CHAPTER 4

Selective and Low Potential Electrocatalytic Oxidation and Sensing of NADH Using 2,2-Diphenyl-1-Picrylhydrazyl Immobilized Graphene Oxide Modified Glassy Carbon Electrode

4.1 Introduction

The development of new redox active organic molecule-stabilized chemically modified electrodes with high stability and a selective electrocatalytic response to target analytes is a continued research interest in the field of electrochemistry. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a nitrogen centered radical species that has often been used as a standard compound for testing the antioxidant activity in various real samples, such as foods (Xie et al., 2014), biological fluids (Perez-Roses et al., 2016), and various plant extracts (Zhang et al., 2011; Singh et al., 2004). In general, DPPH changes its color from deep violet to light yellow in a dark environment, due to the reduction of the DPPH radical to the stable DPPH-H complex (antioxidant feature), which is monitored by comparing the absorbance at $\lambda_{\text{max}} = 517$ nm by UV-vis spectroscopy (Nuutila et al., 2003).

There have been some reports involving electroanalytical studies of DPPH in relation to the antioxidant evaluation of real samples, as an alternative method (Zhang et al., 2017; Amatatongchai et al., 2012; Chua et al., 2013; Wang et al., 2009). Zhang et al. studied the electrochemical antioxidant activity of carbon dots using DPPH with a bare gold electrode surface in 5 mL methanolic phosphate buffer at pH 7.2 (Fisher Scientific pH 2100) (Zhang et al., 2017). The quenching activity of the poor redox peaks observed at $E^{0'} = 0.35$ V (Ag/AgCl), which is due to the radical redox reactions, has been used as an analytical signal for the electrochemical-antioxidant activity. Amatatongchai et al. reported the electroanalysis of the antioxidant features of certain polyphenols using DPPH with a carbon nanotube modified electrode in 0.03 M PBS (pH 7.0) containing 0.03 KCl in 40% (v/v) ethanol (Amatatongchai et al., 2012). Herein, we report, an electro-active DPPH-derivative stabilized graphene oxide modified glassy carbon electrode prepared by a simple drop-casting method for the electrocatalytic oxidation and
sensing of the reduced form of nicotinamide adenine dinucleotide (NADH) in physiological pH solutions.

NADH is a coenzyme used to stimulate the energy production in living cells, such as muscle cells, neurons (Wu et al., 2007), etc. Previous studies reported that a deficiency of NADH in the body provokes fatigue and ATP depletion, causing cell death (Swetha and kumar, 2012; Tig, 2017). Meanwhile, because the NAD$^+/\text{NADH}$ reaction is associated with more than 450 enzymatic systems (Bihar et al., 2016), such as glucosede hydrogenase (Kim et al., 2013), formaldehyde-dehydrogenase (Achmann et al., 2008), nitrite reductase (Quan and Shin, 2010) etc., the development of bio-electrocatalytic materials and electrochemical sensors for NADH is of significant research importance in analytical chemistry (Liu et al., 2017). Indeed, owing to its high over-potential for oxidation (~0.8 V (Ag/AgCl) in pH 7), conventional electrodes, such as GCE and Pt, are not used for NADH oxidation and sensing purposes (Swetha and Kumar, 2012). In order to circumvent this problem, several chemically modified electrodes (CMEs) have been developed. In general, for NADH electrochemical oxidation and sensing, CMEs composed of redox mediators, such as quinone and its related derivatives (Swetha and Kumar, 2012; Canevari et al., 2017; Gligor et al., 2005; Lee et al., 2015), nickel oxide (Sharifi et al., 2013), iron oxide (Roushani et al., 2016), cobalt oxide (Chen et al., 2013), platinum (Guo et al., 2010), silver (Balamurugan et al., 2010), and gold nanoparticles, poly(3,4) ethylenedioxythiophene (PEDOT) (Manesh et al., 2008), polytyrosine (Eguilaz et al., 2016), poly diallylmethylene diamine (Lu et al., 2016), and poly methylene blue (Topcu et al., 2016), have been used. Note that the preparation of the CMEs involves tedious experimental procedures, expensive chemicals and electrode-fouling problems, wherein the surface inactivation difficulties are due to the formation of reactive NAD$^+$ molecules and oxygenated species on the underlying surface (with carbon based electrodes) (Sharfi et al., 2013). For instance, a library of about 20 hydroquinone derivatives was synthesized using a solution phase procedure and they were used as electrode-modifiers on the GCE surface via di-tert-butoxycarbonyl (BOC) protecting agent (Lee et al., 2015). Unfortunately, these quinone functional chemically modified electrodes showed poor stability in neutral pH solution. Upon continuous cycling, about 30% fouling of the active material from the electrode surface was noticed. However, the
DPPH-derivative modified electrode introduced in this work, which required a short preparation time of 10±3 min, showed highly stable and strong redox active features, which enabled it to be used for efficient NADH electrocatalytic oxidation and sensing in physiological pH solutions.

Graphene oxide (GO) has a sp² hybridized honeycomb like lattice packed-carbon structure with oxygen rich functional groups (Balamurugan et al., 2016; Gupta et al., 2017). It possesses an ultra-high surface area and excellent electrical conductivity (Aparecida et al., 2017). Recently, it has been reported that GO has anti-oxidant features (radicals from the sp² sites are involved in proton accession) (Eguilaz et al., 2016). Thus, in this work, GO was chosen as the material for the in situ quenching of DPPH and to develop a stable chemically modified electrode for electroanalytical applications. It was revealed that the nitro-functional group of DPPH is involved in the electrochemical redox reaction on the GO surface, rather than the radical site, with a specific surface-confined pair of redox peaks at $E^0 = 0±0.1$ V (Ag/AgCl) in pH 7 PBS (Scheme 4.1). The surface confined material was characterized by TEM, FT IR, and Raman techniques. The DPPH chemically modified electrode is designated as GCE/GO@DPPH-NH/NH₂. This new redox system showed low potential, efficient and selective electrocatalytic activity towards NADH.

4.2 Experimental Section

4.2.1 Chemicals and Reagents

Graphene oxide-ethanol dispersed stock solution (5 mg mL⁻¹, ~80% carbon basis flake size-0.5–2.0 μm, thickness-0.6–1.2 nm, purity-99%), ethanol (94%), DPPH, pristine-MWCNT (>90% of carbon basis), f-MWCNT (50–70% carbon basis), graphite nanopowder (GNP, size~400nm, 98% purity), graphitized mesoporous carbon (GMC, 99.95%) and NADH (92% pure) were obtained from Sigma Aldrich. The other chemicals, such as NaH₂PO₄ and Na₂HPO₄ were ACS-certified reagents and used without further purification. The aqueous solutions were prepared using deionized distilled water. Unless otherwise stated, pH 7 PBS of ionic strength, $I = 0.1$ M, was used as the
supporting electrolyte. Since dissolved oxygen did not influence the present electrochemical system, all experiments were performed with dissolved oxygen.

4.2.2 Instrumentation

The voltammetric measurements were carried out with a CHI Model 660C electrochemical workstation (USA). The three-electrode system consisted of a GCE or its CMEs as the working electrode (0.0707 cm²), Ag/AgCl as the reference electrode and platinum wire as the auxiliary electrode. Transmission electron microscopy (TEM) experiments were carried out using a Technai, G2 20 Twin FEI instrument (Czech Republic). FTIR analysis was done with a JASCO 4100 Spectrophotometer (Japan) using the KBr method. Raman spectroscopy analysis was performed with a AZILTRON, PRO 532 (USA) using a 532 nm laser excitation source.

4.2.3 Preparation of the Modified Electrode

Initially, the GCE was polished with alumina powder and electrochemically pretreated in the potential window of -0.4 to 1.2 V at a scan rate (ν) of 50 mV s⁻¹. Five times diluted (ethanol) commercial GO solution was used as the stock solution for all of the experiments. The GCE/GO was prepared by casting 5 μL of the GO stock solution on a cleaned GCE surface, followed by 5±1 minutes of air-drying at room temperature (25±2 °C). GCE/GO@DPPH-NH/\textsubscript{2} was prepared by drop casting 3 μL of a dilute DPPH solution (5 mg DPPH dissolved in 500 μL distilled water) on GCE/GO (i.e., GCE/GO@DPPH\textsubscript{ads}, ads=adsorbed), followed by 10±0.5 minutes of air-drying at room temperature and cycling in the potential window of -1 to +1 V at ν = 50 mV s⁻¹ for 20 cycles in pH 7 PBS (optimal) (Scheme 4.1).

The following scheme 4.1 reveals the mechanism of DPPH modification on graphene oxide and its functional changes which is confirmed by the characterization techniques.
Scheme 4.1 Illustrations for the Preparation of GCE/GO@DPPH-NH/NH₂ Electrode: Structure of GO (A), GCE/GO (B), GCE/GO@DPPH_{ads} (C), GCE/GO@DPPH-NHOH (D), GCE/GO@DPPH-NH (E), and GCE/GO@DPPH-NH₂ (F). Steps Between (E) and (F) Represent Redox Reactions between Imine and Amine Groups of GCE/GO@DPPH-NH/NH₂ Electrode. (G) Represents Electro-Inactivity of GCE/DPPH_{ads} (control)
4.3 Results and Discussion

4.3.1 Electrochemical Behavior of DPPH on GCE/GO Modified Electrode

The initial DPPH electrochemical experiments were carried out with an unmodified GCE. In Fig. 1B, curve a represents twenty continuous CV responses of the DPPH casted GCE, i.e., GCE/DPPH$_{ads}$, in pH 7 PBS at $v = 50$ mV s$^{-1}$. The response observed with the GCE/DPPH$_{ads}$ is qualitatively similar to that of the CV response of the unmodified GCE in pH 7 PBS (Fig. 4.1B, curve c), indicating the electro-inactive nature of the DPPH on the GCE (Fig. 4.1B, curve a). This is the reason for the lack of research into DPPH based CMEs in the literature.

![Graph A](image1.png)

**Fig.4.1** (A) Continuous CV responses of GCE/DPPH$_{ads}$ (a) and GCE/GO@DPPH$_{ads}$ (b) in pH 7 PBS. (B) Continuous CV responses of GCE/GO@DPPH-NH$_2$/NH$_2$ Electrode and its Stability in pH 7 PBS. In Fig. 1(a) and (b) curve c: CVs of GCE in pH 7 PBS ($v = 50$ mV s$^{-1}$)

Note that the irreversible peak observed at an anodic potential of $E_{pa} = -0.5$ V in curve a of Fig. 4.1a is due to the dissolved oxygen reduction reaction. Interestingly, when the
DPPH experiment was carried out with the GCE/GO underlying electrode, i.e., GCE/GO@DPPH\textsubscript{ads}, a cathodic peak at $E_{pc} = -0.7 \pm 0.05$ V (C1), followed by a pair of redox peaks (A2/C2) with $E^0 = 0 \pm 0.1$ V, were observed (Fig. 4.1A, curve b). These redox peak currents increased with increasing cycle number up to 20 cycles, after which the saturation of the peak current response was observed.

![Graphs](image_url)

**Fig. 4.2** (A) Effect of $v$ on the CV Response of GCE/GO@DPPH-NH/NH$_2$ in pH 7 PBS. (B) Plot of Anodic ($i_{pa}$) and Cathodic ($i_{pc}$) Peak currents vs $v$. (D) Plot of $E_{pa}$ and $E_{pc}$ vs. log $(v)$.(D) Effect of Solution pH on the CV of GCE/GO@DPPH-NH/NH$_2$ at a Fixed $v = 50$ mV s$^{-1}$. (E) Plot of $E_{pa}$ vs pH

After continuous cycling, when the modified electrode (GCE/GO@DPPH-NH/NH$_2$) was gently washed with distilled water and transferred to fresh pH 7 PBS for the CV
measurements, the redox peaks were retained without any alteration in the peak current and peak potentials, indicating the appreciable stability of the CME (Fig. 4.1B, curve b). When analyzing the effect of the scan rate on the redox peaks, a systematic increase in the peak currents ($i_{pa}$ and $i_{pc}$) was observed with increasing $v$ (Fig. 4.2A).

In these systems, when the electrodes were potential cycled in a window of approximately, -0.1 to 0.6 V (Ag/AgCl) with a millimolar quantity of DPPH, a pair of quasi-reversible redox peaks appeared at $E^{0r} = 0.310\pm0.05$ V (Ag/AgCl) at pH 7 (semi-aqueous/organic medium), which is due to the redox reactions associated with the nitrogen radical species of the DPPH molecule. Surprisingly, no such redox peaks with $E^{nr}$ at around 0.310 V were noticed when GCE/GO@DPPH$_{ads}$ was used as the working electrode in pure pH 7 PBS in this work (Fig. 4.1A and B). It is likely that the surface-confined redox peaks noticed at $E^{nr} = 0\pm0.1$ V on GO in the present work have a structural origin, which is distinctly different from the native DPPH radical-based redox system.

To identify the redox system responsible for the redox peaks at $E^{0r} = 0\pm0.1$ V on the modified GO electrode surface, freshly prepared GCE/GO@DPPH$_{ads}$ electrodes were subjected to continuous potential cycling experiments with different lower and upper potential limits (Fig. 4.3). In the first set of experiments, the starting potential was fixed at 1 V and various lower potential limits were used, viz. -0.6 (a), -0.7 (b), -0.8 (c), -0.9 (d), and -1 V (e). Fig. 4.3A shows that only when the negative potential limit was swept to -0.9 V and beyond, comprising the C1 peak, were specific A2/C2 redox peaks observed. On the other hand, experiments with the same lower potential at -1 V and with various upper potentials, namely 0.6 (a), 0.7 (b), 0.8 (c), 0.9 (d), and 1.0 V (e), showed the A2/C2 peaks in all cases, but with different peak intensities. Two points become clear from these results: (1) DPPH failed to show any faradaic response at $E^{nr} = 0.310\pm0.1$ V on the GO surface (Fig. 4.3), unlike on the GCE and MWCNT electrodes in a semi-organic medium (Zhang et al., 2017; Amatotangchai et al., 2012), indicating that the native DPPH radical-based redox system is inactive on the GO surface; (2) the A2/C2 redox pair with $E^{nr} = 0\pm0.1$ V on the GO electrode appeared only when the more negative cathodic peak C1 occurred at ~ -0.7 V, indicating that the redox reactions of the A2/C2 peaks are closely associated with the C1 peak reduction products.
Nitrobenzene derivatives are reported to undergo irreversible electroreduction at about -0.7 V (Ag/AgCl) forming hydroxyl amine, followed by an electrooxidation addition reaction (at ~ -0.5 V (Ag/AgCl)) to form the corresponding imine derivative (37,38). It has been reported that the later compound is involved in a proton-coupled electron-transfer reaction to form the corresponding amine at about $E^0 = 0$ V (Ag/AgCl) in pH 7 PBS (Madhu et al., 2014). Fig. 4.4 shows the typical CV response of a dilute nitrobenzene-ethanol mixture drop-casted GCE/GO (GCE/GO@nitrobenzene$_{ads}$) in pH 7 PBS, exhibiting results similar to those reported. It is interesting to note that the electrochemical behavior of DPPH on GCE/GO is found to be similar to the electrochemistry of GCE/GO@nitrobenzene$_{ads}$ (Thirumalraj et al., 2014). Based on this information, it is speculated that the imine/amino benzene redox groups of the
electrochemically immobilized DPPH embedded on the GO surface furnished the surface-confined redox peaks at $E^0' = 0$ V (Ag/AgCl) in this work (i.e., GCE/GO@DPPH-NH/NH$_2$).

![Graph](image)

Fig. 4.4 CV of Electrochemical Response of GCE/GO@Nitrobenzene$_{ads}$ in pH 7 PBS at $v = 50$ mV s$^{-1}$

As the amount of the redox active product formed on the GO surface is very low (~ng), being in the composite stage with GO, it is highly difficult to adopt conventional characterizations, such as NMR and mass spectroscopy, to identify the surface product. Therefore, several other physicochemical characterizations of GCE/GO@DPPH-NH/NH$_2$ were done to identify the products formed on the surface.

4.3.2. Physicochemical Characterization of GCE/GO@DPPH-NH/NH$_2$

Fig. 4.5 shows a TEM photograph of GO@DPPH-NH/NH$_2$. Black colored nano-sized spots embedded on a folded sheet like structure were observed. The immobilization of the DPPH electro-reduced product/s on the GO surface, which has a blank sheet like morphology (Zhang et al., 2010), can be inferred.
Fig. 4.5 TEM Image of GO@DPPH-NH/NH₂

Fig. 4.6A shows the comparative Raman spectral response of the GO electrode (a) and GO@DPPH-NH/NH₂ modified electrode (b). In both cases, the characteristic graphitic peaks, namely the D and G bands at ~1350 and 1550 cm⁻¹ corresponding to the disordered sp³ and sp² carbons (Stankovich et al., 2007) respectively, were observed. The intensity ratio of the peaks, $I_D/I_G$, can be taken as a measure of the degree of surface-modification of DPPH-NH/NH₂ on the GO. The calculated $I_D/I_G$ ratios for the unmodified GO and GO@DPPH-NH/NH₂ were 0.46 and 0.55 respectively. The higher $I_D/I_G$ ratio observed with GO@DPPH-NH/NH₂ over the unmodified GO indicates the immobilization of the sp² carbon-containing aromatic units, similar to that resulting from the adsorption of DPPH on the GO. The FTIR spectrum of GO (Fig. 4.6B curve a) shows marked vibrational bands at 3450, 2750, 1620 and 1420 cm⁻¹. This indicates the presence of functional groups, such as carboxyl (–COOH), carbonyl (–C=O) and hydroxyl (–OH) groups, on the GO surface (Acik et al., 2011). In the case of GO@DPPH-NH/NH₂, specific IR bands at 1270, 1600 and 700 cm⁻¹ corresponding to the stretching vibrations of –NH₂ and –C-N, and wagging of –NH₂, respectively were observed (Ali et al., 2017). These observations provide evidence of the surface modification of GO by DPPH in the form of amino-functional groups upon the electrochemical synthesis on the GCE/GO surface, as given in Scheme. 4.1.
4.3.3 Effect of Carbon on Modified Electrode Formation

The nature of the graphitic structure and oxygen functional groups of carbon are expected to influence the electrochemical features of DPPH. In order to examine this influence, various carbon nanomaterials, viz. pristine-MWCNTs (containing ~4% metal impurities and 2% carbonaceous impurity) (Acik et al., 2011), oxygen functionalized-MWCNTs, graphite nanopowder, and graphitized mesoporous carbon, were studied by coating them on the GCE and thensubjecting them to potential cycle electrochemical experiments with DPPH, as shown in Fig. 4.1A, curve b for the GO-coated GCE. Fig. 4.7A-E show the typical CV responses of DPPH immobilized on different carbon-modified GC electrodes in pH 7 PBS in the optimal potential window of -1 to 1 V at v = 50 mV s⁻¹. The base-line corrected peak current values were taken as a quantitative parameter to compare the influences of the carbon materials on the electro-activity of the DPPH. Fig. 6F shows a plot of i_{pa} vs the respective GCE/carbon@DPPH-derivative modified electrodes. As can be seen, the pristine MWCNT-, GMC-, and GNP-modified electrodes showed negligible A2/C2 redox peak generation, whereas the oxygen functionalized MWCNTs and GO (containing rich oxygen functional groups, such as –OH, -C-O-C and –COOH) showed
appreciable A2/C2 redox peak formation. The following conclusions can be drawn from the observations: (i) the metal and carbonaceous impurities in the MWCNT did not have any influence on the immobilization of DPPH and its electro-activity; (ii) the oxygen functional groups on the graphitic structure of the carbons have a strong effect on the A2/C2 redox peaks. It is likely that the –OH and –COOH groups on the f-MWCNTs and GO have strong hydrogen-bonding interactions with the trapped DPPH-NH/NH$_2$, thereby stabilizing and enhancing the redox activity of the modified electrode.

**Fig. 4.7** Effect of Carbon-Type on the Preparation of DPPH-NH/NH$_2$: (A) MWCNT, (B) GMC, (C) GNP, (D) f-MWCNT, and (E) GO in pH 7 PBS at $v = 50$ mV s$^{-1}$. (F) Comparative Bar Graph of Anodic Peak Current ($i_{pa}$) vs Modified Electrode. MWCNT=multiwalled Carbon Nanotubes, GMC=Graphitized Mesoporous Carbon, f-MWCNT= Functionalized Multiwalled Carbon Nanotubes, GNP=Graphite Nano Powder; GO=Graphene Oxide
4.3.4. Electrocatalytic Oxidation and Sensing of NADH

Fig. 4.8A shows the CV responses of the different electrodes in the presence of NADH in pH 7 PBS at \( v = 10 \text{ mV s}^{-1} \). With the GCE/GO@DPPH-NH/NH2 electrode, a profound NADH oxidation starting from \(-0.25 \pm 0.05\) V with a peak current maximum centered near 0 V was observed, particularly at low NADH concentrations. When the NADH experiments were repeated with unmodified GCE/GO (Fig. 4.8A, curve c) and GCE (Fig. 4.8A, curve b) in the same potential window, no NADH oxidation was observed. These observations clearly suggest the efficient electrocatalytic activity of the GCE/GO@DPPH-NH/NH2 electrode for NADH oxidation. The effect of the scan rate on the NADH oxidation showed a regular increase in the oxidation peak current with increasing scan rate (Fig. 4.8B). The plot of \( i_{pa} \) vs \( v^{1/2} \) was linear, passing through the origin, suggesting the diffusion-controlled electron-transfer mechanism for the NADH oxidation at the GCE/GO@DPPH-NH/NH2 electrode (Fig. 4.8C). This is typical of an ECc regeneration mechanism and the electrooxidation of NADH can be expressed as follows:

\[ \text{Electrochemical Oxidation Reaction} \]
**Table 4.1** Comparative Chemically Modified Electrodes for the Electrochemical Detection of NADH along with Sensitivity and Detection Limits Reported.

<table>
<thead>
<tr>
<th>CME</th>
<th>$E^{0'}$ (V)</th>
<th>pH</th>
<th>Sens. (µA µM⁻¹ cm⁻²)</th>
<th>Tech.</th>
<th>L. R (µM)</th>
<th>Interf.</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 FeNP-N/GO</td>
<td>0.35</td>
<td>7</td>
<td>0.028</td>
<td>Amp i-t</td>
<td>0.4—718</td>
<td>H₂O₂</td>
<td>(Balamurugan et al., 2016)</td>
</tr>
<tr>
<td>2 SWCNT-Polymersine</td>
<td>0.20</td>
<td>7.4</td>
<td>0.217</td>
<td>CV</td>
<td>15—0.83</td>
<td>---</td>
<td>(Eguilaz et al., 2016)</td>
</tr>
<tr>
<td>3 rGO/MB/AgNP</td>
<td>0.29</td>
<td>9</td>
<td>0.161</td>
<td>CV</td>
<td>0.25—400</td>
<td>H₂O₂</td>
<td>(Balamurugan et al., 2010)</td>
</tr>
<tr>
<td>4 PDDA-rGO</td>
<td>0.24</td>
<td>7</td>
<td>0.463</td>
<td>CV</td>
<td>10—2900</td>
<td>AA</td>
<td>(Lu et al., 2016)</td>
</tr>
<tr>
<td>5 Pt/Fe₃O₄/rGO</td>
<td>0.50</td>
<td>7</td>
<td>0.108</td>
<td>CV</td>
<td>300—3000</td>
<td>Glucose (Roushani et al., 2016)</td>
<td></td>
</tr>
<tr>
<td>6 SWCNT-rGO</td>
<td>0.50</td>
<td>7-8</td>
<td>0.026</td>
<td>CV</td>
<td>5—800</td>
<td>Hyd</td>
<td>(Adhikari et al., 2017)</td>
</tr>
<tr>
<td>7 AZ@MWCNT</td>
<td>-0.06</td>
<td>7</td>
<td>0.078</td>
<td>CV</td>
<td>0—7000</td>
<td>H₂O₂</td>
<td>(Swetha and Kumar, 2012)</td>
</tr>
<tr>
<td>8 NP/C-dots</td>
<td>0.5</td>
<td>7</td>
<td>0.150</td>
<td>CV</td>
<td>20—5</td>
<td>AA, DA, UA</td>
<td>(Canevari et al., 2017)</td>
</tr>
<tr>
<td>9 Au-AgNP/p(L-cys)-rGO</td>
<td>0.35</td>
<td>7</td>
<td>0.020</td>
<td>Amp i-t</td>
<td>17—1800</td>
<td>---</td>
<td>(Tig, 2017)</td>
</tr>
<tr>
<td>10 Co₃O₄</td>
<td>0.1</td>
<td>6</td>
<td>0.021</td>
<td>CV</td>
<td>10—100</td>
<td>UA</td>
<td>(Chen et al., 2013)</td>
</tr>
<tr>
<td>11 GO/DPPH- -NH/NH₂</td>
<td>0</td>
<td>7</td>
<td>0.168</td>
<td>CV</td>
<td>10—2000</td>
<td>-nil-</td>
<td>This work</td>
</tr>
</tbody>
</table>

NP= Nano particles, FeNP= Iron nanoparticle, SWCNT=Single walled Carbon nanotubes, rGO=Reduced graphene oxide, AgNP= Silver nanoparticles, MB=Methylene blue, PDDA= Poly diallyldimethyl ammonium chloride, Pt=Platinum, Fe₃O₄= Iron Oxide Nanoparticles, Co₃O₄=cobalt oxide nanoparticles, C-dots= Carbon Quantum dots, MWCNT= Multiwalled carbon nanotubes, L-Cys = L-Cysteine, MB=Meldola blue, AZ = Alizarin, DA= Dopamine, AA= Ascorbic acid, UA= Uric acid, Hyd-hydrazine.
When the effect of the NADH concentration was analyzed, a linear increase in the anodic peak current was observed with current sensitivity and regression coefficient values of 11.87 µA mM⁻¹ (0.168 µA µM⁻¹ cm⁻²) and 0.9975, respectively (Inset Fig. 4.8A). Table 4.1 shows the comparative data for the electrochemical oxidation of NADH at different representative CMEs reported in the literature. As can be seen, the newly developed GCE/GO@DPPH-NH/NH₂ electrode showed linear range and detection limits comparable to the CMEs, such as SWCNT-Polytyrosine (Eguilaz et al., 2016), PDDA-rGO (Lu et al., 2016), and Co₃O₄ nanosheet (24). On the other hand, the present modified electrode showed enhanced performance than the CMEs based on Pt/Fe₂O₃/rGO, magnetically confined NP/C-dots. Au-AgNP/p(L-Cys)-rGO (Tig, 2017), etc. It is noteworthy that most of the CMEs listed in Table 1 showed the oxidation peak for NADH located at peak potentials in the range, 0.1—5 V (Ag/AgCl), in pH 7 PBS, whereas the GCE/GO@DPPH-NH/NH₂ electrode of the present work showed the NADH oxidation peak centered at around 0 V (Ag/AgCl) with significant electroanalytical performance. Fig. 4.9A shows the amperometric i-t response of the GCE/GO@DPPH-NH/NH₂ electrode at an applied potential of 0 V (Ag/AgCl) upon spiking 50 μM (final concentration) of NADH in pH 7 PBS. A systematic increase of the current signals when increasing the number of spikes of NADH was observed. The calibration plot was linear in the concentration range of NADH of 50 to 450 μM with regression coefficient and sensitivity values of 0.9968 and 34.48 nA μM⁻¹, respectively. The control experiments of the amperometric i-t sensing of NADH on GCE/GO showed an approximately ten times lower current sensitivity than that of the GCE/GO@DPPH-NH/NH₂ electrode. Note that the current sensitivity obtained in this work is markedly higher than the values obtained on various carbon nanomaterial based electrodes (Balamurugan et al., 2016; Tig, 2017).

The influences of some important interfering biochemicals such as ascorbic acid (AA), hydrazine (Hyd), glucose (Glu), cysteine (Cys), citric acid (CA), nitrate (NO₃⁻) and uric acid (UA), were studied (Fig. 8B). As can be seen in the figure, there were no significant alterations in the current signals upon spiking with these biochemicals, unlike the conventional CMEs, such as FeNP-N-doped GO (Balamurugan et al., 2016), rGO/MB/AgNPs (Balamurugan et al., 2010), PDDA-rGO (Lu et al., 2016), Pt/Fe₂O₃/rGO (Roushani et al., 2016), AZ@MWCNT (Swetha and Kumar, 2012), magnetically confined
NP/C-dots (Caneveri et al., 2011), and Co₃O₄ nanosheets (Acik et al., 2011), which showed marked interferences from the above tested biochemicals, indicating the highly selective sensing feature of the GO/DPPH modified electrode. This was further evidenced from a separate amperometric i-t experiment, in which a pH 7 PB solution containing a mixture of all the above interferants (each at 50-μM concentration) was titrated successively with 50 μM of NADH (Fig. 4.9C). The NADH sensing current in successive measurements was highly reproducible. Indeed, the current measured for 50-μM NADH in the interferent-containing pH 7 PB solution is close to the current value measured for the same NADH concentration (50 μM) in interferent-free pure pH 7 PB solution. This particular behavior suggests conclusively that the GCE/GO@DPPH-NH/NH₂ electrode is highly selective for NADH with respect to the other compounds, which makes an amperometric sensor electrode with complete freedom from the above electroactive interference species.

**Fig. 4.9** Comparative Amperometric i-t responses of (A) GCE/GO@DPPH-NH/NH₂ (curve b), GCE/GO (curve a) at E_{app} = 0 V vs Ag/AgCl for Sensing of 50 μM of NADH Additions in a Stirred Solution. Inset is the Calibration Plot of Current vs [NADH]. (B) Effect of Interference of Various Biochemicals (Glucose, Nitrate, Citric acid, Hydrazine, Ascorbic acid, Uric acid and Cysteine; 50 μM each) on the Amperometric i-t Response of GCE/GO@DPPH-NH/NH₂. (C) Successive additions of 50-μM NADH at GCE/GO@DPPH-NH/NH₂ in a pH 7 PBS containing all the above interferents each at 50 μM.
4.3.5 Real Sample Analysis of NADH

An antiaging cream, diluted with pH 7 PBS and tap water at a 1:4 ratio, was tested as a real sample system for the NADH analyte by a standard addition method using the GCE/GO@DPPH-NH/NH$_2$-modified electrode. NADH is referred to as a key antiaging chemical in cosmetics. NADH is believed to stimulate and boost the human immune system and repair cells, tissues, and most importantly enhance the production of important brain chemicals, such as dopamine. Fig. 4.10 is a typical amperometric i-t response of the real sample, where R denotes the real sample and R + S$_n$ denotes the real sample + added standard concentration of NADH (n = 1, 2, 3). A variable current sensitivity was observed against the standard (Fig. 4.10) due to the matrix in the real system. Table 4.2 lists the analytical data obtained with real sample analysis. The values obtained were found at the micromolar level with good recoveries. In both cases, a recovery of $100 \pm 5\%$ was observed, indicating the potential of the present system to various other real sample analysis.

Table 4.2 Real sample calculation of NADH by standard addition method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Analysis result</th>
</tr>
</thead>
<tbody>
<tr>
<td>R+S1</td>
<td>R+S2</td>
</tr>
<tr>
<td>1. Linear Equation</td>
<td>Y = 0.1500 + 0.0430x</td>
</tr>
<tr>
<td>2. Regression</td>
<td>0.995</td>
</tr>
<tr>
<td>3. Original Detected (µM) (R)</td>
<td>15.5</td>
</tr>
<tr>
<td>4. Added (µM)</td>
<td>50 100 150</td>
</tr>
<tr>
<td>5. Found (µM)</td>
<td>51.5 100.9 152.1</td>
</tr>
<tr>
<td>6. Recovery (%)</td>
<td>102 92.6 101.5</td>
</tr>
</tbody>
</table>

Therefore, taking into account of the sensor activity of the GO-DPPH-modified electrode, some specific molecular interactions with strong bonding between NADH and the hydrogen-bonded GO@DPPH-NH/NH$_2$ network, along with a proper tuning of the NH/NH$_2$ redox potential (i.e., 0 V (Ag/AgCl)) by the particular method of electrode
preparation adopted in this work, appear to confer strong electrocatalytic activity to achieve a stable low potential NADH detection, good selectivity, and significant sensitivity.

**Fig. 4.10** Real Sample Analysis of NADH in an Anti-aging cream using GCE/GO@DPPH-NH/NH₂ Electrode by Standard Addition Approach. Other Conditions are as in Fig. 4.9

4.4 Conclusion

The electrochemical potential cycling of a DPPH adsorbed GCE/GO modified electrode, i.e., GCE/GO@DPPH_{ads}, in the potential window of -1 to +1 V (Ag/AgCl) resulted in the successive reduction of the DPPH molecule’s nitro group to hydroxylamine and then to the respective imine-functional group in pH 7 PBS. The resulting GCE/GO@DPPH-NH/NH₂ modified electrode showed well-defined surface-confined redox peaks with \( E^0 \) = 0 V (Ag/AgCl), due to the proton-coupled electron-transfer involving the imine/amine functional groups on the surface. The redox peaks were found to be stable upon multiple potential cycling and over the solution pH range of 2-11. Physicochemical characterization by TEM, Raman, and IR techniques revealed the immobilization of the amine functional groups on the modified electrode surface. The modified electrode showed efficient electrocatalytic activity towards NADH oxidation with the peak current
maximum located near the $E^0$ of the modified electrode (0 V (Ag/AgCl)). No such beneficial electrochemical response was observed when GCE and GCE/GO were used as the working electrodes for NADH oxidation. The amperometric i-t sensing of NADH was demonstrated with current sensitivity and linear detection range values of 34.48 nAµM$^{-1}$ and 50-450 µM, respectively. The modified electrode showed tolerable current signals in response to other interfering biochemicals such as ascorbic acid, hydrazine, glucose, cysteine, citric acid, nitrate, and uric acid, on the detection of NADH in pH 7 PBS. Since the preparation of the modified electrode is simple and the electrode is highly selective, it can be utilized for various practical applications.