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ORIGINAL ARTICLE

Electrochemical detection of dihydronicotinamide adenine dinucleotide using Al_2O_3-GO nanocomposite modified electrode

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Abstract NADH plays a vital role in the electron transfer processes between metabolites in the cellular energetic reactions. Therefore, there is a crucial need to develop analytical techniques for detecting NADH levels with the metabolism of glucose. In the present study, a nanocomposite of alumina (Al_2O_3) nanoparticles confined graphene oxide (GO) sheet acts as a modifier for carbon paste electrode (CPE) for a sensitive detection of NADH level in a mediator-less detection scheme. Our findings after optimization of experimental conditions reveal that, there is a remarkable enhancement in the direct electron transfer through the Al_2O_3-GO nanocomposite surface with high electrocatalytic activity towards NADH oxidation. Results show that, there is a linear increase in NADH detection from 30 μM to 330 μM, together with linear regression coefficient of 0.98 and LOD 4.5 μM. These results confirm that, the developed Al_2O_3-GO based CPE electrode is a promising electrode for real NADH level detection in practical enzymatic applicability.

1. Introduction

Dihydronicotinamide adenine dinucleotide (NADH) plays a very important role in the enzymatic reactions. NADH and its oxidized form NAD^+ handle hydrogen and electron transfer between metabolites in the cellular redox energetic reactions. Moreover, they are the coenzymes for numerous dehydrogenase enzymes and components of biomarker systems. Several studies showed that, NADH is essential for the

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regeneration of glutathione following its oxidation where the levels of glutathione changed dramatically based on NADH levels (Limoges et al., 2006). Furthermore, NADH behaves as a crucial co-factor of other enzymes in biological, clinical and pharmaceutical samples (Wei et al., 2008, Wu et al., 2007). Thus, there is a high demand to develop suitable analytical techniques that helps in sensitive screening and detecting NADH level along with the metabolism of glucose, which considered among the important detections of diabetic and cancer cells (Moradi et al., 2013). However, the direct oxidation of NADH occurs associated with high overpotential along with non-desired oxidation products. That leads to surface fouling of the used sensor and hence decreases the detection sensitivity.

So far, many research trials focused on NADH detection, including electrochemical investigations (Serban and Murr, 2004, Lee and Compton, 2013), analytical methods based on colorimetric (Liu et al., 2012), photo-electrochemical (Li et al., 2014, Wang et al., 2009), enzymatic assay (Ricci et al., 2007) and chemiluminescence techniques (Downey and Nieman, 1992, Devadoss et al., 2012). Among those techniques, electrochemical methods received significant considerations due to their high sensitivity, fast response, ease of sample preparation and lower cost (Chen and Shah, 2013). Much research effort directed towards new materials which can help to develop and improving the electrochemical biosensors based on composite electrode for more sensitive and rapid response results (Kimmel et al., 2012).

In recent years, design of graphene based nanomaterials received much interest towards their applicability as electrocatalysts for NADH oxidation without the aid of redox mediators (Li et al., 2014). Zhang and co-workers (Zhang et al., 2011) reported about the non-mediated use of screen printed carbon electrode (SPCE) which was modified using graphene oxide to detect NADH in a neutral solution with the concentration ranging from 0.8 to 500 μM and the detection limit of 0.10 μM. On the other hand, Lin and co-workers (Lin et al., 2014) reported about the detection of NADH using graphene oxide and multi-walled carbon nanotube (GO-MWCNT) composites via hybridization of poly-luminol and poly-neutral red as electron mediators with a linear range from 1.33×10^{-8} to 1.95×10^{-4} M and a detection limit of 1.33×10^{-8} M. Most recently, Roushani and co-workers (Roushani et al., 2016) reported about using the ternary composite of metallic Pt, Fe_3O_4 metal oxide and graphene oxide for NADH detection using a glassy carbon electrode with a detection limit of 5 nM. However, to date, there are no reports for NADH detection using non-mediated Al_2O_3/GO nanocomposite modified carbon paste electrodes.

Graphene oxide (GO) consists of a 2*D* single layer of sp^2-hybridized carbon atoms and possesses unique features such as high surface area, superior charge transport at room temperature, outstanding mechanical stability, easiness of mass production and functionalization. However, some of these unique features can be deteriorated due to its ease of aggregation and poor solubility which hinder its real applicability in electrochemical biosensors. To overcome these limitations, the oxide containing groups in GO can be used for surface anchoring of noble metals and/or metal oxide particles to enhance the electrochemical sensing ability (Novoselov et al., 2012). In other words, it is necessary to modify GO surface to have a multifunctional composite which possesses both superior properties of graphene as well as its functionalizing materials (Shao et al., 2010).

Nano-structured metal oxides have been extensively applied for the energy and environmental applications due to their high surface area, good biocompatibility and catalytic activity, chemical stability and relatively low toxicity (Pratima et al., 2011). Although, some metal oxides possess moderate electrical conductivity, having them as a combined nanocompsite electrochemical biosensor with GO is expecting to help reducing the produced overpotential from the investigated analyte and decreasing the current density. In this regard, several research works have been done by our group and others using metal oxide/graphene oxide nanocomposites (MO/GO) for the non-enzymatic detection of H_2O_2 (Salih et al., 2016, Li et al., 2010, Huang and Li, 2013), and monitoring of the bioelectrochemical response from the stimulated living microbial cells (Rabeay et al., 2017).

The aim of present study is to reveal the real applicability of novel Al_2O_3-GO nanocomposite modified carbon paste electrode (CPE) to be used in the real detection of NADH level in a mediator-less detection scheme. To the best of our knowledge, there are no reports in literature on the detection of NADH using Al_2O_3-GO nanocomposite. Advantages of this innovative method include (i) an easy and fast synthesis strategy; (ii) formation of uniform Al_2O_3 nanoparticles-GO nanocomposite, where GO has thick layer, and (iii) enhancement of the direct electron transfer through the composite surface groups, where Al_2O_3-GO nanocomposite shows high electrocatalytic activity and stability for NADH oxidation without any need for additional surface modification. This exhibits an excellent performance for the sensitive detection of NADH.

2. Experimental

2.1. Materials

Graphite flakes, sulfuric acid (H_2SO_4), potassium permanganate ($KMnO_4$), Alumina (Al_2O_3), phosphoric acid (H_3PO_4), hydrochloric acid (HCl), ethanol, alcohol dehydrogenase from *Saccharomyces cerevisiae* and NAD^+ were purchased from Sigma-Aldrich., Tris-Base and Tris-HCl were purchased from Fisher Scientific. Miller Lite Highlife beer was purchased from local liquor store in Riverside, California, USA.

2.2. Synthesis of graphene oxide (GO)

GO was synthesized using modified Hummer's method (Marcano et al., 2010) where 75 ml of a concentrated mixture H_2SO_4/H_3PO_4 (4:1 vol ratio) was added to 3.0 g of purified natural graphite in a round bottom flask. 10.5 ml $KMnO_4$ were added to the flask under temperature preservation at 4 °C. The mixture was subjected to control stirring for 12 h while increasing slowly the temperature to 60 °C. Afterwards, 100 ml of deionized water was added to the mixture, followed by further addition of 5 ml of 30% H_2O_2. The yield precipitate of GO was collected, washed thoroughly with 500 ml of 1.0 M HCl using vacuum filtration and subjected to additional washing with ethanol and deionized water. Finally, the suspended GO was

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