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ORIGINAL ARTICLE

Modification of Glucose biosensor using Pt/MWCNTs electrode and optimization by application of taguchi method

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Abstract

In this paper, multi-wall carbon nanotubes (MWCNTs), gold nanoparticles (GNp) and glucose oxidase (GOD) was developed for the specific detection of glucose. MWCNTs were chemically modified with the H_2SO_4–HNO_3 pretreatment to introduce carboxyl groups which were used to interact with the amino groups of poly(allylamine) (PAA) and cysteamine via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/ N-hydroxysuccinimide cross-linking reaction, respectively. A cleaned Pt electrode was immersed in PAA, MWCNTs, cysteamine and GNp, respectively, followed by the adsorption of GOD, assembling the one layer of films on the surface of Pt electrode (GOD/GNp/MWCNTs/Pt electrode) and was used as working electrodes (anode) along with a platinum auxiliary electrode and the reference electrode Ag/AgCl (cathode). Working electrode was containing the Phosphate-buffered saline (PBS) with pH = 4, 6, 8 enzyme. To evaluate the performance of the constructed biosensor, it was put into a dish containing 0.05, 0.5 and 1 mmol lit^{-1} (mM) glucose concentrations which were dissolved in doubly distilled water. The produced current and its rate were 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. Glucose concentration and PBS pH design has been tested and analyzed by QUALTEK-4 software measure. According to the performed experiments and software analysis, 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 1 mmol lit^{-1} and pH =6, respectively. After confirmation tests in optimum conditions, the rate of production was obtained, 21.67 mA, which with respect to the expected error rate of application, it was calculated to be 8.1%. This error rate demonstrates that the accuracy of tests is with high sensitivity and accuracy.

Keywords: *Carbon nanotubes; Enzyme Glucose Oxidase; Gold nanoparticles; Platinum electrode; Software QUALTEK-4.*

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INTRODUCTION

Platinum has been extensively used in many applications, chiefly as a fuel cell technology and of catalyst for CO oxidation in catalytic commutation [1].Carbon nanotubes (MWCNTs) have become the one of utmost researched materials in the last decades because of their eventual applications in any aspect of nano technology [2]. Potential applications of carbon nanotubes including catalyst supports in heterogeneous catalysis, mechanical strength, high-strength engineering fibers, components of composites and sensors, that result mainly from theirs high surface area, electrical conductivity, chemical and thermal stability. Due nano scale size with a low weight mainspring hollow cylindrical convoluted graphite sheets that possess a hollow core suitable for impounding guest molecules and large surface area of carbon nanotubes with particular properties of noble (including Pt) metal nanoparticles in one segregate structure there is a great deal of interest in linking nanoparticles

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to nanotube surface, leading to modern and prospective applications [3]. In the application as fortification of fillers in polymer matrix, MWCNTs must evince good dispersion, high interfacial stress transfer and better equilibrium of MWCNTs in the polymer matrix before they can be of notable use. However, in this application, the functionalities of MWCNTs are frequently limited by their irresoluble in most organic solvent [4].

Several methods of chemical functionalization have been promoted to increase the solubility of MWCNTs. The most widely accepted accession to increase the solubility of MWCNTs is through wet chemical oxidation process. This accession is initiated by mixing MWCNTs with strong acids such H_2SO_4, HNO_3 or a mixture of the two. In the process, Carboxylic groups (=O) are introduced so that MWCNTs could link with longer chain Functional groups such as Amine groups (-NH_3, -NH_2 or -NH that Amine groups of 3, 2 or 1, respectively), the charge balance around the MWCNTs is disquiet and the electronic polarity is induced in order to enhance the solubility of MWCNTs in the organic solvents [5]. Several methods have been successfully promoted and practically applied for fabricating electrochemical sensors and in many others [6-9]. Among all the enzyme-based biosensors, chiefly glucose biosensors, is necessary for an authentic monitoring of blood glucose levels. Recently, Wang *et al.* [10], delineated the probably applications of MWCNTs in constructing such devices. GOx is the uttermost common glucose oxidizing enzyme used in linkage with biosensors, in other forms of bio analytical devices and for biofuel research [11-15]. GOx is correlation selective for glucose, however, some other glucose derivatives and sugars are also oxidized by this enzyme [16].

EXPERIMENTAL

Reagents

Multi-wall carbon nanotubes (MWCNTs, No. ART 99685-96-8, 5gr, 10- 20 nm) Product of Academy of America and glucose oxidase (GOD, No. ART 6125, 50KU mg^{-1} protein), 1 - ethyl -3 - (-3dimethylaminopropyl) Carbodiimide (EDC, No. ART 424331, 5gr), poly allyamine (PAA, No. ART 479136, 5gr, M_w=17000), N - hydroxysuccinimide (NHS, No. ART 130672, 5gr), gold nanoparticles (GNP, CA. about 24nm) was purchased from Sigma-Aldrich. Alumina powder (Code: 6, 2802, 000) and glucose products Metrohm (No ART 1204667, 1 gr, M_w = 180.155 gr mol^{-1}) and potassium dehydration phosphate (KH_2PO_4, M_w = 136.09 gr mol^{-1}, 1 kg pack Code: 1000-5101-1) was purchased from Merck. All other reagents: HCL (M_w = 46.36 gr mol^{-1}, density = 19.1 kg lit^{-1}, purity 37%), sulfuric acid (H_2SO_4 ,M_w = 98 gr mol^{-1}, density = 84.1 kg lit^{-1}, purity 98 %), nitric acide (HNO_3, M_w = 63.9gr mol^{-1} density = 1.40 kg lit^{-1}, purity 65%), NaOH (M_w=40.08 gr mol^{-1}), doubly distilled water and cysteamine 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°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 (5,000 rpm) until the pH of result MWCNTs solution became up to 7. The resulting MWCNTs were inside place for 10 min a 1:1 (v/v) EDC/NHS mixture intermediate (25 mg ml^{-1} EDC and 25 mg ml^{-1} NHS) until aromatic

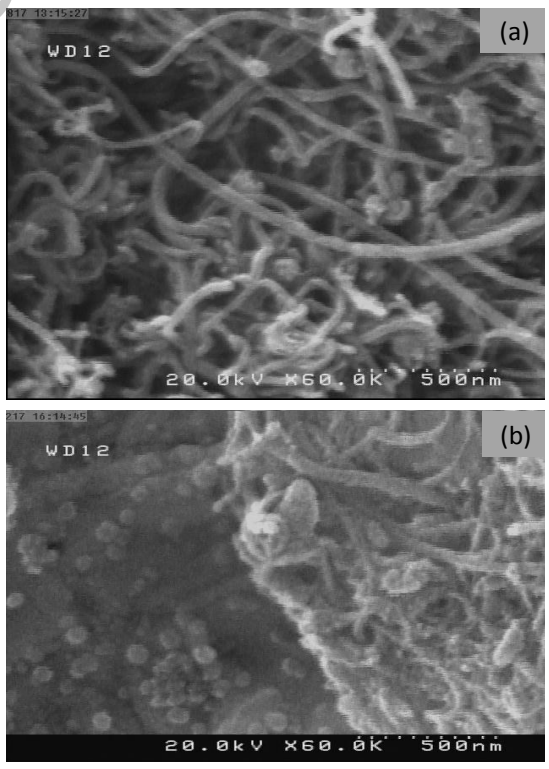

Fig. 1: SEM (a) and TEM (b) image of MWCNTs

loop confirm up MWCNTs, and then washed with doubly distilled water by centrifugation. Scanning Electron Microscopy (SEM-as you can see in Fig. 1a) and Transmission Electron Microscopy (TEM-as you can see in Fig. 1b) was employed to study MWCNTs and the surface morphology of the modified electrode.

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 given to the electrode surface 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 10 min in doubly distilled water, if the surface of Alumina Electrode remains lost, and serve the consolidation not occur.

The cleaned Pt electrode was soak in PAA solution for 25 mins and abluented in PBS for 5 min which was exerted at the end of each assembly dethrone for separating the languid adsorption (Unless it is another form) without drying-procedure, and then ordinal transferred to MWCNTs, Cysteamine and GNp, respectively for about 25 mins. The Carboxyl on the MWCNTs could create an active ester via EDC/NHS, which was used to aggregate by the Carboxyl–Amine coupling. PAA and Cysteamine 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 aggregating the same couple mechanism as that for PAA on the MWCNTs surface. So MWCNTs modified by Thiol function group is assembled on the Pt electrode surface. Then GNp can be chemisorbed to the MWCNTs surface modified by Thiol groups. GOD is finally adsorbed onto the surface of GNp, assembling the one layer of films modified Pt electrode (Fig. 2) so modified by Thiol function group is aggregate on the Pt electrode surface and see IR spectra of electrode in Fig. 3.

Preparing Biosensors

Pt electrode coated by MWCNTs as a working electrode (W.E), Ag/AgCl the reference electrode and Platinum electrode (diameter of 1 mm) as an auxiliary electrode sequential applied. Voltamogram for W.E was realized by the injection of different solution of glucose at the (GOD/GNp/MWCNTs) /Pt electrode in different pH of PBS at room temperature, which was a dependence of the glucose current response measured at different applied potential. The biosensor put on analyte

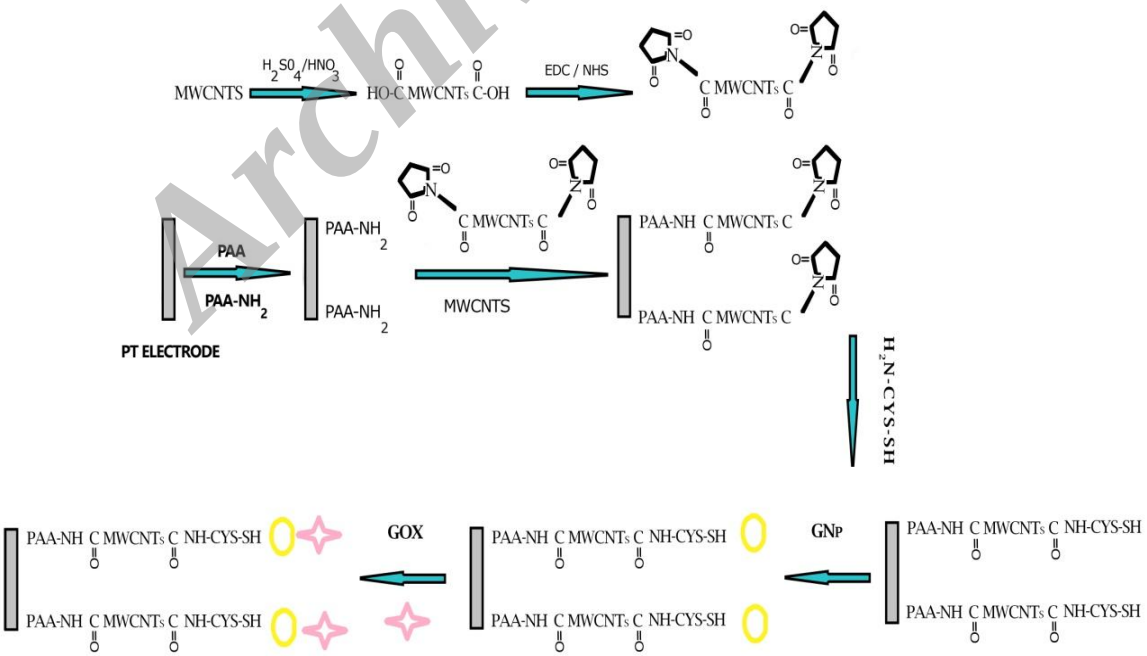

Fig. 2: Schemes of the pretreatment of MWCNTs (top) the stepwise fabrication process of the films on a Pt electrode (bottom)

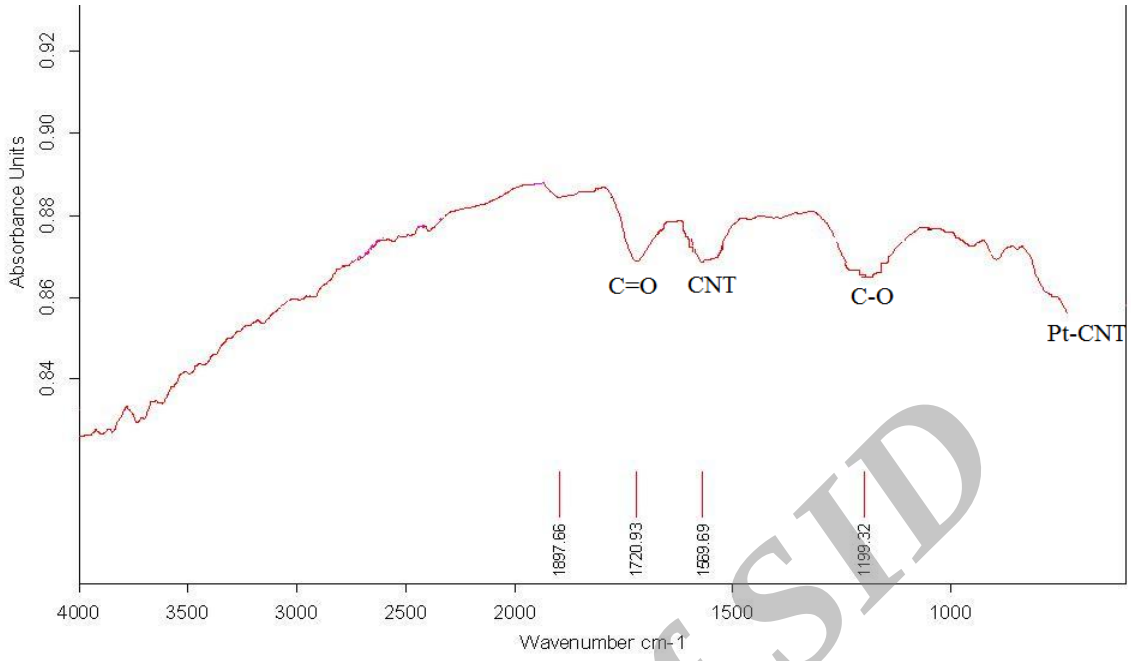

Fig. 3: Indicate that carboxylic groups are present on the surface electrode before regarded as the one layer of films (IR spectra of electrode)

solution (various concentration 0.001, 0.01 and 0.1 mmollit^{-1}) for detection of glucose. After diffuse of glucose the reaction was started and productive electron transferred 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.

Design of experiments

Qualitek-4 software for design of experiments using the Taguchi approach was used in the present paper. Taguchi approach has established Orthogonal Arrays (OA) to describe a large number of experimental situations, mainly to reduce experimental errors and to enhance the efficiency and reproducibility of laboratory experiments. For all experiments, we should follow the three basic steps outlined below (Fig. 4). Whenever we utilize Qualitek-4 design and analysis capabilities, it would be assumed that you have completed the planning session and have all necessary information related to the experiment [17].

The software uses the An L_9 Orthogonal Arrays, (According to Step 1 in flowchart), with selection of two factors of pH and C at three levels per factor that has been chosen based on the number of factors and levels mentioned in Table 1 (According to Step 2 in flowchart), Due to the experiment number, the test results are recorded in Table 2.

The choice of optimal conditions through nominal (Eq.1), smaller (Eq.2) and bigger the best (Eq.3), S/N analysis was done. (According to Step 3 in flowchart) [18]. According to the analysis for the case of bigger the best, software calculates the values of S/N ratio at the optimum condition. The experiment with S/N ratio implicitly is bigger experiment optimal (Fig. 5).

Average effect of facts for factors and interaction are seen in main effects. According to Percent (P%) at ANOVA table, found that changes in glucose concentration was much easier than changes in pH. In this Table, P% is the percentage contribution of each factor. Other parameters in this table include: degree of freedom for each factor (DOF), Sum of Sqrs. (S), Variance (V), F-ratio (F), Pure Sum the numbers for each that show the same results (Table 3). Finally the software estimate expect result S/N_{opt}, Y_{exp}, performance at any arbitrary condition and contribution level of each factor [19].

$$n = -10\log_{10}[\frac{\text{mean of sum of squares}}{\text{mesured ideal}}] \quad (1)$$

$$n = -10\log_{10}[\frac{\text{mean of sum of squares}}{\text{reciprocal of mesured datal}}] \quad (2)$$

$$n = \log_{10}[\frac{\text{square of mean}}{\text{variance}}] \quad (3)$$

The optimum process conditions for producting the current modified electrode, were determined according to the Taguchi experimental design method using Qualitek-4 software [20-21].

That uses the an L_9 orthogonal array (OA) in software, along with a selection of two factors of pH and C at three levels that per factor has been chosen based on the number of factors and levels mentioned in Table 1 .

In this paper, the bigger-is-best (Eq.4) was used. The performance statistics was evaluated by using the following equations:

$$\frac{S}{N} = -10\log(MSD) \quad (4)$$

$$MSD = 10[\frac{-(\frac{S}{N})}{10}] \quad (5)$$

Table 1: Denote Factor and level in Design of Experimental

Parameters		Levels		
		1	2	3
A	pH	4	6	8
B	C (mM)	0.05	0.5	1

Table 2: Experimental Layout Using an L9 Orthogonal Array

Experiment number	parameters and their levels		Current(mA)	
	A	B		
1	1	1	6.14	8.70
2	1	2	11.23	10.93
3	1	3	17.05	14.63
4	2	1	11.30	12.91
5	2	2	13.06	14.92
6	2	3	20.07	19.84
7	3	1	6.71	9.33
8	3	2	12.07	13.56
9	3	3	18.08	18.97

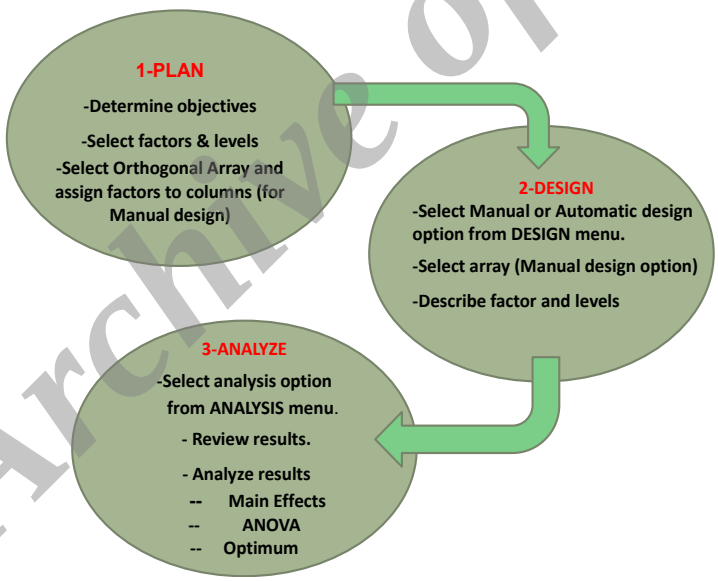

Fig. 4: Flowchart of the Taguchi method

Table 3: Result Analysis ANOVA

Parameters		DOF	sum of squares (S)	Variance (V)	F-Ratio (F)	Percent P (%)
A	PH	2	12.55	6.27	10.55	13.62
B	C(mM)	2	60.92	30.55	58.93	76.07
Other/error		4	3.98	0.11		10.29
Total		8	77.46			100 %

$$MSD = [\frac{\left(\frac{1}{Y_1}\right)^2+\left(\frac{1}{Y_2}\right)^2+\cdots+\left(\frac{1}{Y_n}\right)^2}{n}] \quad (6)$$

$$Y_{exp} = \sqrt{\frac{1}{MSD}} \quad (7)$$

RESULTS AND DISCUSSION

Analysis of results

After stabilization of carbon nanotubes on platinum electrode is needed to help glucose oxidase enzyme in concentration 0.05 mmollit^{-1} NaCl and varying pH= 4, 6, 8 PBS, than the analyte (glucose in solvent water different concentrations) put this collection together with electrode platinum auxiliary electrode Ag/AgCl, the analyte, to help Poteantystat device manufacturing process which was examined (Fig. 6a, 6b, 6c respectively). It can be seen from Fig. 7, as a concentration as 1 mM, and close to neutral pH, the most productive has happened. In addition, it was found in very low pH, such as pH = 4, as compared to higher pH (pH=8) flow rate of production was lower. This is due to the presence of the enzyme, oxidation of glucose to gluconolactone in the presence of oxygen.

While enzyme position with natural pH, low quantity happen or wile PBS with larger than the buffer, natural term extinct. With increasing concentration, the flow rate is increased production and reduced flow diverts neutral pH range. The peak current, voltage, often near to the 0/7, respectively (Fig. 8).

Withal the results of experiments has been indicated according to the test design in Table 2 and the optimal conditions are obtained according to the Tables 2 and 3, based on S/N analysis on the basis of the highest value and the ANOVA analysis. According to Table 2, Experiment 6 (pH = 6 and glucose concentration= 1 mmollit^{-1}) has been chosen as the optimal analysis and value S/N =26 (Fig. 5) and mean current was obtained Y =19.95 mA respectively. The contribution of each factor on the table analysis ANOVA (Table 3) is expressed in a different way, and shows to achieve optimum shift, which factor is more appropriate.

ANOVA analysis showed that the change in the glucose concentration has the highest effect in current produce. In a way that Sum of Sqrt., Variance, F-Ratio and P-Percent are 60.92, 30.55, 58.93 and 76.07 which are more than the value calculated for the effects of change in the pH.

In simple terms, work with changes in its concentration is easier and changes are more effective on the production. Also observed that the obtained error rate is 10.29, which is less than 15%, thus it can be concluded that the experimental design is acceptable. Finally, software calculates two digits as Y_{exp} and S/N_{OPT}. The more the digits are closer to the S/N and Y digits, the error percentage is less.

Values obtained from the software:

S/N_{OPT} = 26.67 mA Y_{EXP} = 21.57

By using Potentiostat, the same amount of glucose 1 mmollit^{-1} and pH=6 (confirmation test

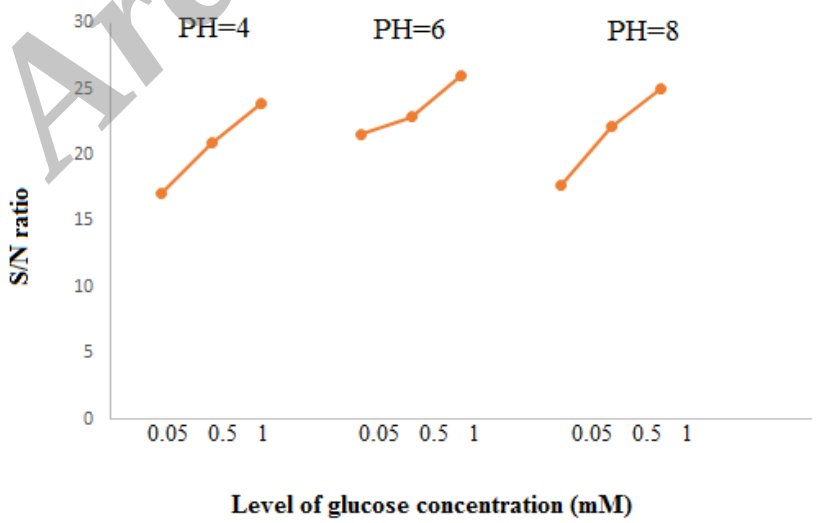

Fig. 5: The plot of S/N ratio versus level of glucose concentration variables

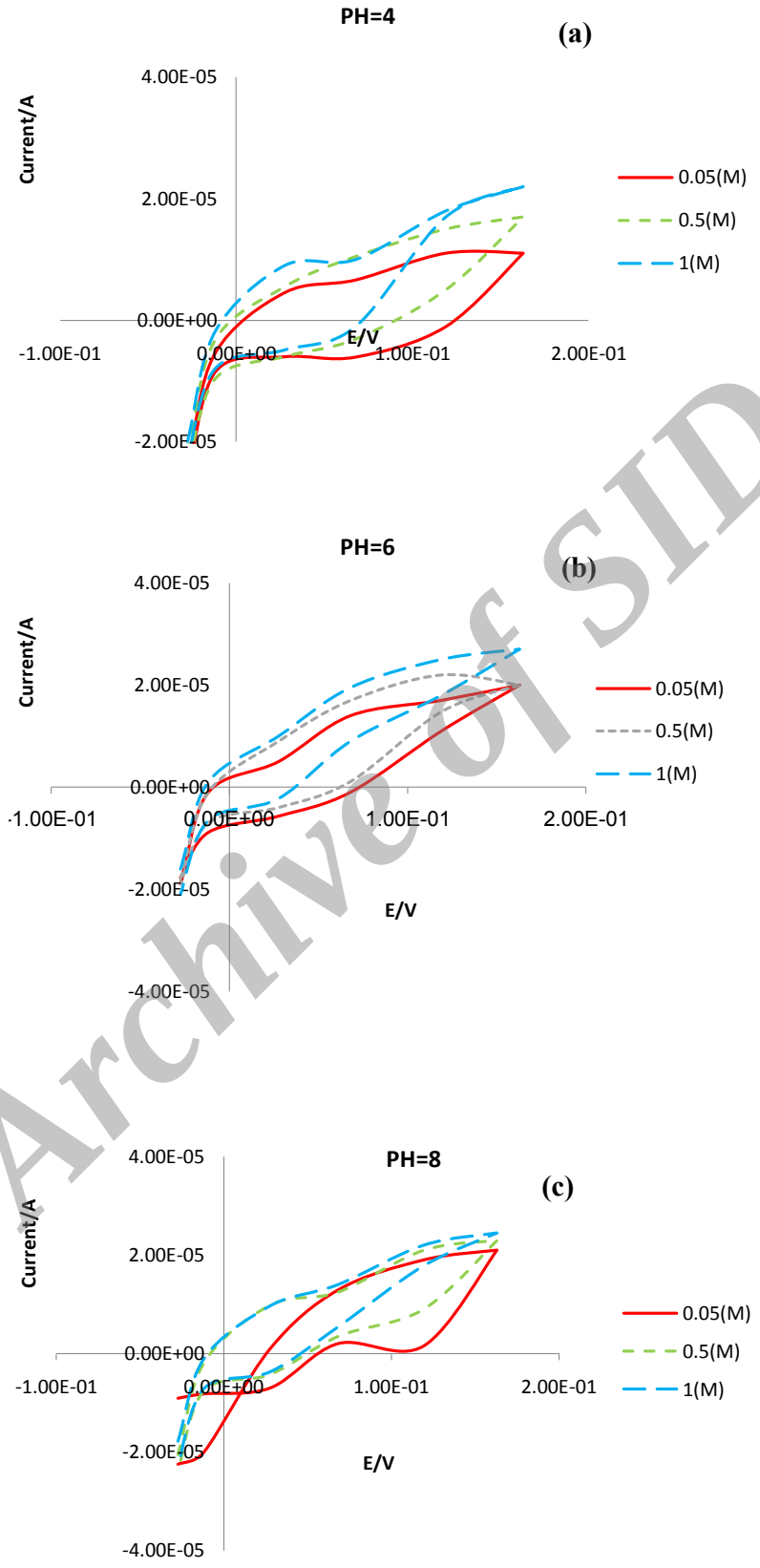

Fig. 6: Flow measurement biosensor for pH=4(a), pH=6(b), pH=8(c) and different concentration (mmol lit^{-1})

conditions) for platinum electrode uncoated carbon nanotube, current production measuring was evaluated, and $6.5e^{-5}$ = 0/00437 mA is obtained. In summary, According to software forecasts, optimized conditions are with glucose concentration=1 mmollit^{-1} and pH=6 (that see in Figs. 6 and 7 the similar). In this condition, current production is obtained 21.57 that was tested again in the same conditions and the current production is obtained 20.07 mA that relative error percentage was 7.4%. Table 4 is given for better comparison.

Based on the above table, the relative percentage error between current response predicted and current response measured is low and this claim can be a credit for the accuracy of results and the low error percentage.

The relative percentage error in the production current, between the measured and predicted condition are about 8.12% and the relative percentage error between predicted current response and current response experimental

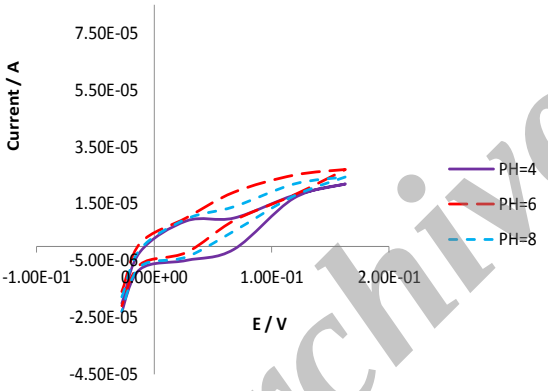

Fig. 7: Current at concentration 1 mM and different PH

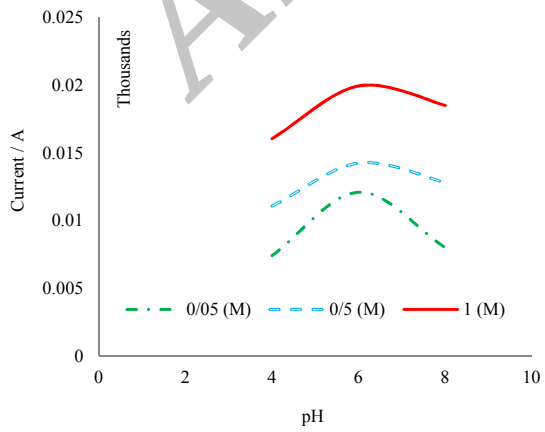

Fig. 8: Curve: pH at constant voltage V =0/7 (V) and different Concentrations (mmol lit^{-1})

Table 4: Results of the predicted and measured

Parameters		Value current(mA)		
		value	Level	Contribution
A	pH	6	2	1.521
B	C (mM)	1	3	3.192
total contribution			4.713	
Observed value of current (mA)			19.95	
predicted value of current (mA)			21.57	

confirmation is about 7.47% which respectively both relative percentage errors are less than 15%. Also, after correction of enriched carbon nanotubes on the platinum electrode, it is determined that the optimal production is about 21.57 mA (predicted value) but for the case of the pure platinum electrode was made 4.3 mA which demonstrates that enriching carbon nanotubes electrodes increases surface area in the working electrodes and this increase in the surface increases electron transfer and increases current production. The reason is that Glucose biosensors in very low concentration of glucose, all of the enzyme places don't fill with substrate (glucose) and the enzyme action is very low. With gradual increment in glucose concentration, enzyme action is increased until in a specific concentration, all of active places in enzyme are filled and enzyme action is reached to maximum in operating position. With increasing in glucose, the reaction is reached to a stage in which its speed doesn't change with increment in glucose concentration and it is stayed stable. When all of the active places in enzyme are filled, this phenomenon is occurred.

CONCLUSION

According to The results performance of biosensor, the optimum conditions for pH and glucose concentration are 6 and 1 mmollit-1, respectively. The loss of current production in acid solution (pH=4) is higher than alkaline solution (pH=8). The current transition using working electrode made in this paper (GOD/ GNp/MWCNTs/Pt electrode) is bigger than pure platinum electrode about 5.01 times in optimum conditions. According to the result of ANOVA analysis, the glucose concentration factor is more effective than the pH factor in the efficiency biosensor. By comparing the observed values of current and the predicted value, a little error was observed.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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