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Selective Electrochemical Nanosensor based on Modified Carbon Paste Electrode for Determination of NADH in the presence of Uric Acid

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Abstract- The electrochemical properties of a modified carbon paste electrode with the synthesized compound of 2,2'-[1,7–heptanediylbis(nitrilomethylidene)]-bis(4-hydroxyphenol) (DHBH) and graphite nanoparticle (GN) were studied by cyclic voltammetry (CV), chronoamperometry and differential pulse voltammetry (DPV) methods. The proposed electrode shows excellent electrocatalytic activity towards the oxidation of NADH under the optimum pH of 7.0. The modifier and nanoparticle simultaneously lead to a reduce overpotential of NADH oxidation about 250 mV and enhance current about 6 μA of the unmodified CPE. This electrochemical sensor exhibited a detection limit (3σ) of 13.4 nM with two linear dynamic ranges (0.01-6.0 and 6.0-400.0 μM) for the determination of NADH. The modified electrode can detect well NADH (180 mV) in the presence of Uric acid (360 mV) simultaneously. Also, the performance of the proposed sensor was evaluated in real samples.

Keywords- Modified electrode, Electrocatalyst, Over potential, NADH, Uric acid

1. INTRODUCTION

The use of chemically modified electrodes as an effective and sensitive electrochemical manner for the determination of biologically essential material has been expanded [1]. These electrodes have been modified with different materials such as enzymes, polymers and nanoparticles. Carbon paste electrodes (CPEs) has many advantages such as low cost, very low background current, wide potential window, and facility in preparation [2]. Therefore, CPEs have been widely used in electroanalysis and electrochemical studies. Among different methods, the voltammetric method as a sensitive, compact, and efficient method is very popular for analysis [3].

b-Nicotinamide adenine dinucleotide (NADH) is an essential enzyme that plays an important role in energy production in the systems of living organisms, which presents in various dehydrogenases enzymatic reactions. Some researchers have shown that NADH is useful for the cure of Parkinson's, Alzheimer's, and depression disease [4]. According to the investigations, NADH is necessary for the reproduction of glutathione (GSH) following its oxidation. When GSH levels fall, NADH depleted and facilities the regeneration of GSH to its active form [5]. Bioelectrochemical technique for dehydrogenases, such as biosensors, biofuel cells, and bioreactors are very important. so NADH detection is very considered [6]. generally, the direct oxidation of NADH is almost irreversible and accompanied with coupled side reactions, which products such as benzyl, propyl and methyl reactants on the electrode surface and adsorbs [7]. Among different research for determination of NADH such as enzymatic assay [8], high-performance liquid chromatography [9], colorimetry [10], and photoelectrochemistry [11], electrochemical method is best NADH sensor, due to the cost-effectiveness, rapid response and straight forward operation [12]. At ordinary electrodes, overvoltage of NADH oxidation is large and associated with the aggregation of reaction products [7]. To solve the problem, many efforts have been made toward the design new electrode materials that decrease the oxidation overpotential of NADH.

Uric acid (UA) is the main nitrogenous source in the urine and a primary substance of purine metabolism [13]. Its aggregation in body leads to many diseases, such as pneumonia, leukemia [14] Lesch–Nyhan, gout and hyperuricemia disease [15].

In the research, we prepared the modified CPE with DHBH and GN (DHBH-GN/CPE) and then study performance of NADH in a modified electrode at optimizing condition. We also study the electrocatalytic behavior of the introduced electrode in determination of NADH and UA simultaneously with acceptable potential separate. To evaluate how applicable, the modified sensor was successfully evaluated in the real samples. The results indicate that DHBH-GN/CPE contains many benefits such as low detection limit, high apparent charge transfer rate constant, good stability and high repeatability.

2. EXPERIMENTAL

2.1. Chemicals and apparatus

Electrochemical studies were performed with a galvanostat/potentiostat (electroanalyzer system, SAMA 500, Iran). Experiments were carried out in a three-electrode system with

DHBH-GN/CPE as the working, a platinum wire as counter and a standard calomel electrode as reference electrodes. The DHBH-GN/CPE was prepared by mixing graphite powder: DHBH: GN (96: 1: 3) and ~0.7 mL of paraffin into mortar for 0.5 h. later the product was packed into the glass tube (10 cm long and ca. 3.7 mm i.d) and copper wire as electrical connecter inserted. Also, CPE electrode without GNs (DHBH/CPE), without DHBH (GN/CPE), and without DHBH and GN (CPE) were prepared with the same method.

UA, graphite nanoparticle, paraffin oil (0.88 $\mathrm{gcm^{-3}}$, DC 350) and other reagents were purchased from Merck (Germany, Darmstadt). The procedure of synthesis of DHBH was reported in our prior work [16]. pH values of Phosphate buffers solution (PBS) were tested by a Metrohm 691 pH Meter.

2.2. Electrochemical studies

CV studies: Using Laviron equation, k_s as apparent charge transfer rate constant were determined for the case of electroactive species in surface with $\Delta E_p > \frac{200}{n}$ mV [17]:

$$\log(k_s) = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\left(\frac{RT}{nFv}\right) - \alpha(1-\alpha)nF\frac{\Delta Ep}{2.3\,RT} \quad (1)$$

The transfer coefficient (α) is found from the slope of the plot of E_p *vs.* log t.
equation of Sharp et al. [18] was used for the calculation of Γ as the surface coverage of the electrode:

$$I_p = n^2F^2A\Gamma^2v/4RT \quad (2)$$

where A, n and ν are the surface area (0.01 $\mathrm{cm^2}$), the number of reaction electrons and scan rate, respectively.

Chronoamperometry studies: On the basis of the Cottrell method [19], the mean value of diffusion coefficient (D) calculated:

$$I = nFAC_b\,(D/\pi t)^{1/2} \quad (3)$$

Using the method of Galus [19] were evaluated the catalytic rate constant (k) between NADH and DHBH-GN/CPE:

$$I_C/I_L = \pi^{1/2}\,(kCb_t)^{1/2} \quad (4)$$

where I_L and I_C are limited catalytic current in the absence and presence of NADH at the DHBH-GN/CPE, respectively. Also, C_b and t are the bulk concentration of NADH and the time elapsed, respectively.

3. RESULTS AND DISCUSSION

3.1. Electrochemical performance of DHBH-GN/CPE

The voltammetry behavior of DHBH-GN/CPE was investigated in aqueous solutions and pH 7 was selected as buffer optimal conditions. According to optioned slope of the $E^{0'}$ *vs.* pH (47.0 mV) at pH range from 4-10, which is close to the Nernstian value of 59.2 mV, were predicted translate system of two electrons and two protons [19].

Potential of DHBH-GN/CPE electrode was considered from 0.0 to 0.35 V at 100 mV/s and $\Delta E_p=E_{pa}(0.192)–E_{pc}(0.091)=0.101\ V \geq (0.059/n)\ V$ for a reversible system. Fig. 1b demonstrates a linear relationship between the potential scan rate of the DHBH-GN/CPE and the current of CV response in the range 20–2000 mV/s indicates that diffusion-phenomena controls the redox process at surface. Also, the changes of E_p relative logν at scan rates above 90 mV is shown in Fig. 1c and slopes of two straight lines are $2.3RT/(1-\alpha)nF$ for the anodic peak and $2.3RT/\alpha nF$ for the cathodic peaks and followed α was obtained value of 0.32 and 0.68, respectively. Furthermore, using Eq. (1), the mean value of k_s as apparent charge transfer rate constant was obtained to be 5.67 s^{-1}.

Γ as the surface coverage of the modified electrode was estimated value of 6.8×10^{-10} mol/cm^2 while n=2 based on Eq. (2).

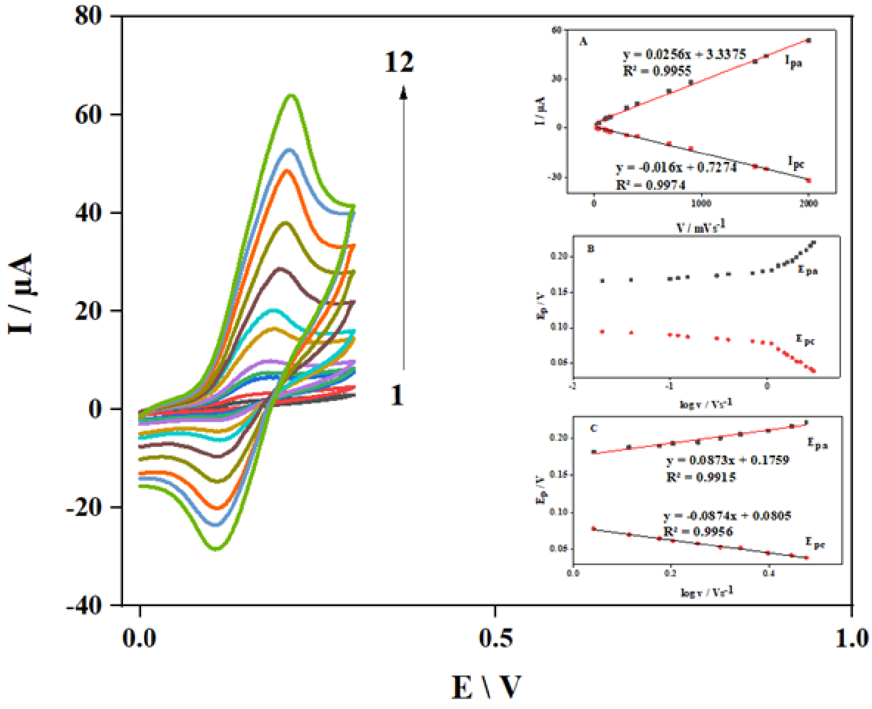


Fig. 1. CV of DHBH-GN/CPE electrode in 0.1 M PBS (pH=7.0) at different scan rates 20, 40, 100, 120, 150, 300, 400, 700, 900, 1500, 1600 and 2000 mV/s, respectively; (A) plot of I_p vs. υ, (B) variation of E_p vs. log υ, (C) variation plot of E_p vs. log υ

3.2. Electrocatalytic properties of NADH at the DHBH-GN/CPE

Fig. 2 demonstrates the oxidation of NADH 0.4 mM at the bare CPE, GN/CPE, DHBH/CPE, and DHBH-GN/CPE. In this figure, oxidation potential of NADH has different values so that in the absent DHBH is about 490 mV and in the present DHBH is about 240 mV. Therefore, these results showed CPE modified with DHBH decrease of the peak potential of NADH oxidation at about 250 mV. Generally, the CPE modified with GN has the current of peak rather than unmodified CPE electrodes regardless of the oxidation of NADH and DHBH. Therefore, DHBH-GN/CPE proved excellent electrocatalytic behavior for NADH oxidation. In addition, the strong electrocatalytic effect of the DHBH-GN/CPE the presence of NADH leads to a decrease of E_{pc} [19].

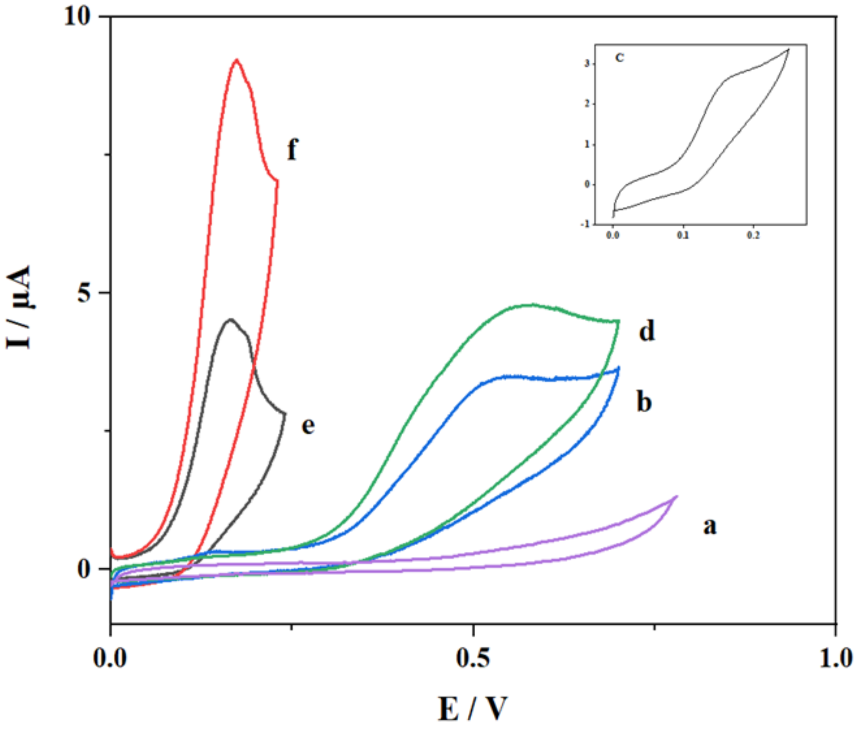


Fig. 2. Cyclic voltammograms of (a) CPE in 0.1 M PBS at scan rate of 40mV/s; (b) as (a) 0.4 mM NADH; (c) as (a) at the surface of DHBH/CPE; (d) as (b) at the GN/CPE; (e) as (b) at the DHBH/CPE; (f) as (b) at the DHBH-GN/CPE

Fig. 3a shows the electrocatalytic oxidation of NADH at different scan rates at the DHBH-GN/CPE. The linear dependence of peak current (I_p) and the $\nu^{1/2}$ indicate that the oxidation of NADH at the surface of modified electrode is diffusion rather than surface controlled. Relation with the scan rate normalized current and scan rate (Fig. 3c) improved of an EC' electrocatalytic mechanism (Scheme 1).

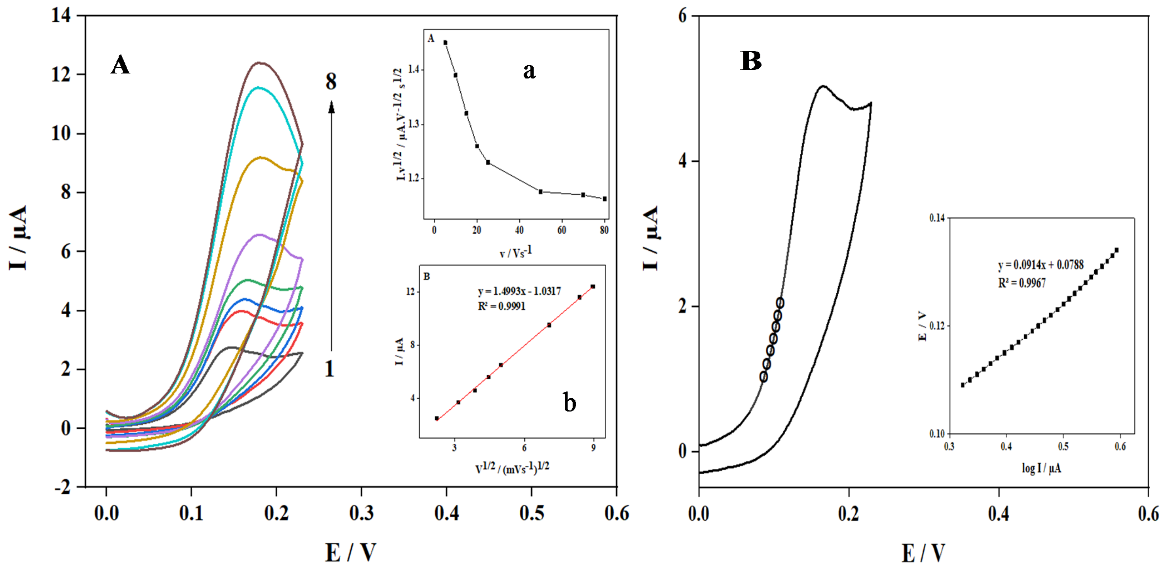
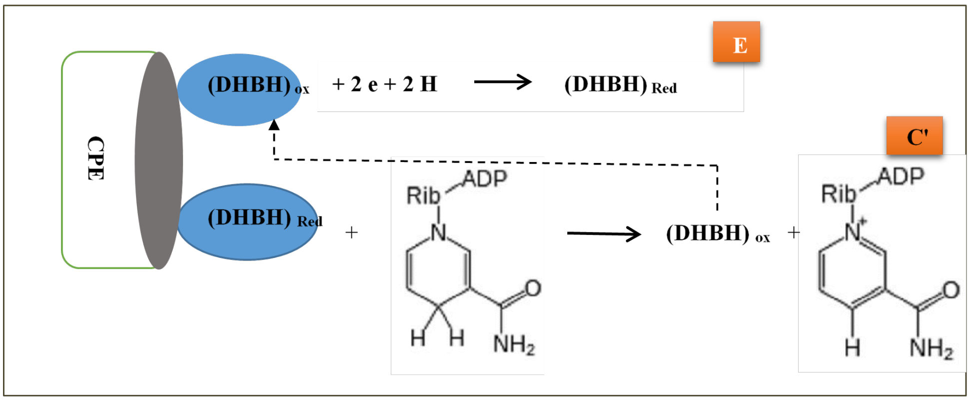


Fig. 3. (A) Cyclic voltammograms of a DHBH in 0.1 M PBS containing 0.4 mM NADH at different scan rates; 5, 10, 15, 20, 25, 50, 70 and 80 mV/s scan rates, Insets: (a) plot of the electrocatalytic currents *vs.* υ^2 and (b) plot of the scan rate normalized current *vs.* υ; (B) Tafel plot derived from CV recorded at a scan rate of 20 mV/s



Scheme 1. Reaction mechanism between DHBH and NADH

According to data of the increasing part of the CV curve of the DHBH-GN/CPE (Fig. 3B), the plot of E_p versus the log I was recorded and the slope of 0.0914 V/decade obtained. Considering slope and one electron transfer as electron of rate-limiting, α was calculated value of 0.35.

3.3. Chronoamperometric measurements

Fig. 4 shows chronoamperometric investigations of NADH at the DHBH-GN/CPE for various concentrations of NADH at the potential of 300 mV. In Fig. 4b the slope obtained from straight lines of Fig. 4a at different concentrations of NADH was employed. According to the slopes and Cottrell equation [17] (Eq. 3), mean diffusion coefficient of D for NADH as an electroactive material was obtained. 6.43×10^{-5} cm^2/s.

Using chronoamperometry measurements also can determine k as catalytic constant. From the values of the slopes of I_C/I_L versus. $t^{1/2}$ in Fig. 4c, the mean value of k was calculated to be 1.67×10^4 L/(mol.s).

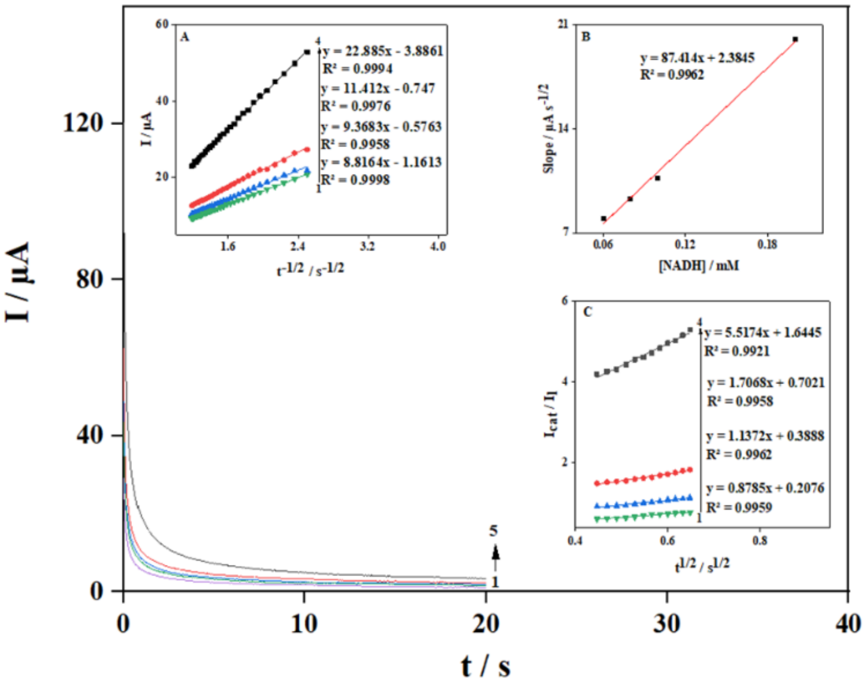


Fig. 4. Chronoamperograms of the DHBH-GN/CPE in 0.1 M PBS for different concentrations of NADH: 0, 0.06, 0.08, 0.1 and 0.2 mM of NADH. Insets: (A) plots of I vs. $t^{-1/2}$ (B) Variation of the slope of the straight lines *vs.* the concentration of NADH. (C) Dependence of I_{cat}/I_l on $t^{1/2}$

3.4. Differential pulse voltammetry measurement

DPV measurements was applied to explore oxidation of NADH at the DHBH-GN/CPE electrode. In Fig. 5, the DPVs of various concentrations of NADH was showed. It is clear that the peak current of oxidation increase when concentration of NADH increased. The inset of Fig. 5 shows the dependence of peak current to the concentration of NADH with two linear segments with different slopes at ranges 0.01-6.0 and 6.0-400.0 µM. The decrease in sensitivity in the linear range with higher concentrations is probably due to the kinetic limitation. The lower limit of detection of NADH was determined 13.4 nM.

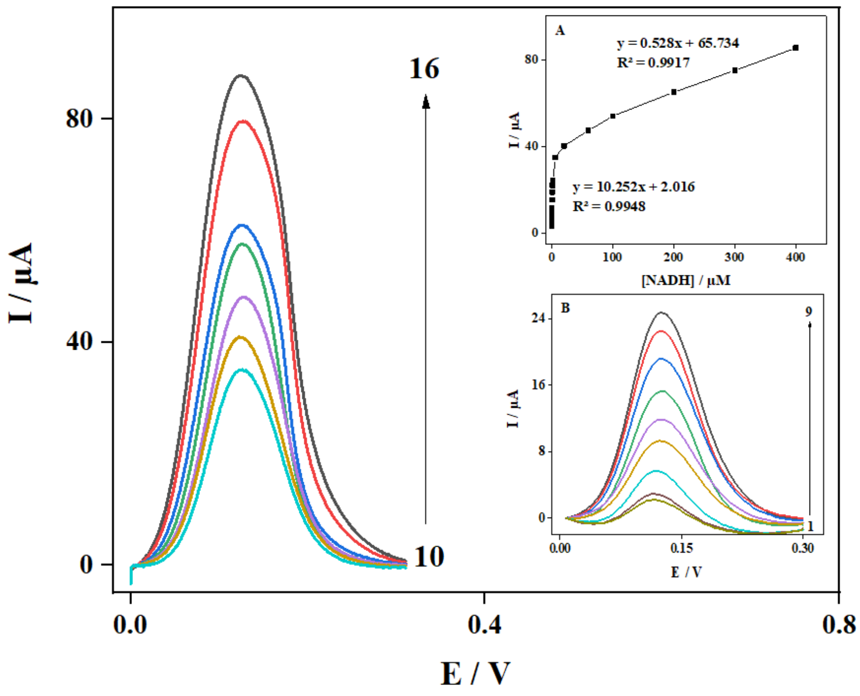


Fig. 5. DPVs of DHBH-GN/CPE in 0.1 M PBS containing various concentrations of NADH in µM. The numbers 1-16 correspond to 0.01, 0.02, 0.04, 0.1, 0.4, 0.6, 0.8, 1.0, 2.0, 6.0, 20.0, 60.0, 100.0, 200.0, 300.0 and 400.0 µM, respectively

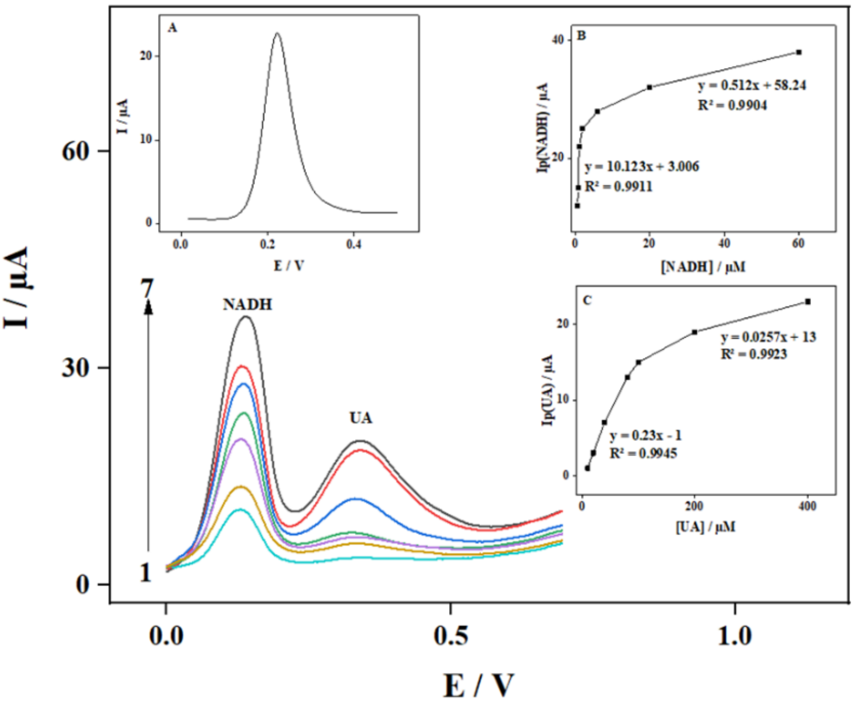


Fig. 6. DPVs of DHBH-GN/CPE in 0.1 M PBS solution containing various concentrations of NADH-UA in µM. The numbers 1-7 correspond to 0.6-10, 0.8-20 1-40, 2-80, 6-100, 20-200 and 60-400, respectively

In the DPV measurements, the DHBH-GN/CPE electrode showed a very good resolution for detecting NADH (180 mV) in the presence of UA (360 mV) at different concentrations (Fig. 6). As shown in the inset A, only one peak at 230 mV is observed if the unmodified CPE is applied in a mixture of 400.0 µM UA and 60.0 µM NADH. This demonstrates the performance of the modified CPE as well for the simultaneous determination of UA and NADH. As the concentration of the NADH and UA increases, the oxidation rate increases in the DHBH-GN/CPE, followed by the oxidation peak current of NADH (Inset B of Fig. 6).

The sensitivities of the DHBH-GN/CPE for determination of NADH in the presence and absence of UA were obtained to be 10.123 and 10.252 µA/µM, while the unmodified electrode exhibits the same sensitivity in the presence and absence of UA. Therefore, the presence of UA in the DHBH-GN/CPE does not interfere with the measurement of NADH.

In Table 1, values reported linear range and detection limit of NADH by other chemically modified electrodes has been compared.

Table 1. Comparison of some electrochemical modified electrodes used in the determination of NADH

Substrate	Modifier	Method	Limit of detection (M)	Dynamic range (M)	Ref.
GCE	NiONPs	Amp	0.106	0.11-1000.0	[20]
GCE	rGO-AuNPs	Amp	0.00113	0.05-50.0	[21]
GCE	Gr-PQQ-CTS	Amp	0.16	0.32-220.0	[22]
GCE	NDG	Amp	0.37	0.5-12.0	[23]
GCE	SWCNTs (oxidized)-Polytry	Amp	0.0079	0.15-83.0	[24]
CPE	DHBH	DPV	0.0134	0.01-400.0	This work

NiONPs: nickel oxide nanoparticles. **rGO:** reduced graphene oxide. **AuNPs:** gold nanoparticles. **PQQ:** pyrroloquinoline quinone. **CTS:** chitosan. **NDG:** nitrogen-doped graphene. **SWCNTs:** single-walled carbon nanotubes. **Polytry:** poly-tyrosine. **DHBH:** 2((7-(2, 5dihydrobenzylideneamino) heptylimino) methyl) benzene-1, 4-diol

3.5. Repeatability and stability of DHBH-GN/CPE electrodes

To evaluate the reproducibility and stability of the electrode, differential pulse voltammetry method was used. Five separate electrodes were prepared under optimum conditions for NADH measurement. A relative standard deviation of 3.8% was obtained for the electrode response, indicating appropriate electrode reproducibility.

For electrode stability, the modified electrode was kept at room temperature and after one day, three days and one-week flow of NADH oxidation differential pulse voltammograms were 98.7%, 97.3% and 96.5% similar to the initial signal, respectively, Indicating good electrode stability.

3.6. Real sample test

To determine the applicability of the proposed electrode for the detection of NADH, it should be evaluated in real biological samples. So, according to spike method NADH was evaluated 6 times in the Blood serum solutions. For this purpose, plasma was diluted 1: 100 by phosphate buffer with pH=0.7. Subsequent concentrations of NADH were then added to the plasma and the corresponding pulse voltammograms obtained. Then the resulting peak current and calibration diagram were obtained and NADH concentrations were calculated.

The percentage of recovery associated with the measurements was calculated. The results of this study are presented in Table 2.

Table 2. Determination of NADH in real samples

Number	**Substrate (µM)**	**Found (µM)**	**Recovery (%)**
1	0	-	-
2	0.8	0.82	102.5
3	2.0	2.04	102.0
4	20.0	20.50	102.5
5	60.0	58.80	98.0
6	200.0	201.20	100.6

4. CONCLUSION

The DHBH-GN/CPE with excellent reproducibility and long stability was fabricated. CV studies at different scan rates were obtained anodic transfer coefficient, also was showed the surface coverage of the electrode that its value for this electrode is very proper. Chronoamperometric investigation revealed an amount of 6.43×10^{-5} cm^2/s for diffusion coefficient. Using DVP the modified electrode exhibited excellent electrocatalytic activity towards the simultaneous detection of NADH and UA with wide potential differences. Also, simultaneous as well as independent electrochemical determinations of these species in mixture are possible without electrochemical interference from each other. The modified electrode has been shown to be promising for determination of NADH with many desirable properties including, high stability, selectivity, good reproducibility and repeatability, high sensitivity, low detection limit, fast response time, antifouling property, together with the ease of preparation, low cost and surface regeneration, makes the proposed modified electrode very useful for accurate determination of NADH and UA their oxidation products and was successfully used in real samples.

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