Int. J.Nano Dimens. 6(1): 23-30, Winter 2015
ISSN: 2008-8868

Contents list available at **IJND**

International Journal of Nano Dimension

Journal homepage: *www.IJND.ir*

The obtain optimum production conditions for Glucose Oxidase biosensor using software Qualtek-4

O. Ramezani Azghandi[1,*]
A. Farahbakhsh[2]

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

Received 01 August 2013
Accepted 12 October 2013

ABSTRACT

The Glucose Oxidase (Gox) electrochemical sensors were used to detect a little amount of glucose. In this paper, the pure platinum plate (length of 5 cm and a width of 3 mm), the Ag/AgCl electrode and platinum electrode (diameter of 1 mm) were applied as a working electrode (W.E), reference electrode and auxiliary electrode sequential. Factors measured, glucose concentration and pH is Phosphate-buffered saline (PBS) that design software QUALTEK-4 has been tested. The experiments performed tests of the software, with increasing concentration, the current output is increased. Optimal conditions were optioned in neutral pH and maximum glucose concentrations. After confirmation tests in optimum conditions the expected error rate of application lower than 10 %. It shows the true error rate test with high sensitivity and accuracy.

Keywords: *Sensors; Software Qualtek-4; Biosensors; Current; Glucose Oxidase; PBS.*

INTRODUCTION

The real impress of enzymes as analytical dean, however, was not sense until the mid-1960s and early 1970s, thought the fact that in 1951 Stetter usaged applications of gross enzymes to a species of analytical problems. Uppermost as 1845, Osann appoint hydrogen peroxide using peroxidase but in fact the operation of various enzymes provided as analytical dean, both soluble and immobilized on habitation vehicle, has debut descriptively since the 1970. Use of glucose oxidase (GOD) has been in the quantification of unihibit glucose in solvent, agricultural products, food processing and fermentation [1-2]. Mainspring Biosensors, particularly enzyme-based amperometric sensors, have been studied expandly because of their scientific quantification and commercial potential in both academic and applied fields [3-4].

* Corresponding author:
Omid Ramezani Azghandi
Young Researchers Club, Quchan Branch, Islamic Azad University, Quchan, Iran.
Tel +98 9153091381
Fax +98 5856236161
Email omid_r64@iauq.ac.ir

Glucose electrochemical biosensors based on the enzymatic oxidation of glucose oxidase have author creation considerable due to their advantages of high sensitivity and selectivity, quick response time, etc. for example, in these sensors, the immobilized GOD enzyme catalyzes the oxidation of glucose to gluconolactone in the attendant of oxygen, while coenzyme flavin adenine dinucleotide (FAD) is abated to $FADH_2$.propensity enzymatic reaction, molecular oxygen allegiance as an electron acceptor for $FADH_2$ and reoxidizes $FADH_2$ to FAD, whereas O_2 is abated to H_2O_2. The H_2O_2 is then discovering by amperometric measurement, allowing the determination of the accordance glucose concentration of the solution [4-5].

$$\text{Glucose} + O_2 \longrightarrow \text{Gluconolactone} + H_2O_2 \quad (1)$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

Amperometric glucose biosensor is the most ordinary used method for glucose detection, because of its advantages, such as naivete and quickness, but there are still some problems, such as tender linear range, low sensitivity and stability, which can't reconcile the detection necessary with high accuracy. In order to progress the efficiency of the glucose biosensor, remarkable research and too try have been appropriated to this field by many methods, such as the addition of redox mediators, nanoparticles, conducting polymer, carbon nanotubes, etc. [6-7]. Among the various MWCNTs engaged because of their singular property for novel biosensors amperometric by Layer-by-Layer (LBL) method [8].

Addition auspices has been paid to the use of MWCNTs as a promising electrode material due to the singular properties of MWCNTs, such as: enhanced electron transfer, high electrical conductivity, high mechanical and chemical and catalytically property, ability to grow on different substrates, particle geometrical: nanoscale 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, excellent chemical stability and high thermal capacity , good mechanical strength ,make increasing auspices and dutying with Biosensors of researchers in the field of electrochemical - Biosensors amperometric [9-15] .

The layer-by-layer (LbL) method has become the prime choice for fabrication of nanostructured films in which contribute between distinct materials may be acceded in a suitable, low-cost manner. With the LbL technique a wide variety of materials may be engaged, and film fabrication is done under temperance conditions, which is especially significant for preserving activity of biomolecules. Among such materials are those with electroactive properties which prompted researchers to engage LbL films for species usage, including: catalysis, electrochromism, electrochemical sensing and biosensing, among others. Remarkable, the LbL method has also lent itself for inquest of basal studies in electrochemistry, as in the use of the charge transport mechanisms and redox mediator immobilization [16-19].

The Taguchi method of experimental design is one of the widely accepted techniques for off line quality assurance of products and processes. This method is a traditional approach for robust experimental design that seeks to obtain a best combination set of factors/levels with lowest cost societal solution to achieve customer requirements.

Robust Design method, also called the Taguchi method, pioneered by Dr. Genichi Taguchi, greatly improves engineering productivity. Taguchi parameter design is based on the concept of fractional factorial design. The main objective of parameter design is to minimize the process or product variation and to design robust and flexible processes or products that are adaptable to environmental conditions Taguchi's approach to design of experiments is easy to adopt and apply for users with limited knowledge of statistics; hence it has gained a wide popularity in the engineering and scientific community. Many companies around the world have saved hundreds of millions of dollars by using the method in diverse industries: automobiles, xerography, telecommunications, electronics, software, etc [20].

In summary in this paper, we had that fabricated, glucose oxidase Biosensor, based on

modified Pt by carbon nanotube by LBL method, which was the applied potential of 0.7 V versus Ag/AgCl and the final Qualitek-4 software for design of experiments using the Taguchi approach was used.

EXPERIMENTAL

Reagents

Glucose oxidase (GOD, No. ART 6125, 50 KU / 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%), H_2SO_4 (M_w = 98 gr / mol, density = 84.1 kg / lit, purity 98 %), 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 ˚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 intermediate (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 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 a blunted 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 1). So MWCNTs modified by thiol function group is aggregate on the Pt electrode surface.

Preparing Biosensors

Platinum electrode coated by MWCNTs as a working electrode (W.E), Ag/AgCl the reference electrode and platinum electrode (diameter of 1 mm) applied as an auxiliary electrode sequential. The platinum working electrode was dipped in Semi-permeable membrane made of cellophane, contain of 0.01 M Gox/ PBS solution and the glucose nanobiosensor was make. The biosensor put on analyzes solution (various concentrations 0.01, 0.05 and 0.1 mM) for detection of glucose. After diffuse of glucose was started in membrane the reaction 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.

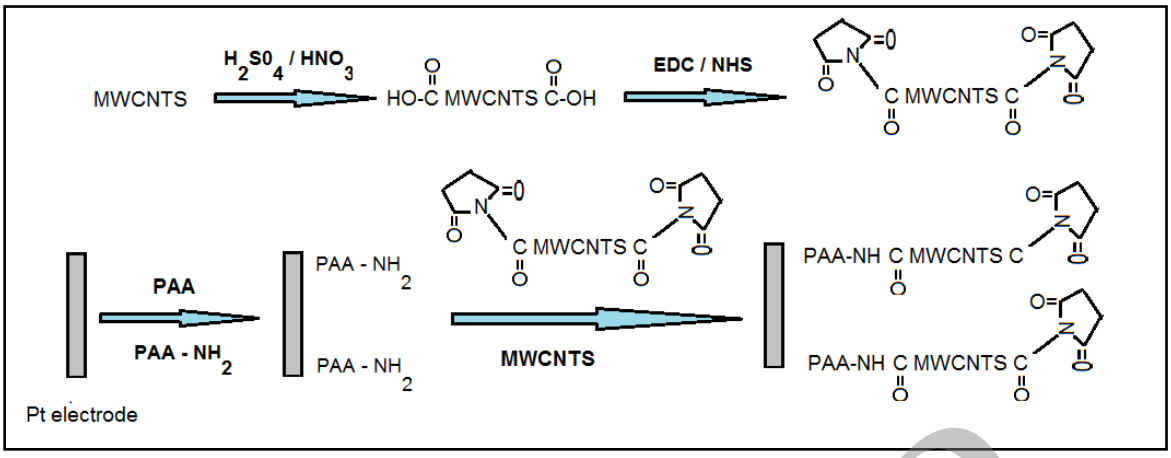

Fig. 1. Schemes of the pretreatment of MWCNTs (top) the stepwise fabrication process of the films on a Pt electrode (bottom)

Design of experiments

Dr. Taguchi has developed a method based on "ORTHOGONAL ARRAY" experiments which gives much reduced "variance" for the experiment with "optimum settings "of control parameters. Qualitek-4 software for design of experiments using the Taguchi approach was used in the present study [20-21].

Overall, design of the experiment has three steps:

1-planning phase (design)

2-conducting phase (result)

3-analysis phase (analysis)

Planning phase includes second subsection: automatic design and manual design that in automatic design, you simply indicate what are your factors and levels; it selects the array and assigns the factors to the appropriate columns. You also have a limited selection of interactions and outer array designs. While automatic design can handle most of your common experiment designs, the manual design option allows you to create the special designs to suit your needs also conducting phase. Analyses can be performed using Standard or S/N (Signal-to-Noise ratios) for Smaller (Eq. 3), Bigger (Eq. 4), Nominal (Eq. 5) or Dynamic Characteristics This case arises when a specified value is MOST desired, meaning that neither a smaller nor a larger value is desirable. Analysis phases consist of three basic steps: main effect, ANOVA and optimum studies. [22]

$n = -10\text{Log}_{10}$ [mean of sum of squares of {measured - ideal}]

(3)

$n = -10\ \text{Log}_{10}$ [Mean of sum squares of reciprocal of measured data]

(4)

$$n = 10\ \text{Log}_{10}\frac{\text{square of mean}}{\text{variance}} \qquad (5)$$

According ANOVA table, P% is the percentage contribution of each factor that obtains through the relation (Eg.) of 6.

$$P_F = \frac{SS_F - (DOF_F V_{Er})}{SS_T} \times 100 \qquad (6)$$

Where, DOF_F is the degree of freedom for each factor that is DOF_F = L-1 (L is the number of level for each factor). SS_T is the total sum of squares that is determined according to relation 7. In this equation, m and n present the number of experiments and the number of the repetitions, respectively. Y_T is the total average value of the measured results that can be computed by equation 8.

$$SS_T = \sum_{j=1}^{m}(\sum_{i=1}^{n} Y_i^2)_j - mn(\overline{Y}_T)^2 \qquad (7)$$

$$\overline{Y}_T = \frac{\sum_{j=1}^{m}(\sum_{i=1}^{n} Y_i)_j}{mn} \qquad (8)$$

Y_i is the response of experiment that in this work, the current production (output) has been chosen as Y_i. Meanwhile, SS_F is the factorial sum of squares, which was estimated using the following relation:

$$SS_F = \frac{mn}{L}\sum_{k=1}^{L}(\overline{Y_k^L} - \overline{Y_T})^2 \qquad (9)$$

Y_K is the average value of the results for each factor measured at K_{th} level. V_{Er} is the error variance that was computed by relation 10 [21-24].

$$V_{ER} = \frac{SS_T - \sum_{F=A}^{D} SS_F}{m(n-1)} \qquad (10)$$

The optimum process conditions for the production current the modified electrode were determined according to the Taguchi experimental design method using Qualitek-4 software. 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.

Finally, the software estimate of expect result S/N_{opt} and Y_{exp} and performance at any Arbitrary condition and contribution level of each factor. In this paper, the bigger-is-best (Eq.11) was used. The performance statistics was evaluated by using the following equations:

$$\frac{S}{N} = -10 \times \log(MSD) \qquad (11)$$

MSD = 10^[-(S/N)/10] (12)

MSD=[(1/y1)^2 + (1/y2)^2 + ... + (1/yn)^2] / n =[Avg. (1/yi)^2] = 1/Yexp^2

(13)

Yexp = SQR (1/MSD) (14)

RESULTS AND DISCUSSION

Analysis of results

According to the design which has been done by Qualitek- 4 for two factors of pH and C at three levels, Table1 has been obtained.

Table 1. Denote Factor and level in Design of Experimental

Factors	Level 3	Level 2	Level 1
pH	4	7	9
C(mM)	0.01	0.05	0.1

The results of experiments has been shown according to the test design in Table 2 and the optimal conditions are obtained according to the Table 2 and 3, based on S / N analysis on the basis of the highest value and the ANOVA analysis.

Table 2. Result experimental and S/N ratio

Number of examine	Current for recur 1 (mA)	Current for recur 2 (mA)	value S/N ratio
1	6.737	8.523	17.471
2	10.780	11.454	20.907
3	13.475	12.984	22.426
4	12.802	14.117	22.549
5	14.824	16.414	23.839
6	26.957	25.128	28.297
7	10.106	13.476	21.163
8	12.069	15.114	22.501
9	20.213	19.342	22.786

According to Table 2, Experiment 6 (pH=7 and glucose concentration= 0.1 mM) has been chosen as the optimal analysis and value S / N =28.297 and respectively mean current was obtained Y= 26.04.

The contribution of each factor on the by table analysis ANOVA (Table 3) is expressed in a different way, and shows to achieve optimum shift, which factor is more appropriate.

Table 3. Result Analysis ANOVA

Col # /Factor	DOF (f)	Sum of Sqrs. (S)	Variance (V)	F-Ratio (F)	Percent P (%)
pH	2	32.86	16.43	24.641	41.503
C(M)	2	40.434	20.217	30.32	51.474
Error	4	2.66	0.665		7.02
Total	8	51.838			100 %

ANOVA analysis delineate that change in the glucose concentration has more effect on the current produced higher rather than the change in pH. In a way that Sum of Sqrt., Variance, F-Ratio and P-Percent are 40.434, 20.217, 30.32 and 51.474 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 7.02, 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} = 27.659

Y_{EXP} = 24.143 mA

By using Potentiostat, the same amount of glucose 0.1 mM and pH =7 (confirmation test conditions) for platinum electrode uncoated carbon nanotube, current production measuring was evaluated, and 0.00539 A is obtained.

In summary, According to software forecasts, optimized conditions are with glucose concentration= 0.1 mM and pH =7 (As you can see in Figure 2).

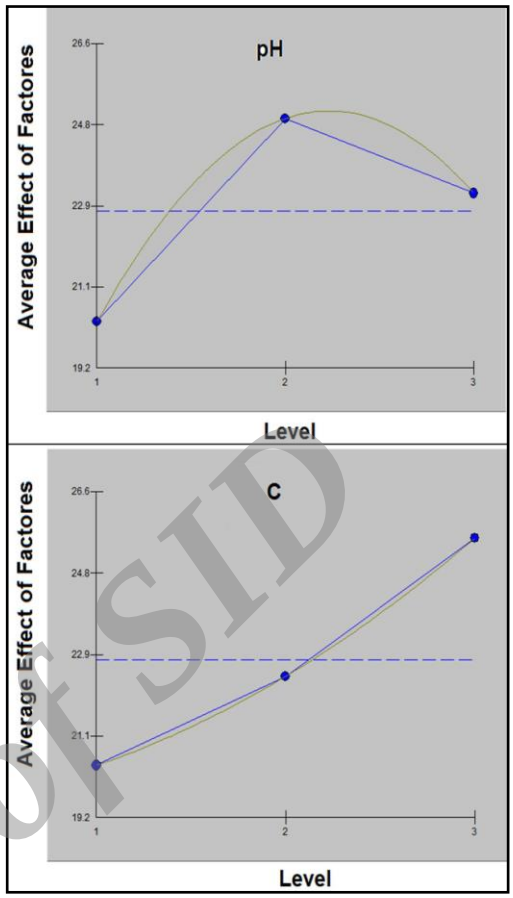

Fig. 2. Influence of different levels of each factor

It is also known as the level 2 of PH factor and level 3 of glucose concentration factor that has the greatest impact and confirms that bigger current the best. In this condition was tested again in the same conditions and the production current is obtained 25.21 mA. Table 4 is given for better comparison.

Table 4. Results of the predicted and measured

Results of the measured	Results of the predicted	
26.04	24.143	current/ mA Value
28.297	27.659	S/N value

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 error. The relative percentage error in the production current, between the measured and predicted condition are about 7.28 % and the relative percentage error between predicted current response and current response experimental confirmation is about 4.23% which respectively both relative percentage errors are less than 15% .

Also, after correction of enriched carbon nanotubes on the Pt electrode, it is determined that the optimal production is about 24.143 mA (predicted value) but for the case of the pure platinum electrode was made 5.3 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.

CONCLUSIONS

According the results of the performance of biosensor, it was demonstrated that in the optimal condition, the PH=7 and the concentration is at the maximum state. The percentage of the decrease of production in the optimal condition at acidity circle (PH=4), is higher than the alkali circle (PH=9). The amount of current production in the optimal conditions (pH =7 and C=0/1 mM for working electrode enriched by carbon nanotubes higher than net platinum working electrode also According to the result of ANOVA analysis, the glucose concentration factor is more effective than the pH factor in the efficiency biosensor. Also used to compare the observed values and predicted value of current was determined that the result error is little that shows the higher sensitivity of the made biosensor.

ACKNOWLEDGMENTS

This work was financially supported by research institute of petroleum industry of Iran (RIPI). The authors would like to express their sincere gratitude to all those who contributed to this research.

REFERENCES

[1] Wang H., Zhou C. H., Liang J., Yu H., Pen F., (2008), An enhanced Glucose Biosensor Modified by Pt/sulfonated-MWCNTs with Layer by Layer Technique. *Int. J. Electrochem. Sci.* 3: 1180-1185.

[2] Tasviri M., Rafiee-Pour H. A., Ghourchian H., Gholami M. R., (2011), Amine functionalized TiO_2-carbon nanotube composite:Synthesis, characterization and application to glucose biosensing. *Appl. Nanosci*. 1: 189-195.

[3] Zhu J., Kamiya A., (2002), Surface tension, wettability and reactivity of molten titanium in Ti/yttria-stabilized Zirconia system. *Mater. Sci. & Eng. A.* 327: 117-127.

[4] Le T. T., Tran P. D., Pham X. T., Ton D. H., Dang CH. M., (2010), Glucose oxidase immobilization on different modified surfaces of platinum nanowire for application in glucose detection. *Adv. Nat. Sci. Nanosci. Nanotech*. 1: 1-4.

[5] Garjonyte R., Malinauskas A., (2000), Amperometric glucose biosensors based on Prussian Blue and polyaniline glucose oxidase modified electrodes. *Biosen. & Bioelectronics.* 15: 445-451.

[6] Wang H. J., Zhou C. M., Peng F., (2007), Glucose biosensor based on platinum nanoparticles supported sulfonated-carbon nanotubes modified glassy carbon electrode. *Int. J. Electrochem. Sci.* 2: 508-516.

[7] Balasubramanian K., Burghard M., (2006), Biosensors based on carbon nanotubes. *Anal. Bioanal. Chem*. 385: 452-468.

[8] Olek M., Ostrander J., (2004), Layer-by-layer assembled composites from multiwall

carbon nanotubes with different morphologies. *Nano Lett.* 4: 1889-1895.

[9] Chen J., Rao A. M., (2001), Dissolution of Full-Length Single-Walled Carbon Nanotubes. *J. Phys. Chem. B.* 105: 2525-2528.

[10] Tahermansouri, H., khoei D. Ch., Meskinfam M., (2010), Functionalization of carboxylated multi-wall nanotubes with 1,2-phenylenediamine. *Int. J. Nano Dimens.* 2: 153-158.

[11] Iijima S., Ichihashi T., (1993), Single-shell carbon nanotubes of 1-nm diameter. *Nat. Vol*. 363: 603-605.

[12] Antiochia R., Gorton L., (2007), Development of a carbon nanotube paste electrode osmium polymer-mediated biosensor for determination of glucose in alcoholic beverages. *Biosensors Bioelect.* 22: 2611-2617.

[13] Odaci D., Telefoncu A., Timur S., (2008), Pyranose oxidase biosensor based on carbon nanotube (CNT)-modified carbon paste electrodes. *Sens. Actuat. B.* 132: 159-162.

[14] Guo D.J., Li H.L., (2005), Highly dispersed Ag nanoparticles on functional MWNT surfaces for methanol oxidation in alkaline solution. *Carbon*. 43: 1259-1264.

[15] Anik U., Cubukcu M., (2008), Examination of the Electroanalytic Performance of Carbon Nanotube (CNT) Modified Carbon Paste Electrodes as Xanthine Biosensor Transducers. *Turk. J. Chem*. 32: 711-719.

[16] Zhang Y., Wang Y., Zhang Sh., Zhang L., (2011), Fabrication of Multilayers Film of Myoglobin and Glutaraldehyde on the Surface of Gold Electrode Based on Covalent Interaction and Its Application. *Am. J. Biomed. Sci*. 3: 210-217.

[17] DeLongchamp D.M., Kastantin M., Hammond P.T., (2003), High contrast electrochromism from layer-by-layer polymer films. *Chem. Mater*. 15: 1575-1586.

[18] Cheng L., Dong S., (2000), Comparative studies on electrochemical behavior and electrocatalytic properties of heteropolyanion-containing multilayer films prepared by two methods. *J. Electroanal. Chem.* 481: 168-176.

[19] Crespilho F. N., Zucolotto V., Oliveira O. N., Nart F. C., (2006), Electrochemistry of Layer-by-Layer Films. *Int. J. Electrochem. Sci*. 1: 194-214.

[20] Upadhye R. A., (2012), Optimization of Sand Casting Process Parameter Using Taguchi Method in Foundry. *Int. J. Eng. Res. & Tech.* 7: 1-11.

[21] Madaeni S. S., Koocheki S., (2006), Application of taguchi method in the optimization of wastewater treatment using spiral-bound reverse osmosis element. *Chem. Eng. J.* 119: 37-44.

[22] Farahbakhsh A., Taghizadeh M., Yakhchali B., Movagharnejad K., (2011), Stabilization of Heavy Oil-Water Emulsions using a Bio/Chemical Emulsifier Mixture. *Chem. Eng. Technol.* 34: 1807-1812.

[23] Kim S. T., Yim B., Park Y., (2010), Application of Taguchi Experimental Design for the Optimization of Effective Parameters on the Rapeseed Methyl Ester Production. *Environ. Eng. Res*. 15: 129-134.

[24] Mehravar R., Jahanshahi M., Najafpour Gh. D., Saghatoleslami N., (2011), Applying the Taguchi Method for Optimized Fabrication of Lactalbumin Nanoparticles as Carrier in Drug Delivery and Food Science. *Iranica. J. Ener. Environ.* 2: 87-91.

Cite this article as: O. Ramezani Azghandi *et al.*: The obtain optimum production conditions for Glucose oxidase biosensor using software Qualtek-4.
Int. J.Nano Dimens. 6(1): 23-30, Winter 2015