T. R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCE
DEPARTMENT OF CHEMISTRY

VOLTAMMETRIC DETERMINATION OF IDARUBICIN USED IN CANCER TREATMENT ON BORON-DOPED DIAMOND ELECTRODE

M. Sc. THESIS

PREPARED BY: Hemn Abdulazeez Hakeem BARZANI
SUPERVISOR: Prof. Dr. Zühre ŞENTÜRK

VAN-2019

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ACCEPTANCE AND APPROVAL PAGE

This thesis entitled "Voltammetric Determination of Idarubicin used in Cancer Treatment on Boron-doped Diamond Electrode" presented by Hemn Abdulazez Hakeem BARZANI under supervision of Prof. Dr. Zühre ŞENTÜRK in the department of Chemistry has been accepted as a M.Sc. thesis according to Legislations of Graduate Higher Education on 25/07/2019 with unanimity / majority of votes members of jury.

Chair: Prof. Dr. Zühre ŞENTÜRK Signature:

Member: Prof. Dr. Yavuz YARDIM Signature:

Member: Prof. Dr. Abdülkadir LEVENT Signature:

This thesis has been approved by the committee of The Institute of Natural and Applied Science on .01../08./2019 with decision number 2019/411I

Signature

Prof. Dr. Suat ŞENSOY
Director of Institute

THESIS STATEMENT

All information presented in the thesis obtained in the frame of ethical behavior and academic rules. In addition all kinds of information that does not belong to me have been cited appropriately in the thesis prepared by the thesis writing rules.

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Hemn Abdulazeez Hakeem BARZANI

ABSTRACT

VOLTAMMETRIC DETERMINATION OF IDARUBICIN USED IN CANCER TREATMENT ON BORON-DOPED DIAMOND ELECTRODE

BARZANI, Hemn Abdulazeez Hakeem
M.Sc. Thesis, Chemistry Science
Supervisor: Prof. Dr. Zühre ŞENTÜRK
August 2019, 71 pages

Idarubicin (4-demethoxy-daunorubicin) is a commercially available antineoplastic agent which belongs to the family of anthracycline antibiotics. This thesis examined the use of an anodically pretreated boron doped diamond electrode without any chemical modifications for the electrochemical determination of Idarubicin by using cyclic and square-wave voltammetry in aqueous solutions over the pH range of 2.0-8.0. The compound was mainly oxidized in two steps at high positive potentials, resulting in the formation of redox couples with reduction waves at less positive potentials. Using square-wave voltammetry, Idarubicin could be determined in the concentration range 0.5-25µg mL^{-1} (9.4×10^{-7}-4.6×10^{-5} M) in Britton-Robinson buffer (pH 2.0) at +1.10 V (vs. Ag/AgCl). The limit of detection was 0.0174 µg mL^{-1} (3.2×10^{-8} M). The practical applicability of the developed method was demonstrated on the determination of Idarubicin in pharmaceutical formulation, with results similar to those obtained using a high-performance liquid chromatography with diode-array detection.

Keywords: Anodic pretreatment, Boron-doped diamond electrode, Idarubicin, Injectable solutions, Square-wave voltammetry.

ÖZET

KANSER TEDAVİSİNDE KULLANILAN İDARUBİSİN′İN BOR-KATKILI ELMAS ELEKTROT ÜZERİNDE VOLTAMETRİK TAYİNİ

BARZANI, Hemn Abdulazeez Hakeem
Yüksek Lisans Tezi, Kimya Bölümü
Danışman: Prof. Dr. Zühre ŞENTÜRK
Ağustos 2019, 71 sayfa

İdarubisin (4-demetoksi-daunorubisin), antrasiklin antibiyotik ailesine ait, ticari olarak temin edilebilen antineoplastik bir bileşiktir. Bu tez çalışması, İdarubisin′in elektrokimyasal tayini için 2.0-8.0 pH aralığındaki sulu çözeltilerde dönüşümlü ve kare-dalga voltametrisi teknikleri yardımıyla anodik olarak ön işlem görmüş bor-katkılı elmas elektrotunun herhangi bir kimyasal modifikasyon uygulamadan kullanılabilirliğini araştırmıştır. Bileşik temel olarak yüksek pozitif gerilim değerlerinde iki basamakta yükseltgenmekte olup bu olay daha az pozitif gerilim değerlerinde indirgenme basamağını içeren redoks çiftlerin oluşumu ile sonuçlanmaktadır. İdarubisin, kare-dalga voltametrisi kullanılarak Britton-Robinson tamponu (pH 2.0) içerisinde +1.10 V (Ag/AgCl′e karşı) gerilim değerinde 0.5-25µg mL^{-1} (9.4×10^{-7}-4.6×10^{-5} M) derişim aralığında tayin edilmiştir. Gözlenebilirlik sınırı, 0.0174 µg mL^{-1} (3.2×10^{-8} M) olarak hesaplanmıştır. Geliştirilen yöntemin pratik olarak uygulanabilirliği, farmasötik formülasyondaki İdarubisin tayininde gösterilmiş olup sonuçlar diyot-dizisi dedektörlü yüksek performanslı sıvı kromatografisi tekniği kullanılarak elde edilenlerle uyumlu bulunmuştur.

Anahtar kelimeler: Anodik ön işlem, Bor-katkılı elmas elektrot, Enjektabl çözelti, İdarubisin, Kare-dalga voltametrisi.

ACKNOWLEDGEMENTS

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Finally I would like to thank all of my friends especially Mr.Kovan Dilawer Issa for nonstop encouragements and support.

2019

Hemn Abdulazeez Hakeem BARZANI

TABLE OF CONTENTS

Pages

Pages

LIST OF TABLES

Table **Pages**

LIST OF FIGURES

Figure **Pages**

SYMBOLS AND ABBREVIATIONS INDEX

Some symbols and abbreviations used in this study are presented below, along with descriptions.

Symbols	Description
NaH_2PO_4	Sodium dihydrogenphosphate
Na_2HPO_4	Disodium hydrogenphosphate
NaCl	Sodium chloride
CH_3COOH	Acetic acid
H_3PO_4	Phosphoric acid
NaOH	Sodium hydroxide
H_3BO_3	Boric acid
HCl	Hydrochloric acid
mL	Milliliter
M	Molarity
mV s^{-1}	Milivolt per second
Hz	Hertz
S	Second

Abbreviations	Explanation
LOD	Limit of detection
LOQ	Limit of quantification
Log	Logarithm
C	Molar concentration
E	Electrode potential
E^0	Standard electrode potential
A	Charge transfer coefficient
N	Number of electrons

Abbreviations	Explanation
R	The gas constant (8.314 J K^{-1} mol^{-1})
T	Temperature (^{0}K)
t_R	Retention time
E_p	Peak potential
E_{pa}	Anodic peak potential
E_{pc}	Cathodic peak potential
i_p	Peak current
i_{pc}	Cathodic peak current
i_{pa}	Anodic peak current
N	Scan rate
F	Square-wave frequency
ΔE_s	Scan increment
ΔE_{sw}	Square-wave amplitude
LC	Liquid chromatography
HPLC	High performance liquid chromatography
UHPLC	Ultra high performance liquid chromatography
GC	Gas chromatography
CE	Capillary electrophoresis
MEKC	Micellar electrokinetic chromatography
UV	Ultra-viole
MS	Mass spectrometry
ES-MS	Electrospray mass spectrometry
FIA	Flow-injection analysis
DAD	Diode-array detection
FT-IR	Fourier transform infrared
NMR	Nuclear magnetic resonans
SPR	Surface plasma resonance
ICP	Inductively coupled plasma
FL	Fluorescence
LIF	Laser-induced fluorescence

Abbreviations	Explanation
CV	Cyclic voltammetry
SWV	Square-wave voltammetry
DPV	Differential pulse voltammetry
AdSV	Adsorptive stripping voltammetry
GCE	Glassy carbon electrode
CPE	Carbon paste electrode
CFE	Carbon fiber electrode
DME	Dropping mercury electrode
EPPG	Edge plane pyrolytic graphite electrode
BDD	Boron doped diamond
APT	Anodically pretreated
MWCNT	Multiwalled carbon nanotube
PGE	Pencil graphite electrode
Gr	Graphene
SPE	Screen printed electrode
CME	Chemically modified electrode

1. INTRODUCTION

Today, cancer is one of the leading public health problems in the world, which significantly affects public health and quality of life. It is undeniable that there is an increased interest in therapeutic drugs, as it is a class of disease with low recovery and survival rates. On the other hand, due to the possible toxic properties of antineoplastic drugs, it is of great importance to protect the healthcare professionals who apply this treatment from the risk of contact with these drugs and provide a safe working environment. Therefore, the importance of the development of a new method for the detection and determination of compounds used in cancer treatment from different drug forms, biological samples (urine, blood, tissue, etc.) and environmental samples is of great importance.

Analyzes in pharmaceutical (drug) and clinical samples are an important part of analytical chemistry. Single or simultaneous determination of compounds present in different matrix media plays an extremely important role in medical and pharmaceutical science. In addition to being reliable and sensitive, the analytical methods used for such analyzes should be applicable over a wide range of analytical concentrations, be fast, simple (do not require highly experienced personnel) and cost-effective in terms of equipment. Pharmaceutical and clinical analyzes performed by electrochemical methods, especially voltammetric techniques, have strengthened its place in the literature with its advantages such as simplicity, cheapness, fast analysis along with high sensitivity. On the other hand, it is extremely important to study the oxidation and reduction processes of the pharmacologically important compounds. These investigations make it possible to understand the interaction of molecules of interest with complex biological systems.

In the light of the above knowledge, in this study it is aimed to develop and apply a sensitive and accurate voltammetric method for the determination of Idarubicin, which is one of the drugs using very effectively in cancer treatment in recent years. It should be underlined that its redox properties have been studied very little. In the first part of the study, the electrochemical properties of the selected compound will be examined in detail by voltammetric method in various supporting electrolytes in a wide

pH range on bare boron-doped diamond electrode. In the light of the positive findings in the first part, an effective voltammetric technique will then be described for the determination of Idarubicin. In the last part, the proposed voltammetric technique will be tested in pharmaceutical (drug) sample, and the results will be compared with the results of high performance liquid chromatography.

2. LITERATURE REVIEW

2.1. An Overview of Cancer Chemotherapy and Antineoplastic Drugs

In this section, initially, it will be briefly given information about the drugs used in cancer treatment and Idarubicin which is selected for this study. Then, it will be focused on the analytical methods used for the determination of idarubicin.

Cancer is the second cause of death after cardiovascular disease in the death statistics of developed countries, and accepted as a global epidemic. It appears to be almost equal with the animal kingdom. In archaeological and paleopathological studies, cancer findings were found in dinosaur fossils and mummies of thousands of years. According to the written sources, the term cancer (Greek carkinos = crab) is thought to have been used by Hippocrates (BC 460-375) for the first time, not by Galen (AD 129-216), contrary to misunderstanding (Temel, 2015).

Cancer can be summarized as an uncontrolled proliferation of the cells of the body, non-differentiation, loss of function, spread to the adjacent tissue (invasion) and its spread to distant tissues (metastasis) (Figure 2.1) (Weinberg, 1996; Shewach and Kuchta, 2009).

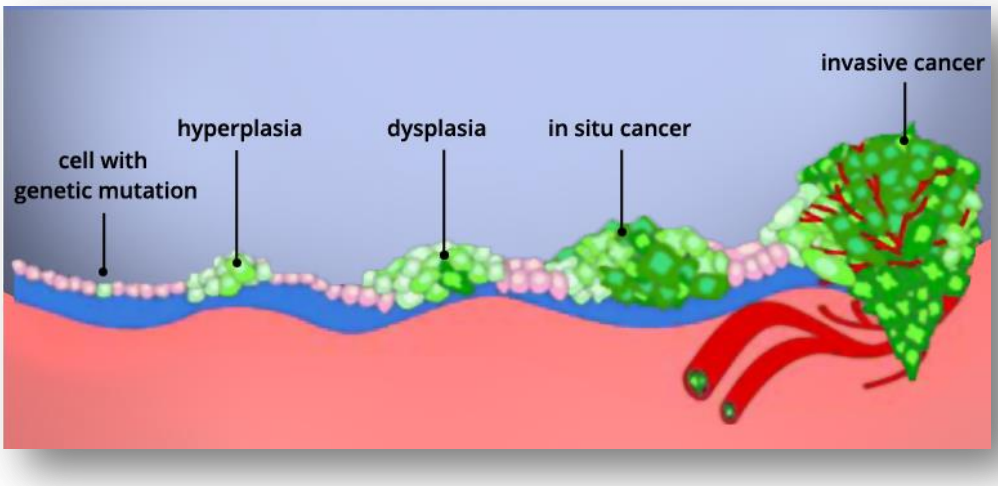


Figure 2.1. The stages of cancer.

There are different approaches to the treatment of cancer: (*i*) surgical intervention, (*ii*) radiation therapy, (*iii*) drug use (chemotherapy), (*iv*) hormone therapy, (*v*) immunotherapy, (*vi*) targeted smart drug treatment, (*vii*) hyperthermia. Today, successful results in the treatment of cancer are achieved through the combination of the above mentioned treatment methods.

Chemotherapy is a word derived from German physician Paul Ehrlich in the 1900s with regard to the treatment of syphilis. Today, the main principle of chemotherapy is the treatment of antineoplastic drugs, also known as cytotoxic drugs (Bökesoy et al., 2000; Moosa et al., 2003; Thurston, 2009; Kayaalp, 2012). What is expected of the treatment is to stop or destroy the growth and proliferation of cancer cells without damaging the normal cells of the patient. There is a close relationship between the efficiency of antineoplastic drugs and the cell cycle. There are four major periods in the cell cycle (Figure 2.2): (*i*) the period of preparation for DNA synthesis (**G1**). It is also called the ″rest period″ symbolized as **G0** because of the longest duration and the most variable one; (*ii*) DNA synthesis or replication period (**S**) which takes 4-24 hours; (*iii*) mitosis preparation period (**G2**) taking approximately 2 hours in which the mitotic strand occurs; (*iv*) mitosis (cell division) period (**M**) which takes less than 1 hour.

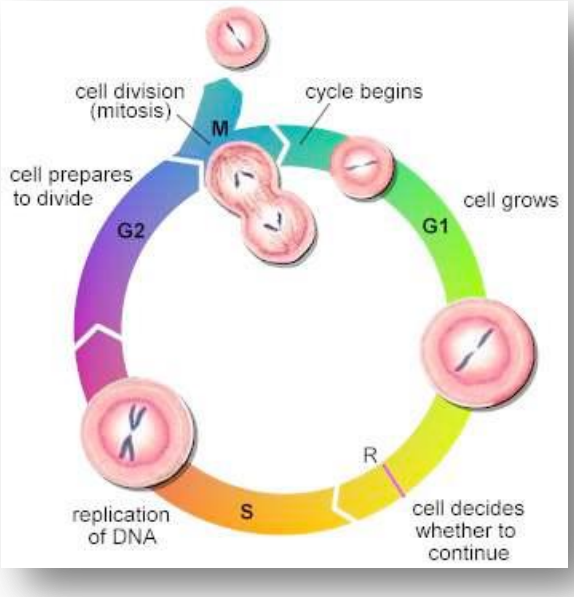


Figure 2.2. Phases of cell proliferation (cell cycle) (adaptation from Weinberg. 1996).

An important condition limiting the efficacy of antineoplastic drugs; drug effect is not specific to the period of the cell cycle. Some drugs act only during a specific period of the cell cycle (*cell-cycle specific drugs*); others are effective in all periods of cell life (*cell-cycle nonspecific drugs*). The general characteristic of the drugs in the second group is the direct degradation of the DNA structure.

While antineoplastic drugs show a cytotoxic effect against the cancer cell, they can also destroy the rapidly growing normal cells. Therefore, the drugs used today are more anti proliferative (cell proliferation inhibitor) drugs than anticancer. Treatment with antineoplastic drugs can lead to undesirable and even life-threatening side effects. These drugs cause general side effects such as suppression of bone marrow, which reduces the construction of one or all of the shaped elements forming the blood, lymphotoxic effect and immunosuppression (suppression of the immune system), nausea and vomiting, oral and intestinal ulceration, diarrhea, and alopecia (hair loss). These drugs are mutagenic (creating permanent structure change on the DNA in the cell nucleus), teratogenic (forming deformity and deficiency in the womb) and carcinogenic effects. In addition, they can also create very specific side effects. For this reason, the drug treatment should be done by experienced specialists.

Another condition that limits the effectiveness of antineoplastic drugs is that the cancer cell is resistant to the drug or has gained resistance over time. In order to prevent resistance during the treatment, the drugs are used together or the single drug is administered in sufficient time and dose.

Antineoplastic drugs are generally administered (*i*) orally in the form of tablets or capsules, (*ii*) by intramuscular or subcutaneous injection, (*iii*) by intravenous infusion. Since many drugs cannot be absorbed by the digestive tract, intravenous administration is preferred. Since the drugs given by the vein are added to the circulation, their effects are fast.

Table 2.1 shows the classification of antineoplastic drugs that have been used today according to their general mechanisms of action or sources.

Table 2.1. Classification of antineoplastic drugs

I. Alkylating agents
- A. Nitrogen mustard
  - 1. Mechlorethamine (nitrogen mustard)
  - 2. Cyclophosphamide
  - 3. Chlorambucil
  - 4. Melphalan
  - 5. Ifosfamide
- B. Alkyl sulfonate
  - 1. Busulfan
- C. Nitrosoureas
  - 1. Carmustine
  - 2. Lomustine
  - 3. Semustine
  - 4. Streptozotocin
- D. Ethylenimine
  - 1. Thiotepa
- E. Triazine
  - 1. Dacarbazine

II. Antimetabolites
- A. Folate antagonists
  - 1. Methotrexate
- B. Anti-purines
  - 1. Thioguanine
  - 2. Mercaptopurine
  - 3. Fludarabine
  - 4. Pentostatin
  - 5. Cladribine
- C. Anti-pyrimidines
  - 1. Cytarabine
  - 2. 5-fluorourasile
- D. Cytosine analogs
  - 1. Decitabine
  - 2. Gemcitabine

III. Cytotoxic antibiotics
- A. Anthracyclines
  - 1. Doxorubicin (Adriamycin)
  - 2. Daunorubicin (Daunomycin)
  - 3. İdarubicin
  - 4. Epirubicin
- B. Bleomycin
  - 1. Bleomycin sulfate
- C. Mitomycin C
- D. Dactinomycin (Actinomycin)
- E. Plikamisin (Mitramisin)

IV. Herbal drugs
- A. *Vinca* alcaloids
  - 1. Vincristine
  - 2. Vinblastine
- B. Epipodophyllotoxins
  - 1. Etoposide
  - 2. Teniposide
- C. Tacsanes
  - 1. Paclitaxsel
  - 2. Docetaxel
- D. Captothecin analogs
  - 1. Irinotecan
  - 2. Topotecan

V. Enzymes
- A. L-Asparaginase

VI. Steroid Hormones and Antagonists
- A. Glucocorticoids
- B. Estrogens, antiestrogens
  - 1. Tamoxifen citrate
  - 2. Estramustin sodium phosphate
- C. Androgens, antiandrogens
  - 1. Flutamide
- D. Progestins
- E. Luteinizing hormone-secreting hormone antagonists
  - 1. Buserelin
  - 2. Leuprolide
- F. Octreotide acetate (Sandostatin)

VII. Monoclonal antibodies
  - 1. Cetuximab
  - 2. bevacizumab
  - 3. Ritucsimab

VIII. Immunomodulatory
- A. Levamisole
- B. İnterferons
  - 1. İnterferon alfa-2a
- C. Interleukins: aldesleukin (interleukins-2)

IX. Other antineoplastic drugs
- A. Hydroxyurea
- B. Mitotane
- C. Hexamthylmelamine
- D. Cisplatin
- E. Carboplatin
- F. mitoxantrone

The alkylating agents contained in this table are the most commonly used drug group and are not cell-cycle-specific. They can affect cells every time. One of the important features; because they are prodrugs, they are inactive *in vitro*. The initiation of modern cancer chemotherapy occurs at the end of the 1940s with the use of meclorethamine and its derivatives (nitrogen mustard) for treatment. Antimetabolites are commonly used antineoplastic drugs, such as alkylating drugs. One of their major differences is that these compounds are cell-cycle specific drugs, which show their maximum effects in S-phase, and are often effective in high-type tumors of the proliferative fraction. Cytotoxic antibiotics are from the group of drugs that are not related to cell cycle and are effective for both fast and slow dividing tumors. Herbal-derived drugs are metaphase (M) periodic drugs of mitosis. Currently, an average of 70 varieties in Turkey has antineoplastic drugs used in chemotherapy.

2.1.1. Anthracyclines

Anthracyclines, a group of antitumor antibiotics have been used since their discovery in 1960, and can be considered among the most useful antineoplastic agents. These compounds are used to treat many cancers, including leukemia, lymphomas, breast, stomach, uterine, ovarian, bladder cancer, and lung cancers (Minotti et al., 2004; Mc Gowan et al., 2017). Some of them are natural products extracted from *Streptomyces peucetiusor* or *Streptomyces galilaeus*. In order to decrease their toxicity, and the multi-drug resistance, many hundred derivatives of anthracyclines have also been synthesized for more than 50 years.

Chemically anthracyclines have a tetracyclic molecule with an anthraquinone backbone (aglycon), which is connected to an amino sugar moiety (daunosamine) by a glycosidic linkage. The central anthraquinone gives the compounds their characteristic orange-red color and intense fluorescence. The compounds lose their fluorescence when intercalated in DNA. Figure 2.3 shows the basic chemical structure of anthracyclines. Most of them bear a morpholino group at position 3′ or 4′ of the sugar residue, while others have a hydrophilic group attached to the side chain in position 9. Chemical structures of some selected anthracyclines are shown in Table 2.2. Nowadays, the clinically most used anthracyclines are Daunorubicin, Doxorubicin, Epirubicin and

Idarubicin.

The anthraquinone head is quite lipophilic and aids anthracyclines binding to targets molecules through hydrophobic interactions. The amino sugar helps to make the molecules soluble in water. All the anthracyclines are currently marketed as hydrochloric acid salt since this formulation is soluble in both water and polar organic solvents.

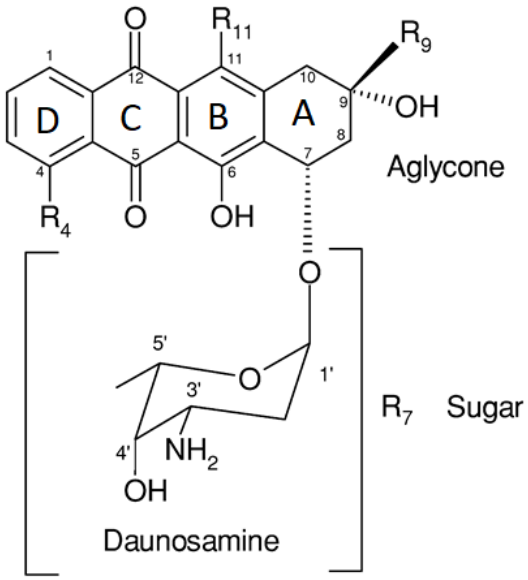
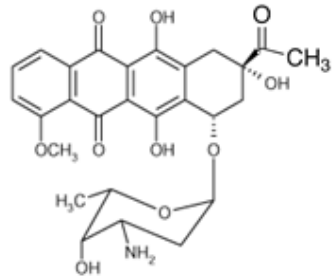
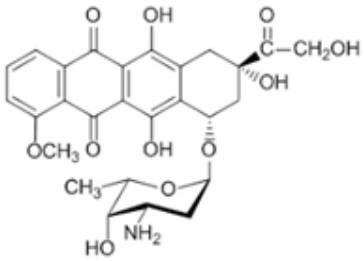
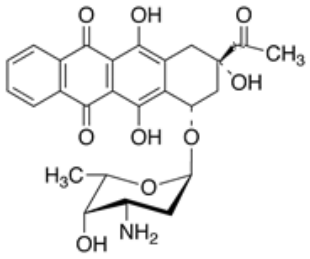
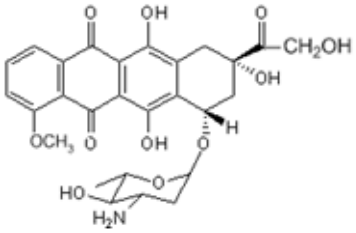
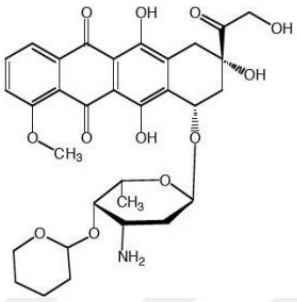
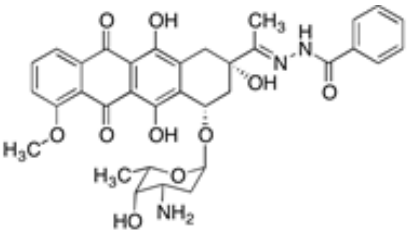


Figure 2.3. Basic anthracycline structure.

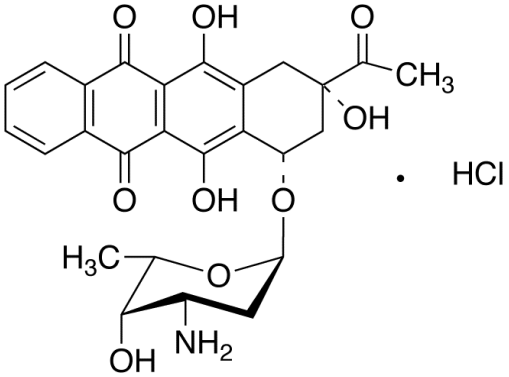
Table 2.2. Chemical structures of some representative anthracyclines

 Daunorubicin	 Doxorubicin	 Idarubicin
 Epirubicin	 Pirarubicin	 Zorubicin

2.1.1.1. Idarubicin

Idarubicin, which is selected for this study, is a member of anthracyclines. The molecular structure of Idarubicin is given in Table 2.3, together with its physical and chemical properties.

Table 2.3. Molecular structure, chemical name and general properties of Idarubicin

Molecular structure	General properties
	IUPAC name: (7*S*,9*S*)-9-acetyl-7-[(2*R*,4*S*,5*S*,6*S*)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-8,10-dihydro-7*H*-tetracene-5,12-dione Molecular formula: $C_{26}H_{27}NO_9$ Molar weight: 497.5 g/mol (base), 534.0 g/mol (in hydrochloride salt) Melting point: 183-185 °C Water Solubility: 0.772 mg/mL (base), highly soluble (as hydrochloride salt)

Idarubicin is a 4-demethoxy synthetic analogue of Daunorubicin (the first anthracycline isolated from the pigment-producing actinobacteria *Streptomyces peucetius* in 1963). The compound was approved by the FDA in 1990. The absence of a methoxy group gives more lipophilic character than Daunorubicin, which improves the absorption across the gastrointestinal mucosa and enhances uptake into tumour cells in vitro. Due to its lipophilic property, this compound can also be administered orally among the several anthracycline derivatives. Idarubicin is 5 to 6 times more potent and less cardiotoxic than Daunorubicin It shows the action mechanism by inhibiting the topoisomerase II enzyme, DNA and RNA synthesis (Fukushima et al., 1993; Zahraei and Rabbani-Chadegani, 2007). It binds to DNA at high capacity resulting in cell death and DNA damage (Fields and Koeller, 1991; Bigioni et al., 1994). It is taken into the cell quickly from Daunorubicin (Gallois et al.,1998). It is administered intravenously by dissolving in saline. Its half-life is approximately 14-15 hours, reaching the highest plasma concentration after approximately 1-5 hours. Its bioavailability is approximately 30%. In particular, it is used in the treatment of resistant breast cancer, acute myeloid leukemia, chronic lymphocytic leukemia and non-lymphocytic leukemia (Hollingshead and Faulds, 1991). Its major side effects are myelosuppression, nausea, mucositis, abdominal pain, diarrhea and alopecia (Błasiak et al. 2000; Newman and Cragg, 2016).

2.1.1.1.1. Overview of analytical methods for idarubicin

Today, with the increase in cancer incidence, the need for high sensitivity, selective and reliable analytical methods which allow the determination of antineoplastic drugs, in particular anthracyclines is of outmost importance. Its reason can be summarized as follows (Tjaden and De Bruijn, 1990; E.A., 1990agotto et al., 2001; Rauf et al., 2005; Kosjek and Heat, 2011; Nussbaumer et al., 2011; Negreira et al., 2013):

(*i*) Analysis in pharmaceutical formulations:

♣ Quality control in the production phase of the drug: A method allowing simultaneous determination of the basic drug, its impurities and degradation products should be used. The quality control process required for all drugs should be compatible with pharmaceutical regulations. LC and CE are commonly used methods in this step.

MS is preferred as a detection system. Although LC-UV is used routinely, the sensitivity of the method may not be sufficient in degradation or impurity studies.

♣ Quality control of the prepared formulation prior to treatment: The commercial formulation is diluted by dissolving in sodium chloride (NaCl, 0.9%) or glucose (5%) before patient administration. The durability of dilute solutions is generally limited (or unknown) and should be prepared by experienced personnel just before application. Even though drug regulations do not require this final control, analysis can be applied to establish the correct concentration of drugs and to reduce the risk of errors that could result in death. One of the analytical approaches is FIA with DAD, and the other is FT-IR spectroscopy.

♣ Formulation studies: Compatibility of different formulations prepared from the antineoplastic drug agent with the material (glass or plastic) in which it is contained is very important to prevent adsorption or degradation of the active substance. The most commonly used method in these studies is LC-UV technique

(*ii*) Analysis in biological samples:

♣ Because of the many side effects caused by this group of compounds, their determination with basic metabolites is of primary importance in order to obtain information about their pharmacokinetic properties. Thus; both doses and exposure times can be optimized to enhance treatment, prolong life and prevent disease progression. For this purpose, GC and LC techniques with MS detector are the most commonly used methods in the analysis of biological samples (plasma, urine, feces, tissue homogenate, tumor cells).

♣ The biological activity of chemotherapeutic agents depends on their binding to DNA; in this way, they block the DNA replication and cell division, resulting in the death of the cancerous cell. Therefore, the association with DNA can be used to understand the mechanism and toxicity of these drugs. Furthermore, it is of great importance to study the synthesis of antineoplastic drug-DNA interaction in terms of the design and synthesis of new chemotherapeutics with broad-spectrum activity, with low side effects and reduced resistance. The techniques used for this purpose include DNA-footprint, NMR, MS, FT-IR, Raman spectroscopy, molecular modeling techniques, SPR and CE.

(*iii*) Analysis in environmental samples:

♣ Surface and air contamination: A wide range of chemotherapy formulations prepared by different drugs and techniques are produced especially in hospital units. In order to detect pollution in these environments, selective and sensitive (LC-MS/MS) methods are required.

♣ Wastewater: After treatment of this group of medicines, a significant amount of cytotoxic component is eliminated by urine without metabolizing to reach the wastewater. Because of their possible toxicity to human and environmental health, even at very small concentrations, analysis of antineoplastic drugs and their metabolites in hospital flows and in wastewater samples is needed. The methods used frequently for this purpose are the ICP with MS detector, CE-UV, LC with FL detector, and LC-MS/MS.

It will be focused on the determination of Idarubicin later in this section.

Separation methods for Idarubicin

To determine this compound in samples of complex composition, separation methods such as LC with different types of detection as well as CE are commonly used. Some examples are presented in Table 2.4.

Table 2.4. Some examples of the determination of Idarubicin in various samples by separation methods

Identified Compounds	**Technique**	**Detector**	**Sensitivity**	**Sample**	**Reference**
Idarubicin and antineoplastic agents	UHPLC	MS	-	Drugs Wiping samples	Guichard et al., 2019
Idarubicin and antineoplastic agents	CE	UV	-	Injectable formulations	Guichard et al., 2018
Idarubicin and other anthracyclines	MEKC	LIF	-	Cancer cells	Mbuna et al., 2011
Idarubicin and other Anthracyclines	HPLC	FL	0.30 ng/mL (LOD)	Plasma and saliva	Maudens et al., 2009
Idarubicin and other anthracyclines	HPLC	UV	162 ng/mL (LOD)	Drugs	Badea et al., 2005
Idarubicin and other Anthracyclines	HPLC	MS/MS	0.01 ng/mL (LOD)	Drugs and urine	Sottani et al., 2004
Idarubicin and other anthracyclines	CE	LIF	<0.9 ng/mL (LOD)	Serum	Pérez-Ruiz et al., 2001
Idarubicin and other Anthracyclines	HPLC	ES-MS	1 ng/mL (LOD)	Human serum	Lachâtre et al., 2000
Idarubicin	CE	Amperometric	8×10^{-8} M	Human urine	Hu et al., 2000
Idarubicin and idarubicinol	HPLC	FL	0.25 ng/mL (LOD)	Rat plasma	Kuhlmann et al., 1999
Idarubicin, and other compounds	HPLC	UV	-	-	Zhang et al., 1998
Idarubicin and idarubicinol	CE	LIF	0.5 ng/mL (LOQ)	Plasma	Hempel et al., 1997
Idarubicin	HPLC	UV	-	-	Mo and Guan, 1994
Idarubicin and its metabolites	HPLC	FL	0.2 ng/mL (LOD)	Human plasma and urine	Camaggi et al., 1992

Electroanalytical methods for Idarubicin

Although most of these separation techniques, especially LC with MS detection, offer a high degree of sensitivity and selectivity; they often require higher cost of instrumentation (especially expensive detectors such as MS or MS/MS), long analysis times, complicated extraction processes, and higher consumption of environmentally unfriendly solvents. In this respect, one of the electroanalytical methods, voltammetry can offer the advantages such as simplicity, low cost, rapidity and suitability for on-site analysis as well as allowing for the investigation of redox behaviors of antineoplastic agents in order to use their additional therapeutic potentials (Uslu and Özkan, 2011; Farghaly et al., 2014; Lima et al., 2018).

Electron transfer and oxidative stress are known to play a major role in the therapeutic and at the same time negative effects of antineoplastics. Because of the presence of redox active groups in the molecular structure of many of the related compounds; in addition to electroanalytical investigations, findings from electrochemical studies will provide important information about both mechanisms of action of these drugs (Kovacic, 2007).

Apart from the aforementioned classical approaches, electrochemical techniques are also used to monitor the real-time effects of the drug at the cell level in patients (Yong and Qi-Long, 2001; Wooley et al., 2002; Chen et al., 2005).

On the other hand, in recent years there is great interest in the electrochemical investigation of the relationship between antineoplastic drugs and DNA. Electrochemical signals obtained in drug-DNA interaction provide important information about the action mechanism. Additionally, this interaction (DNA biosensors) can be used to determine the relevant drugs or DNA-targeted new developed drugs (Rauf et al., 2005).

Despite the electroactive nature of Idarubicin, a survey of the literature indicates that very little attention (only two papers) has been paid to its voltammetric investigation. Both of them were published in the last five years, and involve the use modified electrodes for its analysis. Its reason will be explained later (in Results and Discussion Section). The analytical performance characteristics of these two electrochemical sensors used in the determination of Idarubicin are summarized in Table 2.5.

Table 2.5. Comparison of the analytical performances of two electrochemical sensors previously reported for Idarubicin determination

Electrode	CPE	GCE, EPPG
Modifier	TiO_2/carbon nanofiber	MWCNT
Technique	SWV	DP-AdSV
Supporting electrolyte	Phosphate buffer, pH 2.0	Phosphate buffer, pH 3.0
Linear range, M	1.2×10^{-8} -1.0×10^{-5}	9.36×10^{-8} - 9.36×10^{-6} (GCE) 9.36×10^{-8} - 9.36×10^{-7} (EPPG)
LOD, M	3×10^{-9}	1.87×10^{-8} (GCE) 3.75×10^{-8} (EPPG)
Sample	Serum, urine	Drug
Reference	Arkan et al., 2017	Kurbanoğlu et al., 2013

On the other hand, an analytical procedure has been reported for the determination of Idarubicin and other which involves CE (Hu et al., 2000), in combination with amperometric detection.

Apart from the classical analytical methods mentioned above, electrochemical biosensors have also been used for the determination of Idarubicin (Chandara et al., 2011; Evtugyn et al., 2015) or for the explanation of its interaction mechanism with DNA (Ding et al., 2005; Hajian and Panahi, 2013; Satana Kara, 2014).

2.2. General Knowledge on Analytical Methods Used in This Study

The objective of this section is to give a general overview on voltammetry which is selected for the determination of Idarubicin in the present study. Then, it will be given a brief description on high performance liquid chromatography that is used for comparison.

2.2.1. Voltammetry at solid electrodes

The first battery was invented 219 years ago that is accepted the birth of practical electrochemistry. 97 years ago -around the 2nd World War- the polarography that is a special case of voltammetry using hanging mercury electrode was invented by Czech chemist Jaroslav Heyrovsky (for which he received the 1959 Nobel Prize in chemistry) -the starting point of the electroanalysis. It was also the shift from the classical analysis methods to instrumental analysis methods. The last century coincided with the rise and maturation of electroanalytical chemistry. During the last five decades, with the advent of microelectronics and computer technologies, a large number of different methods were developed, instruments were designed and constructed, and also a rigorous theoretical and mathematical framework was established (Bard and Zoski, 2000; Lubert ve Kalcher, 2010).

Electroanalytical methods are the general names of a group of quantitative analytical methods that examine the relationship between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential or charge, and their relationship to chemical parameters. The electroanalytical methods used today are shown in Figure 2.4 (Bard and Faulkner, 2000; Brainina, 2001; Wang, 2006; Farghaly et al 2014). A diversity of measured signals (potential, current, resistance, impedance) opens up wide possibilities for their application.

Because a sensor (electrode) constitutes the most basic part of any measuring system, electrochemical sensing has a very important role in the development of electroanalytical methods including potentiometry, amperometry and voltammetry (Brett and Brett, 2011).

In a voltammetric system, a known potential value is applied to the polarizable working electrode to provide oxidation and/or reduction of the analyte (i.e., the ion or molecule involved in the electrode reaction). This redox process results in a current whose magnitude is a measure of analyte concentration. In modern systems, in addition to the working electrode, the electrochemical cell includes the reference electrode, the auxiliary (counter) electrode, and a N_2-purge line for removing dissolved O_2 (Figure 2.5).

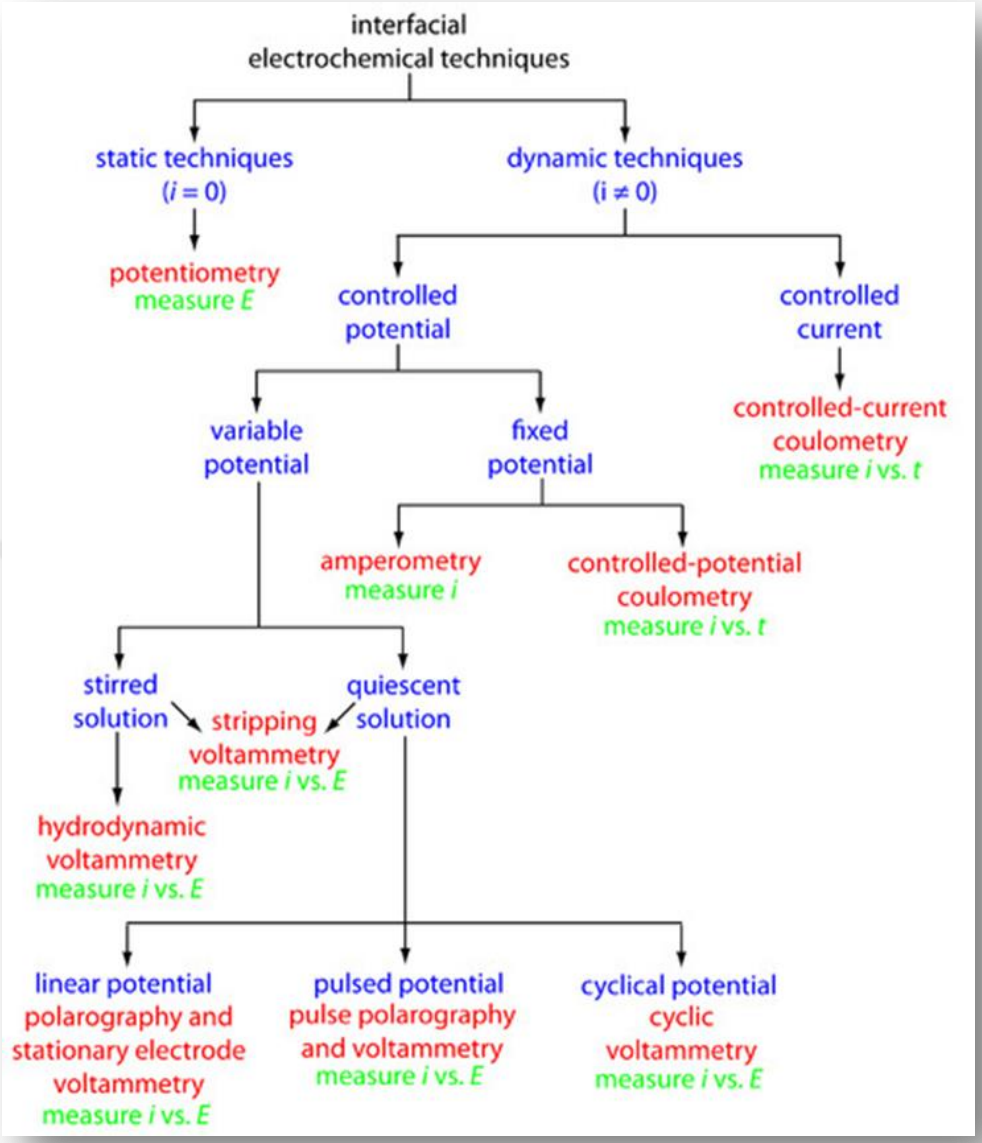


Figure 2.4. Family tree of electroanalytical techniques.

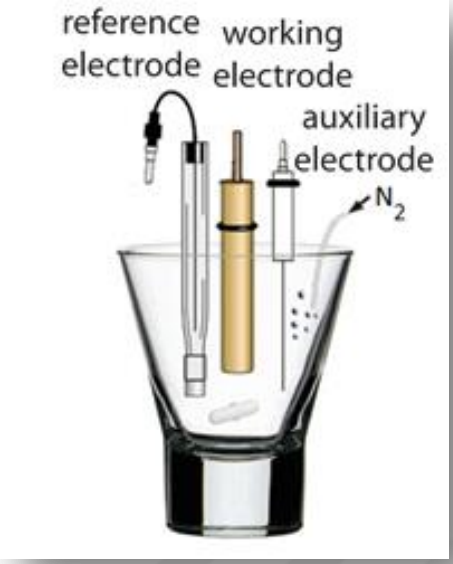


Figure 2.5. Electrochemical cell and its components (adaptation from Özkan et al., 2015).

The performance of the voltammetric method is strongly influenced by the material used as the working electrode. In the selection of the working electrode, criteria such as redox behavior of the target analyte, background current in the potential region required for measurement, potential working range, electrical conductivity, surface reproducibility, mechanical properties, cost, applicability and toxicity of the electrode material should be considered (Özkan et al., 2015).

The first application of voltammetry was performed using a dropping mercury electrode (DME), the first example of mercury-based electrodes, and the method was specifically named ″polarography″. Since mercury has a smooth and easily renewable surface, surface contamination is reduced, and thus the reproducibility of the method is increased. In addition, since mercury-based electrodes allow a wide negative potential range, they are used in the determination of many metal ions and organic compounds with reducible functional groups (Heyrovsky, 2011; Zuman, 2011).

However, it is clear that different electrode materials will be required to perform the analysis of the molecules having oxidation or oxidation/reduction properties. Therefore, the term ″voltammetry″ was first used in 1940 to describe experiments carried out on "solid electrodes" and other types of mercury-based electrodes other than DME.

Despite the difficulty of controlling the reproducible response on the solid electrode surface, solid electrodes have advantages over mercury-based electrodes in terms of mechanical strength and ease of use. In addition to their analytical uses, solid electrodes also allow to investigate the redox properties of pharmacologically, biologically and environmentally important compounds and, therefore, to elucidate their very complex in vivo oxidation pathways (Uslu and Özkan, 2007a; Kalcher et al., 2009; Svancara et al., 2009; Cavalheiro et al., 2012).

The working solid electrodes commonly used in voltammetry are given in Figure 2.6.

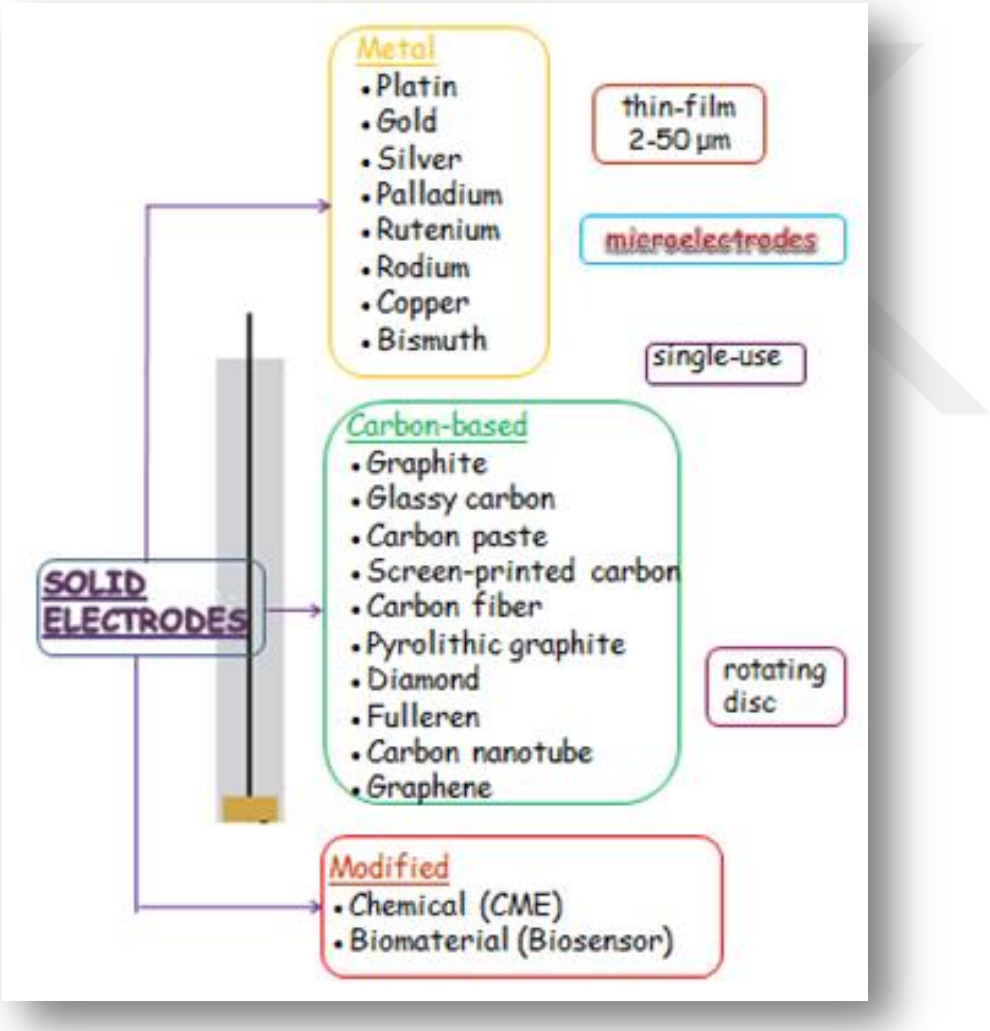


Figure 2.6. Some samples of solid working electrodes.

Solid electrodes in the form of wires, plates, discs are available in stationary, rotating or vibrating types. Among these electrodes, metal electrodes (Pt, Au, Bi, Pd, Rh, Cu, Ru, Ni, Cd, Sn, In) are used for specific electroanalytical applications. Electrode materials based on different carbon forms (carbon-based) have found widespread application in electroanalytical chemistry as they have a wide potential window, low cost, rich surface chemistry, low background current, chemical inertness and suitability for many difficult experiments. Carbon-based electrodes are also suitable for detection applications in some techniques (HPLC and CA with electrochemical detectors). These include graphite, glassy carbon (GC), carbon paste (CP), carbon nanotube (CNT), carbon-fiber (CF), boron-doped diamond (BDD), screen-printed carbon (SPC), graphene (Gr), fulleren, pencil graphite (PG) electrodes. In the 1990s, screen-printed technology opened a new horizon for single-use electrochemical sensor (SPE) applications. A very important development in solid electrodes is the application of chemical modified electrodes (CME), which began in the mid-1970s. Their applications offer excellent performance in terms of sensitivity and selectivity for the determination of various important biologically active compounds (Bard and Zoski, 2000; Diaz-Ballote t al., 2007; Uslu and Özkan, 2007a, b, Özkan et al., 2015).

It should be pointed at this point that, during the electrochemical oxidation of compounds which contain phenol moieties such as Idarubicin at the surface of conventional bare carbonaceous electrodes (graphite GC, CP), it can be observed a noticeable decrease in the current by the polymeric film formation that may promote a gradual passivation of the electrode surface (Ghanema et al., 2007; Hoyer and Jensen, 2007). To overcome this problem with electrode surface fouling by the analyte, one possible strategy is to use CME as mentioned above (Chao and Ma, 2014; Deng et al., 2015; Moyo et al., 2015; Li et al., 2017; Zhang et al., 2018). However, surface modification may present some drawbacks, such as long-time preparation, short-term stability of response, poor reproducibility, high cost, and well-trained personnel requirement. Another way of enhancing the sensitivity of the voltammetric method is the application of novel and perspective bare electrode materials.

By the end of 20th century, boron-doped diamond (BDD) received great attention as an eco-friendly carbonaceous electrode material (Luong et al., 2009; Peckova et al, 2009; Peckova and Barek, 2011; Cobb et al., 2018; Baluchova et al.,

2019; He et al., 2019; Souse et al., 2019). BDD has been shown to have minimal passivation problems (the presence of sp^3 hybridized of diamond carbon atoms (He et al., 2019). Moreover, for many analytes, the adsorbed reaction by oxidation products is easily removed from the electrode surface by using an appropriate electrochemical pretreatment (cathodic or anodic one) (Peckova et al, 2009). These procedures enhance the particular voltammetric signals, and ensure repeatable and reproducible response of analytes. Moreover, BDD has some other prestigious electrochemical properties without the need of any chemical modifications, such as having a widest usable potential window in aqueous solutions (up to +3.5 V) among all metal and carbon electrode materials, low residual current, low sensitivity to dissolved oxygen, and high corrosion resistance in extremely aggressive media.

During the past decades, our research group has reported some papers dealing with the application of BDD electrode pretreated by different polarization ways for the determination of some biologically active compounds important in the field of environmental, agricultural, pharmaceutical, clinical and food analyses (Yardım et al. 2011, Talay Pınar et al., 2013; Yardım et al. 2013a, Yardım et al 2013b, Levent et al. 2014; Yardım and Şentürk, 2014; Talay Pınar and Şentürk, 2016; Yiğit et al., 2016a,; Yiğit et al., 2016b; Ali et al., 2017; Alpar et al., 2017, Abdullah et al., 2018; Alpar et al., 2018a; Alpar et al., 2018b; Dönmez et al., 2018; Talay Pınar et al., 2018; Seyidahmet et al., 2019).

2.2.1.1. Voltammetric techniques

A general classification of commonly used voltammetric techniques is shown in Figure 2.7.

In this section, it will be briefly discussed voltammetric techniques used in the present study (Fogg and Wang, 1999; Bard and Zoski, 2000; Barek et al., 2001; Wang, 2006; Özkan et al., 2003; Özkan, 2009; Uslu) and Özkan, 2011; Özkan et al., 2015).

2.2.1.1.1. Cyclic voltammetry

Cyclic voltammetry (CV) is one of the most widely used voltammetric techniques, especially in the field of organic chemistry. It is often the first step in an electroanalytical study. It is used to study the mechanisms of complex redox reactions, to investigate intermediate product reactions and the stability of reaction products. Due to its low sensitivity (~ 10^{-5} M), the application areas are limited in quantification studies.

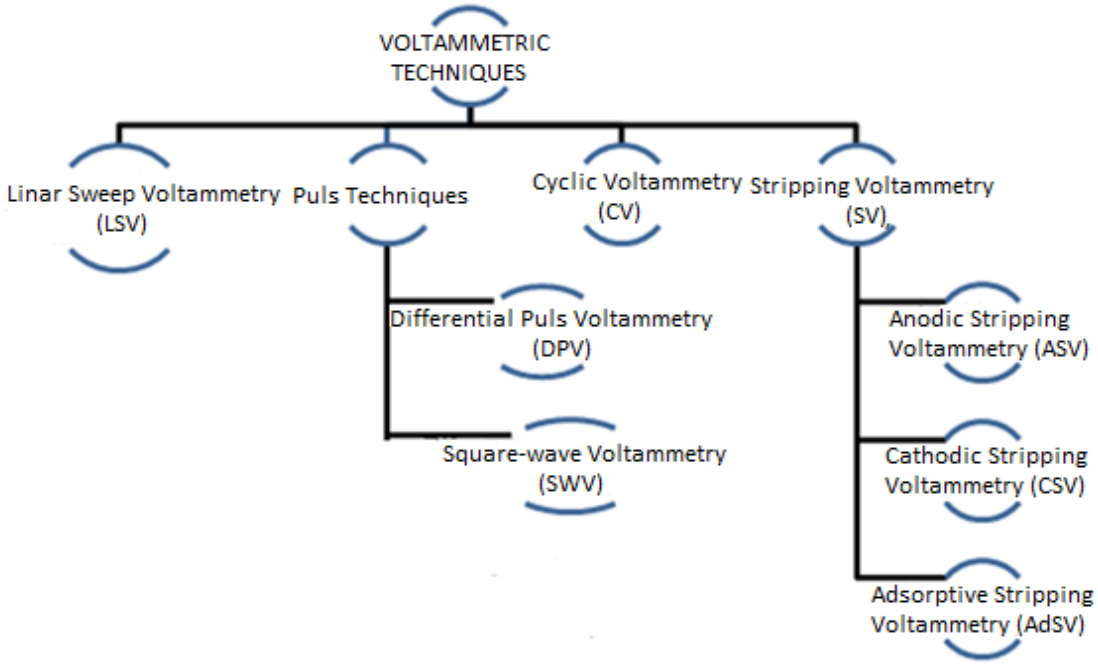


Figure 2.7. A general classification of commonly used voltammetric techniques.

In this technique, the potential applied to the selected working electrode is reversed after changing to a value (positive or negative direction) at certain scan rates (Figure 2.8 A). The return potential can be changed to the initial potential or to a different potential. During this process, the current is recorded as a function of the potential. The current-potential curve obtained under these conditions is called a *"cyclic voltammogram"*. If the same process is repeated multiple times, *"repetetive cyclic voltammogram"* is obtained (Figure 2.8 B).

The important parameters in a cyclic voltammogram are the peak potentials (E_{pa}, E_{pc}) and peak currents (i_{pa}, i_{pc}) of the anodic and cathodic peaks, respectively. For a reversible redox reaction at 25 °C with n electrons ΔE_p ($E_{pc} - E_{pa}$) should be $0.0592/n$ V

or about 60 mV for one electron. As the reversibility of the electrode reaction decreases (Figure 2.9), anodic and cathodic peaks are observed at different potentials and more widely. In an irreversible electrode reaction, the peak is completely lost as the product is consumed very quickly.

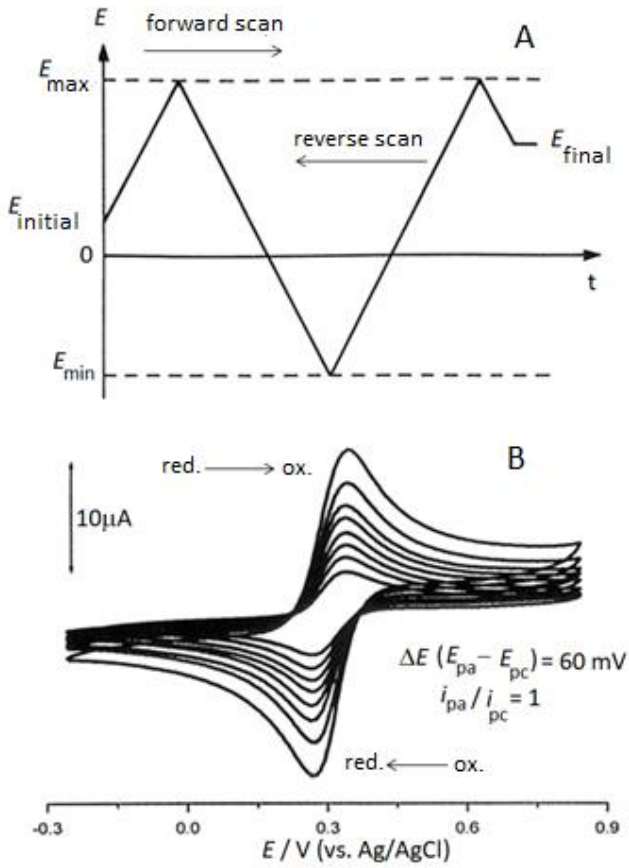


Figure 2.8. Potential waveform and its respective current response for cyclic voltammetry.

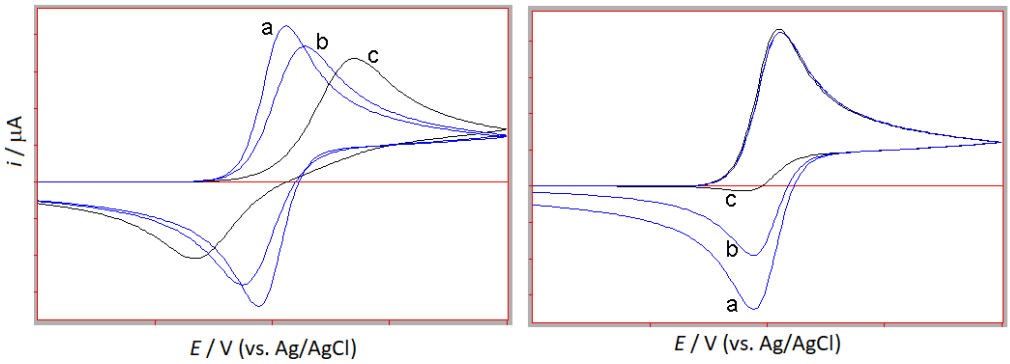


Figure 2.9. Types of reversible (a), quasi-reversible (b) and irreversible (c) electrochemical reactions observed in CV technique.

2.2.1.1.2. Square-wave voltammetry

Many pulse techniques have been tried over the years to increase the speed and sensitivity of the voltammetric method. These techniques are based on sampling the current generated against potential steps. These successive potential steps are applied to the working electrode at desired intervals. One of these pulse techniques, square-wave voltammetry (SWV), has high speed and sensitivity. Besides these advantages, there is no need to remove oxygen from the solution medium when the reduction is not too slow.

In the SWV technique, there are different types of pulses (Barker, Kalousek and Osteryoung). A potential waveform and a typical SW voltammogram for the most widely used SWV technique, Osteryoung SW voltammetry, is shown in Figure 2.10. Using this technique, analyzes can be performed with a sensitivity of 10^{-7}-10^{-8} M.

Due to its high speed, the SWV technique is used in the detection system of flow systems such as FIA, LC and CE.

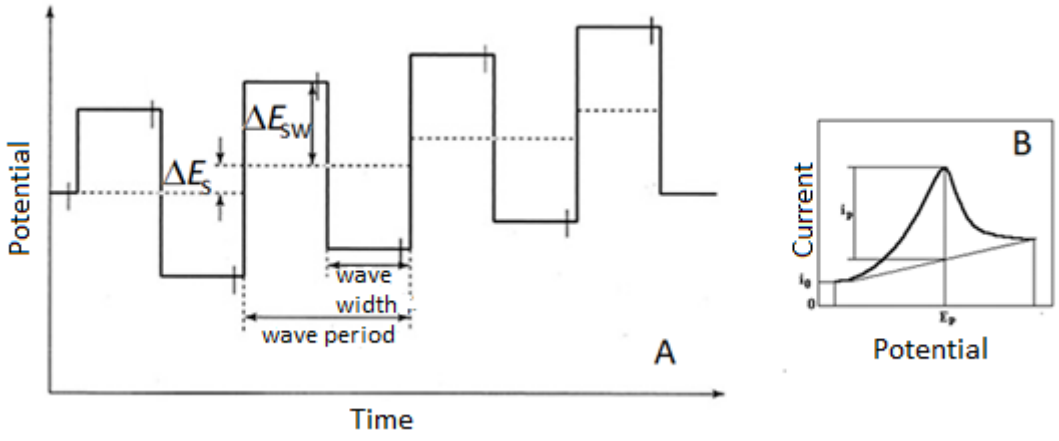


Figure 2.10. Potential waveform (A) and its respective current response (B) for SWV.

2.2.2. High performance liquid chromatography

High Performance Liquid Chromatography (HPLC) is most versatile and modern analytical technique used to separate, identify, and quantify each component in a mixture with its high sensitivity and selectivity ability (Skoog et al. 1998; Moreno-Arribas and Polo; 2003; Scott, 2003; Kupiec, 2004). Since it was introduced in early 1970's, this technique has also been known by high-pressure liquid chromatography or high-resolution liquid chromatography because of high pressures required to force the mobile phase or solvent through the stationary phase, or good resolution achieved using this technique, respectively.

As shown in the schematic diagram in Figure 2.11, the apparatus consists of a pumping system, an injector, a chromatographic column, stationary and mobile phases, connecting tubing and fittings, a detector and a data collection device (computer, integrator or recorder).

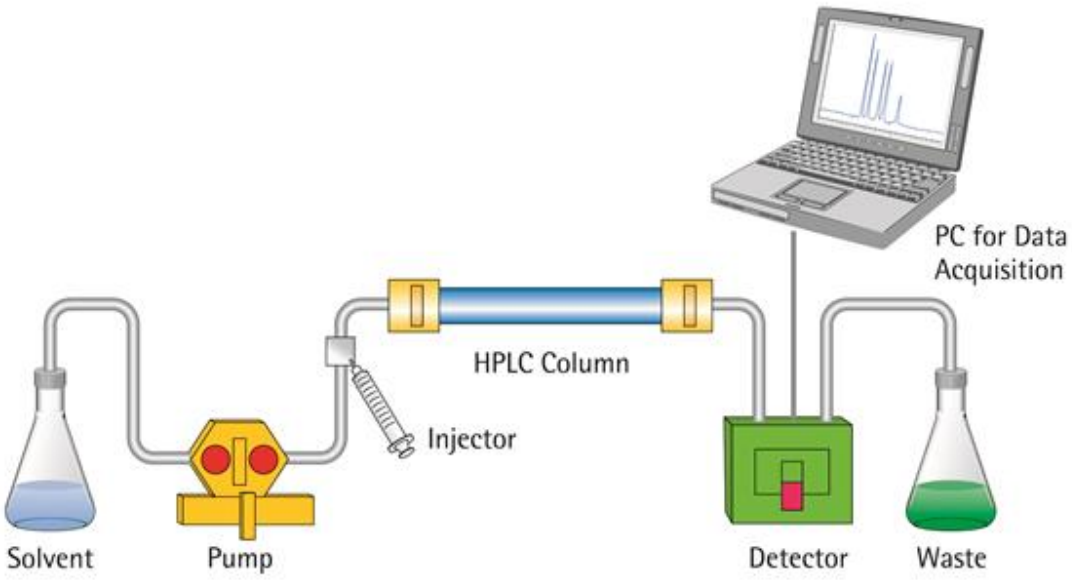


Figure 2.11. Schematic diagram showing components of a typical apparatus for HPLC.

Main separation types in HPLC: (*i*) reversed phase in which stationary phase is apolar whereas mobile phase is polar (water, buffer, methanol, acetonitrile, etc.). (*ii*) normal phase in which stationary phase is polar whereas mobile phase is apolar (hexane, octanol etc.)

Chromatographic process begins by injecting the sample in a discrete small volume into the stream of mobile phase percolating through the column. The sample components move through the column at different velocities, which are a function of specific physical interactions with the adsorbent which is called stationary phase. The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is an identifying characteristic of a given analyte. In the end, each component elutes from the column as a peak on the data display. Elution can be achieved by two different modes: (*i*) isocratic elution, without changing the mobile phase composition, (*ii*) gradient elution, where the mobile phase composition is changed during the course of the separation. Detection of the eluting components is important part, and the method used for detection is dependent on the choice of the detector. The response of the detector to each component is displayed on a chart recorder or computer screen and is known as a chromatogram.

3. MATERIAL AND METHOD

3.1. Chemicals and Solutions

3.1.1. Chemicals

Name of chemicals	Manufacturer name
Idarubicin hydrochloride	Sigma
Acetonitrile	Merck
Acetic acid (100% w/v)	Merck
Hydrochloric acid (37% w/v)	Merck
Phosphoric acid (85% w/v)	Merck
Boric asid	Merck
Sodium hydroxide	Merck
Glucose	Sigma
Fructose	Sigma
Lactose	Sigma
Sucrose	Sigma
Dopamine	Sigma
Uric acid	Sigma
$NaH_2PO_4.2H_2O$	Merck
$Na_2HPO_4.7H_2O$	Merck

3.1.2. Preparation of solutions

3.1.2.1. Preparation of idarubicin solutions

The stock solutions of Idarubicin were prepared in water using its hydrochloride salt corresponding to Idarubicin concentration of 1.0 mg mL^{-1}. The solutions were preserved at +4 ^{o}C when not in use and protected from daylight during use in the laboratory. The working and calibration solutions of Idarubicin were set up from the stock solution by dilution of appropriate volume with supporting electrolyte.

3.1.2.2. Preparation of supporting electrolyte

Three different supporting electrolytes, namely Britton-Robinson buffer (BR, 0.4 M, pH 2.0-8.0), acetate buffer (0.1 M, pH 4.8), and phosphate buffer (0.1 M, pH 2.5, 7.4) solutions were used. They are prepared with deionized water further purified via a Milli-Q system (Millipore) and stored in Pyrex® glass bottles in refrigerator.

For the BR buffer, the solution containing a mixture of 0.04 M H_3BO_3, 0.04 M H_3PO_4 and 0.04 M CH_3COOH was adjusted to the desired pH by adding 5 M NaOH solution.

For the acetate buffer, 5 M NaOH was added to a 0.1 M CH_3COOH solution and adjusted to the desired pH.

The phosphate buffer was adjusted to the desired pH by adding 5 M NaOH or 5 M HCl solution to 0.1 M Na_2HPO_4. $7H_2O$ solution.

3.2. Apparatus

Electrochemical analyzer	●Autolab PGSTAT 128N (EcoChemie, The Netherlands) (GPES 4.9 with software) ●Bioanalytical System (BAS) 100B/W
Electrochemical cell assembly	
A) Electrodes	
Working electrode	BDD working electrode (Windsor Scientific Ltd.; Ø: 3mm, diameter)
Reference Electrode	Ag/AgCl (3 M NaCl) (Model RE-1 BAS, USA)
Auxiliary electrode	Platinum wire (MF 1032 BAS)
B) Electrochemical test cells	10-mL one-compartment voltammetric cell (MR1208)
HPLC apparatus	HP Agilent 1100 autosampler system with an Agilent series G-1328 Diode Array Detector (DAD) at 254 nm
Chromatographic column	A nucleosil C18 (250 mm × 4.6 m, 5 µm)
Mixing system	Magnetic stirrer (ARE) Magnet (Spinb VM micro)
pH meter	WTW, Inolab pH 720

Automatic micro pipettes (Pyrex®)	Eppendorf
Centrifuge	Cooled Hermle Z320
Precision scale	Vibra

Different sizes of Pyrex® pipettes, beakers, flasks and glass tubes.

3.2.1. Voltammetric system

In electroanalytical studies, the electrochemical analyzer (EcoChemie) was used together with three electrode cell unit (Bioanalytical System Inc., BAS). Since the voltammetric system is supported by computer technology, control of the device, data collection and evaluation are easily performed. This system is given in Figure 3.1.

Figure 3.1. The electrochemical analyzer (A) and three electrode cell unit (B).

Voltammetric cell with a volume of 10 mL is a single chamber and cover assembly made of Pyrex® glass, specially manufactured for the electrochemical analyzer (Figure 2.5 and 3.1B). The electrodes are placed in the cell with four entries in the cover. In the fourth entrance, a Teflon pipe is dipped in order to send nitrogen gas if necessary (in cathodic side studies). With the help of this, the dissolved oxygen in the working solution is removed.

In the experiments, BDD electrode (with the diameter of 3 mm and boron content 1000 ppm) was used as the working electrode. An anodic or cathodic (for comparative purposes) pretreatment of BDD electrode was carried out once a day, before starting any other electrochemical experiments, in the presence of 0.5 M H_2SO_4

applying either +1.8 or -1.8V (both for period of 180 s) to get oxygen- and hydrogen-terminated electrode surface. Between individual measurements, an activation program was applied for 60 s under the same conditions in order to obtain reproducible and reliable results.

3.3. Measurement Procedures

3.3.1. Voltammetric procedure

Cyclic voltammetry (CV) was employed customarily for preliminary studies on electrochemical behavior of Idarubicin, followed by a systematic study of square-wave voltammetry (SWV) to assess the analytical performance and the method applicability. All measurements were performed in triplicate at laboratory temperature (25 ± 5 °C).

The initial and final potential values were variable for CV studies, depending on the pH value and the cut-off the electrolyte. The general procedure for SWV of Idarubicin was as follows: The required aliquot of the Idarubicin working solutions was placed in a cell containing a selected supporting electrolyte at a desired pH. The previously treated BDD electrode was placed in the electrochemical cell, and the voltammogram was recorded, while the potential was scanned from +0.0 to +1.6 V (unless otherwise stated) using SW wave form. For analytical application, the following SWV parameters being employed: frequency, 175 Hz; pulse amplitude, 40 mV; scan increment, 12 mV.

3.3.2. HPLC procedure for comparison

For HPLC experiments, the Agilent Technologies HP 1100 software was used to collect, integrate and analyze the chromatographic data. The mobile phase was composed of 0.02 M NaH_2PO_4/acetonitrile mixture (57:43 v/v). The mobile phase was prepared daily and degassed by ultrasonication before use. All solutions were filtered using a vacuum filtration system through 0.45-µm membrane filters from Agilent Technologies. All analysis was done under isocratic conditions at a flow rate of 1.3 mL min^{-1}. The injection volume was 20.0 µL, DAD was set at 254 nm. To get the working

standard solutions of suitable concentrations (corresponding to the linearity range stated later), Idarubicin stock and sample solutions prepared in water was serially diluted with the mobile phase.

3.4. Preparation of Samples

For the present analytical applications, Idarubicin pharmaceutical form, Idamen IV® injection solution labeled as containing 1 mg mL^{-1} Idarubicin HCl was procured from commercial local pharmacy. The content of two vials was mixed thoroughly. The required volume was taken, diluted with the selected buffer solution or mobile phase, and portions of the sample solutions were analyzed according to the SWV or HPLC parameters, respectively.

4. RESULTS AND DISCUSSION

4.1. Electrochemical Investigation of Idarubicin on Boron-doped Diamond Electrode

In order to investigate the electrochemical response of Idarubicin on BDD electrode, the experiments were initially executed by means of CV and SWV.

4.1.1. Cyclic voltammetry

First experiments were focused on the CV behavior of Idarubicin using an anodically pretreated BDD (here referred as APT-BDD, see below for electrochemical pretreatment works) at a scan rate of 100 mV s^{-1} in BR buffer at pH 2.0 (most suitable medium for analytical purposes, as shown later). As can be seen in Figure 4.1, the three consecutive CVs were registered for 50 μg mL^{-1} Idarubicin within the potential window from 0 to +1.6 V. A cyclic voltammogram without Idarubicin was also plotted in the graphs for the sake of comparison.

It is apparent that, in the first cycle, Idarubicin exhibited a small peak (labelled Ia) (ca. + 0.40 V) which was not clearly extracted from the background discharge, while a distinct and well-defined oxidation peak (labelled IIa) that occurred at more positive potential (ca. + 1.10 V). On scanning in the negative direction, two reduction peaks (labelled Ic and IIc) were also obtained with peak potentials of about + 0.30 and + 0.40 V, respectively. This observation at BDD electrode seems to be contradictory to those obtained in the previous investigations for Idarubicin using bare and modified GC and EPPG electrodes at pH 3.0 (Kurbanoğlu et al., 2013), and bare and modified CP electrode at the same pH value (Arkan et al., 2017). In both of those studies, it has been reported only one oxidation and one reduction peaks. The difference of peak potentials of these peaks observed was highly smaller in contrast to that on BDD electrode, which reveals the fact that the reversibility of this process is seriously reduced in the case of BDD electrode. Furthermore, the peak potential of IIa shifted to more positive values, with an increase in its peak current on BDD electrode when compared to previously

reported electrodes. It was demonstrated that higher oxidation overpotential, indicating slower electron transfer kinetics, is required to oxidize Idarubicin on the surface of BDD.

The peak Ic was also observed by inverting the potential scan just before the peak IIa whereas the peak IIc disappeared in this case (data not shown). This observation indicates that peaks Ic and IIc may correspond to reduction of the oxidation products formed in anodic peaks IIa and Ia, respectively.

In the subsequent cycles, surprisingly, it was observed less decrease of oxidation step IIa, indicating low deactivation of electrode surface. Although Idarubicin is a phenolic compound, the effect of adsorption in its electrochemical sensing may be considered as minor, when using BDD (pretreated by the proposed way) as the working electrode due to its well-known adsorption resistivity.

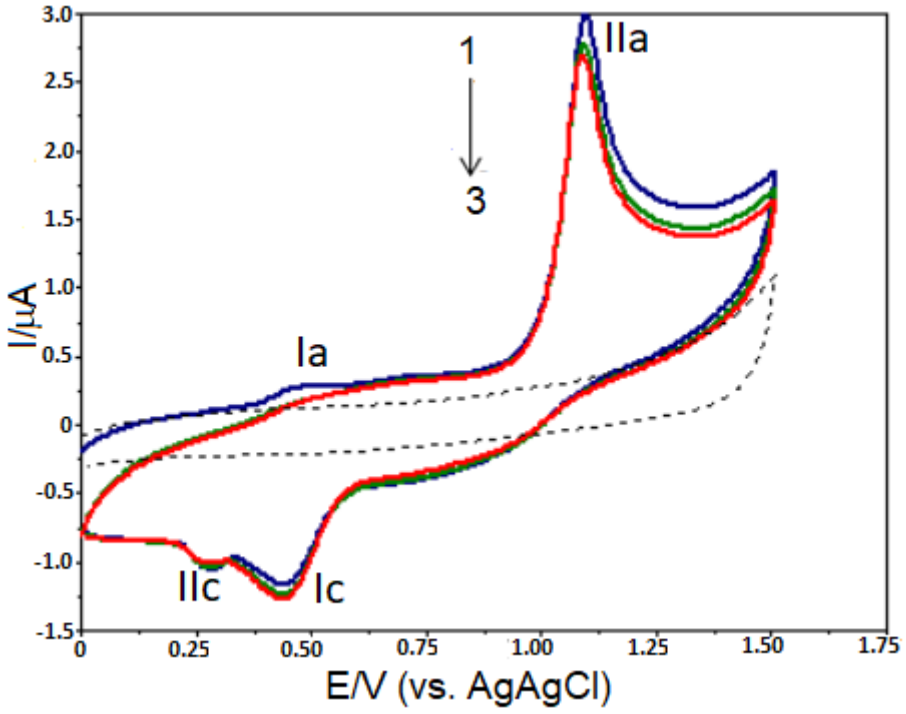


Figure 4.1. The repetitive cyclic voltammograms of 50 μg mL^{-1} Idarubicin in BR buffer at pH 2.0. Electrode, anodically pretreated BDD; scan rate, 100 mV s^{-1}. The numbers 1 through 3 shown within the graphs correspond to the order of the recorded scans. Dashed lines represent background current.

4.1.1.1. Influence of scan rate

In order to obtain further information on the nature of the oxidation process, the CV curves for concentration of 50 μg mL^{-1} Idarubicin in BR buffer at pH 2.0 were carried out for the increasing scan rate (v) values from 25 to 600 mV s^{-1}. It should be noted that the initial oxidation process (Ia) was not clearly observed at low scan rate values, so the secondary oxidation step (IIa) was chosen as an analytical signal in the following studies.

As seen from Figure 4.2, the anodic peak potential (E_{pa}) was shifted slightly to more positive values as scan rate increased. The phenomenon is characteristic for the irreversible or quasi-reversible electrochemical reaction. The plot of the E_{pa} versus log v was also linear expressed by the equations:

E_{pa} (V) = 0.05 log ν (mV s^{-1}) + 1.01 (r = 0.995, n = 9)

For an irreversible electrode process, the relationship between E_p and v is described by the following equation (Laviron, 1979).

$E_p = E^0 + (2.303RT / \alpha nF) \log (RTk^0 / \alpha nF) + (2.303RT / \alpha nF) \log v$

where α is charge transfer coefficient and n the number of electrons involved in the redox reaction, the other terms having their usual meaning. The slope obtained from the E_p vs log v was 0.05. Thus, by means of the above equation, using T = 298 K, R= 8.314 J $K^{-1}mol^{-1}$, and F=96480 C mol^{-1} αn was calculated to be about 1.18. For most systems, the value of α is assumed as equal to 0.5 in totally irreversible electrode process. Thus, the value of n = 2.36 (≈ 2). This result indicates that the oxidation of Idarubicin involves two electrons per molecule on APT-BDD electrode.

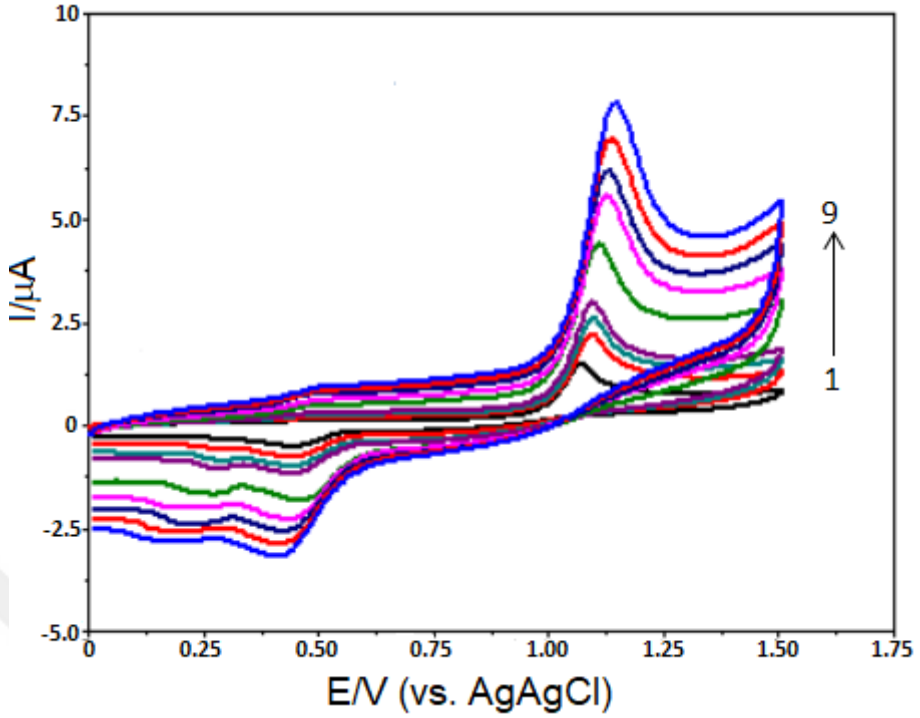


Figure 4.2. Cyclic voltammograms of 50 μg mL^{-1} Idarubicin in BR buffer at pH 2.0 recorded at different scan rates. Electrode, anodically pretreated BDD. The numbers 1-9 correspond to 25, 50, 75, 100, 200, 300, 400, 500 and 600 mVs^{-1}.

On the other hand, IIa showed a linear relationship between the oxidation peak currents (i_p) and square root of scan rate ($\nu^{1/2}$), which indicates that the electrode process is diffusion-controlled (Figure 4.3). The equations are noted below:

$$i_p\ (\mu A) = 0.186\ \nu^{1/2}\ (mV\ s^{-1}) + 0.241 \quad (r = 0.998, n = 9)$$

In order to better understand the Idarubicin oxidation on BDD electrode, plots were constructed between the logarithm of peak current ($\log i_p$) and logarithm of scan rate ($\log \nu$). In this case, it was also obtained a linear relationship (Figure 4.3) according to the following equations:

$$\log i_p\ (\mu A) = 0.454\ \log \nu\ (mV\ s^{-1}) - 0.585 \quad (r = 0.998, n = 9)$$

As can be seen from the equation, the value of the slope is close to the theoretical value of 0.5 (Bard and Faulkner, 2000). This fact indicates that Idarubicin oxidation process can be mainly controlled by diffusion.

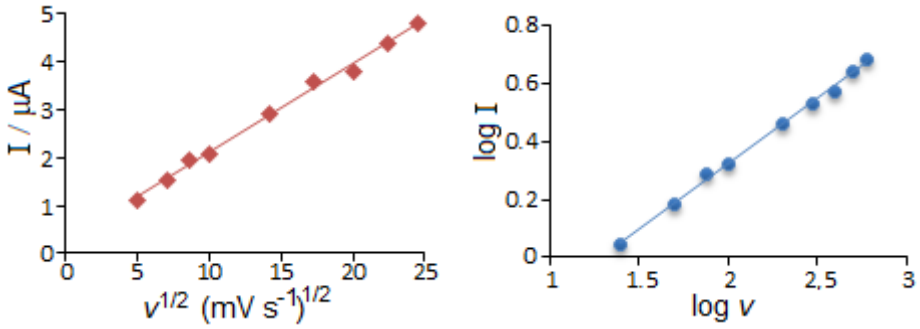


Figure 4.3. The linear relationships between the oxidation peak currents (i_p) and scan rate (ν) Idarubicin, 50 µg mL^{-1}; method, CV; supporting electrolyte, BR buffer at pH 2.0; electrode, anodically pretreated BDD.

4.1.1.2. Influence of electrode pretreatment procedure

The electrochemical response of BDD electrode is strongly affected by the type of pretreatment applied to the surface before experiments. Thus, this effect is of great importance in the case of electroanalytical studies. Although BDD electrode is known to be resistant to fouling, preliminary studies revealed that suitable electroanalytical responses in terms of reproducibility could not be obtained when no activation of BDD electrode was applied. As stated before (in Section 2.2.1), the analytical performance of BDD electrode is strongly affected by the type of pretreatment applied to its surface because anodic pretreatment turns the electrode surface to predominantly oxygen terminated and cathodic pretreatment turns it to predominantly hydrogen terminated. In view of this, CV responses of 25 µg mL^{-1} Idarubicin in BR buffer at pH 2.0 were obtained after the BDD electrode was anodically (APT-BDD, at +1.8 V for 180 s) or cathodically (CPT-BDD, at -1.8 V for 180 s) pretreated (for details on these pretreatments, see Experimental Section), which are presented in Figure 4.4.

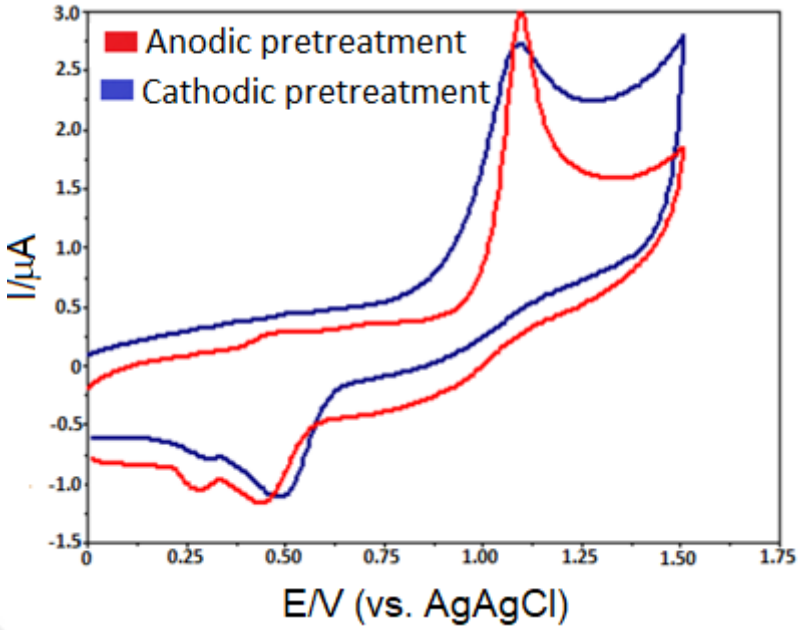


Figure 4.4. CV curves of 50 μg mL^{-1} Idarubicin in BR buffer at pH 2.0 by using different pretreatment procedures. Electrode, BDD; scan rate, 100 mV s^{-1}.

Once the anodic pretreatment was shown to yield best peak definition, more intense current intensity for the peak IIa and higher reproducibility of the measurements, this pretreatment was selected in all following experiments. Note that after stronger polarization processes the electrochemical activity of Idarubicin decreased significantly. This activation protocol was carried out at the beginning of each working day. Between individual measurements, in order to guarantee a clean electrode surface, an activation program was applied for 60 s under the same condition (at +1.8 V).

4.1.2. Square-wave voltammetry

Due to the desirable sensitivity with good separation from the background current, and a speed (analysis time in a few second) obtained by SWV approach in relation to differential pulse voltammetry (DPV), all following experiments were carried out by using this voltammetric wave-form.

4.1.2.2. Influence of pH and supporting electrolyte

In order to broaden the knowledge on electrochemical behavior of Idarubicin, and to find optimal conditions for its sensitive determination, the attention was then turned to study the effect of supporting electrolytes with different pH values (2.0-8.0) and compositions. Experiments were not performed above pH 8, because Idarubicin is extremely unstable in alkaline medium (Kaushik and Bansal, 2013).

In Figure 4.5A, the baseline corrected SW voltammograms of 25 μg mL^{-1} Idarubicin was depicted within the pH range 2.0-8.0 in BR buffer in the potential window from 0 V to +1.5 V. For the whole pH range mentioned above, in addition to the anodic peak IIa, a new oxidation peak IIIa was recorded at more positive potentials than IIa. At pH 2.0-4.0, the peak IIa was predominant, while the peak IIIa was barely detectable. By raising the solution pH, the peak current of IIa decreased significantly at pH value up to 5.0, after which it gradually increased while peak IIIa became more intense. It is clear that the difference between their oxidation peak potentials was higher at all pH values ensuring a good separation. It should be noted at this point that the oxidation peak Ia was barely observable under the studied pH values.

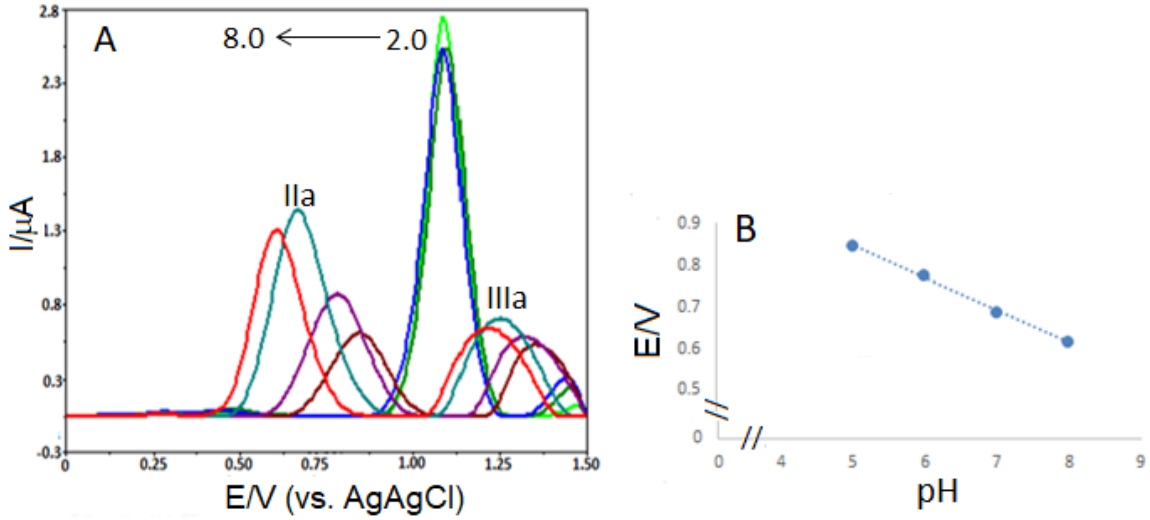


Figure 4.5. SW voltammograms of 25 μg mL^{-1} Idarubicin in BR buffer at pH 2.0-8.0 (A), and related E_p/pH plot (B). Electrode, anodically pretreated BDD; SWV parameters: frequency, 50 Hz; scan increment,6 mV; pulse amplitude, 30 mV.

From the Figure 4.5.A, it is seen that the pH value from 2.0 to 4.0 has little influence on the peak potential of peak IIa (E_p) (from + 1.09 V at pH 2.0 to +1.08 V at pH 4.0), indicating that no proton transfer steps occurs before the electron transfer rate-determining step at these pHs. However, between the pH values from 5.0 to 8.0, E_p of the Idarubicin oxidation peak IIa shifted to less positive potentials. The plot of the E_p versus pH showed a straight line, which can be expressed by the following equations:

E_p (V) = -0.0778 pH + 1.2397 (r = 0.999) (Figure 4.5. B).

This can be explained by involvement of protons in the electrochemical reaction of Idarubicin at APT-BDD electrode. The slope was found to be 77.8 mV per pH unit that is close to the theoretical value of 59 mV/pH which indicates that the numbers of electron and proton taking part in the electrode reaction are equal. Taking into consideration the scan rate results, the electrochemical oxidation of Idarubicin is expected to occur by pH-dependent two electron and two proton transfer process. It confirms the results of this compound obtained in previously published papers (Kurbanoğlu et al., 2013; Arkan et al., 2017).

Next, the influence of the different supporting electrolytes on the electrochemical oxidation of Idarubicin was examined (Figure 4.6). By using phosphate buffer pH 2.5, acetate buffer pH 4.8 and phosphate buffer 7.4, oxidation peak potentials were obtained as +1.13, 1.07 and 0.60 V, respectively, with the peak currents of 0.70, 0.90 and 1.15 μA. As can be seen, E_p values are in agreement with those in BR buffer. However, in the case of simple buffer solutions (acetate and phosphate ones), lower i_{pA} values were obtained (especially in phosphate buffer at pH 2.5). This may be due to the differences of the ionic strength and composition of supporting electrolytes studied at similar pHs.

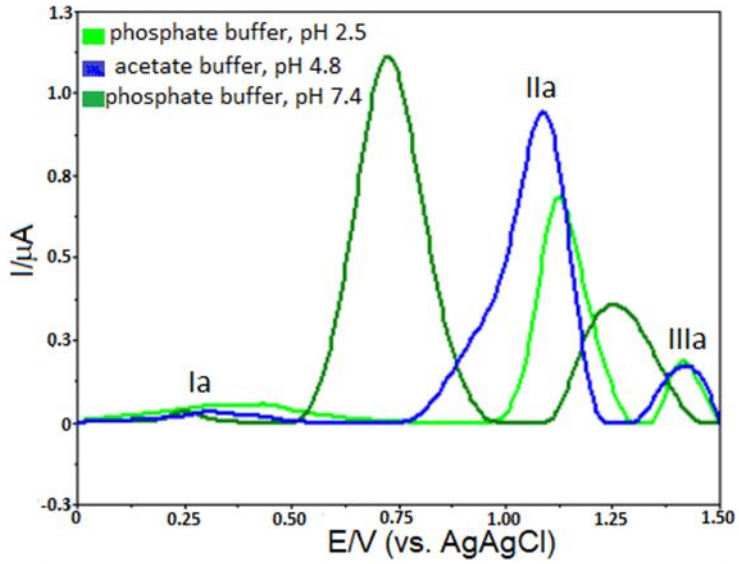


Figure 4.6. SW voltammograms of 25 µg mL^{-1} Idarubicin in various supporting electrolytes at different pH values. Other operating conditions as indicated in Figure 4.5.

Idarubicin contains three potentially ionizable groups thus possibly exhibiting three pKa values at maximum: a tertiary amine group in sugar moiety, and two phenolic hydroxyl groups at C6 and C11 on the anthracycline ring system. In most studies conducted to date for the compounds belong to the anthracycline family; it has been determined two pKa values. Literature values ranging from 7.2 to 8.6 for pKa_1 and from 9.4 to 10.2 for pKa_2 depending on the analyte concentration, pH, ionic strength, temperature and measurement techniques are assigned to the deprotonation of amine group on daunosamine sugar and phenolic hydroxyl group at C11, respectively (Sturgeon and Schulman, 1977; Razzano et al., 1990; Raghunand et al., 2003; Sanli et al., 2014; Matyjaszczyk et al., 2017). Considering that the phenolic group is known to be weakly acidic, indicating strong bonding between the proton and the oxygen donor, the amine nitrogen atom will have a lower pKa than the ligands containing phenolic oxygen. In a previously published report (Razzano et al., 1990), it was concluded that the phenolic hydroxyl group at C6 is the less acidic one. Thus it is expected that this second hydroxyl group does not undergo deprotonation until pH >13.0. Its higher pKa value (pKa_3) is ascribed in part to electronic effects leading to higher strength of the corresponding hydrogen bond with quinone oxygen, and in part to a steric effect from the daunosamine moiety at C7. This explanation receives a support from an earlier investigation of quinizarin which contains two phenolic hydroxyl groups on the

anthraquinone unit (Kiraly and Martin, 1982). The pKa values of quinizarin have been reported to be 9.92, 13.7 which are nearly identical to the pKa_2 and pKa_3 values for investigated anthracyclines.

In some other studies, only one pKa value was found for these compounds, which may be explained by pKa values fairly close to each other. (Marbeuf-Gueye et al., 1999; Garnier-Suillerot et al., 2001; Hu et al., 2003). One of these studies offers alternative pKa values for three anthracycline compounds including Idarubicin (Hu et al., 2003). However, the abnormal pKa values were obtained in the range of 4.64-4.99 both by CE and spectrophotometry. However, until now, there is no evidence in literature for a deprotonation in this range in either Idarubicin or other similar compounds.

In the light of above explanation, we may assume that Idarubicin shows three different species in aqueous solutions, which are cationic, zwitterionic and anionic. It is expected that N-atom (i.e., 3′-N) in the sugar moiety could be protonated at low pHs (pH<6), and therefore carries a positive charge (cationic Idarubicin). When pH is raised from 6 to 7.5, the fraction of positively charged amine groups is reduced in favor of neutral molecular form of Idarubicin. However, above ~pH 7.5 the neutral Idarubicin decreases because of deprotonated phenol group at C11. Part of the neutral Idarubicin molecules might exist in zwitterionic form. In very strong alkaline condition, Idarubicin turns to predominantly its anionic form due to the deprotonation of the less acidic –OH group at C6 (anionic Idarubicin).

At this point it is important to underlined the fact that the surface of BDD pretreated cathodically is hydrophobic whereas anodic polarization process decreases the hydrophobicity of electrode surface and converts it to the relatively negative depending on the polarization potential and period (Ferro et al., 2010). This explains why the highest peak intensity was observed in strongly acidic solutions on APT-BDD electrode because negative charge on the electrode surface attracts to positively charged amino sugar moiety on Idarubicin. On the other hand, in our anodic pretreatment conditions (relatively mild polarization potential of +1.8 V), it may not be enough the transformation of BDD surface to the negative completely, indicating that the surface is still partly hydrophobic. Taking into account the potent lipophilic character of Idarubicin, additionally, hydrophobic interactions between the hydrophobic part of the

compound and electrode surface should be also taken into account. However, when the results obtained on APT- and CPT-BDD electrodes are compared; electrostatic interactions seem to be playing a more important role at lower pH values (see Figure 4.4). As pointed out above, the amount of the uncharged Idarubicin is more significant at pH values between 6 and 8, so it is expected that the hydrophobic interactions between Idarubicin and electrode surface play important role, and therefore an increase in analytical signal should be observed.

To sum up, all experiments were carried out in BR buffer at pH 2.0 for the rest of analytical investigation.

4.1.2.2.1. Possible oxidation mechanism of idarubicin

It should be underlined that only little is known on the electrooxidation mechanism of not only Idarubicin but also structurally similar anthracycline compounds so far. Although the proposed work does not focus to clarify the detailed oxidation pathway of Idarubicin, a short comment can be made. Considering our experimental findings, and taking into account the previously published report dealing with the electrochemical oxidation of quinizarin which is a dihydroxyanthraquinone having the two hydroxy substituents at the 1- and 4-positions, on the surface of GC electrode (Nematollahi et al., 2012), it is believed that the oxidation process is related to hydroxyl groups at C6 and C11 in ring B of the molecule. The oxidation peak (IIa) may correspond to the electrooxidation of this group, resulting from two-electron, two-proton transfer reaction of dihydroxyanthraquinone moiety to form an anthracene-tetraone product. Similar mechanism for the oxidation of Daunorubicin on carbon surfaces has been reported in previous electrochemical study (Oliveira-Brett et al., 2002). The reaction pathway for this oxidation step is shown in Figure 4.7.

As seen from the CV curve (Figure 4.1), the oxidation peak current is significantly higher than reduction one indicating that the generated oxidation product is not stable which is related to the presence of two fused quinonic rings in the structure of anthracene-tetraon.

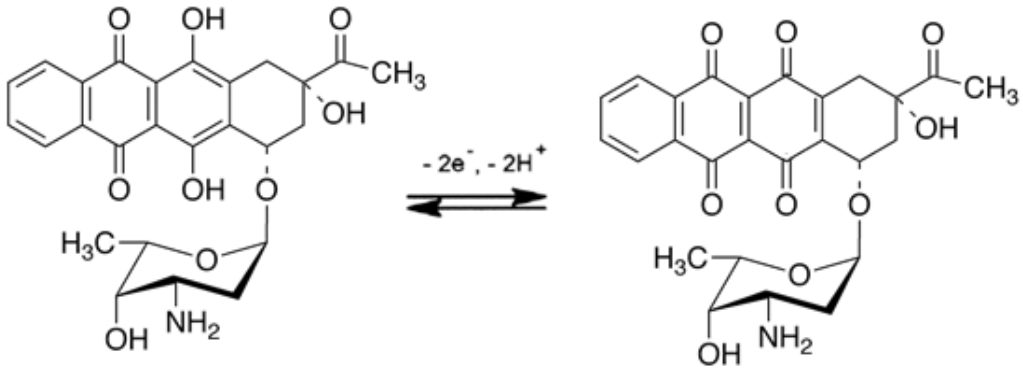


Figure 4.7. The proposed mechanism for Idarubicin oxidation.

The point concerning the nature of the additional oxidation peak (peak IIIa) occurred at more positive potential, which was not observed in previously reported studies, remained obscure. We may assume that the former oxidation is followed by several steps to generate numerous products.

4.1.2.3. Optimization of SWV parameters

To ensure the analytical sensitivity for the determination of Idarubicin (20 μg mL) on APT-BDD electrode, in the following step, it was attempted to analyze the dependence of SW responses on puls parameters such as frequency (f), pulse amplitude (ΔE_{sw}) and step potential (ΔE_s) in BR buffer at pH 2.0. The optimization was undertaken in such a manner that one parameter was always changed within the procedure while the others were kept constant.

An alteration of f in the range of 15-225 Hz (with the ΔE_s and ΔE_{sw} fixed at 12 mV and 40 mV, respectively) indicated that the recorded voltammetric signal increased gradually until the value of 175 Hz, after which it decreased. With regards to ΔE_{sw}, the studied values ranged from 10 to 70 mV (remaining parameters: ΔE_s = 12 mV, f = 175 Hz), the results showed the increase of the voltammetric signal up to 50 mV as the ΔE_{sw} increased. Besides this, the higher values of 40 mV resulted in a considerable widening in the voltammetric signal. The ΔE_s was varied between 4-18 mV with a fixed parameters of f = 175 Hz and ΔE_{sw}= 40 mV. The results showed the increase of current response until the value of 16 mV. This effect was also accompanied by peak

broadening. For the construction of the calibration curve, the optimal values were: f, 175 Hz; ΔE_{sw}, 40 mV and ΔE_s, 12 mV.

4.2. Quantification of Idarubicin

4.2.1. Calibration graph and limit of detection

After optimization of chemical conditions and instrumental parameters, the analytical performance was evaluated by examining the oxidation peak current +1.10 V as a function of concentration of Idarubicin from 0.5-25µg mL^{-1} (9.4×10^{-7}-4.6×10^{-5} M). Figure 4.8A shows SW voltammograms recorded for the addition of Idarubicin standard to the BR buffer at pH 2.0. A linear relationship between peak current and concentration is shown in Figure 4.8B. The oxidation peak current increased proportionally with the Idarubicin concentration to yield a highly linear calibration plot; i_p (µA) = 0.685 C (µg mL^{-1}- 0.181 (r = 0.999, n = 6)], where i_p is the oxidation peak current, C the Idarubicin concentration, r the correlation coefficient and n the number of experiments.

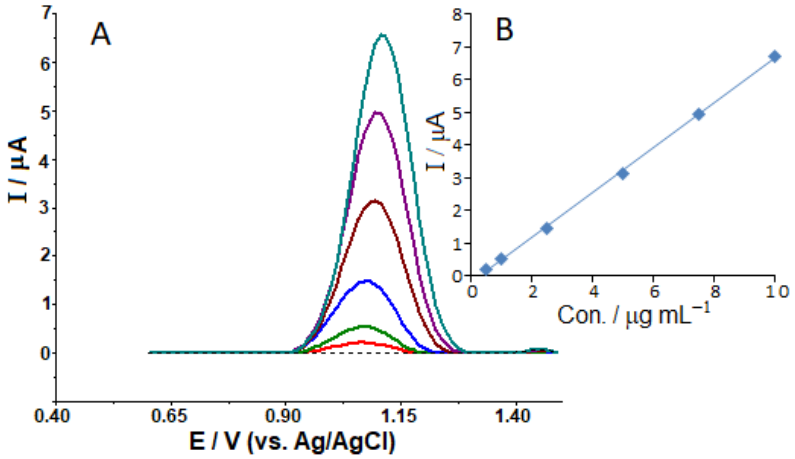


Figure 4.8. (A) SW voltammograms for idarubicin levels of 0.5, 1.0, 2.5, 5.0, 7.5 10,and 25 µg mL^{-1} (from bottom to top) in BR buffer at pH 2.0. (B) corresponding calibration plot for the quantitation of Idarubicin.Electrode, anodically pretreated BDD; SWV parameters: frequency, 175 Hz; scan increment, 12 mV; pulse amplitude, 40 mV.

Limits of detection (LOD) and quantification (LOQ) were calculated as the three and ten times the standard deviation of the peak currents (ten runs) of the lowest

concentration of the related linearity range divided by the slope of the particular calibration curves, respectively (Gümüştaş and Özkan, 2011). LOD and LOQ were found to be 0.0174 µg mL^{-1} (3.2×10^{-8} M) and 0.254 µg mL^{-1} (4.7×10^{-7}M), respectively.

Here it should be mentioned once again that there are only two reports on voltammetric determination of Idarubicin (see Table 2.5). Both of them use modified electrodes and an accumulation step in the methodology. APT-BDD electrode showed equal sensitive voltammetric response in terms of LOD with multiwalled carbon nanotubes modified GC and EPPG electrodes (Kurbanoğlu et al., 2013). On the other hand, CP electrode modified with electrospun carbon nanofibers and TiO_2 nanoparticles (Arkan et al., 2017) declares ten times more sensitivity than the APT-BDD electrode. However, it should be pointed out that the modification process which sometimes can lead to the low reproducible results is often complicated, time-consuming and inconvenient, and the prices of modifying substances are usually high. In our studies, usage of APT-BDD electrode without any chemical modification (except for a simple anodical pretreatment between individual measurements which takes less than 2 min) showed good performance including rapidity, simplicity, sufficient sensitivity, and lso reduced expenses and operational skills of analyst.

4.2.2. Precision and selectivity

To estimate the precision of the proposed method, the intra-day repeatability (in ten measurements in a day), and inter-day repeatability (in three measurements performed over three consecutive days) were investigated for 2.5 µg mL^{-1} of Idarubicin under the optimum experimental conditions. The RSD values were calculated to be 4.2 and 6.1% for intra- and inter-day repeatability, respectively.The RSD values suggest that the APT-BDD electrode has proven to be suitable electrochemical sensor for the repeatable quantification of Idarubicin in the real samples.

Taking into account that Idarubicin is fairly unstable in alkaline solutions, working solutions prepared in alkaline region were protected from light and used within 5 h.

Prior to the analysis of samples, the influence of selected potentially interfering substances, such as inorganic ions and organic compounds were examined by SWV for 5

μg mL^{-1} Idarubicin under the same experimental conditions. The tolerance limit was defined as the maximum concentration of the selected interfering compounds, which caused an approximately ±5% relative error for the oxidation peak current of Idarubicin.

It was found that 100-fold surplus concentrations of inorganic ions such as Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Ca^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Mg^{2+} did not significantly effect on the oxidation peak current under the chosen experiment conditions. With regards to small biomolecules such as fructose, glucose, lactose and sucrose, insignificant effect was also recorded in their 50-fold excess.

The effect of dopamine and uric acid, which could be present in biological fluids such as urine, were evaluated in a molar concentrations at the ratio (Idarubicin:interfering agent) of 1:1. Figure 4.9 shows the respective SW voltammograms. Obviously, the oxidation peak current of Idarubicin was affected in the presence of these molecules in their equimolar concentration to Idarubicin. As a concluded, the method would probably be unsuccessful for the analysis of samples with high concentrations of the interfering compounds. In this case, to eliminate these interferences it can be applied a separation technique before voltammetric determination. However, this limitation was not expected to affect the determination of Idarubicin in the commercial samples.

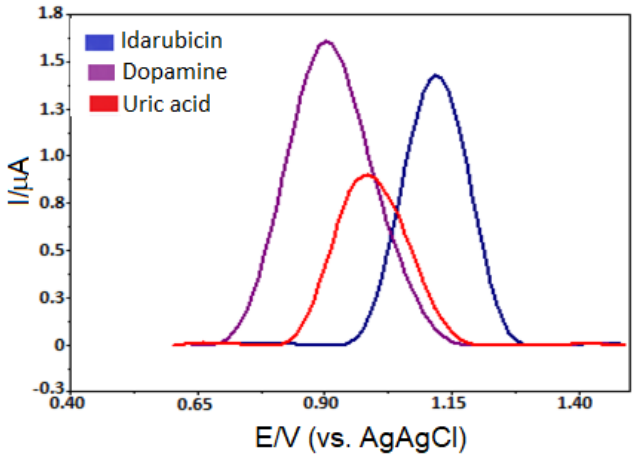


Figure 4.9. SW voltammograms of 5 μg mL^{-1} Idarubicin in presence of equimolar concentration of dopamine and uric acid in BR buffer at pH 2.0 Other operating conditions as indicated in Figure 4.8.

4.2.3. Real sample analysis

In order to ealuate the performance of the proposed methodology in real samples, its applicability was tested in a commercial medicinal sample such as Idamen IV® injectable solution (1 mg mL^{-1} Idarubicin HCl). Voltamograms of the vial solutions prepared in the manner described in Section 4.2.3, that is quick and easy, were recorded under conditions prepared for standard substances. The Idarubicin content in injectable solution was determined by calibration method and the average of triplicate measurements.

The validity of the developed method was evaluated by applying spike/recovery experiments in commercial samples. These experiments were carried out by adding standard Idarubicin solutions (2.5, 5.0 and 7.5 µg mL^{-1}) prepared in supporting electrolyte to 10 mL of sample solution in voltammetric cell, and voltammetric responses were evaluated. Representative SW voltammograms are illustrated in Figure 4.10. The peak at about +1.13 V can be attributed to Idarubicin oxidation, as multiple additions of standards have shown a concomitant increase in peak currents without any distortion of peak potential.

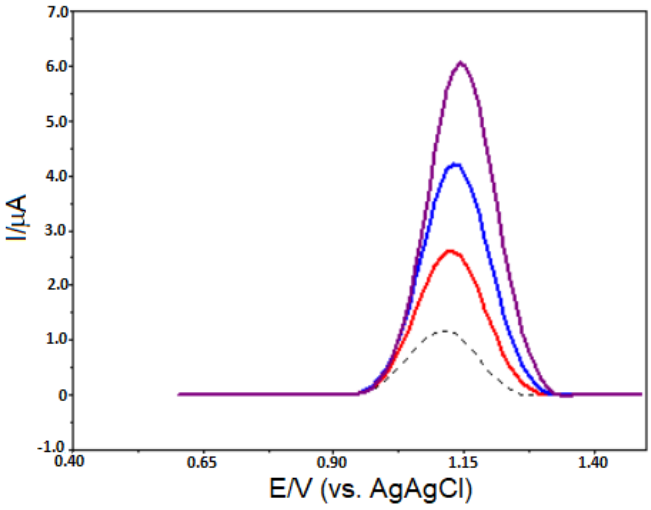


Figure 4.10. SW voltammograms of the diluted commercial sample (dashed lines) and after standard additions of 2.5, 5.0, and 7.5 µg mL^{-1} Idarubicin (from bottom to top). Other operating conditions as indicated in Figure 4.8.

The compound recovery was calculated by comparing the concentration obtained from the spiked samples with those of the pure compound. The recovery values obtained are listed in Table 1. As a result, no significant interference of the compounds present in the commercial samples was observed and the developed method provided good accuracy for the determination of Idarubicin in injectable solution.

Table 4.1. Results of the recovery analysis of Idarubicin in commercial samples.

Idarubicin added ($\mu g\ mL^{-1}$)[a]	Level determined[a,b] ($\mu g\ mL^{-1}$)	Recovery (%) ± RSD(%)
2.5	2.47	98.92±1.46
5.0	4.85	97.01±3.05
7.5	7.40	98.73±4.90

[a]Concentration in the measured solution
[b]Average of three replicate measurements

4.2.4. Comparison by HPLC

The data obtained by the proposed voltammetric procedure for commercial formulations were also compared to those obtained by HPLC-DAD. At first, the standard solution of Idarubicin was injected in triplicate and chromatographed under the previously specified conditions. Typical chromatogram obtained was illustrated in Figure 4.11. As can be seen from the figure, a well-defined peak was obtained with a retention time (t_R) 5.689 min for Idarubicin.

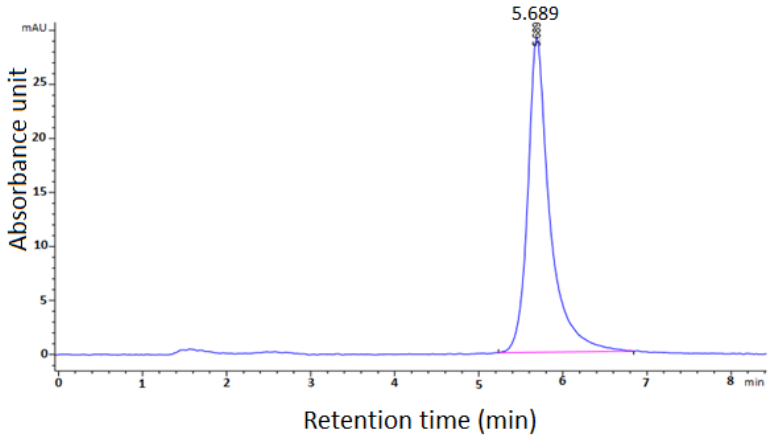


Figure 4.11. The standard solution chromatogram of Idarubicin obtained by HPLC. Chromatographic conditions: mobile phase, 0.02 M NaH_2PO_4/acetonitrile mixture (57:43 v/v); DAD set at 254 nm, flow rate, 1.3 mL min^{-1}.

A calibration curve was obtained by plotting the peak area of the analyte peaks versus the analyte concentration over a range of 2.5–75 µg mL^{-1} (4.6×10^{-6}–1.4×10^{-4} M) (Figure 4.12). The variation of peak area with added Idarubicin concentration (2.5-75µg mL^{-1}) was linear and obeyed the following equation:

y = 54.323x–2.7212 (r= 0.999; n=8); where y is the peak area, x Idarubicin concentration, r the correlation coefficient and n the number of experiments.

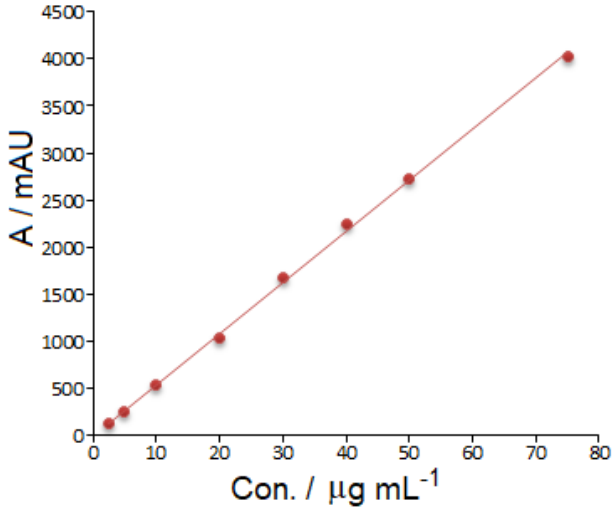


Figure 4.12. Calibration curve of Idarubicin at different concentrations (2.5-75 µg mL^{-1}) using HPLC method.

In following experiments, the comparison HPLC method was also applied for its analysis in commercial drug sample (Idamen IV® injectable solution). As illustrated in Figure 4.13, well-defined peak was obtained at t_R ~5.709 min.

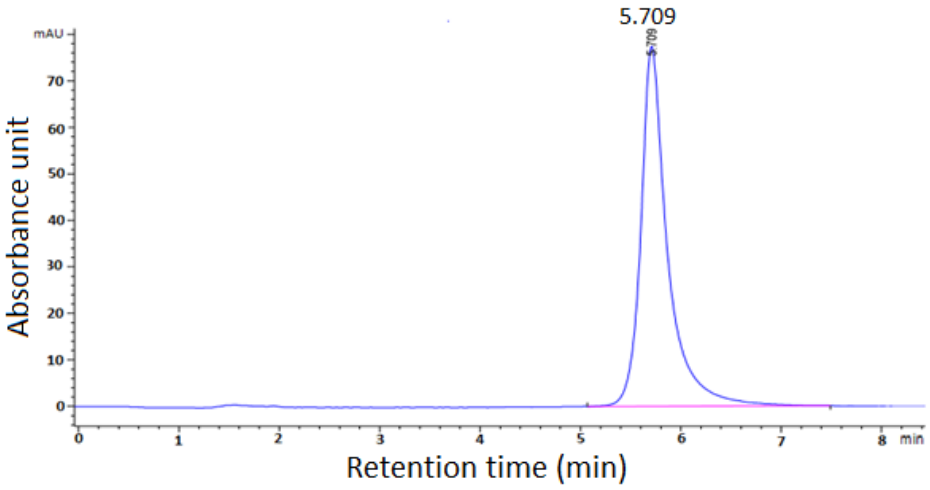


Figure 4.13. The sample solution chromatogram of Idarubicin obtained by HPLC. Other operating conditions as indicated in Figure 4.12.

The assay results examined by proposed (SWV) and comparison (HPLC) methods are summarized in Table 4.2. These results of the analysis of pharmaceutical products indicate the fact that the proposed protocol does not suffer from any considerable matrix effect, and agrees quite well with that obtained by the HPLC method. Moreover, the results obtained by both methods are in good agreement with the label value of 1 mg in 1 mL per vial declared by producer.

Table 4.2. Idarubicin content in in commercial injectabl solutions by using SWV and HPLC

Drug	SWV[a]	HPLC[a]	E(%)[b]
Idamen IV® injectable solution (1mg/mL)	0.975±0.041	1.065±0.0006	-8.4507

[a]Mean ± SD (n = 3)
[b]Relative error, E(%) = [(voltammetric value-HPLC value)/HPLC value] × 100

5. CONCLUSION

As explained in the Literature Reviews section, there are only two articles in the literature based on the voltammetric determination of Idarubicin. Both of them are focused on the application of time and cost demanding chemically modified carbon-based electrodes, and also used an accumulation step in the methodology (AdSV).

In the present study, the suitability of BDD electrode without any chemical modification (except a simple electrochemically pretreatment) in combination with SWV was evaluated for electrochemical behavior of Idarubicin. The proposed approach could be applicable directly to the routine quality control of pharmaceutical products, dispensing any use of organic reagents, complex sample extraction, or expensive apparatuses.

The importance of the findings obtained from this thesis can be summarized as follows:

(*i*) It is clear that the development of a simple, rapid, sensitive and accurate method for the detection and determination of compounds used in cancer treatment from drug formulations, biological (urine, blood, tissue, etc.) and environmental (wastewater) samples will contribute to social health. An important advantage of voltammetric techniques over other methods is that they enables on-site analysis. BDD electrodes are often used as portable electrodes in voltammetric measurements (Wang and Taha, 1991) based on batch injection analysis (BIA) technique, which has very effective features such as speed, sample volume, sensitivity and simplicity (Freitas et al., 2017; De Araujo et al., 2018). Therefore, it is thought that the voltammetric technique on the surface of the BDD described in this study may constitute the first step for in situ measurements of wastes in the hospital environment, not only of Idarubicin, but also of the other structurally similar anthracycline group antineoplastics.

(*ii*) As mentioned before, the electron transfer and oxidative stress are known to play a major role in the therapeutic and adverse effects (toxic) of antineoplastics. Bearing this important knowledge in mind, in addition to the practical application of the proposed voltammetric method, the experimental work presented above may provide important information about both action mechanisms not only of the Idarubicin but also

of the other anthracycline antibiotics used in cancer therapy having redox active groups in their molecular structure.

(*iii*) This work in connection with our previous ones underlines the importance of the antifouling properties of BDD electrode pretreated with different activation protocols may allow the use of this electrode for the determination of some other polyphenolic compounds.

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EXTENDED TURKISH SUMMARY (GENİŞLETİLMİŞ TÜRKÇE ÖZET)

ROMATOİD ARTRİTLERDE OKSİDATİF STRES SEVİYELERİNİN VE BAZI ANTİOKSİDAN ENZİM FAALİYETLERİNİN BELİRLENMESİ

BARZANI, Hemn Abdulazeez Hakeem
Yüksek Lisans Tezi, Kimya Bölümü
Danışman: Prof. Dr. Zühre ŞENTÜRK
Ağustos 2019, 71 sayfa

1. ÖZET

Bu çalışmada, anodik olarak ön-işlem görmüş bor-katkılı elmas elektrot üzerinde kanser tedavisinde kullanılan ve antrasiklin antibiyotikleri içerisinde yer alan İdarubisin bileşiğinin tayini için elektrokimyasal bir yöntem geliştirilmiştir. Kare-dalga voltametri tekniği kulllanılarak Britton-Robinson tamponu (pH 2.0) içerisinde +1.10 V (Ag/AgCl′e karşı) gerilim değerinde, 0.0174 μg mL^{-1} (3.2×10^{-8} M) gözlenebilirlik sınırına sahip 0.5-25μg mL^{-1} (9.4×10^{-7}-4.6×10^{-5} mol L^{-1}) derişim aralığında hayli doğrusal analitik eğri elde edilmiştir. Önerilen yöntem ticari ilaç örneklerinde İdarubisin′in ölçümüne başarıyla uygulanmış ve sonuçlar DAD dedektörlü HPLC yöntemi ile kıyaslanmıştır..

Anahtar kelimeler: Anodik ön işlem, Bor-katkılı elmas elektrot, Enjektabl çözelti, İdarubisin, Kare-dalga voltametrisi.

2. MATERYAL VE YÖNTEM

Voltametrik ölçümler, Autolab Elektrokimyasal Analizörü kullanılarak gerçekleştirilmiş ve genel olarak aşağıdaki yol izlenmiştir:

(i) Analizi yapılacak İdarubisin bileşiğinin farklı çözelti ortamlarında (Britton-Robinson, asetat ve fosfat tamponları), geniş bir pH aralığında (2.0-8.0) ve farklı gerilim tarama hızlarında (25-600 mV s^{-1}) bor-katkılı elmas elektrot (BDD) üzerinde elektrokimyasal davranışları, dönüşümlü voltametri (CV) ve kare-dalga voltametrisi (SW) teknikleri ile araştırılmıştır.

(*ii*) Analiz çözeltisi bileşiminin, pH′nin, voltametrik dalga formu değişkenlerinin (SW) elektrot yanıtına etkisi incelenmiş ve en iyi deney koşulları belirlenmiştir.
(*iii*) Yöntemin (SWV) aletsel performans özellikleri [çalışma aralığı, kesinlik (gün-içi ve günler-arası tekrarlanabilirlik), seçicilik, duyarlılık (gözlenebilme sınırı ve tayin alt sınırı) ve geri kazanım] araştırılmıştır.
(*v*) Geliştirilen voltametrik teknik ile gerçek örnek olarak seçilen enjeksiyon çözeltilerinde İdarubisin içeriği saptanmıştır.

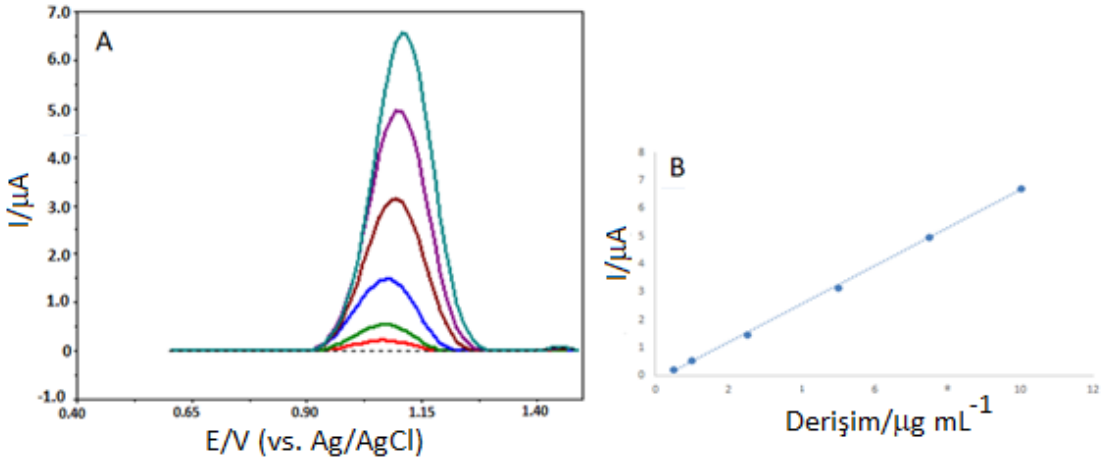
3. BULGULAR VE TARTIŞMA

Bu tez kapsamında sunulan çalışmada, anodik ön-işlem uygulanmış BDD elektrot yüzeyinde İdarubisin bileşiğinin belirlenmesi için CV ve SWV teknikleri kullanılarak yeni bir yöntem geliştirilmiştir.

İlk olarak CV tekniği kullanılarak 50 μg mL^{-1} İdarubisin bileşiğinin Britton Robinson tamponu (pH 2.0) içerisinde, 100 mV s^{-1} tarama hızında, 0 ile +1.6 V gerilim aralığında dönüşümlü voltamogramları elde edilmiştir. Eğriler üzerinde anodik ön-işlem görmüş BDD elektrot üzerinde moleküle ilişkin +0.40 V dolayında çok belirsiz, +1.10 V dolayında ise keskin ve iyi belirmiş iki yükseltgenme basamağı gözlenmiştir. Gerilim taramasına ters yönde devam edildiğinde ise +0.30 ve 0.40 V gerilim değerlerinde birbirinden çok iyi ayrılmamış iki indirgenme basamağı belirmiştir. CV tekniği ile 25-600 mV s^{-1} aralığındaki hız taraması sonuçlarında İdarubisin molekülünün yükseltgenmesine ait elektrot işleminin difüzyon kontrollü olarak yürüdüğü saptanmıştır. Elektrot ön-işlem araştırması sonucunda elektrot yüzeyinin temizleme işleminin anodik yolla yapılmasına (0.5 M H_2SO_4 içerisinde 180 s süreyle +1.8 V gerilim uygulaması) karar verilmiştir.

SWV tekniği kullanılarak ortamın asitliğinin ve destek elektroliti bileşiminin İdarubisin yükseltgenme sinyalleri üzerine etkisi araştırılmış ve pik şiddeti açısından en iyi sonuçlar, Britton Robinson tamponu (pH 2.0) içerisinde elde edilmiştir. BDD elektrot üzerinde kare-dalga değişkenlerinin optimizasyonu çalışması yapıldığında 175 Hz frekans, 12 mV puls adımı 40 mV amplitüd değerlerinin en iyi aletsel koşul değerleri olarak kullanılabileceği saptanmıştır.

Yukarıda gerçekleştirilen araştırma bulgularının ışığında İdarubisin bileşiğinin analitik validasyon araştırmasına geçilmiştir. Bileşik için SWV tekniği kulllanılarak Britton-Robinson tamponu (pH 2.0) içerisinde +1.10 V (vs. Ag/AgCl) gerilim değerinde, 0.0174 µg mL^{-1} (3.2×10^{-8} M) gözlenebilirlik sınırına sahip 0.5-25µg mL^{-1} (9.4×10^{-7}-4.6×10^{-5} mol L^{-1}) derişim aralığında hayli doğrusal analitik eğri elde edilmiştir. (Şekil 1).



Şekil 1. (A) Britton-Robinson tampon (pH 2.0) içerisinde farklı derişimlerdeki (alttan üste doğru; 0.5, 1.0, 2.5, 5.0, 7.5 10, 25 µg mL^{-1}) İdarubisin bileşiğinin kare-dalga voltamogramları. (B) İdarubisin tayininde kullanılan kalibrasyon eğrisi. Kare-dalga değişkenleri; frekans, 175 Hz; adım gerilimi, 12 mV; amplitüt, 40 mV.

Daha sonraki aşamada yöntemin tekrarlanabilirlik ve seçicilik çalışmaları yapılmıştır. Araştırmanın son bölümünde elde edilen bulgular, bileşiğin ticari ilaç şekli olan enjeksiyon çözeltisi örneğine başarıyla uygulanmış ve sonuçlar HPLC-DAD tekniği bulguları ile kıyaslanmıştır.

Bu araştırmanın özgün değeri ve bilimsel açıdan önemi aşağıdaki şekilde vurgulanabilir:

(*i*) Çalışma konusunun birincil önemi; seçilen İdarubisin bileşiğinin niteliğine ilişkindir. Genelde antineoplastik ilaçların özelde İdarubisin bileşiğinin farmasötik formülasyonlardan, biyolojik örneklerden ve çevresel örneklerden tayinine olanak sağlayan yüksek duyarlılığa sahip, seçici ve güvenilir analitik yöntemlere büyük ölçüde gereksinim duyulmaktadır.

Farmasötik formülasyonlarda: Sitotoksik amaçlı kullanılacak örneğin üretiminden hastaya uygulama aşamasına kadar olan süreçte analitik yöntemler; ticari ürünlerin

kalite kontrolü açısından, hastaya uygulama öncesi seyreltik örneklerin kalite kontrolü açısından ve dayanıklılık ve girişim etkilerinin saptanması açısından gereklidir.

Biyolojik örneklerde: Analitik yöntemler; sitotoksik ilaçların biyolojik sıvı ve dokulardan tayini açısından, yeni ilaçların temel araştırmaları açısından, farmakodinamik ve farmakokinetik çalışmalar açısından, ilacın tedaviedici dozunun ya da maruziyetin biyolojik olarak izlenmesi açısından gereklidir.

Çevresel örneklerde: Kanser tedavisinde kullanılan ilaçlar, hastaların idrarıyla elimine olup atık sulara karışmaktadır. Bu nedenle analitik yöntemler, özellikle hastane çevresindeki atık su örnekleri ve malzemelerin maruziyet derecesinin saptanması açısından gereklidir.

(*ii*) Çalışma konusunun ikincil önemi ise; kanser kemoterapisinde kullanılan ilaçların analizi için seçilen yöntem ve bu yöntemde kullanılacak olan elektrotlara ilişkindir. Elektroanalitik çalışmalarda yeni bir elektrot tasarlamak kadar var olan bir elektroda küçük modifikasyonlar yaparak ya da herhangi bir değişiklik yapmadan elektrotun farklı uygulama alanlarının gösterilmesi de önemlidir. İdarubisin′in voltametrik tayinine yönelik olarak yayımlanan iki bilimsel makalede de kimyasal-modifiye elektrotlar kullanılmıştır. Bu tür elektrotların duyarlık ve seçiciliği artırdığı bir gerçektir ancak modifiye elektrot tasarımı zaman alıcıdır ve çoğu kez tekrarlanabilir sonuçlar elde edilemez. Buna karşın bu tez çalışmasında kullanılan BDD elektrot için herhangi birkimyasal modifikasyon işlemi olmaksızın analizler gerçekleştirilmiştir. Bu durumda analitik işlemin hazırlık süresi kısalmış, ölçümlerdeki hata riski azalmış, maliyet düşmüş ve analizcinin özel deneysel becerisine gerek duyulmamıştır.

(*iii*) Elektron aktarımı ve oksidatif stresin antineoplastiklerin tedavi edici ve aynı zamanda olumsuz etkileri (toksik) üzerinde büyük rol oynadığı bilinmektedir. Çalışma konusunun üçüncül önemi ise; ilgili bileşiklerin pek çoğunun moleküler yapısında redoks etkin gruplarının bulunması nedeniyle; elektroanalitik araştırmalara ek olarak, elektrokimyasal çalışmalardan edinilen bulguların, İdarubisin bileşiğinin her iki etki mekanizması hakkında önemli bilgiler sunacak olmasıdır.

4. SONUÇ

İdarubisin bileşiğinin voltametrik tayinine yönelik yeni ve kapsamlı bir çalışmanın kaynakçaya ilk defa kazandırılacak olması tezin temel özgünlüğünü oluşturmaktadır. Bu çalışmada elde edilen bulguların diğer antrasiklin türevi antineoplastik ilaçların BDD elektrot üzerinde gerçekleştirilecek elektrokimyasal tayinine ışık tutacağı açıktır.

CURRICULUM VITAE

Hemn Abdulazeez Hakeem BARZANI was born in Barzan, North Iraq in 1993. He received B.Sc. degree in Chemistry from Salahaddin University in 2016. He started to M.Sc. degree programme in Van Yüzüncü Yıl University (Van, Turkey) in 2017 under the supervision of Prof. Zühre ŞENTÜRK.

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