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Araştırma Makalesi / Research Article

The Coupling of Dihydroxybenzene Derivatives to MWCNT and Electrochemical NADH Oxidation

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Abstract

3,4 and 2,5- dihydroxybenzene compounds were separately and covalently coupled to the multiwalled carbon nanotube (MWCNT) deposited on the surface of glassy carbon electrode through electrochemical reduction of diazonium compound of benzylamine bearing Boc protecting group (tert-butyloxycarbonyl). Following the removal of the Boc group, an amide bond was formed between the amine-terminated surface and the acyl group of the dihydroxybenzene derivatives. The electrochemical properties of dihydroxybenzene-modified MWCNT and the influence of the sweep rate on the voltammogram were elucidated by cyclic voltammetry (CV). Electron transfer kinetics of modified MWCNT by dihydroxybenzene derivatives was also studied using Laviron's theory. As evidenced by an improvement in the anodic peak current and a negative shift in the overpotential of NADH oxidation when compared to the bare MWCNT, 3,4 and 2,5- dihydroxybenzene modified MWCNT electrodes were shown to possess catalytic activity toward NADH oxidation.

Keywords
MWCNT;
NADH;Sensor;
Electrode Modification

Dihidroksibenzen Türevlerinin MWCNT Bağlanması ve Elektrokimyasal NADH Oksidasyonu

Öz

3,4 ve 2,5-dihidroksibenzen bileşikleri, Boc (tert-bütiloksikarbonil) koruyucu grubunu taşıyan benzilaminin diazonyum bileşiğinin elektrokimyasal indirgenmesi yoluyla camsı karbon elektrotun (GC) yüzeyinde biriktirilen çok duvarlı karbon nanotüpe (MWCNT) ayrı ayrı ve kovalent olarak bağlanmıştır. Boc grubunun çıkarılmasının ardından, amin uçlu yüzey ile dihidroksibenzen türevlerinin açil grubu arasında bir amit bağı oluşturulmuştur. Dihidroksibenzen ile modifiye edilmiş MWCNT'nin elektrokimyasal özellikleri ve tarama hızının voltamogram üzerindeki etkisi dönüşümlü voltametri (CV) ile aydınlatılmıştır. Dihidroksibenzen türevleri tarafından modifiye edilmiş MWCNT'nin elektron transfer kinetiği de Laviron teorisi kullanılarak incelenmiştir. Modifiye edilmemiş MWCNT ile karşılaştırıldığında anodik pik akımındaki artış ve NADH oksidasyonunun aşırı-potansiyelindeki negatif kayma ile kanıtlandığı gibi, 3,4 ve 2,5-dihidroksibenzen ile modifiye edilmiş MWCNT elektrotlarının NADH oksidasyonuna karşı katalitik aktiviteye sahip olduğu gösterilmiştir.

Anahtar kelimeler
MWCNT; NADH;
Sensör; Elektrot
Modifikasyonu

1. Introduction

Over the last few decades, carbon nanotubes or carbon-based nanoparticles have been considered a hot research topic among researchers due to their exceptional electronic features (Iijima 1991, Iijima and Ichihashi 1993). Therefore, carbon nanotubes are at the center of many scientific and nano-technological types of research. Besides its extraordinary properties, one of the instinctively most encountered drawbacks of carbon nanotubes is its poor solubility in water. In order to get rid of this problem, the surface of the MWCNT is modified through two methods, non-covalent adsorption and covalent binding of the regarding molecules to the MWCNT (Downard 2000, Karousis *et al.* 2010, Kharisov *et al.* 2009, Marcoux *et al.* 2004). For the covalent modification of MWCNT, chemical or electrochemical reduction of diazonium has widely been utilized for sensor and bioelectronics applications due to its capability to manipulate surface coverages of the components to be coupled and flexibility to bind the wide variety of the compounds to aromatic ring bonded to MWCNT surface (Abiman *et al.* 2008, Barbier *et al.* 1990, Marcoux *et al.* 2004). Bartlett *et al*.(2008) proposed a novel method for the functionalization of carbon electrodes with diamine derivatives to form C-C or C-N bonds through electrochemical oxidation or reduction of the corresponding diamine or diazonium compounds bearing the Boc protecting group. After the deprotection of the amine from the Boc group, it was shown that anthraquinone, nitrobenzene, and dihydroxybenzene compounds could be coupled to the free amino group by applying basic experimental methods designed for solid-phase synthesis (Chrétien *et al.* 2008, Ghanem *et al.* 2008, Ghanem *et al.* 2012). We have also used this mentioned method to obtain functionalized MWCNT in order to form a chemical bond between dihydroxybenzene and the free amino group on the MWCNT surface.

It is well-established in the literature that nicotinamide adenine dinucleotide (NADH) bears a vital role in the electron transfer events that take place in a living organism (Baradoke *et al.* 2019, Carlson and Miller 1985). NADH is therefore considered one of the most significant biomolecules and detection of this corresponding biological compound is extremely important. Consequently, the construction of electrodes with the ability to interchange NADH with NAD^+ has drawn extensive attention in the biosensor field. However, there are two difficulties required to be taken into account for an NADH biosensor. These are the large overpotential belonging to the NADH oxidation triggered by electrochemical inducement and fouling taking place in the electrode surface due to the adsorption of NADH oxidized form, NAD^+ (Jiménez *et al.* 2014, Koçak and Alıcı 2020). If this fouling issue is not overcome, a lack of sensitivity, selectivity, reproducibility, and stability in the biosensor system could be observed. In order to dispose of the above-mentioned drawbacks, numerous types of the mediator to be coupled to the conductive surfaces have extensively been utilized to accelerate electron transfer kinetic between the related surface and NADH. For this purpose, quinone derivatives, (Carlson and Miller 1985), (poly(caffeic acid), Rębiś et al. 2021), nitroaromatic compounds, (Contreras et al. 2020), Porphyrazine (Rębiś et al. 2020) were utilized as mediator.

We have reported here the functionalization of MWCNT immobilized on GC via electrochemical reduction of $-C_6H_4CH_2NHBoc$ linker, followed by the covalent attachment of 3,4 and 2,5 dihydroxybenzene to an amine-terminated MWCNT surface obtained after removal of Boc group through the formation of an amide bond by employing synthesis approach designed for solid surface. Electrochemical properties of dihydroxybenzene-modified MWCNT and their impact on the NADH oxidation were investigated by CV, considering the oxidation potential of NADH at the bare GC and MWCNT deposited on GC.

2. Materials and Method

2,5 and 3,4 dihydroxybenzene chloride, 1,4-dioxane, and dimethylformamide (DMF) were obtained from TCI Chemicals. Tetrabutylammonium tetrafluoroborate (TBATFB), acetonitrile (ACN), HCl (%37), dichloromethane, pyridine, and 1 M BBr_3 in dichloromethane were purchased from Sigma Aldrich. Ethanol and NaOH, Na_2HPO_4, and NaH_2PO_4 were also purchased from Fisher Scientific. Multiwall carbon nanotubes with bamboo-like structures (CNTs) with a diameter of 20–30 nm and length of 10-30 µm was purchased from NanoLab (Boston, MA). As previously mentioned (Chrétien *et al*. 2008, Ghanem *et al.* 2008, Ghanem *et al.* 2013), 4-(N-Boc-aminomethyl) aniline was converted into 4-(NBoc-aminomethyl) benzene diazonium tetrafluoroborate salt in two processes. Ultra-pure water (18 MΩ cm) from New Human Power System was used to prepare all solutions.

Glassy carbon was manually fabricated into the working electrodes (GC). Here are the steps for creating a homemade GC. The GC rod was first divided into segments and placed within a glass tube (0.071 cm^2, HW-Germany). Copper wires were joined to glass-working electrodes made of glassy carbon using indium (Aldrich). Each electrode's conductivity was measured to assume each experiment in order to provide a good electrical connection. Before electrochemical analysis, we initially polished the glassy carbon electrodes with silicon carbide polishing paper, followed by 1 µm and 0.3 µm alumina (Buehler), sonication in acetonitrile and a thorough cleaning procedure with ethanol and ultrapure water, Finally, the surface of the resulting electrode was dried under an argon stream.

All electrochemical measurements stated in this work were performed by employing the SP-50 model Biologic Science Instruments Potentiostat/Galvanostat. A three-electrode system was used and Ag/AgCl electrodes are utilized as reference electrodes, platinum wire, and GC/MWCNT electrodes were employed as counter and working electrodes, respectively. The deposition of MWCNT to a glassy carbon electrode (GC) was performed as follows; 10 mg of carbon nanotube is added to 10 ml of dimethylformamide. The resulting suspension was sonicated for 30 minutes and 5 µL of obtained aliquot was then dropped onto the GC, which was permitted to evaporate at ambient circumstances. The resulting electrode is noted as GC/MWCNT. Electrochemical grafting of diazonium compound to GC/MWCNT was carried out by electrochemical reduction of -$C_6H_4CH_2NHBoc$ in ACN comprising 0.1M tetrabutylammonium tetrafluoroborate at 50 mV s^{-1} through sweeping potential toward negative potential values. Afterward, detachment of the Boc was fulfilled by inserting the functionalized GC/MWCNT in 4M HCl in dioxane, followed by washing the modified electrodes with ethanol and plenty of water. The covalent attachment of 3,4 and 2,5 acetyl chloride at modified MWCNT with free amine group was done by the following method. Acetyl chloride (1 mmol) was added into the solution consisting of dichloromethane-pyridine (1:1). Resulting functionalized GC/MWCNT electrode was inserted into the solution for 16 hours at ambient conditions to react. The alteration of the methoxy to hydroxyl groups was fulfilled by using 1 M of BBr_3 for 1 hour.

3. Results and Discussion

3.1. The Functionalization of MWCNT

Electrochemical grafting of -$C_6H_4CH_2NHBoc$ linker onto MWCNT immobilized GC and plain GC was performed via reduction of 10 mM of diazonium compound of the above-mentioned linker in acetonitrile containing 0.1 M TBATFB at 50 mV s^{-1}. As clearly seen in Figures 1 a and b, a quite large cathodic peak at -600 mV was acquired during the first scan, which could be associated with electrochemical reduction of -$C_6H_4CH_2NHBoc$ and generation of radical that couples to either GC or MWCNT surface to form C-C bond. However, in the case of the following scan, the corresponding peak becomes absent, indicating that the surface is covered by the -$C_6H_4CH_2NHBoc$ linker (Ghanem *et al.* 2012, Ghanem *et al.* 2013). When it comes to GC immobilized with MWCNT, the story slightly differentiates. As shown in Fig. 1b, contrary to GC, two cathodic peaks are appearing at the peak potential of 105 mV and -420 mV. Similar to the modification of bare GC surface as stated above, these two peaks can be associated with electrochemical reduction of -$C_6H_4CH_2NHBoc$ and formation of radical attacking to the carbon surface. The reason for the appearance of two cathodic peaks at the MWCNT surface rather than one may be linked to the discrepancy in the reactivity towards generated aromatic radical of the different surfaces of MWCNT. It is well documented that MWCNT consists of two surfaces with edge and basal sites and the MWCNT used in our work possesses a bamboo-like structure where the density of edge sites is ampler than basal sites (Banks and Compton 2006, Banks *et al.* 2005). It is also widely accepted that edge sites exhibit more reactivity than basal sites for many electrochemical reactions. Based on all these explanations, it can therefore be drawn that peaks that come into view at 105 mV and -420 mV could be assigned to electrochemical reduction of edge and basal location, respectively (Ghanem *et al.* 2012, Kocak *et al.* 2013). In the case of the modification of GC immobilized with MWCNT, related cathodic peaks are diminished on the third scan.

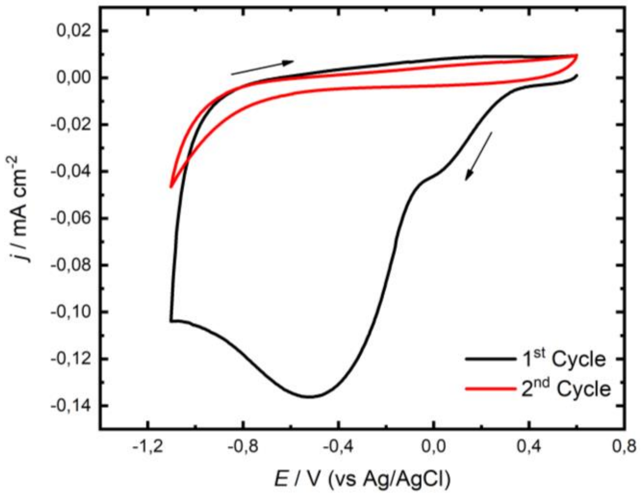

Figure 1 a. The CV of the 5 mM $-C_6H_4CH_2NHBoc$ recorded for GC in ACN containing 0.1M TBATFB at 50 mV s^{-1}.

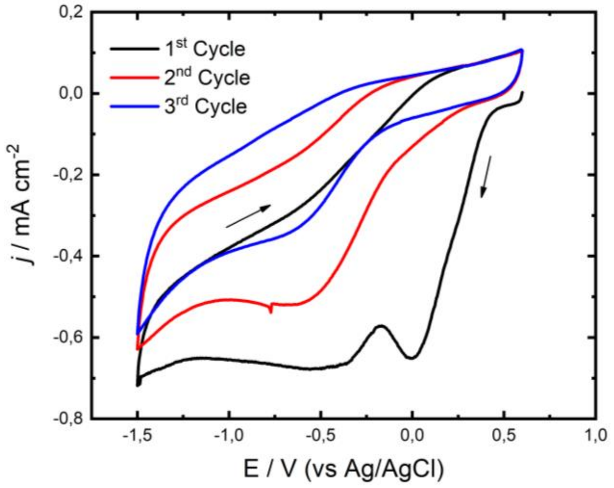

Figure 1 b. The CV of the 5 mM $-C_6H_4CH_2NHBoc$ recorded for GC/MWCNT in ACN containing 0.1M TBATFB at 50 mV s^{-1}.

To confirm whether the covalent attachment of the $-C_6H_4CH_2NHBoc$ linker onto the MWCNT surface is achieved, CV experiments were fulfilled in 1 mM of $Fe(CN)_6^{-4}$ comprising 0.1M KCl as supporting electrolyte for bare and modified MWCNT electrode through $-C_6H_4CH_2NHBoc$ as illustrated in Fig.2. In Fig.2, reversible electrochemical process with the redox potential of 230 mV was attained for the plain MWCNT. However, the CV recorded for modified MWCNT through $-C_6H_4CH_2NHBoc$ shows no either an anodic or cathodic peak, which means the reversible electrochemical process obtained for unmodified GC is completely surpassed. This certainly suggests a successful covalent binding of $-C_6H_4CH_2NHBoc$ to the MWCNT.

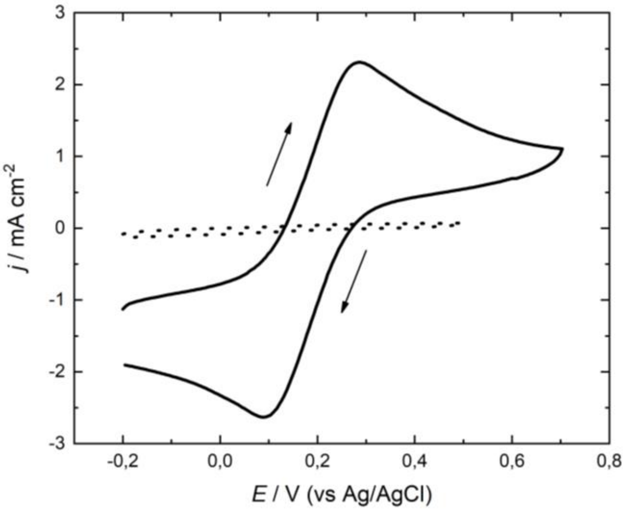

Figure 2. The CV recorded at the sweep rate of 50 mV s^{-1} for 5 mM $K_4[Fe(CN)_6]$ containing 0.1 M KCl as a supporting electrolyte. The solid line and dotted line represent bare and $-C_6H_4CH_2NHBoc$ modified MWCNT, respectively.

After the achievement of the covalent attachment of the $-C_6H_4CH_2NHBoc$ linker to MWCNT, deprotection of the amine group was fulfilled by the removal of Boc in 4M HCl solution in dioxane. Treatment of the modified MWCNT electrode with this procedure leads to the acquisition of an amine-terminated surface that allows the coupling of any chemical compounds with the generation of an amide bond through the solid phase synthesis methodology. The resulting electrode is then treated with acyl chloride derivatives of 3,4 and 2,5 dimethoxybenzene in the presence of pyridine for 16 hours to give a di-HB (dihydroxybenzene) modified electrode (Figure 3). These two dimethoxybenzene compounds are reacted with 1 M BBr_3 in DCM for 60 min to achieve the conversion of 3,4 and 2,5 dimethoxybenzene compounds to corresponding dihydroxybenzene compounds.

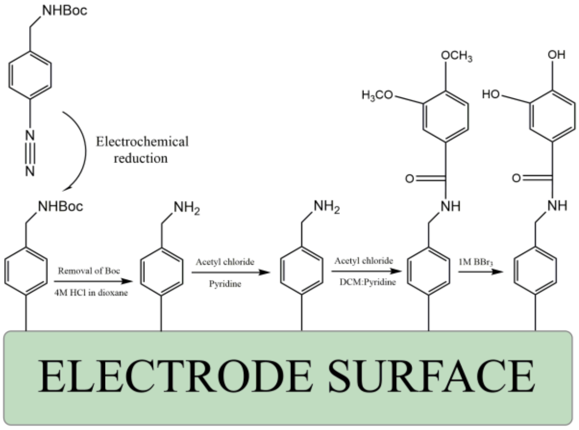

Figure 3. General representation for coupling of dihydroxybenzene compounds to MWCNT deposited on GC.

The FTIR technique was used to better elucidate the respective reactions carried out on the MWCNT surface as illustrated in Figure 4. For this purpose, the self-attachment of the Diazonium salt to the MWCNT surface was carried out. The presence of $C_6H_4CH_2NH$– linker and 3,4-dihydroxybenzene on the MWCNT was confirmed by FTIR spectroscopy. Infrared spectra of modified-MWCNT through $C_6H_4CH_2NH$– after removal of the Boc group and dihydroxybenzene-modified MWCNT through $C_6H_5CH_2NH_2$ were represented in Fig.1. For modified MWCNT through $C_6H_4CH_2NH$– two peaks located at 2930 and 3190 cm^{-1} were assigned to the nitrogen-hydrogen stretching mode of primary amines (Silverstein *et al.* 2005). Strong peaks obtained at 1680 cm^{-1} and 1277 cm^{-1} were assigned to nitrogen-hydrogen bending and carbon-nitrogen stretching vibrations of primary amines. Benzene ring carbon- carbon stretching at 1520 cm^{-1}, and benzene ring C–H out-of-plane bending at 720 cm^{-1} were also observed (G. Giambastini *et al.* 2011). The transmission spectrum of the modified carbon nanotubes also revealed the presence of dihydroxybenzene through $C_6H_5CH_2NH_2$ on the MWCNT. Two other distinct bands at 1430 and 1580 cm^{-1} were also observed, located in the zone related to the (–CONH–), corresponding, respectively, to the (C=O) stretching band and to the (N-H) bending vibration band (N. Follain *et al.* 2008), indicating that an amide bond was formed between dihydroxybenzene and $C_6H_4CH_2NH$–. Peaks at 2990 cm^{-1} and 3070 cm^{-1} are characteristic of the oxygen-hydrogen vibration bands for dihydroxybenzene that is hydrogen-bonded. While the out-of-plane bending of the carbon-hydrogen bonds in a phenyl ring is accountable for the other absorption bands at 690 cm^{-1} and 803 cm^{-1}, the other two peaks located at 1250 cm^{-1} and 1280 cm^{-1} can also be assigned to the carbon-oxygen vibration band (Kafi-Ahmadi and Javanpour, 2020). The intense peak appearing at 1695 cm^{-1} could also be associated with the presence of the carbon-carbon vibration band of the aromatic ring.

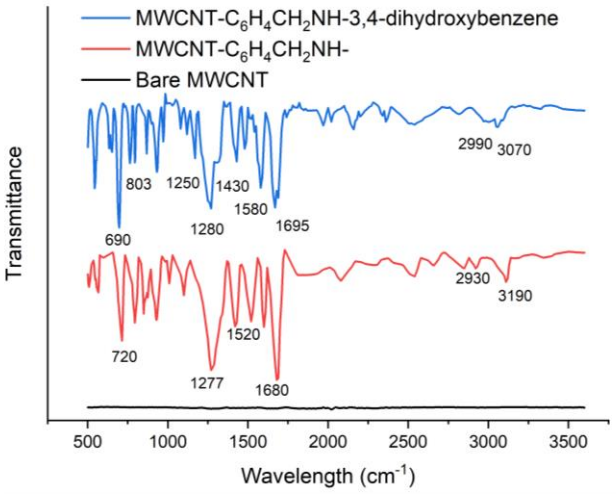

Figure 4. FTIR spectra for bare MWCNT, MWCNT modified by diazonium salt after removal of protecting group (Ghanem *et al.* 2013), and dihydroxybenzene-modified MWCNT via diazonium salt.

After washing the resulting electrode several times with ethanol and water, respectively, electrochemical characterization of MWCNT modified with 3,4, and 2,5 dihydroxybenzene was carried out in PBS (phosphate buffer solution) at 50 mV s^{-1}. Figures 5 and 6 show successive cyclic voltammograms for MWCNT electrodes modified with 3,4 and 2,5 dihydroxybenzene derivatives. Both voltammograms clearly demonstrate characteristic and well-defined peaks for MWCNT electrode modified via both dihydroxybenzene compounds with the redox potential of 170 mV for 3,4 dihydroxybenzene and 190 mV for 2,5 dihydroxybenzene, indicating quasi-reversible electrochemical process and successful covalent coupling and conversion of dimethoxybenzene to dihydroxybenzene compounds. CVs also show that there is a merely neglectable loss in the peak current of related dihydroxybenzene compounds even after 30 cycles, referring to the fact that the stability of the resulting electrodes fairly exhibits good performance. It is also noteworthy that peak separation for 3,4 dihydroxybenzene is larger by 20 mV than 2,5 dihydroxybenzene, which suggests faster electron transfer kinetics at the MWCNT electrode modified by 2,5 dihydroxybenzene. This finding is also supported by applying the Laviron theory to be discussed afterward.

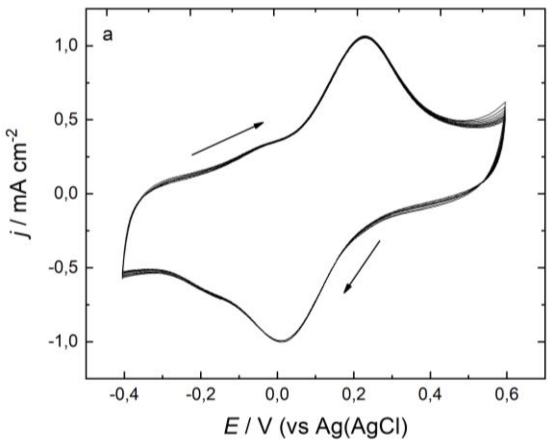

Figure 5. Successive CVs of 3,4-dihydroxybenzene modified GC/MWCNT in PBS buffer at 50 mV s^{-1}.

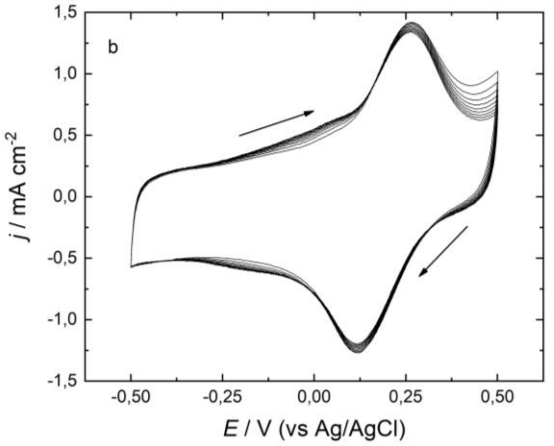

Figure 6. Successive CVs of 2,5-dihydroxybenzene modified GC/MWCNT in PBS at 50 mV s^{-1}.

3.2. The Effect of Scan Rate on Modified MWCNT

Figures 7a and 8a show the CV of the corresponding MWCNT-modified electrode with 3,4 and 2,5 dihydroxybenzene compounds at various sweep rates which range from 5 mV s^{-1} to 500 mV s^{-1} in 0.1M PBS. As clearly seen in both Figures, there is an increment in the anodic and cathodic signal upon the increasing scan rate as expected surface-confined dihydroxybenzene derivatives. Also, a linear straight line between the sweep rate and the anodic and cathodic peak current was attained, which could be regarded as further verification of the successful covalent coupling of dihydroxybenzene derivatives as shown in Figure 7b and Figure 8b. It can also be said that increasing the scan rate brings about a shift in the oxidation peak potential to a more positive value and again a shift in the reduction peak potential to a more negative value. This also indicates redox pair of both modified MWCNT electrodes by dihydroxybenzene derivatives exhibit a quasi-reversible electrochemical process.

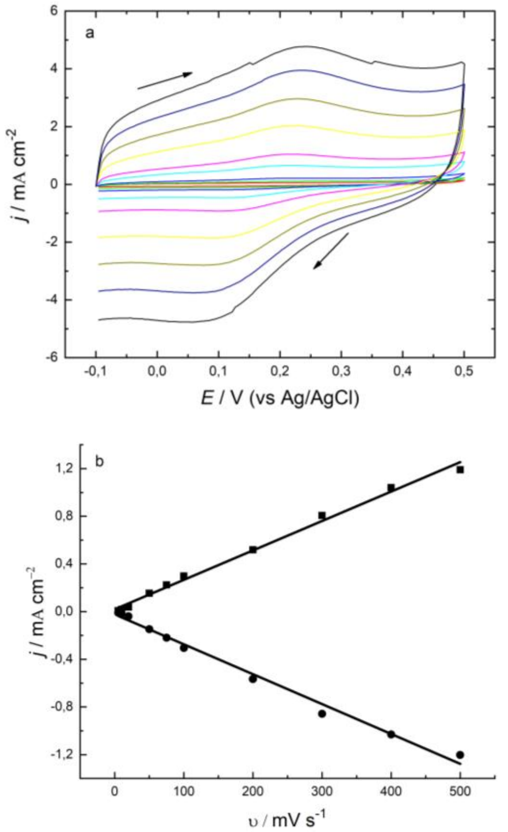

Figure 7. a) CVs of 3,4 -di-HB modified GC/MWCNT in the increasing sweep rate from 5 to 500 mV s^{-1} b) linear relationship displayed for anodic or cathodic peak current vs sweep rate.

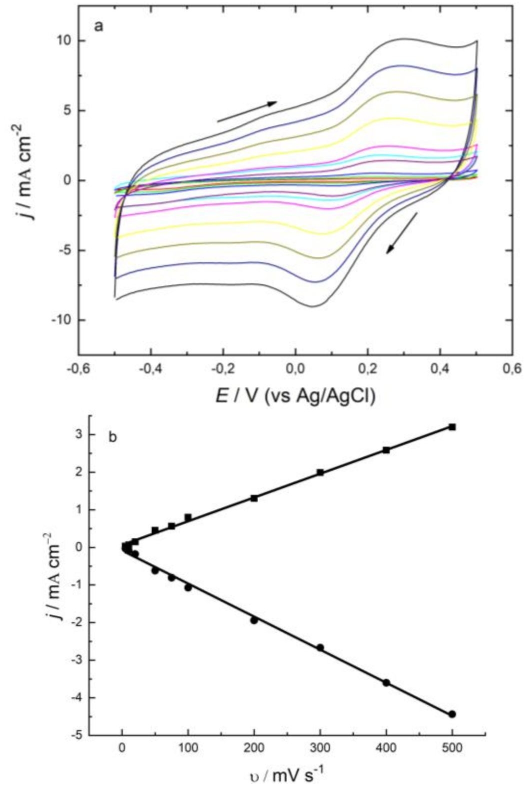

Figure 8. a) CVs of 2,5 -di-HB modified GC/MWCNT in the increasing sweep rate from 5 to 500 mV s^{-1}. b) linear relationship displayed for anodic or cathodic peak current vs sweep rate.

Once the difference between oxidation or reduction peak potential and mid potential is plotted versus the logarithm of sweep rate as illustrated in Figures 9 and 10, a linear relationship is obtained at the relatively higher sweep rates values (v > 200 mV s^{-1}) for the cathodic and anodic branch of corresponding curves. By following Laviron's theory, the electron transfer coefficient (k_s) and apparent rate constant of charge transfer (α) can be calculated by taking the linear part of the anodic and cathodic curves into account (Laviron, 1979). The slope of both straight lines corresponds to $-2.3RT/\alpha nF$ and $-2.3RT/(1-\alpha)nF$ for the anodic and cathodic regions, respectively. α values for MWCNT electrodes modified by 3,4 and 2,5 dihydroxybenzene were calculated to be 0.34 and 0.28, respectively. electron transfer coefficient (k_s) is calculated by the given equation below

$$logk_s = \alpha\log(1-\alpha) + \ (1-\alpha)\log\alpha - log\left(\frac{RT}{nFv}\right) - \frac{\alpha(1-\alpha)nF\Delta E_p}{2.3RT}$$

Where R is the ideal gas constant, F is the faraday constant, and n is the number of electrons transferred. k_s values of MWCNT electrodes modified by 3,4 and 2,5 dihydroxybenzene were calculated to be 0.88 and 2.15, which can be considered a further confirmation of faster electron transfer kinetics at MWCNT electrode modified by 2,5 dihydroxybenzene in comparison to 3,4 dihydroxybenzene.

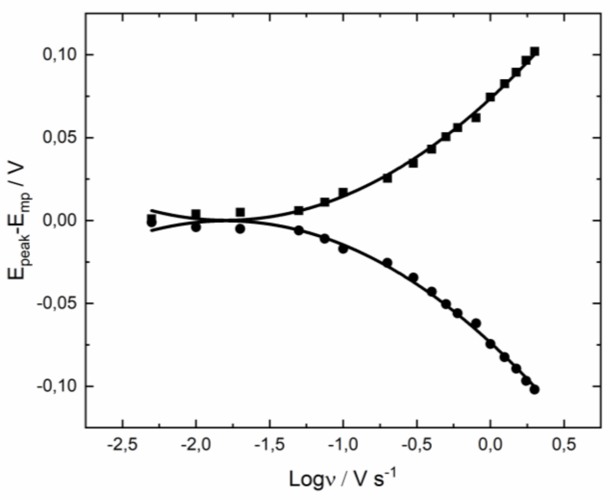

Figure 9. Laviron plot for 3,4- di-HB modified GC/MWCNT.

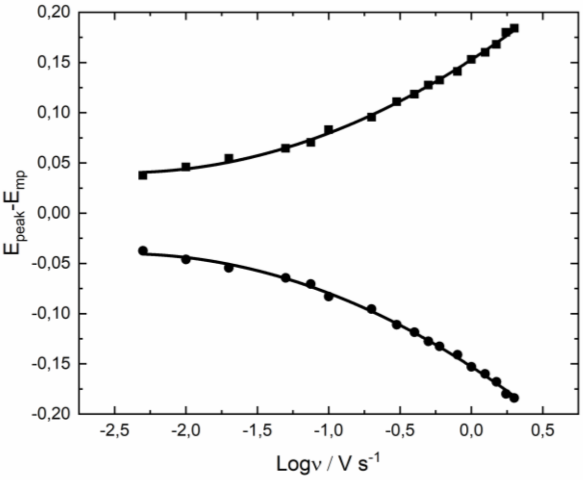

Figure 10. Laviron plot for 2,5- di-HB modified GC/MWCNT.Also, the amount of drop-coated loadings of MWCNT on the GC electrode was investigated. Thus, electrochemical grafting of -$C_6H_4CH_2NHBoc$ linker to deposited MWCNT on GC and the coupling of 2,5 dihydroxybenzene through the formation of amide bond using solid phase synthesis methodology were accomplished as previously detailed in experimental sections. As illustrated in Figure 11, oxidation and reduction peaks response inherently rise upon the addition of more MWCNT on the GC surface. It is also attention-grabbing that peak separation enlarges as the amount of the deposited MWCNT increases. This interesting behaviour could stem from pH changes in the vicinity of the modified surface when the buffer capacity is not capable of compensating for the generation or consumption of proton during the electrochemical redox reaction. Another reason for this experimental finding could be attributed to the fact that all dihydroxybenzene molecules attached to the MWCNT surface cannot be accessed by buffer solution as a consequence of the massive surface area of MWCNT, therefore quite a sluggish electron transfer kinetics takes place in the dihydroxybenzene modified MWCNT.

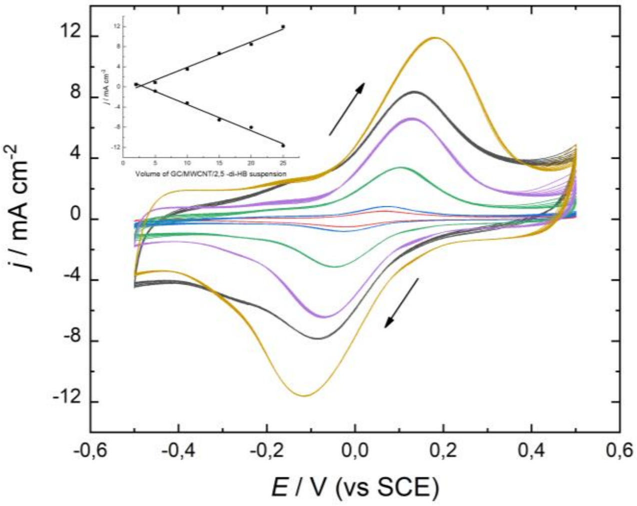

Figure 11. The influence of the amount of MWCNT deposited on GC on the peak current of 2,5 di-HB modified GC/MWCNT. Inset shows a linear relationship obtained for anodic or cathodic peak current and the amount of deposited MWCNT on GC.

3.3. NADH Oxidation at modified MWCNT

The covalent tethering of dihydroxybenzene to the MWCNT surface was considered to have a significant influence on NADH oxidation. As shown in Figures 12 and 13, CV measurements were run in the existence and absence of NADH at 50 mV s^{-1} for 3,4 and 2,5 dihydroxybenzene-modified MWCNT, respectively. As clearly seen on both curves, the introduction of both dihydroxybenzene derivatives to MWCNT leads to an increment in the oxidation peak response and a shift in the oxidation potential of the NADH toward a more negative value for modified-MWCNT electrodes by 2,5 and 3,4 dihydroxybenzene compounds, in comparison to NADH oxidation at plain MWCNT, which makes NADH oxidation happen at 400 mV (not shown). As clearly seen from Fig. 11 and 12, as electrochemical oxidation of NADH oxidation occurs at the modified-MWCNT by 2,5 and 3,4 dihydroxybenzene compounds at 230mV and 240 mV (dotted lines), it can be deduced that the overpotential of NADH oxidation is substantially decreased by both the dihydroxybenzene modified MWCNT electrodes by 170 mV and 160 mV for 2,5 and 3,4 dihydroxybenzene, respectively, compared to NADH oxidation at bare MWCNT. This interesting behavior could be assigned to the surface features and morphology of the MWCNT, which suggest that carbon nanotubes are quite abundant in terms of the proportion of edge planes, which mainly comprise oxygenated functional groups such as carboxylate, phenol, and quinone. Because, as the density of the edge sites increases, it can intrinsically be assumed that any electrochemical reactions between biomolecules and surfaces possessing oxygenated organic groups become more facile. Based on all these experimental findings and deductions, it can be said that the covalent attachment of both dihydroxybenzene derivatives to MWCNT gives rise to more effortless interaction between NADH and both modified MWCNT, referring to the appearance of oxidation peak potential of NADH arises at the lower positive value for both modified MWCNT electrodes compared to plain MWCNT. It is also required to state that both modified-MWCNT electrodes with dihydroxybenzene derivatives have a significant catalytic influence on NADH oxidation because of a noticeable increase in the anodic response and a shift toward negative potential values observed for electrochemical NADH oxidation.

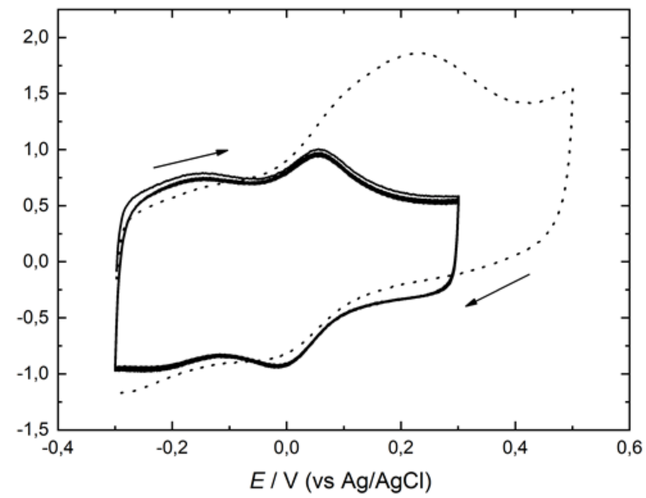

Figure 12. CVs of 2,5- di-HB modified GC/MWCNT in PBS buffer at 50 mV s^{-1} in the absence (solid line) and existence (dotted line) of 1 mM NADH.

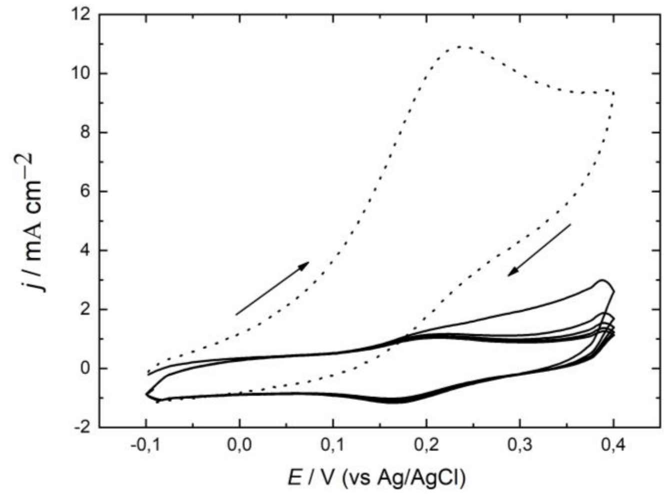

Figure 13. CVs of 3,4- di-HB modified GC/MWCNT in PBS buffer at 50 mV s^{-1} in the absence (solid line) and existence (dotted line) of 1 mM NADH.

4. Conclusion

We have demonstrated that the previously described method for the functionalization of conductive surfaces could be used for the modification of MWCNT with dihydroxybenzene derivatives, For this purpose, coupling of the benzylamine linker via reduction of the corresponding -$C_6H_4CH_2NHBoc$ compound followed by the covalent attachment of 3,4 or 2,5 dihydroxybenzene to free amine group obtained after removal of Boc group via formation of amide bond using conventional solid phase synthesis methodology was accomplished. Both dihydroxybenzene-modified MWCNT electrodes were characterized using cyclic voltammetry and exhibit a quasi-reversible process. di-HB-modified MWCNT electrodes were also shown to have an electrocatalytic activity for NADH oxidation due to an enhancement in the anodic peak response and a negative shift in the anodic peak potential of NADH oxidation. Hence, the proposed modified MWCNT electrodes could be utilized in the detection of NADH in biological samples.

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5. References

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