## Electrochemical determination of catechol in tea samples using anthraquinone modified carbon paste electrode

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#### **ABSTRACT**

Electrochemical investigation of catechol using square wave voltammetry with anthraquinone modified carbon paste electrode was found to be very sensitive. Compared with the unmodified carbon paste electrode, the anthraquinone modified electrode remarkably increases the peak currents of catechol, and greatly lowers the peak potential separation. Two varieties of tea, namely green, and black variety: Wush Wush tea, from Ethiopia, known by its brand name were investigated. Responses for the extracts using ethanol: water (1:4) % v/v 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 μmol·L<sup>-1</sup> of catechol gave a relative standard deviation of 3.65% and linear calibration plots were obtained in the range 6 to 80  $\mu$ mol·L<sup>-1</sup> with (R = 0.998) and the detection limit with (S/N = 3) was as low as 2.155 x 10<sup>-7</sup> mol·L<sup>-1</sup>.

**Keywords:** Square Wave Voltammetry; Anthraquinone; Modified Carbon Paste Electrode; Catechol

#### 1. INTRODUCTION

Catechol (1,2-benzenediol) is a natural polyphenolic compound that widely exists in higher plants such as tea,

vegetables, fruits, tobacco and some traditional Chinese medicines. It has a biological importance such as antioxidation, anti-virus, flower stimulating effect and affecting the activities of some enzymes [1-3]. Reactions between oxidized polyphenols and proteins are thought to be partly responsible for the so called enzymatic browning in plants [4,5]. Catechol is also used in a variety of applications such as a reagent for photography, dyeing fur, rubber and plastic production, pesticides and in pharmaceutical industries [6]. Recent epidemiological studies have shown that the consumption of tea prevent cancer in humans because the tea leaf contains abundant groups of polyphenols [7]. Despite the fact that catechols are ubiquitous and posses multiple advantageous, their toxicity has been observed in a variety of organisms, and toxic concentrations have been found in the environment. Further, catechol is readily absorbed from the gastrointestinal tract causing renal tube degeneration and decreases in liver functions. Even at low concentration in foods and cigarette smokes, catechol may cause mutagenesis and cancerous alterations [8,9]. Many different reactions can occur with biomolecules such as DNA, proteins and membranes, ultimately leading to non repairable damage

Many methods have been reported for the determination of catechols and its derivatives including: IR spectrometry, UV vis spectrophotometry [10], thin layer chromatography, high performance liquid chromatography [3,11] are to mention some. However, these methods are costly, require highly skilled manpower, complicated and time consuming procedures which is difficult to use for routine analysis. Electroanalytical methods are promising approach owning to its sensitivity, rapidity and simplicity [2] though the case of interference. The electrochemical oxidation of catechols in the presence of nu-

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cleophiles such as: ethanol, 2-thiobarbituric acid, b-diketones, 4-hydroxy-6-methyl-a-pyrone, dibuthylamine and diethylamine, 4-hydroxycoumarin, 3-hydroxycoumarin, alkanedithiol, 4,7-dihydroxycounarin and sulfunic acid were studied [4,11,12]. However, the use of anthraquinone modified carbon paste electrode in the determination of catechol has been seldom reported.

In this work, the use of carbon paste modified with anthraquinone as a sensitive electrochemical electrode was established for the determination of catechol using square wave voltammetry. Parameters such as pH of working solution, amount of modifier, frequency, amplitude and step potential were optimized to improve the method efficiency during the experiment. Furthermore, real sample of tea (*Camellia sinensis* L.) varieties green tea and black tea has been proven to produce a good response towards catechol.

#### 2. EXPERIMENTAL

#### 2.1. Reagents

All reagents and chemicals were of analytical grade. Catechol (Sigma Aldrich, 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 during the experiment. Stock solution of catechol (0.10 mmol·L<sup>-1</sup>) was prepared in 0.2 M phosphate buffer of pH 7.5. All experiments were carried out in phosphate buffer of (0.2 M, pH 7.5). The stock solution of catechol prepared was stored in the refrigerator to avoid exposure to air and light to keep its stability. All solutions and subsequent dilutions were prepared using de-ionized water.

#### 2.2. 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 working electrode (anthraquinone modified carbon paste), a silver-silver chloride reference electrode and a platinum wire serving as a counter electrode.

#### 2.3. Preparation of Carbon Paste Electrode

The carbon paste electrode (CPE), first introduced by Adams [13], is one of the most commonly used electrodes. Carbon paste electrodes (CPEs) belong to a special group of heterogeneous carbon electrodes. It is a mixture prepared from carbon (graphite) powder and a suitable liquid binder packed into a suitably designed electrode body. Due to its low cost, ease of fabrication,

high sensitivity and renewable surface, the CPE has been widely applied in the electroanalytical community [14]. 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 minutes 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.

## 2.4. 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 minutes to give a uniform paste. In the preparation of modified carbon paste electrode the following composition were prepared and tested. For 5% (w/w) Anthraguinone modified electrode; 67.5% (w/w) graphite powders and 27.5% (w/w) paraffin liquid was used. For 10% (w/w) Anthraquinone, 65% (w/w) graphite powder and 25% (w/w) paraffin liquid were utilized. And for 15% (w/w) Anthraquinone; 62.5% (w/w) graphite powders and 22.5% (w/w) paraffin liquid were mixed. Similarly, for 20% (w/w) Anthraquinone modified; 60% (w/w) graphite powder, and 20% (w/w) paraffin liquid were homogenized. 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 2.3. The electrode surface was polished on a clean smooth paper before use [15].

#### 2.5. Real Sample Preparation

Two varieties of tea green and black from Wush Wush brand were taken from the Ethio Agri-CEFT factory Addis Ababa, Ethiopia, extracted and treated as indicated in reference [16]. Then it was quantified using square wave votammetry at optimized conditions.

#### Tea Sample Preparation by Infusion

Wush Wush tea samples such as green and black from Ethio Agri-CEFT were taken and 2 gm of tea infusion was made using hot ( $100^{\circ}$ C) de-ionized water. After filtering each infusion, 50 ml was taken and diluted with 100 ml of buffer solution (0.1 M Na<sub>2</sub>HPO<sub>4</sub> + 0.1 M NaH<sub>2</sub>PO<sub>4</sub>) for analysis. This was to internalize the catechol amount in drinking tea, in a cup of tea.

#### 3. RESULTS AND DISCUSSION

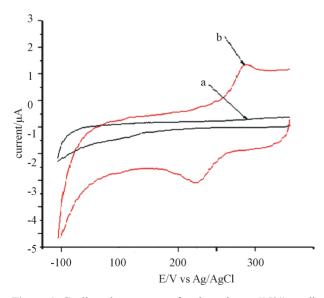
#### 3.1. Electrochemical Behavior of Catechol

The electrochemical behavior of catechol was studied

using cyclic voltammetry (CV) in 0.2 M phosphate buffer. **Figure 1** shows cyclic voltammograms of anthraquinone modified CPE without catechol (curve a) and with standard catechol (curve b). The response in cyclic voltammograms during the experiment revealed that in case of unmodified carbon paste electrode, the oxidation peak was observed at 292 mV (figure not shown) during the anodic sweep. In the reverse scan, the reduction potential peak was at 226 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, inferring the electron transfer reaction is slow. Under similar conditions, when anthraquinone modified CPE was used, the response of 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 modified CPE shows the catalytic activity of anthraquinone for catechol detection.

Quantification of catechol using anthraquinone modified CPE in a square wave voltammetry significantly increases the oxidation peak, attesting that anthraquinone modified CPE greatly improves the detection sensitivity for catechol (**Figure 2**). The magnitude of the signal (peak current) enhancement was found to be 22% ( $\mu$ A/  $\mu$ A) times relative to the unmodified electrode. The enhancement in the peak current is due to the catalytic

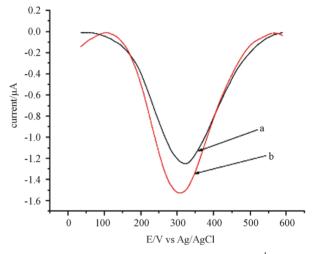


**Figure 1.** Cyclic voltamograms of anthraquinone (15%) modified CPE in 0.2 M phosphate buffer a) in the absence of catechol, b) in 80  $\mu$ mol·L<sup>-1</sup> catechol. Scan rate = 100 mV·s<sup>-1</sup>.

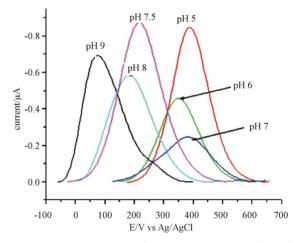
property of anthraquinone which considerably enhances the oxidation signal of catechol although its electric conductivity is poor since it is an organic compound [15,16].

#### 3.2. 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 µmol·L<sup>-1</sup> catechol in the investigated pH range is detailed in (**Figure 3**). 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 9.0) it is thus suggested that the oxidation of catechol is due 1,4-Michael addition reaction with



**Figure 2.** Square wave voltamogram of 70  $\mu$ mol·L<sup>-1</sup> catechol in 0.2 M phosphate buffer a) unmodified CPE, b) anthraquinone modified (15%) CPE, frequency 25 Hz, amplitude 55 mV and step potential 5 mV.



**Figure 3.** Square wave voltammograms on the effect of pH with anthraquinone (10%) modified CPE for 80  $\mu$ mol·L<sup>-1</sup> catechol. Frequency 15 Hz, amplitude 25 mV and step potential 4 mV.

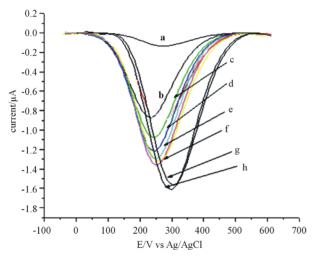
hydroxyl ion which can complicate the catalytic mechanism [17,18].

## 3.3. Optimization of Square Wave Parameters

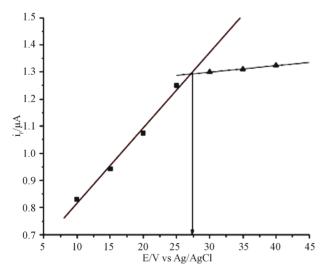
#### 3.3.1. Effect of Square Wave Frequency

As it is shown in **Figure 4**, 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.

As it is seen in (**Figure 5**), the peak current increased linearly up to 25 Hz as the frequency was increased. Af-



**Figure 4.** Square wave voltammograms showing the effect of frequency on the responses for catechol (80  $\mu$ mol·L<sup>-1</sup>) 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.



**Figure 5.** Variation of peak current with the square wave frequency using anthraquinone (10%) modified CPE in catechol (80 µmol·L<sup>-1</sup>). Amplitude 25 mV, step potential 4 mV.

terwards frequency increment was slight losing its linear relation. As a result 25 Hz was selected as optimal for further analysis.

#### 3.3.2. Effect of Square Wave Amplitude

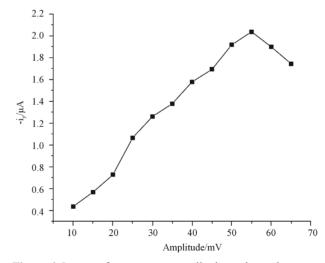
The effect of square wave amplitude on the current response was studied by varying the square wave amplitude from 10 to 70 mV at the frequency of 25 Hz and a step potential of 5 mV. The result showed that as the amplitude was increased from 10 to 55 mV consequently the increase in peak current was observed. Whereas as the amplitude was further increased from 55 mV the peak current slightly decreased as it is indicated in (**Figure 6**) suggesting 55 mV was best for quantifying catechol.

#### 3.3.3. Effect of Square Wave Step Potential

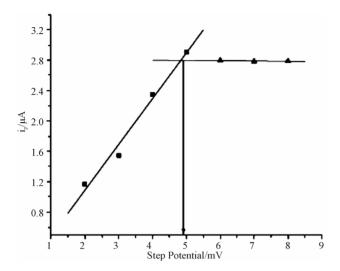
As it is indicated in (**Figure 7**), upon increasing the step potential of the system, an increase in peak current was observed up to 5 mV then after the peak current declined slightly. Hence square wave step potential of 5 mV was chosen as optimal condition for the subsequent experiment.

#### 3.3.4. Effect of Modifier Composition

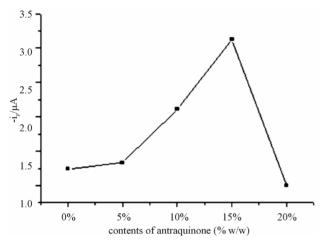
The construction of electrodes by incorporating an electroactive substance into a carbon paste matrix was first reported by Kuwana in (1964) and has been extensively applied till now for the determination of trace amount of elements, preparation of biosensors, and evaluation of electrochemical processes and investigation of electrocatalytic mechanisms [14,19]. The paste composition strongly affects the electrode reactivity, with the increase in pasting liquid content decreasing the electron transferrates [10,13]. The result in **Figure 8** explains the



**Figure 6.** Impact of square wave amplitude on the peak current using 15% anthraquinone modified electrode for 70  $\mu$ mol·L<sup>-1</sup> catechol. Frequency 25 mV and step potential 5 mV.



**Figure 7.** Dependence of peak current on square wave step potential using anthraquionone (10%) modified electrode for 80 µmol/L catechol.



**Figure 8.** The effect of amount of anthraquinone (modifier) on the oxidation peak current of  $80~\mu\text{mol}\cdot\text{L}^{-1}$  catechol. Frequency 25 Hz, amplitude 55 mV, step potential 5 mV.

oxidation peak current as function of modifier composition (amount of anthraquinone (% w/w)) considerably enhances the oxidation signal of catechol. When the content of anthraquinone was increased from 0% to 15% (w/w), with the selected data sets (points) current output showed an increment this is supported by literatures in the detection of catechol amine [20] whereas with further increasing the modifier amount from 15% to 20% (w/w), the oxidation peak current decreased leading 15% to be optimal working condition for the experiment.

# 3.4. 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  $\mu mol \cdot L^{-1}$  catechol solution. To characterize the repeatability of anthraquinone modified CPE, seven successive determinations of 80  $\mu mol \cdot L^{-1}$  standard catechol was made. The result of seven repetitive measurements showed that the relative standard deviation (RSD %) was 3.4 which revealed an excellent repeatability. During the experiment, there has been no significant difference in the peak current responses for the same electrode over a period of three months tested by setting the modified electrode in the refrigerator after trial.

#### 3.5. 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  $\mu$ mol·L<sup>-1</sup> (**Figure 9**) for eight average data points (n = 8). 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 R is approaching positive one (0.998).

#### 3.6. Analysis of Catechol in Tea Samples

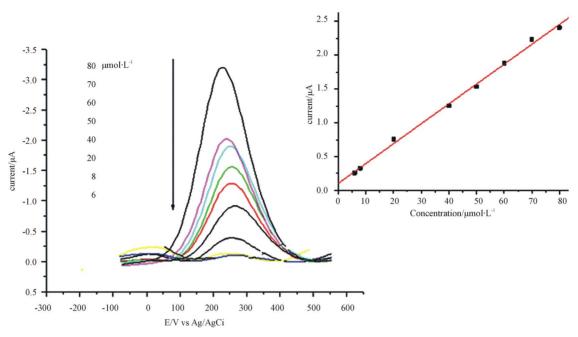
To predict presence of catechol in the green tea, subsequent runs of square wave have been made with diluted extract of green tea and standard catechol added onto a diluted green tea extract. Current responses were recorded after each run that is only green tea and after 1 ml of  $60 \ \mu mol \cdot L^{-1}$  catechol addition.

Peak current enhancement was found on nearly similar potential and with almost same band shape suggesting the presence of catechol in tea sample. This situation has been delineated in (**Figure 10**).

The content of catechol was determined using interpolation, and the result is given in the **Table 1** by comparing detection of catechol with other methods. The sample was determined in triplicate and found higher amount in green tea than in black Wush Wush tea. This might be evidenced by part that catechol my get oxidized during the process of the tea in the factory.

The amount of sample catechol determined using hot water infusion for Wush Wush tea was found to be two magnitudes less than the one obtained by the water: ethanol extraction (**Table 2**). This indicates that catechol is slightly soluble in water as compared to ethanol. This phenomenon was explained by Adam *et al.* [9].

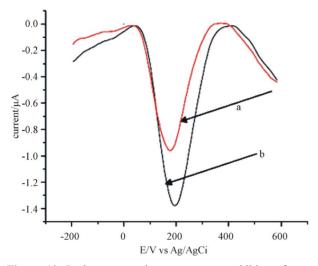
Catechol determination using different methods are possible. Here **Table 1** under is a comparison of different parameters found during catechol detection as detailed in



**Figure 9.** Square wave voltammograms for anthraquinone modified electrode containing different concentration of catechol. Frequency 25 mV, amplitude 55 mV and step potential 5 mV.

**Table 1.** Comparison of the different methods of catechol determination.

Methods	Electrode/modifieror biosensor	LOD	References
Differential pulse voltammetry	Bare induced tin oxide using a redox cycling of hydrazine	1 μmol·L <sup>-1</sup>	[21]
Square wave voltammetry	Clay modified glassy carbon with preanodization	$0.10 \; nmol \cdot L^{-1}$	[22]
Differential pulse voltammetry	CPE modified with mesoporous Al-doped silica	$0.10~\mu\text{mol}{\cdot}L^{-1}$	[12]
Square wave voltammetry	CPE modified with Anthraquinone	$0.2155~\mu mol \cdot L^{-1}$	This work
Square wave voltammetry	A multi-wall carbon nano-tube	$0.25~\mu mol \cdot L^{-1}$	[16]



**Figure 10.** Peak current enhancement upon addition of standard catechol to tea sample a) only green tea b) After addition of 1 ml of 60  $\mu$ mol·L<sup>-1</sup> catechol at anthraquinone (15%) modified CPE. Frequency 25 Hz, amplitude 55 mV, step potential 5 mV.

**Table 2.** Determination of catechol in Wush Wush tea sample in different extraction solvent.

Sample	Amount of catechol (water + ethanol) extraction (g/g)	Hot water infusion (g/g) $\times$ 10 <sup>-2</sup>
Wush Wush	$0.16 \pm 0.001$	$0.123 \pm 6.4 \times 10^{-4}$

the corresponding references given.

#### 4. CONCLUSION

In general, electrochemical analysis employing anthraquinone modified CPE has been developed for the determination of catechol with the detection limit of  $2.155 \times 10^{-7} \text{ mol} \cdot \text{L}^{-1}$ . This system is useful for determination of catechol and it derivatives from beverages like tea. The system has excellent reproducibility, repeatability and high sensitivity. This work can be extended to the use of other modifiers or similar quinines 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 catechol can be integrated with separation techniques such as chromatography. And this remains as the researchers future work or for other researchers interesting in the area.

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