EGE UNIVERSITY

**GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCE**

PREPARATION OF THE SPME FIBERS BY ELECTROPOLYMERIZATION AND ITS APPLICATION IN VOLTAMMETRIC AND CHROMATOGRAPHIC DETERMINATION OF ENDOCRINE DISRUPTOR PESTICIDES

İREMAYDIN

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İren AYDIN tarafından Yüksek Lisans tezi olarak sunulan "***Preparation Of The SPME Fibers By Electropolymerization And Its Application In Voltammetric And Chromatographic Determination of Endocrine Disruptor Pesticides***" başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesinin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve **16.08.2015** tarihinde yapılan tez savunma sınavında aday oybirliği/oyçokluğu ile başarılı bulunmuştur.

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İmzası

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ÖZET

ELEKTROPOLIMERIZASYONLA SPME FIBER HAZIRLANMASI VE ENDOKRIN BOZUCU PESTISITLERIN VOLTAMMETRIK VE KROMATOGRAFIK ANALIZINE UYGULANMASI

AYDIN, İrem

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Tarımda koruyucu olarak kullanılan pestisitlerin gıdalardaki kalıntıları endokrin sistemini etkileyebilmektedir. Geniş spektrumlu bir fungisit olan Boscalid bezelye, çilek ve domates yetiştiriciliğinde yaygın olarak kullanılmaktadır. MRL değeri birçok gıda türü için 1 mg/kg olarak belirlenmiştir ve gıdalarda bu düzeyde analiz yapabilecek duyarlı ve seçimli yöntemlere gereksinim vardır.

Bu tez çalışmasında domates suyu ve salça örneklerinde Boscalid tayinine yönelik elektrokimyasala ve kromatografik bir yöntem geliştirilmesi amaçlanmıştır. Elektrokimyasal çalışmalar Boscalidin kobalt iyonları varlığında dolaylı olarak AdCSV ile nanomolar düzeyinin altında tayinini içermektedir. Mekanistik çalışmalar indirgenmenin katalitik niteliği ortaya konulmuştur.

Kromatografik çalışmalar ticari (PDMS-DVB) ve lab yapımı (PPy) katı faz mikroözütleme(SPME) fiberlerinin kullanımını içermektedir. Deneysel parametreler; pH, karıştırma hızı ve ekstraksiyon süresi domates suyu ortamında optimize edilmiş ve ppb düzeyinde kalibrasyon grafikleri çizilmiştir. Tüm sonuçlar göstermektedir ki Boscalid her iki yöntemle seçimli ve duyarlı olarak saptanabilmektedir ve yöntemler gerçek örneklere uyarlamıştır.

Anahtar Sözcükler: Boscalid, voltammetri, gaz kromatografi, katı faz mikro özütleme, domates suyu ve salça

ABSTRACT

PREPARATION OF THE SPME FIBERS BY ELECTROPOLYMERIZATION AND ITS APPLICATION IN VOLTAMMETRIC AND CHROMATOGRAPHIC DETERMINATION OF ENDOCRINE DISRUPTOR PESTICIDES

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The pesticides are used in agricultural production for food protection but, their residue in food alters the endocrine system. Boscalid is a broad spectrum fungicide for beans, berries and tomatoes. MRL values of Boscalid were established as 1 mg/kg for various types of food and therefore, sensitive and accurate analysis methods are required for BOS determination in food samples.

In this thesis, it was aimed to develop electrochemical and chromatographic methods for Boscalid determination in tomato juice and puree samples. Electrochemical studies include indirect determination of Boscalid in the presence of Co(II) ions by AdCSV in sub nanomolar levels. The catalytic character of the reduction process was established by mechanistic studies.

Chromatographic studies include the use of a commercial (PDMS-DVB) and a lab made (PPy) solid phase microextraction (SPME) fibers. Experimental parameters such as pH, stirring rate and extraction time was optimized in tomato juice sample medium. The calibration graphs were drawn in ppb ranges. Overall results have demonstrated that Boscalid can be determined by both methods sensitively and selectively and the methods developed were applied to the real samples.

Keywords: Boscalid, voltammetry, gas chromatography, solid phase micro extraction, tomato juice and puree

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ABREVIATIONS

EDC : Endocrine Disrupting Chemicals.

PBS : Polychlorinated Biphenyls

LLE : Liquid-Liquid Extraction

SPE :Solid Phase Extraction

GC : Gas Chromatography

LC : Liquid Chromatography

LC–MS/MS : Liquid Chromatography Tandem Mass Spectrometry

dSPE : dispersive solid phase extraction

SPME : Solid Phase Micro Extraction

HS : Headspace

DI HS : Direct İmmersion Headspace

MS : Mass Detector

FID : Flame İonization Detector

ECD : Electron Capture Detector

MSD : Mass Spectrometry Detector

BOS :Boscalid

PLE : Pencil Lead Electrode

GCE : Glassy Carbon Electrode

SEM : Scanning electron microscopic

PTh : Polythiophene

PPy : Polypyrrole

PA : Polyacrylate

PDMS : Polydimethyl Siloxane

CV Cyclic Voltammetry

DPV : Differential Pulse Technique

ASV : Anodic Stripping Voltammetry

AdCSV : Adsorptive Cathodic Stripping Voltammetry

PAH : Polycyclic Aromatic Hydrocarbons

1. INTRODUCTION

1.1 Endocrine Disrupter Chemicals and Their Health Issue

The endocrine system acts as a communication tool for the human body. Any substance that changes the function of this system is termed an endocrine disruptor (Darbre, 2015a). Endocrine Disrupting Chemicals (EDCs) interfere with normal hormone activity by mimicking the sex steroid hormones by binding to their natural receptors, by varying the synthesis and deterioration of natural hormones or by changing the production and functioning of hormone receptors.

EDCs may occur naturally, but the majority is artificial compounds that have been released into the environment. EDCs have been found in many everyday products such as foodproducts (soybeans, flax), pesticides (DDT etc.), household chemicals(detergents including nonylphenol and octylphenol), personal care products (cosmetics), plastic bottles (bisphenol A, phthalates), and industrial chemicals including (polychlorinated biphenyls (PCBs), dioxin and benzo(a)pyrene) and pharmaceuticals (birth control pills) (Cooke, 2015). These compounds may get into foods by environmental pollutants, food-packaging materials, and natural plant components. Therefore, maximum residue limits (MRL) were set by authorities for their presence in food and environment.

EDCs are suspected to be relation with altered reproductive function, increased incidence of breast cancer and neurodevelopmental delays in children along with the changes in immune function (Darbre, 2015b). The compounds that mimic estrogens are termed environmental estrogens. Interestingly, their chemical structures are very different and therefore, it is impossible to predict their effect as an endocrine disruptor by looking at its chemical structure. A pesticide which is used for food protection can be an EDC.

In general terms, a pesticide is a substance intended to control, destroy or repel a pest. Pesticides can be classified by its physical state, chemical structure such as organochlorine, organophosphate, carbamate and synthetic pyrethroid pesticides. They can also be classified according to the target organism such as fungicides, insecticides, herbicides;etc. Depending on the exposure, pesticides can have acute or chronic health effects on human. The toxic substance either accumulates in body tissues or causes minor irreversible damage.

Since agricultural chemicals are widely used in food production, people are exposed to pesticide residues through their diets. In fact, children are particularly subjected to their adverse effects, including neurodevelopmental delays. Therefore, their determination in food samples is important in terms of controlling the dosages used.

1.2Analytical Methods Developed for Pesticide Residues

The development of reliable screening and quantitating methods is alwaysessential for theachievement of high-quality results in monitoring the pesticide residues in food samples. Due to the complex matrixes of food samples, the sample preparation method is equally important as the quantitation method. Early studies employ classical sample preparation techniques such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) prior to the analysis. Following the extraction step, the compounds are separated either on gas chromatography (GC) or liquid chromatography (LC), and then, quantified using various kinds of detectors depending on the type of the molecules.

Nowadays, in addition to the sensitivity and selectivity of the method, other prerequisites such as using less volume of sample, minimizing the solvent consumption and the usage of greener and cost-effective techniques are also being searched (Pizzutti et al., 2009). Multi-residue methodsfor pesticides in fruits and vegetables have been enhanced recently. Generally, QuEChERS (quick, easy, cheap, effective, rugged and safe) is the method of choice in sample preparation, followed by liquid chromatography tandem mass spectrometry (LC–MS/MS).This technique is a practical alternative to traditional LLE and SPE techniques where large volume of solvent is consumed.

The process mainly involves two stepsand in the first step the homogenized fruit or vegetable sample is extracted by using an organic solvent and salt solution. By this means, sugar, lipid, protein, pigment etc. contents of the matrixare removed.Then, the supernatant is cleaned by using a dispersive solid phase extraction (dSPE) technique. Original method has been modified to improve its performances and alternative approaches were compared by Lehotay (2010). Since the first work, new sorbents have also been proposed andtested for the d-SPE step and it was revealed that the method is very flexible and reliable results can be obtained for numerous pesticides when different types ofsolvents, salts and sorbentsare used in both steps.

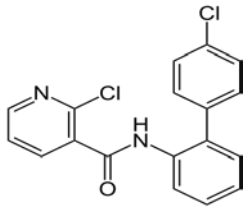
As an alternative approach, solid-phase microextraction (SPME) technique can be used for residue analysis. Since it was introduced by Pawliszyn and co-workers in 1989, SPME has become very popular (Belardi and Pawliszyn, 1989, Pawliszyn and Arthur, 1990). The technique employs a reusable silica fiber which was coated with a polymeric film and where the analytes are extracted into according to their polarity in either in the solution by direct immersion (DI-SPME) or in the head space (HS-SPME) mode (Pawliszyn, 1997). Then, the fiber is injected to the GC where the adsorbed compounds are thermally desorbed and entered the column for effective separation. Several detectors are used for subsequent quantification including flame ionization detector (FID), electron capture detector (ECD) or mass spectrometry detector (MSD). Recent trend in SPME applications is to fabricate the fiber by using electropolymerization technique and modification with several nanoparticles or composite materials.

In this thesis it was aimed to develop a chromatographic and voltammetric method for the determination of pesticide residues in food samples. A relatively new fungicide, Boscalid, which is widely used for tomato cultivation was chosen as the test material.

1.3. Chemical Properties of Boscalid and Analysis Methods

Boscalid (2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide) is a white colored crystalline powder and belongs to the chemical class of carboxamides. Boscalid is a broad spectrum fungicide which is employed to fight against highly destructive plant pathogens. Since it was first registered in 2003, it is widely used fungicide for beans, berries, tomatoes, canola, carrots, grapes, lettuce, peanuts, pistachios, potatoes, strawberries etc.(EPA, 2003). Then after, it was included into the EU positive list of authorized agrochemicals (EC Regulation, 2008.).Table 1.1 summarizes the physicochemical properties of Boscalid.

Table 1.1 The structure and physicochemical properties of Boscalid (González-Rodríguez et al., 2009)

 2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide	**MW:**343.2 g/mol **BP:** decomposes **VP:** $8.2x10^{-8}$ mm Hg @20^{o}C **S:** 4.64 mg/L **H:** 5.18×10^{-8} Pa·m^3/mol **log K_{ow}**: 2.96

Although boscalid is classified as suggestive evidence of carcinogenicity, it is not sufficient to assess human carcinogenic potential. This fungicide is persistent since it has low mobility in soil; however, it may move to surface water. Boscalid is essentially nontoxic to terrestrial animals and is moderately toxic to aquatic animals on an acute exposure basis but, its bioaccumulation in fishwas found not significant. MRL values of Boscalid were established as 1 mg/kg for various types of food (EFSA, 2010). Therefore, sensitive and accurate analysis methods are required for BOS determination in food samples. However, there are a few studies on its determination in food matrixes.

Boscalid determination in a variety of food samples has been carried out gas chromatograph coupled with several detectors orby liquid chromatography with mass spectrometry. Table 1.2 summarizes the analytical characteristics of the methods developed for its determination in several types of food.

Table 1.2 Chromatographic methods developed for Boscalid analysis in food samples*

Food matrix	**Extraction**	**Detection**	**LOD**	**LOQ**	**Recovery**	**Reference**
Cucumber	LLE (MeOH/H_2O/HCl/ n-hexane)	GC-µECD	0.005µg/mL	0.01 µg/mL	86 -104%	Chen, 2007
Soya Grain	LLE (Acetone and ACN)	LC–MS/MS	0.1 ng/mL	10 µg/kg	70 -120%	Pizzutti, 2009
Vineyard	LLE (Acetone /MeOH) → SPE	LC–MS/MS			87-89%	Gabriolotto, 2009
Grapes and Wines	LLE→ SPE PTVInj.	GC-ITMS		2 µg/kg	76-147%	González-Rodríguez, 2009
Blueberries	DI-SPME (PDMS)	GC-µECD	1.33 µg/kg	4.42 µg/kg	104 %	Munitz, 2013
Honeybees	QuEChERS	UHPLC-QToF UHPLC-QqQ		0.1 µg/kg		Jabot, 2016

*µECD: Micro electron capture detector, PTV inj: Programmable thermal vaporization injection, GC-ITMS: Gas chromatography ion trap mass spectrometry, UHPLC-QToF: Ultra high performance liquid chromatography- quadrupole time of flight, UHPLC-QqQ: Ultra high performance liquid chromatography- triple quadrupole

According to the table given above, Boscalid can be determined in several matrixes sensitively by applying different procedures and instrumental approaches. Beside chromatographic techniques there are no other analysis methods developed for Boscalid probably due to the compound is not electroactive.

1.4. The Aim of the Thesis

In this thesis, considering the structure of Boscalid which is a pyridine carboxamide derivative, an alternative approach was tried and literature survey has revealed that pyridine carboxamide complexes with Co(III) ions and become an electroactive complex (Meghdadi, 2008).

In a recent study, the structural and magnetic properties of Co (II) complexes with pyridine carboxamide examined and intermolecular hydrogen bonds in the crystal structures were discussed (Dojer et al., 2014). In this thesis, considering the molecular similarities, Co(II) complexes of boscalid and their possible use in indirect electrochemical determination of the fungicide was searched for the first time. The studies were carried out with glassy carbon electrode (GCE) and pencil lead electrode (PLE) as a cheap alternative. The latter electrode can also be treated in harsher conditions to modify the electrode surface as given in detail (Section 1.8). Initial part of the thesis is dedicated to the chemical treatment of PLEs in a microwave oven to see the performances of the electrode. The change in the surface morphology was revealed by Scanning electron microscopic (SEM) measurements and their voltammetric behavior was also investigated.

In conjunction with the electrochemical studies, the thesis also includesgas chromatographic analysis of Boscalid following the enrichment on an SPME fiber. Laboratory made SPME fibers along with the commercial ones were tested to evaluate their performances. Experimental conditions were optimized and the developed methods were applied to the real samples. Tomato juices and tomato puree which is the main gradient of Turkish cooking was chosen as the test materials. The theoretical basis of the techniques used in Boscalid determination is given in the following chapters.

1.5. Theoretical Aspects of the Chromatographic Methods

Chromatography is a physical separation method where the components are distributed between two phases, one of which is stationary while the mobile phase flows in a definite direction (Poole, 2003). In gas chromatography (GC), as the name implies, the mobile phase is a carrier gas. Generally an inert gas such as helium or nitrogen is employed for this purpose according to the detector. The stationary phase is a thin layer of liquid or polymer on an inert solid support inside a column.

The compounds in gaseous phase interact with inner walls of the column and each compound elutes at a certain time known as the retention time (tr). This can be used for the identification of the compound whereas the quantitation is made by comparing the peak area of each compound with those of standard signal. Modern instruments include an automatic injector placed in an enclosure that is thermostatically controlled. The injector can be a simple valve or can be an automatic injector that is microprocessor controlled.

Most samples are injected as liquids but volatile compounds can be injected by head-space technique. Splitless injection describe the whole sample injected will be transferred into the column. If the column will not tolerate high loads of sample, split injections are made where only a partial amount of sample is transferred to the column.

The column is placed in an oven to control the column temperature which is raised in a predetermined pathway to elute the more retained peaks in a reasonable time. Finally, the detector is situated in its own oven to monitor physicochemical properties of the eluted peaks. There is a wide range of detectors available which are classified as destructive and non-destructive detectors. In his thesis electron capture (ECD) and mass spectrometry detector (MS) detectors were used.

In ECD, a^{63}Ni is used as an electron source in a detector cell and a constant electrode potential is employed to collect all the electrons giving a constant current. The compounds eluted from the column capture these electrons and the current signal decreases according to the amount of the compound.

The most widespread type of MS coupled with a GC is the quadrupole including four parallel metal rods where a radio frequency (RF) voltage with a DC offset voltage is applied between one pair of rods and the other. Ions travel between the rods but, only ions of a certain mass-to-charge ratio (m/z)will enters the detector. Other common detector is the ion trap mass spectrometer (ITMS). Other detectors may be encountered such as time of flight (TOF) and tandem quadrupoles (MS-MS).

1.6. Modern Sample Preparation Techniques

Even though advanced instrumentation is used,the accuracy and selectivity of the method developed for complex matrixes usually depend on the techniques used in sample preparation step (Mitra, 2003). This step is a requirementfor analyte purification as well as the sample clean-up prior to the analysis.

Traditional sample preparation methods such as the LLE,consumelarge amount of toxic organic solvents and laborousprocedures result in loss of analytes. Therefore, more effective and greener techniques are being searched.

Nowadays, many traditional techniques are replaced by SPE where a cartridge is used as an adsorbent for the analytes with matching polarity. For avoiding solvent consumption, modern microextraction techniques are being intensely studied.

Solid phase microextraction (SPME) was introduced in 1989 (Pawliszyn, 1989) and made commercially available in 1993. A polymeric thin film coated fiber is exposed to the sample where the target analytes are extracted on into thisfilm. In-tube version of SPME includes the pumped liquid or gaseous sample. Analytes trapped on the fiber are rapidly delivered to a capillary GC column by thermal desorption. The amount of solute extracted in the film is proportional to the solution concentration and therefore, analytical quantitation can be made.

In comparison to the other microextraction techniques, SPME is fast, simple and greener technique since no solvent is consumed during the process. The technique is very sensitive and cleaner extracts than LLE or SPE can be obtained.

SPME sampling of organic moleculescan be made in two modes; direct and headspace sampling. In direct immersion SPME, the fiber is inserted into the sample matrix under optimal conditions; stirring rate, sample volume, pH etc. In the latter mode, the fiber is placed in the headspace of the sample for volatile analytes. Equilibrium is attained more rapidly in this mode since the analytes can diffuse more rapidly to the coating on the fiber.

In addition to the commercial SPME fibers, recent trend is to fabricate the fibers with desired polarity and conducting polymers are drawing attention for this purpose. Electrochemical polymerization is the method of choice since it is possible to control the thickness of the film by changing the deposition conditions.

Previously in this laboratory, electrochemical polymerization technique was employed to produce polypyrrole (PPy) and polythiophene (PTh) coated fibers for pesticide analysis (Korba et al., 2013, Pelit and Dizdaş, 2013). A recent study carried out has included the development of a novel PTh-ionic liquid modified clay film which gives the most sensitive results for certain pesticides (Pelit et al., 2015). The fibers have proven to give comparable results with the commercial fibers such as polyacrylate (PA) and polydimethylsiloxane (PDMS).

1.7 Theoretical Aspects of Voltammetry

Electroanalytical techniques are dealing with the measurements of electrical quantities, such as current, potential, or charge and their relationship to chemical parameters. Voltammetry is the method group in which the current passed through the electrodesinrelation with the reactions at the electrode surface is monitored against the applied potential according to the reference electrode. This method group has a wide range of applications from environmental monitoring to biomedical analysis (Wang, 2006).

Cyclic voltammetry (CV) is the most widely used technique to obtain qualitative information about electrochemical reactions. CV consists of scanning linearly the potential of a working electrode in a quiescent solution, using a triangular potential waveform while the current resulting from the applied potential is monitored.

For quantitative analysis, other voltammetric techniques developed are used. In direct current voltammetry, the residual current arising from the capacitive current limits the sensitivity and this current can be eliminated by time dependent current sampling techniques. Pulse voltammetric techniques were developed by to improve the performance and lower the detection limits for the electroactive species. The current is measured at a time when the difference between the Faradaic current and the capacitive current is large. In **differential pulse voltammetry** (DPV) the current is measured twice; just before the pulse and near the end of pulse so the difference gives the Faradaic current free from the capacitive component. The current difference is plotted against the potential applied and a peak is obtained where the top of the peak corresponds to E½ and the height of the peak is proportional to the concentration.

The sensitivity of the technique can be increased up to nanomolar or sub ppb by applying stripping methods. In **Anodic Stripping Voltammetry** (ASV) four to six metal ions can be preconcentrated on the electrode surface by applying sufficiently negative potentials in metallic forms. Then, the potential scan is reversed and the oxidation peaks are recorded. In **Adsorptive Cathodic Stripping Voltammetry** (AdCSV), a complex which is adsorbed on the electrode surface at rather positive potentials is reduced by scanning the potential in negative direction. This method gives more sensitive results and sub ppb levels can be detected by this means. Most sensitive results are obtained with catalytic methods.

In voltammetry three-electrode system is used where the potential of working electrode is changed against a reference electrode, usually Ag/AgCl, which has a constant potential in the range studied. An auxiliary electrode, usually platinum in spiral shape, provides the current measurement. These three electrodes are immersed in a cell in which an electrolyte solution is placed.

Working electrodes can be a metallic disk such as gold or platinum electrode, a mercury dropping electrode or more commonly encountered carbon based electrodes. Glassy carbon electrode is the popular electrode since it has a high electrical conductivity and provides a flat surface available for modification. Recent advances in nanotechnology have been utilized to develop catalytic surfaces by modifying the electrode with nanoparticles and other nanomaterials (Özdokur et al., 2016).

Another carbon based electrode is **pencil lead electrode** (PLE). Since it was first introduced by Bond et al., PLE is becoming more popular since it is a very cost effective alternative to expensive electrodes yet providing sensitive results in accordance with the ease of modification of its surfaces (Bond et al., 1997). Table 1.3 summarizes the methods developed by using this electrode for a variety of analytes including metallic ions, pesticides, pharmaceuticals, etc in sub micromolar levels.

Table 1.3 Voltammetric methods developed for various analytes by using PLE

Electrode	**Method**	**Analyte**	**References**
PLE-MFE	ASV	Cd, Pb	Bond, 1997
PLE-BiFE	ASV	Zn, Cd, Pb, Cu	Pierini, 2016
MIP-PPy-PLE	DPV, CV	Phenothiazine	Nezhadali, 2015
Cu-np- MWCNT-PLE	CV, EIS, Amperometry	Hydrazine	Heydari, 2016
PLE	DPV	Acyclovir	Dilgin,2016
MIP-PPy-PLE	EIS, CV	Chlorpyrifos	Uygun, 2013

*MFE: Merury film electrode, BiFE: Bismuth film electrode, MIP-PPy: molecularly imprinted poypyrrole, Cu-np-MWCNT: copper nanoparticle modified multiwall carbon nanotube, EIS: electrochemical impedance spectrometry.

1.8. The Use of PLE in Chromatographic Applications

Pencil lead electrodes also draw attention in chromatographic applications as an adsorbent for volatile or semi-volatile organics. Pencil lead material is usually modified with a chemical accompanied by a thermal treatment. Djozan et al have investigated the performance of PLE as a new SPME fiber material (Djozan and Assadi, 2004).The fibers have been prepared by thermally treating at high temperatures in the stream of an inert gas, in the presence of conc. H_2SO_4, fusing with NaOH at 400^oC, and activation at 600^oC with watervapor. Then, the fibers were used for extraction of trace amounts of polycyclic aromatic hydrocarbons (PAH) from aqueous samples by GC–FID. It was revealed that the fiber treated with water vapor has given the best results.

In a later study, the modified pencil lead SPME was utilized for the determination ofmethamphetamine (MAMP) from aqueous solutionswithout chemical derivatization prior to GC-MS analysis (Djozan and Baheri, 2010). Experimental parameters such as pH, extraction time and temperatureand salt amount were optimized and the performance of the fiber was comparable to the most effective commercial fiber; polyacrylate (PA).

Inspiring by the fact that graphite is both the main component of pencil lead and also the precursor of graphene; Liu et al. have developed a chemical exfoliation strategy to fabricate graphene modified SPME fiber using commercial pencil lead (Liu et al., 2014). The proposed method combines the low cost of pencil lead with the unique extraction capability of graphene. The PLE is placed in an oven where it is treated with strong acid (H_2SO_4) and oxidizing reagent ($KMnO_4$) at 100◦C for 2 h and then, the fibers were washed sequentially with H_2O_2 and HCl for reducing and then removing the Mn(II) species and then, with hydrazine solution to reduce the graphene pieces. Following the washing step, pencil leads were air dried, cut into pieces andstuck to a holder for SPME. The performance of the fiber was evaluated by detecting bisphenol analogs in direct SPME mode. The method developed was successfully applied in the analysis of tap water and waste water with high spike recoveries.

Similar procedure was applied in a microwave oven for exfoliation and reduction of graphite oxide powders and it was shown that reduced graphite oxide materials could be readily obtained ina minute (Zhu et al., 2010). This exfoliated graphite oxide has displayed a high specific capacitance values indicating the potential to be used in an ultra-capacitor cell.

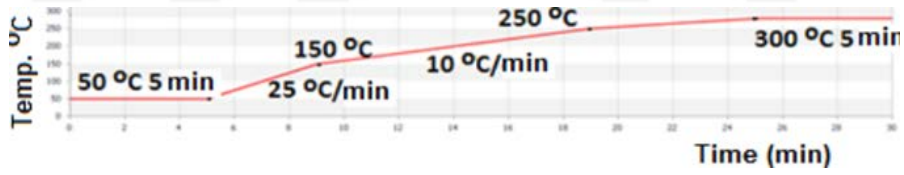
Therefore, in the light of the literature, the use of PLE as an SPME fiber after treatment with strong chemicals in a microwave oven was intended to be studied. The performance of the thermally and chemically treated electrodes were also tested voltammetrically in the presence of $Fe(CN)_6^{4-}$ ions.

2. EXPERIMENTAL

2.1. Instrumentation and Procedures

Chromatographic Boscalid determination was maintained by using an Agilent 7890B gas chromatograph equipped with an Agilent 5977E mass detector (GC-MS) equipped with PAL RSI 85 SPME autosampler. Operational conditions and temperature programming of the instrument are given in Table 2.1.

Table 2.1 Operational conditions of GCMS system used in Bos determination

Column	DB5 (30mx0.25mmx0.25 µm)		
Inlet temp.	250°C	**Injection**	Splitless (1 µL)
Quadrupol	150°C	**Septum purge flow**	15 mL/min
MS source	250°C	**Carrier gas**	He (1 mL/min)
Transfer line	290°C	**Solvent delay**	7 min
Temperature programming			
			

SPME fiber coating was carried out using a Metrohm Autolab PGStat 101voltammetric analyzer with a three-electrode system including stainless steel wire (316 type, i.d. 0.3 mm) as the working electrode, a platinum wire as the auxiliary electrode and a Ag/AgCl electrode as the reference electrode. Figure 2.1 schematically shows the system used for electropolymerization.

The SPME holder for manual sampling was obtained from Supelco (Bellefonte, PA, USA). Commercial SPME fiber PDMS/DVB was supplied from Supelco and conditioned in an oven before use.IKA-RCT (Germany) heater/magnetic stirrer and Nüve BM402 cryostat was used to maintain fixed temperature for head space during adsorption studies.

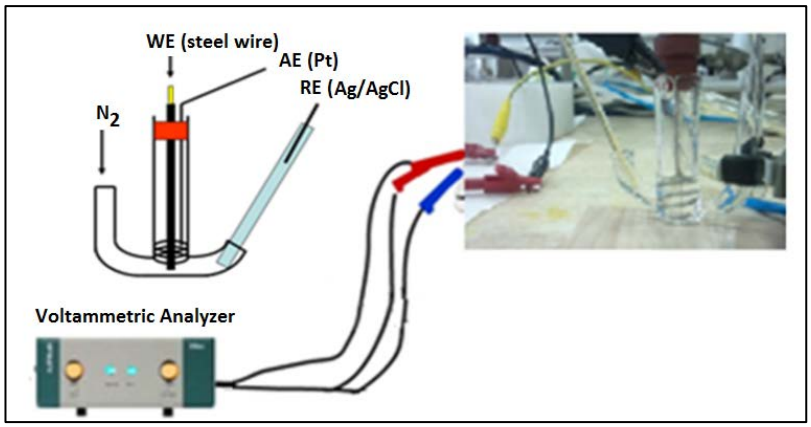


Figure 2.1 Voltammetric system used for preparation of PPy-SPME fiber.

The pH adjustments were made by using Orion 4 Star pH-meter. Pencil lead electrodes were chemically treated in a Mars 5 microwave oven. SEM measurements were accomplished by using Philips XL30 SFEG. FTIR measurements were made with Perkin-Elmer Pyris FTIR spectrophotometer.

2.2 Reagents

All the reagents were of analytical reagent grade. Aqueous solutions were prepared with ultrapure water (18.2 MΩ/cm) from a Milli Pore Milli-Q Gradient water purification system.

Britton Robinson buffer solutions were used to maintain a wide range of pH and it was prepared by mixing 0.08 mol phosphoric, boric and acetic acids with dropwiseaddition of 2.0 mol NaOH to obtain the desired pHs. Standard solutions of the Boscalid were purchased from Dr. Ehrenstarfer-Schafers (Augsburg, Germany) and stored at 4°C prior to use and the stock solutions were prepared in acetone. Tomato juice and puree samples were obtained from local market.

Voltammetric behavior of PLE was studied in pH 3.0 Chloroacetic acid(CAA) solutions. Pencil leads were obtained from local market and their serial numbers are given below;

HB: 0.3mm 05/14 Series HB TombowPLE
2H: 0.3mm 06/04 Series2H TombowPLE
UPH: 0.3mm Ultra-Polymer H SeriesTombowPLE
UPHB: 0.3mm Ultra-Polymer HB Series TombowPLE

2.3. Procedures

2.3.1. Procedures for Chromatographic Analysis

Fiber coating: First of all, the stainless steel wire was cleaned ultrasonically ina test tube containing acetone for 15 minand rinsed with distilled water. Aqueous solution of 0.1 M pyrrole including 7.0×10^{-3}M SDS was placed in the voltammetric cell and after purging the solution with nitrogen gas, the potential was cycled in a range of 0.5–1.2 V with a scan rate of 20 mV/s for subsequent 10 cycles as described elsewhere (Korba et al., 2013).

After the electropolymerization step, the PPy-DS fiber was washed with methanol, acetone, and water, respectively. The fiber was dried under nitrogen gas and then, heated at 100^{o}C for 20 min in an oven before connecting to the SPME holder. Before the use, the fiber was conditioned at 200^{o}C in a GC injection port under helium gas for about an hour until a clear blank was obtained. Characterization of the fiber surfaces were already made in a previous study (Korba et al., 2013).

Direct Immersion SPME Procedure: The performance of the lab-made fiber along with the commercial PDMS-DVB fiber was tested by immersing the SPME fibers directly into the tomato juice sample (Figure 2.2). Experimental conditions such as the pH, stirring rate and adsorption time were optimized in the sample media. For this purpose, tomato juice samples were filtered through 0.45 µm Teflon filter and 25 mL of this sample was diluted with 25 mL of ultrapure water. The mixture was centrifuged at 4000 rpm for 20 min. The pH of the supernatant solution was adjusted to 4.0 and 10 mL of this mixture was placed in a vial where the fiber is directly immersed in ambient temperature (35^{o}C).

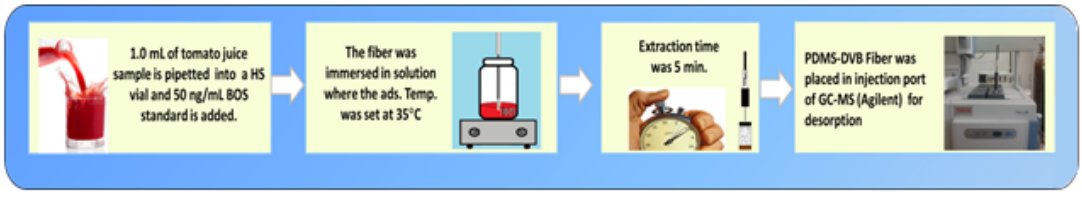


Figure 2.2 The direct immersion SPME-GC-MS procedure used for Boscalid anlaysis

Adsorption time was first selected as 5 min for optimization studies and the stirring rate was changed between 250-400 rpm. After 5 min, the fiber was redrawn and injected into the GC-MS system for subsequent thermal desorption. The chromatograms were recorded according to the conditions given above.

2.3.2. Procedures for Voltammetry

Exfoliation of PLE: PLEs were trimmed with a razor and ultrasonicated for 10 min. Then, they were immersed in a Teflon cell of the microwave oven and 5 mL of 1:1 H_2SO_4 solution and 0.5 g $KMnO_4$ is added (Figure 2.3).Pressure was set to 600 psi and the temperature was risen to 800^oC in 25 min. Then, the Teflon sample container is allowed to cool down and then, treated PLEs are washed with ultrapure water for several times. The electrodes were then washed with H_2O_2 (30%) and 0.1M HCl mixture for 15 min to remove any impurities. Following this step, the electrode surface was treated with a reducing agent such as hydrazine or sodium borohydride or simply rinsed with ultrapure water as described in Fig. 2.2. Then the electrodes were placed in an electrochemical cell and0.01 M K_4FeCN_6 solutions prepared in pH 3.0 CAA solution were added. Cyclic voltammograms were recorded in the potential range of -0.2 – 0.8 V at a scan rate of 100 mV/s.

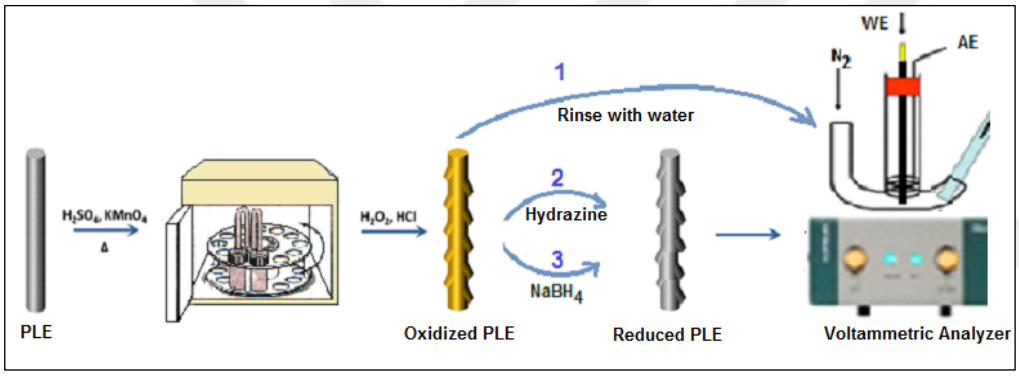


Figure 2.3 Schematic presentation of exfoliation of PLE in microwave oven.

Voltammetric Boscalid Analysis: Before voltammetric measurements, the PLEs were conditioned in pH 7.0 BR buffer solution by applying 0.9 V for 120 s. Then, the electrode was immersed in 2 mL of pH:2.0 BR buffer solutions in the electrochemical cell. After recording the background current, the cell content was spiked with Co(II) ions to be $5x10^{-6}$ M. Then, Boscalid standard was added and deposition was affected 0.2 V for 120 s. The cat-AdCSV peaks were recorded at a scan rate of 1.0 V/s in negative direction.

In sample application step, 5.0 mL tomato juice samples were mixed with 17.5 mL pH 2.0 BR buffer solution and 2.5 mL of acetone is added then, the volume is made 25 mL. The pH is controlled and HCl is added if necessary to maintain the pH. This mixture is mixed for 2 min and then, filtered through a 0.45 µm membrane filter. Similar procedure was applied for tomato puree samples.

3. RESULTS AND DISCUSSION

The main objective of this thesis was to develop sensitive and selective electrochemical and chromatographic methods for Boscalid determination in tomato juice and puree samples. First part of this section is dedicated to the electrochemical studies.

3.1. Electrochemical Studies

This section includes the results of thermally treated PLEs and Adsorptive cathodic stripping voltammetric determination of Boscalid.

3.1.1. Voltammetric Behaviousof Thermally Treated PLEs

Initial studies were conducted to see the performances of thermally treated PLEs. For this purpose, irst of all untreated PLEs were placed in an electrochemical cell containing 0.01 M K_4FeCN_6 solution prepared in pH 3.0 CAA buffer. Figure 3.1 shows the cyclic voltammograms were recorded in the potential range of -0.2 – 0.8 V at a scan rate of 100 mV/s.

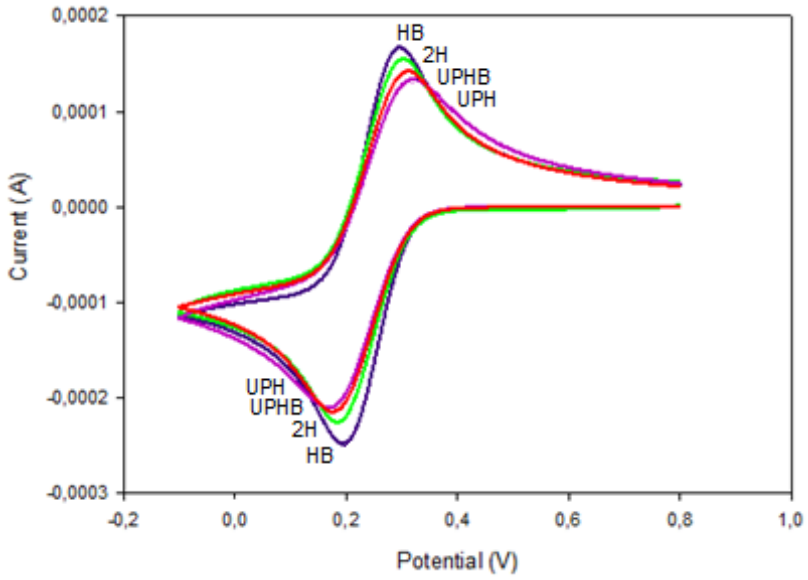


Figure 3.1 Cyclic voltammograms of untreated PLEs in 0.01 M $K_4Fe(CN)_6$in pH 3.0 CAA

As shown in Fig.3.1, reversible peaks were obtained for ferri-ferro redox couple but, different peak heights were obtained for different brand of PLE. The peak characteristics were summarized in Table 3.1.

Table 3.1Peak characteristics of different brand of untreated PLEs

PLEtype	E_p^a (V)	I_p^a(µA)	E_p^c (V)	I_p^c(µA)
HB	0,29	204	0,196	198
2H	0,29	182	0.190	164
UPHB	0,30	168	0,18	154
UPH	0,30	152	0,18	146

Thermal treatment of PLEs following by a washing step withH_2O_2 (30%)and 0.1M HCl mixture, the electrodes were treated with 10 mL of 5% hydrazine solution for an hour. In another experiment, the electrodes were treated with 1.0 g of $NaBH_4$ dissolved in 2 mL of water for an hour. Then, as an alternative to reducing agent, the electrodes were simply rinsed with ultrapure water Figure 3.2 shows the cyclic voltammograms obtained for all kind of treated electrodes as background current. The performance of the electrode in the presence of 0.01 M $Fe(CN)_6^{4-}$ can be seen in Figure 3.3 and overall results are summarized in Table 3.2.

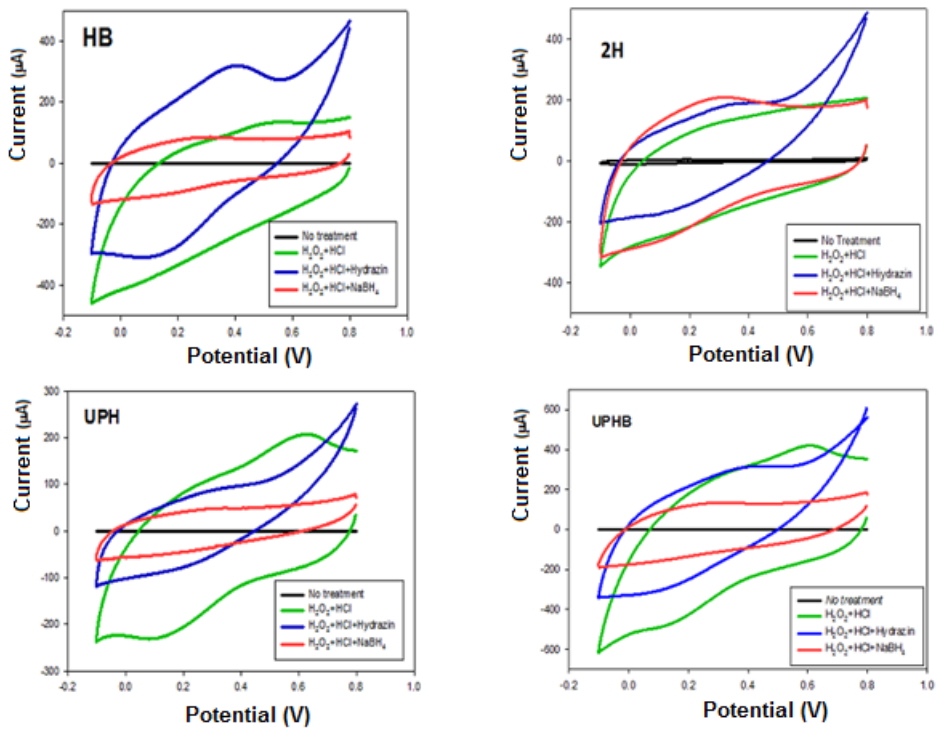


Figure 3.2 Cyclic voltammograms obtained for treated PLEs in comparison to the untreated electrodes in pH 3.0 CAA medium.

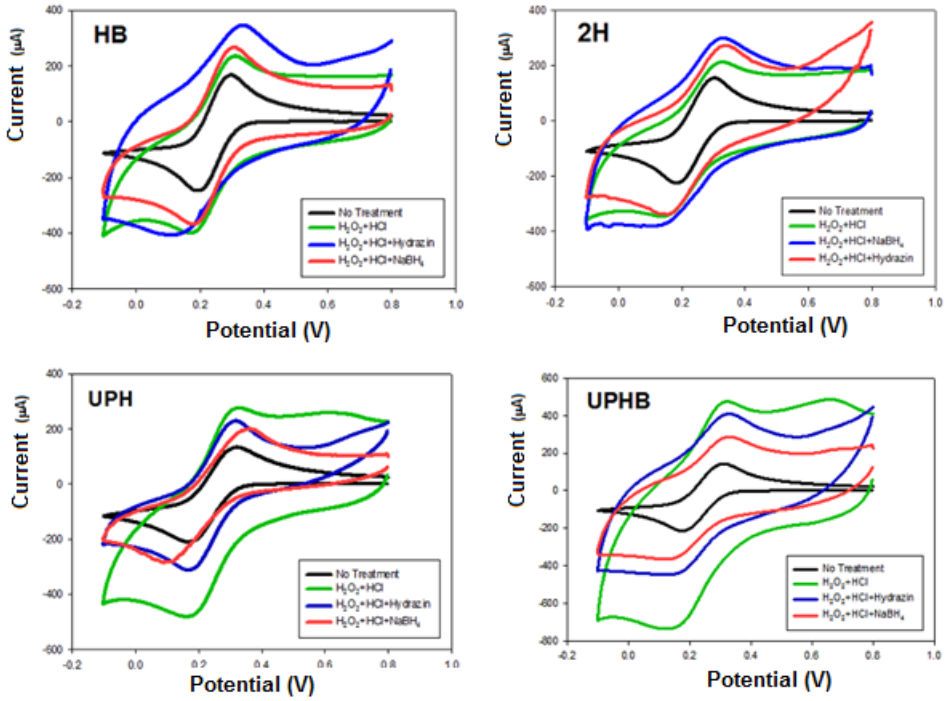


Figure 3.3. Cyclic voltammograms of thermally treated PLEs in the presence of 0.01 M $Fe(CN)_6^{4-}$ ions.

Table 3.2 Peak characteristics of tratedPLEs in the presence of 0.01 M $Fe(CN)_6^{4-}$ ions.

PLE	Treatment		Peak Potential (V)			Peak Current(µA)		Current ratio I_p^a/I_p^c
			E_p^a	E_p^c	ΔE	I_p^a	I_p^c	
HB	**A**	Untreated PLE	0,290	0,196	0,094	204,0	-198,0	1,03
	B	µwave→H_2O_2 + HCl	0,302	0,185	0,117	228,8	-161,4	1,42
	C	µwave→H_2O_2 + HCl→ hydrazine	0,326	0,151	0,175	204,9	-162,8	1,26
	D	µwave→H_2O_2 + HCl→ $NaBH_4$	0,302	0,185	0,117	259,4	-213,3	1,22
2H	**A**	Untreated PLE	0,290	0,189	0,101	182,0	-164,0	1,11
	B	µwave→H_2O_2 + HCl	0,314	0,160	0,154	157,8	-119,4	1,32
	C	µwave→H_2O_2 + HCl→ hydrazine	0,326	0,155	0,171	170,3	-168,0	1,01
	D	µwave→H_2O_2 + HCl→ $NaBH_4$	0,326	0,155	0,171	194,5	-114,7	1,69
UPH	**A**	Untreated PLE	0,300	0,180	0,120	152,0	-146,0	1,04
	B	µwave→H_2O_2 + HCl	0,309	0,182	0,127	186,6	-203,1	0,92
	C	µwave→H_2O_2 + HCl→ hydrazine	0,309	0,180	0,129	194,0	-194,9	0,99
	D	µwave→H_2O_2 + HCl→ $NaBH_4$	0,356	0,114	0,242	200,5	-154,8	1,29
UPHB	**A**	Untreated PLE	0,300	0,180	0,120	168,0	-154,0	1,09
	B	µwave→H_2O_2 + HCl	0,304	0,165	0,139	269,5	-247,2	1,09
	C	µwave→H_2O_2 + HCl→ hydrazine	0,319	0,160	0,159	208,4	-158,4	1,31
	D	µwave→H_2O_2 + HCl→ $NaBH_4$	0,324	0,146	0,178	166,2	-130,9	1,27

According to these results, the peak potential differences (ΔE) were very high between the untreated and treated electrodes indicating the surface modification by thermal and chemical treatment of the surface. In addition, the current ratio of anodic and cathodic peaks was found higher than the untreated electrode. Here, the background current also plays an important role and therefore, the signal evaluation after the background correction would give an insight to the interaction. Figure 3.4 shows the background corrected signals for treated PLEs.

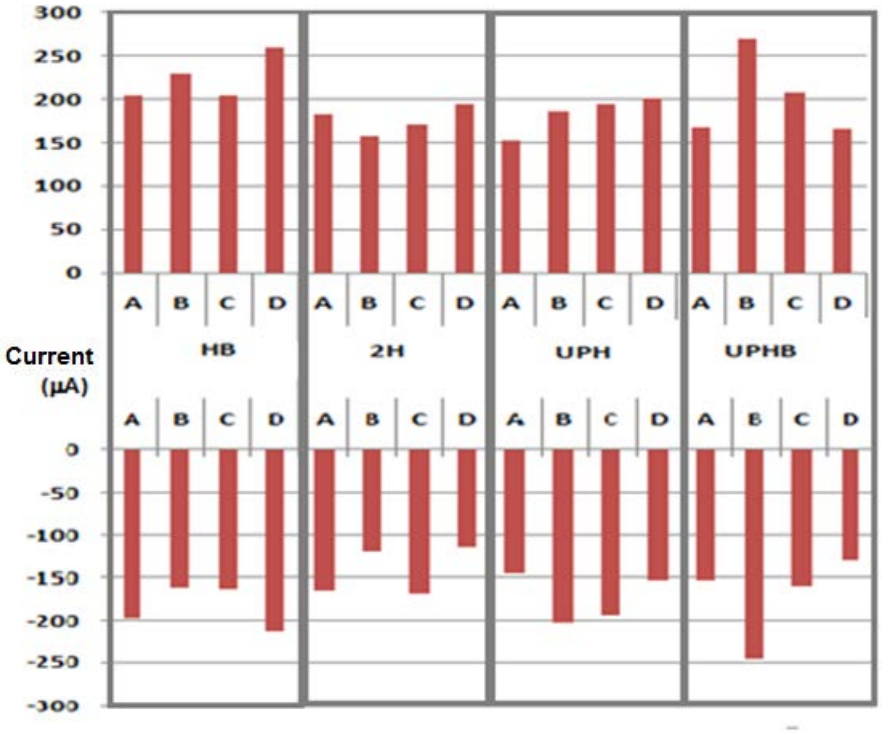


Figure 3.4 Peak current values obtained for thermally and chemically treated PLEs after background correction was made.

As can be clearly seen now, best current values were obtained with HB brand PLE after reducing with $NaBH_4$reagent. Besides, this electrode gives more reversible results since the potential difference of both peaks (ΔE = 0,117 V) are very small in comparison to the other electrodes. Therfore, best performance was obtained with HB electrode and SEM images were given in Figure 3.5 for different magnification.

As can be seen from the figure, surface layer of the electrode is quite different from the untreated electrode. As it is consistent with the literature (Liu et al.,2014), thermal treatment coupled with strong chemicals have resulted exfoliation on the surface by increasing the surface energy and therefore, changing the catalytic activity of the surface.

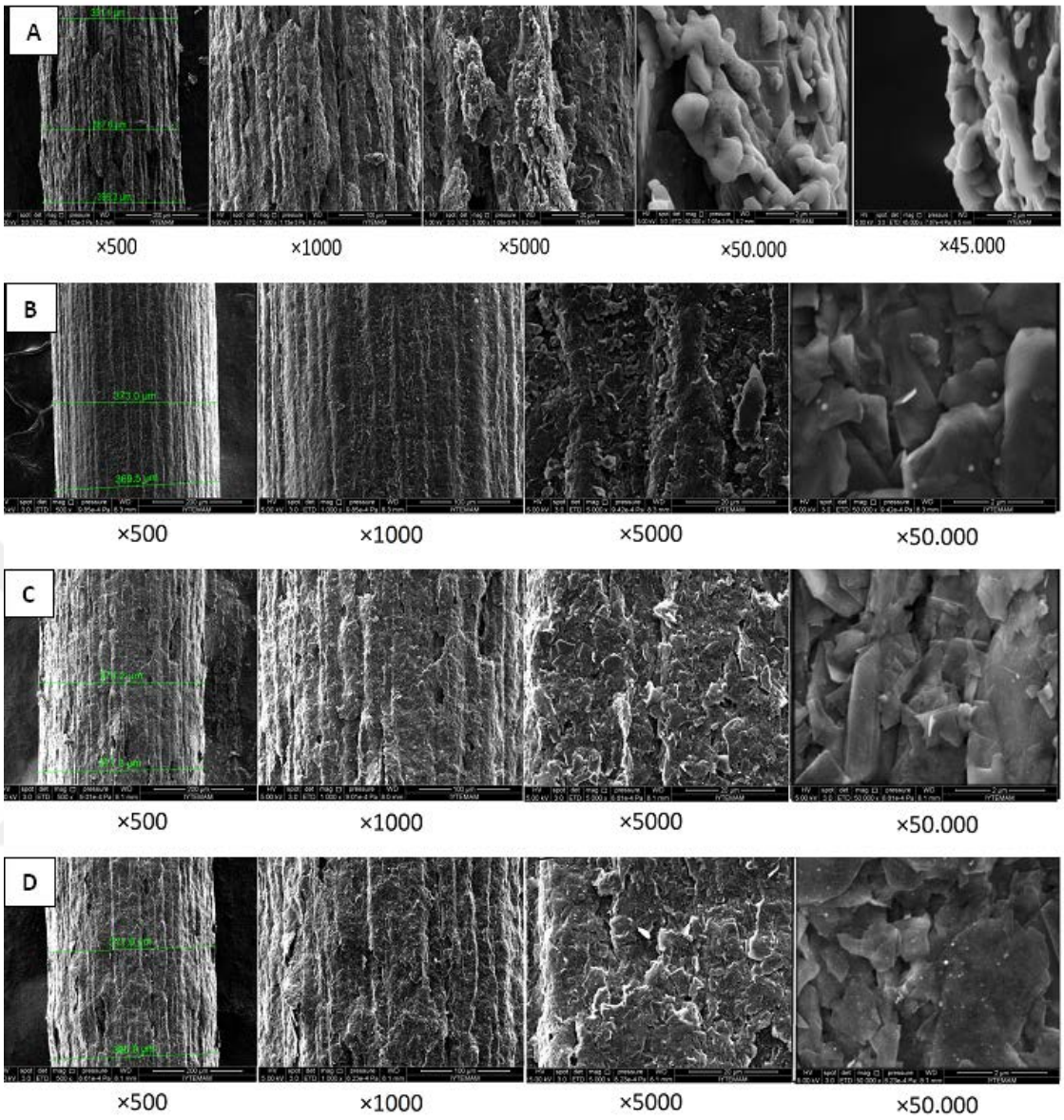


Figure 3.5 SEM images of thermally treated HB brand PLE **A)** Untreated, **B)** Microwave processed and treated with H_2O_2 and HCl, **C)**The PLE in B further reduced with hydrazine, **D)** The PLE in B further reduced with sodium borohydride

However, high background of these treated electrodes limits their use in voltammetric purpose. On the other hand, these surface modifications can increase the adsorption characteristics of the electrode making their use in chromatographic analysis.

3.1.2. Voltammetric Determination of Boscalid

In this thesis, any attempts to investigate the voltammetric behavior of Boscalid have been failed since this molecule is not electroactive in the potential range studied. Therefore, we have searched the literature to find any structural similarity with metallic ions. As described in introduction part, Boscalid is a derivative of pyridine carboxamide which gives complex formation with cobalt ions. Therefore, PLE electrode were used in the presence of Co(II) ions to see any reduction peak belongs to the complex.

Figure 3.6 shows the DP voltammograms recorded in pH 4.0 BR buffer solution. The buffer solution was spiked with Cobalt ions to be $5x10^{-4}$ M and the deposition is affected at -0.5 V for 1 min. By scanning the potential in DP mode in negative direction, a small peak at -1.4 V is observed. Boscalid alone has given no peak (Fig. 3.6b), however upon addition of Boscalid standard to be $1x10^{-5}$ M on to Co(II) ions, a peak at -1.2 V appears. From this figure, it is evident that Boscalid complexes with Co(II) ions and therefore, this complex formation allows us to determine Boscalid indirectly by Adsorptive cathodic stripping voltammetry (AdCSV) after depositing at rather positive potentials.

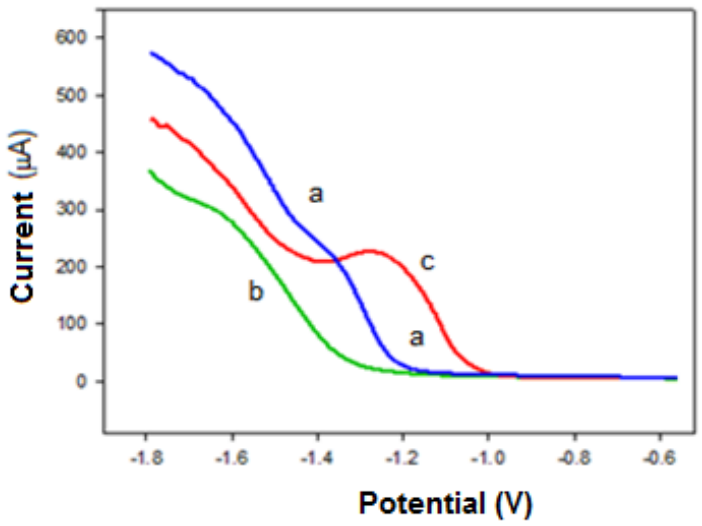


Figure 3.6 AdCSV dp voltammograms obtained for a) $5x10^{-4}$ M Co(II) in pH 4.0BR buffer solution, b) $1x10^{-5}$ M Boscalid alone and c)after addition of Co(II) + Boscalid. Deposition was affected at 0.5 V for60 s.

This AdCSV method can be utilized for Boscalid determination after optimization of the experimental parameters namely, the solution pH, type of PLE, deposition potential and timeand scan rate.

The medium pH was studied in the range of 2.0 -7.0 by using BR buffer solutions. Figure 3.7 shows the dp voltammograms recorded for$5x10^{-4}$ M Co(II) solution including $1x10^{-5}$ M Boscalid afterdeposition at -0.5 Vfor 60 sat a HB type PLE. The peak characteristics were given in inset.

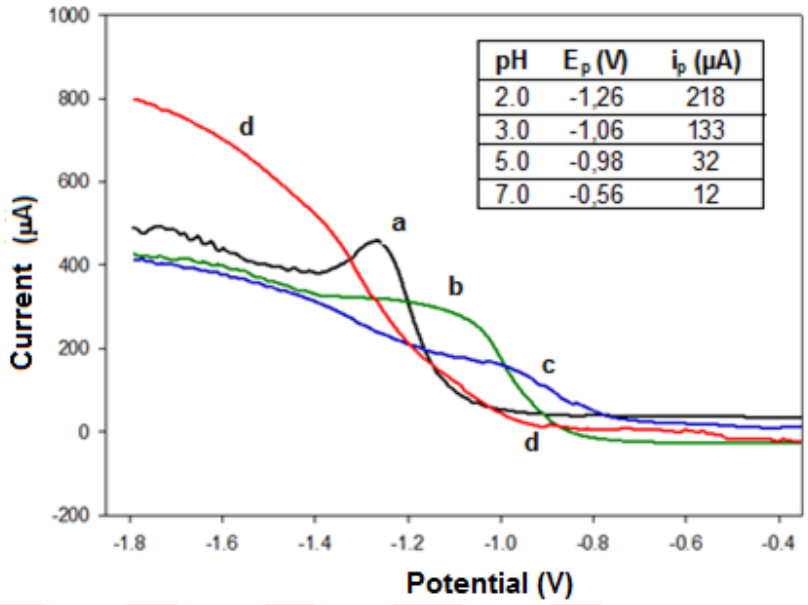
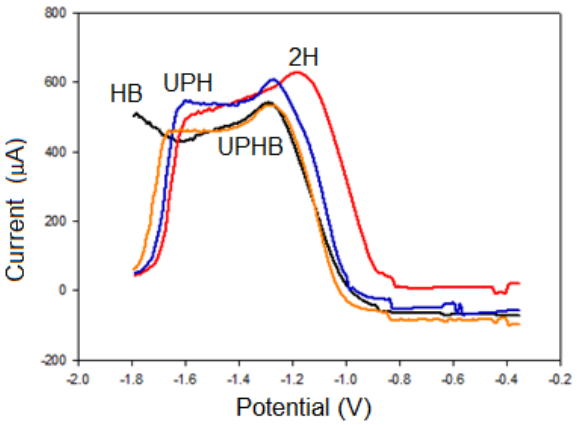


Figure 3.7 The effect of medium pH on AdCSV peaks of $5x10^{-4}$ M Co(II) and $1x10^{-5}$ M Boscalid mixture after deposition at -0,5 V for 60 s in pH a) 2,0; b) 3,0; c) 5,0 and d) 7.0 BR buffer.

As can be clearly deduced from the figure that best peak formation was observed at pH 2.0 and this pH was chosen for further studies. In this part of the study, the performances of other brands of PLE were tested under the same conditions i.e. pH 2.0 BR buffer, deposition potential of -0.2 V for 2 min ad a scan rate of 100 mV/s.

As shown in Figure 3.8, best peak current as well as the peak potential was obtained with 2H brand of PLE. However, due to the high background current obtained with this PLE, further studies were carried out with the second best HB brand of PLE which gives relatively flat baseline.

The performance difference of HB and 2H brand PLE can be attributed to the structural effects. Figure3.9 displays FTIR spectra of powdered HB and 2H materials by using ATR techqniue. From the spectrum, it can be concluded the peak at 1000 cm^{-1} wave number was attributed to the C=C vibration and it is more appearent for HB brand indicating a polymeric structure is involved. Inaddition, the peaks at 3000 cm^{-1} might indicate the silica residue in resin used for the manufacture which explains the difference in the adsorption characteristics.



	i_p (µA)	E_p (V)
HB	344,6	-1,267
2H	370,5	-1,126
UPH	281,2	-1,146
UPHB	329,1	-1,236

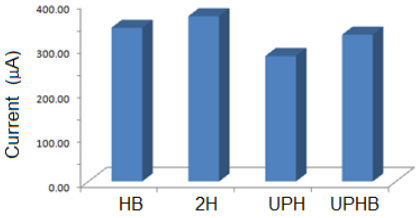


Figure 3.8 The effect of brand of PLE on the AdCSV peaks of Co(II)-Boscalid complex after deposition at -0.2 V for 120 s and scanning at a rate of 100 mV/s.

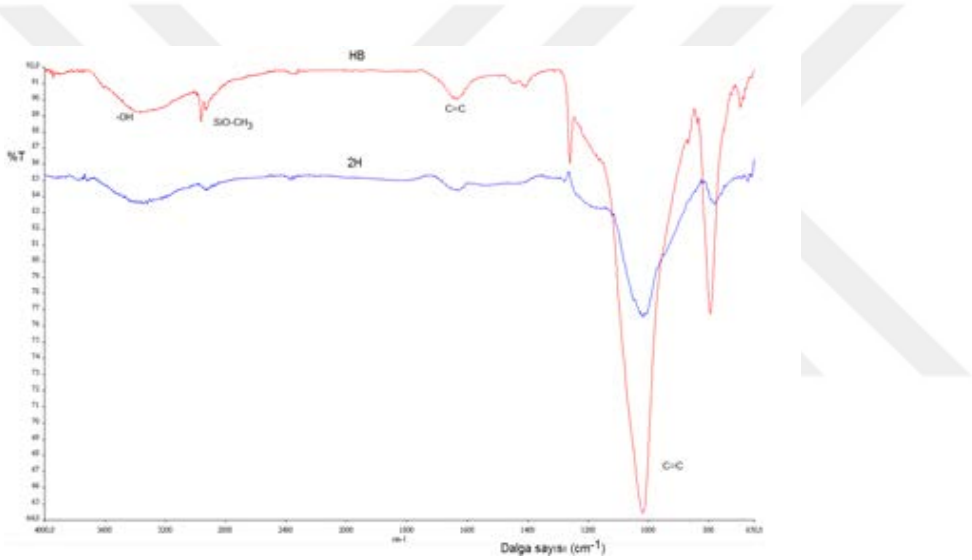


Figure 3.9 ATR-FTIR spectra recorded for HB and 2H PLE powders.

In the next step, the concentration of Co(II) ions was optimized. HB brand PLE was conditioned and placed in an electrochemical cell containing 2.0 mL of pH 2.0 BR buffer solution. The background current was monitored and Boscalid standard was added to be $1x10^{-5}$ M. Then, Co(II) standards were added to be in a concentration range of $1x10^{-6}$- $5x10^{-5}$ M. After deposition at -0.2 V for 120 s, the potential was scanned in negative direction at a rate of 1.0 V/s. Figure 3.10 displays the actual voltammograms and the peak currrents obtained for each Co(II) concentration were given in inset.

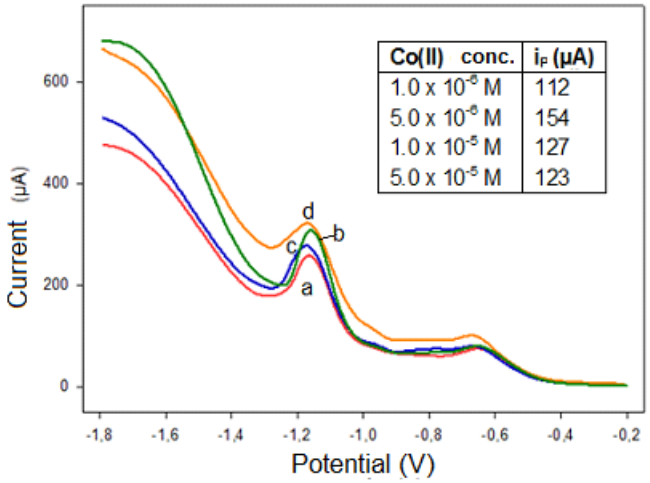


Figure 3.10 The effect of Co(II) concentration of AdCSV peaks of the Bos complex at a) 1.0 x10^{-6} M, b) 5.0 x10^{-6} M, c) 1.0 x10^{-5} M ve d) 5.0 x10^{-5} M

As it is evident from the figure, best peak currents were obtained in the presence of 5x10^{-6} M Co(II) ions. Further studies deal with deposition conditions. Figure 3.11 shows the AdCSV DP signals for various deposition potentials affected for 60 s.

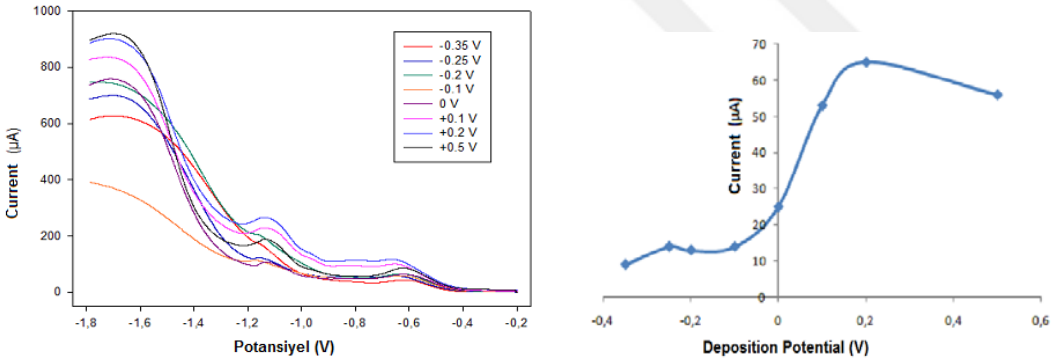


Figure 3.11 The effect of deposition potential on the AdCSV peaks of Co(II)-Boscalid complex after deposition for 1 min at a) -0.5 V, b) -0.35 V, c) -0.2 V, d) 0 V, e) 0.1 V ve f) 0.5 V.

From theplot diagram given in Figure 3.11, it is clear that 0.2 V has given the best signal formation. Next step is to optimize the deposition time. For this purpose the complex was deposited at pH 2.0 BR buffer at 0.2 V for 0-180 s. Figure 3.12 shows the AdCSV DP voltammograms recorded for 5x10^{-4} M Co(II) and 1x10^{-5} M Boscalid mixture.

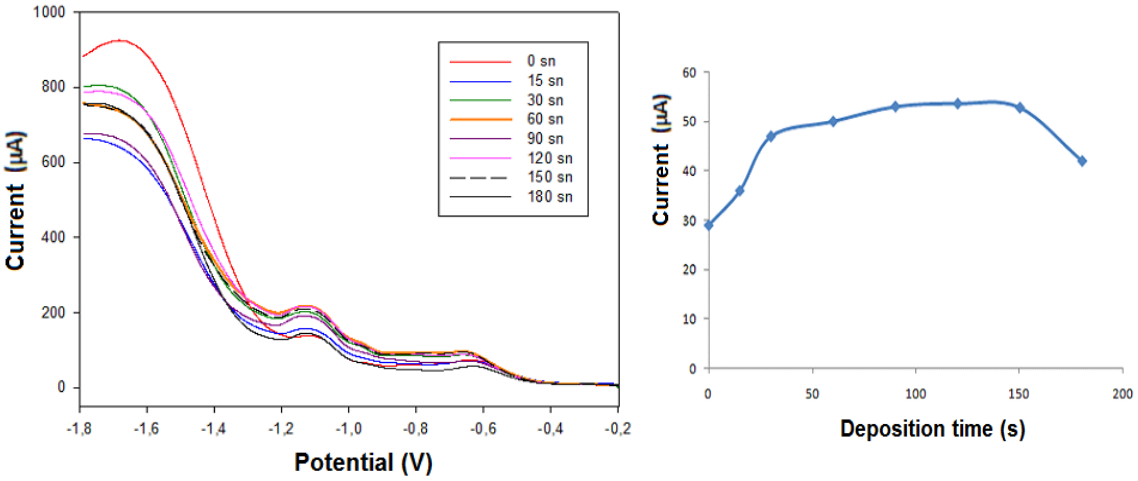


Figure 3.12 The effect of deposition time on the AdCSV peaks of Co(II)-Boscalid complex after deposition at -0.2 V for a) 0, b) 30 s, c) 60 s, d) 90 s, e) 120 s, f) 150 s, g) 180 s

The plot diagram on Figure 3.12 clearly shows that after 150 s deposition time, the electrode surface is saturated and a decrease in the signal is shown also indicates that free Boscalid molecule and the complex competes for the active sites of the surface.

Another parameter to be optimized is the scan rate. For tis purpose, pH 2.0 BR buffer solution is placed in the electrochemical cell and after recording the background current, is Co(II) is added to be 5×10^{-4} M and Bos is added to give $1x10^{-5}$M. After bubling through the solution with nitrogen gas for 2 min, the potential is affected at 0.2 for 120 s. DP voltammograms were recorded between -0,35- -1,8 V range at different scan rates. Figure 3.13 shows the effect of scan rate on the signal formation.

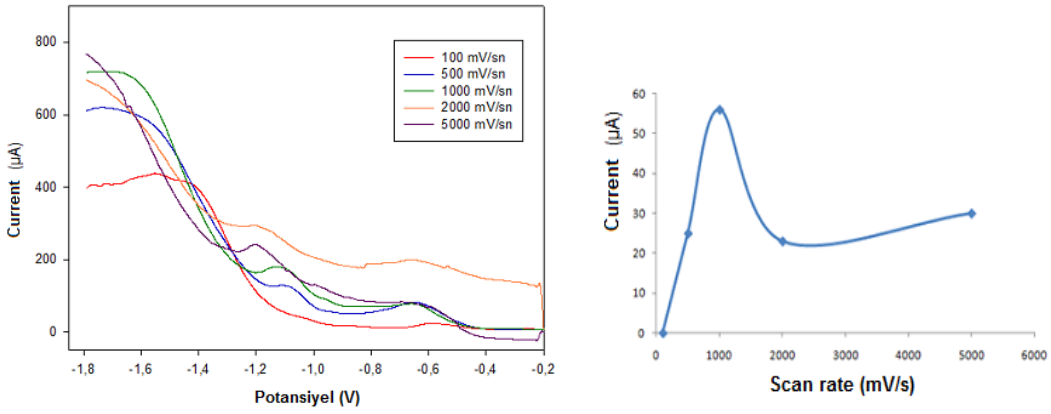


Figure 3.13 The effect of scan rate on the AdCSV peaks of Co(II)-Boscalid complex after deposition at 0.2 V for 120 s at various scan rates.

As can be seen from the figure above, the scan rate has a great effect on the peak formation and the best signal was obtained with as high as 1.0 V/s scan rate. This indicates that complex adsorbes on the electrode surface and upon scanning the potential in negative direction, it is readily reduced. The current changes with scan rate display a peak formation which is characteristic for a catalytic process.

Considering the pyridine moiety in the compound and the high current formation in acidic media, this catalytic effect can be explained. Peak potential, on the other hand, did not show much change by scan rate. In the light of these findings, it can be clearly stated that Boscalid-Co(II) complex can be determined by Cat-AdCSVs method.

To reveal the adsorption character of the complex another experiment was carried out and the peak currents and potentials obtained from the solution of 5×10^{-6} M Co(II) and 1×10^{-8} M Boscalid mixture in pH 2.0 were plotted against the square root of scan rate in the range of 50-900 mV/s. As shown in Figure 3.14, the linear relationship between the peak current and the square root of scan rate indicates that the current is diffusional controlled.

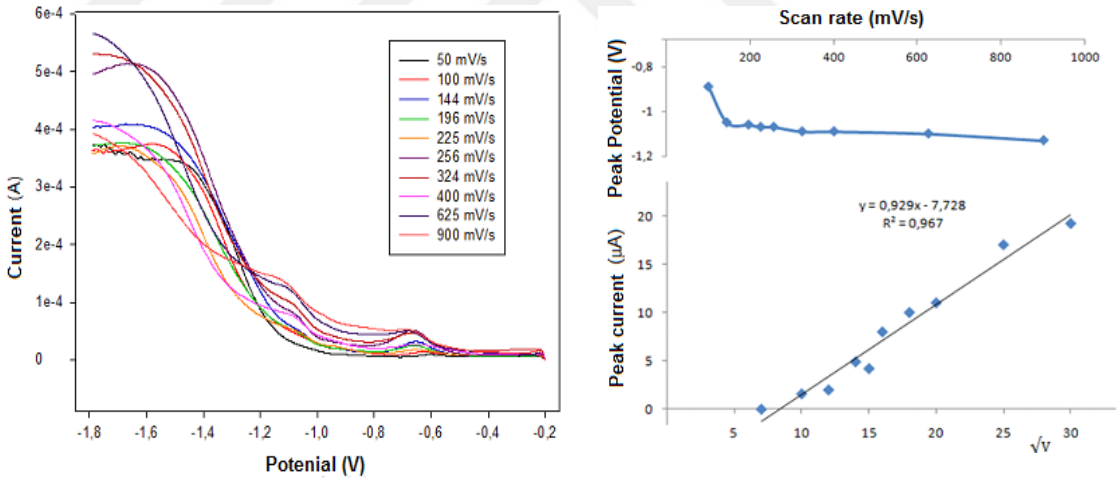


Figure 3.14 The effect of scan rate on the AdCSV peaks of Co(II)-Boscalid complex after deposition at 0.2 V for 120 s and the change in peak current and potential of the complex.

After optimizing the conditions, the studies were conducted to draw calibration curve for Boscalid standard. Considering that deposition time strongly effects the signal magnitude as well as the saturation of the surface, calibration curves were drawn for three different deposition times of 30, 60 and 120 s at 0.2 V in different concentration ranges while Co(II) concentration was kept constant as $5x10^{-6}$ M (Figure 3.15).

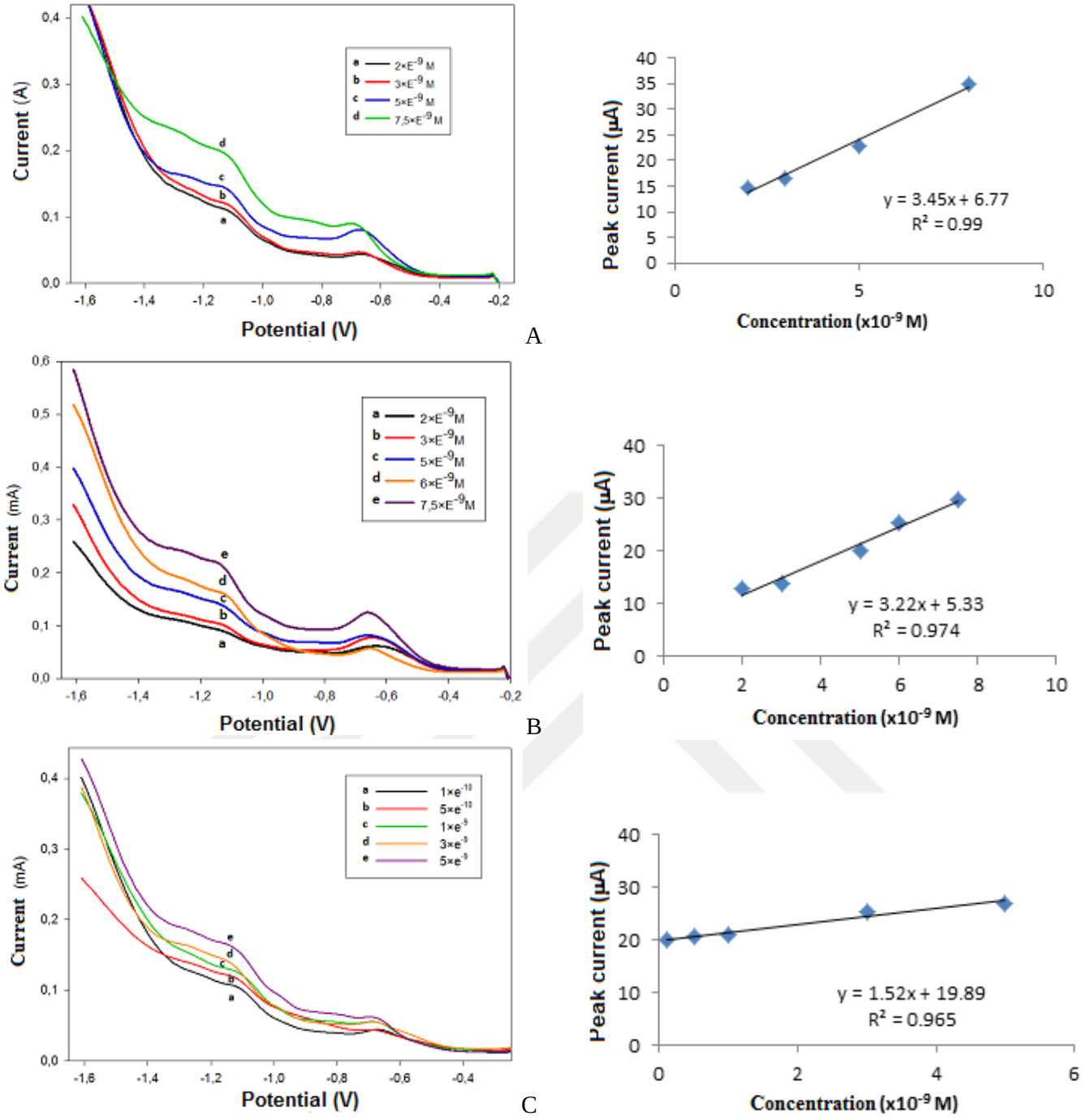


Figure 3.15 Caibration curves obtained for cat-AdCSV peaks of Co(II)-Boscalid complex after depositing at 0.2 V for A) 30, B) 60 and C) 120 s and scanning at 1.0 V/s scan rate.

As can be seen in the figure above, linear calibration curves (R^2> 0.96) can be obtained for nanomolar levels of concentrations for different deposition times. Even sub ppb levels can be reached for 2 min deposition, but regression coefficient was low. Longer deposition time have resulted even worse regression coefficient (data not shown) which is attributed to the self adorption of Boscalid on the surface.

Calibration studies were carried on by using 120 s deposition time for a wider concentration range and as can be seen in Figure 3.16, calibration graph was given as inset.

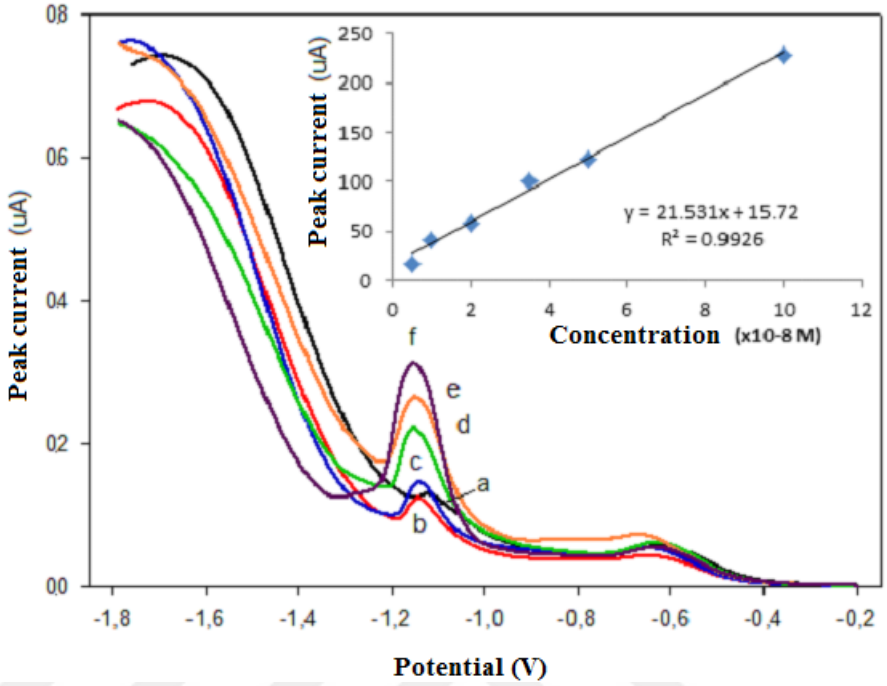


Figure 3.16 Calibration curves obtained for cat-AdCSV peaks of Co(II)-Boscalid complex after depositing at 0.2 V for 120 s for concentration of a) $5x10^{-9}$ M, b) $1x10^{-8}$ M, c)$2x10^{-8}$ M, d)$3.5x10^{-8}$ M, e)$5x10^{-8}$ M ve f)$1x10^{-7}$ M BOS.

To establish the sensitivity of the method, the background signal was recorded subsequently and the mean value was calculated as 4.27 ± 0.31 (n=3). Using this value and making backgraound corrections, thelimit of detection (LOD) according to S/N = 3 principle, was calculated as 4.3 $x10^{-10}$ M. Therefore, the limit of quantitation (LOQ = 3.3 LOD) can be established as 1.4 $x10^{-9}$ M. This high sensitivity of the method can be attributed to the catalytic aspect of the AdCSV signal.

The method was then, applied to the real samples. For this purpose, tomato juice samples obtained from local market was used. Again, the PLEs were conditioned at 0.9 V for 120 s in pH 7.0 BR buffer and then, immersed in pH 2.0 BR buffer solution for background recording. Meanwhile, tomato juice sample was prepared by mixing 10.0 mL of tomato juice with 40.0 mL of ultra pure water and the mixture pH is measured as 4.0.

The pH of tomato juice sample was adjusted to 2.0 by addition of diluted HCl solution. It was added a small amount of acetone and centifuged at 4000 rpm and 2 mL from the supernatant was transferred to the cell for subsequent measurement. Figure 3.17shows the effect of acetone additions on the cat-AdCSV peaks of Boscalid-Co(II) complex.

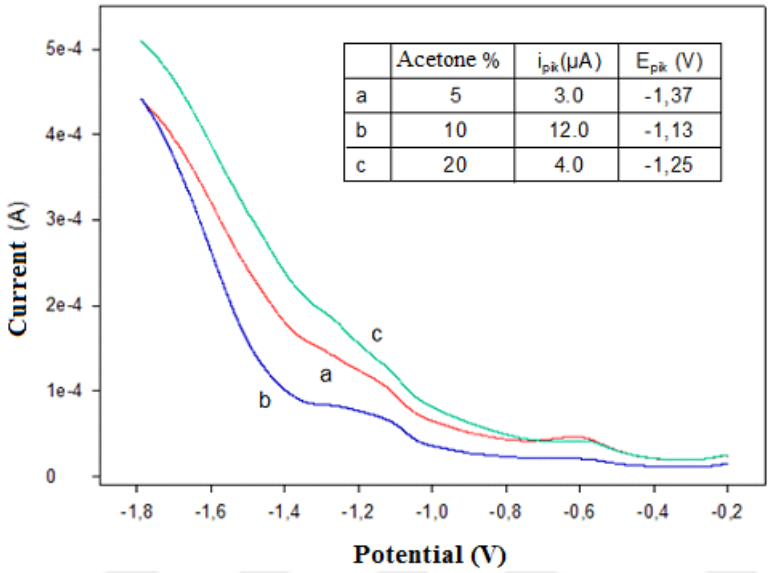


Figure 3.17 The effect of acetone amount on the cat-AdCSV peaks of Co(II)-Boscalid complex a) 5%, b) 10%, c)20% acetone.

As can be followed from the figure, 10% acetone provides a better peak formation. Further studies utilize this percentage. Tomato samples were prepared according the the precedure given in Experimental Section and then, 1.90 mL of the treated sample is placed in the cell where it is mixed with 100 µL of Co(II) standard to be 5×10^{-6} M. Deposition was affected at 0.2 V for 60 s and the potential was scanned from -0.2 to -1.8 V at a rate of 1.0 V/s. Figure 3.18 shows the background corrected voltammograms for standard addition studies. Each measurement was made with a freshly prepared electrode to avoid memory effect.

As can be seen in Figure 3.19, linear standard addition curves were obtained with a regression coefficient close to unity in nanomolar regions. The background current (3.2 µA) was found some what smaller than the aqueous standard solutions and the reproducibility was studied for 1.0 $x10^{-8}$ M Boscalid concentration. The mean signal was calculated as 8.76± 0.59 (n=3). RSD was estimated as 6.7% which is quite satisfactory.

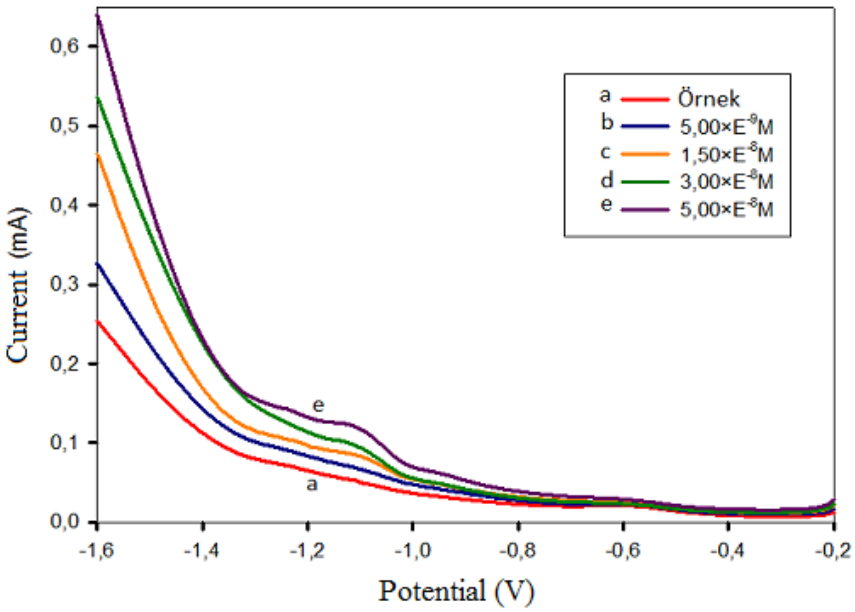


Figure 3.18 Background corrected cat-AdCS Voltammograms obtained for peaks of Co(II)-Boscalid complex in tomato juice sample after depositing at 0.2 V for 60 s.

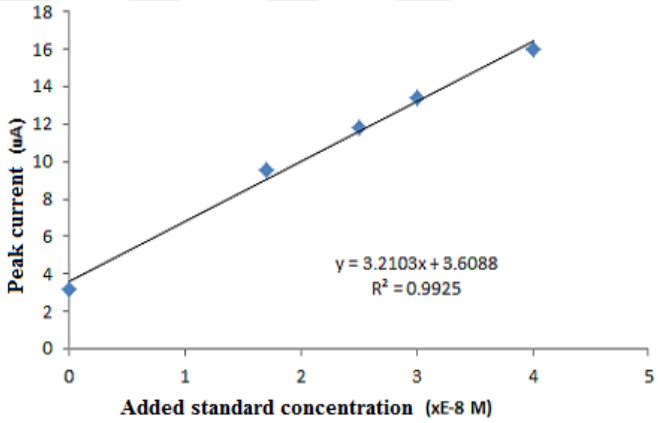


Figure 3.19 Caibration curves obtained for cat-AdCSV peaks of Co(II)-Boscalid complex in tomato juice samples

The accuracy of the method was tested by recovery measurements. Forlow, medium and high concentrations of the calibration curve were chosen and the recovery was calculated as 90.9% for 5.0 x 10^{-9} M, 91.9% for 1.25 x 10^{-8} M and 109.1% for 5.0 x 10^{-8} M.

The method was also applied for tomato puree which is the main ingredient of Turkish cookery. The puree samples were weighed as 0.50 g and dissolved in pH 2.0 BR buffer solution and acetone is added to be 10% before making the volume upto 50 mL. The pH is controlled and the sample is filtred through 0.45 µm Teflon filter. Then, the voltammetric procedure is applied. Figure 3.20 shows the cat-AdCSV peaks and standard addition graph.

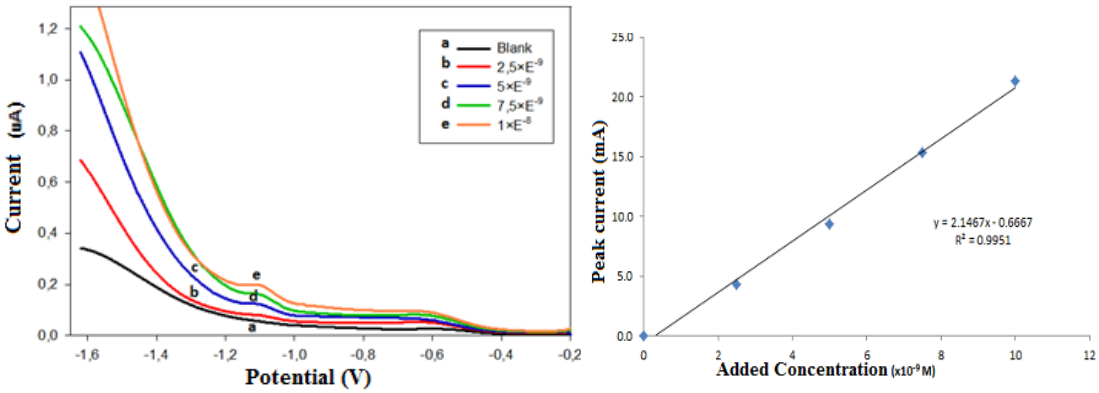


Figure 3.20 Standard addition voltammograms obtained for peaks of Co(II)-Boscalid complex in tomato puree after depositing at 0.2 V for 60 s and standard addition graph.

As can be seen from the figures above, the method can be succesfully applied to tomato puree samples. However, the solution is red colored and the tip of the PLE was observed to be blackened after the measurements. The calibration graph deviates linearity for higher concentrations (data not shown). Therefore, the sample is diluted to maintain a good calibration graph.

3.2. Chromatographic Analysis of Boscalid

In chromatographic determination of Boscalid, initial studies were dedicated to identify the Boscalid fungicide by direct injection of liquid standard to the GC-MS system. Figure 3.21 shows the chromatograms recorded for 5, 10, 20, 40 and 100 ng/mL Boscalid standard at 140 m/z values.

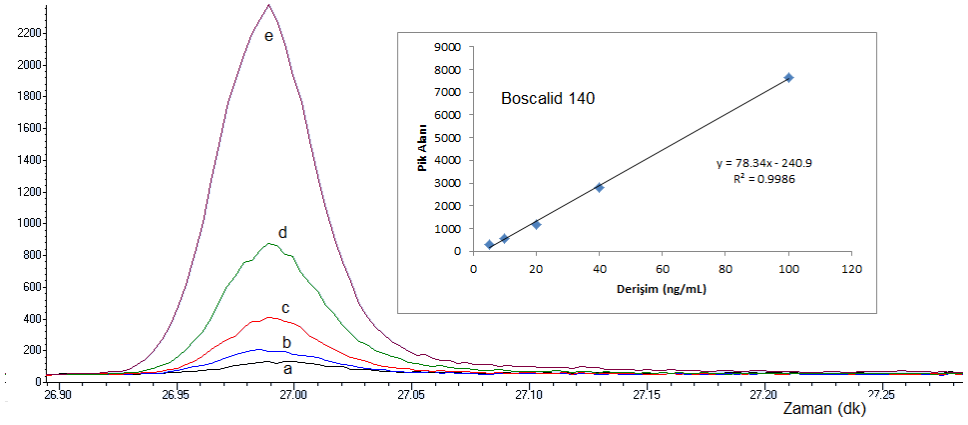


Figure 3.21 GC-MS chromatogram and calibration curve drawn for a) 5, b) 10, c) 20, d) 40 and e) 100 ng/mL Boscalid standard injection.

As can be deduced from the chromatogram, a linear calibration curve can be obtained by exploiting the area of the peak located at 27th min. For more sensitive determination, an enrichment method is necessary for food samples.

Therefore, solid phase micro extraction methods were applied in the direct immersion mode since Boscalid is not very volatile. For this purpose, a commercial (PDMS-DVB) and a laboratory made (PPy) SPME fiber were used.

Optimization of experimental parameters and calibration studies were carried out in tomato juice samples as described in Experimental Section. 1.0 mL of sample is pipetted into the vial and Boscalid standard is added and after pH adjustment, the SPME fiber was immersed in the solution for 5 min and then, the fiber was withdrawn into the needle for subsequent injection into the GC-MS system. Experimental parameters such as solution pH, extraction time and stirring rate were optimized by using PDMS-DVB fiber. Extractions were made at laboratory conditions at ambient temperature (35^{o}C).

The effect of sample pH on the chromatographic peak area was investigated by taking 1:1 diluted tomato juie sample and centrifuging at 4000rpm for 15 min and the supernatant was taken and the pH was adjusted to desired pH by using HCl or NaOH solutions. Boscalid standard is added to be 50 ng/mL and the GC-MS conditions given in Experimental Section was applied. Figure 3.22 shows the pH effect on the peak area.

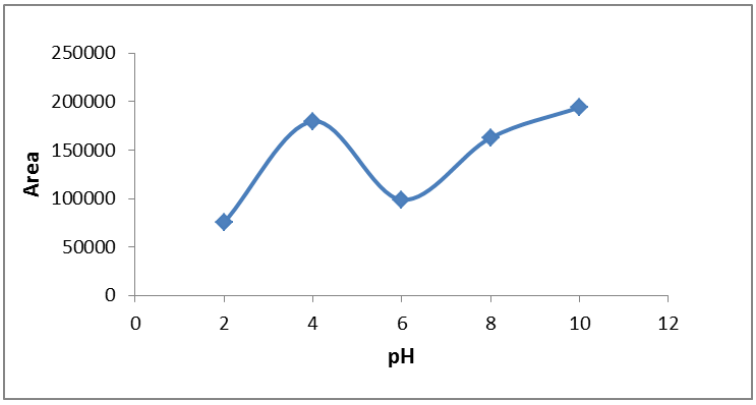


Figure 3.22 The effect of pH on the DI-SPME-GC-MS peak area of 50 ng/mL Boscalid.

As can be seen from the figure, medium pH has a strong effect on the response and best results were obtained with pH 4.0 buffer solution. This pH is also very close to natural pH of tomato juice so no reagent addition is necessary.

Next parameter to be optimized is the stirring rate. Stirring is used to ensure optimum mass transfer to the fiber surface. Therefore, a couple of stirring rated were tested. Table 3.3 shows that 250 rpm has given the best results.

Table 3.3 The effect of stirring rate on the DI-SPME-GC-MS peak area of 50 ng/mL of Boscalid.

Stirring Rate (rpm)	250	350	400
Area	350679	310541	248116

The last parameter is the extraction time. The duration of fiber is exposed to the sample solution is important for a reproducible and sufficient enrichment of the analyte. Therefore, several extraction times were tested and as shown in Figure 3.23, 20 min has given the best results. The peak area has decreased for longer times probably due to desorption of the analytes from the fiber surface.

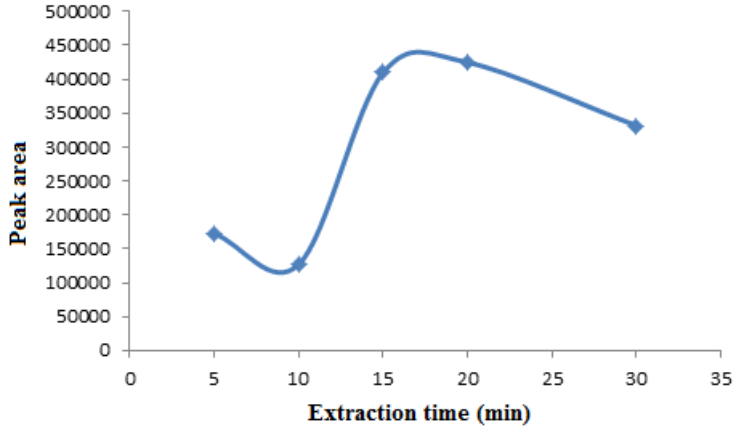


Figure 3.23 Influence of extraction timeon the DI-PDMS-DVB-SPME-GC-MS peak area of 50 ng/mL Boscalid in pH 4.0 tomato juice at 250 rpm.

Under optimized conditions (pH 4.0, extraction time: 20 min and 250 rpm stirring rate) the calibration graph was constructed in ng/mL concentration ranges for tomato juices. Figure 3.24 shows that a linear realationship is obtained.

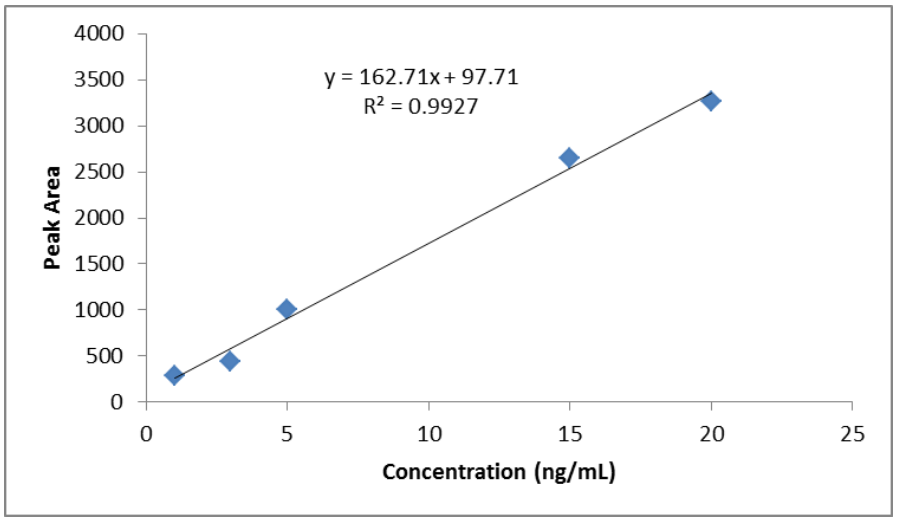


Figure 3.24 The calibration curve obtained for DI-PDMS-DVB-SPME-GC-MS analysis of Bocalid in pH 4.0 for 20 min ads at 250 rpm.

The experiments were reeated with lab-made PPy-SPME fibers under exactly the same conditions i.e; lab temperature (35^{o}C, pH 4.0 BR, adsorption time : 20 min at 250 rpm) and the chromatograms obtained were given in Figure 3.25 and the calibration curve was given in Figure 3.26.

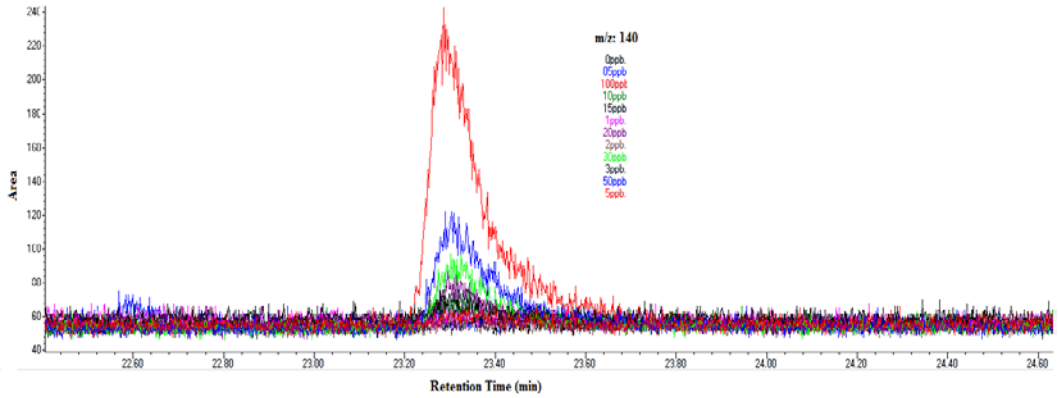


Figure 3.25 The chromatograms obtained for DI-PPy-SPME-GC-MS analysis of Bocalid in pH 4.0 for 20 min adsorption at 250 rpm.

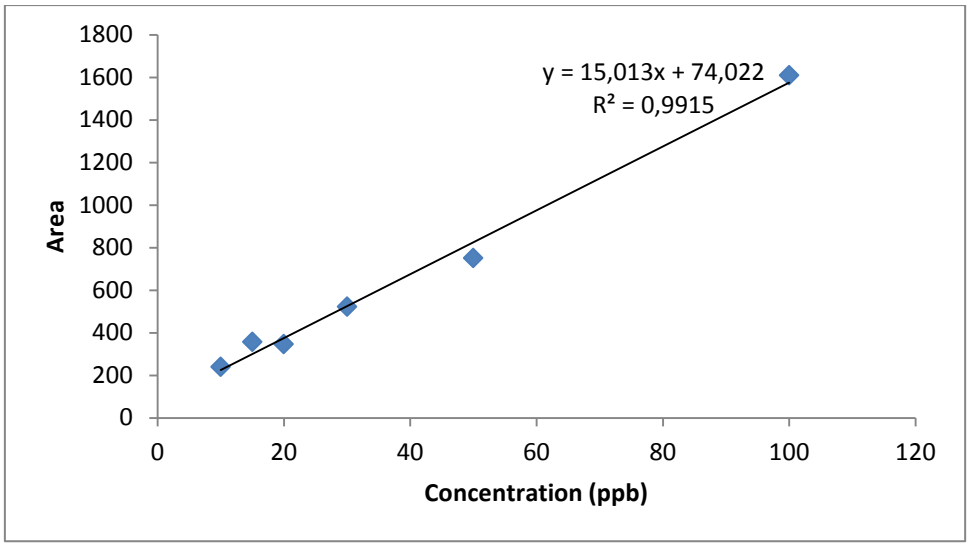


Figure 3.26 The calibration curve obtained for DI-PPy-SPME-GC-MS analysis of Bocalid in pH 4.0 for 20 min adsorption at 250 rpm.

In comparison to the commercial fiber, rather noisy chromatograms were obtained with lab-made fiber. The calibration graph was drawn in a narrower range being 10.0-100 ng/mL.Analytical performance of the fiber was given in Table 3.4 along with the caracteristics of PDMS-DVB fiber and voltammetric method as well.

Table 3.4 Comparison Analytical Results of PPy and PDMS-DVB fiber

Analytical characteristic	**DI-SPME-GC-MS Methods**		**VoltammetricCat-AdCSV**	
	PDMS-DVB	**PPy fiber**	**In Buffer soln**	**In Tomato juice**
Linear range	2.0-20 ng/mL	10-100 ng/mL	0.5-10 x10^{-8} M	1.8 – 4.0 x10^{-8} M
Equation	y = 162.71x + 97.71	y= 15.013x + 74.022	y = 21.531x+ 15.72	y = 3.2103x +3.6088
R^2	0.9927	0.9915	0.9926	0.9925
LOD	1.26 ng/mL	6.73 ng/mL	4.3 x10^{-10} M (0.15 ng/mL)	-
LOQ	4.21 ng/mL	22.4 ng/mL	1.4 x10^{-9} M (48 ng/mL)	-
RSD	-	12% for 50 ng/mL	6.7 % for 1.0 x10^{-8} M	-
Recovery	114% for 5 ng/mL 105.4% for 15 ng/mL	99% for 30 ng/mL 90% for 50 ng/mL	-	90.9% for 5.0 x 10^{-9} M, 91.9% for 1.25 x 10^{-8} M 109.1% for5.0 x 10^{-8} M.

4. CONCLUSION

In this thesis, it was aimed to develop an electrochemical and a chromatographic method for sensitive and selective boscalid determination in tomato juice and puree samples.

Electrochemical studies include the voltammetric investigation of thermally treated PLEs in the presence of $Fe(CN)_6^{4-}$ ions. Even though thermally and chemically treated and reduced PLEs have given better signal, background current was also increased making difficult for electrochemical purposes.

On the other hand, electrochemically inactive Boscalid can be determined adsorptive cathodic stripping voltammetrically in the presence of Co(II) ions. The catalytic character of the reduction process was established by mechanistic studies. The method was optimized and sub nanomolar levels can be determined by this means.

Considering the complicated matrix of the food samples, a solid phase micro extraction technique was used. For this purpose, lab made (PPy) and commercial (PDMS-DVB) fibers were utilized. Experimental parameters such as pH, stirring rate and extraction time was optimized in tomato juice sample medium. The calibration graphs were drawn in ppb ranges.

Overall results have demonstrated that Boscalid can be determined by both methods sensitively and yet, selectively. Future studies will be dealing with applications of the methods for a range of samples.

REFERENCES

Belardi, R. P., Pawliszyn, J. B., 1989, Application of chemically modified fused silica fibers in the extraction of organics from water matrix samples and their rapid transfer to capillary columns, Water Pollution Research Journal of Canada, 24 (1989), p. 179

Bond, A.M., Mahon, P.J., Schiewe, J., Beckett, V. V., 1997, An inexpensive and renewable pencil electrode for usein field-based stripping voltammetry, Analytical Chimica Acta 345 (1997) 67-74

Chen, M.F., Huang, J.W., Chıen, H.P., 2007, Residue Analysis of Fungicide Boscalid in Cucumbers Following Applications of Boscalid 50% Water Dispersible Granule, Journal of Food and Drug Analysis, Vol. 15, No. 2, 2007, Pages 174-177

Cooke, G. M., 2015, Endocrine Disruption and Human Health, Chapter 17 – Regulatory Considerations for Endocrine Disrupters in Food, , Academic Press, Rekha Mehta, 2015, Pages 301–318

Darbre, P.D., 2015 a, Endocrine Disruption and Human Health, Chapter 1 – What Are Endocrine Disrupters and Where Are They Found, Academic Press, 2015, Pages 3–26

Dilgin, D.G., Karakaya, S., 2016, Differential pulse voltammetric determination of acyclovir in pharmaceutical preparations using a pencil graphite electrode, Materials Science and Engineering C 63 (2016) 570–576

Djozan, D., Assadi, Y., 2004, Modified pencil lead as a new fiber for solid-phase microextraction, Chromatographia 60: 313 (2004).

Djozan, D., Baheri, T., 2010, Investigation of Pencil Leads Fiber Efficiency for SPME of Trace Amount of Methamphetamine from Human Saliva Prior to GC–MS Analysis, Journal of Chromatographic Science, Vol. 48, March 2010.

Dojer, B., Pevec, A., Belaj, F., Jaglicic, Z., Kristl, M., Jaglicic, M., Drofenik, M 2014, Structural and magnetic properties of cobalt(II) complexes with pyridine carboxamide ligands, Journal of Molecular Structure 1076 (2014) 713–718

EC Regulation, 2008, Commission Directive 2008/44/EC of 4 April 2008 amending Council Directive 91/414/EEC to include benthiavalicarb, boscalid, carvone, fluoxastrobin, Paecilomyceslilacinus and prothioconazoleas active substances. Official Journal of the European Union, L 94, 13–20.

EFSA, 2010, European Food Safety Authority; Modification of the existing MRLs for boscalid in various crops. EFSA Journal 8 (2010) 1780.doi:http://dx.doi.org/10.2903/j.efsa.2010.1780

EPA, 2003 USA EPA Office of Prevention, Pesticides and Toxic Substances (7501C) Pesticide Fact Sheet Name of Chemical: Boscalid, July 2003

Gabriolotto, C., Monchiero, M., Negre, M., Spadaro, D., Gullino, M.L., 2009, Effectiveness of control strategies against Botrytis cinerea in vineyard and evaluation of the residual fungicide concentrations, Journal of Environmental Science and Health – Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 44 (2009) 389–396.

González-Rodríguez, R.M., Cancho-Grande, B., Simal-Gándara, J., 2009, Multiresidue determination of 11 new fungicides in grapes and wines by liquid–liquid extraction/clean-up and programmable temperature vaporization injection with analyte protectants/gas chromatography/ion trap mass spectrometry, Journal of Chromatography A, 1216 (2009) 6033–6042.

Heydari, H., Gholivand, M. B.,Abdolmaleki, A., 2016, Cyclic voltammetry deposition of copper nanostructure on MWCNTs modified pencil graphite electrode: An ultra-sensitive hydrazine sensor, Materials Science and Engineering C 66 (2016) 16–24

Jabot, C., Daniele, G.,Giroud, B., Tchamitchian, S., Belzunces, L.P., Casabianca, H., Vulliet, E., 2016, Detection and quantification of boscalid and its metabolites in honeybees, Chemosphere 156 (2016) 245-251

Korba, K., Pelit, L, Pelit, F.O., Özdokur, K.V., Ertas, H., Eroglu, A.E., Ertas, F.N., 2013, Preparation and characterization of sodium dodecyl sulfate doped polypyrrole solid phase micro extraction fiber and its application to endocrine disruptor pesticide analysis, J of Chrom. B, 929 (2013) 90–96

Lehotay SJ1, Son KA, Kwon H, Koesukwiwat U, Fu W, Mastovska K, Hoh E, Leepipatpiboon N., Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables, J Chromatogr A.;1217(2010)2548-60.

Liu, Q., Cheng, M., Long, Y., Yu, M., Wang, T., Jiang, G., 2014, Graphenized pencil lead fiber: Facile preparation and applicationin solid-phase microextraction, Journal of Chromatography A, 1325 (2014) 1– 7

Meghdadi, S., Amirnasr, M., Habibi, M.H., Amiri, A., Ghodsi, V., Rohani, A., Harrington, R.W., Clegg, W., 2008, Synthesis, structure, and electrochemistry of pyridinecar- boxamide cobalt(III) complexes; the effect of bridge substituents on the redox properties, Polyhedron 27 (2008) 2771–2778

Munitz, M.S., Resnik, S.L., Montti, M.I.T., 2013, Method development and validation for boscalid in blueberries by solid-phase microextraction gas chromatography, and their degradation kinetics,Food Chemistry 136 (2013) 1399–1404

Mitra, S., 2003, Sample Preparation Techniques in Analytical Chemistry, John Wiley & Sons, New Jersey, USA

Nezhadali, A., Rouki, Z., Nezhadali, M., 2015, Electrochemical preparation of a molecularly imprinted polypyrrole modified pencil graphite electrode for the determination of phenothiazinein model and real biological samples, Talanta144(2015)456–465

Pawliszyn, J., Arthur, C.L., 1990 Solid-phase microextraction with thermal-desorption using fused-silica optical fibers, Journal of Analytical Chemistry, 62: 2145-2148 pp.

Pawliszyn, 1997, Solid Phase Microextration—Theory and Practice.Wiley:New York, J. Pawliszyn, 1997.

Pizzutti, I.R., Kok, A., Hiemstra, M., Wickert, C., Prestes, O.D., 2009, Method validation and comparison of acetonitrile and acetone extraction for the analysis of 169 pesticides in soya grain by liquid chromatography–tandem mass spectrometry, Journal of Chromatography A, 1216 (2009) 4539–4552.

Özdokur, K.V., Tatlı, A.Y., Yılmaz, B., Koçak, S., Ertaş, F.N., 2016, Development of pulsed deposited manganese and molybdenum oxide surfaces decorated withplatinum nanoparticles and their catalytic application for formaldehyde oxidation, Int. Journal of Hydrogen Energy, 41 (2016) 5927-5933

Pelit, L., Dizdas, T.N., 2013, Preparation and application of a polythiophene solid-phase microextraction fiber for the determination of endocrine-disruptor pesticides in well waters, J. Sep. Sci. 36 (2013) 3234–3241

Pelit, F.O., Pelit, L., Dizdas, T.N, Aftafa, C., Ertaş, H., Yalçınkaya, E.E., Türkmen, H., Ertaş, F.N., 2015, A novel polythiophene – ionic liquid modified clay composite solidphase microextraction fiber: Preparation, characterization andapplication to pesticide analysis, AnalyticaChimicaActa 859 (2015) 37–45

Pierini, G.D, Pistonesi, M.F., DiNezio, M.S., Centurión, M.E., 2016, A pencil-lead bismuth film electrode and chemometric tools for simultaneous determination of heavy metals in propolis samples, Microchemical, Journal 125 (2016) 266–272

Poole, C.F., 2003, The Essence of Chromatography, Elsevier, Netherlands, 925 p

Uygun, Z.O., Dilgin, Y., 2013, A novel impedimetric sensor based on molecularly imprinted polypyrrole modified pencil graphite electrode for trace level determination of chlorpyrifos, ,Sensors and Actuators B 188 (2013) 78–84

Wang, J., 2006, Analytical Electrochemistry, Third edition, Wiley& Sons, New Jersey, USA, 262 p.

Zhu, Y., Murali, S., Stoller, M.D., Velamakanni, A., Piner, R.D., Ruoff, R.S., 2010, Microwave assisted exfoliation and reduction of graphite oxide for ultra capacitors, Carbon, 48 (2010) 2106 –2122