Addis Ababa University

School of Graduate Studies

Department of Chemistry

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Simultaneous Determination of paracetamol and *para* -amino phenol using PEDOT Modified Glassy Carbon Electrode

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List of Abbreviations and Symbols

APAP:	N-acetyl *para*-aminophenol
APAP/PAP:	Equimolar mixture of paracetamol and *para*-aminophenol
BAS:	Bioanalytical system
C	Concentration
CILE:	Carbon ionic liquid electrodes
CPEs:	Carbon paste electrodes
CMEs:	Chemically modified electrodes
CV:	Cyclic voltammetry
DME:	Dropping mercury electrode
DPV:	Differential pulse voltammetry
E:	Potential
E^o:	Formal potential
ECPs:	Electrically conducting polymers
EDOT:	3,4-ethylenedioxythiophene
E_{pa}:	Anodic potential
E_{pc}:	Cathodic potential
GC:	Glassy carbon electrode
IP	Peack current
I:	Current
I_a:	Anodic current
I_c:	Cathodic current
HPLC:	High performance liquid chromatography
LOD:	Limit of detection
LC:	Liquid chromatography
n	Number of experiments
mV:	Millivolt
mM:	Millimolar
m:	Slope of calibration curve
mV/s:	Millivolt per second

NPV:	Normal pulse voltammetry
PEDOT:	Poly (3,4-Ethylenedioxythiophene)
PAP:	*para*-aminophenol
PEDOT/GC:	Glassy carbon electrode modified with Poly (3,4-ethylenedioxythiophene)
pka:	Power of dissociation constant
PBS:	Phosphate buffer solution
PC:	Personal computer
PPY:	Polypyrrole
R:	Correlation coefficient
RSD	Relative standard deviation
SCE:	Saturated calomel electrode
SPEs:	Screen printed electrodes
SWV:	Square wave voltammetry
SAMs	Self assembled monolayers
TBATFB:	Tetrabutylammonium tetraflouroborate
UA	Uric acid
µA:	Microampere
µM:	Micromolar
σ:	Standard deviation

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Abstract

In this study reliable and simple electrochemical method has been proposed for the simultaneous determination of paracetamol and *para*-amonophenol in pharmaceutical formulations. The oxidation and reduction peak potentials in of APAP at PEDOT/GC occurred at 381 and 353.65 mV *vs*. Ag/AgCl, respectively at pH 7.0, while those for PAP at PEDOT/GC appeared at 127 mV and 92 mV, respectively. In comparison to bare glassy carbon, the apparent reversibility and potential shift of the electrochemical reactions of APAP and PAP were significantly improved at PEDOT/GC. A peak separation of about 257.48 mV was observed while running the differential pulse voltammogram of APAP and PAP. This allows for the simultaneous determination of APAP and PAP in tablets. The linear range were found to be 1-100 µM for APAP and 4-320 µM for PAP with the detection limits of 0.4048 µM and 1.1880 µM (calculated by 3σ/m) respectively.

Key words: Poly (3,4-ethylenedioxythiophene), modified glassy carbon electrode, paracetamol and *para*-aminophenol.

1. Introduction

Paracetamol (N-acetyl-*para*-aminophenol or acetaminophen) is an effective and safe analgesic agent used to reduce fever, cough and cold. It is also used worldwide for the relief of mild to moderate pain associated with headache, migraine and noninflammatory conditions in patients prone to gastric symptoms. APAP is a synthetic derivative of *para*-aminophenol and is hydrolyzed in inappropriate storage conditions, such as high temperatures and acidic or basic media to PAP. PAP has been detected in APAP as an impurity or a synthetic intermediate, and has been reported to have nephrotoxicity and teratogenic effects due to its structural similarity to aniline and phenol. Due to this fact, a rigorous method of quality control of pharmaceutical fabrication is demanded. Many analytical purposes in simultaneous analysis of pharmacological species are based on modern instrumental techniques, such as HPLC, titrimetry, capillary electrophoresis, spectrofluorometry and spectrophotometry. However, these techniques are generally expensive and time-consuming, so it becomes very difficult to establish an online system. Therefore, chemically modified electrodes were extensively used due to they are highly selective, sensitive, low cost of fabrication and low detection limit. PEDOT/GC was used in this work to determine APAP and PAP simultaneously.

1.1. Objective of the study

1.1.1. General objective

The general objective of this study is simultaneous determination of Para-aminophenol and Paracetamol using PEDOT/GC in tablets.

1.1.2. Specific objectives

The specific objectives of this study are to:

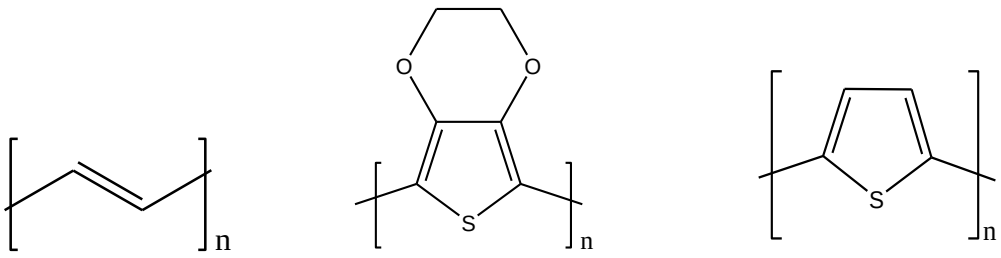
(i) Investigate the significance of PEDOT/GC electrode for determination of APAP and PAP than bare glassy carbon electrode.
(ii) Construct calibration curves for simultaneous determination of APAP and PAP at PEDOT/GC using DPV technique.
(iii) Calculate the LOD of APAP and PAP using PEDOT/GC and
(iv) Calculate the recovery of PAP and APAP from EPHARM paracetamol and PANADOL of Kenya tablets.

2. Literature Review

2.1. Conducting polymers

A polymer (material containing a long chain of molecular structures) is first and foremost an insulator. The idea that polymers or plastics could conduct electricity is considered absurd. Approximately three decades ago, scientists discovered that a type of conjugated polymer called 'polyacetylene' could become highly electrically conductive after undergoing a structural modification process called doping. It is universally agreed that the doping process is an effective method to produce conducting polymers. Doping allows electrons to flow due to the formation of conduction bands. As doping occurs, the electrons in the conjugated system, which are loosely bound, are able to jump around the polymer chain. Electric current will be produced when the electrons are moving along the polymer chains [1, 2].

The conducting polymer is called a 'conjugated polymer' because of the alternating single and double bonds in the polymer chain. Due to the special conjugation in their chains, it enables the electrons to de-localize throughout the whole system and thus many atoms may share them. The de-localized electrons may move around the whole system and become the charge carriers to make them conductive [3].



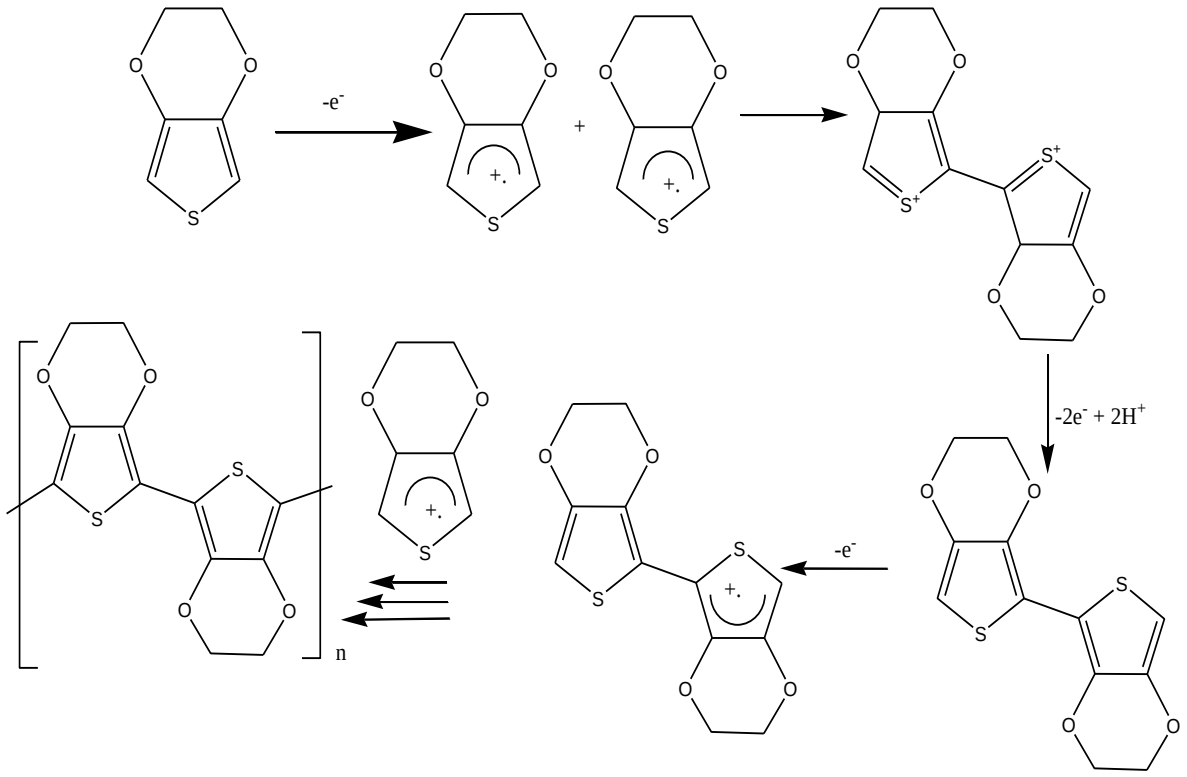
Polyacetylene Poly(3,4-ethyelenedioxythiophene) polythiophene

Fig. 1. Some example of conducting polymers.

2.1.1. Synthesis of conducting polymers

Synthesis of conducting polymers can be classified into two major categories: chemically polymerization and electrochemically polymerization. Via chemical polymerization, conjugated monomers react with an excess amount of an oxidant in a suitable solvent, such as acid. The polymerization takes place spontaneously and requires constant stirring. A major advantage of chemical polymerization concerns the possibility of mass production at a reasonable cost this is often difficult with electrochemical methods [4, 5].

The second method is electrochemical polymerization, which involves placing counter, working and reference electrodes into the solution containing diluted monomer and electrolyte (the dopant) in a solvent. After applying a suitable voltage, the first steps is electro oxidative formation of a radical cation from starting monomer this step is followed by dimerization process, followed by further oxidation and coupling reactions. Well-adhered film can thus be formed on the surface of working electrode. The behavior of electro polymerized film can be controlled by the polymerization conditions such as, the electrolyte, solvent, monomer concentrations, applied potential or current and duration [6, 7]. An important feature of the electropolymerization technique is the direct formation of conducting polymer films that are highly conductive, simple and suitable for use especially in electronic devices. But, not all organic monomers undergo electropolymerizatiton of course. Certain monomers are electropolymerized due to stability of the radical ions generated in the first step and the oxidation potentials for generation of these radical cations [8, 9].



Scheme 1. Mechanism of electropolymerization of EDOT

2.1.2. Conducting polymers in sensor application

Conducting polymers (CPs) exhibit intrinsic electronic conductivity. Their structure contains a one-dimensional organic backbone based on the alternating of single and double bonds, which enables a super orbital to be formed for electronic conduction. Polyacetylene, poly(3,4-ethylene dioxythiophene), polyaromatic and polyheterocyclic chains can provide such a structure. Macroscopic conduction through these polymers takes place by charge hopping both along the polymer chains and between the macromolecules that make up individual fibers and between the fibers themselves [10].

One of the increasingly studied subjects in connection with CPs is their application in sensors, mainly in electrochemical sensors. This is due to their key property that they are remarkable transduction matrices sensitive to gases, vapors, ions and bimolecular systems, resulting in a straightforward with conductance, impedance or redox potential change via the modulation of

their doping level. Polyacetylene, polyaniline, polythiophene, poly(3,4-ethyelenedioxythiophene) and polypyrrole are the most widely used materials [11].

Especially, ionically stabilized poly-*N*-methylpyrrole films with immobilized enzymes have gained attention in biosensor applications for detecting glucose, uric acid and cholesterol. Polyaniline and polypyrrole have successfully been applied in multicomponent gas sensing. Organic conducting polymers sensors are better than inorganic sensors due to they have high selectivity and the ability to operate at ambient temperatures without the necessity of heating [12].

2.2. Chemical sensors

A chemical sensor is a device which responds to a particular analyte in selective way through a chemical reaction between the sensors and the analytes. As a result of chemical interactions, physical property of the sensors was changed, this change causes sensors to transform the concentrations of analytes to other detectable physical signals, such as currents, absorbance, mass or acoustic variables. This leads to the qualitative or quantitative determination of the analyte. Chemical sensors contain two basic functional units: A receptor part and a transducers part. Some sensors may include a separator which for example a membrane, a transducer is a device capable transforming the energy carrying the chemical information about the sample into a useful analytical signal. The transducers as such do not show selectivity [13].

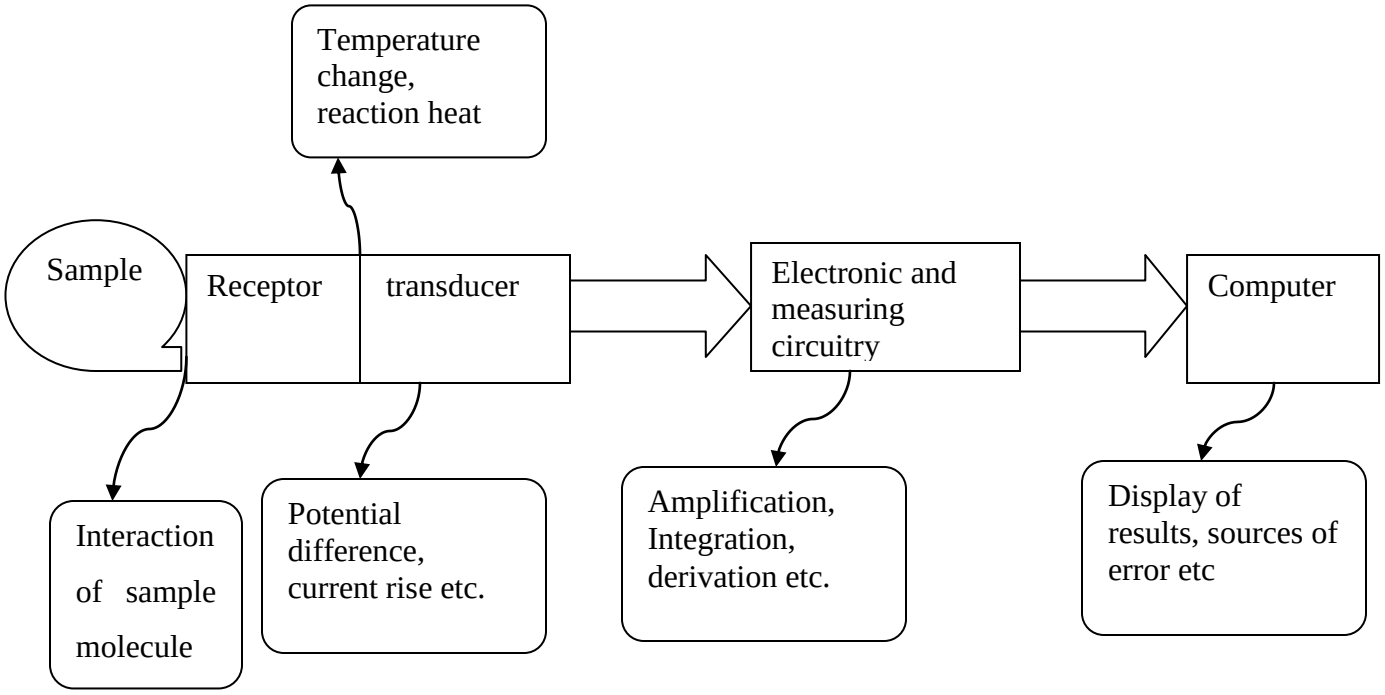


Fig. 2 Typical chemical sensor system

2.3. Chemically modified electrodes (CMEs)

CMEs represent a modern approach to electrode systems. These electrodes rely on the placement of reagent on the surface to impart the behavior of reagent to the surface of the electrode. Such deliberate alteration of the electrode surfaces can thus meet to the need of many electro analytical problems and may form basis for new analytical applications including energy conversion, electrochemical synthesis and microelectronic devices. CME is that generally quite thin film from a molecular monolayer to perhaps a few micrometers-thick multilayers [14].

Electrodes are usually chemically modified by one of the following four approaches.

(1) Chemisorptions-adsorption: Here the forces involved are the valence forces of the same as those operating in the formation of chemical compounds**.** The chemical film is strongly ideally and irreversibly adsorbed (chemisorbed) onto the electrode surface. This approach usually yields monolayer (or less) coverage. This type of modification are the substrate-coupled self-

assembled monolayers (SAMs) in which uncorrelated molecules spontaneously chemisorb at specific sites on the surface of the electrode to form a super lattice.

(2) Covalent bonding: Linking agents, such as, organosilanes or cyanuric chloride are used to covalently attach from one to several monomolecular layers of the chemical modifier to the electrode surface.

(3) Polymer film coating: Electron-conductive and nonconductive polymer films are held on the electrode surface by combination of chemisorptions and low solubility in the contacting solution or by physical anchoring in a porous electrode. The polymer film can be organic, organometallic or inorganic.

(4) Composite: The chemical modifier is simply mixed with an electrode matrix material, as in the case of an electron-transfer mediator (electrocatalyst) combined with the carbon particles (plus binder) of a carbon paste electrode. Alternatively, intercalation matrices such as certain Langmuir-Blodgett films, zeolites, clays and molecular sieves can be used to contain the modifier.

Polymer film-coated electrodes may be further subdivided by the process used to apply the film, namely.

Dip-coating - this procedure consists of immersing the electrode material in a solution of the polymer for a period sufficient for spontaneous film formation to occur by adsorption. The film quantity in this procedure may be augmented by withdrawing the electrode from the solution and allowing the film of polymer solution to dry on the electrode.

Solvent evaporation - a droplet of a solution of the polymer is applied to the electrode surface and the solvent is allowed to evaporate. A major advantage of this approach is that the polymer coverage is immediately known from the original polymer solution concentration and droplet volume.

Spin coating - also called spin casting; a droplet of a dilute solution of the polymer is applied to the surface of a rotating electrode. Excess solution is spun off the surface and the remaining thin polymer film is allowed to dry. Multiple layers are applied in the same way until the desired thickness is obtained.

Electrochemical deposition- also called redox deposition, this procedure relies on the variation of polymer solubility with oxidation (and ionic) state, so that film formation will occur, often irreversibly, when a polymer is oxidized or reduced to its less soluble state.

Electrochemical polymerization- a solution of monomer is oxidized or reduced to an activated form that polymerizes to form a polymer film directly on the electrode surface. This procedure results in few pinholes since polymerization would be accentuated at exposed (pinhole) sites at the electrode surface. Unless the polymer film itself is redox active, electrode passivation occurs and further film growth is prevented.

Radiofrequency polymerization- a polymer-filming method in which vapors of the monomer are exposed to a radiofrequency plasma discharge. The high energetics of the radiofrequency discharge may result in chemical damage, thereby producing unknown functionalities and structural modifications to the polymer.

Cross-linking-a chemical step designed to couple chemical components of a film on an electrode to impart some desired property to the film such as increased stability, decreased permeability, or altered electron transport characteristics. Cross-linked films are often formed by copolymerization of bi-functional and poly functional monomers. Cross-linking may be activated chemically, electrochemically, photolytically, radiolytically, or thermally. These forms of modification are the substrate-decoupled (SAMs) in which adsorbate molecules are arranged on the electrode surface independently of any substrate structure [15].

2.4. Poly (3,4-ethylenedioxythiophene)

Due to their wide possible applications, conducting polymers are the focus of interest in much research; for sensors and biosensors they have been used in order to enhance the response time, sensitivity, stability and versatility.

Poly (3,4-ethylenedioxythiophene) (PEDOT) belongs to a group of very stable conducting polymers and has been synthesized electrochemically on different electrode surfaces, mainly platinum, but also gold and glassy carbon. PEDOT has high electrical conductivity and high chemical stability in aqueous solutions. Researchers have focused great attention to applying PEDOT and its derivatives in industry, including photographic films, solid-state capacitors,

printed circuit board, electroluminescent lamps and light-emitting diodes [16]. Electrochemical properties of PEDOT also reveal that they have the ability to promote electron transfer reactions when used as electrode materials. Researchers have reported electro analysis based on PEDOT-modified electrodes. Chen *et al*. used PEDOT modified glassy carbon electrodes for detecting dopamine and ascorbic acid [14].

Manisankar et al. used PEDOT modified electrodes for determining pesticides [16]. PEDOT-modified screen-printed electrode (SPE/PEDOT) was also used for determining cysteine and APAP [17]. Recently, Su and Cheng reported detection of nitrite at SPE/PEDOT [18] and S. Mehretie, *et al.* use PEDOT glassy crbon electrode for determination of APAP [19]. In this study PEDOT modified glassy carbon was used to detect APAP and PAP simultaneously.

2.5. Paracetamol and *para*-aminophenol

Acetaminophen (APAP), also known as N-acetyl-*para*-aminophenol or paracetamol, is an effective analgesic and antipyretic drug with rather limited anti-inflammatory properties. This substance is commonly used for relieving mild to moderate pain associated with headache, backache, arthritis and postoperative pain and is used universally for reducing fevers of bacterial or viral origin [20]. APAP is the preferred alternative to aspirin, particularly for patients who cannot tolerate aspirin [21]. Overdoses of APAP lead to hepatic toxicity, in some cases associated with liver and kidney damage and even death. Therefore, it is very important to establish a simple, fast, economical, sensitive and accurate detection method for APAP. Determination of APAP, alone and in mixtures, is an important aspect of quality control in pharmaceutical formulations and biological fluids (such as urine, blood, or plasma) in the medical field. This process has been widely studied using titrimetry [22], spectrophotometry [23], spectrofluorometry [24], high performance liquid chromatography [25], capillary electrophoresis [26], flow-injection method [27], and enzyme-based methods [28]. However, liquid chromatography is time-consuming, and titrimetry and spectrophotometry are not convenient. These methods typically involve hydrolysis of APAP to PAP, production of a color compound by an appropriate reaction, and a tedious extraction process prior to the determination. Researchers have used electrochemical detections to overcome these difficulties, which have proven to be promising detection methods for APAP because of its electro activity

at solid electrodes. Besides, electrochemical detection also possesses advantages over conventional methods, such as rapid detection speed, remarkable sensitivity, easy operation, low cost, and the ability to be miniaturized and disposable.

The PAP is a widely used industrial chemical and a metabolite of common household analgesics, such as APAP [29]. PAP is a fundamental material for the production of APAP as one of the most produced pharmaceuticals worldwide. However, if PAP occurs in APAP in trace amounts it shows biochemical and environmental risks. It is a harmful compound for a human organism because it increases body temperature and remains active for a long time [29]. It causes nephrotoxicity and teratogenic effect [30, 31]. Therefore, very sensitive method of determination is needed. PAP, however, on most electrode surfaces is reversibly oxidized to *para* quinoneimine, while it has irreversible behavior on CPE and has quasi reversible behavior on modified CPEs and also it has been reported that PAP has quasi-reversible behavior on ionic liquid modified CPE [32], using 1-heptyl-3-methylimidazolium bromide as a modifier [33].
Simultaneous determination of APAP, PAP or PAP in APAP formulations has also been reported using spectrophotometry and LC. However, there is still a sustained interest in the development of simple and reliable methods for the simultaneous determination of APAP and PAP in samples such as pharmaceutical preparations. Recently, carbon ionic liquid electrodes (CILE) have been proposed and used as convenient electrodes for the electrochemical applications. Using ionic liquid as pasting binder in CPE allows one to construct a new generation of carbon composite electrodes with advantages over conventional CPEs such as high conductivity, fast electron transfer and antifouling properties [32].

paracetamol *para*-aminophenol

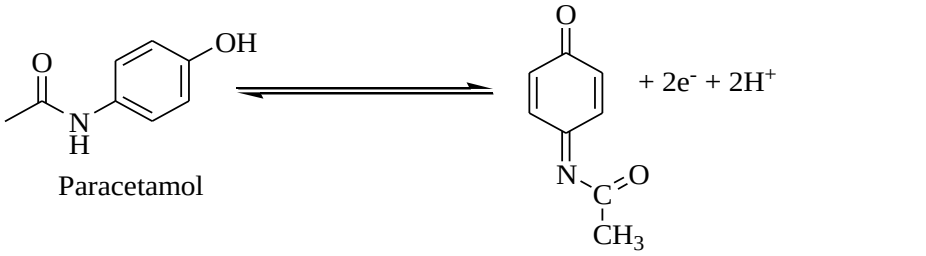
Fig. 3 Chemical structure of paracetamol and *para*-aminophenol

2.5.1. Chemical property of paracetamol *para*-aminophenol

Paracetamol is stable at saturated aqueous solutions. Its pKa value is 9.5 in aqueous media. The stability decreases in acidic or in alkaline conditions. It is slowly broken down into acetic acid and *para*-aminophenol.

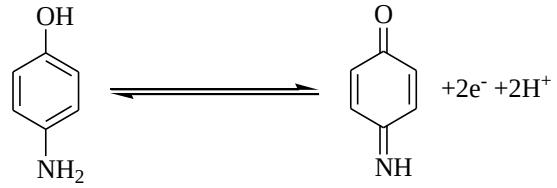
The *para*-aminophenol has strong reducing property. It reacts with oxidizing agents such as peroxides and nitrates. Contact *para*-aminophenol with nitric acid is expected to be highly exothermic. It is slowly decomposes on exposure to air and sunlight. Burning *para*-aminophenol may produce ammonia and nitrogen oxides [34].

The oxidation of both APAP and PAP are two-electron, two-proton transfer process as shown the scheme below.



Paracetamol

N-(4-oxocyclohexa-2,5-dienylidene)acetamide



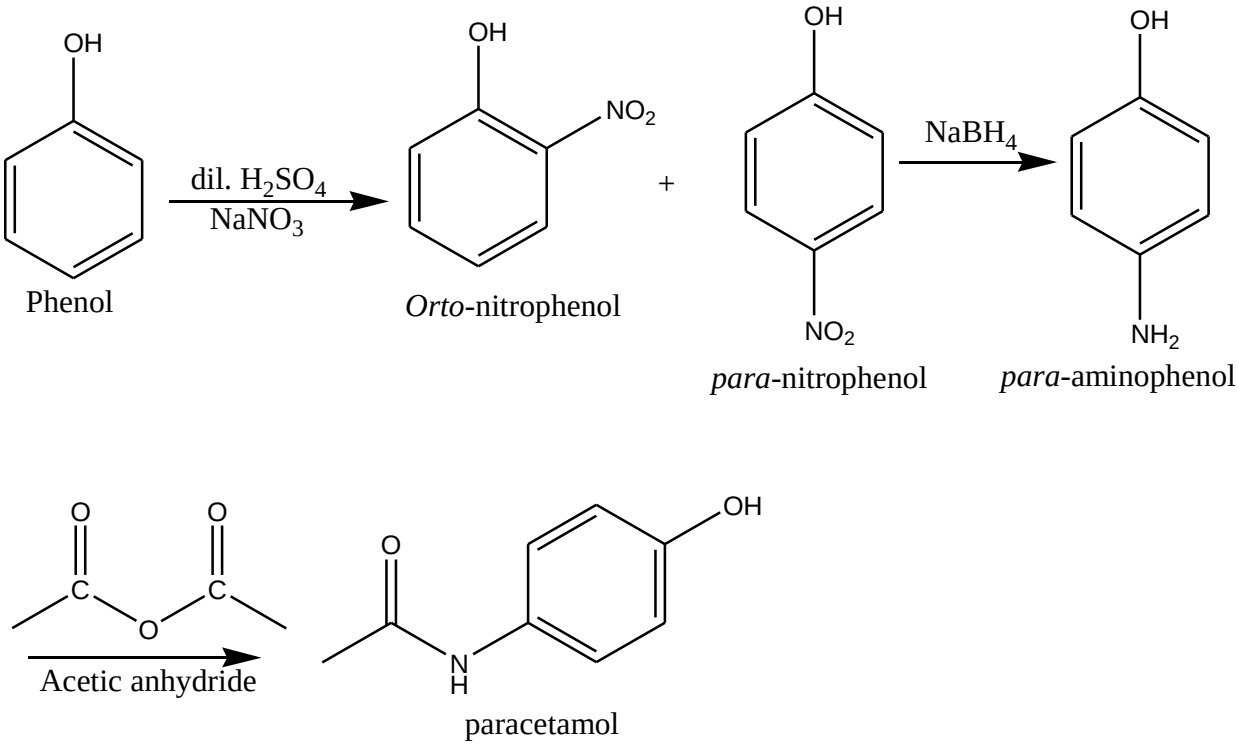
4-aminophenol

4-iminocyclohexa-2,5-dienone

Scheme 2. Oxidation of APAP and PAP [35].

2.5.2. Synthesis of paracetamol and *para*-aminophenol

Compared with many other drugs, paracetamol is much easier to synthesize, because it lacks stereo center. As a result, there is no need to design a stereo-selective synthesis. Industrial preparation of paracetamol usually proceeds from nitrobenzene [36]. A one-step reductive acetamidation reaction can be mediated by thioacetate [37]. Paracetamol may be easily prepared in the laboratory by nitrating phenol with sodium nitrate, separating the desired *para*-nitro phenol from the *ortho*- byproduct, and reducing the nitro group with sodium borohydride. The resultant *para*-aminophenol is then acetylated with acetic anhydride [38]. In this reaction, phenol is strongly activating, thus the reaction requires only mild conditions.



Scheme 3. Syntheses pathway of paracetamol and *para*-aminophenol

2.6. Electroanalytical techniques

Voltammetry is an electroanalytical technique based on the measurement of current flowing through an electrode dipped in solution containing electro active compounds while a potential is imposed upon it [39]. It is typically performed using a three electrode potentiostat, which accurately controls the applied potential. The redox reaction takes places at working electrode, because the working electrode is where the reaction of interest is taking place. The working electrode can be solid (platinum, gold or glassy carbon). If the working electrode is formed by drop of mercury the analytical technique is called polarogaphy [40]. The second electrode is a reference electrode, which maintains a constant potential throughout the experiments and the third electrode the counter electrode, which complete the electrical circuit. The counter electrode also known as the auxiliary electrode, is often much larger than working electrode to minimize current density at the electrode surface [40]. The common characteristic of all technique is that they involve the application of a potential (E) to an electrode and monitoring of the resulting current (I) flowing through electrochemical cell [41].

The analytical advantage of various voltammetric techniques include excellent sensitivity with very large useful linear concentration range for both inorganic and organic species (10^{-12} to 10^{-1}), a large number of useful solvents and electrolytes, a wide range of temperature, rapid analysis times (in seconds) simultaneous determination of analytes, the ability to determine kinetics and mechanistic parameters, a well developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the case with which different potential wave-forms can be generated and small current measured.

The use of the voltammetric techniques is the basis of the comprehension of the laws concerning several electrochemical phenomena and has a great importance in several technological fields like: research of corrosion-proof materials, research of new electrodic process for chemical industries for example, millions of tons of aluminum, chlorine, soda are produced by means of electrochemical reactions and production of new type of batteries that can store rapidly great quantity of energy. It is also used as quantitative analysis of trace metals those of oxidizable or reducible chemicals at μg/L levels or less [42].

2.6.1. Cyclic Voltammetry (CV)

Cyclic voltammetry consists of the most effective and versatile electro analytical technique for the study of electro active species. Its versatility was combined with ease of the measurement has resulted in extensive use cyclic voltammetry in the field of electrochemistry, inorganic chemistry, organic chemistry and biochemistry. The effectiveness of cyclic voltammetry results from its capability for rapid observing redox behavior over a wide potential range [43 - 44]. It was most widely used techniques of all methods by both electrochemist and non electrochemist. It allows the analyst to mechanistically study systems, especially the assignment and characterization of redox couples [43]. Cyclic voltammetry consists of cycling the potential of an electrode, which is immersed in unstirred solution and measuring the resulting current. The potential of the working electrode is controlled verses a reference electrode such as a saturated calomel electrode (SCE) ($Hg/Hg_2Cl_2/Cl^-$) or $Ag/AgCl/Cl^-$. The controlling potential that is applied across these two electrodes can be considered an excitation signal. The excitation signal for cyclic voltammetry is linear potential scan with a triangular wave form as shown in Fig.1. This triangular potential excitation signal sweeps the potential of the electrode between two electrode sometimes called the switching potential [44].

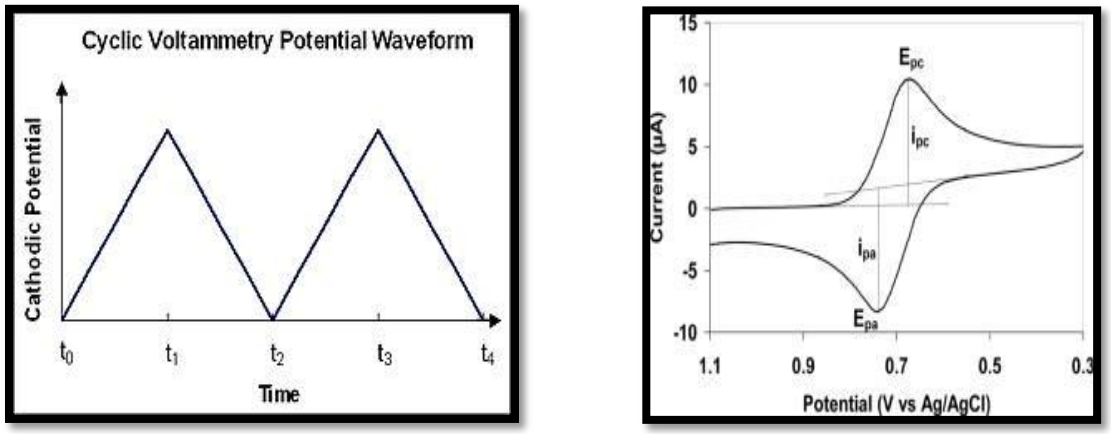


Fig. 4. (**a**) Excitation wave form of cyclic voltammetry (**b**) response obtained for the reversible cyclic voltammetry

A cyclic voltammogram is obtained by measuring the current at the working electrode during the potential scan. The current can be measured considered as the response signal to the potential excitation signal. The voltammogram is a display of current (vertical axis) verses potential (horizontal axis). Because the potential varies linearly with time, the horizontal axis can also be thought as time axis [39-44]. Cyclic voltammetry has become an important and widely used electro analytical technique in many area of chemistry. It is rarely use for quantitative determinations, but it is widely used for the study of redox process, for understanding reaction intermediate, and obtaining stability of reaction products The important parameter of a cyclic voltammogram are the magnitude of the anodic peak current (I_{pa}), cathodic peak current (I_{pc}), anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). A redox couple in which both species rapidly exchange electrons with the working electrode is termed as electrochemical reversible couple. Such couple can be identified from a cyclic voltammogram by measurement of the potential difference between the two peaks potential. The formal reduction potential $E^{o'}$ for reversible couple is centered between E_{pa} and E_{pc} [40, 44, 46].

$$E^{o'} = \frac{E_{pa} + E_{pc}}{2} \tag{1}$$

The number (n) of electrons transferred in the electrode reaction for reversible couple can determined from separation between peak potentials.

$$\Delta E = E_{pa} - E_{pc} = \frac{59}{n} mV \tag{2}$$

Where n is the number of electrons transferred and E_{pa} and E_{pc} is the anodic and cathodic peak potential, respectively, in volts. Thus for reversible redox reaction at 25°C ΔE should be $59mV$ or about 60 mV for one electrons.

$$I_p = 0.4463nFAC(nF\nu DRT)^{1/2} \tag{3}$$

Where, n = number of electrons transferred, F = Faraday constant (96485.339 C/mol), A = the electrode surface area in (m^2), ν = scan rate in (Volt/s), R = gas constant (8.314 J/K), T = Temperature (K) and C = concentration (mol/m^3) [42].

2.6.2. Pulse Methods

The imposition of potential pulse to the electrode leads in most experimental situations to a considerable improvement (increase) in the ratio of the charging and faradic currents compared to that linear scan voltammetry. This is because the faradic current usually decreases with $1/t^{1/2}$, while the charging current decreases much faster. In consequence, decreased lower limits of detection are obtained [43].

2.6.2.1. Normal Pulse Voltammetry (NPV)

This technique uses a series of potential pulses of increasing amplitude. The current measurement is made near the end of each pulse, which allows time for the charging current to decay. It is usually carried out in an unstirred solution at either DME (called normal pulse polagraphy) or solid electrodes. The potential is pulsed from an initial potential (E_i). The duration of the pulse, is usually 1 to 100 msec. and the interval between pulses typically 0.1 to 5 sec. The resulting voltammogram displays the sampled current on the vertical axis and the potential to which the pulse is stepped on the horizontal axis [44, 45]

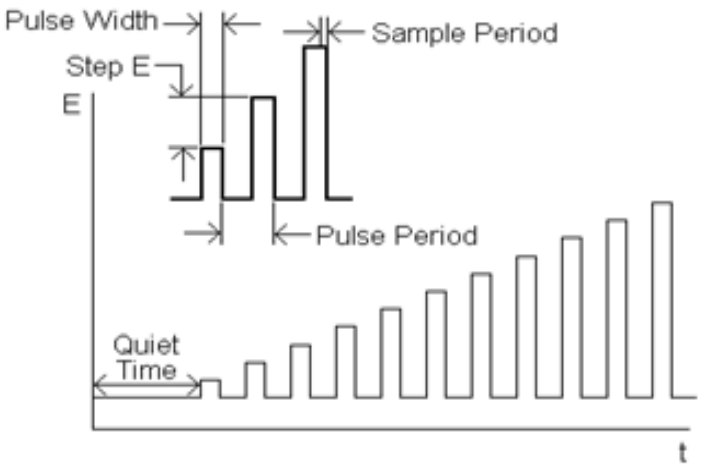


Fig .5 Excitation wave form of Normal pulse Voltammetry

2.6.2.2. Differential pulse voltammetry (DPV)

This technique is comparable to normal pulse voltammetry in that the potential is also scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed, of small amplitude (10 to 100 mV) and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point just before the application of the pulse and the second at the end of the pulse. These sampling points are selected to allow for the decay of the nonfaradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential [45].

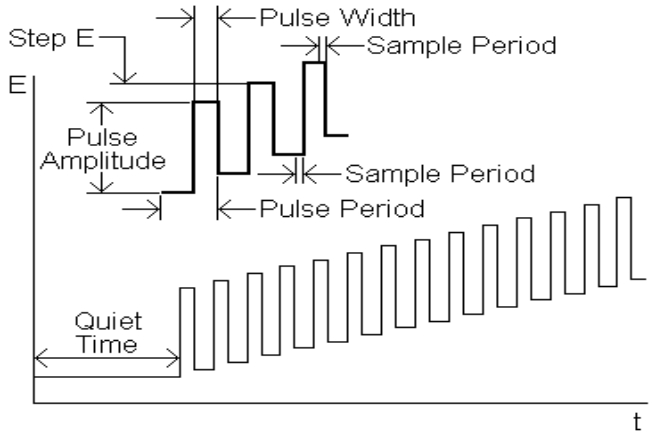


Fig. 6 Excitation wave form of differential pulse Voltammetry

2.6.2.3. Square-wave voltammetry (SWV)

The excitation signal in SWV consists of a symmetrical square-wave pulse of amplitude superimposed on a staircase waveform of step height ΔE, where the forward pulse of the square wave coincides with the staircase step. The net current, I_{net}, is obtained by taking the difference between the forward and reverse currents ($I_{fwd} - I_{rev}$) and is centered on the redox potential. The peak height is directly proportional to the concentration of the electro active species and direct detection limits as low as 10^{-8} M is possible. Square-wave voltammetry has several advantages. Among these are its excellent sensitivity and the rejection of background currents. Applications of square-wave voltammetry include the study of electrode kinetics with regard to preceding,

following, or catalytic homogeneous chemical reactions, determination of some species at trace levels and it is used with electrochemical detection in HPLC [40, 46].

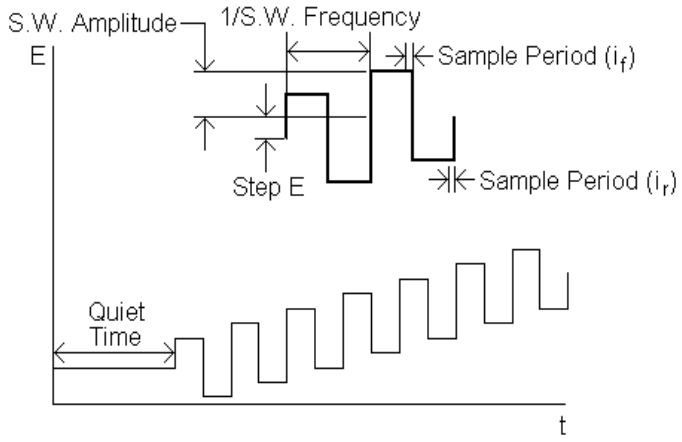


Fig. 7 Excitation wave form of square wave voltammetry

3. Experimental Part

3.1. Chemicals

Paracetamol (Sigma-Aldrich, Germany) tetrabutylammonium tetrafluoroborate (Sigma-Aldrich, Germany), acetonitrile (Scharlau Chemie, Spain), disodium hydrogen phosphate (Techno Pharmchem, India), sodium dihydrogen phosphate (BDH, England), hydrochloric acid (Riedel-deHaen, Germany), and sodium hydroxide (BDH, England) and *para*-aminophenol(Sigma-Aldrich, Germany), 3,4-ethyleneoxythiophene (EDOT), EPHARM paracetamol and PANADOL of Kenya tablets were used without further purification.

3.2. Instrumentation

The electrochemical data were obtained using the BAS Epsilon (Basj bioanalytical system ink. 2701 Kent. Ave. W. Lafayette. Ink. 47906) voltametric analyzer. The portion of the instrument where the actual electron transfer processes occur is in the electrochemical cell.

The cell that used is a three electrode cell consisting of reference, working and counter electrodes, with stirring and purging capabilities. The platinum wire was used as an auxiliary electrode, PEDOT/GC and bare glassy carbon electrode were used as working electrodes and Ag/AgCl sat's Cl^- was used as reference electrode. The three electrode stand is interfaced to the potentiostat which in turn interfaced to the PC. The experiments were conducted in phosphate buffer solution at pH = 7 and at room temperature. Cyclic voltametry and differential pulse voltametry were used for this study.

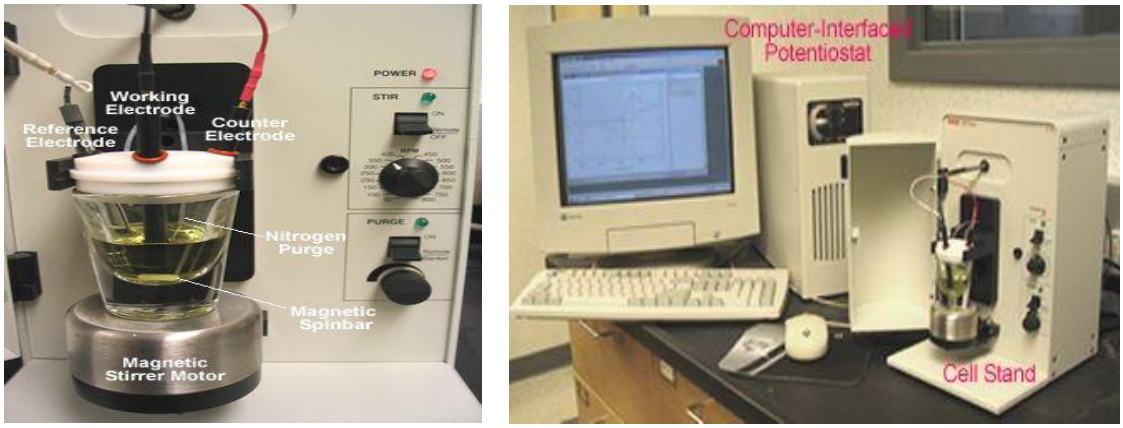


Fig. 8 Epsilon potentiostat with computer interface

Other instruments such as, A JENWAY 3510 pH meter (Barloworld Scintific Ltd, Dunmow Essex, UK), electronic balance (model LA 204) and centerifuge were used.

3.3. Analytical Procedures

3.3.1. Preparation of phosphate buffer and standard solutions of analytes

For analysis of standard paracetamol and *para*-aminophenol 0.1 M buffer solution of $Na_2HPO_4.2H_2O$ at pH = 7 was prepared and pH was adjusted using concentrated HCl or NaOH. Different concentration of paracetamol at constant concentration of PAP and different PAP concentration at constant concentration of APAP were prepared using buffer solution.

3.3.2. Preparation PEDOT/GC electrode

To prepare modified PEDOT/GC electrode first of all working electrode was checked whether pure or not by polishing it by gamma alumina powder with 0.05 micron. Back ground CV was taken using 0.1 M Phosphate buffer solution as supporting electrolyte. Glassy carbon was modified by 0.1 M EDOT between 0 to 1300 mV for 8 cycles. The film was washed by acetonitrile. PEDOT/GC was stabilized by run CV using TBATFB as electrolyte between -300 mV and 600 mV for 10 cycles.

3.3.3. Electrochemical measurements

The electrochemical behavior of APAP and PAP at PEDOT modified GC electrode was investigated using cyclic voltammetry (CV) in range from -100 mV to 700 mV. The determination of APAP and PAP was carried out using differential pulse voltammetry (DPV). DPVs were obtained by scanning the potential in the range from 0 to +500 mV at frequency amplitude of 25 Hz. Before each experiment, the PEDOT/GC was washed with distilled water. 0.05 M NaOH solution was used as electrolyte and its CV was run with a scan rate 50 mV/s until the peak of APAP and PAP disappeared. The APAP and PAP concentrations were obtained by measuring the heights of the oxidative peak currents.

3.3.4. Preparations of Calibration curve

To prepare a calibration curve of PAP and APAP, different concentration of APAP (1.0 μM - 100.0 μM) at 40 μM PAP was prepared by phosphate buffer at pH = 7. In the same way different concentration of PAP (4.0 μM - 320 μM) at 40 μM APAP was prepared by phosphate buffer. DPV at pulse amplitude 25 mV, pulse width 75 msec and with potential window 0 to 500 mV of each prepared solution was measured. Their calibrations curves were drawn using origin 6.

3.3.5. Real sample analysis

The proposed method was applied for the determination of APAP and PAP in two commercial available paracetamol tablets: EPHARM paracetamol tablets and PANADOL Kenya tablets. Five tablets of each sample were accurately weighed and powdered finely in a mortar. The average weight of each tablets powder was added to phosphate buffer solution in 100 ml volumetric flask and was shaked till it was dissolved. The solution was centrifugated then filtered by Whatman 41filter paper. The prepared solution was filled with phosphate buffer and it was further diluted. The diluted solution contains specified amount of APAP (which is already in the tablets) and PAP (which is spiked in a solutions). The DPV was measured by using BAS Epsilon. From calibration curve concentrations of APAP and PAP were extrapolated and recovery was calculated by dividing the obtained concentration to the spiked.

4. Results and Discussions

4.1. Preparation of PEDOT modified glassy carbon electrode

To prepare PEDOT/GC, the electropolymerization of 0.1 M (EDOT) on glassy carbon electrode was carried out using cyclic voltammetry between 0 and 1300 mV for eight cycles. Eight cycles was chosen as the optimum number of cycles to decrease the memory effect due to adsorption of APAP and PAP on the PEDOT/GC surface. This memory effect can be eliminated using 0.05 M NaOH electrolyte and run linear sweep voltammetry between -300 mV and 600 mV repeatedly. Fig. 9 shows that as the number of cycle's increase more PEDOT is grown on the surface of GC. PEDOT/GC was washed with acetonitrile to stabilize radical cations and oligomers on the surface of GC. This is due to acetonitrile is aprotic solvent that the ability to stabilize the radical cations and oligomers on the surface of GC [48]. The inset in Fig. 9 demonstrates the cyclic voltammogram of the PEDOT/GC in the phosphate buffer solution without APAP and PAP.

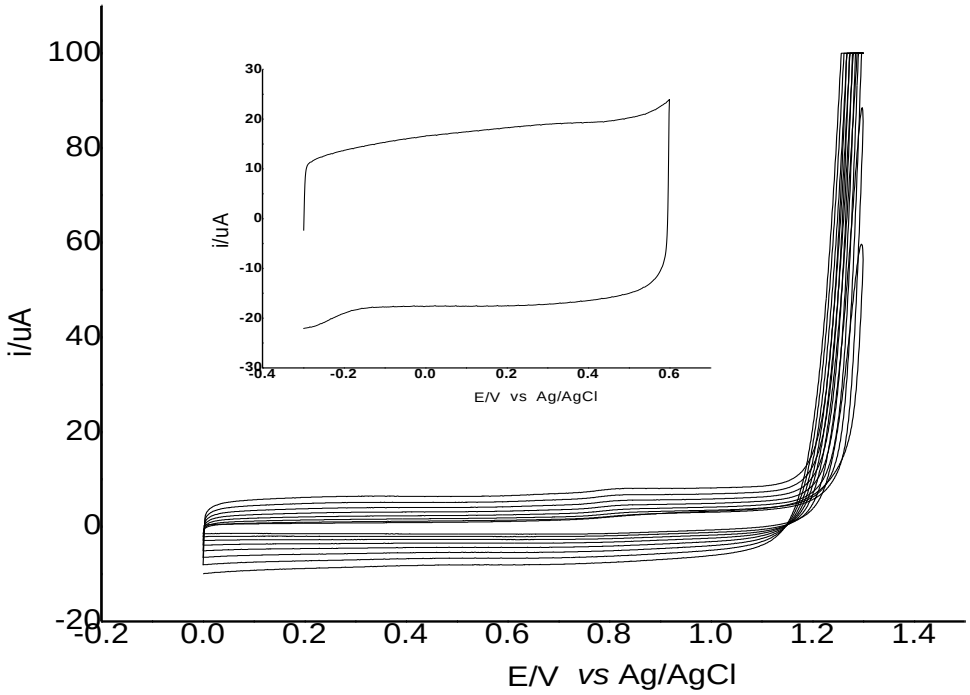


Fig. 9 Electropolymerization of EDOT between 0 and 1300 mV in TBATFB for 8 cycles.

In order to form stable film on the surface of GCE stabilization was carried out in TBATFB. Fig. 10 shows that after modified electrode was washed with acetonitrle cyclic voltametry was run between -300 mV and 600 mV in non aqueous 0.1M TBATFB to remove radical cations and form monomer free PEDOT/GC.

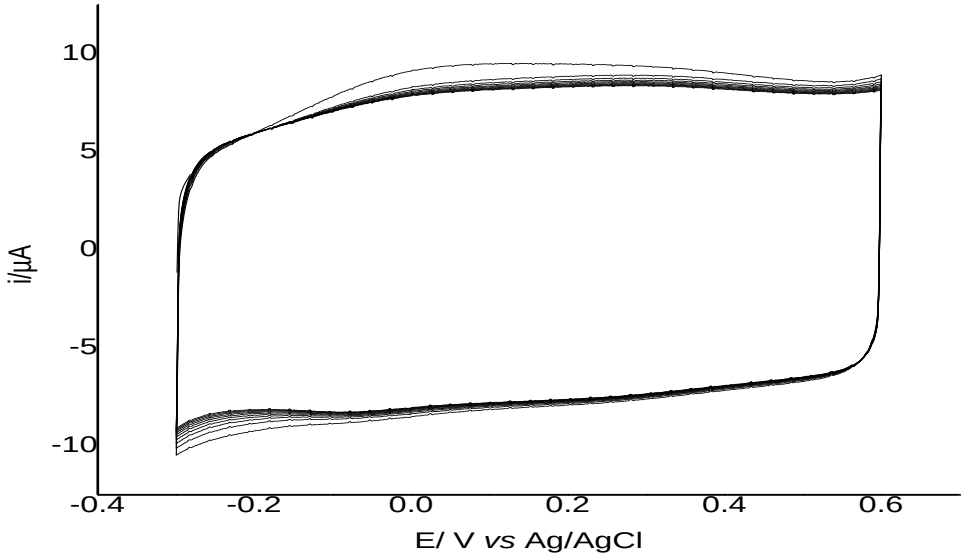


Fig. 10 Stabilization of PEDOT from (-300 mV to 600 mV) for 10 cycles in monomer free TBATFB electrolyte solution.

4.2. Electrochemical behavior of APAP and PAP on PEDOT modified GCE

In order to illustrate the electrocatalytic effect PEDOT/GC than bare GCE towards APAP and PAP, the electrochemical properties of APAP and PAP were examined using CV between -100 mV and 700 mV. Table 1 shows that the oxidation potential of 0.2 mM PAP was 197 mV and its reduction potential was 53.62 mV on bare GCE. This indicates that quasi reversible behavior on bare glassy carbon electrode [32]. APAP oxidation potential was 540 mV and almost has no reduction peak at bare glassy carbon electrode. This shows that it is irreversible and slow electron transfer behavior on bare GCE [32]. The oxidation potential of 0.2 mM PAP was changed to 127 mV and its reduction potential was 92 mV and oxidation potential of 0.2 mM APAP was also shifted to 381 mV and its reduction potential was 353.6 mV on

PEDOT/GC. This shows that the oxidation potential was shifted to more negative and peak to peak separation was decreased for both APAP and PAP. This is due to there is a fast electron transfer rate on PEDOT/GC electrode. Since peak to peak separation was 257 mV for APAP and PAP at PEDOT/GC it is sufficient for simultaneous determination of the two compounds on PEDOT/GC [37]. The peak currents (I_{pa}, I_{pc}) are enhanced on PEDOT/GC than bare GCE.

Table 1. Peak current and peak potential of PAP and APAP on bare GCE and PEDOT/GC taken from Fig. 11 and Fig. 12

Analytes	Bare glassy carbon					PEDOT/GC				
	Peak current (µA)		Peak potential (mV)		ΔE (mV)	Peak current (µA)		Peak potential (mV)		ΔE (mV)
APAP	I_{pa}	2.43	E_{pa}	540.00	539.17	I_{pa}	10.12	E_{pa}	381.00	38.65
	I_{pc}	-0.55	E_{pc}	0.84		I_{pc}	-7.98	E_{pc}	353.65	
PAP	I_{pa}	2.30	E_{pa}	198.26	145.36	I_{pa}	7.40	E_{pa}	127.00	35.00
	I_{pc}	-2.08	E_{pc}	53.62		I_{pc}	-7.37	E_{pc}	92.00	

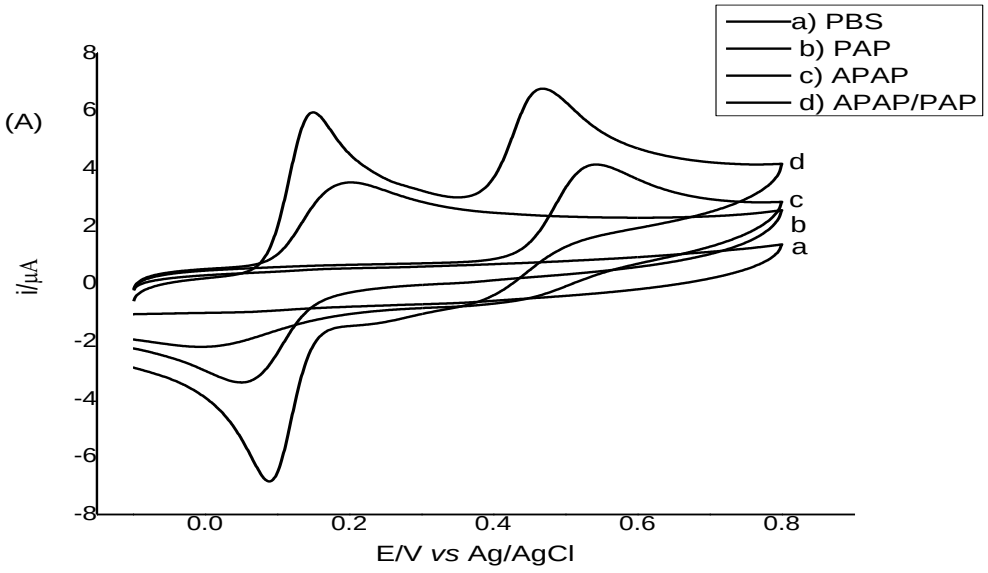


Fig. 11 Cyclic Voltamogram of 0.2 mM APAP, PAP and equimolar mixture of the two compounds in phosphate buffer solution (pH = 7) on bare glassy carbon electrode.

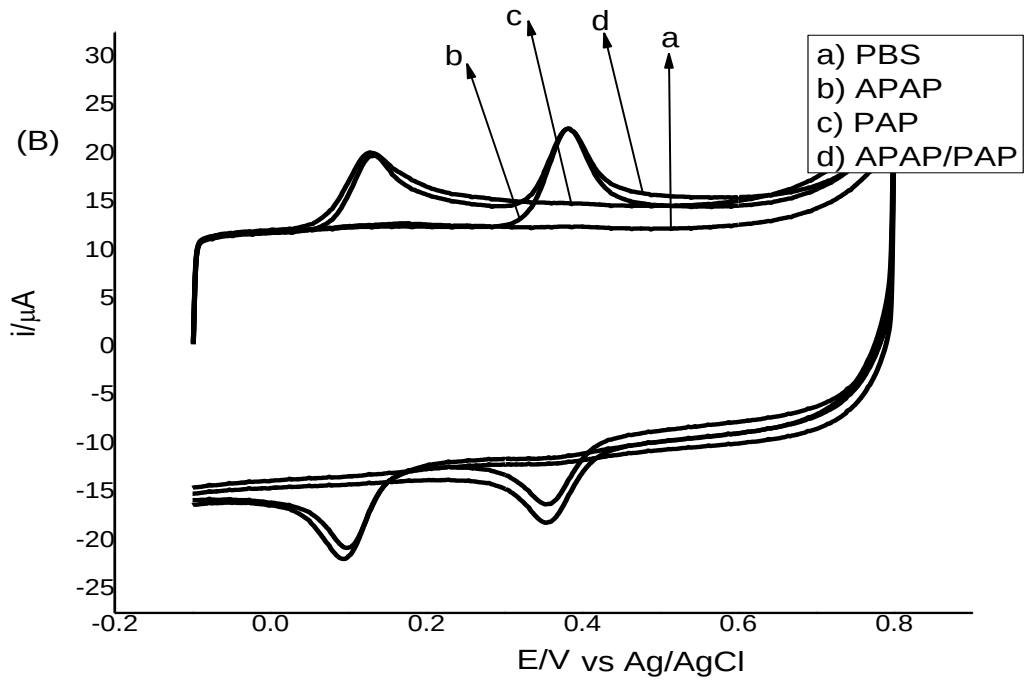


Fig. 12 Cyclic Voltamogram of 0.2 mM APAP, PAP and equimolar mixture of the two compounds in phosphate buffer solution (pH = 7) on PEDOT modified glassy carbon electrode.

In order to investigate the oxidation and reduction behavior of PAP and APAP, the effect scan rate of the oxidative and reductive peak current of 0.2 mM PAP and APAP at PEDOT/GC in 0.1 M phosphate buffer solution (pH = 7) were investigated. Fig. 13 shows both the oxidative and reductive peak current of APAP and PAP increased with scan rate (10 - 500 mV/s).

Fig. 13 also shows that influences of scan rate (10 - 500 mV/s) on peak potentials (E_{pa}, E_{pc}). The potentials (E_{pa} and E_{pc}) shifts toward more negative for E_{pc} and shifts towards more positive for E_{pa}, indicating that both potentials (E_{pa}, E_{pc}) are functions of scan rates. Accordingly, from Fig. 12 plots of E_{pc} and E_{pa} vs ln υ should yield two straight lines whose slopes equals to -RT/αnF and RT/(1 - α)nF respectively [51]. E_{pa} and E_{pc} vs lnυ of APAP and PAP.

(E_{pa} = 0.1923 + 0.016 lnυ, R=0.9908, E_{pc} = 0.077 - 0.014 lnυ, R = 0.9918) and (E_{pa} = 0.1498 + 0.074lnυ, R= 0.9918, E_{pc} = 0.11469 - 0.065 lnυ, R= 0.9916) respectively. From

the slope of E_{pa} and E_{pc} *vs* lnυ of APAP and PAP the electron transfer coefficient (α) was calculated to be 0.446 and 0.533 for APAP and PAP respectively.

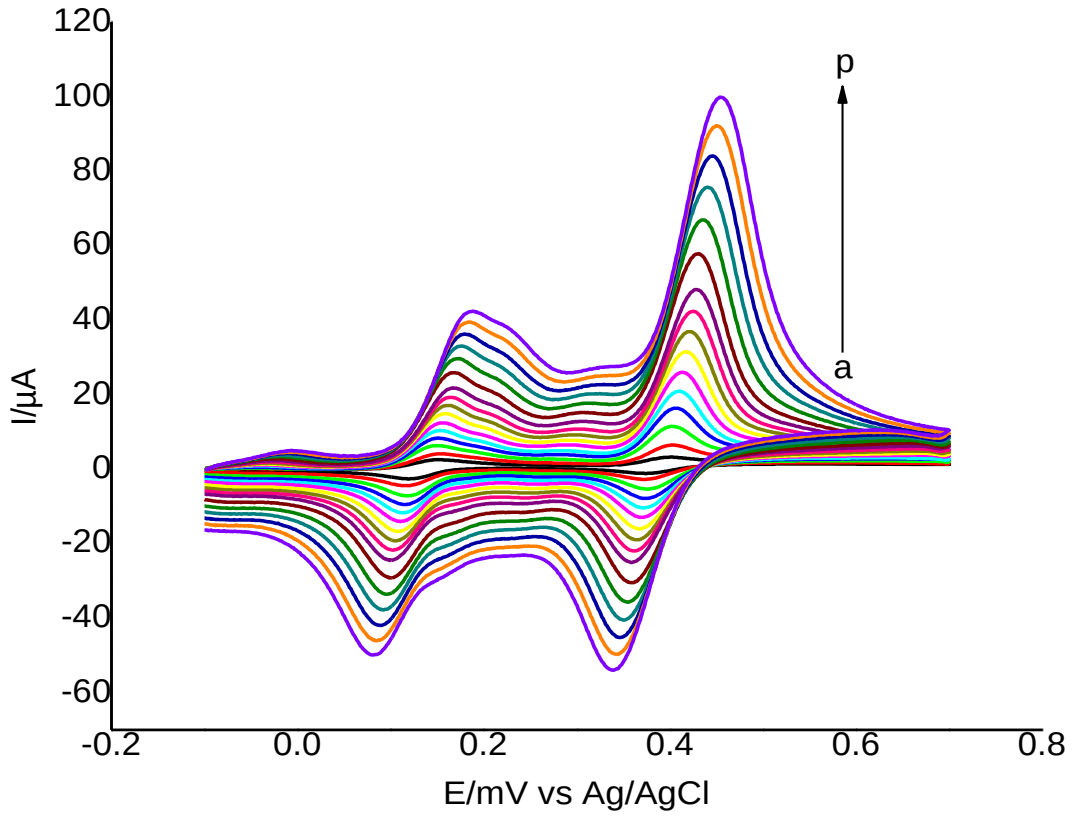


Fig. 13 Peak current of 0.2 mM PAP and APAP on the scan rate in the range (10 mV/s - 500 mV/s) in 0.1 M phosphate buffer solution (pH = 7) at PEDOT/GC.

Fig. 14 and Fig. 15 show that linear relationship between the peak currents (I_{pa}, I_{pc}) and scan rate (10-500 mV/s) with equations for APAP (I_{pa} = 5.201 + 19.59υ, R = 0.9968, I_{pc} = -1.851 - 18.38υ, R = 0.9971) and for PAP (I_{pa} = 3.772 + 81.64υ, R = 0.9951, I_{pc} = -3.888 - 95υ, R = 0.9971). This indicates that the electrode process is adsorption controlled for both APAP and PAP [19, 56].

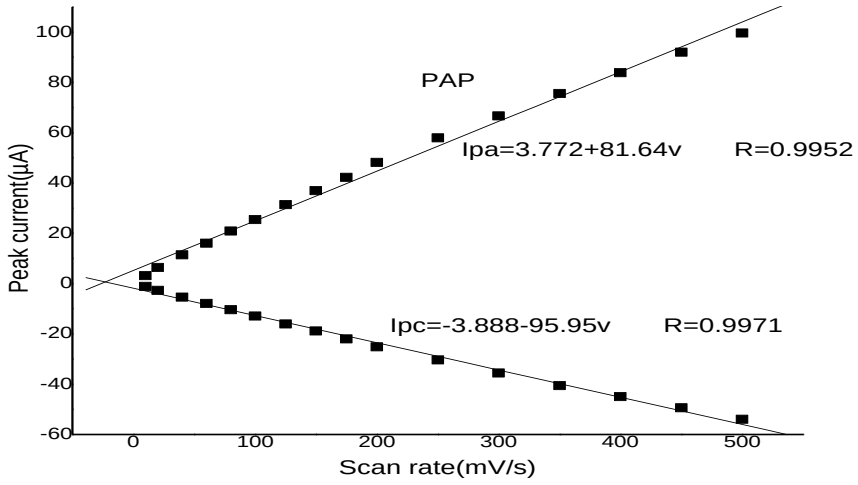


Fig. 14 Peak current Vs Scan rate (10-500 mV/s) of 0.2 mM PAP.

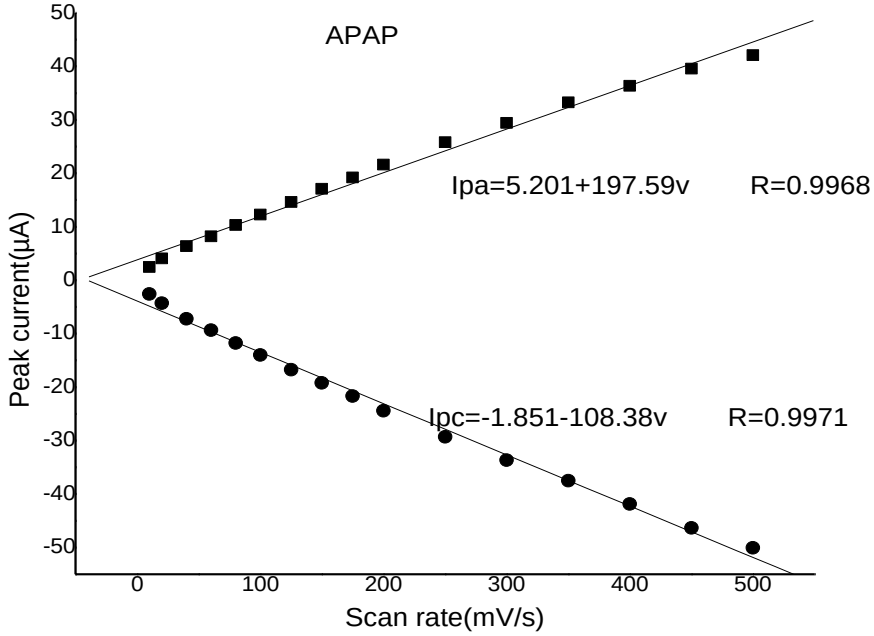


Fig. 15 Peak current Vs scan rate (10 -500mV/s) of 0.2 mM APAP

4.3. Effect of pH and buffer preference

To identify better buffer for the analytes (APAP and PAP) three different buffers (phosphate, acetate and borax) were taken and the analytes were prepared by these buffer and their cyclic voltammetry was run. Fig. 16 shows that potential in phosphate buffer solution was shifted to more negative value and peak current was also enhanced than acetate buffer. Therefore, phosphate buffer was better for the analysis. On the other hand, borax buffer is not suitable since the analytes are not stable in this buffer. The pH value of the solution has a significant influence on the peak current and peak potential of catalytic oxidation and reduction of APAP and PAP Fig. 17 shows that at higher pH current was decreased due to hydroxylation of the mediator [55]. Potential was shifted to more negative due to proton involved in electrochemical reaction of APAP and PAP [32]. At lower pH current was decreased due to common ion effect [19]. Fig. (18 and 19) indicate that peak current of APAP and PAP reaches maximum value at pH = 7.0. Therefore, pH = 7.0 was better for further analysis.

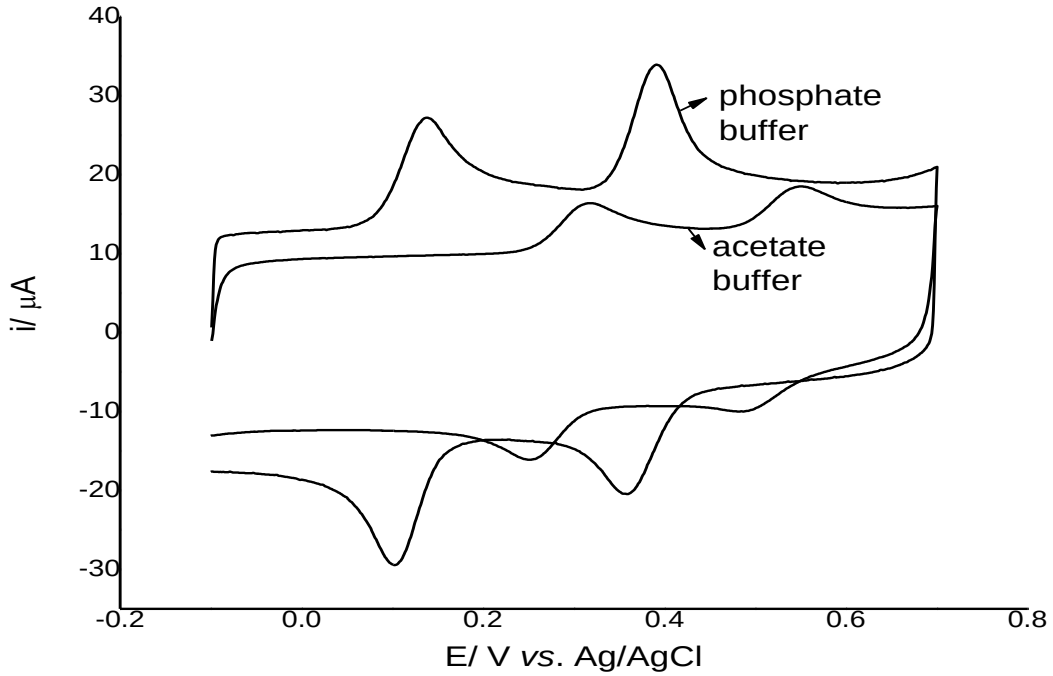


Fig. 16 Cyclic voltamogram of APAP and PAP in acetate and phosphate buffer solution

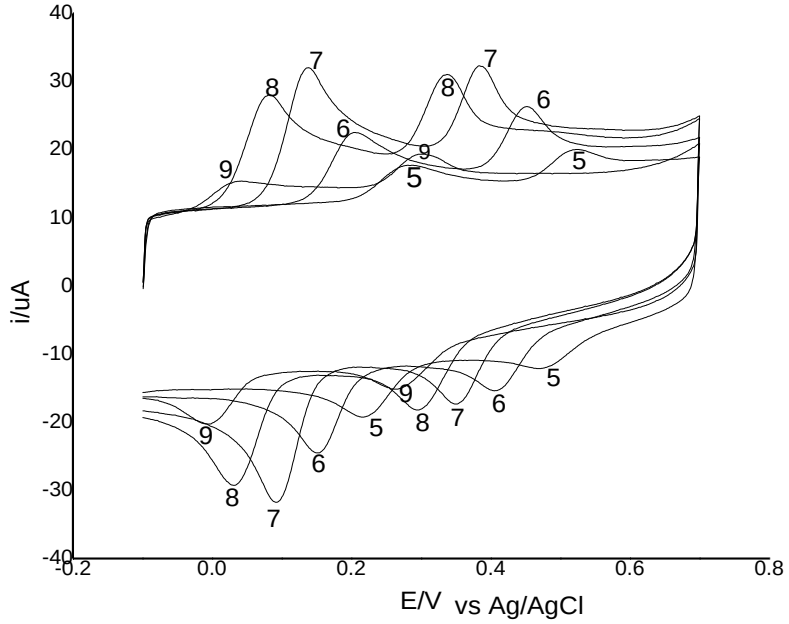


Fig. 17 Cyclic voltammogram of pH values on the peak current and peak potential of 0.5mM APAP and PAP on the PEDOT/GC

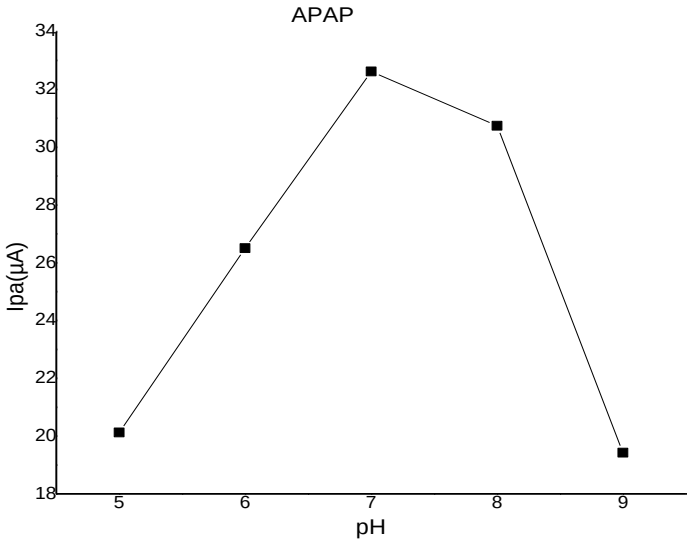


Fig. 18 Peak current versus pH of APAP and PAP

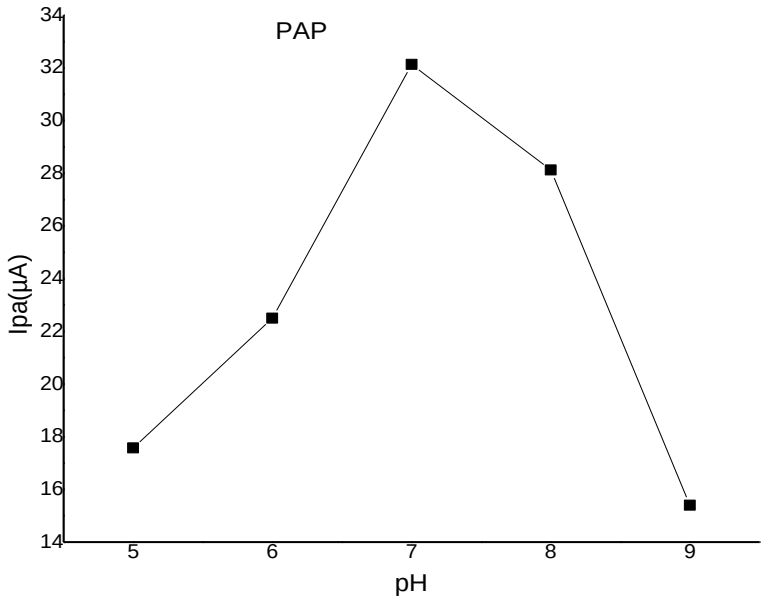


Fig. 19 Peak current versus pH of APAP and PAP

The number electrons involved in electrochemical reaction of APAP and PAP were found from change in potential (ΔE) of APAP and PAP on PEDOT/GC. If the change in potential was found to be 30 mV the reaction is reversible and number of electron involved is two. However, if the reaction is quasi reversible, change in potential (ΔE) is varies 30 mV – 40 mV and number of electron involved was considered to be two [56].

In this study change in potential (ΔE) of APAP and PAP on PEDOT/GC was found to be 38.65 mV and 35.00 mV respectively. Therefore, electrochemical reaction of both APAP and PAP is quasi reversible and number of electron involved for electrochemical reaction of both APAP and PAP is two. The number of protons involved in electrochemical reaction of APAP and PAP was found from formal potential (E^o) *vs.* pH. If the slope of E^o versus pH was around -59 mV/pH the number of protons are two. Fig. (20 and 21) show that the plot of E^o *vs.* pH of PAP and of and APAP respectively. From these plots slope of PAP and APAP is -58.06 mV/pH and -54.00 mV/pH, respectively and number of proton involved in electrochemical reaction PAP and APAP were two. This indicates that number of proton is equals to number of electron [35].

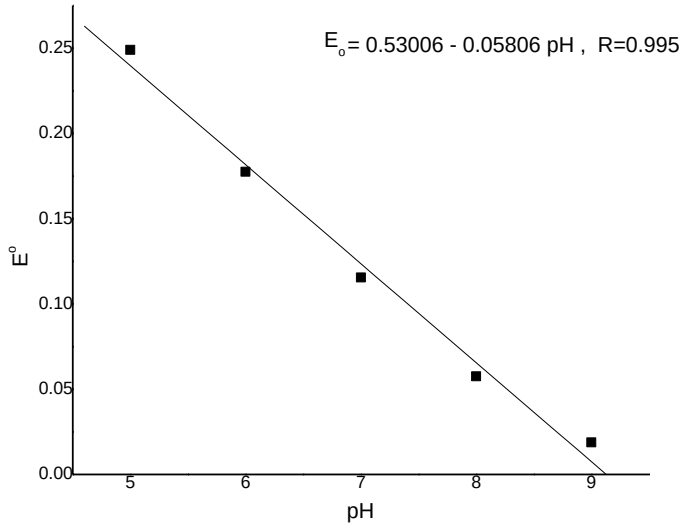


Fig. 20 Formal peak potential (E^o) versus pH of PAP

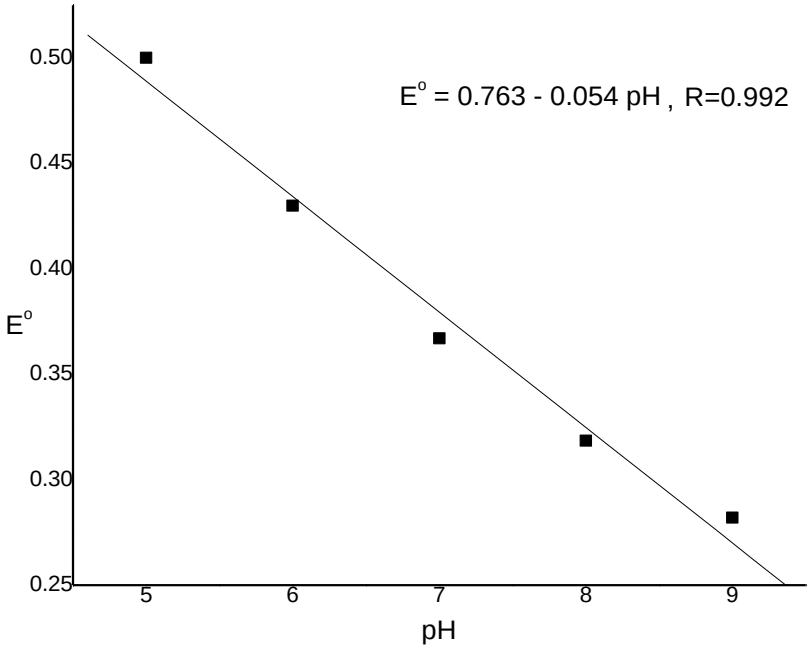


Fig. 21 Formal peak potential (E^o) versus pH of APAP

4.4. Analytical performances

To further enhance sensitivity and lower detection limit, a more sensitive technique differential pulse voltammetry, is used to analytically detect APAP and PAP at PEDOT/GC to evaluate its calibration characteristics. As it can be seen from Fig. 22 and Fig. 24 two well separated peaks corresponding to APAP and PAP appeared at 367 mV and 109 mV respectively. Peak to peak separation is about 257 mV on PEDOT/GC which is sufficient for their simultaneous determination in sample containing these two compounds. The Fig. 22 shows DP voltammogram of APAP from 1 µM to 100 µM at 40 µM of PAP. Fig. 23 shows that linear relationship between concentration of APAP and peak current with equations Ip (µA) = 0.6582 + 0.2844c (µM), R = 0.994). Fig. 24 also shows that DP voltammogram of PAP from 4 µM to 320 µM at 40 µM of APAP. Fig. 25 shows that a linear relationship between concentration of PAP and peak current with equation (Ip (µA) = 0.2103 + 0.0938 c (µM), R = 0.9982). The detection limit of APAP and PAP can be calculated by measuring the DPV of modified electrode without APAP and PAP nine times and find the standard deviation of the nine measurements. Accordingly, the standard deviations of the nine measurements are 3.8375×10^{-8} and 3.716×10^{-8} for APAP and PAP, respectively. From these values the detection limit of the PEDOT modified electrode of the two analytes was calculated by $3\sigma/m$ [50]. Hence, LOD of APA P and PAP was 0.4048 µM and 1.188 µM, respectively.

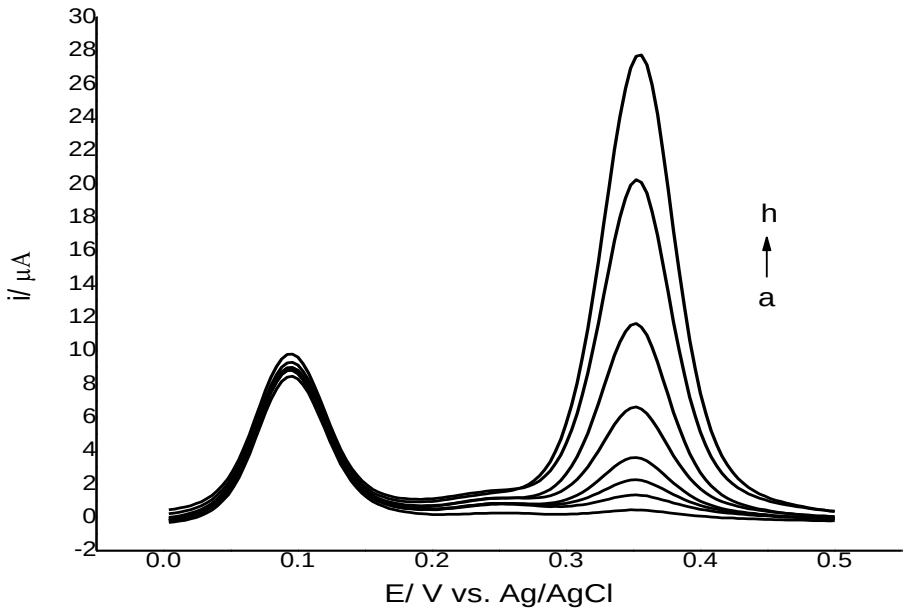


Fig. 22 DPV at the PEDOT-modified GCE in the presence of 40 μM PAP for different Concentrations of APAP (from a to h) 1.0, 4.0, 6.0, 10.0, 20.0, 40.0, 60.0, and 100.0 M at 50 mV/s (Background subtracted).

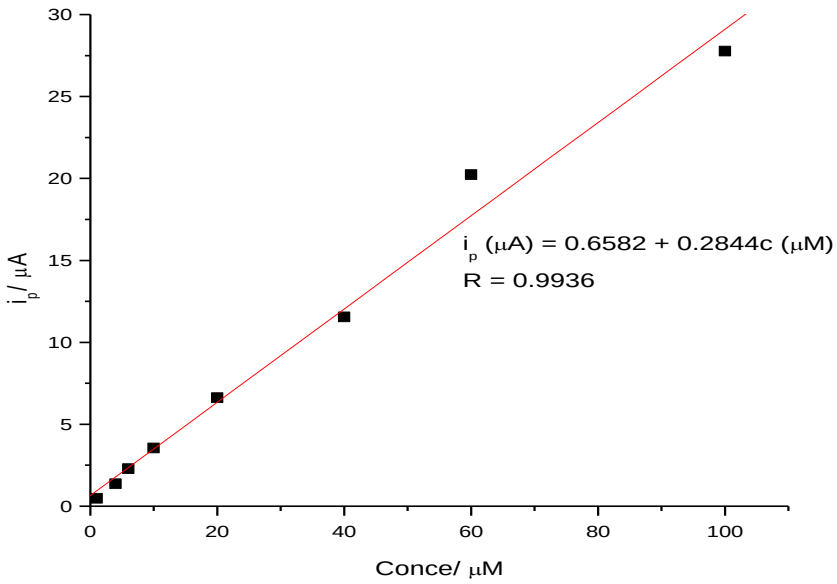


Fig. 23 Calibration curve of APAP.

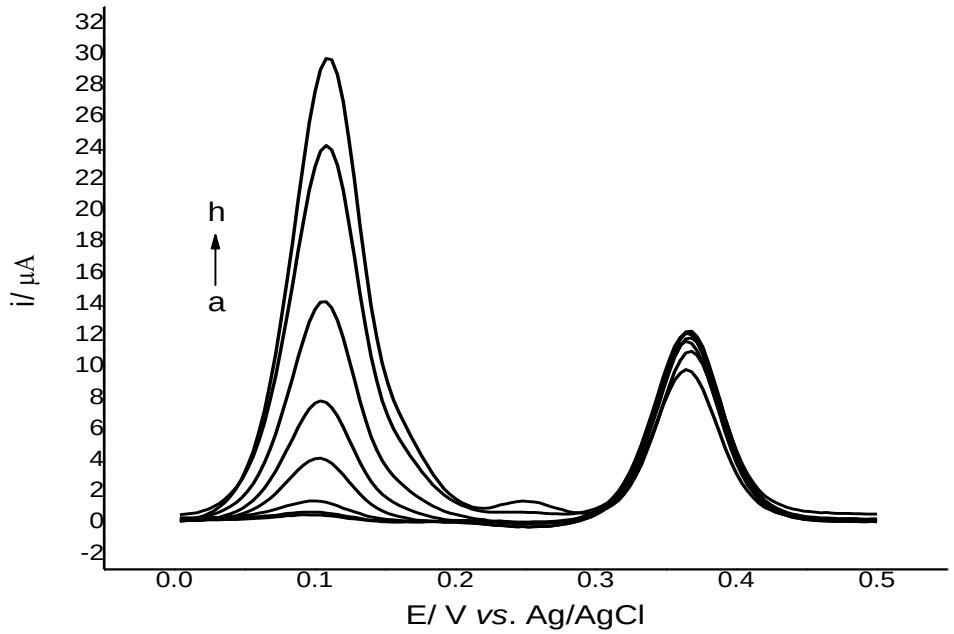


Fig. 24 DPV of the modified electrode in the presence of 40 μM APAP for different Concentrations of PAP (from a to h): 4.0, 6.0 10.0, 40.0, 80.0, 160, 240, and 320 μM at scan rate of 50 mV/s (Background subtracted).

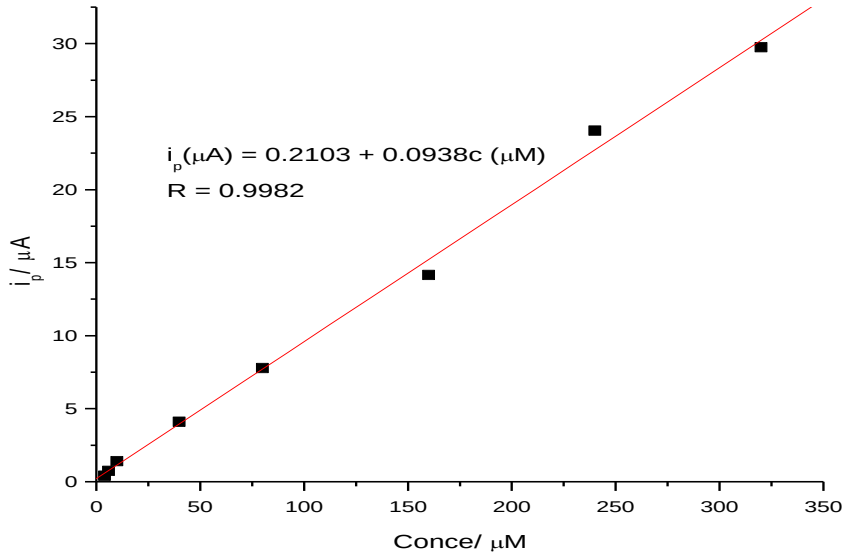


Fig. 25 Calibration curve of PAP

4.5. Reproducibility test

To estimate the repeatability of the proposed method, the RSD of five successive measurements of peak current of 0.1 mM APAP and PAP on single modified electrode was calculated to be 2.1% and 1.9% respectively, which demonstrates that good reproducibility of the method. The electrode to electrode reproducibility was investigated on four PEDOT/GC; the RSD of four average peak current of 0.1 mM APAP and PAP was calculated to be 4.8% and 4.3%, respectively. From these values it is possible to conclude that using single electrode modification is better throughout the experiment.

4.6. Recovery test

The recovery was also studied to evaluate the accuracy of the method, and the results are listed in Table 3. From Table 3 the average recovery of three independent experiments for APAP and PAP in Tablet 1 (EPHARM) were calculated to be 102.55% and 100.7 % respectively and in tablet 2 (PANADOL) average recoveries of APAP and PAP were calculated to be 105.85% and 99.05% respectively. These results indicate that the method was better for analysis of APAP and PAP in EPHARM (Ethiopia) and PANADOL (Kenya) tablets.

Table 2. Determination of APAP and PAP in commercial tablets (EPHARM and PANADOL) using the PEDOT/GCE

Matrix	Added (μM)		Found[a] (μM)		Recovery (%)	
	APAP	PAP	APAP	PAP	APAP	PAP
Tablet 1 (EPHARM)	-	-	5.94 (±0.07)	-	-	-
	25.0	24.0	30.1 (±0.21)	(25.4 ±0.17)	96.6	105.8
	42.0	57.0	51.5 (±0.28)	(54.5 ±0.24)	108.5	95.6
Tablet (PANADOL)	-	-	5.79 (±0.11)	-	-	-
	34.0	54.0	41.1 (±0.27)	(54.9 ±0.22)	103.9	101.7
	49.0	66.0	58.6 (±0.23)	(63.6 ±0.26)	107.8	96.4

[a] Mean value ± standard deviation (n = 3)

4.7. Interference study

Possible interference for the detection of APAP in urine at PEDOT modified electrode is uric acid. 0.5 mM uric acid was added to 0.5 mM APAP and run its cyclic voltammetry between -100 mV and 700 mV, as it can be seen from Fig. 26 peak to peak separation of the two analytes were very small as a result determination of one analyte in the presence of the other is not possible because their oxidation or reduction potentials are extremely close. Therefore, to determine APAP in urine, uric acid should be removed from urine. Other possible interferances to determine APAP in commercial tablets are acetyl salicylic acid, ascorbic acid; saccharine, citric acid and sodium bicarbonate were studied in the previous work [19].

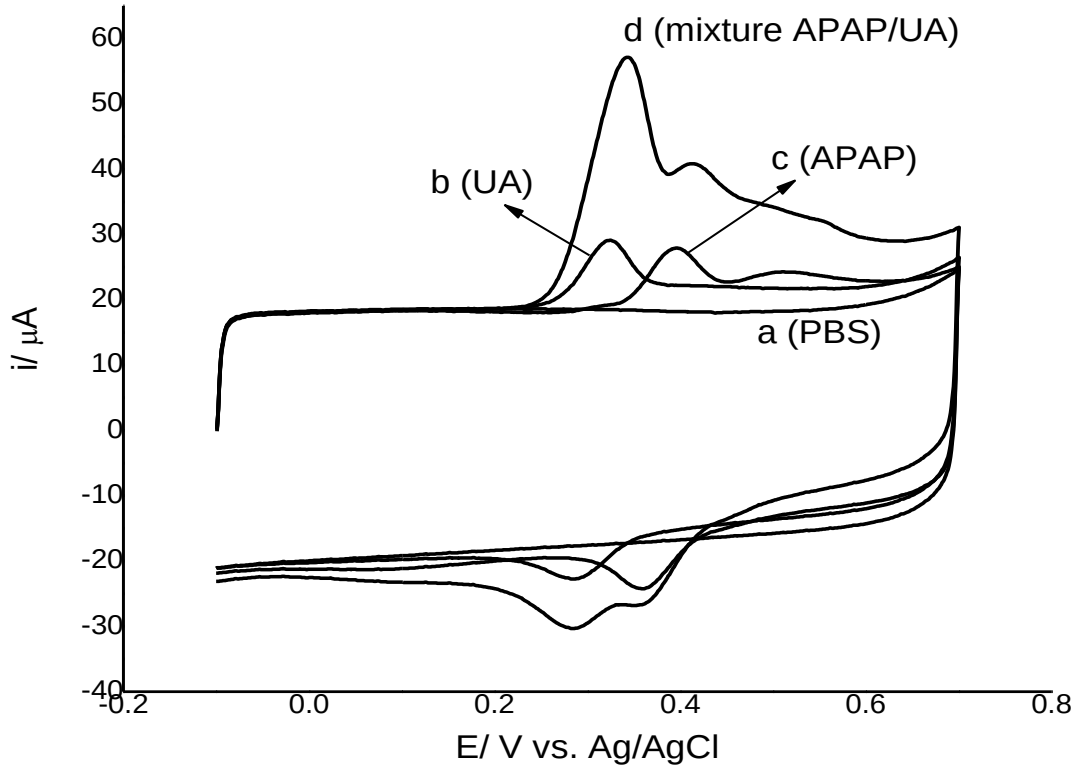


Fig. 26 CVs at the PEDOT-modified GCE for: a) PBS (pH = 7.0), b) UA (0.5 mM), c) APAP (0.5 mM), d) mixture of UA (0.5 mM) and APAP (0.5 mM), at scan rate of 50 mV s^{-1}.

Table 3. Comparison of simultaneous determination of APAP and PAP with other methods

Method	Linear range		LOD		Reference
	APAP	PAP	APAP	PAP	
HPLC	2.5 µg /ml- 20 µg/ml (16.5 µM-132.5 µM)	2.5 µg/ml - 20 µg/ml (22.9 µM -183.5 µM)	0.06 µg/ml (0.4 µM)	0.5 g/ml (4.6 µM)	53
Spectroscopic	0.5 µg/ml-21 µg/ml (3.3 µM -140 µM)	0.1 µg/ml -15 µg/ml (0.92 µM -137 µM)	0.5 µg/ml 3.3 µM	0.1 µg/ml (0.92 µM)	52
CILE/CPE	2 µM -2.2 mM	0.3 µM-1 mM	0.5 µM	0.1 µM	32
PEDOT/GC	1 µM -100 µM	4 µM -320 µM	0.4048 µM	1.188 µM	This work

5. Conclusions

In this study a simple, fast, reproducible procedure was used for fabrication of PEDOT modified glassy carbon electrode for simultaneous determination of APAP and PAP. It was found that the redox peak current of both APAP and PAP was improved significantly and the oxidation peak shifted towards less positive potentials at the PEDOT/GC compared to the bare glassy carbon which suggests that PEDOT/GC displays more excellent electro-catalytic property towards both APAP and PAP oxidation. The obtained results showed that the PEDOT/GC has better analytical performance than the bare glassy carbon in the APAP and PAP determination and LOD of APAP and PAP was 0.4048 µM and 1.188 µM, respectively; the average recoveries of APAP in EPHARM paracetamol and PANADOL Kenya tablets are 102.55% and 105.85%, respectively and that of PAP in EPHARM are 99.05% and 100.7%, respectively.

6. References

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Declaration

I the undersigned confirm that the results reported in this work were obtained by research carried out by me under the supervision of my advisor in College of science, Department of Chemistry, Addis Ababa University in Academic year 2010 - 2011. No part of this work shall be published in scientific journals or reported in the media or presented at a conference without the knowledge and consent of my advisor, who is principal scientist responsible for any publication. Further more if the work is published the institutional address given should be the Chemistry Department, Addis Ababa University.

Name: Tadele Hunde Wondimu Signature: _____

This Thesis work has been submitted for examination with my approval as a university advisor.

Advisor: Shimelis Admassie (Ph.D) Signature: _____

Place and Date of submission: School of Graduate Studies

June, 2011

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