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Simultaneous Determination of Dopamine and Pyridine-2-aldoxime methochloride at Poly (3, 4-ethylenedioxythiophene) Modified Glassy Carbon Electrode

BY

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This is to certify that the thesis prepared by **Abera Demeke Ambaye** entitled: "***Simultaneous Determination of Dopamine and pyridine-2-aldoxime methochiloride at*** **Poly (3, 4-ethylenedioxythiophene)** ***Modified Glassy Carbon Electrode"*** and submitted in partial fulfillment of the requirements of the Degree of Master of Science in Chemistry (Analytical Chemistry) compiles with regulation of the University and meets the accepted standards with respect to originality and quality.

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I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

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List of abbreviations

DA	Dopamine
PAM-2	Pyridine-2-aldoxime methochloride
GCE	Glassy carbon electrode
PEDOT	Poly (3, 4-ethylenedioxythiophene)
CV	Cyclic voltammetry
SWAdSV	Square Wave adsorptive Stripping Voltammetry
L-DOPA	dihydroxyl phenylalanine
ADHD	Attention Deficit Hyperactivity Disorder
AcHE	acetylcholine esterase enzyme
ACh	acetylcholine
CPs	Conjugated polymers
PBS	Phosphate buffer solution
L	Liter
μ	Micro

ABSTRACT

A sensitive and selective method was developed for the simultaneous determination of Dopamine (DA) and Pyridne-2-alidoxime methochiloride (PAM-2) with poly (3, 4-ethylenedioxythiophene) (PEDOT) modified glassy carbon electrode (GCE). Cyclic voltammetry and Square wave adsorptive stripping voltammetry (SWAdSV) were used to investigate the electrochemical reaction of DA and PAM-2 at the PEDOT modified electrode. DA and PAM-2 were showed a diffusion control and adsorption control reaction mechanisms, in phosphate buffer solution of pH 7.0 respectively. The significant anodic peak potential difference (525 mV) between DA and PAM-2 enabled the simultaneous determination of both species based on Square wave adsorptive stripping voltammetry. The voltammetric responses gave linear ranges of 5×10^{-7}–1.0×10^{-4} mol L^{-1} and 5.0×10^{-6} –15.0×10^{-5} $molL^{-1}$, with detection limits of 3.1×10^{-8} $molL^{-1}$ and $1.91\times10^{-7} molL^{-1}$ for DA and PAM-2, and The method was successfully applied for the determination of DA and PAM-2 in biological samples, with in a very good recovery result 101.57 % and 95.17 % respectively.

Keywords: Dopamine (DA), Pyridine-2-aldoxime methochloride (PAM-2), Glassy carbon electrode (GCE), Poly (3, 4-ethylenedioxythiophene) (PEDOT), Cyclic voltammetry (CV) and Square wave adsorptive stripping voltammetry (SWAdSV)

1. Introduction

Catecholamines are a class of biogenic amines that act as neurotransmitters. catecholamine is a monoamine, an organic compound that has a catechol (benzene with two hydroxyl side groups) and a side-chain amine [1]. Catechol can be either a free molecule or a substituent of a larger molecule, where it represents a 1, 2-dihydroxybenzene group. Catecholamines, derived from the amino acid tyrosine, water soluble and are bound to plasma proteins in circulation.

Dopamine (DA) is one type of the Catecholamines which is produced from phenyalanine and tyrosine which is created from phenylalanine by hydroxylation by the enzyme phenylalanine hydroxylase. Catecholamine secreting cells use several reactions to convert tyrosine serially to dihydroxyl phenylalanine (L-DOPA) and then to dopamine. Depending on the cell type, DA may be further converted to norepinephrine or even further converted to epinephrine [2].

Chemically, DA molecule consists of a catechol structure (a benzene ring with two hydroxyl side groups) with one amine group attached via an ethyl chain as shown in Figure 1. The presence of a benzene ring with this amine attachment makes it a phenethylamine, a family that includes numerous psychoactive drugs. Dopamine, like most amines, is an organic base. In acidic environments, it is generally protonated. The protonated form is highly water-soluble and relatively stable, though it is capable of becoming oxidized if exposed to oxygen or other oxidants. In basic environments, dopamine is not protonated. In this free base form, it is less water soluble and also more highly reactive.

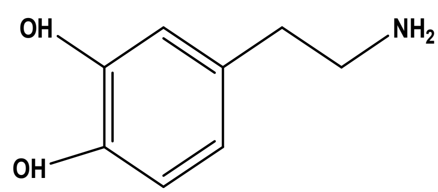


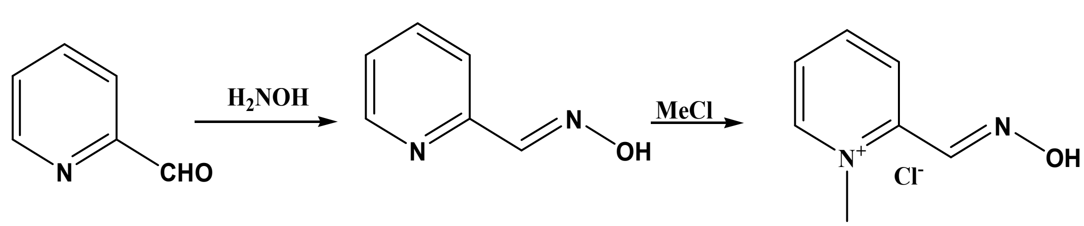
Figure 1. Structure of dopamine

DA is involved in a number of physiological and behavioral processes including cognition, locomotion, mood, motivation, and reward. Abnormalities in the central dopaminergic systems contribute to several neuropsychiatric diseases, including Parkinson’s disease, attention deficit hyperactivity disorder, schizophrenia, bipolar disorder, binge eating disorder, and addiction [3]. In particular, the nigrostriatal system is thought to give us the motivation to seek basic needs, such as food, while the mesolimbic pathway enables us to feel pleasure from them. Our eating behavior and our desire for food is tied in closely with these systems, which receives input from areas of the brain that monitor our nutritional need for food, such as the hypothalamus. Therefore, it is not surprising that dysfunction in DA signaling has been linked to eating disorders and obesity.

In the brain, DA functions as a neurotransmitter a chemical released by nerve cells to send signals to other nerve cells. The brain includes several distinct dopamine systems, one of which plays a major role in reward-motivated behavior. Most types of reward increase the level of dopamine in the brain, and a variety of addictive drugs increase dopamine neuronal activity. Other brain dopamine systems are involved in motor control and in controlling the release of various hormones.

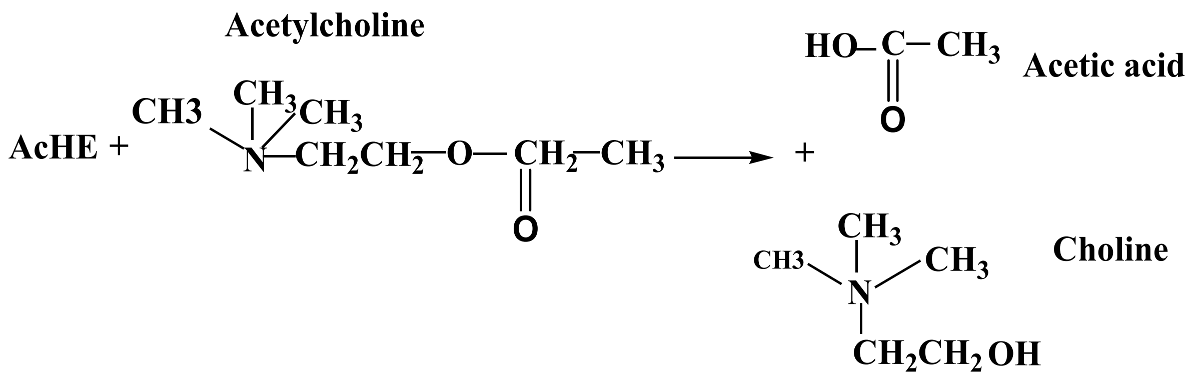
As a potent neuromodulator, it produces effect on emotion, movement, heart rate, blood pressure, memory and so on. Low levels or practically complete depletion of dopamine in the central nervous system is believed to be related to several neurological diseases, such as schizophrenia and Parkinson's disease [4].

On the other hand, Pralidoxime (2-pyridine aldoxime methyl chloride,) (PAM-2) belongs to a family of compounds known to be oximes. PAM-2 is one type of oximes, which is synthesized by reacting picolinaldehyde (2-formyl pyridine) with hydroxylamine producing pyridine-2-aldoxime, which is further reacted with methyl chloride, forming the desired pralidoxime as shown in Scheme 1.



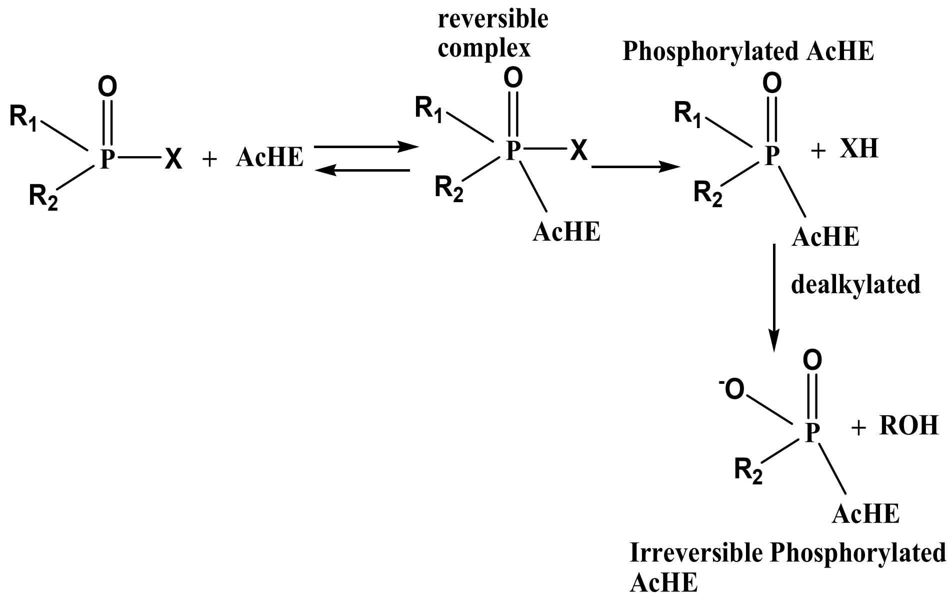
Scheme 1. Chemical synthesis of PAM-2 [5]

The enzyme acetylcholine esterase (AcHE) is found in synaptic membranes, where it degrades through its hydrolytic activity, the neurotransmitter acetylcholine, producing choline and acetate as shown in Scheme 2.



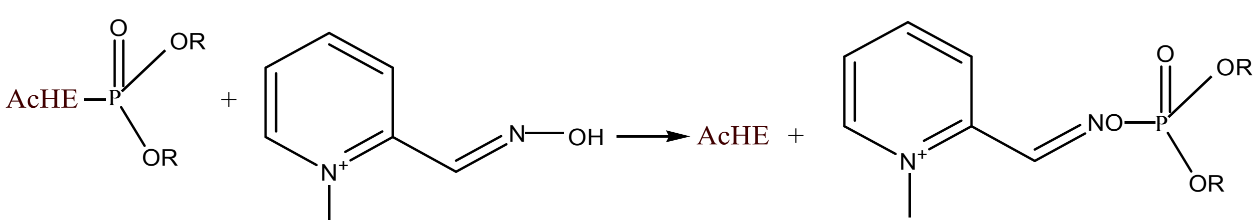
Scheme 2. How the positively charged nitrogen in the acetylcholine molecule is attracted to the ionic site on acetylcholinesterase, and hydrolysis is catalyzed at the esteric site to form choline and acetic acid [6]

This reaction is important for the regulation of synaptic activity in the central and peripheral neural system. Organophosphate pesticides block the function of AcHE, causing the accumulation of excessive acetylcholine in the synaptic cleft. This causes neurotoxic effects such as neuromuscular paralysis (i.e. continuous muscle contraction) throughout the entire body [7]. An organophosphate inhibits AcHE by forming covalent bond between OP and the active site of AcHE as shown in Scheme 3.



Scheme 3. The binding of OP to the active site of AcHE [7]

Pralidoxime is used as an antidote for organophosphate poisoning. The AcHE has two parts to it. An acetylcholine molecule, bound at both ends to both sites of the enzyme, is cleaved in to two, producing acetic acid and choline. Pralidoxime is able to attach to the other half (the unblocked, anionic site) of the AcHE. It then binds to the organophosphate; the organophosphate changes conformation, and loses its binding to the AcHE, see Scheme 4. The conjoined poison/antidote then unbinds from the site, and thus regenerates the enzyme, which is now able to function again.



Schems 4. Reactivation of phosphorylated acetylcholineesterase with PAM-2 through phosphorylated aldoxime formation [8]

Nerve agents or organophosphorus compounds have been manufactured for use in chemical warfare. These agents are known to be present in military stockpiles of several nations. Nerve agents inhibit cholinesterase enzymes in plasma, erythrocytes and at cholinergic nerve endings in tissues. Once tissue cholinesterase is inhibited by the nerve agent, the enzyme cannot hydrolyze the neurotransmitter acetylcholine. Consequently, acetylcholine accumulates and causes prolonged stimulation of the affected tissues.

There is an interrelation between dopamine and acetylcholine. The similarity of the DA and acetylcholine effects could be explained by the fact that dopaminergic and cholinergic neurons serve similar functions and are tightly interconnected in neuronal networks. A major repercussion of this is that it increases the effective acetylcholine/dopamine (ACh/DA) ratio. When this ratio is too high, either by increased acetylcholine (ACh) tone, or decreased dopamine (DA) tone, it can cause problems. Factors which increase acetylcholine tone, such as CI levels and stress, make these problems worse. Factors which increase dopamine tone, such as exercise and positive/empowering life events make these problems better. The following factors likely increase the risk of experiencing negative effects from dietary and/or environmental CI intake. Possible reasons could include age (levels are lower in small children and the elderly), poor nutritional status, or impaired liver function [9]. The principle action PAM-2 is to reactivate cholinesterase enzyme whose active sites are blocked by organophosphate pesticide or nerve gases [8]. The regeneration or reactivation of the AChE allows hydrolyze acetylcholine neurotransmitter at the synapse. Therefore, it is very important to investigate the effect of PAM-2 onto dopamine and vice versa. This interrelation between DA and PAM-2 drives us to investigate those using analytical method.

1.1. Objective of the study

1.1.1. General objective

The general objective of this study is to develop a voltammetric method for the simultaneous determination of dopamine and pyridine-2-aldoxime methochloride (PAM-2) in biological sample.

1.1.2. Specific objectives

To characterize the electrochemical behavior of DA and PAM-2 at the PEDOT modified glassy carbon electrode,

To optimize of the conditions of the PEDOT modified electrode in the simultaneous determination of DA and PAM-2,

To examine the potential interfering substances for the simultaneous determination of DA and PAM-2 at the PEDOT modified glassy carbon electrode, and

To apply the PEDOT modified electrode for the simultaneous determination of DA and PAM-2 in human blood serum.

2. Literature reviews

Several detection methods have been developed in the literature to determine DA such as high performance liquid chromatography (HPLC)[10], capillary electrophoresis[11], fluorimetry[12], chemilumine-scence[13], UV spectroscopy[14], ion-exchange column chromatography[15] and enzymatic methods[16]. However, HPLC method requires fast sample preparation, prolonged analysis time and expensive material. The enzymatic method is lack of stability and very expensive use of enzyme. Capillary electrophoresis method needs expensive apparatus. Such detection methods are often complicated, very expensive, and suffer from sensitivity, selectivity and reproducibility.

On the other hand, various methods have been also reported for the determination of PAM-2, including high performance liquid chromatography [17]. But the chromatographic methods involve laborious and sophisticated procedures. A capillary zone electrophoresis method for the fast and reliable determination of PAM chloride in serum was described [18] and an isotachophoresis method for the determination of PAM chloride in dosage forms was also reported [19]. These methods are time consuming and require expensive and sophisticated instruments. Besides there was a spectrophotometric method for the determination of PAM chloride in aqueous solutions using Pd (II) as the analytical reagent [20]. However, spectroscopic detection requires complex formation to enhance the sensitivity and selectivity of the technique which hampers its suitability for routine analysis. To overcome these problems, electrochemical method is an alternative technique due to its simplicity and selectivity.

Carbon electrodes are commonly employed as a working electrode for electrochemical determination of various analytes of interest. These electrodes are inert material which is useful for oxidation and reduction reactions in both aqueous and non aqueous solutions. Among the various carbon electrodes, glassy carbon electrode has been very popular because of its excellent mechanical and electrical properties, wide potential window, chemical inertness and relatively reproducible performance.

However, electrochemical analysis on the unmodified electrodes, such as glassy carbon has limitations because of the overlapping of redox potentials of analyte species, hence often suffers from fouling effect that results in a poor selectivity and reproducibility [21]. In order to address the problems of unmodified electrode, electrochemical techniques based on chemically modified electrodes have attracted much attention because of their fast response, high sensitivity and selectivity in the determination of trace level of analytes [21].

Conjugated polymers (CPs) have widely been used for surface modification of electrodes. Conjugated polymers have conjugated structures that are characterized by high electrical conductivity. These offer good electrocatalytic behavior, which explains their use as transducers in the preparation of efficient electrochemical sensors [22, 23].

Thin films of CPs can be easily synthesized on the electrode surface by electrochemical methods. Their physicochemical properties strongly depend on the electropolymerization conditions such as solvent type, supporting electrolyte, electrode material, polymerization potential, and electropolymerization method [24].The formation of charge carriers on the conjugated backbone is realized by oxidation (p-doping) or reduction (n-doping) that allows the appearance of a metal-like intrinsic conductivity.

Polymer modified electrodes have been used for the electrochemical studies of DA such as,

β-Cyclodextrin-modified GCE [1], Iron Oxide/Reduced Graphene Oxide Modified Glassy Carbon Electrode [25], indenedione ryptophan with Azure A-interlinked multi-walled carbon nanotube/gold nanoparticles composite modified electrode[26], poly (p-nitrobenzenazo resorcinol) modified glassy carbon electrode [27], Nafion/carbon nanotubes coated poly (3-methylthiophene) modified electrode [28], poly (thionine)-modified GC modified electrodes [29], molecularly imprinted polymer-sensor [30], poly (3, 4 ethylenedioxy) thiophene film modified electrodes [31].

On the other hand, only a few voltammetric methods have been reported for the determination of PAM-2 such as the poly (p-toluene sulfonic acid) modified glassy carbon electrode GCE [32], and multiwalled carbon nanotube modified platinum [33]. Recently our group reported iron doped zeolite modified glassy carbon electrode for the determination of PAM-2 [8].

Polymer-modified electrodes prepared by electropolymerization have received extensive interest in the detection of analytes because of their selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film [34]. Selectivity of the modifier can be attained by different mechanisms such as size exclusion, ion exchange, hydrophobic interaction [35] and electrostatic interaction [36, 37].

Electrochemically active polymers can be classified into several categories based on the mode of charge propagation. The mode of charge propagation is linked to the chemical structure of the polymer. The two main categories are electron-conducting polymers and proton (ion)-conducting polymers. We will focus on electron-conducting polymers here. It can also distinguish between two main classes of electron-conducting polymers based on the mode of electron transport: redox polymers and electronically conducting polymers. Among different CP modifications, the polymer-modified electrode has gained interest in a wide range of areas such as the determination of pesticides [38] and phenolic compounds [39].

In this research, an electron-conducting polymer, poly(3,4- ethylenedioxythiophene), PEDOT is used as a modifier due to its peculiar properties such as high conductivity (600 S/cm), high stability in the oxidized state[40] and easily polymerized electrochemically in the presence of different counter-ions. PEDOT has been used in a wide range of applications, spanning from antistatic and electrically conducting coatings for solar cells [41], photovoltaics [42], fuel cells[43], and actuators[44].

EDOT monomer is electrochemically polymerized at the glassy carbon electrode. The electropolymerization is generally achieved by potentiostatic, galvanostatic or potentiodynamic methods. Potentiodynamic techniques using cyclic voltammetry have been mainly used to obtain qualitative information about the oxidation and reduction processes involved in the early stages of the polymerization reaction and examine the electrochemical behavior of the polymer film after electrodeposition as shown in Figure 2.

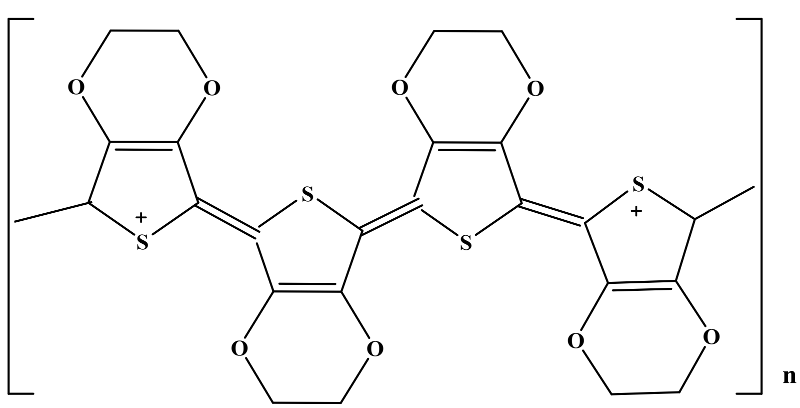


Figure 2. The chemical structure of doped PEDOT

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Recently reported the voltammetric determination of dopamine using PEDOT modified GCE [31]. The modified electrode has been found to show much better results compared to bare GCE and has a lot of potential applications for electroanalytical investigations.

Therefore, it is very important to develop simple and rapid methods for the simultaneous determination of dopamine and PAM-2 in routine analysis. Although these above reviewed modified electrodes demonstrated good sensitivity, selectivity and low detection limit, none of them has been applied for the simultaneous determination of DA and PAM.

To our knowledge, no work has so far been reported on the simultaneous determination of DA and PAM-2 based on PEDOT modified electrodes. The present study reports the preparation of PEDOT modified GCE and its application for the simultaneous determination of DA and PAM-2. The method offers well resolved voltammetric responses for DA and PAM-2. The response for DA and PAM-2 in the presence of other interferences, such as ascorbic acid and uric acid has also been investigated. The PEDOT modified electrode was applied for the determination of DA and PAM-2 in biological samples.

2.1. Electrochemical Techniques

An electroanalytical technique encompasses a group of quantitative analytical methods that are based upon the electrical properties of a solution of the analyte when it is made a part of an electrochemical cell. These techniques are capable of producing exceptionally wealth of characterization information describing electrochemical addressable systems. Electrochemical techniques are powerful and versatile analytical techniques that offer accuracy and precision as well as a large linear dynamic range [45].

Electroanalytical measurements offer a number of important benefits such as selectivity resulting from the choice of electrode material, high sensitivity and low detection limit, possibility of furnishing real time measurement, and application as miniaturized sensors where other sensors may not be useful. The principal criterion for electroanalytical measurements is that the species, which is desired to be measured, should be electroactive, meaning it should undergo a redox reaction at or be adsorbed onto the electrode. Electrochemical measurements can only be carried out in situations in which the medium among the three electrodes making up the electrical circuit is sufficiently conducting. Electroanalytical methods are well established and use relatively inexpensive equipment to produce unique characterization information for molecules and chemical systems: qualitative and quantitative analytical data, thermodynamic data and kinetic data.

2.1.1.Voltammetry

Voltammetry is one type of electroanalytical techniques in which the current potential behavior at an electrode surface is measured. The potential is varied in some systematic manner to cause oxidation or reduction of the electroactive chemical species at the electrode. The resultant current is proportional to the concentration of the electrochemical species.

A three electrode system is used, which includes a working electrode, at which the oxidation or reduction process of interest occurs, a reference electrode, such as the SCE (Saturated Calomel Reference Electrode) or silver-silver chloride electrode and an auxiliary or counter electrode, which carries the bulk of the current. The three electrodes are connected to the power source, which is a specially designed circuit for precise control of the potential applied to the working electrode and often called a potentiostat.

Voltammetric technique is based on the measurement of the current flowing through the working electrode dipped in a solution containing electro active compounds, while a potential scanning is imposed on it. The working electrode could be made with several materials. Usually, it has a very small surface in order to quickly and accurately assumes the potential imposed by the electrical circuit. The electrode can be solid (gold, platinum or carbon) or liquid electrode like mercury drop electrode. In voltammetry, the current corresponding to the quantity of material transported to the electrode and reacting at the electrode surface is usually measured. This current is proportional to the concentration of the electroactive component present in the test solution [46].

2.1.1.1. Cyclic voltammetry (CV)

Cyclic voltammetry is the most versatile electroanalytical techniques for the study of electroactive species. Its versatility combined with ease of measurement has resulted in extensive use of cyclic voltammetry in the field of electrochemistry, inorganic chemistry, and biochemistry. And also cyclic voltammetry has the ability to rapidly provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron transfer reactions, and on coupled chemical or adsorption processes [47].

It offers a rapid location of redox potentials of the electroactive species and convenient evaluation of the effect of solvent upon the redox process. The potential of the working electrode is controlled versus a reference electrode. The resulting triangular waveform is shown in Figure 3a. When the electrode reaction is reversible, the cyclic voltammorgram exhibits peaks on the forward and reverse scans. The parameters of importance are the anodic peak current (i_{pa}), cathodic peak current (i_{pc}), anodic peak potential E_{pa}) and cathodic peak potentials (E_{pc}).

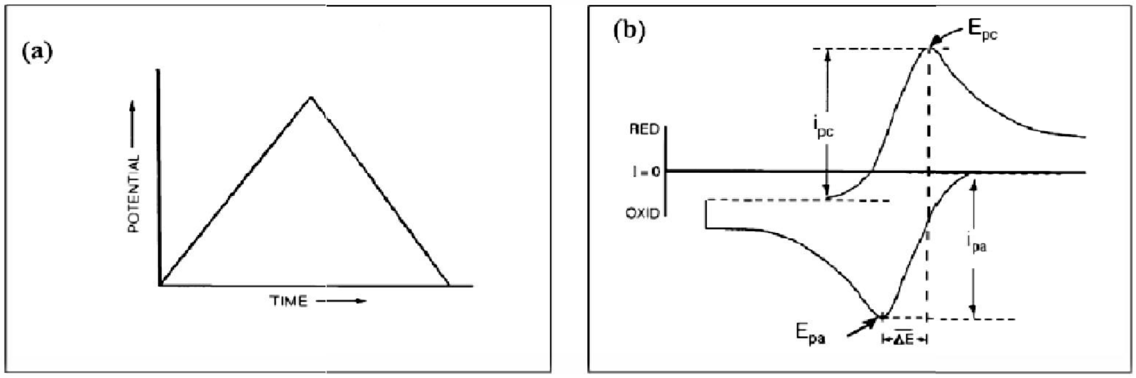


Figure 3. a) Excitation waveform and b) response obtained for the reversible by cyclic voltammetry [47]

A cyclic voltammogram can quickly show the presence of all species that undergo oxidation reduction reactions at the working electrode within the limits set by the solvent electrolysis.

A redox couple in which both species rapidly exchange electrons with working electrode is termed an electrochemically reversible couple. The formal potential E_0 for a reversible is centered between E_{pa} and E_{pc}

$$E_0 = \frac{(E_{pa} + E_{pc})}{2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

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

$$\Delta E = E_{pa} - E_{pc} = \frac{0.059}{n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The peak current for a reversible system is described by the Randles-Sevcik equation for the forward sweep of the first cycle.

$$i_P = 2.69 \times 10^5 n^{\frac{3}{2}} ACD^{\frac{1}{2}} \upsilon^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where i_P is the peak current, n is the electron stoichiometry, A is the electrode area (cm^2), D is the diffusion coefficient ($\frac{cm^2}{s}$), C is the concentration ($\frac{mol}{cm^3}$), and υ is the scan rate ($\frac{V}{s}$). Accordingly, for reversible electrode reaction the ratio of i_{pa} to that of i_{pc} is one. The difference in peak potential for two electron transfer process is 29 mV. i_{pa} Increases with $\upsilon^{\frac{1}{2}}$ and is directly proportional to concentration. For an irreversible process, that is slow (sluggish) electron transfer at the electrode surface, the ratio of i_{pa} to that of i_{pc} is greater than one. The peak current is given by:

$$i_P = 2.69\times10^5 n(na\alpha)^{\frac{1}{2}} ACD^{\frac{1}{2}}\upsilon^{\frac{1}{2}} \qquad (4)$$

Where na is the number of electrons in the rate determining steps, and α is transfer coefficient. All other quantities have the same meaning as equation (3). In the case of a totally irreversible reaction only the peaks on the forward scan is observed. Irreversibility is due to slow electron transfer rate and results in ΔE_P greater than 70 mV for one electron reaction.

For the study of the reaction mechanism of electroactive species the relation of peak current and scan rate is related based on the power law as the equation (5). The slope from the plot of logarithm of peak current versus logarithm of scan rate indicates the electrochemical reaction at the electrode either diffusion controlled (if the value close to 0.50) or adsorption controlled process (if the value approach to 1.0) [48].

$$i = a\upsilon^b \qquad (5)$$

Where i is the current and υ is the scan rate and a and b are constants.

2.1.1.2. Square Wave Voltammetry (SWV)

Square wave voltammetry is a type of pulse voltammetry that offers the advantage of speed and high sensitivity. An entire voltammogram is obtained in a few seconds or less. The excitation signal in SWV consists of a symmetrical square wave pulse of amplitude E_{sw} where superimposed on a staircase waveform of step height ΔE_S, where the forward pulse of the square wave coincides with the staircase as shown in Figure 4. The net current, i_{ne}, is obtained by taking the difference between the forward and reverse currents ($i_2 - i_1$) and is centered on the redox potential. The peak height is directly proportional to the concentration of the electroactive species. Excellent sensitivity is achieved from the fact that the net current is larger than either the forward or the reverse components, since it is the difference between them and detection limits as low as 1 x 10^{-8} $molL^{-1}$ are possible [49].

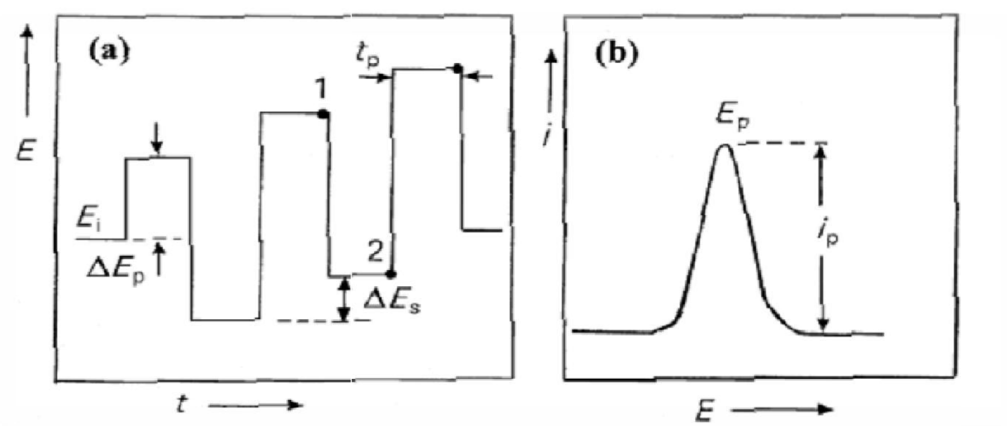


Figure 4. Excitation waveform of square wave voltammetry. b. Response obtained by square wave voltammetry

Square-wave voltammetry has several advantages. Among these are its excellent sensitivity and the rejection of background current. This speed, coupled with computer control and signals averaging, allows for experiments to be performed repetitively and increases the signal to noise ratio. The net peak current for an irreversible system is given by [49].

$$I_p = constat \quad \alpha n^2 \Delta E \Delta E_{SW} \; (fD) \frac{1}{2} C \; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; (6)$$

where ΔE is the step potential, f is square wave frequency, E_{sw} is the square wave amplitude, α is the transfer coefficient, n is the overall electron transfer, C is the bulk concentration, and D is the diffusion coefficient of the electroactive species. The effective scan rate is given by fΔE. Kinetic parameters can also benefit from the rapid scanning and the reversal nature of square wave voltammetry.

Square wave voltammetry possesses the most important features of both pulse techniques and cyclic voltammetry; hence it is one of the most advanced methods in the family of pulse techniques. For this reason, square wave voltammetry is an exceptionally appealing method for electroanalysis. Most important parameters in Square Wave Voltammetry are discussed as follows.

Square Wave Frequency

An increase in square wave frequency results in an increase in the scan rate which in turn increases the peak current [50]. However, at very high frequency, the peak current may become unstable and be obscured by a large residual current. On the other hand very low frequency gives a low but narrow signal, and increases the total analyses time. Hence, the selection of frequency usually requires a compromise among sensitivity, resolution and speed.

Square Wave Amplitude

In square wave voltammetry, the peak currents usually increase with increasing amplitude. However, the width of the peak also increases as the square wave amplitude grows larger and normally one refrains from increasing the square wave amplitude, because resolution may be degraded unacceptably [50].

Square Wave Step Potential

The square wave voltammetric peak current usually increases as the step potential increases with an accompanying peak broadening. At higher step heights, too few points are sampled, thus affecting the reproducibility of the detection [50].

2.1.1.3. Stripping Voltammetry

Stripping voltammetry is a sensitive electroanalytical technique for the determination of trace amounts of metals and organic species in solution. The technique consists of three steps. First, metal ions are deposited onto an electrode which is held at a suitable potential. The solution is stirred during this step to maximize the amount of metal deposited. Second, stirring is stopped so that the solution will become steady. Third, the metal deposits are stripped from the electrode by scanning the potential. The observed current during the stripping step can be related to the amount of the metal in the solution. The stripping step may consist of a positive or a negative potential scan, creating either an anodic or cathodic current respectively.

2.1.1.4. Adsorptive Stripping Voltammetry (AdSV)

In drug analysis, AdSV is popular because of the low limit of determination, its accuracy and precision, as well as the low cost of instrumentation relative to other analytical methods of analysis. For trace analysis of pharmaceutically active organic compounds that cannot be accumulated by electroanalysis another stripping method was proposed: Adsorptive stripping voltammetry (AdSV). In this technique, the analyte is adsorbed on the working electrode by means of a nonelectrolytic process prior to the voltammetric scan. AdSV technique involves the formation, adsorptive accumulation and reduction (potential cathodic scan) of a surface-active complex of the metal.

The principle of AdSV can be compared to the other stripping techniques such as ASV or CSV except that no charge is transferred during the pre-concentration step. Accumulation of the analyte at the electrode surface is performed at open circuit by applying a suitable potential at which no electrochemical reactions occur for set time. After the equilibrium time, the potential is scanned by anodic or cathodic direction depending on the redox properties of the investigated compounds. The adsorptive approach can offer improvements in selectivity or sensitivity for organic compounds and metals that are measurable also by conventional stripping analysis and that exhibit surface active properties. The adsorptive accumulation scheme results in very effective pre-concentration, allowing highly sensitive measurements (about 10^{-10}-10^{-11}M levels) following short adsorption times. To attain such high sensitivity, it is essential to optimize operational variables such as nature of the supporting electrolyte, pH, accumulation potential and time etc favoring strong adsorption [51].

3. Experimental part

3.1. Reagents and apparatus

DA (Sigma, Germany), PAM-2 (Sigma–Aldrich, UK), tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (Sigma–Aldrich, Germany), acetonitrile (Scharlau Chemie, Spain), dipotasium hydrogen phosphate (Techno Pharmchem, India), potassium dihydrogenphosphate (BDH, England), hydrochloric acid (Riedel-deHaen, Germany), sodium hydroxide (BDH, England) Uric acid and ascorbic acid (Aldrich) were used without further purification. Glucose (ARCO) solutions were left to reach mutarotational equilibrium at room temperature for 24 h before use. 3, 4-Ethyleneoxythiophene (EDOT) was distilled repeatedly under vacuum until a colorless liquid was obtained and was kept in the dark. Stock solutions of DA and PAM-2 in phosphate buffer solutions (0.1 $molL^{-1}$ KH_2PO_4 and 0.1 $molL^{-1}$ K_2HPO_4) were prepared by using deionized water. The pH of the phosphate buffer solution was adjusted by adding drops of concentrated hydrochloric acid and sodium hydroxide. The voltammetric experiments were carried out using BASi Epsilon, controlled by a Dell computer with a conventional three-electrode configuration. The PEDOT modified GCE was used as the working electrode, a platinum wire electrode served as the counter electrode with Ag/AgCl/saturated KCl as the reference electrode. The pH of the buffer solutions was measured with a Jenway model 3510 pH meter.

3.2. Preparation of the modified electrode

Prior to modification, the GCE was polished with polished with 0.05 μm alumina on the polishing cloth and rinsed with deionized water in an ultrasonic bath. followed by immersing the polished and dried electrode into a 0.01M EDOT monomer. The electropolymerization of the EDOT monomer at a GCE was made by running successive cycles between -0.20 V and 1.30 V versus Ag/AgCl/saturated KCl at a scan rate of 50 mVs^{-1} .The polymerization was carried out from a non-aqueous solution containing 0.1 $molL^{-1}$ Bu_4NBF_4 in acetonitrile. Finally, the modified electrode was stabilized by running cyclic voltammetry in phosphate buffer solution (pH = 7.0) .

3.3. Human blood serum Sample preparation

The human blood serum was obtained from Ethio tebib hospital, and then the human blood serum sample was transferred to the serum separator vacuum tube with in inserted of gel, and it was kept until the blood sample become clotted as well. The human blood samples were centrifuged at 5000 rpm for 5 min, the serum sample of the blood was separated from the blood cell, and then the serum samples were diluted ten-times with 0.10 $molL^{-1}$ phosphate buffer solution of pH 7.0 and were used without any further treatment [52].

3.4. Electrochemical measurements

The electrochemical behavior of DA and PAM-2 at PEDOT modified GCE was investigated by using cyclic voltammetry. Adsorptive stripping measurements were used for the determination of both analytes by a potential sweep from -0.20 V to 0.80 V. The square wave voltammograms were recorded under quiescent conditions using an optimized step potential 5 mV, amplitude 25 mV and frequency 15 Hz. Prior to each experiment, the modified electrode was regenerated by scanning square wave voltammetry from -0.20 V to 0.80 V in phosphate buffer solution until the peak current for the analyte disappears.

4. Result and discussion

4.1. Electropolymerization of EDOT at GCE

The electropolymerization of the EDOT monomer at a GCE was made by running successive cycles between -0.20 V and 1.30 V vs. Ag/AgCl/ saturated KCl at a scan rate of 50 mV s^{-1} as shown in Figure 5. The polymerization was carried out from a non-aqueous solution containing 0.1 $molL^{-1}$ Bu_4NBF_4 in acetonitrile. The cyclic voltammogram shows the redox peaks that are characteristic for polymer formation [31] and the current increases with each successive cycles, which indicate an enhancement in the film thickness of PEDOT polymer on GC with each cycle. The inset in Figure 5 demonstrates the cyclic voltammogram of the PEDOT modified electrode in the phosphate buffer solution without DA and PAM-2.

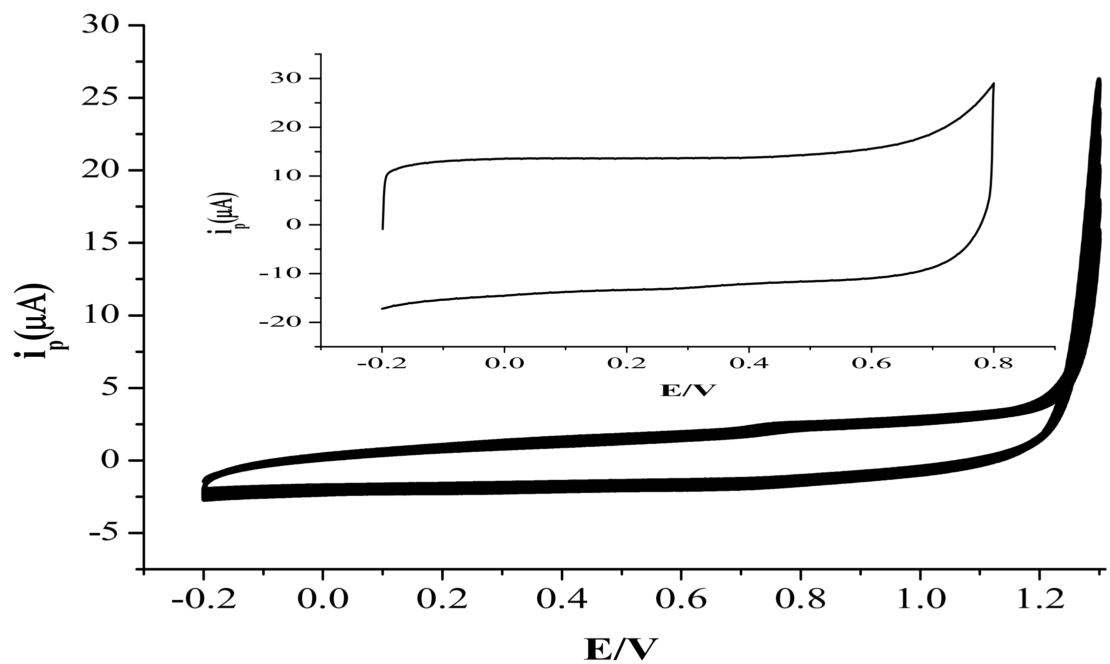


Figure 5. Electropolymerization of 0.01 $mmolL^{-1}$ EDOT at GCE at scan rate 50 mVs^{-1} and the inset is the PEDOT modified electrode in phosphate buffer solution

4.2. Electrochemical behaviors of DA and PAM-2 on PEDOT modified GCE

In order to investigate the electrocatalytic activity of the PEDOT modified GCE, cyclic voltammetry (CV) experiments were performed in phosphate buffer pH 7.0 containing DA and PAM-2 as shown in Figure 6. The oxidation and reduction peak potentials of DA at the modified electrode appeared at 180 and 150 mV, respectively, at a scan rate of 50 mVs^{-1}. The peak-to-peak potential separation is lowered to 30 mV for the modified electrode (as shown in Figure 6d) compared to 210 mV for the bare GC electrode (Figure 6b). The anodic and cathodic peak potentials for 0.05 $mmolL^{-1}$ DA at the modified electrode are independent of scan rate increment, indicating a reversible reaction behavior.

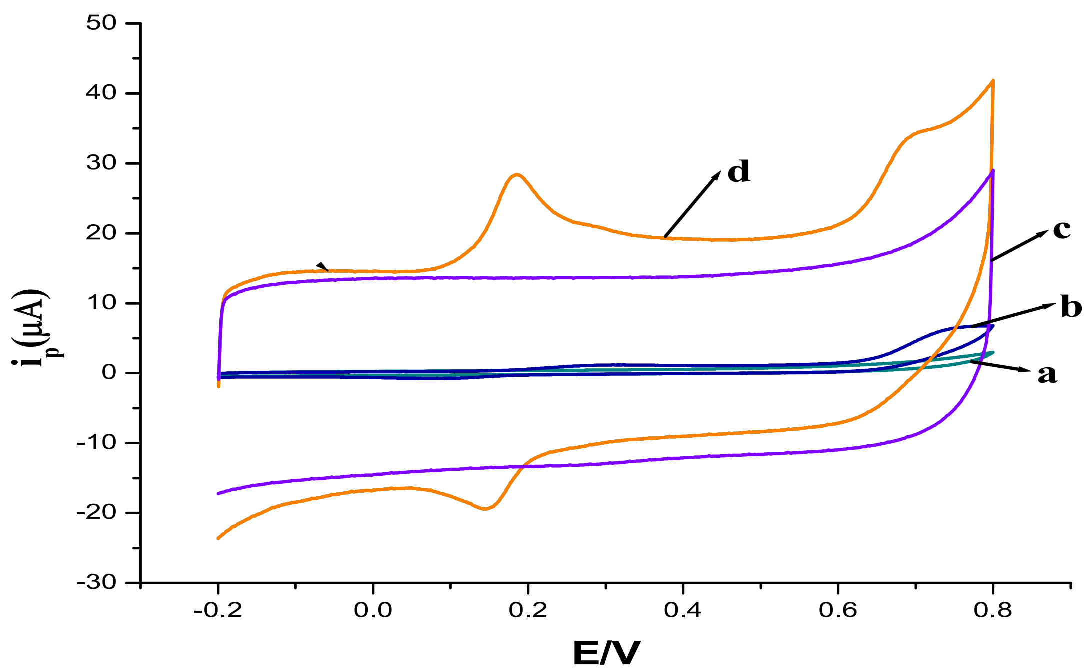


Figure 6. CV of bare GCE and PEDOT modified GCE in pH 7.0 phosphate buffer solution at a scan rate of 50 mVs^{-1}. (a) and (c) are the bare GC and PEDOT modified GCE in phosphate buffer solution without analyte respectively. And also (b) and (d) are bare GC and PEDOT-modified GCE in a mixture of DA (0.05 mmol L^{-1}) and PAM-2 (0.5 mmol L^{-1}) respectively

The electrochemical response for 0.5 $mmolL^{-1}$ PAM-2 in both bare and modified electrode shows anodic peak at 705 mV vs. Ag/AgCl (saturated KCl) but not a reverse peak, indicating that it exhibits an irreversible reaction behavior.

The considerable enhancement of the peak currents (9-10 times) for PAM-2, and for DA (20-23 times) at the modified electrode suggest that the PEDOT modified electrode exhibits an excellent electrocatalytic activity for both species compared to the bare GC electrode (as shown in Figure 6). The electrocatalytic effect of the PEDOT modified electrode could be attributed to the excellent behavior of the PEDOT polymer such as high conductivity, strong adsorptive capability, and significant increment in active electrode area [40].

The PEDOT-modified GCE showed two well-defined redox peaks of DA at a formal potential (E^0) of 165 mV, calculated as the midpoint of anodic and cathodic peak potentials. The anodic potential difference between PAM-2 and DA was found to be 525 mV. Hence, the simultaneous determination of PAM-2 and DA at the PEDOT modified GCE in phosphate buffer is possible.

4.3. Effect of Scan Rate

The effect of scan rate on the anodic peak current of DA and PAM-2 at PEDOT modified GCE was studied. The influence of scan rate on the electrochemical response for PAM-2 was studied by cyclic voltammetry. The logarithm of the peak currents (log i_p) linearly increases with the logarithm of scan rates (log ν) in the range of 25–150 mV s^{-1}, with a linear regression equation: $\log i_p = 0.01 + 0.57 \log \nu$ and R = 0.998. The slope of the linear equation (0.57) is close to the ideal value 0.50, expected to be diffusion controlled process [48].

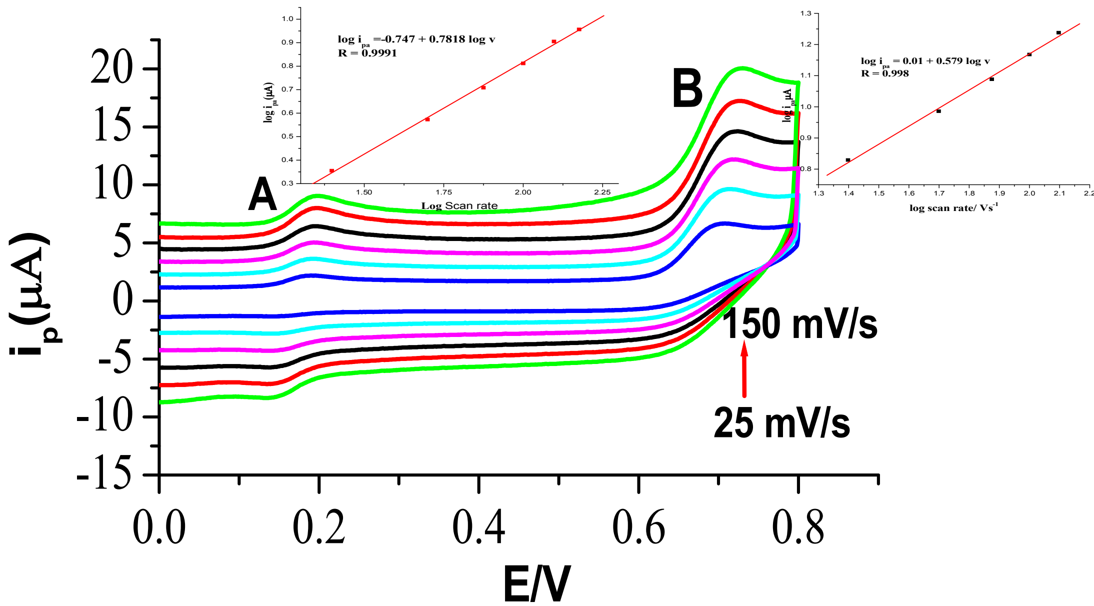


Figure 7. CVs of the PEDOT modified GCE for DA (0.05 $mmolL^{-1}$) and PAM-2(0.5 $mmolL^{-1}$) in 0.1 $molL^{-1}$ phosphate buffer solution (pH 7.0) at scan rates from 25 to 150 mV s^{-1}. The Inset plots of the log of peak currents of (A) DA and (B) PAM-2 vs. log of scan rates

On the other hand, the effect of the scan rate on the peak currents of DA was also investigated in 0.1 M PBS pH 7.0 containing 0.05 $mmolL^{-1}$ DA. The anodic currents increased with increasing the scan rates. The logarithm of peak current was linear with the logarithm of the scan rate over the range of 25–150 mV/s and the linear regression equations were log I_{pa}= −0 .747 + 0.78 log υ and R = 0.9991. These results close to the theoretical value of 1 that indicated the anodic process is dominated by a complete adsorption control process PEDOT modified GCE [48].

4.4. Effect of pH

The cyclic voltammetric responses for PAM-2 (0.5µ$molL^{-1}$) and DA (0.5$mmolL^{-1}$) at the PEDOT-modified GCE were studied in the pH range 5.5–8.0, using 0.1 M phosphate buffer at scan rate of 50 mVs^{-1}, in order to evaluate the ratio of electrons and protons and to obtain the optimum pH value involved in the oxidation of PAM-2 and DA.

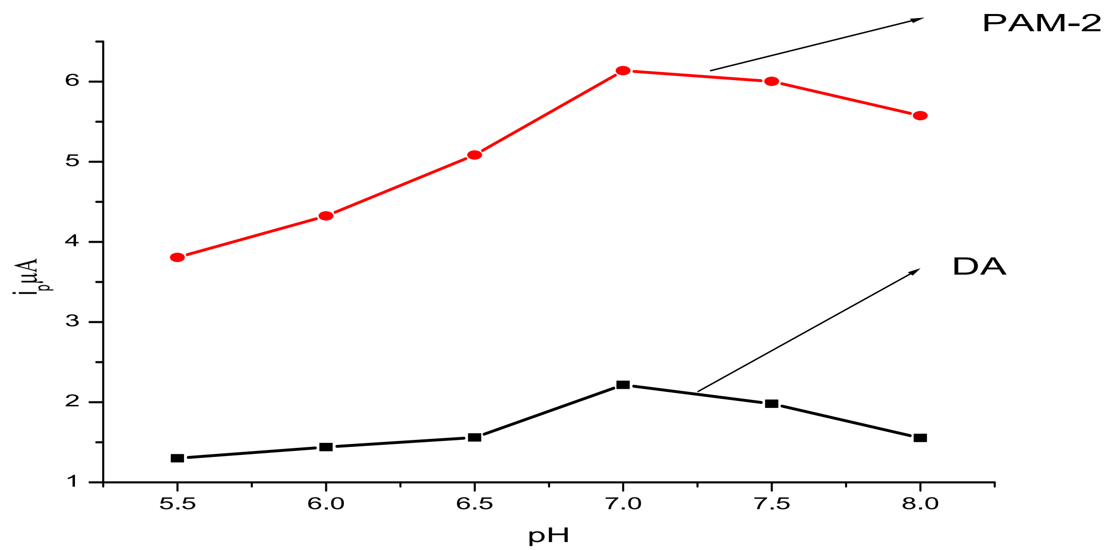


Figure 8. The effect of pH on the anodic peak current of DA and PAM-2

As can be seen in Figure 9 the CV recorded at different pH values. For both analyte, the highest anodic peak current response was observed at about pH 7.0 of phosphate buffer solution, Figure 8 shows that the anodic peak current increased with increasing solution pH until it reached 7. The anodic peak current decreased when the pH > 7. The anodic peak current of DA and PAM-2 were increased with the increase of pH over the range 5.5–7. Then the protonated degree of DA decreased with the increasing pH, because hydroxy group of DA and PAM-2 were easier to be oxidized in alkali. The anodic peak current of DA decreased with the increase of pH over the range 7–8. Since pH 7.0 is the physiological pH value, it was chosen for the electrochemical detection of DA. The anodic peak potential for DA and PAM-2 shift negatively as the pH increases, indicating that proton takes part in the electrode reaction. Hence pH 7 is selected as the optimum pH for both DA and PAM-2 in the subsequent experiments. And also the relation between anodic peak potential and pH of DA and PAM-2 were studied.

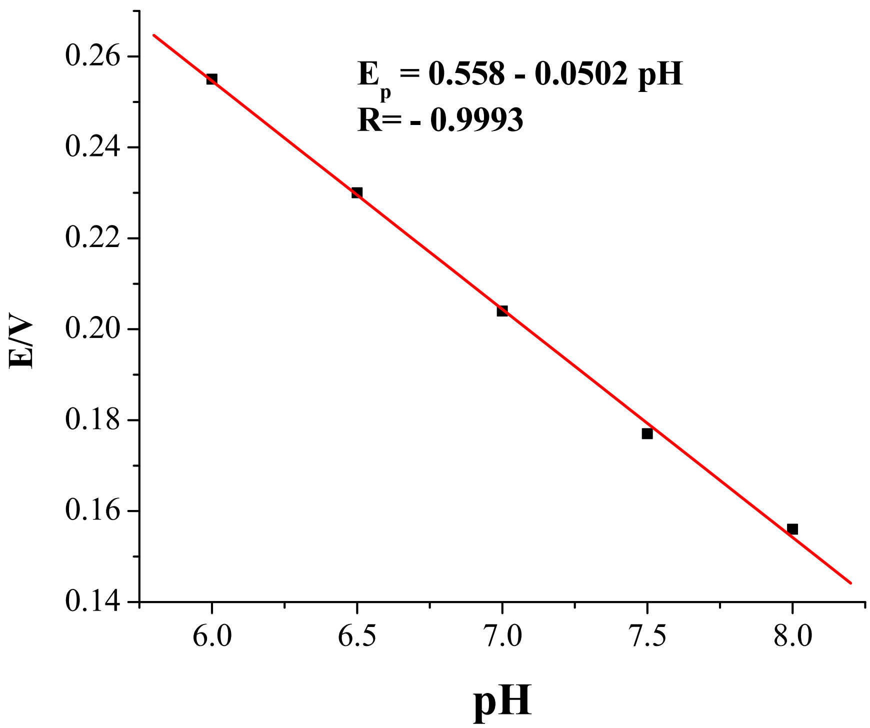
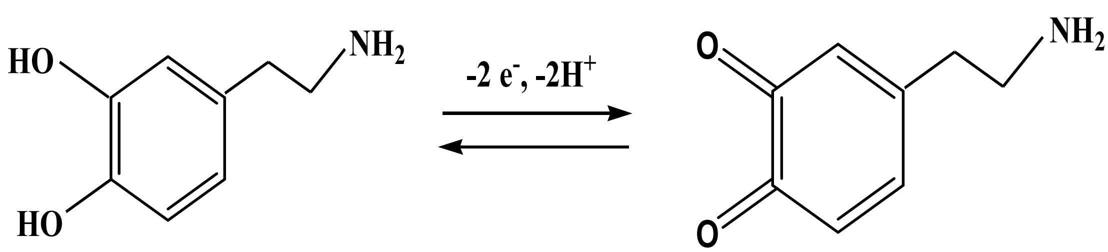


Figure 9. Plot of peak potential versus pH for DA

The variation of peak potential with pH for DA (from 6.0 to 8.0) is depicted in Figure. 9. The equation relating E_{pa} with pH is given by E_{pa}= 0.558−0.050 pH with a linear correlation coefficient -0.9993 for DA The slope of –50.2 mV/pH for DA is close to the theoretical value of –59 mV/pH, suggesting that the electron transfer step is preceded by a protonation with an equal number of protons and electrons, and the reaction mechanism of DA is was demonstreated in scheme 5.



Schems 5. Mechanism of DA oxidation at the PEDOT modified GCE [37]

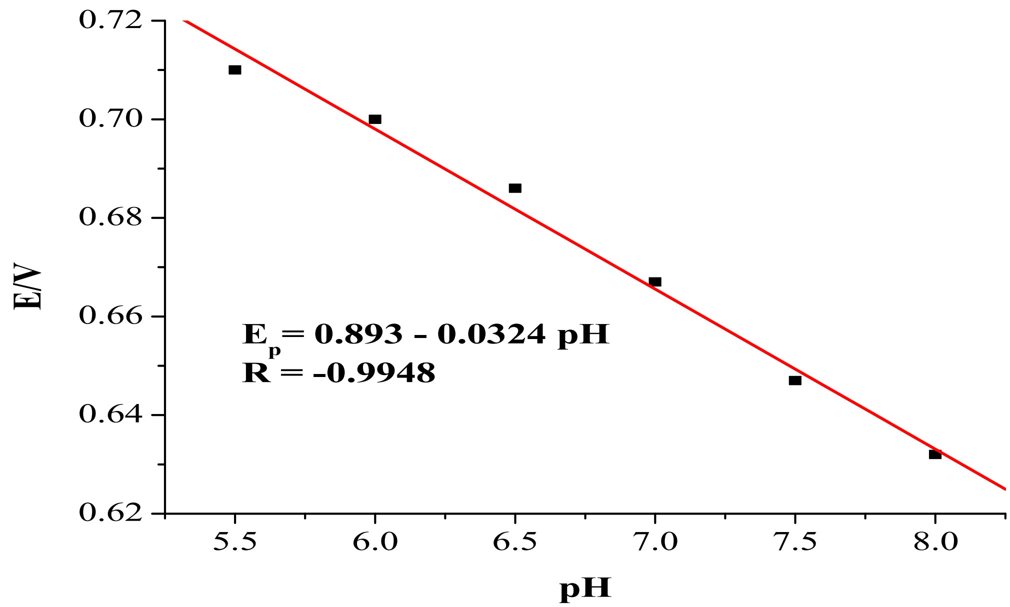
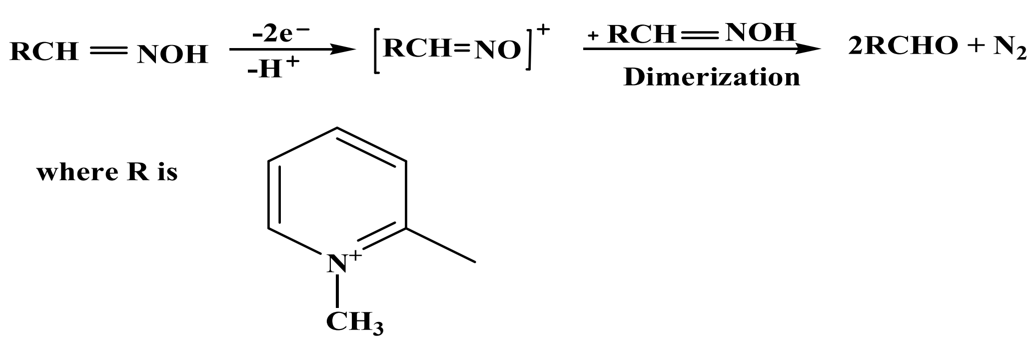


Figure 10. Plot of anodic peak potential versus pH for PAM-2

The variation of peak potential with pH (from 5.5 to 8.0) is depicted in the Figure. 10. The peak potentials linearly change with pH according to the equation: $E_p = 0.895 - 0.0324pH$ with a linear correlation coefficient = -0.9948. The slope 32.4 mV per unit pH increase indicates that the electrochemical reaction involves unequal electrons and protons. The result is consistent with the literature reports [8] and the reaction of PAM-2 at the modified electrode is demonstrated in scheme 6.



Schems 6. Mechanism of PAM-2 oxidation at the PEDOT modified GCE [8]

4.5. Optimization of deposition time and accumulation potential for PAM-2

The voltammetric responses for 0.5mmolL^{-1} PAM-2 at the PEDOT-modified GCE was studied in the time range 30 s–210 s.

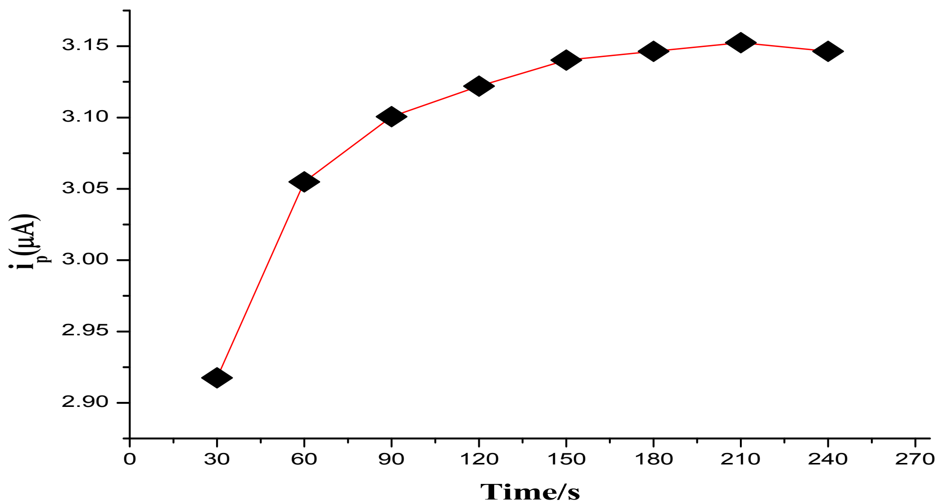


Figure 11. The effect of time of deposition on the anodic peak current

As can be seen in the Figure 11, the square wave voltammograms were recorded at different time values. The square wave voltammograms were recorded with increasing deposition times. The peak current increased up to 150 s and then there was no significant change in the voltammetric peak up to 210 s. Hence, 150 s deposition time was used as the optimum time for subsequent experiments. And also the voltammetric responses for 0.5 mmolL^{-1} PAM-2 at the PEDOT-modified GCE was studied in the accumulation potential range of -50 mV – 700 mV.

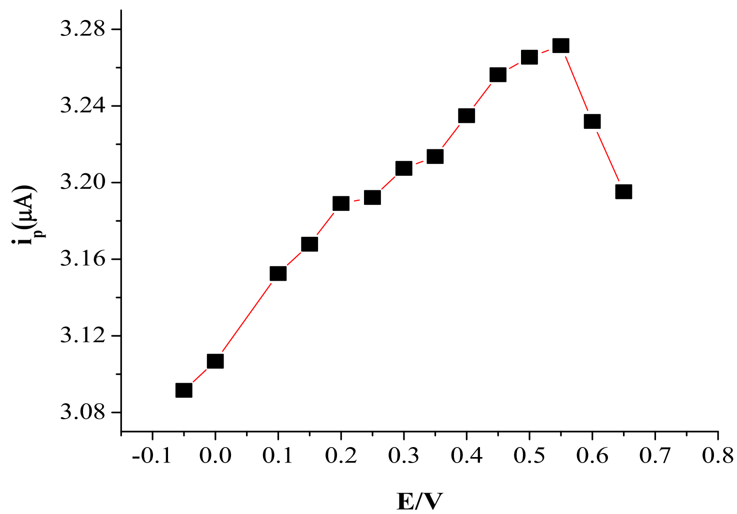
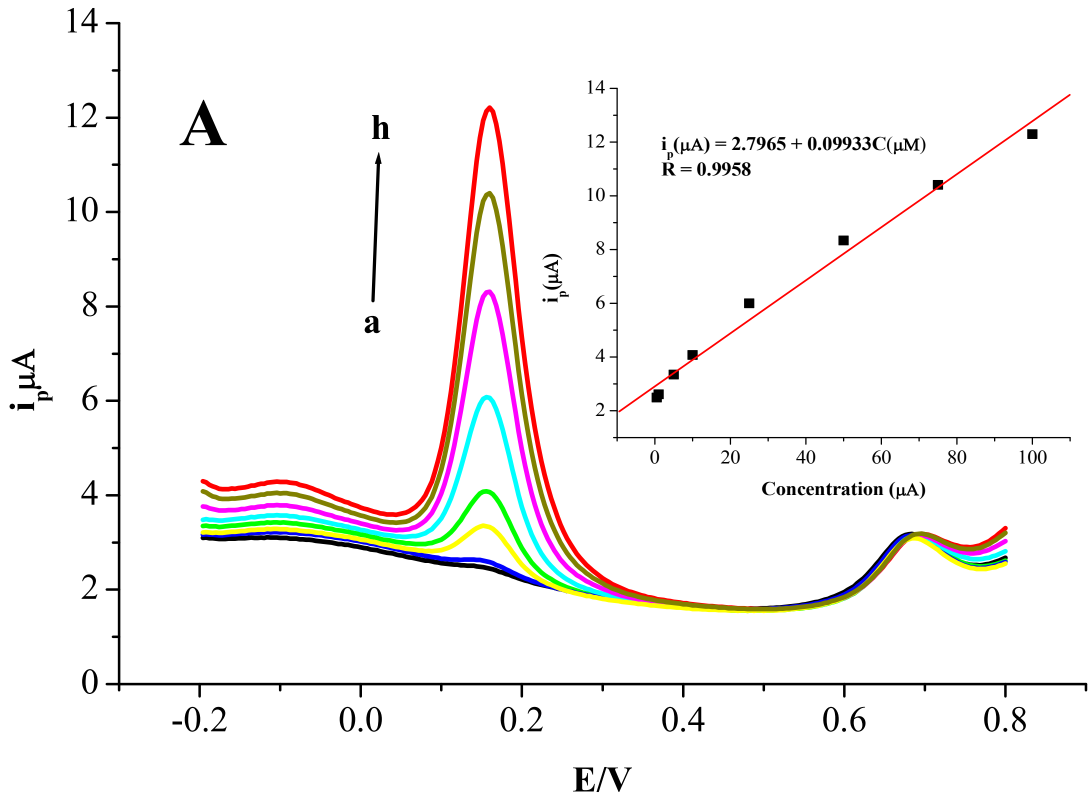


Figure 12. The effect of accumulation potential on the anodic peak current of PAM-2

As can be seen in the Figure 12 the square wave voltammograms were recorded at different accumulation potential values. The square wave voltammograms were recorded with increasing accumulation potential. The effect of accumulation potential on the response of PAM-2 was investigated in the range -50 mV – 700 mV. Figure 13 shows that the anodic peak current increased with increasing deposition potential until it reached 550 mV. The anodic peak current decreased when the deposition potential was greater than 550 mV, which was used for subsequent experiments.

4.6. Analytical performances

In order to increase the sensitivity, SWAdV was selected to examine the analytical performance of the PEDOT modified GCE for the simultaneous determination of DA and PAM-2. As we can see in Figure 13 (A) and (B) show well defined peaks appeared at 157 mV (for DA) and 682 mV (for PAM-2) in the voltammograms; the peak-to-peak potential separation is 525 mV, which is very sufficient to determine the two species simultaneously. The performance of the PEDOT-modified GCE for DA and PAM-2 was studied under the optimized conditions. Figure 13 (A) and (B) shows the SWAdSV responses for the DA and PAM-2 mixture prepared by varying the concentration of either one of the analyte while keeping the other constant.


A
h
a
i_p μA
E/V
i_p(μA) = 2.7965 + 0.09933C(μM)
R = 0.9958
i_p(μA)
Concentration (μA)

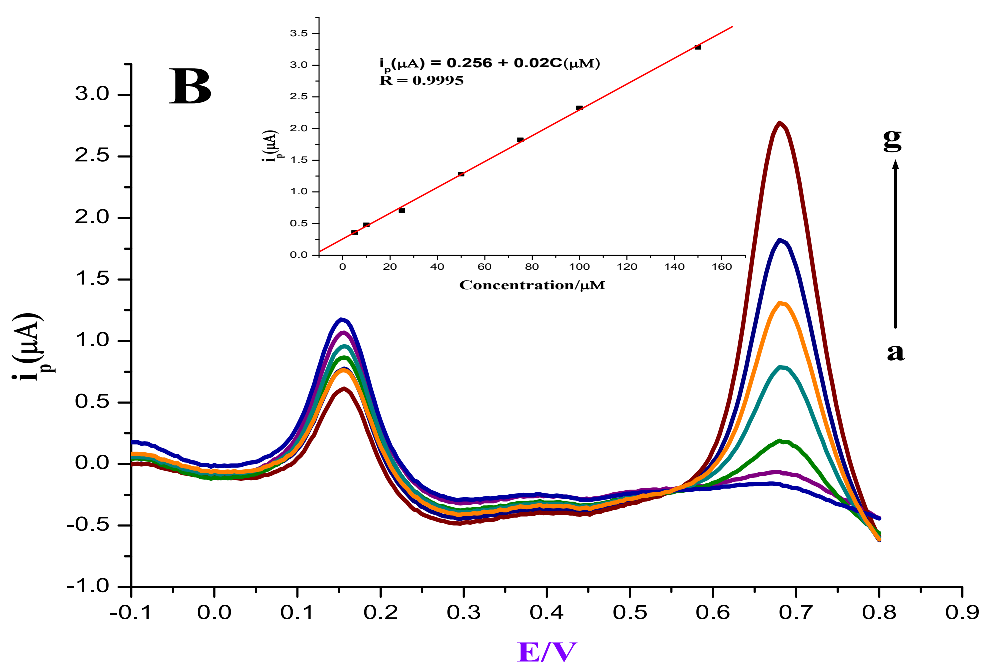

B
g
a
i_p(μA)
E/V
i_p(μA) = 0.256 + 0.02C(μM)
R = 0.9995
Concentration/μM

Figure 13. (A) (SWAdSV) peaks at the PEDOT modified GCE in the presence of PAM-2 (50 μmol L^{-1}) for different concentrations of DA: (a) 0.5, (b) 1, (c) 5, (d) 10, (e) 25, (f) 50, (g) 75 and (h) 100 μmol L^{-1}. (B) SWAdSV of the modified electrode in the presence of DA (5.0 μmol L^{-1}) for different concentrations of PAM-2 (from a to h): (a) 5, (b) 10, (c) 25, (d) 50, (e) 75, (f) 100 and (g) 150 μmolL^{-1}. (For (B) background was subtracted). (The inset shows (A) calibration graph of concentration versus anodic peak current of DA and (B) calibration graph of concentration versus anodic peak current of PAM-2)

The anodic current is linearly related to the DA concentration (0.5–100 μmolL^{-1}) in the presence of 50 μmolL^{-1} PAM-2 (inset Figure 13 (A) and (B). The linear regression equation was i_{pa} (μA) = 2.7965 + 0.0993C (μmolL^{-1}), with a linear correlation coefficient (R) of 0.9958. Similarly, the i_{pa} increases linearly with increasing concentration of PAM-2 (5.0–150 μmolL^{-1}) in the presence of 5.0 μmolL^{-1} DA (inset of Figure 13 (B)). The linear regression equation was i_{pa} (μA) = 0.268 + 0.02C μmolL^{-1}), with a linear correlation coefficient (R) of 0.9995. The calibration plots of the modified electrode for DA with and without PAM-2 show similar sensitivity, indicating the two species do not interfere in the electrochemical response of each other. The reproducibility of three individual PEDOT-modified GCEs for the responses of 75 μmol L^{-1} PAM-2 and 50 μmolL^{-1} DA was evaluated and the relative standard deviations (RSD) obtained were 0.451% and 2.692%, respectively. The RSD for five successive determinations of 75 μmol L^{-1} PAM-2 and 50 μmolL^{-1} DA at the modified electrode were 0.115% and 1.557%, respectively, which demonstrate a very good repeatability of the three performance of the modified electrode.

The comparison of various modified GCE with the present study of PEDOT modified GCE is demonstrated in Table 1. The detection limit for DA was found to be 3.10×10^{-8} μmolL^{-1} based on signal to noise ratio of 3, which is lower than that of Nafion/carbon nanotubes coated poly (3-methylthiophene) modified electrode (5×10^{-9}μmolL^{-1}) [28] and it is comparable with indenedione ryptophan with Azure A-interlinked multi-walled carbon nanotube/gold nanoparticles composite modified electrode (1.4×10^{-8}) [26] μmolL^{-1}) but better than poly (p-nitrobenzenazo resorcinol) modified glassy carbon electrode (3×10^{-7}μmolL^{-1}) [27], Iron Oxide/Reduced Graphene Oxide Modified Glassy Carbon Electrode (1.2×10^{-7} μmol L^{-1}) [25] and β-cyclodextrin GCE (1.5×10^{-6} μmol L^{-1}) [1].

Table 1. Comparison of analytical performance of several modified electrodes for DA and PAM-2 determinations.

Species	Modified electrodes	Method	Linear range (μM)	Detection limit(μM)	Ref.
DA	β-cyclodextrin GCE	DPV	10—170	1.5	[1]
	Fe3O4/rGO/GCE	DPV	0.5 – 100	0.12	[25]
	Nafion/AuNPs/AzA/ MWCNTs	DPV	0.5–50	0.014	[26]
	NBAR	DPV	5–20	0.3	[27]
	Nafion/NTs/P3MT	DPV	1–6	0.005	[28]
	PEDOT MGCE	SWAdSV	0.5–100	0.031	This work
PAM-2	(Fe^{3+}Y) modified GCE	SWASV	0.5–100	0.161	[8]
	p-TSA MGCE	SWV	0.1–1000	0.03	[32]
	CNT modified GCE	DPV	1-1000	0.3	[33]
	PEDOT MGCE	SWAdSV	5–150	0.191	This work

On the other hand, the detection limit for PAM-2 was 1.91×10^{-7} μmolL^{-1} based on signal to noise ratio of 3, which is comparable to that obtained with the carbon nanotube modified GCE (3.0×10^{-7} μmolL^{-1}) [33] and zeolite modified electrode 1.61×10^{-7} μmolL^{-1} [8] but larger than of the modified electrode at the poly (p-toluene sulfonic acid) (p-TSA) modified GCE (3.0×10^{-8} μmolL^{-1}) [32].

4.7. Interference study

The selectivity of the PEDOT modified GCE was studied in the presence of potential interfering species. Possible interfering substances that could exist in human blood serum samples such as ascorbic acid, uric acid and glucose were studied. The interferences of ascorbic acid, uric acid and glucose, in the determination of DA and PAM-2 at the modified electrode were investigated by running Square Wave adsorptive stripping voltammetry. Ascorbic acid and glucose did not exhibit any peak in the working potential window while uric acid (UA) shows a peak in the working potential but it did not overlap with the peaks of DA and PAM-2 as depicted in Figure 15. Hence, the interference of UA on the determination DA and PAM-2 was negligible.

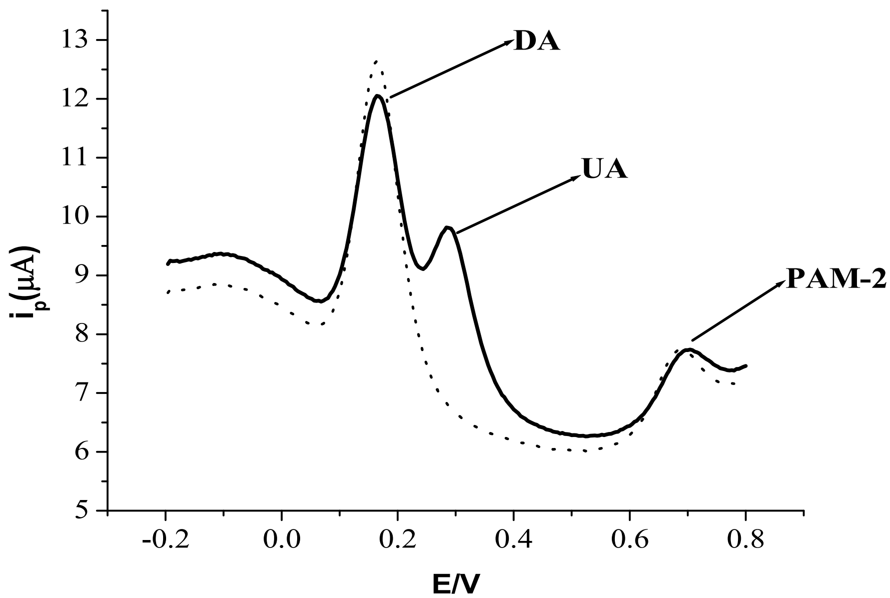


Figure 14. The broken line voltammogram shows the mixture solution of containing 60 μM of each DA and PAM-2 where as the solid line voltammogram shows the mixture solution of containing 60 μM of each DA and PAM-2 and UA

4.8. Analytical application of the modified electrode

The proposed modified electrode was applied for the analysis of human blood serum samples. The serum sample was centrifuged and then 2.5 mL of serum sample was diluted ten times with 0.10 M phosphate buffer solution of pH 7.0 and was used without any further treatment.

The diluted serum sample was spiked with different amounts of DA and PAM-2 by using multiple standard addition method.

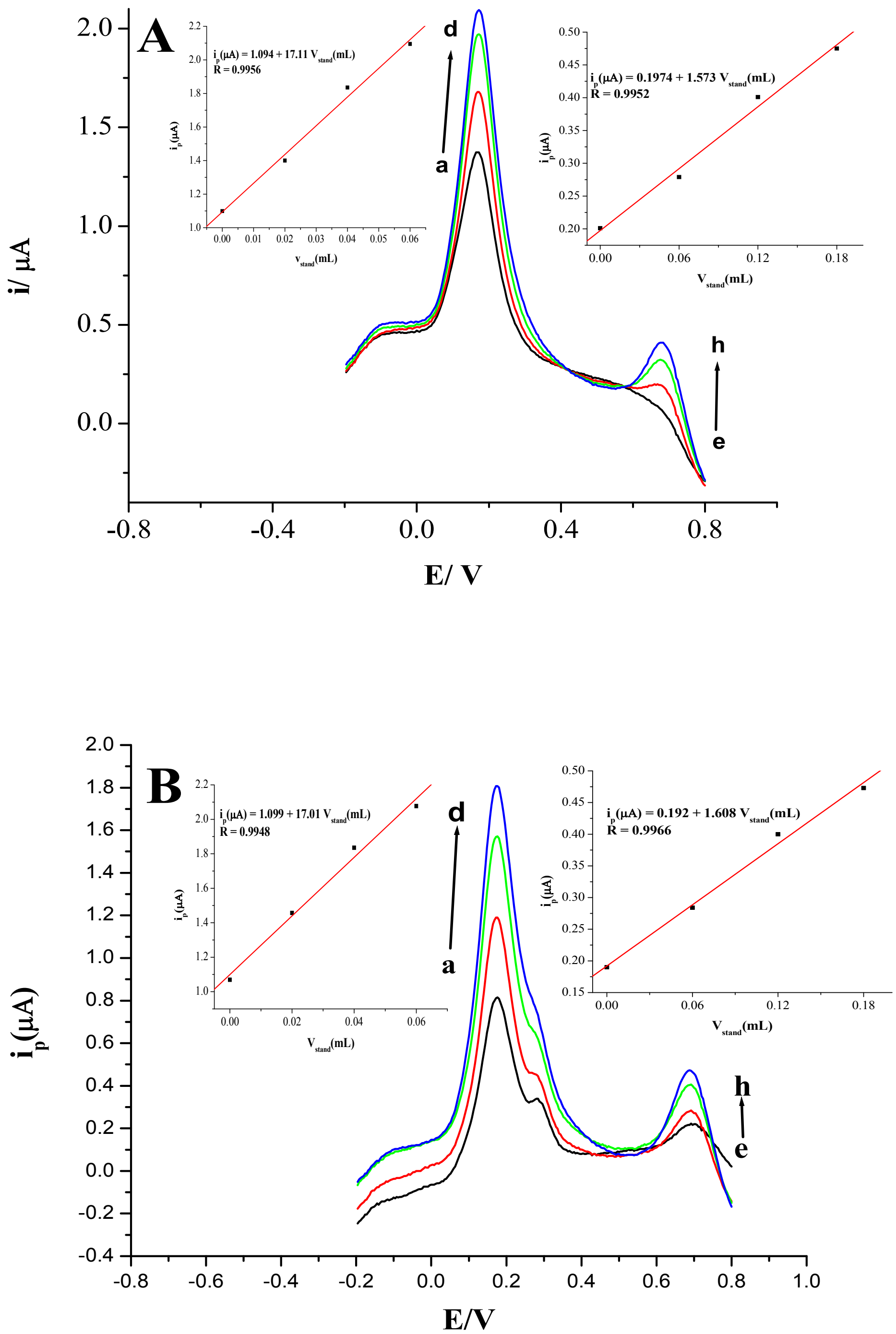


Figure 15. SWAdSV responses for: 8µM DA and 10 µM PAM-2 in 25 mL (A) Phosphate buffer solution and (B) human blood serum sample: (a) sample, spiked with: (b) 20 µL, (c) 40

μL and (d) 60 μL of 5.0 mM DA. And (e) sample, spiked with: (f) 60 μL, (g) 120 μL and (h) 180 μL of 5.0 mM PAM-2(The inset shows (A) calibration graph of volume of standard added of versus anodic peak current of DA and PA M-2 in phosphate buffer solution and (B) calibration graph of volume of standard added versus anodic peak current of PAM-2)

SWAdSVs were obtained by spiking 20, 40 and 60 μL of 5.0 mmolL^{-1} DA, and to the 25 mL of and 25 mL of phosphate buffer solution (pH 7.0) and human blood serum sample; see Figure 15 A and B. The slopes of the plots for the variation of the adsorptive stripping peak current versus the volume of standard added were 17.11μA/mL (linear correlation coefficient (R) = 0.9956) in phosphate buffer solution (inset Figure. 15A) and 17.01μA/mL (linear correlation coefficient (R) = 0.9948) in the human blood serum (as shown in the inset Figure. 15B) indicating that there is no matrix effect blood serum sample in the electrochemical measurements.

On the other hand, adsorptive stripping voltammograms were obtained by spiking 60, 120 and 180 μL of 5.0 mM PAM-2 to the 25 mL of phosphate buffer solution (pH 7.0) and human blood serum sample; as shown in Figure 15 A and B. The slopes of the plots for the variation of the adsorptive stripping peak current versus the volume of standard added were 1.573 μA/mL (linear correlation coefficient (R) = 0.9952) in phosphate buffer solution (inset Figure. 15A) and 1.608 μA/mL (linear correlation coefficient (R) = 0.9966) in the human blood serum (as shown in the inset Figure. 15B).

The results provided a very good recovery for DA and PAM-2 added to the human blood serum sample of 101.57% and 95.17% respectively indicating that the complex matrix in human blood serum did not interfere in the determinations of DA and PAM-2 [36-37].

5. Conclusion

The present study proposes a simple and low-cost PEDOT modified electrode for the simultaneous determination of dopamine and pyridine-2-alidoxime methochiloride. The PEDOT-modified GCE exhibited enhanced electrochemical responses for the simultaneous determination of DA and PAM-2 due to an increase in surface active area of the modified electrode. The high sensitivity, selectivity and very low detection limit (3.1×10^{-8} M) for DA and (1.91×10^{-7} M) for PAM-2 at the modified electrode make the PEDOT-modified electrode very useful for developing a simple electrochemical device. The PEDOT modified electrode is also useful for the simultaneous determination of DA and PAM-2 in biological samples without the interference of coexisting species.

6. References

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