Chapter - II

CuO NANOPARTICLES SENSOR FOR THE ELECTROCHEMICAL DETERMINATION OF DOPAMINE

Sathish Reddy, B.E. Kumara Swamy, H. Jayadevappa,
2.1. Introduction

In the present work, different shaped CuO nanoparticles were synthesized using cetyl trimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) in a co-precipitation method. The CuO nanoparticles were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), infrared absorption spectroscopy (IR) and UV-visible absorption spectroscopy (UV-Vis). The prepared CuO nanoparticles were used for the preparation of modified carbon-paste electrodes (MCPE) for the electrochemical detection of dopamine (DA) at pH 6.0. The MCPE prepared from flake-shaped CuO nanoparticles exhibited an enhanced current response for DA. Electrochemical parameters, such as the surface area of the electrode, the heterogeneous rate constant \( k_h \) and the lower detection limit \( (5.5 \times 10^{-8} \text{ M}) \), were calculated and compared with those of the MCPE prepared from rod-shaped CuO nanoparticles. The MCPE prepared from SDS/polyglycine flakeshaped CuO nanoparticles exhibited a further improved current response for DA and a high selectivity \( (E_{AA} - E_{DA} = 0.28 \text{ V}) \) for the simultaneous investigation of DA and ascorbic acid (AA) at pH 6.0. The modified carbon-paste electrochemical sensors were compared and the MCPE prepared from SDS/polyglycine flakeshaped CuO nanoparticles exhibited better performance than the MCPE prepared from CTAB/polyglycine flakeshaped CuO nanoparticles.

2.2. Chemistry of Dopamine

Dopamine (DA), also known as "4-(2-aminoethyl) benzene-1, 2-diol" belongs to a member of the catecholamine family. Since the discovery of dopamine as a neurotransmitter in the late 1950s, dopamine has become the most widely studied catecholamine [1]. Dopamine plays an important role in the functions of the central nervous system, renal, hormonal, and cardiovascular systems. Dopamine was first synthesized by George Barger and James Ewens in 1910 however; it was not until 1958 when Arvid Carlsson discovered that dopamine was not just a precursor of norepinephrine and epinephrine that dopamine began to gain considerable interest in studies related to its role in the central nervous system and neurological disorders. In the
brain, dopamine functions as a neurotransmitter activating dopamine receptors and is produced in various areas of the brain such as the substantia nigra and the ventral tegmental area. DA is also a neurohormone released by the hypothalamus. Its main function as a hormone is to inhibit the release of prolactin from the anterior lobe of the pituitary. Dopamine can be supplied as a medication that acts on the sympathetic nervous system, producing effects such as increased heart rate and blood pressure. However, since dopamine cannot cross the blood-brain barrier, dopamine given as a drug does not directly affect the central nervous system. To increase the amount of dopamine in the brains of patients with diseases such as Parkinson’s disease and Dopa-Responsive Dystonia, a synthetic precursor to dopamine such as L-DOPA (levodopa) can be given, since this will cross the blood-brain barrier. Dopamine undergoes oxidation to form dopaquinone as shown in scheme 2.1.

\[
\begin{align*}
\text{DA} & \quad \text{Scheme 2.1. Oxidation mechanism for dopamine} \\
\text{DOQ} & \quad +2H^+ + 2e^- 
\end{align*}
\]

2.2.1. Biosynthesis of Dopamine

Dopamine is synthesized by the body and is involved in physical and cognitive functions. The synthesis process of dopamine is shown in scheme 2.2. Dopamine is biosynthesized in the body (mainly by nervous tissue and the medulla of the adrenal glands) first by the hydroxylation of the amino acid L-tyrosine to L-DOPA via the enzyme tyrosine 3-monoxygenase, also known as tyrosine hydroxylase, and then by the decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase (which is often referred to as dopa decarboxylase). In some neurons, dopamine is further processed into norepinephrine by dopamine beta-hydroxylase. Dopaminergic neurons located in the midbrain are the main sources of dopamine in the central nervous system. A basic definition of a neuron is a nerve cell in the nervous system that is responsible in
processing and transmitting messages by electrochemical signalling. The structure of a
typical neuron contains a central cell body called the soma that is surrounded by dendrites
that receives signals from other neurons and is attached to a long thin axon that carries
messages away from the cell body. The incoming messages from the dendrites are passed
to the end terminals of the axons and the neurotransmitters (dopamine) are released into
the synapse. The dopamine molecules diffuse across the synapse and interacts/attaches to
its specialized protein called dopamine receptors which send the message on to other
neurons. After the message is passed on, the receptors release the dopamine particles
back into the synapse where the excess dopamine is recycled for reuse. Therefore the
imbalance or dysfunction of the dopaminergic neuron process can result into various
neurological diseases, sleeping and eating disorders, additive behaviors associated with
drug abuse [2, 3].

2.2.2. Dysfunction of Dopamine

The developments of methods for measuring dopamine in biological systems are
of importance for the analysis and diagnosis of neurological disorders such as
Parkinson’s disease. There are over 1.2 million people in the U.S. who suffer from
Parkinson’s disease with 50,000 new cases reported annually and is one of the most
common neurological diseases in North America [4]. Parkinson’s disease is defined as a
neurological or degenerative disorder of the central nervous system. Patients who are
diagnosed with Parkinson’s disease have a 60 to 80 percent loss of these dopamine
producing neurons when symptoms appear. Diagnosis is normally based on medical
history and a neurological examination which consists of observations using the Unified
Parkinson’s Disease Rating Scale and an interview. Therefore early signs and symptoms
of the disorder can be dismissed as just the effects of normal ageing. Numerous studies
have shown that dopamine affects the brain processes that control movement, emotional
response, and the ability to experience pleasure and pain. Also an imbalance of dopamine
can lead to eating and sleeping disorders and additive behaviors associated with drug
abuse. Therefore, there is an immediate need to develop simple and rapid methods for
selectively determining dopamine in routine analysis.
2.2.3. Biological Relevance of Dopamine

Dopamine has many functions in the brain. Dopamine affects the basal ganglia motor loop which in turn affects the way the brain controls movements. Shortage of dopamine, particularly the death of dopamine neurons in the nigrostriatal pathway, causes Parkinson's disease, in which a person loses the ability to execute smooth, controlled movements. Degeneration of dopamine, decline of cognitive function, motor symptoms, and other problems lead to decreased efficiency and function of the brain and body. This leads to a downward spiral of further decrease in efficiency and function, which results in
degeneration, aging, breakdown, and death. Neurotransmitter release is initiated by an electrical impulse called an action potential. Each neuron has a resting membrane. When an appropriate neurotransmitter binds to receptors on the dendrites or cell bodies, ion channels open, allowing an influx of Na\(^+\) that changes the membrane potential and initiates an action potential or firing. It then propagates down the axon to the terminal at a rate of 0.5 m/s [5]. This firing causes voltage-gated Ca\(^{2+}\) channels to open in the terminals. The resultant Ca\(^{2+}\) influx triggers the vesicles to fuse with the cell membrane and release their contents, a process termed as exocytosis. Because some vesicles are docked adjacent to the membrane, exocytosis occurs on a millisecond timescale [6]. Neurotransmission involves the conversion of an electrical impulse to a chemical event and then to another electrical event, is extremely rapid. Action potentials and neurotransmitters represents the bricks with which the internal representation of the external world is build.

2.3. Chemistry of Ascorbic acid

Ascorbic acid (AA) is a sugar of molecular weight 176.13. The molecule, which is partially ionized at physiological pH, contains two acid-ionized groups (pKa 4.04 and 11.34). Though stable to air and light when dry, in aqueous solution it is powerful reducing agent, with redox potential of about 0.05 V at 30°C and pH 7.4. It readily undergoes reversible oxidation to dehydroascorbic acid (scheme.2.3).

![Scheme 2.3: Oxidation Mechanism of Ascorbic acid](image)

\[ AA \xrightarrow{+2H^+ + 2e^-} \text{Dehydro ascorbate (DHA)} \]

2.3.1. Biosynthesis of ascorbic acid

In mammals such as the rat, synthesis of AA occurs through intermediate formation of D-glucuronic acid, L-gulonic acid and L-gulonolactone. As L-gulonolactone
oxidase activity is confined to the liver, all AA within the central nervous system ultimately derives from the bloodstream. Primates and guinea-pigs are unusual amongst animals in their inability to synthesise AA and are therefore susceptible to the deficiency disease scurvy.

2.3.2. Biological Relevance of Ascorbic acid

This a water-soluble vitamin which is important in forming collagen, a protein that gives structure to bones, cartilage, muscle, and blood vessels. It also helps maintain capillaries, bones, and teeth and aids in the absorption of iron. Ascorbic acid, a reducing agent, is necessary to maintain the enzyme prolyl hydroxylase in an active form, most likely by keeping its iron atom in a reduced state. The precursor molecule to the protein collagen, procollagen, contains an unusual amino acid sequence in that every third amino acid is a glycine and contains a high frequency of two amino acids not found in any other proteins - hydroxyproline and hydroxylysine. These latter two amino acids are converted from proline and lysine, respectively, after the procollagen molecule has been synthesized. The hydroxylation of proline and lysine in procollagen is carried out by the enzyme prolyl hydroxylase using ascorbic acid as a cofactor. The natural form of the vitamin is the L-isomer. Ascorbic acid plays an important role as a component of enzymes involved in the synthesis of collagen and carnitine; however, its most vital role is as a water-soluble vitamin in the human body [7, 8]. Ascorbic acid is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of reactive oxygen and nitrogen oxide species, ascorbic acid has been shown to be effective against the superoxide radical ion, hydrogen peroxide, the hydroxyl radical and singlet oxygen [9]. Ascorbic acid protects folic acid reductase, which converts folic acid to folinic acid, and may help release free folic acid from its conjugates in food. Ascorbic acid facilitates the absorption of iron. It helps maintain elasticity of the skin aids the absorption of iron and improves resistance to infection, treatment of scurvy and may prevent the occurrence and development of cancer.
2.4. Review of CuO, CuO nanoparticles, nanoparticles modified electrode, detection of dopamine and nanoparticles synthesis

Copper (II) oxide is a p-type metal oxide semiconductor with a narrow band-gap that exhibits a versatile range of properties. It has been effectively used in the fabrication of electrical, optical and photovoltaic devices, heterogeneous catalysis, magnetic storage media, gas sensing, field-emission (FE) emitters and lithium ion electrode materials [10, 11].

Recently, the synthesis of leaf-like CuO nanoparticles and a Nafion/GOD/CuO modified electrode for the detection of glucose [12] and the synthesis of shuttle-like CuO nanoparticles and a CuO nanoparticles/poly(thionine) glassy carbon electrode for determination of Hg(II) in water samples have been reported [13]. CuO nanoparticles and their modified gold electrode have been used to investigate rutin [14]. A CuO nanoparticle-modified sol-gel-derived carbon ceramic prepared by microwave irradiation has been applied for the determination of adenine at very low potentials [15]. Additionally, a Au-nanoplates modified ITO electrode for the oxidation of dopamine (DA) and ascorbic acid (AA) exhibits good electrocatalytic activity than spherical-gold-nanoparticle-modified ITO electrode [16].

DA is a prevalent catecholamine neurotransmitter in the brain. Degeneration of dopaminergic neurons is the predominant cause of Parkinson’s disease (PD), with which approximately 500,000 people in the United States have been diagnosed [17], and causes reduced DA concentrations, even DA depletion, in the striatum and probably in other basal ganglia areas [18,19]. DA is electrochemically active (oxidizable), which allows electrochemical techniques to be employed for the detection of DA levels. Electrochemical detection is a simple, sensitive and environmentally friendly detection method that is even suitable for the analysis of colored or turbid samples. However, the electrochemical detection of DA in physiological samples is challenging because of the high concentrations of ascorbic acid (AA) (0.2–0.5 mM) that accompany the low concentrations of DA (10^{-8} to 10^{-7} M) in physiological samples [18, 20, 21]. In addition, DA exists in cationic form, whereas AA is in an anionic form. AA is not only an electrochemically active (oxidizable) compound but is also electrochemically oxidized at a potential close to that of DA at conventional unmodified electrodes [18, 22, 23].
The literature contains several reports that claim to have solved this problem. The reported approaches include the use of an IL N butylpyridinium hexafluorophosphate (BPPFe) MCPE [24], a ZnO MCPE [25], a ZnO/redox mediator composite-film-coated GCE [26], a polyglycine film coated on CPE [27], a poly(calmagite) film coated on CPE [28] and a SDS MCPE [29]. In a solution if the concentration of SDS is greater than that of CMC, a separation between the peaks of AA and DA, $E_{AA} - E_{DA}$, of 0.238 V is possible using a CPE [30]. A cetyl trimethyl ammonium bromide (CTAB) solution has also enabled the separation of the oxidation peak potentials of AA and DA with a tin hexacyanoferrate-modified CPE [31].

Rod-shaped CuO nanoparticles have been synthesized using a one-step annealing process in air with copper plates as the starting material [32], and flake-shaped CuO nanoparticles have been synthesized by a hydrothermal method [33].

2.5. Experimental Methods

2.5.1. Apparatus

The CuO nanoparticles were characterized by various techniques. Powder XRD patterns were recorded on a Philips XRD X'Pert Pro diffractometer equipped with a Cu-Kα radiation ($\lambda = 1.5438 \, \text{Å}$) source. IR absorption spectra were recorded on a Perkin Elmer Spectrum 1000 FTIR spectrometer on thoroughly dried samples using KBr pellets as dilutants. UV-visible spectra were obtained on a Perkin Elmer UV–VIS spectrophotometer by dispersing and sonicating CuO nanoparticles in water from a Millipore purification system. The structural morphology of the CuO nanoparticles was studied using a JEOL JSM-848 scanning electron microscope and a JEOL 2000 Fx-II transmission electron microscope equipped with ultra-thin windows from Oxford Instruments. All of the electrochemical experiments were performed using a single-compartment, three-electrode cell with MCPEs prepared with CuO nanoparticles, polyglycine/flake-shaped CuO nanoparticles, SDS/polyglycine/flake-shaped CuO nanoparticles and CTAB/polyglycine/flake-shaped CuO nanoparticles as the working electrode. An aqueous saturated calomel electrode (SCE) was used as the reference electrode, and a Pt wire served as the auxiliary electrode. All potentials were measured
and reported vs. the SCE. The cyclic voltammetry (CV) measurements and differential pulse voltammetry (DPV) techniques were performed on a model 660c (CH Instruments) potentiostat/galvanostat.

2.5.2. Materials

CuSO₄·6H₂O was purchased from S. D. Fine Chemicals. Absolute ethanol (99.9%), sodium hydroxide (NaOH) and graphite powder were from Merck Chemicals, and DA, CTAB and SDS were purchased from Himedia Chemicals. DA stock solutions were prepared in 0.1 M perchloric acid. Phosphate buffer was prepared, and the pH was adjusted by the addition of 0.2 M NaH₂PO₄ and Na₂HPO₄ solutions. All the aqueous solutions were prepared with double-distilled water.

2.5.3. Preparation of CuO nanoparticles

The rod- and flake-shaped CuO nanoparticles were synthesized according to the co-precipitation method described elsewhere [34, 35], with minor modifications. In a typical experiment, the first solution contained 0.01 M CuSO₄·6H₂O and 0.02 M SDS, and the second solution contained 0.02 M NaOH; all of the solutions were prepared using distilled water. The first solution was added to the second solution with continuous stirring. The resulting brown-colored precipitate was filtered through Whatmann filter paper (grade 41) and dried at 80°C in a hot-air oven for approximately 1 h. The dried precipitate was transferred to a silica crucible and ignited at 400°C for approximately 3 h. The obtained powder was then washed with ethanol three or four times to remove impurities present in the CuO nanoparticles. The same procedure was followed for the preparation of CuO nanoparticles using CTAB as the surfactant.

2.5.4. Preparation of bare carbon-paste electrode and modified carbon-paste electrode

The bare carbon-paste electrode (bare CPE) was prepared by hand mixing 80% graphite powder with 20% silicon oil in an agate mortar for approximately 30 min to produce a homogenous carbon paste. The paste was packed into the homemade cavity and smoothed on a piece of weighing paper. The modified carbon-paste electrode (MCPE) was prepared by the addition of 30 mg of CuO nanoparticles to the previously prepared graphite powder/silicon oil mixture.
2.5.5. Preparation of polyglycine/flake-shaped CuO nanoparticle modified carbon paste electrode

The following procedure was used to pack the paste and apply a polyglycine film onto the MCPE prepared from flake-shaped CuO nanoparticles [26]. Electrochemical polymerization of glycine on the MCPE prepared from flake-shaped CuO nanoparticles was performed using a cyclic voltammetric method in an aqueous solution that contained 0.04 M glycine in 0.2 M acetate buffer solution at pH 5.0. Electropolymerization was achieved by the formation of a film that grew between -0.5 V and 1.8 V at a scan rate of 100 mV/s for five cycles using CV. After polymerization, the electrode was thoroughly washed with distilled water.

2.5.6. Preparation of SDS/polyglycine/flake-shaped CuO nanoparticle and CTAB/polyglycine/flake-shaped CuO nanoparticle modified carbon paste electrode

SDS solution (10 µL) was added to the surface of the MCPE prepared from polyglycine/flake-shaped CuO nanoparticles for 5 min. The electrode was later thoroughly rinsed with water to remove unabsorbed modifier and dried in air at room temperature. The same procedure was followed for the preparation of the MCPE prepared from CTAB/polyglycine/flake-shaped CuO nanoparticles using a CTAB solution (10 µL).

2.6. Results and Discussion

2.6.1. Characterization

The XRD pattern of the obtained CuO nanoparticles is shown in Fig.2.1. All peaks in Figs.2.1.(A, (SDS)) and (B, (CTAB)) can be well indexed to the monoclinic structure of copper oxide (JCPDS PDF, no. 05-0661) with high crystallinity. No impurity peaks of other copper oxides were observed, which indicates the high purity of the products. The crystallite sizes of the CuO nanoparticles were calculated using the Debye–Scherrer formula. The average particle size is tabulated in Table.2.1.
The IR transmittance spectrum for the CuO nanoparticles synthesized using SDS is displayed in Fig. 2.2. (A). The peaks at approximately 2923, 2858, 1439 and 1113 cm\(^{-1}\) are due to C–H stretching and bending. These band vibrations provide evidence for the incorporation of SDS into the copper oxide. Broad peaks at approximately 3421 and 1610 cm\(^{-1}\) are attributed to H–OH stretching, and a small peak at approximately 2350 cm\(^{-1}\) is attributed to O=C=O stretching. The peak at 509 cm\(^{-1}\) is a characteristic peak of Cu–O stretching [36, 37].

The IR transmittance spectrum for the CuO nanoparticles synthesized using CTAB is displayed in Fig.2.2.(B). Bands observed in the 2800–3000 cm\(^{-1}\) region are attributed to the CTAB surfactant [38-40]. The CTAB IR spectra show two intense bands that are assigned to asymmetric (2924 cm\(^{-1}\)) and symmetric (2852 cm\(^{-1}\)) stretching vibrations of C–CH\(_2\) in the methylene chains. The sharp bands in the region of 1439 cm\(^{-1}\) are attributed to the deformation of –CH\(_2\)– and –CH\(_3\) groups [39] in the incorporated surfactants. The weak bands detected in the region of 2924 and 2852 cm\(^{-1}\) were assigned to C–CH\(_3\) asymmetric stretching and N–CH\(_3\) symmetric stretching vibrations of the solid and the surfactant, respectively. These band vibrations provide evidence for the incorporation of CTAB into the copper oxide. The broad band between 3200 and 3600 cm\(^{-1}\) and the band centered at 1630 cm\(^{-1}\) were observed for all samples and assigned to O–H stretching and deformation vibrations of weakly bound water. Furthermore, the presence of bands at 1113 cm\(^{-1}\) clearly indicates the binding of CTAB molecules to the CuO [41,42]. The sharp peak at 509 cm\(^{-1}\) is attributed to the framework vibrations of copper oxide [42].

The surface morphology of the samples obtained using SDS and CTAB was examined using SEM and TEM; the images are shown in Fig.2.3. (A- D). The SEM and TEM images clearly show different morphologies and sizes for CuO nanoparticles prepared in different surfactants. A rod-like morphology was observed for CuO nanoparticles (SDS) in the SEM image in Fig.2.3.(A) and in the corresponding TEM image for the same sample in Fig.2.3.(C). Upon close examination, the particles in the TEM image were observed to be rod-shaped with a width of approximately 100 nm and a length of 200 nm. Flake-like morphology was observed for the CuO nanoparticles (CTAB) in the SEM image in Fig.2.3.(B) and in the corresponding TEM image for the
same sample in Fig.2.3.(D). The particles, upon close inspection in the TEM image, were observed to be round and flake-shaped with a size of approximately 25 nm.

The UV–visible absorption spectra of the prepared CuO nanoparticles dispersed in ethanol solution show broad absorption peaks centered at approximately 366 nm for the flake-shaped CuO nanoparticles and at 394 nm for the rod-shaped CuO nanoparticles, as shown in Fig.2.4 (A) and (B). The UV–visible absorption spectra for both types of CuO nanoparticles show clear evidence that the absorption wavelength of the flake-shaped CuO nanoparticles was blue-shifted compared to that of the rod-shaped CuO nanoparticles. The obtained wavelengths of maximum absorption are tabulated in Table 2.1.

2.6.2. Mechanisms

According to Yin et al. [43], the mechanism of the effect of SDS on the formation of rod-shaped CuO nanoparticles may be explained as follows: first, in aqueous solution, the hydrophilic group will point to the outer surface of the capsule, while the hydrophobic end points inward due to the electrostatic interaction of the sulfonic groups and the Cu\(^{2+}\) ions. The outer surface of the hydrophilic end is occupied by numerous Cu\(^{2+}\) ions. When this solution is subsequently added dropwise to the 0.02 M NaOH solution, it then contains hydroxyl groups that react to form dodecyl sulfate copper hydroxide ion (DS Cu(OH)\(_2\)\(^{4+}\)); these ion nuclei will form active sites to generate DS [Cu(OH)\(_2\)]\(_n\) rod-like structures. After calcination, the water molecules are removed to form DS [CuO]\(_n\) rod-like nanostructures.

The mechanism of the effect of CTAB on the formation of flake-shaped CuO nanoparticles may be explained as follows: first, in aqueous solution, the hydrophilic group will point to the outer surface of the capsule, while the hydrophobic end points inward. After the solutions of the cetyltrimethyl ammonium ion (CTA\(^+\)) group and Cu\(^{2+}\) ions are added dropwise to 0.2 M NaOH solution, it contains hydroxyl groups that react to form Cu(OH)\(_2\)\(^{-4}\) ion nuclei, which surround the CTA\(^+\) groups due to electrostatic interactions. These ion nuclei subsequently form active sites to generate [Cu(OH)\(_2\)]\(_n\) flake-shaped structures with the surrounding CTA\(^+\) groups. After calcination, the water molecules are removed to form the CTA [CuO]\(_n\) flake-like nanostructures.
2.6.3. Effect of pH

The effect of pH on the determination of DA in PBS solution at the MCPE prepared with CuO nanoparticles was carefully investigated in the pH range of 5.5–8.0. Graphs of $i_{pa}(A)$ versus the pH of the solution and $E^0(V)$ versus the pH of the solution for MCPEs prepared with flake- and rod-shaped CuO nanoparticles are shown in Fig.2.5 (A) and (B), respectively. The anodic peak current of DA increases with increasing pH values until the pH reaches 6.0 to 6.5; the anodic peak current then decreases with further increases in the pH. The maximum anodic peak current occurred at pH 6.0. Therefore, PBS with a pH of 6.0 was selected for all subsequent electrochemical DA analyses. The formal potential ($E^0$) of DA decreased with an increase in the pH value. A linear regression equations obtained were $E^0 (V) = -0.03691 \text{ pH } + 0.3968$ (n=6, $Y=0.986$) for the MCPE prepared with flake-shaped CuO nanoparticles and $E^0(V) = -0.0390 \text{ pH } + 0.428$ (n=6, $Y=0.996$) for the MCPE prepared with rod-shaped CuO nanoparticles. The slopes were 0.0369 and 0.039 V/pH, respectively, which were nearly half of the theoretical value of -0.0576 V/ pH at 18°C. The results indicate that two-electron transfer accompanied by two-proton transfer occurred, which was identical to the results reported by Sun et al [14] and by Bath et al [34]. According to the above results, the electrode process for DA on the MCPE prepared with CuO nanoparticles is shown in Scheme.2.1.

2.6.4. Electrochemical response of DA at the bare electrode and the MCPE

The electrochemical responses of $5 \times 10^{-5}$ M DA in 0.2 M phosphate buffer solution of pH 6.0 at the bare CPE and at the MCPE prepared with CuO nanoparticles were measured at a scan rate of 0.200 Vs$^{-1}$. The corresponding peak-potential differences, $\Delta E_p$, of 0.159 V, 0.097V and 0.084V for the bare CPE and the MCPEs prepared from rod- and flake-shaped CuO nanoparticles, respectively, are shown in Fig.2.6.(A-C). The result indicates both CuO nanoparticles exhibit good electrocatalytic activity than bare CPE and among CuO nanoparticles a flake-shaped CuO nanoparticles exhibit enhanced current response with slight reduction of over potential than the rod-shaped CuO nanoparticles. This shows that the MCPE prepared from flake-shaped CuO nanoparticles exhibit good electrocatalytic activity for the detection of DA and the obtained results are consistent with the reported literature [16].
2.6.5. The effect of scan rate

The effect of scan rate for DA in phosphate buffer solution at pH 6.0 was studied by CV at MCPEs prepared from flake- and rod-shaped CuO nanoparticles. The results in Figs.2.7.(A) and (D) show an increase in the redox peak current at a scan rate of 0.01 to 0.800 V s\(^{-1}\). The graph obtained exhibited good linearity between the square root of the scan rate (\(v^{1/2}\)) and the redox peak currents for the MCPE prepared with the flake-shaped CuO nanoparticles, with correlation coefficients of \(r^2=0.999\) and 0.999. The MCPE prepared from rod-shaped CuO nanoparticles exhibited correlation coefficients of \(r^2=0.996\) and 0.998, as shown in Figs.2.7.(B) and (E). These results indicate that the electron-transfer reaction of MCPEs prepared with both rod- and flake-shaped CuO nanoparticles was a diffusion-controlled process. The surface area available for the electron transfer to species in the solution can be estimated by the Randles-Sevcik equation (1) [45, 46]. This equation relates the peak current for an electron-transfer-controlled process with the square root of the scan rate:

\[
i_p = 2.69 \times 10^5 \, n^{3/2} \, A \, D^{1/2} \, C \, v^{1/2}
\]

where, \(i_p\) is the peak current (A), \(A\) is the electroactive area (cm\(^2\)), \(C\) is the concentration of the electroactive species (mol cm\(^{-3}\)), \(n\) is the number of exchanged electrons, \(D\) is the diffusion coefficient (cm\(^2\) s\(^{-1}\)) and \(v\) is the scan rate (V s\(^{-1}\)). The values of the diffusion coefficients were obtained from the slopes of the \(i_{pa}\) versus \(v^{1/2}\) plots shown in Figs.2.7 (B) and (E). The surface area of both of the MCPE electrodes prepared from CuO nanoparticles were calculated using equation (1); the results are presented in Table.2.1.

The MCPE electrodes prepared from CuO nanoparticles shows diffusion-controlled process and The peak-to-peak separation (\(\Delta Ep\)) was 0.097, 0.084V and the ratio of redox peak currents (\(i_{pa}/i_{pc}\)) was 1.53,1.48 which were the characteristics of a quasi-reversible electrode process. Wei Sun et.al.[24], was successfully applied the below equation for diffusion-controlled and quasi-reversible electrode process using equations (2) and (3) calculated the number of electron transferred, charge-transfer coefficient and equation (4) was used for the calculation of electron-transfer rate constant. Therefore the below equations are used for the calculations.
\[ E_{pc} = E^° - \frac{RT}{\alpha n F} \ln v \] \hspace{2cm} (2)

\[ E_{pa} = E^° + \frac{RT}{(1-\alpha) n F} \ln v \] \hspace{2cm} (3)

and

\[ \log k_s = \alpha \log (1-\alpha) + (1-\alpha) \log \alpha - \log \frac{RT}{n F v} - \frac{(1-\alpha)\alpha F \Delta E_p}{2.3RT} \] \hspace{2cm} (4)

Where \( \alpha \) is the charge-transfer coefficient, \( n \) is the number of electrons transferred, \( k_s \) is the electron-transfer rate constant, \( E^° \) is the formal potential, \( F \) is the Faraday constant, \( E_{pc} \) is the cathodic peak potential and \( E_{pa} \) is the anodic peak potential. The redox peak potentials increase as the scan rate increases. The relationship of the peak potentials with the scan rate was constructed and could be used for the calculation of the electrochemical parameters. A linear relationship between \( E_p \) and \( \ln v \) was established according to the Laviron equation [24, 44], and two straight lines were obtained, as shown in Figs.2.7.(C) and (F). The linear-regression equations calculated for the MCPEs prepared with flake- and rod-shaped CuO nanoparticles were \( E_{pa}(V) = 0.01676(Vs^{-1}) \ln v + 0.2973(V) \) \((n = 10, \gamma = 0.990)\), \( E_{pc}(V) = -0.00982(Vs^{-1}) \ln v + 0.13557(V) \) \((n = 10, \gamma = 0.993)\) and \( E_{pa}(V) = 0.0176(Vs^{-1}) \ln v + 0.3036(V) \) \((n = 10, \gamma = 0.990)\), \( E_{pc}(V) = -0.00989(Vs^{-1}) \ln v + 0.12707(V) \) \((n = 10, \gamma = 0.993)\), respectively. The charge-transfer coefficient \( (\alpha) \) and the number of electrons \( (n) \) were calculated according to equations (2) and (3), and the electron-transfer rate constant \( (k_s) \) was calculated using equation (4). All of the calculation results are shown in Table.2.1 and calculated \( k_s \) value for MCPEs prepared with flake-shaped CuO nanoparticles are consistent with the reported literature [24].

2.6.6. The effect of the concentration of DA

The differential pulse voltammetry technique was used for analysis of DA concentration, which was varied from 0.1 to 30 \( \mu M \). The results for the MCPEs prepared from flake- and rod-shaped CuO nanoparticles are shown in Figs.2.8 (A) and (C), respectively. The corresponding graphs of anodic peak current versus concentration of DA shows two linear relationship ranges of 0.3 to 1.4 \( \mu M \) and 2 to 20 \( \mu M \), with linear regression equations of \( i_{pa} (\mu A) = 2.99 (C \mu M/L) + 0.569 (\mu A) \) and \( i_{pa} (\mu A) = 0.303 \).
(CμM/L) + 2.71 (μA), respectively. The correlation coefficient for the first linearity was 0.991, and that for the second was 0.998 for the MCPE prepared with flake-shaped CuO nanoparticles, as shown in Fig. 2.8(B). For DA concentrations of 0.6 to 1.4 μM and 2 to 20 μM, the linear regression equations were \( i_{pa} (μA) = 1.372 (CμM/L) + 0.853 (μA) \) and \( i_{pa} (μA) = 0.193 (CμM/L) + 2.048(μA) \), respectively. The correlation coefficient for the first linearity was 0.996, and that for the second 0.997 for the MCPE prepared with rod-shaped CuO nanoparticles, as shown in Fig.2.8. (D). The decrease in the sensitivity (slope) in the second linear range was due to kinetic limitations [28, 48]. The detection limits for DA in the lower concentration range was \( 5.52\times10^{-8} \) M for the MCPE prepared with flake-shaped CuO nanoparticles and \( 1.8\times10^{-7} \) M for the MCPE prepared with rod-shaped CuO nanoparticles. The limit of detection (LOD) was calculated according to the equation \( LOD = K S^0/S \), where K is a constant related to the confidence level. In accordance with the suggestion of the IUPAC, the value of K is 3 at the 99% confidence level, \( S^0 \) is the standard deviation of ten blank-solution measurements (no added DA), and S is the slope of the calibration graph. The proposed electrode exhibited a relatively lower detection limit than those recently reported elsewhere [27, 49, 50-42] (Table 2.2).

Table 2.1 shows a comparison between the rod shaped CuO nanoparticles and the flake-shaped CuO nanoparticles. The flake-shaped CuO nanoparticles exhibit a smaller average crystallite size and a smaller particle size with a significant blue shift. The MCPE prepared with the flake-shaped CuO nanoparticles exhibits a higher electrode surface area, a higher electron-transfer rate constant and a lower detection limit compared with the MCPE prepared with the rod-shaped CuO nanoparticles. Therefore, the MCPE prepared with flake-shaped CuO nanoparticles may form hydrogen bonds with the hydroxyl groups of DA, which would activate the hydroxyl groups and weaken the bond energy of O–H to form dopaquinone [53]. The MCPE prepared from flake-shaped CuO nanoparticles can therefore be used as an electrochemical sensor for the investigation of DA in the presence of AA.

2.6.7. Simultaneous determination of DA and AA by DPV

According to recent reports [27, 28, 30, 54, 55], the separation of the oxidation peak potentials between DA and AA plays an important role for the analysis of DA in the
presence of AA. Fig. 2.9 (A-D) shows the separation of the oxidation peak potentials between DA and AA. The small separation of 0.12 V for the MCPE prepared with flake-shaped CuO nanoparticles may be due to a small amount of CTAB surfactants present on the CuO nanoparticles, as was clearly explained in recent reports [56,57]. The separations of the other MCPEs were 0.16 V for the MCPE prepared with polyglycineflake-shaped CuO nanoparticles, 0.26 V for MCPE prepared with CTAB/polyglycineflake-shaped CuO nanoparticles and 0.28 V for MCPE prepared with SDS/polyglycineflake-shaped CuO nanoparticles. The MCPE prepared with SDS/polyglycineflake-shaped CuO nanoparticles shows a large peak-potential separation between DA and AA compared with both of other MCPEs in this study and recently reported results in the literature [24, 28, 30, 54, 55]. The DPV analysis of DA in the presence of AA exhibits a linear increase in the anodic peak current with an increase in the concentration of DA, as shown in Fig.2.10.(A). The graph of the peak current versus the concentration of DA also exhibited good linearity, as shown in Fig.2.10.(B). Therefore, SDS/polyglycineflake-shaped CuO nanoparticles modification was prior to the selective detection of DA in the presence of AA. Our results suggested the possibility of a simultaneous multi-detection system based on the DPV method.

2.6.8. Electrochemical current response for DA at different MCPEs

Figure 2.11 shows the electrochemical responses of $1 \times 10^{-5}$ M DA in 0.2 M phosphate buffer solution at pH 6.0 at the MCPE prepared with flake-shaped CuO nanoparticles and at the MCPE prepared with different films of flake-shaped CuO nanoparticles at a scan rate of 0.05 V s$^{-1}$. The MCPE prepared with SDS/polyglycineflake-shaped CuO nanoparticles exhibited an enhanced current response with sharp redox peak for DA compared with the MCPEs prepared with CTAB/polyglycineflake-shaped CuO nanoparticles, polyglycineflake-shaped CuO nanoparticles and flake-shaped CuO nanoparticles. The current enhanced was found to be 18 $\mu$A compared with the MCPE prepared with flake-shaped CuO nanoparticles. This confirm that an SDS/polyglycineflake-shaped CuO nanoparticles exhibit good electrochemical sensing effect on DA and also compared with recent literature [16].
2.6.9. The effect of scan rate for DA at the MCPE prepared with SDS/polyglycine/flake-shaped CuO nanoparticles

The effect of the scan rate for DA was studied by CV at the MCPE prepared with SDS/polyglycine/flake-shaped CuO nanoparticles; the results show an increase in the redox peak currents with an increase in the scan rate (0.01–0.800Vs^{-1}), as shown in Fig. 2.12.(A). The peak current versus scan rate (v) was plotted. The graph obtained exhibited good linearity between the scan rates versus peak currents, as shown in Fig.2.12. (B). In the range from 0.01 to 0.800Vs^{-1}, both the anodic and cathodic peak currents were proportional to the scan rate (v). The correlation coefficient was 0.9994, which indicates the electrode reaction process was adsorption-controlled (58-60). According to an equation previously reported [60] for determining the value of k° from experimental ΔEp values, equation (5) was a valid approximation of such curves for DEp> 10 mV. The values of k° for the DA were determined from the experimental ΔEp values, the data in Table 2.3 and equation (5). The values of k° indicate that strong adsorptions of reactants and products are involved. Here, the k° is the heterogeneous rate constant, and ΔEp is the potential difference between the anodic and cathodic peak potentials. The heterogeneous rate constant (k°) was estimated using eq. (5). The value of k° obtained at a scan rate of 0.100Vs^{-1} for the MCPE prepared with SDS/polyglycine/flake-shaped CuO nanoparticles exhibits a larger heterogeneous rate constant compared with those determined in other scan-rate-variation studies. The calculated data are tabulated in the Table.2.3.

\[ \Delta E_p = 201.39 \log \left( \frac{v}{k^o} \right) - 301.78 \]  

\[ \text{------- (5)} \]
2.7. Conclusions

- The different shaped CuO nanoparticles were prepared by co-precipitation method and the electrochemical parameters are studied.

- The modified carbon paste shows effective sensor towards electrochemical investigation of DA in presence of AA. The prepared CuO nanoparticles MCPE shows low detection limit compared with the previous literatures.

- The SDS/polyglycine/flake shaped CuO nanoparticle MCPE shows significant effect of redox peak currents for DA and good electrochemical signal separation between DA and AA.

- Therefore, the present method could be extended to many metal oxides and ferrites for the synthesis of modified electrodes with good electrocatalytic activities for the simultaneous investigation of DA and AA and for bioactive molecules or neurotransmitters.
Fig. 2.1. XRD patterns for CuO nanoparticle samples prepared using (A) SDS and (B) CTAB.

Fig. 2.2. IR spectra of CuO nanoparticle samples prepared using (A) SDS and (B) CTAB.
Fig. 2.3. SEM images of CuO nanoparticle samples prepared using (A) SDS and (B) CTAB, and the corresponding TEM images of samples prepared using (C) SDS and (D) CTAB.

Fig. 2.4. UV-visible absorption spectra of CuO nanoparticle samples prepared using (A) CTAB and (B) SDS.
Fig. 2.5. Plot of DA oxidation peak current versus PBS solution pH (5.5 to 8.0) and formal potential versus PBS solution pH (5.5 to 8.0) at a scan rate 0.100Vs⁻¹ for (A) the MCPE prepared with flake-shaped CuO nanoparticles and (B) the MCPE prepared with rod-shaped CuO nanoparticles.

Fig. 2.6. Cyclic voltammograms of 5×10⁻⁵ M DA in PBS at pH 6.0 of (A) bare CPE, (B) a MCPE prepared with rod-shaped CuO nanoparticles and (C) a MCPE prepared with flake-shaped CuO nanoparticles at scan rate of 0.200 V s⁻¹.
Fig. 2.7. Results for the MCPE prepared with CuO nanoparticles, (A) and (D) shows cyclic voltammograms of 1.0×10⁻⁵ mol/L DA with different scan rates (0.01, 0.03, 0.05, 0.08, 0.100, 0.200, 0.300, 0.400, 0.500, 0.600, 0.700 and 0.800 Vs⁻¹) in PBS at pH 6.0. (B) and (E) shows a graph of the redox peak current versus the square root of the scan rate. (C) and (F) shows a graph of the peak potential versus the natural log of the scan rate for the with flake-shaped (A,B,C) and rod-shaped (D,E,F) CuO nanoparticles, respectively.

Fig. 2.8. Results for the MCPE prepared with CuO nanoparticles, (A) and (C) shows Differential pulse voltammograms of (a) 4.0×10⁻⁷ M, (b) 6×10⁻⁷ M, (c) 8×10⁻⁷ M, (d) 10×10⁻⁷ M, (e) 2.0×10⁻⁶ M, (f) 4.0×10⁻⁶ M, (g) 6.0×10⁻⁶ M, (h) 8.0×10⁻⁶ M, (i) 1×10⁻⁵ M, (j) 2×10⁻⁵ M, (k) 3×10⁻⁵ M, (l) 4×10⁻⁵ M DA and (a) 6.0×10⁻⁷ M, (b) 8×10⁻⁷ M, (c) 10×10⁻⁷ M, (d) 2.0×10⁻⁶ M, (e) 4.0×10⁻⁶ M, (f) 6.0×10⁻⁶ M, (g) 8.0×10⁻⁶ M, (h) 1×10⁻⁵ M, (i) 2×10⁻⁵ M, (j) 3×10⁻⁵ M, (k) 4×10⁻⁵ M in 0.2 M phosphate buffer solution at pH 6.0. (B) and (D) shows a graph of the peak current versus the concentration of dopamine for the flake-shaped (A,B) and rod-shaped (C,D) CuO nanoparticles, respectively.
Fig. 2.9. Differential pulse voltammograms of $1 \times 10^{-6}$ M DA in 0.2 M phosphate buffer solution of pH 6.0 in the presence of $2.0 \times 10^{-4}$ M AA for peak separation between $E_{AA}$ and $E_{DA}$ at different MCPEs: (A) MCPE prepared from flake-shaped CuO nanoparticles, (B) MCPE prepared with polyglycine/ flake-shaped CuO, (C) MCPE prepared with CTAB/ polyglycine/ flake-shaped CuO nanoparticles and (D) MCPE prepared with SDS/polyglycine/ flake-shaped CuO nanoparticles.
Fig. 2.10. (A) shows Differential pulse voltammograms of (a) $2.0 \times 10^{-6}$ M, (b) $4.0 \times 10^{-6}$ M, (c) $6.0 \times 10^{-6}$ M, (d) $8.0 \times 10^{-6}$ M, (e) $1.0 \times 10^{-5}$ M, and (f) $1.2 \times 10^{-5}$ M DA in 0.2 M phosphate buffer solution at pH 6.0 in the presence of $5.0 \times 10^{-4}$ M AA at the MCPE prepared with SDS/polyglycine/flake-shaped CuO nanoparticles. The Fig. (B) shows a graph of the anodic peak current versus the concentration of DA in the presence of AA.
Fig. 2.11. Cyclic voltammograms of $1 \times 10^{-5}$ MDA at (A) the MCPE prepared with flake-shaped CuO nanoparticles, (B) the MCPE prepared with polyglycine/ flake-shaped CuO nanoparticles, (C) the MCPE prepared with CTAB/ polyglycine/ flake-shaped CuO nanoparticles, and (D) the MCPE prepared with SDS/polyglycine/flake-shaped CuO nanoparticles at a scan rate of 0.050 V s$^{-1}$. 
Fig. 2.12. (A) Variation of the scan rate for DA at the MCPE prepared from SDS/polyglycine/flake-shaped CuO nanoparticles (0.010, 0.030, 0.050, 0.080, 0.100, 0.200, 0.300, 0.400, 0.500, 0.600, 0.700 and 0.800 V s⁻¹) in PBS at pH 6.0. The (B) shows a graph of peak current versus scan rate for DA.
Table 2.1. Comparison between rod- and flake-shaped CuO nanoparticles and their electrocatalytic performances.

<table>
<thead>
<tr>
<th>CuO nanoparticles</th>
<th>Average Crystalline size (nm)</th>
<th>Size of CuO nanoparticles (nm) from TEM image</th>
<th>UV-visible maxima absorption (nm)</th>
<th>Surface area of MCPE (cm²)</th>
<th>Charge transfer coefficient at MCPE (α)</th>
<th>Number of electrons at MCPE (n)</th>
<th>Electron transfer rate constant at MCPE (ks)(l/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flake shaped</td>
<td>37.2</td>
<td>~25</td>
<td>366</td>
<td>0.08081</td>
<td>0.654</td>
<td>2.066</td>
<td>0.5496</td>
</tr>
<tr>
<td>Rod shaped</td>
<td>110</td>
<td>~50 width and 150 length</td>
<td>394</td>
<td>0.0767</td>
<td>0.644</td>
<td>1.88</td>
<td>0.4314</td>
</tr>
</tbody>
</table>

Table 2.2. Comparison of the detection limits of different modified electrodes.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Detection limit (μM)</th>
<th>Techniques</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana/MWCNTs/ MCPE</td>
<td>2.09</td>
<td>DPV</td>
<td>[41]</td>
</tr>
<tr>
<td>CCE/ferrocene carboxylic acid</td>
<td>0.45</td>
<td>SWV</td>
<td>[42]</td>
</tr>
<tr>
<td>Polyglycine/CPE</td>
<td>0.1</td>
<td>CV</td>
<td>[18]</td>
</tr>
<tr>
<td>MEs/SAM-Au electrode</td>
<td>1.1</td>
<td>CV</td>
<td>[43]</td>
</tr>
<tr>
<td>LDH/CILE</td>
<td>5</td>
<td>DPV</td>
<td>[40]</td>
</tr>
<tr>
<td>Rod shaped CuO nanoparticles/ MCPE</td>
<td>0.18</td>
<td>DPV</td>
<td>Present Work</td>
</tr>
<tr>
<td>Flake shaped CuO nanoparticles/ MCPE</td>
<td>0.055</td>
<td>DPV</td>
<td>Present work</td>
</tr>
</tbody>
</table>

82
Table 2.3. Variation of the voltammetric parameters gathered from the plots shown in Fig. 2.12 as a function of the potential scan rate

<table>
<thead>
<tr>
<th>v/mVs⁻¹</th>
<th>ΔEp/mV</th>
<th>k⁰/s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>25</td>
<td>0.238</td>
</tr>
<tr>
<td>30</td>
<td>49</td>
<td>0.537</td>
</tr>
<tr>
<td>50</td>
<td>69</td>
<td>0.707</td>
</tr>
<tr>
<td>80</td>
<td>94</td>
<td>0.870</td>
</tr>
<tr>
<td>100</td>
<td>109</td>
<td>1.023</td>
</tr>
<tr>
<td>200</td>
<td>173</td>
<td>0.878</td>
</tr>
<tr>
<td>300</td>
<td>225</td>
<td>0.727</td>
</tr>
<tr>
<td>500</td>
<td>312</td>
<td>0.449</td>
</tr>
<tr>
<td>600</td>
<td>352</td>
<td>0.342</td>
</tr>
<tr>
<td>700</td>
<td>399</td>
<td>0.234</td>
</tr>
<tr>
<td>800</td>
<td>430</td>
<td>0.186</td>
</tr>
</tbody>
</table>
2.8. References


