

Electrocatalytic determination of NADH by means of electrodes modified with MWCNTs and nitroaromatic compounds



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ABSTRACT

In this work, we have both synthesized and studied 4-phenylbutyl-3,5-dinitrobenzoate (3,5-DNBPh) as a potential precursor of an electrocatalytic mediator for the oxidation of NADH, with the di-nitro compound immobilized in a three-dimensional network of glassy carbon electrodes modified with MWCNTs. This di-nitro compound is activated electrochemically to selectively generate one nitroso-hydroxylamine redox pair, which ultimately acts as a redox mediator of NADH oxidation. Thus, NADH electrocatalysis displaces the oxidation potential of NADH with the formation of a single voltammetric peak, the displacement occurs from $E_p = +0.374$ V in GCE/MWCNT to $+0.001$ V in GCE/MWCNT/3,5-DNBPh (very close to 0 V, $\Delta E_p = 0.373$ V). The 4-phenylbutyl-3,5-dinitrobenzoate mediator is sensitive to NADH concentration, and a linear regression between 0.1 and 0.6 mM NADH ($r^2 = 0.99$) was obtained, with a detection limit of $22.3 \mu\text{M}$ ($S/N = 3$) and a quantification limit of $74.2 \mu\text{M}$ ($S/N = 10$). The developed modified electrode presents low NADH electrocatalytic oxidation potentials, and an interesting and novel advantage is that the preparation of the electrodes is fast, clean and effective, allowing biosensors to be obtained in a few steps.

1. Introduction

Modified electrodes have been extensively studied in electrochemistry due to their characteristics. By incorporating a specific compound, the modified electrodes promote a charge transfer reaction and increase the electrode's reactivity towards an electroactive species [1]. Among the most interesting materials for electrode modification are carbon-based nanostructures, as they show interesting electrochemical results compared to traditional materials [2–3]. There are various forms of carbon-based nanomaterials depending on the type of hybridization and atomic arrangement, including graphene, fullerenes, and carbon nanotubes. Carbon nanotubes (CNTs) have unique electronic properties, such as a large work surface, rapid electronic kinetics, chemical inertia, high sensitivity and selectivity and low detection limits, making them suitable for use as electrochemical sensors and electrocatalytic supports [3–7].

Multiwalled carbon nanotubes (MWCNTs) have been shown to be capable of trapping compounds in their three-dimensional network by physisorption [8], a property that can be used to functionalize the surface of the modified electrode with molecules that can act as potential mediators through covalent bonds or noncovalent interactions

such as van der Waals forces [9–10]. These mediators can act as electrochemical sensors for species of biological interest, such as serotonin [11], hydrogen peroxide [12], tryptophan [13], and NADH [7,14–16].

Nicotinamide adenine dinucleotide (NADH) is an indispensable coenzyme for redox reactions in cells, mostly in cellular respiration, making it an important species in charge transfer at the biological level. Its oxidized form, NAD^+ , is an excellent acceptor of reduced species that are produced by different oxidative processes of cellular substrates, such as glycolysis, and serves as a cofactor of enzyme dehydrogenases [17–19]. On the other hand, the reduced NADH form can be re-oxidized by reactions catalysed by enzymes such as NADH dehydrogenase in the electronic transport chain [17]. Due to its considerable influence on biological systems, NADH is monitored in different processes using biosensors [20]. NADH's oxidation potential on glassy carbon electrodes is > 0.5 V [21], which is problematic because the electrode surface is saturated with mixtures of oxidation products, including radical coupling products [22].

Several researchers have used electrodes modified with CNTs with immobilized compounds in the three-dimensional network, as they allow fast electronic transfer, reducing the oxidation overpotential of NADH [7,19,23]. In previous work, aromatic nitro compounds have

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been shown to be entrapped in MWCNTs and to show electrocatalytic activity towards NADH [8,24]. According to the current research on the preparation of nanostructured electrode platforms with MWCNTs, we have visualized two problems that affect the application of these platforms. First, the drop casting method for applying the MWCNTs coating generates a problem of heterogeneous deposits, which result in limited reproducibility. Second, at the electrocatalytic interfaces that a redox mediator precursor interacts with, the fragility of this precursor interaction with the nanostructured platform affecting on the durability of the redox mediator in the electrocatalytic reaction. In the first case, we proposed to improve the drop casting method by using a new solvent such as 1,3-dioxolane that improved the dispersion of the MWCNTs [8]. In the second case, we propose an improved bond between the nitroaromatic and electrode, while still avoiding the formation of a covalent bond. For this we consider introducing nitroaromatic molecules with a kind of anchor that we call an “adsorptive tail”, which increases the strength of the nitroaromatic-MWCNT interaction [24]. This paper was very important since it opened the door for a paradigm change on how to attack the problem of strengthening the π - π interaction between nitroaromatic and the MWCNTs platform. Thanks to the finding obtained in that paper we began to consider the concept of “adsorptive tail” that we intend to expand in this paper with a simpler but equally adsorptive “adsorption tail” structure.

In this work, we have both synthesized and studied 4-phenylbutyl-3,5-dinitrobenzoate (3,5-DNBPh) (Fig. 1) as a potential precursor of an electrocatalytic mediator for NADH oxidation, with this dinitro compound immobilized in the three-dimensional network of electrodes modified with CNTs. The immobilization of the nitro mediators is achieved by submerging the modified electrode with MWCNTs in a solution of the nitro compound in a non-aqueous medium. Subsequently, the nitro mediator is electrochemically activated to generate the nitroso-hydroxylamine redox pair, which ultimately acts as a redox mediator.

2. Materials and methods

2.1. Reagents and materials

All reagents used were of analytical grade. Reduced nicotinamide adenine dinucleotide (NADH), 1,3-dioxolane and acetonitrile were acquired from Sigma Aldrich and were used without prior purification. MWCNTs (diameter \sim 10 nm, length \sim 1.5 μ m) were acquired from Dropsens. S.L, Spain.

2.2. Solution/dispersion preparation

3,5-DNBPh working solutions were prepared in acetonitrile at a final concentration of 0.3 mM.

NADH solutions (3 mM) were prepared daily in 0.1 M Britton-Robinson buffer (pH 7.0) and purged with extrapure nitrogen before each measurement.

MWCNT dispersions (3 mg/mL) were prepared in 1,3-dioxolane by sonication for 5 min.

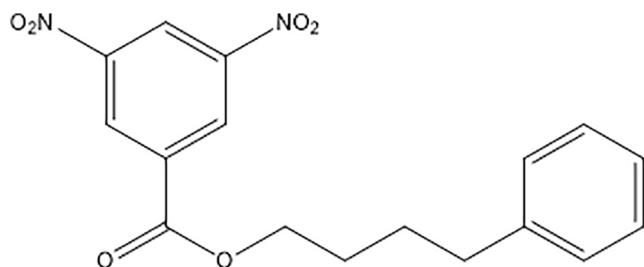


Fig. 1. Chemical structure of 4-phenylbutyl-3,5-dinitrobenzoate (3,5-DNBPh).

2.3. Electrochemical equipment

Voltammetric experiments were carried out with BAS-100 (Bioanalytical systems) equipment. A three-electrode electrochemical cell was used. A glassy carbon working electrode (3 mm diameter, Model CHI104, CH Instruments) was modified with MWCNTs. A platinum electrode (BASiMW-1032) was used as the auxiliary electrode, and a Ag/AgCl electrode (BASi MF-2052) was used as the reference electrode.

2.4. Synthesis of 3,5-DNBPh

First, 3.3 mmol of 4-phenylbutanol was added to 3.3 mmol of 3,5-dinitrobenzoyl chloride [25] in 5 mL of tetrahydrofuran at room temperature for 24 h. A precipitate formed and was evaporated to dryness and then washed with a saturated NaHCO₃ solution, and then the product was extracted with ethyl acetate. After drying, 620 mg of liquid compound was obtained (Fig. 1).

4-Phenylbutyl-3,5-dinitrobenzoate (3,5-DNBPh) C₁₇H₁₆N₂O₆ (melting point 58–59 °C): 54,57%, ¹H NMR (CDCl₃): δ 9,11 (m, 3H, H-Ar-NO₂); 7,19 (m, 5H, H-Ar); 4,41 (d, J = 6,0 Hz, 2H, CH₂-O); 2,65 (d, J = 5,81 Hz, 2H, CH₂-Ar); 1,88 (m, 4H, CH₂-CH₂-). ¹³C NMR (CDCl₃): δ 167,15; 148,63; 134,07; 129,47; 128,51; 128,43; 126,08; 122,39; 66,88; 35,38; 28,11; 27,63.

2.5. Preparation of modified electrodes with 3,5-DNBPh

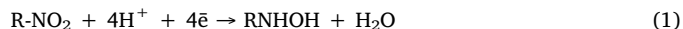
Before any modification, the GCE was polished with 0.05 and 0.3 μ m alumina. The GCEs were modified with a 5 μ L aliquot of a 3 mg/mL dispersion of MWCNTs in 1,3-dioxolane and allowed to dry for 5 min at room temperature, and then a modified GC/MWCNT electrode was obtained. The working electrodes were modified by immersion of the electrode in a solution of 3,5-DNBPh for 10 s. The electrodes were then washed with nanopure water, and GCE/MWCNT electrodes with an entrapped nitro compound (GCE/MWCNT/3,5-DNBPh) were obtained.

2.6. Electrochemical characterization of the precursor (3,5-DNBPh)

Once 3,5-DNBPh was entrapped in the GCE/MWCNT, it was immersed in a cell containing only 0.1 M Britton-Robinson buffer. The electrochemical activation of the precursor consisted of a cathodic sweep from 0 V to -0.7 V, an anodic sweep from -0.7 V to $+0.3$ V, and finally, a cathodic sweep from $+0.3$ V to -0.7 V.

2.7. Electrogeneration of the electrocatalytic mediator

This methodology has been previously described [24]. The GCE/MWCNT with the entrapped precursor was immersed in a cell containing only 0.1 M Britton-Robinson buffer at pH 7 without stirring. Subsequently, to reduce only one nitro group, 3,5-DNBPh was reduced from the nitro precursor to hydroxylamine (Med) *in situ* by applying a potential sweep from 0.0 V to -0.4 V (equation 1), and then the hydroxylamine derivative was oxidized to the nitroso derivative by applying a potential sweep from -0.1 V to $+0.2$ V (Eq. 2).



2.8. Evaluation of electrocatalytic activity

Once the nitroso derivative was obtained, the GCE/MWCNT/Med was immersed in a cell containing a 3 mM NADH solution for one minute, and then a potential sweep from -0.2 V to $+0.6$ V was applied to observe the oxidation potential shift of NADH.

2.9. Analytical curve

A series of 6 solutions of concentrations 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mM of NADH in Britton-Robinson buffer pH 7 were prepared and used as a point on the analytical curve. The voltammetric signal for each concentration was obtained by cyclic voltammetry, with a sweep rate of 100 mV/s, between -0.2 and $+0.2$ V. The NADH oxidation signal and the mediator regeneration signal were recorded. Each measurement was carried out in triplicate with a different electrode in which the mediator was previously encapsulated. Calibration curve validation parameters: linear range: 0.1 to 0.6 mM, sensitivity: 70.46% $\mu\text{cat} / \text{NADH concentration}$; reproducibility: 3.4%.

2.10. NADH determination procedure

By using cyclic voltammetry, the voltammetric signal of the mediator was obtained in the absence of NADH, and then the electrode was placed in a cell with NADH at different concentrations, with an accumulation time of one minute. Then, an anodic scan from -0.2 V to $+0.2$ V was carried out, and finally, a cathodic sweep was conducted from $+0.2$ V to -0.2 V (0.1 V/s). Each measurement was performed in triplicate.

3. Results and discussion

3,5-DNBPh (Fig. 1) has two aromatic rings, one substituted with nitro groups and the other containing a hydrophobic substituent in the ester group. The structural form of 3,5-DNBPh indicates low solubility in polar solvents due to its apolar nature (nitro groups and unsubstituted ring). The carbons with sp^2 hybridization present in the molecule facilitate entrapment in the three-dimensional network of CNTs due to their structural similarities and van der Waals and π - π types of interactions between the aromatic rings and the rings present in the nanotube network [26]. This feature facilitates entrapment of the precursor in modified electrodes.

3.1. Electrochemical characterization of 3,5-DNBPh

The presence of nitro groups in the 3,5-DNBPh structure allows us to obtain species that can serve as electrocatalytic mediators with reversible redox behaviour. To do this, it is necessary to electrochemically characterize 3,5-DNBPh and thus obtain adequate potential for its study.

Fig. 2 shows the voltammetric characterization for 3,5-DNBPh entrapped on a GCE/MWCNT in an aqueous buffer solution. Two reduction peaks (I with $E_p - 0.367$ V and II with $E_p - 0.501$ V) can be observed. Each peak is attributed to the four-electron four-proton reduction of each NO_2 group to form an NHOH group according to equation 1. In the subsequent sweeps, we can observe NHOH/NO redox pairs (III/III' with $E_p + 0.031 / -0.017$ V and IV/IV' with $E_p + 0.112 / +0.061$) corresponding to each nitro group according to equation 2.

From the above results, it is clear that one redox pair is obtained for each nitro group of 3,5-DNBPh. To determine which redox pair corresponds to which nitro group, we selected a shifting potential of -0.42 V in the cathodic scan to selectively reduce only one nitro group. In Fig. 3, we can observe the overlap of two cyclic voltammograms (CV) of 3,5-DNBPh entrapped in a GCE/MWCNT in 0.1 M Britton-Robinson buffer, pH 7. The whole line corresponds to the voltammogram when only one of the nitro groups has been selectively reduced. We can deduce that when only one of the nitro groups is selectively reduced, the voltammogram change shows only peak I and only the corresponding pair redox IV/IV'. Thus, the 3,5-DNBPh compound trapped in the GCE/MWCNT electrode acts as a precursor that, by means of electrochemical activation, generates the redox mediator that will be proven to be able to electrocatalyse the oxidation of NADH.

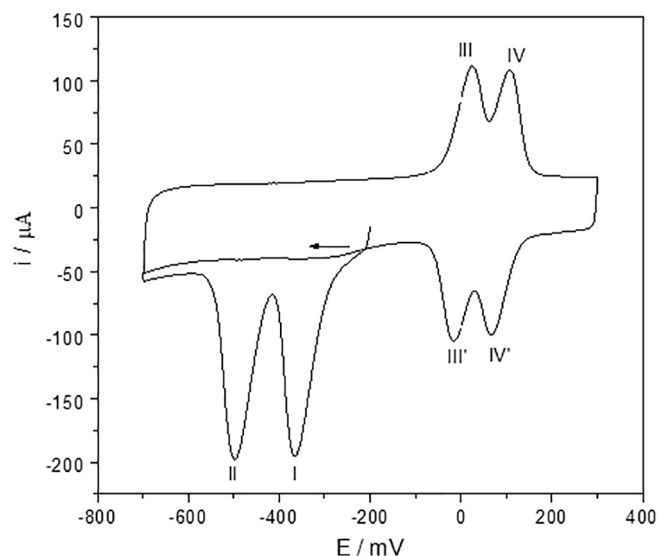


Fig. 2. Cyclic voltammogram of 3,5-DNBPh entrapped on a GCE/MWCNT in 0.1 M Britton-Robinson buffer, pH 7.0. Sweep rate = 100 mV/s, sensitivity = 1×10^{-4} μA , initial potential = $+0.2$ V, low potential = -0.7 V, high potential = $+0.3$ V. Three segments recorded, negative scan.

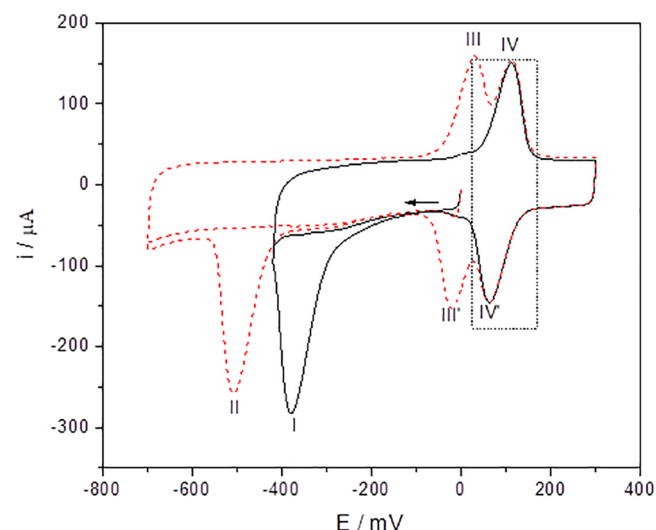


Fig. 3. Overlap of two cyclic voltammograms of 3,5-DNBPh entrapped in a GCE/MWCNT in 0.1 M Britton-Robinson buffer, pH 7.0. The whole line corresponds to CV when only one nitro group was reduced. The dotted line corresponds to the CV when the two nitro groups have been reduced. Sweep rate = 100 mV/s.

3.2. Electrocatalytic activity of 3,5-DNBPh

With the precursor entrapped in GCE/MWCNT, electrochemical activation generates the corresponding mediator redox pair NHOH/NO, which is also entrapped in the electrode, generating GCE/MWCNT/Med. This GCE/MWCNT/Med was subjected to the voltammetric experiment in two modes: a) inserted in a solution containing only buffer and b) immersed in a 3 mM NADH solution containing buffer with an accumulation time of 60 s.

From the dotted line in Fig. 4, we can observe the CV of GCE/MWCNT/Med in only buffer, showing only the mediator redox pair NHOH/NO, according to the above-mentioned mode "a". From the whole line in Fig. 4, we can observe the CV of GCE/MWCNT/Med in a 3 mM NADH solution containing buffer with an accumulation time of 60 s. The irreversible signal I in Fig. 4 corresponds to electrocatalysis of

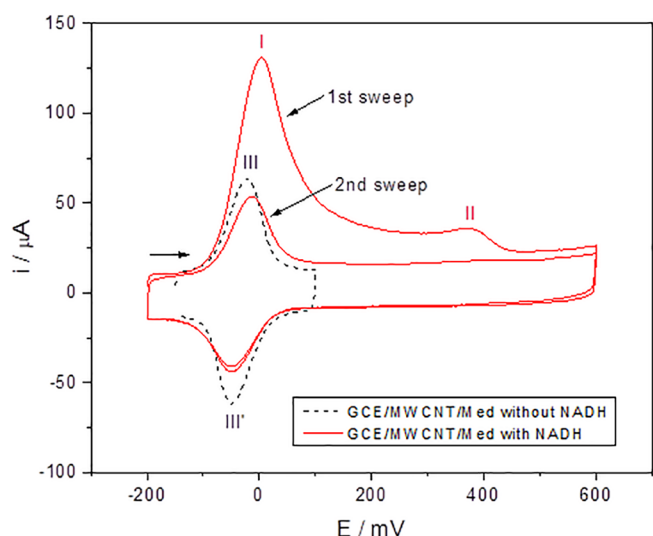


Fig. 4. Cyclic voltammograms showing NADH electrocatalysis on GCE/MWCNT with entrapped 3,5-DNBPh as the precursor. Solid red line: CV of GCE/MWCNT/Med in a 3 mM NADH solution containing buffer with an accumulation time of 60 s. Black dotted line: CV of GCE/MWCNT/Med without the presence of NADH. Britton-Robinson buffer, pH 7.0, $E_0 = -0.2$ V, $E_H = +0.6$ V, $E_L = -0.2$ V. Sweep rate = 100 mV/s.

NADH oxidation, and reversible signal III/III' corresponds to the redox pair of the entrapped mediator. Signal II corresponds to non-electrocatalysed NADH from within the solution. In a second anodic sweep, signal II disappears, as there was no accumulation time and all NADH present on the electrode was already oxidized. Additionally, in Fig. 4, the comparison of the mediator in the presence and absence of NADH, dotted line and solid line from signal III / III', shows that there is a decrease in the oxidation peak current of the mediator when the GCE/MWCNT/Med comes into contact with 3 mM NADH. This occurs due to the displacement of mediator molecules by NADH molecules that move by diffusion from the bulk of the solution to the electrode surface. Signal I in Fig. 4 is composed of the overlap of the redox pair signal III of the mediators plus the electrocatalytic signal of NADH that oxidizes at that same potential. This signal represents the process in which the mediator encapsulated in the nanotubes is oxidized and oxidizes to the NADH that comes from the solution. Oxidation of NADH that interacts with the GCE/MWCNT/Med electrode occurs at a lower overpotential than in GCE/MWCNT. Fig. 6 signal I vs signal II. In summary, the electrocatalytic effect on the overpotential can be reflected by the signal displacement of NADH from + 0.374 V on GCE/MWCNT to potential of 0.001 V on GCE/MWCNT/Med, implying a decrease in the overpotential of 0.373 V. In the active zone of the GCE/MWCNT/Med electrode that is not covered by the mediator, the oxidation of NADH occurs as usual in a GCE/MWCNT electrode. Figs. 4 and 6. signal II.

The electrocatalysis current can be defined as the difference between signals I and III, where signal III is obtained in the absence of NADH. It is possible to express the percentage of electrocatalytic current by the following equation:

$$\%i_{\text{ecat}} = 100 - \frac{i_{\text{NHOH/NO}} * 100}{i_{(\text{NHOH/NO} + \text{NADH}_{\text{ecat}})}} \quad (3)$$

where % i_{ecat} corresponds to the percentage of peak current of electrocatalysed NADH, $i_{\text{NHOH/NO}}$ is signal III in the absence of NADH and $i_{(\text{NHOH/NO} + \text{NADH}_{\text{ecat}})}$ is signal I. Fig. 5 shows a graphic of these values.

During preparation of the electrodes modified with MWCNTs, the amount of CNTs added to each GCE determines the coefficient of inter-electrode variation, causing variation in the measurement with each electrode. As a result, i_{ecat} varies depending on the amount of CNTs since more nanotubes deposited on the glassy carbon electrode results

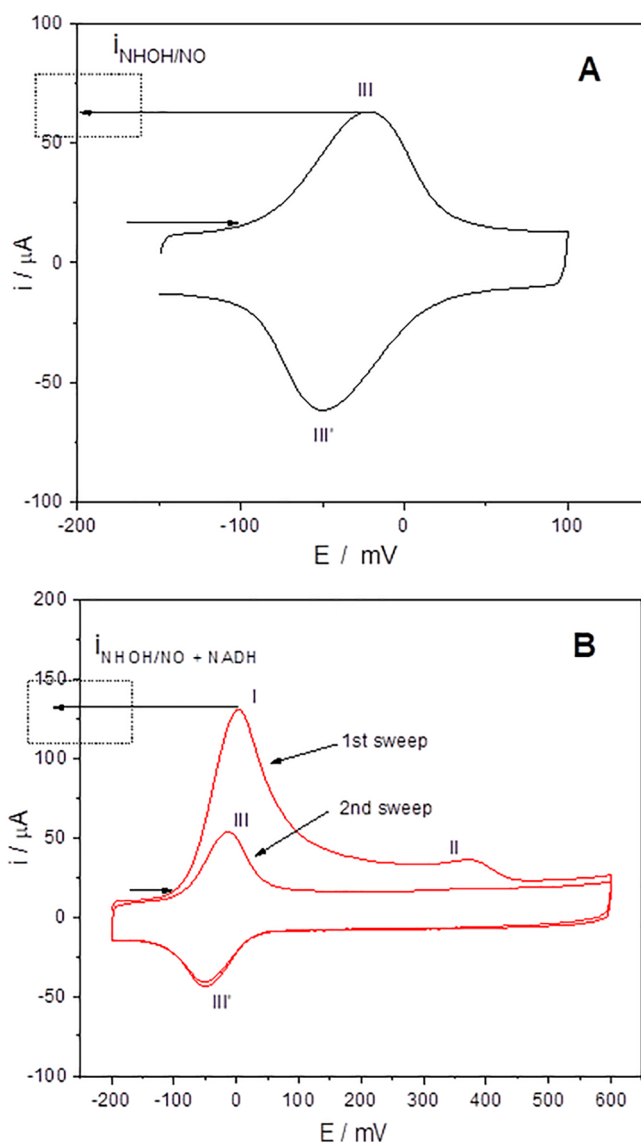


Fig. 5. Graphic of $i_{\text{NHOH/NO}}$ and $i_{\text{NHOH/NO} + \text{NADH}}$. A. The black line corresponds to the mediator in the absence of NADH. B. The red line corresponds to NADH electrocatalysis in the presence of the mediator.

in more entrapped mediator in the three-dimensional network. The advantage of determining the percentage of electrocatalytic current as a normalization factor is that the result is expressed as a percentage fraction of the sum between currents I and III, i.e., the sum of the oxidation current of the hydroxylamine derivative of the mediator and the electrocatalytic current, resulting in normalizing the value of the electrocatalytic current without knowing the area of the electrode; we present this methodology as a simple experimental way to correct the error caused by differences in the number of nanotubes without having to calculate the area of each electrode prior to each measure by traditional methods

In Fig. 6, a general electrocatalysis scheme is presented. In the scheme, it is possible to directly compare the behaviour of the CVs of NADH on GCE/MWCNT, NADH on GCE/MWCNT/Med and GCE/MWCNT/Med in a buffer solution without NADH. Briefly, the electrocatalytic effect on the overpotential can be reflected by the shift of the peak potential of the oxidation of NADH on the GCE/MWCNT electrode (peak II in Fig. 6) with respect to the peak potential of NADH on the GCE/MWCNT/Med (peak I in Fig. 6), which results in a decrease in the overpotential of almost 0.5 V or even more when compared to NADH on a conventional electrode.

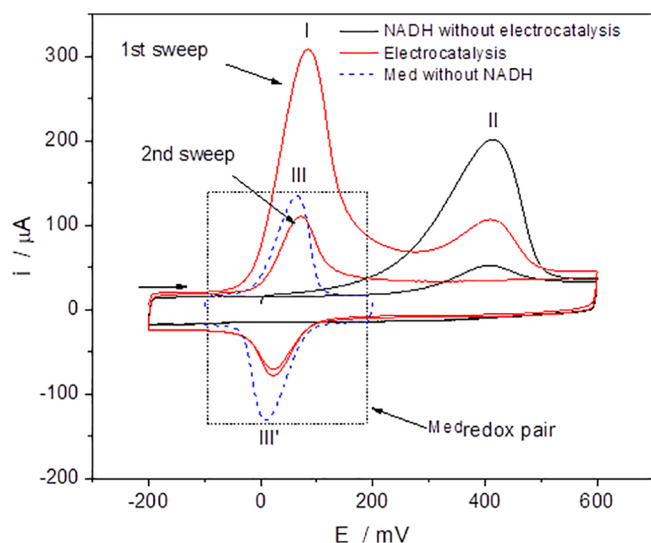


Fig. 6. Overlap of cyclic voltammograms of mediator without NADH (blue dotted line), NADH without mediator (black dotted line) and electrocatalysed NADH on a GCE/MWCNT/Med (red solid line).

3.3. Analytical application

The function of a biosensor is to sense the change in the concentration of a substrate with which it is in contact [27]. Sensitivity is important for a biosensor, as it dictates its usefulness. To determine the range over which an electrocatalytic mediator has a linear response, a study of $\%i_{\text{ecat}}$ based on NADH concentration variation was conducted by using cyclic voltammetry. Therefore, the electrocatalytic peak current (signal I) and the non-electrocatalytic peak signal of the mediator (signal III in the absence of NADH) were obtained. In Fig. 7, the analytical curve obtained for 3,5-DNBPh is presented, with an electrochemical response proportional to the increase in NADH concentration.

For 3,5-DNBPh, a linear regression $\%i_{\text{ecat}} = 70.46 [C]_{\text{NADH}} + 9.19$

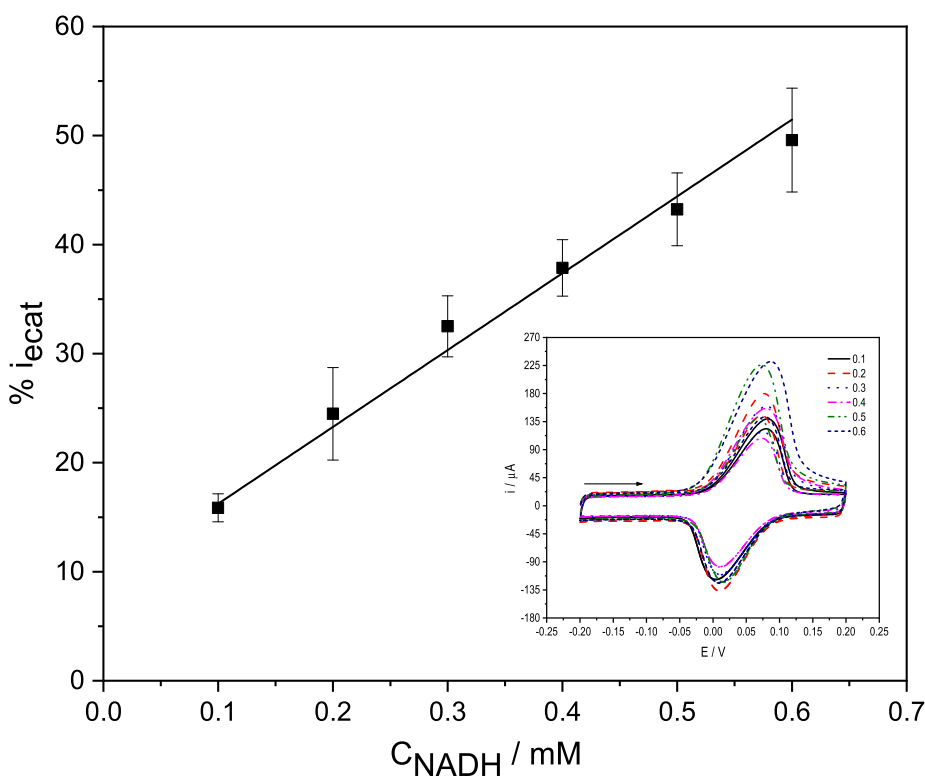


Fig. 7. Analytical curve of NADH in Britton-Robinson buffer pH 7.0 using GCE/MWCNT/3,5-DNBPh. Inset: Cyclic Voltammograms of a series of 6 solutions at 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mM NADH in Britton-Robinson buffer pH 7.0 using GCE/MWCNT/3,5-DNBPh (sweep rate of 100 mV/s, $E_0 = -0.2$ V, $E_H = +0.2$ V, $E_L = -0.2$ V). Each measurement was made in triplicate with different electrodes.

($r^2 = 0.99$) was obtained, with a detection limit of 22.3 μM ($S/N = 3$) and a quantification limit of 74.2 μM ($S/N = 10$). The 3,5-DNBPh mediator is sensitive to changes in the NADH concentration. To compare the analytical values, the values obtained by other authors are shown in Table 1.

On the other hand, to know the stability of the mediator encapsulated in the modified electrode, experiments were carried out by means of CV, isolating the nitroso-hydroxylamine redox pair, to generate the 3,5-DNBPh intermediate in a cell containing 0.1 M Britton-Robinson buffer pH, 7.0. For this purpose, with a single electrode, 37 scans cyclic voltammograms with a waiting time of 5 min after each measurement at 0.1 V/s were carried out. By plotting both the cathodic and anodic peak current intensities, a relationship between the peak current intensity and the waiting time after each measurement was obtained, showing a linear decrease in cathodic and anodic peak current with respect to time, with a decrease in peak current intensity for the redox pair is 0.25 $\mu\text{A}/\text{min}$, whereby the compound is retained for approximately 15 h.

From Table 1, it is noted that other NADH quantification methods had smaller LODs than those reported in this work; however, the developed GCE/MWCNT/Med electrode has the advantage of having low NADH electrocatalytic oxidation potentials, so this process requires less energy. An interesting and novel advantage is that the preparation of the electrodes in this work is fast, clean and effective, allowing biosensors to be obtained in a few steps. In this sense, the working electrodes reported in Table 1 have different preparation methods, such as the electrodeposition of rosmarinic acid on SPCE [33] or the electropolymerization of thionine over GCE and then addition of suspension of magnetic chitosan-coated microspheres and dry for 4 h [34] and cycling the electrode several times in both cases. In other types, the components are mechanically mixed with carbon paste for a prolonged period of time, and then they are filled in a hollow electrode [31,36], others in which a suspension is added on the GCE, with long preparation processes using the electrodes as such or cycling them several times previously [28,30,32,37]. Another type in which after a suspension and filtration process of their components, they are dried and a pellet is

Table 1
Analytical parameters for NADH determination with different modified electrodes.

Electrode	Technique	E_p NADH	Linear range	LOD	Ref.
GCE/CNF	Amp	+ 0.32 V (PBS, pH 7.0)	0.03–2.1 mM	11 μ M	[28]
PVC/TTF-TCNQ/Aucoil Electrodes	Amp	+ 0.4 V (PBS, pH 7.0)	0–500 μ M	4 μ M	[29]
GCE/CNS	LV	+ 0.355 V (PBS, pH 7.0)	0–500 μ M	0.44 μ M	[30]
CPE/NiONPs	Amp	+ 0.4 V (PBS, pH 7.4)	0.1–1000 μ M	0.05 μ M	[31]
SPCE/ERGO/PAH	Amp	+ 0.45 (PBS, pH 7)	0.02–0.8 mM	6 μ M	[32]
SPCE/RA	Amp	+ 0.25 V (PBS, pH 7.25)	18.72–220 μ M	5.62 μ M	[33]
GCE/OMC/NB	Amp	–0.1 V (PBS, pH 6.85)	0.35–1.2 mM	1.2 μ M	[34]
GCE/PTH/MCMS	Amp	+ 0.05 V (PBS, pH 7.0)	2–10 μ M; 10–100 μ M	0.51 μ M	[35]
CPE/IL/NiONPs	SWV	+ 0.63 V (PBS, pH 7.0)	0.03–900 μ M	9 nM	[36]
GCE/IL-G/Chitosan	Amp	+ 0.45 V (PBS, pH 7.4)	0.25–2 mM	–	[37]
GCE/MWCNT/3,5-DNBPH	CV	+ 0.001 V (BR, pH 7.0)	0.1–0.6 mM	22.3 μ M	This work

Amp: amperometry; BR: Britton-Robinson buffer; CNF: carbon nanofiber; CNS: carbon nanosheet; CPE: carbon paste electrode; CV: cyclic voltammetry; ERGO: electrochemically reduced graphene oxide; FIA: flow injection analysis; G: graphene; GCE: glassy carbon electrode; IL-G: ionic liquid-functionalized graphene; LV: linear voltammetry; MCMS: magnetic chitosan-coated microspheres; NB: Nile blue; NiONPs: NiO nanoparticles; OMC: ordered mesoporous carbon; PAH: poly (allylamine hydrochloride); PBS: phosphate-buffered saline; PP: diphenyl alanine peptide; PTH: electrogenerated polythionine; RA: rosmarinic acid; SPCE: screen-printed carbon electrode; SWV: square wave voltammetry.

formed by compression to give rise to electrode [29]. The method of preparing the working electrode most similar to that of this work corresponds to which a dispersion of ordered mesoporous carbon is dropped on GCE, dried under an infrared lamp for 30 min and the modified electrode is prepared by immersing the OMC/GCE into NB aqueous solution for 5 min.

4. Conclusions

The synthesized compound 4-phenylbutyl-3,5-dinitrobenzoate was an effective precursor of an electrocatalytic mediator for NADH oxidation when immobilized in the three-dimensional network of glassy carbon electrodes modified with MWCNTs. The selective electrochemical activation of this di-nitro compound yields one nitroso-hydroxylamine redox pair that acts as a redox mediator of NADH oxidation, and NADH electrocatalysis shifts NADH's oxidation potential to more negative potentials with the formation of a single voltammetric peak. The 4-phenylbutyl-3,5-dinitrobenzoate mediator was sensitive to changes in the NADH concentration, and a linear regression between 0.1 and 0.6 mM NADH was obtained, with detection and quantification limits of 22.3 μ M and 74.2 μ M, respectively. The developed modified electrode presents low NADH electrocatalytic oxidation potentials, and an interesting and novel advantage is that the preparation of the electrodes is fast, clean and effective, allowing biosensors to be obtained in a few steps.

Thanks to the finding obtained in this article, we expanded the previously described concept of “adsorbent tail”, that is, butylpyrene [24], to a simpler but equally adsorbent “adsorption tail” structure such as 4-phenylbutyl.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2020.105422>.

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