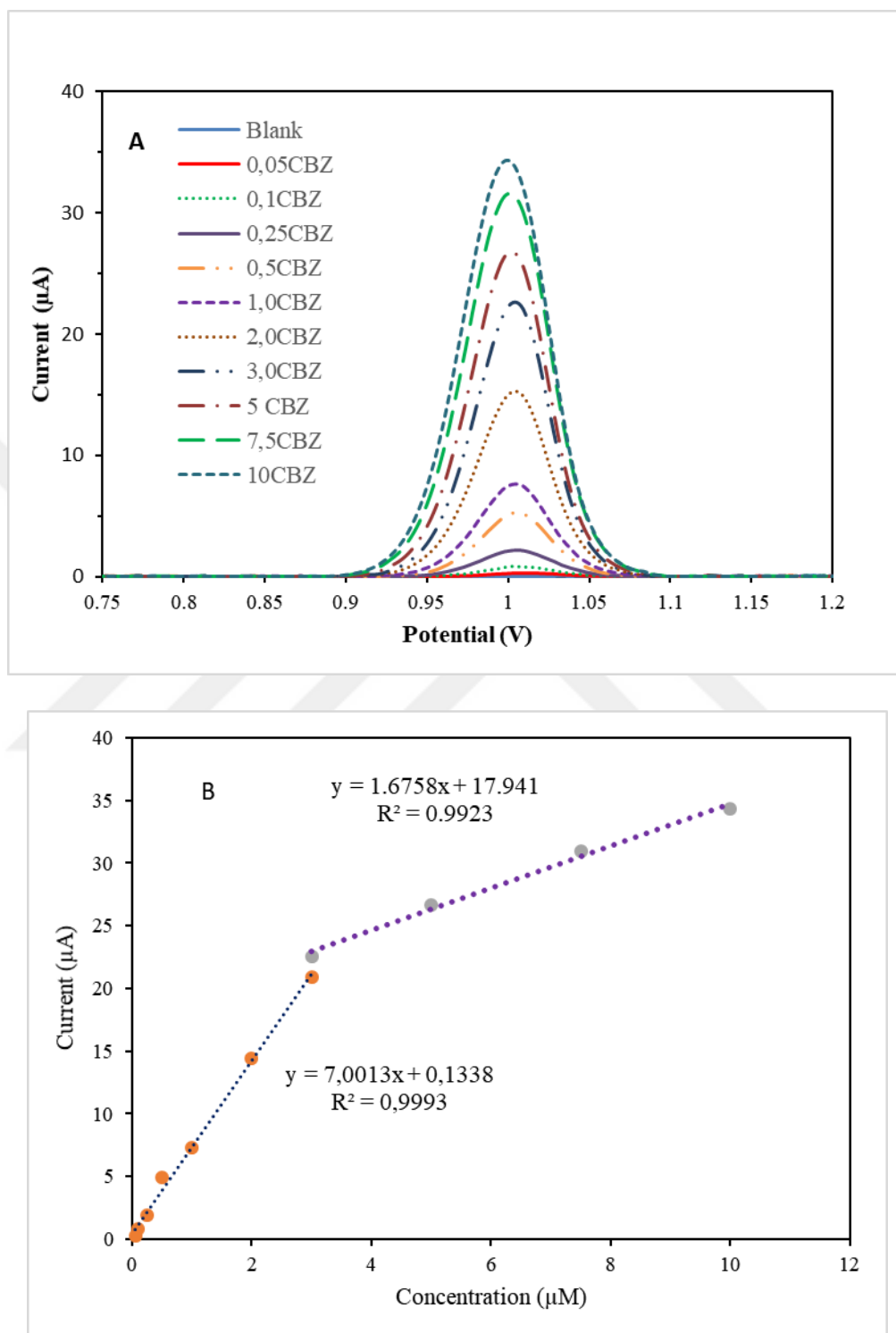
Figure 4.20. *(A) Baseline- corrected differential pulse voltammograms of increase concentration of CBZ .(B) Calibration curve of increase concentration of CBZ , 0.1 M PBS (pH 2.0). Accumulation time: 120 s, stirring rate: 370 rpm*
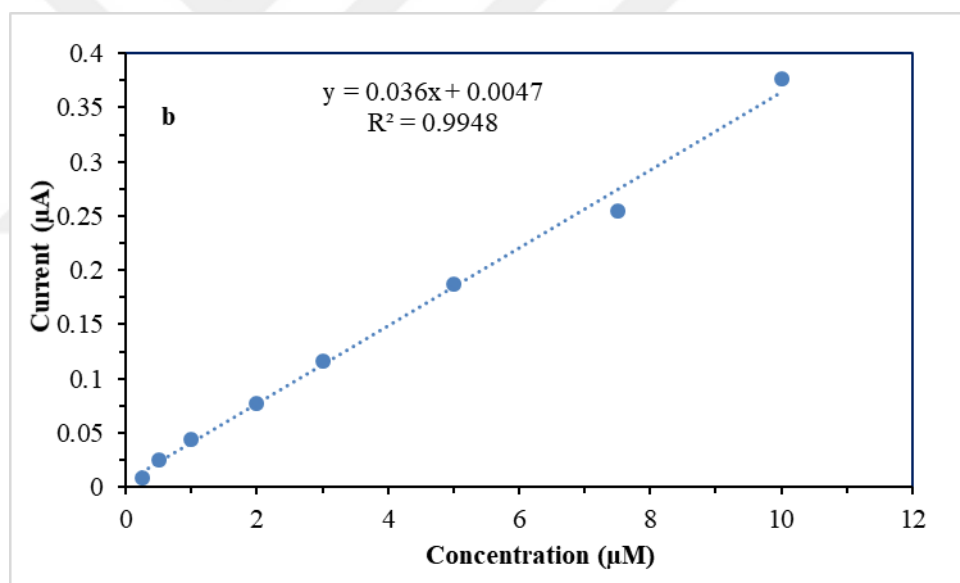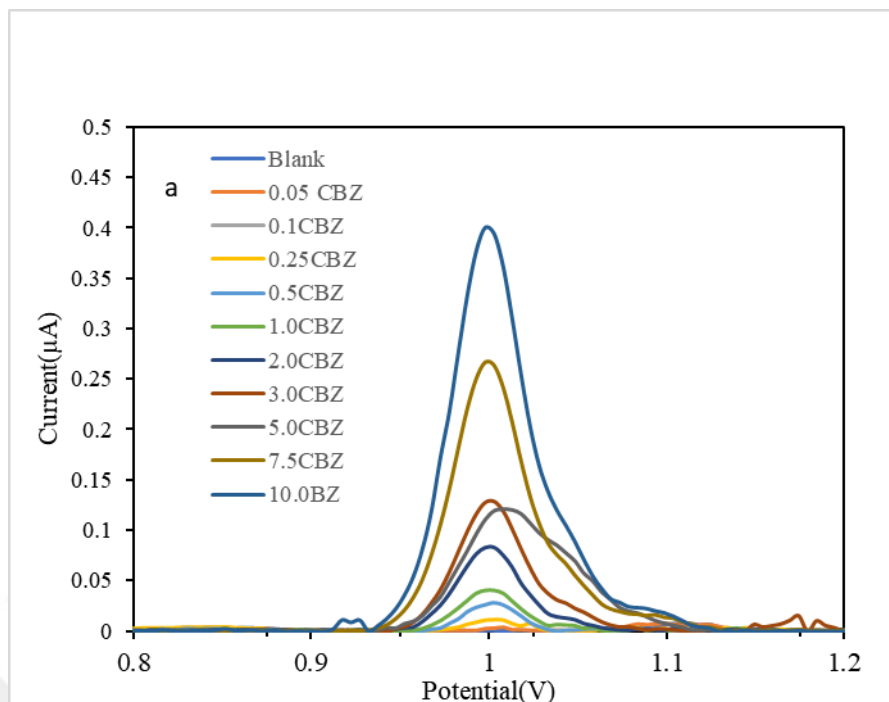
Figure 4.21. *a. Baseline-corrected differential pulse voltammograms; and b. Calibration curve. Differential pulse voltammetry for different CBZ concentration at bare CPE in 0.1 M PBS (pH 2.0). Accumulation time: 120 s, stirring rate: 370rmp*

Table 4.3. *Comparison of analytical parameters of the proposed method with previous studies*

Electrodes	Linear range (µM)	Detection limit (µM)	Literature review
1.SiO$_2$/MWCNT/GCE	0.2 - 4.0	0.056	[72]
2. ZnFe$_2$O$_4$/SWCNTS/GCE	0.5 – 100.0	0.09	[73]
3. NP-Cu/RGO/GCE	0.5 - 30	0.09	[74]

4.BDD	4.95 – 69.0	1.6	[75]
5. TCP/CPE	0.05 - 100	0.03	[76]
6. MWCNTs - BDDE	0.672 - 11.2	0.196	[77]
7. NPG/ GCE	5.0 – 150	0.02	[78]
8.CPE/FS@Ag	0.05 - 10.0	0.000940	This study

1. Silicon dioxide- multi walled carbon nanotubes- glassy carbon electrode
2. Spinel ferrite -single walled carbon nanotubes- glassy carbon electrode
3. Nanoporous copper – reduced graphene oxide - glassy carbon electrode
4. BDD diamond electrode
5. Tricresyl phosphate –carbon paste electrode
6. Boron doped diamond electrode- multiwalled carbon nanotubes
7. Nanoporous gold - glassy carbon electrode.
8. carbon paste electrode- fumed silica decorated silver nanoparticles

4.8. Analysis of CBZ in real sample

In the investigation of the validity and applicability of the proposed method, the CPE/FS@Ag was used to determine CBZ in the real samples including Porsuk river water, tomatoes and commercial fruit juice samples such as apple and orange under optimized conditions. The measurement was conducted with differential pulse voltammetry in 0.1 M phosphate buffer solution (pH = 2). The measurements showed that the samples did not contain CBZ. Therefore, the measurements were continued with CBZ-spiked samples. Differential pulse voltammograms of spiked river water sample in the presence of the increasing concentrations of CBZ shown in Figure 4.22. In Figure 4.22. CBZ- spiked river water showed a well-defined peak at the potential value of +1.0 V, which belongs to the oxidation of CBZ. The current values gradually increased with the addition of CBZ standards into the measurement medium. The recovery values of CBZ were calculated by using current values (Table 4.4). The results indicate that the prepared electrode allows the successful quantitative determination of trace levels of CBZ in the real sample. The experiments were also repeated with CBZ-spiked tomatoes (Fig.4.23) and (Fig.4.24), CBZ-spiked apple (Fig.4.25) and (Fig.4.26), and CBZ-spiked orange samples (Fig.4.27) and (Fig.4.28). The real samples were also analyzed by HPLC analysis and obtained results were compared with results obtained by DPV (Table 4.4). There was a good correlation between the results of both analyses. The HPLC has disadvantages include low sensitivity, take a long time in analysis, expensive because consuming solutions in the mobile phase and need a large volume of solutions, in addition, to require sample pretreatment steps like extraction. But DPV analysis has advantages such as low cost, simple and rapid analysis. The recovery values of the

concentration of CBZ in HPLC was determined in a range from 94.1 to 106.7% and range of 74.1 to 105.6% in DPV.

Table 4.4. *DPV and HPLC determination of CBZ in real samples containing Porsuk river water and commercial orange and apple juice samples and tomatoes sample*

DPV				HPLC		
Sample	Added (μM)	Found (μM)	Recovery (%)	Added (μM)	Found (μM)	Recovery (%)
River water sample	0.166	0.153	92.3%	-	-	-
Tomatoe sample	30	26.92	89.7%	30	28.25	94.1
	15	11.12	74.1%	15	15.6	104.0
Apple juice Sample	30	31.7	105.6%	30	30.2	100.6
	15	12.72	84.8%	15	14.6	97.3
Orange juice sample	30	25.65	85.5%	30	32.1	106.7
	15	12.81	85.4%	15	15.06	100.4

Figure 4.22. *Baseline -corrected DPV_S of CBZ spiked river water sample in the presence of increasing concentrations of CBZ. Accumulation time: 120 s, stirring Rate: 370 rpm*
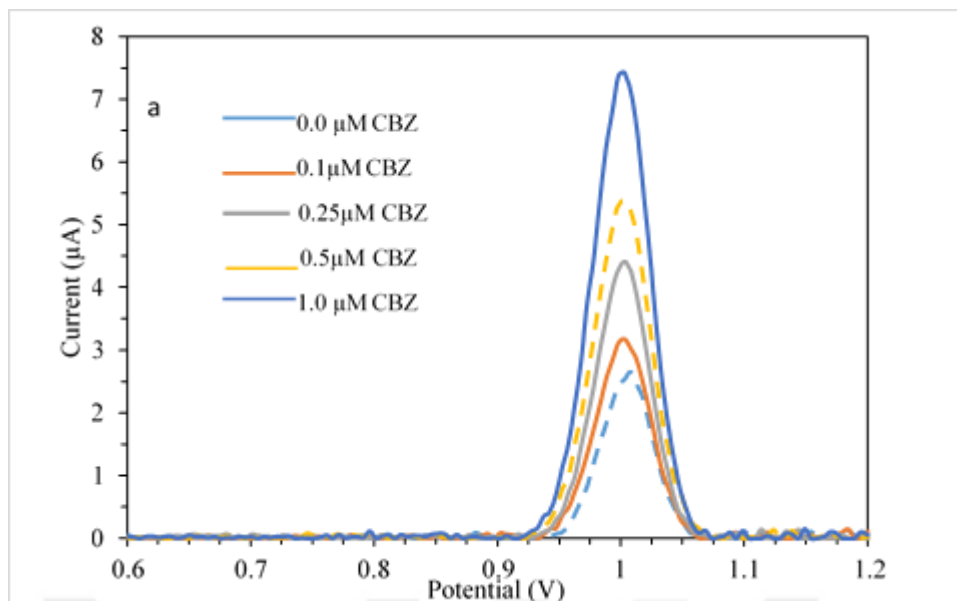
Figure 4.23. *Baseline -corrected DPVs of CBZ spiked (1) tomatoes sample in the presence of increasing concentrations of CBZ. Accumulation time: 120 s, stirring Rate: 370 rpm*
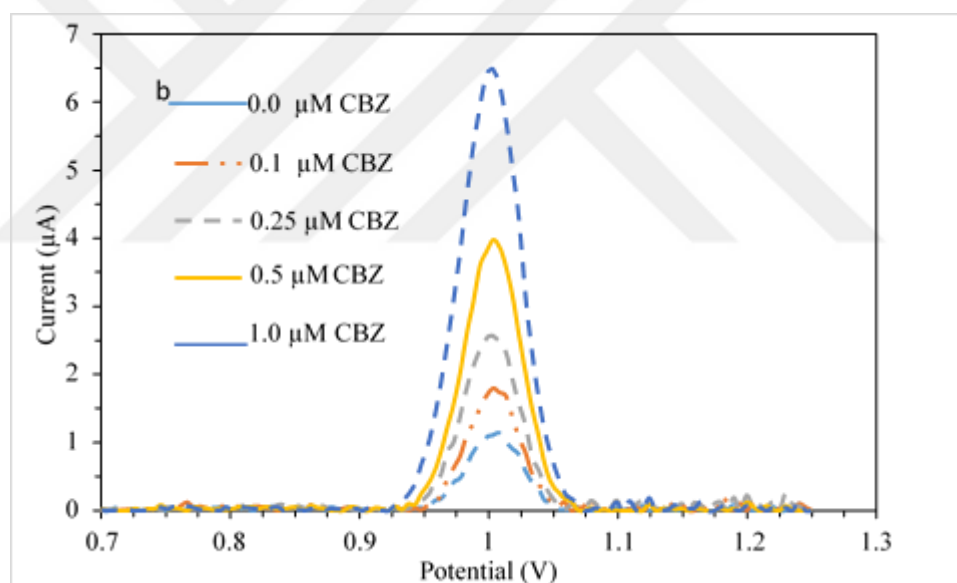
Figure 4.24. *Baseline -corrected DPVs of CBZ CBZ spiked (2) tomatoes sample in the presence of increasing concentrations of CBZ. Accumulation time: 120 s, stirring Rate: 370 rpm*
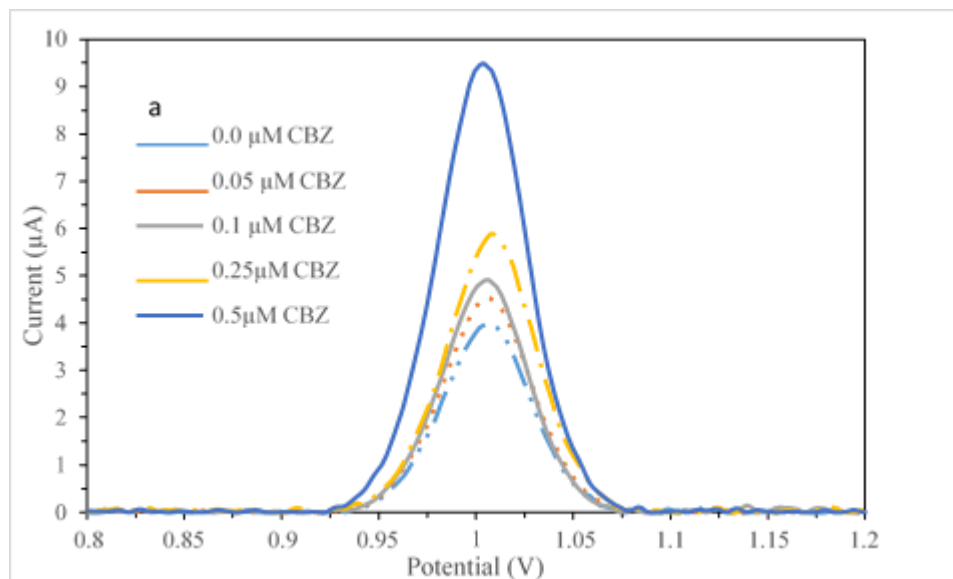
Figure 4.25. *Baseline -corrected DPV$_S$ of CBZ spiked (1) apple sample in the presence of increasing concentrations of CBZ. Accumulation time: 120 s, stirring Rate: 370 rpm*

Figure 4.26. *Baseline -corrected DPV$_S$ of CBZ spiked (2) apple sample in the presence of increasing concentrations of CBZ. Accumulation time: 120 s, stirring Rate: 370 rpm*
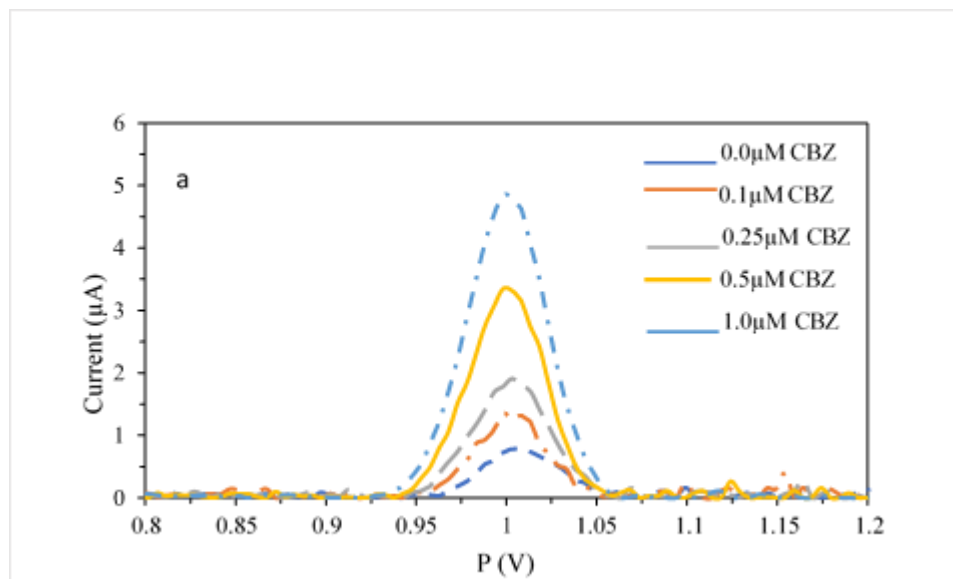
Figure 4.27. *Baseline -corrected DPV$_S$ of CBZ CBZ spiked (1) orange sample in the presence of increasing concentrations of CBZ. Accumulation time: 120 s, stirring Rate: 370 rpm.*
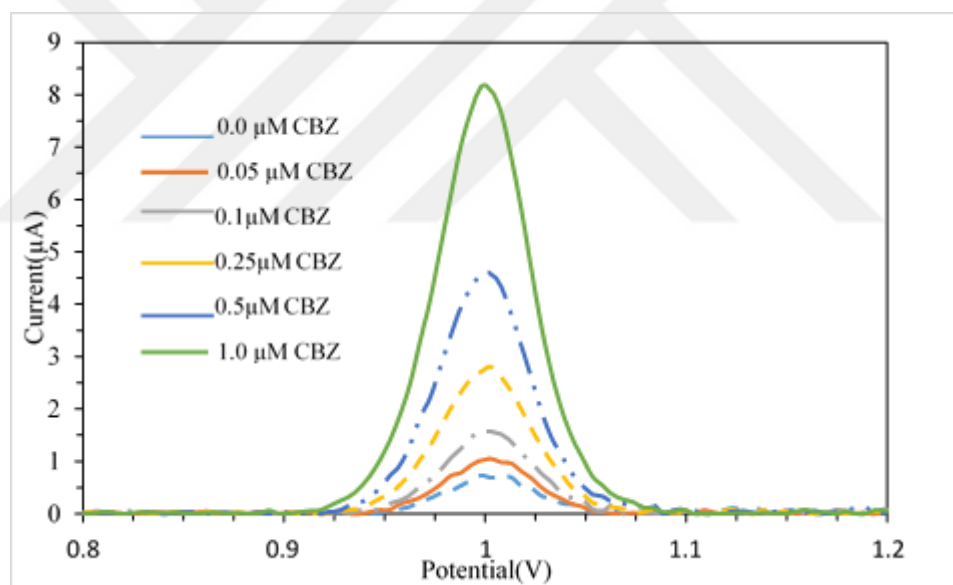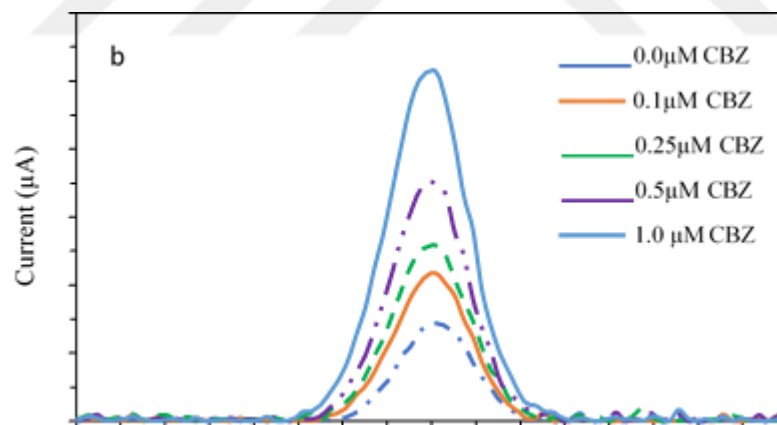

Figure 4.28. *Baseline -corrected DPV$_S$ of CBZ spiked (2) orange sample in the presence of increasing concentrations of CBZ. Accumulation time: 120 s, stirring Rate: 370 rpm*

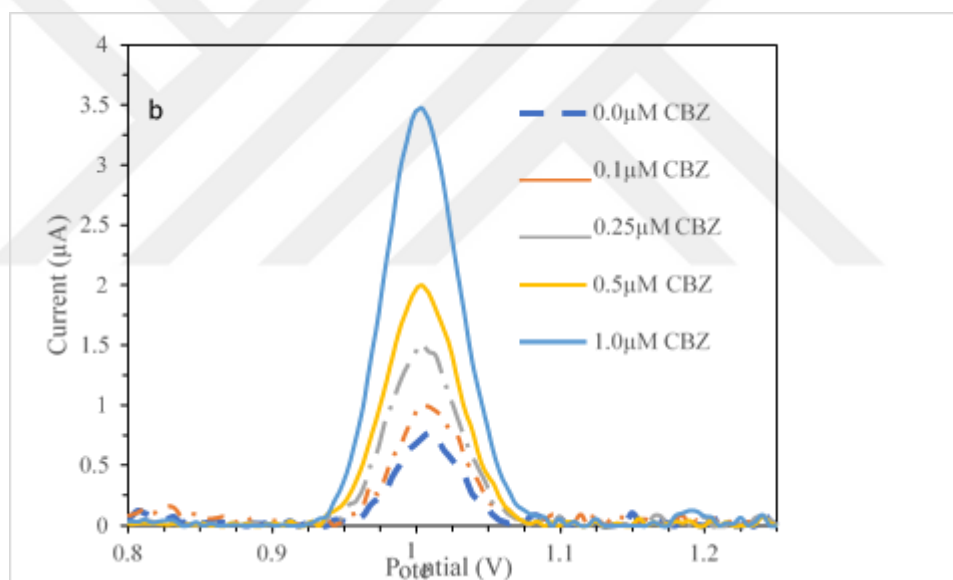## 5. CONCLUSION

Carbendazim is considered as a carcinogenic pesticide used in agriculture. Carbendazim can lead to serious health problems including hormonal and endocrine system disorders. The production and the using of carbendazim should be monitored. Trace detection of carbendazim is very important due to its high toxicity. In this study, we have developed an electrochemical sensor for the voltammetric determination of CBZ. A nanocomposite (FS@Ag) was fabricated and was used in the preparation of modified CPE. The synthesized nanocomposite increased the oxidation peak current of CBZ. The prepared electrode was electrochemically characterized by cyclic voltammetry by using $Fe(CN)_6^{3-/4-}$ and $Ru(NH_3)_6^{3+}$ redox couple. The results showed that FS@Ag has a great effect on the in electrochemical behavior of the CPE. Experimental characterizations of the prepared nanocomposite were obtained by using scanning electron microscopy, X-ray diffraction, and EDX analysis and mapping.

The experimental parameters were investigated and optimized, and a good result was achieved using 0.1 M PBS (pH 2.0), and accumulation time was 120s, and modifying agent percentage was optimized as 15%. The linear response of the oxidation peak current of CBZ was obtained in a wide concentration range of 0.05 μM – 3.0 μM. The limit of detection value and limit of quantification were investigated to be 0.000940 μM and 0.003134 μM respectively. The effects of the interfering substance on the voltammetric behavior of CBZ were investigated. The prepared modified electrode showed sensitive and accurate results in the determination of CBZ in real samples such as river water, tomatoe, apple, and orange juice. The procedure of the developed electrochemical sensor is also less costly, faster and simpler than HPLC. Moreover, the proposed electrochemical sensor showed good selectivity, high sensitivity, low detection limit and stable electrochemical performance, which provides a new approach for the potential research of pesticide toxicity and suggesting its potential utility in routine laboratory tests.

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