ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

VOLTAMMETRIC DETERMINATION OF CAFFEINE IN COFFEE USING ANTHRAQUINONE MODIFIED CARBON PASTE ELECTRODE

By: Kerem Seid

ADVISORS:

Merid Tessema (Ph. D)

Mesfin Redi (Ph. D)

July 2008

VOLTAMMETRIC DETERMINATION OF CAFFEINE IN COFFEE USING ANTHRAQUINONE MODIFIED CARBON PASTE ELECTRODE

A GRADUATE PROJECT SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY: KEREM SEID

JULY 2008

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

VOLTAMMETRIC DETERMINATION OF CAFFEINE IN COFFEE USING ANTHRAQUINONE MODIFIED CARBON PASTE ELECTRODE

BY: KEREM SEID
DEPARTMENT OF CHEMISTRY
FACULITY OF SCIENCE

Approved by:	Signature
Dr. Merid Tessema Advisor	_____
Dr. Mesfin Redi Advisor	_____
Dr. Shimels Admassie Examiner	_____
Dr. Tarekegn Birhanu Examiner	_____

DECLARATION

I, the undersigned, declare that this MSc project is my original work and has not been presented for any degree in any other university and that all sources of material used for this MSc project have been duly acknowledged.

Name: Kerem Seid

Signature:_____

This MSC project has been submitted for examination with my approval as a university advisor.

Name: Dr Merid Tessema

Signature:_____

Dr. Mesfin Redi

Signature:_____

Date and place of submission: Department of chemistry, Addis Ababa university

July 2008

TABLE OF CONTENTS

PAGES

List of figures……..... iii

List of tables ……v

Acknowledgement…….....vi

Abstract……... vii

Abbreviation ……. viii

1. INTRODUCTION……... 1

1.1. Coffee……...…… 1

1.1.1 Introduction…….. 1

1.1.2 Coffee species……..……..... 2

1.1.3 Coffee constituents …….. 3

1.1.4 Physiological effects of coffee…….. 4

1.2 Caffeine ……... 5

1.2.1 Introduction …….. 5

1.2.2 Sources and prevalence of caffeine …….. 6

1.2.3 Metabolism of caffeine in human body ……7

1.2.4 Advantages and disadvantages of caffeine…… 8

1.2.5 Methods of analysis ……. 9

1.2.6 Electrochemical oxidation of caffeine ……. 10

1.3 Carbon paste electrodes …… 13

1.3.1 Introduction ……. 13

1.3.2 Preparation of carbon paste electrodes …… 13

1.3.3 Modified carbon paste electrodes …… 14

2. Objective of the study…… 16

3. Theoritical background …… 17

3.1 Voltammetry …….. 17

3.2 Cyclic voltammetry …… 18

3.3 Square wave voltammetry ……. 21

4. Experimental part …… 24
4.1 Reagents and chemicals ……. 24
4.2 Apparatus ……... 24
4.3 Preparation of Unmodified and Modified Carbon Paste Electrode……. 24
4.3.1 Preparation of Unmodified Carbon Paste Electrode……. 24
4.3.2 Preparation of modified carbon paste electrode …….. 25
4.4 Electrochemical measurements …… 25
5. Result and discussion …… 26
5.1 Cyclic voltammetric investigation ……..……... 26
5.1.1 Cyclic voltammetry of caffeine …… 26
5.1.2 Cyclic voltammetry of anthraquinone ……. 27
5.2 Square wave investigation …...…….. 28
5.3 Optimization of experimental parameters …….. 29
5.3.1 Electrode composition effect ...……. 29
5.3.2 Effect of pH of supporting electrolyte …….. 32
5.3.3 Effect of the square wave frequency …… 36
5.3.4 Effect of square wave amplitude …….. 37
5.3.5 Effect of square wave step potential ……. 38
5.4 Linear range and detection limit …… 40
6. Conclusion …… 42
7. References ……...……. 43

List of figures

Figure 1 The structure of different xanthines..... 5

Figure 2 Electrochemical redox pathway of caffeine ……... 12

Figure 3 Potential Vs time graph and potential versus current graph of cyclic Voltammetry…… 19

Figure 4 Potential-excitation signal and voltammogram for square wave voltammetry…… 22

Figure 5 Cyclic voltammogram of caffeine at the unmodified carbon paste electrode... 26

Figure 6 Cyclic voltammogram of a buffer of pH=12 using UNMCPE and anthraquinone MCPE ……..... 27

Figure 7 Square wave voltammogram of anthraquinone ……..……... 28

Figure 8 Cyclic voltammogram of a buffer pH = 8 using variable composition of anthraquinone ……..... 30

Figure 9 The linear graph of anodic current versus percentage composition of anthraquinone …….. 31

Figure 10 Cyclic voltammogram of anthraquinone at different pH of a buffer……. 32

Figure 11 Square wave voltammogram of anthraquinone at different pH of a buffer……. 33

Figure 12 The dependence of peak current of anthraquinone with pH ……. 34

Figure 13 The stability of the peak current of anthraquinone at pH =12 ……. 35

Figure 14 Dependence of the peak potential of anthraquinone on the pH of the buffer …….. 36

Figure 15 Dependence of the peak current of anthraquinone on the square wave frequency …… 37
Figure 16 Dependence of the peak current of anthraquinone on the square wave amplitude …… 38
Figure 17 Dependence of step potential on the peak current of anthraquinone …… 39
Figure 18 Calibration curve for the effect of caffeine on the redox behavior of anthraquinone …… 40
Figure 19 Calibration curve for the effect of caffeine on the redox behavior of anthraquinone…….…… 41

List of Tables

Table 1 Optimized parameters …… …… 39

Table 2 Comparison of anthraquinone with 1,4-Benzoquinone …….. 41

Acknowledgement

First of all I would like to thank my creature to keep me healthy and reach this stage. I would also thank my father, Ato Seid Endris and my mother, W/O Leila Mohammed, for their infinite support throughout my life, Ato Ahmed Endris, Eshetu Hassen and Seid Mohammed for his financial and moral support.

I would want to express my warmest and heart full acknowledgement to my advisors Dr. Mesfin Redi and Dr. Merid Tessema for their valuable guidance, devoted assistance, encouragement and ultimate help through out the project work.

I would like to thank AAU for its acceptance of me and allowance with suitable payment. Also I would like thank students of M.Sc and PhD in the department of chemistry, Addis Ababa University for their moral help and assistance.

It is also my pleasure to thank all people who has been helping me throughout my life.

Abstract

Anthraquinone modified carbon paste electrode is used for the determination of caffeine in coffee. The optimized pH=12 value gives an advantages of undergoing square wave voltammetry of real sample with less sample pretreatment steps. A linear calibration curve is obtained over 0- 500 μM in 0.1 M NaH_2PO_4 and Na_2HPO_4 buffer solution with a detection limit (3σ) of 5 nM. Determination of caffeine in coffee was done by subtracting the background current.

(Key words: CMCPEs, anthraquinone)

Abbreviations

CP	Carbon paste
CPE	Carbon paste electrode
CMCPE	Chemically modified carbon paste electrode
UMCPE	Unmodified carbon paste electrode
SWV	Square wave voltammetry
CV	Cyclic voltammetry

1. Introduction

1.1. Coffee

1.1.1. Introduction

Coffee is most popular drink around the world. Different people of the world consume coffee in different forms. Historically, consumption of coffee had been related to unhealthy behaviors, such as smoking, large amount alcohol drinking. But recently it becomes popular due to its beneficial activities [1].

All cultivated species of coffee have their origin in Africa. The name coffee is derived from the name of the province "*Keffa" in* Ethiopia, where shepherds discovered the coffee beans in the 6th century [1]. The Arabs introduced coffee from Ethiopia to Yemen during the 13th century, where the habit of drinking coffee was developed in the 15th century. This habit gradually spread to the rest of the world, leading to the increased interest of some countries to produce coffee as a commodity on a large scale.

Coffee has enormous commercial and social importance and is the most important traded commodity in the world after oil [2, 3]. Global output is expected to reach 7.0 million tons by 2010. World consumption of coffee is projected to increase by 0.4% annually from 6.7 million tons in 1998–2000 to 6.9 million tons in 2010 [3].

It is also very important commodity crop for many developing countries, once contributing over 10–11 billion US $ annually and providing a source of income for thousands of small-scale farmers, as well as being a significant source of employments [4].

The production of coffee beans is the base of the economy of several tropical countries, such as Mexico, Guatemala, El Salvador, Nicaragua, Costa Rica, Panama, Venezuela, Colombia, Ecuador, Peru, Bolivia, Paraguay, Brazil, Ethiopia, Kenya, Tanzania, Zambia, and Mozambique. [2, 5**].** More than 50 countries, in which 25 of them in Africa, depend on coffee for their foreign exchange [6].

Ethiopia is the third largest coffee producer in Africa after Uganda and Ivory Coast [6]. It covers 2.5 % of the worlds coffee trade [6]. Coffee covers about 60 % of Ethiopia's export and 10 % of its GDP [7]. It also accounts for 5 % of gross national product, 42% of taxes from foreign trade [6]. In the country 24.5 Kg coffee is consumed annually per household and 4.5 Kg per capita [7, 8].

Coffee grows in most part of Ethiopia. Oromia and SNNP regions comprise largest coffee cultivated areas such as welega, Elu Ababoura, Jimma, Benchi Maji, Sidamo, Gedeo, Guji, East and West Hararge, South and north Omo. About 204500to 683600 hectares of land is under coffee cultivation [7]. This is a small part of areas, which is suitable for coffee production. Around 25% of the country's population directly or indirectly depends on coffee [6]. About 95% of the coffee output is grown by small farmers, most of whom work less than half a hectare of land. An average yield is between 540 to 490 Kg per hectare [7].

1.1.2. Coffee Species

Coffee tree belongs to the large botanic family called *Rubiaceae,* which includes more than 500 genera and 8000 species [9, 10]. The two most economically important and mainly cultivated species of coffee are *Coffea arabica* L. and *Coffea canephora* Pierre, usually known as Arabica and robusta, respectively [10, 11, 12]. Another species of coffee that is cultivated less extensively is *Coffea liberica.*

Coffea arabica is native to the southwestern highlands of Ethiopia. It is the most cultivated coffee species throughout the world [12]. About 90% of world's coffee production is coffee arabica and 9% is robusta [11]. They are the better quality coffees and the most expensive ones [10]. Coffee arabica grows at higher altitudes, 1000 to 2000 meters, and while it has a lower yield and less caffeine content (0.8 to 1.4%), it is widely recognized to be superior to robusta. Arabica has a delicate acidic flavor, a refined aroma and a caramel aftertaste. It mainly grows in Central America (Mexico, Guatemala, El Salvador, Nicaragua, Costa Rica, and Panama), South America (Venezuela, Colombia,

Ecuador, Peru, Bolivia, Paraguay, and Argentina), India, Eastern Africa (Ethiopia, Kenya, Tanzania, Zambia, and Mozambique), and Papua New Guinea. It is the most important foreign currency earner for more than 80 developing countries. It was responsible for the transfer of over US$13 billion from developed countries to developing countries in 1983. *C*. arabica is the only species occurring in Ethiopia.

Coffea Canephora is second to Coffea Arabica in terms of commercial importance. It is widely grown in Africa and Indonesia. It is originated in the humid lowland forests of tropical Africa, which stretch from Guinea to Uganda and Angola. It is grown at lower altitudes, 0 to 700 meters. Its caffeine content is higher than Arabica. It has a stronger flavor than arabica with a full body and a woody aftertaste, which is useful in creating blends and especially useful in instant coffee. Compared with arabica, robusta is generally vigorous and productive [12]. It mainly grows in Western and Central Africa (Ivory Coast, Cameroon, Uganda, Angola, etc.), Malaysia (Vietnam, Sri Lanka, Sumatra, Java, etc.), Brazil, India. (Ivory Coast, Cameroon, Uganda, Angola, etc.), Malaysia (Vietnam, Sri Lanka, Sumatra, Java, etc.), Brazil, India.

1.1.3. Coffee Constituents

Coffee contains a multitude of substances, many of which are potentially biologically active. The chemical composition of coffee varies for different reasons such as the species and variety of coffee beans and to a lesser extent other factors such as agricultural activities, degree of maturation and storage conditions [1, 6].

In the order of their abundance, coffee contains phenol polymers 8%, polysachrides 6%, chlorogenic acids 4%, minerals 3%, water 2%, caffeine 1%, organic acids 0.5%, sugar 0.3%, lipids 0.2% and aroma 0.1% [6]. Organic acids such as oxalic, succinic, fumaric, malic, tartaric, citric and quinic acids are considered to play an important role in coffee flavor [13].

Coffee has the highest and the most variable caffeine content among dietary products, which contain this alkaloid. The value differs from 30-175 mg of caffeine per cup (150mL) coffee. The standard value has been suggested to be 85 mg of caffeine per cup of ground roasted coffee [1].

Coffee is also enriched with many other ingredients that may contribute to its biological activities such as niacin, potassium, magnesium and anti oxidants such as tocopherols, phenols and chlorogenic acids. The two diterpinoids, *cafestol* and *kahweol,* are also found in significant level. They are a natural constituent of green coffee beans, are realised from roast, and ground coffee by hot water [1].

1.1.4. Physiological Effects of Coffee

Coffee contains several biologically active substances, which have some capability of causing physiological effects. One constituent of coffee, *caffeine* is a mild stimulating agent. Besides this, it has physiological effects, such as diuretic action, gastric secretion potentiating action etc [14, 15, 16].

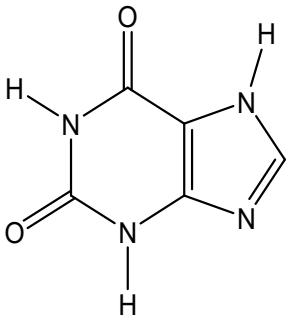
Coffee constituents, such as the lipid soluble heterocyclic compounds including furans, pyrolls and maltol have been found to exhibit higher antioxidant activities. Some researches showed that coffee has an inverse relationship with a risk of type 2 Diabetes Mellitus [1].

Non-filtered boiled coffee increases the serum cholesterol level and thus increases the coronary heart disease. The method of brewing is an important factor for the hypercholesterolemic effect. It is also noted that unfiltered coffee increases plasma homocysteine, which is a risk factor for cardiovascular diseases when elevated, concentration by 10% in subjects who drink six cups of unfiltered coffee per day for two weeks. The effect of coffee on homocisteine concentration could be due to a decrease in blood vitamin b_6 concentration mediated by caffeine [1].

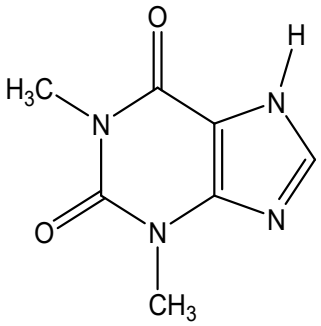
1.2 Caffeine

1.2.1 Introduction

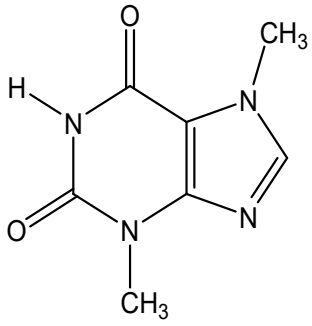
Caffeine, 1,3,7-trimethylxanthine, is a natural alkaloid, which is a class of naturally occurring compounds containing nitrogen and having the properties of an organic amine base. It is found in a family of compounds called *alkylxanthines (3,7-dihydro-1H-purine-2,6-dione)*, which belongs to the purine group. The remaining N-Methyl derivatives of xanthine are *theophylline* and *theobromine* [14, 17].



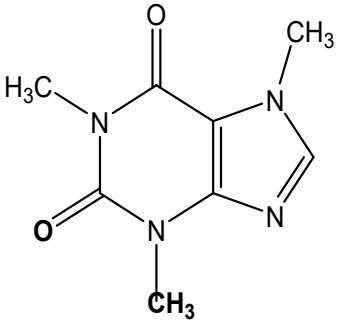
Xanthine



Theophylline



Theobromine



Caffeine

Figure-1- The structure of different xanthines

Caffeine was first isolated from coffee in 1820. In its pure state, it is a crystalline white powder and is moderately soluble in water and organic solvents, such as ethanol, ethyl acetate, methanol, benzene etc [18]. It has a slightly bitter taste in some concentrations, but it is virtually without taste in dilute solutions [19].

1.2.2. Sources and Prevalence's of Caffeine

Caffeine is probably the most frequently ingested pharmacologically active substance in the world [20]. It is obtained from foodstuffs (e.g. coffee, tea, chocolate), carbonated beverages and some medications [16, 21, 22, 23, 24]. Coffee bean, tea leaf, cola nut, guarana, cocoa bean, mate etc are the major natural sources of caffeine [16, 21, 22 25]. More than 60 plants in the world contain caffeine [22]. Coffee is the major sources of caffeine (60-75 %) in the adult diet in northern America and European countries such as Finland, Sweden, Denmark and Switzerland. Where as caffeinated soft drinks and chocolate are the major sources of caffeine in the diet of children [20].

Caffeine is found in coffee associated to chlorogenic acids [2]. It contributes to a particular proportion of the perceived bitterness of the cup of coffee and also makes small contribution to espresso's strength and body. It also plays an important role in determining the quality of coffee beverages [5].

Coffee has both the highest and the most variable caffeine content among dietary products containing this alkaloid [1]. The variability is due to variety of the coffee beans, methods of preparation (e.g. the brewing of coffee), volume of a cup, analytical methods utilized for the determination of caffeine and strength of the infusion [20, 23, 26]. Brewed coffee has the highest caffeine content (56-100mg/100 mL), followed by instant coffee and tea (20-73 mg/100 mL) and cola (9-19 mg/100 mL). Cocoa and chocolate products are also important sources of caffeine (e.g. 5-20 mg/100g in chocolate candy). Although the level of caffeine in chocolate is less variable, it depends on the origin of the beans [20].

Carbonated beverages are the major sources of caffeine for all ages of human being [20]. Caffeine is added to soft drinks as part of a flavor profile as its bitterness taste enhances other flavors. The variability of caffeine content in carbonated beverages occurs among the brands, since most of the caffeine content of these products is added from other natural sources [23, 27]. The caffeine content in these products is about 0.01 %; a 200 mL serving contains about 20 mg caffeine [26].

Caffeine is also the major constituent of many prescription and non-prescription drug preparations such as cold remedies, diet pills, painkillers, anti-migraine pharmaceuticals, diuretics and other stimulants [21, 24]. The caffeine content is (30-100 mg/tablet or capsule) in prescriptions and (15-200 mg/tablet or capsule) in non-prescriptions [20].

1.2.3. Metabolism of Caffeine in Human Body

Caffeine enters into human body through different sources such as foodstuffs, beverages and medications, which are mentioned on previous sections. Following to ingestion, caffeine is rapidly, essentially and completely absorbed from the gastrointestinal tract into the blood stream. Maximum caffeine concentration in blood reaches within 1-1.5 hrs following ingestion [20, 28]. The absorbed caffeine is readily distributed through out the entire body. It passes across the blood brain barrier, through the placenta into amniotic fluid and the fetus, and into the breast milk. Caffeine is also detected in semen [20].

Liver is the primary site of caffeine metabolism [20, 24, 28]. Caffeine is metabolized in the liver through a series of N-demethylation and purine ring oxidation reaction to yield a mixture of mono and dimethylxanthines and methyl uric acids [20, 24]. The main dimethylxanthine metabolites are theophyline (4%), theobromine (12%) and paraxanthine (1,7- dimethylxanthine) (84%) [20, 24, 29]. Paraxanthine is not found in foods but is the main metabolite of caffeine. The mechanism of metabolism of caffeine differs from species to species [29].

The half-life of caffeine, the time required for the body to eliminate one-half of the total amount of caffeine consumed at a given time, varies widely among individuals according to factors, such as repose, age, sex, liver function, pregnancy, some concurrent

medications, smoking, and the level of enzymes in the liver needed for caffeine metabolism. Caffeine has a half life, which is faster than theobromine. It is completely metabolized by human body; only 1-5 % of ingested caffeine is recovered unchanged in the urine. Infants up to the age of 8-9 months have a greatly reduced ability to metabolized caffeine, excreting about 85 % of the administered caffeine in the urine unchanged. The half life in the newborn ranges from 50-100 hrs, but it gradually approaches that of an adult by 6 months of age. Caffeine's half life is about 20-30 % shorter in females than in males. The half life in females using oral contraceptive steroids is approximately twice that observed for ovulatory females [20]. Pregnancy slows down the metabolism of caffeine. The metabolic half life increases steadily from 4 hr during the first trimester to 18 hr during the third trimester. Obesity also slows down caffeine metabolism. Smoking is another factor, which affects the metabolism of caffeine. It accelerates the rate at which caffeine is eliminated [20, 28].

1.2.4. Advantages and Disadvantages of Caffeine

Caffeine is a drug that has been widely used for centuries. Its main effect is a mild stimulant of the central nervous system (CNS), helping to reduce feelings of drowsiness and fatigue. However, regular use may lead to "habituation"; that is no net benefit from use but, rather, a negative effect if the drug is not taken.

Besides the above mentioned CNS stimulant effect, caffeine also temporarily increases heart beat, blood pressure, gastric acid secretion and stimulates the action of lung; increases basal metabolic rate (BMR), promotes urine production and relaxes smooth muscles, notably the bronchial muscles. It is extensively used in medicines most commonly in head ache medications and treating migraine, either alone or in combination. It enhances the action of the ergot alkaloids used for the treatment of this problem, and increases the potency of analgesics, such as aspirin. It can some what relief asthma attacks by dilating the bronchial airways. Caffeine also decreases type two diabetes mellitus.

Since 1970s, xanthine derivatives, including caffeine have been reported to inhibit DNA repair and to enhance the anti tumor effect of cisplatin and be a risk factors of kidney

malfunction [14]. Caffeine was considered to be risk factors of cardiovascular diseases but recent studies showed that this effect is not due to caffeine but is the result of two compounds found on coffee, cafestol and kahweol [1].

The effect of caffeine varies from person to person: some individuals can drink several cups of coffee in an hour and notice no effect, while others may feel a strong effect after just one serving.

1.2.5. Methods of Analysis

Since caffeine is found in different frequently ingested beverages, foods and medications and plays important roles in some physiological activities, determination of its content is vital. Different separation and detection methods are applied for the determination of caffeine in various mixtures. Chromatographic methods specially, HPLC, are a common technique which is used for the determination of caffeine in various sources of caffeine [2, 23, 30]. Their detection limits are better. Paraskevas D. and et.al used a high-throughput high-performance liquid chromatographic assay for the determination of caffeine in food samples using a monolithic column and found a linear range of 0–200 mg L^{-1} and detection limit of 0.10 mg L^{-1} [30]. Too long extraction and analysis time and very large solvent consumption makes them difficult [31].

Several other analytical methods have been used for the determination of caffeine, such as titrimetry, Spectrophotometry, polarography, GC, biosensing methods [18, 32]. Capillary electrophoresis [2, 33, 34], thin layer chromatography and gas chromatography [15], combined with detection methods such as mass spectroscopy [35], FTIR spectrophotometry and others have also been used. Abebe Belay and et.al used a UV/Visible spectrometer for the determination of caffeine in coffee [6].

Electrochemical method is preferred due to several advantages, such as excellent sensitivity with a very large useful linear range for both inorganic and organic species, a large number of useful solvents and electrolytes, wide range of temperature, rapid analysis time, simultaneous determination of several analytes, the ability to determine kinetics and mechanistic parameters, a well developed theory and the ability to

reasonably estimate the value of unknown parameters and the case with which different potential unknown form can be generated and small currents measured. Low cost of the method is another benefit.

Several authors have used electrochemical methods of determination of caffeine. Barbara Brunetti and her colleagues used a nafion covered glassy carbon electrode for voltammetric determination of caffeine [36]. Jhy-Myng Zen and et.al used a nafion ruthenium oxide pyrochlore chemically modified electrode for the determination of caffeine in beverages [31]. They obtained a linear range of 5-200 μM with detection limit (3σ) of $2\mu M$. Anodic voltammetry of caffeine at conductive boron doped diamond electrode was used as a detection method by Nicolae Spãtaru [14]. Muluken, in his graduation thesis, used a 1,4-Benzoquinone modified carbon paste electrode for the determination of caffeine in coffee. He used both cyclic and square wave voltammetry and found a detection limit of 0.33 μM [37].

Adavntages, such as very low background current, individual polarizability, specific reaction kinetics, electrode activity at the carbon paste surface as well as in the carbon paste bulk, variability in utilizing various mechanisms and their synergic effect at both CPEs and CMCPEs, various alternative procedures for pre treating, conditioning and regenerating the electrode surface and carbon paste itself and low cost makes carbon paste electrode to be used as working electrode.

1.2.6. Electrochemical Oxidation of Caffeine

The electrochemical oxidation of caffeine at graphite electrode is a four electron process overall [38, 39]. An overall oxidation mechanism for caffeine can be represented by an initial potential controlling two electron oxidation of the $–N_9= C_8-$ double bond to give the appropriate methylated uric acid (II, in figure 2). A further two electron oxidation of the $–C_4=C_5-$ double bond occurs with the formation of such uric acid -4,5-diol (III, in figure 2) since the uric acid is more readily oxidized. These preliminary electrochemical products very rapidly decompose as the result of steric factors to yield a methylated alloxan (VI, in fig) and a methylated allantoin (VII, in figure 2) and carbon dioxide: and

so these intermediates can't be detected by cyclic voltammetry. A very small quantity of the uric acid-4,5-diol is further oxidized to the appropriate parabanic acid (IV, in figure 2), which is derived from the pyrimidine ring moiety of the initial caffeine and is the house of the appropriate amount of methyl urea and CO_2.

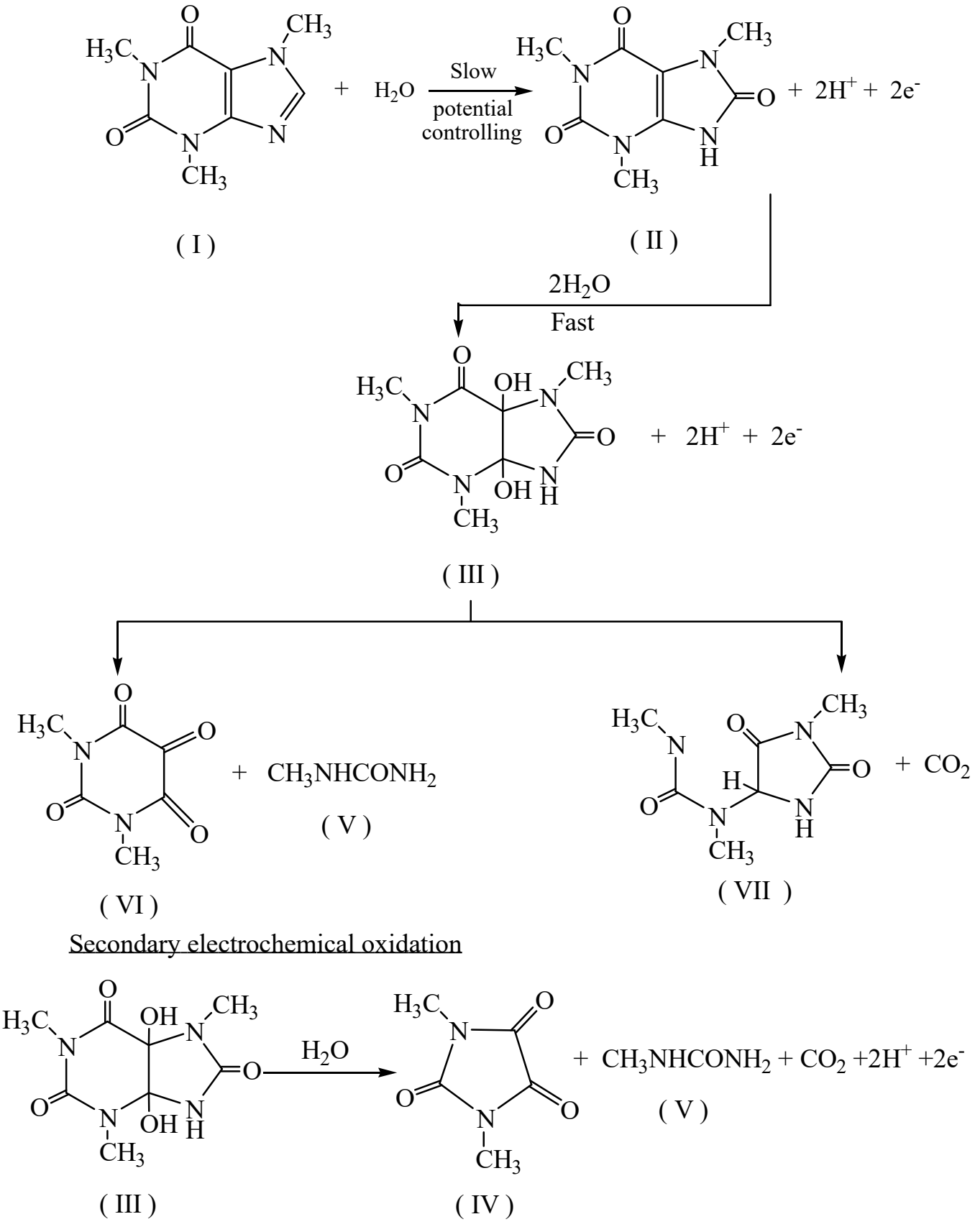


Figure 2**.** Electrochemical redox pathway of caffeine

1.3. Carbon Paste Electrodes

1.3.1. Introduction

New electrode materials with favorable analytical and electrochemical performances are persuaded all along by research workers in order to meet the needs for modern electrochemical sensing and biosensing devices. Carbon, due to its diverse merits, such as low background current, broad potential window, inertness, low cost, high activity and conductivity has been the most popular electrode material in the field of electrochemistry. Various Forms of carbon such as *graphite*, glassy carbon, amorphous carbon powders, ordered mesoporous carbon (OMC), carbon fiber, carbon nanotubes and boron doped diamond are extensively employed as indicators in the fundamentals and application of electrochemistry [40, 41, 42].

Historically, the carbon paste electrode was prepared to produce dropping carbon electrode [43]. The Adams group introduced the first carbon paste electrode around 1959-63. They postulated first basic characteristics of CPEs and some rules for their usage. Around 1964-65. C. they introduced also the first modified CPE which was prepared by rubbing the modifier into the paste. The replacement of common non-electro active pasting liquids by electrolyte solutions opened the avenue for a specific branch of the electrochemistry of CP electro active electrodes in 1974 [44].

1.3.2. Preparation of Carbon Paste Electrode

Carbon paste electrode is an electrode which is prepared by mixing carbon powder with a liquid, which is used as a binder. At a moment these electrodes are known as *bare* or *("virgin")* or more often, as *unmodified carbon paste electrodes*. The proper electro active moiety in carbon paste is the graphite powder with micrometric particles of high purity. The liquid is used to bind all the particles of the graphite and it must fulfill the criteria's, such as *chemically inertness*, *insulating*, *non-volatile, water immiscible,* and *forming a paste mixture of fine consistency.* Paraffin oils, such as nujol and uvasol are more extensively used as the binder. These materials have some less favorable

characteristics, like their vulnerability in medias with organic solvents. Silicon oil based and an organophosphate binder also becomes well known. High ion pairing ability is an attractive feature of organophosphate binders.

1.3.3. Modified Carbon Paste Electrodes

A modified carbon paste electrode is composed of the graphite powder, binder and modifier. The modifier is usually one substance but there are cases where the modifiers can be two or more components. The main reason for the modification of CPEs is to obtain qualitatively new sensor with desired, often predefine properties. Carbon has a high surface activity, which explains its susceptibility to poisoning by organic compounds. Bonds with hydrogen, hydro and carboxyl groups, and sometimes quinones, can be formed at the carbon surface. The presence of these groups signifies the behavior of the CPEs. Due to these, the electrodes can be pH sensitive.

The preparation of CMCPEs is very simple. The modifier can be dissolved directly in the binder or admixed mechanically to the paste during its homogenization. It is also possible to soak graphite particle with a solution of a modifier. Already made up CPEs can be modified *in situ.*

According to Kalcher [45], the purpose of the modifier can be classified to four

- Preferential entrapment of desired species (e.g. preconcentration in stripping analysis)
- Mediation of electrode reactions via immobilized molecules or their fragments
- Acting in catalytic phenomena (catalytic electrochemical responses)
- Alteration of the surface characteristics of a CPE

Various chemical reagents have been used as a modifier. Single compounds, sophisticated chemical agents, special inorganic materials and matrices, or living organisms are widely used type of modifiers. Classic analytical reagents like dimethylglyoxime, 8-hydroxyquinoline, derivatives of 2-nephtol have been used as selective modifiers for adsorptive accumulation of selected ions. An ion exchanger type

modifiers like cetyltrimethylammoniumbromide (CTAB) used as a reliable modifier to preconcentrate and detect some less common metal species. Clay materials such as zeolites are well known both naturally occurring and artificially prepared groups of hydrated crystalline alluminosilicates that are capable of acting as ion exchangers and ion traps according to the molecular size. Substrate from living organisms have been used as a modifier since CPE is a convenient material to be modified with natural biomass.

Construction of CPEs depends on the type of the electrode. The holder of the paste can be made from glasses, PVC tubes, or Teflon rods. For common CPEs, The actual diameter of the end hole forming the proper carbon paste surface is being chosen from 2 to 10 mm, which is convenient for most electrochemical measurements. The preparation and packing of the paste into the holder allows easy and quick surface renewal of CPEs, which gives a good surface reproducibility. The traditional manual procedure of homogenizing pastes by a mortar are preferred for a reason that the hand mixing of the paste gives the analyst to choose the individual components as well as their mutual ratio. The amount of the modifier in the paste usually varies between 10 to 30 % (w/w) depending on the character of the modifying agent and its capability of forming enough active sites in the modified paste.

The benefits of the carbon paste electrodes are their very low background current, individual polarizability (with variable potential window), specific reaction kinetics, electrode activity at the carbon paste surface as well as in the carbon paste bulk, variability in utilizing various mechanisms and their synergic effect at both CPEs and CMCPEs, and various alternative procedures for pre treating, conditioning and regenerating the electrode surface and carbon paste itself.

Carbon paste and their modifications have several and wide applications. Their applications in inorganic analysis, such as in the determination of Au(III), Ag(I), Hg(I) and Au(II), Cu(I) and Cu(II) with very good detection limits, has been reported. Application of UMCPES and CMCPEs has shown a very good result in the determination of organic materials. They are applied in the determination of glucose, ascorbic acid, caffeine, uric acid, proteins, DNA and RNA, hydroquinone and etc.

2. Objective of the Study

2.1 General Objectives

- To develop cheap, more sensitive and validated voltammetric determination of caffeine

2.2 Specific Objectives

- To develop a suitable voltammetric method for the determination of caffeine with anthraquinone modified carbon paste electrode
- To analyze the optimum experimental parameters such as pH, Square wave frequency, Square wave amplitude, square wave step potential, and effect of modifier percentage in the CPE

3. Theoretical Background

3.1. Voltammetry

Historically, a branch of electrochemistry we now call voltammetry was developed from the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky, for which he received the 1959 Nobel Prize in chemistry. Voltammetry is an analytical technique which works based on the measurement of the current flowing through the electrochemical cell containing electro active substances, while the potential is applied to an electrode. In voltammetry a time-dependent potential is applied to an electrochemical cell, and the current flowing through the cell is measured as a function of that potential. A plot of current as a function of applied potential is called a voltammogram. They are considered to be active techniques because the applied potential forces a change in the concentration of an electro active species at the electrode surface by electrochemically reducing or oxidizing it [45, 46].

In voltammetric measurements, a time-dependent potential excitation signal is applied to the working electrode, changing its potential relative to the fixed potential of the reference electrode. The resulting current between the working and auxiliary electrodes is measured. In many cases, the applied potential is varied and the current is monitored over a period of time (t). Therefore all voltammetric techniques can be described as a function of *E, i* and *t.* Since the reaction of interest occurs at the working electrode, the classification of current is based on this reaction. A current due to the analyte's reduction is called a cathodic current and, by convention, is considered positive. Anodic currents are due to oxidation reactions and carry a negative value [47].

The voltammetric techniques are preferred for their benefits such as excellent sensitivity with a very large useful linear range for both inorganic and organic species (10^{-12} to 10^{-1}), a large number of useful solvents and electrolytes, wide range of temperature, rapid analysis time (in seconds), simultaneous determination of several analytes, the ability to determine kinetics and mechanistic parameters, a well developed theory and the ability to reasonably estimate the value of unknown parameters and the case with which different potential unknown form can be generated and small currents measured [49, 50].

These techniques have wide applications in several fields of science. Analytical chemists routinely use them for the quantitative determination of a variety of dissolved inorganic and organic substances. Inorganic, physical and biological chemists widely use them for a variety of purposes such as fundamental studies of oxidation and reduction processes in various media, adsorption processes or surfaces, electron transfer processes, and transport, speciation, and thermodynamic properties of solvated species. They are also applicable in the determination of compounds of pharmaceutical interest. Coupling with some separative techniques, such as chromatography, they are effective tools for the analysis of complex mixtures.

The electrochemical cell, where the voltammetric experiment is carried out, consists a working electrode (indicator or sensor), a reference electrode and usually a counter electrode (auxiliary electrode). The working electrode provides the interface across which a charge can be transferred or its effect felt. The reduction or oxidation of a substance at the surface of a working electrode, at appropriate applied potential, results in the mass transport of a new material to the electrode surface and generation of current [48, 49]..

3.2. Cyclic Voltammetry

Cyclic voltammetry is a particular linear sweep voltammetry, which is a technique where the monitoring of a current is applied while a potential scanning is varying linearly. It is based on varying the applied potential at a working electrode in both forward and reverse directions (at some scan rate) while monitoring the current. In this way a redox couple in solution is exposed before to an oxidation and afterwards to a reduction (or vise versa). And finally a potential versus time graph of the scanning will be recorded and this graph is known as cyclic voltammogram. The plot of a cyclic voltammetry consist on a close curve: reversible redox couples show both as cathodic and anodic peak, while irreversible redox systems show only one peak [49,51]. Cyclic voltammetry is carried out in unstirred quiescent solution to insure that the redox system is diffusion control.

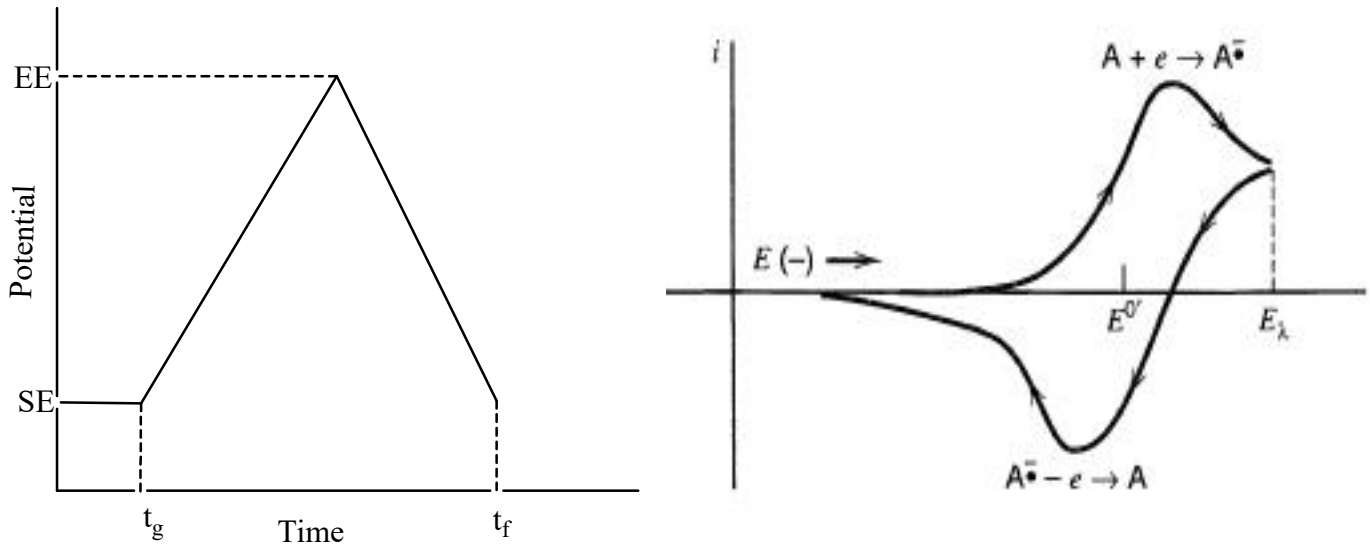


Figure-3- Potential Vs time graph and potential versus current graph of cyclic voltammogram [49].

Cyclic voltammetry is often the first experiment performed in an electro analytical study. It offers a rapid location of redox potentials of the electro active species, and convenient evaluation of the effect of media upon the redox process. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on thermodynamics of redox processes, on the kinetics of heterogeneous electron transfer reactions, and on coupled chemical reactions or adsorption processes [48].

The important parameters in cyclic voltammogram are the peak potentials (E_{pc}, E_{pa}) and peak currents (i_{pc}, i_{pa}) of the cathodic and anodic peaks, respectively. The separation of the peaks for reversible electron transfer is expressed by

$$\Delta E_p = \left| E_{p,a} - E_{p,c} \right| = \frac{2.303RT}{nF} \quad \text{..... 1}$$

For reversible redox reaction at temperature of 25 ^oC ΔE_p is

$$\Delta E_p = \frac{0.0592}{2} \quad \text{.....2}$$

The position of the peaks on the potential axis (E_p) is related to the formal potential of the redox process. The formal potential for a reversible couple is centered between $E_{p,a}$, and $E_{p,c}$:

$$E^o = \frac{E_{p,a} + E_{p,c}}{2} \quad \text{.....3}$$

Thus, the peak separation can be used to determine the number of electrons transferred, and as a criterion for a Nernstian behavior. The concentration of an electro active species in the cell in reversible reaction is related to the current by the Randles-Sevcik equation:

$$i_p = 2.686 \times 10^5 n^{\frac{3}{2}} A C^o D^{\frac{1}{2}} \nu^{\frac{1}{2}} \quad \text{.....4}$$

Where, i_p is then peak current in amperes, A is the electrode area in cm^2, D is the diffusion coefficient in cm^2s^{-1}, C^o is the concentration in mol cm^{-3}, and ν is the scan rate in V s^{-1}.

For irreversible processes, the individual peaks are reduced in size and widely separated. Totally irreversible systems are characterized by a shift of the peak potential with the scan rate:

$$E_p = E^o - \frac{RT}{\alpha n_a F}\left[0.78 - \ln\frac{k^o}{D^{\frac{1}{2}}} + \ln\left(\frac{\alpha n_a F \nu}{RT}\right)^{\frac{1}{2}}\right] \quad \text{.....5}$$

Where α is the transfer coefficient and n_a is the number of electrons involved in the charge transfer step. Thus, E_p occurs at potentials higher than E^o, with the over potential related to k^o and α. Independent of the value k^o, such peak displacement can be compensated by an appropriate change of the scan rate. The peak potential and the half peak potential (at 25°C) will differ by $\frac{48}{\alpha n m V}$. Hence the voltammogram becomes more drawn out as αn decreases.

The peak current of the irreversible system is given by

$$i_p = \left(2.99 \times 10^5\right) n(\alpha n_a)^{\frac{1}{2}} A C D^{\frac{1}{2}} \nu^{\frac{1}{2}} \quad \text{..... 6}$$

Even though cyclic voltammetry has some merits its poor sensitivity makes it less applicable in quantitative analysis.

3.3. Square Wave Voltammetry

Square wave voltammetry is one of the pulse techniques. It is amplitude differential technique in which a wave form composed of a symmetrical square wave, superimposed on a base staircase potential, is applied to a working electrode. In SWV technique, current is sampled twice per cycle at the end of each pulse. The forward current sample, i_f, arises from the first pulse per cycle, which is in the direction of the staircase scan. The reverse current sample, i_r, is taken at the end of the second pulse, which on the opposite direction. The difference between the two measurements is plotted versus the base staircase potential.

The net peak current for the irreversible system is given by [59];

$$I_p = \text{constant } \alpha n^2 \Delta E\, E_{sw}\, (fD)^{1/2}\, C \quad \text{.....7}$$

Also the net peak current for the reversible system is given by [27];

$$I_p = \text{constant } n^2 \Delta E\, E_{sw}\, (fD)^{1/2}\, C \quad \text{.....8}$$

Consequently, the result of a single SWV run is three voltammograms, showing the forward, reverse and difference currents versus the potential on the corresponding staircase tread. The resulting peak shaped voltammogram is symmetrical about the half wave potential, and the peak current is proportional to the concentration.

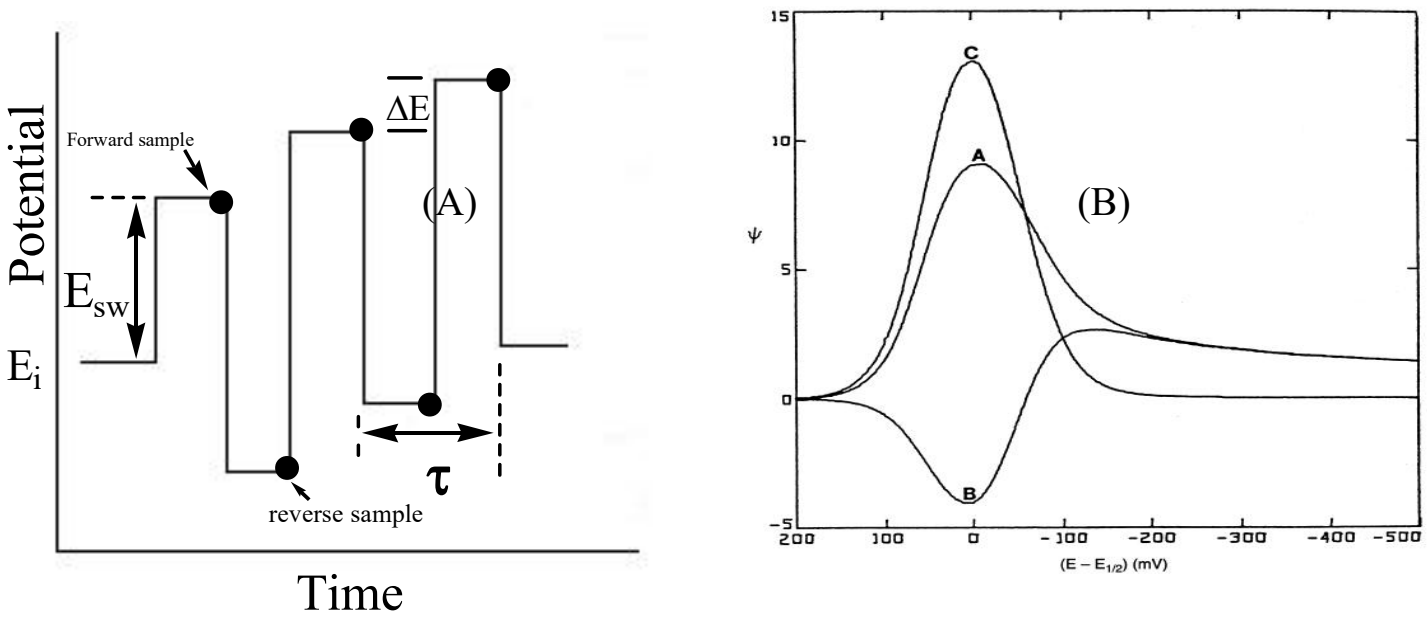


Figure -4- Potential-excitation signals (A) and voltammograms (B) for square voltammetry. Current is sampled at the time intervals indicated by the solid circles (●) [48].

The square wave voltammetry is characterized by a pulse height, E_{sw}, measured with respect to the corresponding tread of the staircase, and a pulse width τ. Alternatively, the pulse width can be expressed in terms of the square wave frequency,

$$f = 1/2\tau \ldots\ldots\ldots\ldots\ldots\ldots 9$$

The staircase shifts by ΔE at the beginning of each cycle; thus the scan rate

$$\nu = \Delta E/2\tau = f\Delta E \ldots\ldots\ldots\ldots 10$$

Frequencies of 1 to 100 cycles per second permit the use of extremely fast potential scan rates. The scan begins at an initial potential, E_i, which can be applied for an arbitrary time to initialize the system as desired.

Square wave voltammetry has several advantages. Among these are its excellent sensitivity and the rejection of background current. Excellent sensitivity is accrues from the fact that the net current is larger than either the forward or reverse components (since it is the difference between them). The sensitivity of this technique can be increased by enhancing the amplitude of the square wave or the frequency. The limit of the enhancing

is strictly related to the kinetic aspect of the redox system. The interference due to capacitive current are lowered to minimum because the current is sampled just at the end of the half waves, when the current of the double electrical layer is the least. Coupled with the effective discrimination against the charging background current, very low detection limits can be attained. Another advantage of SWV is its speed. A complete voltammogram can be recorded with in seconds. This speed coupled with computer control and signals averaging, allows experiments to be performed repetitively and increases the signal-to-noise ratio [46, 47, 48, 49, 50, 51].

4. Experimental Part

4.1. Reagents and Chemicals

The chemicals and reagents used were graphite powder and paraffin oils (USP, d = 0.845g/ml). (Fluka, Switzerland), Caffeine (Evans, UK), disodium hydrogen phosphate (Wagtech International Ltd, UK), sodium dihydrogen phosphate (Riedel-de Haen AG), HCl (Riedel-de Haen AG) and NaOH (BDH Poole, England) and anthraquinone (BDH Poole, England).

4.2. Apparatus

The electrochemical measurements were performed by using CV-50W voltammetric analyzer (electroanalytical systems, USA) with BAS 50 W electrochemical analyzer software, using a standard cell with three electrodes. The three electrode system consists of a carbon paste electrode (UNCPE or CMCPE), a $Ag/AgCl/K^+, Cl^-//$ reference electrode and a platinum counter electrode. All measurements were carried out at the laboratory temperature.

4.3 Preparation of Unmodified and Modified Carbon Paste Electrode

4.3.1 Preparation of Unmodified Carbon Paste Electrode

Unmodified carbon paste electrode was made of 70 % (w/w) graphite powder and 30 % (w/w) paraffin oil. 100 mg of graphite powder was weighed and mixed with 51 µL of paraffin oil. The mixture was homogenized by using a piston and mortar for 20 minutes. The paste was then housed in a tip of an insulin syringe with diameter of 2 mm that was purchased from local pharmacies by pushing the syringe on the paste. A copper wire was used as an electrical conductor between the paste and the potentiostat. Finally the CPE was kept in a refrigerator for 24 hrs. The electrode surface was polished before each determination with a white paper prior to measurement. The already prepared CPE was used for repeating measurements by extruding some paste from the syringe and polishing the surface.

4.3.2. Preparation of Modified Carbon Paste Electrodes

Modified carbon paste electrodes were prepared by mixing the modifier with graphite powder and paraffin oil. The required amounts of anthraquinone was weighed and added to the weighed graphite and mixed by mortar for five minutes. A certain amount of paraffin oil was added to the mixture and homogenized by a piston for 20 min. The final mixture was packed at the tip of the syringe and kept in the refrigerator for 24 hrs. The ratio of the graphite and paraffin oil was decreases by the same ratio while the ratio of the modifier increases. The electrode was applied to the measurements by polishing the surface prior to measurement.

4.4. Electrochemical Measurements

A 0.1 M of NaH_2PO_4/Na_2HPO_4 phosphate buffer was used as a supporting electrolyte. A pH of 6. 7 and 8 was prepared from a standard by taking a certain ratio of the two buffer constituents. pHs greater than 8 was prepared by adding 2 M sodium hydroxide (NaOH) on 0.1 M pH=8 buffer.

A 250 mL of 10 mM caffeine stock solution in 0.1 M of phosphate buffer of pH=8 was prepared. The required pHs was obtained by adjusting the pH using 2 M NaOH. The concentration of caffeine solution below 10 mM was prepared by diluting the calculated amount of the stock solution with the buffer in which the stock solution was prepared.

The cyclic voltammetry measurements were performed in the potential range of -2 V to 2V, at a scan rate of 100 mV/s. The square wave voltammograms were recorded by scanning the potential from 200 mV to 1800 mV versus $Ag/AgCl/k^+$, Cl^- reference electrode.

5. Result and Discussion

5.1. Cyclic Voltammetric Investigation

5.1.1. Cyclic Voltammetry of Caffeine

The cyclic voltammogram of a pH = 12 buffer and Caffeine was scanned at the unmodified carbon paste electrode to the positive and negative maximum potential in order to determine the free potential window is shown below in figure 5.

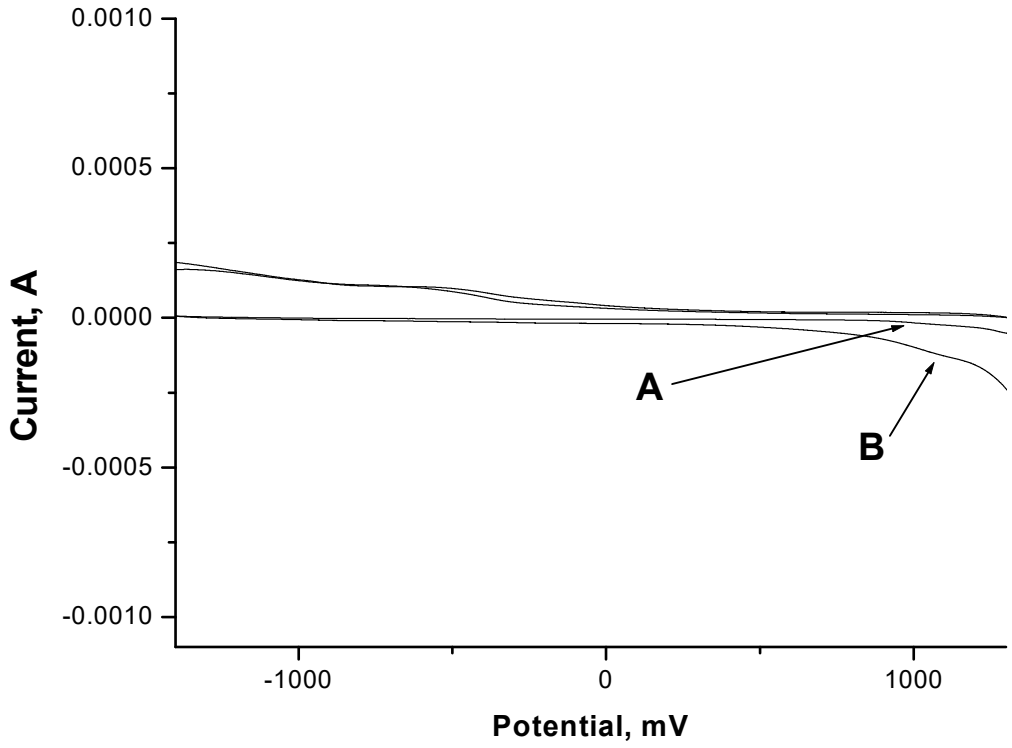


Figure -5 - Cyclic voltammogram of (A) 0 mM caffeine and (B) 2 mM caffeine at the unmodified carbon paste electrode at scan rate of 100 mV/s.

Based on the previous figure caffeine is an electro active substance in which it irreversibly oxidized at the surface of unmodified carbon paste electrode at a potential of 1.45 V. At the absence of caffeine such peak did not appear. So it is convenient to study caffeine redox behavior using NaH_2PO_4 and Na_2HPO_4 buffer system.

5.1.2. Cyclic Voltammetry of Anthraquinone

The cyclic voltammogram of a buffer of pH =12 at the potential range of -2 V to 2 V was recorded with unmodified and anthraquinone modified carbon paste electrode at scan rate of 100 mV/s. Two reversible peaks at the potentials of -0.59 V and – 1.17 V was observed for the anthraquinone modified carbon paste electrode. These peaks are due to the redox behavior of anthraquinone within the modified electrode. When the cyclic voltammogram of the buffer recorded at the same conditions such two reversible peaks doesn't appear, as shown in A in figure 6

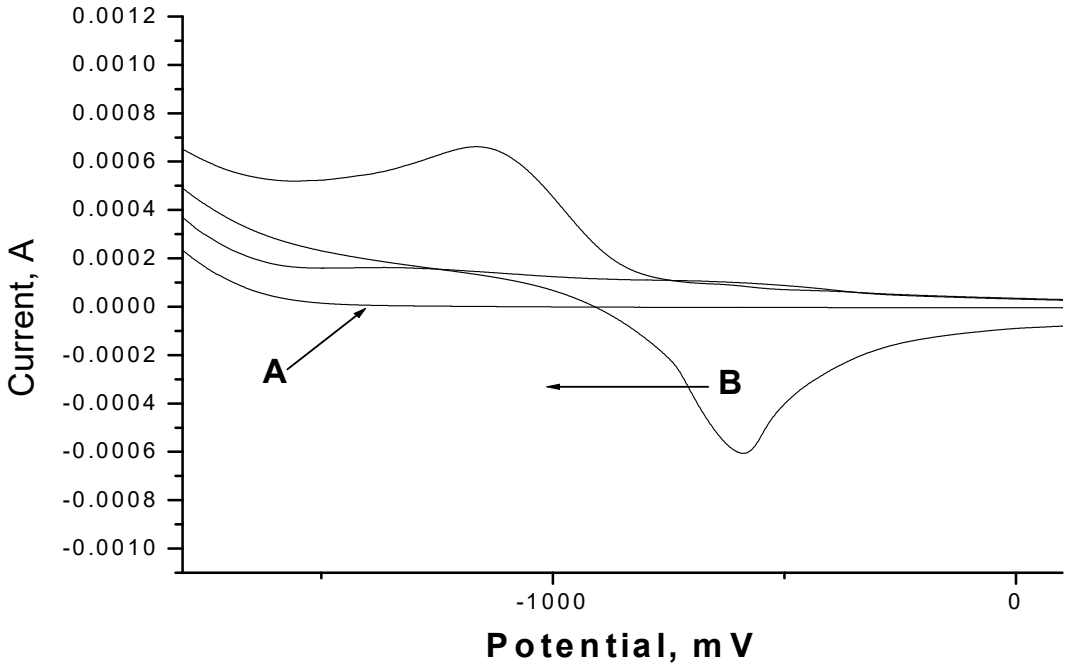


Figure -6- Cyclic voltammogram of a buffer of pH=12 using (A) UNMCPE and (B) Anthraquinone MCPE at scan rate of 100 mV/s

5.2. Square Wave Investigation

The square wave voltammogram for the redox behavior of anthraquinone in the absence of caffeine is given below in figure 7

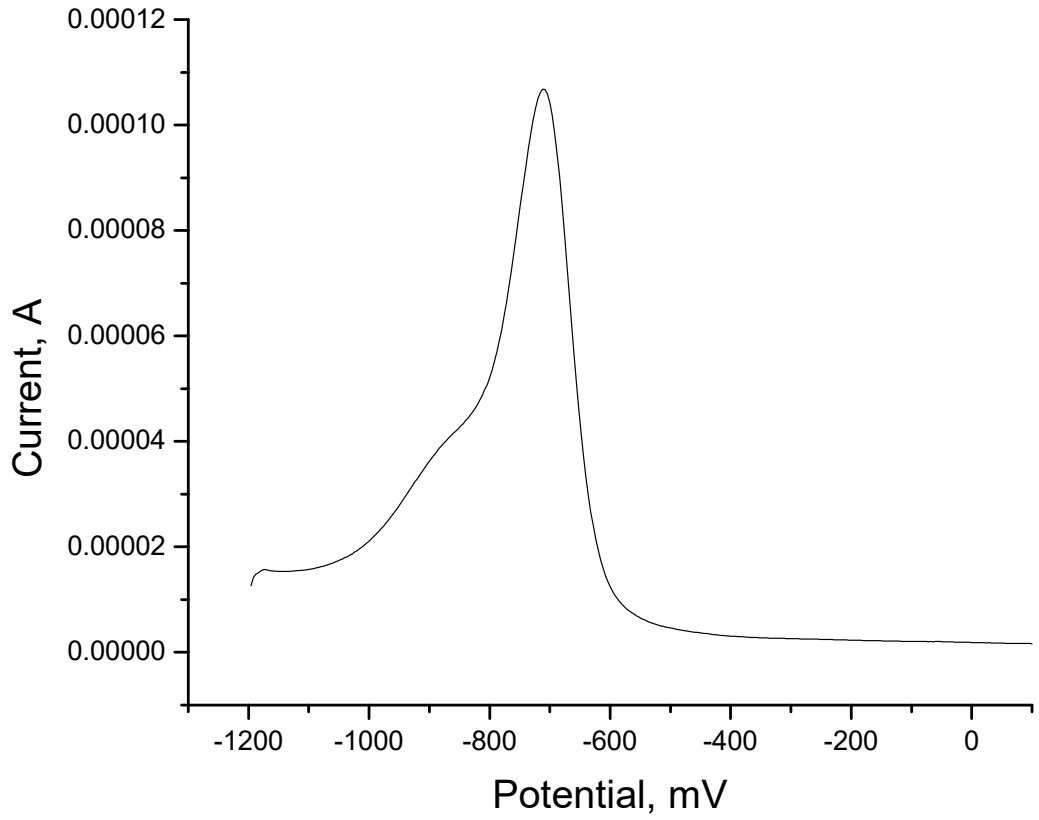


Figure -7- Square wave voltammogram of anthraquinone in the electrode

The effect of caffeine on the square wave voltammogram of anthraquinone was studied by preparing different caffeine concentrations in 0.1 M pH=12 buffer solution. In the presence of caffeine within the buffer solution, the square wave voltammogram of anthraquinone shows a distinct and broadened peak. As the concentration of caffeine in the buffer increases, an increase in the peak current of anthraquinone was observed. The increase in the peak currents of anthraquinone reveals that the presence of caffeine in the solution creates an electro active species.

The effect of caffeine on the peak potential redox behavior of anthraquinone was studied and found to be less variable. The peak potential varies randomly i.e. there is no an increase or a decrease in the peak potential. This may tell us that there is no catalytic effect within each other but the presence of caffeine enhances the peak current.

5.3. Optimization of Experimental Parameters

5.3.1. Electrode Composition Effect

The effect of the composition of anthraquinone within the carbon paste electrode is studied. The composition of the anthraquinone was varied from 5 % (w/w) to 20 % (w/w) in a total of 200 mg paste and is mentioned below

- 5 % anthraquinone, 67.5 % graphite powder and 27.5 % paraffin oil by weight
- 10 % anthraquinone, 65 % graphite powder and 25 % paraffin oil by weight
- 15 % anthraquinone, 62.5 % graphite powder and 22.5 % paraffin oil by weight
- 20 % anthraquinone, 60 % graphite powder and 20 % paraffin oil by weight

The result is shown in figure 8 below.

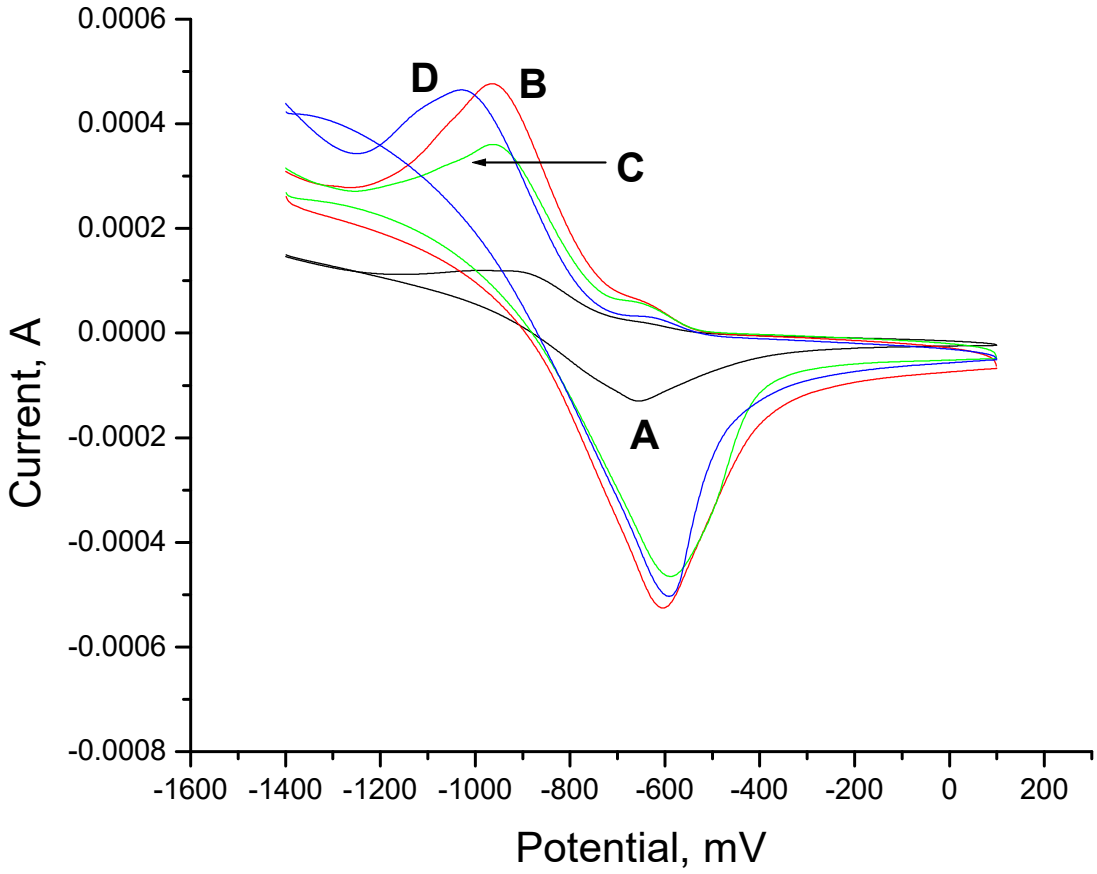


Figure -8- Cyclic voltammogram of a buffer pH = 8 using variable composition of anthraquinone: A) 5 % B) 10 % C) 15 % D)20 %.

The linear graph is shown below in figure 9.

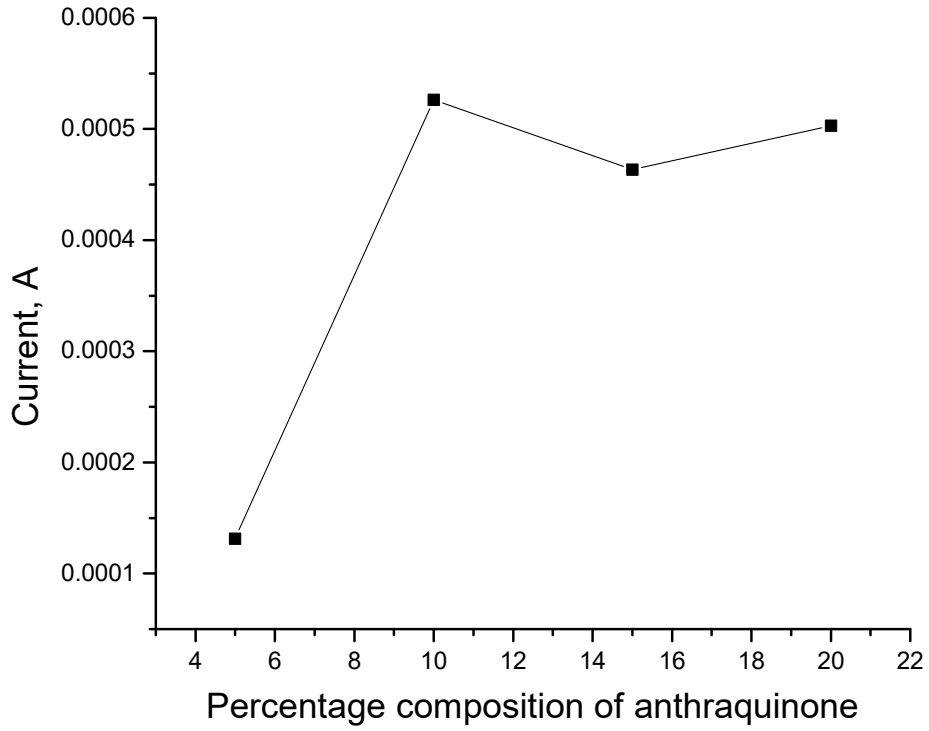


Figure -9- The linear graph of anodic current versus percentage composition of anthraquinone

As it can be seen from the above figure, then highest current was observed for the electrode composition of 10 % anthraquinone, 65 % graphite powder and 25 % paraffin oil. The smallest current was observed for 5 % anthraquinone, which may be due to the amount of the modifier is too low. For further experiments, the 10 % composition electrode was selected for further experiment.

5.3.2. Effect of pH of Supporting Electrolyte

The effect of pH on the cyclic voltammetric response of anthraquinone was studied in from pH 6 up to pH 12, which is shown in the figure below. This range was selected because of the besicity of caffeine. In acidic medium caffeine response is very low.

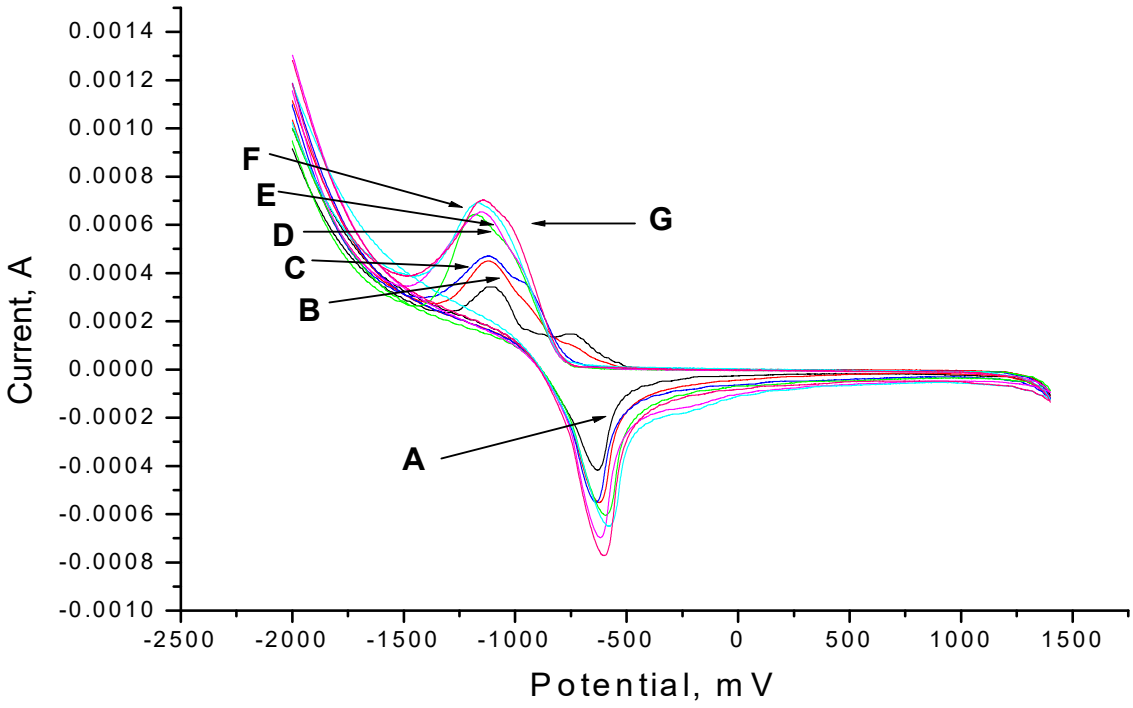


Figure -10- Cyclic voltammogram of anthraquinone at different pH value: A) 6 B) 7 C) 8 D) 9 E) 10 F) 11 G) 12

The effect of pH on square wave voltammogram of anthraquinone was studied within a range of 8 to 12, which is shown below in figure 11.

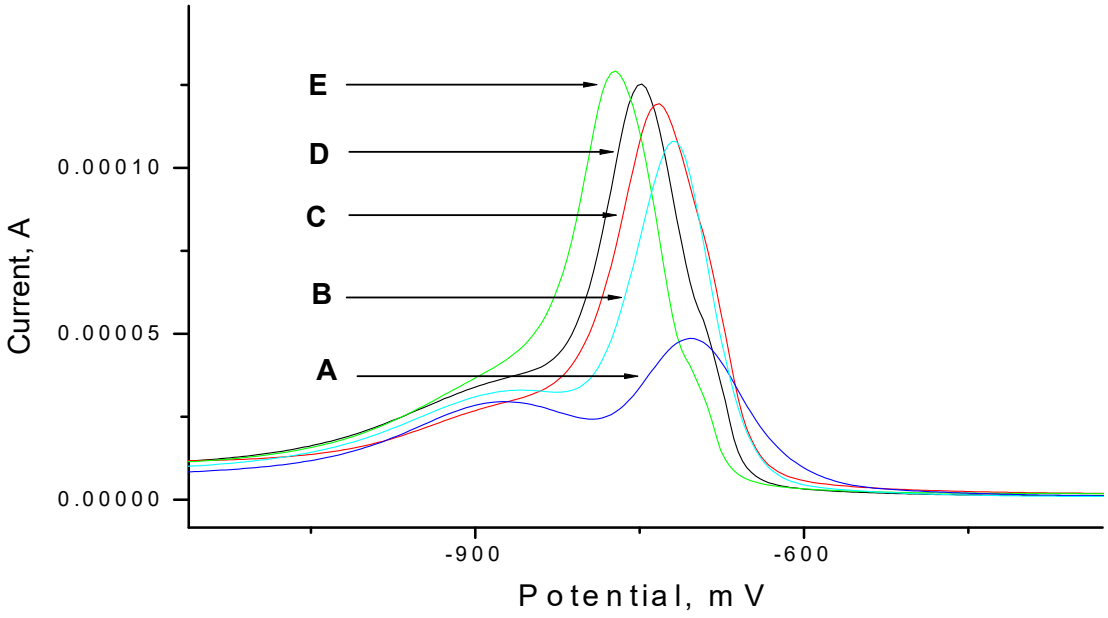


Figure -11- Square wave voltammogram of anthraquinone at different pH values: A) 8 B) 9 C)10 D) 11 E) 12

The plot of the peak current with pH value is shown below in figure 12

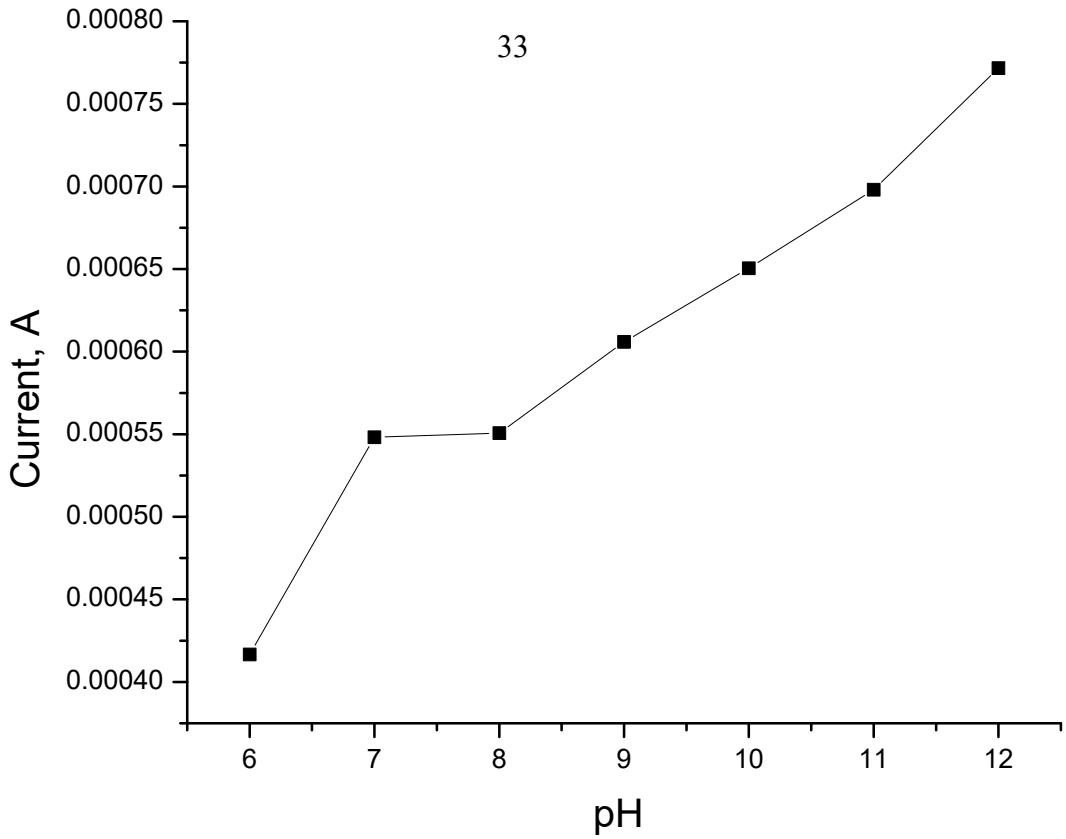


Figure -12- The dependence of peak current of anthraquinone with pH

As it can be seen from the plot of current with pH, the peak current of anthraquinone increases with the increasing of pH. It increases sharply from pH 9 to 12. After a cyclic voltammogram with 15 cycles was recorded for each pHs from6 to 12, the peak currents of pH =12 gives a stable current with small variations. The stability of the current at the pH value of 12 gives advantages in the square wave study of real sample because in alkaline medium the other components of coffee (acids) are suppressed due to neutralization. Therefore sample pretreatment system can be minimized and the square wave voltammogram of coffee sample can be recorded without further extraction of the sample.

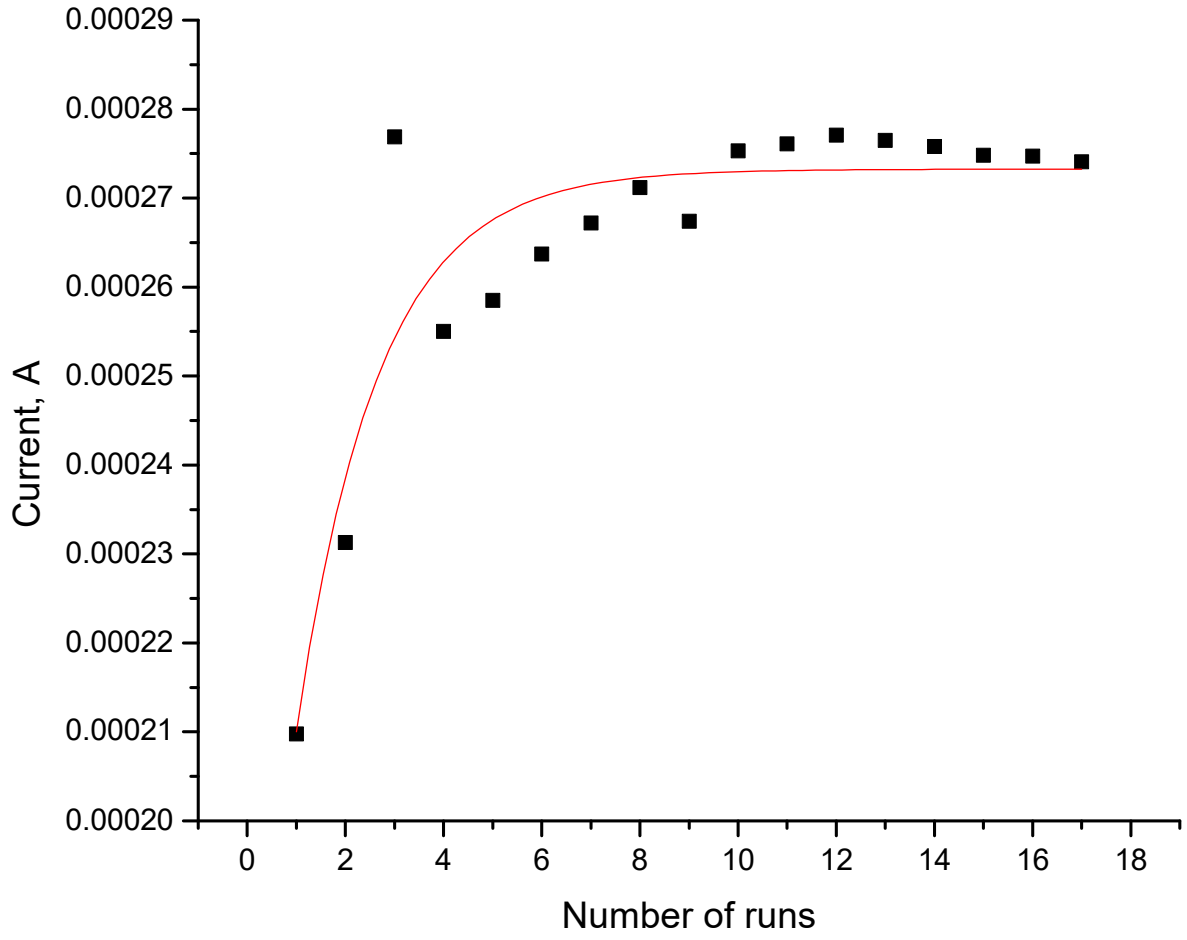


Figure -13- The stability of the peak current of anthraquinone at pH =12

The influence of pH on the peak potential of anthraquinone is shown in figure 14. As the pH increases the potential of anthraquinone shifted toward more negative values.

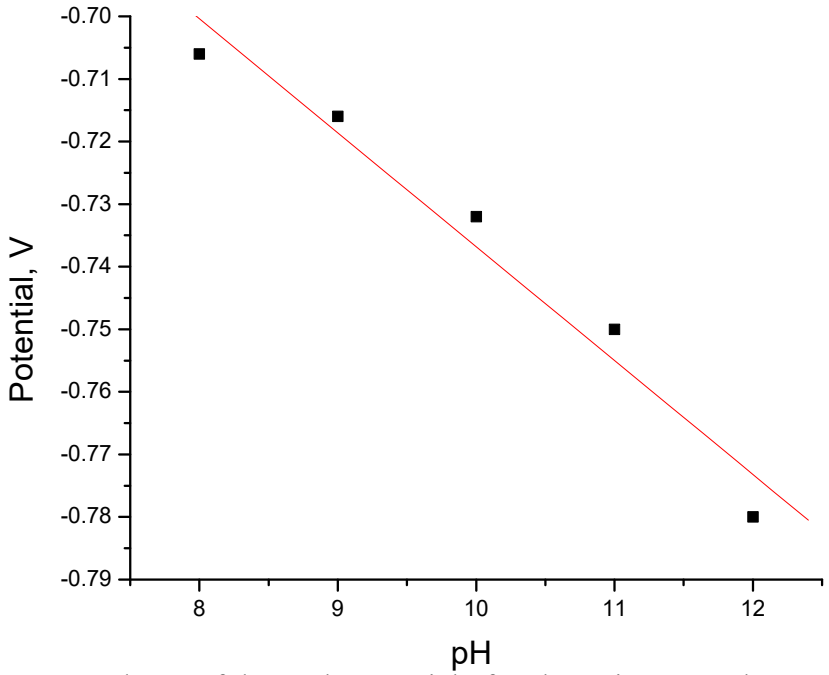


Figure -14- Dependence of the peak potential of anthraquinone on the pH of the buffer

5.3.3. Effect of the Square Wave Frequency

The effect of square wave frequency on the peak current of anthraquinone was studied by varying the frequency from 15 Hz to 50 Hz at a step potential of 4 mV and amplitude of 25 mV in the absence of caffeine. However at very high frequency, the peak current is unstable and masked by large residual current. On the other hand very low frequency gives a low but narrow signal in the total analyte time. Hence, the selection of the frequency usually requires a compromise among sensitivity, resolution, and speed. The square wave frequency of 30 Hz was chosen for the subsequent experiments.

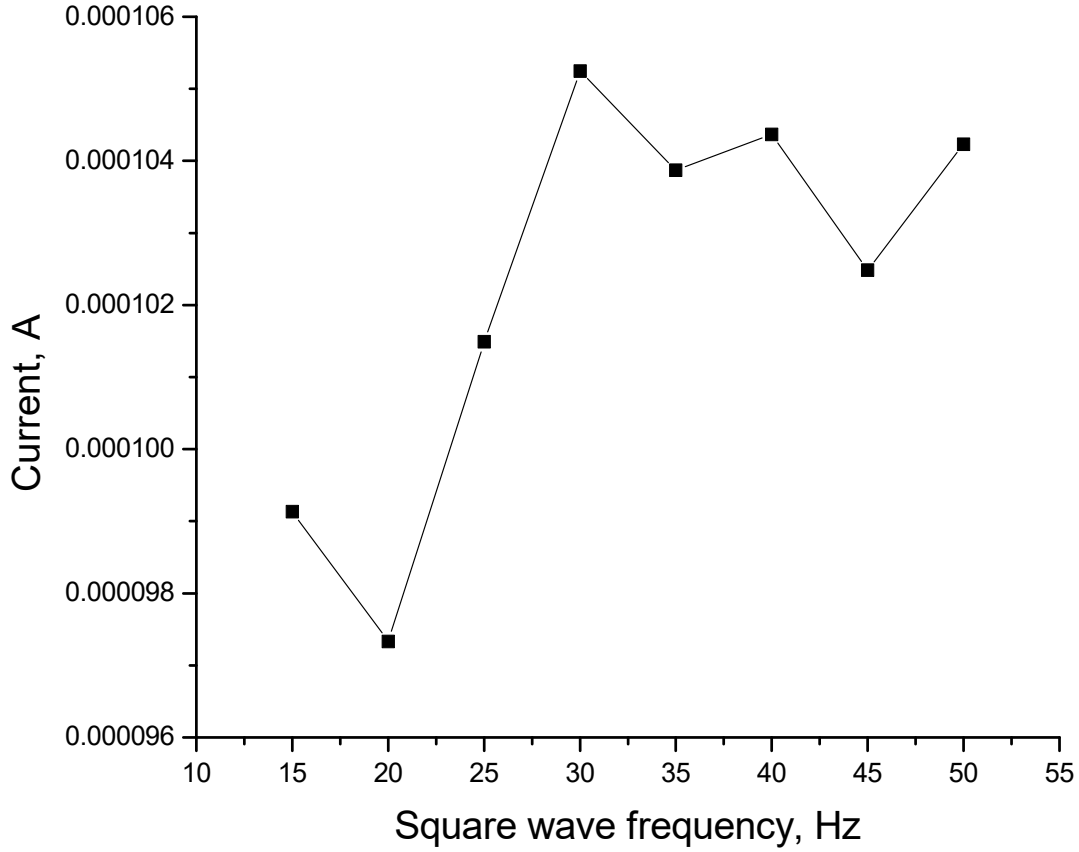


Figure -15- Dependence of the peak current of anthraquinone on the square wave frequency

5.3.4. Effect of Square Wave Amplitude

The effect of pulse amplitude on the peak current of anthraquinone was studied by varying the SW amplitude from 5 mV to 85 mV. At the SW frequency of 15 Hz and SW step potential of 4 mV. Upon the increasing of amplitude increasing of the peak current was observed. But the linearity of the line was kept until 20 mV step potential, after this value the linearity of the graph decreases and the electrode became saturated. The value where the linearity ends was selected for further experiment.

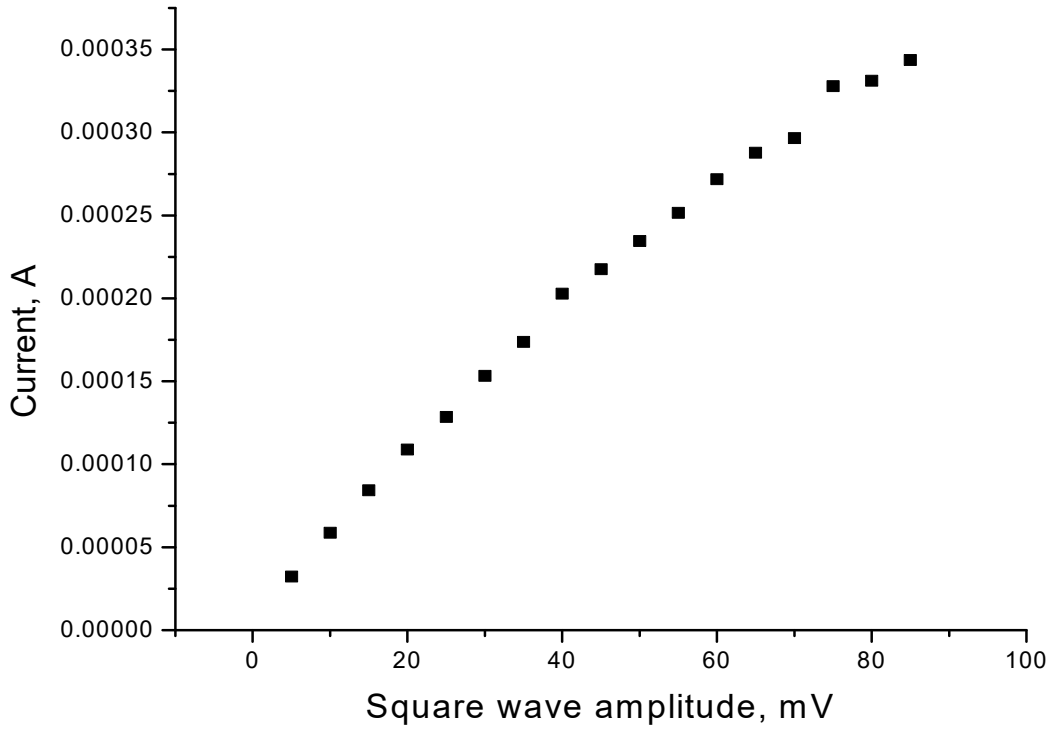


Figure -16- Dependence of the peak current of anthraquinone on the square wave amplitude

5.3.5. Effect of Square Wave Step Potential

The effect of square wave step potential on the peak current of anthraquinone was studied in the following step potentials; 2, 4, 6, 8 and 10 mV, at the square wave frequency of 15 Hz and amplitude 25 mV. The maximum peak current was observed at the step potential of 4 mV and this value was selected for subsequent experiment.

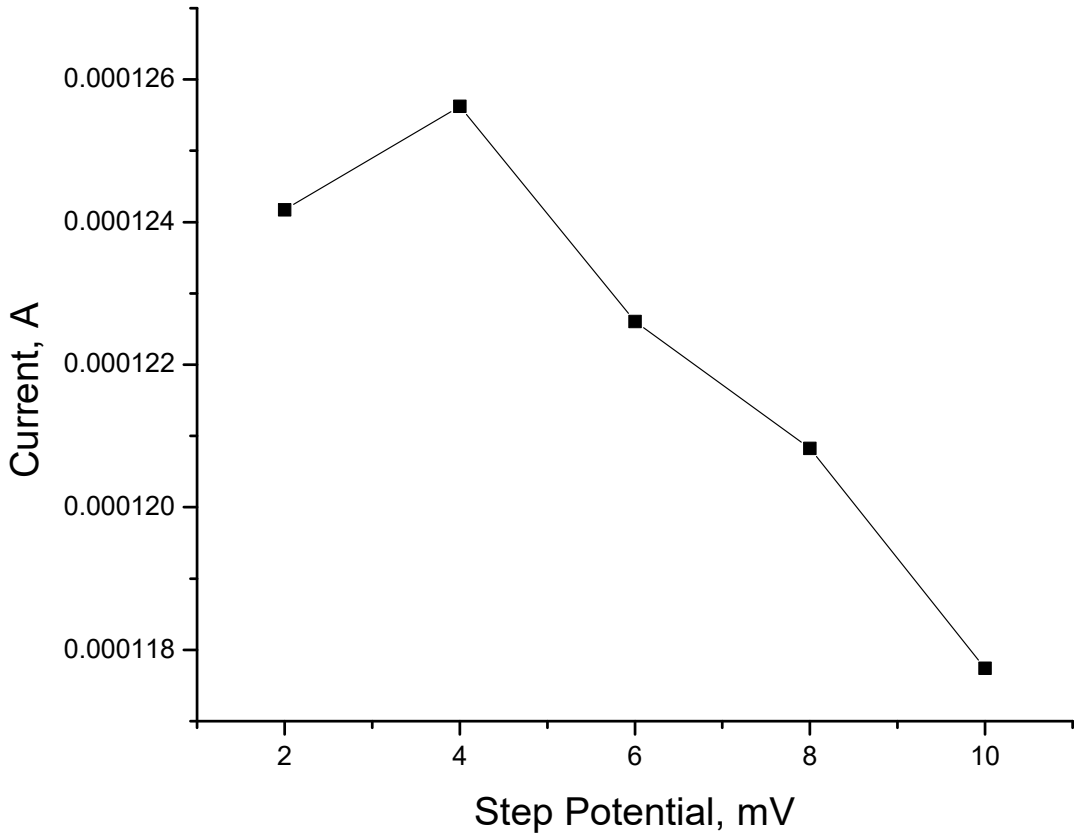


Figure -17- Dependence of step potential on the peak current of anthraquinone

The optimized parameters are summarized in the table 1

Parameters	Optimum values
Composition of anthraquinone	10 % (w/w)
pH of supporting electrolyte	12
Square wave frequency (Hz)	30
Square wave amplitude (mV)	20
Square wave step potential (mV)	4

Table -1- Optimized parameters for real sample analysis

5.4. Linear Range and detection Limits

The linear range was determined for voltammetric determination of caffeine with anthraquinone modified carbon paste electrode was obtained by recording the current by varying the of standard caffeine concentration from 100 μ M to 6 mM by using the procedures listed in section 4.5.1. This experiment was done three times and showed almost similar results. The range from 0 to 500 μM was taken as the linear range with slope of 53 μA/mM and linear regression coefficient, 0.99994.

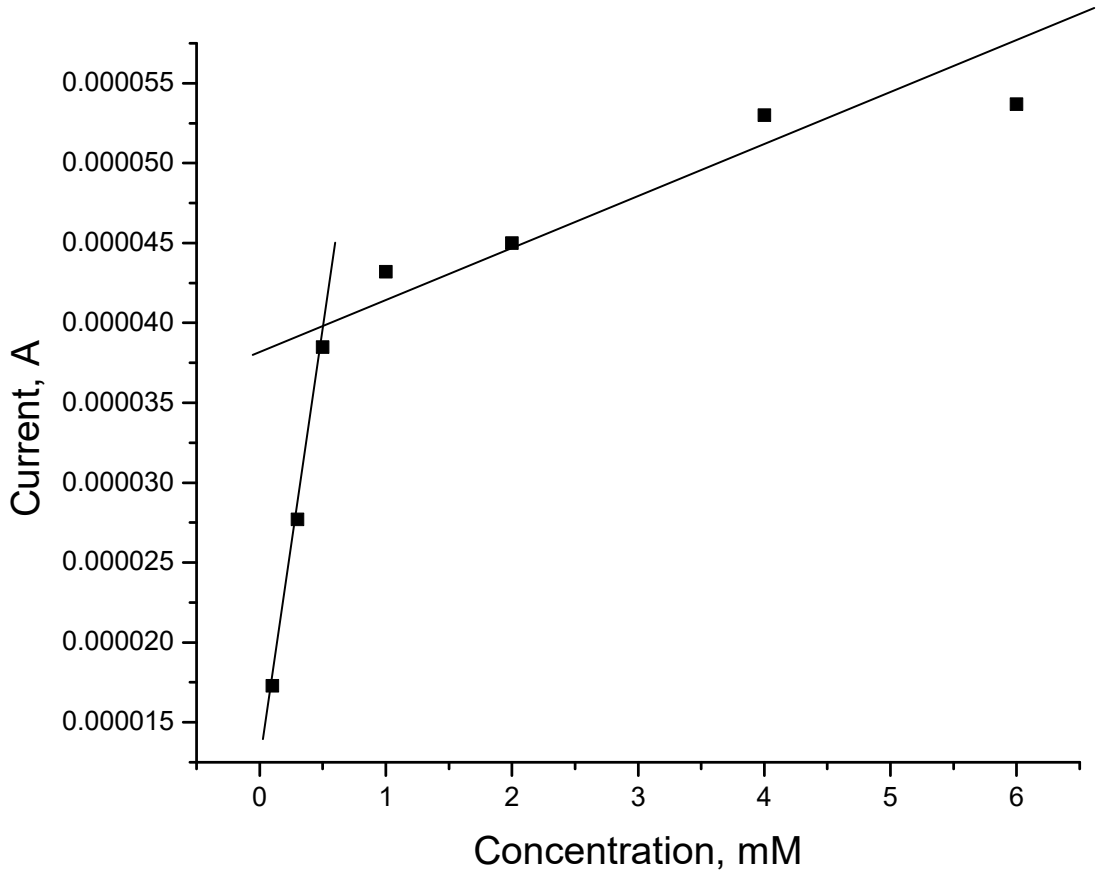


Figure -18- Calibration curve for the effect of caffeine on the redox behavior of anthraquinone

The following graph (figure 20) was used as a calibration curve for the real sample analysis.

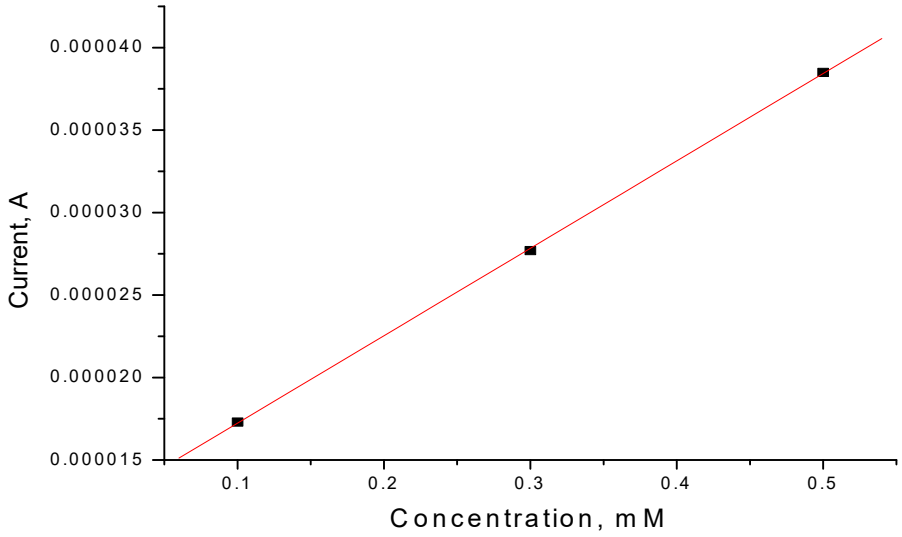


Figure -19- Calibration curve for the effect of caffeine concentration (concentration range 0 – 500 µM) on the redox behavior of anthraquinone: (Fitness values, slope = 5.3E-5± 5.7735E-7 A/mM and linear regression coefficient, 0.9994).

The detection limit of this method was obtained by three times the standard deviation of the peak current of five determination of 2 mM caffeine and found to be 5 nM. This value is smaller than the one obtained by Muluken Using 1,4- Benzoquinone as a modifier. It was better from some methods listed in section 1.2.4. Less time and less costy and less labor requirement made this method useful in the determination of caffeine.

	Anthraquinone	1,4 Benzoquinone
Sensitivity	53 µA/mM	77.8 µA/mM
Linear Range	0 – 500 µM	0 – 500 µM
Detection limit	5 nM	0.33 µM

Table -2- Comparison of anthraquinone with 1,4-Benzoquinone obtained by Muluken

6. Conclusion

This study revealed that anthraquinone can be applied in the square wave voltammetric determination of caffeine in coffee. Anthraquinone`s peak current and peak potential is pH dependence and showed a direct relation with pH. It also showed. The presence of caffeine in the buffer solution enhances the peak current and showed an increase in peak current of anthraquinone. Application of this method based on the optimized parameters give a good linear range of 0 – 500 μ M and very good detection limit, 5 nM. Determination of caffeine using this method has many advantages. In the future using more time and effort this method can be applied more than this and improved.

7. References

1. T. Ranheim and B. Halvorsen, *Mol. Nutr. Food Res.*, 2005, 49:274-284
2. T. Nogueira and C. Lucio de Lago, *Electrophoresis,* 2007, 28: 3570-3574
3. K. Fujioka and T. Shibamato, *Food Chemistry,* 2008, 106;217-221
4. F**.** Engelmann, M.E. Dulloo, C. Astorga, S. Dussert and F. Anthony (editors), *Conserving coffee genetic resources,* Topical Reviews In Agricultural Biodiversity
5. A. Pizzariello, J. Švorc, M. Stred`ansky and S. Miertuš, *J. Sci. Food Agri.,* 1999, 79:1136-1140
6. A. Belay, K. Ture, M. Redi and A. Asfaw, *Food Chemistry,* 2008, 108:310- 315
7. F. Gemech and J. Struthers, *J. International Development,* 2007, 19:1131-1142
8. Diriba Muleta, Acta Universitatis Agriculturae Sueciae, doctoral thesis, Swedish University of Agricultural Sciences/Uppsala, Sweden, 2007:117
9. C. Campa, S. Doulbeau, S. Dussert, S. Hamon, M. Noirot, *Food Chemistry,* 2005 91:633–637
10. M. J. Martin, F. Pablos, G. Gonzaleza, *Analytica Chimica Acta,* 1998, 358(2):177 183
11. F. Carrera, M. León-Camacho, F. Pablos and A. G. González, *Analytica. Chimica. Acta,* 1998, 370:131-139
12. P. Moncada and S. McCouch, *Genome,* 2004, 47:501-509
13. G. N. Jham, ,S. A. fernandes, C. F. Garcia, A.A. da Silva, *Phytochem. Anal.,* 2002, 13(2):99-104
14. N. Spãtaru, B. V. Sarada, D. A. Tryk and A. fujishima, *Electroanalysis,* 2002, 14 (11): 721-728
15. T. Hirai and H. Kondo, *Analytical sciences,* 1985, 1: 191-194
16. C. Alford, J. Bhatti, T. Leigh, *Human Phycopharmacology,* 1998, 11(3):185-198
17. A. Wang, L. Jun Li, F. Zang and Y. Fang, *Analytica Chimica Acta,* 2000, 419(2):235-242
18. V.R. Sarath Babu, S. Patra, N.G. Karanth, M.A. Kumar, M.S. Thakur, *Analytica Chimica Acta*, 2007,582:329–334
19. E. Leong Way, *Ann. Rev. Pharmacol. Toxicol.,* 1985, 25:769-775

20. P. Nawrot, S. Jordan, J. Eastwood, J. Rotstein, A. Hugenholtz and M. Feeley, *Food Additives and Contaminants,* 2003, 20(1):1-30
21. A. Sergio, G. Salvador, D. G. Miguel, *Analytica Chimica Acta,* 2005, 547(2):197-203
22. A. Quiroz –Neto and Et.al, *J. applied Toxicology,* 2001, 21(3):229-234
23. M. C. R. De Camargo and M. C. F Toledo, *J Sci Food Agric,* 1999, 79:1861-1864
24. I. Pérez-Martínez, S. Sagrado, M. J. Medina-Hernández, *Analytica Chimica Acta,* 1995, 304:195-201
25. Bewketu, *Further improvement of voltammetric determination of caffeine by using 1,4-Benzoquinone modified carbon paste electrode,* 2007, AAu graduate project
26. P. B. dews, *Ann. Rev. Nutr.,* 1982, 2:323-341
27. R. Lucena, S. Cárdenas, M. Gallego and M. Valcárcel, *Analytica Chimica Acta,* 2005, 530:283-289
28. IOCCC, Methylxanthines, December,1996
29. T. Kumazawa and Et. al, *Analytica Chimica Acta,* 1999, 387(1):53-60
30. P. D. Tzanavaras, D. G. Themelis, *Analytica Chimica Acta*, 2007, 581:89–94
31. Jyh-Myng Zen, Yuan-shih ting and Y. Shih, *Analyst,* 1998, 123:1145-1147
32. A. Pizzariello, J. Švorc, M. Stred`ansky and S. Miertuš, *J. Sci. Food Agri.,* 1999, 79:1136-1140
33. F. Regan, Y. Shakalisava, *Analytica Chimica Acta*, 2005, 540:103–110
34. K. A. Swinney and D. J. Bornhop, *J. Microcolumn Separations*, 1999, 11(8):596-604
35. C.W. Huck, W. Guggenbichler, G.K. Bonn, *Analytica Chimica Acta, 2005,* 538:195–203
36. B. Brunetti, E.Desimoni, P. Casati, *electroanalysis,* 2007, 19(2):385-388
37. Muluken, Addis Ababa university graduate project, 2005
38. A. Bazzi, J. Montogomory and G. Alent, *Analyst,* 1988, 113: 121-124
39. B. H. Hansen and G. Dryhurst, *J. Electro. Anal. Chem.,* 1971, 30:407-416
40. L. Zhu, C. Tian, D. Zhu and R. Yang, *Electroanalysis,*2008,
41. M. E. Rice, Z. Galus and R. N. Adams, *J. Electroanal. Chem.,* 1983, 143:89-102
42. C. Urbaniczky and K. Lundstrőm, *J. Electroanal. Chem.,* 1984, 176:169-182

43. I. Švancra, K. Vytřas, J. Barek and J. Zima, *Critical Reviews in Analytical Chemistry,* 2001, 31(4):311-345

44. F. A. Schultz and t. Kuwana, *J. Electroanal. Chem.,* 1965, 10:95-103

45. K. Kalcher, *Electroanalysis,* 1990**,** 2, 419

46. S. P. Kounaves, *Voltammetric Techniques,* Tufts university chemistry Department

47. P. Protti, Introduction to Modern Voltammetric and Polarographic Analysis Techniques, 4th Ed., 2001

48. Joseph Wang, *Analytical chemistry*, 2nd Ed. Wiley-VCH, 2001, pg-28-74

49. A. J. Bard, L. R. Faulkner, *Electrochemical Methods Fundamentals And Applications,* 2nd Ed., John Wiley & Sons, 2001, pg- 226-304

50. C. M. A. Brett and A. M. O. Brett**,** *electrochemistry principles, methods and applications,2nd Ed.,* Oxford University press, 1994, pg- 174-224

51. J. Koryta, J. Dvořák and L. Kavan, *Principles of Electrochemistry,* 2nd Ed. John Wiley and sons, chichester, England, 1993, pg- 288- 289