ELECTROCHEMICAL DETERMINATION OF CAFFEINE IN LAAGEE COFFEE (ETHIOPIA) WITH ELECTROPOLYMERIZATION OF ALIZARIN ON GLASSY CARBON ELECTRODE

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ABSTRACT

Alizarin was deposited on the surface of glassy carbon electrode by scanning between -1.6 to +2.0 V electrode potential for six cycles. An oxidative peak with an improved peak current appeared at alizarin electropolymerized glassy carbon electrode which showed that the electrocatalytic activity of the modifier towards the oxidation of caffeine compared to the response of unmodified glassy carbon electrode. Cyclic Voltammetric technique was exploited for investigation of linear dependence of oxidative peak current on caffeine concentration in the range 20×10^{-6} to 1000×10^{-6}mol $L^{-1}$with determination coefficient (R^2) and method detection limit (LoD = 3s/slope) of 0.998 and 3.7 x 10^{-6}M, respectively. An attempt was made to determine the caffeine content of 'Laagee' coffee extracts of Abe Dongoro coffee grown in Horo Guduru wollega zone, Ethiopia. Comparisons between analytical (UV-visible spectrophotometry) and electroanalytical (CV) methods were done for the investigation of quantitative information of caffeine in coffee extracts. The concentration of caffeine determined at optimized parameters by UV-visible spectrophotomery and cyclic voltammetry was 0.144% and 0.148% respectively.

Key Words: Modified electrode, Electropolymerization, Lage Coffee, Caffeine Sensor.

1. Introduction

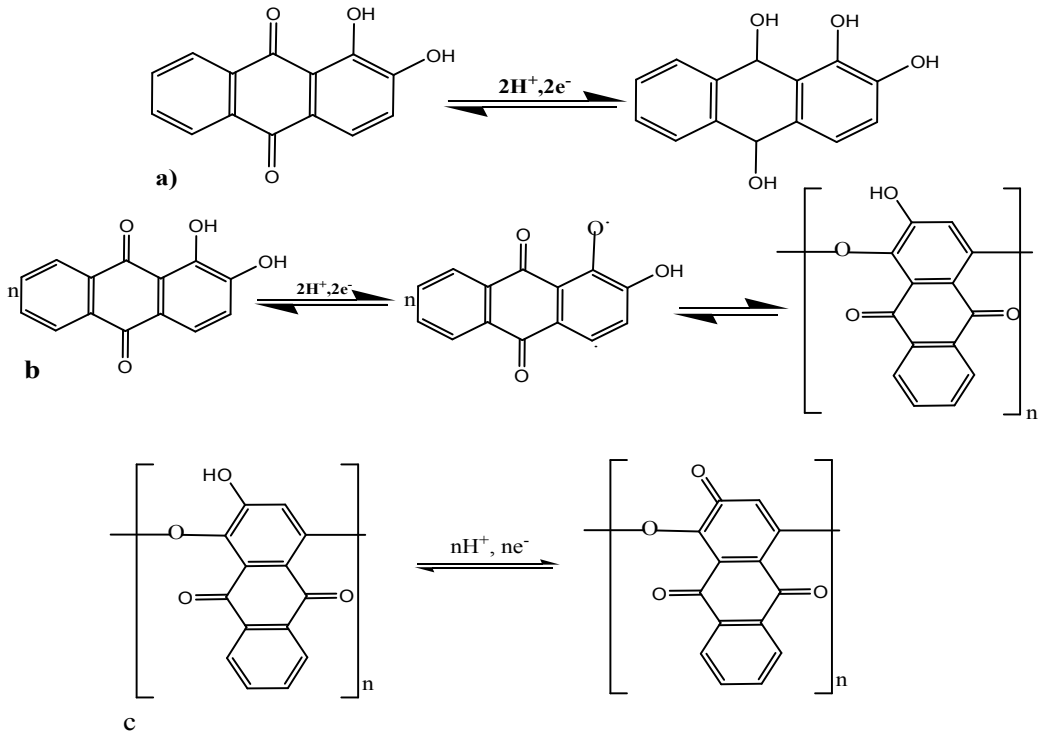
The development of a simple, sensitive, rapid, and reliable method for the determination of caffeine in different source is considered great importance. Because Caffeine (1,3,7-trimethylxanthine) is a natural alkaloid which occurs in various food and beverages; even in various drugs (Wondimkun et al., 2016). Among many possible sources, coffee is composed of the highest caffeine concentration and it is the most utilized source of caffeine (Ashoka et al., 2015). Thus, it is imperative to develop a method which is likely reliable, stable, sensitive, rapid and less expensive for the determination of caffeine in coffee is a significant field of research.

Capillary Chromatography(Regan & Shakalisava, 2005 ; Wang et al., 2000), High performance liquid chromatography(Patil,2012; Aly,2013), and spectroscopic methods (Gebeyehu & Bikila, 2015 ; Wondimkun et al., 2016 ; Demissie et al., 2016) are reported analytical methods used for the determination of caffeine in different real samples. These methods have low linear concentration range, low selectivity, time-consuming in sample preparation procedures, high expense instrumentation, and complexity in techniques (Tadesse et al., 2013 ; Zhang et al., 2011). Electroanalytical methods have been used due to their simplicity, satisfactory sensitivity, wide linear concentration range, cost effective of instrument, and suitability for fast detection. Although, the use of bare working electrode for the electrochemical caffeine determination is not as such effective due to the higher positive oxidation potential of CAF, which may be overlap with the discharged electrolytic solution potential (Švorc, 2013). Modification of the working electrode and selection of electrolyte solution is mandatory in order to avoid such impact (Tadesse et al., 2013 ; Švorc, 2013).

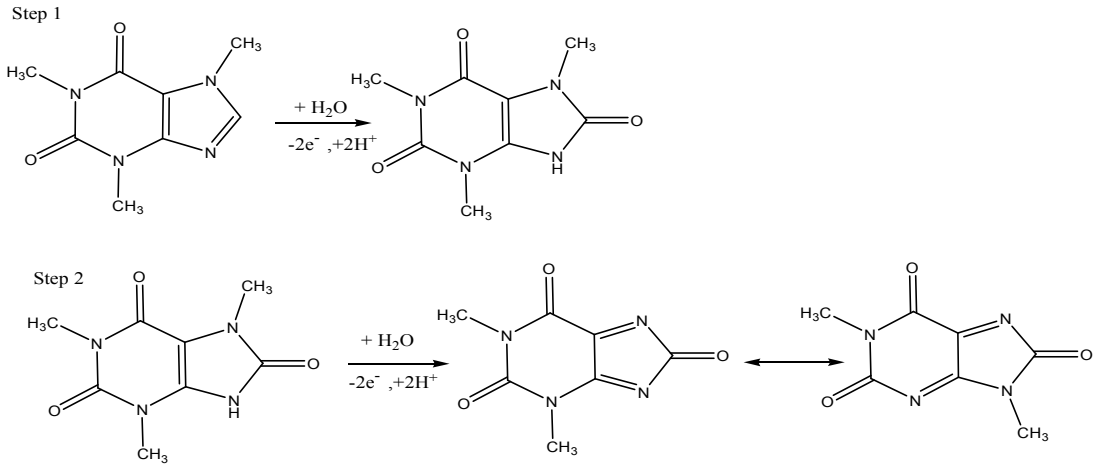
Hence to improve the sensitivity, reproducibility and selectivity, solid electrodes (CPE, Graphite, GCE) have been modified using several modifiers such as Nafion (Torres et al., 2014 ; Zhao et al., 2011), carbon nanotubes and graphene oxide(Wang et al., 2015 ; Habibi et al., 2012), conducting polymers(Amare & Admassie, 2012 ; Filik et al., 2016; Chitravathi* & Munichandraiah, 2016 ; Amare & Aklog, 2017), and metal nanoparticles (Efa & Soreta, 2017), for the determination of CAF. Electropolymerized electrodes by monomer is the simple and forward approach among different modification methods; are interesting because of their stability, reproducibility, and active site increases on the electrode(Amare & Admassie, 2012; Apaydın et al., 2015; Mahanthesha et al., 2010).

Alizarin compound consists of a functional group of quinine and double hydroxyl groups from its structure, which serve as hydrogen acceptor that improves charge transfer reactivity between the working electrode and the analyte (Zhang et al., 2014). The electrochemical reaction of alizarin, formation of polyalizarin and electropolymerization of alizarin monomer on bare GCE have been proposed(**scheme1**)(Jiang et al.,2017).

Coffee grown at different localities of Ethiopia were studied using several methods, but coffee that is known as "Laagee coffee" which is grown in Horo Guduru Wollega Zone, Ethiopia is not reported until this work has been done. The aim of this research is to report the qualitative and quantitative information of Laagee coffee grown in, Horo Guduru, Ethiopia. Thus, alizarin monomer electropolymerized on glassy carbon electrode potentiostatically was exploited for determination of caffeine in Lage coffee using cyclic voltammetric technique.



Scheme 1: the electrochemical polymerization of alizarin. a)oxidation-reduction reaction of alizarin b) Formation of polyalizarin c) Redox reaction of poly(alizarin)



Scheme 2: Electrochemical reaction mechanism of caffeine

2. Experimental

2.1. **Reagents and Chemicals.**

Alumina powder, polishing paper, Caffeine standard ($C_8N_4O_2H_{10}$, anhydrous powder 99% (sigma Aldrich, Germany), Alizarin($C_{14}H_8O_4$ (99%)), sulphuric acid (H_2SO_4, 98%, Merck), sodium hydroxide (99.3%), potassium nitrate (KNO_3, 99%,NICE), potassium chloride (KCl, 99.5%, Finkem), potassium hexacyanoferrate ($K_3Fe(CN)_6$, 98%, Blulux) were used. All chemicals were analytical grade and used without further purification. Double distilled water was used for the preparation of all solutions. All reagents used were of analytical or chemical grade.

2.2.Instrumentation

All the voltameteric measurements were carried out in a BASi Epsilion EC-Version 1.40.67 voltammetric analyzer (Bio-analytical Systems, USA) containing three electrode system, Ag/AgCl saturated with 3M KCl as reference electrode, platinum wire as counter electrode and glassy carbon electrode (3mm diameter, covered with Teflon) as working electrode. All electrochemical measurements were carried out at room temperature of 22-25C°. All potentials are given against Ag/AgCl (3.0 MKCl). pH measurements were performed with an Elico LI 120pH meter (Elico Ltd., Hyderabad, India).

2.3 Fabrication of the Polyalizarin Modified Glassy Carbon Electrodes

The modification of glassy carbon electrode with polyalizarin (AZ) was done as stated elsewhere (Mahanthesha et al., 2014 ; Amare & Admassie, 2012). The bare GCE (3mm diameter) was first rinsed with distilled water, polished carefully with a polishing paper and then further polished with alumina slurries having particle size 3µm to a mirror finish surface. Then it was repetitively rinsed with distilled water to remove the residue and sonicated in distilled water for five minutes. Then the polished GC electrode was electrochemically conditioned by potential scanning from - 0.1 to +1.4 V in 1 M KNO_3 for five complete scans at 50mV/s, to diminish background current due to oxidation of the electrode. The electrode surface modification were prepared by scanning the potential of the polished GCE in a 0.1M H_2SO_4 solution containing 2.0mM alizarin between −1.6 and+2.0V at 100mVs^{-1} for six cycle's. The modified electrode, then rinsed with distilled water to remove physically adsorbed and unreacted species from the electrode surface. Subsequently, the modified electrode stabilized in 0.5 M HNO_3 by scanning the potential between -1.6 and +2.0 V until a steady cyclic voltammogram is obtained. Finally, the modified electrode was dried in air and made ready for use.

2.4 Preparation of Standard Solutions

Standard stock solution of caffeine ($1x10^{-3}$molL^{-1}) was prepared in 0.1molL^{-1} H_2SO_4 and stored at 4°C. The prepared solution was used for the voltammetric investigation of caffeine at both the bare and modified GCE. The pH of the solution was adjusted either by 1molL^{-1} H_2SO_4 or 3molL^{-1}NaOH. Working standard solutions of lower concentrations (20, 40, 60, 80, 100, 200, 400, 600, 800 and 1000µM) were prepared from 10mM CAF solution through serial dilution immediately before use.

2.5 Real Sample Preparation (Analytical Procedure)

Laagee Coffee sample was obtained from Abe Dongoro Woreda. Acid digestion and chloroform extracts of the coffee samples was prepared according to the procedure outlined before (Efa & Soreta, 2017). Briefly: For voltammetric analysis, 4g of raw powder coffee was added into 150mL beaker, and then dissolved in 100mL of 6 mol L^{-1} H_2SO_4. The mixture was diluted with 50mL of 0.1molL^{-1} H_2SO_4 to the mark after 4 min acid digestion and filtered. Then, the pH of the extracted sample solution (filtrate) optimized to 0.80 by 3molL^{-1} NaOH and was ready for CV analysis.

For spectrophotometric determination of caffeine, a weighed 4 g of sieved raw coffee sample powder was dissolved in 250mL distilled water and boiled in the temperature range 80-90^{o}C for 30 min while stirring using magnetic stirrer. The water-extracted caffeine was separated from the residue by decantation. Then the solution was cooled to room temperature and caffeine was extracted using chloroform. The caffeine was collected after removing the solvent using rotavapor and diluted to 150ml of solution for absorbance measurement. For this purpose 1000ppm caffeine was prepared by dissolving 200mg of caffeine standard in 200mL of distilled water. Working standards of caffeine prepared in 100mL of volumetric flasks: 50, 40, 30, 20, and 10ppm. The absorbance of each standard solution and the extracted sample solution was measured using a UV-Vis spectrophotometer at maximum absorption of 272nm. The absorbance values were then plotted against concentrations to generate a calibration curve.

3 RESULTS AND DISCUSSIONS

3.1 Electropolymerization of Polyalizarin on Glassy Carbon Electrode

Cyclic voltammograms of 2.0mM alizarin in a 0.1M H_2SO_4 at the polished GCE was obtained between −1.6 and +2.0V at scan rate of 0.1Vs^{-1}. **Figure 1** shows electropolymerization of alizarin on polished glassy carbon electrode at different segments in order to optimize effect of the number of potential cycles for film performance. The resulting coverage of Alizarin on the electrode surface was then monitored as a function of potential cycle's number at several scans. The anodic peak current of alizarin modified glassy carbon electrode increases with increasing the number of potential cycles, in the range of 10–12 segment and starts to decrease for the potential cycles more than 12 segments, thus this value was considered as optimum. **Figure 2** shows CVs of the electropolymerization of the modifier on glassy carbon electrode. In the first scan two anodic and one cathodic peaks were observed : anodic peak(1), anodic peak (2) and cathodic peak (3). Anodic peaks (1&2) are peaks of alizarin film formation on the electrode while cathodic peak (3) was the individual peak due to reduction of hydrogen from solution used for electropolymerization of alizarin (0.1molL^{-1} H_2SO_4). The peaks' current increased occur upon continuous scanning cycles; this reflects the continuous growth of the alizarin electropolymerization film on the surface of glassy carbon electrode.

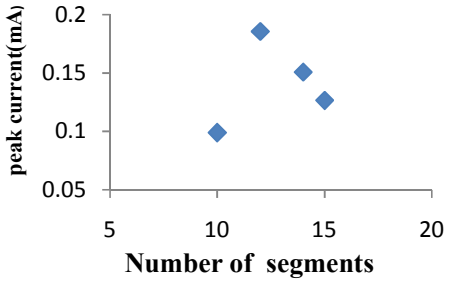


Figure 1**:** Optimization for electropolymerization of 2mM alizarin (in 0.1M H_2SO_4) on glassy carbon electrode at different segments (10-15 segments).

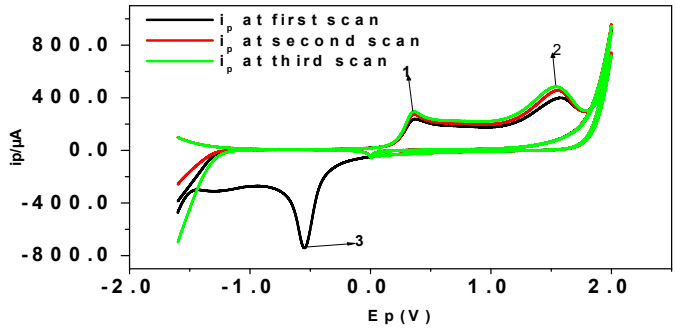


Figure 2: CV of electropolymerization of 2mM alizarin (in 0.1M H_2SO_4) on glassy carbon electrode; scanning potential: -1.6 to +2.0V; scan rate: 100mVs^{-1}, number of cycle: 6

2.3 Characterization of the PAZMGCE by Potassium Hexacyanoferrate

The prepared polyalizarin modified glassy carbon electrode was characterized by exploiting cyclic voltammetry using potassium hexacyanoferrate probe in potassium nitrate. As shown in **Figure 3** pair of well-defined redox peaks current for $K_3Fe(CN)_6$ probe were observed at the bare GCE, but after the modification of the surface with polyalizarin, the anodic and cathodic current peaks were decreased, showing that polyalizarin acted as a hole blocking layer for electron and mass transfer that hindered the diffusion of ferric cyanide toward the surface of PAZMGCE. This is due to the electrostatic repulsive interaction between polyalizarin and $K_3Fe(CN)_6$.

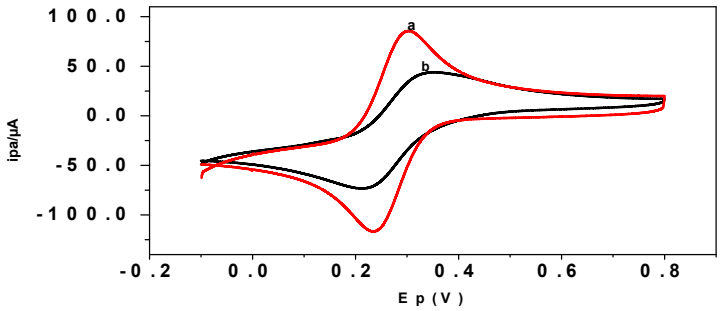


Figure 3: CV of 10mM $K_3Fe(CN)_6$ on bare (a) and Polyalizarin modified(b) GCE(electrolyte 1M KNO_3 (v = 50 mVs^{-1})).

3.3 Electrochemical Behavior of Caffeine

The electrochemical behavior of caffeine at the polyalizarin modified glassy carbon electrode was investigated using cyclic voltammetry. **Figure 4** shows the cyclic voltammograms of supporting electrolyte in the absence of caffeine(curve a), bare GCE (curve b) and polyalizarin modified GCE in the presence of $1\times10^{-3}molL^{-1}$ of caffeine (curve c) recorded under similar conditions. At the bare GCE, caffeine exhibited an irreversible anodic peak potential with low anodic peak current (curve b). But at the modified electrode (curve c), a well-defined, irreversible anodic peak potential with an enhanced anodic peak current was observed. In Comparison to the bare GCE, the PAZMGCE a much more enhanced current (c) was observed for caffeine oxidation, indicating the electrocatalytic activity for the performance of the modified electrode. An irreversible voltammogram caffeine oxidation exhibited at both unmodified and modified electrodes in this work confirms that the detection of caffeine is only upon oxidation process; which is similar with previously reported investigations (Amare & Admassie, 2012; Torres et al., 2014; Wang et al., 2015; Amare & Aklog, 2017).

Polyalizarin(PAZ) was protonated and formed as $PAZH^+$(**Scheme 3a**), and the electrochemical-reactions occurring at the modified electrodes may be accompanied by the exchange of protons with the solution. The possible electrode reactions can be expressed as follows though detailed mechanism might be more complicated:

$$PAZH^+ + CAF + H_2O \rightarrow PARH^+\cdot CAF$$

$$PARH^+\cdot CAF + H_2O \rightarrow PARH^+\cdot CAF - oxo + 2H^+ + 2e^- \text{ (unstable)}$$

$$PAZH^+\cdot CAF - oxo + H_2O \rightarrow PAZH^+ + oxo - CAF - oxo + 4H^+ + 4e^-$$

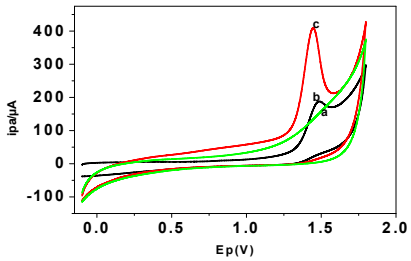


Figure 4: Cyclic voltammograms:(a) of supporting electrolyte in the absence of CAF, (b) BGCE and (c) PAZMGCE, in the presence of 1x 10^{-3} $molL^{-1}$ CAF in 0.1$molL^{-1}$ H_2SO_4 (pH 0.80), at a scan rate of 100mVs^{-1}.

2.3 Optimization of the Method Parameters

Effect of pH

The effects of pH of supporting electrolyte on the oxidation peak current of caffeine were studied from pH range of 0.7 to 2.0 in 0.1$molL^{-1}$ H_2SO_4. Figure 5 shows the cyclic voltammetry of 1mM Caffeine at these pH ranges. As pH increases from 0.9 to 2.0, the anodic peak current decreases resulting from decrease in electrostatic attraction between polyalizarin modified glassy carbon electrode and caffeine. At pH 0.8 the maximum peak current was obtained with wide potential window, thus used for further analysis of caffeine in this work.

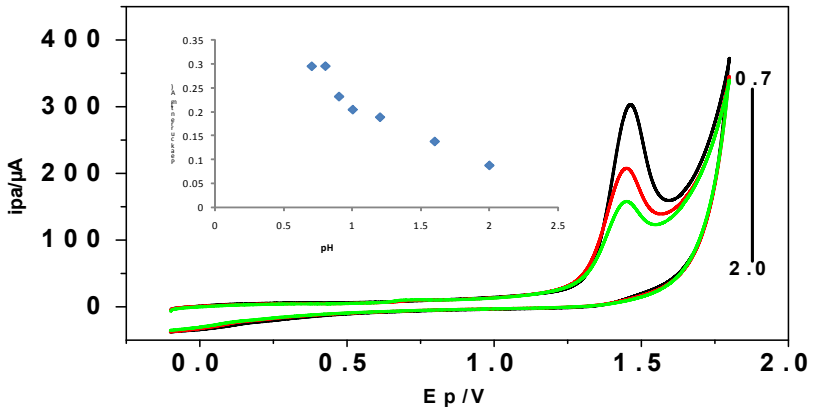


Figure 5: The cyclic voltammogram of 1mM CAF at PAZMGCE in 0.1M H_2SO_4 at different pH values: 0.7, 0.8, 0.9, 1.0, 1.2, 1.6 and 2.0(at 100mVs^{-1}); Inset: calibration curve for peak current versus pH.

Effect of Scan rate

In order to investigate the type of reaction kinetics the caffeine follows at the surface of polyalizarin modified glassy carbon electrode, the correlation coefficients (R^2) for the plots of peak current as a function of scan rate and square root of scan rate were compared in the scan rate range 20 to 500mVs^{-1}. As shown in **Figure 6**, oxidation peak currents of caffeine are gradually increased as the scan rates increased. A higher correlation coefficient (R^2= 0.999) is occurred for the scan rate square root dependence of peak current than for the scan rate dependence of peak current. This indicates the caffeine oxidation at the PAZMGCE follows diffusion controlled electrochemical kinetics which is exactly confirmed according to previous literature findings (Efa & Soreta, 2017; Amare & Aklog, 2017).

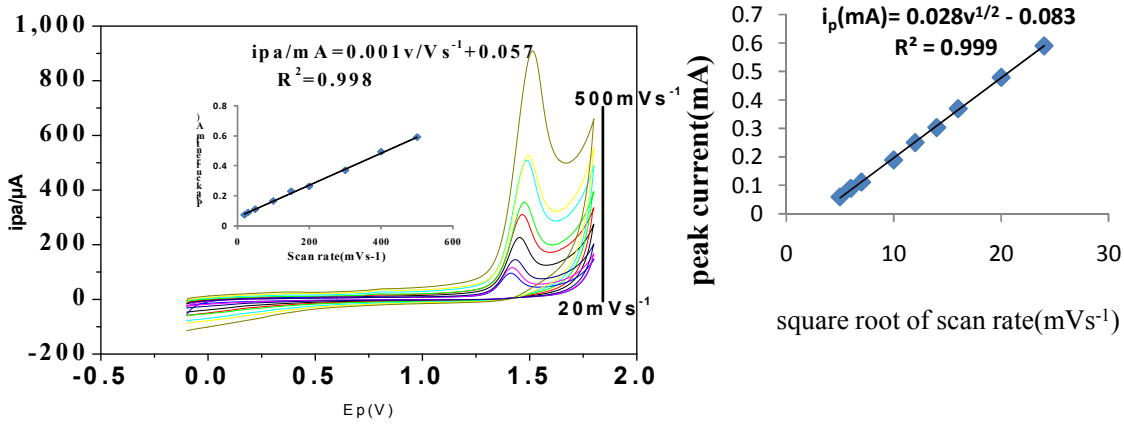


Figure 6: Cyclic voltammograms of PAZMGCE in 0.1M H_2SO_4 (pH 0.80) containing 1mM of CAF, at scan rate of 20, 30, 50, 100, 150, 200, 300, 400, and 500mVs^{-1}; insert: plot of peak current versus scan rate.

2.4 Calibration Curve and Detection Limit of Caffeine at PAZMGCE

Under the optimized solution and method parameters, anodic peak current of caffeine at polyalizarin modified GCE was linearly proportional to the caffeine concentration in the range of 20 to 1000 $\times 10^{-6}$mol L^{-1} (**Figure 7**) with a linear regression equation and correlation coefficient of $Ipa/\mu A$= 31.5 + 0.180[Caffeine] M and R^2 = 0.998, respectively. Method limit of detection (LoD = 3S/slope); where S is standard deviation of peak current of lowest concentration from linear concentration range for n=7) was calculated to be 3.7 x 10^{-6} M.

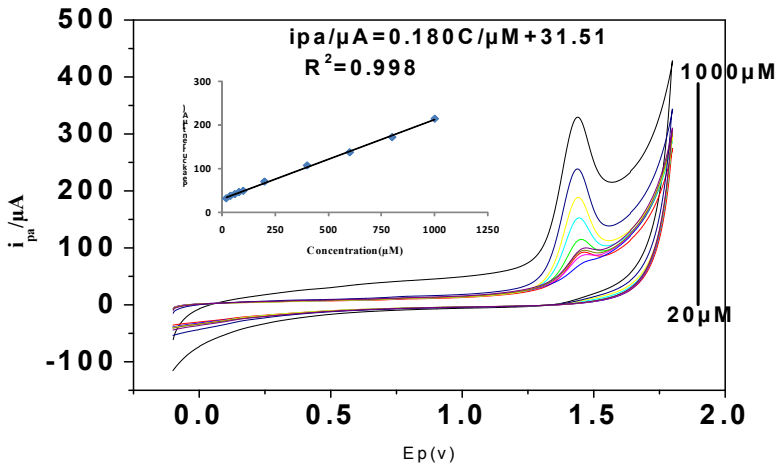


Figure 7: Cyclic voltammogram of different concentration of caffeine on PAZMGCE; 20, 40, 60, 80, 100, 200, 400, 600, 800 and 1000µM CAF (pH =0.80, v=0.1Vs^{-1});Insert: plot of anodic peak current versus concentration of caffeine.

2.5 Application of the Method for the Determination of Caffeine in Coffee Samples

Sample coffee extract was prepared by acid digestion and chloroform as described in the procedure under the experimental part for electroanalytical (CV) and analytical (UV-Visible spectrophotometry) methods respectively. To determine the concentration of caffeine in real coffee sample, the cyclic voltammetric peak current and UV-visible spectrophotometric absorbance for each extract sample was recorded. Then, converted to concentration units using the regression equation of the calibration curve. Comparison of caffeine determined from sample coffee extract by cyclic voltammetry and UV-visible spectrophotometry was done in this work.

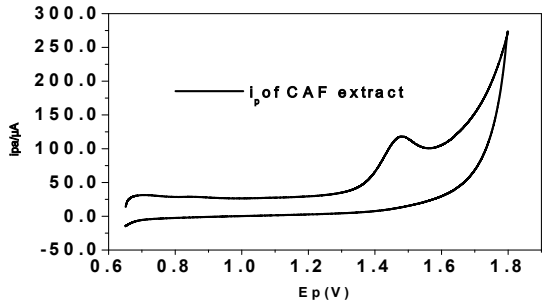


Figure 8: Cyclic voltammetric response for acid digestion extracted coffee sample

Analysis of UV/Vis spectrophotometer result

For validation purpose, the result of electrochemical method was compared with that obtained by UV/Vis spectrophotometer. The absorbance of the working standards and samples were measured at 272nm. The caffeine concentration of the samples was calculated from linear regression equation of absorbance versus standard concentration as it can be seen from calibration curve (**Figure 9**). The amounts of caffeine obtained by UV/Vis spectrophotometric method was lower than those obtained by electrochemical method (**Table 2**). This suggests that the new developed method is more sensitive than UV/Vis spectrophotometer method for determination of caffeine.

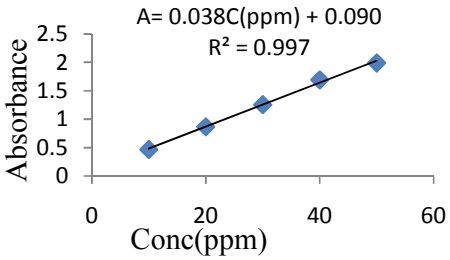


Figure 9: Calibration curve for absorbance versus concentration

Table 1: Measured absorbance values of working standard solutions

Concentration(ppm)	Absorbance
50	1.99
40	1.692
30	1.254
20	0.864
10	0.466

Table 2: Concentration of caffeine obtained from raw coffee by different methods

Methods	%w/w
Cyclic voltammetry	0.148
Spectrophotometry(n=3)	0.144

3.7 Repeatability, Reproducibility and Stability

The repeatability of the PAZMGCE was evaluated with repeated current responses (n = 7) with the same modified electrode for 2.0mM caffeine in 0.1 M H_2SO_4 pH 0.80 solution and the relative standard deviation (RSD) of the peak current was calculated to be 3.7%, which suggests good repeatability of the measurement at the modified electrode. Similarly, the reproducibility of the electrodes was determined by using two modified glassy carbon electrodes under the same working conditions and the RSD was 2.27% (n=8). The results indicated that the PAZMGCE showed good reproducibility for the detection of caffeine.

Stability

To determine the stability of PAZMGCE toward the oxidation peak current of caffeine, the electrode was modified with the modifier and stored at 4^0C in supporting electrolyte (0.1M H_2SO_4). The two peak current responses are recorded under the same conditions at first day, seventh day and fifteenth day as shown in **Table 3**. The calculated mean current for 15 durations was demonstrating the stability of the modified electrode. The peak current of caffeine retained 96.9% of its initial response current after 15 days. This shows the modified electrode has good stability toward the peak current response.

Table 3: Peak currents recorded at different days to check stability of the modified electrode for 1mM caffeine

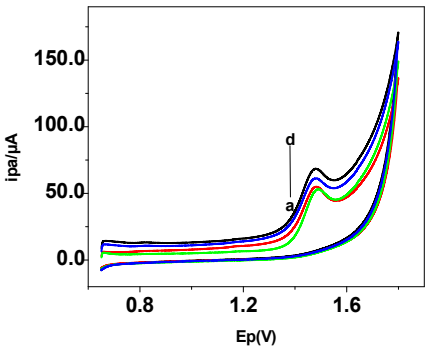
Peak current at first day		Peak current at seventh day		Peak current at fifteenth day	
E_p	i_p	E_p	i_p	E_p	i_p
1453	0. 1987	1457	0.1968	1463	0.1907
1452	0. 1984	1458	0.1921	1463	0.1940

3.8 Recovery Study of the Developed Method

To evaluate the accuracy and applicability of the developed cyclic voltammetric method using polyalizarin modified GCE, the recovery of spiked standard caffeine in acid digestion extracts of coffee samples was checked. The results are summarized in **Table 4**. The recovery was acceptable, showing that the developed method could be effectively used for the determination of caffeine in extract coffee samples.

Table 4: Percentage recoveries of coffee extract samples spiked with standard caffeine of different concentrations ((a–d): 0, 4, 6 and 8 × 10^{-6}mol l^{-1}), respectively.

Sample	Initial(µM)	Spiked(µM)	Detected(µM)	Recovery (%)
Coffee extract(a)	38.83	-	-	-
Coffee extract(b)	38.83	4	42.72	97.25
Coffee extract(c)	38.83	6	44.39	92.67
Coffee extract(d)	38.83	8	48.27	118.00



Figur 10: CVs of Lage coffee extract samples spiked with different concentrations of standard caffeine (a-d): 0, 4, 6 and 8 x 10^{-6} mol L^{-1}, respectively.

Interference study

The possible interference from compounds of similar structures (which is N-methyl derivatives of xanthine) in the determination of caffeine was further studied. **Figure11** showed cyclic voltammetry at PAZMGCE in 0.10 $molL^{-1}$ H_2SO_4 with $2\times10^{-3}molL^{-1}$ caffeine and theophylline concentrations. Two well-defined peaks appeared at the potentials of 1309mV and 1459mV, corresponding to theophylline and caffeine respectively, which offered a promising possibility for the simultaneous determination of theophylline and caffeine. This confirms that analysis of caffeine in coffee extract using the PAZMGCE is not affected by structurally similar compounds like theophylline.

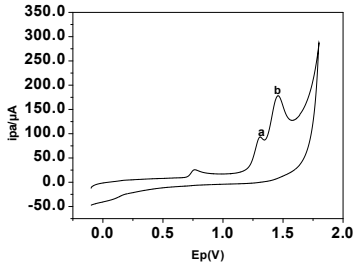


Figure 11: Cyclic voltammogram of mixture of **(a)** theophylline (2·0 × 10^{-3}mol L^{-1}) and **(b)** caffeine (2·0 × 10^{-3}mol L^{-1}) peaks.

3.9 Comparisons of Caffeine Content of Coffee Grown at Different Localities of Ethiopia

The caffeine content for Lage coffee sample observed was below 0.5 % for both cyclic voltammetric and UV-Visible spectrophotometric method which is 0.148% and 0.144% respectively. The caffeine concentrations depend principally on the genus or variety of coffee, the geographical origin (agro-ecology) and the caffeine extraction method from

coffee beans. Furthermore, the caffeine contents determined in this work was compared with the caffeine contents of coffee beans reported by similar analytical techniques. In general, caffeine concentrations in coffee beans reported ranges between 0.9 % - 1.2 % (w/w) (Petracco, 2005) and 1.0 - 1.2 % (w/w) (Belay et al., 2008). Caffeine content of coffee grown in different areas of Hararghe region, Ethiopia ranges from 0.601 % to 0.903 % (Demissie et al., 2016). Therefore, it is clear that the concentrations of caffeine in coffee collected from Abe Dongoro woreda Lage area was noticeably lower than their counterpart (0.601 - 1.2 %) grown in other parts of Ethiopia.

4. Conclusion

The newly developed cyclic voltammetric sensor at polyalizarin modified glassy carbon electrode(PAZMGCE) showed good sensitivity, selectivity, reproducibility and stability for the detection of caffeine. The method is relatively cheaper than those previously reported depending on the cost of modifier used in this work. Excellent recoveries with acceptable values were achieved for the determination of spiked standard caffeine samples in coffee extracts. Therefore, the method developed can be applied for the direct analysis of caffeine content in real samples. In this study both UV/Vis-spectrophotometry and Cyclic voltammetric methods were used for the determination of caffeine content of Laagee coffee. The result of the methods indicated that the concentration of caffeine in coffee beans (% w/w) was 0.144% and 0.148% respectively. Therefore, from this research work the new developed electroanalytical method(CV) is more sensitive than analytical (UV/Vis-spectrophotometry) method.

Recommendation

Further study is required to confirm the morphology of the modified electrode by SEM and to improve the caffeine content of Laagee coffee by another method such as SWV, which can detect smaller than this value.

References

[1] A.Carolina Torres, Madalina M. Barsan, Christopher M.A. Brett (2014). Simple electrochemical sensor for caffeine based on carbon and Nafion-modified carbon electrodes. *Food Chemistry, 149* , 215–220.

[2] A.N. Golikand, J. B.Raoof, M. Baghayeri, M. Asgari, and L. Irannejad (2009). Electrochemical Reduction of Dioxygen on Alizarin Modified Glassy Carbon Electrode in Acidic Medium. *Russian Journal Of Electrochemistry,* 45(8),881– 886.

[3] A.Wang, L. Li, F. Zang, and Y. Fang (2000). Amperometric detection of three purine alkaloids following their separation by micellar electrokinetic capillary chromatography. *Analytica Chimica Acta ,* 419, 235-242.

[4] Abebe Belay, Kassahun Ture, Mesfin Redi, Araya Asfaw (2008). Measurement of caffeine in coffee beans with UV/vis spectrometer. *Food Chemistry* , 310–315.

[5] Ahmed A. Aly (2013). Determination of caffeine in roasted and irradiatedcoffee beans with gamma rays by high performance liquid chromatography. *Food Science and Quality Management* , 28-34.

[6] Belete Tewabe Gebeyehu and Solomon Libsu Bikila. (2015). Determination of Caffeine Content and Antioxidant Activity of Coffee. *American Journal of Applied Chemistry ,* 3(2), 69-76.

[7] Biuck Habibi, Mehri Abazari, Mohammad Hossien Pournaghi-Azar(2012). A Carbon Nanotube Modified Electrode For Determination Of Caffeine By Differential Pulse Voltammetry. *Chinese Journal Of Catalysis,* 33(11), 1783–1790.

[8] Ephrem G. Demissie, Girma W. Woyessa, Arayaselassie Abebe (2016). Uv/vis spectrometer determination of caffeine in green coffee beans from hararghe,ethiopia, using beer-lambert's law and integrated absorption coefficient techniques. *Scientific Study & Research ,* 17(2), 109 – 123.

[9] Esra Rukiye Apaydın, Gulsen Saglikoglu, Selehattin Yilmaz, Mustafa Yildiz (2015). Sensitive Electrochemical Determination of Oxcarbazepine using Polymer Film Modified Glassy Carbon Electrode. *Int. J. Electrochem. Sci.,* 10, 1904 - 1915.

[10] F.Regan, and Y. Shakalisava. (2005). Rapid simultaneous determination of alkylxanthines by CZE and its applications in analysis of pharmaceuticals and food samples. *Analytica Chimica Acta , 540*, 103-110.

[11] Fangyuan Zhao, Fei Wang, Weining Zhao, Jing Zhou, Yang Liu, Lina Zou & Baoxian Ye (2011). Voltammetric sensor for caffeine based on a glassy carbon electrode modified with Nafion and graphene oxide. *Microchim Acta,* 174, 383–390.

[12] Hayati Filik, Asiye Aslıhan Avan & Yasemin Mümin (2016,). Simultaneous Electrochemical Determination of Caffeine and Vanillin by Using Poly(Alizarin Red S) Modified Glassy Carbon Electrode. *Food Anal. Methods*.

[13] K.R. Mahanthesha, B.E. Kumara Swamy*, Umesh Chandra, Yadav D. Bodke, K. Vasanth Kumar Pai and B.S Sherigara (2010). Cyclic Voltammetric Investigations of Alizarin at Carbon Paste Electrode using Surfactants. *Int. J. Electrochem. Sci.,*4,1237 - 1247.

[14] Kurangalara R. Mahanthesha, B. E. (2014). Poly (alizarin) Modified Glassy Carbon Electrode for the Electrochemical Investigation of Omeprazole: A Voltammetric study. *Anal. Bioanal. Electrochem, 6*, 234 - 244.

[15] Ľubomír Švorc. (2013). Determination of Caffeine: A Comprehensive Review on Electrochemical Methods. *Int. J. Electrochem. Sci.*, 8, 5755 - 5773.

[16] Meareg Amare and Senait Aklog (2017). Electrochemical Determination of Caffeine Content in Ethiopian Coffee Samples Using Lignin Modified Glassy Carbon Electrode. *Journal of Analytical Methods in Chemistry* , 1-8.

[17] Meareg Amare and Shimelis Admassie(2012). Polymer modified glassy carbon electrode for the electrochemical determination of caffeine in coffee. *Talanta ,* 93, 122-128.

[18] Mulugeta Tesema Efa and Tesfaye Refera Soreta (2017). Voltametric Sensor for Determination of Caffeine Using Nanohole Modified Glassy Carbon Electrode. *International Journal of Modern Chemistry and Applied Science ,* 4, 492-503.

[19] Pandurang N. Patil*(2012). Caffeine in Various Samples and Their Analysis With HPLC. *International Journal of Pharmaceutical Sciences Review and Research,*16(2),76-83.

[20] Petracco M. (2005). Our every day cup coffee. *The chemistry behind its magic,journal of Chemical Education* , 1161-1167.

[21] Pradeep S., G. N. Rameshaiah, Hadagali Ashoka (2015). Caffeine extraction and characterization. *Int J Cur ,* 7, 16-19.

[22] Qing Zhanga, Dawei Pan, Haiyun Zhang, Haitao Han, Qi Kang (2014). Development of a poly(alizarin red S)/ionic liquid film modified electrode for voltammetric determination of catechol. *Electrochimica Acta,*133, 23–29.

[23] S.Chitravathi* and N.Munichandraiah (2016). Voltammetric determination of paracetamol, tramadol and caffeine using poly(Nile blue) modified glassy carbon electrode. *Journal of Electroanalytical Chemistry ,* 764, 93-103.

[24] Yan Jiang[1] , Tianci Niu[1], Zhihua Wang[1], Wensheng Tan[2], Feng Liu[1], & Yong Kong[1] (2017). Electrochemical polymerization of alizarin and the electrochemical properties of poly(alizarin). *Springer*, DOI 10.1007/s11581-017-2288-2.

[25] Yan Wang, Ting Wu & Chun-yan Bi (2015). Simultaneous determination of acetaminophen, theophylline and caffeine using a glassy carbon disk electrode modified with a composite consisting of poly(Alizarin Violet 3B), multiwalled carbon nanotubes and graphene. *Microchim Acta,*doi 10.1007/s00604-015-1688-0.

[26] Yemane Tadesse, Abraha Tadese, R. C. Saini, and Rishi Pal(2013). Cyclic Voltammetric Investigation of Caffeine at Anthraquinone Modified Carbon Paste Electrode. *International Journal of Electrochemistry* , 1-7.

[27] Zewdu Tadesse Wondimkun1, Abdisa Gebisa Jebessa, Liboro Hundito Molloro, Tesfaye Haile (2016). The Determination of Caffeine Level of WolaitaZone,Ethiopia Coffee Using UV-visible Spectrophotometer. *American Journal of Applied Chemistry,*4(2)59-63.

[28] Zhang, J.; Wang, L. P.; Guo, W.; Peng, X. D.; Li, M.; Yuan, Z. B (2011). Sensitive differential pulse stripping voltammetry of caffeine in medicines and cola using a sensor based on multi-walled carbon nanotubes and nafion. *International Journal of Electrochemical sciences* , 6(4), 997-1007.

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