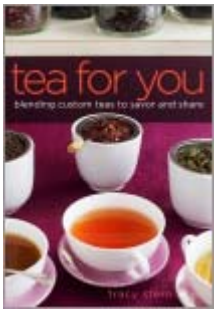
ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

ELECTROCHEMICAL DETERMINATION OF CATECHOL IN TEA SAMPLES USING ANTHRAQUINONE MODIFIED CARBON PASTE ELECTRODE

BY: FEKADU MELAK



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A PROJECT SUBMMITED TO THE DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY.

BY FEKADU MELAK

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Declaration

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

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Abstract:

The electrochemical behavior of catechol was investigated using square wave voltammetry with anthraquinone modified CPE and was found to be very sensitive. The modified electrodes showed an enhancement of current response for catechol as compared to the unmodified carbon paste electrode. Two varieties of tea, namely Green tea, and Wush Wush tea were investigated. Responses for the extracts using ethanol: water mixture showed green tea to be superior in catechol content.

Optimization of different variables such as pH of working solution, modifier composition and square wave parameters such as frequency, amplitude and step potential were made to improve the method efficiency during the experiment. The reproducibility for the nine repeated analysis of 80 $\mu molL^{-1}$ of catechol gave a relative standard deviation of 3.65%, indicating excellent reproducibility of the method. Linear calibration plots were obtained in the range 6 to 80 $\mu molL^{-1}$ with (R = 0.99853) and the detection limit with (S/N=3) was as low as $2.155x10^{-7}$ $molL^{-1}$.

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Abbreviations and symbols

SWV	square wave voltammetry
CV	cyclic voltammetry
CPE	carbon paste electrode
mCPE	modified carbon paste electrode
CMEs	chemically modified electrodes
t_p	pulse width
LOD	limit of detection
RSD	relative standard devation
E_{sw}	amplitude potential

1. Introduction

1.1 Origin and History of Tea

Tea is one of the most consumed beverages in the world and was first used in China for medical properties 5000 years ago [1]. There are two recognized varieties or groups referred to as assamica or Assam and China, from their origins in Assam and China. Assam and China tea differ in morphological features and quality. As far as the Western tea trade is concerned, Assams have been regarded as producing the best fermented tea. In recent years, however, jats of China origin or hybrids with Assam have also been found that produce high quality fermented tea. Legends, and most sources, acknowledge the Chinese Emperor Shen Nung (28th century B.C.) with the discovery of tea. The tea plant originated in South-East Asia, probably in the region incorporating sources and high valleys of the Brahmagutra, the Irrawaddy, the Salween and the Mekong rivers at the border separating India, China and Burma. The Chinese are familiar with camellia leaves, which they used in vegetable relishes and quite probably as part of medicinal compounds. But until the emperor's discovery, the leaves had never been considered an ingredient of a hot, refreshing drink [2, 3].

Historically, the cultivation of tea plant in other countries was started at different time. The first cultivation introduced to various countries was Indonesia in 1684, India in 1780, Russia in 1833, Sri Lanka in 1839, Malawi in 1875, Iran in 1900, Kenya in 1903, and Turkey and Argentina in 1924. In the late 17th century, tea became a popular beverage served in numerous tea houses in London. Its popularity was rapid and today it is the most widely consumed beverage in the world next to water [3].

1.2 Botany of Tea Plant

When left unpruned, tea grows in to a small tree about 10 m high with a conical shape. In cultivation, it is pruned to form a hedge of convenient height for hand plucking or

machine harvesting. Much of the tea produced in tropical countries is grown on steep hillsides at high elevations [3].

The tea shrub is a perennial evergreen plant. It is under the Theaceae family and the Camellia species (Camellia sinensis). In fact there are other varieties in addition to the previous two types of varieties. These varieties differ in the height of the tea bush, the number of stems and characteristics of their leaves. Leaves are produced alternatively on branches which originate in the leaf axils lower in the canopy. The leaves have a finely serrated margin. All tea leaves are somewhat glabrous and shiny and they contain thickened stone cells (scleroids). These are of importance in the tea-trade because their presence in made tea to determine its quality. With the tea crops, leaf growth is of central importance because the leaves provide the yield. Tea flushes at regular frequency which is not strongly related to climatic changes, though the quantity of leaf produced at flush is greatly affected by rainfall and temperature as well as by nutrition. The frequency of plucking depends on the flushing and growth rates; it will vary from one to two weeks in tropics, during the period of flushing. In general, for quality of tea two leaves and a bud are the ones to be plucked. It is very important for continued production not to pluck the mature leaves below this; these are called maintenance leaves [4].

1.3 Climate and Soil for Tea Growth

Climate and soil characteristics are the most important ecological factors in growing tea. The variety of the tea plant is also another factor in growing tea. For instance, the Assam variety is less hardy than the China variety, which can tolerate a longer dry season or lower temperature. It grows under a variety of climate conditions from the humid tropical lowlands to regions of high latitudes in Russia and Japan. In tropical countries, tea leaves are harvested all year around. In temperate countries, harvesting is seasonal. There are many different kinds of products of different quality arising from different cultivation practices, growing conditions and processing methods. Application of fertilizer is also important for the growth of the tea plant and the quality of the black tea leaves. For instance, an increase in the potassium application rate increases the polyphenol and

amino acid content of the tea. The failure to apply fertilizer resulted in depletion of the organic matter status of the soil [3].

1.4 Types of Tea

There are several major categories of tea, which are distinguished by different processing methods and, consequently, different concentrations of the chemical components in tea. Depending on the degree of fermentation prior to further heating and processing teas are categorized in to three major varieties: green tea, Oolong tea and black tea. The fermentation process involves an enzymatic oxidation of polyphenols, leading to the formation of chemical compounds that generate both the aroma and color of black tea.

The Green tea is a yellow to green color which is preferably manufactured from the Camellia sinensis variety Sinensis. Its chemical constituents are not altered by fermentation and the enzyme is inhibited by tea. In general, green tea doesn't involve the withering processes as well as fermentation.

The Oolong tea is a semi-fermented tea which is manufactured by letting the tea leaf to undergo incomplete fermentation. The Oolong tea, with a large twisted leaf, brownish in color with white tips, produces a light green, slightly coppery infusion. Its chemical composition is in between the green tea and the black tea [2].

The Black tea is a completely fermented tea leaves. Fresh tea leaves are rich in polyphenolic compounds known as catechins. When tea leaves are intentionally broken or rolled during processing catechins become oxidized through the action of polyphenol oxidase enzymes present in the tea leaves. The oxidation of catechins, known as fermentation in the tea industry, causes them to polymerize and to form larger, more complex polyphenols known as theaflavins and thearubigins. This brings a difference in the color, aroma and chemical composition of the tea leaf as compared to green tea and oolong tea [5, 6].

In fact there are other two types of tea, the white and yellow teas that have been regarded as two subclasses of green tea. These two types of tea are different from green tea due to differences in variety, processing, geographical and traditional distributions [3].

1.5 Tea in Ethiopia

The production of tea-leaves in Ethiopia has got a history of more than half a century. Nevertheless, it is only three decades since the consolidated and organized development of tea production began. The production of tea-leaves was begun in 1984 with the support of the state and up to 1996 not more than 2,000 hectares were produced. In Ethiopia, tea is mostly grown in the highland dense forest regions where the land is fertile and thus the use of fertilizer is very minimal.

Moreover, the availability of abundant and cheap labor in the country has made the use of manual weeding, instead of chemical weeding, possible. Because of this mostly organic cultivation, Ethiopian tea is increasingly sought for its aroma and natural flavors [2, 5].

Table 1: Facts and figures about Wush Wush and Gumaro tea plantations as detailed in reference [5].

	Wush Wush tea plantation	**Gumaro tea plantation**
Location	Kaffa Zone, Gimbo Wereda, SNNPR	Illubabor Zone, Ale Dido Wereda, Oromya Region
Distance from Addis Ababa	460 km southwest of Addis Ababa	637 km southwest of Addis Ababa
Altitude	1900 m	1718 m
Annual rainfall	1820 mm	2089 mm
Temperature	12–24 °C	12–27 °C
Area under tea	1249 ha	860 ha
Soil	Fertile, good drainage, red brown color	Same as at Wush Wush

1.6 Economic Importance of Tea

A Chinese document published in 347 A.D. states that people in Southwest China used teas for paying tribute to the Chinese emperors as early as 1066 B.C. In the essay "Tong Yue", written by a country landlord Wang Bao and published in 59 B.C., there mentioned the making and sale of tea. It showed that tea was commercially available in the local country market, suggesting that tea processing and marketing as early as 59 B.C. in Southwest China.

Tea is the second most consumed beverage in the world with an estimated 18-20 billion cups consumed daily. The principal teas produced and consumed in the world are black and green teas, with small amount of other types. Black tea represents approximately 78% of total consumed tea in the world, whereas green tea accounts for approximately 20%. The major producers of tea are India, China, Sri Lanka, and Kenya, while the major consumers are India, China, Turkey, and Japan. During the 1990s, the world production and consumption of tea has increased steadily with occasional fluctuation in some years. Thus, Camellia sinensis has become a very important agricultural and commercial product, with unique horticultural and processing methods. It is easy to see how tea is commercially important. For instance, tea is the leading export crop in Kenya, which places Kenya to be the third largest producer of black tea after India and Srilanka and large amount of money is earned. Ethiopia also exports teas regularly to different countries nowadays [3, 5].

1.7 Health Benefits of Tea

The chemical components in black tea have a health benefit to human. Epidemiological and animal studies suggest that tea is protective against certain cancers, cardiovascular diseases, and neurodegenerative diseases [6]. Although the oxidization process modifies the type of flavonoid present, the total level and their overall antioxidant activity, is similar in both green tea and black tea. Teas are also used in folk medicine for headaches, body aches and pains, digestion, diuretics, enhancement of immune defenses, and detoxification, as an energizer, and to prolong life [2].

1.8 Black Tea Manufacturing

The green, glossy leaves and young new shoots two leaves and a bud for quality tea are plucked and immediately processed for optimal freshness. The first step in the manufacturing of black tea is withering, which removes a large proportion of the water from the fresh leaf by evaporation. The leaves become limp and suitable for rolling and undergoing fermentation.

Rolling consists of twisting or breaking up the leaves so that preparing them for fermentation and transforming them into particles corresponding to the type of commercial tea required.

Fermentation is the most important stage in the manufacture of black tea. It involves the enzymatic oxidation (fermentation) of the polyphenols which are converted into theaflavines and thearubigines, with the leaves turning from green to coppery brown color. In this process, the monomeric flavan-3-ols undergo polyphenol oxidase-dependent oxidative polymerization leading to the formation of bisflavanols, theaflavines, thearubigines. These chemical compounds possess benzotropolone rings with dihydroxy or trihydroxy substitution systems, which give the characteristic color and taste of black tea [3].

Firing will follow the fermentation step which stops the fermentation and reduce the water content of the tea which makes handling and transportation easy. The firing leads to the destruction of the polyphenol-oxidases. Then the tea will be packed after sorting, which consists of extracting the fibers with the aid of winnowing machines and grading the tea by size and volumetric weight [7].

1.9 Chemical Composition of Tea

The chemical composition of tea may vary depending on different parameters such as the variety of leaf, growing environment, application of fertilizers, manufacturing, particle size of ground tea leaves and infusion preparation. Tea leaves contain many compounds, such as polysaccharides, volatile oils, vitamins, minerals, purines, alkaloids (eg.caffeine) and polyphenols (catechins and flavonoids). Although all three tea types have

antibacterial and free radical capturing (antioxidising) activities, the efficacy decreases substantially when the tea becomes darker. This is due to lower contents of anti-oxidising polyphenols remaining in the leaves.

Flavonoids (polyphenols): Proven medicinal properties include antioxidant, anti-inflammatory, anti-allergic, antibacterial and antiviral effects. They also have the ability to strengthen veins and decrease their permeability. It is widely believed that the antioxidising effects of both black and green varieties are reduced when taken with milk. This is thought to be due to the effective binding of flavonoids by proteins [8].

However, a recent ex vivo study concluded that flavonols are absorbed from tea and their bioavailability is not affected by milk [8, 9].

Tea tannins - called catechins (polyphenols): Appear to be the most potent therapeutic plant-derived chemicals, in that, aside from their antiseptic and antioxidant properties, they are able to form complexes with other molecules, thereby detoxifying the system. Catechins include gallocatechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (EGC) and epigallocatechin gallate (EGCG). Catechins make up approximately one-quarter of fresh dried green tea leaves, of which EGCG comprises 60 %.

Vitamin C: A recent study by du Toit et al, showed that black, green and oolong tea are all extremely good sources of vitamin C. They found that one or two cups a day provide the equivalent of three glasses of orange juice or two capsules [8, 9].

Though there is personal difference, among the characteristics of a good cup of black tea they are red or rosy color and briskness without bitterness. The chemical entities in tea that are generally believed to cause variations in color and bitterness are chiefly the theaflavins (3-6%) and thearubigins (12-18%) of tea solids by weight, respectively. The quantities of each, and the ratio of their quantities, are said to determine both the color characteristics of the tea beverage (tea infusion). For example, a tea with a high level of theaflavins and a low level of thearubigins would tend to give a beverage with a yellow-orange color and a high degree of briskness. Conversely, a tea with a low level of theaflavins and a high level of thearubigins would be expected to give a tea infusion with a brown color and little briskness (a soft tea).A tea with an optimum level

of each of these chemical groups may give a tea infusion with a rosy color and appropriate briskness [6].

2 Catechol in Tea

Tea and coffee are popular beverages which provide significant source of phenolic compounds in the diet. The major organic compounds in tea belong to the catechin family, also known as flavan-3-ols which constitute upto 30% of tea solids by weight, while various flavonols are also present up to 4% [1]. Catechol (1,2-benzenediol) is a natural polyphenolic compound that widely exists in higher plants such as tea, vegetables, fruits, tobacco and some traditional Chinse medicines. Catechol has been widely studied due to its biological importance such as anti-oxidation, anti-virus, flower stimulating effect and affecting the activities of some enzymes. In addition, catechol and catchol derivatives affect the taste of teas and tobaccos and their quality can be improved by changing the content of these compounds [10-12].

Recent epidemiological studies have shown that the consumption of tea prevent cancer in humans because the tea leaf contains abundant groups of polyphenols. The benefits from the tea polyphenols are e.g., anti-inflammatory, anti-obesity, reduced incidence of a variety of cancers and cardiovascular diseases [1].

Aromatic hydroxyl phenols are common chemicals used in industries as well as in clinical and biochemical applications. Such chemicals often exist as a mixture of polyphenols in the working matrix. Due to human exposure to aromatic hydrocarbons, various phenolic derivatives were found in clinical blood and urine samples. Among these, catecholamines of o-diphenol derivatives are well known for their neurotransmission [13]. In 1950, it was suggested that benzene metabolites were responsible for the benzene toxicity in mammals and humans. According to Porteous and Williams catechol was already identified as one of these metabolites at the end of (1948). The toxicities of catechols for micro-organisms have been demonstrated in the last years, and have been suggested to be the reason for the difficulties in cultivating microorganisms on benzene, toluene or chlorobenzene. Several studies indicated that the toxicity of catechol for water flea, zebra fish, trout, rabbit, cat, rat, mouse and human cell

lines. Moreover, catechol is readily absorbed from the gastrointestinal tract causing renal tube degeneration, decrease in liver function. It is a significant environmental pollutant with high toxicity and it exists with hydroquinone in environmental samples. Even at low concentration in foods and cigarette smokes, catechol may cause mutagenesis and cancerous alterations. Indeed, in the environment toxic concentrations of catechol have been found though the modes of action causing toxicity are hardly understood [13, 14].

2.1 Environmental Effects of Catechol

Catechol (1, 2-dihydroxybenzene) is used in a variety of applications. It is used as a reagent for photography, dyeing fur, rubber and plastic production, pesticides and in pharmaceutical industries. Substituted catechols especially chlorinated and methylated catechols, are by-products in pulp and oil mills. Catechols are intermediary products from the degradation of aromatic compounds and lignin by microorganism.

In humans and mammals, catechols can occur as metabolites in the degradation of benzene or estrogens or as endogenous compounds such as neurotransmitters and their precursors [adrenaline, noradrenaline, dopamine and L-dopa]. Additionally, catechols can be taken up in the form of tobacco smoke (as catechol, catechol-semiquinones and polymerized catechols) or as food component (e.g., catechol, dopamine, caffeic acid, tea catechin) [11, 12, 15].

2.2 Chemical Properties of Catechol

Catechols can undergo a variety of chemical reactions, such as complex formation and redox chemistry of catechols. In presence of heavy metals such as iron or copper, stable complexes can be formed. In the presence of of oxidizing agents, catehols can be oxidized to semiquinone radicals and in the next step to o-benzoquinones. Heavy metals may catalyse radox reactions in which catechols are involved. As a consequence of the chemical properties and the chemical reactions of catechols, many different reactions can occur with biomolecules such as DNA, proteins and membranes, ultimately leading to non repairable damage. Reactions with nucleic acids such as adduct formation and strand

breaks are discussed in different literatures. Interactions with proteins causing protein and enzyme inactivation are also delineated [13]. The oxidation of catechol is a fairly reversible $2e^-$ processes leading to the o-quinone. The interest in catechols centers on the high reactivity of this o-quinone and it has provided an excellent substrate for their measurement of rapid 1, 4-addition reaction. The first such reaction involved the important class of biogenic catecholamine, adernaline, noradrenaline, also known as epiphrine & norepiphrine respectively, dopamine and related substances [16]. Due to its biological importance and toxicity effects catechol needs rapid and sensitive methods to detect it.

2.3 Methods of Catechol Detection

Many analytical methods have been reported for the determination of catechols and its derivatives including: IR spectrometry, UV vis spectrophotometry, thin layer chromatography, high performance liquid chromatography, electrochemical luminescent inhibition [12] and flow injection molecularly imprinted solid phase extraction for selective spectrophotometric detection of catechol [17].

However, these methods are costly, require highly skilled manpower, complicated and time consuming procedures which is difficult to use for routine analysis. In general, due to the biological importance and its negative effect of catehol, it is important to establish a sensitive, rapid and simple method for the determination of catechol as voltammetric detection. Catechol contains Polyphonic hydroxyl group and possesses excellent electrochemical activity [11]. To increase the sensitivity and other parameters of voltammetric techniques with different modifiers has been cited [11]. Of the modifiers reported; a penicillamine modified electrode with limit of detection 7.5 $X10^{-7}$ $molL^{-1}$, mesoporous Al-doped Silica with limit of detection $1X10^{-7}$ $molL^{-1}$, a multi-wall carbon nanotubes with limit of detection of $2.5X10^{-7}$ $molL^{-1}$ [12], a nano Au/alkanedithiol self assembled gold electrode with limit of detection 10^{-6} $molL^{-1}$ [18], mesoporous platinum electrode with limit of detection 10^{-6} $molL^{-1}$ [19] and a nanostructured titanium oxide modified electrode [20] were employed in the detection of catechol electrochemically.

2.3.1 Voltammetric Methods

Voltammetry comprises a group of electroanalytical methods in which information about the analyte is derived from the measurement of current as function of applied potential obtained under conditions that encourage polarization of an indicator electrode. Historically, the field of voltammetry developed from polarography, which is a particular type of voltammetry that was discovered by the Czechoslovakian chemist Jaroslav Heyrovsky in early 1920s [21, 22].

2.3.2 Cyclic Voltammetry

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions but is rarely used for quantitative determinations, and it is widely used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics processes and the kinetics of heterogeneous electron transfer reactions and also on coupled chemical reactions or adsorption processes. It is often the first experimental technique to be performed. In particular, it offers a rapid location of redox potentials of the electroactive species, and convenient evaluation of the effect of media on the redox process.

Depending on the information sought, single or multiple cycles can be used. During the potential sweep, the potentiostat measures the current resulting from the applied potential. The resulting current-potential plot is termed cyclic voltammogram [16]. As the potential approaches the E^0 for redox process a cathodic current begins to increase, until the peak is reached. After traversing the potential region in which the reduction process takes place (at least 90/n mV beyond the peak), the direction of potential sweep is reversed. In general, cyclic voltammogram is characterized by several parameters. Four of these observables are the two peak currents; the two peak potentials provide the basis of diagnosis. The reverse-to-forward peak current ratio, ipa/ipc, is unity

for simple reversible couple. This peak ratio can be strongly affected by chemical reactions coupled to the redox process. And also the separation between the peak potentials (for reverse couple) is given by $\Delta E = E_{pa} - E_{pc} = 0.059/n$ V [22]. For a reversible reaction, the concentration is related to peak current by the Randles–Sevcik expression (at 25 °C

$$i_p = 2.686 \cdot 10^5 n^{3/2} A C_0 D^{1/2} v^{1/2} \quad (1)$$

Where ip is the peak current in amperes, A is the electrode area (cm^2), D is the diffusion coefficient ($cm^2\ s^{-1}$), C_0 is the concentration in mol cm^{-3}, and n is the scan rate in V s^{-1}.

2.3.3 Pulse Voltammetric Techniques

The various pulse voltammetric techniques are all based on the difference in the rate of the decay of the double layer charging currents and the faradic currents following a potential step. After the potential is stepped, the charging currents, decay exponentially with time which is faster than the faradic currents, decay as a function of the square root of time. Since the current is sampled late in the pulse life, an effective discrimination against the charging current is achieved. Therefore, in pulse voltammetric techniques the measured current is mainly of the faradic current. The discrimination against the charging current that is inherent in these techniques leads to lower detection limits and higher sensitivity [21, 23]

2.3.4 Square Wave Voltammetry

Square wave voltammetry is a large-amplitude differential technique in which the wave form composed of a symmetric square wave, superimposed on a base staircase potential, is applied to the working electrode. The current is sampled twice during each square wave cycle, once at the end of the forward pulse and once at the end of the reverse pulse. Since the square wave modulation amplitude is very large, the reverse pulses cause the reverse reaction of the product [22]. Below are the potential versus time wave form of square wave system.

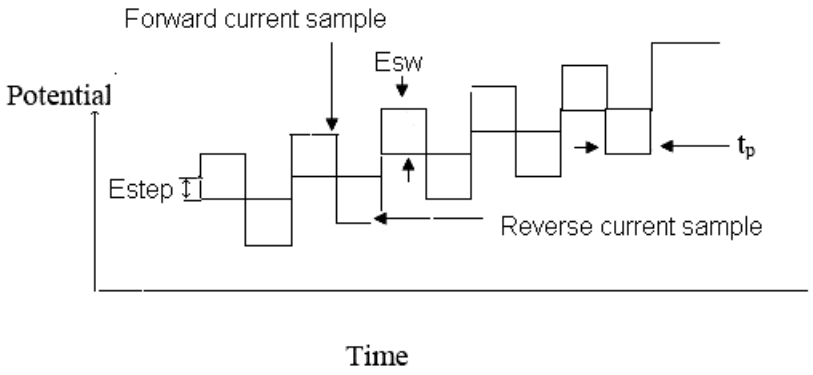

Fig. 1: The applied potential wave form for square wave voltametry.

In general, square wave voltammtry posses excellent sensitivity. The sensitivity is higher than from differential polarographic techniques coupled with the effective discrimination against the charging background current, very low detection limit can be attained. The major advantageous of square wave voltammtry is its speed. The effective scan rate is given by fΔEs. The analysis time is very short, complete voltammogram can be recorded within a few seconds, as compared with 2-3 min. in other differential pulse voltammetry. An additional advantage of square wave voltammtry is that its fast scans capability to determine changes in voltammetric responses with time nondestructively. These may include changes due to homogeneous kinetics, as in synthetic reactions, to the heterogeneous kinetics as in dissolution reactions, or to the mode of sample presentation, as in flow injection analysis and in chemical detectors for HPLC [21, 22].

2.4 Carbon Electrodes

Solid electrodes based on carbon are currently in widespread use in electroanalysis, primarily because of their broad potential window, low background current, low cost, chemical inertness, and suitability for various sensing detection applications. In contrast electron transfer rates observed at carbon surfaces are often slower than observed at metal electrodes. A variety of electrode pretreatment procedures have been proposed to increase the electron transfer rates. The type of carbon, as well as the pretreatment method, thus has a profound effect on the analytical performance. The most popular carbon electrode

materials are those involving glassy carbon, carbon paste, carbon fiber, screen printed carbon strips, carbon films, or other carbon composites [16, 22].

2.5 Carbon Paste Electrodes

Carbon paste electrodes developed from an attempt to prepare a fluid of suspended carbon particles to be used in the sense of a dropping electrode for anodic oxidations. Such a mixture can be prepared and dropped from a capillary bore slightly greater than that of DME. However, the conditions for successful operation of dropping carbon paste electrodes are far from ideal, and it soon became apparent that a thick paste packed in the pool configuration and used either stationary or rotated. Carbon paste electrodes have particularly zero residual current over the entire anodic potential range. Carbon paste electrodes are made by simple hand mixing of powdered graphite with an organic liquid sufficiently immiscible with water to keep the electrode matrix from dissolving when immersed in the test solution. The pasting liquids offer an easily renewable and modified surface, low cost, and very low background current contributions. A wide choice of pasting liquids are possible, but practical considerations of low volatility, its purity, and economy narrow the choice to a few liquids. These include Nujol (mineral oil), paraffin oil, silicone grease, bromoform and bromonaphthalene [16].

2.6 Modified Carbon Paste Electrode

Chemically modified electrodes (CMEs) represent a modern approach to electrode system. These electrodes rely on the placement of reagents on to the surface to impart the behavior of that reagent to the modified surface. Such deliberate alteration of electrode surfaces can meet the needs of many electroanalytical problems, and may form the basis for new analytical applications and different sensing devices. The immobilization of elelectrocatalysts has also been done by incorporating an electroactive substance in the electrode matrix and carbon paste electrode spiked with catalysts may be suitable. The construction of electrodes by incorporating an electroactive substance in to a carbon paste

matrix was first reported by Kuwana in 1964 and has been extensively applied till now [24].

The development and application of chemically modified CPEs electrodes have received considerable attention in recent years due to many advantageous, such as easily manufacture, renewable surfaces, accelerating electron transfer reaction, preferential accumulation or selective membrane permeation thus, imply higher selectivity, sensitivity, or stability on electrochemical reactions.

As it is indicated in the literature, physisorbed or chemisorbed monolayer of several quinines, including duroquinones, anthraquinone, and dopamine itself are catalytic towards dopamine oxidation and reduction. The most plausible mechanism is "self-catalysis" by adsorbed quinine, which remained adsorbed during electron transfer to a redox couple in solution. Thus, the above results delineate a variety of catechols and hydroquinone chemistry, as well as provide more fundamental insights into quinine electron-transfer mechanism [24, 25]. The figure below is the theoretical catalytic pathway of anthraquinone on catechol redox system.

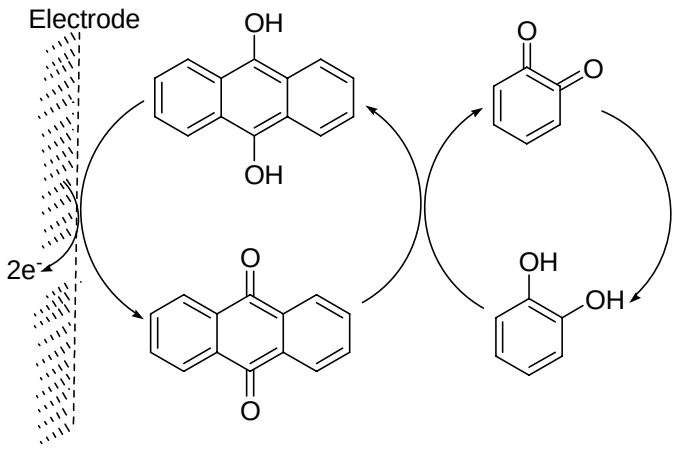

Fig. 2: Schematic diagram for catechol electroxidation and reduction using anthraquinone.

Quinones are a well-known and important functional moiety in various biological systems, for example, an electron-proton carrier in respiratory assembles and in

photosynthetic electron flow systems. The application of quinines and its derivatives such as polymeric quinines and quinine modified macrocycles is attracting new interest in the development of molecular electronics and potentiometeric sensors [26].

3. Objectives of the Study

3.1 General Objective

To develop an electrochemichal determination method for the routine analysis of catechol in tea (Camellia sinensis).

3.2 Specific Objective

To introduce a novel modifier for catechol determination in electrochemical measurements. To optimize factors that have major impact on the sensitivity of the method such as square wave parameters (amplitude, frequency, step potential) as well as composition of the modifier and pH of working solution. To determine the amount of catechol Ethiopian green tea and Wush Wush tea.

4. Experimental

4.1 Reagents

Catechol (Indian industries), graphite powder (BDH, UK), paraffin oil (FINE CHEM Industries, India), di-potassium phosphate and di-hydrogen phosphate (Fulka, Germany), anthraquinone (BDH chemicals Ltd, England) and ethanol alcohol (Changshu Yangyuan chemicals, China), NaOH (Labmerk chemicals, India) and HCl (Riedel-De Haen, Germany) were used in the experiment. Stock solution of catechol (0.10 $mmolL^{-1}$) was prepared in 0.2 M phosphate buffer of pH 7.5. All experiments were carried out in phosphate buffer (0.2 M, pH 7.5). The stock solution of catechol prepared was stored in the refrigerator to avoid exposure to air and light. All solutions and subsequent dilutions were prepared using de-ionized water.

4.2 Instruments and Apparatus

The voltametric experiments were performed using BAS-50 W potentiostat/galvanostat analyzer coupled with KTC personnel computer with conventional three electrode configuration consisting of anthraquinone modified carbon paste working electrode, a silver-silver chloride reference electrode and a platinum wire serving as a counter electrode.

4.3 Preparation of Carbon Paste Electrode

To prepare carbon paste electrode for this experiment 70% (w/w) of graphite powder and 30% (w/w) of paraffin oil were mixed homogeneously for 20 min. with a mortar and pestle. The homogenized mixture was kept in the refrigerator for 24 hrs, and then the paste was housed in a tip of an insulin syringe by introducing a conducting copper wire that extends between the tip and the back of the syringe.

4.4 Preparation of Anthraquinone Modified CPE

The modified electrode containing the following percentage ratio of graphite powder, anthraquinone, and paraffin liquid were homogeneously mixed for 20 min. to give a uniform paste. For 10% (w/w) Anthraquinone, 65% (w/w) graphite powder, and 25 %(w/w) paraffin liquid utilized. Similarly, for 5% (w/w) Anthraquinone; 67.5 %(w/w) graphite powder, and 27.5 %(w/w) paraffin liquid used. For 15 %(w/w) Anthraquinone; 62.5 %(w /w) graphite powders, and 22.5 %(w/w) paraffin liquid mixed. For 20 %(w/w) Anthraquinone; 60 %(w /w) graphite powder, and 20 %(w/w) paraffin liquid mixed. After the modified paste was prepared, it was kept for 24 hrs in a refrigerator. The modified paste was introduced in to the tip of the syringe by removing part of the unmodified paste filled before as in section 4.3. The electrode surface was polished on a clean smooth paper before.

4.5 Sample Preparation

Five varities of tea were purchased from local markets and supermarkets and treated as indictated in reference [11]. From each tea samples 0.2 gm was exactly weighed and the catechol was extracted with 60 ml of 20% (v/v) ethanol solution for 20 min at 80 0C. Then the mixture was filtered and the volume was made up to 100 ml with phosphate buffer for further voltammetric analysis.

4.5.1 Sample Preparation by Infusion

Wush wush tea was purchased from local markets and about 2 gm of tea infusion was made using hot (100 °C) de-ionized water. After filtering each infusion, 50 ml was taken and diluted with 100 ml of buffer solution (0.1 M Na_2HPO_4+ 0.1 M NaH_2PO_4) for analysis.

5. Results and Discussion

5.1 Electrochemical Behavior of Catechol

The electrochemical behavior of catechol was studied using cyclic voltammetry (CV) in 0.2 M phosphate buffer. Fig.3 shows the cyclic voltammograms of anthraquinone modified CPE at modified CPE without catechol (curve a) and with standard catechol (curve b).

The the response in the cyclic voltammograms during the experiment revealed that in case of unmodified carbon paste electrode, the oxidation peak was observed at 292 mV during the anodic sweep. In the reverse scan, the reduction potential peak was at 26 mV. The peak potential separation, which can indicate the reversibility behavior of the reaction, is very large (266 mV) at the unmodified carbon paste electrode, indicating that the electron transfer reaction is slow. Under similar conditions, when anthraquinone modified CPE was used the response potential for of catechol redox system showed different behavior: The oxidation peak potential shifted negatively to 278 mV while the reduction peak potential shifted positively to 36 mV, which gives separation in potential of 251 mV at the anthraquinone modified carbon paste electrode, suggesting the electrochemical behavior of catechol with slight reversibility than before as one utilizes anthraquinone as modifier. This decrease in separation potential using mCPE shows the catalytic activity of anthraquinone for catechol detection.

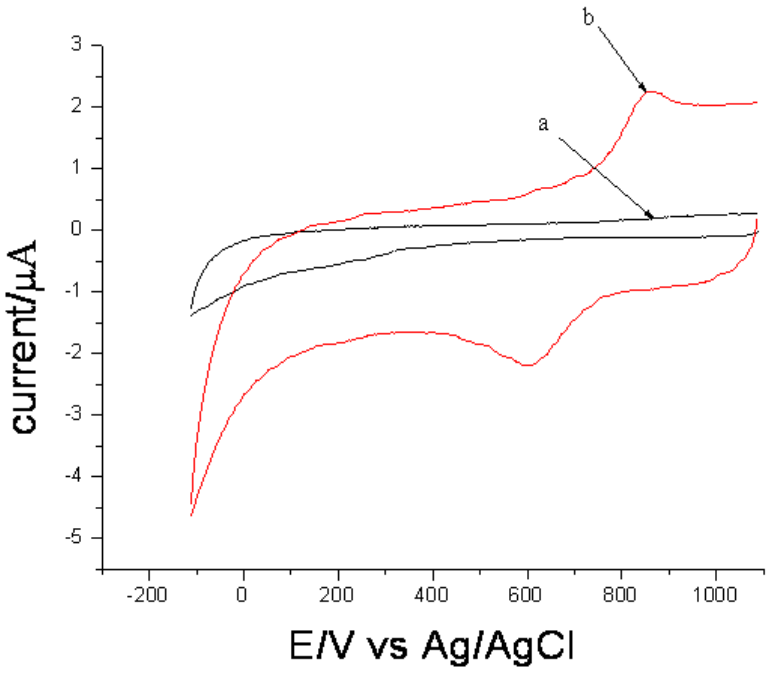

Fig. 3: Cyclic voltamograms of anthraquinone (15%) modified CPE in 0.2 M phosphate buffer a) in the absence of catechol b) in 80 μmolL^{-1} catechol. Scan rate =100 mVs^{-1}.

Quantification of catechol using anthraquinone modified CPE in a square wave voltammetry significantly increases the oxidation peak, attesting that anthraquinone mCPE greatly improves the detection sensitivity for catechol as dictated in the (Fig. 4).

As indicated in (Fig. 4), the response for catechol at the unmodified CPE (curve a is low suggesting that the activity of unmodified CPE is less as compared to anthraquinone modified CPE (curve b). The magnitude of the signal (peak current) enhancement was calculated and was found 22% (μA/μA) relative to the unmodified electrode.

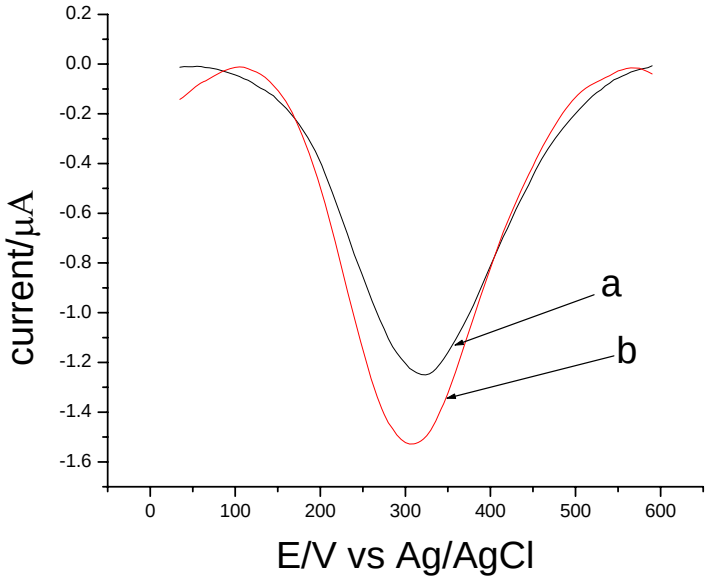

Fig. 4: Square wave voltammograms of 70 μmolL^{-1} catechol in 0.2 M phosphate buffer a) unmodified CPE b) anthraqunione (15 %) modified CPE. Frequency 25 Hz, amplitude 55 mV, and step potential 5 mV.

This enhancement in the peak current is due to the catalytic property of anthraquinone as indicated in different literature. Anthraquinone as a modifier considerably enhances the oxidation signal of catechol although its electric conductivity is poor since it is an organic compound [25].

5.2 Effect of pH

The influence of pH on the electrochemical reaction or response of catechol was examined using phosphate buffer of pH ranging between pH 5 to 9. The position and peak current for 80 μmolL^{-1} catechol in the investigated pH range is detailed in (Fig. 5).

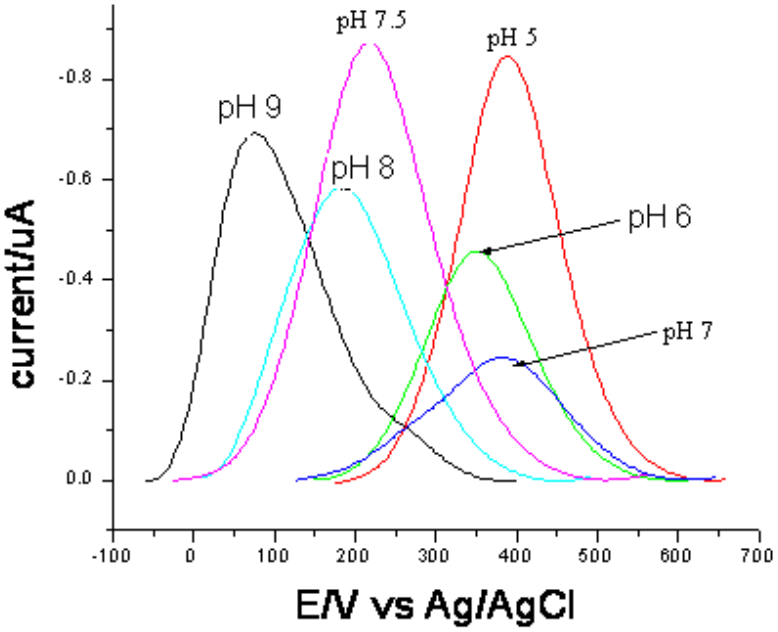

Fig. 5: Square wave voltammograms showing the effect of pH with anthraquinone (10 %) modified CPE for 80 μmolL^{-1}catechol. Frequency 15 Hz, amplitude 25 mV and step potential 4 mV.

As it is seen in the graph, beyond pH 7.5 the peak current decreases as the pH increases this may be due to o-benzoquinone can undergo a hydroxylation reaction. Further in more alkaline solution (e.g., pH 9.00) it is thus suggested that the oxidation of catechol is followed by 1,4-Michael addition reaction with hydroxyl ion which can complicate the catalytic mechanism [27, 28].

5.3 Optimization of Square Wave Parameters for Catechol Determination

5.3.1 Effect of Square Wave Frequency

As it is shown in Fig 6, it was found that the catechol signal increased as the frequency increased from 10 Hz to 100 Hz; however, during the square wave run beyond 25 Hz at potential around 245 mV the peak current still showed increment though not considerable.

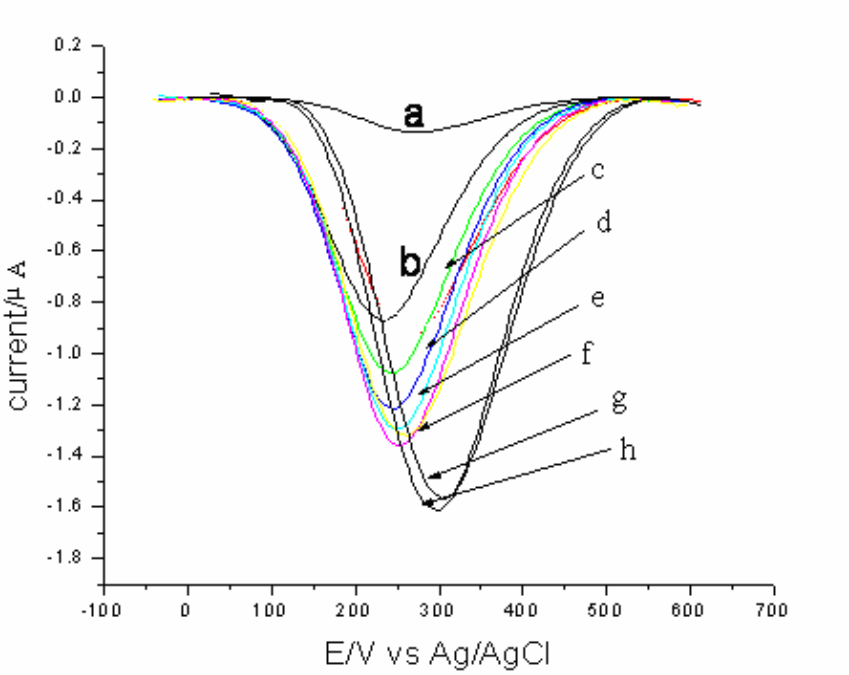

Fig. 6: Square wave voltammograms showing the effect of frequency on the responses for catechol (80 μmolL^{-1}) using 10 % modifier. a) at 10 Hz, b) at 15 Hz c) at 25 Hz d) at 30 Hz e) at 35 Hz f) at 40 Hz g) 65 Hz h) at 70 Hz . Amplitude of 25 mV and step potential set at 4 mV.

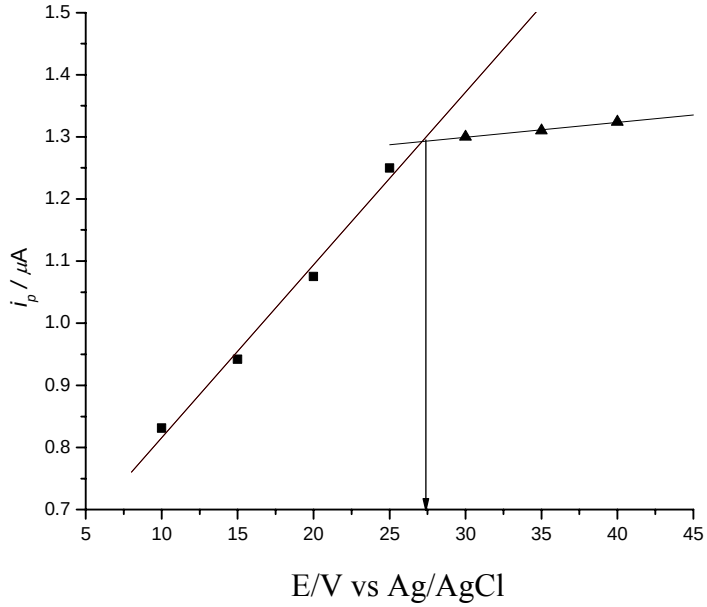

Fig. 7: Variation of peak current with the square wave using anthraquinone (10%) modified CPE in catechol (80 μmolL^{-1}). Amplitude 25 mV, step potential 4 mV.

As it is seen in (Fig. 7), the peak current increased linearly up to 25 Hz as the frequency was increased. After that as the frequency was increased the increment was slight losing its linear relation. As a result 25 Hz was selected as optimal for further analysis.

5.3.2 Effect of Square Wave Amplitude

The effect of square wave amplitude on the current response was studied by varying the square wave amplitude from 10 to 70 mV at the frequency of 25 Hz and a step potential of 5 mV. The result showed that as the amplitude was increased from 10 to 55 mV consequently the increase in peak current was observed. Whereas as the amplitude was further increased from 55 mV the peak current slightly decreased as it is indicated in (Fig. 8) suggesting 55 mV was best for quantifying catechol.

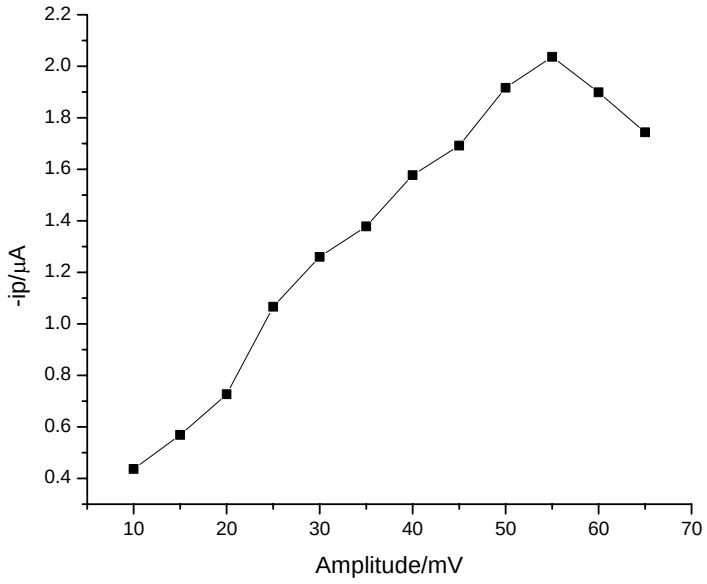

Fig. 8: Impact of square wave amplitude on the peak current using 15% anthraquinone modified electrode for 70 μmolL^{-1} catechol. Frequency 25 mV, step potential 5 mV.

5.3.3 Effect of Square Wave Step Potential

As it is indicated in (Fig. 9), upon increasing the step potential of the system, an increase in peak current was observed up to 5 mV then after the peak current declined slightly. Hence square wave step potential of 5 mV was chosen as optimal condition for the subsequent experiment.

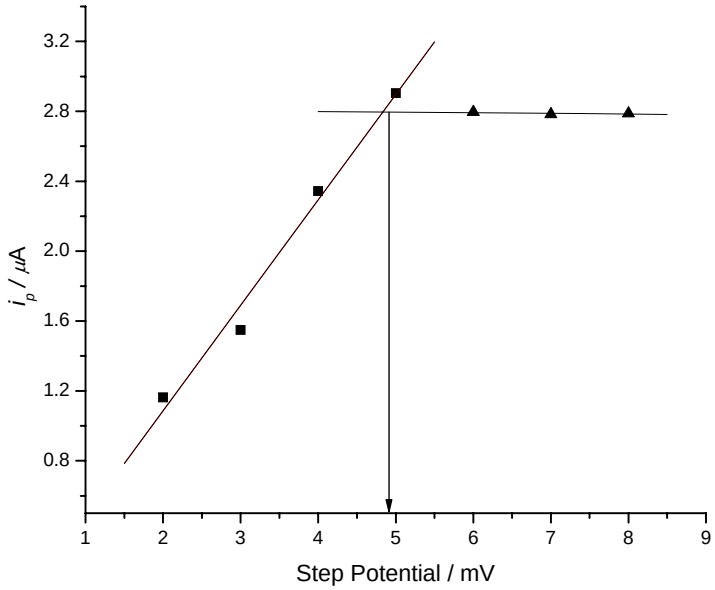

Fig. 9: Dependence of peak current on square wave step potential using anthraquinone (10%) modified electrode for 80 μmolL^{-1} catechol.

5.4 Effect of Modifier Composition

The construction of electrodes by incorporating an electroactive substance into a carbon paste matrix was first reported by Kuwana in (1964) and has been extensively applied till now for the determination of trace amount of elements, preparation of biosensors, and evaluation of electrochemical processes and investigation of electrocatalytic mechanisms [25].

The chemistry of anthraquinone has received much attention because of its relevance to some important technological process. Anthraquinone and anthraquinone derivatives have been used in analytical chemistry, mainly as chelating agents and

chromophores. They also display interesting electrochemical behavior because of their quinoid structure. Anthraquinone as a modifier considerably enhances the oxidation signal of catechol although its electric conductivity is poor since it is an organic compound. The paste composition strongly affects the electrode reactivity, with the increase in pasting liquid content decreasing the electron transfer rates [16, 25]. Fig. 10 explains the oxidation peak current as function of modifier composition (amount of anthraquinone (% w/w)). When the content of anthraquinone was increased from 0% to 15%(w/w), with the selected data sets (points) showed an increment. Here, the response did not appear robust so additional data sets should be considered, i.e points in between 10% and 15%. Whereas with further increasing the modifier amount from 15% to 20 %(w/w), the oxidation peak current decreased. Hence 15% was selected as a working condition for the experiment.

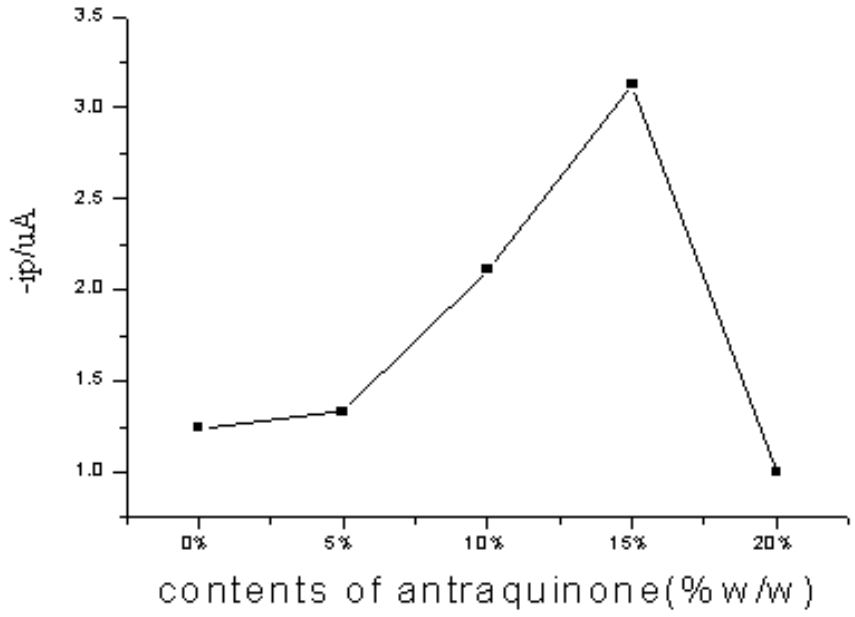

Fig. 10: The effect of amount of anthraquinone (modifier) on the oxidation peak current of 80 μmolL^{-1} Catechol. Frequency 25 Hz, amplitude 55 mV, step potential 5 mV.

5.5 Repeatability, Reproducibility and Stability of Modified Carbon Paste Electrode

In this experiment, reproducibility was investigated by considering three modified electrodes prepared independently by taking triplicate measurements using the three electrodes. The reproducibility expressed in relative standard deviation was found to be 3.65 % for 80 $\mu molL^{-1}$ catechol solution showing excellent reproducibility of the method. To characterize the repeatability of anthraquinone modified CPE, ten successive determinations of 80 $\mu molL^{-1}$standard catechol was made. The result of ten repetitive measurements showed that the relative standard deviation (RSD %) was 3.4 which revealed an excellent repeatability. The modified CPE showed high stability. As it is shown during the experiment, there has been no significant difference in the peak current responses for the same electrode over a period of three months.

5.6 Calibration Plots for Catechol

Using the optimal square wave parameters described the calibration curve for the determination of catechol concentration was established. The peak height for catechol was found to increase with increasing concentration from 6 to 80 $\mu molL^{-1}$ (Fig. 11). The calibration curve for eight average data points (n = 8) was found to be linear with R = 0.99853 with the regression equation of $Y = A + B*C$. Where A = -9.68771×10^{-8} and B = 0.02756 = 0.99853. In analytical practice, calibration graphs frequently give numerical R-values greater than 0.99, and R-values less than about 0.90 are relatively uncommon. It can be shown that R can take values in the range $-1 \leq R \leq +1$ [29]. As it is indicated using the numerical value of coefficient of variation ® for this experiment, the data sets showed a good linear fit because the value of ® is approaching positive one. The detection limit for catechol, considering signal-to-noise ratio of three was found to be $2.155x10^{-7}molL^{-1}$.

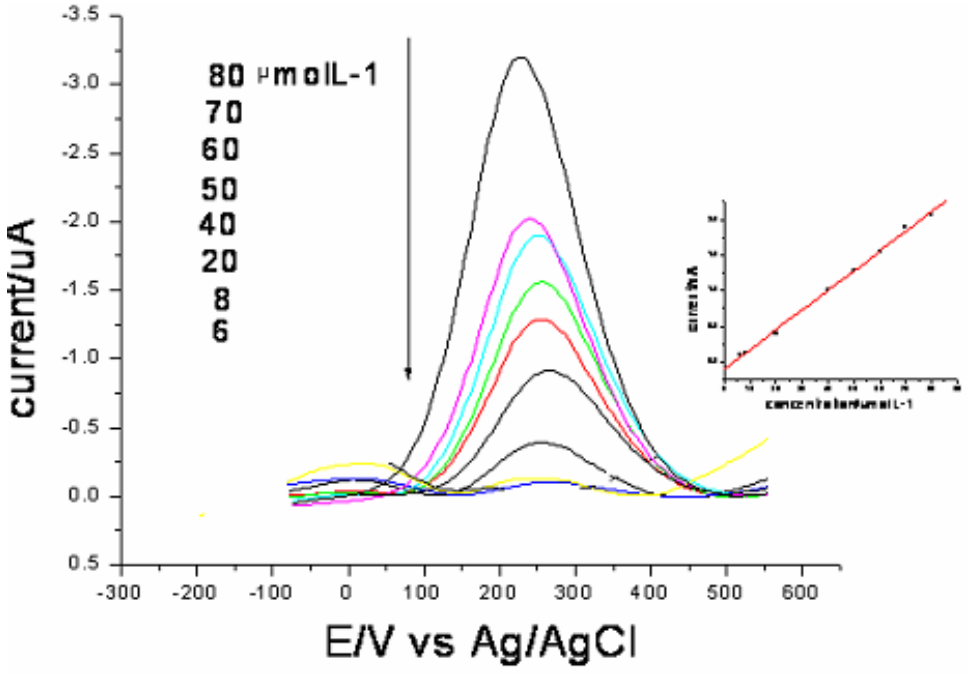

Fig. 11 Square wave voltammograms for anthraquinone modified electrode containing different concentration of catechol. Frequency 25 mV, amplitude 55 mV and step potential 5 mV.

5.7 Analysis of Catechol in Tea Samples

At the first glance, to examine catechol in real sample (Camellia sinensis) standard catechol of (60 μmolL^{-1}) was added on a diluted green tea sample to estimate the presence of catechol in the tea sample. Results were recorded after 1ml addition of 60 μmolL^{-1} catechol and a peak current enhancement was found on nearly similar potential and with almost same band shape as of the standard catechol suggesting the catechol in tea sample. This situation has been delineated in (Fig.12). If there are interfering compounds in similar potential the peak expected would broaden but in this experiment no peak broadening was observed on adding catechol onto the tea sample. And also as it is explained by Adam et. al, if catechol derivatives or other groups are attached with the

precursor catechol, such as saccharides or other functionalities the potential would vary as large as 100 mV[8].

During the experiment, repeated extraction was made for the different varieties of tea samples and estimation of catechol amount in each variety of tea using (SWV) was studied. And it was found that according to the literature based extraction and dilution, wush wush tea catechol level which was found to be 0.16 ± 0.001(g/g) lies within the linear range of the method, whereas Ethiopian green tea is out of the linear range until nearly 100 ml times dilution than the preparation of tea samples as indicated by Kilmartin et. al.

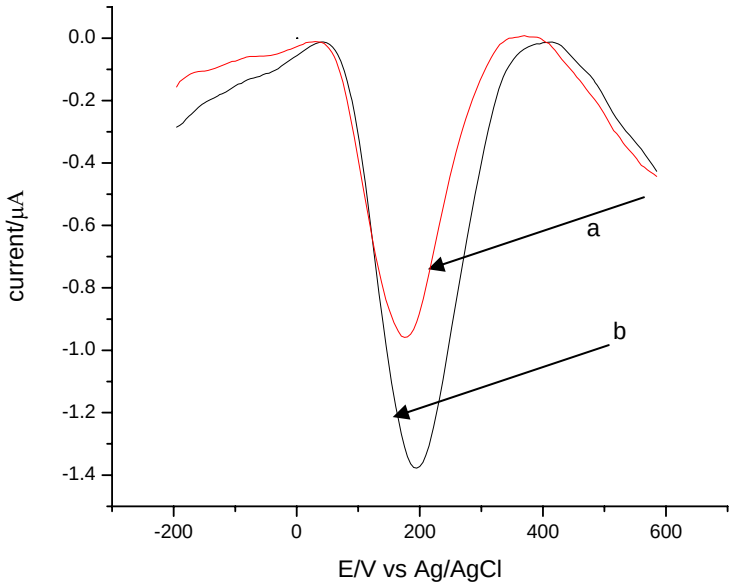

Fig. 12: Peak current enhancement upon addition of standard catechol to tea sample a) only green tea b) After addition of 1 ml of 60 μmolL^{-1} catechol at anthraquinone (15%) modified CPE. Frequency 25 Hz, amplitude 55 mV, step potential 5 mV.

Table 2: Methods of determination of catechol in tea samples

		Methods	
Sample	By HPLC (g/g)	By DPV using mesoporous Al-doped silica modified electrode (g/g)	**This method** (g/g)
A	0.184	0.168	**0.16 ± 0.001** (Wush Wush tea)

[11].

This method was applied to determine catechol in several tea samples. The content of catechol was determined using interpolation, and the result is given in the Table 2 by comparing detection of catechol with other methods. The sample was determined in triplicate, and the RSD is below 5.0%. The result of this method is comparable with the one obtained in reference [11] which is by DPV using mesoporous Al-doped silica modified electrode. However, the result can be used to have a rough approximation for the detection of catechol for this particular case because the sample (type of tea) is not known in the two methods used for comparison, besides variation in the conditions of the measurements taken.

When investigation was made using the infusion of wush wush tea, the following result was obtained.

Table 3: Determination of catechol in wush wush tea samples.

Sample	Using water + ethanol extraction (g/g)	Using hot water infusion (g/g) x 10^{-2}
Wush wush	0.16 ± 0.001	0.123 ± 6.2 x 10^{-4}

The amount of catechol determined with ethanol: water mixture was found to be 0.16 ± 0.001 (g/g). The same experiment was conducted using hot water infusion of the tea samples; the quantity found was (1.23 x10^{-3} ± 6.2 x10^{-4}) g/g for wush wush tea which is two magnitudes less than that obtained by the water: ethanol extraction. This indicates that catechol is slightly soluble in water as compared to ethanol. This phenomenon was explained by Adam et.al [8].

Catechol determination using different methods are possible. Here under is a comparison of different parameters found during catechol detection as detailed in the corresponding references given.

Table 4 Comparison of the different methods of catechol determination

Method	Electrode/modifier or biosensor used if any	LOD	Linear range	Reference
Differential pulse voltammetry	Bare indium tin oxide electrode using a redox cycling of hydrazine	1 $\mu molL^{-1}$	20-100 $\mu molL^{-1}$	[15]
Square wave voltammetry	Clay modified glassy carbon with preanodization	0.10 $nmolL^{-1}$	0.7-15 $\mu molL^{-1}$	[14]
Differential pulse voltammetry	CPE modified with mesoporous Al-doped silica(Al/SiO_2)	0.1 $\mu molL^{-1}$	0.5-50 $\mu molL^{-1}$	[11]
Square wave voltammetry	**CPE modified with anthraquinone**	**0.2155 $\mu molL^{-1}$**	**6-80 $\mu molL^{-1}$**	**This Work**
Square wave voltammetry	A multi-wall carbon nano-tubes modified electrode	0.25 $\mu molL^{-1}$		[11]

As it is indicated in the Table 4, square wave voltammetric detection of catechol with clay modified glassy carbon electrode followed by preanodization is the most sensitive method but requires preanodization. Differential pulse voltammetric determination using CPE modified with mesoporous Al-doped silica (Al/SiO2) is more sensitive than this method but the modifier is expensive. This method is more sensitive than the rest of the methods and also simpler.

6. Conclusion

In general, electrochemical analysis employing anthraquinone modified CPE has been developed for the determination of catechol with the detection limit of 2.155×10^{-7} molL^{-1}.This system is useful for determination of catechol in drinking tea. The system has excellent reproducibility, repeatability and high sensitivity. This work can be extended to the use of other modifiers to apply for the determination of catechol in tea and other food samples. The method is simple, rapid and applicable in routine analysis. To exclude potential interferences in environmental and food analysis the electrochemical determination of chatechol can be integrated with separation techniques such as chromatography and the researcher left for other researchers interested in the area.

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