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RESEARCH ARTICLE

IMMOBILIZATION OF GLUCOSE OXIDASE IN MULTI-WALL CARBON NANOTUBES ON PT MODIFIED ELECTRODE FOR HIGH-SENSITIVITY GLUCOSE DETECTION

[1]Omid ramezani azghandi and *,[2]Afshin Farahbakhsh

[1]Young Researchers Club, Quchan Branch, Islamic Azad University, Quchan, Iran
[2]Department of Chemical Engineering, Quchan Branch, Islamic Azad University, Quchan, Iran

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ABSTRACT

Glucose oxidase sensor is one of the best methods for measuring small amounts of glucose. this sensor can be modified using multi wall carbon nanotubes as complementary substance in the electrode structure with the aim of increasing efficiency.In this paper, Pt electrode, was enriched with carbon nanotubes by an intervening material of amino groups of poly (allylamine) (PAA), and was used as working electrodes (anode) along with a platinum auxiliary electrode and the reference electrode Ag / AgCl (cathode). Working electrode was surrounded by a semi-permeable membrane containing the Phosphate-buffered saline (PBS) with Ph = 4,6,9 enzyme. To evaluate the performance of the constructed biosensor, it was put into a dish containing 0.01, 0.05 and 0.1 M glucose concentrations which were dissolved in doubly distilled water. The produced current and its rate was measured by Potentiostat with transferring the electron from the working electrode to reference electrode which was released by the reaction of glucose in the presence of the enzyme. According to the performed experiments, with increasing concentration, the flow rate of current production is increased and pH deviance from neutral range reduces the flow. Optimal conditions was obtained in concentrations 0.1 M and pH=6, respectively.

INTRODUCTION

Diabetes mellitus is one of the principal causes of death and disability in the World, and is highly responsible for heart disease, kidney failure, and blindness. Million s people in the world are afflicted with diabetes mellitus. This figure is expected to rise up to more than three hundred million by 2030 (Gavin, 2007, Reach, *et al*., 1992). Frequent testing of physiological blood glucose levels to avoid diabetic emergencies, is crucial for the confirmation of effective treatment. Such metabolic disorder results from insulin deficiency and hyperglycemia (Wang, *et al*., 2008, King, *et al*., 1998, Wild, *et al*., 2004). The challenge of providing such reliable and tight glycemic control remains the subject of a considerable amount of research. Therefore, the development of high sensitive, low-cost, reliable glucose sensors having an excellent selectivity has been the subject of concern for decades, not only in medical science but also in the food industries (Wang, 2008, Wilson, *et al*., 2005). Electrochemical biosensors for glucose play a leading role in this direction. Amperometric enzyme electrodes, based on glucose oxidase (GOx) bound to electrode transducers, have thus been the subject of substantial research (Wang, 2001). Biosensors are portable analytical devices designed to be used by the general public, directly in the field without any specific training or without requiring any processing steps. For a portable analytical device to achieve this goal, which essentially means there cannot be any sample preparation step, requires the device to have exquisite selectivity for the target analyte because invariably complex samples such as blood will have a myriad of other compounds present. To achieve this specificity biosensors borrow from nature using the recognition molecules of living things such as enzymes, antibodies, peptides or DNA (Hall, 1990). Thus a biosensor comprises a biorecognition molecule integrated with a signal transducer to give a reagentless analytical device (see Figure 1). The signal transducer determines the extent of the biorecognition event and converts it into an electronic signal which can be outputted to the end user. Common transducers include amperometric electrodes, optical waveguides or mass sensitive piezoelectric crystals. The final biosensor is a solid-state device which is exposed to a solution sample and hence the biorecognition reaction is an interfacial reaction. The classic example of a biosensor is the glucose monitors used by diabetics where the biorecognition molecule is the enzyme glucose oxidase and the transducer is an electrode (Thevenot, *et al*., 2001, Wink, *et al*., 1997). Enzymatic biosensors are attractive as they have many potential applications in various fields that include medical diagnosis, pharmaceutical and environmental controls. Electrochemical sensors hold much promise among the enzymatic biosensors.

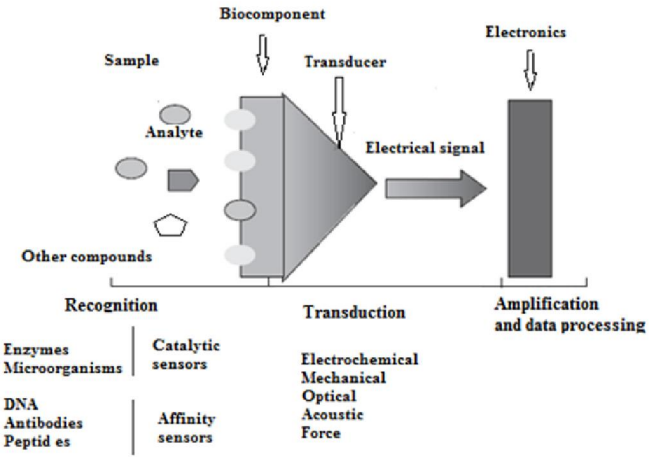


Figure 1. A schematic of a component biosensor

Sensitivity and stability are the key features for using the biosensors in practical analysis and commercial developments. Electrode

Corresponding author: Afshin Farahbakhsh, Department of Chemical Engineering, Quchan Branch, Islamic Azad University, Quchan, Iran

modifying materials (Manesh, *et al*., 2007) and techniques for enzyme immobilization (Qiu, *et al*., 2009) have been developed to achieve these key features. A wide variety of electrodes modifying materials such as carbon nanotubes (CNTs) (Gopalan, *et al*., 2009, Manesh, *et al*., 2008), polymers (Santhosh, *et al*., 2009) and silica (Nadzhafova, *et al*., 2007) have been used for the fabrication of biosensors. The recent discovery of the carbon nanotube (CNT) has attracted considerable attention due to their dimensions and structure-sensitive properties (Pumera, *et al*., 2006). CNTs consisting of cylindrical graphite sheets with nanometer diameter are relatively novel materials that have attracted increasing attention when used as electrode materials (Kirgoz, *et al*., 2007). Because of their unique properties, such as enhanced electron transfer, high electrical conductivity, high mechanical properties, ability to grow on different substrates, and nanoscale size with a high aspect ratio, CNTs have been intensively researched for electrocatalytic and sensing applications (Yun, *et al*., 2006). GOx is the most common glucose oxidizing enzyme used in conjunction with biosensors and, in other forms of bioanalytical devices and for biofuel research (Olea, *et al*., 2007, Yin, *et al*., 2007). GOx is relatively selective for glucose, however, some other sugars and glucose derivatives are also oxidized by this enzyme (Timur, *et al*., 2006).

Experimental

Reagents

Glucose oxidase (GOD, No. ART 6125, 50KU / mg protein), poly allyamine (PAA, No. ART 479136, 5gr, M_w=17000), 1 - ethyl -3 - (-3dimethylaminopropyl) Carbodiimide (EDC, No. ART 424331, 5gr), N - hydroxysuccinimide (NHS, No. ART 130672, 5gr) was purchased from Sigma-Aldrich. Multi-wall carbon nanotubes (MWCNTs, No. ART 99685-96-8, 5gr, nm 10- 20) Product of Academy of America and alumina powder (Code: 6,2802,000) and glucose products Metrohm (No ART 1204667, 1 gr, M_w = 180.155 gr / mol) and potassium dehydration phosphate (KH_2PO_4, M_w = 136.09 gr / mol, 1 kg pack Code: 1000-5101-1) was purchased from Merck,. All other reagents: HCL (M_w = 46.36 gr / mol, density = 19.1 kg / lit, purity 37%), sulfuric acid (H_2SO_4,M_w = 98 gr / mol, density = 84.1 kg / lit, purity 98 %), nitric acide (HNO_3,M_w = 63.9gr / mol density = 1.40 kg / lit, purity 65%), NaOH (M_w = 40.08 gr / mol), doubly distilled water and has been prepared within the country. All aqueous solutions were prepared with doubly distilled water and All experiments were performed in PBS at room temperature, approximately 25 $^{\circ}C$.

Preparation of carbon nanotubes

MWCNTs (diameter: about of 20 nm) were chemically abridge by ultrasonic Agitation in a mixture of nitric acid and sulfuric acid (1:3) for about 6 h initially oxidized by acid procedure to nominate carboxyl groups on their jag and any vitiate in the lateral walls. The resulting MWCNTs were separated and washed with doubly distilled water by centrifugation (6,000 rpm) until the pH MWCNTs up to 7. The resulting MWCNTs were inside place for 10min a 1:1 (v/v) EDC/NHS mixture intemediate (25 mg/ml EDC and 25 mg/ml NHS) until aromatic loop confirm up MWCNTs, and then washed with doubly distilled water by centrifugation. Afterward the enrichment the working electrode can be explained by MWCNTs.

Modified electrodes

The Pt electrode (length and width cm 5 mm 3 and a small thickness, grade 9.99) was utterly polished using an alumina powder is given to the electrode surface is completely smooth .then etched for 4 min in a 1:3:4 (in volume) mixture of acid sulfuric / acid chloride / water and then sonicated for 6 min in doubly distilled water the if surface of alumina electrode remains lost, and serve the consolidation not occur. The Pt electrode cleaned was soak in PAA solution for 25 min and abluented in PBS for 5 min which was exerted at the end of each assembly dethrone for separation the languid adsorption (Unless is another form) without drying-procedure, and then serial transferred to MWCNTs for about 25 min. Happen, The carboxyl on the MWCNTs could create an active ester via EDC/NHS, which was used to aggregate by the carboxyl–amine linking. PAA has the amino groups, as a highly positively charged material, which can first aggregate on the surface of Pt electrode. The MWCNTs modified by EDC/NHS can aggregate on the PAA surface by the carboxyl–amine connect, followed by the aggregate the same couple mechanism as that for PAA on the MWCNTs surface (see Figure 2). So MWCNTs modified by thiol function group is aggregate on the Pt electrode surface.

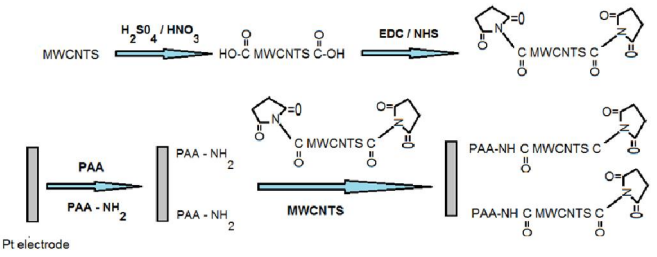


Figure 2. Schemes of the pretreatment of MWCNTs (top) the stepwise fabrication process of the films on a Pt electrode (bottom)

Preparing Biosensors

Pt electrode coated by MWCNTs as a aworking electrode (W.E), Ag/AgCl refrence electrode and platinum electrode (diameter of 1 mm) as a auxiliary electrode sequential applied. the platinum working electrode was dipped in Semi-permeable membrane made of cellephone, contain of 0.01 M Gox/ PBS solution and the glucose nanobiosensor was maked.the biosensor put on analyte solution (various concentration 0.01, 0.05 and 0.1 mM) for detection of glucose. After the diffuse of glucose in membrane the reaction was started and productive electron transfe on the working electrode and that is quantifiable by Potentiostat (product AMEL, model 7050 and use hardware M70). This device is marked with the voltage and frequency to detect glucose in a specific area.

RESULTS AND DISCUSSION

After stabilization of carbon nanotubes on platinum electrode is needed to help glucose oxidase enzyme in pH= 4, 6, 9 PBS, and put it in the membrane than the analyte (glucose in solvent water different concentrations) and put this collection together with semi-permeable electrode platinum auxiliary electrode Ag / AgCl, the analyte, to help Poteantystat device manufacturing process was examined (see Figure 3).

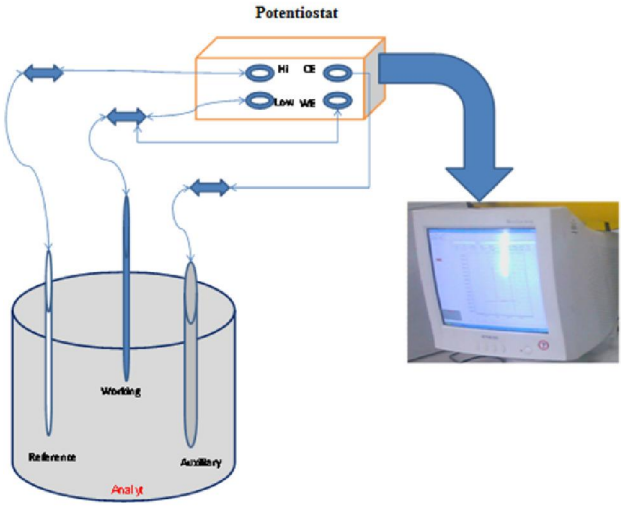


Figure 3. Schematic diagram of measurement biosensor system

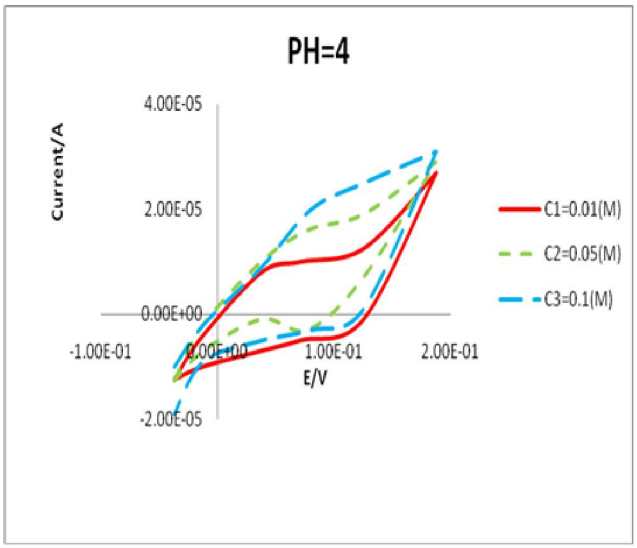
We done with twice recur for current measurement in different pH and concentration analyte, that get Table 1 of sequal. According to

current output obtained for modified electrodes to achieve better analysis, the results of statistical analyzes and graphs were analyzed.

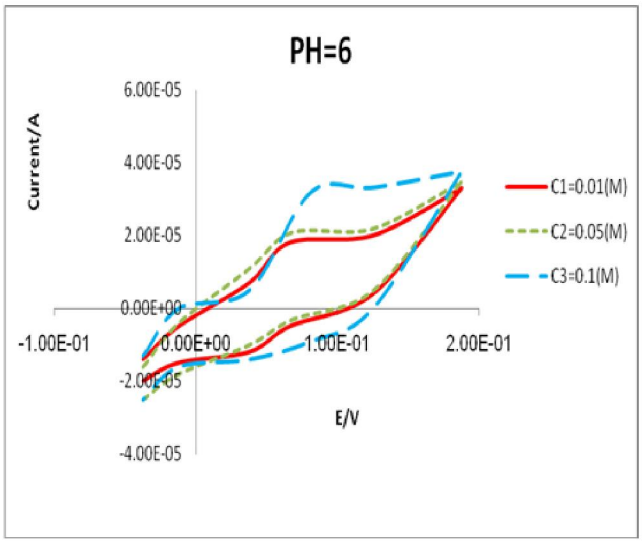
Table 1. Analize result for current output

pH	Concentraition(M)	Current for recur 1 (mA)	Current for recur 2 (mA)
4 =pH	C_1=0.01	6.737= $^{5-}$ e	8.26=$^{5-}$ 1.265e
4 =pH	C_2=0.05	10.780=$^{5-}$ 1.61e	11.454=$^{5-}$ 1.70e
4 =pH	C_3=0.1	13.475=$^{5-}$2e	12.984=$^{5-}$ 1.927e
6 =pH	C_1=0.01	12.128=$^{5-}$ 1.80e	11.845=$^{5-}$ 1.758e
6 =pH	C_2=0.05	14.149=$^{5-}$ 2.11e	12.991=$^{5-}$ 1.928e
6 =pH	C_3=0.1	21.561=$^{5-}$ 3.2e	19.654=$^{5-}$ 2.917e
9 =pH	C_1=0.01	10.106=$^{5-}$ 1.50e	13.476 =$^{5-}$ 2e
9 =pH	C_2=0.05	12.069=$^{5-}$ 1.79e	15.114=$^{5-}$ 2.243e
9 =pH	C_3=0.1	20.213=$^{5-}$ 3 e	19.344=$^{5-}$ 2.871e

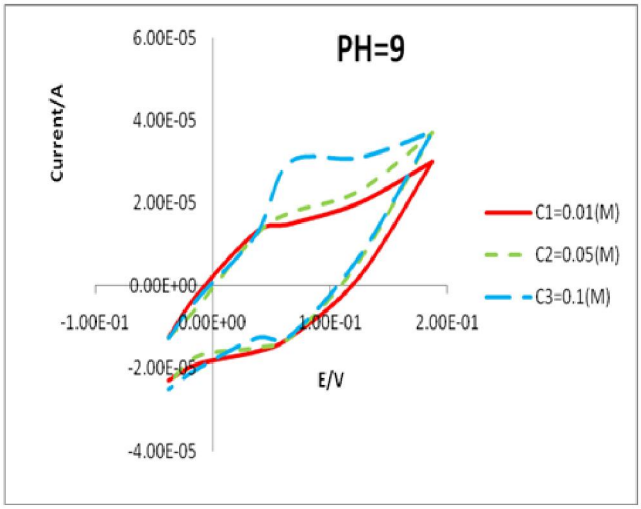
Figure 4 (A) was plotted of the evaluation current output biosensor for phosphate buffer in pH =4 and concentrations 0.01, 0.05 and 0.1 M. we can be seen, with the increase from 0.01 to 0.1 M, production flow rate increases so that the concentration of 0.1 compared to the other concentrations, increasing the flow of cases the product is specified. Figure 4 (B) evaluation current biosensor for phosphate buffer pH =6 and the concentrations were plotted. With these Figures, the previous results obtained for the case that produced the most current has the highest concentration. Of Figures 4 (C) plotted for PBS with pH =9 confirming the increase in production is responsible for increasing glucose concentration.



A



B



C

Figure 4. Flow measurement biosensor for pH =4 (A), pH =6 (B) and pH = 9 (C) and different concentration (M)

This is because at low concentrations, due to the lack of substrate, enzyme active sites is not full yet, and the low enzyme activity. It is time that the so-called excess glucose remains in the free form. With increasing concentration, the flow rate is increased production and reduced flow is diverted neutral pH range. The peak current, often near the voltage (v) 0.7, respectively. Results to another can be seen in Figure 5. We observe in Figure 5, that pH = 6 and concentration 0.1 M, the maximum flow rate is produced. Also, after correction of enriched carbon nanotubes on the pelatinum electrodes, it is determined that the optimal production is about 21.56 mA (predicted value) but for the case of the pure platinum electrode was made 4.7 mA which demonstrates that enriching carbon nanotubes electrodes increases surface area in the working electrod and this increase in the surface increases electron transfer and increases current production.

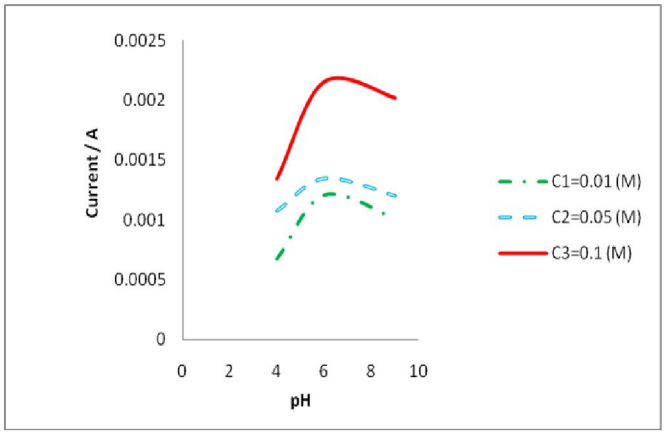


Figure 5. Curve A- pH at constant voltage V =0.7 (V) and different Concentrations (M)

Conclusion

According the results of the performance of biosensor, it was demonstrated that in the optimal condition, the PH=6 and the concentration C=0.1 M. also demonstrates that enriching carbon nanotubes electrodes increases surface area in the working electrod and this increase in the surface increases electron transfer and increases current production. Optimum voltage is V=0.7 v. Also see, that amount of current production (current output or production flow rate) at pH=9 bigger than the amount of current production in pH=4 but both current production under current production in ph=6 and with the increase concentration from 0.01M to 0.1 M, production flow rate is incremented.

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