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Optimization of glassy carbon electrode based graphene/ferritin/glucose oxidase bioanode for biofuel cell applications

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ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form
19 February 2014
Accepted 28 February 2014
Available online 27 March 2014

Keywords:
Glassy carbon electrode
Ferritin
Glucose oxidase
Bioanode
Bifuel cells

ABSTRACT

A glassy carbon (GC)/graphene/ferritin/glucose oxidase (GOx) anode was developed by using graphene/ferritin biocomposite as an electron transfer enhancer and mediator, respectively. The electrode exhibited good electrocatalytic activity towards the oxidation of glucose. The electrocatalytic oxidation of glucose using GOx modified electrode increased with increasing the concentration of glucose upto 45 mM. The results showed that the graphene/ferritin biocomposite mediator provides enhancement in electron transfer generated at the active cites of GOx to the electrode. All electrochemical measurements were carried out by cyclic voltammetry (CV) and linear sweep voltammetry (LSV). A saturation current density of 66.5 ± 2 mA cm^{-2} at scan rate 100 mV s^{-1} for the oxidation of 45 mM glucose was achieved.

Introduction

In recent years researchers have been focused on the study and development of biofuel cells (BFC's), and their applications in various fields such as biomedical and genetic engineering and biotechnology [1]. Biofuel cells are the cells that generate electricity by an electrochemical oxidation reaction occurring at the electrodes. Biofuel cells are totally different from conventional energy systems in terms of cost effectiveness or enabling competitiveness for the small scale power supply. The biofuel cells are generating electrical energy by using renewable substances such as glucose, ethanol etc. as fuel [2–4]. Various types of enzymes are being utilized for the biocatalytic conversion of chemical energy associated with the fuel into electrical energy [5,6]. Contrarily, conventional energy systems are using expensive metal catalysts such as Pd, Pt, or Ru [5] having high redox potential and also are not specific for the conversion of particular fuel. These are the major obstacle encountered in conventional energy systems and can be resolved by using enzyme based BFCs. Biofuel cells have the possibilities to miniaturize into small power supply devices and also work closer to the redox potential of the enzyme [7]. These advantages of biofuel cells make them suitable for implantable devices such as pacemaker, cochlear implants, insulin pump, biosensors [8], drug delivery systems, nano biobatteries [9,10] *vivo* biosensor [6,11,12], and also remote sensing and communication devices in bioelectronics [13].

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http://dx.doi.org/10.1016/j.ijhydene.2014.02.171

The enzyme electrode bearing oxidase/hydrogenase enzymes on its surface have been used as an effective fuel oxidizing anode. Because of complex 3-Dimensional structure of enzymes, the electron transferring units are intensely buried inside the structures which enable a poor electrical communication between the redox active cites of the enzyme and the electrode [14,15]. To overcome this barrier, enzymes can be transformed to be conductive *via* chemical modification or by using redox mediators with conducting polymers [16,17]. These redox active conducting polymers with electron transfer mediators facilitate the transportation of electrons by shuttling between the enzyme active sites and the surface of electrodes. Graphene is the allotropes of carbon in which sp^2 bonded carbon atoms is 2D planar in structure [18]. There is no doubt that graphene has risen as a shining star [19] due to it's extraordinary properties such as electrical, mechanical, thermal and optical. The electrical connection of the enzyme with the GC electrode is growing area of research for the advancement of biofuel cells [20]. The electrical property of the graphene is utilized for making biofuel fuel cell anode [21]. The graphene is used to provide electrical communication between GC electrode and enzyme GOx. The immobilization of the enzyme and the use of redox mediators on the graphene modified GC electrode provide an efficient path to generate electron transfer between the enzyme and conductive surface of the electrode due to the presence of redox mediator.

A number of non-biocompatible redox mediators have been widely used to boost the electron transfer rate [22,23]. However, mediator molecules must also be environmentally inert so that they can be easily disposed of (with the spent cell) and/or implanted as part of a medical device with- out harming the patient. Ferritin is a redox protein which is potentially redox active, biocompatible and environmentally inert mediator and work close to the oxidation potential of enzyme [19,24]. Therefore, ferritin is utilized as electron transfer mediator to the electron generated by the oxidation of fuel from the active cites of GOx to the GC electrode surface. In this paper, GC/graphene/ferritin/GOx anode is developed in which ferritin acted as an electron transfer mediator while graphene electrons transfer enhancer and can easily provide electrical communication between GOx and GC electrode.

Experimental

Chemicals and reagents

The ferritin (10 mg ml^{-1}) from horse spleen, glutaraldehyde, purified graphene used were obtained from Sigma Chemicals, India. Phosphate buffer solutions of pH 5.0 and pH 7.0 (Otto Pvt. Ltd. India), glucose oxidase (GOx), Central Drug House (CDH), India, the anionic surfactant, sodium dodecyl sulphate (SDS) (Qualigens Fine Chemicals, India) and D-(+)-glucose anhydrous (Himedia Laboratories Pvt. Ltd. India) were used as received.

Instrumental

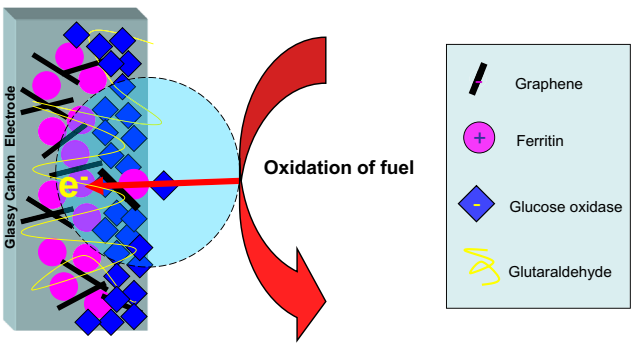
All electrochemical measurements were performed using a computer controlled Potentiostat/Galvanostat (302N Autolab, Switzerland). A conventional three electrode system including a working electrode of as prepared of glassy carbon (GC) biocomposite electrode, an Ag/AgCl reference electrode and a platinum wire counter electrode were used for all electrochemical measurements in phosphate buffer solution (PBS) at pH 7.0 in the presence of glucose and in the absence of glucose at room temperature (25 ± 3 °C) in air. The electrode was cleaned with S15H ultrasonic cleaner (Elmasonic, Germany).

Preparation of graphene dispersion

Graphene dispersion was prepared by mixing 2 mg of graphene with 10 ml aqueous solution of 200 ppm sodium dodecyl sulfate (SDS), a cationic surfactant. The mixture was then ultrasonicated for 40 min. The performance of dispersant was measured by a UV–Vis spectrophotometer (Perkin Elmer, USA; Model- Lambda 25) and absorption spectrum between 300 and 700 nm was recorded.

Preparation of graphene/ferritin/GOx electrodes

A 3 mm diameter GC electrode was polished with 0.05 μm alumina slurry using a velvet pad. The electrode was ultrasonicated for a period of 10 min and washed with deionized water and allowed to dry at room temperature (25 ± 3 °C). After drying of the electrode, a 4.5 μl of graphene dispersion (as prepared above) was deposited on the GC electrode and is allowed to dry at a room temperature (25 ± 3 °C) for 6 h. Further, a 4.5 μl of (10 mg ml^{-1}) ferritin was cast on the dried graphene modified GC electrode. The modified electrode is allowed to dry at room temperature for 30 min. GOx (10 mg ml^{-1}) was dissolved in a phosphate buffer saline (PBS) solution pH 5.0 to maintain the activity of the enzyme during immobilization process. An 8.5 μl of GOx solution was cast on the dried graphene/ferritin biocomposite and allowed to absorb at room temperature (25 ± 3 °C) for 1 h. Finally, a 10 μl of a 2% glutaraldehyde aqueous solution was drop casted to cross link the graphene/ferritin/GOx biocomposite electrode firmly and left to dry for a period of 15 min. The electrode was also dipped in deionized water for a period of 2 min to remove any non-bounded GOx and glutaraldehyde. The electrode was allowed to dry at room temperature and was placed in refrigerator until the measurements were taken. The proposed design for the



Scheme 1 – The design for the layer-by-layer immobilization of biomolecules and their electrostatic interaction.