



Fabrication of a sensitive amperometric sensor for NADH and H₂O₂ using palladium nanoparticles-multiwalled carbon nanotube nanohybrid



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ABSTRACT

Palladium nanoparticles decorated multiwalled carbon nanotubes (PdNPs-MWCNTs) were synthesized and simply cast on the surface of a glassy carbon electrode (GCE) to prepare an amperometric sensor. The fabricated sensor (PdNPs-MWCNTs/GCE) showed excellent electrocatalytic activity towards NADH and H₂O₂ oxidation and H₂O₂ reduction. A fast, linear and highly sensitive response was observed for NADH in the concentration range between 0.1 and 200 μM with a detection limit ($S/N = 3$) of 32 nM. Also, the sensor exhibited fast and sensitive responses (<2 s) towards H₂O₂. The sensitivity and detection limit for H₂O₂ at the operating potential of +0.35 V were 167 nA μM⁻¹ cm⁻² and 1.2 μM, respectively and better than those obtained at the operating potential of -0.25 V (68 nA μM⁻¹ cm⁻² and 14 μM). Moreover, further modification of the proposed sensor by glucose oxidase led to the fabrication of a glucose biosensor with satisfactory performance.

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1. Introduction

Dihyronicotinamide adenine dinucleotide (NADH) and its oxidized form, NAD⁺, are very important coenzymes in all living cells and play key roles in many biological electron transfer reactions [1]. These nucleotides are responsible for the catalytic reactions of all NAD(H)-dependent enzymes. The formal potential of the redox reaction of NAD⁺/NADH couple is relatively low ($E_{\text{NAD}^+/\text{NADH}}^0 = -315$ mV vs. NHE at pH 7) [1] and consequently any (bio)sensor based on NAD⁺ reduction needs an applied potential of less than $E_{\text{NAD}^+/\text{NADH}}^0$. This potential is too low for practical applications, since it is susceptible for the reaction of many reducible interfering compounds present in real samples. So, electrochemical biosensors/sensors based on NADH oxidation are relevant for the fabrication and further development. Both reactions of NAD⁺ reduction and NADH oxidation show pronounced irreversibility with overpotentials of up to 1 V. In the past 20 years, many attempts have been made to reduce the large overpotential needs for NADH oxidation at the electrode, either directly or by the use of an appropriate mediator. Since the first paper published by Tse and Kusana [2], many types of materials such as quinones, redox dyes, conducting polymers, metal complexes, nanoparticles and nanomaterials have been proposed

for the electrocatalytic oxidation of NADH [1]. But, due to the importance of these biological species, there is still continuing interest for the development of new types of NAD⁺/NADH sensors with improved analytical performance.

Hydrogen peroxide is the by-product of many enzymatic reactions which is catalyzed by oxidases. Over the past decade, numerous electrochemical based oxidase biosensors have been developed for the quantification of their relevant substrate by determination of the produced H₂O₂. In addition, sensitive determination of H₂O₂ is of great importance not only in pharmaceutical and clinical but also in industrial and environmental analyses [3]. Among different analytical methods, electrochemical (bio)sensors have received significant attention due to their high sensitivity and selectivity, fast response, practicality, simplicity, low-cost and convenient operation. In electrochemistry, H₂O₂ can be either oxidized or reduced directly at ordinary solid electrodes. However, these processes in analytical applications are limited by slow electrode kinetics and high overpotential. Thus, the current research on H₂O₂ detection is mainly focused on electrode modifications in order to decrease the overpotential and increase the electron transfer kinetics. For these considerations, a large range of materials such as redox proteins, transition metals, metal oxides, metal phthalocyanines, metal porphyrins, redox polymers, and nanomaterials have been employed to conduct electrocatalytic H₂O₂ detection [3–9]. So, there is a great interest to modify conventional electrodes with the new novel materials for the sensitive determination of H₂O₂.

Carbon nanotubes (CNTs) have been widely used as a novel material for the preparation of modified electrodes due to their high surface area,

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high electrical conductivity and unique physical properties [10]. Recent findings indicate that CNTs have been successfully applied for the electrocatalytic oxidation of NADH [11] and electrocatalytic oxidation and reduction reactions of H_2O_2 [12,13]. The surface of CNTs can be decorated with metal nanoparticles (NPs) to create a new class of nano-hybrid material with the integrated properties of two components. These nano-hybrid materials can be easily prepared by chemical or electrochemical deposition of metal NPs on the surface of CNTs as the support [14–16]. CNTs decorated with metal NPs show integrated electrochemical, mechanical and catalytic properties which are not available to the respective components alone. Recent studies show that noble metal NPs together with CNTs have been extensively applied for the fabrication of new modified electrodes with the aim of NADH and/or H_2O_2 detection at the reduced redox potentials and low concentration levels [14,15,17–21].

Nanostructured materials such as metal NPs provide higher effective surface area, improve the rate of electron transfer and enhance the response time of (bio)sensor. Palladium NPs (PdNPs) have been applied for the determination of glucose [22], oxygen [23] and ethanol [24] which exhibited sluggish redox processes on conventional electrodes and also for NADH and H_2O_2 with the improved electrocatalytic performance [14,15,17,20,25–28]. Previous studies in our group revealed that PdNPs decorated multiwalled carbon nanotubes (PdNPs-MWCNTs) exhibited excellent electrocatalytic activity towards hydrazine oxidation [29] and luminol- O_2 (luminol- H_2O_2) electrochemiluminescence (ECL) reactions [30]. In this study, the electrocatalytic efficiency of PdNPs-MWCNTs nanohybrid towards NADH and H_2O_2 redox reactions is demonstrated. Also, the biosensing capability of the proposed nanohybrid is examined by the immobilization of glucose oxidase (GOx) as a typical model of H_2O_2 producing enzyme on a GCE modified with PdNPs-MWCNTs (PdNPs-MWCNTs/GCE).

2. Experimental

2.1. Materials and reagents

All chemicals were of analytical reagent grade and used as supplied without further purifications except for multiwalled carbon nanotubes (MWCNTs). Palladium(II) acetate ($\text{Pd}(\text{CH}_3\text{COO})_2$, 47% Pd), hydrogen peroxide, sulfuric acid, sodium hydroxide, and N,N-dimethylformamide (DMF) were obtained from Merck (Darmstadt, Germany). MWCNTs (95% purity, OD = 10–30 nm, ID = 5–10 nm, and length = 0.5–500 μm) were obtained from Aldrich (Steinheim, Germany). Reduced disodium hydrate salt of NADH, glucose oxidase (GOx, EC 1.1.3.4, type VII from *Aspergillus niger*, 221 U mg^{-1}) and D-(+)-glucose (97%) were obtained from Sigma (St. Louis, MO, USA). Nafion perfluorinated ion-exchange (5% solution in 90% light alcohol) was obtained from Fluka (Buchs, Switzerland). The stock solution of Nafion (5%) was diluted with absolute ethanol five times and neutralized with NH_4OH aqueous solution (25%) in absolute ethanol [29].

2.2. Apparatus

Cyclic voltammetry and amperometric studies were performed using an Autolab potentiostat–galvanostat model PGSTAT30 (Utrecht, the Netherlands) with a conventional three-electrode setup in which glassy carbon modified electrodes, an $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{sat}}$ electrode, and a platinum rod served as working, reference, and auxiliary electrodes, respectively. The working potential was applied to the working electrode in the standard way and the output signal was acquired by Autolab NOVA software. The test solutions were purged with argon gas (Ar) at least for 20 min to prepare Ar-saturated test solutions and to remove dissolved oxygen prior to the cyclic voltammetric and amperometric measurements. All measurements were performed at room temperature. A Metrohm 691 pH meter was used for pH adjustments. Transmission electron microscopy (TEM) measurements were

performed using a Philips CM 120 Cryo-TEM instrument (Eindhoven, The Netherlands) at 120 kV. Scanning electron microscopy (SEM) was performed with a Philips instrument, Model X-30 at 15 kV.

2.3. Preparation of the PdNPs-MWCNTs

MWCNTs were decorated with PdNPs according to the method reported previously [31] with a slight modification. The treatment of MWCNTs was conducted by refluxing with HNO_3 (70%) for 16 h, followed by filtering and thorough washing of the material with deionized water to reach pH of 7 for the filtrate and then dried in a vacuum oven. The pretreated MWCNTs (8 mmol carbon equivalent) were dry mixed with $\text{Pd}(\text{CH}_3\text{COO})_2$ powder (0.08 mmol) using a mortar and pestle for 30 min, 15 min more than that proposed by Lin et al. [31], under ambient conditions. The mixture was then transferred to a glass vial and heated in an oven to 300 °C over 1 h and held isothermally at 300 °C for extra 3 h under nitrogen. The product was then collected as PdNPs-MWCNTs.

2.4. Fabrication of the PdNPs-MWCNTs/GCE

The surface of a glassy carbon electrode (Sigradur K, HTW Hochttemperature Werkstoffe GmbH, Thierhaupten, Germany) was polished successively with 0.3 and 0.1 μm alumina paste (Struers, Copenhagen, Denmark) to obtain a mirror finish and then cleaned in ethanol and water under ultrasonication. One milligram of the PdNPs-MWCNTs was dispersed in 2 mL of DMF with ultrasonic agitation for an hour to achieve a well-dispersed suspension. One micro-liter of the prepared PdNPs-MWCNTs suspension was cast on the surface of GCE and then the prepared sensor, PdNPs-MWCNTs/GCE, was dried in an oven at 50 °C. For comparison purposes, MWCNTs/GCE was prepared through a similar procedure.

2.5. Fabrication of the Nafion/GOx/PdNPs-MWCNTs/GCE

A 5 μL aliquot of the GOx solution ($0.18 \text{ U } \mu\text{L}^{-1}$) was placed on the surface of the PdNPs-MWCNTs/GCE and allowed to adsorb for 20 min at room temperature. The surface of GOx adsorbed PdNPs-MWCNTs/GCE (GOx/PdNPs-MWCNTs/GCE) was then covered with a layer of Nafion using 5 μL of Nafion solution (0.5%). The prepared biosensor, Nafion/GOx/PdNPs-MWCNTs/GCE, was dried at room temperature and stored in a refrigerator at 4 °C when not in use.

3. Results and discussion

3.1. Characterization of PdNPs-MWCNTs

TEM image of the prepared PdNPs-MWCNTs revealed the formation of uniform PdNPs on MWCNTs surface with a diameter about 5 nm (Fig. 1). SEM images of GCE/nanoPd-MWCNTs and GCE proved the successful modification of electrode surface with PdNPs-MWCNTs (Fig. S1). Cyclic voltammetry at a scan rate of 50 mVs^{-1} by PdNPs-MWCNTs/GCE in 0.5 M H_2SO_4 solution revealed the characteristic current features of Pd oxide formation at about 0.65 V and Pd oxide reduction at about 0.38 V, similar to that reported previously [29] (Fig. S2). Furthermore, the appearance of two anodic peaks at about -0.092 and 0.036 V and two cathodic peaks at about -0.09 V and -0.28 was attributed to the adsorption and desorption of hydrogen atoms at the electrode surface, respectively. The results clearly confirmed that thermal decomposition of palladium acetate salt created uniform PdNPs on the surface of MWCNTs.

3.2. The effect of amount of PdNPs-MWCNTs on GCE

The effect of the loading of PdNPs-MWCNTs at the surface of GCE on the voltammetric response was evaluated by cyclic voltammetry in

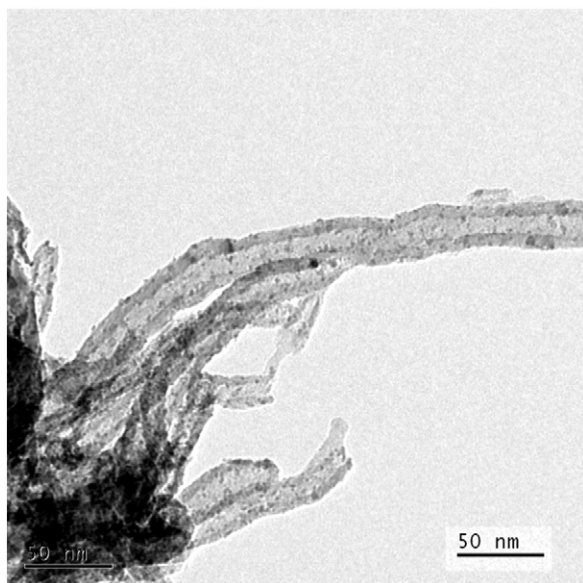


Fig. 1. TEM image of the prepared PdNPs-MWCNTs nanohybrid.

0.1 M KCl solution containing 1 mM of $\text{K}_3\text{Fe}(\text{CN})_6$ as a well-known electrochemical probe. Fixed volumes of PdNPs-MWCNTs suspension (1 μL) with different concentrations were cast on the surface of GCE. The plot of peak currents versus the amount of PdNPs-MWCNTs (mg mL^{-1} in DMF) showed that the response increased with increasing the concentration of nanomaterials and then reached a plateau at a concentration about 0.5 mg mL^{-1} (Fig. S3).

3.3. Electrochemical detection of NADH using PdNPs-MWCNTs/GCE

Fig. 2 demonstrates cyclic voltammograms of a GCE (dotted line), MWCNTs/GCE (dashed line) and PdNPs-MWCNTs/GCE (solid line) recorded in Ar-saturated phosphate buffer solution (0.1 M, pH 7.4) containing 2 mM of NADH. As seen in Fig. 2, an irreversible oxidation peak is observed for NADH on GCE at the potential about 0.70 V vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{sat}}$, whereas the oxidation potentials for NADH on MWCNTs/GCE and PdNPs-MWCNTs/GCE shift to the lower potentials and appear at about 0.60 and 0.39 V, respectively. It seems that the catalytic effect of MWCNTs in MWCNTs/GCE and the synergic effect of

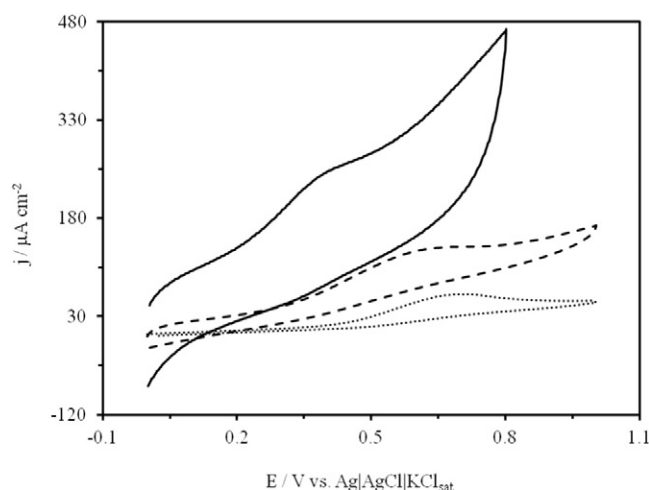


Fig. 2. Cyclic voltammograms of a GCE (dotted line), MWCNTs/GCE (dashed line) and PdNPs-MWCNTs/GCE (solid line) in the presence of 2 mM of NADH. Conditions: supporting electrolyte, Ar-saturated phosphate buffer solution (0.1 M, pH 7.4); scan rate, 50 mV s^{-1} .

PdNPs-MWCNTs as a result of direct attachment of PdNPs on MWCNTs in PdNPs-MWCNTs/GCE towards NADH oxidation are responsible for the overpotential decrease.

The influence of pH on the amperometric response of sensor towards NADH was investigated in the range between 4 and 8. The sensor response increased with the increase of pH and reached to the plateau at the pH about 7 (Fig. S4). The plot of current density versus time was recorded for successive additions of NADH in Ar-saturated phosphate buffer solution (0.1 M, pH 7.4) using PdNPs-MWCNTs/GCE at the applied potential of +0.45 V (Fig. 3). The relationship between response (current density) and NADH concentration was linear in two concentration ranges of 0.1–40 μM (Fig. 3A, inset) and 50–200 μM (Fig. 3B, inset) with a correlation coefficient better than 0.9996. The slope of calibration curves for the first and second concentration ranges was 596 and 423 $\text{nA } \mu\text{M}^{-1} \text{ cm}^{-2}$, respectively. The detection limit for NADH measurement ($\text{S/N} = 3$) was estimated to be 32 nM. The relative standard deviation (RSD%) for ten times determination of 1 μM of NADH was better than 3%. The reproducibility for five NADH sensors, prepared and used in different days was about 8%. The response time of the sensor towards NADH was less than 3 s. The electroanalytical features of some reported NADH sensors are summarized in Table 1 and compared with those obtained in this study. As seen, the sensitivity and detection limit of the fabricated PdNPs-MWCNTs/GCE were remarkably better than those reported for the other electrodes modified with various nanocomposites and different modification strategies. The improvement in sensitivity and detection limit of the proposed sensor can be attributed to the synergic effect of PdNPs-MWCNTs towards NADH oxidation.

3.4. Electrochemical detection of H_2O_2 using PdNPs-MWCNTs/GCE

Cyclic voltammetry was performed in Ar-saturated phosphate buffer solution (0.1 M, pH 7.4) using a GCE, MWCNTs/GCE and PdNPs-MWCNTs/GCE in the absence and presence of 5 mM of H_2O_2 (Fig. 4). The electrochemical reduction and oxidation reactions of H_2O_2 on GCE and MWCNTs/GCE were started at the potentials about -0.1 and $+0.6$ V vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{sat}}$, respectively (Fig. 4, insets A and B). Also, the current signal intensities for reduction and oxidation of H_2O_2 on MWCNTs/GCE were almost 10 times of those observed on GCE. But, a noticeable oxidation peak at about $+0.4$ V and a pronounced catalytic reduction peak at about -0.2 V were observed on PdNPs-

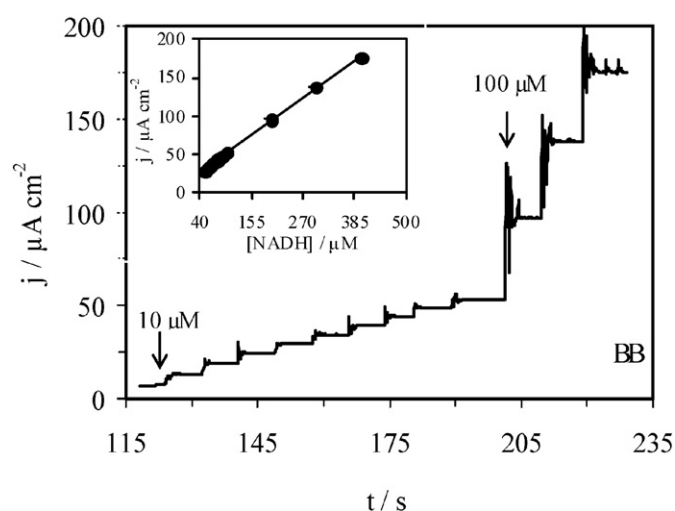


Fig. 3. The plots of current density versus time recorded using PdNPs-MWCNTs/GCE for successive additions of NADH in the concentration range between 0.1–40 μM (A) and 50–200 μM (B). Numbers above the steps denote concentration of added NADH. Insets A and B: calibration curves for NADH in the concentration range between 0.1–40 μM and 50–200 μM . Conditions: supporting electrolyte: Ar-saturated phosphate buffer solution (0.1 M, pH 7.4); operating potential: +0.45 mV versus $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{sat}}$; rotating speed: 2000 rpm.

Table 1Electroanalytical characteristics of various electrodes modified with nanomaterials towards H_2O_2 and NADH detection.

Modified electrode	H_2O_2				NADH				Refs.
	E_{app} (mV)	LDR (μM)	DL (μM)	Sensitivity $\mu\text{A mM}^{-1} \text{cm}^{-2}$	E_{app} (mV)	LDR (μM)	DL (μM)	Sensitivity $\text{nA } \mu\text{M}^{-1} \text{cm}^{-2}$	
PDDA-t-GO-Pd/GCE	−100	10–10,000	0.22						[27]
PtPd/MWCNTs/GCE	+250 ^b	2.5–125	1.2	414.8					[14]
Pd/PEDOT/GCE	−200 ^b	2.5–1000	2.84	215.3					[26]
GNPs-OMC/GCE	−150	2–3920	0.49	22					[41]
Nafion/Pt NPs-MWCNTs/GCE	+700	0.5–2900	0.1	591.33 ^a					[21]
GCE/PVA-MWCNTs-PtNPs	0	2–3.8	0.7	122.63					[18]
Pd/MCNs-GCE	−300	7.5–10,000	1.0	307.5					[28]
Nafion®/MWCNTs-Pd/GCE	−200	1–10,000	0.3	23 ^a					[15]
PdNPs/AuNPs/CNTs/chit/GCE	−200	0.1–6000	0.1						[17]
Au-TiO ₂ /graphene/GCE		10–200	0.7			10–240	0.2		[42]
rGO/SWCNT/GCE	−500	50–5000	1.3	2732.4	+100	20–400	0.078	204	[43]
Pd/CNF-CPE	−200	0.2–20,000	0.2	4.15	+500	0.2–716.6	0.2	2.34 ^a	[25]
PEDOP/MWCNTs-Pd/GCE					+420	1–13,000	0.18	12.72 ^a	[20]
Au _{coll} -MWCNTs-Teflon					+300	10–1000	4.6	37.3 ^a	[19]
Au-RGO/Chit/GCE					+350	1.5–320	1.2	12	[44]
GN-AuNRs/GCE					+400	20–160	6	10.27	[45]
						160–480		27.07	
PdNPs-MWCNTs/GCE	+350	2–60	1.2	167		0.1–40		596	This work
	−250	20–1000	14	68	+450	50–200	0.032	423	

LDR, linear dynamic range; DL, detection limit; chit, chitosan; CNF, carbon nanofibers; GN, graphene; MCNs, mesoporous carbon nanospheres; MWCNTs, multi walled carbon nanotubes; OMC, ordered mesoporous carbon; PEDOT, poly(3,4-ethylenedioxythiophene); PDDA, poly(diallyldimethylammonium chloride); PVA, poly(vinyl aniline); rGO, reduced graphene oxide; SWCNTs, single walled carbon nanotubes; t-GO, thiolated graphene oxide.

^a $\text{nA } \mu\text{M}^{-1}$.

^b SCE.

MWCNTs/GCE for the oxidation and reduction of H_2O_2 , respectively. It seems that the excellent catalytic activity of PdNPs towards H_2O_2 oxidation and fast electron-transfer ability of MWCNTs caused to observe a noticeable oxidation peak for H_2O_2 at a lower potential on PdNPs-MWCNTs/GCE. Additionally, the electrocatalytic reduction of H_2O_2 with a pronounced catalytic reduction peak at about -0.2 V was ascribed to the electrochemical reduction of Pd oxide. On the basis of an EC mechanism, Pd oxide nanoparticles are first reduced electrochemically (started at about 0.0 V) to PdNPs, which react chemically with H_2O_2 resulting in the reduction of H_2O_2 and in the regeneration of the electrocatalyst i.e. Pd oxide nanoparticles.

The influence of pH on the amperometric response of sensor towards H_2O_2 was investigated in the range between 2 and 9. The sensor response increased with the increase of pH and reached to the plateau at the pH about 7 (Fig. S5). The electroanalytical characteristics

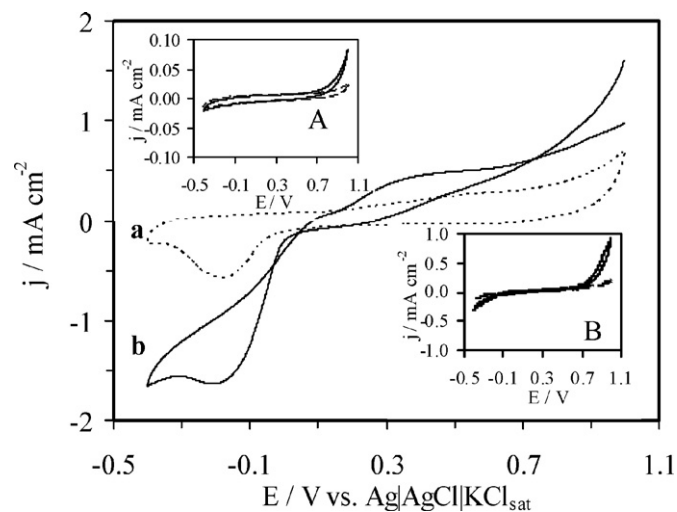


Fig. 4. Cyclic voltammograms of a GCE (inset A), MWCNTs/GCE (inset B) and PdNPs-MWCNTs/GCE in the absence (dashed line) and the presence (solid line) of 10 mM of H_2O_2 . Conditions: supporting electrolyte, Ar-saturated phosphate buffer solution (0.1 M, pH 7.4); scan rate, 50 mV s^{-1} .

of the proposed sensor were evaluated for the determination of H_2O_2 using chronoamperometry (Fig. 5). Since, both reduction and oxidation reactions of hydrogen peroxide were catalyzed on PdNPs-MWCNTs/GCE, the plots of current density versus time were recorded for successive additions of H_2O_2 in Ar-saturated phosphate buffer solution (0.1 M, pH 7.4) at the applied potentials of -0.25 (Fig. 5A) and $+0.35$ (Fig. 5B) V vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{sat}}$. The calibration curve for H_2O_2 detection based on its reduction was linear in the concentration range between 20 and 1000 μM with a correlation coefficient of 0.9999 (Fig. 5A, inset). The sensitivity and detection limit ($S/N = 3$) were 68 $\text{nA } \mu\text{M}^{-1} \text{cm}^{-2}$ and 14 μM , respectively. Also, calibration curve for H_2O_2 detection based on its oxidation was linear in the concentration range between 2 and 60 μM with a correlation coefficient of 0.9998 (Fig. 5B, inset). The sensitivity and detection limit ($S/N = 3$) were 167 $\text{nA } \mu\text{M}^{-1} \text{cm}^{-2}$ and 1.2 μM , respectively. The response time of the sensor towards H_2O_2 at both applied potentials was less than 2 s. The relative standard deviation (RSD%) for H_2O_2 detection at the applied potentials of -0.25 and $+0.35$ V was better than 8.5% (20 μM , $n = 10$) and 4.5% (2 μM , $n = 5$), respectively. The reproducibility for five H_2O_2 sensors, prepared and used in different days was about 8% .

The electroanalytical features of the proposed sensor towards H_2O_2 are summarized in Table 1 and compared with those reported for some other nanohybrid material based sensors prepared using different modification strategies.

3.5. Electrochemical detection of glucose using Nafion/GOx/PdNPs-MWCNTs/GCE

Excellent electrocatalytic activity of PdNPs-MWCNTs/GCE towards NADH and H_2O_2 led us to examine its possible application for the fabrication of enzymatic base biosensors. Glucose oxidase (GOx) enzyme was selected due to its low cost and ease of access and used as a typical model of H_2O_2 producing enzyme. GOx was immobilized on PdNPs-MWCNTs/GCE surface by a layer of Nafion. The layer of Nafion not only stabilizes the immobilized GOx and prevents its leaching but also it can act as a barrier for easily oxidizable compounds such as ascorbate and urate through electrostatic repulsion.

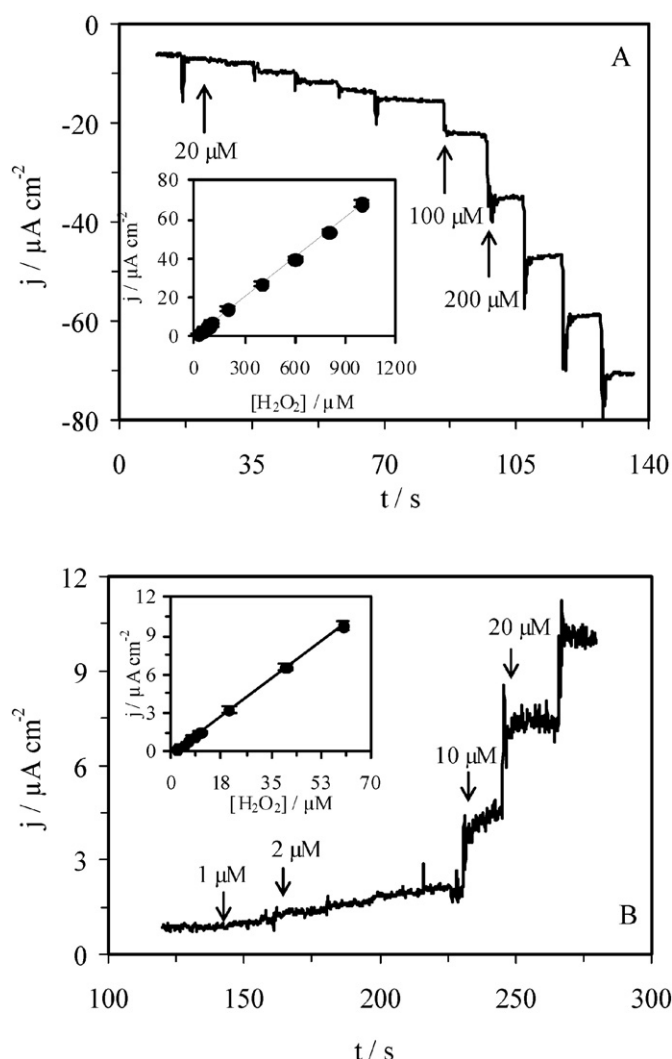


Fig. 5. The plots of current density versus time recorded using PdNPs-MWCNTs/GCE for successive additions of H_2O_2 at the applied potentials of -0.25 V (A) and $+0.35\text{ V}$ (B). Numbers above steps denote concentration of added H_2O_2 . Insets A and B: calibration curves for H_2O_2 at the applied potentials of -0.25 and $+0.35\text{ V}$. Conditions: supporting electrolyte: Ar-saturated phosphate buffer solution (0.1 M, pH 7.4); rotating speed: 2000 rpm.

The plot of current density versus time was recorded for successive additions of glucose in air-saturated phosphate buffer solution (0.1 M, pH 7.4) using the fabricated biosensor, Nafion/GOx/PdNPs-MWCNTs/GCE, at the applied potential of $+0.35\text{ V}$ vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{sat}}$ (Fig. 6). The response of the biosensor was linear towards glucose in the concentration range between 20 and $1000\text{ }\mu\text{M}$ with a correlation coefficient of 0.9999 (Fig. 6, inset). The sensitivity and detection limit ($S/N = 3$) of the biosensor for glucose detection were $7.1\text{ nA }\mu\text{M}^{-1}\text{ cm}^{-2}$ and $11.7\text{ }\mu\text{M}$, respectively. The response time of the biosensor towards glucose was less than 2 s. The relative standard deviation (RSD%) for repetitive measurements ($n = 10$) of $50\text{ }\mu\text{M}$ of glucose was 0.3%. The reproducibility for five glucose biosensors, prepared and used in different days was about 10%. The stability of glucose biosensor was investigated by successive injections of $100\text{ }\mu\text{M}$ of glucose for 60 min. The proposed biosensor retained approximately 85% of their initial response after 60 min of successive determinations, which must be regarded as good operational stability. The electroanalytical characteristics of the fabricated glucose biosensor were comparable or even better than those reported for some other biosensors comprising of metal NPs and MWCNTs [32–36]. The apparent Michaelis–Menten constant, K_m^{app} , for the

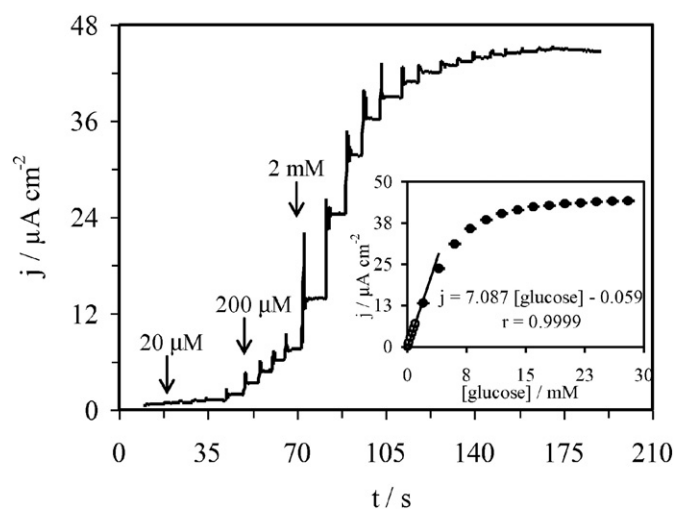


Fig. 6. The plot of current density versus time recorded using Nafion/GOx/PdNPs-MWCNTs/GCE for successive additions of glucose at the applied potential of $+0.35\text{ V}$. Numbers above steps denote concentration of added glucose. Inset: calibration curve for glucose. Conditions: supporting electrolyte: Air-saturated phosphate buffer solution (0.1 M, pH 7.4); rotating speed: 2000 rpm.

immobilized GOx was calculated using Lineweaver–Burk plot (Fig. S6) [37]. The calculated K_m^{app} was 5 mM and less than the those values reported for the native GOx in solution (27 mM) [38], the GOx immobilized on the electrodes modified with Aucoll-CNT-Teflon (14.9 mM) [39] and PtNPs-TH-MWCNTs (6.7 mM) [40]. The smaller K_m^{app} value for the immobilized GOx in Nafion/GOx/PdNPs-MWCNTs/GCE indicates that the enzyme, GOx, shows higher affinity towards glucose.

To evaluate the selectivity of the proposed biosensor, the effect of the presence of potent interfering species such as dopamine, urate and ascorbate ($100\text{ }\mu\text{M}$) on the current signal intensity of the biosensor towards glucose ($100\text{ }\mu\text{M}$) was investigated. A 5% error criterion was adopted. No significant interference was observed for urate, dopamine and ascorbate at the concentration of $100\text{ }\mu\text{M}$.

The fabricated GOx base biosensor was successfully applied for determination of glucose in normal human serum to examine its applicability for real sample analysis. The concentration of glucose in serum was determined and compared with that determined in local hospital. The concentration of glucose in normal human serum determined by local hospital and by the proposed biosensor was 5.41 and 5.25 mM, respectively. The recovery of the analysis was about 97%. Also, in order to examine the reliability of the proposed biosensor certain amounts of standard glucose solution (100, 200 and $300\text{ }\mu\text{M}$) was added to the normal human serum solution (9 mL phosphate buffer containing 1 mL of the human serum) and analyzed using the proposed biosensor. Recovery of each measurement was calculated by comparing the results obtained before and after adding of standard glucose solution. Recoveries for the spiked samples were between 96% and 104%.

4. Conclusion

A glassy carbon electrode was modified with PdNPs-MWCNTs nanohybrid by a simple drop and dry method and then it was successfully applied for the catalytic oxidation of NADH and H_2O_2 and electrocatalytic reduction of H_2O_2 at the reduced overpotentials. The fabricated sensor showed excellent ability towards H_2O_2 and NADH detection with many desirable features such as high sensitivity, low detection limit, short response time and satisfactory linear dynamic range. The capability of the sensor for biosensing purposes was successfully exemplified by incorporation of GOx as a model enzyme with PdNPs-MWCNTs modified GCE for the fabrication of a glucose biosensor.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.msec.2016.01.058>.

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