

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

**VOLTAMMETRIC DETERMINATION OF HEAVY METALS IN
ENVIRONMENTAL SAMPLES BY CARBON ELECTRODES**



Ph.D. THESIS

Gül Şirin USTABAŞI

Department of Chemistry

Chemistry Programme

JULY 2022

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**Thesis Advisor: Prof. Dr. Mustafa ÖZCAN
Thesis Co-Advisor: Dr. Ebru ÇETİNKAYA**

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

**ÇEVRESEL ÖRNEKLERDE KARBON TEMELLİ ELEKTROTLARLA
VOLTAMETRİK AĞIR METAL ANALİZİ**

DOKTORA TEZİ

**Gül Şirin USTABAŞI
(509172018)**

Kimya Anabilim Dalı

Kimya Programı

**Tez Danışmanı: Prof. Dr. Mustafa ÖZCAN
Eş Danışman: Dr. Ebru ÇETİNKAYA**

TEMMUZ 2022

Gül Şirin Ustabaşı, a Ph.D. student of ITU Graduate School student ID 509172018, successfully defended the dissertation entitled “VOLTAMMETRIC DETERMINATION OF HEAVY METALS IN ENVIRONMENTAL SAMPLES BY CARBON ELECTRODES”, which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor : **Prof. Dr. Mustafa ÖZCAN**
Istanbul Technical University

Co-advisor : **Dr. Ebru ÇETİNKAYA**
Yildiz Technical University

Jury Members : **Prof. Dr. İsmail YILMAZ**
Istanbul Technical University

.....
Dr. Öğretim Üyesi Ferah ÇALIŞIR
Istanbul Technical University

.....
Prof. Dr. Dilek BAKIRCIOĞLU
Trakya University

.....
Prof. Dr. Birsen DEMİRATA ÖZTÜRK
Istanbul Technical University

.....
Doç. Dr. Dilek ÖZYURT
Istanbul University

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Gül Şirin USTABAŞI
(Chemist)



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ABBREVIATIONS

AAS	: Atomic Absorption Spectrometry
AdSV	: Adsorptive Stripping Voltammetry
ASV	: Anodic Stripping Voltammetry
CBE	: Carbon Based Electrode
CV	: Cyclic Voltammetry
DME	: Dropping Mercury Electrode
DPAdSV	: Differential Pulse Adsorptive Stripping Voltammetry
DWT	: Discrete wavelet transform
GCE	: Glassy Carbon Electrode
HMDE	: Hanging Mercury Drop Electrode
ICP	: Inductively Coupled Plasma
LSV	: Linear Sweep Voltammetry
MFE	: Mercury Film Electrode
MWCNT	: Multiwall Carbon Nanotubes
N	: Nafion
OP	: o-phenanthroline
PGE	: Pencil Graphite Electrode
SEC	: Spectroelectrochemical
SPCE	: Screen-printed Carbon Electrode
SWASV	: Square Wave Anodic Stripping Voltammetry



SYMBOLS

A	: Absorbance
C₀	: Concentration in the solution
C_{ox}[*]	: Bulk concentration of oxidised complex
C_{red}	: Concentration of reduced complex
D or D₀	: Diffusion coefficient
d	: Optical path
E	: Potential
E_d	: Deposition potential
F	: Faraday constant
f_R	: Rugosity coefficient
I	: Current
I_p	: Peak current
n	: Number of electrons involved in the process
n	: Number of transferred electrodes per mole
p	: Light intensity in sample
p₀	: Light intensity in reference
S	: Surface area of the electrode
t	: Time
V	: Scan rate
Z	: Impedance
ε	: Molar absorptivity



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VOLTAMMETRIC DETERMINATION OF HEAVY METALS IN ENVIRONMENTAL SAMPLES BY CARBON ELECTRODES

SUMMARY

Quantification of heavy metals such as lead and cadmium is a significant pursuit to accurately assess the contamination levels in the environment. Usually, they are emitted from anthropological and industrial sources and can be found in the environment at trace levels. Nevertheless, heavy metals tend to accumulate both in the environmental media and the living organisms, which in turn causes more significant harm. These pollutants are most commonly determined via spectrometric methods like atomic absorption spectrometry (AAS) and inductively coupled plasma (ICP) due to their sensitivity and accuracy. However, thanks to the recent developments in the field, electrochemical methods can generate results much faster with comparable sensitivity and accuracy, when suitable methods and electrodes are chosen. Electrochemical methods not only save valuable time for the analyst but also allow for on-site measurements with lower instrumental costs than the abovementioned spectrometric methods.

There are plenty of electroanalytical methods to employ. Among them, voltammetric methods, especially anodic stripping voltammetry (ASV) and adsorptive stripping voltammetry (AdSV), offer a significant advantage in the determination of heavy metals as they allow ppb-level analysis in a relatively short time with good reproducibility. Achieved analytical performances can be improved by coupling ASV or AdSV with square wave (SWASV/SWAdSV) or differential pulse (DPASV/DPAdSV) techniques.

Regardless of the quantification method, the choice of the working electrode is a crucial factor for the overall analytical performance. In the last decade, promising results were reported in the literature with carbon-based electrodes (CBE) in various electroanalytical applications. They have become one of the main alternatives to hanging mercury drop electrodes because they can easily be modified with numerous materials to achieve an outstanding performance without having any toxic effects. Other than being non-toxic, CBE offer many advantages like having an outstanding surface structure, versatile electrochemical properties, and low costs. There are different types of CBE with various properties that are fit for different purposes. Some of the popular ones for heavy metal analysis are glassy carbon electrodes (GCE), carbon paste electrodes (CPE), graphite electrodes (GrE), and screen-printed carbon electrodes (SPCE). They all have unique properties, and they allow the user to conduct multi-element analysis in diverse matrices such as wastewaters, food samples, and environmental water samples.

In the scope of this thesis, two CBE with two particularly distinct structures were studied: pencil graphite electrodes (PGE) and SPCE. Both electrodes were utilized in different ways to detect several metals of interest: with PGE lead, cadmium, and zinc were determined via SWASV in water samples collected from the Ergene basin, and with SPCE copper and iron were determined via DPAdSV in certified wastewater

reference material. Additionally, some future aspects and possibilities were considered after some studies conducted with the SPELEC instrument, which presents a brand new technique that allows for simultaneous spectrometric and electrochemical measurements. The studies related to PGE were carried out in Turkey in the laboratories of Istanbul Technical University and Mir Research and Development company, while the studies related to SPCE were conducted in the laboratories of the University of Barcelona, Spain.

When the PGE was employed as the working electrode, two main modification strategies were applied: dip-coating for multi-wall carbon nanotube (MWCNT) and Nafion, and in-situ plating for bismuth. Once the dip-coating process was complete, the electrodes were left to dry at room temperature and then used in the analysis. Bismuth was collected on the electrode surface during the stripping step along with the analytes. Moreover, when using bare SPCE, copper and iron ions were determined simultaneously in the presence of ortho-phenanthroline and $K_4(Fe)CN_6$ at a large linear range with DPAdSV technique. This method displayed a greater sensitivity toward the copper(I) o-phenanthroline complex compared to iron(II) o-phenanthroline complex. The studies carried out for this thesis also includes a literature survey, which mainly focuses on the four most preferred CBE in voltammetric heavy metal analysis: glassy carbon electrodes, carbon paste electrodes, graphite electrodes, and screen-printed carbon electrodes.

The flow of this thesis includes firstly an introduction of the thesis subject and the relevant analytical techniques. Following the introduction, an in-depth literature review is presented in Chapter 4. After the review, in Chapter 6, optimization and validation studies conducted with Nafion, MWCNT, and bismuth modified PGE to determine lead and cadmium in contaminated waters is revealed. In Chapter 7, the analytical performance of PGE modified with only MWCNT and bismuth was investigated. For this purpose method optimization and validation studies were conducted with three analytes: lead, cadmium and zinc. In Chapter 8, iron and copper determination with bare SPCE in the presence of $K_4(Fe)CN_6$ and o-phenanthroline was studied and obtained satisfying results were presented. Finally, in Chapter 9, by using the iron and o-phenanthroline complex as a model substance, perspectives on spectroelectrochemistry instrument applications are discussed. Employed methods and specific conditions are displayed separately for each study.

AĞIR METALLERİN ÇEVRESEL ÖRNEKLERDE KARBON ELEKTROTTLARLA VOLTAMETRİK TAYİNİ

ÖZET

Kurşun ve kadmiyum gibi ağır metallerin kantitatif tayini, çevresel kontaminasyon seviyelerini doğru bir şekilde değerlendirmek adına önemli bir çalışma konusudur. Ağır metaller genellikle antropolojik ve endüstriyel kaynaklardan salınırlar ve çevrede eser düzeyde bulunurlar. Bununla birlikte, ağır metaller hem çevresel ortamlarda hem de canlı organizmalarda birikme eğilimindedir. Örneğin, cıva ve kadmiyum gibi bazı ağır metaller zamanla vücutta birikir ve belirli seviyelerden daha yüksek konsantrasyonda bulunmaları canlılarda ölümcül hastalıklara sebep olur. Öte yandan, demir ve bakır gibi bazı metaller ise enzimatik tepkimelerde kofaktör görevi üstlendiklerinden vücutta belirli bir düzeyin üstünde bulunmaları gerekir ve eksiklikleri hastalıklara yol açabilir. Bu kirleticilerin analizi, yaygın olarak yüksek hassasiyet ve doğruluk ile sonuç ürettiklerinden, atomik absorpsiyon spektrometrisi (AAS) ve endüktif eşleşmiş plazma (ICP) gibi spektrometrik yöntemlerle belirlenir. Ancak, elektrokimya alanındaki son gelişmeler sayesinde, uygun yöntemler ve elektrotlar seçildiğinde elektroanalitik yöntemlerin, karşılaştırılabilir hassasiyet ve doğrulukla çok daha hızlı sonuçlar üretebildiği görülmüştür. Elektrokimyasal yöntemler, yalnızca analist için değerli zamandan tasarruf etmekle kalmaz, aynı zamanda yukarıda adı geçen spektrometrik yöntemlerden daha düşük enstrümantal maliyetlerle yerinde (on-site) ölçüm yapmaya da olanak tanır. Böylece, tercih edilen hassasiyetle ve seçicilikle elde edilen sonuçlar çok daha hızlı ve düşük bir maliyetle üretilmiş olur.

Ağır metal analizlerinde kullanılabilecek elektroanalitik yöntemler son derece çeşitlidir. Bunlar arasında voltametrik yöntemler, özellikle anodik sıyırma voltametrisi (ASV) ve adsorptif sıyırma voltametrisi (AdSV), nispeten daha kısa sürede, ppb düzeyinde, tekrarlanabilir sonuçlar üreten yöntemler olduğundan ağır metallerin belirlenmesinde son derece avantajlıdır. Sıyırma voltametrisiyle elde edilen analitik performanslar, anodik sıyırma voltametrisi veya adsorptif sıyırma voltametrisi yöntemlerini kare dalga (SWASV/SWAdSV) veya diferansiyel puls (DPASV/DPAAdSV) gibi ileri tekniklerle birleştirerek geliştirilebilirler. ASV tekniğinde temel olarak başlangıçta uygulanan potansiyel ile metaller elektrot yüzeyinde indirgenerek biriktirilir. Ardından yükseltgenme potansiyeli uygulanarak yüzeyden sıyrılırlar ve bu sıyırma esnasında ürettikleri akım ile konsantrasyonları belirlenir. AdSV tekniğinde ise biriktirme basamağında analitler yüzeye adsorbe olurlar ve sıyırma basamağında desorbe olarak elektrot yüzeyinden ayrılırlar.

Diğer yandan, çalışma elektrodunun seçilmesi, tayin yönteminden bağımsız olarak, analitik performans açısından büyük önem taşıyan bir faktördür. Geçtiğimiz on yılda, çeşitli elektroanalitik uygulamalarda karbon temelli elektrotların (CBE) kullanılması ile pek çok umut vaat eden çalışma literatürde raporlanmıştır. Karbon temelli elektrotlar, toksik etkiye sahip olmadıkları ve olağanüstü analitik performansla çok çeşitli malzemelerle kolayca modifiye edilerek ulaşabildikleri için, asılı cıva damla

elektrotlarına alternatif olarak görülmektedirler. Karbon temelli elektrotlar, toksik olmamalarının yanı sıra olağanüstü bir yüzey yapısına, çok yönlü elektrokimyasal özelliklere ve düşük maliyete sahip olmaları gibi birçok avantaja sahiptirler. Farklı amaçlara uygun, çeşitli özelliklere sahip farklı karbon elektrot türleri piyasada mevcuttur. Ağır metal analizi için sıklıkla tercih edilen bazı karbon temelli elektrotlar arasında camı karbon elektrotlar (GCE), karbon pasta elektrotları (CPE), grafit elektrotlar (GrE) ve ekran baskılı karbon elektrotlar (SPCE) yer alır. Hepsinin benzersiz özellikleri vardır ve kullanıcının atık sular, gıda numuneleri ve çevresel su numuneleri gibi çeşitli matrislerde çoklu element analizi yapmasına olanak tanır.

Bu tez kapsamında, birbirlerinden farklı yapılara sahip iki adet karbon temelli elektrot ile çalışılmıştır: kalem ucu grafit elektrotlar (PGE) ve ekran baskılı karbon elektrotlar (SPCE). Kalem ucu elektrotlar son derece düşük maliyetli ve her yerde bulunabilen bir elektrot olmasına rağmen grafit yapısında olduğundan son derece üstün yüzey özelliklerine sahiptir. İletken ve inert yapıdaki grafit, aynı zamanda geniş bir yüzey alanına da sahiptir ve kolayca modifiye edilerek çeşitli özellikler kazandırılabilir. PGE ile çalışmanın temel zorluğu tekrarlanabilir sonuçlar elde etmek için temas yüzeyinin ayarlanması ve sabit tutulması gerekliliğidir. SPCE da ise tekrarlanabilirlik çok daha yüksektir ve yapısı gereği yüzey alanı sabit ve belirlidir. Yüzeyinde referans elektrotu, karşıt elektrotu ve çalışma elektrotunu bir arada bulundurur. Çeşitli malzemelerden üretilebilir ve yüzeyi bazı tekniklerle kolayca modifiye edilebilir. Her iki elektrot da çeşitli ağır metalleri tespit etmek için farklı şekillerde kullanılmıştır: Ergene havzasından toplanan su numunelerinde PGE ile kurşun, kadmiyum ve çinko SWASV yöntemiyle tayin edilmiştir ve SPCE ile sertifikalı atık su referans materyalinde DPAdSV ile bakır ve demir tayin edilmiştir. Ek olarak, kullanıcının eşzamanlı olarak spektrometrik ve elektrokimyasal ölçümler yapmasına izin veren, yakın zamanda piyasaya sürülmüş SPELEC cihazı ile tamamlanan bazı deneyler ile gelecekte yapılabilecek muhtemel çalışmalar ve çeşitli olasılıklar değerlendirilmiştir. PGE ile ilgili deneyler Türkiye’de, İstanbul Teknik Üniversitesi ve Mir Araştırma ve Geliştirme firmasının laboratuvarlarında tamamlanmış, SPCE ile ilgili deneyler ise İspanya’da, Barselona Üniversitesi laboratuvarlarında gerçekleştirilmiştir.

Çalışma elektrodu olarak PGE kullanıldığında iki ana modifikasyon stratejisi uygulanmıştır: çok duvarlı karbon nanotüp (MWCNT) ve Nafion için daldırma ile yüzey modifikasyonu ve bizmut için in-situ kaplama. Daldırma işlemi için MWCNT ve Nafion etanol içinde ultrasonikasyon yardımıyla dağıtılmıştır. Ardından, bu süspansiyonun içine PGE süspansiyona yüzeyine dik bir şekilde daldırılmıştır. Daldırma işlemi tamamlandıktan sonra, elektrotların analizlerde kullanıma hazır hale gelmesi için, elektrotlar oda sıcaklığında kurumaya bırakılmıştır. Bizmut ise elektrot yüzeyine, diğer analitlerle birlikte, sıyırma işlemi sırasında, sisteme yapılan sabit potansiyel uygulamasıyla toplanmıştır. Diğer yandan, çıplak SPCE kullanıldığında, ise eş zamanlı bakır ve demir iyonlarını tayini o-fenantrolin ve $K_4(Fe)CN_6$ varlığında DPAdSV tekniği kullanılarak gerçekleştirilmiştir. Bu yöntemin o-fenantrolin ile kompleks haline getirilmiş bakıra (Cu(I)) olan hassasiyetinin o-fenantrolin ile kompleks oluşturmuş demire (Fe(II)) olan hassasiyetinden daha yüksek olduğu gözlemlenmiştir. Tez için yürütülen çalışmalar aynı zamanda bir derinlemesine bir literatür incelemesi de içermektedir. Bu literatür incelemesi, çoğunlukla voltametrik ağır metal analizlerinde en çok tercih edilen dört karbon temelli elektrot üzerine yapılan çalışmalara odaklanmaktadır: camı karbon elektrotlar, karbon pasta elektrotlar, grafit elektrotlar ve ekran baskılı karbon elektrotlar.

Bu tezin akışı öncelikle tez konusunu ve kullanılan analitik tekniklere ait teorik bilgileri içeren bir girişı içerir. Girişin ardından, dördüncü bölümde kapsamlı bir literatür taraması sunulmaktadır. Ardından altıncı bölümde Nafion, MWCNT ve bizmut ile modifiye edilen kalem ucu grafit elektrot kullanarak eş zamanlı kurşun ve kadmiyum analizi için geliştirilen voltametrik metotun optimizasyon ve validasyon çalışmaları anlatılmıştır. Yedinci bölümde, yalnızca MWCNT ve bizmut ile modifiye edilmiş kalem ucu grafit elektrot kullanarak tamamlanan eş zamanlı kurşun, kadmiyum ve çinko analizine ait modifikasyon ve metot optimizasyonu ve metot validasyonu çalışmaları aktarılmıştır. Sekizinci bölümde ise $K_4(Fe)CN_6$ ve o-fenantrolin varlığında çıplak SPCE kullanılarak eş zamanlı, voltametrik demir ve bakır analizi için geliştirilen voltametrik yöntemle ait metot optimizasyonu ve validasyonu çalışmaları anlatılmış ve üzerine tartışılmıştır. Son olarak dokuzuncu bölümde ise demir ve o-fenantrolin kompleksini model alarak spektroelektrokimyasal cihaz uygulamaları üzerine derinlemesine bir inceleme yer almaktadır. Kullanılan yöntemler ve özel koşullar her çalışma için ayrı ayrı açıklanmıştır. Sonuç kısmında ise tamamlanan tüm araştırmalar ve deneyler üzerine genel bir inceleme yapılmış ve tez çalışması genel bir çerçevede yorumlanmıştır.

Sonuç olarak, bu tez çalışmasında çevre üzerinde farklı etkilere sahip çeşitli ağır metallerin hızlı ve doğru sonuç üreten voltametrik yöntemlerle nicel tayini üzerine araştırmalar yapılmıştır. Elde edilen verilerin ışığında, literatüre karbon temelli elektrotların modifikasyonu ve bu modifiye elektrotların çeşitli yöntemlerle kullanılması hakkında katkı sağlanmıştır.



1. INTRODUCTION

Heavy metals are often released to the environment via anthropological sources. Their effects on living beings vary according to their structures and chemical interactions. Among them lead and cadmium pose a great threat to many organisms, even in trace amounts. These toxic metals tend to accumulate in different parts of the body and harm vital organs and systems. Cadmium is known to have carcinogenic effects on humans and it damages the respiratory and skeletal system too [1]. Lead mainly causes neurological damage, but it also impairs cardiovascular and gastrointestinal systems [2]. Because of these harmful impacts, their concentrations are limited to certain concentration levels in drinking waters, and these levels need to be monitored regularly. Contrary to lead and cadmium, some other metals are essential and can be allowed at low ppm levels in drinking waters like zinc, copper, and iron [3]. Essential metals tend to be involved in certain metabolic and enzymatic reactions in many organisms and their deficiency may cause growth inhibition or various other complications. Therefore, environmental concentrations of these metals should be observed and controlled periodically.

Because of the sensitivity and accuracy they provide, the most common techniques to monitor the the concentrations of heavy metals are spectrometric methods such as atomic absorption spectrometry (AAS) and inductively coupled plasma (ICP) [4]. Even though these methods provide reliable results, they involve very expensive instruments with sophisticated structures that require expert knowledge to operate. Moreover, the instrumentation of AAS and ICP are often made up of large and heavy parts. Thus, they cannot be moved around and it is necessary to transport the samples to the lab and store them properly until the time of analysis, which in the end takes up a lot of time. Alternatively, electrochemical methods, such as voltammetry, are portable, user-friendly, and relatively cheap [5]. Electrochemical methods are also quite versatile with various types of electrodes and techniques that they offer. Among the many options of electrochemical methods for heavy metal determination, voltammetric methods seem to be a great fit the purpose and provide satisfying results.

During a voltammetric analysis using a pre-concentration step followed by a stripping step is a common practice that allows for a highly sensitive determination in various matrices. Basically, this method involves concentrating the analyte on the electrode surface by applying a certain voltage for some time and then by changing the applied potential gradually, analytes leave the surface at a characteristic potential value. The current that they generate during this separation is proportional to their concentration. Depending on the mechanism of pre-concentration and stripping, stripping voltammetry has three main types: anodic, cathodic, and adsorptive [6]. In Anodic stripping voltammetry (ASV) analytes are reduced onto the electrode surface and then oxidized back to the solution during stripping. In cathodic stripping voltammetry (CSV) the process works in the opposite direction as the analytes are oxidized during pre-concentration and reduced during stripping. In adsorptive stripping voltammetry (AdSV) the analytes would be adsorbed on the electrode surface during pre-concentration and released during stripping. Hence, ASV and AdSV are common voltammetric approaches for trace determination of heavy metals. They can also be coupled with square wave (SW) and differential pulse (DP) techniques to obtain even more superior results.

Moreover, finding the right type of electrode for the analysis is a crucial factor that determines the analytical performance of the chosen method. For many years scientists used mercury electrode, because it provided excellent data for diverse types of analytes in various sample media. However, due to the harmful impacts of mercury electrodes, researchers focused on finding substitute electrodes that could provide satisfying results [7]. Subsequently, it was discovered that carbon-based electrodes (CBE) were non-toxic alternatives to the mercury electrodes that possessed outstanding surface and electrochemical properties [8]. Also, by making use of the allotropes of carbon, even more desirable features can be attained.

For voltammetric heavy metal determination, some of the most popular types of CBE are namely glassy carbon electrodes (GCE), carbon paste electrodes (CPE), graphite electrodes (GrE), and screen-printed carbon electrodes (SPCE). In the scope of this thesis, two types of disposable CBE were investigated: pencil graphite electrodes (PGE) and SPCE. PGE are ubiquitous, cheap, and easy-to-use. They maintain the benefits that graphite materials possess, as they hold a cost-effective advantage too. Recent studies show that modified PGE is capable of detecting heavy metals at low

ppb levels [9,10]. Similarly, SPCE are also easy-to-use. Their main advantage lies in the uniformity of their surface and the fact that they have a known, constant surface area. There are plenty of studies that both modified and unmodified SPCE can perform trace level quantification of heavy metals [11-14].

This thesis focused on simultaneous analysis of lead, cadmium, and zinc via two modified variations of PGE, along with determinations of iron and copper with bare SPCE. Detection of the abovementioned analytes were achieved with high sensitivity in a wide linear range. Necessary modifications were accomplished with simple strategies and in relatively short times. SWASV method was utilized when using PGE, while DPAdSV method was employed with SPCE. Overall low detection values and good reproducibility values were observed with both electrodes and techniques, and the analytical performances were not affected by the presence of interfering ions. The proposed methods were also tested with certified reference materials and environmental water samples via application of standard addition. The generated results were in compliance with the certified values and the comparative measurements acquired with graphite furnace atomic absorption spectrometry (GFAAS). Thus proving that these methods were fit to analyze several heavy metals simultaneously in complex environmental media with satisfying reproducibility, sensitivity and accuracy.



2. HEAVY METALS

Heavy metals are elements with high atomic weights and they reside between the 4th and 6th periods of the periodic table. Even though they are all naturally occurring pollutants with various toxic properties, they all have different characteristics and uses in various industrial applications. Some of them exhibit carcinogenic and toxic properties at certain levels, while some of them are essential metals and are necessary for healthy growth in organisms. Their concentrations in the environment are usually determined with spectrometric methods, such as atomic absorption spectrometry and inductively coupled plasma, due to the sensitivity and accuracy they provide. Nevertheless, electrochemical methods seem to provide similar benefits in shorter time and with lower costs. In the scope of this thesis, five heavy metals with unique properties have been examined: lead, cadmium, zinc, iron, and copper.

2.1 Lead

Lead is one of the most extensively investigated heavy metals so far. Yet, it still has not lost its significance throughout the years. It has been used in various industrial and anthropologic applications: from plumbing and fuels in the past to ammunitions and lead-acid batteries today [15]. More specifically, lead can penetrate to environmental media via applications of metal plating, dye pigment additives, ore smelting, and industrial wastes [16]. This widespread exploitation of lead has caused large-scale contaminations all around the world.

The most common path for lead intake is from respiratory and digestive systems but it is also possible to absorb through skin [17]. Lead exposure harms neurological, respiratorial, and cardiovascular systems in human body. Lead toxicity is mainly observed through biochemical processes that involve calcium, which is a cofactor in many cellular activities. It can even replace calcium in the bone structure and mimic its actions in several vital processes as well [18]. Some other toxic effects include complications with oxidative stress, inter- and intra-cellular signalling, ion transfer, and protein folding [16].

2.2 Cadmium

Cadmium is a silver white metal that is resistant to corrosion and insoluble in water, except for some of its salts. It is mostly used in alloys, coatings, and in batteries. It is known for being rapidly oxidized in air. Furthermore, cadmium emissions mostly originate from anthropogenic sources like smelting and municipal waste incineration. It is also emitted to air via coal combustion and nonferrous metal production [19]. Once cadmium is emitted to water or soil, it is likely to accumulate in foodstuff such as plants, seeds, and shellfish [20]. Moreover, it tends to accumulate in different parts for different plants [21].

Cadmium is known to be toxic to organisms at all concentration levels with a long biological half-life. In human body, it accumulates the most in liver and kidneys [19] and displays carcinogenic effects. Therefore, long time exposures cause severe renal damage and even result in kidney failures. Besides the nephrotoxicity, some cardiovascular diseases and respiratory complications are also observed, even though reportedly respiratory symptoms can be reversed when the exposure stops [22].

2.3 Zinc

Zinc is an essential type of heavy metal that acts as cofactor and is necessary for many vital functions in the human body like tissue repair and growth [23]. However, excess zinc would cause toxic effects that may result in nausea, vomiting, and lethargy [24]. Excess zinc can be emitted to the environment via industrial activities and poor waste management strategies. It is also possible to be exposed to zinc via inhalation, in which case zinc would cause inflammation in the respiratory tract [25]. On the other hand, its deficiency would inhibit growth, damage the immune system, and cause appetite loss [26].

Additionally, zinc may interact with other metals [27] and organic substances [28] in the environment, which in turn affects many biological activities. Zinc maybe released to the environmental via both anthropological and natural routes. Once it is released, it is more likely to stay in the soil, and in the water bodies it tends to be adsorbed on sediment surfaces. Zinc does not accumulate in plants but it is known to bioconcentrate in aquatic organisms; mainly in crustaceans and bivalve species [29].

2.4 Iron

Being the second most abundant metal on earth's crust, iron is a vital element for the growth of most of the complex organisms. It serves many crucial purposes such as contributing to processes of oxygen carrying proteins, various biological reductions and oxidations, and involving in enzymatic reactions [16]. Monitoring the environmental concentrations of iron is significant because it is involved in many biological cycles and processes [30]. Soluble iron can be found in the form of Fe(II) and Fe(III) and the transition between these ions is a vital factor for the many life forms in environmental waters [31,32].

As opposed to other notorious heavy metals, iron is a necessary element and its toxic properties are only observed at high concentrations, while its deficiency might be fatal. Lack of iron eventually may lead to anemia, which is a disease linked to decrease in red blood cells and causes some other complications as well [33]. On the other hand, in the case of excess iron, generation of reactive oxygen species and tissue damage can be observed [34].

2.5 Copper

Copper is an essential element with reddish shiny color. As a malleable metal with high heat and electrical conductivity, it is commonly used in wires, plating and coin making. Similar to iron, copper is crucial for many enzymatic reactions in human body, yet, in the case of excess exposure hepatocellular degeneration and necrosis [35] as well as some psychiatric problems like anxiety and insomnia [36]. Copper is known to be involved in several redox reactions, which lead to the generation of several reactive oxygen species. Thus, its toxicity also extends to its ability to break down DNA strands, and it can achieve that even more than iron ions can [35].

In the environment, copper ions are reportedly mobile [37,38]. It is mainly released to the environment via natural occurrences like dusts blown by winds and flora decay. It can also be discharged by anthropological sources such as municipal solid waste and burnt fossil fuel [39]. Even though copper plays significant roles in nature by contributing to vital processes like photosynthesis [40], it can also interfere with metabolic activities of the microorganisms and may depreciate the decomposition of organic matter [41].



3. VOLTAMMETRIC TECHNIQUES FOR HEAVY METAL DETERMINATION

3.1 Instrumentation

Electrochemical processes generally do not require extremely complicated or expensive instrumentation. The devices are relatively cheaper and more user friendly compared to methods like AAS. The fundamental instruments for electrochemical applications are galvanostats and potentiostats. Galvanostats keep the current constant while observing the changes in potential. Contrarily, potentiostats control the voltage and observe the changes in current; it is commonly used in many electroanalytical applications. On the other hand, these devices can be combined and commercialized as potentiostats/galvanostats. Moreover, a more recent technology involves bringing two different measurement techniques together to enable applications of spectroelectrochemistry. Spectroelectrochemical applications can be conducted by coupling a potentiostat to a spectrometer or using a spectroelectrochemical device, which is specifically designed for such purposes.

3.1.1 Potentiostat

In general sense, the potentiostat is an instrument that controls the potential of the working electrode in an electrochemical cell and measures the current response generated in return [42]. It is not only used in electroanalytical applications, but also in corrosion, batteries, plating, and electrocatalysis studies. In the electroanalytical applications, it works by connecting three electrodes (reference, counter, and working electrode) as a complete system. Potentiostats are quite versatile, they can work with many techniques like pulse, stripping, voltammetric, and potentiometric techniques. Some brands allow the user to combine various techniques or add modules to conduct more complicated procedures. As an example, if the potentiostat does not already possess these features, extra modules can be added to the system to conduct electrochemical impedance spectroscopy, to control and observe reactions kinetics,

and even to carry out two different procedures in two different cells simultaneously. A potentiostat and its modules are usually controlled by a user-friendly software from a computer and there would only be cable connection points and an ON/OFF button on the instrument.

3.1.2 Spectroelectrochemical instrument

Combining spectrometric and electrochemical measurements in one instrument and carrying them out simultaneously is relatively a new concept. A spectroelectrochemical instrument consists of a potentiostat/galvanostat along with a spectrometer. This technique works with all three electrodes that is printed on a screen-printed electrodes and it gathers both spectrometric and electrochemical data simultaneously [43]. There are several modes of measurement in spectroelectrochemistry that can be applied to diverse analytes and matrices. If the method calls for a transparent electrode, the most common electrode choice is polyethylene terephthalate coated with indium tin oxide. However, there are times when the required electrode cannot be transparent. In this case, reflection mode should be chosen, which measures the light reflected from the electrode [44]. Thanks to their reflective surface, gold electrodes can be employed in reflection mode. Considering the high cost of gold electrodes SPCE can be an alternative choice, even though they produce lower counts. A scheme of a reflection cell setup is presented in Figure 3.1.

There are also various cells manufactured for spectroelectrochemistry depending on the requirements of the chosen method. For instance, there are cells that only require a small amount of sample (app. 100 μL) to be compressed between the electrode and the light source/detector. This type of cell is convenient for determination of non-fluorescent substances. If the analyte is to be determined by its fluorescent properties, then a cell that measures the emitted light with a 90° diversion from the light source should be chosen.

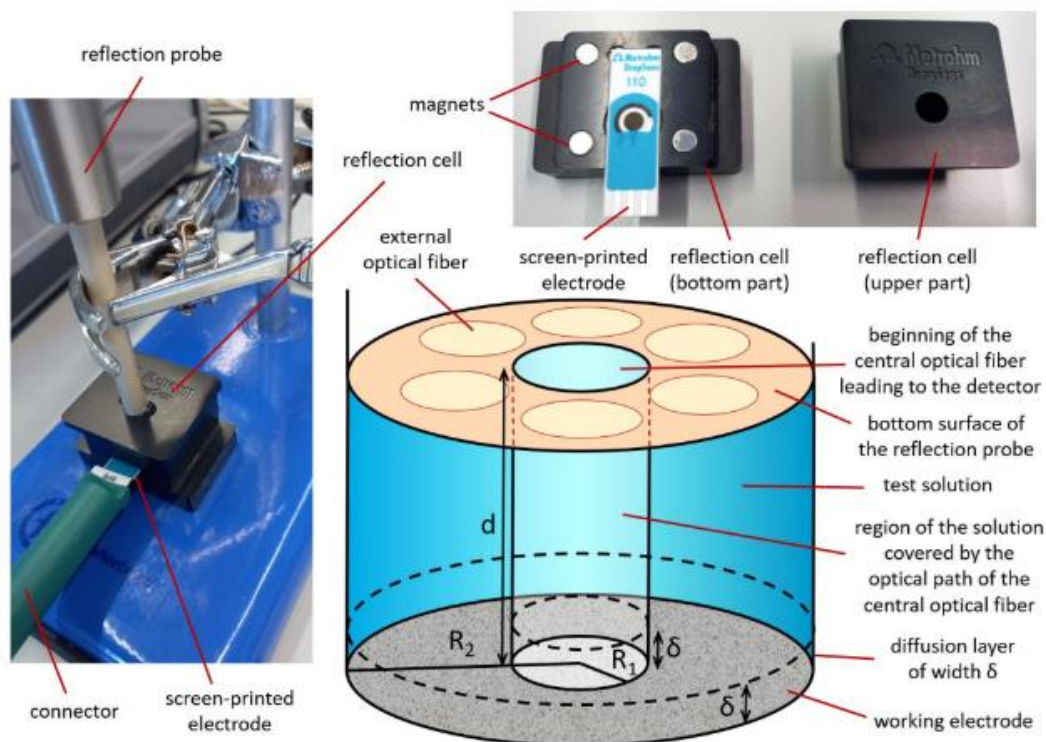


Figure 3.1 : Scheme of a reflection cell setup showing the space comprised between the optical probe and the screen-printed unit. The sizes of the central and the six external optical fibres and the diffusion layer have been, so that in practise $R_1 \ll R_2$ and $\delta \ll d$. The light entering the central fiber comes from the reflection of the light provided by the optical fibres surrounding the central one, which cover a radius quite bigger than R_1 .

3.2 Electrodes and Electrochemical Cell

The electrochemical cell contains the electrodes and the working solutions and it is where all the reactions take place during the analysis. Modern electroanalytical procedures often use a three electrode system with a reference electrode, a counter electrode, and a working electrode. Choosing the right type of electrodes for the desired analytes and techniques is significant because it directly affects the analytical performance of the method.

3.2.1 Reference electrodes

Reference electrodes provide a stable potential during reactions taking place in the cell so that the only change in the measured potential or current would be caused by the analyte. Standard hydrogen electrodes can be considered as a basic type of reference

electrode with a declared potential of 0 V at any temperature, half-cell potential of other elements are calculated in reference to hydrogen electrodes [45]. Main drawbacks of this electrode are first, they are very fragile and second, ionization of generated gas may complicate the redox reactions [46]. Saturated calomel electrodes, on the other hand, does not involve any gas formation and more practical to use. It is known to provide a highly reproducible potential by the reversible reaction of solid calomel (Hg_2Cl_2) and liquid drop of mercury (Hg), in the presence of saturated KCl [47]. Finally, the most commonly used reference electrode in the recent applications of electrochemical systems is silver/silver chloride (Ag/AgCl) electrode. In this type of electrode, Ag/AgCl wire is kept in concentrated KCl solution. Similar to saturated calomel electrodes, Ag/AgCl electrodes supply a constant potential that does not fluctuate with time or temperature. Due to its simple and stable structure it can also be miniaturized and used in microelectrode applications [48].

3.2.2 Counter electrodes

Counter electrodes can also be referred to as auxiliary electrodes and they complete the electrical circuit so that the electrodes can flow freely in the system [49]. They are generally made from inert substances like platinum so that they do not interfere with the chemical reactions taking place in the electrochemical cell. Since the current flows between the working and counter electrodes, the surface area of the counter electrode has to be significantly larger than the working electrode. Thus, the polarization of the counter electrode would be avoided and the possibility of the size of the counter electrode to be a limiting factor in the kinetics of the reactions taking place in the cell would be eliminated [50].

3.2.3 Working electrodes

The choice of working electrodes is crucial for all types of electrochemical analysis because their surface functions as an interface, where all the reactions or adsorptions related to the analysis take place. A reliable working electrode provides sensitive and reproducible results and allows working at a wide potential range [51]. Moreover, ideal working electrode should be inert under the analysis conditions, as well as being low-cost, non-toxic, and with a uniform surface. Working electrodes can be made up of

diverse substances from metals to carbonaceous materials [52]. The most popular working electrodes for heavy metal determination are mercury electrodes, gold electrodes and carbon based electrodes [7]. Gold electrodes are very useful, yet, due to some limitations like high cost and their surface structure being compromised in the positive potential range, they will not be further discussed in this section. Instead, mercury and carbon electrodes will be explained in more detail.

3.2.3.1 Mercury electrodes

Mercury electrodes have been extensively used in many electroanalytical applications for decades. They provide exceptional sensitivity and selectivity for many types of analytes. Nevertheless, due to the toxic properties of mercury the researchers started to search for more environmentally friendly and less hazardous alternatives for working electrodes.

The most commonly preferred mercury electrodes are dropping mercury electrodes (DME), hanging mercury drop electrodes (HMDE), and mercury film electrodes (MFE). Both DME and HMDE are successfully employed in numerous applications and they are known to produce sensitive and reproducible results with organic and inorganic analytes [53]. The main advantage of HMDE over DME is the fact that HMDE requires less use of mercury. Additionally, HMDE allows the utilization of techniques that are related to both adsorption and electrolytic reactions to collect the analytes on electrode surface. However, there are drawbacks to using HMDE too: the mercury drops generated should regularly be adjusted for precision, modification of HMDE is not practical, and since the mercury drops can easily be displaced, it cannot be easily transported to contamination site for environmental analysis [53]. Finally, with MFE, the amount of mercury necessary is minimized. Even though they are smaller in size, they provide larger surface areas. Due to their stable structure, they can also be modified more easily compared to abovementioned mercury electrodes and can be utilized in flow analysis [54]. Yet, they require laborious cleaning and reactivation steps and they provide comparatively poor precision and reproducibility.

3.2.3.2 Carbon-based electrodes

Carbon is an abundant material and it does not pose a threat to human health as mercury electrodes do. Using electrodes made of carbon allows for many possibilities because there are different types of allotropes of carbon that can be utilized for different purposes. CBE can broadly be categorized in two groups: homogeneous and heterogeneous electrodes. CBE like GCE, SPCE, and diamond electrode are homogeneous, while CPE and some modified electrodes are considered heterogeneous electrodes. Moreover, they are reported to provide outstanding analytical performances with a variety of analytes: from pharmaceuticals and organic pollutants to heavy metals and biochemical compounds [55]. They are also suitable for electroanalytical applications in many complex sample matrices [4,56].

Surface of CBE are often modified to achieve superior analytical performances. It is relatively easy to modify their surface but usually a conditioning or a cleaning step is necessary prior to modification, and these steps can be troublesome depending on the type of CBE [57]. It is also possible to obtain composite electrodes with outstanding properties like high stability, sensitivity, and, resolution, when materials like carbon nanotubes are used [58]. More detail on the performances and properties of popular CBE will be revealed in Chapter 4, Literature Survey. In the scope of this thesis only two types of CBE were studied: PGE and SPCE. Therefore, only these two types of CBE is further discussed in this section.

Pencil graphite electrodes

The main advantage of choosing disposable pencil graphite electrodes (PGE) is that they provide the outstanding surface properties of graphite with a minimal cost. They are quite abundant and useful. On the other hand, it might be challenging to sustain the repeatability and the reproducibility with PGE because it requires some type of special electrode design to keep the same surface area of the electrode inserted into the working solution and maintain a vertical stance. Once the constant surface area is achieved, it is possible to obtain satisfying electroanalytical results. Additionally, this feature can also be turned into an advantage, since the surface area can be adjusted by the user [59].

Moreover, the type of the pencil lead that is utilized for such electrochemical purposes makes significant differences in terms of electroanalytical determinations. The work of Kariuki [60] showed that harder pencil leads exhibit higher disorder in Raman spectroscopy and they contain slightly higher amounts of impurities like aluminum and oxygen. A more recent study from Prasertying et al. [61] also reported that 2B 0.5 cm pencil lead provided an outstanding performance in terms of reversibility with potassium ferricyanide redox reaction in cyclic voltammetry, compared to other tested types. The softness or hardness of a pencil lead is indicated by letters H and B: the letter H means hard as the letter B means black and soft. The letter indication is typically accompanied by a number to signify the degree of softness of the graphite. For example, an 4B lead is softer and leaves a darker mark on the paper compared to 3B. The order of softness would go on as $4B > 3B > 2B > B > HB$.

Finally, modifying PGE via electrochemical methods does not require a lot of effort and it is commonly practiced. Application of dip-coating is also possible and can be conducted easily. However, it is not feasible to apply methods like drop-casting or spin-coating due to the geometry of pencil leads.

Screen-printed carbon electrodes

Screen-printed carbon electrodes (SPCE) are products of a more recent technology and they are very practical and useful tools for electrochemical applications. They can be manufactured from different allotropes of carbon such as carbon nanotubes, graphite, and graphene. They contain all three electrodes (reference, counter, and working) on their surface but the user may choose to only use the working electrode of SPCE and work with different reference and counter electrodes by electrically connecting these electrodes to the system. To produce SPCE, the inks or pastes of electrodes must be

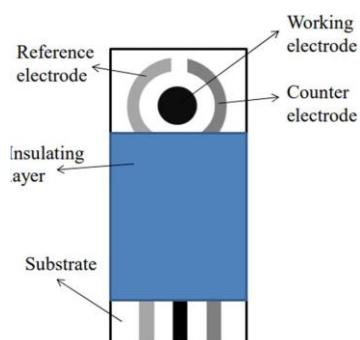


Figure 3.2 : Conventional structure of a SPCE.

printed on a surface referred to as substrate. This surface is typically made of either a polymeric material or ceramic [62]. Usually, the conventional structure of a SPCE consists of three electrodes imprinted on a substrate and an insulating layer on top that leaves two open ends for electrical connection, as represented in Figure 3.2.

The surface area of this type of electrodes are constant and they can be easily modified with various methods such as electrografting, spin-coating, and drop-casting. However, care must be taken during such modifications (especially spin-coating and drop-casting) to avoid altering the surface of reference or counter electrodes, which are placed relatively close to the working electrode in certain types of SPCE. Modified SPCE can quantify diverse types of analytes, including even the radioactive elements and bacteria [63] with satisfying sensitivity and reproducibility.

3.3 Voltammetric Techniques

Voltammetric techniques measure the generated current in an electrochemical cell when a certain potential profile is applied on the working electrode. Two of the most common voltammetric techniques that find a wide range of applications are linear sweep voltammetry (LSV) and cyclic voltammetry (CV). LSV measures the current in an electrochemical cell, while the applied potential on the working electrode is linearly swept. The resulting voltammogram presents a peak from which the analyte concentration can be integrated. It gives valuable information for both reversible and irreversible reactions and the electrochemical behaviors of the species of interest [64]. On the other hand, CV scans between two potential values in both directions, so it provides data for both oxidation and reduction processes. The voltammogram obtained from a CV scan shows the number of electrons involved in the reactions and whether or not these reactions are reversible. If the reactions are reversible the upper and lower part of the voltammogram would be symmetrical. Additionally, concentration of analytes can be calculated from the peaks. Moreover, the positions of the peaks also present significant information about the reaction kinetics: if the position of a peak shifts toward a more extreme potential due to a change made in the conditions (i.e. by changing the pH), it is said that the oxidation or the reduction occurs slower. Thus, the equilibrium could not be achieved rapidly.

Other than voltammetry there are several other methods that are employed for various types of analysis. Some of these are amperometry, potentiometry, coulometry, and chronoamperometry. In amperometry, as opposed to voltammetry, a fixed oxidizing or reducing potential is applied on the working electrode, and the generated current from the electrochemical activity of the species on the electrode surface is measured. It is commonly used in the analysis of biological samples [65]. Potentiometry measures the changes in the potential in an electrochemical cell, during a redox reaction taking place at the interface. It is an effective method that is applicable in many fields; it is even used in titrations instead of an indicator that changes color. Coulometry measures the charge necessary to carry out a redox reaction on the surface of the electrode, and the most noteworthy application is the Karl Fischer method, which is used to determine the water content [66]. Finally, chronoamperometry measures the changes in the current in a time-dependent manner while a certain potential profile is applied on the working electrode.

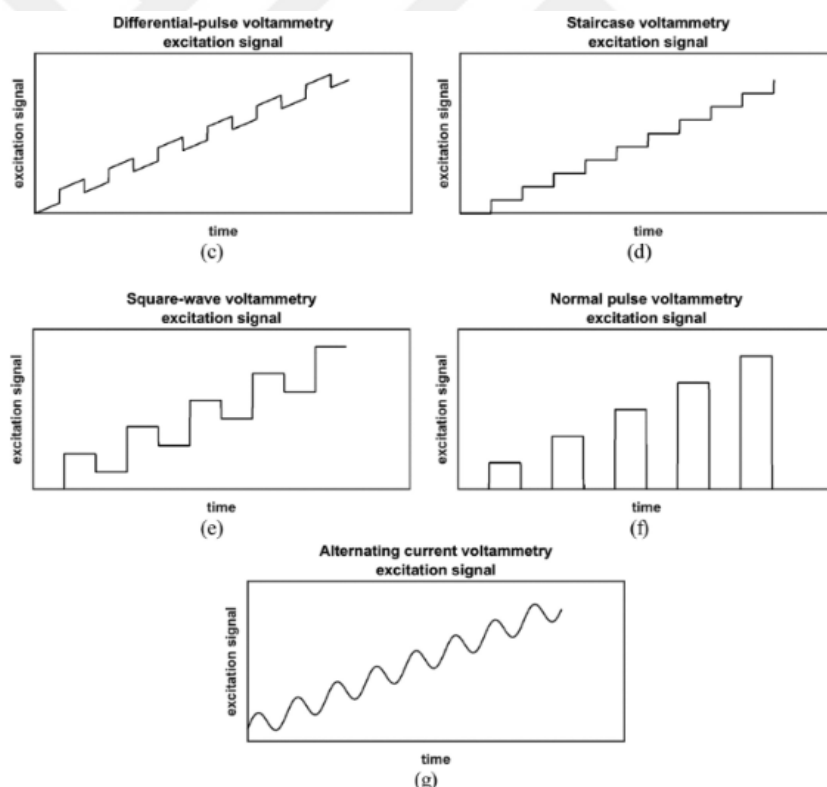


Figure 3.3 : Typical excitation signal profiles obtained from some of the common voltammetric techniques: (c) differential pulse voltammetry, (d) staircase voltammetry, (e) square wave voltammetry, (f) normal pulse voltammetry, and (g) alternating current voltammetry. Image retrieved from an open access article [67] distributed under Creative Commons Attribution License.

Voltammetric methods can also be coupled with pulse and stripping techniques to achieve superior analytical performances. Some of the common pulse and stripping techniques utilized in heavy metal analysis are described in the following section. Additionally, the typical excitation signal profiles that some of the common voltammetric methods produce are displayed in Figure 3.3.

3.3.1 Pulse techniques

The main advantage of the pulse techniques is that they offer higher sensitivities compared to methods like LSV, allowing determinations at low ppb levels. Some of the popularly used pulse techniques are described below.

3.3.1.1 Normal pulse voltammetry

In normal pulse voltammetry, voltage pulses are applied with an increasing amplitude and a fixed frequency. The current would be measured right after each pulse. The key point in normal pulse voltammetry is that the electrode surface should remain constant even though applied potential could possibly cause alterations. Therefore, the potential is left to return to its initial value after each pulse, so that the electrode surface can maintain its original structure [68]. This method can be used with both solid electrodes and HMDE or DME. Normal pulse voltammetry is usually employed for quantitative analysis, but it can also be applied for kinetics and mechanism studies.

3.3.1.2 Differential pulse voltammetry

The potential profile of differential pulse voltammetry involves starting from a base potential and linearly increasing the applied potential with 50-100 ms pulse time. Due to the small step sizes in differential pulse voltammetry, narrow peaks can be obtained and analytes with similar oxidation behaviors can easily be separated [69]. Analyte concentrations can be integrated from the peaks observed in the potential to current graphs. Differential pulse voltammetry is already a sensitive method but its sensitivity can be further improved by coupling it with stripping methods. Even though it may provide some information on the kinetics and mechanism of electrochemical reactions,

it is often used in quantitative determination. Best performances can be achieved with solid electrodes [70], yet, it is also possible to use mercury electrodes.

3.3.1.3 Square wave voltammetry

Similar to differential pulse voltammetry, square wave voltammetry starts from a base potential and applies an gradually increasing potential profile to the electrode. However, the amplitude is large with square wave voltammetry and the increases in the excitation signal appear as symmetrical square waves [70]. In many cases, resulting peaks observed in potential to current graphs are symmetrical with significant peak heights. Satisfying peak heights can be achieved with this technique, because the current is calculated as the difference between two currents generated at the forward step and the return step of the square wave. Since these measured currents have opposite signs, their absolute difference results in a larger value [68].

Square wave voltammetry provides fast results with excellent sensitivity and selectivity to diverse types of analytes, therefore, it finds applications in the determinations of pharmaceuticals, heavy metals, biomarkers, and various environmental contaminants [70]. It allows detection limits at very low ppb levels and depending on the applied method and the employed electrode, quite large linear working ranges are also available with this technique. Finally, it can be equipped with various types of electrodes: from paper-based and solid electrodes to HMDE and rotating disk electrodes.

3.4 Pre-concentration and Stripping Methods

Stripping methods involve a pre-concentration step prior to a potential sweep application that oxidizes or reduces the analytes in a selective manner. As the name infers, the pre-concentration step (also referred to as the deposition step) collects or deposits the analytes onto the electrode surface for a designated amount of time, either by anodic or cathodic or adsorptive reactions. This step often includes a constant stirring during the voltage application, and then 1-5 seconds of waiting period before the potential scan so that the working solution in the electrochemical cell would stabilize. Coupling a voltammetric technique with a stripping method significantly

increases the sensitivity and allows the user to work with enhanced analytical performance. The three main stripping methods are anodic stripping voltammetry (ASV), cathodic stripping voltammetry (CSV), and adsorptive stripping voltammetry (AdSV).

3.4.1 Anodic stripping voltammetry

Anodic stripping voltammetry is a highly sensitive and versatile method that especially finds use in heavy metal determinations. It is also applicable to small biomolecules and proteins [6]. ASV process involves application of a negative enough potential for a certain amount of time during the pre-concentration step to ensure the analyte is reduced onto the electrode surface. Similar to other stripping methods, ASV consists of three main steps. In the first one, during the pre-concentration step, analytes would be accumulated onto the working electrode under a negative potential with a fixed stirring rate. In the second step, the stirring and the potential application would come to a halt for a few second to allow the working solution to settle down. Finally, a potential scan that starts from the applied potential in the first step would occur and the analytes would cause current peak signals as they are stripped from the surface.

By using ASV, many metals can be determined with desirable sensitivity and selectivity, even in complex matrices like soils and natural waters. The method is resistant to the interferences arise from alkali metals, so it is also useful in the analysis of seawaters [6]. Such outstanding features would be even more pronounced when ASV is coupled with voltammetric techniques like square wave or differential pulse voltammetry.

3.4.2 Cathodic stripping voltammetry

It is a technique that measures the current generated during a potential scan toward more negative potentials [71]. It allows trace analysis of various types of analytes such as halides, sulphur containing organic compounds, and trace metals precipitated as hydroxides [6]. Typically, the analytes are collected to the electrode surface during the pre-concentration step and they are released back to the solution during the potential scan. The pre-concentration step typically involves anodic reactions, even precipitations [72] that occur under positive potentials.

3.4.3 Adsorptive stripping voltammetry

Adsorptive stripping voltammetry is an electrochemical technique that concentrates the analytes on the electrode by adsorption instead of a reduction or an oxidation reaction before the stripping step [73]. It can be utilized in the analysis of many analytes but mainly it is useful for large molecules and complexes. It is also quite useful in the trace analysis of metals when the metals are paired with an appropriate ligand. As described by Adeloju [74], there are three main steps in this type of metal determination. First, the metal and the ligand would bind together and the resulting complex would accumulate on the electrode surface under constant potential during pre-concentration. Second, the solution would be left to reach equilibrium by stopping the stirring and the application of potential. In the final step, the deposited complex would be stripped from the electrode surface by reduction.

The main cause for interferences in an AdSV measurement would be caused by the competition between the analytes and other substances (surface-active substances or other metals) during the complex formation. Possible solutions for such interferences involve removing the organic and inorganic impurities before the analysis by appropriate digestion techniques, using masking agents, and choosing a more selective potential for the pre-concentration step [75].

3.5 Analytical Parameters

For the validation of the method certain parameters were assessed. To begin with, linear ranges of each method were evaluated. Linear range of a method signifies the concentration range in which the obtained signals increase linearly with increasing concentration. Once this range is determined repeatability and reproducibility of the method can be investigated. Repeatability obtained with modified PGE was calculated from the standard deviations of 10 consecutive measurements taken with the same electrode, while reproducibility was calculated from the standard deviations of 10 measurements taken from 10 separately prepared electrodes. On the other hand, due to the high uniformity of bare surface of the SPCE, repeatability and reproducibility calculations were done with 5 measurements in the simultaneous determination of iron and copper.

Other important parameters that determine the analytical performance of a method are limit of detection (LOD) and limit of quantification (LOQ). LOD is the lowest possible analyte concentration that can be separated from the baseline, and in the studies conducted with modified PGE it is calculated as 3-fold of the standard deviation obtained from 10 repetitive blank measurements divided by the slope from the calibration graph, while LOQ is calculated as 10-fold of the standard deviation obtained from 10 repetitive blank measurements divided by the slope from the calibration graph. In the analysis conducted with SPCE, LOD and LOQ were calculated with a different method, where LOD and LOQ are $3\sigma/s$ and $10\sigma/s$, respectively. In this way, σ is the standard deviation of the y-intercept and s is the slope. The details of this calculation method is revealed in Chapter 8.

4. LITERATURE SURVEY¹

4.1 Brief Introduction

Among the numerous electrochemical techniques, voltammetric methods, especially Anodic Stripping Voltammetry (ASV), have proven to allow for sensitive multi-element analysis of heavy metals in a simpler and uncostly manner [77]. Voltammetric methods simply measure the generated current in a cell, as a function of applied potential during a time interval. Peaks obtained at certain potential values provide qualitative information, while peak height or area gives quantitative information. Modern voltammetric methods tend to utilize a three-electrode system including a working electrode, counter electrode, and reference electrode. The accuracy and sensitivity of voltammetric methods depend highly on the working electrode on which the analytes are either oxidized or reduced. Recent studies discovered that carbon-based electrodes offer a huge potential for effective heavy metal analysis and can even replace mercury electrodes since they provide a less toxic alternative.

For a long time, hanging drop mercury electrodes (HDME) were commonly preferred in electrochemical analysis of various types of analytes, including heavy metals. The reason why HDME was frequently preferred is that they provided outstanding analytical performance. Locatelli and Torsi [78] managed to detect and quantify 5 metals (copper: Cu, lead: Pb, cadmium: Cd, zinc: Zn, and antimony: Sb) simultaneously with an HDME and achieved LOD levels between 0.009–0.096 $\mu\text{g g}^{-1}$ in food samples, even though there were some overlapping peaks. In a more recent study, Melucci et al. [79] were able to determine Cu, Pb, Cd, and Zn in tea matrices by exploiting HDME along with the standard addition method. Even when mercury was used as a modifier, it was reported to increase the sensitivity of the electrode. Ping et al. [80] asserted that modifying glassy carbon electrode with mercuric oxide allowed them to determine lead and cadmium with 0.08 and

¹This chapter is based on the paper “Ustabasi, G., Ozcan, M., Yilmaz, I. (2021). Review – Voltammetric Determination of Heavy Metals with Carbon-Based Electrodes, *Journal of the Electrochemical Society*, 168, 097508.” [76]

0.05 $\mu\text{g L}^{-1}$ detection limits, respectively. Nevertheless, due to the toxic nature of mercury, recent studies started to search for more eco-friendly materials [81]. Carbon, being an abundant, nontoxic, and versatile element, was found to be extremely beneficial in terms of heavy metal analysis. In that regard, carbon could be used either on its own, in powder form, to create carbon paste electrodes or its allotropes (especially graphite and graphene) could be employed, since their vast properties (such as conductivity, high surface area, etc.) make them more attractive materials for electrochemical purposes. Their adsorptive behavior towards heavy metals is also noted in the literature and can be considered as a significant indicator for their interactions [82,83].

In terms of voltammetric analysis of heavy metals by carbon-based materials, some highly promising results have been reported in previous studies: Hwang et al. [84] compared how carbon nanotube (CNT), glassy carbon, activated carbon, and graphite performed in simultaneous Pb, Cd, Zn analysis after modifying their surfaces with Bismuth (Bi), and found the CNT to be the most sensitive towards chosen metals. Obtained LOD values for Pb, Cd, and Zn were 1.3, 0.7, and 12 $\mu\text{g L}^{-1}$, respectively. Aragay et al. [85] determined Cu, Pb, Cd, and mercury (Hg) in seawaters using heated graphite nanoparticles (NP) as the working electrode, with a linear range of 5–100 $\mu\text{g L}^{-1}$ for Pb, Cd, and Cu, and 1–10 $\mu\text{g L}^{-1}$ for Hg. Lastly, Ashrafi et al. [86] were able to reach quite low LOD values in wheat flour certified reference material (CRM) (0.77 $\mu\text{g L}^{-1}$ for Cd and 0.65 $\mu\text{g L}^{-1}$ for Pb) by employing a nanocomposite electrode consisted of multi-walled carbon nanotube (MWCNT) modified with Sb NP.

4.2 Carbon Based Electrodes: Types, Properties, and Applications

There are various types of carbon-based electrodes (CBE); however, this study mainly focuses on the four most common types of CBE in heavy metal analysis: glassy carbon electrodes (GCE), carbon paste electrodes (CPE), graphite electrodes (GrE), and screen-printed carbon electrodes (SPCE). This categorization does not necessarily rely on the electrode shape or constituents, but rather on their production methods. Therefore, when CPE or SPCE are mentioned in the text, CPE or SPCE that contain graphite in their structure are also included, even though GrE are discussed in a separate section. From here on in the text, electrodes that are made of graphitic rods

fall into the category of GrE, while electrodes that are made of graphite powder to obtain ink or paste may fall either into CPE or SPCE.

CBE not only provides low-cost, rapid multi-element analysis but also allows the user to work with a wide range of methods and matrices. Table 4.1 summarizes the recent studies employing CBE for heavy metal analysis in diverse sample matrices. As it is evident from the table, when dealing with heavy metal analysis ASV dominates other methods. In ASV, metals undergo reduction during deposition and experience anodic dissolution during desorption. Basically, ASV involves a pre-concentration step, which accumulates the positively charged heavy metals on the electrode surface either via Faraday's reaction or adsorption. When Faraday's reaction takes place, heavy metal ions first undergo reduction under the influence of constant negative potential and reach zero charge. Then during the dissolution phase heavy metals are oxidized again, while the potential moves in the anodic direction. In the case of adsorption, reduction of heavy metals onto the electrode surface is actualized by ligands that can form complexes with the analytes [4]. ASV is usually utilized in combination with the square wave (SW) and differential pulse (DP) methods, which makes it more efficient. Utilizing SWASV for heavy metal determination at ppb levels has been a common practice in literature throughout the years [10,84,87,97,106,107]. It is also possible to reach ppb levels with DP-ASV [90,108,109], but usually SW-ASV provides better resolution [9]. In most studies, results obtained from voltammetric methods are usually validated by AAS and ICP measurements. Even though these established spectrometric methods require a long time for determination, do not allow on-site analysis, and generate high operational costs, they are commonly utilized for heavy metal analysis because they provide high accuracy and low LOD values. However, with the recent advances in the field, voltammetric methods provide similar benefits without demanding the high cost, the long time, and the obligation of immobility. These advantages are more emphasized when voltammetric methods are coupled with CBE; which are diverse, versatile, and efficient.

Table 4.1 : Summary of several studies determining heavy metals with four common types of CBE (carbon based electrodes). (SWASV: square wave anodic stripping voltammetry, DPASV: differential pulse anodic stripping voltammetry, ASV: Amodic stripping voltammetry, CV: cyclic voltammetry, LSASV: linear sweep anodic stripping voltammetry, LSV: linear sweep voltammetry).

Electrode	Method	Analyte	Matrix	Results	References
GCE	SWASV	Pb, Cd	Tap water	Interferences caused by Cu were eliminated by adding potassium hexacyanoferrate.	[87]
	SWASV	Zn	Food samples ultra-sonicated in water	Analytical parameters were observed to be improved during simultaneous measurements with Pb and Cd.	[88]
	SWASV	Cd, Pb, Hg, Zn, Cu	(Spiked) Tap and lake water and rice samples	Measurements were in agreement with ICP and AFS results.	[89]
	DPASV	Pb, Cd, Zn, Cu	River samples	In-situ coating GCE surface with Bi-film led to higher stability and repeatability as well as low LOD.	[90]
	DPASV	Pb, Cd, Zn	Tap water	GCE surface modified with graphene-Bi film allowed for simultaneous analysis of Pb, Cd, and Zn with high sensitivity and low cost. At high analyte concentrations, IR drop was observed due to the lowered reversibility of oxidation.	[91]

Table 4.1 (continued) : Summary of several studies determining heavy metals with four common types of CBE (carbon based electrodes). (SWASV: square wave anodic stripping voltammetry, DPASV: differential pulse anodic stripping voltammetry, ASV: Amodic stripping voltammetry, CV: cyclic voltammetry, LSASV: linear sweep anodic stripping voltammetry, LSV: linear sweep voltammetry).

Electrode	Method	Analyte	Matrix	Results	References
GrE	ASV	Pb, Cd	Herbal food supplements	In the case of Cd, graphite electrodes obtained from waste batteries provided results that complied with AAS, however, results were not accurate for Pb.	[92]
	ASV	Pb, Cd	UHT milk	Results were compatible with the ones obtained via AAS. High analytical performance with low cost.	[93]
	ASV	Pb, Cu	Bovine seminal plasma	Results conform with ICP-AES measurements.	[94]
	SWASV	Pb, Cd	CRM water	Presence of Cu caused interferences.	[9]
	SWASV	As, Hg, Pb	Industrial water samples	Bi-modified exfoliated GrE provided sensitive and reproducible results, which also correlated with ICP-OES.	[95]

Table 4.1 (continued) : Summary of several studies determining heavy metals with four common types of CBE (carbon based electrodes). (SWASV: square wave anodic stripping voltammetry, DPASV: differential pulse anodic stripping voltammetry, ASV: Amodic stripping voltammetry, CV: cyclic voltammetry, LSASV: linear sweep anodic stripping voltammetry, LSV: linear sweep voltammetry).

Electrode	Method	Analyte	Matrix	Results	References
CPE	CV	Sn(II)	Aqueous solutions	Relatively easy, green, and cheap. Dichromate, permanganate, iodate, and bromate caused interferences.	[96]
	SWASV	Pb, Cd	Well water	High selectivity and repeatability. Presence of Cu causes interferences by forming intermetallic species with Pb, Cd, and Zn. Adding ferricyanide diminished this affect.	[97]
	LSASV	Pb, Cd	Aqueous solutions	Carbon paste modified with MWCNT and 3% Sb ₂ O ₃ provided a better analytical performance. Higher sensitivity was observed with simultaneous measurements.	[98]
	SWASV	Hg (II)	Tap water	Graphite based CPE modified with EDTA allowed for a low LOD (8.6 x 10 ⁻⁹ M), whit 5 min preconcentration time.	[99]
	SWASV	Pb, Cd, Zn, Cu, Ag	Industrial wastewater	CPE modified with Cr ₂ O ₃ allowed for sensitive determination of 5 metals, simultaneously. They also developed a SPCE from the same CPE and observed similar LOD and linear range values (except for Cd and Zn).	[100]

Table 4.1 (continued) : Summary of several studies determining heavy metals with four common types of CBE (carbon based electrodes). (SWASV: square wave anodic stripping voltammetry, DPASV: differential pulse anodic stripping voltammetry, ASV: Amodic stripping voltammetry, CV: cyclic voltammetry, LSASV: linear sweep anodic stripping voltammetry, LSV: linear sweep voltammetry).

Electrode	Method	Analyte	Matrix	Results	References
	SWASV	Cd, Zn	Soil samples	Sequential detection of Cd and Zn was made possible by covering the surface of modified electrode (graphene oxide/graphitic carbon nitride) first with Bi then with Ga via pH adjustments.	[101]
	DPASV	Cd, Pb	Estuarine water samples	Even though exfoliated Bi modification on carbon electrode surface was heterogeneous, due to the exploitation of drop casting, high trueness and reproducibility was achieved in CRM and sample measurements.	[102]
SPCE	SWASV	Tl(I)	Various water CRMs	Obtained signals were highest when Bi-film was applied on carbon electrode. Addition of EDTA significantly decreased the interferences caused by Pb and Cd. Amberlite XAD-7 resin was found to be effective for removing organic content in samples.	[103]
	SWASV	Cd, Zn	Tap water and bottled water	Addition of Ga and/or potassium ferrocyanide was helpful for elimination Cu interferences.	[104]
	LSV	Cr(VI)	River water samples	Sn NP modification allowed achieving sub-ppb level analysis. Surface of the working electrode was modified with Au NP to enhance selectivity and sensitivity. Obtained portable system provided results that were in line with AAS.	[105]

Even though CBE offer many advantages in heavy metal analysis they are not exempt from interferences. Some modification methods may lower or prevent some extent but usually heavy metals interfere with each other and cause combined peaks, lowered signals, and compromised accuracy. On the other hand, standard addition method is employed to overcome such effects. Studies that applied this method frequently report accurate results and well separated distinct peaks in voltammograms [9,110-112].

4.2.1 Glassy carbon electrodes

GCE are one of the most common electrodes used in sensing and quantifying heavy metals. They are produced by the pyrolysis of polymeric resins and are unreactive, resistant to high temperature, and impermeable to gasses and liquids [113]. They provide excellent analytical performance with a wide range of metals and are easily modified. Quite low LOD values can be achieved when GCE is used. For instance, Thanh et al. [90] simply coated the electrode surface with Bi-film and they were able to reach 1.07, 0.93, 0.65, and 0.94 ppb for Zn, Cd, Pb, and Cu, respectively, while maintaining high accuracy and repeatability within measurements. In the work of Lee et al. [91], after modifying the surface with electrodeposition of graphene oxide and in situ plating bismuth film, Pb, Cd, and Zn were determined simultaneously between 1–100 μL^{-1} . In a recent study conducted by our research group [114], voltammetric Hg^{2+} determination was made possible via multi-channel receptors of naphthoquinone-aniline/pyrene union incorporated GCE at a linear range of 3.5–20.5 ppm, using DP technique. Various studies report that lower LOD levels and broader linear ranges are possible via surface modification. Baghayeri et al. [115] modified the surface of GCE with dendrimer functionalized magnetic graphene oxide and simultaneously detected Pb and Cd with LODs 130 and 70 ng L^{-1} and linear ranges 0.4–120 and 0.2–140 $\mu\text{g L}^{-1}$, respectively, using SWASV. Hassan et al. [116] measured Pb in the linear range of 10.0–120.0 $\mu\text{g L}^{-1}$, and Cd and Zn in the linear range of 0.0–50 $\mu\text{g L}^{-1}$ (respective LOD values: 3.18 ng L^{-1} , 0.107 $\mu\text{g L}^{-1}$, and 0.037 $\mu\text{g L}^{-1}$) in tap water simultaneously via SW-ASV employing Hg-Bi film electrodeposited on GCE in the presence of poly(1,2-diaminoanthraquinone).

4.2.2 Graphite electrodes

Graphite electrodes, such as graphitic rods and pencil graphite electrodes, exhibit promising features for electrochemical analysis with their large surface areas, great conductive properties, and low costs. Especially, disposable pencil graphite electrodes are commonly used for diverse analytes due to their ubiquity, low price, and efficiency. Plus, usually, there is no need for a polishing step before the analysis with pencil graphite electrodes, even though it is needed for many other electrodes [59]. Moreover, it is an efficient tool in the electroanalysis of heavy metals, mostly preferred in Pb, Cd, and Cu detection. For instance, Donmez et al. [9] were able to simultaneously determine Pb and Cd in water samples up to 0.46 and 1.11 $\mu\text{g L}^{-1}$. Skiba and Gou [94] investigated the levels of Pb and Cu (with respective LODs: 3.6 and 40.0 $\mu\text{g L}^{-1}$) in bovine seminal plasma and found that the samples contained 390–2400 $\mu\text{g L}^{-1}$ and 240–1170 $\mu\text{g L}^{-1}$ of Pb and Cu, respectively. GrE can also be used in the analysis of other metals: Mafa et al. [95] were able to detect As, Hg, and Pb in industrial water samples by utilizing exfoliated GrE modified with Bi, in the concentration range of 1–250 $\mu\text{g L}^{-1}$.

4.2.3 Carbon paste electrodes

Carbon paste electrodes are commonly used in the electroanalysis of heavy metals due to the simplicity of preparation and modification. To prepare a CPE one has to add the carbonaceous material in powder or dust form to highly viscous oil, which is usually paraffin, mineral oil, or silicon oil. They are often preferred because it is relatively easy to incorporate diverse materials into that mixture, to increase the metal deposition onto the surface, such as nanoparticles, [98,117] waste materials, [118,119] and various chemicals. [99,100]. In a study conducted by Sivan et al. [120] Pb ions were determined in plastic toys via a CPE prepared by adding Gum Arabic and TiO_2 NP to the carbon paste made of graphite powder and silicon oil. They obtained a LOD value of 506 ppb and the resulting electrode maintained its stability and reproducibility for 12 days. Moutcine et al. [121] incorporated ZnO NP into the formulation of CPE and detected Cd and Hg in drinking water samples in the linear range of 3–21 μM with LODs 0.46 and 0.43 nM, respectively. Nonetheless, lower LOD levels can be achieved when working with CPE, given that the right modifiers are chosen. Ping et al. [122] were able to quantify Pb and Cd with 0.21 and 0.15 $\mu\text{g L}^{-1}$ LODs in a complex matrix

like milk via a novel electrode that contains ionic liquid, bismuth NP, and graphite powder. Zheng et al. [123] detected Pb and Cd simultaneously with mesoporous alumina modified CPE and obtained respective LODs of 0.2 and 2.0 nM. Additionally, Malakootian et al. [124] modified CPE with Eu⁺³ doped NiO and measured Pb and Cd in food samples with 0.1 and 0.4 µg L⁻¹ LODs, respectively.

4.2.4 Screen-printed carbon electrodes

SPCE are relatively new tools for electrochemical analysis, which allows the user to analyze with lower sample volume than usual, and in a shorter time. The basic structure of SPCE involves a three-electrode system immobilized on a surface, two open ends for sample introduction and electrical connection, and an insulating layer. To create a SPCE, electrodes must be prepared as pastes or inks so that they can be printed on the pre-determined surface. This surface is called the substrate and it can be made of ceramic or a polymeric material like polyethylene terephthalate (PET). SPCE are very convenient for quick, on-site analysis. In one of the earlier studies, Injang et al. [125] utilized ASV in sequential injection analysis for plant samples using a SPCE that has a Bi-modified working electrode that consists of MWCNT and carbon ink. They achieved high sensitivity for simultaneous quantification of Pb, Cd, and Zn. In more recent studies, higher analytical performance is achieved in simultaneous heavy metal analysis via more intricate surface modifications. Trachioti et al. [104] were able to detect Zn and Cd in tap and bottled water samples at sub-ppb levels, after integrating Sn NP on the carbon surface via sparking. McEleney et al. [101] modified the surface of their electrode made of graphene oxide and graphitic carbon nitride with bismuth and gallium by changing the pH within the same cell to determine Cd and Zn (pH 4.6 and 5.1, respectively).

4.2.5 Other carbon electrodes

Even though most of the studies employ one of the four electrodes described above, there are also other alternatives. For instance, variations of microelectrodes are highly promising in terms of simultaneous ultra-trace heavy metal detection. Lv et al. [126] reported that with the microelectrode prepared by assembling nano frameworks of porous carbon nitride nanosheets and MWCNT on a carbon-fiber microelectrode, they were able to measure Cu, Pb, and Hg in local water samples with the following LOD values: 1.0×10^{-13} , 1.8×10^{-11} , 8.0×10^{-12} , respectively. Diamond electrodes are also

effective, especially when modified with bismuth and utilized in anodic stripping voltammetry [127] or combined with other carbonaceous materials like graphite [128]. Moreover, Xuan and Park [107] developed a Bi-modified working electrode with micro-patterned reduced graphene oxide and CNT on a gold substrate, which was able to detect Pb and Cd in drinking water samples in the concentration range of 20 ppb to 200 ppb. They noted that sensitivities and LOD values could have been improved by increasing the deposition time. They also found that the presence of Cu in samples lowered the signals obtained from Pb and Cd. Scandurra et al. [129] prepared a disposable graphene paper electrode modified with Nafion and Bi to determine Pb and Cd in drinking water samples. They were able to conduct ultra-trace analysis with SWASV due to modification with Nafion, which attracted positively charged metals, and Bi, which bound Cd and Pb by forming metallic alloys. Lastly, carbon cloth electrodes are also employed in the heavy metal analysis as a low-cost, portable material that has the potential for being mass-produced. Reported results on electrochemical determination of heavy metals with carbon cloth electrodes also seem encouraging: Shi et al. [130] simultaneously determined Pb and Cd in water samples with LOD values reaching 1.90 and 3.42 ppb, respectively, and Foo et al. [131] were able to detect Cu ions with 0.05 μM LOD.

4.3 Modified Carbon Electrodes for Better Analytical Performance

Surfaces of CBE are often modified to achieve higher sensitivity, obtain measurements with minimum interference, and better overall analytical performance. Table 4.2 shows some examples of the materials used for this purpose and the LOD and linear range values obtained in the respective studies. Some modifications also allow for simultaneous multi-element determination with high sensitivity. One of the promising materials that provide such desired results is Nafion, which is a non-crosslinked polymeric substance that contains sulfonate groups in its structure. For instance, Lee et al. [145] reported that they were able to determine Pb, Cd, and Zn simultaneously in standard solutions with a linear range of 1–50 ppb, using a SPCE that is modified with Bi and Nafion. Later, Palisoc et al. [146] stressed that modification with $([\text{Ru}(\text{NH}_3)_6]^{+3})$ doped Nafion could be utilized in various voltammetric heavy metal analysis. In a more recent study, Tan et al. [147] developed an effective GCE electrode for simultaneous heavy metal (Zn, Cd, Pb, Cu, and Hg) detection in agricultural food

samples. Modifying mixture was obtained by synthesizing fluorinated graphene, dispersing it in 0.5% Nafion, sonicating, adding synthesized gold nano-cage electrode, and sonicating again. They were able to achieve wide linear ranges (6–7000, 4–6000, 6–5000, 4–4000, 6–5000 $\mu\text{g L}^{-1}$) and low LOD (0.08, 0.09, 0.05, 0.19, 0.01 $\mu\text{g L}^{-1}$) values (for Zn, Cd, Pb, Cu, and Hg respectively).

On the other hand, bismuth seems to be a very common choice of modification material when it comes to CBE utilized for heavy metal analysis [143,148]. It is so apt for this purpose; it can even be used as a working electrode in heavy metal analysis [149,150]. There are plenty of studies that report how in situ plating of bismuth on carbon electrodes increase the sensitivity and improve the resolution in simultaneous heavy metal analysis [9,151,152]. CBE modified with bismuth show significant enhancement in terms of their analytical performance compared to unmodified or bare electrodes. Kilic and Kizil [10] showed that using graphene bismuth nanocomposite film modified pencil graphite electrode allows for trace-level analysis of Pb and Cd in tap water samples, in the linear range of 5–100 $\mu\text{g L}^{-1}$. Maria-Hormingos et al. [153] successfully investigated the levels of Pb and Cd in mineral water and river water samples, using polystyrene sulfonate, bismuth, and carbon nanopowder composite in their SPCE and compared three modification methods for bismuth. The first method involved immersion of the composite electrode in bismuth solution for several hours. The second and third methods are about drop-casting the modifier solution on the surface of SPCE: in the second method, solid Bi_2O_3 is used in the making of the composite, while in the third method Bi^{2+} is incorporated in the mix in its ionic form. Among them, the immersion method led to better performance, with LOD levels as low as 0.012 and 0.029 $\mu\text{g L}^{-1}$ for Cd and Pb, respectively.

It should be acknowledged that the performance of bismuth film electrodes, just like all electrodes, highly depend on the pH and the supporting electrolyte, especially because the potential window of bismuth varies according to the acidity of a given medium [154]. Furthermore, structure of the bismuth film surface is also a crucial factor that determines the analytical performance of the electrode. According to Nunes et al. [155], bismuth films that maintain a more homogeneous surface provide higher electrochemical signals for lead detection.

Finally, incorporating nanomaterials in electrochemical sensors have been frequently reported in the literature to contribute to the outstanding performance in heavy metal

detection [156]. A wide range from metals to polymer-based nanomaterials can be employed to achieve superior selectivity, sensitivity, and stability with CBE [157]. Ma et al. [133] were able to detect iron (Fe(II)) with 3 nM LOD and 0.01–2 μ M linear range via GCE modified with platinum NP, graphene oxide, and leucomethylene blue. Amare et al. [117] integrated silver NP in the structure of CPE and conducted simultaneous determination of Pb and Cd in 5–160 ppm range.



Table 4.2 : Summary of analytical performances achieved in several studies determining heavy metals with modified CBE (carbon based electrodes). (GCE: glassy carbon electrode, GrE: Graphite electrode, CPE: carbon paste electrode, SPCE: screen printed carbon electrode).

Electrode	Modification	Analyte	LOD	Linear Range	Reference
GCE	Amino acids (best performance was observed with alanine)	Zn	8.92 pM	10 nM-100 μ M	[132]
		Cd	5.77 pM	7.5 nM-75 μ M	
		Cu	3.01 pM	5 nM-50 μ M	
		Hg	5.89 pM	7.5 nM-75 μ M	
	Graphene oxide, leucomethylene blue, and Pt NP	Fe(II)	3 nM	0.01-2 μ M	[133]
	Sodium carboxymethyl cellulose and Bi	Cd	0.75 nM	0.001-1 μ M	[134]
	Nano-fibrillated cellulose	Pb	0.5 nM	10^{-5} - 3.2×10^{-8} M	[135]
		Cd	5 nM		
		Cu	0.5 nM		
		Hg	5 nM		
	Bi and activated graphene-Nafion composite	Pb	0.05 μ g L ⁻¹	5-100 μ g L ⁻¹	[136]
		Cd	0.07 μ g L ⁻¹		
		Zn	0.57 μ g L ⁻¹		

Table 4.2 (continued) : Summary of analytical performances achieved in several studies determining heavy metals with modified CBE (carbon based electrodes). (GCE: glassy carbon electrode, GrE: Graphite electrode, CPE: carbon paste electrode, SPCE: screen printed carbon electrode).

Electrode	Modification	Analyte	LOD	Linear Range	Reference
GrE	L-methionine was electropolymerized on pencil graphite electrode before Au NP were electrochemically entrapped and deoxyribonucleic acid was immobilized on the surface	Hg(II)	0.004 aM	0.1 aM-0.1 nM	[137]
	Pencil graphite electrode was modified by CV to obtain graphene structure on the surface, which then was modified in-situ with Bi.	Pb	0.29 $\mu\text{g L}^{-1}$	5-100 $\mu\text{g L}^{-1}$	[10]
		Cd	0.12 $\mu\text{g L}^{-1}$		
	Graphite pencil electrode modified with porous d-penicillamine functionalized nanocellulose	Cu	0.048 pM	0.2-50 pM	[138]
	Hydrophilized graphite felt modified with alginic acid	Pb	8.6×10^{-8} M	0.5-0.9 μM	[139]
		Hg	1.1×10^{-7} M	2-5 μM	
		Pb	4.83×10^{-7} M		
	Paraffin impregnated graphite electrode was combined with HNO_3 and MWCNT	Cd	2.98×10^{-7} M	$1.96\text{-}5.66 \times 10^{-6}$ M	[140]
		Cu	3.81×10^{-7} M		
		Hg	6.79×10^{-7} M		

Table 4.2 (continued) : Summary of analytical performances achieved in several studies determining heavy metals with modified CBE (carbon based electrodes). (GCE: glassy carbon electrode, GrE: Graphite electrode, CPE: carbon paste electrode, SPCE: screen printed carbon electrode).

Electrode	Modification	Analyte	LOD	Linear Range	Reference
CPE	Spent coffee grounds	Pb	90 μM	30-189 μM	[119]
		Cd	89 μM	30-269 μM	
	Ag-NP	Pb	0.9996 ppm	5-160 ppm	[117]
		Cd	0.9976 ppm		
	Al_2O_3 NP	Pb	0.75 nM	3.3-21 μM	[141]
		Hg	0.52 nM		
	CNT, Fe_3O_4 , and eggshell composite	Cd	2.4 ng mL ⁻¹	3-250 ng mL ⁻¹	[118]
	Polypyrrole, MWCNT, Bi particles	Pb	99 ng L ⁻¹	0.11-120 $\mu\text{g L}^{-1}$	[142]
		Cd	157 ng L ⁻¹	0.16-120 $\mu\text{g L}^{-1}$	

Table 4.2 (continued) : Summary of analytical performances achieved in several studies determining heavy metals with modified CBE (carbon based electrodes). (GCE: glassy carbon electrode, GrE: Graphite electrode, CPE: carbon paste electrode, SPCE: screen printed carbon electrode).

Electrode	Modification	Analyte	LOD	Linear Range	Reference
SPCE	Single-walled carbon nanohorn and Bi	Pb	0.4 $\mu\text{g L}^{-1}$	1.0-60 $\mu\text{g L}^{-1}$	[143]
		Cd	0.2 $\mu\text{g L}^{-1}$		
		Zn	25 $\mu\text{g L}^{-1}$		
	Graphite modified with Cr (III), using ethyl cellulose as binder and alpha terpineol as solvent	Cd	3 $\mu\text{g L}^{-1}$	10-800 $\mu\text{g L}^{-1}$	[100]
		Pb	3 $\mu\text{g L}^{-1}$	10-800 $\mu\text{g L}^{-1}$	
		Cu	3 $\mu\text{g L}^{-1}$	10-800 $\mu\text{g L}^{-1}$	
		Ag	3 $\mu\text{g L}^{-1}$	10-500 $\mu\text{g L}^{-1}$	
	Selenocystine on aryl diazonium salt monolayers	Pb	3.2 $\mu\text{g L}^{-1}$	10.8-150 $\mu\text{g L}^{-1}$	[111]
		Cd	3.2 $\mu\text{g L}^{-1}$	10.7-150 $\mu\text{g L}^{-1}$	
	Carbon ink modified with Nafion, ionic liquid, and graphene composite	Pb	0.08 ng L^{-1}	0.1-100 ng L^{-1}	[144]
		Cd	0.06 ng L^{-1}		
		Zn	0.09 ng L^{-1}		
	Glutathione modified screen-printed carbon nanofiber electrode	Pb	3.0 $\mu\text{g L}^{-1}$	10.1-150.1 $\mu\text{g L}^{-1}$	[112]
		Cd	3.2 $\mu\text{g L}^{-1}$	10.8-150.1 $\mu\text{g L}^{-1}$	



5. EXPERIMENTAL SECTION

5.1 Instruments and chemicals

In the studies related to PGE, Gamry (Warminster, PA, USA) reference 600 electrochemical workstation was employed for all the measurements. The three electrode system that was equipped in those studies consisted of an Ag/AgCl reference electrode, a Pt wire counter electrode and a modified PGE as the working electrode. PGE (diameter: 0.5 mm, softness: 2B) was purchased from Faber-Castell (Stein, Germany). Scanning electron microscope (SEM) images were obtained via field-emission SEM (FESEM, QUANTA FEG-250, FEI). To verify the results obtained via proposed methods, samples were also analyzed with Agilent GTA 120 Graphite Tube Atomizer (Santa Clara, CA, USA).

In the studies related to SPCE, all electrochemical measurements were obtained with a SPCE (diameter: 4 mm) supplied by Metrohm DropSens (DRP-110, Spain). All chemicals used in the studies were of analytical grade. All the solutions sample preparations were done daily with ultrapure water.



6. CONSTRUCTION OF PENCIL GRAPHITE ELECTRODE MODIFIED WITH NAFION, MWCNT, AND BISMUTH FOR SIMULTANEOUS LEAD AND CADMIUM DETECTION²

6.1 Experimental Procedure

6.1.1 Chemicals and apparatus

All of the chemicals used in this study were of analytical grade and bought from Merck (Darmstadt, Germany). Pb^{2+} , Cd^{2+} , and bismuth standards were prepared from 1000 mg L⁻¹ ICP standard solutions (Merck, Darmstadt, Germany). 20% Nafion solution dispersed in ethanol was purchased from BilveTek (Istanbul, Turkey). MWCNT having an outside diameter of 48–78 nm and inside diameter of 5–15 nm, with an electrical conductivity of 98 S cm⁻¹ was acquired from Nanografi (Ankara, Turkey). Supporting electrolyte was chosen as 0.1 M acetate buffer having a pH value of 4.5, which was prepared from NaCH₃COO and CH₃COOH. AWTW (Weilheim, Germany) 7310 pH meter was employed when pH determination was necessary. Each solution was prepared daily with ultrapure water. Altogether measurements were carried out at room temperature and dissolved gasses were not eliminated in the electrochemical cell. Gamry (Warminster, PA, USA) Reference 600 electrochemical workstation, which was employed in all electrochemical processes, was equipped with a three electrode system that had Ag/AgCl as the reference electrode, Pt wire as the counter electrode, and PGE as the working electrode. PGE (diameter: 0.5 mm, softness: 2B) was bought from Faber-Castell (Stein, Germany). In the course of electrochemical procedures, a 0.5 cm part of PGE was dipped into the electrochemical cell, maintaining a surface area of 8.05 mm². Electrochemical impedance spectroscopy measurements were carried out with 10 mV amplitude in a frequency range of 0.1 Hz–100000 Hz. Scanning electron microscope (SEM) visuals

²This chapter is based on the paper “Ustabasi, G.S., Yilmaz, I., Ozcan, M., Cetinkaya, E. (2021). Construction of Pencil Graphite Electrode Modified with Nafion, MWCNT, and Bismuth for Simultaneous Lead and Cadmium Detection, *Journal of the Electrochemical Society*, 168, 106513.” [158]

were obtained via field-emission SEM (FESEM, QUANTA FEG-250, FEI) to assess the morphologies of electrode surfaces. Environmental water samples from Ergene Basin (Trakya, Turkey) were also analyzed via Agilent GTA 120 Graphite Tube Atomizer (Santa Clara, CA, USA) to verify the results attained with the proposed voltammetric method.

6.1.2 Electrode modification

Optimized modification process involves suspending 0.25 ml of 20% Nafion and 1.0 mg of MWCNT in 5 ml of ethanol. This suspension was sonicated for 2 h to achieve a homogeneous dispersion before dip-coating the PGE. Prior to dipcoating, PGE were also left to be sonicated for 2 h in pure ethanol to clean the electrode surface. After the sonication was complete, 4 cm parts of PGE were vertically immersed in aforementioned suspension for 3 s, and then leftover ethanol was evaporated at room temperature. Coated electrodes were finally in situ plated with 0.3 ppm Bi during simultaneous Pb^{2+} and Cd^{2+} detection.

6.1.3 Characterization of electrode properties

Investigation and characterization of the surface and electrochemical properties were achieved by interpreting the results of cyclic voltammetry, electrochemical impedance spectroscopy and SEM measurements of both PGE and MWCNT/N/PGE. To obtain a high level of resolution in SEM images of electrodes, samples were sputter-coated with Au under vacuum. Cyclic voltammetry was applied to electrodes in 0.1M KCl solution containing 2.5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$. Electrochemical impedance spectroscopy (EIS) measurements were conducted in a medium of 0.1 mol L^{-1} H_2SO_4 solution to estimate the charge transfer capacities.

6.1.4 Preparation of samples

Environmental water samples were collected from a river in Trakya, Northwestern region of Turkey, called Ergene Basin. These water samples were maintained at +4 °C till the time for the analysis come. They were brought to room temperature before the sample preparation steps were applied. First, approximately 50 ml portion of the samples were filtered with 391 grade Sartorius Stedim Biotech (Gottingen, Germany) filter papers. After the filtration, samples were exposed to UV radiation for 2 h, in a closed environment. Thus, possible interferences caused by the organic matter content

would be eliminated. Following the sample preparation, samples were stored at +4 °C, in polypropylene centrifuge tubes, until the analysis.

6.1.5 Voltammetric measurements

Voltammetric analysis were conducted via a Gamry potentiostat equipped with a three electrode system as described in Chemicals and apparatus section. SWASV method was chosen to determine Pb^{2+} and Cd^{2+} in this study and parameters of this method were optimized to achieve the highest possible currents. Optimized method, which was employed in each validation and sample measurements steps, had the following values set as constant in pH 4.5 acetate buffer supporting electrolyte: 120 s pre-concentration time, -1.0 V pre-concentration potential, followed by a scan between -1.0 and 0.4 V, with 5 mV step size, 40 mV pulse size and 25 Hz frequency. Before the voltammetric method was optimized the following parameters were held constant: pH 4.5 acetate buffer solution as supporting electrolyte; pre-concentration for 120 s at -1.2 V; square wave scan between -1.4 and 0.4 V, with pulse size 40 mV and frequency 25 Hz. All electrochemical measurements were carried out with three replicates.

6.2 Results and Discussion

6.2.1 Optimization of electrode preparation process

Before each parameter was optimized certain values were held constant: amount of MWCNT in modification suspension was 1.0 mg, Nafion content in modification suspension was 1% , concentration of bismuth was 0.2 ppm, and duration of dipping was 5 s. Figure 1 visualizes the results obtained from the optimization of electrode modification process. Tested concentrations of modifiers were chosen from various relevant studies [9,88,93,144,145]. The aim of the optimization was to determine the minimum amount of modifier required for the maximum level of generated analyte signal. Thus, the cost and toxicity of the fabricated electrode would be minimized. Additionally, the immersion time necessary to achieve the highest performance was also investigated so that time efficiency of the method would not be compromised. As shown in Figure 6.1, increasing the amounts of chosen materials did not significantly enhance the obtained analyte signals after certain modifier concentrations such as 1.0 mg for MWCNT and 1% for Nafion to be suspended in 5 ml of ethanol, and 0.3 ppm for in situ coating of bismuth. Moreover, the analyte signals obtained after varying

durations of dipping reached their maximum level with 3 s of dipping of the PGE in modifier suspension, which demonstrates a great advantage in terms of time management.

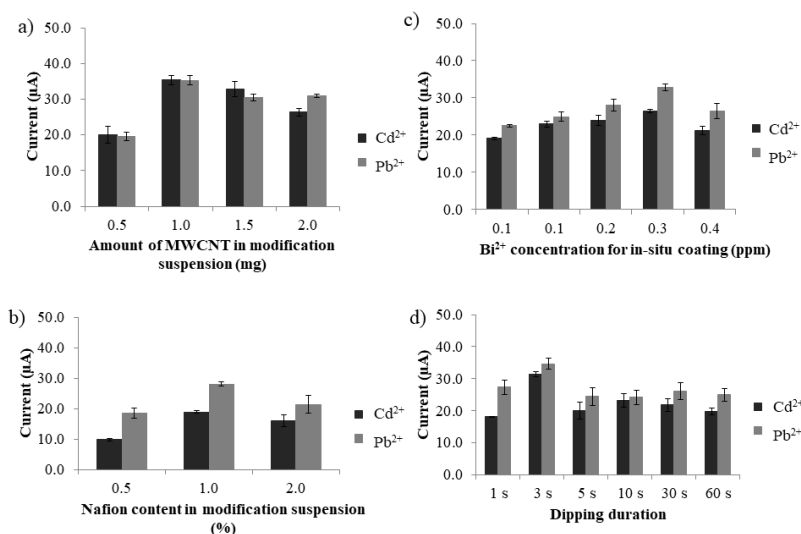


Figure 6.1 : Optimization of modification parameters by comparing obtained analyte peak heights: (a) amount of MWCNT in modification suspension, (b) Nafion content in modification suspension, (c) bismuth concentration for in-situ coating, (d) duration of dip coating.

Furthermore, when the performances of bare and modified electrodes are compared, the great improvement in sensitivity has been observed as presented in Figure 6.2. While bare PGE is able to quantify Pb^{2+} in the electrolyte medium, it fails to detect Cd^{2+} simultaneously. When the bare electrode is modified with MWCNT and Nafion it is also able to detect Cd^{2+} , however, it still performs significantly poorer relative to MWCNT/N/Bi/PGE as it generate higher currents for both $100 \mu\text{g L}^{-1}$ Cd^{2+} and Pb^{2+} .

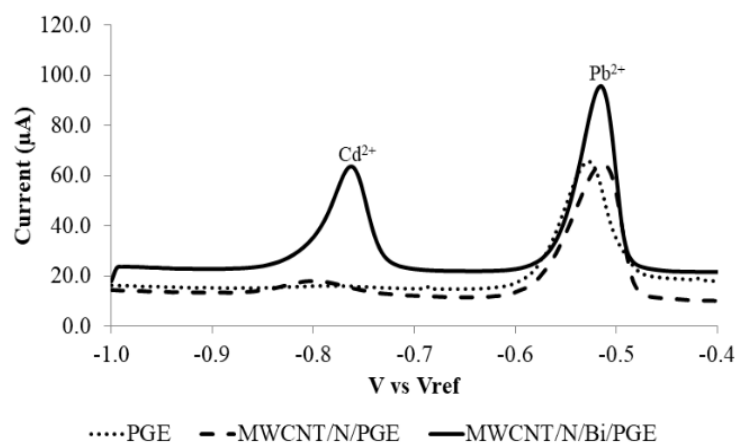


Figure 6.2 : Sensitivity comparison of the bare and modified electrodes via measurements of $100 \mu\text{g L}^{-1}$ Pb^{2+} and Cd^{2+} with pH 4.5 acetate buffer as supporting electrolyte.

6.2.2 Optimization of pre-concentration parameters

Pre-concentration parameters to be optimized were pre-concentration time and potential. In Figure 6.3, the results of this optimization are revealed. Figure 6.3a shows that setting the pre-concentration potential to -1.0 V leads to the highest current peaks for both Pb^{2+} and Cd^{2+} . Furthermore, in Figure 6.3b, increasing the pre-concentration time also increases the analyte signals at first, but after 120 s this increase is observed no more, and the trend became stagnant. Consequently, 120 s was chosen as pre-concentration time.

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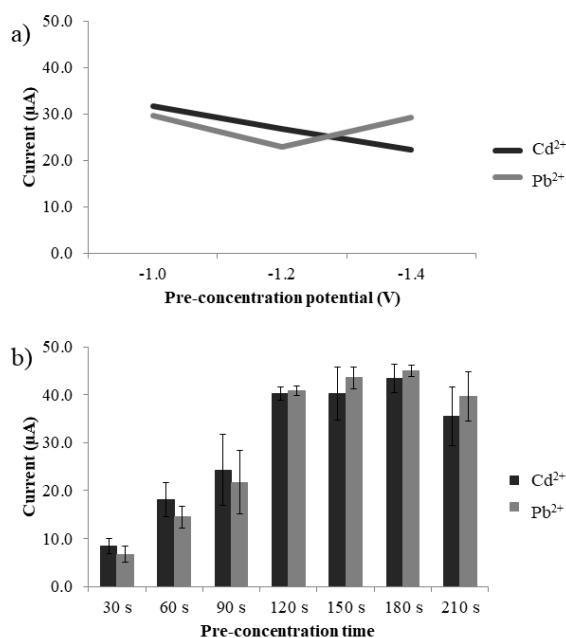


Figure 6.3 : Optimization of pre-concentration parameters: (a) pre-concentration potential, (b) pre-concentration time.

6.2.4 Electrode characterization

Cyclic voltammetry voltammograms and Nyquist plots of EIS measurements of bare and modified electrodes are presented in Figure 6.4. In Figure 6.4a, anodic and cathodic peaks of $\text{Fe}(\text{CN})_6^{3-/4-}$ is accentuated with bare PGE, whereas with MWCNT/N/PGE Faradaic reactions cannot be observed. Even though the interaction between the electrode and the electrolyte is visible, negatively charged Nafion may have inhibited the access of $\text{Fe}(\text{CN})_6^{3-/4-}$ to the electrode [159] by blanketing the electrode surface with its polymeric structure and preventing the electron exchange [160]. On the other hand, in Figure 6.4b Nyquist plot of MWCNT/N/PGE appears closer to Warburg impedance with 45° angle, which indicates that diffusion is the main driving force for the metals' accumulation on the electrode surface [161]. The results of cyclic voltammetry and EIS measurements complement one another, because they both indicate that determination mechanism of MWCNT/N/PGE depends on diffusion rather than Faradaic reactions. Moreover, EIS results also show that electrical resistivity of MWCNT/N/PGE is significantly lower than PGE, which provides a great advantage over bare electrode.

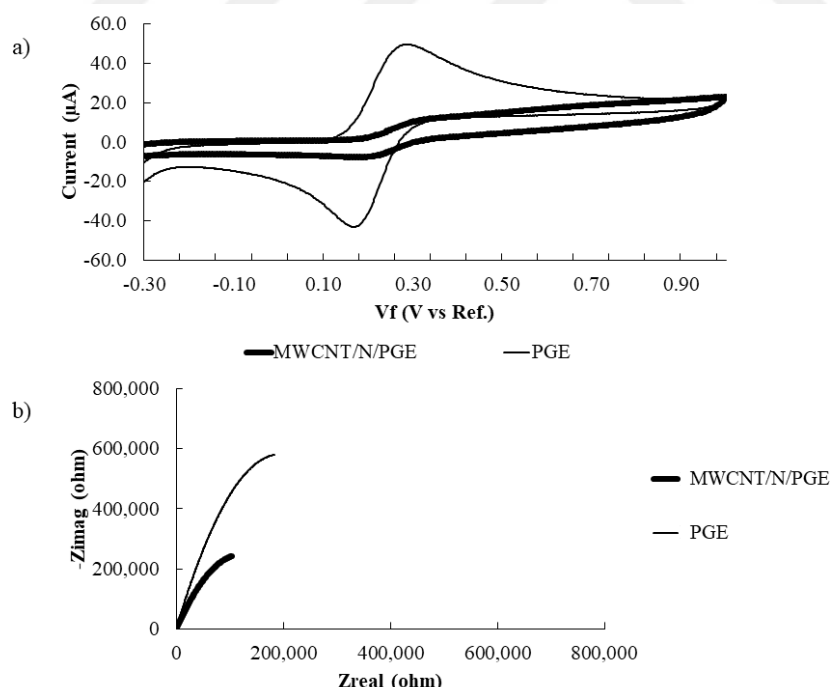


Figure 6.4 : Characterization of electrochemical properties of PGE and MWCNT/N/PGE: (a) Cyclic voltammograms of PGE (thin line) and MWCNT/N/PGE (thick line) in 0.1 M KCl solution with 2.5 mM $\text{Fe}(\text{CN})_6^{4-/3-}$ (scan rate: 100 mVs^{-1}), (b) Electrochemical impedance spectra of PGE (thin line) and MWCNT/ PGE (thick line) in $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ (frequency range: 0.1 Hz–0.1 MHz, amplitude: 10 mV).

SEM visuals of bare and modified electrodes are in Figure 6.5. The tubular structured entities in Figures 6.5c–6.5f are MWCNT, and the glossy look recognized in Figures 6.5e and 6.5f is caused by Nafion. MWCNT's curly appearance and the shiny, film like presence of Nafion matches the previous descriptions reported in literature [160,162,163]. By assessing the morphological changes on graphite surface, it can be inferred that dip-coating was successfully executed and the anticipated modification was accomplished, though a homogeneous distribution could not be achieved. Nevertheless, this heterogeneity did not compromise the repeatability and reproducibility of MWCNT/N/Bi/PGE. Moreover, deposition of bismuth onto the electrode surface is not permanent in this method of in situ plating: It is collected onto the surface during the pre-concentration step under -1.0 V and it is transferred back to the supporting electrolyte medium as the potential is being scanned from -1.0 to -0.4 V, just like the analytes. Therefore, the presence of bismuth could not be visualized via SEM imaging, but the voltammetric peaks generated by bismuth ions are presented in Fig. S1 (available online at stacks.iop.org/JES/168/106513/mmedia).

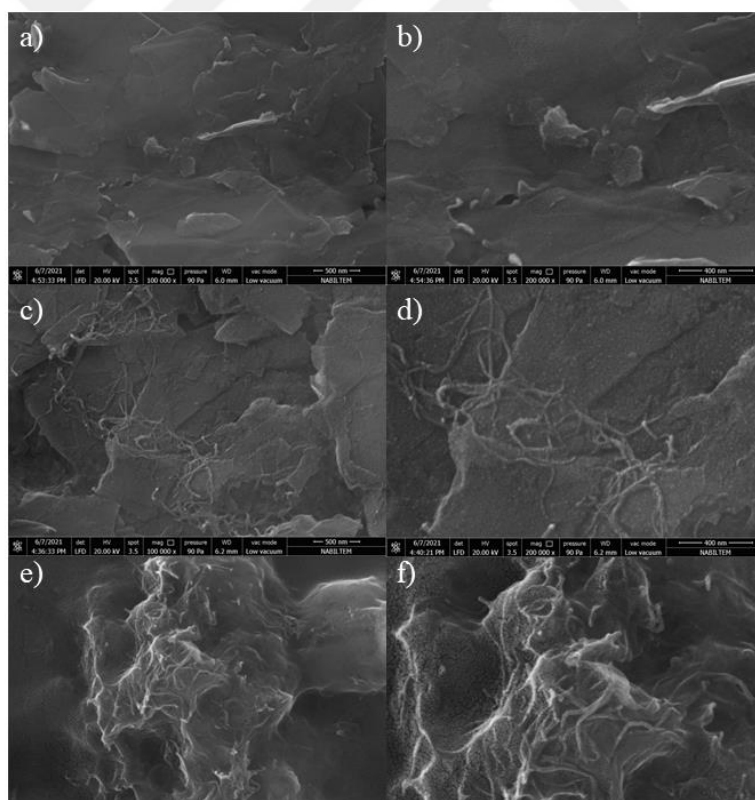


Figure 6.5 : SEM visuals of (a) 100000 times magnified bare PGE, (b) 200000 times magnified bare PGE, (c) 100000 times magnified MWCNT/PGE, and (d) 200000 times magnified MWCNT/PGE, (e) 100000 times magnified MWCNT/N/PGE, (f) 200000 times magnified MWCNT/N/PGE.

Repeatability was calculated by examining the standard deviation of 10 replicates of $80 \mu\text{g L}^{-1}$ Pb^{2+} and Cd^{2+} measurements carried out under optimized conditions. Reproducibility was evaluated by taking the standard deviation of the averages of three times replicated results obtained from $80 \mu\text{g L}^{-1}$ Pb^{2+} and Cd^{2+} measurements carried out under optimized conditions using 10 different MWCNT/N/Bi/PGE. Both of the standard deviations calculated for repeatability and reproducibility parameters of MWCNT/N/Bi/PGE were smaller than 5% for Pb^{2+} and Cd^{2+} , indicating high stability for the modified electrode. On the other hand, even though PGE are considered disposable due to their ubiquity and inexpensiveness, the stability of MWCNT/N/Bi/PGE was tested by taking measurements of $80 \mu\text{g L}^{-1}$ Pb^{2+} and Cd^{2+} under optimized conditions with 24 h intervals. Finally, it was found that the standard deviation calculated from MWCNT/N/Bi/PGE measurements were less than 10% for three consecutive days. In the later measurements, obtained results were significantly altered, meaning that the stability of the electrode is compromised after three days.

6.2.5 Calibration data

To evaluate the analytical performance of the MWCNT/N/Bi/PGE, parameters like detection limits and dynamic linear range were investigated. LOD and LOQ values were calculated by multiplying the standard deviations of ten repetitive measurements of blank solution with 3 for LOD, and with 10 for LOQ, and dividing by the slope of the corresponding calibration curve of analytes. Obtained LOD and LOQ values for Pb^{2+} and Cd^{2+} were 0.37 and $0.32 \mu\text{g L}^{-1}$, and 1.25 and $1.07 \mu\text{g L}^{-1}$, respectively. As displayed in Figure 6.6, the dynamic linear ranges for Pb^{2+} and Cd^{2+} were $1\text{--}100 \mu\text{g L}^{-1}$ and $1\text{--}90 \mu\text{g L}^{-1}$, respectively. In Table 6.1, the comparison of LOD values and linear ranges obtained in this study and in the recent studies that conducted simultaneous lead and cadmium analysis via CBE is present. By comparing those studies it can be concluded that MWCNT/N/Bi/PGE had a favorable performance in terms of a wide linear working range. On the other hand, in terms of detection limits, the constructed electrode seems to perform slightly better than average. There are several studies incorporating Nafion, MWCNT and bismuth, along with other materials, that report LOD values smaller than $0.1 \mu\text{g L}^{-1}$ for both lead and cadmium, like the work of Zhao and Liu [164] and Zhao et al. [165] However, their choice of CBE is glassy carbon electrodes, which are relatively expensive and require polishing, whereas PGE do not [59].

6.2.6 Analysis of the certified reference material

Both the certified and measured values of Canadian certified reference water sample (TM-254) are in Table 6.2. Standard addition method was employed with the proposed method. As it is evident from the table, the proposed voltammetric method provided accurate results.

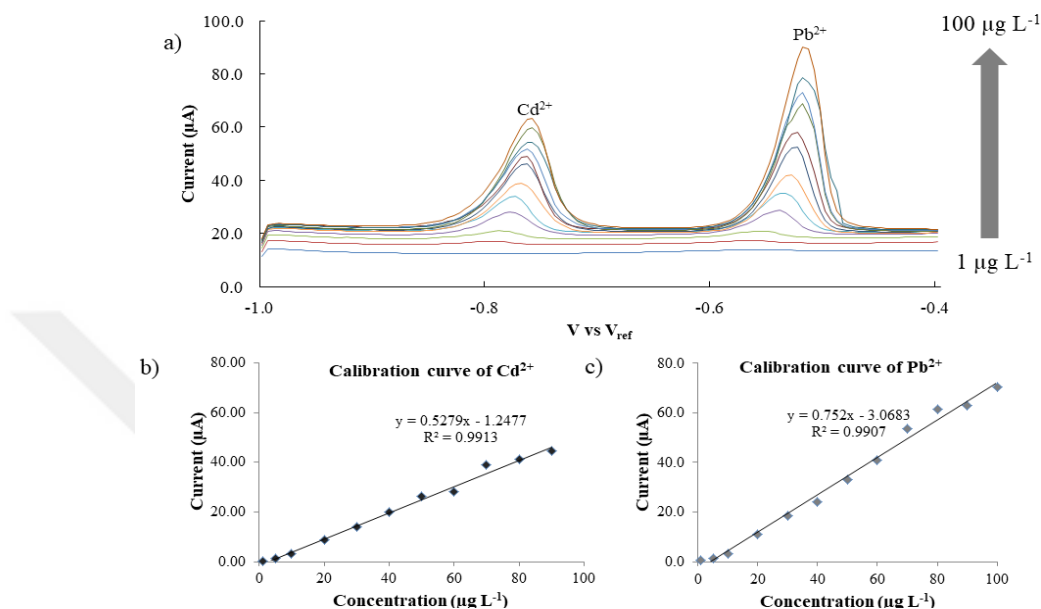


Figure 6.6 : Assessment of dynamic linear range: (a) voltammograms obtained from 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg L⁻¹ Pb²⁺ and Cd²⁺ analyte concentrations via MWCNT/N/Bi/PGE, (b) calibration curve of Cd²⁺, (c) calibration curve of Pb²⁺.

Table 6.1 : Analytical performances reported in relevant studies utilizing carbon based electrodes to detect Pb²⁺ and Cd²⁺, simultaneously.

Reference	Electrode*	Method	LOD (μg L ⁻¹)		Linear range (μg L ⁻¹)	
			Pb ²⁺	Cd ²⁺	Pb ²⁺	Cd ²⁺
[90]	Bi-F/GCE	DPASV	0.65	0.93	5-110	5-110
[107]	Au/rGO/CNT/Bi	SWASV	0.2	0.6	20-200	20-200
[98]	Sb ₂ O ₃ /MWCNT paste	LASV	6.12	16.77	5-35	80-150
[97]	BiPs-CNFs/IL/CPE	SWASV	0.12	0.25	2-120	2-120
[87]	2D-Bi/G/GCE	SWASV	0.3	0.3	1-30	1-30
This study	MWCNT/N/Bi/PGE	SWASV	0.37	0.32	1-100	1-90

*Electrode abbreviations: Bi-F: Bismuth film, GCE: Glassy carbon electrode, Au: Gold, rGO: reduced graphene oxide, CNT: Carbon nanotubes, Bi: Bismuth, MWCNT: Multi wall carbon nanotube, BiPs: Bismuth particles, CNF: Carbon nanofibers, IL: Ionic liquid, CPE: Carbon paste electrode, 2D-Bi: Two dimensional bismuthene, G: Graphene, N: Nafion, PGE: Pencil graphite electrode.

Table 6.2 : Certified and determined concentration values of Pb^{2+} and Cd^{2+} from Canadian Water Sample (TM-25.4) (N: 3).

	Mean concentration $\pm$ RSD ($\mu\text{g L}^{-1}$)	
	Certified value	Measured value
Pb^{2+}	27.0 $\pm$ 2.35	26.1 $\pm$ 4.46
Cd^{2+}	23.5 $\pm$ 1.80	25.2 $\pm$ 1.31

6.2.7 Interference study

Interferences that may be caused by some common ions found in environmental sample media were explored. Na^+ , K^+ , NH_4^+ , Mg^{2+} , Ca^{2+} , Ni^{2+} , NO_3^- , Mn^{2+} , Fe^{2+} , PO_4^{3-} ions were added to the measurement medium with a concentration that is 100 times greater than that of analytes. As can be seen from Table 6.3, addition of these common ions did not lead to significant interferences, which means deviations from measurements with no additional ions were less than 5%. However, Cu^{2+} with a concentration that is 2 times greater than the analytes caused drastic changes in obtained signals. This interference caused by Cu^{2+} ion in lead and cadmium determination is frequently announced in the literature [9,10,87]. Adding ferrocyanides to the medium is a typical method for defeating the interference caused by Cu^{2+} ion [166]. It is also worth acknowledging that standard addition method also helps to eliminate certain interferences and has a vital significance in electrochemical analysis applications.

6.2.8 Determination of analytes in environmental samples

Environmental water samples were collected in the year 2020, from a riverbed at the northwestern region of Turkey named Ergene Basin. The area is reportedly contaminated by industrial activities [167]. In Table 6.4, the coordinates of the sample collection points and the analyte concentrations determined by two different methods are given. In all SWASV measurements of samples, standard addition method was employed to exclude possible interferences. Findings indicate that the voltammetric method proposed in this study is a satisfactory method for heavy metal analysis, which is compliant with a commonly trusted method in the field that is GFAAS. Consequently, MWCNT/N/Bi/PGE is an efficient electrode that allows rapid

determination of Pb^{2+} and Cd^{2+} , simultaneously in environmental water samples that have complex matrices, when employed in SWASV technique.

Table 6.3 : Investigation of possible interferences caused by some common ions to the analyte signals obtained from $80 \mu\text{g L}^{-1}$ Pb^{2+} and Cd^{2+} .

Interfering ion	Pb^{2+} (μA)	Cd^{2+} (μA)
None	59.4 ± 7.7	43.2 ± 8.1
Ca^{2+}	59.3 ± 2.4	45.3 ± 3.8
Mg^{2+}	56.6 ± 6.4	45.1 ± 6.7
K^{+}	60.9 ± 9.6	45.2 ± 9.3
Na^{+}	61.1 ± 3.3	45.7 ± 9.9
NO_3^{-}	62.1 ± 6.5	41.8 ± 9.1
NH_4^{+}	56.4 ± 3.7	45.8 ± 4.4
Mn^{2+}	57.8 ± 2.6	42.6 ± 4.6
Fe^{2+}	56.8 ± 4.5	41.8 ± 4.7
Ni^{2+}	60.3 ± 6.9	41.8 ± 9.5
PO_4^{3-}	61.8 ± 8.3	41.2 ± 8.4
Cu^{2+}	1.5 ± 1.0	6.4 ± 2.6

Table 6.4 : Comparative concentrations of Pb^{2+} and Cd^{2+} detected in environmental water samples by GFAAS and SWASV equipped with the MWCNT/N/Bi/PGE (N:3).

Sample coordinate	Analyte	GFAAS results ($\mu\text{g L}^{-1}$)	SWASV results ($\mu\text{g L}^{-1}$)
N: 41.23177	Pb^{2+}	138 ± 6.92	140 ± 14.3
E: 27.85027	Cd^{2+}	4.97 ± 0.25	4.62 ± 0.60
N: 41.231768	Pb^{2+}	84.3 ± 4.21	84.2 ± 1.15
E: 27.647017	Cd^{2+}	4.25 ± 0.21	4.36 ± 0.24
N: 41.2512	Pb^{2+}	32.7 ± 1.64	34.1 ± 2.45
E: 27.124375	Cd^{2+}	2.79 ± 0.14	2.71 ± 0.43



7. SIMULTANEOUS, SELECTIVE, AND HIGHLY SENSITIVE VOLTAMMETRIC DETERMINATION OF LEAD, CADMIUM, AND ZINC VIA MODIFIED PENCIL ELECTRODES³

7.1 Experimental

7.1.1 Chemicals and instruments

All of the chemicals used in this study were of analytical grade and bought from Merck (Darmstadt, Germany). Pb^{2+} , Cd^{2+} , Zn^{2+} , and Bi^{3+} standards were prepared from 1000 mg L⁻¹ ICP standard solutions (Merck, Darmstadt, Germany). MWCNT with 48–78 nm outside diameter, 5–15 nm inside diameter, and electrical conductivity 98 S/cm was purchased from Nanografi (Ankara, Turkey). 0.1 M Acetate buffer (pH 4.5, prepared from CH_3COONa and CH_3COOH) was chosen as the supporting electrolyte. The working solutions were prepared daily with deionized water. Experiments were conducted at room temperature and dissolved gasses were not eliminated. Gamry (Warminster, PA, USA) Reference 600 electrochemical workstation was utilized in all electrochemical procedures, including a three electrode system having PGE as the working electrode, Pt wire as the counter electrode, and Ag/AgCl as the reference electrode. Disposable PGE with 0.5 mm diameter and 2B softness were purchased from Faber-Castell (Stein, Germany). During the measurements, only a 0.5 cm part of each PGE was immersed in the working solution, which provided a surface area of 8.05 mm². Frequency range of 0.1 Hz–100000 Hz and amplitude of 10 mV were set for electrochemical impedance spectroscopy experiments. pH measurements were conducted with AWTW (Weilheim, Germany) 7310 pH meter. Fourier transform infrared spectroscopy (FTIR) analyses of the electrodes were conducted with Thermo Scientific Smart ITX FTIR (MA, USA). Electrode surfaces were morphologically characterized by Field-emission scanning electron microscope (FESEM, QUANTA FEG-250, FEI). Agilent GTA 120 Graphite Tube Atomizer (USA) was equipped to analyze water samples.

³This chapter is based on the paper “Ustabasi, G.S., Yilmaz, I., Ozcan, M., Cetinkaya, E. (2022). Simultaneous, selective and highly sensitive oltammetric determination of lead, cadmium, and zinc via modified pencil graphite electrode. *Electroanalysis*, 34.” [168]

7.1.2 Electrode preparation

1.0 mg of MWCNT were suspended in 5 mL of ethanol and sonicated for 2 h to allow uniform dispersion before the PGE were immersed in it. To obtain a clean surface, PGE were sonicated for 2 h in ethanol prior to modification as well. Once the sonication was over, 4 cm parts of electrodes were vertically dipped in MWCNT suspension for 5 s, then they were left to dry at room temperature on their own. After the electrodes were dried, they were coated in-situ with bismuth and employed in simultaneous Pb^{2+} , Cd^{2+} , and Zn^{2+} determination. It should be noted that even though a 0.5 cm part of the pencil lead is employed during the analysis, 4 cm part of a 6 cm long pencil lead is dipped into MWCNT suspension to maintain a homogeneous structure throughout, in terms of electrochemical properties such as conductivity. Then the leads were cut at the 4 cm length by carefully measuring via digital caliper before they were placed inside the electrode skeleton, which consists of a clutch from a mechanical pencil and copper wire for electrical conduction. In the end, the PGE had MWCNT all over its surface, but bismuth was only plated at the tip that is immersed into the sample solution during SWASV.

7.1.3 Electrode characterization

To investigate the surface properties and electrochemical changes caused by the modification FTIR spectra, electroactive surface area (ESA), electrochemical impedance spectroscopy and SEM visuals of both the bare PGE and MWCNT/PGE were evaluated. No pretreatment or sample preparations were needed for FTIR analysis, except for the gentle scraping off of the particles from the electrode surface. Electrodes were sputter-coated with gold under vacuum prior to SEM imaging to achieve better resolution at higher magnifications. Electroactive surface area (ESA) was determined by applying cyclic voltammetry for both bare and modified electrodes in 0.1 M KCl solution that includes 2.5 mM $\text{Fe}(\text{CN})_6^{4-/3-}$. Electrochemical impedance spectroscopy (EIS) analysis was performed in 0.1 mol L⁻¹ H₂SO₄ solution to evaluate the charge transfer capacities.

7.1.4 Electrochemical measurements

SWASV measurements were conducted via Gamry potentiostat, utilizing a three electrode system consisting of Ag/AgCl electrode, Pt wire, and modified PGE as the

reference electrode, counter electrode, and working electrode, respectively. Optimized pre-concentration parameters were 180 s for pre-concentration time and -1.4 V for pre-concentration potential with an I-Limit of 3 A. This optimized method was employed in all validation and sample analysis measurements. Before the voltammetric method was optimized the following parameters were held constant: pH 4.5 acetate buffer solution as supporting electrolyte; pre-concentration for 120 s at -1.4 V with I-Limit 3 A; SWASV between -1.4 and 0 V, with pulse size 40 mV and frequency 25 Hz. Finally, all electrochemical measurements were conducted thrice.

7.1.5 Sample preparation

Water samples collected from Ergene Basin, Turkey, were kept at +4 °C until the analysis. They were left to arrive at room temperature before necessary sample preparation steps. Primarily, approximately 50 mL of the samples were filtered through 391 grade Sartorius Stedim Biotech (Gottingen, Germany) filter discs. Then the filtered samples were subjected to continuous UV radiation for 2 h in a closed environment to eliminate organic substances. Finally, they were placed in 50 mL polypropylene centrifuge tubes and kept at +4 °C until the analysis.

7.2 Results and Discussion

7.2.1 Optimization of electrode preparation process

Both Bi^{3+} and MWCNT are frequently preferred materials in the literature as electrode modifiers in heavy metal determination studies [5,169]. They might either be combined with other materials or used on their own. Figure 7.1 shows how the different combinations of PGE, MWCNT (1.0 mg in 5 mL ethanol), and Bi^{3+} (0.2 ppm, insitu) performed in the determination of Pb^{2+} , Cd^{2+} , and Zn^{2+} in the supporting electrolyte of acetate buffer (pH 4.5), in this study. The highest signals were obtained when both Bi^{3+} and MWCNT were utilized to modify PGE. Moreover, they seem to have a synergistic effect in the simultaneous determination process, improving both sensitivity and reproducibility. As can be seen from the figure, the deviations are much higher when only one of them is used in the modification combination. On the other hand, even though the average value of lead signal with MWCNT/PGE is similar to that of MWCNT/Bi/ PGE, this is not the case for the other simultaneously determined analytes. When both MWCNT and bismuth are incorporated in the electrode structure

obtained peaks are significantly higher for all three analytes, simultaneously. Therefore, their concentrations had to be optimized to ascertain the most advantageous combination of modifiers.

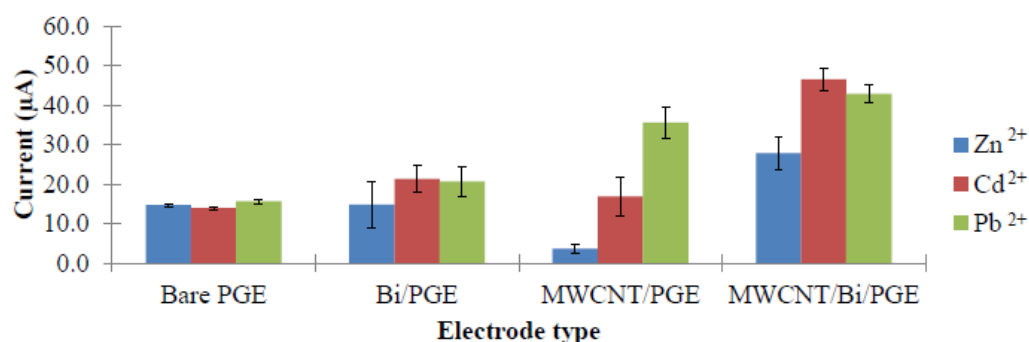


Figure 7.1 : Anodic current peaks obtained from 100 µg L⁻¹ of analytes in pH 4.5 acetate buffer solution with bare PGE, PGE modified with in-situ Bismuth (0.2 ppm), PGE modified with MWCNT (1.0 mg), and PGE modified with MWCNT (1.0 mg) and in-situ bismuth (0.2 ppm) (N:3).

Optimal MWCNT and Bi³⁺ concentrations were determined by testing the analytical performances of electrodes modified with various concentration levels. Amount of MWCNT dispersed in ethanol was varied between 0.5 and 2.0 mg, while the concentration of Bi³⁺ to be in situ plated on the electrode was varied between 0.1 and 0.5 ppm. During the optimization of MWCNT, Bi³⁺ concentration was kept constant at 0.2 ppm, whereas in Bi³⁺ concentration optimization amount of MWCNT was kept at 1.0 mg. Concentrations of MWCNT and Bi³⁺ to be tested were chosen from the relevant studies in the literature [9,88,93,145]. Highest analyte signals were obtained when 1.0 mg and 1.5 mg of MWCNT was added to 5 mL of ethanol. To maintain the cost and material efficiency, the minimum amount of substance that provided the highest performance was selected. Therefore, optimum MWCNT concentration was chosen as 1.0 mg. With the same logic in mind, Bi³⁺ concentration was set at 0.3 ppm. Voltammograms obtained during MWCNT and bismuth optimization are presented in Figure 7.2.

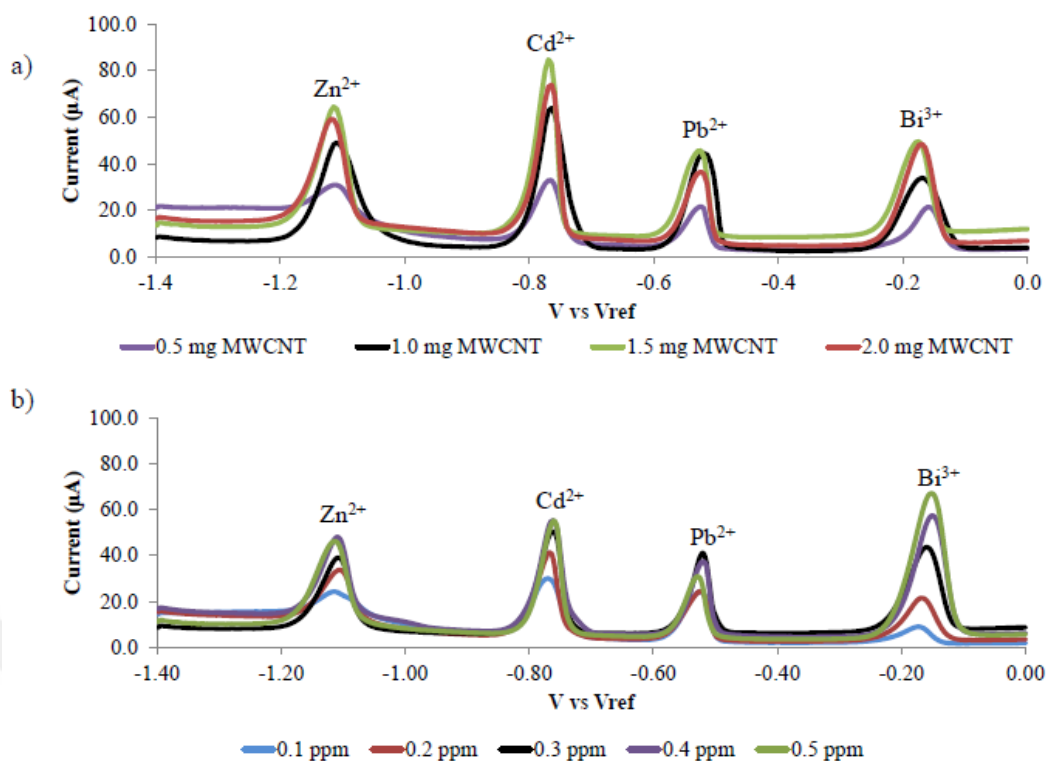


Figure 7.2 : Optimization of (a) MWCNT (using 0.2 ppm bismuth) and (b) bismuth (using 1.0 mg MWCNT) concentrations for electrode preparation. Concentrations of lead, cadmium and zinc were held constant at $100 \mu\text{g L}^{-1}$.

7.2.2 Electrode characterization

To characterize the surface and electrochemical properties of the modified electrode, firstly, FTIR spectra of both PGE and the MWCNT/PGE were assessed. As seen in Figure 7.3, PGE exhibits a relatively simple FTIR spectra due to its all carbon structure, while MWCNT/PGE displays a small O–H stretch peak around $3200\text{--}3400 \text{ nm}^{-1}$, alkyne C–H stretch around 3300 nm^{-1} , C–H stretch with a double peak around $2580\text{--}3100 \text{ nm}^{-1}$, and C=O stretching between $1650\text{--}1800 \text{ nm}^{-1}$. Sonicating and suspending MWCNT in ethanol right before modification seems to oxygenate the structure and add –COOH groups to several sites on the surface of the MWCNT/PGE. Addition of this functional group increases the overall polarity and allows for electrons to flow more freely on the electrode surface, which lowers the electrical resistivity [170,171]. Moreover, the presence of higher number of active sites on the electrode also increases the amount of metals deposited on the surface.

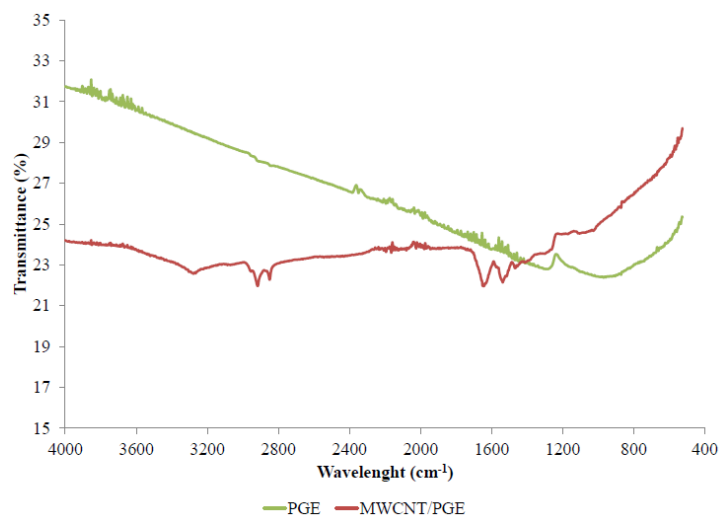


Figure 7.3 : FTIR spectra of MWCNT modified electrode (red line) and bare electrode (green line).

SEM visuals of both the bare and the modified electrodes are in Figure 7.4. As it is evident from the figure, after the immersion process, surface of the electrode is covered with a tubular structured substance, which is MWCNT. This curly shape of MWCNT was also reported in several other studies [172-174]. Outputs of SEM analysis reveal that immersion of PGE in MWCNT ethanol suspension is a facile technique that allows modifying the surface in a short time. It is worth acknowledging that Figure 7.4b also demonstrates the uneven distribution of MWCNT on the surface. Nevertheless, this phenomenon did not affect the performance of the electrode, since it exhibits acceptable repeatability and reproducibility as described below.

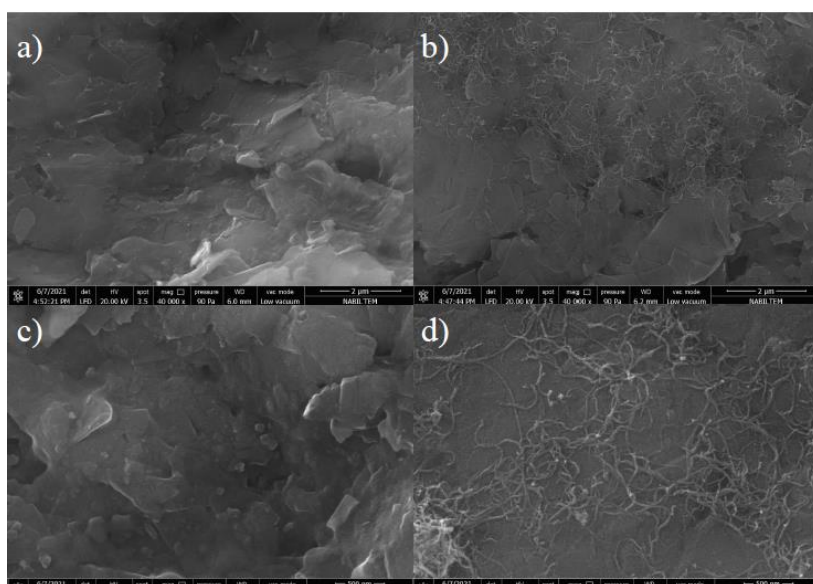


Figure 7.4 : SEM visuals of (a) bare PGE under 40000x magnification, (b) MWCNT/PGE, under 40000x magnification, (c) bare PGE under 100000x magnification, and (d) MWCNT/PGE under 100000x magnification.

To determine the repeatability parameter of MWCNT/Bi/PGE, standard deviation of 10 repetitive analyte measurements in 80 µg L⁻¹ standard solution of lead, cadmium, and zinc (under optimized conditions) were calculated and found less than 5%. Also, the standard deviation of 10 measurements, conducted with three replicates each, in 80 µg L⁻¹ standard solution of lead, cadmium, and zinc (under optimized conditions) were conveyed with 10 separately prepared MWCNT/Bi/PGE to determine the reproducibility parameter. Obtained standard deviation of measurements obtained from separately prepared electrodes was found to be less than 10%. This indicates stability and reusability for MWCNT/Bi/PGE developed in this study. Furthermore, PGE are considered disposable because of their abundance and low cost. Nevertheless, the durability of the constructed MWCNT/Bi/PGE was determined by measuring 80 µg L⁻¹ Pb²⁺, Cd²⁺, and Zn²⁺ under optimized conditions with 24 h intervals. Standard deviation was less than 10% for seven consecutive days. Obtained currents were significantly lowered after the 8th day, which indicates disruption in the stability of the modified electrode. The voltammograms acquired from ESA and EIS measurements are presented in Figure 7.5a and 7.5b, respectively. ESA values were calculated via the Randles-Sevcik equation as follows:

$$I_p = (2.69 \times 10^5) n^{2/3} S D_0^{1/2} C_0 V^{1/2} \quad (7.1)$$

In this equation, S stands for the surface area of the electrode (cm²), n stands for the number of transferred electrons per mole, D₀ stands for the diffusion coefficient of the molecules in the solution (cm²s⁻¹), C₀ stands for the solution concentration (mol cm⁻³), V stands for the scan rate (Vs⁻¹), and I_p stands for the peak current of the redox couple. The ESA (abbreviated as S in the equation) is calculated from this equation by using the following constants for both the bare and modified electrodes: n=1, C₀=2.5 molcm⁻³, D₀=7.6×10⁻⁶ cm²s⁻¹ [175,176], and V=150 Vs⁻¹. The ESA values of PGE and MWCNT/PGE were calculated as 0.0517 cm² and 0.0426 cm², respectively. Modified electrode had a slightly lower ESA compared to the bare electrode, which is an unexpected drawback. However, as it is evident from Figure 5b, the charge transfer resistance of the modified electrode is remarkably lower than the bare electrode, making it much more conductive. This drastic increase in conductivity due to modification with MWCNT compensates for the slight reduction in the ESA and agrees with the FTIR results.

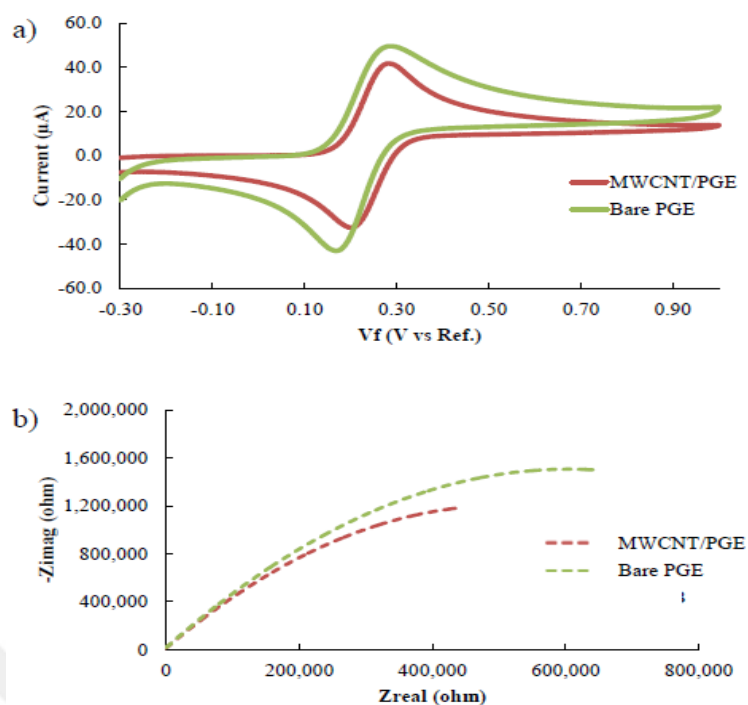


Figure 7.5 : Electrochemical characterization of electrodes: (a) Cyclic voltammograms of PGE (green line) and MWCNT/PGE (red line) in 0.1 M KCl solution containing 2.5 mM $\text{Fe(CN)}_6^{4-/3-}$ (scan rate: 100 mV s^{-1}), (b) Electrochemical impedance spectra of PGE (green dotted line) and MWCNT/PGE (red dotted line) in $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ (frequency range: 0.1 Hz– 0.1 MHz, amplitude: 10 mV).

7.2.3 Optimization of voltammetric parameters

Four different supporting electrolytes were evaluated in terms of analyte signal generation: 0.01 M HClO_4 , acetate buffer (pH 4.5), 0.1 M NaCl, and 0.5 M HCl. Among them pH 4.5 acetate buffer provided the most desirable results, which is a highly common type of electrolyte choice in heavy metal determination. Finally, the optimum deposition time was scanned between 30 and 240 s, and it was found that obtained peaks did not significantly increase after 180 s of deposition.

7.2.4 Calibration curve and detection limits

Linear range, LOD and LOQ values were determined to assess the analytical performance of MWCNT/Bi/PGE. To determine LOD and LOQ values, standard deviations of 10 repetitive measurements of blank solution was multiplied with 3 for LOD, and 10 for LOQ, and then the results were divided by the corresponding calibration curve slope for the analyte. Calculated LOD values for Pb^{2+} , Cd^{2+} , and Zn^{2+} are 0.27, 0.43, $1.63 \mu\text{g L}^{-1}$, respectively, and LOQ values for Pb^{2+} , Cd^{2+} , and Zn^{2+} are

0.89, 1.44, 5.43 $\mu\text{g L}^{-1}$, respectively. Obtained current peaks were linearly increased with increasing analyte concentrations between 1–80 $\mu\text{g L}^{-1}$, 5–80 $\mu\text{g L}^{-1}$, and 10–80 $\mu\text{g L}^{-1}$ for Pb^{2+} , Cd^{2+} , and Zn^{2+} , respectively. Figure 7.6 contains the voltammograms of varying analyte concentrations and the calibration curves of each analyte. The voltammograms show how distinctly separated and welldefined the peaks are, indicating a high level of resolution. Furthermore, Table 7.1 provides a comparison of deposition times, linear ranges and LOD levels of some relevant studies analyzing Pb^{2+} , Cd^{2+} , and Zn^{2+} simultaneously via various carbon based electrodes. This comparison shows that on the basis of detection limits this study had a somewhat average performance but it is desirable in terms of wide linear ranges. Additionally, the proposed method does not offer a significant advantage over other studies in terms of deposition time. Yet, due to the simplicity and the rapidity of the modification process, it is still able to save time for the analyst.

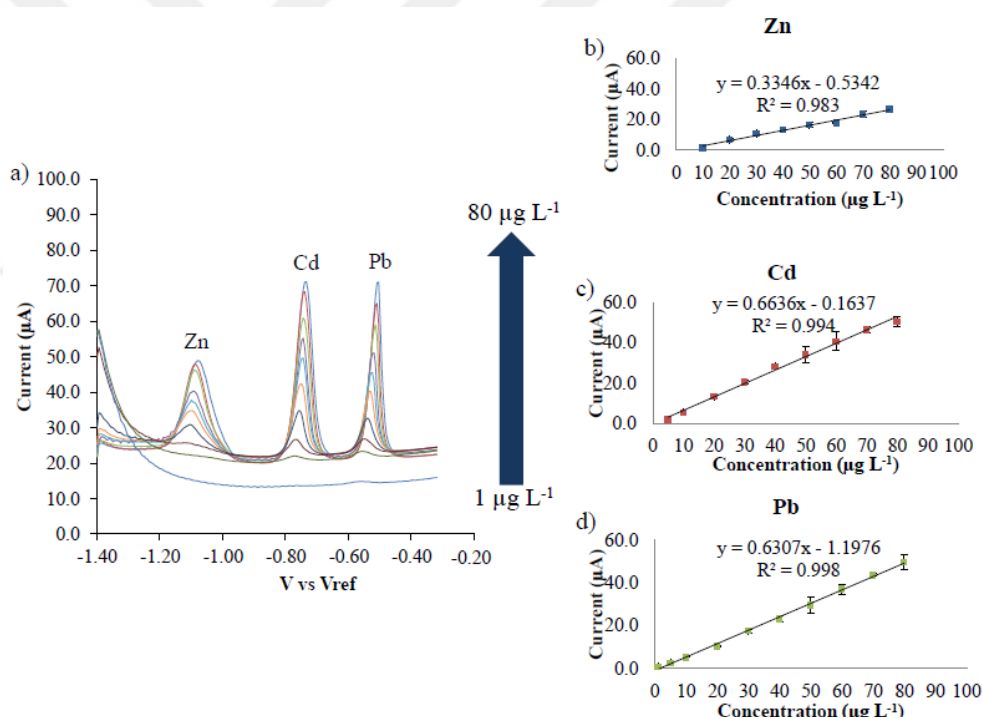


Figure 7.6 : Results of linear range investigation: (a) voltammograms obtained with optimized electrode and under optimized conditions, (b) calibration curve of Zn, (c) calibration curve of Cd, (d) calibration curve of Pb (N:3).

Table 7.1 : Comparison of the analytical performances of carbon based electrodes detecting Pb²⁺, Cd²⁺, and Zn²⁺ simultaneously.

Ref.	Electrode ^[a]	Method & deposition time	LOD ($\mu\text{g L}^{-1}$)			Linear range ($\mu\text{g L}^{-1}$)		
			Pb	Cd	Zn	Pb	Cd	Zn
[145]	N/Bi/SPCE	SW-ASV (180 s)	0.17	0.09	0.6	1-50	1-50	1-50
[136]	AG/N/Bi/G CE	DP-ASV (300 s)	0.05	0.07	0.57	5-100	5-100	5-100
[144]	Bi-F/N/IL/G/SPCE	SW-ASV (120 s)	0.08	0.06	0.09	1-100	1-100	1-100
[84]	Bi-CNT SPE	SW-ASV (300 s)	1.3	0.7	12	2-100	2-100	2-100
[89]	Fe ₃ O ₄ /Fl-MWCNT/G CE	SW-ASV (180 s)	1.7	1.6	0.78	3-2125	5-3372	6-6216
[117]	O/Ag/CPE	SW-ASV (40 s)	48	89	-	5000-160000	5000-160000	-
[148]	Bi/Gr-E-CE	SW-ASV (120 s)	15	5.6	3.9	98-883	56-281	41-352
[143]	Bi-F/SWCNH/SPE	SW-ASV (150 s)	0.2	0.4	-	1-60	1-60	-
[90]	Bi-F/GCE	DP-ASV (110 s)	0.65	0.93	1.07	5-110	5-110	5-110
This study	MWCNT/Bi/PGE	SW-ASV (180 s)	0.27	0.43	1.63	1-80	5-80	10-80

[a] Electrode abbreviations: N: Nafion, Bi: Bismuth, SPCE: Screen printed carbon electrode, AG: Activated graphene, GCE: Glassy carbon electrode, F: Film, IL: Ionic liquid, G: Graphene, CNT: Carbon nanotube, SPE: Screen printed electrode, Fl: Fluorinated, MWCNT: Multi wall carbon nanotube, O: Ocimum sanctum leaf extract, Ag: Silver, CPE: Carbon paste electrode, Gr-E-CE: Graphite epoxy composite electrode, SWCNH: Single walled carbon nanohorn.

7.2.5 Certified reference material measurement

Certified reference Canadian water sample (TM-254) was analyzed with the optimized electrode and method. Standard addition method was applied for the measurements. The proposed method coupled with the MWCNT/Bi/PGE generated reliable results with a less than 5% deviation from the certified values of the Canadian water sample (TM-254).

7.2.6 Interference study

Effects of several ions that may be present in the sample medium were investigated. In the presence of 100-fold analyte concentration of Na⁺, K⁺, Mg²⁺, Ca²⁺, NH₄⁺, NO₃⁻, Mn²⁺, Fe²⁺, PO₄³⁻ ions did not cause more than 5% deviation. However, the presence of 2-fold analyte concentration of Ni²⁺ and Cu²⁺ significantly decreased the analyte

signals. The presence of Cu^{2+} ions is frequently reported in the literature to cause interferences in Pb^{2+} , Cd^{2+} and Zn^{2+} analysis [9,10,87]. The most common approach to eliminate the interferences caused by Cu^{2+} is the addition of ferrocyanides [166]. On the other hand, Ni^{2+} ions are not usually reported to cause interferences for Pb^{2+} , Cd^{2+} and Zn^{2+} determination. However, there are some studies that indicate this phenomenon similar to our study [90]. Interferences caused by nickel can be avoided by adding dimethylglyoxime (DMG) to the sample media beforehand and remove the Ni-DMG complex precipitate prior to analysis [177]. Moreover, standard addition is a very effective and essential application in electroanalysis, which also helps to overcome such interferences.

7.2.7 Sample measurements

Environmental water samples were collected in 2020 from Ergene Basin that resides in the northwestern region of Turkey, Trakya, which is subjected to contamination by industrial effluents [167]. Table 7.2 shows both the coordinates of sample collection points and the analyte concentrations in the samples determined via both the proposed method and GFAAS. Standard addition method was applied throughout all the SWASV measurements to overcome aforementioned interferences. Moreover, 1.5 mL of samples was placed in the electrochemical cell containing electrolyte to reach a total volume of 15 mL, resulting in a 10-fold dilution of both the analytes and the interfering components. Voltammograms and standard addition plots of these samples are presented in Figure S1, Figure S2, and Figure S3. When the results acquired via GFAAS and the proposed SWASV are compared it is seen that the two methods generate results in compliance to one another. Thus, it is established that when the MWCNT/Bi/PGE constructed in this study with a simple modification process is coupled with the optimized SWASV method, the combination allows for accurate simultaneous heavy metal determination in environmental water samples.

Table 7.2: The results of real sample measurements obtained by GFAAS and SWASV equipped with the MWCNT/Bi/PGE (N: 3).

No	Coordinates	Pb ($\mu\text{g L}^{-1}$)		Cd ($\mu\text{g L}^{-1}$)		Zn ($\mu\text{g L}^{-1}$)	
		GFAAS	SWASV	GFAAS	SWASV	GFAAS	SWASV
1	N:41.23161 E:27.644962	119+5.98	112+3.95	4.13+0.21	4.31+0.40	35.2+2.47	35.7+4.60
2	N:41.1809672 E:27.7773610	103+5.18	104+3.59	6.48+0.32	6.90+0.29	56.3+3.94	53.7+7.97
3	N:41.1802627 E:27.77143	15.8+0.79	14.7+0.79	13.6+0.68	13.1+1.26	52.5+3.67	52.3+2.87

8. SIMULTANEOUS DETERMINATION OF IRON AND COPPER USING SCREEN-PRINTED CARBON ELECTRODES BY ADSORPTIVE STRIPPING VOLTAMMETRY WITH O-PHENANTHROLINE⁴

8.1 Experimental

8.1.1 Chemicals

All chemicals used in this study were of analytical grade. o-phenanthroline (Phen) was purchased from J.T. Baker (Pennsylvania, USA), $K_4Fe(CN)_6$ was supplied by Panreac (Barcelona, Spain) and all other products were acquired from Merck (Darmstadt, Germany). Stock solutions of Cu(II) and Fe(III) were prepared from their salts and standardized complexometrically. All solutions were prepared with water obtained from Milli-Q Reference A + Water Purification System (Millipore, France) with an electrical resistivity of 18.4 M Ω . Certified wastewater reference material (SPS-WW2, Waste Water Level 2, Batch no: 112) was obtained from Spectrapure Standards AS (Oslo, Norway). The certificate of analysis provided by the producer states that this reference material contains 13 elements with the following concentrations: 10000 $\mu g L^{-1}$ Al, 500 $\mu g L^{-1}$ As, 100 $\mu g L^{-1}$ Cd, 300 $\mu g L^{-1}$ Co, 1000 $\mu g L^{-1}$ Cr, 2000 $\mu g L^{-1}$ Cu, 5000 $\mu g L^{-1}$ Fe, 2000 $\mu g L^{-1}$ Mn, 5000 $\mu g L^{-1}$ Ni, 5000 $\mu g L^{-1}$ P, 500 $\mu g L^{-1}$ Pb, 500 $\mu g L^{-1}$ V, 3000 $\mu g L^{-1}$ Zn. All reagents and prepared solutions were stored at + 4°C until their use.

8.1.2 Voltammetric measurements

Voltammetric measurements were carried out via the NOVA 2.1.3 (Metrohm Autolab, Utrecht, Netherlands) electrochemical software system, and employing an Autolab PGSTAT204 Potentiostat/Galvanostat by Metrohm (Utrecht, Netherlands). Electrochemical procedures were conducted at room temperature and without deaeration with a three electrode system that consisted of Ag/AgCl/KCl (3 mol L⁻¹)

⁴This chapter is based on the paper “Ustabasi, G.S., Pérez-Ràfols, C., Serrano, N., Díaz-Cruz, J.M. (2022). Simultaneous determination of iron and copper using screen-printed carbon electrodes by adsorptive stripping voltammetry with o-phenanthroline. *Microchemical Journal*, 179, 107597.” [178]

reference electrode, a Pt counter electrode from Metrohm, and a working carbon electrode with 4 mm diameter from a screen-printed carbon electrode (SPCE) supplied by Metrohm DropSens (DRP-110, Spain).

Voltammetric measurements were carried out in a solution containing $0.1 \mu\text{mol L}^{-1}$ $\text{K}_4\text{Fe}(\text{CN})_6$ and 100 mg L^{-1} Phen in acetate buffer (pH 4.5). Metal accumulation was performed by applying a deposition potential (E_d) of -0.4 V for 240 s under constant stirring. The oxidation step takes place from -0.4 V to 1.0 V with 0.05 s modulation time, 2 mV step potential, and 50 mV modulation amplitude. A new SPCE was employed for every experiment and three repetitive blank measurements were initially taken in order to obtain a stable background current. Blank correction was applied in all measurements.

Certified wastewater reference material was analyzed using the standard addition calibration method. pH was adjusted in the vessel by the addition of pH 4.5 acetate buffer, resulting in a dilution factor of 1/20; then Phen and ferrocyanide were added and voltammetric measurements were carried out at the above-mentioned conditions. Three successive metal additions were performed and the corresponding voltammograms were recorded.

8.2 Results and discussion

Figure 8.1 shows a tentative mechanism for the AdSV determination of iron and copper assisted by Phen and ferrocyanide. In the stripping step a deposition potential of -0.4 V is applied for 240 s, resulting in a twostep process. Initially Fe(III) and Cu(II) are reduced to Fe(II) and Cu(I) , respectively with the aid of $\text{K}_4\text{Fe}(\text{CN})_6$. Next, Fe(II) and Cu(I) are complexed with the Phen present in solution and adsorbed onto the electrode surface. It is worth noting that the presence of $\text{K}_4\text{Fe}(\text{CN})_6$ as reducing agent is imperative for the AdSV iron determination whereas the reduction of Cu(II) is possible without the presence of ferrocyanide, albeit to a slightly lower extension. Then, during the anodic scan, the oxidation of Fe(II)-Phen and Cu(I)-Phen complexes is recorded, showing two oxidation peaks at ca. -0.2 V and 0.8 V for copper and iron, respectively, as well as a third signal at ca. 0 V related to the oxidation of ferrocyanide from the solution (Figure 8.2).

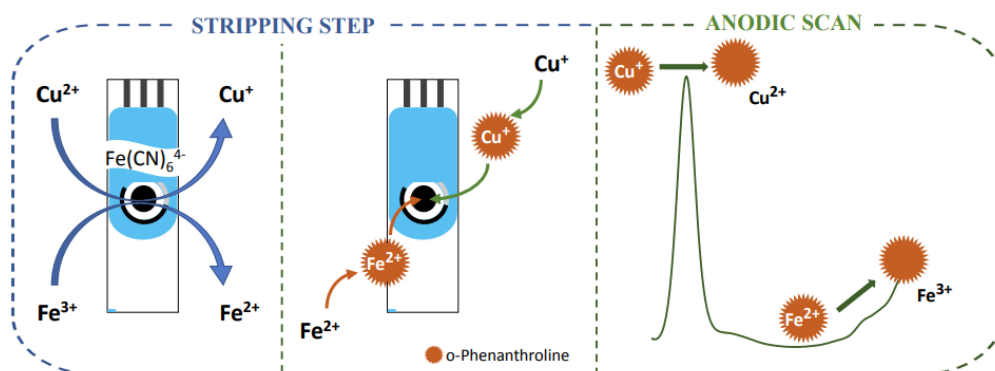


Figure 8.1 : Schematic description of suggested mechanism for iron and copper reduction on the electrode surface assisted by o-phenanthroline and ferrocyanide.

8.2.1 Optimization of complex formation conditions and voltammetric parameters

As a first step, the parameters related to the stripping step were optimized to ensure the proper reduction of Fe(III) and Cu(II). Figure 8.2a displays the effect of $K_4Fe(CN)_6$ concentration on the AdSV determination of iron and copper. As it can be seen, increasing concentrations of $K_4Fe(CN)_6$ give rise to higher voltammetric peaks for both metal ions. This is in agreement with the above-proposed mechanism as higher concentrations will lead to a more effective reduction of Fe(III) and Cu(II). However, large concentrations of $K_4Fe(CN)_6$ result in an excess of ferrocyanide that is also oxidized in the anodic scan, showing a voltammetric peak at ca. 0.05 V that partially overlaps with copper peak. Thus, $0.1 \mu\text{mol L}^{-1}$ of $K_4Fe(CN)_6$ was selected as a compromise concentration for further measurements.

Deposition potential and time were also optimized considering E_d from -0.4 V to -0.6 V and t_d from 120 to 360 s. An E_d of -0.4 V provided the largest peaks for both analytes and a t_d of 240 s was selected as a compromise between peak current and time analysis.

Then, in order to improve the formation of metal ion complexes, Phen concentration and pH were also optimized. Three different concentrations were tested for Phen: 10, 100, and 1000 mg L^{-1} . As it can be seen in Figure 8.2b, high concentrations of Phen result in higher copper voltammetric peaks but lower iron peaks. In addition, the peak related to the oxidation of ferrocyanide considerably increases at high Phen concentrations. This fact can be explained because high amounts of Phen hinder the reduction of Fe(III) to Fe(II), even in the presence of $K_4Fe(CN)_6$. As a consequence,

iron peak decreases and ferrocyanide is not consumed, leading to a higher oxidation peak.

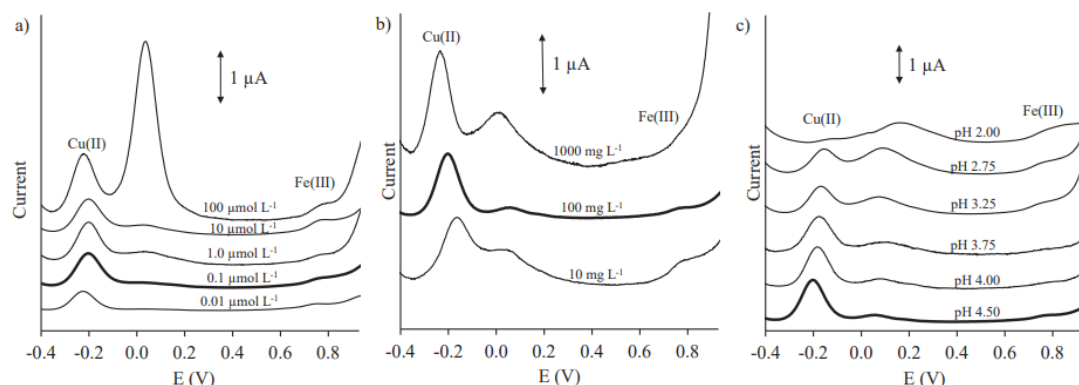


Figure 8.2 : Optimization of complex formation conditions: (a) concentration of $\text{K}_4\text{Fe}(\text{CN})_6$, (b) concentration of o-phenanthroline, and (c) pH. Optimization was always performed using a solution containing $50 \mu\text{g L}^{-1}$ of $\text{Cu}(\text{II})$ and either 100 (for a) or 50 (for b and c) $\mu\text{g L}^{-1}$ of $\text{Fe}(\text{III})$ and applying an E_d of -0.4 V for 120 s. Concentration of o-phenanthroline was fixed to 100 mg L^{-1} in (a) and (c); pH was set to 4.5 in (a) and (b) and $2 \mu\text{mol L}^{-1}$ $\text{K}_4\text{Fe}(\text{CN})_6$ was employed in (b) and (c).

Finally, pH is also a crucial parameter because it affects the complexation process. pH values from 2.0 to 4.5 were evaluated (Figure 8.2c). For this purpose, several buffers were prepared: acetate buffer (pH 3.75 – 4.50), citrate buffer (pH 2.75 – 3.25) and hydrochloric acid (pH 2). Higher pH was not considered to avoid the formation of hydroxocomplexes, which is particularly important in the case of iron. The highest and best-defined voltammetric peaks were achieved at pH 4.5. Lower pH values lead to the protonation of Phen ($\text{pK}_a = 4.27$), which hinders the complexation process.

8.2.2 Analytical performance

Once the optimal conditions for the determination of copper and iron were established, both individual (Figure 8.3) and simultaneous (Figure 8.4) calibrations of target metal ions were carried out considering concentrations from 0.5 to 400 and from 5 to 400 $\mu\text{g L}^{-1}$ for copper and iron, respectively. Each calibration study was conducted in triplicate and their average values and standard deviations were computed. A large and well-defined oxidation peak was observed for copper, whereas the voltammetric peak of iron is much lower. This could be due to the positive charge of both $\text{Cu}(\text{I})$ and $\text{Fe}(\text{II})$ complexes with Phen. Although both of them are accumulated onto an electrode submitted to a negative potential, the adsorbed complex of copper is reoxidized at a slightly less negative potential, which ensures that it remains at the electrode surface until it is stripped from it. In contrast, the oxidation of the adsorbed $\text{Fe}(\text{II})$ -Phen

complex takes place at a very positive potential (due to the higher stability as compared to the complex of Fe(III)) and electrostatic repulsion may cause a desorption of most of the accumulated complex before it can be reoxidized. Anyway, as it will be explained later, the remaining adsorbed iron is enough to provide an acceptable sensitivity. Moreover, both peaks increase linearly with metal ion concentration as it can be observed in the calibration plots (insets in Figures 8.3 and 8.4).

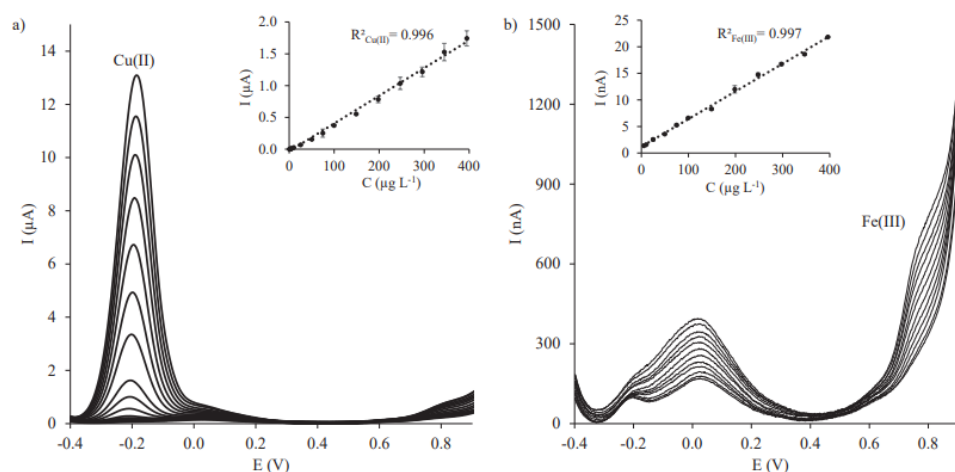


Figure 8.3 : Individual calibration plots and corresponding calibration curves (insets) of (a) Cu(II) and (b) Fe(III) in pH 4.5 acetate buffer acquired by applying an E_d of -0.4 V for 240 s in the presence of $0.1 \mu\text{mol L}^{-1}$ $\text{K}_4\text{Fe}(\text{CN})_6$ and 100 mg L^{-1} o-phenanthroline.

The analytical performance was assessed in terms of sensitivity (as the slope obtained from the calibration curves), linearity and limits of detection (LOD) and quantification (LOQ) (Table 8.2). LOD and LOQ were calculated as $3\sigma/s$ and $10\sigma/s$, respectively, where “ σ ” represents the standard deviation of the y-intercept and “s” represents the slope attained from the lower part of the corresponding calibration curves ($0.5\text{--}25 \mu\text{g L}^{-1}$ for copper and $5\text{--}100 \mu\text{g L}^{-1}$ for iron). LOQ was stated as the lower limit of the linear range.

As expected from the obtained AdS voltammograms, copper sensitivity is much higher than that observed for iron. According to the previous discussion, this could be attributed to copper complex being more easily accumulated and retained on the electrode surface until reoxidation. Moreover, the sensitivities achieved in both individual and simultaneous calibration were mainly the same, which indicates that iron and copper do not interfere with each other during simultaneous measurements. It should be highlighted that previous works dealing with the determinations of iron and copper reported important problems associated to these interactions [179-182].

We hypothesize that the lack of interactions in this method could be attributed to the presence of the reducing agent $\text{K}_4\text{Fe}(\text{CN})_6$, which reduces both $\text{Fe}(\text{III})$ and $\text{Cu}(\text{II})$, preventing the coexistence of $\text{Fe}(\text{II})$ and $\text{Cu}(\text{II})$ and avoiding redox processes among them. Thus, the lack of interaction between iron and copper represents a significant improvement in the simultaneous voltammetric determination of these metal ions.

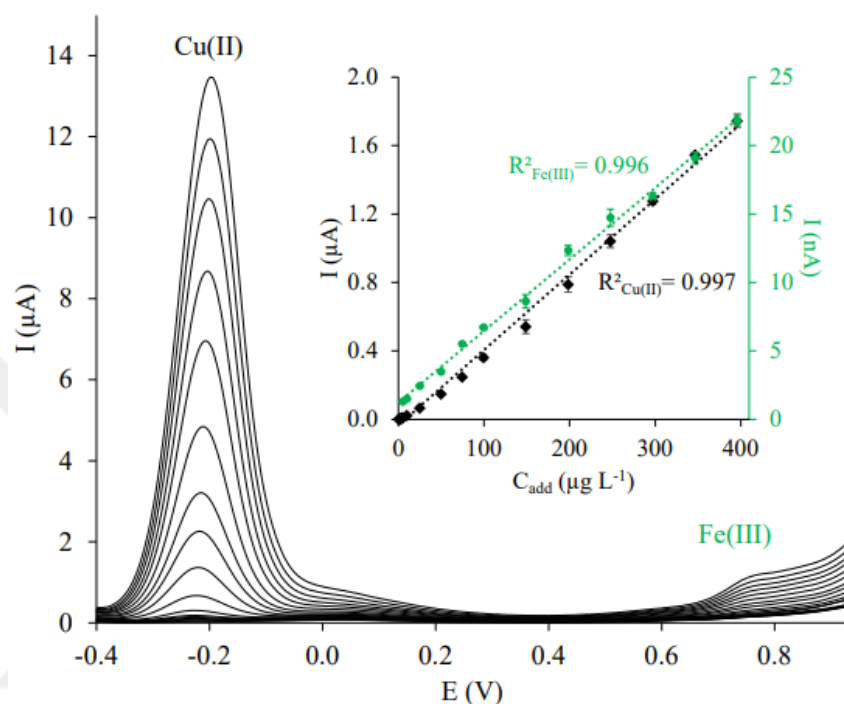


Figure 8.4 : Simultaneous calibration voltammograms of $\text{Cu}(\text{II})$ and $\text{Fe}(\text{III})$ acquired by applying an E_d of -0.4 V for 240 s, in the presence of $0.1 \mu\text{mol L}^{-1}$ $\text{K}_4\text{Fe}(\text{CN})_6$ and 100 mg L^{-1} o-phenanthroline in pH 4.5 acetate buffer. Inset: Calibration curves for $\text{Cu}(\text{II})$ (diamond, black) and $\text{Fe}(\text{III})$ (round, green).

A good linearity up to $400 \mu\text{g L}^{-1}$ was achieved for both metal ions, providing a wide linear range that covers both low and high concentrations expected in the environment. This fact is a strong point of this method as the majority of works reported in the literature mainly deal with either low concentrations [180] or large concentrations [183], particularly in the case of iron. Moreover, linear ranges were not altered in the simultaneous calibration, demonstrating that iron and copper are not competing for the working electrode surface and again confirming the lack of interaction between both species.

LODs and LOQs from individual and simultaneous calibrations are summarized in Table 8.2. As it can be seen, LODs around 0.3 and $3.7 \mu\text{g L}^{-1}$ were achieved for copper and iron, respectively. It should be highlighted that LODs were practically the same in

separate and joint calibrations, confirming the suitability of this method for the simultaneous determination of iron and copper.

The analytical performance reported in the present study was also compared to other relevant studies in the literature that investigated simultaneous iron and copper determination via voltammetric methods. This comparison is revealed in Table 8.1. Ferreira et al. reported a borondoped diamond electrode [184] which was able to determine 5 metals simultaneously in a short deposition time. Obtained LODs were slightly lower and slightly higher than those achieved in our study for copper and iron, respectively. However, this worked focused only in low metal ion concentrations, reporting narrow linear ranges (up to $40 \mu\text{g L}^{-1}$) with the additional drawback that diamond electrodes are highly expensive as compared to the disposable SPCE employed in this study. On the other hand, lower LOD values, particularly for iron, were reported with hanging mercury drop electrode (HMDE) [185,186], yet the extremely low values reported in the work of Santos-Echeandia [186] were achieved using a longer deposition time and were associated to a really narrow linear range. Even though HMDE is known for its exceptional performance, the concerns regarding its toxicity cause the recent trends in electrochemistry to lean toward greener alternatives [7]. As for the carbon nanotubes modified glassy carbon electrode [187], although the linear range of iron is much wider than that obtained in our method, the LOD values for both Cu and Fe are one order of magnitude higher, with the additional disadvantage of using an electrode modified with carbon nanoallotropes that increases the economical cost. Lastly, in the work of Ghoneim [188], remarkably low LOD values were obtained with a carbon paste electrode (CPE) when determining copper and iron as complexes of 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol. This work of Ghoneim seems to adopt a similar approach to

our proposed method by using an unmodified electrode and adding the ligand directly into the measurement solution. Despite its simplicity, the drawback of their method is that it only allows detection at trace levels of analytes within a relatively narrow linear range, whereas copper and iron can be found at various concentration levels in the environment.

Due to the wide linear range obtained for copper and iron, repeatability and reproducibility were evaluated at two different concentration levels (50 and $250 \mu\text{g L}^{-1}$) of both analytes to make sure that the proposed method provides satisfying

repeatability and reproducibility throughout the whole concentration range. Repeatability was calculated from the relative standard deviation (RSD%) of five readings taken from a single electrode and presented as intervals to incorporate the results obtained for three different electrodes. On the other hand, reproducibility was computed from five repetitive measurements taken from three different electrodes. From the repeatability and reproducibility results presented in Table 8.3, it can be deduced that the method provided satisfying repeatability and reproducibility with less than 7 and 5% RSD for copper and iron, respectively.

8.2.3 Certified reference material measurement

The suitability of the developed method for the simultaneous determination of copper and iron in real samples was assessed through the analysis of a wastewater reference material using the standard addition method and in triplicate. Fig. 8.5a shows the obtained AdS voltammograms, which display a similar voltammetric behavior as that observed in synthetic samples. Calibration plots for copper and iron are presented in Fig. 8.5b and 8.5c, respectively and the determined concentrations are reported in Table 8.4. As it can be seen, good reproducibility and excellent accuracy (relative errors equal or lower than 1%) were attained for both metal ions.

Thus, these good values, coupled to the well-defined peaks obtained, show that the proposed method performs well even in a complex sample medium that also contains other ions at high concentrations (see experimental section for detailed values).

Table 8.1: Reported findings from studies that investigated simultaneous voltammetric analysis of iron and copper.

Ref	Electrode	Analyte	Technique	Deposition time	Applied samples	Peak potentials (V)		LOD ($\mu\text{g L}^{-1}$)		Linear range ($\mu\text{g L}^{-1}$)	
						Cu	Fe	Cu	Fe	Cu	Fe
[184]	B-doped diamond electrode	Fe, Cu, Zn, Pb, Cd	SWASV	60 s	Fuel ethanol	0.05	0.53	1.42	1.9	2-40	2-40
[185]	HMDE	Fe, Cu, Cd	DPAdSV	60 s	Tap water	-0.28	-0.46	0.4	0.5	0.5-100	1-80
[186]	HMDE	Co, Cu, Fe, Ni, V	AdCSV	60 s/300 s ^a	Estuarine pore water	-0.18	-0.35	0.006	0.07	Up to 5.08	Up to 16.8
[187]	N/CNT/GCE	Fe and Cu	LSSV	270 s	Fuel ethanol	0.05	0.5	3.2	40	6.4-57	56-2792
[188]	CPE	Mn, Cu, Fe	SWAdCSV	240 s	Ground, tap, and bottled water	-0.05	-0.36	0.11	0.09	0.36-45	0.31-25
This study	Bare SPCE	Fe and Cu	DPAdSV	240 s	Wastewater CRM	-0.2	0.76	0.34	3.74	1.14-400	12.5-400

Abbreviations: B, Boron; SWASV, square wave anodic stripping voltammetry; HMDE, hanging mercury drop electrode; DPAdSV, differential pulse adsorptive stripping voltammetry; N, Nafion; CNT, carbon nanotube; GCE, glassy carbon electrode; LSSV, linear sweep stripping electrode; CPE, carbon paste electrode; SWAdCSV, square wave adsorptive cathodic stripping voltammetry; AdCSV, adsorptive cathodic stripping voltammetry; SPCE, screen printed carbon electrode.

^a 300 s of deposition for LOD and 60 s deposition for linear range calculation.

Table 8.2: Comparison of the analytical performances obtained with individual and simultaneous determination of Cu(II) and Fe(III) ions. Analytes were determined using E_d of -0.4 V for 240 s in the presence of $0.1 \mu\text{mol L}^{-1}$ $\text{K}_4\text{Fe}(\text{CN})_6$ and 100 mg L^{-1} o-phenanthroline in pH 4.5 acetate buffer. The standard deviations are shown within brackets. ($n = 3$).

	Individual		Simultaneous	
	Cu(II)	Fe(III)	Cu(II)	Fe(III)
Sensitivity ($\text{nAV } \mu\text{g}^{-1} \text{L}$)	4.35 (0.06)	0.0521 (0.0006)	4.40 (0.04)	0.0523 (0.0006)
Intercept ($\mu\text{g L}^{-1}$)	-30 (10)	1.2 (0.1)	-34 (8)	1.2 (0.1)
Linear range ^a ($\mu\text{g L}^{-1}$)	1.05-400	12.4-400	1.14-400	12.5-400
R^2	0.996	0.997	0.997	0.996
LOD ($\mu\text{g L}^{-1}$)	0.315	3.71	0.341	3.74

^aThe initial value of linear range is established from the calculated LOQ.

Table 8.3: Results obtained from repeatability and reproducibility studies. Analytes were determined by using $E_d - 0.4$ V for 240 s deposition time in the presence of $0.1 \mu\text{mol L}^{-1}$ $\text{K}_4\text{Fe}(\text{CN})_6$ and 100 mg L^{-1} o-phenanthroline in pH 4.5 acetate buffer.

	Repeatability (%)		Reproducibility (%)	
	Cu(II)	Fe(III)	Cu(II)	Fe(III)
$50 \mu\text{g L}^{-1}$	2.5-4.3	1.5-2.6	2.7	3.5
$250 \mu\text{g L}^{-1}$	2.4-6.8	2.3-3.7	2.8	4.8

Table 8.4: Certified and measured concentrations of copper and iron ions in the wastewater reference material. Analytes were determined using E_d of -0.4 V for 240 s in the presence of $0.1 \mu\text{mol L}^{-1}$ $\text{K}_4\text{Fe}(\text{CN})_6$ and 100 mg L^{-1} o-phenanthroline in pH 4.5 acetate buffer. The standard deviations are shown within brackets. ($n = 3$).

	Certified values	Measured values	Relative error
	($\mu\text{g L}^{-1}$)	($\mu\text{g L}^{-1}$)	(%)
Cu	2000 (10)	2020 (10)	1.0
Fe	5000 (25)	5030 (60)	0.6

9. CONSIDERATIONS ON THE USE OF SPECTROELECTROCHEMISTRY IN REFLECTION MODE FOR QUANTITATIVE ANALYSIS: STUDY OF THE Fe(III)/Fe(II) – ORTHOPHENANTHROLINE SYSTEM⁵

9.1 Materials and Methods

9.1.1 Chemicals

Analytical grade chemicals were utilized in all experiments. These chemicals were acquired from Merck (Darmstadt, Germany), except for orto-phenanthroline, which was purchased from J.T. Baker (Pennsylvania, USA). Stock solutions of Fe(III) and Cu(II) were prepared from their salts and standardized. Purified water (with electrical resistivity of 18.4 MΩ) was obtained from Milli-Q Reference A+ Water Purification System (Millipore, France) to prepare all solutions. Finally, all of the prepared solutions were kept at +4°C until their use.

9.1.2 Spectroelectrochemical measurements

Spectroelectrochemical (SEC) measurements were conducted with a SPELEC equipment from Metrohm DropSens (Oviedo, Spain) controlled by the DropView SPELEC software system (Version 3.2.2). All measurements were carried out in a SEC cell (ref. DRP-REFLECELL 70055) in normal configuration, i.e., with the screen-printed electrode and the optical probe placed horizontally and vertically, respectively.

Samples were inserted into the measuring device by first placing 50 µL of sample onto the working electrode of the screen-printed carbon electrode (SPCE) unit, fixed onto the lower part of the cell (Figure 3.1). The cell was closed inserting the upper part, which remained fixed with a set of eight magnets. Then, an additional volume of 50 µL was added through the hole on the top of the cell. Presence of any air bubbles was checked and prevented prior to the measurement. Once the sample was

⁵This chapter is based on the paper “Ustabasi, G.S., Bastos Arrieta, J., Pérez-Ràfols, C., Serrano, N., Díaz-Cruz, J.M. (2022). Considerations on the use of spectroelectrochemistry in reflection mode for quantitative analysis: study of the Fe(III)/Fe(II) –orthophenanthroline system. *Microchemical Journal*, 107678, 2022.” [189]

placed in the cell, the optical probe was introduced slowly in the hole and some extra drops were allowed to spill out from the sides of the probe. The cell was thoroughly washed with water after each measurement and a fresh drop was used every time. Also, a new SPCE was used for each set of measurements. SPCE devices, with a 4 mm diameter working electrode, were provided by Metrohm DropSens (DRP-110, Spain) and connected by means of a flexible cable provided by Metrohm DropSens (ref. CAST).

Unless otherwise indicated, SEC measurements were conducted in solutions containing 25 mmol L⁻¹ of OP in acetate buffer 0.1 mol L⁻¹ (pH 4.5). Cyclic voltammetric (CV) measurements were scanned between -0.3 V and 1.1 V with a scan rate of 50 mV s⁻¹. Chronoamperometric (CA) measurements were carried out at a fixed potential of -0.4 V every 0.5 s during 90 s. Integration time was set to 1000 ms for all spectrometric measurements considering a wavelength range from 200 nm to 900 nm.

9.1.3 Data treatment

Raw experimental data were imported from DropView software to Excel® and Matlab® environments and processed with home-made programs written in Matlab language [190]. Raw optical spectra in counts acquired at 2007 wavelengths were submitted to four-level discrete wavelet transform (DWT) using Daubechies 4 function, which provided good results in the compression and denoising of spectra acquired with SPELEC in a previous study [191]. Absorbance spectra were computed from raw spectra or DWT-denoised spectra in counts using as a reference the corresponding spectra measured for the sample solution prior to the SEC measurement. In both cases, Equation 9.1 was applied to get the absorbance value for a given wavelength as $A = \log_{10} (p_0/p)$, where p_0 , p are the counts measured before starting the electrochemical measurement and at the time when the absorbance is going to be computed, respectively. In practical terms, this means applying the equation above, element by element, to couples of vectors of 2007 or 258 points to get single absorbance vectors of 2007 or 258 points.

$$A = \log_{10} \frac{p_0}{p} = \varepsilon \Delta c_{red} d \quad (9.1)$$

9.2 Results and Discussion

9.2.1 Preliminary voltammetric study

CV was used for a preliminary study of the Fe(III)-OP system. Figure 9.1 compares the CV signals obtained at different concentrations of both substances. Figure 9.1a shows in red colour a CV of Fe(III) in the absence of OP. In the forward scan there is a reduction peak of Fe(III)-ion at ca. -0.2 mV, whereas in the backward scan there is a broad peak at ca. 0.2 V corresponding to the reoxidation of the Fe(II)-ion generated in the forward scan. The CV of OP alone, in blue colour, presents a significant capacitive current (large vertical distance between the currents in both scans as compared to the blank of the buffer) and several cathodic signals: one relatively small and very broad at ca. 0.2 mV and another very large starting at ca. -0.4 V. It is not clear whether these signals are due to the reduction of OP or to an enhanced reduction of the solvent. Anyway, the second signal restricts the working potential to values more positive than -0.4 V. Finally, the CV of a mixture of Fe(III) and OP, in green colour, shows a cathodic peak in the forward scan at ca. 0.2 V and an anodic peak in the backward scan at ca. 0.9 V. The cathodic peak can be undoubtedly assigned to the reduction of the oxidised form of the iron complex (Fe(OP)_3^{3+}) present in the solution. As for the anodic peak, it is due to the reoxidation of the reduced complex generated previously in the forward scan and which remains in the diffusion layer. If we compare both peaks with those obtained for the same concentration of Fe(III) in the absence of OP, we see that they are quite bigger, and much better shaped in the case of the anodic one. This fact suggests that the formation of OP complexes with Fe(II) and Fe(III) favours the electrochemical processes of this redox pair. Moreover, both peaks are dramatically moved to more positive potentials (more than 0.4 V the cathodic peak and more than 0.7 V the anodic one). Figure 9.1b shows that this shift is progressive as the concentration of OP increases. This can be explained considering that the 1:3 complex of OP with Fe(II) (predominant at large excess of OP) is quite more stable than the analogous complex of Fe(III), which stabilises the reduced species as compared to the oxidised one. Thus, it is not surprising that the addition of OP makes Fe(III) easier to be reduced (cathodic peak potential less negative) and Fe(II) more difficult to be oxidised (anodic peaks more positive). At the view of these results, an intermediate concentration of 25 mmol L⁻¹ of OP was selected for further experiments, in order to assure enough excess of ligand and to prevent too large interfering cathodic signals.

Figure 9.1c shows that, for a fixed concentration of OP 25 mmol L⁻¹, successive additions of Fe(III)-ion cause a progressive increase of both peaks, thus confirming that they are caused by the reduction and oxidation of Fe(III) and Fe(II) complexes, respectively. A slight negative shift of the potentials is also observed as the concentration of iron increases (in opposition to the trend shown in Figure 9.1b at increasing concentrations of OP). Figure 9.1d evidences a very good linear relationship between the anodic peak and the concentration of Fe(III), whereas linearity is not as good in the case of the cathodic peak, probably because of the interference of the cathodic signals of OP mentioned above.

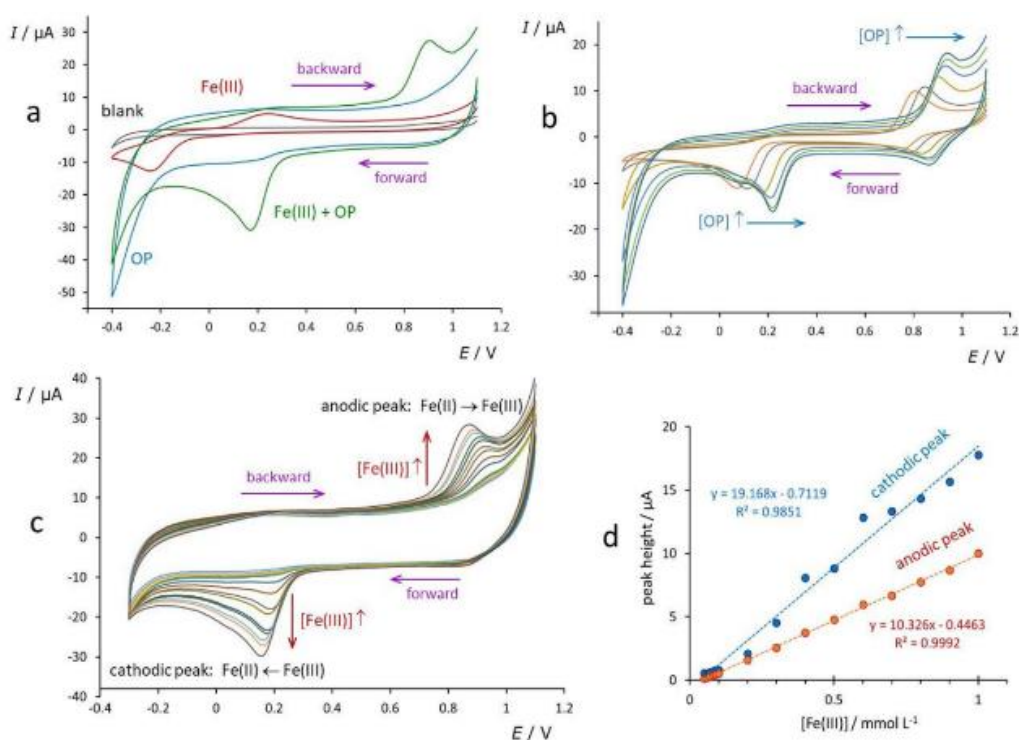


Figure 9.1 : Results of the preliminary CV study. a) CV signals obtained for the buffer alone, and solutions of Fe(III) 1.0 mmol L⁻¹, OP 25 mmol L⁻¹, and Fe(III) 1.0 mmol L⁻¹ + OP 25 mmol L⁻¹; b) evolution of CV signals for a Fe(III) solution 1.0 mmol L⁻¹ and concentrations of OP 5, 10, 25, 50, 75 and 100 mmol L⁻¹; c) evolution of CV signals for a OP solution 25 mmol L⁻¹ and increasing concentrations of Fe(III) within the range 0.05–1.0 mmol L⁻¹; d) calibration plots obtained from the heights of cathodic and anodic peaks shown in c. The directions of the scans are denoted with purple arrows.

An additional study was made by differential pulse voltammetry (DPV), a very popular technique for analytical purposes. However, obtained peaks are quite distorted and noisy, probably due to the above-mentioned cathodic signals of OP. Moreover, there is not a linear dependence of the peak height with the concentration of Fe(III). Consequently, DPV was discarded for the further studies.

9.2.2 SEC study by means of cyclic voltammetry (CV)

A series of SEC experiments were carried out in the reflection cell by means of CV at a fixed concentration of OP 25 mmol L^{-1} and increasing concentrations of Fe(III) in the range $0.01 - 1.0 \text{ mmol L}^{-1}$. Figure 9.2 shows some typical results obtained in this way for three different concentrations of Fe(III): 0.2, 0.5 and 1.0 mmol L^{-1} . The potential scan (Figure 9.2a) is the same as in Figure 9.1c and the obtained voltammograms present a similar shape. Figure 9.2b shows the counts of the 57 spectra registered in every experiment during the evolution of the CV scan. Although the counts at the different wavelengths are practically the same, there is a narrow region around 500 nm where the evolution of the potential produces significant changes in the number of counts. Moreover, the changes become more notorious as the concentration of Fe(III) increases. This strongly suggests that the decrease in the counts is due to the absorption of radiation in the region around 500 nm by the complex of Fe(II) with OP, which is released at the electrode as a consequence of the CV scan. The complex of Fe(III) does not seem to have a significant absorption in this region and, if it was not the case, the calculation of absorbance taking the initial spectrum as a reference would minimise such contribution.

A key point in the treatment of UV-VIS spectral data is the signal-to-noise ratio. Working with a low reflectance electrode such as SPCE is more versatile and affordable than using gold screen-printed units. However, the counts achieved are quite lower and this produces a considerable noise which becomes more relevant as the concentration of Fe(III) decreases. The application of a DWT to the raw spectra in counts can be a very useful strategy covering two main objectives: compressing the data with minimal loss of information and reducing noise. In this work we have applied DWT using the function ‘Daubechies 4’ with 4 compression levels.

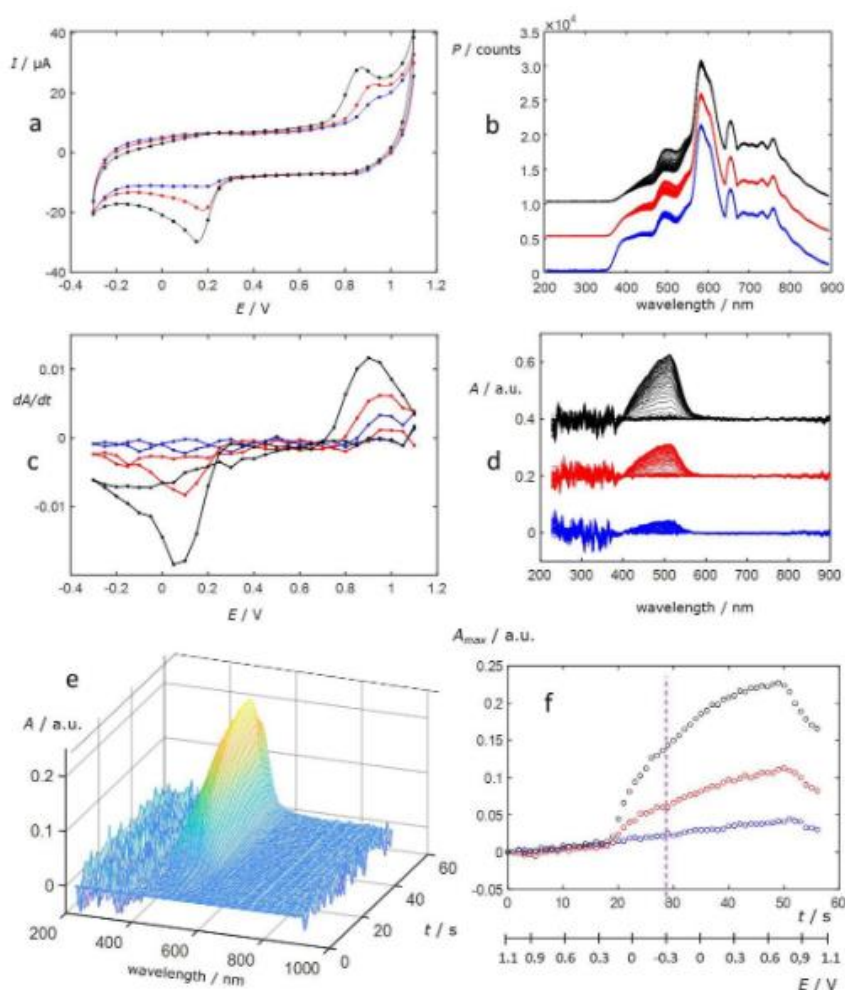


Figure 9.2 : Typical results of SEC experiments based on CV scans in solutions containing 25 mmol L⁻¹ of OP and different concentrations of Fe(III): 0.2 mmol L⁻¹ (blue), 0.5 mmol L⁻¹ (red) and 1.0 mmol L⁻¹ (black). a) CV signals (solid lines) denoting with solid circles the points in which spectra were registered (one spectrum per second); b) radiation power measured by the detector, in counts, as a function of the wavelength; d) absorbance spectra, obtained by dividing every spectra in counts by the first spectrum, just before SEC measurement; c) derivative of the maximum absorbance with respect to the time (solid circles, connected by straight lines); e) 3D plot of absorbance versus wavelength and time for the solution 1.0 mmol L⁻¹ Fe(III) and 25 mmol L⁻¹ OP; f) maximum absorbance versus time and potential (second axis) for the three solutions above, with a dashed purple line separating forward and backward scans. In figures b and d, red and black spectra have been shifted along the y axis for comparison purposes.

Using these compressed and denoised spectra, still in counts, it is possible to obtain reasonable absorbance plots dividing the successive spectra measured in every SEC experiment by the first one (that acquired just before the CV scan). Figure 9.2d compares the absorbance spectra obtained for the three concentrations of Fe(III) considered, showing a maximum in the absorbance at ca. 510 nm whose height increases with the concentration of Fe(III). It is important to notice the relatively high

level of noise registered in the UV region, where counts are scarce. Anyway, this noise is much lower than that obtained when absorbance is computed from raw, not compressed spectra in counts (not shown). Moreover, the level of noise is comparable at the different concentrations of Fe(III) and, as a consequence, the signal-to-noise ratio increases as the Fe(III) concentration grows and the absorbance peaks go higher (Figure 9.2d). Figures 9.2e and 9.2f show a more detailed picture of the evolution of the absorbance spectra along the CV scan as a function of the variable time. In both 3D and 2D plots it is clear that the absorbance only starts to grow when the forward scan approaches potentials sufficiently negative to reduce the Fe(III)-OP complex and the growing continues until the backward scan approaches potentials positive enough to reoxidise it. In good agreement with Equation (9.2), the derivative with respect to the time of the absorbance achieved at 510 nm (Figure 9.2f) generates a curve (Figure 9.2c) with a similar shape as the CV plot (Figure 9.2a). Besides the higher dispersion of optical data, it is interesting to notice the absence of the capacitive contribution which appears in the middle of CV curves (capacitive currents have no optical effects).

$$\frac{dA(t)}{dt} = \frac{D^{1/2}\varepsilon}{\pi^{1/2}} c_{ox}^* t^{-1/2} = I(t) \frac{\varepsilon}{nFS} \quad (9.2)$$

Some experiments were made applying a negative deposition potential to accumulate the reduced complex at the electrode surface prior to the CV scan, but no significant improvement of the SEC signals was noticed. This can be due to several reasons: the inefficient mass transport towards the electrode, just by diffusion, the possible electrostatic repulsion at positive potentials between the electrode and the positively charged complex, and the fact that the optical measurements detect the coloured complex not only at the electrode surface, but at any distance inside the optical path. Indeed, deposition at the electrode only shifts the distribution of reagents and products from the bulk to the electrode surface and the diffusion layer. Only species that are being produced or consumed at the electrode are able to change the absorbance, independently of the place where they remain, on the electrode surface or in the solution.

9.2.3 SEC study by means of chronoamperometry (CA)

A set of chronoamperometric (CA) measurements was carried out at a fixed potential of -0.4 V which, according to the CV plots of Figures 9.1 and 9.2, should provide a potential sufficiently negative to leave the reduction process of the complex of Fe(III)

controlled by diffusion. Then, under these conditions and for a planar working electrode such as that of SPCE, the Cottrell expression of Equation (9.3) should hold.

$$I = \frac{nFSD^{1/2}c_{ox}^*}{\pi^{1/2}t^{1/2}} \quad (9.3)$$

CA measurements were made at the same concentrations of OP and Fe(III) used in the CV study summarised in Figure 9.2, and similar SEC effects were detected along the raw spectra in counts (not shown). Nevertheless, a better signal-to-noise ratio was achieved, which allowed us to obtain reasonable data at lower concentrations of Fe(III) than in the case of CV. Figure 9.3 shows some examples of the signals obtained in this CA study. It is remarkable, on one hand, the higher difference between the absorbance evolution at different concentrations (Figure 9.3d) as compared to the small variations found in the current evolution (Figure 9.3a), and, on the other hand, the better signal-to-noise ratio of CA absorbance spectra (Figure 9.3c) as compared to these obtained by CV (Figure 9.2d).

Figure 9.4 deals with possible linearisations, using Cottrell Equation. in (9.3) for electrochemical data (Figure 9.4a) and Equation (9.4) for absorbance data (Figure 9.5b).

$$A = \frac{2D^{1/2}\varepsilon}{\pi^{1/2}} c_{ox}^* t^{1/2} \quad (9.4)$$

In the first case, linear dependence with $t^{1/2}$ is achieved relatively late, when the current has decreased in a significant way and sensitivity is low. On the contrary, in the second case, a perfect linearity with $t^{1/2}$ is achieved very early and the absorbance is continuously growing with time. This means that, as time goes by, the current is decreasing forced by the low efficiency of mass transport whereas the absorbance is increasing due to the accumulation of the absorbed reduced complex in the diffusion layer. Especially interesting is the study of the slopes calculated from figures 9.4a and 9.4b. Results obtained from currents are linear even at relatively low concentrations of Fe(III). However, slopes only become concentration-dependent at more than 0.10 mmol L⁻¹ of Fe(III). In contrast, slopes computed from absorbances are perfectly linear with respect to the concentration of Fe(III), inside two concentration ranges, shown in Figures 9.4c and 9.4d.

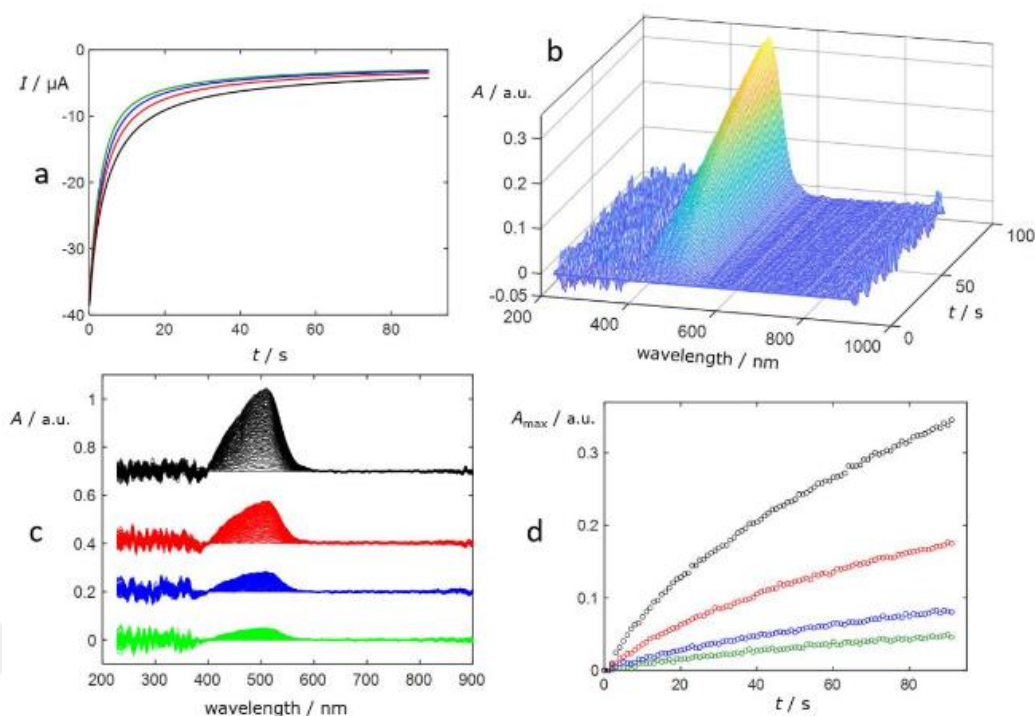


Figure 9.3 : Typical results of SEC experiments based on chronoamperometric (CA) scans carried out in solutions containing 25 mmol L⁻¹ of OP and different concentrations of Fe(III): 0.1 mmol L⁻¹ (green), 0.2 mmol L⁻¹ (blue), 0.5 mmol L⁻¹ (red) and 1.0 mmol L⁻¹ (black). a) Currents as a function of time measured when a fixed potential of -0.4 V is applied while one UV-vis spectrum per second is registered; b) absorbance measured as a function of wavelength and time in the CA experiment carried out for a concentration of 1.0 mmol L⁻¹ of Fe(III); c) absorbance spectra obtained at the different concentrations above; d) maximum absorbance with respect to the time. In figure c some spectra have been shifted along the y axis for a better comparison of the four data sets.

At this point, it must be noted the key denoising role of the DWT applied to compress optical data. Such impression is confirmed when absorbance spectra are computed from the measured counts. Figure 9.5 and Table 9.1 compare the linear plots of the maximum absorbance as a function of $t^{1/2}$ at different concentrations of Fe(III) when absorbance is computed from raw data or from DWT-compressed data. It is clear that compressed data are substantially less noisy and provide more precise slope values than in the case of raw data. Moreover, the differences are more evident as the concentration of Fe(III) decreases.

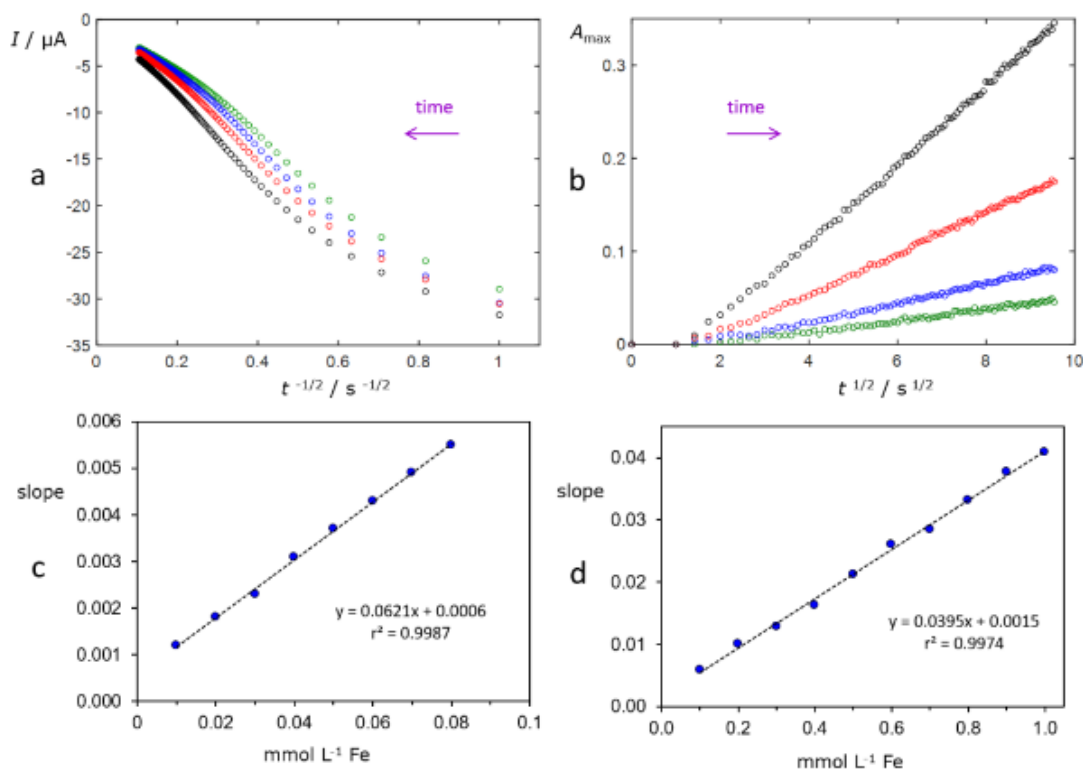


Figure 9.4 : Linearisation of SEC data using CA scans for a OP concentration 25 mmol L^{-1} and concentrations of Fe(III) 0.1 mmol L^{-1} (green), 0.2 mmol L^{-1} (blue), 0.5 mmol L^{-1} (red) and 1.0 mmol L^{-1} (black): a) Current as a function of $t^{-1/2}$; b) Maximum absorbance as a function of $t^{1/2}$; c and d) Slopes of the A_{max} vs. $t^{1/2}$ plots as a function of the concentration of Fe(III) inside two different linearity ranges. Purple arrows indicate the evolution of time in the experiments. In c and d the equations and the r^2 coefficients of the calibration lines are indicated inside the plots.

Table 9.2 compares some relevant calibration parameters obtained using different strategies in SEC experiments. In the case of CV measurements, the anodic peak measured in the backward scan is much more reliable for calibration than the cathodic scan, notoriously affected by reduction signals of OP. As for chronoamperometric (CA) measurements, the use of Cottrell Equation to carry out calibration from currents is not especially precise (LOD is quite higher than using the anodic CV peak). In contrast, absorbance data acquired along CA measurements provide quite better results, with detection limits at the level of $\mu\text{mol L}^{-1}$. This can be due, on one hand, to the progressive increase of the concentration of the absorbing species with time and, on the other hand, to the denoising effect of DWT. This is especially true for the use of A_{max} vs. $t^{1/2}$ slopes, where the additional denoising effect of the second regression (getting a ‘slope of slopes’) can play a key role.

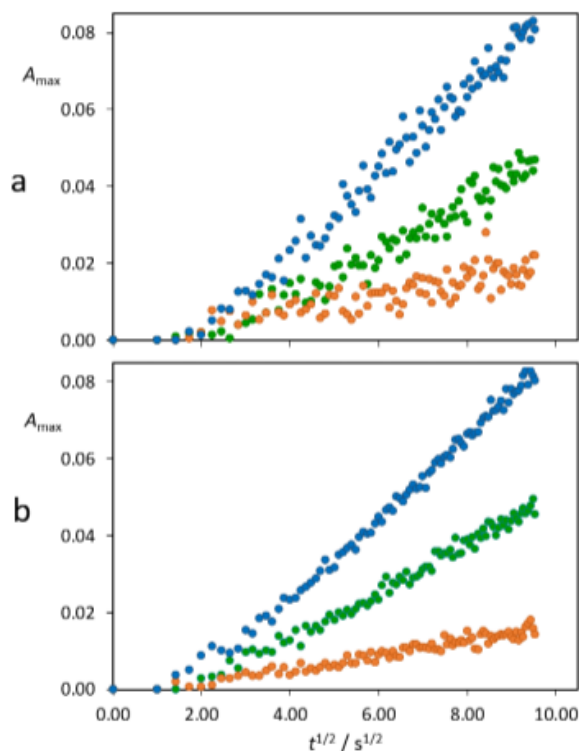


Figure 9.5 : Effect of DWT on the noise of chronoabsorptometric data measured for a OP concentration of 25 mmol L⁻¹ and Fe(III) concentrations of 0.02 mmol L⁻¹ (orange), 0.1 mmol L⁻¹ (green) and 0.2 mmol L⁻¹ (blue). Results in a were obtained from the raw spectra measured at 2007 wavelengths, whereas results in b were achieved from the compressed spectra resulting from DWT.

Finally, Equation (9.5)-(9.8) have been applied to the estimation of the diffusion coefficient of the complex Fe(III)-OP. First, chronoamperometric experiments were carried out with potassium ferricyanide and Equation (9.7) was used to estimate the fR roughness factor by using the diffusion coefficient of ferricyanide, 7.26 10⁻⁶ cm²s⁻¹, determined in [192]. A fR value of 1.15 was found, suggesting that the roughness of SPCE is not especially high (the effective surface was only 15% higher than the geometrical one). With this fR value, Eqns. (9.7) and (9.8) were applied to chronoamperometric and chronoabsorptometric data acquired in solutions containing Fe(III) in the range 0.1-0.9 mmol L⁻¹ and OP 25 mol L⁻¹ and diffusion coefficients of 7.67 10⁻⁶ cm²s⁻¹ and 7.73 10⁻⁶ cm²s⁻¹ were obtained, respectively, for the Fe(III)-OP complex. Both estimates are very similar to each other and also to that of ferricyanide complex, which suggests a good reliability of chronoabsorptometric data for the estimation of diffusion coefficients of spectroelectrochemically active substances.

$$f_R = S'/S \quad (9.5)$$

$$I = \frac{nFS'D^{1/2}c_{ox}^*}{\pi^{1/2}t^{1/2}} = \frac{nFSf_RD^{1/2}c_{ox}^*}{\pi^{1/2}t^{1/2}} \quad (9.6)$$

$$J = \frac{I}{nFS} = \frac{D^{1/2} f_R c_{ox}^*}{\pi^{1/2} t^{1/2}} \quad (9.7)$$

$$\text{slope } (A \text{ vs. } t^{1/2}) = \frac{2 f_R D^{1/2} \varepsilon}{\pi^{1/2}} c_{ox}^* \quad (9.8)$$

Table 9.1: Comparison of the slopes, their relative standard deviations and the r^2 coefficients obtained from chronoabsorptometric data ($A_{\max}$ vs. $t^{1/2}$) at three concentrations of Fe(III) using the raw spectra and the spectra compressed by DWT. Linear regression was carried out with points at times higher than 4 s.

$c_{\text{Fe(III)}}$ (mmol L ⁻¹)	Slope (mmol ⁻¹ L t ^{-1/2})		RSD slope (%)		r^2	
	raw spectra	compressed spectra	raw spectra	compressed spectra	raw spectra	compressed spectra
0.02	1.92 10 ⁻³	1.89 10 ⁻³	8.02	3.04	0.6437	0.9265
0.10	6.22 10 ⁻³	6.15 10 ⁻³	2.54	1.27	0.9474	0.9864
0.20	1.08 10 ⁻²	1.04 10 ⁻²	1.57	0.84	0.9793	0.9940

Table 9.2: Calibration parameters resulting from data acquired from SEC experiments in different ways. Linear ranges are computed from LOQ to the end of linearity.

Type of data	Fe(III) alone			Fe(III) + 0.1 mmol L ⁻¹ Cu(II)		
	linear range (mmol L ⁻¹)	r^2	LOD (mmol L ⁻¹)	linear range (mmol L ⁻¹)	r^2	LOD (mmol L ⁻¹)
CV current (cathodic peak)	0.17 – 1.00	0.9851	0.052	-	-	-
CV current (anodic peak)	0.04 – 1.00	0.9992	0.012	0.2 – 1.0	0.9835	0.064
CA current I vs. $t^{1/2}$	0.12 – 0.90	0.9967	0.037	0.6 – 1.0	0.9358	0.194
CA $A_{\max}$	0.02 – 0.08	0.9896	0.006	0.06 – 0.09	0.9395	0.018
	0.10 – 1.00	0.9977	-	0.10 – 1.00	0.9649	-
CA slope $A_{\max}$ vs. $t^{1/2}$	0.007 – 0.08	0.9987	0.002	0.02 – 0.09	0.9890	0.007
	0.10 – 1.00	0.9974	-	0.10 – 1.00	0.9574	-

9.2.4 Chronoamperometric study in the presence of Cu(II)-ion

In order to evaluate the effect of electrochemical interferences on the performance of the studied methodologies, several experiments were carried out for the Fe(III)-OP system under the same conditions of the previous section but in the presence of 0.1 mmol L⁻¹ of Cu(II)-ion.

As Figure 9.6 shows, the CV signals measured under these conditions still present the same cathodic and anodic peaks of Fe(III) but with two new overlapped signals, corresponding to the reversible reduction and reoxidation of Cu(II) species. The sensitive overlapping between the reduction signals of Cu(II) and Fe(III) hinders the use of this peak for the determination of Fe(III). However, the presence of copper not only affects this signal, but also the LOD values, which seem to be raised as shown in Table 9.2. Yet, the strategies based on the measurements of absorbance along CA experiments are much less affected by the interference of Cu(II)-ions, surely due to the increased selectivity provided by the combination of selective potentials and selective wavelengths. Indeed, the slope of the calibration plot in the presence of Cu(II) is $0.061 \mu\text{A s}^{-1/2} \text{mol}^{-1}\text{L}$, very close to that of $0.062 \mu\text{A s}^{-1/2} \text{mol}^{-1}\text{L}$ obtained in the absence of Cu(II).

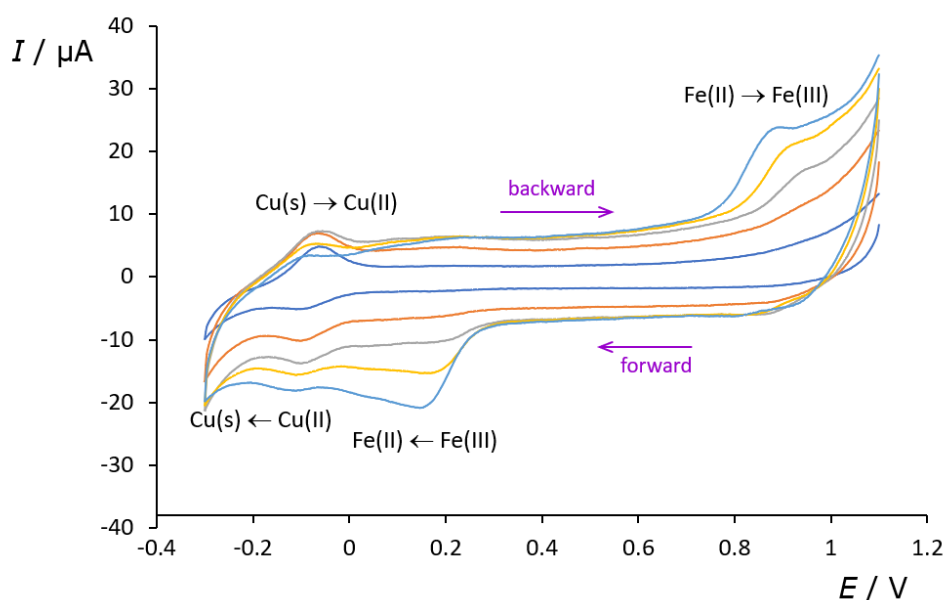


Figure 9.6 : CV scans registered for a solution containing 25 mmol L^{-1} of OP, 0.1 mmol L^{-1} of Cu(II) and increasing concentrations of Fe(III) (0.02 , 0.05 , 0.2 , 0.5 and 0.8 mmol L^{-1}).



10. CONCLUSION

In conclusion, this thesis study dealt with voltammetric determination of various heavy metals that play significant roles in the environment. For this purpose, two carbon based electrodes with different properties were employed: screen-printed carbon electrode and pencil graphite electrode. These two electrodes with unique properties provided outstanding sensitivity and selectivity toward chosen metals. They proved that they were able to perform well, even in the presence of interfering ions that can naturally occur in the environmental samples. As a result, several effective and useful electrochemical methods were developed and contributions were made to the literature. The main advantages of electrochemical methods over conventional spectrometric methods (namely AAS and ICP) include:

- (i) Simple instruments that even the non-experts can use,
- (ii) Shorter analysis time,
- (iii) Lower capital and operational costs,
- (iv) Portable devices.

Future outlook on this thesis subject would involve research and development for potential on-site analysis technologies for heavy metals, as well as improvements on the modification strategies and possibilities of creating novel spectroelectrochemical methods. Conducting such on-site analysis is crucial in terms of rapid quantification of hazardous metals to avoid spreading the contamination in soils and waters. In case of on-site soil analysis, it is also possible to provide recommendation for fertilizer or soil micronutrients to increase crop yields or maintain soil health.



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CURRICULUM VITAE

Name Surname : Gül Şirin USTABAŞI

EDUCATION :

- **B.Sc.** : 2015, Istanbul Technical University, Faculty of Science and Letters, Department of Chemistry
- **M.Sc.** : 2018, Istanbul Technical University, Graduate School, Department of Chemistry

PROFESSIONAL EXPERIENCE AND REWARDS:

- 02.2020-06.2022, Mir Research and Development, R&D Assistant Engineer in the frame of TUBITAK 2244 Industrial PhD Fellowship Programme.
- 10.2021-03.2022, University of Barcelona, Visiting Researcher with the scholarship provided by TUBITAK 2214-A International Research Fellowship Programme for PhD Students.
- 07.2019, Young Researcher grant to join the 2nd ICNSME with oral presentation, which took place in Aveiro, Portugal
- 11.2015-02.2020, Istanbul Aydin University, Research Assistant.

PUBLICATIONS ON THE THESIS:

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