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PREPARATION OF A NOVEL MODIFIED ELECTRODE FOR SENSITIVE DETERMINATION OF METHYLDOPA

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ÖZET

Yüksek Lisans Tezi

METILDOPANIN DUYARLI BIR ŞEKILDE SAPTANMASI IÇIN YENI BIR MODIFIYE ELEKTROTUN HAZIRLANMASI

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Karbon nanotop ve neodmiyum oksit nanoparçacıklar ile yeni bir malzeme hazırlanmıştır. Elde edilen malzeme elektrot kaplama da kullanılmış ve yeni bir modifiye elektrot hazırlanmıştır. Hazırlanan elektrot platform (Nd_2O_3/CNBs/GCE) metildopa saptanması için kullanılmıştır, Nd_2O_3/CNBs/GCE sistemi metildopa elektrot reaksiyonunu hızlandırmıştır. Nd_2O_3/CNBs/GCE sistemi, metildopa için $2.0x10^{-7}$ M ile $1.8x10^{-5}$ M aralığında doğrusal bir ilişki göstermiştir. Modifiye elektrot ile $7.24\ x10^{-8}$ M'lık bir saptama sınırı elde edilmiştir. Ayrıca, yeni sensörün tekrarlanabilirlik, kesinlik ve doğruluk parametreleri mükemmeldir.

ANAHTAR KELİMELER: Metildopa, neodmiyum oksit, voltametri, elektroanaliz, sensör

ABSTRACT

MSc Thesis

PREPARATION OF A NOVEL MODIFIED ELECTRODE FOR SENSITIVE DETERMINATION OF METHYLDOPA

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A novel material was prepared using carbon nanoballs and neodymium oxide. The material was used for coating an electrode and a new paltform has been obtained. The electrode prepared was used for the detecting methyldopa. The Nd_2O_3/CNBs/GCE system accelerated the electrode reaction of methyldopa. The Nd_2O_3/CNBs/GCE system exhibited a linear relationship between $2.0x10^{-7}$ M and $1.8x10^{-5}$ M for methyldopa. A detection limit of 7.24 $x10^{-8}$ M was obtained for methyldopa at proposed modified electrode. In addition, the analytical parameters of novel sensor such as reproducibility, precision and accuracy are excellent.

KEY WORDS: Methyldopa, neodymium oxide, voltammetry, electroanalysis, sensor

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LIST OF SYMBOLS and ABBREVIATIONS

CNB	Carbon Nanoballs
CNT	Carbon Nanotubes
CTS	Chitosan
CV	Cyclic Voltammetry
Ep	Peak Potential
GCE	Glassy Carbon Electrode
MD	Methyldopa
mV	Milivolt
n	Number of Electrons
nM	Nanomolar
Nd_2O_3	Neodymium oxide
PAR	Paracetamol
PBS	Phosphate Buffer
R.S.D	Relative Standard Deviation
XRD	X-Ray Diffraction

1. INTRODUCTION

Drugs are important substances that are used for the relief of pains or preventing and treatment of diseases (Karaman, 2013). It has been reported that drug is derived from dry herb and mainly extracted from plants (Wadud et al., 2007). Historically, some unconventional methods based on plants, animal products and minerals were utilized for treating diseased. The analysis of drugs consists of tests for materials, raw application or application applications, and animal product. assortment, including blood, birth and tissues

Analysis of drug includes tests on pharmaceutical or veterinary formulations, and more complex matrices including foods of animal origin, beverages, and food items that are performed for clinical and forensic purposes which involve a variety of matrices such as samples of blood, urine, and tissues (Martinez Calatayud, 2005). The key issue for ensuring the safety of drug therapy is the control and minimization of their effects. Because side effects of a substance are inherent characteristics of drug, drug analyzers cannot be affected by them. In addition, drug analysts exhibit a key role to ensure the quality of pharmaceuticals which is related to the safety of drugs (Görög, 2008).

High blood pressure which is an major issue for human being effects on the daily life of a large number of people (Vahedi et al., 2013). Hypertension associated with structural deformation in blood vessels and heart may lead to various complications such as cardiovascular disease and mortality. The blood pressure should take values between 90 mmHg and 140 mmHg (Chien et al., 2016). A number of people on the globe have high blood pressure problem and about seven million of them die each year (Ramírez et al., 2016). Other complications related to the blood pressure can arise such as damages to the arteries, heart and brain workload and failure of kidney (Sarnak et al., 2005). A notable case may be observed for those who experience the period of pregnancy. However, the prescribed medicines should be less harmful on during pregnancy (Reed,

1985). Thus, scientists have proposed many drugs to solve the problem of high blood pressure.

Methyldopa is reported to be one of the key drugs that can be used to treat high blood pressure (Vahedi et al., 2013). Methyldopa, a dopamine analogue and acts on the nervous system for lowering blood pressure and is specifically indicated for the treatment of hypertension for people suffering renal insufficiency or during pregnancy as indicated in Figure 1.1 is a catecholamine-containing amine-containing antihypertensive chemical attached to a benzene ring and containing two hydroxyl groups (Reed, 1985; Kutluay and Aslanoglu; 2016, Dehnavi and Soleymanpour, 2020). The hypotensive effect of methyldopa is associated with being converted to α-methylnorepinephrine which stimulates central α receptors reducing the activity of nerves and leading to a decrease in blood pressure (Conway et al., 1979). Common side effects can include drowsiness, liver damages, cell damages, and unexpected allergic reactions (Hoyumpa and Connell, 1973).

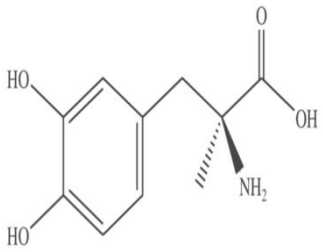

Figure 1.1. Chemical structure of methyldopa

Electrochemical sensors offer promising devices for monitoring and controlling of a wide range of analytes as a result of their sensitivity, specificity and the ease of use, fast analysis, low-cost, reliable and reproducible measurements with miniaturized and portable devices (Wang, 2002; Patel et al., 2020), the interest in their application in the clinical, pharmaceutical and environmental field substantially has increased. The stability, sensitivity and selectivity of voltammetric platforms towards target species are mostly related on surface area and the type of modifying materials (Săndulescu, 2011).

It is clear that detecting and quantifying methyldopa is a vitally important feature for pharmacy and medicine (Myhre et al., 1982). Several quantifying procedures have appeared in the literature to analyze methyldopa in pharmaceuticals or biological liquids. The procedures include titrimetry (Pathak et al., 1982), chromatography (Li et al., 2010), and spectrophotometry (Abdulrahman et al., 2005). Some of the previously mentioned procedures have time consuming issues and may involve strict experimental conditions. Some also suffering from interferences are not appropriate for carrying out routine analysis of drugs. Electrochemical procedures offer alternative means for determination of methyldopa and provide advantages such as high sensitivity and portable devices for rapid monitoring purposes (Shahrokhian et al., 2011; Salmanipour et al., 2012).

Voltammetry was subjected to the analysis of samples more than other analytical techniques for the determination of analytes, because of several benefits i.e. low cost, high efficiency, rapid response, simplicity of analytical procedures, speed, higher sensitivity and reproducibility (Bard and Faulkner, 2001). Voltammetry, a family of electroanalytical techniques, is based on electrolytic reduction or oxidation mainly for the analyte detection or investigation of mechanism and kinetics of redox reactions. It measures current as a function potential. Initially, Jaroslav Heyrovsky introduced the first voltammetric technique in the early 1920s for which uses only two electrodes. However, modern voltammetry uses a three-electrode system. Results for a voltammetric experiment are presented by a voltammogram which is the based on the current generated by the analyte versus applied potential (Bard and Faulkner, 2001). An oxidation/reduction reaction which occurs at any electrode is related to the applied potential. The potential forces the ions in the solution to gain or lose electrons (Joshi and Sutrave, 2018). Thus, cyclic voltammetry is a potential technique to acquire the redox behavior of chemicals through a systematic study of the measurement of current versus applied voltage of a particular electrochemical cell as given in Figure 1.2. The graph of current-potential is termed a cyclic voltammogram.

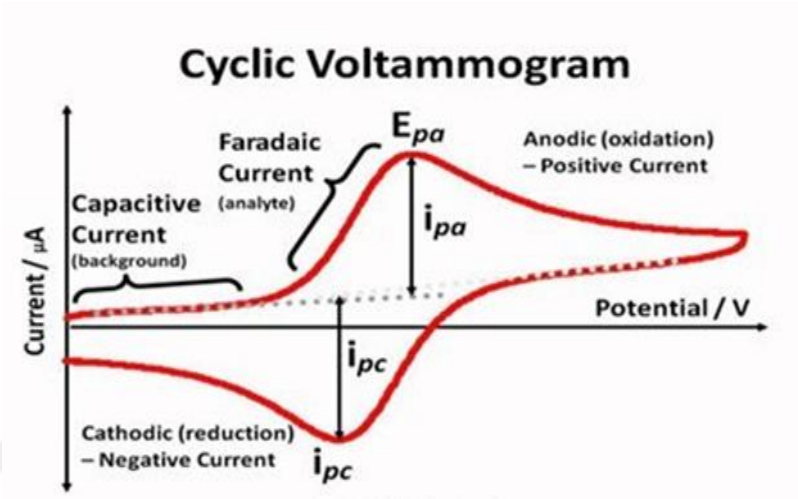

Figure 1.2. CV graph with forward and reverse scan

An electrochemical cell represented in Figure 1.3 consisting of working, auxiliary, and reference electrodes is a three electrode voltammetric system (Elgrishi et al., 2018; Gomaa et al., 2018). The auxiliary electrode is usually a platinum rod. However, silver/silver chloride or saturated calomel electrode can serve as reference electrode. However, gold, mercury, silver, platinum and carbon are be used as working electrodes (Wang et al., 2019; Lee et al., 2017). The voltammetric cell also includes a N_2-purge line for removing dissolved oxygen.

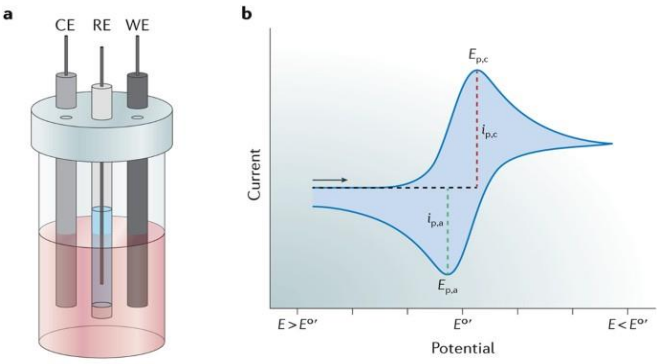

Figure 1.3. Autolab electrochemical workstation

Voltammetry is characterized by large dynamic range, accuracy, high sensitivity and simplicity. Also, a number of nanostructured materials such as metallic nanoparticles, and nanotubes have recently been utilized to improve properties of the electrodes (Shahmiri et al., 2013; Fouladgar and Mohammadzadeh, 2014). However, the selectivity for electrochemical platforms may be sometimes restricted in the presence of interferences that display similar redox behavior with methyldopa (Kutluay and Aslanoglu 2013; Hosseinzadeh et al., 2009). An important strategy for the minimization of interferences from other molecules is to modify the surface of electrodes using various materials (Raoof et al., 2007; Keyvanfard et al., 2013)

Voltammetric procedures at various platforms have been extensively applied as accurate and precise analytical tools in detecting trace amounts of species. The most important characteristics of platforms is the capability to stimulate redox reactions through the significant drop in overvoltage when compared to the conventional electrodes (Beitollahi et al., 2011b; Luo et al., 2013; Taleat et al., 2008; Shangguan and Li, 2013; Thomas et al., 2013a; Raoof et al., 2006; de Oliveira et al., 2013, Beitollahi et al., 2011a; Thomas et al., 2013b; Mohammadi et al., 2013; Sanghavi et al., 2013; Beitollahi et al., 2014a; Li et al., 2012; Ghoreishi et al., 2012; Yildiz et al., 2014).

Carbon nanotubes have promising properties and can be used as attractive modification materials for fabricating voltammetric platforms due to their ability to provide high catalytic activity and reduce surface contamination (Keyvanfard et al., 2014). Modification of electrodes with carbon nanotubes has also been documented for use in analytical sensing resulting in low detection limits, good accuracy, reduced overpotentials, large surface area, good physical and chemical properties, excellent conductivity. They are very attractive as a support for heterogeneous catalysis (Lu and Tsai, 2011; Campbell and Compton, 2010; He et al., 2004; Vairavapandian et al., 2008). In addition, the uniform dispersion of nanoparticles of metals or metal oxides on carbon nanotubes surfaces enable promise as nanoelectrocatalysts for use in electroanalysis (Yánez-Sedeno et al., 2010; Wang et al., 2007).

Materials such as multiwalled carbon nanotubes (MWCNTs) were utilized as the sensing material with various working electrodes such as glassy carbon electrode, pencil graphite electrode, carbon paste electrode for electrochemical sensors which all were utilized for the voltammetric analysis (Wilson and Gifford, 2005; Väärtnõu and Lust, 2006; Chao and Ma, 2014; Tomac et al., 2017). Compared with the other electrodes made of noble metals, the glassy carbon electrode prevents surface fouling which is caused by adsorption of oxidation substances on electrodes, resulting in reduced sensitivity and deterioration of the time-dependent response (Natale et al., 2015).

The development of nanoparticles exhibited significant advantages such as higher electroactive surface area for electrochemical reactions. They also presented good selectivity and improved electrocatalytic activity over conventional electrodes in sensing applications (Arai et al., 2004; Han et al., 2004). Experimental parameters such as deposition potential, period of duration, and solution consistency are optional for controlling growth rate of nanoparticles for the electrodeposition of metallic nanostructures (Day et al., 2007; Wildgoose et al., 2006).

Nanomaterials have recently gained potential interest of research as having unique structure-dependent behavior and therefore can be utilized in potential applications in many basic and applied fields (Jia et al., 2013; Beitollahi et al., 2014a). Materials of metallic oxide nanostructures have been utilized in potential applications great interest owing to their non-toxicity, chemical stability, ease of preparation and good electrocatalytic effect (Mohammadi et al., 2011).

Carbon nanospheres or nanoballs (CNBs) are simply carbon materials available in spherical or nearly spherical shapes with a diameter of 100 nanometers. Various shapes of carbon nanospheres such as balls, microbeads, carbon black, onion and microporous beads have been utilized in electroanalysis as modifiers (Calderón-Moreno et al., 2007; Mao et al., 2018). Recent studies have demonstrated potential importance of carbon nanoballs in critical applications such as batteries, cells, capacitors, boosters, field

emission cathode materials, catalyst support materials, composites and refining processes. The carbon balls normally exist as agglomerates of several nanoballs through van der Waals bonding. They have a brown to black colour as the thickness increases. The carbon balls have a BET surface area ranging from as low as 2 to 1200 m^2 g -1 (Jin et al., 2005; Deshmukh et al., 2010). The density ranges from as low as 0.4 to 1.6 g cm-3 depending on the synthesis procedures (Mhlanga et al., 2010). The chemical reactivity of carbon spheres is somewhat similar to those of graphitic materials. They appear to be insoluble in non polar solvents and sparingly soluble in most solvents because of their hydrophobic nature. However, solvents such as chloroform, benzene, methanol and toluene have resulted in miscibility (Sobkowicz et al., 2009). The presence of dangling bonds has enabled carbon balls to be functionalised by acids like nitric acid (Kang and Wang, 1996).

2. LITERATURE REVIEW

The main issue for ensuring the safety of drug therapy is the control and minimization of their effects. Because side effects are inherent characteristics of a substance, drug analyzers cannot be affected by them. In addition, drug analysts exhibit a key role to ensure the quality of drug materials and pharmaceuticals which reflects to the safety of drugs (Görög, 2008). Analysis of pharmaceuticals is traditionally related to chemical analysis that deals with drugs as drug substances and pharmaceutical products (Dispas et al., 2021). The literature reveals that several analytical procedures were used to analyze methyldopa such as chromatography (Abbasi et al., 2019), spectrophotometry (Behera et al., 2012), flow injection analysis (Mestre et al., 2019), and electroanalysis (Asadpour-Zeynali and Mollarasouli, 2016; Moccelini et al., 2011), and various voltammetric platforms were studied for the determination of methyldopa based on carbon nanotubes (Rezaei et al., 2013), electro deposition of platinum-rutenium nanoparticles on carbon nanotubes modified platforms (Shahrokhian and Rastgar, 2011) and/or at carbon paste electrode (Karimi-Maleh et al., 2012, Mohammadi et al., 2008). Some of previously studied procedures are time consuming and may involve strict conditions of measurements or suffer from interferences and are therefore not appropriate for daily analysis of drugs. Electrochemical procedures offer alternative means for determination of methyldopa and provide advantages such as high sensitivity and portable devices for rapid monitoring purposes (Shahrokhian et al., 2011; Salmanipour et al., 2012).

A flow injection spectrophotometry procedure for determining methyldopa in pharmaceuticals has been proposed by Ribeiro et al. (2005). The procedure is simple, fast, relatively inexpensive, accurate, precise and sensitive, using the fewest number of reagents and reaction sequences and contains the formation of a yellow-colored product which is measured at wavelength of 410 nm upon complexing of methyldopa with molybdate.

Shahrokhian and Rastgar, (2011), prepared a coated vitreous carbon electrode by efficient deposition of nanoparticles of ruthenium-platinum on a layer of carbon nanotubes, in order to accurately quantify traces of methyldopa in drugs and biological samples. The procedure found to provide a highly sensitive quantification for determining submicromolar amounts of methyldopa with a range of 0.05-40 μM and a detection limit of 10 nM.

Rezaei et al. (2013), developed a multi-walled coated glass electrode for sensitive quantification of methyldopa in biological and drugs. The study showed that methyldopa oxidation on surface of GCE coated with carbon nanotubes significantly improved peak response compared to the bare electrode. A dynamic working range with two ranges which were 0.1–30 and 30.0–300.0 μM and a detection limit of 0.08 μM was reported.

Beitollahi et al. (2014b), developed a platform for the electroanalysis of pharmaceutical, food, agricultural, and environmental samples. The study exhibited that peak current for methyldopa exhibited a working range of 9.0×10^{-8} to 5.0×10^{-4} M. The authors also reported an LOD of 5.0×10^{-8} M.

Molaakbari et al. (2014), reported a novel platform using an electrode coated with a mixture of ferrocene and TiO_2 nanoparticles. The response of the proposed novel sensor to the catalytic current for the concentration of methyldopa exhibited a linear relationship over a range from 2.0×10^{-7} to 1.0×10^{-4} M. Molaakbari et al. (2014), also presented an LOD of 8.0×10^{-8} M.

Ensafi et al. (2015), proposed a methyldopa-sensitive identification nanosensor using carbon nanotubes decorated with ferrite nickel nanoparticles ($NiFe_2O_4$). The composite material based nanosensor exhibited a synergistic activity for methyldopa electrode reaction. The authors reported peak current was linear with a range of 0.5-900 μM for methyldopa and a detection limit of 0.08 μM.

Baytak et al. (2016), reported a new ultrasonically prepared sensing platform by modifying a GCE with terbium oxide nanoparticles (Tb_4O_7NPs) and CNTs. The novel sensing platform was applied for quantification of methyldopa. The procedure yielded working ranges from 5.0×10^{-9} M to 1.0×10^{-6} M for methyldopa and paracetamol, respectively. The detection limit presented for methyldopa was 1.5×10^{-9} M.

Ramírez et al. (2016), developed a fluorine-doped SnO_2 material for the oxidation and the determination of methyldopa in pharmaceuticals. The pH-dependent electrochemical behavior of methyldopa exhibited two linear plots between 2 μM and 30 μM, 30 μM and 60 μM with a detection limit of 0.15 μM.

Kutluay and Aslanoglu, (2016), utilized carbon nanotubes coated electrode to determine methyldopa content in pharmaceuticals. A linear range from 0.005 to 0.388 μM was reported with a detection limit of 1.0 nM using voltammetric methods..

Fouladgar and Ahmadzadeh, (2016), described a new method based on NiO and ionic liquid coated electrode. The procedure was successfully applied for determining methyldopa in a solution containing folic acid, with a linear response of concentration from 0.1 to 700.0 μM and a detection limit of 0.06 nM.

The Fe_3O_4@SiO_2/GO nanocomposite has also been synthesized to modify the surface of electrode which was applied for electrochemical determination of methyldopa in a media containing uric acid. The authors reported a calibration curve of 0.1 to 400.0 μM for methyldopa with detection limit of 86.0 nM (Movlaee et al., 2017),

Keyvanfard et al. (2017), manufactured an electrostimulation-based platform for SWV of methyldopa in a solution containing tyrosine. To achieve this goal, the carbon paste electrode was coated with nanoparticles of nickedl and 2-(3,4-dihydroxyethyl)isoindoline-1,3-dione. The sensor showing improved electrocatalytic oxidation of methyldopa yielded a linear range of 0.08-500 μM.

A nanocomposite based platform was prepared based on graphene-1-butyl-3-methylmidazolium hexafluorophosphate which exhibited an improvement in the electrooxidation peak of methyldopa with a range of 0.04-750.0 μM. In addition, the proposed platform yielded a detection limit 0.01 μM (Sanati and Faridbod, 2017).

Beitollahi et al. (2018), presented a novel procedure for determining methyldopa in a solution containing species of phenylephrine and guaifenesin. The authors prepared a platform based on modifying electrodes with graphene acetate-ethyl-2- (4-ferrocinyl [1,2,3] triazole acetate. The platform showed two linear working ranges which were 0.4–30.0 μM and 30.0–500.0 μM. The reported detection limit was 0.08 μM.

Ghodsi et al. (2019), developed and applied a modified electrode composed of horseradish peroxidase (HRP) trapped on silica/carbon nanotubes on carbon electrodes for determination of methyldopa by electrocatalytic oxidation of methyldopa with HRP for the first time. The proposed biosensor showed linear response in two ranges of 1 μM to 3.5 mM and 3.5 mMto 12 mM, and detection limit was obtained about 25.3 nM.

Pourtaheri et al. (2019), developed a coated carbon electrodes with nanoflowers of Li+3/ZnO and N-(ferrocenylmethylidene)-fluorene-2-amine to determined methyldopa and phenylephrine simultaneously. A linear response of concentrations of methyldopa of 0.2 to 500.0 μM was obtained. The presence of phenylephrine did not alter the sensitivity of proposed sensor to methyldopa.

A modified graphite pencil electrode was also reported by Dehnavi and Soleymanpour, (2020). The platform was constructed using graphenes and phosphomolybdic acid. It was peformed for analyzing human blood, urine and milk samples. The results indicated an increment of surface area, catalytic effect and a linear working range of 4.9×10^{-10}–1.0×10^{-7} M was obtained with a detection limit of 1.2×10^{-10} M was reported.

The aim of the present study is to develop a sensing material for determination of methyldopa. A glassy carbon electrode was utilized as the platform which was modified with neodymium (III) oxide (Nd_2O_3) nanoparticles, and carbon nanoblack (CNBs) to enhance the stability, sensitivity and selectivity in analysis of samples containing methyldopa. Cyclic voltammetry was successfully performed as the analytical technique with the proposed electrode /CNBs Nd_2O_3-NPs /GCE) for the analysis of drug samples.

3. MATERIAL and METHOD

3.1. Material

There are several tools used for the current study. Some of these are listed below;

- An Epsilon brand potentiostat (BASi, US) was used for voltammetry.
- A glassy carbon electrode (GCE) (BASi, US) served as working electrode, A Metrohm silver/silver chloride was served as reference electrode however a Pt wire served as counter electrode.
- The pH measurements were made with Metrohm 744 pH Meter (Metrohm, Switzerland).
- Composite of carbon nanoballs-metal nanoparticules was prepared using a KUDOS (SK3301OHP) model ultrasonic bath.
- SHIMADZU (AY-220) model balances was used for analtytical weighing.
- X-ray diffraction was used for the characterization of carbon nanoballs and neodymium oxide

The reagents used without additional purification and obtained from Sigma–Aldrich, Fluka or Merck and are listed below:

- Acetic acid (≥ 99.8 %, Merck),
- Acetonitrile (99.9 %, Merck),
- Acetone (≥ 99.8 %, Merck),
- Alumina powder, (0.3; 1.0 micron, Buehler),

- Methyldopa powder(99.0%, Sigma Aldrich),
- Chloroform (99.0 %,Merck),
- Carbon black nanoballs (95.0%, Nano Lab),
- Na_2HPO_4 (99.5 %, Merck),
- Ethanol (≥99.5%, Merck),
- Neodymium(III) oxide Nano powder (99.995%, US-Nano),
- Alfamet tablet (Local pharmacy)
- Hydrochloric acid, (37-38 %, Merck),
- Multiwalled carbon nanotubes (MWCNTs) (95.0 %, Nano Lab),
- Nitric acid (65.0 %, Merck),
- Paracetamol (98.0%, Alfa Aesar),
- KCl (99.5 %, Merck),
- KH_2PO_4 (99.5 %, Merck),
- NaOH (≥99.0 %, Merck),

3.2. Method

3.2.1. Preparation of solutions

Phosphate buffer (PBS) was prepared upon addition of 6.8 g/L of KH_2PO_4, 8.8 g/L of $Na_2HPO_4.2H_2O$, and 0.75 g/L of KCl into a 1 L flask. The solution was then diluted to 1 L using ultra pure water. Then, the pH of solutions was adjusted by hydrochloric acid or sodium hydroxide. Stock solutions of methyldopa and paracetamol were prepared using 0.1 M PBS. The working solution was deoxygenated using N_2 gas before each run. Ionic strength of solutions was kept constant. Fresh stock solutions were prepared before use and stock solutions were kept in amber bottles for protecting from day-light. Dilutions of stock solutions were made using phosphate buffer. Electrochemical experiments were carried out under oxygen-free nitrogen.

3.2.2. Activation of electrodes

Prior to develop an electrochemical platform, GCEs were first washed and polished with alumina of 1 μm and 0.3 μm on polishing pad. A simple way to polish electrodes is using alfa motion of electrode in alumina slurry on the polishing pad (Elgrishi et al., 2018) as illustrated in Figure 3.1. Then, polished glassy carbon electrodes were sonicated in a mixture acetone-ethanol for 10 min. The electrode was then rinsed with double-distilled water. The activation of electrode was carried out in 0.1 M PBS by cyclic voltammetry. The range of potential varies from −0.2 to +1.0 V at 0.1 V/s. The activation was completed upon the observation of a stable voltammogram.

Figure 3.1. Polishing glassy carbon electrode

3.2.3. CNBs/ Nd_2O_3-NPs /GCE platform preparation

Prior to coating surface of electrode, carbon black nanoballs neodymium oxides nanoparticle were weighed and dispersed in chloroform. The mixture was then sonicated for 45 minutes for obtaining a black suspension. Afterwards, 5 μL of black suspension (CNBs/ Nd_2O_3-NPs) was dispersed on the electrode by using a micropipette. Then, the electrode left to dry in air for 5 min. The platform was denoted as CNBs/ Nd_2O_3-NPs /GCE. The platform was washed with ultra-pure water before use. Then, the cyclic voltammetry was carried out in phosphate buffer for the activation of the electrochemical platform in range of -0.2 to +1.0 V for obtaining a stable voltammogram. A schematic preparation of the platform has been illustrated in Figure 3.2.

Figure 3.3 shows the XRD spectrum of Nd_2O_3/CNBs. Peaks at 2θ = 30.5°, 32.0°, 35.0°, 44.0° and 56.0° correspond to the facets of Nd_2O_3, and peak at 2θ = 26° corresponds to the diffraction facet of carbon nanoballs.

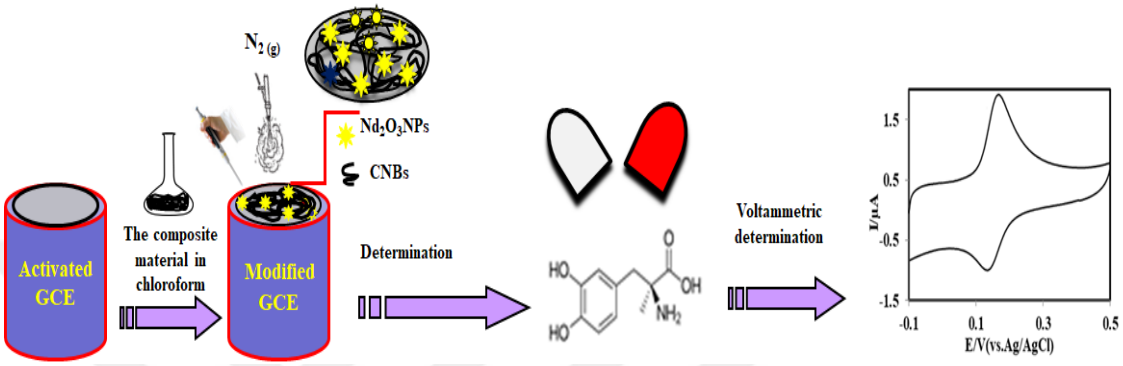

Figure 3.1. Schematic illustration of the proposed electrode

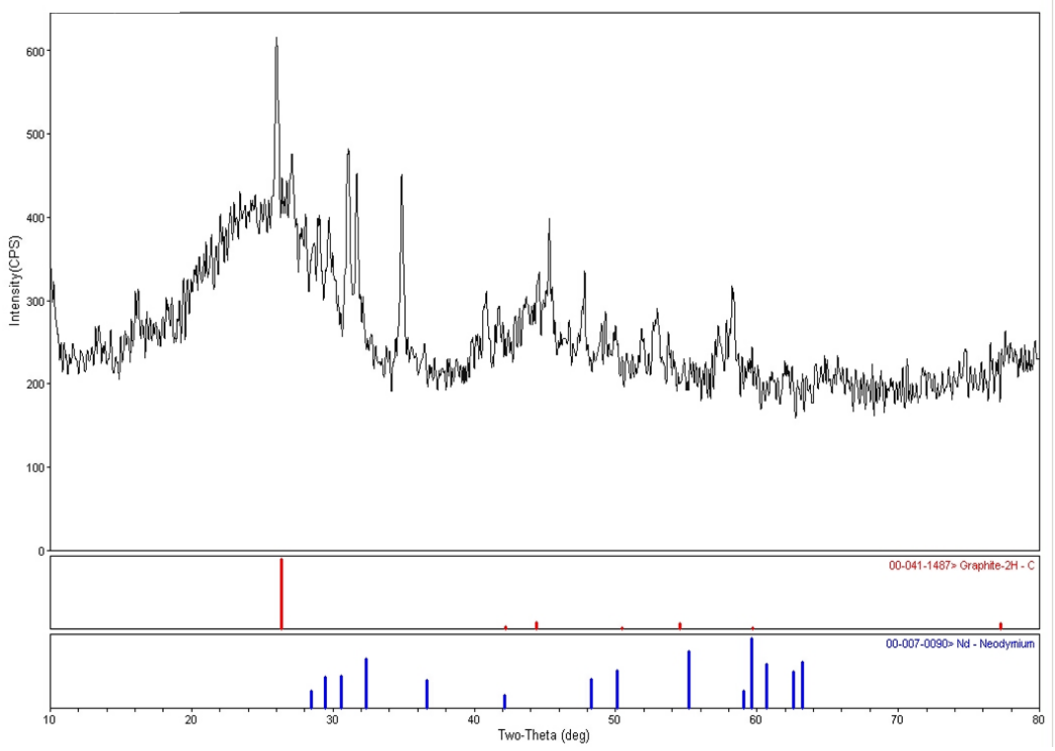

Figure 3.3. XRD pattern of the proposed electrode

3.2.4. Optimization of platform

The sensitivity of the method was high when the ratio of 1:10 for the mixture of Nd_2O_3NPs and carbon nanoballs was dispersed in chloroform. The sonication of Nd_2O_3 and CNBs should not be less than 45 min. In addition the electrode should be coated with only 5 µL of black suspension for high response towards methyldopa.

3.2.5. Voltammetric measurement

The electrode was activated by cyclic voltammetry in phosphate buffer in a potential range of -0.2 to +1.0 V. The procedure was completed upon the observation of a stable background voltammogram. Voltammograms of methyldopa were recorded at at several electrodes for comparison. The mechanism of methyldopa at CNBs/Nd_2O_3-NPs/ GCE was determined by the effect of scan rate. Furthermore, influence of pH on peak potential was investigated to determine the redox reaction of methyldopa. Then, linear working range was obtained for methyldopa using a plot of peak current against the various concentrations by cyclic voltammetry.

4. RESULTS and DISCUSSIONS

4.1. Cyclic Voltammetry of Methyldopa

Cyclic voltammograms of 6.0×10^{-6} M methyldopa studied at various electrodes by cyclic voltammetry are presented in Figure 4.1. Electrodes such as (a) GCE, (b) GCE modified with CNBs and (c) GCE coated with CNBs/Nd_2O_3-NPS are compared for a sweep rate of 50 mV/s in 0.1 M buffer at pH 4.

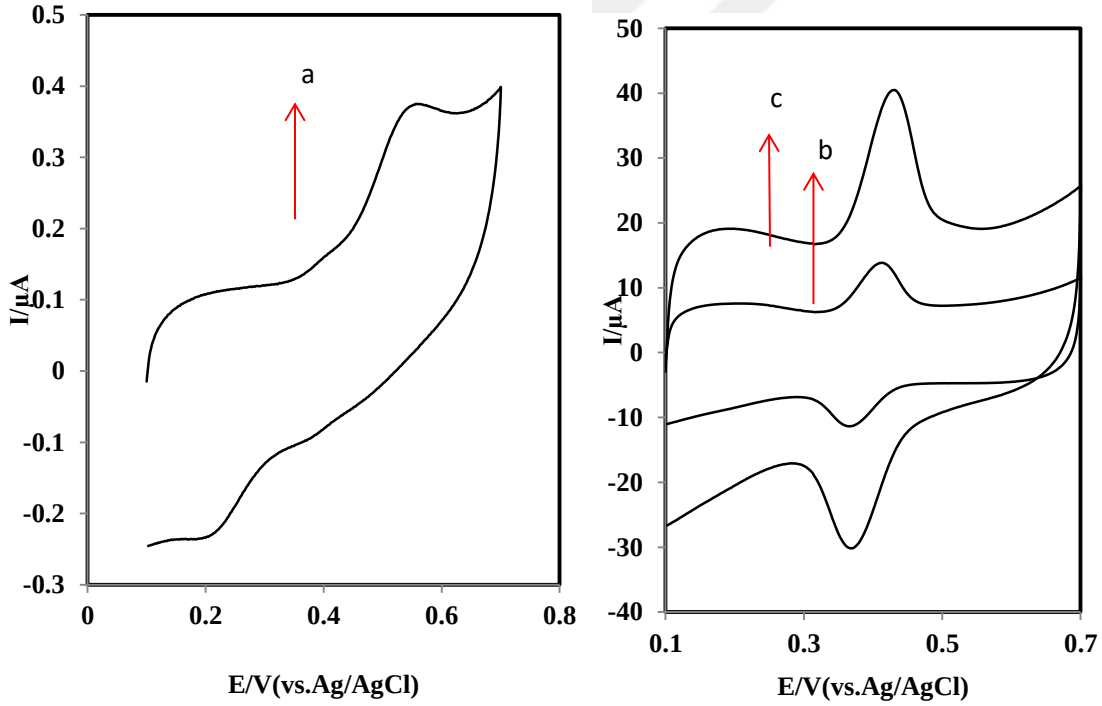

Figure 4.4. CVs of 6.0×10^{-6} M methyldopa at GCE (a); CNBs/GCE (b) and CNBs/Nd_2O_3/GCE (c)

Data indicated that bare GCE showed a poor voltammogram towards Methyldopa. The separation between oxidation/reduction peak potentials (ΔEp) was found to be 339 mV (Epa = 0.539V, Epc = 0.202 V) as shown in Figure 4.1a. The electrode coated with carbon nanoballs (CNB/GCE) showed an oxidation peak at 0.40 V. The CNB/GCE also

produced a redection peak at 0.36 V. The ΔEp was 50 mV for MD at GCE coated with carbon nanoballs as presented in Figure 4.1b. However, an electrode coated with a carbon nanoballs and neodmyium oxide nanoparticles (CNBs/Nd_2O_3-NPs /GCE) exhibited an improved peak at 0.40 V. The reversed peak was observed at a potential of 0.38 V using the proposed electrode (Figure 4.1c). ΔEp was 20 mV for MD process. Obviously, ΔEp was decreased to 20 mV at CNBs/Nd2O3-NPs /GCE when compared to other electrodes. The decrease in ΔEp at the proposed electrode system was a clear indication of acceleration of electrochemical reaction of MD. Decrease in ΔEp and high response for methyldopa at the proposed electrode indicated that the CNBs/Nd_2O_3-NPs /GCE was more appropriate for studying electrode reaction of methyldopa , such an improvement of methyldopa process is due to the increase of surface area with carbon nanoballs and neodymium oxide nanoparticles (Teker and Aslanoglu, 2019; Kutluay and Aslanoglu, 2016; Fathi et al., 2020).

4.2. Effect of scan rate

Voltammetry of methyldopa at various scan rate was studied. The peak potential and peak response was dependent on scan rate using the proposed platform. The experimental results were used to determine possible reaction mechanism for methyldopa. Voltammograms for 6.0×10^{-6} M methyldopa in phosphate buffer for various scan rates are given in Figure 4.2. The response was increased linearly with sweep rate. This indicated an adsorption controlled mechanism for methyldopa (Figure 4.3). The calibration equation was Ipa (μA) = 0.2584 υ (mV s^{-1})+7.36. A correlation coefficient of 0.9916 was obtained. Peak potential shifted with increase in scan rate. This is an indicative of a quasi-reversible electrode reaction at voltammetric platform (Rezaei et al., 2013).

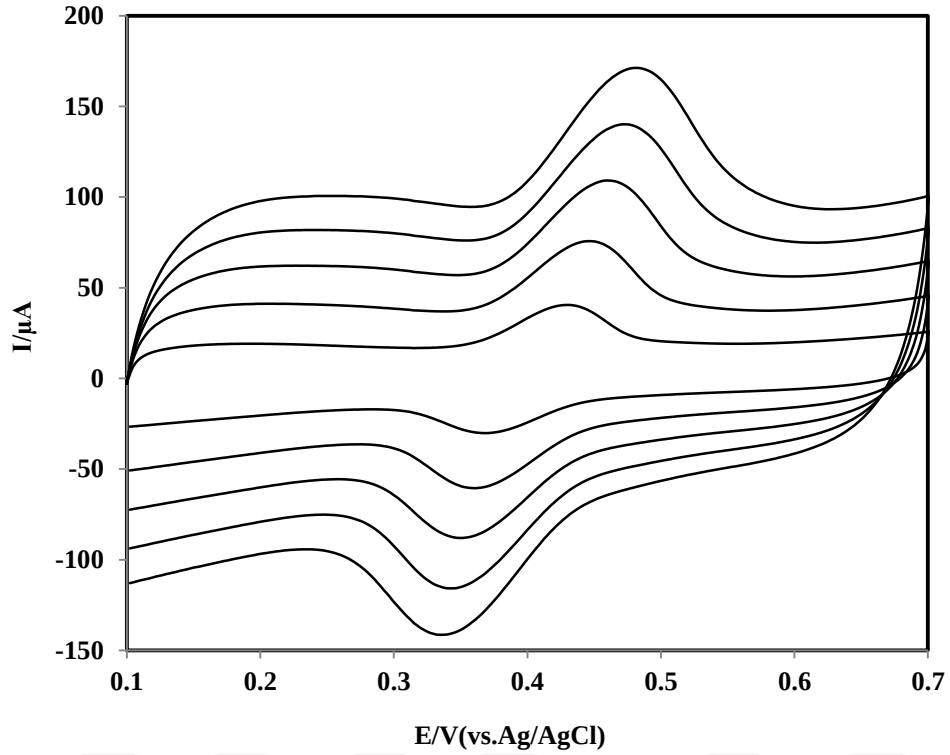

Figure 4.5. CVs of 6.0×10^{-6} M methyldopa at CNBs/Nd_2O_3/GCE. Scan rate: 50, 100, 150, 200, 225 mV/s and 250 mV/s. (bottom to top)

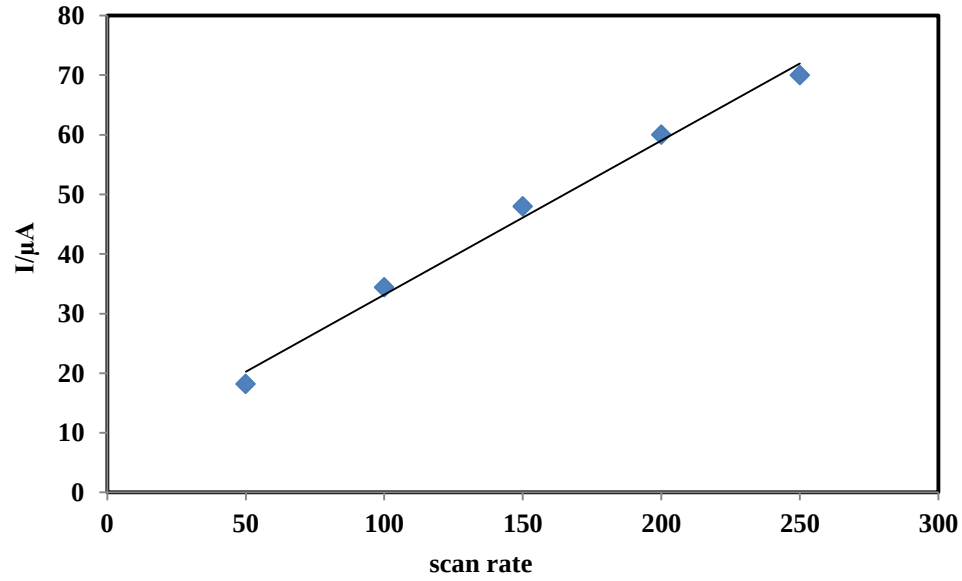

Figure 4.6. Plot of current response of 6.0×10^{-6} M methyldopa versus scan rates

4.3. Effect of pH

Peak potential for methyldopa is influenced by solution pH, Therefore, the oxidation of methyldopa was performed to obtain the significant pH value for the further studies and to acquire the number of protons involved in its reaction by cyclic voltammetry. Figure 4.4 shows the proposed electrode reaction for methyldopa. For this purpose, various pH solutions containing 6.0×10^{-6} M methyldopa at the proposed electrode are presented in Figure 4.5. An equation of E_{pa} (v) = 0.6645 – 0.0653pH was obtained for MD at the proposed electrode. The correlation coefficient was 0.979. The potential shifted toward the negative voltage with increasing pH values indicated that methyldopa oxidation included proton transfer. The anodic peak potential (E_{pa}) was plotted versus pH and a slope -0.0653 V/ pH^{-1} was obtained (Figure 4.6). This indicates that the ratio for electrons and protons participating in redox couple is 1:1. This reveals equal number of electron and proton. Thus, it can be concluded that two protons are involved in electrode reaction of methyldopa (Kutluay and Aslanoglu, 2016).

The results of pH studies at the proposed electrode also showed that the highest current value was obtained at pH 4 (Fouladgar and Ahmadzadeh, 2016). Therefore, pH 4 was chosen for further studies of methyldopa.

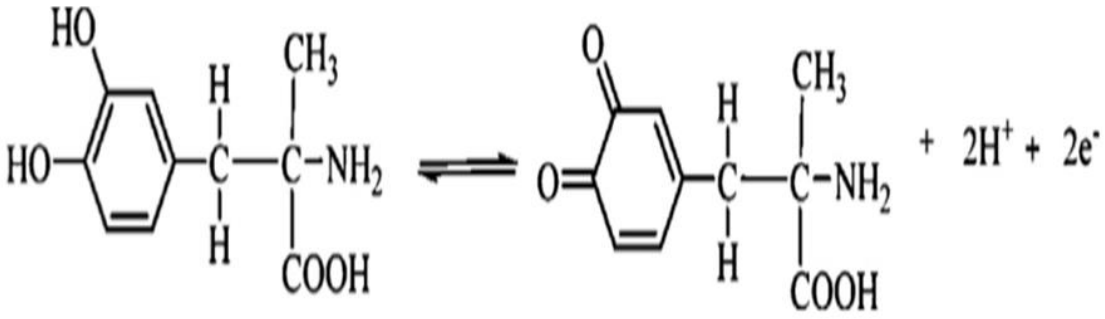

Figure 4.4. The electrode reaction of methyldopa

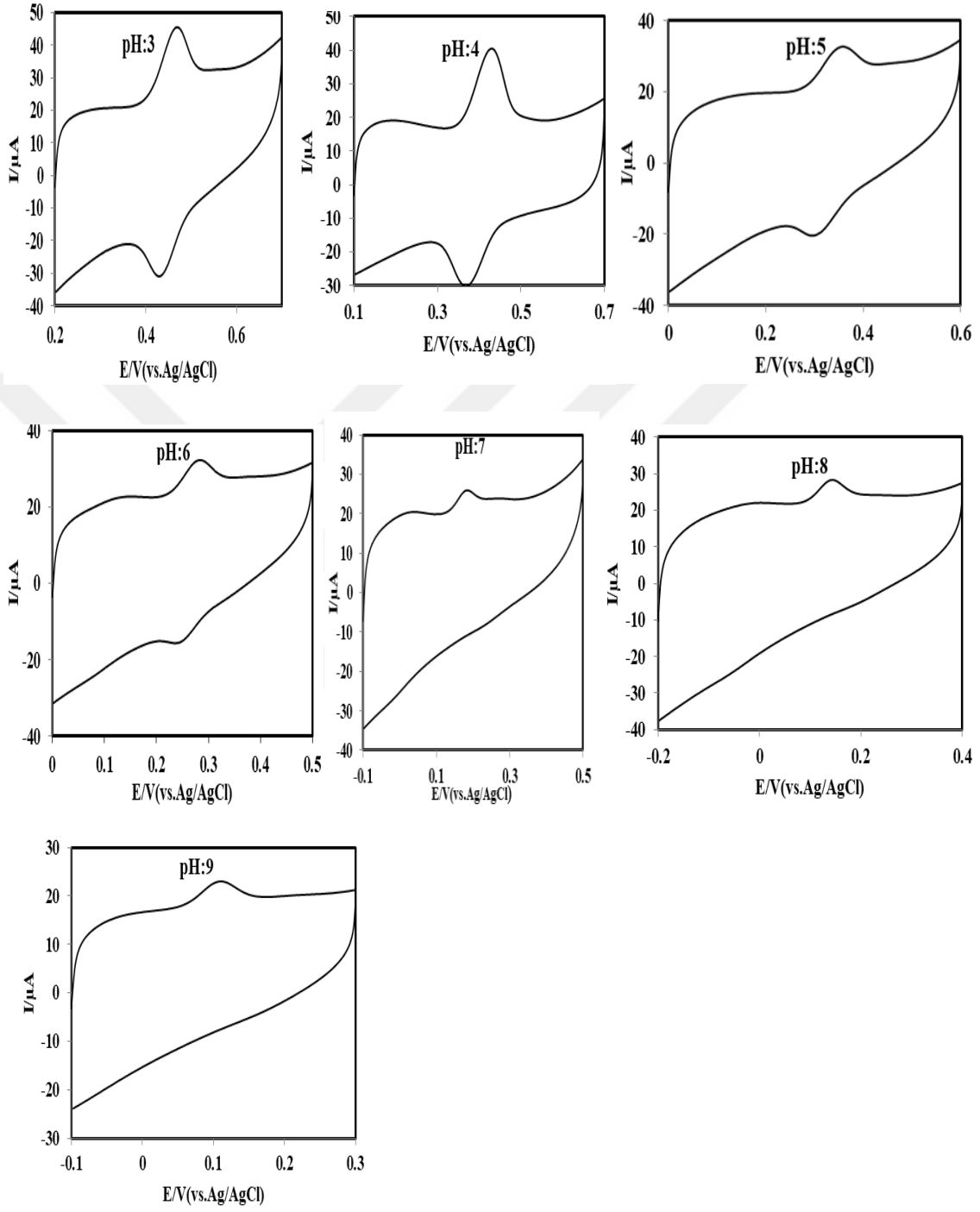

Figure 4.5. CVs of 6.0×10^{-6} M methyldopa at CNBs/Nd_2O_3/GCE in phosphate buffer at various pH values

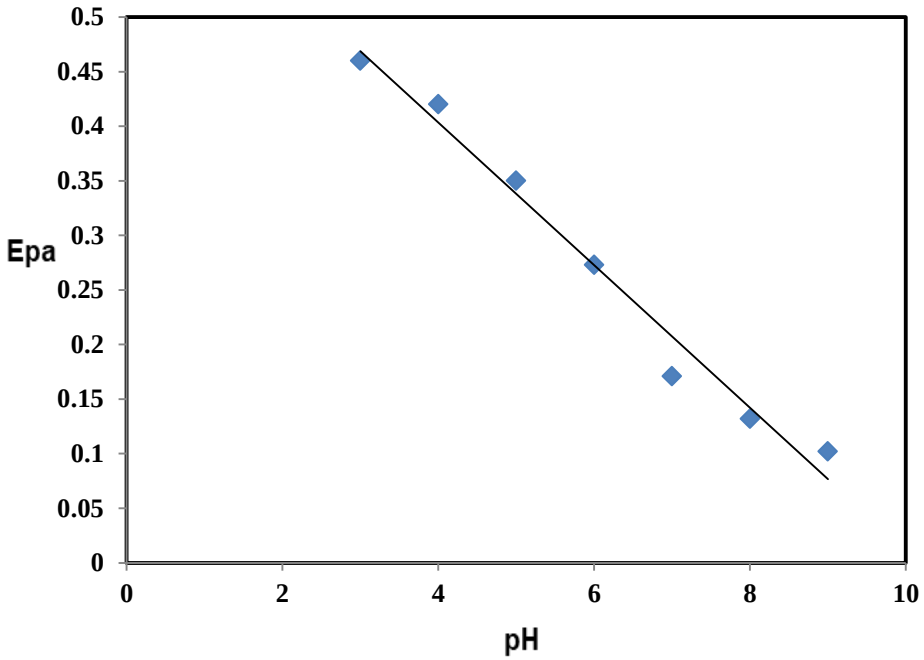

Figure 4.6. A plot of peak potentials of methyldopa versus solution pH

4.4. Selectivity and Repeatability

Voltammetry is a sensitive approach to evaluating many drugs. Methyldopa and paracetamol have unique electrochemical properties. Although they have similar structures but their pharmacological properties are different. Methyldopa is an α2 inhibitor. However, paracetamol behave as an anti-inflammatory drug (Vieira et al., 2003; Uesawa and Mohri, 2010; Calas-Blanchard et al., 2015; Kutluay and Aslanoglu, 2016). CVs for a number concentrations of MD in a solution containing paracetamol at CNBs/Nd_2O_3-NPs/ GCE in 0.1 M phosphate buffer exhibited two improved peaks for methyldopa, and paracetamol as shown in Figure 4.7. The response of peak current of methyldopa increased linearly with its concentration. This indicates that it does not interfere with paracetamol determination. Therefore, results showed great selectivity of the proposed platform methyldopa in drugs.

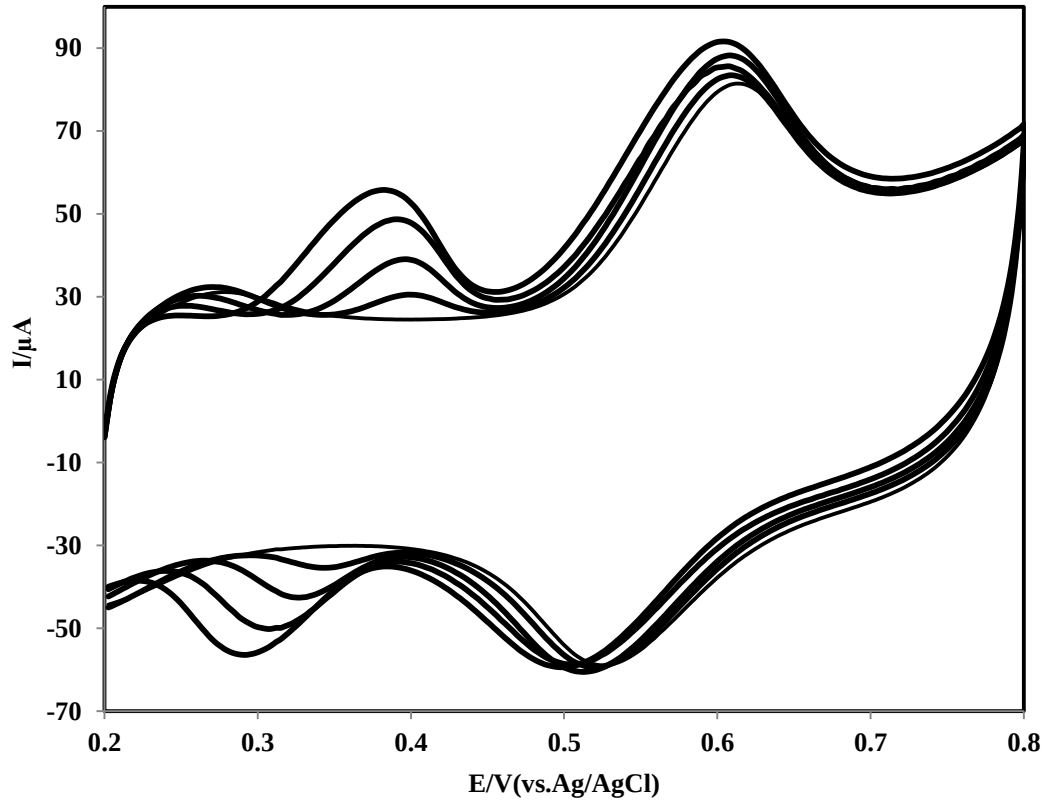

Figure 4.7. Selectivity studies of methyldopa in the presence of 8.0×10^{-6} M PAR CNBs/Nd_2O_3/GCE. Methyldopa: 6.0×10^{-6} M; 1.2×10^{-5} M; 1.7×10^{-5}M; 2.3×10^{-5} M

Repeatability of the proposed voltammetric platform CNBs/Nd_2O_3/GCE was also excellent for the electrode process of methyldopa because an RSD of RSD% = ≈1.4% was obtained for 1.8×10^{-5} M MD in 0.1 M PBS at pH 4.0 for10 scans.

4.5. Calibration

Cyclic voltammetry at CNBs/Nd_2O_3NPs/GCE was performed for study of calibration plot of methyldopa. Voltammograms for various concentrations of MD are given in Figure 4.8. The peak current was plotted for methyldopa concentration as shown in Figure 4.9. The response was linear in the range of 2.0×10^{-7}M - 1.8×10^{-5} M. The corresponding equation with a correlation cofficient were represented as Ip(µA) =

0.90(μM)+ 0.556, R^2 = 0.990 respectivly. A detection limit (LOD) = 7.24×10^{-8} M was obtained for methyldopa.

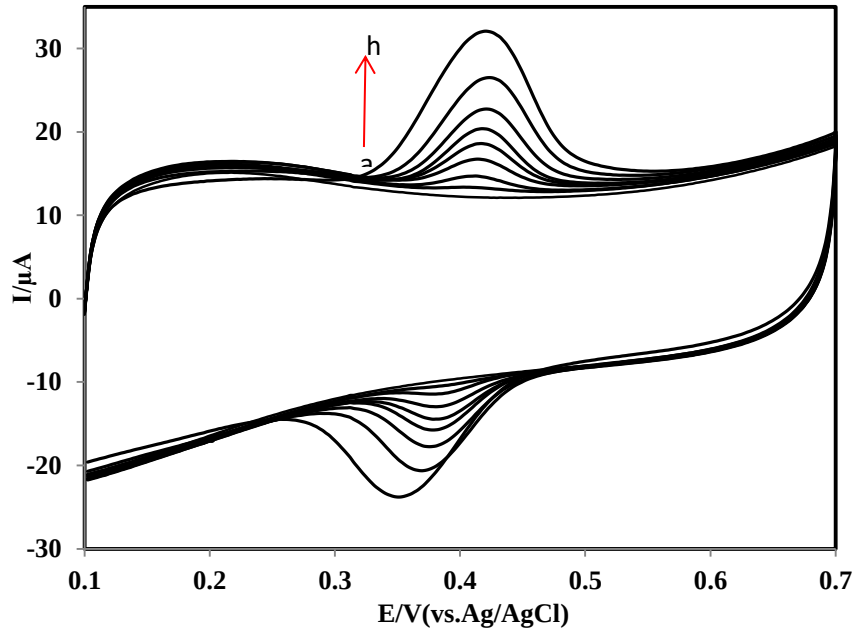

Figure 4.8. Voltammograms for methyldopa concentrations at CNBs/Nd_2O_3/GCE. Methyldopa: a) 2.0×10^{-7}; b) 6.0×10^{-7} M; c) 1.2×10^{-6} M; d) 1.8×10^{-6} M; e) 3.0×10^{-6} M; f) 6.0×10^{-6} M; g) 1.2×10^{-5} M; h) 1.8×10^{-5} M

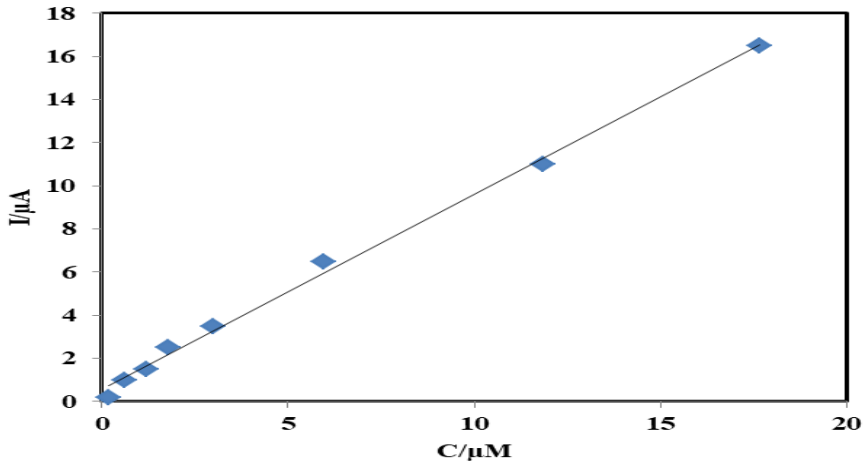

Figure 4.9. Calibration plot of methyldopa

4.6. Analysis of Drug Sample

The determination of cyclic voltammetric (CV) of methyldopa in drugs refers to regression equation. Data are summarized in Table 4.1. The average recovery was 99.4% with an RSD of 2.8%. The drug analysis at proposed platform showed good agreement to the declared value These results showed that the CNBs/Nd_2O_3/GCE is accurate and precise for quantifying methyldopa in drug sample.

Table 4.1. Determination of methyldopa in drugs

Sample	Added (mg)	Found (mg)	Recovery%	RSD%
Alfamet Tablet	250	248.5	99.4	2.8

5. CONCLUSIONS and RECOMMENDATIONS

In this study, a new voltammetric method for sensitive quantification of methyldopa in drug samples was developed using a GCE modified with CNBs and neodymium oxide nanoparticles. The electrochemical platform exhibited remarkable electrocatalytic activity methyldopa. Compared with conventional naked GCE and CNBs/GCE, the CNBs/Nd_2O_3/GCE showed high performance in point of peak potential difference and current response for methyldopa. The proposed electrode was used for detecting methyldopa in a sample containing paracetamol. The results showed that high amount of paracetamol did not interfere with methyldopa. The platform was successfully performed for determination of calibration equation for MD using CV. The peak current increased linearly with of MD concentration. A linear range of 2.0×10^{-7} M - 1.8×10^{-5} M was obtained. The proposed sensor showed good sensitivity with a LOD of 7.24×10^{-8}. The platform of CNBs/Nd_2O_3/GCE was successfully utilized in drug sample.

Further measurements may be conducted using other materials e.g. carbon nanotubes to improve the sensitivity of platforms and enable the study of methyldopa in samples containing ascorbic acid and uric acid. Also, proposed electrode can also be applied to other real samples.

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