HAWASSA UNIVERSITY

SCHOOL OF POSTGRADUATE STUDIES

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF CHEMISTRY MASTER OF SCIENCE PROGRAM OF ANALYTICAL CHEMISTRY

1,4-BENZOQUINONE MODIFIED CARBON PASTE ELECTRODE FOR VOLTAMMETRIC INVESTIGATIONOF DOPAMINE

BY:

KASSA BEKO

APRIL 2025

HAWASSA, ETHIOPIA

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1,4-BENZOQUINONE MODIFIED CARBON PASTE ELECTRODE FOR VOLTAMMETRIC INVESTIGATION OF DOPAMINE

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIESIN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY (SPECIALIZATION: ANALYTICAL CHEMISTRY)

BY:

KASSA BEKO

ADVISOR: MELAKU ZIGDE (Asst. Prof. of Analytical Chemistry)

APRIL 2025

HAWASSA, ETHIOPIA

DECLARATION

Here I declare that this thesis entitled **"1,4-benzoquinone modified carbon paste electrode for voltammetric investigation of dopamine"** is my own work. Dopamine has been investigated by using different electrodes but it has not been done by 1,4-benzoquinone Modified Carbon Paste Electrode so far and presented in any other university. This work was submitted in partial fulfillment of the requirements for the MSc degree in chemistry with a specialization in Analytical chemistry at Hawassa University. The experimental work is my own work and the collaborative contributions have been clearly identified and dully acknowledged.

Name: Kassa Beko

Signature: ———————————

Date and place of submission: April 2025, Hawassa.

This MSc thesis has been submitted for examination with my approval as university advisor.

Melaku Zigde (Assistant professor)

Signature: ———————————

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APPROVAL SHEET 1

The undersigned hereby certify that they have read and recommend to the College of Natural and Computational Science, School of Graduate Studies for acceptance a MSc thesis entitled"**1,4-benzoquinone modified carbon paste electrode for voltammetric investigation of dopamine"** by Kassa Beko in partial fulfillment of the requirements for the degree of Master of Science in Analytical Chemistry.

Approved by:	Signature	Date
Advisor: Melaku Zigde	_____	_____

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EXAMINERS' APPROVAL SHEET 2

We, the undersigned members of that the Board of Examiners of the final open defense by Kassa Beko have read and evaluated his thesis entitled **"1,4-benzoquinone modified carbon paste electrode for voltammetric investigation of dopamine"**, and examined the candidate. This is, therefore, to certify that the thesis has accepted in partial fulfillment of the requirements for the degree of Masters of Science in Chemistry with a specialization in Analytical Chemistry.

_____	_____	_____
Name of Major Advisor	Signature	Date
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Name of Internal examiner-I	Signature	Date
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Name of Internal examiner-II	Signature	Date
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Name of External Examiner	Signature	Date
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SGS Approval	Signature	Date

Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the School of Graduate Studies (SGS) through the Department/School of Graduate Committee (DGC/SGC) of the candidate's department.

Stamp of SGS Date: _____

ACKNOWLEDGEMENTS

First, I would like to thank my God for his help to complete this study peacefully and to reach to this point in my life after passing difficulties to precede this work.

I would like to express my deepest gratitude to my advisor Mr. Melaku Zigde (Assistant Professor of Analytical Chemistry) for his valuable guidance, consistence encouragement and positive attitude throughout my thesis work.

I would like to thank the Hawassa University,Department of Chemistry, and Hawassa University Academic V/P for helping me to proceed my experimental work in other institution.

I would like to thank Adama science and technology university, (ASTU) for accepting and allowing me to do my experiment, as well as laboratory technician for his guidance during my lab session.

I am grateful thank to my beloved wife Tesfaynesh Mukura for her encouragementsuggestions and her father Kes Mukura Mune, Brother Tucha Tunuka, and Mamush Tunuka for financial support.

Lastly, but not least, I would like than Mr Hamisale Hatiya and his colleagues for supporting me copying and printing necessary copies.

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ABBREVIATIONS

AA	Ascorbic acid
AADC	Aromatic amino acid decarboxylase
ADHA	Attention deficit hyperactivity disorder
BAS	Bioanalytical system
BQMCPE	Benzoquinone modified carbon paste electrode
CMEs	Chemical modified electrodes
CPE	Carbon paste electrode
CV	Cyclic voltammetry
DA	Dopamine
DCE	Dropping carbon electrode
DDC	Dopa decarboxylase
DME	Dropping mercury electrode
DPV	Differential pulse voltammetry
GCE	Glassy carbon electrode
GO	Graphene oxide
HQ	Hydroquinone
L-DOPA	Levorotatorydihydroxyphenylalanine
LOD	Limit of detection
MNPs	Metal nanoparticles
MWCNTs	Multi-walled carbon nanotubes

NPs	Nanoparticles
NE	Norepinephrine
PA	P. nitroaniline
PBS	Phosphate buffer solution
PHMoFBP	1-phenyl-3-methyl-4-orthofluorobenzoyl-5-pyrazolone
rGO	Reduced grapheme oxide
SWCNTs	Single-walled carbon nanotubes
SWV	Square wave voltammetry
TH	Tyrosine hydrolase
TY	Tyrptophan
UA	Uric acid

ABSTRACT

Dopamine (DA) is one of electrochemically active molecule; electrochemical techniques currently have received great interest for their investigation due to their simplicity, cost effective, low detection limit, and fast response time. This study investigates the electrochemical oxidation of dopamine using a low-cost and sensitive voltammetric method based on a 1,4-Benzoquinone modified carbon paste electrode. Cyclic voltammetry revealed significant enhancements in the oxidative peak current for dopamine at the modified electrode compared to unmodified carbon paste electrode, indicating its electrocatalytic properties. Scan rate, pH, differential pulse amplitude, and concentration of dopamine were optimized for the investigation of dopamine. The optimized values were 100 mV/s, 7, and 240mV, respectively. The voltammogram resulted from those parameters showed that quasireversible nature of the analyte towards modified electrode with the transfer of two electrons per molecule of the analyte. The dependence of anodic peak current on the square root of scan rate with high correlation coefficients (R^2=0.99907 and scan rate (R^2=0.99103) indicated that, the diffusion control is dominant, but adsorption also plays a role. Key kinetic parameters were calculated: anodic transfer coefficient (α =0.798), diffusion coefficient (D=2.18×10^{-7} cm^2/s), and heterogeneous electron transfer rate constant (Ks,h=2.26×10^{-4} cm/s). Anodic transfer coefficient (α =0.798) value is closer to one this suggests that anodic process is more favorable. Optimization of experimental conditions led to be a linear response for DA concentration ranging from 1.0 × 10^{-3} M to 3.5 × 10^{-3} M, with a detection limit of 1.39 × 10^{-4} M using cyclic voltammetry and 7.64 × 10^{-5} M with differential pulse voltammetry. The result suggests that 1,4-BQMCPE is a promising electrode material for the development of electrochemical sensors for DA detection.

Keywords: 1,4-Benzoquinone,carbon paste electrode, cyclic voltammetry,dopamine, electrochemical oxidation, kinetic parameter

1. INTRODUCTION

1.1. Background of the study

The biomolecules like dopamine (DA)are playing a significant role in the human body. They are involved in metabolic processes, and some of them act as neurotransmitters, neuroactive molecules, etc. Among the biomolecules, DA is one of the predominated ones, which is located naturally in the human body [1]. In the first time, Arvid Carlson discovered dopamine in 1957 [2]. Dopamine (DA) affects the cardiovascular, central nervous, endocrine and renal systems [3]. DA is a catecholamine neurotransmitter that directs the signal from the body to the brain. DA controls the movement and emotional responses of humans [4, 5].

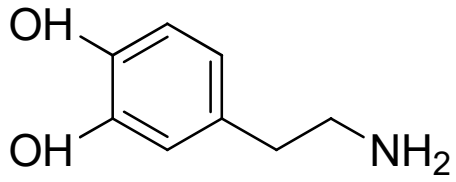

Figure 1. Structure of dopamine (DA)

The normal level of DA in human serum and blood is 10^{-6} -10^{-9}M. The excess level of DA can lead of euphoria whereas when dopamine concentration levels are depleted from the normal level in the human body, it causes to some neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, Schizophrenia [6–8]. Alzheimer's disease [9] arises from the formation of amyloid plaques that hinder the DA-ergic neurons and consequently their capability to produce DA. Parkinson's, the second most common neurodegenerative disease, which is linked to the degeneration of the nerve cells of the basal ganglia (substantia nigra) of the brain [10], which results in a decrease of DA production.

DA is also related to hyperactivity and attention, deficit hyperactivity disorder (ADHD) found in children [11]. ADHD is attributable to the alterations in some specific areas of the brain (pre-frontal cortex, part of the cerebellum and some of the basal ganglia, clusters of nerve cells located deep in the brain) that regulate attention and which are smaller in people with this disorder.

Furthermore, dopamine regulates all brain functions; it may protect the human heart racing and widen blood vessels in the viscera, which is effective in the treatment of cardiogenic shock and kidney failure [12]. Therefore, the precise investigation of DA levels with simple, low cost, sensitive and selective methods is highly required for analytical application and diagnostic research.

At present, a series of several analytical methods namely Fluorimetry [13], Spectrophotometry [14], Chemiluminescence [15], Capillary electrophoresis [16], Liquid chromatography [17], and Electrochemical methods [18, 19] have been developed and reported for determination of DA in the study of physiology mechanism and clinical diagnoses. However, these conventional methods mentioned above involve continuing too long sample pre-treatments, expensive and are time consuming [20, 21].Since, DA is one of electrochemically active molecule, electrochemical techniques currently have received great interest for their investigation due to their simplicity, cost effective, low detection limit, fast response time, selectivity, high sensitivity, and reproducibility [22, 23].

However, the detection of the DA via electrochemical methods can be difficult when DA co-exists with other redox-active biomolecules that can be oxidized at close oxidation potentials, such as AA or UA. To overcome this problem and perform selective detection of DA, many materials have been developed and used to obtain selective modified electrodes [24]. Indeed, several modifier materials like polymers [25], nanocomposites [26], carbon nanotubes, and grapheme

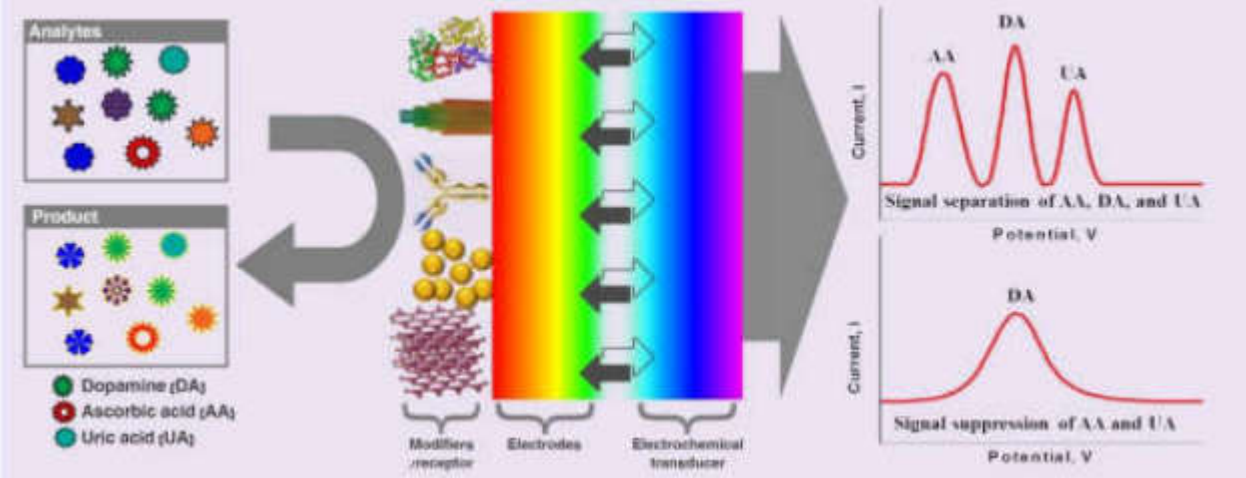

Figure 2. Electrochemical dopamine sensor presentation (Source: Int. J. Electrochem. Sci., 2020, 15, 599-612)

In this study, 1,4-BQMCPE sensor will be prepare and the use of chemically modified electrodes can help in selective, sensitive, and reproducible investigation of DA in the presence of other interferences by reduction of ohmic resistance associated with the wide potential window.

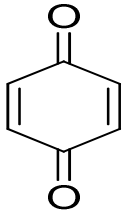

Figure 3. Structure of 1,4 Benzoquinone

1.2. Statement of the Problem

The direct electrochemical oxidation of bioactive molecule such as DA using unmodified electrode is possible but requires high over potentials. These high over potential results in electrode fouling, poor reproducibility, low selectivity and poor sensitivity and therefore this technique is infrequently employed analytically. The poor reproducibility of direct electrochemical oxidation of DA have led to the interest in the use of mediators and modified electrodes to catalyze the electrochemical oxidation of bioactive molecules. Focusing on the solutions, different researchers have tried different strategies including the use of chemically modified electrodes [27].

However, the issue of selectivity against potential interferents such as (AA, UA, glucose, urea and so on) has retained as a key challenge and continued to attract the interest of analytical chemists. The use of carbon electrodes including CPEs have advantages as they are response rapidly, direct and miniaturized in clinical samples and field applications. Nevertheless, modification of the electrode surface with suitable modifier is important to improve sensitivity and selectivity. Therefore, in this study a 1,4-BQMCPE will prepared for the investigation of DA.

1.3. Research Questions

This study, therefore, will try to answer the following basic research questions:

- How 1,4-Benzoquinone is used in the preparation of modified carbon paste electrode?
- What is the use of 1,4-Benzoquinone in the preparation of modified carbon paste electrode?
- Is 1,4-BQMCPE is effective for the electrochemical investigation of DA using CV and differential pulse voltammetry?
- What are the optimum experimental conditions for the investigation of DA using 1,4-Benzoquinone/CPE?
- Does the developed electrochemical sensor is effective compared to other spectroscopic methods?

1.4. Objectives of the study

1.4.1. General objective

- The general objective of this study was to develop 1,4-Benzoquinone modified carbon paste electrode(1,4-BQMCPE)for the voltammetric investigation of dopamine.

1.4.2. Specific objectives

The specific objectives of this study were:

- To prepare bare/unmodified carbon paste electrode;
- To modify the carbon paste electrode with 1,4-Benzoquinone or BQMCPEs;
- To characterize the modified electrode using CV and DPV;
- To assess the electrochemical behavior(reversibility)of the oxidation of DA;

- To determine the kinetic parameters like anodic transfer coefficient (α), diffusion coefficient (D) and heterogeneous transfer rate constant (k_s,h).
- To optimize the experimental conditions for the determination DA on 1,4-BQMCPE;

1.5. Significance of the study

This study is intend to give clear information on the concepts, important features and application of electrochemistry for biological molecules such as DA and to give safety information on the level DA in human urine to stakeholders including hospitals and consumers.

Therefore, the outcome of the research can contribute advanced knowledge in use of miniaturized techniques for sensing species and can add value to the existing literature of health quality control in detecting and determining DA level in clinical samples. This study can also serve to generate baseline information for further studies in related areas of study.

1.6. Scope of the study

In this study, much of the work described was concerned fabrication of electrode, electrode modification with 1,4-BQ, characterization of electrode by CV and DPV technique, determine kinetic parameters and optimize the optimum conditions were selected based on response to the intensity of DA reduction current during fabrication of 1,4-BQMCPE.

These are effects of composition of the modifiers, effects of pH buffer solutions, scan rate, pulse amplitude and concentration of analyte.

1.7. Limitation of Study

The limitation of the study due to financial constraints the carbon paste electrode was characterized only by CV and DPV. Scanning electron microscopy (SEM), which was used to study morphological features of electrode, was not included in the study.

2. REVIEW OF LITERATURE

2.1. Introduction

The main thrust of this thesis deals with the implementation of the development of modified carbon paste electrodes suitable for DA analysis. Therefore, the rest of this chapter was concerned with the overview of DA synthesis, its deficiencies, and recent literature utilizing different substrate materials as a sensor for biological quality and carbon electrodes.

Dopamine (DA) is a representative of neurotransmitters (neuromediators) responsible for the transfer of neural signals in humans and animal bodies. The level of DA in the human body is crucial for learning and memory, for cardiovascular and renal systems, and for human behavior. Deviations from the normal levels of DA can leads schizophrenia, Parkinson's disease, and a number of other health problems, including drug addiction. Normal levels of DA concentration, dependent on the age, are from 0.1 to 0.4 nM in the blood, variation in the urine is wider: from 0.1 to 2 μM [28, 29]. Accordingly, the control and fluctuations of the amount of DA are extremely important in monitoring the analytical systems in the human brain.Because of such several associated disease the determination of DA in biological samples, especially in serum and urine, is clinically important. Therefore, monitoring the concentration of DA in body fluids is essential to have a healthy life. In view of this, various analytical techniques have been proposed to estimate dopamine and to obtain a simple, sensitive, and fast detection method such as Fluorimetry[13], Spectrophotometry [14], Chemiluminescence [15], Capillary electrophoresis [16], Liquid chromatography [17], and Electrochemical methods [18, 19].

2.2. Dopamine

Dopamine (DA) was first synthesized in 1910 by George Barger and James Ewens at welcome Laboratories in London, England, but 40 years passed before it was discovered in mammalian tissue and almost another 10 years before it was accepted as a neurotransmitter.

Dopamine is a monoamine whose precursor in the Barger-Ewens synthesis is 3,4-dihydroxyphenylalanine (Levodopamine or L-DOPA).DA, or 3-hydroxytyramine, is a simple organic chemical in the catecholamine family derived from the amino acid tyrosine and was discovered in the sheep heart and adrenal medulla.

At the time, DA was discovered in mammalian tissue, it was theorized to function primarily as an intermediate in the production of the neurotransmitters, epinephrine, and norepinephrine (NE). But by the late 1950's, Arvid Carlsson and Nils-Ake Hillarp was first recognized the function of DA as a neurotransmitter at the laboratory for chemical pharmacology of the National institute of Sweden. Carlsson was awarded the 2000 Nobel Prize in physiology or medicine for showing that DA is not only a precursor of norepinephrine (noradrenalline) and epinephrine (adrenaline), but also a neurotransmitter.

Dopamine has the chemical formula $C_6H_3\ (OH)_2{-}CH_2{-}CH_2\ {-}NH_2$. Its chemical name is 4-(2-aminoethyl) benzene-1,2-diol and its abbreviation is DA. In the brain, DA functions as a neurotransmitter - a chemical released by nerve cells to send signals to other nerve cells. DA can act either as an inhibitory mechanism or as an excitatory mechanism in the nervous system, depending on the location of DA neurons, and the receiving characteristics of the next neuron in the chain [30, 31].

2.2.1. Physical and chemical properties of dopamine

DA is an important neurotransmitter located in the substantia nigra part of the brain. DA has a molecular formula of $C_8H_{11}NO_2[C_6H_3(OH)_2{-}CH_2{-}CH_2{-}NH_2]$ and a molecular weight of 153.18 g/mol. DA is a gray-white, odorless, fine powder. It is very soluble element in water and its solubility is 60.0g/100mL. The melting point of this compound is 128 ºC and the boiling point of this compound is 337.69 ºC at 760 mmHg. Its density is 1.26 g/cm^3. It is a member of the catecholamine family, which has a characteristic benzene ring with hydroxyl groups in the third and fourth positions (**Figure 3**). Its name derives from its chemical structure, which consists of an amine ($-NH_2$) group linked to a catechol structure called dihydroxyphenethylamine, the decarboxylated form of dihydroxyphenylalanine (L-DOPA). It is a stable compound under normal temperature and pressure [30].

2.2.2. Biosynthesis of dopamine

DA is synthesized in the body (mainly by nervous tissue and adrenal glands) first by the dehydration of the amino acid tyrosine to dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase (TH) and then by the decarboxylation of L-DOPA by aromatic -L- amino acid decarboxylase (AADC).

Tyrosine usually considered as the starting point in the biosynthesis of DA and this amino acid is abundant in dietary proteins. In addition, dietary phenylalaninethen converted to tyrosine both in the liver by phenylalanine hydroxylase and in the dopamine neuron by tyrosine hydroxylase.

Blood-borne tyrosine is taken up into the brain by a low-affinity amino acid transport system and subsequently from brain extracellular fluid into dopaminergic neurons by high and low affinity amino acid transporters. Once tyrosine has entered the neuron, its conversion to dihydroxyphenylalanine (L-DOPA), driven by the cytosolic enzyme tyrosine hydroxylase (TH), which found in all cells that synthesize catecholamine has and is a mixed-function oxidase that uses molecular oxygen and tyrosine as its substrates and biopterin as its cofactor, is normally the rate-limiting step in dopamine biosynthesis.

Tyrosine availability does not influence the rate of tyrosine hydroxylation in vivo under normal conditions in most dopamine-ergic neurons, but when the enzyme is activated, or in dopamine neuronal systems that have a relatively high basal firing rate (e.g., dopamine neurons projecting to the medial prefrontal cortex), tyrosine levels can affect the rate of conversion to L-DOPA. Aromatic amino acid decarboxylase (AADC, dopa decarboxylase) is the enzyme responsible for the cytosolic conversion of L-DOPA to dopamine. This enzyme so avidly decarboxylates L-DOPA that results in very low level of this amino acid in the brain under normal conditions.

Dopa decarboxylase (DDC) is a pyridoxine dependent enzyme that has a low Km and a high Vmax with respect to L-DOPA; thus, endogenous L-DOPA efficiently converted to dopamine. DDC can also decarboxylate, 5-hydroxytryptophan, the precursor of serotonin, as well as other aromatic amino acids; accordingly, it also called aromatic amino acid decarboxylase (AADC). DDC is widely distributed throughout the body, where it is found both in catecholamine and serotonin containing neurons and in non-neuronal tissues, such as kidney and blood vessels. In DA containing neurons, this enzyme is the final step in the pathway and this exploited clinically in the treatment of Parkinson’s disease by giving patients L-DOPA to boost the production of dopamine in the remaining dopaminergic axon terminals [32, 33].

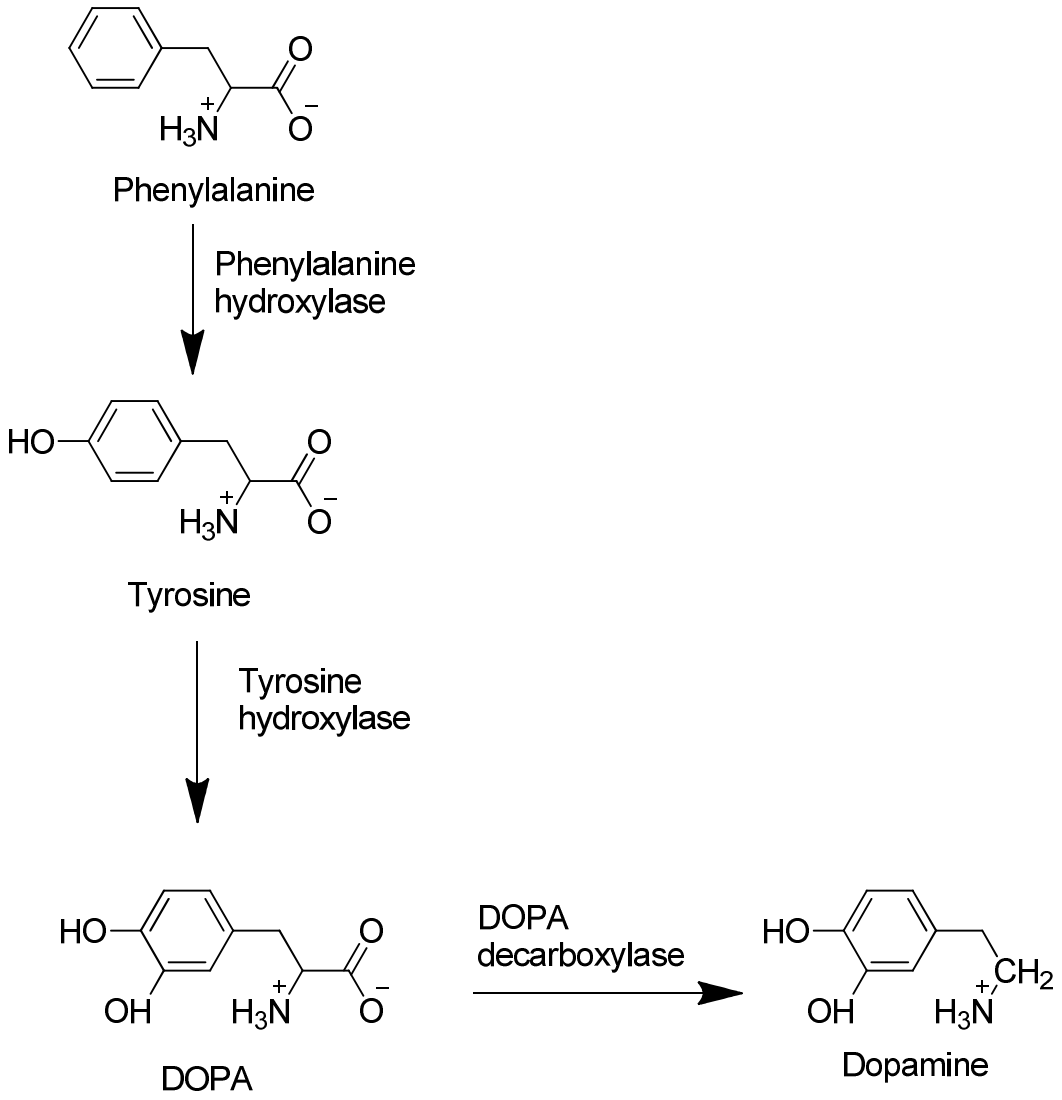

Figure 4. Biosynthetic pathway of dopamine

2.2.3. Health benefits of dopamine

DA has many functions in the brain, including important roles in behavior and cognition, voluntary movement, motivation, punishment and reward, inhibition of prolactin production (involved in lactation and sexual gratification), controlling the flow of information to the frontal lobe from other parts of the brain, sleep, mood, attention, working memory, and learning [30]. DA plays a significant role in the cardiovascular, renal, hormonal, and central nervous systems. DA is useful in the management of states of low cardiac output, associated with compromised renal function as with cardiogenic and hypovolemic shock.

DA also causes the release of norepinephrine from nerve terminals, which contributes to its effects on the heart. DA usually increases the systolic and pulse pressure and either has no effect on the diastolic blood pressure or increases it slightly. DA produces positive chronotropic and inotropic effects on the myocardium, resulting in increased heart rate and cardiac contractility. Aside from controlling movement, DA is also greatly involved in feelings of reward and alertness and purposeful behavior. In addition, DA has shown to play an important role in drug addiction and attention disorders, and it has been associated with HIV infection [34].

2.2.4. Dopamine and deficiencies

Especially, DA deficiency linked to feelings of depression, as DA is widely held to be linked to basic body movement and emotions. When there is a DA deficiency, there is too little of this neurotransmitter to correctly regulating emotions, meaning that the often-assuaging mental impulses that mitigate intense feelings of sadness inhibited by a lack of neurotransmission, causing sometimes-severe cases of depression. Since movement is also impaired, this causes physical depression as well, which leads to loss of sleep, diminished impulse to exercise, and a lack of willingness to take action for one self.

DA deficiency can manifest in a wide variety of symptoms, most of which are also associated with clinical depression (and specifically major depressive disorder). This can include a lack of interest in one's life, diminished motivation and inability to feel pleasure, procrastination, and an overabundance of sleep or a paradoxical insomnia, especially in waking up very early. Sometimes, DA deficiency can be linked to other anxiety or mood disorders, and be marked by extreme mood swings, hopelessness, guilt, impulsive behavior, poor memory, inability to focus, impaired concentration, and diminished ability to think in abstract ways [35].

2.3. Electrochemical Oxidation of Dopamine

The electrochemical oxidation of dopamine occurs via an ECE mechanism (**figure5**). DA is first oxidized to an open-chain ortho-quinone, which is a transient intermediate in the sequence, through a two-electron exchange reaction. Following the initial oxidation, a pH dependent, 1,4-addition reaction can occur, forming a cyclized product called a leucodopaminechrome.

At low pH values, the open-chain quinones are protonated largely, and the cyclization reaction is unfavorable. At higher pH values, a sufficient amount of unprotonated quinone is available so that cyclization is observed. The cyclized product can then undergo oxidation via another two-electron transfer reaction to dopaminochrome. In this scheme, '**E**' denotes the electrochemical reactions and '**C**' denotes the chemical reactions [36]. It was shown that oxidized DA cyclized at about one-tenth the rate of oxidized norepinephrine at pH 7.0. At physiological pH, the rate of cyclization is 0.263/s for the oxidized DA. Cyclization however is not the only reaction that the o-quinone may undergo. It will also react very rapidly (at 1800 times the rate of cyclization) with nucleophiles such as glutathione [37].

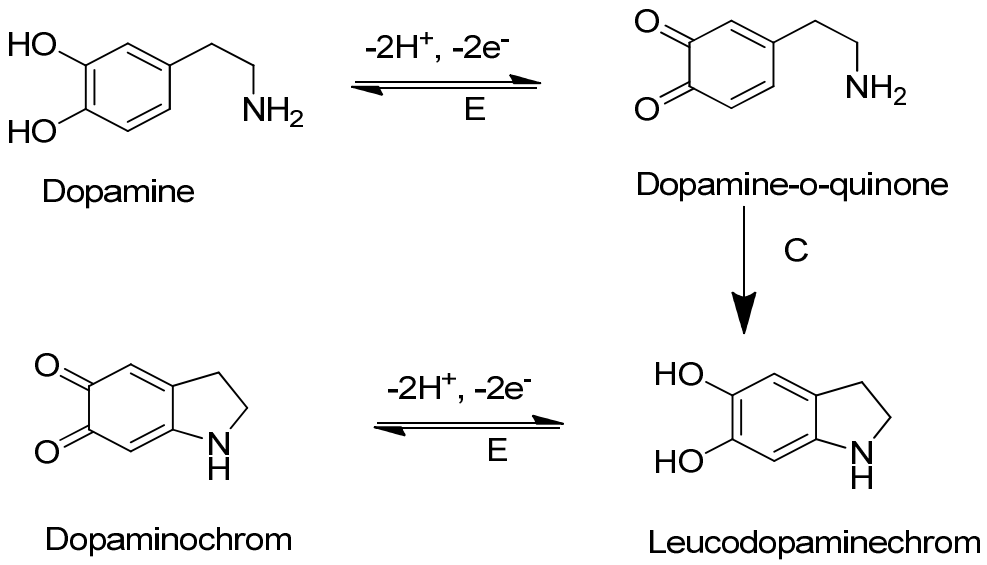

Figure 5. The electrochemical mechanisms of oxidation of dopamine

2.4. Chemically Modified Electrode

Chemically modified electrodes (CMEs) are electrodes that have their surfaces chemically modified to change the electrode's physical, chemical, electrochemical, optical, electrical, and transport properties. These electrodes are used for advanced purposes in research [38]. CMEs have increased the scope of electrochemical sensing by providing improvements in electrode selectivity and enabling the detection of number of electro inactive materials. This is the result of the modifiers present in electrode surface, which enable the use of a wide range analyte-electrode interaction in the generation of an analytical signal [39].

CMEs emerged in 1973 when Lane and Hubbard modified various olefin compounds on clean platinum electrode through chemisorptions, which significantly changed the electrochemical response of the electrode [40]. Since then, CMEs have been developing rapidly to investigate the direct electrochemistry of immobilized molecules and the mechanisms of redox reactions.

The fabrication of CMEs is to immobilize molecules with specific functions on the ordinary electrode surface by chemical or physical methods. For instance, proteins deposited on the surface of the CMEs can then retain their biological activities to some extent; thus, electrochemical performance of the electrode can be improved for the analysis of proteins. Similarly, thiol compounds can be covalently attached to the surface of gold electrode through the formation of metal–sulfur (Au–S) bond [41]. This process has then introduced some new functional groups not only for the improvement of biocompatibility but also for the later electrostatic interaction. Therefore, biologic analysis by using the CMEs can be conducted [42].

Now days, electrochemists have become interested in purposely modifying an electrode by adsorbing, coating, or attaching specific molecules to the surface. This deliberate and controlled modification of the electrode surface can produce electrodes with new and interesting properties that may form the basis of new applications of electrochemistry. Fundamental studies of such modified electrodes have also provided a better insight into the nature of charge transfer and transport processes in the thin films and electrode-electrolyte interfaces [43]. As described earlier, the electrochemical determination of DA concentration is challenging because of the closeness of oxidation potentials and the accumulation of the oxidation products at the electrode surface, which leads to electrode poisoning [44]. Consequently, it results in loss of reusability, selectivity, and reproducibility of these electrode surfaces. In order to overcome such problems and improve the determination of DA concentration, many materials have been developed and used to fabricate modified electrodes. However, electrochemical methods of the DA control attract attention because electrochemical sensing is promising in view of rapid, sensitive, selective, and low-cost detection of various biomolecular analytes.

Since DA is an electrochemically active compound it can be easily determined by electrochemical methods. However, there are two problems in the electrochemical detection of DA. A major problem for the electrochemical detection of DA in real biological matrices is the coexistence of some interfering compounds. Among these ascorbic acid (AA) is of particular importance. Because AA exist at much higher concentration than that of DA and oxidizes at a near potential with DA on bare carbon electrode surface which result in an overlap of their voltammetric response.

Another problem is the fouling of the electrode surface by the adsorption of oxidation products, which results in rather poor selectivity and reproducibility. Thus, it is difficult to detect DA in the presence of high level of AA in real biological samples, hence it is important to construct suitable electrode and to establish a sensitive and selective detection method for DA [45]. One of the most common routes to resolve the above problems is to use a chemically modified electrode, which has the ability to eliminate the interfering substances from DA determination.

The study of electrochemical determination with different modified electrodes such as polymer-modified electrode, carbon ionic liquid electrode, nanomaterials modified electrodes, self-assembled monolayer's and chemically modified carbon paste electrodes for sensitive and selective determination of DA have been reported to solve the problems [46]. Thanks to the progress in electronics and computer sciences, from which electrochemical instrumentation has gained considerable benefits in terms of precision, accuracy, sensitivity, selectivity and automation, the electro analysis of electrochemically bioactive compounds is currently actively involved in new research areas of voltammetric techniques. However, in general at bare electrodes their direct redox reactions take place at very similar potentials, which results in rather poor selectivity of voltammetric response. Consequently, chemical modification of electrode surface should improve their sensitivity and selectivity.

2.5. Carbon Based Electrode

Good electrical conductivity of the electrodes is an important factor. Carbon-based electrodes usually have a wider potential range than the other solid electrodes because of their broad potential window, low background current; rich surface chemistry, chemical inertness, low cost and suitability for various sensing and detection applications.

Carbon electrodes may be classified as homogenous (glassy carbon, graphite, vitreous carbon, screen printed, fullerenes, carbon nanotubes and diamond) or heterogeneous (carbon paste and modified carbon paste).

Carbon-based electrodes are generally inexpensive and they are available in a variety of forms. Carbon-based electrodes have slow kinetics leading to a wide useful potential range; especially in the anodic direction. Successful applications of carbon-based electrodes in voltammetry depend on the high chemical and electrochemical stability of carbon materials, a relatively high oxygen and hydrogen overvoltage on the materials, a wide working potential range, simplicity of renewal of the electrode surface, and availability of carbon-based materials. Carbon-based materials do not interact with analytes or deposited compounds during electrolysis, a feature that rules out the appearance of a systematic error caused by such interactions [47].

The type of carbon-based electrodes can play an important role on the analytical performance. The best-known carbon-based electrodes are those involving glassy carbon, carbon paste, carbon fiber, screen-printed carbon strips, carbon films and diamond, pyrolytic graphite, fullerenes, wax impregnated graphite, Kelgraf, carbon nanotube, and reticulated vitreous carbon. A variety of electrode pretreatment procedures has been proposed to increase the electron transfer rates. The type of carbon-based electrode and the using pretreatment method have a highly effective contribution on the electrode surface [48].

2.5.1. Metal and metal oxide nanoparticles

Because of metal nanoparticles high reactivity and surface area, it exhibits electronic, physical and chemical properties better than that of bulk materials. The precursor materials of metal nanoparticles are significantly determining the properties of metal nanoparticles. In addition, the stability, shape, size, and charge of metal nanoparticles can be controlled by using stabilizers.

Many properties are qualified using metal nanoparticles in electrochemical sensors, such as the ability to convert any small redox activity to a signal. This property, which is because of the high sensitivity of metal nanoparticles to any surface changes, shows fast electron transfer with the analyte due to high surface area.

However, the bare electrodes show slow electron transfer and thus affect the ability of modified electrodes with metal nanoparticles to resolve overlapped potentials of mixed compounds. From many reports, it is noticed that the enhancement of analytes electrochemical detection is reached by the combination of different materials with metal nanoparticles.S. Palanisamy *et al.* (**2019**) reported the determination of DA in the presence of AA & UA with graphene oxide modified with Pd nanoparticles. Therefore, Pd nanoparticles can be loaded on the surface of graphene oxide (GO) in order to resolve the overlapped peaks by the attraction between DA positive charge and GO negative charge. Therefore, a good support material like cellulose microfibers supported with Pd nanoparticles was loaded over a semi-conductive GO to select DA with LOD = 23 nM [49].

Bikila Negasa *et al*. (**2017**) reported that determination of DA at modified glassy carbon electrode (GCE) with a film produced by reduction of diazonium generated from p-nitroaniline (PNA).Pores were created purposely by stripping pre-deposited gold nanoparticles (AuNPs) in the modifier film. The modified electrodes were characterized electrochemically by common redox probes: hydroquinone (HQ), hexacyanoferrate $[(Fe(CN)_6)]^{3-}$ and hexamine ruthenium(III) $[Ru(NH_3)_6]^{3+}$. Comparison was made for the cyclic voltammetric and amperometric response of DA using the modified electrodes against the bare GCE in phosphate buffer solution (PBS) of pH 7.5. The bare and modified GCE showed a linear response to DA in the concentration range of 0.2- 2.2 mM and 5-35 μM with detection limit of 0.015 mM and 0.6112 μM, respectively. The modified electrode showed high sensitivity, well selectivity, good anti-interference ability, durable stability and good electrode reproducibility for determining DA. The reported modified electrode is a promising sensor for use in electroanalysis of DA [50].

2.5.2. Metal Nanostructures

Metal nanoparticles (MNPs) are an advantageous material for biosensing owing to their excellent conductivity, signal amplification, and facilitation of both electron transfer and electrical contact between the redox center of a biomolecule and the electrode surface. These electrocatalytic properties of the metal nanoparticles are far superior to the bulk metals due to their high surface area and improved reactivity. In particular, gold nanoparticles have been used in various electrochemical biosensors since gold enhances cell adhesion and growth

[51]. Thus, a nanostructured gold surface consisting of closely packed outwardly growing spikes showed a significant electrocatalytic effect for the electro oxidation of DA due to the presence of numerous surface-active sites [52]. The resulting biosensor exhibited a linear range of 1–100 μM, with a detection limit of 5 μM using differential pulse voltammetry.

In another study, nanostructured gold surfaces was prepared by electro deposition and used for the determination of DA. The square wave voltammetry peak current was linearly dependent on DA concentration up to 10 μM, with a detection limit of 0.57 μM[53].

MNPs have attracted significant interest in the modification of electrodes. However, proper control of the size and shapes of NPs is a challenging task. Maintaining NPs with the same size and shape during different batches of synthesis is another major concern. Moreover, homogeneous dispersion of NPs on the surface of electrode cannot be easily controlled and achieved. Therefore, it is difficult to develop rigid and reproducible electrodes for obtaining reproducible and sensitive electrode.

2.6. Carbon Materials Used as Modifier

Carbon materials are used in various fields due to their thermal stability, chemical resistance, and excellent mechanical properties. They have tremendous potential for sensing target biomolecules, such as DA, due to their excellent electrical conductivity, fast electron transfer kinetics, and reasonable biocompatibility. Carbon materials are also low-cost nanomaterials that can be used alone or in combination with other materials.

2.6.1. Carbon nanotubes

Carbon-based materials such as carbon nanotubes (CNTs) are used as modifier either directly, or by hybridizing with other NPs or organic materials. Because of the extraordinary characteristics of CNTs, such as low electrode fouling, high electron-transfer rate, a large effective detection surface and high electro catalytic activity, numerous researchers have investigated their potential applications in recent years. Although available in different forms and shapes, SWCNTs and MWCNTs are more widely used as a sensing surface over bare electrodes. The metallic behavior of MWCNTs can be attributed to the presence of a single metallic layer, while SWCNTs are classified as metallic and semiconducting; separation of the metallic ones will be more beneficial for modifying electrodes [54].

It has been noted from numerous examples that researchers have combined SWCNTs or MWCNTs with other emerging materials to take advantage of the synergistic effects [55].

CNTs are frequently used to decrease the oxidation potential of DA and facilitate its biosensing. For instance, Beitollahi, H. *et al* (**2012**) fabricated highly selective and sensitive sensor based on 5-amino-3,4-dimethyl-biphenyl-2-ol functionalized multi-walled carbon nanotubes was used to modify the carbon paste electrode (CPEs) surfaces to simultaneously detect DA, UA and Tryptophan by SWV. The SWV data showed that the obtained anodic peak currents were linearly proportional to concentration in the range of 1.2–800 μM DA, with a detection limit of 0.16 μM, and in the range of 0.1- 500 μM and with a detection limit of 0.07 μM for UA and for TY 1-1000 μM with detection limit 0.03 μM [56]. In addition, Li, Y *et al* (**2012**) developed SWCNTs modified with sodium dodecyl sulfate possessed a negative charge that allowed a successful determination of DA in the presence of AA and UA and showed good recovery in some biological fluids. The catalytic peak currents obtained by voltammetry increased linearly with the increase in DA concentrations in the range of 5–100 μM with a detection limit of 200 nM [57].

Negles, E. *et al* (**2017**) reported a novel electrochemical sensor for detection of DA and UA in the presence of AA using SWCNTs and ionic liquids. SWCNTs dispersed in chitosan and treated by ionic liquids allowed the determination of DA for concentration range between 0.5 and 30 μM within real samples (detection limit: 0.16 μM). In this case, the use of SWCNTs and an ionic liquid increased significantly the anodic peak current intensity, thus facilitating the detection of the neurotransmitter [58].

Balasubramanian, P. *et al* (**2020**) developed MWCNTs modified with Au-Ag nanoparticles for monitoring extracellular dopamine in neuronal cells. The presence of metal nanoparticles increased the electron transfer rate and the sensing performances of the sensor, leading to a linear range of 3 nM–2.3 μM and a detection limit of 0.23 nM [59].

Hang, Q. *et al* (**2020**)fabricated another biosensor permitted the monitoring of extracellular DA in neuronal cells by combining MWCNTs with graphene nanoparticles that increased the surface area of the sensor leading to a linear range of 5 nM–100 μM and a detection limit of 0.87 nm [60].

Emi N, Mohamad M. *et al* (**2023**) developed a simple, rapid, and sensitive method for the electrochemical detection of DA utilising 1-phenyl-3-methyl-4-orthofluorobenzoyl-5-pyrazolone (PHMoFBP)/multiwalled carbon nanotube (MWCNT) carbon paste electrode (CPE).

The electrochemical behavior of DA was performed through cyclic voltammetry and square wave voltammetry. PHMoFBP/MWCNT showed a higher current at the lower potential for the oxidation of DA compared to bare MWCNT. The sensor's improved electrocatalytic activity observed to detect in a 0.1 M phosphate buffer saline (PBS) solution at pH 8.0. A good linear regression analysis was observed between electrical response and the concentration of DA in the range of 1 to 1000 μM. Under optimized experimental conditions, 1.0×10^{-7} M has been determined as the limit of detection (LOD). The sensor has expressed considerable sensitivity towards DA detection without interference and successfully used to determine DA in dopamine hydrochloride injection [61].

Even though, the properties of CNTs make them attractive as substrates for the detection of bioactive molecules, there has been main drawback that they aggregate in almost all aqueous and organic solvents.

2.6.2. Graphene and its derivatives

Graphene is a 2-D nanomaterial consisting of a single layer of sp^2 network of carbon atoms. Graphene and its derivatives graphene oxide (GO) and reduced graphene oxide (rGO) has emerged as promising materials for wide practical applications. These includes electrocatalysis and electroanalysis, due to their beneficial characteristics, including high thermal and electrical conductivity excellent mechanic performances, a wide electrochemical potential window (2.5 V), and the possibility to be easily functionalized by covalent or non-covalent binding or modified with elemental dopants [62]. In addition, graphene possesses several specific advantages for DA electrochemical detection since: (i) Dopamine molecules are capable of thermodynamic adsorption on a graphene surface through π–π stacking interactions; (ii) graphene possesses a very high specific surface area that can support the adsorption and diffusion processes of dopamine, and (iii) the presence of oxygen-containing groups on the GO or rGO surface accelerates the electron transfer during electrochemical biosensing and generally leads to a better selectivity, sensitivity, and limit of detection [63].

Bernardo P. *et al* (**2021**) fabricated electrochemical DA sensors based on reduced graphene oxide coupled with Au or Pt nanoparticles. Sensors were developed by co-electrodeposition onto a flexible substrate, and a systematic investigation concerning the electrodeposition parameters (concentration of precursors, deposition time and potential) was carried out to maximize the sensitivity of the dopamine detection. Square wave voltammetry was used as an electrochemical technique that ensured a high sensitive detection in the nM range. Sensors were challenged against synthetic urine in order to simulate a real sample detection scenario where DA concentrations are usually lower than 600 nM. This sensors show a negligible interference from UA and AA, which did not affect sensor performance.

A wide linear range (0.1–20 μm for gold nanoparticles, 0.1–10 μm for platinum nanoparticles) with high sensitivity (6.02 and 7.19 μA μM^{-1} cm^{-2} for gold and platinum,respectively) and a low limit of detection (75 and 62 nM for Au and Pt, respectively) were achieved. Real urine sample were also assayed, where the concentrations of DA detected aligned very closely to measurements undertaken using conventional laboratory techniques. Sensor fabrication employed a cost-effective production process with the possibility of also being integrated into flexible substrates, thus allowing for the possible development of wearable sensing devices [64].

2.7. Polymer Materials used as modifier

2.7.1. Conducting Polymers

Conducting polymers are very common modifiers, which can chemically or electrochemically, deposited over bare electrodes from their monomer solutions. They have an extended π-conjugated structure with alternating single and double bonds across the polymeric chain, which causes delocalization of the electrons in the polymeric backbone, and is responsible for their outstanding electrical and optical properties.

Conducting polymers forms a protective layer to avoid surface fouling, high biocompatibility, and the possibility to be selective towards target bioanalytes by avoiding the interfering species through hydrophobic, hydrophilic, ion exchange, or electrostatic interactions.

For all these reasons, conducting polymers and their nanocomposites are widely used for biomedical applications [65], in particular for electrochemical sensing of biomolecules [66], including neurotransmitters such as DA [67].

Several works have been dedicated to the use of electrodes modified by a conducting polymer and gold nanoparticles to detect DA. For example,Atta, N.F et al. (**2012)** developed Gold nanoparticles modified poly(3,4-ethylene-dioxythiophene) electrode for the selective determination of sub-nano concentrations of DA in presence of sodium dodecyl sulfate. This modified electrode showed a higher catalytic activity due to the rich electron cloud in the polymer and electro catalytic properties of the nanoparticles and sodium dodecyl sulfate. The DA concentration could measure in the linear range of 0.5–140 μM, with a low detection limit of 0.39 nM. The modified electrode was validated for the determination of DA in human urine [68].

In addition to this, Mahalakshmi, S. et al. (**2021**) developedanother electrode, which was modified by electrodeposition of gold nanoparticles over polyaniline using linear voltammetry leading to an efficient loading of nanoparticles in the polymer matrix. The resulting electrode showed enhanced electrocatalytic activity in the working linear range of 20–100 μM and a detection limit of 16 μM. Such enhanced electrocatalytic response was attributed to a synergistic interaction between the polymer film and the nanoparticles [69].

2.8. Methods of Analysis of Dopamine

A number of quantitative analytical methods have been reported for DA determination in the study of physiology mechanism and clinical diagnose. Accordingly, the control and fluctuations of the amount of DA are extremely important in monitoring the analytical systems in the human brain. In view of this, various analytical techniques have been proposed to estimate DA and to obtain a simple, sensitive, and fast detection methods described in introduction part.

2.8.1. Electro analytical Methods

When target biomolecules are captured by a sensing material deposited on the working electrode of an electrochemical biosensor, an analytical measurable signal is generated (**Figure 6A**).

In the case of DA, many electrochemical methods (e.g., amperometry, cyclic voltammetry, differential pulse voltammetry) have been developed since dopamine can be oxidized easily [70], leading to the formation of dopamine-o-quinone through a two-electron process [71]. The electrons released by DA during its oxidation generate currents that may be linearly dependent on the concentration of the electro activeDA biomolecules, thus enabling the quantification of these compounds.

Electrochemical methods have many advantages for DA detection: the low cost of electrochemical instrumentation, the size of the electrodes that can be conveniently implanted in living cells, the short response time, and the capacity to monitor DA in real-time. However, the detection of DA via electrochemical methods can be difficult when DA co-exists with other redox-active biomolecules that can be oxidized at close oxidation potentials, such as AA or UA. To overcome this problem and perform selective detection, many materials have been developed and used to obtain selective modified electrodes.

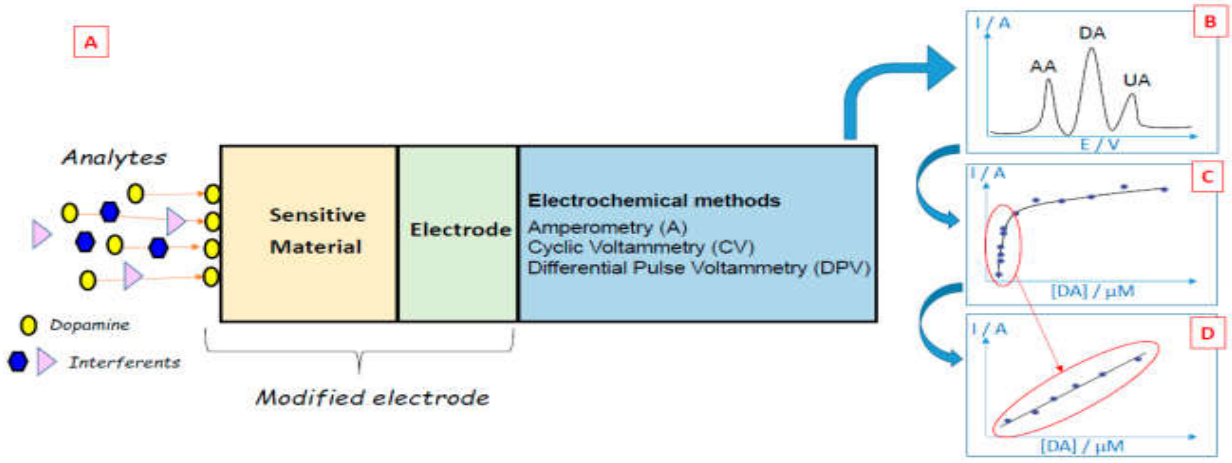

Figure 6. Principles of dopamine (DA) electrochemical biosensors, (B) Voltammetric curve of DA in the presence of Ascorbic acid and Uric acid, (C) Oxidation of DA as a function of DA concentration, (D) Calibration curve of DA

2.8.1.1. Amperometry

In amperometric biosensors, the current produced during the oxidation or reduction of an electroactive biological element at a constant potential applied between a working electrode and a reference electrode is measured, providing specific quantitative analytical information

[72-74]. These biosensors are inspired by the first amperometric biosensor developed by Clark in 1956, who fabricated an amperometric oxygen sensor producing a current proportional to the oxygen concentration when the potential was applied to a platinum electrode [75]. In the case of DA biosensors, a constant potential is applied, potential that is sufficient to oxidize dopamine to dopamine-o-quinone through a two electron process. The current is proportional to the DA concentration over a more or less wide concentration range, thus allowing the quantification of the DA concentration in the sample [76-78]. However, amperometry is not selective since all electroactive compounds that can oxidize at the applied potential produce an amperometric response.

2.8.1.2. Cyclic Voltammetry (CV)

During cyclic voltammetry experiments, a current is produced by sweeping the potential applied between two electrodes over a range that is associated with the redox reaction of the analyte. This redox reaction generates a change in peak current that can be correlated to the concentration of the analyte, thus leading to specific quantitative analytical information [72, 79]. This method has the advantage of providing both qualitative information deduced from the potential location of the current peak and quantitative information deduced from the intensity of the peak current. For example, the oxidation of DA using cyclic voltammetry leads to an oxidation peak, which is characteristic of this biomolecule. By studying the evolution of the intensity of the oxidation peak present in the cyclic voltammograms for different dopamine concentrations, it is possible to draw calibration curves and quantify this compound (**Figure 6 C, D**).

2.8.1.3. Differential Pulse Voltammetry (DPV)

Differential pulse voltammetry is a derivative of the linear sweep voltammetry technique with a series of regular voltage pulses superimposed on the potential linear sweep. In DPV, a base potential value is chosen at which there is no faradaic reaction, and this potential is applied to the electrode. The base potential is increased between pulses with equal increments. The current is measured just before each potential change, and the current difference is plotted against the potential. Sampling the current immediately before the potential is modified reduces the effect of the charging current.

In the linear sweep technique, an oxidation process leads to the formation of a wave in the voltammogram, but in the DPV technique, an oxidation process originates a peak. This sharper shape facilitates the interpretation of the voltammogram and renders DPV more accurate than linear voltammetry. This is particularly useful in the case of electrochemical biosensors since it is easier to discriminate the sharp peaks attributed to dopamine, ascorbic acid or uric acid when applying the DPV technique compared to the large waves obtained by linear voltammetry (**Figure 6 B**).

2.9. Fabrication of CPEs for the electrochemical analysis of neurotransmitters (dopamine)

CPE has been used for the electrochemical determination of different neurotransmitters by adopting various electrochemical strategies. Several studies have been reported by modifying CPE in order to detect the level neurotransmitters such as DA. For instance, Umesh et al. (**2013**) prepared CPEs coated with poly (malachite green) film for sensitive and selective measurement of DA in pH 7 phosphate buffer. A fully enhanced redox peak was observed with a detection limit of 2.5×10^{-7} M by cyclic voltammetry.

Further studies have shown that DA oxidation shifts negatively by increasing pH. The effects of concentration and scan rate resulted in a linear response and the entire electrode process was diffusion controlled. The fabricated CPE acts as suitable and sensitive electrode for measuring DA. Simultaneous studies have shown excellent potential differences between DA and other neurotransmitters using both DPV and CV techniques [80]. In addition, Jasmine et al. (**2021**) used Perovskite materials such as LaNiO3, LaFeO3, and LaCoO3 were used for modifying the CPEs for the analysis of neurotransmitters such as dopamine. These materials significantly increased the electrochemical active area of the electrode and reduced the charge transfer resistance of the modified electrode. Further, the perovskite modified electrode could enhance the thermal stability and surface features such as phase formation and morphology. The modified electrodes showed a high current response, enabling nanomolar sensing of neurotransmitters such as DA. The perovskite-modified CPE (LNO MCPE) showed a wide linear range, high sensitivity, excellent selectivity, and wide linear concentration range are the superiority of the electrode and this enables simultaneous detection and quantification of four neurotransmitters [81].

Hadi et al. (**2019**) reported a chemically modified CPE containing bis-hydroquinone (DOH) for the electrode-catalyzed oxidation of DA. Kinetic parameters of the modified CPE such as the oxidation diffusion coefficient and electron transfer coefficient ($\alpha = 0.33$) on the surface of DOH were also incorporated. Under optimum conditions (pH=7.0), DA oxidation on the surface of the fabricated electrode has been found to occur at a positive potential about 290 mV lower than the bare CPE.

The oxidation peak current exhibited a linear variation depends on DA concentration, and the linear analysis curve was obtained in the SWV range of 3.0×10^{-5}–2×10^{-3} M DA. The detection limit was determined to be 3.2×10^{-6} M. This method was also used to determine the DA of a drug injection using a standard addition method [82].

2.9.1. Unmodified carbon paste electrodes

In 1958, R.N. Adams discovered a new type of electrode by using a mixture of carbon powder with a liquid non-electroactive binder and called it as carbon paste. His original idea was to develop a dropping carbon electrode (DCE) that could be constructed similarly like the drooping mercury electrode (DME). Although practical experiments with DCE failed, the mixture of carbon powder and a binder prepared in thicker consistency presented as a new type of electrode material [83, 84].

Carbon paste electrodes are an example of the more general class of composite electrodes, in which chemically useful functionalities introduced during physical mixing of graphite and pasting liquids. Carbon paste electrodes are mixtures prepared from graphite powder and various water-immiscible non-conducting organic binders. This kind of carbon-based electrode offers an easily renewable and modified surface at low cost and very low background current contributions.

Carbon paste electrodes are classified as bare or unmodified carbon electrodes. The pasting liquid was chosen for inertness, low solubility in the studied solvent and low volatility. Mineral oil (Nujol), paraffin oil, silicone grease and organophoshate, etc. are some examples of the pasting liquids. After thorough mixing the graphite powder and the desired pasting liquid, the paste is packed into a small holder (usually 2-5 mm deep, 3 mm diameter, Teflon) with electrical contact at the back.

The paste composition and ratio of pasting liquids strongly affect the electrode reactivity. The amount of the pasting liquid controls the electron transfer rates as well as the background current contributions. A higher amount of pasting liquid decreases electron transfer rates, as well as the background current contributions. Very rapid electron transfer rates occur on the dry and pure graphite carbon [47, 48, and 85].

2.9.2. Modified carbon paste electrode

The effort to make use of the favorable mechanical and electrochemical properties of carbon pastes for the preparation of a new design of sensors started at the beginning of the 1980s. Modification of a carbon paste by impregnating the carbon particles with methanolic solution of dimethylglyoxime represents a milestone in the history of carbon paste electrodes. It was a first effort when a classic analytical reagent had served as selective modifier, thus initiating a very successful role of chemically modified carbon paste electrodes in electrochemical analysis.

Hand in hand with a rapid development of chemically modified carbon paste electrodes, the favorable mechanical and electrochemical properties of carbon paste was tested for the preparation of special sensors containing enzymes allowing one to examine some enzymatically catalyzed reactions of biological substances. This way of attaching enzymes to an electrode material immediately attracted bioanalysts and carbon paste-based enzymatic biosensors had rapidly come to the front [83]. A modified carbon paste is a mixture of powdered graphite, nonelectrolytic liquid binder and a modifying agent. The modifying agent is usually one substance, but the pastes also can be modify with two or even more components, which is the case of carbon paste based biosensors containing an enzyme together with the appropriate mediator. The amount of the modification agents in the carbon paste usually varies between 10 to 30% (w/w), depending on the characteristics and the capability of the modification agent of forming enough active sites in the modified carbon paste. The modifier is either dissolved in the pasting liquid or physically mixed with the paste while preparing a modified carbon paste electrode. The modifier itself could be electroactive or may be a complexing agent, which can extract an electroactive analyte into the surface of the paste electrode. It is also possible to soak graphite particles with a solution of a modifier. After evaporating the solvent, the impregnated carbon powder is ready to use.

The use of modifiers for the preparation of modified carbon paste electrodes can be divided into several groups as follows: Chemical compounds and analytical reagents (e.g., HgO, 8-hydroxyquinoline); ion exchangers; clay minerals (e.g., zeolite and similar compounds); silica and silica-containing matrices and substrates such as peat moss, algae, or bacteria.

2.9.3. 1,4-Benzoquinone modified carbon paste electrodes

Quinones are a class of compounds consisting of conjugated cyclic diketone organic systems. Naturally, quinones function in cellular respiration, photosynthesis, and blood coagulation that occur in certain plants and animals. Their biological functions are mostly relates to their electron transfer rates and redox potentials [86]. In aprotic solvents, 1,4-benzoquinone, is reduced in two consecutive one electron steps to form hydroquinone radical anion and hydroquinonedianion as shown by the following general reaction equation:

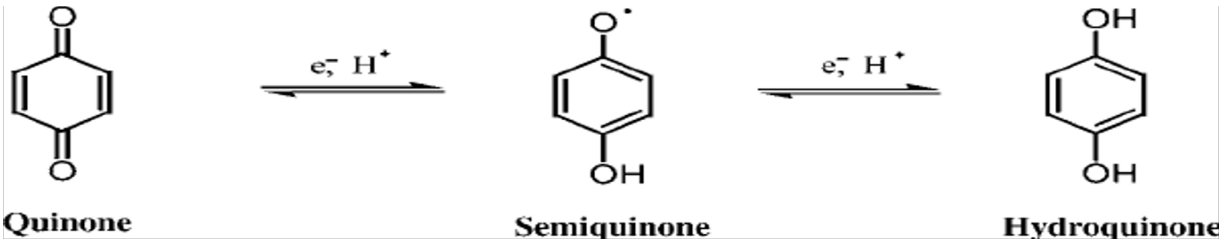

Figure 7. Redox reaction steps for quinone/hydroquinone inter-conversion

Benzoquinone and hydroquinone redox couples have been widely used in electrochemical studies. Moreover, BQ have been found important as modifying agents for the preparation of modified carbon paste electrodes. An oligosaccharide dehydrogenase based amperometric sensor by using a BQ bulk modified carbon paste electrode has been used for the determination of low molecular weight saccharides including glucose [87]. The BQMCPE had shown remarkable advantages from its low noise levels. In another study, the electrochemical behavior of a tetrabromo-p-benzoquinone modified carbon paste electrode has been investigated. The function of the BQ modified electrode for the electrocatalytic oxidation of ascorbic acid, dopamine and uric acid was found to be attractive. In addition, the simultaneous determination of the three components in the mixture was made possible based on differential pulse voltammetric technique by using the BQMCPE.

3. MATERIALS AND METHODS

3.1. Chemicals

The chemicals used for this experiment were dopamine hydrochloride (India), graphite powder (BDH, England), paraffin oil (Nice, India), disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$)(BDH, England),sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$)(Nice, India), sodium hydroxide, (Scharlau, Spain), hydrochloric acid,(Nice chemicals, India), perchloric acid (Nice, India), 1,4- Benzoquinone (Riedel-DeHaen, Germany), distilled water. All chemicals used were in analytical grade. Distilled water was used throughout the experiment and all stock serial solutions of dopamine were freshly prepared.

3.2. Instruments

The electrochemical measurement was performed by using Epsilion EC-Ver 1.40.67 voltammetric analyzer (BAS, USA),which was connected to DellPentium desktop computer using a standard cell with three electrodes. The three electrode system consists of unmodified carbon paste electrode (UMCPE) or 1,4-benzoqunone modified carbon paste electrode (1,4-BQMCPE) used as working electrode, Ag/AgCl as reference electrode and a platinum wire as a counter electrode, all measurements were carrying out at the laboratory temperature.Digital pH meterwas used to measure the pH of the buffer solution. Measuring cylinder, dropper and micropipettes was used for measuring different volumes of sample solution, acid reagents and dopamine standard solutions.

3.3. Study Methodology

The study was conducted by the application of cyclic voltammetry and differential pulse voltammetry techniques.The electrochemicalperformance studies of the target electrode (1,4-BQMCPE) were evaluated by the application ofcyclic voltammetry.The performance characteristics of the modified carbon paste electrode including the limit of detection (LOD) and kinetic parameters for investigation of dopaminewas studied by differential pulse voltammetry.

3.4. Procedure for Preparation of Solution

3.4.1. Buffer solution

Supporting electrolyte of phosphate buffer (PBS) solution in different pH ranging from 4.0-8.0 was prepared from a mixture of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 buffer solution in distilled water. 0.1 M HCl and 0.1 M NaOH solutions were added to adjust the pH of the buffer solutions.

3.4.2. Standard preparation

A stock solution of 25 mM was prepared by dissolving 2.40 mL of dopamine HCl injection in 0.1M perchloric acid solution and all other solution was prepared with double distilled water. The required concentrations of dopamine were prepared by diluting the stock solution with 0.1M solution of perchloric acid.

3.5. Preparation of Electrodes

3.5.1. Preparation of carbon paste electrode

Graphite powder and paraffin oil of high purity were used in the preparation of a carbon paste. Unmodified carbon paste (0.1 g) was prepared by mixing graphite powder with paraffin oil. The composition of the paste is 70% (w/w) graphite powder and 30% (w/w) paraffin oil. The mixture was homogenized with mortar and pestle for 30 minutes and allowed to rest for 24 hours. The homogenized paste is packed in the tip of Teflon tube (3 mm diameter, 7 mm deep). A copper wire was inserted from the backside of the Teflon tube to provide electrical contact. Then the surface of the electrode was smoothed against a smooth white paper until a shiny surface was emerged.

3.5.2. Preparation of 1,4-BQMCPE

The modified electrode containing the following percentage ratio of graphite powder, 1,4-Benzoquinone (1,4-BQ) and paraffin oil were mixed, and homogenized with mortar and pestle for 30 minute to give a uniform paste. For 5% (w/w) 1,4-BQ, 65% (w/w) graphite powder, and 30% (w/w) paraffin oil utilized. In the same way, for 10% (w/w), 1,4-BQ; 70%(w/w) graphite powder, and 20% (w/w) paraffin oil used. For 15% (w/w), 1,4-BQ; 65% graphite powder, 20% (w/w) paraffin oil mixed. For 20% (w/w), 1,4-BQ; 60%(w/w) graphite powders, and 20%(w/w) paraffin oil mixed.

For 23% (w/w), 1,4-BQ; 57% (w/w) graphite powders and 20% (w/w) paraffin oil mixed. For 25% (w/w), 1,4-BQ; 55% (w/w) graphite powders, and 20% (w/w) paraffin oil mixed. The homogenized paste was packed at the tip of Teflon tube and kept at room temperature for 24 hrs. The electrode surface was smoothed against smooth white paper until a shiny surface was emerged before electrochemical measurements [88].

3.6. Optimization of different experimental conditions for CV investigation of DA

3.6.1. Effect of modifier Composition

The characterization of 1,4-BQMCPE was investigated by using cyclic voltammetric technique. 1,4-BQMCPE was prepared in different ratios by adding different amounts of 1,4-Benzoquinone in milligrams.

3.6.2. Effect of pH

The electrochemical behavior of 1,4-BQMCPE was studied over a pH range of 4-8 in a solution containing 1.0 mM of DA in 0.1 M PBS as supporting electrolyte at a scan rate of 50mV/s to optimize the electrochemical response of MCPE for the oxidation and reduction of DA.

3.6.3. Effect scan rate

The effect of varying scan rates on the cyclic voltammograms of 1.0 mM DA using 1,4-BQMCPE in 0.1 M PBS of pH=7.0 supporting electrolyte was studied by varying the scan rate from 50-500 mV/s.

3.6.4. Determination of kinetic parameters

Anodic transfer coefficient (α), heterogeneous rate constant (ks,h), and diffusion coefficient (D) was determined.

3.6.5. Effect of Concentration of Dopamine

The effect of varying concentrations of DA was studied at 1,4-BQMCPE in 0.1 M PBS of pH=7.0 at a scan rate of 50 mV/s.

3.7. Optimization of experimental conditions involved in DPV for DA investigation

The optimization of experimental variables in the investigation of DA was made using a single working electrode system. It makes the technique suitable for quantitative analysis. This device consists of three electrodes: Ag/AgCl as the reference electrode, platinum as the counter electrode and CPE working electrode. For the investigation of DA, the carbon paste working electrode was modified with 1,4-Benzoquinone.

3.7.1. Effect of scan rate

The effect of the scan rate on the DPV oxidation peak current of 1.0 mM of DA in 0.1 M PBS of pH=7.0 at 1,4-BQMCPE was studied by varying the scan rate from 25 to 125 mV/s.

3.7.2. Effect of pulse amplitude

The effect of differential pulse amplitude on the oxidation peak current of 1.0 mM of DA in 0.1 M PBS of pH=7.0 at 1,4-BQMCPE was studied by varying the differential pulse amplitudes from 60 mV to 240 mV at a scan rate of 100 mV/s.

3.7.3. Effect of Concentration of Dopamine

The effect of varying DA concentrations on the differential pulse voltammetric peak current response of DA was studied at 1,4-BQMCPE.

4. RESULTS AND DISCUSSION

4.1. Cyclic Voltammetric Investigation

4.1.1. Electrochemical response of dopamine at 1,4-BQMCPE and UMCPE

Dopamine being an easily oxidizable catecholamine, its voltammogram was recorded in the potential range from 0 to 800 mV with supporting electrolyte of 0.1 M PBS of pH=7.0 at a scan rate of 50 mV/s. Cyclic voltammograms of a 1,4-BQMCPE in 0.1 M PBS of pH=7.0 in the back ground (a) and presence (b) of 1.0 mM DA at a scan rate of 50 mV/s (Figure 8).

The appearance of the anodic and cathodic peaks at the blank supporting electrolyte solution, revealed that there was peak interferences of the modifier 1,4-benzoquinone at the oxidation and reduction peak current of DA. This may due to the redox reaction properties of 1,4-benzoquinone. Therefore, in order to overcome the interference background current should be subtracted from maximum peak current.

With the addition of DA, the oxidation peak current was increased significantly from -2.325×10^{-6} A to -6.381×10^{-6} A, when compared with that obtained at the modified electrode in the absence of DA (Figure 8). The peak separation (ΔEp) from the cyclic voltammogram of modified electrode in the absence of DA was found to be 262 mV, which clearly shows that the electrochemical reaction of 1,4-benzoquinone in the bulk of the carbon paste is quaisireversible.

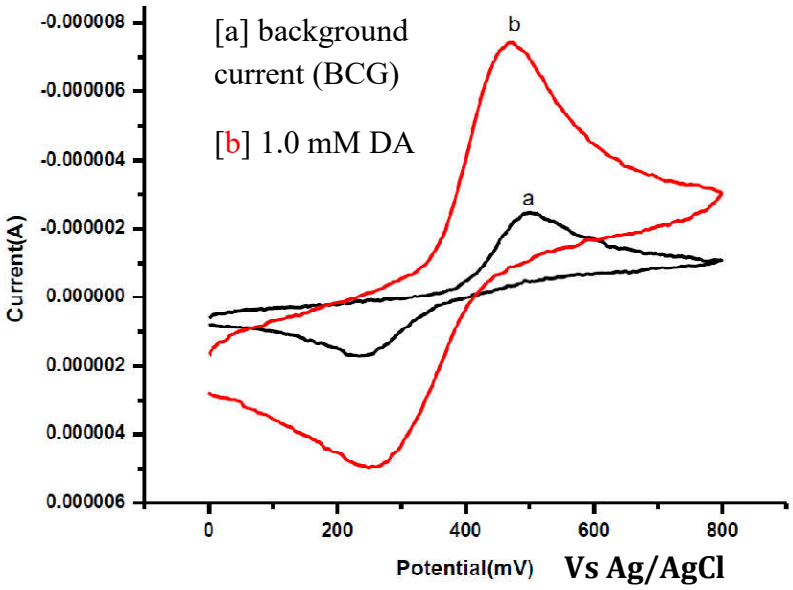

Figure 8. Typical cyclic voltammograms of 1,4-BQMCPE in 0.1 M PBS (pH = 7.0) at a Ag/AgCl electrod with a scan rate of 50 mV/S in the background (a) and presence of 1.0 mM DA.

The electrochemical properties of DA at the UMCPE and 1,4-BQMCPE were examined using cyclic voltammetry, and the result was shown in Figure9. At theUMCPE, the cyclic voltammogram of DA shows an oxidation peak potential at 497mV and reduction peak potential at 268 mV with low current signal of -2.193×10^{-6}A (**Figure 9, curve a**).

In comparison to UMCPE, electrochemical response of DA at1,4-BQMCPE after modification with 40 mg of 1,4-benzoquinone the electrode shows improvement in enhancement of both anodic and cathodic peak current, -6.381×10^{-6} Aand $+5.470\times10^{-6}$A respectively. The oxidation peak potential occurs at 468 mV and reduction peak potential at 262 mV (Vs. Ag/AgCl) (**Figure 9, curve b**). The peak-to-peak separation (ΔEP) was found to be 206 mV and the ratio of redox peak current (Ipa/Ipc)was 1.17, which were the characteristics of quasireversible electrode process. The modified electrode exhibited a strong promoting effect and a high stability towards the electrochemical oxidation of DA at pH 7 in phosphate buffer solution (PBS).

It was also observed that the peak currents enhanced at the 1,4-BQMCPE which provides more evidence for asserting that the 1,4-Benzoquinone in the carbon paste electrode possessed high electro catalytic activity towards the DA investigation.

Therefore, 1,4-BQMCPE is used for the entire studies to determine the electrochemical properties of DA by cyclic and differential pulse voltammetry. To do so, some optimization parameters such as pH, electrode composition, scan rate and pulse amplitude are incorporated to enhance theperformance of the modified electrode up on investigation of DA.

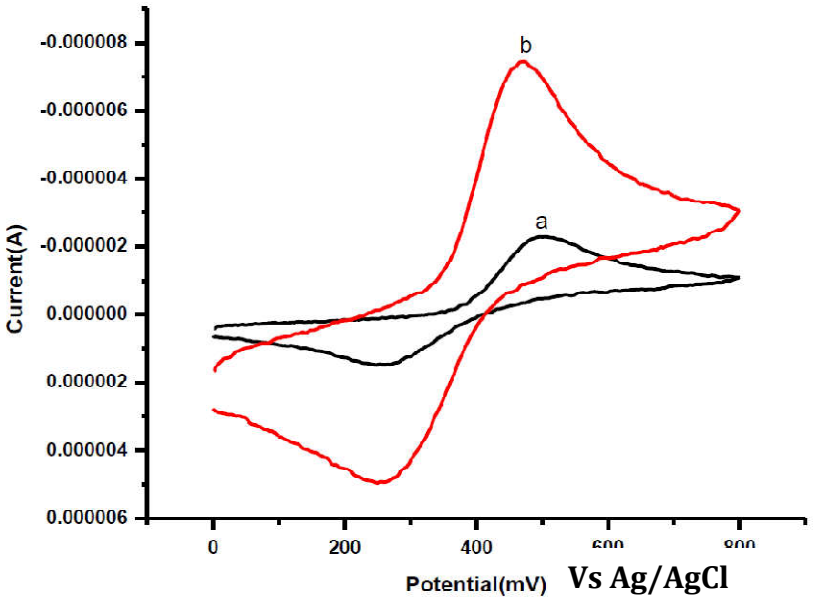

Figure 9. Cyclic voltammograms of 1.0 mM DA obtained at UMCPE (Curve a) and 1, 4-BQMCPE (Curve b) in 0.1 M PBS (pH = 7.0) using Ag/AgCl electrode, scan rate = 50 mV/s.

4.1.2. Effect of modifier composition

The effect of modifier composition strongly influences the voltammetric response of the electrode in the investigation of the analyte. The characterization of 1,4-BQMCPE was investigated by using cyclic voltammetric technique. 1,4-BQMCPE was prepared of different ratio by adding different amounts of 1,4-Benzoquinone in milligrams. By increasing the amount of 1,4-Benzoquinone from 20 mg to 40 mg in the carbon paste electrode, the electrochemical redox peak current of 1.0 mM DA goes on increasing in 0.1 MPBS of pH=7.0 as a supporting electrolyte.

Further increase of 1,4-Benzoquinone decreases the current signal of DA. This occurs due to a decrease in the graphite content in the paste and, consequent reduction of the conductive electrode area.

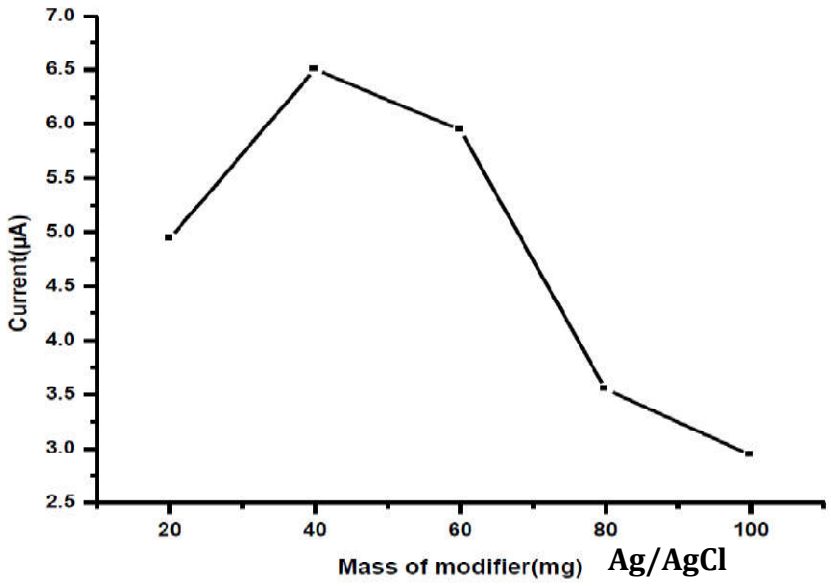

Figure 10. Effect of amount of 1,4-Benzoquinone on the anodic peak current of 1.0 mM DA in 0.1 M PBS (pH = 7.0), Ag/AgCl electrode at scan rate = 50 mV/s.

The graph of anodic peak current versus different amounts of 1,4-Benzoquinone in the carbon paste was plotted and the maximum current signal was noticed in 40 mg of 1,4-BQMCPE. So, 40 mg of 1,4-BQMCPE was chosen as optimum for the study of all other parameters (**Figure 10**).

4.1.3. Effect of pH

The electrochemical behavior of the carbon paste electrode modified with 1,4-Benzoquinonewas studied over a pH range of 4-8 in solution containing 1.0 mM of DA in0.1 M PBS as supporting electrolyte at a scan rate of 50 mV/s to optimize the electrochemical response of MCPE for the oxidation and reduction of DA. The graph of the anodic peak current as a function of different pH values at 1,4-BQMCPE in 0.1 M PBS is shown in **figure 11**.

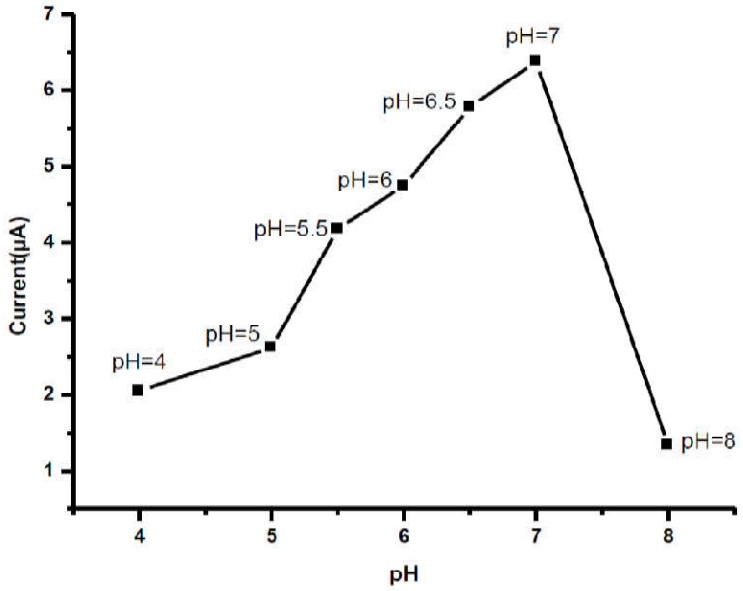

Figure 11. Effect of variation of pH on the anodic peak current of 1.0 mM DA in 0.1 M PBS at 1,4-BQMCPE at a scan rate of 50 mV/s

The effect of pH on anodic peak current in electrochemical processes depends on the specific reaction and analyte, but generally, changes in pH can influence the rate of electrochemical reaction steps, potentially leading to shifts in peak current and potential. The anodic peak current was increased with increasing pH from 4.0 to 7.0 and then decreased for higher pH values and moreover the electrode was not stable and the results were not reproducible at higher pH values, especially above pH=8.0. The better sensitivity and shape of the voltammogram was observed at pH=7.0 suggested it as optimal pH value (**Figure 11**). Therefore, pH=7.0 was chosen for the entire experiment.

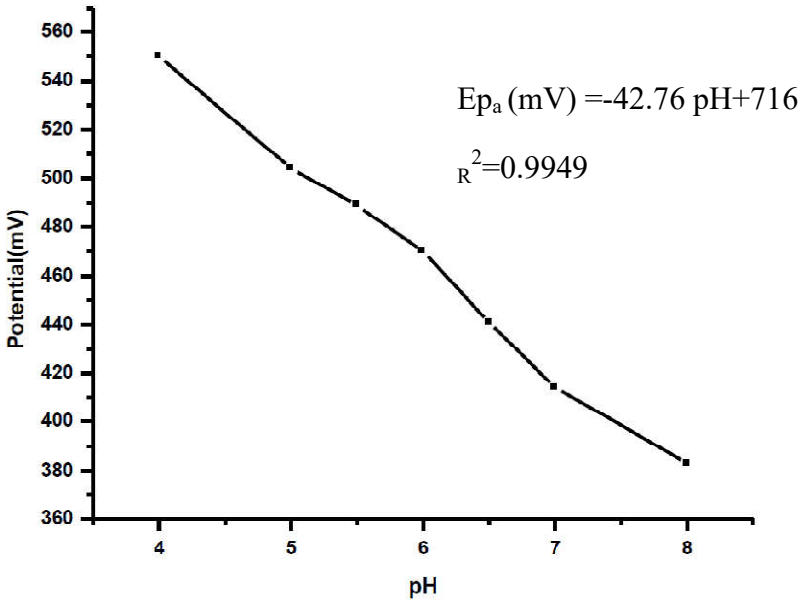

Figure 12. Effect of variation of pH on the anodic peak potential of 1.0 mM DA in 0.1 M PSB at 1,4-BQMCPE at a scan rate of 50 mV/s

The graph of anodic peak potential versus pH was plotted and the result shows that the anodic peak potential was shifted linearly towards less positive side with increasing in the pH values. The anodic peak potential of DA was shifted from 550 mV to 383 mV with respect to the pH change from 4.0 to 8.0 (**Figure12**). This linearity indicates that equal number of protons and electrons were involved in the electrochemical oxidation of DA (**figure 13**).

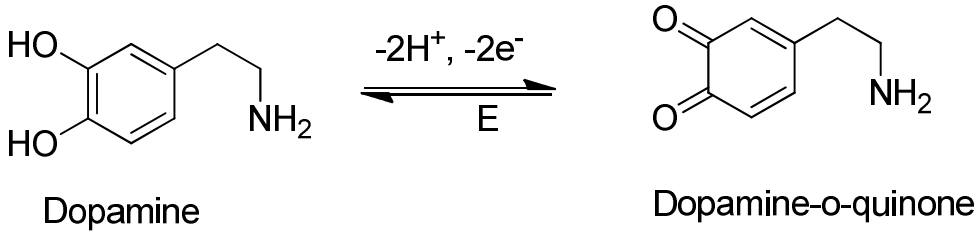

Figure 13. Oxidation reaction of dopamine

4.1.4. Effect of scan rate

The effect of varying scan rates on the cyclic voltammograms of 1.0 mM DA using1,4-BQMCPE in 0.1 M PBS of pH=7.0 supporting electrolyte was studied by varying the scan rate from 50-500 mV/s as shown below in **figure 14**.

It was found that with the increase of the scan rate, the oxidation peak current increased gradually and the oxidation peak potential shifted towards more positive potential, which is also one of the characteristics of quasireversible electrode process. Each curve was almost the same from 150 mV/s_500 mV/s but it is clearly seen that the peak current increases with increasing scan rate (**Figure 14**). This can be rationalized by diffusion layer and the time taken to record the scan. It is obvious that the cyclic voltammogram will take more time to record as the scan rate is decreased. Therefore, the size of the diffusion layer above the 1,4-benzoquinone electrode surfaces is different depending up on the voltage scan rate used. In a slow voltage scan rate the diffusion layer will grow much further from the electrode in comparison to a fast scan rate. From these results, a scan rate of 50 mV/s was chosen for the entire studies.

Peak shape appears slightly broadened and there is shit in peak potential at higher scan rates (**figure 14**). Peak broadening indicates that the electrochemical reaction is not happening quickly enough to keep up with the increasing scan rate, leading to a slower and less defined peak. If the electron transfer process is slow, the reaction cannot keep up with the faster scan rates, leading to the observed shifts and broadening. There was two potential shifts observed: Anodic shift: a shift of the oxidation peak (anodic) to more positive potentials with increasing scan rate suggests that the process is not fully reversible and that the kinetics of the electron transfer are slow. Cathodic shift: a shift of the reduction peak (cathodic) to more negative potentials with increasing scan rate suggests that the process is not fully reversible and that the kinetics of the electron transfer are slow.

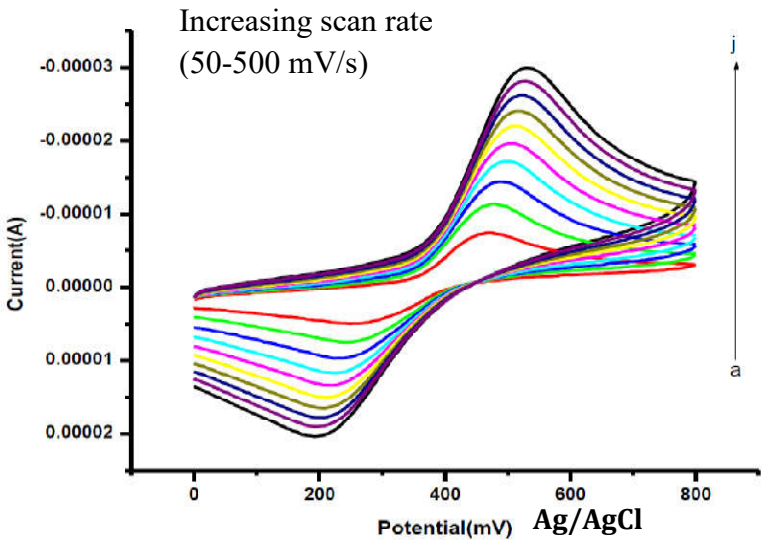

Figure 14. Cyclic voltammograms of different scan rate in the presence of 1.0 mM DA in 0.1 M PBS (pH = 7.0), Scan rate (a-j; 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mV/s)

The graph of anodic peak current (i_{pa}) versus scan rate (v) and square root of scan rate ($v^{1/2}$) were plotted and the graph obtained were nearly straight lines. In the range from 50 mV/s to 500 mV/s the anodic peak currents were proportional to the scan rate (v) and also the square root of scan rate ($v^{1/2}$) with linear regression coefficient, r, 0.99103 and 0.99907 for i_{pa} vs. v and ipa vs. $v^{1/2}$ respectively (**Figure 15** and **Figure 16**). When we compare the correlation coefficient of scan rate and the square root of scan rate, r-value for square root of scan rate is greater than that of r-value of scan rate. This means that peak current is directly proportional to the square root of the scan rate. The dependence of anodic peak current on the square root of scan rate and scan rate indicated that, the diffusion control is dominant, but adsorption also plays a role.

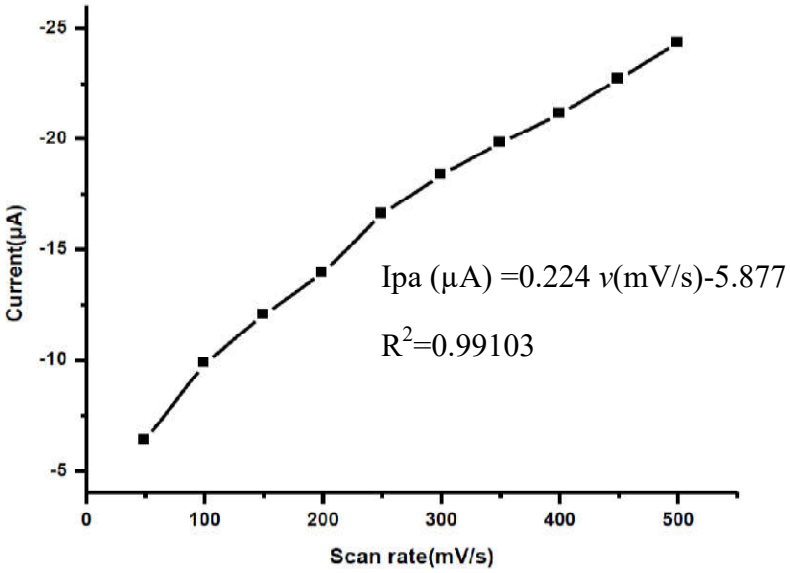

Figure 15. Linear plot of the anodic peak current of 1.0 mM in 0.1 M PBS of pH = 7.0, versus square root of scan rate: 50-500 mV/s.

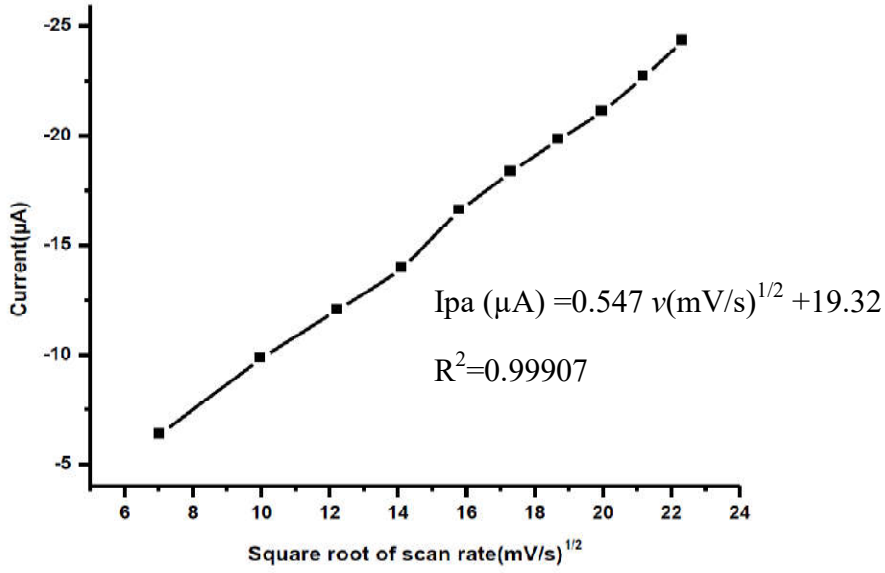

Figure 16. Linear plot of the anodic peak current of 1.0 mM DA in 0.1 M PBS of pH= 7.0, versus square root of scan rate (50-500 mV/s)

4.1.5. Determination of kinetic parameters

The transfer coefficient (α) can be calculated from the slope of the resulted curve of Ep vs. log *v* using equation 4.2.

$$Epa = K + \frac{2.3RTlogv}{2(1-\alpha)n\alpha F} \qquad \textbf{Equation 4.1}$$

$$\text{Slope} = \frac{2.3RT}{2(1-\alpha)n\alpha F} \qquad \textbf{Equation 4.2}$$

Where $\alpha$is transfer coefficient, n$\alpha$is the number of electrons involved in the rate determining step, *v* is scan rate, R is gas constant, E_{pa} is peak potential.

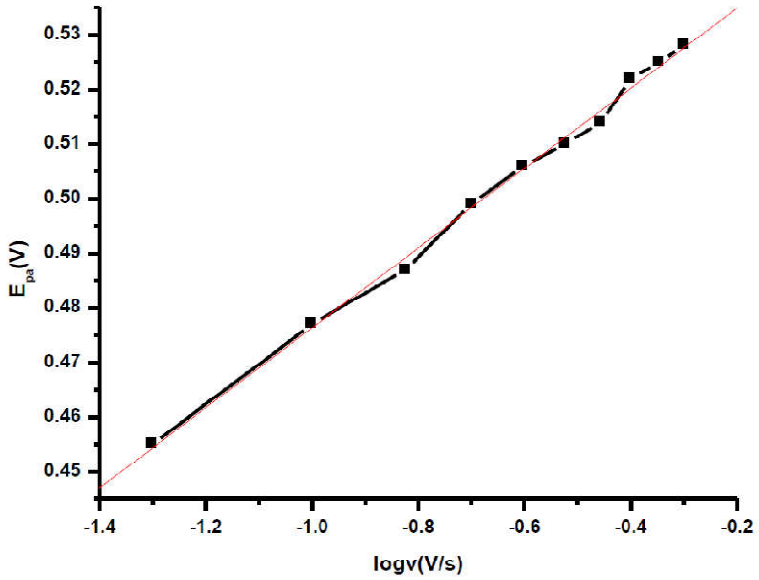

Figure 17. Plot of Ep versus logV

Based on Figure 16 and Eq. (4.2), the value of transfer coefficient (α) was calculated as,

$$0.07313 = \frac{2.3RT}{2(1-\alpha)n\alpha F}$$

By rearranging the above equation, the value of transfer coefficient (α) from this calculation is 0.798. Higher value of transfer coefficient (α) indicates deviation from reversible system. By calculating α from the slope of Ep vs. log*v* curve, k can be obtained from (**equation 4.3**)

$$K = E^{\circ} + \frac{RT}{(1-\alpha)n\alpha F} \times \left(0.78 + \frac{2.3}{2}\log\left(\frac{(1-\alpha)n\alpha FD}{((Ks,h))^2 RT}\right)\right)$$ Equation 4.3.

Where,

$\alpha$is anodic transfer coefficient;

nα is the number of electrons involved inthe rate-determining step;

E^0 is formal electrode potential $E^0 = (E_{pa}+E_{pc})^2 = 0.365V$;

Ks,h is heterogeneous electron transfer rate constant.

Based on Figure 17 and eq. (4.2), the value of α was calculated as **0.798**; and from Randless-Sevcik equation describes the relationship between peak current and scan rate for a reversible, diffusion-controlled process;

$i_p = (2.99 \times 10^5)n(\alpha n\alpha)^{1/2}ACD^{1/2}v^{1/2}$.; Ip is the peak current; n is the number of electrons transferred; A is the electrode area (cm^2); D is the diffusion coefficient (cm^2/s); C is the concentration of analyte (mol/cm^3); v is the scan rate (mV/s)

The calculated values are; $D = 2.18 \times 10^{-7}$ cm^2s^{-1}, $A = 0.07$ cm^2, and n=2.

The experimental intercept K from the graph was obtained as **0.5495**. By substituting the above values in eq. (4.3), we found that the heterogeneous electron transfer rate constant ks,h = $2.26 \times 10^{-4}cms^{-1}$. The calculated values of the heterogeneous rate constant (ks,h),diffusion coefficient (D), and anodic transfer coefficient (α) are summarized in table 4.1below.

Table 4.1: The Kinetic parameters of DA at 1,4-BQMCPE.

S. №	**Concentration of DA(M)**	**α**	**D (cm^2s^{-1})**	**$K_{h,s}$(cms^{-1})**
1	1.0×10^{-3}	0.798	2.89×10^{-7}	2.61×10^{-4}
2	1.5×10^{-3}	0.798	2.53×10^{-7}	2.43×10^{-4}
3	2.0×10^{-3}	0.798	2.16×10^{-7}	2.23×10^{-4}
4	2.5×10^{-3}	0.798	1.98×10^{-7}	2.17×10^{-4}
5	3.0×10^{-3}	0.798	1.85×10^{-7}	2.10×10^{-4}
6	3.5×10^{-3}	0.798	1.70×10^{-7}	2.00×10^{-4}
7	…….	Average	**2.18×10^{-7}**	**2.26×10^{-4}**

4.1.6. Effect of concentration of dopamine

The effect of varying concentration of dopamine was studied at 1,4-BQMCPE in 0.1 M PBS of pH=7.0 at a scan rate of 50 mV/s. The successive enhancement of cyclic voltammograms peak current on increasing DA concentration from 1.0×10^{-3}M to 3.5×10^{-3}M was shown **Figure 18**.

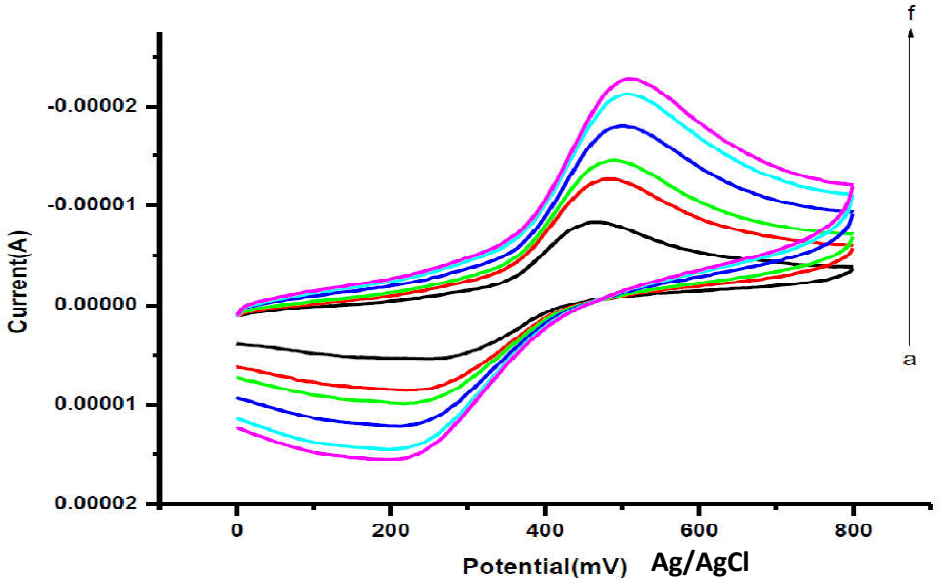

Figure 18. Cyclic voltammogram of different concentration of DA at 1,4-BQMCPE in 0.1 M PBS of pH = 7.0 with a scan rate of 50 mV/s, a-f, (a) 1.0, (b) 1.5; (c) 2.0; (d) 2.5; (e) 3.0; (f) 3.5 mM

By increasing the concentration of dopamine, the I_{pa} and I_{pc} goes on increasing with shifting E_{pa} towards more positive potential and E_{pc} with negligible shifting (Figure 18).

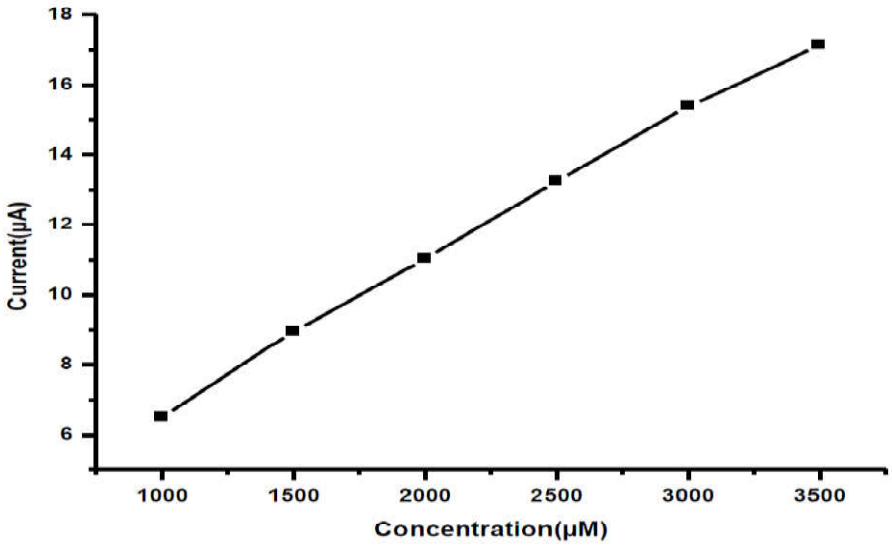

Figure 19. Calibration curve for the determination of DA in 0.1 M PBS of pH = 7.0 ata scan rate of 50 mV/s at 1,4-BQMCPE

The plot of peak current versus the respective concentrations of DA was found to be linear in the range of 1.0×10^{-3} M to 3.5×10^{-3} M with a correlation coefficient of r = 0.99901 (n=6) and a standard deviation (S) of **0.198** (Figure 19). The linear regression equation was found to be Ip(μA) = 2.449+0.0043C(μM). The enhancement of cyclic voltammograms peak current on increasing DA concentration is due to the presence of more ions in the solution, which makes the flow of electrons easy, and the molecules collide together. The detection limit of DA was calculated by using the formula (eqn 4.4) and it was found to be 1.39×10^{-4} M.

$$LOD = \frac{3S}{M} \qquad \textbf{Equation 4.4}$$

Where, **S** stands for standard deviation and **M** stands for mean concentration of analyte.

4.2. Differential Pulse Voltammetry Investigation

The DPV peak for the oxidation of DA at 1,4-BQMCPEis larger than that obtained at the UMCPE. In addition, the oxidation peak potential shifts from 439 mV of the UMCPE to 376 mV of the 1,4-BQMCPE indicating that 1,4-BQMCPEaccelerates the electron transfer reaction at the electrode surface (**Figure 20**).

This was confirmed earlier by cyclic voltammetry investigation part. Hence, 1,4-BQMCPE was further systematically studied by DPV for the investigation of DA in the similar potential range from 0 to 800 mV.

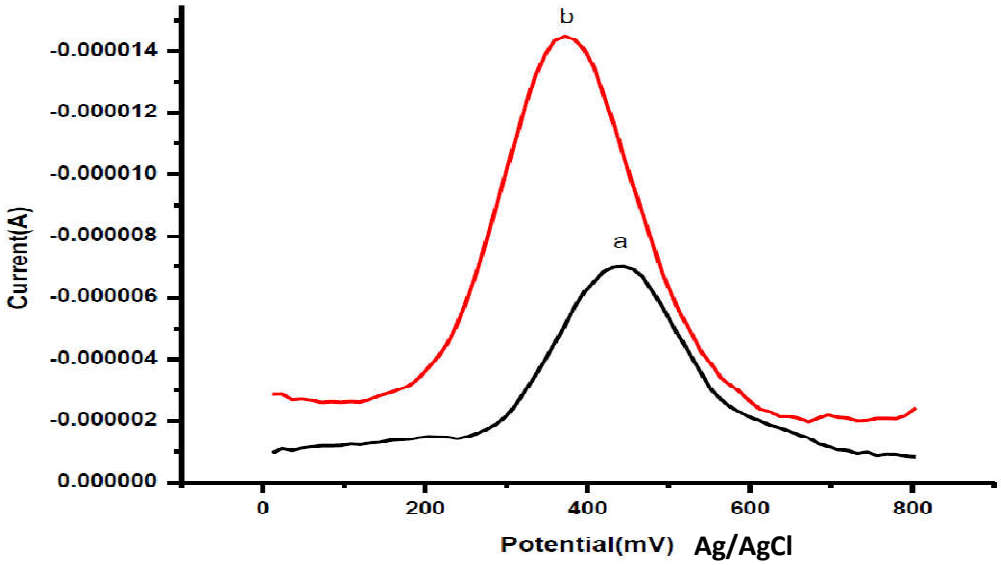

Figure 20. Typical DPV of 1.0 mM DA at (a) UMCPE and (b) 1,4-BQMCPE in 0.1 M PBS of pH =7.0 at a scan rate of 100 mV/s and pulse amplitude of 240 mV.

Detection of analytes using electrochemical techniques depends on the parameters of the techniques used. With regard to DPV, detection of the analytes depends on parameters such as pulse amplitude, scan rate, and pH. Hence, optimizations of these parameters are required for evaluation of the analytical performance of 1,4-BQMCPE for the electrochemical determination of DA.

4.2.1. Effect of scan rate

The effect of scan rate on the oxidation peak current of 1.0 mM of DA in 0.1 M PBS of pH=7.0 at 1,4-BQMCPE was studied by varying the scan rate from 25 to 125 mV/s as shown(**figure 21**).

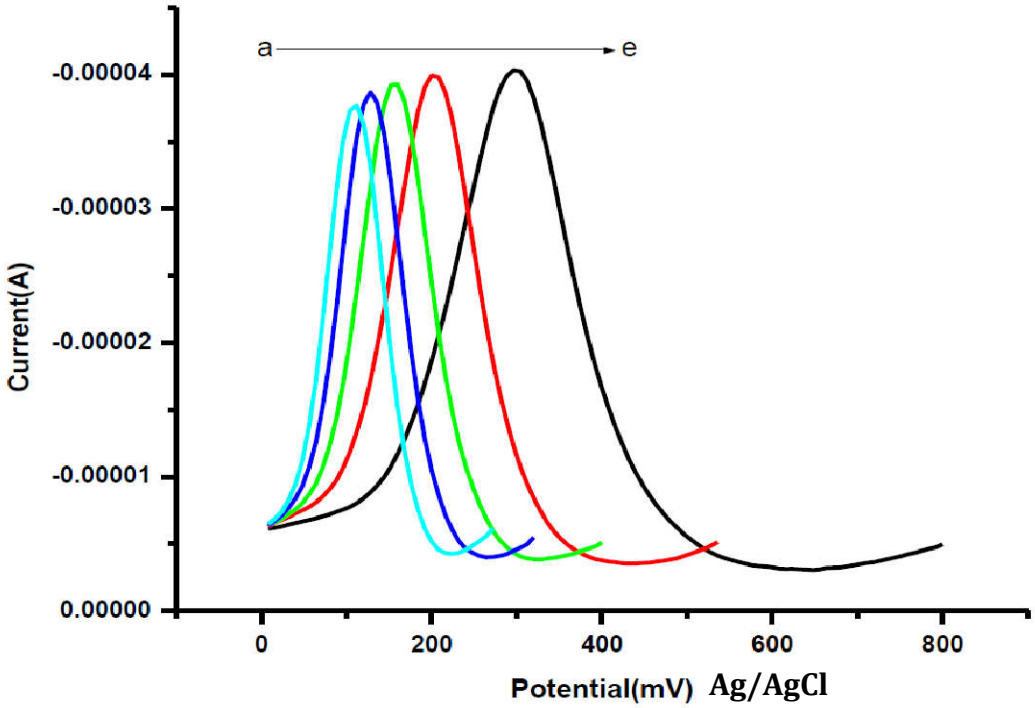

Figure 21. Differential pulse voltammograms for 1.0 mM of DA in 0.1 M PBS of pH = 7.0 at 1,4-BQMCPE at different scan rate: (a) 25, (b) 50, (c) 75, (d) 100 and (e) 125 mV/s using pulse amplitude of 240 mV.

The peak current was increased with increasing of scan rate. A scan rate of 100 mV/s was chosen for subsequent experiments because above 100 mV/s of scan rate the peak current became leveled off. The probable reason was the peak broadening above 100 mV/s of scan rate. When the peak currents were plotted against the square roots of scan rate, the following relationship was obtained.

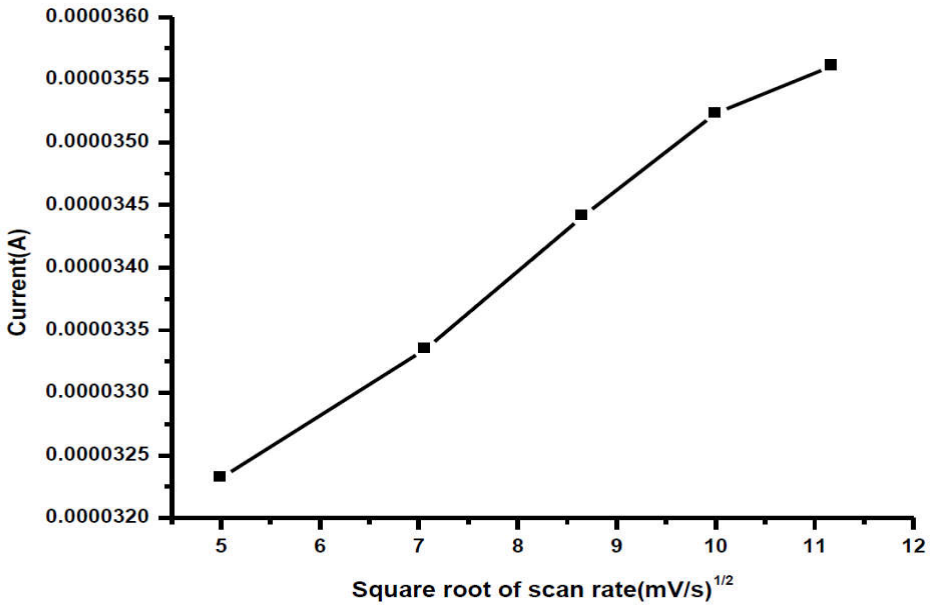

Figure 22. Plot of the DPV peak current of 1.0 mM of DA in 0.1 M PBS of pH = 7.0 at a pulse amplitude of 240 mV versus square root of scan rate

The DPV peak current was increased linearly as the square root of scan rate ranges from 5 to 10 $(mV/s)^{1/2}$ with a correlation coefficient of r=0.99628. However, for higher scan rates the graph was deviated from linearity which confirms that the peak current was leveled off and broadened (**Figure 22**). The linearity above allows concluding that the reaction was diffusion controlled.

4.2.2. Effect of pulse amplitude

The effect of differential pulse amplitude on the oxidation peak current of 1.0 mM of DA in 0.1 M PBS of pH=7.0 at 1,4-BQMCPE was studied by varying the differential pulse amplitudes from 60 mV to 240 mV at a scan rate of 100 mV/s (**Figure 23**). Upon increasing the differential pulse amplitude, a linear increase in the peak current was observed and hence, 240 mV was chosen as the pulse amplitude for the subsequent experiment (**Figure 23**).

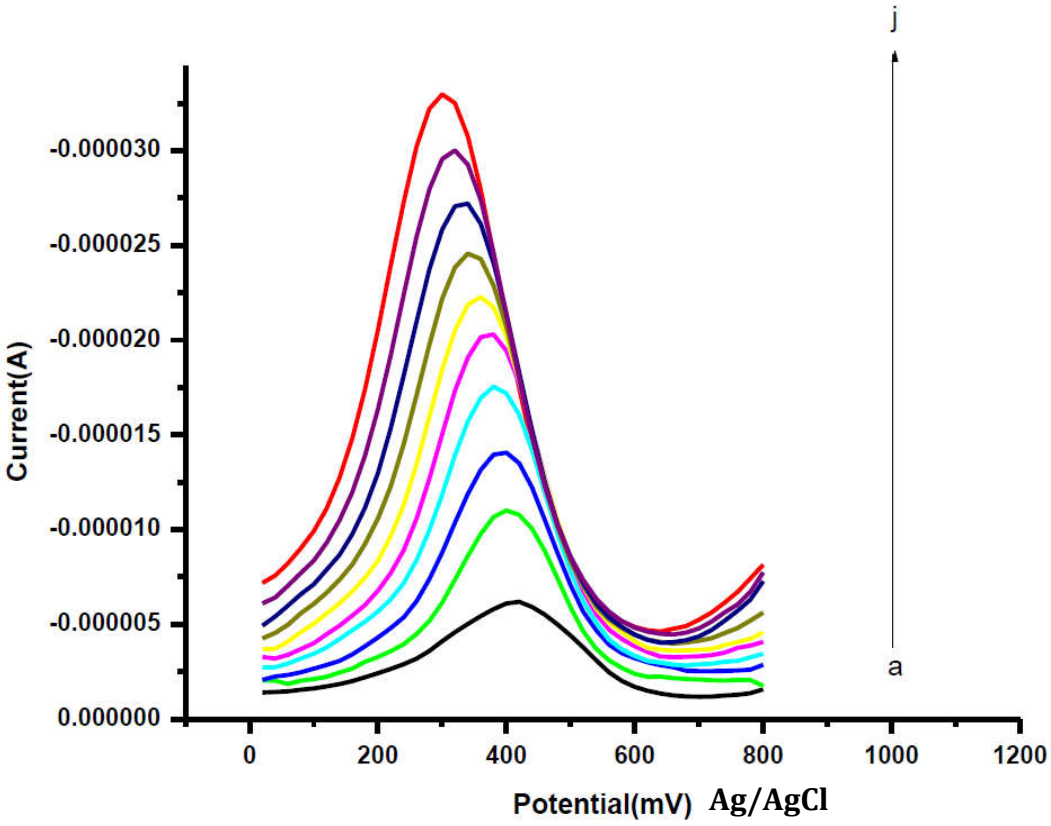

Figure 23. DPV of 1.0 mM of DA in 0.1 M PBS of pH = 7.0 at a scan rate of 100 mV.s and differential pulse amplitudes of (a) 60, (b) 80, (c) 100, (d) 120, (e) 140, (f) 160, (g) 180, (h) 200, (i), 220, and (j) 240 mV.

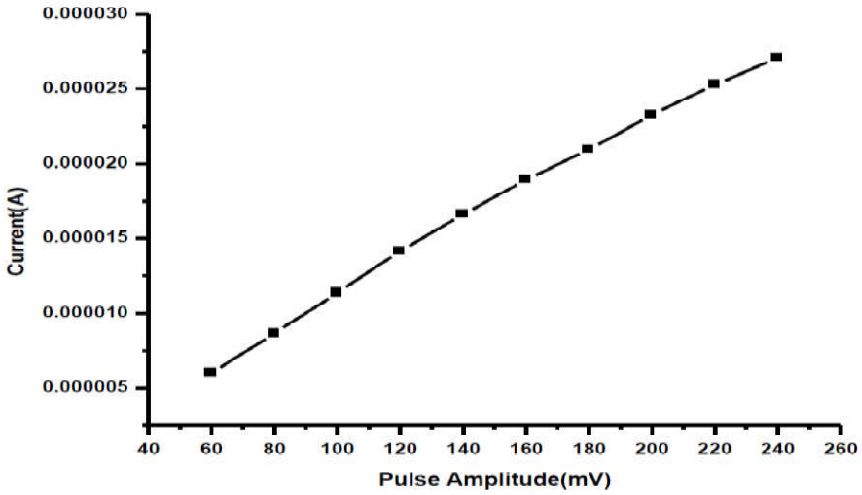

Figure 24. DPV peak currents of 1.0 mM of DA in 0.1 M PBS of pH = 7.0 at 1,4-BQMCPE at a scan rate of 100 mV/s and differential pulse amplitudes of 240 mV.

4.2.3. Effect of concentration of DA

Based up on the optimum experimental conditions shown in table 4.2 below, the effectof varying DA concentration on the DPV peak current response of DA was studied at 1,4-BQMCPE.

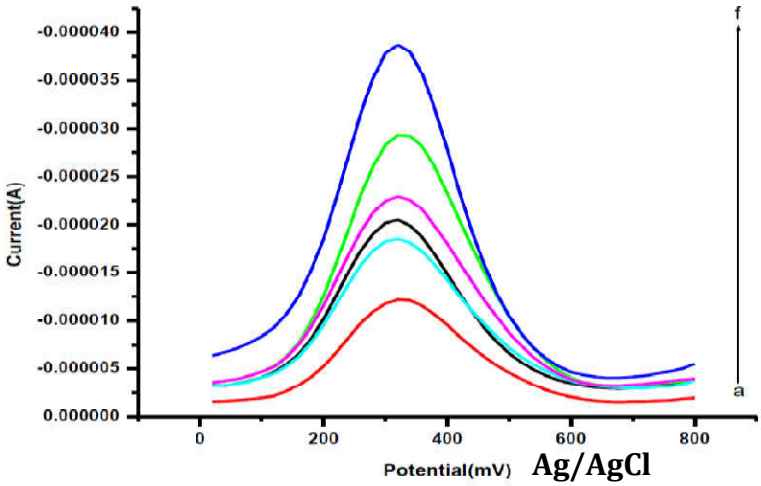

Figure 25. DPV of different DA concentrations of (a) 1.0, (b) 1.5, (c) 2.0, (d) 2.5, (e) 3.0, and (f) 3.5 mM in 0.1 M PBS of pH = 7.0 at a scan rate of 100 mV/s and pulse amplitude of 240 mV at 1,4-BQMCPE.

The successive enhancement of the DPV peak current response of DA on increasing DA concentration from 1.0 × 10−3 M to 3.5 × 10−3 M was shown (Figure 25).

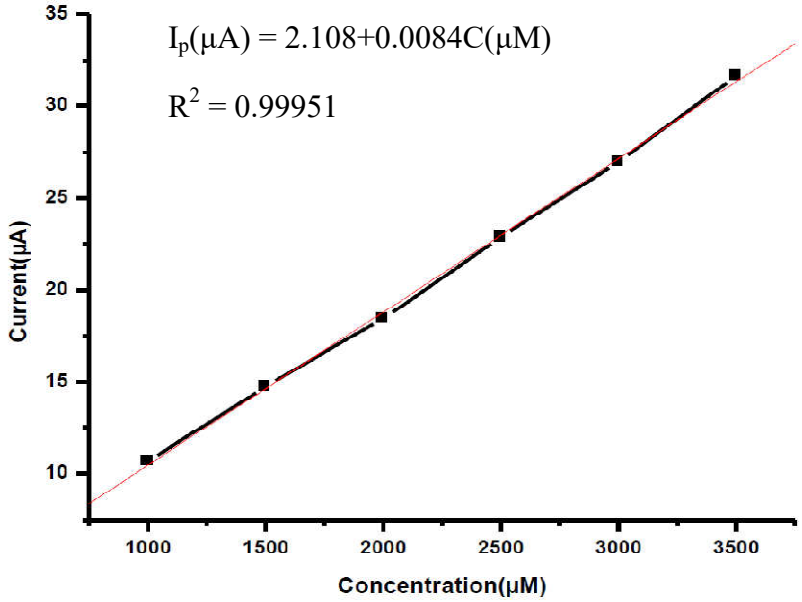

Figure 26. Linear plot for the determination of DA in 0.1 M PBS of pH = 7.0 at 1,4-BQMCPE at a scan rate of 100 mV/s and pulse amplitude of 240 mV with different DA concentrations: (a) 1.0, (b) 1.5, (c) 2.0, (d) 2.5 (e) 3.0, and (f) 3.5 mM.

The plot of DPV peak current versus the respective concentrations of DA was found to be linear in the range of 1.0×10^{-3} M to 3.5×10^{-3} M with a correlation coefficient of r = 0.99951 (n=6) and a standard deviation (S) of 0.2735 (**Figure 26**). The linear regression equation was found to be $I_p(\mu A) = 2.108+0.0084C(\mu M)$ with the limit of detection 7.64×10^{-5} M. The enhancement of the differential voltammograms peak current on increasing DA concentration is due to the presence of more ions in the solution, which makes the flow of electrons easy, and the molecules collide together.

4.3. Optimum Experimental Conditions

The optimum experimental conditions from experimental parameters for both CV and DPV determination of DA at 1,4-BQMCPE can be summarized as follows in **table 4.2**:

Table 4.2: Optimum experimental conditions for the determination of DA by CVand DPV at 1,4-BQMCPE.

Parameters	**Optimum value**	**Method**
pH Buffer Solution	7.0	CV, DPV
Scan rate, mV/s	50	CV
	100	DPV
Pulse amplitude, mV	240	DPV
Composition of modifier, mg	40	CV, DPV

5. CONCLUSSIONS

The electrochemical oxidation of dopamine was successfully investigated by CV and DPV with 1,4-BQMCPE. The optimum experimental conditions for the oxidation of dopamine were determined. The effect of composition of modifier, pH, scan rate, pulse amplitude, as well as concentration of analyte was observed on the voltammetric responses. The anodic transfer coefficient and the mean diffusion coefficient and heterogeneous electron transfer rate constant were determined. The voltammogram resulted from those parameters showed that quasireversible nature of the analyte towards modified electrode with the transfer of two electrons per molecule of the analyte. The linear scan rate dependence showed that the system undergoes both diffusion and adsorption controlled electrode process.

The method presented in this study is simple and fast, the linear working range was lower, and the detection limit was greatly improved to allow a sensitive detection of dopamine. The low detection limit and its high sensitivity suggest that the modified 1,4-BQMCPE can act as a useful electrode material for the development of electrochemical sensor for DA. The modified electrode acts as good sensor for dopamine and can be further applied for the investigation of other neurotransmitter. The proposed method can be applied to the determination of DA in urine samples.

6. REFERENCES

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