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Voltametric Sensor for Determination of Caffeine Using Nanohole Modified Glassy Carbon Electrode

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Abstract: In this work, nanohole modified glassy carbon electrode was electrochemically fabricated and used for determination of caffeine in coffee. The surface modification procedure involved electrochemical nucleation gold nanoparticles on a glassy carbon electrode that served as a template for the nanoholes fabrication. After the electronucleation step, the electrode surface was passivated with P-notroanaline to produce molecular insulation to the electrode. The nanohole modifeied electrode was produced when the nucleated gold nanoparticle were electrochemically stripped off from the electrode surface. The developed electrode helps to minimize oxidation potential of caffeine and also to increases mass transport of caffeine to electrode surface thereby increasing sensitivity and selectivity of the modified electrode for determination of caffeine. The method enables determination of caffeine in the range from 1.0×10^{-7} to 1.0×10^{-3} mol L^{-1},with limit of detection of 7.28×10^{-8} mol L^{-1}. The effect of theophylline and theobromine on the determination of caffeine was studied and found to be minimal. Besides, the sensor displayed good stability and reproducibility.

***Keywords*:** caffeine, nanohole electrode, Gold nanoparticles, electro nucleation, glassy carbon electrode

1. Introduction

Electroanalytical methods have found a vast number of applications in different sample matrixes [1]. The major improvement in electroanalysis relies mainly on the electrode modification strategies for tailored applications. Hence, electrode surface modification remains an active research area. There are a number of surface modification strategies such as chemical modification of carbon electrodes [2 -4], electrodeposition of metal and nanoparticles on electrode surface, polymerization and the use of surfacantant as modifiers. The electrochemical techniques utilize tailor made chemically modified electrode for sensitive and selective analytical applications [5]. Surface modification of conventional electrodes are very important in electroanalysis for the enhancement of current responses of the electrode and developing stable and highly target specified interfaces [6].

Nanomaterials have been widely used in electrochemistry as elements of a chemical sensor [7]. Modifying the surface of the electrode with nanomaterials has the possibility of controlling how electrode interacts with its environments [8 - 9]. Even though many electrochemical methods have been used surface modified electrodes for the detection of caffeine, no investigation has been reported on the electroanalysis of caffeine by nanhole modified electrodes glassy carbon electrode [9]

Caffeine (1, 3, 7-trimethylxanthine) is a natural alkaloid, which is found in various kinds of food and beverages that we consume in daily life. It can have some adverse effects on health if consumed in excess and it is also considered to be a risk factor for cardiovascular diseases. That's why there have been numerous studies with the target to develop reliable methods for determining caffeine, such as spectrophotometery, chromatography and biosensing [10 -12]. Nevertheless, these methods are generally more expensive, time-consuming and complicated than electroanalytical techniques. Accordingly, interest in electrochemical methods has increased for its simplicity, portability, high sensitivity, and moderate-cost instrumentation [13].

However, the electrochemical determination of caffeine at the more common electrode materials is generally not feasible because the oxidation of caffeine occurs at a very positive potential, thus overlapping with the discharge of the background medium. So various modified electrodes have been developed for the determination of caffeine recently [14].

In this work, a voltammetric sensor for determination of caffeine based on nanohole modified glassy carbon electrode is reported. The sequential deposition approach developed by Soreta *et al* [15] was employed in the electrode surface modification procedure.

2. Experimental

Chemicals

Caffeine reagent ($C_8N_4O_2H_{10}$, anhydrous powder 99%, , theophylline ($C_8N_4O_2H_8$, hydrous powder 99%, theobromine ($C_8N_4O_2H_8$, hydrous powder, 99%, potassium tetera chloroaurate ($KAuCl_4$, 99.995%, hexamine ruthenium chloride, ($Ru(NH_3)_6Cl_3$, 98%), sodium perchlorate ($NaClO_4$) and 2-mercaptoethanol ($HSCH_2CH_2OH$, 99%,) were purchased from sigma Aldrich, germany.

Para- nitro aniline ($C_6H_6N_2O_2$, 99%, Kiran), sodium nitrite ($NaNO_2$, 96%, Nice), hydrochloric acid (HCl, 37%, Riedel dehaen), , sulphuric acid (H_2SO_4, 98%, Merck), hydroquinone, ($C_6H_6O_2$, 99%, Kiran), potassium nitrate (KNO_3, 99%,NICE), potassium chloride (KCl, 99.5%, Finkem), potassium hexacyanoferrate ($K_3Fe(CN)_6$, 97%, Labmerk) chloroform ($CHCl_3$, 99.5% NICE), calcium carbonate ($CaCO_3$, 99% NICE), sodium sulphate (Na_2SO_4, 99%, Finkem) and Sodium hydroxide (NaOH, 99.3%, Eines) were obtained from stated suppliers and used as received without any pre-treatment. Deionised water was used to prepare all solutions.

Methods

Solution preparation:

Standard stock solution of caffeine (2.0×10^{-2} $molL^{-1}$) was prepared in deionised water and diluted with 0.01$molL^{-1}$ H_2SO_4 and stored at 4 °C. The pH of the solution was adjusted either by 0.1 $molL^{-1}$ H_2SO_4 or diluted NaOH Working standard solutions of lower concentrations were prepared immediately before use.

Instrumentation

Cyclic voltammetry and amperometric experiments were performed out using BASi Epsilion EC-Version 1.40.67 voltammetric analyzer (Bio-analytical Systems, USA) containing three electrode system, Ag/AgCl as reference electrode (BASi, MF 2079), platinum wire as counter electrode BASi, MW 1032) and glassy carbon electrode (3 mm diameter, BASi, MF 2012) as working electrode. All potentials were reported with respect to this reference electrode. Magnetic stirrer (BASi C3 Cell stand at 500 rms) was used for stirring.

Electrode Preparation

The bare GC electrode (3 mm diameter) was first polished with a polishing paper and micro cloth (BAS, Bioanalytical Systems USA) and then further polished to mirror finish with alumina slurries of 3 µm (BAS, USA) and rinsed thoroughly with distilled water. Then the polished GC electrode was electrochemically conditioned by potential scanning from 0 to 1.4 V in 0.1 MKCl for at least five complete scans at 50mV/s, to diminish background current due to oxidation of the electrode. Electrodes with a high background current above a selected reference were excluded.

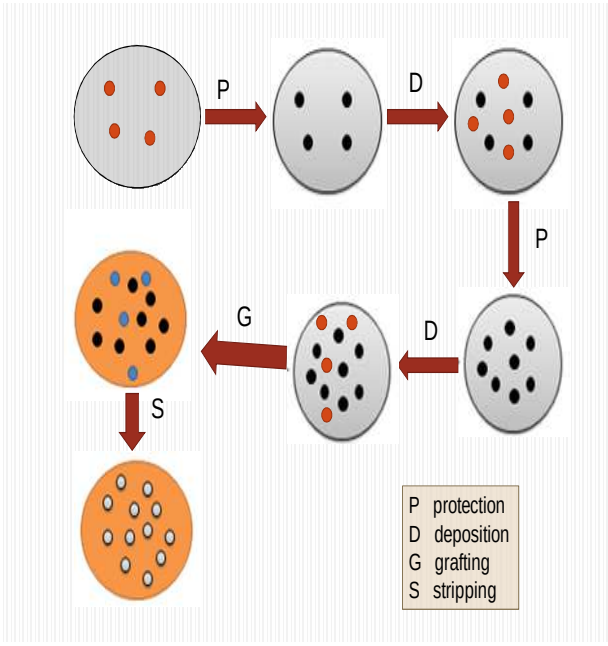
Surface modification of the electrode

Following the polishing and electrochemically conditioning steps, the electrodes were modified by the electronucleation of gold nanoparticles according to the literature report by Soreta et al [15].

Fabrication of the random nanohole arrayed electrode has been carried out using three-step surface modification strategy as depicted in Scheme 1. These were; (i) electrochemical deposition of gold nanoparticles (ii) Grafting of p-nitroaniline film and (iii) Stripping of gold nanoparticles

(*i) Electrochemical nucleation of Gold Nanoparticles*.

Gold nanoparticles were electrodeposited from solution of 0.1 mmol L^{-1} $KAuCl_4$ in 0.5mol L^{-1} H_2SO_4 according to the procedure reported by Soreta etal [15]. After the first and second gold electronucleation steps, the particles were capped with self assembled monolayer of 2-mercaptoethanol [15].



(ii) ***Grafting of p-nitroaniline Film***. P-nitroaniline film was grafted on GC electrode from 3 mmol L^{-1} of p-nitroaniline diazonium salt solution that was prepared according to literature [16]. Briefly solution of 3 mmol L^{-1} of p-nitroaniline and 0.1molL^{-1} sodium nitrite in 0.5mol L^{-1} of hydrochloric acid were kept separately in an ice jacketed beaker for 1 h. Then 400 μL of 0.1 mol L^{-1} $NaNO_2$ was added to 20 mL of 3 mmol L^{-1} paranitoaniline (PNA) under stirring at room temperature and then CV was used to graft the film on bare GCE and AuNPs deposited GC electrode from 0.6 to -0.2 V for 3 cycles at scan rate of 100 mVs^{-1}[16].

iii) ***Stripping of Gold Nanoparticles.*** Gold nanoparticles was stripped from P-nitroaniline grafted AuNPs deposited GC electrode using CV from 0 mV to 1400 mV for eight complete scans at a scan rate of 0.1Vs^{-1} in 0.1 mol L^{-1} KCl solution [15].

Scheme 1 Nanoarrayed electrode fabrication steps

Sample preparation

Raw coffee obtained from shops in Jimma Town, Ethiopia. The level of caffeine was determined in powdered and roasted coffee sample amperometricallyy using the following sample preparation procedure. For voltammetric analysis, four gram of raw powder coffee was added into 150 mL beaker, and then dissolved in 100 mL of 6 mol L^{-1} H_2SO_4 [17. The mixture was diluted with 50 mL of 0.1mol L^{-1} H_2SO_4 to the mark after 4 min, 5 min, and 10 min acid digestion and filtered, and then the pH of the solution adjusted to 1.92 by 0.1mol L^{-1} NaOH. Amperometric determination of caffeine at the modified electrode was done by standard addition method. Working standards of caffeine *2 µ* mol L^{-1}, *4µ* mol L^{-1}, *6 µ* mol L^{-1}, *8µ* mol L^{-1}, *10 µ* mol L^{-1}, *12µ* mol L^{-1},was successive ejected in to 5 mL of each sample solution for the determination of caffeine.

For spectrophotometric determination of caffeine in coffee, first the roasted and podwered coffee was brewed in 100 mL of boiling water. Then the solution was cooled to room temperature and caffeine was extracted using chloroform. The caffeine was collected after removing the solvent using rotavapor [17]. For this purpose 1000 ppm caffeine was prepared by dissolving 200 mg of caffeine standard in 200 mL of distilled water. Working standards of caffeine 10, 20, 30, 40, 50 and 100 ppm ejected to aliquots 1mLof sample solution for determination of caffeine [18]

3. Results and Discussion

Gold nanoparticles were deposited from 0.1 mmol L^{-1} $KAuCl_4$ in 0.5 mol L^{-1}aqueous H_2SO_4. As shown in Figure 1 AuNPs was oxidized at 1.2 V indicated pure deposition AuNPs. The cathodic peak observed around 0.6 V showed the reduction of AuNPs. In addition, deposition of AuNPs were checked by running CV of electrode only in supporting electrolyte after depositing gold on GCE. The appearance of sharp cathodic peak at 0.955V (Inset), is an indication of deposited AuNPs and it is in good agreement with the experimental results reported by *Soreta, et al* [15].

Chronoamperometric Deposition of Gold nanoparticles

Next to cyclic voltammetry, gold nanoparticles was electrodeposited using chronoamperometry by applying 0 V stepped to 1.1 V for 5s from solution 0.1 mmol $L^{-1}$$KAuCl_4$ in 0.5 mol L^{-1} H_2SO_4 [15]. To increase number nanoparticles (avoid secondary nucleation) a sequential deposition technique using self assembling monolayer of 2-mercaptoethanol was used based on *Soreta, et al* [15].

Grafting of P-nitroaniline Diazonium Film

Next to chronoamperometry deposition of AuNPs, GC electrodes grafted with P-nitroaniline (NPA) moiety was performed using CV for 3 cycles from - 0.2 V to 0.6V, at scan rate of100 mVs^{-1} [19]. The film formation on the surface of bare and nanohole modified electrode was confirmed using common redox probes such as $K_3Fe(CN)_6$, $Ru(NH_3)_6Cl_3$ and hydroquinone (HQ)**.**

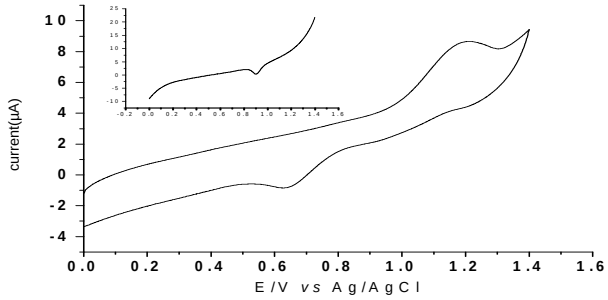


Figure 1 Cyclic voltammogram of 0.1 mmol L^{-} $KAuCl_4$ in 0.5mol L^{-1} H_2SO_4 at bare GC electrode. Inset, reduction of a gold oxide layer at scan rate100 mVs^{-}.[1]

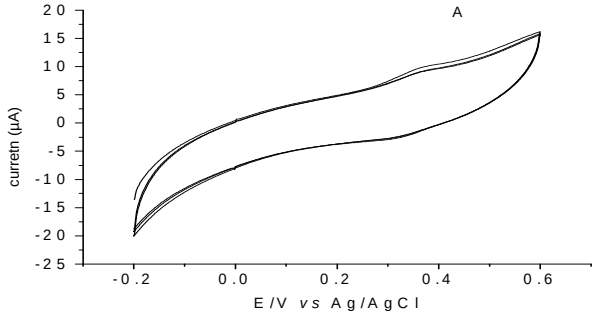


Figure 2 Cyclic voltammogram of 3 mmol L^{-1} PNA in 0.5 mol L^{-1} HCl on bare GCE (A) and nanohole/GCE (B) after addition of 0.1 mol L^{-1} $NaNO_2$ on AuNPs deposited GC (V = 100 mVs^{-1})

Stripping of Gold Nanoparticles

After grafting NPA film on GCE, the next step was to produce nanohole. This was achieved by stripping the nucleated AuNPs using CV from 0 mV to 1400 mV with eight cyclic scans at a scan rate of 0.1 Vs^{-1}. As shown in Figure 3 the anodic peak current observed in the first cycle around 950 mV is due to oxidation of AuNPs and its magnitude decreased in the successive cycles indicate complete removal AuNPs [15].

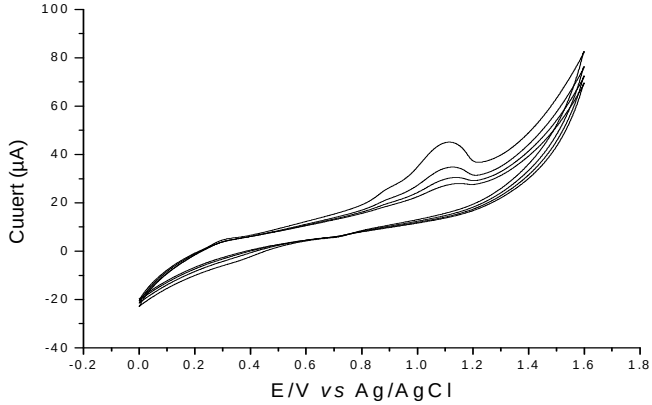


Figure 3 Cyclic voltammogram of gold nanoparticles nucleated nitro phenyl modified GC in 0.1 mol L^{-1} KCl (V = 100 mVs^{-1})

Electrochemical Characterization the Fabricated Electrode

The prepared nanohole modified electrode was characterized using cyclic voltammetry using hydroquinone, hexamine ruthenium chloride and potassium hexacyanoferrate probes. The characterizations of nanoarrayed electrode were further investigated using cyclic voltammetry. As shown in Figure 4 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 nanostructure, the anodic and cathodic current peaks were almost disappeared, showing that PNA acted as a blocking layer for electron and mass transfer that hindered the diffusion ferric cyanide toward the electrode surface. This is due to the electrostatic repulsive interaction effect between PNA and $K_3Fe(CN)_6$ [20]

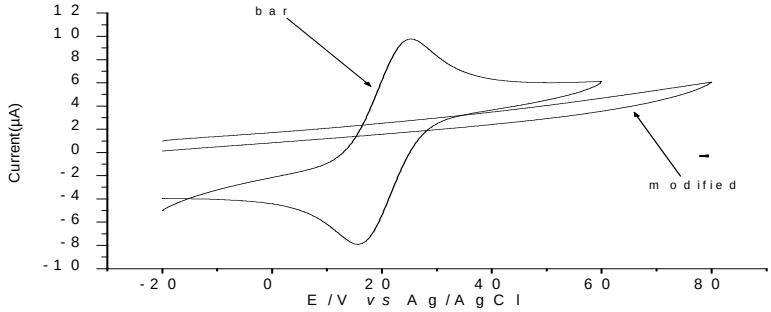


Figure 4 Cyclic voltammogram of 10 mmol L^{-1} Fe $(CN)_6^{-3}$ on bare and NP modified GCE (electrolyte 0.1 mol L^{-1} KCl (V = 50 mVs^{-1}).

Redox peak current of Ru $(NH_3)_6^{3+}$ at nanohole modified glassy carbon electrode was compared to at PNA grafted AuNPs deposited GCE [16]. The current response increased at nanohole modified electrode due to the electrostatic interaction attractions between negatively charged film with $Ru(NH_3)_6^{+3}$ and also increased mass transfer contribution to the electrode due to their large surface area than film grafted on gold nanoparticles modified GCE(Figure5) [21].

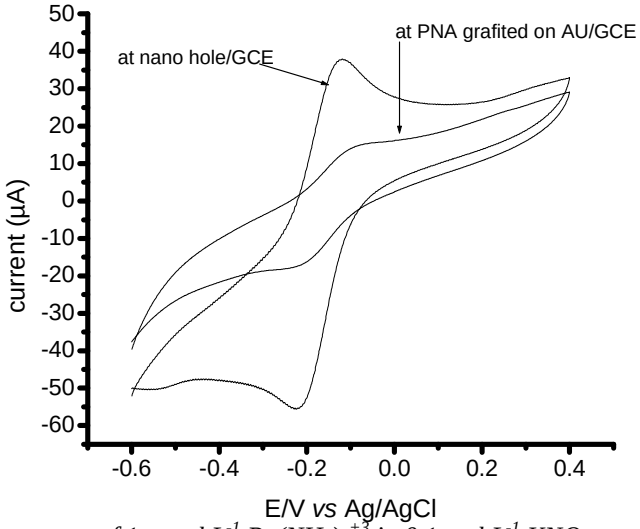


Figure 5 Cyclic voltammogram of 1 mmol L^{-1} $Ru(NH_3)_6^{+3}$ in 0.1 mol L^{-1} KNO_3 at nanohole modified /GCE and PNA grafted on AuNPs

The cyclic voltammogram presented in Figure 6 is the voltammetric response of HQ solution at nanohole modified GCE, bare GCE and nitro phenyl modified GCE [22]. However, the minimized redox potential separations observed, at nanohole modified glassy carbon electrode than bare and PNA modified bare electrode is due to improved enhanced mass transport [23].

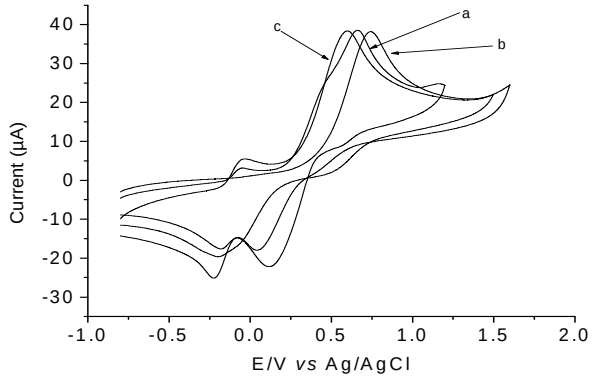


Figure 6 Cyclic voltammogram of 10 mmol L^{-1} HQ in 0.1 mol L^{-1} $NaClO_4$ on (a) nanohole modified GCE, (b) bare GCE and (c) PNA/ GCE (V = 100 mVs^{-1}).

Voltammetric Behaviour of Caffeine at Nanohole Modified GCE

Figure 7 depicts the cyclic voltammogram of 2.0×10^{-5} mol L^{-1} caffeine in 0.01mol L^{-1} H_2SO_4 at nanohole modified GCE (pH 1.92). There was no obvious peak current corresponding to the oxidation caffeine, at bare GCE within the potential window studied. This is in line with experimental results reported [24], but an anodic peak of caffeine was observed around 1490 mV at nanohole/GCE, this shows the modified electrode decrease over potential oxidation of caffeine, due to enhanced mass transport of caffeine to the surface the electrode. The result shows the proposed method displayed good feasibility for determination of caffeine as compared to previous report [24].

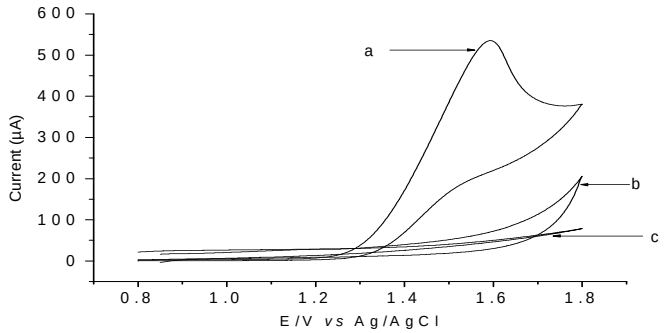


Figure 7 Cyclic voltammogram 2.0×10^{-5} mol L^{-1} Caffeine in 0.01mmol L^{-1} H_2SO_4 recorded at the nano hole /GCE (a), only in supporting electrolyte (b) and at bar GCE in the presence of caffeine (c), (all at scan rate of 50 mVs^{-1}).

Optimization of Experimental Parameters

Effect of Supporting Electrolyte

Various types of electrolyte solutions were tested as supporting electrolytes [25]. This because electrochemical behaviour of caffeine is can be affected by supporting electrolyte solutions [25]. As Figure 8 shows, out of these electrolytes, sulphuric acid solution is the most suitable medium yielding the best peak separation for caffeine oxidation from the background currents. So, sulphuric acid solution is used as supporting electrolyte for caffeine analysis throughout this work.

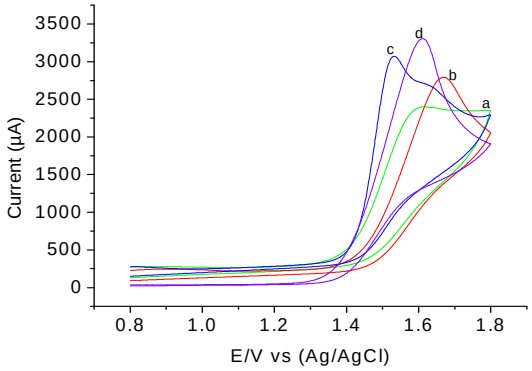


Figure 8 Cyclic voltagram of the nanohole modified/GCE in the presence of $2x10^{-5}$ mol L^{-1} caffeine in 0.01mol L^{-1} different supporting electrolytes: CH_3COO (a), HCl (b), HNO_3 (c) and H_2SO_4 (d) at scan rat of 50 mVs^{-1}

Effect of pH

The pH of the electrolyte is also an important parameter that can influence the response of the electrode in the electroanalysis of caffeine. The effects of pH of supporting electrolyte on the oxidation peak current of caffeine were studied from pH range of 0.8 to 7.5 in 0.01mol L^{-1} H_2SO_4. As shown in Figure 9 magnitude of the peak current decreased at higher pH values and the decrease in anodic peak current is more pronounced as the pH increased further [25], the sharpness of the peaks also decreases along with increasing the pH of the solution.

This observation could be due to dominant hydroxyl ions at high pH that repelled by the negative film modify the electrode surface, this minimize diffusion of the caffeine to the electrode surface and also due to the electrostatic attraction of caffeine with electrode surface since it was protonated. To remove unreacted species from its surface, electrodes was conditioned by cycling the potential between -0.2 and 1.2 V (10 cycles, 0.1 Vs^{-1} in the supporting electrolyte solution).

At pH 0.8 where maximum peak current was obtained with narrow potential window, however; this voltammogram suffers from poor back ground current. The one at pH 1.92 showed the next maximum peak current with wide potential window, thus used throughout the analytical determination of caffeine in this work [25].

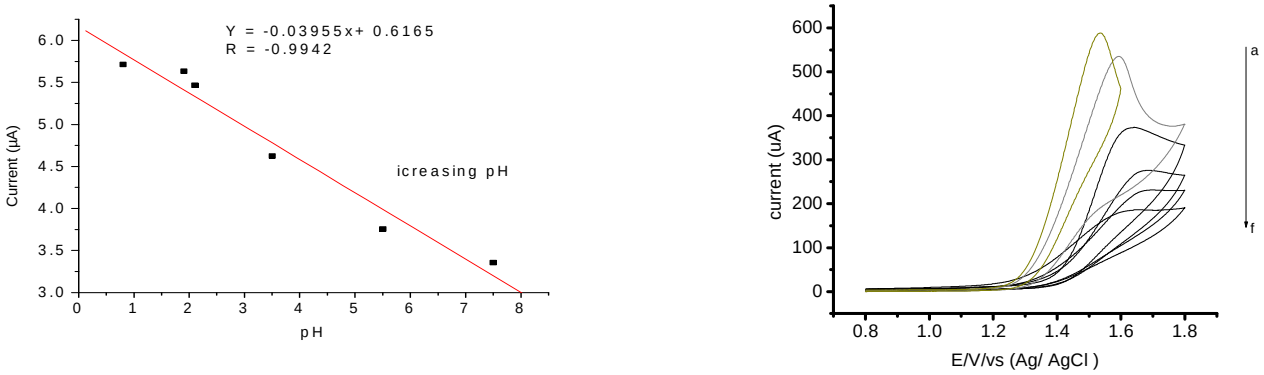


Figure 9 The cyclic voltammogram of 1.0×10^{-3} mol L^{-1} caffeine at nano hole /GCE in 0.01mol L^{-1} H_2SO_4 at different pH values (a → f): 0.8, 1.9, 2.1, 3.5, 5.5 and 7.5

Effect of Concentration

The relationship between oxidation peak currents and caffeine concentrations was studied within the range of $1x10^{-7}$ to $1x10^{-3}$ mol L^{-1}. As can be seen from Figure 10, oxidation peak current of caffeine increased with increase in concentration [26]. Regression linear equation of peak current vs concentration is i_p (µA) = 0.11 x + 0.130 (µM), and correlation coefficient R for the equation is 0.998

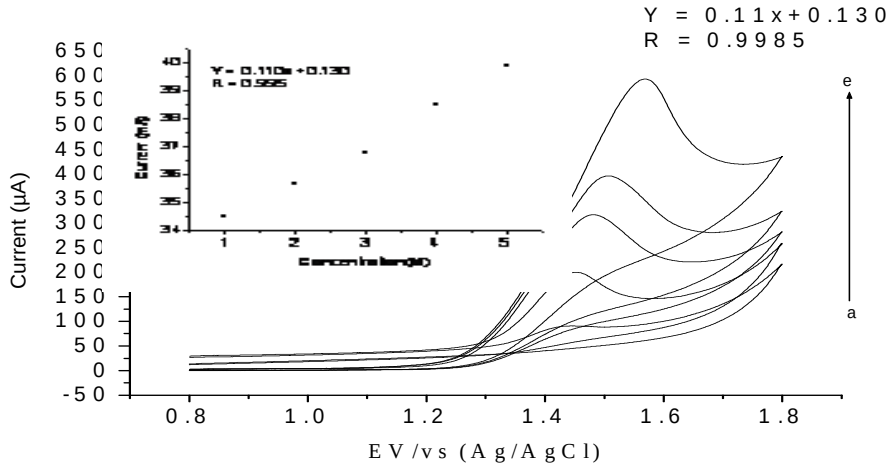


Figure 10 Cyclic voltammogram gram of different concentrations caffeine at nanohole GCE in 0.010 mol L^{-1} H_2SO_4 (a) 1×10^{-7}, (b) 1×10^{-6}, (c) 1×10^{-5}, (d) 1×10^{-4},(e) 1×10^{-3} mol L^{-1} at scan rat of 50 mVs $^{-1}$.

Effect of Scan rate

In order to investigate whether the oxidation caffeine at the nanohole/GCE is diffusion control or not, the effect scan rate has to be studied. The scan rate study was done both in low and high scan rate ranges. The effect of scan rate on the oxidative peak current of caffeine was studied within the ranges of 0.03 to10 Vs^{-1}. As shown in Figure 11, oxidation peak currents of caffeine at low scan rate (0.03 – 1 V s^{-1}) is gradually increased as the scan rates increased from 0.03 to 1 Vs^{-1}. The best linear fit (R = 0.997, Figure 12) was observed for plot of peak current vs to the square root of the scan rate within this range, indicating electrode reaction is diffusion controlled [27].

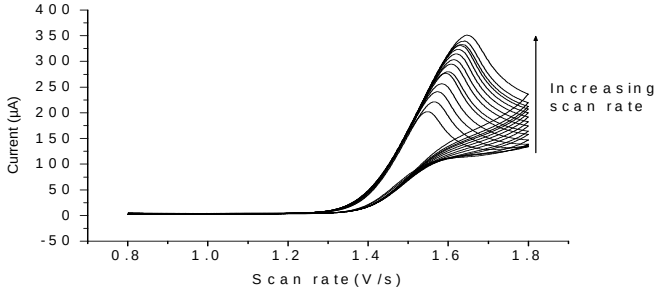


Figure 11 Cyclic voltammogram of caffeine at different scan rate of: 0.03.0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 1Vs^{-1}

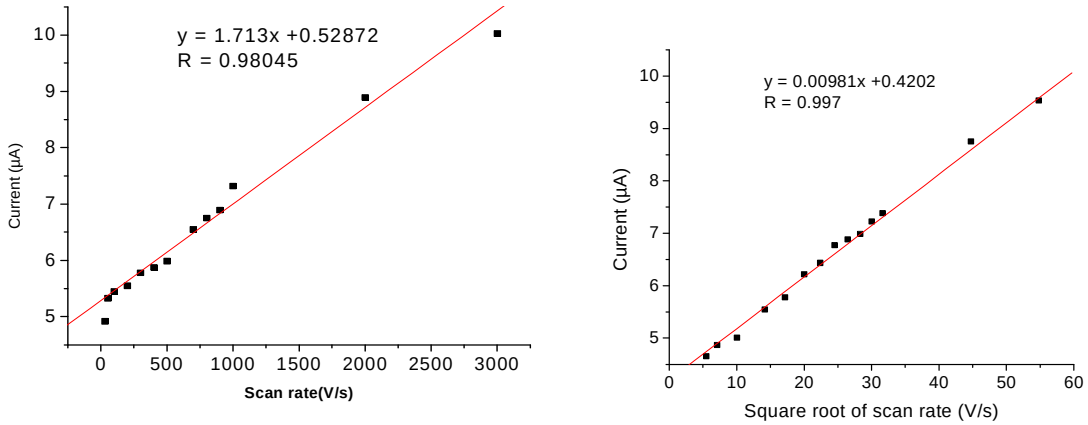


Figure 12 Calibration curve caffeine at of low scan rate.

From 1 to 10 Vs^{-1} oxidation peak potentials increases and even becomes more stable with further increase of scan rates.

This observation indicated that absorption processes occurred onto nano hole modified GCE surface [27]. This can be rationalized by considering the size of the diffusion layer and the time taken to record the scan. Form this we concluded that electrochemical oxidation of caffeine on the surface of nanohole/GCE is not a fully diffusion-controlled process; rather it is adsorption reaction Figure13, and the peak current increases linearly with increasing scan rate. So absorption current increases more rapid than diffusion current [27].

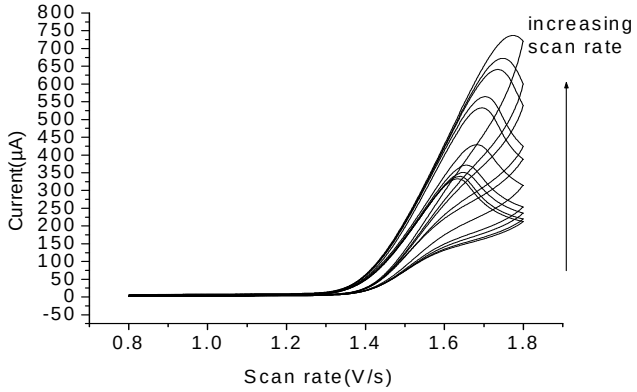
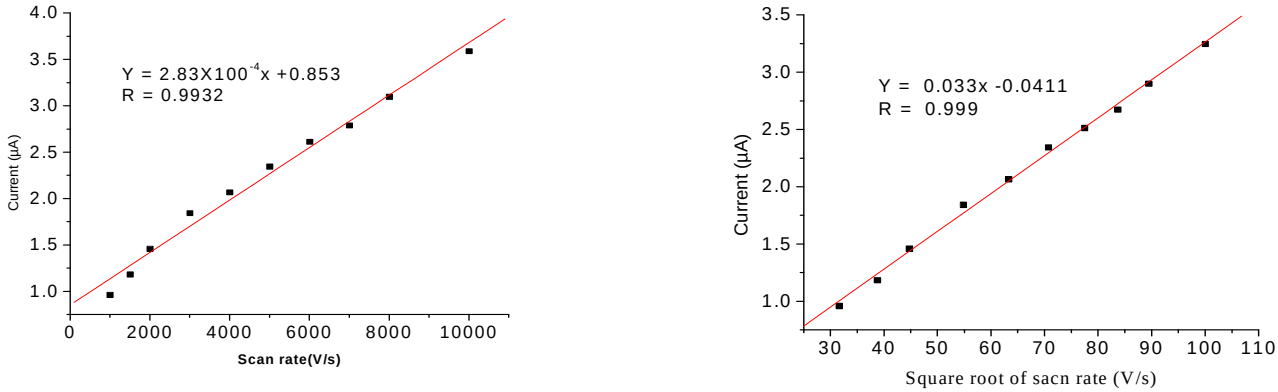


Figure 13 Cyclic voltammogram of caffeine at high scan rate of: 1, 2, 3, 4, 5, 6, 7, 8, and 10 Vs^{-1}



1Figure 14 Calibration curve caffeine at high scan rat of: 1, 2, 3, 4, 5, 6, 7, 8, and 10 Vs^{-1}

Interferences Study

The interference of some compounds that have chemical structure closer to caffeine [28] and those that co-exist with caffeine in different samples were evaluated at the nanohole modified glassy carbon sensor. The selected compounds for this test were theophylline and theobromine. The test was performed in 0.01 mol L^{-1} H_2SO_4 solution. The influence of interfering species is shown below; theobromine oxidized at 1335 mV (Figure 15) and theophylline oxidized at 1355 mV

(Figure 16) on the determination of caffeine at nanohole/GCE (a) and bar GCE(b) [28].

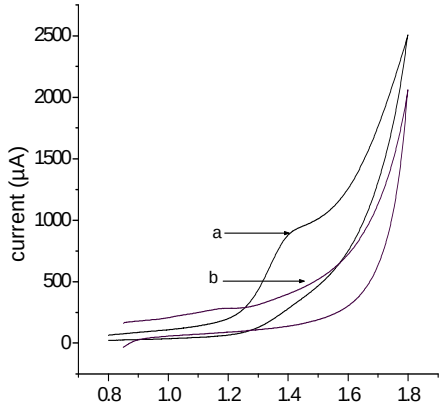


Figure 15 Cyclic voltammogram of theobromine at Nanohole/GCE (a) and bare (b).

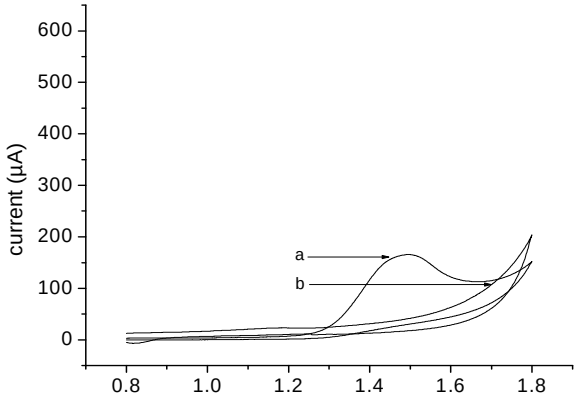


Figure 16 Cyclic voltammogram of theophylline at Nanohole/GCE (a) and bare (b).

The experimental results showed that the presence of these interferents did not significantly influence the determination of caffeine in the coffee since they oxidized at lower potential of caffeine at nanohole modified GCE.

Even when a fixed amount of caffeine mixed with theobromine and theophylline, under the same experimental conditions, the results showed two anodic responses for both interferents and caffeine [29]. The following potential were obtained from mixture of theobromine and caffeine 1328 mV and 1486 mV (Figure 17), theophylline and caffeine 1348 and caffeine 1470 mV were obtained (Figure 18) respectively. These indicated that nanohole/GCE had good selectivity for determination of caffeine in the presence of these two interferenets [30].

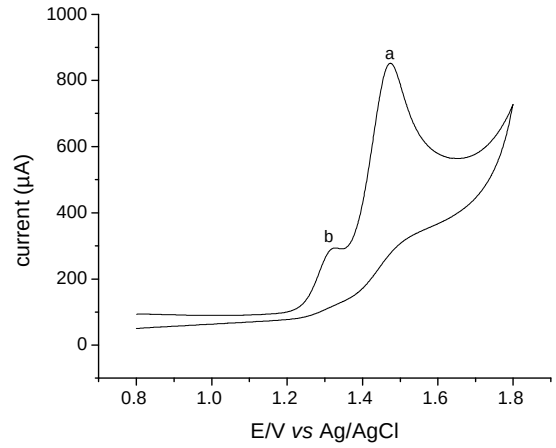


Figure 17 Cyclic voltammogram of mixture of Caffeine (2·0 × 10^{-3} mol L^{-1}) and theobromine (2·0 × 10^{-3} mol L^{-1}) caffeine and (b) theobromine peaks

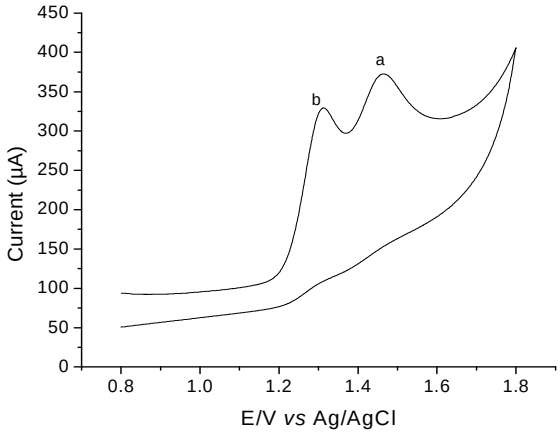


Figure 18 Cyclic voltammogram of mixture of Caffeine (2·0 × 10^{-3} mol L^{-1}) and theophylline (2·0 × 10^{-3} mol L^{-1}) caffeine and (b) theophylline peaks

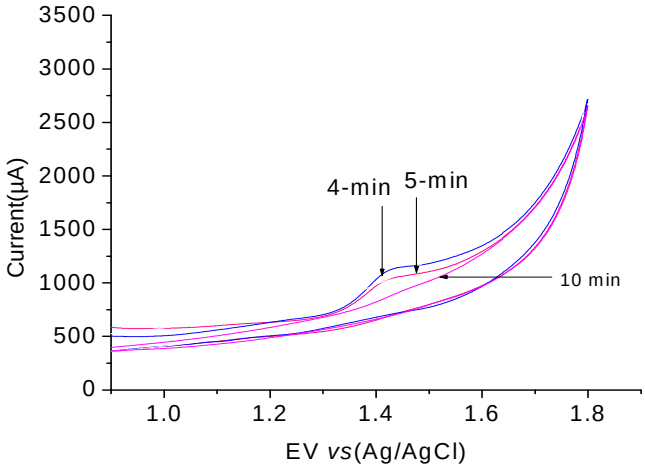


Figure 20 Cyclic voltammogram of raw coffee bean at different time of extraction.

Preliminary experiments were performed to optimize the best time of caffeine extraction from coffee. As shown in Figure 20 the voltammetric peak current recorded at nanohole modified glassy electrode decreased with time extraction. The significant difference in peak current of caffeine recorded at different time of extractions is due to high protonation reactions could affect the oxidation caffeine. So, 4 min was optimized for extraction of caffeine from coffee throughout this work [31-32].

Reproducibility and Stability of the Modified Electrode

Stability and reproducibility are the two vital characteristics for the modified electrode. Five GC electrodes were modified based on optimized conditions and then oxidation peak current of $2{\cdot}0 \times 10^{-3}$ mol L^{-1} caffeine was measured in each case. The peak current kept almost unchanged. The RSD of the peak current was 4.3% (n = 5), revealing good reproducibility of the modified electrode [32].

Sstability of the nanohole modified GC electrode toward oxidation of caffeine was tested. The nanohole/GCE was stored in 0.01 mol L^{-1} supporting electrolyte at 4°C for 7 days and the peak current was recorded. The modified GC electrode was then scanned repeatedly in supporting electrolyte until the steady current was attained. Then, the stabilized electrode was used to run CV of caffeine and the measurements were recorded again after 15 days storage of electrode in supporting electrolyte. The peak current showed almost no change. Each measurement proceeded by linear scanning of the electrode and again we extended the storage time to 23 days in supporting electrolyte. The calculated mean current for 45duration was demonstrating the stability of the modified electrode. The peak current of caffeine retained 97% of its initial response current after 45 days. This shows the modified electrode has long term current response.

Table1. The peak current recorded after 45 days storage of electrode in supporting

Current recoded at first day		Current recoded after 45 days storage of electrode in 0.01 mol L^{-1} H_2SO_4	
Average Peak current	Peak potential	Average Peak current	Peak potential
2.773	1489	2.560	1486
2.808	1490	2.558	1485
2.808	1490	2.600	1487

Amperometric Response of Caffeine

Amperometry under stirred condition is much more sensitive than cyclic voltammetry; this method is usually employed for determination of lower concentrations of analytes [31$32]. The chronoamperometric curve of the sensor in sulphuric acid solution containing various concentrations of caffeine was obtained using optimized potential (1.5V) by CV. In this work, amperometric measurements were made for determination of caffeine at the nanohole modified electrodes and their analytical performance was compared with respect to bare GCE. Fig 19 displays the current-time response of the nanohole modified GCE with successive injection of 2μ mol L^{-1} to16 μ mol L^{-1} with limit of detection (LOD = 3δ/slope) of 7.28×10^{-8} mol L^{-1} caffeine at an applied potential of 1.5 V. Current response of caffeine increase with caffeine concentration at nanohole modified GCE

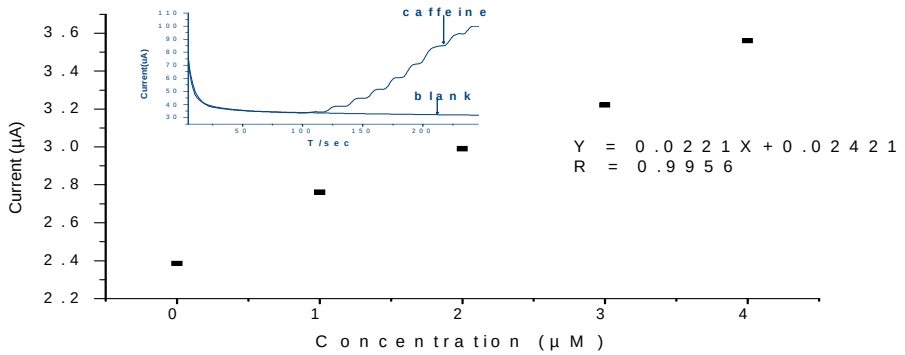


Figure 19 Amperometric response of different concentration of caffeine: 2 µ mol L^{-1}, *4µ* mol L^{-1}, *6 µ* mol L^{-1}, *8µ* mol L^{-1}, *10 µ* mol L^{-1}, *12µ* mol L^{-1}, *14µ* mol L^{-1}, *and 16 µ* mol L^{-1}

Real Sample Analysis

It was hoped that complete extraction of caffeine from coffee could be effected by acid digestion procedure. This extraction procedure has advantage that, the method takes less time than the others, has ability to penetrate through the coffee beans than other solvents, and environmental it is not pollutant. Three different coffee samples were individually analyzed. The samples were prepared as reported in the experimental section and analyzed by using the standard addition method (by analyzing the sample as received after five standard additions). The highest amount of caffeine in samples analyzed was found in raw coffee sample while the lowest was found in roasted and brewed coffee sample. The resulting caffeine content reported in Table 3 and is in good agreement with pervious literature [32].

Table 2. Concentration of caffeine obtained by cyclic voltammetric analysis

Coffee samples	w/w %
raw coffee	0.39
roasted coffee	0.32
brewed coffee	0.28

Analysis of Chloroform ($CHCl_3$) Extracts of coffee

Extraction of caffeine from coffee was achieved by using chloroform as an extracting solvent. However the result indicated that, the concentration of caffeine obtained by chloroform extraction is much lower than those obtained by acid digestion method (Table 3) [33]. This is because as it is known caffeine has no hydrogen atoms than those found on methyl group. The four nitrogen atoms have a lone pair of electron that forms polar hydrogen bonds which tends to increase solubility of caffeine in acidified water than chloroform and in turn decrease yield of chloroform extraction [33].

Melting point measurements are usually used to characterize purity of extracts. Pure caffeine melts at 238 °C [34]. The melting points of the $CHCl_3$ extracts were within the range of materials 232 – 238 ^{0}c showing slightly lower melting point than pure caffeine. The slight disagreement with the pure caffeine melting point could be due to presence of impurities in the extracted product.

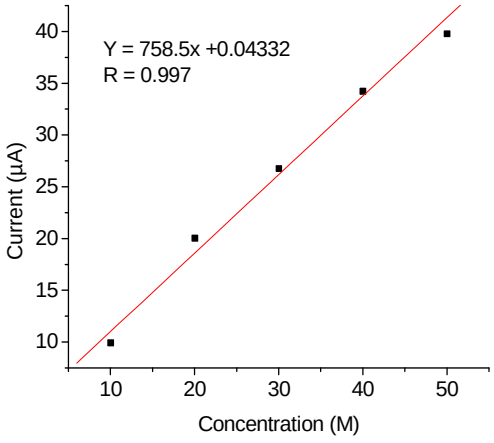


Figure 21 calibration curve for Caffeine as obtained from cyclic voltammetric method for determination of caffeine from recovered mass.

Table 3 Concentration caffeine obtained from raw coffee by different procedures

Procedures	w/w %
Acid digestion (normal procedure)	0.390
Chloroform extraction (controlled procedure)	0.333

Analysis of UV/Vis spectrophotometer result

For validation purpose, the result of electrochemical method was compared with the one obtained by UV/Vis spectrophotometer **[**32**]**. The absorbance of the working standards and samples were measured at 272 nm. 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 22) with LOD 0.1µg/m [32]. The amounts of caffeine obtained by UV/Vis spectrophotometeric method were lower than those obtained by electrochemical method which used to achieve lower LOD (Table 5). This suggests that the present method is more sensitive than UV/Vis spectrophotometeric method for determination of caffeine.

Table 4. Measured absorbance values of standard solution

Caffeine (ppm) Standard	Absorbance
10	1.1703
20	1.372
30	1.555
40	1.735
50	1.899
100	2.811

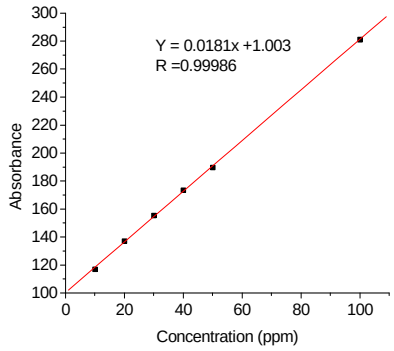


Figure 22 Caffeine calibration curve for UV/ Vis Spectrophotometeric method

Table 5. Concentration caffeine obtained from raw coffee by different methods

Methods	w/w %
Cylicvoltametry	0.39
UV/VIS Spectrophotometeric method (n = 3)	0.36

Caffeine determination using different methods are possible. Here under this, a comparison of different parameters found during caffeine detection as detailed in the corresponding references given.

As it is indicated in the table 6, nanohole modified glassy carbon electrode detection of caffeine is the most sensitive method than the rest of the methods.

Table 6. Comparison between the newly developed caffeine sensors and other reported methods

Methods	Analytica range(mol L^{-1})	Limits of detections (mol L^{-1})	Ref
Nanohole modified GCE	1×10^{-7}– 1×1^{-3}	7.28×10^{-8}	This work
Voltammetric sensor for caffeine based on glassy carbon electrode modified with Nafion and graphene oxide	4.0×10^{-7} – 8.0×10^{-5}	2.0×10^{-7}	25
Amperometric detection of three purine alkaloids following their separation by micellar ectrokinetic capillary chromatography	4.0×10^{-6}– 5.0×10^{-4}	2.0×10^{-6}	34
Utilization of electrochemical methods in determination of trace elements in beverages	$4 \times 10^{-7} – 2.5 \times 10^{-5}$	1.5×10^{-7}	35

4. Conclusions

This study demonstrated a new caffeine voltammetric sensor at nanohole modified glassy carbon electrode, showing good sensitivity and selectivity for the detection of caffeine and thereby minimized oxidation of caffeine compared to previous report table 6. Nanohole modified glassy carbon electrode exhibited significant advantages of wide linear range and low detection limit for caffeine compared with previous works. Besides, the modified electrode showed good reproducibility and stability, and offered a good possibility for extending the technique in routine analysis of caffeine.

5. References

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