

AN ELECTROCHEMICAL BIOSENSOR DEVELOPED FOR THE ONLINE MONITORING OF H₂O₂ BASED ON THE REDUCED GRAPHENE OXIDE-CERIUM DIOXIDE NANOCOMPOSITE

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Abstract

A biosensor was developed for monitoring H₂O₂ based on reduced Graphene Oxide-Cerium Oxide (rGO-CeO₂) nanocomposite (NC). The NC shows catalytic effect on the electrochemical reduction of H₂O₂ at -0.3 V. The effect of the NC on the electrochemical reduction of H₂O₂ was studied by cyclic voltammetry and compared with bare glassy carbon (GC) and GC/r-GO. Moreover, to improve the signal a complex containing copper was incorporated in the dispersion of the nanocomposite which acts as a mediator. It was found that the electrochemical reduction of H₂O₂ takes place at less negative potential with increased peak current attitude at GC/r-GO-mediator. The developed sensor GC/r-GO-mediator, was applied for the continuous monitoring of H₂O₂ by chronoamperometry. The applied potential was -0.3 V which results in the enhanced sensitivity of the developed biosensor. The developed biosensor shows good stability, and reproducibility. The reproducibility of the developed electrode was evaluated by calculation of relative standard deviation value 6.77%. The linear range was found to be 3.4 - 23.48 µg/ml. Furthermore, limit of detection and limit of quantification were 0.68 µg/ml and 2.27 µg/ml respectively.

Keywords: Nanocomposite, reduced graphene oxide-cerium dioxide, biosensor, biomimetic, chronoamperometry

1. INTRODUCTION

Hydrogen peroxide (H₂O₂) a simple molecule in nature belongs to the class of reactive oxygen species with greater significance in the field of pharmaceutical, environmental, mining, textile, food manufacturing and processing industry, clinical and diagnostic field [1]. Physiologically and pathological importance of H₂O₂ is associated with many important cellular process like signal transduction, immune cell activation, apoptosis, etc. [2,3]. H₂O₂ is widely used in the food and processing industry as preservative due to its bactericidal activities and used as sterilization in the packaging industry [4]. However excessive use of H₂O₂ in food packaging and processing is harmful for health as it acts as an adulterants leading to severe gastrointestinal problem [5]. In rainwater, H₂O₂ can be formed due to photochemical reaction and present in industrial waste to contaminate the ground and surface water, a threat to the environment [6]. Therefore, continuous monitoring and sensitive detection of H₂O₂ is important for environmental protection, food industry, clinical and diagnostics application [7-10]. In recent years, real-time monitoring of H₂O₂ is significant to study the physiological and pathological changes leading to investigate the process of oxidative stress *in vivo* and *in vitro* due to its chemical properties. Several methods like spectrophotometric, fluorimetric methods were used for the detection, analysis and estimation of H₂O₂ [11-13]. Several disadvantages like expensive chemical, require skilled technicians, extended time for the analysis and suffering from interferences are common issues with these conventional methods for continuous monitoring. Electrochemical detection is selective, sensitive,

simple, easy to use, accurate, cost-effective, and quick [6]. Moreover, amperometric methods can be applied to measure accurate kinetic information in real-time to image the continuous release of H₂O₂ [14]. The detection and estimation of H₂O₂ by using electrochemical amperometric techniques have attracted great attention for its application in biosensors and online monitoring. The development and preparation of sensors and biosensors were enriched after the discovery of the carbon nanomaterials. Different carbon nanocomposites (NC) such as single-walled carbon nanotubes, multiwalled-carbon nanotubes, graphene, graphene oxide (GO) and reduced graphene oxide (rGO) were used to promote and enhance electron transfer reaction in the development of biosensors [15,16]. Different types of sensors are used for the detection of biologically complex molecule like enzyme based biosensor, non-enzymatic sensors, nucleic acid based sensors, peptide based sensors. Enzymatic biosensors possess various challenges regarding the instability of the enzyme, immobilisation on the electrode surface leading to leakage and low effective binding on the surface of the electrode which causes low activity of the sensor. Moreover, enzymes increase the overall cost for the preparation of the sensor and put obstacles for the preparation of cost-effective sensors [17,18]. These serious challenges lead to the development of NC possessing biomimetic properties with stability, long lifetime, and cost-effective characteristics. For fast detection and successful analysis amperometric method will be relied on the applied potential. The applied potential was selected at less negative potential to achieve better sensitivity and stability of the developed sensor. The main aim of this work is to develop a non-enzymatic, cost effective, sensor for the rapid detection and analysis of H₂O₂ with higher sensitivity, stability which is cost-effective as well. A copper containing complex, tris(2,2'-bipyridyl)copper(II) dichloride was synthesized and modified accordingly to be used as a mediator together with rGO-CeO₂ to achieve sensitive analysis of H₂O₂ at low potential [19,20].

2. MATERIALS AND METHODS

Graphite flakes (100 mesh, ≥75% min), Sulfuric acid (H₂SO₄), Potassium permanganate (KMnO₄) Nafion 117 (NF), Cerium trinitrate (Ce(NO₃)₃), Sodium chloride (NaCl), Potassium chloride (KCl), Sodium phosphate dibasic (Na₂HPO₄), Potassium dihydrogen phosphate (KH₂PO₄), N,N-Dimethylformamide (DMF), Hydrogen peroxide (H₂O₂) are analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). The water (18.20 MΩ·cm) used for solution preparation was first double distilled by an Aqua Osmotic 02 (Aqua Osmotic, Tisnov, Czech Republic) and then deionized by using a Millipore RG (MilliQ water, Millipore Corp., Billerica, MA, USA). An Autolab electrochemical analyzer model PGSTAT-101 operated via NOVA 2.1 software (Metrohm Autolab, Utrecht, Netherland) was used for the voltammetry experiments. 0.01 M Phosphate buffer saline (PBS) was prepared with 0.132 M of NaCl, 0.0027 M KCl, 0.01 M of Na₂HPO₄ and 0.0018 M of KH₂PO₄ were mixed to prepare 1.0 l buffer solution. The pH was adjusted at 7.4 by 1.0 M NaOH. The entire study was conducted by using this buffer. The conventional three-electrode configuration was used in which a platinum electrode used as a counter electrode, Ag/AgCl 1.0 M KCl as the reference electrode and a modified glassy carbon electrode (GCE) served as the working electrode.

2.1 Preparation of nanocomposite (NC)

Following Hummer's procedure GO was prepared by the chemical oxidation of graphite flakes in a mixture of concentrated H₂SO₄ and KMnO₄ [21]. The modified NC of rGO-CeO₂ was synthesized after getting inspired from the previous study [22]. Modified NC with mediator was prepared after mixing 1.0 ml of rGO-CeO₂ with 0.1 mg of copper mediator (M) tris(2,2'-bipyridyl)copper(II) dichloride sonicated for 40 min.

2.2 Preparation of modified electrode

The bare GCE was polished on a polishing pad by using diamond suspension in water (0.5 micron) for 2 min followed by sonification in MilliQ water for 5 min and in absolute ethanol for another 5 min. The electrode cleaning procedure was repeated with polishing with alumina slurry of (1.0 micron) and (0.5 micron)

respectively. The polished electrode was modified by using different composition of NC. **Figure 1** presents modification of electrode by drop casting 10.0 μl of the NC on the bare electrode and allowed to be dried in room temperature (RT). 5.0 μl of 1.0% NF was drop casted and left for drying in RT. The NF was prepared by neutralization of NF using 8.0% ammonium solution. 0.1 M of H_2O_2 was freshly prepared as stock solution as analyte.



Figure 1 Preparation of modified electrode

2.3 Electrochemical measurement

The electrochemical behaviour of H_2O_2 at the developed electrode was studied by cyclic voltammetry (CV). The CV parameters were start potential 0.0 V, stop potential +0.4 V, scan rate 50 mV/s, upper vertex potential +0.5 V and lower vertex potential -1.0 V. The potential was scanned towards anodic direction. The rapid electrochemical detection of analyte was conducted by chronoamperometry at the selected applied potential. The chronoamperometric study was carried at a selected potential of -0.3 V at working electrode for 3000 s. The current directed between the working and counter electrode was sampled every 1 s.

3. RESULTS AND DISCUSSION

3.1 Study of electrochemical behaviour of H_2O_2 by CV

The CV study was conducted with bare GCE, GCE modified with rGO, GCE modified with rGO-CeO₂, GCE modified with rGO-CeO₂ and M as different NC in blank and 0.1 M H_2O_2 . As seen in **Figure 2** with addition of 250 μl of 0.1 M H_2O_2 in PBS, a broad reduction current appeared at rGO. The attitude of the reduction increased at rGO-CeO₂ indicating an improved electro catalytic properties of the synthesized NC. However, including the M into the rGO-CeO₂ as rGO-CeO₂+M resulted in a shift in the potential of the reduction peak to less negative potential and an increase in the reduction peak current. This might be due to the synergic electro catalytic and biomimetic properties of the NC along with the mediating activity that of the mediator.

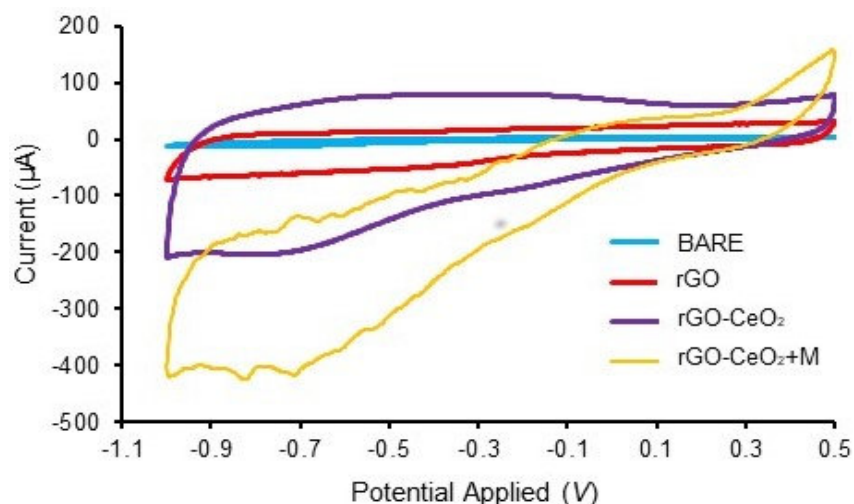


Figure 2 Electrochemical behaviour of different modified GCE using CV at 0.1 M H_2O_2

As depicted in **Figure 3** copper is first electrochemically reduced at the electrode surface from Cu(II) to Cu(I). Cu(I) in solution reduces the H₂O₂ to water while itself oxidises to Cu(II) again. Therefore, the reduction current of Cu(II) to Cu(I) is related to the amount of the H₂O₂ which reduces to water. The high surface, electro-catalytic and biomimetic properties of the NC results in an enhanced reduction current at such small potential magnitude.

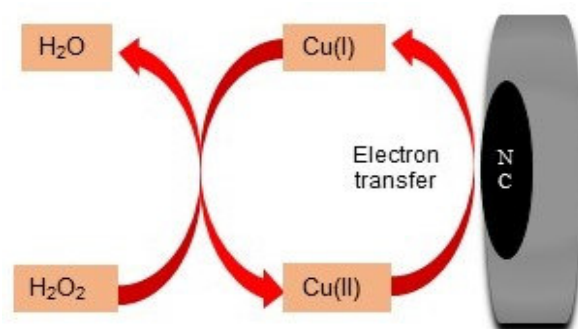


Figure 3 Expected mechanism of the NC

3.2 Amperometric study

The sensitivity of the different developed electrodes was studied at an applied potential corresponding to the reduction of H₂O₂. For comparing the sensitivity, different calibration curves obtained by the chronoamperometry at -0.3 V were plotted and the slopes of the calibration curves are represented in **Table 1**. As can be observed the highest sensitivity belongs to that of rGO-CeO₂+M.

Table 1 Detail sensitivity of different NC on GCE at -0.3 V

Nanocomposites (NC)	rGO	rGO-CeO ₂	rGO-CeO ₂ +M
Sensitivity ($\mu\text{A}\cdot\text{ml}/\mu\text{g}$)	0.067	0.801	2.956

At -0.3 V, the sensitivity was found to be highest with GCE/rGO-CeO₂+M/NF. The reproducibility of the sensor was studied by continuous injection of 20 μl of 0.1 M H₂O₂. All the experiments were carried out thrice to check the stability of the NC on the electrode surface along with the reproducibility of the sensor.

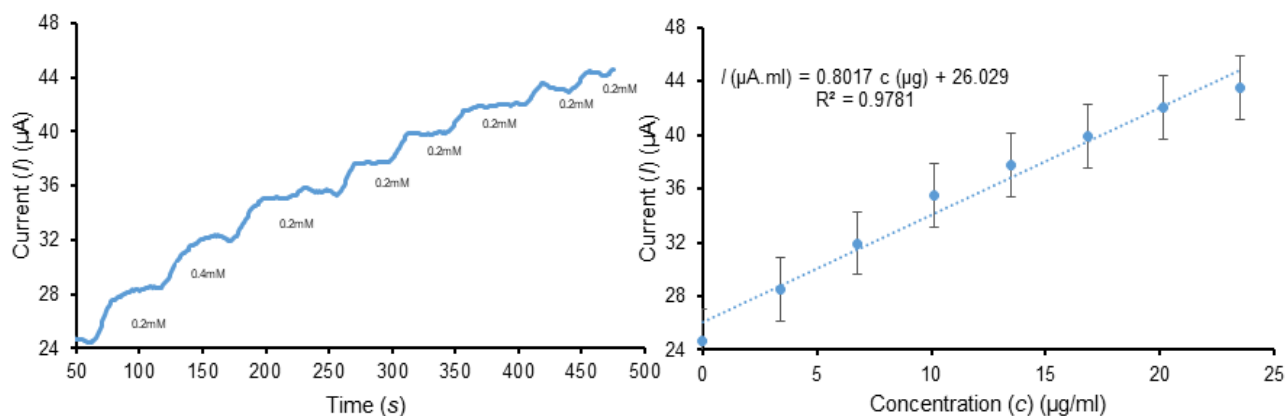


Figure 4 Chronoamperometry and calibration curve of GCE/rGO-CeO₂/NF at 0.01 M PBS

The calibration curve of GCE/rGO-CeO₂/NF and GCE/rGO-CeO₂+M/NF with their corresponding chronoamperometric study were presented in **Figure 4** and **Figure 5** respectively. A good linearity between

the reduction current and the continuous injection of H₂O₂ in the reaction buffer was observed. The sensitivity of the GCE/rGO-CeO₂+M/NF was found to be better in-comparison with the GCE/rGO-CeO₂/NF electrode. Very low response was obtained with the GCE modified with GCE/rGO/NF.

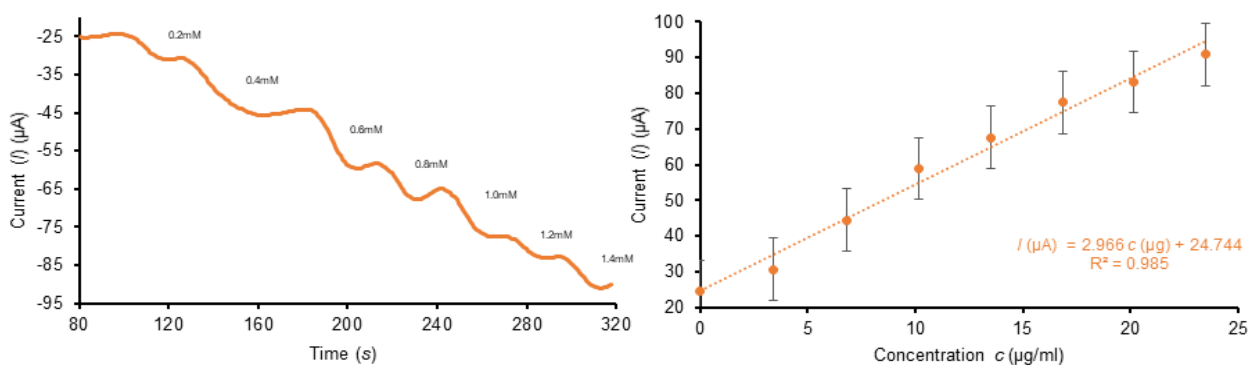


Figure 5 Chronoamperometry and calibration curve of GCE/rGO-CeO₂+M/NF at 0.01 M PBS

The limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 0.68 $\mu\text{g/ml}$ and 2.27 $\mu\text{g/ml}$ with linear range of detection 3.4 - 23.48 $\mu\text{g/ml}$. The RSD% of the modified electrode was calculated as 6.77.

5. CONCLUSION

The proposed study was conducted to develop a non-enzymatic sensor using modified carbon NC, able to detect H₂O₂ with high sensitivity at small potential magnitude. The combining effect of the carbon NC with the copper mediator increases the electron transfer capacity and electrical conductivity to acts as a better transducer material. Increase in sensitivity, higher repeatability obtained with a simple, rapid and inexpensive method were the advantages of the prepared sensor. This preliminary study confirms the biomimetic property of the modified carbon NC to be used for the fabrication, modification, detection and analysis of analyte by the sensor. For future perspective this sensor can be modified into biosensor by immobilisation of enzyme which will be able to detect some complex biological molecule as analyte. Due to low applied potential, the stability and integrity of the enzyme will remain intact and expected to perform at highest activity. The selectivity and interference study will be performed in future to check the biomimetic activity of the modified carbon NC at different conditions.

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