

Full Paper

The NADH Electrochemical Detection Performed at Carbon Nanofibers Modified Glassy Carbon Electrode

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Abstract

In this work, the capability of carbon nanofibers to be used for the design of catalytic electrochemical biosensors is demonstrated. The direct electrochemistry of NADH was studied at a glassy carbon electrode modified using carbon nanofibers. A decrease of the oxidation potential of NADH by more than 300 mV is observed in the case of the assembled carbon nanofiber-glassy carbon electrode comparing with a bare glassy carbon electrode. The carbon nanofiber-modified electrode exhibited a wide linear response range of 3×10^{-5} to 2.1×10^{-3} mol L⁻¹ with a correlation coefficient of 0.997 for the detection of NADH, a high specific sensitivity of 3637.65 (μA/M cm²), a low detection limit (LOD = 3σ) of 11 μM, and a fast response time (3 s). These results have confirmed the fact that the carbon nanofibers represent a promising material to assemble electrochemical sensors and biosensors.

Keywords: Carbon nanofibers (CNFs), Chemically modified electrodes (CMEs), NADH direct electrochemistry, Biosensor

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1. Introduction

The continuous progress of nanotechnology in material science has led to the development of nanostructure materials with unique chemical, physical, and mechanical properties. Since the discovery of carbon nanotubes in 1991 [1] and based on the results obtained from the characterization of these nanostructures [2–8], many other carbon-based nanomaterials have been developed. Among these, carbon nanofibers (CNFs) are the subject of extensive experimental and theoretical studies for specific applications, mainly as catalyst supports [9, 10].

CNFs are cylindrical nanostructures with graphene layers arranged as stacked cones, cups, or plates. They have lengths in the order of micrometers, while their diameter varies between some tens of nanometers up to ca. 200 nm. Their mechanical strength and electric properties are similar to carbon nanotubes [11] while their size and graphite ordering can be well controlled [12]. Since the first report on a CNF-based biosensor [13], CNFs have been extensively used in the design of electrochemical sensors and biosensors [14–17]. Their use in these devices is based on the fact that CNFs can play dual roles. They can be used as immobilization matrixes for biomolecules, while at the same time they can relay the electrochemical signal acting as transducers. Carbon-based nanomaterials have been shown to be ideal

for biosensor applications since they are conductive, biocompatible, possess very large surface areas, and easily functionalized. Especially considering the larger nominal surface area of CNFs, the density of the surface active groups [18] results higher than that observed for other conventional electrode materials, allowing a selective immobilization of several biomolecules, such as enzymes [19], antibodies, and DNA.

The determination of β-nicotinamide adenine dinucleotide (NADH) is very important because NADH and its oxidized form (NAD⁺) are the coenzymes for a large number of dehydrogenase enzymes (>300) and components of biomarker systems. However, direct oxidation of NADH at bare electrode occurs at high overpotential (0.6–0.8 V) and is usually encountered by low sensitivity and the fouling of the electrode surface by its oxidation products [20–22]. Consequently, considerable effort has been devoted toward the goal of identifying new electrode materials or mediators to allow the stable low potential determination of NADH. Carbon nanotubes (CNT) are one such novel material utilized for the stable low potential detection of NADH. Musameh et al. [5] found that a randomly dispersed CNT layer lowered the detection voltage by 490 mV compared to a bare glassy carbon electrode, whilst Chen et al. [23] have found that a vertically aligned of CNT layer significantly decreased this overpotential, compared to the

randomly coated electrodes. Considering these properties, in this work a new functionalized nanomaterial, based on carbon nanofibers, has been used to investigate the direct electrochemistry of NADH at GC modified electrode. This paper reports on the preparation and electrochemical characterization of NADH sensor using the CNF-modified glassy carbon electrode (CNF/GCE).

2. Experimental

2.1. Reagents

CNFs were purchased from Electrovac AG (Austria) [24]. Dibasic phosphate, monobasic phosphate, KCl, and NADH were obtained from Sigma (St. Louis, MO). All chemicals, from commercial sources, were of analytical grade and used as received. The NADH solutions were prepared daily. All the solutions were prepared in distilled water.

2.2. Apparatus and Analytical Procedures

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were performed using an Autolab electrochemical system (Eco Chemie, Utrecht, The Netherlands) consisting in PGSTAT-12 and GPES software. The electrochemical cell was assembled with a conventional three-electrode system: a glassy carbon working electrode (AMEL GC/492/3; 3 mm diameter, Milan, Italy), an Ag|AgCl reference electrode and a Pt counter electrode. All experiments were carried out at room temperature.

Cyclic voltammetry experiments were performed at a scan rate of 100 mV/s over the relevant potential range using 0.2 M phosphate buffer containing 0.2 M KCl, pH 7.0.

DPV measurements were performed on the following conditions: a modulation time: 70 ms; modulation amplitude: 25 mV; interval time: 0.4 s; step potential: 1.65 mV; scan rate of 5 mV/s; initial potential: 0.2 V; end potential: 0.7 V for NADH determinations.

Amperometric measurements were performed with a METROHM, 641 VA-Detector (Switzerland), combined with an AMEL 868 recorder. A magnetic stirrer provided the convective transport during the amperometric measurements.

2.3. GC Electrode Surface Preparation and Modification

CNF were dispersed into CH₃CN using an ultrasonic bath for 1 h in order to obtain a stable and homogeneous dispersion of 1 mg/ml. Prior to the electrode surface modification, the GC electrode was polished using 1, 0.3 and 0.05 μm aluminum slurries, respectively, then rinsed with distilled water, and finally sonicated in distilled water for ca. 15 min, to remove the residual aluminum slurries from the GC electrode surface. Finally, the GC electrode surface was coated with 2 μL of 1 mg/mL CNF/CH₃CN

suspension, followed by the solvent evaporation at room temperature.

3. Results and Discussion

3.1. Morphological Characterization of the CNF Deposit

The deposition procedure used here, produces deposits having thickness ranging from 2 to 5 μm, as displayed in Figure 1a. The morphology of the CNF coating on the GC electrode appears as a net of fibers, randomly connect one another on the electrode surface (Fig. 1b). The net pores are characterized by diameters ranging from 150–300 nm (see Fig. 1c) producing a higher porosity-based electrode compared with the GC bare conventional probe.

3.2. The Oxidation Process on the CNF Modified Electrode

In order to characterize the electrochemical behavior of the CNF-GC electrode in a 0.2 M phosphate buffer solution, pH 7, a cyclic voltammogram was recorded in a wide potential region (Fig. 2, curve c). When the measurements were performed in 1 mM NADH buffered solution (Fig. 2, curve b), the oxidation process of NADH appeared in the positive scan at ca. 0.3 V. For the same concentration of NADH, the cyclic voltammogram recorded on the bare-GC electrode shows an oxidation peak at 0.6 V vs. Ag/AgCl (Fig. 2, curve a).

Cyclic voltammetric experiments for NADH with CNF-GC electrode (Fig. 2) indicated an improved electrochemical reactivity towards the oxidation of this molecule compared to common GC electrodes.

The voltammetric behavior of the CNF-GC electrode in NADH solution was further studied by differential pulse voltammetry. DPV was chosen as an experimental technique that offers a better resolution and signal-to-noise ratio compared to CV.

The peak potential is negatively shifted when the electrode is modified by the CNF layer (Fig. 3, curve b) compared to the unmodified electrode (Fig. 3, curve a), from 0.6 V to 0.32 V. Katekawa et al. [25] reported the same positive potential value in their study of oxidation of NADH on a bare glassy carbon electrode using DPV in phosphate buffer pH 7.0. There is no peak at 0.32 V in the absence of NADH (Fig. 3, curve c). Therefore, the peak current at 0.32 V can be assigned to the catalytic oxidation of NADH by carbon nanofibers.

Such a large decrease in the overpotential for the NADH oxidation at a CNF-modified electrode could be ascribed to the high density of electronic states of CNFs (the tunneling effect) and make the CNF-electrodes suitable for the amperometric determination of NADH at lower potential. In addition, considering the higher CNF's surface density of functional groups (–COOH–), the NADH electrochemical oxidation could be reasonably catalyzed, according to the

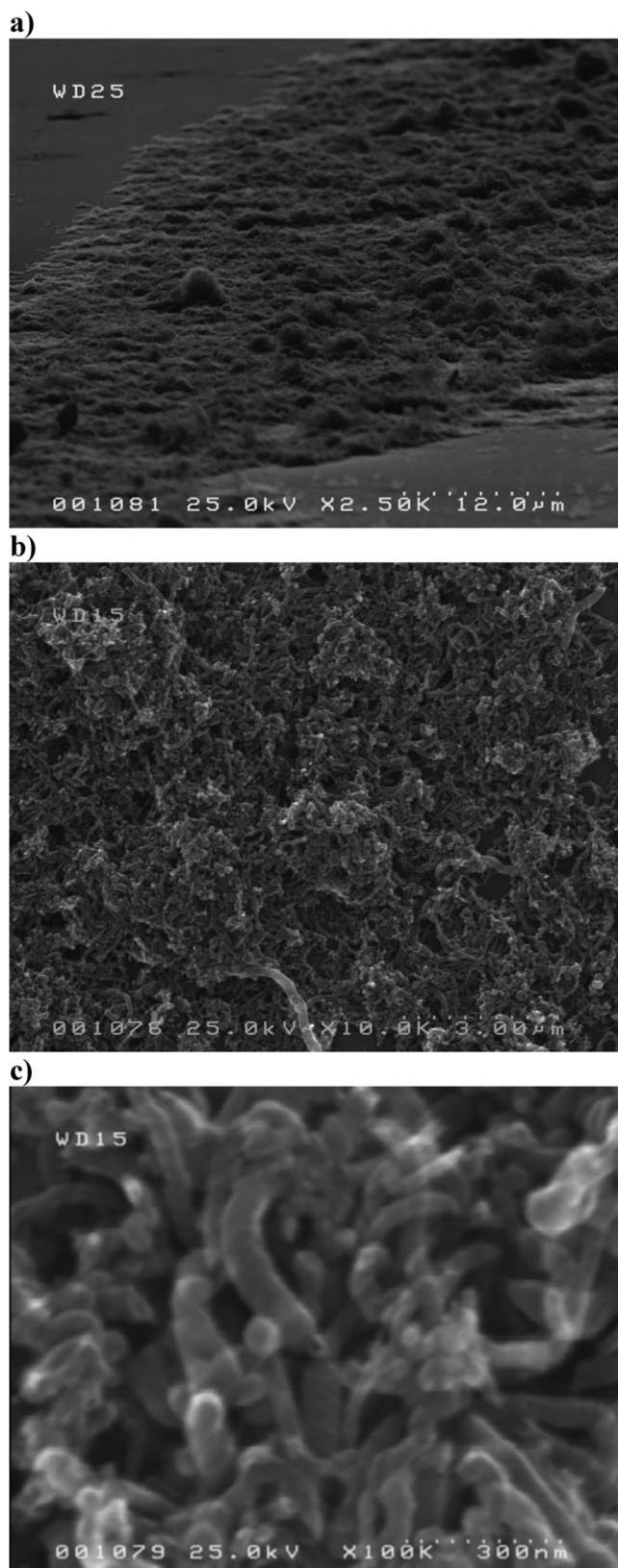


Fig. 1. CNF's FESEM micrographs: in a) a tilted view of the CNF-modified GC electrode surface; in b) the typical morphology of the CNF coating on the GC electrode surface; and in c) a higher magnification view of the CNF net coating, previously deposited by casting on the GC electrode surface.

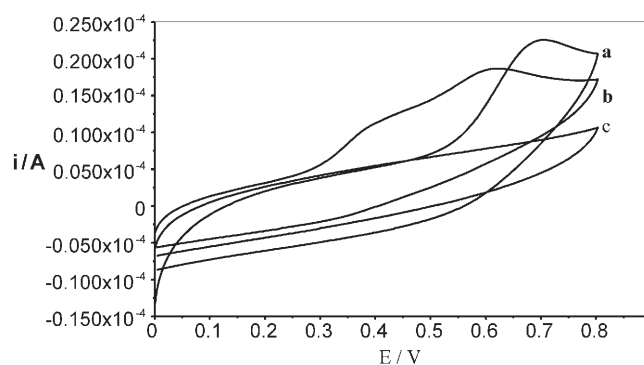


Fig. 2. Cyclic voltammograms on: a) bare GC electrode and 1 mM NADH; b) CNF-modified GC electrode and 1 mM NADH; c) CNF-modified GC electrode and phosphate buffer. Scan rate: 100 mV/s.

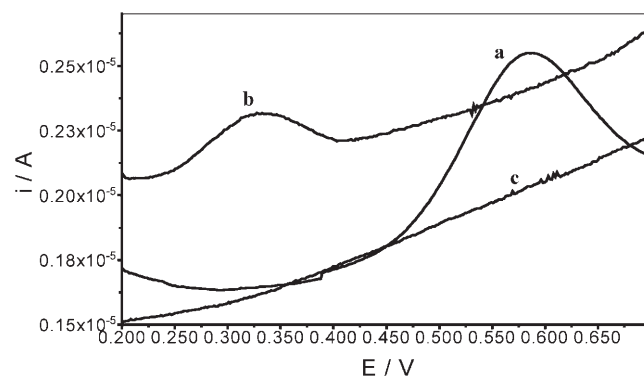


Fig. 3. Differential pulse voltammograms obtained on a) bare GC and 0.2 mM NADH; b) CNF-GC electrode and 0.2 mM NADH; c) CNF-GC electrode and phosphate buffer. Scan rate: 5 mV/s, modulation time: 70 ms; modulation amplitude: 25 mV; interval time: 0.4 s.

literature [26, 27]. In fact, it is very well noted that the presence of some active species on the electrode surfaces, can mediate the charge transfer reactions [26, 27]. Moreover, as displayed on the Figure 1 about the CNF's deposit architecture, it is possible to consider that the open net structure could be responsible for easier electron-transfer processes because of a higher density of electroactive sites, if compared with a flat electrode surfaces.

Both cyclic voltammetry and differential pulse voltammetry experiments on CNF electrodes have demonstrated the improved electrochemical reactivity towards the oxidation of NADH compared to the conventional GC electrode.

3.3. Influence of NADH Concentration on the Peak Parameters

Figure 4A shows a set of differential pulse voltammograms recorded in a wide range of concentrations of NADH in phosphate buffer solution (from 0.0 to 1.0 mM). It is important to note that concentrations as low as 0.1 mM

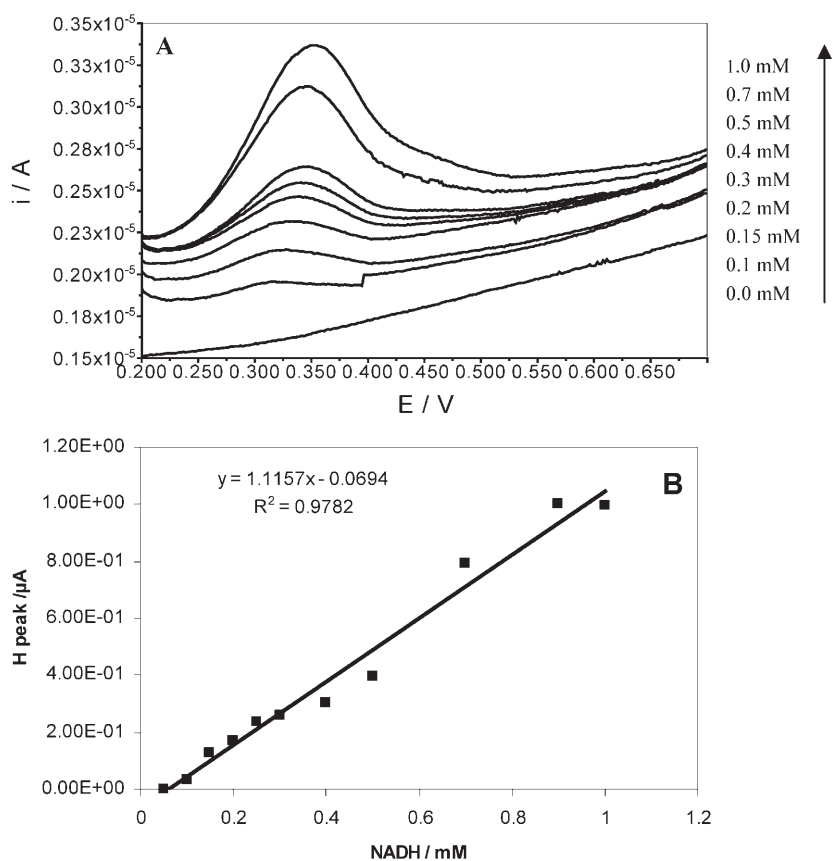


Fig. 4. A) Differential pulse voltammograms recorded at CNF-GC electrode for different concentrations of NADH. B) Dependence of the peak current on the NADH concentration. Other experimental conditions as described in Figure 2.

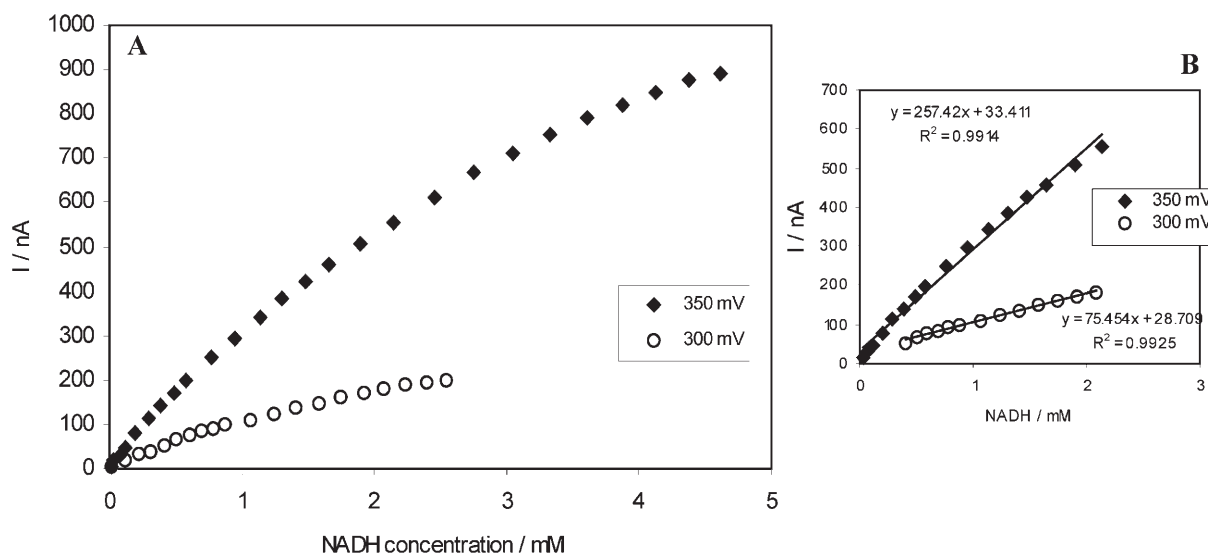


Fig. 5. A) Calibration graphs for NADH at CN-modified-GC electrodes by amperometry in stirred solution at 300 and 350 mV; B) NADH linear response ranges.

were detected with high accuracy using the CNF-GC electrode, indicating that the modified GC electrode could be used as a sensitive sensor for the NADH detection. This

dependence is presented in Figure 4B and shows that the peak current (H peak) varies linearly with concentration. Using this method 13.4 μM NADH can be detected.

Table 1. Analytical parameters for the NADH amperometric detection at CNF-modified-GC electrode ($n = 3$ electrodes).

Potential (V)	Sensitivity ($\mu\text{A/M}$)	Specific sensitivity ($\mu\text{A/M cm}^2$)	LOD (3σ) (mM)	Linear range (mM)	R^2	Response time (s)
0.3	75 ± 11.32	1061 ± 160	0.040	0.4–2	0.9925	4
0.35	257 ± 8.34	3637 ± 118	0.011	0.03–2.14	0.9914	3

3.4. NADH Calibration at CNF Modified-GC Electrode

The choice of the best working potential for the NADH amperometric measurements should require a less positive potential value to achieve a good selectivity (toward the most common interferences) and at the same time, a higher signal/noise ratio for a significant sensitivity of the resulting sensors. Taking into account all these considerations, and also the sensitivity shown in Figure 5, the selected potential value of 0.35 V fulfills all these conditions.

The amperometric response (calibration curves) of CNF-GC electrodes towards NADH was assessed at two potentials: +0.3 V and +0.35 V (Fig. 5A). Nanofibers-based electrodes display a well-defined current dependence over concentration range 0.03–2.14 mM, with a good sensitivity for 0.35 V applied potential (Fig. 5B). In the Table 1, the analytical parameters of CNF-GC electrodes are listed.

4. Conclusions

This paper reports that the modification of GC bare electrode by a layer of CNFs could provide an electrode surface capable to oxidize the NADH cofactor at lower potential, compared to the GC unmodified electrodes. The developed sensor for NADH exhibits a good sensitivity on a wide linear range of concentration, for an applied potential of 0.35 mV versus Ag/AgCl, a low detection limit and a short response time.

This work also confirmed that CNFs represent a promising nanomaterial for several interesting electro-analytical applications. The noticeable electrocatalytic activity towards the NADH oxidation, could offer a great possibility to assemble low-potential biosensors for numerous important substrates detection.

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6. References

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