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Research Paper | Medical Science

Studies on Designing, Optimisation and Application of Biosensor using Glucose Oxidase of Aspergillus niger F- C405-2

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ABSTRACT

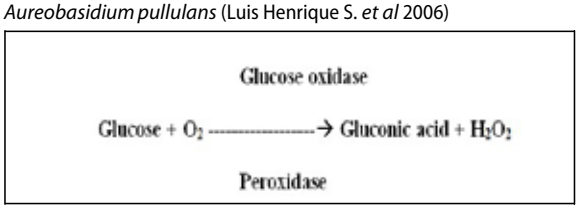
Biosensor is a device which detects and combines with a biological component analysed with a physicochemical detector component. In present work we have designed a glucose biosensor using immobilised glucose oxidase enzyme on cellulose acetate produced by Aspergillus niger F- C405-2. This glucose oxidase (GO) has shown thermal stability at 600C for 120 minutes. Glucose oxidase was immobilized on pyrrol-cellulose acetate (Py-CA) film over Indium tin oxide (ITO). Platinum and silver-silver chlorides are used as reference electrode and suitable for estimating blood sugar with commercial glucometer. The biosensor prepared was optimised and calibrated against standard glucose solutions of variable concentration (0.5 to 6 mg/ml) with change in electric current measured in ampere. The amount of glucose present in patient's blood was estimated using our designed biosensor and diagnosed for diabetes. Our instrumental results were found to be correlated with that measured using commercial glucometer. No significant deviation was observed.

KEYWORDS : Blood Sugar, Biosensor, Immobilization, PY-CA-GO Film.

Introduction:

Glucose oxidase (E.C. 1.1.3.4) is a very important non-hydrolytic enzyme that belongs to the oxidoreductase family. Glucose oxidase is also called as glucose aerodehydrogenase. Glucose oxidase acts as a catalyst and oxidizes β-D-glucose to gluconic acid. It is produced by number of organisms, especially Fungi such as *Aspergillus niger, Aspergillus flavus, Penicillium variabile, Penicillium adametzii, Penicillium amagasakiene, Pleurotus ostreatus* and *Yeast*

Aureobasidium pullulans (Luis Henrique S. *et al* 2006)



Glucose-oxidase (GO) is an important diagnostic enzyme in Food and Pharmaceutical industry (Shazia Khurshid 2008). GO is also used extensively in detection of glucose from blood, food, and fermented products. For follow up treatment, blood glucose of diabetic patients should be monitored and checked periodically. Therefore, in such cases a reliable method of glucose determination is required, which should ideally be accurate, precise, specific, rapid, sensitive and inexpensive.

Many different methods have been described for the determination of glucose in biological fluids. There are two Methods - chemical and enzymatic. Enzymatic methods have been developed to increase specificity, sensitivity, and also reduce interference. Recently immobilized enzyme technology is used where enzyme itself is immobilized on a support, so it is not discarded with reaction mixture after analysis has been completed. Consequently, the enzyme can be recovered and recycled (Sudarat Manochiopinij *et al* 1985).

The immobilization technique for localizing enzyme at the surface of various electrodes plays a very important role in the research of glucose biosensor. The conducting polymers are being widely used in biosensor applications because it provides stable and porous matrix for immobilization of bio component and it also facilitates the electron transfer process. The widely used conducting polymers for immobilization of enzyme are polyaniline, polypyrrole and polythiophene.

In this paper we have focused on use of immobilized GO in biosensor which is suitable for detection of Glucose. In addition, an evaluation of sensitivity, accuracy and precision of glucose determination with prepared membrane has been made and comparative studies of glucose determination using prepared membrane and using the lab certified glucometer has also been carried out.

Material Methods:

Isolation, Screening and Optimization of GO production:

Total 42 isolates were isolated from various agriculture lands and hot water spring at Unkeshwar Nanded, MS, India. They are subjected for primary screening by plate assay and 16 GO producers were assed quantitatively. During secondary screening *Aspergillus niger* strain 18 was then selected as an efficient GO producer at 50^0C and identified by means of morphological characters and 16/23/28S rDNA sequence analysis.

The optimum temperature and pH for growth and production of glucose oxidase from screened culture of *Aspergillus niger* strain F-C405-2 was determined by growing them at various temperatures (40-50-60^{0}C) and pH (5-6-7-8-9). Similarly the production of glucose oxidase was monitored periodically giving higher yield after 72 hours of incubation and in shake flask agitated at 180 rpm.

Production of glucose oxidase was carried using various carbon and nitrogen sources. Similarly to make the cost effective production of glucose oxidase various natural carbon sources such as beet root, mix fruit extract, sorghum and molasses were used. As metal ions were found to be playing important role in production and regulation the enzyme productions was carried out using different metals.

Strain improvement studies were performed by developing strains using physical mutagenic

agents' viz. UV, gamma radiations and chemical mutagenic agents like ETBR and Sodium azide. Immobilisation of whole cells of *Aspergillus niger* strain F-C405-2 was carried out using various immobilising agents viz. Sodium alginate, Gelatine, Polyvinyl Chloride (Wong Fuizing and Azila Abdul, 2004) and Cellulose acetate and cross linking with glutaraldehyde. Highest production was noted in case of cellulose acetate.

Impact of immobilization on the enzyme activity was studied and compared with the free enzyme. Among the various agents cellulose acetate showed its maximum enzyme activity. The effect of temperature and pH on activity of free enzyme and the immobilized enzyme produced were studied. Free and immobilized form of enzyme is optimally active at 40^0 and 50^0C and pH - 6.00.

Primarily the immobilized Glucose-oxidase (GO) on cellulose acetate was used to measure blood sugar level from various patients as

shown in Figure No.1. The next level was designing glucose oxidase biosensor by preparing glucose sensitive electrode as shown in Figure No.4. (Shirale D. J., 2006).

Biosensor Preparation

In a typical procedure, Cellulose acetate was dissolved in Pyrrole (Py) with 14 wt % of Cellulose acetate (CA) in the Pyrrole solvent. The Py–CA viscous solution was stirred overnight at room temperature until a homogenous solution was obtained.

The CA film was then prepared using wet cast technique as follows:

The resultant Py–CA viscous solution was spread on a glass plate (75 x 25 mm) with about

100 um thickness. The plate with the sprayed Py–CA solution layer was immersed immediately in aqueous solution containing 80mM concentrations of $FeCl_3$ at 25^0C, which coagulates CA and polymerizes Py. The Polypyrrole (PPy) formed in the CA film matrix on the glass plate turns black after a certain time. The resultant black film was washed using distilled water as per Figure No.2.

These membranes are then placed in glucose oxidase solution prepared in 0.1M phosphate buffer (pH 6.0) at 25^0C for 24 hours. The membranes are washed thoroughly with de-ionized water in order to remove any unbound enzyme**.** The change in response potential of the active device is of interest for sensor application. The conductivity of PY-CA-GO electrode depends on several factors, such as analyte, pH, temperature, polymer film potential, substrate concentration and enzyme loading. The GO is immobilized on PY-CA film by cross-linking via glutaraldehyde. The potential-time relationship of PY-CA-GO electrode when applied current of enzyme was set 0.5 mA in phosphate buffer as shown in Figure No.3 and 4.

Results and Discussion:

We have isolated various thermophilic (thermoduric) and alkalophilic microorganisms. By applying various strategies we have selected a strain of *Aspergillus* (identified at molecular level by

28S rDNA technology) as *Aspergillus niger* F-C405-2 similar to Luis Henrique *et al.* (2006). The

fermentation medium was modified and newly designed by using native components and other supplementary carbon and nitrogen sources which were found to increase the production. Similarly other physiological and environmental parameters were optimised and used for large scale production in the form of stirred batch fermentation (Sherbeny G.AEL 2005). Similarly strain

improvement was carried out and random mutagenesis with UV radiations and treatment with ethidium bromide gave satisfied results. By considering associated risk we have selected UV mutagenesis further for programme mutagenesis as observed by Muhammad Ramzan and Tahir Mehmmod (2008).

From the point of biotechnology it is every important to introduce novel methods. The

production of glucose oxidase was carried out using different immobilising matrices. Immobilisation has lot of advantages as to keep the product intact and pure; one can reuse the intact cells for many times for production, protecting the cells as well as enzyme against various corrosive agents in fermentation medium etc. In present study we were successful in getting near about all these advantages. Immobilised cells with cellulose acetate produced GO in short period of time (48 hours) as compared to intact cells, enhanced thermal (50^0C for 180 min) and pH stability and many other properties. The physico-chemical characters of our glucose oxidase were found to be matching with what is recorded by various scientists as per their reviews.

In vitro utility of our enzyme was studied in various fields. It was found to be a novel preservative and stabiliser as glucose oxidase results into synthesis of gluconic acid. It not only reduces alcohol content (by 2%) in wine but also increases shelf life of wine. Similarly we have also used this enzyme in baking, production of gluconic acid etc.

In our last phase of research work more emphasis is given on designing biosensor. The biosensor we have designed was used to determine blood sugar of different patients.

Sensitivity and Stability: Optimum sample volume was 100µl. A calibration curve using series of standard glucose solution was constructed. Figure No.4 shows straight line relationship

500-5000µg/ml. With the increasing concentration of glucose, the response potential also increases and finally reached steady state value. Standard graph of glucose is plotted (Figure No.4). Different concentration of glucose from normal, diabetic and severe diabetic persons was determined (Table No.2). Efficiency of glucose oxidase biosensor is determined by correlating our outputs with the results obtained using commercial biosensor (Glucometer form) prepared using glucose oxidase synthesised chemically. In present case assuming that the enzyme is uniformly distributed throughout the film, the reaction takes place predominantly on surface of the film. It was found that prepared membrane remained stable enough to allow at least 100 consecutive analyses over 8 hours and was therefore suitable for routine services.

The analytical accuracy of our own designed and prepared biosensor of glucose oxidase was

measured by estimating blood sugar. The results were presented in Table No.2. Blood sugar level monitored from patient's blood sample using our own designed biosensor of glucose oxidase a product of our research. To prove the efficiency and accuracy of the designed biosensor results were compared with that determined using commercially used glucometer in all pathological laboratories. While comparing it was observed that the deviations obtained were not statistically significant. When correlation studies were carried out it was observed that both data positively correlate with each other. Similar results were observed by Sudarat Manochiopinij in 1985 in proposed method of membrane bound biosensor. They offer same precision and accuracy of analysis as that of glucometer from pathological laboratories.

The advantages of our biosensor is its thermo stability by which it will help to increase durability, long time use and economical.

Figure No.1: Glucose oxidase immobilization film

Figure No.2: Pyrrole and Cellulose acetate

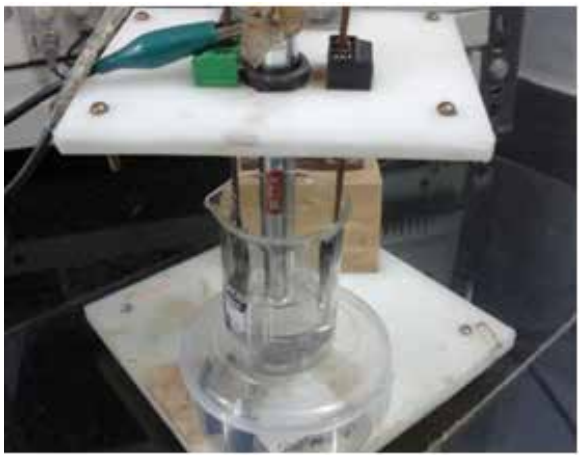


Figure No.3: Assembling of Py-CA-GO electrode

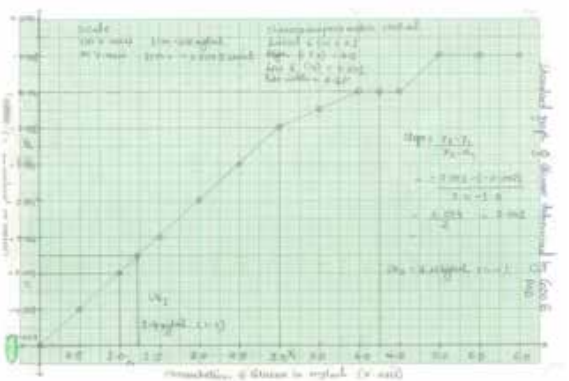


Figure No.4: Standard graph of glucose using GO biosensor

Table No.1: Standard graph of Glucose using GO biosensor

Sr. No.	Concentration of glucose (ug/ml)	Current (Ampere)
1	500	-0.008
2	1000	-0.007
3	1400	-0.0065
4	1500	-0.006
5	2000	-0.005
7	2500	-0.004
8	3000	-0.003
9	3500	-0.0025
10	4000	-0.002
11	4250	-0.002
12	4500	-0.002
13	5000	-0.0015
14	6000	-0.0015

Table No.2: Blood sugar detection by GO biosensor

Sr. No.	No. of patients	Blood sugar estimated in mg/dl	
		Commercial Glucometer	GO based designed biosensor
1	2F	77	74
2	75F	105	110
3	3F	124	130
4	5P	148	147
5	3P	171	169
7	4P	291	287
8	1F	339	335
9	6P	370	386
10	1P	495	490
11	SD	143.88	143.68
12	CV	61.05	60.07

Statistical analysis revealed that there is no difference in coefficient of variation for blood sugar measured by glucometer (61.05%) and lab made biosensor (60.07%). Hence the entire experiment performed using biosensor was statistically accepted with the same accuracy and precision as commercial glucometer.

In our last phase of research work more emphasis is given on designing biosensor. The biosensor we have designed was used to determine blood sugar from different patients. As per our observations, statistically we have proved that the efficiency of our biosensor by comparing its efficiency with that of commercially available glucometer.

REFERENCES

1. Bodade Ragini C., Chandarahas N. Khobragade and Saiful Arfeen (2010), Optimization of culture conditions for glucose oxidase production by a Penicillium chrysogenum SR19 strain. Eng. Life Sci. 10, No.1, 35-39. | | 2. Chudobova I., Vrbova E., Kodicek M., Janovcova J.and Kas J. (1996), Fibre optic biosensor for the determination of D-glucose based on absorption changes of immobilized glucose oxidase. Anal Chemical Acta; 319(1):103–10. | | 3. Chang D. A. and Yen B. K. Gale (2006), Design and Fabrication of a multianalyate capable optical Biosensor using a multiphysics approach. Journal Micro/Nanolith MEMSMOEMS 5(2), 021105. Doi:10.117/1.2203666. | | 4. D. J. Shirale, V. K. Gade, and P. D. Gaikwad (2006), Studies of immobilized glucose oxidase on galvanostatistically synthesized poly (N-methyl pyrrole) film with PVS-NaNo3 composit dopant | 5. Gothoskar V.S. and S.M Dharmadhikari,. (2013), Screening and cultural studies of Aspergillus niger | for production of Glucose oxidase published in I.J.A.R. 3(6), ISSN-2249-555X, PP 79-80. | | 6. Luis Henrique, Guimarae S. and Simone C. (2006), Screening of Filamentous Fungi for production of | Enzyme of Biotechnological Interest. Brazilian Journal of Microbiology , 37: PP 474-480.ISSN 1517- | 8382 | | 7. Muhammad Ramzan and Tahir Mehmmod (2011), Enhanced production of glucose oxidase from UV | mutant of Aspergillus niger. African Journal of Biotechnology Vol.10 (65). PP14522-14533, 24 October | ISSN1684-5315. | | 8. Shazia Khurshid, Muhammad Akram Kashmiri, Zahid Quershi and Waqar Ahmad (2011), Optimization of glucose oxidase production by Aspergillus niger. African Journal of Biotechnology. Vol. 10(9), PP 1674- | 1678. | | 9. Sherbeny G.A.EL, Shindia A.A., and Sheriff (2005), Optimization of various factors affecting glucose oxidase activity produced by Aspergillus niger. International Journal of Agriculture and Biology 1560- | 8530/2005/07-6- PP 953-958. | | 10. Sudarat Manochiopinij, Chamras, Sapsammarnwong and Kulnaree Sirisali (1985), Detection of glucose using immobilized glucose oxidase, Journal of Sciences Soc. Thailand 11 PP 79-85. | | 11. Wong Fuizing and Azila Abdul Aziz(2004), Immobilization glucose oxidase on polyvinyl alcohol., Department of Bioprocess Engineering Faculty of Chemical and Natural resources Engineering University Technology Malaysia |