

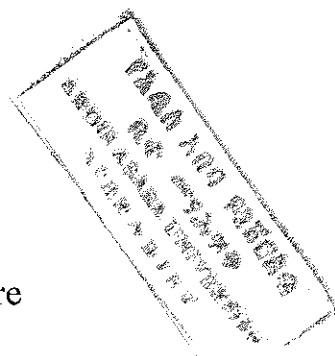
Addis Ababa University
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**Indirect Voltammetric Determination of Caffeine Content in
Coffee with 1,4-benzoquinone Modified Carbon Paste Electrode**

BY

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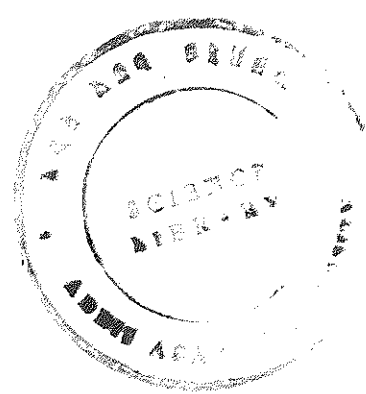
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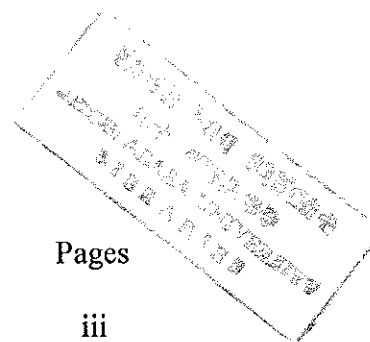
I would like to express my thanks to all my instructors, laboratory assistants and the store keeper at the Department of Chemistry.

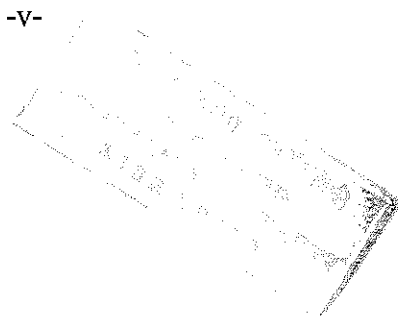
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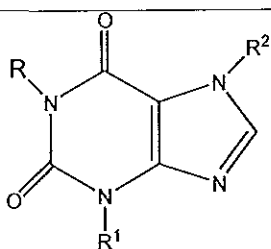
Abstract

1,4-benzoquinone modified carbon paste electrode is used for the determination of caffeine in coffee by square wave voltammetry. Voltammetric studies showed that the peak current of 1,4-benzoquinone decreases with increasing the concentration of caffeine. The effects of pH, electrode composition, step potential, pulse amplitude and square wave frequency on the voltammetric response were thoroughly investigated, and it was found that 1,4-benzoquinone exhibits excellent behavior, interms of very well-defined, reproducible voltammetric peaks for caffeine determination. The results enabled the measurement of the peak current of 1,4-benzoquinone for the determination of caffeine content in coffee. Promising results were obtained for caffeine determination in real samples

Introduction

Caffeine is an alkaloid; a class of naturally occurring compounds containing nitrogen and having the properties of an organic amine base. It belongs to a class of organic compounds called Xanthines. Other common members of this class include theophylline and theobromine [1–14]

Table 1: Structure of Xanthine and its naturally occurring *N*-methyl derivatives.

Structure	R	R ¹	R ²	Compound
	CH ₃	CH ₃	H	Theophylline
	H	CH ₃	CH ₃	Theobromine
	CH ₃	CH ₃	CH ₃	Caffeine

Caffeine is a typical distillation or sublimation product from coffee during the roasting process. In pure form, it is composed of long, white, silky crystals with a melting point of 278 °C, sublimation point of 178 °C and a density of 1.2 g/cm³. It has a slightly bitter taste in some concentrations, but is virtually without taste in dilute proportions [1 – 3]. It is moderately soluble in water and a wide range of organic solvents as shown in table 2 [1, 2]

Table 2 Solubility of caffeine in water and organic solvents at 25 °C [3].

Solvent	Solubility g/100 g solvent
Water	2.13
Ethanol	1.88
Methanol	1.14
Acetone	2.32
Benzene	0.91
Carbon tetrachloride	0.091
Chloroform	12.9
Dichloromethane	9



Caffeine is found naturally in such foods as coffee, tea, cola nuts, yerbamate, guarana berries, and (in small amounts) cacao beans. For the plant, caffeine acts as a natural pesticide since it paralyzes and kills some of the insects that attempt to feed on the plant [1, 15 - 18].

The roasting of green coffee beans yields the commercial coffee, which is the basis of the worldwide bitter and dark coffee beverages, such as espresso. Caffeine contributes to a particular proportion of the perceived bitterness of a cup of coffee and also makes a small contribution to espresso's strength. Moreover, the importance of the mild stimulating properties of caffeine should also not be neglected [16]. One common source of caffeine is the coffee plant, the beans from which are used to produce coffee. The caffeine content of green coffee varies depending on the species of beans, where they are grown, and the way they are processed. Environmental and agricultural factors are considerably less important than genetic variation in controlling the caffeine content of green coffee [1, 2].

1.1 Coffee species

Coffee is a member of large *Rubiaceae* family, within which it constitutes the *coffee genus* that was created by *C. Linnaeus*. There are more than 6,000 species of coffee plant, with at least 25 major types, all independent to tropical Africa and certain islands in the Indian Ocean, notably Madagascar. Difficulties in classification and even in designation of a plant as a true member of the coffee genus arise because of the great variation in the plants and seeds. *Chealier* tried to group the species within the *genus coffee* into the following four sections, namely: *Argocoffea*, *Paracoffea*, *Mascarocoffea* and *Eucoffea*. The section *Eucoffea*, now more correctly named *coffea*, and *Mascarocoffea* include most of the presently known coffee species. All species of coffee are woody, but they range from small shrubs to large trees over 10 meters; the leaves can be yellowish, dark green, bronze or tinged with purple. The two most economically important species of coffee are coffee Arabica and coffee Robusta. Two other species, which are grown on a much smaller scale, are coffee Liberica and Excelsa coffee [1 - 9].

1.1.1 Arabica coffee

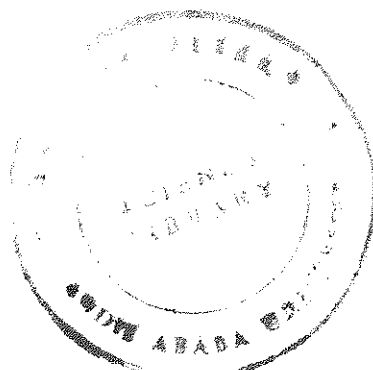
Coffee Arabica was first described by Linnaeus in 1753. It is an evergreen plant, having about 8 to 10 m tall. The size and the shape of beans of this type of coffee differ depending upon the varieties, environmental conditions and cropping practices. These species, which is autogamous (self-fertilizing), has relatively uniform characteristics. The average Arabica coffee plant is a large bush with dark green oval leaves. It is genetically different from other coffee species, having four sets of chromosomes rather than two. The fruits are oval and mature in 7 to 9 months. They usually contain two flat seeds (the coffee beans)-when only one been develops, it is called a pea berry. Arabica trees do best at altitudes of 3,000 to 6,500 feet where the slower growing process concentrates their flavors. An Arabica tree yields only 1 to 1.5 pounds of green coffee per year. Because the Arabica tree is susceptible to disease, frost, and drought, it requires very careful cultivation with just the right climatic conditions. It accounts for over 70% of world production [1-9].

1.1.2 Robusta coffee

The term 'Robusta' is actually the name of a widely grown variety of this species. It is a robust shrub or small tree growing up to 10 meters in height, but with a shallow root system. The fruits are rounded and take up to 11 months to mature; the seeds are oval in shape and smaller than those of Arabica coffee. Robusta coffee accounts for close to 30 % of world production. It is more resistant to disease than Arabica coffee and is grown between sea level and 800 m above. It yields 2 to 3 pounds of green coffee a year. This plant is used for the lower grades of coffee that are sold in the market. Although generally not found in gourmet shops, Robusta beans are often used in the processing of soluble instant coffees and popular commercial blends [1-9].

1.1.3 Liberica

Liberica coffee is the third recognized commercial variety. It grows as a large tree, up to 18 meters in height, with large leathery leaves. The fruits and seeds (beans) are also large.



According to a review of extensive studies, the caffeine content of green coffee beans in % on dry mass basis are given as coffee arabica (0.6-1.6 %), coffee liberica (1.4-1.8 %), and coffee robusta (1.3-2.4 %). Generally, dark roast coffee has less caffeine than lighter roasts since the roasting process reduces the caffeine content of the bean [4].

1.2 Green Coffee Processing

Green coffee is prepared from the cherries of the coffee tree by removing the various covering layers in the most efficacious manner. Two main processes have been used for many years, traditionally distinguished as the dry processes and the wet processes, which differ fundamentally in their approach. Apart from differentiation by species and geographical origin, green coffee prepared by these processes is known as either dry process ('natural') or wet process ('washed'). In addition, certain cleaning and polishing processes may be additionally adopted, together with various grading and sorting techniques to provide the finished coffees for export [1].

1.2.1 The Dry Processing Method

This method consists of three basic steps, these are:

- The harvested cherries may be subjected to some form of classification, but then are dried in their entirety, most usually in the sun.
- The dried cherry coffee is then subjected to a milling operation to separate out the green beans.
- With the initial drying of the cherries, the final beans are sufficiently dry for subsequent storage and bagging for export [1].

1.2.2 The Wet Processing Method

The wet process is more sophisticated than the dry process and, by general consent, leads to a better quality coffee and commands a higher price. The process carried out correctly, when applied to Arabica coffee provides the so-called parchment coffee and finally hulling provides

the dry green beans. This method requires the use of substantial quantities of water. In this method, the cherries are squeezed in a pulping machine, which removes the outer fleshy material leaving a bean covered in mucilage. This mucilage is fermented and dispersed [3].

Pulping involves the removal of the outer red skin (exocarp) and the white fleshy pulp (mesocarp) and the separation of the pulp and beans. It is recommended that this stage should be completed within 12–24 hours after picking; otherwise, the cherries should be kept under water for another day. The cherry coffee arrives at the hoppers of the pulpers with water, where its flow is regulated. The pulping machine needs to be adjusted, for the average size and ripeness of the coffee cherry being presented. The waste pulp should be thrown out at the rear of the machine and conveyed well away; but some may inevitably go forward with the beans. The pulped beans therefore need to go next to some form of vibrating screens when the larger pieces of pulp and imperfectly pulped cherries are sent to another smaller pulper. The separated pulped beans should then pass in short water-washing channels (usually made of concrete) where a further floatation separation can be arranged by means of sluice gates.

The other stage of wet processing method is fermentation. It is necessary to remove any residual adhering pulp, and importantly to remove a very adhesive mucilaginous layer, leaving only the parchment covering, enclosing the green beans, or seeds. Fermentation in large scale practice is a batch process taking place in rectangularly sectioned concrete tanks of which a number will be needed in a typical pulper, for both capacity reasons, and the need to process separately and simultaneously any different qualities of coffee in process at that stage. As pulping proceeds, so the water outlet from a tank is closed until the tank is filled with its complement of pulped coffee. Fermentation takes place whilst the coffee lies in a sticky mess (say one meter thick) with the water drained away. The time of fermentation allowed varies according to the location, with temperature being an important factor, from about six hours to as long as eighty hours, though a time of 24 hours may be regarded as typical in a pulper at any 900 meters altitude [1, 2].

1.3 Coffee and caffeine consumption

Coffee is a widely and extensively consumed recreational beverage. Whatever effects are attributed specifically to the consumption of coffee, one must assume that they are associated with compounds, which in dietary terms are found almost only in coffee beverage. Immediately

this focuses attention upon caffeine, the chlorogenic acids, possibly the sparingly water soluble diterpenes, kahweol and cafestol and possibly the ill defined and numerous miscellaneous products of roasting [1].

Caffeine is readily and rapidly absorbed after both enteral and parental application. It is rapidly distributed throughout all tissues as a function of their water content. In humans, caffeine appears in all tissue fluids about 5 minutes after application, and peak blood plasma levels are reached after about 20 to 30 minutes. The substance also penetrates easily into the different organ systems including the brain, gonadal tissue, placenta, fetal tissue, and maternal milk. It is almost entirely metabolized; only about 1 % is excreted via urine in its non – metabolized form. The main metabolite in man belongs to the classes of demethylated Xanthines, uric acids or uracid derivatives of caffeine [1, 3, 14, 19].

The half-life of caffeine in human plasma has been reported to be about three to six hours, but varies widely between individuals. Several different conditions have been found to alter the time course of elimination considerably, where as caffeine clearance is relatively slow in patients with alcoholic liver disease, it is accelerated up to more than two fold in heavy smokers [1, 2].

In women, the use of contraceptives has been reported to double the half – life, as is also the case for the second and third trimesters of pregnancy, a finding suggested to be due to a decrease in biotransformation caused by the altered hormonal milieu. A roughly tenfold increase in the half – life has been observed in neonates, with gradual normalization by the age of about 6 months. The slow clearance in neonates has been ascribed to the fact that neonates are deficient in N–demethylation, which causes them to excrete caffeine in the urine mainly as the unchanged compound.

Studies on the effects of xanthines on intracellular translocation of calcium have concentrated mainly on the effects of caffeine on skeletal muscle. The substance increases the twitch response of the isolated frog sartorius muscle preparation. It is believed that this effect is due to increased release of calcium from the terminal cisternae of the sarcoplasmic reticulum. This effect has, however, been shown to occur at relatively moderate dose levels only under particular experimental circumstances. Normally, the effect can be observed only at dose ranges, which by far exceed the therapeutic range.



The exact mechanism for calcium translocation is not yet known, and besides a direct action on the release mechanism the possibilities of a decreased calcium re-uptake or a direct action on the sarcoplasmic reticulum have also been proposed. Furthermore, it also seems likely that caffeine facilitates transmitter release by increasing calcium release at neuro muscular junctions [1, 2]

1.4 Metabolism and Toxicology of Caffeine

Caffeine is ingested to have many physiological effects such as gastric acid secretion, diuresis and stimulation of the central nervous and the cardiovascular systems. It is considered to be a risk factor for cardiovascular diseases and may affect behavior effects of depression and hyperactivity [12, 13].

Caffeine is metabolized by demethylation and oxidation. The major human path way results in paraxanthine (1,7-dimethylxanthine), leading to the principal urinary metabolites, 1-methylxanthine, 1-methyluric acids, and an acetylated uracil derivative. Minor degradation pathways involve the formation and metabolism of theophylline and theobromine. No evidence exists to suggest that methylxanthines are converted to uric acid or that their injection can exacerbate gout [14].

In most cases, metabolism obeys first order elimination kinetics within therapeutic range. At higher concentrations, however, zero order kinetics occurs with the saturation of metabolic enzymes. This prolongs the decline of caffeine's concentration. The metabolism of methylxanthines is also influenced by the presence of other agents or specific diseases. For example, the half-life of theophylline can be prolonged significantly in patients with hepatic cirrhosis, congestive heart failure, or acute pulmonary congestion. In the liver, the first metabolic products of caffeine are three dimethylxanthines: theophylline (4%), theobromine (12%), and paraxanthine (84 %). Theophylline relaxes smooth muscles of the bronchii and has been used as a treatment of asthma [20]. Theobromine, the principal alkaloid in cocoa (chocolate) can increase blood vessel dilation and urine volume. Paraxanthine increases lipolysis, leading to elevated glycerol and free fatty acid in the blood plasma. Three basic cellular actions of methylxanthines are probably responsible for their diverse effects. In order of increasing importance, they are [1]:

1.5 Beneficial Effects of Caffeine

According to medical studies, caffeine is a mild stimulant and habit forming, with a good effect that is both temporary and transitory. It is specially valued because; accompanying its stimulatory action, there is no depression either initially or eventually. Taken in a reasonable measure it reduces a craving for sweets by stimulating the production of those adrenal hormones that cause blood sugars to be increased. Stimulatory effects of caffeine results in increased capacity for both mental activity and muscular work. Through caffeine stimulation, work is made more of a pleasure and, if it is not abused, this stimulus can be of great help. The weakness, general malaise, and discomfort from excess of alcohol are nullified with black coffee or hypodermic injections of caffeine. Insensibility from hashish or opium is said to be ended by the use of caffeine medication. Even the dullness and sense of depression from a little too much tobacco is helped by coffee [2].

It has been reported that a number of biochemical reactions in our body generate reactive oxygen species mainly comprising free radicals and excited states, capable of damaging crucial biomolecules. Caffeine has been reported to have significant abilities to scavenge highly reactive free radicals and excited states of oxygen and to protect crucial biological molecules against these species [10]. The other beneficial effects of caffeine are [3, 23]:

- Reduced risk of parkinson's disease;
- Reduced risk of colon cancer; and
- Reduced risk of stone formation (calculi).

1.6 Methods of Analysis

Because of the relatively important role of caffeine in determining the quality of coffee beverages, the development of a sensitive, fast and cost-effective method for monitoring caffeine is greatly needed [13]. Normally, high performance liquid chromatography separation and UV-spectrophotometric detection method is applied to both regular decaffeinated green and roasted coffees for caffeine content determinations. Also, other methods such as capillary electrophoresis, thin layer chromatography and gas chromatography are used for separation of caffeine in the analysis of mixtures, combined with several other detection methods such as mass



spectroscopy and FTIR spectrophotometric measurements. However, very costly instrumentation, high skilled technicians and complicated and time-consuming procedures are required for such methods [16, 24, 25].

The development and application of new caffeine detection methods remain an active area of investigation, particularly in food and clinical chemistry. Glassy carbon (GC) electrodes have been used for the amperometric detection of caffeine, theophylline and theobromine in beverages, either directly or after solid phase extraction, and for the voltammetric determination of xanthine within the concentration ranges 50 to 200 μM . Compared to bare GC electrodes, GC electrodes modified with a lead-ruthenium pyrochlore oxide in a Nafion matrix exhibited a marked enhancement of the current response for caffeine determination by a square-wave voltammetry, over the 5-200 μM ranges [12, 16]. Significant advantages have been achieved with the electrocatalytic function of ruthenium oxide pyrochlore catalyst [16]. Thus, chemically modified electrodes have been used for several determinations with excellent sensitivity and are further applied to the determination of caffeine in tea, decaffeinated coffee, and cola beverages.

The use of carbon (or graphite) and platinum electrodes in the anodic determination of xanthines is rendered difficult by interference from oxidation background currents that are not precisely reproducible. This is because relatively large positive potentials (>1.4 V versus SCE) are required for reasonable sensitivity, both in amperometry for GC and voltammetry for GC and pyrolytic graphite. The optimization of the peak current is important for analysis, but this relies on the availability of electrode materials that have the ability to detect compounds that are difficult to oxidize. Due to its excellent electrochemical features such as wide potential window in aqueous solutions, low background current and long term stability of response conductive diamond is an electrode material that has attracted great interest, especially in electroanalysis [12].

Based on cyclic voltammetry, coulometry and chromatographic measurements the overall mechanisms of oxidation of caffeine involves four protons and four electrons [3,12]. The mechanism proceeds by two electron potential controlling oxidation of carbon-nitrogen double bond to give trimethyl substituted uric acid at 1.4 V. This intermediate is more readily oxidized than the parent compound in two-electron process to uric acid 4,5, diol. This primary electrochemical product is extremely unstable as a result of steric factors so that it rapidly fragments to give the dimethyl-substituted alloxan, trimethyl-substituted allantoin and methyl urea [3]. The electrochemical process is shown in figure 1.

1.7 Carbon Paste Electrodes

Due to the rapid development of various types of modified carbon pastes, it was necessary to introduce a new adequate specification. Classically, all carbon pastes were binary mixtures prepared from carbon powder and organic liquid of non-electrolytic character. Now, such mixtures are classified as bar ("virgin") or, more often, as unmodified carbon pastes. For a long time, the choice of the main constituents for carbon pastes was rather monotonic and, in fact, there was no reason to seek new alternative materials. The properties of conventional paste mixtures from spectroscopic graphite and paraffin oils were found satisfactory for a majority of applications, including very popular modifications. Nevertheless, also in this area, the recent years have brought some interesting innovations. The proper electroactive moiety in carbon pastes is still graphite powder with micrometric particles of high purity and distribution uniformity usually; graphite powders are not pretreated further prior to use. Some procedures made in the past which concentrated on lowering undesirable absorption capabilities of some commercial graphites seem to be too complicated compared to their benefit. From a present day's point of view, it is more effective to perform some test experiments with newly made pastes that reveal excessive absorptiveness of some graphites. Then, it is better to choose some commercially available powders with specially pretreated surface exhibiting only negligible absorption capability [26].

Carbon paste electrodes, prepared from graphite powder, mixed with various water immiscible organic binders (pasting liquids) offer an easily renewable and modified surface, low cost and very low background current contributions. A wide choice of pasting liquids is possible, but practical considerations of low volatility, purity, and economy narrow the choice to a few liquids. These include Nujol (mineral oil), paraffin oil, silicon grease, and bromonaphthalene. The paste composition strongly affects the electrode activity, increase in the pasting liquid content decreasing the electron transfer rates, as well as the background current contributions. The optimum composition is reported to be 70 to 80 % by weight of graphite powder [26-28].

Carbon paste electrode has been widely applied in electrochemistry mainly as a substitute for noble metals. It is advantageous over dry graphite because the later has higher residual current. Carbon paste electrodes have practically zero residual current over the entire anodic potential range. This is because there is no oxidation current due to dissolution of oxygen or desorption of hydrogen.

Carbon paste electrode has many advantageous. It has a wider potential window than common metallic electrodes, which means more redox reactions can be observed at carbon paste electrodes than at metallic electrodes. In addition, it is inexpensive, easy to prepare and renew. A disadvantage of carbon pastes is the tendency of the organic binder to dissolve in solutions containing an appreciable fraction of organic solvents [26, 27].

1.8 Modified Carbon Paste Electrodes

The attempts to utilize favorable mechanical and electrochemical properties of carbon pastes for the preparation of a new generation of sensors culminated at the beginning of the 1980s. Modification of a carbon paste by impregnating the carbon particles with methanolic solution of dimethylglyoxime represents another milestone in the history of CPEs. It was a first attempt when classic analytical reagent had served as selective modifier, thus initiating a very successful role of chemically modified carbon paste electrodes (CMCPEs) in electrochemical analysis, as shown in a review covering this period. Since that time, the number of publications dealing with chemically modified carbon paste-based electrodes started to grow exponentially and this trend continued over the next decade when other review articles had appeared. Hand in hand with a rapid development of CMCPEs, favorable mechanical and electrochemical properties of carbon pastes were tested for the preparation of special sensors containing enzymes allowing one to monitor some enzymatically catalyzed reactions of biologic substances. This way of anchoring enzymes to an electrode material immediately attracted biochemists/bioanalysts and carbon paste-based enzymatic biosensors had rapidly come to the fore. Retrospectively, they have occupied a very significant position in the family of CPEs when having finally formed a specific independent area similarly like electroactive carbon pastes in the electrochemistry of solids [26].

Chemically modified electrodes (CMEs) represent a modern approach to electrode systems. Such deliberate alteration of electrode surfaces, through the incorporation of an appropriate surface modifier, can meet the needs of many electroanalytical problems, and may form the basis for new analytical applications and different sensing devices [29-33].

There are different directions by which CMEs can benefit analytical applications. These include acceleration of electron-transfer reactions, preferential accumulation, or selective membrane permeation. Such steps can impart higher selectivity, sensitivity, or stability to electrochemical devices. These analytical applications and improvements have been extensively

reviewed. Many other important applications, including electrochromic display devices, controlled release of drugs, electrosynthesis, and corrosion protection, should also benefit from the rational design of electrode surfaces [13, 26, 27, 30].

The base of modified carbon pastes is usually a mixture of powdered graphite and non-electrolyte binder. Another constituent in the mixture is then a modifier itself. A modifying agent is usually one substance, but the pastes can also be modified with two or even more components, which is the case of carbon-paste based biosensors containing enzyme (or its carrier) together with appropriate mediator or chemically modified electrodes with a mixture of two modifiers. The amount of modifier in the paste usually varies between 10 to 30 % (w/w), depending on the character of modifying agent and its capability of forming enough active sites in the modified paste (eg. Functional groups immobilized at the electrode surface or molecules of an extractant in the bulk). The possible functions of modifiers can be summarized in the following way [26, 28]

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

When considering these possibilities in combination with the above-mentioned flexibility of carbon pastes, it is not surprising that in the last decade the number and the diversity of substances used for the preparation of chemically modified carbon paste electrodes have grown in a geometric order. Among modifiers recently used, one can find single compounds, sophisticated chemical agents, special inorganic materials and matrices, or living organisms. In general, the main reason to modify an electrode is to obtain a qualitatively new sensor with desired, often predefined properties [26].

In this study, we attempted to fabricate 1,4-benzoquinone modified carbon paste electrode for the indirect voltammetric determination of caffeine in coffee with the aim of raising the selectivity and sensitivity of the carbon paste electrode.

1.10 Objective of the Study

One of the requirements of a good coffee is whether it carries a reasonable satisfying quantity of the stimulant caffeine [1]. Therefore the determination of the level of caffeine in green coffee is very important.

The general objective of this research is to develop a less expensive, validated voltammetric method for the determination of the level of caffeine in green coffee grown in Ethiopia.

The specific objective of this study includes:

- Development of a suitable method for square wave voltammetric determination of caffeine by using 1,4-benzoquinone modified carbon paste electrode.
- Analysis of the level of caffeine in coffee grown in Ethiopia.

2. Theoretical Background

2.1 Cyclic Voltammetry

Cyclic voltammetry is perhaps the most versatile electroanalytical technique for the study of electroactive species. Its versatility combined with ease of measurement has resulted in extensive use of cyclic voltammetry in the fields of electrochemistry, inorganic chemistry, organic chemistry and biochemistry. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron transfer reactions, and on coupled chemical reactions or adsorption processes [29, 32, 37, 38].

Cyclic voltammetry is often the first experiment performed in an electroanalytical study. In particular; it offers a rapid location of redox potentials of the electroactive species and convenient evaluation of the effect of solvent upon the redox process. It consists of cycling the potential of an electrode, which is immersed in unstirred solution, and measuring the resulting current. The potential of this working electrode is controlled versus a reference electrode such as an SCE or Ag/AgCl electrode. The controlling potential that is applied across these two

electrodes can be considered an excitation signal. The excitation signal for cyclic voltammetry is a linear potential scan with a triangular waveform.

This triangular potential excitation signal sweeps the potential of the electrode between two values, sometimes called the switching potentials. During the potential sweep, the potentiostat measures the current resulting from the applied potential. The resulting plot of current versus potential is termed a cyclic voltammogram. The voltammogram is a display of current (vertical axis) versus potential (horizontal axis). Because the potential varies linearly with time, the horizontal axis can also be thought as a time axis. This is helpful in understanding the fundamentals of the technique [16, 29, 30 32].

A cyclic voltammogram can quickly show the presence of all species that undergo oxidation-reduction reactions at the electrode within the limits set by solvent electrolysis and electrode. The important parameters of a cyclic voltammogram are the magnitudes of the anodic peak current (i_{pa}), cathodic peak current (i_{pc}), anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) [24]. A redox couple in which both species rapidly exchange electrons with the working electrode is termed an electrochemically reversible couple. The formal reduction potential E^0 for a reversible couple is centered between E_{pa} and E_{pc} :

$$E^0 = \frac{(E_{pa} + E_{pc})}{2} \quad (1)$$

The number (n) of electrons transferred in the electrode reaction for a reversible couple can be determined from the separation between the peak potentials:

$$\Delta E = E_{pa} - E_{pc} = 0.059/n \quad (2)$$

The peak current for a reversible system is described by the Randles-Sevcik equation for the forward sweep of the first cycle.

$$i_p = 2.69 \times 10^5 n^{3/2} ACD^{1/2} \nu^{1/2} \quad (3)$$

where i_p is the peak current (A), n is the electron stoichiometry, A is the electrode area (cm^2), D is the diffusion coefficient (cm^2/s), C is the concentration (mol/cm^3) and ν is the scan rate (V/s). Accordingly, i_p increases with $\nu^{1/2}$ and is directly proportional to concentration. The relationship

2.2.1 Square Wave Voltammetry

Square wave voltammetry is a large amplitude differential technique in which a waveform composed of a symmetrical square wave, superimposed on a base staircase potential, is applied to the working electrode. The square wave signal is defined by its frequency f , the amplitude E_{sw} , and scan increment ΔE . The square wave amplitude is one half of the peak-to-peak amplitude and the potential increment ΔE is the step height of the staircase waveform [29, 30, 32].

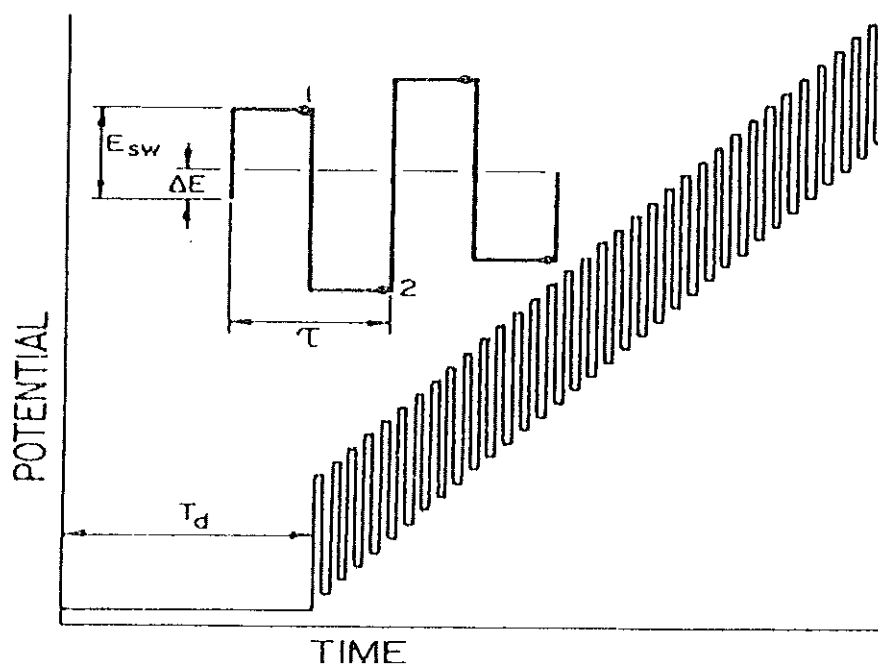


Figure 2. Excitation signal for square-wave voltammetry

The current is measured at the end of each half cycle, and the current measured on the reverse half-cycle (i_r) is subtracted from the current measured on the forward half-cycle (i_f). This difference current ($i_f - i_r$) is displayed as a function of the applied potential. The resulting peak shaped voltammogram is symmetrical about the half wave potential, and the peak current is proportional to the concentration. Excellent sensitivity is achieved from the fact that the net current is larger than either the forward or the reverse components (since it is the difference between them); coupled with the effective discrimination against the charging background current, very low detection limits near 1×10^{-8} M can be attained [28, 29, 30, 40].

The net peak current for irreversible system is given by:

$$i_p = \text{const.} \alpha n^2 \Delta E E_{sw} (fD)^{1/2} C \quad (5)$$

where: ΔE is the step potential, f is square wave frequency, E_{sw} is the square wave amplitude, α is the transfer coefficient, n is the over all electron transfer, C is the bulk concentration, D is the diffusion coefficient of electroactive species

The effective scan rate is given by $f\Delta E$. Kinetic parameters can also benefit from the rapid scanning and the reversal nature of square wave voltammetry. This method possesses both pulse techniques and cyclic voltammetry; hence it is one of the most advanced methods in the family of pulse techniques. For this reason, square wave voltammetry is an exceptionally appealing method for analytical usage, as well as for mechanistic or kinetic studies of the electrode mechanism and has already been widely verified experimentally.

2.3 Choice of Operating Parameters in Square Wave Voltammetry (SWV)

2.3.1 Effect of Supporting Electrolyte

Electrochemical measurements are commonly carried out in a medium, which consists of a solvent containing a supporting electrolyte. The choice of the solvent is dictated primarily by the solubility of the analyte and its redox reactivity, and by solvent properties, such as electrical conductivity, electrochemical activity, and chemical reactivity. The solvent should not react with the analyte (or products) and should not undergo electrochemical reactions over a wide potential range. Supporting electrolytes are required in controlled-potential experiments to decrease the resistance of the solution, to eliminate electromigration effects, and to maintain a constant ionic strength. Electrolytic media of high conductivity are desirable since they prevent internal heating due to I^2R power dissipation and permit relative error free control of electrode potential. The solution must contain a sufficient concentration of the conductive species so that a large ohmic drop is prevented, migration current is suppressed, and constancy of activity and diffusion coefficient is maintained. When acids or bases are formed during the redox process the electrolyte may be chosen to serve as buffer. Buffer systems such as acetate or phosphate are used when pH control is essential. Care must be taken in that the electrolyte is truly inert in the

potential range of the experiment, not reacting with the electrode. Solution conditions in particular pH have profound effects [3].

2.3.2 Square Wave Frequency

It is found that an increase in square wave frequency results in an increase in the effective scan rate which in turn increases the peak current. However, at very high frequency, the peak current is unstable and obscured by a large residual current [13]. On the other hand very low frequency gives a low but narrow signal, and increases the total analysis time. Hence, the selection of frequency usually requires a compromise among sensitivity, resolution and speed.

2.3.3 Square Wave Amplitude

It is found that, when the amplitude is increased, the peak currents increase. However, the peak width also increases with increasing pulse amplitude. Therefore, the optimum pulse amplitude must be found to maximize, i_p , and resolution (small peak width) [3, 13, 40].

2.3.4 Step potential

The net current increases as step potential increases, however, accompanied by peak broadening and loss of peak symmetry [13]. At higher step heights, too few points are sampled, thus affecting the reproducibility of the detection. Therefore, the optimum step potential must be found to maximize sensitivity and to obtain better peak symmetry.

3.4. Voltammetric Procedure

Cyclic voltammetric experiments for caffeine solution were conducted with 1,4-benzoquinone modified carbon paste electrode. Cyclic voltammograms were recorded between -1.8 V to 2.4 V with scan rate of 100 mV/s. Square wave voltammograms were obtained by scanning the potential from -500 mV to 500 mV versus Ag /AgCl reference electrode with the following square wave parameters: Frequency 25 Hz, amplitude 30 mV, step height 2 mV. Caffeine quantification was achieved by measuring the current of the oxidation peak of 1,4-benzoquinone after background subtraction. For all of the experiments, a mixture of 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 solution was used as supporting electrolyte. Stock solution of caffeine was prepared by dissolving 1.25 g of caffeine in 250 ml of deionized water. The required amounts of caffeine working solutions were prepared by diluting the stock solution with the supporting electrolyte. The working solutions were placed in a cell containing the supporting electrolyte solution at the selected pH. The pH of the solution was varied between 1 and 12 to get the best measurement condition.

3.5 Application of the Method to Real Samples

3.5.1 Sample Preparation

The coffee bean was milled and grounded with an analytical grinder suitable for grinding green coffee bean. The ground bean was sieved in woven wire cloth with a mesh size of 500 μm . 100 mg of ground coffee was added in to a 125 mL Erlenmeyer flask fitted with a stopper. 25 ml of distilled water was added to the flask and boiled for 1 hour in a hot plate with stirring. After allowing the residue to settle, the solution was filtered while hot. For removal of interferents; liquid – liquid extraction using dichloromethane was performed using 250 ml separatory funnel. The extraction was performed with 100 ml of dichloromethane three times by gentle swirling of the mixture to mix the layers and carefully inverting the funnels several times. The extracts were mixed and passed through 0.25 g anhydrous Na_2SO_4 for drying. Dichloromethane was removed to dryness by transferring in to vials and allowing to dry in the hood. The residue was dissolved

As can be seen in figure 5, caffeine is an electroactive compound because it shows an oxidation peak current around 1.45 V. In the absence of caffeine, such peak was not observed. When the concentration of caffeine was increased from 2 mM to 8 mM, the peak currents increased successively as shown in figure 6

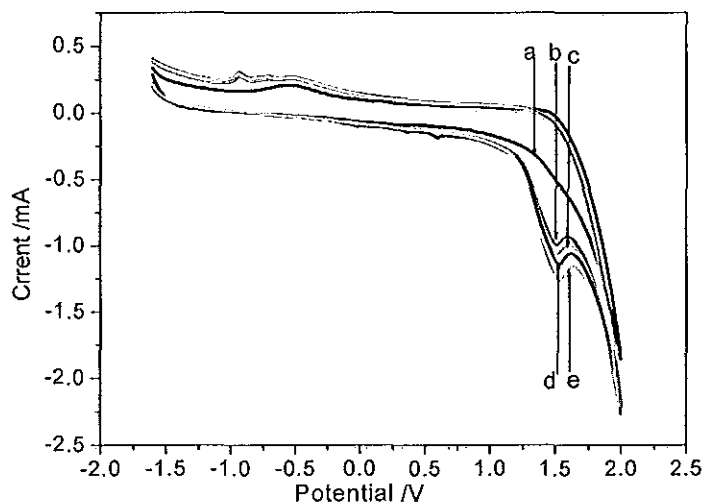


Figure 6 Cyclic voltammogram of: (A) 0 mM Caffeine (B) 2 mM Caffeine (C) 4 mM Caffeine (D) 6 mM Caffeine (E) 8 mM Caffeine at unmodified electrode

The relationship of caffeine concentration and the peak current is linear as it is given in Randles-Sevcik equation. The dependence of the peak current on caffeine concentration is depicted in the calibration curve shown in Figure 7.

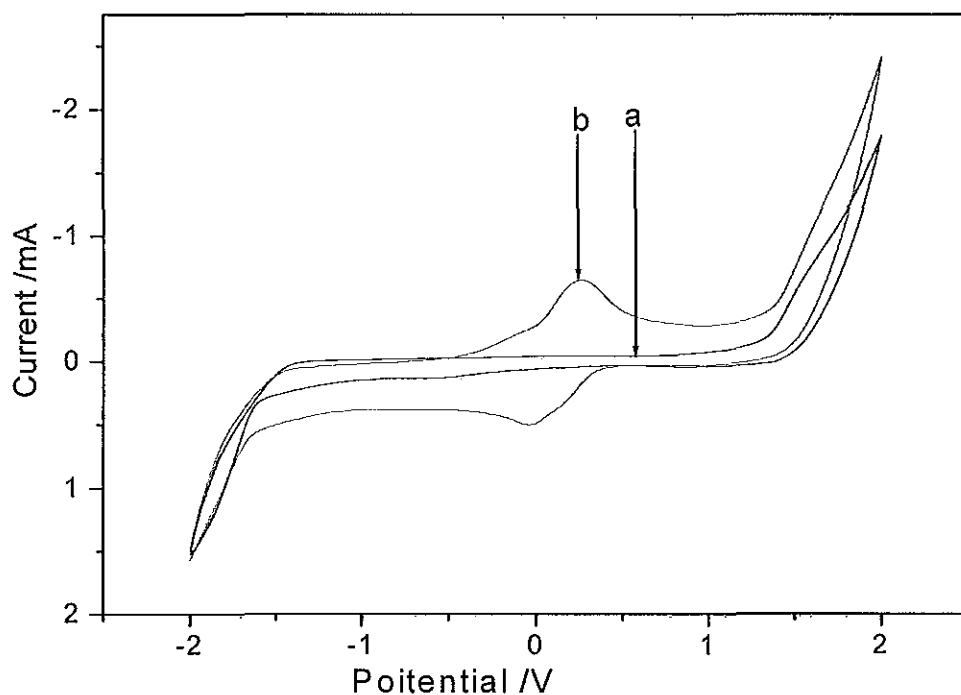
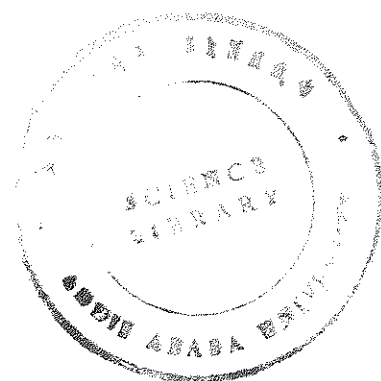


Figure 8. Cyclic voltammogram for the redox behavior of 1,4-benzoquinone: a) Unmodified electrode b) 1,4-benzoquinone modified electrode

These peaks which are due to the redox behavior of 1,4-benzoquinone within the modified electrode decreases when caffeine was added to the solution. However change in peak potentials was not observed. Further addition of caffeine in the solution resulted in a successive decrease of the peak currents in the voltammogram as shown in figure 9



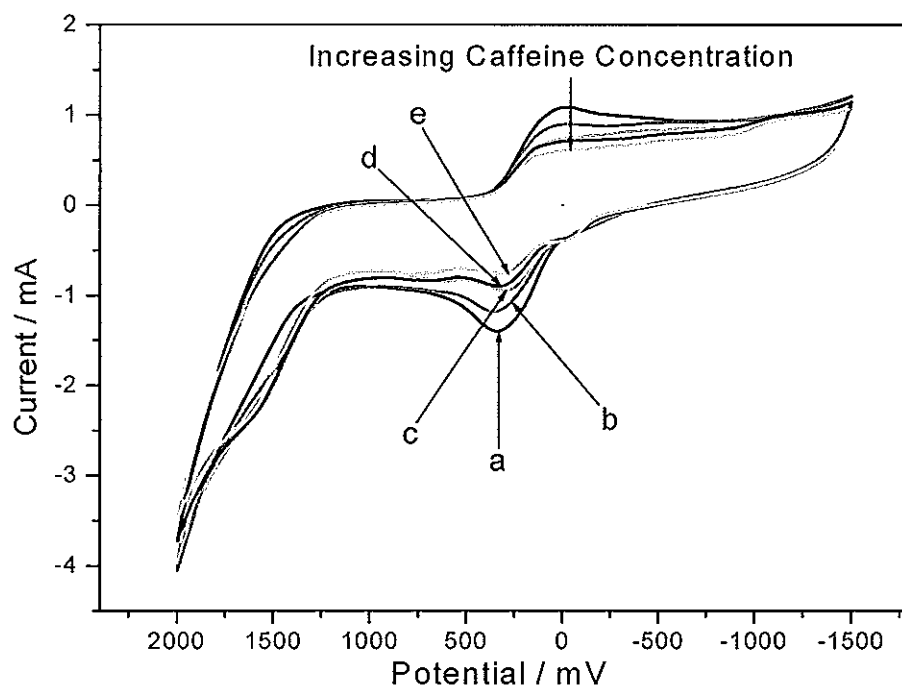


Figure 9. Cyclic voltammogram for the redox behavior of 1,4-benzoquinone at different caffeine concentrations: a) 0 b) 2 c) 4 d) 6 e) 8 mM caffeine.

The redox behaviour of 1,4-benzoquinone as well as the effect of adding caffeine on the peak currents obtained for 1,4-benzoquinone was further investigated using Square Wave Voltammetry, as the peaks are symmetric and thus the forward and back ward currents will add up to give better sensitivity for a given concentration..

4.1.2 Square Wave Investigation

The square wave voltammogram for the redox behavior of 1,4-benzoquinone within the electrode is shown in figure 10.

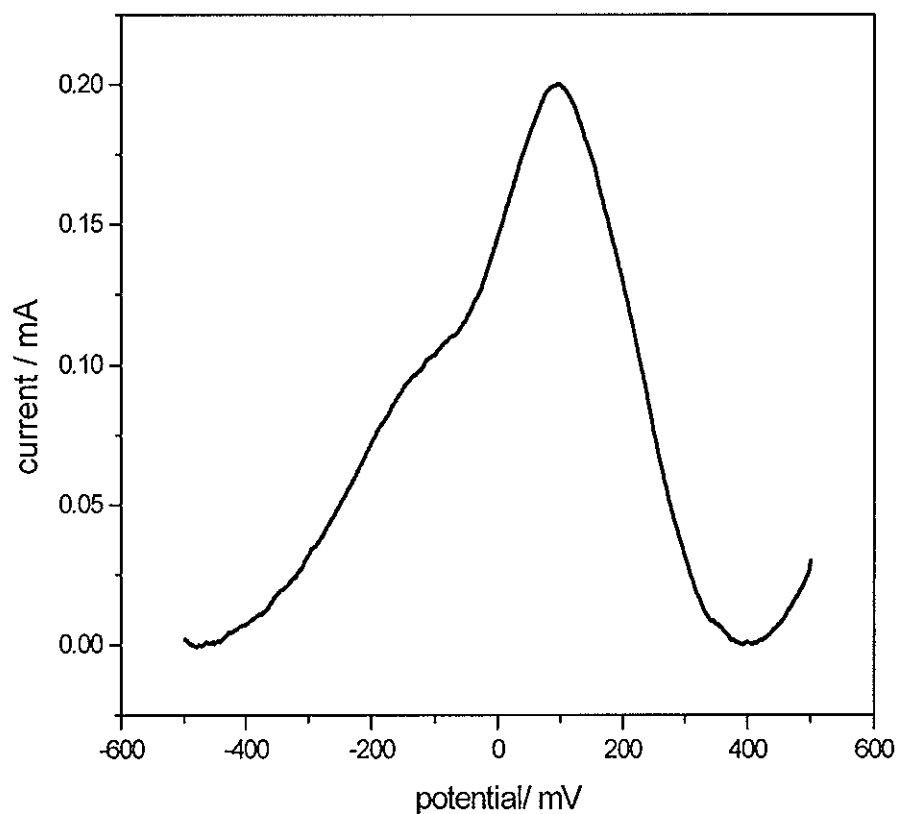


Figure 10. Square wave voltammogram for the redox behavior of 1,4-benzoquinone.

In the presence of caffeine within the solution, the square wave voltammogram of 1,4-benzoquinone displays a distinct reduction of the peak current. Further increase in the concentration of caffeine resulted in a successive decrease of 1,4-benzoquinone redox peak, as observed in cyclic voltammetry.

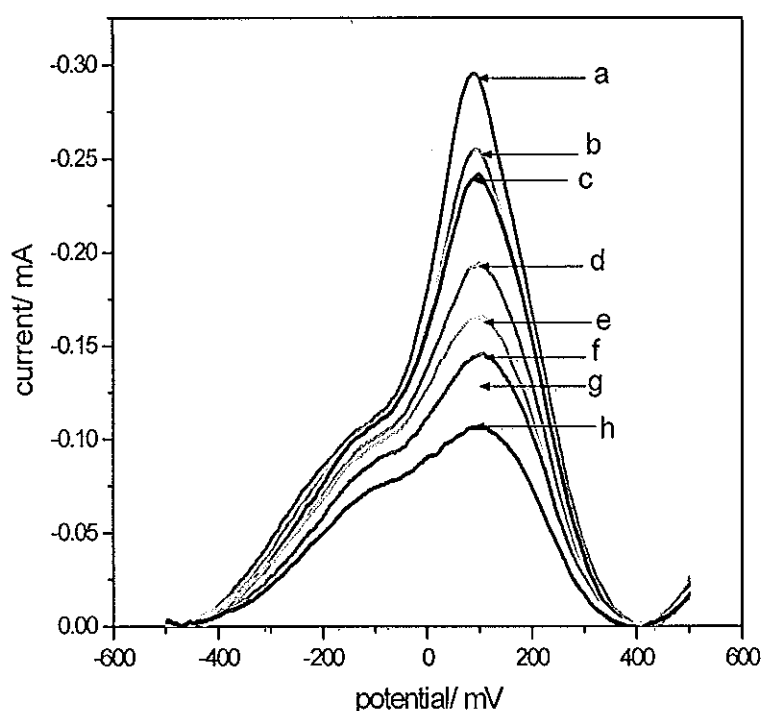


Figure 11. Square wave voltammogram for the redox behavior of 1,4 - benzoquinone at different caffeine concentrations: a) 0 b) 0.01 c) 0.05 d) 0.2 e) 0.5 f) 2 g) 4 h) 6 mM caffeine.

The relation of the decrease in the peak current of 1,4-benzoquinone and increase in the concentration of caffeine can be given by the following expression:

$$i_{pBQ} = KC_{BQ} \quad \text{initial condition} \quad (6)$$

$$-i_{pBQ} = KC_{Ca} \quad \text{observed} \quad (7)$$

Where C_{BQ} is initial concentration of 1,4-benzoquinone, C_{Ca} is concentration of caffeine, K is $2.69 \times 10^5 n^{3/2} AD^{1/2} v^{1/2}$

$$i_{pBQ} + (-i_{pBQ}) = K_{BQ}C_{BQ} + K_{Ca}C_{Ca} \quad (8)$$

$$\Delta i_p = K_1 + K_{Ca}C_{Ca} \quad (9)$$

Where $K_1 = K_{BQ}C_{BQ}$

between caffeine and the reduced species of 1,4-benzoquinone and it might also be competitive surface adsorption

In order to further check the interaction of caffeine with the reduced species of 1,4-benzoquinone, the experiment was conducted using 1,4-benzoquinone in solution at different caffeine concentrations with unmodified carbon paste electrode. The result shows that the peak current decreases with increasing caffeine concentration as we observed previously when 1,4-benzoquinone was within the electrode

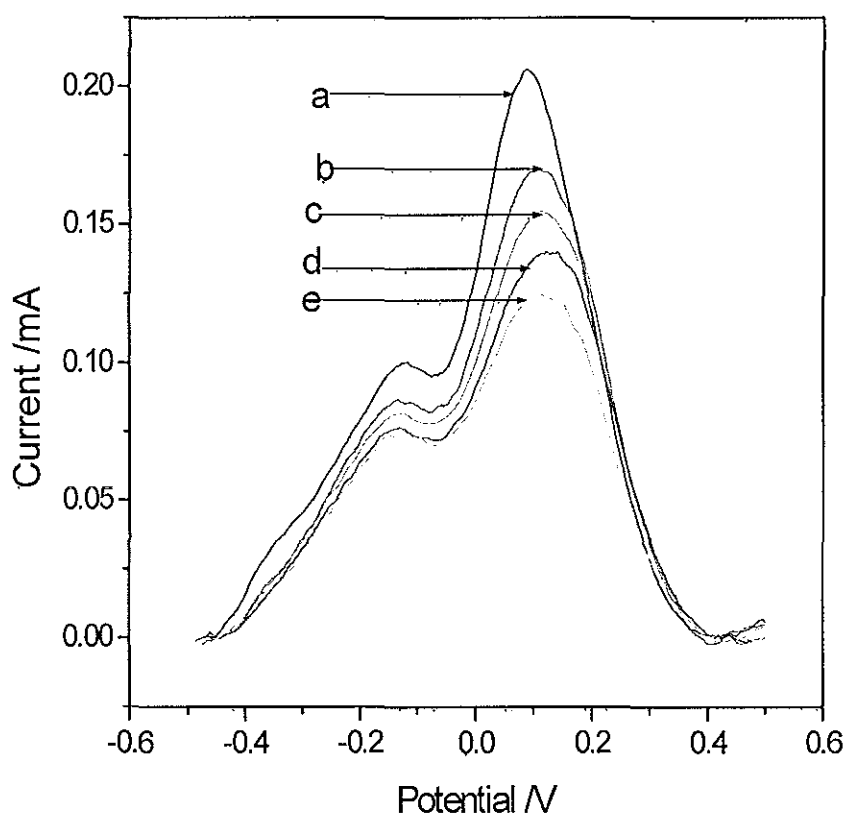


Figure 13. Square wave voltammogram for the redox behavior of 1,4-benzoquinone in solution at different caffeine concentrations: a) 0 b) 2 c) 4 d) 6 e) 8 mM caffeine.

There may also be current suppression in a similar manner as fluorescence quenching. If this may be the case, then there could be some change in the bonding strength in 1,4-benzoquinone and/or caffeine. To check this phenomenon, the IR- spectrum for caffeine, 1,4-benzoquinone and

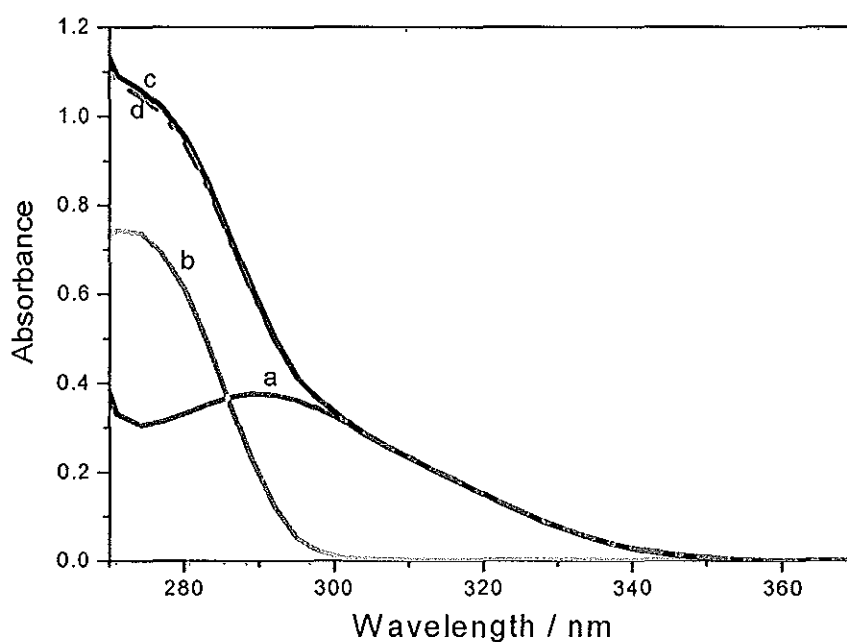


Figure 16. Absorption spectrum of: a) 1,4-benzoquinone b) caffeine c) 1,4-benzoquinone and caffeine solution, and d) the sum of 1,4-benzoquinone (a) and caffeine (b) spectra.

As we observe in figure 16, curve c is the absorption spectrum of caffeine and 1,4-benzoquinone solution. This curve is equal to curve d, which is the convolution of caffeine (b) and 1,4-benzoquinone (a) spectra. This implies that the absorption spectrum of caffeine and 1,4-benzoquinone solution is not for a new compound resulting from the reaction between caffeine and 1,4-benzoquinone. This was taken as a very important justification for the absence of reaction between caffeine and 1,4-benzoquinone.

In order to check the interaction of the reduced species of 1,4-benzoquinone with a compound that contains N-atom as in caffeine, square wave voltammetry experiments were conducted with *p*-nitroaniline. The result is shown in figure 17. The peak currents of 1,4-benzoquinone decreases with increasing the concentration of *p*-nitroaniline. This decrease in peak current might be due to the interaction of the intermediate species of 1,4-benzoquinone with *p*-nitroaniline through hydrogen bonding or exciplex formation. This confirms that the reduced species of 1,4-benzoquinone interacts with a compound that contains N-atom like caffeine.

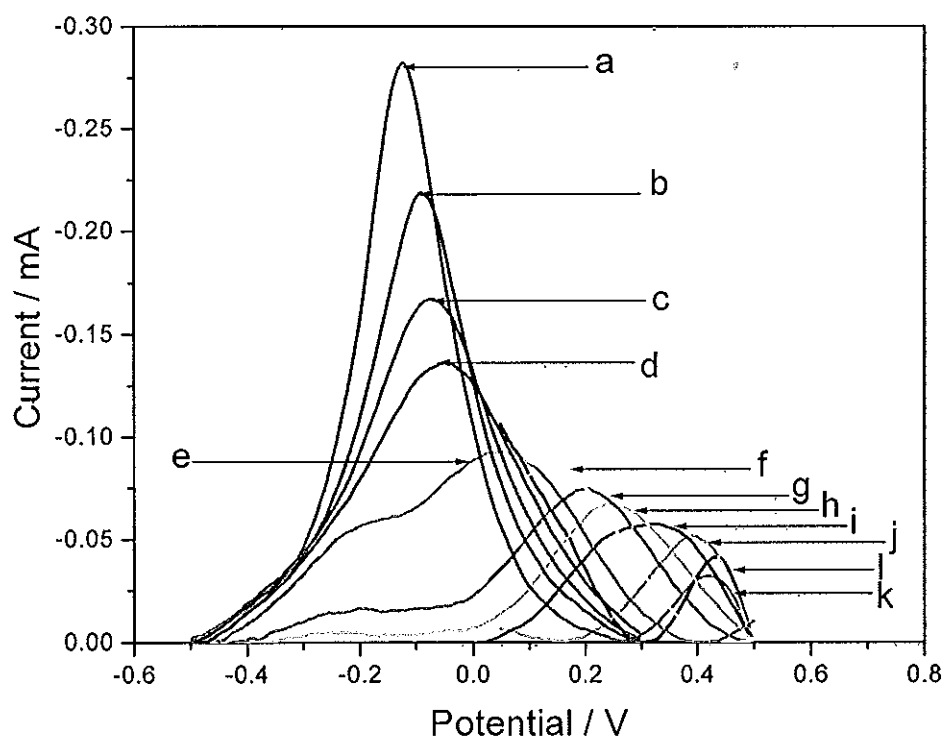


Figure 18. Effect of pH on the peak current of 1,4-benzoquinone with 2 mM caffeine. pH values of the peaks are: a) 12 b) 11 c) 10 d) 9 e) 8 f) 7 g) 6 h) 5 i) 4 j) 3 k) 2 l) 1.

The dependence of the peak current and the peak potential of 1,4-benzoquinone on pH are shown in figure 19 and 20 respectively. The influence of pH on the peak current of 1,4-benzoquinone was investigated over the pH range 1-12. In the pH range 2-8, the peak current of 1,4-benzoquinone increases linearly, with a slope $(\partial I/\partial \text{pH})_{C,T}$ equal to 8.72×10^{-6} . Further, the current increases sharply, in the pH range 9-12 with a slope $(\partial I/\partial \text{pH})_{C,T}$ equal to 4.895×10^{-5} . Since current is proportional to the rate of the electrochemical reaction, one could say that the rate of the electrochemical reaction of 1,4-benzoquinone increases with increasing pH. Furthermore the change in the slope of current versus pH implies that the mechanism for the electrochemical reaction of 1,4-benzoquinone changes at about pH 8.

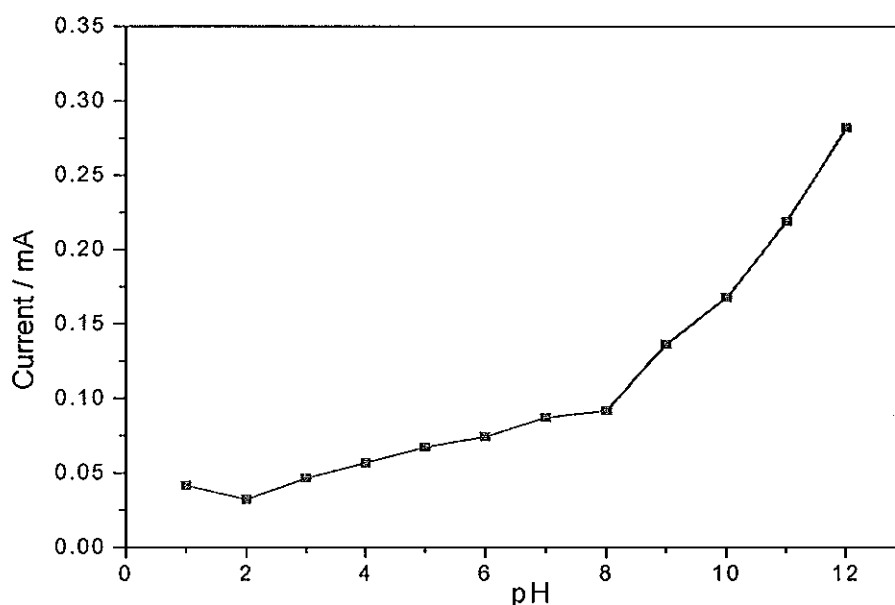


Figure 19 Dependence of the peak current of 1,4-benzoquinone on pH for 2 mM caffeine

For the redox behavior of 1,4-benzoquinone, the equilibrium position of 1,4-benzoquinone/hydroquinone is proportional to the square of the hydrogen-ion concentration. This implies that the electrode potential is sensitive to pH; a change of one unit of pH in water solution changes the potential of the electrode by 0.059 V [43]. Therefore, the shift in the square wave peak potential as a function of pH was studied and a linear dependence was observed. As can be seen from figure 20, it is observed that the peak potential of 1,4-benzoquinone shifts towards negative potential as the pH increases. This indicates that the hydrogen ion takes part in the electrode reaction. According to the literature [42], $E_p = K - (0.059\gamma/n)pH$, where γ is the number of hydrogen-ions that take part in the electrode reaction and n is the number of electrons, for a reversible reaction. There are two linear plots in the figure that are described by the following equations:

$$E_{p(V)} = -66.1143 \text{ pH} + 594; \quad R = -0.99937 \text{ (pH 3-8)} \quad (10)$$

$$E_{p(V)} = -25.3 \text{ pH} + 181; \quad R = -0.99964 \text{ (pH 9-12)} \quad (11)$$

The dependence of the peak potential on pH has slopes of 66 mV and 25 mV per pH unit, respectively. This implies that the number of protons involved in the redox process changes from two to one as the pH increases (for $n = 2$). Electrode process involving a weak acid or base have potential-pH variations which show a change in slope at $\text{pH} = \text{pK}_a$. From the intersection of the linear parts of the plots, the pK_a value of 1,4-benzoquinone was calculated to be 10. This is in a very good agreement with the literature value of 10.16 [45].

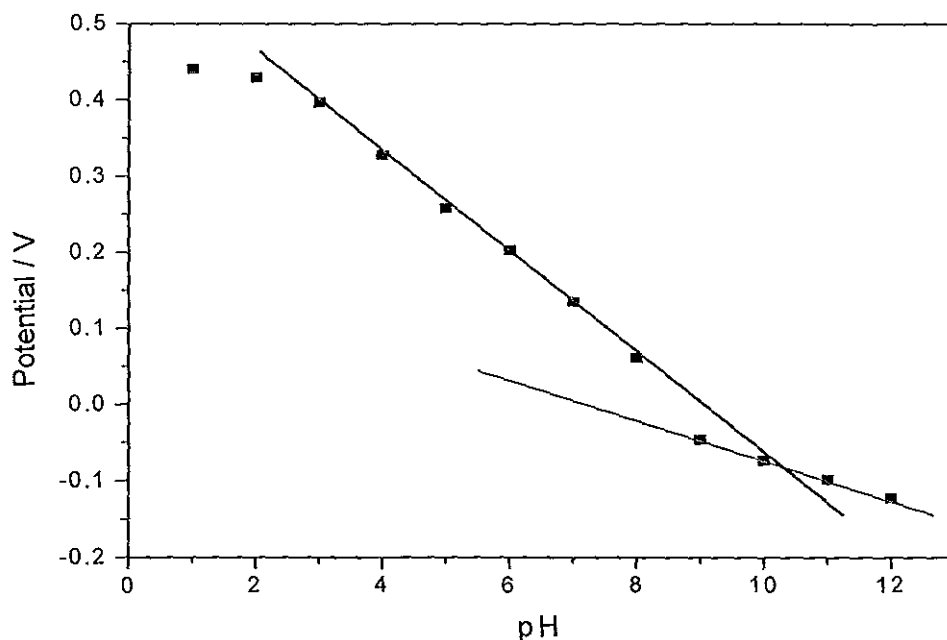


Figure 20 Dependence of the peak potential of 1,4-benzoquinone on pH for 2 mM caffeine

To check whether the peak currents observed at different pH values are due to 1,4-benzoquinone or caffeine, the experiment was conducted with 1,4-benzoquinone in solution in the absence of caffeine with unmodified carbon paste electrode in the pH range of 2 to 11. The resulting voltammogram is similar to the one obtained using caffeine solution with 1,4-benzoquinone modified carbon paste electrode. From this it is possible to conclude that pH changes affect the redox behavior of 1,4-benzoquinone and the presence of caffeine in the solution does not alter the effect of pH on the redox behavior of 1,4-benzoquinone.

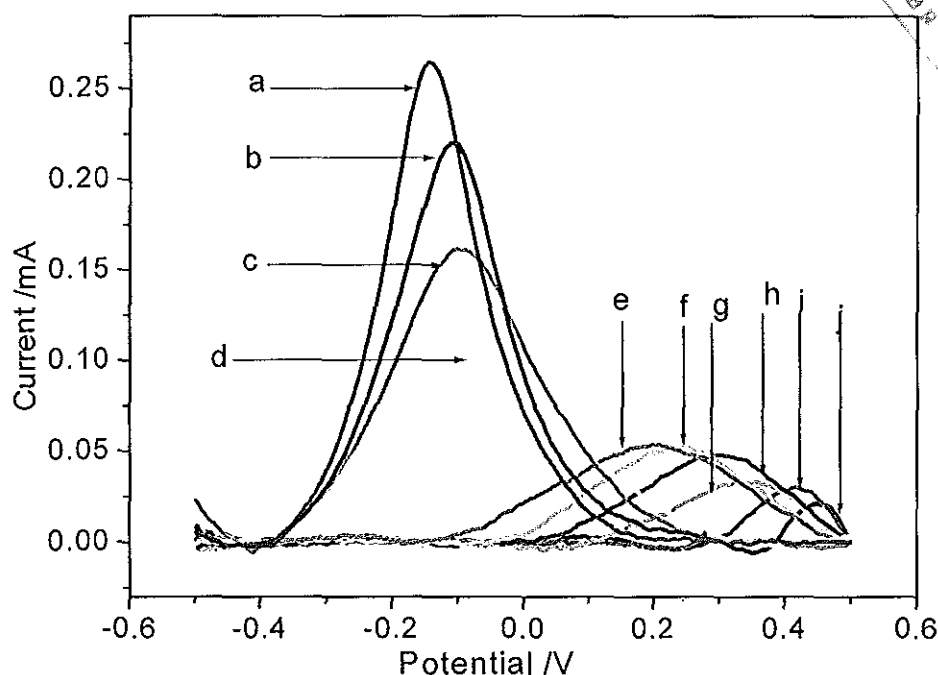


Figure 21. Effect of pH on the peak current of 2 mM 1,4-benzoquinone in the absence of caffeine. pH values of the peaks are: a)11 b)10 c)9 d)8 e)7 f)6 g)5 h) 4 i) 3 j) 2.

Figure 22 and 23 respectively show the dependence of the peak current and the peak potential on the pH value of 1,4-benzoquinone in solution and in the absence of caffeine. Similar trends are observed for both like the ones obtained for 1,4-benzoquinone incorporated in the paste. It can be concluded that integration of pH effects on peak current and peak potential are alike regardless of whether we have 1,4-benzoquinone in solution or in the electrode.

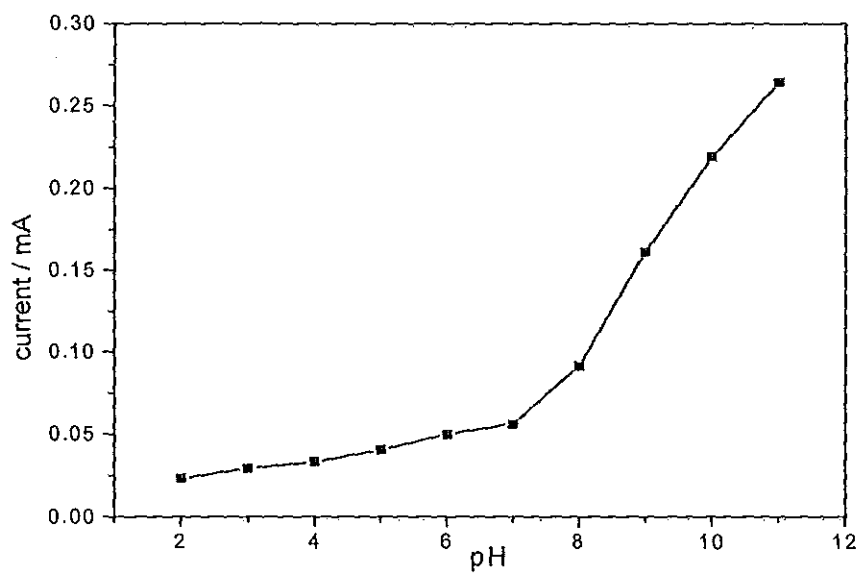


Figure 22 Dependence of the peak current of 1,4-benzoquinone in solution on pH in the absence of caffeine

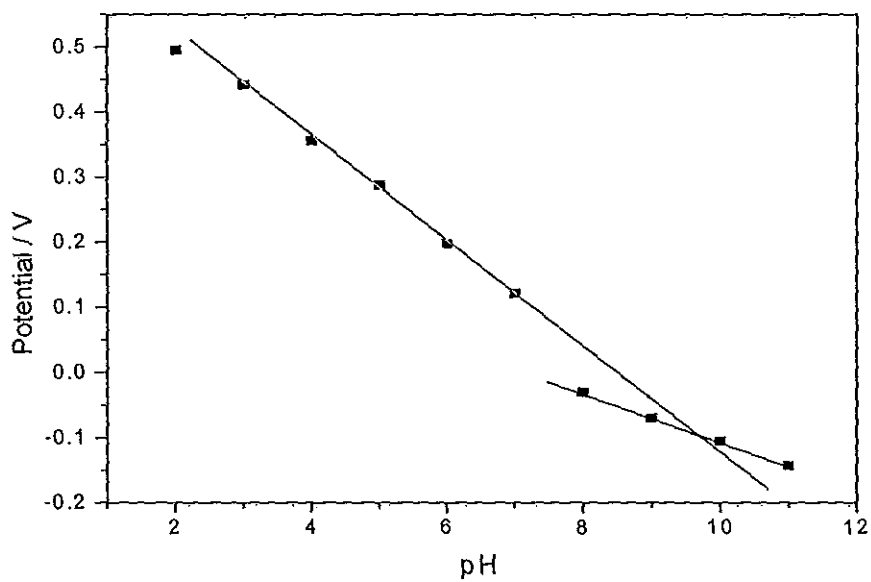


Figure 23 Dependence of the peak potential of 1,4-benzoquinone in solution on pH in the absence of caffeine

In our experiment, the electrode was not stable at higher pH values and the result was not reproducible. This is because the method for the electrochemical reduction of 1,4-benzoquinone is not very useful above pH 9 and 10 because 1,4-benzoquinone reacts irreversibly with alkali [43]. Therefore, we used pH 6 throughout the experiment.

4.2.2 Effect of Square wave frequency

The effect of square wave frequency on the peak current of 1,4-benzoquinone was studied by varying the frequency from 25 Hz to 50 Hz at step potential of 2 mV and amplitude of 30 mV for 2 mM caffeine solution. The maximum peak current obtained was at 25 Hz and thus it was chosen for subsequent experiments. The effect of square wave frequency on peak current of 1,4-benzoquinone is shown in figure 20.

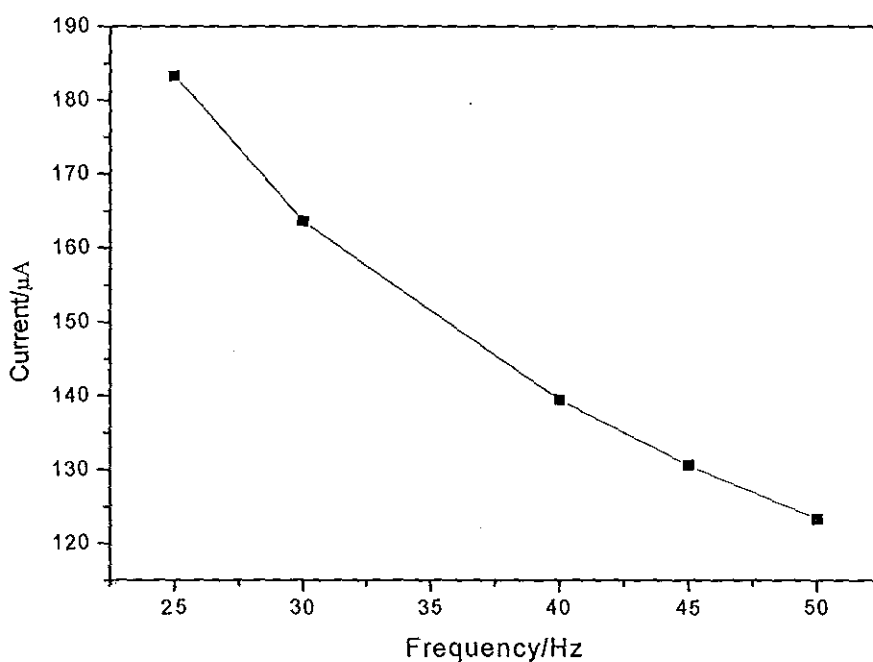


Figure 24. Dependence of peak current of 1,4-benzoquinone on the square wave frequency for 2 mM caffeine.

Square wave frequency has also a diagnostic value, as it indicates whether the electrochemical process is diffusion or adsorption controlled. This can be evaluated by plotting i_p versus $f^{1/2}$.

The plot of i_p versus $f^{1/2}$ is linear with slope 2.17×10^{-5} as shown in figure 25. This confirms that the electrode process is diffusion controlled, which is in agreement with the previously reported results for the determination of caffeine at both chemically modified and unmodified electrode.

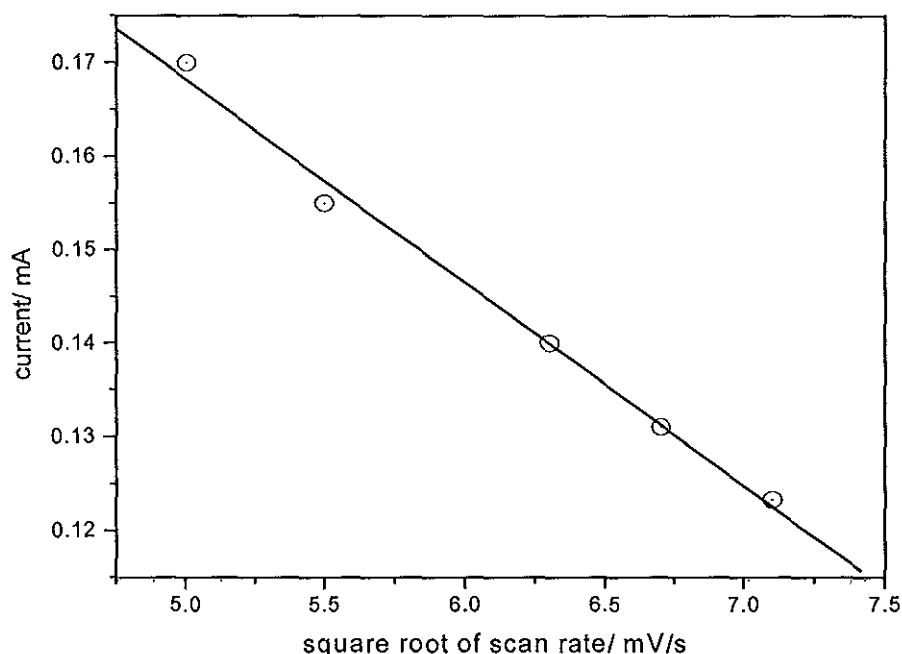


Figure 25. Plot of peak current of 1,4-benzoquinone versus square root of frequency for 2 mM caffeine.

4.2.3 Effect of Square Wave Pulse Amplitude

The effect of pulse amplitude on the peak current of 1,4-benzoquinone was studied by varying the pulse amplitude from 5 mV to 85 mV at frequency of 25 Hz and step potential of 2 mV. Upon increasing the pulse amplitude a linear increase in the peak current was observed accompanied by peak broadening in particular when the amplitude was greater than 30 mV. Hence, 30 mV was chosen as the square wave pulse amplitude for the subsequent experiment. The peak current for 1,4-benzoquinone as a function of the square wave pulse amplitude is shown in figure 26.

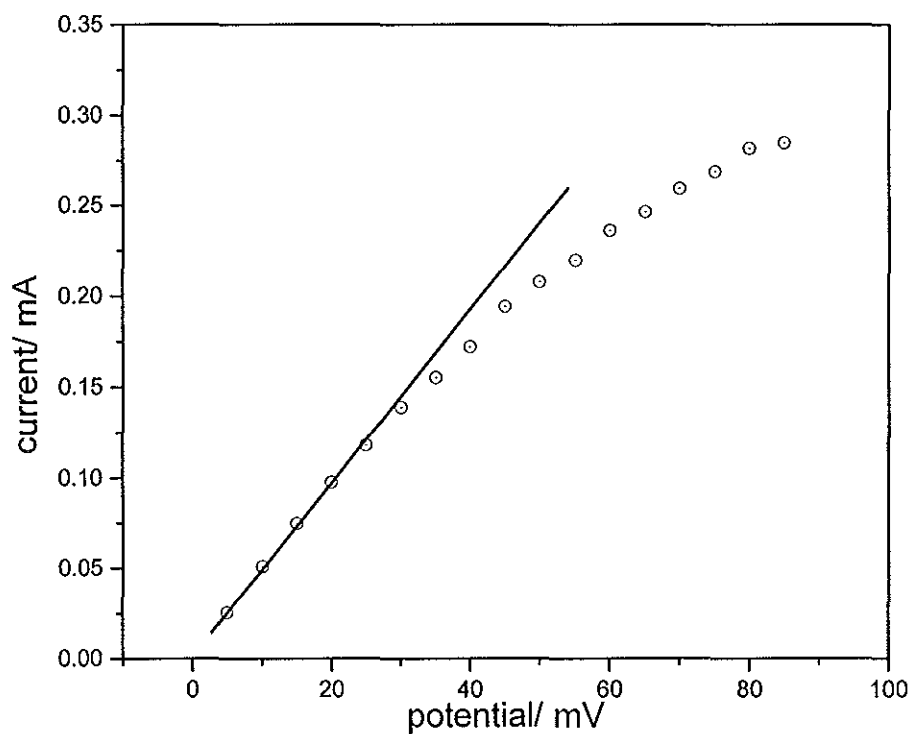


Figure 26. Square wave voltammetric peak currents of 1,4-benzoquinone at various square wave amplitude.

4.2.4 Effect of step potential

The effect of step potential on the peak current of 1,4-benzoquinone was studied by varying the step potential from 2 mV to 10 mV at a frequency of 25 Hz and amplitude of 30 mV. The maximum peak current was observed at 2 mV and thus it was chosen for the subsequent experiment. The peak current for 1,4-benzoquinone as a function of the square wave step potential is shown in figure 26.

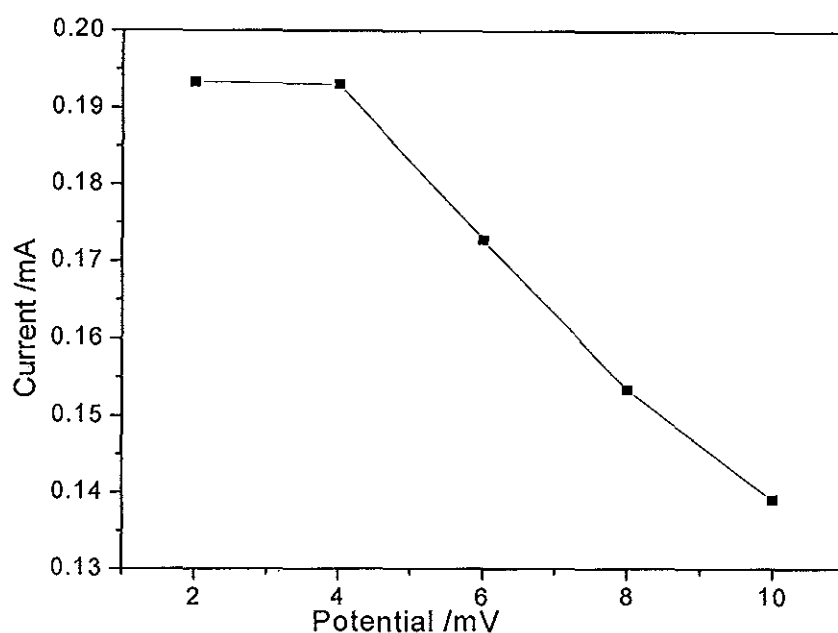


Figure 27 Square wave voltammetric peak current of 1,4-benzoquinone at various step potentials.

4.2.5 Electrode composition effect

The effect of the amount of 1,4-benzoquinone in the carbon paste on the voltammetric response of the modified electrode was studied by varying the amount of 1,4-benzoquinone. The peak currents increased with increasing the amount of 1,4-benzoquinone up to 10 % (w/w). For 1,4-benzoquinone amounts higher than 10 % (w/w) the peak currents decreased significantly. This occurs due to a decrease in the graphite content in the paste and, consequently reduction of the conductive electrode surface [11, 22]. The best composition was found for an electrode composition of 10 % (w/w) 1,4-benzoquinone, 25 % (w/w) paraffin oil, and 65 % (w/w) graphite powder.

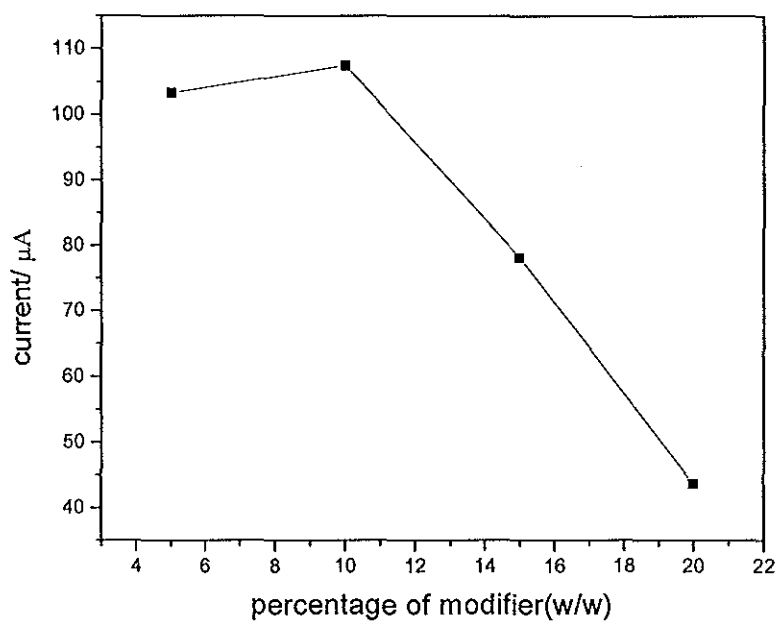


Figure 28.Effect of electrode composition on the voltammetric peak current of 1,4-benzoquinone .

The optimum parameters used are summarized in Table 3.

Table 3: Optimum values of the experimental parameters.

Parameters	Optimum values
pH of supporting electrolyte	6
Square wave frequency (Hz)	25
Square wave amplitude (mV)	30
Step potential (mV)	2
Composition of 1,4-benzoquinone	10 % (w/w)

4.3. Linear Range and Detection Limit

The analytical utility of a given procedure depends on obtaining well-defined dependence of current responses on concentration. The dependence of the voltammetric signal of 1,4-benzoquinone on the concentration of caffeine and the inherent sensitivity of this method is illustrated by square wave voltammetry for different concentration of caffeine based on the optimum experimental conditions (Table 3), and the procedure described in section 3.4. The relationship between the square wave voltammetric peak current of 1,4-benzoquinone and caffeine concentration was examined. A calibration curve was constructed by measuring the peak currents of 1,4-benzoquinone for each calibration standard.

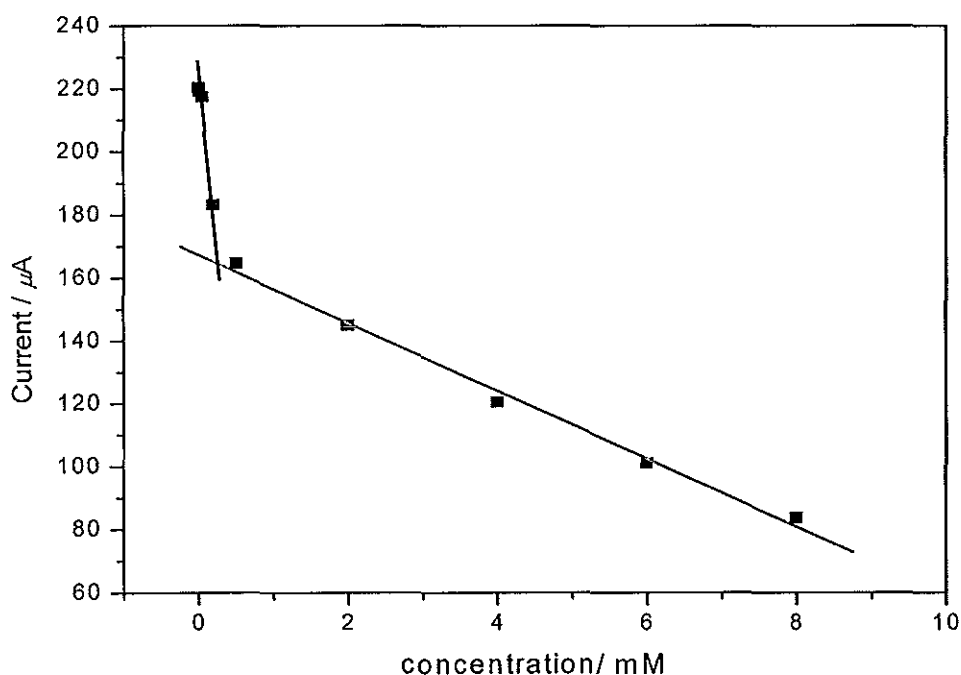


Figure 29. Calibration curve for the effect of caffeine on the redox behavior of 1,4-benzoquinone. Fitness values: slope $-10.8134 \mu\text{A}/\text{mM}$ (-10.8 ± 1.712) $\mu\text{A}/\text{mM}$. Regression coefficient: -0.9982 .

Figure 11 and 29 show square wave voltammograms and calibration graph for the dependence of the square wave peak current of 1,4-benzoquinone on the concentration of caffeine respectively. All the peak currents below caffeine concentration of 0.5 mM lie above the straight line. Repeated measurements were carried out to confirm this observed pattern. Many repetitions show that these points are consistently above the straight line. Therefore the linear range for the determination of caffeine with 1,4-benzoquinone modified carbon paste electrode extends from 0.5 mM to 8 mM caffeine.

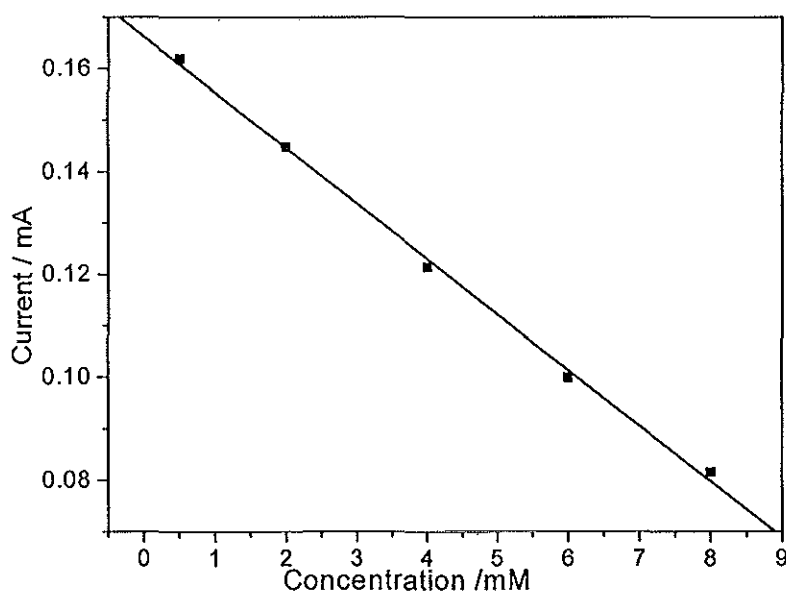


Figure 30 calibration curve for the effect of caffeine (concentration range 0.5-8 mM) on the redox behavior of 1,4-benzoquinone. Fitness values: slope $-10.8134 \mu\text{A}/\text{mM}$ (-10.8 ± 1.712) $\mu\text{A}/\text{mM}$ Regression coefficient: -0.9982

The detection limit, concentration equal to three times the standard deviation of the peak current for five determinations of 2 mM of caffeine was found to be $5.136 \mu\text{g}/\text{mL}$. All the peak currents below caffeine concentration of 0.5 mM can form a linear region. This region can also be used for analytical purpose. This increases the detection limit of 1,4-benzoquinone modified carbon paste electrode

Table 4: Comparison between BQMCPE and other methods.

Method	Detection limit	Linear range
BQMCPE	0.33 μ M	0 – 0.5 mM (0 – 97 mg / L)
	5.136 μ M	0.5 mM – 8 mM (97 mg/L – 1552 mg/L)
HPLC method [3]	0.3 μ M	0 – 300 mg/L
Nafion CME method [13]	2 μ M	0 – 100 mg/L

4.4 Real sample analysis

To obtain the calibration curve presented in figure 24, a series of standard solutions of caffeine were used. The standard solutions were prepared using the same procedure used to prepare the unknown sample solution. The peak current of the unknown sample solution prepared in the same way fall within the region covered by the standards. With that the validity of the calibration was established and the quantity of caffeine in the unknown sample was determined from the calibration curve. Thus, the quantity of caffeine obtained using this method was (4.2845 ± 0.001712) mM. The result is in good agreement with the value 4.1 mM determined by UV–VIS spectroscopic method.

5. Conclusion

In this study, it was demonstrated that 1,4-benzoquinone chemically modified electrode can be applied to the determination of caffeine in coffee using square wave voltammetric technique. During the study, it was observed that the voltammetric peak current of 1,4-benzoquinone decreases with increasing caffeine concentration. The relation of decreasing in 1,4-benzoquinone peak current and increasing caffeine concentration is linear. This suggests that 1,4-benzoquinone modified carbon paste electrode acts as a useful material for the development of the technique to determine caffeine content from coffee. But the technique must be developed by further work to apply for quantitative analysis.

6. References

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