2019-12-04

Electrochemical Determination of Paracetamol Using Glycine Modified Carbon Paste Electrode

Aragaw, Nega

BAHIR DAR UNIVERSITY

OFFICE OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

Electrochemical Determination of Paracetamol Using Glycine Modified Carbon Paste Electrode

By: Aragaw Nega

BAHIR DAR, ETHIOPIA

September 2019

Electrochemical Determination of Paracetamol Using Glycine Modified Carbon Paste Electrode

By: Aragaw Nega Mekonnen

A Thesis Submitted to the College of Science, Post Graduate Programe, Department of Chemistry in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

Advisor Name: Dr. Yonas Beyene

BAHIR DAR, ETHIOPIA

September 2019

LETTER OF APPROVAL

I, the undersigned certify that this thesis work which entitles "**Electrochemical Determination of Paracetamol Using Glycine Modified Carbon Paste Electrode"** as part of the work recommended in fulfillment of the requirement of a master of science in chemistry at Bahir Dar University.

Submitted by: Aragaw Nega

Approved by: Board of examiners

	Name	Signature	Date
1.	Dr._____ **Advisor**	_____	_____
2.	**Dr.**_____ External examiner	_____	_____
3.	Dr. _____ Internal examiner	_____	_____
4.	Dr._____ chairman	_____	_____

DECLARATION

I, the undersigned, declare that the thesis entitled ‘‘**Electrochemical Determination of Paracetamol Using Glycine Modified Carbon Paste Electrode**’’ submitted in partial fulfillment of the requirement for the degree of master of science in chemistry to the post graduate program of college of science, Bahir Dar university is an authentic work conducted by Aragaw Nega under supervision of Dr. Yonas Beyene.

Name: Aragaw Nega

Signature: _____

Place: Bahir Dar

Adivisor: Dr. Yonas Beyene

Signature: _____

ACKNOWLEDGEMENT

Most importantly, I would like to laud almighty God for giving me the stamina to culminate my research work successfully. My second gratitude goes to my conscientious advisor, Dr. Yonas Beyene for his continual and indispensable professional support and stringent follow up throughout the research work. My third gratitude goes to my family and the staff of department of chemistry of Bahir Dar University for their cordial collaboration in conducting all of the experiments in research Laboratory.

ABSTRACT

In this study the electrochemical behavior of GlyCPE was compared with bare CPE for the determination of PCT using CV and DPSV. After cyclic voltammetric investigation of the electrochemical behavior of paracetamol and dependence of peak current on scan rate and pH; differential pulse stripping voltammetric method based on glycine modified carbon paste electrode was developed for direct determination of paracetamol in pharmaceutical tablet samples. In contrast to the peak potential at the unmodified electrode, enhancement of the oxidative peak at a lower potential at the modified electrode indicated a catalytic property of the modifier towards paracetamol oxidation. While the observed peak potential shift with scan rate confirmed the irreversibility of the reaction, comparable correlation coefficients for the dependence of peak current of square root of scan rate and the slop of log ν versus log I_{pa} indicated that the irreversible oxidation reaction was diffusion controlled. The peak current response of the developed method showed a linear dependence on the paracetamol concentration in the range 5 to 1000 μM. The recoveries from 90 to 106%, wide dynamic range, low limit of detection (0.01 μM), and limit of quantification (0.04 μM). Good recovery results for spiked PCT in tablet samples and selective determination of PCT in tablet formulations in the presence of selected potential interferents such as ascorbic acid confirmed the potential applicability of the developed method for the determination of PCT in real samples. Finally, the proposed methods were applied for paracetamol determination in three brands of pharmaceutical tablet samples such as Julphar aldol, Panadol adva, and Para denk.

Keywords: paracetamol, tablet formation samples, glycine modified carbon paste electrode, cyclic voltammetry, differential pulse stripping voltammetry.

TABLE OF CONTENTS **PAGE**

Contents

ABBREVIATIONS AND ACRONYMS

ASV..... Anodic Stripping Voltammetry

CPE .....Carbon Paste Electrode

CV ..... Cyclic Voltammetry

DPSV ..... Differential Pulse stripping Voltammetry

E_{acc}.....Accumulation potential

GLY .....Glycine

GlyCPE .....Glycine modified carbon paste electrode

I_{pa}.....Anodic peak current

I_{pc}.....Cathodic peak current

LOD .....Limit of Detection

LOQ ..... Limit of Quantification

MCPE..... Modified carbon paste electrode

NSAID .....Non-Steroidal Anti-Inflammatory Drugs

PBS .....Phosphate Buffer Solution

PCT .....Paracetamol

QC.....Quality control

SWV.....Square Wave Voltammetry

t_{acc}.....Accumulation time

UCPE .....Unmodified carbon paste electrode

V---------- Scane rate

LIST OF FIGURE **PAGE**

LIST OF TABLES **PAGE**

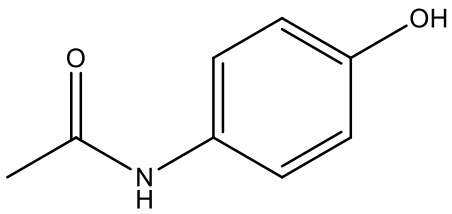
1. INTRODUCTION

Drug analysis is undertaken during various phases of pharmaceutical development [1], such as formulation and stability studies, quality control (QC) and toxicology and pharmacological testing in animals and man [2-4]. In hospitals, drug analysis is performed on patient samples in support of clinical trials, i.e. bioavailability and pharmacokinetic studies and in monitoring therapeutic drugs and drugs abuse [5, 6]. All these investigations require reliable and validated analytical methods in order to measure drugs in complex media such as formulation and biofluids.

PCT is an acylated aromatic amide that was firstly introduced in medicine by Von Mering in 1893 as an antipyretic/analgesic. It has low toxicity when used at recommended doses. The drug is of worldwide application for the relief of postoperative pain as well as mild to moderate pain associated with headache, backache and arthritis [7-9]. PCT is also known as an anticancer drug that tends to supplant aspirin for patients who are allergic to aspirin. In the case of drug overdose, accumulation of toxic products leads to severe kidney and liver problems [10, 11]. Nausea, vomiting, perspiration, and tiredness are the other reported mal-effects of PCT overdose [12, 13].

Paracetamol (PCT) is one of the most common oral analgesics and antipyretics used for the relief of fever, headache, menstrual cramps and other minor aches and pains [1], [14]. It is also useful in the management of more severe pains, where it allows lower dosages of additional Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) or opioid analgesics to be used, thereby minimizing overall side-effects [15].

Although PCT (Scheme 1) is renowned to have excellent safety profile at recommended therapeutic doses, acute overdose or misused in at-risk populations is also known to exhibit few side effects including fatal hepatotoxicity, often heightened with use of alcohol [6], [13], [14]. Therefore, there is a clear need to find a sensitive and selective analytical technique that enables to monitor the PCT content of the various sources including pharmaceuticals and body fluids.



Scheme 1 Chemical structure of paracetamol (acetaminophen).

Highperformance liquid chromatography [15, 16] spectrometry [17-19] and amperometry [20-23] are among the reported methods for determination of PCT in real samples including pharmaceutical formulations. Although most of them are standard methods due to their high sensitivity and reproducibility, these conventional methods are also known to have some limitations including high instrumental and analysis cost, skilled man power, and most of them are not environmentally-friendly. In contrast to these conventional analytical methods, electroanalytical methods offer remarkable sensitivity, accuracy, and precision in addition to a large dynamic range, with relatively low instrumentation cost [24, 25]. Thus, there is a clear need for development of suitable electroanalytical methods for determination of PCT in various matrices. Voltammetric techniques using modified electrodes have been reported [26-34] for determination of PCT in pharmaceutical formulations.

1.1. Statement of the Problem

Paracetamol or acetaminophen (N-acetyl-p-aminophenol) is an acetylated aromatic amide. It is commonly used as over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is generally used for the relief of headaches and other minor aches and pains such as muscular aches, chronic pain, migraine headache, back ache, tooth ache, and other aches and pains [7-9].

When administered combined with opioid analgesics, paracetamol can also be used to alleviate more cruel pain such as post-surgical pain as well as offer palliative care in advanced cancer patients. However, overdose and the chronic use of paracetamol produce toxic metabolite accumulation that will cause nervousness, trembling, nausea, seizures,

insomnia, headaches, kidney and liver damages [10, 11]. Therefore, assessment of the level of paracetamol content in any matrix containing paracetamol including tablet formulation is crucial to introduce the possible side effects of overdoses.

To the best of our knowledge, no work has been reported on the application of glycine modified carbon paste electrode (GlyCPE) for determination of paracetamol in tablet formulations. Therefore, in this study very simple and cheap glycine modified carbon paste electrode (GlyCPE) was conducted to determine PCT content in three brands of tablet samples by using cyclic and differential pulse stripping voltammetric techniques.

1.2. Objective of the study

1.3. General objective

Investigate the electrochemical behaviors of paracetamol at glycine modified carbon paste electrode.

1.4. Specific objectives

1. To investigate the electrochemical behavior of paracetamol at glycine modified carbon paste electrode using cyclic voltammetric.
2. To determine the optimum pH of buffer solution needed for the study ofparacetamol at glycine modified carbon paste electrode.
3. To study the effect of scan rate on the peak current and peak potential of paracetamol at glycine modified carbon paste electrode.
4. To construct a calibration curve using standard paracetamol solutions.
5. To determine paracetamol in tablet using DPSV.

1.5. Significant of the study

The main significance of this study is to assess the PCT content of pharmaceutical tablet samples available in markets and to create the awareness of negative effects of PCT on human health. Thus, this study will contributed to the scientific community a precise and accurate method for determining the PCT content in real samples characterization by complex matrix.

2. LITERATURE REVIEW

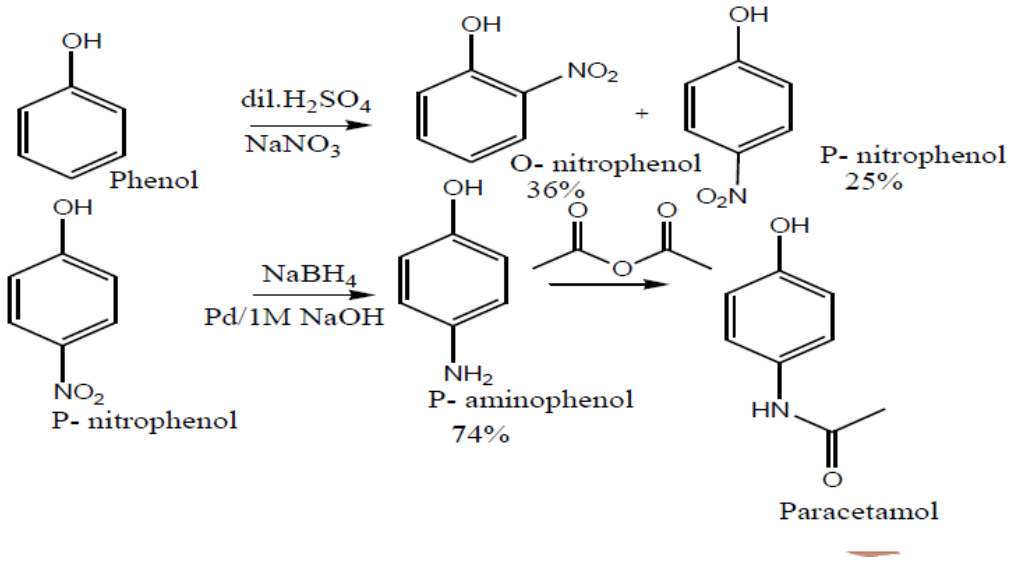
2.1. Electrochemical Activation and Chemical Modification of Electrode

Chemical modification and surface treatment of a solid electrode is method to extensively improve the electrochemical performance of the electrode. Especially, electrochemical pretreatment is used for cleaning and activating surface of electrode. The surfaces of the metal and carbon electrodes can be oxidized, and thus various kinds of oxygenous groups, such as phenolic, quinoidal, and carboxyl functionalities,can be added on the surfaces [35-37].

The goals of chemical modification are to acceleration electron transfer reactions at the electrode surface, changing transport properties to the electrode surface, creating selective membrane permeation, and interferent exclusion. However, its responses are depend on the preparation method of the chemically modified electrode, and in particular, factors such as surface coverage, film composition and morphology. Electropolymerisation, chemisorptions, covalent (chemical) attachment (silanisation or direct bonding), sol-gel encapsulation, physical adsorption, and the Langmuir-Blodgett method (creating highly ordered monolayer films) can be employed to attach modifiers to solid electrode surfaces [38].

2.2. Synthesis of Paracetamol

The original method for production of PCT involves the nitration of phenol with sodium nitrate gives a mixture of two isomers, from which wanted 4-nitrophenol (bp 279 °C) can easily be separated by steam distillation. In this electrophilic aromatic substitution reaction, phenol's oxygen is strongly activating, thus the reaction requires only mild conditions as compared to nitration of benzene itself (scheme 2). The nitro group is then reduced to an amine, giving 4-aminophenol. Finally, the amine is acetylated with acetic anhydride. Industrially direct hydrogenation is used, but in the laboratory scale sodium borohydride serves [39, 40].



Scheme 2 Synthesis of PCT from phenol in the laboratory

An alternative industrial synthesis developed by Hoechst–Celanese involves direct acylation of phenol with acetic anhydride catalyzed by hydrogen fluoride, conversion of the ketones to a ketoxime with hydroxylamine, followed by the acid-catalyzed Beckmann rearrangement to give the amide (PCT)

2.2.1. Physical properties of paracetamol

PCT is a white, odorless crystalline powder with a bitter taste which melts at 169-171^{o}c. its solubility in cold water is 1.43g/100cm^{3}, but it is much more soluble in hot water (5g/100cm^{3}) and in ethanol (14g/100cm^{3}). It is also soluble in acetone, chloroform, methyl alcohol and alkali hydroxide, but insoluble in benzene and ether.

2.2.2. Chemical Properties of Paracetamol

Paracetamol is most stable in saturated aqueous solution. It is a moderately water and lipid soluble weak organic acid with Pka value of 9.5 and is therefore largely unionized over the physiological range of pH. The stability decreases in acidic or alkaline conditions. It is slowly broken down in to acetic acid and p-aminophenol.

2.3.Electrochemical Methods

There are different types of electrochemical methods. Some of these methods are: cyclic voltammetry (CV), square wave stripping voltammetry (SWSV), differential pulse stripping voltammetry (DPSV) and linear sweep voltammetry (LSV). Cyclic voltammetry and linear sweep voltammetry are the two commonly used potential sweep techniques while differential pulse stripping and square wave stripping voltammetry techniques are pulse techniques and they are renowned for determining heavy metals in food and water matrices [41, 42].

To grasp electrochemistry, the following five important and interrelated concepts need to be appreciated: (1) the potential of the electrode determines the form of the analyte (reduced and oxidized form) at the surface of the electrode; (2) The concentration of analyte at the surface of the electrode may not be the same as its concentration in bulk solution; (3) The analyte may participate in other chemical reactions as well as reduction-oxidation reaction; (4) current is a measure of the rate of oxidation or reduction of analyte; and (5) we cannot control simultaneously current and potential [41].

2.4.Cyclic Voltammetric Technique

Cyclic voltammetry (CV) is a sweep technique in which potential is scanned positively and negatively (oxidative scan and reductive scan). Hence, it is a better technique to study the electrochemical behavior of analyte because the analyte is scanned twice [41].

Cyclic volltammetry is effective for it observes swiftly the redox behavior of analyte over a wide potential range. Cyclic voltammetry consists of cycling the potential of an electrode, which is immersed in a quiescent solution, and measuring the resulting current. The potential of this working electrode is controlled versus a reference electrode such as a saturated calomel electrode (SCE) or a silver/silver chloride electrode (Ag/AgCl). The controlling potential which is applied across these two electrodes can be considered an excitation signal [41].

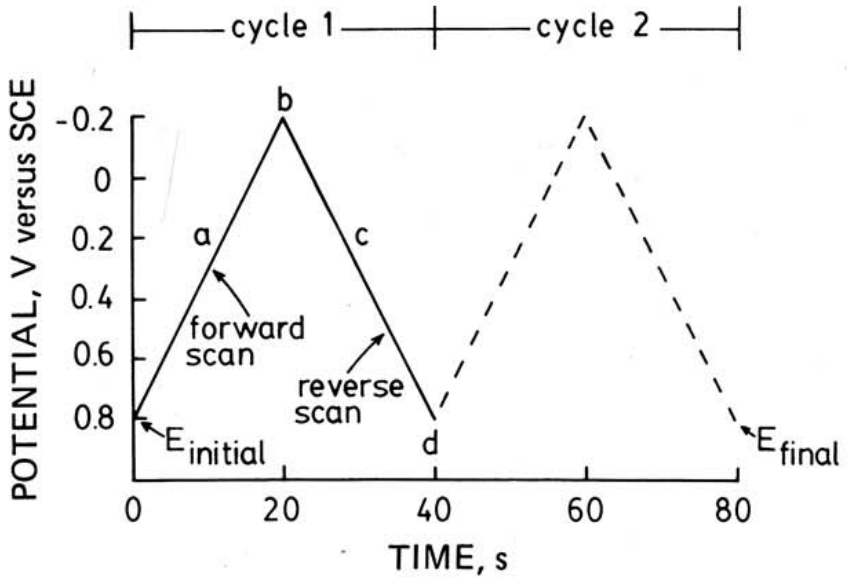
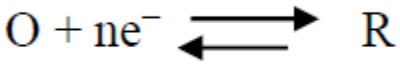


Fig.1: Cyclic Voltammetry

In the voltammetric techniques, the potential can be scanned in one direction, either to more positive potentials or to more negative potentials. As it is shown above in figure 0ne, in cyclic voltammetry a scan is completed in both directions. Firstly, the potential is scanned to more positive values, resulting in the following oxidation reaction for the species

$$R \rightleftharpoons O + ne^-$$

When the potential reaches a predetermined switching potential, the direction of the scan is reversed towards more negative potential. Because oxidized species is generated on the forward scan during the reverse scan it is reduced back to R [41].



2.5.Differential Pulse Voltammetry (DPV)

It is a voltammetric method that is used to make electrochemical measurements and it is the derivative of linear sweep voltammetry or staircase voltammetry with a series of regular voltage pulses superimposed on the potential linear sweep or stair steps [43, 44].
This technique is similar to normal pulse voltammetry except for two important differences. The base potential is increased between pulses with equal increment. Current is measured twice prior to applying pulse and at the end of the pulse. The difference between the two currents is measured. Pulses that are superimposed on a potential ramp have also been employed. For microprocessor control the staircase wave form is clearly simple to put in to 13 operation. Since DPV is a differential technique, the response is similar to the first derivative of a conventional Voltammogram that is a peak. The peak potential can be approximately identified with El/2. With increasing irreversibility, E moves away from El/2 (reversible system), at the same time as peak width increases and its height diminishes. The degree of reversibility of an electrode reaction is similar to that observed in NPV [43].

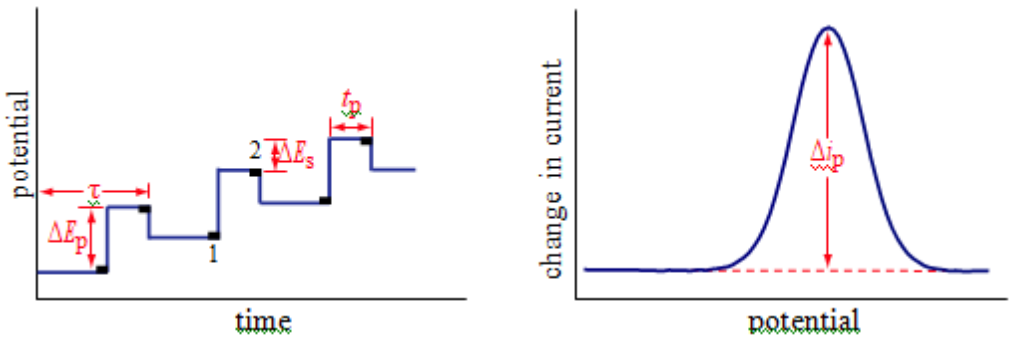


Fig. 2**:** Potential-excitation signals and voltammograms for differential pulse polarography

2.6.Square Wave Voltammetry

Square wave voltammetry has exceptional versatility, which was invented by Ramaley and Krause but has been developed extensively in recent years by the Osteryoungs and their coworkers. It can be observed as combining the best aspects of several pulse voltammetric methods, including the background suppression and sensitivity of differential pulse voltammetry, the diagnostic Value of normal pulse voltammetry, and the ability to interrogate products directly in much the manner of reverse pulse voltammetry. It also gives access to a wider range of time scales than can be achieved by any of the pulse polarographic techniques [45].

Square wave voltammetry is normally carried out at a stationary electrode; such as HMDE, and involves the wave form and measurement scheme. As in other forms of pulse voltammetry, the electrode is taken through a series of measurement cycles; however there is 14 no renewal of the diffusion layer between cycles. Square wave voltammetry has no true polarographic mode unlike NPV, RPV, and DPV [45, 46].

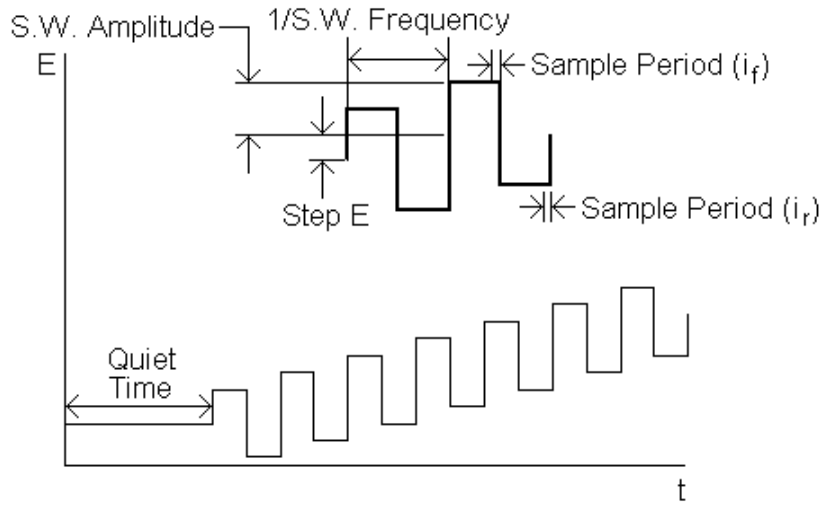


Fig. 3: Potential wave form for square wave voltammetry

2.7.Stripping Voltammetry

Another important voltammetric technique is stripping voltammetry. There are three types of stripping voltammetry. Namely: anodic stripping voltammetry, cathodic stripping voltammetry and adsorptive stripping voltammetry. Anodic stripping voltammetry is the most widely used technique and much emphasis is given to it. In anodic stripping voltammetry, three types of potential are applied on the working electrode. The first potential is the potential that can preconcentrate or bring the electroactive species closer towards the electrode solution interface from the bulk of the solution, the second potential is the potential that is strong enough to adsorb the analyte or the electroactive species on the surface of the electrode and eventually the third potential is scanned positively so that the analyte is stripped ofthe surface of the electrode [41].

2.8.**Solvents and supporting electrolyte**

In electrochemistry, according to an IUPAC definition, is an electrolyte containing chemical species that are not electroactive (within the range of potential used) and which has an ionic strength and conductivity much larger than those due to the electroactive species added to the electrolyte. Supporting electrolyte is also sometimes referred to as inert electrolyte or inactive electrolyte.

Supporting electrolytes are widely used in electrochemical measurements when control of electrode potentials is required. The choice of the solvent is dictated primarily by the solubility of the analyte and its redox activity, and by solvent properties such as the electrical conductivity and electrochemical activity. The solvent should not react with the analyte or products and should undergo electrochemically reaction over a wide potential range. This is done to increase the conductivity of the solution (to practically eliminate the so-called IR drop), to eliminate the transport of electroactive species by ion migration in the electric field, to maintain constant ionic strength, to maintain constant pH, etc. The inert supporting electrolyte may be an inorganic salt, a mineral salt, or buffer. Buffer systems(such as acetate, phosphate, citrate, or BRB) are used when a pH control is essential. The composition of the electrolyte may affect the selectivity of voltammetric measurement. The supporting electrolyte should be prepared from a highly purified reagent, and should not be easily oxidized or reduced [49].

2.9.Reference Electrodes

Voltammetric methods are those in which current passing in an electrochemical cell is measured as a function of the potential applied to the working electrode. Potential, by definition, is not something that can be directly measured. Rather, the measurement of applied potential requires that a reference point first be established, and individual potentials be measured relative to that reference point. This is accomplished by placing a second electrode, called the reference electrode, in the cell and measuring potential as the energy difference between the two electrodes. As Kissinger and Bott have perfectly

expressed, “electrochemistry with a single electrode is like the sound of one hand clapping”.

Reference electrodes should be constructed using half-cell components that are stable over time and with changing temperature, present at well-defined values of activity. They should possess fixed, reproducible electrode potentials. The reference half-cell with which most of us are familiar is the standard hydrogen electrode (SHE), composed of an inert solid like platinum on which hydrogen gas is adsorbed, immersed in a solution containing hydrogen ions at unit activity. Tables of electrode potential values for many redox couples relative to the SHE are commonly available.

Practical application of the SHE is limited by the difficulties in preparing solutions containing H^+ at unit activity and maintaining unit activity for H_2 (g) in the half-cell. The most commonly used reference electrodes for aqueous solutions are the saturated calomel electrode (SCE), with potential determination by the reaction:

$$Hg_2Cl_2(s) + 2e^- \rightarrow 2Hg(1) + 2Cl^-$$

or the silver-silver chloride electrode (Ag/AgCl),with potential determination by the reaction:

$$AgCl(s) + e^- \rightarrow Ag(s) + Cl^-$$

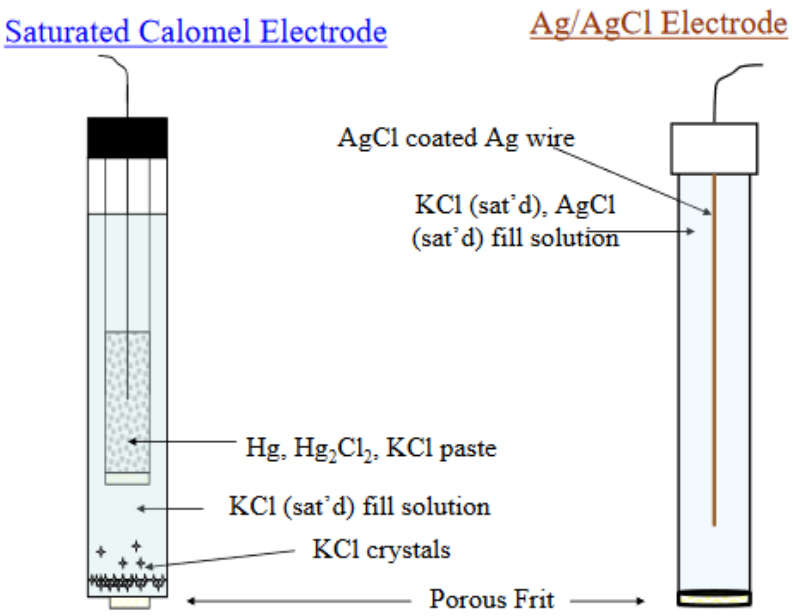


Fig. 4: Saturated Calomel Electrode (SCE) and Silver-Silver Chloride Electrode (Ag/Agcl).

2.10. Working Electrode

The performance of the voltammetric procedure is strongly influenced by the working electrode materials. The working electrode should provide high signal-to-noise characteristic, as well as reproducibility response. Thus its selection primarily depends on two factors: the redox behavior of the target analyte and the background current over the potential region for the measurement. Other consideration includes the potential window, electrical conductivity, mechanical property, cost, availability, and toxicity. The most popular are those involving mercury, carbon or metals.

2.11.Counter Electrode

The counter electrode, often also called the auxiliary electrode used in a three electrode electrochemical cell for voltammetric analysis. The purpose of the auxiliary electrode (AE) is to provide a pathway for current to flow in the electrochemical cell without passing significant current through the reference electrode. There are no specific material requirements for the electrode beyond it not adversely influencing reactions occurring at the working electrode (WE). Remember that if a reduction occurs at the WE, there must be an oxidation that takes place at the AE. Care should be taken that electrode products formed at the AE do not interfere with the WE reaction. The AE can be physically separated from the WE compartment using a fritted tube, but one should be aware that under certain circumstances this can have a deleterious effect.

The most commonly used material for the auxiliary electrode is platinum, due to its inertness and the speed with which most electrode reactions occur at its surface. Other, less expensive materials may also be used as auxiliary electrodes. Choices include carbon, copper, or stainless steel if corrosion is not an issue for a particular electrolyte solution or reaction.

As discussed in the previous section on electrochemical cells, the AE should supply current density and potential that is constant across the length of the WE. Many times this means fashioning the AE such that it "mirrors" the shape of the WE, as is the case for a rectangular WE being located near a rectangular AE of similar area. Wire is convenient in that it can be coiled in a symmetrical arrangement around the WE. In some instances, the electrochemical cell can be fashioned from the material chosen for the AE, and the cell itself can serve that purpose.

2.12.Carbon Paste Electrodes (CPEs)

CPEs are among the most popular types of carbon electrodes which have been widely used in electroanalysis, mainly due to such interesting properties as chemical inertness, low cost, wide potential window and suitability for a variety of sensing and detection

applications. The performance of the carbon paste electrodes depends on the properties of the modifier materials used to impart selectivity towards the target species.

2.13.Chemically Modified Carbon Paste Electrodes (CMCPEs)

The modification of CPE begins in 1964 with the fundamental studies on the placement of a reagent onto the surface, to impart behavior of that reagent to the modified surface. Such deliberate alteration can thus meet the needs of solution to many electroanalytical problems.

There are various ways in which the CMCPE can benefit analytical applications. These include the acceleration of electron transfer reactions, preferential accumulation or selective membrane permeation. Such steps can impart higher sensitivity, selectivity or stability.

CPE is chemically modified by means of various techniques. Some of the techniques include direct mixing, the modifier can be dissolved directly in the binder or admixed mechanically to the paste during its homogenization, soak graphite particles with a solution of a modifier, and after evaporating the solvent use the impregnated carbon powder (solvent volatilization) and the prepared pastes can be modified in situ [46]. Whereas direct modifications obviously provide special sensors for one purpose use, but in situ approaches offer a possibility to employ the same carbon paste for repetitive modifications with different modifiers.

3. EXPERIMENTS

3.1. Chemicals

All chemicals that have been used in this experiment were of analytical grade. graphite powder (Blulux), paraffin oil (BDH), paracetamol (Kenya, Ethiopia, Germany), K_2HPO_4 (98–101%, BDH, England), KH_2PO_4(Titar, India), NaOH (Blulux, India), HCl (85%, India), glycine (98.8%, India). Distilled water was used for solution preparation.

3.2. Apparatus and instruments

CHI 760 Electrochemical Workstation (Austin, Texas, USA), Nimbus analytical electronic top loading balance (USA), pH meter Adwa, mortar and pestle with a three-electrode system glycine modified carbon paste electrode as a working electrode, platinum coil as a counter electrode, and Ag/AgCl as a reference electrode) was used for voltammetric measurements.

3.3. Procedures

3.4. Preparation of unmodified and Modified Carbon Paste Electrode

3.4.1. Preparation of Unmodified Carbon Paste Electrode

Unmodified carbon paste electrode (UCPE) was prepared following a procedure that was reported elsewhere [38, 52]. Briefly: graphite powder and paraffin oil were mixed in the ratio 70:30, respectively (%w/w). 1.0 g of graphite powder that was mixed with 0.429 g (density = 0.88 gm^{-3}) of paraffin oil was homogenized thoroughly with a mortar and pestle for 40 minutes. The mixture was then left for further 24 hrs. Finally, the carbon paste was crammed in to a teflon tube by tapping and using copper wire to make electrical contact

3.4.2. Preparation of glycine modified carbon pate electrodes (GlyCPE)

Four modified carbon paste electrodes were prepared. In preparing glycine modified carbon paste electrode (GlyCPE), the mass of graphite powder was kept constant and that of glycine was variable. The mass of the graphite powder that was taken was one gram. One gram of graphite powder was added to each of four mortars. 0.5, 1.0, 1.5, 2.0 and 2.5 mg glycine was added in to the first, second, third, fourth and fifth mortar respectively. The mixture was homogenized with mortar and pestle. After a mixture of glycine and graphite powder has been homogenized, 0.429 g of paraffin oil was added to each mortar. The fifth mortars containing a mixture of graphite powder, glycine and paraffin oil were homogenized for 40 minutes one after the other. Each of the fifth mortars containing the mixture was left for 24 hours. After 24 hours the carbon paste was crammed in to fife teflon tubes by tapping and using copper wire. The purpose of adding paraffin oil was to bind the graphite powder strongly

3.5. Preparation of supporting electrolyte

Supporting electrolyte of phosphate buffer solutions (PBS) were prepared by mixing equi-molar (0.1 M) KH_2PO_4 and K_2HPO_4 in distilled water. The PBS of the required pH was prepared by mixing appropriate volumes of the solutions followed by adjusting the pH using drops of HCl (0.1 M) and NaOH (0.1 M).

3.6. Preparation of standard solution of paracetamol

33 mM stock solution of paracetamol was prepared by dissolving 0.5 g of paracetamol in 100 ml of pH 4.5 PBS. From the stock solution, while 0.5 mM paracetamol solution was used for the cyclic voltammetric investigations, working solutions of different concentrations of paracetamol (5, 10, 50, 100, 200, 300, 400, 500, 700, and 1000 μM) in pH 6 PBS were prepared from 33 mM stock solution and 0.5 mM intermediate solution through serial dilution.

3.7. Tablet sample preparation

Paracetamol tablets of three brands; Adol Julphar (Ethiopia), Panadol adva (Kenya), and Para-Denk (Germany) all labeled 500 mg PCT/tablet were purchased from a pharmacy in Bahir Dar city for analysis of their PCT content using the developed method. Four weighed tablets from each brand were powdered using mortar and pastel and homogenized. 33 mM stock solution of PCT tablet was prepared by dissolving 0.5 g of the powder in pH 4.5 PBS in each brand tablet. Furthermore, 0.5 mM working tablet solution was prepared by dissolving 0.37 mL of the tablet stock solution in pH 6 PBS and kept in a refrigerator for its PCT content analysis. To further validate the applicability of the developed method for determination of PCT in real samples like tablet formulations and recovery studies were conducted.

4. RESULTS AND DISCUSSION

Cyclic voltammetric and differential pulse stripping voltammetric techniques were used to investigate the electrochemical behaviors of paracetamol and determine paracetamol content in tablet samples, respectively.

4.1. Cyclic Voltammetric Behaviour of PCT at Bare CPE and GlyCPE

Cyclic voltammetric behavior of 0.5 mM PCT at UCPE and GlyCPE was studied in 0.1 M PBS, pH 6.0 at a scan rate of 50 mV/s. Fig. 5 showed that PCT exhibit an irreversible behavior in both electrodes. However, at GlyCPE (Fig. 5b), an improved peak potential difference and anodic current enhancement at the modified electrode confirmed the modification of the electrode surface with a material that possesses electrocatalytic activity towards the oxidation of PCT. Therefore, the addition of glycine in carbon paste electrode could highly enhance the electrochemical performance of the electrode.

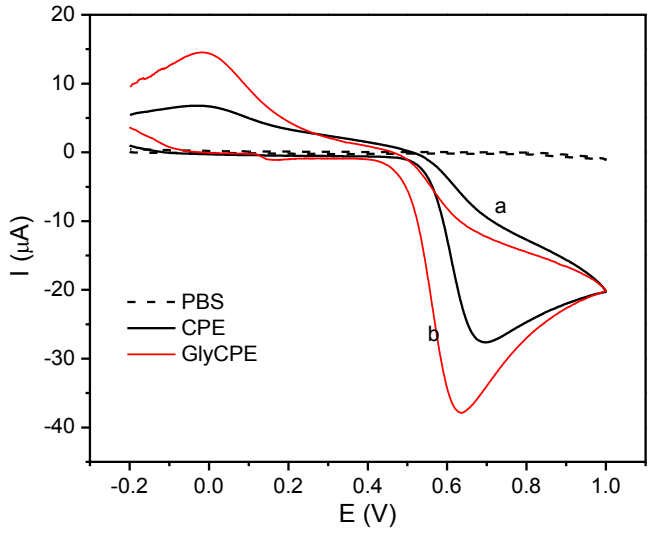


Fig. 5: CVs of the UCPE (a) and the GlyCPE (b) recorded in 0.1 M PBS (pH = 6) in the absence (dashed Line) and in the Presence of 0.5 mM PCT (solid lines) at scan rate of 50 mVs^{-1}

4.2. Effect of Glycine(Gly) modifier on the peak current of paracetamol

The characterization of GlyCPE was investigated by using cyclic voltammery technique. GlyCPE was prepared of different ratio by adding different amount of Gly in milligrams. By increasing the amount of Gly from 0.5 mg to 1.5 mg in the carbon paste electrode, the electrochemical oxidative peak current of 0.5 mM PCT was increased at 0.1 M PBS. Further increase of Gly the current signal of PCT was decreases as shown in Fig. 6.

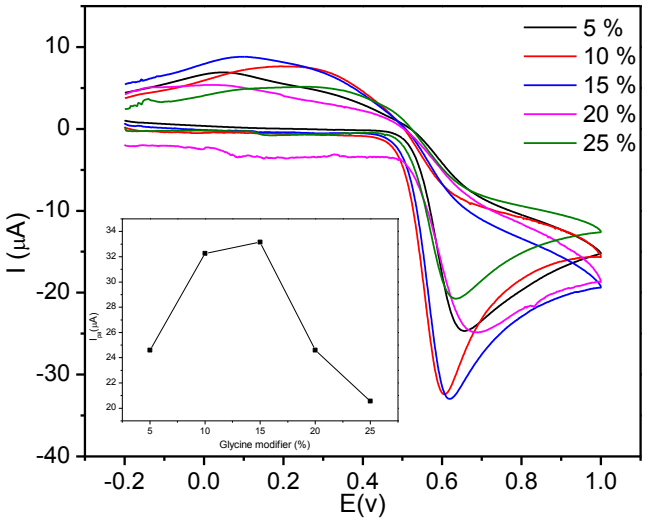


Fig. 6: CVs of different amount of Gly modifiers in PBS at 0.5 mM PCT. Inset Plot of anodic peak current versus Gly modifiers

4.3. Effect of pH of PBS on the peak current and peak potential of PCT at glycine modified carbon paste electrode

The effect of pH on the oxidation of paracetamole at GlyCPE was studied in the pH range 2, 3, 4, 5, 6, 7, 8 and 9. The cyclic voltammograms of 0.5 mM of paracetamole in PBS of various pH are shown in Fig. 7. As shown from the Fig. 8a, the anodic peak current of PCT initially increases from pH 2.0 to 3.0 and become stable till 4.0 and decrease from pH 4.0 to 5.0 then increases till pH 6.0 and then decreased at pH values beyond 6.

Therefore, pH 6 was chosen as the optimum pH in the subsequent experiments which is an agreement with the previous report [47].

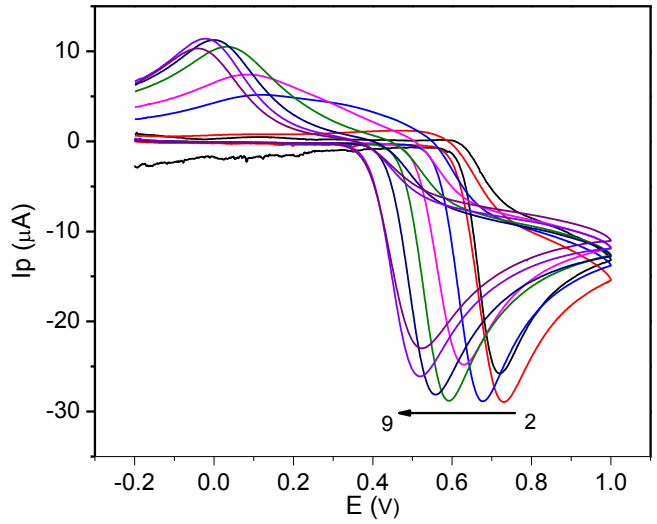


Fig.7: CVs of GlyCPE in PBS of different pH values (a-h: 3, 4, 5, 6, 7, 8, and 9, respectively) containing 0.5 mM PCT. Scan rate 50 mVs^{-1}.

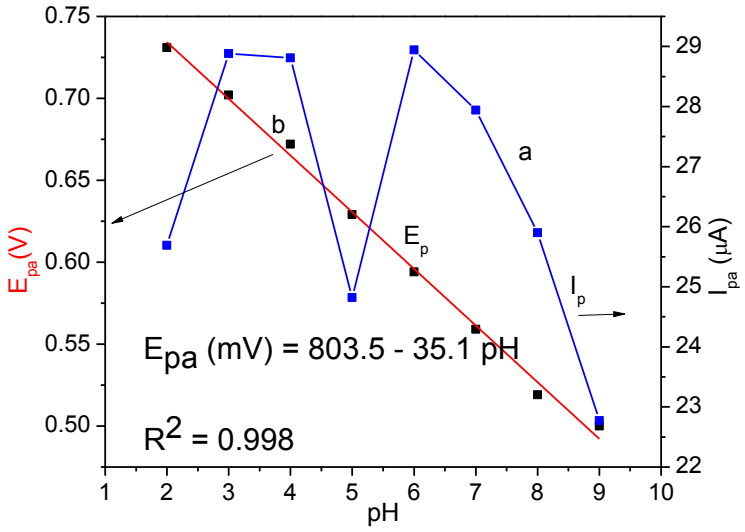
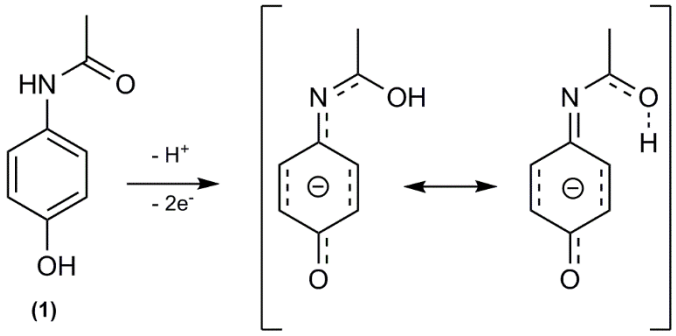


Fig. 8: Plot of (a) oxidative peak current response versus pH and (b) peak potential as a function of the pH of PBS solution containing 0.5 mM PCT

The influence of pH on the peak potentials of paracetamole was also examined. With increasing pH, the oxidative peak potential of paracetamol at GlyCPE shifted in the negative potential direction indicating the participation of protons in the oxidation of paracetamole at the surface of the modified electrode [39]. As can be observed from Fig. 8b, a linear relationship between the peak potential and solution pH with a linear equation and correlation coefficient of E_{pa} (mV) = 803.5 – 35.1/pH and R^2 = 0.998, respectively was obtained.

As paracetamol oxidation is known to involve two protons and two electrons, the slope would be expected to be 59-60 mV/pH. The 35 mv/ pH slope obtained in the present studies indicates that the electrode process is more complex and is caused by the fact that small quantity of PCT is oxidised to N-hydroxy acetaminophen [48]. Therefore this study was shown that electrode reaction process was more number of electron and small number of proton participated in the oxidation.



Scheme 3. Electrochemical oxidation of PCT

4.4. Effect of scan rate on the oxidative peak current of paracetamol at glycine modified carbon paste electrode

In order to investigate the reversibility of PCT and type of reaction kinetics its reaction at the GlyCPE, the effect of scan rate on the peak potential and peak current was studied in the range 10-200 mVs^{-1}. The observed peak potential shift in the positive direction with increasing scan rate confirms irreversibility of the oxidation reaction of paracetamol at the modified electrode with increasing scan rate shown in Fig. 9.

In order to investigate whether the oxidation kinetics of paracetamol at GlyCPE is predominantly diffusion controlled or surface confined process, the dependence of peak current on square root of scan rate was studied. The high correlation coefficients for the dependence of peak current on the square root of scan rate (Fig. 10) indicated that the involvement of diffusion mode of mass transport. Therefore, according to the Nernst- equation a better correlation coefficient for the linearity of the dependence peak current on the square root of scan rate shows the oxidation kinetics is predominantly diffusion controlled [49, 50].

The logarithm of peak current versus logarithm of scan rate was also plotted for PCT in order to decide wither the electrode reaction process is diffusion or adsorption controlled. As shown in the (Fig. 11) the slope (0.45) of log I_{pa} versus log ν was more close to 0.5 indicated that the electrode reaction process is diffusion controlled [40].

Generally correlation coefficient of curve between peak current versus square root of scan rate (Fig. 10) and the slope of log I_{pa} versus log ν (Fig. 11) suggesting that the oxidation reactions of PCT compounds at GlyCPE, are diffusion-controlled process.

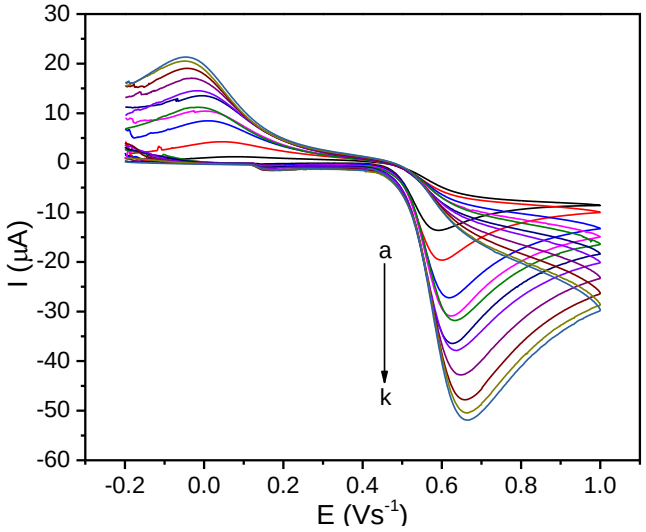


Fig. 9: CVs of GlyCPE in pH 6 PBS containing 0.5 mM PCT at various scan rates (a–k: 10, 20, 40, 50, 60, 80, 100, 125, 150, 175, and 200 mV s^{-1}, respectively)

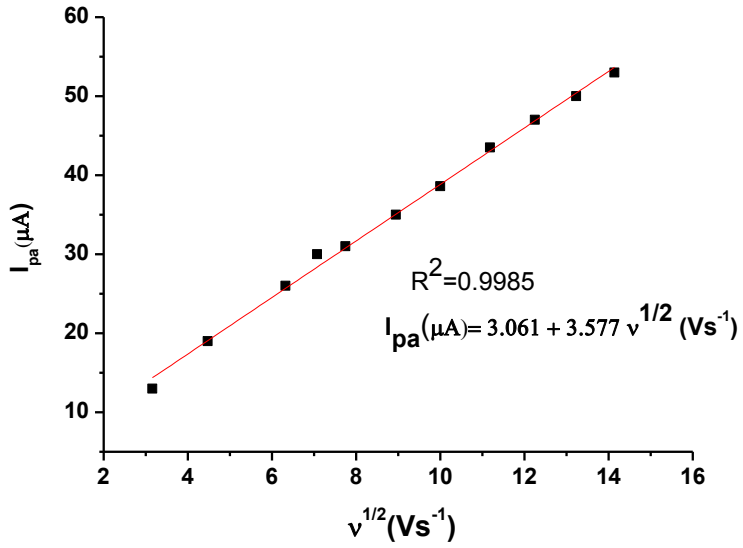


Fig. 10: Plot of peak current versus square root of scan rate

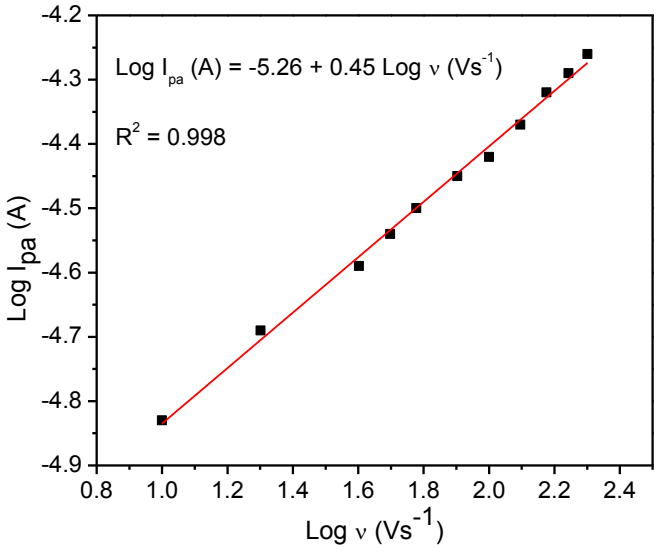


Fig. 11: Plot of log of Peak current versus log of scan rate

4.5. Effect of concentration

The Fig. 12 shows the cyclic voltammograms at GlyCPE of PCT with different concentration (10-1000 μM) in 0.1 M PBS of pH 6 at scan rate 50 mVs^{-1}. The anodic current of PCT was increase with increase in concentration. Therefore GlyCPE shows good selectivity and sensitivity in the electrochemical studies of PCT. The plot I_{pa} versus concentration of PCT gives correlation co-efficient value of 0.9962 as in Fig. 13. This revealed that good linearity. The detection limit of PCT at GlyCPE was 0.18 μM. The detection limit was calculated by using the formula (1) at (n=8) [51] where S is the standard deviation and M is the slope obtained from the calibration curve.

LOD = 3S/M---------- (1)

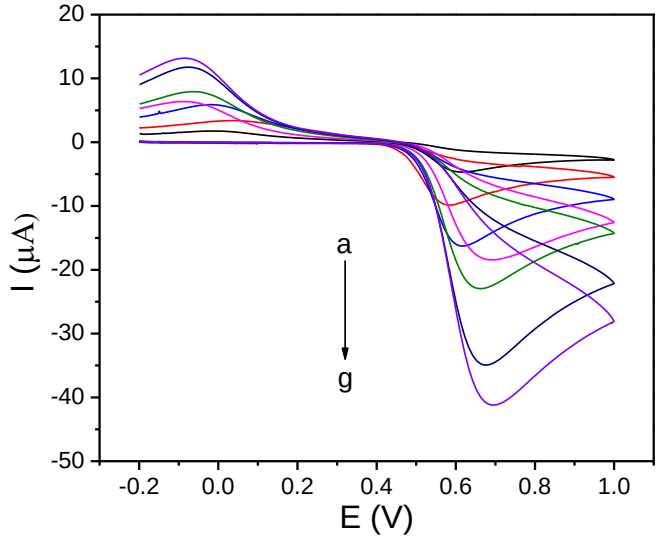


Fig. 12: CVs of glycpe in pH 6 PBS containing various concentrations of PCT (a-g: 10, 50, 100, 200, 400, 700, and 1000 μM, respectively)

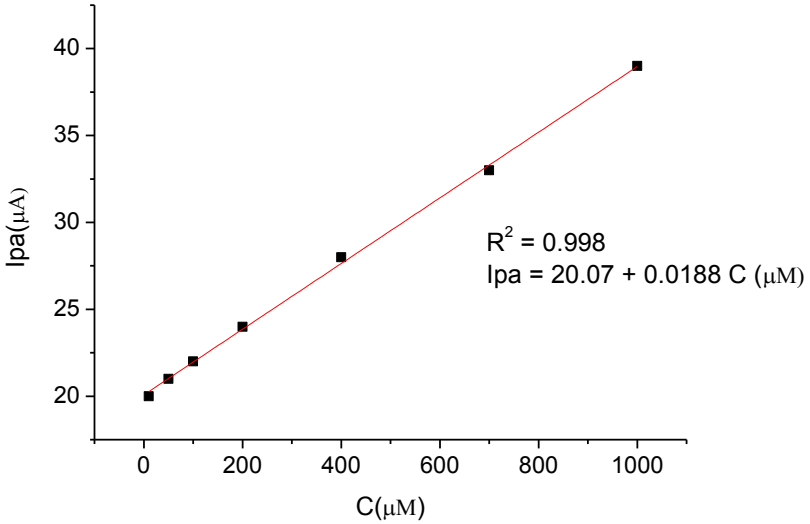


Fig. 13: plot of oxidative peak current versus concentration of PCT

4.6. Differential pulse stripping voltammetric investigation

The electrochemical oxidation of paracetamole at glycine modified carbon paste electrode was studied using differential pulse stripping voltammetry. Fig. 14 represents the differential pulse stripping voltammograms of GlyCPE in pH 6 PBS in the absence (a) and in the presence (b) of 0.5 mM paracetamole. No peak is observed at the voltammogram of GlyCPE in PBS solution while there is an irreversible oxidative peak in the presence of paracetamol.

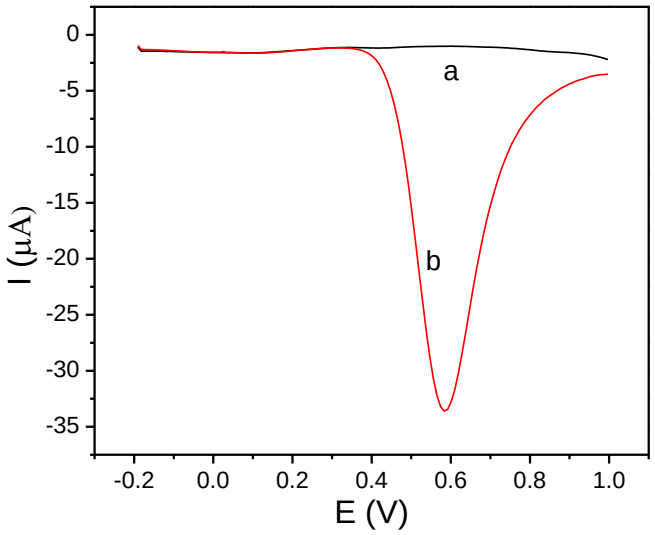


Fig. 14: DPSVs of GlyCPE in pH 6 PBS solution containing (a) no PCT and (b) 0.5 mM PCT.

Since the kinetics of the oxidation of paracetamol at GlyCPE is diffusion controlled, the potential accumulation (E_{acc}) and accumulation time (t_{acc}) were optimized.

4.7.Optimization of Deposition parameters

4.7.1. Deposition potential (E_{acc}) and Time (t_{acc})

The effect of accumulation potential and time on the magnitude of peak current response of GlyCPE for 0.5 mM paracetamole was investigated. The effect of accumulation potential (E_{acc}) over the potential range of 400 to 650 mV on the oxidative peak current of PCT at a constant accumulation time of 30 s, the resulting voltammograms are (not shown). As can be seen from the Fig. 15a, the peak current increased with increasing the accumulation potential from 400 to 500 mV. A peak current decrease was observed at accumulation potentials higher than 500 mV and hence, a preconcentration potential of 500 mV was taken as the optimum accumulation potential throughout the present work.

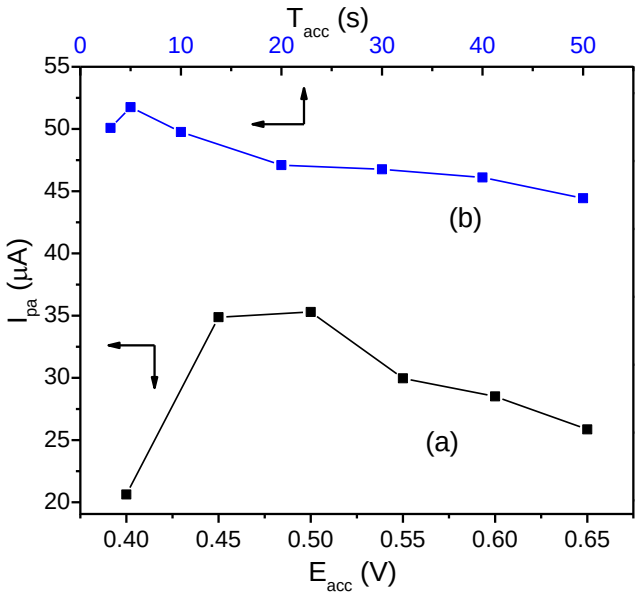


Fig. 15: Plot of oxidative peak current of 0.5 mM PCT solution versus (a) accumulation potential and (b) accumulation time. Scan rate: 50 mV/s and pulse amplitude: 50 mV

The effect of accumulation time on the peak current of 0.5 mM paracetamol in pH 6 PBS using GlyCPE was studied by varying the time from 3 to 50 s at accumulation potential 500 mV, pulse amplitude 50 mV and scan rate 50 mVs^{-1}. The resulting voltammogram are (not shown).

As can be seen from the Fig. 15b, the peak current increased with increasing the accumulation (deposition) time until it reached its maximum at 5 s. An accumulation time longer than 5 s, the peak current was decrease which could be ascribed to the saturation of the electrode surface. Thus, an accumulation time of 5 s was selected as an optimum (deposition) time for this work.

4.8. Linear Range and Limit of Detection

Under the optimum experimental conditions (pH, accumulation potential (E_{acc}), accumulation time (t_{acc}), pulse width, amplitude, and pulse period of: 6.0, 0.5 V, 5 s, 0.025 s, 0.05 V, and 0.05 s respectively), the dependence of oxidative peak current on the concentration of PCT and the inherited sensitivity of the method was investigated in the concentration rang 5-1000 μM. Fig. 16 shows the back ground corrected differential pulse striping voltammograms of various concentrations of PCT in pH 6 PBS at the GlyCPE. The oxidative peak current increases with increase the concentration of paracetamol.

The dependence of peak current as the function of the concentration of paracetamol is shown in Fig. 17. The liner regression equation and correlation coefficient were found to be I_{pa} (μA) = 6.04 + 0.26 C (μM) and R^2= 0.999, respectively. The limit of detection (LOD) and limit of quantification (LOQ) calculated using the equation (1) and (2) were 0.012 μM and 0.043 μM respectively.

LOQ = 10S/M----------2

Where s is the standard deviation of the blank (n =9) and m is the slope of the calibration curve.

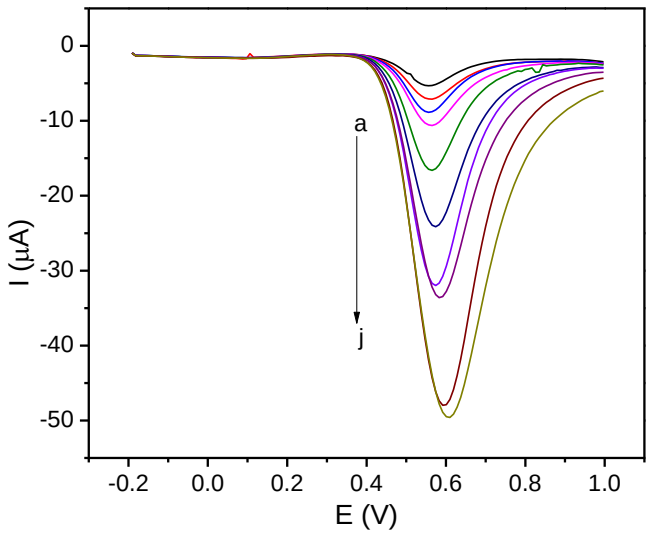


Fig. 16. DPSVs of GlyCPE in pH 6 PBS containing various concentrations of PCT (5, 10, 50, 100, 200, 300, 400, 500, 700, and 1000 μM, respectively)

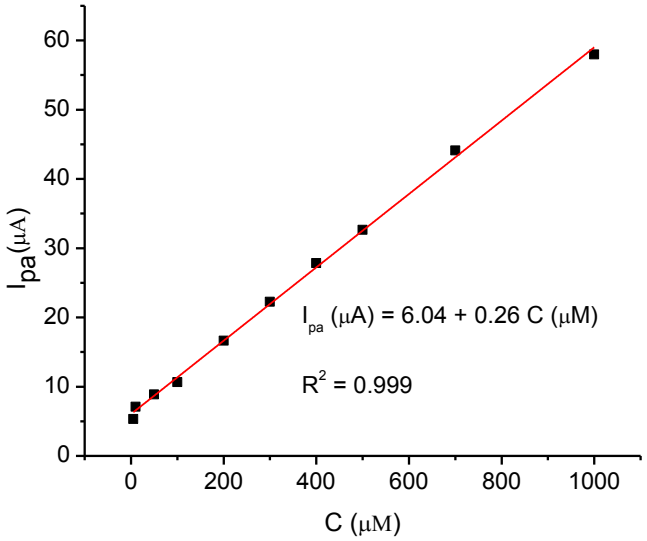


Fig. 17: Plot of oxidative peak current versus concentration

4.9. Determination of PCT content in tablet sample

The selectivity and the accuracy and hence the validity of the GlyCPE for the determination of PCT in real samples were demonstrated by evaluating its application for the determination of PCT content in some pharmaceutical tablets. In this study, three brands of paracetamol tablets (Panadol adva Kenya, Julphar aldol Ethiopia, Para denk Germany) available at a local pharmacy were analyzed for their labeled paracetamol contents.

Fig. 18 presents the differential pulse stripping voltammogram for the studied three tablet brands, the detected PCT content in each brand tablet and hence the percent detected relative to the theoretical label and corresponding tablet mass are summarized in Table 1. In each of the three brands of tablet sample 55 μM PCT were prepared following the procedure under the experimental section. Table 1 presents the detected paracetamol content of each brand of tablet calculated using the regression equation compared against the theoretically expected paracetamol content as per to the label. As can be seen from the Table 1, the method enabled to detect paracetamol in the range of 97.6 to 103 % of what is expected. The deviation of the detected from the expected may be accounted for

either low efficiency of the method used or inconsistency of the factories to maintain the paracetamol content per tablet during production.

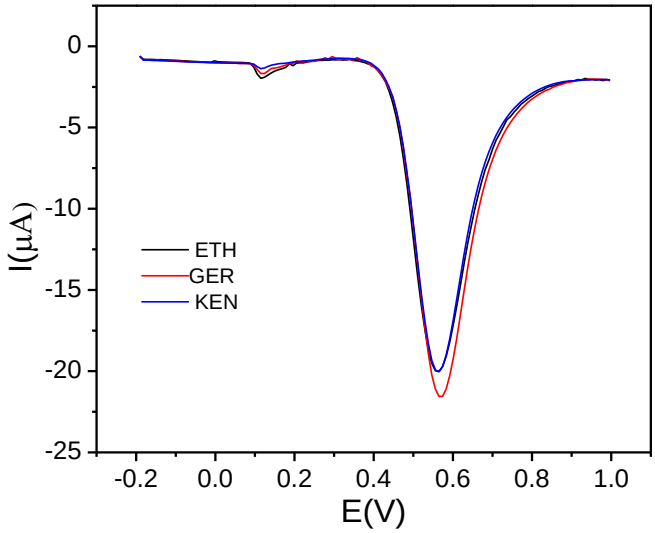


Fig. 18**:** DPSVs of GlyCPE in pH 6 PBS containing in three brands of paracetamol tablets (Ethiopia, Germany, and Kenya).

Table 1: Summary of detected paracetamol content per tablet sample using the method compared with expected PCT content

Tablet sample	Nominal PCTcontent (μM)	Detected PCT*		Declared PCT content in mg per tablet	% detected
		μM	mg/tablet		
Panadol Adva (Kenya)	55	53.69	488	500	97.6
Adol Julphar (Ethiopia)	55	54.3	493	500	98
Para Denk (Germany	55	57	518	500	103

*mean of triplicate measurement

4.10. Recovery and Interference study of the developed method

To evaluate the accuracy of the developed DPSV method for its applicability for determination of paracetamol in real sample where matrix effect could be pronounced, recovery studies for spiked paracetamol in tablet sample solution was conducted. For this purpose, the three brand tablet sample solution of paracetamol content of the tested brands was selected. From each brand two tablet solutions of 0.11 mL paracetamol content were prepared to one of which, 33 μM of standard paracetamol was spiked while the other unspiked. The resulting voltammogram are shown in Fig. 19. Recovery results in the range of 90 to 106 %, indicated excellent accuracy and precision of the developed method [52, 53]. From this, it could be concluded that the low paracetamol content detected in the paracetamol brands of Panadol Adva (Kenya) tablets (Table 1) is not due to low performance of the method but due to lower paracetamol content than the labels.

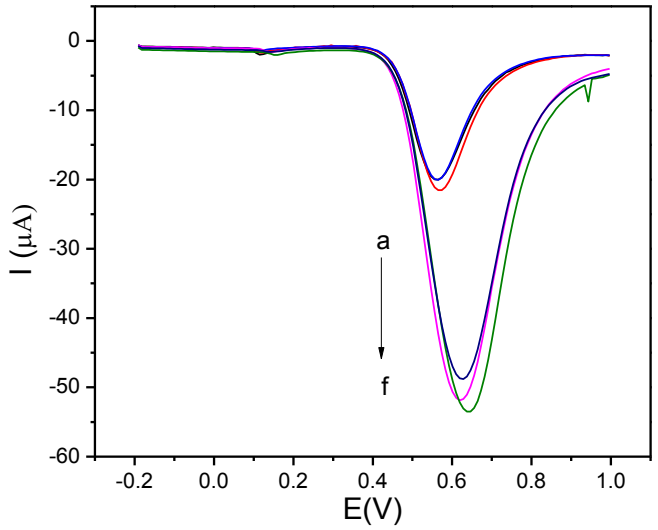


Fig. 19: DPSV of pH 6 PBS containing (a) KEN, (b) ETH, (c) GER PCT tablet solution, (d) a + 33 μM, (e) b + 33 μM, and (f) c + 33 μM standard PCT

Table 2: Percentage recovery of PCT from pharmaceutical tablets using GlyCPE

Tablet brand	Present PCT(μM)	Added PCT (μM)	Expected PCT (μM)	Detected PCT (μM)*	% Recovery ± %RSD
Adol Julphar (Ethiopia)	142	33	175	176	103 ± 0.52
Panadol Adva (Kenya)	142	33	175	172	90 ± 0.86
Para Denk (Germany	144	33	177	179	106 ± 0.23

*mean of triplicate measurement.

To further elaborate the potential applicability of the method, the selectivity of the method for PCT in the presence of potential interference was studied. For the interference studies ascorbic acid were selected. The effect of each selected potential interferent was investigated at various concentrations of the interferents added to 100 μM PCT. The voltammograms are shown in Fig. 20. As can be observed from Table 3, the presence of different concentrations of ascorbic acid with a fixed concentration of PCT did not significantly affect the peak current response or concentration of PCT.

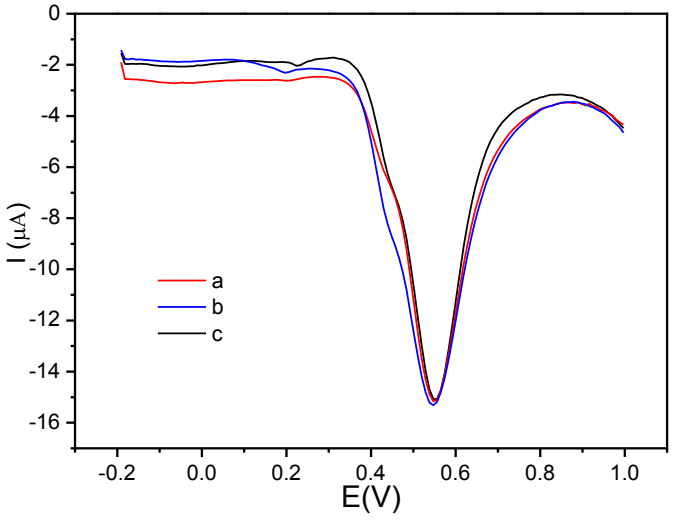


Fig. 20. DPSVs of pH 6.0 PBS containing (a) Para Denk (Germany) PCT tablet solution, (b) a + 50 μM AA (c) a + 100 μM AA

Table 3: Interference study of PCT with different concentrations of Ascorbic acid

Tablet brand	Initially present PCT μM	Added AA (μM)	Expected PCT (μM)	Detected PCT(μM)*	% detected
Para Denk (Germany	100	-	100	98.644	-
	100	50	100	100.16	100.16
	100	100	100	97.79	97.79

*mean of triplicate measurement

4.11.Performance of the developed method compared to reported works

The performance of the developed electrode in this work was compared with selected previously reported electrodes in terms of the linear range, limit of detection, nature of the substrate and cost of material used for modification. As can be seen from Table 4, the present electrode showed the list limit of detection except the electrode modified with palladium which is has toxic effect on human health. Therefore, the glycine modifier showed a comparable performance even with the methods that have used expensive otherwise toxic electrode modifiers.

Table 4: Comparison of proposed technique with others

Electrode	**Detection limit (µM)**	**Techniques**	**Reference**
MCPE/PR	0.53	DPV	[47]
Graphite and polyurethane screen-printed composite electrode	1.6	DPV	[56]
Bi_2O_3/GCE	0.2	DPV	[49]
DLC:VAMWCNT	0.367	SWV	[55]
MWCNT/$TiO_{2//}$/GCE	11.77	CV	[21]
Poly(AHNSA)/GCE	0.79	SWV	[54]
Palladium/GO	0.0022	DPV	[20]
Poly(taurine)/TiO2-Gr/GCE	0.5	DPV	[57]
Glycine/CPE	0.012	DPSV	This work

5. CONCLUSIONS

Cyclic voltammetric investigation of PCT at CPE and GlyCP revealed an irreversible oxidation peak over the studied potential window. While the peak potential shift with scan rate confirmed the irreversibility of the reaction, peak potential shift with pH also indicated the involvement of protons in the oxidation process. In contrast to the unmodified carbon paste electrode, glycine modified carbon paste electrode showed catalytic property towards oxidation of PCT. A differential pulse stripping voltammetric method using the glycine modified carbon paste electrode was used for determination of PCT even in a tablet formulation with a complex matrix. Wide dynamic concentration range, low detection limit, excellent recovery results and hence accuracy, high recovery results demonstrating its precision validated the applicability of the developed method for determination of PCT in tablet samples. Detected paracetamol content of the studied three brands of tablet samples were in the range between 97.6 % - 103% of what is the difference expected according to the label might be ascribed to possible matrix difference among the factories or failure of the companies in maintaining the 500 mg/tablet description.

.

REFERENCES

1. Jóźwiak-Bebenista, M. and Nowak, J.Z., 2014. Paracetamol: mechanism of action, applications and safety concern. Acta poloniae pharmaceutica, 71(1), pp.11-23.

2. Berridge, J.C., 1993. Pharmaceutical analysis in a multidisciplinary development environment. The Journal of pharmacy and pharmacology, 45, pp.361-366.

3. J. Chamberlain. The Analysis of Drugs in Biological Fluids, 2nd ed., CRC Press, Boca Raton, FL, 1995. 104 N. Abo El-Maali Bioelectrochemistry.2004;64: 99–107

4. Baselt, R.C., 2000. Disposition of toxic drugs and chemicals in man (Vol. 59). Chemical Toxicology Institute.

5. De Martino, M. and Chiarugi, A., 2015. Recent advances in pediatric use of oral paracetamol in fever and pain management. Pain and therapy, 4(2), pp.149-168.

6. Vidhya Malar, H.L., 2012. Mary Mettilda Bai S (2012) Beware of Paracetamol Toxicity. J Clinic Toxicol 2: 142. doi: 10.4172/2161-0495.1000142 doses or extended periods of use. Bloody urine and stools are also a side effect of Paracetamol and suggest irritation in the stomach [5]. The most common effects of Paracetamol poisoning are nausea and vomiting (Doctoral dissertation, these effects usually appear within 24 hours after the medication).

7. Keeley, G.P., McEvoy, N., Nolan, H., Kumar, S., Rezvani, E., Holzinger, M., Cosnier, S. and Duesberg, G.S., 2012. Simultaneous electrochemical determination of dopamine and paracetamol based on thin pyrolytic carbon films. Analytical Methods, 4(7), pp.2048-2053..

8. Babaei, A., Dehdashti, A. and Afrasiabi, M., 2011. Development of a method for a sensitive simultaneous determination of dopamine and paracetamol in biological samples and pharmaceutical preparations. International Journal of Electrochemistry, 2011.

9. Alothman, Z.A., Bukhari, N., Wabaidur, S.M. and Haider, S., 2010. Simultaneous electrochemical determination of dopamine and acetaminophen using multiwall carbon nanotubes modified glassy carbon electrode. Sensors and Actuators B: Chemical, 146(1), pp.314-320.

10. Tadayon, F., Naghinejad, R. and Daneshinejad, H., 2016. A sensitive and selective electrochemical method for the simultaneous determination of dopamine and paracetamol based on a multiwalled carbon nanotubes/poly (l-lysine)-modified glassy carbon electrode. *Chemistry Letters*, *45*(8), pp.1006-1008.

11. El-Maali, N.A., 2004. Voltammetric analysis of drugs.*Bioelectrochemistry*, *64*(1), pp.99-107.

12. Ruth, R.L., Carol, T.W., Rochelle, D.S., Pharmacology, DrugActions and Reactions, Fifth ed. The Parthenon Publishing Group,International Publishers in Medicine, Science and Technology,1996.

13. Boopathi, M., Won, M.S. and Shim, Y.B., 2004. A sensor for acetaminophen in a blood medium using a Cu (II)-conducting polymer complex modified electrode. *Analytica Chimica Acta*,*512*(2), pp.191-197.

14. Holubek, W.J., Kalman, S. and Hoffman, R.S., 2006. Acetaminophen-induced acute liver failure: Results of a United States multicenter, prospective study. *Hepatology*, *43*(4), pp.880-880.

15. Narwade, S.S., 2014. Qualitative and quantitative analysis of paracetamol in different drug samples by HPLC technique. *J. Appl. Chem*, *7*(8), pp.46-49.

16. Anuar, N.S., Basirun, W.J., Ladan, M., Shalauddin, M. and Mehmood, M.S., 2018. Fabrication of platinum nitrogen-doped graphene nanocomposite modified electrode for the electrochemical detection of acetaminophen. *Sensors and Actuators B: Chemical*, *266*, pp.375-383.

17. Ogunneye A.L., Adewuyi G.O., Omoboyowa D.A., Saraye T.K. Spectrophotometric determination of acetaminophen content of different brands of paracetamol tablets from South-West Nigeria. J. Res. Environ. Sci. Toxicol. 2012;1(10):251–257.

18. Kumar, G.P., Kumar, G.B., Sekhar, T.C. and Murthy, S.B., 2012. Spectrophotometric determination of paracetamol using sodium bismuthate as chromogen. *Int. J. Res. Chem. Environ*,*2*(1), pp.231-235.

19. Hoang, V.D., Ly, D.T.H., Tho, N.H. and Minh Thi Nguyen, H., 2014. UV spectrophotometric simultaneous determination of paracetamol and ibuprofen in combined tablets by derivative and wavelet transforms. *The Scientific World Journal*, *2014*.

20. Xu, Z., Yue, Q., Zhuang, Z. and Xiao, D., 2009. Flow injection amperometric determination of acetaminophen at a gold nanoparticle modified carbon paste electrode. *Microchimica Acta*, *164*(3-4), pp.387-393.

21. Felix, F.S., Brett, C.M. and Angnes, L., 2007. Carbon film resistor electrode for amperometric determination of acetaminophen in pharmaceutical formulations. *Journal of pharmaceutical and biomedical analysis*, *43*(5), pp.1622-1627.

22. dos Santos, W.T., Gimenes, D.T., de Almeida, E.G., Eiras, S.D.P., Albuquerque, Y.D. and Richter, E.M., 2009. Simple flow injection amperometric system for simultaneous determination of dipyrone and paracetamol in pharmaceutical formulations. *Journal of the Brazilian Chemical Society*, *20*(7), pp.1249-1255.

23. Vaughan, P.A., Scott, L.D. and McAller, J.F., 1991. Amperometric biosensor for the rapid determination of acetaminophen in whole blood. *Analytica chimica acta*, *248*(2), pp.361-365.

24. Rawat, A., Chandra, S. and Sarkar, A., 2010. Easy way of sample monitoring: Chemical sensors and biosensors. Anal Bioanal Chem, 2, pp.212-216.

25. Shrivastava, A., Sharma, J. and Soni, V., 2013. Various electroanalytical methods for the determination of uranium in different matrices. Bulletin of Faculty of Pharmacy, Cairo University, 51(1), pp.113-129..

26. Pasban, A.A., Hossein Nia, E. and Piryaei, M., 2017. Determination of acetaminophen via TiO2/MWCNT modified electrode. Journal of Nanoanalysis, 4(2), pp.142-149.

27. Dai, Y., Li, X., Lu, X. and Kan, X., 2016. Voltammetric determination of paracetamol using a glassy carbon electrode modified with Prussian Blue and a molecularly imprinted polymer, and ratiometric read-out of two signals. *Microchimica Acta*, *183*(10), pp.2771-2778.

28. Kalambate, P.K. and Srivastava, A.K., 2016. Simultaneous voltammetric determination of paracetamol, cetirizine and phenylephrine using a multiwalled carbon nanotube-platinum nanoparticles nanocomposite modified carbon paste electrode.*Sensors and Actuators B: Chemical*, *233*, pp.237-248..

29. Li, J., Liu, J., Tan, G., Jiang, J., Peng, S., Deng, M., Qian, D., Feng, Y. and Liu, Y., 2014. High-sensitivity paracetamol sensor based on Pd/graphene oxide nanocomposite as

an enhanced electrochemical sensing platform. *Biosensors and Bioelectronics*, *54*, pp.468-475..

30. Tanuja, S.B., Swamy, B.K. and Pai, K.V., 2017. Electrochemical determination of paracetamol in presence of folic acid at nevirapine modified carbon paste electrode: A cyclic voltammetric study. *Journal of Electroanalytical Chemistry*, *798*, pp.17-23.

31. Yilmaz, S., Bas, Z., Sadikoglu, M., Yagmur, S. and Saglikoglu, G., 2016. Sensitive voltammetric determination of paracetamol on poly (4-Aminobenzene Sulfonic Acid) modified glassy carbon electrode. *Int. J. Electrochem. Sci*, *11*, pp.6244-6255.

32. Umasankar, Y., Unnikrishnan, B., Chen, S.M. and Ting, T.W., 2012. Effective determination of acetaminophen present in pharmaceutical drug using functionalized multi-walled carbon nanotube film. *Int. J. Electrochem. Sci*, *7*(1), pp.484-498..

33. Švorc, Ľ., Sochr, J., Tomčík, P., Rievaj, M. and Bustin, D., 2012. Simultaneous determination of paracetamol and penicillin V by square-wave voltammetry at a bare boron-doped diamond electrode. *Electrochimica Acta*, *68*, pp.227-234.

34. Ahmadpour-Mobarakeh, L. and Nezamzadeh-Ejhieh, A., 2015. A zeolite modified carbon paste electrode as useful sensor for voltammetric determination of acetaminophen. *Materials Science and Engineering: C*, *49*, pp.493-499.

35. Kassa, H., Geto, A. and Admassie, S., 2013. Voltammetric determination of nicotine in cigarette tobacco at electrochemically activated glassy carbon electrode. *Bulletin of the Chemical Society of Ethiopia*, *27*(3), pp.321-328.

36. Fagan, D.T., Hu, I.F. and Kuwana, T., 1985. Vacuum heat-treatment for activation of glassy carbon electrodes. *Analytical chemistry*, *57*(14), pp.2759-2763.

37. Qiao, J.X., Luo, H.Q. and Li, N.B., 2008. Electrochemical behavior of uric acid and epinephrine at an electrochemically activated glassy carbon electrode. *colloids and surfaces B: Biointerfaces*, *62*(1), pp.31-35.

38. Zen, J.M., Senthil Kumar, A. and Tsai, D.M., 2003. Recent updates of chemically modified electrodes in analytical chemistry. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*,*15*(13), pp.1073-1087.

39. Guo, D.J. and Jin, M., 2013. Electrocatalytic oxidation and the mechanism of dopamine on a MWNT-modified glassy carbon electrode. *Russian Journal of Electrochemistry*, *49*(2), pp.200-202.

40.Tasdemir I.H., Ece A., Kilic E. Experimental and theoretical study on the electrochemical behavior of zefenopril and its voltammetric determination. Curr. Pharmaceut. Anal. 2012; 8(4):339–348.

41. Harvey, D., 2000. *Modern analytical chemistry*. McGraw-Hill..

42. Thomas W. Electrochemical Methods of Analysis, Department of Chemistry,Bates College,Lewiston ME 04240twenzel@bates.edu

43. Brett, C. and Oliveira Brett, A.M., 1993. *Electrochemistry: principles, methods, and applications* (No. 544.6 BRE).

44. Dogan-Topal, B., Kul, D., Ozkan, S.A. and Uslu, B., 2011. Anodic behaviour of fulvestrant and its voltammetric determination in pharmaceuticals and human serum on highly boron-doped diamond electrode using differential pulse adsorptive stripping voltammetry. *Journal of Applied Electrochemistry*, *41*(11), p.1253.

45. Bard, A.J., Faulkner, L.R., Leddy, J. and Zoski, C.G., 1980.*Electrochemical methods: fundamentals and applications* (Vol. 2). New York: wiley.

46. Lara, J., Torres, J.F., Beltrán, O.G., Nagles, E. and Hurtado, J., 2017. Simultaneous determination of lead and cadmium by stripping voltammetry using in-situ mercury film glassy carbon electrode coated with Nafion-macrocyclic ester. *Int. J. Electrochem. Sci*, *12*, pp.6920-6929.

47. Câmpean, A., Tertiş, M. and Săndulescu, R., 2011. Voltammetric determination of some alkaloids and other compounds in pharmaceuticals and urine using an electrochemically activated glassy carbon electrode. *Open Chemistry*, *9*(4), pp.688-700.

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

49. Bard, A.J. and Faulkner, L.R., 2001. Fundamentals and applications. *Electrochemical Methods*, *2*, p.482.

50. Maleki, N., Safavi, A. and Tajabadi, F., 2007. Investigation of the role of ionic liquids in imparting electrocatalytic behavior to carbon paste electrode. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*, *19*(21), pp.2247-2250..

51. Kutluay, A. and Aslanoglu, M., 2013. Modification of electrodes using conductive porous layers to confer selectivity for the voltammetric detection of paracetamol in the presence of ascorbic acid, dopamine and uric acid. *Sensors and Actuators B: Chemical*, *185*, pp.398-404.

52. Braggio, S., Barnaby, R.J., Grossi, P. and Cugola, M., 1996. A strategy for validation of bioanalytical methods. *Journal of pharmaceutical and biomedical analysis*, *14*(4), pp.375-388.

53. Laviron, E., Roullier, L. and Degrand, C., 1980. A multilayer model for the study of space distributed redox modified electrodes: Part II. Theory and application of linear potential sweep voltammetry for a simple reaction. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, *112*(1), pp.11-23..

54. Yilmaz, S., Bas, Z., Sadikoglu, M., Yagmur, S. and Saglikoglu, G., 2016. Sensitive voltammetric determination of paracetamol on poly (4-Aminobenzene Sulfonic Acid) modified glassy carbon electrode. *Int. J. Electrochem. Sci*, *11*, pp.6244-6255.

55. Silva, T.A., Zanin, H., Corat, E.J. and Fatibello-Filho, O., 2017. Simultaneous Voltammetric Determination of Paracetamol, Codeine and Caffeine on Diamond-like Carbon Porous Electrodes. *Electroanalysis*, *29*(3), pp.907-916..

56. Abu-Qare, A.W. and Abou-Donia, M.B., 2001. A validated HPLC method for the determination of pyridostigmine bromide, acetaminophen, acetylsalicylic acid and caffeine in rat plasma and urine. *Journal of pharmaceutical and biomedical analysis*,*26*(5-6), pp.939-947.

57. Xiong, X.Q., Huang, K.J., Xu, C.X., Jin, C.X. and Zhai, Q.G., 2013. Glassy carbon electrode modified with poly (taurine)/TiO2-graphene composite film for determination of acetaminophen and caffeine. *Chemical industry and chemical engineering quarterly/CICEQ*, *19*(3), pp.359-368.