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Single-Walled Carbon Nanotube and Hemoglobin used in a Dopamine Biosensor

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Planning a dopamine biosensor by single-walled carbon nanotube (SWNTs) additionally hemoglobin (Hb) experimented. Cyclic voltammetry (CV) for entirety electrochemical research were functioned. A standard three-electrode cell was operated during the analyses, Hb– carbon paste electrode (CPE) or SWNTs-Hb changed CPE as a working electrode, a saturated calomel electrode (SCE) as a reference electrode, and a platinum electrode as a counter electrode. Oxidation peak potential (Epa) as well as reduction peak potential (Epc) were between −350 also −400mV, respectively, furthermore the peak-to-peak disjunction (ΔEp) was 50mV. To check the catalytic behavior of Hb, cyclic voltammograms of the SWNTs-Hb changed carbon paste electrode were examined in a solution with various concentration of dopamine. The outcomes demonstrate that dopamine displayed a linear association for diverse concentrations of dopamine in the level of 2 to 11 mM.

Keywords: Single-walled carbon nanotube (SWNTs), Dopamine, Bioelectrochemical sensing, Hemoglobin

1. INTRODUCTION

Nanomaterial is the hottest area in nanotechnology that analyzes fabrication, characterization, and analysis of substances with morphological form on the nanoscale in at least one dimension. The nanoscale is frequently described as the size that is smaller than 100 nm [1-3]. Although, it is sometimes enlarged to a size smaller than 1 mm. The most important aspect of nanomaterial is their particular characteristics related with nanoscale geometries. The best basic difference of nanomaterial is the high external region to volume ratio, which results in several unusual physical as well as chemical characteristics like as high molecular adsorption, large external tension force, improved chemical and biological behaviors, large catalytic functions, also severe mechanical strength, however another peculiar property of nanomaterial and modernly best analyzed is the quantum size outcome that directs to their discrete electronic band composition identical those of molecules[4-5]. This quantum property of nanomaterial can lead to an extraordinary high sensitivity additionally selectivity of biosensors also can be encouragement to the area of diagnostics. Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure. These cylindrical carbon molecules have unusual properties, which are valuable for nanotechnology, electronics, optics and other fields of materials science and technology. Most single-walled nanotubes (SWNT) have a diameter of close to 1 nanometer, with a tube length that can be repeated millions of courses longer [6-9]. Modern benefit in synthesis as well as fundamental believing of properties of nanomaterial has led to significant advancement of nanomaterial-based biosensors [10-11]. Electrochemical biosensors have been extensively studied because of their specific features: fast response, easy fabrication, high sensitivity, selectivity, quantification of analyte target, and additionally they give the probability of portable analyzes that could be applied in point-of-care instruments. To correct the characteristics of electrochemical sensors, modern conceptions of signal amplification are desirable for ultrasensitive biosensors [12-14]. The design of new electrochemical biosensors combining an electrode with nanomaterial is recently gaining importance. In this study we designed a dopamine biosensor with SWNTs and Hb. Dopamine (3, 4 dihydroxyphenylethylamine, DAH_2) is a critical brain neurotransmitter molecule of catecholamine also its inadequacy directs to brain diseases such Parkinsonism, where dopamine (DA) levels are reduced and schizophrenia, which can be related to excess activity of dopamine [15-17]. It used by neurons in several brain regions involved in motivation and reinforcement, Foremost critically the nucleus accumbens. Bioelectrochemical action of dopamine recreations crucial roles in its physiological acts also is an essential agent in diagnosis of some disorders in clinical medicine [18-20]. Hence, it is desirable to develop an electrochemical method to study electron transfer processes. Schematic structure of SWNTs, molecular structure of hemoglobin and molecular structure of dopamine shown in figure 1.

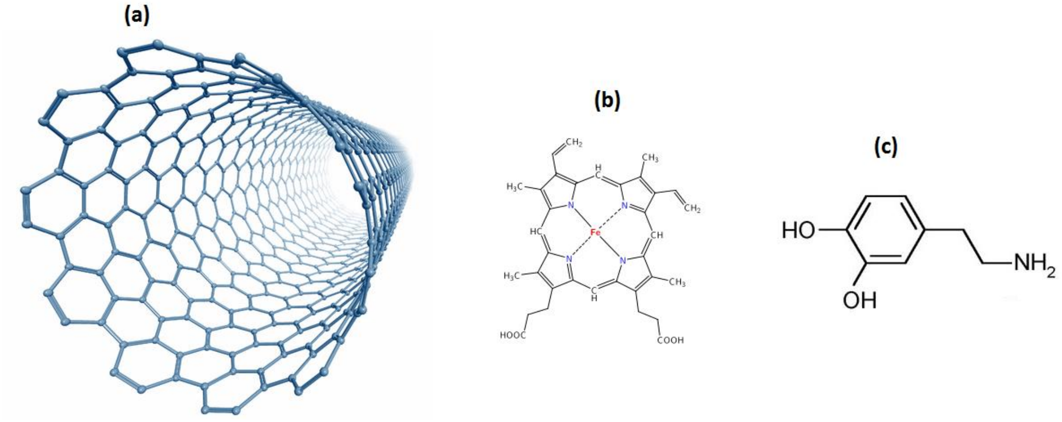


Figure 1. (a) Schematic structure of SWNTs; (b) molecular structure of hemoglobin; (c) molecular structure of dopamine

2. MATERIALS AND METHODS

2.1. Materials

Entire objective includes dopamine, $Fe(NO_3)_3$.$9H_2O$, $Co(NO_3)_2$.$6H_2O$, MgO, HCL, HNO_3, hemoglobin also difference reagents purchased from Sigma-Aldrich Co. (USA), additionally used without furthermore purification. The supporting electrolyte used for all experiments was 0.1 M pH 7 phosphate buffer solution (PBS), which prepared by using a potassium phosphate solution (KH_2PO_4 and K_2HPO_4; 0.1 M general phosphate) at pH 7.0. All the reagents applied were of analytical grade also all aqueous solutions were composed applying doubly distilled water generated by a Barnstead water system.

2.2. Apparatus

Cyclic voltammetry (CV) analysis were appeared using an Autolab potentiostat PGSTAT 302 (Eco Chemie, Utrecht, The Netherlands) acted by the average aim Electrochemical systems data processing software (GPES, software version 4.9, Eco Chemie). A conventional three-electrode cell was employed throughout the experiments, with Hb–CPE or SWNTs modified carbon paste electrode (4.0mm diameter) as a working electrode, a saturated calomel electrode (SCE) as a reference electrode, also a platinum electrode as a counter electrode. The experimental solutions were de-aerated using highly pure nitrogen for 30 min. and a nitrogen atmosphere was kept over the solutions during the measurements. All the electrochemical measurements were carried out in 0.1 M PBS, pH 7.0 at 25 ± 1 ^{o}C. All experimental solutions were deoxygenated by bubbling highly pure nitrogen for 20min and a nitrogen atmosphere accepted all along calculations. Structural and morphological analysis of the samples was done using SEM (scanning electron microscopy), XRD (x-ray diffraction), and UV-Visible spectroscopy analytical methods. XRD was measured using the instrument of Germany's Siemens and CuKα was obtained 1.54056 $\overset{\circ}{A}$. Ultraviolet-Visible spectroscopy (UV-VIS) was obtained by Shimadzu UV 160 Spectrophotometer. A fixed bed flow and a ceramic boat (length, wide, height: 1.5×1×8) were used for SWNTs preparation.

2.3. Synthesis of SWNTs

In this research, SWNTs along with Fe/Co metal catalyst were grown on MgO basis using CVD (Chemical Vapor Deposition). Then, grown nanotubes were putrefied in two processes of under atmospheric oxidation and acidic stew with HCL. SWNTs synthesis through this method consists of two stages: 1) catalyst preparation, and 2) SWNTs synthesis. Bimetallic catalyst of Fe/Co was prepared using wet chemical saturation method on MgO basis. 500 mg MgO was dispersed in 10 ml distilled water and the resulting suspension for 60 min in an ultrasonic device was changed into homogeneous suspension. Metallic nitrates of $Fe(NO_3)_3.9H_2O$ and $Co(NO_3)_2.6H_2O$ with the ratios of Fe/Co/MgO : 15/15/70 were added to suspension and exposed to ultrasonic for 30 minutes. Then, this mixture was dried at the temperature of 120°C and milled to a soft powder. Next, the obtained catalyst was use as a basis for the growth of nanotubes.

2.4. The growth of SWNTs

First of all, the catalyst was revived using hydrogen gas and in the temperature of 500-600ºC. To this end, the prepared catalyst was spread into a ceramic boat, was placed inside a fixed bed flow, and after being revived by hydrogen gas the gas flow was interrupted, and nitrogen gas with volumetric flow rate of 150ml/min and the temperature of 800ºC was remained for 30 minutes. To grow SWNTs, methane gas with volumetric flow rate of 30ml/min and the temperature of 870ºC was entered into the system for 60 minutes. Finally, methane gas flowing was stopped and the boat's product under a nitrogen flow was cooled to room temperature [12-13]. In this project, high purity (99%) hydrogen, nitrogen, and methane gases were used.

2.5. SWNTs purification

Synthesized nanotubes, in order to be purified, were at first oxidized for 60 minutes in the temperature of 400º to remove amorphous carbon. In order to separate MgO and metallic nanoparticles of Fe and Co, 200 mg of the sample underwent acidic stew in 50ml HCL 3N for 6 hours. After separating purified SWNTs and washing them with distilled water, in order to remove the acidic effect, the nanotubes were dried at 100ºC for 24 hours [10-14].

2.6. Activation of SWNTs

SWNTs were activated using the method introduced by Louis et al [15-16]. According to this method, 2 mg SWNTs were added to 10 ml saturated H_2SO_4 and HNO_3 with the ratio of 3:1. This solution was placed for 4 hours in sonication bath with the power of 55 Hz. Then, nanotubes were washed using deionized distilled water, and were collected by a 0.45 µm diameter filter. This process continues till the water used in washing has a neutral pH. In this method, by oxidizing SWNTs through using acid, formation of Co_2 gas and its getting out of the solution, activated carboxyl groups are formed at the end of the SWNTs, and thus, these nanotubes get activated.

2.7. Preparation of bare carbon paste electrode

The carbon powder (particle measurement 50 mm, density 20-30g/100mL) was combined with the binder, silicone oil; in an agate mortar also homogenized employing the pestle. The electrode consisted of a Teflon well, mounted at the end of a Teflon tube. The prepared paste was filled into the Teflon well. A copper wire fixed to a graphite rod and inserted into the Teflon tube served to establish electrical connection with the outward circuit. The electrode surface of the working electrode was recovered mechanically by smoothing several pastes off and next polishing on a piece of transparent paper before conducting each of the experiments. The experiments were performed in unstirred solutions.

2.8. Preparation of SWNTs modified carbon paste electrode

The SWNTs modified carbon paste electrode was developed by hand combining of carbon powder, binder also 25 mg SWNTs with silicon oil in an agate mortar to make a homogenous carbon paste. Other steps of produced modified carbon paste electrode were similar to preparation of bare carbon paste electrode.

2.9. Preparation of Hb- SWNTs modified carbon paste electrode

The modified electrode that produced in previous section was used for production of Hb-SWNTs modified carbon paste electrode. In this domain, the Hb was immobilized by falling 4 µl of 10 mg/ml of the protein solution onto the SWNTs modified carbon paste electrode also dried for about 30 min at room temperature. The electrode was then gently washed with de-ionized double distilled water and put at 4 ºC when not in use.

3. RESULTS AND DISCUSSION

3.1. Scanning electron microscopy study

As it is well known, the properties of a broad range of materials and the performance of a large variety of devices depend strongly on their surface characteristics. Scanning electron microscopy (SEM) study showed in Figure 2 (a, b). The scale bare was 100 nm. Figure 2 (a) show unmodified carbon paste electrode and Figure 2 (b) show modified carbon paste electrode with SWNTs and Hb.

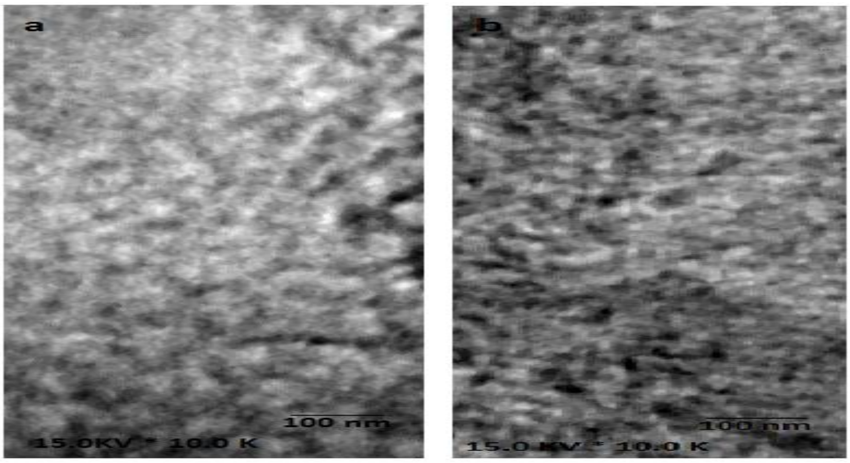


Figure 2. Scanning electron microscopy; a) unmodified carbon paste electrode, b) modified carbon paste electrode with SWNTs and Hb; in both state scale bare was 100 nm.

3.2. UV-Visible spectrum characteristics of synthesized nanotubes

UV-Visible spectroscopy is a useful technique for determining the amount of the SWNTs functionalizing and examining how nanotubes are dispersed in aqueous environment. Free л electron in initial nanotubes has a special absorption in UV-Visible spectrum. These nanotubes have a special peak in the wavelength of 170nm [17-20] (figure 3).

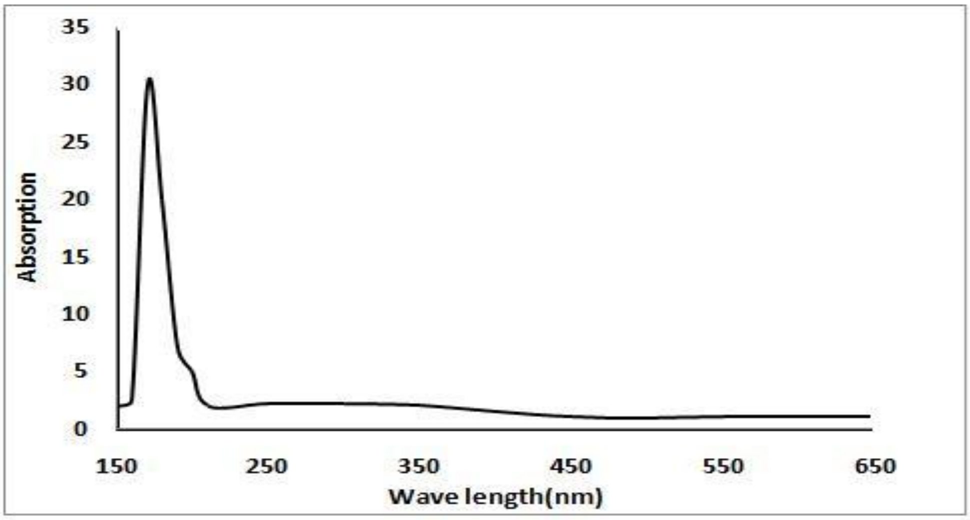


Figure 3. UV-Visible spectroscopy of synthesized SWNTs after purification

As a result of SWNTs activation, a particular peak is observable in the wavelength of 230nm. The peak observed in the wavelength of 230nm is due to the SWNTs carboxylation. This peak is created because of the two free electrons of -c=o existed in carbonyl group (-coo) (figure 4).

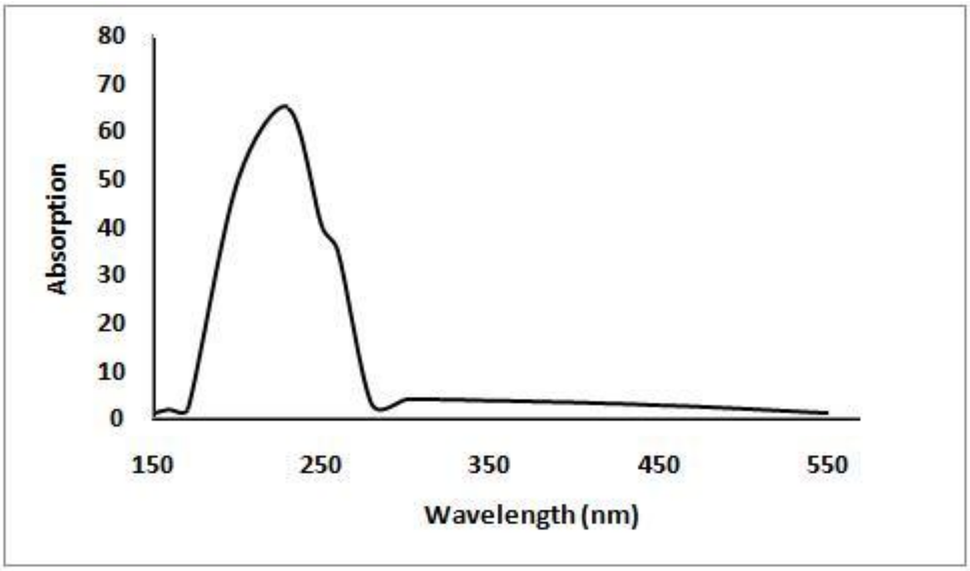


Figure 4. UV-Visible spectroscopy of synthesized SWNTs after activation

UV-Visible spectrum of synthesized SWNTs, after purification and functionalizing, indicates the high quality of produced nanotubes (there is a sharp peak at a wavelength of 170nm), and the good functionalization of the nanotubes.

3.3. X-Rey diffraction pattern of synthesized SWNTs

The X-ray diffraction data were recorded by using Cu Kα radiation (1.5406 A°). The intensity data were collected over a 2θ range of 20–80°. The average grain size of the samples was estimated with the help of Scherrer equation:

$$D = 0.89\lambda / \beta \cos\theta \quad (1)$$

Where λ is the wavelength (Cu Kα), β is the full width at the half-maximum (FWHM) of the SWNTs line, and θ is the diffraction angle. For further exploration of the properties of synthesized crystalline nanotubes, XRD pattern was used that is shown in figure 5.

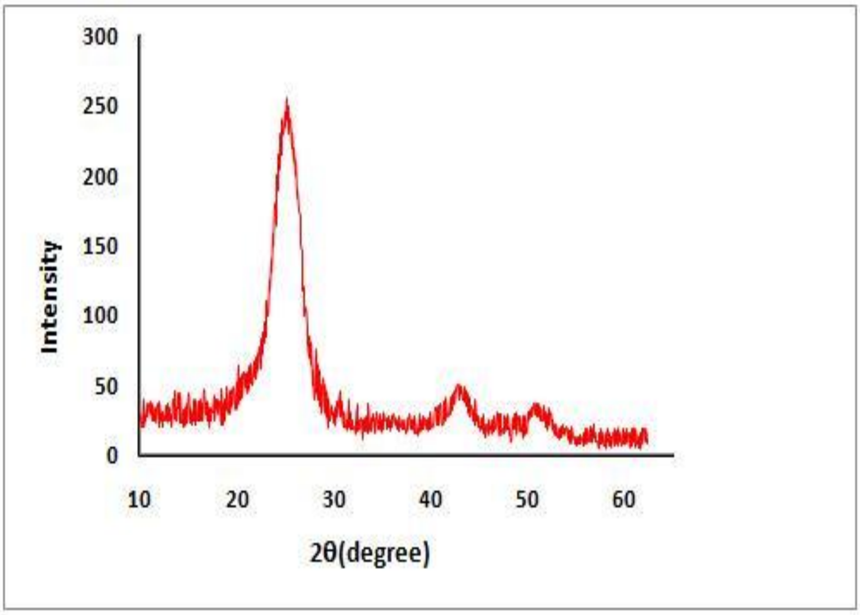


Figure 5. X-Rey diffraction pattern of synthesized SWNTs

As shown in figure 5, the peak at 2θ=25.1° is related to the SWNTs, and the peak at the angles 43° and 52° is related to SWNTs.

3.1. Electrochemical study of bare or SWNTs- Hb modified carbon paste electrode

The electron transfer of redox protein heme categorize onto the surface of an electrode is a critical concept in the area of bioelectrochemistry [21-22]. It can be applied to assay the process of biological electron transfer, and also provide an important platform for the development of biosensors.

The electron transfer changes Fe (III) in the heme of Hb to Fe (II). This mechanism can catalyzes some chemical reaction such as the redox reactions in neurotransmitter. Unfortunately, strong adsorption of Hb on the electrode surface could cause denaturation. On the other hand, the electrochemically energetic centers in Hb are continually buried deeply in its enlarged three-dimensional composition, which causes direct electron transfer between Hb also the electrode surface very difficult. In recent years, some nanomaterials have been used in electrochemical sensors based on proteins as facilitator. Figure 6 (a & b) shows the cyclic voltammograms obtained of the Hb–CPE or SWNTs-Hb modified carbon paste electrode in 0.1M PBS (pH 7.0).

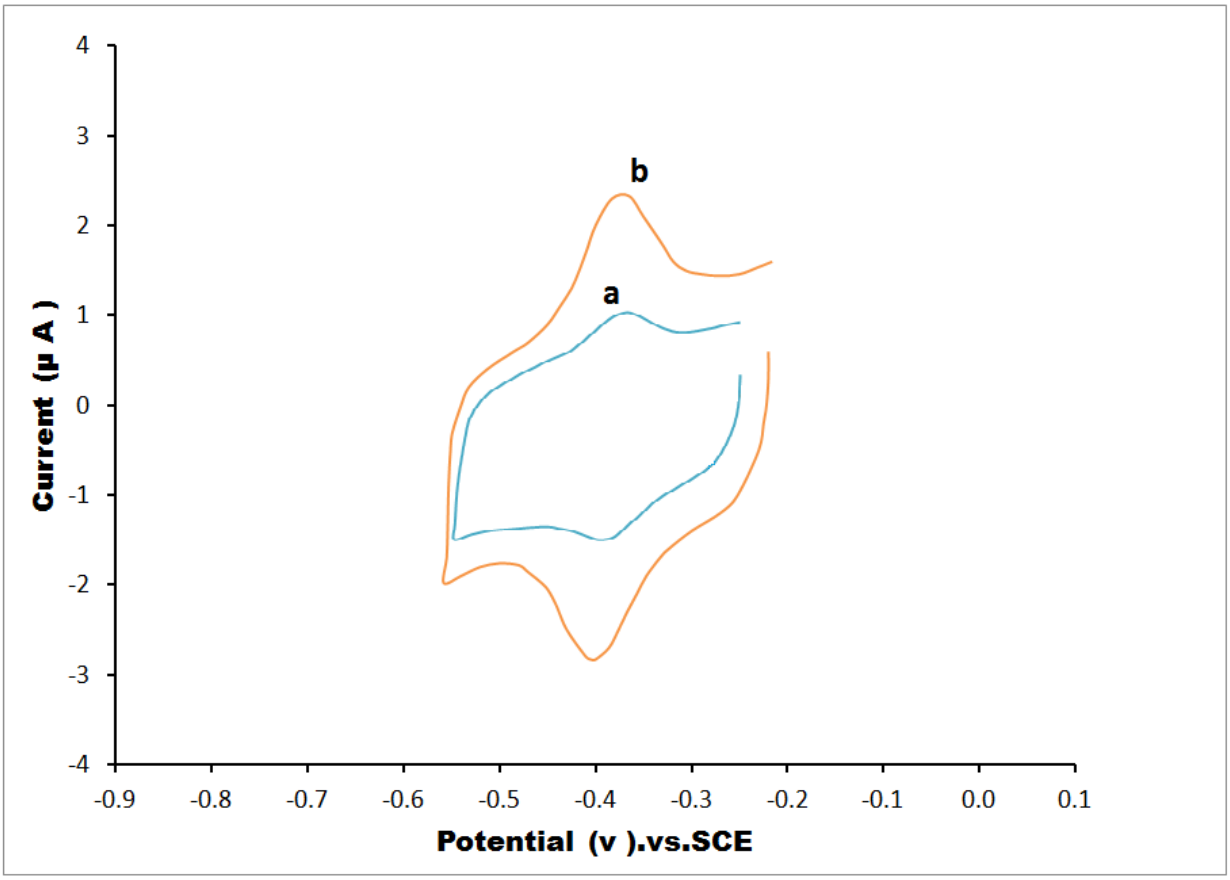


Figure 6. a) Cyclic voltammograms of Hb–CPE; b) Cyclic voltammograms of SWNTs-Hb modified carbon paste electrode; Conditions: 0.1 mol L^{-1} PBS (pH 7.0), scan rate of $50mVs^{-1}$

It can be observed that only a weak redox peak displayed at the Hb–CPE electrode (curve a). At the SWNTs- Hb-CPE, the analytical effects showed a well-defined quasireversible and reproducible anodic and cathodic peaks (curve b). These results showed that the direct electron transfer of Hb onto the surface of the electrode is modified by the introduction of SWNTs. On other hand events demonstrated that Hb changes its molecule to a propitious direction for direct electron transfer and SWNTs may play an important role in the electron transfer between Hb and electrode and this event may be due to the enhanced accessible surface area to the electrolyte.

As demonstrated in Figure. 2, curve b, the oxidation peak potential (Epa) also reduction peak potential (Epc) are between −350 additionally −400mV, respectively, and the peak-to-peak separation

(ΔEp) was 50mV. The ratio of reduction peak current (Ipc) to oxidation peak current (Ipa) is near 1.14. Entire of these electrochemical limitations define that the reaction of Hb happening at the electrode modified by SWNTs is a quasireversible redox process. The formal potential (E°), which is estimated as the midpoint between E_{pa} and E_{pc}, is about −375mV.

3.2. Electrochemical study of SWNTs- Hb modified carbon paste electrode at various scan rates

The effect of different scan rates on the answer of immobilized SWNTs- Hb modified carbon paste electrode is demonstrated in Figure. 7.

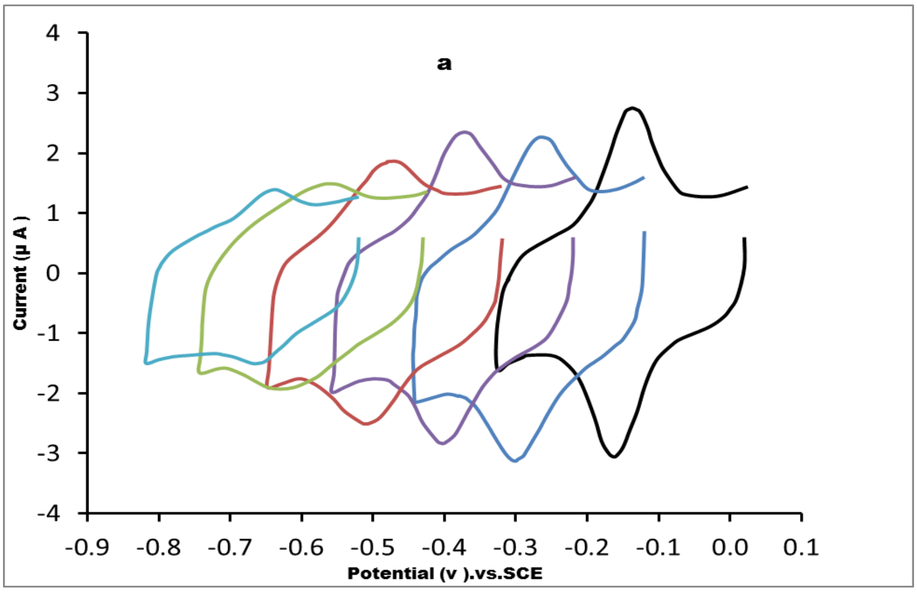


Figure 7. Cyclic voltammograms of SWNTs-Hb modified carbon paste electrode at different scan rate, from inward to outward; 5, 10, 25, 50, 100 and 200 mVs^{-1}.

With the increasing of scan rate, E_{pa} and E_{pc} were shifted slightly toward the positive and the negative direction of potential, respectively, while Ipa and Ipc developing linearly. The effects display that the electrochemical reaction is a surface-controlled mechanism. For one surface-controlled process, surface coverage concentration (г) and the number of electrons transported (n) can be obtained according to Lavrion's equation. From The curves in Figure. 7, Q can be calculated as $1.76\times10^{-7\ C}$. The effective surface area A of the electrode was obtained as $0.065cm^{2}$ by using SWNTs-Hb modified carbon paste electrode. So, n can be measured as 1.3 from the slope of Figure. 7(inset), demonstrating that a single electron reaction happens at the interface. That time the average surface coverage of Hb on the surface, г can be obtained as 1.34×10^{-11} mol cm^{-2}. The electron transfer rate ks of Hb can be calculated with the formula ks = nmFv/RT when nEp < 200 mV, where m is a parameter related with nEp [23]. Acquiring the charge transfer coefficient as 0.5, an average ks value of $5.80\pm0.70\ s^{-1}$ could be produced with the peak- to-peak separation at diverse scan rates. This effect demonstrates that a resultant promotion could be behaved by SWNTs in the mechanism of electron transfer between Hb also carbon paste electrode. All former researches proved that ks rate depends on the substances applied to immobilize the proteins; for nano-structured electrodes, the events are perhaps improved.

3.3. pH effect on electrochemical behaiour of SWNTs-Hb modified carbon paste electrode

Following that, the effect of pH on the electrochemical process of SWNTs-Hb modified carbon paste electrode was observed. Clearly, a maximum peak current is present at pH 7.0, which is like with soluble Hb [24], demonstrating that the optimal pH value for electron transfer of immobilized Hb on SWNTs modified carbon paste electrode does not alteration.

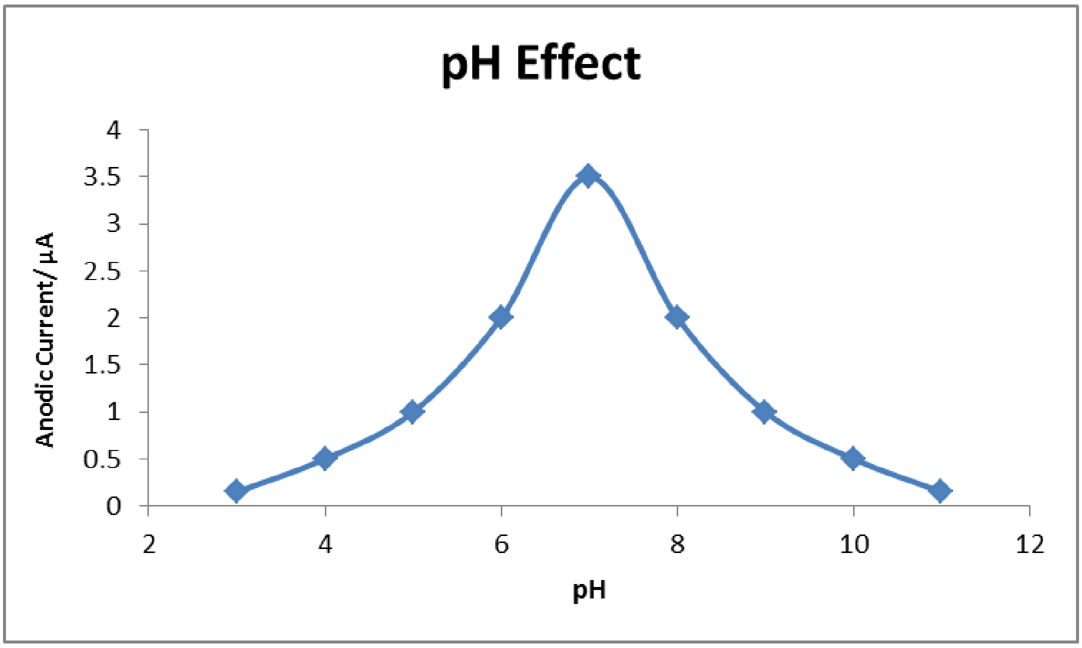
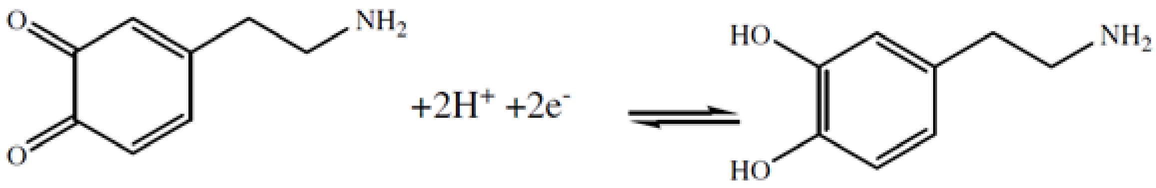


Figure 4. Effect of pH on electrochemical behavior of SWNTs-Hb modified carbon paste electrode

3.4. Design dopamine biosensor by SWNTs-Hb modified carbon paste electrode

In order to check the catalytic action of Hb, cyclic voltammograms of the SWNTs-Hb modified carbon paste electrode were analyzed in different solutions with various concentration of dopamine. The events demonstrate that the cathodic peak currents spread along with amplifying dopamine concentration (Figure. 5 (a)). Although, the managed analyses display that, however the electrodes are modified with Hb, that is to say, the immobilized Hb on the electrode still maintains its electrocatalytic activity only when SWNTs are immobilized on surface of carbon paste electrode. In this study, anodic current showed a linear increase for various dopamine concentrations in the range of 2 to 11 mM (Figure. 5. (b)). The additional mechanism was according below equations:



$\text{Hb (Fe}_{\text{III}}\text{) + substrate} \rightarrow \text{Hb (Fe}_{\text{II}}\text{) + product}$

At electrode:

$\text{Hb (Fe}_{\text{II}}\text{)} \rightarrow \text{Hb (Fe}_{\text{III}}\text{)} + e^-$

The linear equation was expressed as $i_{pc}(\mu A) = 0.4739 + 0.0451$ (mM) ($R^2 = 0.9961$) with a detection limit of 1 mM for this interval.

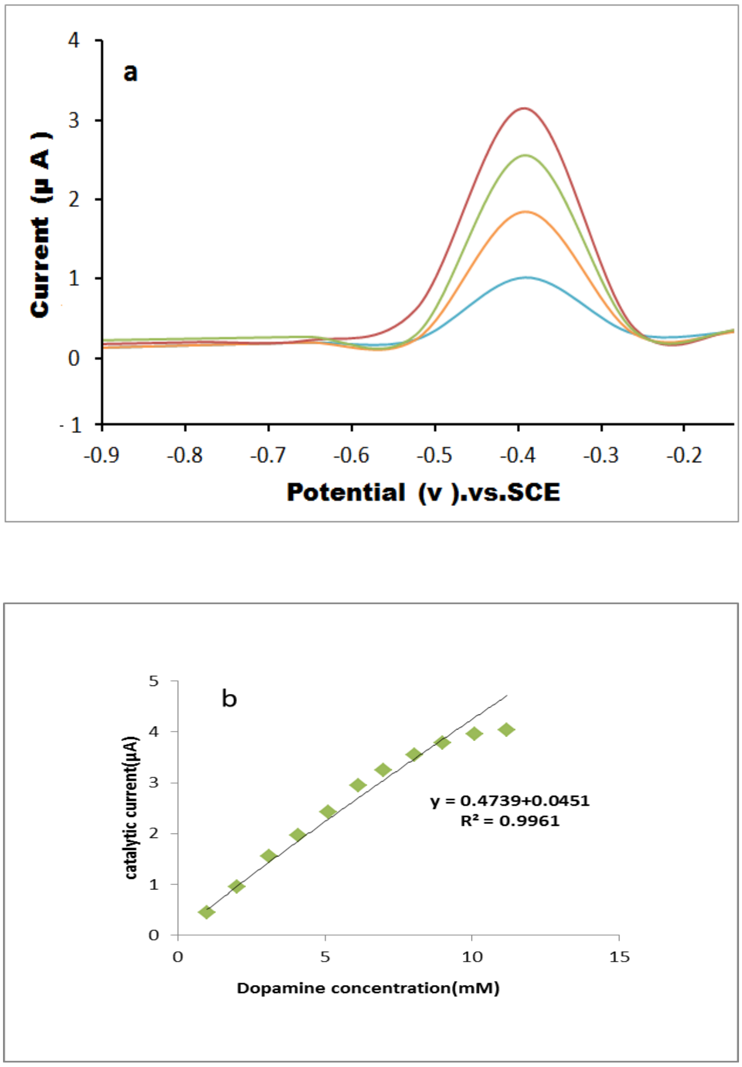


Figure 5. (a) Cyclic voltammograms obtained at an SWNTs-Hb modified carbon paste electrode in 0.1M phosphate buffer solution (pH 7.0) for different concentrations of dopamine and (b) the relationship between anodic peak current of Hb and different concentrations of dopamine (scan rate: 50 mVs^{-1}).

The reaction has certain kinetic specialties of the protein catalyzed reaction due to the current tends toward consistency when the concentration is higher than 1.0×10−5 M. The reciprocals of steady-state current and dopamine concentration have a good linear relationship with a correlation coefficient of 0.9961. So, the apparent Michaelis–Menten constant (*K*app M) for the electrocatalytic activity of the SWNTs-Hb modified carbon paste electrode can be measured as 0.34mM from the Lineweaver–Burk equation [25], which describes that Hb immobilized on the SWNTs modified carbon paste electrode exhibits a high degree of catalysis. It is also confirmed that Hb could keep its biological performance when exchanging electrons with the electrode straightly.

4. CONCLUSION

The revolution of nanotechnology in biology offers a chance to determine notable factors at the small category. Nanoparticles have abundant probable assignments in biosensors. In this study, we planned a dopamine biosensor by single-walled carbon nanotube as well as hemoglobin. The Sensitivity of mentioned biosensor to the reduction of dopamine demonstrated that it owns favorable constancy.

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